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Separation of cationic *cis–trans* (*Z–E*) isomers and diastereoisomers using non-aqueous capillary electrophoresis

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Abstract

The separation of cationic *cis–trans* (*Z–E*) isomers as well as cationic diastereomers is demonstrated in non-aqueous capillary electrophoresis (NACE). No addition of surfactants, cyclodextrins or other complexing agents are necessary. Solvent mixtures of methanol and acetonitrile are used for the electrophoresis media and different electrolytes have been investigated. The separation is not dependent on differences in the pK_a values of the substances but rather on the apparent volume of the conformers which is a result of intramolecular interactions and solvation. As long as the solutes are protonated good separation selectivity can be obtained. The separation is shown to be very much dependent on the type and concentration of the electrolyte used. The technique has been used for purity testing of drug substances. © 1997 Elsevier Science B.V.

Keywords: Diastereomer separation; *cis–trans* Isomers; Enantiomer separation; Pharmaceutical analysis

1. Introduction

The separation of molecules with similar or very similar structure possesses a challenge in capillary electrophoresis (CE). Such separations are most often achieved by addition of different reagents. The separation of enantiomers requires a chiral environment and often also a micellar pseudostationary phase is incorporated into the electrophoretic buffer in order to increase selectivity [1–3]. Diastereomers, *cis–trans* isomers and molecules of similar structure with identical mass-over-charge ratios which all have different physico-chemical characteristics may be separated using micellar electrokinetic chromatography (MEKC) or other pseudostationary phases [4–10] or by adding cyclodextrins to the electrophoresis buffer [5,7,10–14]. A few examples of the separation of diastereomers and *cis–trans* isomers of proline-containing peptides without using complexing agents have been reported [15–19].

Recently, the advantage of non-aqueous capillary electrophoresis (NACE) for the separation of molecules with similar structure and with the same mass-over-charge ratio has been demonstrated [20,21]. In the present paper we demonstrate how high separation selectivity of *cis–trans* cationic isomers and diastereomers may be obtained under acidic condition using NACE without the addition of any surfactants, cyclodextrins or other complexing agents.

2. Experimental

2.1. Chemicals

Chlorprothixene hydrochloride, clopenthixol hydrochloride and flupenthixol hydrochloride and

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flupenthixol decanoate were all obtained from H. Lundbeck (Copenhagen, Denmark). Clomiphene was extracted from Clomid tablets (Paranova, Ballerup, Denmark) with methanol (MeOH)–acetonitrile (MeCN) (1:1, v/v) and thiothixene was extracted from Navane tablets (Pfizer, Copenhagen, Denmark) in a similar way.

Quinine, quinidine, cinchonine and cinchonidine were obtained from Fluka (Buchs, Switzerland).

L-Ala–L-Phe was obtained from Sigma (St. Louis, MO, USA) and L-Ala–D-Phe from Bachem (Bubendorf, Switzerland).

Acetic acid (AcOH), formic acid (HCOOH), ammonium acetate ($AcONH_4$), methanesulfonic acid (MSA) and trifluoroacetic acid (TFA) and all other chemicals were of analytical-reagent grade and were obtained from E. Merck (Darmstadt, Germany). The solvents and all other chemicals were used as received from the supplier.

2.2. Apparatus

An HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with on-column diode array detection was used. The following parameters were used for all the analysis unless otherwise stated: the detection wavelength was 214 nm. The separations were performed in fused-silica capillaries [64 cm (55.5 cm to detector)×50 μm I.D.] from Polymicro Technologies (Phoenix, AZ, USA). The capillaries were thermostated at 25°C with air. Samples were kept at ambient temperature in the autosampler and injected by applying a pressure of 5 kPa (50 mbar) for 3 s. A voltage of 30 kV was applied during analysis.

