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SHORT COMMUNICATION

Monolithic LC method applied to fesoterodine fumarate low dose extended-release tablets: Dissolution and release kinetics

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Abstract A dissolution test for fesoterodine low dose extended-release tablets using liquid chromatographic (LC) method equipped with a C_{18} monolithic column was developed and validated. LC system was operated isocratically at controlled temperature (40 °C) using a mobile phase of acetonitrile: methanol:0.03 M ammonium acetate (pH 3.8) (30:15:55, v/v/v), run at a flow rate of 1.5 mL/min and detected at 208 nm. The best dissolution conditions for this formulation were achieved using a USP apparatus 2 (paddle) at 100 rpm and 900 mL of phosphate buffer at pH 6.8 as the dissolution medium. Validation parameters such as the specificity, linearity, accuracy, precision, and robustness were evaluated according to international guidelines, giving results within the acceptable range. The kinetic parameters of drug release were also investigated using model-dependent methods and the dissolution profiles were best described by the Higuchi model. The validated dissolution test can be applied for quality control of this formulation.

1. Introduction

The drug release kinetics follows a well defined behavior in order to supply the maintenance dose enabling the attainment of the desired drug concentration. Thus, the use of mathematical modeling turns out to be very useful for the prediction of release kinetics. There are a number of kinetic models, which describe the overall release of drug from the dosage forms. Therefore, developing tools that facilitate product development by reducing the necessity of bio-studies are always desirable [1–3].

Antimuscarinic medications are the first-line pharmacotherapy for overactive bladder (OAB). Fesoterodine (FESO; Fig. 1) is an effective and well-tolerated antimuscarinic agent, in an extended-release preparation in 4 mg and 8 mg once-daily doses, licensed for the treatment of symptoms that may occur in patients with OAB [4]. OAB disorder is a collection of symptoms, in particular, urinary urgency with or without urgency urinary incontinence, usually accompanied by increased micturition frequency and nocturia [5].

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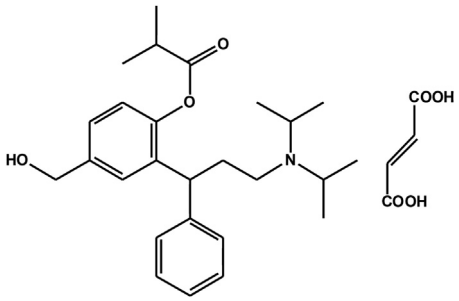

Fig. 1 Chemical structure of fesoterodine fumarate.

FESO functions as a prodrug and gets rapidly and extensively hydrolyzed by nonspecific esterases to its primary active metabolite 5-hydroxymethyl tolterodine (5-HMT), such that it is undetectable in blood after oral administration [4], and presented in bioequivalence study of FESO [6]. Then, the antimuscarinic activity of FESO is solely due to 5-HMT. Besides, there is a further hepatic metabolism of 5-HMT, forming three inactive metabolites [7], and flip-flop pharmacokinetic in that the terminal half-life of 5-HMT is observed due to the extended-release rate from the formulation [4]. Thus, to compare responses of 5-HMT in bioassays and parent drug in physicochemical test is complex and more studies are required to establish an *in vitro–in vivo* correlation [8–10].

Some analytical methods were developed and validated for FESO determination in pharmaceutical formulation [11–14]. Therefore, the aim of the present study was to develop and validate a dissolution test for FESO tablets using monolithic liquid chromatography (LC) method. The monolithic columns represent an approach that provides high rates of mass transfer at lower pressure drops as well as high efficiencies even at elevated flow rates [11]. Moreover, the release kinetics was determined using model-dependent approaches.

2. Experimental

2.1. Chemicals

FESO fumarate reference substance was purchased from Toronto Research Chemicals Inc. (Toronto, ON, Canada). Toviaz® (Pfizer Inc., Zwickau, Germany) extended-release tablets, containing 8 mg of FESO fumarate (6.2 mg of FESO base), were obtained from commercial sources. All chemicals used were of pharmaceutical or special analytical grade. Ultrapure water (Milli Q Gradient System, Millipore Corp., Bedford, MA, USA) was used for all the analyses.

2.2. Instrumentation

A Vankel® VK 7010 (Vankel Technology Group, Cary, USA) dissolution station multi-bath ($n=8$) with a VK 8000 dissolution auto-sampling station, VK type bidirectional peristaltic pump and VK 750D digitally controlled heater/circulator was used.

