Detecting Hesperidin in Pharmaceutical Forms via Sensitive and Eco-Friendly Cloud Point Extraction Method Utilizing Spectrophotometry

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Abstract: *A spectrophotometric method was developed for the determination of hesperidin (HSP) in pharmaceutical preparations and bulk powder, characterized by sensitivity, simplicity, and ecofriendliness. The method involves diazotization of* p-*chloroaniline (PCA), followed by coupling with HSP in an alkaline medium. The resulting azo product is extracted using the cloud point extraction (CPE) with Triton X-114, and the absorbance was measured at 437 nm. Two approaches were used, i.e., the batch spectrophotometric method and the CPE technique. The batch method showed a detection limit of 0.409 μg/mL, while the CPE method achieved a significantly lower detection limit of 0.023 μg/mL. The linearity ranges were 3–40 and 1–8.5 μg/mL of HSP for the batch and CPE methods. Both methods demonstrated high precision (RSD < 1.17%) and excellent recovery rates, 99.18 to 102.27% for the batch method and 97.32 to 104.28% for CPE, with an enrichment factor of 4.7 using CPE. The methods were successfully applied to the analysis of HSP in supplement formulations and spiked urine samples without significant interference. A greenness evaluation using AGREE software confirmed its environmentally friendly nature. The proposed method offers a reliable, green, low-cost analytical approach suitable for routine pharmaceutical quality control in laboratories to analyze HSP in dosage forms.*

Keywords: *cloud point extraction; hesperidin;* p-*chloroaniline; TritonX-114; AGREE*

■ INTRODUCTION

Cloud point extraction (CPE) is a method that involves extracting organic or inorganic compounds from chemical or biological systems using extractants such as non-ionic surfactants. These surfactants tend to phase-separate and form micellar aggregates when heated to a critical temperature or above [1]. These techniques are also referred to as micelle-extraction, micelle-mediated extraction, or liquid-concentration technique. The surfactant is often adsorbed at the interface between different phases, with the polar head facing the aqueous component and the hydrophobic tail facing the lipophilic layer. The shape of micelles can range from roughly spherical to ellipsoidal, depending on the surfactant and solution conditions. The quantity of surfactants included within a micelle is called the aggregation number. The value of this number is influenced by various factors, including the type of surfactant, the structure of the groups involved, the properties and concentration of the electrolyte, the nature of the solvent, the temperature, and the pH of the solution [2].

CPE, referred to as a green extraction method, has emerged as the superior extraction technique compared to other methods due to its numerous advantages. These include simplicity, efficient recovery, high enrichment factor, minimal use of organic solvents, and the ability to extract a wide range of chemicals and metals. Surfactants and micelles are becoming more commonly employed in analytical procedures for extraction and preconcentration. When compared to other methods of extracting liquids from liquids, CPE demonstrates environmentally friendly characteristics [3-4]. Traditional extraction methods used in pharmaceutical analysis, such as liquid-liquid and solid-phase extraction, often involve drawbacks, including large volumes of organic solvents, high operational costs, and

time-consuming procedures. In contrast, CPE offers high selectivity and preconcentration efficiency [5-7].

Hesperitin-7-rutinoside, commonly known as hesperidin (HSP, $C_{28}H_{34}O_{15}$), appears as a yellow to brown powder with a molecular weight of 610.56 g/mol. It is practically insoluble in water but dissolves in pyridine, DMC, and NaOH. HSP exhibits a melting point of 250–255 °C and has a distinctive absorption spectrum at 285 nm (Fig. 1) [8]. HSP is a flavanone glycoside that is predominantly derived from plants in the Rutaceae family, such as grapefruit, lemon, orange, and tangerine. It can also be found in smaller quantities in other plant species, including peppermint and Welsh onion [9-10]. HSP has been documented to exhibit a wide range of actions, including antioxidant, anti-inflammatory, antiviral, and anticarcinogenic effects. It has also been shown to protect against DNA damage and lipid peroxidation. The supplements containing micronized flavonoids, specifically HSP, along with other bioflavonoids like Diosmin, are primarily used to treat lymphoedema and blood vessel disorders such as varicose veins, venous stasis, and hemorrhoids. There are various analytical techniques for determining HSP, these techniques include the RP-HPLC [11], rapid LC-MS/MS [12], UV-vis spectrophotometric [13], HPLC-UV [14], and HPTLC [15] methods. The main aim of this article is to establish a spectrophotometric method for the determination of HSP in supplements. The analytical process involves the coupling reaction between HSP and diazotized *p*-chloroaniline (PCA) in an alkaline medium to form a colored azo complex. To improve the method's sensitivity, the CPE technique used non-ionic surfactant Triton X-114.

