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Journal of Chromatography A, 903 (2000) 271–278

JOURNAL OF CHROMATOGRAPHY A

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Short communication

Separation and determination of haloperidol, parabens and some of their degradation products by micellar electrokinetic chromatography

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Received 6 August 1999; received in revised form 21 October 1999; accepted 16 August 2000

Abstract

A micellar solution containing phosphate buffer, anionic surfactant, and water-miscible organic solvent was employed as a migration solution for the separation and the quantification of eleven analytes by micellar electrokinetic chromatography (MEKC): the analytes examined were haloperidol, methylparaben, ethylparaben, *n*-propylparaben, *iso*-propylparaben, *n*-butylparaben, *iso*-butylparaben, *sec*-butylparaben, 4-(4-chlorophenyl)-4-hydroxypiperidine, 4-fluorobenzoic acid and 4-hydroxybenzoic acid. In order to provide good separation between micelle and haloperidol, which showed strongest interaction with the micelle among the analytes, surfactant concentrations and organic modifier percentages were studied with phosphate buffer at pH 7.0. All the analytes were successfully resolved when 10 m*M* sodium dodecylsulfate and 15% ethanol were contained in the migration solution; the time window was very wide in the range from 14.8 to 65.5 min. Optimized applied voltage at 30 kV and capillary temperature at 45°C enable analyze all compounds in less than 17 min with the best resolution, the shorter migration time window, the highest precision and lowest detection limit. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Micellar electrokinetic chromatography; Haloperidol; Parabens; Benzoic acids

1. Introduction

Haloperidol belongs to the butyrophenone group of drugs and is widely used as a major tranquilizer in psychiatric fields [1]. It is usually commercialized in the presence of parabens in oral and injection solutions. It has been reported that the haloperidol is unstable when exposed to elevated temperature and light [2,3]. Several analytical techniques were reported to quantify the haloperidol and haloperidol with methylparaben and propylparaben in drugs, by nuclear magnetic resonance spectrometry [4], liquid chromatographic methods [5–7] and derivative spectrophotometry [8]; or with its metabolites by gas chromatography [9–11], liquid chromatography [12–14], combined capillary electrophoresis–mass spectrometry [15] and free solution capillary electrophoresis [16]. However no one has dealt with other kinds of parabens in the presence of degradation products.

Since the development of micellar electrokinetic chromatography (MEKC) technique [17], it has been proved that the separation method can provide high

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PII: S0021-9673(00)00869-4

resolution efficiency for a wide diversity of chemical structures [18]. In the present work, we develop a MEKC method in order to resolve and quantify haloperidol, methyparaben, ethylparaben, *n*-propylparaben, *iso*-propylparaben, *n*-butylparaben, *iso*-butylparaben, *sec*-butylparaben, 4-(4-chlorophenyl)-4-hydroxypiperidine, 4-fluorobenzoic acid as hydrolytic products of haloperidol and 4-hydroxybenzoic acid as plausible hydrolytic product of parabens. Chemical structures of the objective analytes are shown in Fig. 1.

A systematic study of the parameters that influence selectivity, mobility and interaction with anionic surfactant micelles is presented. The proposed method was optimized for several experimental variables, and the optimal conditions are: a migration solution containing 10 m*M* sodium dodecylsulfate, phosphate buffer (pH 7.0), and 15% ethanol as organic modifier and apparatus conditions of 30 kV applied voltage, 45°C capillary temperature, and 200 nm detection wavelength. The optimized method is preferably applied to the analysis of haloperidol, parabens and their impurities in commercial oral and injection solutions, which present photosensitivity especially to daylight and temperature. In the present work we propose an application to quantify haloperidol in commercial tablet that we dispose.

