ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
22 (2000) 413–421

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

The analysis of basic and acidic compounds using non-aqueous CE and non-aqueous CE-MS

John Senior [a,*], Delphine Rolland [b], David Tolson [c], Sotiris Chantzis [d], Vern De Biasi [a]

[a] *Analytical Sciences, SB Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow CM19 5AW, UK*
[b] *Ecole de Chimie Polymeres et Materiaux de Strasbourg, Strasbourg, France*
[c] *Computational and Structural Sciences, SB Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow CM19 5AW, UK*
[d] *University of Kent, Canterbury, UK*

Received 10 May 1999; received in revised form 24 September 1999; accepted 26 September 1999

Abstract

It has been shown that non-aqueous capillary electrophoresis (NACE) can provide improved separations in comparison to those obtained using conventional CE under aqueous conditions [1] (ACE). Previous work carried out in our laboratories involving initial investigations into the technique have been reported [2]. Based on the findings of that work it was possible to separate a variety of basic pharmaceuticals from selected impurities and to obtain the successful separation of some hydrophobic sulphonic acids. The successful coupling of NACE to mass spectrometry (NACE-MS) has also been demonstrated. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Non-aqueous capillary electrophoresis; Non-aqueous capillary electrophoresis-mass spectrometry; Compounds

1. Introduction

The use of organic solvents as the basis of the electrophoretic media ('run buffer') in capillary electrophoresis (CE) has been reported [3–5] as a powerful technique for achieving highly selective and fast separations. The speed and selectivity of the non-aqueous capillary electrophoresis (NACE) separation is thought to be primarily due to exploitation of the physiochemical properties of the run buffer, e.g. viscosity (η) and their acid–base behaviour, provided by use of solvents other than water.

The improved selectivity obtained in NACE is derived, in part, from differences in the ionised–unionised equilibrium of analyte molecules in aqueous and non-aqueous solvents. The difference in this equilibrium for two closely related structures is often larger in non-aqueous conditions and thus contributes to the enhanced selectivity observed in NACE. Reduced migration times for analyte and buffer ions are obtained, in part, by a

* Corresponding author. Tel.: +44-1279-627410; fax: +44-1279-627404.
E-mail address: john_p_senior@sbphrd.com (J. Senior)

0731-7085/00/$ - see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(00)00234-X

reduction in the viscosity of the run buffer. For example the substitution of water ($\eta = 0.89$ cP at 25°C [6]) by acetonitrile ($\eta = 0.34$ cP at 25°C [6]). As η is inversely proportional to the magnitude of electroosmotic flow (EOF) ($\mu_{EOF} = (\varepsilon\zeta/\eta)$ the rate of migration of solute ions should, all other factors being equal, be reduced by approximately one third. However, in practice observed run times are often reduced by a factor of 4 or 5. Thus other physiochemical properties of the run buffer such as its dielectric constant are also thought to be important. However, the interdependancy of the majority of the experimental variables within the NACE experiment, make it difficult to design and perform studies for investigation into the effect of these variables on observed separations.

The vast majority of NACE separations reported in the literature are for compounds of a basic nature. The analysis of strong acids is hampered by a relatively low EOF and a relatively high electrophorectic migration. This problem was overcome by increasing the EOF by employing the use of run buffers with a high apparent pH (pH*) [7]. An alternative approach has been proposed which assumes that at lower pH* some acids have sufficient electrophoretic mobility to overcome the EOF. In these cases analysis can be achieved by reversal of the EOF or by injection of the sample at the outlet end of the capillary. This second approach utilises the electric field strength generated by the full length of the capillary (from 20 cm) but results in reduced migration times as the separation is performed in the short end of the capillary (≈ 7 cm).

Successful coupling of NACE to mass spectrometry instruments operating in electrospray ionisation mode has been reported [8,9]. The use of volatile non-aqueous solvents provides a convenient matrix for introduction of samples into a mass spectrometer than provided by aqueous solvents. NACE-mass spectrometry (MS) has been shown to provide better detection sensitivity [8] than aqueous capillary electrophoresis (ACE)-MS and this is thought to be due to a more efficient electrospray process.

