Scientia Pharmazeutica (Sci. Pharm.) **68**, 281–296 (2000)

Kinetic Method for the Determination of Cisapride in Pharmaceutical Preparations

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ABSTRACT

An accurate kinetic method for the determination of cisapride is described. The method is based on the reaction of cisapride with potassium dichromate in 2.5 M sulphuric acid at room temperature for a fixed-time of 10 min, afterwards the absorbance of the reaction product is measured at 527 nm. The concentration of cisapride is computed using the corresponding calibration equation for the fixed-time method. The method is applied successfully to commercial tablets and oral suspension dosage forms. The results obtained are compared statistically with the British Pharmacopoeia method. The determination of cisapride by other kinetic methods is feasible with calibration equation obtained but the fixed-time method proves to be more applicable.

Keywords: Kinetic method, cisapride, pharmaceutical preparations.

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INTRODUCTION

Cisapride, chemically known as (±)-cis-4-amino-5-chloro-N-[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide monohydrate (see Fig. 1), is used in the treatment of disorders of the gastrointestinal tract. It is believed to facilitate acetylcholine release from the myenteric plexus of the gut[1,2] and to activate 5-HT_4 receptors in the intestinal wall[3]. It has been shown to be effective in treating gastro-oesophageal reflux disease in adults, children and neonates, and it is promising in the management of functional dyspepsia, gastroparesis, intestinal pseudo-obstruction and irritable bowel syndrome[4-6]. Few methods have been reported for the determination of cisapride. These include HPLC methods for determination of the drug in neonatal plasma[3] and animal tissues[1]. Bioequivalence studies have been performed in healthy human volunteers using reversed-phase HPLC[2]. Cisapride enantiomers have been separated using capillary electrophoresis[7]. In pharmaceutical formulations, cisapride has been assayed using fluorimetric method[8]. Few spectrophotometric methods have been reported for assay of cisapride in pharmaceutical preparations. These include extraction spectrophotometric method, based on the formation of chloroform soluble-ion associates with some sulfophthalein dyes[9] or with Suprachen violet-3B, Erioglaucine A, Naphthalene blue 12 BR and Tropaeolin 000[10]. Other spectrophotometric methods have been reported for determination of cisapride in pharmaceutical preparations based on oxidative coupling with 3-methyl-2-

benzothiazolinone hydrazone (MBTH) in presence of ferric chloride, oxidation with Fe (III) and subsequent chelation of Fe (II) with 1,10-phenanthroline or through the formation of colored charge transfer complex with chloranilic acid[11]. A stability-indicating HPTLC method has been reported for the assay of cisapride and related impurities in tablets[12]. In British[13] and European[14] Pharmacopoeias, cisapride has been assayed using potentiometric titration with 0.1 M perchloric acid.

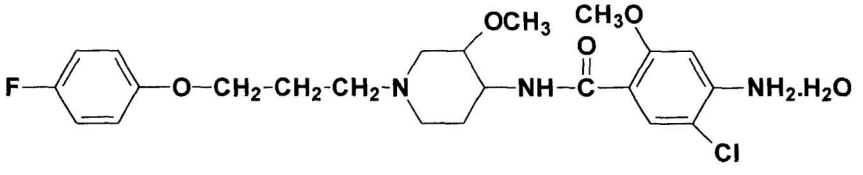


Fig. 1: Chemical structure of cisapride.

In the present work, a kinetically -based method is proposed for the determination of cisapride in pharmaceutical preparations. The proposed method depends on the oxidation of the drug with potassium dichromate in sulphuric acid medium at room temperature. The absorbance of the colored solution is measured at 527 nm. The results obtained by the proposed method are compared with those obtained by the British Pharmacopoeia potentiometric method.

EXPERIMENTAL

Apparatus:-

The spectrophotometric measurements were made on PYE Unicam PU 8800 UV / Visible spectrophotometer (Philips) using quartz cells (1cm) .

Materials:-

Reference cisapride was kindly supplied by Janssen Pharmaceutica and used without further treatment. Prepulsid tablets labeled to contain 10 mg cisapride per tablet and prepulsid suspension labeled to contain 1 mg . ml^{-1} cisapride were obtained from local market in Riyadh, Saudi Arabia.

Reagents:-

All reagents used were of analytical grade. Cisapride standard solution (0.2% w/v) in acetone . Potassium dichromate solution (0.5% w/v) was prepared in 2 M sulphuric acid. Sulphuric acid (4 M), this was prepared and kept as a stock solution.

Sample solution of cisapride in formulations:-

Twenty tablets were powdered and mixed. An accurate weight equivalent to 50 mg cisapride was transferred into 25 ml volumetric flask, sonicated for 15 min, diluted to volume with acetone and filtered.