2. Experimental

2.1. Chemicals and reagents

Haloperidol, sodium dodecyl sulfate (SDS), potassium dihydrogenphosphate and disodium hydrogenphosphate were purchased from Wako (Osaka, Japan). Parabens, benzoic acids, 4-(4-chlorophenyl)-4-hydroxypiperidine and Sudan III were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Deionized and distilled water was used for the preparation of the solutions. Individual stock solutions of haloperidol and Sudan III at concentrations of 5×10^{-4} *M* were prepared in ethanol. All other analytes were prepared to give 10^{-3} *M* in SDS (10^{-2} *M*) solution. The pH of the buffer was adjusted by mixing a potassium dihydrogenphosphate solution (10^{-1} *M*) with a disodium hydrogenphosphate solution (10^{-1} *M*). The working mixture standard was prepared by diluting the stock solutions with water; it contains 5×10^{-5} *M* of each analyte, 12 m*M* SDS, 10 m*M* phosphate buffer, 5×10^{-6} *M* Sudan III and 3% ethanol.

2.2. Apparatus

Electophoretic separations were made by an Applied Biosystems 270A (Foster City, USA) capillary electrophoresis system, equipped with a UV detector. The dimensions of capillary (GL Sciences, Tokyo) attached to the system were 72 cm in total length, 50 cm effective length from the sample injection point to the detector, and 50 μm I.D. All the electropherograms were recorded by a Hitachi D-2000 Chromato-Integrator.

2.3. Electrophoretic measurement

The capillary is regenerated daily with sodium hydroxide solution (10^{-1} *M*) for 3 min, followed by washing with purified water for 3 min. To ensure the reproducibility, the capillary was prewashed for 2 min with the running buffer before each sample injection. Injection of the sample solutions was carried out at the anodic end of the capillary for 3 s by a vacuum system (injection volume: about 9 nl). The analytes were detected at 200 nm at the cathodic end.

2.4. Standard procedure

The separation and the quantification of different analytes were performed with a migrating solution containing 10 m*M* SDS, 10 m*M* phosphate buffer at pH 7.0 and 15% ethanol. Optimum electrophoretic conditions were found at 30 kV applied voltage, 45°C capillary temperature, 200 nm detection wavelength and 3 s injection period.

2.5. Mobility calculation

The electrophoretic mobility of an analyte, μ_n, was calculated in a usual manner from the observed migration time with the equation:

