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Titrimetric And Spectrophotometric Determination Of Salbutamol Sulphate In Pharmaceuticals Using Chloramine-T And Two Dyes

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ABSTRACT

Four new methods are described for the assay of salbutamol sulphate(SBS) in bulk drug and in dosage forms using chloramine-T and two dyes, methyl orange and indigocarmine, as reagents. In direct titrimetry (method A), aqueous solution of SBS is titrated with chloramine-T(CAT) in hydrochloric acid medium and in the presence of excess of potassium bromide, using methyl orange indicator. Back titrimetry(method B) involves treating of SBS with a measured excess of chloramine-T in hydrochloric acid medium, and after the oxidation of SBS is judged to be complete, the unreacted oxidant is determined iodometrically, In both methods, the reaction follows a 1:2 (SBS:CAT), reaction stoichiometry method A is applicable over 2.5-9.5 mg, and 3-10 mg of SBS can be determined by method B. Spectrophotometric methods entail the addition of a known excess of CAT to SBS in hydrochloric acid medium followed by determination of residual oxidant by reacting 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 CAT reacted corresponds to the amount of SBS. In spectrophotometric methods, the systems obey Beer's law for 0.5-4.5 and 1.25-12.5 μg/ml for method C and method D, respectively. Apparent molar absorptivity values are calculated to be 6.89×10^4 (method A) and 2.46×10^4 l/mol/cm (method B). The limits of detection and quantification are reported for both methods. Intra-day and inter-day precision and accuracy of the developed methods were evaluated. The methods were successfully applied to the assay of SBS in tablet and capsule formulations and the results were compared with those of a reference method by applying Student's t-and F-tests. No interference was observed from common tablet adjuvants.

KEYWORDS Salbutamol sulphate; Assay; Titrimetry; Spectrophotometry; Chloramine-T; Tablets.

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INTRODUCTION

Salbutamol sulphate (SBS) whose structure is given in figure 1 is a selective beta-2-agoinst antiasthmatic. Its primary action is to stimulate adenyl cyclase which catalyses the formation of cyclic adenocin mono phosphate (AMP). The cyclic AMP thus formed mediates smooth muscle relaxation and branchodilation.. Among the techniques reported for the determination SBS in pharmaceuticals, methods using high performance liquid chromatography (HPLC)[1-7] are tedious, time-consuming, and require special and expensive apparatus. Besides, all the reported HPLC methods are relatively insensitive. The other chromatographic method, thin-layer chromatography[8], although simpler than HPLC methods, is also less sensitive with the linear range being 20-580 μg/ml. Non-chromatographic methods such as derivative ultra-violet spectrophotometry[9], derivative difference spectrophotometry[10], capillary electrophoresis[11] and a-c oscillopolarography[12] are also relatively complicated in terms of assay procedure or equipment required for analysis.

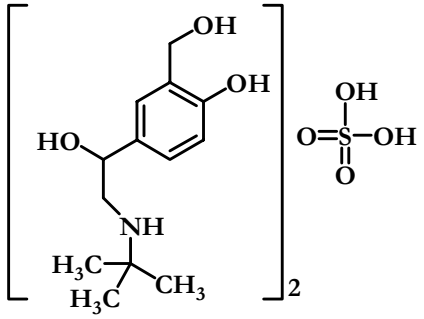

Figure 1: Structure of salbutamolsulphate

The most widely used technique for the assay of SBS in pharmaceuticals has been the visible spectrophotometry, and procedures based on such varied reactions as redox[13,14], reduction followed by chelation[15], oxidative coupling[16,17], diazotization and coupling[18,19], nitrosation[20], nitration[21], nitration followed by Meiscnheiner complex formation[22] and charge-transfer complex formation[23] are found in the literature. However, many of these procedures suffer from one or the other disadvantage (TABLE 1) such as poor sensitivity, heating or extraction step, critical working conditions or the use of organic solvents; and hence are unsatisfactory for routine analysis. The only visual titrimetric method[24] reported

TABLE 1: Comparison of reported spectrophotometric methods with the proposed method for the assay of SBS

