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Application of a modified central composite design to optimize the capillary electrochromatographic separation of related S-oxidation compounds

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Abstract

Capillary electrochromatography was employed to separate the antibacterial 3-[4-(methylsulfinyl) phenyl]-5S-acetamidomethyl-2-oxazolidinone from its related S-oxidation products. The separation was optimized by a systematic search for the optimal separation conditions employing a modified central composite design. The variables examined in the optimization were in terms of applied potential, volume fraction of acetonitrile and buffer (Tris hydrochloride) concentration. A response surface mapping for selected variables at an applied potential of 25 kV was also performed.

Keywords: Central composite design; Optimization; Electrochromatography; DUP105

1. Introduction and theory

Capillary electrochromatography (CEC) has been suggested as a unique alternative to conventional liquid chromatography. The technique has been presented as a separation method similar to capillary liquid chromatography. However unlike capillary liquid chromatography, no high pressure is required to elute solutes [1]. Instead, in CEC, solute elution is dependent of the electro-osmotic flow created by an applied potential.

CEC has also proved to be a useful electrophoretic technique for the separation of neutral compounds, especially those for which conventional capillary electrophoresis and micellar electrokinetic electrochromatography present separation difficulties.

The neutral 3-[4-(methylsulfinyl) phenyl]-5S-acetamidomethyl-2-oxazolidinone (DUP105 **I**) is characterized by the presence of the 4- (methylsulfinyl) phenyl group bonded to the N at position 3 of the oxazolidinone ring. The desired enantiomer of the sulfoxide being generated by asymmetric sulfoxidation of the prochiral methyl sulfide precursor [2–5]. In vivo the sulfoxide **I** is either reduced to the methyl sulfide **II** or oxidized to the sulfoxide **III** (see Fig. 1 for molecular structures). The three compounds have been separated by several chromatographic methods. An isocratic octadecylsilane (ODS)-based, liquid chromatographic method employing an acetonitrile–water mixture [6] and a gradient elution method employing an acetonitrile–water gradient on a phenyl column [7] have been developed. A supercritical fluid chromatography method for the separation of the diastereoisomers of DUP105 has also

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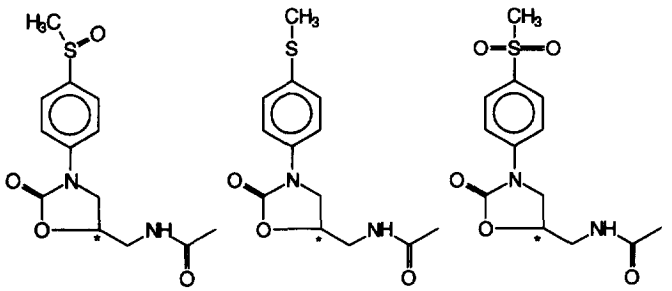

I II III

Fig. 1. Molecular structure for DUP105 and related compounds. (**I**) DUP105, (**II**) DUP105 precursor, (**III**) DUP105 sulfone.

been reported [8]. The objective of this exercise was to develop a CEC method, for the separation of the model compounds, DUP105 and its related oxidation products. The method developed was to be optimized in terms of analysis time and quality of separation.

A modified central composite design [9] was employed to examine the influence of three variables, namely applied potential, buffer concentration and volume fraction of buffer on the quality of separation (see Table 1). This was followed by profiling the influence of selected factors on the quality of separation obtained.

2. Experimental

Tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl) and thiourea (thiocarbamide) were both purchased from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile (ACN) was purchased from EM Science (Gibbstown, NJ, USA). All water was deionized though a Millipore Milli-Q System (Bedford, MA, USA). The DUP105 lot No. INS 7735-30, DUP105 sulfone, lot No. BP0181-161 and DUP105 Precursor, lot No. 57732-4 were all synthesized at DuPont Merck.

Table 1
Listing of experiments forming central composite design

Experiment	Voltage (kV)	Volume fraction (%)	Buffer concentration (m*M*)	Runs
1	30	50	30	3
2	20	50	30	3
3	30	50	50	3
4	20	50	50	3
5	30	40	30	3
6	20	40	30	3
7	20	40	50	3
8	30	40	50	3
9	25	45	40	3
10	15	45	40	3
11	25	55	40	3
12	25	35	40	3
13	syslimit[a]	45	40	3
14	25	45	25	3
15	25	45	55	3

[a] System limit.

