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Application of micellar electrokinetic chromatography for analyzing antiviral drugs in pharmaceutical semisolid formulations

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Abstract

The application of micellar electrokinetic chromatography (MEKC) to the determination of the antiviral drugs brivudin (BV) and aciclovir (AC) in pharmaceutical semisolid formulations was studied. A method was developed for separating AC and BV both from hydrophilic and from lipophilic semisolid formulations and for the rapid determination of BV and AC using MEKC. The detection limit, the effective mobility and the relative standard deviation of the migration times and of the peak areas were determined. © 1998 Elsevier Science B.V.

Keywords: MEKC; Analysis of aciclovir and brivudin; Semisolid formulations; Vaseline; Hydrogel; Drug content

1. Introduction

Determination of antiviral drugs in semisolid topical formulations is important concerning standardization and monitoring of stability of such formulations. In recent years, mainly HPLC [1,2] and HPTLC techniques have been used for this purpose. However, nowadays interest is focused on the use of capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC) and isotachophoresis (ITP) for the determination of drugs in pharmaceutical formulations due to their high efficiency [3,4]. Brivudin (BV) and aciclovir (AC) (for the chemical structure see Fig. 1) are well known antiviral drugs which are used in semisolid formulations. Although their chemical structures are different capillary electrophoretic methods should be very useful in determining these water-soluble drugs. Up to date in the literature the determination of the antiviral drugs mentioned above by means of high-performance electrophoretic techniques has not yet been described. Therefore, it was necessary to develop methods for determining water-soluble antiviral drugs such as BV and AC in both hydrophilic and lipophilic semisolid formulations. The performance of the developed MEKC method for the determination of BV and AC in semisolid formulations was tested with regard to detection limit and standard deviation.

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2. Experimental

2.1. Apparatus

Capillary electrophoresis experiments were performed on a Hewlett Packard Model G1600A (Waldbronn, Germany) ^{3D}CE system with diode–array detector from 190 to 600 nm. CE ChemStation equipped with a HP Vectra 486/66U workstation was used for instrument control, data acquisition, and data analysis. The system was controlled by windows software, which was modified to the HP system. The detection wavelengths were 200 and 250 nm. The capillaries, normal and light path (fused-silica), obtained from Hewlett Packard (Germany) with a total length (64.5 cm), length to detector (56 cm) and internal diameter (50 μm) with a 150 μm extended light path (bubble cell) were used for the determination and for the separation of the antiviral drugs.

2.2. Chemicals

Brivudin (BV; 9-[(2-hydroxy)methyl]guanine), aciclovir (AC; (E)-5-(2-bromovinyl)-2′-deoxyuridine), vaseline and the hydrogel were obtained from Berlin–Chemie (Berlin, Germany).

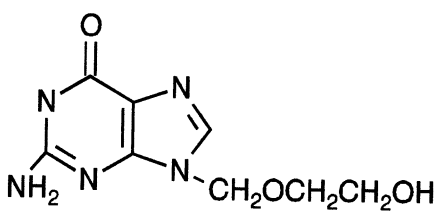
Boric acid and NaOH for the preparation of borate buffer pH 10.0 (20 mM), dodecyltrimethylammoniumbromide (DTB), sodium dodecyl sulfate (SDS), petrolether and ethanol were obtained from Fluka (Buchs, Switzerland). Doubly distilled water was used throughout.

2.3. Sample and buffer preparation

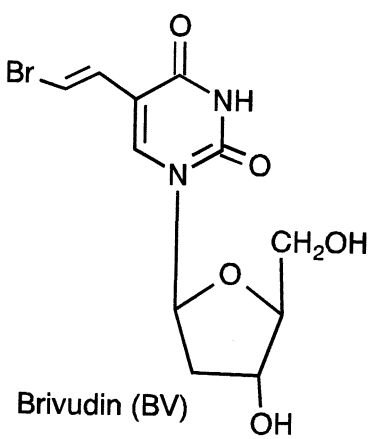
To prepare borate buffer 2.48 g boric acid and 0.8 g NaOH were dissolved in water filling up to a volume of 1000 ml. 59 ml of this solution was then diluted with 0.2 N NaOH to give a volume of 100 ml, such that the final pH was approximately 10.0. For separating BV and AC from the vaseline 10 mg of the ointment were shaken for 1 h with a mixture consisting of 2.0 ml petrolether, 1.5 ml borate buffer pH 10.0 and 1.5 ml ethanol. After shaking the aqueous phase was separated and filtered for the MEKC measurement. In order to determine BV and AC content in the hydrogel 10 mg of the formulation were shaken for 1 h with 2.0 ml borate buffer pH 10.0. After filtration and separation the MEKC measurement was carried out. Run buffer contains additionally 10 mM sodium dodecyl sulfate for the determination and 5 mM dodecyltrimethylammoniumbromide for the separation of the drugs. The standard BV and AC concentrations were measured in the described solutions in a range 1–500 μg ml^{-1}. All samples and buffers were filtered through a 0.2 μm syringe filter and injected immediately. Buffers were degassed by ultrasound at least 10 min before use.

