Ion-Pair Cloud Point Extraction Method for Dapsone Determination using Salbutamol as a Green Reagent: Application of Environmental Assessment Tools BAGI, CACI, and AGREES

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In this study, a simple and eco-friendly spectrophotometric method was developed for the determination of dapsone (DAP) in pharmaceutical formulations using an ion-pair cloud point extraction (IP-CPE) approach. The IP-CPE method is based on the coupling reaction of dapsone with salbutamol (SUB) in an alkaline medium, producing a stable orange DAP-SUB azo dye with a maximum absorption wavelength at 457 nm. This is followed by the formation of ion-pair associates between the negatively charged DAP-SUB dye and the cationic surfactant CTAB. Triton X-114 was then employed as a non-ionic surfactant to facilitate the cloud point extraction (CPE) procedure. The method exhibited a linear range of 0.25–6 mg/L, with limits of detection (LOD) and quantification (LOQ) of 0.0808 mg/L and 0.245 mg/L, respectively. Good sensitivity was achieved, with a distribution ratio (D) of 3.22 and a preconcentration factor (PF) of 20. Salbutamol was used as a green reagent according to the PBT (persistent, bioaccumulative, and toxic) criteria, and only a small volume of ethanol was required for desorption, significantly reducing solvent waste. The IP-CPE method was further validated using environmental assessment tools BAGI, CACI, and AGREES, all of which confirmed its compliance with green analytical chemistry principles. Overall, the proposed method provides a simple, low-cost, environmentally friendly, and accurate procedure for the preconcentration and determination of dapsone.

Keywords: dapsone, salbutamol, green reagent, Triton X-114, BAGI, CACI, AGREES

Introduction

Dapsone (DAP) has the molecular formula $C_{12}H_{12}N_2O_2S$ and a molecular mass of 248.30 g/mol. It corresponds to the chemical structure 4,4′-sulfonyldianiline or 4,4′-diaminodiphenyl sulfone. DAP is an antibiotic used primarily in the treatment of dermatitis herpetiformis and leprosy. Also known as 4,4-diaminodiphenyl sulfone, it is medically important due to its antimalarial and anti-inflammatory properties [1–3]. Salbutamol (SAL), a selective β_2-adrenergic receptor agonist (SABA), has the molecular formula $C_{13}H_{21}NO_3$ and a molecular mass of 239.32 g/mol. It is widely used as a reliever drug for acute cases of chronic lung diseases and bronchospasm in asthma. Salbutamol is listed among the World Health Organization's (WHO) essential medicines due to its safety, lack of severe side effects, and strong efficacy profile [4,5].

Numerous methods have been developed for the determination of DAP, including LC–MS/MS in meat and milk [6], TLC and HPLC for oxidative degradants (using 30% hydrogen peroxide) [7], GC–MS in raw sewer water samples [8], UPLC–MS/MS with positive electrospray ionization in residues from poultry, porcine, ovine, and bovine muscle tissue [9], HPLC–MS/MS in muscle tissue and milk [10], LC–MS/MS in milk, honey, eggs, and muscle [11], HPLC with UV detection and LC–MS/MS for impurities of dapsone [12], electrochemical sensors (e.g., polyaniline film on Fe_3O_4NPs/Pt) in pharmaceutical samples [13], molecularly imprinted polypyrrole membranes in human serum and urine [14], Co-MOF/MIP-based electrochemical sensors in solution [15], RGO/AuNPs/PANI-MIP nanocomposites in real samples [16], poly(methylene green–Fe_2O_3NP) sensors in tablets and river water [17], flow injection chemiluminescence sensors (FI–CL–MIPs) in urine [18], spectrophotometry using sodium 1,2-naphthoquinone-4-sulfonic as reagent in pharmaceuticals [19], oxidative coupling with pyrocatechol in the presence of KIO_4 in tablets [20], and o-chloranil-based spectrophotometry in pharmaceutical preparations [21].

Most of these methods are complex, requiring substantial amounts of hazardous materials such as toxic organic solvents and specialized chemicals, as well as advanced and expensive instrumentation.

