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New Titrimetric And Spectrophotometric Methods For The Assay Of Promethazine In Pharmaceuticals Using N-Chlorosuccinimide And Two Dyes

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ABSTRACT

Titrimetric and spectrophotometric methods, two each, are described for the assay of promethazine hydrochloride (PH) in bulk drug and in dosage forms using N-chlorosuccinimide (NCS) and two dyes, methyl orange and indigo carmine as reagents. In direct titrimetry (method A), aqueous solution of PH is titrated directly with a standard solution of NCS in acid medium and in the presence of potassium bromide (KBr). Indirect titration (method B) involves the addition of a measured excess of standard NCS to PH solution in acid medium followed by iodometric back titration of unreacted oxidant. Spectrophotometric methods entail the addition of a known excess of NCS to a solution of PH in acid medium and in the presence of KBr followed by determination of residual bromine by reaction with a fixed amount of either methyl orange and measuring the absorbance at 520 nm (method C) or indigo carmine and measuring the absorbance at 610 nm (method D). In all the methods, the amount of NCS reacted corresponds to the amount of PH. In both titrimetric methods, the reaction stoichiometry was found to be 1:1 (PH : NCS) and are applicable over 2-20 mg (method A) and 1.5-10 mg (method B) ranges. In spectrophotometric methods, the absorbance was found increase linearly with the correlation coefficients of 0.9998 and 0.9995 for method C and method D, respectively. The systems obey Beer's law for 0.5-6.0 µg/ml (method C) and 1-10 µg/ml (method D). The calculated apparent molar absorptivity values are found to be 3.92×10^4 and 1.69×10^4 l/mol/cm for method C and method D, respectively, and the corresponding Sandell sensitivity values are 0.0082 and 0.0019 µg/cm². The limits of detection and quantification are reported for both spectrophotometric methods. Intra-day and inter-day precision and accuracy of the methods were evaluated as per ICH guidelines. The methods were successfully applied to the determination of PH in tablets, injections and syrup, and the results were statistically compared with those of a reference method by applying Student's t- and F-tests. No interference was observed from common additives and excipients found in dosage forms. The accuracy and reliability of the methods were further ascertained by performing recovery test *via* standard addition method.

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KEYWORDS

Promethazine; N-Chlorosuccinimide; Spectrophotometry; Titrimetry; Dosage forms.

INTRODUCTION

Promethazine hydrochloride (PH) is a member of phenothiazine group of drugs that are widely used as antihistamines, hypnotics and tranquillizers^[1]. Analytical methods for the determination of phenothiazines including PH have recently been reviewed^[2]. In the past one and a half decades, various titrimetric^[3-7], UV-spectrophotometric^[8-11], visible spectrophotometric^[12-25], fluorimetric^[26], phosphorimetric^[27], voltammetric^[28], thin layer chromatographic^[29], gas chromatographic^[30] and high performance liquid chromatographic^[31-33] methods have been reported for the determination of PH. The official methods usually involve a non-aqueous titration or a UV-spectrophotometric procedure^[34].

This paper describes the titrimetric and spectrophotometric determination of PH in bulk drug and in dosage forms using N-chlorosuccinimide(NCS) and two dyes methyl orange and indigo carmine as reagents. The methods, besides being sensitive, have been demonstrated to be both accurate and precise.

EXPERIMENTAL

Apparatus

A Systronics model 106 digital spectrophotometer provided with 1-cm matched quartz cells was used for absorbance measurements.

Reagents and solutions

All chemicals used were analytical reagent grade and distilled water was used to prepare solutions.

N-chlorosuccinimide (0.01 and 0.005 M)

A stock standard solution equivalent to 0.01 M N-chlorosuccinimide was prepared by dissolving about 1.5 g of the chemical (S. D. Fine Chem. Ltd., Mumbai, India) in water and diluting to 1 litre and standardized^[35], and used in direct titration. The solution was diluted one fold and used in indirect titrimetric method.

Sodium thiosulphate (0.01M)

An approximately 0.01 M solution was prepared by dissolving about 2.5 g of the chemical in one liter of water and standardized iodometrically with pure

dichromate^[36]

Hydrochloric acid (4M)

Concentrated acid (354 ml) (S. D. Fine Chem. Ltd., Mumbai, India; Sp.gr.1.18) was diluted to 1 litre with water.