Reagent/s used	λ_{max}, nm	Beer's Law limit μg ml^{-1}	Molar absorptivity $l\ mol^{-1}\ cm^{-1}$	Remarks	References
F-C reagent*	760	0.0-6.0	-	-	13
F-C reagent*	750	1-15	-	Uses on-line solid phase extraction and flow injection analysis apparatus	14
Iron(III) – 1, 10 –phenanthroline	510	400-4000	-	Least sensitive	15
Ferricyanide-4-aminophenazone	505	25-175	-	Involves heating; waiting time of 30 min	16
Cerium(IV) - MBTH*	530	Upto 15	2.4×10^4	Involves extraction and an expensive reagent	17
$NaNO_2$ - PHSA	440	Upto 10	-	-	18
$NaNO_2$-3-amino pyridine	440	1-10	-	-	19
$NaNO_2$	410	5-60	-	Involves boiling for 30 min	20
$KNO_3 – H_2SO_4$	420	0-48	-	Involves boiling for 30 min	21
HNO_3-H_2SO_4/Meisenheimer complex formation	386	4.8-16.0	-	Involves boiling for 20 min	22
DCQO*		1-30	-	Uses organic solvent	23
TCNQ*		2-20	-	Uses organic solvent	23
CAT / Methyl orange,	520	0.5-4.5	6.89×10^4	No heating, extraction step involved.	Present methods
Indigo carmine	610	1.25-12.5	2.46×10^4		

* F-C reagent - Folin-Ciocalteu reagent; MBTH - 3-methyl benzothiazolin-2-one hydrozone; PHSA - phenylhydrazine sulphonic acid; DCQ - 2,6 – Dichloroquinone chlorimide; TCNQ - 7, 7, 8, 8 – tetracyano quinodimethane

employs N-bromosuccinimide as the oxidimetric titrant which is unstable in solution and requires daily standardization[25]. Recently Issa et al[26]. have reported a conductometric titration method using phosphotungstic and phospho molybdic acids as titrants. Even these procedures are time consuming and less sensitive.

This paper describes four assay methods for SBS in tablets and capsules. The methods are based on two techniques and employ N-Bromosuccinimide as an oxidizing agent, methyl orange and indigocarmine dye as reagents. The proposed methods have the advantages of being rapid and simple and are free from interference from common tablet and capsule excipients. The results obtained were closely comparable to those of a reported method, and recovery tests were also found to be satisfactory.

EXPERIMENTAL

Reagents and materials

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Chloramine-T solution (0.01M) was prepared by dissolving about 2.8 g of the chemical (Qualigens fine chem., Glaxo India Ltd., Mumbai) in water and diluting to 1 litre, and used for method B after standardization[27]. The solution was diluted to 0.005 M for method A. For spectrophotometric investigation, the above solution was diluted appropriately with water to get 50 and 225 μg/ml concentrations for method C and method D, respectively. To prepare 50 μg/ml methyl orange for method A, first, a 500 μg/ml dye solution was prepared by dissolving 59 mg of dye (s.d.fine-chem Ltd.,Mumbai, assay 85%) in water and diluting to 100 ml in a calibrated flask, and filtered using glass wool. This was diluted tenfold with water to get the required concentration. For method B, first, a 1000 μg/ml indigo carmine solution was prepared by dissolving 112 mg of dye (s.d.fine-chem Ltd.,Mumbai, 90 % assay) in water and diluting to 100 ml, and filterd. This was appropriately diluted with water to get 200 μg/ ml. Hydrochloric acid (5M) was prepared by diluting 221 ml of concentrated acid (s.d.fine-chem Ltd.,Mumbai, Sp gr 1.18) to 500 ml of water and this was further diluted to get 1M for method A. Methyl orange indicator (0.05%) was prepared by disolving 25 mg of the dye (S.D. Fine Chem, Mumbai, India) in 50 ml of water for method A. Sodium thiosulphate solution (0.02 M) was prepared by dissolving about 5 g of the chemical (SISCO Chem, Industries, Mumbai) in 1 litre of water and standardized with pure potassium dichromate iodometrically[28]. Aqueous solutions of potassium iodide (10 %) and starch indicator (1 %) were prepared in the usual way. Pharmaceutical grade SBS, certified to be 99.7% pure was procured from Cipla India Ltd, Mumbai India,and was used as received. A 1 mg/ml solution of SBS was prepared by dissolving accurately weighed 250 mg of pure drug in water and diluting to 250 ml with water in a calibrated flask and used for assay by titrimetry. This stock solution (1000 μg/ml) was diluted with water to get working concentrations of 10 and 50 μg/ml SBS for method C and method D, respectively.

Methods

Direct titrimetry. (Method A)

A 10 ml aliquot of standard drug solution containing 2.5-9.5 mg of SBS was accurately measured into a 100 ml titration flask and the solution was acidified by adding 5 ml of 5 M-hydrochloric acid. After adding 2 ml of 10% KBr solution, the total volume was adjusted to 20 ml with water and titrated with 0.005 M CAT using one drop of methyl orange indicator to a colourless end point. An indicator blank was run and the volume of CAT consumed in the blank titration was subtracted from that consumed in the sample titration. The amount of drug in the aliquot was calculated based on the formula:

$$\text{Amount (mg)} = \frac{VSMw}{n}$$

Where V = volume of CAT reacting with the drug in ml, S = molarity of CAT solution, Mw = relative molecular mass of drug, and n = number of moles CAT reacting with each mole of SBS.

