Journal of Chromatography, 596 (1992) 95–100
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 933

Micellar electrokinetic chromatographic study of hydroquinone and some of its ethers

Determination of hydroquinone in skin-toning cream

Inna K. Sakodinskaya*, Claudia Desiderio, Annalisa Nardi and Salvatore Fanali*

Istituto di Cromatografia del CNR, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo (Rome) (Italy)

(First received October 9th, 1991; revised manuscript received December 5th, 1991)

ABSTRACT

The separation of hydroquinone and some of its ether derivatives was studied by micellar electrokinetic chromatography with sodium dodecyl sulphate as an anionic surfactant in the background electrolyte. The optimized method was used for the determination of hydroquinone in a sample of skin-toning cream. On-column detection at 254 nm with caffeine as an internal standard gave good quantitative results.

INTRODUCTION

Hydroquinone (HQ) is used as an antioxidant, as a photographic reducer and developer, etc. HQ and some of its ethers are also used in cosmetic creams as depigmenters, the mechanism of action being the inhibition of melanin formation [1–4]. HQ is toxic at concentrations higher than 2%, causing dermatitis [1,3]. Hence a knowledge of its content in consumer products (*e.g.*, in cosmetics) is important in order to avoid health problems. Several methods have been used for the determination of hydroquinone, *e.g.*, high-performance liquid chromatography (HPLC), spectrophotometry and thin-layer chromatography (TLC) etc. [3–8].

Micellar electrokinetic capillary chromatography (MECC) [9,10] is an electrophoretic technique in which the separation is performed with the same apparatus as used for capillary zone electrophoresis (CZE). Both charged and neutral compounds have been analysed succesfully by using MECC [11–16]. Usually negative surfactants such as sodium dodecyl sulphate or sodium decyl sulphate added to the background electrolyte (BGE) will improve the selectivity of the separation. The separation mechanism is based on the partitioning of the solute between micelles, moving in the opposite direction to the electroosmotic flow, and the bulk aqueous phase.

In this work we used MECC for the determination of hydroquinone in a sample of skin-toning cream. In order to optimize the method we studied the effects of the pH of the BGE and the amount of sodium dodecyl sulphate (SDS) on the migration time (t_m) and the capacity factor (k') of hydroquinone and some of its ether derivatives.

The determination of HQ in a real sample was performed by using the internal standard method by measuring the peak-area ratio.

EXPERIMENTAL

Apparatus

Electrophoretic experiments were performed in a laboratory-made apparatus consisting of a modi-

* Present address: Chemical Department, Moscow State University, GSP-119899 Moscow, USSR.

0021-9673/92/$05.00

fied Varian UV 2550 detector and a Glassman LH60R power supply able to deliver either constant voltage or constant current. A fused-silica capillary tube (50 cm × 75 μm I.D., 38 cm to the detector) (SGE, Victoria, Australia) was used for the separations. The modified detector cell and the construction of the capillary connection block, the electrolyte vessel and the capillary holder have been described previously [17]. Detection was carried out at 254 nm. Electropherograms were recorded with an LKB (Bromma, Sweden) Model 2210 recorder. A Chromatopac C-R5A integrator (Shimadzu, Kyoto, Japan) was used for quantification. Sampling was done by the hydrodynamic method (6 s at a height of 10 cm). The constant-voltage mode was chosen for electrophoretic analysis.

Chemicals

The compounds investigated were hydroquinone (HQ), hydroquinone monomethyl ether (MHQ), hydroquinone dimethyl ether (DMHQQ), hydroquinone monopropyl ether (PHQ), hydroquinone monobenzyl ether (BHQ) and hydroquinone monophenyl ether (PhHQ) (all from Fluka, Buchs, Switzerland). Caffeine, used as an internal standard (I.S.), and anthracene, used to mark the maximum retention time, were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulphate (SDS) was obtained from Sigma (St. Louis, MO, USA). Methanol (Carlo Erba, Milan, Italy) was of HPLC grade. All other reagents were of analytical-reagent grade and were used as received. The water used was doubly distilled. The BGE containing methanol was prepared from stock solutions of borate buffer of adjusted pH in order to obtain a borate concentration of 0.01 *M* after the addition of methanol. The appropriate amount of SDS was added to the BGE.

Epocler skin-toning cream (Laboratory Whitehall, New York, USA), declared to contain HQ, was used as a real sample for quantitative analysis.

