Summer 2000

Investigations on the Use of Ion Mobility Spectrometry for Clinical Chemistry Applications

Henri Parson Patten
Old Dominion University

INVESTIGATIONS ON THE USE OF ION MOBILITY SPECTROMETRY FOR CLINICAL CHEMISTRY APPLICATIONS

by

Henri Parson Patten
B.A. December 1994, Hampton University

A Dissertation Submitted to the Faculty of
Old Dominion University and Eastern Virginia Medical School
in Partial Fulfillment of the Requirement for the Degree of

DOCTOR OF PHILOSOPHY

BIOMEDICAL SCIENCES

OLD DOMINION UNIVERSITY
August 2000

Approved by:

Edward J. Poziomek (Director)

Barbara Hargrave (Member)

Patricia A. Pleban (Member)

Mark S. Elliott (Member)

ABSTRACT

INVESTIGATIONS ON THE USE OF ION MOBILITY SPECTROMETRY FOR CLINICAL CHEMISTRY APPLICATIONS

Henri Parson Patten
Old Dominion University, 2000
Director: Dr. Edward J. Poziomek

The major objective of this research is to examine ion mobility spectrometry as a rapid screening tool for specific application to clinical chemistry research and laboratory use. Methodology was developed for target analytes representing several classes of physiologically active substances, including anesthetics, illicit drugs, and their metabolites. The IMS characteristics of animal tissues and other compounds such as amino acids and proteins were determined. Quality assurance and control procedures were developed for specific quality data objectives. Criteria were established relating to use of IMS for assessing the precision and accuracy of data, qualitative screening, and semi-quantitative analyses.

It was found that animal tissues and plasma harvested from rabbits can be characterized using IMS. The mobility spectra of these tissues were also found to contain peaks assigned to the anesthetics, Rompun and Ketaset, heparin, and ecgonine methyl ester (EME), a cocaine metabolite. Enhancement and retardation effects were identified with cocaine and its metabolites as well as with heroin, and its metabolite 6-acetylmorphine. A nonspecific interaction of heroin and morphine with animal tissues and different proteins was also identified using IMS. It was concluded that the

use of IMS for clinical applications is feasible. The benefits and limitations of using IMS for clinical chemistry applications were identified.

This thesis is dedicated to my family.

ACKNOWLEDGMENTS

All honor, praise, and glory is due to God. I offer this dissertation as an act of worship. Through this experience, God has patiently guided me with love and mercy, providing me with the strength, peace of mind, and endurance to see it to completion.

This accomplishment would not have been possible without the unending support of my family. To my mother, Ada Wilson, who walked with me through this journey, from the first step to the last, constantly lifting me up to God in prayer. To my father, Wayman Parson, who taught me that there is nothing I cannot achieve through hard work and determination. To my sister, Angela Parson, who is one of my best friends and has suffered through lots of hard work and many sleepless nights. To my husband, Edward Patten Jr., you are my soul mate and comforter, and my son, Edward Patten III, you give me inspiration and constantly remind me that you are watching the examples that I set.

I would like to extend my heartfelt thanks to my step-father Milton Wilson for his wisdom and assistance on the many challenge of life. To Willar White-Parson and Davida White-Pettaway, I offer my gratitude. My warmest appreciation also goes to my extended and church family, who are too numerous to name.

Dr. Poziomek, you have been an excellent mentor to me. You have provided me opportunities that have facilitated my growth as a scientist, speaker, and analytical thinker. Your extensive knowledge, innovative ideas, and organizational skills have shown me the meaning of scientific excellence. I am grateful and thankful for your patience, sincere interest, and involvement in this educational endeavor. Dr. Hargrave,

thank you for the use of your laboratory, materials, skill, and willingness to participate in this project. Dr. Pleban, you have guided me through my coursework and have provided me with knowledge to be an effective clinical chemist. Dr. Elliott, your humor and unique ways of viewing science will always be fondly remembered.

To all of my co-workers who have contributed in so many ways to this research project, I could not have done it without you. Sincere thanks is given to Grazyna Orzechowska, Julie Patrick, Juliana Homstead, Vangielynn Tersol, Hidayah Kendall, Saeed Almeer, Marsha Galicia, Andrea Chambliss, Anne Reamy, and Maricel Eucebio.

A special note of appreciation is given to those who have supported me through their sheer generosity and willingness to give. Dr. Lester Pretlow, you introduced me to this program and made sure that I started off on the right foot. Hidayah, I cannot count the times when you have offered your support to me and have been a true friend. Brian Austin, without your computer expertise, I would still be working on my dissertation. To Lennis Whitaker and Lorne Dennis, you helped me to keep everything in perspective and provided me with smiles and laughter when I wanted to cry.

Support from the Counterdrug Technology Development Office (CDTDPO) – John J. Pennella, under Contract DAAB10-95-C-0021 through the U.S. Army Communications – Electronics Command, the Army Research Office under Grant No. DAAH04-96-1-0211, and the Virginia Council of Higher Education is gratefully acknowledged.

TABLE OF CONTENTS

Chapter Page

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

Figure Page

CHAPTER I

INTRODUCTION

Ion Mobility Spectrometry for Clinical Chemistry Applications

Ion mobility spectrometry (IMS) has historically been used to analyze environmental hazards, chemical warfare agents, explosives, and drugs on surfaces and in ambient air. This fast and sensitive technique has not, however, been extended for use in clinical laboratories. Clinical chemistry, which incorporates many aspects of physiology and diagnostic medicine, is a dynamic science which continually needs new measurement technologies. IMS is a simple technique that offers promise for clinical chemistry applications in view of its diversity as an analytical tool. IMS offers the potential of detecting target analytes in the timely, efficient, and cost effective manner required in a clinical chemistry setting.

Ion Mobility Spectrometry (IMS)

IMS is an analytical technique that characterizes chemical substances through gas-phase ion mobilities. These substances, initially in gas, liquid, or solid form, are vaporized and allowed to flow into the reaction region of a drift tube at atmospheric pressure. Most IMS instruments use ambient air pumped into the spectrometer through a semipermeable membrane. Molecules are ionized and the product ions are allowed to enter into the drift region under a weak electric current for mobility analysis. Ionization preferences of analytes, and mobility differences of the ions or ion clusters give

---

The model for this dissertation was Analytica Chimica Acta

specificity. Analysis and characterization are based on analyte separations due to ionic mobilities rather than masses; this distinguishes IMS from mass spectrometry (MS). During analysis, the product ions are measured when they collide with a detector; current is recorded continuously versus time, and produces a spectrum [1]. An example using cocaine is given in Fig. 1.

Although the required chemistry and physics of ions in ambient air were developed in the early 1900's, it was 1970 when IMS was first introduced (Cohen and Karasek [2,3]) as a technique for organic chemical analysis. Initially thought of as a type of chromatography, many researchers looked for IMS to become an alternative for mass spectrometry. Due to the lack of information concerning ion molecule chemistry, ion behavior at atmospheric pressure, and applicability to mixtures, many publications of the 1980s rejected IMS, deeming the instrument to be confusing and too complicated for practical application. Nevertheless, research continued, and IMS was successfully developed by military agencies as a sensitive detector for chemical warfare agents [4]. Most exploratory research done during this period was conducted by a small number of universities and government laboratories. During the 1990's a renewed interest in IMS technology emerged due to refinements in instrumentation and an increased understanding of its fundamental principles. When compared to conventional analytical techniques, IMS provides an abundance of information, without the power, size, and weight demands of other techniques such as gas chromatography-mass spectrometry (GC/MS) and Fourier-transform infrared (FT/IR) spectroscopy. Today, IMS is being employed to detect and analyze environmental analytes, [5-7] illicit drugs, [8-11] and

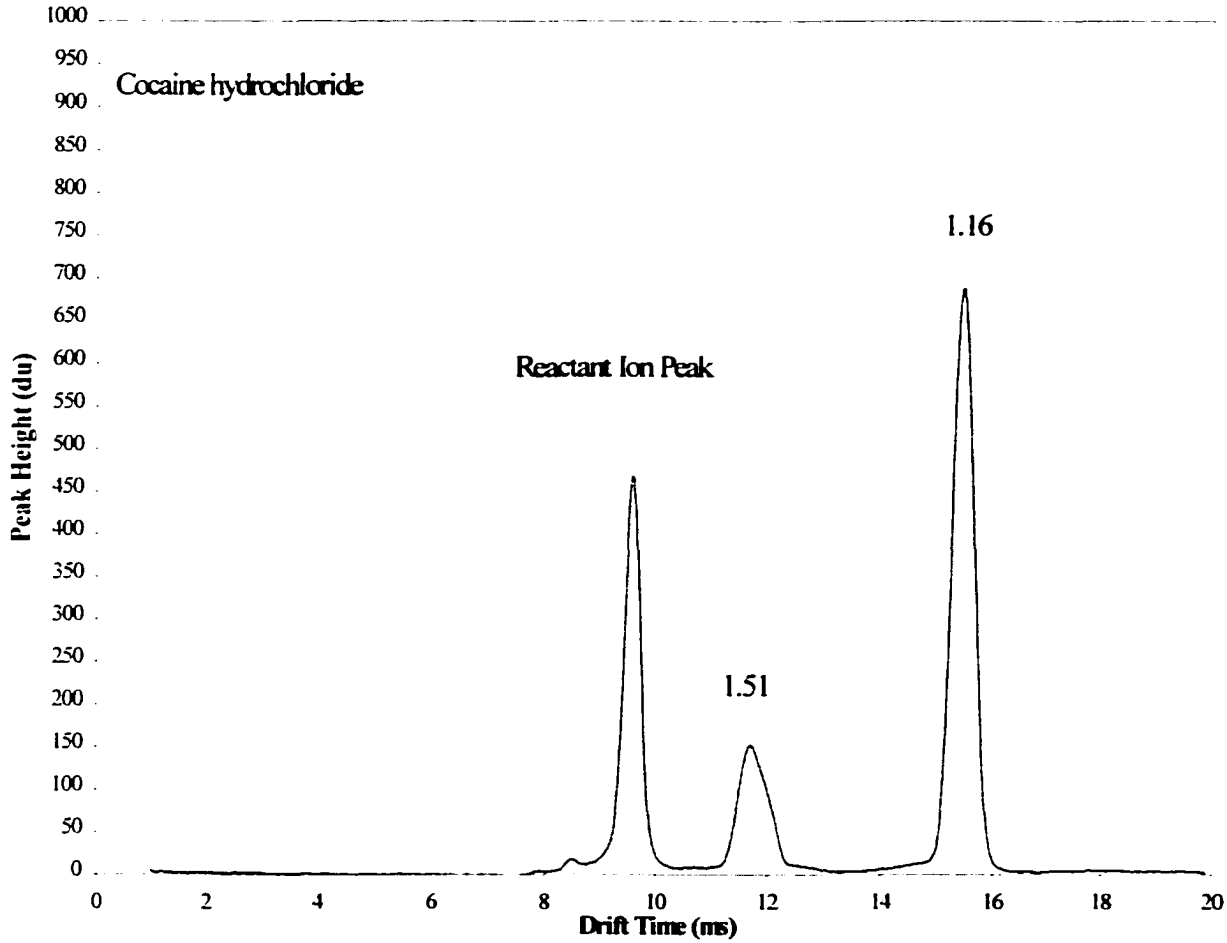


Fig. 1. IMS mobility spectrum of 75 ng cocaine hydrochloride. The characteristic peak of cocaine has a K_o value of 1.16 $cm^2V^{-1}s^{-1}$. The peak at K_o, 1.51 $cm^2V^{-1}s^{-1}$ is a thermal decomposition product of cocaine formed in the spectrometer.

explosives [12-17]. It continues as an important field method for chemical warfare agents. Table 1 gives a few examples of the types of chemicals that have been detected with IMS.

Table 1
Examples of chemicals reported to be detected using IMS [2]

Organophosphorus esters	Phthalic Acids
C Organophosphorus esters	Aromatic hydrocarbons
S Chloropentafluorobenzene	Alkyl amines
E SF_6	Anesthetics
N Explosives	Ethylcellosolve acetate
B Narcotics	Dicarboxylic acids
C Benzene	Nitrobenzaldehyde
N Chloroform	Alkyl esters
Nitrobenzene	

IMS technology continues to be reviewed for new applications in analytical chemistry [4, 18-22]. Attributes of small size, low weight, and low power have led IMS to be considered for on-site analytical applications. Ionization chemistry can be modified to focus IMS selectivity and to allow an analyzer to be developed for specific scenarios [1]. Research on ionization methodology, behavior of ions in air at atmospheric pressure, and data processing continues. Improvements in instrument design, and the coupling of IMS technology with other analytical instruments, such as gas chromatography (GC-IMS) and mass spectrometry (IMS-MS), hold much promise for increased utility and new applications.

Instrumentation. Available IMS instrumentation ranges from sophisticated laboratory models to hand-held field units. IMS electronics include a high voltage supply, shutter control, a signal processor, and a data system. The basic utilities or

mechanics include inlet control, temperature control, and flow control [23]. A typical IMS system can be divided into several sections: sample introduction, reaction region, ion drift region, and ion collection/detection. A schematic of the Barringer IONSCAN IMS system is shown in Fig. 2 [24].

Analyte vapors are introduced either by direct sampling of vapors or by thermal desorption from a solid matrix such as a swipe material. In the Barringer IONSCAN, the solid and liquid samples are introduced through a desorber inlet. The sample is deposited on a membrane (e.g., Teflon, fiberglass, or paper). The membrane is placed into the IONSCAN where the analyte is desorbed as vapors. These vapors pass into the reaction chamber in a flow of carrier gas, where they are ionized using ^{63}Ni. Product ions are gated into the drift tube where the drift times in an electric field are measured. Operations are normally performed at atmospheric pressure using ambient air as the carrier and drift gases, though other gases can also be used.

Drift tubes are constructed with different designs, dimensions, operating parameters and materials. Most drift tubes are made of stainless steel as a conducting surface, and insulating components (machinable glass, Teflon, ceramic, or other plastics) [23]. The electric field in a drift tube is provided by a high voltage supply (typically ±1-10 kV). Electric pulses are applied to an ion shutter to create multiple ion mobility spectra.

The ionization source, normally housed in the reaction region of the drift tube, must be insensitive to oxygen, moisture, and other components of sampled air. Ionization methods include use of radioactive sources, photoionization, corona-spray

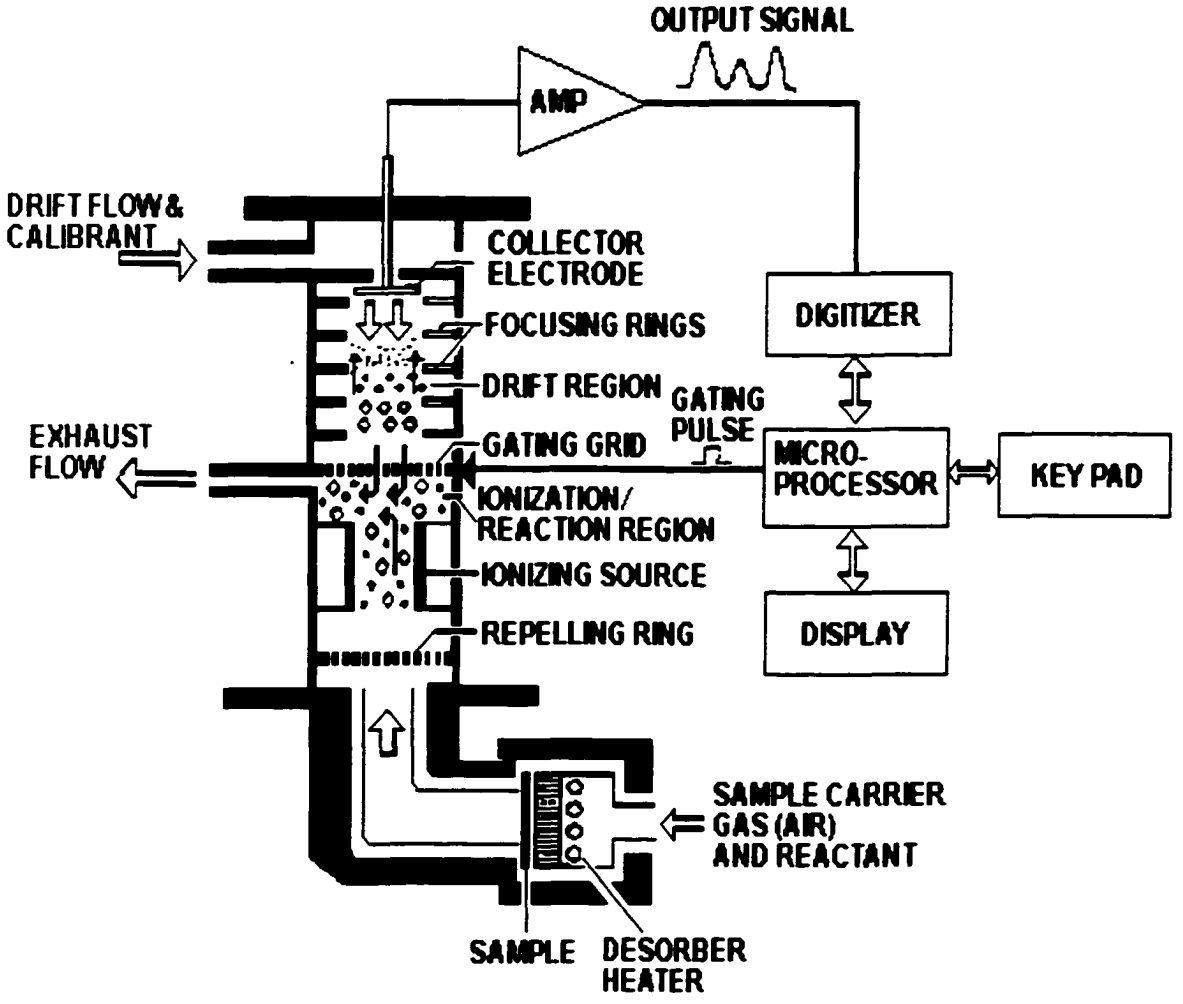


Fig. 2. Schematic of the Barringer IONSCAN 400 [24]

ionization, flame ionization, and corona discharge [1]. A very common detection method to measure ion current utilizes a circular collector plate.

Chemistry. There are two major chemical processes in IMS analysis. The first is the gas-phase ionization of target analyte molecules through charge exchanges or ion-molecule reactions at atmospheric pressure [1]. The second is analyte ion mobility in a weak electric field.

Target molecules are ionized by either proton-transfer reactions (Equation 1), and negative ion reactions such as ion transfer (Equation 2), charge transfer (Equation 3), or dissociative charge transfer (Equation 4) [1].

$$T + H(H_2O)_n^+ \longrightarrow TH^+ + nH_2O;\ n = 1\text{-}3 \quad (1)$$

$$T + (H_2O)_nO_2^- \longrightarrow T \bullet O_2^- + nH_2O;\ n = 1\text{-}3 \quad (2)$$

$$T + (H_2O)_nO_2^- \longrightarrow T^- + O_2 + nH_2O \quad (3)$$

$$T\text{-}X + (H_2O)_nO_2^- \longrightarrow X^- + T + O_2 + H_2O \quad (4)$$

During the chemical ionization, other reactions, such as nucleophilic attachment and hydride abstractions may also occur. The ion clusters shown in equations 1-4 are formed from ambient air in a series of reactions initiated by beta particles when Ni^{63} is the ionization source.

If nicotinamide is chosen as a reagent molecule to increase selectivity, protonated nicotinamide may react with the target analyte (Equation 5 and 6).

$$(H_2O)_nH^+ + \text{Nicotinamide} \longrightarrow (H_2O)_n(\text{Nicotinamide})H^+ \quad (5)$$

$$(H_2O)_n(\text{Nicotinamide})H^+ + T \longrightarrow TH^+ + nH_2O + \text{Nicotinamide} \quad (6)$$

The product ions that are formed through these processes are generally long-lived, robust, and low energy species. Also, since they rarely dissociate or fragment, they can be related directly to the original neutral molecule [1]. There is preferential and competitive distribution of the available charge among neutral molecules in the sample according to their gas-phase electron or proton affinities. Selectivity of IMS for different analytes is based on the atmospheric pressure ionization events themselves, the polarity of the ions and the mobility of the ions. Target analytes with high electron or proton affinities can be detected more readily than those with low affinities. The ionization chemistry can be modified through the use of specific reaction ions such as protonated nicotinamide mentioned above.

After the target molecules are ionized, they are differentiated according to their gas-phase mobilities. These are calculated from drift time across a fixed distance in an electric field. Mobility is related to the electric field strength by the ion drift velocity. The drift velocity (v_d) (Equation 7) is inversely proportional to drift time and is calculated from the mobility (K) and electric field (E) [23].

$$v_d = K \bullet E \qquad (7)$$

In most cases, product ion migration is related to reduced mobility constant, K_o ($cm^2/V^{-1}s^{-1}$), which is K expressed under standard temperature and pressure conditions. (Equation 8)

$$K_o = \frac{L}{t_d E} \bullet \frac{273}{T} \bullet \frac{760}{P} \qquad (8)$$

Where,

L = length of ion drift region (cm)

t_d = drift time (s)

E = electric field strength (V/cm)

T = temperature (K)

P = pressure (torr)

Target analytes with product ions of different masses can be differentiated very readily through drift times (different mobility constants). The signal amplitude can be used as a basis for semiquantitative or quantitative analyses, depending on how carefully the conditions are controlled.

The method chosen to ionize target molecules could affect the mobility spectrum of compounds due to differences in ionization chemistry. The use of ^{63}Ni has been popular due to its physical stability; instruments have been produced with noise-free operation, low power demands, small size, cost effectiveness, and capability to operate in explosive atmospheres [1]. Although the use of ^{63}Ni is still common, other ionization techniques are gaining popularity. Photoionization represents an interesting alternative with added selectivity and a large linear working range. Selectivity can be tuned through the use of photodischarge lamps with different ionization energies. Corona-discharge is another method currently being investigated, which allows a broad range of organic compounds to be ionized [25]. Alternatives to radioactive ionization sources are being investigated by several research groups.

It is possible to control the ion chemistry, through the selection of different ionization sources as well as reactant ions. This should lead to much higher sensitivity and selectivity, and more quantitative results. It should help to better focus IMS toward specific scenarios in applications such as clinical chemistry.

State of the Technology in Clinical Chemistry

Clinical chemistry encompasses various bioanalytical techniques used to diagnose medical disorders. This branch of laboratory medicine includes, but is not limited to, the assessment of the endocrine system, acid-base balance, hematology, and renal, liver, gastric, pancreatic and intestinal functions. It also incorporates many aspects of pathophysiology, chemistry, statistics, instrumental analysis, as well as principles of quality control and management. Clinical chemistry has become a vital part of health care management, and continues to be used by medical technologists and practicing physicians.

Clinical laboratories rely heavily on automated instrumental analysis and require strict quality control management. Chemical analyses of tissues and body fluids are performed to elucidate molecular interactions and pathways, and to verify different diseases. Major techniques used in today's clinical laboratories are listed in Table 2.

Table 2
Examples of techniques utilized in clinical laboratories [26]

Clinical Chemistry Departments	Automated Analysis
General Chemistry	Immunoassay
Immunoassay	Chemiluminescence and bioluminescence assays
Off-site Automation	High-performance liquid chromatography (HPLC)
Hematology	Capillary electrophoresis
Microbiology	Electroanalysis and biosensors
Urinalysis	Flame, flameless, and plasma spectroscopy
Blood Bank	Mass spectrometry
Reference	Infrared spectroscopy

Clinical instruments for general chemistry integrate sample processing, chemical analyses, and data management as part of total laboratory automation. Emphasis is

placed on cost effectiveness and efficiency with expanded menus of assays. There is a new focus of combining measurement techniques such as traditional chemistry (electrolyte assays, enzymatic assays, etc.) and immunoassays in a single analyzer. This type of analyzer may utilize robotics and data management to form an integrated system [26]. Efficiency of immunoassay analyzers may be enhanced by long-term calibration, autodilution, barcoding, stat and repeat capabilities, and clot detection. The current trend is toward modular automation, where two or more instruments are linked by a single controller, with additional instruments added as needed. In this scenario, analyzers share sample and reagent handling preparation, and transport systems. Another trend is toward bedside analysis whenever possible. Thus, there is an increased interest in on-site automation and the development of miniature analytical devices, microchips, and microscopic channels. Disposable devices that eliminate the manipulation of manual reagents offer convenience, quality assurance, rapid results, and cost effectiveness [26].

Some clinical analyzers include test panels for a comprehensive blood analysis, consisting of white cell, hemoglobin, red cell, and platelet counts. There is an increased interest in limiting manual review for cell counting. Advances have been made in automated slide markers, scanning devices, and image analysis. There have been several advances in molecular diagnostics for the detection of infectious diseases. Amplification methods in microbiology and virus testing have been enhanced through polymerase chain reactions (PCR), ligase chain reactions (LCR) and nucleic acid-based sequence amplification (NASBA) [26]. These techniques are available for a variety of

organisms and offer increased sensitivity and turn-around time when compared to culture-based or other direct immunologic methods.

Among the instruments in the clinical laboratory, are those dedicated for specific analytes, such as in urinalysis and blood bank analyses. Often these tests are handled in individual departments to increase quality control. In addition, there are reference laboratories that perform non-routine assays or offer specialized techniques.

Immunoassays utilize antibodies or antibody-related reagents for the determination of specific analytes. The use of antibodies allows very high selectivity, though some cross-reactivity may be encountered. Immunoassays, as categorized by the labels employed, include radioimmunoassays (RIA), enzyme immunoassays (ELISA), fluoresence immunoassays (FPIA), and chemiluminescence immunoassays (CIA). Other immunoassay variations include nonlabeled, electrochemical, liposome, chromatographic, and elcetrophoretic techniques [26].

Methods based on chemiluminescence and bioluminescence utilize light emission to label compounds in the analysis of body fluids and tissues. Light is produced from the decay of excited species to electronic ground states in chemical reactions, and from reactions utilizing materials of biological origin. Such techniques are used to analyze DNA, iron (III) in blood, hormones, and nitric oxide in exhaled air from patients with lung cancer, and hormones, among others [26]. Chemiluminescence has become a routine technique in the clinical laboratory. The use of bioluminescence, light emission with biomolecules, is increasing.

Electrophoresis carried out in a small-bore fused silica capillary is termed capillary electrophoresis [27]. Clinical laboratories routinely use this method to assist in

diagnosing disorders such as arthritis, phenylketonuria, organic acidurias, and neuropsychiatric diseases. Capillary electrophoresis is also used in DNA and RNA analysis, drug monitoring, protein analysis, and single-cell analysis [28].

High-performance liquid chromatography (HPLC) can be used to measure many of the analytes mentioned above, although it is utilized mainly as a confirmatory method in clinical chemistry. HPLC is also used to detect markers in diagnosis, treatment and prognosis of diseases. HPLC may be coupled to mass spectrometry (MS) and nuclear magnetic resonance (NMR) to increase reliability in the identification of bioanalytes [26].

The trend in analytical chemistry toward miniaturization extends to clinical chemistry as well. This includes research and development on genosensors (gene-based detectors), enzyme electrodes, biocatalytic and affinity biosensors, immunosensors, DNA biosensors, optical sensors, ion selective electrode, and voltammetric and amperometric devices [29,30].

Many clinical laboratories use MS to identify the molecular composition and structure of bioanalytes. This includes electrospray ionization (EI) and matrix-assisted laser desorption/ionization (MALDI) techniques. MS may also be coupled with other methods such as GC and HPLC to provide very rigorous and reliable analysis. Such combinations are used in illicit drug and metabolite analyses, and in complicated tasks such as protein characterization, sequencing of DNA, quantification of disease markers, and detection of genetic mutations [31,32].

Flame, flameless, and plasma spectroscopy are used primarily for the analysis of metals in biological specimens. Flame atomic absorption is relatively simple and is

used for the determination of metals such as lead, copper, iron, manganese, and zinc at ng/mL levels [33]. Flameless atomic absorption techniques have been transitioned from the laboratory to the bedside with the development of portable analyzers. The target analytes are chromium, cadmium, and magnesium [34]. Inductively coupled plasma atomic emission spectrometry (ICP-AES) and ICP-MS are powerful tools which can be used for multielement determination, although high cost is a limitation [26].

Infrared (IR) spectroscopy may be useful in organic structure determination in specific clinical scenarios such as in diagnosing liver disease, monitoring cancer progression, and monitoring oxy- and deoxyhemoglobin in the brain, blood, and other tissues [26].

Selecting Instruments for the Clinical Laboratory. The clinical laboratory represents a dynamic environment in which measurement technologies are continually being updated and new methods are being introduced [35]. As dictated by new advances in medical sciences, needs for higher sensitivity, increased selectivity, cost effectiveness, and rapid response are increasing.

Information on selecting instruments for clinical chemistry is available [36]. Table 3 was compiled to illustrate some of the important considerations. Clinical-specific requirements based on the chemistry and biochemistry needs would fall under performance objectives.

As mentioned earlier, IMS technology has proven itself in many applications not directly related to clinical chemistry. IMS has been chosen over other technologies because of its unique advantages. This thesis addresses the question of IMS relevance to current and evolving needs in the clinical laboratory.

Table 3
Important considerations in choosing instruments for the clinical laboratory

Cost Effectiveness
Acquisition costs
Operating costs
Reagents
Disposable items
Analysis time per sample
Sample throughput
Preventive maintenance costs
Service contract costs
Service call costs
QA/QC
Ability to meet/exceed data quality objectives
Performance
Ability to meet performance objectives
Minimization of interferences
Minimization of hazardous reagents and wastes
Multianalyte capability
Operations
User friendliness
Training requirements
Availability of vendor training
Adequacy of manuals and documentation
Automation
Computer capability
Computer requirements
Capability to flag anomalies
Selectable tests for profiling
Multisample capability
Adequacy of sample identification system
Adequacy of reports

Table 3
Continued

Other
Portability
Ruggedness
Space requirements
Facility/utility requirements
Maintenance requirements

Statements of Hypotheses and Specific Aims

The major goal is to examine IMS as a rapid screening tool for specific application to clinical chemistry research and clinical laboratory use. Methodology will be developed for target analytes representing several classes of physiologically active substances. Emphasis will be placed on potential interferences and quantification of data. IMS characteristics of animal tissues, amino acids and proteins will be determined. Information will be obtained on potential interactions between selected physiologically active substances and other molecules. *The research is meant to narrow the gap between clinical chemistry needs for a rapid, inexpensive, easy-to-use analytical technique and the high potential of IMS to meet those needs.*

Our hypotheses and specific aims are as follows:

> **Hypothesis 1:** We hypothesize that quality assurance and quality control procedures for IMS use in the clinical laboratory and clinical chemistry research can be established. The specific aim is to perform background checks and calibration curves from controlled standards, thus establishing criteria for assessing the accuracy of data.

Hypothesis 2: We hypothesize that IMS can be used to analyze materials of biological origin in clinical chemistry. The specific aim is to analyze plasma and tissues harvested from rabbits and to determine whether the potential exists to characterize them using IMS.

Hypothesis 3: We hypothesize that information useful to clinical chemistry can be obtained from a data compendium of IMS mobility spectra and associated reduced mobility constants of compounds related to biological materials can be compiled. The specific aim is to illustrate potential utility of IMS information through a compilation of IMS data from a variety of substances including animal tissue, drugs, proteins, amino acids, as a few examples.

Hypothesis 4: We hypothesize that IMS can be used to characterize a variety of physiologically active chemicals in biological media. The specific aim is to identify anesthetics and drugs in rabbit tissues and plasma as a demonstration of the vast analytical potential of IMS in such applications.

Hypothesis 5: We hypothesize that there may be enhancement and retardation effects that affect the utility of IMS in clinical chemistry. The specific aim is to identify such anomalies using the IMS of cocaine, heroin, and several of their metabolites as an illustration of what might be encountered in the clinical laboratory, and in clinical chemistry research.

Hypothesis 6: We hypothesize that guidelines can be formulated relative to the use of IMS for clinical chemistry. The specific aim is to assess the advantages and disadvantages of IMS use in a clinical chemistry setting based on the thesis research.

There are many areas can be explored relative to the viability of IMS in the clinical laboratory. However, it is judged that the research as outlined above will form a sufficient information base to allow both researchers and developers to make decisions on whether to pursue IMS in clinical chemistry applications.

CHAPTER II

EXPERIMENTAL

Introduction

The research designs and methods for this project focus on the development of procedures to analyze biological materials and physiologically active substances using IMS, while seeking quality results. Quality assurance issues are addressed in the next chapter, but are implemented throughout the experimental protocol covered here.

The experimental design incorporates IMS in the analysis of various animal tissues for the detection of physiologically active substances introduced *in vitro* and *in vivo*. It should be noted that the IMS of animal tissues has not been reported in the literature. The IMS measurement methods were adapted from well-known IMS techniques utilizing solid samples. Though improvements are envisioned, the techniques described here are judged to be sufficient for the data quality objectives of the thesis and illustrate how easily IMS can be adapted.

The Barringer IONSCAN 400 IMS was available for this research and represents technology which is well proven in the field and ports of entry for illicit substances, both drugs and explosives.

The use of biological materials in the research represents a partnership between the Department of Biological Sciences, and the Department of Chemistry and Biochemistry at Old Dominion University.

The Old Dominion University Chemical Hygiene Plan was followed.

Instrumentation

Barringer IONSCAN ion mobility spectrometer, Model 400 settings were as follows for the positive mode: analysis time - 8 seconds; drift tube: 233 °C; inlet: 275 °C; and desorber: 288 °C. For negative mode studies, the settings were: analysis time: 6.4 seconds; drift tube: 105 °C; inlet: 237 °C; and desorber: 247 °C. The sample membranes were Barringer Teflon™, 50 μm pore size. The instrument software allows calculation of peak amplitudes as either maximum, cumulative, or average values. Average amplitudes were utilized throughout for the calibration curves and semiquantitative analyses.

Animal Tissues

Subsections below detail the surgical procedures that were used by co-investigators to harvest animal tissues. The tissue storage and handling procedures employed are standard, and were supervised by Dr. Barbara Hargrave in the Old Dominion University Biological Sciences Department.

Animals and Materials

Male and female New Zealand White rabbits were purchased from Blue and Gray Rabbitry, Unionville, VA. Rompun (100 mg/mL xylazine, 0.9 mg methylparaben, 0.1 mg propylparaben) was obtained in aqueous solution at pH of 5.5±0.3. Citric acid and sodium citrate were added for pH adjustment. Ketaset (100 mg/mL ketamine HCl and 0.1 mg/mL benzethonium chloride) was obtained in water (pH 3.5-5.5). Rompun (5 mg/kg) and Ketaset (40 mg/kg) were used to induce general anesthesia during surgical procedures. A pentobarbital solution (324 mg/mL) was used as the

euthanizing agent. The above drugs were purchased from Henry Schein Port Washington, NY. Heparin sodium (1000 units/mL), purchased from Eikins-Sinn Inc., Cherry Hill, NJ, was used for intravenous or intraarterial injections as an anticoagulant. Heparinized saline (8.6 mg sodium chloride, 0.01 mL benzyl alcohol in water at pH 5.0 – 7.5; NaOH and/or HCl were added to adjust pH) was also used as an anticoagulation agent. Heparinized saline was placed in all catheters before and after obtaining blood samples.

Surgical Procedures

Each rabbit was fasted 12 hours prior to surgery and had free access to water (protocol approved by the Animal Care and Use Committee, Old Dominion University). The intramuscular administration of Ketaset and Rompun induced general anesthesia. A polyvinyl catheter, containing heparinized saline, was placed in the carotid artery. The heparin-saline filled catheter was closed and run subcutaneously through the nape of the neck. All incisions were sutured and the catheter was placed in a polyvinyl pouch and sewn to the nape of the neck for protection against accidental removal. Each rabbit was allowed at least 24 hours to recover from surgery before experiments. All animals were housed in individual cages, and allowed free access to food and water. It should be noted that the animals were used by Dr. Hargrave for physiological measurements which are not part of the thesis research. Those results are not reported here. An example is a thesis by Andrea Chambliss [35].

On the day of an experiment, the rabbits were allowed to acclimate to their surroundings for approximately 60 minutes. The catheter was opened and filled with a solution of fresh heparinized saline. Each rabbit was then given an intra-arterial

injection of saline (1 mL), heroin (200 μg), or ecgonine methyl ester (EME) (2 mg/kg). Acute as well as chronic studies were performed. In acute studies, arterial blood samples were taken five and thirty minutes after the drug is injected. In chronic studies, the drug was administered on a timed device for 14 days via an Alzet pump. After the completion of each experiment, pentobarbital (1 mL) was administered to euthanize the animal. Adult rabbit liver, lungs, kidneys, renal arteries, heart, ovaries, and testicles were removed and placed in appropriately labeled sterile tubes. The tissues were then placed into sterilized containers and snap-frozen with liquid nitrogen. All tissues were stored at -70°C until analysis. Samples of fetal liver, lungs, kidney, and heart were also removed, pooled, and frozen with liquid nitrogen and stored under the same conditions. Designation numbers and information concerning each animal are given in Table 4.

Tissue Preparation

To prepare tissue samples for analysis by IMS, a fraction of adult and fetal tissues were thawed and dried using a speed vac centrifuge. After the tissue was dried, samples were ground into a powder using a mortar and pestle. Each sample was labeled with the appropriate animal number, date, and tissue type.

Procedures for In Vitro Studies

Tissue samples were stored in a refrigerator until the day of IMS analysis. Small amounts of dried tissue from rabbits given the saline control were weighed and analyzed by IMS to establish a control background. The Teflon membranes were pre-cleaned by analyzing each separately with the IMS until there were no peaks present other than the reactant ion peak. Approximately 0.5-1 mg of dried tissue from the organs (liver, lungs, kidneys, renal arteries, heart, and ovaries) of animals receiving

Table 4
Classification of experimental conditions for each control animal, which received injections of heparinized saline

Animal Number	Type of Study	Pregnancy Condition	Initial Weight (kg)	Anesthesia	Euthanasia
C1	acute	non-pregnant	3.5	Ketaset and Rompun	used
C2	acute	non-pregnant	4.2	none	used
C3	acute	non-pregnant	3.3	Ketaset and Rompun	none
C4	acute	pregnant	4.0	none	used
A27	acute	pregnant	3.8	Ketaset and Rompun	none
A43	acute	pregnant	4.2	Ketaset and Rompun	used
A44	acute	pregnant	4.6	Ketaset and Rompun	used
A39	chronic	pregnant	5.8	Ketaset and Rompun	used
A40	chronic	pregnant	4.1	Ketaset and Rompun	used

saline, heroin or EME was placed on Teflon membranes for IMS analysis. This procedure was performed to indicate the presence or absence of each drug or its metabolites. All samples were analyzed in the positive ion mode by IMS, with selected samples being analyzed in the negative ion mode.

In order to optimize the IMS experimental protocol used to evaluate each animal tissue, 0.5-1 mg of C3 brain tissue samples (harvested from a rabbit exposed to heparinized saline) were individually analyzed on pre-cleaned Teflon membrane at varying desorber temperatures and sampling times. The desorber temperature of the IMS was reprogrammed from the standard temperature of 288°C to 100°C, 150°C,

200°C, 250°C and 300°C during analyses. The sampling time was also changed from an 8 second analysis to 5, 10, 15, and 20 second analyses.

Aliquots of several concentrations of different drugs were added directly to various tissues to assess potential interactions between the tissues and each drug. (Procedures for each drug class are given below under the name of each drug.) The solvent was allowed to evaporate and the tissue was analyzed for the target analyte using IMS.

Biologically Related Materials

Amino Acids

Amino acids were analyzed using both positive and negative modes of the IMS. This allowed fingerprints for each amino acid to be established. Compounds included glycine, phenylalanine, tryptophan, glutamine, tyrosine, cysteine, arginine, histidine, and glutamic acid. Prescreening involved placing small amounts on Teflon membranes and analyzing with IMS. The amino acids that had distinct IMS signatures were selected for further analysis.

Small amounts (approximately 0.5-1.0 mg) of each amino acid was placed on a pre-cleaned Teflon membrane and analyzed under standard conditions. The two amino acids (tyrosine and glutamine) with prominent signatures were also analyzed on S&S paper (Schleicher and Schuell, 6.2 cm diameter) and QMA (Whatman glass microfiber filters, 4.7 cm diameter). Solutions of tyrosine were made by dissolving 10 mg of tyrosine in 9 mL of water and 1 mL of HCl (11.6 N). Diluting with 5 mL water and 5 mL methanol gave concentrations of 1000 ng/μL and 100 ng/μL. One μL of each tyrosine solution was placed on S&S paper and QMA and analyzed in both the positive

and negative modes. A similar procedure was employed in making glutamine solutions and obtaining IMS mobility spectra. The amino acids were tested with analytes using the same procedures as with proteins mentioned below.

Proteins

Vegetable protein powder (Sundown, 94%), containing calcium, phosphorus, iron, magnesium, and an amino acid profile, were evaluated for effects on the IMS of heroin and its metabolites. Small amounts of solid were analyzed with and without analyte. Aliquots of various concentrations of heroin were applied directly to the amino acid or protein. The solvent was allowed to evaporate for approximately 1 minute and the mixture was analyzed for any noticeable differences.

An IMS background of vegetable protein was obtained by placing 0.5-1 mg of protein powder on a Teflon membrane and analyzing it in the positive mode of IMS. The analytes, 100 ng of heroin HCl in methanol, 25 ng of morphine in methanol, and 25 ng of 6-acetylmorphine in acetonitrile, were pipetted directly onto 0.5-1 mg of protein powder and analyzed. Also, adding 25 ng, 50 ng, 75 ng and 100 ng of heroin solution onto individual Teflon membranes with 0.5-1 mg of vegetable protein allowed a calibration curve for heroin in the presence of protein powder to be obtained. Triplicate analyses were performed.

Glycine Peptides

Glycine peptides (Sigma Chemical Co.) were investigated for their IMS properties and effects on the IMS of heroin. Specifically, diglycine, triglycine, tetraglycine, pentaglycine, and hexaglycine peptides were analyzed to determine if the

number of repeating units made a difference in the results. In addition, polyglycine peptides of molecular weights 4,600 and 5,200 were evaluated.

A background of each glycine peptide was obtained by placing 0.5-1 mg of the solid on a Teflon membrane and analyzing with IMS. Solutions of 25 ng and 50 ng of heroin-HCl in methanol were placed directly onto each peptide powder. The solvent was allowed to evaporate for approximately 1 minute and the sample was scanned in the positive mode. Experiments were performed in triplicate. Significant changes in the peak height of heroin were recorded.

Rompun and Ketaset

Both Rompun (5 mg/kg) and Ketaset (40 mg/kg) were administered to the New Zealand White rabbits to induce general anesthesia during surgical procedures. These compounds were also examined by IMS in both the positive and negative modes to for control purposes. Calibration curves for each anesthetic were obtained using methanolic solutions. A stock solution of 100 ng/μL was prepared by adding 495 μL of methanol to 5 μL of anesthetic solutions received, with subsequent dilutions to 50 ng/μL, 25 ng/μL, 10 ng/μL, 5 ng/μL, and 1 ng/μL concentration levels. IMS analyses were performed using pre-cleaned Teflon membranes.

Tissues harvested from rabbits that received injections of Rompun and Ketaset were analyzed for xylazine and ketamine, respectively. This was done to determine if these anesthetic agents could be detected in tissues using IMS. Other studies included the deposition (via syringe) of 100 ng/μL of Rompun or Ketaset directly on tissue from the heart, liver, lungs, kidney, renal arteries, and ovaries of control animal C3, who

received only pentabarbitol. This helped to confirm that IMS peaks assigned to the anesthetic from *in vivo* tissue correlate with the same peaks from *in vitro* studies.

An extraction study was also performed to determine if anesthetics could be extracted from tissues harvested from animals exposed to Rompun and Ketaset. Representative samples of 50 mg – 70 mg of atrial, lung and ventricle C3 animal tissue were placed into vials of 1 mL with methanol or 1 mL of acetonitrile. These samples were shaken and allowed to stand in a refrigerator overnight. The supernatant liquid was decanted the next day and allowed to warm to room temperature. Five μL of the supernatant were allowed to evaporate on a pre-cleaned Teflon membrane using standard conditions, and analyzed by IMS. The solvent was allowed to evaporate from the remaining tissue samples at room temperature and residues were also analyzed.

The euthanizing agent was composed of pentobarbital sodium, isopropyl alcohol, propylene glycol, edetate disodium, and benzyl alcohol. The euthanizing agent and benzyl alcohol (Mallinckrodt Chemical Co.) were checked for potential IMS signatures. A heparin sodium solution (1000 USP units/mL) was also used in the surgical protocol for each animal. One μL of this solution was analyzed to detect any IMS signature.

2-Chloroethyl Ethyl Sulfide and Methyl Iodide

Stock solutions of 2-chloroethyl ethyl sulfide (1 mg/mL) were prepared by adding 2 μL of the neat chemical to 2 mL of acetonitrile. Dilutions were made to 100 ng/μL and 500 ng/μL. Analyses were performed in negative and positive modes using Teflon membranes. The negative mode was chosen for tissue studies because of the more prominent signature. Aliquots of each concentration of the analyte were added to

C3 brain tissues previously lyophilized and ground. The mixture was analyzed by IMS to determine any changes or interactions between the tissue and 2-chloroethyl ethyl sulfide. Each concentration of 2-chloroethyl ethyl sulfide was also added to 100 ng/μL of Rompun and 5 ng/μL of cocaine to examine any potential interactions between compounds. Methyl iodide (99%), another alkylating agent, was also examined in a similar manner. Aliquots (1 μL and 5 μL amounts) of neat compound were examined in negative and positive IMS modes. Methyl iodide solutions were added to 0.5-1 mg of C3 brain tissue and 100 ng/mL of Rompun followed by IMS analysis to detect any potential interaction.

Identification of Factors that Influence the IMS Analysis of Contraband Drugs

Reagents

Anhydroecgonine methyl ester (AEME) standard in acetonitrile (1.0 mg/mL) – Radian International, 99% purity; ecgonine methyl ester (EME) standard in acetonitrile (100 μg/mL) – Radian International, 99% purity; heroin hydrochloride standard in methanol (100 μg/mL) – Sigma Chemical Co.; 6-acetylmorphine standard in acetonitrile (1.0 mg/mL) - Radian International, 99% purity; cocaine hydrochloride standard in methanol (1.0 mg/mL) – Sigma Chemical Co.; morphine; cocaethylene; codeine; ecgonine hydrochloride standard in methanol (1.0 mg/mL) – Radian International; benzoylecgonine standard – Radian International.

Cocaine Hydrochloride and its Decomposition Products

Two studies were performed to determine effects that cocaine decomposition products might have on the IMS characteristics of cocaine. In one study, IMS was used

for the identification and analysis of AEME and EME in the presence of each other. The second study involved the analysis of cocaine in the presence of AEME alone.

AEME and EME exhibit ion mobility values which are relatively close (reduced mobility constants, K_o: 1.505 and 1.485 $cm^2V^{-1}s^{-1}$, respectively). Calibration curves were obtained using 1 μL each of acetonitrile solutions: 0.2, 0.5, 1, 5, 10, 25, 40, 50, and 100 ng/μL (AEME) and 0.5, 1, 5, 10, 20, 25, 30, 40, 50, and 100 ng/μL (EME). The aliquots were applied individually to Teflon membranes using a 1 μL syringe. The syringe was cleaned before each deposition first with acetonitrile or methanol, then with the particular test solution. In each deposition, the solution was spread evenly on the membrane with the tip of the syringe needle. The solvent was allowed to evaporate for approximately one minute before the IMS analysis. The IMS analysis was repeated, usually one or two times, until no residual sample was detected on the membrane. Though all results were recorded, only results from the first run were used for the calibration curve. Each sample was analyzed in triplicate.

AEME/mL acetonitrile (100 μg), EME/mL acetonitrile (100 μg), and acetonitile were pipetted (Eppendorf™ pipettes) into vials making up solution volumes of 100 μL in testing IMS response with mixtures. The ratios by weight (AEME/EME) for individual samples were: 10 ng/10 ng, 10 ng/20 ng, 10 ng/40 ng, 10 ng/60 ng, 10 ng/80 ng, 20 ng/10 ng, 40 ng/10 ng, and 60 ng/10 ng. After each solution was applied to the Teflon membrane, the solvent was allowed to evaporate before analysis.

To determine the effects of AEME on cocaine hydrochloride, calibration curves were established for each compound. Aliquots of cocaine solutions with 1 ng/μL, 5 ng/μL, 10 ng/μL, 15 ng/μL, 30 ng/μL, 50 ng/μL, 75 ng/μL, and 100 ng/μL

concentrations were prepared in methanol and analyzed by IMS in the positive mode. AEME calibration concentrations of 0.2 ng/μL, 0.5 ng/μL, 1 ng/μL, 5 ng/μL, 10 ng/μL, 25 ng/μL, 50 ng/μL, 75 ng/μL, and 100 ng/μL were prepared in acetonitrile and analyzed. Ratios of 10:1, 10:5, 5:1, 5:5, 5:1, 0.2:5, 0.5:5, 1:5, 1:1, 1:10, and 1:25 of AEME to cocaine-HCl were also analyzed. Aliquots of both compounds were applied to a pre-cleaned Teflon membrane one after another, with AEME being applied after cocaine-HCl. The order of addition was important. Other compounds, such as 25 ng/μL benzoylecgonine, 25 ng/μL codeine, 25 ng/μL cocaethylene, 25 ng/μL 6-acetylmorphine, 25 ng/μL morphine, 25 ng/μL ecgonine, 25 ng/μL heroin and 25 ng/μL EME were also analyzed in the presence of cocaine to determine their effects, if any. In addition, a 10:5 ratio of cocaine HCl to AEME were examined on surfaces of S&S paper, QMA, Whatman filter paper, and Barringer, 3.0 cm diameter fiber glass filters in lieu of Teflon membranes, to determine any matrix effects in the desorption process.

Heroin and its Metabolites

The effects of 6-acetylmorphine on the IMS detection of heroin were examined. Aliquots of heroin hydrochloride were applied to a Teflon membrane. This was followed with aliquots of 6-acetylmorphine in various concentrations. Studies with various mixtures of 6-acetylmorphine and heroin hydrochloride were analyzed. The solvent was allowed to evaporate prior to analysis. Each analysis was performed in triplicate.

The analysis of heroin hydrochloride and 6-acetyl-morphine by IMS was accomplished by applying 1 μL aliquots of heroin hydrochloride to Teflon membranes using microcapillary pipettes followed by aliquots of solutions containing different

amounts of 6-acetylmorphine. Each solution was spread evenly on the membranes using the tip of the microcapillary pipette. The solvent was allowed to evaporate prior to analysis. The heroin hydrochloride methanolic solutions contained 25 ng, 50 ng, 75 ng or 100 ng heroin freebase per μL. Each amount of heroin was analyzed in the presence of 25 ng, 50 ng, 75 ng and 100 ng of 6-acetylmorphine. These amounts of 6-acetylmorphine were contained in 1 μL, 2 μL, 3 μL, and 2 μL acetonitrile solutions, respectively. Each combination was analyzed in triplicate.

To evaluate the interaction of opiates on animal tissues, 25 ng/μL and 100 ng/μL heroin-HCl, 25 ng/μL 6-acetylmorphine, and 25 ng/μL morphine were added *in vitro* to 0.5-1 mg A39 brain tissue. Each sample was weighed on pre-cleaned Teflon. One μL aliquots of each opiate were added individually to each tissue. The samples were then analyzed in the positive mode using IMS.

GC/ MS of Animal Tissues Exposed to Heroin

Gas chromatography-mass spectrometry (GC-MS) was used to analyze samples of animal tissues exposed to heroin *in vivo* and *in vitro*, as well as each separately to assist in understanding interactions with animal tissues. A gas chromatograph (Hewlett Packard model HP-3840) coupled with a quadrapole mass spectrometer was used in the experiments. The capillary column was J&W DB-5 column (30 m, 0.25 mm I.D., 0.25 μm film thickness). The temperature of the injection port was 250°C and the temperature of the oven was programmed: 50°C held for 1 minute, then ramped with 3 steps: 50°C/minutes to 200°C held 3 minutes, 20°C/min to 250°C held 2 minutes, 20°C/min to 300°C held 3 minutes. Helium was used as a carrier gas with a constant pressure of 12 for the chromatographic process. A purge time to vent was set for 0.75

minutes to allow a complete desorption of analytes. Aliquots of 10 ng, 100 ng, and 1000 ng of heroin-HCl in methanol were manually introduced to the split/splitless injection port of the GC-MS with a 10 μL syringe. Small amounts (0.5-1mg) of animal tissue were analyzed by placing the tissue in a deactivated GC liner. Silanized glass wool was placed at both ends of the liner to prevent any of the solid particles from entering the column.

CHAPTER III

ION MOBILITY SPECTROMETRY QUALITY ASSURANCE AND QUALITY CONTROL

Introduction

Quality assurance and quality control (QA/QC) procedures are necessary to ensure that the IMS data generated are as precise and accurate as possible. Such procedures assist in the daily monitoring of instrument performance, providing an early indication of potential problems, as well as keeping maintenance records up to date for any necessary corrective actions. QA is a system of activities required to provide the quality of data needed. High quality in one situation may be unacceptable in another. QC procedures ensure the quality and integrity of the data [36]. QC refers to the actual procedures employed to ensure that the data generated is accurate and precise enough for its intended use. Although the QC protocol may be similar using a particular technology such as IMS, the overall QA plans may differ depending on the data quality objectives. The plans implemented in clinical chemistry incorporate extra measures to ensure data accuracy.

A QA/QC program measures and verifies laboratory performance, while generating accurate and precise data. Accuracy is the indication of closeness of agreement between an observed value and the true value, while precision is the expression of the reproducibility of the value [37]. Basic elements of QA/QC include suitable equipment, good laboratory and measurement practices, standard operations procedures, documentation, and training. To assess the efficacy of a QA/QC plan, internal and external assessment techniques may be utilized. The repetition of

measurements, control charts, measurements using different instruments, and the interchange of operators is an example of internal techniques. External techniques may involve analyzing standard reference material and collaborative tests with other laboratories [36].

The quality needed for the data obtained in the present research varied with each study. In experiments involving animal tissues and biologically related materials, the emphasis was more on qualitative aspects rather than quantitative assessment. In the identification of factors that influence the IMS of contraband drugs, more importance was placed on the semi-quantitative analysis of the data obtained. Instrument performance was also monitored very closely to ensure quality data throughout.

The quality control plan in most clinical laboratories incorporates the practices mentioned above, but may include extra measures to ensure that data quality objects are met. In many cases, physicians base their diagnoses heavily on the laboratory results. Measures, such as delta checks, are examples of quality control procedures to ensure the integrity of the data. Delta checks are performed by comparing values from previous specimen from the same patient with current results and determining if they are significantly different (by 15%). Proficiency testing utilizes external quality control samples, which allows the performance of different laboratories to be followed for long-term accuracy of analytical results [38]. The thesis research did not address any specific clinical laboratory need, but focused on obtaining the best IMS data possible under the constraints of specific experiments.

Quality Control Procedures

Specific QC procedures that may be implemented include instrument calibration, the analysis of reference standards, replicates and blanks. Also included are sample handling and preparation, complete documentation, proper data generation, and corrective actions for factors which might adversely affect the quality of the data.

The components of the QC protocol used for generating IMS data in this thesis included [37]:

- Analyzing a QC standard at both the beginning and end of a day's analyses.
- Obtaining daily 3 point calibration curves and comparing results to the most recent and more extensive curve for target analytes when data is to be used in a semi-quantitative analyses.
- Maintaining a laboratory standard log which contains information on all quality control maintenance and calibration standards.
- Adhering to a regular instrument maintenance schedule.

Preparation of Quality Control Standards.

Cocaine hydrochloride standards (for the positive IMS mode) and 2,4,6-trinitrotoluene (TNT) (purchased from Radian) (for the negative IMS mode) were prepared in methanol from certified standards available commercially. The QC standards were prepared solely for the purpose of evaluating instrument performance. All standards received were marked with the date received and date opened. Silanized (deactivated) glass vials were utilized for all narcotics to prevent degradation.