$$\mu_n = \frac{L_d L_t}{V}\left(\frac{1}{t_R} - \frac{1}{t_{eo}}\right)$$

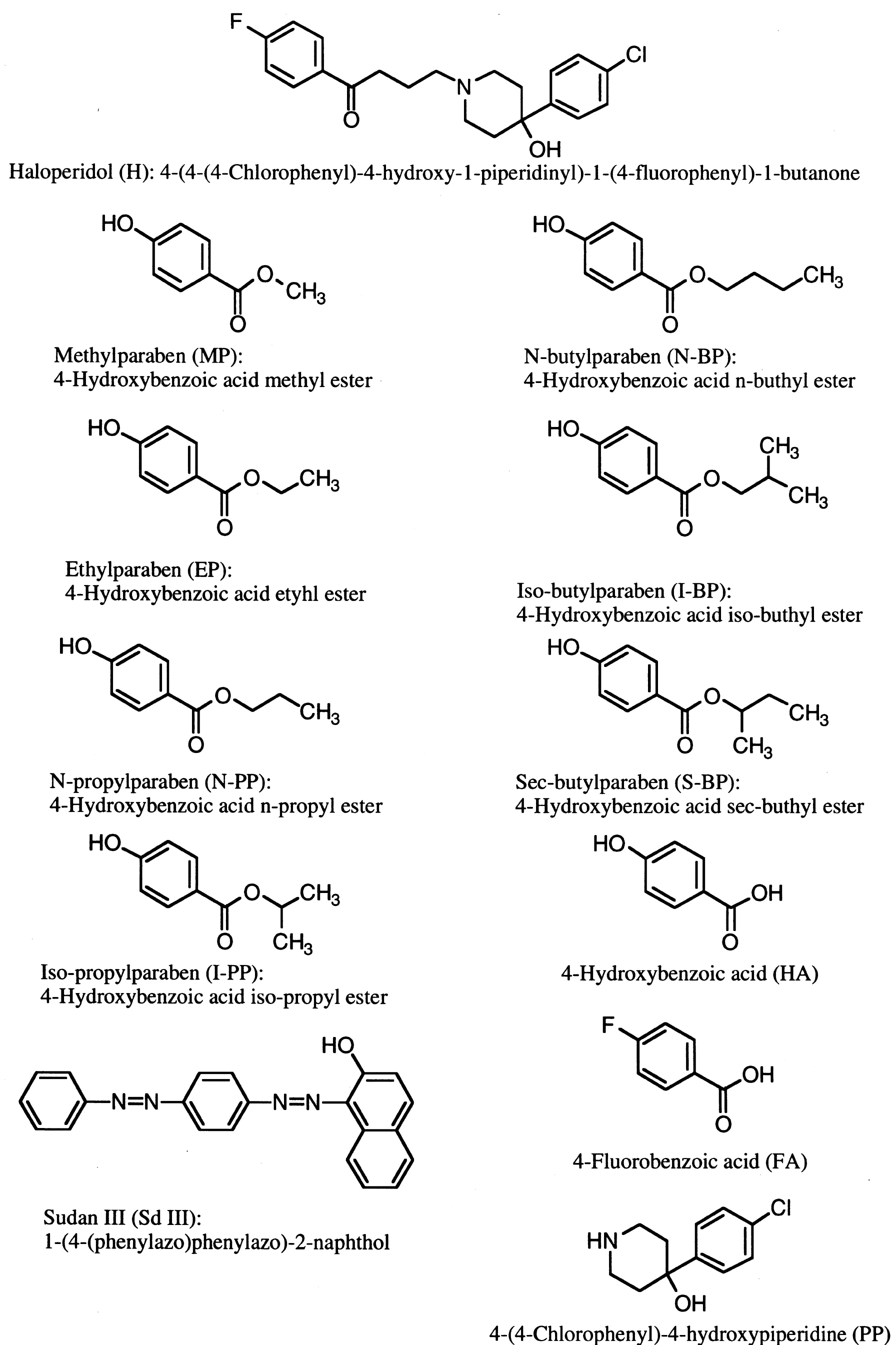

Fig. 1. Representative structures and abbreviations of the analytes.

where t_R is the migration time of a certain analyte, t_{eo} is the migration time of the solute which does not interact with micelle (ethanol as neutral marker), L_t is the total length of capillary, L_d is the length of capillary between an injection end (anode) and a detector, and V is the applied voltage.

3. Results and discussion

3.1. Migration behavior of haloperidol, parabens, benzoic acids and piperidine by capillary zone electrophoresis

In consideration of a suitable buffer for capillary zone electrophoresis (CZE) separation of the analytes, their mobilities in aqueous buffer systems are dependent upon their apparent charge. The structural diversity of the analytes can be classified into three groups according to their ionizable functional groups: parabens (phenols), benzoic acids (carboxylic acids), and haloperidol and piperidine (amines). In the present study, we firstly investigated CZE separation of the analytes with phosphate buffer (pH 7.0). It was observed that the parabens co-migrated and were eluted with ethanol.

In the case of benzoic acids, basicity of the buffer used leads to their ionization and results in anionic character. They are present as anions in the migrating solution and migrate slower than the EOF, with different mobility depending on the degree of the ionization and molecular mass: migration of hydroxybenzoic acid was faster than that of fluorobenzoic acid. Haloperidol and piperidine ($pK_a>7.5$) possess basic functional group (amine), they are protonated at pH 7.0 and have positive charge. They migrate to the cathodic direction electrophoretically and are faster than the EOF. From the CZE experiments, the addition of a surfactant to the migrating solution was required to enhance the solubility of the non-polar compounds and to improve the selectivity for parabens by utilizing the micelle solubilization.

