New Spectrophotometric Methods for the Determination of Cisapride

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Three simple and sensitive spectrophotometric methods have been described for the determination of cisapride, based on the coupling of the diazotised drug with the reagents such as chromotrophic acid (method A), phloroglucinol (method B) and N- (1-naphthyl) ethylene diamine dihydrochloride, NED (method C) to give coloured products having maximum absorbances at 530nm, 450nm and 540nm, respectively. These methods are extended to the analysis of pharmaceutical formulations and results compared with the UV reference method.

CISAPRIDE (CPD), is official in B.P.[1]. The reported methods for its determination are fluorimetry,[2] HPLC[3,4], TLC[5] and a fluorescent polarisation immuno assay method.[6] No colorimetric method has been reported so far for its determined. It is, therefore, of interest to develop fast and simple procedures for the determination of CPD in pharmaceutical formulations. In the present communication, three spectrophotometric methods have been described for the determination of CPD, which involve diazotisation of CPD followed by coupling with the above mentioned reagents.

A systronics model 106 digital spectrophotometer with 1cm matched glass cells and an Elico-LI-120 digital pH meter were used for all absorbance and pH measurement. All solutions were prepared in doubly distilled water and all chemicals of analytical grade were used.

Aqueous solutions of sodium nitrite (0.1%), hydrochloric acid (0.1M), sodium hydroxide (1.0 M), ammonium sulphamate (5.0%), chromotrophic acid (0.5%), phloroglucinol (0.5%) and NED (0.2%) were used.

A 1mg/ml stock solution of CPD was prepared by dissolving 100 mg of the drug in a minimum amount of glacial acetic acid and made upto 100 ml with distilled water. This stock solution was diluted stepwise with distilled water to obtain the working standard solution (100 μg/ml for all three methods).

Aliquots of standard drug solution (0.25-3.0 ml, 100 μg/ml, method A: 0.25-4.0 ml 100 μg/ml, methods B and C) were placed in a series of 25 ml calibrated tubes. Solutions of 1.0 ml each of sodium nitrite and HCl were added and allowed to stand for 3 min to complete the diazotisation. Then 5.0 ml of ammonium sulphamate was added and shaken thoroughly to destroy the excess nitrous acid. 4.0 ml of chromotrophic acid and 1.5 ml of NaOH (method A), 4.0 ml of phloroglucinol (method B) or 1.0 ml of NED (method C) were added successively and diluted to mark with distilled water. Absorbances of the coloured species were measured against their corresponding reagent blanks at 530 nm, 450 nm and 540 nm for methods, A,B and C respectively during the stability period (1 min - 4 h, method A; 1 min-2h, method B; and 1 min-3h, method C). The amount of CPD present was computed from the respective calibration curves.

A portion of any pharmaceutical preparation (tablets, chewable tablets and suspension) equivalent to 100 mg of active ingredient was treated with about 40 ml of chloroform and the insoluble portion was

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Table 1: Assay and Recovery of cisapride in pharmaceutical formulations

Formulation	Labelled amount (mg)	Found by proposed methods* A	B	C	found by UV reference method+	Recovery in proposed methods** % A	B	C
Tablets								
T_1	10	10.05±0.095	10.15±0.076	9.94±0.084	9.97±0.088	99.7±0.89	100.1±0.98	99.5±0.14
T_2	10	9.91±0.064	10.29±0.059	9.76±0.050	10.21±0.092	99.9±0.84	99.6±1.05	99.5±0.87
Chewable Tablets								
T_1	10	9.94±0.106	10.05±0.087	9.95±0.084	9.95±0.134	99.9±0.26	99.7±0.78	99.4±0.24
T_2	10	9.94±0.047	10.09±0.051	9.97±0.075	9.93±0.072	99.5±0.55	100.2±0.95	99.9±0.74
Suspension								
S_1	30	29.92±0.57	30.12±0.87	31.05±0.62	30.12±0.972	99.7±0.93	100.4±0.78	101.2±0.93
S_2	60	59.72±0.89	60.21±0.81	61.07±0.86	59.90±1.02	99.5±0.68	100.3±0.63	101.8±0.21

** After adding 10mg: each values is an average of three determinations.

* Average of six determinations.