Starch indicator(1%)

One g of starch was made into a paste with little water and poured into 100 ml boiling water, boiled for 1 min and cooled.

Potassium bromide (10% and 2%) and potassium iodide (10%)

Prepared by dissolving appropriate amounts in water.

Standard solution of PH

Pharmaceutical grade PH certified to be 99.6% pure was received from Rhone-Poulenc as gift and was used as received. A stock standard solution containing 2 mg/ml PH was prepared by dissolving calculated amount of pure drug in water and kept in an amber colored bottle and stored in refrigerator. The solution was used in titrimetric work. The solution (2000 µg/ml PH) was appropriately diluted with water to get working concentration of 20 and 50 µg/ml for spectrophotometric method C and method D, respectively.

Procedures

Direct titration(Method A)

A 10 ml aliquot of pure drug solution containing 2-20 mg of PH was accurately measured and transferred into a 100 ml titration flask and acidified by adding 5 ml of 4 M hydrochloric acid. Two ml of 10% KBr was added and the solution titrated with 0.01M NCS to a colourless end point. At this point, 5 ml of 10% KI solution was added and the liberated iodine titrated against 0.01M thiosulphate solution using starch as indicator. The amount of drug in the measured aliquot was calculated from:

$$\text{Amount(mg)} = \frac{\left[V_{\text{NCS}} \times M_{\text{NCS}} - \left(\frac{V_{\text{thio}} \times M_{\text{thio}}}{2} \right) \right]_{M_w}}{n}$$

where

V_{NCS} = volume of NCS consumed, ml

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M_{NCS} = Concentration of NCS consumed, mol/l

V_{thio} = Volume of thiosulphate consumed, ml

M_{thi} = Concentration of thiosulphate, mol/l

M_w = Relative molecular mass of drug

n = Number of moles of NCS required to react with each mole of PH.

Back titration (Method B)

A 10 ml aliquot of pure drug solution containing 1.5-10.0 mg of PH was accurately measured and transferred into a 100 ml titration flask and acidified by adding 5 ml of 4 M HCl. Ten ml of 0.005 M NCS was added by means of a pipette, the content mixed and let stand for 10 min with occasional swirling. Lastly, 5 ml of 10% KI solution was added and the liberated iodine titrated with 0.01 M thiosulphate solution using starch indicator. A blank titration was performed under identical conditions. The amount of PH in the measured aliquot was calculated from:

$$\text{Amount(mg)} = \frac{(B-S)M_w R}{n}$$

where

B= Volume of thiosulphate consumed in the blank titration, ml

S= Volume of thiosulphate consumed in the sample titration, ml

R= Concentration of NCS, mol/l

Spectrophotometry using methyl orange (Method C)

Different aliquots (0.25, 0.5, 1.0.....3.0 ml) of 20 µg/ml pure PH solution were accurately measured and transferred into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was brought to 3 ml by adding water. To each flask were added 1 ml of each of 4 M HCl and 2% KBr and 40 µg/ml NCS successively; the flasks were stoppered, the content mixed and allowed to stand for 10 min with intermittent shaking. Finally, 1 ml of 50 µg/ml methyl orange solution was added to each flask, the volume was diluted to the mark with water mixed well and absorbance measured at 520 nm against a reagent blank after 5 min.

Spectrophotometry using indigo carmine (Method D)

Varying aliquots (0.25, 0.5, 0.75---2.0 ml) of 50 µg/ml standard PH solution were accurately mea-

sured into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was adjusted to 2 ml by adding water. One ml of 4 M HCl was added to each flask followed by 1 ml each of 2% KBr and 150 µg/ml NCS, the last being measured accurately. The flasks were stoppered, content mixed and let stand. After 10 min, 1 ml of 200 µg/ml indigo carmine was added by means of a micro burette, the volume was diluted to the mark with water, mixed well and absorbance measured at 610 nm against a reagent blank.

In both spectrophotometric methods the concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the Beer's law data.

Procedure for dosage forms

Tablets. Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 100 mg of PH was accurately weighed into a 100 ml calibrated flask, 60 ml water added and shaken for 20 min. Then, the volume was made up to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 ml portion of the filtrate was discarded, a convenient aliquot of the subsequent portion was assayed by titrimetric methods. The tablets extract(1000 µg/ml in PH) was diluted with water to get 20 and 50 µg/ml concentration for assay by spectrophotometric methods described earlier.

