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## Original Article

# Simultaneous Estimation of Salbutamol Sulphate and guaiphenesin in their Combined Liquid Dosage Form by HPTLC Method

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ABSTRACT

A simple, specific, sensitive and validated high-performance thin layer chromatographic (HPTLC) method was developed for the simultaneous analysis of Salbutamol sulphate and Guaiphenesin. Spectro-densitometric scanning-integration was performed at an absorbance wavelength 280 nm. A TLC aluminium sheet pre coated with silica gel 60 $F_{254}$ was used as the stationary phase. The mobile phase system containing Ethyl acetate: Methanol: Ammonia (25% w/v) (75: 15: 10 v/v) gave a good resolution of Salbutamol sulphate and Guaiphenesin with $R_f$ values of 0.47 and 0.65, respectively. The calibration plot of Salbutamol sulphate exhibited good linear regression relationship (r = 0.9987) over a concentration range of 200-1000 ng/spot. The calibration plot of Guaiphenesin exhibited good polynomial regression relationship (r = 0.9997) over a concentration range of 10-50 µg/spot. Detection and quantitation limit was found to be 70 ng and 100 ng respectively, for Salbutamol sulphate and 30 ng and 50 ng, for Guaiphenesin. The proposed method was used for determination of both drugs in Ventorlin and Asthalin Syrup containing Salbutamol sulphate and Guaiphenesin with satisfactory precision (Intraday) [2.67-4.46% for Salbutamol sulphate and 2.39-4.42% for Guaiphenesin] and accuracy [100.97± 0.50% and 100.45 ± 0.58% RSD, for Salbutamol sulphate and Guaiphenesin respectively]

**Key words:** Salbutamol sulphate Guaiphenesin, Calibration, polynomial regression precision

## 1. INTRODUCTION

Salbutamol sulpahte (SAL) is the selective prototypic $\beta_2$-adrenoceptor agonist. It is used as an anti-asthmatic in the treatment of bronchial asthma, bronchospasm, in the patients with reversible obstructive airway and in prevention of exercise induced bronchospasm. [1-3] It may be used in uncomplicated premature labour. SAL

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is chemically (*RS*)-1-(4-hydroxy-3-hydroxy- methyl phenyl)-2-(*tert*-butyl amino) ethanol sulphate. [2, 3] Guaiphenesin (GUA) is used as an expectorant in the symptomatic management of coughs associated with the common cold, bronchitis, pharyngitis, influenza, measles etc. It is chemically *(RS)*-3-(2-methoxyphenoxy)-1,2- propanediol. SAL and GUA combinations are available in the market for the respiratory disorders where bronchospasm and excessive secretion of tenacious mucus are complicating factors, for example bronchial asthma, chronic bronchitis & emphysema. Chemical structures of GUA and SAL are shown in Figure 1.

SAL (API) is official in the Indian Pharmacopoeia, [2] British Pharmacopoeia [4], and US Pharmacopoeia [5], and SAL syrup and tablets are official in British Pharmacopoeia [4]. GUA (API) is official in the Indian Pharmacopoeia [2], British Pharmacopoeia [4], and US Pharmacopoeia [5], and GUA tablets, capsules and injection are also official in US Pharmacopoeia. [5] However, the combination of SAL and GUA is not official in any pharmacopoeia. Several methods have been reported in literature for individual estimation of the drugs but very few methods have been reported for simultaneous estimation of SAL and GUA in combined dosage form, which includes chemo metrics-assisted spectrophotometry [6], Electro kinetic chromatography and Gas chromatography-Mass spectrometry [7] and Micellar electrokinetic chromatography [8]. HPLC, though accurate and precise method, is time consuming, costly and requires skilled operator. Therefore the aim of this study was to develop and validate simple, specific, inexpensive, rapid, accurate and precise High Performance Thin Layer Chromatography (HPTLC) method for simultaneous estimation of SAL and GUA in their combined dosage form. The proposed method was successfully applied to two marketed cough syrups Ventorlin® and Asthalin® and the contents were determined without any interference of excipients.

