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Determination of cisapride in pharmaceutical preparations using derivative spectrophotometry

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Abstract

Derivative spectrophotometric and high performance liquid chromatographic methods (HPLC) were described for the determination of cisapride in pharmaceutical preparations. Spectrophotometrically, cisapride was determined by measuring the ^1D-values at 264, 300 nm and ^2D-values at 276, 290 and 276–290 nm. Beer's Law was obeyed in the range 2–12 μg ml^{-1}. The HPLC method depends upon using micropack-Si-10 column at ambient temperature with a mobile phase consisting of methanol–concentrated ammonia (99.25: 0.75) at a flow rate of 1 ml min^{-1}. Quantitation was achieved by UV detection at 272 nm using quinine as internal standard. Calibration curve was linear over the concentration range 2–10 μg ml^{-1}. Both derivative spectrophotometry and HPLC methods showed good linearity, precision and reproducibility. No interference was found from tablet or suspension matrices at the selected derivative wavelengths and chromatographic conditions. The proposed methods were successfully applied to the assay of commercial tablets and suspension. The procedures were rapid, simple and suitable for quality control applications.

Keywords: Cisapride; Derivative spectrophotometry; HPLC

1. Introduction

Cisapride is used in various gastrointestinal motility disorders. It is believed to facilitate acetylcholine release from the mynetric plexus of the gut and to activate $5HT_4$ receptors in the intestinal wall. Cisapride has been shown to be effective in treating gastro-oesophageal reflux diseases in adults, children and neonates [1–3]. Various assay methods have been reported for the determination of cisapride. In British [4] and European [5] pharmacopoeias cisapride has been assayed in bulk drugs using potentiometric titration with 0.1 M perchloric acid. In pharmaceutical preparations, cisapride has been determined by measuring the intrinsic fluorescence of the drug at 355.2 nm with excitation wavelength at 310 nm [6]. Few spectrophotometric methods have been reported for determination of cisapride in pharmaceutical preparations. These included extraction spectrophotometric methods [7,8], reaction with *p*-dimethylamino cinnamaldehyde and phos-

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phoric acid [9], oxidative coupling with 3-methyl 2-benzothiazolinone hydrazone (MBTH) or oxidation of the drug with Fe(III) and subsequent chelation of Fe(II) with 1,10-phenathroline or through the formation of charge transfer complex with chloranilic acid [10]. A stability indicating HPTLC method has been reported for assay of cisapride and related impurities in tablets [11]. HPLC methods have been reported for determination of cisapride in pharmaceutical preparation [12], plasma [13–15], serum [16] or animal tissues [17]. Bioequivalence studies have been performed using reversed phase HPLC [18].

Based on the above reported methods, it was thought necessary to develop simple, fast and accurate spectrophotometric and HPLC methods for the determination of cisapride in pharmaceutical preparations. This work presents first- and second-derivative spectrophotometric and HPLC methods for determination of cisapride in pharmaceutical preparations without any interference from the pharmaceutical excipients.

2. Experimental

2.1. Materials and reagents

Reference cisapride was kindly supplied by Janssen Pharmaceutica, Belgium and used without further treatment. Quinine was obtained from Riedel-de Häen AG, Germany. Prepulsid tablets, containing 10 mg cisapride; prepulsid suspension, containing 1 mg ml^{-1} cisapride were obtained from local market in Riyadh. All experiments were performed with analytical reagent grade. Methanol used for HPLC separation was HPLC grade, Riedel-de Häen AG, Germany.

2.2. Apparatus

The spectrophotometric measurements were performed on a UNICAM UV-VIS spectrophotometer He λ 10Sα, using 1.0 cm quartz cells, connected to PC computer fitted with vision scan software (UNICAM) and Hewlett–Packard DeskJet printer. The spectral band width was 2 nm and the wavelength scanning speed was 3800 nm min^{-1}. The derivative spectra of test and reference solutions were recorded over the range 340–220 nm with $\Delta\lambda = 4$ nm.

The HPLC was composed of Waters-Millipore, with M-45 pump and solvent delivery system equipped with 712 WISP autoinjector and connected to a multiple wavelength detector (Model 481). The peak area integration were performed using a chromatographic data module.

2.3. Chromatographic conditions

Chromatographic separation and quantitation was performed on micropack-Si-10 (30 cm × 3.9 mm i.d.) column from Waters, with a mobile phase consisting of methanol–concentrated ammonia (99.25: 0.75) at ambient temperature. Detection was made at 272 nm and sensitivity was set at 0.1 a.u.f.s. The samples were injected 10 μl at a flow rate 1 ml min^{-1}. The chart speed was 1 cm min^{-1}.

2.4. Preparation of standard solution

A stock standard solution was prepared by dissolving cisapride in methanol to obtain 200 μg ml^{-1}.

