Evaluation of salbutamol in pure form and pharmaceutical formulations using spectrophotometry and green nonionic surfactant of cloud point extraction

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Abstract. The purpose of the study was to identify a quick, simple and sensitive spectrophotometric technique for the assessment of salbutamol (SAL). The proposed approach depends on SAL’s azo-coupling reaction with BEN. The orange color of the reaction product was analyzed at λ<sub>max</sub> (480 nm) against a blank solution. It was obeyed to beer-lambert law over concentration between (2.5-17.5 mg.L⁻¹) with LOD (1.283 mg.L⁻¹), LOQ (4.234 mg.L⁻¹) and molar absorptivity (12922.74 L.mol⁻¹.cm⁻¹). The procedure revealed high sensitivity for assessment of chosen drug. The new cloud point extraction technique was also successfully utilized for the extraction of pharmaceutically pure SAL drug, (also known as 2-[4-(2,4,4-trimethylpentan-2-yl) phenoxy] ethanol), was selected as green extraction solvent due to its properties and structure. The influence of various parameters, such as the kind and volume of surfactant, salt, temperature and incubation time on the CPE of salbutamol was investigated in detail and a set of perfect conditions was established. A correlation coefficient (R²) was 0.9914 for the calibration curve was obtained. The LOD, LOQ and molar absorptivity were 0.041 mg.L⁻¹, 0.12 mg.L⁻¹ and 47024.61 L.mol⁻¹.cm⁻¹, respectively. We believe that our suggested method is rapid, very convenient, and cost effective for the determination of salbutamol drug in the various samples.

Keywords: nonionic surfactant, salbutamol, spectrophotometry, cloud point extraction, pharmaceutical formulations.
1. Introduction

Salbutamol (SAL), (commonly known as albuterol), is a synthetic β2-adrenergic agonist. (Figure 1) illustrates its chemical structure, [ 2-( tert- butyl amino) -1- ( 4- hydroxyl-3- hydroxymethyl)phenyl ethanol [1]. It is utilized for the alleviation of bronchospasm in the condition such as, chronic obstructive pulmonary disease [2,3] and asthma [4,5]. Different techniques for the evaluation of (SAL) in biological samples or pharmaceutical preparations have recently been reported. including fluorescence and chemiluminescence, radioimmunoassay, HPLC, potentiometry, flow injection analysis, capillary electrophoresis, GC-MS and enzyme-linked immunosorbent assay [6-16]. Although these techniques are generally accepted, but they need progressed technical expertise and are costly. It is essential, along these lines, to develop a simple, rapid and precise method for the analysis of salbutamol. Accordingly, we used a new method for evaluating salbutamol using azo-coupling reaction with benzidine as a reagent and extracting it via the technique of cloud point extraction (CPE). CPE procedure has become more popular compared with another extraction techniques due to the benefit of low consumption of organic solvent, high recovery, low cost, high enrichment factor and quick phase separation. It is alluring that diminishes the utilization and exposures to the solvent and also, It reduces the cost of extraction and removal time that was used for salbutamol pre-concentration after the formation of azo compound that was poorly soluble in H2O[17-27]. The purpose of the current work is to combine and refine the technique of cloud point extraction with a spectrophotometric method to evaluate the highly sensitive and selective method of salbutamol.

![Figure 1. Salbutamol structure.](image)

2. Materials and methods

2.1. Apparatus.

All spectrophotometric measurements were achieved utilizing UV-Vis spectrophotometer single beam (160) and 1 cm quartz cells. The pH solutions were adjusted using metlar pH meter. Sartorius digital balance was used for weighing.

2.2. Chemicals and reagents.

All chemicals used were of analytical grade. The pure form of salbutamol (99.8%) was produced by Samarra (SDI), IRAQ while benzidine (BEN) was purchased from MERCK Company. The stock solution of salbutamol (250 mg. L⁻¹), was prepared by dissolving 0.025 gm of it in adequate amount of D.W, stirred then the final volume was adjusted up to 100 mL in a volumetric flask. Similarly, the stock benzidine solution (250 mg. L⁻¹) was prepared. The 10% TritonX-100, 25% Sodium hydroxide, 4% Urea solution and 1.0 % of NaNO2 solution were used.

2.3. Preparation of SAL tablet solution.
20 tablets of butalin (2 mg) obtained from SDI (Samarra, Iraq) were powdered and equivalent doses (100 mg L\(^{-1}\)) were transferred to a volumetric flask (100 mL) and dissolved in MeOH, up to 25 mL final volume. The solution was centrifuged and filtered for 4 min at 2000 rpm, then diluted to (100 mL) of final volume using D.W.

2.4. Preparation of SAL syrup solution.
65 mL of butalin (2 mg : 5 mL) equivalent to 0.026 gm salbutamol provided from SDI (Samarra, Iraq) was transferred into a 100 mL volumetric flask, then D.W was added up to 100 mL to prepare 260 mg L\(^{-1}\) solution of SAL.