A Shimadzu LC system (Shimadzu Corp, Kyoto, Japan) equipped with an SCL-10AVP system controller, an LC-10ADVP binary pump, an SIL-10ADVP autosampler, a CTO-10ACvp column oven, and an SPD-M10AVP photodiode array (PDA) detector was used. The detector was set at 208 nm and peak areas were integrated automatically by computer using Class VP software (v 6.12).

2.3. Analytical conditions

A reversed-phase Phenomenex Inc. (Torrance, CA, USA) Onyx C_{18} monolithic column (100 mm × 4.6 mm i.d.) was used. A security guard holder was used to protect the analytical column. The Shimadzu LC system was operated isocratically at controlled temperature (40 °C) using a mobile phase of acetonitrile:methanol:0.03 M ammonium acetate (pH 3.8) (30:15:55, v/v/v), run at a flow rate of 1.5 mL/min and detected at 208 nm. The injection volume was 25 µL. Published LC method was optimized to improve the sensitivity [12].

2.4. Dissolution test conditions

Dissolution testing was performed in compliance with USP 34 [15] using 900 mL of dissolution medium pre-heated at 37 ± 0.5 °C. The effects of rotation speed, filters and dissolution medium were evaluated using a paddle (USP apparatus 2). Automatic sampling was performed using 5 mL aliquots and these solutions were immediately filtered through 70 µm filters connected into the equipment. The dissolution samples were analyzed by the LC method at predetermined time intervals (1, 2, 4, 6, 8 and 12 h). The cumulative percentage of drug release was plotted against time, in order to obtain the release profile and to calculate the *in vitro* dissolution data ($n=6$).

2.5. Determination of sink conditions and filter suitability

FESO sink conditions were determined in water, 0.01 and 0.1 M HCl, sodium acetate buffer pH 4.5, and phosphate buffer pH 6.8 and pH 7.5, using an equivalent to three times of the dose in the pharmaceutical formulation. Vessels ($n=3$) containing 250 mL of medium were preheated in a thermostatically controlled water bath at 37 ± 0.5 °C, before adding an excess of FESO. The suspensions were gently agitated. Aliquots of 5 mL were removed from each vessel after 16 h and filtered. The filtered samples were directly analyzed by LC method. The sink conditions were desirable, but not mandatory.

The filter evaluation is essential to determine if it can be used in the dissolution test without adsorption of the drug and if it removes insoluble excipients that may otherwise cause high background or turbidity [15]. FESO sample solution was prepared in dissolution medium proposed with a final concentration of 6.89 µg/mL. The solution was transferred to the vessel that was gently rotated for 16 h at 37 ± 0.5 °C. Aliquot of this solution was withdrawn and filtered using 10 µm and 70 µm porous cannula filters manufactured from ultra-high molecular weight polyethylene. The same procedure was performed with another aliquot of the same solution, but this was centrifuged for 5 min at 3000 rpm instead of being filtered. The solutions were analyzed by LC method.

2.6. Dissolution method validation

To demonstrate that the method was adequate for dissolution test purposes, it was validated by LC through the analyses of stability, specificity, linearity, accuracy, precision, robustness and filter

suitability parameters according to USP 34 and ICH guidelines [15,16].

2.7. Evaluation of release kinetic

To evaluate FESO release kinetics, five model-dependent approaches were applied: zero-order, first-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas, whose equations are shown in Table 1 [1,2]. The curves were constructed applying the kinetic models cited, considering the sampling time for $\geq 80\%$ of drug dissolution ($t_{80\%}$). Frequently, pharmacopeias use this parameter as an acceptance limit for the dissolution test. The mathematical model that best expressed the dissolution profile of FESO tablets was selected based on the coefficient of determination (R^2) [1]. The experimental data were plotted and evaluated according to each model [1,2].

3. Results and discussion

3.1. Development of the dissolution test

Dissolution tests were initially performed with each dissolution medium at the stirring rate of 75 rpm to investigate the drug release. The evaluation of sink conditions for FESO demonstrated that the drug is soluble in all dissolution media evaluated due to high aqueous solubility of the drug.

The evaluation of the 10 and 70 μm filters does not interfere in the results of the analysis, giving values within 98–102% for the filtered samples compared with the centrifuged solutions, as specified [17]. The 70 μm filters were used in all analyses.

