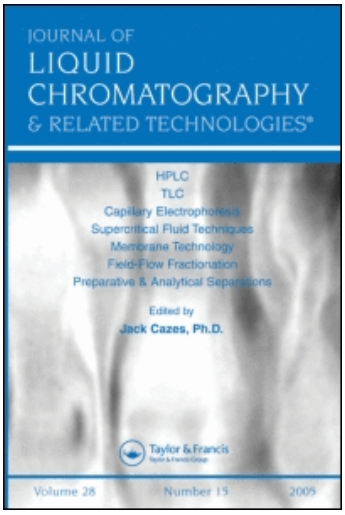

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Purity Determination of Alprostadil by Micellar Electrokinetic Chromatography with Signal Enhancement Involving Field-Amplified Sample Stacking and Extended Path Length Detection

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Abstract: Two related procedures based on micellar electrokinetic chromatography (MEKC) were developed and validated for purity determination of alprostadil. Alprostadil is the active ingredient in Caverject DCS, indicated for erectile dysfunction. The techniques of field-amplified sample stacking and high sensitivity optical cell enhancement were used. For the former, the sample was injected for 20 s under vacuum followed by a sample buffer zone backout at −10 kV for 0.9 min. For the latter, which relies on extended path length detection via a capillary/z-cell configuration, the sample was injected under vacuum for 5 s with no stacking of the sample zone. All process and degradation impurities were separated from the internal standard and from PGE_1 except for 11-epi-PGE_1, which appears as a shoulder on the front edge of the PGE_1 peak. The precision for both techniques meets current validation expectations, generally below ±1%, always below ±2%. Linearity/recovery from 70–120% using reversed polarity electrostacking resulted in a mean recovery of 100.0% with an RSD of 0.81%. For the capillary/z-cell the mean recovery was 100.9% with an RSD of 1.3%. Results obtained by both MEKC procedures on two lots of alprostadil were comparable to HPLC. There was no statistical difference

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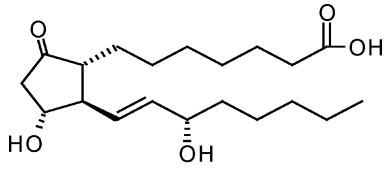
between means at the 95% confidence interval. Hence, the two variants on MEKC provide an orthogonal means to HPLC for assessing the purity of alprostadil.

Keywords: MEKC, Capillary/z-cell, Extended path length detection, Field-amplified sample stacking, Alprostadil, PGE_1, Electrostacking, Pharmaceutical analysis

INTRODUCTION

Alprostadil, or PGE_1, a prostaglandin, is the active ingredient in Caverject® Dual Chamber Syringe (DCS), a product marketed by Pfizer for treatment of erectile dysfunction. The lack of a chromophore in alprostadil has presented challenges to purity measurement by HPLC. Historically, it has been reacted with bromo-2′-acetonapthone (α-BAN) to produce a colored derivative, which is then quantitated by normal phase LC. More recently, a reversed phase procedure has been developed that utilizes end absorption at either 200 or 214 nm (unpublished). Although an improvement over the earlier method, the reversed phase procedure suffers from the vagaries of short wavelength detection, principally related to the long pathlength required.



alprostadil; PGE_1

By comparison, capillary electrophoresis (CE) and related techniques are more immune to this difficulty because of a much shorter pathlength (equal to the internal diameter of the capillary). Offsetting this gain, however, is the poorer solution concentration sensitivity of CE, which is 2–3 orders of magnitude poorer than that of HPLC.[1]

Various approaches have been devised to address this loss in sensitivity. One has been to bend a portion of the capillary and, thereby, configure it as a z-cell.[2,3] Perkin-Elmer-Applied Biosytems achieved an approximate 14-fold signal enhancement using a z-cell with a 75 μm i.d. capillary (cell made by LC Packings). A drawback to the LC Packings design is that the capillary/z-cell is a nondetachable unit. Hence, it is fragile, and, furthermore, costs nearly $1000 apiece. In contrast, Hewlett-Packard (HP) introduced a capillary/z-cell assembly that may be disassembled.[4] A more widely adopted approach is embodied by the bubble cell,[1,5] commercialized by HP. However, due to engineering constraints, the gain realized has been limited to about three.

An alternative approach to sensitivity enhancement is through use of on-line concentration techniques. The coupling of capillary isotachophoresis to CE has

been extensively explored, but can be instrumentally complex, and may require considerable method development in choosing leading and terminating electrolytes.[6] An experimentally more tractable approach is to invoke variations on the CE experiment and perform all steps in the same capillary.[7] An early review of the various schemes proposed was given by Chien and Burgi.[7] More recent reviews have been presented by Chien[8,9] and others.[10–12]

Sample stacking, the simplest and most straightforward concentration scheme, occurs whenever the sample is injected in a buffer solution of lower ionic strength than the run buffer. Its drawback is that the concentration effect is small. In field-amplified injection, a large sample is injected electrokinetically in a low concentration sample buffer, then concentrated at the sample/run buffer interface as the sample buffer is removed electroosmotically from the capillary under the influence of a reversed polarity electric field. The primary drawback to this technique is that, because the sample is injected electrokinetically, the injected sample is no longer representative of the original sample since the concentration factor for each component depends on its electrophoretic mobility.

Polarity-reversed sample stacking differs from field-amplified injection in that the sample is injected hydrodynamically (by pressure or vacuum) rather than electrokinetically; hence, there is no sample discrimination.

We used polarity-reversed sample stacking as applied to anions in the present project. Publications by Chien[8,9] and Chien and Burgi[7] may be consulted for illustrations that show how sample stacking works. The concentration factor that may be realized is given by

$$C_{i\,run} = \gamma C_{i\,spl}$$

where $C_{i\ spl}$ is the concentration of i in the sample buffer, $C_{i\ run}$ is the concentration in the run buffer, and γ is the ratio of resistivities, $\gamma = \rho_{spl}/\rho_{run}$, in the sample and run buffers. The resistivities in turn are inversely proportional to concentration (conductivity). Coincident with the concentration factor is a reduction in the width of the sample zone, given by $x_i = x_{inj}/\gamma$. Although it may appear that virtually any concentration factor can be achieved simply by making γ large, that is not the case. For large γ, a difference in localized electroosmotic flows (EOFs) sets up an electroosmotic pressure differential, which in turn induces a laminar flow profile at the interfacial boundary.[7] Since it is the electroosmotically induced flat flow profile that is responsible for the high efficiencies in CE, formation of a parabolic flow profile leads to self-defeating band broadening. Hence, since stacking and broadening work against one another, a compromise is therefore necessary. According to Burgi and Chien, originators of the technique, the sample should be prepared in a buffer that is about 10 × less concentrated than the run buffer.[13] The techniques of field-amplified injection and polarity-reversed sample stacking are sometimes collectively referred to as field-amplified sample stacking (FASS).