Stock solutions of 25, 30, 40, 50 and 55 m*M* Tris·HCl (M_r 157.6) in deionized water were prepared. From these stock solutions, the various mobile phases were prepared by mixing the appropriate volumes of stock solution with acetonitrile, shaken to ensure mixing prior to filtration through a 0.45 μm membrane.

The sample mixture contained DUP105, its precursor and sulfone each at a concentration of 1 mg ml^{-1}. Thiourea at a concentration of 1.0 mg ml^{-1} was included in the mixture as a marker compound. During sample preparation an acetonitrile–deionized water (1:1) mixture was employed as solvent for the drug substances and marker compound.

All CEC experiments were performed on the Hewlett-Packard 3D capillary electrophoresis ($HP^{3D}CE$) system with a fused-silica capillary of 35 cm (effective length 25 cm)×100 μm I.D. packed with 3 μm octadecylsilane particles (Hewlett-Packard, Waldbronn, Germany). The columns were preconditioned prior to each run by a high pressure flush using the mobile phase at 6.0 bar for 5 min, while applying a +15 kV potential across the electrodes. All runs were performed in the CEC mode, at a 25°C (cassette temperature) while applying a pressure of 10 bar at both ends of the packed capillary. All sample solutions were injected hydrodynamically (50.0 mbar, 6.0 s). The electrophoretic run was typically 20 min except where all four solutes known to elute in less than 10 min. All signals from 190–700 nm were recorded on-line using the diode array detection system. The current, pressure and absorption signals at 220 nm (bandwidth 16 nm), 254 nm (bandwidth 30 nm) and 280 nm (bandwidth 40 nm) all referenced against 450 nm (Bandwidth 80 nm) were viewed on-line. For experimental purposes the data obtained for measurements at 254 nm was employed.

For the optimization, emphasis was placed on those system and mobile phase variables reported to have a significant effect on selectivity [10,11]. The optimization of the CEC method was performed in two stages. First a modified central composite design was applied to three controlled variables namely applied potential, buffer concentration and proportion of buffer (volume fraction in mobile phase). This was done to obtain a general feel for quality of separation. Three optimization criteria were drawn to describe the quality of separation. The first being

Table 2
Correlation coefficients obtained from multivariate analysis of optimization response criteria examined

Criterion	r^2
α_{av}	0.78
Cr	0.98
COF	0.97

Table 3
Statistical data for central composite design result analysis

Model term	Cr		COF	
	Estimate	*p*-value	Estimate	*p*-value
Intercept	1.43	<0.001	11.5	<0.001
App-Pot	0.345	<0.001	2.0	<0.001
Buffer	−0.111	<0.001	−0.424	0.014
Molarity	−0.04	0.05	0.1992	0.18
App-Pot·Buffer	−0.02	0.37	0.52	0.02
App-Pot·Molarity	0.0475	0.07	−0.063	0.71
Buffer·Molarity	0.052	0.05	0.37	0.07
App-pot·App-pot	0.047	0.19	0.12	0.64
Buffer·Buffer	0.026	0.10	−0.35	0.02
Molarity·Molarity	0.006	0.79	−0.31	0.15

App-Pot = Applied potential.
Buffer = volume fraction of buffer.
Molarity = Molarity of buffer.

average selectivity α_{av}, the second optimization criterion Cr was given by

$$Cr = 10 \cdot \left(\frac{\alpha_{av}}{t_m} \right) \cdot f \quad (1)$$

where α_{av} is the average selectivity, the divisor t_m being the migration time for the slowest eluting solute. This divisor served as a means of favoring separations with shorter migration times. The multiplication factor f to take into account the number of solutes that emerged separately from the column. The factor was assigned a value of unity when all four solutes emerged separately and zero if any one of the four failed to emerge or coeluted. The

