Spectrophotometric Determination of Salbutamol Sulphate by Coupling with Diazotized 5-Amino-2-chlorobenzotrifluoride – Application to Pharmaceutical Preparations

Nabeel S. Othman                               Ali M. Asaad
Department of Chemistry/ College of Science/ University of Mosul

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ABSTRACT
A simple, rapid and sensitive spectrophotometric method for the estimation of trace amounts of salbutamol sulphate (SBS) in pharmaceutical preparations has been proposed. The method is based on the coupling reaction of the intended compound with diazotized 5-amino-2-chlorobenzotrifluoride in alkaline medium and in the presence of Triton X-100 to form a yellow-orange dye that shows maximum absorption at 467 nm. Beer’s law is obeyed over the range 5 – 300 µg / 10 ml (i.e.,0.5-30 ppm) with a molar absorptivity of $4.20 \times 10^4$ l.mol$^{-1}$.cm$^{-1}$ and Sandell’s sensitivity index of 0.0139 µg.cm$^{-2}$, a relative error of +1.08 to +3.46 % and a relative standard deviation of ±0.76 to ±3.50 %, depending on the concentration. The method has been applied to estimate salbutamol sulphate in syrup, tablet and ventolin.

Keywords: Salbutamol sulphate, diazotized 5-amino-2-chlorobenzotrifluoride, diazo-coupling, Triton X-100, spectrophotometry

INTRODUCTION
Salbutamol sulphate is the racemic form of salbutamol, it is an important drug, indicated for the relief of severe bronchial spasm associated with asthma (Fattah et al., 1998). Salbutamol
sulphate is \([\text{di[(RS)-2-(1,1-dimethyl) ethylamino-1-[4-hydroxy-3-(hydroxymethyl)phenyl] ethanol] sulphot}]\) a white or almost white, crystalline powder, freely soluble in water, slightly soluble in alcohol and in ether, very slightly soluble in methylene chloride and has the following structure (British Pharmacopoeia, 2007).

![Chemical Structure](image)

\[\text{M.wt } = 576.7 \text{g/mol}\]

**Salbutamol sulphate**

The assay of Salbutamol sulphate officially listed in British Pharmacopoeia describes a potentiometric titration procedure (British Pharmacopoeia, 2007).

Several analytical methods for the determination of salbutamol were developed and reported such as TLC (Dave et al., 2011), GC and GC-MS (Caban et al., 2011; Wang et al., 2010), cyclic voltammetry (Ganjali et al., 2005), adsorptive stripping voltammetry on a carbon paste electrode (Attaran et al., 2012), Capillary electrophoresis coupled with electrochemiluminescence (Bao et al., 2012), flow injection method (Al-Abachi and Subhi, 2013), HPLC and RP-HPLC (Mukesh and Ranjit, 2011; Pai et al., 2009; Ghulam et al., 2009; Li et al., 2010), HPLC-chemiluminescence (Zhang et al., 2011), solid phase extraction (SPE)-HPLC (Liu and Wang, 2011; Yan et al., 2012), and fluorescence (Tang et al., 2010).

Various spectrophotometric procedures have been reported for the determination of salbutamol sulphate as a pure and in dosage form using different reagents such as diazotized sulphanilic acid (Othman and Zakaria, 2004), diazotized p-nitroaniline (Othman and Hamdoun, 2005), Folinciocalteu (Basavaiah and Prameela, 2003), iron(III) with ferricyanide (Kanakapura and Huiikal, 2003), hydroxyl ammonium chloride in alkaline medium (Manasa, 2013), p-phenylenediamine in presence of sodium meta periodate (Al-Hafith, 2005), 2,6-dichloroquinonechlorimide and 7,7,8,8-tetrayanoquindimethan (Mohamed et al., 2002), and sodium hydroxide (Eswarudu et al., 2012). The present work describes a spectrophotometric method for the determination of salbutamol sulphate. The method is based on coupling the salbutamol sulphate with diazotized 5-amino-2-chlorobenzotrifluoride to form a stable and soluble azo dye product.

**THE EXPERIMENTAL**

**Apparatus:**

The spectrophotometric measurements were carried out on Jasco V-630 using 1cm glass cells.

**Reagents**

All chemicals used are of the highest purity available.

