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Original Research Article

Development of Dissolution Test Method for Drotaverine Hydrochloride/Mefenamic Acid Combination Using Derivative Spectrophotometry

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Abstract

Purpose: *To develop and validate a dissolution test method for tablets containing 80 mg of drotaverine hydrochloride (DRT) and 250 mg of mefenamic acid (MEF).*
Methods: *Sink conditions, drug stability and specificity in different dissolution media were tested to optimize a dissolution test method using a USP paddle type dissolution test apparatus set at a speed of 50 rpm. The dissolution medium consisted of 900 ml of phosphate buffer (pH 6.8) containing 0.25% w/v cetrimide at 37 ± 0.5 °C and 45 min time-point. To determine both drugs simultaneously, a first derivative UV spectrophotometric method was developed and validated. Drug release was analyzed by first derivative UV method at 253.8 nm and 304 nm for DRT and MEF respectively. The dissolution method was validated as per ICH guidelines.*
Results: *The two brands each showed 98% of drug release for both drugs when the developed dissolution method was used. The regression plot was linear in the concentration range 4 - 24 µg/mL for each of the drugs and regression coefficient (r^2) was greater than 0.999 for each drug. Relative standard deviation (% RSD) for precision and accuracy of proposed method was < 2.*
Conclusion: *The proposed dissolution method is simple, cost-effective, precise, accurate and specific. It can be successfully employed in routine quality control of DRT and MEF combination tablets.*

Keywords: *Drotaverine hydrochloride, Mefenamic acid, First derivative spectrophotometry, Dissolution, Validation*

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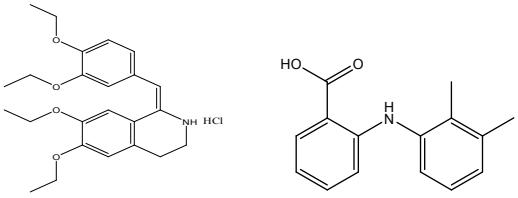
INTRODUCTION

Drotaverine hydrochloride (DRT, Figure 1A), chemically 6,7,3',4'-Tetraethoxy-1-benzal-1,2,3,4 -tetrahydroisoquinoline hydrochloride, is an analogue of papaverine with smooth muscle relaxant properties. Mefenamic acid (MEF, Figure 1B), chemically, 2-[(2, 3-Dimethylphenyl) amino] benzoic acid, is an agent which exert its analgesic, anti-inflammatory, and antipyretic properties by inhibition of cyclooxygenase [1-6]. The combined dosage form of DRT and MEF is therapeutically used for uterine irritability, as well as primary and secondary dysmenorrhoea.

Dissolution testing of poorly soluble compounds in immediate release solid dosage forms presents several challenges. Single dissolution

method for the analysis of combined dosage forms is preferred for simplification of quality control testing [7-13]. However, development of a single dissolution method for DRT and MEF is practically challenging due to the pH dependent dissolution of these two drugs.



A B

Figure 1: Chemical structures of (A) drotaverine hydrochloride (DRT)and (B) mefenamic acid (MEF)

Presently, assay of DRT is not described in any pharmacopoeia and there is no specification for its dissolution test, but a dissolution medium, tris buffer (pH 9.0) containing 1 %w/v sodium lauryl sulphate, is recommended for MEF. This dissolution medium is not suitable for in vitro dissolution test of immediate release dosage forms since the pH of gastro-intestinal tract is not anywhere near 9.0. To the best of our knowledge, no single *in vitro* dissolution test has been reported for DRT and MEF in a combined tablet dosage form. Therefore, the objective of the present work was to develop and validate a single dissolution test method for DRT and MEF in a combined tablet dosage form using first derivative UV spectrophotometric method.

EXPERIMENTAL

Materials

DRT and MEF were obtained as a gifts from Aurobindo Pharmaceutical and Wan Bary Ltd, Hyderabad, India. Hydrochloric acid, methanol, ortho-phosphoric acid, potassium dihydrogen orthophosphate, sodium hydroxide and sodium chloride were purchased from SD Fine Chemicals Ltd, Mumbai, India; sodium lauryl sulfate (SLS), Tween 80 and cetrimide were purchased from Himedia Ltd, Mumbai, India. Double distilled water was used throughout the study. DRT and MEF combination tablet formulations - Doverin-M (Intas Pharmaceuticals Ltd, India) and Drofem (FDC Ltd, India) - were purchased from the local market.

Instruments

Electrolab-TDT-08L dissolution test apparatus, double beam 1800 UV-Visible spectrophotometer (Shimadzu, Japan), analytical balance (Shimadzu AUX 220, Japan), orbital shaker bath (Bio-Technics, India), pH meter (Elico, Hyderabad) and ultrasonic cleaner (Sonica, Italy) were used for the study.

