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Short communication

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

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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

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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 (μg ml^{-1})	0.38	0.82	0.53
LOQ (μg 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 μg ml^{-1} of KP, 25 μg ml^{-1} of MP, 12.5 μg ml^{-1} of PP and 625 μg 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 ($\approx$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, $t_c = 2.571$, ($\nu = 2n - 2$).