Indian Journal of Chemical Technology
Vol. 23, September 2016, pp. 425-432

New visible spectrophometric methods for the assay of cintapride

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Received 13 June 2014; accepted 11 August 2015

In this study, four new simple, accurate and highly sensitive spectrophotometric methods have been developed for the determination of Cinitapride (CIN), in both pure and in pharmaceutical preparations. The method M_1 [Metol-Cr (VI)] and M_2 (DMPD – CAT) are direct methods. The method M_3 (NBS/Br-/metol - SA) where the drug is oxidised with excess of N-bromosuccunimide in acid medium, followed by the determination of unreacted N-bromosuccunimide with the dye Celestin Blue and M_4 (EDDP/KIO_3), where the coloured species formation appears to be due to the formation of coloured charge-transfer complex are indirect methods. Regresion analysis of Beer's law plots show good correlation in the concentration range of 4.0-16, 2.0-12.0, 4.0-14 and 2-10 µg/mL for methods M_1, M_2, M_3 and M_4 respectively, and the corresponding molar absorptivity values are 0.6741×10^4, 3.3708×10^4, 2.5155×10^4 and 2.435×10^4 L $mol^{-1}cm^{-1}$. All variables have been optimized and the results were statistically compared with those of literature methods by employing the student's ***T***-test and ***F***-test. No interference has been observed from excipients normally added to the tablets. All the methods are new and superior to the existing methods in terms of λ max and molar absorpitivity. The methods can be applied to the routine pharmaceutical analysis of cinitapride in the formulations.

Keywords: Cinitapride (CIN), Visible spectrophotometry, Metol-Cr (VI), DMPD–CAT, NBS/Br-/metol–SA, EDDP/KIO_3.

Cinitapride (CIN) (RS)-4-amino-N-[1-(1-cyclohex-3-enylmethyl)-4-piperidyl]-2-ethoxy-5-nitro-benzamide (Fig. 1) is yellowish crystalline powder sparingly soluble in water and soluble in chloroform, methanol and glacial acetic acid. It is a gastro intestinal drug that has action against to the serotoninergic 5-HT2 and D2 dopaminergic receptors that has been indicated in the gastro esophageal reflux and in the functional disorders of gastrointestinal motility treatment. Pharmacological effect of cinitapride has been studied[1]. Polarographic[2], derivative spectophotometric[3], validated extractive spectrophotometric method[4], LC-MS/MS[5,6], RP-HPLC[7,8], HPLC[9-12], new visible spectrometric methods[13,14], U.V[15], were available in the literature for the estimation of the drug. Relatively little attention was paid to the development of visible spectrophotometric methods for this drug. The chemical features of the drug molecule offers a lot of scope for the development of new methods with better sensitivity, specificity, precision and accuracy. The reported chromatographic techniques (HPLC or GC) require expensive experimental set-up and are not affordable in every laboratory for routine analysis. Although visible spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, for their simplicity, selectivity and sensitivity there is only a single report so far for the determination of Cinitapride. Therefore, the need for a fast, low cost and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing Cinitapride. This paper describes the development of sensitive and rapid spectrophotometric methods using Metol-chromium (Cr) (VI) (method M_1), ***p***-N,N-dimethyl phenylene diamine (DMPD)-chloramine T (CAT) (method M_2), N-bromosuccinamide (NBS)/Metol-sulphanilamide (S.A) (method M_3) and 2,2'ethane diamino diphenol (EDDP)/KIO_3 (method M_4) which have been found to be satisfactory for the determination of Cinitapride in pure and pharmaceutical formulations.

Experimental Section

Apparatus

All spectral and absorbance measurements were made on a systronics 106 model visible spectrophotometer with 1 cm matched quartz cells. An Elico 120 digital ***p***H meter was used for ***p***H measurements.

Reagents and standards

All chemicals and reagents used were of analytical reagent grade and distilled water was used throughout the investigation.