Standard solutions were kept in the freezer at 0°C (temperatures are monitored)

when not in use and were allowed to warm to room temperature before analyses. Dilutions were performed using Eppendorf™ pipettes and volumetric glassware. All glassware was rinsed with solvent before use. All vials were labeled with compound name, concentration, solvent, date of preparation, and initialed and stored in a freezer.

Instrument blanks were performed by analyzing positive and negative mode standards, supplied by Barringer Instruments. These standards contain verification compounds, with peaks at K_o values of 1.53, 1.21, and 1.00 $cm^2V^{-1}s^{-1}$ for the positive mode and peaks at K_o values of 1.45, 1.39, 1.31, 1.15, and 1.10 $cm^2V^{-1}s^{-1}$ for the negative mode [39]. Analyzing an empty tray slide allowed the IONSCAN 400 baseline to be checked. A background of the Teflon sample membrane (50 μm pore size) was performed before each analysis. The Teflon membrane was analyzed using standard conditions for the IONSCAN 400 until a satisfactory flat base line was achieved with only the reactant ion peak being present. Additional procedures included analyzing acetonitrile and methanol on separate Teflon membranes to ensure contamination-free solvents.

A five point calibration curve (concentrations ranging from 1 ng/μL to 25 ng/μL) was generated for cocaine hydrochloride in order to determine a concentration sufficient for quality control checks (Fig. 3). Quality control standards within the linear range of the calibration curve (1 ng – 50 ng cocaine hydrochloride) were analyzed prior to the day's analyses and the signal obtained was compared to those obtained in previous days. Corrective actions were taken as necessary. A five point calibration curve (concentrations ranging from 1 ng/μL to 20 ng/μL) was also generated for TNT.

A sample calibration curve is shown in Fig. 4. TNT standards were used for the quality control checks in the negative mode in the same manner described above.

Assessment and Maintenance of Instrument Performance. Routine maintenance procedures for the IMS included cleaning of the inlet liner and condenser tube monthly with methanol. Silanized glass wool was placed inside the inlet liner to prevent the introduction of solid particles into the IMS drift tube. The dessicant was changed in the air purification tube when the indicator changed color. The sintered and dust filters on the back of the detector and pump modules were also cleaned regularly [37]. If unexplained problems were noted, changing the dessicant was the first corrective action pursued.

All information regarding instrument operation and service was recorded in the instrument log book. The log book was dedicated to the IONSCAN 400 and updated after each use. The log book was also used to note all service records and maintenance information.

Problems that have been encountered in using IMS include decrease in target analyte signal, presence of extraneous peaks, shift in K_o values, and absence of reactant ion peak. Possible causes for the signal decreases and extraneous peaks are dirty inlet, leaks, degradation of sample, contamination of the drift tube, and competition in reactant ion chemistry with another compound [40]. A shift in K_o values may indicate the presence of water in the system and/or spent dessicant in the air purification unit. An absence of the reactant ion peak may indicate a leak, contamination, or the glass wool being packed too tightly [40]. Corrective actions are taken appropriate to each situation and recorded in the instrument log book.

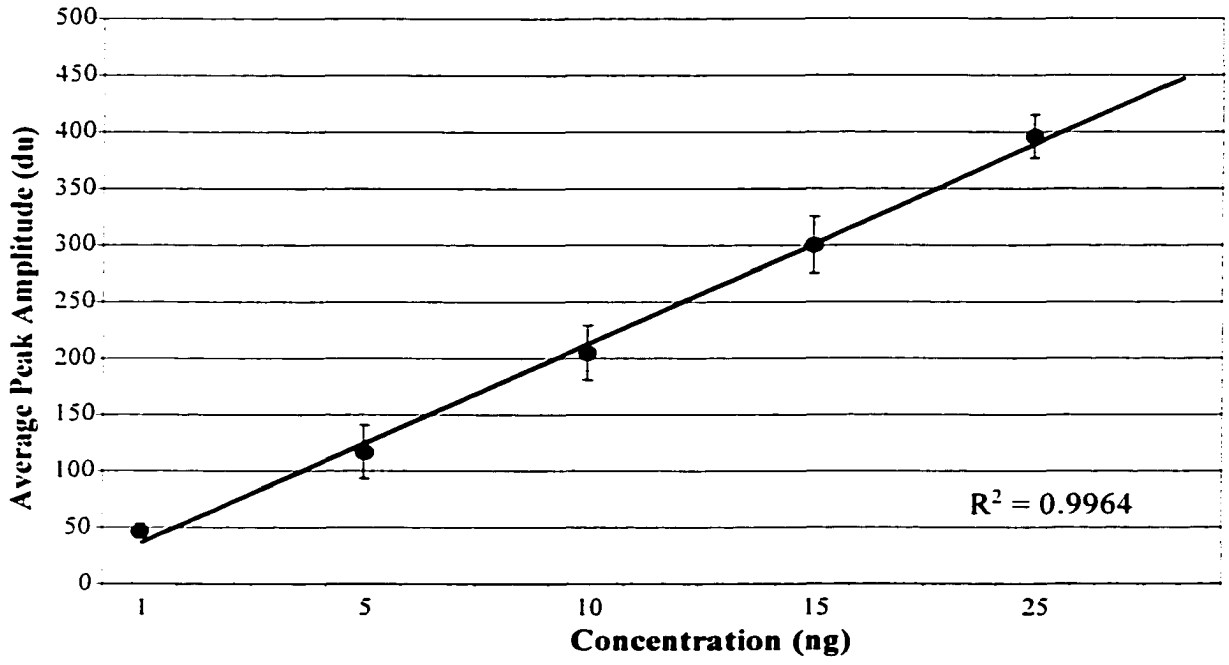


Fig. 3. IMS calibration curve for 1 – 25 ng cocaine hydrochloride (K_o, 1.16 $cm^2V^{-1}s^{-1}$).

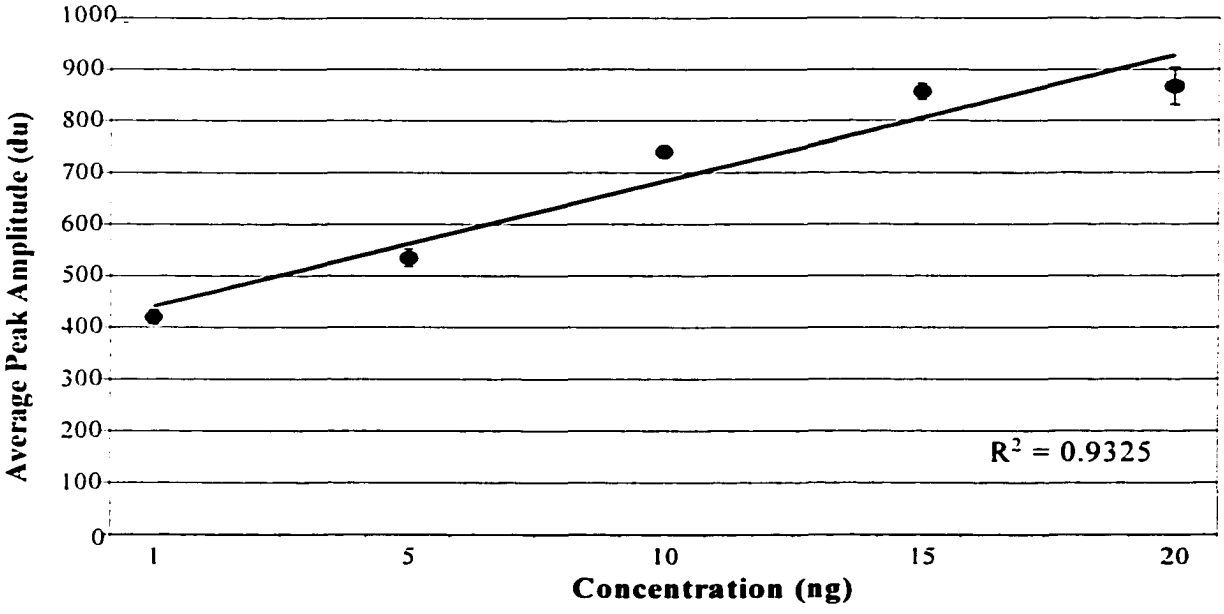


Fig. 4. IMS calibration curve for 1 – 20 ng 2,4,6- trinitrotoluene (TNT) (K_o, 1.45 $cm^2V^{-1}s^{-1}$).

Results

Data Variability

Experiments were performed to determine data variability within daily, day to day, month to month, and year to year time frames. Fig. 5 illustrates 25 ng/μL of cocaine hydrochloride analyzed in the morning (approximately 10 AM), afternoon (approximately 1 PM) and evening (approximately 5 PM) on four different days within one month. The instrument was left on during the day between each set of analyses. It was turned off at night. Samples were analyzed on different days using diluted cocaine standards from a certified solution of cocaine hydrochloride. The one exception was sample D, which was made from solid cocaine hydrochloride of high purity. Fig. 5 illustrates that there can be significant variation during the day. Samples A, B, and C show higher instrument sensitivity at the end of the day. This variation is an exception and was noted during a period in which unexplained variations were observed. It is being brought out as an example why QC checks are important on a daily basis. It was concluded that the variation was due to the instrument rather than sample integrity especially since the sensitivity was increasing rather than decreasing. One possibility with the morning samples might be that the desorption temperature had not reached the set level. However, there is an instrument control which indicates the all settings have been reached after the warm-up period. Perhaps there was a malfunction; however, the exact cause was not found.

Figs. 6 and 7 show the month to month variations for the average amplitude and K_o values for 25 ng/μL cocaine hydrochloride run in 9 consecutive months. The data in

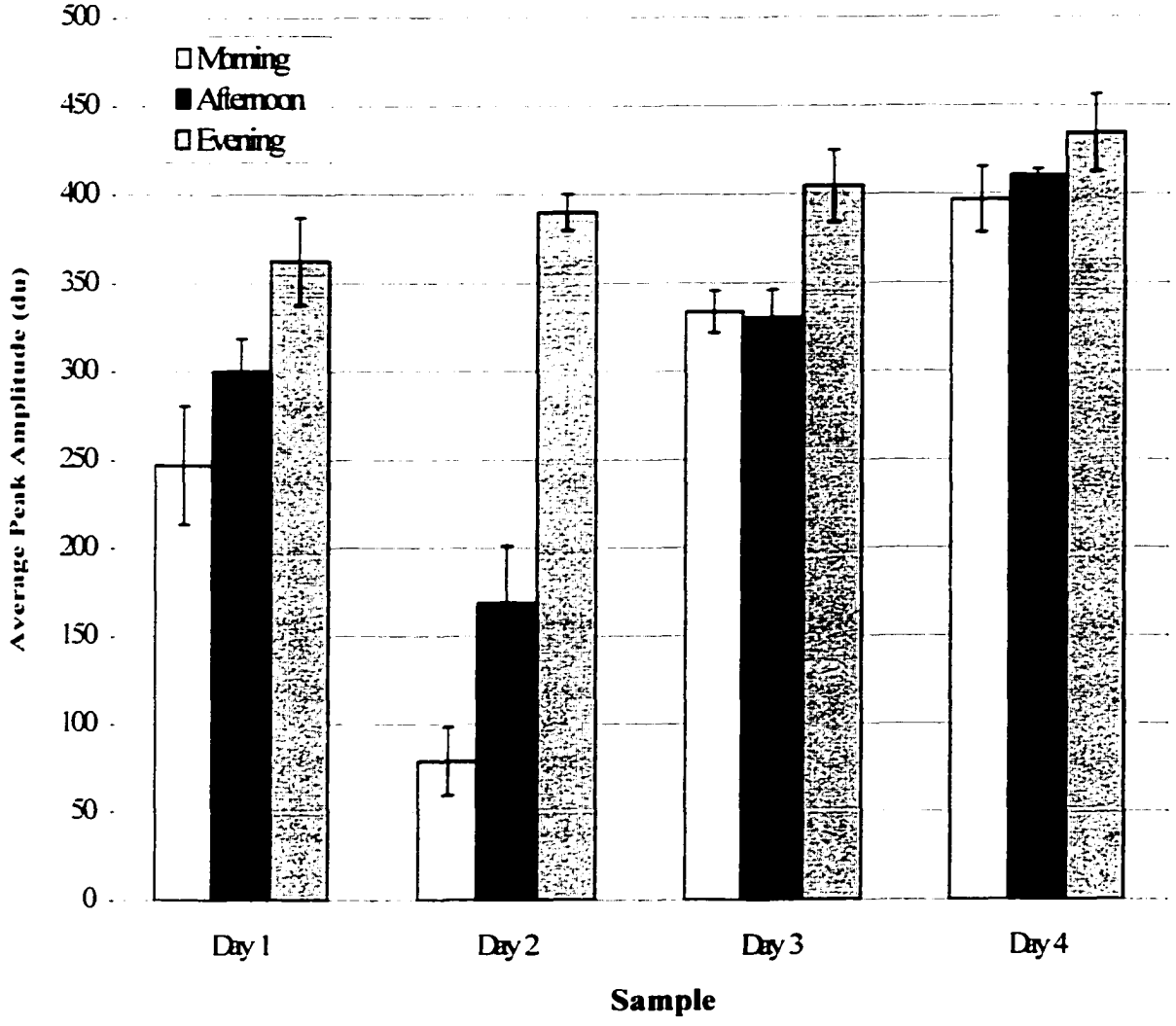


Fig. 5. An illustration of within day variations for four different days. Twenty-five ng of cocaine hydrochloride were analyzed in the morning (approximately10 AM), afternoon (approximately 1 PM), and evening (approximately 5 PM).

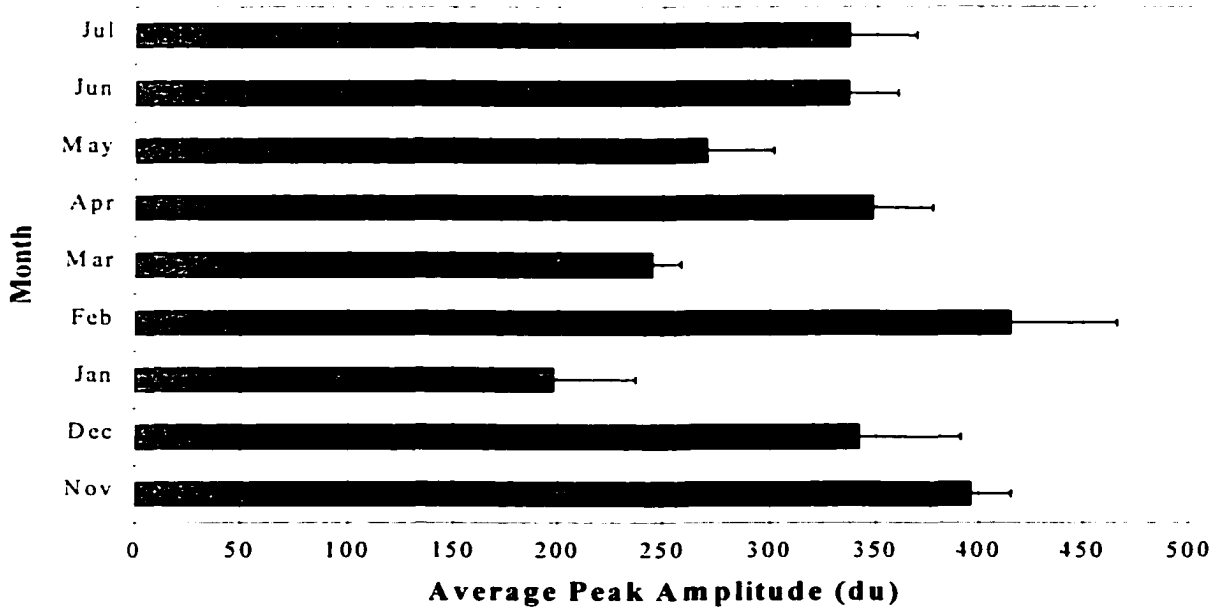


Fig. 6. An illustration of month to month variations for the average amplitude of 25 ng/ μL cocaine hydrochloride (K_o, 1.16 $cm^2V^{-1}s^{-1}$).

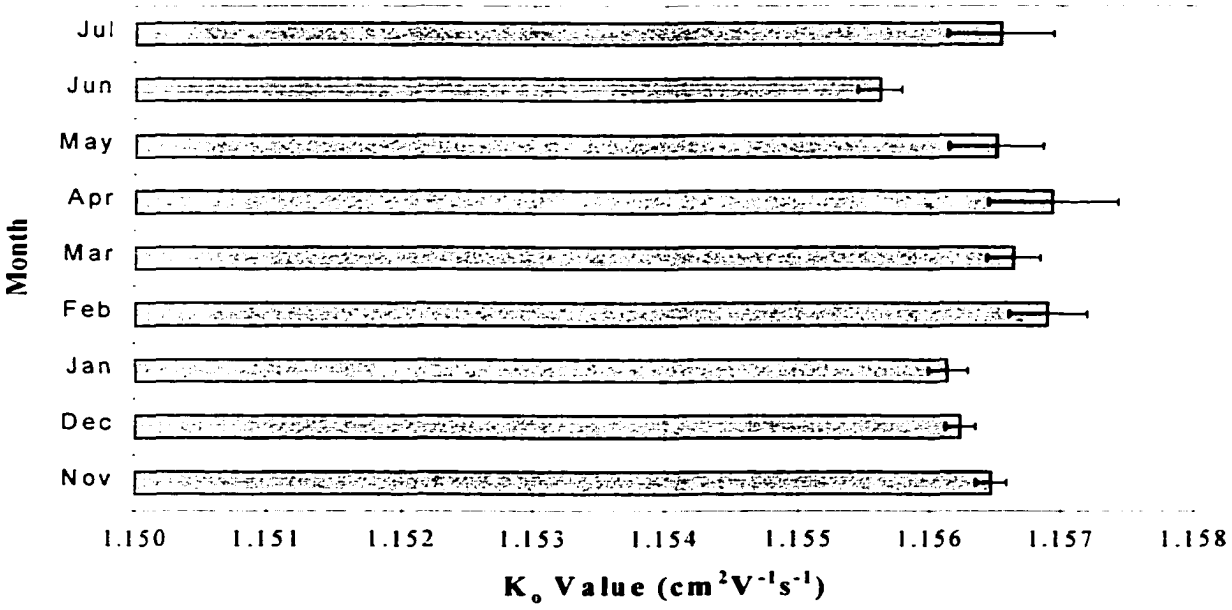


Fig. 7. An illustration of month to month variations for the average K_o value for 25 ng/μL cocaine hydrochloride.

Fig. 6 represents the averages of triplicate analyses for the daily QC checks. The month to month variation is significant for the average amplitudes, which may range from 200 du to 425 du. The K_o variations (Fig.7) are very small, with the K_o value ranging from 1.155 to 1.157 $cm^2V^{-1}s^{-1}$. We have been using three significant figures for K_o which brings the average to 1.16 for every month.

Year to year variations are shown in Fig. 8. Calibration curves for 1995, 1996, 1997, and 1998 are given for 1, 2, 5, 10, 25, and 50 ng of cocaine hydrochloride. Curves obtained in 1995 and 1997 show little difference. The 1998 calibration curve performed in 1998 also seems to correlates well with those from 1995 and 1997 except for the point from 50 ng of cocaine hydrochloride. The calibration curve obtained in 1996 exhibits the highest amplitudes for all concentrations. It should be pointed out that different operators were involved. It is clear that calibration curves should be obtained often for specific compounds when semi-quantitative analyses are an objective.

Determination of Precision

Precision was determined using 25 ng/μL cocaine hydrochloride, 10 ng/μL of AEME and EME, and 0.5-1 mg of animal tissue. Each set of analyses comprised fifteen consecutive replicates with different samples of the same analytes. The time to complete the fifteen runs ranged from one half-hour to one hour. Fig. 9 exhibits the results for cocaine. The average amplitude for was 507 du with a standard deviation (SD) of ± 39 and a relative standard deviation (RSD) of 7.7%. Fig 10. shows data for AEME with an average amplitude of 483 du, SD of ± 44, and RSD of 9.0%. Data for EME is shown in Fig. 11. The average for 10 ng/μL of EME is 628 du, with a SD of ± 46 and a RSD of 7.3%. Fig. 12 illustrates data from fifteen analyses for A-43 brain tissue. The two ion

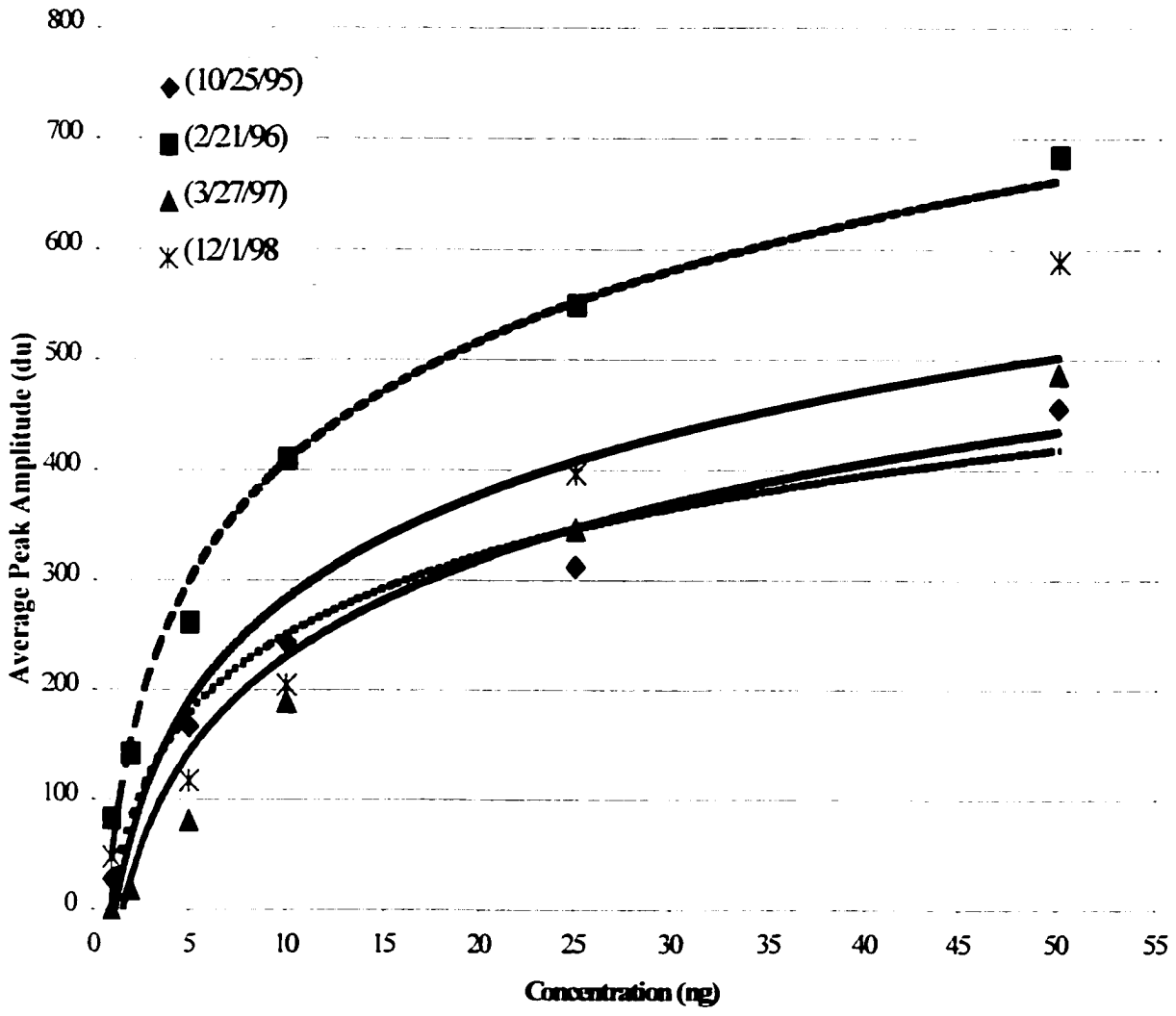


Fig. 8. An illustration of the year to year variations for 25 ng/μL cocaine hydrochloride (K_o, 1.16 $cm^2V^{-1}s^{-1}$). The calibration curves are based on using 1- 50 ng of cocaine hydrochloride.

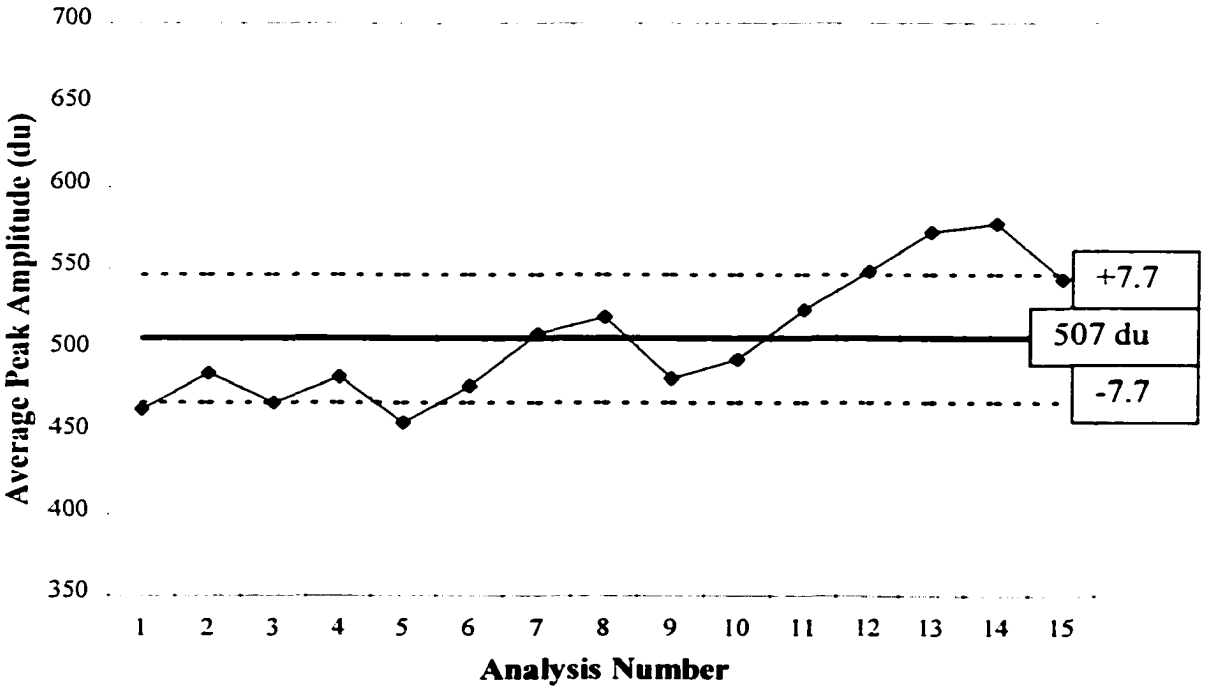


Fig. 9. Precision results for fifteen consecutive analyses of 25 ng/μL cocaine hydrochloride (K_o, 1.16 $cm^2V^{-1}s^{-1}$). The average amplitude is 507 du, with a SD of ±39 and a RSD of 7.7%.

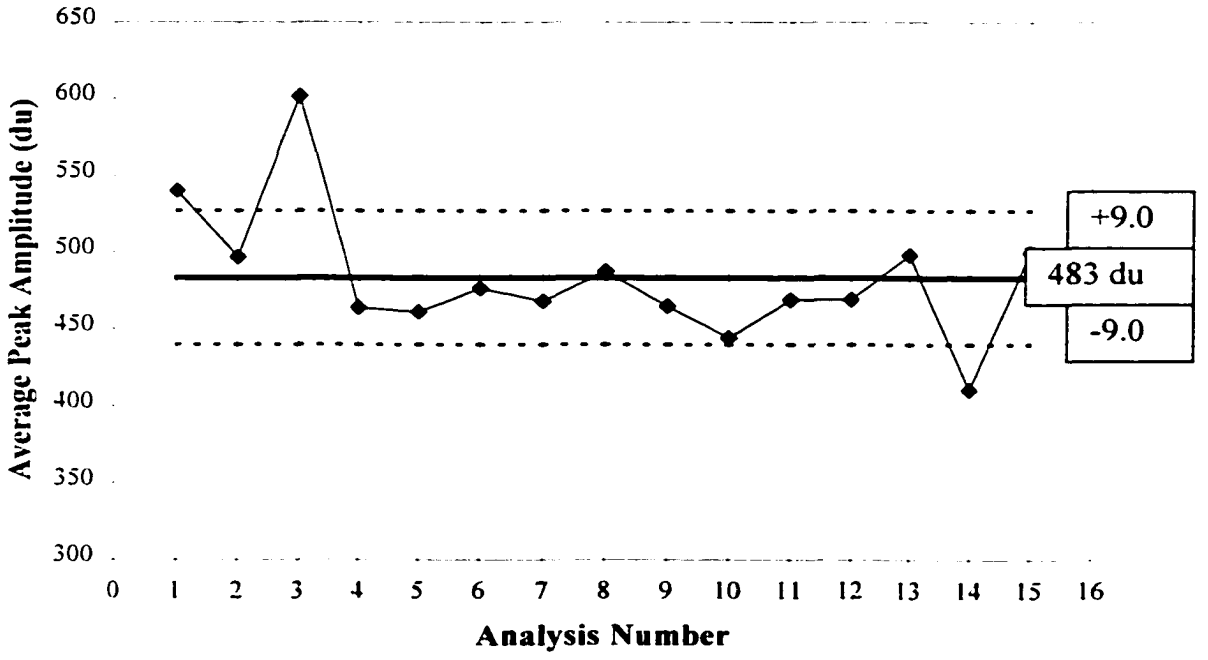


Fig. 10. Precision results for fifteen consecutive analyses of 10 ng/μL AEME (K_o, 1.50 $cm^2V^{-1}s^{-1}$). The average amplitude is 483 du, with a SD of ±44, and RSD of 9.0%.

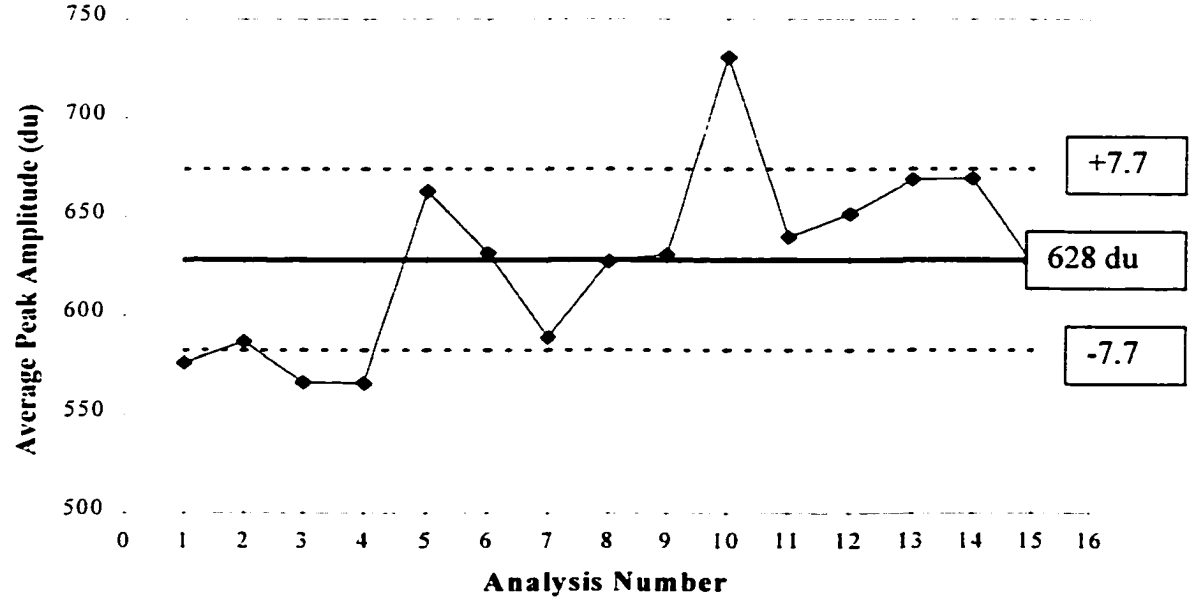


Fig. 11. Precision results for fifteen consecutive analyses of 10 ng/μL EME (K_o, 1.48 $cm^2V^{-1}s^{-1}$). The average amplitude is 628 du, with a SD of ±46, and RSD of 7.7%.

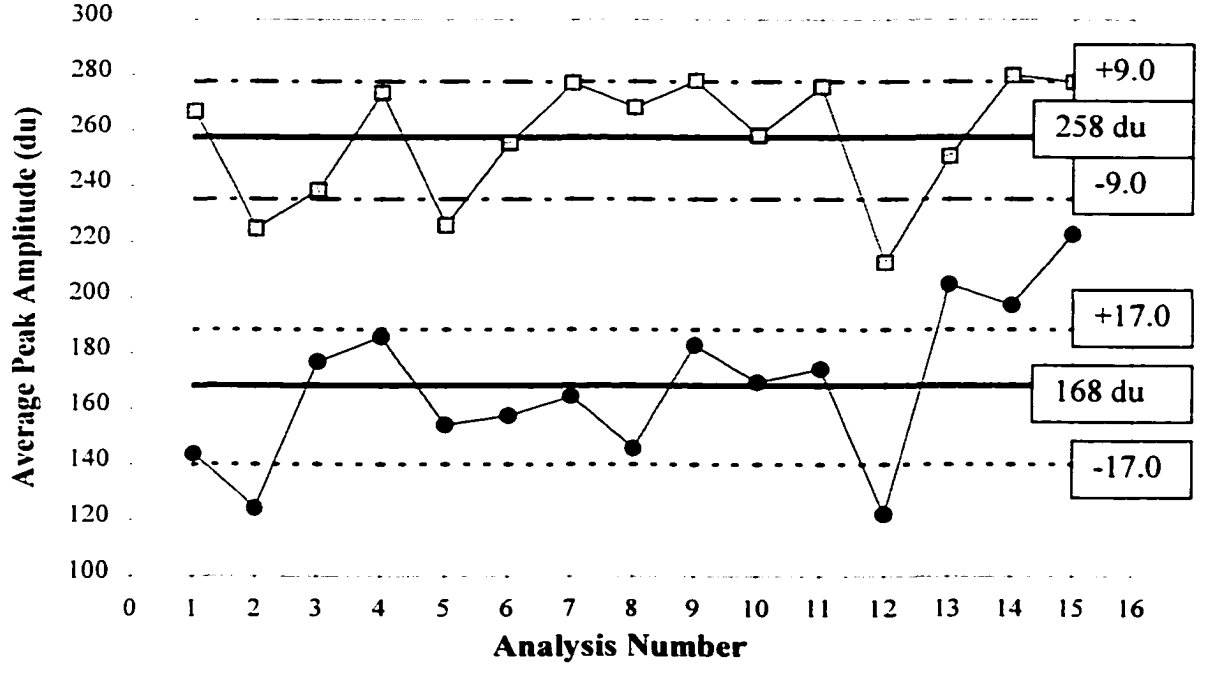


Fig. 12. Precision results for fifteen consecutive analyses of 0.5-1 mg A43 brain tissue. The peak at K_o 1.65 $cm^2V^{-1}s^{-1}$ has an average amplitude of 258 du, with a SD of ±22, and RSD of 9.0%. The peak at K_o 1.57 $cm^2V^{-1}s^{-1}$ has an average amplitude of 168 du, with a SD of ±28 and a RSD of 17%.

peaks inherent to the tissues as described in Chapter IV were measured. The peak at K_o 1.65 $cm^2V^{-1}s^{-1}$ has an average of 258 du, with a SD of ± 22 and a RSD of 9.0%. The other peak at K_o 1.57 $cm^2V^{-1}s^{-1}$ has an average amplitude of 168 du, a SD of ± 28 and a RSD of 17%. The statistical data for K_o values for cocaine, AEME, EME, and animal tissues are given in Table 5.

Table 5
Statistical data for the K_o values of cocaine, AEME, EME, and A-43 brain tissue.

Analyte	Average K_o value ($cm^2V^{-1}s^{-1}$)	Relative Standard Deviation[a]
Cocaine hydrochloride	1.17 ± 0.00	0.01%
AEME	1.51 ± 0.00	0.02%
EME	1.49 ± 0.00	0.05%
A-43 Brain tissue Peak 1	1.66 ± 0.00	0.02%
A-43 Brain tissue Peak 2	1.53 ± 0.00	0.01%

[a]Calculated using four significant figures for K_o values rather than three shown in the table.

Discussion and Conclusions

QC procedures were established to ensure that IMS data generated is accurate and precise enough for their intended use, i.e., screening vs. semi-quantitative analysis. Cocaine standards supplied, by a manufacturer, were used to determine whether the IMS procedures were under statistical control. The results were excellent indicators of instrument variability. Diluted solutions of the primary standards were used to ensure that calibration stays in control and that detection limits are satisfactory. Peak amplitudes and K_o values were established for QC check solutions that must be met each day before proceeding further. For example, if the peak amplitude of the QC solution

was significantly lower than the amplitudes observed form the calibration curve of the same solution, or there was a significant shift in K_o values of the solution, corrective procedures were implemented. Any deviation from the criteria requires assessment of the problem and corrective action. The procedure was found helpful in maintaining data quality, and in providing an early indication of potential problems.

It is concluded that cocaine is a useful standard for IMS QC in the positive mode. Cocaine hydrochloride, (as well as the freebase) when analyzed by IMS, decomposes with the appearance of an ion peak corresponding to the formation of AEME. The extent of decomposition depends on temperature, heating time, and nature of the IMS desorption surface. Greater than expected decomposition of cocaine standard has served as an important indicator that instrument checks need to be performed before proceeding further.

Precision of IMS analyses was determined for cocaine hydrochloride, AEME, EME, and 0.5-1 mg of brain tissue from rabbits. Based on the results, it was concluded that the results justify development of semi-quantitative methods based on IMS for cocaine, AEME, and EME with appropriate calibration curves.

The precision data obtained from the IMS of brain tissue were interesting. It is concluded that the reproducibility of the tissue background will facilitate analysis of drugs and metabolites in animal tissues.

CHAPTER IV

ION MOBILITY SPECTROMETRY OF ANIMAL TISSUES

Introduction

Today's clinical laboratory has the capability of analyzing many substances including proteins, trace metals, tumor markers, and different drugs in tissues harvested from diseased areas of the body. Useful methods also include immunoassays, titration assays, colorimetric assays, centrifugation analysis, chromatographic methods, and immunocytochemical assays just to a mention several. Such analyses have provided information concerning malignant organs as well as organs damaged by chronic drug use or autoimmune reactions.

IMS may be an attractive alternative and/or a useful adjunct in the analysis of tissues and other substances of biological origin. IMS was used in the present research to detect the presence of cocaine and other physiologically active substances in tissues harvested from New Zealand white rabbits. The specific details of these studies are addressed in chapters V and VI. The present chapter describes the IMS of control tissues under different conditions as baseline information for studies of tissues from animals exposed to drugs.

Control tissues from non-pregnant rabbits, pregnant rabbits, and their fetuses were analyzed for ion peaks innate to the animal tissues themselves and to establish whether such peaks coincided with peaks from drugs or metabolites. This served as a basis for establishing windows which could be used for compounds of interest.

The tissues examined from the maternal and nonpregnant adult rabbits were brain, lung, kidney, renal artery, atria, ovary, and liver. The fetal and cotyledon, heart,

spectra might be due to these compounds. Peaks appearing in positive-mode mobility spectra kidney, lung, and liver were also analyzed. Assay reproducibility between animals, and assay variations within organs were determined. Optimizations of IMS parameters, desorption temperature, analysis time, and sample amount for tissue analyses were also defined.

Results

IMS Signatures of Animal Tissues: Positive and Negative Mode Fingerprints

The mobility spectra obtained from analyzing animal tissues in the positive mode generally exhibited at least four peaks with K_o values 1.65, 1.52, 1.38, and 1.31 $cm^2V^{-1}s^{-1}$. An example using C3 brain tissue is shown in Fig. 13. Other examples are given in Appendix A. The ion peaks are not necessarily inherent to the tissues since the animals and harvested tissues were exposed to various chemicals including euthanizing agent (main ingredient - pentabarbitol, minor ingredient - benzyl alcohol), anesthetics (Ketaset and Rompun) and heparin (Fig. 14). The K_o values of tissues harvested from animal C3, and compounds used with animals and/or tissues, are given in Tables 6 and 7, respectively.

The ion peaks of highest amplitude shown in Table 7 arise from the anesthetics Rompun and Ketaset at K_o values 1.38 $cm^2V^{-1}s^{-1}$ and 1.37 $cm^2V^{-1}s^{-1}$, respectively. The next highest is from heparin at 1.31 $cm^2V^{-1}s^{-1}$. The concentrations of all of the compounds are much higher relative to the sensitivity that IMS is generally capable of. High amplitudes such as observed when analyzing Rompun and Ketaset alone may also reflect saturation of the spectrometer. Lower concentrations were also run and are

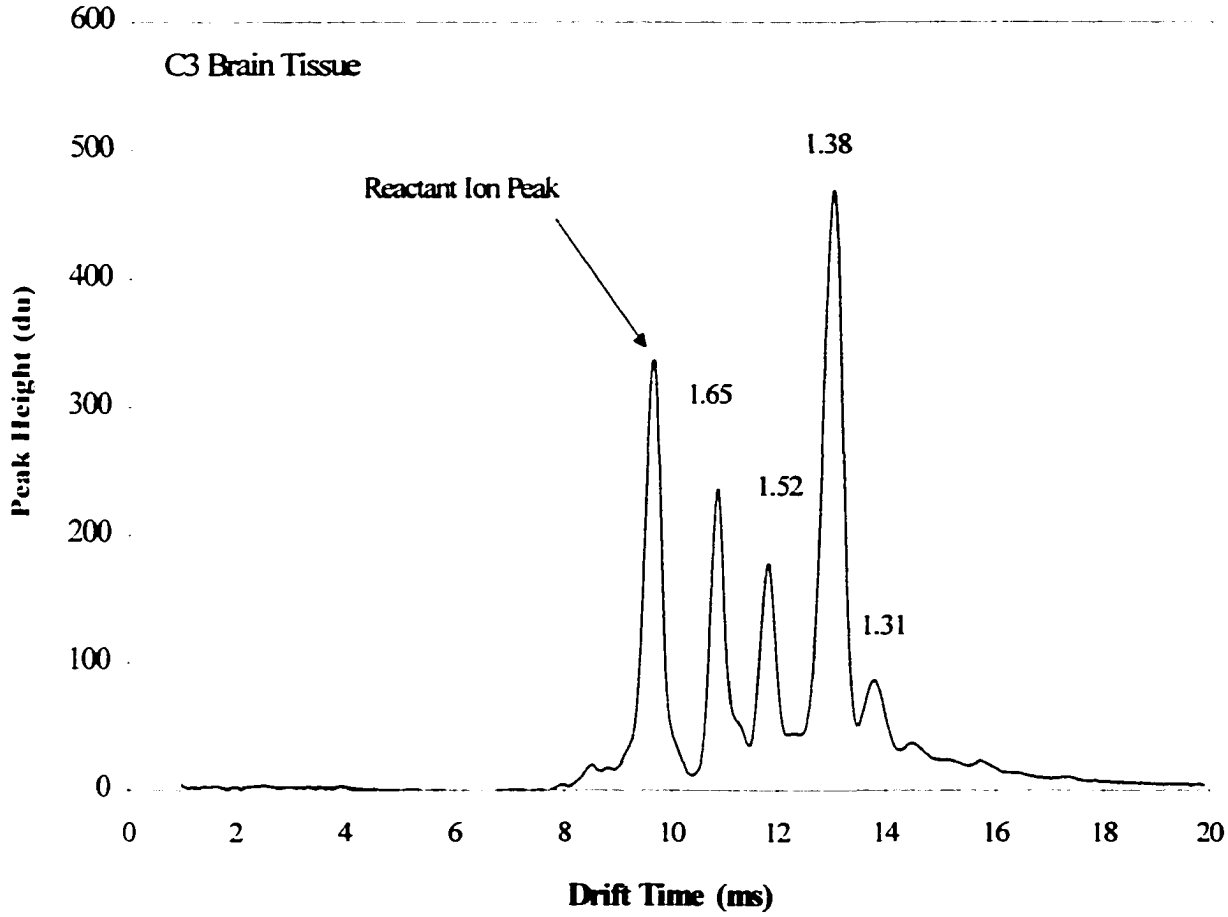
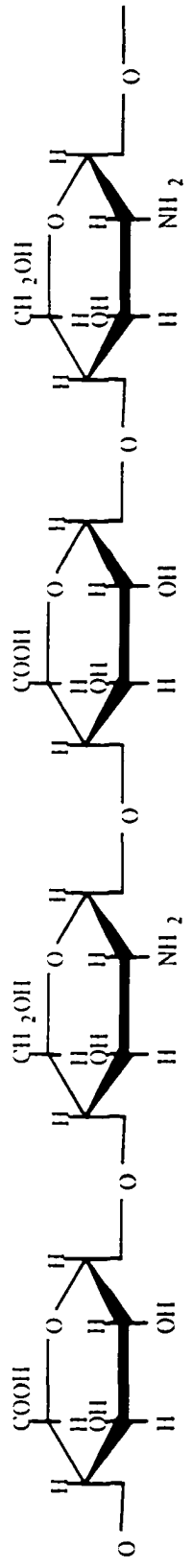
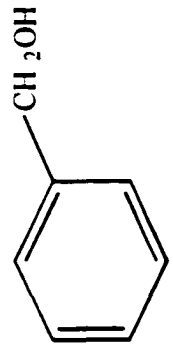


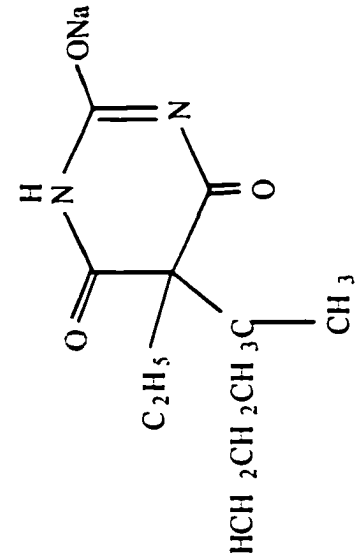
Fig. 13. The positive mode IMS mobility spectrum of brain tissue from animal C3 (control animal given vehicle saline in an acute study). K_o values 1.65 and 1.52 $cm^2V^{-1}s^{-1}$ are native to the tissue. A K_o value 1.38 $cm^2V^{-1}s^{-1}$ is attributed to the anesthetic given during surgery.



Heparin



Benzyl Alcohol



Pentobarbitol Sodium

Fig. 14. Structures of heparin, benzyl alcohol, and pentobarbitol sodium.

Table 6
IMS peaks of tissues obtained from animal C3. This animal received saline in an acute study

Organ	Peak Number	K_o ($cm^2V^{-1}s^{-1}$)	Amplitude (du)
Renal Artery	1	1.38	269
	2	1.31	60
Brain	1	1.65	204
	2	1.51	146
	3	1.38	438
	4	1.31	65
Adrenal	1	1.65	46
	2	1.51	503
	3	1.31	28
Liver	1	1.65	53
	2	1.38	90
Kidney	1	1.65	95
	2	1.38	577
Atria	1	1.38	251
	2	1.31	734
Lung	1	1.65	233
	2	1.51	105
	3	1.38	517
	4	1.31	73
Ovary	1	1.65	237
	2	1.51	73
	3	1.38	336
	4	1.31	43
Ventricle	1	1.65	267
	2	1.51	57
	3	1.38	401
	4	1.31	259

Table 7
The IMS K_o values and amplitudes of 1 μL of compound solutions used routinely with the animals or tissues

Compound	Concentration (μg/μL)	Peak Number	K_o ($cm^2V^{-1}s^{-1}$)	Amplitude (du)
Rompun	100	1	1.38	884
		2	0.91	49
Ketaset	100	1	1.47	42
		2	1.37	978
		3	1.03	63
Euthanasia	3.24[b]	1	1.44	219
		2	1.38	184
Benzyl Alcohol	100%	1	1.40	256
		2	1.45	86
Heparin	1 unit/μL[a]	1	1.31	663

[a] Each mL of heparin contains 1000 USP units. USP is a measurement of anticoagulant activity.
[b]Calculated as pentabarbitol, the major component.

described in Chapter V. However, the results with high concentrations give a first indication that any peaks with same K_o values appearing in tissue sample mobility of animal tissues (Table 6) that coincide with peaks from compounds listed in Table 4, have K_o values of 1.38 $cm^2V^{-1}s^{-1}$ and 1.31 $cm^2V^{-1}s^{-1}$. These could arise from the anesthetics, euthanizing agent and heparin.

The anesthetics, Rompun and Ketaset, though different compounds (xylazine and ketamine, respectively) have K_o values which overlap and are not distinguishable using IMS. The relatively low molecular weights of Rompun and Ketaset (220.3 and 237.7, respectively), low melting points (140 – 142 °C and 92-93 °C, respectively), [41,42] and the presence of a secondary amine function (high proton affinity) would lead to the expectation of strong IMS peaks as was found (Table 7).

A weak peak with K_o 1.38 $cm^2V^{-1}s^{-1}$ was found in the mobility spectra of brain tissue from an animal that received euthanasia but no anesthesia (Fig. 15A). This ion peak may be inherent to the brain tissue or may arise from the euthanasia. A weak peak was found for a relatively high amount of euthanasia (Table 7). There were two animals that died spontaneously and received anesthesia, but not euthanasia. Tissues from these animals exhibit relatively high peaks at K_o 1.38 $cm^2V^{-1}s^{-1}$. Though these peaks can not be assigned unequivocally, the strong IMS response to Ketaset and Rompun coupled with the strong peaks found in the tissues of animals treated with anesthesia, leads one to conclude that the peak at 1.38 $cm^2V^{-1}s^{-1}$ is largely due to anesthesia, with the euthanizing agent playing a minor role. Further evidence was obtained when Rompun was added in vitro to brain tissue. The mobility spectrum (Fig. 15B) appeared identical to that of brain tissue from an animal receiving anesthetic (Fig. 13). This also means that the mobility spectrum region at or in the vicinity of K_o 1.38 $cm^2V^{-1}s^{-1}$ cannot be used for making conclusions as to the presence of a particular drug or metabolite for animals receiving Rompun and Ketaset.

Assignment of the peak at K_o 1.31 $cm^2V^{-1}s^{-1}$ in animal tissues also could not be done unequivocally although heparin did exhibit the peak (Table 6). Heparin was injected into the animal systemically (intravenously or intra-arterially) and was used as an anticoagulant when obtaining blood samples. Heparin is a heterogeneous mixture of variable sulfated polysaccharide chains composed of repeating units of D-glucosamine and either L-iduronic or D-glucuronic acids. The molecular weight ranges from 6,000 – 30,000 Da [43]. The IMS signal is most likely due to thermal decomposition which might be avoided at lower IMS desorption temperatures, but was not investigated.

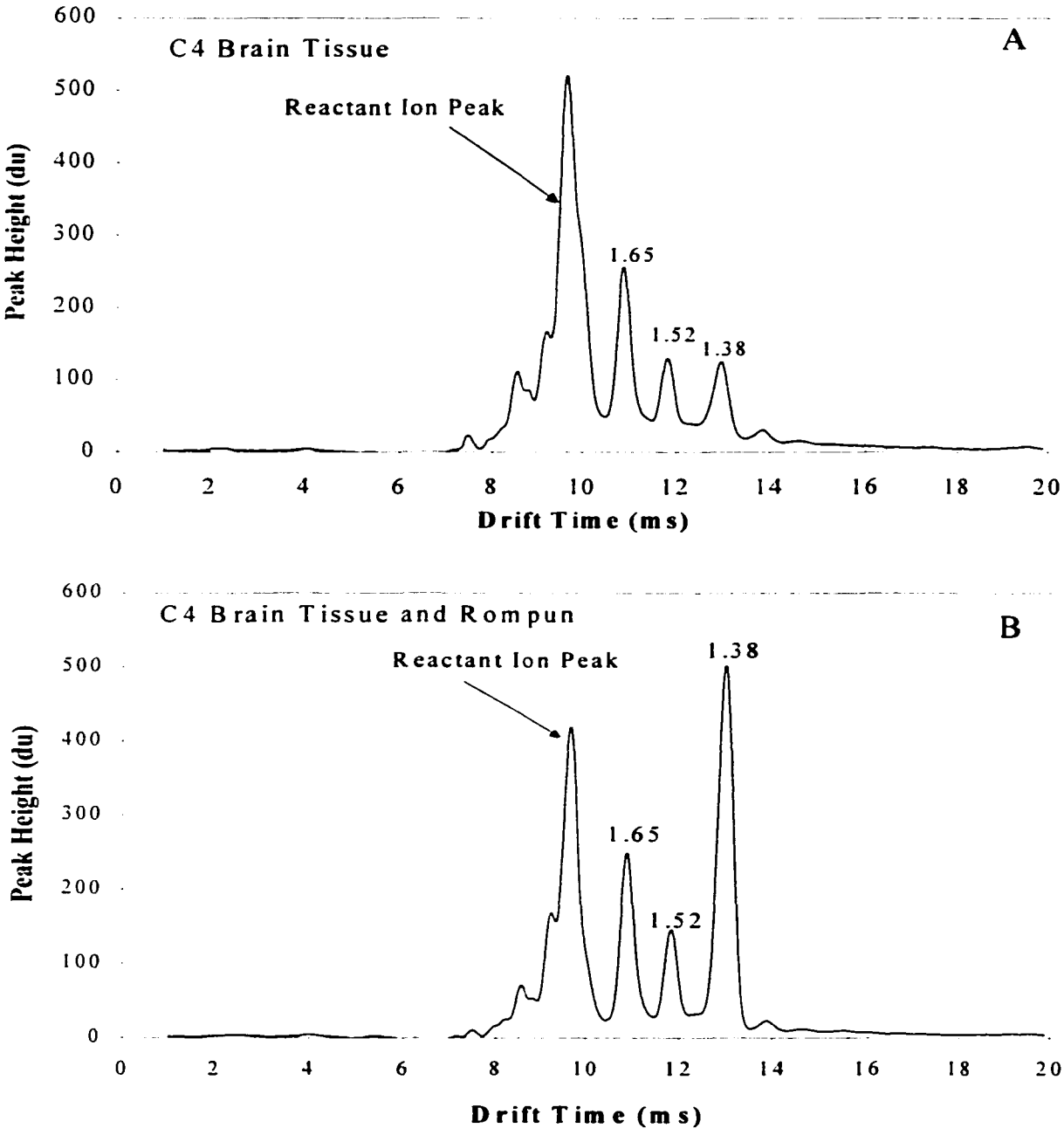


Fig. 15A and 15B. The positive mode IMS mobility spectra of brain tissue from animal C4. (A) Represents brain tissue that was not exposed to anesthetic. (B) Represents 1 μg/μL of Rompun added to brain tissue *in vitro*.

The negative IMS mode (negative ions) was examined also as a means of characterizing the tissues. Fig. 16 illustrates an example of results from brain tissue. The K_o values and amplitudes for C3 animal tissue analyzed in the negative mode are given in Table 8.

The euthanizing agent and anesthetics were also analyzed in the negative mode. However, there did not seem to be any direct correlation between peaks attributed to these compounds and peaks present in the animal tissue spectra.

Optimization of Sampling Time and Desorption Temperature

Sampling times (during thermal desorption) were varied, from 5 to 20 seconds, for brain tissue and anesthetics as representative materials for optimization of sensitivity (K_o 1.38 $cm^2V^{-1}s^{-1}$). Sampling times of 8 and 5 seconds for brain tissue and anesthetic, respectively, in a mixture of brain tissue and anesthetic, gave the highest peak amplitude (Fig. 17). No new peaks were observed with the longer times.

