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High-speed determination of drug related impurities by capillary electrophoresis employing commercial instrumentation

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ABSTRACT

High-speed capillary electrophoresis (HSCE) separations of pharmaceuticals are presented with high efficiency separations being achieved typically within 2 min. These results are obtained by applying high voltages across relatively short capillaries. Separation selectivity is shown to be similar to normal-speed CE. Quantitative HSCE impurity results compare well to those achieved by CE, TLC and HPLC. Analytical method validation criteria for HSCE such as linearity, precision and limits of detection are assessed.

INTRODUCTION

Currently the analysis of pharmaceuticals is predominantly performed by HPLC which can offer a high degree of automation, reliability, selectivity, good precision and reasonable analysis times. These factors are all fundamental requirements in pharmaceutical analysis where many hundreds, or thousands of samples, of each individual drug candidate, are analysed to provide chemical stability data.

HPLC can offer the potential of reducing analysis times by employing short and efficient packed columns. However, microbore HPLC necessitates the use of specialised equipment since the volumes of liquid eluting from a microbore column are small and travel through the detector rapidly. Any dead volumes present in the system have an extremely deleterious effect on both separation efficiency and resolution [1]. Numerous successful high-speed HPLC separations have been published including the separation of catecholamines [2]. The on-column detection mode employed in CE offers no opportunity for dead volumes to arise. However, despite its potential advantages, microbore HPLC is not widely used in pharmaceutical analysis, possibly due to instrumentation considerations and requirements.

CE has been investigated for use in the area of drug analysis by several groups [3–8]. The simultaneous use of HPLC and CE make a powerful combination when used to assess the validity of results generated using either technique. This joint approach has been demonstrated for the determination of the active content in pharmaceutical formulations [3], drug-related impurities [4] and chiral analysis [8].

It has been suggested [9] that CE, in comparison to HPLC, can potentially offer advantages in terms of resolution, separation efficiencies and reduced sample volumes and consumables. However, HPLC is generally considered superior in terms of injection precision and sensitivity.

According to Green and Jorgenson [10] the migration time of the solute is proportional to the length of capillary to the detector:

$$t = \frac{L^2}{(\mu + \mu_{osm})V} \quad (1)$$

where L = length of capillary (cm)
μ = electrophoretic mobility
μ_{osm} = electroendosmotic flow velocity
V = applied voltage (V)

The maximum applicable voltage with commer-

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cial instrumentation is limited to 30 kV, largely for reasons of electrical insulation. Therefore, the use of short capillaries is the best means of reducing analysis times. However, the length of capillary employed is limited by the joule heat which is generated when a voltage is applied. The level of this heat is proportional to several factors including electrolyte conductivity and the capillary length and bore. This heat, not fully dissipated, is detrimental to the separations achieved. This problem may be overcome by the use of narrow-bore (25 μm) capillaries where the thermal heat dissipation is good given the relatively high surface areas compared to the 50- or 75-μm bore capillaries widely used in CE.

The use of narrow-bore capillaries, though solving the heat dissipation problem, present an alternative difficulty in that the sensitivity achieved is proportional to the bore of the capillary employed [11]. Since the relative performance of CE, compared to HPLC, in terms of sensitivity was earlier highlighted as an area of weakness, this is not the best approach to reducing analysis times.

The standard lengths employed in CE are in the range 50–100 cm. In addition, the length of capillary used is often dictated by the physical constraints of particular commercial instruments.

Typical power outputs for low-molarity buffers are in the region of 1–2 W/m whilst for more concentrated buffers (*ca.* 100–200 m*M*) values in the region of 6–8 W/m can be expected. Eqn. 2 shows that the power output increases with reduced capillary length [12]. This would indicate that the problems of adequate heat dissipation would be increased when using concentrated electrolytes in high-speed CE (HSCE).

$$W = \frac{K c r^2 V^2}{L} \quad (2)$$

where W = Watts per metre
K = molar conductivity
c = electrolyte concentration
r = capillary radius
V = applied voltage
L = total capillary length

When working with high power levels the heat generated within the capillary cannot be fully dissipated which can cause boiling, or outgassing, of the electrolyte. This would then result in electrical breakdown of the system. Therefore, it is necessary to select operating conditions of electrolyte concentration and applied voltage to maintain power output at an acceptable level.

