Analysis of Oseltamivir in Tamiflu® Capsules Using Micellar Electrokinetic Chromatography

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Received January 24, 2008; accepted August 14, 2008; published online September 16, 2008

A simple and rapid micellar electrokinetic chromatography (MEKC) method was developed for the analysis of an antiviral drug, oseltamivir, and its hydrolyzed product in Tamiflu® capsules. Background electrolytes consisted of boric acid 10 mM, pH 10, and sodium dodecyl sulphate (SDS) 40 mM. The limit of detection (LOD) and limit of quantification (LOQ) of oseltamivir were 1.7 and 8.0 μg/ml, respectively. MEKC sweeping in a high electroosmotic flow environment for neutral analytes was also utilized to improve the sensitivity of the assay. In MEKC-sweeping mode, a buffer comprising boric acid 30 mM, pH 10, and SDS 50 mM was used. A 17-fold increase in detection sensitivity was achieved with the MEKC-sweeping method compared with the MEKC mode. Unlike in MEKC, the LOD and LOQ for oseltamivir were 0.1 and 0.3 μg/ml, respectively, using the MEKC-sweeping method. Both methods were successful in determining oseltamivir concentration in its capsule formulation, and the MEKC-sweeping method was capable of determination of the drug at lower concentrations. The hydrolyzed product of oseltamivir (oseltamivir carboxylate) was also detected using the MEKC method. Our observations revealed that the prodrug could be hydrolyzed to the active compound at alkaline pH within *ca.* 60 min.

Key words capillary electrophoresis; micellar electrokinetic chromatography; oseltamivir; Tamiflu®; analysis; sweeping

Oseltamivir (Ro 64-0796 or GS4104) is an ethyl ester prodrug of oseltamivir carboxylate (Ro 64-0802 or GS4071) with the chemical name of ethyl (3*R*,4*R*,5*S*)4-acetylamino 5-amino-3(1-ethylpropoxy)1-cyclohexene-1-carboxylate phosphate (1 : 1). Its chemical structure is shown in Fig. 1. It inhibits neuraminidase activity in influenza A and B viruses. The activity of the enzyme is essential for the spread of infectious virus in the body. Oseltamivir is an effective therapeutic agent against pandemic influenza strains, including H5N1 that caused pandemics during recent years around the world. Since 2003, H5N1 has had a devastating impact on domestic and wild bird populations in many parts of Southeast Asia, Europe, the Middle East, and some parts of Africa. The virus was transmitted to humans, infecting more than 230 people, of whom over 50% died.[1)]