Many on-line concentration schemes have been published utilizing these concepts, e.g., refs.[14–21] including extension to neutral species.[17,22–25]

Most applications are to trace analysis, in physiological media[26–29] or for environmental analysis.[17,30,31] Presently, considerable effort is being directed at achieving large preconcentration factors in microfabricated devices.[32,33] Application to pharmaceutical dosage forms appears to have been rare despite the apparent benefit for impurities determinations. However, in the presence of a great excess of a charged drug, the requirement of a low conductivity sample zone is violated, thereby precluding large preconcentration factors. The higher the concentration in the original sample, the harder it is to achieve meaningful concentration, especially without peak distortion. In the present instance only moderate amplification was required for a drug substance that possesses sparing water solubility. We report here on two variants of an MEKC procedure developed to determine the purity of alprostadil bulk drug. One utilizes field-amplified sample stacking (FASS), the other extended path length detection (EPLD) through use of the capillary/z-cell.

EXPERIMENTAL

Instrumentation

Experiments were conducted on a PE-ABI 270A and on a PE-ABI 270HT electrophoretic analyzer. The former was used only for development while the latter was used both for development and validation. For most studies the 270HT autosampler was cooled to 6°C using a Neslab RTE-5DD refrigerated circulating bath. Cooling is required in order to inhibit degradation of PGE_1 to PGA_1. PGE_1 degrades to PGA_1 at the rate of 0.24 μg mL^{-1} h^{-1} at room temperature. In the present case, at a concentration of 200 μg/mL (prescribed in the method), this would lead to more than 1% degradation in 10 hours, hence the use of a refrigerated autosampler.

Capillaries

Untreated fused silica capillaries were purchased with precut detection windows. Nearly all experiments were conducted on 50 μm i.d. capillaries. For enhanced sensitivity detection via path length extension, an integrated capillary/z-cell combination marketed as a High Sensitivity Optical Cell Assembly (LC Packings, San Francisco) was used; the diameter was 50 μm. Capillaries were preconditioned with 1 N NaOH for at least 30 min, then rinsed with water for an equivalent period. A 0.1 N NaOH solution was employed as a rinse between samples. Our normal regimen consisted of rinsing the capillary with 1 N NaOH daily for about 15 min prior to commencing runs, then storing in water overnight. In this fashion we were able to achieve reasonably consistent elution times. The nominal total length was 72 cm and the length-to-detector 50 cm.

Reagents

The buffer salts sodium phosphate, dibasic, anhydrous powder ('Baker Analyzed'), sodium phosphate, dibasic, crystal ('Baker Analyzed'), and sodium phosphate, monobasic, monohydrate, crystal ('Baker Analyzed') were purchased from J.T. Baker. Lauryl sulfate (sodium dodecyl sulfate) was obtained from Sigma (SigmaUltra). Tetramethylammonium perchlorate (TMAP), cholic acid, and β-cyclodextrin, sulfated were obtained from GFS, Sigma, and Aldrich, respectively. All internal standards investigated were obtained from Aldrich, including 3-hydroxycinnamic acid ($HOC_6H_4CH{=}CHCO_2H$, 99%), used initially, and 3,4-dihydroxyhydrocinnamic acid ($(HO)_2C_6H_3CH_2CH_2CO_2H$, 98%), adopted for the final method. Purified water was obtained from a Milli Q water purification system, isopropanol from Burdick & Jackson, and ethanol from Quantum Chemical.

The prostaglandins were obtained either from the Pharmacia & Upjohn Control Reference Standards (CRS) group or from Cayman Chemical (Ann Arbor, MI). PGE_1, PGA_1, and PGB_1 were obtained from the CRS laboratory; all are solids. Of the remaining prostaglandins, all were solids except for 15-keto-PGE_1 and 13,14-dihydro-PGE_1. These two were furnished by Cayman as solutions in methyl acetate. To prepare (semi-quantitatively) standards of these two, an appropriate volume was microlitered into a vial, the methyl acetate solvent then blown to dryness.

Preparation of Solutions

In preparing a run buffer, separately prepared 100 mM stock solutions of sodium phosphate, monobasic, and sodium phosphate, dibasic, were mixed in appropriate amounts to achieve the desired pH of 7.0. Sodium dodecyl sulfate (SDS), isopropanol, and the 100 mM phosphate stock were then combined and the solution diluted with purified water to achieve the final composition: {pH 7.0, 30 mM phosphate, 35 mM SDS, 10% IPA}. This solution was filtered through a 0.45 μm filter, then ultrasonicated with vacuum daily, prior to use.

The sample buffer consisted of 2 mM phosphate, prepared by diluting 10 mM phosphate, pH 7.6, five-fold. The internal standard solution was composed of 0.11 mM 3,4-dihydroxyhydrocinnamic acid (3,4-DHHCA) dissolved in sample buffer. The internal standard solution was prepared daily, as 3,4-DHHCA degrades at room temperature. The sample was subsequently prepared by weighing 4.0 mg of alprostadil into a suitable vial, adding 1.0 mL of ethanol, swirling/ultrasonicating to dissolve, then adding 19.0 mL of internal standard solution and mixing well; the sample was not filtered. Approximately 500 μL aliquots of the sample preparation were transferred to 0.6 mL polypropylene microcentrifuge tubes, then placed in the refrigerated autosampler tray.

Procedure

The nominal (total) capillary length was 72 cm (50 cm to detector), and the diameter 50 μm. For polarity-reversed electrostacking, vacuum injection (5 in Hg) was applied for 20 s followed by a 0.9 min sample buffer backout at −10 kV. For the capillary/z-cell, vacuum injection (5 in Hg) was applied for 5 s, but with no concentration of the sample zone. After injection (and backout), normal polarity voltage of 25 kV was applied; the current was about 29 μA. The time constant was 0.5 s and detection was at 200 nm.

Method Development/Optimization

General method development was conducted on a 50 μm capillary without application of either field-amplified sample stacking or extended path length detection. The development had principally to do with buffer selection, sample preparation, and optimization of capillary and instrumental parameters. Further optimization was then conducted separately for each of the two procedures.

Data Acquisition and Integration

AccessChrom (PE) with a PE Nelson 900 interface box was used for data acquisition and workup. Peak areas were divided by their elution times to obtain adjusted peak areas ($area_{adj}$). $Area_{adj}$ compensates for area bias due to shifts in migration time. Quantitation was by internal standard.

