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Short communication

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

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Abstract

In this study, micellar electrokinetic chromatography (MEKC) method was developed for the determination of clotrimazole (CLO), methylparaben (MP) and propylparaben (PP) in a pharmaceutical preparation. Separation was carried out in a fused silica capillary (60 cm × 75 μm i.d.) at 25 kV with UV detection at 212 nm. Optimized background electrolyte (BGE) was 15 mM phosphate buffer (pH 7.2) containing 30 mM sodium dodecyl sulfate (SDS) as a surfactant. Rectilinear calibration ranges were 50–500 mg l^{-1} for CLO, 10–100 mg l^{-1} for MP and 2.5–25 mg l^{-1} for PP. The total analysis time was <12 min.

Keywords: MEKC; Clotrimazole; Methylparaben; Propylparaben

1. Introduction

Clotrimazole 1-[(2-chlorophenyl) diphenylmethyl]-1H-imidazole (CLO) is a broad-spectrum antifungal agent that like other antimycotic imidazoles, interferes in the lipid synthesis of fungi and thus causes an alteration of the permeability of the cell walls. The spectrum of its efficacy includes all human pathogenic non-invasive fungi: dermatophytes (species of microsporum, trichophyton, and epidermophyton) and yeasts (Candida group and *Malassezia furfur*). CLO is also active against gram-positive bacteria and trichomonas. So far, there have been no indications for the development of a resistance [1].

Methylparaben (MP) and propylparaben (PP) are effective antibacterial and antifungal agents, which are commonly used as preservatives in pharmaceuticals. MP is effective especially against bacteria; PP has more pronounced effect against yeasts. Their spectrum of efficacy is close to the spectrum of benzoic acid, but they are effective not only in acidic, but also in alkaline and neutral media. They are also less toxic than benzoic acid [2].

There have been numerous publications describing various methods for the quantification of these compounds individually or in combination with other drugs. CLO has been determined in cream and ointment using TLC with silicagel F_{254} as a stationary phase [3,4]. Some papers dealing with spectrophotometric determination are also published [5–8]. Another widely used method is RP-HPLC [9–12]. MEKC method with UV detection at 254 nm was reported for the analysis of a cream containing CLO, betamethasone dipropionate and their related substances [10]. CZE was used for the determination of CLO in solutions, tablets and vaginal tablets [13,14].

RP-HPLC with UV detection is usually the method of choice for the analysis of MP and PP [15–20]. RP-HPLC with electrochemical detection was used for the determination of parabens in ophthalmological solution [21]. For the determination of parabens in shampoo CZE method was reported [17]. MEKC was used for the determination of imidurea,

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MP and PP in a pharmaceutical ointment [22]. Another MEKC method dealt with the determination of haloperidole, parabens and some of their degradation products [23].

Only one method for the determination of CLO, MP and PP was found in the literature—the RP-HPLC method with UV detection at 210 nm. The chromatographic separations were carried out on a Purospher RP-18e column (125 mm × 4 mm × 5 μm); the mobile phase was a mixture of acetonitrile:water (70:30). This method was applied for the analysis of a topical cream [24]. To our best knowledge, CLO, MP and PP have not yet been simultaneously determined by MEKC.

The aim of this work was the development of capillary MEKC method with UV detection for the assay of CLO, MP and PP in a multicomponent pharmaceutical formulation.

2. Experimental

2.1. Instruments

The experiments were performed on P/ACE MDQ 2200 capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) equipped with a diode array detector and a liquid cooling device. Data acquisition and collection were performed by 32 Karat Software, version 4.01. The separation capillary used was a fused silica capillary 60 cm in total length (50 cm to the detector) and 75 μm i.d. maintained at 25 ± 0.1 °C. Samples were injected hydrodynamically at a pressure of 3.45 kPa for 5 s. The voltage of 25 kV was applied and UV detection was set at 212 nm. Before the beginning of the measurements, the capillary was rinsed with 1 M NaOH for 10 min, water for 10 min and buffer for 10 min. Between analyses the capillary was rinsed with 0.1 M NaOH for 5 min, ultra-pure water for 5 min and running buffer for 5 min.