A limited number of reports [13–18] concerning the use of short capillaries have appeared in the literature. Chen [13] reported the use of a 25 cm × 25 μm capillary for the rapid and efficient separation of proteins using relatively concentrated buffer (150 m*M* borax). The good heat dissipation ability of the narrow-bore capillary allowed use of the high ionic strength buffer to achieve separations within 200 s. A relative standard deviation (R.S.D.) of less than 1% was obtained for migration times of 9 replicate injections, and efficiencies of up to 100 000 theoretical plates were obtained. Bushey and Jorgenson [14] separated a peptic digest within 55 s by applying 22 kV across a 38 cm long capillary (6.5 cm to detector). This separation formed the second dimension of a HPLC–CE instrument.

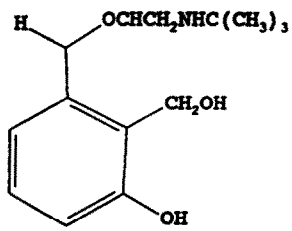
Other reports of HSCE include chiral analysis [15], separations of inorganic anions [16] and underivatised sugars [17].

Desty [18] has defined the use of "number of theoretical plate per second" as a criterion of speed and efficiency in rapid separation techniques. When employing specialised capillary GC equipment 2000 plates/s [18] can be achieved. It is considered appropriate to employ this performance criterion measurement in HSCE.

EXPERIMENTAL

Sodium citrate (20 m*M*, pH 2.5) was obtained from Applied Biosystems (San Jose, CA, USA) and inorganic chemicals were purchased from Aldrich (Poole, UK). Water was obtained from a Millipore Milli-Q system (Watford, UK). CE was performed on a P/ACE 2000 CE instrument (Beckman, Palo Alto, CA, USA) which was connected to a Hewlett-Packard HP 1000 (Bracknell, UK) data collection system.

The fused-silica capillaries used in this study were purchased from Beckman. The minimum capillary length that it is possible to operate with on the commercial CE instrument available in our laboratory is 27 cm long (20 cm to the detector). Capillaries of these dimensions were used for all HSCE separations described in this paper. Test samples were obtained from within Glaxo Group Research, the chemical structures are given in Fig. 1.



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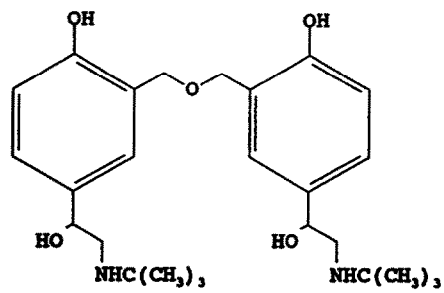

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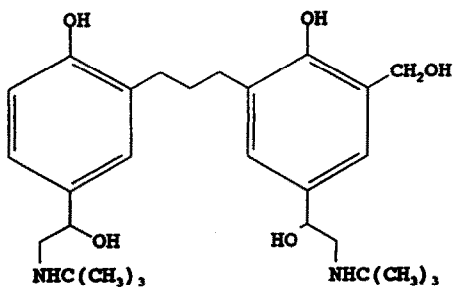

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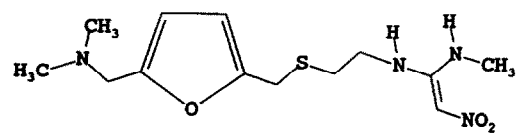

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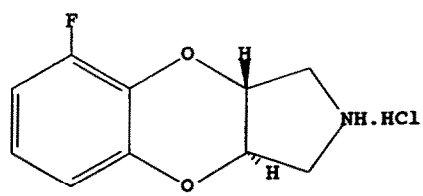

V

Fig. 1. Chemical structures of test solutes. I = Salbutamol; II = salbutamol bis-ether; III = salbutamol dimer; IV = ranitidine; V = fluparoxan.