Oseltamivir is prescribed for both the treatment and prevention of influenza and is marketed as its phosphate salt by Roche Company under the trade name, Tamiflu®. Tamiflu® is available in both capsule and oral suspension formulations. In addition, the drug is supplied as an active pharmaceutical ingredient (API) for pandemic stockpiling. The API can be dissolved in water and distributed for widespread use in a pandemic outbreak. Brewster *et al.* showed the bioequivalence of API solution and capsule formulation.[2)] Oseltamivir is rapidly hydrolyzed *in vivo* to oseltamivir carboxylate by hepatic carboxylesterase. Both the prodrug and oseltamivir carboxylate are excreted unchanged in the urine.[3)] Bioassay,[4)] chromatographic methods with fluorescence,[5)] mass spectrometric[6,7)] and UV[8—13)] detection have been described for the analysis of oseltamivir, but to the best of our knowledge, there is no report on the capillary electrophoresis (CE) method for the analysis of oseltamivir. To provide more information on the reported methods to analyze oseltamivir and its hydrolytic product, the related articles are briefly reviewed in chronological order. Eisenberg and Cundy[5)] reported an HPLC method for the analysis of oseltamivir carboxylate in rat plasma using pre-column derivatization with naphthalendialdehyde and fluorescence detection. The recovery, limit of detection (LOD) and limit of quantification (LOQ) of their proposed method were 68%, 20 ng/ml and 50 ng/ml, respectively.[5)] An HPLC-MS method was developed for analyses of oseltamivir phosphate and oseltamivir carboxylate in plasma and urine samples with LOQs of 1 and 10 ng/ml for the prodrug and the active compound, respectively.[6)] A simple and rapid analysis of oseltamivir was reported using the HPLC-UV method in Tamiflu capsules.[8)] Another HPLC-UV method was presented for the analysis of oseltamivir phosphate in Tamiflu® capsules and two generic versions of the drug.[9)] None of these latter methods[8,9)] dealt with the possibility of separation of oseltamivir carboxylate. Lindegårdh *et al.*[7)] developed a solid-phase extraction and gradient HPLC coupled with a tandom mass spectrometer for analyses of oseltamivir phosphate and oseltamivir carboxylate in human plasma, saliva, and urine samples. The reported LOQs for oseltamivir phosphate were 1, 1, and 5 ng/ml, respectively for plasma, saliva, and urine samples. The corresponding values for oseltamivir carboxylate were 10, 10, and 30 ng/ml.[7)] Bahrami and coworkers[10)] developed a solid-phase extraction HPLC-UV method for the determination of oseltamivir carboxylate in human serum. The reported LOD and LOQ were 5 and 15 ng/ml, respectively. The method was successfully applied to a bioequivalence study in 24 healthy volunteers. Fuke *et al.*[11)] developed an HPLC-UV method for the determination of oseltamivir carboxylate in

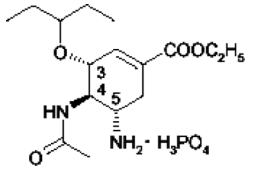

Fig. 1. Chemical Structure of Oseltamivir Phosphate

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biological materials with the LOD of 0.04 ng/ml. They used the method to analyze oseltamivir carboxylate in different biological samples collected during the autopsy of a patient who died after ingestion of 75 mg Tamiflu. The cause of death was falling down from a height of 25 m. Green *et al.*[12] reported simple colorimetric and liquid chromatographic (LC) methods to assay the quality of oseltamivir phosphate in its formulations. The colorimetric assay is based on-ion pair complexes of oseltamivir with Congo red and bromochlorophenol blue which were extracted from aqueous solutions with ethyl acetate. In the LC method, carbonate buffer and acetonitrile (70 : 30) were used as the mobile phase. The analyte was detected using a UV detector at 254 and 220 nm.[12] The latest report dealt with the stability assay of oseltamivir using HPLC.[13] The drug was exposed to acidic, alkaline, and oxidative degradation processes and the solutions were analyzed using a gradient HPLC system. The reported LOD and LOQ were 0.05 and 0.3 ng/ml, respectively.[13]

The main advantages of CE methods are speed, resolution, efficiency, analyte solubility and stability, minimal reagent and solvent consumption, compatibility with mass spectrometry, and the availability of several modes which have all made CE a popular analytical technique in the field of pharmaceutical and biomedical analysis. The applications of various aspects of CE in analysis of small-molecule pharmaceuticals were reviewed by Altria and coworkers.[14] There are many advantages to developing a CE-based analytical method for analysis of drugs. This is more evident in investigations of purity analysis of raw materials, degraded drugs in pharmaceutical formulations, and of drug metabolism. In such cases, the analytes that should be simultaneously analyzed in the presence of the main drug are compounds structurally related to the main drug for which a single analytical technique can not provide complete separation of the analytes, and therefore different analytical methods employing various separation mechanisms should be employed by pharmaceutical analysts. Although HPLC is the main analytical method recommended by different pharmacopeia, other techniques are valid, especially when the orthogonality concept is considered.[15] Orthogonality can be achieved when the findings of analytical techniques with different mechanism are combined, *e.g.*, CE with HPLC.