3.2. Resolution of haloperidol, parabens, benzoic acids and piperidine by MEKC

Important alternatives for the optimization of MEKC parameters are the nature of the surfactant and its concentration. The most widely used micelle is anionic sodium dodecylsulfate. Sudan III, which can distribute largely into the micelle and migrates together, was added to detect the migration time of the micelles. It was observed that haloperidol (H) was greatly retained into the micelle and migrated with Sudan III when 12 mM of SDS mixed with 10 mM phosphate buffer was used at 10 kV and 35°C. Mobility of the analytes in the presence of SDS is shown in Fig. 2. Mobility of benzoic acids changed with increasing concentration of SDS. It indicates

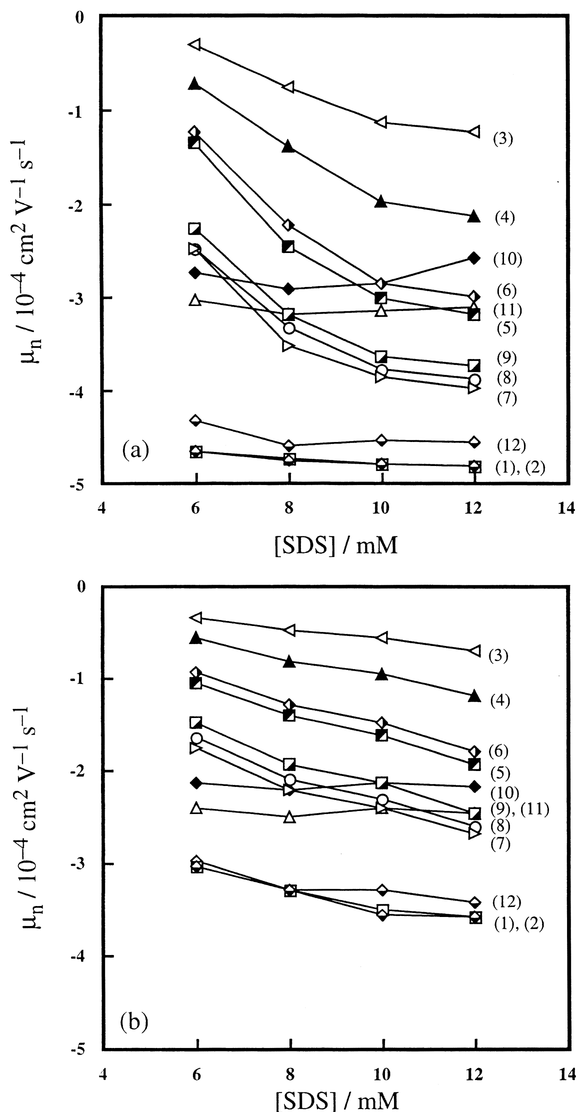

Fig. 2. Effect of SDS concentration on the mobility of analytes. Migrating solution: (a) SDS mixed with 10 mM phosphate buffer (pH 7.0); (b) SDS mixed with 10 mM phosphate buffer (pH 7.0) and 10% ethanol. Sample solution: 5×10^{-5} M of each analytes, 12 mM SDS, 10 mM phosphate buffer (pH 7.0), 5×10^{-6} M Sudan III, and 3% ethanol. CE conditions: applied voltage, 10 kV; temperature, 35°C; detection wavelength, 200 nm; injection period, 3 s. (1), Sd III; (2), H; (3), MP; (4), EP; (5), N-PP; (6), I-PP; (7), N-BP; (8), I-BP; (9), S-BP; (10), HA; (11), FA; (12), PP.

that the migration mechanism is changing from CZE to MEKC. The capacity factor of the parabens is related to their solubility to the micelle; the absolute values of the mobility are larger in such a reagent as the alkyl group is greater, and they increase with increasing concentration of SDS. Binding of the analytes should be dominated by their hydrophobic character. Migration of piperidine and haloperidol required longer migration time and strong interaction of haloperidol with SDS micelle was suspected. It would be attributed to the hydrophobic interaction between the analytes and micelle as well as the formation of ion pair.

As a first approach to resolve haloperidol from Sudan III, the effect of SDS concentrations was tested by varying their concentrations from 6 to 12 m*M*. The decrease in SDS concentrations does not affect the micelle–haloperidol interaction as is shown in Fig. 2(a). However the addition of ethanol in the micellar solution resulted in reducing the partition of haloperidol between the hydrophobic micellar phase and the aqueous phase.

