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COMPARATIVE REVIEW OF SPECTROSCOPIC AND CHROMATOGRAPHIC METHODS FOR SALBUTAMOL ANALYSIS IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL SAMPLES

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ABSTRACT

Salbutamol (albuterol) is an extensively administered agonist of the beta 2-adrenergic receptor also used in the treatment of bronchospasm of asthma and chronic obstructive pulmonary disease (COPD) **[1]**. Due to the pharmacological significance of salbutamol and its widespread use in clinical practice, the need of precise, accurate and validated methods of analysis has turned out to be significant to quality control and regulatory assurance **[4]**. High-Performance Liquid Chromatography (HPLC) is one of the most frequently used and, moreover, it is highly sensitive and reproducible. HPLC allows the successful quantification of both bulk and formulated form of drug **[1]**. High-Performance Thin Layer Chromatography (HPTLC) provides a low-cost and fast method of routine analysis, particularly well suited to qualitative identification, and stability studies **[1]**. Salbutamol is not volatile and so Gas Chromatography (GC) is not used commonly, but it becomes efficient with derivatization methods increasing its detectability in complex mixtures **[2]**. Liquid Chromatography-Mass Spectrometry (LC-MS) takes the benefits of both excellent separation capacity of liquid chromatography and the high specificity and sensitivity of mass spectrometry; thus, it is suitable in pharmacokinetic and bioanalytical applications of biological samples such as plasma and urine **[1]**. Ultraviolet (UV) spectroscopy is also an important measure used in the initial examinations because of its affordability, and simplicity of use. It is widely applied in ascertaining the concentration of

drugs in their formulas by calculating absorbance at its specific wavelength (λmax ~ 276 nm) **[1]**. This combination of analytical methodologies guarantees thorough analysis of the purity, contents, stability and possible degradation products of salbutamol. The various methods have their own merits which are applied in analysis depending on the requirements of the study, i.e., quality control routine or advanced pharmacokinetics research implementation, and therefore play their part in the proper therapeutic use of salbutamol **[3, 4]**.

Keywords: Spectroscopic And Chromatographic Methods, Salbutamol Analysis, Pharmaceutical Formulations, Biological Samples

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are respiratory diseases that impact the quality of life of millions of people and impose a high cost on healthcare systems **[4]**. Such conditions are marked by inflammation, constriction, and obstruction of the airways that cause symptoms of wheezing, limited pulmonary functioning, and shortness of breath **[4]**. Salbutamol belongs to several medications applied in the treatment of these conditions; however, it is a key element of pharmacotherapy because of its quick-acting bronchodilatory effect that produces a beneficial result almost immediately **[1]**. Salbutamol is a short-acting β2-adrenergic receptor agonist that relaxes bronchial smooth muscle and relieves acute bronchospasms in a brief period **[1]**. It has extensive applications in emergent and maintenance treatment and is generally utilized by inhalation, though oral and intravenous routes also exist **[1]**. Because of its therapeutic efficacy, along with its sensitivity to regulations—particularly under conditions of doping control—precise and dependable methods of analysis are a prerequisite for its quantification in both pharmaceutical formulations and biological samples **[2]**. Simple and cost-effective methods of analysis of a fixed-dose combination such as UV-Visible spectrophotometry can be used, especially in cases where validation has been made as per ICH (1). HPLC is one of the cornerstones of pharmaceutical analysis because of its high precision and resolution, as well as being suitable as a routine quality control method **[1]**. To perform trace-level detection in complex biological matrices such as urine and plasma, gas chromatography MS/MS (GC-MS/MS) and liquid chromatography MS/MS (LC-MS/MS) offer better sensitivity and specificity, and are therefore indispensable in bioanalytical and doping investigations **[2, 3]**. Such sophisticated methods allow revealing the presence of salbutamol even in nanogram amounts, meeting international standards like those established by the World Anti-Doping Agency (WADA) **[1]**.

Overall, the variety of analytical procedures established to examine salbutamol, their development, and validation highlight the importance of this drug in modern respiratory treatment, guaranteeing a high level of quality, safety standards, and regulatory compliance in today's pharmaceutical science **[1, 4]**.

