Vincenzo Pucci[1]
Roberto Mandrioli[1]
Maria Augusta Raggi[1]
Salvatore Fanali[2]

[1]University of Bologna, Department of Pharmaceutical Sciences, Bologna, Italy
[2]Institute of Chemical Methodologies, National Council of Research, Rome Research Area, Monterotondo Scalo, Rome, Italy

Reversed-phase capillary electrochromatography for the simultaneous determination of acetylsalicylic acid, paracetamol, and caffeine in analgesic tablets

The separation and simultaneous determination of caffeine, paracetamol, and acetylsalicylic acid in two analgesic tablet formulations was investigated by capillary electrochromatography (CEC). The effect of mobile phase composition on the separation and peak efficiency of the three analytes was studied and evaluated; in particular, the influence of buffer type, buffer pH, and acetonitrile content of the mobile phase was investigated. The analyses were carried out under optimized separation conditions, using a full-packed silica capillary (75 μm ID; 30.0 cm and 21.5 cm total and effective lengths, respectively) with a 5 μm C_8 stationary phase. A mixture of 25 mM ammonium formate at pH 3.0 and acetonitrile (30:70 v/v) was used as the mobile phase. UV detection was at 210 nm. Good linearity was found in the range of 50–200, 20–160, and 4–20 μg/mL for acetylsalicylic acid (r^2 = 0.9988), paracetamol (r^2 = 0.9990) and caffeine (r^2 = 0.9990), respectively. Intermediate precision (RSD interday) as low as 0.1–0.8% was found for retention times, while the RSD values for the peak area ratios ($A_{analyte}/A_{IS}$) were in the range of 1.9–2.9%. The optimized CEC method was applied to the analysis of the studied compounds present in commercial tablets.

Keywords: Acetylsalicylic acid / Caffeine / Capillary electrochromatography / Paracetamol
DOI 10.1002/elps.200305631

1 Introduction

Acetylsalicylic acid (ASA) and paracetamol (PAR) are currently used as analgesic and antipyretic agents, often associated with caffeine (CAF) in many commercial formulations including tablets and capsules [1]. Different methods have been reported for the simultaneous assay of ASA, PAR, and CAF, including spectrophotometry [2], second-derivative spectrophotometry [3], planar chromatography [4–6], infrared (IR) spectroscopy [7], capillary electrophoresis (CE) [8–10], and high-performance liquid chromatography [11–15]. Among the CE techniques employed for the analysis of ASA, PAR, and CAF, interesting results were obtained using capillary electrochromatography (CEC) coupled with nuclear magnetic resonance (NMR) [16], however, the analytical method is very expensive and requires experience in the field.

The usefulness of CEC for the analysis of ASA or PAR was demonstrated by Altria *et al.* [17], while a mixture of ASA and three of its metabolites was separated under alkaline conditions using anion-exchange CEC [18]. The analysis of CAF alone was carried out using silica [19] or cellulose acetate fiber [20] as the stationary phase, and in combination with other correlated substances and PAR using a normal-phase CEC [21]. CEC is a recently developed separation technique combining the best properties of capillary electrophoresis (high efficiency) with those of HPLC, *i.e.*, high selectivity and increased sample loading which has recently generated great interest especially in the analytical field [22–24]. In CEC the mobile phase and the analytes, under the influence of a relatively strong electric field, are driven to the detector through a capillary usually packed with a stationary phase employed in HPLC. Solutes are separated according to their partitioning coefficient between the two phases and, when charged, their electrophoretic mobility should also be considered. A strong electroosmotic flow (EOF) is necessary in order to speed up the CEC analysis and this can be easily obtained by using classical reversed-phase stationary phases where free silanol groups are present [25, 26]. As in CE, the EOF has a nearly flat flow velocity profile with reduced dispersion compared to LC, leading to a high efficiency [23]. Reversed-phase CEC has been successfully used for the separation of neutral drugs [27–32] and acidic compounds [17, 33–36]. The major factor holding back its further development has been the perceived inability of

Correspondence: Dr. Salvatore Fanali, Institute of Chemical Methodologies, National Council of Research, Rome Research Area, P.O. Box 10, I-00016 Monterotondo Scalo, Rome, Italy
E-mail: fanali@imc.cnr.it
Fax: +39-06-90672-269

Abbreviations: ASA, acetylsalicylic acid; **CAF**, caffeine; **IS**, internal standard; **PAR**, paracetamol

CEC to analyse basic compounds. In fact, the analysis of basic compounds often results in broad peaks and serious peak tailing. The same residual silanol groups that are essential for creating the EOF cause this tailing. Pharmaceuticals (and their impurities) are often basic, and, therefore, the application of CEC to pharmaceutical analysis can be often problematic. However, the addition of a competing amine to the mobile phase at low pH, has been proposed to improve the separation of basic substances by reversed-phase CEC [25, 37–39].

