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Review Article

A Review on Simple, Rapid and Validated Methods for Estimation of Salbutamol Sulphate from Pharmaceutical Formulations

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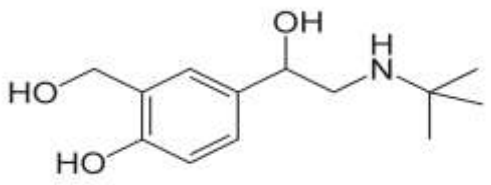
Abstract: Various methods which are simple, sensitive and rapid are described for the estimation of salbutamol sulphate (SBS) in bulk drug and in tablet dosage forms using N-bromosuccinimide (NBS), bromate–bromide solution (BBS), four dyes, rhodamine-B and methylene blue, DONA & DPNA, as reagents and β-Cyclodextrin (BCD) as complexing agent. These methods allow semimicro and microlevel determination of SBS in authentic samples and in dosage forms. The methods were successfully applied to the assay of SBS in tablet and capsule formulations and the results were statistically compared. The accuracy and reliability of the methods were further accompanied by recovery experiments via the standard-addition technique.
Keywords: Salbutamol sulphate, N-bromosuccinimide, bromate–bromide solution, DONA & DPNA spectrophotometry, , β-Cyclodextrin.

INTRODUCTION

Salbutamol, *RS*-[4-[2-(*tert*-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol] is a short acting β2-adrenergic receptor agonist used for the relief of Broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease[1,2,3]. Salbutamol is still commonly delivered as a racemic mixture (+,-). Salbutamol, even though S-Salbutamol is known to have a detrimental effect on asthma sufferers (in fact the exact opposite effect of the R Isomer)[4].

Salbutamol sulphate (SBS) whose structure is given in Fig. 1 is a selective β-2-agonist antiasthmatic. Its primary action is to stimulate adenyl cyclase which catalyzes the formation of cyclic adenosin monophosphate. The drug is official in *European Pharmacopoeia* [5], which describes a potentiometric titration in non-aqueous medium, *British Pharmacoeia* [6] and *Indian Pharmacopoeia* [7]. Some different methods of analysis have been reported for the determination of SBS, including HPLC [8-10] and UV-spectrophotometry [11, 12], but most of them require extensive sample preparation prior to the measurement step, some are less sensitive and some other are relatively complicated in terms of assay procedure or equipment required for analysis.



SBS in pharmaceuticals has been assayed by visible spectrophotometric methods based on reactions such 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 Meisenheiner complex formation [22] and charge-transfer complex formation [23]. However, many of these procedures suffer from some disadvantage, such as poor sensitivity, heating or extraction step, critical working conditions or the use of organic solvents, and are hence unsatisfactory for routine analysis. The only visual titrimetric method [24] reported employs NBS as the oxidimetric titrant in the presence of potassium bromide and using methyl red as indicator. However, the method is applicable over a macro scale. Recently, Issa *et al*. [25] have reported a conductometric titration method using phosphotungstic and phosphomolybdic acids as titrants. Even these procedures are time consuming and less sensitive.

This review describes TEN assay methods for SBS in tablets, capsules and syrups. The methods employ N-bromosuccinimide[26], bromate–bromide solution[27] as an oxidizing agents, rhodamine-B and methylene blue dyes[26,27] as reagents for spectrophotometric analysis. Diazotised o-nitroaniline (DONA)[28] & diazotised p-nitroaniline (DPNA) [29] as a for colour formation, Continuous and Stop flow methods [30] &, Spectroflourometric Estimations[31].

The methods have the advantages of being rapid and simple and are free from interferences 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

METHODS AND PROCEDURES

Titrimetry Using NBS

A 10-mL aliquot of pure drug solution containing 1.0–5.0 mg of SBS was accurately measured and transferred into a 100-mL titration flask. The solution was acidified by adding 5 mL of 5 mol/L acetic acid followed by the addition of 10 mL of 0.01 mol/L NBS. The content was mixed well and the flask was kept aside for 15 min under occasional swirling. Then, 5 mL of 10% potassium iodide was added to the flask and the liberated iodine was titrated with 0.01 mol/L sodium thiosulphate to a starch end point. A blank titration was run under the same conditions [26].