Preconcentration is therefore a crucial step for the determination of analytes such as drugs, heavy metals, and pesticides in complex matrices. Cloud point extraction (CPE) is one of the most widely used and simple methods for both preconcentration and determination. Recent modifications have enhanced its advantages [22, 23], including the use of smaller volumes of less toxic, biodegradable nonionic surfactants, aligning with green chemistry principles. The method also offers improved selectivity, shorter extraction times (with desorption sometimes taking only a few seconds), and better cost-effectiveness due to reduced solvent consumption, lower energy requirements, and minimal instrumentation. Consequently, the reduced use of hazardous solvents makes CPE consistent with the principles of green chemistry [24, 25].

In this study, a new eco-friendly spectrophotometric method has been developed for the determination of dapsone (DAP) in pharmaceuticals using an ion-pair cloud point extraction (IP-CPE) procedure. The method employs salbutamol as a green reagent and requires only a small volume of ethanol for desorption, significantly reducing solvent waste. Its greenness is further supported by the use of non-hazardous reagents and low energy consumption. The method has been validated using environmental assessment tools such as BAGI, CACI, and AGREES, all confirming its compliance with the principles of green analytical chemistry. Overall, this procedure offers a simple, low-cost, eco-friendly, and accurate approach for the preconcentration and determination of dapsone.

Experimetal part

Apparatus

All absorbance measurements for the ion-pair CPE method were performed using a Shimadzu UV-1800 UV-Vis spectrophotometer (Japan), equipped with quartz cells of 1.0 cm and 0.5 cm path length.

Chemicals and reagents

High-purity dapsone (DAP) ($C_{12}H_{12}N_2O_2S$, M.wt. = 248.30 g/mol, >99%) and salbutamol (SAL) ($C_{13}H_{21}NO_3$, M.wt. = 239.32 g/mol, >99%) were obtained from the State Pharmaceutical Industries and Medical Devices Company (SDI, Samarra, Iraq). Stock solutions of each drug (1000 mg/L) were prepared separately by dissolving 0.1 g of the compound in 1 mL of 1 M HCl, followed by dilution to the mark in a 100 mL volumetric flask. Cetyltrimethylammonium bromide (CTAB) was purchased from Sigma-Aldrich. Solutions of 25% (w/v) NaOH, 1:1 (v/v) HCl, 10% (v/v) Triton X-114, and 1% (w/v) $NaNO_2$ were obtained from BDH. All chemicals and reagents were of analytical grade and used without further purification.

General procedure of ion-pair CPE

A 1 mL aliquot of dapsone solution (0.25–6 mg/L, primary arylamine) was treated with 0.5 mL of 1% $NaNO_2$ in the presence of 0.75 mL of 1:1 HCl and cooled in an ice bath (0–5 °C) for 10 min to form the diazonium salt. Subsequently, 0.5 mL of 10% urea was added, the mixture was shaken well and left for several minutes. Then, 1 mL of salbutamol solution (1000 mg/L) was added, followed by 1 mL of 25% NaOH. The yellow color developed immediately. The solution was diluted to 20 mL with distilled water.

For the CPE procedure, different concentrations of the azo dye (0.25–6 mg/L) were prepared in 20 mL centrifuge tubes to construct a calibration curve. To each tube, 0.75 mL of 1% CTAB, 0.5 mL of 10% (v/v) Triton X-114, and 0.5 mL of 5% Na_2SO_4 were added. The tubes were tightly sealed to prevent evaporation and transferred to a water bath at 90 °C for 45 min until complete phase separation occurred. The tubes were then cooled in an ice bath for 5 minutes to increase viscosity, resulting in the formation of a cloudy layer. The aqueous phase was carefully decanted, leaving the surfactant-rich phase at the bottom. Finally, 1 mL of ethanol was added to dissolve the micellar layer containing the azo dye, and the absorbance of the product was measured at 457 nm against the corresponding blank (Figure 1) [26].

Determination of dapsone in pharmaceutical products

A 0.1 g sample of DAP tablets (500 mg formulation from the USA, 200 mg formulation from India) was accurately weighed, dissolved in 1 mL of 5 M HCl, and diluted to 100 mL in a volumetric flask after filtration to remove insoluble particles.