STRUCTURE

A benzene ring with specific functional groups attached, with hydroxymethyl group, a hydroxyl group and an isopropyl- aminoethyl group

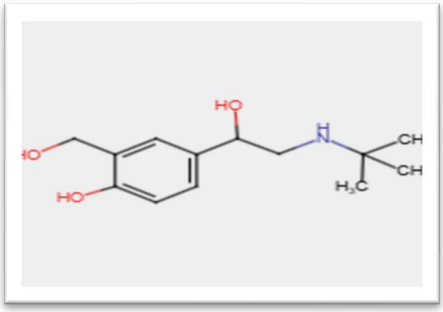

Figure 1: Structure of salbutamol

- Phenolic Ring: backbone consisted of a benzene ring with hydroxyl groups on it, which determines the hydrophilicity and receptor binding capability.
- Hydroxymethyl (-CH 2 OH): This group, bound to the aromatic ring improves water solubility as well as those related to pharmacokinetics
- Ethanolamine Side Chain Ethanolamine side chain attached to the aromatic ring, has a secondary alcohol and an amine group, essential in activation of 82-adrenergic receptors.
- Tert-Butylamino Group: The tert-butyl group connected to the nitrogen heightens β 2 selectivity compared to β 1 selectivity and, thereby, reduces cardiac side effects.

UV Spectroscopy for salbutamol

The estimated UV spectrophotometric multimethod is systematic and was developed to excel in the estimation of salbutamol sulphate despite the interference of proteins **[5]**. The desired principle used was the simple, correct and precise UV-Vis spectrophotometric method of analysis using the simultaneous equation approach which worked on the basis of two wavelengths—242 nm and 272 nm. These wavelengths were selected on the basis of values of maximum absorbance (λmax) of salbutamol at 244 nm and the spectral nature of salbutamol under various conditions **[5]**. Stock solutions of salbutamol sulphate were made up to standard concentrations in distilled water and the range of 1–10 µg/mL studied. This procedure showed linearity within this range in accordance with Beer-Lambert law **[5]**. It was able to determine drug concentration fairly accurately by use of simultaneous equations with absorptivity coefficients of the chosen wavelengths. The method was validated according to ICH guidelines with appropriate precision, linearity, and recovery. Sensitivity of the method was established by determining that the limit of detection (LOD) of salbutamol was 0.95 µg/mL and the limit of

quantification (LOQ) was 0.18375 µg/mL **[5]**.

With regard to a similar methodology to combat biological matrix interference, the derivative UV spectrophotometry technique was used to quantitatively determine salbutamol sulphate in bovine serum albumin (BSA) **[6]**. Albumin has been reported to affect standard zero-order UV absorbance measurements because of overlapping protein signals, especially UV-absorbing moieties such as flavins. The interference signals were minimized using second-order (2D) and fourth-order (4D) derivative spectrophotometric methods. The 2D technique was particularly useful at low levels of salbutamol concentrations—as little as 2 µg/mL could be quantified precisely in the presence of 1 µg/mL albumin with good linearity and precision **[6]**. At very low concentration levels, however, the 4D technique proved less reliable due to higher spectral noise. Nevertheless, the 4D method produced results comparable to 2D when 20 µg/mL of salbutamol was analyzed in the presence of up to 20 µg/mL of albumin **[6]**.

All in all, these derivative techniques provided accurate and interference-free measurement of salbutamol in biological specimens. These two UV-based analytical methods (conventional UV-Vis spectrophotometry with simultaneous equations and derivative spectrophotometry) together yield a strong and effective platform for accurate quantification of salbutamol sulphate. The former is optimal for pharmaceutical formulations because of its simplicity and reproducibility, whereas the derivative techniques are valuable in analysis of salbutamol in biomatrix form where protein interference may be problematic. Their combined use provides a complete solution for quality control, pharmacokinetics, and therapeutic drug monitoring of salbutamol in various types of samples **[5, 6]**.