2.4. Analysis conditions

A new capillary was washed for 15 min with NaOH (1.0 M) at 40°C, followed by washing for



Acyclovir (AC)



Brivudin (BV)

Fig. 1. Chemical structure of the drugs used.

Table 1
Analytical parameters for the determination of antiviral drugs studied at pH 10

Compound	μ_e	R.S.D. (t) %	R.S.D. (f) %	Detection limit μg ml^{-1}
BV	-15.84 ± 0.11	1.9	3.2	3
AC	-15.57 ± 0.13	1.4	2.1	3

μ_e Effective mobility (10^{-5} cm^2 V^{-1} s^{-1}).
R.S.D. (t) Relative standard deviation of the migration times.
R.S.D. (f) Relative standard deviation of the peak areas.

Table 2
Determination of brivudine and acyclovir in semisolid formulations

Compound	Formulation	Content in %		S.D.R. (%)	Detection limit (μg ml^{-1})
		Declared	Measured		
BV	Hydrogel	0.5	0.32	6.36	5.74
	Vaseline	0.5	0.64	8.51	70.3
		1.0	1.20	14.4	
		2.0	2.95	12.1	
		6.0	6.53	14.7	
		10	9.89	11.1	
AC	Hydrogel	0.5	0.63	7.16	3.12
	Vaseline	0.5	0.51	12.9	10
		1.0	1.13	8.7	

10 min with water at the same temperature and for 5 min with water at 25°C. Before each injection, the capillary was flushed with 0.1 M NaOH for 3 min and with the actual buffer solution for 5 min. The temperature was kept at 25°C, separation potentials of -30 kV using DTB and $+30$ kV using SDS were used. Acetone was used as a marker substance for the determination of the electroosmotic mobility. The samples [buffer–acetone (99:1)] were injected at 200 mbar s (hydrodynamic injection). Detailed experimental conditions are listed in the Figures.

3. Results and discussion

3.1. Analytical parameters

In this paper the determination and the separation of antiviral drugs in semisolid formulation using the micellar electrokinetic chromatography were studied. Acyclovir (pK_a 2.52, 9.35 [5]) and brivudine (pK_a 9.3 [5]) exhibited in buffer solutions at pH values from 3 to 8.3 very low solubility. Sufficient solubility was achieved for both BV and AC first at pH 10 in borate buffer. For this reason 20 mM borate (pH 10) containinig 10 mM sodium dodecyl sulfate (SDS) was used as the electrophoresis buffer for the determination of the drugs. For the optimization of the separations experiments were carried out with light path capillaries and with normal capillaries. The results showed that the sensitivity of the method was increased a threefold using the capillaries with light path.

Table 1 summarizes estimated quantitative analytical parameters of the drugs using the electrophoresis buffer. To determine the linearity plots of peak area versus concentrations over the range of 5–500 μg ml^{-1} were made for the compounds. To control the reproducibility of the peak areas and of the migration times, five injections of the drugs (15 μg ml^{-1}) were made. Very good reproducibility of the peak areas and of the migration times (Tables 1 and 2) was obtained for the antiviral drugs. The detection limits for all

analytes were determined at 250 nm using the following equation (Table 1): $L = 3NC/h$ where N = noise, C = sample concentration, h = peak height.

