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Pharmacokinetics of Dexamethasone Delivered via Iontophoresis

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Doctor of Philosophy

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ABSTRACT

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Study Design: Controlled laboratory study. **Objectives**: To determine the time course of dexamethasone sodium phosphate (Dex-P) iontophoresis delivery to underlying tissues using microdialysis. **Background**: The efficacy of iontophoresis at delivering Dex-P through the skin is unknown in humans because of the lack of minimally invasive measurement techniques. **Methods**: Sixty-four healthy male participants (age = 24.4 ± 3.3 yrs, height = 71.8 ± 2.5 in, weight = 181.8 ± 26.1 lbs) were randomly assigned into one of six groups: 1) 1 mA current, 1 mm probes depth; 2) 1 mA current, 4 mm probes depth; 3) 2 mA current, 1 mm probes depth; 4) 2 mA current, 4 mm probes depth; 5) *in vivo* retrodialysis; and 6) skin perfusion flowmetry. Microdialysis probes assess the combined recovery (Dex_{total}) of Dex-P, dexamethasone (Dex) and its metabolite. *In vivo* calibration of the microdialysis probes occurred via retrodialysis. Laser Doppler flowmetry assessed skin perfusion. **Results**: There was no difference of Dex_{total} between current intensities ($P = 0.99$) but a greater amount of Dex_{total} was recovered by the 1 mm probe ($P < 0.0001$) compared to the 4 mm probe. Peak means for the 1 and 2 mA at 1 mm were 10.8 ± 8.1 and 7.7 ± 5.5 μg/ml and at 4mm being 2.0 ± 0.8 and 1.3 ± 0.9 μg/ml, respectively. Skin perfusion rapidly increased during both current intensity treatments, but significantly decreased before the conclusion of the 1 mA treatment ($P < 0.0001$). Peak skin perfusion was 741.4 ± 408.7% and 711.6 ± 260.8% baseline for 1 and 2 mA intensities, respectively. **Conclusion**: Iontophoresis delivery of Dex-P was successful measured *in vivo* through human skin. Significant concentrations of Dex_{total} were found regardless of current intensity. Though current induced vasodilation occurred, it did not significantly affect the tissue accumulation of Dex_{total}.

Keywords: transdermal drug delivery, iontophoresis, dexamethasone, microdialysis

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INTRODUCTION

Iontophoresis is a transdermal drug delivery system which uses a direct, or galvanic, current to transfer ions through the skin to underlying tissues. Dexamethasone sodium phosphate (Dex-P) delivered via iontophoresis is commonly used in physical medicine and rehabilitation to treat tendinopathies, however, this procedure has inconsistent clinical outcomes.[1,8,18,21-23,25,28,33] Research designed to examine Dex-P iontophoresis efficacy has been lacking due to methodological limitations.

Using animal models, initial research has demonstrated the effectiveness of Dex-P iontophoresis at delivering the drug across the epidermis[6,12,24] but without accumulation in venous blood.[5] However, the human epidermis has structural differences from animal models making a true evaluation of Dex-P iontophoresis effects on humans unclear with animal models.

In vivo human studies are limited by an inability to monitor tissue levels of Dex-P with minimally invasive techniques. Researchers use indirect methods such as assessing skin blanching characteristics[2] and venous blood draws[31] to examine Dex-P delivery. One group of researchers directly measured the concentration of Dex-P in underlying tissues after an iontophoresis treatment using tissue biopsies.[13,14] After a 40 mA*min iontophoresis treatment with Dex-P to the semitendinosus, Gurney et al[13] found Dex-P in some but not all biopsy samples.

The inability to adequately characterize Dex-P delivery to underlying tissue during iontophoresis leaves us with insufficient data to help optimize iontophoresis parameters and thereby limits our ability to explain the inconsistencies in clinical outcomes. We use a minimally invasive technique, intradermal microdialysis, to sample the accumulation of Dex-P, its biologically active form (dexamethasone, Dex) and its metabolites during iontophoresis delivery.

Microdialysis is a method used to sample local exogenous or endogenous compounds from interstitial fluid. Microdialysis involves perfusing a probe, which has a semi-permeable membrane, with a physiological solution (perfusate) allowing equilibration between the perfusate and interstitial fluid. Equilibration of an analyte occurs through a diffusion gradient between the interstitial fluid and perfusate resulting in a dialysate containing our analyte of interest.[30] Our lab has used this technique successfully to examine transdermal drug delivery of lidocaine.[9,10]

One aim of this study was to quantify the time course of Dex-P iontophoresis delivery through the skin (at 1 and 4 mm depth) at current intensities of 1 and 2 mA using intradermal microdialysis. Based upon current theories for drug delivery with iontophoresis we tested the well accepted hypothesis[15] that the tissue accumulation of Dex in the underlying skin will follow a similar time course when plotted as a function of iontophoresis dose (mA*min) regardless of the applied current intensity.

METHODS

Participants

We recruited 64 healthy males (age = 24.2 ± 3.3 y, height = 181.8 ± 26.1 cm, mass = 82.4 ± 11.8 kg, subcutaneous fat thickness = 0.61 ± 0.19 cm). Females were excluded from this study because of potential side-effects of Dex-P on fetal development in pregnant women. Male participants were also kept from participating if they had one or more of the following conditions: a known allergy to Dex, diabetes, an infection or open wound on the posterior lower leg, decreased circulation or sensitivity in the area to be treated, or an injury to either lower extremity within the previous 2 mo.

The study was approved by Brigham Young University's Institutional Review Board before participant recruitment. All participants provided written informed consent before individual data collection began.

Instruments and Dialysate Analysis

We used the Triviarion iontophoresis delivery kit (ActivaTek Inc., Salt Lake City, UT) to deliver Dex-P to the treatment site. The drug-delivery (cathode) electrode (12.56 cm^2) was prepared with 2 ml of 0.4% Dex-P while the center of the dispersive (positive) electrode (37.5 cm^2) was placed 15 cm distal to the Dex-P filled cathode electrode. We used an Iontophor-II phoresor (model: 6111PM/DX, Life-Tech Inc., Stafford,TX) to deliver the iontophoresis treatment.