Validation/Quantitation

Separate validation was conducted for 1) field amplified sample stacking (FASS) on a normal 50 μm capillary, and for 2) the high sensitivity optical cell. More extensive validation was conducted for FASS, which we addressed first. Linearity and recovery were assessed from 70–120% of the assay concentration. Precision testing consisted of system precision, precision for replicate preparations, and precision at 80% of the target concentration. Selectivity and signal enhancement for both FASS and extended path length detection were assessed. The developed procedures were tested on two lots of alprostadil. Six replicates of each were assayed. These results were then compared to HPLC results generated in-house using a validated reversed phase procedure (vide supra).

RESULTS AND DISCUSSION

Method Development

Alprostadil has a pK_a of ~4.95 ($K_a = 1 \times 10^{-5}$). A pH where the prostaglandins are ionized would be a logical first choice for separation by CE. Although CZE proved largely ineffective in separating the prostaglandins of interest, MEKC showed considerable promise. In fact, it proved difficult to realize any meaningful separation of the prostaglandins without inclusion of a surfactant in the run buffer. Earlier work conducted by us on a freeze-dried formulation of alprostadil suggested that MEKC, in conjunction with polarity-reversed sample stacking, would be workable for the determination of PGE_1 and PGA_1 (unpublished results). A preliminary electropherogram resulting from this earlier work is shown in Figure 1. This electropherogram shows only PGE_1, PGA_1, the principal degradation impurity, and PGB_1, another possible impurity. The buffer consisted of pH 7.0, 20 mM phosphate, 5 mM TMAP, 30 mM SDS, and 10% IPA; 3-hydroxycinnamic acid was identified as a possible internal standard. The purpose of the TMAP was to enhance separation between PGA_1 and PGB_1 through ion-pairing. However, we later concluded that TMAP was unnecessary.

The impurities shown in Figure 2 are the known process and degradation impurities of alprostadil. Of these, only PGA_1 is a significant degradation impurity. Although we sought to separate all of these potential impurities from one another, for the purposes of determining the purity of alprostadil bulk drug, it was only necessary to separate them from the major component. Upon attempting to separate the compounds of Figure 2 using

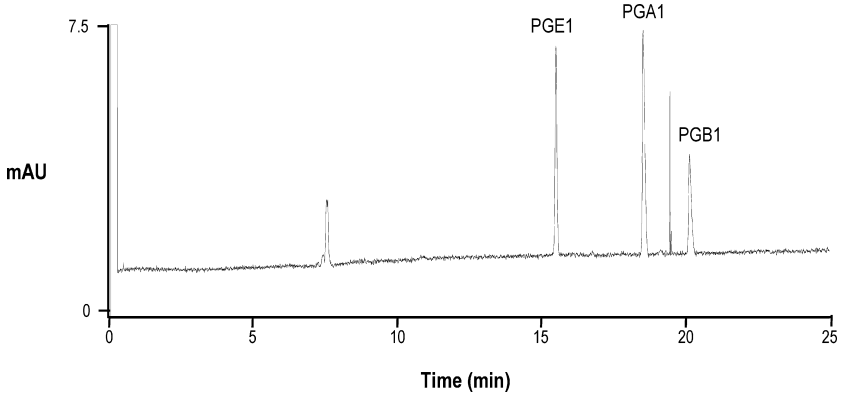


Figure 1. An early separation of prostaglandins by MEKC. The run buffer consisted of pH 7.0, 20 mM phosphate, 5 mM TMAP, 30 mM SDS, and 10% IPA. The applied potential was 25 kV for a 72 cm (50 cm to detector) × 50 μm i.d. capillary. Detection was at 200 nm.

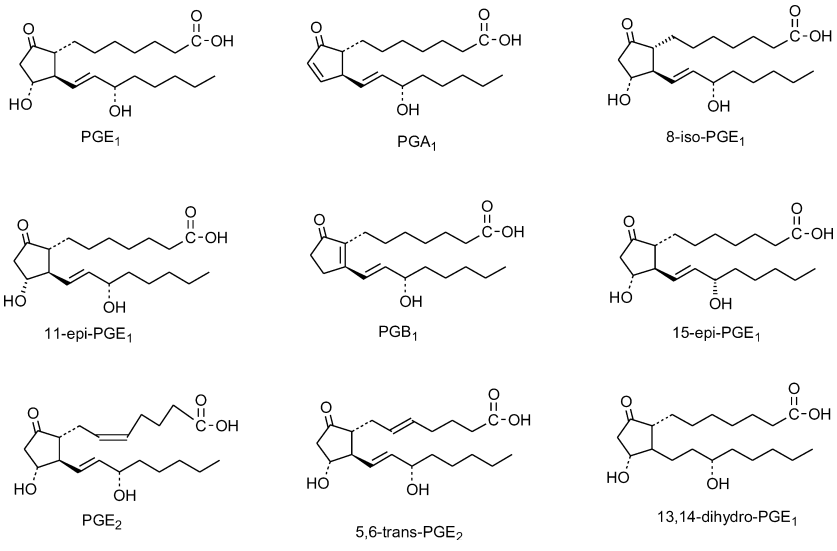


Figure 2. Structures of alprostadil (PGE_1) and possible process and degradation impurities.

{pH 7.0, 30 mM phosphate, 30 mM SDS, 10% IPA} as the run buffer and 3-hydroxycinnamic acid (3-HCA) as the internal standard, 3-HCA coeluted with PGE_2 and was just baseline resolved from 8-iso-PGE_1. Additionally, 11-epi-PGE_1 was unresolved from PGE_1, although there was some separation when the SDS concentration was increased. The corresponding separations achieved at 20 mM and 40 mM SDS are shown in Figure 3.

As an alternative approach, we attempted to separate these two compounds by incorporating a sulfated β-cyclodextrin (CD) into the run buffer. Although both 11-epi-PGE_1 and PGE_1 formed a guest-host complex with the CD (apparent by an increase in the migration time), there was no differentiation, i.e., no change in selectivity, hence no improvement in the separation. For {pH 7.0, 30 mM phosphate, 30 mM SDS, 10% IPA} as the run buffer, substitution of 3,4-dihydroxyhydrocinnamic acid (3,4-DHHCA) for 3-HCA resulted in selective, earlier elution of the internal standard and PGE_2 and 8-iso-PGE_1 were now well separated from the internal standard. However, 3,4-DHHCA coeluted with the earliest eluting impurity, 15-keto-PGE_1. In order to achieve separation of the internal standard from 15-keto-PGE_1, two approaches were investigated: 1) search for a different internal standard; and 2) selectively retard 15-keto-PGE_1 relative to 3,4-DHHCA by increasing the concentration of SDS in the run buffer.