## 2. MATERIALS

### *2.1 Reagents and Materials*

*(a) Solvents*: Analytical reagent grade Ethyl acetate (Finar Chemicals, India) and methanol (RFCL Limited, India) and ammonia (25% w/v) (s. d. Fine Chem Limited, India); Iso propyl alcohol (s. d. Fine Chem Limited, India); Sodium bicarbonate (s. d. Fine Chem Limited, India)

*(b) Standards*: SAL and GUA were a gift sample from Preet Pharma, Gujarat, India.

(c) Ventorlin® syrup (GSK Pharmaceutical Ltd, India) – Batch 02053, labeled 2 mg SAL and 100 mg GUA in each 5 ml of syrup, were purchased commercially.

(d) Asthalin® syrup (Cipla Pharmaceuticals, Mumbai, India) – Batch 060305, labeled 2 mg SAL and 100 mg GUA in each 5 ml of syrup, were purchased commercially.

### *2.2 Apparatus*

*(a) HPTLC Plate*: 20×20cm, percolated with silica gel 60 $F_{254}$, 0.2 mm layer thickness ( E.Merck, Germany)

*(b) Spotting device*: Linomat IV Semiautomatic sample applicator (Camag, Switzerland)

*(c) Chamber*: Twin trough chamber for 20 × 10 cm (Camag)

*(d) Densitometer*: TLC Scanner-3 linked to win CATS software (Camag). Scanner mode- absorbance-reflectance; Scanning Wavelength: 280 nm; lamp: Deuterium; measurement type: remission; measurement mode: absorption; detection mode: automatic. Scanner setting- Slit dimension: 3.00 × 0.1 mm

*(e) Syringe*: 100 µl (Hamilton, Switzerland)

*(f) Analytical balance*: Shimandzu Libror AEG – 220 balances

## 3. METHODS

### *3.1 Preparation of SAL and GUA standard solutions*

Stock solution of SAL (equivalent to 2 mg/ml) was prepared by dissolving 20 mg SAL pure substance in 10 ml methanol. Working stock solution of SAL (equivalent to 0.2 mg/ml) was prepared by transferring 1.0 ml of above stock solution in 10.0 ml methanol. Stock solution (10 mg/ml) of GUA was prepared by dissolving 100 mg GUA pure substance in 10.0 ml methanol, separately. These solutions were stored under refrigeration at $4^0$C. A mixture of the drugs was prepared by transferring 1.0 ml of stock solutions of each compound to 10 ml volumetric flask and diluting to volume with methanol. (Final concentrations of SAL, 0.02 mg/ml and GUA, 1 mg/ml)

***3.2 Preparation of calibration curve***

10-50 micro liters of standard solutions of combined standard solution of SAL (0.2, 0.4, 0.6, 0.8 and 1.0 µg/spot) and GUA (10, 20, 30, 40, and 50 µg/spot) and 2 sample solutions (20 µl; corresponding to 0.4 µg SAL and 20 µg GUA/spot) were applied onto a pre coated HPTLC plate using the semiautomatic sample spotter (bandwidth: 3 mm, distance between the tracks: 5 mm). The plate was developed to a distance of 45 mm in a HPTLC chamber containing the mobile phase, i.e., Ethyl acetate-methanol-ammonia (7.5+1.5+1.0 v/v/v), at 25 ± 2 $^0$C. The plate was dried at room temperature. The substances on the silica gel layer were identified densitometrically at 280 nm. The chromatograms were scanned at 280 nm with slit dimensions of 0.1 mm × 3 mm; 400 nm was used as the reference wavelength for all measurements. Concentrations of the compounds chromatographed were determined from changes in the intensity of diffusely reflected light. Evaluation was via peak area with linear regression for SAL and polynomial regression for GUA.