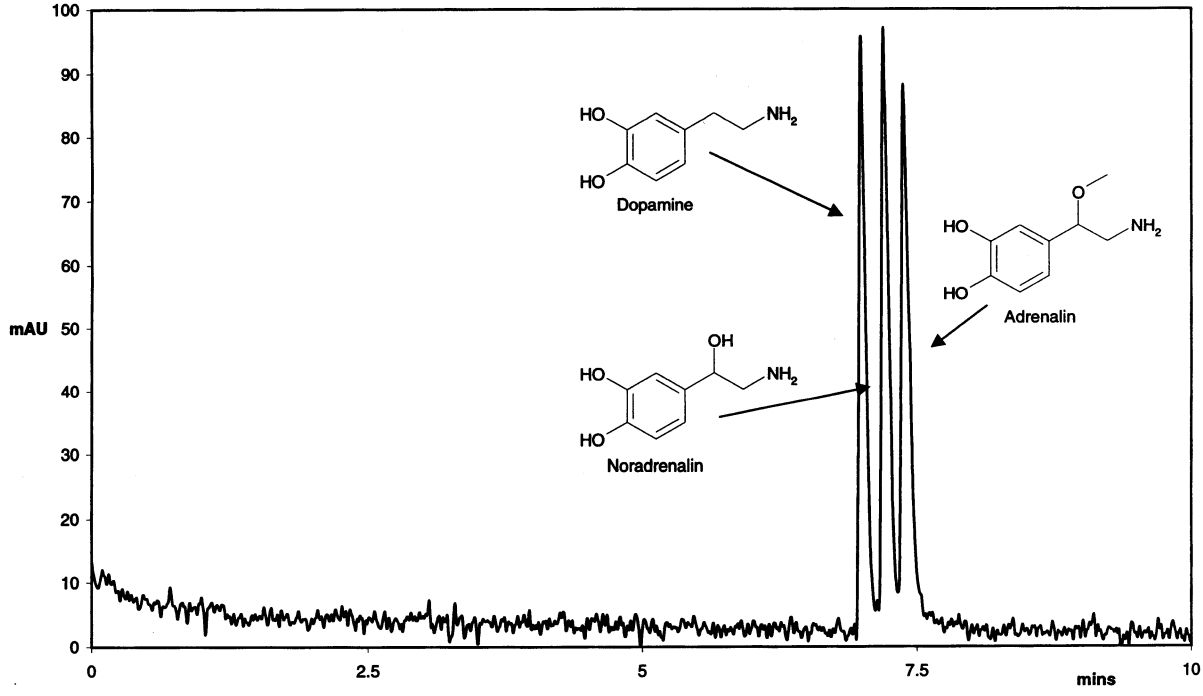

Fig. 1. Separation of test mixture 1 using sodium acetate (50 mM, pH 4.5).

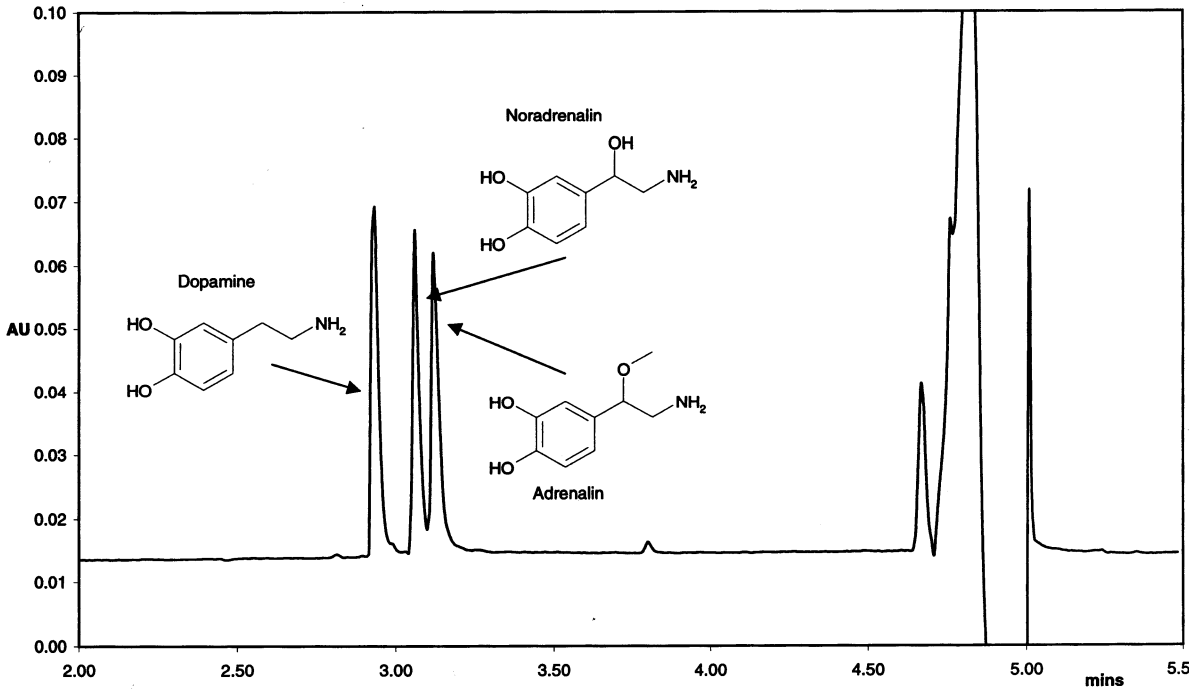

Fig. 2. Separation of test mixture 1 components using ammonium acetate (25 mM) and acetic acid (1 M) in acetonitrile–methanol (75:25 v/v).