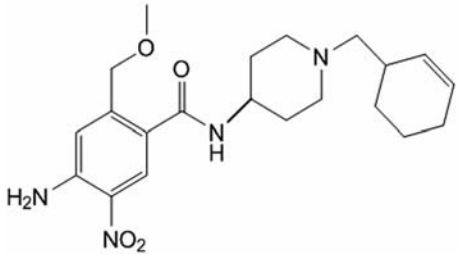


Fig. 1 — Cinitapride

Standard CIN solution

Pharmaceutical grade CIN was obtained from Ranbaxy India, as a gift sample. A stock standard solution equivalent to working standard was prepared from the stock solution. One mg mL^{-1} stock solution of CIN in aqueous medium was prepared by dissolving 100 mg of CIN in 10 mL of 0.1M HCl followed by dilution to 100 mL with distilled water (method M_1, M_2, M_3 and M_4). Working standard solution is prepared by further diluting the stock solutions suitably where ever necessary with appropriate solvents. 100 µg/mL M_1, 100 µg/mL M_2, 100 µg/mL M_3 and 100 µg/mL M_4 with distilled water. Pharmaceutical formulations of Cinmove 1 mg (Cipla), Kinpride 1 mg (Dr.Reddy's) Cintapro 1 mg (Zydus Alidac) were purchased from local markets in India.

Method M_1

Metol solution (BDH, 0.15%, w/v 4.35×10^{-3} M): Prepared by dissolving 150 mg of metol in 100 mL of distilled water

Cr (VI) (Reechem; 0.3%, w/v 1.02×10^{-2} M): Prepared by dissolving 300 mg of dichromate in 100 mL of distilled water.

Buffer solution (***p***H 3.0): Prepared by dissolving 40.846 g of $KHPO_4$ in 100 mL distilled water and 408 mL of 0.1 N HCl are mixed and brought to 200 mL with water

Method M_2

DMPD solution (Merck; 0.1%, w/v 4.68×10^{-3}M):

Prepared by dissolving 100 mg of DMPD in 100 mL of distilled water

CAT solution (Loba 0.1% w/v $4.39 \times l0^{-3}$M): Prepared by dissolving 100 mg in 100 mL distilled water.

Buffer solution: (***p***H 7 buffer): Prepared by mixing 61.2 mL of Na_2HPO_4 (0.067 M) and 38.8 mL KH_2PO_4 (0.067 M) and ***p***H of the solution was adjusted to ***p***H 7

Method M_3

NBS solution (Lob a; 0.088%, w/v 4.94×10^{-3}M): Prepared by dissolving 88 mg of NBS in 100 mL of distilled water

KBr solution (Wilson Labs; 0.5%, 4.2×10^{-2}M): 5.0 mL of AcOH was made upto 100 mL of distilled water.

Metol solution (Wilson Labs; 0.3%, 8.71×10^{-3}M):

Prepared by dissolving 500 mg of KBr in 100 mL of distilled water.

SA solution (Wilson Labs; 0.2%, $1.16 \times l0^{-2}$M: Prepared by dissolving 300 mg of Metol in 100 mL of distilled water.

Method M_4

EDDP solution (0.05%, 2.05×10^{-3}M): Prepared by dissolving 200 mg of SA in 100 mL of distilled water.

Potassium iodate (A.R. grade; 0.02 M): Prepared by dissolving 4.26 g of KIO_3 in L of distilled water

Hydrochloric acid (E. Merck) 0.25 M and: Prepared by diluting 21.5 mL of Conc. HCl to 1000 mL with distilled water. 0.1 M: Prepared by diluting 8.6 mL of concentrated hydrochloric acid to 1000 mL with distilled water and Standardized.

Method M_1 [Metol - Cr (VI)]

Aliquots of standard drug solution (1.0-4.0 mL, 100 µg /mL) were transferred into a series of 25 mL graduated tubes containing 15 mL of ***p***H 3.0 buffer and 1 mL each of metol (4.35×10^{-3}M) and potassium dichromate (1.02×10^{-2}M) were added successively and diluted to the mark with distilled water. The absorbance was measured at 560 nm after 10 min against a reagent blank prepared in a similar manner. The drug concentration was deduced from a calibration curve (Fig. 2).