Prior to use, the capillaries were rinsed with 1 *M* sodium hydroxide for 60 min, 0.1 *M* sodium hydroxide for 20 min, distilled water for 20 min and the final electrophoresis medium for 10 min. Between analyses, the capillaries were flushed with electrophoresis medium for 2 min.

pH was measured in the electrophoresis media using a pH meter (Radiometer, Copenhagen, Denmark) equipped with a glass–calomel electrode. The electrode were calibrated using standard aqueous buffers.

2.3. Electrophoretic parameters

In this paper the separation is expressed as the resolution R_S between the isomers:

$$R_S = \frac{1.17(t_{R_1} - t_{R_2})}{w_{h_2} + w_{h_1}} \quad (1)$$

using the observed migration times (t_R) and peak widths at half height (w_h). The selectivity is expressed as the relationship between the electrophoretic mobilities μ_{ep} of the two isomers:

$$\mu_{ep} = \mu_{obs} - \mu_{EOF} \quad (2)$$

where μ_{obs} is the observed mobility and μ_{EOF} is the mobility corresponding to the electroosmotic flow.

The mobilities are calculated from the formula:

$$\mu = \frac{L \cdot l}{t_R V} \quad (3)$$

where L is the total and l is the effective length of the capillary, t_R is the observed migration time and V the voltage applied.

2.4. Sample preparation

All test substances were dissolved in a mixture of methanol–acetonitrile (1:1, v/v) to a concentration of 0.1 mg/ml. The test substances obtained as tablet preparations were extracted from the tablets with a mixture of methanol–acetonitrile (1:1, v/v) to give an expected concentration of 0.1 mg/ml. Prior to analysis the extracts were centrifuged at 18 000 *g*. Clomiphene, thiothixene and chlorprothixene were only obtained in the *cis*-form. In order to isomerise these test solutes a part of the test solutions were exposed to diffuse day light for a few hours.

In tests for isomeric purity the substances were dissolved in methanol–acetonitrile (1:1, v/v) to a concentration of 5.0 mg/ml.

3. Results and discussion

A number of drug substances exhibiting *cis–trans* isomeric properties are marketed as only one of the isomers—often the *cis* isomer. It is therefore of

interest to be able to quantify minor amounts of one isomer in the other. *cis–trans* Isomers have been separated by high-performance liquid chromatography (HPLC) due to the differences in their physico-chemical properties leading to differences in their partition ratios in the chromatographic system. However, separation in particular of lipophilic cationic substances are often accompanied by problems like severe peak tailing and thus less efficient separations are obtained.

CE of smaller cationic solutes in free solution is characterised by showing high efficiency and short migration times. The use of NACE further improves selectivity and therefore this technique should be ideal if *cis–trans* isomers in general can be separated in such systems.

As diastereomers also have different physico-chemical properties but have the same mass-over-charge ratios we have included such test substances as well. All test solutes are shown in Fig. 1.

