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Journal of Pharmaceutical and Biomedical Analysis
19 (1999) 945–954

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Comparison between micellar electrokinetic chromatography and HPLC for the determination of Betamethasone Dipropionate, Clotrimazole and their related substances

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Received in revised form 7 October 1998; accepted 13 October 1998

Abstract

The complete separation of a composite mixture that consisted of Betamethasone Dipropionate (BMD), Clotrimazole and their derivatives in a pharmaceutical dosage form was achieved within 15 min using sodium dodecyl sulfate (SDS) micellar electrokinetic chromatography (MEKC). For the MEKC separations, electrophoretic media consisting of SDS-phosphate buffer and various concentrations of alcohols or acetonitrile were used. The optimal condition for separating BMD, Clotrimazole and their analogues was found to be 50 mM SDS–15% acetonitrile–5% butanol at pH 7.2. The results demonstrated that the method was valid for the quantitation of BMD, Clotrimazole and analogues with selectivity and precision comparable to that of High-Performance Liquid Chromatography (HPLC). © 1999 Elsevier Science B.V. All rights reserved.

Keywords: MEKC; CE; HPLC; Clotrimazole; Betamethasone Dipropionate; SDS

1. Introduction

Recent developments in capillary electrophoresis (CE) are mainly focused on the improvement of method precision and using CE for quantitative applications [1–8]. Based on the availability of commercial instruments, developing better CE methodology has become a significant area. The lack of method precision and ruggedness is a major drawback with the technology.

HPLC is a powerful analytical tool that has been used for decades in pharmaceutical laboratories. Although HPLC is a rapid, precise and rugged alternative to other routine chemistry techniques it does have limitation such as separation efficiency attainability with high molecular weight solutes. Efficiency improvement in traditional LC is commonly to go to very high pressures or to wait very long times. Utilizing CE it has been shown that multicomponent excipients and

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PII: S0731-7085(98)00288-X

degradants can be easily separated and quantitated.

Micellar electrokinetic chromatography (MEKC) has been successfully applied to a broad range of charged and neutral species [9–16]. There are various factors which can directly affect the precision of quantitative analysis in CE, such as sample concentration, temperature control, electrolyte ionic strength as well as sample injection modes. It has been reported that less than 1% relative standard deviation (RSD) can be achieved by carefully controlling subtle factors [17,18]. In a CE system, subtle factors include the liquid levels in the sample or buffer vials, the number of injections per buffer vial, rinsing of the capillary or refreshing the running buffer vials between injections.

The purpose of this study is to investigate the feasibility of utilizing a simple and rapid SDS–MEKC method for the separation and determination of Betamethasone Dipropionate (BMD), Clotrimazole and their related degradation products (Scheme 1a and Scheme 1b). BMD and Clotrimazole are the active components in Clotrimazole and Betamethasone Dipropionate cream, an anti–fungal and anti-inflammatory drug for dermatological use [19]. The MEKC method was applied in the separation and quantitation of the real drug products. The results were evaluated and compared with those obtained by HPLC.

