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Short communication

Development and validation of a rapid capillary electrophoresis method for the determination of oseltamivir phosphate in Tamiflu® and generic versions

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ABSTRACT

A rapid and reliable capillary zone electrophoresis method was developed and validated for the assay of oseltamivir phosphate in capsules. Separation was carried out in fused silica capillary (60.2 cm total length and 10.0 cm effective length, 75 μm i.d.) by applying a potential of −15 kV at 25 °C. The selected electrophoretic buffer consisted of 50 mM sodium phosphate, pH 6.3 (direct UV detection, 226 nm). A short electrophoretic analysis time (less than 1.5 min) was obtained using the short end injection mode. The method was validated in terms of specificity, linearity, precision and accuracy. The RSD values were 0.94 and 0.98% for repeatability and intermediate precision, respectively. Recovery determinations allowed the calculation of a confidence interval from 98.64 to 100.26% with a relative standard deviation value of 0.38%. LOD and LOQ were estimated at 0.97 and 3.24 μg/mL, respectively. The validated method was successfully applied to the determination of oseltamivir in three commercially available capsules (Tamiflu®, Saiflu® and Flufy®). The results were in good agreement with those obtained by a HPLC method previously developed in our laboratory. This method presents advantages including short run time, simple and rapid sample preparation and no use of non-aqueous solvent throughout the analysis.

1. Introduction

Oseltamivir phosphate (OP) belongs to a new class of drugs termed neuraminidase inhibitors, which are active against both influenza viruses type A and B [1–5]. OP is a prodrug that is subsequently metabolized via hepatic esterases into oseltamivir carboxylate, the active form. The phosphate salt enables to develop effective oral treatment which is recommended both for treatment and prophylaxis of influenza. The recommended doses for adults according to World Health Organization (WHO) are 75 mg twice a day for 5 days (treatment) and 75 mg once a day for at least 7 days (prophylaxis) [6].

OP was developed by Gilead Sciences and introduced into the market by Hoffmann La Roche. Large orders hit the market in a short time because of worldwide epidemic of influenza with significant morbidity and mortality. As Roche pharmaceuticals was unable to supply the orders, some laboratories were allowed to produce their own version under a sub-licensing agreement. So, OP is available as 75 mg capsules and a 12 mg/mL suspension under the brand name Tamiflu® and several generic names.

The analytical determination of OP has been carried out by colorimetry [7], by HPLC using UV detection [7–13], fluorescence detection [14,15] or MS detection [16–18]. Micellar electrokinetic chromatography (MEKC) was recently applied [19].

The aim of this work was to carry out a rapid determination of OP in capsules by capillary zone electrophoresis (CZE) which is now well established as an analytical technique for the assessment of small pharmaceutical molecules [20]. Short end injection technique is envisaged to shorten analysis time [21–23]. The proposed method was validated and successfully applied to the determination of OP in pharmaceutical formulations.

2. Experimental

2.1. Chemicals

Na_2HPO_4, $2H_2O$ and H_3PO_4 85% were Ph. Eur. grade (Merck, Darmstadt, Germany). Phenoxyethanol and lidocaine were purchased from Sigma (St Quentin Fallavier, France). OP standard sample was provided by Heterodrugs (Mumbai, India). Ultra pure water was used for buffer solutions preparation and assays.

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Tamiflu® capsules were purchased from a pharmacy. Generic versions, Saiflu® and Flufy® capsules, were supplied by Saidal Group (Algerian Pharmaceutical Industry) and Julphar company (Gulf Pharmaceutical Industries), respectively. One capsule is labeled as containing 75 mg oseltamivir free base (i.e. 98.5 mg OP).

2.2. Apparatus and method

Electrophoresis was carried out on a P/ACE System MDQ equipped with a diode array detection system from Beckman Coulter (Fullerton, CA, USA). A P/ACE station Software package (32 Karat version 5.0) was used to control the system. An uncoated fused silica capillary with a total length of 60.2 cm × 75 μm i.d. from Agilent was used. Samples were hydrodynamically injected into the capillary for 5 s at 0.5 psi at the detector side. Consequently, the effective length of the capillary was 10 cm. The capillary was pre-conditioned prior to its first use by successively conducting a 5-min rinse with 0.1 M HCl, a 2-min rinse with water, a 10-min rinse with 0.1 M NaOH, a 2-min rinse with water and finally a 20-min rinse with electrolyte buffer. Separations were carried out at −15 kV at 25 °C using a 50-mM sodium phosphate buffer, pH 6.3. UV detection was carried out at 226 nm. Conditioning between runs consisted of a 2-min rinse with phosphate buffer. Buffer solutions were used within one week and filtered using 0.45 μm micro syringe filters before use.