HPLC (High Performance Liquid Chromatography) for salbutamol

Salbutamol sulphate is among the extensively quantified drugs within pharmaceutical formulations and biological samples using a series of validated HPLC methods, particularly reversed-phase HPLC. C18 reversed-phase columns were consistently used to separate the analytes, most commonly with dimensions of 250 × 4.6 mm and a particle size of 5 µm, which provided a balance between resolution and run time. The chromatographic systems were run isocratically to maintain constant mobile phase composition during analysis. Typical mobile phases included aqueous buffers such as phosphate buffer at acidic pH (3.0–4.0) or 50 mM ammonium acetate at neutral pH (7.0). Triethylamine was occasionally added to improve peak symmetry and reduce tailing. These buffers

were combined with acetonitrile in ratios around 80:20 v/v, which gave optimal separation and retention of salbutamol and related compounds. The mobile phase was generally delivered at a rate of 1.0 mL/min, ensuring reproducibility and consistent retention times across repeated injections. Detection of salbutamol varied depending on the application: UV detection was employed at 220–224 or 276 nm in pharmaceutical formulations, while fluorescence (excitation 276 nm, emission 310 nm) was used for biological assays. In certain cases, diode array detection provided additional spectral confirmation at 220 nm **[7, 8, 10]**.

In the bioanalysis of plasma and urine samples, solid-phase extraction (SPE) was a crucial step to improve purity, reduce protein interference, and maximize analyte recovery. The adoption of SPE enhanced sensitivity by minimizing matrix effects that interfere with direct injection of biological fluids. Internal standards such as terbutaline and bambuterol hydrochloride were commonly employed to correct for recovery efficiency and minor variations in chromatographic performance, ensuring reliability and reproducibility of quantification. For pharmaceutical dosage forms such as tablets, direct dilution and filtration were generally sufficient before injection, making sample preparation simpler than for biological fluids **[11, 12]**.

Calibration curves were constructed over application-specific ranges. For pharmacokinetic and doping-control studies, linear concentration ranges between 25 and 1000 ng/mL were employed, while pharmaceutical quality control studies extended calibration to the microgram per milliliter level. In advanced methods using diode array detection, linear ranges up to 50 µg/mL were validated, with limits of detection as low as 0.15 µg/mL, demonstrating remarkable sensitivity. Across all protocols, calibration curves exhibited excellent linearity, with correlation coefficients consistently above 0.998 **[8, 12]**.

Validation of these methods followed ICH guidelines. Accuracy was consistently high, with recovery values between 98% and 100%, indicating minimal bias. Precision was confirmed by relative standard deviation (RSD) values below 2%, demonstrating reproducibility across analysts and days. Suitability tests performed prior to sample analysis confirmed adequate system performance, with resolution > 2, tailing factors < 2, and theoretical plate counts > 2000, all indicative of efficient separation and peak symmetry. Robustness was also verified, as deliberate minor changes in pH, flow rate, or temperature did not significantly affect chromatographic performance. Sensitivity was a notable strength of these methods,

with limits of detection and quantification in the low nanogram per milliliter range, allowing reliable measurement of trace concentrations in biological fluids **[9, 11]**.

Taken together, these methodologies have demonstrated wide applicability in pharmaceutical, clinical, and regulatory settings. In drug quality control, validated HPLC assays were successfully applied to routine analysis of salbutamol in tablets either alone or in combination with other bronchodilators, ensuring correct dosage and product consistency. In clinical and pharmacokinetic studies, they were employed to quantify salbutamol in plasma and urine following inhalation or oral administration, generating key data for therapeutic monitoring, optimization of treatment, and bioequivalence studies. Furthermore, the sensitivity and reliability of the urine assays rendered them valuable for doping control, where detection of low-level excretion is critical. The combination of powerful chromatographic separation, reliable sample preparation, high sensitivity detection, and rigorous validation ensures that these HPLC techniques provide a robust platform for the accurate estimation of salbutamol across a range of applications **[7, 11, 12]**.

HPTLC (High Performance Thin Layer Chromatography) for salbutamol

High-Performance Thin Layer Chromatography (HPTLC) is an apt analytical technique that can be effectively used to analyze salbutamol sulphate, especially in quality control routine of pharmaceutical formulations; both in inhalation and tablet dosage forms. HPTLC has numerous merits as compared to traditional modes of chromatographic analysis, including speed, cost-efficiency, efficiency of sampling, and the capacity to consider several samples in parallel using one plate. It is performed with the utilisation of aluminium-backed silica gel 60 F254 plates which are thinly coated with a consistent stationary phase that permits improvement in both sensitivity and resolution. They are popular since these plates have good separation capability as well as reliable performance **[13, 17]**.