2.2. Chemicals

MP, PP, sodium dodecyl sulfate (SDS) and methanol were obtained from Sigma–Aldrich (Milwaukee, WI, USA); phosphoric acid, sodium hydrogen phosphate and sodium hydroxide were obtained from Merck (Darmstadt, Germany); hydrochloric acid and Sudan III were purchased from Lachema (Brno, Czech Republic). Clotrimazole was purchased from Amoli Organics (Mumbai, India). A Millipore Milli-Q RG ultra-pure water was used for the preparation of the solutions. Commercially available cream (Clotrimazol HBF, Herbacos-Bofarma, Pardubice, Czech Republic) containing CLO, MP and PP was used for the quantitative analysis.

2.3. Running buffer

15 mM sodium hydrogen phosphate with an addition of 30 mM SDS was used as running buffer. It was prepared by dissolving a calculated amount of sodium hydrogen phosphate and SDS in ultra-pure water. The pH was adjusted to 7.2 by 0.5 M phosphoric acid.

2.4. Standard solutions

Stock solutions containing 2.5 g l^{-1} CLO, 0.5 g l^{-1} MP and 0.125 g l^{-1} PP were prepared by weighing an appropriate amount of the corresponding standard substance and dissolving in methanol. After that, the solution was diluted to the required concentration by the running buffer.

2.5. Sample preparation

Approximately 2.5 g of the Clotrimazol HBF was weighed on analytical balance and treated with 30 ml of methanol; the mixture was stirred intensively for 10 min and thereafter it was diluted with water to 50.0 ml. The resultant mixture was filtered through Millipore membrane filter (pore size 0.45 μm). 1 ml of the filtered solution was diluted by running buffer to 25 ml and injected for analysis.

2.6. HPLC conditions

The HPLC system, comprising a binary pump LCP4100 (Ecom, Prague, Czech Republic), an autosampler Waters 717 Plus, a variable wavelength detector waters 486 (Waters, Milford, USA) and PC for data processing was controlled by a chromatographic software CSW v1.7 for Windows (Data Apex s.r.o., Prague, Czech Republic). The chromatographic separations were carried out on a Purospher RP-18e column (125 mm × 4 mm × 5 μm); the mobile phase was a mixture of acetonitrile:water (70:30). The UV detector was set at 210 nm. An accurately weighed portion (0.5 g) of the pharmaceutical cream was transferred to a 50 ml centrifuge tube, supplemented with 20 ml of acetonitrile and sonicated. Then the tube was cooled in a freezer for 10 min and promptly centrifuged at 3000 rpm for 15 min. 10 μl of the supernatant liquid were injected for analysis.

3. Results and discussion

The scope of this work was the simultaneous separation and determination of CLO, MP and PP. MEKC—as a potent modified electrophoresis method allowing excellent separations of neutral, hydrophobic or water insoluble species was used with respect to the acid base properties of CLO (base), MP and PP (weak acids) with pK_a 6.12, 8.30 and 8.23, respectively [25].

3.1. Method optimization

The separations were examined in a buffer containing sodium hydrogen phosphate and SDS as a surfactant. In this system, following parameters have been optimized:

3.2. SDS concentration

The critical micellar concentration (CMC) of SDS is 8.2 mM [26]. The SDS concentrations tested were between 10 and 50 mM and the buffer used was 25 mM phosphate buffer, pH 7.2. At higher concentrations of SDS (50 and 40 mM), the results were unsatisfactory. During 35 min only two asymmetric peaks appeared (CLO migrated probably with PP) and the results were not reproducible. With the concentration 30 mM of SDS and lower, all three peaks appeared on the electropherogram and the migration times significantly changed. With the decreasing concentration of SDS migration times of MP and PP decreased, but the migration time of CLO increased. The reason for this electrophoretic behavior of CLO can be explained as a consequence of steric hindrance by the substituents groups of the largest CLO. Therefore, CLO would be less solubilized by the micelles and the capacity factor should be smaller with the higher surfactant concentration. The optimal concentration of SDS was 30 mM.