Method M_2 (DMPD - CAT)

Aliquots of standard drug solutions ranging from (0.5-3.0 mL, 100 µg/mL), were placed into a series of 25 mL graduated tubes, 9 mL of ***p***H 7 buffer, 1.0 mL (of $4.68 \times l0^{-3}$M DMPD) solution, 1.0 mL of CAT (4.39×10^{-3}M) were added successively. The volume was made upto the mark with distilled water and the kept aside for 15 min to allow full colour development. The absorbance was measured at 660 nm against a reagent blank. The coloured species was stable for 2 h. The drug concentration was deduced from a calibration curve (Fig. 3).

Method M_3 (NBS/metol-SA)

Aliquots of the standard drug solution (1.0-3.5 mL, 100 µg /mL) were transferred into a series of 25 mL

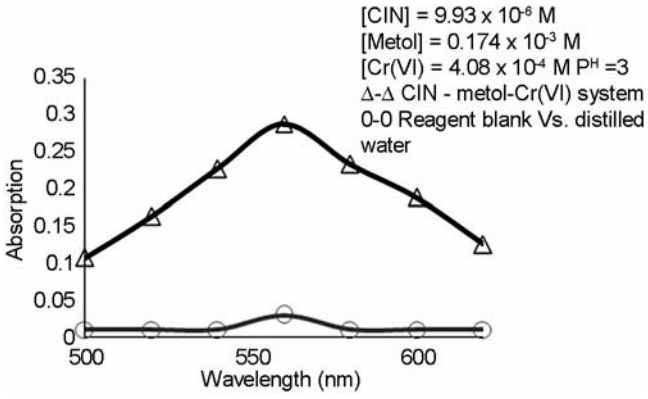


Fig. 2 — Absorption spectra of CIN- Metol - Cr(VI) M1

calibrated tubes containing 1.0 mL of AcOH (8.74 × $l0^{-1}$ M), 0.5 mL of KBr (4.2 × $l0^{-2}$ M), and 1.0 mL of NBS (4.9 × $l0^{-3}$ M) solutions. The volume was brought to 10 ml with distilled water. The tubes were kept aside for 15 min at room temperature. Then 1.0 mL of (8.71 × $l0^{-3}$ M) metol solution and after 2 min 2.0 mL of (1.16 × $l0^{-2}$ M) SA solutions was added. The volume was made upto 25 mL with distilled water and the absorbance was measured after 10 min at 520 nm against reagent blank. The amount of drug present was calculated from its calibration graph (Fig. 4).

Method M_4 (EDDP - IO_3^-)

Aliquots of the standard drug solution (0.5-2.5 mL, 100 µg/mL) 15 mL of ***p***H 3 buffer, 2 mL of EDDP (2.05 × 10^{-3} M) and 3 mL of KIO_3 (0.02M) were added successively and diluted to the mark with distilled water. The absorbance of the coloured species was measured after 10 min and before 30 min at 520 nm against reagent blank prepared in a similar manner. The amount of drug was calculated from its calibration graph (Fig. 5).