***3.3 Preparation of sample solutions***

A 5 ml aliquot of the Commercial syrup (Ventorlin® or Asthalin®) was transferred into 10 ml volumetric flask. The volume was adjusted with methanol. From this solution, 2 ml was pipetted and transferred into another 10 ml volumetric flask. The volume was adjusted to the mark with methanol. The methanolic solution was used for chromatographic analysis. (SAL 20 µg/ml and GUA 1 mg/ml)

***3.4 Method validation***

The method was validated in compliance with International Conference on Harmonization guidelines. [9]

*(a) Specificity*._ The specificity of the method was established by comparing the chromatograms and measuring the peak purities of SAL and GUA from standard and sample solutions of liquid dosage forms. The peak purity of SAL and GUA were assessed by comparing spectra obtained at the peak start (S), peak middle (M) and peak end (E) of a spot. Correlation between SAL and GUA spectra from standard and sample was also obtained.

*(b) Accuracy*._ The accuracy of the method was determined by standard addition method and calculating the recoveries of SAL and GUA . Prequantified sample stock solution of SAL and GUA ( 1 mL equivalent to 200µG/ml of SAL and 10mg/ml of GUA) was transferred into a series of 10 mL volumetric flasks. Known amounts of standard stock solution of SAL(0, 1,2 and 3 mL equivalent to 200, 400, 600 ng/spot ) and GUA ( 0, 1, 2 and 3 mL equivalent to 0, 10,20 and 30 µg/spot) were added to this prequantified working sample solutions and diluted up to the mark with methanol. Each solution (10 µL) was applied on plates in triplicate. The plates were developed and scanned as described above, and the recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the calibration curves.

*(c) Precision*._ The intraday and interday precision of the proposed method was determined by estimating the

corresponding responses five times on the same day and on five different days over a period of one week for three different concentrations of SAL (200, 400, 600 ng/spot) and GUA (10, 20, 30 µg/spot). The repeatability of sample application was checked by repeatedly measuring the area of seven spots having same concentration of SAL (400ng/spot) and GUA (20 µg/spot) applied on the same plate, while the repeatability of measurement of peak area was checked by repeatedly measuring the area of one spot of SAL (400ng/spot) and GUA (20 µg/spot) for seven times. The results were reported in terms of RSD.

*(d) LOD and LOQ*._ The LOD and LOQ of SAL and GUA were calculated by preparing a series of solutions containing decreasing concentrations of SAL from 0.02 to 0.004 mg/ml and GUA from 1 to 0.001 mg/ml by appropriate dilution of the stock solutions of these drugs (SAL 0.02 mg/ml and GUA 1 mg/ml).

*(e) Robustness*._ The robustness of the method was studied by changing the composition of the mobile phase by ± 0.2 mL of organic solvent, development distance by ±1 cm, and temperature by ± 2˚C.

### *3.4 Determination of SAL and GUA in Liquid Dosage Form*

The responses of sample solutions were measured at 280 nm for quantification of SAL and GUA by the proposed method. The amount of SAL and GUA present in the sample solutions were determined by fitting the responses into the regression equation of the calibration curve for SAL and GUA, respectively.