Four additional internal standards were evaluated: 4-hydroxyphenylacetic acid (4-HPAA), 2-hydroxyphenylacetic acid (2-HPAA), phenylacetic acid (PAA), and 3,4-dihydroxyphenylacetic acid (3,4-DHPAA). It turned out that

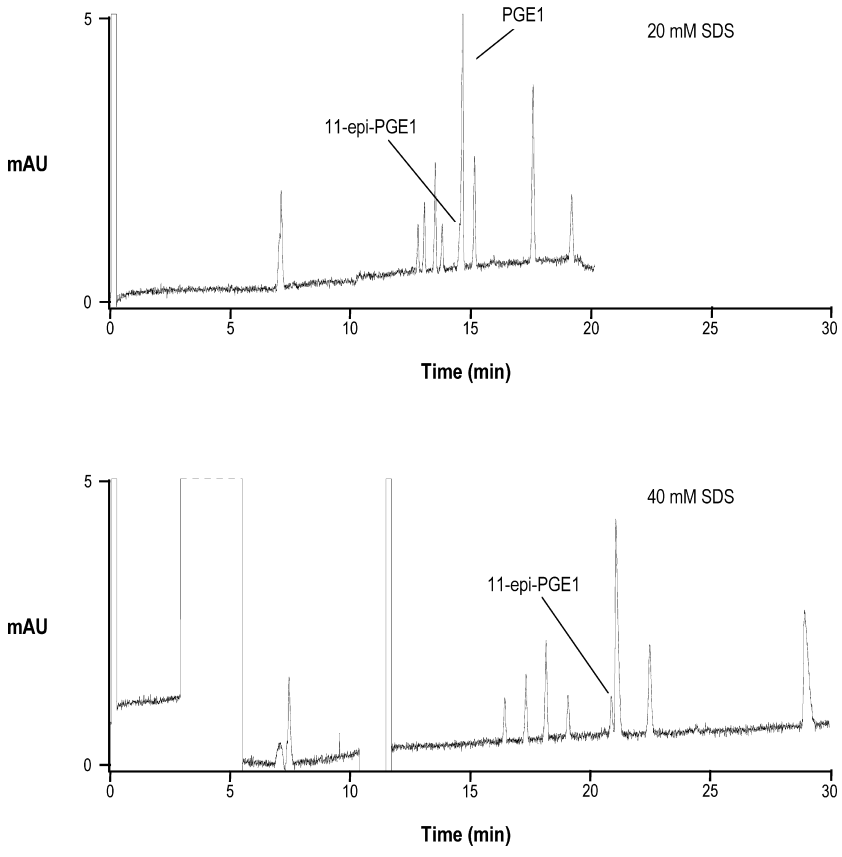


Figure 3. Separation of 11-epi-PGE_1 from PGE_1 as a function of SDS concentration: 20 mM (top), and 40 mM (bottom). The remaining conditions are as in Figure 1.

each of these compounds eluted after 15-keto-PGE_1, and hence, were less satisfactory than 3,4-DHHCA. Keeping 3,4-DHHCA as the internal standard, we next examined the effect of varying the concentration of SDS. Increasing the concentration from 30 to 35 mM resulted in the electropherogram shown in Figure 4. 3,4-DHHCA is seen to be well separated from 15-keto-PGE_1. The small peak on the front side of the 3,4-DHHCA peak is an unidentified degradation product of 3,4-DHHCA. (3,4-DHHCA must be prepared daily.) The small, spurious peaks in Figure 4 were the result of general degradation of an impurities mixture that was several weeks old. Note that the electropherogram of Figure 4 was generated using optimized FASS conditions (vide infra).

As noted earlier, PGE_1 possesses only end absorption. Hence, detection at short wavelength was mandated; we selected 200 nm. ε_{200} was calculated to be 3.8×10^3 L cm^{-1} mol^{-1}. As molar absorptivities go, this represents a rather weak chromophore. Hence, in order to achieve an acceptable S/N,

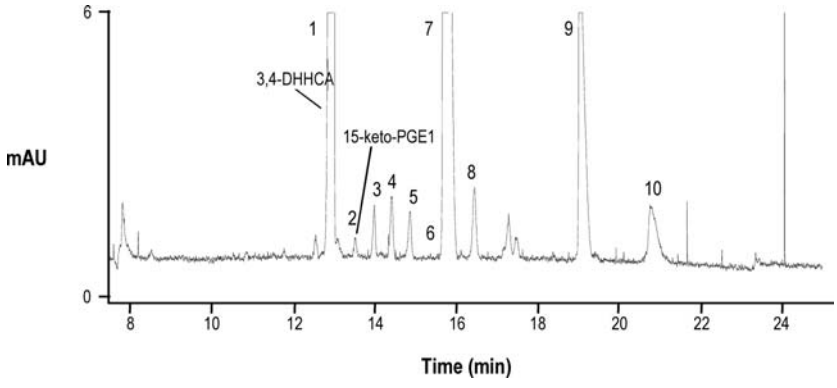


Figure 4. Separation of 3,4-DHHCA from 15-keto-PGE_1 achieved with 35 mM SDS. Run buffer: pH 7.0, 30 mM phosphate, 35 mM SDS, 10% IPA. Optimized FASS conditions were applied: 20 s injection, −10 kV reversal potential, 0.9 min reversal time. The separation was then achieved at 25 kV. Peak identification: **3**, 15-epi-PGE_1; **4**, PGE_2; **5**, 8-iso-PGE_1; **6**, 13,14-dihydro-PGE_1 (not detected); **7**, PGE_1 + 11-epi-PGE_1; **8**, 5,6-trans-PGE_2; **9**, PGA_1; **10**, PGB_1 (+ an unknown interference).

either a concentrated sample preparation or some sort of signal enhancement was required. In exploring the former option, we determined that at a sample preparation concentration greater than about 0.5 mg/mL, PGE_1 precipitated from solution. Although a PGE_1 concentration of 0.5 mg/mL could be used without deleterious effect, it was deemed insufficient to ensure accurate and precise quantitation, hence the current project in which both field amplified sample stacking and extended path length detection were investigated as means of signal enhancement.

Reversed Polarity Electrostacking Optimization

Various combinations of sample plug length (injection time), reversal voltage, and reversal time were examined. The parameters monitored were signal enhancement, efficiency, and reproducibility. With respect to the last, the system had to be sufficiently repeatable so that no sample was lost in the reversal step. Sample plug length (t) was varied from 15–60 s, $V_{reversal}$ between −10 and −20 kV, and $t_{reversal}$ from 0.4–2.0 min.

Initial testing for a 30 s sample plug with −20 kV reversal showed 1.0 min to be a suitable reversal time. For longer sample plugs, the signal enhancement was greater than at 30 s; however, peak distortion set in. At the longest backout time (2 min), some of the sample was lost. The effectiveness of the backout may be gauged by noting the EOF disturbance in the electropherogram. If an EOF upset is not discernible, then the reversal time is too long, i.e., the entire sample buffer zone was backed out of the capillary.