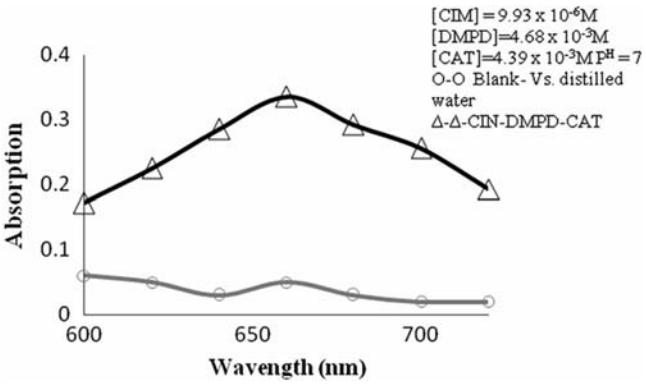


Fig. 3 — Absorption of CIN -DMPD-CAT M2

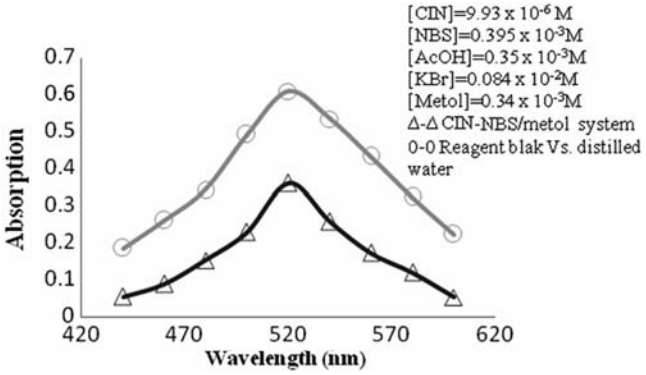


Fig. 4— Absorption spectra of CIN-Metol-S.A M3

Procedure for tablets

Accurately weighed 100 mg of pure or pharmaceutical preparation (tablet was dissolved in 20.0 mL methanol and filtered to remove the insoluble portion (if any) , the filtrate was made upto 100ml with methyl alcohol (1 mg/mL). The final concentration of CIN was brought upto 100.0 µg/mL with methyl alcohol and mixed well and filtered using a Whatman No.41 filter paper. An appropriate dilute solution was subjected to analysis by the procedures described above.

Results and Discussion

An aqueous solution of the dye maintaining the ***p***H suitable for charge-transfer complex formation in procedures for method M_1 [CIN- Metol/Cr (VI)] is represented in Scheme 1, method M_2 (CIN- DMPD) Oxidative coupling reactions is represented in Scheme 2.