Standard solutions

The standard solutions used for electrophoretic experiments were prepared by dissolving the standard compounds in water–methanol (3:1, v/v). HQ standard solutions ($5 \cdot 10^{-5}$–$8 \cdot 10^{-4}$ *M*), containing a fixed concentration of caffeine (I.S.), were prepared in water–methanol (3:1) and used for calibration.

Assay of depigmenters in skin-toning cream

The extraction procedure was similar to that described by Gagliardi *et al.* [3]. A carefully weighed amount of cream (*ca.* 0.1 g) was heated in 40 ml of an aqueous solution containing 4 ml of methanol and 4 ml of caffeine ($4 \cdot 10^{-3}$ *M*) as I.S. at 50°C until it was completely dissolved. After cooling, the extract was filtered through a Millex-GS filter (Millipore) and injected for electrophoretic analysis.

RESULTS AND DISCUSSION

We first tried to separate a mixture of HQ and some of its ethers by using CZE in BGE at different pH values. CZE is a powerful analytical technique with a high resolving power and high efficiency, which is mainly used for the separation of ionic compounds even when the separation of uncharged compounds has been shown [18,19].

As expected, our attempts failed because the analyte compounds moved with similar effective mobilities under the operating conditions used. In order to modify selectively the electrophoretic mobility, it is necessary to change the composition of the BGE, *e.g.*, by adding cyclodextrins for isomer separations [20] or metal cations for peptides [21]. Therefore, considering the properties of the analyte compounds, we used SDS, added to the BGE above the critical micellar concentration (CMC) [12], as a selective agent.

Optimization of separation

Fig. 1 shows the dependence of the capacity factor (k') of HQ and its ethers on the concentration of SDS in 0.01 *M* borate buffer (pH 9.5). The k' values were calculated in accord to Terabe *et al.* [9]. In the absence of SDS all the compounds migrate with approximately the same velocity near to that of electroosmotic flow (methanol). By increasing the amount of SDS, hydrophobic compounds, such as phenyl and benzyl ether, moved with a longer migration time whereas there was no noticeable effect on methanol and only a very weak effect on HQ, which can be considered to be relatively hydrophilic compounds. This is in accord with the pK_a values of 10.35 for HQ, 10.24 for MHQ, 10.9 for BHQ, 10.7 for PrHQ and 9.9 for PhHQ [22,23], which should be neutral compounds at pH 9.5. Hence the separation achieved at high SDS concentrations is due

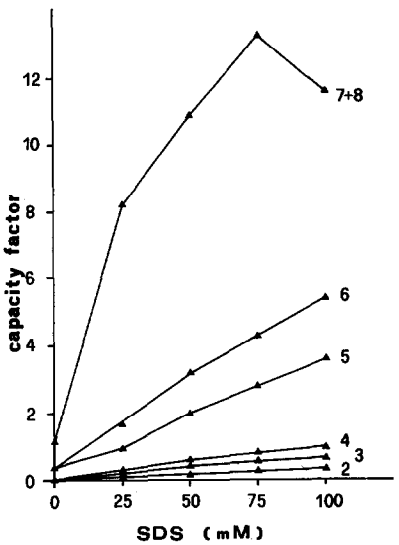

Fig. 1. Effect of SDS added to the background electrolyte [0.01 *M* borate buffer (pH 9.5)] on the capacity factor (k'). 1 = Methanol 2 = hydroquinone; 3 = caffeine (I.S.); 4 = hydroquinone monomethyl ether; 5 = hydroquinone dimethyl ether; 6 = hydroquinone monopropyl ether; 7 = hydroquinone monobenzyl ether; 8 = hydroquinone monophenyl ether. Electrophoresis, 10 kV, 20μA; sampling, hydrodynamic, 6 s per 10 cm standard mixture about 10^{-4} *M* each; volume injected, *ca.* 10 nl.

to the difference in the hydrophobicities of the analytes. The compounds investigated migrate in the same order as in reversed-phase HPLC separation [3], which is also due to the difference in their hydrophobicities.

The main criterion for the choice of the conditions was the ability to determine HQ concentration and therefore the separation of HQ from methanol and its negative peak should be carefully followed as some methanol is inevitably present in the extracts from the cream sample [3]. Experiments performed at higher voltage decreased the migration time of all the analyte compounds, owing to a higher electroosmotic flow [18]. The HQ peak was very close to the negative peak of methanol, making quantification difficult.