We attained optimal performance using a 20 s injection plug with a −10 kV reversal potential applied for 1.0 min. The signal enhancement was 5.5-fold when compared to a 2.5 s vacuum injection without sample reversal. The sample buffer consisted of either 2 or 5 mM phosphate. Although only a modest gain, 5.5-fold amplification resulted in acceptable performance as gauged by subsequent method validation (vide infra).

The effect of concentrating the sample via FASS can be seen in Figure 5. The top electropherogram shows PGE_1 injected from the run buffer with 2.5 s of vacuum without sample reversal, hence no sample concentration. The middle panel is of PGE_1 injected from sample buffer with 2.5 s of vacuum applied and no sample reversal, thereby achieving sample concentration via electrostacking only. The bottom electropherogram shows PGE_1 injected from the sample buffer with reversed-polarity electrostacking. The highest efficiency was obtained for injection from the sample buffer (electrostacking, middle panel), but the greatest response was achieved by FASS (bottom panel).

As was noted, there is a practical upper limit to the difference in concentration between the sample and run buffers as a result of the electroosmotic back pressure that develops, which in turn produces a laminar flow profile that leads to reduced plate count. A rule of thumb states that a roughly 10-fold difference is considered optimal.[13] Using a run buffer phosphate concentration of 30 mM at pH 7.0, we examined the effect of sample buffer concentrations of 1, 2, and 5 mM phosphate (PGE_1 present at $\sim$200 μg/mL). Although the peak height was nearly the same at 2 and 5 mM, the peak shape was better at the lower concentration. We therefore selected 2 mM for the sample buffer concentration.

The pH in the sample buffer zone also needed to be optimized. Calculation of the fraction of PGE_1 ionized vs. pH reveals that above pH 7.0 little is gained by raising the pH, but that below pH 7.0 the fraction ionized drops off sharply with decreasing pH. This is shown in Table 1.

The electrophoretic mobility, μ, is directly proportional to charge (fraction ionized) through the expression $\mu = q/6\pi\eta r$, where η is viscosity, and r the ionic radius. In turn, the concentration factor achieved in reversed polarity electrostacking is proportional to μ. Hence, the pH of the sample must be controlled in order to achieve a repeatable concentration factor. Based on pK_as for structurally similar compounds, we would expect the pK_a for 3,4-DHHCA, the internal standard, to be lower than that for PGE_1. Hence, we can expect it to be essentially fully ionized at pH 7.0 or above. However, for a 2 mM sample buffer, originally at pH 7.0, and also containing 5% ethanol to aid in dissolution of PGE_1, the apparent pH is pulled down to 6.38 upon addition of sample and internal standard. Clearly, based on Table 1, this is undesirable. Although the effect would be less for a 5 mM sample buffer, better electrophoretic behavior was obtained at 2 mM than at 5 mM (vide supra).

In the general case, for a sample matrix whose composition could vary, 2 mM would be an unacceptable choice because of its low buffering capacity. However, in the present instance, the sample matrix was defined

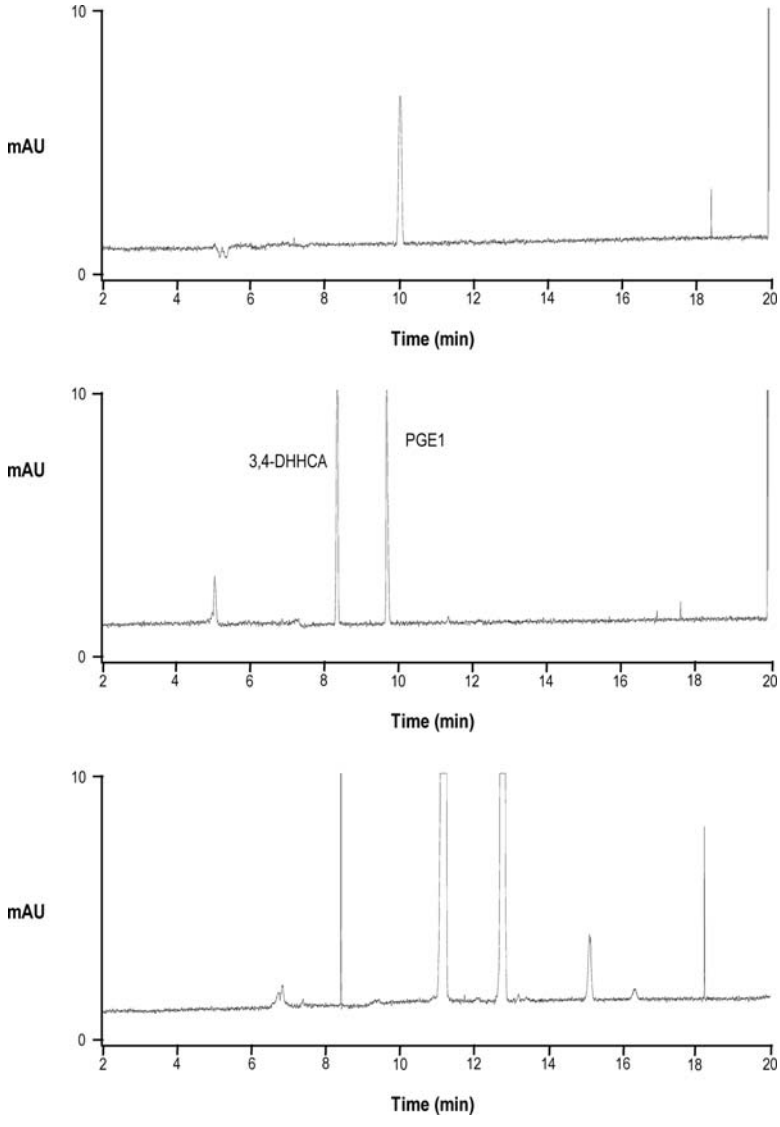


Figure 5. Comparative electropherograms for sample injected from run buffer (top), sample buffer (middle), and sample buffer with FASS (bottom). The FASS conditions were 20 s injection, −10 kV reversal potential, 1.0 min reversal time. [PGE_1]: ∼200 μg/mL.

(and fixed). Through trial and error, we determined that if the pH of the sample buffer was adjusted to 7.6 prior to addition of PGE_1 and the internal standard, then the pH remained at 7.0 or above after addition, which assures at least 99% ionization (Table 1).