Selection of wavelength

Standard solutions of DRT and MEF were diluted appropriately with phosphate buffer (pH 6.8) to obtain a solution containing DRT (12 µg/mL) and MEF (12 µg/mL). The spectra of these diluted solutions were scanned in the spectral region of 200 to 400 nm using phosphate buffer pH 6.8 as blank. The zero-order spectra of DRT and MEF were transformed to corresponding first-derivative spectra in the range of 200 to 400 nm.

Derivative conditions

First-order derivative spectra of DRT (12 µg/mL) and MEF (12 µg/ mL) were overlapped. The zero-crossing point (ZCP) values of MEF at which the DRT showed some derivative response were recorded. The wavelength, 253.8 nm, was selected for the quantification of DRT (where the derivative response for MEF was zero) while 304.0 nm was selected for the quantification of MEF (where the derivative response for DRT was zero). The optimized wavelengths of 253.8 nm and 304 nm for DRT and MEF, respectively were confirmed by checking the derivative absorbance of various concentrations of both drugs in the range of 4 - 24 µg/mL.[14].

Dissolution test conditions

Dissolution testing of combined DRT and MEF tablet formulation (Doverin-M) was performed in a paddle type USP tablet dissolution apparatus, in 900 mL of various buffer and surfactant media at 50 rpm and 37 ± 0.5 °C for 45 min. Aliquots of 5.0 mL were withdrawn at 5 min interval up to 45 min, and replaced with an equal volume of fresh medium to maintain sink conditions. At the end of the test, the withdrawn samples were filtered, diluted with phosphate buffer pH 6.8 and quantified by the developed and validated spectrophotometric derivative method (n = 3). The amount of dissolved drugs was computed from the respective calibration curves and then plotted against time.

The media in which highest drug release occurred for Doverin-M, and this medium was chosen for *in vitro* dissolution studies in DROFEM.

Method validation

The dissolution method was validated as per ICH guidelines. The parameters assessed were specificity, linearity, precision and accuracy.

Specificity

Specificity is the ability of the method to accurately measure a compound in the presence of other components such as impurities, degradation products and matrix components. The specificity of the proposed method was evaluated through the analysis of a placebo solution, which it was prepared with the common excipients (lactose, starch, microcrystalline cellulose, magnesium stearate and talc) of the pharmaceutical formulation. Thus, the mixture of component inert was prepared in their usual concentration employed in tablets (concentrations were determined based in Handbook of pharmaceutical Excipients and calculated for medium weight of content). The developed method was applied to in order to check if any component of the formulation could generate a response or a read with absorption band similar to the drugs.

Linearity

Appropriate aliquots of standard stock solutions of DRT (100 µg/mL) and MEF (100 µg/ mL) were taken in two different sets of 10 mL volumetric flasks and diluted up to the mark with phosphate buffer (pH 6.8) to obtain final concentrations of 4 - 24 µg /mL for both drugs. First-derivative spectra were recorded using the prepared solutions against phosphate buffer (pH 6.8) as blank. The values of the first-derivative absorbance for DRT and MEF were obtained using six different concentrations by measuring each concentration against solvent blank at the chosen wavelength 253.8 nm and 304 nm for DRT and MEF, respectively. The values of first-derivative absorbance were plotted against corresponding concentrations to construct the calibration curves.

Precision

The precision of the method was determined by repeatability (intra-day) and reproducibility (inter-day precision). It was evaluated based on the relative standard deviation (% RSD) [18]. The intra- and inter-day precision were assessed after subjecting six tablets to the dissolution test conditions, on the same day and on three different days, respectively.

Accuracy

Accuracy was evaluated by adding known amounts of the reference substance to the placebo sample in the dissolution medium at 80 %, 100 % and 120 % of the nominal assay value of DRT and MEF. The accuracy was calculated as the the % drug recovered from the formulation matrix.

Statistical analysis

The dissolution data of the two formulations were subjected to statistical analysis using Student's t-test by Prism 4.0 software, and differences between data were considered significant at $p < 0.05$.

RESULTS

Development of dissolution test method

Selection of dissolution test method was based on screening studies using USP apparatus 2 at a paddle speed 50 rpm: operating conditions are listed in Table 1.Selection of a dissolution medium to accommodate adequate solubility and stability of both DRT and MEF was critical for the selected dissolution method. Several compendia dissolution media were screened; these include various media - buffer (pH 1.2 to 9.0), surfactant and buffer/surfactant. The addition of surfactants to the buffer solution was also investigated [15,16], Cummulative DRT and MEF release (Figure 2) in phosphate buffer (pH 6.8) containing 0.25 %w/v cetrimide was 98.8 and 101.3 %, respectively, from Doverin-M tablets. Therefore, the selected dissolution test conditions were: USP apparatus 2 at paddle speed 50 rpm in a medium of phosphate buffer (pH 6.8) containing 0.25 %w/v cetrimide. Dissolution studies on Drofem tablets were performed at these dissolution test conditions. These results, shown in Figure 2, reveal that 99.01 and 101.16 % of DRT and MEF were released, respectively, from Drofem tablets in the optimized medium. Statistical analysis of the dissolution profile data for the two formulations indicate that the profiles were similar (t_{cal} = 4.0 and 0.15 for DRT and MEF, respectively, which is less than t_{cri} = 4.303 ($p \leq 0.05$).