The aim of this paper was to investigate the possibility of using the high efficiency and selectivity power of CEC technique for the analysis in reversed-phase mode of pharmaceutical compounds employing UV detection. The effect of several experimental parameters, such as buffer type and pH and the concentration of organic modifier, on resolution, retention time and retention factor were studied analyzing in the same run ASA, PAR and CAF. The optimized method was applied to the analysis of the three active principles present in two analgesic pharmaceutical preparations.

2 Materials and methods

2.1 Chemicals

All chemicals were of analytical grade and used without further purification. Formic acid, citric acid, phosphoric acid (85%), acetic acid, ammonia solution (30%), ammonium acetate, sodium hydroxide, and dimethylsulfoxide (DMSO) were purchased from Carlo Erba (Milan, Italy). Acetonitrile (ACN) and methanol (MeOH) were from Sigma (St. Louis, MO, USA). ASA, PAR, CAF, and propyl-4-hydroxybenzoate, used as internal standard (IS), were obtained from Carlo Erba (Milan, Italy). For their chemical structures, see Fig. 1.

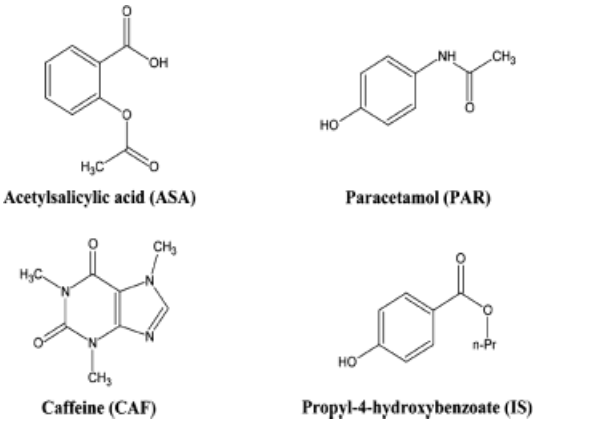

Figure 1. Chemical structure of the analytes and IS.

2.2 Electrochromatography

Experiments were carried out with a HP^{3D} CE apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with an UV-visible diode array detector operated at 210 nm and an air thermostating cooling system. The CEC packed capillary was positioned into the appropriate cartridge after removing the layer of polyimide (about 0.5 cm) for on-line detection. CEC packed columns were prepared in our laboratory. The fused-silica capillaries 75 μm ID (375 μm OD) were purchased from Composite Metal Services (Hallow, Worcester, UK) and packed with LiChrospher 100 RP8 (5 μm) (Merck, Darmstadt, Germany). The fused-silica capillary was connected with one end to an HPLC column frit (temporary frit) and with the opposite side to a steel HPLC precolumn, containing the slurry, connected to a LC 10 HPLC pump (Perkin-Elmer, Norwalk, CT, USA). The precolumn and part of the capillary were dipped into an ultrasonic bath in order to keep the particles of the stationary phase in suspension. Methanol was pumped at ~2000 psi until the capillary was packed (35 cm). Then, after removing the slurry reservoir, distilled water was pumped (~ 3000 psi) into the capillary for about 1 h. A heating coil was used for the preparation of both the inlet and the outlet frits by sintering the C_8 particles at ~600°C × 60 s. Detection window was therefore made at 8.5 cm from the outlet frit with a razor. After removing the temporary frit, the column was cut close to the inlet and outlet frits. The total length of the capillary (completely packed) used in this investigation was 30 cm while 21.5 cm was the effective length. The packed capillary was equilibrated with the aqueous-organic mobile phase for 1 h by using the HP^{3D} instrument applying 12 bar pressure at the inlet end of the capillary and then both pressure (8 bar) and voltage (25 kV) applied until a stable current and baseline signal were monitored (about 15 min). The mobile phase was 25 mM formic acid, titrated to pH 3.0 with ammonia solution, and acetonitrile (70% v/v). CEC experiments were carried out applying 25 kV and 8 bar pressure at both ends of the capillary. Injection was done at the anodic end of the capillary by high-pressure application (12 bar × 30 s) followed by a mobile phase plug (12 bar, 12 s). The capillary temperature was maintained at 25°C. DMSO was used as the EOF marker in all method development assays.