The CE instrument utilised in this work has an arrangement in which the capillary is housed in a cartridge. This cartridge is filled with cooling fluid which helps to control temperature and to enhance dissipation of excess heat generated within the capillary.

The CE separation method employed is given below, the method consists of five automated steps:

Step I	Rinse cycle 1: 0.5 *M* NaOH, 1 min
Step II	Rinse cycle 2: run buffer, 1 min
Step III	Set detector: 0.02 a.u.f.s.
Step IV	5.0 s hydrodynamic sampling
Step V	Operating voltage: +30 kV
	Operating temperature: 25°C
	Capillary dimensions: 27 cm (20 cm to detector) × 50 μm
	Run time: 100 s
	Wavelength: 200 nm

Rinse steps are recommended to condition the capillary [13]. This flushing is achieved hydrodynamically, therefore the use of a shorter capillary results in a higher pressure difference across the capillary resulting in more flushing solvent passing through the capillary per unit time. This allows the use of shorter rinsing times. The peak widths obtained in HSCE may be fractions of a second therefore it is essential to select the fastest time constant available on both the instrument and data handling system (16 Hz). All peak areas used in these studies were normalised to compensate for their differential peak velocities [19]. This normalisation consists of dividing the measured peak area by the migration time of the corresponding peak.

RESULTS AND DISCUSSION

Analysis time reduction

To experimentally confirm the predicted reduction in analysis time a separation was conducted using a standard capillary length of 75 cm long (68 cm to the detector) and applying 30 kV, resulting in a field strength of 400 V/cm. The separation was then repeated applying 30 kV across a 27 cm capillary (20 cm to the detector) which generated a field strength of 1111 V/cm. The migration speed of an ion is directly related to both the field strength and the length of capillary to the detector.

The reduction in analysis time transferring the separation between different dimension capillaries can be calculated using eqn. 3.

$$\text{Reduction factor} = \frac{E_1 l_2}{E_2 l_1} \quad (3)$$

where E_1 = field strength (V/cm) applied across capillary 1
E_2 = field strength (V/cm) applied across capillary 2
l_1 = length to detector along capillary 1 (cm)
l_2 = length to detector along capillary 2 (cm)

In the example given above it would be expected that the migration times would be approximately reduced 10-fold changing from the 75 cm long capillary to the shorter column. Other factors such as comparisons of the internal temperature within the respective capillaries have not been included in this treatment.

The example selected was the CE separation of selected dimeric impurities of salbutamol, which are present at low levels in drug substance [20]. The chemical structures, and identities, of the compounds concerned are given in Fig. 1. Fig. 2A shows the separation of the dimeric impurities from each other, and from salbutamol, within an analysis time of 10 min. Fig. 2B shows the HSCE separation with a 1 min analysis time. This represents the predicted approximately 10-fold reduction in analysis time. It should be noted that there is slight loss in resolution when using the short capillary, however quantitation of the impurities is still readily achieved.