The desorber temperatures were varied from 100°C to 350°C to examine effects on peak amplitude for brain tissue and anesthetic. The highest amplitudes for the analysis of brain tissue and anesthetic were found at 250°C and 200°C, respectively. (Fig. 18) A sampling time of 8 seconds and desorption temperature of 285°C were chosen for the remaining experiments in the study as reasonable conditions to detect major changes to the IMS characteristics of both animal tissue and anesthetic. This is not meant to imply that these are the best choices for every purpose. For example, a much lower desorption temperature may eliminate tissue background peaks and yet allow specific analytes to be readily analyzed. Investigators need to optimize sampling time and desorption temperature consistent with objectives of each specific study.

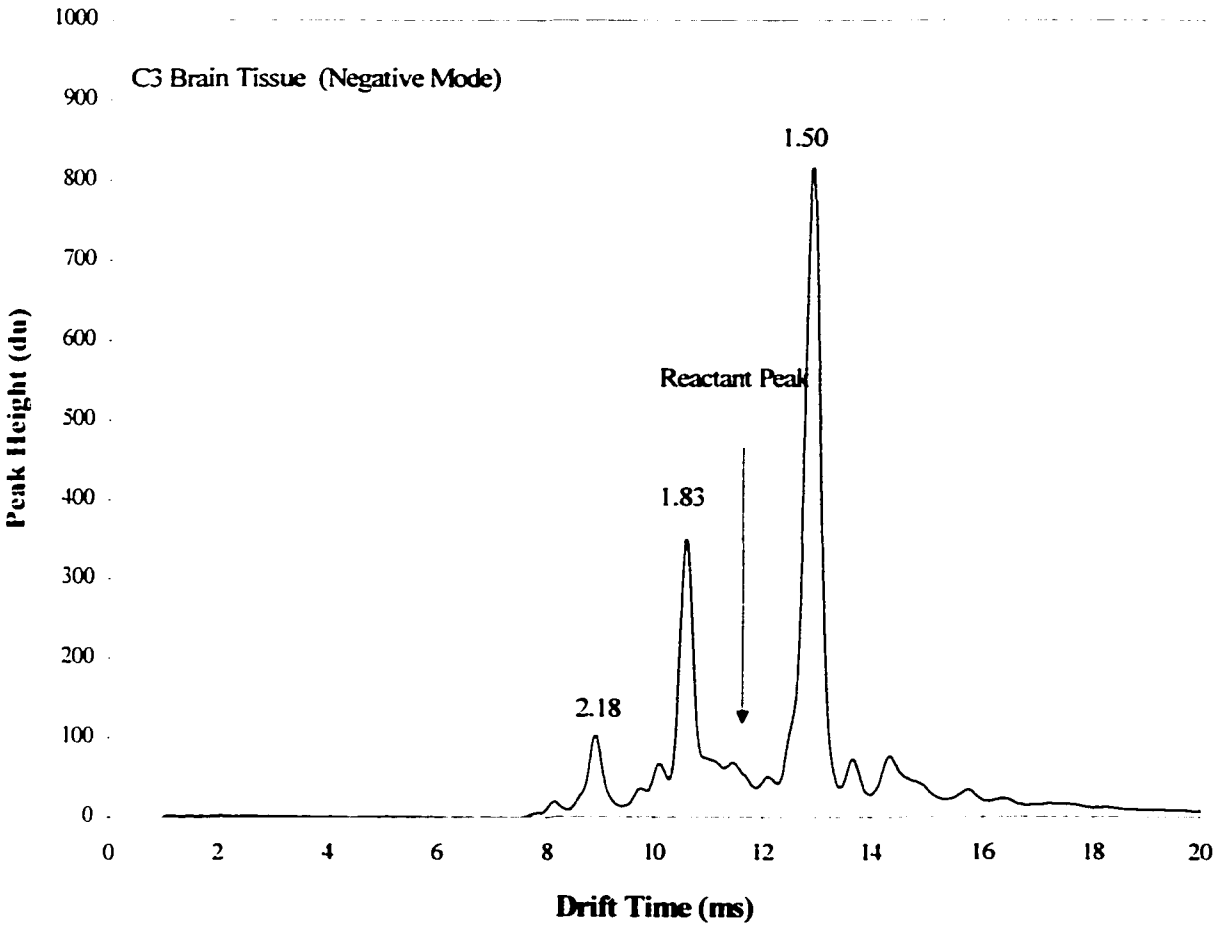


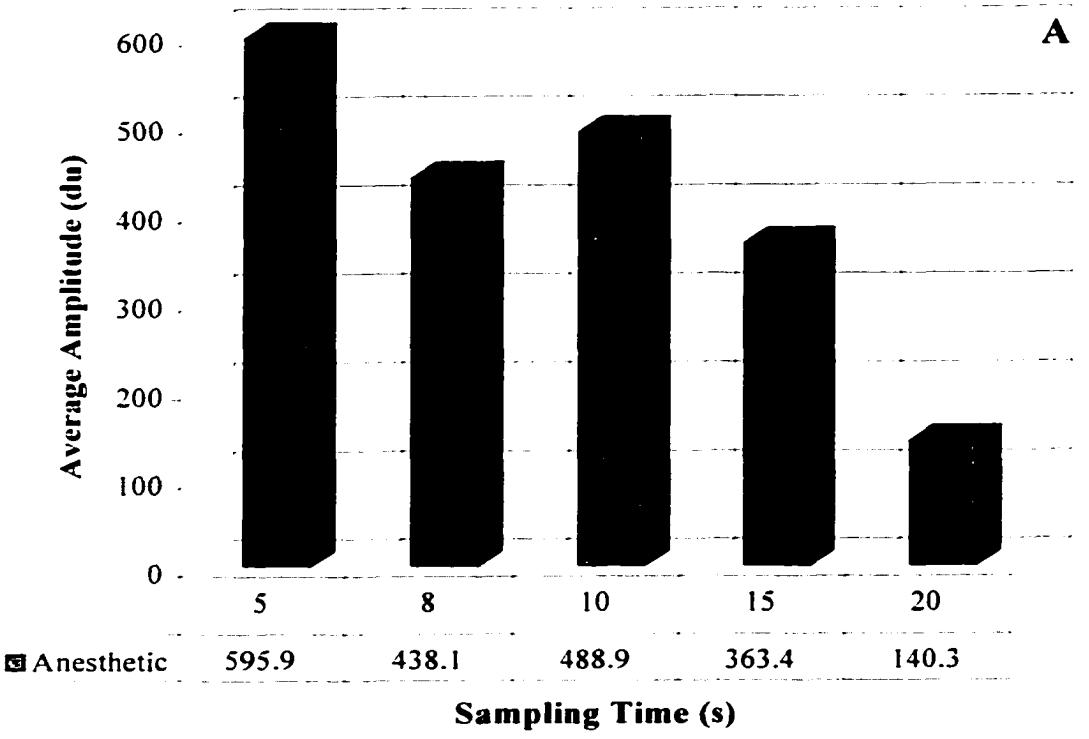
Fig. 16. The negative mode IMS mobility spectrum of brain tissue from animal C3.

Table 8
IMS peaks from C3 animal tissue in the negative mode

Organ	Peak Number	K_o ($cm^2V^{-1}s^{-1}$)	Amplitude (du)
Renal Artery	1	2.18	326
	2	1.83	206
	3	1.50	93
	4	1.20	165
Brain	1	2.18	178
	2	1.83	841
	3	1.69	235
	4	1.50	306
Adrenal	1	2.18	229
	2	1.83	232
	3	1.77	179
	4	1.69	270
	5	1.57	62
	6	1.50	142
	7	1.42	91
Liver	1	2.18	164
	2	1.83	266
	3	1.77	127
	4	1.69	104
	5	1.49	223
	6	1.41	53
Kidney	1	2.19	185
	2	1.95	170
	3	1.83	135
	4	1.73	113
	5	1.60	66
	6	1.50	66
Atria	1	2.18	220
	2	1.83	627
	3	1.77	189
	4	1.69	101
	5	1.60	48
	6	1.50	76

Table 8
Continued

Organ	Peak Number	K_o ($cm^2V^{-1}s^{-1}$)	Amplitude (du)
Lung	1	2.18	179
	2	1.83	745
	3	1.69	184
	4	1.50	367
	5	1.42	53
Ovary	1	2.18	193
	2	1.83	504
	3	1.69	81
	4	1.58	72
	5	1.50	179
			177
Ventricle	1	2.18	
	2	1.83	
	3	1.77	
	4	1.69	
	5	1.50	
	6	1.18	



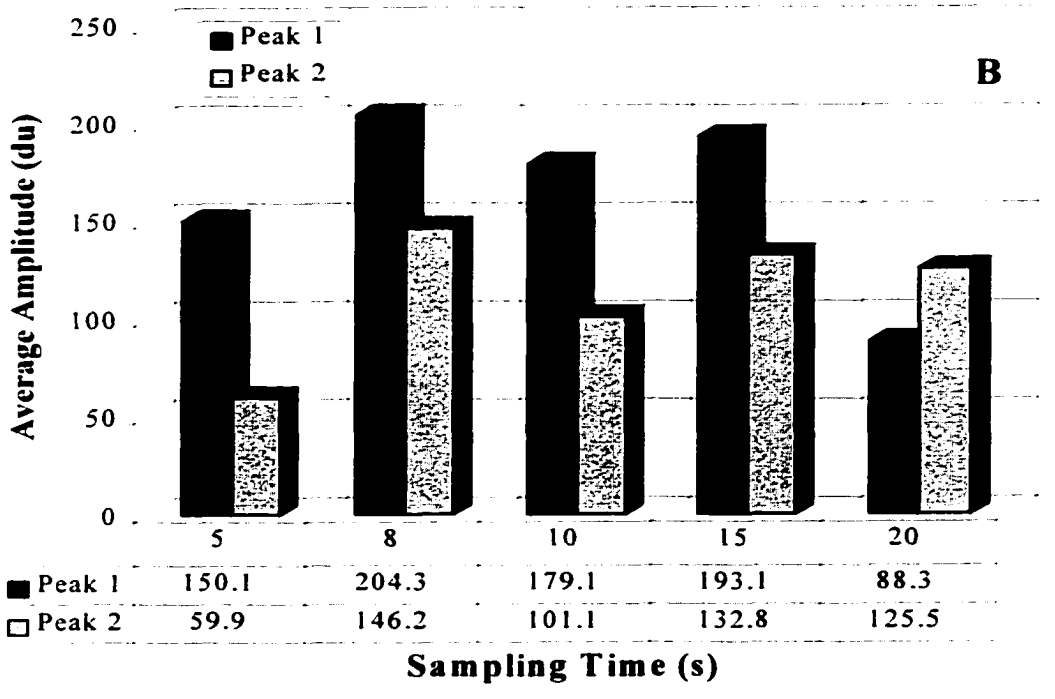


Fig. 17A and 17B. Optimization of sampling time for the IMS analysis of C3 brain tissue with anesthetic. (A) The optimal sampling time for anesthetic is 5 seconds. (B) The optimal sampling time for compounds innate to brain tissue is 8 seconds.

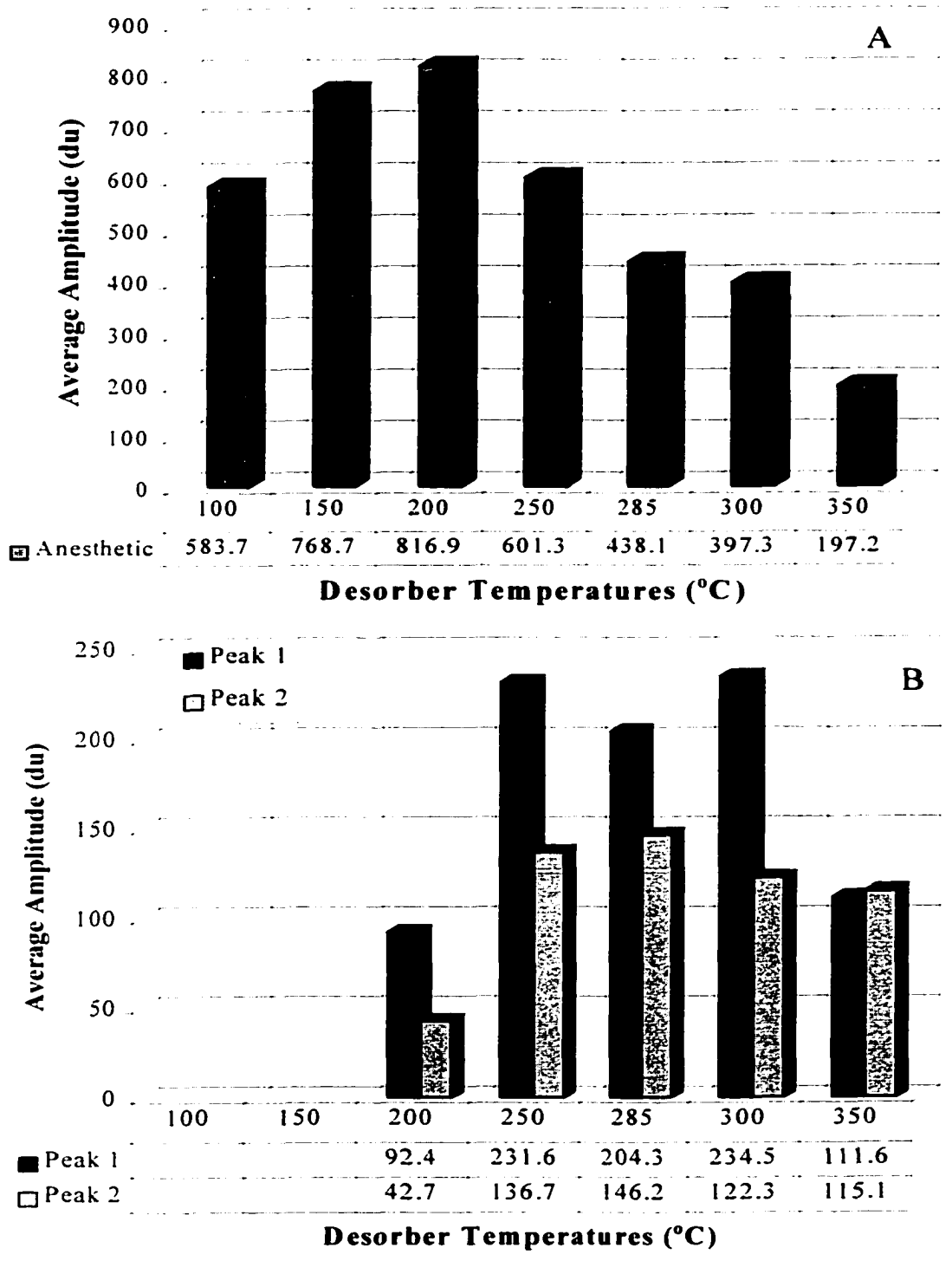


Fig. 18A and 18B. Optimization of desorber temperature for the IMS analysis of C3 brain tissue with anesthetic. (A) The optimal desorption temperature for anesthetic is 200°C. (B) The optimal desorption temperature for compounds innate to brain tissue is 250°C.

IMS of Plasma

Plasma, urine, and amniotic fluids were collected from rabbits and analyzed using IMS. All animals received anesthetic, heparin, and in some cases EME. Plasma samples were taken at times ranging from 0 minutes to 30 minutes after a drug or sterile water was administered.

Fig. 19 illustrates a plasma sample from animal C1, collected directly after the injection of sterile water. In this mobility spectrum, there is a peak at K_o 1.38 $cm^2V^{-1}s^{-1}$, which corresponds to the mobility constant of Rompun. Fig. 20 shows a peak of 1.49 $cm^2V^{-1}s^{-1}$, which corresponds to EME. It was interesting that a peak for Rompun was absent. This animal (A15) was pregnant and received an intramuscular injection of 2 mg/kg EME. The plasma sample was collected 5 minutes after EME was injected. received an intramuscular injection of 2 mg/kg EME. The plasma sample was collected 5 minutes after EME was injected.

The time of collection of the plasma affects the amplitude of the IMS drug peak. Fig. 21 illustrates the collection of plasma from animal C3 at times ranging from 0 to 20 minutes. This chart shows that anesthesia is detectable immediately after the injection of the drug. The peak amplitude of anesthesia decreases with increasing sampling time. This trend was subjected to regression analysis, which indicated that it was significant at $P < 0.01$.

It was found that both anesthetic and EME could be detected in a urine sample collected for 24 hours from animal A3 after injection with EME. Fig. 22 shows that there are distinct peaks at 1.38 and 1.49 $cm^2V^{-1}s^{-1}$, assigned to anesthesia and EME, respectively.

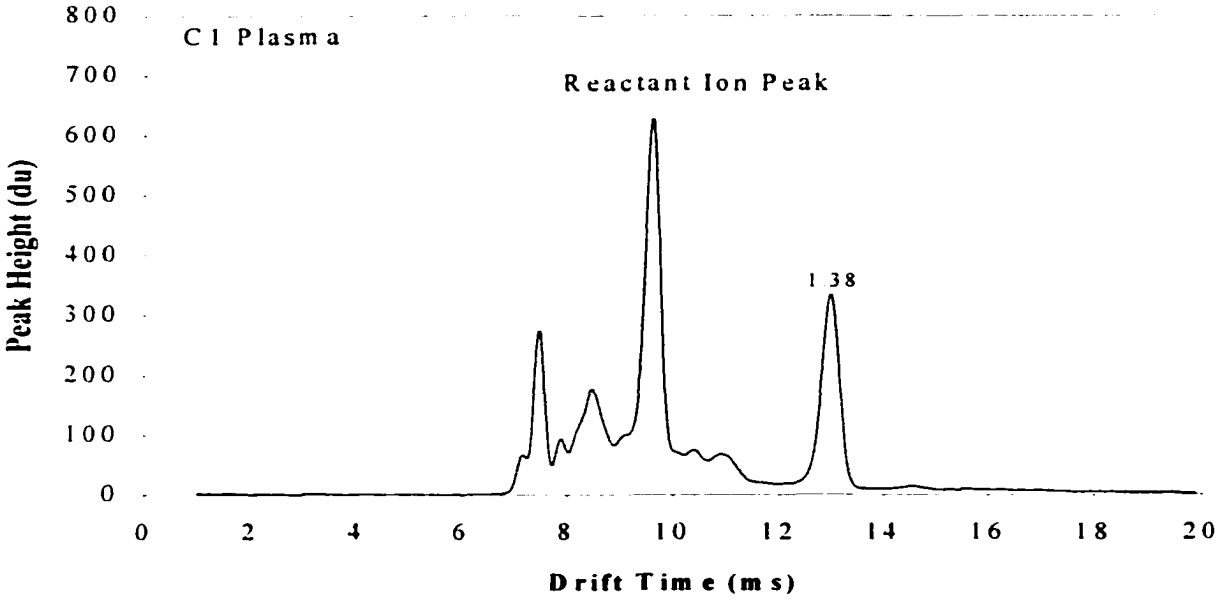


Fig. 19. IMS mobility spectrum of plasma from animal C1 collected directly after injection of sterile water. The peak at K_o 1.38 $cm^2V^{-1}s^{-1}$ indicates the presence of anesthetic.

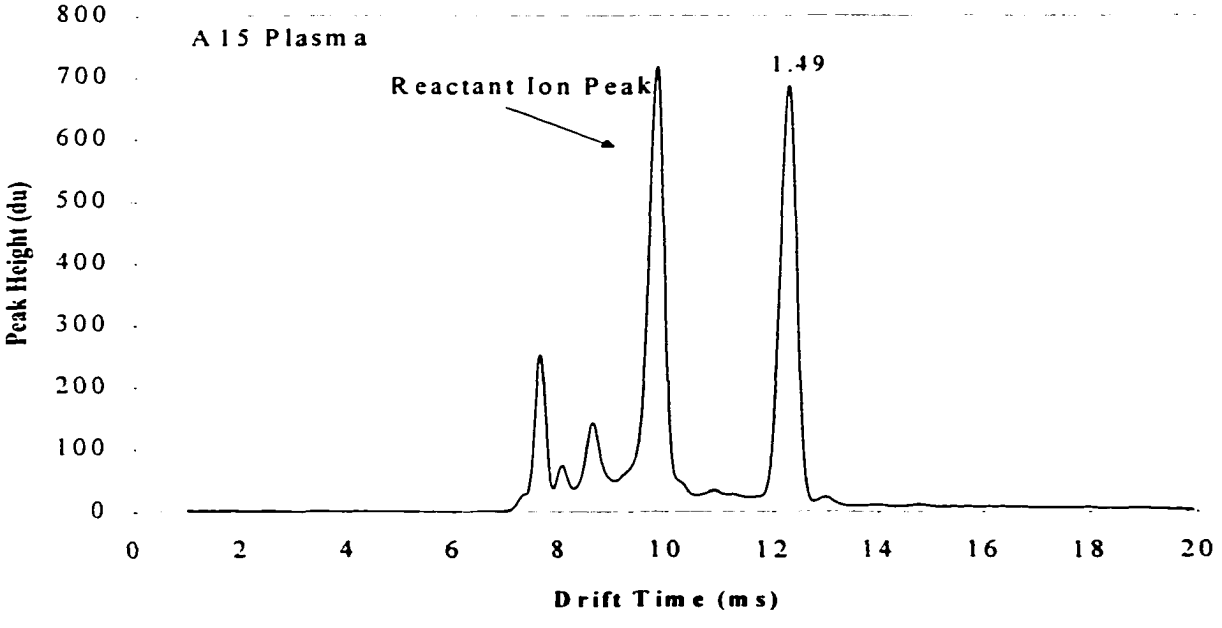


Fig. 20. IMS mobility spectrum of plasma from animal A15 which received an intramuscular injection of 2 mg/kg EME. The plasma sample was collected 5 minutes after EME was administered. The peak at K_o 1.49 $cm^2V^{-1}s^{-1}$ indicates the presence of EME.

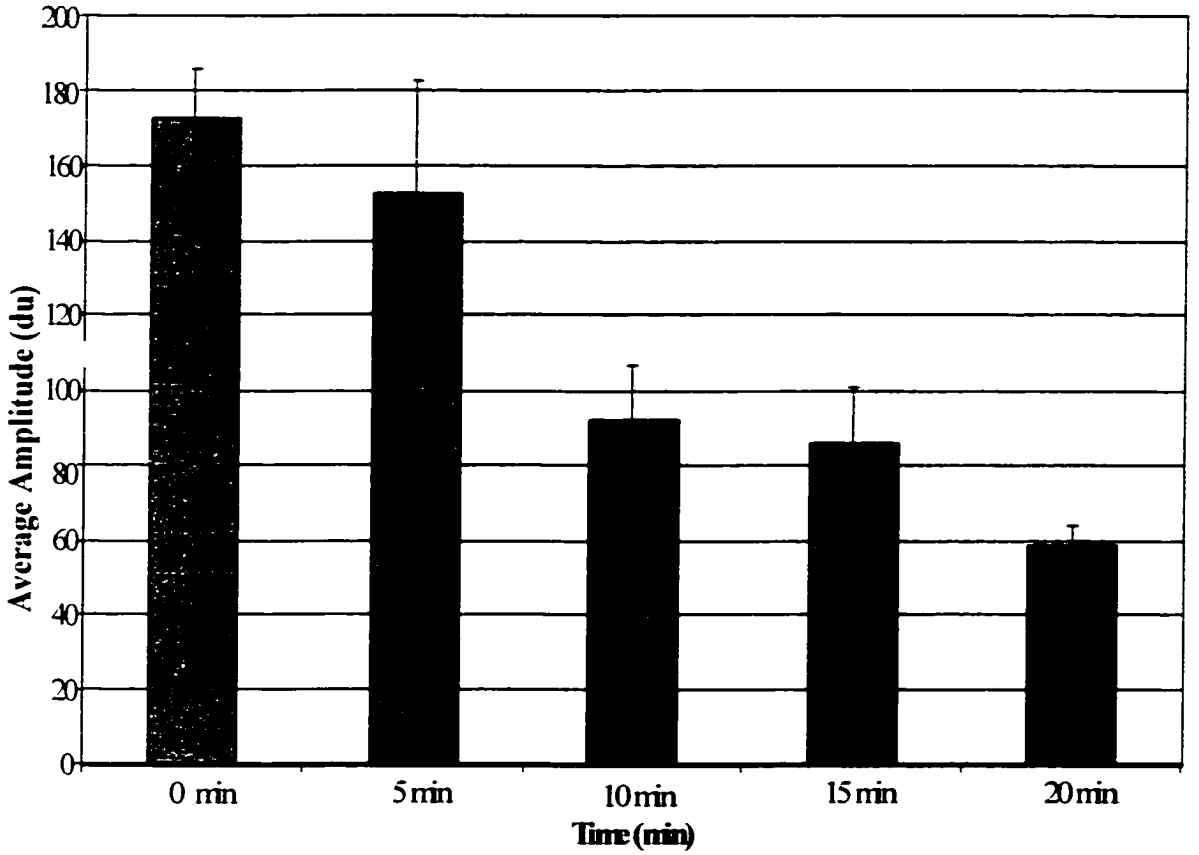


Fig. 21. Amplitudes of the ion peak corresponding to anesthetic (K_o, 1.38 cm^2V^{-1} s^{-1}) from the IMS analysis of plasma (animal C3) collected at different time intervals.

Amniotic fluid was collected from an animal in a chronic study, with cocaine being administered via an Alzet pump for 14 days. In the mobility spectrum of this fluid, there is a peak at K_o 1.38 $cm^2V^{-1}s^{-1}$, which corresponds to the mobility constant of Rompun (Fig. 23).

Based on these preliminary results, it appears that IMS may be very useful in analyzing drugs and metabolite directly in biological fluids. Useful information may be obtainable relative to metabolic rates by varying sample collection time. There appears to be great potential in the use of IMS to analyze biological fluids directly. Additional work is necessary including gathering data with control animals to fully appreciate the possibilities.

Acute vs. Chronic Studies

In acute studies, the animals were sacrificed approximately 24 hours after surgery, and in chronic studies after 10 days. In some cases, it appears that the presence of any drug given in the chronic study has been completely metabolized and does not appear in the mobility spectra. The analysis of tissue from an acute study generally exhibits medium or large amounts of anesthetic. Additional information and specific examples are given at the end of this chapter.

Animal to Animal Variations

Animal to animal differences in tissue results may depend on animal gender and age. There appears to be no overall differences between tissue from a non-pregnant animals and tissue from a pregnant one. Fetal tissue and adult tissue differ in the relative amplitudes of the peaks seen in their mobility spectra. Fetal tissue also exhibits several peaks that are lower in ion mass and are not present in adult tissue.

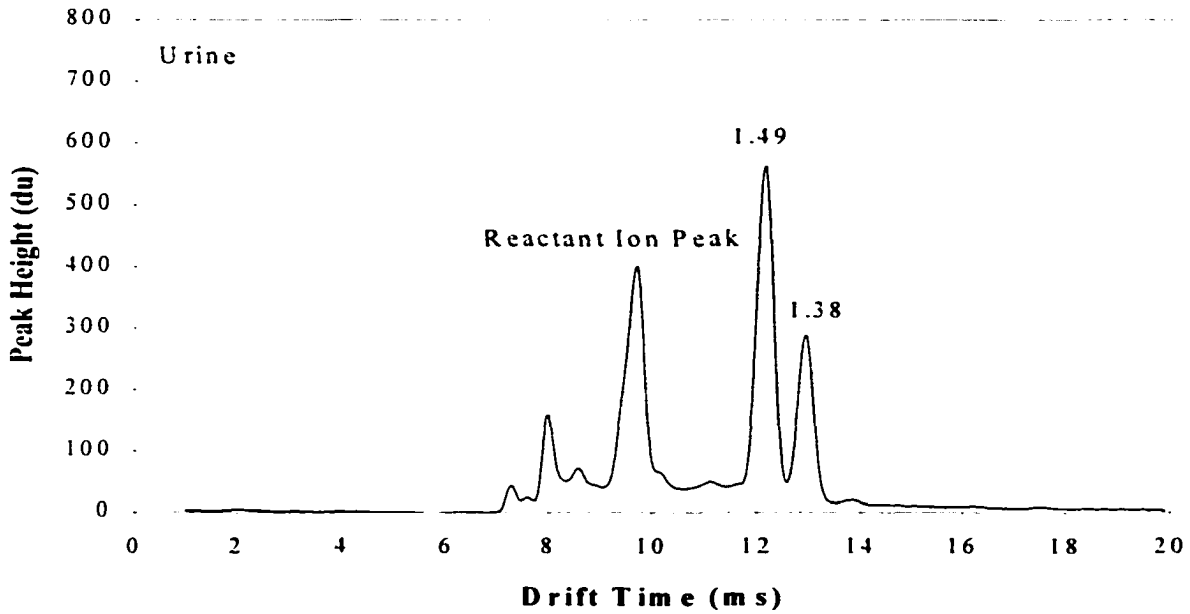


Fig. 22. IMS mobility spectrum of urine from animal A3 collected over 24 hours after injection with EME. The peaks at K_o, 1.49 cm^2V^{-1} s^{-1} and K_o, 1.40 cm^2V^{-1} s^{-1}) indicate the presence of EME and anesthetic, respectively.

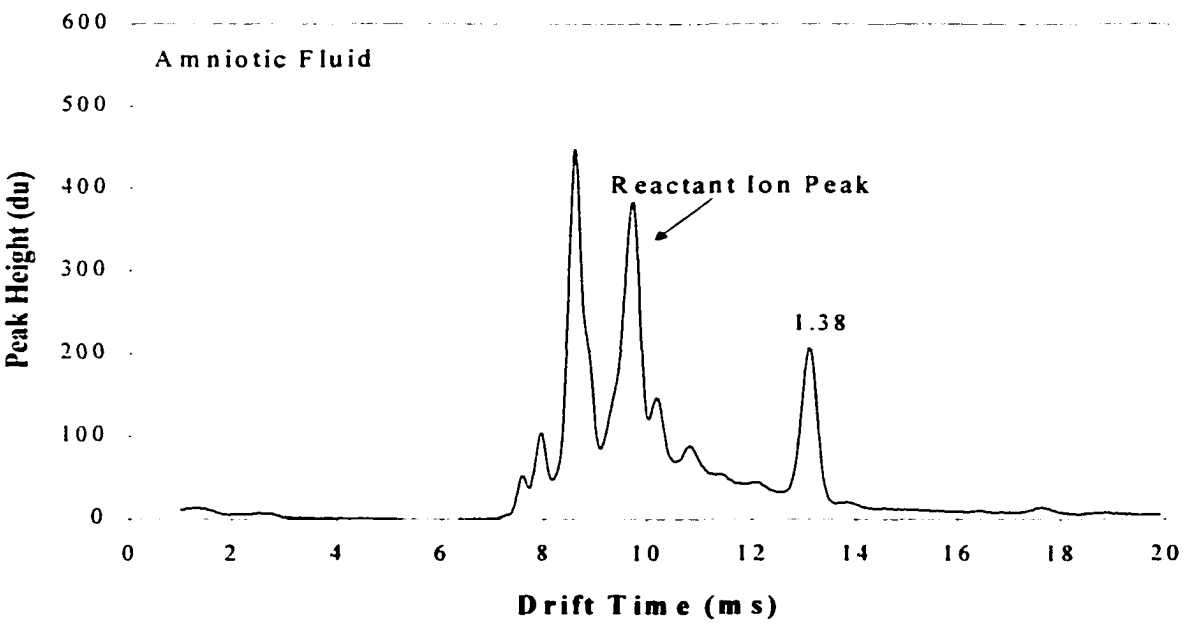


Fig. 23. IMS mobility spectrum of amniotic fluid from animal A28. The peak at K_o, 1.38 $cm^2V^{-1}s^{-1}$ indicates the presence of anesthetic.

Evidence suggests that anesthetic given to a pregnant rabbit appears in the tissue of her fetuses (Fig. 24). However, no control fetal tissues were available from pregnant rabbits not receiving anesthetics. Brain tissue was chosen as a representative organ for most of the animal tissues examined. A comparison (Fig. 25) of tissues from different animals shows that there are two peaks native to brain tissue in each case (K_o 1.65 and 1.51$cm^2V^{-1}s^{-1}$). Anesthetic peaks (K_o 1.38 $cm^2V^{-1}s^{-1}$) appeared with all of the samples except two (A43 and A44). A peak at K_o 1.31 $cm^2V^{-1}s^{-1}$ was present at low intensities in all of the tissues excluding C1 and A44. A new peak (K_o 1.16 $cm^2V^{-1}s^{-1}$) was observed in animal tissues A39 and A44.

Discussion

The specific aim of this study was to analyze rabbit brain, lung, kidney, renal artery, atria, ovary, and liver harvested from animals and to determine whether the potential exists to characterize them using IMS. Another aim was to determine whether a sufficient number of windows were open in the IMS mobility spectra to allow drugs and/or their metabolites to be directly identified in the tissues without the need of performing extractions or employing special recovery procedures.

Rabbits were utilized since tissues were available from an independent study in the Department of Biological Science, Old Dominion University, (Principal Investigator: Professor Barbara Y. Hargrave) on the effect of illicit drugs on the cardiovascular and hormonal system of pregnant New Zealand white rabbits.

The IMS analysis of tissues from a variety of organs under the condition chosen, demonstrated the feasibility of characterizing animal tissues. Analyses were performed both in the positive and negative modes.

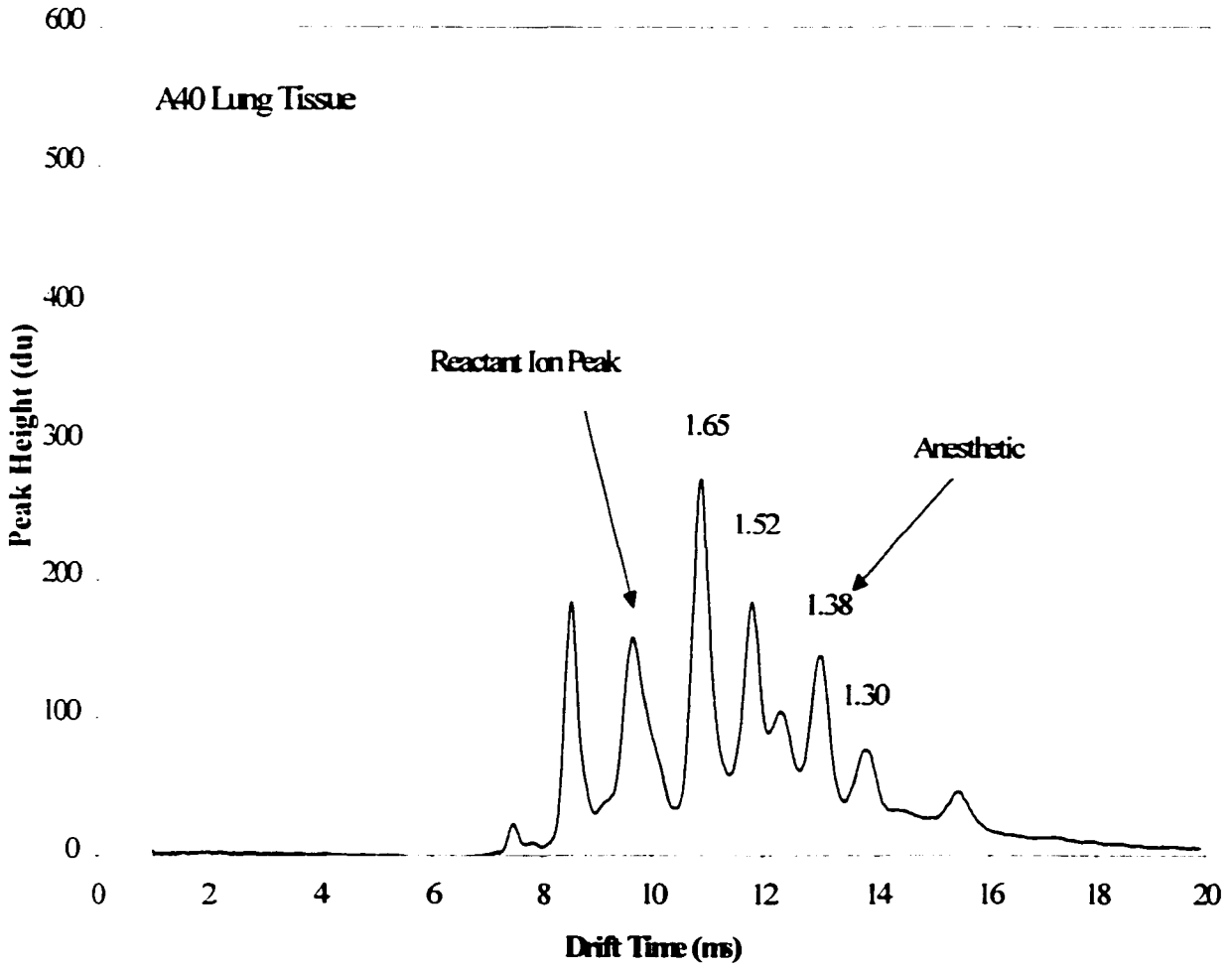
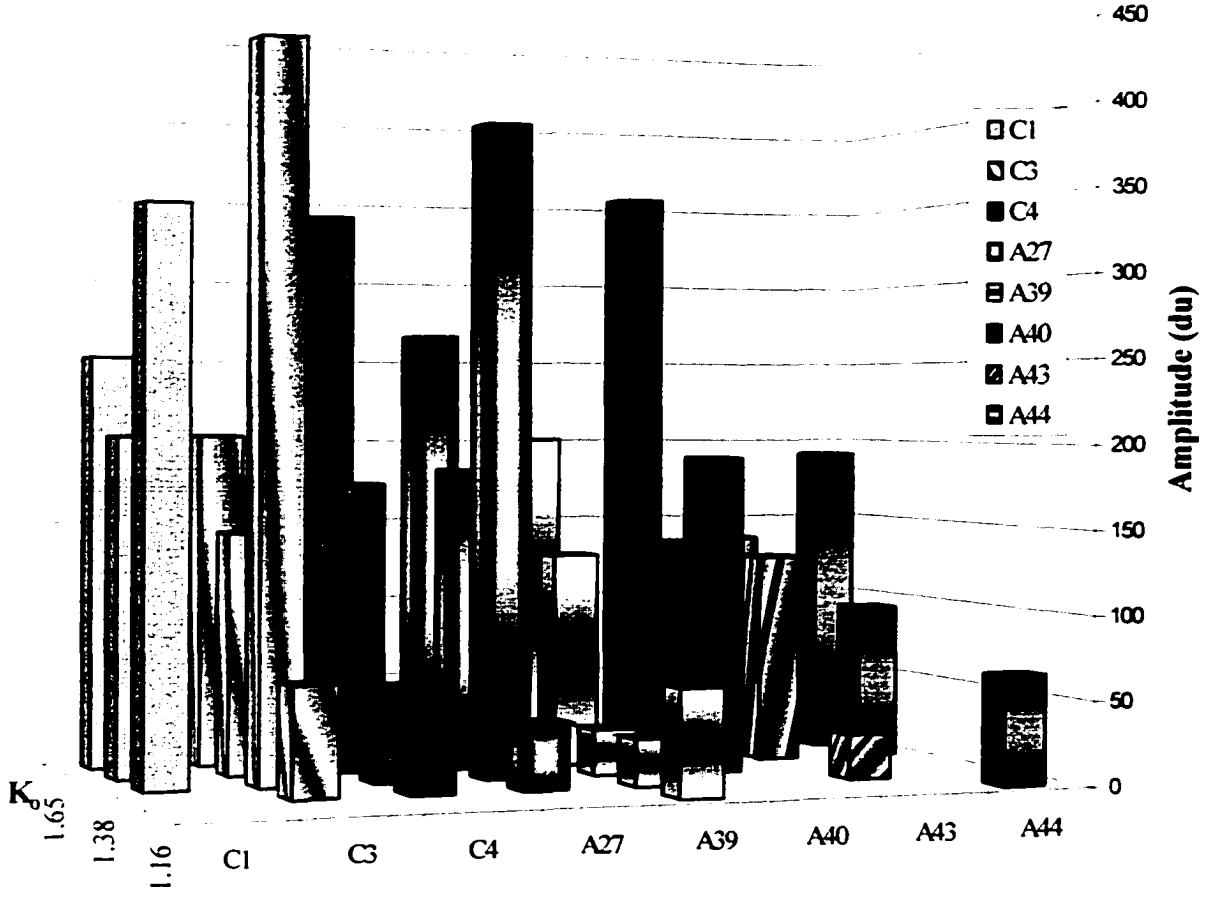


Fig. 24. The positive mode IMS mobility spectrum of fetal lung tissue from animal A40 (a control animal given vehicle saline in a chronic study). K_o value 1.38 $cm^2V^{-1}s^{-1}$ is attributed to anesthetic, given to the mother during surgery, which has passed through the cotyledon to the fetus.



	K_o				
	1.65	1.51	1.38	1.31	1.16
Animal number	**Amplitude**				
C1	251.9	136.7	308.2		
C3	204.3	146.2	438.1	64.8	
C4	339.8	174.8	56.8	20.3	
A27	265.7	182.6	390.2	39.1	
A39	201.1	129.9	27.7	28.2	63
A40	354.1	153.5	30.8	29.8	
A43	137.4	127.5		26.9	
A44	191.2	94			65

Fig. 25. A comparative analysis of the IMS K_o values and amplitudes of the brain tissue analyzed for the animals included in the study.

It was interesting to find that the positive ion mobility spectra of the tissues had two ion peaks that were common to most of the tissues analyzed (K_o 1.65 and 1.52 $cm^2V^{-1}s^{-1}$). As shown in Table 6, all of the tissues (nine organs) except for the ones from renal artery and atria, exhibited the 1.65 $cm^2V^{-1}s^{-1}$ peak. The liver and kidney tissue mobility spectrum showed the 1.65 $cm^2V^{-1}s^{-1}$ peak, but not the one at 1.51 $cm^2V^{-1}s^{-1}$. For tissues exhibiting both peaks, the one at 1.65 $cm^2V^{-1}s^{-1}$ was more intense than the one at K_o 1.51 $cm^2V^{-1}s^{-1}$ except for adrenal tissue. In the latter case, the K_o 1.51 $cm^2V^{-1}s^{-1}$ peak intensity was one of the highest found among all organ tissues. The peaks with highest amplitudes among all the tissues, irrespective of K_o value, were atria, 734 du (K_o, 1.31 $cm^2V^{-1}s^{-1}$); kidney 577 du (K_o, 1.38 $cm^2V^{-1}s^{-1}$); lung 517 du (K_o, 1.38 $cm^2V^{-1}s^{-1}$); and adrenal, 503 du (K_o, 1.51 $cm^2V^{-1}s^{-1}$).

Two other peaks appeared in the positive ion mobility spectrum of most of the tissues (K_o 1.38 and 1.31 $cm^2V^{-1}s^{-1}$). As indicated in the Results section, it is very likely that they are from the anesthetic agents and heparin, respectively. It was interesting to note that the amplitude at K_o 1.38 $cm^2V^{-1}s^{-1}$ varies from 0 to 578 du among the nine different tissues. As mentioned earlier this peak is most likely due to the anesthesia (Rompun or Ketaset). Sufficient information is not available to determine the physiological significance, however, it does identify a potential area for additional research, and how IMS may be useful in clinical chemistry research.

The peak with K_o 1.31 $cm^2V^{-1}s^{-1}$ may be due to heparin. However, with most of the tissues the intensity was very low. Tissues from atria and ventricle exhibited higher intensities, 734 du and 259 du, respectively. It is not surprising that heparin would

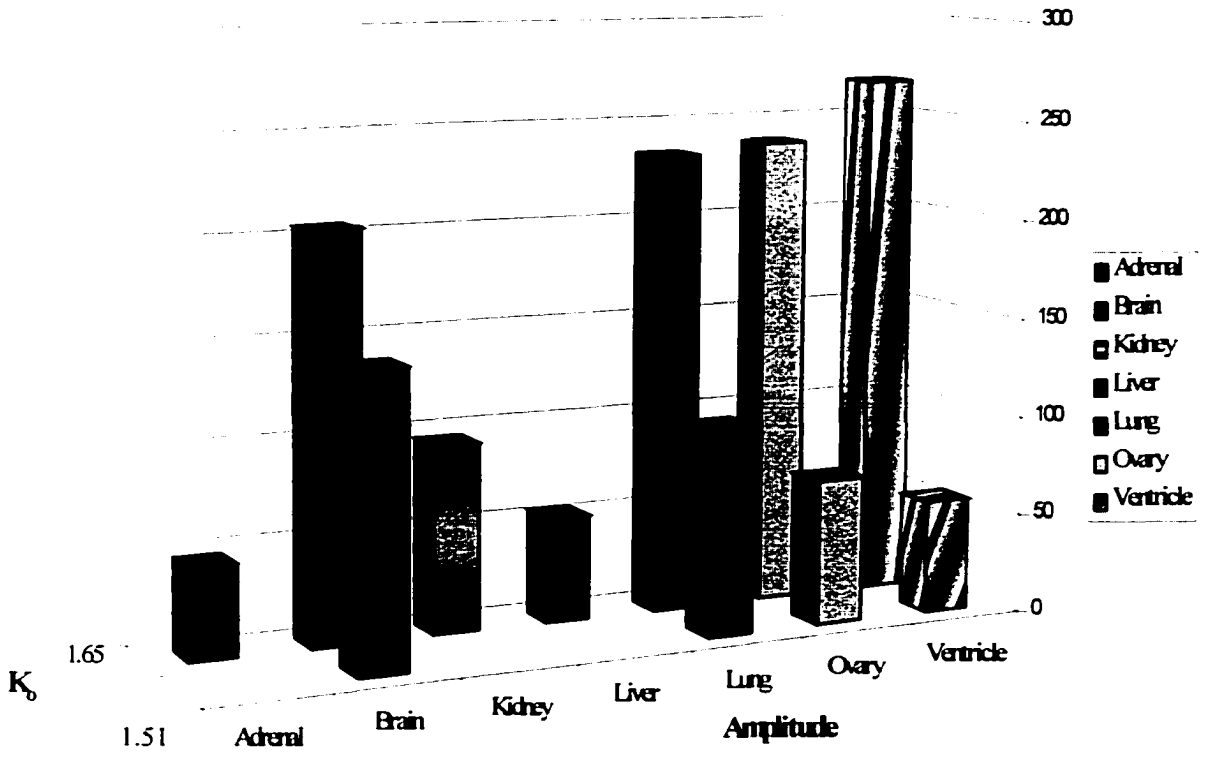
accumulate in heart tissues, but assignments of the K_o 1.31 $cm^2V^{-1}s^{-1}$ are not unequivocal. No control was available since all animals received heparin.

The distribution of anesthetic and heparin among tissues from animals receiving these compounds may be useful indicators for physiological purposes and may help to differentiate the various tissues. However, we also examined the tissue patterns between tissues in which only peaks with K_o 1.65 $cm^2V^{-1}s^{-1}$ and K_o 1.51 $cm^2V^{-1}s^{-1}$ were present (Fig. 26).

The distinguishing features of negative ion mobility spectra are the number of low intensity peaks. This implies that the tissues decompose under thermal desorption to give a number of products eventually leading to negative ions as a result of the IMS chemistry. It is also interesting to note that the reactant ion peak is absent in Fig. 16. This indicates that the reaction ion chemistry was not completed. It also implies that $(H_2O)_nO_2^-$ is not specific enough for tissue samples. Reactant ions with higher electron affinity may give more useful mobility spectra.

The major negative ion peak among all tissue samples has a K_o value of 1.83 $cm^2V^{-1}s^{-1}$. However, this is not true of every sample. In the case of renal artery and kidney tissues, the ion peak with K_o 2.19 $cm^2V^{-1}s^{-1}$ is the most intense. (These two peaks appear in every sample.) The second most intense peak in brain, liver, lung, and ovary tissues has a K_o value of 1.50 $cm^2V^{-1}s^{-1}$.

The K_o values in the negative mode for tissue correspond to negative ions of lower mass than the positive ions found using the positive mode. The negative mode is usually preferred for analytes with high electron affinity. Drugs such as cocaine and heroin (and their metabolites) have high proton affinity and are normally analyzed in



	K_o	
	1.65	1.51
Tissue type	**Amplitude**	
Adrenal	46.4	
Brain	204.3	146.2
Kidney	94.6	
Liver	53.1	
Lung	233.1	104.7
Ovary	237.3	73.1
Ventricle	266.7	56.8

Fig. 26. A comparison of IMS K_o values (1.65 and 1.51 $cm^2V^{-1}s^{-1}$) and amplitudes for organs from C3 animal tissue.

the positive mode. Since the present work focuses on cocaine, heroin and related compounds, research on optimizing the negative mode conditions and choice of reactant ion for clinical chemistry applications were not pursued. Some experiments, however, were performed in the negative mode and are described in Chapter V.

The origin of the ion peaks innate to animal tissues (e.g., K_o 1.65 and 1.51 $cm^2V^{-1}s^{-1}$) in the positive mode), is not clear. These could be compounds within the tissue (e.g., hormones) or they could be compounds formed from tissue decomposition during thermal desorption in the IMS sample chamber. The source in the latter case might be proteins. The ions contained in mobility spectra can be identified using IMS-MS, however, this equipment was not available. Such research will undoubtedly be accomplished as interest in IMS of animal (and human tissues) grows.

Much IMS data can be gathered within a relatively short period of time. There are many instrumental parameters that can be varied including the major ones of sampling time and desorption temperature. A choice of low desorption temperature may eliminate ion peaks due to the tissue and open up the entire mobility spectrum window for target analytes. In our studies, much lower temperatures could have been used to analyze anesthetics in animal tissues. There are many choices to be made in terms of selecting tissues from a single organ or many organs. One also has a choice of targeting negative or positive ions. The present work utilized air as a source of reactant ions in the negative mode, and nicotinamide as a source of reactant ions in the positive mode. The choices of ion mode and reactant ion chemistry allow much flexibility in targeting analytes of interest. As more experience is gained with IMS of tissues, use of pattern recognition techniques should be very helpful in helping to develop specific

IMS methods. It is also clear that IMS of tissues is not limited to clinical chemistry applications. Use in pathology and forensic applications are very promising as well.

Applications of IMS Animal Tissue Research

As mentioned above, the IMS animal tissue research was done in collaboration, between the Department of Biological Sciences and the Department of Chemistry and Biochemistry with project leaders being Professors Hargrave and Poziomek, respectively. The students from the Biological Sciences Department were Barbara B. Guinn and Andrea Chambliss. Details of the biological part of the research can be found in their Master of Science theses [35, 44]. They represent early examples of how IMS of animal tissues can be applied in biological sciences research.

Effect of Cocaine on Plasma Renin Activity in Male Rabbits: Cocaine Detection in Male Plasma and Tissue Using Ion Mobility Spectrometry (IMS)

Animal tissues were harvested from male New Zealand White (NZW) rabbits in studies where each rabbit was given an intra-arterial injection of 2 mg/kg cocaine hydrochloride. Tissues and plasma prepared in the manner described in the Experimental section. Results of these experiments indicated that EME was consistently detected in the mobility spectra of all plasma samples five minutes after the injection of cocaine. EME was also detected by IMS in the tissues from lungs, liver, kidneys, and testicles harvested from these animals. GC/MS was use to validate the IMS assignment of EME in plasma samples.

IMS calibration curves of tissues and plasma were spiked with cocaine, EME, and BE were used to determine whether IMS results could quantify the amount of cocaine or EME present in plasma and tissue. It was found that BE could not be

identified in the presence (or suspected presence) of cocaine because of the similarity of K_o values between the compounds. The ion peak from BE is hidden under the cocaine peak because the IMS sensitivity to BE is less than that of cocaine.

A study varying the optimal sample size of animal tissues for IMS was also performed. It was determined that varying the sample size did not significantly change the mean IMS amplitudes of tissue samples. These results led to a standard sample size of 0.5-1 mg animal tissue for *in vitro* experiments in which calibration curves for mixtures of target analytes and tissue were being generated and for qualitative *in vivo* studies. Calibration curves were obtained for tissues from atria, ventricles, lungs, kidneys, liver, and testicles, each spiked with cocaine hydrochloride or EME.

It was also found that EME can be detected in most tissues from animals treated with cocaine hydrochloride. Highest amounts were found in kidneys and lungs. Very little was found in heart tissues. EME was consistently detected in all plasma samples five minutes after the injection of cocaine hydrochloride. This research represents the first example of using IMS of animal tissues from *in vivo* studies.

Effect of Cocaine on Renin Angiotensin System: Cocaine Detection in Adult and Fetal Tissue and Adult Plasma Using Ion Mobility Spectrometry (IMS)

In a related study, IMS was used to detect cocaine hydrochloride and/or its metabolite, EME, in both maternal and fetal tissues and maternal plasma of pregnant rabbits. EME was consistently detected in all plasma samples five minutes after intra-arterial injection of cocaine hydrochloride (2 mg/kg). EME was also detected in all maternal and fetal tissue samples analyzed. Cocaine and EME were detected in the

tissues of the two young fetuses analyzed. Control tissues were also spiked in these studies and GC/MS was used to confirm the IMS results.

More specifically, cocaine was detected in 57% of maternal kidneys, while lesser amounts were found in the atria and ventricle, 17% and 40%, respectively, from seven pregnant rabbits. EME was detected in all of the maternal liver, kidneys, and ventricle tissues, while 67% of the atria and 80% of the lung tissues contained EME. For fetal tissues, cocaine was detected in 20% of the livers, 40% of the kidneys, 20% of the lungs, 20% of the hearts, and 40% of the cotyledons. EME was detected in all fetal tissue samples. BE was not detected in any of the tissue or plasma samples.

Effect of Illicit Drugs on the Cardiovascular and Hormonal Responses in Pregnant Rabbits

Another study focused on giving injections of EME (2 mg/kg) and heroin (2 μg) instead of cocaine to pregnant rabbits. EME was studied since the biological and pharmacological activity of this compound has not been proven and recent data interpretations are still controversial. The IMS results should indicate the presence of EME in all maternal and fetal tissue samples. Strong EME IMS signals were found in maternal adrenal glands, kidney, brain, and heart. Lesser, but detectable amounts were found in all fetal tissues. However, generation of quantitative data will require additional method development.

IMS analysis of tissues harvested from animals exposed to heroin, showed no ion peaks at the mobility constants expected for heroin (K_o 1.04 and 1.14 $cm^2V^{-1}s^{-1}$). Also, no ion peaks for heroin were observed in the mobility spectra of control tissues spiked with heroin at concentrations below 200 ng.

Additional studies were conducted where morphine was added to control tissues. Results indicated that at least 8000 ng of morphine had to be added to tissue before a peak corresponding to morphine was observed. Confirmatory tests with GC/MS, however, indicated the presence of heroin in the animal tissues. More detailed discussion of the heroin and morphine retardation effects is given in Chapter VI.

All of the studies described above point out the feasibility of developing IMS as a useful adjunct to physiological studies, but additional research needs to be conducted to refine the methodology for specific applications, especially if semi-quantitative information is required.

CHAPTER V

ORIGIN OF ION PEAKS IN THE MOBILITY SPECTRA OF ANIMAL TISSUES

Introduction

It was illustrated in the previous chapter that IMS has much potential in the analysis of drugs and metabolites in animal tissues. This also applies to the characterization of the animal tissues. It was discovered that the IMS of tissues harvested from animals can reveal the compounds/drugs that the animals were exposed to. Ion peaks in the plasmagrams of tissues from various organs were easily assigned to the anesthesia (Rompun and Ketaset) used with the animals.

It was also found that the tissues themselves had characteristic ion peaks which varied in amplitude between different organ tissues from the same animal. On the other hand, the ion peaks for a specific tissue (brain from rabbits, as an example) had amplitudes that were reasonably consistent between a number of animals. It is not known whether the ion peaks found with animal tissues also appear in tissues from different animal species.

The origin of the two characteristic ion peaks (K_o 1.65 and 1.51 $cm^2V^{-1}s^{-1}$) is of interest. The compound(s) could be a relatively non-volatile tissue component or a thermal decomposition product from heating the tissue samples in the IMS desorber unit.