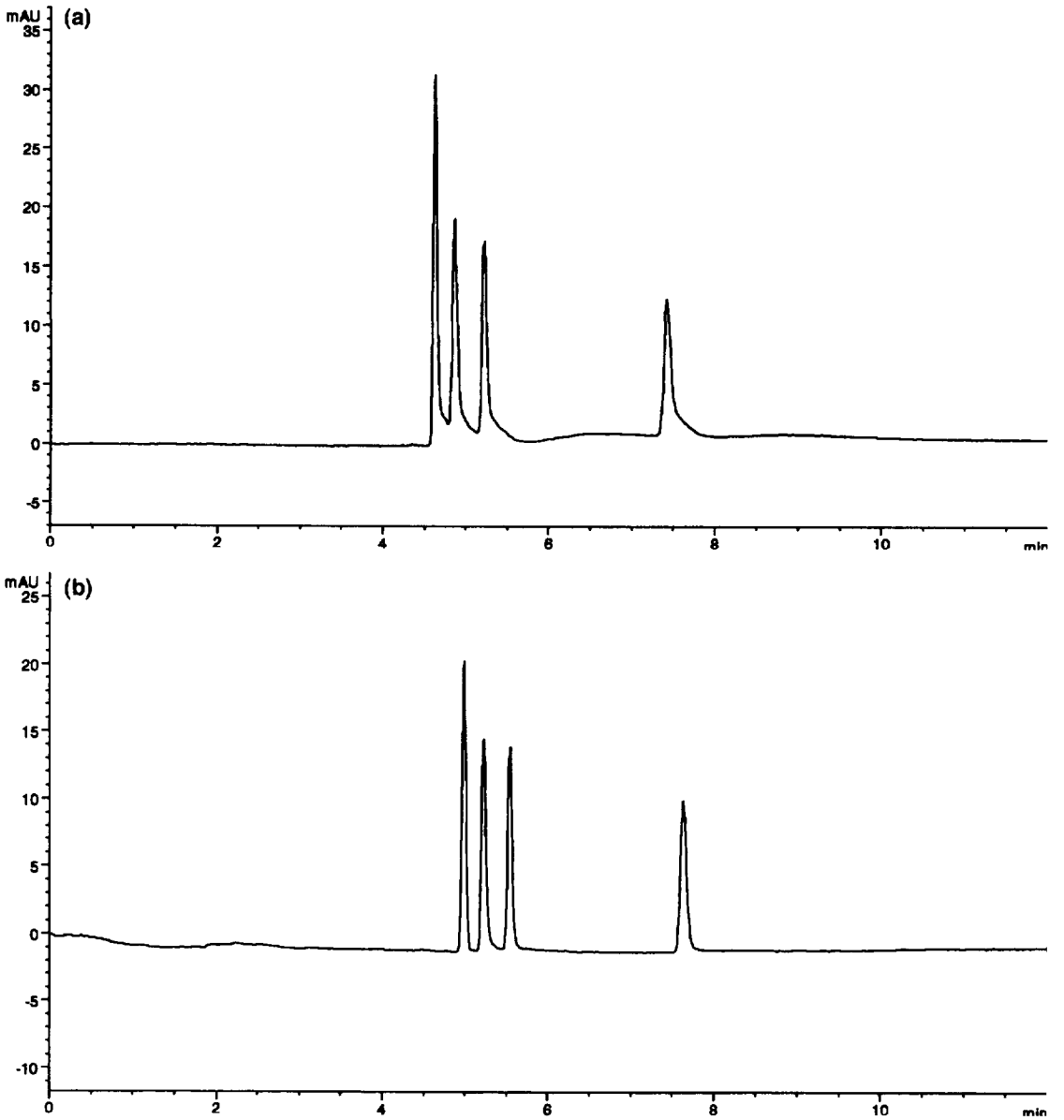

Fig. 2. Effect of variation of buffer concentration on the chromatograms. Elution order thiourea, DUP105, DUP105 sulfone and DUP105 precursor. (a) Applied potential 25 kV mobile phase ACN–Tris·HCl (25 m*M*) (55:45). (b) Applied potential 25 kV mobile phase ACN–Tris·HCl (40 m*M*) (55:45). (c) Applied potential 25 kV mobile phase ACN–Tris·HCl (55 m*M*) (55:45).