2.3 Standard solutions

Standard stock solutions (1 mg/mL) were prepared in acetonitrile and diluted with a mixture water:acetonitrile (50:50 v/v) to the desired concentrations prior to injection. Calibration standard solutions were prepared by mixing appropriate aliquots of stock solutions and IS with a mix-

ture of water:acetonitrile (50:50 v/v) to give ten different solutions in the concentration range of 50–200 μg/mL for ASA, of 20–160 μg/mL for PAR, and of 4–20 μg/mL for CAF. The final concentration of IS was 50 μg/mL.

2.4 Sample preparation

The two commercialized pharmaceutical formulations analyzed were: (A) Drin® (Alfa Wassermann S.p.A., Alanno, PE, Italy) tablets containing 300 mg ASA, 125 mg PAR, and 25 mg CAF, and as excipients microcrystalline cellulose, starch, castor oil, glyceryl palmitate/stearate, and silicium dioxide. (B) NeoNisidina® (Boehringer Ingelheim Italia S.p.A. Firenze, Italy) tablets containing 250 mg ASA, 200 mg PAR, and 25 mg CAF, and as excipients: starch, lactose, and stearic acid. The three active ingredients were extracted from the tablets using the following procedure. First, twenty tablets were accurately weighed, finely ground to a powder and thoroughly mixed. Then, an aliquot of this powder corresponding to 20 mg of declared ASA was weighed and transferred into a volumetric flask. 20 mL of a mixture of water:acetonitrile (30:70 v/v) was added, the mixture was agitated for 15 min on an ultrasonic bath and then centrifuged for 15 min at 3000 rpm. The supernatant (with a final concentration of 1 mg/mL of ASA) was used for preparing the working solutions diluting the stock solution with the mixture water:acetonitrile (1:1).

2.5 Precision assays

Standard and tablets solutions of the ASA, PAR, and CAF were prepared and analyzed six times within the same day to obtain the repeatability, and six times over different days to obtain the intermediate precision, according to United States Pharmacopeia (USP) requirements [40]. Each assay was carried out on a different extraction from each commercial tablet. The percentage relative standard deviations (RSD%) of the data obtained were calculated for three analytes. The limits of detection (LODs) and quantitation (LOQs) were calculated according to USP guidelines [40], as the analyte concentrations which give rise to signals equal to 3 and 10 times the baseline noise, respectively.

2.6 Accuracy

The accuracy of the methods was evaluated by means of recovery determinations, adding a known quantity of the three reference powders to a certain amount of each pharmaceutical formulations, in order to obtain three different levels of addition for each analyte. The samples were analyzed and the mean recovery as well as the repeatability were calculated on six assays for each concentration added.

3 Results and discussion

3.1 Method optimization

In CEC the pH of the buffer is one of the experimental parameters that has to be carefully selected in order to find the optimum experimental conditions for the baseline resolution of analytes. The dissociation degree of charged/chargeable analytes and the electroosmotic flow are pH-dependent, *i.e.*, the movement of analyzed compounds (ASA, pK_a = 3.5; PAR, pK_a = 9.5, and CAF, pK_{a1} = 14, pK_{a2} = 0.6) is influenced not only by the EOF but also by their effective mobility. Therefore, experiments were carried out using mobile phases composed of 80% ACN and 25 mM ammonium acetate in the pH range 2.5–6.0. At buffer pH values higher than 3.0, CAF and PAR were eluted within a reasonable time ($<$ 6 min) while ASA did not appear at the detection window after 60 min probably due to its acidic properties (migration against the EOF). Lowering the buffer pH to 3.0 allowed for the baseline separation of the three studied compounds with the PAR peak eluted just before the EOF. At this pH the analytes were not charged and therefore the separation was based on their different partitioning between mobile and stationary phases. Figure 2 shows the CEC separation of CAF, ASA, and PAR using the above-mentioned mobile phase at pH 3.0. As can be observed, a relatively high EOF was present despite the low pH value due to the high content of acetonitrile. However, due to the elution of PAR very close to EOF, quantitative analysis seems to be difficult and therefore we further investigated the effect of the buffer type on analyte resolution.