2.5. A general procedure of diazotization.
We used a developed procedure to prepare the azo coupling solution by adding (1 mL) of salbutamol 250 mg L\(^{-1}\) in the volumetric flask (10 mL) immersed in an ice bath (0-5 °C), to which 1 mL of hydrochloric acid (1:1) was added followed by the step by step addition of (1.2 mL) of (1%) NaNO\(_2\) solution and left for 25 min. Then, (1.4 mL) of 4% urea solution was added with stirring to remove the excess of nitrite followed by the addition of 1 mL of 250 mg L\(^{-1}\) benzidine. Finally, 1 mL of sodium hydroxide (25%) was added and the final mixture was diluted with D.W to 10 mL. The azo dye solution has orange colored which have absorbance at 480 nm.

2.6. Cloud point extraction technique of salbutamol (SAL).
The main method is based on azo-coupling reaction of SAL with nitrous acid coupled with BEN. Various concentrations ranging from 1.0-6.0 mg L\(^{-1}\) of azo compound formed (SAL) were putted in the centrifuge tubes (10 mL), then (1.4 mL) of 10% TX-100 was added and the final volume was adjusted to 100 mL using D.W. The prepared solutions were immersed in the water bath for 40 min at 80 °C. In order to improve the viscosity of the surfactant-rich phase, the solutions were centrifuged for 10 min at 4000 rpm and these solutions cooled for 25 min in an ice bath. The organic phase (surfact-rich phase) was dissolved with MeOH and diluted to 2 mL and transferred to a quartz cell of 1 cm. At \(\lambda_{max}\) (480 nm), then, the solution absorbance was measured. The blank solution was presented without a (SAL) drug using the same procedure.

3. Results and Discussion
The fundamental study appears the diazotization reaction of salbutamol with nitrous acid and coupling with BEN as a reagent and formation of the orange colored at \(\lambda_{max}\) 480 nm. The absorption spectra of product against the blank as showed in figure 2.
3.1. Optimization of the system.

To fully utilize our technique, reaction conditions were optimized. In order to achieve optimal experimental conditions, various parameters were investigated. By setting these parameters to be fixed and optimizing one per time, the parameters were optimized. The effect of various acids, such as HCl, HNO₃, H₂SO₄ and acetic acid was studied for the production of diazonium salt and the results are shown in table 1. The effect of acid volume on a fixed concentration of azo solution was investigated in the range of 0.2-1.4 mL. The best volume of HCl was 1.0 mL, Figure 3.

| Type of acid | HCl  | H₂SO₄ | HNO₃ | CH₃COOH |
|--------------|------|-------|------|---------|
| Abs. λ_max | 480  | 0.330 | 0.255 | 0.150   | 0.105   |

It was observed that the volume of sodium nitrite can affect the maximum absorbance signal, so different volumes were added in the range of 0.2-1.6 mL (1.0 %). The measurements showed that the best absorbance signal was achieved by adding 1.2 mL of NaNO₂ (1.0 %) solution and it was therefore used to perform subsequent measurements, Figure 4.

Figure 2. The UV-Visible spectrum of salbutamol, a) blank solution, b) SAL solution c) azo compound solution (SAL+BEN).
A range (0.2-1.8 mL) of 4% of urea solution was added to eliminate the excess of nitrous acid. The results showed that the maximum absorbance was given by 1.4 mL of urea solution and was used to achieve the accompanying measurements, Figure 5. The absorbance was recorded utilizing various alkalis such as NaOH, KOH and NH₄OH, among which sodium hydroxide was the best used alkali. The influence of base volume can affect the maximum absorbance, therefore, various volumes in the range of 0.2-1.8 mL of 25% sodium hydroxide were added. The results showed that the best absorption signal was given by 1.0 mL of NaOH solution and it was therefore used to achieve the next steps, Figure 6.
The influence of reagent volume (BEN) can affect the maximum absorbance, as a result, various volumes in the range of 0.2-1.8mL of benzedine solution were added. The results showed that 1.2 mL of BEN solution can achieve best absorbance signal, it was therefore used to accomplish the subsequent steps, Figure 7.

A major effect on the form of the product compound may be caused by the sequential addition. Sundry studies have been accomplished with different sequential additions. The results revealed that our sequential of (T), (D) and (B), had the best absorbance signal, Table 2.

### Table 2. Effect of addition sequence on absorbance of azo dye.

| Order of Additions | Abs.λ<sub>max</sub> 480 nm. |
|--------------------|---------------------------|
| T+R+B              | 0.744                     |
| T+B+R              | 0.731                     |
| T+M                | 0.410                     |

T: (HCl+SAL+Urea+NaNO<sub>2</sub>), R:BEN, B:Base, M:(R+B)

Figure 7. Effect of reagent volume on absorbance of azo dye.