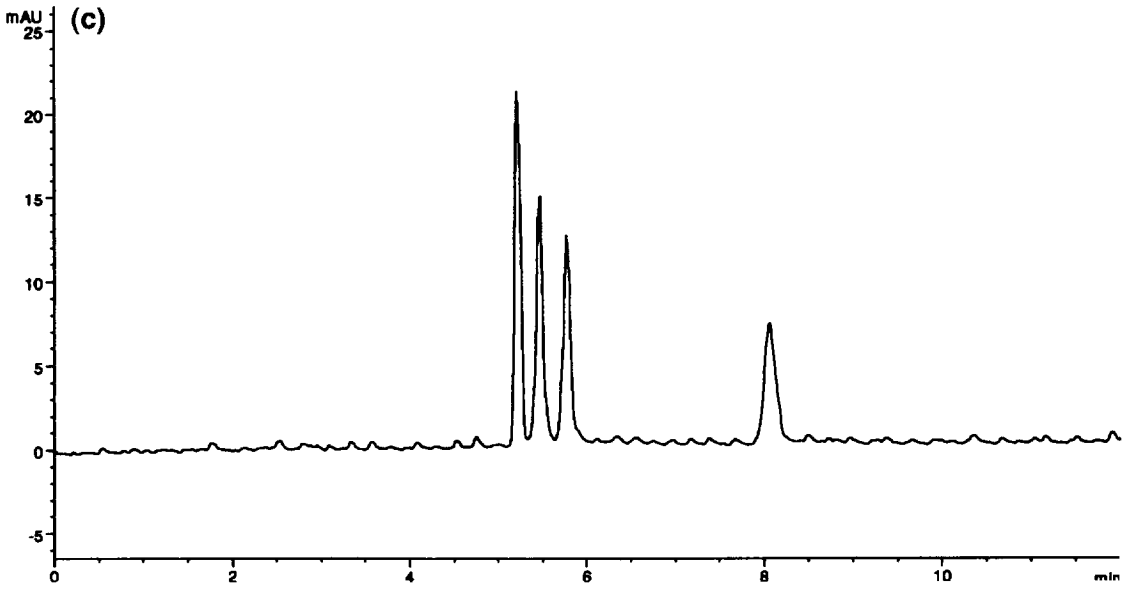

Fig. 2. (*continued*)

multiplier ten served to yield values greater than unity. The third optimization criterion was the chromatographic optimization function proposed by Glajch et al. [12].

$$\mathrm{COF} = \sum_{i=1}^{n} A_i \ln(R_i/R_{id}) + B(t_m - t_n) \quad (2)$$

where R_i is the resolution for the ith pair and R_{id} the desired resolution for the ith pair set at unity. The term t_m referring to the desired maximum analysis of 10 min and t_n the time of the last detected peak.

At this stage pH was excluded from the design for two reasons. First, DUP105 and its related compounds do not ionize and therefore the effects of pH on solute ionization would not complicate the separation. Second, there would be a difficulty in modeling the data, to take into account the potentially sigmoidal relationship between mobility and pH for ionizable solutes [13].

Multivariate analysis of the data was performed using both the RSREG module and the PROCREG module of SAS version 6.08, and JMP ver. 3.16 (Statistical Analysis Systems, Cary, NC, USA) employing a quadratic model. A quadratic model was applied purely on the grounds of the inverse relationship between electrophoretic mobility (μ) and viscosity (η).

$$\mu = \frac{Q}{6\pi\eta r} \quad (3)$$

where Q is the charge on the solute and r the solute radius.

3. Results and discussion

The applied voltage ranged from 20 to 30 kV yielding a field strength ranging from 300–450 V cm^{-1}. The measured current for almost all the runs was just under 10 μA. The buffer proportion was varied between 35% and 55% and the concentration of buffer varied between 25 and 55 m*M*.

The correlation coefficients generated from the multivariate regression of the central composite design data were employed as measures of suitability for the optimization criterion. From Table 2 average selectivity (α_{av}) performed poorly as an optimization criterion when compared to the in-house composite criterion Cr or the chromatographic optimization function of Glajch et al. [12]. This was not entirely unexpected as the use of averages assumes a normal as opposed to a skewed distribution, for the latter the average is not a suitable measure of central tendency. Furthermore, the choice of average selectivity as an

optimization criterion did not make allowance for analysis time [12]. On the other hand multivariate regression of the data employing either the composite optimization criterion (Cr) or the chromatographic optimization function (COF) yielded significantly better correlation coefficients. The two criteria (Cr and COF) were therefore considered to be more appropriate indices of separation quality than average selectivity (Table 2).

Employing either optimization criterion a significant linear correlation was demonstrated between either criterion and both applied potential or buffer proportion [statistical significance level (p) less than 0.01], a feature not exhibited with average selectivity α_{av} (Table 3).