Injections. The contents of ten ampules of injections were pooled together and a measured volume containing 100 mg of PH was diluted to 100 ml with water in a calibrated flask and assayed as described under tablets.

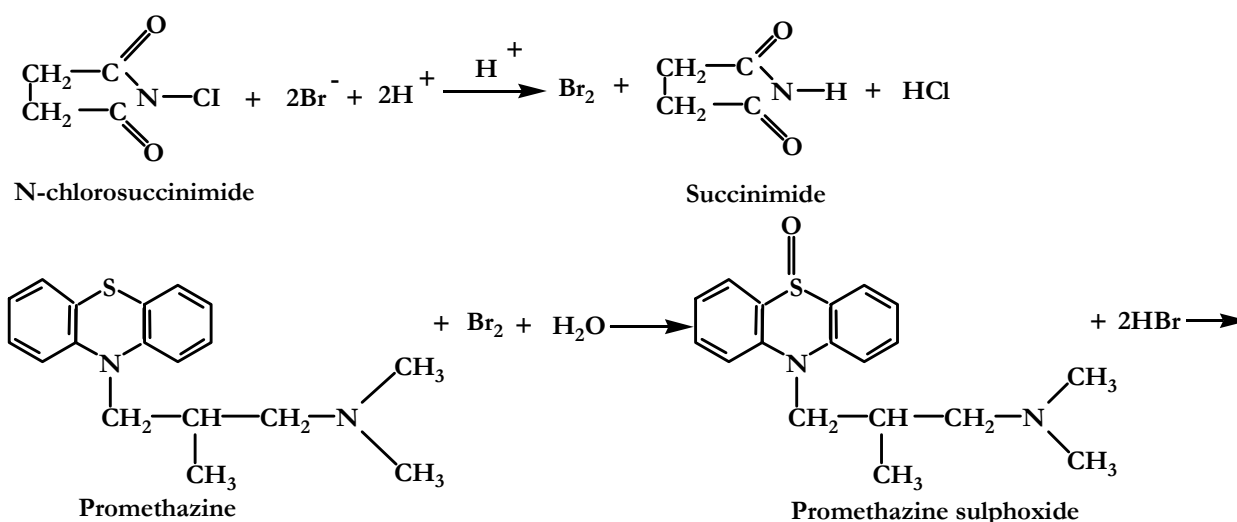
Syrup. The contents of two bottles of syrup were pooled and an accurately measured volume containing 100 mg of PH was transferred into a separating funnel and rendered alkaline with 6 M ammonia solution. The promethazine base was extracted with chloroform; the extracts were pooled, washed with water evaporated to dryness and the residue dissolved in 1M HCl and diluted to volume in a 100 ml calibrated flask. The syrup solution was then subjected to analysis by the proposed method as described earlier.

RESULTS AND DISCUSSION

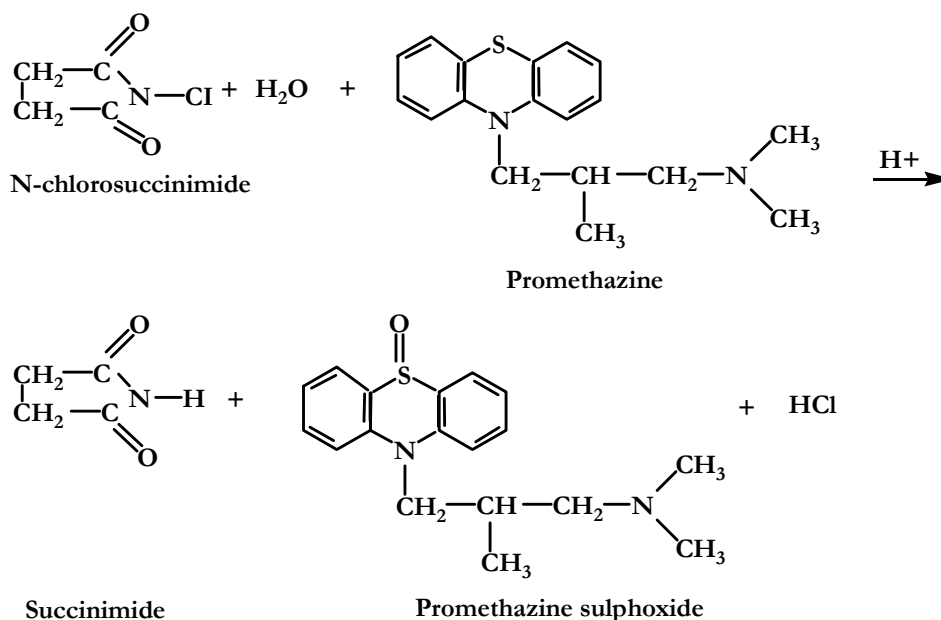
Promethazine, being a phenothiazine derivative, is prone to easy chemical oxidation. The present titrimetric methods are based on the fact that promethazine undergoes oxidation by NCS or insitu generated bromine first to promethazinium free radical and finally to the colourless sulphoxide^[37,38]. Direct titration with NCS was not successful, However, it could