The USP apparatus 2 (paddles) was chosen due to its acceptance as a standard procedure for tablets. Paddles are most used at 50 or 75 rpm, rates outside 25–150 rpm are usually inappropriate and 100 rpm may be used, especially for extended-release products [15]. Experimentally, an agitation speed of 100 rpm showed a more rapid release profile than 50 and 75 rpm, maintaining the suitable sink conditions and maximum differentiation of drug release. Therefore, $t_{80\%}$ was obtained in 12 h, in accordance with extended-release formulations and suitable for pharmacokinetic studies such as *in vitro–in vivo* correlation. FDA recently published a dissolution method with phosphate buffer pH 6.8 at 75 rpm and 20 h test. In our tests, the same condition was performed showing time-consuming and principally less differentiation response, which can affect pharmacokinetic studies.

The dissolution medium was selected based on a screening study with paddles, at stirring rate of 100 rpm, with 900 mL of water, 0.01 and 0.1 M HCl, sodium acetate buffer (pH 4.5), phosphate buffer pH 6.8 and pH 7.5. The results showed that phosphate buffer pH 6.8 was the best dissolution medium, since it provided the highest drug release percentage and ensured sink conditions. Therefore, the method screening was desired in order to obtain an adequate differentiation of the drug release, improving the potential discriminative power of the method.

Based on these results, USP apparatus 2 at stirring rate of 100 rpm was selected as the dissolution apparatus and 900 mL of phosphate buffer pH 6.8 was chosen as the dissolution medium.

Table 1 Mathematical models used for kinetics of drug release.

Mathematical models	Equations
Zero-order	$Q_t = Q_0 + k_0 t$
First-order	$\log Q_t = \log Q_0 + (k_1 t)/2.303$
Higuchi	$f_t = k_H t^{1/2}$
Hixson–Crowell	$W_0^{1/3} - W_t^{1/3} = k_{HC} t$
Korsmeyer–Peppas	$Q_t/Q_\alpha = k_K t^n$

Q_t, amount of drug dissolved in time t; Q_0, initial amount of drug in the solution; k_0 and k_1, zero order and first order release constants, respectively; f_t, amount of drug released in time t by surface unity; k_H, Higuchi dissolution constant; W_0, initial amount of drug in the pharmaceutical dosage form; W_t, remaining amount of drug in the pharmaceutical dosage form at time t; k_{HC}, a constant incorporating the surface–volume relation; Q_α, amount of drug released at infinite time t; k_K, Korsmeyer–Peppas dissolution constant; n release exponent (indicative of drug release mechanism).

3.2. Validation of the method

The stability test of the reference substance solution and samples demonstrated that FESO was stable in the test conditions for up to 16 h at temperature of 37 ± 0.5 °C and after 24 h at room temperature (24 ± 2 °C), using freshly prepared solutions as reference. There was no evidence of degradation of FESO under these conditions. According to the literature [15], the acceptable range for solution stability was 98–102% of the initial value.

The specificity of the dissolution test by the LC method demonstrated no interference of pharmaceutical excipients. No chromatographic peak from the placebo formulation was observed with the same retention time of FESO (Fig. 2). According to the USP 34 and Pharmacopeial Forum [15,16], the lack of chromatographic peaks from the placebo formulation demonstrates the specificity of the method.

The three analytical curves constructed for FESO were found to be linear in the range of 1.0–8.0 μg/mL (1.0, 2.0, 4.0, 6.0 and 8.0 μg/mL). The value of the determination coefficient calculated ($R^2 = 0.9974$, $y = 34328x + 6634.8$, where x is the concentration and y is the peak absolute area) indicated the linearity of the analytical curve for the method.

Moreover, according to Pharmacopeial Forum the linearity should be evaluated from about 20% of expected concentration to about 20% above the maximum possible concentration [17]. Commercial tablets containing 6.2 mg FESO base have a release maximum of the 6.89 μg/mL. As $t_{80\%}$ was evaluated, relative to 5.51 μg/mL, 20% and 120% of this are in the range performed.

The accuracy was demonstrated by the recovery of known amounts of FESO to the dissolution vessels. Concentrations of 1.38, 6.89, and 8.27 μg/mL (corresponding respectively to 20%, 100%, and 120% of the nominal assay concentration) were evaluated and mean recoveries were 1.35, 7.19 and 8.57 μg/mL, respectively, corroborating the accuracy of the method. Percentage recoveries from 95% to 105% were recommended for the accuracy test [15,16].

Intra-day precision was determined by triplicate injection of each solution and the intermediate precision was evaluated at three different concentration levels (1.38, 6.89, and 8.27 μg/mL) in 3 days. The low relative standard deviation (RSD) ($\leq 5\%$) demonstrated adequate precision of the method. The results of accuracy and precision are presented in Table 2.

