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Separation of basic drugs with non-aqueous capillary electrophoresis

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Abstract

Capillary zone electrophoresis (CZE) was investigated in non-aqueous media. Efficient, rapid and versatile electrophoretic conditions were obtained with 20 m*M* ammonium acetate in acetonitrile–methanol–acetic acid (49:50:1). Using this non-aqueous medium, the baseline separation of nine morphine analogues, eleven antihistamines, eleven antipsychotics and ten stimulants could each be achieved in 6 min. The migration order observed was very different from one expected for an aqueous medium. The migration time repeatability for individual components was between 0.8 and 3.7% R.S.D. The migration time-normalized peak area had a poor precision; however, with one of the components as an internal reference, the quantitative repeatability could be improved to between 2.2 and 9.1% R.S.D. The precision data appeared to be instrument dependent, as excellent results could be obtained from an instrument with better evaporation and temperature control. Alternatively, much improved speed, efficiency and precision were also achieved with tetra-*n*-butylammonium tetrafluoroborate as the electrolyte, albeit with reduced selectivity. The effects of the electrolyte, non-aqueous medium and applied voltage on the separation are discussed.

Keywords: Pharmaceutical analysis; Basic drugs; Antihistamines; Morphine analogues

1. Introduction

Capillary electrophoresis (CE) has been one of the fastest growing separation techniques in the last decade. It is rapid and efficient, and has been applied to many types of analysis [1,2]. However, acceptable precision data are not reported as frequently for CE as for routine chromatographic techniques, and further development seems to be necessary to bring CE to full maturity. Moreover, most CE investigations have been performed in an aqueous solution. The analytes had to be hydrophilic enough to remain dissolved and to migrate in the electrolyte medium. The addition of surfactant modifiers and organic solvents to the aqueous phase obviously extended the range of suitable analytes, but a certain degree of solubility in the aqueous medium is still necessary. Adsorption on the capillary wall can also be significant for analytes with very limited solubility.

Published work on CE in essentially non-aqueous media is rare. The first such separation was briefly reported in 1984 by Walbroehl and

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Jorgenson [3], who analysed quinoline-type charged compounds in acetonitrile containing hydrochloric acid and tetraethylammonium perchlorate. In 1986, Walbroehl and Jorgenson [4] also separated several neutral and non-polar compounds in acetonitrile in the presence of tetrahexylammonium perchlorate and 0–50% water. They suggested a mechanism involving unequal solvophobic association of the neutral analytes with the electrolyte to explain the separation. More recently, Tanaka et al. [5] mentioned the incomplete separation of triphenylene and *o*-terphenyl in methanol in the presence of 0.05% acetic acid and cationic starburst dendrimers, Naylor and co-workers [6–9] described the CE and CE–MS analyses of drugs and metabolites in methanol containing ammonium acetate and acetic acid and Sahota and Khaledi [10] reported the separation of six dipeptides in formamide containing sodium dihydrogenphosphate. Precision data were not reported in any of these investigations.

In this paper, we report our findings on capillary zone electrophoresis (CZE) in non-aqueous media, and on the selection of an essentially non-aqueous electrolytic medium for the high-speed analysis of many basic drug mixtures. Precision data for non-aqueous CE investigations are presented for the first time.

2. Experimental

2.1. Apparatus

Unless indicated otherwise, CE separations were performed on a Waters (Milford, MA, USA) Quanta 4000 capillary electrophoresis system with a built-in 0–30 kV high-voltage power supply, a fixed-wavelength UV detector near the cathodic end and a forced-air cooling system. The CE system was placed beneath a hood so that harmful solvent vapours can be safely eliminated from the laboratory environment. Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 75 or 50 μm I.D. and of variable lengths were used. A detector window was made by burning the capillary at the appropriate position (7.5 cm from the outlet) and the burned polyimide was removed by an acetone wash. About 1 cm of the polyimide coating at each end of the capillary was similarly removed in order to prevent it dissolving in the non-aqueous media. Unless indicated otherwise, the UV detector was set at 214 nm (using a zinc lamp and a 214-nm filter) and hydrostatic injections (lasting 5 s at a height of 10 cm) were used throughout. Data processing was carried out with either a VAX-based Waters Expert Ease 860 chromatography system or a Waters Millennium 2010 chromatography system. A Beckman (Fullerton, CA, USA) P/ACE 5500 CE system was used for the investigation of instrument-related imprecision.

2.2. Reagents and chemicals

The basic drug standards were obtained from Alltech (Deerfield, IL, USA). Optima-grade acetonitrile (with 0.01% of water) and methanol (with 0.07% of water) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Reagent-grade ammonium acetate was obtained from Nacalai Tesque (Kyoto, Japan), tetraethylammonium bromide and tetra-*n*-butylammonium tetrafluoroborate from Sigma (St. Louis, MO, USA) and acetic acid (with less than 0.2% of water) and formamide from Riedel-de Haën (Seelze, Germany). Other solvents and chemicals were of reagent grade or better.

2.3. Procedures

The non-aqueous running medium was prepared by simply mixing the appropriate amount of ammonium acetate in methanol with other solvent components such as acetonitrile, acetic acid or additional methanol. Other running media were prepared similarly. Solutions of various drug mixtures were prepared by dissolving the appropriate amounts of individual drug standards in the running medium and stored at −40°C until used. All samples and media in contact with the capillary were filtered through 0.45-μm PTFE syringe filters before use.

At the beginning of a working day, a vacuum

(about 50–60 kPa) was applied to the detector end and the capillary was purged consecutively for 5 min each with 0.5 *M* sodium hydroxide, deionized water, acetonitrile and the running medium. The same treatment was carried out before each run during reproducibility studies (which gave slightly better results), whereas for qualitative investigations the capillary was merely purged with the running medium between runs. When not in use, the capillary was washed with acetonitrile and deionized water and then stored in air.

The electroosmotic mobilities were determined by injecting a neutral marker (naphthalene or phenanthrene). For the reproducibility studies, a total of eight consecutive and identical runs were performed, and the electrolyte medium was renewed between runs.