Other anionic surfactants with different alkyl chain length instead of SDS was also investigated. Sodium octyl sulfate (SOS) at 50 m*M* in the presence of 10 m*M* phosphate buffer (pH 7.0) was examined. The similar migration behavior of the analytes as in the CZE method was observed. This result can be explained by the fact that the micelle is not formed in the migrating solution, and that interactions between the analytes and the surfactant is negligible. A solution of 2.5 m*M* of sodium tetradecylsulfate (STS; CMC=2.5 m*M* at 40°C in water [19]) mixed with 10 m*M* phosphate buffer (pH 7.0) was prepared. It was observed that this amount of STS is not soluble in the used buffer at the ambient temperature.

3.3. Improvement of the resolution by the addition of ethanol

The effect of SDS concentration was re-examined when the amount of 10% ethanol was added to the micellar solution. As can be seen in the Fig. 2(b), the haloperidol-micelle selectivity was greatly improved in the presence of 10 m*M* SDS. Ethanol percentage in the migrating solution was investigated by varying ethanol percentage from 5 to 15% in 5% steps at fixed SDS concentration and phosphate buffer concentration at 10 m*M*. The best selectivity and resolution for all analytes were achieved by using 15% ethanol at 35°C as capillary temperature with 4 μA as recorded current as shown in Fig. 3(a). The presence of the organic solvent miscible with water caused a reduction in EOF and subsequently an extension in the elution window.

3.4. Optimization of electrophoretic conditions

A further optimization by varying the running voltage in term of analysis speed was carried out. As expected the migration times decreased dramatically as the applied voltage increased from 10 to 30 kV. Up to 30 kV, the selectivity between the analytes were almost unaffected as the voltage was increased, though some comigration was observed between the n-butylparaben and the hydroxybenzoic acid. The electropherogram is shown in Fig. 3(b).

The effect of temperature from 30 to 45°C in 5°C steps was examined. As is known, higher temperatures cause small buffer viscosity, reduce the solutes–SDS hydrophobic interaction and lower the selectivity as a result. Lesser interactions between SDS and the different analytes, which can be induced by increase in temperature, can results a different extent of interaction; therefore different electrophoretic mobility and better resolution can be achieved. An electropherogram was obtained under the optimised conditions as shown in Fig. 4; a good separation of all the analytes was achieved in less than 17 min with a high sensitivity.

3.5. Calibration data, precision and limit of detection by the proposed method

To evaluate the stability of the separation of the proposed method, studies on the variations of peak area, height and mobility were investigated for all analytes. As summarised in Table 1, RSD values less than 2% were obtained by injecting the mixture of eleven standards at different concentrations. A good linear correlation was also obtained for the eleven compounds, with correlation coefficient higher than 0.995. Detection limits of all the analytes were determined at a signal-to-noise ratio of three; they were found to be in the ranges from 2×10^{-6} to 4×10^{-6} *M*.

