Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 452–455
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Separation and determination of ketoprofen, methylparaben and propylparaben in pharmaceutical preparation by micellar electrokinetic chromatography

Jiří Šafra*, Marie Pospíšilová

Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Department of Analytical Chemistry, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic

Received 3 October 2007; received in revised form 2 January 2008; accepted 17 January 2008
Available online 29 January 2008

Abstract

Simple micellar electrokinetic chromatographic (MEKC) method was developed for the determination of ketoprofen as the active substance and methylparaben and propylparaben as preservatives in a semisolid pharmaceutical preparation. Separation was carried out with a fused silica capillary and UV detection at 200 nm. Optimized background electrolyte was 50 mM tricine buffer containing 30 mM sodium dodecyl sulfate as surfactant and 15% (v/v) of methanol. Single separation took about 13 min. No statistically significant differences were found when comparing the results with those of RP-HPLC method reported in literature.

Keywords: MEKC; Ketoprofen; Methylparaben; Propylparaben

1. Introduction

Ketoprofen (KP) is a propionic acid derivative, namely 2-(3-benzoylphenyl)propanoic acid and it has shown anti-inflammatory, analgesic and antipyretic activity [1]. Methylparaben (MP) and propylparaben (PP) are effective antibacterial and anti-fungal agents that are commonly used as preservatives in food, beverages, cosmetics and pharmaceuticals [2]. MP and PP are used together since they have a synergistic effect [3]. Recently KP was determined in tablets by flow injection analysis [4], in gel by HPLC [5–8], in suppositories by micellar liquid chromatography [9], in blood plasma by RP-HPLC [10] and in wastewater by LC–MS/MS [11]. KP and six other anti-inflammatory drugs were also determined in pharmaceutical preparations by CZE with UV detection (borate buffer of pH 8.15 containing 15% of methanol was employed) [12]. The comparison of capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) has been done for the determination of different pharmaceuticals containing NSAIDs. The CZE method utilized phosphate buffer of pH 8.0 and in the MEKC method borate buffer of pH 9.0 containing 40 mM SDS was used. Both CZE and MEKC method were found to be suitable for the assay of anti-inflammatory drugs [13]. Only two HPLC methods for simultaneous determination of KP in the presence of MP and PP have been published so far [6,8]. To our best knowledge, KP, MP and PP have not yet been determined simultaneously by MEKC.

The aim of this work was the development of capillary MEKC method with UV detection for the assay of KP, MP and PP in a multi-component gel.

2. Experimental

2.1. Electrophoretic system

All experiments were performed by using a computer-controlled P/ACE MDQ electrophoretic analyzer equipped with a photodiode array detection system (Beckman Instruments, Fullerton, CA, USA). Electrophoretic separations were performed in fused silica capillaries with total length of 60 cm, effective length 50 cm and I.D. 75 μm, maintained at 20 °C. The capillary was conditioned with 1 M NaOH for

* Corresponding author. Tel.: +420 495 067 482; fax: +420 495 518 718.
E-mail address: jiri.safra@faf.cuni.cz (J. Šafra).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.01.030

Table 1
Analytical and validation parameters of the MEKC method

	Methylparaben	Ketoprofen	Propylparaben
SST-parameters			
Number of theoretical plates	48971	8772	49064
Peak asymmetry	0.90	0.62	0.86
Resolution	MP – KP: 4.84	KP – DF: 2.28	DF – PP: 9.89
Repeatability–migration time[a] (intra-day R.S.D., %)	0.73	0.52	0.72
Repeatability–migration time[a] (inter-day R.S.D., %)	1.11	0.82	1.33
Repeatability–peak area[a] (intra-day R.S.D., %)	0.76	0.77	2.15
Repeatability–peak area[a] (inter-day R.S.D., %)	0.98	0.87	2.82
Validation data			
Linearity–slope; S.D. of the slope	0.2712 ± 0.0022	0.3087 ± 0.0140	0.3299 ± 0.0202
Linearity–intercept; S.D. of the intercept	0.0032 ± 0.0053	3.2690 ± 1.7285	0.0115 ± 0.0251
Linearity–correlation coefficient	0.9999	0.9969	0.9944
Linearity–residual S.D.	0.0064	2.0824	0.0302
Accuracy (% recovery)	103.86	96.56	102.35
Selectivity	No interference	No interference	No interference
LOD ($\mu g\,ml^{-1}$)	0.38	0.82	0.53
LOQ ($\mu g\,ml^{-1}$)	1.28	1.78	2.74

[a] $n = 6$.

