ELSEVIER

Journal of Chromatography A, 887 (2000) 489–496

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Analysis of hydroquinone and some of its ethers by using capillary electrochromatography

Claudia Desiderio, Luigia Ossicini, Salvatore Fanali*

Istituto di Cromatografia del Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo, Rome, Italy

Abstract

Capillary electrochromatography (CEC) was used for the analysis of relevant compounds in cosmetic preparation. Hydroquinone (HQ) and some of its ethers (methyl-, dimethyl-, benzyl-, phenyl-, propyl-HQ derivatives) were analyzed by using an octadecylsilica (ODS) stationary phase packed in fused-silica capillary (100 μm I.D.; 30 cm and 21.5 cm total and effective lengths, respectively). 20 m*M* Ammonium acetate pH 6–acetonitrile (50–70%) were the mobile phases used for the experiments. The acetonitrile (ACN) content strongly influenced the resolution of the studied compounds as well as the efficiency and the retention factor. Baseline resolution for the studied analytes was achieved at both the lowest and the highest percentage of ACN, the last one providing the shortest analysis time. Mobile phase containing 70% of ACN was therefore used for the analysis of an extract of skin-toning cream declared to contain HQ. Good repeatability of both retention times, peak areas and peak areas ratio ($A_{sample}/A_{internal\ standard}$) was found. The calibration graphs were linear in the concentration range studied (5–90 μg/ml) with correlation coefficients between 0.9975 and 09991. The analysis of the cosmetic preparation revealed the presence of HQ (1.72%, w/w) and of two additional peaks (not identified). © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Electrochromatography; Hydroquinone

1. Introduction

Hydroquinone (HQ) is a compound mainly used as antioxidant in the photography industry as well as depigmenter agent in cosmetic products such as skin-toning creams. The HQ mechanism of action in the biological process is based on the inhibition of melanin formation and due to the toxicological effects of HQ, it can cause dermatitis, EU regulations allow its content in cosmetics within the 2% (w/w) level. It has shown that HQ and some of its derivatives (not allowed by European Directive) were present in analyzed skin-toning creams [1]. Thus the analytical determination of HQ and its derivatives in cosmetics is very important for the human health protection and consumers safeguarding.

Several analytical methods have been so far used for the analysis of HQ in toning creams including high-performance liquid chromatography (HPLC), spectrophotometry, thin layer chromatography (TLC), micellar electrokinetic chromatography (MEKC) etc. [1–7].

Capillary electrochromatography (CEC) is a recent analytical technique performed in fused-silica capillaries packed with chromatographic particle materials immobilized into the column by an inlet and an outlet frits. In CEC both the electrophoretic and chromatographic mechanism are combined and

*Corresponding author. Tel.: +39-6-9067-2256; fax: +39-6-9067-2269.

E-mail address: fanali@nserv.icmat.mlib.cnr.it (S. Fanali)

0021-9673/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(99)01197-8

the analytes can be separated according to their different partition between the mobile and the stationary phases. The electroosmotic flow, generated by the silanol groups, present in both the packed particles and the capillary wall, when voltage is applied, pumps the mobile phase and the analytes through the column towards the on line detection cell.

CEC can be advantageously used for the analysis of either neutral and charged analytes [8]. In the last case the separation will occur according to the compounds mobility and chromatographic partition.

So far several studies have been published dealing with CEC theory [9–11], column preparation [12–14] as well as some applications [8,13,15–17].

The aim of this report is to optimize a CEC separation method for the analysis of hydroquinone in skin-toning cream. HQ was separated from some of its ethers, namely hydroquinone monomethyl ether (MHQ), hydroquinone dimethyl ether (DMHQ), hydroquinone monopropyl ether (PHQ), hydroquinone monobenzyl ether (BHQ) and hydroquinone monophenyl ether (PhHQ) studying the effect of the organic modifier, present in the mobile phase, on retention factor (k') and efficiency (N). Repeatability of retention time and peak area, linearity, limit of detection (LOD) and limit of quantitation (LOQ) were also investigated.

2. Experimental

2.1. Instrumentation

Experiments were performed in a HP^{3D} Automatic Electrophoresis Instrument (Hewlett-Packard, Waldbronne, Germany) equipped with an UV-visible diode array detector operated at 205 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.