Poor mass sensitivity is often an undesirable characteristic of CE employing optical detectors. There are two possibilities to overcome this limitation. i) The first approach is to use the detection methods with higher sensitivity than the most common detection method, *i.e.*, UV absorption. This is the case *e.g.* by using laser-induced fluorescence or mass spectrometric detection. The first method is directly applicable only in rare cases—most pharmaceuticals have no intrinsic fluorescence and necessitate thus derivatization—mass spectrometry can be directly used, whereas it needs sophisticated instrumentation. (ii) The second possibility to overcome the low mass sensitivity is to concentrate the analytes. This can be carried out with the advantage of on-column sample stacking methods prior to separation which is recently reviewed by Simpson and co-workers.[16]

Oseltamivir is a fairly polar drug ($\log P = 0.36$), while oseltamivir carboxylate is highly polar with $\log P$ value of -2.10.[17] The difference in the polarity of the analytes makes them suitable compounds for analysis using MEKC. As noted above, no published report on CE analysis of this drug is available in the literature. The purposes of this study are to present the MEKC and MEKC-sweeping methods for the analysis of oseltamivir and its hydrolyzed product in a pharmaceutical formulation. The LOD and LOQ of MEKC method were improved using the sweeping technique.

Experimental

Chemicals and Solutions Oseltamivir was kindly donated by Starway Company (Shenzhen, China). Sodium diclofenac and atenolol were gifts from Sobhan Pharmaceutical Company (Rasht, Iran). Sodium dodecyl sulphate (SDS) and boric acid were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). Sodium hydroxide was purchased from Merck (Darmstadt, Germany). Tamiflu® capsules were donated by the Health Services Section of Tabriz University of Medical Sciences, Iran. Stock solution (2 mg/ml) of oseltamivir was freshly prepared in distilled water prior to use. Stock solutions of sodium diclofenac (1 mg/ml) and atenolol (2 mg/ml) as internal standards (IS) were prepared in distilled water and used in the MEKC and MEKC-sweeping methods, respectively. Both stock solutions were stored at +4 °C. Stock solution of oseltamivir carboxylate was prepared by dissolving a suitable quantity of the drug in NaOH 0.1 M (pH 13) to obtain a solution of 2 mg/ml. For MEKC method, standard solutions were prepared daily by diluting stock solutions with 10-fold diluted background electrolyte (BGE) to yield final concentrations of oseltamivir 20 μg/ml and sodium diclofenac 10 μg/ml. Standard solutions for the MEKC-sweeping method were prepared from stock solutions using BGE without SDS and contained oseltamivir and atenolol at concentrations of 2 μg/ml each.

Tamiflu® Capsule Content Preparation The capsule content preparation procedure for analysis was similar to that described by Lindegårdh *et al.*[8] In brief, one capsule of oseltamivir (Tamiflu®) was transferred to a 50 ml volumetric flask and approximately 40 ml of deionized water was added. The volumetric flask was sonicated at 40 °C for 28 min to dissolve the capsule completely. Approximately 9 ml of deionized water was added and the solution was left to cool to room temperature (about 30 min) before the flask was filled up to the mark to yield a theoretical concentration of 1.5 mg/ml of oseltamivir. The flask was inverted a few times to mix the solution, and 1 ml was then transferred to an Eppendorf cup and centrifuged at 13000 rpm for 8 min on a microcentrifuge. Then the standard solutions were obtained by dilution of the aliquot with 10 fold diluted BGE (MEKC method) and BGE without SDS (MEKC-sweeping method).