**Salbutamol sulphate solution, 100 µg. ml\(^{-1}\).**

A 0.0100g amount of salbutamol sulphate was dissolved in distilled water, then the volume was completed to 100 ml in a volumetric flask with distilled water.

**Diazotized 5-amino-2-chlorobenzotrifluoride solution 5x10\(^{-3}\) M.**

This solution was prepared daily by dissolving 0.0978 g of 5-amino-2-chlorobenzotrifluoride in 10 ml of ethanol then a 3.0 ml of concentrated HCl was added, followed by dilution to 80 ml with distilled water, then the solution was transferred into a 100-ml volumetric flask and cooled to (0 - 5) °C in an ice-bath, a 0.0345 g of sodium nitrite was added then stirred vigorously, after 5 minutes the solution was made up to 100 ml with cooled distilled water and stored in a dark bottle.
Sodium hydroxide solution, 2 M.
This solution was prepared by the dilution of the concentrated volumetric solution (1Ampoule, Fluka) to 500 ml with distilled water and then transferred to a plastic bottle.

Triton X-100, (1%).
A 1.0 g of Triton X-100 was dissolved in 100 ml of distilled water.

Interferences solutions, 1000 µg.ml$^{-1}$.
These solutions were prepared by dissolving 0.1 g of each of them in 100 ml of distilled water.

Butadine syrup solution, 100 µg.ml$^{-1}$.
This solution was prepared by diluting 25 ml of butadine syrup (2 mg salbutamol sulphate per 5 ml) to 100 ml with distilled water in a volumetric flask.

Butadine tablets solution, 100 µg.ml$^{-1}$.
Finely 5 powdered tablets of butadine drug (each tablet contains 2 mg salbutamol sulphate) were dissolved in 80 ml of distilled water, and the solution was shaked and warmed. The solution was filtered into a 100-ml volumetric flask, the residue was washed with distilled water and diluted to volume with distilled water to obtain 100 mg/l salbutamol sulphate.

Solution, 100 µg.ml$^{-1}$.Salb. Vent.
A 2 ml of salb. vent. (5mg salbutamol sulphate per 1ml) diluted to 100 ml with distilled water in a volumetric flask to obtain 100 µg.ml$^{-1}$ salbutamol sulphate solution.

RESULTS AND DISCUSSION

Principles of the method
The method included the following steps:
- Preparation of diazotized 5-amino-2-chlorobenzotrifluoride.

Optimum reaction conditions
The effect of different factors on the formation of the colored dye is investigated and the reaction conditions have been optimized.

For the subsequent experiments, 100 µg of salbutamol sulphate is taken in 10 ml as a final volume and absorbance measurements are achieved directly after dilution with distilled water at 451.5 nm.
Selection of diazotized reagent

Some diazotized reagents (1 ml of 5x10^{-3} M) have been selected for optimum conditions. The results in Table (1) show that 5-amino-2-chlorobenzotrifluoride gives the highest intensity with high value of color contrast, therefore, it has been selected for the subsequent experiments.

Table 1: Selection of diazotized reagent

| 1 ml of (5x10^{-3}M) diazotized reagent | λ_{max}(nm) | ∆λ_{max}(nm)* | Absorbance |
|----------------------------------------|-------------|----------------|------------|
| 5-Amino-2-chlorobenzotrifluoride        | 451.5       | 141.5          | 0.3068     |
| 3-Aminobenzotrifluoride                | 437         | 138            | 0.2023     |

- ∆λ_{max}, = \lambda_{maxS} - \lambda_{maxB}  where S = Sample, B = Blank

Optimum amount of diazotized 5-amino-2-chlorobenzotrifluoride reagent

The effect of different amounts (1.0 – 6.0 ml) of 5-amino-2-chlorobenzotrifluoride (5x10^{-3} M) reagent on the absorbance of solutions containing different amounts of salbutamol sulphate (50-300 µg/10ml) has been studied; the results in Table (2) show that 4 ml of diazotized 5-amino-2-chlorobenzotrifluoride reagent gives highest absorbance and with best determination coefficient (R^2 = 0.999), therefore this volume of diazotized reagent was selected for the subsequent experiments.