2.2. Chemicals

All chemicals used in this study were of analytical grade and used without further purification. Ammonia solution (30%) and acetic acid were purchased from Carlo Erba (Milan, Italy). Acetonitrile (ACN) and methanol (MeOH) were from BDH (Poole, UK). Hydroquinone (HQ), hydroquinone monomethyl ether (MHQ), hydroquinone dimethyl ether (DMHQ), hydroquinone monopropyl ether (PHQ), hydroquinone monobenzyl ether (BHQ) and hydroquinone monophenyl ether (PhHQ) were obtained from Fluka (Buchs, Switzerland). Skin-toning cream, declared to contain hydroquinone, was purchased in a pharmacy and used for quantitative analysis.

2.3. Electrochromatography

CEC packed columns were prepared in our laboratory. The fused-silica capillaries 100 μm I.D. (375 μm O.D.) were purchased from Composite Metal Services (Hallow, Worcs., UK) and packed with LiChrospher 100 RP_{18} (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 peek HPLC pre-column, containing the slurry, connected to a LC 10 HPLC pump (Perkin-Elmer). The pre-column and part of the capillary were dipped into an ultrasonic bath in order to keep in solution the particles of the stationary phase. Methanol was pumped at ~2000 p.s.i. until the capillary was packed (35 cm). Then, after removing the slurry reservoir, double distilled water was pumped (~ 3000 p.s.i.) into the capillary for about 1 h.

An heating coil was used for the preparation of both the inlet and the outlet frits by sintering the C_{18} particles at ~600°C×60 s. Detection window was therefore made at 8.5 cm from the outlet frit by polyimide removal at ~300°C×30 s.

After removing the temporary frit, the column was cut close to the inlet and outlet frits. The total length (completely packed) used in this study 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 (12 bar) and voltage (25 kV) applied until a stable current and baseline signal were monitored (about 15 min).

The mobile phase used for the experiments was 20

mM acetic acid, titrated to pH 6 with ammonia solution, and different concentrations of acetonitrile (50–70%, v/v). CEC experiments were carried out applying 25 kV and 5 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 BGE plug (12 bar, 12 s). The capillary temperature was maintained at 25°C.

2.4. Standard solutions

Standard stock solutions (1 mg/ml) were prepared in acetonitrile and diluted with water to the desired concentrations prior to injection with water.

Calibration standard solutions were prepared by mixing appropriate aliquots of stock solutions and internal standard (I.S.) with water to give 10 different solutions in the concentration range of 5–90 μg/ml for all studied compounds. The final concentration of I.S. (2-phenyl-1,2-propanediol) was 55 μg/ml.

2.5. Sample preparation

The procedure for the extraction of hydroquinone from the skin-toning cream was similar to that previously described [7]. Aliquots of skin toning cream were weighted, added with the I.S. and then extracted with aqueous solution containing 10% (v/v) methanol in order to obtain 2.5 mg of cream per ml of solution and 50 μg/ml I.S. concentration.

The mixture was vortex mixed at 50°C for 15 min. After cooling the solution was filtered through a 0.45 μm nylon filters (Lida, Kenosha, WI, USA) and injected for the CEC experiments.

3. Results and discussion

In CEC the electroosmotic flow (EOF) is the main driving force responsible for the movement of analytes and mobile phase through the stationary phase towards the detector. In comparison to pressure driven LC separations, where the mobile phase moves with a parabolic profile, electroosmotic transport of the mobile phase is associated with a flat flow profile and high efficiency of the separation process is maintained [8].

Based on our previous experience in the analysis of HQ and its ethers using micellar electrokinetic chromatography (MEKC) [7] and according to the physico-chemical properties of these compounds, all electrically neutral, a packed capillary with 5 μm octadecyl silica particles was used for the study. Preliminary experiments were carried out using as mobile phase a mixture of ammonium acetate at pH 6 and acetonitrile as organic modifier in order to have high electroosmotic flow. In fact it was reported that the presence of ACN in the BGE in CEC provides, compared with other organic solvents, strong electroosmotic flow even at low buffer pHs [8].

The mobile phase containing 20 mM ammonium acetate at pH 6 and acetonitrile (40:60, v/v) allowed to achieve the separation of HQ and its ethers in less than 10 min, however co-elution of DMHQ and PhHQ was observed. In order to find the optimum experimental conditions for the separation of all the studied compounds in mixture, the effect of ACN concentration, added to the ammonium acetate buffer, on migration times, retention factor (k') and efficiency was investigated.