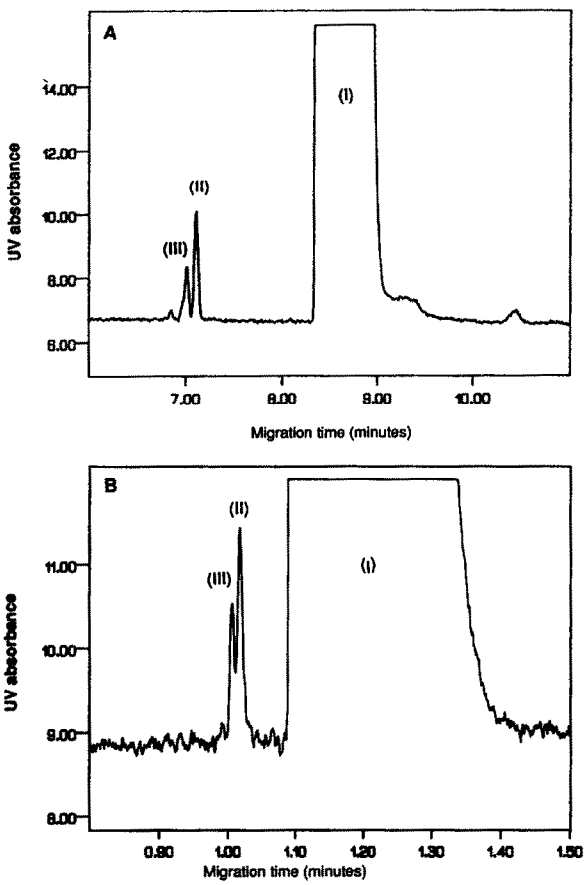

Fig. 2. (A) CE separation of selected salbutamol dimeric impurities. (B) HSCE separation of selected salbutamol dimeric impurities. Separation conditions: 50 m*M* borax pH 2.2 with conc. H_3PO_4, sample concentration 1 mg/ml in water, 200 nm, 2 s pressure injection, 30 kV. Peaks: I = salbutamol; II = salbutamol bis-ether; III = salbutamol dimer. Absorbance in arbitrary units.

Determination of drug-related impurities

High-speed separation of the related impurities of three pharmaceuticals, ranitidine, salbutamol and fluparoxan, are described below as illustrative examples.

Salbutamol

Salbutamol (Fig. 1) is a bronchodilator employed for the treatment of asthma. Thin-layer chromatographic (TLC) and HPLC methods have been developed for the determination of salbutamol related impurities [21]. The levels of selected dimeric impurities present in a range of salbutamol drug substance batches were simultaneously analysed by TLC, HPLC and CE [20]. The results generated (Table I) show good agreement between the three techniques [20]. These batches were further tested by HSCE and the results (Table I) obtained compare well with those previously obtained. Fig. 2A and B shows the CE and HSCE separations, respectively.

Impurity levels were determined in HSCE by use of external standard of the bis ether compound. An R.S.D. of 3.50% was obtained on 16 injections of two calibration solutions. This is considered an acceptable level of precision given the low levels of impurities being determined.

The results obtained by HSCE are comparable with those generated by CE, HPLC and TLC. The spread of results for the replicate injections in HSCE is acceptable when compared to those previously generated by CE and HPLC. This analysis demonstrates that HSCE is capable of generating useful information regarding drug purity within a relatively short analysis time. This ability would be of considerable importance within a quality control

TABLE I

COMPARISON OF BIS-ETHER AND DIMER LEVELS IN EXPERIMENTAL SALBUTAMOL SULPHATE DRUG SUBSTANCE BATCHES AS DETERMINED BY HSCE, CE, HPLC AND TLC

Batch	Salbutamol-bis-ether (%, w/w)				Salbutamol dimer[a]			
	HSCE	CE	HPLC	TLC	HSCE	CE	HPLC	TLC
1	0.171	0.144	0.156	0.18	0.089	0.077	0.076	0.09
	0.171	0.137	0.159		0.092	0.086	0.082	
2	0.132	0.101	0.110	0.19	0.091	0.062	0.066	0.12
	0.139	0.101	0.113		0.089	0.067	0.060	
3	0.215	0.201	0.191	0.24	0.137	0.133	0.111	0.16
	0.205	0.202	0.190		0.153	0.135	0.098	
4	0.140	0.121	0.134	0.20	0.088	0.073	0.058	0.13
	0.137	0.145	0.135		0.078	0.080	0.052	
5	0.132	0.129	0.135	0.12	0.070	0.081	0.056	0.08
	0.150	0.115	0.131		0.093	0.073	0.053	
6	0.295	0.308	0.281	0.32	0.204	0.179	0.171	0.23
	0.321	0.309	0.257		0.216	0.188	0.151	
7	0.116	0.068	0.093	0.12	0.068	0.045	0.035	0.06
	0.090	0.081	0.103		0.066	0.055	0.032	
8	0.413	0.384	0.378	0.45	0.244	0.199	0.181	0.28
	0.393	0.439	0.382		0.241	0.216	0.188	
9	0.376	0.372	0.375	0.30	0.219	0.191	0.173	0.30
	0.373	0.366	0.348		0.220	0.186	0.172	
10	0.618	0.754	0.660	0.69	0.395	0.388	0.334	0.45
	0.605	0.766	0.667		0.392	0.397	0.353	