In the ideal conditions mentioned above, the nature of the azo solution was demonstrated using the methods of continuous variation and molar ratio. Inflection at molar ratio 1.0 appeared in the plot of absorbance versus molar ratio of SAL to BEN, obtained by various BEN concentrations, Figure 8. Moreover, the job method showed a ratio of BEN to SAL = 1.0, Figure 9. Therefore, the results indicated which the stoichiometric ratio was (1:1).
3.2. Calibration curve.
Following the previous optimized conditions of salbutamol evolution, a linear calibration curve was established by plotting absorbance versus concentration of SAL (2.5-17.5 mg.L⁻¹), Figure 10.

3.3. Investigation of optimization of cloud point extraction for SAL.
The effect of various surfactants, such as TX-100, TW80, TW20 & CTAB was studied for the separation and extraction of azo solution and the results are showed in Table 3. The effect of TX-100 on the extraction of azo solution was studied in the volume range 0.2-1.8 mL. The absorbance
increased by increasing TX-100 volume up to 1.4 mL and decreased at higher volumes. The results demonstrated that 1.4mL gave the best absorbance as shown in Figure.11.

Table 3. Effect type of surfactant on absorbance

| Surfactant | Abs.λmax 480 nm. |
|------------|------------------|
| Triton-x100 | 0.905            |
| Tween 80   | 0.233            |
| Tween 20   | -----            |
| CTAB       | -----            |

The influence of temperature on the efficiency of extraction of SAL is illustrated in Figure.12. The CMC of nonionic surfactant diminished with temperature, while in the increase of temperature, hydrophobic micelles number in the surfactant phase become higher, due to an increase in the separation and extraction ability of TX-100 towards SAL because of the dehydration in the external layer of micelles [28]. Figure.12 reveals the evidence where the absorbance of SAL increases from 60 to 80 °C, while beyond 80 °C, the absorbance decreases due to the increase of viscosity. Cloud point extraction is a kind of equilibrium extraction. The perfect efficiency of extraction was acquired once the equilibrium was established. As short time of incubations, can influence the CPE, we therefore, studied their influence on the efficiency of extraction of SAL in the range of 20 to 50 min. The results shown in Figure.13 indicate that the absorbance of SAL drug decreased during long incubation time (longer than 40 min), however, the extraction equilibrium can be carried out within 40 min. Also, a 10.0 min and centrifugation at 4000 rpm was found to be sufficient for successful CPE, Figure .14 and 15.

Figure 11. Effect volume of (10% v/v) Triton X-100.

Figure 12: Effect of Temperature on CPE of AZO-SAL.

Figure 13: Effect of Time on CPE of AZO-SAL.
Different types of solvents including methanol, ethanol, water, DMF and CHCl₃ was investigated to decrease the viscosity of surfactant-rich phase. Appropriate amount (2 mL) of sample was used for absorbance measurement at 480 nm. The procedure carried out as follow, 1 mL of the sample solution, including SAL drug and 1 mL (1000 mg.L⁻¹) of maltose, sucrose, glucose, galactose, fructose and Gum arabic, was separated and extracted under the ideal experimental conditions. The obtained results in Tabl.4 along with the values of recovery demonstrated that there is no observable interference by the diverse compounds present at moderate concentration. The results show good selectivity and applicability of the suggested procedure in the accurate assessment of SAL drug in pharmaceutical formulations.

| Compound      | Recovery% |
|---------------|-----------|
| Maltose       | 97.8      |
| Sucrose       | 99.3      |
| Glucose       | 98.4      |
| Galactose     | 96.2      |
| Fructose      | 97.7      |
| Gum arabic    | 96.8      |

3.4. Analytical performance of the method.

The calibration curve was obtained in the range of 1.0-5.5 mg. L⁻¹ with a correlation coefficient (R²) of 0.9914 under ideal conditions. Using the mean of absorbance versus SAL concentration of 3 replication experiments, the calibration graph was prepared. Using (10s / b) and (3s / b), where s is the standard deviation and b is the calibration graph slope, the quantification limit (LOQ) and detection limit (LOD) were determined. The LOQ calculated was 0.12 mg. L⁻¹ with a LOD of 0.041 mg. L⁻¹.
Figure 16. Calibration curve of CPE of SAL drug.