Table 1. Fraction of PGE_1 ionized vs. pH

pH	Fraction ionized
6.4	0.965
6.5	0.972
6.6	0.978
6.7	0.982
6.8	0.986
6.9	0.989
7.0	0.991
7.1	0.993
7.2	0.994

Finally, repeated use of the same pair of buffer vials leads to degraded performance due to changes in pH, ion depletion, solution contamination, etc. Hence, we needed to determine how many consecutive runs could be made from a given buffer vial pair without ill effect. Tracking theoretical plate count, peak width, peak symmetry, and tailing factor as figures-of-merit, we determined four to be the maximum number of consecutive runs that could be made for a single buffer vial pair on the PE-ABI 270HT.

Capillary/z-Cell Method Optimization

Both a 50 and 75 μm i.d. capillary/z-cell were available from PE-ABI (manufactured by LC Packings). Use of the 75 μm i.d. capillary/z-cell is more common, as it is easier to work with and is less prone to plugging. Also, the signal enhancement attainable on a 75 μm capillary/z-cell is about 13, whereas it is about 7 on the 50 μm capillary/z-cell.[34] Although we had successfully used a 75 μm capillary/z-cell on another project, we have always achieved superior electrophoretic performance on 50 μm capillaries. We, therefore, pursued development on the 50 μm capillary/z-cell.

As no sample stacking was used with the z-cell, the principal parameter to optimize was injection time to balance detectability against efficiency. Accordingly, we varied injection time (vacuum injection), first from 2.5–20 s, then over the narrower range 2.5–5.0 s. The largest signal response at both 25 and 30 kV was obtained for a 20 s injection. However, as expected, the efficiency at 20 s was also the poorest. A value of 5.0 s was selected on the basis of its enhanced response with respect to 2.5 s, while at the same time suffering only a small loss in efficiency. The height increased by almost 60% while losing only 25% of the plate count.

A separation of the impurities mix on the capillary/z-cell is shown in Figure 6. As shown above for on-line concentration (Figure 4), all impurities are separated from PGE_1 and from the internal standard and also from one another except for 11-epi-PGE_1, which coelutes with PGE_1. Comparison

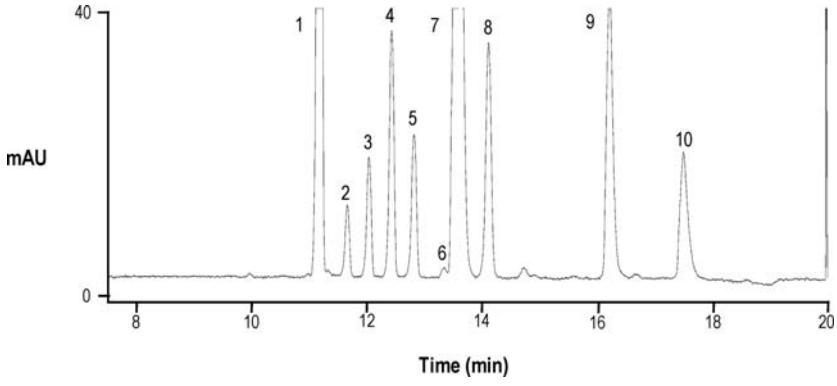


Figure 6. Separation of 3,4-DHHCA and PGE_1 from prostaglandin impurities on the capillary/z-cell. The capillary dimensions were 72 cm total length (50 cm to detector) × 50 μm i.d. The applied potential was 25 kV. Peak identifications are as in Figure 4.

with Figure 4 reveals similar performance for on-line concentration and for enhancement via the z-cell.

Validation Results

Field-Amplified Sample Stacking (FASS)

Samples were run according to the scheme detailed in the optimization section above. Final conditions were as follows (also see Experimental section): capillary, untreated, 72 cm (50 cm to detector) × 50 μm i.d.; rise time, 0.5 s; data acquisition rate, 5 pt s^{-1}; detection, 200 nm; applied voltage, 25 kV; sample injection, 20 s vacuum (5 in Hg), then apply −10 kV for 0.9 min; run buffer, 30 mM sodium phosphate, pH 7.0, 35 mM SDS, 10% IPA (filtered and degassed); internal standard solution, 0.105 mM 3,4-DHHCA in sample buffer; sample buffer, 2 mM sodium phosphate prepared by diluting 10 mM, pH 7.60 sodium phosphate five-fold; sample preparation, 4.0 mg alprostadil dissolved in 1.0 mL ethanol, then add 19.0 mL of internal standard solution. Validation was conducted with respect to linearity, recovery, and precision. The method was additionally tested on samples that had been assayed by HPLC and the results compared.

Linearity/Recovery

Linearity and recovery were assessed together over the range 70–120% of the targeted assay concentration, 200 μg/mL. The results are presented in Table 2. The mean recovery was 100.0% with an RSD of 0.81%. First-order regression yielded a correlation coefficient (r^2) of 0.9992 with an intercept of 0.0632. The 95% confidence limits for the intercept (0.0126 → 0.114) did not

Table 2. Linearity/recovery results by FASS

Assay conc., %	Amount added[a]	Amount found[a]	Recovery, %
70	1.400 mg	1.417 mg	101.2
80	1.591	1.595	100.3
80	1.591	1.602	100.7
90	1.791	1.800	100.5
100	2.006	2.003	99.9
100	2.006	1.988	99.1
110	2.200	2.174	98.8
120	2.399	2.392	99.7
			x = 100.0
			RSD = 0.81%

[a]In 10 mL.

quite encompass zero, nor the slope (0.939 → 0.992) quite one. Failure to do so may relate to the high correlation obtained. The lower 95% intercept value corresponds to 0.5% of the targeted assay concentration and is less than the standard error (0.0207). The upper 95% confidence limit for the slope is greater than 99% of the theoretical with the difference less than the standard error (0.0109). From a practical standpoint, these differences from zero and one are not significant. The RSD for the standards (n = 6) used to calculate recovery was 0.64%.

Precision

Instrumental or system precision was tested by injecting a single sample preparation six times, each injection made from a different vial. The experiment was conducted twice, and on different days. To measure precision for replicate preparations, single injections were made of six different preparations. In the event that significant degradation occurs, precision data at 80% of the labeled amount is often collected. Six replicate preparations were prepared at 80% of the targeted assay concentration, each injected once. For each precision measurement, a standard factor (equal to the sample weight divided by the adjusted area) was calculated.