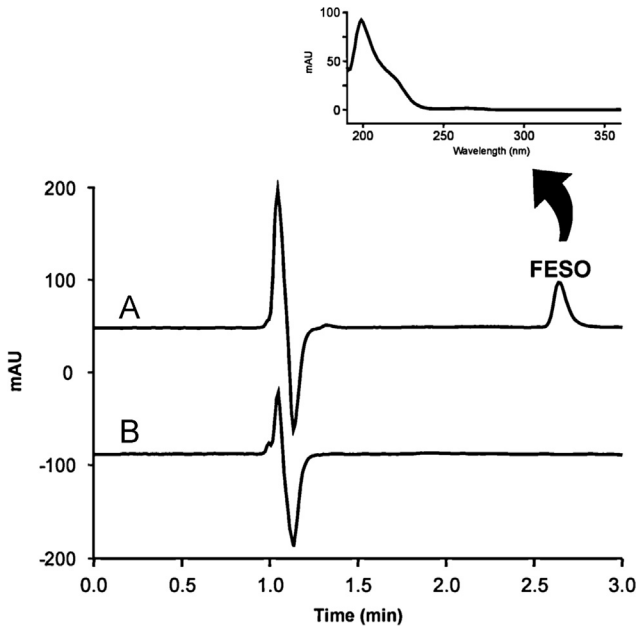

Fig. 2 Chromatograms of fesoterodine reference solution with UV spectra (A) and placebo sample solution (B) in dissolution medium. Chromatographic conditions: Phenomenex Onyx C_{18} monolithic column (100 mm × 4.6 mm i.d.), 40 °C; mobile phase: acetonitrile: methanol:0.03 M ammonium acetate (pH 3.8) (30:15:55, v/v/v); flow rate: 1.5 mL/min; detection: 208 nm.

Table 2 Accuracy and precision of the dissolution the method.

Concentration (μg/mL)	Day	Accuracy (recovery, %)		Precision (RSD, %)	
		Intra-day	Inter-day	Intra-day	Inter-day
1.38	1	97.93	97.98	4.74	3.51
	2	97.88		3.02	
	3	98.14		4.20	
6.89	1	104.76	104.42	3.66	2.26
	2	103.60		0.96	
	3	104.91		2.14	
8.27	1	102.95	103.74	2.90	1.97
	2	103.32		0.70	
	3	104.96		1.85	

RSD = relative standard deviation.

For robustness evaluation, results obtained from different pH values of buffer solution (6.6 and 7.0) were analyzed by ANOVA at each time, showing no significative difference among the FESO released from Toviaz® by LC method ($F_{calculated} < F_{critical} = 5.14$; $P > 0.05$) (Table 3). This data indicated that the proposed method was robust under the conditions tested.

3.3. *Kinetics of drug release*

The dissolution profile (Fig. 3) was used to evaluate the kinetics of drug release. After mathematical modeling of dissolution profile data, graphical plots and the model selection criteria represented by the determination coefficients and dissolution constants are presented (Fig. 4). Considering these values, dissolution profiles were better described by the Higuchi model. Higuchi describes drug release as a diffusion process based in Fick's law, square root time dependent. This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water soluble drugs, as the Toviaz® [1,2,18,19]. Experimentally, after 16 h of dissolution test, the matrix physically swelled forming a gel, enabling the drug dissolution into the matrix and its exit through the outer surface of gel.

Table 3 Drug release of fesoterodine in different pH values of buffer solution during robustness testing.

Time (h)	Drug release (%)			$F_{calculated}$ [b]
	pH 6.6 [a]	pH 6.8 [a]	pH 7.0 [a]	
1	16.84	16.81	17.57	2.99
2	26.59	28.34	27.18	3.86
4	43.96	44.62	45.93	2.52
6	56.69	60.35	59.73	4.70
8	66.62	69.05	70.83	4.79
12	79.42	83.17	83.94	4.25

[a] Mean of three tablets.
[b] $F_{calculated} < F_{critical} = 5.14$ ($P > 0.05$).