From Table 3 it is also evident that buffer concentration exerts minimal influence on the quality of separation as measured by either criterion when compared to applied potential or buffer proportion. This was consistent with the primary purpose for

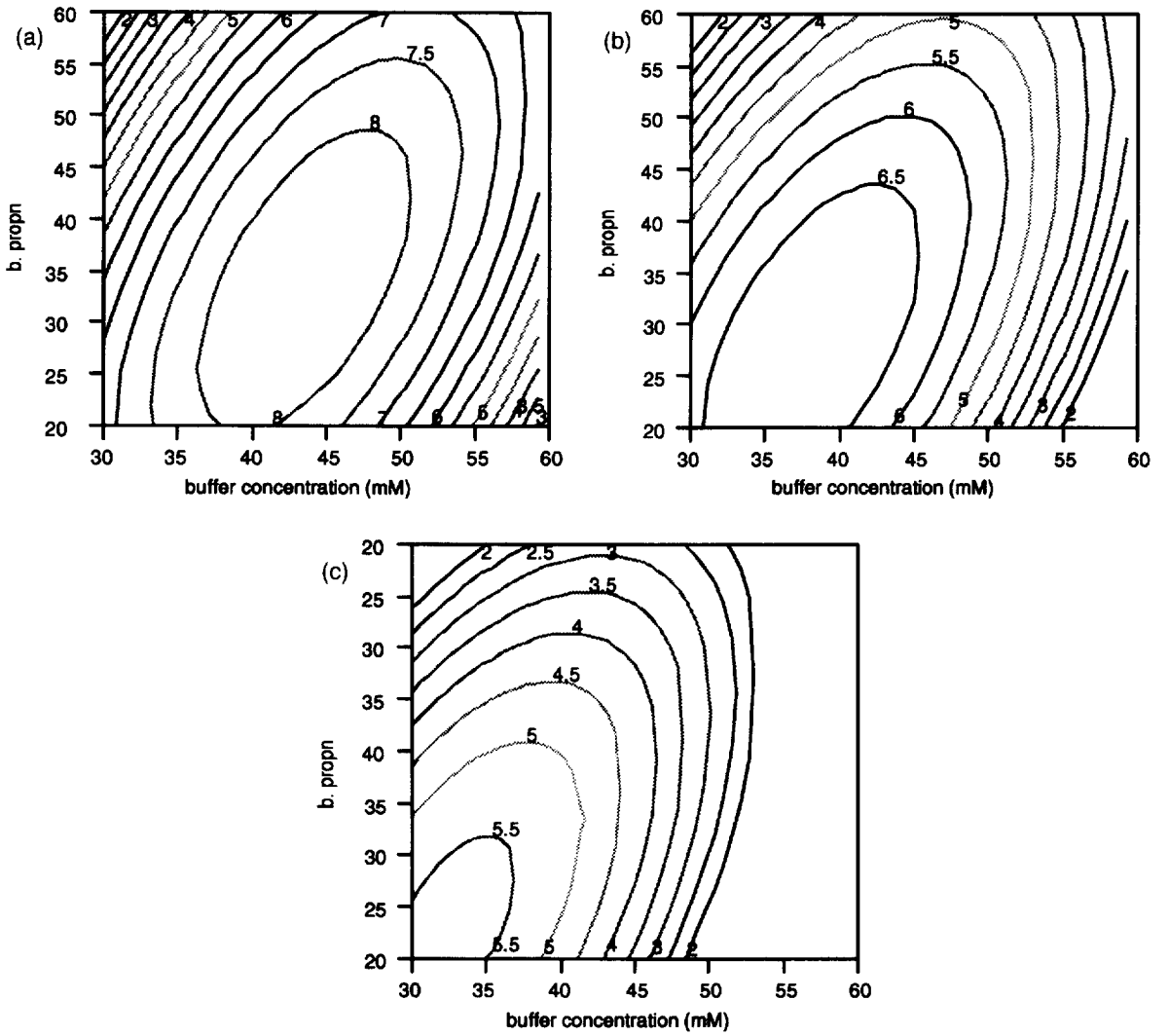

Fig. 3. Contour plots for influence of buffer concentration, proportion and applied potential on the COF. (a) Contour plot for COF at 30 kV applied potential. Optimum ~35% buffer, 43 m*M* Tris·HCl. (b) Contour plot for COF at 25 kV applied potential. Optimum ~30% buffer, 37 m*M* Tris·HCl. (c) Contour plot for COF at 20 kV applied potential. Optimum ~23% buffer, 33 m*M* Tris·HCl.

inclusion of the buffer, to provide a conductive medium though the largely non-aqueous mobile phase. This observation can also be inferred from examination of the chromatograms which also demonstrate the limited influence of buffer concentration on the quality of separation (Fig. 2).