Results for the precision testing are shown in Table 3. An example electropherogram (taken from the instrumental precision run) is shown in Figure 7. On day 1 for system precision testing, the RSD was 0.22%, while on day 2 the RSD was 0.71%. For replicate preparations injected once each, the resulting RSD was 1.23%, revealing that the sample preparation contributes significantly to overall variability. At 80% of the targeted assay concentration, the calculated recovery was slightly high, and with a somewhat higher RSD as well. The RSD for the standards used to calculate the recovery registered 0.40% (n = 6). These results, taken together, demonstrate the repeatability of the in-line sample concentration scheme used. The overall precision,

Table 3. Precision results by FASS

Type of precision	n	Result
Instrumental	6[a]	0.22 and 0.71%
Replicate preparations	6	1.23%
80% of targeted concentration	6	1.99%

[a]On each of two days.

falling within the required $\pm 2\%$ window, is acceptable for purity determination of a pharmaceutical active ingredient.

Quantitation of Two Lots and Comparison with HPLC Results

We assayed two lots using the developed FASS procedure, comparing the results to those obtained using the reversed phase HPLC procedure. Six replicate preparations were made of each lot. Four mg of alprostadil was weighed into a screw-cap vial, 1.0 mL of ethanol added, the drug dissolved with gentle swirling and/or ultrasonication, and finally 19.0 mL of internal standard solution added. The results of the comparison are given in Table 4. The two sets of results by $MEKC_{FASS}$ for lot B are for the same samples/standards set run twice in succession. A representative electropherogram of lot B and of a corresponding reference standard are shown in Figure 8. Clearly, the agreement is excellent between the (referee) HPLC results and the FASS results for both lots. t-Testing revealed no statistical difference between means at the 95% confidence interval. The RSDs for standards were all under 1%.

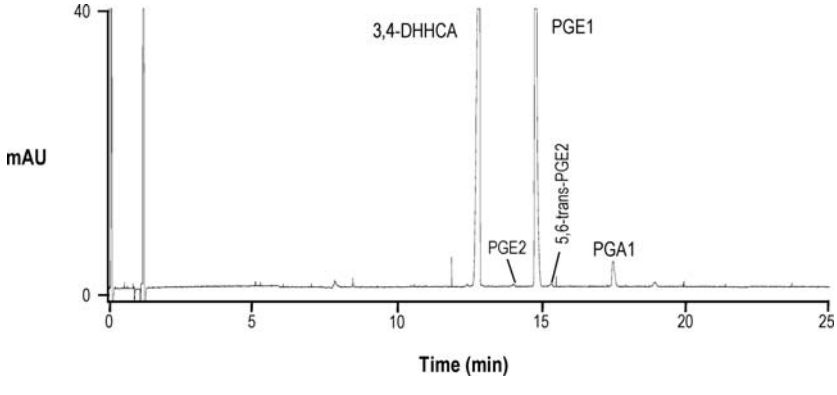


Figure 7. An illustrative electropherogram for precision obtained by FASS. The conditions are as given in the text under Validation Results, Field-amplified sample stacking (FASS).

Table 4. Assay results for two lots of alprostadil run by FASS and comparison to HPLC

Lot A		Lot B		
HPLC	MEKC	HPLC	$MEKC_{FASS}1$	$MEKC_{FASS}2$
98.2%	98.1%	98.4%	97.8%	97.8%
98.0	98.1	97.8	97.6	98.2
97.7	98.0	98.6	98.1	98.2
97.6	98.6	98.4	97.8	97.9
98.6	98.7	96.0	97.7	98.3
	99.1	97.5	96.7	97.3
x = 98.0	x = 98.4	x = 97.8	x = 97.6	x = 97.9
RSD = 0.41%	RSD = 0.44%	RSD = 0.99%	RSD = 0.50%	RSD = 0.37%

Capillary/z-Cell

As noted, we conducted less extensive validation on the capillary/z-cell than by FASS. This is a less demanding assay from the standpoint that no in-line concentration takes place. Hence, we did not separately assess precision, but rather gauged it from the replicate runs made on the two comparison lots. Linearity/recovery was assessed the same as for FASS. The final conditions were as above for FASS, with the following exceptions: capillary/detection, High Sensitivity Optical Cell Assembly, 50 μm i.d. utilizing extended path length detection; sample injection, 5.0 s vacuum (5 in Hg).

Linearity/Recovery

Linearity/recovery was assessed over the range 70–120% of the targeted assay concentration, the same as for FASS. The results obtained are shown in Table 5. The mean recovery was 100.9%, the RSD 1.33%. First-order regression yielded a correlation coefficient (r^2) of 0.9986; the intercept was 0.2307, higher than for FASS. The same as for FASS, the 95% confidence interval for the intercept (0.0444 → 0.418) does not cross the origin, nor does the slope (0.898 → 0.994) encompass one. The lower 95% intercept value corresponds to 0.5% of the targeted assay concentration, which is smaller than the standard error of the intercept (0.0673). The standard error of the slope (0.0174) is greater than the difference between one and the upper 95% value for the slope. Nevertheless, there appears to be a slight bias. The same as for FASS, we do not regard these small differences as significant from a practical standpoint.

Precision/Comparison with HPLC

Precision was gauged from the comparative data generated vs. HPLC. The results obtained for the capillary/z-cell for the two lots of alprostadil

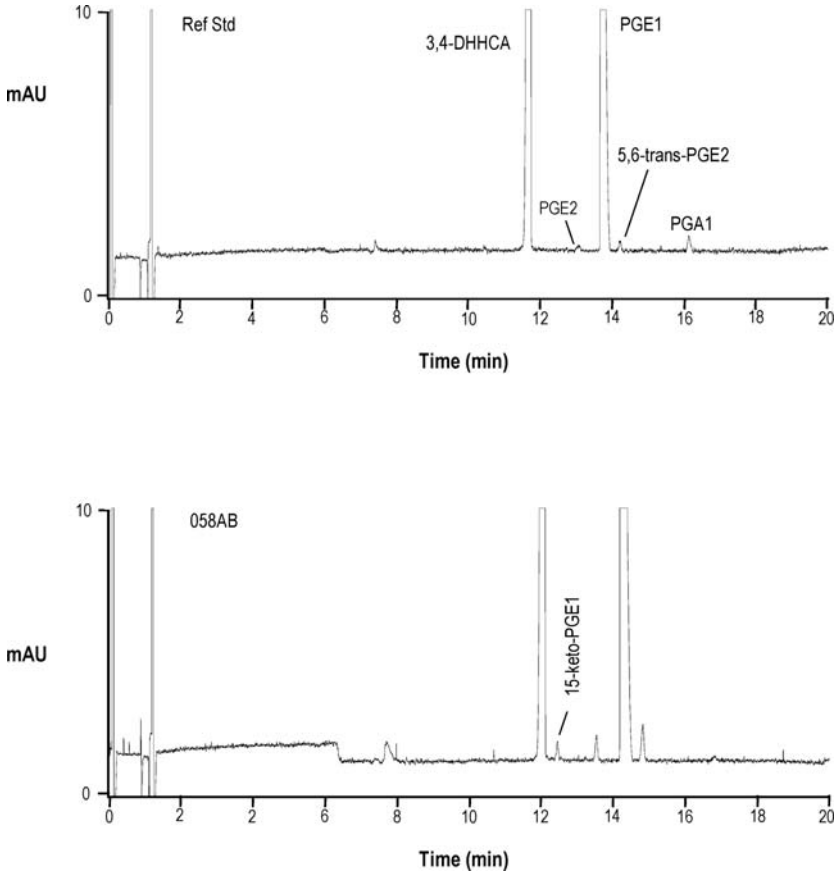


Figure 8. Electropherograms of a reference standard (top) and of lot B (bottom) generated by FASS. The conditions are the same as in Figure 7.