The analysis itself is started with putting the salbutamol sulphate standard sample and the test ones on the HPTLC plates as the thin bands. These bands are deposited with highly controlled, computer-controlled sample applicator devices like the CAMAG ATS (Automatic TLC Sampler) or the Linomat applicators. These tools allow the accurate and reproducible application of analyte in specific volumes and locations, and thus they minimize the risk of human error and increase precision **[17]**.

The plates are then usually washed with methanol before application is carried out to wash off any impurities on the surface and left to dry in an oven to provide clean active

surface to carry out the separation. Normally, the chromatographic separation of inhalable dosage forms is optimized by the mobile phase of methanol, ethyl acetate, toluene and ammonia in volumetric ratio of approximately 3:1:3:0.15 (v/v/v/v). This composition of the mobile phase is critically chosen so that efficient migration of salbutamol sulphate could take place on the basis of satisfactory separation among the excipients and other co-formulants used in the formulation. In these circumstances, salbutamol has a reproducible retention factor (Rf) of about 0.38 ± 0.02, and accurate Rf values aid in the simple identification of the compound and establish authenticity of the formulation under test **[16]**.

Growth of the chromatographic steps is done in twin-trough saturated development chambers which have been lined with filter paper and saturated using the mobile phase. Development may take place after 20 to 30 minutes of equilibrium inside the chamber, which provides a controlled and homogenous atmosphere providing even migrations of the solvent front. This is an important measure against variation in separation, and to enhance plate-to-plate reproducibility **[14, 17]**.

The plates are dried after development and scanned densitometrically, usually at or near 232–275 nm. Densitometric scanning determines the quantification of the drug by the area under the peak of the salbutamol band and strength of the band. The peak area analysis can be used to quantify the quantity of drug in every sample accurately. The construction of calibration curves is made with the help of standard solutions of salbutamol with the concentration within the range of 100–500 ng per spot, and the method has been proven to be linear within this range. Under the given conditions, the limit of detection (LOD) is about 40 ng per spot, whereas the limit of quantification (LOQ) equals to 112 ng per spot, which shows the sensitivity of the method to be good in terms of very frequent analyses **[13, 14, 16, 17]**.

LC-MS method for salbutamol

Liquid chromatography Mass spectrometry (LC-MS/MS) is possibly the most proficient and delicate procedure to be used in the quantification of salbutamol in multicomplex biological matrices, like plasma and urine. The normal procedure entails liquid–liquid extraction (LLE), usually with ethyl acetate, to separate salbutamol and an internal standard (e.g., acetaminophen or deuterated analogues) in the sample. The analytes thus extracted are separated by reverse-phase ion-pair chromatography on a C18 column with a mobile phase of acetonitrile and ammonium acetate (ratio of the mobile phase is about 30:70 v/v). The quantification is done under positive electrospray ionization (ESI)

following accurate multiple-reaction monitoring (MRM) transitions including m/z 240.2→148.1 in case of salbutamol **[18]**. This technique facilitates very low levels of quantitation (LLOQ)—as low as 0.02 ng/mL in plasma and approximately 1 ng/mL in urine—with superb levels of accuracy, precision, linearity, and repeatability which satisfy the strict needs of pharmacokinetic studies **[20]**. This protocol has been confirmed in studies that have used nebulized administration, or co-administration with such agents as clenbuterol, at broader dynamic ranges, 0.05 to 100 ng/mL in plasma and up to 135 ng/mL in urine, with reduced inter- and intra-assay variation (less than 8%) **[21]**. New UPLC approaches, however, share the major structures of extraction and determination and have been able to shorten analysis considerably to under 3.5 minutes, allowing high-throughput analysis in anti-doping and clinical applications **[20]**. In order to overcome ion suppression and matrix effects associated with ESI-MS, method development involves solid-phase extraction (SPE) efficiencies or optimal LLE clean-up, internal standards, isotopically labeled standards, and matrix-matched calibration curves **[22]**. Also, it has been found that researchers have used LC-MS/MS approaches (chiral stationary phases of teicoplanin or similar) to separate the R- and S-enantiomers of salbutamol at LLOQ near 0.3 ng/mL with recoveries of 84–92% **[19]**. By using these well-designed LC-MS/MS approaches, analysts are able to attain very high sensitivity and selectivity in analysis of salbutamol suitable to pharmacokinetic, forensic, doping-control and therapeutic monitoring purposes.