The applied potential clearly has a significant influence on the criterion, and generally it was found

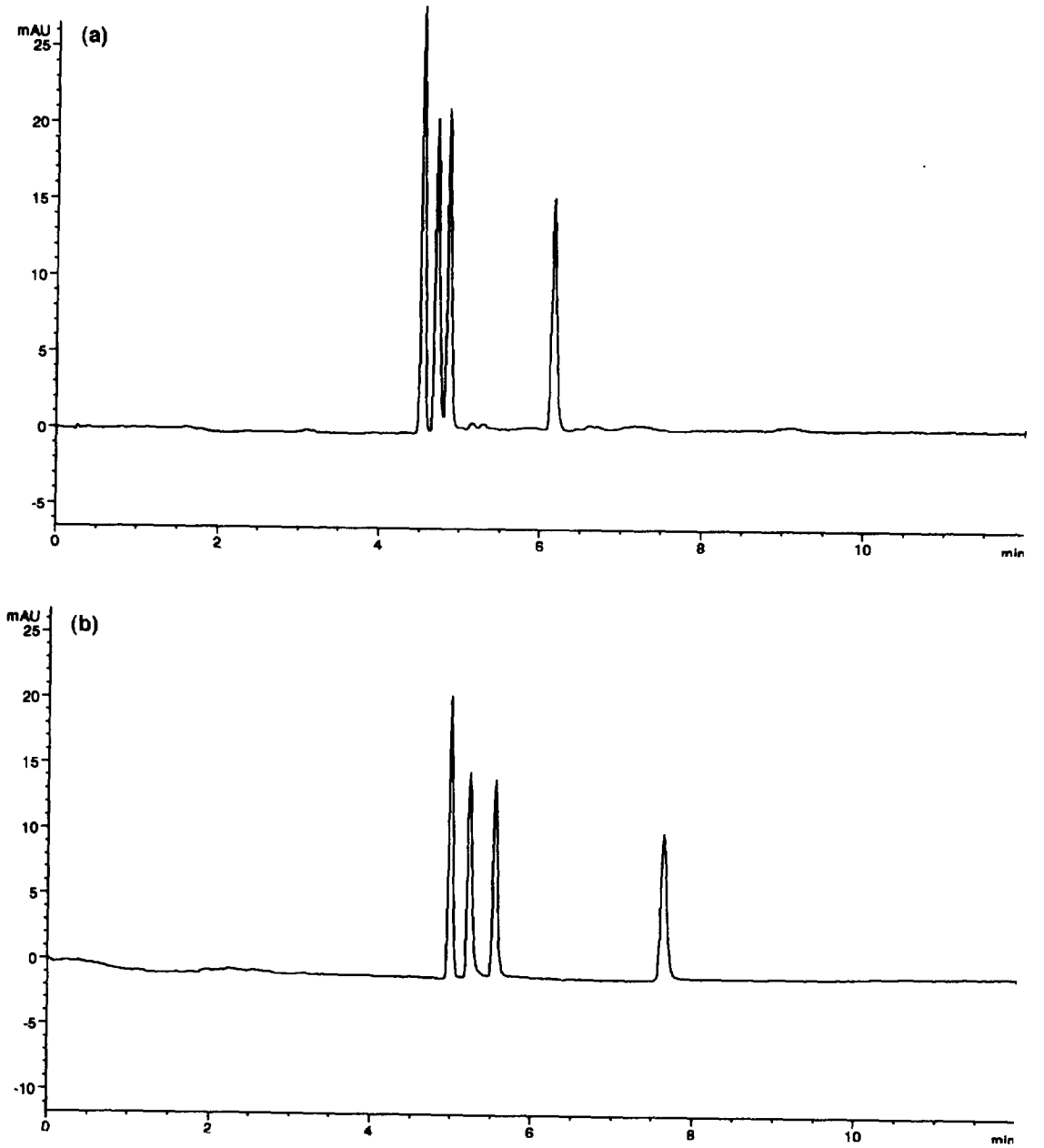

Fig. 4. Influence of buffer proportion on the chromatograms obtained. Elution order thiourea, DUP105, DUP105 sulfone and DUP105 precursor. (a) 35% Tris·HCl (40 m*M*) in ACN, applied potential 25 kV. (b) 45% Tris·HCl (40 m*M*) in ACN, applied potential 25 kV. (c) 55% Tris·HCl (40 m*M*) in ACN, applied potential 25 kV.

