

## Complexometric-Spectrophotometric Determination of Cisapride and Tiapride in Their Formulations†

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AJC-11680

Simple and sensitive visible spectrophotometric methods were developed for the quantitative determination of cisapride and tiapride in their pharmaceutical formulations. The methods are based on the formation of ion association complexes with acidic dyes, which are extractable into chloroform. Cisapride was determined by its reaction with bromocresol green or bromophenol blue at pH 2.5 to yield yellow coloured complexes, peaking at 414 nm and 412 nm with bromocresol green and bromophenol blue respectively. Tiapride was determined by its reaction with tropaeolin OO(TOO) at pH 5 to yield a yellowish ion-pair complex measured at 412 nm. Good linearities were achieved in the range of 5-22.5 µg/mL cisapride with both bromocresol green and bromophenol blue and 4-20 µg/mL tiapride with mean recoveries of 100.0 ± 0.28, 100.4 ± 0.94 and 99.9 ± 0.72 %, respectively. The proposed methods were successfully applied to analyze both drugs in their tablets.

**Key Words:** Cisapride, Tiapride, Bromocresol green, Bromophenol blue, Tropaeolin OO, Ion pair complexes, Pharmaceutical formulations, Spectrophotometry.

### INTRODUCTION

Cisapride and tiapride are substituted benzamides, which exhibit antipsychotic properties<sup>1</sup>. They are antagonist of the dopamine D<sub>2</sub> receptors, which distinguishes these compounds from other antipsychotic agents. This singular feature may explain the very low incidence of side-effects on the extrapyramidal system. In addition to their anti-psychotic action, substituted benzamides present antiemetic, antidiabetic and antihypertensive action<sup>1</sup>.

A review of the literature revealed that few methods have been described for determination of cisapride and tiapride. The two drugs and their formulations are officially determined in the British pharmacopoeia<sup>2</sup> by non-aqueous titration with HClO<sub>4</sub>. Cisapride is also official in the European pharmacopoeia<sup>3</sup>. The reported methods for determination of cisapride either in pharmaceuticals or in biological fluids include high-performance liquid chromatography (HPLC)<sup>4-9</sup>, thin-layer chromatography (TLC)<sup>10</sup>, polarography<sup>11</sup>, differential pulse and square wave voltammetry<sup>12</sup>, spectrofluorimetry<sup>13</sup> and derivative UV spectrophotometry<sup>14,15</sup>.

Few reports on the use of visible spectrophotometry were found in the literature for the determination of cisapride in pharmaceuticals<sup>16-20</sup>. Tiapride was determined in pharmaceuticals or in biological fluids by few techniques including

titrimetry in non-aqueous medium with HClO<sub>4</sub><sup>21</sup>, HPLC<sup>22-24</sup>, hydrophilic interaction liquid chromatography-tandem mass spectroscopy<sup>25</sup>, TLC<sup>26</sup>, GC<sup>27</sup>, capillary electrophoresis<sup>28</sup>, IR-spectroscopy<sup>29</sup>, differential pulse anodic voltammetry<sup>30</sup>, selective membrane sensors<sup>31</sup>, flow-injection chemiluminescence<sup>32</sup> and spectrofluorimetry<sup>24</sup>. Derivative spectrophotometric methods<sup>24</sup> were used for the determination of tiapride in pharmaceutical preparations and human plasma, but no visible spectrophotometric methods were found in the literature for its determination.

The aim of the present study was to develop new, simple, sensitive and reliable visible spectrophotometric methods for the fast control analysis of cisapride and tiapride in pure forms and in their dosage forms based on the formation of ion-pair complexes.

### EXPERIMENTAL

A Unicam UV-VIS spectrometer, Helios Alpha: Helios Beta model with 1 cm cuvette (Biochrom, England) was used for all spectra and absorbance measurements.

Pure cisapride and tiapride were obtained from Janssen Pharmaceutica Co./Belgica. Pharmaceutical formulations were obtained from local markets, as Prepulsid tablets (10 mg cisapride/tablet), B.N. 98 F22/611 produced by Janssen Pharmaceutica Co./Beerse /Belgica and Tiapridal tablets (100

†Presented at International Conference on Global Trends in Pure and Applied Chemical Sciences, 3-4 March, 2012; Udaipur, India

mg tiapride/tablet) B.N. 20024, produced by Synthelabo laboratories.