The next step was diazotization and azo dye formation. An aliquot of 1 mL of the prepared sample solution was transferred into a 20 mL centrifuge tube and reacted with 0.5 mL of 1% $NaNO_2$ and 0.75 mL of 1:1 HCl in an ice bath (0–5 °C) for 10 min to form a diazonium salt. Subsequently, 0.5 mL of 10% urea was added to eliminate excess nitrite, followed by 1 mL of salbutamol solution (1000 mg/L) and 1 mL of 25% NaOH, resulting in the formation of an orange DAP–SUB azo dye. The solution was then diluted to 20 mL with distilled water.

For the cloud point extraction step, 0.75 mL of 1% CTAB, 0.5 mL of 10% (v/v) Triton X-114, and 0.5 mL of 5% Na_2SO_4 were added. The mixture was heated in a water bath at 90 °C for 45 min, producing a dense, surfactant-rich cloudy layer containing the concentrated dye. After cooling in an ice bath, the aqueous phase was decanted, leaving the surfactant-rich phase. Finally, the concentrated DAP–SUB azo dye layer was re-dissolved in 1 mL of ethanol, and its absorbance was measured at 457 nm.

The results demonstrated good reproducibility and accuracy, confirming that the proposed method is efficient and reliable for the determination of DAP in pharmaceutical formulations from different manufacturers (India and USA). No significant matrix interferences were observed.

Results and discussion

In this study, the orange DAP–SUB azo dye

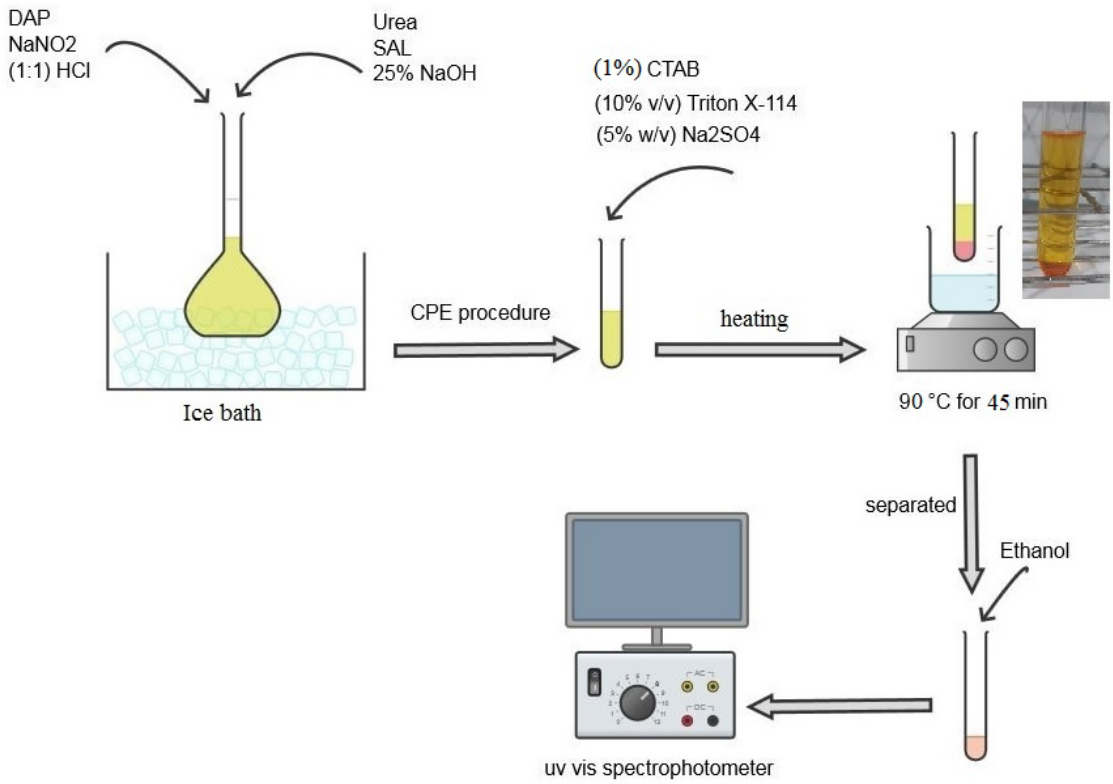
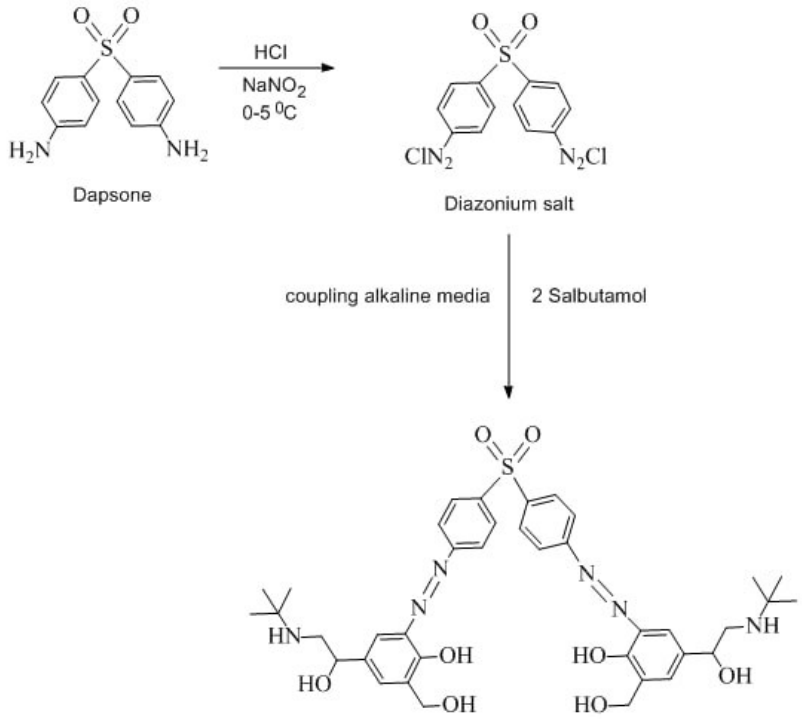