[a] Dimer by HPLC expressed as % (w/w) salbutamol, CE results expressed as % (w/w) bis-ether, TLC results expressed as % (w/w) dimer.

environment where high sample throughput is essential.

It is essential that consistent migration times are obtained to establish peak identification. Literature reports indicate [7] that the precision obtained for peak migration times is in the order of 0.5–1% R.S.D.

A single salbutamol sample was injected nine times under the HSCE conditions and the variation in the migration times was calculated. The performance data are given in Table II. In this study the capillary was flushed between runs with base and then electrolyte. The effect of eliminating these steps has been shown to be detrimental to the reproducibility of migration times in HSCE [13].

Linearity. Linearity of detector response with salbutamol concentration was demonstrated over the range 0.002–0.1 mg/ml (correlation coefficient 0.9995). This linearity is similar to that reported for other CE analyses [3].

Limit of detection. The limit of detection obtained was 2 μg/ml of salbutamol in solution which is equivalent to $1 \cdot 10^{-7}$ *M*, this figure is in agreement with that reported elsewhere [3] for salbutamol as determined by CE.

Ranitidine

Ranitidine is a compound used for the treatment of peptic ulcers. There are a range of possible synthetic and degradative impurities [22]. Various analytical methods have been described for the separation and quantitation of ranitidine and its related impurities. A low pH, CE method has been developed which is capable of resolving ranitidine related impurities. The electrolyte employed in this separation contains 2 m*M* dimethyl-β-cyclodextrin to obtain the required selectivity. Other workers [23,24] have reported the use of cyclodextrin additives to alter the separation selectivity in CE.

TABLE II

REPRODUCIBILITY OF MIGRATION TIMES

Peak identity	Migration time range (min)	R.S.D. ($n = 9$) (%)
Dimer	0.94–0.96	0.6
Bis-ether	0.95–0.97	0.6
Salbutamol	1.07–1.09	0.5

A feature and attraction of CE is that samples with a complex matrix can often be directly injected onto the capillary without fouling the capillary. This ability can often overcome the need for elaborate sample pretreatment. Direct injections of urine [25], plasma [26] and liquid pharmaceutical formulations [25] have been reported.

Fig. 3 shows the separation of a degraded ranitidine syrup sample. The formulation (15 mg/ml) was directly loaded onto the capillary to assess the levels of impurities. Up to 500 000 plates are obtained in this separation which is equivalent to 10 000 plates/s. This analysis demonstrates that HSCE can be used for the high throughput testing of formulated products during stability evaluation or in quality control testing.

Fluparoxan

Previously, we have reported [5] the use of CE to separate fluparoxan from one of its related impurities. A drug substance batch, prior to purification, containing high levels of related impurities was analysed by both CE and HSCE.

Fig. 4 shows both the conventional CE and HSCE analysis. This sample was analysed five times using both techniques to give the data in Table III. The impurity profiles and levels are similar in both instances. The precision of main peak area are 0.7 and 1.1% R.S.D. for conventional CE and HSCE,