be easily accomplished in the presence of KBr, the reacting species being insitu generated bromine following the reaction between NCS and KBr in acid medium. The possible reaction schemes are shown in figure 1^[39,40].

In both methods HCl medium was found to produce accurate and stoichiometric results. Five ml of 4M acid in a total volume 20-25 ml was used in the assay although 3-8 ml acid did not affect the reac-



SCHEME A (Direct titration)



SCHEME B (Back titration)

Figure 1: Possible reaction schemes in titrimetry

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tion stoichiometry. In direct titrimetry, the red color of promethazinium radical cation served as the self-indicator. Titration to colourless sulphoxide end point resulted in slightly higher values of 'n' and this was traced to presence of unreacted bromine at the end point. When this bromine was determined iodometrically and correction applied, correct 'n' value, and accurate and precise results were obtained. In indirect titrimetry, the reaction was complete in 10 min and from 10th to 30th min, a very small amount of NCS was consumed but without resulting any significant molar-ratio. Hence, 10 min reaction time should be strictly adhered to. In both methods, a 1:1 (PH : NCS) reaction stoichiometry was obtained which was used for calculations. PH in the ranges 2-20 and 1.5-10 mg can be determined by direct and back titrimetric procedures, respectively. The relation between the titration end point and the amount of the drug was also evaluated, and the relation was found to be linear as shown by the calculated correlation coefficients of 0.9972 and -0.9986 for direct and back titration methods, respectively.

In the proposed spectrophotometric methods, the ability of insitu generated bromine to cause oxidation of PH and irreversibly destroy methyl orange and indigo carmine to colourless products in acid medium has been used. PH when added in increasing concentrations to a fixed concentration of NCS in the presence of bromide, consumes the insitu generated bromine proportionately and there will be a concomitant fall in the concentration of bromine. When a fixed concentration of either dye is added to decreasing concentration of bromine, a concomitant increase in the concentration of dye results. A proportional increase in the absorbance at the respective λ_{\max} is thus observed with increasing concentration of PH.

Preliminary experiments were performed to fix the upper limits of the dyes that could be determined spectrophotometrically, and these were found to be 5 and 20 $\mu\text{g}/\text{ml}$ for methyl orange and indigo carmine, respectively. A NCS concentration of 4.0 $\mu\text{g}/\text{ml}$ in the presence of excess of bromide was found to irreversibly destroy the red color due to 5 $\mu\text{g}/\text{ml}$ methyl orange in acid medium whereas 15 $\mu\text{g}/\text{ml}$ NCS was required to cause similar action on 20 $\mu\text{g}/$

ml indigo carmine in acidic conditions. Hence, different amounts of PH were reacted with 1 ml of 40 $\mu\text{g}/\text{ml}$ NCS in method C and 1 ml of 150 $\mu\text{g}/\text{ml}$ NCS in method D followed by determination of residual oxidant as described under respective procedures.

For the oxidation of PH by bromine and the bleaching of dye by the latter, hydrochloric acid was found to be ideal. One ml of 4 M acid in a total volume of about 6-7 ml was adequate for the oxidation step, which was complete in 10 min, and the same acid concentration was maintained for the bleaching step. Neither the acid concentration nor the reaction time was critical; 0.5-3.0 ml (method C) and 0.5-1.5 ml (method D) of 4M HCl and reaction times up to 30 min (method C) and up to 20 min (method D) produced the same absorbance values for a given PH concentration. A 5 min contact time was found necessary for bleaching of dye color by the residual bromine. The absorbance of either dye color was stable for several hours in the presence of the reaction product.