Figure 1. Schematic representation of the general procedure of the ion-pair cloud point extraction (IP-CPE) method for the determination of the DAP-SUB azo dye.

was found to be negatively charged in an alkaline medium. Salbutamol molecules, which couple with the diazonium salt of dapsone, contain phenolic hydroxyl groups (–OH). The coupling reaction is carried out in alkaline conditions (due to the addition of NaOH). Under these conditions, the –OH groups lose their protons (H^+) and are converted into negatively charged phenoxide ions (O^-). Since the final dye product contains two –OH groups, it acquires a total of two negative charges (Scheme 1) [27,28]. For this reason, cetyltrimethylammonium bromide (CTAB), a cationic (positively charged) surfactant, was employed to form ion-pair associates with the negatively charged DAP–SUB azo dye. This strong electrostatic attraction significantly enhances the partitioning of the dye into the surfactant-rich phase of micelles formed by the nonionic surfactant Triton X-114, thereby improving the efficiency of the cloud point extraction (CPE) procedure. The chemical formula of the DAP–SUB azo dye, $C_{38}H_{48}N_6O_8S$ (M.wt. = 748.90 g/mol), was determined by combining all atoms from one dapsone molecule and two salbutamol molecules, while accounting for the hydrogen atoms lost during the coupling reaction.

The absorbance increased as the volume of 1% CTAB solution was raised from 0.2 mL to 0.75 mL. Beyond 0.75 mL, the absorbance began to decrease due to the formation of a larger and less dense micellar layer caused by excess CTAB, which lowered the preconcentration factor and reduced the absorbance (Figure 2A) [29]. As shown in Figure 2B, the maximum absorbance for the 10% Triton X-114 solution was obtained at 0.5 mL. Excess Triton X-114 increased the volume of the surfactant-rich phase, diluting the extracted orange azo dye and consequently decreasing both absorbance and the preconcentration factor [30]. The role of Na_2SO_4 is to act as an electrolyte, increasing the ionic strength and enhancing the “salting-out” effect. This forces the nonpolar azo dye and Triton X-114 molecules out of the aqueous phase, reducing the volume of the surfactant-rich phase and thereby making it denser. An optimal volume of 0.5 mL of 5% Na_2SO_4 was selected, as it provided the best balance for this salting-out effect (Figure 2C) [31]. Triton X-114 has a cloud point of approximately 22–23 °C. Heating the solution to a higher temperature (90 °C) ensures faster and more complete separation of the micellar phase from the aqueous phase [32]. However, temperatures above 90 °C may cause decomposition of the orange azo dye product. Therefore, 90 °C was chosen as the optimal temperature for this study (Figure 2D). Finally, sufficient time is required for the cloud point extraction process to reach equilibrium and achieve complete extraction of the orange azo dye. An extraction time of 45 min was selected as optimal, as it corresponded to the maximum absorbance observed (Figure 2E).