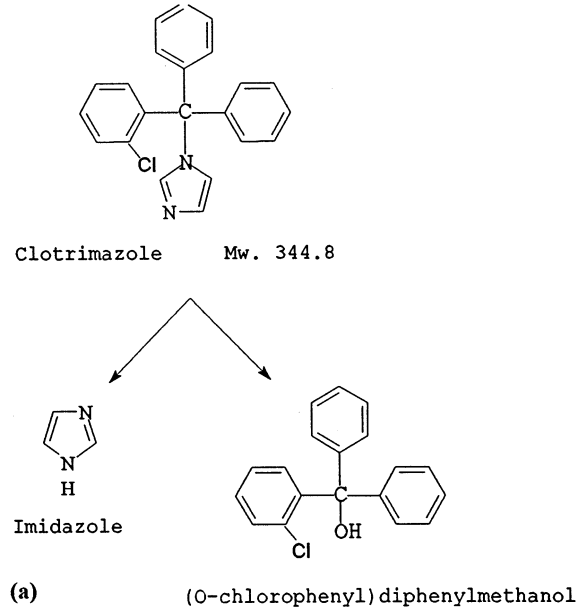
2. Experimental and methods

2.1. Materials

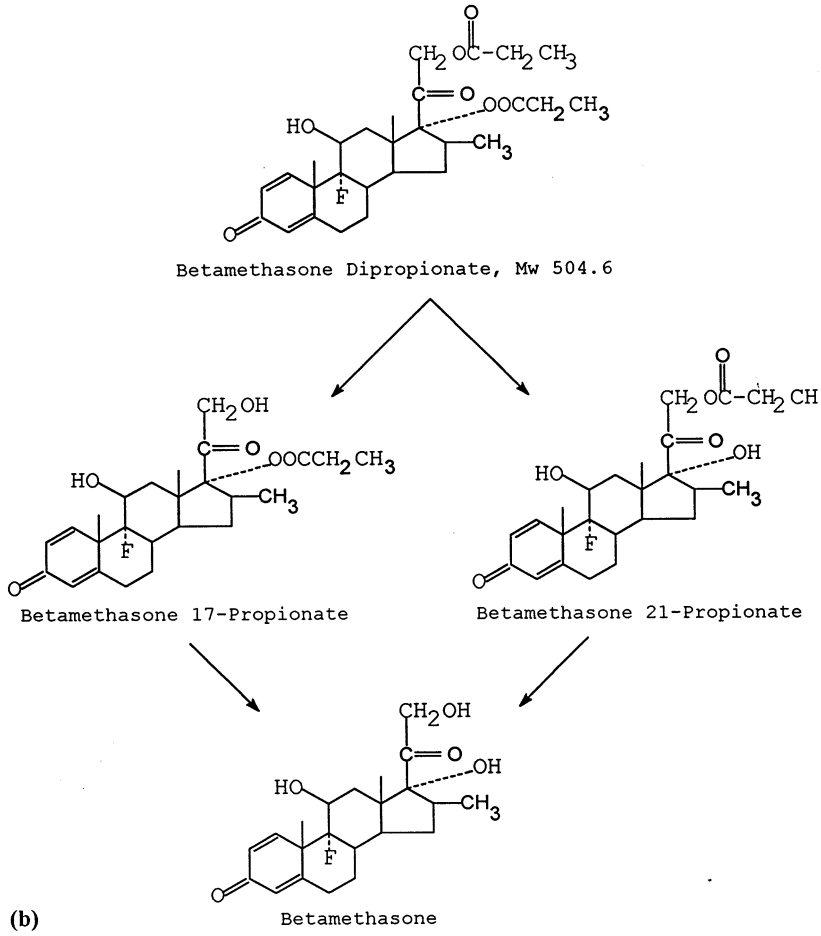
Methanol, acetonitrile and *n*-butanol (HPLC grade) were from Baxter (McGaw Park, IL, USA). Analytical sodium phosphate salt (Na_2HPO_4) and sodium dodecyl sulfate (SDS) were from Sigma (St. Louis, MO, USA). SDS was recrystallized from methanol before use. Betamethasone Dipropionate, Clotrimazole, (O-chlorophenyl)diphenylmethanol and Progesterone were obtained from US Pharmacopeia (Rockville, MD, USA). The drug product, Lotrisone cream (the cream contains 1% Clotrimazole and 0.064% BMD), was from Schering–Plough (Kenilworth, NJ, USA). Other related substances were from Sigma or Sicor (Milan, Italy).

2.2. CE apparatus and conditions

Two CE systems were used in the experiments: an in-house-made instrument consisting of an acrylic box designed with a safety-interlocked door to prevent operator contact with a 30 kV high-voltage power supply (Glassman High Voltage, Whitehouse Station, NY), which was connected to the buffer reservoirs with platinum electrodes to effect CE separation. For on-line UV absorption detection, a detection window was made by burning off a small section of the polyimide coating from the capillary. Absorption of analytes, which migrated pass the detection window, were measured using a Spectra-100 UV-Vis detector (Spectra Physics, San Jose, CA, USA), the output signals were fed into a Chromjet integrator (Spectra-Physics). All method precision measurements were carried out on a second instrument, HP^{3D} CE system (Hewlett–Packard, Palo Alto, CA), set at 254 nm. The CE system was controlled using computer software supplied by the manufacturer of the IBM-PC.