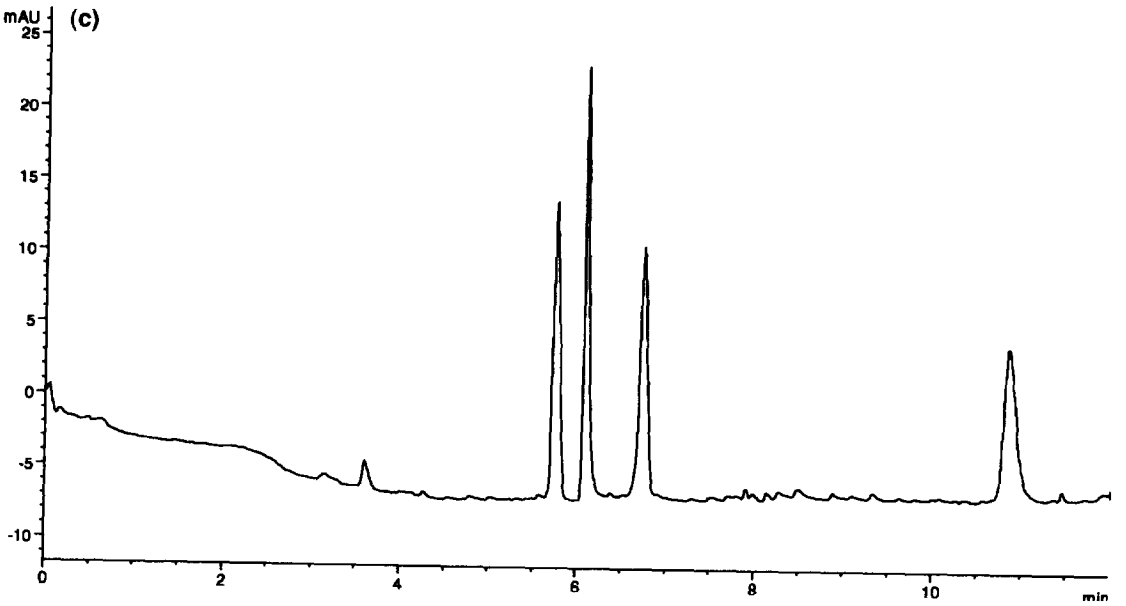

Fig. 4. (*continued*)

that the higher the potential the more desirable the numerical value of the criterion (Fig. 3).

In general better separations as defined by a higher COF were achievable by the application of higher potentials. However, it should be noted that higher potentials would also be associated with a greater risk of separation failure due to bubble formation. For this reason, 25 kV was identified as the preferred applied potential. The proportion of buffer however had a negative influence on the optimization criterion. High buffer proportions generally decreased numerical value of the criterion, largely by increasing selectivity, but more importantly, the migration time and hence analysis times (Fig. 4). As expected the converse, that is, an increase in the proportion of acetonitrile decreased analysis time but more significantly compromised selectivity.

In fact coelution of DUP105 and DUP105 sulfone occurred (poor baseline resolution) when the proportion of acetonitrile was greater than 60%. The incorporation of methanol into a 60% acetonitrile mobile phase was also found to result in the coelution of DUP105 and it sulfone.

The contour surfaces in Fig. 3a–c reveal smooth response surfaces that exhibit a gradual increase in the optimization criterion with a decrease in either the buffer concentration or the volume fraction of buffer. A characteristic feature of the response surface was the presence of a maximum, the amplitude of which was larger at the upper than at the lower end of applied potential range. This was assumed to be a consequence of second order interactions between the proportion of buffer with either applied potential or buffer concentration. The amplitude was also expected to influence both method precision and ruggedness.

The tendency towards an undesirable criterion value at the lower buffer concentration and proportion was presumed to be a consequence of the shorter migration times associated with low proportion of buffer. At the other extreme the high buffer proportion and concentration, facilitated better selectivity (α) as shown by the improved peak separation. Indeed a preliminary heuristic search for desirable electrochromatographic conditions ended at a 40% buffer (50 m*M*) in acetonitrile for an applied potential of 25 kV (Fig. 5).

A saddle point was predicted lie outside the parameter space employed in the optimization. The significance of the saddle point was not examined.