2. Experimental

NACE experiments were carried out using a Hewlett Packard HP3DCE system. Unless stated otherwise separations were performed using an untreated fused capillary (length to detector 57 cm, internal diameter 50 μM). All capillaries were conditioned prior to use by rinsing with 1.0 M NaOH for 15 min followed by 15 min with 0.1 M NaOH and finally for 15 min using the run buffer. The capillary was reconditioned on changing the run buffer but was not reconditioned prior to each injection. All separations were performed at 30 kV and the capillary was maintained at 25°C. Samples were loaded into the capillary by pressure injection (2 s at 50 mbar) and detection was at by UV at 200 or 214 nm.

NACE-MS experiments were carried out using a Beckman P/ACE system MDQ equipped with a capillary cassette adapted for MS. Unless stated otherwise separations were performed using an untreated fused capillary (length to detector 77 cm, internal diameter 50 μM). All capillaries were conditioned prior to use by rinsing with 1.0 M NaOH for 15 min followed by 15 min with 0.1 M NaOH and finally for 15 min using the run buffer. The capillary was reconditioned on changing the run buffer but was not reconditioned prior to each injection. All separations were performed at 25 kV and the capillary was maintained at a temperature of 25°C. Samples were loaded into the capillary by pressure injection (2 s at 50 mbar). MS was achieved using a Finnigan MAT LCQ. The electrospray needle was held at +4.5 kV. The sheath gas was 20 U of N_2 and the sheath liquid was methanol–ammonium formate (pH 2.5, 200 mM) (50:50 v/v)) set at 5 μl min^{-1}.

Test mixture 1 contained dopamine (DA, Fluka), adrenaline (A, Sigma) and noradrenaline (NA, Sigma). Test mixture 2 contained BRL29060 and BRL57138A, test mixture 3 contained SB202026A and BRL57259A and Test mixture 4 contained impramine and desipramine. Samples of these compounds were obtained in house. Test mixture 5 contained 2-napthalenesulphonic acid (Aldrich), 1,5-napthalenesulphonic acid (Aldrich)

and 1,3,6-napthalenesulphonic acid (Aldrich). All test mixtures were prepared in methanol to a final concentration of 0.2 mM of each component.

Materials used in preparation of NACE run buffers were as follows; methanol 205 (Romil), acetonitrile 190 far UV grade (Romil), acetic acid glacial (Fisher), formic acid (Fluka), sodium acetate (Fisher), sodium formate (Fluka).

Specific details of the composition of the run buffer used for each experiment are given in the relevant figures.

Apparent pH (pH*) values were measured using a pH meter calibrated with pH 4 and pH 10 aqueous buffer solutions.

3. Results and discussion

Test mixture 1 was chosen to include structurally similar basic compounds. Test mixture 5 was chosen to include structurally similar acidic compounds. All other test mixtures were chosen so as to include the major compound of interest in the presence of a structurally similar impurity.

3.1. Separation of basic test mixtures

An electropherogram illustrating the separation of test mixture 1 using optimum ACE and NACE separations are given in Figs. 1 and 2 respectively. Good separation of DA, NA and A was obtained using either an ACE or NACE method. The order of migration for the test solutes was the same in both methods and the resolution obtained in their separation was similar. However, the migration time for the latest migrating component (A) was 8.1 min with the ACE method compared to 3.2 min with the NACE method. This is thought to be primarily due to an increase in the rate of migration of the test solutes and an increase in the rate of EOF. These increased rates of migration can be attributed in part to a reduction in the viscosity of the run buffer.

Optimum separation of the components in test mixtures 2, 3 and 4 were obtained from NACE experiments with the use run buffers composed of either sodium acetate or sodium formate in acetonitrile/methanol mixtures.