Scheme 1a. Clotrimazole and its related substances



Scheme 1b. BMD and its related substances

2.2.1. CE conditions

The parameters employed for the operation of the HP[3D] CE instrument were as follows: the detector was set at 254 nm with a rise time of 0.3 s, the injection was set at 60 μA for electrokinetic mode at 6.0 s injection time, the column temperature was maintained at 30°C, the voltage was set at 15 kV and a positive polarity. The run times were set at 14 min with a 90 μA current using 50 mM SDS and 10 mM Na_2HPO_4 buffer (~ pH 7.2) solutions (15% acetonitrile and 5% butanol were added, v/v) or 35 min with a 80 μA current using 40 mM SDS and 10 mM Na_2HPO_4 buffer (~ pH 7.2) solutions containing 15% methanol and 5% butanol. The capillary was washed between runs using running buffer for 3 min. The electrolyte reservoirs were switched to another pair of fresh running buffer vials after every four injections.

2.2.2. Capillary conditions

Untreated 41 cm (35 cm to the detection window) × 75 μm ID fused-silica capillary tubes (Polymicro Technologies, Phoenix, AZ) were used for all separations. New capillaries were treated by purging with 0.5 M NaOH for ≈ 0.5 h, rinsing with distilled water and then filling the capillary with run buffer for at least 6 h before use.

2.2.3. Buffers

To prepare the run buffer, appropriate amounts of SDS were dissolved in 10 mM sodium phos-

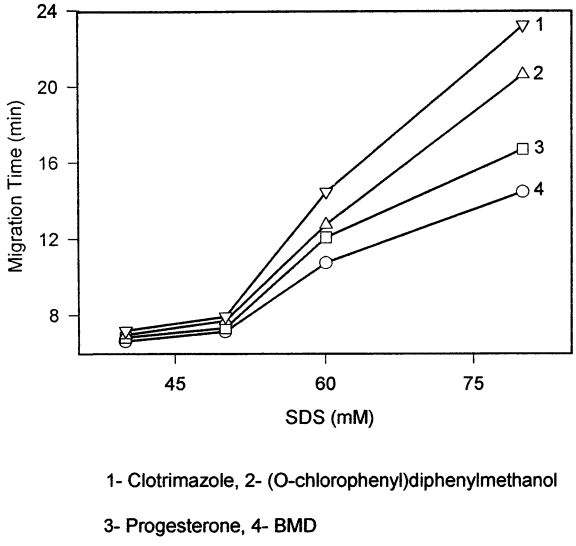


Fig. 1. Influence of SDS concentrations on MEKC migration time. Sample conditions as in Fig. 2. All run buffers were made in 10 mM sodium phosphate buffer (pH 7.2) solution containing 15% methanol and 5% butanol.

phate (Na_2HPO_4). Next H_3PO_4 was added to obtain the appropriate pH values, and methanol or acetonitrile and butanol were added to obtain solutions of the desired concentrations. All solutions were filtered through a 0.45 μm membrane before use.

2.3. HPLC apparatus and conditions

The HPLC system used consisted of a Waters 510 liquid chromatography (Waters, Milford, MA), a photodiode array detector (model 996), an auto sampler (model 717 plus) and a system controller. The chromatographic software interfaced with the system controller was 'Millennium' (Version 2010, Waters) available for the IBM-PC. The absorption wavelengths were set at 254 nm for detecting drug components. The analytical HPLC column was a 5 μm Waters Symmetry™ C_8 (3.9 × 150 mm) column. This column was protected by a 20 mm refillable Symmetry™ C_8 guard column. A mobile phase consisting of $\sim$1.8 volume of methanol and 1.0 volume of 25 mM potassium phosphate adjusted with phosphoric acid to an apparent pH of $\sim$7.0. Other chromatographic conditions included 1.2 ml min^{-1} flow rate and 20 μl injection volume and ambient column temperature.

