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Determination of Fexofenadine in Tablets by Capillary Electrophoresis in Free Solution and in Solution with Cyclodextrins as Analyte Carriers

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ABSTRACT Capillary electrophoresis (CE) methods for the determination of fexofenadine (FEX) in commercial pharmaceuticals were developed. It was demonstrated that FEX could be effectively analyzed in free solution cationic CE at low pH. Another analytical approach studied was based on cyclodextrin (CD) modified CE where highly charged CD derivatives served as analyte carriers. In this way, the separation range was spread to physiological pH region and a CE analysis of FEX, present actually in its zwitterionic form, could be accomplished. Several parameters affecting the separations were studied, including the type and concentration of carrier ion, counterion, analyte carrier, and pH of the buffer. The methods based on the free solution CE and CD-modified CE were compared each other, validated, and applied for the determination of FEX in tablets.

KEYWORDS Fexofenadine, Drug, Zwitterion, Charged cyclodextrins, Capillary electrophoresis

INTRODUCTION

The recently introduced H-1 receptor antagonist fexofenadine (FEX), Fig. 1, belongs to the second-generation antihistamines. It is approved for treatment of seasonal allergic rhinitis and urticaria. The probability that cardiotoxic side-effects occur in connection with FEX is assessed as being extremely low. Since metabolism is not effected by cytochrome P 450 no interaction with other drugs is assumed. FEX does not have a negative effect on the psychomotor efficiency and it shows a high degree of daily-life suitability. The substance has anti-inflammatory characteristics what presents a modern approach to allergy therapy (Amon & Zollinger, 2001).

Although widely used in the treatment of allergic diseases, FEX is not listed in any pharmacopoeia, and there are few methods in the literature for its

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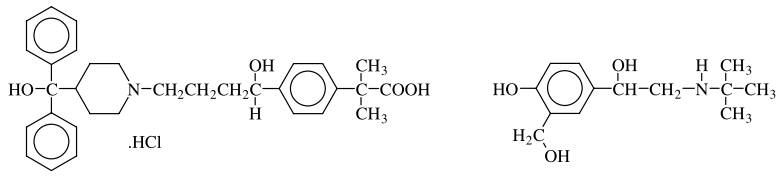

FIGURE 1 **Chemical Structures of the Drugs Used.**

quantitation in pharmaceutical dosage forms (Breier et al., 2004; Gazy et al., 2002; Mahgoub et al., 2003). Analysis of biological samples includes determination of FEX in human plasma and urine (Fu et al., 2004; Hofmann et al., 2002; Uno et al., 2004). FEX exists as a zwitterion in aqueous media at physiological pH, and it was successfully analyzed by high performance liquid chromatography (HPLC) (Breier et al., 2004; Fu et al., 2004; Hofmann et al., 2002; Uno et al., 2004). Capillary electrophoresis (CE) has not been used for the analysis of FEX so far. A rare use of CE for analysis of zwitterionic drugs can be assigned to the specific migration properties of such analytes due to counteraction of the oppositely charged functionalities. Here, derivatization of analyte or extreme pH of separation buffer is required for sufficient migration of zwitterionic compounds (Lee & Lin, 1994; Mikuš & Kaniansky, 2000). On the other hand, an approach being used in CE to provide a movement of analyte with an effective charge near to zero is based on its transport with the aid of suitable carrier molecules like charged micelles and charged cyclodextrins (CDs) (El Rassi, 2000; Evans & Stalcup, 2003; Terabe, 2004). For the present, the applications have been performed in hydrodynamically open CE separation systems in narrow bore capillaries (mostly 50 μm I.D.). However, no work was devoted to use the analyte carrier systems (micellar or cyclodextrin) in hydrodynamically closed CE mode despite the fact that such CE analyzers are commercially available and the range of their application is wide (Kaniansky, 2000).