In method M_3 (CIN- metol – SA) the drug with a known excess of oxidizing agent [NBS] and the second step is the reaction of the excess oxidant with a standard amount of chromogenic reagent (metol-SA) is represented in the Scheme 3. In method M_4 (CIN- EDDP) Oxidation of the individual primary intermediates give rise to coloured products to produce dye species is represented in the Scheme 4.

The optimum conditions for the development of methods M_1 (Scheme 1), M_2 (Scheme 2), M_3 (Scheme 3) and M_4 (Scheme 4) were established by varying parameters one at a time and observing the effect produced on the absorbance of the coloured species.

Method validation

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

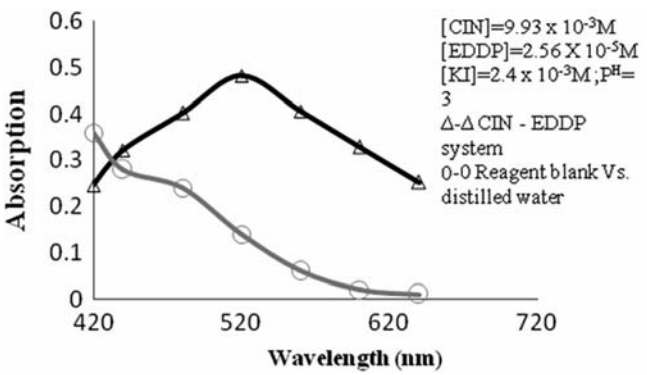
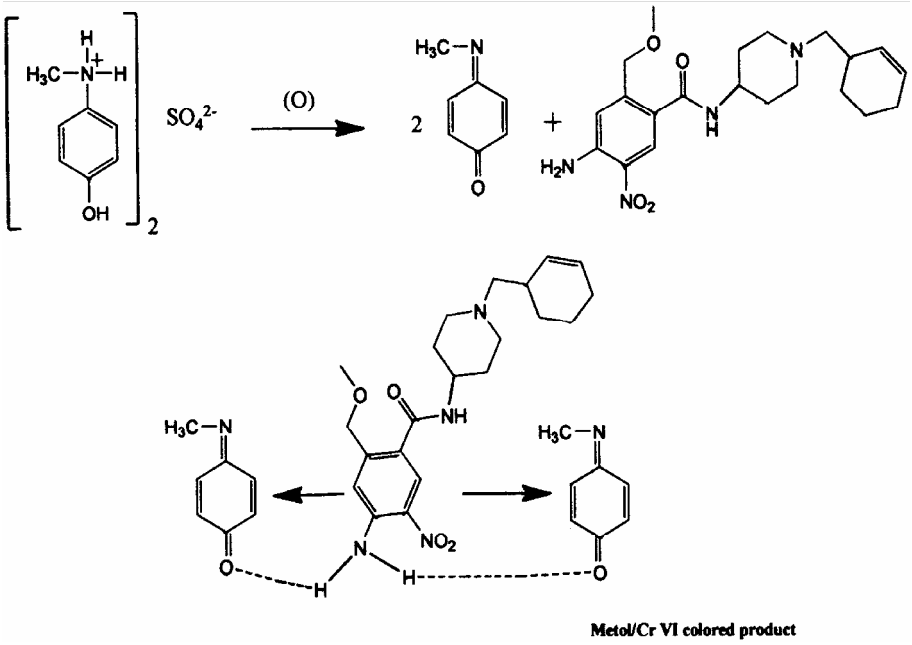
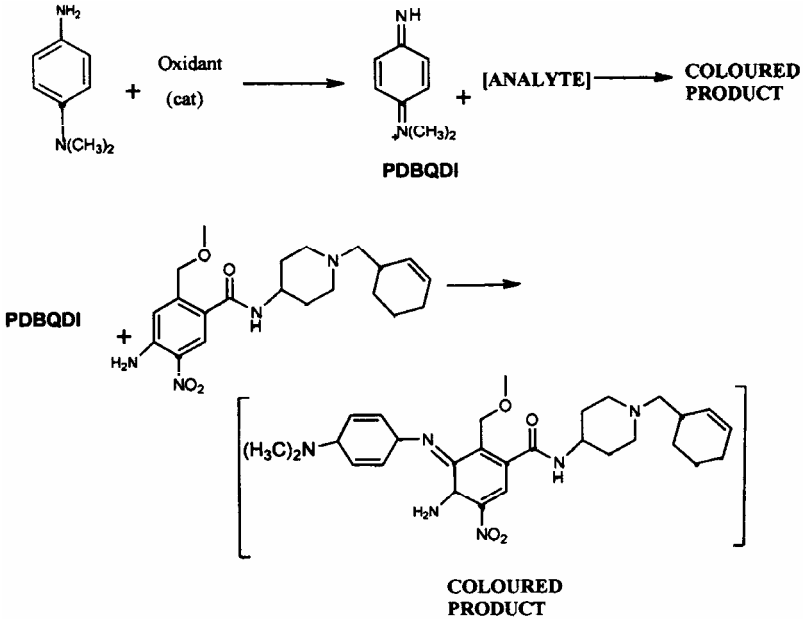


