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Journal of Chromatography A, 1053 (2004) 217–226
JOURNAL OF CHROMATOGRAPHY A
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Determination of impurities in the drug 5-aminosalicylic acid by micellar electrokinetic capillary chromatography using an electrolyte pH that approaches the isoelectric point of the parent compound

Lee Allen [a], Julie Weinberger [b], Robert Weinberger [c,*]

[a] *Procter and Gamble Pharmaceuticals, Norwich, NY 13815, USA*
[b] *Horace Greeley High School, Chappaqua, NY 10514, USA*
[c] *CE Technologies Inc., P.O. Box 140, Chappaqua, NY 10514, USA*

Available online 28 July 2004

Abstract

Process impurities found in the drug substance 5-aminosalicylic acid were determined by micellar electrokinetic capillary chromatography (MECC). In order to enhance the detection of trace impurities, the parent drug was dissolved to an unusually high concentration of 5 mg/mL. To reduce the dispersive processes of electromigration dispersion and anti-stacking, both of which occur at high solute concentration, the electrolyte pH was adjusted to be close to the apparent isoelectric point (p*I*) of the zwitterionic drug. In this fashion, the net charge on the solute should approach zero thereby minimizing the aforementioned sources of band-broadening. Two additional developments are reported. Short sodium hydroxide washes were used to optimize the MECC reproducibility. The elimination of anti-stacking permitted the use of peak heights to quantitate low level impurities with improved precision. The method was compared to the high-performance liquid chromatographic (HPLC) method found in the United States Pharmacopoeia (USP). Both the MECC and HPLC methods were found to be similar with regard to migration-retention time reproducibility, peak area-height reproducibility, linearity and limit of quantitation. The MECC separation is 2× faster than HPLC. Both methods meet the system suitability requirements described in the USP.

Keywords: Pharmaceutical analysis; pH effects; Aminosalicylic acid

1. Introduction

High-performance capillary electrophoresis (CE) has not been routinely used for the determination of impurities in pharmaceutical products. Methods must have sufficient sensitivity to detect impurities, relative to the main compound, at or below the 0.1% level and below. This sensitivity requirement is sometimes problematic when CE is employed. In some applications, isotachophoretic techniques such as sample self-stacking [1] can be used to improve limits of detection (LODs) but these methods are often too complex for routine use. Indeed, one of the attractive features of CE is simple methodology.

One problem limiting the detection of impurities using CE is the need to use relatively low concentrations (0.5–1 mg/mL) of the main drug substance. Higher concentrations can result in electromigration dispersion of the main component and anti-stacking of the trace impurities. The end-result is increased band-broadening beyond what is normally expected when optimal conditions for CE are used. This band-broadening can prevent the observation of impurities or degradants that have migration times near that of the main component.

The separation of trace impurities in phenol process streams as neutral compounds by micellar electrokinetic capillary chromatography (MECC) has been reported [2]. By selecting an electrolyte pH of 7, the main component is effectively neutral (the pK_a of phenol is 10) and electrodispersive band-broadening is eliminated. The absence of band-broadening allows 10 mg/mL solutions of the main component to be prepared and separated which results in

* Corresponding author. Tel.: +1 9142380514; fax: +1 9142380514.
E-mail address: robertweinberger@aol.com (R. Weinberger).

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doi:10.1016/j.chroma.2004.05.052

sub-ppm LODs for the impurities and a linear dynamic range of 10^5.

Reaching solute neutrality for phenol is relatively easy because of its high pK_a. The normal working pH range for CE is 2–12 making it difficult to achieve solute neutrality for any acid with a pK_a of less than 5 or any base with a pK_a of greater than 9. Since these boundaries are well-defined, we decided to test the applicability of this method for determining impurities and degradants in a zwitterionic drug. In this case, the ideal pH to minimize band-broadening would be equal to the pI of the zwitterion. Note that it is not necessary for the impurities to be neutral, only the main component. The impurities are too dilute to incur or cause any band-broadening.

5-Aminosalicylic acid (5-ASA, generic name: mesalamine) is marketed for the treatment of ulcerative colitis. The potential process impurities are available as well-characterized and purified standards. The structures of the main drug and selected impurities are shown in Fig. 1. These impurities can be determined by high-performance liquid chromatographic (HPLC) [3] but a 1 h run time is required to resolve all impurities. With this information in hand, we decided to test the suitability of the neutral solute concept for the separation of and determination of impurities in 5-ASA.

Four of the drug-related impurities of 5-ASA were previously separated by MECC using a relatively complex alkaline electrolyte on a short 8.5 cm capillary [4]. In the cited paper, the authors report a limit of quantitation (LOQ) of 0.05% for 3-ASA and this is usually adequate for quantitation of most impurities. A short capillary will not likely be suitable to separate a full complement of impurities due to limited resolving power. When a longer capillary is used with the correspondingly longer separation time, the LOQ will be proportionately higher because of solute diffusion.

2. Experimental

2.1. Reagents

The main drug, 5-ASA and all impurities were standard-grade material obtained from Procter & Gamble Pharmaceuticals (Norwich, NY, USA). Phosphoric acid, sodium dodecyl sulfate (SDS), and sodium 1-octanesulfonic acid were obtained from Sigma (St. Louis, MO, USA). Borate buffer, pH 9.3 and borate–SDS were obtained from Agilent Technologies (Wilmington, DE, USA). Methanol, acetonitrile and potassium phosphate, monobasic were obtained from J.T. Baker. Phenol was obtained from Dakota Gasification (Beulah, ND, USA).

