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**A SYSTEMATIC REVIEW OF ANALYTICAL METHODOLOGIES FOR  
ESTIMATION AND CHARACTERIZATION OF SALBUTAMOL IN BULK  
DOSAGE FORM**

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**ABSTRACT**

Salbutamol is a potent anti-asthmatic agent, is a short-acting  $\beta$ 2-adrenergic receptor agonist act, Salbutamol and terbutaline are similar  $\beta$ -2 adrenoceptor agonists that are believed to exert their maximal therapeutic effect through bronchodilation. Stimulation of  $\beta$ -2 receptors in airway smooth muscle induces the cyclic AMP (c-AMP) pathway. In the present study, an attempt has been made to study various analytical methods for determination of salbutamol from different dosage form, these methods can be successfully applied for determination of salbutamol. The HPLC, UV, AAS, HPTLC, capillary electroscopic methods are available for assessment of salbutamol.

**Keywords:  $\beta$ 2-agonist HPLC, HPTLC, COPD, analysis**

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**INTRODUCTION:**

Salbutamol is also known as albuterol, it is a short-acting  $\beta$ 2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and COPD. It is usually given by the inhaled route for direct effect on bronchial smooth muscle. This is usually achieved through a metered dose inhaler, Nebulizer or other proprietary delivery devices. In these forms of delivery, the effect of Salbutamol can take place within 5 to 20 minutes of dosing. It can also be given orally or intravenously. Despite the fact that SS is well absorbed, its systemic bioavailability is only 50% due to extensive presystemic metabolism in the gastrointestinal tract and liver [1].

Salbutamol Sulphate in pharmaceuticals has been assayed using visible spectrophotometric methods based on reactions such as redox, reducing and then chelating, oxidative coupling, diazotization and coupling, nitrosation, nitration, nitration followed by Meisenheimer complex formation and charge-transfer complex formation [2-3].

Literature survey revealed that several techniques have been adopted for the determination of salbutamol. These include, high-performance liquid chromatography (HPLC), HPLC–mass spectrometry (MS),

gas chromatography–MS, electrokinetic chromatography, MS, LC, immunoassay, capillary electrophoresis, spectrophotometry, polarography, Potentiometry using ion-selective electrodes, and Voltametry [4–5].

**PHARMACOLOGY OF SALBUTAMOL**

Salbutamol and terbutaline are similar  $\beta$ -2 adrenoceptor agonists that are believed to exert their maximal therapeutic effect through bronchodilation. Stimulation of  $\beta$ -2 receptors in airway smooth muscle induces the cyclic AMP (c-AMP) pathway. c-AMP is a molecule with various cellular functions. Increased activity of c-AMP-dependent protein kinase A inhibits myosin phosphorylation and lowers intracellular calcium concentration, which in turn relaxes smooth muscle and causes bronchodilation.4 Increased intracellular c- AMP may also inhibit mast cell inflammatory mediator release (**Figure 1**; adapted from refs. 5 and 6). Severe airway obstruction may restrict delivery of inhaled salbutamol to the airway epithelium, thus providing a theoretical rationale for the use of intravenous preparations. Because adrenoceptors are found in various organs and tissues,  $\beta$ -2 agonists can also cause various extra pulmonary adverse effects [6-7-8].

**PHARMACOKINETICS**

The half-life of salbutamol is 4–6 h, and it is excreted renally. The bronchodilatory effects of salbutamol are believed to occur at blood concentrations of between 5 and 20 ng/mL, and higher concentrations are thought to result in a greater risk of toxicity. There are limited data regarding the ideal dosing schedule for intravenous salbutamol in children.

A recent review commented on the relatively high doses per unit of weight advised for use in children compared with adult regimens and recommended further research into the pharmacodynamics and pharmacokinetics of intravenous salbutamol in the paediatric population. [9].

#### ADEVERC EFFECT

The most common side effects are fine tremor, anxiety, headache, muscle cramps, dry mouth, and palpitation. Other symptoms may include tachycardia, arrhythmia, flushing of the skin, myocardial ischemia (rare), and disturbances of sleep and behaviour. Rarely occurring, but of importance, are allergic reactions of paradoxical bronchospasms, urticaria (hives), angioedema, hypotension, and collapse. High doses or prolonged use may cause hypokalemia, which is of concern especially in patients with kidney failure and those on certain diuretics and xanthine derivatives.