2.4. Sample preparation and pretreatment

An accurately weighed portion ($\approx$1.0 g for HPLC and $\approx$1.5 g for CE) of the cream was transferred into a screw-capped, 50 ml centrifuge tube. 6.0 ml of progesterone (the internal standard, 0.05 mg ml^{-1}) methanol solution was added. The tube was capped and heated at 60°C in a water bath for 10 min with occasional shaking. The tube was removed from the bath and cooled in an ice-bath for 20 min and promptly centrifuged at 4000 rpm for 5 min. A portion of the supernatant liquid was filtered through a 0.45 μm PTFE membrane filter before injection. The final concentrations of the sample or reference standards were $\approx$1.7 mg ml^{-1} of Clotrimazole and 0.11 mg ml^{-1} of BMD. Additional steps were taken for CE sample preparation; the supernatant liquid was transferred into a 5.0 ml volumetric

Table 1
Precision of migration time (based on eight ($n = 8$) consequent injections)

Compound	Migration time range (min)	Mean	%RSD
Imidazole	3.01–3.19	3.10	2.1
Benzyl alcohol	3.81–3.94	3.85	1.4
Betamethasone (BM)	5.51–5.60	5.56	0.6
BM-21-Propionate	6.83–6.96	6.89	0.8
BMD	8.86–9.04	8.94	0.6
Progesterone	9.46–9.79	9.63	1.2
(O–chlorophenyl)diphenylmethanol	10.89–11.37	11.15	1.6
Clotrimazole	11.40–11.95	11.70	1.8