Analytical parameters of the spectrophotometric methods

Linear relation is found between absorbance and concentration in the ranges given in TABLE 1. The graph showed negligible intercept and are described by the equation:

$$Y = a + b X$$

TABLE 1: Quantitative parameters of the spectrophotometric methods

Parameter	Method C	Method D
λ_{\max} , nm	520	610
Beer's law limits, $\mu\text{g}/\text{ml}$	0.5-5.0	1.0-10.0
Molar absorptivity, $l/\text{mol}/\text{cm}$	3.92×10^4	1.69×10^4
Sandell sensitivity, $\mu\text{g}/\text{cm}^2$	0.0082	0.0019
Limit of detection, $\mu\text{g}/\text{ml}$	0.05	0.09
Limit of quantification, $\mu\text{g}/\text{ml}$	0.15	0.27
Regression equation, Y^*	-	-
Intercept (a)	-0.0058	0.0069
Slope (b)	0.1272	0.0513
Sa	5.6×10^{-3}	5.4×10^{-3}
Sb	1.1×10^{-3}	7.7×10^{-3}
Correlation coefficient (r)	0.9998	0.9995

* $Y = a + bX$ where Y is the absorbance and X concentration in $\mu\text{g}/\text{ml}$
Sa=Standard deviation of intercept Sb= Standard deviation of slope.

TABLE 2: Intra-day accuracy and precision of the methods

Method*	ALB taken	ALB found**	Range	Relative error, %	SD	SDM	RSD %	ROE %
A	5.0	4.99	0.321	0.04	0.1107	0.041	2.21	±2.20
	10.0	9.91	0.322	0.95	0.1223	0.046	1.23	±1.22
	15.0	14.95	0.28	0.33	0.1097	0.041	0.73	±0.73
B	3.0	3.01	0.08	0.1	0.043	0.016	1.42	±1.418
	6.0	5.98	0.096	0.33	0.0428	0.0162	0.72	±0.715
	9.0	8.95	0.12	0.56	0.0429	0.0162	0.48	±0.47
C	1.5	1.48	0.08	1.33	0.0095	0.004	0.64	±0.63
	3.0	2.97	0.09	1.00	0.035	0.0132	1.19	±1.18
	4.5	4.49	0.05	0.22	0.028	0.0106	0.62	±0.61
D	2.0	1.95	0.08	2.5	0.026	0.0099	1.33	±1.32
	5.0	5.05	0.099	0.98	0.036	0.0136	0.27	±0.26
	8.0	7.97	0.064	0.38	0.029	0.1096	1.38	±1.37

**Mean value of seven determinations

*In methods A and B, PH taken/found, range, SD and SDM are in mg, and in methods C and D, they are in mg/ml

SD. Standard deviation; SDM. Standard deviation of mean; RSD. Relative standard deviation and ROE. Range of error at 95% confidence level for six degrees of freedom.

TABLE 3: Results of assay of formulations by the proposed methods

Dosage form and brand name	Nominal amount mg per tablet/ml	Reference method	% found*± SD			
			Method A	Method B	Method C	Method C
Tablets Phenar	10	98.64±0.62	97.71±0.92	99.81±1.21	97.66±0.85	97.54±1.49
			t=2.19	t=1.73	t=2.11	t=1.65
			F=2.20	F=3.81	F=1.88	F=5.78
gan ^a	25	102.48±0.84	101.1±0.98	103.3±1.51	100.5±1.31	100.6±1.32
			t=2.39	t=1.10	t=1.84	t=2.75
			F=1.36	F=3.23	F=2.43	F=2.47
Promasum ^b	25	97.84±1.24	98.55±0.92	99.12±0.88	96.01±1.31	98.89±1.18
			t=2.40	t=1.91	t=2.27	t=1.37
			F=1.82	F=1.98	F=1.12	F=1.10
Emin ^c	25	99.12±0.76	100.5±0.59	98.11±0.96	98.79±0.89	97.91±0.69
			t=3.23	t=1.86	t=0.63	t=2.64
			F=1.66	F=1.59	F=1.37	F=1.21
Injection Primozynd ^d	12.5	98.75±0.36	97.9±0.84	99.21±0.71	97.01±0.75	97.58±0.68
			t=2.24	t=2.85	t=4.95	t=3.56
			F=5.44	F=3.89	F=4.34	F=3.57
Phena ^e	25	100.66±1.14	99.01±1.21	101.89±1.31	98.99±1.16	99.34±0.99
			t=2.22	t=1.59	t=2.29	t=1.27
			F=1.13	F=1.32	F=1.04	F=1.33
Syrup Phenargan ^a		101.54±1.17	99.68±0.85	102.85±1.35	99.98±0.96	100.01±1.42
			t=2.91	t=1.64	t=0.66	t=1.87
			F=1.89	F=1.33	F=1.49	F=1.47