Apparatus and MEKC Analyses All analyses were performed using a Prince capillary electrophoresis (Prince Technologies B.V., Emmen, the Netherlands) equipped with a Jasco CE-971 UV/Vis detector (Jasco Corporation, Tokyo, Japan) adjusted to 214 nm. The digital outputs of the detector were collected using Teifgostar software (Teifgostar Co., Tehran, Iran) for further numerical analyses. Separation column was an uncoated fused-silica capillary (50 μm ID, 375 μm OD; GL Sciences Inc., Japan) with total length of 90 cm and effective length of 72 cm. For MEKC, sample injections were hydrodynamically conducted at 100 mbar for 18 s. In the MEKC-sweeping mode, the samples were injected at 250 mbar for 1 min. A voltage of +30 kV was applied and the temperature was 25 °C. BGE was prepared using boric acid and NaOH 2 M containing various concentrations of SDS and then filtered through a membrane filter (GHP, 0.45 μm, 47 mm, Pall Corporation). Before each run, the capillary was rinsed with NaOH 0.1 M, water, and running buffer for 3.5 min each. The new capillary was conditioned with NaOH 1 M, water, and BGE for 20 min each.

Results and Discussion

MEKC Method Since oseltamivir is a basic drug with pK_a of 7.75—8.80,[8] it is assumed to be less charged at pH values above its pK_a. Series of borate buffers with pH values between 9.0—10.5 were applied for oseltamivir analysis. The migration times were decreased with increasing pH values, alyhough bubble formation was observed at pH values higher than 10, for which reason pH 10 was selected as the optimum pH for this study.

Ionic strength of the electrolyte affected the resolution and migration time of the analytes. To study this effect, borate

buffer concentrations of 5 and 10 mM were evaluated in order to obtain shorter migration times with suitable repeatability. The results indicated that at a low borate buffer concentration (5 mM), repeatability was poor whereas at the buffer concentration of 10 mM reproducibility was acceptable and analyte and IS peaks were well resolved.

SDS concentrations of 15, 40, and 50 mM were evaluated to improve resolution. Although shorter migration times were achieved with low SDS concentrations, repeatability was drastically decreased. When a BGE with SDS 50 mM was applied to the separation, migration times were increased with no improvement in the resolution of the peaks. Therefore the SDS concentration of 40 mM which yielded better resolution in an appropriate time was employed for further analyses.

To study the influence of sample solvent on peak shape, two stock solutions were prepared in methanol and water. Standard solutions were prepared using methanol, water and 10-fold diluted BGE from each stock solution. Although a small reduction in migration time was observed using methanolic sample solution, the peak heights were markedly decreased.

The shortest migration time was obtained with aqueous sample from each stock solution, but a tailing in the oseltamivir peak was also observed. The most favourable results were obtained when standard solution was prepared by dilution of aqueous stock solution with 10-fold diluted BGE.

Validation of the method was carried out according to ICH guidelines.[18] The calibration plot of oseltamivir standard solution was linear in the range of 5.0—70.0 μg/ml ($r^2=0.99$). The LOD was 1.7 μg/ml with a signal-to-noise ratio of 3 : 1 and the LOQ calculated with a signal-to-noise ratio of 10 : 1 was 5.0 μg/ml. Relative standard deviation (RSD) values of peak normalized areas [oseltamivir area/diclofenac (IS) area] and migration times were calculated for repeatability and intermediate precision (for details, see Table 1).

To check the capability of the proposed method to separate oseltamivir and its hydrolyzed product, a 2 mg/ml stock solution was prepared by dissolving a suitable amount of oseltamivir in NaOH 0.1 M (pH 13), the standard solution was then prepared by diluting stock solution with 10-fold diluted BGE and injected at different time intervals. As seen in Fig. 2, the peak height of oseltamivir appearing at 13.5 min was reduced while that of the hydrolyzed product (appearing at 7 min) was increased, indicating the hydrolysis rate of oseltamivir. The required time for complete *in vitro* hydrolysis of oseltamivir at pH 13 is 1 h, as shown in Fig. 3. To check the stability of the hydrolyzed product, the peak characteristics (area and height) of the oseltamivir hydrolyzed product in a standard solution was evaluated on two different days and the results showed that this product is stable at high pH.