Titrimetry Using bromate–bromide solution

A 10-mL aliquot of pure drug solution(3.0–8.0 mg SBS) was accurately measured and transferred into a 100 mL titration flask, and the solution was acidified by adding 5 mL of 1M HCl. Ten milliliters of bromate–bromide solution (5 mM $KBrO_3$ –50 mM KBr) were pipetted into the flask, the flask was stoppered, the contents were mixed well, and left to stand for 15 min with occasional swirling. Finally, 5 mL of 10% potassium iodide solution were added and the liberated iodine titrated with 0.03 M thiosulphate solution using a starch indicator. A blank titration was performed under similar conditions [27].

Spectrophotometric method A (Using NBS)

Aliquots of pure SBS solution (0.5 to 3.5 mL, 5 mg/mL) were transferred into a series of 10 mL calibrated flask and the total volume was adjusted to 4 mL with water. To each flask, 1 mL of 1 mol/L HCl was added, followed by 1 mL of NBS solution (70 mg/mL). The contents were mixed and the flasks were set aside for 10 min under occasional shaking. Finally, 1 mL of 50 mg/mL rhodamine B solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 555 nm against a reagent blank after 10 min [26].

Spectrophotometric method B (Using NBS)

Varying aliquots (0.5–5.0 mL) of standard 10 mg/mL SBS solution were accurately measured and delivered into a series of 10mL calibrated flasks, and the total volume was made up to 5.0 mL with water. To each flask, 1 mL each of 5 mol/L HCL and 150 mg/mL NBS solution were added successively; the flasks were let stand for 10 min under occasional shaking. Then, 1 mL of 40 mg/mL methylene blue solution was added to each flask, the volume was adjusted to the mark with water and mixed. The absorbance of each solution was measured at 665 nm against a reagent blank after 5 min [26].

Spectrophotometric method A using bromate–bromide solution

Different aliquots (0.5, 1.0, …, 5.0 mL) of standard 5 µg/mL SBS solution were delivered into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 5 mL by adding water. To each flask, 1 mL of 5 M HCl and 1 mL of $KBrO_3$ –KBr solution (10 µg/mL $KBrO_3$) were added. The flasks were stoppered, the contents were mixed, and then left to stand for 15 min with occasional shaking. Finally, 1 mL of 50 µg/mL rhodamine-B solution was added to each flask, the volume was brought up to the mark with water, mixed well, and the absorbance of each solution was measured at 555 nm against a reagent blank after 5 min [27].

Spectrophotometric method B using bromate–bromide solution

Varying aliquots (0.5, 1.0, …, 5.0 mL) of standard 15 µg/mL SBS solution were accurately measured into a series of 10 mL calibrated flasks with the help of a micro burette and the volume was brought to 5 mL by adding water. To each flask, 1 mL of 5 M HCl was added followed by 1 mL of the bromate–bromide reagent solution (25 µg/mL with respect to $KBrO_3$). The flasks were stoppered, the contents were mixed well, and then left to stand for 10 min with occasional shaking. Lastly, 1 mL of 40 µg/mL methylene-blue solution was added to each flask, the volume was brought up to the mark with water and mixed well, and the absorbance was measured at 665 nm against a reagent blank after 5 min [27].

7] <u>DONA method</u>: - An aliquot of sample containing 50-1000 µg of SBS was transferred into a series of 25 ml standard flasks to cover the range of 2-40 µg/ml. A volume of 3ml of 3×10^{-3}M DONA solution and 1ml of 0.1M sodium hydroxide solution were added. The contents of flasks were diluted to the mark with distilled water, mixed well and left for 20 min. The absorbance was measured at 448 nm (at room temperature 20°C). The color of the formed dye is stable for more than 3hr. For optimization of conditions and in all subsequent experiments, a solution of 500 µg was used and the final volume was 25 ml (i.e.20 µg/ml). Fifty to 100 tablets were accurately weighed and powdered .An amount to tablets equivalent to 100 mg of the pure drug, was dissolved in distilled water and transferred into a 100 ml calibrated flask and completed to the mark with the same solvent. The flask with its contents was shaked well and filtered. Samples of 20 and 30 ppm of SBS were taken and the measurements were carried out as described earlier under general procedure [28].

DPNA method

To a series of 25ml volumetric flasks transferred 10 –160µg (0.4–6.4 ppm) of SBS, 1 ml diazotized *p*-nitroaniline (5 mM) and 4 ml of 1N NaOH were added, then the volumes were made to the mark with distilled water. The absorbances were read against a reagent blank prepared in the same manner but without salbutamol sulphate at 488 nm using 1cm cells. The colour is formed immediately and is stable for at least 1 hour. The calibration graph is linear over the

range 0.4 – 4.8 ppm, and higher concentrations show negative deviation. The molar absorptivity, calculated in the region of least photometric error and at the wavelength of maximum absorption, is found to be 3.13 $\times 10^4$ l/mol/cm [29].