Salbutamol metered dose inhalers have been described as the “single biggest source of carbon emissions from NHS medicines prescribing” due to the propellants used in the inhalers. Dry powder inhalers are recommended as a low-carbon alternative [10].

#### PHYSICOCHEMICAL PROPERTTEIS: [11-12-13-14]

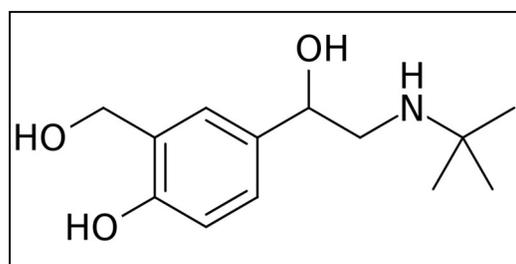


Figure 1: Structure of salbutamol [11]

**IUPAC Name:** [1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*t*-butylamino) ethanol]

**Molar mass:** 239.311 g/mol

**Formula:** C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub>

**CAS ID:** 18559-94-9

**Other names:** Albuterol (USAN US)

**Onset of action:** <15 min (inhaled), <30 min (pill)

**Duration of action:** 2–6 hrs

**Metabolism:** Liver [13].

**Melting Point:** 157-158°C (with decomposition)

**Description:** A white or almost white, crystal clear solution in methanol, very pale clear yellow solution.

**Solubility:** Sparingly soluble in water; soluble in ethanol (96%); slightly soluble in ether.

**Presence of ring:** Benzen ring present

**P(Octanol/water):** 1.46

**Chiral centers:** 1

**MOA SALBUTAMOL: [14]**

Salbutamol relaxes the smooth muscles of all airways, from the trachea to the terminal bronchioles. Salbutamol acts as a functional antagonist to relax the airway irrespective of the spasmogen involved, thus protecting against all bronchoconstrictor challenges.

**Analytical Methods on Salbutamol**

**Uv-visible spectroscopy**

All chemical used were of analytical grade. Standard solutions of salbutamol and bromhexine were prepared by dissolving 50 mg in 10 ml absolute ethanol following by the dilution to 50 ml with distilled water. Further dilution of standard solutions was prepared to obtain final concentration ranging between 2–50 µg/ml. The absorption measurements were recorded along 200–350 nm with 1 nm intervals against a blank of 20% ethanol. To assay tablets, not less than 10 tablets were finely powdered and a weight equivalent to one tablet was placed in a beaker containing 10 ml absolute ethanol. The solution was shaken for a while, then filtered through Whatman filter paper No.41

to a 50 ml measuring flask. The filtrate was diluted with water to the mark. The experiment was repeated as described before. The HPLC determination was performed on a microBondpack Phenyl (30 × 0.4 cm i.d.) as described. Salbutamol and bromhexine solutions were prepared by dissolving appropriate amounts in aqueous 75% methanol containing 5 mM sulphanic acid as internal standard. The mobile phase was 5 mM pentanesulfanic acid in aqueous 75% methanol, flow rate was 0.5 ml min<sup>-1</sup> and spectrophotometric detection was carried at 280 nm [15-19].

**HPLC**

A rapid and simple high-performance liquid chromatography UV method was developed for the separation and quantification of salbutamol. The analyses were carried using an Agilent Technologies HP1200 series HPLC system equipped with a quaternary gradient pump, a diode array detector, and a Chemstation data-analysis system (Agilent, Palo Alto, CA, USA). Chromatographic separation was performed at 25C on Agilent Eclipse C18 (250 mm 4.6 mm i.d., 5 mm particle) analytical columns (Agilent) at a flow rate of 0.8 mL/min via a ternary gradient. Eluent A consisted of 0.1% formic acid, eluent B is 0.1% formic acid in acetonitrile solution, and eluent C is 50

mmol/L ammonium acetate aqueous solution. Separation was performed with a gradient, as shown in Table 1. The injection volume was 20 mL, and the detection wavelength of the detector was set at 225 nm. Complete baseline separation of three  $\beta$ -agonists was achieved in < 20 minutes; the linear range is 0.2e50 mg/L with a correlation coefficient  $R^2$  value of > 0.99. Excellent method reproducibility was found by intra- and interday precisions with a relative standard deviation of < 3%. The detection limit (S/N  $\frac{1}{3}$ ) was found to be <0.05 mg/L [20].