2.2. Capillary electrophoresis

An Agilent CE system was used for all studies. The capillary was 72 cm (length to detector) × 50 μm i.d. with an extended pathlength (bubble factor) of 3. The optimized

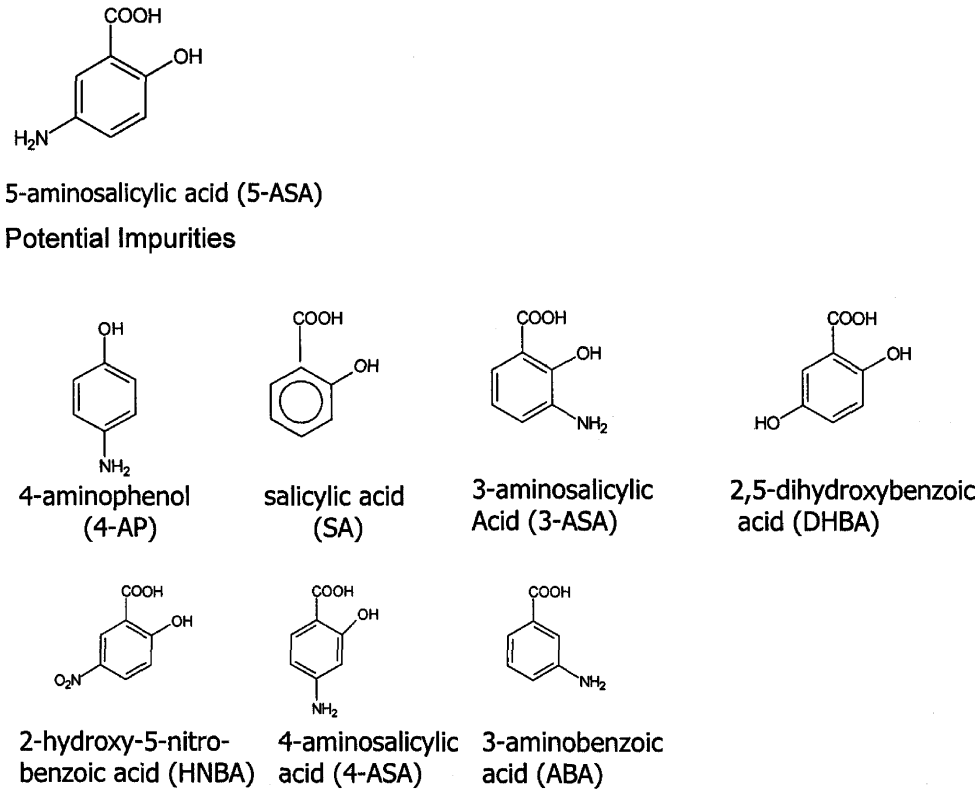

Fig. 1. Structures of 5-ASA and its impurities and degradation products.