Mobile phases with citrate, phosphate, and formate buffers were used, while maintaining the amount of acetonitrile and the ionic strength of the mobile phase constant. The effect of different buffer types on the retention times of the analytes and EOF is reported in Fig. 3. As can be observed, the use of a formate buffer allowed for the fastest separation of the analytes, however, ASA and CAF were not separated from each other. The use of the other buffers caused a peak broadening effect and required longer analysis times. Therefore, a formate buffer at pH 3.0 was selected for further experiments to study the influence of acetonitrile concentration on analyte resolution.

The amount of acetonitrile added to a 25 mM formate buffer, pH 3.0 was varied over the range 60–80% in order to manipulate the extent of hydrophobic interactions occur-

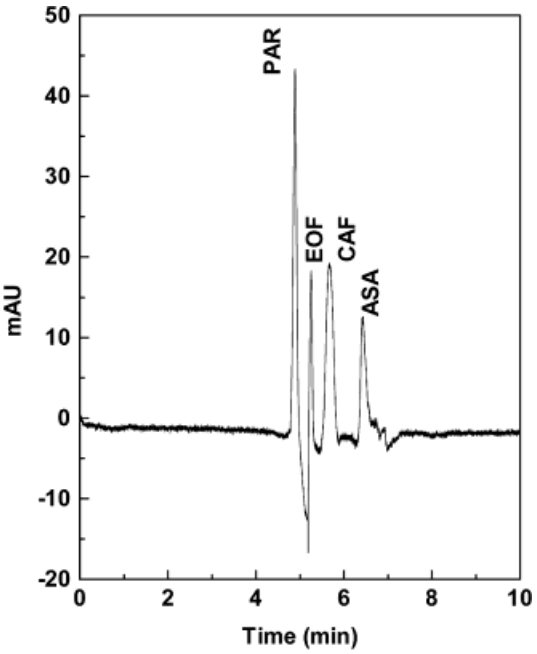

Figure 2. CEC reversed-phase analysis of the analytes in 5 mM (final concentration) ammonium acetate buffer at pH 3.0 containing 80% of acetonitrile. Capillary: 30 cm total length (21.5 cm effective length) × 75 μm C_8 fully packed; voltage, 25 kV; injection, 12 bar for 0.5 min followed by mobile phase injection at 12 bar for 0.2 min. Analytes concentration: 50 μg/mL each.

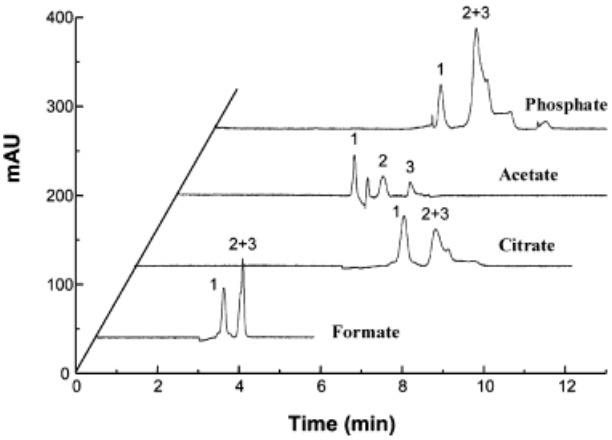

Figure 3. Chromatograms of the separation of ASA, PAR, and CAF in mixture (50 μg/mL concentration), obtained using a mobile phase 80% of ACN/20% of 25 mM, pH 3.0, of ammonium formate, citrate, acetate, and phosphate, respectively. Peaks: 1, PAR; 2, CAF, 3, ASA. Other experimental conditions as in Fig. 2.

ring between the analytes and the stationary phase. A graph reporting the log of the retention factor as a function of acetonitrile content is shown in Fig. 4. As can be seen, *k* decreased almost linearly by increasing