#### RP-HPLC

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of salbutamol sulphate and bromhexine hydrochloride. The separation was carried out using a mobile phase consisting of acetonitrile, methanol and phosphate buffer, pH 4 in the ratio 60:20:20 v/v. The column used was SS Wakosil-II C-18 with a flow rate of 1 ml/min and UV detection at 224 nm. The described method was linear over a concentration range of 10-110  $\mu\text{g/ml}$  and 20-140  $\mu\text{g/ml}$  for the assay of salbutamol sulphate and bromhexine hydrochloride, respectively. The mean recovery was found to be 95-105% for salbutamol sulphate and 96.2-102.1% for

bromhexine hydrochloride when determined at five different levels [21, 22].

#### Atomic Absorption Spectrophotometric Methods

Two sensitive kinetic and atomic absorption spectrophotometric (AAS) methods were developed for the determination of salbutamol sulfate (SLS) in its dosage forms. The kinetic method was based on the bromination reaction of the drug by bromine generated in-situ by the reaction of bromate with bromide in acidic medium. The reaction was followed spectrophotometrically by measuring the decrease of bromine color at 380 nm. The reaction was carefully studied and optimized. Under optimum conditions, the stoichiometry and the order of the reaction were determined. The initial rate and fixed time methods were utilized for the determination of salbutamol sulfate concentrations. The AAS method was depended on the oxidation of iron (II) with excess bromine from the bromination reaction of the drug. A new method for separation iron (III) was used. Then iron (II) in aqueous layer was aspirated into air-acetylene flame and determined by AAS. The linear ranges for the proposed methods were 2.0-10.0 and 0.2-2.0  $\mu\text{g mL}^{-1}$  with detection limit of 0.30 and 0.012  $\mu\text{g mL}^{-1}$  for kinetic and AAS, respectively. The proposed

methods were validated; the mean recovery ranges from 98.0 to 102.0% with RSD < 2.1%. Common excipients did not interfere the measurements of SLS. The methods were successfully applied to determined SLS in dosage forms; there was no significant difference between the proposed methods and official one [23].

### Capillary Electrophoresis

This Method describes the separation and quantification of salbutamol in pharmaceutical products (salbutamol syrups) by capillary electrophoresis (CE) with contactless conductivity detection (C(4)D). The system was studied by micellar electrokinetic capillary chromatography (MEKC) and free solution capillary electrophoresis (FSCE), being the latter chosen in function of best resolution and sensitivity in comparison with the MEKC method. CE-C(4)D was applied to analysis of salbutamol in syrups utilizing  $1.0 \times 10^{-2}$  molL<sup>-1</sup> acetic acid/sodium acetate buffer (pH 4.9) as running electrolyte. Tetraethylammonium (TEA) solution was used as internal standard. The results obtained include a linear dynamic range from  $7. \times 10^{-5}$  to  $3.0 \times 10^{-4}$  molL<sup>-1</sup> and good repeatability (R.S.D.=4.7% for n=10 for a  $7.0 \times 10^{-5}$  molL<sup>-1</sup> salbutamol solution). The detection limit was calculated as  $1.0 \times 10^{-5}$

molL<sup>-1</sup>) and the limit of quantification was estimated as  $3.3 \times 10^{-5}$  molL<sup>-1</sup>). For syrups analysis the reproducibility presented deviations between 1.5% and 2.5% (three different days) obtained for measurements in triplicate [24].

### LC-MS

A sequential solid-phase extraction (SPE) method was developed and validated using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) for the detection and quantification of salbutamol enantiomers in porcine urine. Porcine urine samples were hydrolysed with  $\beta$ -glucuronidase/arylsulfatase from *Helix pomatia* and then subjected to a double solid-phase extraction (SPE) first using the Abs-Elut Nexus SPE and then followed by the Bond Elut Phenylboronic Acid (PBA) SPE. The salbutamol enantiomers were separated using the Astec CHIROBIOTIC™ T HPLC column (3.0mm×100mm; 5 $\mu$ m) maintained at 15°C with a 15min isocratic run at a flow rate of 0.4mL/min. The mobile phase constituted of 5mM ammonium formate in methanol. Salbutamol and salbutamol-tert-butyl-d9 (internal standard, IS) was monitored and quantified with the multiple reaction monitoring (MRM) mode. The method showed good linearity for the range

of 0.1-10ng/mL with limit of quantification at 0.3ng/mL. Analysis of the QC samples showed intra- and inter-assay precisions to be less than 5.04%, and recovery ranging from 83.82 to 102.33% [25].