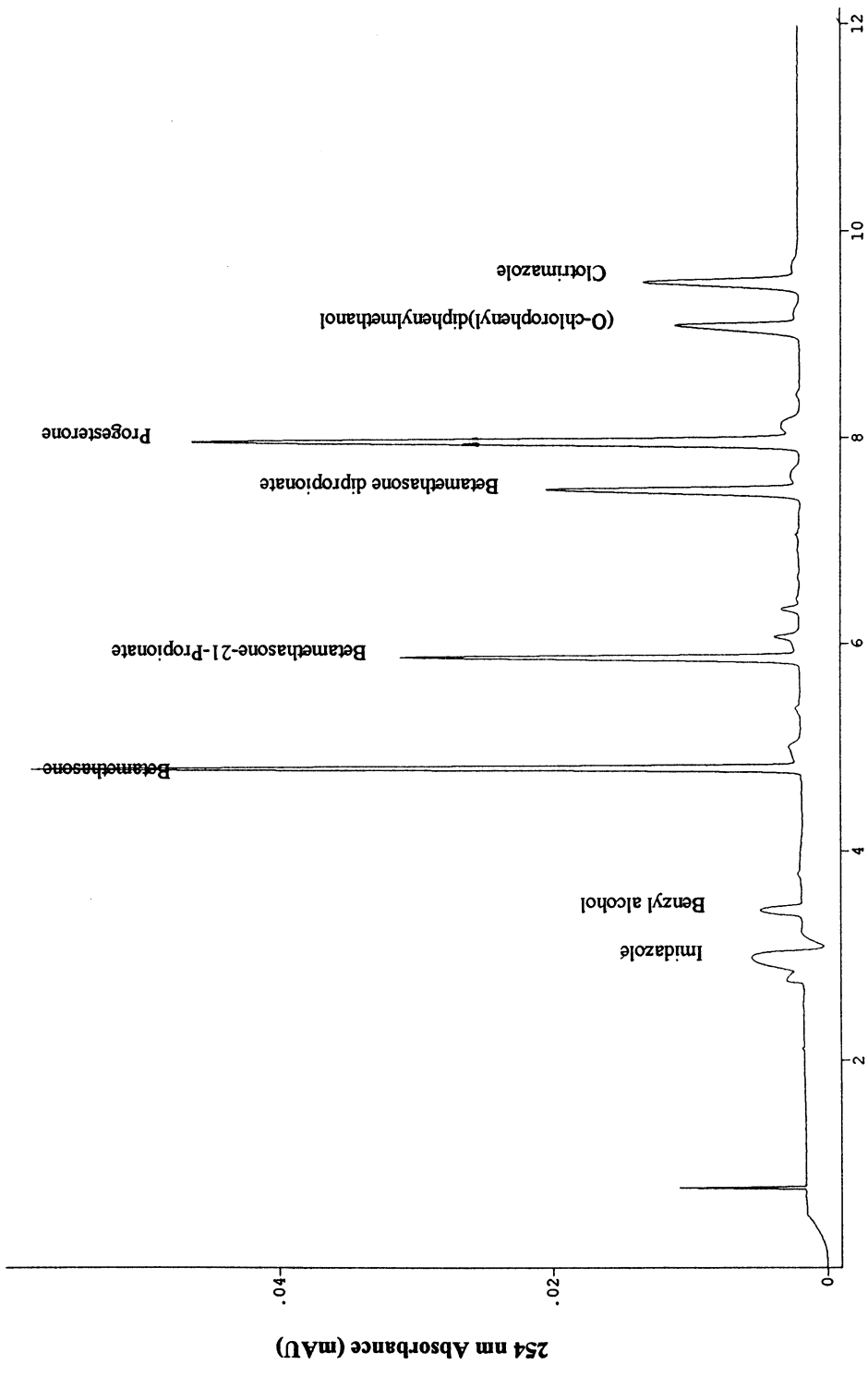


Fig. 2. MEKC electropherogram of reference standards mixture of 1-Imidazole (0.30 mg ml^{-1}), 2-Benzyl alcohol (1.33 mg ml^{-1}), 3-Betamethasone (0.61 mg ml^{-1}), 4-BM-21-Propionate (0.29 mg ml^{-1}), 5-BMD (0.25 mg ml^{-1}), 6-Progesterone (0.23 mg ml^{-1}, internal standard), 7-O-chlorophenyl)diphenylmethanol (0.97 mg ml^{-1}) and 8-Clotrimazole (2.24 mg ml^{-1}). Capillary length = 41 cm (effective length = 35 cm), run buffer solution: 50 mM SDS in 10 mM sodium phosphate buffer (pH 7.2), 5% butanol and 15% acetonitrile added. Applied voltage = 15 kV. Detection: $\lambda = 254$ nm. See the text for the detail of MEKC conditions.

Table 2
Precision of peak area (based on eight ($n = 8$) consequent injections, $\lambda = 254$ nm)

Compound	Peak area range (μAU)	Mean	%RSD
Imidazole	79026–82123	80646	1.2
Benzyl alcohol	38563–40897	39746	2.1
Betamethasone (BM)	78052–81267	79746	1.6
BM-21-propionate	31205–33204	32327	2.4
BMD	34881–36826	36078	1.8
Progesterone	38836–40273	39444	1.8
(O–chlorophenyl)diphenylmethanol	29400–30901	30444	1.7
Clotrimazole	31991–34294	33175	2.1

flask and evaporated in a water bath at 40°C under a steam of nitrogen to $\approx 2/5$ of the volume, the flask was then diluted to volume with 50 mM SDS solution and mixed reference standards for the CE experiments were prepared in 50 mM SDS solution containing 40% methanol. The final concentrations of the sample or reference standards were ≈ 2.5 mg ml^{-1} of Clotrimazole and 0.17 mg ml^{-1} of BMD for the CE experiments.

3. Results and discussion

3.1. MEKC Separation

MEKC Separation was affected by the amount of surfactant in the run buffer. Optimal conditions for separating BMD and Clotrimazole from their analogues were obtained with a running buffer containing 50 mM SDS and 10 mM sodium phosphate (pH 7.2) with 15% acetonitrile and 5% butanol added as the modifiers. The resolution between the peaks of Clotrimazole and (O–chlorophenyl)diphenolmethanol was improved by adding butanol which can enhance the solubilities of these analytes. If methanol was added instead of acetonitrile the running time was almost tripled. This may be due to effects of electroosmotic flow, which is largely suppressed by the alcohols. Additionally, with a relatively large organic composition in the running buffer, SDS micelles may be less tortured in acetonitrile than in methanol [20]. The migration time of each analyte increased with a increase in concentration of SDS from 40 to 80 mM as demonstrated in Fig. 1. Under optimal conditions the theoretical plate number measured greater than 16 000 for the Clotrimazole peak. In comparison, HPLC separation typically obtained a plate number of less than 1300. The reference standard mixture was injected eight times with RSDs of 2.1% or less for peak area and migration time for both BMD and Clotrimazole. The precision results are summarized in Tables 1 and 2.

3.2. MEKC Electropherogram

Fig. 2 shows the mixture of BMD and Clotrimazole with four other related drug substances were well resolved in 15 min using a 50 mM SDS solution with 15% acetonitrile and 5% butanol as the run buffer modifiers on a bare silica capillary. Fig. 3 shows the HPLC chromatogram of the same sample mixture at different concentrations.