MEKC-Sweeping Method The MEKC method was applied successfully to determine oseltamivir, but its sensitivity was low as expected from the UV detectors, and thus it was necessary to improve the sensitivity of the method. Oseltamivir is a basic polar molecule, and therefore the applicability of three common methods, the reversed electrode polarity stacking mode (REPSM), stacking with reverse migrating pseudo-stationary phase (SRMP), and MEKC-sweeping method at a high electroosmotic flow (EOF) environment for

Table 1. Repeatability and Intermediate Precision Data for Oseltamivir in MEKC and MEKC-Sweeping Methods

	MEKC		MEKC-sweeping	
	35.0 μg/ml		1.0 μg/ml	
	Normalized area	Migration time	Area	Migration time
Repeatability (RSD)	1.14	1.70	1.70	0.21
Intermediate precision (RSD)	1.09	1.68	3.94	4.51

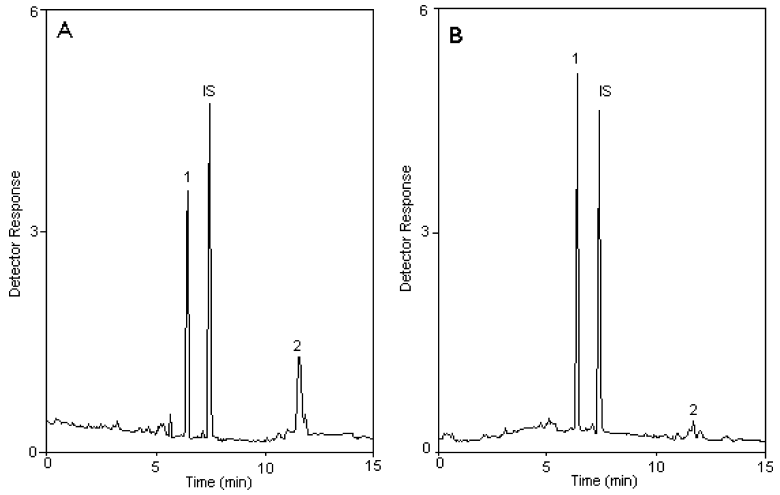

Fig. 2. Electropherograms of Oseltamivir and Its Hydrolyzed Product in Two Subsequent Injections Showing Oseltamivir Conversion to Its Hydrolyzed Product

(A) Electropherogram obtained immediately after mixing fresh oseltamivir and its hydrolyzed product. (B) Electropherogram of the same solution after 30 min. BGE: boric acid 10 mM, pH 10, SDS 40 mM. Sample: 1. Oseltamivir hydrolyzed product 20 μg/ml. 2. Oseltamivir 20 μg/ml diclofenac 10 μg/ml (IS).

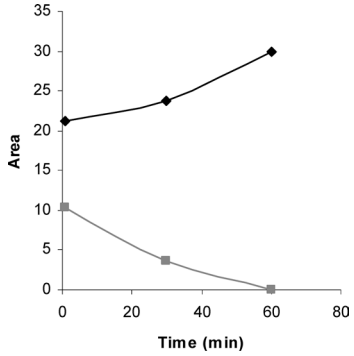

Fig. 3. Time Profile of Oseltamivir (■) Conversion to Its Hydrolyzed Product (◆)

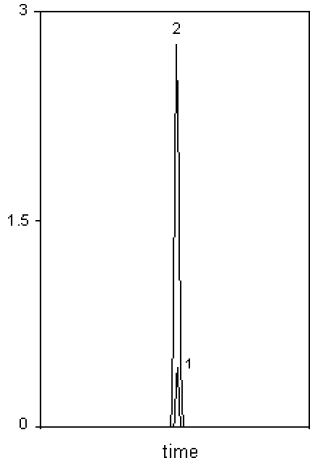

Fig. 4. Overlay of Electropherograms

Oseltamivir 2 μg/ml in 10 times diluted BGE of boric acid 30 mM, pH 10.0, peak 1: MEKC mode, BGE: boric acid 10 mM, pH 10.0, SDS 40 mM. Peak 2: MEKC-sweeping mode, BGE: boric acid 30 mM, pH 10.0, SDS 50 mM.

neutral analytes[19—21] were investigated to increase the sensitivity of the method.