3. Results and discussion

3.1. Selection of a non-aqueous medium

Our objective was to determine if CE in an essentially non-aqueous medium could be a practical, reproducible and versatile technique for the analysis of basic drugs. In an acidic medium, the different electrophoretic mobilities of the protonated form of the basic drugs should provide the basis for their separation by CZE. However, the ratio of the protonated and neutral forms of a basic drug in an acidic but totally non-aqueous medium may be smaller, and the extent of electroosmotic flow (EOF) is therefore expected to have a significant influence on the apparent mobility of the basic drug in such a medium. We therefore first determined the EOF of some organic solvents.

The electroosmotic mobility (μ_{eo}) can be defined as

$$\mu_{eo} = -\zeta\varepsilon_0 D/\eta \quad (1)$$

where ζ is the zeta potential of the interior capillary wall, ε_0 the permittivity of vacuum ($8.854 \cdot 10^{-12}$ F m^{-1}), D the dielectric constant of the medium, and η viscosity of the medium. Although the value of the zeta potential changes with the type of the medium and its ionic strength, it would seem from Eq. 1 that non-aqueous solvents having a large D/η value could give rise to appreciable electroosmotic mobilities. In this regard, acetonitrile, with a dielectric constant of 37.5 and a viscosity of 0.34 mPa s, has perhaps the most favourable D/η value (110 mPa^{-1} s^{-1}), even higher than that for water (78.5 mPa^{-1} s^{-1}). Other organic solvents having high D/η values are acetone (69 mPa^{-1} s^{-1}) and methanol (59 mPa^{-1} s^{-1}) [11]. Since the electrolyte concentration is often a sensitive parameter and UV detection is often used, acetone was discarded owing to its low boiling point (56°C) and high UV cut-off (330 nm), whereas both acetonitrile and methanol were considered good candidates for studying non-aqueous CE (boiling points 82 and 65°C, respectively, and UV cut-offs at 190 and 205 nm, respectively) [11]. Both early and recent findings [3–9] supported our selections. Acetonitrile and methanol in various proportions were therefore adopted as the media in which the electroosmotic mobilities of a neutral marker would be measured and compared.

The electroosmotic mobility, μ_{eo}, can be determined from the following equation by injecting a neutral marker:

$$\mu_{eo} = L_t L_d / V t_{eo} \quad (2)$$

where L_t is the total length of the capillary, L_d is the length of the capillary to the detector (i.e., $L_t = 7.5$ cm in our system), V is the applied voltage and t_{eo} is the migration time of the neutral marker. Initial experiments conducted with 50 m*M* tetraethylammonium bromide revealed reasonable EOF in acetonitrile or acetonitrile-based solvent mixtures. For instance, the electroosmotic mobility of a neutral marker in acetonitrile was found to be $2.8 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} in a 50-μm capillary (60 cm long). However, the running medium at the inlet end was found to turn yellowish in a relatively short time, suggesting that some of the bromide ions might be undergoing oxidation at the anodic surface.

Prompted by the encouraging results of Benson et al. [6], who used methanol containing ammonium acetate and acetic acid to analyze

pyrazoloacridine and derivatives, we turned our attention to non-aqueous media containing 20 m*M* ammonium acetate and 1% acetic acid in different amounts of acetonitrile and methanol. Such volatile electrolytic media could be useful not only in CE but also in CE–MS analysis. The results of electroosmotic mobility determination are summarized in Table 1. Each of the media featured in the table gave a reasonable EOF, with 20 m*M* ammonium acetate in acetonitrile–methanol–acetic acid (69:30:1) giving the fastest EOF.

It was observed that a very low current is encountered in a totally non-aqueous medium (less than 30 μA for the conditions listed in Table 1). Sahota and Khaledi [10] reasoned that this would reduce Joule heating. However, the reduction in heat resulting from a reduction in the current may be countered by a smaller heat capacity and lower thermal conductivity of the non-aqueous medium. Thus, the actual effect of working with a particular non-aqueous medium upon the quality of a CZE analysis (analysis time, precision, efficiency and selectivity) is better determined empirically with a test mixture.

A standard mixture of 100 ppm each of nine morphine analogues was then analysed using the five running media and other conditions listed in Table 1. Assuming that the retention effect due to solute–wall interaction is relatively small, the electrophoretic mobility, μ_{ep}, of each component was calculated for each individual medium as follows:

$$\mu_{ep} = \mu_{app} - \mu_{eo} \quad (3)$$

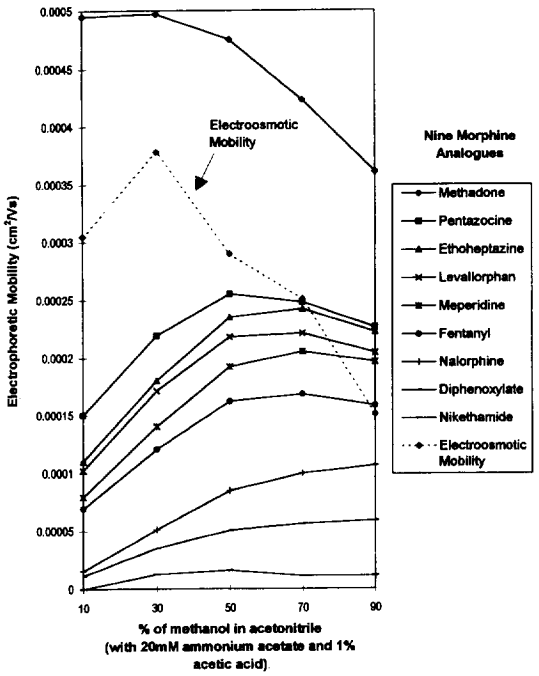

Fig. 1. Dependence of electrophoretic mobilities of nine morphine analogues on the composition of the non-aqueous medium. Applied voltage, 20 kV; capillary, 60 cm × 75 μm I.D.