Several electrolyte systems containing 75 m*M* SDS at different pH values ranging from 7.5 to 11.5 were used in order to study the effect of pH on the selectivity of the separation. Increasing pH should convert HQ and its ethers into a charged form and provide separations due to the differences in the charge-to-mass ratio and differences in hydrophobicity. However, the studied compounds are unstable in basic media owing to rapid oxidation [1]. We therefore decided to select a relatively low pH value in order to ensure stability of the analytes. Fig. 2 shows the pH dependence of the capacity factors of HQ and its ethers. The influence of pH in the range 7.5–10.5 on the capacity factor of HQ seems weak, corresponding to its neutrality and hydrophilicity, whereas those compounds which are more hydrophobic are sufficiently retarded with increase in pH owing to the absorption in the negatively charged micelles of SDS. In fact, at pH 10.5 for HQ we obtained more than one peak, probably owing to decomposition of sample. Therefore, despite the availability of an additional separation mechanism at higher pH values, it is preferable to use less basic media.

All the ethers and HQ show sufficient differences in migration times at SDS concentrations above 0.05 *M* (Fig. 1). However, BHQ and PhHQ mixtures form one peak at pH 7.5–9.5 and 0.075 *M* SDS. The two compounds were partially resolved when the electrolyte at pH 10.5 containing 0.075 *M* SDS was used. The addition of an organic co-solvent has been shown to improve the selectivity of separation in MECC [24]. We therefore tried to use

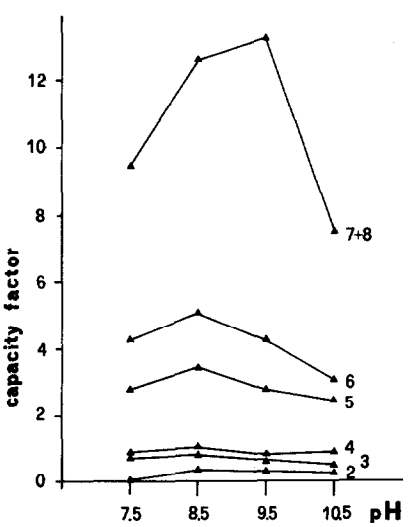

Fig. 2. Effect of carrier pH on the capacity factor of HQ and some of its ether derivatives. SDS concentration = 0.075 *M*; for experimental conditions and compounds, see Fig. 1.

a micellar buffer solution (MBS) containing different amounts of methanol ranging from 10 to 40% (v/v). The migration time of all the compounds increased with increasing amount of methanol in the MBS (results not shown); this is mainly due to the decrease in the electroosmotic flow.

The optimum buffer solution for the separation of the studied compounds was that containing 10% of methanol, which combines complete resolution with a shorter analysis time. In order to study the effect of the amount of SDS on the migration time of HQ and its ether derivatives, we performed several electrophoretic separations in borate buffer (pH 9.5) containing 10% of methanol and different amount of SDS. Table I shows the effect of SDS on the migration times when 10% methanol–borate buffer was used. There is a general increase in t_m and k' with increasing amount of SDS (except for methanol), and this effect is more evident for BHQ and PhHQ (the most hydrophobic compounds). In this electrolyte system (methanol mixture), SDS was found to be a better selective agent for the analyte compounds than in aqueous micellar solution. This may be due to the improved repartitioning of the solute between SDS and the buffer. The complete separation of BHQ and PhHQ was achieved.

Fig. 3 shows a typical electropherogram for the separation of HQ and its ether derivatives when a micellar buffer solution without and with methanol was used. Experiments performed in a micellar buffer solution containing 0.01 *M* borate buffer (pH 9.5), 10% methanol and 75 m*M* SDS in order to verify the reproducibility of the method for the analysis of HQ failed; the reproducibility of the method was poor compared with that obtained in buffer without methanol.

As in preliminary experiments carried out by analysing a sample of skin-toning cream we did not find BHQ and PhHQ, we selected a micellar buffer solution containing 0.01 *M* borate buffer (pH 9.5) and 0.075 *M* SDS for the analysis of HQ in a real sample.

Ten electrophoretic runs, performed by injecting standard solutions containing $4 \cdot 10^{-4}$ *M* caffeine (I.S.) and different amounts of HQ, were carried out and the calibration graph was found to be linear from $5 \cdot 10^{-5}$ to $8 \cdot 10^{-4}$ *M* with a correlation coefficient of 0.9997. The relative standard deviation, calculated by measuring the HQ/I.S. peak-area ratio by analysing the same sample ($2 \cdot 10^{-4}$ *M* HQ and $4 \cdot 10^{-4}$ *M* caffeine) six times, was found to be 1.8%.