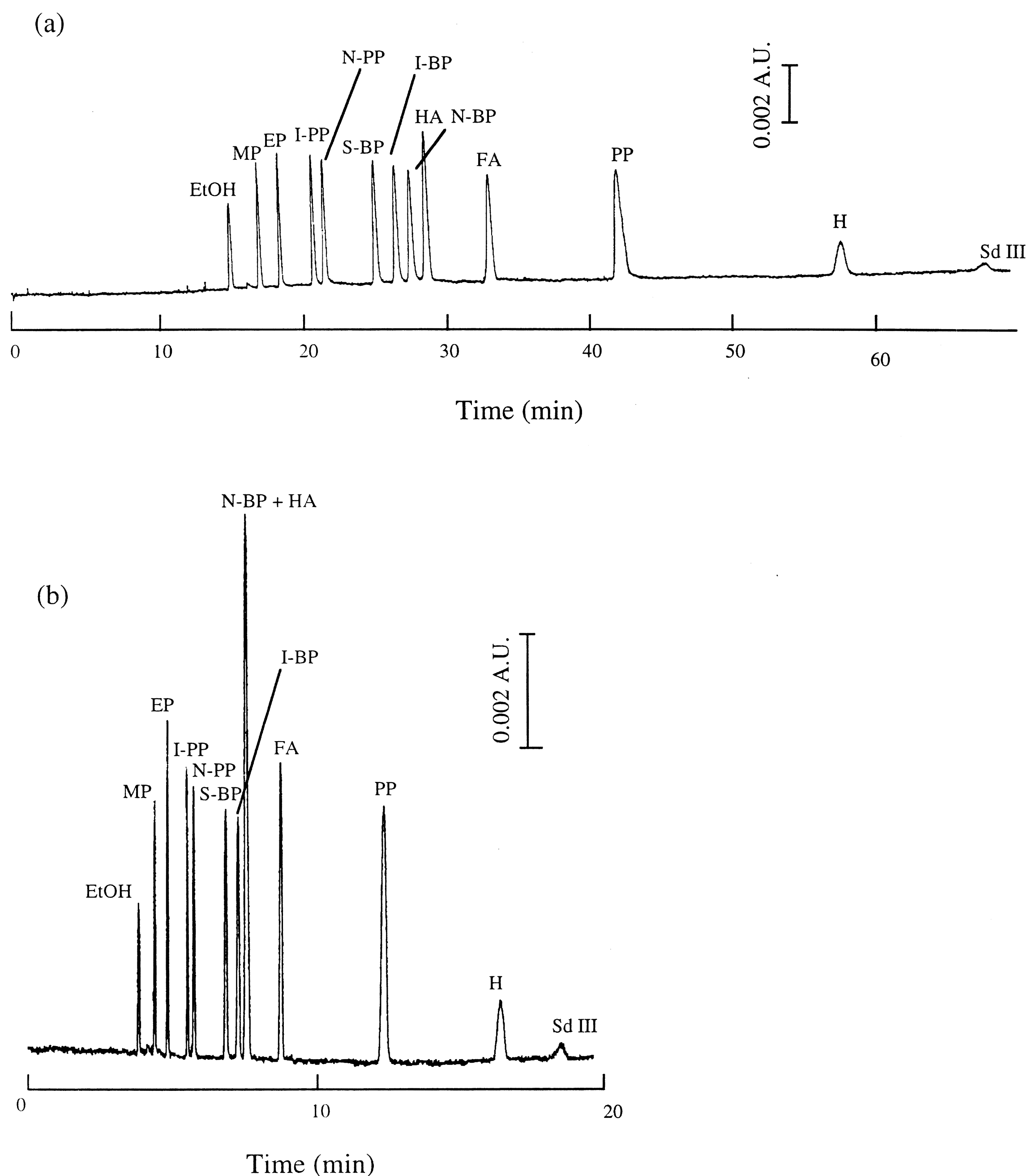

Fig. 3. Electropherograms for eleven analytes in the presence of ethanol in the migrating solution. Applied voltage: (a), 10 kV; (b), 30 kV. Current: (a), 4 μA; (b), 14 μA. Migrating solution: 10 m*M* SDS + 10 m*M* phosphate buffer (pH 7.0) + 15% ethanol. Sample solution: 5×10^{-5} *M* of each analyte, 12 m*M* SDS, 10 m*M* phosphate buffer (pH 7.0), 5×10^{-6} *M* Sudan III, and 3% ethanol. CE conditions: capillary temperature, 35°C; detection wavelength, 200 nm; injection period, 3 s.