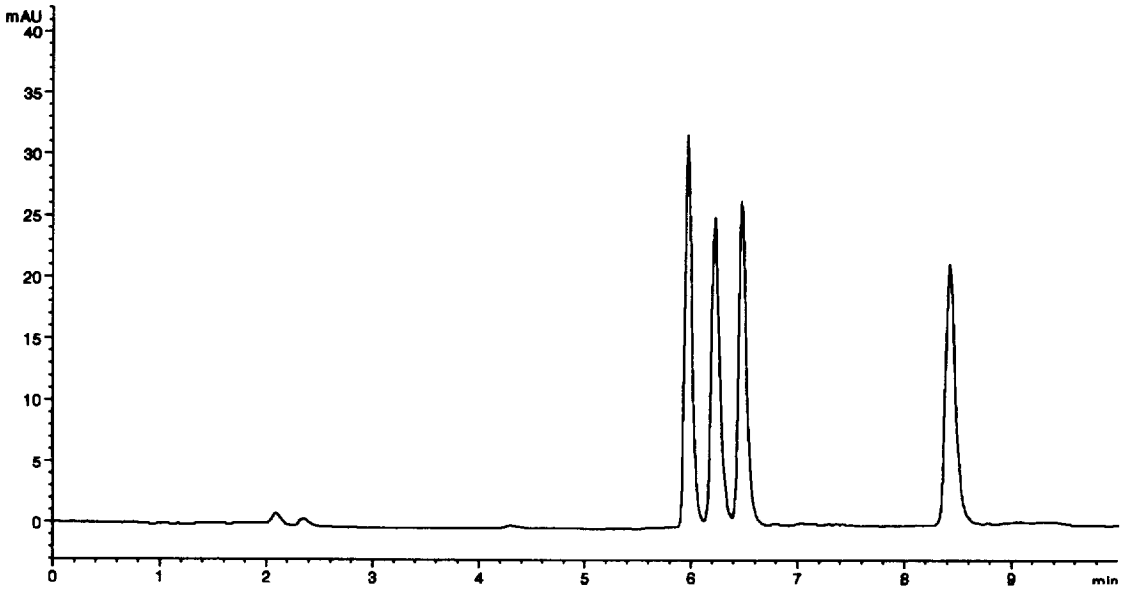

Fig. 5. Chromatogram for ACN–Tris·HCl (50 m*M*) (60:40) mobile phase. Elution order thiourea, DUP105, DUP105 sulfone and DUP105 precursor.

4. Conclusions

The separation of DUP105 from its sulfone and sulfide by CEC is feasible. The response surfaces for the separation reveal a smooth variation in the quality of separation with both the buffer proportion and concentration. The response surfaces are characterized by the presence of a maximum, whose position was dependent on the applied potential. The maximum represented the combination of buffer concentration and proportion affording the better separations as defined by the optimization criterion. The volume fraction of buffer has a greater influence on the quality of separation than does the buffer capacity. This permits the use of high buffer concentrations, with the attendant advantage of longer intervals between vial replenishment. Higher applied potentials diminish the influence of buffer concentration and proportion on the quality of separation.

References

[1] G. Ross, M. Dittmann, F. Bek and G. Rozing, Am. Lab., March (1996).
[2] P. Pitchen, E. Dunack, M.N. Desmukh and H.B. Kagan, J. Am. Chem. Soc., 106 (1984) 8188.
[3] E. Difuria, G. Modena and R. Seraglia, Synthesis, 4 (1984) 325.
[4] T. Sugimoto, T. Kokubo, J. Miyazaki, S. Tanimoto and M. Okano, J. Chem. Soc. Chem. Commun., 9 (1979) 402.
[5] S. Colonna, S. Banh, F. Fontana and M. Sommaruga, J. Org. Chem., 50 (1985) 769.
[6] J. Miyawa, M. Alasandro and C. Riley, in preparation.
[7] M. Alasandro, G. Bennett, R. Boucher, J. Brown and T. Harris, unpublished results.
[8] M. Alasandro, J. Pharm. Biomed. Anal., 35 (1996) 1156.
[9] C.K. Bayne and I.B. Rubin, in Practical Experimental Designs and Optimization Methods for Chemists, VCH Publishers, Deerfield Beach, FL, 1986.
[10] M. Jimidar, Q Yang, J. Smeyers-Verbeke and D.L. Massart, Trends Anal. Chem., 15 (1996) 2.
[11] R.M. Lopes Marques, P.J. Schoenmakers, C.B. Lucasius and L. Buydens, Chromatographia, 36 (1993) 83.
[12] J.L. Glajch, J.J. Kirkland, K.M. Squire and J.M. Minor, J. Chromatogr., 199 (1980) 57.
[13] D.R. Baker, in Capillary Electrophoresis, Wiley, New York, 1995.