The aim of the present work was to elaborate method for the determination of FEX in pharmaceuticals and, at the same time, to show a possibility of using an analyte carrier system in hydrodynamically closed CE separation mode. Two analytical approaches were used for the determination of FEX. The first one was based on free solution cationic CE at low pH, serving as a reference method, while the second one was based on CD-mediated CE with highly charged CDs serving as analyte carriers in reversed polarity mode. Several parameters affecting the separations were studied, including the type and concentration of carrier ion, counterion, analyte carrier, and the pH of the buffer. A hydrodynamically closed CE separation system, minimizing impacts of non-separative transport processes (hydrodynamic, electroosmotic) on the migration velocity of the separated constituent, was expected to produce highly reproducible migration velocity of FEX that is desired for peak area based quantitation of the analyte. Moreover, the advantage of using columns of larger I.D. (here 300 μm) includes significantly reduced contribution of electromigration dispersion to the separation efficiency. At higher conductivities of the running electrolytes this gain, however, can be partially lost due to increased thermal dispersive effects (Kaniansky et al., 1997a).

EXPERIMENTAL

Instrumentation

A CS Isotachophoretic Analyzer (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was used in a single-column configuration of the separation unit. The separation unit consisted of the following modules: 1) a capillary zone electrophoresis (CZE) injection valve with a 100 nl internal sample loop drilled in a rotating disc (the sample loop is filled by a syringe); 2) a column provided with a 300 μm I.D. (650 μm O.D.) capillary tube made of fluorinated ethylene-propylene copolymer (FEP) of 210 mm total length (160 mm to the photometric detector); 3) a counter-electrode compartment with a hydrodynamically (membrane) closed connecting channel to the separation compartment.

The CZE column was provided with a LCD 2083 on-column photometric detector with variable wavelengths, 190–600 nm (Ecom, Praha, Czech Republic). In this work the photometric detector was set at a 231 nm detection wavelength. The signal from the detector

was led to a PC via a Unilab data acquisition unit (Villa-Labeco). ITP Pro32 Win software (version 1.0) obtained from KasComp (Bratislava, Slovak Republic) was used for data acquisition and processing.

Prior to the use, the capillary was not particularly treated to suppress an electro-osmotic flow (EOF). A dynamic coating of the capillary wall by means of a 0.2% (w/v) methylhydroxyethylcellulose (m-HEC) in background electrolyte solutions served for this purpose (Kaniansky et al., 1997b). CE analyses were carried out in cationic as well as anionic regime of the separation with direct injections of the samples. The experiments were performed in constant current mode (Kaniansky et al., 1997a) in the range of 50–250 μA (corresponding voltages ranged in the interval of 1.2–8.0 kV) at a 20°C temperature.

Chemicals and Samples

The carrier electrolyte solution was prepared from chemicals obtained from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland) in water demineralized by a Rowapure-Ultrapure water purification system (Premier, Phoenix, Arizona, U.S.A.). All chemicals used were of analytical grade or additionally purified by the usual methods. The solutions of the electrolytes were filtered before use through disposable membrane filters (a 1.2 μm pore size) purchased from Sigma (St. Louis, MO, U.S.A.).

Water-soluble polymer, m-HEC 30 000, was obtained from Serva (Heidelberg, Germany). β-cyclodextrin sulfated sodium salt (S-β-CD) was purchased from Fluka. Carboxyethyl-β-cyclodextrin (CE-β-CD, DS 3, CE purity) is a commercial product of Cyclolab (Budapest, Hungary).

Analytical reference standards of FEX and salbutamol (SAL) were obtained from Aventis Pharma (Prague, Czech Republic) and Sigma-Aldrich (Seelze, Germany). Analyzed pharmaceutical tablets, Telfast® 180 (180 mg of FEX hydrochloride per tablet), were obtained commercially (Hoechst, Frankfurt am Main, Germany).

Procedures for Sample and Standard Solution Preparations

Standard Solutions

Pure standard stock solutions of FEX and SAL (serving as an internal standard) were prepared in demineralized water at 1 mg/ml concentrations and stored at −8°C in the freezer. Working solutions at a 80 μg/ml concentration of FEX and 20 μg/ml SAL, unless otherwise stated, were made by dilution of the stock solution with demineralized water and directly injected into the CE equipment.