10 min, 0.1 M NaOH for 10 min and with water for 10 min daily before the first sample injection and it was washed with 0.1 M NaOH for 2 min and water for 2 min between the runs. The samples were injected hydrodynamically at a pressure of 50 mbar for 6 s. Detection was carried out at 200 nm and all measurements were performed at a constant voltage of 30 kV.

2.2. Reagents

MP, PP, KP, tricine, sodium dodecyl sulfate (SDS), sodium diclofenac (DF) and methanol were obtained from Sigma–Aldrich (Milwaukee, WI, USA). A Millipore Milli-Q RG ultra-pure water was used for the preparation of the solutions. Ketoprofen gel 2.5%, Herbacos–Bofarma Ltd., Bochemie Group (Pardubice, Czech Republic) was analyzed.

A 50 mM tricine buffer containing 30 mM SDS and 15% (v/v) of methanol was used as running buffer. The pH* was adjusted to 8.3 by 0.1 M NaOH.

The model mixture for the development of the CZE method and for the optimization experiments contained 625 $\mu g\,ml^{-1}$ of KP, 25 $\mu g\,ml^{-1}$ of MP, 12.5 $\mu g\,ml^{-1}$ of PP and 625 $\mu g\,ml^{-1}$ of DF as internal standard (I.S.). It was prepared by dissolving the compounds in 15% (v/v) methanol.

2.3. Sample preparation

An accurately weighed portion (≈2 g) of the pharmaceutical gel was treated with 25 ml of 30% (v/v) methanol; the mixture was stirred intensively for 5 min and thereafter it was sonicated for 15 min at 40 °C. The resultant mixture was centrifuged at 3000 rpm for 15 min. A 5 ml aliquot of the supernatant was diluted with water to 10 ml and injected for analysis.

Identification of peaks in the gel samples was based on comparison of migration times of compounds in standard solutions. Peak identity was confirmed by UV spectra.

3. Results and discussion

MEKC as a potent modified electrophoretic method allowing excellent separations of neutral, hydrophobic or water insoluble species was used with respect to the acid base properties of KP, MP and PP with pK_a 4.23, 8.30 and 8.23, respectively [14]. The use of CZE in too high pH aqueous background electrolytes was not suitable for this purpose because of the fact that the paraben esters undergo hydrolysis in alkaline pH∼9.8 [15].

3.1. Method optimization

Several electrolyte systems were examined: borate buffer, phosphate buffer, borate–phosphate buffer, TRIS adjusted by different sulfonic acids (MOPSO, HEPES, MES, BES, ACES and TAPS), tricine, bicine and glycylglycine buffers. All mentioned buffers contained 40 mM SDS as the surfactant. The pH* was adjusted to 8.5.

Unsatisfactory separation of analytes, long migration times, peak asymmetry, improper baseline drifting and high current

Table 2
Results of MEKC determination of MP, PP and KP in ketoprofen gel 2.5% ($n = 6$)

Analyte	Declared (g/100 g)	Found (g/100 g) ± R.S.D.% MEKC ($n = 6$)	Found (g/100 g) ± R.S.D.% HPLC ($n = 6$)	Student's t-test[a] ($n = 6$)
Methylparaben	0.05	0.049 ± 1.70	0.050 ± 1.92	0.1235
Ketoprofen	2.5	2.49 ± 0.52	2.45 ± 1.90	0.4126
Propylparaben	0.025	0.027 ± 1.87	0.026 ± 1.92	0.3900

[a] 95% confidence level, $t_c = 2.571$, ($\nu = 2n - 2$).