As can be observed in Fig. 1a–e, the increase of the organic modifier concentration provided shorter migration times for all the analytes and increased the height of the peaks due to the smaller peak width obtained. The variation of the organic modifier content in the BGE influences the residence time of the analytes in the reversed-phase silica particles differently modulating their affinity based partition. When the ACN content rised the elution order stayed constant for almost all the analyzed compounds with the exception of DMHQ which eluted before BHQ at 50% of ACN and was instead the most retained compounds at 70% of organic modifier.

Fig. 2 shows the plot of the log of retention factor ($k'=(t_R - t_o)/t_o$, t_R and t_o are the retention and electroosmotic time respectively) versus the acetonitrile concentration.

The logarithmic function of k' slightly decreased by increasing the content of ACN in the mobile phase for most of the studied compounds due to a weaker interaction of analytes with the modified silica packed material. This effect was not remarkable for HQ and MHQ (k' stayed at almost a constant value) probably due to their polarity.

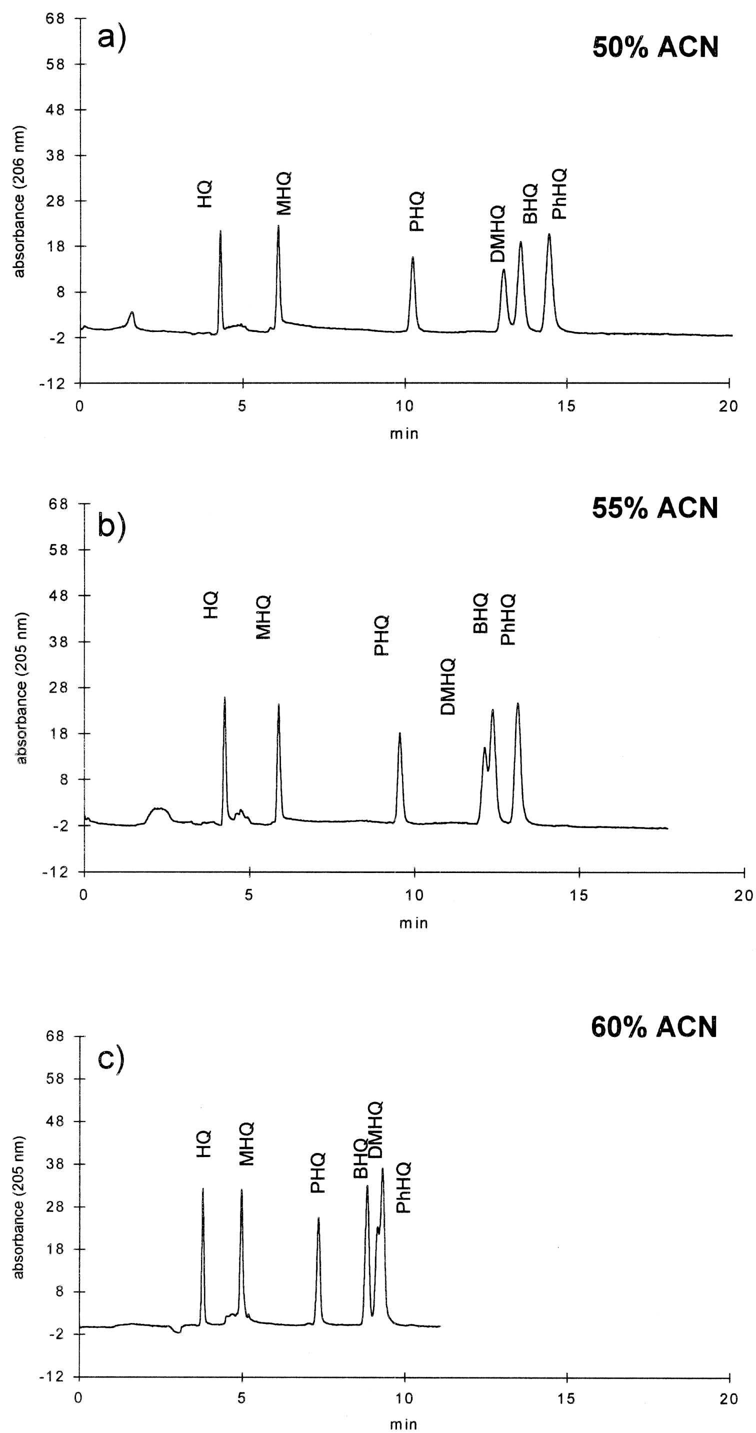