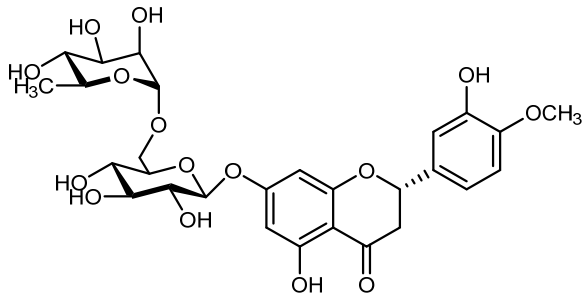


Fig 1. The chemical structure of hesperidin

■ EXPERIMENTAL SECTION

Materials

All chemicals used in this study were of analytical reagent grade. HSP (≥ 95.0%) was purchased from Carl Roth (Germany). PCA (99.0%), HCl (36.0 w/w, 36.46%), $NaNO_2$ (99.0%), and NaOH (99.0%) were all supplied from BDH (England). Triton X-114 (> 99.9%) was purchased from Amresco, Inc. (USA).

Instrumentation

Absorption spectra and absorbance measurements were carried out using a Shimadzu UV–vis 260 digital single beam spectrophotometer (Shimadzu, Kyoto, Japan), with a wavelength range of 190–1100 nm and a fixed spectral band width of 1.0 nm. A quartz cuvette with a 1 cm path length and an internal volume of 50 µL was utilized for spectrophotometric analysis. A thermostatically controlled water bath (Haake F3, England) was employed to maintain precise temperature conditions during the experiments. The separation process was performed using a Hettich EBA 21 centrifuge, equipped with 20 mL calibrated centrifuge tubes.

Procedure

Standard solution preparation

Preparation of HSP solution. The stock standard solution of HSP (200 µg mL^{-1}) was prepared by dissolving 0.02 g of HSP in 5 mL of 0.1 M NaOH, followed by dilution with distilled water to the mark in a 100 mL calibrated flask.

Diazotized PCA reagent solution. A solution of 0.08929 g of PCA was dissolved in 3 mL of ethanol in a 100 mL beaker. Then 3 mL of 1 M HCl was added, followed by placing the beaker in an ice bath. A 0.0483 g of $NaNO_2$ was added with stirring, and after 5 min, the solution was transferred into a volumetric flask and the volume was adjusted to the mark with distilled water to obtain the stock solution, resulting in a solution with a concentration of 0.007 M.

NaOH solution. A solution was prepared by dissolving 1.2 g of NaOH in a 100 mL volumetric flask with distilled water and then diluting to the mark with distilled water, resulting in a 0.3 M solution.

Triton X-114 solution. This solution with a concentration of 10% v/v was prepared by dissolving 10 mL of Triton X-114 in distilled water and diluting to the mark in a 100 mL volumetric flask with the same solvent.

Pharmaceutical sample preparation for HSP detection

Ten capsules of HSP supplement (Vitamins Because, LLC, USA, 1000 mg) were weighed. The average weight of the content of one capsule, equivalent to 200 µg/mL, was dissolved in 5 mL of 0.1 M NaOH. The solution was then transferred into a 100 mL volumetric flask, and the volume was adjusted to the mark using distilled water, followed by filtering. Serial dilutions were employed to prepare working solutions. The solutions of the interferences (1000 µg/mL) were prepared via dissolving 0.1 g of each interference (talc, glucose, PVP, Mg stearate, and starch) in distilled water using a 100 mL volumetric flask. Serial dilutions were employed to prepare working solutions (100 µg/mL of each interference). A urine sample was obtained from a healthy female, about 15 years old and stored frozen without any preservatives until used. Before applying the suggested method, the urine sample was kept below room temperature until it reached the ambient temperature. The urine sample was then spiked with varying amounts of standard solution of HSP supplement and centrifuged for 15 min at 2000 rpm. The centrifuged sample was collected in a clean beaker, diluted to 25 mL with distilled water, and the spiked urine samples were prepared according to previous research published [16].

CPE and estimation procedure

As much as 2 mL of a 0.007 M solution of diazotized PCA reagent was poured into 10 mL calibrated flasks. HSP solution in a range of concentrations from 1 to 8.5 µg mL^{-1} were prepared, and 1.5 mL of 0.3 M NaOH and 2 mL of Triton X-114 (10% v/v) were added to the prepared HSP solutions. The contents of the flasks were combined and diluted with distilled water. Then, they were transferred to a centrifuging tube with a volume of 10 mL, which equilibrated at 60 °C for 15 min in the thermostatic bath. The tubes were then centrifuged for 10 min at 2000 rpm to separate the two phases. Subsequently, the tubes were cooled using an ice bath to assist the separation process, and the aqueous phase was then decanted, while the surfactant-rich phase (micelles surrounding the azo-dye) was dissolved in 2 mL of ethanol and then determined using spectrophotometry at a wavelength of 437 nm, as shown in Fig. 2.