+ UV reference method, developed in our laboratory.

filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in minimum amount of glacial acetic acid, followed by dilution to 100 ml with distilled water to obtain a solution of 1 mg/ml, which was further diluted with distilled water to the working concentration range.

Optimum operating conditions used in the procedure were established by adopting variation of one variable at a time (OVAT)[7] method. The optical characteristics such as Beer's law limits (μg/ml), molar absorptivity (1 mol^{-1} cm^{-1}) and Sandell's sensitivity (μg/cm^2/0.001 absorbance unit) were found to be 1.0-10.0, $3.48x10^4$, 0.013; 1.0-14.0, $2.62x10^4$, 0.018; and 1.0-14.0, $3.11x10^4$, 0.015 for methods A, B and C, respectively. The slope, intercept, and correlation coefficient obtained by linear least squares treatment of the results were found to be $7.22x10^{-2}$, $0.91x10^{-3}$, 0.999; $5.35x10^{-2}$, $2.13x10^{-3}$, 0.999; and $6.29x10^{-2}$, $3.39x10^{-3}$, 0.999 for methods A,B and C, respectively. The precision and accuracy of these methods were tested by estimating six replicate samples of CPD within the Beer's law limits. The percent standard deviation and the percent range of error at 95% confidence level have been found to be 0.43, 0.45; 0.48, 0.51; and 0.51, 0.54 for methods A, B and C, respectively. The λ_{max} and ε_{max} values of the proposed methods are in the order C>A>B and A>C>B respectively. All the three methods are equally important for the determination of CPD in pharmaceutical formulations, since the possible interferences will be removed by extracting CPD selectively into chloroform prior to its estimation.

Formulations were successfully analysed by the proposed methods. The values obtained by the proposed and UV reference (which is developed in our laboratory, showing characteristic λ_{max} at 308 nm in methanol) methods for pharmaceutical preparations are listed in Table 1. Recovery experiments were performed using the standard addition method and the results are shown in Table 1. The excipients and additives usually present in the dosage forms of CPD did not interfere in the proposed methods.

The proposed methods are simple, rapid and sensitive with reasonable precision and accuracy and offer advantage in that only trace amounts of

drug or dosage formulation (even upto 1.0 μg/ml) is required for analysis. The proposed methods can be used for the routine determination of CPD in the pure form and in pharmaceutical formulations depending upon the availability of chemicals.

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Colorimetric Determination of Vitamin-A with 4- Hydroxy-3-Methoxy-Benzaldehyde

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A simple Spectrophotometric method in the visible region is described for the estimation of vitamin A or its esters. The method is based on the formation of a coloured condensation product with 4-hydroxy-3-methoxy benzaldehyde which shows maximum absorption at 610 nm.

A few colorimetric methods of assay have been reported for a analysis of vitamin A or its ester in bulk samples and pharmaceutical formulations[1-7] H.P.L.C. procedures[8-9] were also developed for the estimation of vitamin A. The present work describes a new, simple, rapid, selective and sensitive method which is based on the reaction with 4-hydroxy-3-methoxy benzaldehyde which gives an intense blue colour with maximum absorption at 610 nm.

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Ethanolic solution of 4 hydroxy-3-methoxy benzaldehyde (0.5% W/V) and ethanolic solution of potassium hydroxide (9% w/v) were prepared. Analytical grade ethanol, sodium sulphate, solvent ether, isopropanol and sulphuric acid were used in the investigation.

Chemito 2500 u.v. visible scanning spectrophotometer was used for all absorbance measurements.

The standard solution of vitamin A was prepared by transfering an accurately weighed portion of vi-