Table 5. Linearity/recovery results for the capillary/z-cell

Assay conc., %	Amount added[a]	Amount found[a]	Recovery, %
70	2.800 mg	2.858 mg	102.1
80	3.216	3.268	101.6
90	3.591	3.677	102.4
100	3.984	3.997	100.3
110	4.393	4.365	99.4
120	4.804	4.774	99.4
			x = 100.9
			RSD = 1.33%

[a]In 20 mL.

Table 6. Assay results for two lots of alprostadil run by z-cell enhancement and comparison to HPLC

Lot A			Lot B			
HPLC	$MEKC_{FASS}$	$MEKC_{z\text{-}cell}$	HPLC	$MEKC_{FASS}$		$MEKC_{z\text{-}cell}$
98.2%	98.1%	98.4%	98.4%	97.8%	97.8%	97.4%
98.0	98.1	99.5	97.8	97.6	98.2	97.6
97.7	98.0	98.5	98.6	98.1	98.2	97.7
97.6	98.6	99.3	98.4	97.8	97.9	98.3
98.6	98.7	99.5	96.0	97.7	98.3	99.4
	99.1	97.1	97.5	96.7	97.3	98.6
x = 98.0	x = 98.4	x = 98.7	x = 97.8	x = 97.6	x = 97.9	x = 98.2
RSD = 0.41%	RSD = 0.44%	RSD = 0.94%	RSD = 0.99%	RSD = 0.50%	RSD = 0.37%	RSD = 0.77%

are shown in Table 6. The earlier results by FASS (Table 4) are repeated for convenience along with the HPLC results. Electropherograms for the standard and for lot B are shown in Figure 9. These may be compared with Figure 8 for the corresponding runs by FASS. Just as for FASS, agreement with HPLC is seen to be excellent for the capillary/z-cell. t-Testing of the capillary/z-cell results vs. HPLC results again showed that, at the 95% confidence interval, there was no statistical difference between means. The RSD for the six replicate measurements on lot A was 0.94%, and on lot B 0.77%. For the analogous experiment by FASS, the RSD for lot A was 0.44%, and for lot B 0.50%. Hence, these two approaches are comparable, and both demonstrate acceptable precision.

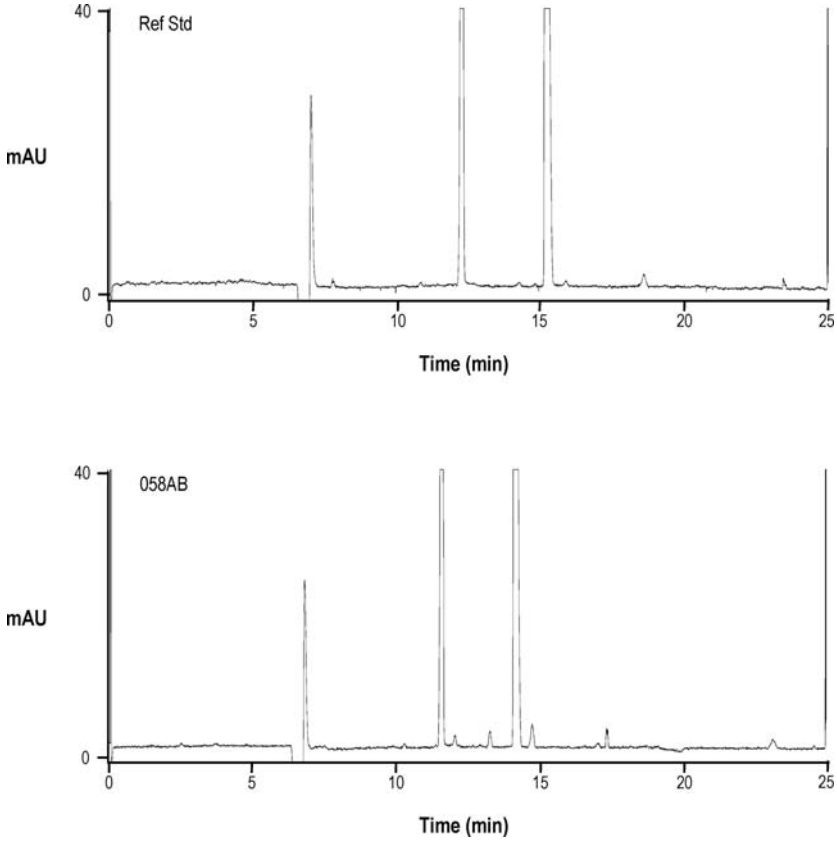


Figure 9. Electropherograms of a reference standard (top) and of lot B (bottom) on the capillary/z-cell. The conditions are as given in the text under Validation Results, Capillary/z-cell.

CONCLUSIONS

Two related MEKC procedures were developed and validated for purity determination of alprostadil. Alprostadil presents a quantitation challenge for CE because of lack of a chromophore and because of low water solubility. The techniques of field-amplified sample stacking (FASS) and high sensitivity optical cell enhancement were used to obtain the necessary signal response, the former based on large volume sample injection with subsequent on-line concentration, the latter on extended path length detection via a capillary/z-cell configuration.

The precision for both techniques was within current validation expectations, generally less than $\pm 1\%$, always less than $\pm 2\%$. Linearity/recovery by FASS from 70 to 120% of the assay concentration resulted in a mean recovery of 100.0% with an RSD of 0.81%. For the capillary/z-cell the mean recovery was 100.9% with an RSD of 1.3%. All process and degradation impurities are separated from the internal standard and from PGE_1 except for 11-epi-PGE_1, which appears, under best conditions, as a shoulder on the front edge of the PGE_1 peak. Comparison of results by FASS and z-cell enhancement with HPLC for two lots of alprostadil revealed excellent agreement. There was no statistical difference between means at the 95% confidence interval. An advantage of the MEKC procedures relative to HPLC is a shorter analysis time, about 20 min total for separation and capillary rinsing/refilling. The MEKC-based procedures serve the additional function of furnishing an orthogonal means for purity assessment of alprostadil.

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