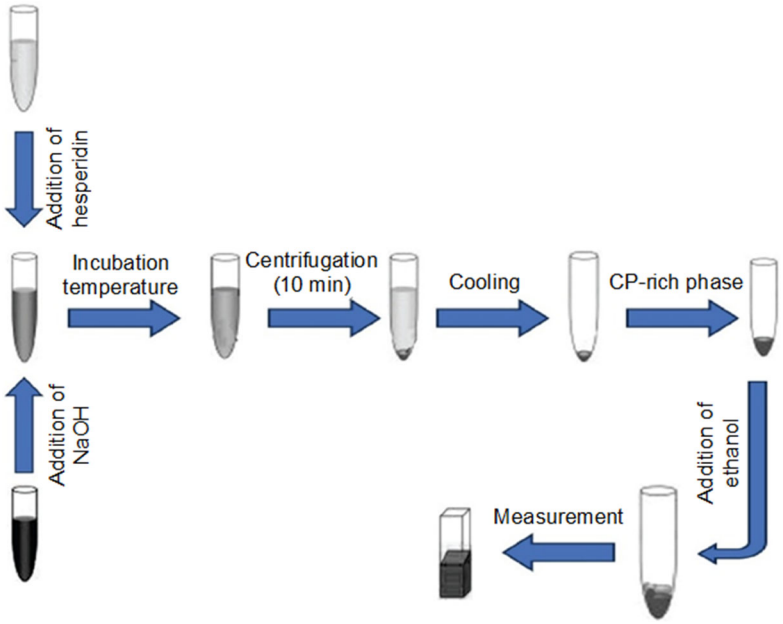


Fig 2. Schematic illustration procedure for hesperidin estimation using the CPE method

■ RESULTS AND DISCUSSION

The diazotization-coupling reaction between the diazotized reagent (PCA) and HSP yielded an orange-colored azo-dye, with maximum absorbance at 437 nm. As shown in Fig. 3, using CPE significantly enhanced the sensitivity of the HSP-azo complex compared to the direct method. The sensitivity achieved through the CPE method can be attributed to the selective partitioning of the hydrophobic HSP-azo complex into the surfactant-rich phase formed by Triton X-114 above its cloud point temperature. This results in effective analyte preconcentration in a small volume, enhancing the analytical signal. Unlike conventional extraction techniques, CPE minimizes hazardous organic solvents and relies on biodegradable surfactants. The factors influencing reaction, such as the effect of surfactant concentration, the influence of type and volume of base solution, the effect of centrifuge time and rate, sensitivity, and extraction efficiency, were investigated. The freshly prepared nitrous acid reacted with the amino group in the reagent (PCA) to form diazonium salts. Then, it was coupled with HSP at the para position of the polyphenol compound to produce an azo dye. This reaction was performed in a slightly alkaline medium, which was used to convert HSP into a more stable phenoxide ion form, instead of the phenol form. Scheme 1 illustrates the mechanism of the proposed reaction.

Optimal Variables Selected for Batch and CPE Procedures

The study focused on analyzing the variables that impact the reaction product of the azo-dye, intending to improve its sensitivity. Both batch and CPE procedures were thoroughly examined. Various factors were investigated in this study, including the quantities of diazotized PCA, NaOH, and Triton X-114, as well as the incubation temperature and time for extraction were studied by changing one variable, and keeping the others constant. A concentration of 8 µg/mL of HSP was utilized in all optimization studies. The absorbance was measured at a wavelength of 437 nm against the blank for