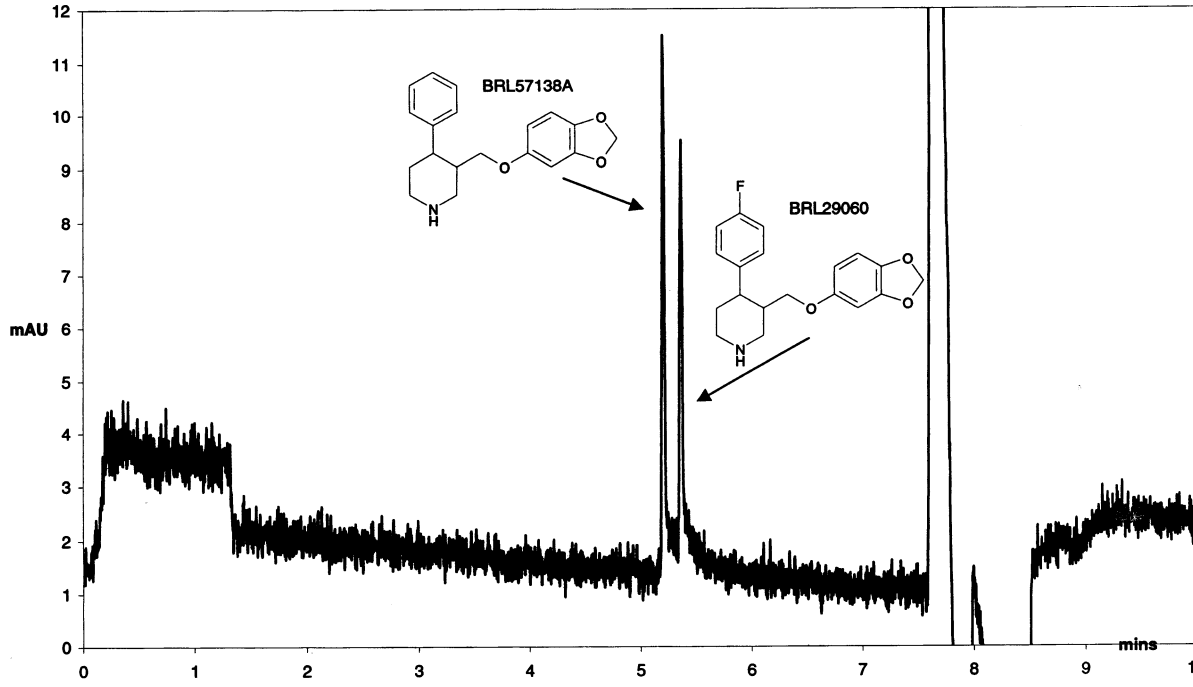

Fig. 3. Separation of test mixture 2 components using sodium acetate (75 mM) and acetic acid (0.5 M) in acetonitrile/methanol (50:50 v/v).

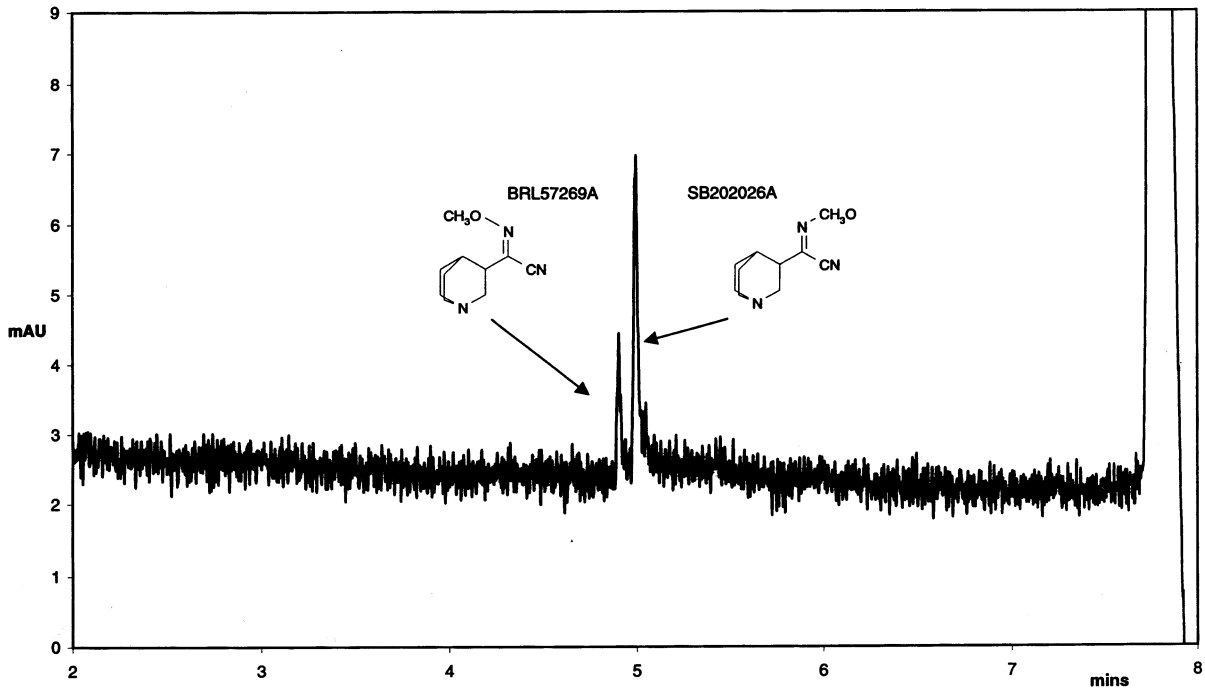

Fig. 4. Separation of test mixture 3 components using sodium acetate (75 mM) and acetic acid (0.5 M) in acetonitrile–methanol (50:50 v/v).

An electropherogram illustrating the separation of BRL29060 and BRL57138A is given in Fig. 3. Good separation was obtained using NACE within 6 min, compared to a run time of 25 min required to obtain separation under optimum ACE conditions.

An electropherogram from illustrating the separation of SB202026A and BRL57259A is given in Fig. 4. Baseline separation was obtained using NACE within 5.5 min, compared to a run time of 20 min required under optimum ACE conditions.