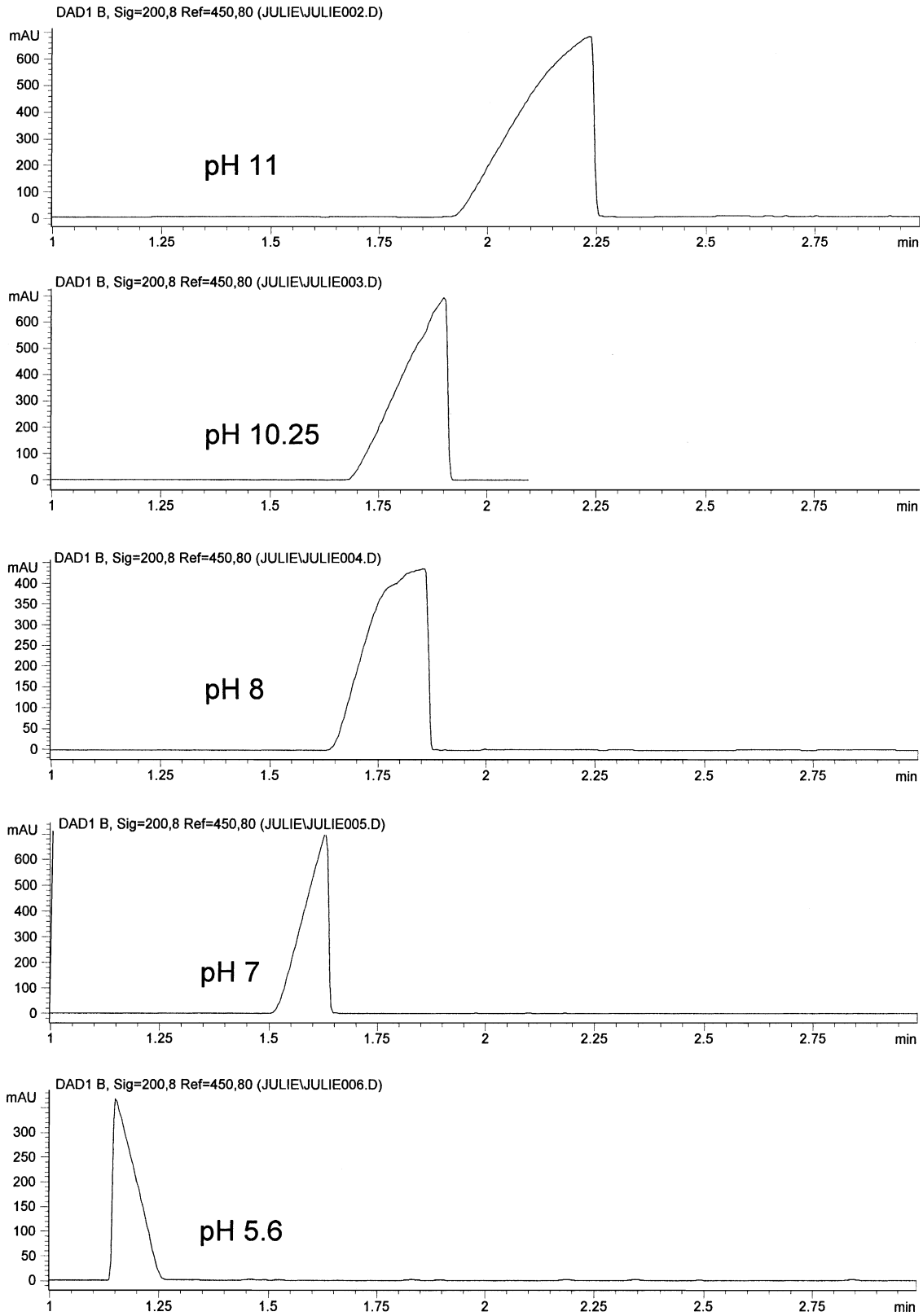

Fig. 2. Impact of pH on peak shape of 5-ASA (5 mg/mL). Capillary: 80.5 cm (effective length 8.5 cm) × 50 μM i.d.; BGE: 50 mM phosphate, 50 mM SDS, pH as specified on figure; injection: −250 mbar s; temperature: 30 °C; voltage: 30 kV; detection: UV, 200 nm.

background electrolyte (BGE) was 50 mM phosphate, pH 6.3 with 75 mM SDS. The voltage was 30 kV and the temperature was 30 °C for all studies. Detection at 200 nm was used for most studies though any wavelength from 200 to 230 nm could be employed. The lower wavelengths provided two to three times improved detection at the expense of a slightly less stable baseline and the potential for more baseline artifacts.

Prior to initial use, the capillary was conditioned with 1 M sodium hydroxide for 15 min. In between runs, the capillary was conditioned with 0.1 M sodium hydroxide for 6 s followed by BGE for 5 min.

For all scouting runs, the short-end of the capillary (8.5 cm) was used and the injection size was −50 mbar s. The long-end of the capillary (72 cm) was used for all analytical separations with an injection size of 100 mbar s.

2.3. Electrolyte preparation

For the initial scouting runs, 490 mg of phosphoric acid and 1.44 g SDS were added to 100 mL of water to yield 50 mM phosphate and 50 mM SDS. The pH was coarsely adjusted with 5 M sodium hydroxide and then fine adjusted with 1 M sodium hydroxide to prepare solutions with pH's of 2, 3.65, 5.6, 7, 8, 10.23 and 11. The optimal BGE composition consisted of 75 mM SDS, 50 mM phosphate, pH 6.3.

2.4. Sample preparation

A 5 mg/mL solution of 5-ASA in 50 mM phosphate, pH 7, with 50 mM SDS was used for most studies. The sample was heated in 50 °C water and sonicated for 1 min to completely dissolve the sample. The solute remained in solution at room temperature but precipitated upon refrigeration. For the scouting runs, the sample was dissolved in each individual electrolyte.

2.5. Estimation of isoelectric point (pI)

Electrolytes without SDS were prepared by titrating 50 mM phosphoric acid with 5 M and then 0.1 M sodium hydroxide to pH 3, 3.7, 4.2 and 6.3. The short-end of the capillary was employed and the solute was dissolved in 50 mM phosphate, pH 6.3 at a concentration of 0.8 mg/mL. Phenol, present at 0.3%, served as a neutral marker. The UV spectrum of each component was used to track solute migration.

2.6. High-performance liquid chromatography (LC)

A Waters model 2695 pump and autosampler coupled to a Waters 2487 UV detector set at 220 nm was used for all studies. The column was a 150 mm × 4.6 mm i.d. Restek Ultra C_8 packed with 5 μm particles. The mobile phase flow rate was 1.2 mL/min. Separation was performed at ambient temperature.

Aqueous buffer was prepared by dissolving 1.36 g of potassium phosphate monobasic and 2.2 g sodium 1-octanesulfonate into 890 mL of purified water and filtering through a 0.5 μm filter. The pH was adjusted to 2.2 with phosphoric acid. The final mobile phase consisted of 80 mL of methanol and 30 mL of acetonitrile added to the filtered, aqueous buffer.

3. Results and discussion

3.1. Impact of pH on the peak shape of 5-ASA

Fig. 2 shows electropherograms of 5-ASA, 5 mg/mL in 50 mM phosphate with 50 mM SDS, at various pH values. At pH 8–11, broadened saw-tooth peaks were obtained that were characteristic of electromigration dispersion. The peak sharpened at pH 7 but showed some evidence of asymmetry. At pH 5.6, a sharpened peak was found but with an asymmetry opposite to pH 7. The drug substance in the pH 5.6 buffer precipitated after a few hours. It has been reported that the solubility of 5-ASA is minimized between pH 2 and 5.5 [5].