The purpose of this chapter is to extend the tissue research described in the previous chapter by examining relevant chemistry of animal tissue using IMS. The specific aim is to learn more about the origin of the four ion peaks (K_o 1.65, 1.51, 1.38,

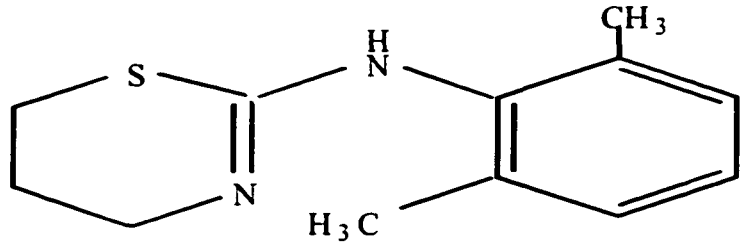
and 1.31 $cm^2V^{-1}s^{-1}$). The overall strategy includes learning more about the properties of Rompun and Ketaset, examining the potential of quantifying the anesthetics contained in tissues, extracting animal tissues with solvents of different polarities in attempting to isolate sources of IMS peaks, reacting animal tissues with alkylating agents to examine effects on the IMS mobility spectra, and screening vegetable proteins, synthesized proteins, various amino acids, and naturally occurring complex materials for comparison to the IMS mobility spectra of animal tissues. Brief descriptions (not covered earlier) of the chemicals used follow.

Anesthesia Agents

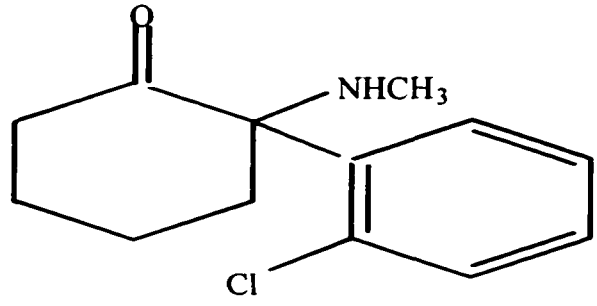
Rompun and Ketaset were used as anesthesia agents with the animals from which tissues were harvested. Both compounds contain nitrogen moieties (Fig. 27) which undoubtedly provide sufficient proton affinity to give high detection sensitivity in the IMS positive mode.

Xylazine, the active ingredient of Rompun, is a non-narcotic compound that acts as a sedative and analgesic as well as a muscle relaxant. It is generally used in veterinary medicine. Rompun's sedative and analgesic activity is related to central nervous system depression, while its muscle relaxant effect is based on inhibition of the intraneural transmission of impulses in the central nervous system [41].

Ketamine, the active ingredient of Ketaset, is a rapid-acting nonnarcotic, nonbarbiturate, dissociative anesthetic used mainly in small animals, but can be used in humans. It is also classified as a psychedelic drug and is often abused with phencyclidine (PCP) [45]. Ketaset is characterized by profound analgesia, mild cardiac



Xylazine



Ketamine

2-Choroethyl ethyl sulfide

Fig. 27. Structures of xylazine (major compound in Rompun), ketamine (major component in Ketaset), and 2-chloroethyl ethyl sulfide, an alkylating agent.

stimulation and respiratory depression, but maintains protective reflexes such as coughing and swallowing [42]. Toxic doses may cause severe muscle rigidity, an increase in blood pressure, and seizure activity. This drug is detoxified by the liver and excreted by the kidneys. The mechanism of action is thought to alter the interactions of dopamine, serotonin, and norepinephrine with their receptors. Ketamine also binds to the sigma opiate receptor in the hippocampus of the limbic system [46].

Alkylating Agents

Two alkylating agents were used, 2-chloroethyl ethyl sulfide (Fig. 27) and methyl iodide. 2-Chloroethyl ethyl sulfide is commercially available (Aldrich) and is commonly used as a simulant for bis (2-chloroethyl) sulphide, a general cell poison that has been used as a chemical warfare agent (mustard). Mustard can severely damage any tissue, but its effects are readily seen on the eyes and skin. Inhalation of its vapors causes major corrosive effects, producing long-lasting lesions, bronchitis, bronchopneumonia, and eventually fatal lung edema [47]. We used 2-chloroethyl ethyl sulfide was used in *in vitro* studies with animal tissues.

Methyl iodide is another well-known alkylating agent used in our studies with animal tissues. It is a neurotoxin and narcotic that may produce severe pulmonary, skin and eye irritation. It also depresses the central nervous system and may cause convulsions, paralysis, hypotension, and coma.

Vegetable and Synthesized Proteins

The vegetable protein (soy isolate) (Protein 94) used contains 94% protein and is manufactured by Sundown®. The amino acid profile is given in Table 9. The vegetable protein also contains 5% calcium, 15% phosphorus, 6% magnesium, and 15% sodium.

Table 9
Amino acid profile for Protein 94

Amino acid	%	Amino acid	%
Isoleucine	3.7	Histidine	2.0
Leucine	6.4	Arginine	5.2
Lysine	5.1	Aspartic acid	9.5
Methionine	1.1	Serine	4.1
Cysteine	1.1	Glutamic acid	16.0
Phenylalanine	4.2	Proline	4.3
Threonine	2.9	Glycine	2.8
Tryptophan	1.0	Alanine	2.8
Valine	3.8	Tyrosine	3.2

Slim Fast® (chocolate), purchased from a retail store, contains vitamins A, C, D, E, B_6 and B_{12}. It also contains a variety of materials such as calcium, iron, thiamin, riboflavin, niacin, folate, biotin, panthenic acid, phosphorus, iodine, magnesium, zinc, and copper. The protein content is 5 g/28 g and is a soy protein isolate. The glycine peptides, purchased from Sigma, are listed in Table 10.

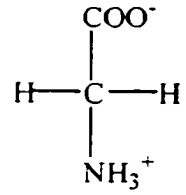
Amino Acids

Amino acids represent the most fundamental building blocks of protein. It was important to check whether any of these materials or their thermal decomposition products were the source of the ion peaks found in IMS. A variety of amino acids were selected (Fig. 28).

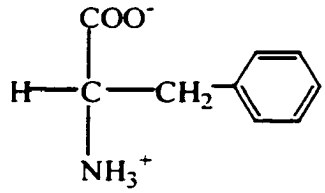
Table 10
Information for glycine peptides supplied by the distributor, Sigma Chemical Co.

Glycine Peptide	Formula Weight	Stored Temperature	Catalog Number	Other
Glycine	75.07	Room temp.	G7126	99% TLC
Diglycine	132.1	Room temp.	G3028	pKa 8.2 at 25°C
Triglycine	189.2	Room temp.	G1377	
Tetraglycine	246.2	0°C	G3882	
Pentaglycine	303.3	0°C	G5755	
Hexaglycine	360.3	0°C	G5630	H_2O content 0.5 mol/mol
Polyglycine	4,600(vis)[a]	>0°C	P8790	DP(vis) 80[a]
Polyglycine	5,200(vis)[a]	>0°C	P3548	DP(vis) 91[a]

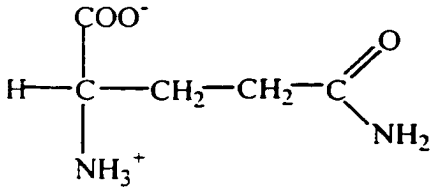
[a]The glycine peptides are a mixture of polymer chains differing in the degree of polymerization (DP). Both the molecular weight and DP are characterized using solution viscosity.



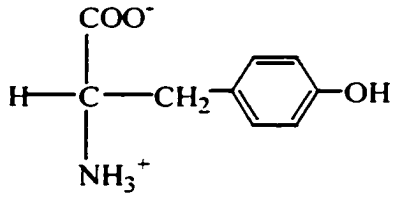
Glycine



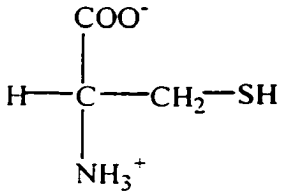
Phenylalanine



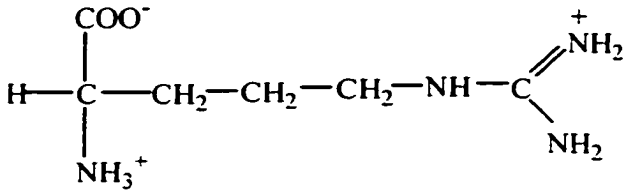
Glutamine



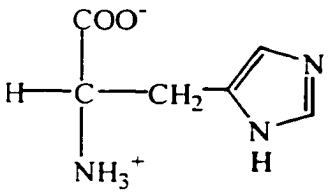
Tyrosine



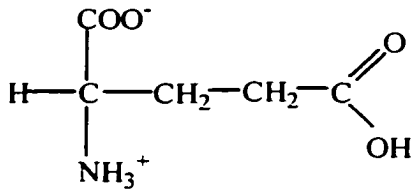
Cysteine



Arginine



Histidine



Glutamic acid

Fig. 28. Structures of amino acids analyzed using IMS.

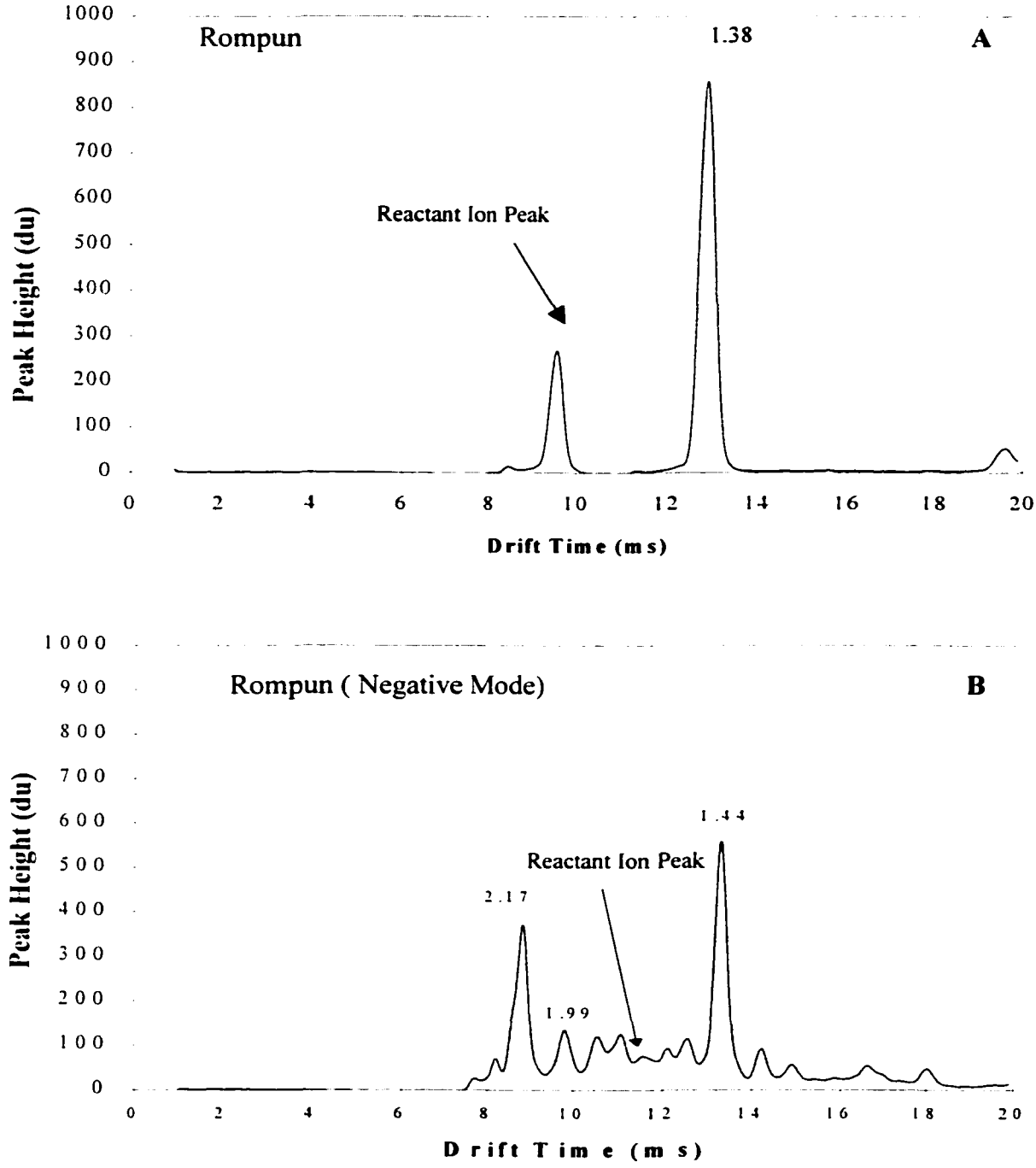
Results

Rompun and Ketaset

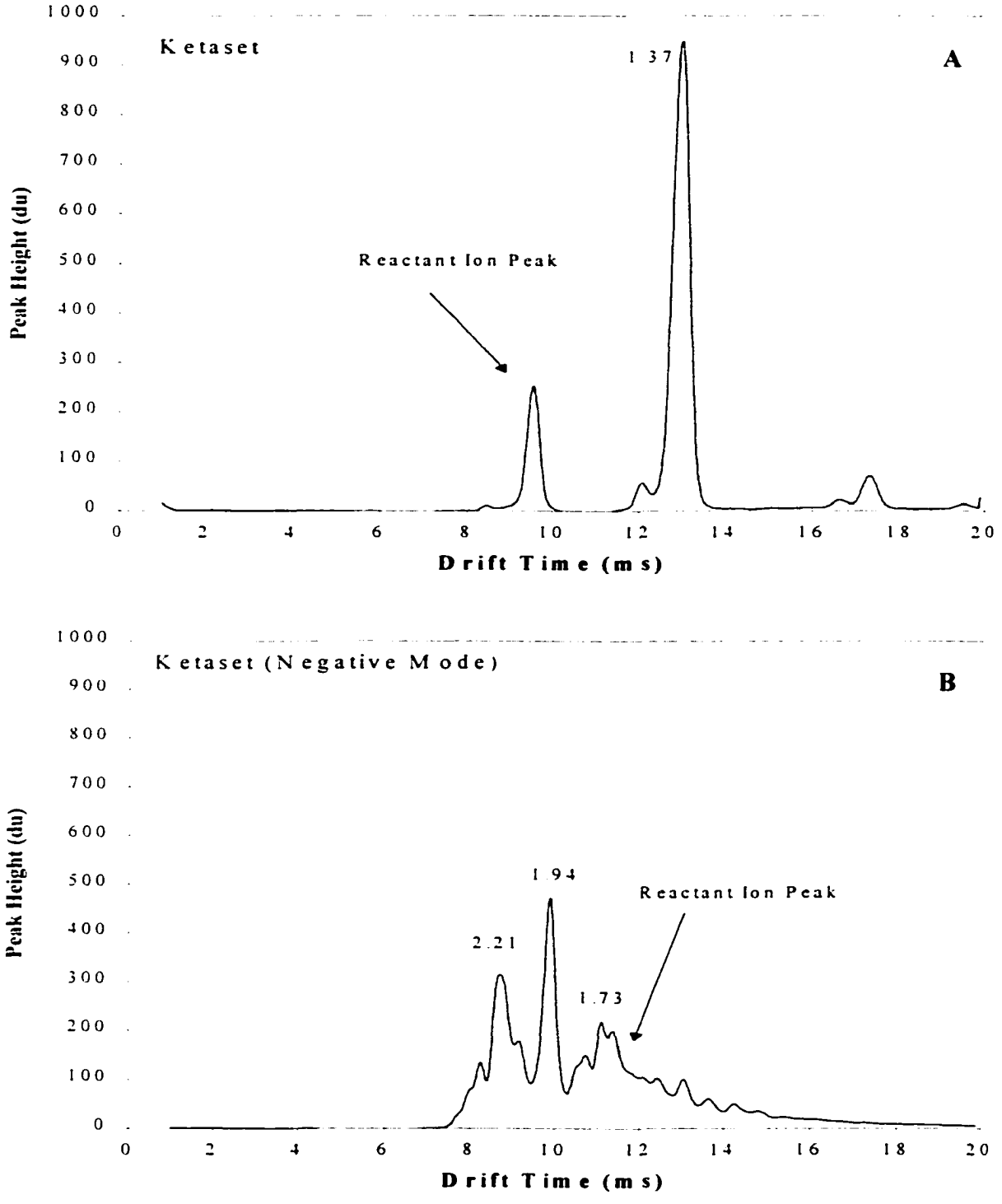
Rompun and Ketaset were examined by IMS in both the positive and negative modes to assist in establishing a background control for animal tissues (Figs. 29 and 30). The negative ion mobility spectrum shows a strong peak at K_o 1.44 $cm^2V^{-1}s^{-1}$ which may be characteristic of Rompun, however, the reactant ion peak is absent. The K_o 1.44 $cm^2V^{-1}s^{-1}$ peak is absent in the negative ion spectrum of Ketaset. Additional work would need to be performed with a more selective reactant ion to better understand the mobility spectrum. A preliminary calibration curve for each anesthetic was obtained in the positive mode and is shown in Fig. 31. The concentrations ranged from 1ng/uL to 100 ng/μL. Additional runs are needed using specific matrices to match experimental conditions before quantitative work is attempted.

To determine if Rompun and Ketaset (and possibly other components) could be extracted from tissues, 50 –70 mg of C3 atrial, ventricle and lung tissue were gently shaken in 1 mL of methanol and/or 1 mL of acetonitrile and allowed to stand for approximately 18 hours. Five μL of supernatant from each mixture as well as the tissue residues were analyzed by IMS (Table 11). It appears that Rompun and/or Ketaset can be recovered from the tissues under the conditions described based on the K_o, 1.38-1.39 $cm^2V^{-1}s^{-1}$ peaks found in the extract. Methanol appears to be a better solvent than acetonitrile based on the higher peak amplitudes.

Another peak (K_o 1.30 $cm^2V^{-1}s^{-1}$) was observed in the extracts. In this case, acetonitrile appears to be the better solvent with higher amplitudes being observed than



Figs. 29A and 29B. IMS mobility spectra of 100 mg/mL Rompun in the positive ion (A) and negative ion (B) modes.



Figs. 30A and 30B. IMS mobility spectra of 100 mg/mL Ketaset in the positive ion (A) and negative ion (B) modes.

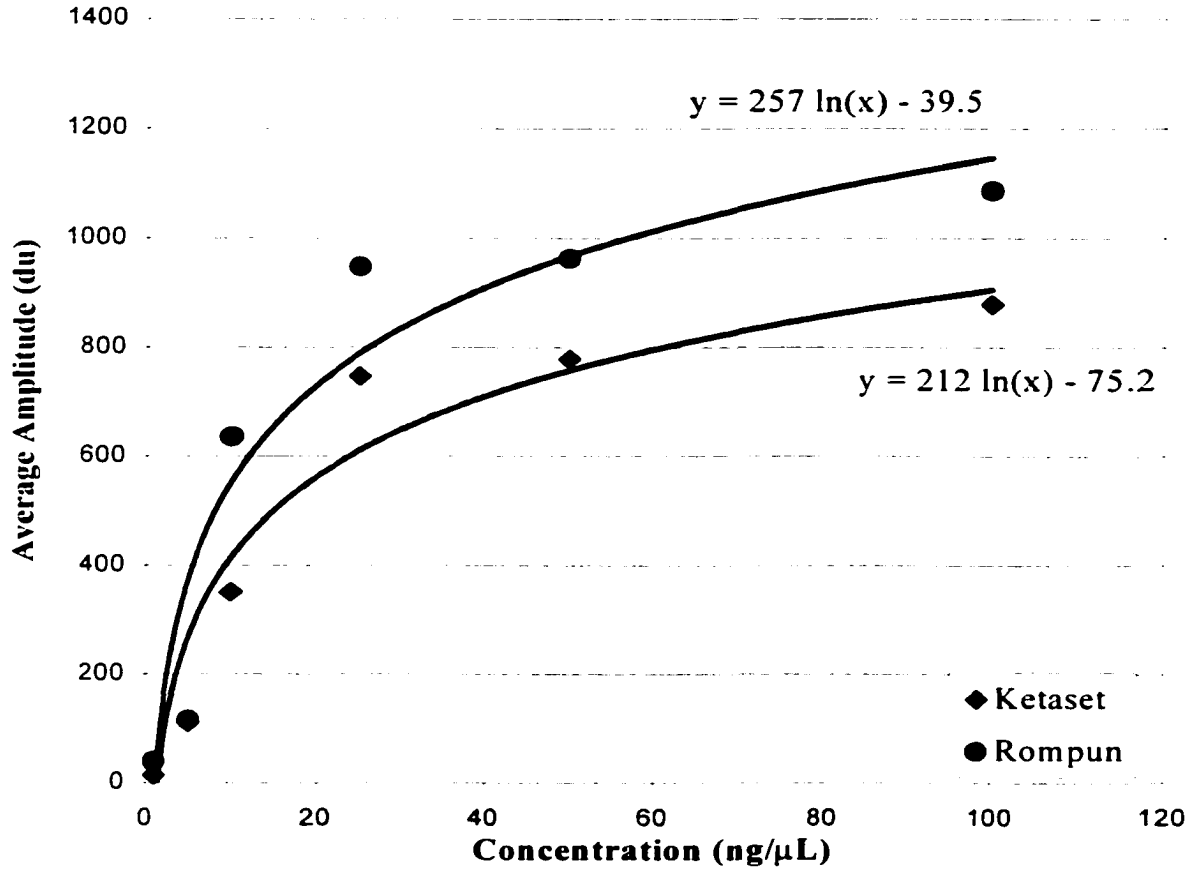


Fig. 31. Calibration curves for 1, 5, 10, 25, 50, and 100 ng/μL of separate solutions of Rompun and Ketaset in saline.

Table 11
Peak K_o values and amplitudes for C3 animal tissues extracts

Animal Tissue Extract	Solvent	Peak Number	K_o $cm^2V^{-1}s^{-1}$	Amplitude (du)
Atrial Supernatant	Methanol	1	1.39	169
		2	1.30	257
	Acetonitrile	1	1.38	107
		2	1.30	419
Atrial Tissue Residue	Methanol	1	1.38	162
		2	1.30	284
	Acetonitrile	1	1.39	204
		2	1.30	557
Lung Supernatant	Methanol	1	1.39	339
	Acetonitrile	1	1.38	128
		2	1.30	328
Lung Tissue Residue	Methanol	1	1.65	62
		2	1.30	159
	Acetonitrile	1	1.65	181
		2	1.52	73
		3	1.38	388
		4	1.30	46
Ventricular Supernatant	Methanol	1	1.39	461
		2	1.30	254
	Acetonitrile	1	1.38	222
		2	1.30	23
Ventricular Tissue Residue	Methanol	1	1.38	124
		2	1.30	439
	Acetonitrile	1	1.65	84
		2	1.38	340
		3	1.30	419

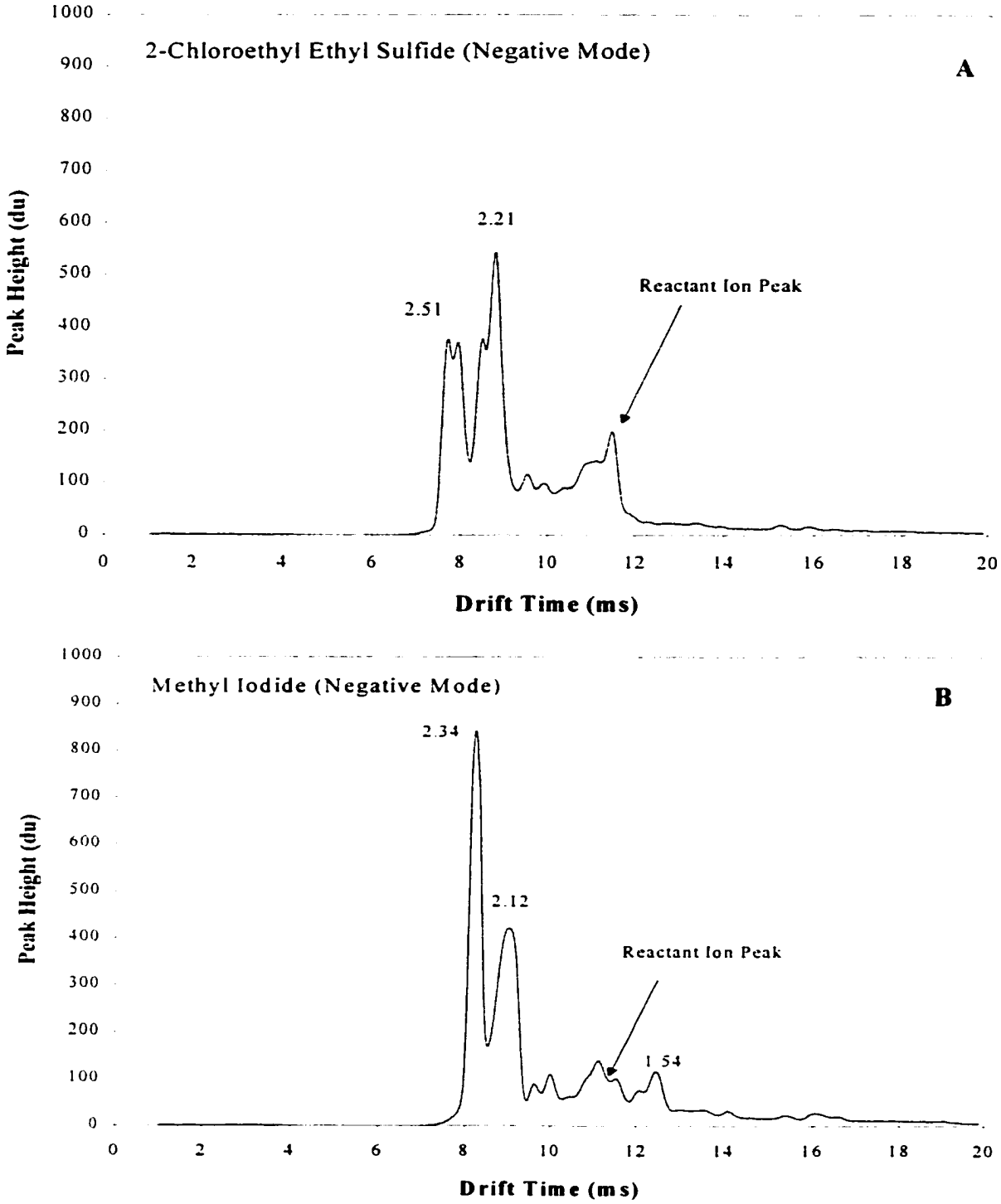
from methanol. As discussed in Chapter IV, the source of the 1.30 $cm^2V^{-1}s^{-1}$ peak appears to be from heparin administered to animals. However, heparin is also biosynthesized and stored in mast cells of various animal tissues, particularly liver, lung

or gut [43]. Tissues were not available from animals that had not received heparin to check whether 1.30 $cm^2V^{-1}s^{-1}$ the peak was inherent to the tissue.

Alkylating Agents

Mobility spectra of 2-chloroethyl ethyl sulfide and methyl iodide (as received without dilution) were measured in both negative (Fig. 32) and positive modes (Figs. 33 and 34). Mixtures of the alkylating agents and brain tissue were analyzed by IMS to determine any changes in the tissue mobility spectra. Two brain tissues were examined, C3 (from animals that had received excess Ketaset), and C4 (from an animal that did not receive anesthetic). The negative mode mobility spectra of C4 brain tissue alone and after exposure *in vitro* to 2-chloroethyl ethyl sulfide are shown in Figs. 35A and 35B, respectively. Although, there was not a shift or elimination of peaks, the spectrum does show a major decrease in the intensity of one of the tissue peaks (K_o 1.54 $cm^2V^{-1}s^{-1}$) implying alkylation.

It is interesting to note that the negative ion mobility spectrum of C4 brain tissue (no anesthetic) shows peaks with K_o 1.54 and 1.50 $cm^2V^{-1}s^{-1}$ (Fig. 35A), whereas the negative ion mobility spectrum of C3 brain tissue (excess Ketaset) shows only K_o 1.50 $cm^2V^{-1}s^{-1}$. This is another example that the negative ion IMS should be examined in more detail with animal tissues. A similar experiment was performed with undiluted methyl iodide and C4 brain tissue. No changes were apparent (Fig. 36), however, the results were inconclusive. Reactions of methyl iodide occurred as evidenced by the very intense peak at K_o 2.34 $cm^2V^{-1}s^{-1}$ most likely iodide ion. The electron affinity of the iodide coupled with its high concentration did not allow the ion peaks from the tissue to be visible due to competitive reactions. C3 brain tissue with anesthetic was



Figs. 32A and 32B. IMS mobility spectra of (A) 2-chloroethyl ethyl sulfide (500 ng/μL) and (B) undiluted methyl iodide in the negative ion mode.

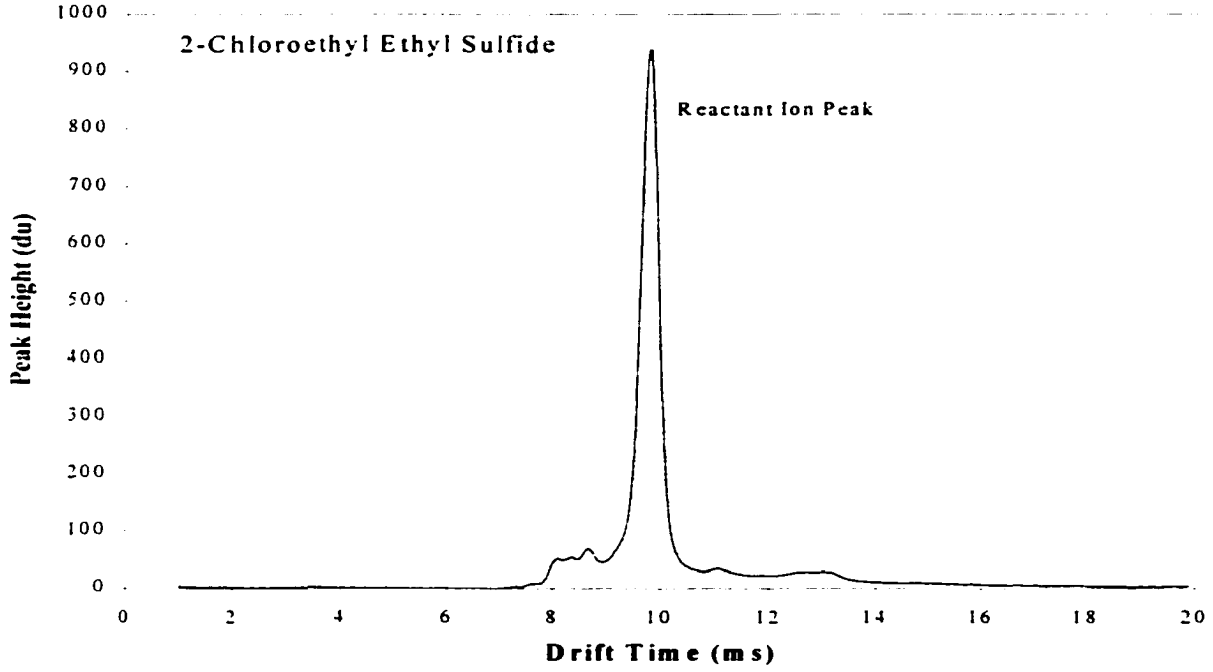


Fig. 33. The positive mode mobility spectrum of 500 ng/μL 2-chloroethyl ethyl sulfide.

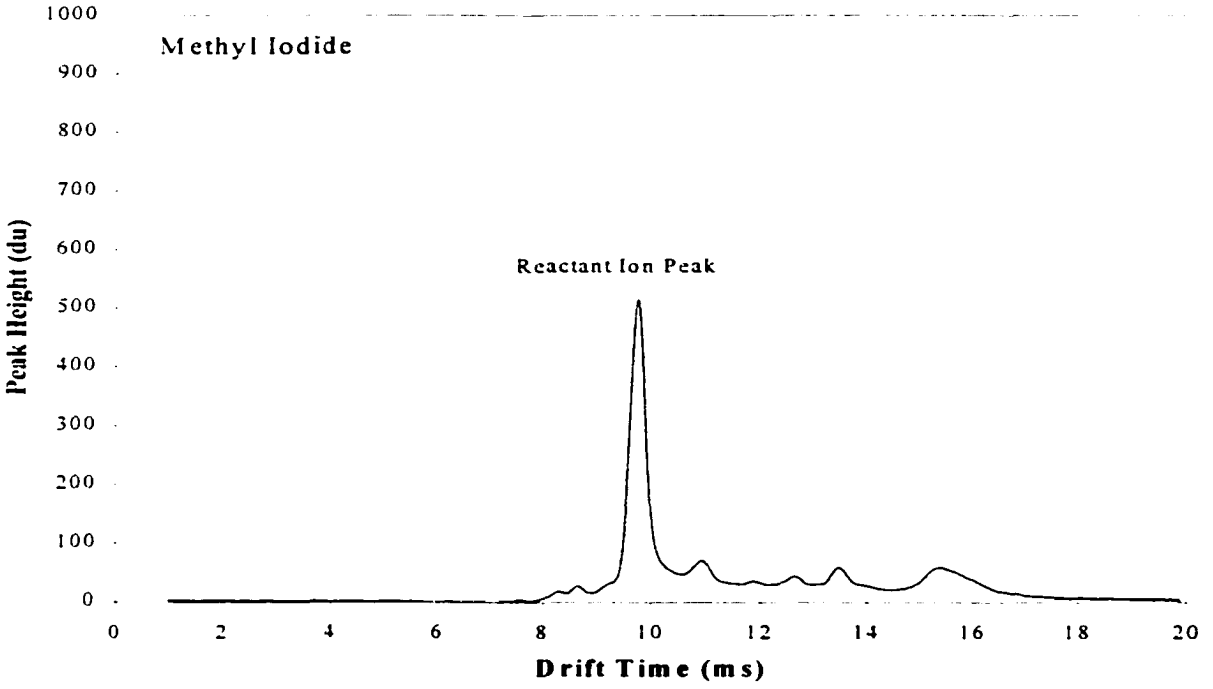
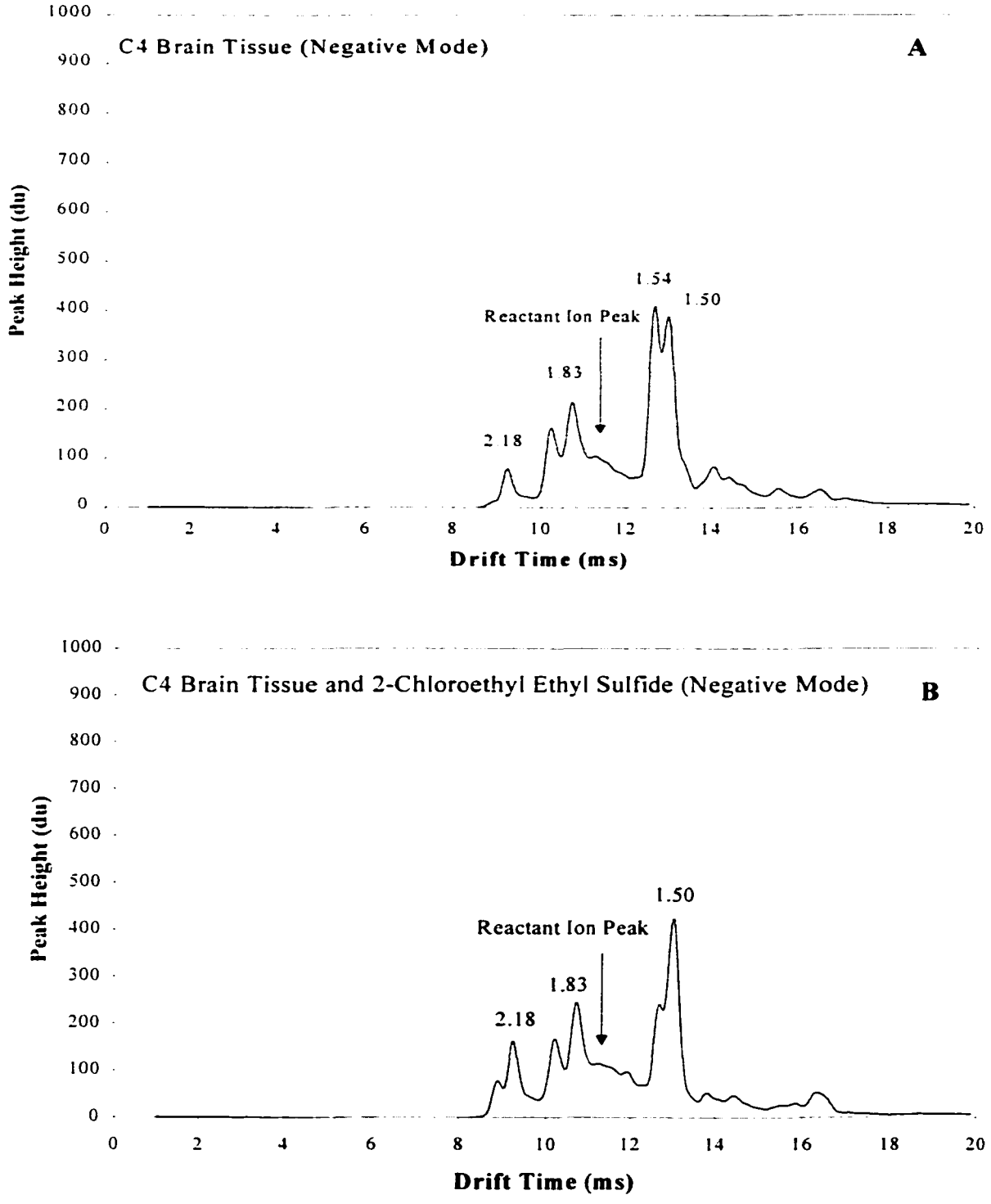


Fig. 34. IMS mobility spectrum of undiluted methyl iodide in the positive ion mode.



Figs. 35A and 35B. The negative mode mobility spectra of (A) C4 brain tissue alone and (B) after exposure *in vitro* to 500 ng/μL 2-chloroethyl ethyl sulfide.

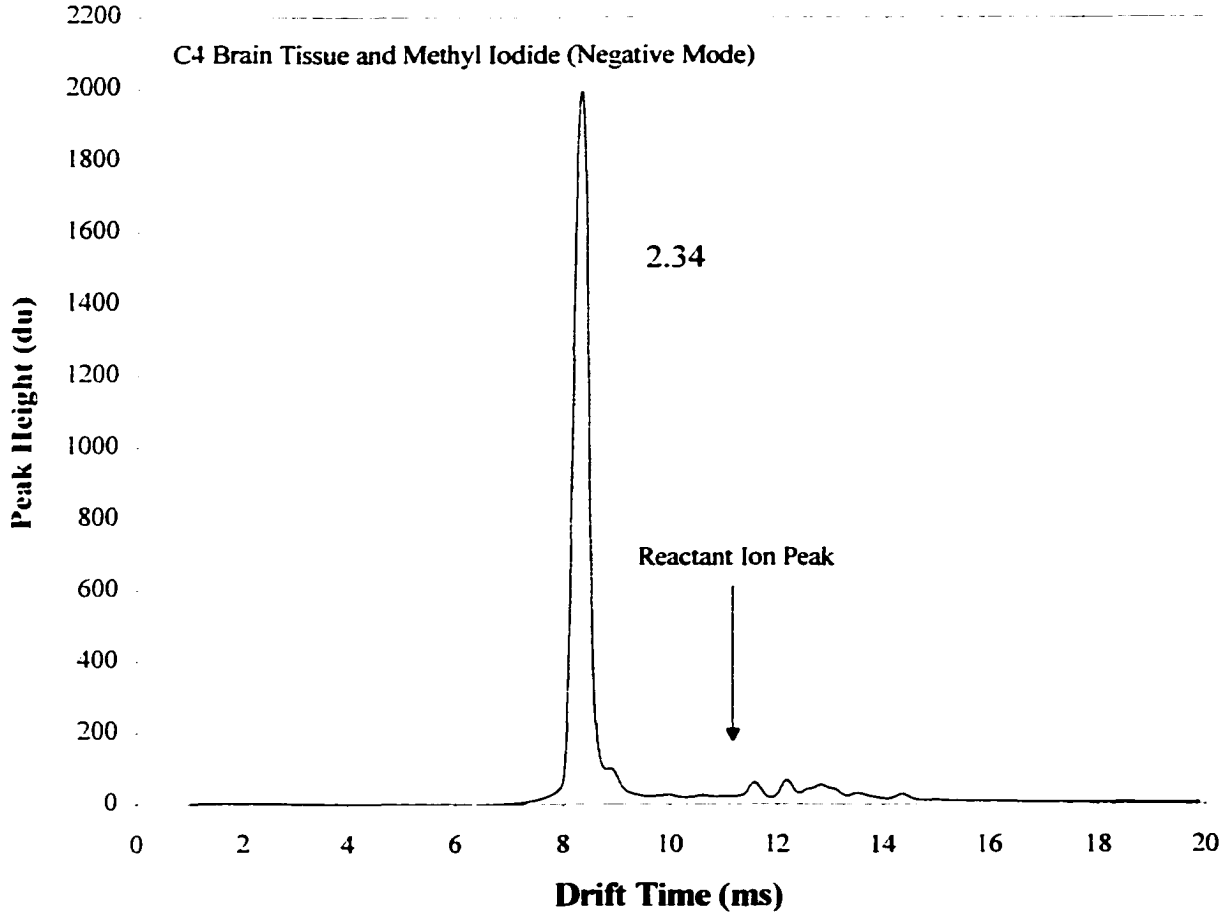


Fig. 36. Negative ion mobility spectrum of undiluted methyl iodide mixed with C4 brain tissue.

exposed *in vitro* to methyl iodide and 2-chloroethyl ethyl sulfide. These results are shown in Figs. 37A, 37B, and 37C. Some methyl iodide reacted as judged by the appearance of the peak at K_o 2.33 $cm^2V^{-1}s^{-1}$.

Reactions of C3 brain tissue with 2-chloroethyl ethyl sulfide were also checked using the positive mode of IMS. Figs. 29, 38, 39, and 40 show Rompun alone, Rompun with 2-choroethyl ethyl sulfide, C3 brain tissue control, and C3 brain tissue with 2-chloroethyl ethyl sulfide. It is clear from Figs. 29 and 38 that addition of 2-chloroethyl ethyl sulfide leads to a decrease of the peak amplitude at K_o 1.38 $cm^2V^{-1}s^{-1}$. However, no decrease of the same peak was noted in a similar treatment of C3 brain tissue.

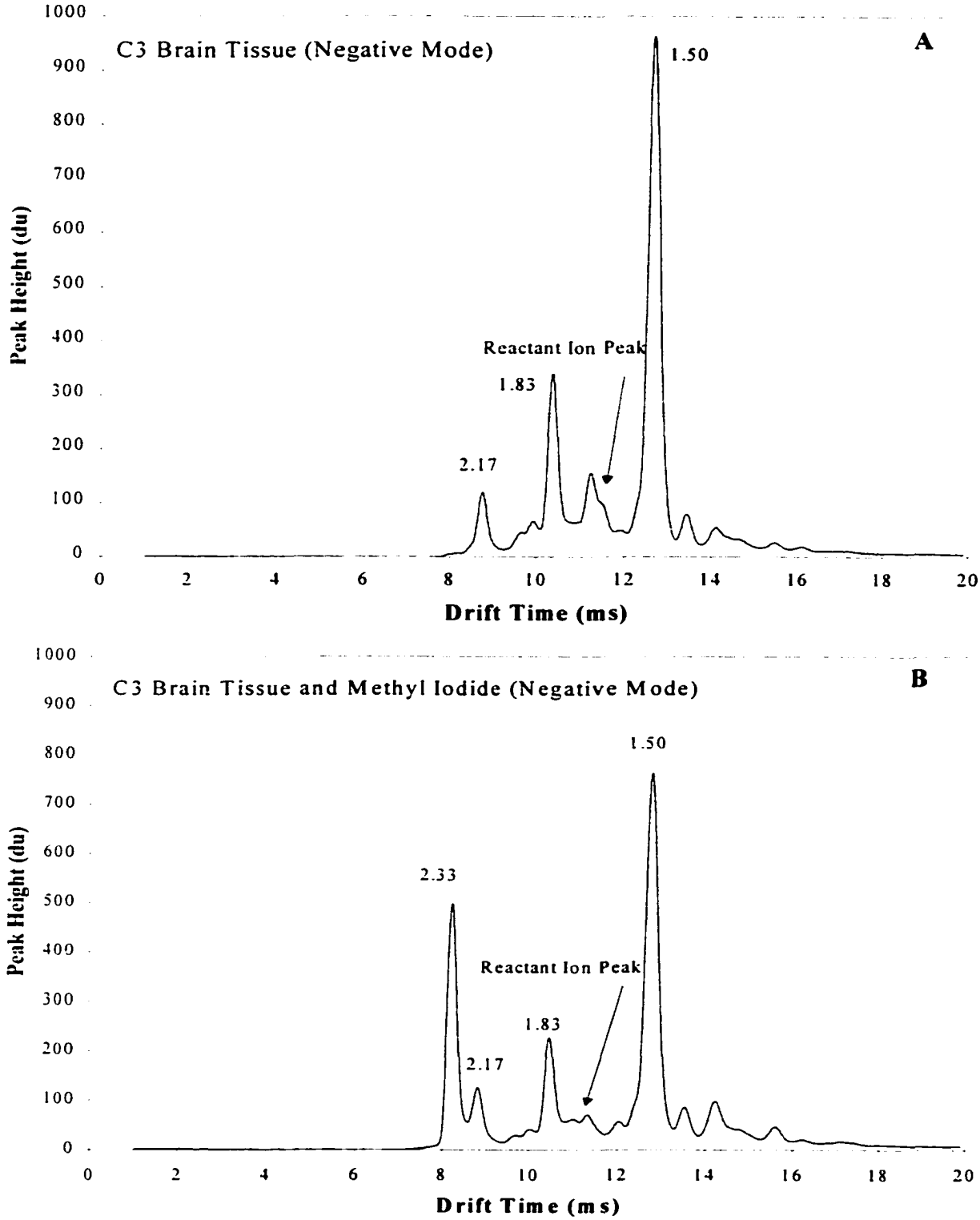
A new peak (K_o 1.16 $cm^2V^{-1}s^{-1}$) appeared in the mobility spectrum for C3 brain tissue control. Checking several samples gave the same results. The Teflon membranes used for the particular sampled did not show the peak. Though the tissues were stored in the freezer they were several months old. It appears that the peak may be due to tissue decomposition in storage or the introduction of an impurity. The K_o of 1.16 $cm^2V^{-1}s^{-1}$ corresponds to that of cocaine but the animals were not treated with cocaine.

Vegetable Proteins and Polyglycines

The mobility spectrum for Protein 94 is shown in ChapterVI. The spectra for polyglycines and Slim Fast, are given in Appendix B.

Amino Acids

The positive and negative mode K_o values and amplitudes of several amino acids are listed in Table 12. As expected, the positive and negative ion mobility spectra are different for the same amino acid. In the case of arginine and tyrosine, no ion peaks



Figs. 37A and 37B. Negative ion mobility spectra of (A) C3 brain tissue and (B) C3 brain tissue exposed *in vitro* to undiluted methyl iodide.

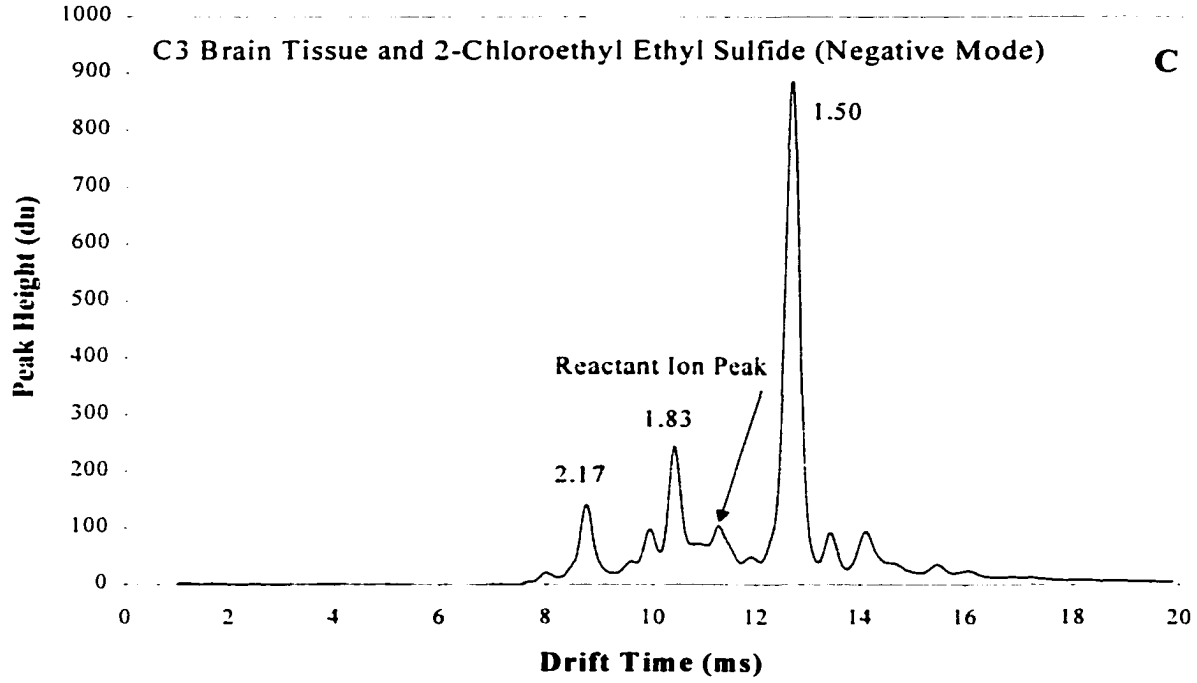


Fig. 37C. The negative ion mobility spectrum of C3 brain tissue exposed *in vitro* to 500 ng/μL 2-chloroethyl ethyl sulfide.

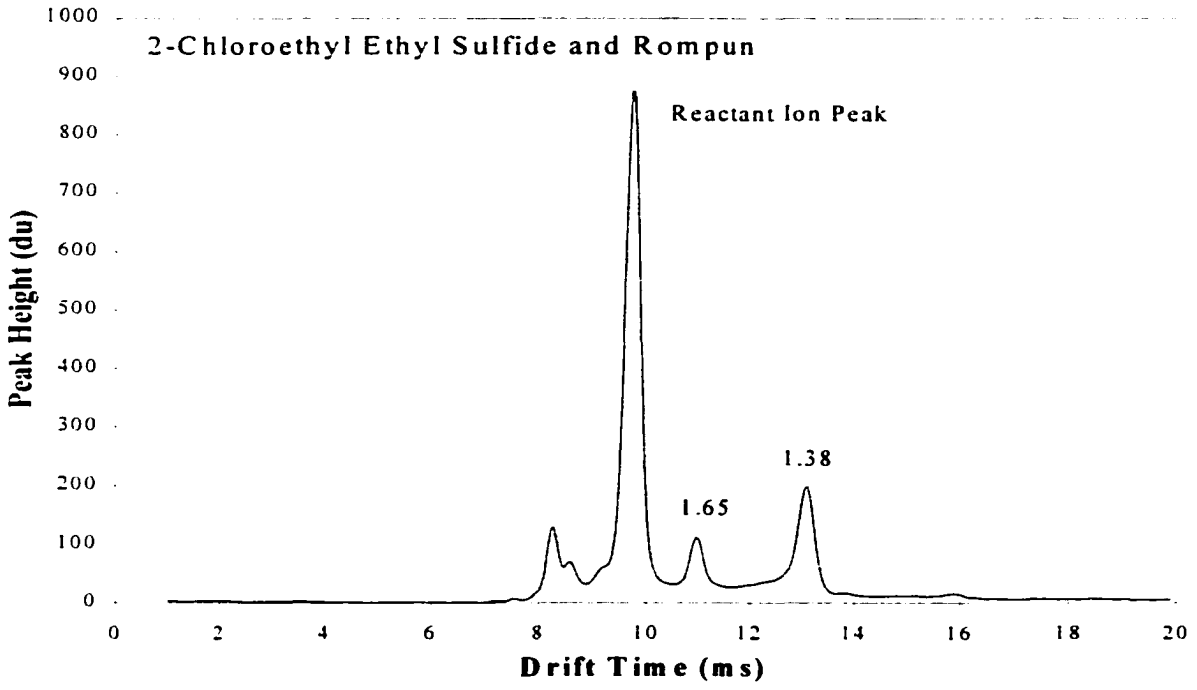


Fig. 38. Positive mode spectrum of a mixture of 100 ng/μL Rompun and 500 ng/μL 2-chloroethyl ethyl sulfide.

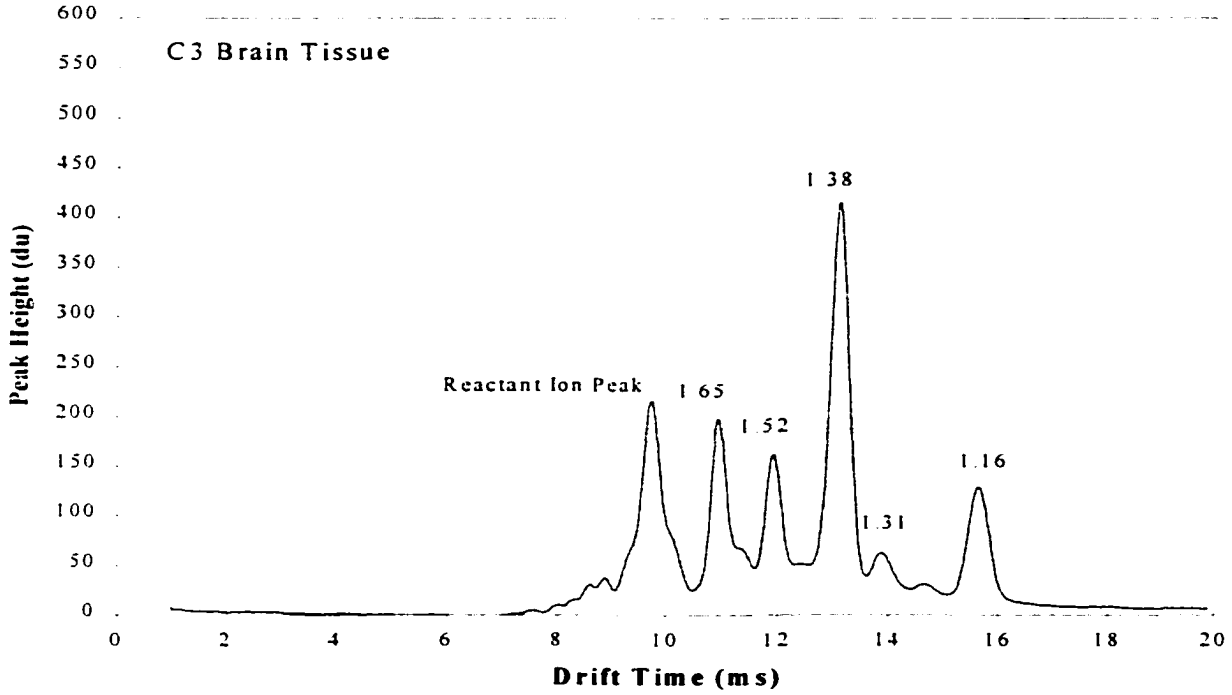


Fig. 39. Positive mobility spectrum of C3 brain tissue.