Scheme 1. Proposed reaction mechanism between dapsone (DAP) and salbutamol (SAL) leading to the formation of the DAP-SUB azo dye.

Method validation

Under the optimum conditions, the maximum absorption spectra of the DAP–SUB azo dye at 457 nm, both before and after extraction, are shown in Figure 3. The analytical characteristics of the IP-CPE method for the determination of the DAP–SUB azo dye demonstrated robust performance (Figure S1, Table 1).

Table 1. Validation parameters of the IP-CPE method for the determination of the DAP-SUB azo dye.

Parameter	Value
Calibration equation	Y = 0.082x – 0.023
Linearity range (mg/L)	0.25 – 6
Correlation coefficient (R^2)	0.999
Accuracy (mean ± SD)	100.403 ± 2.234
RSD (%)	2.23
LOD (mg/L)	0.0808
LOQ (mg/L)	0.245
Preconcentration factor (PF)	20
Distribution ratio (D)	3.22

The method exhibited an excellent correlation coefficient (R^2 = 0.999), with a low limit of detection (LOD = 0.0808 mg/L, calculated as 3.3 × SD / slope) and a limit of quantification (LOQ = 0.245 mg/L, calculated as 10 × SD / slope), confirming the high sensitivity of the IP-CPE method for detecting DAP–SUB azo dye at low concentrations. The preconcentration factor (PF = 20) was obtained from the ratio of the initial DAP–SUB azo dye solution volume (20 mL) to the desorption solvent volume of ethanol (1 mL) [33]. In addition, the distribution ratio (D = 3.22) was calculated as the ratio of the dye concentration after extraction (1.29×10^{-5} mol/L, 9.670 mg/L) to that before extraction (4.01×10^{-6} mol/L, 3 mg/L) [34]. The molecular weight of the DAP–SUB azo dye was determined to be 748.90 g/mol.

Accuracy, precision, and application

The accuracy and precision of the method were evaluated by measuring the absorbance at least three times for DAP concentrations of 0.5, 2, and 4 mg/L under the optimized conditions to assess intra-day repeatability. As shown in Table S1, the low RSD% values (all less than 3.451%) and the satisfactory mean recovery values (ranging from 100.325% to 101.626%) confirm that the CPE method provides good accuracy and is essentially free from random errors. Furthermore, the CPE method was successfully applied to the determination of DAP in commercial tablets containing 200 mg (India) and 500 mg (USA) of the active ingredient. The samples were analyzed at different concentrations (0.5, 2, and 4 mg/L), and the obtained results showed good agreement with the labeled values (Table S2), confirming the applicability of the proposed method for routine pharmaceutical analysis.

Environmental evaluation

The aim of this study was to apply the IP-CPE method for the determination and preconcentration of dapsone as an eco-friendly analytical procedure, assessed using established environmental evaluation tools (BAGI, CACI, and AGREE). The Ion-Pair CPE method was first evaluated with the Blue Applicability Grade Index (BAGI), where it achieved a score of 75, indicating strong performance as a green methodology [35]. The Click Analytical Chemistry Index (CACI) was also applied, yielding a score of 65, which further supports the environmental compatibility of the method [36]. In addition, the method was assessed using the AGREE (Analytical Greenness) metric, which operates on a 0–1 scale, and obtained a score of 0.6, confirming adherence to the principles of green analytical chemistry [37].

The favorable scores of the IP-CPE method (BAGI = 75, CACI = 65, AGREE = 0.6) are primarily attributed to the use of reagents such as salbutamol, CTAB, Triton X-114, and ethanol, none of which are classified as hazardous under PBT (Persistent, Bioaccumulative, and Toxic) criteria or listed in the EPA's Toxic Release Inventory. Moreover, the method requires only a small volume of desorption solvent (1 mL ethanol) and operates with low energy consumption (≤0.1 kWh per sample using UV-Vis spectrophotometry) [38, 39].