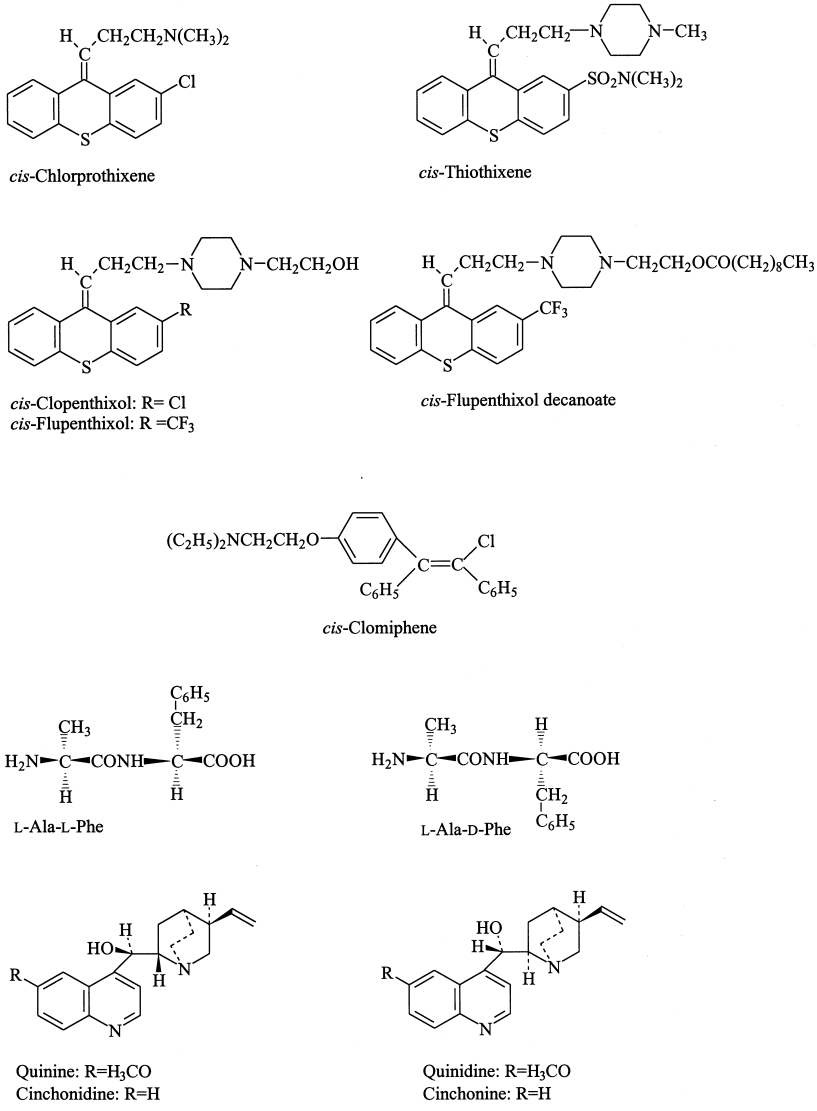

Fig. 1. The molecular structures of some of the test substances. For the *cis–trans* isomers only the *cis* isomer is shown.

3.1. The organic solvent

In order to make this technique generally suitable for testing of conformers and in order to be able to obtain low detection limits of e.g. impurities in drugs and drug substances we decided only to use solvents with high transparency to UV light. Thus, MeOH and MeCN were chosen for these investigations. These solvents have previously been shown to provide high selectivities of compounds with equal or almost equal mass-over-charge ratios [21]. However, only mixtures of the two solvents have been investigated as the solubility of some test solutes and especially of some electrolytes in neat MeCN may be a problem, and in MeOH the migration times are long due to a low EOF.

In Table 1 some characteristics of the separation of the test solutes *cis*- and *trans*-flupenthixol are given. No separation was obtained using only ammonium acetate as the electrolyte but addition of acid results in separation of the *cis–trans* isomers.

The data in Table 1 show that the separation was influenced by the solvent composition and that the best resolution and selectivity is obtained in methanol–acetonitrile (1:1, v/v). Therefore, this solvent mixture was chosen for further investigations.

3.2. pH* and nature of the electrolyte

The dependence of changes in pH* and of changes in the nature and concentration of the electrolyte on the separation selectivity was investigated. Table 2 shows the pH* value of a number of electrophoresis media. The pH values are assigned as pH* as the measurements are performed in a non-aqueous medium. The choice of electrolytes are governed by their solubility in methanol–acetonitrile (1:1, v/v) and by their UV transparency.

The separation obtained is not dependent on the pH* value despite the necessity to protonate the test solutes in order to be able to achieve electrophoretic migration. At pH* about 8 flupenthixol is not fully protonated (the electrophoretic mobility is low, see Table 1) and no separation is obtained. At pH* about 6.5 and below the molecule seems to be fully protonated and the separation obtained is not due to differences in pK_a values as good separation is still obtained at low and very low pH* (using TFA and MSA, respectively). Furthermore, when using increasing amounts of $AcONH_4$ together with 1 *M* AcOH in the electrophoresis media pH increases concomitantly with an increase in selectivity.