Table 2: The optimum amount of diazotized reagent

| ml of (5x10^{-3}M) diazotized reagent solution | Absorbance / µg of salbutamol sulphate | R^2 |
|-----------------------------------------------|---------------------------------------|-----|
|                                               | 50 | 100 | 200 | 300 |
| 1.0                                           | 0.1701 | 0.3068 | 0.5642 | 0.6468 | 0.947 |
| 2.0                                           | 0.1963 | 0.4054 | 0.6835 | 1.0793 | 0.994 |
| 3.0                                           | 0.2121 | 0.3984 | 0.7602 | 1.3131 | 0.988 |
| 4.0                                           | 0.2272 | 0.4596 | 0.9413 | 1.3872 | 0.999 |
| 5.0                                           | 0.2200 | 0.4272 | 0.9258 | 1.3430 | 0.998 |

Effect of pH

Salbutamol sulphate undergoes complete diazo-coupling reaction in alkaline medium (Othman and Zakaria , 2004), so that several bases have been tested Table (3) for optimum conditions (1 ml of 2 M of each base was added).

Table 3: Selection of base

| 1ml of Base (2 M) | λ_{max}(nm) | ∆λ_{max}(nm) | Absorbance | Final PH |
|-------------------|-------------|--------------|------------|---------|
|                   |             |              | Sample Vs. Blank | Blank Vs.DW. |
| NaOH              | 452         | 143          | 0.4775    | 0.0746  | 12.01  |
| KOH               | 361.5       | 46           | 0.4724    | 0.1877  | 12.23  |
| NaHCO₃            | Turbid      |              |            |         |       |
| Na₂CO₃            | Turbid      |              |            |         |       |

The experimental data in Table (3) showed that the reaction needs a strong alkaline medium and NaOH gives a highest sensitivity with best color contrast, therefore it has been fixed for the subsequent experiments.
The optimum amount of sodium hydroxide

The results in Table (4) indicate that 1ml of NaOH (2 M) gives the highest intensity of the colored dye and the value of determination coefficient (0.999), therefore this volume has been recommended for the subsequent experiments.

| ml of NaOH (2M) | Absorbance / µg of salbutamol sulphate | Determination coefficient (R²) |
|-----------------|----------------------------------------|-------------------------------|
| 0.5             | 0.0338 0.0610 0.0882 0.2092 0.2092     | 0.903                         |
| 0.8             | 0.2203 0.4821 1.0587 1.5800           | 0.999                         |
| 1.0             | 0.2384 0.5056 1.0518 1.5946           | 0.999                         |
| 2.0             | 0.2342 0.5001 0.9011 1.1977           | 0.987                         |
| 3.0             | 0.2415 0.3838 0.8325 1.0311           | 0.974                         |

Effect of surfactant

The effect of several types of surfactants with different orders of addition on color intensity and color contrast of the dye has been investigated. (Table 5 and Fig. 1).

| Order of addition | Absorbance/ 1 ml of surfactant solution |
|-------------------|----------------------------------------|
|                   | CTAB (1x10⁻³M) | SDS (1%) | Triton X-100 (1%) |
|                   | Abs.  λmax.(nm) | Abs.  λmax.(nm) | Abs.  λmax.(nm) |
| I                  | 0.4563 440.5 | 0.5244 452 | 0.7033 466.5 |
| II                 | 0.4895 441 | 0.6022 451.5 | 0.7090 467 |
| III                | 0.5016 438 | 0.5138 452.5 | 0.6045 467 |

Note: Absorbance = 0.5259 without surfactant and λmax = 451.5 nm
I. Salbutamol sulphate (S) + surfactant (C) + Reagent (R) + NaOH (B)
II. S + R + C + B
III. S + R + B + C

Fig. 1: The effect of Triton X-100 on absorbance (order II )
A-Sample with Triton X-10
B-Sample without Triton X-100

The results in Table (5) and (Fig. 1) indicate that the addition of Triton X-100 in order (II) increases the intensity of the formed dye and color contrast from 141.5 nm To 158 nm, therefore it has been recommended in subsequent experiments.
The optimum amount of Triton X-100

From the results in Table (6), it was found that 1 ml of (1%) TritonX-100 solution was adequate for the maximum absorbance, therefore it has been used in the subsequent experiments.