Similarly, a sample solution of cisapride in suspension was prepared by transferring accurately measured 50 ml suspension into 500 ml separating funnel and extraction of the drug was performed with three successive portions of chloroform (3 x 125 ml). The combined chloroformic extract was dried by

passing over anhydrous sodium sulphate, collected in a round bottom flask and evaporated to dryness under vacuum. The residue was dissolved in acetone, transferred quantitatively into 25 ml volumetric flask and diluted to volume with acetone.

Procedure:-

A 6 ml volume of 4 M sulphuric acid and 5 ml of 0.5% w/v potassium dichromate solution were placed into a series of 25 ml calibrated flasks. An appropriate volume of cisapride (1.00 – 4.75 ml) was added and the solutions were diluted to volume with 2 M sulphuric acid. At a fixed time of 10 min, the absorbance was measured directly at 527 nm against appropriate blank solution. The concentration of cisapride was then computed from the corresponding equation of the calibration graph for the fixed-time method.

RESULTS AND DISCUSSION

Kinetics and optimization of the reaction conditions:-

The reaction between cisapride and potassium dichromate in acidic medium yields a red colored product with maximum absorbance at 527 nm. As the intensity of color increases with time, it was deemed useful to elaborate a kinetically-based method for the determination of cisapride. To realize this, the reaction was investigated under various conditions of reagent concentrations and acidity.

At room temperature, the reaction rate increased substantially as revealed by the intensification of the developed color, suggesting reasonable sensitivity.

The reaction rate and maximum absorbance increased with increase in potassium dichromate concentration, and ultimately the adoption of 0.1% w/v potassium dichromate in the final solution proved to be adequate for the maximum concentration of cisapride used in the calibration curve.

The influence of sulphuric acid concentration on the reaction rate was studied in order to find a concentration of sulphuric acid which permits moderate oxidation of the drug. It was found that increasing acidity increases the reaction rate with the maximum absorbance being reached in shorter time, probably owing to the rise in the oxidation potential of the dichromate. It was observed that there was no significant difference in the absorbances of reactant solutions, at sulphuric acid concentration above 2.3 M. Therefore, 2.5 M sulphuric acid was chosen as the most suitable medium.

The reaction rate was found to be cisapride dependent. The rates were followed at room temperature with various concentrations of cisapride in the range 80-620 $\mu g \cdot ml^{-1}$, keeping potassium dichromate concentration constant at 0.1% w/v and sulphuric acid concentration 2.5 M . The graphs obtained, depicted in Fig. 2, clearly indicate that the reaction rate obeys the following equation:

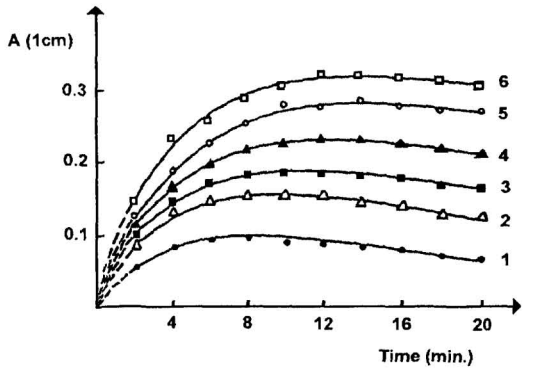


Fig. 2: Absorbance versus time graphs for the reaction between cisapride and potassium dichromate; showing the dependence of the reaction on cisapride concentration, 80,140,200,260,320,380,440 μg .ml^{-1} (curves 1-6) at room temperature, potassium dichromate 0.1% w/v and sulphuric acid concentration 2.5 M.

$$r = k' [\text{cisapride}]^n \quad (1)$$

Where k′ is the pseudo-first order rate constant and n is the order of reaction.

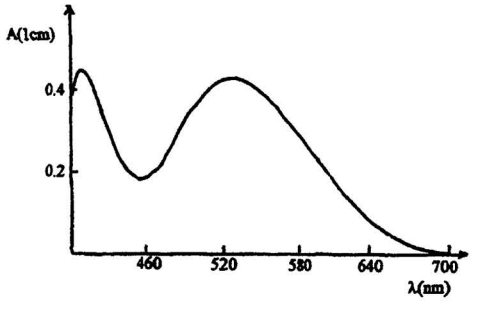
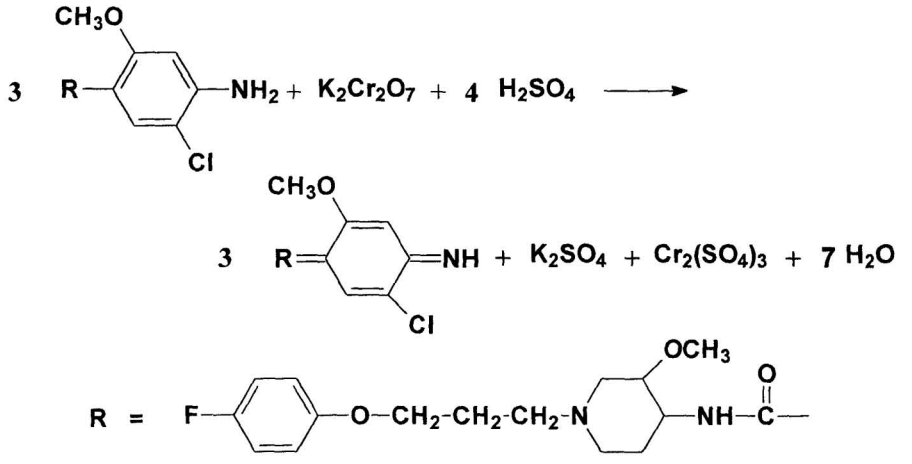