The effective mobilities of the drugs were determined at pH 10 in borate buffer [6]. BV and AC had similar electrophoretic mobilities and migrated in the direction of the cathode (Table 1). Furthermore, the separation of the antiviral drugs BV and AC was studied at different conditions. In pure borate buffer at pH 10 and in borate buffer containing 10 mM SDS BV and AC were not resolved under these conditions. The influence of tetraalkylammmonium compounds on the separations of anionic substances was investigated by Terabe et al. [7]. In the present paper we studied the influence of the concentrations of dodecyltrimethyl ammonium bromide on the separation of BV and AC as well as on the electroosmotic flow (EOF). The results showed that the velocity of the EOF was increased by increasing the surfactant concentrations. A concentration of 30 mM of DTB in run buffer led to overlapping of the EOF with BV. Good separation was achieved at a DTB concentration of 5 mM (Fig. 2). At this concentration BV and AC had different ionic mobilities and migrated into the direction of the anode (Table 3).

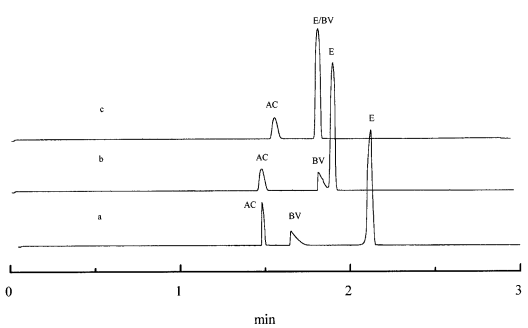


Fig. 2. Influence of the concentrations of dodecyltrimethyl ammonium bromide on the separation of the BV and AC as well as on the electroosmotic flow. Buffer: pH 10, 20 mM borate buffer containing: (a) 5 mM, (b) 15 mM, (c) 30 mM dodecyltrimethyl ammonium bromide; capillary with bubble cell: 64.5 (56 cm to detector) × 50 μm i.d.; field strength: −30 kV; temperature: 25°C, pressure injection: 200 mbar s; detection: 250 nm. E: marker substance (acetone).

Table 3
Analytical parameters for the antiviral drugs brivudine and acyclovir

Compound	μ_e	UV (maxima) nm
BV	-10.76 ± 0.08	200, 250
AC	-15.65 ± 0.13	200, 250

μ_e Effective mobility (10^{-5} cm^2 V^{-1} s^{-1}).

3.2. Applications

MEKC was used to determine BV and AC in a hydrophilic and lipophilic semisolid formulation which were pharmaceutically administered (Fig. 3). Quantification was effected by calibration from standard drug solutions. The contents of the drugs measured in the semisolid formulations were in good accordance with those given by the producer (see Table 2). As shown in Table 4 the detection limits were at the low ppm level (3–10 μg ml^{-1}) except for the determination of BV in vaseline (70 μg ml^{-1}). The reproducibility of the methods described was 7–8% for the hydrogel and 8–14% for the vaseline measured as R.S.D. This parameter for both BV and AC in vaseline was higher due to the separation of the hydrophilic drugs from this lipophilic vehicle system. Furthermore, two samples of the Zovir® creme 5% were analyzed. Table 4 contained the results of the analysis.

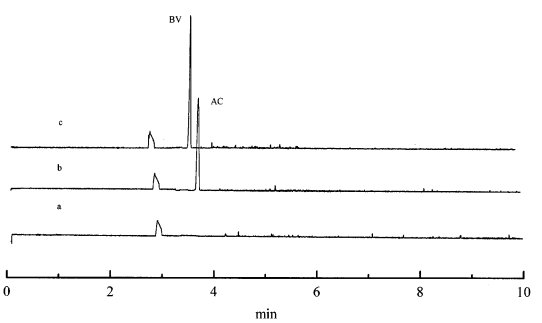


Fig. 3. Electrophergram of antiviral drugs. (a) blank extract, (b) extract containing 30 μg ml^{-1} acyclovir, (c) extract containing brivudin 30 μg ml^{-1}. Buffer: pH 10, 20 mM borate containing 10 mM sodium dodecyl sulfate; field strength 30 kV; other conditions see Fig. 2.

Table 4
Determination of acyclovir in Zovirax® creme 5%

Weight (mg)	AC—amount declared (μg)	Peak areas (mAUs)	AC—amount measured (μg ml^{-1})	Recovery (%)
12.7	635	36.55 ± 0.75	50.68 ± 1.18	93.90 ± 1.8
13.9	695	40.35 ± 0.34	65.85 ± 0.56	94.74 ± 0.8

4. Conclusions

Using the advantages of MEKC such as high separation efficiency, short analysis time and ease of instrumentation and preconditioning we were able to employ this method for determining antiviral drugs both in hydrophilic and in lipophilic ointments. The detection limits of MEKC were in the low ppm range. The method offered good reproducibility depending on the formulation used.

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