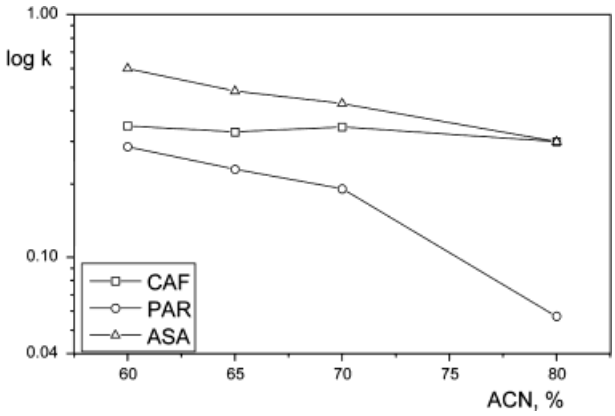

Figure 4. Logarithmic retention factor (log *k*) of the analytes *versus* percentage of acetonitrile present in the mobile phase (25 mM ammonium formate pH 3.0 and 60–80% ACN). For other conditions see Fig. 2.

the acetonitrile concentration, clearly showing the typical reversed phase mechanism for ASA and PAR. On the contrary, this effect was not remarkable for CAF (*k* remained almost constant), probably due to its polarity at this pH. This analyte behavior means that the resolution between CAF and ASA tends to increase, while that between CAF and PAR tends to decrease, with decreasing acetonitrile concentration. Therefore, it can be concluded that a mobile phase with an acetonitrile content of 70% provides the best compromise concerning separation and analysis time.

The use of an IS for quantitative analysis in CE improves [41] detector linearity data as it reduces imprecision in the data points. The internal standard was selected among chemical substances with a molecular structure similar to that of ASA, such as benzoic acid, *p*-hydroxybenzoic acid, salicylic acid, propyl-4-hydroxybenzoate, phenyl salicylate, benzyl alcohol, and *p*-hydroxy phenylpropionic acid. Among these, propyl-4-hydroxybenzoate (Fig. 1) was chosen as the IS and used at a concentration of 50 μg/mL.

3.2 CEC analysis of standard solutions

From the above described results a mobile phase containing 25 mM ammonium formate at pH 3.0/ACN (30:70 v/v) was selected for the analysis of CAF, PAR, and ASA. This mobile phase allowed to achieve baseline resolution of all studied compounds in a short analysis time (less than 8 min) with good efficiency (Fig. 5). The standard calibration curves were established by plotting the ratio between the area of analytes and of the IS against the analyte concentration. The linear correlation coefficients (r^2) in the 50–200, 20–160, and 4–20 μg/mL concentration

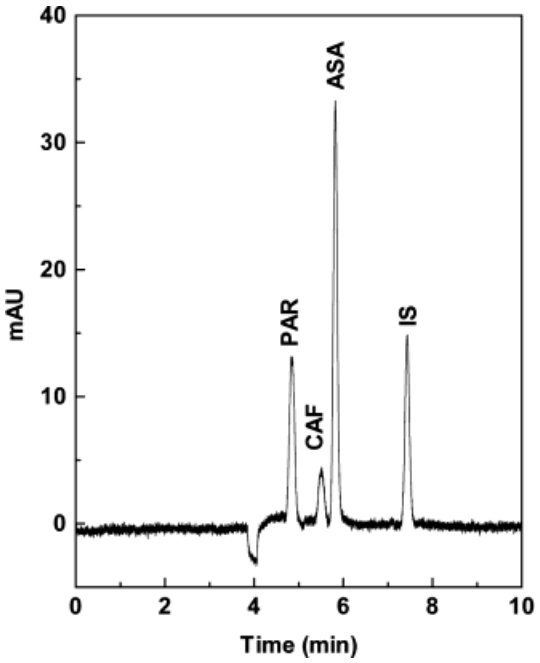

Figure 5. Typical electrochromatogram of the separation of a 100 μg/mL ASA, 40 μg/mL PAR, 8 μg/mL CAF, and 50 μg/mL IS standard solution, under the optimum experimental conditions. Mobile phase, 7.5 mM (final concentration) ammonium formate buffer pH 3.0, containing 70% of acetonitrile. Other conditions as in Fig. 2.

ranges for ASA, PAR, and CAF were 0.9988, 0.9990, and 0.9990, respectively (see Table 1). Table 1 shows the precision data obtained analyzing standard solutions at different concentrations within the same day and over different days. Precision expressed by the mean relative standard deviation (RSD%) values ranged from 1.2 to 1.9 for repeatability and from 1.9 to 2.9 for intermediate precision. The LOD and the LOQ for all the studied compounds were 1 and 3 μg/mL, respectively.