An electropherogram from illustrating the separation of impramine and desipramine is given in Fig. 5. Good separation was obtained using NACE within 12.5 min. The separation of these two compounds was not possible using ACE in this particular study.

3.2. MS data of components in test mixtures 2, 3 and 4

When performing the NACE-MS experiments the NACE run buffer used was ammonium acetate in an acetonitrile/methanol mixture. MS spectra obtained from analysis of test mixtures 2, 3, 4 are given in Figs. 6–8, respectively. These data are compatible with detection of test mixture components. There was insufficient separation in the NACE-MS experiment for test mixture 2 to clearly distinguish the two resolved geometric isomers.

3.3. The separation of acidic compounds

Having demonstrated that NACE is an appropriate technique for the separation of some simple bases attention turned to the analysis of acidic compounds.

Although it is possible to separate acidic compounds with the use of NACE run buffers with pH* > 9.0 in order to have an EOF which permits migration of the anions within an acceptable run time [7], it is also possible to separate acids using NACE buffers with pH* < 7.0. This alternative approach involves injection of the sample at the outlet end of the capillary. The conditions for

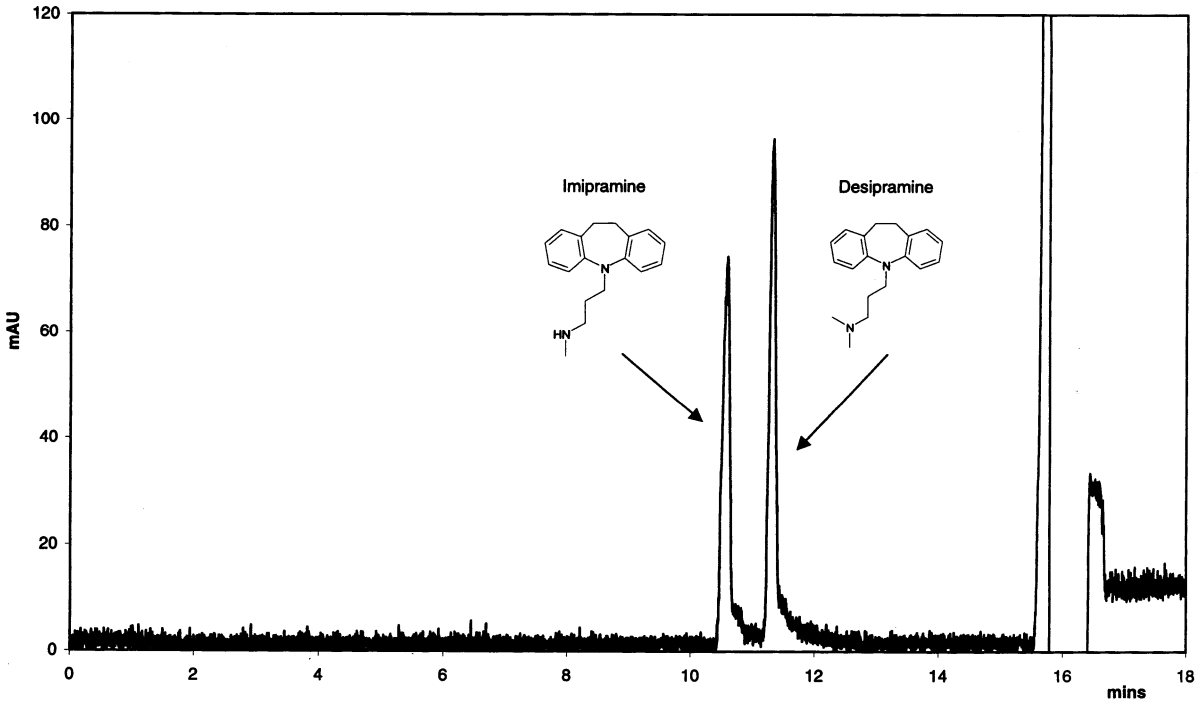

Fig. 5. Separation of test mixture 4 components using sodium formate (75 mM)–formic acid (0.5 M) in acetonitrile–methanol (50:50 v/v).