TABLE I

EFFECT OF SDS CONCENTRATION IN THE BUFFER ELECTROLYTE [0.01 *M* BORATE BUFFER (pH 9.5) CONTAINING 10% (v/v) METHANOL ON THE MIGRATION TIMES (RETENTION TIMES) AND CAPACITY FACTORS OF THE STUDIED COMPOUNDS

Experimental conditions as in Fig. 1.

Compound[a]	[SDS] (m*M*)							
	25		50		75		100	
	t (min)	k'	t (min)	k'	t (min)	k'	t (min)	k'
1	4.1	0	4.5	0	4.6	0	4.6	0
2	4.5	0.2	5.0	0.2	5.0	0.2	5.5	0.3
3	4.5	0.2	5.0	0.2	5.5	0.3	6.0	0.4
4	4.7	0.2	5.8	0.5	6.2	0.6	7.0	0.8
5	5.5	0.6	7.6	1.3	9.0	2.0	10.4	3.3
6	6.0	1.0	8.8	2.3	10.4	3.2	12.2	4.5
7	8.4	2.8	12.4	8.9	14.7	15.7	17.1	24.0
8	8.7	3.2	12.7	10.2	15.0	18.4	15.5	30.0
AN	13.4	∞	15.4	∞	17.1	∞	19.3	∞
t_o/t_{mc}	0.306		0.325		0.269		0.238	

[a] Compounds 1–8 as in Fig. 1; AN = anthracene.

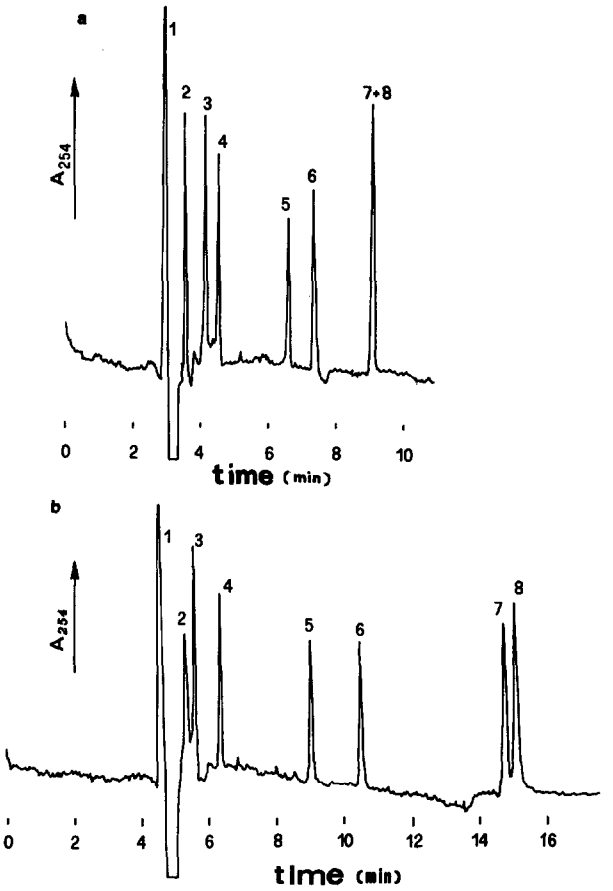

Fig. 3. Electropherogram of the separation of standard mixtures of HQ and its ether derivatives. 0.01 *M* borate buffer (pH 9.5) + 0.075 *M* SDS, (a) without methanol and (b) with 10% (v/v) methanol. Volume injected, *ca.* 10 nl.

Analysis of cream sample

Qualitative analysis of the extract of the skin-toning cream showed not only the peak corresponding to HQ but also two small peaks with migration times of 5.36 and 6.65 min corresponding to none of the studied ethers. We failed to identify the last peak, but the first has the same t_m as the positional isomer of HQ *o*-dihydroxybenzene (catechol), and when the mixture was spiked with catechol the peak with t_m = 5.36 min increased in area. Quantitative analysis of HQ showed that its content was $1.80 \pm 0.05\%$ (w/w), *i.e.*, not more than is permitted by legislation of the European Community, and almost the same as in the cream analysed by HPLC previously [3]. The presence of catechol may be due