These data were consistent with the hypothesis that a neutral zwitterion will provide a sharp peak, even at a

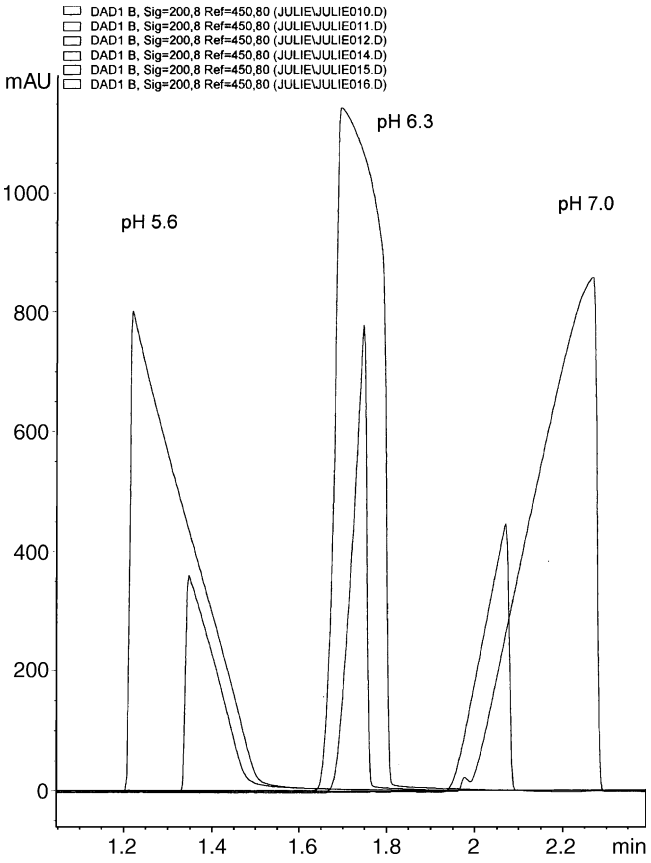

Fig. 3. Impact of pH on peak shape of 5-ASA at 50 and 200 mbar s injection. Other conditions as in Fig. 1.

concentration of 5 mg/mL. The reversal of peak asymmetry was consistent with the reversal of charge that was expected when the pH of the BGE crosses the p*I* of the solute. However, the presumed p*I* range (pH 5.6–7.0) fell outside of the range of limited solubility.

Fig. 3 shows electropherograms of 5-ASA at two injection levels and three different pH values, 5.6, 6.3 and 7. Saw-tooth peaks were found at the outer pHs whereas pH 6.3 yields a symmetrical peak, particularly for the smaller injection size.

A series of experiments using capillary zone electrophoresis (CZE) were performed to estimate the p*I* of 5-ASA. Buffers at pH 3, 3.7, 4.2 and 6.3 were used as electrolytes. Surfactants were not used for this study. The drug concentration was reduced to 0.8 mg/mL to preserve solubility. Phenol served as a neutral marker. The results are shown in Fig. 4. At pH 6.3, phenol elutes well before 5-ASA. This means that 5-ASA was negatively charged at that pH. As the pH was decreased to 4.2, phenol eluted

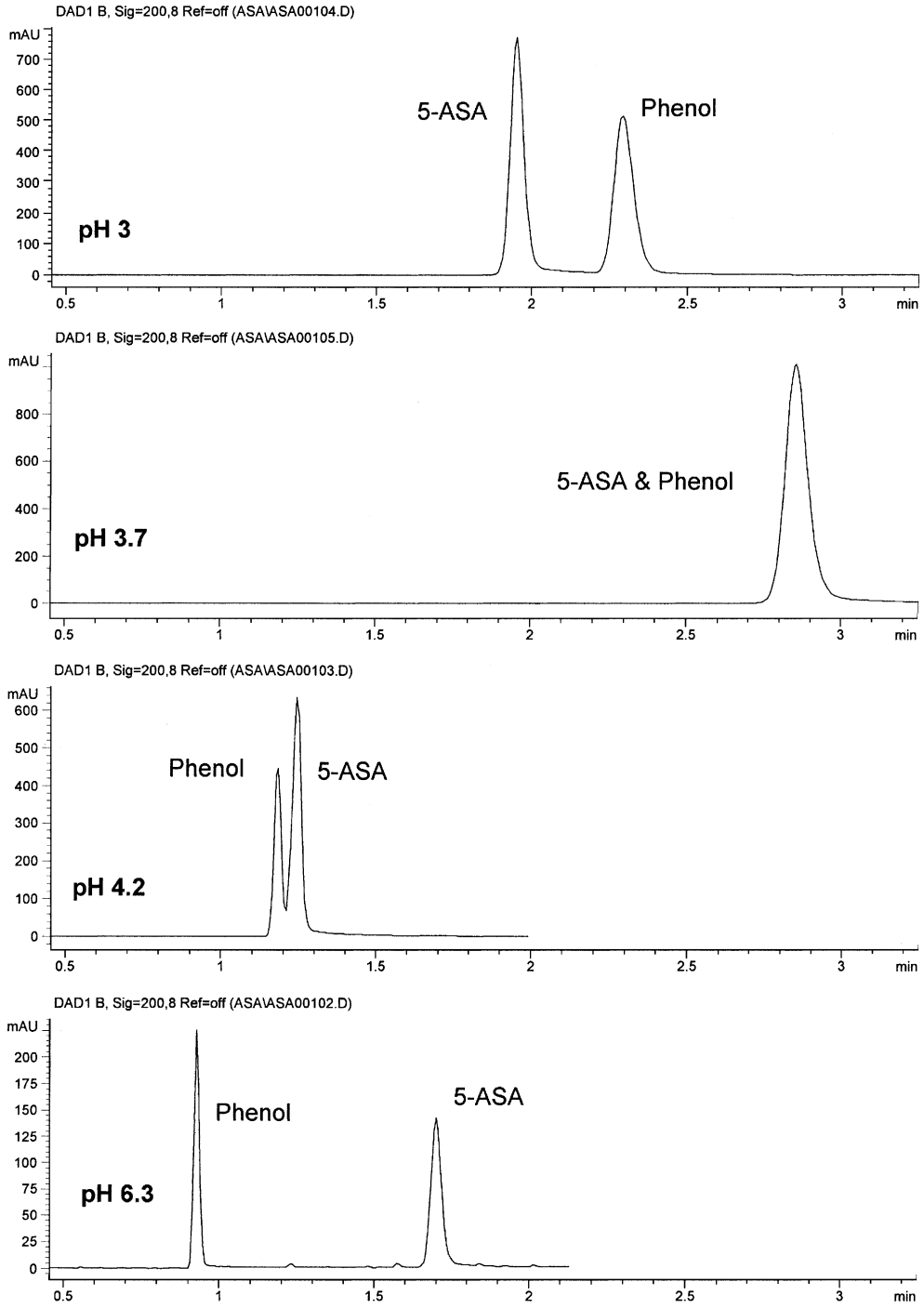

Fig. 4. Determination of p*I* of 5-ASA in the absence of SDS. Other conditions as in Fig. 1.