Fig. 1. Separation of hydroquinone (HQ) and its ethers [methyl hydroquinone (MHQ), propylhydroquinone (PHQ), benzylhydroquinone (BHQ), phenylhydroquinone (PhHQ) and dimethylhydroquinone (DMHQ)] by electrochromatography. Mobile phase: 20 m*M* ammonium acetate/acetonitrile (ACN, 50–70% v/v); applied voltage 25 kV, 2.5–4.1 μA and assisted pressure (both ends of the capillary) 5 bar. For other experimental conditions see text.

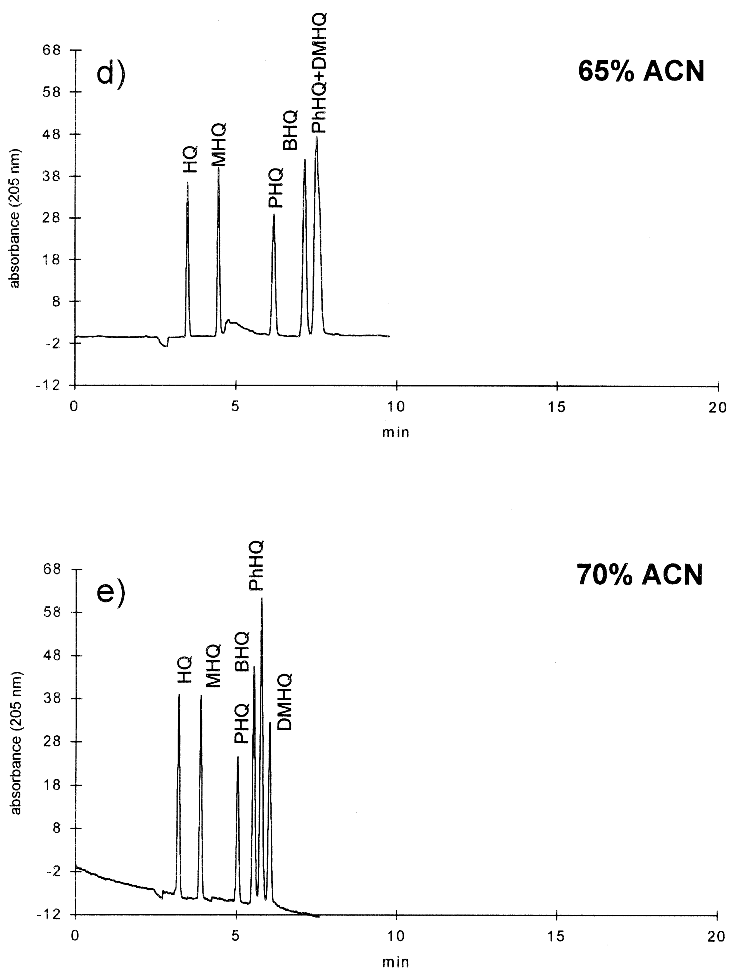

Fig. 1. (*continued*).

The negative peak of the solvent was used for the measurement of the EOF (t_o) because thiourea was slightly retained by the stationary phase; other authors observed similar behaviour [18]. The increase of the % of ACN in the mobile phase caused an increase of EOF from 1.23×10^{-4} to 1.60×10^{-4} cm^2 V^{-1} s^{-1} at 50% and 70% ACN, respectively which contributed to shorten the analyte migration times. Thus our results agree with previously published data [16,19].

Besides the effect above discussed the increase of ACN concentration also influenced the number of theoretical plates×meter (N) (see Fig. 3). This effect was studied for only HQ, MHQ and PHQ that exhibited a baseline resolution at all the ACN concentrations studied. N was calculated by using the following equation:

$$N = 5.54[t_R/w_{1/2}]^{1/2} \quad (1)$$

where t_R and $w_{1/2}$ are the retention time and the peak width at half height, respectively.