3.3 Application to pharmaceutical formulations

A feasible and simple procedure was implemented for the extraction of the three analytes from the two analgesic formulations under investigation. Powdered tablets (A and B, see Section 2.4) were treated with mixtures of acetonitrile and water at different acetonitrile concentrations, ranging from 50 to 100%. Best results were obtained using a mixture of water and acetonitrile (30:70 v/v) assuring good efficiency and selectivity. In fact, no interference from the excipients was revealed. The water:acetonitrile extracts obtained from the preparations A and B were diluted to obtain solutions at different nominal concentrations and analyzed by means of CEC. The chromatogram relative to tablets B is reported in Fig. 6. The analytes and the IS are well separated and no interference from the matrix was detected.

Quality control assays for both preparations were carried out by the optimized CEC method. The mean amount found of the declared value and the relative mean precision, assessed as RSD % values, are detailed in Tables 2 and 3, for each active principle. Satisfactory mean amounts were obtained, *e.g.*, 97%, 102% and 97% for ASA, PAR, and CAF, respectively. Accuracy was assessed by means of recovery assays at three different concentrations for each analyte; the mean recovery values, varying from 94% to 102%, were satisfactory and are reported in Table 4.

Table 1. Precision data and linearity results for the determination of the analytes in standard solutions

Compound	Concentration (μg/mL)	Repeatability RSD%	Intermediate precision RSD%	t_R (min)	Intermediate precision RSD%	Linearity data[a]: $y = a + bx$		
						a	*b*	r^2
ASA	50	1.7	2.8	5.8	0.7	0.0333	0.0176	0.9988
	100	1.2	2.5		0.4			
	200	1.4	2.2		0.2			
PAR	20	1.9	2.8	4.8	0.8	0.1254	0.0208	0.9990
	50	1.9	2.9		0.4			
	160	1.4	2.1		0.2			
CAF	4	1.9	2.9	5.5	0.5	0.0055	0.0364	0.9990
	8	1.8	2.6		0.3			
	20	1.3	2.5		0.2			
IS	50.00	1.2	1.9	7.4	0.1	–	–	–

a) Data from regression curve calculation based on compound/IS peak area ratio

Table 2. Quantitative determination of the three active principles in the pharmaceutical preparations A by means of CEC

Compound	Concentration (μg/mL)	% found of declared (intraday)[a)]	Repeatability RSD%	% found of declared (interday)[a)]	Intermediate precision RSD%	t_R (min)	Intermediate precision RSD%
ASA	50.00	96	0.9	96	1.8	5.8	0.1
	100.00	97	1.1	98	1.5		0.1
	200.00	99	0.7	99	1.1		0.1
PAR	20.83	103	1.7	102	2.2	4.8	0.1
	41.67	100	1.2	101	1.9		0.2
	83.33	103	0.8	103	2.0		0.1
CAF	4.17	94	2.3	95	2.9	5.5	0.2
	8.33	99	2.5	98	2.7		0.2
	16.67	98	1.0	99	2.6		0.2
IS	50.00	100	1.3	100	1.8	7.4	0.2

a) $n = 6$

Table 3. Quantitative determination of the three active principles in the pharmaceutical preparations B by means of CEC

Compound	Concentration (μg/mL)	% found of declared (intraday)[a)]	Repeatability RSD%	% found of declared (interday)[a)]	Intermediate precision RSD%	t_R (min)	Intermediate precision RSD%
ASA	50.00	99	1.2	101	1.8	5.8	0.1
	100.00	100	0.8	99	1.2		0.1
	200.00	101	0.5	102	0.9		0.1
PAR	40	104	0.8	104	1.5	4.8	0.1
	80	102	0.6	103	1.2		0.1
	160	103	0.5	104	1.1		0.1
CAF	5	96	2.5	96	2.7	5.5	0.2
	10	98	1.2	100	2.1		0.2
	20	97	0.8	96	1.6		0.2
IS	50.00	100	1.6	100	1.9	7.4	0.1

a) $n = 6$

Table 4. Accuracy of the CEC method

Formulation	Analyte and concentration added (μg/mL)		Recovery%[a)]	Repeatability RSD%[a)]
A	ASA	50	99	1.8
		75	101	1.5
		100	102	1.3
	PAR	20	94	1.7
		30	98	1.6
		40	99	1.4
	CAF	4	97	2.1
		6	101	2.3
		8	102	2.1
B	ASA	50	99	1.9
		75	101	1.8
		100	98	1.7
	PAR	20	98	1.8
		30	99	1.7
		40	101	1.5
	CAF	4	109	2.3
		6	100	2.0
		8	99	1.7