where μ_{app} is the apparent mobility of a particular component and is given by

$$\mu_{app} = L_t L_d / V t_m \quad (4)$$

with t_m being the observed migration time for that component. The results are presented in Fig. 1. From these data, it was concluded that ace-

Table 1
Electroosmotic mobilities in various non-aqueous media

Electrolytic medium	EOF peak (min)	Electroosmotic mobility ($cm^2 V^{-1} s^{-1}$)
MeCN–MeOH–HOAc (9:90:1)–20 m*M* ammonium acetate	17.00	$1.54 \cdot 10^{-4}$
MeCN–MeOH–HOAc (29:70:1)–20 m*M* ammonium acetate	10.50	$2.50 \cdot 10^{-4}$
MeCN–MeOH–HOAc (49:50:1)–20 m*M* ammonium acetate	9.00	$2.92 \cdot 10^{-4}$
MeCN–MeOH–HOAc (69:30:1)–20 m*M* ammonium acetate	7.00	$3.75 \cdot 10^{-4}$
MeCN–MeOH–HOAc (89:10:1)–20 m*M* ammonium acetate	8.65	$3.03 \cdot 10^{-4}$

Applied voltage, 20 kV; capillary dimensions, 60 cm × 75 μm I.D.

tonitrile–methanol–acetic acid (49:50:1) gave the best resolution for the nine morphine analogues tested, and all components migrated reasonably fast, all of them migrating before the EOF peak, which was at about 9 min.

Decreasing the electrolyte concentration to 10 m*M* resulted in the expected decrease in the migration times, but the resulting resolution was poorer, with pentazocine and ethoheptazine incompletely resolved. Increasing the electrolyte concentration to 30 m*M* resulted in longer migration times with no improvement in resolution (data not shown).

3.2. Separation of basic drug mixtures with the selected medium

The non-aqueous medium chosen for the separation of various basic drug mixtures was therefore 20 m*M* ammonium acetate in acetonitrile–methanol–acetic acid (49:50:1). Because of the very low current observed in this medium, we were able to use very high applied voltages with little problem of Joule heating, thereby achieving high efficiency and speed. After optimizing the applied voltages, the medium provided a relatively fast baseline separation of nine morphine analogues, eleven antihistimines, eleven antipsychotics and ten stimulants, as shown in Figs. 2–5. The mixtures were all separated in ca. 6 min. The structures of the individual components are shown in Figs. 6–9.

In our previous study on the analysis of a mixture of seventeen basic drugs by CZE in an aqueous acidic medium, we observed a migration order that was generally related to the basicity of the drugs [12]. In the present study with a non-aqueous acidic medium, many of the drugs with very similar structures and nearly identical aqueous basicities could be separated. The electrophoretic mobility of an analyte is dependent on its effective charge density. Irrespective of the medium, the migration order of basic drugs separated by CZE under acidic conditions should therefore depend primarily on their ability to protonate and their effective size. As both parameters can be strongly influenced by the

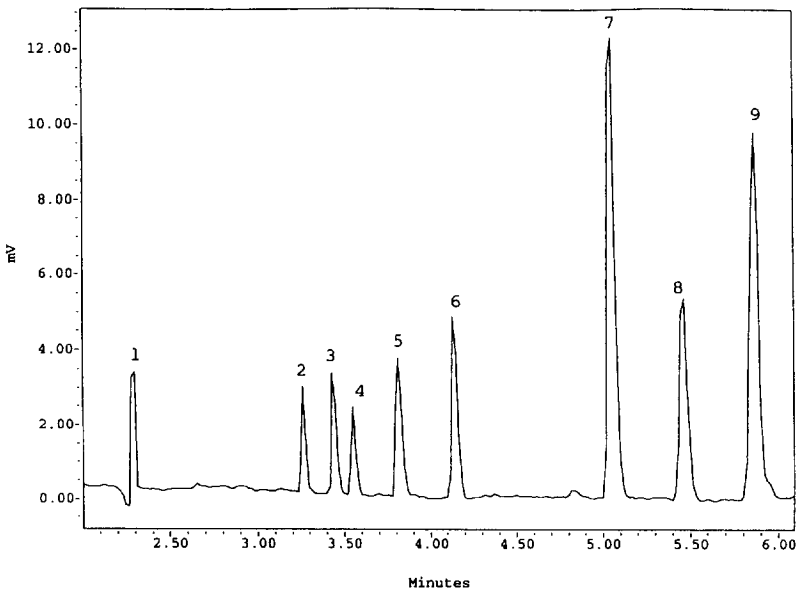

Fig. 2. Electropherogram of a mixture of nine morphine analogues (100 ppm each). Peaks: 1 = Methadone; 2 = pentazocine; 3 = ethoheptazine; 4 = levallorphan; 5 = meperidine; 6 = fentanyl; 7 = nalorphine; 8 = diphenoxylate; 9 = nikethamide. Running medium, acetonitrile–methanol–acetic acid (49:50:1)–20 m*M* ammonium acetate; capillary, 59.6 cm × 75 μm I.D.; applied voltage, 30 kV.