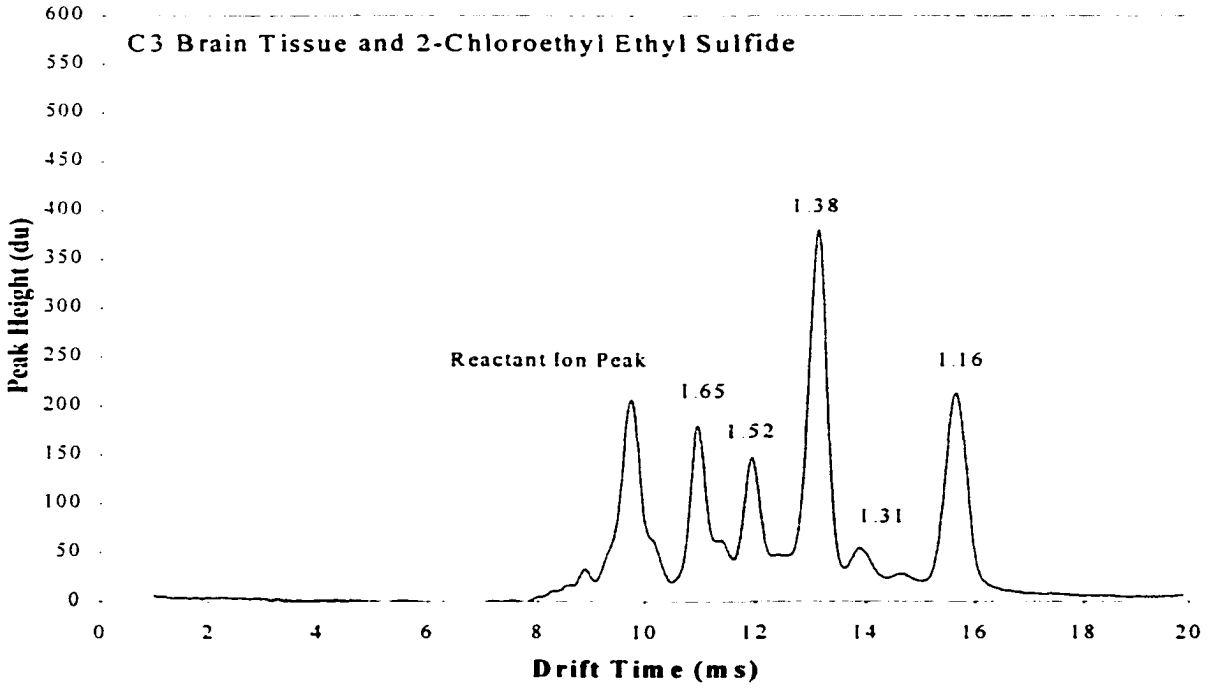
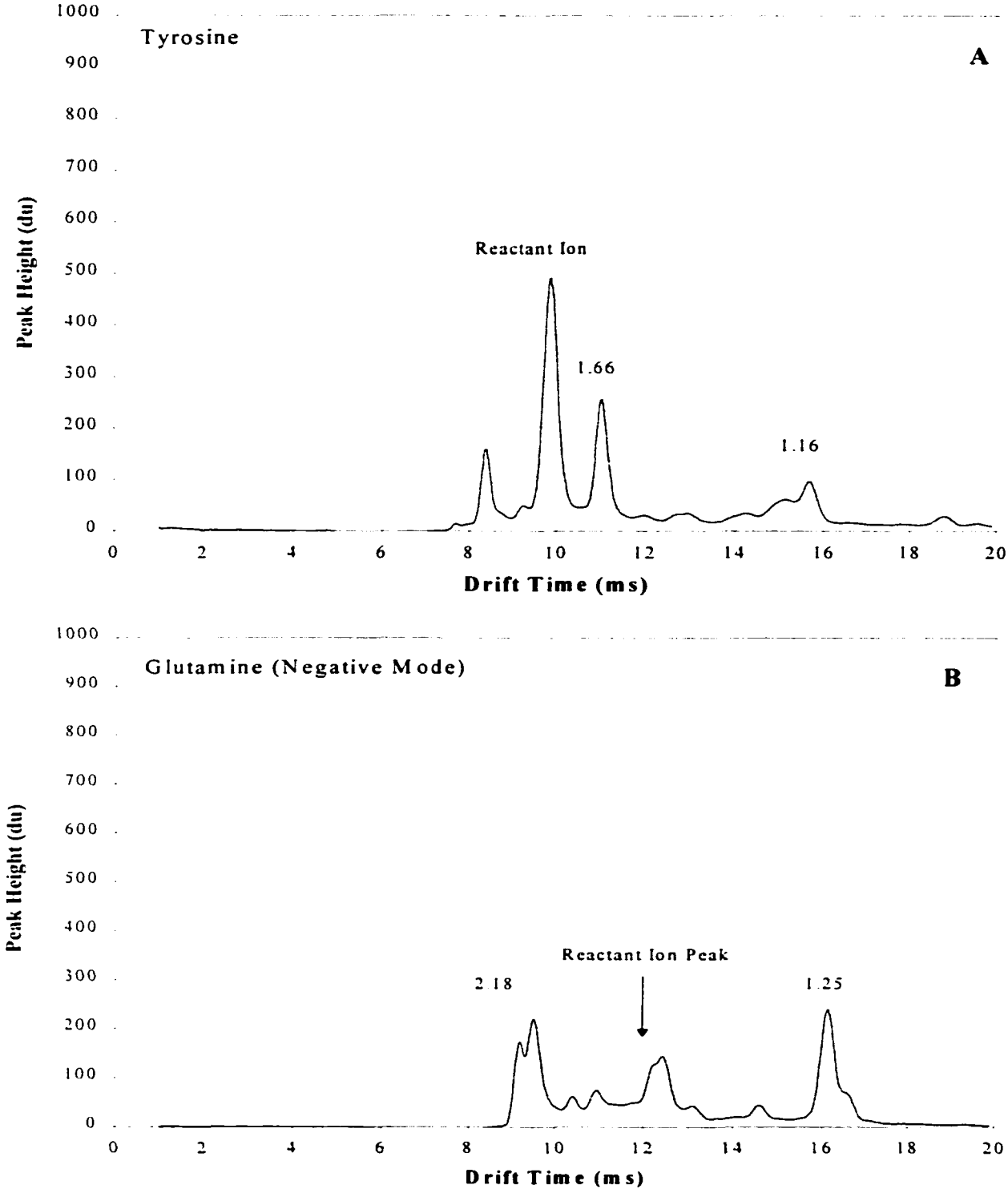


Fig. 40. Positive mode mobility spectrum of C3 brain tissue exposed *in vitro* to 500 ng/μL 2-chloroethyl ethyl sulfide.

Table 12
IMS K_o values and amplitudes of selected amino acids (0.5-1 mg) analyzed in positive and negative modes

Amino Acid	Peak Number	Positive Mode		Negative Mode	
		K_o ($cm^2V^{-1}s^{-1}$)	Amplitude (du)	K_o ($cm^2V^{-1}s^{-1}$)	Amplitude (du)
Glycine	1	1.12	22.2	1.85	136.6
	2			1.22	58.9
Phenylalanine	1	1.36	127.0	1.87	99.0
	2	1.32	38.2	1.81	62.2
	3	1.26	32.3	1.40	45.1
	4	1.06	117.0	1.06	41.0
Glutamine	1	No signal detected		1.25	197.2
Tyrosine	1	1.66	216.3	No signal detected	
	2	1.22	39.7		
	3	1.17	58.9		
Cysteine	1	1.71	72.2	1.46	263.4
	2	1.61	45.1	1.37	95.2
	3	1.46	75.8	1.30	133.1
Arginine	1	1.67	46.2	No signal detected	
	2	1.42	16.4		
Histidine	1	1.70	63.9	1.53	183.0
	2	1.43	27.7	1.25	79.1

were noted in the negative mode. In some cases, a signal was observed in only one of the modes. The amino acids with peaks of highest intensity were tyrosine and glutamine in the positive and negative modes, respectively (Figs. 41A and 41B). These amino acids were dissolved to determine any difference in mobility spectra in comparison to the solid. Tyrosine was dissolved in a dilute HCl solution and glutamine was dissolved in water. Both solutions were hydrophobic to the Teflon membrane used, and were analyzed instead on S&S paper and QMA. A positive ion mobility spectrum similar to that from solid was observed with tyrosine, but no signal was found for glutamine. The concentrations were 1000 ng/mL for tyrosine and glutamine in solution.



Figs. 41A and 41B. IMS mobility spectra of (A) tyrosine in the positive ion mode and (B) glutamine in the negative ion mode.

Discussion

Chemistry studies using IMS have shed additional light on the origin of the four ion peaks found in the tissues from rabbit organs. Solvent extractions of tissue samples showed that the compounds associated with ion peaks of K_o 1.38 and 1.30 $cm^2V^{-1}s^{-1}$ easily extractable. Mobility spectra that illustrate extraction results with C3 atria, lung and ventricle are shown in Appendix C. As shown in Table 11 and Fig. 42 control atrial tissue does not exhibit ion peaks with K_o 1.65 and 1.51 $cm^2V^{-1}s^{-1}$. However, there is a medium intensity peak at K_o 1.37 $cm^2V^{-1}s^{-1}$ and a strong ion peak at 1.29 $cm^2V^{-1}s^{-1}$. The IMS of both methanol and acetonitrile extracts of atrial tissue showed peaks at K_o 1.38 and 1.30 $cm^2V^{-1}s^{-1}$, although the components responsible for the peaks were not completely extracted. The same ion peaks, though lower in intensity, were noted in the IMS of the tissue residues. Results with lung tissue were similar. Use of methanol seems to be more efficient than acetonitrile in extracting the components corresponding to K_o 1.38 and 1.30 $cm^2V^{-1}s^{-1}$. No ion peaks associated with K_o 1.65 and 1.52 $cm^2V^{-1}s^{-1}$ were noted for the residues (supernatant). Methanol seems to exhibit a retardation effect on the IMS of tissue residues after extraction (Table 12). This might be protein denaturization resulting in blocking components from volatizing in the IMS desorption process. Retardation effects were also noted in residual tissues from methanol and acetonitrile extraction of ventricle tissues especially with the ion peaks of K_o 1.38 and 1.30 $cm^2V^{-1}s^{-1}$.

The extraction data are consistent with assignment of ion peaks with K_o 1.38 and 1.31 $cm^2V^{-1}s^{-1}$ to Rompun/Ketaset and heparin, respectively. Both compounds should be soluble in methanol and acetonitrile. The components associated with K_o 1.65 and

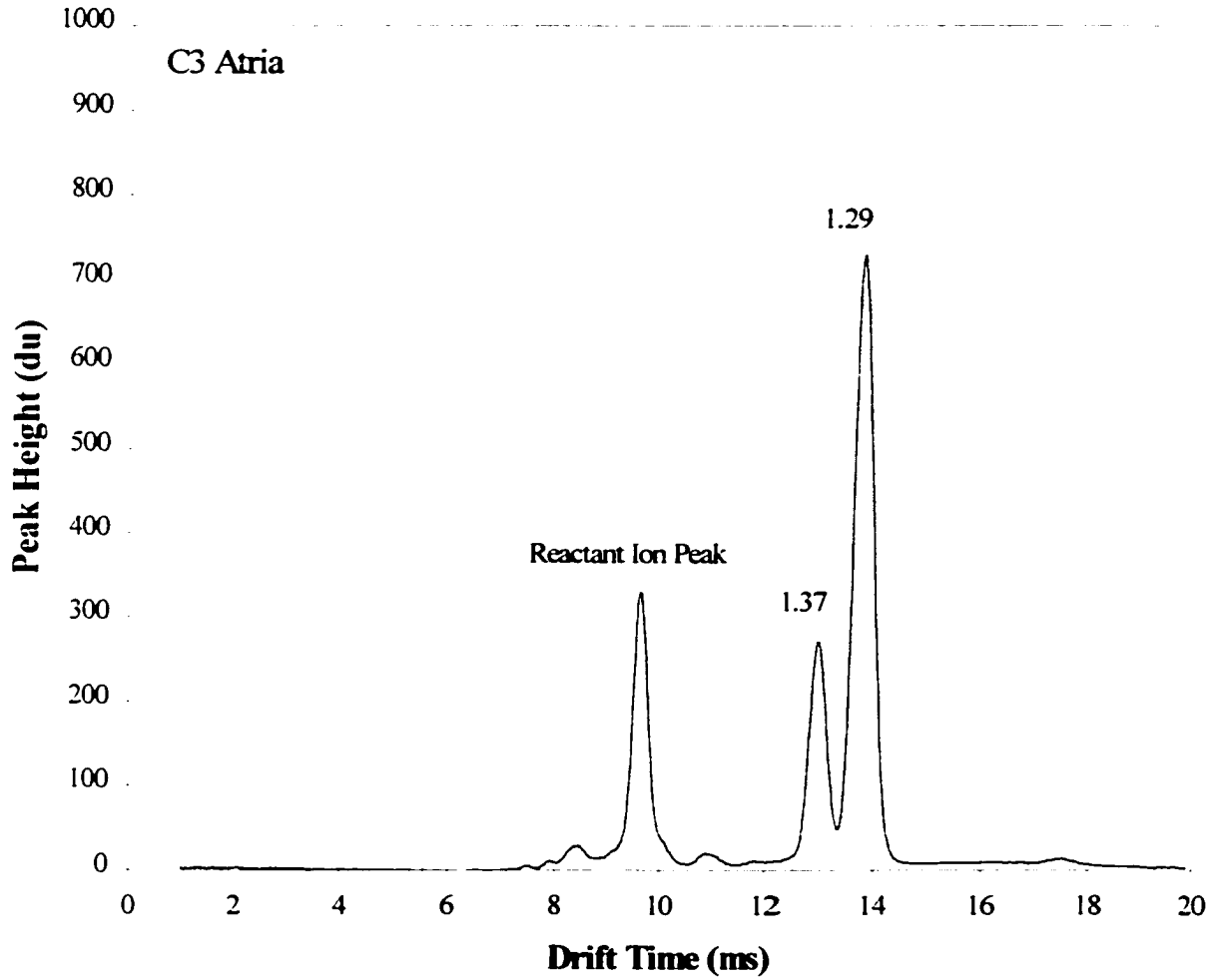


Fig. 42. Positive ion mobility spectrum of C3 atria.

1.52 $cm^2V^{-1}s^{-1}$ did not extract. This adds evidence that they are innate to the tissues and probably arise through thermal decomposition during desorption. Solubility in the methanol and acetonitrile would have been expected.

A number of solid materials were screened with IMS to check whether any ion peaks similar to those from rabbit tissues were found. These included vegetable and synthesized proteins, sugars, humic substances and amino acids. A listing of these mobility spectra are shown in Appendix D. A summary of the IMS results for the amino acids screened are given in Table 12. Four of the amino acids showed positive ion peaks between 1.66 and 1.71 $cm^2V^{-1}s^{-1}$. As mentioned above, the strongest peak in the positive mode was exhibited by tyrosine. This could be the source of the 1.65 $cm^2V^{-1}s^{-1}$ peak in animal tissues. The amino acid with the closest peak to the animal tissue peak of 1.50 $cm^2V^{-1}s^{-1}$ was cysteine with a K_o of 1.46 $cm^2V^{-1}s^{-1}$. It should be noted that the Teflon control membrane exhibited a very weak peak with a K_o value of 1.65 $cm^2V^{-1}s^{-1}$ (Fig. 43). It was surprising to find that vegetable protein (soy protein isolate) showed no peaks, however, the content of tyrosine was only 3.3%. Another product (Slim Fast ®) containing yet smaller amounts of vegetable protein but many other components shows several very weak peaks were found, but nothing corresponding to those found with animal tissue. Three humic substances were also screened (Appendix D). A single ion peak with low mass was found (K_o1.97 $cm^2V^{-1}s^{-1}$). However, it did not correspond to those observed with animal tissues.

Alkylation Agents

The reaction of animal tissues with alkylating agents was examined using IMS in both the negative and positive modes. The idea was to allow two different, but well

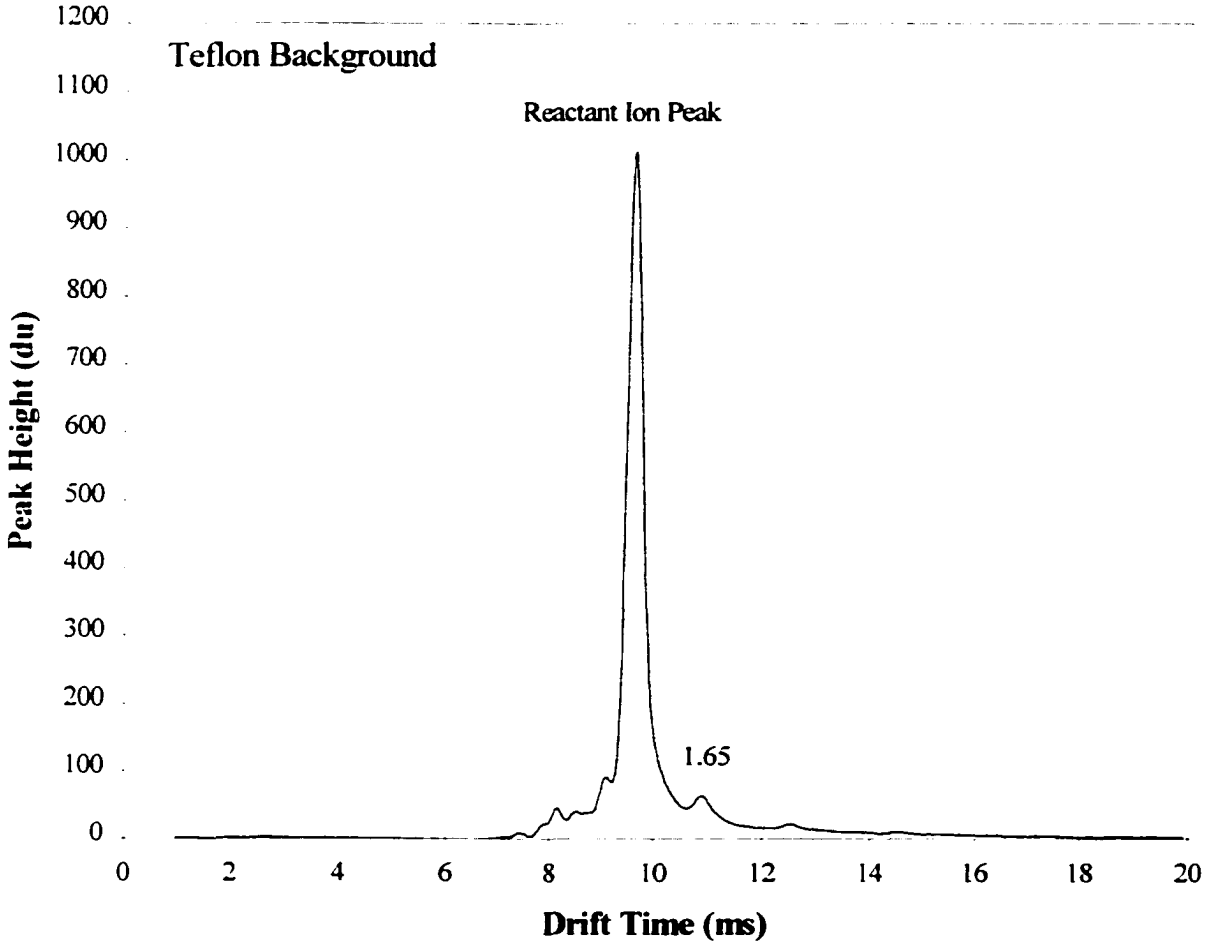
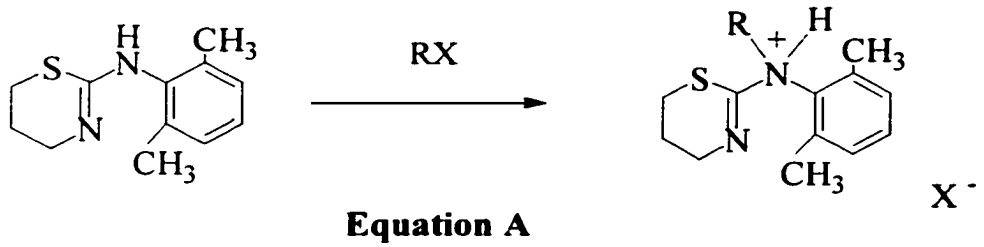


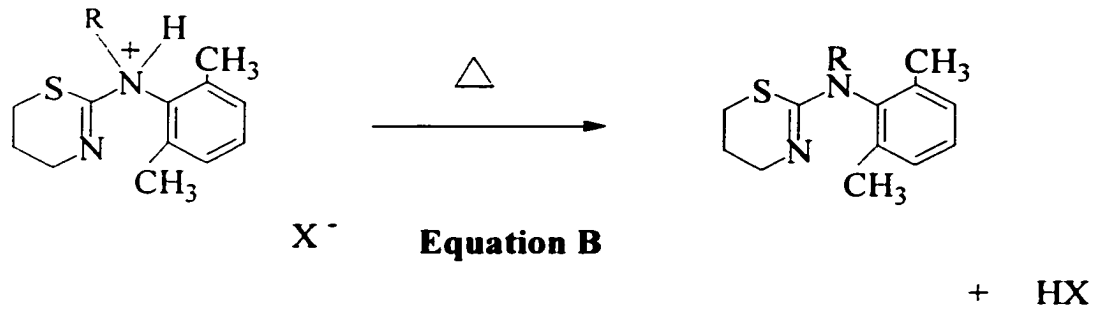
Fig. 43. Positive ion mobility spectrum of Teflon.

known alkylating agents, 2-chloroethyl ethyl sulfide and methyl iodide, to react with high electron density centers in the animal tissues and follow effect on ion peaks in the tissues. For example, alkylation of accessible Rompun in tissues should lead to a salt which would be much less volatile (Fig. 44A). The K_o 1.38 $cm^2V^{-1}s^{-1}$, ion peak in the positive mode should decrease proportionately. If the salt dissociates during thermal desorption to a tertiary amine, the K_o should shift to a lower value due to the increased ion mass (Fig. 44B).

Negative ion mobility spectra of brain tissue C4 after the reaction with 2-chloroethyl ethyl sulfide and methyl iodide gave indication that reaction occurred, but shed no light on reaction sites. The mobility spectra of brain tissue C3 in the negative mode was interesting in view of the strong ion peak at K_o 1.50 $cm^2V^{-1}s^{-1}$ (Fig. 37A). However, addition of methyl iodide to the tissue did not affect the intensity (Fig. 37B and 37C). The peak is not due to Rompun as verified by its negative mode mobility spectra (Fig. 29). It might also arise from a metabolite of Rompun or Ketaset. It is interesting and needs to be explored further.



Equation A



Equation B

Fig. 44. Equation A represents the alkylation of Rompun. Equation B represents the dissociation of the salt to a tertiary amine.

CHAPTER VI

IDENTIFICATION OF FACTORS THAT INFLUENCE THE ION MOBILITY SPECTROMETRY DETECTION OF CONTRABAND DRUGS

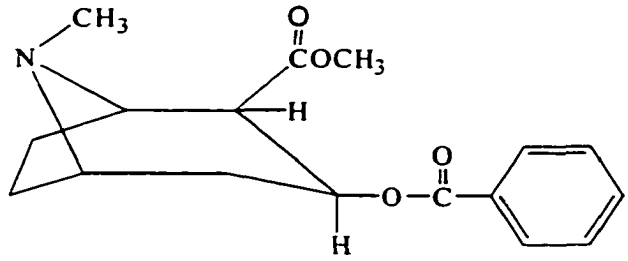
Introduction

We have hypothesized that there may be enhancement and retardation effects that impact the utility of IMS in clinical chemistry. The specific aim of the present chapter is to identify factors that influence IMS analysis using cocaine, heroin, and their metabolites as an illustration of what might be encountered in the clinical laboratory and in clinical chemistry research. Experiments were performed to determine IMS properties of mixtures of cocaine and its decomposition products. Effects of proteins, amino acids, and small glycine peptides on the IMS characteristics of heroin were also examined.

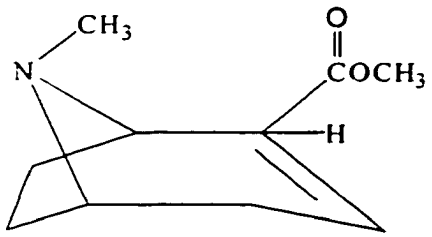
Cocaine and its Metabolites

Cocaine is an alkaloid and potent CNS stimulant that elicits a state of increased alertness and euphoria. CNS effects are thought to occur when cocaine blocks neurotransmitter reuptake, and prolongs the action of dopamine. Although cocaine is an effective local anesthetic and vasoconstrictor, it can produce sympathomimetic responses that include an increase in blood pressure, heart rate and body temperature [48].

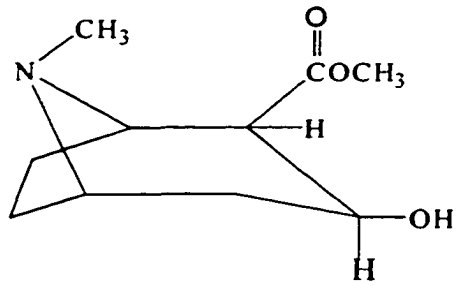
Cocaine is rapidly metabolized by liver esterases into the metabolites, ecgonine methyl ester (EME) and benzoylecgonine (BE) (Fig. 45). Cocaine may also be converted to EME by the action of serum pseudocholinesterase and to BE by



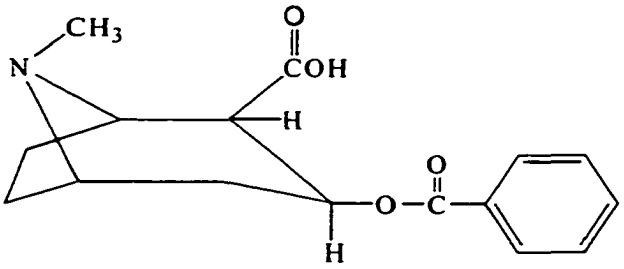
Cocaine



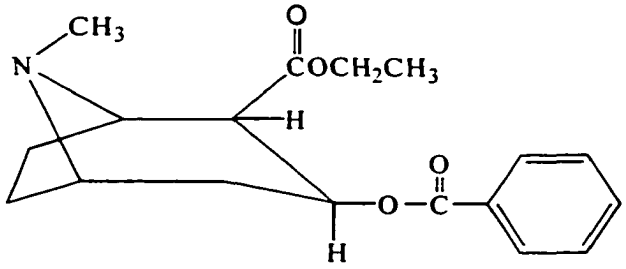
Anhydroecgonine Methyl Ester (AEME)



Ecgonine Methyl Ester (EME)



Benzoylecgonine



Cocaethylene

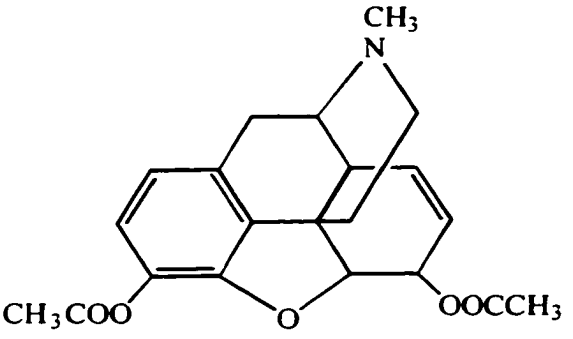
Fig. 45. Structures of cocaine and its common metabolites/decomposition products.

spontaneous hydrolysis [49]. A thermodegradation product of cocaine is anhydroecgonine methyl ester (AEME) (Fig. 45), and is often found in the urine of those who smoke cocaine [50]. In the presence of ethanol, liver carboxyesterase catalyzes the tranesterification of cocaine to cocaethylene (also called ethylcocaine) (Fig. 45). Cocaethylene has the same effects of cocaine and may enhance the stimulatory effects on the CNS.

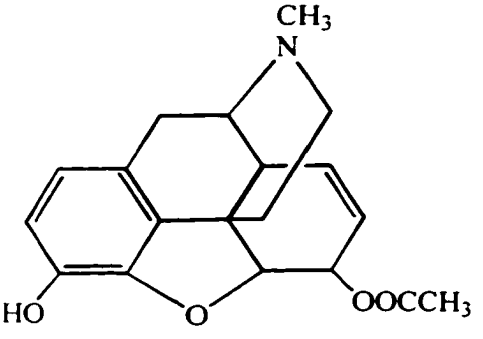
Cocaine's metabolites can be identified using for example, enzyme multiplied immunoassay technique (EMIT) (300 ng/μL cut-off) [48]. Another technique used for confirmatory analysis is gas chromatography/mass spectrometry (GC/MS). This requires liquid-liquid extraction, solvent evaporation, and treatment with N-methyl-N-trimethylsilyltriflouroacetamide (MSTFA) to form EME and BE derivatives [49]. Much information is available in the literature on cocaine detection, identification, and analysis [9].

Heroin and its Metabolites

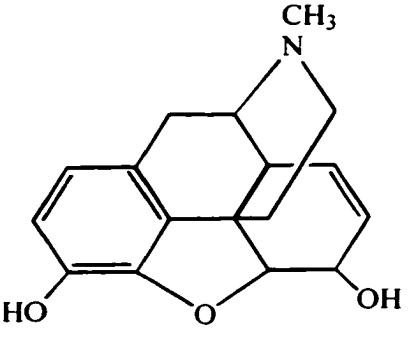
Morphine and heroin are two drugs in the class of opiates, which are very effective painkillers (Fig. 46). Morphine is an active ingredient of opium. Opiates slow down the central nervous system and can reduce hunger. Specifically, opiate derivatives stimulate opiate kappa receptors and depress the release of acetylcholine [35]. Morphine is targeted for excretion from the body through glucuronide conjugation at one of its hydroxyl groups. Heroin (diacetylmorphine) is preferred by opiate abusers because of its rapid onset of action, although this derivative of morphine is itself not active. (Heroin is rapidly converted to 6-acetylmorphine and then to



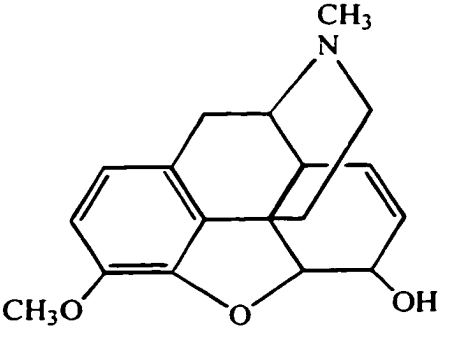
Heroin



6-Acetylmorphine



Morphine



Codeine

Fig. 46. Structures of heroin metabolites or derivatives.

morphine). 6-Acetylmorphine is a metabolite and a hydrolysis product of heroin (Fig. 46). Both heroin and 6-acetylmorphine are hydrolyzed to morphine.

Heroin is synthesized from morphine base by heating with acetic acid anhydride; its hydrochloride is very soluble in water [39]. Heroin and its metabolites, as in the case of cocaine and its metabolites, are currently analyzed in the clinical laboratory by EMIT (300 ng/μL cut-off for morphine) and GC/MS [48]. Commercial immunoassays are designed to primarily detect morphine and codeine (synthesized by the 3-methylation of morphine, (Fig. 46). Some cross-reactivity with morphine-3-glucuronide and other opiates) is reported [49]. GC/MS procedures include treatment with β-glucuronidase, extraction with a solid-phase absorbent, elution, solvent evaporation, and treatment with MSTFA reagent to form trimethylsilyl derivatives of opiates [49].

Results

Cocaine and its Decomposition Products

It has been shown that IMS is a convenient tool to detect and identify cocaine reaction products [9]. K_o values for cocaine and its common reaction products are given in Table 13.

The compounds can be differentiated based on K_o values though detection limits vary widely. Two studies were performed to identify factors that may effect the IMS analysis cocaine. In one case, mixtures of AEME and EME (two decomposition products of cocaine) were analyzed in the presence of each other. In the other case, the same compounds were analyzed in the presence cocaine.

Table 13
K_o values for cocaine and its common reaction products [39]

Compound	K_o ($cm^2V^{-1}s^{-1}$)
Cocaine	1.16
Benzoylecgonine	1.18
EME	1.48
AEME	1.50 [17]
Ecgonine	1.55
Cocaethylene	1.12 and 1.42

IMS of Anhydroecgonine Methyl Ester (AEME) and Ecgonine Methyl Ester (EME) Mixtures. IMS was used for the identification and analysis of AEME and EME in the presence of each other. As shown in Table 13, these compounds exhibit ion mobility values which are relatively close (reduced mobility constants, K_o: 1.50 and 1.48 $cm^2V^{-1}s^{-1}$, respectively). Studies involving cocaine reactions may require differentiation of AEME and EME. Calibration curves for AEME and EME are shown in Figs. 47A and 47B. Analytical information can be obtained from less than a nanogram to approximately 30 ng. Above 30 ng, the system becomes saturated and little increase in peak intensity occurs.

It was found that IMS peaks from AEME and EME mixtures could not be deconvoluted using the instrument software. The K_o values and average amplitudes for these mixtures are given in Table 14. The peak amplitudes from AEME/EME mixtures were about 50% lower than expected by adding amplitudes of individual components using their calibration curves. This is not surprising with higher amounts because of saturation effects but was unexpected for lower amounts. It is probable that amounts of

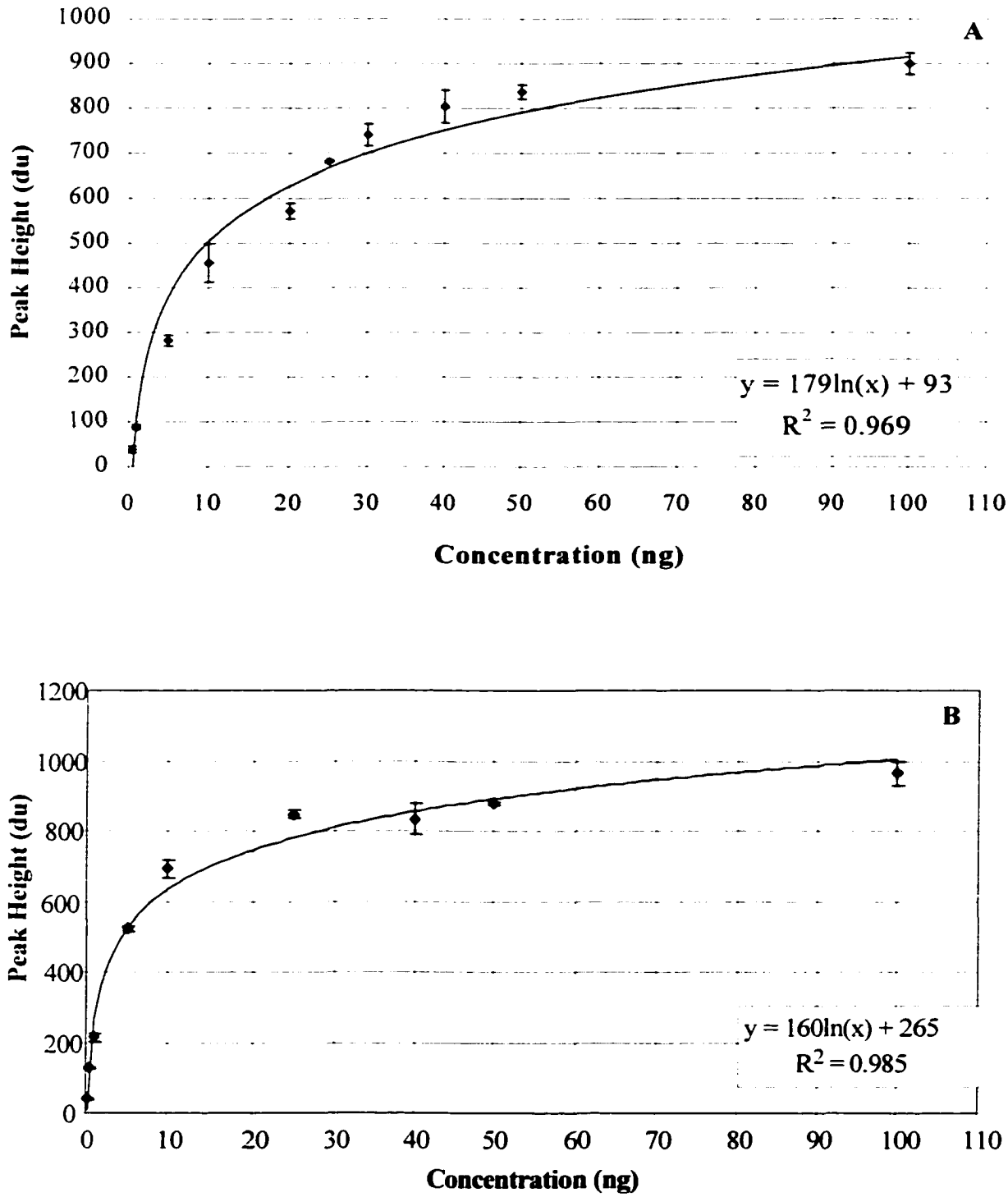


Fig. 47. Ion mobility spectrometry calibration curve for (A) EME (0-100 ng) and (B) AEME (0-100 ng).

Table 14

Ion mobility spectrometry results using mixtures of anhydroecgonine methyl ester (AEME) and ecgonine methyl ester (EME)

Weight Ratio ng AEME/ ng EME	K_o $cm^2V^{-1}s^{-1}$	Average Amplitude (du)
10 / 0	1.504 ±0.000	692 ±25
10 / 10	1.502 ±0.002	531 ±13
10 / 20	1.490 ±0.003	547 ±27
10 / 40	1.485 ±0.000	692 ±27
10 / 60	1.484 ±0.000	815 ±25
10 / 80	1.483 ±0.000	856 ±7
0 / 10	1.485 ± 0.000	406 ±22
10 / 10	1.502 ±0.000	531 ±13
20 / 10	1.493 ±0.000	684 ±19
40 / 10	1.495 ±0.000	750 ±12
60 / 10	1.494 ±0.000	772 ±7
80 / 10	1.496 ±0.000	801 ± 24

either AEME or EME may be underestimated when both compounds are present. This is may be due to effects of peak shape but was not investigated further. AEME shows higher sensitivity to IMS than does EME (Figs. 47A and 47B) but is of little consequence in attempting to analyze mixtures.

Table 14 shows K_o values for combinations in which one component is held at 10 ng and the other is varied up to 80 ng. As EME amounts are increased (holding AEME at 10 ng) the K_o values decrease until the K_o of EME is reached. The peak amplitudes decrease for AEME/EME ratios of 10 ng/10 ng and 10 ng/20 ng, and then increase. The observed decrease may be due to factors such as the lower sensitivity of IMS to EME (Figs. 47A and 47B), peak shape, and/or instrument variability. As

AEME amounts are increased (holding EME at 10 ng), the highest K_o values were found at AEME/EME ratios of 10 ng/10 ng and 80 ng/10 ng. An interesting finding is that at a 10 ng/10 ng weight ratio of AEME/EME, the K_o value (1.50 $cm^2 V^{-1}s^{-1}$) indicates the presence of AEME. A K_o value of 1.49 $cm^2V^{-1}s^{-1}$ is difficult to interpret as to relative amounts of AEME and EME that might be present.

It is clear from the data in Table 14 that K_o values cannot be used to differentiate AEME from EME in their mixtures. (It may also be difficult to identify these compounds using IMS in the presence of other substances which have similar ion mobilities.) Unequivocal identification of AEME and EME would require use of an independent technique such as GC-MS. In some applications, it may be very important to differentiate AEME from EME for identification of cocaine. One might be able to separate IMS peaks for AEME and EME using carrier and drift gases other than air, however, this was not investigated.

Enhancement of IMS Sensitivity to Cocaine. IMS was used to examine the effect of AEME and other compounds on cocaine sensitivity. Studies were also performed using different sample membranes to detect surface effects on cocaine IMS sensitivity.

It was observed that the addition of a little as 1 ng AEME may significantly increase cocaine hydrochloride IMS sensitivity. Fig. 48 illustrates results from adding 1 ng of AEME to 1, 5, 10, and 25 ng each of cocaine hydrochloride. In each case there was a relative increase in the amplitude of the cocaine ion peak. Addition of 1 ng AEME to 5 ng cocaine hydrochloride resulted in a 50% increase of the cocaine peak

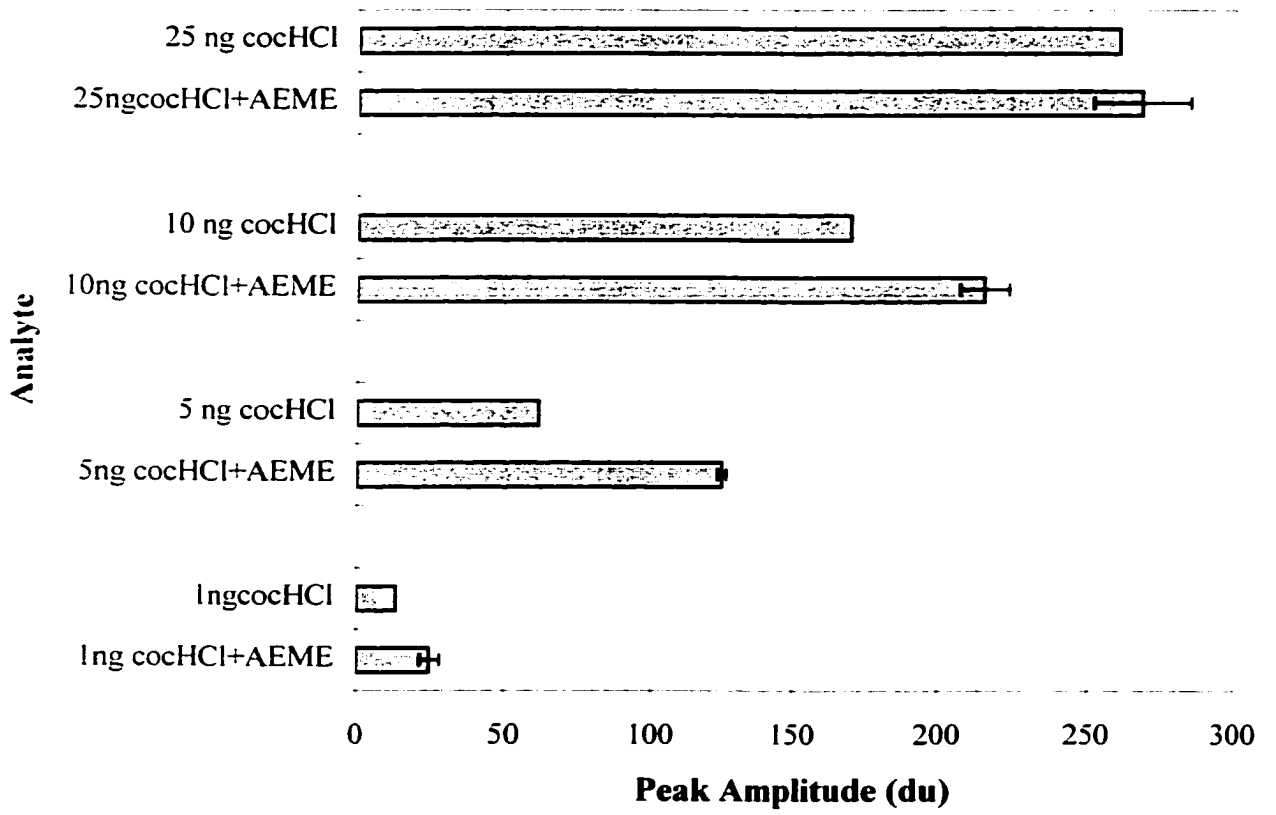


Fig. 48. IMS peak amplitude average increases as a result of adding 1 ng AEME to 1, 5, 10, and 25 ng cocaine hydrochloride (K_o, 1.16 $cm^2V^{-1}s^{-1}$).

amplitude. Addition of more than 1 ng of AEME to 5 ng of cocaine hydrochloride did not lead to further increases of the cocaine signal. This is illustrated in Fig. 49.

The effect of adding cocaethylene, benzoylecgonine, ecgonine hydrochloride, morphine, 6-acetylmorphine, codeine, and heroin on IMS sensitivity of cocaine was also examined. In these experiments 25 ng of each substance was added to 5 ng of cocaine hydrochloride. There was an increase in the IMS sensitivity of cocaine in all cases except cocaethylene and only a slight increase was seen with the addition of benzoylecgonine (Fig.50). This demonstrates that compounds other than AEME may increase cocaine hydrochloride IMS sensitivity as well. It is important to note that such effects may also be caused by solvent. For example when 1 μL of acetonitrile was individually applied to nanogram amounts of cocaine (5 - 25 ng), small increases in cocaine IMS sensitivity were observed (Fig. 51). The acetonitrile was allowed to evaporate for one minute before the analyses were performed.

Four membranes/papers were compared relative to overall effects on IMS sensitivity to cocaine. These included Barringer fiber glass membranes, Whatman filter paper, and Schleicher & Schell (S&S) papers and Barringer Teflon membranes (normally used). Fig. 52 shows that the sample matrix used may significantly affect the amplitude of cocaine. Barringer fiber glass appears to retard the cocaine peak the most, with S & S paper having the least effect, both relative to Teflon. An increase in the cocaine signal was still observed, however, with the addition of 10 ng AEME to 5 ng cocaine, with each sample matrix. The best effect was noted with Whatman filter paper. However, in no case was the amplitude increase greater than that observed with

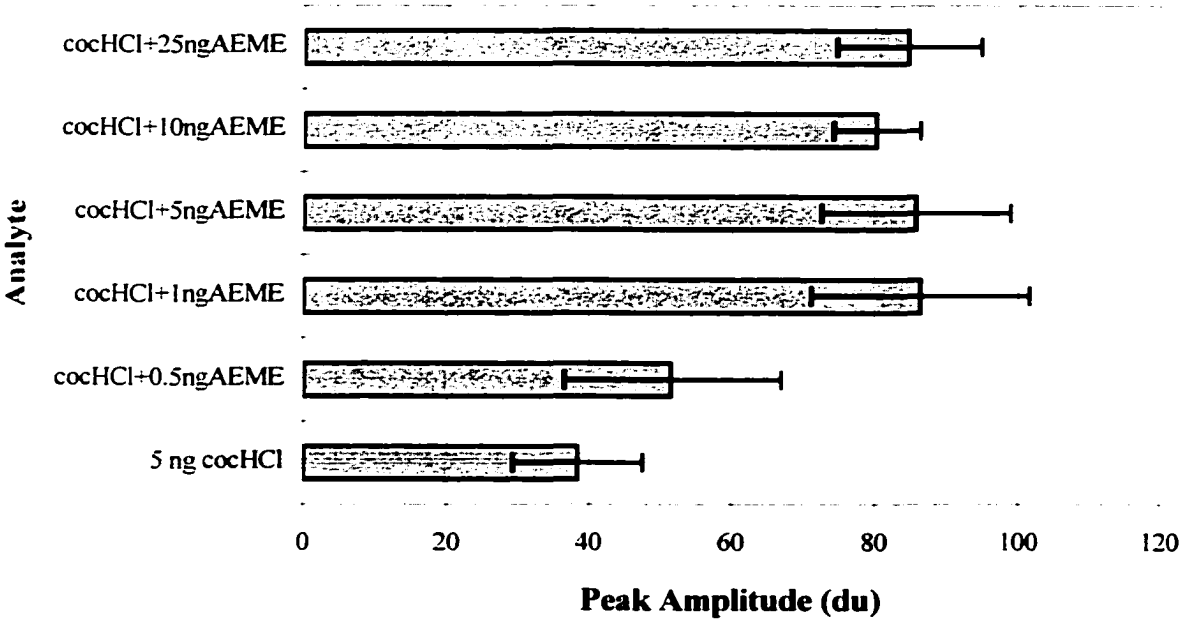


Fig. 49. IMS peak amplitude changes as a result of adding AEME (0.5, 1, 5, 10, and 25 ng) to 5 ng cocaine hydrochloride (K_o, 1.16 $cm^2V^{-1}s^{-1}$).

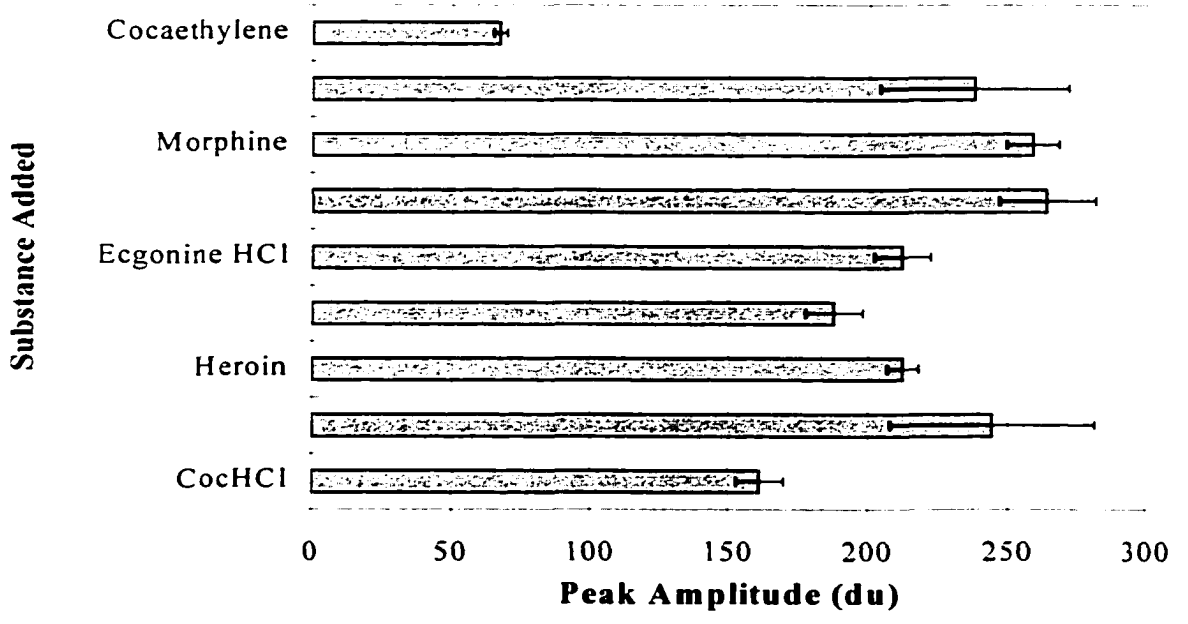


Fig. 50. IMS peak average amplitude changes as a result of adding various substances to 5 ng cocaine hydrochloride (K_o, 1.16 $cm^2V^{-1}s^{-1}$).

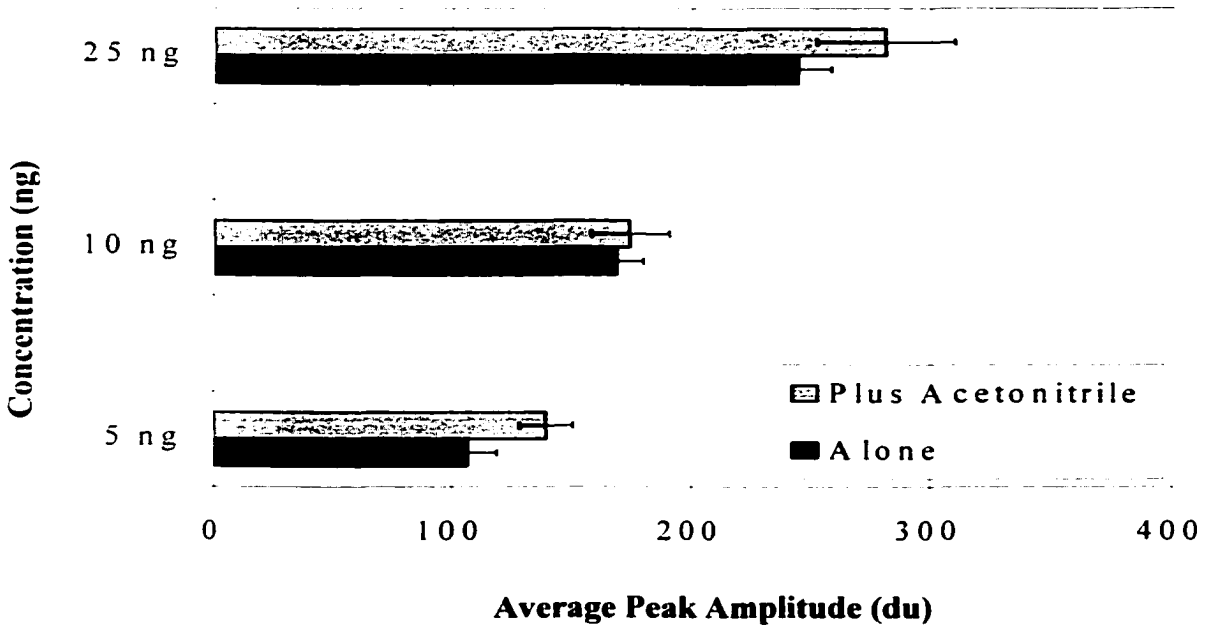


Fig. 51. IMS peak amplitude change as a result of applying 1 μL of acetonitrile to 5, 10, and 25 ng of cocaine hydrochloride prior to analysis.

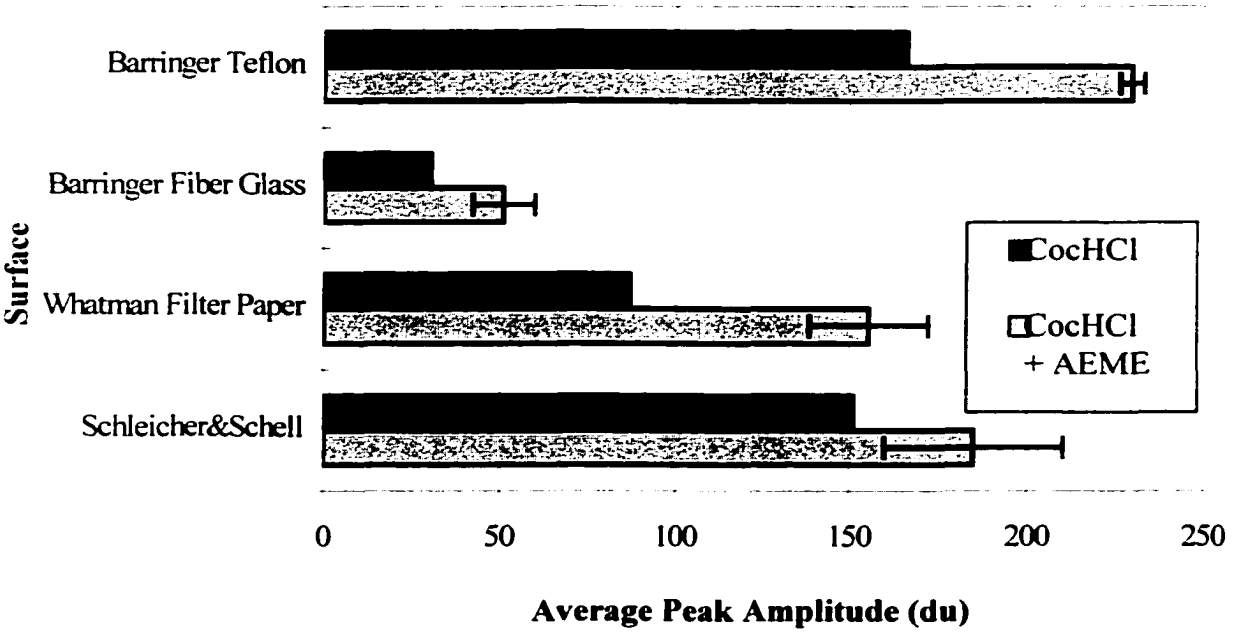


Fig. 52. Effect of sample membrane on the IMS peak amplitude of cocaine hydrochloride alone and in the presence of AEME.

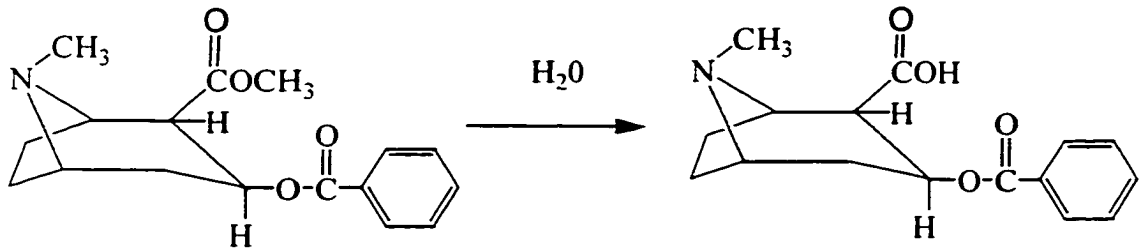
Teflon and AEME. It is clear that clinical laboratory practitioners and researchers need to choose a sample matrix that best suits their needs. For example, though Teflon may provide the highest sensitivity for cocaine, an aqueous sample would better be analyzed with a paper filter or other surface which easily absorbs water.