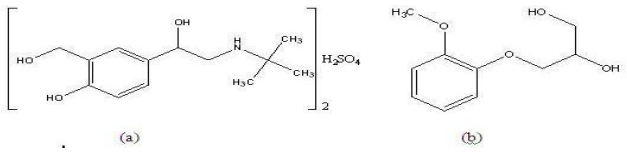


**Fig 1: Chemical Structures of (a) SAL and (b) GUA**

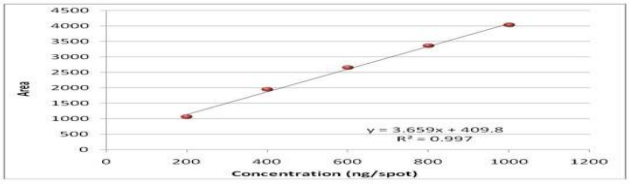


**Fig 2: Calibration curve of SAL**

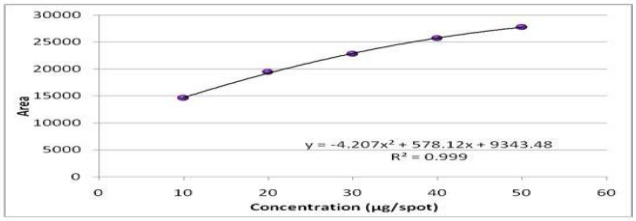


**Fig 3: Calibration curve of GUA**

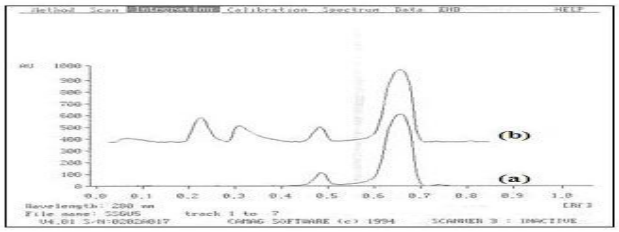


**Fig 4: (a) HPTLC chromatogram showing separation of SAL and GUA in their combined standard solution at 280 nm , with Rf 0.47 and 0.65, respectively. (b) Chromatogram showing the separation of SAL and GUA in Ventorlin Syrup**

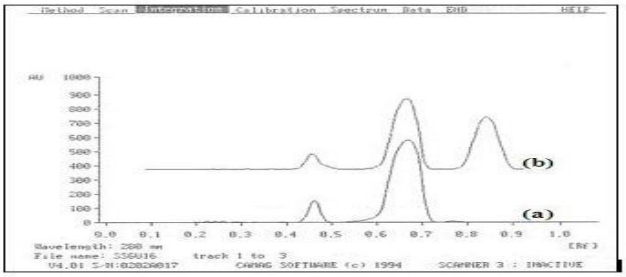


**Fig 5: (a) HPTLC chromatogram showing separation of SAL and GUA in their combined standard solution at 280 nm , with Rf 0.47 and 0.65, respectively. (b) Chromatogram showing the separation of SAL and GUA in Asthalin Syrup.**

**Table 1: Data indicating various validation parameters of the developed method**

| Parameters | SAL | GUA |
|---|---|---|
| Linearity range (n=6) | 200-1000 ng/spot | - |
| Polynomial regression range (n=6) | - | 10-50 µg/spot |
| Linearity equation | y = 3.659x + 409.8 | - |
| Polynomial regression equation: | - | $y = -4.207x^2 + 578.12x + 9343.48$ |
| Correlation coefficient ($r^2$ >0.99) | 0.997 | 0.999 |
| Limit of Detection | 70 ng | 30 ng |
| Limit of Quantification | 100 ng | 50 ng |
| Specificity | Specific | Specific |

**Table 2: Results of precision study for SAL and GUA determination by the proposed HPTLC method**

| Precision (RSD, %) | SAL | GUA |
|---|---|---|
| Intraday (n=5) | 2.56-4.57 | 1.95-4.20 |
| Interday (n=5) | 2.67-4.46 | 2.38-4.42 |
| Repeatability (n=7) | 1.86[a] | 1.50[a] |
| | 0.47[b] | 0.18[b] |

[a] Repeatability of sample application.
[b] Repeatability of measurement of peak area.

**Table 3: Data for the recovery study of SAL and GUA**

| Drug | Amount taken | Amount added | Recovery, % | RSD, % |
|---|---|---|---|---|
| SAL, ng/spot | 200.44 | 0 | 100.66 | 0.48 |
| | 200.44 | 200.44 | 103.91 | 0.39 |
| | 200.44 | 400.88 | 100.99 | 0.52 |
| | 200.44 | 601.32 | 98.66 | 0.56 |
| | 200.44 | 801.76 | 100.65 | 0.59 |
| GUA, µg/spot | 10 | 0 | 101.82 | 0.67 |
| | 10 | 10 | 100.08 | 0.45 |
| | 10 | 20 | 100.38 | 0.80 |
| | 10 | 30 | 97.25 | 0.52 |
| | 10 | 40 | 102.73 | 0.47 |

**Table 4: Analysis results for SAL and GUA liquid dosage forms by the proposed HPTLC methods (n=5)**

| Formulation | SAL | | | GUA | | |
|---|---|---|---|---|---|---|
| | Labeled amount, mg | Amount found, mg | SAL, % ± SD | Labeled amount, mg | Amount found, mg | GUA, % ± SD |
| Ventorlin | 2 | 2.123 | 106.15±4.43 | 100 | 99.17 | 99.17±4.94 |
| Asthalin | 2 | 2.201 | 110.05±3.64 | 100 | 105.17 | 105.17±4.64 |