first and was rapidly followed by 5-ASA. At pH 3.7, both peaks coeluted. At pH 3.5, ASA eluted before neutral phenol proving that the drug was positively charged at that pH.

Contrary to the MECC data which indicated the p*I* of 5-ASA is 6.3, these latter data suggest a p$I = 3.7$. Several effects may account for these differences. First of all, 5-ASA at 5 mg/mL acts as a weak acid and will reduce the pH of the sample solution when dissolved in a buffer. That is why pH 7 was selected as the optimal sample diluent. Using 50 mM phosphate, pH 7 as diluent, a pH of 6.1 was found for a 5 mg/mL solution. In 50 mM phosphate, pH 6.3, the pH dropped to 5.4 after drug addition and the drug was not fully soluble. At a concentration of 0.8 mg/mL, the concentration used for the CZE separations, the pH lowering effect was less pronounced and solubility was maintained. This permitted accurate measurement of the p*I* when using buffers without surfactant.

The micellar model for ionic surfactants describes four zones [6]. The core which is formed by the surfactant's hydrophobic tail has a diameter of 10–28 Å. The next layer, known as the Stern layer is several angstroms thick and contains the head groups and their counterions. The largest portion of the micelle is the Gouy–Chapman layer. This double layer of counterions is several hundred angstroms thick and the counterion-concentration, as H^+ (for an anionic surfactant) can be 100× greater than in the fourth layer, the bulk surrounding water. In this region, the pH can be 1–2 units lower compared to the bulk aqueous solution [7]. This explanation coupled to the reduction of electrolyte pH by 5-ASA are consistent with the apparent p*I* as determined by CZE.

Compared to the previous work with phenols [2], the peak width and peak shape of 5-ASA, while quite acceptable, was not as good as that observed for the phenols. Phenol is always neutral at pH 7, whether it is associated with the

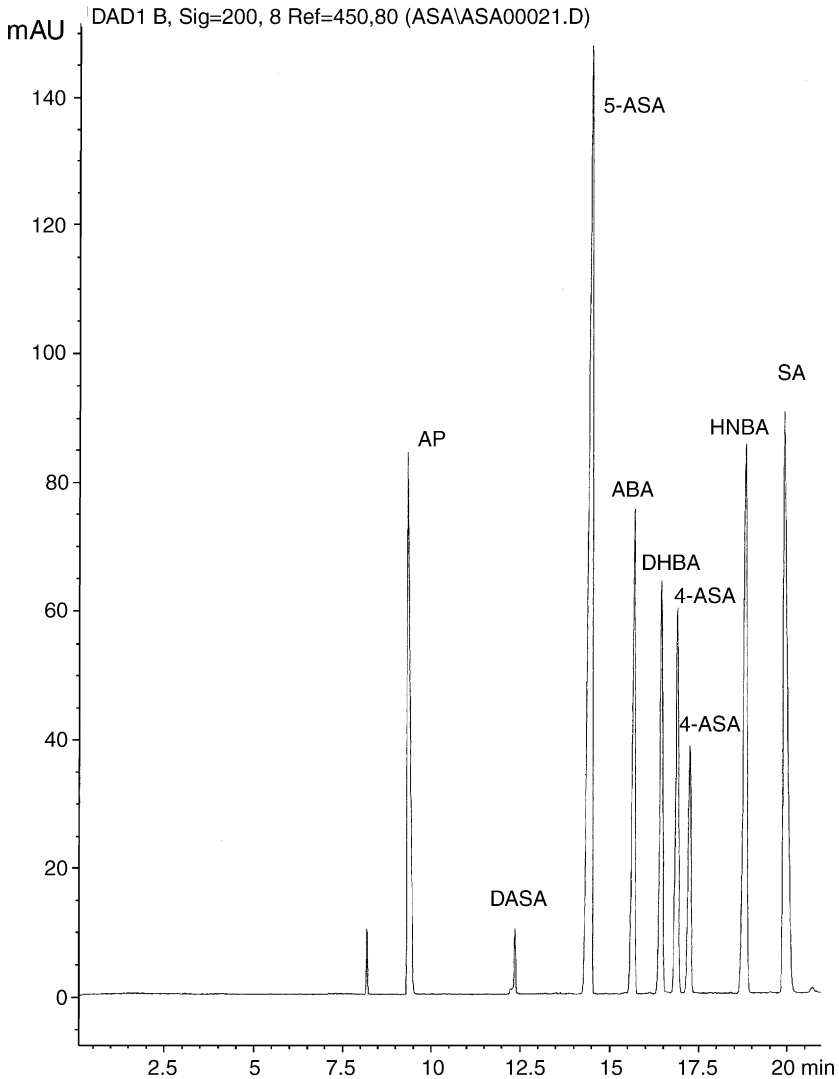

Fig. 5. Separation of 5-ASA from its impurities and degradation products. Capillary: 80.5 cm (effective length 72 cm) × 50 μM i.d.; bubble factor 3; BGE: 50 mM phosphate, 75 mM SDS, pH 6.3; injection: 100 mbar s; temperature: 30 °C; voltage: 30 kV; detection: UV, 200 nm.