Fig. 5 — Absorption spectra of CIN-EDDP-KIO3 system M4



Scheme 1

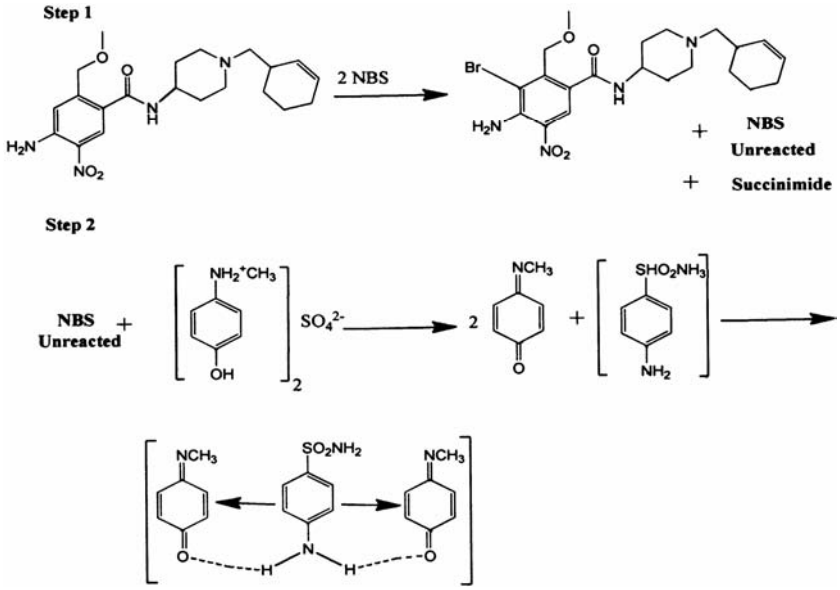


Scheme 2

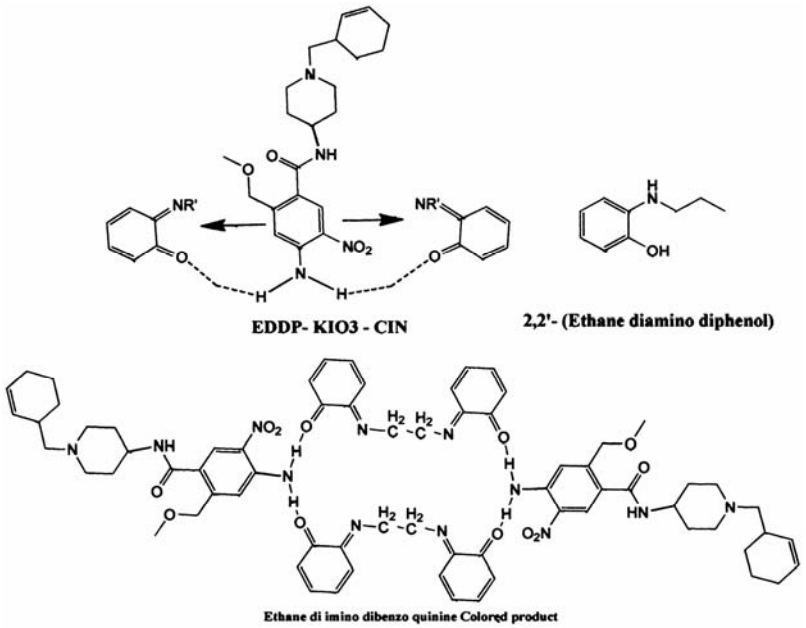
Linearity and sensitivity

Under optimum conditions, a linear relation was obtained between the absorbance and concentration of CIN in the range 0-2.5 μg mL^{-1}. The calibration graph is described by the equation: $\boldsymbol{Y} = \boldsymbol{a} + \boldsymbol{bx}$, where $\boldsymbol{Y}$ = absorbance, $\boldsymbol{a}$ = intercept, $\boldsymbol{b}$ = slope and $\boldsymbol{x}$ = concentration, obtained by the method of least squares. The correlation coefficient (***r***), intercept (***a***) and slope (***b***) for the calibration data and sensitivity parameters, such as apparent molar absorptivity and Sandell sensitivity values, the limits of detection and quantification are compiled in Table 1.

To evaluate the accuracy of the method, one often compares the method being investigated or 'test method' with an existing method called the 'reference method'.



Scheme 3



Scheme 4

Student t-test

Student t t-test is used to compare the means of two related (paired) samples analysed by reference and test methods. It gives answers to the correctness of the null hypothesis with a certain confidence such as 95% or 99%. If the number of pairs (n) are smaller than 30, the condition of normality of x is required or at least the normality of the difference (d_1). If this is the case the quantity is determined by using equation and the results are incorporated in Table 2.

$$t = \frac{\bar{d}}{\frac{S_d}{\sqrt{n}}}$$

F-test

By the F-test we can test the significance of the difference in variances of reference and test methods. Let us suppose that one carries out n_1 replicate measurements by using test method and n_2 replicate measurements by using reference method. If null

Table 1 — Optical and regression characteristic, precision and accuracy of the proposed methods for CIN

Parameter (CIN)	Metol-Cr M_1	DMPD M_2	NBS-Metol M_3	EDDP M_4
λ max(nm)	560	660	520	520
beer's law limit µg/mL	4.0- 16	2.0- 12.0	4.0 - 14	2 - 10
detection limit µg/mL	4.19×10^{-3}	8.1×10^{-2}	2.8×10^{-2}	0.12
Sandle sensitivity	0.149	0.03	0.04	0.02
€ max	0.6741×10^{4}	3.3708×10^{4}	2.5155×10^{4}	2.435×10^{4}
Regretion equation Y=a+bC				
Slope (b)	0.02715	0.055471429	0.055971429	0.0597
Standard deviation on slope(S_b)	0.003098387	0.001727922	0.002195233	0.002280351
Intercept (a)	-0.04	0.1122	0.02592381	0.0032
Standard deviation on intercept (S_a)	0.3463×10^{-3}	0.206×10^{-3}	0.2623×10^{-3}	0.36×10^{-3}
Standard deviation on estimation (S_e)	0.3796×10^{-4}	0.1498×10^{-4}	0.52416×10^{-3}	2.391×10^{-3}
Correlationcoefficient (r)	0.99983	0.99997	0.9995	0.9994
Relative standard deviation (%) *	0.49	0.65	0.52	0.47
%Range of error (Confidence limit)	0.56	0.74	0.59	0.54
0.05 level	0.88+0.05	1.17+0.12	0.93+0.17	0.84+0.4

Table 2 — Results of determination of CIN in capsules and statistical comparison with the reference method.