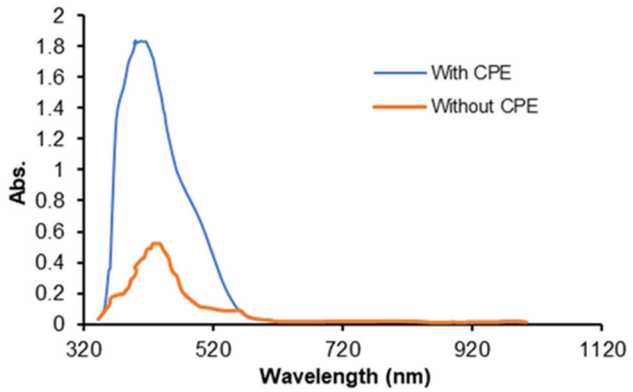
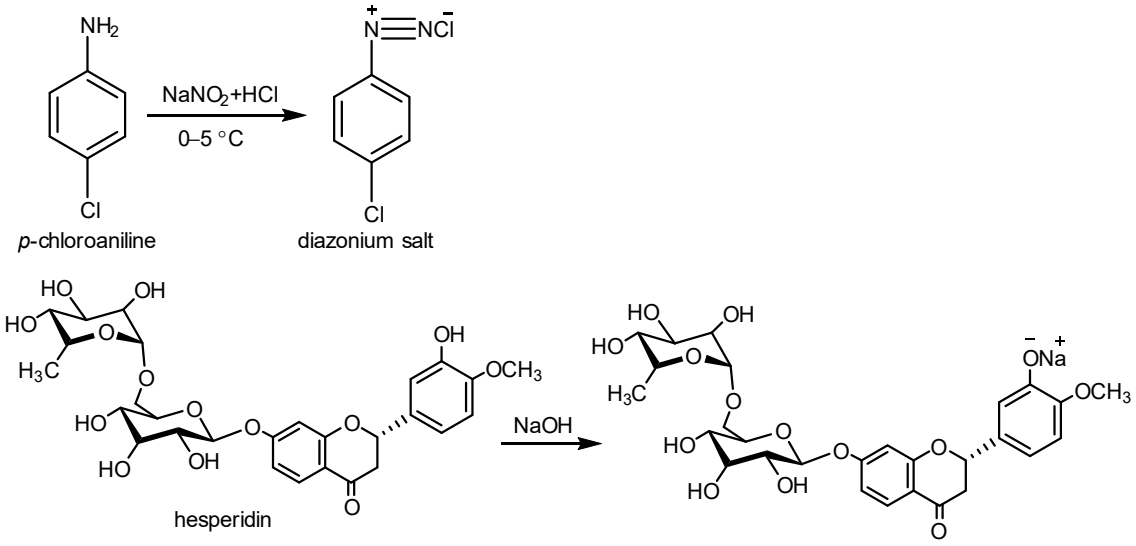
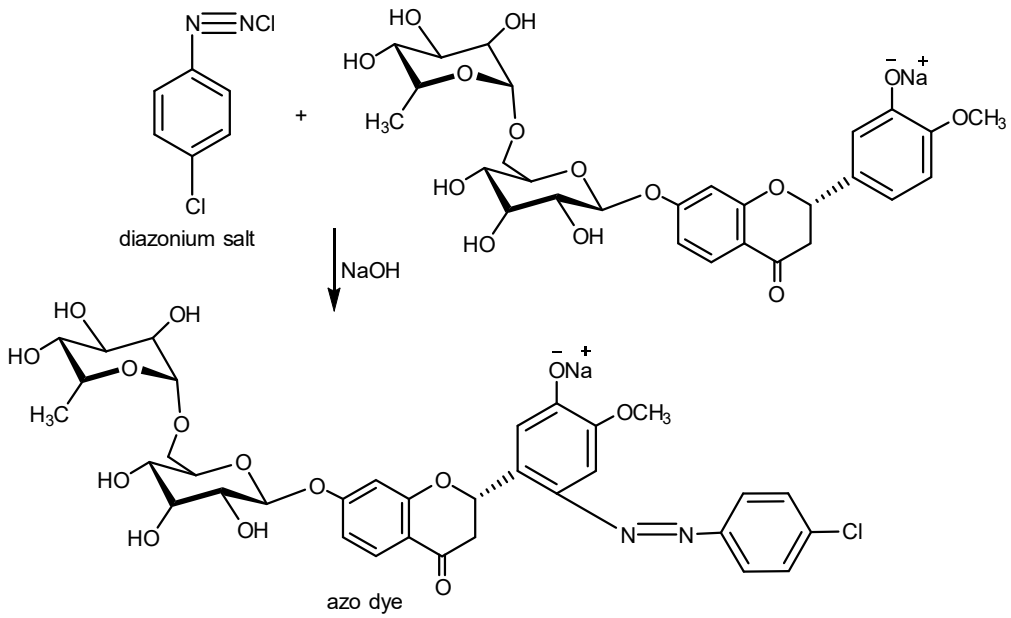


Fig 3. The absorption spectra of 8 µg/mL of HSP treated with 1 mL of a 0.007 M diazotized PCA reagent, 1 mL of 0.3 M NaOH, with and without CPE





Scheme 1. The probable mechanism for the diazo coupling reaction of HSP with the reagent

both methods, respectively.

Study of the chemical variable

Influence of volume of acidic and diazotized PCA reagent. The previous study has shown that the diazotization process is typically performed by utilizing sodium nitrite and acid to generate nitrous acid, which then converts the amino group of the reagent into a diazonium salt. Various types of acids were tested for the diazotization reaction, but only hydrochloric acid yielded a satisfactory outcome. The impact of several quantities of 1 M acid, ranging from 2 to 5 mL, was investigated. The findings showed that the acid solution (consisting of 4 mL of 1 M concentration in a total volume of 10 mL) yielded the most favorable outcome for both techniques (as shown in Fig. 4(a)). The impact of varying concentrations of diazotized PCA reagent was examined by using different volumes (ranging from 0.5 mL to 3 mL) of PCA, while keeping all other conditions constant. The results, as depicted in Fig. 4(b), showed that both the absorbance and extraction recovery increased as the volume of the reagent increased. The maximum values were observed at a volume of 2 mL. However, further addition of the reagent led to an immediate decrease in both absorbance and extraction recovery. Consequently, a volume of 2 mL of a 0.007 M solution of diazotized PCA was chosen as optimal for the complete coupling reaction.