IMS sensitivity effects on cocaine ion peak amplitude were explored in the presence of AEME and animal tissues. Weight ratios of 1:5, 5:1, 2:5, and 5:2 of cocaine hydrochloride to AEME were separately deposited on 0.5-1 mg A43 brain tissue. The controls were cocaine hydrochloride alone and cocaine hydrochloride in the presence and absence of tissues. It was found that the presence of animal tissue decreases the cocaine signal amplitude by as much as two thirds. However, in the presence of AEME and brain tissue the cocaine signal amplitude decrease is much less and in cases falls within the standard deviation. Additional work is required to more fully understand the effect. It is clear however, that based on preliminary results, analysis of cocaine and its decomposition products using IMS may be limited to qualitative applications.

Benzoylecgonine. BE is the major hydrolysis product formed in the metabolism of cocaine (Fig. 53). It is the major cocaine metabolite found in hair and urine [8]. The IMS mobility spectrum of 200 ng of BE from methanol solution is shown in Fig. 54. A calibration curve is given in Fig. 55. The IMS response to BE was found to be about 4-5 times lower than that for cocaine (see Fig. 8 for comparison).

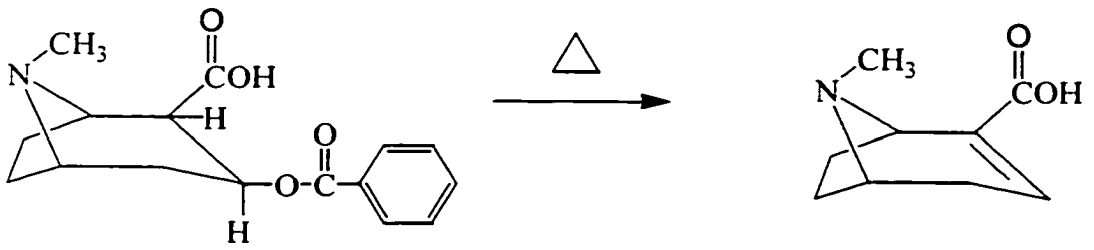
The peak at K_o, 1.18 $cm^2V^{-1}s^{-1}$, is attributed to BE with an ion mass of 289 [39]. At BE concentrations of 100 ng and above, a second peak appeared (K_o 1.63 $cm^2V^{-1}s^{-1}$).

A



Cocaine **Benzoylecgonine**

B



Benzoylecgonine **Ecgonidine**

Fig. 53. (A) Benzoylecgonine is a major hydrolysis product formed in the metabolism of cocaine. (B) Elimination of benzoic acid from benzoylecgonine gives ecgonidine.

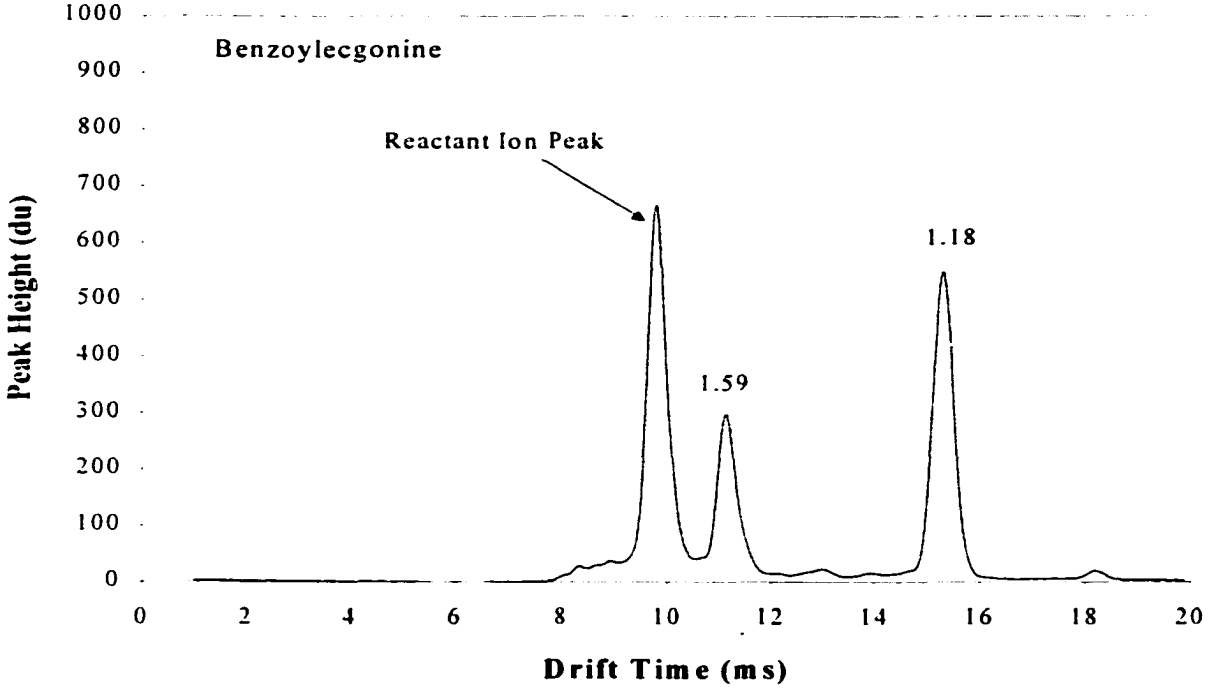


Fig. 54. IMS mobility spectrum of 200 ng benzoylecgonine in methanol.

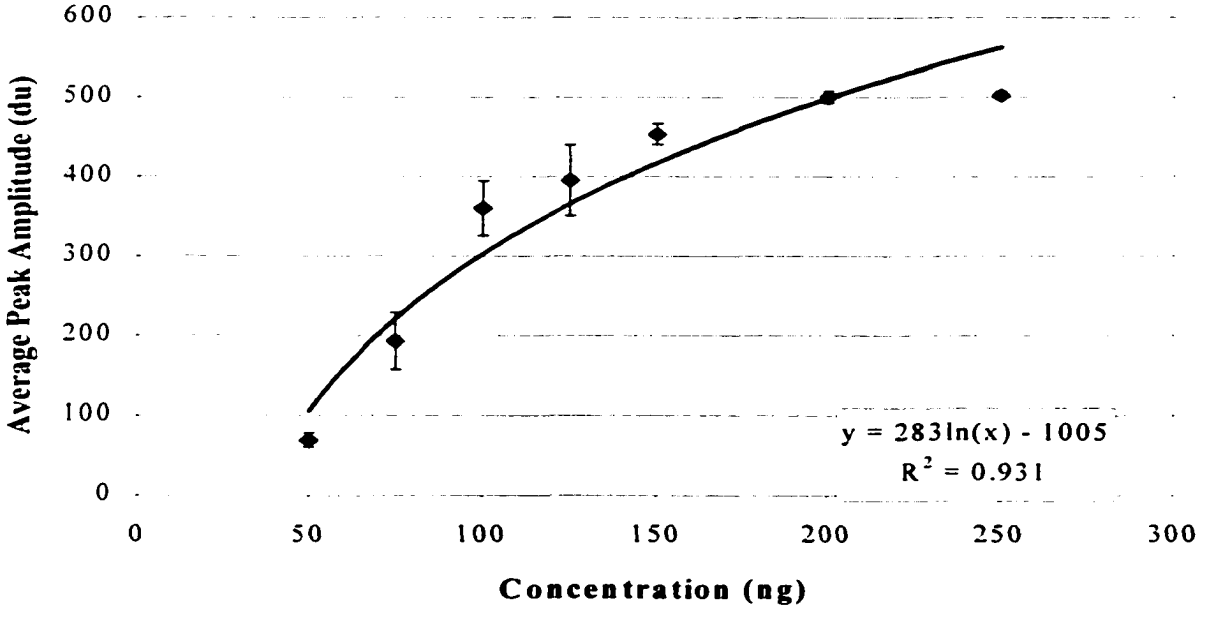


Fig. 55. IMS calibration curve for benzoylecgonine.

This may be a decomposition product formed during IMS thermal desorption or an impurity in the sample.

We speculate that the K_o, 1.63 $cm^2V^{-1}s^{-1}$, peak is from ecgonidine formed in the elimination of benzoic acid from BE (Fig. 53). This is analogous to the elimination of benzoic acid from cocaine to form AEME. This peak also appeared during a time in which the QA/QC daily checks with cocaine showed above average amounts of AEME being formed. It appears that a contaminant in the IMS system was facilitating the benzoic acid elimination.

Hydrolysis of BE to ecgonine was eliminated as a source of the 1.63 $cm^2V^{-1}s^{-1}$ peak since the K_o for ecgonine is 1.55 $cm^2V^{-1}s^{-1}$ (Table13). However, deconvoluting the K_o, 1.63 $cm^2V^{-1}s^{-1}$, ion peak for 200 ng of BE did show an unidentified peak with lower intensity at K_o, 1.59 $cm^2V^{-1}s^{-1}$.

Experiments were performed with mixtures of BE and cocaine to detect any major anomalies in the resulting IMS mobility spectra during a period in which difficulties were being experience with the ion mobility spectrometer. Fig. 56 shows the mobility spectrum for 25 ng cocaine hydrochloride. The cocaine peak is much lower than usual, and the AEME peak is higher.

The major peak in the mobility spectrum (Fig.57) of a mixture of 25 ng cocaine hydrochloride and 200 ng BE was deconvoluted into two peaks, K_o, 1.19 $cm^2V^{-1}s^{-1}$ (amplitude 56), and K_o, 1.17 $cm^2V^{-1}s^{-1}$ (amplitude 535) (cocaine). It is interesting to note that the BE peak is appreciably retarded, but with an increase in the ecgonidine/BE peak ratio. On the other hand, the cocaine peak is significantly enhanced with a major

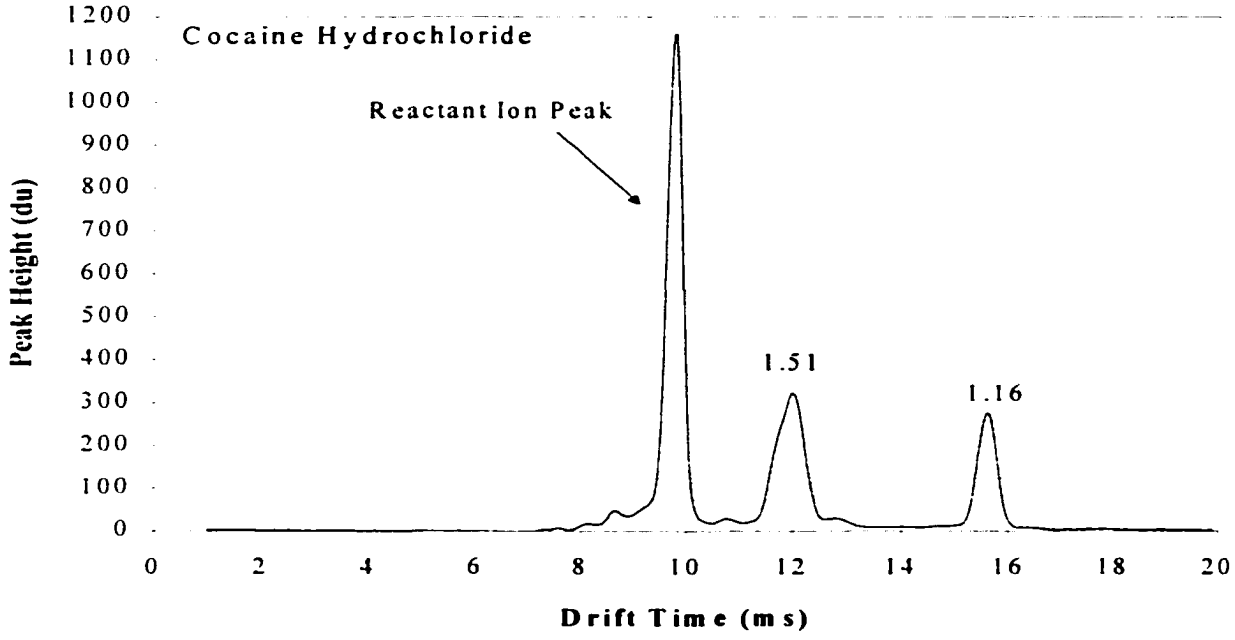


Fig. 56. IMS mobility spectrum for 25 ng cocaine hydrochloride. The cocaine peak (K_o, 1.16 $cm^2V^{-1}s^{-1}$) is much lower than usual and the AEME peak (K_o, 1.51 $cm_2V^{-1}s^{-1}$).

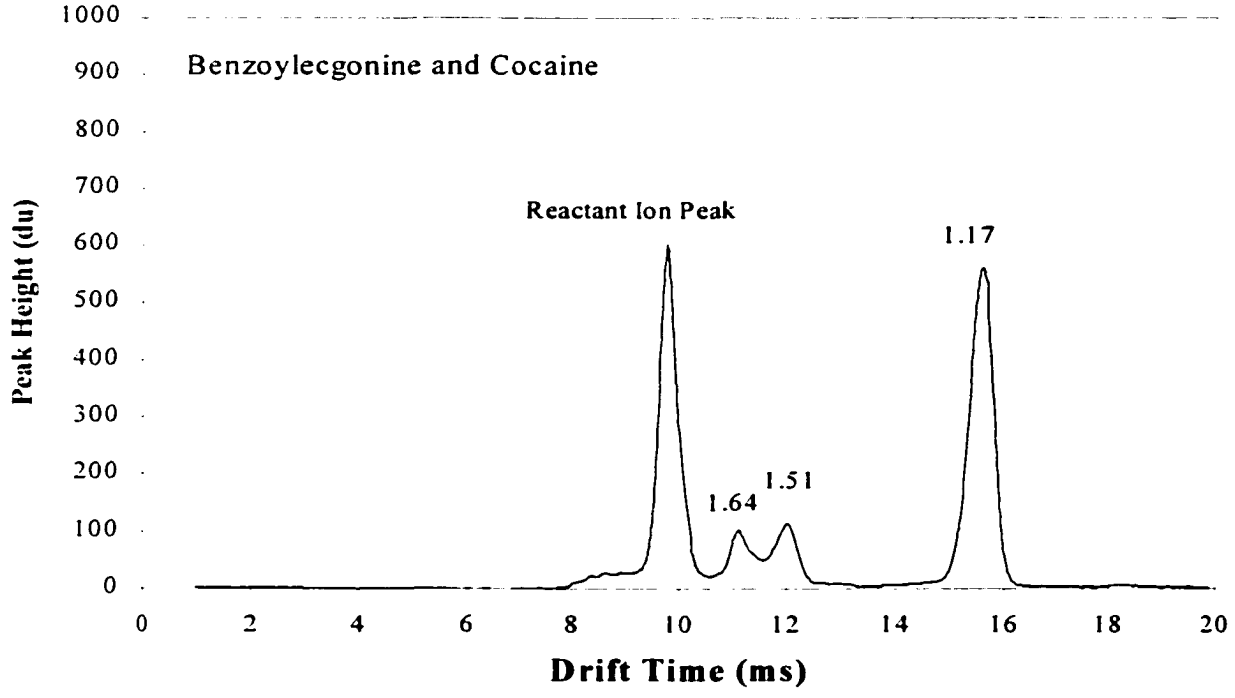


Fig. 57. IMS mobility spectrum of 200 ng benzoylecgonine and 25 ng cocaine hydrochloride. The peak at K_o, 1.17 $cm^2V^{-1}s^{-1}$ can be deconvoluted into two peaks.

decrease in the intensity of the AEME decomposition product of cocaine. The experiment was repeated several times with the same result. The conclusion is that BE is being decomposed preferentially to ecgonidine by a contaminant in the IMS system. It brings out the need for QA/QC protocols to establish how well the ion mobility spectrometer is working. Cocaine is a good candidate to use in such protocols since it is thermally labile giving AEME. BE appears to be similarly labile, appearing to produce ecgonidine. We used cocaine in QA/QC protocols as described in Chapter III, not realizing the potential of BE in QA/QC until the above experiments were performed. Additional experiments need to be added to allow differentiation of signal suppression/enhancement and chemical reactions such as the elimination of benzoic acid.

Heroin and its Metabolites

Heroin was studied in the presence and absence of various materials to identify factors that influence the IMS of this compound and to better understand mobility spectra of tissues harvested from animals receiving heroin. K_o values for heroin and its common metabolites are given in Table 15. The mobility spectra of heroin show two peaks with K_o values of 1.04 and 1.14 $cm^2V^{-1}s^{-1}$.

One study involved analyzing heroin and 6-acetylmorphine in the presence of each other. Another focused on the effect of animal tissues, proteins and amino acids on the IMS analysis of heroin.

Table 15
K_o values for heroin and related compounds [39]

Compound	K_o ($cm^2V^{-1}s^{-1}$)
Heroin	1.04 and 1.14
6-Acetlymorphine	1.13 and 1.26
Morphine	1.22[17]
Codeine	1.18 and 1.21

IMS of Heroin and 6-Actelymorphine Mixtures. As indicated in Table 15 and shown in Fig. 58, heroin has two IMS peaks, as does 6-acetylmorphine. One peak of heroin (K_o,1.14 $cm^2V^{-1}s^{-1}$) is very close to one of 6-acetylmorphine (K_o, 1.13 $cm^2V^{-1}s^{-1}$). This overlapping needs to be taken into account when analyzing samples that contain a mixture of heroin and 6-acetylmorphine. More specifically, the main IMS peak of 6-acetylmorphine (K_o, 1.13 $cm^2V^{-1}s^{-1}$) cannot be used alone to either identify or analyze 6-acetylmorphine when heroin is also present. One must use the K_o, 1.26 $cm^2V^{-1}s^{-1}$, peak.

The effect of various amounts of 6-acetylmorphine on the IMS detection and analysis of heroin hydrochloride was examined (Fig. 59). The K_o, 1.04 $cm^2V^{-1}s^{-1}$ heroin peak was evident, however, its amplitude decreased with increasing amounts of 6-acetylmorphine. The percentage decrease depended somewhat on the amount of heroin hydrochloride. The trend is clear with the largest amount of 6-acetylmorphine (100 ng). Decreases in IMS peak amplitude (K_o, 1.04 $cm^2V^{-1}s^{-1}$) for different amounts of heroin hydrochloride were found to be: 62% (25 ng heroin), 57% (50 ng heroin), 48% (75 ng heroin), and 32% (100 ng heroin).

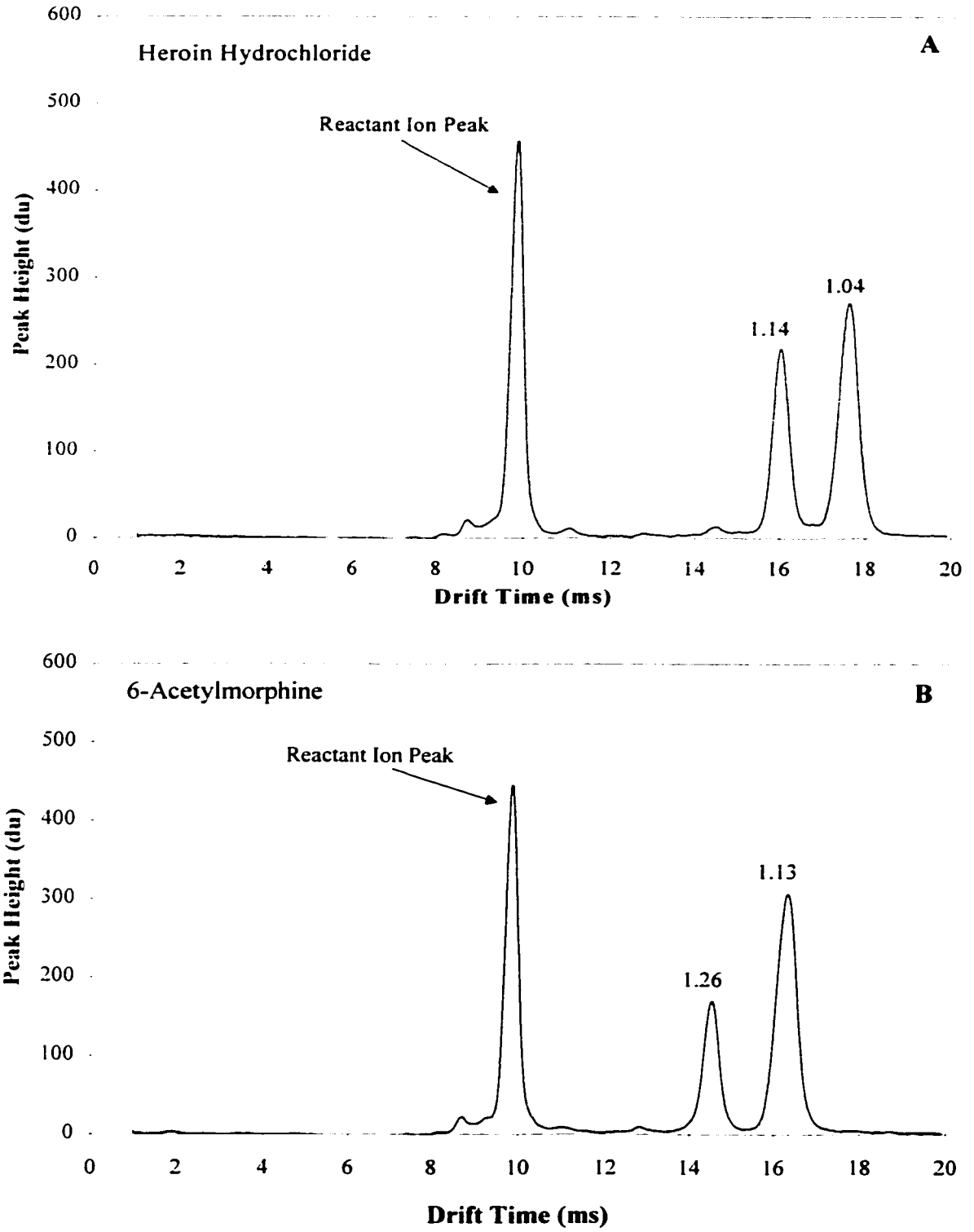


Fig. 58. IMS mobility spectra of (A) heroin hydrochloride and (B) 6-acetylmoprhine.

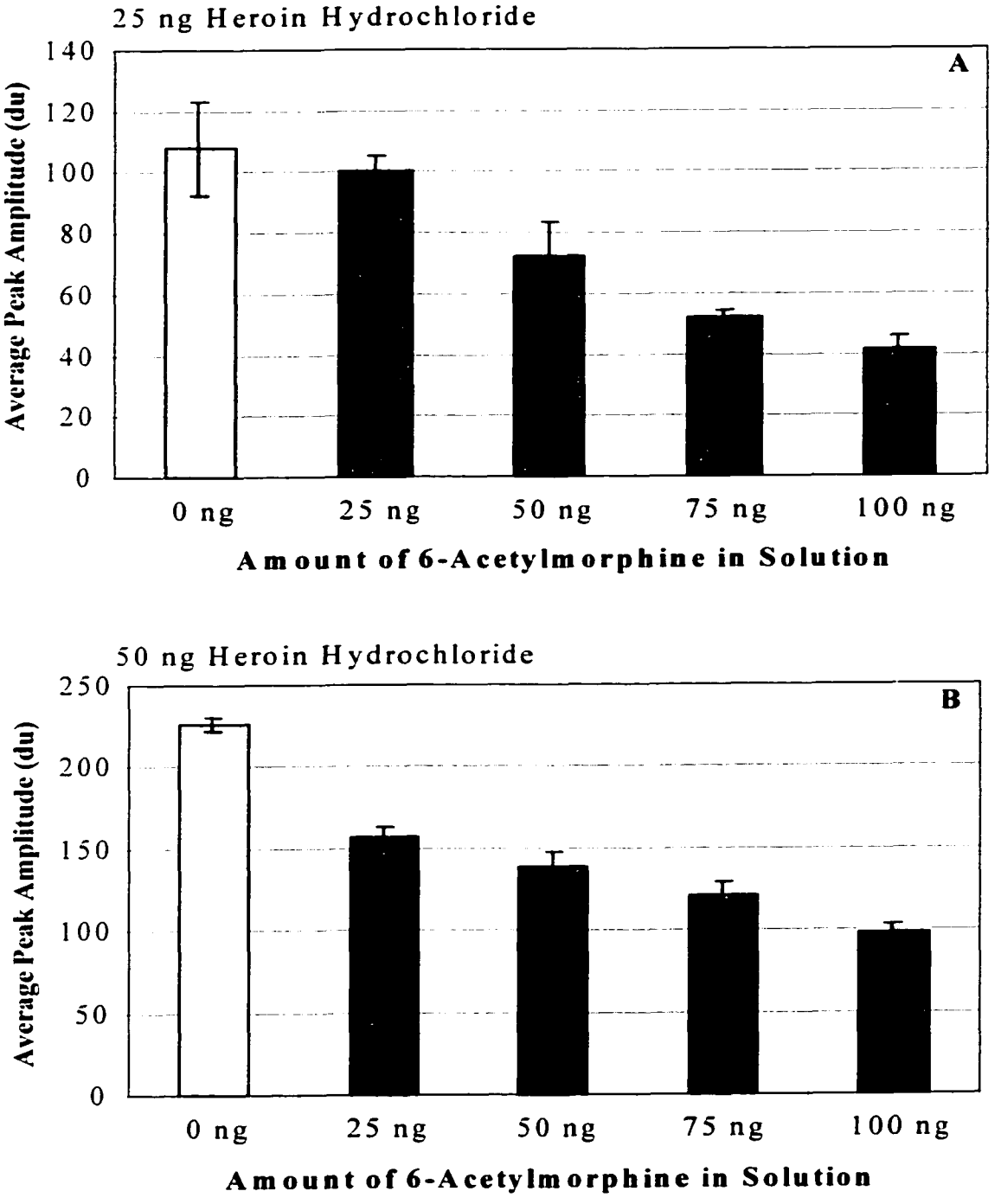


Fig 59. Effects of different amounts of 6-acetylmorphine on the average IMS amplitude of the heroin hydrochloride peak with K_o, 1.04 $cm^2V^{-1}s^{-1}$. (A) 25 ng heroin hydrochloride (B) 50 ng heroin hydrochloride).

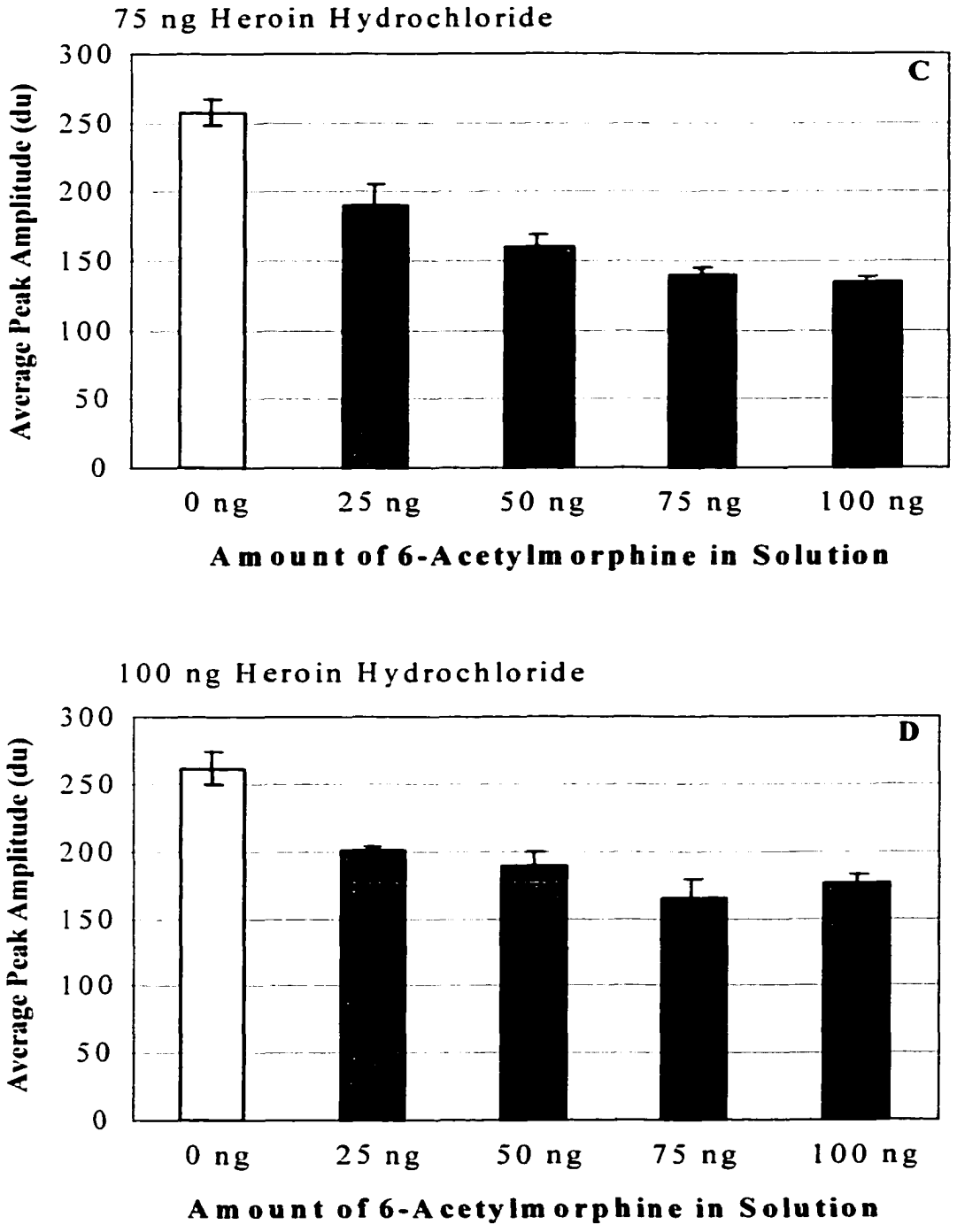


Fig 59. Effects of different amounts of 6-acetylmorphine on the average IMS amplitude of the heroin hydrochloride peak with K_o, 1.04 $cm^2V^{-1}s^{-1}$. (C) 75 ng heroin hydrochloride (D) 100 ng heroin hydrochloride).

It was interesting to note that as IMS saturation levels were approached (above 50 ng heroin hydrochloride), appreciable amplitude decreases were still apparent for the heroin peak (K_o, 1.04 $cm^2 V^{-1}s^{-1}$). For example, with 75 ng heroin hydrochloride, the amplitude measured in the presence of 100 ng 6-acetylmorphine indicates only 25 ng heroin as determined from the heroin IMS calibration curve (Fig. 60). The results imply there is competition between heroin and 6-acetylmorphine for charge with 6-acetylmorphine showing higher proton affinity.

Using the same data but processing it differently allows one to examine any effects of varying amounts of heroin hydrochloride on the amplitude of the 6-acetylmorphine peak (K_o, 1.26 $cm^2 V^{-1}s^{-1}$). The amplitude changed slightly from sample to sample within a series. However, when the amplitudes from all four samples in a particular series were averaged, the values remained approximately the same, regardless of the amount of heroin hydrochloride present.

Effects of Animal Tissues on the IMS Analysis of Heroin and Morphine. An *in vitro* study of heroin in the presence of animal tissues, was conducted to better understand results obtained in the IMS analysis of tissues from animals that received heroin. This research, conducted by Andrea Chambliss of Dr. Barbara Hargrave's research team, was important since IMS showed no heroin metabolites and only a very weak K_o of 1.04 $cm^2 V^{-1}s^{-1}$ for heroin (Fig. 61). Plasma samples from these studies showed no peaks for either heroin or its metabolites (Fig. 62). *In vitro* studies were conducted in which concentrations from 1 ng to 200 ng of heroin hydrochloride were

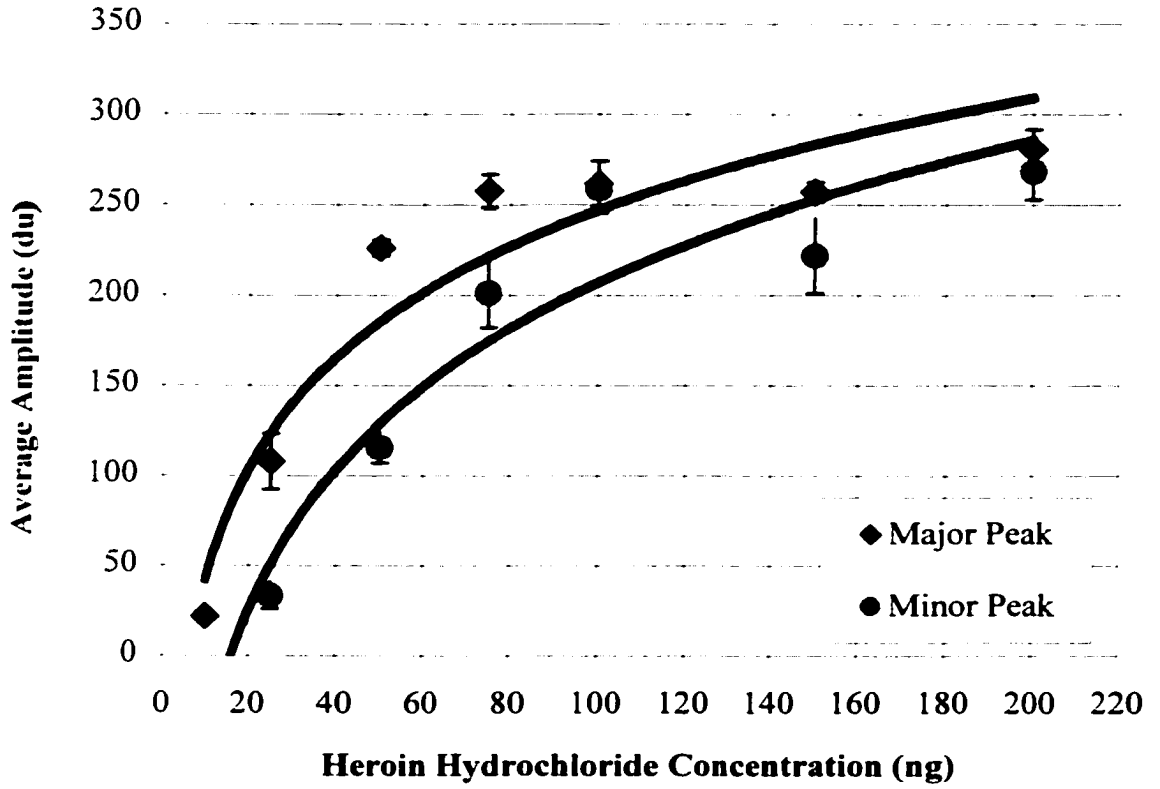


Fig. 60. IMS calibration curves for the major and minor peaks of heroin, K_o 1.04 and 1.14 $cm^2V^{-1}s^{-1}$, respectively.

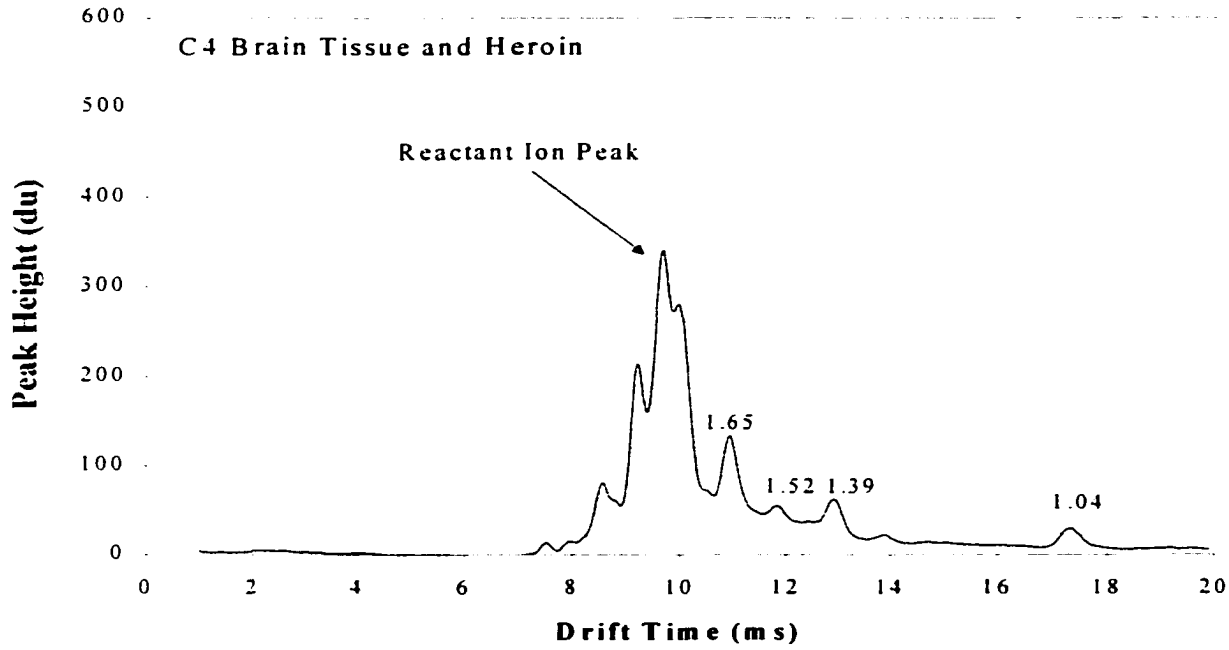


Fig. 61. IMS mobility spectrum of animal tissues harvested from animals that received heroin *in vivo*. The peak at K_o 1.04 $cm^2V^{-1}s^{-1}$ is attributed to heroin. Peaks for heroin metabolites are absent.

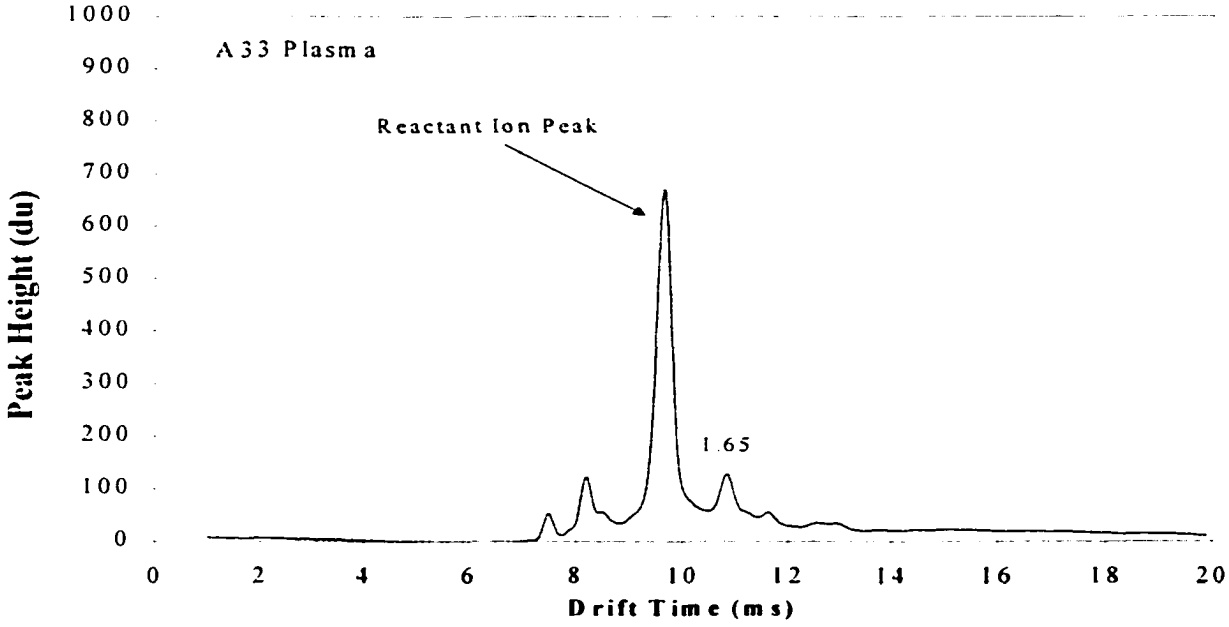


Fig. 62. IMS mobility spectrum of plasma from animals that received heroin *in vivo*. Peaks for heroin and its metabolites are absent.

added to animal tissue. A heroin signal was not observed until 200 ng were added (Table 16).

Table 16
Ion peak amplitudes for heroin (K_o, 1.04 $cm^2V^{-1}s^{-1}$) alone its mixtures with C4 brain tissue

Heroin hydrochloride (ng)	Heroin peak amplitude (du)	Heroin and brain tissue peak amplitude (du)
0	0	0
1	36	0
5	90	0
10	139	0
15	154	0
20	211	0
30	237	0
40	223	0
50	273	0
75	287	0
100	279	0
200	N/A	21

The heroin results led to experimentation with morphine. A mobility spectrum of morphine is shown in Fig. 63. In mixtures with animal tissue, 8000 ng of morphine were needed before an IMS signal was evident (Fig. 64). The results parallel those found with mixtures of animal tissue and heroin.

Effects of Vegetable Protein on the IMS Analysis of Heroin. Proteins from a vegetable source (soy) (Protein 94) were also examined for possible retardation of the IMS signal of heroin. This was an attempt to shed more light on the heroin results given above. The IMS mobility spectrum of Protein 94, is shown in Fig. 65. No peaks

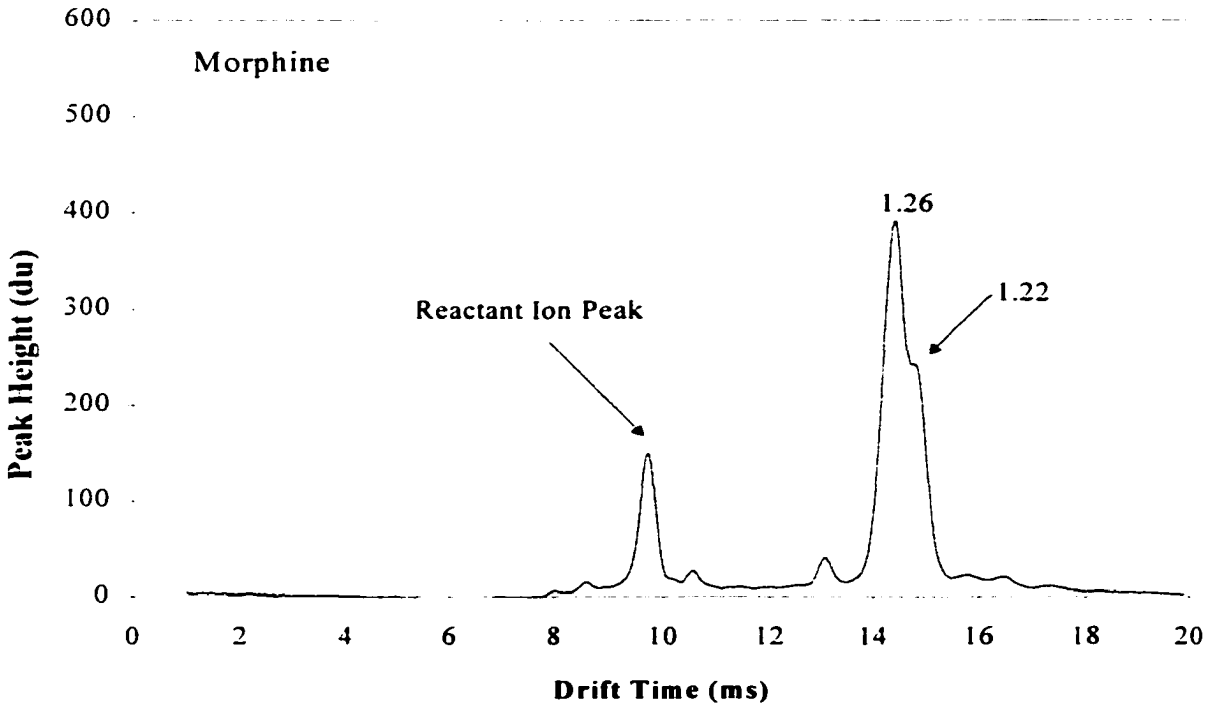


Fig. 63. IMS mobility spectrum of 8000 ng of morphine.

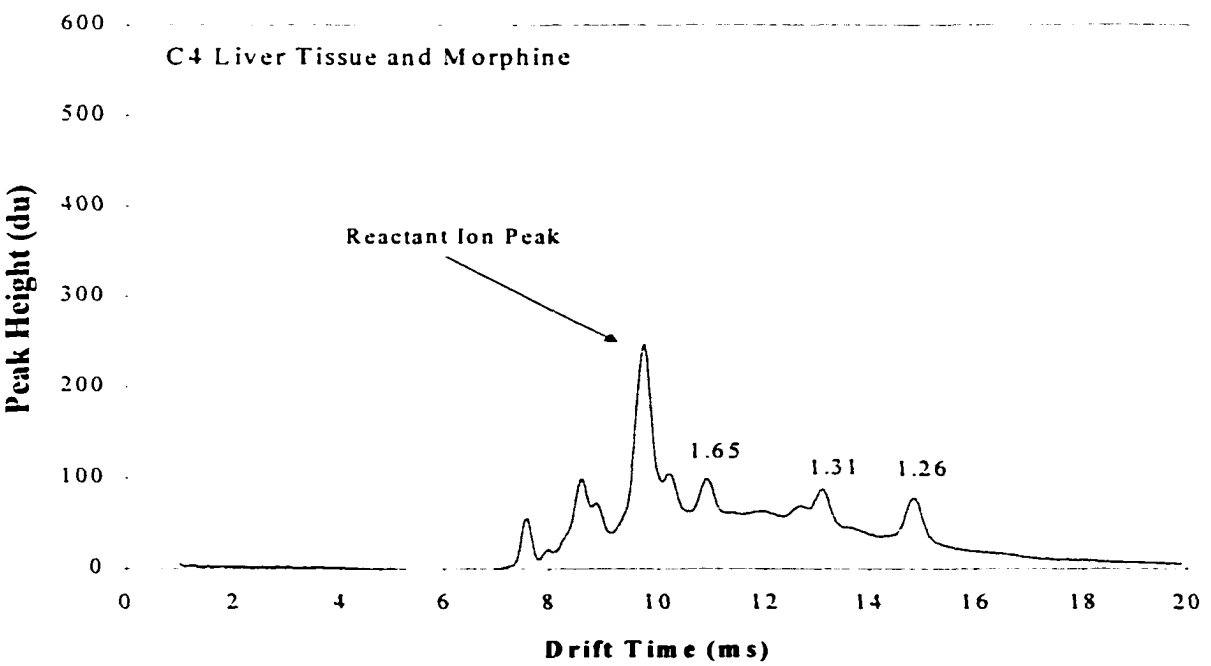


Fig. 64. IMS mobility spectrum liver tissue exposed *in vitro* to 8000 ng of morphine.

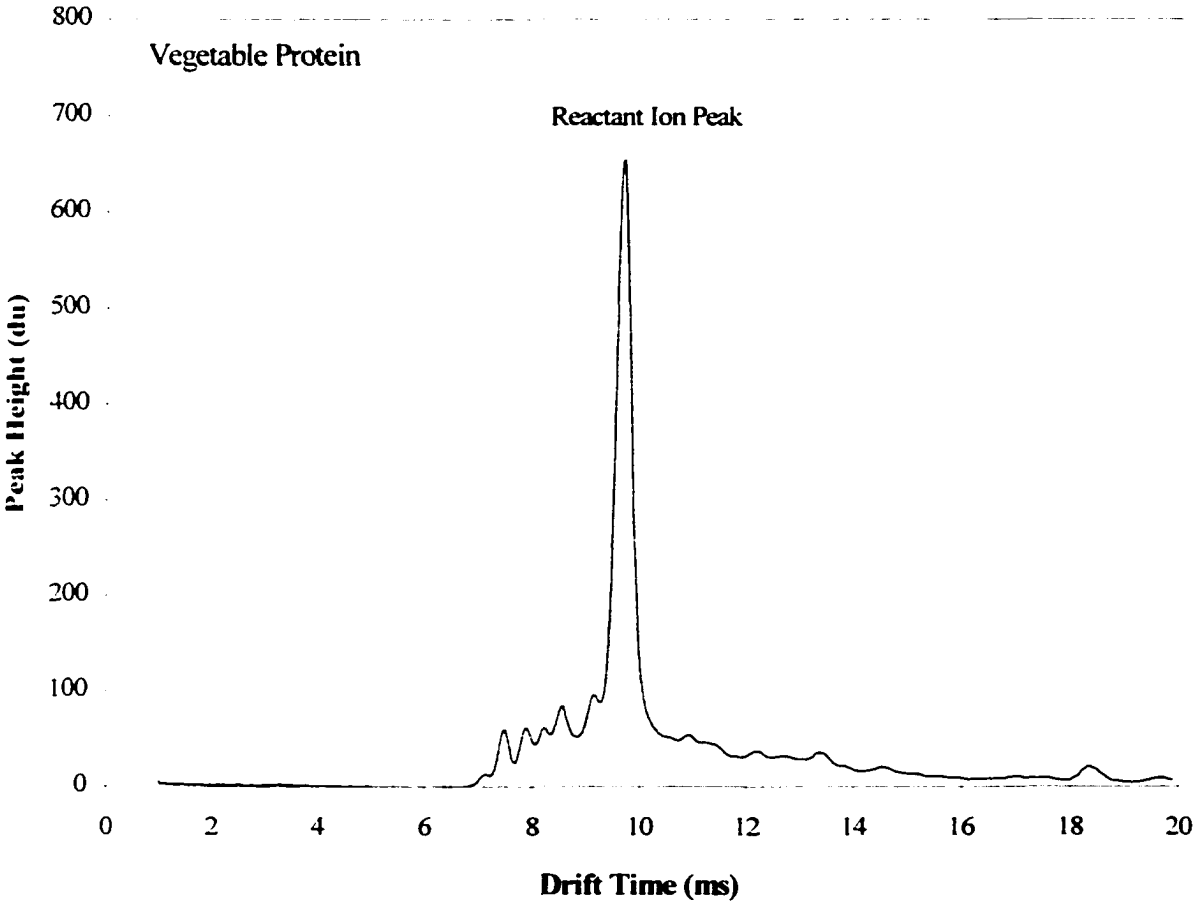


Fig. 65. IMS mobility spectrum of vegetable protein (Protein 94).

were evident other than the reactant ion peak. When attempting to analyze 100 ng of heroin in the presence of vegetable protein, both heroin peaks were retarded.

Fig. 66 illustrates the effect of vegetable proteins on 25 ng, 50 ng, 75 ng, and 100 ng of heroin hydrochloride. The K_o, 1.04 $cm^2V^{-1}s^{-1}$ peak is significantly retarded. The K_o, 1.14 $cm^2V^{-1}s^{-1}$ is also retarded (and in some cases absent) but is not shown in Fig. 66.

Effects of Glycine Peptides on the IMS Sensitivity of Heroin. Another study which examined the effects of proteins on the IMS of heroin, was with glycine peptides. Glycine was chosen because it is a simple amino acid and should give less complicated proteins. Glycine and its analogs may be useful models of the interaction of other proteins with heroin. The IMS mobility spectra of the glycine peptides are shown in Appendix B. Figs. 67A and 67B illustrate the effects of glycine peptides on the IMS sensitivity of heroin (K_o, 1.04 $cm^2V^{-1}s^{-1}$). Glycine itself almost completely retards IMS peaks from 25 ng of heroin. A small signal (18 du) remains when 50 ng of heroin is analyzed with glycine. It was interesting to find less retardation with diglycine, triglycine, tetraglycine, and pentaglycine. However, as the number of repeating glycine units increases, the retardation increases (Fig. 67A). When heroin is analyzed with hexaglycine, the IMS heroin signal decreases. Higher molecular weight polyglycines also retards the IMS signal of heroin, but not as completely (Fig. 67B). The mobility spectra of the analyses of heroin in the presence of glycine peptides are shown in Appendix B.

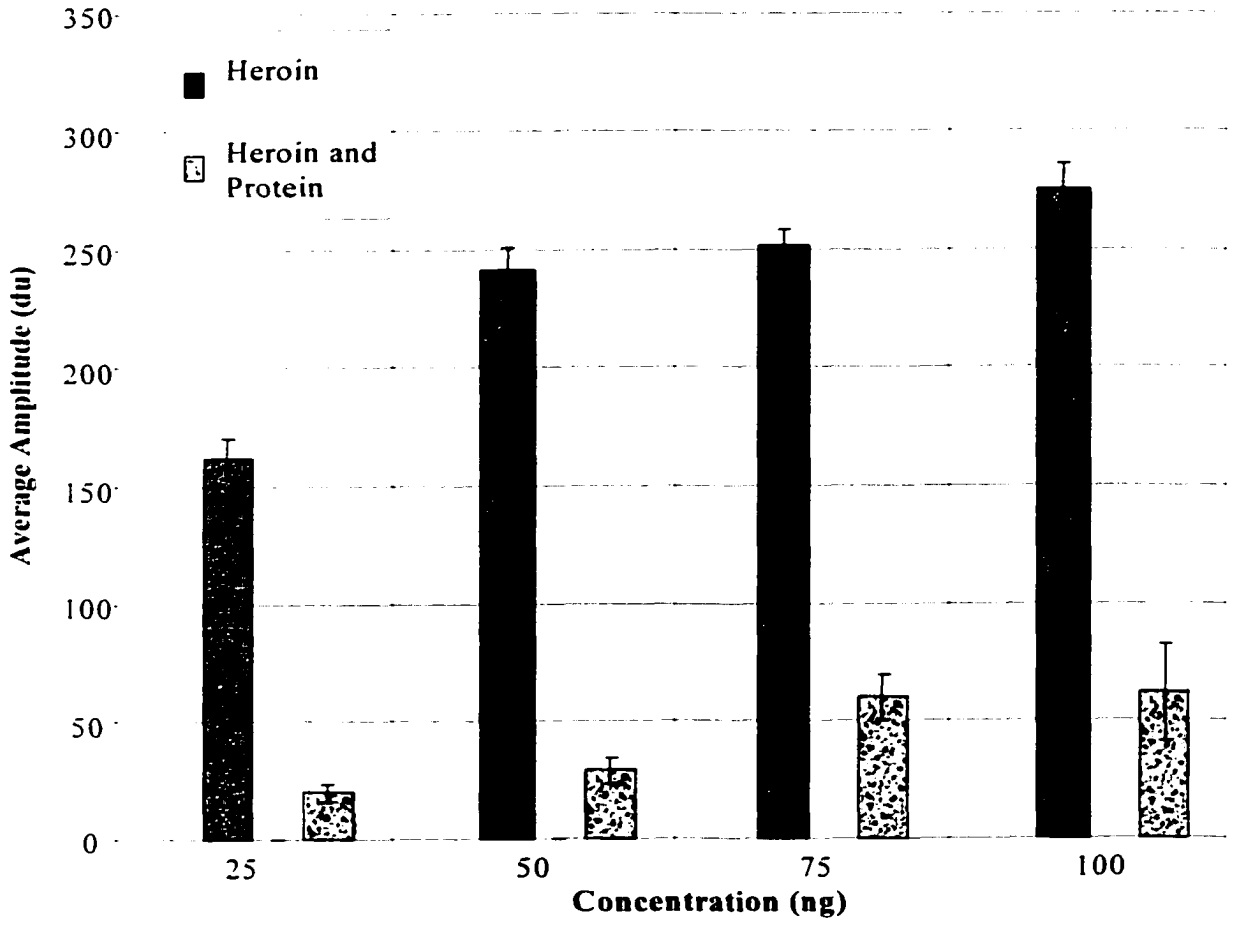


Fig. 66. Effect of vegetable protein (Protein 94) on the IMS peak amplitude (K_o, 1.04 $cm^2V^{-1}s^{-1}$) of heroin hydrochloride.