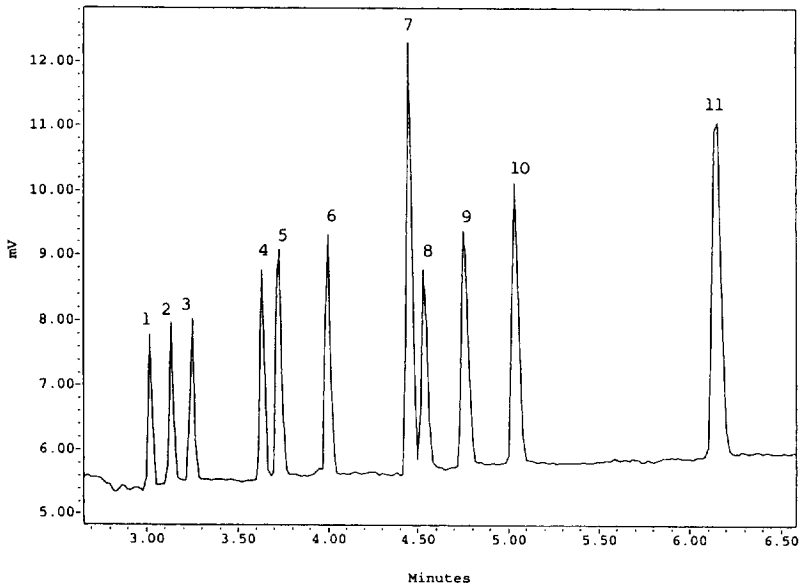

Fig. 3. Electropherogram of a mixture of eleven antihistamines (100 ppm each). Peaks: 1 = Tripelennamine; 2 = pyrilamine; 3 = promethazine; 4 = dimenhydrinate; 5 = pheniramine; 6 = chlorpheniramine; 7 = pyrrobutamine; 8 = methaphenilene; 9 = cyclizine; 10 = chlorcyclizine; 11 = buclizine. Running medium, acetonitrile–methanol–acetic acid (49:50:1)–20 m*M* ammonium acetate; capillary, 62 cm × 75 μm I.D.; applied voltage, 30 kV.

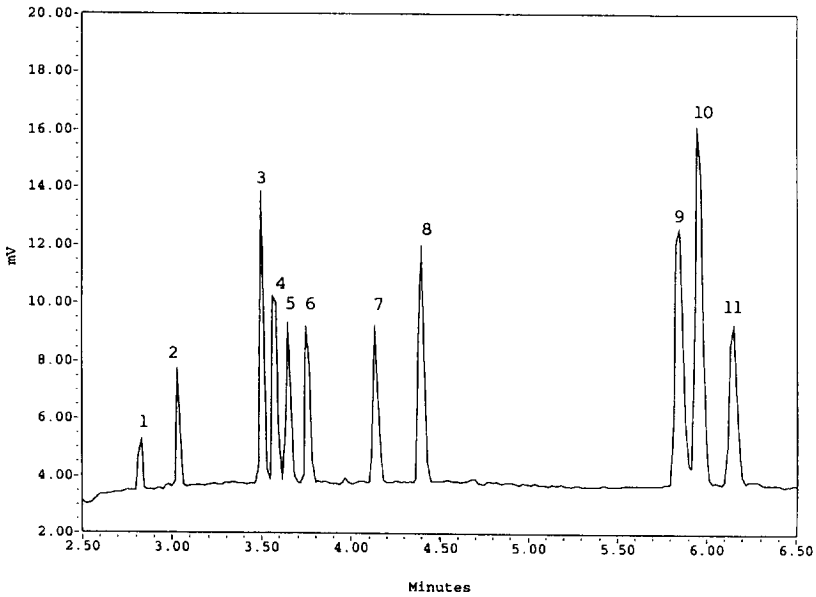

Fig. 4. Electropherogram of a mixture of eleven antipsychotic drugs (100 ppm each). Peaks: 1 = Promethazine; 2 = ethopropazine; 3 = methotrimeprazine; 4 = promazine; 5 = thioridazine; 6 = mesoridazine; 7 = molindone; 8 = thiothixene; 9 = reserpine; 10 = deserpidine; 11 = benzquinamide. Running medium, acetonitrile–methanol–acetic acid (49:50:1)–20 m*M* ammonium acetate; capillary, 59.6 cm × 75 μm I.D.; applied voltage, 30 kV.

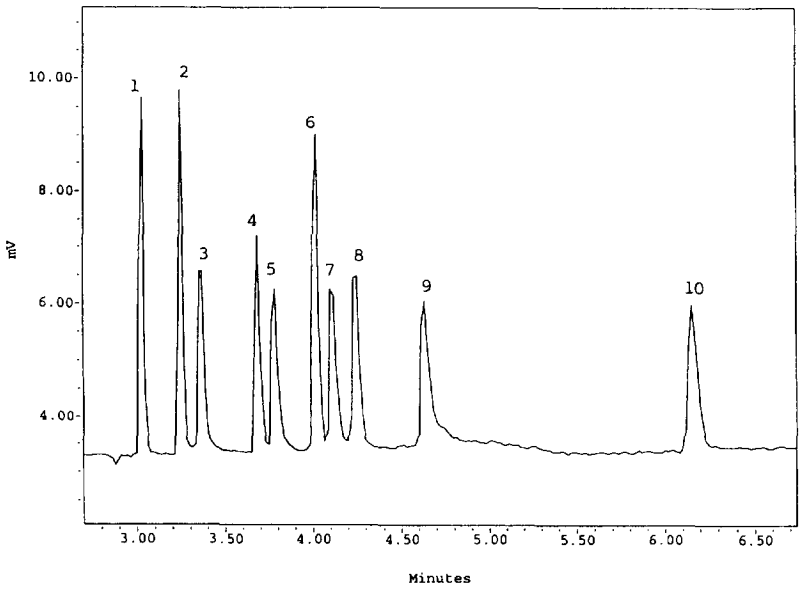

Fig. 5. Electropherogram of a mixture of ten stimulants (75 ppm each). Peaks: 1 = Xylometazoline; 2 = oxymetazoline; 3 = amphetamine; 4 = ephedrine; 5 = phenylpropanolamine; 6 = benzphetamine; 7 = phenylephrine; 8 = nylidrin; 9 = phenmetrazine; 10 = phendimetrazine. Running medium, acetonitrile–methanol–acetic acid (49:50:1)–20 m*M* ammonium acetate; capillary, 58.2 cm × 75 μm I.D.; applied voltage, 25 kV.

medium, unusual selectivity could be expected when much of the water in the electrolytic medium is removed.