The 18 kilo-Dalton linear (3.0 cm) hollow fiber microdialysis probes (150 micron OD) were manufactured in our laboratory per instructions described previously by other researchers[9,10] and gas sterilized with ethylene oxide. We used an infusion pump (model: Pump 11 VPF; Harvard Apparatus, Holliston, MA) to perfuse the microdialysis probes with sterile saline. The depth of the inserted microdialysis probe was verified using musculoskeletal ultrasound imaging (model: LogiQ P5, probe type: 12L, General Electric Company, Fairfield, CT).

The concentrations of Dex-P, Dex and its metabolites in each dialysate sample was determined by reverse-phase high performance liquid chromatography (RP-HPLC) using a previously established method.[7] A diode array detector (model: 1260 Infinity, Agilent Technologies, Inc., Santa Clara, CA) with a wavelength of 239 nm was used to measure the peaks of Dex-P and DEX at 4.2 and 12.4 min, respectively. The lower limit quantification of

Dex-P and Dex was 100 ng/mL and 50 ng/mL, respectively. The standard curves for each analyte is shown in FIGURE 1. Analysis of dialysis samples by RP-HPLC revealed a peak at 5.8 min which represented the Dex metabolite, dexamethasone-21-oic acid (Dex-21-oic acid), and was quantified using a combination of the Dex-P and Dex calibration curve.

Skin perfusion was measured via laser Doppler flowmetry (LDF). Four laser Doppler skin probes (model: VP7a, Moore Instruments, Wilmington, DE) were interfaced with a laser Doppler monitor (Moore Instruments, Wilmington, DE). The laser Doppler output was monitored using Powerlab (ADInstruments Inc., Colorado Springs, CO) in order to measure changes in skin perfusion.

Procedures

Participants reported to the lab for a single visit. Participants were screened for the inclusion and exclusion criteria. Each reviewed and signed the Institutional Review Board approved consent form. Once officially enrolled in the study, the participants were randomly assigned to one of the six groups: 1) 1 mA current intensity with probes depth of 1 mm, n = 8; 2) 1 mA current intensity with probes depth of 4 mm, n = 8; 3) 2 mA current intensity with probes depth of 1 mm, n = 8; 4) 2 mA current intensity with probes depth of 4 mm, n = 8; 5) *in vivo* retrodialysis of Dex-P and Dex, n = 8 ; and 6) skin blood flowmetry, n = 24. We chose to insert the deep probes at a depth of 4 mm because the approximate depth of commonly treated tendons is 4 mm below the surface of the skin.[4]

Microdialysis Probe Placement for Dex-P and Dex Recovery during Iontophoresis

Three linear microdialysis probes were inserted in the left posterior lower leg at the depth based on the participant's group assignment. During the microdialysis probe placement, participants lay prone on a treatment table. An 8" x 8" area on the left posterior lower leg of the

participant was shaved. We visualized the largest girth of the participant's lower leg. Marks were made on the participant's posterior lower leg indicating where the guide needles would be inserted and exit. From the surface of the skin on the posterior lower leg, we measured down the medial side 1 or 4 mm (based on group assignment) and made a mark with a felt marker. From this mark, we measured lateral 5 cm and made a mark where the probe would exit the lower leg. The insertion and exit sites were then cleansed with an iodine swab.

Using aseptic procedures, three sterile 3.5 in. 27 gauge pediatric spinal tap needles (reference 405081, BD Company, Franklin Lakes, NJ) were inserted into the subcutaneous tissue of the participant's left posterior lower leg at the marked sites, either 1 or 4 mm below the surface of the skin (actual depth = 1.3 ± 0.3 or 4.0 ± 0.7 mm). The depth of each needle was verified using musculoskeletal ultrasound imaging. The microdialysis probes were fed through each guide needle and the guide needles were removed leaving the probes in place in the lower leg. Once the probes were inserted, sterile saline solution was perfused through the probe at 1.2 µl/min for 60 min and collected as the pretreatment dialysate. This period allowed the tissue to recover from the mild trauma associated with the guide needle insertion.

Once the 60 min recovery period was complete, the prepared drug-delivery electrode was placed on the skin directly over the microdialysis probes. The dispersive electrode was placed 15 cm distally from the drug delivery electrode. The iontophoresor leads were attached to the respective electrode and the unit was turned on to deliver the iontophoresis treatment based on the participant's group assignment (1mA or 2mA for 120 mA*min). Starting with the initiation of Dex-P iontophoresis, dialysate from each microdialysis probe was collected in 15 min intervals (1mA group = 8 samples and 2 mA group = 4 samples) with the perfusion rate maintained at 1.2 µl/min. After the treatment was completed, we collected an additional 4

dialysate samples at 15 min intervals. Immediately after each collection period, dialysate samples were stored at -20°C until they were analyzed using RP-HPLC.

Microdialysis Probe Placement for Dex-P and Dex Retrodialysis

Retrodialysis is an *in vivo* microdialysis calibration technique based on the theory that *in vivo* loss represents *in vivo* recovery. During retrodialysis, the microdialysis probe was perfused with the analytes of interest (2 µg/mL of Dex-P and Dex) at the same perfusion rate (1.2 µl/min) and the dialysate was monitored for the disappearance of the analyte from the probe. The relative recovery (RR) was then calculated as a percent.[30,32]

$$RR = \left(1 - \frac{[Drug_{dialysate}]}{[Drug_{perfusate}]}\right) x\ 100$$

Participants randomly assigned to the retrodialysis group had a single microdialysis probe inserted into their left posterior lower leg using the same insertion techniques as described earlier (depth = 3.2 ± 1.4 mm). This microdialysis probe was perfused with sterile saline at 1.2 µL/min for 60 min as a recovery period. Following the recovery period, sterile saline with 2 µg/mL of Dex-P and 2 µg/mL of Dex-P was perfused through the microdialysis probe at 1.2 µL/min. Three dialysates were collected at 45 min intervals and were analyzed with RP-HPLC.

RP-HPLC Analysis

For consistency, standard curves of Dex-P and Dex concentrations were analyzed both prior to and at the conclusion of collecting all dialysis samples. The mean standard curves were used to determine the concentrations of Dex-P and Dex in each dialysis sample.