Table 6: The effect of TritonX-100 amount on absorbance

| ml of (1%) TritonX-100 | 0.5 | 1.0 | 2.0 | 3.0 |
|------------------------|-----|-----|-----|-----|
| Absorbance             | 0.6756 | 0.6913 | 0.6715 | 0.6710 |

Effect of time and amount of salbutamol sulphate on absorbance

A study of the time effect on color development showed that the color formed immediately and remained stable for at least 90 minutes, Table (7).

Table 7: Stability of azo dye

| Time/min. | Absorbance/µg of Salbutamol sulphate present |
|-----------|---------------------------------------------|
|           | 50              | 100              | 200              |
| After dilution | 0.3678         | 0.7061         | 1.3129         |
| 10        | 0.3652         | 0.6903         | 1.2981         |
| 20        | 0.3636         | 0.6933         | 1.2939         |
| 30        | 0.3670         | 0.6964         | 1.2940         |
| 40        | 0.3727         | 0.6916         | 1.2958         |
| 50        | 0.3721         | 0.6946         | 1.2906         |
| 60        | 0.3752         | 0.6949         | 1.2969         |
| 90        | 0.3754         | 0.6962         | 1.2952         |
| 120       | 0.3904         | 0.6821         | 1.2390         |
| Over night | 0.1130         | 0.4103         | 0.9259         |

Final Absorption Spectra

Absorption spectra of the colored dye formed from treatment salbutamol sulphate with diazotized 5-amino-2-chlorobenzotrifluoride reagent in basic medium, in the presence of TritonX-100, according to the above recommended procedure, showed that the maximum absorption is obtained at 467 nm (Fig. 2).

![Absorption spectra](image)

Fig. 2: Absorption spectra of 100 µg salbutamol sulphate/10 ml treated according to the recommended procedure and measured against (A) blank, (B) distilled water and (C) blank measured against distilled water.
Procedure and calibration graph

To a series of 10-ml volumetric flasks, increasing volumes of aqueous solution containing 5–300 µg salbutamol sulphate are transferred, 4 ml diazotized 5-amino-2-chlorobenzotrifluoride (5x10⁻³ M) followed by the addition at 1 ml of (1%) Triton X-100 and 1 ml NaOH (2 M) then the volumes were completed to the mark with distilled water. The absorbance for each flask was measured directly after dilution at 467 nm against blank. The calibration graph is linear over the range 0.5 – 30 µg.ml⁻¹ and higher concentrations show negative deviation from Beer’s law (Fig. 3). The apparent molar absorptivity referred to salbutamol sulphate, has been found to be 4.2 ×10⁴. l. ol⁻¹.cm⁻¹.

![Fig. 3: Calibration graph for salbutamol sulphate determination using the proposed method](image)

Nature of the dye

Continuous variations (Job’s method) and mole – ratio methods (Delevie, 1997) indicate that the dye has a composition of 1:1 salbutamol sulphate [SBS] to diazotized 5-amino-2-chlorobenzotrifluoride [Diaz.] reagent (Fig. 4 and 5).

![Fig. 4: Job’s method plot](image)
Hence the dye may have the following structure.

\[
\begin{align*}
\text{Cl} & \quad \text{N} & \quad \text{N} & \quad \text{HO} & \quad \text{CH}_2\text{OH} \\
\text{F}_3\text{C} & \quad \text{N} & \quad \text{N} & \quad \text{OH} & \quad \text{CH}_2\text{OH} \\
\text{HO} & \quad \text{CH}_2\text{OH} \\
\end{align*}
\]

\[
\text{Triton X-100}
\]

**Interference**

The extent of interferences by some excipients which often accompany pharmaceutical preparations as studied by measuring the absorbance of solutions containing 100 µg ml\(^{-1}\) of salbutamol sulphate and various amounts (50, 100 and 1000) of exaplents in a final volume of 10 ml. It was found that the studied excipients do not interfere in the determination of salbutamol sulphate in its dosage forms. Typical results are given in Table (8).