Gas Chromatography Method for salbutamol

Urine is one of the biological samples where determination of salbutamol is important in areas like clinical diagnostics, pharmacokinetics, and anti-doping analysis. A precise and reliable method has been designed to obtain this through a validated method of Gas chromatography-Mass spectrometry in tandem (GC-MS/MS). The technique is characteristically sensitive, specific and very robust, and enables the quantification of salbutamol with high precision at trace levels **[25]**.

Enzymatic hydrolysis will be the analytical procedure start point. When it is excreted in urine, salbutamol may appear in glucuronide conjugate form that is not even directly identifiable using GC-MS. These conjugates are hydrolyzed by an enzyme—β-glucuronidase—to liberate the free drug. This procedure makes sure that the whole amount of salbutamol is provided to be extracted and measured **[26]**.

The cleanup of the sample is achieved following the hydrolysis by using solid-phase extraction (SPE). It incorporates the

use of an XAD-2 resin column that helps the separation of the analyte in the complex biological matrix. With this resin, it is possible to retain salbutamol and release the unwanted biological materials. Distilled water is then used to wash the sample further to eliminate any form of contamination remaining and the methanol used to elute the target compound **[26]**.

In order to make the sample compatible with the GC analysis it is important to use derivatization. Salbutamol is not volatile or thermally stable in nature to inject into the GC. It uses a derivatization cocktail that is composed of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), iodo-trimethylsilane and dithioerythritol. It is this mixture which transforms salbutamol into a more volatile and thermally stable derivative that allows its gas chromatography. The samples are then placed in a 60 °C incubator after the reagents have been added to the samples to facilitate full reaction **[24]**.

After derivatization, a sample is introduced into gas chromatograph inlet in GC vials. The separation is performed on an HP Ultra 1 column (17 m × 0.22 mm × 0.11 μm) that is aimed carefully to separate the volatile components in high resolution. The total injected volume is around 2 μL, with the constraint of 1:11 split ratio; this has aided in regulating the sample volume that passes through the column and avoids overloading. Tandem mass spectrometry is utilized in Multiple Reaction Monitoring (MRM) mode in order to detect it. MRM can be accurately specific with high levels of specificity tracking preselected precursor and product ion transitions, with high specificity, and although other co-eluting materials are present, salbutamol can be specifically detected **[27]**.

GC-MS/MS technique features a relatively swift run time of about 13.67 minutes which makes it suitable mainly on a routine basis. The technique displays linearity within the concentration range of 250–2000 ng/mL and the limit of detection (LOD) as low as 10 ng/mL which reflects the sensitivity of the method. In addition, the recovery values are within the global parameters and the technique has high intra- and inter-day replicates making it reproducible and reliable among various runs of samples **[27]**. Overall, this method of GC-MS/MS is a potent salbutamol quantitation method in complicated biologic samples such as urine. Its mixture of enzymatic hydrolysis, efficient extraction, selective derivatization and selective MRM detection allows quantitation of the considerable amount of drug that could be below the threshold of detection. This lends it very well to be applied in clinical monitoring, therapeutic drug handling, and regulatory testing in the area of sports anti-doping **[25]**.

DISCUSSION

One of the simplest and cheapest techniques applied in the characterization of salbutamol, particularly pharmaceutical preparation is UV-Visible spectrophotometry. The technique utilises particular wavelengths- typically 244 nm and 272 nm- under the simultaneous equation method of determining the content of salbutamol sulphate in solution. The method has a positive linearity in an assay range of 1 to 10 microus per milliliter, according to the law of Beer-Lambert and is validated as proposed according to ICH concerning precision, accuracy, and sensitivity. Sub-techniques of the spectrophotometry such as 2D or 4D derivatives make use of derivative spectrophotometry that can overcome obstructions such as protein interferences with biological samples. They can be quantified precisely in the presence of interfering agents such as bovine serum albumin (BSA) with the 2D technique being especially sensitive at lower doses. UV-based procedure is hence used both in the routine analysis of formulations and the complicated analysis of biological samples depending on the degree of sophistication of the process involved.