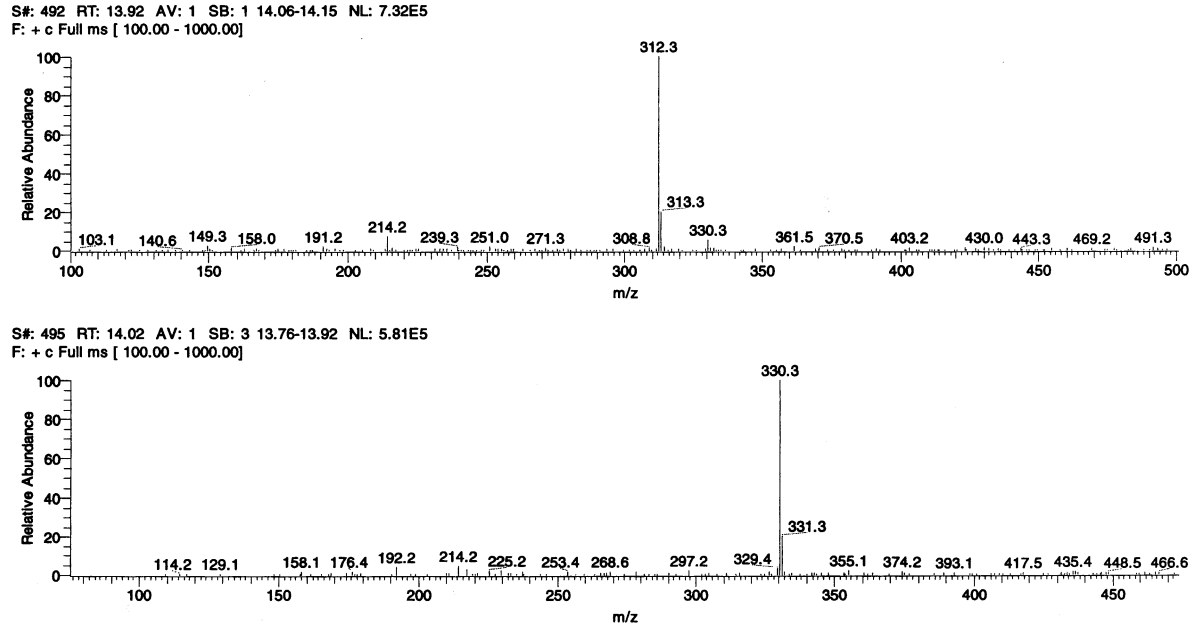

Fig. 6. Mass spectra of test mixture 2 components. The top spectrum is for BRL57138A and the bottom spectrum is for BRL29060.

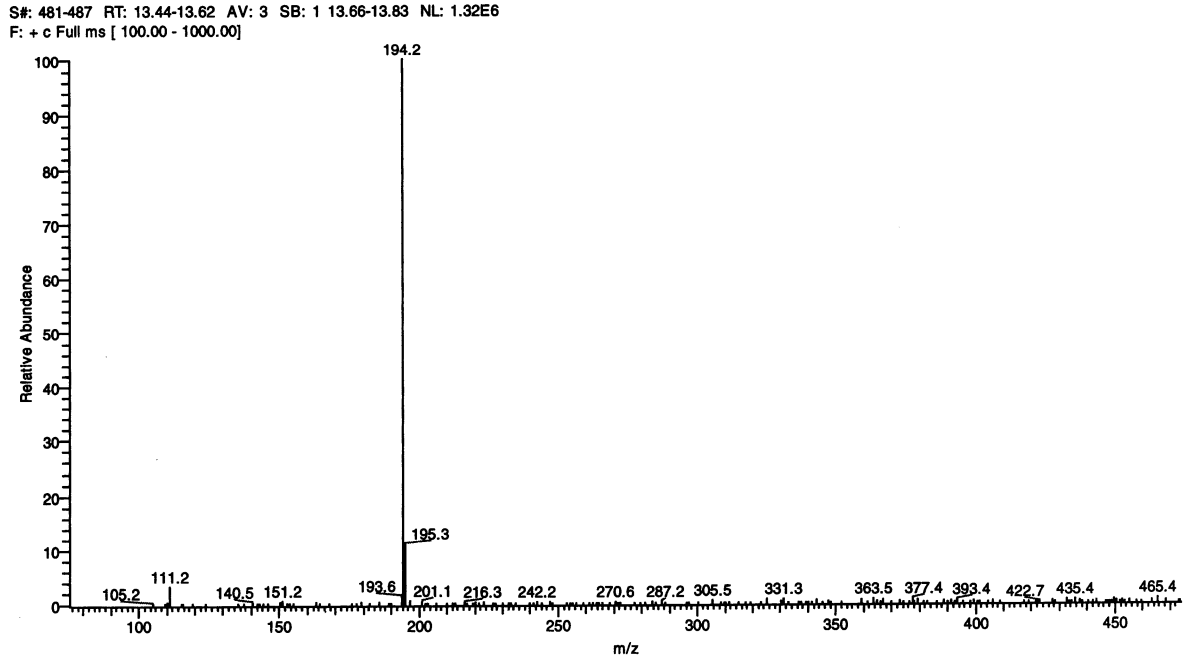

Fig. 7. Mass spectra of test mixture 3 components. The spectrum illustrated is for a peak representing poorly resolved SB202026A from its geometric isomer BRL57269A.