HPLC

An aliquot of sample containing 10–350µg of SBS was transferred into a series of 10mL standard flasks. A volume of 1 mL of 20mM of HAH solution, 1.5mL of 0.2% (w/v) of SNP solution and 0.5mL of 100mM sodium carbonate solution were added. The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 10min. The absorbance was measured at 683nm (at room temperature 25°C) against reagent blank containing all materials except SBS. A calibration graph was drawn and the regression equation was calculated. For the optimization of conditions and in all subsequent experiments, a solution of 200µg was used and the final volume was 10mL.

General cFIA procedure

Working solutions of SBS in the range of 10 to 350µg/mL were prepared from stock solutions. A 150µl portion of SAL was injected into the stream of the mixture of 30mM SNP and 50mM of HAH and was then combined with a stream of 200mM sodium carbonate with a flow rate of 0.5mL/min in each channel. The resulting absorbance of the green dye was measured at 683 nm. Optimization of conditions was carried out on 100µg/mL of SBS.

General sFIA procedure

Accordingly, the flow system with an individual flow rate of 1.08mL/min, was consisted from three stream lines; the first one to deliver a 30mM SNP solution which merges with 70mM HAH stream. A 200µl portion of SAL (working solutions in the range 2-180µg/mL) was injected into these combined lines and was then combined with a stream of 200mM of sodium carbonate to form a green complex product which was then directed towards the flow cell in the detector. The pump was stopped for 80sec via the control unit at 17sec after each injection, when the sample zone was in the detector flow cell. Then the flow was restarted to push the zone out of the detector (this step would need 25sec) and the absorbance was monitored spectrophotometrically at 683 nm. Optimization of conditions was carried out on 100µg mL−1 of SBS [30].

Flourimetry

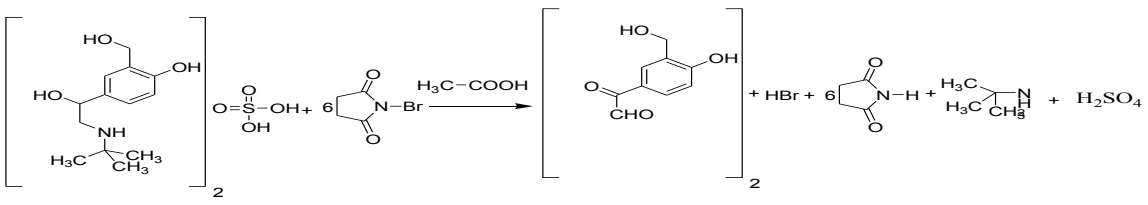
For selection of excitation and emission wavelength of inclusion complex, excitation spectra was scanned between 220-400 nm, while emission spectra was scanned between 400-700 nm. The wavelengths selected for analysis was 279.6 nm as excitation wavelength and 609.8 nm as emission wavelength. Fluorescence intensity of standard and sample solutions determined at selected excitation and emission wavelength.

Twenty tablets were weighed and crushed. Tablet powder equivalent to 20 mg of SBS was accurately weighed and transferred to volumetric flask; 10 ml water was added into crushed powder and was sonicated for 20 minutes. Above solution was filtered using Whatman filter paper 41. Filtrate was collected in crucible and was allowed to evaporate in vacuum dryer until the constant weight was obtained. Collected dry powder was dissolved in 5 ml DMSO and mixed with BCD solution (1 mg/ml, 24 ml) with continuous agitation and kept aside for 20 min and diluted up to 250 ml with water. An aliquot was further diluted with water to obtain final concentration 9.6 µg/ml.

Ten ml of syrup containing 4 mg of SBS was accurately pipetted out and mixed with 25 ml 0.05 M H_2SO_4. Aqueous solution was extracted twice with 50 ml diethyl ether. Aqueous extract was collected in 250 ml volumetric flask. Ether extract was washed with 10 ml water. All aqueous extract was collected together and passed through charcoal to remove coloring matter. DMSO (5 ml) was added in aqueous solution and then BCD solution (1mg/ml, 4.8 ml) was added with continuous agitation and kept aside for 20 min. Volume was adjusted upto the mark with water in 250 ml volumetric flask. An aliquot was further diluted with water to obtain final concentration 9.6 µg/ml [31].