HPLC is low detection limit, specific and repeatable method of analysis that has been used in pharmaceutical and bio- analytical analysis of salbutamol. HPLC generally uses a reversed-phase C-18 column and applied isocratic mobile phase consisting of aqueous buffer with acetonitrile which provide powerful resolution and specificity. Solid-phase extraction (SPE) to biological samples such as plasma and urine is frequently applied as a sample preparation procedure and usually terbutaline is utilised as an internal standard. The technique is stringently verified on important parameters such as linearity, precision, accuracy, specificity, LOD, LOQ, and robustness. It is also remarkably sensitive in cases of detector in trace level, as per the international regulatory set compliances like that of the International Council for Harmonisation (ICH). HPLC is one of the foundations of both standard quality control and more sophisticated pharmacokinetic analysis of salbutamol because it is reliable and scalable.

HPTLC is a sensitive and economical method utilized in the qualitative and quantitative determination of salbutamol, especially in drug formulations such as tablets, inhalers among others. This technique has a high throughput character since analysis of many samples can be performed on one silica gel covered plate. Analysis involves the employment of automated sample applicators to guarantee accuracy and repeatability whereas chromatographic separation occurs under a customized mobile phase program to guarantee the best division. Once developed,

salbutamol is quantified by densitometric scanning at 232 nm using the peak area. Having a linear range of 100-500 ng/spot and LOD and LOQ of about 40 ng and 112 ng/spot respectively, HPTLC appears as a possible alternative when it comes to fast and routine quality analysis. The most sensitive and potent method that can be used in the quantification of salbutamol on complex biological matrices like plasma and urine is LC-MS/MS. Usually, this is carried out through the liquid-liquid extraction (LLE) with solvent such as ethyl acetate and then reverse- phase chromatography mass and positive electrospray ionization. Multiple reaction monitoring (MRM) is used to detect the analyte such as m/z 240.2 148.1 in the case of salbutamol. LC-MS/MS is highly sensitive, as low levels of 0.02 ng/mL in plasma can be analyzed with such quantification and it is an ideal method of pharmacokinetic profiling, doping control, and therapeutic monitoring. The improvement that took place recently with ultra-performance liquid chromatography (UPLC) has also made the analysis experience speed rather than accuracy. Besides, the technique is compatible with chiral analysis to distinguish between enantiomers of salbutamol, which also increases its application in sophisticated research and forensics-related studies.Salbutamol is not naturally volatile or stable so that it could be easily analyzed by GC; however, GC-MS/MS proves to be effective with the introduction of derivatization. The method has the specific application in urine testing in anti-doping and clinical diagnostics. The analytical procedure is started by enzymatic hydrolysis, which transforms salbutamol glucuronide conjugates in the form of free drugs, and then followed by cleanup in an SPE procedure with XAD-2 resin. The formation of a volatile compound involves a derivatization step using MSTFA and similar agents so that it can be analyzed using GC. High specificity and sensitivity is achieved by the sample being injected and separated in the HP Ultra 1 column and then detected in MRM mode. The procedure provides the linearity at the range, 250-2000 ng/mL with LOD of approximately 10 ng/ml. It can be reproduced, precise and applicable to sport medicine and clinical pharmacokinetics regulatory testing.