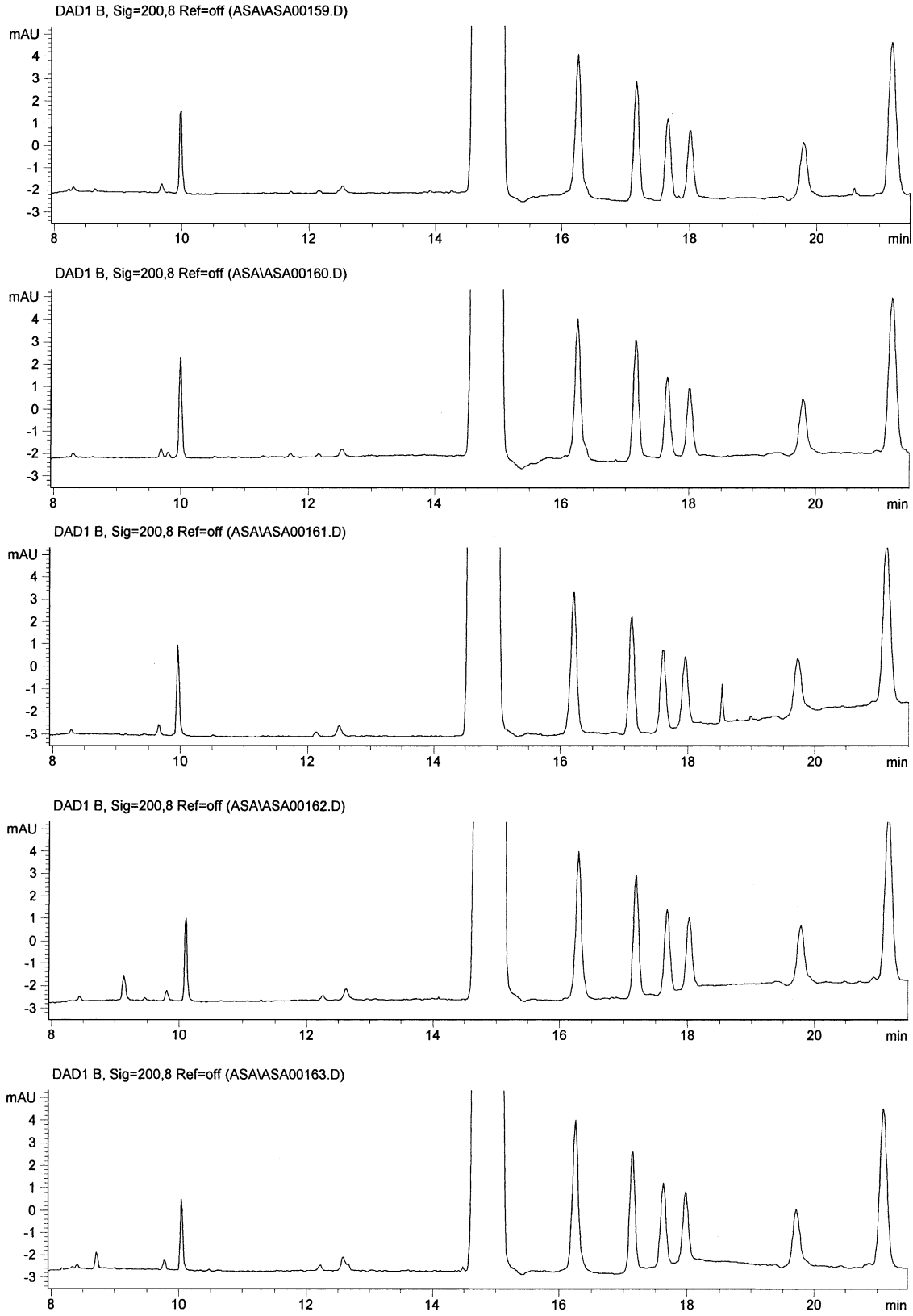

Fig. 6. Five consecutive runs of 5-ASA (5 mg/mL) and impurities (10 μg/mL, 0.2%). Conditions as in Fig. 5.