Fig. 3: Absorption curve of the colored product formed by reaction of cisapride (600 μg.ml^{-1}) with potassium dichromate (0.1% w/v) in 2.5 M sulphuric acid.

Fig. 3 shows the absorption spectrum of the colored product, obtained by reaction of cisapride with potassium dichromate at room temperature. The reaction is proposed to proceed as presented by the following equation:



Apparently, the reaction proceeds in two steps, the first step being fast resulting in the formation of an intermediate product and the second step is the rate-determining step.

From Fig. 2, the rate may be estimated as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds. Taking logarithms of rate and concentration (see Table 1), equation (1) is transformed into:

$$\log (\text{rate}) = \log \frac{\Delta A}{\Delta t} = \log k' + n \log [\text{cisapride}] \qquad (2)$$

Regression of log [cisapride] versus log (rate) by least squares method yielded the regression equation:

$$\log (\text{rate}) = -1.126 + 0.73 \log c$$

with correlation coefficient (r) = 0.9971, hence $k' = 7.48 \times 10^{-2}\ s^{-1}$ and the reaction is first order (n ~ 1) with respect to cisapride.

Evaluation of kinetic methods:

The quantitation of cisapride under the optimized experimental conditions outlined above, where the potassium dichromate and sulphuric acid concentration were about 4-20 times of the initial concentration of cisapride, would result in a pseudo-zero order reaction with respect to their concentrations.

Table 1: Logarithms of rates for different concentrations of cisapride at constant concentration of 2.5 M sulphuric acid and 3.4×10^{-3} M potassium dichromate at room temperature.

log cisapride [M]	log (rate), $\Delta A/\Delta t$
-3.78	-3.87
-3.54	-3.73
-3.38	-3.60
-3.27	-3.52
-3.18	-3.44
-3.10	-3.37
-3.04	-3.33
-2.94	-3.29

However, the rate will be directly proportional to cisapride concentration in a pseudo-first order rate equation as follows:

$$r = k' [\text{cisapride}] \qquad (3)$$

Where k' is the pseudo-first order rate constant. Equation (3) was the basis for several experiments, which were carried out to obtain cisapride concentration using the rate data. Initial-rate, rate-constant, fixed-concentration and fixed-time methods[15,16] were tried and the most suitable analytical method was selected taking into account the applicability, the correlation coefficient (r) and the intercept.

Rate-constant method:

Graphs of log (absorbance) versus time for cisapride concentration in the range of $1.65 \times 10^{-4} - 9.09 \times 10^{-4}$ M (80 – 440 μg . ml^{-1}) were plotted and all appeared to be rectilinear. Pseudo-first order rate constants (k') corresponding to different cisapride concentrations (c) were calculated from the slopes multiplied by –2.303 and are presented in Table 2. Regression of (c) versus k' gave the equation:

$$k' = 2.776 \times 10^{-4} - 2.604\ c\ , \qquad r = 0.9730$$

the value of (r) indicates poor linearity, which is probably due to changes in k' as a result of temperature variation or fluctuation.

Table 2: Values of k′ calculated from slopes of log A versus t graphs multiplied by –2.303 for different concentrations of cisapride at constant concentrations of 2.5 M sulphuric acid and 3.4×10^{-3} M potassium dichromate at room temperature.

Cisapride[M]	$k'.s^{-1}$
1.65×10^{-4}	-3.26×10^{-4}
2.89×10^{-4}	-4.22×10^{-4}
4.13×10^{-4}	-5.57×10^{-4}
5.37×10^{-4}	-1.02×10^{-3}
6.61×10^{-4}	-1.61×10^{-3}
7.85×10^{-4}	-1.92×10^{-3}
9.09×10^{-4}	-1.99×10^{-3}

Initial-rate method.