For example, among the morphine analogous (Fig. 2), methadone, the only acyclic tertiary amine, migrated first, followed by many cyclic tertiary amines, with nikethamide (which has a pyridine ring and should be the least basic), migrating last. It should be noted that in an aqueous solution methadone is not the most basic (pK_a 8.3 for its conjugate acid), compounds such as ethoheptazine and its lower homologue meperidine being more basic (pK_a 8.5 and 8.7, respectively, for their conjugate acids) [13], and they also have lower molecular masses. The migration order between ethoheptazine and meperidine was also interesting: the faster moving ethoheptazine is larger in size and less basic in an aqueous medium. By way of comparison, the migration order for methadone, ethoheptazine and meperidine in an aqueous medium (20 m*M* ammonium acetate with 1% acetic acid) was confirmed to be the reverse of that observed in our non-aqueous medium (data not shown).

Distinct size effects were observable from the present data. For example, chlorcyclizine migrated faster than its analogue buclizine, which carries an additional butylphenyl group (Fig. 3). The presence of an electronegative chlorine atom, say in chlorpheniramine as opposed to pheniramine and in chlorcyclizine as opposed to cyclizine (Fig. 3), increases the molecular size and decreases the basicity, invariably resulting in a slower migration. In general, good selectivity was achieved. Even promethazine and promazine, which are isomers with very similar structures, were well separated (Fig. 4).

Precision data are given in Tables 2–5. The migration time repeatability for most components was below 3% R.S.D. To our knowledge, these are the first reported precision data for any CE analysis in non-aqueous media. Our results have established non-aqueous CZE as a simple, rapid and reproducible technique for the qualitative analysis of basic drugs.

However, the quantitative precision was poor. We focused our attention first on ways to improve the precision data derived from the same

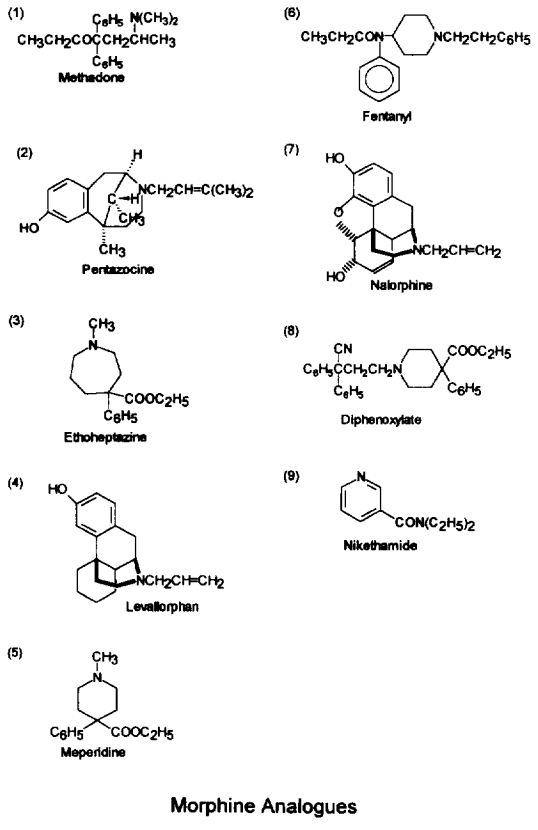

Fig. 6. Structures of the nine morphine analogues (migration order as numbered).

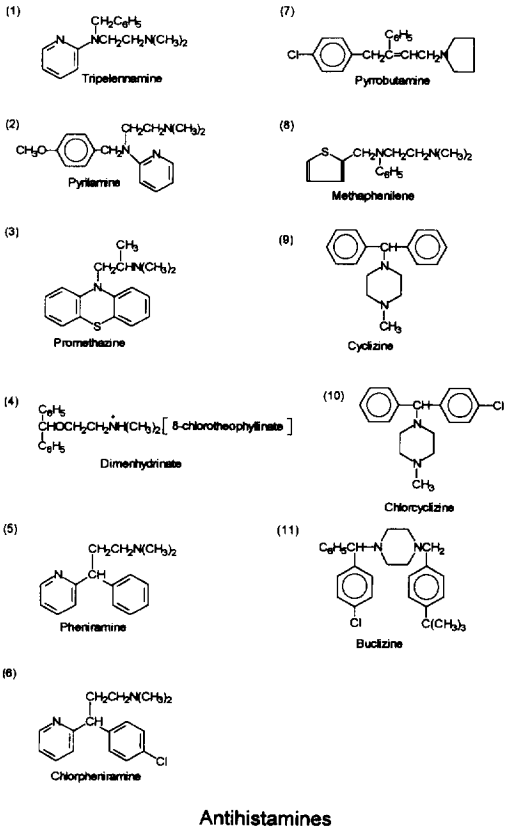

Fig. 7. Structures of the eleven antihistamines (migration order as numbered).

air-cooled instrument, which had no effective evaporation and temperature controls (the sample and electrolyte containers were loosely covered and the temperature of the separation chamber was not thermostated) but had nevertheless provided good precision data for the analysis of basic drugs in an aqueous acidic medium [12]. In order to eliminate the uncertainty due to migration time drifts, the peak area was normalized with migration time before comparison [14] and, as a result, the repeatability of individual components was found to be above 15% R.S.D. for the analyses carried out at 30 kV (Tables 2–4) and between 7% and 13% R.S.D. for the stimulants analysed at 25 kV (Table 5). At 30 kV the observed current was 36–38 μA, whereas at 25 kV it was only 30–32 μA. As expected, the uncertainty appeared to be more pronounced at a higher applied voltage and a higher observed current (i.e., with more Joule heat produced).