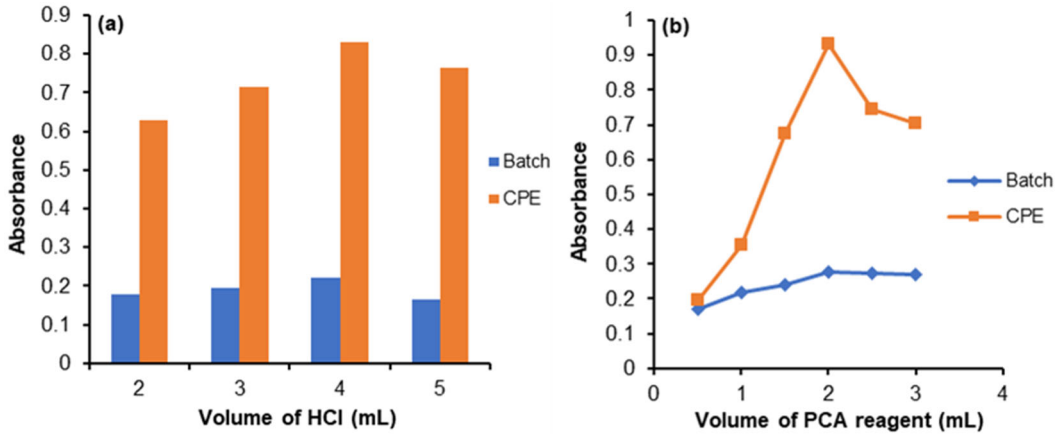


Fig 4. Effect of (a) HCl volume (1 M) and (b) diazotized PCA volume (0.007 M)

Influence of type and volume of base solution. The coupling reaction between the phenolic drug (HSP) and the diazotized reagent requires an alkaline medium. Therefore, it is necessary to investigate the type of alkaline medium to be used. Various types of bases such as NH_4OH, Na_2CO_3, NaOH, and KOH were studied. The determination procedure described in Fig. 5(a) was followed. The results obtained using 0.3 M NaOH showed a higher absorbance compared to the other bases. This is because NaOH, being a strong base, provides a suitable medium for the reaction. Consequently, NaOH (0.3 M) was selected for further experiments. However, it was observed that increasing the volume of NaOH led to a decrease in the absorbance of the colored product, as shown in Fig. 5(b). This is due to a side reaction occurring between the reagent molecules and the high basic medium. Therefore, 1.5 mL and 1 mL of NaOH were used for experiments with and without extraction, respectively, resulting in the maximum reaction.

Study and optimization of CPE parameters

Effect of surfactant concentration. Among various types of surfactants, Triton X-114 is considered the most reactive and efficient surfactant used to extract different organic and inorganic species. The surfactant amount typically affects the efficiency of the separation process. Various volumes of 1–5 mL of 10% (v/v) surfactant were examined by extracting different samples of 8 µg/mL of HSP to study this effect. Fig. 6 showed that maximum sensitivity was attained with 2 mL of surfactant and was chosen for further use.

Effect of temperature and time of heating. In order to achieve extraction and separation, it is necessary to examine the optimal incubation temperature. The impact of temperature on the extraction of the azo-dye was evaluated within the temperature range of 20–80 °C, throughout a 15-min incubation period. The optimal temperature for obtaining the most accurate analytical signal was 60 °C, as shown in Fig. 7(a). The extraction efficiency and the equilibrium between the two phases are mainly affected by the incubation time. The study investigated the incubation period required to complete separations within a temperature range of 60 °C, ranging from 5 to 30 min. The results (Fig. 7(b)) demonstrated that the absorbance of the azo-dye extract increased as the incubation period increased up to 10 min, after which it slightly decreased. Therefore, 10 min extraction time was optimal for achieving complete extraction.

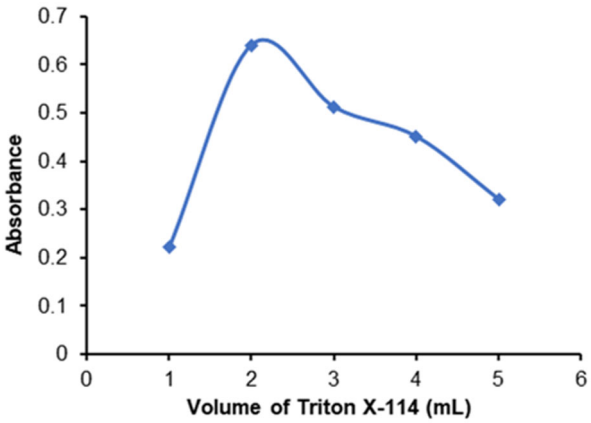


Fig 6. Effect of Triton X-114 volume (10%)