As can be seen in Fig. 3 the efficiency of analytes was differently influenced by the ACN concentration. Although a general decrement of N was observed by rising the concentration of the organic modifier, different trends of the curves were noticed. 65% of ACN was a critical value at which were

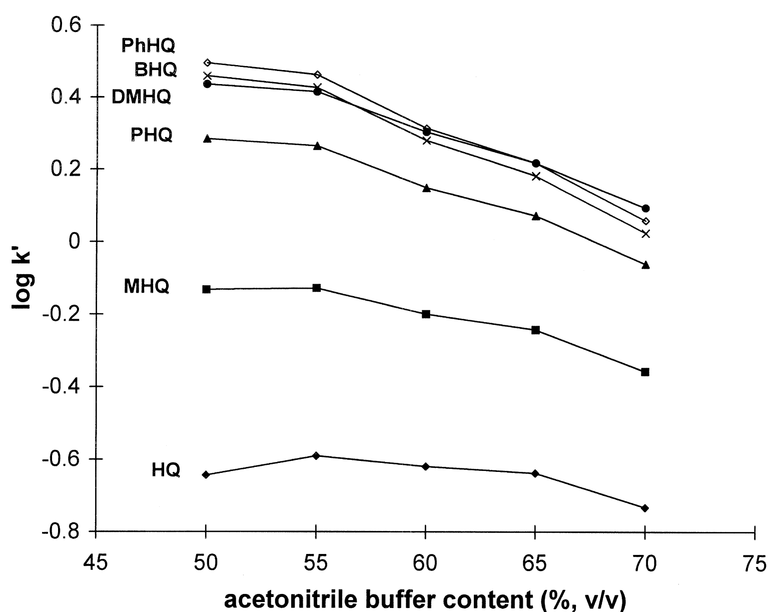

Fig. 2. Logarithmic retention factor (log k') of hydroquinone and some of its ethers versus percentage of acetonitrile present in the mobile phase (20 mM ammonium acetate pH 6 and 50–70% ACN). For other experimental conditions see Fig. 1.

recorded the highest N values for HQ and MHQ, while PHQ showed the lowest.

The measured efficiencies, not very high, were better than those usually obtained in HPLC, however an improvement of this parameter can be achieved by decreasing the diameter of the packed stationary phase particles.

From the above described results the mobile phase, containing 20 mM ammonium acetate at pH 6/ACN (30:70, v/v), was selected for the analysis of

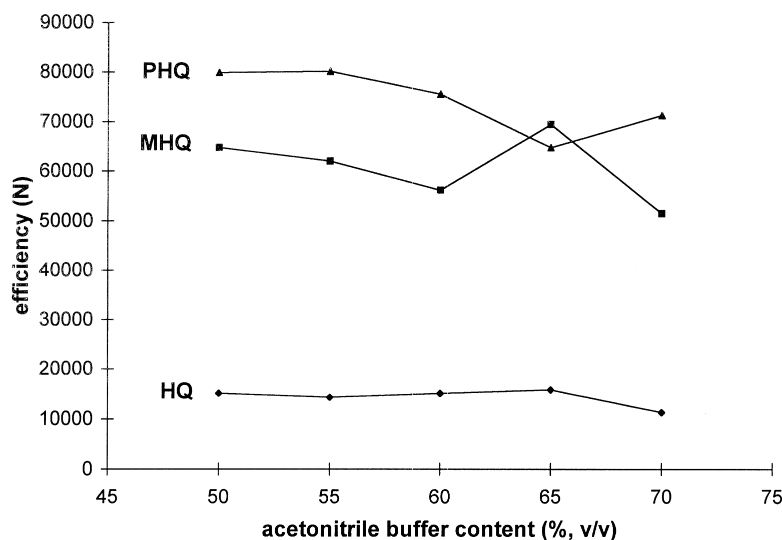

Fig. 3. Effect of % of acetonitrile present in the mobile phase on the number of theoretical plates (N) of studied compounds.

HQ and its ethers. This mobile phase allowed achieving baseline resolution of all studied analytes in the shortest analysis time (less than 6 min) with good efficiency. For quantitative analysis 2-phenyl-1,2-propanediol was added to the analyzed mixture as the internal standard showing an elution time between HQ and MHQ.

The standard mixture containing the I.S., HQ and its ethers was injected and analyzed 10 times in order to verify the repeatability of the CEC method.

The same mixture was analyzed in different days for inter-day repeatability. The results, for both intra-day and inter-day repeatability for retention time, peak areas and peak areas ratio are reported in Table 1. Since in most cases the best repeatability was achieved using the peak area ratio, we selected this parameter for quantitative analysis of skin toning cream.