a) $n = 6$

4 Concluding remarks

A CEC method, using a reversed-phase mode, was applied to the determination of the active ingredients of two analgesic formulations. Optimization of the separation requires a careful investigation of different physicochemical parameters of the mobile phase, such as, *e.g.*, organic modifier concentration, buffer type, and its pH. These parameters influence the driving force of the EOF, as well as the analyte retention time values, elution order and partition separation mechanism. The optimized experimental conditions (25 mM ammonium formate pH 3.0/ACN, 30:70 v/v) allowed for the complete separation of the analytes in less than 8 min. After validation, the CEC method was successfully applied to the analysis of the active principles of two different kinds of analgesic pharmaceutical tablets, providing accurate and precise results in a wide range of amounts, which vary from 25 mg/unit for caffeine to 300 mg/unit for ASA. For all the aforementioned reasons the developed procedure can be useful for the quality control of pharmaceuticals.

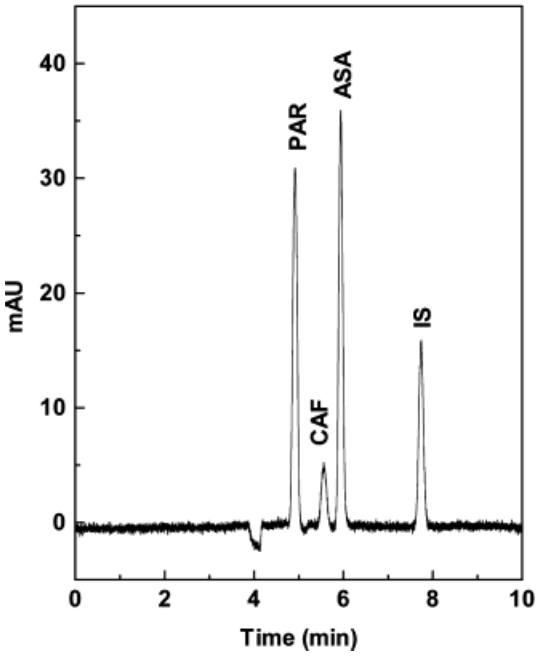

Figure 6. Electrochromatogram of the analysis of tablets B extract. The peaks correspond to the following nominal concentrations: ASA, 100 μg/mL; PAR, 80 μg/mL; CAF, 10 μg/mL. For experimental conditions see text and Fig. 2.

This investigation has been supported by a grant (60%) from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica – Italy. We would like to thank Mr. Massimo Brighenti for his experimental support and assistance with the sampling procedure.

Received May 20, 2003

5 References

[1] *United States Pharmacopeia*, 36th Ed., United States Pharmacopeial Convention, Rockville, MD 2003, pp. 20–21.

[2] Glombitza, B. W., Schmidt, P. C., *J. Pharm. Sci.* 1994, *83*, 751–757.

[3] Kokot, Z., Burda, K., *J. Pharmaceut. Biomed.* 1998, *18*, 871–875.

[4] Argekar, A. P., Sawant, J. C., *J. Planar Chromatogr.* 1999, *12*, 361–364.

[5] Kamble, V. W., Garad, M. V., Dongre, V. G., *J. Planar Chromatogr.* 1996, *9*, 280–281.

[6] Krzek, J., Starek, M., *J. Planar Chromatogr.* 1999, *12*, 356–360.

[7] Bouhsain, Z., Garrignes, S., de-la-Guardia, M., *Fresenius' J. Anal. Chem.* 1997, *357*, 973–976.

[8] Lee, K. J., Heo, G. S., Kim, N. J., Moon, D. G., *J. Chromatogr.* 1992, *608*, 243–250.

[9] Booukerd, S., Lauwers, M., Detaevdernier, M. R., Michote, Y., *J. Chromatogr. A* 1995, *695*, 97–102.

[10] Gfrorer, P., Schewitz, J., Pusecker, K., Tseng, L. H., Albert, K., Bayer, E., *Electrophoresis* 1999, *20*, 3–8.

[11] Franeta, J. T., Agbaba, D., Eric, S., Pavkov, S., Aleksic, M., Vladimirov, S., *Farmaco* 2002, *57*, 709–713.