Skin Perfusion Monitoring

Four LDF probes were attached to the posterior lower leg of the participants randomly assigned to the skin blood flow group. The flow probes were placed within the drug delivery chamber (adjacent to the center electrode, 0 cm), on the periphery of the drug chamber (2 cm

away from center of electrode), on the periphery of the electrode (4 cm away from center of electrode) and between the active and dispersive electrodes (6 cm away from center of electrode). The placements of the probes are visualized in FIGURE 2. The skin of the posterior lower leg was cleaned with isopropyl alcohol and the iontophoresis electrodes and LDF probes were then applied. Baseline skin perfusion was established during a 5-min stabilization period. Then, a 120 mA*min iontophoresis treatment with Dex-P was applied with a current intensity of either 1 (n = 12) or 2 (n = 12) mA. The treatment was randomly assigned and lasted 120 or 60 min, respectively. Skin perfusion was monitored and instantaneously recorded throughout the treatment and for an additional 60 min after the treatment. Heart rate and blood pressure was also measured and recorded every 10 min throughout the treatment and posttreatment.

Data Analysis

We used the area under the RP-HPLC generated peaks at migration times of 4.2, 5.9 and 12.4 min to represent the concentration of Dex-P, Dex-21-oic acid, Dex, respectively, in each dialysate sample. Using the RP-HPLC standard curves and our retrodiaylsis RR, we calculated the total amount of Dex delivered to the tissue or Dex_{total} (Dex_{total} = Dex-P + Dex + $Dex_{metabolite}$) for each sample. Skin perfusion was normalized by dividing the flux reading (mV) by the mean arterial blood pressure (mmHg). Changes in skin perfusion were expressed as a percent change in skin perfusion relative to baseline. All variables (Dex_{total} and skin perfusion) were plotted as a function of iontophoresis dose.

Statistical Analysis

A 4 x 5 (group x dose) mixed model ANOVA was used to determine differences between the intensities and depths of Dex_{total} over the iontophoresis treatment. Due to our timing of sample collection, we could only compare the groups at 30 mA*min dose increments.

A 2 x 13 (intensity x dose) mixed model ANOVA was used to determine differences between skin perfusion and intensities over the iontophoresis dose within the drug delivery electrode (0 cm). We only show the data for skin perfusion at the 0 cm site because there was no visible change in skin perfusion at the other sites. We compared the group differences in skin perfusion at 10 mA*min dose increments.

We used JMP Pro 10 (SAS Inc., Cary, NC) for all statistical analyses and alpha was set at $P < 0.05$.

RESULTS

The retrodialysis recovery indicated that the microdialysis probes collected 27.4 ± 2.0% of Dex_{total} in the extracellular fluid *in vivo*. Using the standard curve and retrodialysis recovery values, the accumulation of Dex_{total} as a function of iontophoresis dose is shown in FIGURE 3 for the different current intensities and probe depths.

We did not recover a substantial amount of Dex-P from the underlying tissues. We recovered Dex in only six of 32 participants with mean concentration of 109.9 ± 88.8 ng/mL representing only 2.92% of all Dex_{total}. The majority of Dex_{total} recovered by intradermal microdialysis was the Dex metabolite, Dex-21-oic acid. The Dex metabolite was recovered in all subjects across all samples.

A greater amount of Dex_{total} was recovered by the 1mm probes compared to the 4mm probes across the final 60 mA*min to 120 mA*min of the iontophoresis treatment ($F_{12,112} = 5.21$, $P < 0.0001$). Peak Dex_{total} at 120 mA*min and 1 mm probe depth for the 1 and 2 mA treatments were similar and averaged 10.8 ± 8.1 and 7.7 ± 5.5 µg/ml, respectively. Averaged between intensities, this represents approximately 0.23% of the Dex-P placed in the iontophoresis chamber. At 4 mm probe depth and 120 mA*min dose Dex_{total} was similar and averaged

2.0 ± 0.8 and 1.3 ± 0.9 μg/ml, for 1 and 2 mA current intensities, respectively. At any given probe depth Dex_{total} was similar for both 1 and 2 mA current intensities across all iontophoresis dosages ($P > 0.05$).

Skin perfusion increased rapidly during both iontophoresis treatment intensities, however, the 1 mA current intensity group showed a significant decrease in skin perfusion before the iontophoresis treatment was over ($F_{12,264} = 23.17$, $P < 0.0001$) (FIGURE 4). Peak skin perfusion of 741.4 ± 408.7% baseline during 1 mA treatments occurred at 40 mA*min. During 2 mA treatments, peak skin perfusion of 711.6 ± 260.8% baseline occurred at 110 mA*min. Skin perfusion returned to baseline values during the 1 mA treatment intensity at 110 mA*min. While skin perfusion returned to baseline in the 2 mA intensity group 60 min after terminating the iontophoresis protocol.

DISCUSSION

By combining intradermal microdialysis and RP-HPLC, we successfully measured *in vivo* extracellular Dex_{total} accumulation at 1 and 4 mm depth in the dermis during iontophoresis in human participants. Earlier studies monitored only Dex concentrations and found little or no accumulation of Dex in tissue underlying the iontophoresis site. For example, Gurney and Wascher,[13,14] used tissue biopsies to measure *in vivo* Dex accumulation. However, only 7 out of 16 participants had any measureable levels of Dex in the biopsy sample of the semitendinosus tendon 85-235 min after a 40 mA*min iontophoresis treatment. In the 7 samples that Dex was detected the mean concentration was 6.6 ± 3.2 ng/g.[14] Similarly, we recovered Dex in only 6 of 32 participants with a mean concentration of 109.9 ± 88.8 ng/mL. However, this represented only a small fraction (< 3%) of Dex_{total}. The major Dex component of our dialysate samples was the Dex metabolite, Dex-21-oic acid.