| Parameters                           | Before CPE                          | After CPE                          |
|--------------------------------------|-------------------------------------|------------------------------------|
| \( \lambda_{\text{max}} \) (nm)     | 480                                 | Orange                             |
| Color                                | Orange                             | Orange                             |
| Regression equation                   | \( Y = 0.0488X + 0.2916 \)         | \( Y = 0.1846X + 0.0081 \)         |
| Linearity range (\( \mu \text{g/mL} \)) | 2.5-17.5                           | 1.0-5.5                            |
| Correlation Coefficient (R\(^2\))    | 0.9963                              | 0.9914                             |
| \( \varepsilon (\text{L.mol}^{-1}\text{.cm}^{-1}) \) | 12922.74                           | 47024.611                          |
| Sandell's sensitivity, (\( \mu \text{g . cm}^{-2} \)) | 0.018                              | 0.005                              |
| Slope (b)                            | 0.0488                              | 0.1846                             |
| Intercept (a)                        | 0.2916                              | 0.0081                             |
| Limit of detection, (\( \mu \text{g/mL} \)) | 1.283                              | 0.041                              |
| Limit of quantification, (\( \mu \text{g/mL}^{-1} \)) | 4.234                              | 0.12                               |
| C.L. for the slope, (b±ts\(_b\)) at 95% | 0.0488±4.682*10^{-3}               | 0.1846±0.0792                      |
| C.L. for the intercept, (a±ts\(_a\)) at 95% | 0.2916±8.143*10^{-6}               | 0.0081±0.385                      |
| Standard error for regression line, (S\(_x\)) | 0.062                              | 0.054                              |
| *C.L. for Conc.X\(_1\), \( \mu \text{g ml}^{-1} \) at (95%) | 9.351±0.0979                      | 1.14±0.015                         |
| *C.L. for Conc.X\(_2\), \( \mu \text{g ml}^{-1} \) at (95%) | 15.062±0.249                      | 2.12±0.023                         |
| *C.L. for Conc.X\(_3\), \( \mu \text{g ml}^{-1} \) at (95%) | 20.512±0.875                      | 3.83±0.017                         |

*Before CPE (X\(_1\)=10, X\(_2\)=15, X\(_3\)=20) and after CPE (X\(_1\)= 1.0, X\(_2\)= 2.0, X\(_3\)=4.0)
Table 6. Application of the proposed CPE for the evaluation of Salbutamol drug.

| Drug          | Conc. of drug mg.L⁻¹ | Relative Error% | Recovery % | Average Recov% | RSD% (n=3) |
|---------------|-----------------------|-----------------|------------|----------------|------------|
| **Butalin(tablet)** |                      |                 |            |                |            |
| Taken         | Found                 |                 |            |                |            |
| 10            | 9.87                  | -1.3            | 98.7       | 100.12         | 3.02       |
| 15            | 14.75                 | -1.6            | 98.33      |                | 0.64       |
| 20            | 20.67                 | 3.39            | 103.35     |                | 1.41       |
| **Butalin(syrup)** |                      |                 |            |                |            |
| 10            | 9.55                  | -4.444          | 95.5       | 100.67         | 2.63       |
| 15            | 15.89                 | 5.96            | 105.93     |                | 1.79       |
| 20            | 20.12                 | 0.64            | 100.6      |                | 1.21       |

3.5. Comparison with literature studies.
The outcomes of the suggested approach and the methods reported here were compared with previously reported methods, Table 7 shows thus our methods have some advantages (such as lower LOQ & LOD) over the previously reported methods.

Table 7. Comparison of the values of LOD and LOQ of the CPE method with various methods reported in literature.

| Method                        | LOD mg/L | LOQ mg/L | Ref. |
|-------------------------------|----------|----------|------|
| Flow injection method         | 3.547    | 11.824   | [29] |
| Colorimetric method           | 0.5510   | 1.3412   | [30] |
| Spectrophotometric method     | 0.55     | 1.66     | [31] |
| Ion pair liquid chromatography| 0.295    | 0.991    | [32] |
| SPE&LC-MS                     | 0.01 mg/kg | 0.05mg/kg | [33] |
| Spectrophotometric method     | 1.349    | 4.088    | [34] |
| Liquid-liquid extraction      | 0.002    | -        | [35] |
| HPLC                          | 0.08     | -        | [36] |
| RP-HPLC                       | 0.16     | 0.54     | [37] |
Cloud point extraction with spectrophotometric method  

|          |          |          |
|----------|----------|----------|
|          | 1.283, 0.041 | 4.234, 0.12 |
| Present work |          |          |

Figure 17. The proposed mechanism of diazotization reaction.

6. Conclusions
The proposed method offers a simple, sensitive, and low-cost spectrophotometric technique for SAL drug evaluation that can be applied to different types of samples. The surfactant has been employed for separation and pre-concentration of SAL drug in pharmaceutical preparations. A comparison between the proposed procedure and the methods previously reported using different instrumental techniques appears to be more sensitive, safe, easy, quick, fast and inexpensive for this procedure.

Acknowledgments
The authors are grateful to Diyala University and Mustansiriyah University, Science faculty, Department of Chemistry staff for their help in sample runs and data analysis.

7. References
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