3.3. Phosphate buffer concentration

Five different concentrations of phosphate buffer were tested in the range 10–30 mM. Migration times as expected significantly decreased with the lower concentrations of phosphate but the resolution between MP–PP and PP–CLO remained practically identical (Fig. 1). Finally, the concentration of 15 mM phosphate buffer was selected; symmetric peaks of all analytes with good resolutions and acceptable migration times were achieved with this concentration.

3.4. pH

The separation of the analytes was examined with buffers containing 15 mM phosphate and 30 mM SDS adjusted to the pH values 6.7–7.8. The influence of the pH on the migration time, resolution and the quality of separation of analytes was not very significant. Therefore, a buffer with pH 7.2 was chosen for the following measurements.

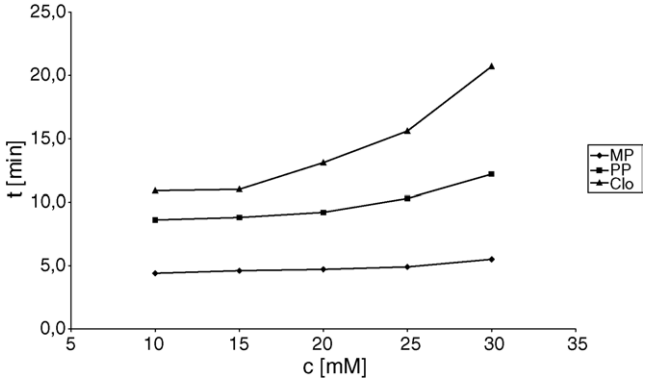

Fig. 1. The effect of the phosphate buffer concentration on the migration time of the analytes. The concentration of SDS was 30 mM, pH 7.2.

3.5. Voltage

A further optimization by varying the running voltage was carried out. The applied voltage ranged from 20 to 30 kV. With the increasing voltage, migration times significantly decreased while the separation was not affected. The value of 25 kV was selected because too high current was generated using the higher voltages.

3.6. Temperature

The effect of temperature was examined between 20 and 30 °C. As expected, with the increasing temperature, migration times decreased and the quality of separation was not significantly changed. Finally the temperature of 25 °C was selected to avoid high current intensity values.

The final conditions for the separation were: 15 mM phosphate buffer (pH 7.2) with the addition of 30 mM SDS. The separation was carried out at 25 kV, 25 ± 0.1 °C and the UV detection was set at 212 nm. The electrophoreogram is shown in Fig. 2. It can be seen that the solutes elute in increasing order of hydrophobicity. Since at pH 7.2 all the examined molecules are practically in their neutral form, their migration times differ only because they have different micelle/water partition coefficients. CLO migrates slower than MP and PP and co-eluted with Sudan III, which was used as micellar velocity marker. All the compounds were identified by comparing the spectrum and the migration time of each peak with those recorded for solutions of the pure standards.

3.7. Method validation

The optimized method was validated by standard procedure. The linearity of the method was tested in the concentration range 10–100 mg l^{-1} for MP, 2.5–25 mg l^{-1} for PP and 50–500 mg l^{-1} for CLO (each concentration level in triplicate). The repeatability was evaluated by performing six successive injections with the concentrations 60 mg l^{-1} for MP, 15 mg l^{-1} for PP and 300 mg l^{-1} for CLO and expressed as R.S.D.% for both migration times and peak areas. The accuracy of the method had to be statistically checked by