Indirect titrimetry (Method B).

A 10 ml aliquot of pure drug solution equiva-

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lent to 3.0-10 mg of SBS was measured accurately and transferred into a 100 ml titration flask. Five ml 1 M hydrochloric acid followed by 10 ml of 0.01 M chloramine-T solution were added and kept aside for 10 min with occasional swirling. Then, 5 ml of 10 % potassium iodide solution were added to the flask and the liberated iodine was titrated with 0.02 M sodium thiosulphate to a starch end point. A blank titration was run under same conditions. The drug content in the aliquot was calculated from:

$$\text{Amount (mg)} = \frac{(B - S)\, M_w\, R}{2}$$

Where

B = volume of thiosulphate solution consumed in the blank titration, ml

S= volume of thiosulphate solution consumed in the sample titration, ml,

M_w = relative molecular mass of the drug and

R = strength of chloramine-T solution, mol/l

Spectrophotometric method using methyl orange (Method C)

Aliquots of pure SBS solution (0.5 to 4.5 mL; 10 μg/ml) were transferred into a series of 10 ml calibrated flasks and the total volume was adjusted to 4.5 ml with water. To each flask were added 1 ml each of 5 M hydrochloric acid followed by 1 ml of chloramine-T solution (50 μg/ml). The contents were mixed well and the flasks were set aside for 10 min with occasional shaking. Finally, 1 mL of 50 μg/ml methyl orange solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 520 nm against reagent blank after 5 min.

Spectrophotometry with indigo carmine (Method D)

Varying aliquots (0.25-2.5 ml) of standard 50 μg/ml SBS solution were measured and accurately delivered into a series of 10 ml calibrated flasks and the total volume was brought to 2.5 ml with water. To each flask were added 1 ml each of 5 M hydrochloric acid and 225 μg/ml chloramine-T solutions successively; the flasks were let stand for 10 min with occasional shaking. Then, 1 ml of 200 μg/ml indigo carmine solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 610 nm against a reagent blank after 5 min.

In either spectrophotometric method, the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

Assay procedure for formulations

Twenty tablets were weighed and ground into a fine powder. Powder equivalent to 100 mg of SBS was weighed accurately into a 100 ml calibrated flask, 60 ml of 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. First 10 ml portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1 mg/ml SBS) was taken for assay by titrimetric procedures. The filtrate was diluted appropriately to get 10 and 50 μg/ml concentrations for analysis by spectrophotometric method C and method D, respectively. The contents of 20 capsules were weighed accurately and powdered. An amount of powder equivalent to 100 mg of SBS was treated as described under tablets and analysed.

RESULTS AND DISCUSSION

The proposed methods are based on the oxidation of SBS by CAT in HCl medium and the reaction is followed by titrimetry and spectrophotometry for quantization purposes. Except direct titrimetry rest of the methods are indirect and are based on the determination of surplus chloramine-T after allowing the reaction between SBS and oxidant to occur. In titrimetry, the unreacted chloramine-T is determined iodometrically, and in spectrophotometric methods, the same is determined by reacting with a fixed amount of either methyl orange or indigocarmine. The latter methods make use of the bleaching action of chloramine-T on either dye, the decolouration being caused by the oxidative destruction of the dye.

Optimisation of experimental conditions

Titrimetry

Direct titration of SBS with CAT was not found to be feasible. However, in the presence of excess of KBr, the titration was feasible with a sharp and reproducible methyl orange end point. A definite and constant reaction stoichiometry was obtained in HCl medium and the reaction was found to be unaffected when 2-7 ml of 5 M HCl was in a total volume of 20-25 ml at the end point. However, in back titrimetry, the reaction was quantitative at a lower acid concentration of 0.2 M although the reaction stoichiometry was found to be unaffected in 0.12-0.28 M HCl concentration. The oxidation reaction was complete and quantitative in 6 min, and contact times up to 15 min had no effect on the stoichiometry and the results. Beyond 15 min and up to 30 min a very small amount of CAT was consumed but without resulting in any significant reaction stoichiometry. Hence, it is necessary to terminate the oxidation step in method B at the end of 10 min to obtain accurate and precise results. A 10 ml aliquot of 0.01 M chloramine-T solution was found adequate for quantitative oxidation of SBS in method B. Employing 0.005 M chloramine-T solution, 2.5-9.5 mg of SBS could be conveniently determined in method A; simillarley by using 0.01 M chloramine-T solution, 3-10 mg of SBS could be estimated in method B. The relation between the amount of drug and titration end point was examined. The linearity is apparent from the calculated correlation coefficient of 0.999 and -0.9954 for method A and B, respectively, and suggests that the reaction between SBS and chloramine-T proceeds stoichiometrically in the ratio 1:2. in both methods.