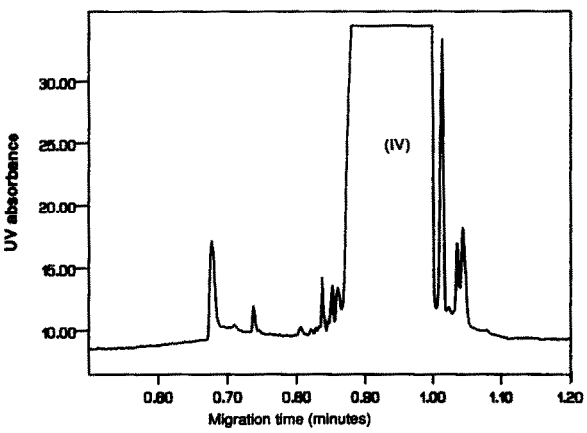

Fig. 3. HSCE separation of degraded ranitidine syrup sample. Separation conditions: 25 m*M* borax with 2 m*M* dimethyl-β-cyclodextrin, pH 2.4 with conc. H_3PO_4, sample concentration 15 mg/ml, 230 nm, 2 s pressure injection, 15 kV. Peak: IV = ranitidine.

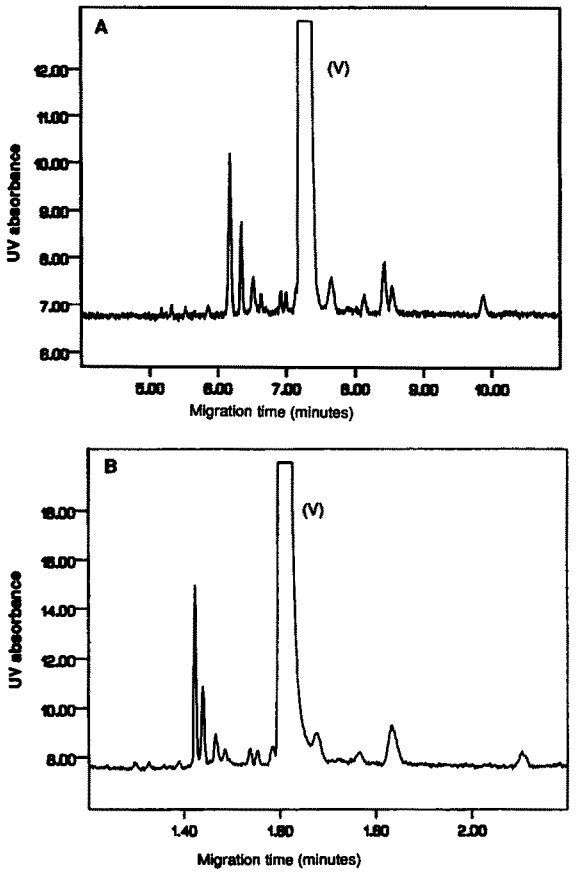

Fig. 4 (A) CE separation of fluparoxan drug substance prior to purification. (B) HSCE separation of fluparoxan drug substance prior to purification. Separation conditions: 50 m*M* borax pH 2.2 with conc. H_3PO_4, sample concentration 0.5 mg/ml in water, 214 nm, 10 s pressure injection, 10 kV. Peak: V = fluparoxan.

respectively, which indicates that quantitative determinations could be considered.

Advantages and limitations of HSCE

The principal advantages of HSCE are the decreased analysis time, and higher sample throughput. In addition, HSCE is a useful option for rapid method development as a wide range of electrolyte compositions can be evaluated within a given time period.

There are, however, some disadvantages employing HSCE. These are that some loss in resolution occurs, and that the analysis time reductions possible may be limited by the electrolyte strength required for a particular analysis.

TABLE III

COMPARISON OF CE AND HSCE FLUPAROXAN IMPURITY DATA

No. of injections = 5. %a/a = %area/area (using normalised peak areas); R.S.D. = relative standard deviation; RMT = relative migration time of impurity compared to fluparoxan.