3.3. Recovery study

Recovery study was evaluated at ≈ 80, 100 and 120% of the nominal concentration for Clotrimazole (1%) and BMD (0.064%) in the drug product. Each concentration was injected in triplicate with the overall range of recovery for the nine solutions determined by peak area (to the internal standard-progesterone peak) response ratio. Mean values ($n = 3$) of 99.7, 99.1 and 99.9% at 80, 100 and 120% level with RSDs of 2.2, 2.6 and 1.6% were obtained for Clotrimazole and mean values ($n = 3$) of 99.0, 100.7 and 100.1% at 80, 100 and 120% level with RSDs of 1.7, 1.1 and 1.3% were obtained for BMD, respectively. The relatively

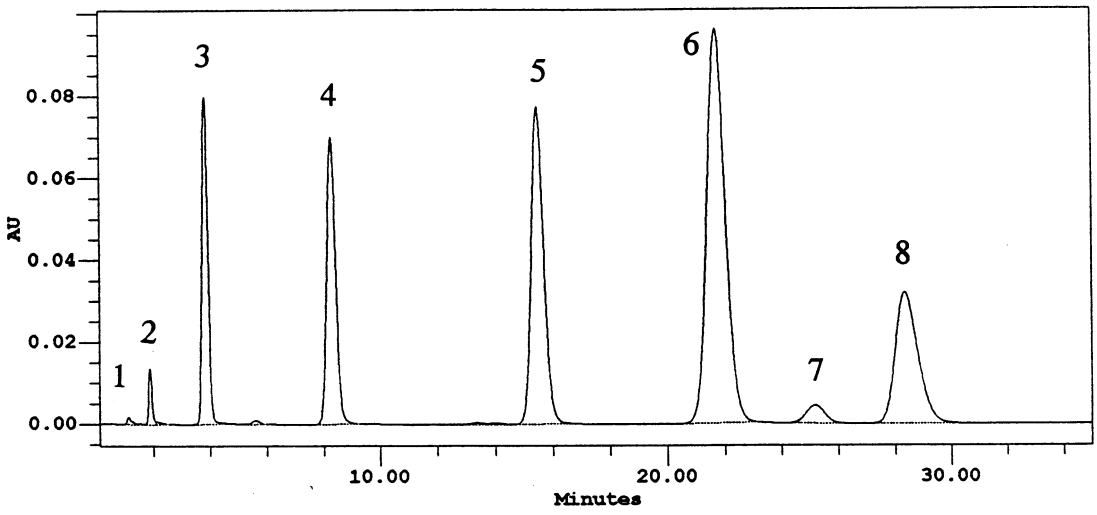


Fig. 3. HPLC chromatogram of reference standards mixture of 1-Imidazole (0.15 mg ml^{-1}), 2-Benzyl alcohol (0.19 mg ml^{-1}), 3-Betamethasone (0.17 mg ml^{-1}), 4-BM-21- Propionate (0.14 mg ml^{-1}), 5-Progesterone (0.16 mg ml^{-1}), 6-BMD (0.18 mg ml^{-1}), 7-O-chlorophenyl)diphenylmethanol (0.20 mg ml^{-1}) and 8-Clotrimazole (0.76 mg ml^{-1}); chromatogram monitoring at 254 nm. See the text for the detail of chromatographic conditions.

higher RSDs that were obtained for Clotrimazole compared to those obtained for BMD may be due to the less sensitive detection setting since 254 nm was not at the maximum absorption of Clotrimazole (Fig. 4).

3.4. System precision

System precision was demonstrated by comparing MEKC with HPLC. RSDs of 2.0 and 1.9% by peak area response ratio were obtained for Clotrimazole and BMD in MEKC, and RSDs of 1.1 and 0.2% for Clotrimazole and BMD in HPLC were obtained. We found that electrokinetic injection with a controlled current setting gave better overall precision than other injection modes (i.e. pressure or voltage control injection). This may be attributed to the fact that constant current can miminize the variability of ionic strength of the sample or running buffer solution from run to run.