Specificity

The derivative spectra of placebo, DRT and MEF in the dissolution medium, shown in Figure 3, reveal that there was no interference from the excipients in the tablets (placebo) with derivative response of either of drugs (DRT and MEF) at their respective analytical wavelengths of 253.8

and 304 nm, respectively. Hence, the method is specific [17].

Table 1: Dissolution efficiency in various dissolution media based on paddle method, stirring rate of 50 rpm, temperature of 37 ^{0}C ± 0.5 and time point of 45 min

Dissolution media	DE[a] ± SD (n = 3)	
	DRT	*MEF*
Doverin-M (Brand-I)		
Distilled water	38.79±1.89	17.19±0.19
Hydrochloric acid	62.93±0.39	4.99±0.42
Tris buffer, pH 4.0	87.60±0.38	4.86±0.01
Acetate buffer, pH 4.6	81.82±0.53	4.86±0.36
Phosphate buffer, pH 6.8	48.61±0.19	22.00±1.19
Phosphate buffer, pH 7.4	36.44±0.74	42.62±0.37
Tris buffer, pH 9.0	18.59±0.66	88.75±0.42
0.25%w/v cetrimide	95.46±1.05	87.14±0.37
0.25%w/v SLS*	35.32±0.68	11.54±0.42
0.25%w/v Tween 80	35.06±0.40	25.66±0.21
Phosphate buffer, pH 6.8 + 0.25%w/v cetrimide	98.84±0.42	101.1±0.76
Phosphate buffer, pH -6.8 +0.25% w/v SLS	62.09±0.67	21.92±0.02
phosphate buffer, pH -6.8 +0.25%v/v Tween 80	34.87±0.39	24.99±0.222
Drofem (Brand II)		
Phosphate buffer (pH 6.8) + 0.25%w/v cetrimide	99.01±0.34	100.16±0.37

**Sodium lauryl sulphate; [a]Dissolution efficiency*

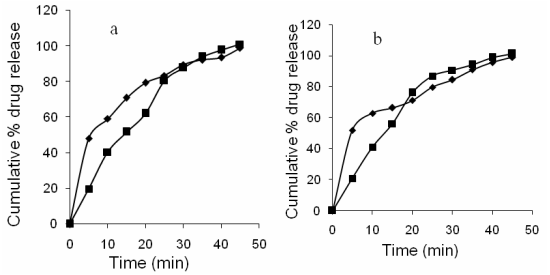


Figure 2: Dissolution profiles of DRT (♦) and MEF (■) for (a) Doverin-M and (b).Drofem tablets

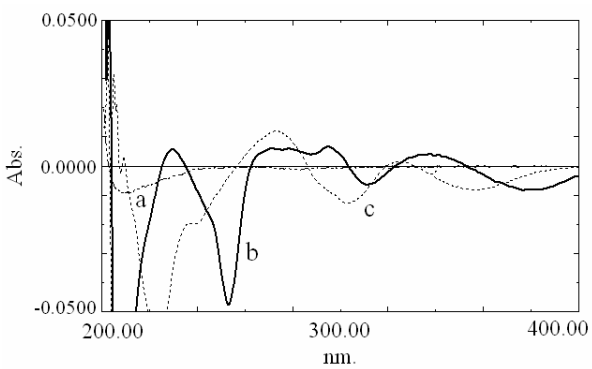


Figure 3: First order spectra of placebo (a), drotaverine hydrochloride (b) and mefenamic acid(c)

Linearity

Linearity plots for DRT and MEF are shown in Figure 4. The results reveal that both DRT and MEF show a linear relationship in the concentration range of 4 - 24 µg/mL for both drugs, with regression coefficient (r^2) of 0.999 for both DRT and MEF.