Table 1								
Matrix	**Technique**	**Detection**	**Mobile phase / Solvent**	**Column / Instrument**	**Flow rate (mL/min)**	**Linearity (range)**	**LOD / LOQ**	**Reference**
Tablet formulation	**RP-HPLC**	**UV**	**Methanol : Water (0.1% TEA) 50:50 v/v**	**Nucleosil C18, 250 × 4.6 mm (C18)**	**0.7 mL/min**	**SLS 1–5 µg/mL**	**0.152 µg/mL/0.002 µg/mL**	**[7]**
Tablet formulation	**RP-HPLC**	**UV**	**Phosphate buffer (pH 3) : Acetonitrile 55:45 v/v**	**Inertsi10DS—3V (250 × 4.6 mm, 5 µm)**	**0.9 mL/min**	**0.5 – 3.0 µg/mL**	**0.021 µg/mL/ 0.065 µg/mL**	**[8]**
cough-syrup	**RP-HPLC**	**UV / PDA**	**Methanol : Acetonitrile (50:50 v/v)**	**C18 type**	**1.0 mL/min**	**–**	**0.036 µg/mL/-**	**[9]**
Salbutamol drug	**RP-HPLC**	**UV**	**ACN : 0.5% phosphoric acid (10:90) + TEA (~0.07%) — pH 2,.5**	**Brisa LC2 C18, 150 × 4.6 mm, 5 µm**	**0.7 mL/min**	**0.999 µg/mL**	**8.05 µg/mL/ 24.15 µg/mL**	**[10]**
Urine or bio samples	**GC–MS or GC–MS/MS with derivatization**	**MS / MS-MS**	**Derivatization reagents and extraction solvents; final injected solvent variable**	**HP Ultra-1 / HP-5 capillary columns**	**Carrier gas flow ~1.2–1.25 mL/min**	**250–2000 ng/mL**	**10 ng/mL; 100 ng/mL**	**[26]**
Human Urine	**GC–MS/MS with enzymatic hydrolysis, SPE (XAD2)**	**Tandem mass spectrometry**	**GC separation; organic solvent: TBME; derivatization reagent: MSTFA/IODO–TMS/DTE)**	**HP Ultra-1 column**	**1.25ml/min**	**250–2000 ng/mL**	**10 ng/mL; 100 ng/mL**	**[25]**
Pure drug & pharmaceutical formulations (tablets, syrup)	**Spectrophotometry with cloud point extraction (CPE)**	**UV-Vis spectroscopy**	**Methanol ; Triton X-100 surfactant system**	**UV-Vis spectrophotometer**	**—**	**2.5–17.5 µg/mL (before CPE), 1.0–5.5 µg/mL (after CPE)**	**LOD: 1.283 µg/mL (before), 0.041 µg/mL (after); LOQ: 4.234 µg/mL (before), 0.12 µg/mL (after)**	**[17]**
Combined dosage form	**UV-Vis spectroscopy (simultaneous equation method)**	**UV-Vis spectroscopy**	**Water**	**Double beam UV-visible spectrophotometer**	**—**	**1–5 µg/mL**	**LOD: 0.95 µg/mL; LOQ: 0.18375 µg/mL**	**[5]**
Salbutamol in presence of bovine albumin	**Derivative UV spectroscopy**	**UV spectroscopy**	**Water**	**UV-260 double beam spectrophotometer**	**—**	**1–80 µg/mL (in absence of interference); 1–8 µg/mL with fixed albumin (1 µg/mL)**		**[6]**