The separation is therefore more likely to be due to differences in the apparent volume (hydrodynamic volume) of the molecules. The size of isomers may be influenced by intramolecular interactions and also by the solvation of the molecules. For all the thioxanthene derivatives the *cis* isomer has the fastest migration rate probably due to a tighter packing of the molecule being a result of in-

Table 1
Capillary electrophoresis of *cis*- and *trans*-flupenthixol in electrophoresis media with different solvent composition and different electrolyte composition

Solvent MeOH–MeCN (v/v)	Electrolyte	μ_{ep} ($cm^2/s\ V$)		R_S	μ_{ep2}/μ_{ep1}
		cis Isomer	*trans* Isomer		
	25 m*M* $AcONH_4$	$2.6\cdot10^{-5}$	$2.6\cdot10^{-5}$	0.00	1.00
75:25	‘ +100 m*M* TFA	$2.4\cdot10^{-4}$	$2.2\cdot10^{-4}$	3.73	1.05
	‘ +30 m*M* MSA	–	–	–	–
	‘	$4.3\cdot10^{-5}$	$4.3\cdot10^{-5}$	0.00	1.00
50:50	‘ +100 m*M* TFA	$2.3\cdot10^{-4}$	$2.1\cdot10^{-4}$	3.54	1.07
	‘ +30 m*M* MSA	$1.8\cdot10^{-4}$	$1.7\cdot10^{-4}$	4.25	1.08
	‘	$2.1\cdot10^{-5}$	$2.1\cdot10^{-5}$	0.00	1.00
25:75	‘ +100 m*M* TFA	$1.7\cdot10^{-4}$	$1.6\cdot10^{-4}$	3.18	1.10
	‘ +30 m*M* MSA	$1.3\cdot10^{-4}$	$1.2\cdot10^{-4}$	2.13	1.11

The electrophoretic mobility is given and the separation is expressed as the resolution R_S and the selectivity μ_{ep2}/μ_{ep1}.

Table 2
Separation of *cis–trans*-flupenthixol expressed as the resolution R_S and the selectivity μ_{ep2}/μ_{ep1} in a number of electrophoresis media based on methanol–acetonitrile (1:1, v/v) as solvent

Electrolyte in MeOH–MeCN (1:1, v/v)	pH*	R_S	μ_{ep2}/μ_{ep1}	μA
25 m*M* $AcONH_4$	8.02	0.00	1.00	13
‘ +100 m*M* AcOH	6.80	0.92	1.03	18
‘ +250 m*M* AcOH	6.31	1.59	1.05	18
‘ +1 *M* AcOH	5.31	2.92	1.06	18
‘ +2 *M* AcOH	4.58	2.78	1.06	17
5 m*M* $AcONH_4$ +1 *M* AcOH	4.76	1.50	1.04	6
10 m*M* $AcONH_4$ +1 *M* AcOH	4.99	1.70	1.04	9
50 m*M* $AcONH_4$ +1 *M* AcOH	5.57	2.99	1.06	30
5 m*M* NH_4Cl	6.08	0.00	1.00	6
10 m*M* NH_4Cl	5.82	2.30	1.11	12
25 m*M* NH_4Cl	4.86	4.82	1.11	26
50 m*M* NH_4Cl	5.08	5.80	1.09	46
25 m*M* $AcONH_4$ +30 m*M* MSA	−0.35	4.25	1.08	27
‘ +100 m*M* TFA	2.05	3.54	1.07	26
‘ +100 m*M* HCOOH	5.54	2.15	1.04	22

The pH* of the electrophoresis medium and the current when 30 kV is applied are given.

tramolecular interactions between groups in the side chain and the functional group on the ring system which are the cause of the *cis–trans* isomers. The tighter packing of the molecule may also to some extent prohibit the association to solvent molecules. Both phenomena will tend to increase the difference in apparent volume of the isomers and thus tend to increase the difference in migration rate.