Pharmaceutical Sample

A total of 10 tablets were weighed and finely powdered. A portion of the powder equivalent to 8 mg FEX was weighed accurately into a 10 ml volumetric flask and suspended in 5 ml of demineralized water and 0.5 ml of 1 mol/l acetic acid. An appropriate amount (2 mg) of the internal standard was added. The flask was placed in ultrasonic water bath for 10 min before completion to the mark with the water. The mixture was centrifuged (8000 rpm) for 10 min. The resulting solution was transferred into a proper flask and stored in a freezer at −8°C. It was properly diluted (80 μg/ml FEX, 20 μg/ml SAL) with demineralized water prior to the analysis and filtered (1.2 μm pore size) before an injection into the CE equipment.

Calibration Graph

Appropriate amounts of the FEX standard stock solution were diluted with demineralized water yielding concentrations of 50, 60, 70, 80, 90, and 100 μg/ml of FEX in the samples. Eight replicate injections of each were made.

Recovery Test

A known amount of powder of pharmaceutical (Telfast) was weighed accurately into a 10 ml volumetric flask, mixed with acid demineralized water and appropriate amount of FEX standard stock solution was added. Further procedure was the same as in the pharmaceutical sample for tablets. After an appropriate dilution of resulting solution, the concentration of added FEX standard varied as follows: 40, 50, and 60 μg/ml (that represented 80, 100, and 120% of FEX compared to the labeled drug amount). Eight replicate injections of each sample were made.

RESULTS AND DISCUSSION

Separation in Free Solution

FEX, possessing both acidic and basic moieties, was analyzed in cationic as well as anionic regime of the

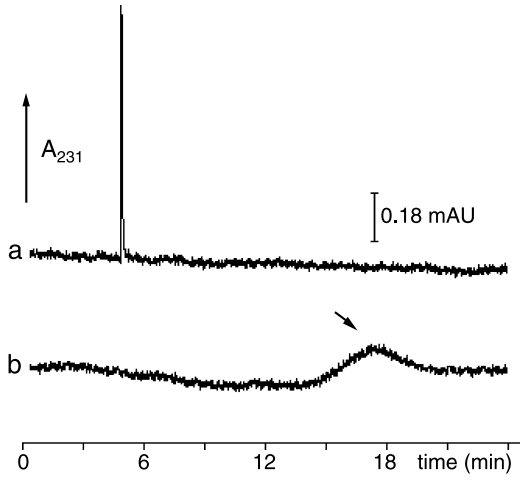

FIGURE 2 **CE Analysis of FEX in Free Solution at Different pH. (a) The Cationic Analysis was Carried Out in ES 1 and Driving Current 150 μA. (b) The Anionic Analysis was Carried Out in ES 3 and Driving Current 80 μA (the Concentration of the Analyte in the Sample was 1 mg/ml). No Peak was Obtained in ES 2 up to 30 minutes.**

separation. The main optimizing parameters of separation in free solution were carrier ion (type, concentration) and pH of the buffer.

The experiments revealed that a cationic regime of the separation and acidic pH (3.2) were convenient for the analysis of FEX (Fig. 2a). On the other hand, alkalic pH (9.0) was less convenient as deprotonated analyte probably tended to adsorb more strongly (Fig. 2b). At pH 5.2, the migration velocity of the analyte was near to zero due to its zwitterionic nature and it could not be analyzed in free solution (see legend to Fig. 2). Although an increase of H^+ or OH^- in the separation electrolyte enhanced ionization/migration of the analyte, increasing conductivity of the electrolyte and Joule heat generated limited this approach.