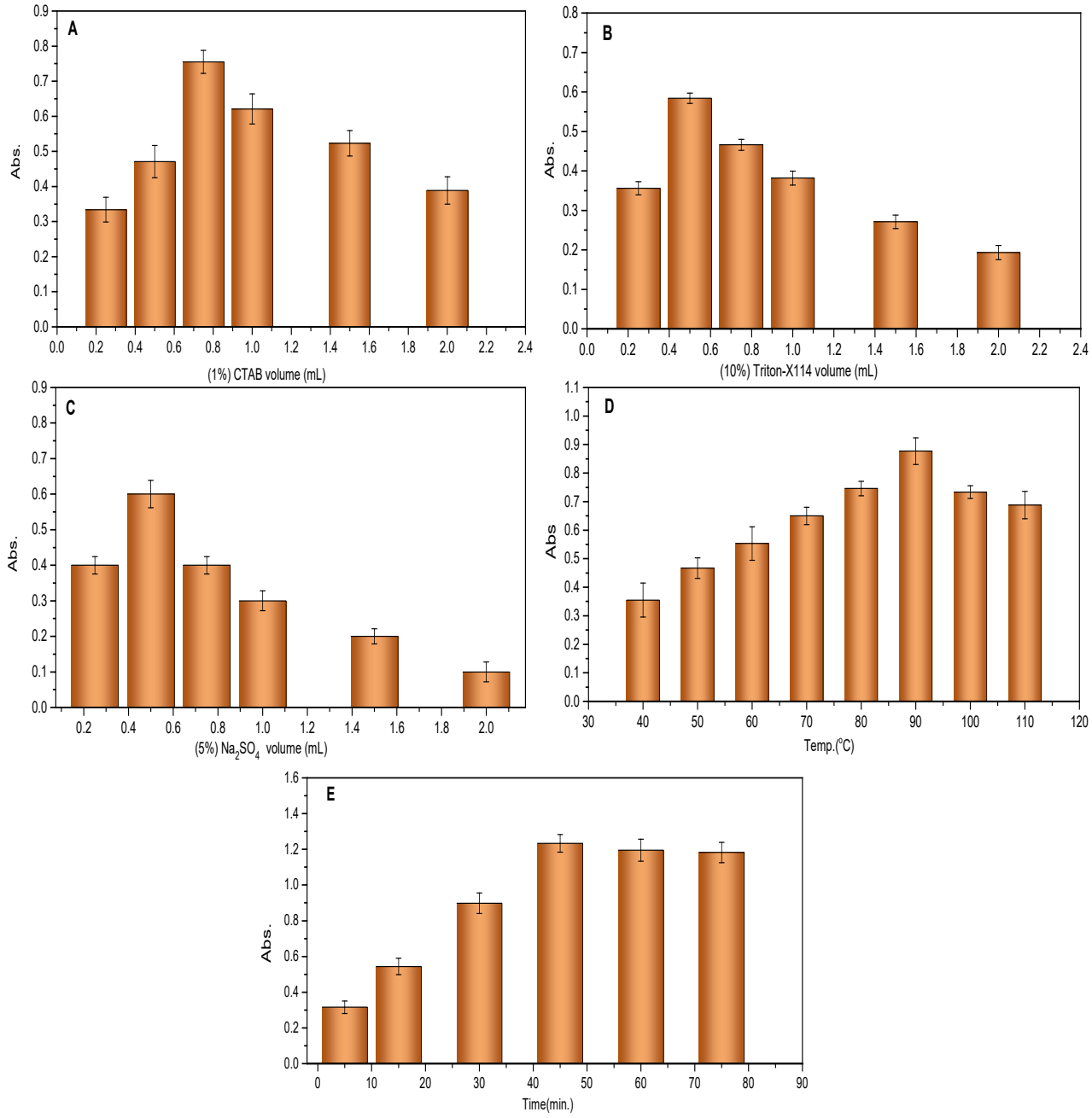


Figure 2. Effect of experimental conditions on the IP-CPE method for the determination of the DAP-SUB azo dye: (A) volume of CTAB, (B) volume of Triton X-114, (C) volume of Na_2SO_4, (D) extraction temperature (°C), and (E) extraction time (min).