2.5. Derivative spectrophotometric method

The standard solutions were prepared by dilution of different volumes of the stock solution to a constant volume with methanol and then diluted with 0.05 M sulphuric acid to reach a concentration range of 2–12 μg ml^{-1}. The ^1D and ^2D curves of the working standard solutions were scanned in the range 340–220 nm against a similarly prepared blank. The observed values of the ^1D amplitudes at 264 and 300 nm and the ^2D amplitudes at 276, 290 and 276–290 nm were plotted against the corresponding concentrations to obtain the calibration graphs.

2.6. HPLC method

Standard solutions of cisapride, containing 2–10 μg ml^{-1} and fixed concentration of 125 μg ml^{-1} quinine were prepared in the mobile phase.

The solutions were filtered through 0.45 μm membrane filter. Triplicate 10 μl injections were made for each concentration and chromatographed under the conditions described above. The peak area ratios of drug to internal standard were plotted against the corresponding concentrations to obtain the calibration graphs.

2.7. Analysis of pharmaceutical preparations

2.7.1. Assay of tablets

Twenty tablets were weighed and finely powdered. A portion of the mixed powder equivalent to 20 mg cisapride was accurately weighed, transferred to 100 ml calibrated flask and dispersed in 50 ml methanol. The flask was placed in ultrasonic bath for 5 min. The resulting suspension was diluted to volume with methanol and filtered. Further dilutions were performed to suit the calibration graphs for the derivative spectrophotometric or HPLC methods.

2.7.2. Assay of suspension

Accurately measured 10 ml of suspension were transferred into 50 ml calibrated flask, extracted with methanol by shaking for 5 min, adjusted to volume with methanol and filtered. Suitable dilutions were made with 0.05 M sulphuric acid or with the mobile phase to suit the calibration graphs for derivative measurements or HPLC method.

3. Results and discussion

3.1. Derivative spectrophotometric method

The zero-order, ^1D and ^2D spectra of cisapride in 0.05 M sulphuric acid are shown in (Figs. 1–3). The zero-order spectrum of cisapride (Fig. 1a) displayed two absorption bands with maxima at 272 and 308 nm. The ^1D curve displayed two maxima at 264 and 300 nm and a minimum at 284 nm (Fig. 2a), while the ^2D curve showed a maximum at 290 nm and a minimum at 276 nm (Fig. 3a). At the same concentration of the drug, a solution prepared from tablet extract showed few spectral changes in the absorption spectrum;

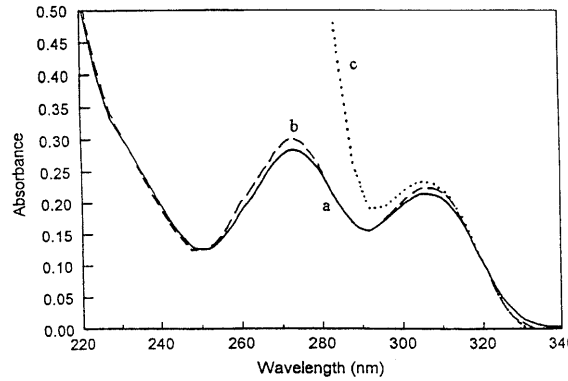

Fig. 1. Zero-order spectra for cisapride 10 μg ml^{-1} (a); tablets (b) and suspension (c); in 0.05 M sulphuric acid.

which resulted in unreliable results in the determination of the drug by the conventional UV method (Fig. 1b). However, a similar solution, prepared from suspension extract showed significant interference with slightly higher absorbance value at 308 nm (Fig. 1c). Application of the first-derivative mode allowed complete elimination of the background absorption due to tablet excipients at 264 and 300 nm (Fig. 2b) and due to suspension matrix at 300 nm (Fig. 2c). Using the second-derivative mode revealed no interference from tablet excipients at 276 and 290 nm (Fig. 3b), while higher ^2D values were obtained for solution prepared from suspension extract at the same concentration (Fig. 3c).

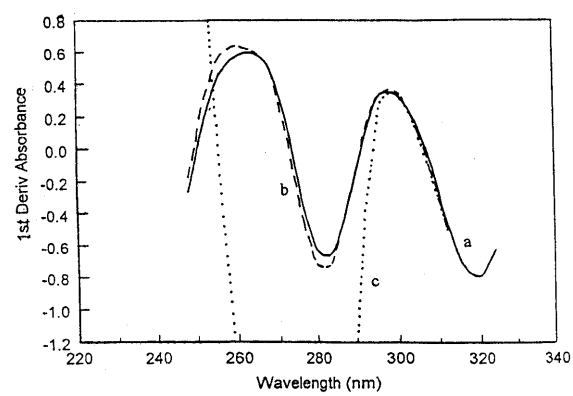

Fig. 2. First-derivative spectra for cisapride 10 μg ml^{-1} (a); tablets (b) and suspension (c); in 0.05 M sulphuric acid.