Slowly migrating organic ions like glycine or 4-morpholineethanesulfonic acid (MES) (carrier ions) and 1,3-bis[tris(hydroxymethyl)methylamino]propane (BTP), ε-aminocaproic acid (ε-ACA), or acetic acid (counterions) were used as buffer constituents (electrolyte systems ESs 1–3, Table 1) instead of fast migrating inorganic ions (e.g., phosphates, hydrochloric acid, sodium hydroxide) in order to minimize electromigration and thermal dispersion effects. From the same reason, lower concentrations of the carrier ions were preferred (a concentration range tested was 20–50 mmol/l).

An optimized method for the free solution CE analysis of FEX was based on the use of ES 1 in cationic regime of the separation. It provided good migration and detection of the analyte and high separation efficiency (Table 2).

Separation in Presence of CD Additives as Analyte Carriers

The low mobility buffers, tested in the first part of our work, acquitted well in analyte carrier separation systems as they effectively compensated increased conductivity of such systems after an addition of highly charged CD derivatives.

Highly substituted CD derivatives (CE-β-CD, S-β-CD) were used as analyte carriers in order to provide transport of FEX to the detector at mediate pH. It was demonstrated that CDs studied formed stable

TABLE 1 **Electrolyte Systems**

Parameter	ES 1	ES 2	ES 3
Solvent	Water	Water	Water
Carrier ion	Glycine	MES	Glycine
Concentration (mmol/l)	25	25	25
Counter ion	Acetic acid	ε-ACA	BTP
pH	3.2	5.2	9.0
EOF suppressor	m-HEC	m-HEC	m-HEC
Concentration (%, w/v)	0.2	0.2	0.2

	ES 4	ES 5	ES 6	ES 7
Electrolyte	ES 2	ES 3	ES 2	ES 3
Complexing agent	CE-β-CD	CE-β-CD	S-β-CD	S-β-CD
Concentration (mg/ml)	5.0	5.0	0.5–5.0	0.5–5.0

TABLE 2 **Effect of the Separation Conditions on the Separation Efficiency and Peak Height of FEX**

Electrolyte system	Separation efficiency[a]	Relative peak height (%)[b]
ES 1	37 390[e]	100.00
ES 2[c]	—	—
ES 3	~0	~0
ES 4	2 593	30.77
ES 5	5 483	50.12
ES 6*	18 791[e]	77.13
ES 7[d]	5 052	36.20

[a]The separation efficiency (N) of the CE system was estimated using the equation $N = 5.54(t/w_{0.5})^2$, where t is the migration time of the maximum of the peak and $w_{0.5}$ the width of the peak at 50% peak height (Heiger, 2000).

[b]A peak height obtained in ES 1 was considered to be 100% and it served as a reference for the calculations.

[c]FEX did not migrate.

[d]Concentration of CD was 5 mg/ml.

[e]The conditions for the optimal efficiencies were indicated by Ohm's law plots (Heiger, 2000). The dependence between controlled current and corresponding voltage was tested in the range of 50–250 μA [optimized currents were 150 μA (ES 1) and 200 μA (ES 6*)]. A disproportionate increase in voltage (separation efficiency) with current indicated a temperature increase and thus, possible temperature gradient affecting the separation efficiency.

inclusion associates with the analyte so that it could be successfully transferred and detected. The stability of the associates depended on CD type and ionization properties of both interacting partners.

The experiments clearly showed that the effectivity of CE-β-CD as a PHM carrier significantly increased with its effective charge so that the useful range for its use was at higher pH (Fig. 3 and Table 2). On the other hand, permanently charged S-β-CD could be

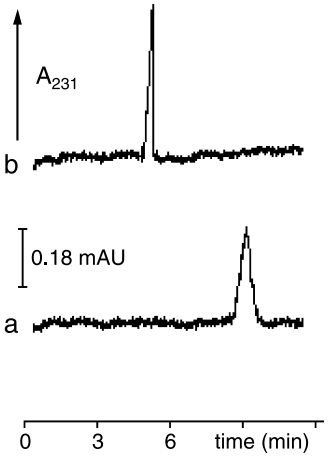

FIGURE 3 **CE Analysis of FEX with CE-β-CD as an Analyte Carrier at Different pH. The Separations were Carried Out in (a) ES 4 (100 μA) and (b) ES 5 (150 μA).**