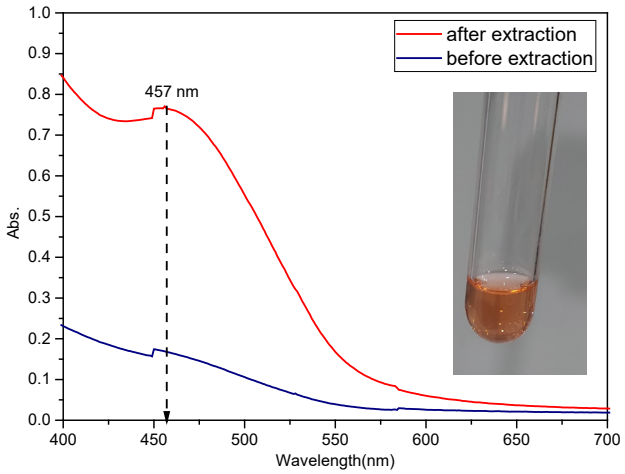


Figure 3. Absorption spectra of the DAP-SUB azo dye (4.01×10^{-6} mol/L) before extraction and (1.29×10^{-5} mol/L) after extraction.

In terms of operational steps, the IP-CPE procedure is relatively straightforward and involves two main stages:

(A) Pre-extraction (sample treatment):

1. A solution of dapsone is treated with $NaNO_2$ in the presence of HCl.
2. The mixture is cooled in an ice bath (0–5 °C) for 10 min to form the diazonium salt.
3. Urea is added to remove excess nitrite
4. Salbutamol and NaOH are added, resulting in the formation of the yellow DAP–SUB azo dye.

(B) Extraction (CPE procedure):

1. Reagent addition: CTAB, Triton X-114, and Na_2SO_4 are added to centrifuge tubes.
2. Heating: Tubes are placed in a water bath at 90 °C for 45 min to achieve complete phase separation.
3. Cooling: Tubes are transferred to an ice bath for 5 min to increase the viscosity of the cloudy layer.
4. Separation: The aqueous phase is removed by simple decantation.
5. Redissolution: 1 mL ethanol is added to dissolve the micelle layer containing the azo dye.

The throughput of the IP-CPE method can be estimated from these steps. The critical times include 10 min for diazonium salt formation, 45 min for the heating step, and 5 min for cooling to increase viscosity, giving a total of 60 min per batch. Since multiple tubes (up to 12) can be processed simultaneously in a single beaker, the throughput is calculated as:

$$\text{Throughput} = \text{Number of samples in batch} / \text{Time (hours)}$$

For a batch of 12 samples, the throughput equals 12 samples per hour, demonstrating that the method combines environmental compatibility with practical efficiency for routine laboratory applications.

Conclusion

This study successfully developed an efficient, simple, and environmentally friendly ion-pair cloud point extraction (IP-CPE) method for the preconcentration and determination of dapsone (DAP). The method is based on the diazotization of DAP followed by coupling with salbutamol (SUB) to form a stable orange DAP-SUB azo dye. This dye subsequently forms an ion-pair complex with the cationic surfactant CTAB and is extracted using the non-ionic surfactant Triton X-114. The proposed IP-CPE method demonstrated strong analytical performance, with favorable values for LOD, LOQ, preconcentration factor (PF), and distribution ratio (D), confirming its accuracy, sensitivity, and suitability for routine DAP determination. The environmental sustainability of the method was further validated using three recognized green chemistry assessment tools - BAGI, CACI, and AGREE, all of which confirmed the eco-friendly nature of the procedure. Overall, this work presents a low-cost, accurate, green, and user-friendly analytical method for the determination of dapsone in pharmaceutical formulations, aligning with the principles of green analytical chemistry.

Declaration of compating interst

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Data availability

The data are available upon request.