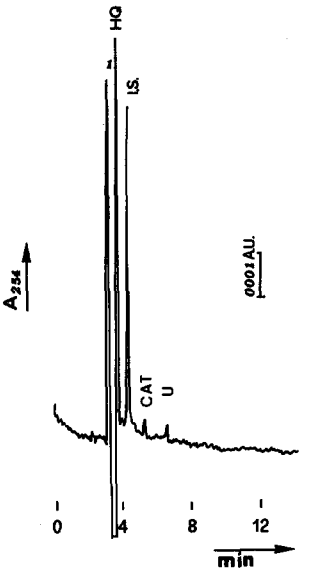

Fig. 4. Analysis of skin-toning cream. Experimental conditions as in Fig. 1. (1) methanol; (HQ) hydroquinone; (I.S.) caffeine; (CAT) catechol; (U) unknown.

to the impurities in the HQ added; this compound is known to have similar toxic effects on skin as phenol and HQ [1], but it is not claimed to be a depigmenter.

CONCLUSIONS

The separation of HQ and its ethers can be performed by using MECC. The use of an organic modifier (methanol) improved the resolution; with this electrolyte system we obtained poor reproducibility. The optimized analytical method was applied to the determination of HQ in cosmetic samples. The results showed its content to be similar to that declared by the producer. The studied method allowed the analysis to be performed in a relatively short time (less than 10 min) with good results. Further studies will be made in order to optimize the quantitative analysis with electrolyte systems containing organic modifiers.

ACKNOWLEDGEMENTS

Thanks are due to Mr. M Cristalli and G. Caponecchi for technical assistance.

REFERENCES

1 S. Budavari (Editor), *The Merck Index*, Merck, Rahway, NJ, 11th ed., 1989, pp. 762–763.
2 E. A. Swinyard, in L. S. Goodman and A. G. Gilman (Editors), *The Pharmacological Basis of Therapeutics*, Macmillan, New York, 5th ed., 1975, p. 955.
3 L. Gagliardi, A. Amato, G. Cavazzutti, F. Chimenti, A. Bolasco and D. Tonelli, *J. Chromatogr.*, 404 (1987) 267.
4 M. Motonok, *Jpn. Kokai Tokkyo Koho JP*, 01 199 916 [89 199 916], 1989; *C.A.*, 112 (1990) P223137g.
5 A. Teglia, *Cosmet. Toiletries, Ed. Ital.*, 10, No. 4 (1989) 10.
6 F. Buhl, E. Dul-Zarycta and M. Chwistek, *Chem. Anal. (Warsaw)*, 33 (1988) 819; *C.A.*, 112 (1990) 24542h.
7 P. Davidkova, J. Kopecek and J. Gasparic, *J. Inf. Rec. Mater.*, 17 (1989) 117; *C.A.* 112 (1990); 90956z.
8 W. G. Burkert, C. N. Owensby and W. L. Hinze, *J. Liq. Chromatogr.*, 4 (1981) 1065.
9 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
10 D. E. Burton, M. J. Sepaniak and M. P. Maskarimec, *J. Chromatogr. Sci.*, 24 (1986) 347.
11 R. A. Wallingford and A. G. Ewing, *J. Chromatogr.*, 441 (1988) 299.
12 H. Nishi and S. Terabe, *Electrophoresis*, 11 (1990) 691.
13 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 2773.
14 H. T. Rasmussen, L. K. Goebel and H. M. McNair, *J. High Resolut. Chromatogr.*, 14 (1991) 25.
15 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
16 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.
17 S. Fanali, L. Ossicini, F. Foret and P. Bocek, *J. Microcol. Sep.*, 1 (1989) 190.
18 F. Foret and P. Bocek, in A. Chrambach, M. J. Dunn and B. J. Radola (Editors), *Advances in Electrophoresis*, Vol. 3, VCH, Weinheim, 1989, p. 271.
19 A. Nardi, S. Fanali and F. Foret, *Electrophoresis*, 11 (1990) 774.
20 S. Fanali and P. Bocek, *Electrophoresis*, 11 (1990) 757.
21 R. A. Mosher, *Electrophoresis*, 11 (1990) 765.
22 R. C. Weast, S. M. Selby and C. D. Hodgman (Editors), *Handbook of Chemistry and Physics*, CRC Press, Cleveland, OH, 46th ed., 1965–66, p. D-78.
23 N. B. Chapman and J. Shorter (Editors), *Correlation Analysis in Chemistry*, Plenum Press, New York, 1978.
24 J. Corse, A. T. Balchunas, D. F. Swaile and M. J. Sepaniak, *J. High Resolut. Chromatogr. Chromatogr. Column*, 11 (1988) 554.