The calibration graphs were linear in the studied concentration range (5–90 μg/ml) with correlation coefficients between 0.9975 and 0.9991 (see Table 1).

The detection limit (LOD) and the limit of quantification (LOQ) for all the studied compounds were 1.37 and 3.35 μg/ml, respectively.

Fig. 4 shows the electrochromatogram of the analysis of the extract of skin-toning cream where is clearly present HQ and two unidentified additional peaks (t_m = 3.586 and 3.825 min) with UV spectra different from any recorded for the standard analytes. The recovery of the method was tested by spiking the skin-toning cream with three different levels of HQ standard solution (corresponding to final concentration of 10, 40 or 80 μg/ml) and analyzing the mixture in duplicate. The recovery values were in the range 78–90%.

The CEC analysis of the extracted cream sample was performed in triplicate and the HQ content was 1.72±0.02% (w/w). Thus the concentration of HQ in the analyzed cosmetic was lower than permitted by legislation of the European Union and similar to that found in samples analyzed by HPLC and MEKC [1,7].

4. Conclusions

A CEC method was optimized for the separation of hydroquinone and some of its ethers using a packed ODS stationary phase. The packed capillaries, produced in laboratory, gave a stable performance for a relatively long time (for about 300 runs no remarkable changes in efficiency and repeatability were recognized). An aqueous buffer at pH 6 mixed with acetonitrile was used as mobile phase providing a relatively high EOF. The optimum experimental conditions for the complete separation of analyzed compounds in less than 6 min were found using 20 m*M* ammonium acetate pH 6/ACN (30:70, v/v).

The efficiency and the repeatability data for retention time, peak area, peak area ratio demonstrated the validity of the method for quantitative analysis of HQ and ethers in skin-toning cream sample. The content of HQ found was 1.72% (w/w) showing that the cosmetic product was prepared in accordance with European Union legislation. The

Table 1
Intra- and inter-day repeatability, linearity results for the determination of hydroquinone and some of its ethers

Compound	Intra-day repeatability data (n=10) STD%			Inter-day repeatability data (8 days) STD%			Linearity data[a]: concentration range 5–90 μg/ml		
	T_m	A	A/A_{IS}	T_m	A	A/A_{IS}	a	b	r^2
HQ	0.50	0.94	1.54	2.49	3.18	5.61	29.3483	−0.0517	0.9988
MHQ	0.42	1.12	1.13	2.35	3.69	3.25	29.5134	0.0200	0.9975
PHQ	0.20	1.65	1.33	3.03	5.28	3.91	24.7861	0.0019	0.9986
BHQ	0.22	1.62	1.40	3.32	6.89	4.89	44.0335	−0.0581	0.9986
PhHQ	0.23	1.88	1.79	3.34	8.24	5.65	49.2271	−0.0216	0.9986
DMHQ	0.25	1.84	2.17	3.20	7.56	5.71	31.3787	−0.0307	0.9991
IS(1-ph-1,2-propanediol)	0.33	1.13	–	2.58	4.27	–	–	–	–

[a] Data from regression curve calculation based on compound/IS peak area ratio.