The poor precision obtained might be attributed in part to the intolerance of the volatile medium towards the instrument's inadequate evaporation and temperature controls. A steady increase of more than 1°C in the ambient temperature of the separation chamber was observed after a few consecutive runs. Significant variations in the amount injected would then be expected from electrolyte evaporation, solvent evaporation or a temperature change, as these processes would affect the viscosity of the medium or the concentration of the sample. To minimize the injection variation, one of the drug components was selected as an internal refer-

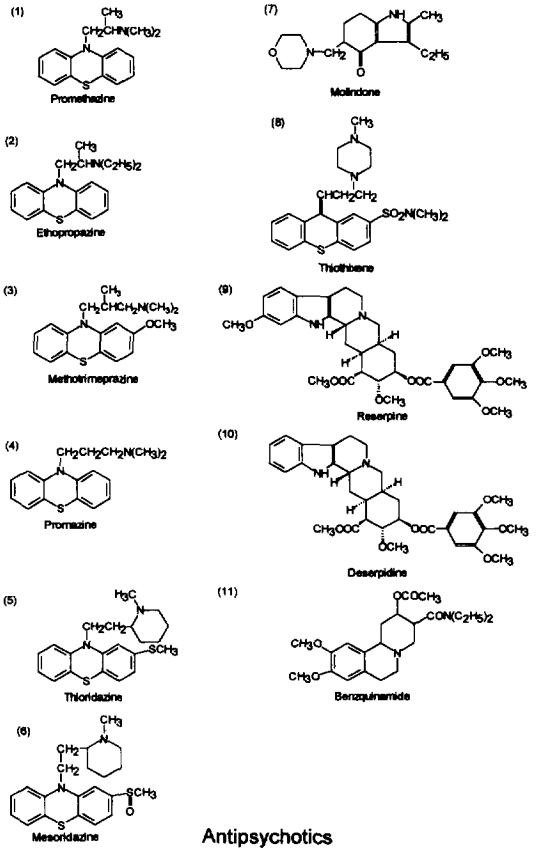

Fig. 8. Structures of the eleven antipsychotics (migration order as numbered).

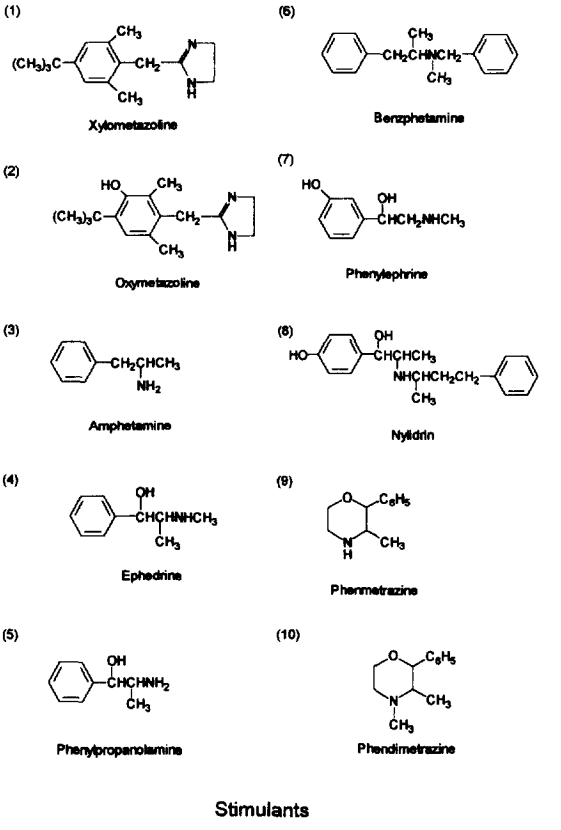

Fig. 9. Structures of the ten stimulants (migration order as numbered).

ence, and the repeatability was improved to between 2 and 9% R.S.D. for the relative normalized areas (Tables 2–5).

Subsequently, it was confirmed that much of the imprecision associated with the present non-aqueous CZE method was instrument related: using a different CE system equipped with liquid-cooled thermostatic control and septum-capped sample/electrolyte vessels, much improved repeatability data (i.e., 0.6–1.0% R.S.D. for the migration times, 0.3–2.3% for the migration-time-normalized areas and 0.7–1.2% R.S.D. for the relative normalized areas) could be obtained for a mixture of six antidepressants which were completely separated in 4 min using the same volatile medium [15].

3.3. Tetra-n-butylammonium tetrafluoroborate as electrolyte

The effect of the electrolyte was also examined briefly using the air-cooled instrument: 20 m*M* tetra-*n*-butylammonium tetrafluoroborate was used instead of ammonium acetate. The non-aqueous medium of acetonitrile–methanol–acetic acid (49:50:1) and other conditions remained unchanged. Initially, the mixture of eleven antihistamines used previously was analysed. Although the speed of analysis and separation efficiency were improved, the selectivity was compromised (with peaks overlapped and a different migration order obtained). Using a mixture of only six antihistamines, baseline separation was achieved at 30 kV within 2.7 min (Fig.

Table 2
Precision data for the separation of nine morphine analogues

Drug	No.	Migration time		Peak area/migration time		Relative peak area/migration time	
		Mean (min)[a]	R.S.D. (%)	Mean (mV)[a]	R.S.D. (%)	Mean[a]	R.S.D. (%)
Methadone	1	2.32	1.16	64.68	19.50	0.4239	9.10
Pentazocine	2	3.30	0.89	31.65	22.17	0.2061	3.29
Ethopheptazine	3	3.47	0.96	38.96	21.54	0.2543	4.94
Levallorphan	4	3.59	0.84	26.01	25.22	0.1691	7.40
Meperidine	5	3.84	0.98	48.35	20.46	0.3160	4.46
Fentanyl	6	4.15	1.36	53.54	21.30	0.3496	5.61
Nalorphine	7	5.07	1.52	153.05	19.59	1.0000	I.S.
Diphenoxylate	8	5.53	1.26	61.49	20.31	0.4020	4.43
Nikethamide	9	5.99	1.93	113.73	20.06	0.7437	4.66

Nalorphine (peak 7) was used as an internal standard for the calculation of the relative normalized peak area.
[a] $n = 8$.

10). About 200 000 and 130 000 theoretical plates were obtained for tripelennamine and buclizine respectively, as compared with about 80 000 and 90 000 plates obtained for the same components when 20 m*M* ammonium acetate was used (Fig. 3).