Further investigations of the influence of the nature of the electrolyte on the separation were performed using the test solutes shown in Fig. 1. Two of the most promising systems listed in Table 2 were used. In Table 3 the resolution R_S of the conformers obtained in two electrophoresis media are given. It is obvious from these data that there is no optimal electrophoresis medium for all conformers. When a separation of a particular set of conformers is needed, the separation has to be optimized with respect primarily to solvent composition and nature and concentration of electrolyte.

4. Application

NACE separation of conformers without using additives like surfactants and cyclodextrins may be used for testing of the isomeric purity of substances. Especially if it comes to substances which are only

Table 3
The separation expressed as the resolution R_S for a number of test solutes obtained in two electrophoresis systems

Test solute	Electrophoresis medium: MeOH–MeCN (1:1, v/v) containing	
	25 m*M* NH_4Cl	50 m*M* $AcONH_4$ +1 *M* AcOH
Chlorprothixene	1.15	3.09
Thiothixene	3.05	3.72
Clopenthixol	2.76	1.65
Flupenthixol	3.32[a]	2.30[a]
Flupenthixol decanoate	5.41	1.80
Clomiphene	0.48	1.29
L-Ala–L-Phe/L-Ala–D-Phe	1.79	2.97
Quinine/quinidine	0.27	1.97
Cinchonine/cinchonidine	0.61	0.76

[a] These data are a little different from those listed in Table 2 due to the use of another capillary.