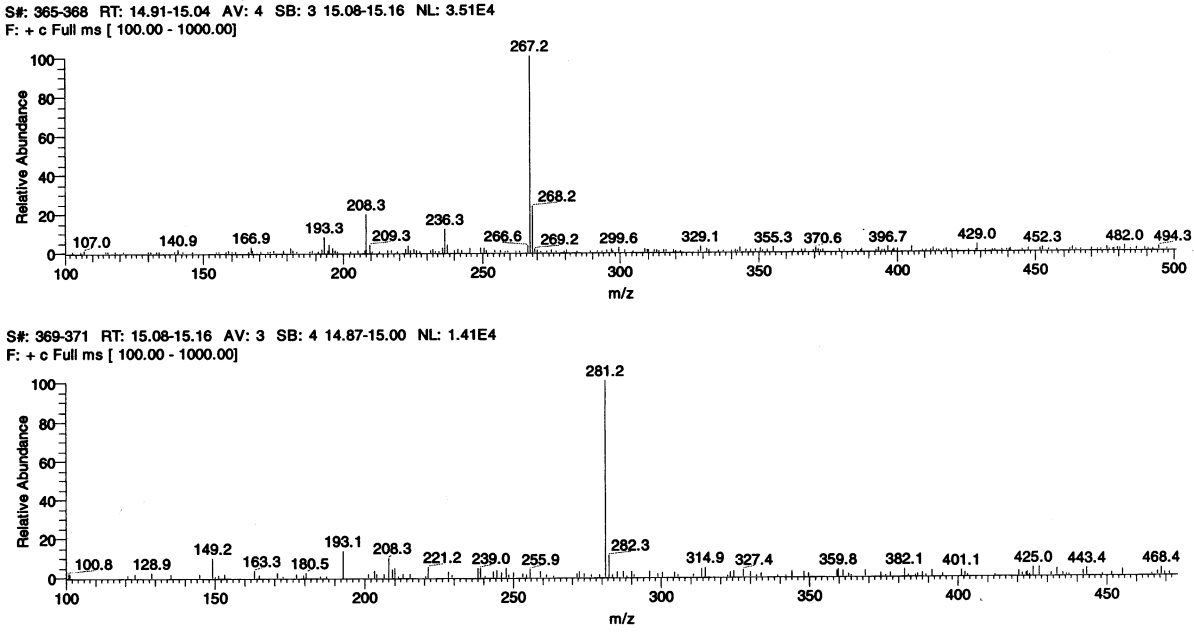

Fig. 8. Mass spectra of test mixture 4 components. The top spectrum is for imipramine and the bottom spectrum is for desipramine.

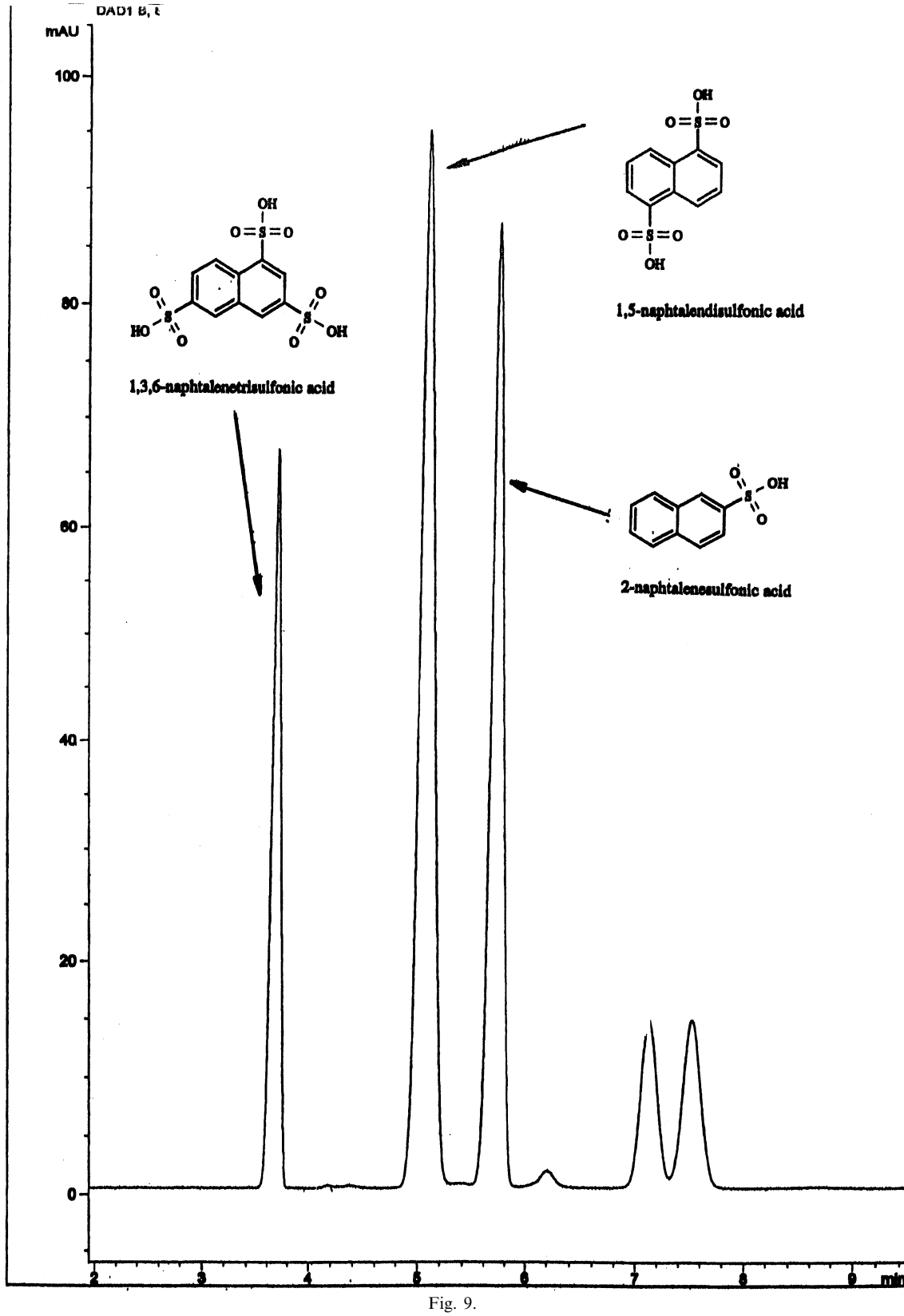
DAD1 B, t
mAU
100
80
60
40
20
0
2
3
4
5
6
7
8
9
min
1,3,6-naphtalenetrisulfonic acid
1,5-naphtalendisulfonic acid
2-naphtalenesulfonic acid