2.3. Solutions preparation

2.3.1. Standard solutions

Stock solutions of OP (400 μg/mL) and lidocaine (240 μg/mL) as internal standard (IS) were prepared separately in water. Working standard solutions of OP (60–140 μg/mL) and IS (120 μg/mL) were prepared by diluting stock solutions in water. Both stock and working standard solutions were prepared daily for the validation.

2.3.2. Sample preparation

The contents of one capsule was weighed and transferred into a 1-L volumetric flask. An accurate mass of IS close to 120 mg was added and then 600 mL of water were added. The mixture was sonicated for 10 min and made up to the volume with the same solvent. The use of water as a dissolving agent allowed the sample stacking effect to occur. The sample solution was filtered through a nylon membrane of 0.45 μm and injected in triplicate. Three capsules were evaluated for each batch.

3. Results and discussion

3.1. Preliminary investigation

Since oseltamivir is a basic drug with pK_a 7.75 [24], phosphate buffer at pH 7.5 was firstly used for OP analysis. A short end injection procedure was envisaged to reduce total analysis time. As expected, the migration time was decreased fivefold compared with conventional injection. However, injection of phenoxyethanol as electroosmotic flow (EOF) marker showed that EOF occurred just after the OP peak. The correct resolution between OP and EOF was reached by decreasing the buffer pH value to 6.3. The migration time (t_m) of OP was not altered whereas the t_m corresponding to EOF was increased. Increasing buffer concentration from 20 to 50 mM resulted in better symmetry factor (1.70–1.21). A 50-mM concentration was thus selected leading to a 1.5-min analysis time. Using an electric field of −249 V cm^{-1}, an acceptable current of 90 μA was produced.

Lidocaine (pK_a 8.00) was used as internal standard. Fig. 1C shows the separation of OP and lidocaine. A good resolution was obtained (i.e. *Rs* = 2.8) and the electrophoretic analysis time remained unchanged.

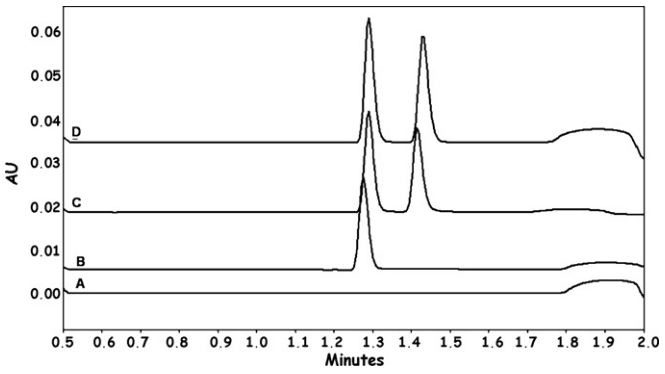

Fig. 1. Electropherograms of placebo (A); IS (120 μg/mL) (B); IS (120 μg/mL) and OP (100 μg/mL) standard solution (C); Tamiflu® solution (98.5 μg/mL of OP) and IS (120 μg/mL) (D). Short end injection (L_{eff} = 10.0 cm; separation voltage −249 V cm^{-1}) using 50 mM sodium phosphate buffer at pH 6.3.

Table 1
R.S.D.% values of t_m and peak areas (n = 40 injections).

	OP	IS
R.S.D.% of t_m	0.88	0.77
R.S.D.% of migration time ratio[a]	0.16	
R.S.D.% of peak area	3.98	2.74
R.S.D.% of peak area ratio[b]	1.59	

[a] The migration time ratio is defined as OP t_m divided by IS t_m.
[b] The peak area ratio is defined as OP peak area divided by IS peak area.

3.2. Validation

The developed CZE method was validated with respect to CZE system repeatability, specificity, linearity, accuracy, precision and limits of detection (LOD) and quantification (LOQ).

3.2.1. CZE system repeatability

CZE system repeatability was determined by injecting several times a standard solution (100 mg/L OP and 120 mg/L lidocaine) using the same set of the two buffer separation vials. Migration times and peak areas do not change significantly up to 40 injections (Table 1). Beyond this number, the electrolyte solution was replenished.

3.2.2. Selectivity

The selectivity of the method was assessed by analysing a placebo solution containing all the components of capsules except OP. The electropherogram (Fig. 1A) was free of any peak between 0.60 and 1.75 min. Both OP and lidocaine migrated in this interval (Fig. 1B and C). Consequently no excipient interferes with analytes of interest and the CZE method was applicable for OP analysis in Tamiflu® and generic drugs as shown in Fig. 1D.