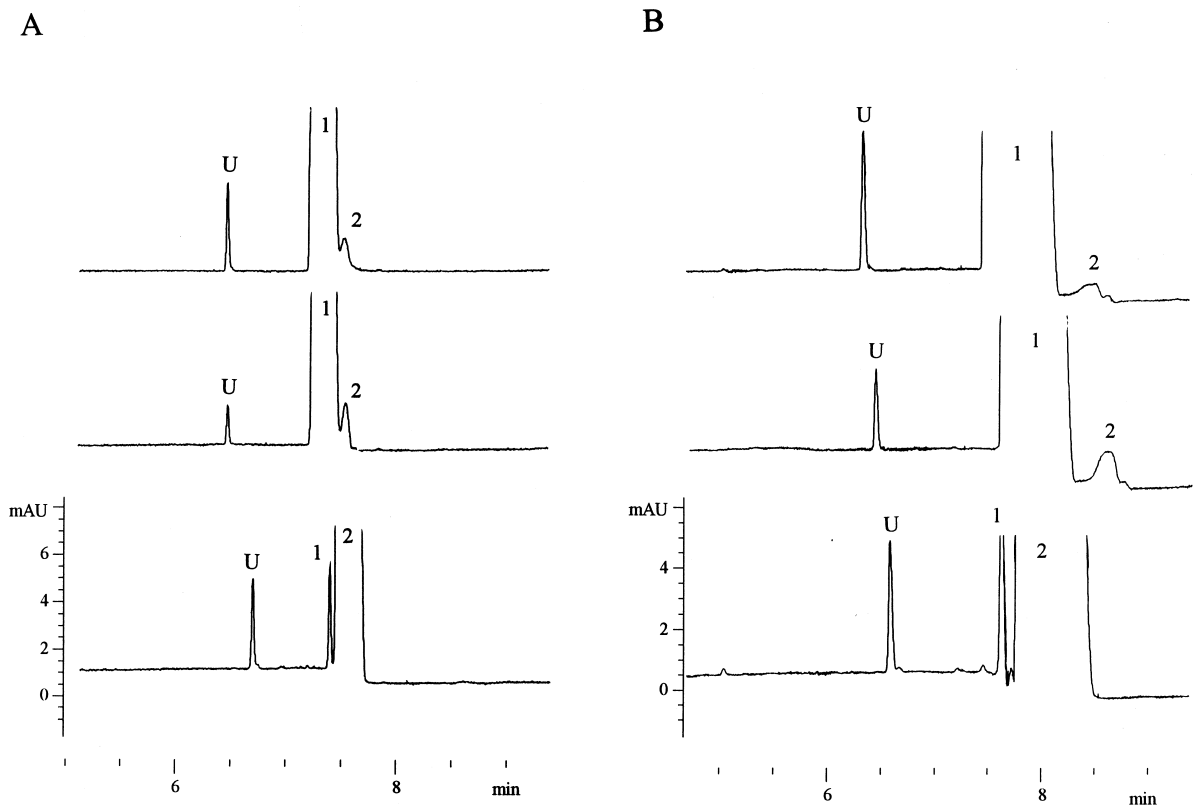

Fig. 2. Electropherograms of *cis*- and *trans*-flupenthixol decanoate obtained in MeOH–MeCN (1:1, v/v) containing (A) 50 m*M* $AcONH_4$ + 1 *M* AcOH and (B) 25 m*M* NH_4Cl. Above: *cis*-flupenthixol decanoate; middle: *cis*-flupenthixol decanoate with 0.5% of *trans*-flupenthixol decanoate added; below: *trans*-flupenthixol decanoate. Capillary: 64 cm (55.5 cm to detector)×50 μm I.D.. Injection 3 s at 5 kPa (50 mbar). Voltage: 30 kV. Detection: 230 nm. Test solution: 5.0 mg/ml of the test sample dissolved in MeOH–MeCN (1:1, v/v). Peak identity: 1, *cis*-flupenthixol decanoate; 2, *trans*-flupenthixol decanoate; U, unknown.

slightly soluble in water the organic solvents facilitates the dissolution of the substances. In Fig. 2 the electropherograms of *cis*- and *trans*-flupenthixol decanoate obtained in the two electrophoresis systems used in Table 3 are shown. The *cis* isomer was found to contain 0.3% of the *trans* isomer using standard addition with *trans*-flupenthixol decanoate. The limit of detection (signal-to-noise ratio = 2) was found to correspond to 0.1% of the *trans* isomer in the *cis* isomer. The electropherogram of the *trans*-flupenthixol decanoate is also shown and it was found to contain the *cis*-isomer (not quantified). A resolution of at least 1.7 is needed if the impurity to be determined has a longer migration time than the main component.

In Fig. 3 examples of the separations of the diastereomers quinine/quinidine and L-Ala–L-Phe/L-Ala–D-Phe are shown.

5. Conclusions

NACE using acidic media has been shown to provide fast and efficient separations of cationic *cis–trans* isomers and diastereomers without using additives like surfactants, cyclodextrins or other complexing agents to the electrophoresis medium. The separations are highly dependent on the solvent of the electrophoresis media as well as on the type and concentration of the electrolytes used. The technique is especially useful for analysing less water soluble substances.