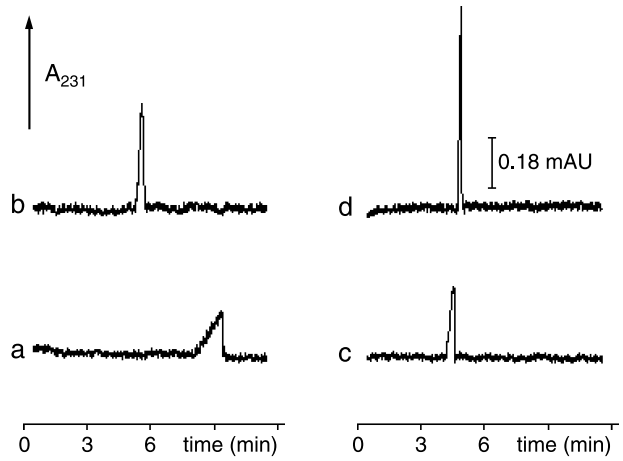

FIGURE 4 **CE Analysis of FEX with S-β-CD as an Analyte Carrier Present at Various Concentrations and Different pH. The Separations were Carried Out in ES 7 and (a) 0.5 mg/ml S-β-CD (100 μA), (b) 5.0 mg/ml S-β-CD (200 μA) and ES 6, and (c) 2.5 mg/ml S-β-CD (200 μA), (d) 5.0 mg/ml S-β-CD (200 μA).**

used universally as it provided successful migration of the analyte in wide range of pH (Fig. 4).

The migration of the analyte and the separation efficiency significantly increased with the concentration of S-β-CD (compare the lower and the upper traces in Fig. 4). A concentration range useful for the analysis of FEX in hydrodynamically closed CE separation system (~3.0–5.0 mg/ml) was limited by dispersion effects, including 1) thermal dispersion at higher concentrations of S-β-CD and 2) diffusion and/or adsorption dispersion at lower concentrations of S-β-CD. The lower separation efficiency with S-β-CD at higher pH (see Table 2 and compare electropherograms b and d in Fig. 4) could be explained in terms of a lower stability of inclusion associate due to the suppressed protonation of FEX.

Optimized conditions, suitable for a highly effective analysis of FEX in analyte carrier hydrodynamically closed CE system, consisted of ES 6 and 5 mg/ml S-β-CD (ES 6*).

Validation

After optimization of the separation conditions, some analytical characteristics of the developed CE methods were investigated using standard solutions (validation samples), as given in the Experimental section. The parameters involved were sensitivity, linearity, precision (run-to-run, day-to-day), and accuracy. In all cases, the peak areas of the peaks were corrected to their migration times to compensate for their

TABLE 3 Precision Data for Repeat Injections of FEX

	RSD (%), n = 8			
	Run-to-run		Day-to-day	
Factor	ES 1	ES 6*	ES 1	ES 6*
Migration time	0.51	0.82	0.77	1.14
Peak area	1.02	1.43	1.44	1.96

TABLE 4 Determination of FEX in Tablets[a]

	ES 1			ES 6*		
Parameter	1	2	3	1	2	3
Purity (%)[b]	99.3	99.8	98.6	98.8	99.5	99.1
RSD (%), n = 8	0.84	0.96	0.87	1.38	1.48	1.51

[a]FEX was determined in 3 different batches of Telfast.
[b]Relative response data used in calculations were obtained using SAL as an internal standard.

differential detector residence times. The validation data given below were obtained using ES 1 and ES 6*.

The detection limits (estimated as 3σ) of FEX using ES 1 and ES 6* were 7.34 and 9.86 μg/ml while the quantitation limits (estimated as 10σ) were 24.47 and 32.87 μg/ml, respectively. The concentration of the analyte in validation sample, corresponding to the limit of quantitation, was determined with acceptable precision (average RSD = 1.19% and 1.62%, $n = 8$) and accuracy (absolute relative error 2.23% and 2.65%, $n = 8$) under the stated conditions.