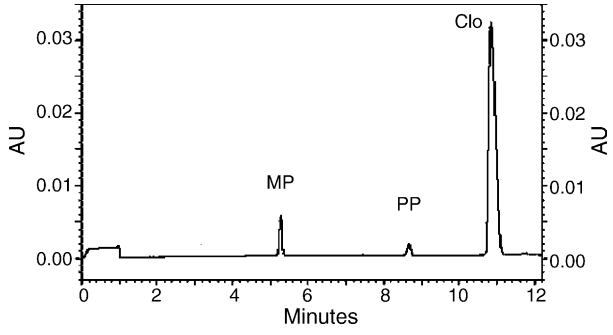

Fig. 2. Electrophoreogram of the pharmaceutical preparation Clotrimazol HBF; BGE: 15 mM phosphate buffer (pH 7.2) with 30 mM SDS; UV detection at 212 nm.

Table 1
Analytical parameters of the proposed method

Analyte	Methylparaben	Propylparaben	Clotrimazole
Linear regression equation $A = a \times c + b$	$1300c + 90$	$1804c - 40$	$3677c + 41$
Correlation coefficient	0.99988	0.99980	0.99989
R.S.D.% area[a]	1.94	2.06	1.72
R.S.D.% migration time[a]	0.70	1.08	1.40
Recovery% (recovery R.S.D.%)	102.96 (1.66)	102.18 (2.16)	101.36 (1.07)
LOD [mg l^{-1}]	0.12	0.10	0.04

For details see the text. $A = a \times c + b$, where A is the peak area (a.u.), c the analyte concentration in mg l^{-1}, a the slope and b is the intercept. The linearity of the method was tested in the concentration range 10–100 mg l^{-1} for MP, 2.5–25 mg l^{-1} for PP and 50–500 mg l^{-1} for CLO (each concentration level in triplicate).
[a] $n = 6$, for concentrations: 60 mg l^{-1} for MP, 15 mg l^{-1} for PP and 300 mg l^{-1} for CLO.

Table 2
Determination of methylparaben, propylparaben and clotrimazole in Clotrimazol HBF

Analyte	Declared amount [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]
Methylparaben	0.20	0.2017 ± 1.66	0.1982 ± 1.61	1.692
Propylparaben	0.05	0.0489 ± 2.16	0.0484 ± 1.72	0.832
Clotrimazole	1.00	0.9993 ± 1.07	0.9927 ± 0.99	1.016

[a] 95% confidence level; $t_c = 2.228$; ($\nu = 2n - 2$).

comparison with the standard official RP-HPLC technique [24] according to the Student's t-test. No significant differences were found between the results obtained by MEKC and official HPLC method for the same batch at 95% confidence level. For the unavailability of the appropriate placebo, the recovery has been checked by the standard addition technique, by analyzing six real samples spiked with a known amount of analyte and six replicates of original non-spiked samples. Detection limits of the analytes were determined as a signal-to-noise ratio of three. The method validation results obtained under the final conditions are given in Table 1.

4. Determination of MP, PP and CLO in the formulation Clotrimazol HBF

The developed method was used for the determination of the three analytes in a pharmaceutical preparation.

The quantitative results were in good agreement with their nominal content. The other constituents of the cream 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.

5. Conclusion

The proposed MEKC method permits the quality control of pharmaceutical preparations containing clotrimazole, methylparaben and propylparaben as active substances in a single run. The total analysis time was <12 min using 15 mM phosphate buffer (pH 7.2) with 30 mM addition of SDS. The method has been validated and the results obtained are precise and accurate. The developed method was successfully applied to the qualitative analysis of active substances in Clotrimazol HBF. 1% and the results obtained by the proposed methods have been statistically compared by means of Student's t-test. T-values indicated the absence of systematic errors at 95% confidence level. Comparing with the published HPLC method [24], sample preparation is straightforward and simple and no organic solvents have to be used in the running buffer. The proposed assay shows lower sensitivity to the HPLC method but it is still fully sufficient for the analysis of pharmaceutical preparations containing CLO, MP and PP as active constituents.

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