[12] Xu, X., Stewart, J. T., *J. Liq. Chromatogr. Relat. Technol.* 2000, *23*, 769–779.

[13] Mamolo, M. G., Vio, L., Maurich, V., *Farmaco* 1985, *40*, 111–123.

[14] Indriyanto, G., Sunarto, A., Adriani, Y., *J. Pharmaceut. Biomed.* 1995, *13*, 1555–1559.

[15] Di Pietra, A. M., Gatti, R., Andrisano, V., Cavrini, V., *J. Chromatogr. A* 1996, *729*, 355–361.

[16] Gfrorer, P., Tseng, L. H., Rapp, E., Albert, K., Bayer, E., *Anal. Chem.* 2001, *73*, 3234–3239.

[17] Altria, K. D., Smith, N. W., Turnbull, C. H., *J. Chromatogr. B* 1998, *717*, 341–353.

[18] Scherer, B., Steiner, F., *J. Chromatogr. A* 2001, *924*, 197–209.

[19] Gillott, N. C., Euerby, M. R., Johnson, C. M., Barrett, D. A., Shaw, P. N., *Chromatographia* 2000, *51*, 167–174.

[20] Jinno, K., Wu, J., Sawada, H., Kiso, Y., *J. High Resolut. Chromatogr.* 1998, *21*, 617–619.

[21] Lai, E. P. C., Dabek-Zlotorzynska, E., *Electrophoresis* 1999, *20*, 2366–2372.

[22] Mistry, K., Krull, I., Grinberg, N., *J. Sep. Sci.* 2002, *25*, 935–958.

[23] Dermaux, A., Sandra, P., *Electrophoresis* 1999, *20*, 3027–2065.

[24] Deyl, Z., Svec, F., *Capillary Electrochromatography*, Elsevier Science, Amsterdam 2001, pp. 87–106.

[25] Gillott, N. C., Euerby, M. R., Johnson, C. M., Barrett, D. A., Shaw, P. N., *Anal. Commun.* 1998, *35*, 217–220.

[26] Zimina, T. M., Smith, R. M., Myers, P., *J. Chromatogr. A* 1997, *758*, 191–197.

[27] Euerby, M. R., Johnson, C. M., Bartle, K. D., Myers, P., Roulin, S. C. P., *Anal. Commun.* 1996, *33*, 403–405.

[28] Smith, N. W., Evans, M. B., *Chromatographia* 1994, *38*, 649–657.

[29] Euerby, M. R., Gilligan, D., Johnson, C. M., Roulin, S. C. P., Myers, P., Bartle, K. D., *J. Microcol. Sep.* 1997, *9*, 373–387.

[30] Reilly, J., Saeed, M., *J. Chromatogr. A* 1998, *829*, 175–186.

[31] Wang, J., Schaufelberger, D. E., Guzman, N. C., *J. Chromatogr. Sci.* 1998, *36*, 155–160.

[32] Desiderio, C., Ossicini, L., Fanali, S., *J. Chromatogr. A* 2000, *887*, 489–496.

[33] Robson, M. M., Cikalo, M. G., Myers, P., Euerby, M. R., Bartle, K. D., *J. Microcol. Sep.* 1997, *9*, 357–372.

[34] Strickmann, D. B., Chankvetadze, B., Blaschke, G., Desiderio, C., Fanali, S., *J. Chromatogr. A* 2000, *887*, 393–407.

[35] Desiderio, C., Fanali, S., *J. Chromatogr. A* 2000, *895*, 123–132.

[36] Lurie, I. S., Meyers, R. P., Conver, T. S., *Anal. Chem.* 1998, *70*, 3255–3260.

[37] Lurie, I. S., Conver, T. S., Ford, L., *Anal. Chem.* 1998, *70*, 4563–4569.

[38] Hilhorst, M. J., Somsen, G. W., de Jong, G. J., *J. Chromatogr. A* 2000, *872*, 315–321.

[39] Dittmann, M. M., Masuch, K., Rozing, G. P., *J. Chromatogr. A* 2000, *887*, 209–221.

[40] *United States Pharmacopeia*, 36th Ed., United States Pharmacopeial Convention, Rockville, MD 2003, pp. 2440–2442.

[41] Altria, K. D., Bestford, J., *J. Capil. Electrophor.* 1996, *3*, 13–23.