3.5. Method precision

Method precision was determined by the assay of five separated sample preparations of Clotrimazole and Betamethasone Dipropionate Cream. Mean values ($n = 5$) of 98.3 and 99.3% with RSDs of 2.0 and 1.2% were obtained for Clotrimazole and BMD, respectively by the MEKC method compared to mean values of 102.8 and 102.1% with RSDs of 0.7 and 0.7% for Clotrimazole and BMD from the HPLC method.

3.6. Linearity

Linearity was demonstrated by comparing MEKC and HPLC systems for Clotrimazole and BMD. A set of standards over the range of 20–120% of the nominal concentrations of Clotrimazole (MEKC: 0.4–2.6 mg ml^{-1} and HPLC: 0.4–2.0 mg ml^{-1}) and BMD (MEKC: 0.03–0.2 mg ml^{-1} and HPLC: 0.04–0.2 mg ml^{-1}) were prepared and analyzed by each system. All plots of analyte concentrations versus average peak area response ratios have correlation coefficients of 0.990+ for MEKC and 0.999 or above for HPLC, respectively.

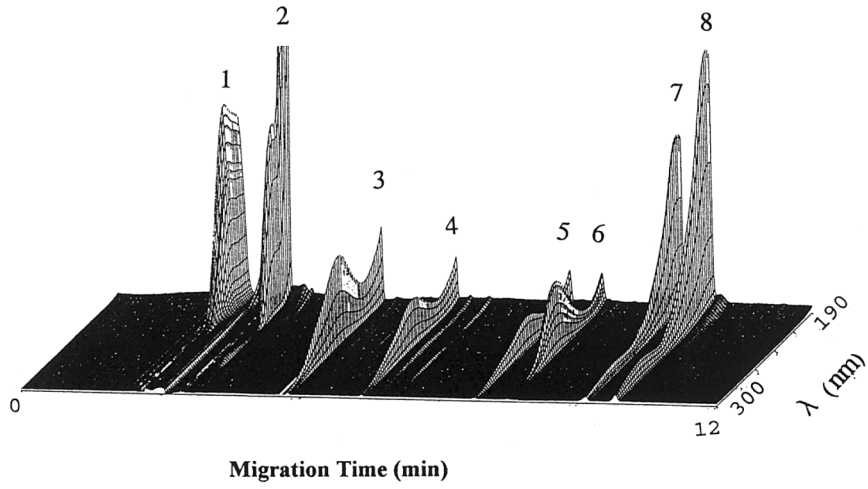


Fig. 4. 3D-plot of the MEKC electropherogram of reference standards mixture. Sample and MEKC conditions as in Fig. 2.

3.7. Limits of detection (LOD)

Limits of detection (LOD) for Clotrimazole and BMD were determined to be ≈ 13 μg ml^{-1} for Clotrimazole (S/N = 3) and 0.34 μg ml^{-1} for BMD (S/N = 3) by peak area response compared to 0.5 μg ml^{-1} for Clotromazole and 7 ng ml^{-1} for BMD from the HPLC method. Linearity at this level was demonstrated by preparing and analyzing a set of standards spanning 0.5–2.5% of the standard assay values. Plot of analyte concentration versus average peak area response generated straight lines with correlation coefficients of 0.991 or higher.

3.8. System suitability

System suitability for the chosen MEKC conditions was found to be very reproducible and rugged. The overall MEKC of this method was good as demonstrated by a typical electropherogram of a sample solution (Fig. 5). Baseline resolution was observed between all peaks with typical resolutions greater than 2.5 and peak asymmetry (tailing factor) less than 1.2 for Clotrimazole, respectively.

4. Conclusion

The experimentation performed exhibits how CE can be a very effective analytical tool with great potential. The data supports the claim that CE methodology can compete with well-established techniques such as HPLC for the quantitative analysis of pharmaceutical samples with regard to time and expense of analysis. Under optimal conditions CE can achieve things that no other analytical instrument has been able to accomplish to date. This study has demonstrated a simple and rapid MEKC procedure which makes possible the quantitative determination of drug substances and their related substances in a pharmaceutical dosage form. With the increased integrity of the data generated by CE, there will be a higher acceptance of its use in the pharmaceutical industry. The minimum use of organic solvents or solutions and its expeditious analysis time makes CE an attractive alternative or partner to other analytical instruments such as HPLC. As a result, it has been shown that MEKC method precision can be greatly improved by carefully controlling the CE operating conditions.