Spectrophotometry

In the proposed spectrophotometric methods, the ability of chloramine-T to effect oxidation of SBS and irreversibly destroy methyl orange or indigo carmine to colourless products in acid medium has been used. SBS when added in increasing concentrations to a fixed concentration of chloramine-T, consumes the latter and there will be a concomitant decrease in its concentration. When a fixed concentration of either dye is added to decreasing concentrations of chloramine-T, a concomitant increase in the concentration of dye results and a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of SBS.

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 μg/ml for methyl orange and indigo carmine, respectively. A chloramine-T concentration of 5 μg/ml was found to irreversibly destroy the red colour of 5 μg/ml methyl orange whereas 22.5 μg/ml chloramine-T was required to bleach the blue colour due to 20 μg/ml indigo carmine. Hence, different amounts of SBS were reacted with 1 mL of 50 μg/ml chloramine-T in method A and 1ml of 225 μg/ml chloramine-T in method B followed by determination of the residual oxidant as described under the respective procedures.

For both oxidation of SBS and bleaching of dye steps, hydrochloric acid medium was found to be ideal. one ml of 5 M hydrochloric acid in a total volume of ~4-5 ml was adequate for the oxidation step which was complete in 10 min for method A, 15 min for method B, and the same quantity of acid was employed for the estimation of the dye. Contact time of 10 and 15 min are not critical and any delay up to 25 min had no effect on the absorbance. A 5 min standing time was found necessary for the complete bleaching of the dye colour by the chloramine-T. The absorbance of either dye colour was stable for several hours in the presence of reaction products.

Analytical data

A linear correlation was found between absorbance at λ_{max} and concentration of SBS. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values of both methods are given in TABLE 2. Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in TABLE 2. The graph showed negligible intercept as described by the regression equation $Y = a + bX$ where Y is the absorbance and X concentration in μg/ml. The limit of detection and quantification calculated according to ICH guidelines[30] are also given in TABLE 2 and reveal the

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TABLE 2: Analytical parameters of the spectrophotometric methods

Parameter	Method A	Method B
λ_{max}, nm	520	610
Beer's Law limits, μg mL^{-1}	0.5-4.5	1.25-12.5
Molar absorptivity, L/moL/cm	6.89x 10^4	2.46 x10^4
Sandell sentivity, μg/cm^2	0.0084	0.0234
Limit of detection, μg mL^{-1}	0.062	0.78
Limit of quantification, μg mL^{-1}	0.18	0.26
Regression equation (Y)*		
Intercept (a)	0.0022	-0.0072
Slope (b)	0.119	0.044
S_a	0.008	0.007
S_b	0.0019	0.006
Correlation co-efficient(R)	0.9989	0.9978

S_a=Standard deviation of intercept
S_b= Standard deviation of slope.

very high sensitivity of the spectrophotometric methods.

Method Validation

Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solution at three different levels (within the working limits) was analysed, each determination being repeated seven times. The relative error (%) and relative standard deviation (%) were less than 1.5 and indicate the high accuracy and precision for the methods (TABLE 3). Titration method with a relative error of less than 1 % and relative standard deviation of less than 1.5 % is the most accurate and precise compared to other three methods. For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values were in the range of 2.2-2.7% and represent the best appraisal of the methods in routine use.

Interference study

To investigate the effect of tablet fillers on the measurements involved in the methods, placebo analysis was carried out. A mixture containing lactose, starch, talc, magnesium stearate, sodium alginate and calcium gluconate in the ratio 80: 7 : 2.5: 0.5: 1:9 was extracted with water and filtered using a quantitative filter paper. The filtrate was subjected to analysis by the proposed methods and it was found that a relative error of 1.5- 2.5 was obtained. From this study, it is apparent that the usual co-formulated substances seldom interfere in the methods.