**Marketed value of five determinations

*Marked by: a. NPIL; b. Sun Pharmaceuticals; c. Cipla; d. Mandar; e. Ind. Swift

**Mean value of five determinations. Tabulated value of t at 95% confidence level is 2.77 Tabulated value of F at 95% confidence level is 6.39

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(where Y = absorbance, a = intercept, b = slope and X = concentration in $\mu\text{g/ml}$) obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are also presented in TABLE 1. Sensitivity parameters such as molar absorptivity and Sandell sensitivity values, and the limits of detection and quantification, are also presented in TABLE 1.

Method validation

Evaluation of accuracy and precision

Intra-day precision and accuracy were assessed from the results of seven replicate analyses on pure drug solution. The mean values and relative standard deviation (RSD) values for replicate analyses at three different amount/concentration levels were calculated. To calculate the inter-day precision, analysis was performed over a period of five days preparing all solutions afresh each day. The accuracy of the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error (RE). TABLE 2 summarizes the intra-day precision and accuracy data for the determination PH by the proposed methods which were within 2.5%. The inter-day relative standard deviation was less than 3%.

Application to dosage forms

The proposed methods were applied to the analysis of PH in tablets, injections and syrups are presented in TABLE 3. The same batch formulations were determined by the reference method^[34] and the results were statistically evaluated by applying the Student's t -test and F -test. The calculated t - and F -values were less than the tabulated values at 95% confidence level revealing that the proposed methods and the reference method^[34] have similar accuracy and precision.

The accuracy and reliability of the methods were further ascertained through recovery experiments. To a fixed and known amount of drug in the formulation, pure PH was added at three different levels, and the total was found by the proposed methods. The recoveries of the pure drug added to tablet powder were in the range of 96.74 - 104.5 as shown in TABLE 4 and reveal that neither the end point detection in titrimetry nor absorbance measurement in

TABLE 4: Results of recovery study

Method	Formulation studied	PH in formulation	Pure PH added	Total found	Pure PH recovered %
A	Promasun tablets (25 mg)	4.93	2.5	7.38	97.85
		4.93	5.0	9.99	101.3
		4.93	10.0	15.35	104.2
B	-	2.48	2.0	4.45	98.66
		2.48	4.0	6.35	96.74
		2.48	6.0	8.56	101.3
C	-	19.20	10.0	29.65	104.5
		19.20	20.0	39.07	99.34
		19.20	30.0	49.74	101.8
D	-	39.56	20.0	59.49	99.67
		39.56	40.0	80.48	102.3
		39.56	60.0	98.23	97.78

**Mean value of Three determinations

In methods A and B, PH in tablet/added, total found, are in mg, and in methods C and D, they are in μg .

spectrophotometry was affected by tablet excipients such as talc, starch, lactose, magnesium stearate, sodium alginate, calcium gluconate and calcium dihydrogenorthophosphate.

CONCLUSION

The proposed titrimetric and spectrophotometric methods using N -chlorosuccinimide and two dyes as reagents are simple, accurate and reasonably precise. The titrimetric methods are applicable over micro-scale compared to many titrimetric procedures previously reported which are applicable for macro levels. The spectrophotometric methods are superior to earlier methods in terms of the stability of the reaction product, optimum conditions and sensitivity. The methods have been successfully applied to the determination of the drug in dosage forms with satisfactory results.

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