RESULTS AND DISCUSSIONS

Methods i.e. 1 & 2 are based the reaction between SBS and a known amount of bromine. In titrimetry, unreacted bromine is determined iodometrically, and in spectrophotometric methods, the same is determined by reacting with a fixed amount of either rhodamine-B or methylene-blue dye. The latter methods make use of the oxidative destruction by bromine on either dye. The propabable mechanism for the reaction is shown below-



According to *Basavaiah et al*, the stoichiometry of the reaction for all calculations is 1:6. The linearity is apparent from the calculated correlation coefficient of –0.9954 and suggests that the reaction between SBS and NBS proceeds stoichiometrically in the ratio 1:6. They got non-stoichiometric results in a hydrochloric acid and sulphuric acid. An acetic acid medium gives quantitative results and a 1.0 mol/L acetic acid concentration is optimal. Although the reaction stoichiometry was unaffected in the range 0.2–2.0 mol/L acetic acid. The oxidation reaction was found to be complete and quantitative in 15 min and contact times up to 25 min had no effect on the stoichiometry and the results. A 10 mL aliquot of 0.01 mol/L NBS (0.1 mmol) solution was found adequate for quantitative oxidation of SBS in the range determined 0.1–0.5 mg/mL [26].

Basavaiah et al also reported that concentration of HCl equal to 0.2 M was maintained for the reaction between SBS and bromine and iodometric back titration of the latter although the results were unaffected when 0.16 to 0.36 M HCl concentrations were used. The reaction was complete in 5 min. Under the experimental conditions, SBS (3.0 to 8.0 mg) could be determined with a fair degree of accuracy and precision the reaction stoichiometry was found to be 1 : 2 (SBS : $KBrO_3$) [27].

In the spectrophotometric methods, SBS was added to a fixed and known amount of NBS or BBS, and after the completion of reaction, the unreacted NBS or BBS was determined by reacting with a fixed amount of either rhodamine B or methylene blue. SBS is added in increasing amounts to a fixed amount of NBS or BBS. The reaction was observed as a proportional increase in the absorbance at the respective λ_{max} with increasing concentration of SBS, as shown by the correlation coefficients of 0.9993 (method A) and 0.9988 (method B) [26,27].

Table-1:

Parameter	Method A	Method B
λ_{max}, nm	555	665
Beer's law limits, µg/mL	0.25–2.5	0.75–7.5
Molar absorptivity, L/mol/cm	8.96×10^4	4.67×10^4
Sandell sensitivity, ng/cm^2	6.4	12.34
Limit of detection,µg/mL	0.07	0.12
Limit of quantification, µg/mL	0.21	0.38
Regression equation (Y)*		
Intercept (a)	0.005	0.0001
Slope (b)	0.166	0.092
Sa	0.0089	0.0092
Sb	0.0039	0.0014
Correlation co-efficient (R)	0.9978	0.999

According to Hind Hadi et al, when various concentrations of DONA solution were added to a fixed concentration of SBS, 3ml of 3×10^{-3} M DONA solution was enough strong to develop the color to its full intensity and give minimum blank value. Above 3ml the absorbance of blank value increased which causes a decrease in the absorbance of sample. Therefore, 3ml of 3×10^{-3} M DONA solution was found optimum and was used in all subsequent experiments [28].

Preliminary results indicated that the presence of an alkaline in the reaction mixture is essential for developing a more intense yellow orange colour. For this, sodium hydroxide, potassium hydroxide, sodium acetate, ammonium hydroxide and sodium carbonate were examined by these authors. Best results were obtained with sodium hydroxide and hence they chose sodium hydroxide and 1ml of 0.1M solution was added as optimum after the diazotized reagent as it gives a high sensitivity and minimum blank value . The absorption spectra of the colored product are given in Figure 1.