(above 100 μA) were observed when inorganic buffers were used. For this reason, a biological buffer with lower co-ion effective mobility was examined. However, the separation of MP and KP was incomplete when TRIS-based buffers were tested. Essential improvement of separation has been achieved when buffer based on tricine was employed. Several additional modifications of tricine buffer (sodium cholate instead of SDS, acetonitrile instead of methanol) brought no improvement. DF migrating between KP and PP was chosen as an optimum internal standard.

The effect of pH* 7.7–8.9 was examined in buffers containing 50 mM tricine and 30 mM SDS. At all pH* values (except of pH* ~ 8.9) all compounds were fully separated. Best results were achieved at pH* 8.3.

The SDS concentrations tested ranged between 10 and 60 mM and the buffer of pH* 8.3 contained 50 mM tricine and 20% (v/v) of methanol. The optimal concentration of SDS was found to be 30 mM. At higher concentrations of SDS (50 and 60 mM), the total time of analysis was unsatisfactory. At ≤20 mM SDS the quality of separation and peak shape deteriorated.

The effect of 20–60 mM tricine was examined. The best results in terms of the time of analysis, resolution of analytes and the current generated were obtained with 50 mM tricine.

The optimum content of methanol was found to be 15% (v/v). With lower content of methanol shorter migration times were achieved but the resolution of all analytes deteriorated. If the separation was performed with content of methanol above 15% (v/v) no improvement of the resolution of analytes was attained. Methanol added to BGE to achieve the separation of the solutes slowed down the electro-osmotic flow and thus all the migration times were lengthened.

The final optimum conditions for the separation were: 50 mM tricine buffer (pH* 8.3) containing 30 mM SDS and 15% (v/v) of methanol. The separation was carried out at 30 kV, 20 ± 0.1 °C. The UV detection wavelength was set at 200 nm with regard the highest sensitivity of the method for all analytes. The effective mobility values u_{eff} (10.84 for MP, 14.02 for KP, 15.24 for DF and 18.00 10^{-9} m^2 V^{-1} s^{-1} for PP) in the optimum BGE of pH* 8.3 were calculated by using the equation [16].

3.2. Validation of the method

Validation parameters were evaluated according to [17,18]. The method validation covers the estimation of validation parameters such as accuracy, precision, selectivity, linearity, LOD and LOQ. The R.S.D. values were calculated for repeated ($n = 6$) standard solution sampling to check the method repeatability as well as for repeated injections gel extract to check the method precision. To determine the intermediate precision, the same experiments were performed during six consecutive days. Linearity was confirmed in the concentration range 100–2000 μg ml^{-1} of KP, 2–40 μg ml^{-1} of MP, and 1–20 μg ml^{-1} of PP at five different analyte concentration levels; the method of internal standard (DF) was used. The LOD and LOQ values were calculated as a measure of the method sensitivity by the signal-to-noise ratio routine.

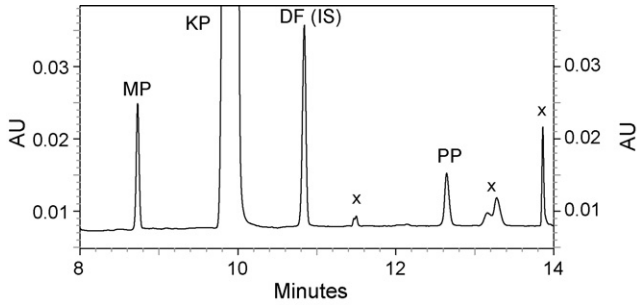

Fig. 1. Electropherogram of ketoprofen gel 2.5%; MP—methylparaben, KP—ketoprofen, DF—diclofenac, PP—propylparaben, x—compounds of essential oil; BGE: 50 mM tricine, 30 mM SDS, 15% (v/v) of methanol (adjusted to pH* 8.3 with NaOH).

The accuracy of the method was statistically checked by comparison of the results with those of the standard official HPLC technique [6] with use of the Student's *t*-test. No significant differences were found between the results obtained by MEKC and official HPLC method for the same batch of ketoprofen gel 2.5% at 95% confidence level.