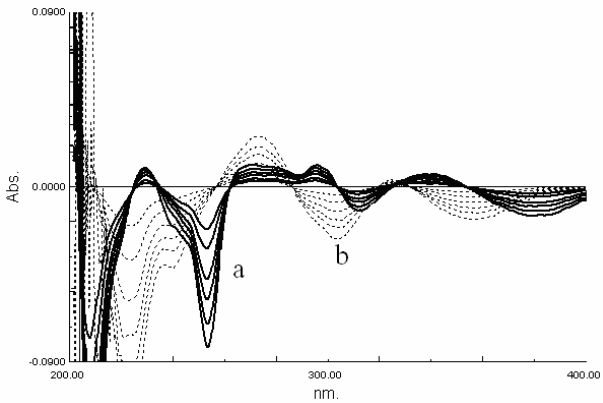


Figure 4: First order spectrum of linearity range of (a) drotaverine hydrochloride and (b) mefenamic acid

Precision

The % relative standard deviation values were found to be less than 2 for intra and inter day precision studies and there is no significant difference was observed between intra and inter day values, which indicates that the proposed method was precise and reproducible. Results are reported in Table 2.

Accuracy

The results are reported in Table 3. The %RSD values obtained for DRT and MEF were < 2, indicating that the method is accurate for its intended use [18].

DISCUSSION

In order to develop single dissolution method for simultaneous quantification of the DRT and MEF, initial trials were performed with the objective to accommodate adequate solubility and stability of both drugs. Sink conditions, stability of the drugs and specificity in different dissolution media were tested to optimize a dissolution test. The screening results showed that the dissolution rate of DRT is higher in pH 4.0 tris buffer than in the other buffers and also that if the dissolution rate of MEF increased with increasing medium pH, then a single pH buffer would be unsuitable for the method chosen. Both drugs exhibited higher dissolution rate in 0.25 %w/v cetrimide than in 0.25 %w/v sodium lauryl sulphate and

Table 2: Precision data for the proposed method

Product	DRT				MEF			
	Intra-day(%)		*Inter-day (%)*		*Intra-day (%)*		*Inter-day (%)*	
	Mean±SD	*%RSD*	*Mean±SD*	*%RSD*	*Mean±SD*	*%RSD*	*Mean±SD*	*%RSD*
Doverin	99.06±0.47	0.476	100.20±1.25	1.247	100.79±0.21	0.208	99.98±0.99	0.990
Drofem	99.29±0.20	0.203	99.10±0.98	0.988	100.9±0.137	0.135	98.89±1.25	1.264

Table 3: Results for accuracy for the proposed method

Analyte	% level of recovery	*Amount of reference (µg/ml)*			
		Added	*Recovered*	*%Recovery*	*%RSD*
DRT	80	7.11	7.02	98.73	1.10
	100	8.80	8.65	98.29	0.89
	120	10.60	10.62	100.18	1.24
MEF	80	22.20	22.00	99.09	1.02
	100	27.70	27.80	100.03	0.98
	120	33.30	33.20	99.69	0.85

0.25 %v/v Tween 80, due to electrostatic interaction between anionic drugs with cationic surfactants (cetrimide). Thus, the *in vitro* dissolution of DRT and MEF was significantly dependent on the type of surfactant used

Finally, 900 mL of phosphate buffer (pH 6.8) containing 0.25 %w/v cetrimide as dissolution media, USP paddle type dissolution apparatus at a speed of 50 rpm and temperature of 37 ± 0.5^0 C, and 45-min time point were selected as the optimum dissolution test conditions. Acidic drugs might dissociate into an anionic form before solubilizing in micelles, which were formed by the cationic surfactant at pH 6.8. This anionic form of acidic drug could have interacted with the cationic surfactant, which will facilitate its partitioning into micelles, and increase its solubility and further dissolution when compared to that of the media containing non-ionic and anionic surfactants. The pH of the dissolution medium (pH 6.8) is within the range of GI-tract pH levels and low concentration of surfactant (0.25 % w/v cetrimide). This medium is convenient and suitable for the study of the dissolution profiles of DRT and MEF when combined in a tablet dosage form.

Generally, for simplification of quality control testing, single dissolution methods are preferred for the analysis of combined drug in a dosage form. Therefore, our goal was to develop a cost-effective, simple and specific single dissolution method for the simultaneous quantification of both drugs (DRT and MEF). To evaluate both drugs simultaneously, a first derivative UV method was developed and validated. The method is selective for the simultaneous determination of DRT and MEF since no interfering peaks appeared near the absorption peaks of the compounds of interest. The developed dissolution method was successfully employed for two different brands of tablets. The data obtained also indicate that the method is accurate and precise within the desired range. Student's t-test analysis indicates that the dissolution profiles of the two test formulations were similar under the dissolution test conditions.

CONCLUSION

A dissolution test for the simultaneous determination of DRT and MEF in tablets has been successfully developed and validated according to ICH guidelines. The proposed dissolution test conditions should enhance laboratory efficiency and is specific, cost-effective, accurate and precise. Thus, the proposed dissolution method can be used in the routine quality control analysis of DRT and MEF when combined in tablets.

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