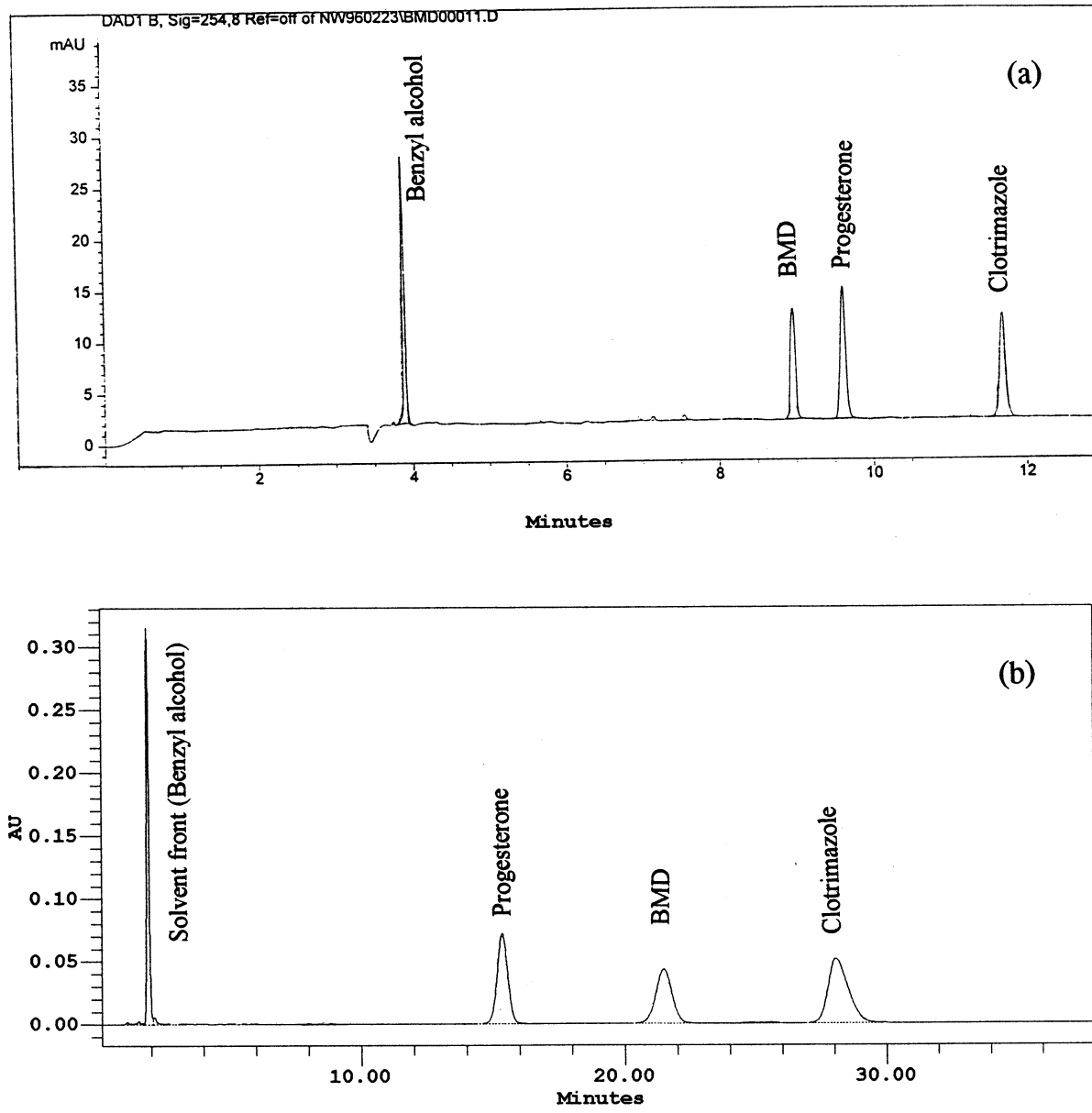


Fig. 5. Separation of BMD/Clotrimazole cream samples; (a) MEKC electropherogram (1.3 g of sample); (b) HPLC chromatogram (0.9 g of sample). Detection: $\lambda = 254$ nm.

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