All chemicals used were of analytical reagent-grade quality and solvents were of spectroscopic grade. Distilled water was used throughout this work. Aqueous solutions of the following acid dyes were prepared: 0.1 % (w/w) Bromocresol green (BDH Chemicals Ltd. Poole, England), 0.1 % (w/w) Bromophenol blue (Prolabo products) and 0.05 % (w/w) Trapaeolin OO (Carlo Erba), Division Chimica Industriale-Milano). Britton and Robinson Buffers of pH = 2.5 and pH = 5 were prepared<sup>33</sup>. Other chemicals used were: anhydrous sodium sulphate (BDH), methanol (BDH) and chloroform (BDH).

**Standard solutions:** Standard stock methanolic solution of pure cisapride (0.5 mg/mL) and tiapride aqueous solution (0.4 mg/mL) were prepared.

**Determination of cisapride:** Accurately measured aliquots of the standard cisapride solution equivalent to 5-22.5 µg/mL, were transferred into a series of 25 mL separating funnels, 2 mL of pH 2.5 buffer solution and 3 mL of 0.1 % BCG dye were added to each separating funnel. Then 10 mL of chloroform was added to each and the contents were shaken for 2 min for BCG or 3 min for BPB. The two phases were allowed to separate and the separated chloroform layer was filtered through anhydrous sodium sulphate. The absorbance of the chloroform layer was measured at 414 nm for BCG or at 412 nm for BPB against reagent blanks. The calibration curves obtained by plotting the absorbance against the concentration of cisapride were utilized to determine the amount of cisapride contained in a given sample.

**Determination of tiapride:** Into a series of 25-mL separating funnels, accurately measured aliquots of standard tiapride solution equivalent to 4-20 µg/mL were introduced. 2 mL of pH5 buffer solutions and 3 mL of 0.05 % TOO were added to each separating funnel. Then 10 mL of chloroform was added to each and the contents were shaken for 4 min. The two phases were allowed to separate and the separated chloroform layer was filtered through anhydrous sodium sulphate. The absorbance of the chloroform layer was measured at 412 nm against a reagent blank. The calibration curve obtained by plotting the absorbance against the concentration of tiapride was utilized to determine the amount of tiapride contained in a given sample.

**Application to pharmaceutical formulations:** Ten Prepulsid tablets were weighed accurately and ground into fine powder. An accurately weighed amount of the powder equivalent to 12.5 mg of cisapride was transferred into a small conical flask and dissolved in 10 mL methanol. The solution was filtered into a 25-mL volumetric flask and completed to volume with methanol. The obtained solution labeled to contain 0.5 mg/mL cisapride was analyzed by the proposed method as detailed above using the standard addition method.

Similarly, a weight of the fine powder of tiapridal tablets equivalent to 10 mg of tiapride was transferred into a small conical flask and dissolved in 10 mL of distilled water. The solution was filtered into a 25 mL volumetric flask and completed to volume with water. The obtained solution labeled to contain 0.4 mg/mL tiapride was analyzed by the proposed method for tiapride determination as described above using the standard addition method.

## RESULTS AND DISCUSSION

BPB and BCG are dyes of sulphonaphthalein type and the colour of such dyes is due to the opening of lactoid ring and subsequent formation of quinoid group<sup>34</sup>. Cisapride and tiapride form ion-pair complexes with acidic dyes such as BPB, BCG and TOO since they contain basic nitrogen atoms, which can be protonated easily. After protonation of the drug, the protonated drug forms ion-pair complexes with these anionic dyes.

**Absorption spectra:** The reaction of BPB or BCG with cisapride or TOO with tiapride results in the formation of intense yellow coloured products. The absorption spectra of these complexes were recorded at 200-800 nm against the corresponding blank solutions as shown in Fig. 1. The resulting ion-pair complexes showed maximum absorbance at 414, 412 and 414 nm for BCG, BPB and TOO methods respectively.