**Table 8: Effect of foreign compounds on the determination of 100 µg salbutamol sulphate**

| Foreign compound | Recovery (%) of 100 µg salbutamol sulphate per µg foreign compound added |
|------------------|--------------------------------------------------|
|                  | 100      | 500      | 1000       |
| Glucose          | 98.36    | 99.86    | 97.21      |
| Arabic Gum       | 102.52   | 100.53   | 101.46     |
| Lactose          | 96.29    | 95.55    | 98.04      |
| Starch           | 101.19   | 97.95    | 101.97     |

**Application of the method**

The proposed method was applied to determine salbutamol sulphate in its pharmaceutical preparations (Butadin syrup, tablet and Salbu. Vent.). The results shown in Table (9) indicated that a good recovery and the RSD% was better than ±3.50%.
Table 9: Analytical applications of the proposed method

| Pharmaceutical preparation | Amount taken (µg) | Amount measured (µg) | Recovery, (%)  | RSD, (%)  |
|----------------------------|------------------|---------------------|--------------|----------|
| Butadin syrup             |                  |                     |              |          |
| 2 mg salbutamol sulphate/ 5 ml (S.D.I Iraq) | 50               | 51.72               | 103.44       | 3.17±    |
|                            | 100              | 101.08              | 101.08       | 0.76±    |
| Butadin tablet            |                  |                     |              |          |
| 2 mg salbutamol sulphate/ tablet (S.D.I Iraq) | 50               | 51.69               | 103.38       | 1.73±    |
|                            | 100              | 102.16              | 102.16       | 2.47±    |
| Salbu. Vent.              |                  |                     |              |          |
| 5 mg salbutamol sulphate/ 1 ml (Diamond pharma-syria) | 50               | 50.90               | 101.80       | 3.50±    |
|                            | 100              | 101.63              | 101.63       | 2.35±    |

*Average of five determinations

The validity of the method was confirmed by applying the standard addition procedure (Al-Abachi, and Al-Ghabsha, 1986) (Fig. 6).
Fig. 6: Standard addition plot for the recoveries of 10,20 µg of salbutamol sulphate in butadin syrup, tablet and ventolin respectively.

The recoveries calculated by using the equations of the linearity in (Fig. 6) and the results obtained are in agreement with the certified value Table (10).

Table 10: The results of standard addition method

| Pharmaceutical preparation | Amount taken (µg.ml⁻¹) | Amount measured (µg.ml⁻¹) | Recovery, % |
|----------------------------|------------------------|--------------------------|-------------|
| Butadin syrup              | 10                     | 10.45                    | 104.50      |
| 2 mg salbutamol sulphate/ 5 ml (S.D.I Iraq) | 20                   | 20.78                    | 103.90      |
| Butadin tablet 2 mg salbutamol sulphate/tablet (S.D.I Iraq) | 10                   | 10.14                    | 101.40      |
| Salbu. Vent. 5 mg salbutamol sulphate/ 1 ml (Diamond pharma-syria) | 20                   | 20.26                    | 101.30      |

Comparison of the methods

Table (11) shows the comparison between some of analytical variables obtained from the present method with other spectrophotometric methods.

Table 11: Comparison of the methods

| Parameter                  | Present method          | Othman and Zakaria, 2004 | Othman and Hamdoun, 2005 | Al-Hafith (2005) |
|----------------------------|-------------------------|--------------------------|--------------------------|-----------------|
| Reagent                    | Diazotized 5-amino-2-chlorobenzotriflour-ide | Diazotized sulphanilic acid | Diazotized p-nitroaniline | p-Phenylenediamine in presence of meta per iodate |
| pH                         | 12.01                   | 12.2                     | 12.2                     | 12.2            |
| Temperature (°C)           | R.T.                    | R.T.                     | R.T.                     | R.T.            |
| Development time (min.)    | After dilution          | 10                       | After dilution           | 50              |
| λ_max (nm)                 | 467                     | 445                      | 488                      | 552             |
| Beer’s law range (ppm)     | 0.5 - 30                | 0.8 – 4.0                | 0.4 – 4.8                | 0.8 – 40        |
| Molar absorptivity (L.mol⁻¹.cm⁻¹) | 4.15×10⁴            | 3.53×10⁴                 | 3.13×10⁴                 | 2.30×10⁴        |
| Colour of the dye          | Yellow - orange         | Yellow                   | Orange                   | Violet          |
| Application of the method  | Syrup, tablet and ventoline | Syrup and tablet         | Syrup and tablet         | Syrup, tablet and ventoline |
CONCLUSION

The advantage of the proposed method compared to the reference methods was a higher sensitivity than the refered methods which were linear from 0.5-30 µg.ml⁻¹. Moreover, the proposed method could be applied successfully to the determination of the salbutamol sulphate in a pure form as well as in its dosage forms.

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