Dex-P is an anionic prodrug with an ester link at the 21 carbon to increase the aqueous solubility of Dex. Mammalian skin contains esterases which hydrolyzes Dex-P into Dex.[24] Based on our HPLC analysis,[7] the metabolite product of the iontophoresis delivery of Dex-P was exclusively Dex-21-oic acid. As such, Dex-P does not appear to completely hydrolyze into pure Dex, but instead into an acid ester form. Dex-21-oic acid has a lower affinity for binding to the glucocorticoid receptor and has a diminished anti-inflammatory potential compared to Dex.[17] To our knowledge, our study was the first to report a metabolite conversion of Dex-P when delivered through human skin. Animal models have reported a more pure conversion rate of Dex-P to Dex in cerebrospinal fluid and plasma.[20,29]

During the iontophoresis treatment, tissue levels of Dex_{total} easily exceeded the minimally effective dose for Dex. Heiss et al[16] established the ED_{50} (minimally effective dose) of Dex as 75 ng/g indicating that small concentrations of Dex have anti-inflammatory effect. Dex_{total} concentrations at both measurement depths surpassed the minimally effective dose of Dex within the first 15 mA*min. However, Dex-21-oic acid has a lower affinity for the glucocorticoid receptor. Though we noted Dex_{total} greater than the minimally effective dose for Dex, it is unclear what the minimal dose of Dex-21-oic acid is needed to provide sufficient anti-inflammatory action.

The assumption of iontophoresis is that the drug concentration delivered through the skin is linearly proportional to the applied iontophoresis dose, which is defined as the current intensity multiplied by the treatment time.[26] Under this assumption, Dex_{total} should be similar at the same iontophoresis dose, regardless of current intensity. We found similar tissue levels of Dex_{total} during 1 and 2 mA treatment intensities. These data provide *in vivo* data to support the widely held expectation that transdermal drug delivery by iontophoresis is depended almost

exclusively by iontophoresis dose. Our data refutes speculation by Anderson et al[2] that smaller iontophoresis intensity may drive more Dex into the tissue. Anderson et al[2] indirectly assessed Dex delivery by measuring skin blanching, a vasoconstriction reaction attributed by Dex, after a 40 mA*min iontophoresis treatment, they noted that greater skin blanching occurring for a longer period of time and with greater magnitude when lower current intensities (0.05 to 0.16 mA) were used.

During the constant influx of drug ions during iontophoresis, passive diffusion and washout by blow flow is to be expected. In animal models, iontophoresis delivery creates a depot of the drug in the outer layers of the epidermis.[24] In addition, it appears that the delivery of anionic drugs via the cathode produces a greater depot development than anode delivery of drugs.[19,27] The anionic drug Dex-P competes with chloride ions during iontophoresis. The small molecular weight of chloride ions facilitates the direct current to carry these ions deeper into the tissue thereby leaving the larger Dex-P ions to form a depot in the epidermis.[2,34] Deeper penetration of Dex-P is hypothesized to occur by passive diffusion and/or convective transport by the microvasculature system.[2] There was a noticeably smaller amount of Dex_{total} at the 1 mm site, but not an increase in Dex_{total} at the deeper 4 mm site after the 120 mA*min iontophoresis treatment. It is likely that tissue clearance of Dex_{total} by the microvasculature system prevented any immediate diffusion mediated increase in Dex_{total} at the 4 mm sample site. Gurney et al[14] noted an association between extraction time and Dex concentration. At the semitendinosus tendon, greater Dex was associated with a longer extraction time, indicating that it may take several hours for passive diffusion of the drug to occur.

Cathodal iontophoresis produces cutaneous vasodilation. Berlinger[3] reported that the superficial vasculature increased 700% from baseline during cathode iontophoresis. The large

increase in skin perfusion at the cathode is approximately 75% of the maximum cutaneous vascular conductance.[11] These results are similar to our findings. However, in the 1 mA current intensity group skin perfusion began to return to baseline at approximately 40 mA*min and eventually reached baseline levels before the end of the iontophoresis treatment. In contrast, skin perfusion remained elevated throughout the 2 mA current treatment and returned to baseline only after the iontophoresis was terminated, requiring at least 60 min to return to baseline.

Current induced vasodilation, noted in cathode iontophoresis, is thought to be mediated by an axon reflex. The current induces primary afferent fiber excitation of C fibers creating a release of its vasodilator neurochemicals, such as substance P and calcitonin gene-related peptides.[35] Also, the production of prostaglandins is stimulated during direct current iontophoresis leading to vasodilation. Prostaglandins are also involved in hyper-sensitizing receptors located on small sensory neurons lowering the firing threshold of these neurons, creating an amplified current induced vasodilation.[35] As noted, we found differences in skin perfusion between our current intensities. It is possible that the 1 mA current stimulated afferent nerves fibers and resulted in the initial vasodilator response, but habituation occurred resulting in a reduced vasodilator signal. Conversely, the 2 mA current maintained current induced vasodilation throughout the treatment. More interesting is that the differences in current induced vasodilation between our two current intensities did not significantly affect the concentration of Dex_{total} in the tissues. Future research is needed to understand if greater current intensities would produce sufficient vasodilation to actually impact drug delivery to the underlying tissues.

Variations in our methods from previous studies[13,14,31] allowed us to monitor several time points during iontophoresis delivery to characterize the rate of accumulation of Dex in human tissue. First, we used a longer iontophoresis dose than the majority of studies. Anderson

et al[2] and Gurney and Wascher[13] used 40 mA*min iontophoresis doses, while we used a 120 mA*min dose. Our iontophoresis delivery system did not produce any skin burns with this high dose. However, our maximum current intensity was only 2.0 mA (current density = 0.16 mA/cm^2). Second, we measured Dex_{total} found in the extracellular fluid space using microdialysis. Microdialysis provided us a method to measure the time course of tissue accumulation of Dex. The lower leg provided us a safe place to insert the microdialysis probes, but some assumptions of the iontophoresis delivery and pharmacodynamics were made. We assumed that the iontophoresis delivery and pharmacodynamics of Dex-P through the stratum corneum at the posterior lower leg would be similar as if done at common treatment sites over a tendon. We also assumed a greater concentration of Dex_{total} would lead to greater cellular and clinical outcome responses.

Limitations

Our study has limitations. We used healthy individuals in order to describe the delivery characteristics of Dex-P when delivered via iontophoresis at two different current intensities. We assume that the delivery of Dex-P through the skin would be similar in an injured population. Our results are limited to inferences made between the two current intensities, 1 and 2 mA, which we selected.

Future Research

A comprehensive examination of all iontophoresis parameters in an effort to optimize drug delivery has yet to be performed in human participants. Future studies using different current intensities and different iontophoresis devices (i.e. wired phoresors vs. wireless patches) is required to establish effective parameters for clinical iontophoresis of Dex-P.