Table 2
Linearity study parameters and regression results.

Linearity	std value	rdf value
Nominal concentration of OP (μg/mL)	98.50	98.50
Concentration range (μg/mL)	60–140	60–140
Regression results		
Determination coefficient (r^2)	0.996	0.997
y-Intercept ±S.D.	0.0053 ± 0.0028	0.0065 ± 0.0042
Slope ±S.D.	0.0085 ± 0.00014	0.0083 ± 0.00012

Table 3
Results obtained for determination of OP in commercial capsules by both CZE and HPLC methods.

Sample	Batch	CZE method			HPLC method	
		OP[a] (mg/capsule) (determined value)	Recovery[b] (%)	R.S.D. (%)	Recovery[b] (%)	R.S.D. (%)
Tamiflu®	B1078	97.5	99.0	1.1	100.5	1.5
	B1065[c]	94.8	96.2	1.5		
Saiflu®	N°001	100.4	101.9	0.3		
	N°004	96.7	98.2	0.9	100.9	1.2
Flufy®	N°019	98.7	100.2	0.9	96.8	1.9

[a] Theoretical value of OP is 98.5 mg/capsule (75 mg of free base).
[b] Mean of three determinations on three capsules.
[c] Expired batch.

3.2.3. Linearity, limit of detection and limit of quantification

According to SFSTP validation guideline [25], linearity was tested on 3 different days at 5 concentration levels of OP in the range 60–140 μg/mL. Both standard (std) and reconstituted dosage formulation (rdf) solutions were tested. Regression curves were obtained by plotting the peak area ratio between OP and lidocaine vs. OP concentration (Table 2). Relationships were linear. A *t*-test was applied to statistically determine if the difference between the slopes and the intercepts of std and rdf calibration curves was significant. The *t*-values (0.97 and 0.25, respectively) were found to be less than the critical *t*-value at 95% confidence level (2.06). Therefore, the determination of OP content in capsules can be performed using the std calibration curve.

The limit of detection (calculated as $3\sigma/S$, where σ is the standard deviation of the *y*-intercept and *S* the slope of the analyte calibration curve) and the limit of quantification ($10\sigma/S$) were determined. LOD and LOQ were estimated at 0.97 and 3.24 μg/mL, respectively. These values are better than those obtained by MEKC [19]. Only the use of MEKC-sweeping mode allows to improve the LOD and LOQ values.

3.2.4. Accuracy

Accuracy was estimated from linearity studies of the rdf solutions (60–140 μg/mL). Recovery determinations allowed the calculation of a confidence interval from 98.64 to 100.26% with a relative standard deviation value of 0.38%. As 100% was included in this confidence interval and RSD was lower than 2%, the CZE method was accurate.

3.2.5. Precision

Precision was tested on 6 independent rdf samples at the nominal concentration (98.5 μg/mL) on 3 consecutive days. The RSD values were 0.94 and 0.98% for repeatability and intermediate precision, respectively. They were within the acceptance criteria of 2% and showed that the method is precise.

Better intermediate precision was obtained by CZE compared to MEKC [20], especially when MEKC-sweeping mode is used. In that case, the intermediate precision (RSD) on area values is almost equal to 4%. This high value limits the use of MEKC for analytical control.

3.3. Assays of capsules

The developed CZE method was applied to the determination of OP in various capsules (Table 3). Two batches of Tamiflu® and three batches of generic formulations (Saiflu® and Flufy®) were analyzed. These results are in close agreement with the theoretical contents of OP in capsules except for Tamiflu® B1065 which is an expired batch. They comply with content requirements (98–102%). In all cases, RSD values were lower than 2%, attesting to the good precision of the method. Comparison with the values obtained by the HPLC method previously developed in our laboratory [8] confirmed the reliability of the CZE method since no significant difference on the recovery values with regard to Student's *t*-test (t-test = 0.57 < t° (0.05, 5) = 2.57) was observed. However the advantage of the electrophoretic method is to be environmentally friendly since the analytical procedure is free of non-aqueous solvent.

4. Conclusion

The use of "short end injection" technique in CZE allows a very short analysis time with respect to validation requirements (i.e. selectivity, linearity, accuracy and precision). The results obtained for OP determination in commercial pharmaceutical formulations attest to the precision and the accuracy of the method. Furthermore, low cost and simple sample pretreatment strengthen its potential applicability for routine analysis in the quality control of capsules with an advantage of no organic solvent consumption compared to HPLC method.

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