Even though the observed current (66–69 μA) was much higher than that found with ammonium acetate as the electrolyte, indicating a larger extent of Joule heating in the present case, the repeatability with respect to migration times and migration time-normalized areas was markedly improved. Using one of the components as an internal reference, further improvement in the quantitative precision was only marginal (Table 6). However, when the concentration of tetra-*n*-

Table 3
Precision data for the separation of eleven antihistamines

Drug	No.	Migration time		Peak area/migration time		Relative peak area/migration time	
		Mean (min)[a]	R.S.D. (%)	Mean (mV)[a]	R.S.D. (%)	Mean[a]	R.S.D. (%)
Tripelennamine	1	2.97	1.50	24.85	18.44	0.4711	8.41
Pyrilamine	2	3.08	1.61	23.99	15.95	0.4556	5.59
Promethazine	3	3.20	1.59	24.04	16.62	0.4571	8.38
Dimenhydrinate	4	3.58	1.67	28.51	19.81	0.5382	2.80
Pheniramine	5	3.66	1.86	42.51	19.08	0.8031	4.86
Chlorpheniramine	6	3.93	1.90	37.92	19.64	0.7160	5.18
Pyrrobutamine	7	4.36	2.08	59.07	19.54	1.1146	3.06
Methaphenilene	8	4.46	1.95	32.66	20.22	0.6163	6.84
Cyclizine	9	4.68	2.04	38.42	18.45	0.7262	2.22
Chlorcyclizine	10	4.95	2.13	43.03	18.55	0.8134	3.07
Buclizine	11	6.05	2.43	52.98	19.37	1.0000	I.S.

Buclizine (peak 11) was used as an internal standard for the calculation of the relative normalized peak area.
[a] $n = 8$.

Table 4
Precision data for the separation of eleven antipsychotics

Drug	No.	Migration time		Peak area/migration time		Relative peak area/migration time	
		Mean (min)[a]	R.S.D. (%)	Mean (mV)[a]	R.S.D. (%)	Mean[a]	R.S.D. (%)
Promethazine	1	2.88	1.31	15.29	19.22	0.3955	8.43
Ethopropazine	2	3.09	1.43	29.67	19.38	0.7669	6.66
Methotrimeprazine	3	3.55	1.63	67.70	17.93	1.7525	4.63
Promazine	4	3.62	1.55	57.84	18.67	1.4949	4.81
Thioridazine	5	3.70	1.67	41.03	21.45	1.0562	4.39
Mesoridazine	6	3.82	1.81	43.22	19.23	1.1164	3.45
Molindone	7	4.20	2.39	35.90	23.61	0.9218	7.74
Thiothixene	8	4.48	2.12	63.17	19.71	1.6303	3.81
Reserpine	9	6.02	3.06	66.79	19.93	1.7257	6.89
Deserpidine	10	6.14	3.24	86.01	18.61	2.2267	6.64
Benzquinamide	11	6.36	3.43	38.82	20.43	1.0000	I.S.

Benzquinamide (peak 11) was used as an internal standard for the calculation of the relative normalized peak area.
[a] $n = 8$.

butylammonium tetrafluoroborate was reduced to 10 m*M*, the current observed was 32–35 μA, the same analysis could be completed in 2.4 min and precisions (R.S.D.s) of 0.6–1.1% for the migration times, 7.0–8.7% for the migration time-normalized areas and 2.0–4.4% for the relative normalized areas were obtained.

The above results indicate that the use of tetra-*n*-butylammonium tetrafluoroborate as the electrolyte could provide a rapid, efficient and repeatable separation. An acceptable quantitative precision could be obtained with this non-volatile electrolyte even when the non-aqueous CZE technique was applied using an instrument

Table 5
Precision data for the separation of ten stimulants

Drug	No.	Migration time		Peak area/migration time		Relative peak area/migration time	
		Mean (min)[a]	R.S.D. (%)	Mean (mV)[a]	R.S.D. (%)	Mean[a]	R.S.D. (%)
Xylometazoline	1	3.01	1.63	66.20	7.30	1.0000	I.S.
Oxymetazoline	2	3.23	1.65	60.22	7.67	0.9101	3.80
Amphetamine	3	3.33	1.80	44.70	8.94	0.6753	5.37
Ephedrine	4	3.65	1.93	40.30	7.46	0.6091	3.68
Phenylpropanolamine	5	3.74	2.14	39.68	12.99	0.5983	8.07
Benzphetamine	6	3.96	2.33	58.06	8.88	0.8772	5.24
Phenylephrine	7	4.06	2.29	39.04	11.60	0.5890	7.16
Nylidrin	8	4.21	2.65	39.95	10.08	0.6033	6.60
Phemetazine	9	4.57	2.65	36.79	10.04	0.5560	7.82
Phendimetrazine	10	6.04	3.68	27.29	7.88	0.4122	2.89

Xylometazoline (peak 1) was used as an internal standard for the calculation of the relative normalized peak area.
[a] $n = 8$.

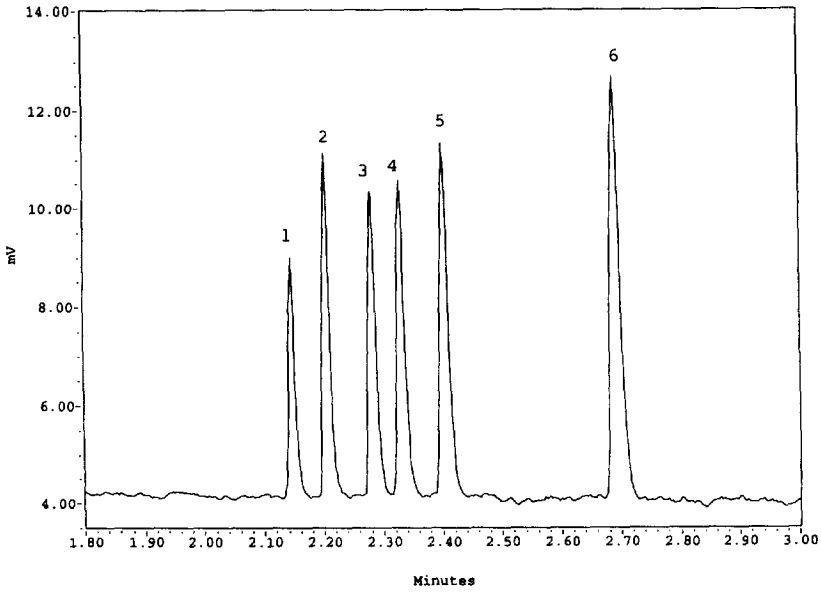

Fig. 10. Electropherogram of a mixture of six antihistamines (100 ppm each). Peaks: 1 = Tripelennamine; 2 = pheniramine; 3 = chlorpheniramine; 4 = cyclizine; 5 = chlorcyclizine; 6 = buclizine. Running medium, acetonitrile–methanol–acetic acid (49:50:1)–20 m*M* tetrabutylammonium tetrafluoroborate; capillary, 60 cm × 75 μm I.D.; applied voltage, 30 kV.