Table 1
Analytical data of the calibration graphs for the determination of cisapride by derivative spectrophotometry and HPLC methods

	^1D		^2D			
Parameters	$^1D_{264}$	$^1D_{300}$	$^2D_{276}$	$^2D_{290}$	$^2D_{276-290}$	HPLC
Linearity range (μg ml^{-1})	2–12	2–12	2–12	2–12	2–12	2–10
Regression equation ($y = a + bC$)[a]	$y = 1.55 \times 10^{-3} + 0.063C$	$y = -1.26 \times 10^{-4} + 0.038C$	$y = 2.26 \times 10^{-5} + 1.19 \times 10^{-3}C$	$y = -1.00 \times 10^{-5} + 1.04 \times 10^{-3}C$	$y = -7.30 \times 10^{-5} + 2.25C$	$y = 0.0022 + 0.027C$
r[b]	0.9997	0.9998	0.9999	0.9999	0.9999	0.9999
$S_{y/x}$[c]	6.31×10^{-3}	2.82×10^{-3}	4.05×10^{-5}	5.79×10^{-5}	1.48×10^{-4}	1.28×10^{-3}
S_a[d]	5.44×10^{-3}	2.43×10^{-4}	3.49×10^{-5}	4.99×10^{-5}	1.27×10^{-4}	1.32×10^{-3}
S_b[e]	7.54×10^{-4}	3.37×10^{-4}	4.84×10^{-6}	6.92×10^{-6}	1.77×10^{-5}	3.96×10^{-3}

[a] y = derivative value (^1D or ^2D) or peak area ratio versus concentration in μg ml^{-1}, $n = 6$.
[b] Correlation coefficient.
[c] $S_{y/x}$ = standard deviation of residuals.
[d] S_a = standard deviation of intercept of regression line.
[e] S_b = standard deviation of slope of regression line.

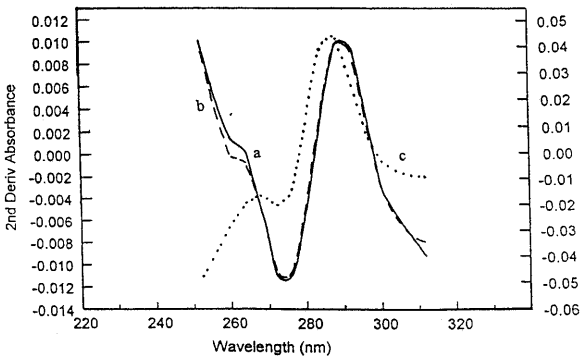

Fig. 3. Second-derivative spectra for cisapride 10 μg ml^{-1} (a); tablets (b) and suspension (c); in 0.05 M sulphuric acid, left scale for curve (a & b); right scale for curve (c).

For quantitative analysis, the ^1D values at 264 and 300 nm and the ^2D values at 276, 290 and 276–290 nm were chosen for the development of a simple and specific procedure for analysis of cisapride in tablets and suspension dosage forms. Linear calibration graphs with negligible intercepts were obtained between the measured ^1D and ^2D values and the corresponding concentrations over the range 2–12 μg ml^{-1}. The statistical parameters, regression equations, calculated from the calibration graphs along with the standard deviations of the slope (S_b) and the intercept (S_a) on the ordinate and the standard deviation of residuals ($S_{y/x}$) are given in Table 1. The linearity of the calibration graphs and conformity of ^1D and ^2D values to Beer's Law were proved by the high values of the correlation coefficients (r) for the regression equations. The detection limits [19] were 0.235 and 0.022 μg ml^{-1} using ^1D measurements at 264 and 300 nm respectively and 0.07, 0.15 and 0.20 μg ml^{-1} using ^2D measurements at 276, 290 or 276–290 nm, respectively. Meanwhile, the quantification limits [20] were 0.84, 0.68 μg ml^{-1} using ^1D-measurements at 264, 300 nm and 0.27, 0.49, 0.60 μg ml^{-1} using ^2D-measurement at 276, 290, 276–290 nm, respectively. The described method was further evaluated by assaying the drug in pure form. Four replicate experiments of different concentrations of pure cisapride were carried out. The within day coefficients of variation were less than 2%, indicating the high reproducibility of the method (Table 2).