Formulation[a]	Labelled amount (mg)	Amount found by reference method	Amount found (mg) by proposed methods[b]			
			M_1 [Metol-Cr (VI)]	M_2 (DMPD – CAT)	M_3 (NBS/Br-/metol - SA)	M_4 (EDDP/KIO_3)
TAB 1	1	1±0.14	1±.20 F=2.04 t=1.47	1±0.22 F=2.46 t=0.54	1±0.17 F=1.47 t=1.76	1±0.12 F=1.36 t=1.73
TAB 2	1	1±0.15	1±0.17 F=1.28 t=1.91	1±0.16 F=1.13 t=1.97	1±0.23 F=2.35 t=1.73	1±0.12 F=1.56 t=2.01

[a]Difference batches of tablets from four different pharmaceutical companies.

[b]Average ± standard deviation of six determinations, the t- and F- test values refer to comparison of the proposed method with the reference method.

Theoritical values at 95% confidence limit , F= 5.05 , t= 2.57

hypothesis is true, then the estimates S_T^2 (variance of test method) and S_R^2 (variance of reference methods) do not differ very much and their ratio should not differ much from unity. One uses the ratio of the variances

$$F = \frac{S_T^2}{S_R^2}$$

It is conventional to calculate the F-ratio by dividing the large variance by the smallest in order to obtain a value equal or larger than unity and the results are incorporated in Table 2.

Recovery study

The accuracy and precision of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed capsule powder was spiked with pure drug at three different concentrations and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co-formulated substances such as talc, dextrose, alginate, acacia, etc. did not interfere in the determination. The results of recovery study are given in Table 3.

Optimum conditions for Method M_1 (metol- Cr VI)

The method involves the reaction of CIN with metol in presence of an oxidant, potassium dichromate. The effect of various parameters such as ***p***H of buffer, volume of buffer, volume of metol, effect and nature of the oxidant on colour development, volume of the oxidant, order of addition of reagents, nature of the solvent for final dilution, and stability of the coloured species were studied by varying one parameter at a time. The optimum conditions are incorporated in Table 4.

Table 3 — Results of recovery experiments via the standard addition technique

Tablet name	brandCIN tablet ($\mu g\ mL^{-1}$)	Method M_1			Method M_2		
		Pure CIN added ($\mu g\ mL^{-1}$)	Total found ($\mu g\ mL^{-1}$)	Pure CIN recovered[C] ($\mu g\ mL^{-1}$)	Pure CIN added ($\mu g\ mL^{-1}$)	Total found ($\mu g\ mL^{-1}$)	Pure CIN recovered[C] ($\mu g\ mL^{-1}$)
Tablet 1	1	0.5	1.55	100.12±0.12	0.5	1.52	99.89±0.23
	1	1	1.99	100.21±0.31	1	2.1	100.04±0.12
	1	1.5	2.52	100.04±0.14	1.5	2.51	99.85±0.32

[C]Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations).