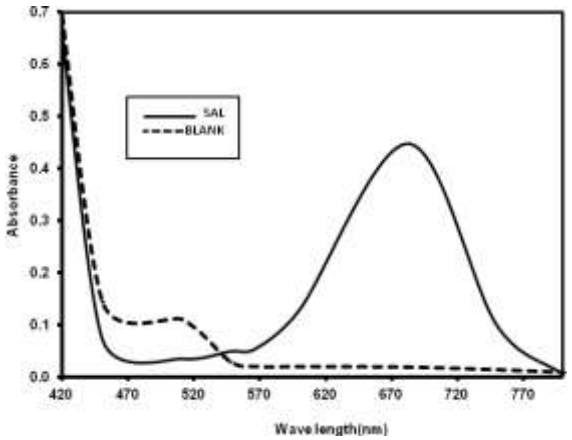


Fig. 1: Absorption spectra of (20 µg/mL) of SAL treated as described under procedure and measured against reagent blank and the reagent blank measured against distilled water

The maximum absorbance is at 683nm for SBS and the blank signal is insignificant in the wavelength chosen for taking measurements. The obtained results from Job's method in Figure 2 showed that a 1:2 (SBS: SNP) product was formed between drug and sodium nitroprusside at 683nm.

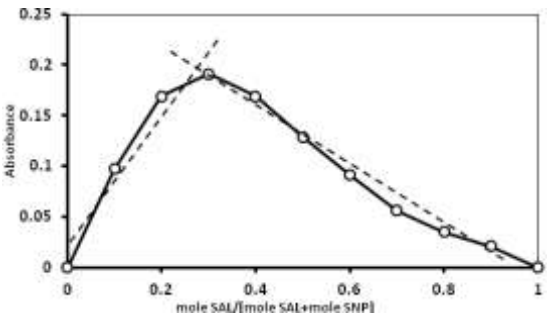


Fig. 2: Study of the mole ratio of the reaction between SAL and SNP

SBS is freely soluble in water but it gives very less fluorescence due to quenching effect [Figure 3]a and b. Literature survey revealed that BCD forms inclusion complex with benzene derivatives and decreases quenching effect. Formation of inclusion complex of SBS with BCD did not show any significant wavelength shift but it enhanced peak intensity. This may be because the hydrophobic cavity of BCD increases solubility, decrease quenching effect and improve fluorescence intensity; hence BCD was chosen as complexing agent in this experiment. Different complexation methods were used to prepare SBS: BCD inclusion complex. The inclusion complex ratio was also optimized and it was observed that 1:1 ratio of SBS and BCD gave maximum fluorescence intensity [Figure 4]. From the scanned spectra it was observed that SBS gives maximum absorbance at 279.6 nm as excitation wavelength and emission wavelength observed was 609.8 nm [Figure 5]a and b.

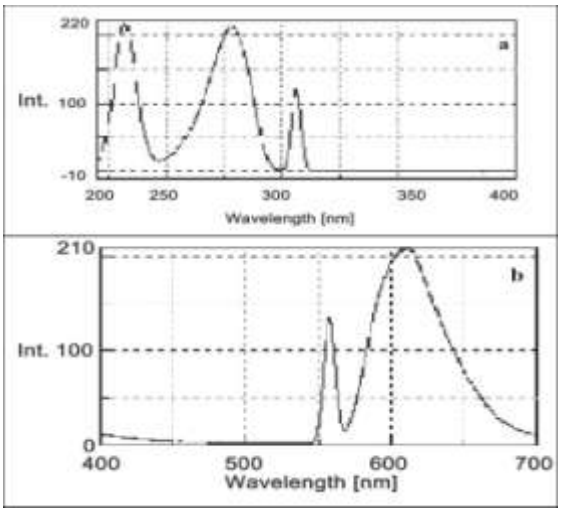


Figure 3: Spectrofluorimetric Spectra of SAL before complexation (a) Excitation spectra (b) Emission spectra

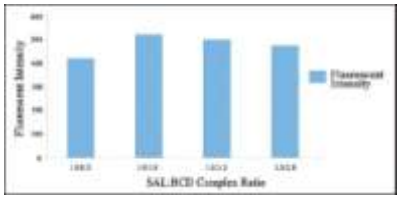


Figure 4: Effect of different ratios of SAL:BCD on fluorescent intensity

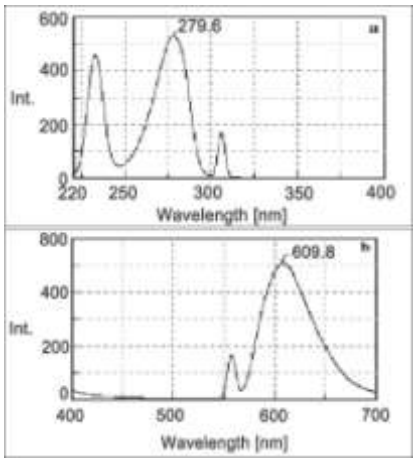


Figure 5: Spectrofluorimetric spectra of SAL: BCD inclusion complex (a) Excitation spectra showing λExmax 279.6 nm (b) Emission spectra showing λEmmax 609.8 nm