Graphs of absorbance versus time for cisapride concentration in the range of $1.65 \times 10^{-4} - 7.85 \times 10^{-4}$ M (80 – 380 μg . ml^{-1}) were plotted and the slopes were calculated. The slopes corresponding to different cisapride concentrations, c, are presented in Table 3. Regression of (c) versus slopes gave the equation:

$$\text{Slope} = 0.1003 + 161.06\ c, \qquad r = 0.9932$$

the value of (r) indicates poor linearity, indicating that the first step is too fast and not rate-determining.

Table 3: Values of slopes calculated for different concentrations of cisapride at 2.5 M sulphuric acid and 3.4 x 10^{-3} M potassium dichromate at room temperature.

Cisapride [M]	k′.s $^{-1}$
1.65 x 10^{-4}	0.133
2.89 x 10^{-4}	0.142
4.13 x 10^{-4}	0.167
5.37 x 10^{-4}	0.183
6.61 x 10^{-4}	0.203
7.85 x 10^{-4}	0.233

Fixed-concentration method:

A preselected value of the absorbance was fixed for different concentrations of cisapride in the range of 2.89 x 10^{-4} – 7.85 x 10^{-4} M (140 – 380 μg.ml^{-1}), and the time was measured in seconds. The reciprocal of time (1/t) versus the initial concentrations of cisapride (Table 4) was plotted and the following equation was obtained by linear regression:

$$\frac{1}{t} = -1.19 \times 10^{-3} + 11.75\ c\ , \qquad r = 0.9947$$

The range of cisapride concentration giving the most satisfactory calibration curve with the above equation was 140 – 380 μg.ml^{-1} with poor linearity and therefore this method was abandoned.

Table 4: Values of reciprocal of time taken at a fixed absorbance for different rates of various concentrations of cisapride at 2.5M sulphuric acid and 3.4×10^{-3} M of potassium dichromate at room temperature.

Cisapride[M]	$\frac{1}{t}.s^{-1}$
2.89×10^{-4}	2.34×10^{-3}
4.13×10^{-4}	3.66×10^{-3}
5.37×10^{-4}	5.00×10^{-3}
6.61×10^{-4}	6.25×10^{-3}
7.85×10^{-4}	8.33×10^{-3}

Fixed-time method:

Reaction rates were determined for different concentration of cisapride. At a preselected fixed-time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance (A) versus initial concentration of cisapride (c) were established at fixed times of 4, 6, 8, 10 and 12 min in the concentration range of $1.65 \times 10^{-4} - 7.85 \times 10^{-4}$ M (80 – 380 μg $.ml^{-1}$), with the regression equations assembled in Table 5. It is clear that the slope increases with time and the most acceptable values for the correlation coefficient (r) and the intercept were obtained for a fixed-time of 10 min. This was, therefore, chosen as the most suitable time interval for measurement.

Table 5: Regression equations adopting the fixed time method for cisapride concentrations over the range of 1.65-7.85 x 10^{-4} M (80 – 380 μg. ml^{-1}) at room temperature.

Time[min]	Calibration equation	Correlation coefficient (r)
4	A = 0.052 + 180.88 c	0.9977
6	A = 0.048 + 226.04 c	0.9967
8	A = 0.037 + 264.52 c	0.9991
10	A = 0.032 + 282.49 c	0.9992
12	A = 0.026 + 300.69 c	0.9981

Applications:-

The fixed-time method was applied to the determination of cisapride in pharmaceutical preparations. The concentration of cisapride was calculated using the corresponding regression equation, shown in Table 5, at the fixed time of 10 min.

The performance of the kinetic spectrophotometric method was statistically compared with the British Pharmacopoeia potentiometric method[13] by Student's t-test and variance ratio test. The calculated t- and F- values did not exceed the theoretical values, indicating no significant difference between the two methods (Table 6).

Table 6: Statistical comparison of the results obtained by the fixed-time method after 10 min with those obtained by the British Pharmacopoeia method[13].

Pharmaceutical Preparation	% Recovery ± S.D.		t†	F†
	Kinetic method*	B.P. Method**		
Tablets	99.79 ± 0.95	99.86 ± 0.53	0.14	3.21
Suspension	101.02 ± 0.61	100.93 ± 0.40	0.38	2.33

* Mean of four separate determinations.
** Mean of five separate determinations.
† Theoretical values of t- and F- are 2.36 and 6.59 at 95% confidence level.

In conclusion, the kinetically based method, proposed in this work, for the determination of cisapride is a direct, simple and accurate method. The simplicity of the method makes it convenient for routine quality control analysis of cisapride. In addition, the proposed method is more sensitive than the British Pharmacopoeia potentiometric method.

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Received May 9th, 2000
Accepted July 21th, 2000