## 4. RESULTS AND DISCUSSION

Since both SAL and GUA have nearly same wavelength maxima, interference becomes prominent in UV-Visible spectrophotometry. Also the estimation of any component at its null point is not that much reliable as the estimation at maximum wavelength. Consecutively for highly specific methods like HPLC and HPTLC, physical separation of those substances is usually necessary before quantitative determination of those substances. So, attempt has been made to develop a validated separation technique for the separation of SAL and GUA in the mixture by HPTLC. The chromatographic conditions were adjusted in order to obtain an efficient and simple routine method. Different mobile phases were tried for the separation of the above substances. The optimized solvent system was Ethyl acetate: methanol: ammonia (25 %w/v) (7.5:1.5:1;v/v/v). The Rf values were found to be 0.47 for SAL and 0.65 for GUA. (Figure 2)

The maximum wavelength of SAL was found to be 279nm-280nm and the maximum wavelength of GUA was 274nm-275nm. As both compounds have nearly same $\lambda_{max,}$ 280 nm was selected for simultaneous scanning of SAL and GUA. In this way, SAL can be detected at low concentrations in the presence of GUA at high concentrations.

### *4.1 Preparation of calibration curve*

As the concentration range of SAL is from 200 to 1000 ng, direct proportionality (linearity) of the concentration with its absorbance was obtained. Linear regression analysis is applied to analyze calibration curve of SAL. The equation is y = 3.659x + 409.8 (Figure 2)

With the objective to allow simultaneous analysis by developing method in wider concentration range, non-linear regression analysis mode was utilized for estimation of GUA. Polynomial regression mode is applicable if wide concentration ranges (1:50 to 1:100) are worked out and with high amount of substance measured in non-linear detector range. The equation for calculation is $y = -4.207x^2 + 578.12x + 9343.48$ (Figure 3)

### *4.2 Method Validation*

*Specificity._* The excipients present in the liquid dosage form did not interfere with the chromatographic responses of SAL and GUA as the peak purities r(S, M) = 0.997 and r (M, E) = 0.9996 for SAL and r(S, M) = 0.997 and r(M,E) = 0.9996 for GUA. Also, good

correlation (r= 0.9999 for SAL and 0.9998 for GUA) were obtained between standard and sample spectra.

*Accuracy.*_ The mean recoveries obtained for SAL and GUA were 100.07± 0.49% and 100.04 ± 0.63% RSD, respectively. The accuracy results are shown in Table 2

*Precision.*_ The values of RSD for intraday and interday variations were found to be in the range of 2.56-4.57% and 2.67-4.46% for SAL and 1.95-4.20% and 2.39-4.42% for GUA. RSD for repeatability of sample application were found to be 1.86 and 1.48 for SAL and GUA respectively, while the repeatability of peak area measurement was 0.47 and 0.18% for SAL and GUA respectively.

*LOD and LOQ.*_ The LOD and LOQ were 70 and 100 ng for SAL and 30 and 50 ng for GUA.

*Robustness.*_ The method was found to be robust, as the results were not significantly affected by deliberate but slight variation in the method parameters.

***4.3 Determination of SAL and GUA in Liquid Dosage Form***

The proposed HPTLC method was applied successfully for the determination of SAL and GUA in liquid dosage form. The results obtained for SAL and GUA were comparable with the corresponding labeled claim values. (Table 4)

## 5. CONCLUSIONS

Due to the absence of an official method for this binary mixture, the high-performance thin layer chromatographic method proposed in this article could represent an alternative to chemo metrics-assisted spectrophotometry, Electro kinetic chromatography and Gas chromatography-Mass spectrometry previously published. This method has been validated for linearity, precision, accuracy, and specificity, and has proved to be convenient and effective for the quality control of SAL and GUA in marketed syrups, without any interference of excipients.

## 6. ACKNOWLEDGEMENTS

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