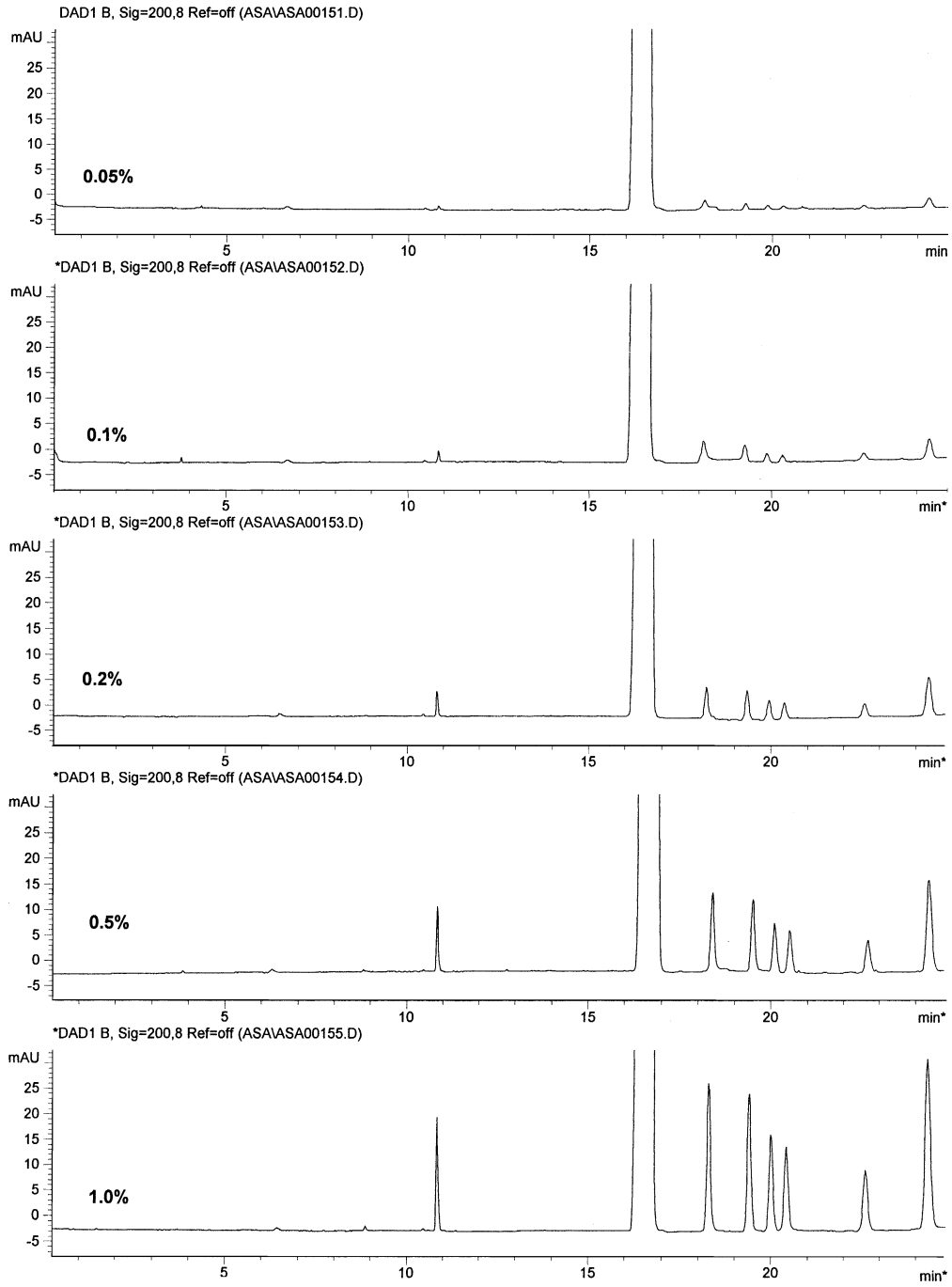

Fig. 7. Calibration runs of 5-ASA (5 mg/mL) and impurities ranging from 0.05 to 1%. Conditions as in Fig. 5.

micelle or in bulk solution. The drug, 5-ASA may approach neutrality in the MECC BGE at pH 6.3 when associated with the micelle but in the bulk solution, it will be anionic. This leads to a mass transport problem (multiple solute velocities) which may account for the increase in peak width versus that observed for phenol.

3.2. Separation of 5-ASA and its impurities by MECC

The separation of 5-ASA and its impurities is shown in Fig. 5. A surfactant concentration of 50–75 mM was found to be adequate for separation. Baseline resolution was achieved for all solutes at the 75 mM level surfactant level. Using 75 mM phosphate, loss of 4-AP was noted at low levels, presumably due to adsorption on the capillary surface. One impurity, 3,5-diaminosalicylic acid (DASA) was unstable in solution and was not quantitated.

Solute selectivity was better at pH 6.3 compared to borate buffer, pH 9.3 (data not shown). To achieve separation at pH 10.2, it was necessary to add an ion-pairing reagent such as tetrabutyl ammonium bromide to the electrolyte [4].

Table 1
Analytical figures of merit as determined by CE

Solute	R.S.D. (%)			LOQ (%)	Linearity (peak height)		
	Area	Height	Time		*r*	Slope	*y*-Intercept
AP	16	12	0.6	0.03	0.9980	24	0.037
5-ASA	1.1	0.95	0.3	na	na	na	na
ABA	3.3	2.6	0.2	0.02	0.9995	30	0.414
DHBA	3.5	1.7	0.1	0.02	0.9998	28	0.04
4-ASA	2.2	2.1	0.2	0.03	0.9999	20	0.15
3-ASA	4.7	2.2	0.2	0.03	0.9998	17	0.2
HNBA	4.9	1.5	0.2	0.05	0.9997	13	0.096
SA	2.7	3.3	0.2	0.01	0.9994	35	0.29

Peaks were identified by standard addition. All of the solutes had characteristic UV spectra (data not shown) that also made peak tracking simple. 5-ASA was stable for at least 24 h at room temperature when dissolved in 50 mM phosphate pH 7, 50 mM SDS. Degradation products were noted for a sample stored for 1 week at room temperature.

3.3. Analytical figures of merit as determined by MECC

Table 1 shows the analytical figures of merit for this application. The relative standard deviations (R.S.D.) were calculated from five consecutive runs.

3.3.1. Migration time reproducibility

A series of five consecutive runs with impurities at the 0.2% level are shown in Fig. 6. Good reproducibility of migration time was found under three conditions: (i) fresh buffers were used for each run, (ii) 1 mL buffer vials were employed, and (iii) a short 6 s. 0.1 M sodium hydroxide wash was used. In the absence of the hydroxide wash, the migration times increased by a few seconds/run. Using a 1 min hydroxide wash and a 5 min electrolyte flush, the migration times decreased by a few seconds/run. The changes were caused by the unstable electroosmotic flow (EOF). It was quickly determined that the short hydroxide wash was sufficient to stabilize the EOF. The wash also reduced the number of baseline artifacts observed.