The linearity of detector response (peak area) for FEX was assessed over the range 30–100 μg/ml. This represented an interval suitable for evaluation of the drug in the analyzed tablets. The straight-line equations and determination coefficients for FEX using ES 1 and ES 6* were $y = 0.0024 + 0.0301x$, $R^2 = 0.9986$, and $y = 0.0019 + 0.0243x$, $R^2 = 0.9986$, respectively.

The method was validated by evaluation of run-to-run and day-to-day precision. The results given in Table 3 clearly indicated that CE separations in a hydrodynamically closed system provided highly reproducible migration and response data. Here, the high precision obtained can be attributed mainly to the suppression of non-separative transport processes and precise injection of the sample.

Application

The validated methods were successfully applied for the determination of FEX in pharmaceutical tablets, as given in Table 4. The contents of FEX obtained by the proposed methods were in a good agreement with those declared. Quantitative estimations of the drug brought consistent results and the absolute differences between determined and declared values ranged from 0.2 to 1.4% (ES 1) and from 0.5 to 1.2% (ES 6*).

The results from the recovery test are presented in Table 5. The mean absolute recoveries using ES 1 and ES 6*, determined by adding known amounts of FEX reference substance to the sample at the beginning of the process, were found to be 98.65% and 98.54%, respectively. Corresponding means of absolute relative errors, 1.35% (ES 1) and 1.46% (ES 6*), indicated good accuracy of the both proposed methods.

The electropherogram from the analysis of pharmaceutical sample is in Fig. 5. No detection interferences from the sample solvent, impurities, and dosage form excipients were occurred separating FEX and SAL as an internal standard at a 231 nm detectection wavelength.

TABLE 5 Recovery of Drug from Samples with Known Concentrations

Amount of standard (μg/ml)				
Added	Found		Recovery (%), $n = 8$	
ES 1, ES 6*	ES 1	ES 6*	ES 1	ES 6*
40.00	39.09	38.99	97.73	97.48
50.00	49.78	49.46	99.56	98.92
60.00	59.20	59.54	98.67	99.23

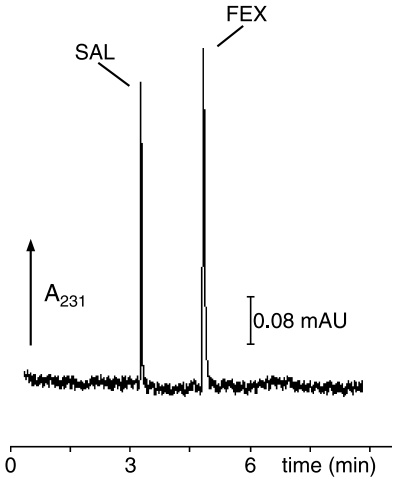

FIGURE 5 Electropherogram from the Determination of FEX in Commercial Pharmaceutical Preparation (tablets Telfast). The Separation was Carried Out in ES 1 (150 μA) in Presence of Internal Standard (SAL). For the Sample Preparation and Other Conditions See the Experimental Section.

CONCLUSION

This work showed that CE is a highly effective analytical tool for the separation and determination of FEX in commercial pharmaceuticals. It was demonstrated that this biologically active compound of zwitterionic nature could be successfully analyzed as the ionic form with the own electrophoretic mobility as well as the zwitterionic form moving with the aid of suitable analyte carriers (here, highly charged CD derivatives). The separation methods proposed, differing each other in the separation mechanism, brought consistent results. The hydrodynamically closed CE separation system provided high precision and accuracy of the analyses, reasonable separation efficiency, and short analysis time, so that it is convenient for routine assay of FEX in pharmaceuticals. The method based on CDs should be advantageous in situations when a different selectivity and/or specific pH of the analysis are required.

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