In the REPSM, BGE contains a high-conductivity micellar buffer and the sample solvent is a buffer with less conductivity than BGE. After sample injection, negative polarity is applied, and the EOF moves toward the inlet and pumps out the sample solvent from the capillary. This polarity permits the anionic analytes to move toward the outlet and stack at one side of the boundary, although the cations and neutral species tend to move to the inlet and then exit the capillary. When the electrophoretic current reaches 95–99% of its original value, the voltage is quickly switched to positive polarity and the separation of the analytes occurs in the MEKC mode. Although the polarity switching procedure removes a large volume of sample matrix, a delicate operation is required to monitor the current carefully, or otherwise poor reproducibility will be obtained.[19—21] In this study, the REPSM method was studied for oseltamivir enrichment, but the reproducibility was not acceptable since in several injections, various quantities of neutral oseltamivir were removed from the capillary during negative voltage application.

When applying stacking with the SRMP method, an acidic micellar BGE is used to minimize EOF and the voltage is negative.[19—21] This method was also evaluated to improve the sensitivity of the oseltamivir assay. With application of the reversed polarity, oseltamivir, which was a cation at low pH, moved toward the inlet. Anionic micelles pushed the analyte to the outlet, but because of its low retention factor, poor stacking occurred, and SRMP was not suitable for the sensitivity enhancement of oseltamivir.

In the MEKC-sweeping method in a high EOF environment, after injection of a large volume of the analyte prepared in BGE without a pseudostationary phase, a positive voltage is applied and micelles enter the sample zone from the outlet (detector end) and sweep the analytes.[20,21] Preliminary studies with this method showed good results regarding reproducibility and sweeping, and therefore development and validation processes were conducted to make the method suitable for determination of the drug at lower concentrations.

Since diclofenac, the internal standard of the MEKC method, is an anionic drug at high pH values, it would exhibit different behavior in the sweeping process. Atenolol, which is a basic drug with pK_a of 9.6, was therefore selected as the internal standard. The concentration of boric acid in BGE was optimized using buffers containing boric acid 10, 30, and 60 mM, among which 30 mM yielded the best resolution of analytes. The effect of SDS concentration on the degree of sweeping was also investigated. When using SDS 50 mM, the migration times were shorter and the peak heights were higher when compared with SDS concentrations of 75, 100, and 150 mM.

To obtain the best injection condition, constant pressure of 250 mbar was applied during injections for 0.50, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, and 4.00 min. With increasing injection time, the migration time and resolution were decreased, resulting in broader peaks, while the area-to-height ratio was increased. Based on these results, the injection condition of 250 mbar for 1 min which filled approximately 14 cm of the capillary was selected for further investigations.

The BGE for this purpose consisted of boric acid 30 mM, pH 10, with SDS 50 mM and yielded a 17-fold enrichment in oseltamivir detection (Fig. 4). The main limitation of the MEKC-sweeping method is that the hydrolyzed product of oseltamivir could not be concentrated because of its different chemical characteristics and higher polarity ($\log P = -2.1$) compared with oseltamivir ($\log P = 0.36$).

Calibration curve for oseltamivir was obtained in the concentration range of 0.05—2.00 μg/ml. The LOD obtained considering a signal-to-noise ratio of 3 : 1 was 0.10 μg/ml. The LOQ was 0.30 μg/ml based on a signal-to-noise ratio of 10 : 1.[18] The RSD of migration time and peak area of oseltamivir in different injections were determined for repeatability and intermediate precision. The results are presented in Table 1. A summary of the analytical methods from the literature and the results from this work are presented in Table 2.