Application to analysis of commercial samples

In order to check the validity of the proposed methods, SBS was determined in some commercial formulations. TABLE 4 gives the results of the determination from which it is clear that there is close

TABLE 3: Intra-day precision and accuracy of the methods

Method	SBS taken	SBS found	Range	RE %	SD	RSD %	ROE
Titrimetric method A	3.0	2.97	0.11	1.0	0.038	1.28	±1.27
	6.0	6.01	0.23	0.17	0.069	1.14	±1.138
	9.0	9.01	0.38	0.14	0.116	1.29	±1.288
Titrimetric method B	4.5	4.49	0.29	0.02	0.03	0.73	±0.729
	6.5	6.48	0.44	0.31	0.082	1.26	±1.258
	8.5	8.51	0.68	0.15	0.071	0.83	±0.829
Spectrophotometric method C	1.0	0.99	0.029	1.4	0.01	1.01	±1.008
	2.0	1.97	0.025	1.5	0.029	1.48	±1.478
	3.0	2.96	0.05	1.33	0.045	1.51	±1.508
Spectrophotometric method D	3.0	2.96	0.07	1.33	0.02	0.76	±0.759
	6.0	5.97	0.06	0.50	0.084	1.40	±1.398
	9.0	9.09	0.09	1.00	0.124	1.37	±1.368

RE. Relative error; SD. Standard deviation; RSD. Relative standard deviation and
ROE. Range of error at 95% confidence level for seven degrees of freedom.
In methods A&B, amount taken/found, range and SD are in mg; the same parameters are in μgml^{-1} in methods C & D.

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TABLE 4: Results of assay of tablets by the proposed methods

Brand name of tablet*	Nominal amount, mg	Reference method	Titrimetric Method A	Found Titrimetric Method B	(% label claim* * ± SD) Spectrophotometric Method C	Spectrophoto Metric Method D
Asmanil[a]	4	99.12±0.76	100.5±0.59 t=3.23 F=1.66	98.11±0.96 t=1.86 F=1.59	101.4±0.89 t=4.37 F=1.37	97.91±0.69 t=2.64 F=1.21
Salmaplon[b]	2	97.84±1.24	98.55±0.92 t=2.40 F=1.82	99.12±0.88 t=1.91 F=1.98	96.01±1.31 t=2.27 F=1.12	98.89±1.18 t=1.37 F=1.10
Ventorlin[c]	4	100.66±1.14	99.01±1.21 t=2.22 F=1.13	101.89±1.31 t=1.59 F=1.32	98.99±1.16 t=2.29 F=1.04	99.34±0.99 t=1.27 F=1.33

***Marked by: a. Igna; b. Khandelwal; c. GSK** ****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**

agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically by a Student's t- test for accuracy and variance ratio F- test for precision with those of the reference method[29] at 95 % confidence level. The calculated t- and F-values (TABLE 4) did not exceed the tabulated values (t=2.77, F=6.39) for four degrees of freedom indicating that there was no significant difference between the proposed methods and the reference method in respect to accuracy and precision.

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analysed tablet or capsule powder was spiked with pure SBS at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative (96.7 - 103.4%) and revealed that co-formulated substances such as talc, starch, gum acacia, lactose, sodium alginate, magnesium stearate, calcium carbonate, calcium gluconate and calcium dihydrogenorthophosphate did not interfere in the determination. The results of recovery study are compiled in TABLE 5.

TABLE 5: Results of recovery study by standard-addition method

Formulation Studied Salmaplon, 2mg	Amount of drug in formulation, mg	Amount of pure drug added, mg	Total found, mg	Pure drug recovered %
Titrimetry Method A	2.96	2.0	4.89	96.7
	2.96	2.0	6.93	99.2
	2.96	2.0	8.99	100.6
Method B	1.98	2.5	4.51	101.2
	1.98	5.0	7.15	103.4
	1.98	7.5	9.37	98.6
Spectrophotometry Salmaplon, 2mg	**Amount of drug in formulation, µg**	**Amount of pure drug added, µg**	**Total found, µg**	**Pure drug recovered %**
Method C	19.20	15.0	34.12	99.5
	19.20	20.0	39.60	102.1
	19.20	25.0	43.78	98.3
Method D	49.5	25.0	74.67	100.9
	49.5	50.0	98.35	97.8
	49.5	75.0	123.02	98.1

Average of three measurements.

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CONCLUSIONS

Four useful micro methods for the determination of SBS have been developed and validated. The methods are simple and rapid taking not more than 15-20 min for the assay. The titrimetric methods which are applicable over 2.5-10.0 mg range employ a reagent(CAT) which is exceptionally stable in solution unlike the reported method using N-bromosuccinimide which requires daily standardization. Both the spectrophotometric methods are more sensitive than the existing ones (TABLE 1) and are free from such experimental variables as heating or extraction step and are free from critical experimental conditions. The methods rely on the use of simple and cheap chemicals and techniques but provide a sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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