Fig. 9.

these experiments are as listed in Section 2 with the exception that the length of capillary used for separation is 7 cm. An electropherogram from the analysis of test mixture 5 is given in Fig. 9. All three components of the test mixture were baseline separated. The three smaller peaks were found to be impurities present in 1,5-napthalendisulfonic acid. This approach relies on the migration of negatively charged species against the EOF and is aided by the relatively low EOF obtained using a NACE buffer at pH* 4.0.

4. Conclusions

It has been shown that the separation of a mixture of A, DA and NA can be achieved under aqueous and non aqueous conditions, however the run time required in NACE mode is approximately 2.5 times less than that required in ACE mode. This can be explained, in part, by differences in the physical properties of the run buffers used in both methods. In particular the differences in viscosity and di-electric constant for ACE and NACE buffers are thought to be important. It has been demonstrated that NACE can provide good separation of the structurally similar basic compounds; BRL29060/BRL57138A and impramine/desipramine and the geometric isomers SB202026A and BRL57259. In the case of BRL29060/BRL57138A and SB202026A and BRL57259 NACE provides separations in a quarter of the run time required for separation under ACE conditions.

It has also been shown that NACE can provide a convenient approach to the analysis of acidic compounds. This was demonstrated by the separation of closely related naphthalene sulphonic acids, achieved by injection at the outlet end of the capillary and therefore by migration against the EOF.

It has also been demonstrated that NACE can be conveniently hyphenated to MS. NACE-MS data has been used qualitatively for confirmation of peak identity and could be used for 'peak tracking' in method optimisation. The NACE-MS data obtained cannot be used conclusively to evaluate the technique as a quantitative tool as the MS parameters were not fully optimised, further work would be required to achieve this objective.

Further work is required in order to gain a deeper understanding of the mechanisms which affect separation in NACE. Based on the data available in literature and from work in the laboratories, it is believed that this further work could lead to the widespread adoption of NACE as a useful separation tool which is capable of providing rapid separations and different selectivity than that given by ACE and HPLC.

References

[1] I. Bjornsdottir, S. Hansen, J. Pharm. Biomed. Anal. 15 (1996) 1083–1089.
[2] J. Senior, S. Chantzis, V. De Biasi, HPLC98 Poster Presentation, St Louis, US, 1998.
[3] J. Tjornelund, S. Hansen, J. Biochem. Biophys. Methods 38 (2) (1999) 139–153.
[4] S. Porras, P. Simo, I. Valko, E. Istvan, P. Jyske, M. Riekkola, J. Biochem. Biophys. Methods 38 (2) (1999) 89–102.
[5] G. Leung, H. Tang, P. Hubert, T. Tso, T. Wan, J. Chromatogr. A 738 (1) (1996) 141–154.
[6] L. Snyder, J. Kirkland, Introduction to Modern Chromatography, Wiley, New York, 1979.
[7] K. Altria, S. Bryan, Chromatographia 46 (3/4) (1997).
[8] W. Lu, G. Poon, P. Carmichael, R. Cole, Anal. Chem. 68 (4) (1996) 668–674.
[9] A. Tomlinson, L. Benson, J. Gorrod, S. Naylor, J. Chromatogr. B: Biomed. Appl. 657 (2) (1994) 373–381.

Fig. 9. Separation of test mixture 5 using ammonium acetate (50 mM)–acetic acid (pH* 4.0) in acetonitrile–methanol (75:25 v/v).