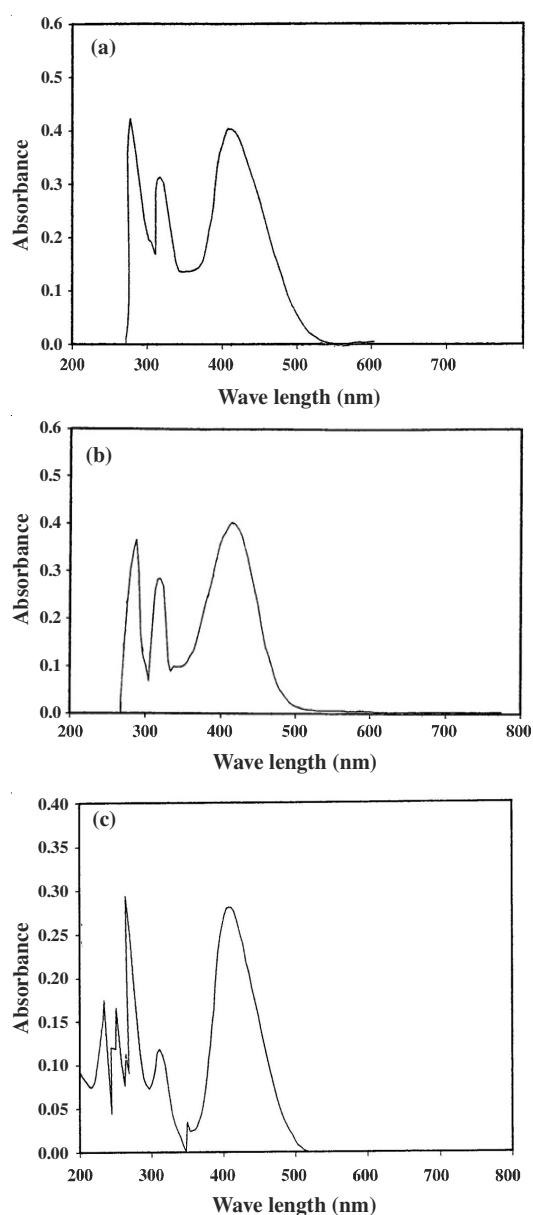


Fig. 1. Absorbance spectra of ion-pair complexes of: (a) Cisapride (10 µg/mL) with BCG against reagent blank. (b) Cisapride (10 µg/mL) with BPB against reagent blank. (c) Tiapride (4 µg/mL) with TOO against reagent blank

**Optimization of reaction variables:** Optimum reaction variables for quantitative determination of the formed ion-pair complexes were established *via* various preliminary experiments such as choice of organic solvent, concentration and volume of the buffer solution, concentration of the dye, shaking period and reaction time.

The effect of buffer solutions was studied by using buffered solutions in the range of pH between 2-8. It was found that pH = 2.5 and pH = 5 were the suitable pH values for cisapride with both dyes and for tiapride with TOO respectively, which gave the highest absorbance of the ion association complexes formed. In order to establish the optimum volumes of the buffered solutions for the proposed methods, cisapride was allowed to react with BCG or BPB and tiapride with TOO in range of volumes between 1.0-5.0 mL of the aqueous buffered solutions (pH = 2.5 for cisapride and pH = 5 for tiapride) and the complexes formed were extracted into the chloroform layer for measurements. A volume of 2.0 mL of each buffer solution was required for higher absorbance value of the complexes in the organic phase at the wavelength of maximum absorbance.

The efficiency of extraction was also affected by dye concentration. The effect of the dye-concentration on the intensity of the colour developed at the selected wavelengths was studied by measuring the absorbances of solutions containing different amounts of the reagents BPB and BCG and fixed concentrations of 5 µg/mL cisapride or similarly using different amounts of the reagent TOO and 4 µg/mL tiapride. The results showed that maximum colour intensity of the complex was achieved with 3 mL of 0.1 % BCG, 2 mL of 0.1 % BPB and 3 mL of 0.05 % TOO solutions and any excess dyes did not affect the absorbance of the complexes. Optimum shaking periods were also investigated and were found to be: 2, 3 and 4 min for BCG, BPB and TOO respectively.

The addition of the dye solution resulted in an immediate full colour development at room temperature and the formed ion-pair complexes were stable for at least 1.0 h in all methods. The reaction was found complete and quantitative when the reactants were added and any delay in the absorbance measurements of the formed ion-pair complexes up to 1.0 h had no pronounced effect on the measured absorbance.

**Composition of the ion-pair complexes:** Job's method<sup>35</sup> of continuous variations of equimolar solutions was employed to establish the composition of the ion-pair complex formed between cisapride with BPB/ BCG and between tiapride with TOO. In this method, solutions of  $1.4 \times 10^{-3}$  mol/L standard cisapride solution and  $1.4 \times 10^{-3}$  mol/L BCG dye or  $1.49 \times 10^{-3}$  mol/L cisapride and  $1.49 \times 10^{-3}$  mol/L BPB dye or  $1.22 \times 10^{-3}$  mol/L standard tiapride solution and  $1.22 \times 10^{-3}$  mol/L TOO dye were mixed in varying volume ratios in such a way that the total volume of each mixture was kept the same at 10 mL. The absorbance of each solution was plotted against the mole fraction of cisapride or tiapride. The plot reached a maximum value at a mole fraction of 0.5, which indicated the formation of 1:1 (drug:dye) ion-pair complexes and confirm the presence of one basic nitrogen containing group.