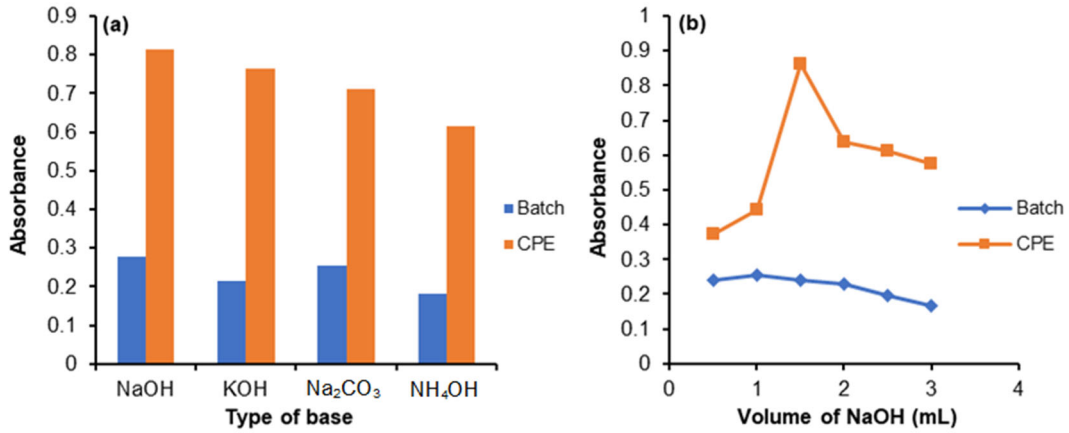


Fig 5. Effect of (a) type of alkaline medium and (b) NaOH volume (0.3 M)

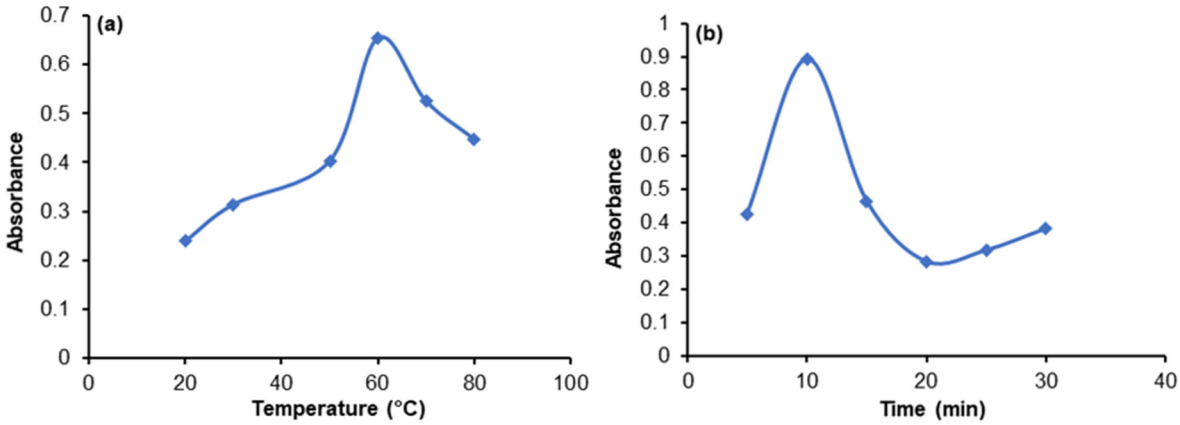


Fig 7. Effect of (a) temperature and (b) incubation time on dye extraction

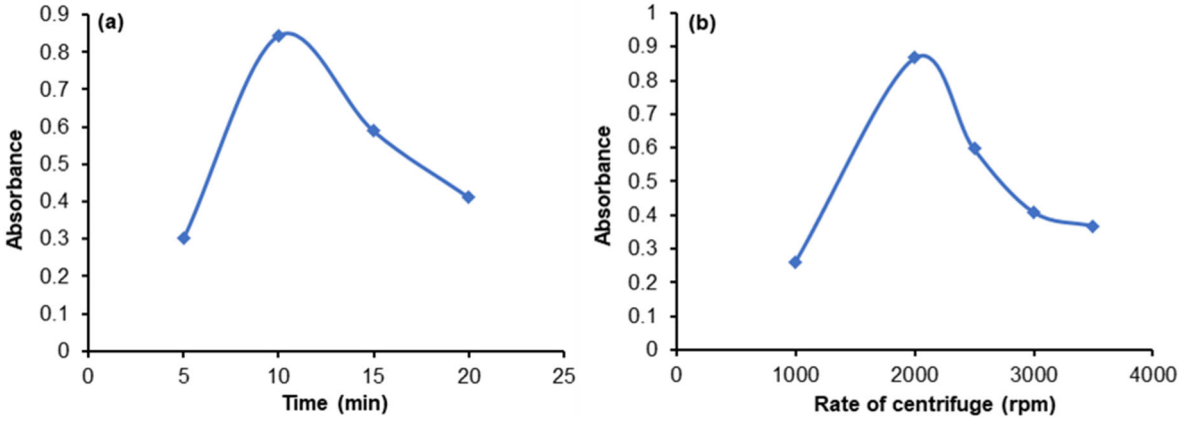


Fig 8. Effect of (a) extraction time at cloud point and (b) centrifugation speed on phase separation