CONCLUSION

Using microdialysis, we successfully measured transdermal drug delivery of Dex-P over the course of an iontophoresis treatment *in vivo* through human skin. When delivered through human skin Dex-P accumulated primarily as the hydrolyzed and less potent Dex metabolite, Dex-21-oic acid. However, based on the significant concentrations of Dex_{total}, compared to the minimally effective dose of Dex, which was recovered at 1 and 4 mm tissue depths, we believe Dex-P iontophoresis to be an effective modality at treating tendonitis conditions. Dex_{total} recovery increased throughout the iontophoresis treatment at similar rates between the 1 and 2 mA current intensities indicating no difference in drug delivery between our high and low current intensities. Current induced vasodialation was prolonged throughout the treatment when treating with 2 mA current intensity, but did not significantly affect the tissue accumulation of Dex_{total}.

REFERENCES

1. Amirjani N, Ashworth NL, Watt MJ, Gordon T, Chan KM. Corticosteroid iontophoresis to treat carpal tunnel syndrome: a double-blind randomized controlled trial. *Muscle Nerve.* 2009;39(5):627-633.

2. Anderson CR, Morris RL, Boeh SD, Panus PC, Sembrowich WL. Effects of iontophoresis current magnitude and duration on dexamethasone deposition and localized drug retention. *Phys Ther.* 2003;83(2):161-170.

3. Berliner MN. Skin microcirculation during tapwater iontophoresis in humans: cathode stimulates more than anode. *Microvasc Res.* 1997;54(1):74-80.

4. Bjordal JM, Demmink JH, Ljunggren AE. Tendon thickness and depth from skin for supraspinatus, common wrist and finger extensors, patellar and Achilles tendons: ultrasonography study of healthy subjects. *Physiotherapy.* 2003;89(6):375-383.

5. Blackford J, Doherty TJ, Ferslew KE, Panus PC. Iontophoresis of dexamethosone-phosphate into the equine tibiotarsal joint. *J Vet Pharmacol Ther.* Aug 2000;23(4):229-236.

6. Cázares-Delgadillo J, Balaguer-Fernández C, Calatayud-Pascual A, et al. Transdermal iontophoresis of dexamethasone sodium phosphate in vitro and in vivo: effect of experimental parameters and skin type on drug stability and transport kinetics. *Eur J Pharm Biopharm.* 2010;75(2):173-178.

7. Chen Q, Zielinski D, Chen J, Koski A, Werst D, Nowak S. A validated, stability-indicating HPLC method for the determination of dexamethasone related substances on dexamethasone-coated drug-eluting stents. *J Pharm Biomed Anal.* 2008;48(3):732-738.

8. Cleland JA, Abbott JH, Kidd MO, et al. Manual physical therapy and exercise versus electrophysical agents and exercise in the management of plantar heel pain: a multicenter randomized clinical trial. *J Orthop Sports Phys Ther.* 2009;39(8):573-585.

9. Coglianese M, Draper DO, Shurtz J, Mark G. Microdialysis and delivery of iontophoresis-driven lidocaine into the human gastrocnemius muscle. *J Athl Train.* 2011;46(3):270-276.

10. Draper DO, Coglianese M, Castel C. Absorption of iontophoresis-driven 2% lidocaine with epinephrine in the tissues at 5 mm below the surface of the skin. *J Athl Train.* 2011;46(3):277-281.

11. Durand S, Fromy B, Bouyé P, Saumet JL, Abraham P. Current-induced vasodilation during water iontophoresis (5 min, 0.10 mA) is delayed from current onset and involves aspirin sensitive mechanisms. *J Vasc Res.* 2002;39(1):59-71.

12. Glass JM, Stephen RL, Jacobson SC. The quantity and distribution of radiolabeled dexamethasone delivered to tissue by iontophoresis. *Int J Dermatol.* 1980;19(9):519-525.

13. Gurney AB, Wascher DC. Absorption of dexamethasone sodium phosphate in human connective tissue using iontophoresis. *Am J Sports Med.* 2008;36(4):753-759.

14. Gurney B, Wascher D, Eaton L, Benesh E, Lucak J. The effect of skin thickness and time in the absorption of dexamethasone in human tendons using iontophoresis. *J Orthop Sports Phys Ther.* 2008;38(5):238-245.

15. Guy RH, Kalia YN, Delgado-Charro MB, Merino V, López A, Marro D. Iontophoresis: electrorepulsion and electroosmosis. *J Control Release.* 2000;64(1-3):129-132.

16. Heiss JD, Papavassiliou E, Merrill MJ, et al. Mechanism of dexamethasone suppression of brain tumor-associated vascular permeability in rats. Involvement of the glucocorticoid receptor and vascular permeability factor. *J Clin Invest.* 1996;98(6):1400-1408.

17. Hong SU, Park KH, Chae JS, Lee JW, Kim CS, Kim JK. Pharmacological evaluation of five novel dexamethasone heneicosoic (21) acid derivatives. *Drugs Exp Clin Res.* 1989;15(11-12):511-520.

18. Jain R, Jain E, Dass AG, Wickstrom O, Walter N, Atkinson PJ. Evaluation of transdermal steroids for trapeziometacarpal arthritis. *J Hand Surg [Am].* 2010;35(6):921-927.

19. James MP, Graham RM, English J. Percutaneous iontophoresis of prednisolone: a pharmacokinetic study. *Clin Exp Dermatol.* 1986;11(1):54-61.

20. Kroin JS, Schaefer RB, Penn RD. Chronic intrathecal administration of dexamethasone sodium phosphate: pharmacokinetics and neurotoxicity in an animal model. *Neurosurgery.* 2000;46(1):178-182.

21. Neeter C, Thome R, Silbernagel KG, Thome P, Karlsson J. Iontophoresis with or without dexamethazone in the treatment of acute Achilles tendon pain. *Scand J Med Sci Sports.* 2003;13(6):376.

22. Nirschl RP, Rodin DM, Ochiai DH, Maartmann-Moe C. Iontophoretic administration of dexamethasone sodium phosphate for acute epicondylitis: a randomized, double-blind, placebo-controlled study. *Am J Sports Med.* 2003;31(2):189-195.