Application of the Methods to Analysis of Tamiflu® Capsules The solutions prepared from Tamiflu® capsules as described in Materials and Methods showed good recovery (*ca.* 100%) with both the MEKC and MEKC-sweeping methods. Figure 5 illustrates the typical electropherograms of oseltamivir in capsules obtained using both methods. The sensitivities of the MEKC and MEKC-sweeping methods using UV detection for the determination of oseltamivir in Tamiflu® capsules were higher than that of HPLC method

Table 2. Summary of Analytical Methods for Determination of Oseltamivir in Different Matrices from the Literature and Present Work

Method	Analyte and sample	Mobile phase	Stationary phase	Detection	LOD	LOQ	Retention time	Ref.
SPE-HPLC	Oseltamivir carboxylate in rat plasma	Acetate buffer + acetonitrile (73 : 27)	ODS-2 150×4.6 mm, 5 μm	F, ex 420, em 472, Naphthalenedialdehyde	20 ng/ml	50 ng/ml	5.2 min	5
SPE-HPLC	Oseltamivir carboxylate and oseltamivir phosphate in human and animal serum and urine	Formate buffer +methanol (50 : 50)	Nova-Pak CN 100×5 mm, 4 μm	MS		Oseltamivir carboxylate in plasma 10 ng/ml, oseltamivir carboxylate in urine 30 ng/ml, OP in plasma 1 ng/ml and OP in urine 5 ng/ml	Ooseltamivir carboxylate: 3.5 min, oseltamivir phosphate 5 min	6
SPE-HPLC	Oseltamivir phosphate and oseltamivir carboxylate in human plasma, saliva and urine	Gradient flow with ammonium acetate buffer+acetonitrile	ZIC-HILIC 50×2.1 mm, 5 μm	MS	Oseltamivir carboxylate in plasma 2.5 ng/ml, oseltamivir carboxylate in saliva 2.5 ng/ml, oseltamivir carboxylate in urine 7.5 ng/ml, oseltamivir phosphate in plasma 0.25 ng/ml oseltamivir phosphate in saliva 0.25 ng/ml, oseltamivir phosphate in urine 1.25 ng/ml	Oseltamivir carboxylate in plasma 10 ng/ml, oseltamivir carboxylate in saliva 10 ng/ml, oseltamivir carboxylate in urine 30 ng/ml, oseltamivir phosphate in plasma 1 ng/ml oseltamivir phosphate in saliva 1 ng/ml, oseltamivir phosphate in urine 5 ng/ml		7
HPLC	Oseltamivir phosphate in formulations	Phosphate buffer+methanol (50 : 50)	Hypersil Gold 150×4.6 mm	UV 220 nm	—	—	6.4 min	8
HPLC	Oseltamivir phosphate in formulations	Formate buffer+methanol (50 : 50)	Zorbax CN 150×4.6 mm, 5 μm	UV 226 nm	—	—	3.4 min	9
SPE-HPLC	Oseltamivir carboxylate in human serum	Phosphate buffer (+triethylamine) +acetonitrile (70 : 30)	Shimpack CLC-ODS 150×4.6 mm, 5 μm	UV 215 nm	5 ng/ml	15 ng/ml	9.1 min	10
SPE-HPLC	Oseltamivir carboxylate in different biological materials	Phosphate buffer +acetonitrile (92 : 8)	XTerra MS C_{18} 150×2.1 mm, 3.5 μm	UV 230 nm	0.04 μg/ml	0.1 μg/ml in blood	18.8	11
HPLC	Oseltamivir phosphate in formulations	Bicarbonate buffer +acetonitrile (70 : 30)	C_{18}	UV 254 and 220 nm	4.2 ng at 254 nm and 2.2 ng at 220 nm	—	4 min	12
HPLC	Oseltamivir phosphate in formulation (stability indicating assay)	Gradient flow with acetonitrile+triethylamine buffer (pH 3.0)	Kromasil C_{18} 250×4.6 mm, 5 μm	UV 215 nm	0.05 μg/ml	0.3 μg/ml	10.6 min	13
Colorimetry	Oseltamivir phosphate in formulations	—	—	Congo red (507 nm), Bromochlorophenol (589 nm)	—	—	—	12
MEKC	Oseltamivir phosphate in formulations, oseltamivir carboxylate in standard solutions	Borate buffer+SDS	Fused silica 90 cm×50 μm (72 cm effective length)	UV 214 nm	1.7 μg/ml	5.0 μg/ml	Oseltamivir carboxylate 7 min, OP 13.5 min	This work
MEKC-sweeping	Oseltamivir phosphate in formulations	Borate buffer+SDS	Fused silica 90 cm×50 μm (72 cm effective length)	UV 214 nm	0.10 μg/ml	0.30 μg/ml	Ooseltamivir phosphate 13.5 min	This work