Table 4 — Optimum conditions established in methods

Method	Conditions of the chemicals for method development		
M_1	***p***H of the buffer ranging from 2.8 to 3.1 and volume from 10-15 mL was studied. ***p***H 3.0 and 15 mL of buffer is required to get best results.	Metol solution ($4.35 \times l0^{-3}$ M) ranging from 0.75-1.25 mL was studied 1.0 mL of metol solution was required	Oxidants: oxidants such as Chloramine-T, metaperiodate, hypochlorite, Fe III, potassium ferri cyanide and Cr(VI) were studied and 1 mL of Cr(VI) ($1.02 \times l0^{-2}$ M) was found to be the best oxidant.
M_2	Phosphate buffer ***p***H 7.0-9.0 range was studied and ***p***H 7.0 was found to be suitable.	DMPD solution (4.68×10^{-3} M) of 0.5-3 mL was studied and 1.0 mL was required.	Oxidants: various oxidants tried in combination with DMPD, CAT was found to be best suited for stability of color formation. 1.0 mL of CAT was found to be best.
M_3	Volume of NBS ($4.94 \times l0^{-3}$ M), KBr (4.2×10^{-2} M) acetic acid (8.75×10^{-1} M), metol (8.71×10^{-3} M) and SA (1.16×10^{-2} M) solutions ranging from 0.5-2.2 mL was studied and max absorbance appeared at 1.0, 0.5, 1.0, 1.0 and 2.0 mL, respectively	Time 15-20 min and temp. 25-50°C was checked for oxidation. Room temperature for 15 min is suitable for oxidation.	KBr solution (4.2×10^{-2}M) 0.5-1.0 mL was studied and found that 0.5 mL is required
M_4	***p***H of the buffer ranging from 2.8 to 3.0 and volume from 10-15 was studied ***p***H 3.0 and 15 mL of buffer is required to get best results	Volume of EDDP (2.05×10^{-3} M) ranging from 1.0-2.0 mL was studied and 2.0 mL was suitable	Oxidants such as CAT, $S_2O_8^-$, I_2, IO_4^-, $K_3Fe(CN)_6$, idosobenzoate and KIO_3 were tried and KIO_3 was found to be suitable oxidant.

Optimum conditions for Method M_2 (DMPD)

This method involves the reaction of CIN with DMPD in the presence of oxidant (CAT). The effect of various parameters such as nature and volume of oxidant, volume of DMPD solution, the order of addition of reagents, effect of solvents on the colour development and stability of the final coloured products were studied. The optimum conditions developed and actual conditions chosen for the procedure are recorded in Table 4.

Optimum conditions for Method M_3 (NBS/Metol- SA)

This is an indirect spectrophotometric method which involves two steps, oxidation of the drug with NBS (first step) and estimation of the unconsumed NBS with metol- SA reagent (second step). In the first step, the volume of NBS required for oxidation of drug, the time and temperature for oxidation of drug, volume of acetic acid were established through controlled experiments. In the second step, the volume of metol and the intermittent time between additions, volume of SA and the solvent for final dilution were found by varying one parameter at a time and the optimum conditions are incorporated in Table 4.

Optimum conditions for Method M_4 (EDDP - KIO_3)

The effect of various parameters such as ***p***H of buffer, volume of buffer, volume of EDDP, effect and nature of the oxidant, volume of the oxidant, order of addition of reagents, nature of the solvent for final dilution and stability of the coloured species were studied by varying one parameter at a time. The optimum conditions are incorporated in Table 4.

Four reagents (Metol-Cr (VI), DMPD-CAT, NBS/metol-SA and EDDP-KIO_3) have been used for the determination of CIN in four methods (M_1, M_2, M_3, M_4). The λ_{max} and ε_{max} values of coloured species formed by four methods for CIN in descending order are $M_2 > M_1 > M_3 = M_4$ and $M_1 < M_4 < M_3 < M_2$ respectively. The ascending order of precision for CIN is $M_4 < M_1 < M_3 < M_2$. The descending order of

sensitivities of proposed visible spectrophotometric methods for each drug is as follows $M_1 < M_4 < M_3 < M_2$. The accuracy of these methods is about 1.5%. There is a good agreement between the values obtained in the reported and proposed methods of CIN in pharmaceutical preparations.

Conclusion

A significant advantage of visible spectrophotometric method is that it can be applied to the determination of individual compounds in a multicomponent mixture. The instrument is simple and is not of high cost. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. In the present study, Cinitapride (CIN) was determined (Method M_1–M_4) successfully as pure compound as well as component in representative pharmaceutical formulations by exploiting different functional groups present. The ingredients usually present in the pharmaceutical formulations of Cinitapride (CIN) did not interfere in the proposed methods. Thus the proposed methods are simple, rapid and sensitive, can be used in the routine determination of pharmaceutical formulations depending upon the need of specific situation.

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