3.3.2. Peak area and peak height reproducibility

The peak area R.S.D. were acceptable for all solutes except for 4-AP. It is unclear why that solute was less precise but it was observed under several sets of experimental conditions, presumably due to the aforementioned adsorptive effect. Injection was not a source of significant error since the main component, 5-ASA, gave a 1% R.S.D. Except for 4-AP, the peak height R.S.D. ranged from 1.5 to 3.3%.

Surprisingly, the peak height data was better compared to peak area for the impurities. This was reproducible under various experimental conditions. Peak area is usually best employed for quantitative CE since both electrodispersion and anti-stacking/stacking effects on peak height/width are compensated for. Since these effects were reduced by the experimental design, peak height became advantageous under low signal/noise conditions.

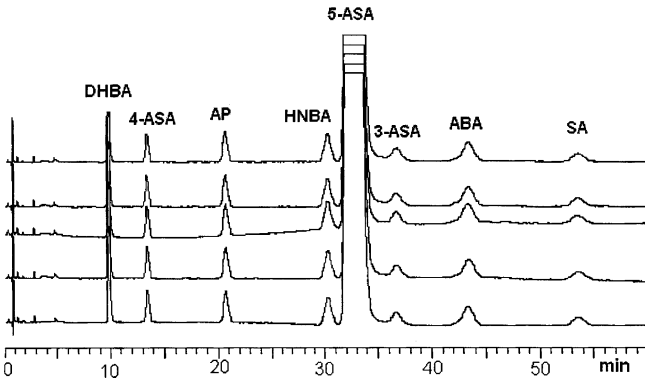

Fig. 8. HPLC of 5-ASA and impurities at the 0.2% level. Overlay of five replicate runs. See text for experimental conditions.

3.3.3. Linearity and limits of detection

A five-point calibration set for each impurity at 0.05, 0.1, 0.2, 0.5 and 1%, relative to the main drug, was run and the electropherograms are shown in Fig. 7. The calibration data is presented in Table 1. Correlation coefficients were 0.998 or better. All of the regression lines passed close to the origin. The calibration data was tested using the 0.2% run and was shown to be accurate (range: 0.19–0.21%). The limits of quantitation at 10× the baseline noise ranged from 0.01 to 0.05%.

3.4. HPLC of 5-ASA and its impurities

Liquid chromatography of 5-ASA and its impurities was performed following the impurity assay procedure described in the United States Pharmacopoeia (USP). A series of five replicate runs at the 0.2% impurity level is shown in Fig. 8. The analytical figures of merit are given in Table 2. The system was calibrated as per Section 3.3.3.

In order to meet the USP system suitability requirements for resolution, a 1 h run was needed. The retention time re-

Table 2
Analytical figures of merit as determined by LC

Solute	R.S.D. (%)			LOQ (%)	Linearity (peak area)		
	Area	Height	Time		*r*	Slope	*y*-Intercept
AP	0.32	0.59	0.04	0.05	0.9999	287000	1600
5-ASA	0.2	0.49	0.07	na	na	na	na
ABA	6.1	2.2	0.09	0.08	0.9998	408000	−6400
DHBA	0.5	0.35	0.05	0.04	0.9999	449000	210
4-ASA	3.8	2.5	0.06	0.05	0.9998	195000	−2400
3-ASA	4.7	1.5	0.14	0.05	0.9979	184000	15560
HNBA	1.1	0.45	0.08	0.05	0.9999	379000	−2500
SA	4.8	3.3	0.06	0.03	1.0000	190000	−1600

producibility is outstanding as is often seen using LC. In a similar fashion to CE, the peak height precision is superior to peak area precision at the 0.2% impurity level. The peak height precision using LC is slightly superior to the same data obtained with CE. The LOQ data were comparable using either technique with a slight advantage to CE.

4. Conclusions

This work has shown that CE separations can be comparable to LC for the determination of impurities in drug substances with regard to the analytical figures of merit. As usual, CE enjoys the advantage of speed and low operating costs. There are three limitations of the CE method. The main drug substance must be soluble in a CE compatible electrolyte at high concentration. The second limitation is the main component must become neutral at a CE compatible pH. The third is that the presence of micelles can influence main component neutrality versus the bulk solution. The normal working range for CE is pH 2–12. For compatible separations, CE can offer a rapid, useful and orthogonal method for the determination of impurities in a variety of bulk chemicals.

Acknowledgements

The authors thank Agilent Technologies for providing the CE instrument used for this project. We also thank Professor Laurence Romsted, Professor of Chemistry, Rutgers University for his helpful discussions on pH effects at the surface of micelles.

References

[1] E.K.M. Andersson, J. Chromatogr. A 846 (1999) 245.
[2] K. Sayler, R. Weinberger, J. Chromatogr. A 1014 (2003) 179.
[3] B.S. Kersten, T. Catalano, Y. Rozenman, J. Chromatogr. 588 (1991) 187.
[4] R. Gotti, R. Pomponio, C. Bertucci, V. Cavrini, J. Chromatogr. A 916 (2001) 175.
[5] D.L. French, J.W. Mauger, Pharm. Res. 10 (1993) 1285.
[6] M. Gratzel, J.K. Thomas, in: E. Wehry (Ed.), Modern Fluorescence Spectroscopy, Plenum Press, New York, 1976 (Chapter 4).
[7] C.A. Bunton, F. Nome, F.H. Quina, L. Romsted, Acc. Chem. Res. 24 (1991) 357.