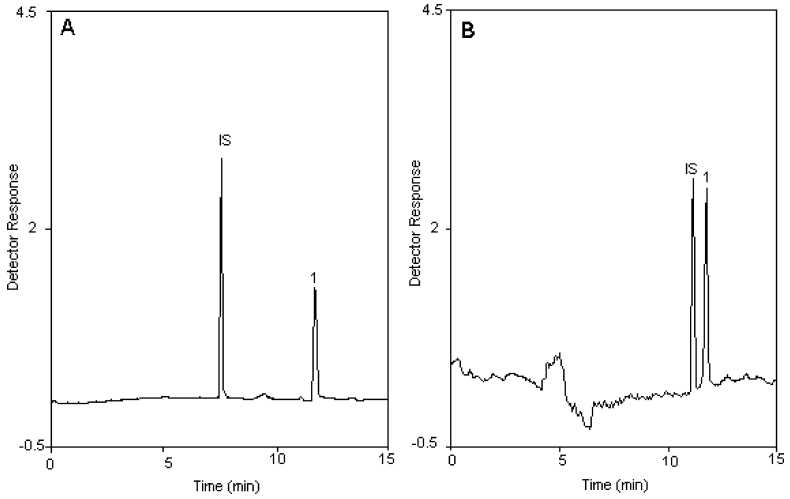

Fig. 5. Electropherogram of Oseltamivir in Tamiflu®

(A) MEKC mode: oseltamivir 20 μg/ml and diclofenac 10 μg/ml (IS). (B) MEKC-sweeping mode: oseltamivir 2 μg/ml and atenolol 2 μg/ml (IS).

with the same detection technique.[8)]

Conclusion

The MEKC mode using BGE of boric acid 10 mM, pH 10, and SDS 40 mM was able to detect oseltamivir and its pharmacologically active hydrolyzed product, oseltamivir carboxylate. In addition, compared with the MEKC mode, under optimized MEKC-sweeping conditions consisting of boric acid 30 mM, pH 10, and SDS 50 mM, a 17-fold improvement in sensitivity was achieved. This enrichment allowed us to determine oseltamivir concentrations of greater than 0.30 μg/ml with UV detection which could be considered as an important issue in the practical applications of CE-based methods. The proposed methods were successfully applied to the analysis of oseltamivir in Tamiflu® capsules. However, the hydrolyzed product of oseltamivir, oseltamivir carboxylate, was detectable only with the MEKC method. The proposed method was partially validated, and full validation is required to make it a suitable analytical method to be used in the pharmaceutical industry. The obtained results were comparable with the results of HPLC analyses of pharmaceutical formulations with the general advantages of the CE methods noted in the introduction. In addition, the developed method is compatible with green chemistry since no organic solvents were used throughout the analysis.

Acknowledgments Financial supports from the Drug Applied Research Center and Zakaria Pharmaceutical Company (grant no. 85—91) is gratefully acknowledged. The authors would like to thank Prof. Kenndler and Dr. Uboh for their helpful comments.

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