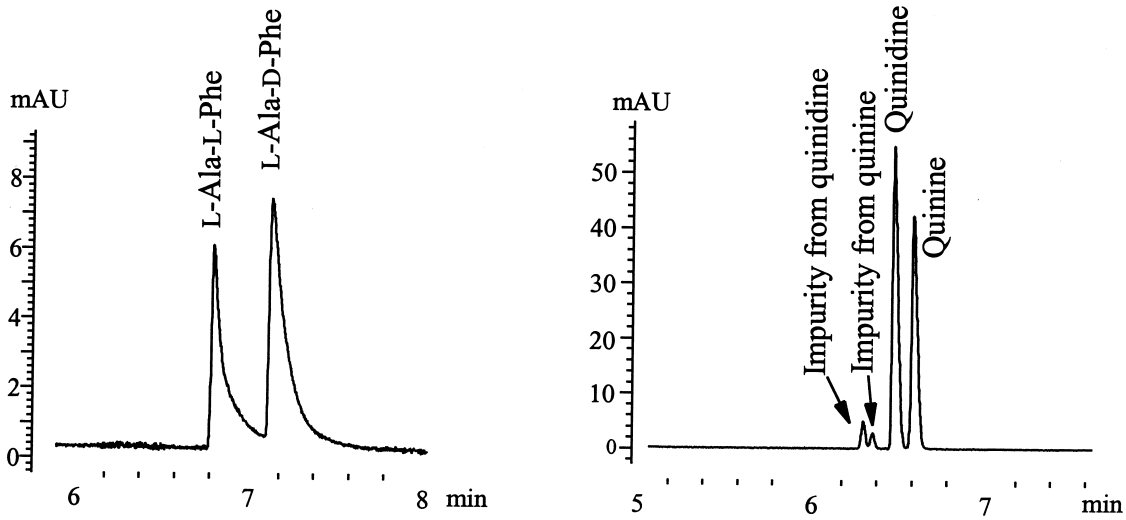

Fig. 3. Separation of diastereomers. Electrophoresis system as in Fig. 2A, with a detection wavelength of 214 nm.

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References

[1] S. Terabe, K. Otsuka, H. Nishi, J. Chromatogr. A 666 (1994) 295–319.
[2] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245–276.
[3] Y. Mori, K. Ueno, T. Umeda, J. Chromatogr. A 757 (1997) 328–332.
[4] M.G. Quaglia, A. Farina, F. Kilar, S. Fanali, E. Bossu, C. Dell'Aquila, J. Liq. Chromatogr. 17 (1994) 3911–3923.
[5] H. Wan, P.E. Anderson, A. Engstrøm, L.G. Blomberg, J. Chromatogr. A 704 (1995) 179–193.
[6] X.Z. Qin, D.P. Ip, E.W. Tsai, J. Chromatogr. 626 (1992) 251–258.
[7] D.K. Bempong, I.L. Honigberg, N.M. Meltzer, J. Pharm. Biomed. Anal. 11 (1993) 829–833.
[8] R.J.H. Houben, H. Gielen, S. Van der Wal, J. Chromatogr. 634 (1993) 317–322.
[9] W. Schutzner, S. Fanali, A. Rizzi, E. Kenndler, J. Chromatogr. A 719 (1996) 410–420.
[10] H. Wan, L.G. Blomberg, J. Chromatogr. A 758 (1997) 303–311.
[11] L. Cellai, C. Desiderio, R. Filippetti, S. Fanali, Electrophoresis 14 (1993) 823–825.
[12] F. Thunecke, H. Hartenstein, D. Sicker, C. Vogt, Chromatographia 38 (1994) 470–474.
[13] L. Zhang, S. Jin, X. Zhou, J.L. Gu, R.N. Fu, Chromatographia 42 (1996) 385–388.
[14] D.K. Bempong, I.L. Honigberg, J. Pharm. Biomed. Anal. 15 (1996) 233–239.
[15] D.A. Kirby, C.L. Miller, J.E. Rivier, J. Chromatogr. 648 (1993) 257–265.
[16] C. Vogt, A. Georgi, G. Werner, Chromatographia 40 (1995) 287–295.
[17] S. Meyer, A. Jabs, M. Schutkowski, G. Fischer, Electrophoresis 15 (1994) 1151–1157.
[18] A.W. Moore, J.W. Jorgenson, Anal. Chem. 67 (1995) 3464–3475.
[19] S. Ma, F. Kálmán, A. Kálmán, F. Thunecke, Cs. Horváth, J. Chromatogr. A 716 (1995) 167–182.
[20] I. Bjørnsdottir, J. Tjørnelund, S.H. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 489–495.
[21] S.H. Hansen, J. Tjørnelund, I. Bjørnsdottir, Trends Anal. Chem. 15 (1996) 175–180.