Effect of centrifuge time and rate. To ensure the thorough separation of the two phases, rich-surfactant and aqueous, tests with centrifugation times ranging from 5 to 20 min at speeds ranging from 1000 to 3500 rpm. As depicted in Fig. 8(a) and 8(b), complete separation was achieved after 10 min at 2000 rpm. These parameters were selected as they were deemed optimal for achieving efficient separation of the aqueous-surfactant phases.

Validation of Method

The calibration curves for both approaches were constructed using the optimal variables from Table 1, which were previously utilized for estimating HSP using batch and CPE procedures. The linearity ranges for batch and CPE were 3–40 and 1–8.5 µg/mL of HSP, respectively. Furthermore, the detection limit was determined using the formula LOD = 3SD/b, where SD represents the standard deviation of 10 replicates of the blank and b represents the slope of the calibration curve. The estimated detection limits for the recommended reaction with and without extraction were 0.409 and 0.023 µg/mL, respectively. The low values of LOD, particularly for the CPE technique, demonstrate the high sensitivity of the methodologies. The low values of the analytical parameters, such as the standard deviation of the residual ($S_{y/x}$), the intercept (Sa), and the slope (Sb), indicate a minimal spread of the calibration points and high accuracy of the current procedures. The enrichment factor (EF) was utilized to assess the extraction efficiency under different conditions. The EF, which is defined as the ratio between the slope of the calibration curve obtained with extraction and the slope obtained without extraction [17], was determined to be 4.7. The calibration graphs of batch and CPE method are shown in Fig. 9(a) and 9(b), respectively.

Accuracy and precision

The repeatability and accuracy of the proposed procedures by conducting five replicates for three different

Table 1. Analytical features for the proposed method, both with and without the extraction

Parameter	Value	
	Batch method	CPE method
Regression equation	Y = 0.0412x + 0.0899	y= 0.1967x + 0.0013
Correlation coefficient, r	0.99925	0.99920
Linearity percentage, R^2 (%)	99.85	99.84
Slope, b (μg mL^{-1})	0.0412	0.1967
Intercept, a	0.0899	0.0013
Linearity range (μg mL^{-1})	3–40	1–8.5
Standard deviation of intercept, Sa	6.08×10^{-1}	1.00×10^{-1}
Standard deviation of the slope, Sb	6.82×10^{-4}	8.40×10^{-3}
Molar absorptivity, ε (L mol^{-1} cm^{-1})	25156.720	120099.119
LOQ, (μg mL^{-1})	1.366	0.079
LOD, (μg mL^{-1})	0.409	0.023
Preconcentration factor. $V_{aq\ phase}/V_{end}$	-----	10
Enrichment factor, slope CPE/slope without CPE	-----	4.7
Standard deviation of the residuals, $S_{y/x}$	2.83×10^{-2}	5.40×10^{-1}
Sandell's sensitivity (μg cm^{-2})	0.0242	0.0050