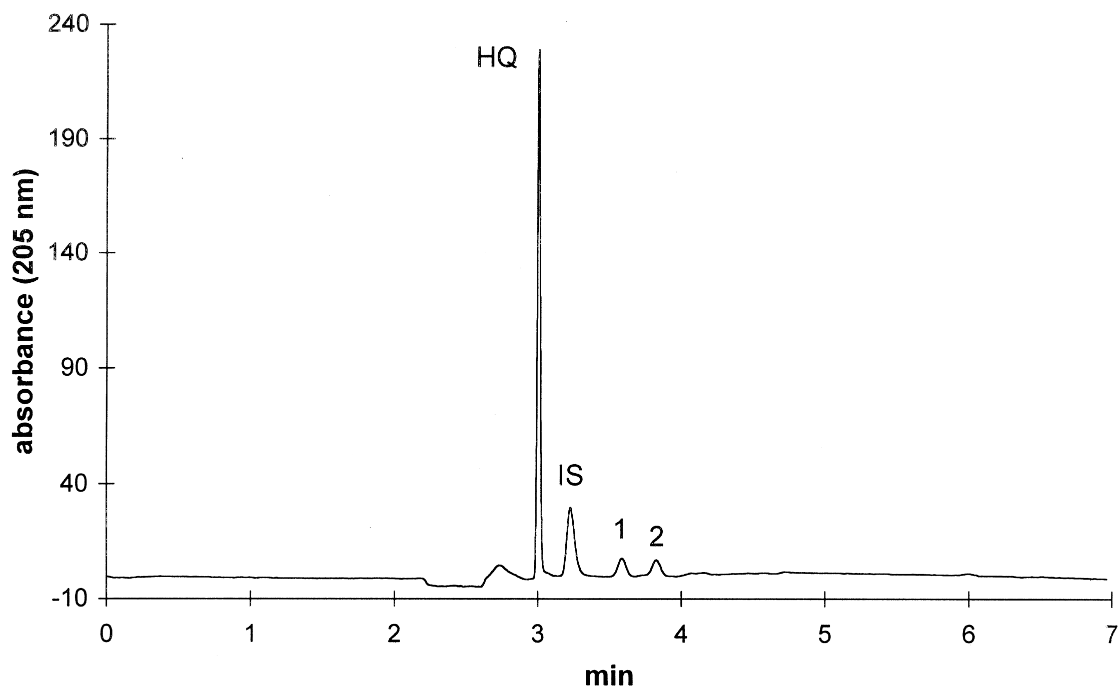

Fig. 4. Electrochromatogram of the analysis of a skin-toning cream extract. For experimental conditions see text and Fig. 1.

CEC analysis of the real sample also revealed the presence of two small impurities that we were not able to characterize. However, due the compatibility of the mobile phase with mass spectrometry (MS), the optimized method can be easily used for CEC-MS measurements in order to characterize the unknown peaks found in the cream sample.

Compared to HPLC, the proposed CEC method can offer some advantages, e.g., higher efficiencies and shorter analysis time (the separation of analytes was obtained in less than 6 min).

Acknowledgements

Thanks are due to Mr Gabriele Caponecchi for his technical assistance.

References

[1] L. Gagliardi, A. Amato, G. Cavazzutti, F. Chimenti, A. Bolasco, D. Tonelli, J. Chromatogr. 404 (1987) 267.
[2] M. Motonok, Jpn. Kokai Tokkyo Koho JP, 1 199 916, (1989); C.A., 112 (1990) P223137g.
[3] A. Teglia, Cosmet. Toiletries, Ed. Ital. 10 (1989) 10.
[4] F. Buhl, E. Dul-Zarycta, M. Chwistek, Chem. Anal. (Warsaw) 33 (1988) 819.
[5] P. Davidkova, J. Kopecek, J. Gasparic, J. Inf. Rec. Mater. 17 (1989) 117.
[6] W.G. Burkert, C.N. Owensby, W.L. Hinze, J. Liq. Chromatogr. 4 (1981) 1065.
[7] I.K. Sakodinskaya, C. Desiderio, A. Nardi, S. Fanali, J. Chromatogr. 596 (1992) 95.
[8] M.M. Robson, M.G. Cikalo, P. Myers, M.R. Euerby, K.D. Bartle, J. Microcol. Sep. 9 (1997) 357.
[9] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[10] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[11] A.S. Rathore, Cs. Horváth, J. Chromatogr. A 781 (1997) 185.
[12] C. Yan, D. Schaufelberger, F. Erni, J. Chromatogr. A 670 (1994) 15.
[13] N.W. Smith, M.B. Evans, Chromatographia 38 (1994) 649.
[14] M. Zhang, Z. El Rassi, Electrophoresis 19 (1998) 2068.
[15] P. Sandra, A. Dermaux, V. Ferraz, M.M. Dittmann, G. Rozing, J. Microcol. 9 (1997) 409.
[16] P.D.A. Angus, E. Victorino, K.M. Payne, C.W. Demarest, T. Catalano, J.F. Stobaugh, Electrophoresis 19 (1998) 2073.
[17] M. Meyering, D. Strickmann, B. Chankvetadze, G. Blaschke, C. Desiderio, S. Fanali, J. Chromatogr. B 723 (1999) 255.
[18] C. Yang, Z. El Rassi, Electrophoresis 19 (1998) 2061.
[19] M.M. Dittmann, G.P. Rozing, J. Microcol. Sep. 9 (1997)