Acknoledgement

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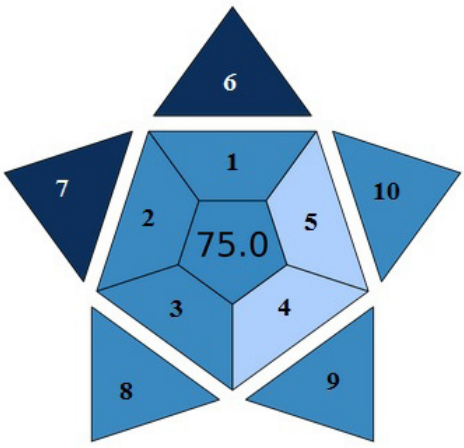
Table 4. Evaluation of the IP-CPE method for the determination of the DAP–SUB azo dye using BAGI, CACI, and AGREE assessment tools.

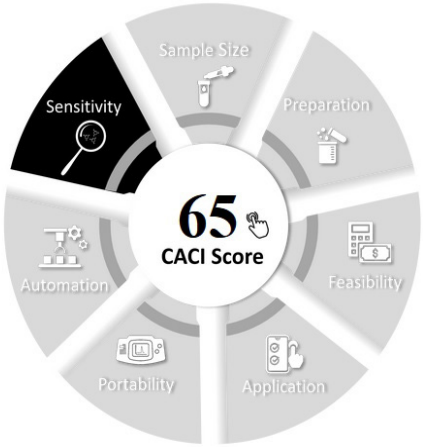
BAGI Tool

1. Type of analysis: Quantitative
2. Multi- or single-element analysis: Multi-analyte
3. Analytical technique: UV-Vis spectrophotometry (simple instrumentation available in common laboratories)
4. Simultaneous sample preparation: 2–12 samples
5. Sample preparation: Minimal extraction using cloud point extraction (CPE)
6. Samples per hour: >10
7. Reagents and materials: Common, commercially available reagents (e.g., ethanol)
8. Preconcentration: Required, one-step preconcentration
9. Degree of automation: Semi-automated with standard laboratory devices (UV-Vis spectrophotometer)
10. Amount of sample: 20 mL of DAP-SUB orange azo dye + 1 mL ethanol as desorption solvent



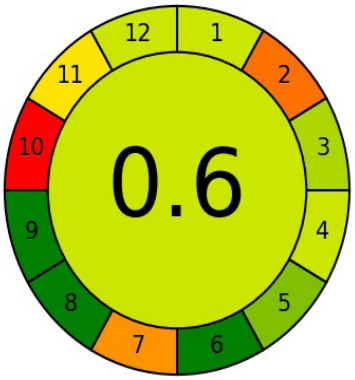
CACA tool

1. Sample Size: Required sample size: 1 mL.
2. Sample Preparation: Level of preparation: Minimal. Time required: ~60 minutes.
3. Feasibility: Chemicals and reagents: Commercially available. Instruments: Available in standard laboratories. Cost of analysis per sample: USD 10–100 (including reagents and instrumentation).
4. Application: Type: Quantitative. Number of analytes: Up to 12 (using 12 tubes in one beaker for heating). Number of matrices: One (tablet sample).
5. Portability: Instrumentation: Miniaturized but not portable.
6. Automation: Level: Semi-automatic (UV-Vis spectrophotometer).
7. Sensitivity: LOD: 0.0808 mg/L. Lowest tested concentration: 0.25 mg/L. Sensitivity: 32.32% (calculated as LOD / lowest concentration × 100).
8. Sample Analysis Time: Very fast (<1 min per sample)



AGREE Tool

1. Sample treatment: External and batch analysis with a reduced number of steps
2. Sample amount: 20 mL DAP-SUB orange azo dye sample + 1 mL ethanol as desorption solvent
3. Device positioning: Off-line (UV-Vis spectrophotometer)
4. Sample preparation stages: 5 steps
5. Automation/miniaturization: Semi-automatic and miniaturized
6. Derivative: None
7. Waste: ≈20 mL
8. Analysis throughput: Moderate (batch-based, >10 samples per hour)
9. Energy consumption: Low (UV-Vis spectrophotometer ≈ 0.1 kWh per sample)
10. Source of reagents: Non bio-based reagents
11. Toxicity: Yes (0.5 mL of 1% $NaNO_2$, 0.75 mL of 1:1 HCl, 1 mL of 25% NaOH; total ≈ 1.25 mL)
12. Operator safety: Ethanol (highly flammable) and $NaNO_2$, HCl, NaOH (toxic to aquatic life)



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