**Methods validation:** Under optimum experimental conditions, the methods were tested for linearity, specificity, precision and reproducibility. With the above spectrophotometric

methods, linear regression equations were obtained ( $A = 0.0543C + 0.0105$ ,  $A = 0.0372C + 0.0302$  and  $A = 0.0507C + 0.1148$  for BPB, BCG and TOO respectively). The regression plots showed that linear dependence of the relative absorbance intensities on the concentrations of the studied drugs were in the ranges of 5.0-22.5 µg/mL for cisapride with both BPB and BCG dyes with LOD of 0.75 and 1.05 µg/mL and LOQ of 2.5 and 3.5 µg/mL respectively and in the range of 4.0-20.0 µg/mL for tiapride with TOO dye with LOD of 1.17 µg/mL and LOQ of 3.9 µg/mL. Statistical evaluation of the experimental data regarding standard deviation of the slope ( $S_b$ ) and standard deviation of the intercept ( $S_a$ ) were calculated and were found to be:  $S_b = 0.001$  for both BPB and BCG and 0.003 for TOO and  $S_a = 0.010, 0.013$  and 0.039 for BPB, BCG and TOO respectively. The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the correlation coefficients 0.9999 for cisapride with both BPB and BCG and 0.9995 for tiapride with TOO (close to 1 in all cases).

The validity of the methods could be proved by analyzing authentic samples of the drugs. The results obtained in Table-1 are in good agreement with those given by the comparison methods<sup>20,32</sup>.

TABLE- 1  
STATISTICAL COMPARISON BETWEEN THE RESULTS OF THE PROPOSED METHODS FOR DETERMINATION OF CISAPRIDE AND TIAPRIDE AND PUBLISHED METHODS

Value	Cisapride		Tiapride		
	BPB	Published	BCG	TOO	Published method <sup>32</sup>
Mean ±	99.9	99.3	100.0	100.4	99.6
S.D.	0.72	0.70	0.28	0.94	0.53
n	9	5	8	8	5
Variance	0.52	0.49	0.08	0.88	0.28
t-test	1.579 (2.179)*		1.749 (2.201)*	1.611 (2.201)*	
F-test	1.067 (6.04)*		3.582 (4.12)*	3.117 (6.09)*	

\*Figures in parentheses are the theoretical t-and F- values at P = 0.05 confidence limit.

The specificity of the methods were investigated by observing that no interferences were encountered from common tablet excipients.

The accuracy and validity of the proposed methods were ascertained by performing the recovery experimental *via* the standard addition procedure. Pre-analyzed tablet powder was spiked with pure drug at five different concentration levels and the total was measured using the proposed methods. The determination with each level was repeated three times and the results of this study presented in Table-2 indicated that the commonly excipients present in the formulations did not interfere in the assay.

## Conclusion

The proposed methods are simple, accurate, inexpensive, less time consuming, sensitive and require minimum equipments and chemicals. The results are reproducible.

TABLE- 2  
RESULTS OF RECOVERY STUDY BY STANDARD-ADDITION METHOD

Cisapride							
Formulation	BCG Method				BPB Method		
	Amount found (mg)	Pure added ( $\mu\text{g/mL}$ )	Pure found ( $\mu\text{g/mL}$ )	Pure recovered (%)*	Amount found (mg)	Pure found ( $\mu\text{g/mL}$ )	Pure recovered (%)*
Prepulsid tablets (10 mg/tablet) B.N.98F22/611	10.04	5.00	4.95	99.00	9.973	5.07	101.40
		7.50	7.50	10.00		7.62	101.60
		10.00	9.97	99.70		10.17	101.70
		12.50	12.39	99.12		12.68	101.44
		15.00	14.87	99.13		15.28	101.87
Mean $\pm$ S.D.				99.39 $\pm$ 0.436			101.60 $\pm$ 0.193
Tiapride/TOO method							
	Found (mg)	Pure added ( $\mu\text{g/mL}$ )		Pure found ( $\mu\text{g/mL}$ )		Pure recovered (%)*	
Tiapridal tablets (100 mg/tablet) B.N. 20024	100.50	4.00		3.96		99.00	
		6.00		5.97		99.50	
		8.00		8.11		101.38	
		10.00		9.91		99.10	
		12.00		12.05		100.42	
Mean $\pm$ S.D.							99.88 $\pm$ 1.008

\*Mean value of three determinations

### ACKNOWLEDGEMENTS

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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