Human plasma	**LC-MS/MS**	**ESI+ MRM, m/z 240.1→148.1 (SAL), m/z 243.1→151.0 (IS)**	**Methanol–water with 10 mM ammonium acetate + 0.1% formic acid**	**Luna C18**	**0.5ml/min**	**0.100–10.0 ng/mL**	**LOQ: 0.100 ng/mL**	**[16]**
Human urine	**Reversed-phase HPLC with SPE**	**Fluorescence (Excitation 269 nm, Emission 312 nm)**	**MeCN:THF:MeOH:Buffer (10:8:14:68, v/v)**	**Zorbax ODS column (5 µm, 25 cm × 0.46 mm ID) with guard cartridge; Shimadzu autosampler + detector**	**1**	**25 – 300 µg/L**	**LOD 4.0 µg/L, LOQ 12.1 µg/L;**	**[11]**
Bulk drug & Pharmaceutical formulation	**RP-HPLC**	**UV detection at 224 nm**	**Methanol : Water (90:10, v/v)**	**C18 column (250 × 4.6 mm, 5 µm)**	**1**	**10 – 50 µg/mL (r^2 = 0.999)**	**LOD 0.207 µg/mL, LOQ 0.691 µg/mL**	**[12]**
Bulk drug & Pharmaceutical formulation	**HPTLC**	**Densitometric detection at 276 nm**	**n-Butanol : Acetic acid : Water (8:1:1, v/v/v)**	**Precoated silica gel 60 F254 TLC plates (10 × 10 cm, 0.2 mm thickness)**		**200 – 1200 ng/spot (r^2 = 0.999)**	**LOD 50 ng/spot, LOQ 150 ng/spot**	**[13]**
Liquid dosage forms (Ventorlin® & Asthalin® syrups)	**HPTLC**	**Densitometric scanning at 280 nm**	**Ethyl acetate : Methanol : Ammonia (25% w/v) (75:15:10, v/v/v)**	**TLC aluminium sheet precoated with silica gel 60 F254 (20 × 20 cm, 0.2 mm layer); Camag Linomat IV applicator & TLC Scanner 3**	**–**	**200 – 1000 ng/spot (r^2 = 0.997)**	**LOD: 70 ng/spot; LOQ: 100 ng/spot**	**[14]**
Human plasma & urine	**HPTLC with absorption microdensitometry at 650 nm (indoaniline dye derivative)**	**Chloroform–ethyl acetate (60:40, v/v)**	**Merck glass-backed silica gel 60 HPTLC plates**	**Camag TLC horizontal development chamber; densitometer**	**–**	**Plasma: ~1–10 ng/mL Urine: ~30–700 ng/mL**	**1 ng/mL 20 ng/mL**	**[15]**

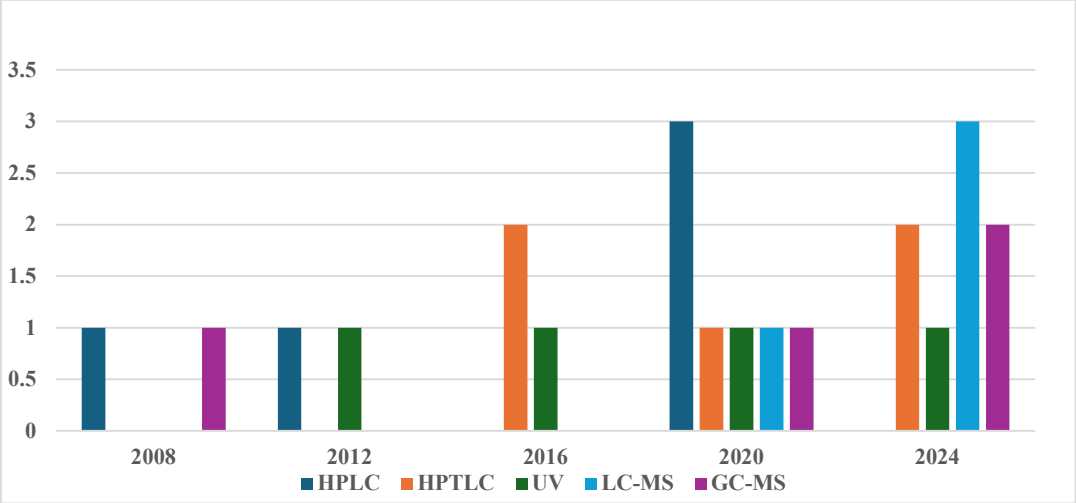

Figure 2: Distribution of analytical methods reported for determination of salbutamol

COCLUSION:

Numerous methods of various complexity have been established and validated to measure salbutamol (both in pharmaceutical products and in bio fluids) correctly: by using simple UV-visible spectrophotometry to challenging LC-MS/MS. Each technique has its own unique benefits: UV-based are cost effective and used routinely, HPLC and HPTLC are rugged, precise and scalable quality control applications, LC-MS/MS offers ultra-trace level sensitivity required in pharmacokinetics, doping and forensics and GC-MS/MS is highly specific when derivatized in a complex setup like urine. Method selection is based on the desired analytical goals, the complexity and the sensitivity demands of the sample. Taken together, these methods will make sure that quality, safety, and regulatory standards are observed, which will make salbutamol therapeutic use effective and responsible

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CONFLICT OF INTEREST:

The authors declare that NO conflict of interest among us.

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