3.2. HPLC method:

To validate the derivative spectrophotometric method, a HPLC procedure was developed. The method involved the use of a micropack-Si-10 column and a mobile phase consisting of methanol–concentrated ammonia (99.25: 0.75, v/v). The mobile phase was chosen after several trials with other solvent combinations. System suitability parameters, calculated under the optimized experimental conditions were: capacity factor (k') 0.83; selectivity (α) 1.95; symmetry factor 1 and column efficiency (n) 18 266.89 plates/m. The chromatographic system described allowed an adequate resolution $R_s = 6.52$ between cisapride ($t_r = 3.23$ min) and the internal standard, quinine ($t_r = 4.59$ min) in a reasonable time (Fig. 4) (R_s, resolution; t_r, retention time). For quantitative determinations, linear calibration graph was plotted between peak area ratios and corresponding concentration over the range 2–10 μg ml^{-1}. The results of the statistical analysis of the experimental data, the regression equation calculated from calibration graph, along with S_b, S_a and $S_{y/x}$

Table 2
Within-day precision of cisapride analysis by derivative spectro-photometry and HPLC

Method	Concentration added (μg ml^{-1})	Mean[a] (CV%)
$^1D_{264}$	2.0	99.50 (0.40)
$^1D_{300}$	2.0	100.37 (0.41)
$^2D_{276}$	2.0	99.26 (0.4)
$^2D_{290}$	2.0	99.52 (0.56)
$^2D_{276–290}$	2.0	100.09 (0.60)
HPLC	4.0	99.72 (0.54)
$^1D_{264}$	8.0	99.93 (0.73)
$^1D_{300}$	8.0	100.16 (0.64)
$^2D_{276}$	8.0	99.95 (0.69)
$^2D_{290}$	8.0	100.60 (0.63)
$^2D_{276–290}$	8.0	99.94 (0.40)
HPLC	7.0	100.42 (0.61)
$^1D_{264}$	12.0	100.64 (0.57)
$^1D_{300}$	12.0	100.34 (0.92)
$^2D_{276}$	12.0	100.18 (0.68)
$^2D_{290}$	12.0	99.64 (0.39)
$^2D_{276–290}$	12.0	99.59 (0.37)
HPLC	10.0	100.16 (0.64)

[a] Mean of four replicate determinations.

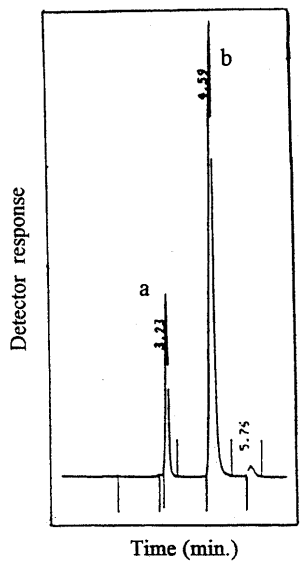

Fig. 4. Typical chromatogram of a 10 μl of a mixture of (a) cisapride 10 μg ml^{-1} and (b) quinine (125 μg ml^{-1}).

are shown in Table 1. The value of the correlation coefficient (r) of the regression equation, indicated good linearity and conformity to Beer's Law (Table 1). The detection limit [19] was 0.064 μg ml^{-1} and the quantification limit [20] was 0.41 μg ml^{-1}, indicating the sensitivity of the proposed HPLC method. Furthermore, the accuracy and precision of the method was evaluated by replicate determination of different concentrations of pure cisapride. The within-day coefficient of variation was 0.64% (Table 2).

3.3. Analysis of pharmaceutical preparations

The proposed methods were applied to the determination of cisapride in pharmaceutical preparations. The results obtained, presented in Table 3, indicate that there is no interference from any excipients, which are normally present in tablet or suspension formulations.

The performance of the derivative spectrophotometric methods was statistically compared with that of the HPLC method by Student's t-test and the F-ratio test at 95% confidence level. The calculated t- and F-values (Table 3) did not exceed the theoretical values, indicating that there was no significant difference between derivative spectrophotometric and HPLC methods with regard to accuracy and precision.

4. Conclusion

The proposed derivative spectrophotometric and HPLC methods provide simple quantitative analysis for the assay of cisapride in pharmaceutical preparations. Due to the rapidity of the proposed methods, they can be conveniently used for the routine determination of cisapride in tablets and suspension dosage forms.

Table 3
Determination of cisapride in pharmaceutical preparations by derivative spectrophotometry and HPLC methods

	Tablets						Suspension	
Method	$^1D_{264}$	$^1D_{300}$	$^2D_{276}$	$^2D_{290}$	$^2D_{276\text{-}290}$	HPLC	$^1D_{300}$	HPLC
Mean[a] ± SD	98.94 ± 0.43	99.51 ± 0.42	99.59 ± 0.74	100.47 ± 0.62	99.09 ± 0.58	99.65 ± 0.63	100.16 ± 0.61	100.69 ± 0.26
t[b]	2.08	0.41	0.14	2.07	1.13		1.13	
F[b]	2.15	2.25	1.38	1.03	1.18		5.50	

[a] Mean of five determinations.
[b] The theorectical t- and F-values at P = 0.05 are 2.31 and 6.39, respectively.

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