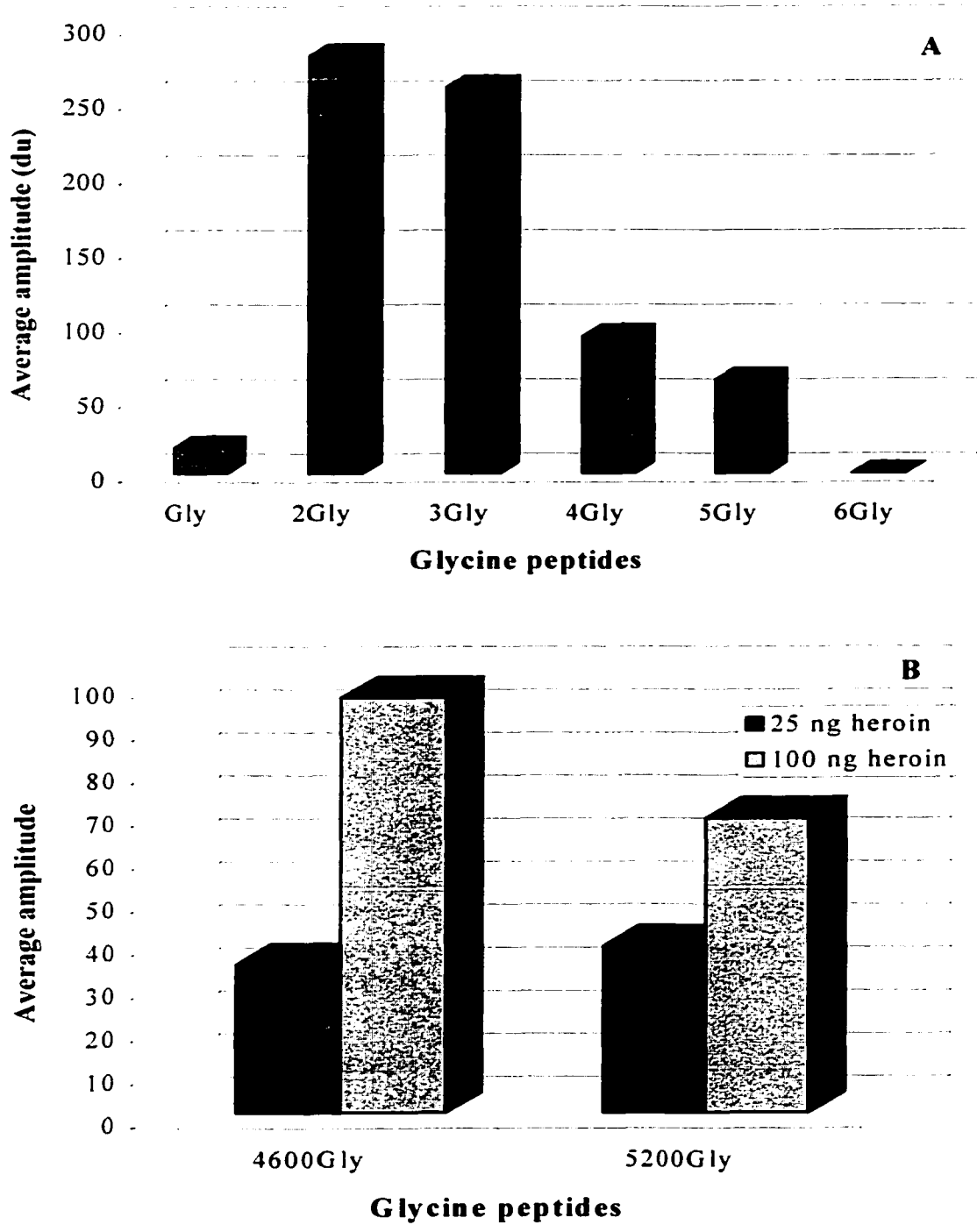


Fig. 67. The effect of (A) glycine and low molecular weight analogs, and (B) glycine polymers on the IMS peak amplitude of heroin hydrochloride (K_o,1.04 $cm^2V^{-1}s^{-1}$).

Discussion and Conclusions

It is clear that IMS will continue to be an important tool for the detection, identification and semi-quantitative analysis of contraband drugs and their reaction products. However, it is apparent that use of IMS in semiquantitative analysis may be limited depending on the specific circumstances.

Cocaine and its Decomposition Products

Mixtures of AEME and EME standards led to a single IMS peak which could not be resolved. Amounts of either AEME or EME may be underestimated when the other compound is present. IMS does not appear to be useful in estimating total AEME-EME.

Caution needs to be taken in reaching conclusions on compound identification and analysis with IMS when both AEME and EME may be present. Unexpected results may be explained on the basis of the similar K_o values for AEME and EME, differences in IMS sensitivity, and differences in proton affinity.

The IMS sensitivity to cocaine can be increased by the addition of AEME. AEME decomposition/reaction in IMS does not produce ion masses similar to those observed in the IMS of cocaine. Therefore, the mechanism of signal enhancement is not clear. Possible factors in observing the sensitivity increase for cocaine include: an increase of cocaine vapor pressure, altered vapor phase chemistry in the ionization chamber, modification of the Teflon surface, cooling effects by AEME on the desorber, and/or the neutralization of cocaine by AEME.

Heroin and its Metabolites

6-Acetylmorphine was found to interfere in the IMS semiquantitative analysis of heroin hydrochloride, but not in its detection and identification. The presence of 6-acetylmorphine significantly decreases the IMS amplitude of the heroin peak (K_o, 1.04 $cm^2V^{-1}s^{-1}$) leading to an underestimation of the amount of heroin hydrochloride actually present in the sample. The effects of heroin hydrochloride on the detection of the 6-acetylmorphine peak (K_o, 1.26 $cm^2V^{-1}s^{-1}$) are minimal. The same amount of 6-acetylmorphine is detected in those samples that contain heroin as those that do not. Semiquantitative analysis of heroin hydrochloride when 6-acetylmorphine is present may not be possible using IMS. Alternative methods of analysis need to be considered.

IMS is not the instrument of choice in detecting heroin and its metabolites in animal tissues and vegetable proteins at low concentrations. Concentrations less than 200 ng of heroin and 8000 ng of morphine were not observed in IMS analysis in the presence of animal tissues. Concentrations less than 100 ng of heroin did not result in peaks in the IMS spectra of heroin analyzed in the presence of vegetable proteins. Simple proteins such as glycine peptides also greatly retard the IMS signal of heroin. It is unclear why the IMS signal of heroin is completely retarded with glycine and hexaglycine, but not with diglycine, triglycine, tetraglyice, pentaglycine or polyglyinces. The mechanism behind IMS signal quenching of heroin is most likely due to either a decrease in the volatility of the heroin (e.g. by tissue interactions) or by ion chemistry in which volatile decomposition products of polyglycine neutralize heroin's positive ions.

It is speculated that a major cause of the heroin peak retardation is non-specific interaction which has not been noted previously in literature to the best of our knowledge. GC/MS was used to analyze 0.5 mg animal tissue mixed with 100 ng of heroin placed in the GC liner. The retention times for heroin and 6-acetylmorphine are 13.2 and 12.5 minutes, respectively. A weak heroin peak was seen in the GC/MS chromatogram. Data from this study suggests that the peak quenching of heroin in the presence of animal tissues is influenced by a combination of non-specific interactions and ionization chemistry. However, it is important to note that the IMS conditions (temperature, etc.) could not be exactly duplicated in the GC/MS experiments.

CHAPTER VII

PROGNOSIS ON THE USE OF ION MOBILITY SPECTROMETRY IN CLINICAL CHEMISTRY

Introduction

Criteria for selecting instruments for the clinical laboratory, are listed in Table 3 (Chapter I). IMS offers many advantages related to these criteria and could be used in clinical laboratories and clinical research, especially in screening applications. Although, IMS has some limitations, its technology is constantly being improved with a view toward expanding applications. IMS has typically been used for field screening for non-biological agents, but the technology has advanced to the point that it is very timely to consider uses in different areas, one of which is clinical chemistry.

The need for increased effectiveness in health care diagnostics has created a higher demand for a greater variety chemistry of services, more rapidly and at lower costs. The selection process of evaluating an instrument for clinical use considers economic aspects, testing rate, methodology, accuracy and precision [36]. The amount of sample consumed per test, methodology, the instrument's stat (short turn around time) capability and user-friendliness are very important criteria. The manpower requirements, personnel training requirements, selectable tests for profiling, computer compatibility, the ability of the instrument to flag anomalies, and the sample identification system employed, are also important considerations. Economic considerations include operating and acquisition costs, reagent and disposable item costs, preventive maintenance costs, manpower costs, and downtime costs. In order for an instrument to be considered for a clinical laboratory, the principles of operation of

the instrument should to be well defined and potential interferences must be understood. The assessment of analytical performance is conducted through evaluations to assure that the sensitivity, specificity, accuracy, and precision claimed can be achieved. In, addition, the instrument should be rugged and provide adequate operator manuals. IMS offers many advantages in most of these criteria, especially those that are cost related.

Benefits and Limitations

A review of IMS technology versus the clinical chemistry technologies currently being utilized and the needs of the clinical laboratory brought out the potential benefits to be gained from the use of IMS. The major benefits are expected to be:

- Cost-effectiveness
- Ability to meet data objectives for rapid screening or semiquantitative analysis
- Multianalyte capability
- High level of user friendliness
- Minimum training requirement
- Low level of operator expertise
- Portability
- Ruggedness
- Minimum space requirements

Advantages of simplicity, portability, low cost, and multianalyte capability offer much promise in meeting performance objectives for a variety of analytes in scenarios involving screening and semiquantitative analysis. IMS involves simple techniques that can be applied easily in screening compounds with low vapor pressures such as would

be encountered in the clinical laboratory. This would allow screening for compounds of interest before utilizing sophisticated, time consuming and more expensive techniques such as GC-MS. Little training would be needed with IMS. Smaller or less-equipped laboratories can especially benefit from screening by minimizing the number of samples needed to be sent to reference laboratories.

IMS offers the potential of detecting target analytes in the timely, efficient and cost effective manner required in the clinical chemistry setting. Rapid analysis, coupled with high sensitivity and selectivity, makes IMS technology an ideal candidate to explore in meeting clinical chemistry needs. Relatively low cost and low consumables may allow large volume screening with the potential for automation.

The use of IMS in clinical chemistry will not be without limitations. Hill and Simpson [22] described potential problems of using IMS for field screening applications. Important limitations include competitive ion/molecule reactions, low resolution, a limited dynamic response range, ease of contamination, and use of a radioactive ionization source, ^{63}Ni. Currently, researchers are focused on providing solutions to IMS limitations. For example, ^{63}Ni has many advantages as an efficient ionization source, as mentioned earlier, but it also has drawbacks due to its radioactivity. Clark, et.al., [25] have reported a surface ionization source as an alternative to ^{63}Ni. This non-radioactive detector is a pulsed corona discharge ionizer. The study reports identical ion chemistry and comparable spectral responses with the corona source and a ^{63}Ni source in the same instrument.

Other examples of improvements in IMS technology include coupling extraction techniques, such as solid phase microextraction (SPME) with IMS [50]. This method

utilizes SPME to sample the headspace of solids and liquids by sorbing compounds on a stationary phase coated on a fused silica fiber. This paper also reports that SPME can be conveniently used in the IMS analysis of cocaine vapors. SPME-IMS has been applied in our laboratory in confirmation with the Barringer IONSCAN 400 to bypass thermal degradation problems, such as the decomposition of cocaine to AEME. This illustrates the inherent flexibility of IMS technology to solve operating problems with simple changes of technique. Another example of the flexibility IMS exhibits is illustrated in an article by Homstead and Poziomek [51]. In this case, research was performed to examine the chemistry of contraband drugs in the sample chamber of the IONSCAN 400 by manually controlling the flow of the air (carrier gas) while the sample is being heated. This technique provided information on the stability and decomposition products of drugs at various temperatures on different surfaces. The use of different drift gases to enhance the separation of compounds using IMS has been recently demonstrated by Asbury and Hill [52]. Different drift gases (helium, argon, nitrogen, and carbon dioxide) were chosen to separate compounds with similar mobility constants. Although, the current limitations favor the use of IMS for screening, rather than quantitative work, these limitations are rapidly being overcome. It is predicted that IMS will be used in quantitative analysis of complicated biological molecules in the near future.

Benefits and Limitations of IMS in the Present Research

It has been shown that IMS can be used in the characterization of animal tissues, identification of drugs and metabolites in tissues, and in various clinical chemistry applications, such as analysis of anesthetics, controlled substances, and metabolites.

cocaine, EME, and heroin [1]. The high sensitivity and selectivity of the IMS allows specific chemicals to be detected at nanogram levels and provides excellent qualitative data. The analysis procedures and the operation of the instrument were easy to understand and implement. The durability of the instrument made maintenance procedures simple and left few problems requiring repair. When there was a need for troubleshooting, the technical support received from Barringer was always timely and efficient. In addition, the IMS software allowed data to be easily accessed for processing. In identifying factors which could influence the IMS detection of contraband drugs, the ion chemistry observed provided data on novel interactions between compounds. The information contained in the thesis opened many windows for future research, e.g., medicinal applications. Expense of consumables (e.g.,Teflon membranes) were minimal. Also, the IMS procedures generated little waste.

Disadvantages included the ion mobility proneness to contamination. When this was encountered, the potential for data error increased as well as created the need for long clearance times. Another limitation was the relatively small dynamic range. The linear range for calibration curves often did not extend beyond 50 ng. Low separation efficiency, such as the case with AEME and EME, and competition in ion chemistry caused some difficulty in interpreting data. However, on the overall, IMS provided reproducible, quality data in a timely, efficient manner.

Potential of IMS with Current Clinical Chemistry Techniques

The potential to couple IMS with techniques used in clinical chemistry has already been established with the design of Fourier-transform (FT)-IMS [22], ion trap (IT)-IMS [53], IMS-MS, GC-IMS, and SPME-IMS. Pursuing tandem technology in

clinical chemistry will expand capability, but usually with much higher cost (not true with SPME-IMS) IMS has been used to analyze most of the drugs that are routinely analyzed using GC or GC/MS. Other instruments used for drug detection include the HPLC. IMS can also be interfaced with this instrument to provide substance confirmation and quantitative data. IMS may also play a role in the development of novel immunoassays techniques. Most immunoassays rely on the detection of the product of the antibody-antigen complex. Methods could be developed using an IMS detector with appropriate choices of detection.

IMS has mainly been used to detect compounds with high vapor pressures. Many of the drugs analyzed in the clinical laboratory have this characteristic. A number of the analytes sent to the laboratory for diagnostic testing [54] could be screened using IMS. The fast detection of narcotics in human hair, urine, and saliva using IMS has already been reported [8]. In addition, the preliminary screening using IMS of patients arriving with drug overdoses at hospital emergency room has also been reported [1]. Table 17 lists IMS data for prescription and illicit drugs and the method currently being used to analyze these substances in the clinical laboratory. This table further illustrates the potential of incorporating IMS in clinical chemistry. Ammonia, analyzed by spectrophotometric techniques, caffeine, detected by HPLC, and acetone, determined by an enzymatic assay, also have the potential to be analyzed using IMS technology [55]. IMS has also been used to detect the hormones, testosterone and progesterone, which are currently determined by immunoassay in the clinical laboratory [22]. However, it is important to understand that one ion reaction scheme will not be applicable to all analytes. IMS can be tuned to many analytes using different reactant

Table 17
K_o values and clinical chemistry assay used to characterize prescription and illicit drugs

Drug	Reported K_o of Major Ions[1] $cm^2V^{-1}s^{-1}$	Clinical Chemistry Technique [55]
Cocaine	1.16	Immunoassay, GC/MS [49]
Opiates		
Heroin	1.04, 1.14	Immunoassay, GC/MS [49]
Morphine	1.22	
Codeine	1.18, 1.21	
Barbiturates		
Phenobarbital	1.44	Capillary GC
Pentobarbital	1.38	
Amobarbital	1.36, 1.53	
Secobarbital	1.31, 1.48	
Amphetamine	1.66	Immunoassay, GC/MS
Methamphetamine	1.63	
Cannabinoids		
THC	1.04	GC/MS
Benzodiazapines		
Chlordiazepoxide (Librium)	1.18	Immunoassay, GC/MS, HPLC
Diazepam (Valium)	1.21	
Flurazepam (Dalmane)	1.03	
Lorazepam (Ativan)	1.19, 1.22	
Oxazepam (Serax)	1.23, 1.28	
Nitroglycerine	1.32	Enzymatic Assay
Amitriptyline (Elavil)	1.19	GC with a nitrogen/ phosphorus detector
Acetominophen	1.70, 1.76, 1.97	Immunoassay

ions. The choice of reactant ion is defined by the analytical objective.

The clinical-related research efforts from this thesis illustrate that IMS is an important technology to pursue in meeting specific objectives. A number of research

opportunities have surfaced as a result of the research efforts. One is the apparent ability of IMS to detect interactions between tissues and drugs. Continued research may lead to convenient methods to study the mechanism of drug action. The identification of IMS peaks inherent to animal tissues may help to develop novel clinical assays for the rapid detection of disease markers.

A recent report indicates that a simple protein test may better predict heart disease than the current methods to screen for cholesterol [56]. Researchers were using the detection of C-reactive protein (CRP), levels to identify person prone to developing heart disease. Another recent finding indicates that beta-amyloid and tau proteins tangles and causes sticky plaque in brain nerve fibers an explanation of Alzheimer's disease [57]. Our research has shown that IMS may be used to characterize and detect interactions with proteins. Expanding the research may lead to simple tests which can provide useful clinical information in a rapid, inexpensive, and unique manner. The prognosis for IMS use in clinical chemistry is judged to be high.

CHAPTER VIII

SUMMARY

The major goal of this thesis research was to examine IMS as a rapid screening tool for specific application to clinical chemistry research and laboratory use. Methodology was developed for target analytes representing several classes of physiologically active substances including anesthetics, and illicit drugs and their metabolites. IMS characteristics of animal tissues and various compounds such as amino acids and proteins were determined.

QA/QC procedures were developed for quality data objectives relating to use of IMS in clinical chemistry studies involving qualitative screening and semiquantitative analyses. Criteria were established for assessing the precision and accuracy of data in the IMS positive ion mode, using cocaine standards. Cocaine was found to be a useful indicator of instrument contamination and malfunctions through a reaction involving elimination of benzoic acid to form AEME. Though most of the IMS research was performed in the positive mode, QA/QC procedures were also developed in the negative ion mode using TNT.

It was found that animal tissues harvested from rabbits can be characterized easily using IMS. It was found that two positive ion peaks with the same K_o values appear in IMS mobility spectra of the majority of the tissues examined. These peaks disappear as the IMS desorption temperature is lowered, leaving the IMS window completely open for studies of any drugs and/or metabolites distributed in the tissues. The mobility spectra were also found to contain peaks assigned to the anesthetics, Rompun and Ketaset, and to heparin, which was used with all of the test animals. This

provided excellent evidence of the potential use of IMS in clinical chemistry. The peaks innate to the tissues were not identified. They varied in relative intensity between organs. The ion peaks may be compounds inherent to the tissue or the result of thermal decomposition in the IMS desorption chamber. The IMS of tissues represents an excellent area for future research and may lead to simple techniques to differentiate healthy from diseased tissues.

A number of interesting enhancement and retardation effects were discovered that affect the utility of IMS in clinical chemistry. They need to be kept in mind in IMS method development to gain maximum benefits from IMS technology. An example is the attempt to analyze two cocaine reaction products (AEME, and the biological metabolite, EME) in the presence of each other. These compounds are similar in molecular weight and structure, can be analyzed semiquantitatively using IMS and have different K_o values. However, when the compounds are in the same mixture, the ion peaks overlap to the extent that they can not be deconvoluted. Also, the peak amplitude is much less than expected based on amplitudes from individual calibration curves of AEME and EME. It was also found in IMS studies performed with heroin and its metabolite, 6-acetylmorphine, that this metabolite interferes with the semiquantitative analysis of heroin, but not its identification. Such problems can be solved if important to the data objectives. However, they serve to bring out the importance of examining effects of expected components on each other when developing IMS methods.

Benzoylecgonine, another important metabolite of cocaine, was found to decompose using IMS, most likely with the elimination of benzoic acid to form ecgonidine. This occurred during a period in which it was suspected, based on QA/QC

procedures, that the instrument was contaminated with a material that catalyzed/enhanced the decomposition. It served to reaffirm the need for QA/QC procedures to be in place.

A nonspecific interaction of heroin an morphine with animal tissues and different proteins was discovered using IMS This was part of collaborative experiments with the Department of Biological Sciences to analyze tissues harvested from animal treated with heroin. This discovery may shed additional light on the physiological mechanism of opiates. It opens up an interesting use of IMS in research and a new method of detecting interactions between tissues/proteins and physiologically active substances.

There are advantages and disadvantages in the use of IMS in clinical chemistry. As the technology improves, especially in sample handling and separation using a simple combination of SPME and/or GC with IMS, more and more applications will unfold. The thesis research provides an insight of what is possible.

REFERENCES

[1] G.A. Eiceman, Z. Karpas, Ion Mobility Spectrometry, CRC Press, Boca Raton, Florida, 1994, pp. 2-18.

[2] M.J. Cohen, F.W. Karasek, Int. J. Chromatogr. Sci. 8 (1970) 330-338.

[3] F.W. Karasek, Anal. Chem. 46 (1974) 710A-720A.

[4] G.A. Eiceman, Crit. Rev. Anal. Chem. 22 (1991) 17-36.

[5] E.J. Poziomek, G.A. Eiceman, Envir. Sci. Tech. 26 (1992) 1313-1316.

[6] J. Stach, J. Flachowsky, M. Brodacki, H.R. Döring, Field Screening of Volatile Organochlorine Compounds Using Ion Mobility Spectrometry, in: Proceedings of 4th International Symposium on Field Screening Methods for Hazardous Wastes and Toxic Chemicals, Las Vegas, Nevada, 1995, pp. 1046-1052.

[7] Y.T. Long, Y. Guo, M.Q. Lu, Anal. Chem. 70 (1999) 347-352.

[8] L. Cacciacarro, L.L. Danylewych-May, L. Kim, Fast Detection of Narcotics in Human Hair, Urine, and Saliva by Ion Mobility Spectrometry, in: Proceedings of Counterdrug Law Enforcement: Applied Technology for Improved Operational Effectiveness International Technology Symposium, Part 1, Nashua, New Hampshire, 1995, pp. 5-27.

[9] L.M. Fytche, M. Hupe, J.B. Kovar, P. Pilon, Int. J. Foren. Sci. 37 (1992) 1550-1566.

[10] P. Pilon, P. Neudorfl, M. Hupe, A. Lawrence, Drug Vapors Sampling Methodologies: Application to Cargo Containers, in: Proceedings of Counterdrug Law Enforcement: Applied Technology for Improved Operational Effectiveness International Technology Symposium, Part 2, Nashua, New Hampshire, 1995, pp. 16-37 – 16-50.

[11] J.R. Jadamec, C. Su, S. Ridgon, L. Norwood, A Novel Approach to Increasing Cocaine Detection Confidence Utilizing Ion Mobility Spectrometry, in: Proceedings of the 3rd International Workshop on Ion Mobility Spectrometry, Galveston, Texas, 1994, pp. 229-237.

[12] G.E. Spangler, J.P. Carrico, S.H. Kim, Analysis of Explosives and Explosives Residues with Ion Mobility Spectrometry (IMS), in: Proceedings of the International Symposium on the Analysis and Detection of Explosives, Quantico, Virginia, 1983, pp. 409-413.

[13] D.D. Fetterolf, T.D. Clark, Int. J. Forensic. Sci. 38 (1993) 28-39.

[14] A.H. Lawrence, P. Neudorfl, Anal. Chem. 60 (1988) 104-109.

[15] F. Garofolo, F. Marziali, V. Migliozzi, A. Stama, Rapid Communications in Mass Spectrometry 10 (1996) 1231-1236.

[16] S.F. Hallowell, J.P. Davies, B.L. Gresham, Qualtitative/Semi-Quantitative Chemical Characterization of the Auburn Olfactometer in: A.H. Lawrence (Ed.), Cargo Inspection Technologies, SPIE 2276, San Diego, California, 1994, 437-448.

[17] E. Bender, A. Hogan, D. Leggen, G. Miskolczy, S. MacDonald, Int. J. Forensic Sci. 37 (1992) 1673-1678.

[18] H.H. Hill, Jr., W.F . Siems, R.H. St. Louis, D.G. McMinn, Anal. Chem. 62 (1990) 1201A-1209A.

[19] R.H. St. Louis, H.H. Hill, Jr., Crit. Rev. Anal. Chem. 21 (1990) 321-355.

[20] J.E. Roehl, Appl. Spectroscopy Rev. 26 (1991) 1-57.

[21] J.A. Reategui, Sensors 59 (1994) 14-20.

[22] H.H. Hill, G. Simpson, Field Anal. Chem. Technol. 1 (1997) 119-134.

[23] J.L. Baunbach, G.A. Eiceman, App. Spec. 53 (1999) 338A-342A.

[24] R.K. Ritchie, F.J. Kuja, R.A. Jackson, L.L. Danylewich-May, L.H. Kim, Improvements in Narcotics Detection Using Ion Mobility Spectrometry, in: Proceedings Tactical Technologies and Wide Area Surveillance International Symposium, Chicago, Illinois, 1993, pp. 273-282.

[25] A. Clark, P.D. Arnold, A.H. Brittain, R. Wilson, The Graseby NARCOTEC™ Narcotics Detector: a Non-Radioactive Version, in: Proceedings Harnessing Technology to Support the National Drug Control Strategy, Chicago, Illinois, 1997, pp. 17-23 - 17-33.

[26] J. Anderson, Anal. Chem. 71 (1999) 293-358.

[27] C.A. Burtis, E.R. Ashwood, Tietz Textbook of Clinical Chemistry, W.B. Saunders Company, Philadelphia, Pennsylvania, 1994, pp. 314-346.

[28] J. Anderson, Anal. Chem. 69 (1997) 165-230.

[29] J. Anderson, L. Coury, J. Leddy, Int. J. Anal. Chem. 70 (1198) 519R-526R.

[30] J. Janata, M. Josowiez, P. Vanysek, D. DeVaney Anal.Chem. 70 (1988) 179R-183R.

[31] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Science, 246, (1989) 64-69.

[32] M. Kara, F. Hillenkamp. Anal. Chem. 60 (1988) 2299-2304.

[33] L. Elci, U. Sahin, A. Ulgen, Trace Elem. Electrolytes 15 (1998) 106-111.

[34] P. Bermejo-Barrera, A. Pinerro-Morida, J. Moreda-Pinerro, A. Bermejo-Barrera, Fresenius J. Anal. Chem. 30 (1998) 193-199.

[35] A.A. Chambliss, The Effect of Illicit Drugs on the Cardiovascular and Hormonal System of Pregnant New Zealand White Rabbits, Master's Thesis. Old Dominion University, Norfolk, Virginia, May 1999.

[36] J.K. Taylor, Quality Assurance of Chemical Measurements, Lewis Publishers, Chelsea, Michigan, 1987, pp. 107-123.

[37] E.J. Poziomek, J. Homstead, S.H. Almeer, Quality Issues in Use of Ion Mobility Spectrometry, in: Proceedings on Enforcement and Security Technologies, SPIE 3575, Boston, Massachusetts, 1998, pp. 403-413.

[38] C.A. Burtis, E.R. Ashwood, Tietz Textbook of Clinical Chemistry, W.B Saunders Company, Philadelphia, Pennsylvania, 1994, pp. 585-588.

[39] Barringer Research Limited, Drugs Plasmagram Library, Rexdale, Ontario, Canada, 1995.

[40] Miles, Inc., Agriculture Division, Rompun Package Insert, Kansas, October 1992.

[41] Fort Dodge Laboratories, Inc., Ketaset Package Insert, Iowa, August 1994.

[42] S. Budavari, ed. The Merck Index: An Encyclopedia of Chemical, Drugs, and Biologicals, Merck & Co. Inc., Whitehouse Station, New Jersey, 1996.

[43] B.B. Guinn, Effect of Cocaine on Renin Angiotensin System: Cocaine Detection in Adult and Fetal Tissue and Adult Plasma Using Ion Mobility Spectrometry, Master's Thesis. Old Dominion University, Norfolk, Virginia, May 1997.

[44] R. Seymour, D.E. Smith, Guide to Psychoactive Drugs: An Up-to-the-Minute Reference to Mind-Altering Substance, Harrington Park Press, New York, New York, 1987, pp. 3-82.

[45] R.M. Julien, M.D., A Primer of Drug Action, W. H. Freeman and Co., New York, New York, 1988, pp. 40-145.

[46] The Problem of Chemical and Biological Warfare: CB Weapons Today, Stockholm International Peace Research Institute, Stockholm, Sweden, 1973, pp. 48-52.

[47] C.A. Burtis, E.R. Ashwood, Tietz Textbook of Clinical Chemistry, W. B. Saunders Company, Philadelphia, Pennsylvania, 1994, pp. 1115-1235.

[48] C.A. Burtis, E.R. Ashwood, Tietz Textbook of Clinical Chemistry, W. B. Saunders Company, Philadelphia, Pennsylvania, 1994, pp. 1200-1208.

[49] G.E. Orzechoroska, E.J. Poziomek, V. Tersol, J. Homstead, Evaluation of Solid-Phase Microextraction in Detection of Contraband Drug Vapors, in: P. Pilon and S. Burmeister (Eds.), Proceedings of Chemistry- and Biology-Based Technologies for Contraband Detection, SPIE 2937, Boston, Massachusetts, 1997, pp. 8-18.

[50] J. Homstead, E.J. Poziomek, Contraband-Drug Surface Chemistry at Nanogram Levels, in: P. Pilon and S. Burmeister (Eds.), Proceedings of Chemistry- and Biology-Based Technologies for Contraband Detection, SPIE 2937, Boston, Massachusetts, 1997, pp. 245-250.

[51] G.R. Ashbury, H.H. Hill, Jr., Anal. Chem., 72 (2000) 580-592.

[52] W. McGann, V. Bradley, A. Borsody, S. Lepine, A New High Efficiency Ion Trap Mobility Detection System for Narcotics and Explosives, in: Proceedings Cargo Inspection Technologies, SPIE 2276, San Diego, California, 1994, pp. 64-68.

[53] J.L. Kee. Handbook of Laboratory and Diagnostic Tests with Nursing Implications, Appleton & Lange, Norwalk, Connecticut, 1994, pp. 375-382.

[54] C.A. Burtis, E.R. Ashwood, Tietz Textbook of Clinical Chemistry, W.B. Saunders Company, Philadelphia, Pennsylvania, 1994, pp. 1155-1999.

[55] P.M. Ridker, C.H. Henekens, J.E. Buring, N. Rifai. N. Engl. J. Med. 342 (2000) 836-842.

[56] D.J. Selkoe, J. Am. Med. Assoc. 283 (2000) 1615-1622.

APPENDIX A

IMS MOBILITY SPECTRA OF C3 ANIMAL TISSUES

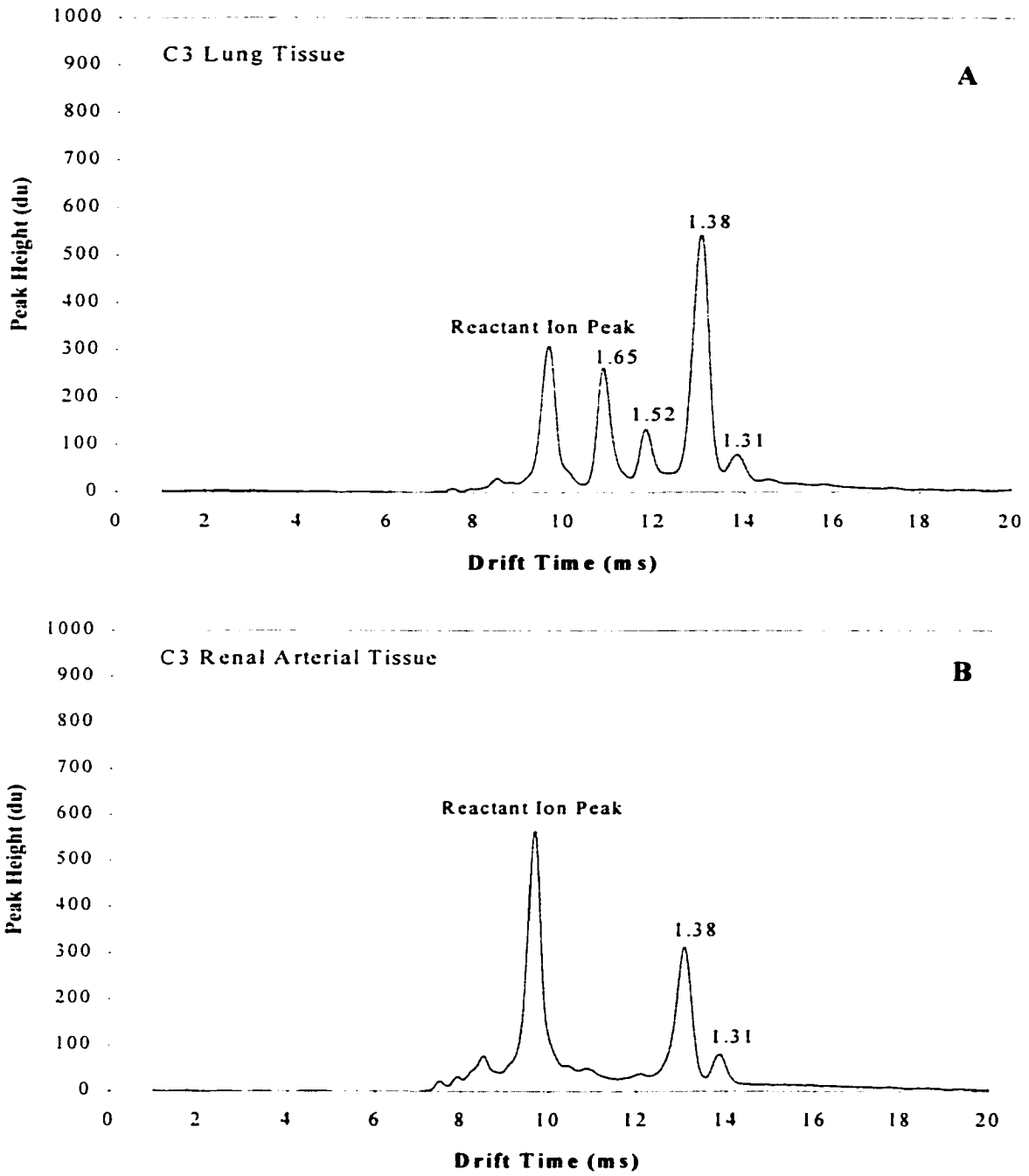


Fig. A-1. Positive mode IMS mobility spectra of (A) lung tissue and (B) renal arterial tissue from animal C3. This animal was given vehicle saline, anesthetic, and euthanasia in an acute study.

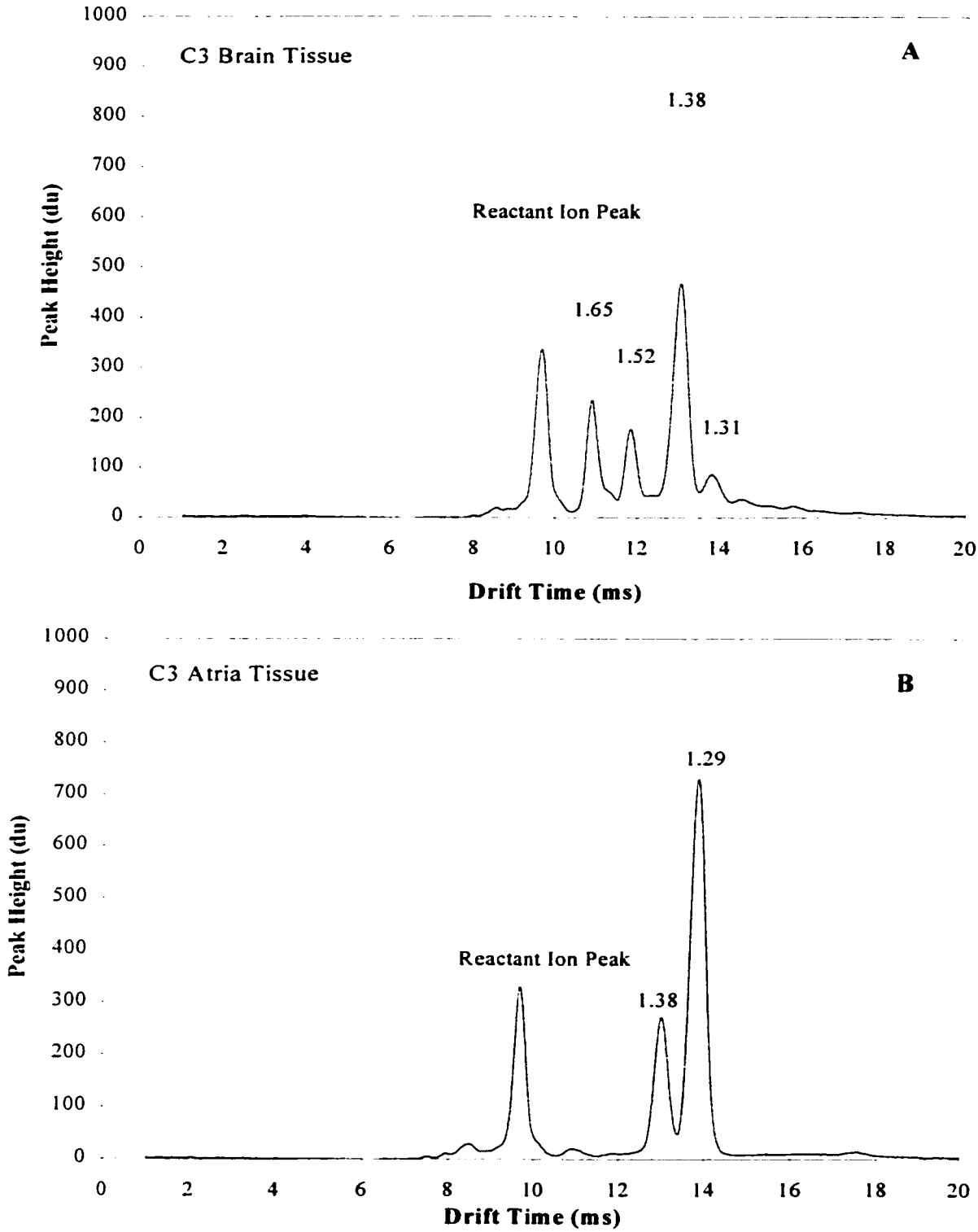


Fig. A-2. Positive mode IMS mobility spectra of (A) brain tissue and (B) atrial tissue from animal C3. This animal was given vehicle saline, anesthetic, and euthanasia in an acute study.

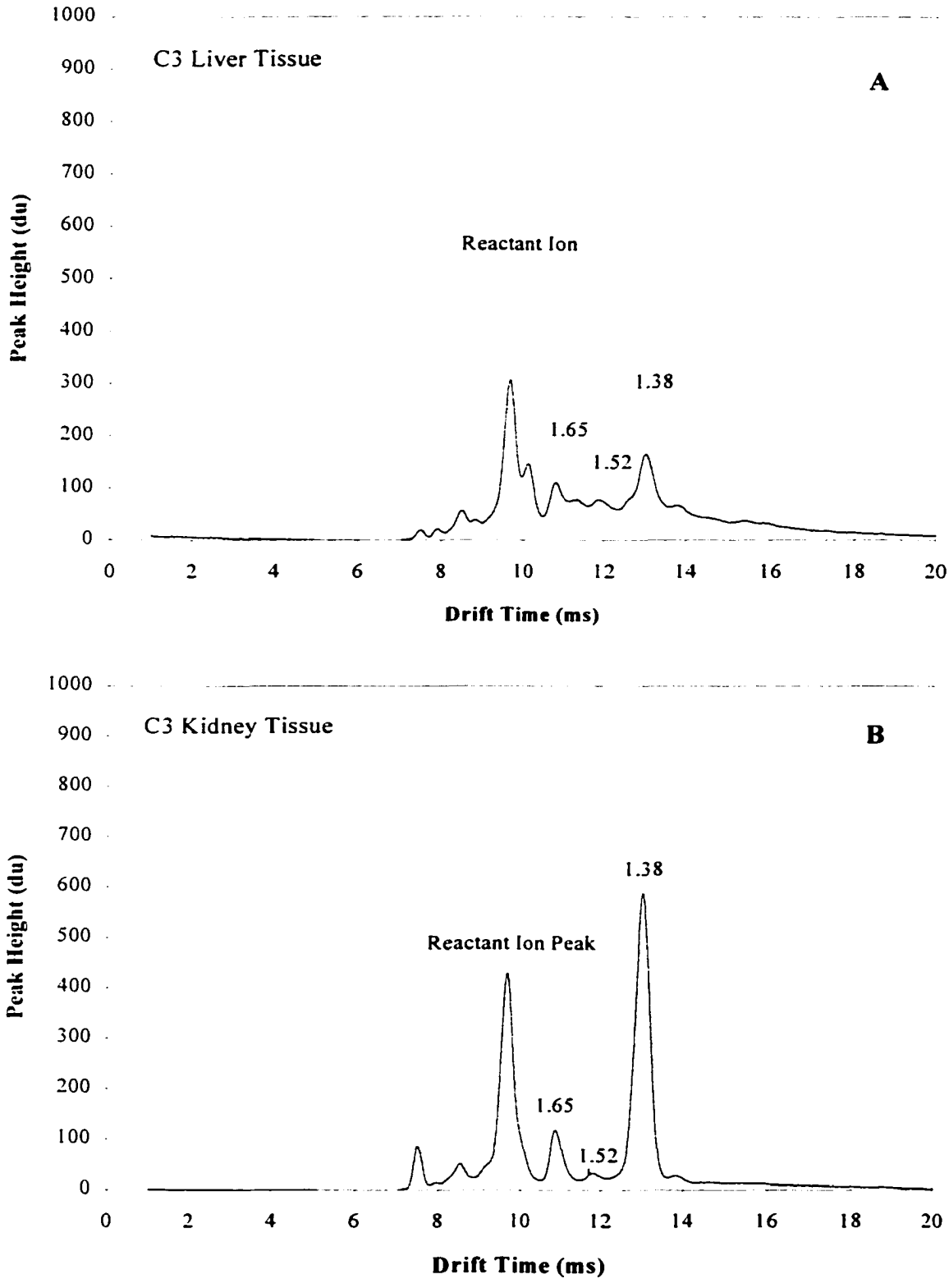


Fig. A-3. Positive mode IMS mobility spectra of (A) liver tissue and (B) kidney tissue from animal C3. This animal was given vehicle saline, anesthetic, and euthanasia in an acute study.

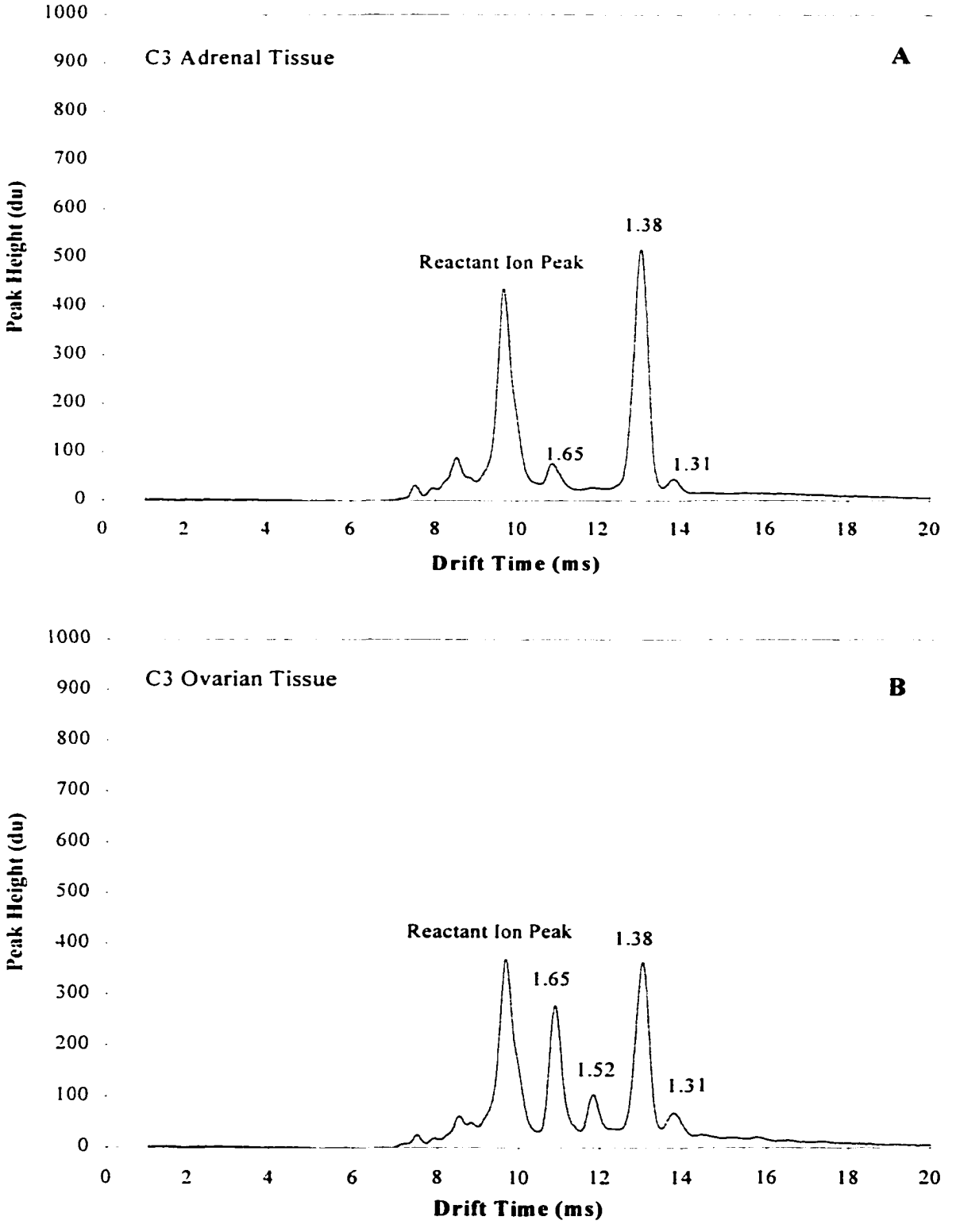


Fig. A-4. Positive mode IMS mobility spectra of (A) adrenal tissue and (B) ovarian tissue from animal C3. This animal was given vehicle saline, anesthetic, and euthanasia in an acute study.

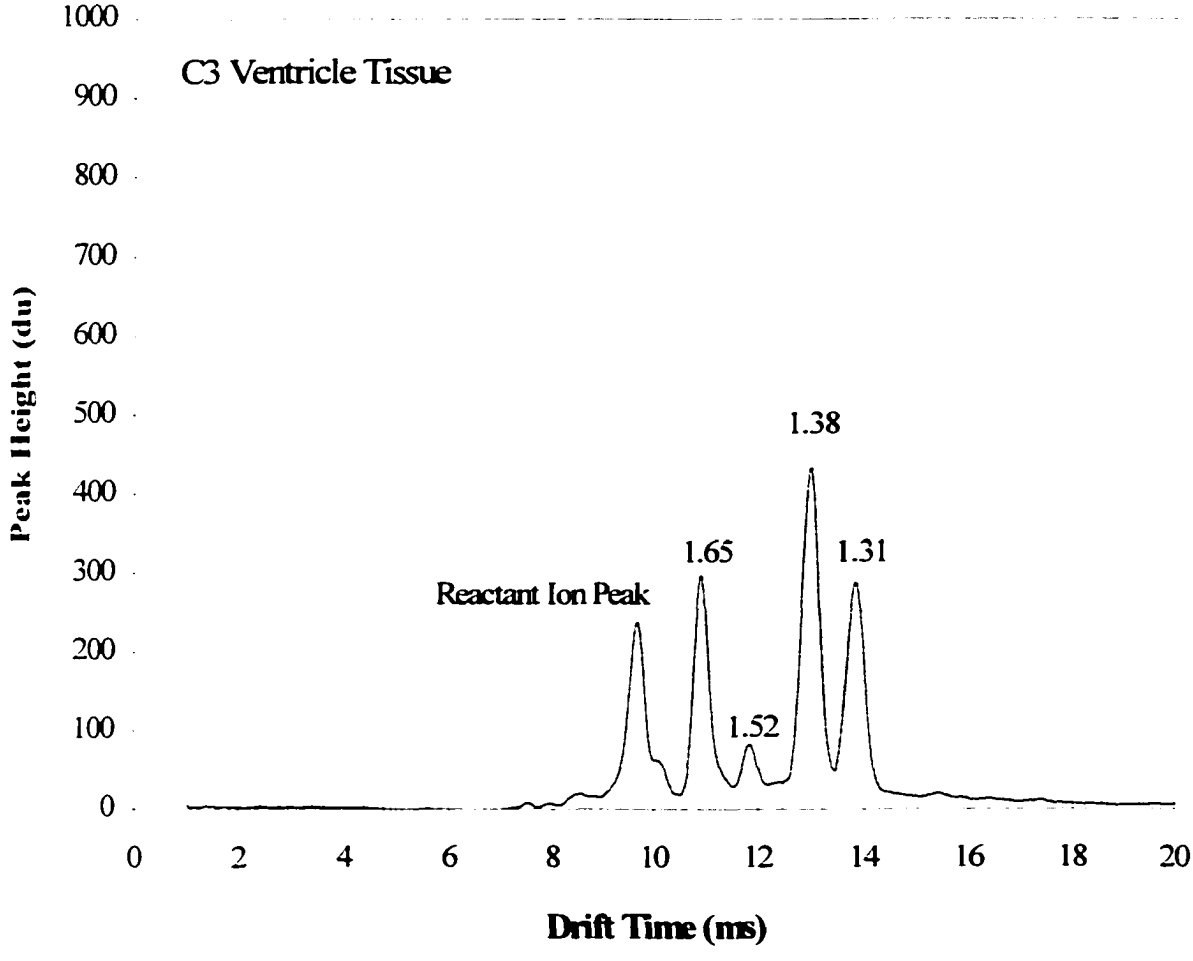


Fig. A-5. Positive mode IMS mobility spectra of ventricle tissue from animal C3. This animal was given vehicle saline, anesthetic, and euthanasia in an acute study.

APPENDIX B

MOBILITY SPECTRA OF GLYCINE PEPTIDES AND THEIR EFFECTS ON THE IMS OF HEROIN HYDROCHLORIDE

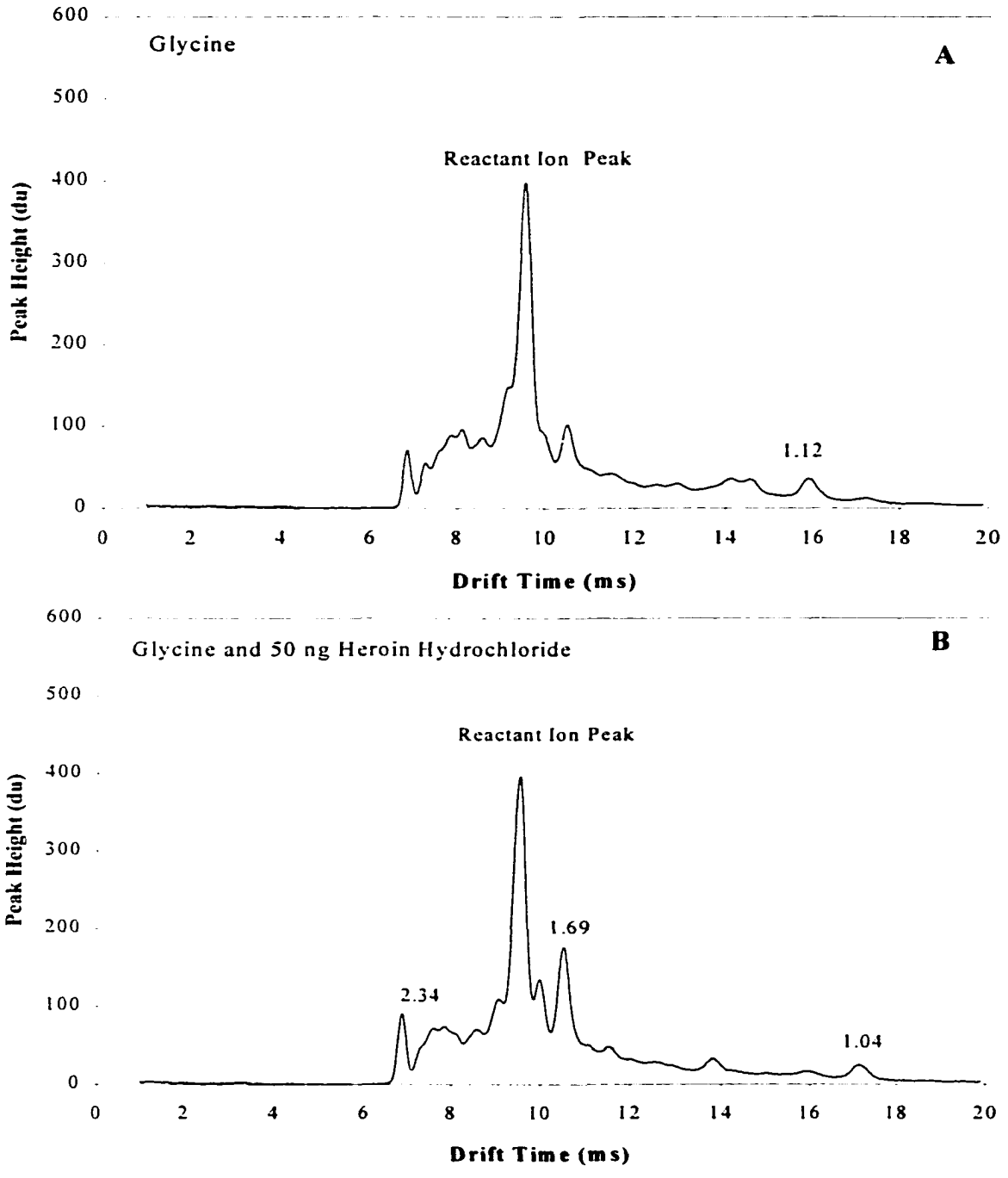


Fig. B-1. IMS mobility spectra of (A) 0.5-1 mg glycine and (B) 50 ng of heroin hydrochloride analyzed with glycine.