23. Osborne HR, Allison GT. Treatment of plantar fasciitis by LowDye taping and iontophoresis: short term results of a double blinded, randomised, placebo controlled clinical trial of dexamethasone and acetic acid. *Br J Sports Med.* 2006;40(6):545-549.

24. Paturi J, Kim HD, Chakraborty B, Friden PM, Banga AK. Transdermal and intradermal iontophoretic delivery of dexamethasone sodium phosphate: quantification of the drug localized in skin. *J Drug Target.* 2010;18(2):134-140.

25. Pellecchia GL, Hamel H, Behnke P. Treatment of infrapatellar tendinitis: a combination of modalities and transverse friction massage versus iontophoresis. *J Sport Rehabil.* 1994;3(2):135-145.

26. Phipps JB, Padmanabhan RV, Lattin GA. Iontophoretic delivery of model inorganic and drug ions. *J Pharm Sci.* 1989;78(5):365-369.

27. Riviere JE, Monteiro-Riviere NA, Inman AO. Determination of lidocaine concentrations in skin after transdermal iontophoresis: effects of vasoactive drugs. *Pharm Res.* 1992;9(2):211-214.

28. Runeson L, Haker E. Iontophoresis with cortisone in the treatment of lateral epicondylalgia (tennis elbow): a double-blind study. *Scand J Med Sci Sports.* 2002;12(3):136.

29. Samtani MN, Jusko WJ. Stability of dexamethasone sodium phosphate in rat plasma. *Int J Pharm.* 2005;301(1–2):262-266.

30. Schnetz E, Fartasch M. Microdialysis for the evaluation of penetration through the human skin barrier - a promising tool for future research? *Eur J Pharm Sci.* 2001;12(3):165-174.

31. Smutok MA, Mayo MF, Gabaree CL, Ferslew KE, Panus PC. Failure to detect dexamethasone phosphate in the local venous blood postcathodic iontophoresis in humans. *J Orthop Sports Phys Ther.* 2002;32(9):461-468.

32. Stahl M, Bouw R, Jackson A, Pay V. Human microdialysis. *Curr Pharm Biotechnol.* 2002;3(2):165-178.

33. Stefanou A, Marshall N, Holdan W, Siddiqui A. A randomized study comparing corticosteroid injection to corticosteroid iontophoresis for lateral epicondylitis. *J Hand Surg [Am]*. 2012;37(1):104-109.

34. Sylvestre JP, Díaz-Marín C, Delgado-Charro MB, Guy RH. Iontophoresis of dexamethasone phosphate: competition with chloride ions. *J Control Release.* 2008;131(1):41-46.

35. Tartas M, Bouyé P, Koïtka A, et al. Cathodal current-induced vasodilation to single application and the amplified response to repeated application in humans rely on aspirin-sensitive mechanisms. *J Appl Physiol.* 2005;99(4):1538-1544.

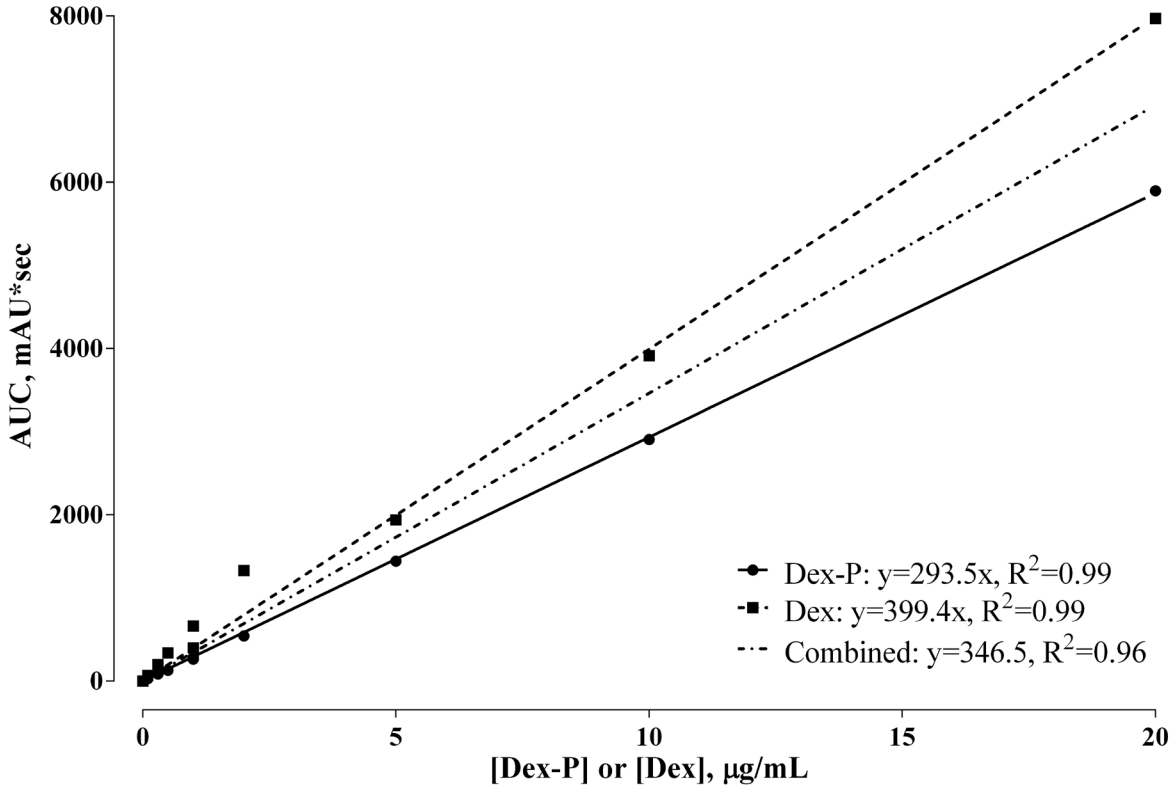

FIGURE 1. RP-HPLC standard curves of dexamethasone sodium phosphate (Dex-P), dexamethasone (Dex) and their combined standard curve for Dexamethasone-21-oic acid calculation. Each point represents the mean area under the absorbance (239 nm) time curve (AUC) of duplicate runs.