### HPTLC

Samples were applied 0.5 cm from the edge of the HPTLC plate. Development was carried out, immediately after spotting, in a Camag horizontal development chamber (Camag). Two solvent systems were used: system A (urine analysis):

chloroformethylacetate (60:40, v/v); system B (plasma or urine analysis): ethyl acetate chloroform-methanol (60:40:1, v/v). Plates were developed for 3.5 cm beyond the origin. The derivative (III) had an RF of 0.3 to 0.36 in system A and 0.3 in system B. Chromatograms could be preserved by dipping the plate in a solution of liquid paraffin in hexane (20%,v/v), HPTLC plate absorption maxima were observed at 250 and 650 nm [26, 27].

**Table 1: A summary of research work on the analytical methods for the estimation of Salbutamol alone and in the combination**

Sr. No.	Drug	Method	Instrument, Mobile phase, RT, Flow Rate & Result of Validation
1.	Salbutamol	UV Method	Instrument: JASCO UV-VISIBLE 450 spectrophotometer. M.Phase: Ethanol and distilled water. $\lambda_{max}$ : 200–350nm. Result : 0.986 Slope and Intercept : 1.126 and –2.534 Detection Limit: 2–50 $\mu\text{g/ml}$ . Recovery: 134% [16-19]
2.	Salbutamol	HPLC Method	Instrument: Agilent Technologies HP1200 M.Phase: formic acid, acetonitrile solution, ammonium acetate Column: Eclipse C18 (250 mm $\times$ 4.6 mm i.d., 5 mm particle) $\lambda_{max}$ : 225 nm Flow Rate : 0.8 mL/min. RSD: < 3% Detection limit: <0.05 mg/L Result : R <sup>2</sup> >0.99 [20]
3.	Salbutamol	RPLC Method	Instrument: M.Phase: acetonitrile, methanol and phosphate buffer(pH 4) Column: SS Wakosil-II C-18 $\lambda_{max}$ : 224 nm. Flow Rate : 1 ml/min Recovery : 95-105% RSD: 0.13% Linearity Range: 10-110 $\mu\text{g/ml}$ [21]
4.	Salbutamol	Dissolution Method	Apparatus: Bio-Dis dissolution apparatus( VK 750D). Temp: 37 $\pm$ 0.5 $^{\circ}\text{C}$ Agitation rate: 10 rpm Media: 200 ml (6.8 phosphate buffer) Analysed by : HPLC Method M.Phase: ACN, phosphate buffer( pH = 4), sodium octane sulphonate. Column: Phenomenex <sup>®</sup> Hyperclone <sup>®</sup> column, 5 $\mu\text{m}$ , 150 mm $\times$ 4.6 mm $\lambda_{max}$ : 220 nm. Flow Rate: 1.0 mL/min Release: After 12 hr approximately 83% to complete drug release. [23]
5.	Salbutamol	UV Method	Instrument: UV-visible spectrophotometer (Shimadzu, model pharm spec 1800) Solvent: Methanol. $\lambda_{max}$ : 257 nm. Recovery: 98.82%–101.19% %RSD: 1.30% LOD: 0.55 $\mu\text{g/ml}$ LOQ : 1.66 $\mu\text{g/ml}$

### CONCLUSION

This review article discusses various analytical methods for Salbutamol in pharmaceutical and biological specimens.

This review could serve as a foundation for scientists and researchers in academia and industry to investigate the development of analytical techniques for Salbutamol. Many

research publications containing spectrophotometric, hyphenated, and chromatographic methods were discussed in this article. With the help of voltammetry, UV/Vis spectrophotometric, HPLC, FT-IR, HPTLC, X-ray diffraction, and DSC equipment, researchers and scientists were able to design a method. Because of higher sample throughput and a higher level of automation, HPLC procedures were found to be the most sensitive and produced accurate and repeatable findings. In spectroscopic methods, methanol, ethanol, and a mixture of methanol and water were commonly used to prepare standard solutions. The problem arises because of the solvent used, which is typically buffers. While none of the studies used an appropriate statistical tool to improve the analytical process.

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