	CE	HSCE
Fluparoxan area	613 792	750 578
Fluparoxan area R.S.D. (%)	0.7	1.1
Total No. of impurities	15	14
Total %area/area (%a/a)	11.04	11.95
Total %area/area R.S.D. (%)	1.8	2.2
Impurity RMT 0.85 (%a/a)	3.4	3.4
Impurity RMT 0.85 %a/a R.S.D. (%)	1.6	1.8
Impurity RMT 0.87 (%a/a)	1.6	1.8
Impurity RMT 0.87 %a/a R.S.D. (%)	3.0	4.9

CONCLUSIONS

Use of high voltages coupled with short separation capillaries can greatly reduce the separation times involved in CE. These advantages can be obtained using commercially available equipment with no specific modifications being required. Typical analysis times may be less than 100 s, with high separation efficiencies of up to be 10 000 plates/s being obtained with similar selectivity to conventional speed CE. This aspect, coupled with the automated method development facilities incorporated in commercial CE instrumentation, makes CE an attractive option when considering a technique for routine, high throughput testing of pharmaceuticals.

It is suggested that HSCE will have a considerable role for in-process control and the determination of drug related impurities.

REFERENCES

1 C. E. Reese and R. P. W. Scott, *J. Chromatogr. Sci.*, 18 (1980) 479.
2 R. P. W. Scott, *J. Chromatogr. Sci.*, 23 (1985) 47.
3 M. T. Ackermans, J. L. Beckers, F. M. Everaerts and I. G. J. A. Seelen, *J. Chromatogr.*, 590 (1992) 341.
4 G. M. Mclaughlin, J. A. Nolan, J. L. Lindahl, J. A. Morrison and T. J. Bronzert, *J. Liq. Chromatogr.*, 15 (1992) 961.
5 K. D. Altria and N. W. Smith, *J. Chromatogr.*, 538 (1991) 506.
6 H. Nishi and S. Terabe, *Electrophoresis*, 11 (1990) 691.
7 M. E. Swartz, *J. Liq. Chromatogr.*, 14 (1991) 923.
8 K. D. Altria, D. M. Goodall and M. M. Rogan, *Chromatographia*, 34 (1992) 19.

9 W. Steuer, I. Grant and F. Erni, *J. Chromatogr.*, 507 (1990) 125.
10 J. S. Green and J. W. Jorgenson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 529.
11 J. P. Chervet, R. E. J. van Soest and M. Ursem, *J. Chromatogr.*, 543 (1991) 439.
12 H. J. Isaaq, G. M. Janini, I. Z. Atamana and G. M. Muschik, *J. Liq. Chromatogr.*, 14 (1991) 817.
13 F. T. A. Chen, *J. Chromatogr.*, 559 (1991) 445.
14 M. M. Bushey and J. W. Jorgenson, *J. Chromatogr.*, 480 (1989) 301.
15 M. J. Sepaniak, R. O. Cole and B. K. Clark, *J. Liq. Chromatogr.*, 15 (1992) 1023.
16 W. R. Jones and P. J. Jandik, *J. Chromatogr.*, 546 (1991) 445.
17 J. Pawliszn and J. Wu, *J. Chromatogr.*, 559 (1991) 111.
18 D. H. Desty, *Adv. Chromatogr.*, 1 (1965) 100.
19 D. M. Goodall, S. J. Williams and D. K. Lloyd, *Trends Anal. Chem.*, 10 (1991) 272.
20 K. D. Altria, *J. Chromatogr.*, in press.
21 M. Mulholland and J. Waterhouse, *Chromatographia*, 25 (1988) 769.
22 M. B. Evans, P. A. Haywood, D. Johnson, M. Martin-Smith, G. Munro and J. C. Wahlich, *J. Pharm. Biomed. Anal.*, 7 (1989) 1.
23 S. K. Yeo, C. P. Ong and S. F. Y. Li, *Anal. Chem.*, 63 (1991) 2222.
24 S. Terabe, Y. Miyashita, O. R. N. Shibata, E. R. Barnhart, L. R. Alexander, D. G. Patterson, B. L. Karger, K. Hosoyo and N. Tanaka, *J. Chromatogr.*, 516 (1990) 23.
25 K. D. Altria and M. M. Rogan, *J. Pharm. Biomed. Anal.*, 8 (1990) 1005.
26 H. Nishi, T. Fukuyama and M. Matsuo, *J. Chromatogr.*, 515 (1990) 245.