Since appropriate placebo was unavailable the recovery was checked by the standard addition technique, i.e., by analyzing six real samples spiked with a known amount of analyte and six replicates of original non-spiked samples. The results are summarized in Table 1 indicating good values for this method.

3.3. Determination of KP, MP and PP in the ketoprofen gel 2.5% HBF formulation

The method developed was used for the determination of the three analytes in ketoprofen gel 2.5% HBF. The results were in good agreement with the nominal label content. Other constituents of the gel (such as aromatic oil) did not interfere in the determination of the analytes and no degradation products of preservatives were found. The results of the analysis are given in Table 2. The electropherogram is shown in Fig. 1.

4. Conclusion

The proposed MEKC method permits the quality control of pharmaceutical preparations containing KP, MP and PP as active substances in a single run. The total analysis time is <13 min. The method was validated and the results obtained were precise and accurate. The developed method was successfully applied to the quantitative analysis of active substances in ketoprofen gel 2.5% and the results obtained were statistically compared with those of published HPLC method [6] by the Student's *t*-test. The *t*-values indicated the absence of systematic errors at 95% confidence level. The proposed assay shows lower sensitivity compared to the HPLC method (but it is still fully sufficient for the analysis of real preparations), on the other hand MEKC analysis was less reagent consuming and hence more economic and more ecological than HPLC method [6]. The results suggest that MEKC coupled with a simple extraction procedure is suitable for a simultaneous determination of KP, MM and PP in pharmaceutical preparation.

Acknowledgements

This work was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic, No. NR/8964-3, by the MSM Project 0021620822 of the Czech Ministry of Education and by the Grant Agency of Charles University 107707/B/2007/FaF.

References

[1] D. Lincová, H. Farghali, Fundamental and Applied Farmacology, Galén, Prague, 2002.
[2] H. Kang, H. Kim, J. Pharm. Biomed. Anal. 15 (1997) 1359–1364.
[3] M.D. Kreuz, A.L. Howard, D. Ip, J. Pharm. Biomed. Anal. 19 (1999) 725–735.
[4] H.Y. Aboul-Enein, A.G. Dal, M. Tuncel, Farmaco 58 (2003) 419–422.
[5] D.K. Bempong, L. Bhattacharyya, J. Chromatogr. A 1073 (2005) 341–346.
[6] J. Dvořák, R. Hájková, L. Matysová, L. Nováková, M. Koupparis, P. Solich, J. Pharm. Biomed. Anal. 36 (2004) 625–629.
[7] A. Panusa, G. Multari, G. Incarnato, L. Gagliardi, J. Pharm. Biomed. Anal. 43 (2007) 1221–1227.
[8] L. Nováková, L. Matysová, D. Solichová, M.A. Koupparis, P. Solich, J. Chromatogr. B 813 (2004) 191–197.
[9] C. Martinez-Algaba, L. Escuder-Gilabert, S. Sagrado, R.M. Villanueva-Camanas, M.J. Medina-Hernandez, J. Pharm. Biomed. Anal. 36 (2004) 393–399.
[10] M.J. Martin, F. Pablos, A.G. Gonzalez, Talanta 49 (1999) 453–459.
[11] M. Gros, M. Petrovic, D. Barcelo, Talanta 70 (2006) 678–690.
[12] Y. Chen, S. Wu, Anal. Bioanal. Chem. 381 (2005) 907–912.
[13] M.G. Donato, W. Baeyens, W. Van Den Bossche, P. Sandra, J. Pharm. Biomed. Anal. 12 (1994) 21–26.
[14] SciFinder Scholar (Ed.), Chemical Abstracts Service, American Chemical Society, Ohio, USA, 2004.
[15] R. Shija, V.B. Sunderland, C. McDonald, Int. J. Pharm. 80 (1992) 203–211.
[16] F. Foret, L. Křivánková, P. Boček, Capillary Zone Electrophoresis, VCH, Weinheim, 1993.
[17] International Conference on Harmonisation, Validation of Analytical Procedures: Text and Methodology, ICH Q2(R1). Fed. Reg. 60, 1995, 11260–11262.
[18] Information Bulletin of State Institute of Drug Control 11, Skarnitzl Foundation, Prague, 1995.