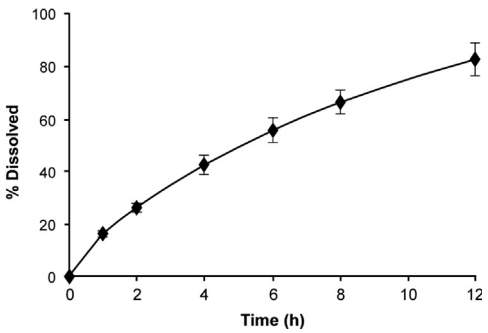

Fig. 3 Dissolution profile of fesoterodine low dose extended-release tablets ($n = 6$) in phosphate buffer pH 6.8 medium (37 ± 0.5 °C) using apparatus 2 rotating at 100 rpm.

4. Conclusions

The dissolution test for FESO tablets was developed and validated according to the USP 34 and ICH guidelines. *In vitro* dissolution profile for FESO was obtained using 900 mL of phosphate buffer pH 6.8 as dissolution medium, paddle at 100 rpm. The percentage of drug dissolved was determined by the monolithic-column LC method and the kinetics of drug release was better described by the Higuchi model. The proposed method demonstrated to be adequate for quality control of FESO dosage form, since there is no official monograph, contributing to assure the therapeutic efficacy of the drug.

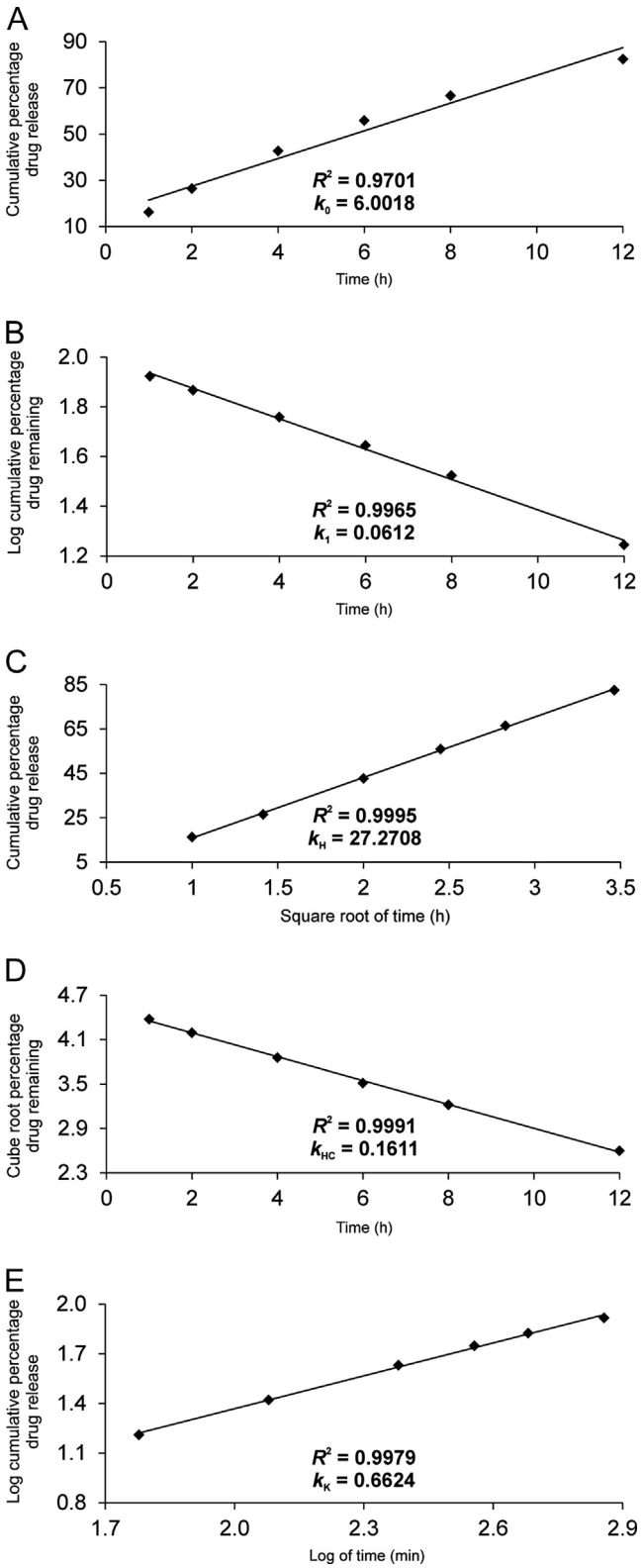

Fig. 4 Graphical plots obtained by fitting experimental release data of fesoterodine to (A) zero-order, (B) first-order, (C) Higuchi, (D) Hixson–Crowell, and (E) Korsmeyer–Peppas models. R^2, coefficient of determination; k, dissolution constants of respective mathematical models.

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