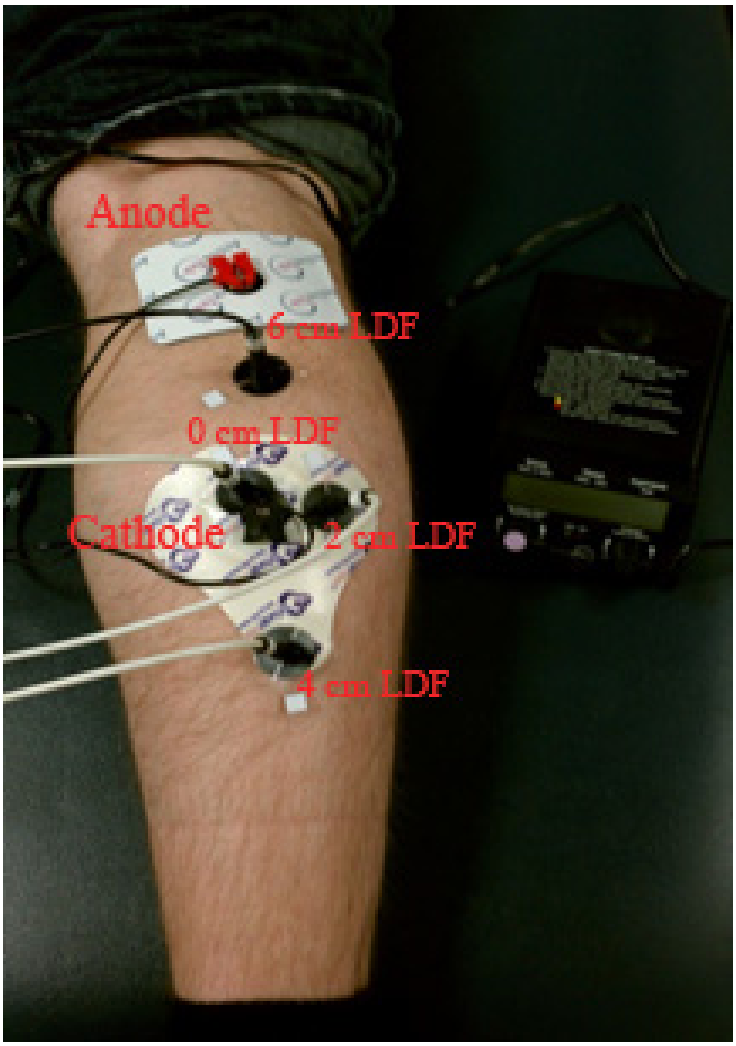

FIGURE 2. Placement of laser Doppler flowmeter probes to measure skin perfusion during iontophoresis treatment. Laser Doppler flowmeter probes were placed inside the drug chamber (0 cm), just outside the drug chamber (2 cm) and 4 and 6 cm from the center of the drug electrode.