This method was validated by the authors for linearity and range, precision, LOD, LOQ, accuracy as per ICH guidelines for analytical method validation [32]. The SBS: BCD inclusion complex showed linear response in the concentration range of 4-20 µg/ml with good correlation coefficient (r) value of 0.9982. The results of linearity, LOD, LOQ and precision study are given in [Table 2]. The accuracy study was performed by standard addition method. [Table 3] shows the percentage of drug recovered (98.52-101.45%) which are in good agreement with the added amount and label claim. Recovery experiment indicated the absence of interference from commonly encountered pharmaceutical additives and excipients

Table 2: Summary of results of method validation of SAL by proposed method

Validation parameter	Results
Linearity range (µg/ml)	4–20
Regression equation	y = 41.89x + 57.84
Correlation coefficient (r)	0.9982
Intra-day precision (RSD) (n = 3)	0.67%
Inter-day precision (RSD) (n = 3)	1.16%
LOD (µg/ml)	0.60
LOQ (µg/ml)	1.81

Table 3: Results from the accuracy study of SAL by standard addition method

Concentration of SAL in (µg/ml)	% addition w.r.t. test concentration	Fluorescence intensity	Amount of SAL recovered (µg/ml)	Recovery (%)
4	80	348.6738	7.25	100.69
	80	344.9235	7.17	99.58
	80	350.9282	7.30	101.39
4	100	381.9254	7.99	99.89
	100	387.5295	8.12	101.45
	100	377.1492	7.89	98.57
4	120	420.5925	8.84	100.55
	120	415.8293	8.74	99.35
	120	412.5254	8.66	98.52

Precision and accuracy

Intra-day precision was 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 levels (amounts/concentrations) were calculated. Analysis was performed over a period of five days to evaluate the inter-day precision by preparing all solutions afresh each day.

Application to dosage forms

Different types of marketed formulations of SBS are available and according to that sample preparation can vary for estimation. Initially IP procedure was followed for sample preparation and modification were incorporated as per developed method requirement. The obtained results, indicates that the developed and validated method can be successfully applied for estimation of SBS from all type of marketed formulations like tablet, syrup and aerosol.

The proposed methods were applied to the analysis of SBS in tablets and capsules and the results were statistically compared with those obtained by the reported method (9), which consisted of measuring the absorbance of blue chromogen at 670 nm after treating tablet extract with Folin-Ciocalteau reagent in alkaline medium. The calculated *t* and *F*-values were lower than the tabulated values at 95% confidence level, revealing that the proposed methods and the reference method have similar accuracy and precision. In a few cases the *t*-calculated values are deviant and this can be ascribed to random errors.

Recovery Experiments

Pure SBS was added at three different levels to a fixed and known amount of drug in the tablet/capsule powder (pre-analysed) and the total was found by the proposed methods, from which the percent recovery of pure drug added was calculated. From the recovery experiment, it was found that the percent recovery of the pure drug added to tablet/capsule powder ranged from 97.5 to 104.5, as shown in Table 4, and that neither the end point detection in titrimetry nor absorbance measurement in spectrophotometry was affected by tablet excipients such as talc, starch, lactose, magnesium stearate, sodium alginate, calcium gluconate and calcium dihydrogenorthophosphate.

Table 4: Recovery of SBS from different methods

Method	Total SBS Recovered (%)
Titrimetry (NBS)	99.2 ± 2.1
Titrimetry (BBS)	98.01 ± 1.21
Spectrophotometric method A (NBS)	98.5 ± 2.6
Spectrophotometric method A (BBS)	99.01 ± 0.97
Spectrophotometric method B (NBS)	97.9 ± 3.7
Spectrophotometric method B (BBS)	98.95 ± 1.21
DONA	98.844 ± 1.992
DPNA	98.200 ± 1.992
HPLC	99.50± 0.97
Flourometry	98.54 ± 2.09

CONCLUSION

The proposed methods can be used to determine the content uniformity of tablets and capsules, as well as the purity of salbutamol raw material. Besides the simplicity of the procedures, the relative cheapness of apparatus demonstrates their advantageous characteristics in addition to their high accuracy and precision. These methods can also be applied for the determination of salbutamol in urine and blood samples.

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