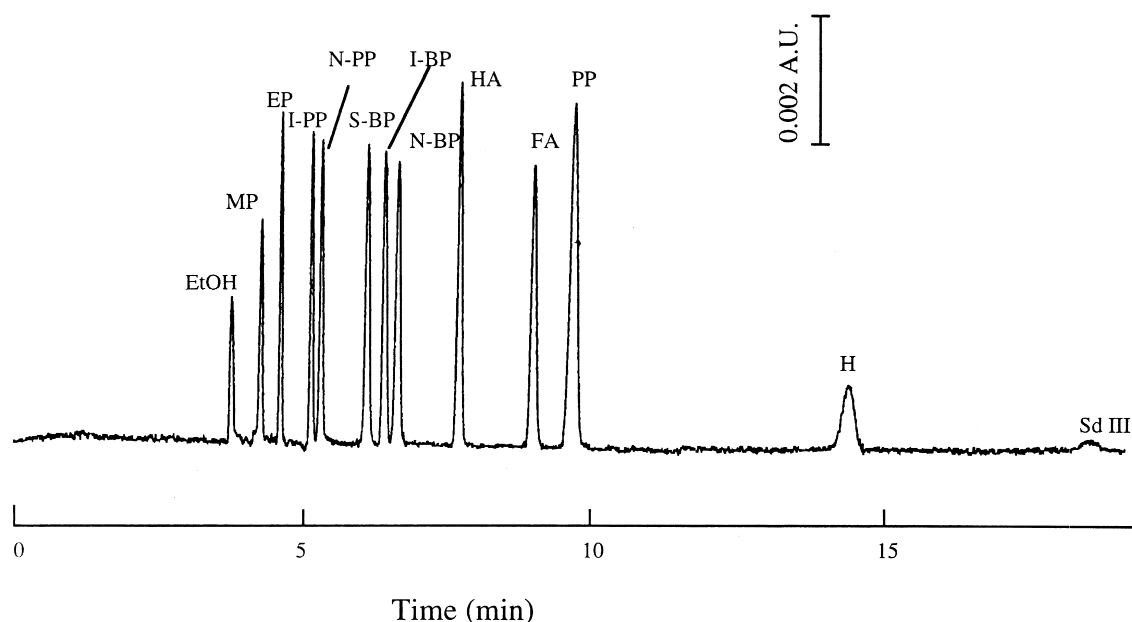

Fig. 4. Electropherogram of eleven analytes obtained under the optimized conditions. Migrating solution: 10 m*M* SDS + 10 m*M* phosphate buffer (pH 7.0) + 15% ethanol. Sample solution: 5×10^{-5} *M* of each analyte, 12 m*M* SDS, 10 m*M* phosphate buffer (pH 7.0), 5×10^{-6} *M* Sudan III, and 3% ethanol. CE conditions: applied voltage, 30 kV; current, 16 μA; capillary temperature, 45°C; detection wavelength, 200 nm; injection period, 3 s.

3.6. Application to a practical sample

The objective of the developed method was not only to obtain good resolution and short time analysis but also to provide the detection and the quantification of active ingredient, excipients and contaminants at very low level.

The analysis of a practical sample of a commercial

Table 1
Precision, calibration data and limits of detection by the proposed method

Analyte	Relative Standard Deviation (%)[a]			Correlation of calibration graph[b]	Detection limit[c] (μ*M*)
	Area	Height	Mobility		
H	0.586	0.435	0.214	0.999	3
MP	0.924	0.780	0.091	0.995	4
EP	1.866	1.185	0.098	0.995	4
N-PP	1.535	0.912	0.101	0.997	4
I-PP	1.818	0.690	0.285	0.999	4
N-BP	1.605	0.631	0.236	0.996	4
I-BP	1.841	0.894	0.214	0.999	4
S-BP	1.697	0.979	0.398	0.998	4
HA	1.682	1.000	0.209	0.995	3
FA	1.574	0.435	0.167	0.997	3
PP	1.123	1.068	0.173	0.999	2

[a] Values analyzed with 10 measurements.
[b] Concentration range: 1×10^{-5}–5×10^{-5} M.
[c] Values at $S/N=3$.

Table 2
Comparison of determination results of haloperidol by MEKC and UV

Method	MEKC ($n=3$)	UV[a] ($n=3$)
Percent claim found±SD	98.91±1.29	100.89±2.20

[a] USP XXII Method (Ref. [21]).

tablet, containing 0.75 mg of haloperidol in one tablet was performed by spectrophotometry [20] and proposed MEKC method. Results are summarized in Table 2, the Standard Deviation (SD) obtained by using the US Pharmacopeia method is greater than the S.D of the MEKC method, while the results of the dosage of haloperidol obtained by both methods are within the specification required [21]. Furthermore, no interferences from the excipient, as well as no degradation products, were observed in the electropherogram.