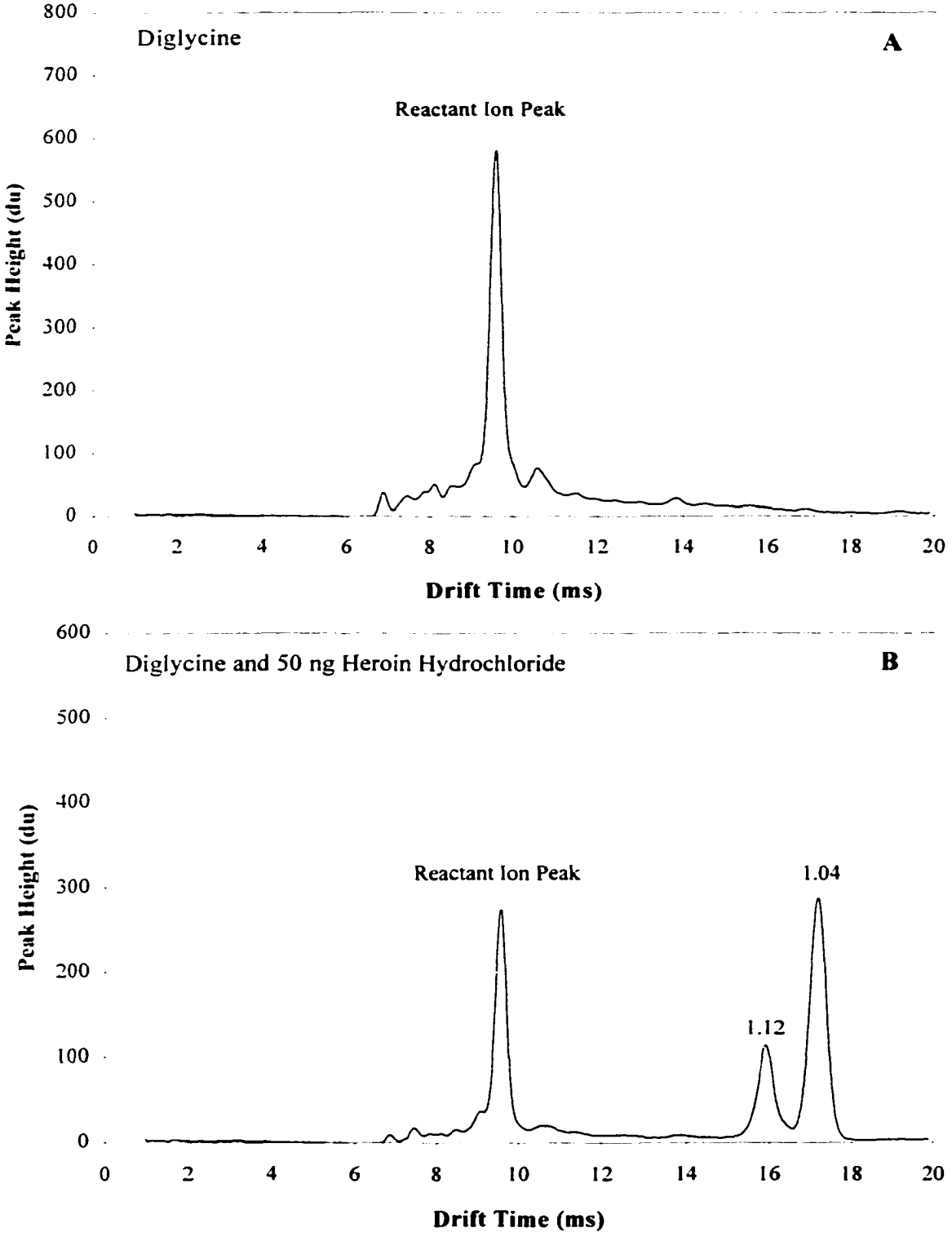


Fig. B-2. IMS mobility spectra of (A) 0.5-1 mg diglycine and (B) 50 ng of heroin hydrochloride with diglycine.

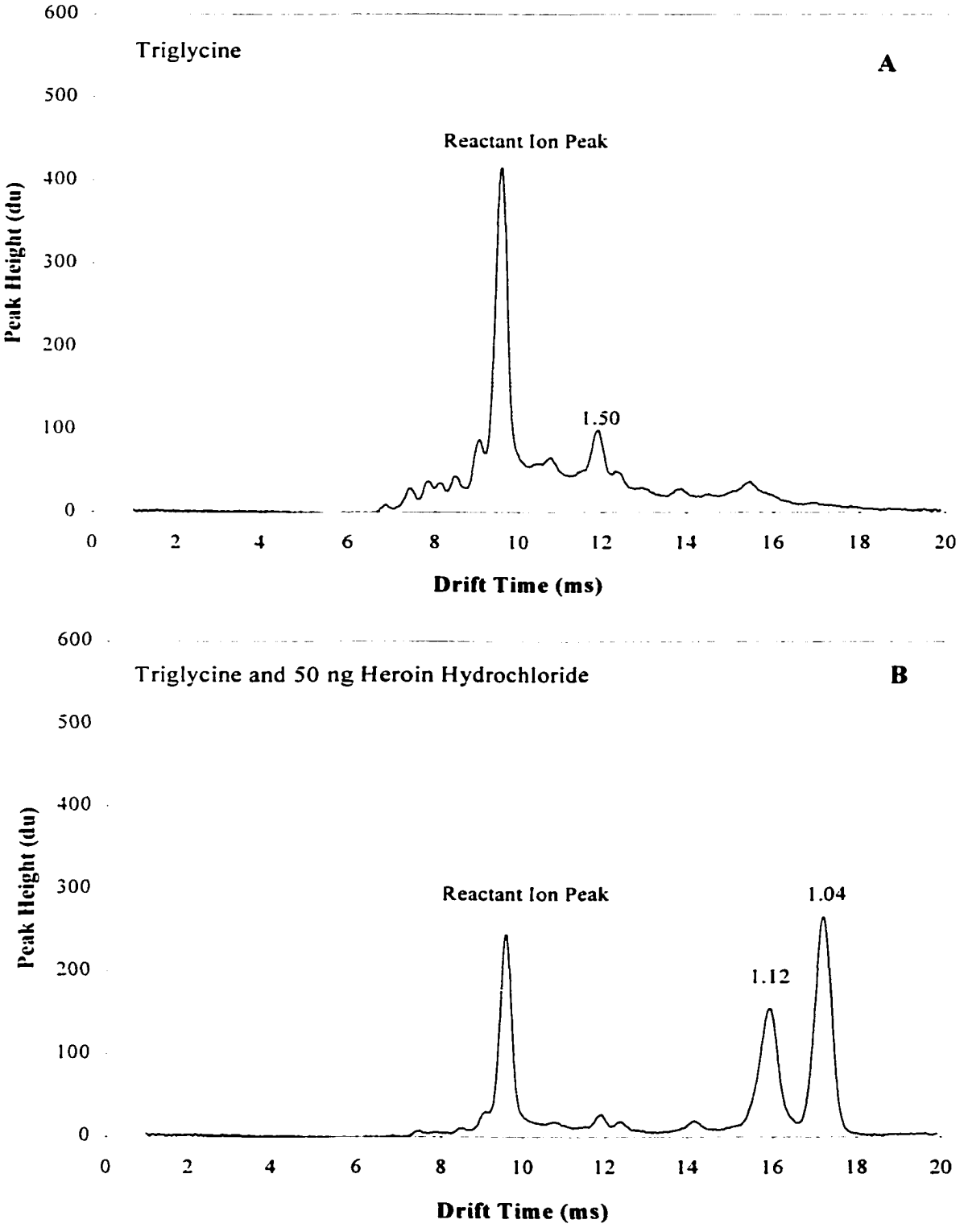


Fig. B-3. IMS mobility spectra of (A) 0.5-1 mg triglycine and (B) 50 ng of heroin hydrochloride analyzed with triglycine.

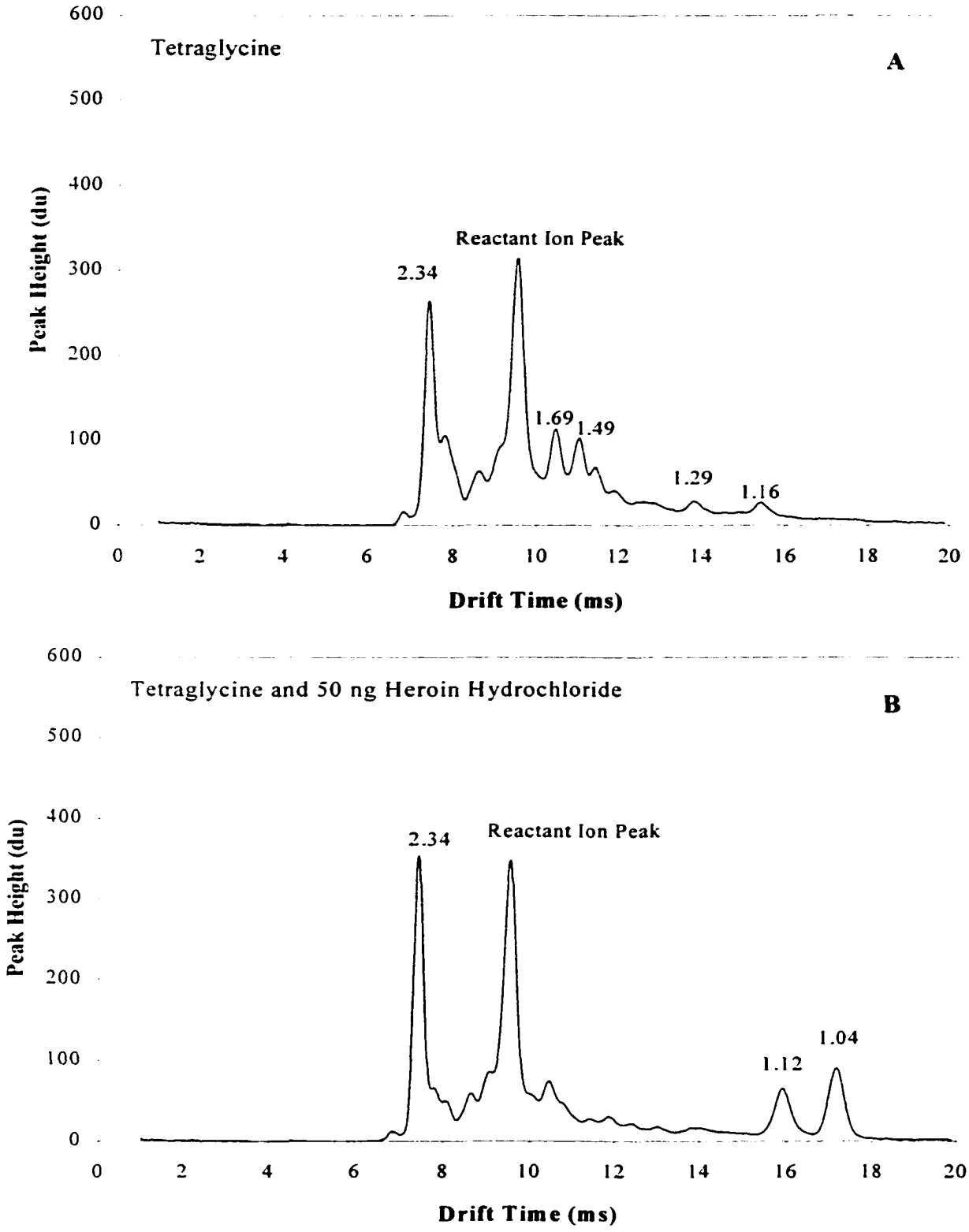


Fig. B-4. IMS mobility spectra of (A) 0.5-1 mg tetraglycine and (B) 50 ng of heroin hydrochloride analyzed with tetraglycine.

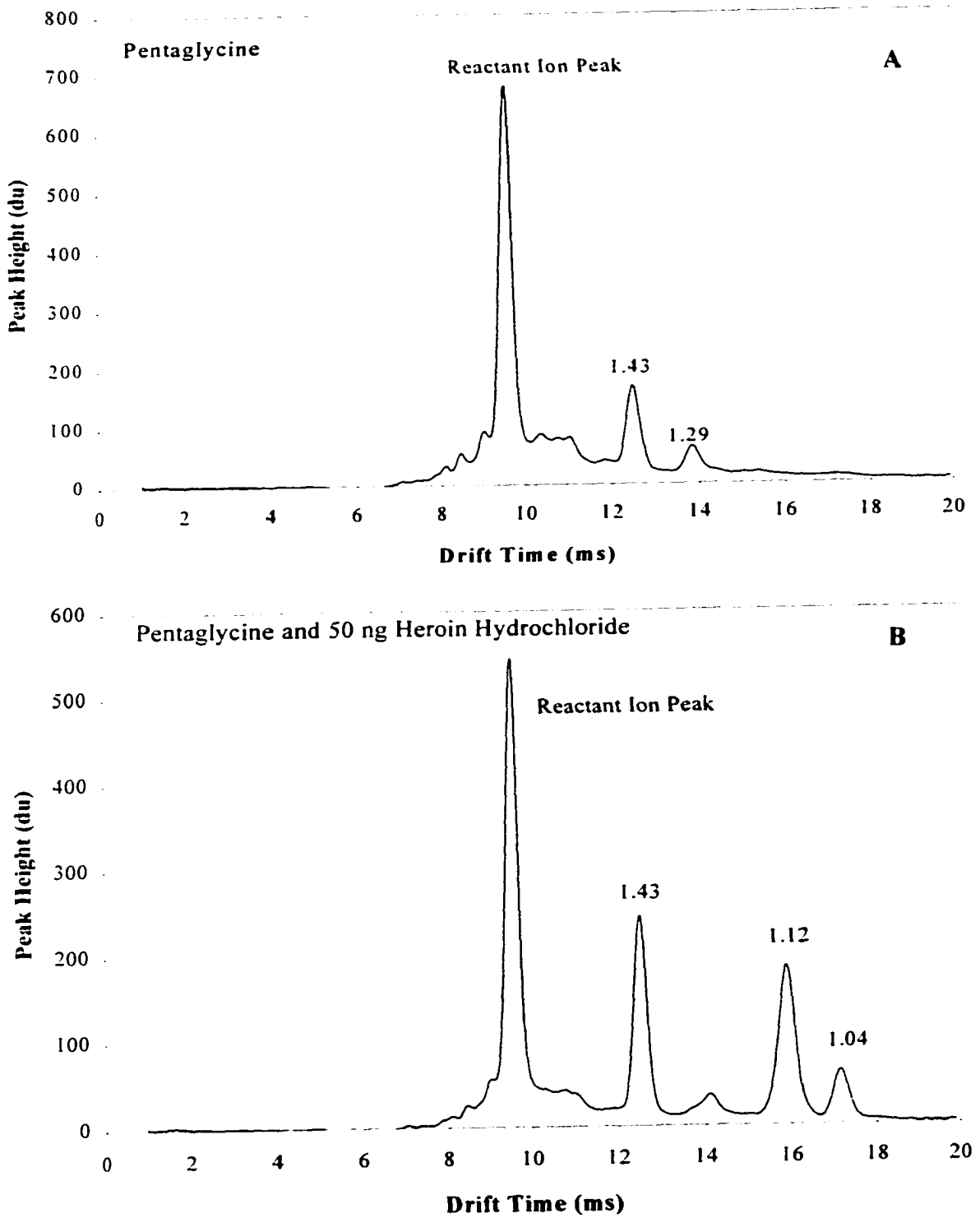


Fig. B-5. IMS mobility spectra of (A) 0.5-1 mg pentaglycine and (B) 50 ng of heroin hydrochloride analyzed with pentaglycine.

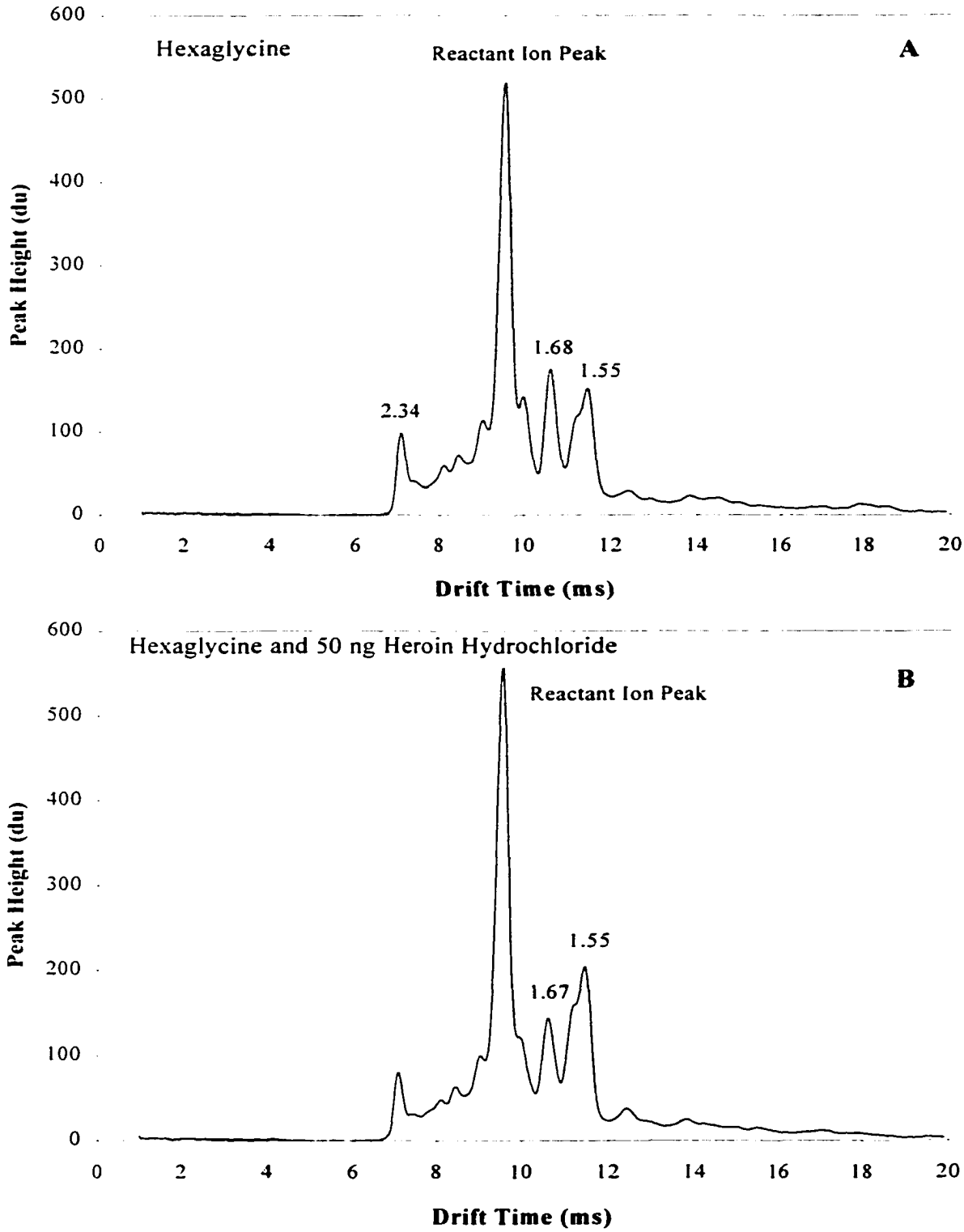


Fig. B-6. IMS mobility spectra of (A) 0.5-1 mg hexaglycine and (B) 50 ng of heroin hydrochloride analyzed with hexaglycine.

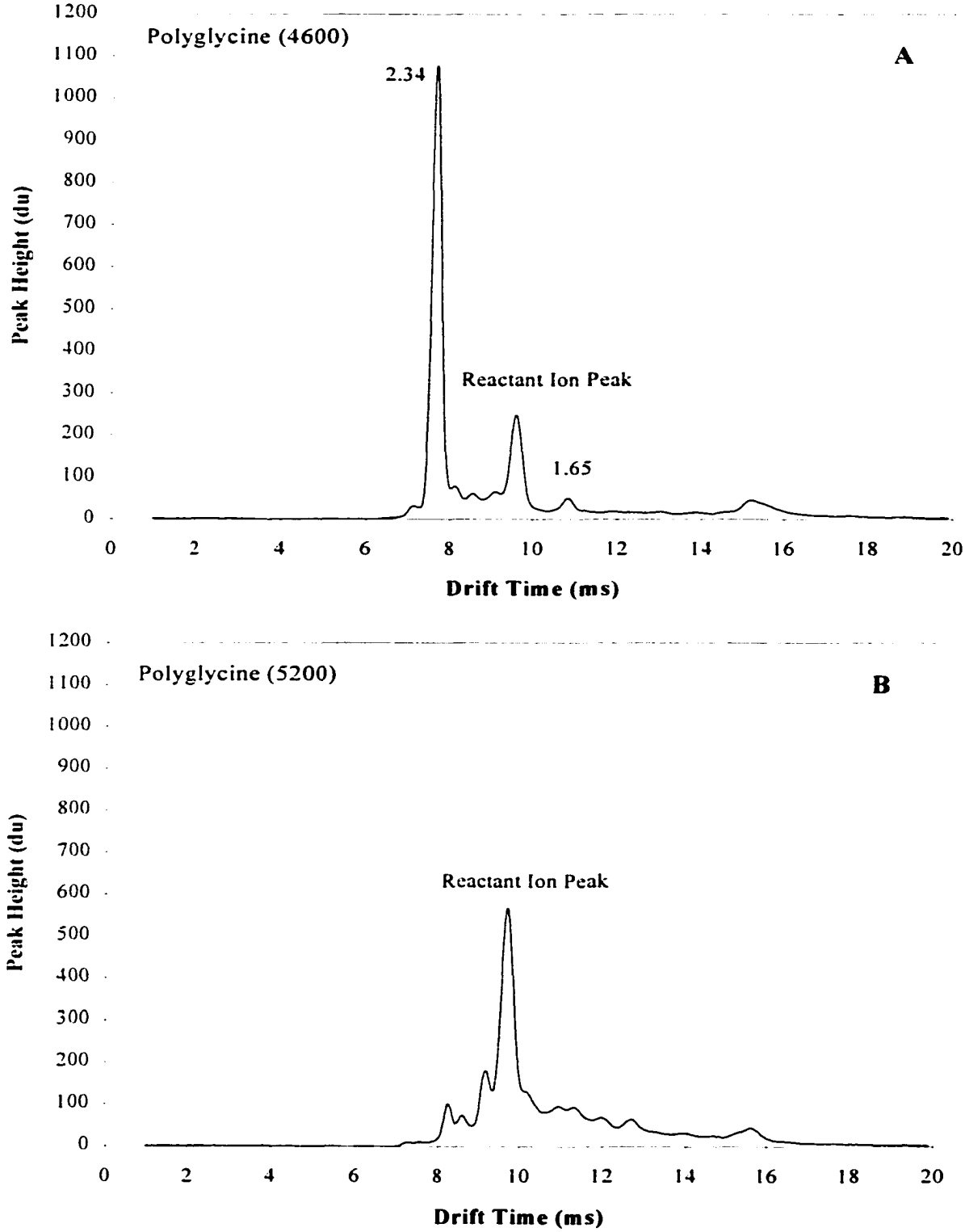


Fig. B-7. IMS mobility spectra of (A) a 4600 MW polyglycine and (B) a 5200 MW polyglycine.

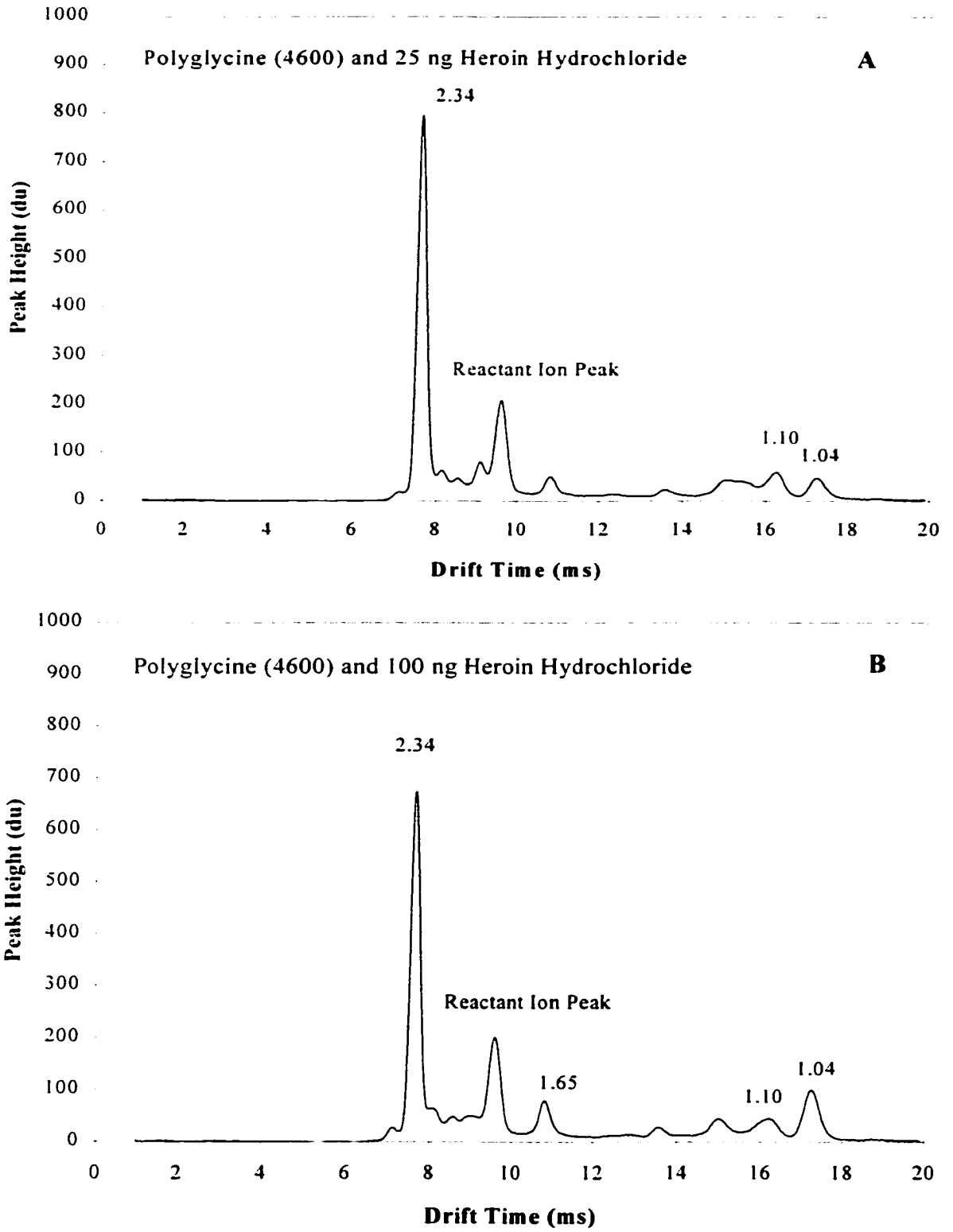


Fig. B-8. IMS mobility spectra of 0.5-1 mg polyglycine (4600) analyzed with (A) 25 ng of heroin hydrochloride and (B)100 ng of heroin hydrochloride.

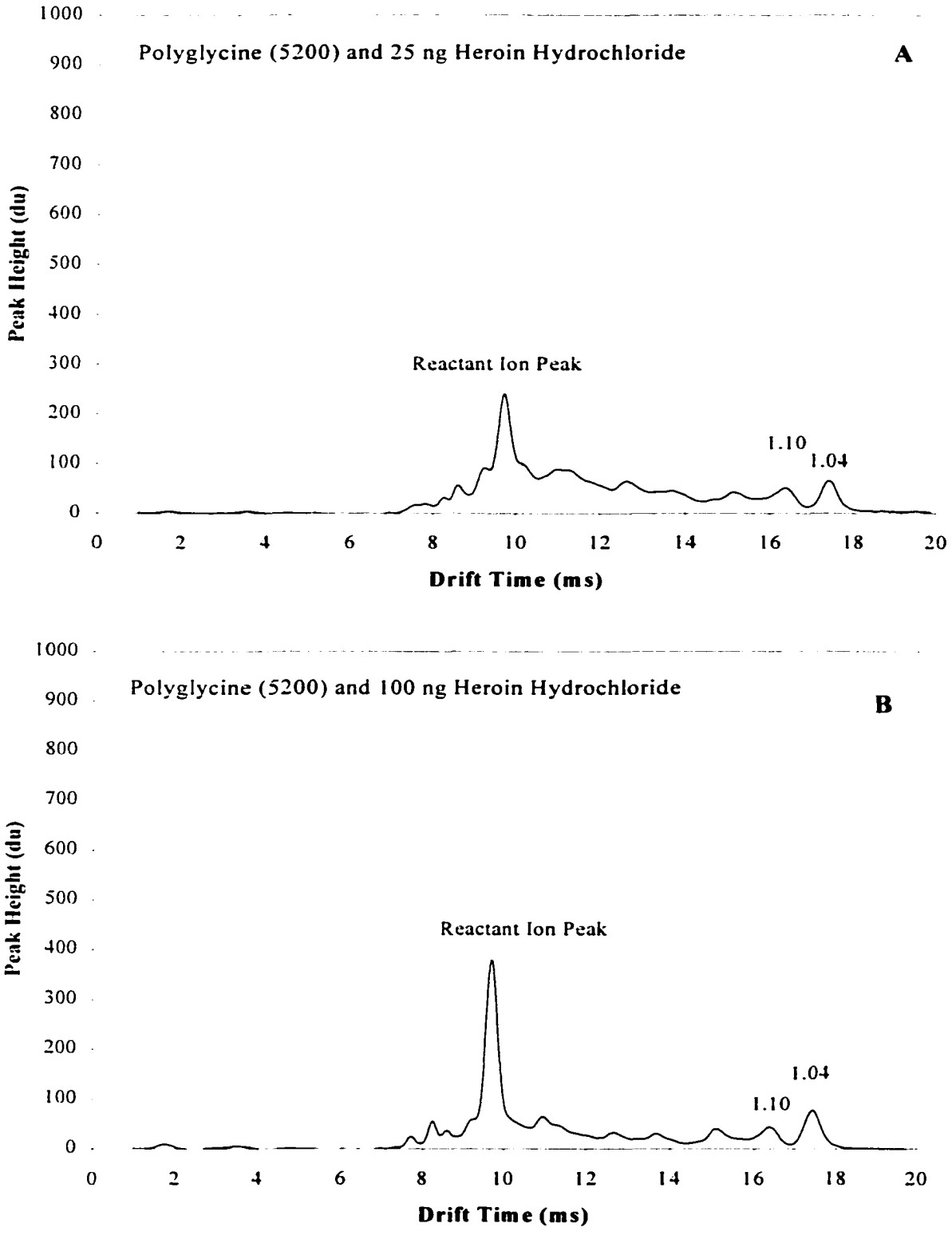


Fig. B-9. IMS mobility spectra of 0.5-1 mg of polyglycine (5200) analyzed with 25 ng heroin hydrochloride and (B) 50 ng of heroin hydrochloride.

APPENDIX C

IMS MOBILITY SPECTRA OF C3 ANIMAL TISSUES EXTRACTED WITH ACETONITRILE AND METHANOL

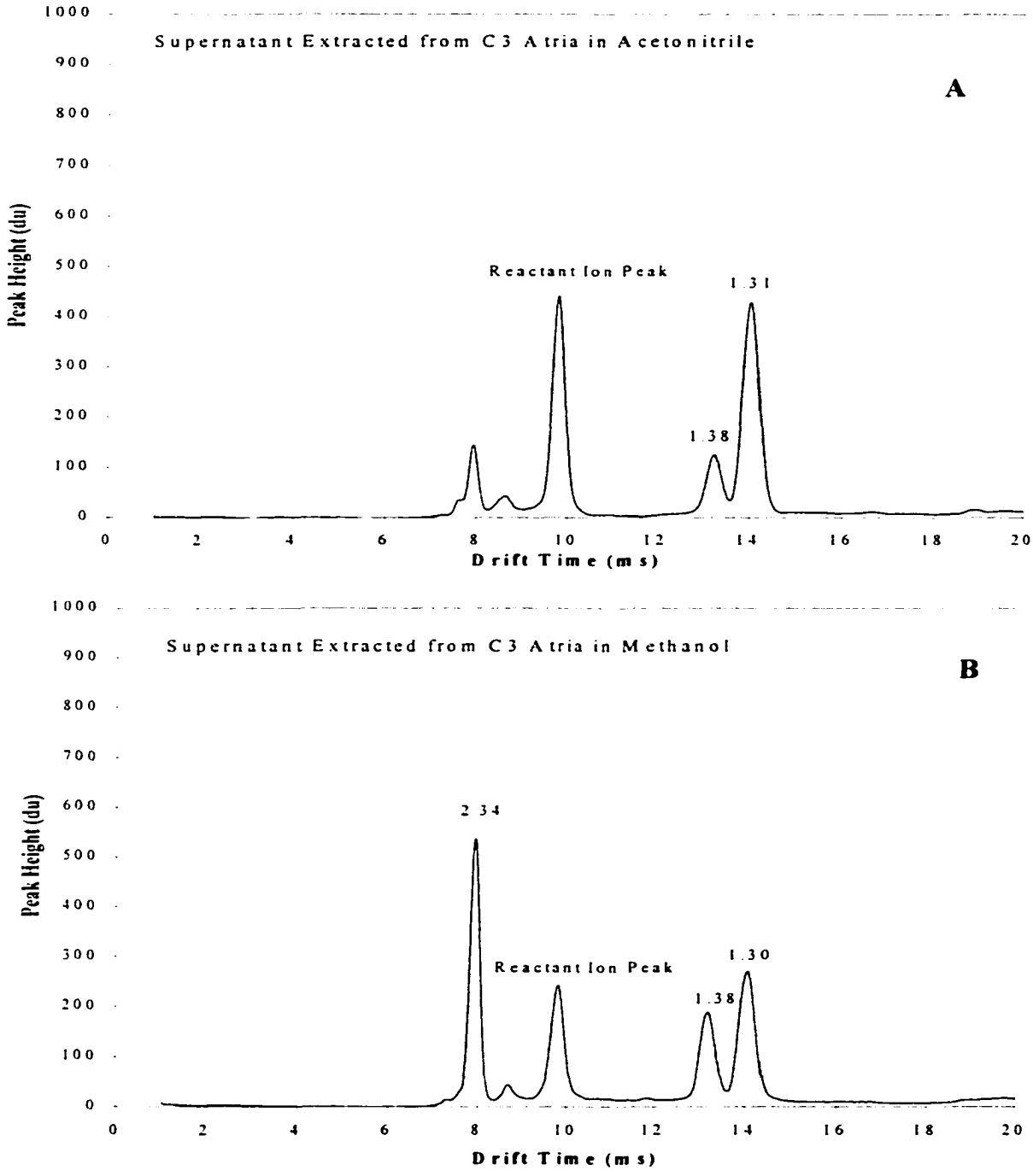


Fig. C-1. IMS mobility spectra for solvent extractions of (A) C1 atrial tissue in acetonitrile and (B) C1 atrial tissue in methanol.

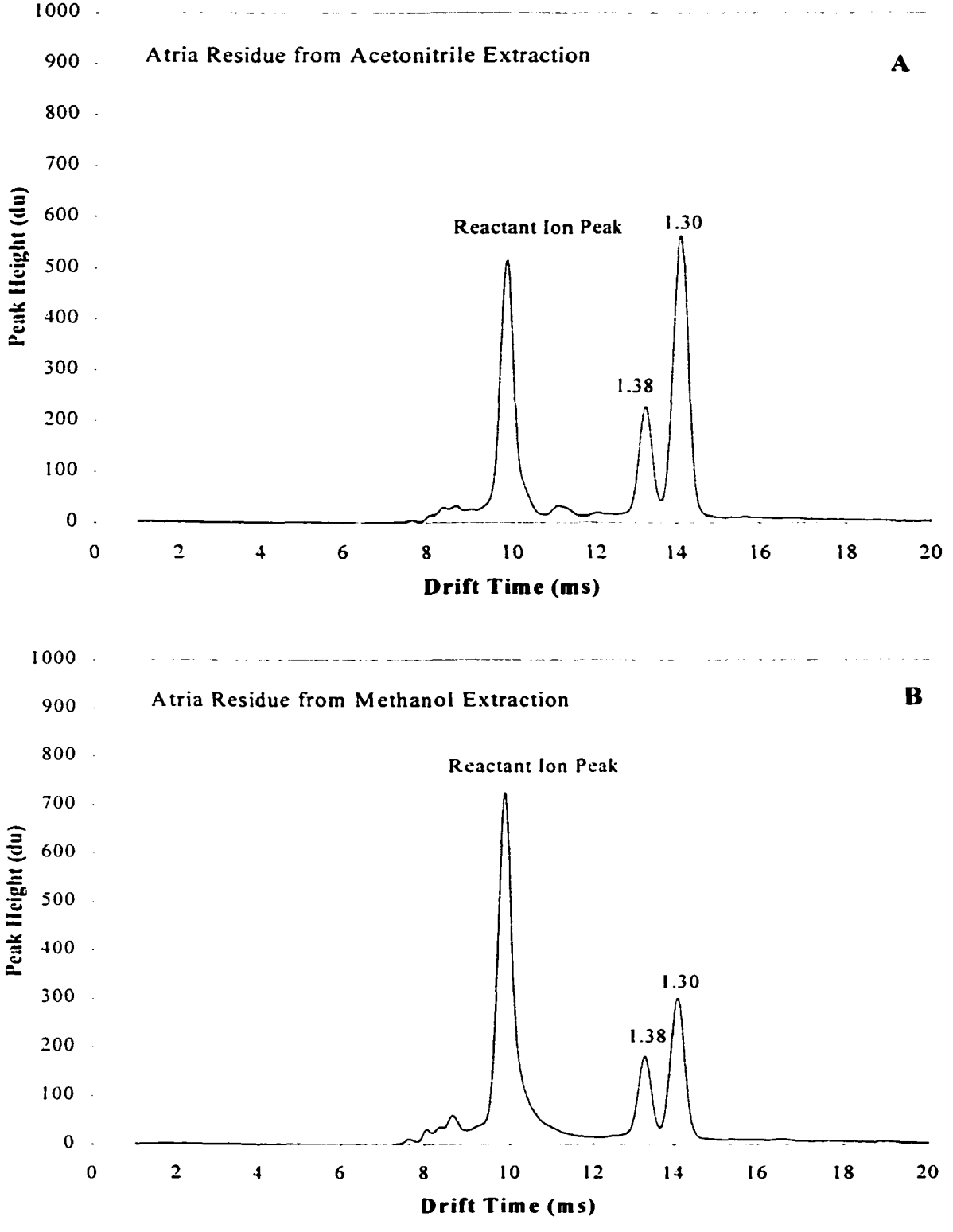


Fig. C-2. IMS mobility spectra of atrial tissue extracted with (A) acetonitrile and (B) methanol.

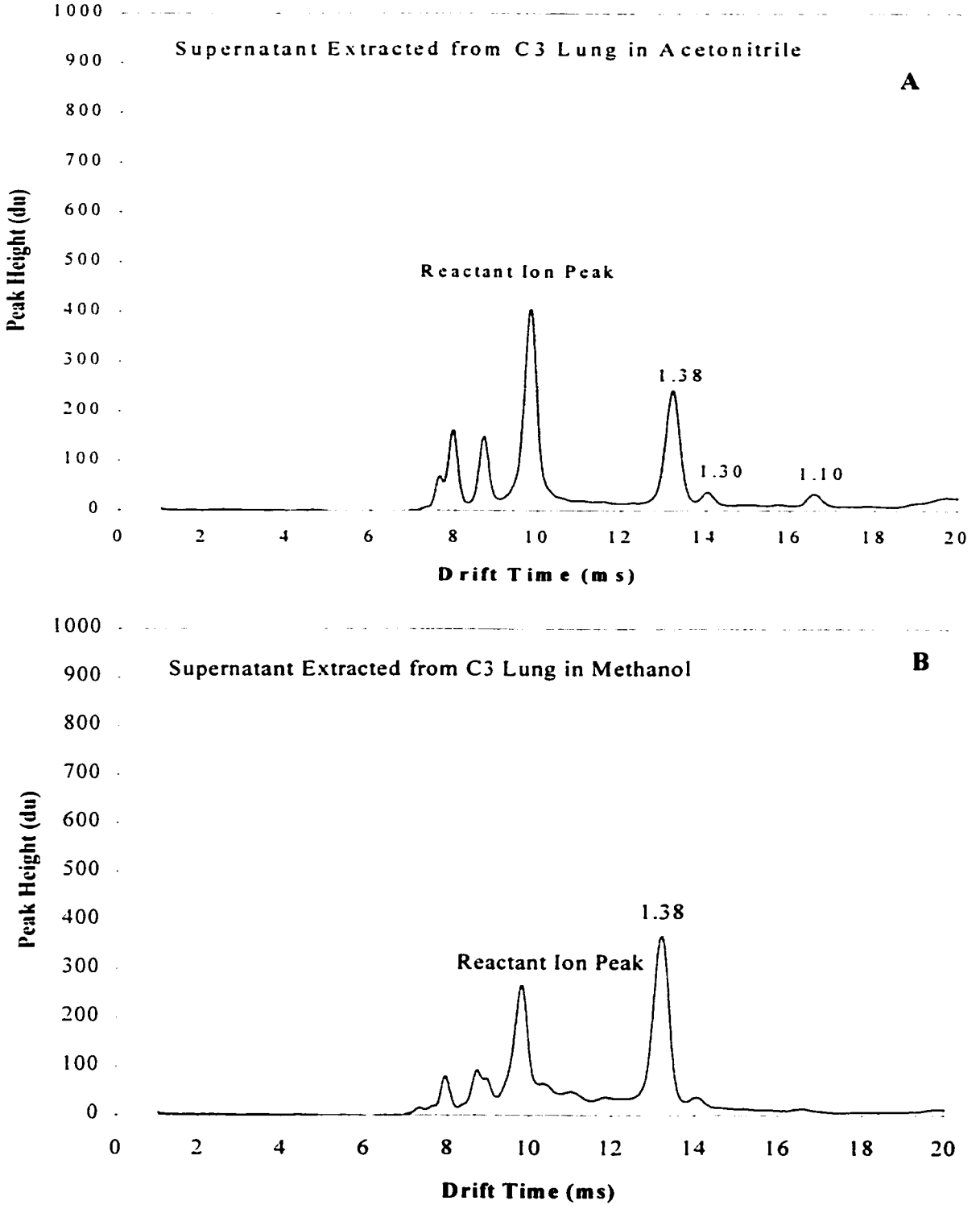


Fig. C-3. IMS mobility spectra for solvent extractions of (A) C1 lung tissue in acetonitrile and (B) C1 lung tissue in methanol.

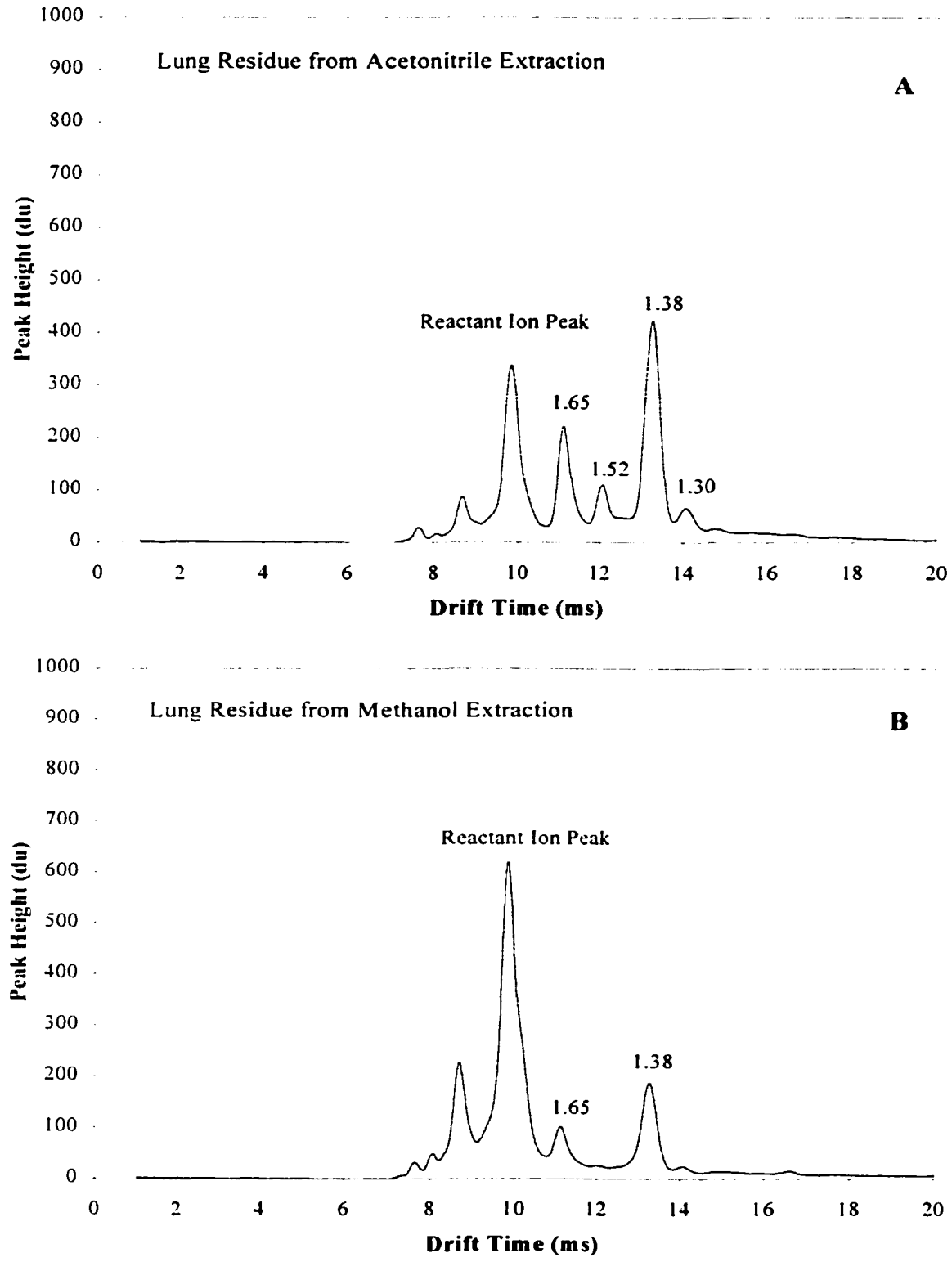


Fig. C-4. IMS mobility spectra of lung tissue extracted with (A) acetonitrile and (B) methanol.

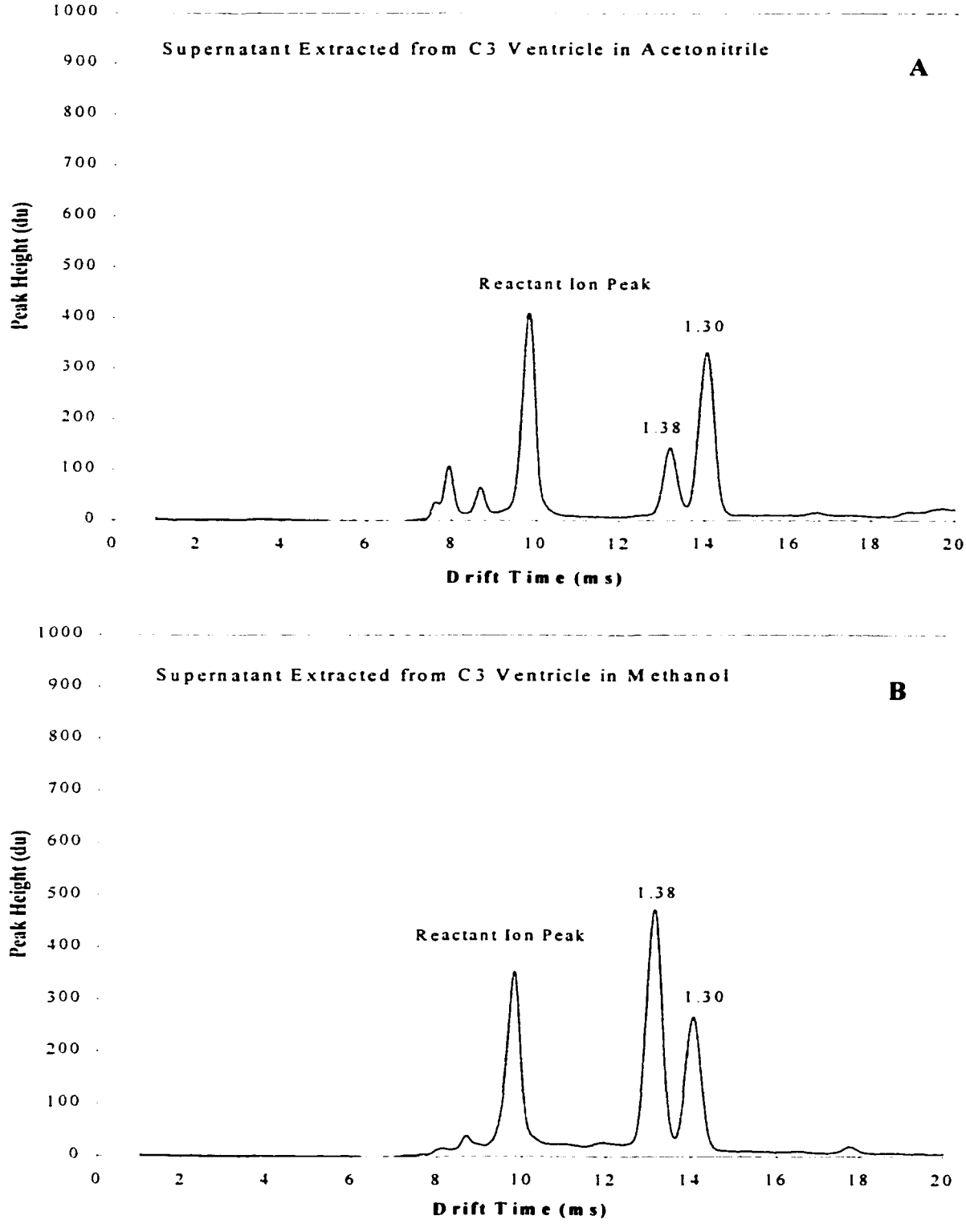


Fig. C-5. IMS mobility spectra for solvent extractions of (A) C1 ventricular tissue in acetonitrile and (B) C1 ventricular tissue in methanol.

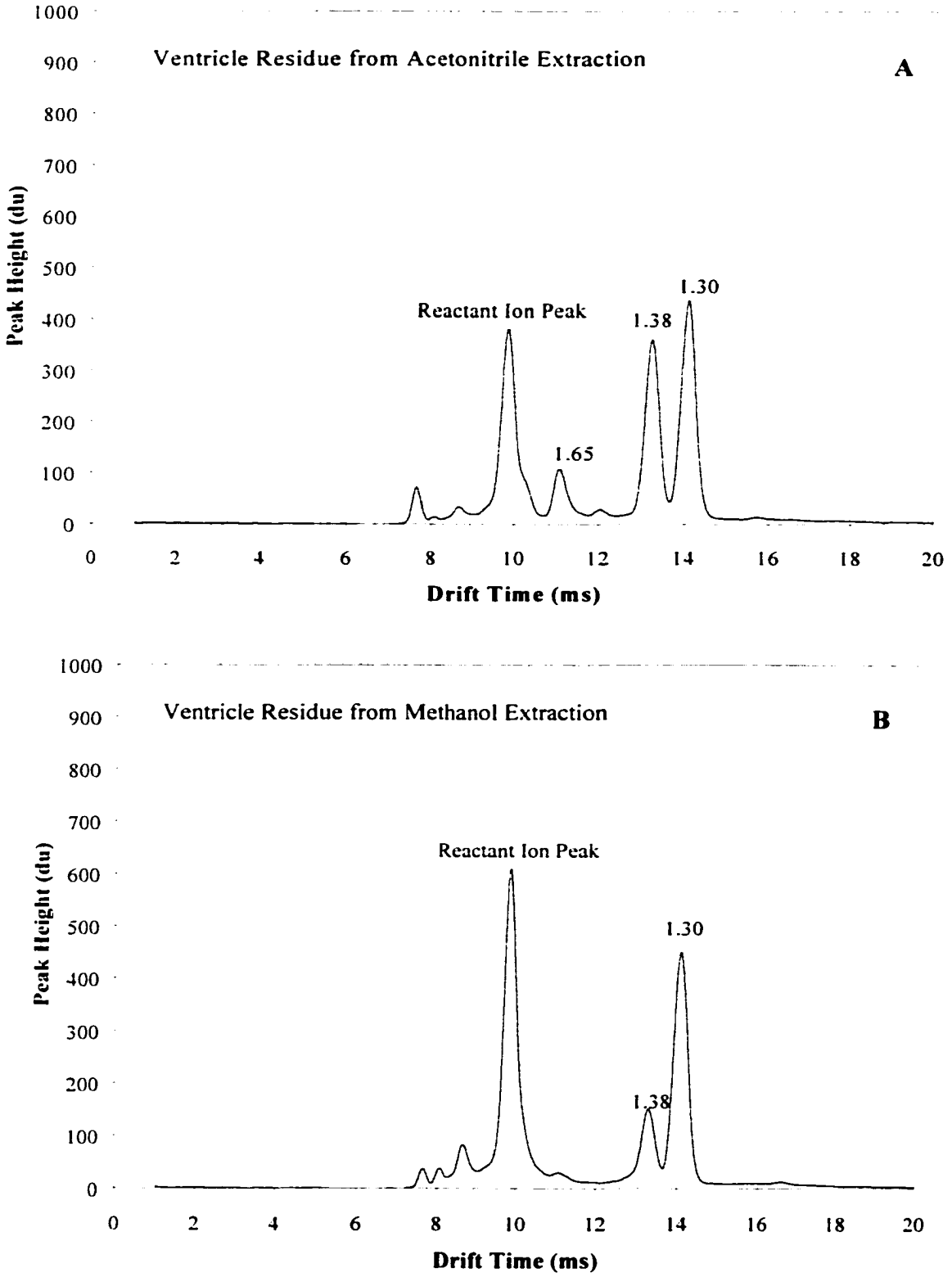


Fig. C-6. IMS mobility spectra of ventricular tissue extracted with (A) acetonitrile and (B) methanol.

APPENDIX D

IMS SPECTRAL INFORMATION FOR SYNTHESIZED PROTEINS, SUGARS, AND HUMIC SUBSTANCES

Table D-1
IMS spectral information for synthesized proteins, sugars, and humic substances

Analyte	Weight (mg)	Peak Number	K_o ($cm^2V^{-1}s^{-1}$)	Amplitude (du)
Proteins				
Vegetable (Protein 94)	0.5-1	N/A	N/A	N/A
Slim Fast	0.5-1	1	1.67	69
		2	1.47	29
Sugars				
Honey	50	1	1.65	56
		2	1.56	20
		3	1.44	20
Corn Syrup	50	1	1.66	30
Brown Sugar	0.5-1	1	1.65	36
Maple Sugar	0.5-1	1	1.67	47
Pure Cane Sugar	0.5-1	1	1.65	33
Maple Syrup	50	1	16.5	72
Humic Substances				
Leonardite (IHSS)	3.3	1	1.97	921
		2	1.66	37
Aldrich (sodium salt)	0.5-1	1	1.97	179
		2	1.51	53
Fluka	0.5-1	1	1.97	39

VITA

HENRI PARSON PATTEN
429 Saddle Rock Road
Norfolk, VA 23502

Education:

◆**Old Dominion University**
Norfolk, VA
Ph.D. Clinical Chemistry

May 2000 GPA: 3.76/4.0

◆**Hampton University**
Hampton, VA
Major: Chemistry
Minor: Mathematics
B.A. December 1994 GPA: 3.0/4.0

Experience:

◆**Old Dominion University - Interdisciplinary Research Laboratory**
Graduate Research Assistant (8/96 - Present)
Work with a team of eight researchers to analyze biological materials for the presence of physiologically active substances using an Ion Mobility Spectrometer (IMS).

◆**Children's Hospital of the King's Daughters Practicum, Norfolk, VA**
Intern (5/99 - 6/99)
Worked with a hospital supervisor and laboratory technicians to learn the instruments used in a clinical laboratory.

◆**Research Enhancement Enrichment Program (REEP), Hampton University**
Undergraduate Research Assistant (6/92 - 8/94)
Worked with an inorganic research scientist in the synthesis of hydrazinium and DABCOnium metal complexes.

◆**James Madison University, Harrisonburg, VA**
Summer Intern (6/93 - 8/93)
Selected to work with an inorganic chemistry to purify buckministerfullerene (C_{60}) and synthesize metal complexes.

Honors:

- ◆Virginia Council of Higher Education Fellowship – Three year tuition
- ◆ National Collegiate Natural Science Award
- ◆ Merit Scholarship – Recognized for academic achievement
- ◆ Sarah Cohen Scholarship – Awarded for academic achievement
- ◆ Research Enhancement Enrichment Program Scholarship – Two-year tuition
- ◆ Daughter's of the American Revolution Scholarship

Presentations:

Nine oral and poster presentations and one article published in a proceedings.