without adequate evaporation and temperature controls. Compared with the separation achieved with ammonium acetate as the electrolyte, the reduced selectivity obtained here could be due to the absence of a delicate proton transfer equilibrium between the ammonium ion and the various amino groups of the basic drugs.

3.4. Other media for the separation of basic drugs

Pure acetonitrile was not tested as a non-aqueous electrolyte medium because ammonium acetate is insoluble in it.

When methanol was used as the solvent system

Table 6
Precision data for the separation of six antihistamines in a running medium with 20 m*M* tetrabutylammonium tetrafluoroborate

Drug	No.	Migration time		Peak area/migration time		Relative peak area/migration time	
		Mean (min)[a]	R.S.D. (%)	Mean (mV)[a]	R.S.D. (%)	Mean[a]	R.S.D. (%)
Tripelennamine	1	2.15	0.70	26.48	4.67	0.6248	4.64
Pheniramine	2	2.21	0.67	42.49	7.38	1.0000	I.S.
Chlorpheniramine	3	2.29	0.64	36.66	7.65	0.8644	6.56
Cyclizine	4	2.33	0.55	38.85	7.39	0.9157	5.79
Chlorcyclizine	5	2.40	0.65	47.31	7.40	1.1154	6.16
Buclizine	6	2.68	0.90	62.26	6.24	1.4684	5.83

Pheniramine (peak 2) was used as an internal standard for the estimation of the relative normalized peak area.
[a] $n = 8$.

instead of a mixture of methanol and acetonitrile [i.e., methanol–acetic acid (99:1)–20 m*M* ammonium acetate], an even higher efficiency could be obtained at 25 or 30 kV (about 28 and 34 μA, respectively) for the separation of the mixture of eleven antihistamines, although the electroosmotic mobility was much reduced ($9.8 \cdot 10^{-5}$ $cm^2\ V^{-1}\ s^{-1}$), the run times were longer (11 and 9.5 min, respectively) and the baselines were considerably noisier (data not shown). Since methanol has in fact a more favourable thermal conductivity and volume-based heat capacity than acetonitrile (0.2 versus 0.188 $W\ m^{-1}\ K^{-1}$, and 1.99 versus 1.74 $J\ ml^{-1}\ K^{-1}$, respectively) [16][1], it was not clear whether the noisy baseline, also observable in previous work [6,8,9], was due to its lower boiling point or to its higher UV cut-off.

Because of the high dielectric constant and good solvent properties, formamide has been investigated previously as a solvent for non-aqueous CE [10]. When we used formamide in place of acetonitrile (i.e., the electrolyte, 20 m*M* ammonium acetate, and other solvent components, 1% acetic acid and different percentages of methanol, remained unchanged), detection at 280 nm had to be used, and only four of the nine morphine analogues with good absorption at 280 nm, namely methadone, pentazocine, levallorphan and nalorphine, were tested. The migration times of these compounds were much decreased. With 30% or more formamide present, pentazocine and levallorphan were completely merged (data not shown).

Compared with other recently reported non-aqueous methods [5–9], it would seem that the use of an acetonitrile–methanol mixture instead of methanol alone could provide a faster and more robust analysis. In addition, the solvent mixture would allow a wider range of hydrophobic compounds to be analyzed. The drawbacks of using formamide as a CE medium have already been pointed out by Sahota and Khaledi [10]; these problems, which include solvent instability, noisy baseline and background absorption, are absent in our significantly faster non-aqueous system.

Ong et al. [17] reported the CE separation of nine antihistamines in 6 min using an aqueous electrolytic medium with sodium dodecyl sulphate, β-cyclodextrin and tetrabutylammonium hydrogensulphate as mixed carriers. No reproducibility data were given. In comparison, the present non-aqueous system is equally fast, reproducible and much simpler to use.

4. Conclusion

Through the use of a non-aqueous medium composed of 20 m*M* ammonium acetate in acetonitrile–methanol–acetic acid (49:50:1) for the separation of different types of basic drug mixtures, the speed, efficiency, selectivity, precision, simplicity and versatility of non-aqueous CZE have been confirmed. A significant improvement in the repeatability was possible with a CE instrument that minimized the evaporation of the volatile solvents or electrolyte and provided good control of the operating temperature. Improvements in precision, speed and separation efficiency could also be achieved with tetra-*n*-butylammonium tetrafluoroborate as the electrolyte, although the selectivity was compromised. It is expected that the selectivity could be enhanced or manipulated by varying the amount or type of acid used, thereby extending the scope of this technique. The use of a stronger acid, such as trifluoroacetic acid, could be useful for the less basic compounds, and the use of an amine base, such as triethylamine, could lead to the separation of acidic compounds.

Non-aqueous CZE will be particularly useful in analyzing hydrophobic substances or substances which are difficult to separate in the aqueous media. In addition, as has already been demonstrated [7–9], the technique is easily interfaced with an electrospray ion source for performing on-line CE–MS. We are currently applying this technique to the analysis of very hydrophobic substances [18], examining inter-day

[1] The volume-based heat capacity is obtained by multiplying the specific heat capacity by the density.

variations and investigating the effects of trace amounts of water present in the organic media.

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