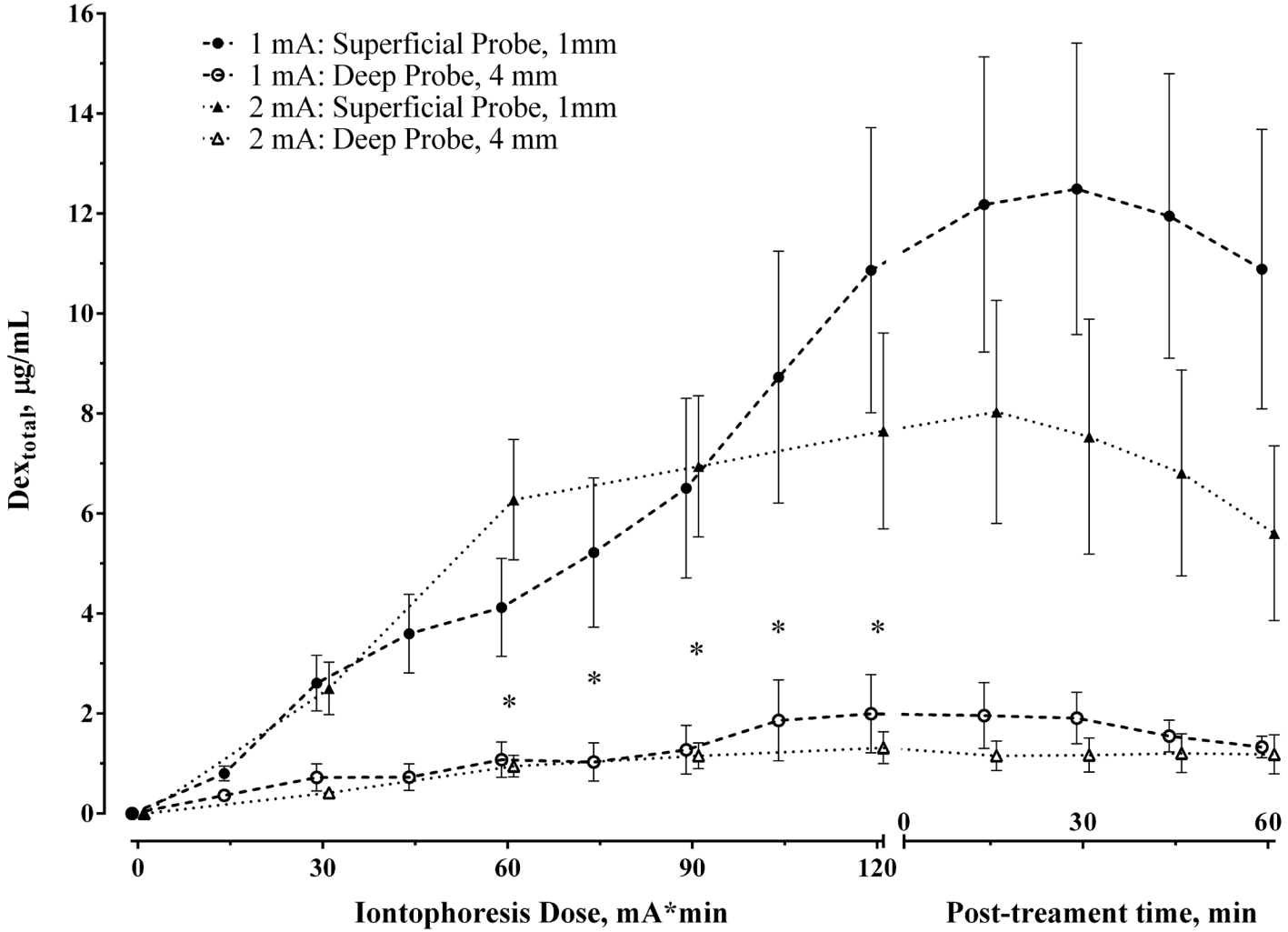

FIGURE 3. Dex_{total} concentrations (Dex_{total} = Dexamethasone sodium phospate+Dexamethason+Dexamethasone-21-oic acid) between 1 and 2 mA intensities and different depth over a 120 mA*min iontophoresis dose. Values are mean ± 1 SEM for 8 participants in each group. *= Indicates significant difference of Dex_{total} between 1 and 4 mm at a given current intensity ($p<0.05$).

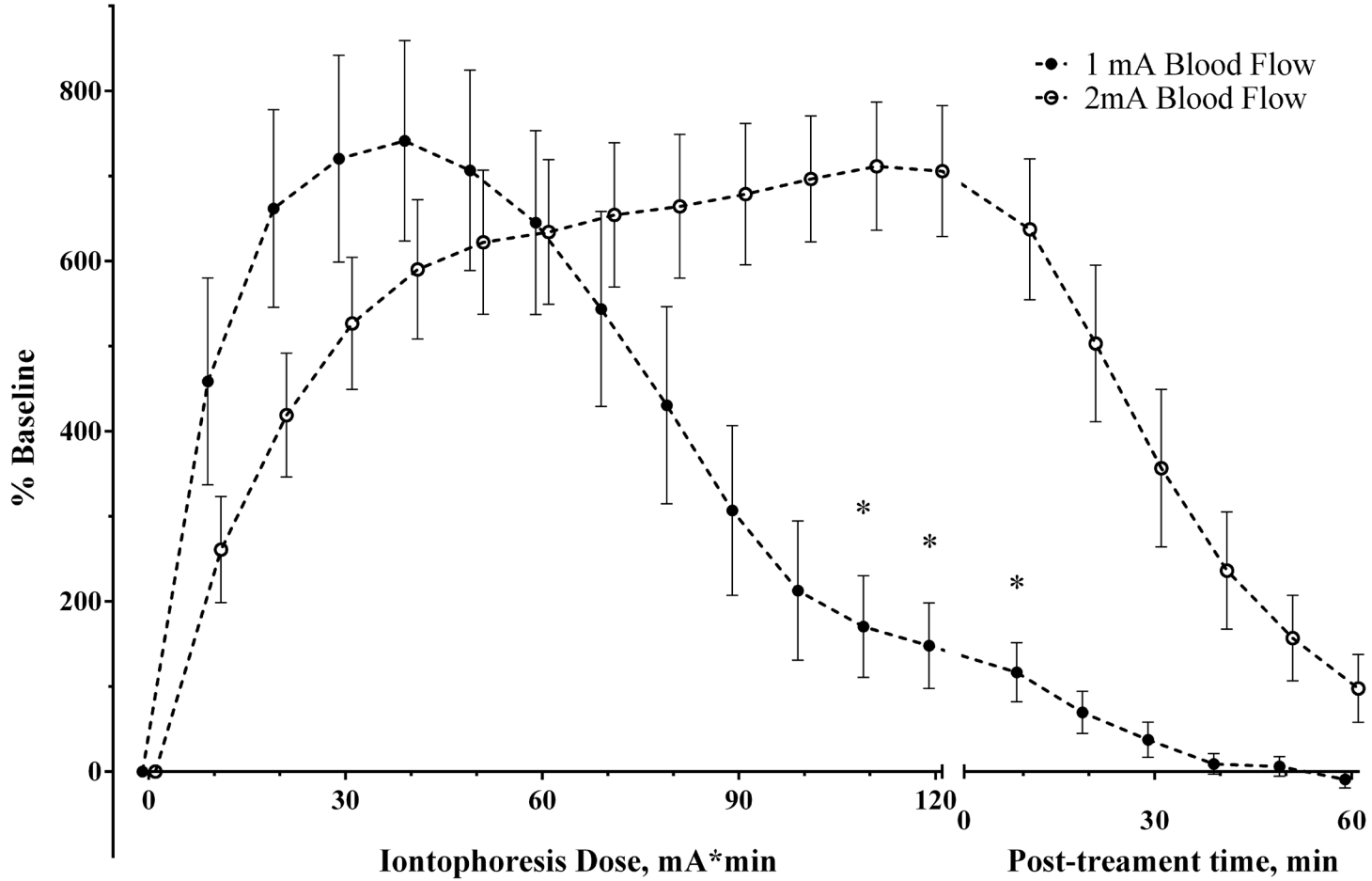

Figure 4. Superficial skin perfusion response of 1 and 2 mA current intensities during a 120 mA*min iontophoresis dose. Vales are mean ± 1 SEM for 12 participants in each group. * = Indicates significant difference between 1 and 2 mA treatment intensities ($p<0.05$).

Appendix A
Reverse-Phase Higher Performance Liquid Chromatography Methods

Instrument Model: Agilent 1260 (Agilent Technologies Inc., Santa Clara, CA)
Column: Zorbax eclipse XDB-C8 4.6 x 150 mm 5µm

Binary Pump (model: G1212B)
Flow: 0.650 mL/min
Use solvent types: Yes
Low pressure limit: 0.30 bar
High pressure limit: 600.00 bar
Maximum flow gradient: 100.00 mL/min^2

Stop time: 17.00 min
Post time: 1.00 min

Solvent: Aminium formade (20mM) / acetonitrile (70/30) in pH 3.8 adjusted with formic acid

Sampler (model: G1329B)
Draw speed: 200.00 µL/min
Ejection speed: 200.00 µL/min
Injection volume: 20.00 µL

Column Comp.
Temperature: 45.0° C

DAD (model: G4212B)
Peakwidth: >0.10 min (2.0 sec response time)
Signals
A. Wavelength: 239.0 nm (reference 360 nm)