In order to more fully valid the MEKC method, the precision was evaluated by ten determinations in the same day (Table 3). The RSD are less than 0.5% for the area, height and mobility. The linearity was determined by varying the sample concentrations from 0.6×10^{-5} to 1.4×10^{-5} *M* in step of 0.2×10^{-5} *M*. Linear regression and correlation showed that the method is linear and gave correlation coefficient of 0.998 (Table 3).

4. Conclusion

A simultaneous determination of haloperidol, and its degradation products, 4-(4-chlorophenyl)-4-hydroxypiperidine and 4-fluorobenzoic acid in the presence of seven different parabens and their hydrolytic product: 4-hydroxybenzoic acid, has been developed. This method provides excellent reproducibility, good linear correlation and appropriate sensitivity. It can be indicated to be useful for routine simultaneous analysis of haloperidol and parabens and research of hydrolytic products in pharmaceutical application.

Table 3
Precision and linearity study of the proposed method applied to the determination of haloperidol in tablet

Relative Standard Deviation (%)[a]			Correlation coefficient[b]
Area	Height	Mobility	
0.366	0.448	0.192	0.998

[a] Values analyzed with 10 measurements.
[b] Concentration range: 0.6×10^{-5}–1.4×10^{-5} *M*.

References

[1] R.J. Baldessarini, in: A.G. Goodman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), The Pharmacological Basis of Therapeutics, Pergamon Press, New York, 1991, p. 383.
[2] A. Pannaffio, D.S. Greene, Drug Devel. Ind. Pharm. 9 (1983) 485.
[3] C.A. Janicki, C.Y. Ko, Analytical Profiles of Drug Substances, Academic Press, New York, 1980.
[4] J.W. Turczan, C.A. Lau-cam, Drug Devel. Ind. Pharm. 15 (1989) 107.
[5] H. Szumilo, M. Przyborowska, G. Misztal, L. Przyborowski, Chem. Anal. 33 (1988) 957.
[6] J.E. Kountourellis, C.K. Markapoulou, J. Liquid Chromatogr. 14 (1991) 2969.
[7] H. Trabelsi, F. Raouafi, A. Saddam, K.A. Bouzouita, J. Pharm. Belg. 52 (1997) 145.
[8] S. Ouanas, M. Kallel, H. Trabelsi, F. Safta, K.A. Bouzouita, J. Pharm. Biomed. Anal. 17 (1998) 361.
[9] R.F. Tyndale, T. Iniba, J. Chromatogr. 529 (1990) 182.
[10] Y. Nagai, H. Arimoto, T. Fujii, J. High Resolut. Chromatogr. 13 (1990) 614.
[11] J. Fang, G.B. Baker, R.T. Coutts, J. Chromatogr. B 682 (1996) 283.
[12] K. Igrashi, N. Castognal Jr., J. Chromatogr. 579 (1992) 277.
[13] T. Ohkubo, R. Shimoyama, K. Sugawara, J. Pharm. Sci. 81 (1992) 947.
[14] J. Fang, J.W. Gorrod, J. Chromatogr. 614 (1993) 267.
[15] A.J. Tomlinson, S. Nayalor, L.M. Benson, K.L. Johnson, J. Chromatogr. 621 (1993) 239.
[16] A.J. Tomlinson, L.M. Benson, J.P. Landers, G.F. Scanlan, J. Chromatogr. A 652 (1993) 417.
[17] S. Terabe, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[18] J.P. Landers, in: Handbook of Capillary Electrophoresis, CRC Press, 1994, pp. 43–93, Chapter 3.
[19] M.J. Rosen, in: Surfactants and Interfacial Phenomena, 2nd ed, Wiley-Interscience Publication, 1989, p. 122, Chapter 3.
[20] The United States Pharmacopeia (USP) XXIII, US Pharmacopeial convention: Rockville, MD, 1995, 731.
[21] The United States Pharmacopeia (USP) XXII, US Pharmacopeia convention: Rockville, MD, 1990, 629.