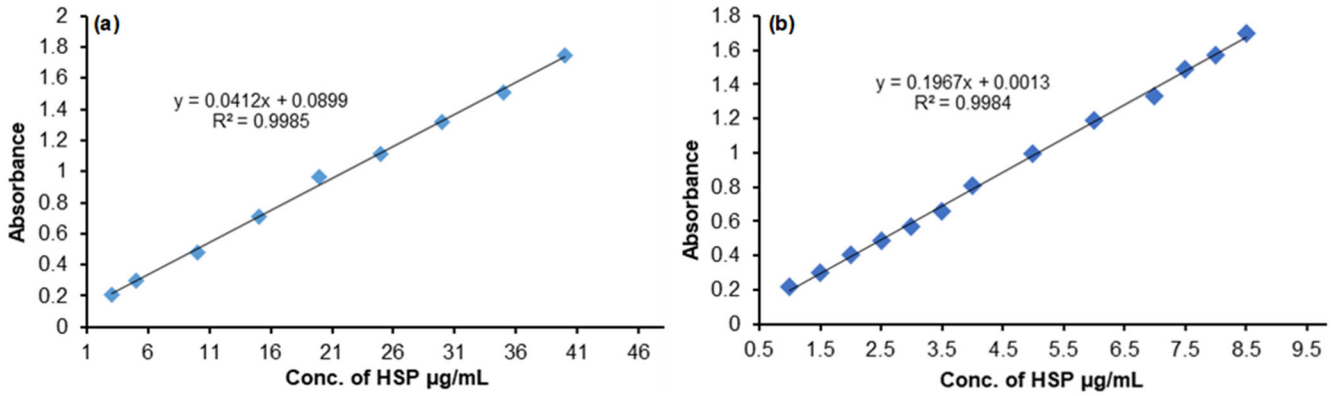


Fig 9. Calibration graph for HSP using (a) batch and (b) CPE method

concentrations of HSP solutions using batch and CPE methods (with and without extraction). Table 2 demonstrates that both methods exhibited high accuracy and repeatability, as evidenced by the acceptable relative standard deviation values and low error rates (excellent recovery values). These results are comparable to or better than those reported for conventional UV and FIA methods, highlighting the method's reliability. The CPE method enhanced sensitivity without compromising reproducibility, supporting its suitability for routine pharmaceutical quality control.

Pharmaceutical applications

The investigation involved the evaluation of five duplicates for three different concentrations of HSP in both batch and CPE systems under optimal conditions. Using the developed batch and CPE methods, HSP quantification in pharmaceutical capsules (HSP capsules, Vitamins Because, LLC, 1000 mg) was conducted. The results, summarized in Table 3, provide a comparative evaluation of both techniques. In the standard addition procedure, a fixed concentration of the supplement (HSP) was mixed with increasing quantities of the standard solution (10, 5, 15, 25, and 1.5, 2, 2.5 μg/mL) for method A and method B, respectively (as shown in Table 4). The two methods proved effective in determining the HSP content in supplement capsules.

The proposed technique was successfully employed to assess HSP in a human, intentionally contaminated

Table 2. Accuracy and precision of batch and CPE methods for HSP determination

Method	Conc. of HSP (μg mL^{-1}) Present	Found*	Rec.%	RSD%	SD
Batch	10	9.76	97.64	0.67	0.0033
	15	15.24	101.60	0.46	0.0033
	25	25.17	100.68	0.75	0.0085
CPE	1.5	1.56	104.28	1.17	0.0036
	2.5	2.44	97.83	0.98	0.0047
	3.0	2.91	97.32	0.54	0.0031

*Average of five repetitions

Table 3. Determination of HSP in supplement formulation using batch and CPE methods

Supplement preparation	Method	Conc. of HSP μg mL^{-1}		Rec.%	RSD%	SD
		Present	Found			
Hesperidin capsules 1000 mg Vitamins Because, LLC. USA	Batch	10	9.96	99.62	0.84	0.0041
		15	15.08	100.58	0.36	0.0026
		25	25.02	100.11	0.41	0.0047
	CPE	1.5	1.45	96.67	1.46	0.0036
		2.5	2.51	100.58	0.77	0.0047
		3.0	2.96	98.85	0.48	0.0031

*Average of five repetitions

Table 4. Evaluation of batch and CPE methods using the standard addition

Supplement preparation	Method	Conc. of HSP μg mL^{-1}		REC.%	RSD%	SD
		Present	Found*			
Hesperidin capsules 1000 mg Vitamins Because, LLC. USA	Batch	10	10.39	103.93	0.81	0.0039
		15	15.45	103.01	0.64	0.0045
		25	25.61	102.46	0.35	0.0038
	CPE	1.5	1.51	101.11	0.85	0.0025
		2.0	1.94	97.40	0.80	0.0032
		2.5	2.46	98.45	0.74	0.0036

*Average of five repetitions

Table 5. Determination of HSP in spiked human urine samples using the proposed methods

Supplement preparation	Applications	Conc. of HSP μg mL^{-1}		Rec.%	RSD%
		Taken	Found		
HSP capsules 1000 mg Vitamins Because, LLC. USA	Urine	1.5	1.42	94.67	0.92
		2.0	1.90	95.04	1.12

urine sample. Two different concentration levels (1.5 and 2 μg/mL) were evaluated for accuracy and precision, with each concentration being tested three times. The precision and accuracy of the urine samples were found to be satisfactory, as indicated in Table 5.

Effect of interferences

In order to assess the effectiveness and selectivity of the proposed methods for routine analysis of HSP, a potential interference study was conducted. Familiar excipients typically used in pharmaceutical formulations, such as starch, glucose, talc, polyvinylpyrrolidone (PVP), and magnesium stearate, were tested individually at a concentration ten times higher (100 μg/mL) than that of HSP (4 μg/mL). The recovery values ranged from 98 to

Table 6. HSP analysis employing CPE in the presence of common excipients

Excipients (100 µg/mL)	Amount of HSP (µg/mL)		(Rec. ± SD)% added found (n = 5)
	Added	Found	
Glucose	4	4.06	101.69 ± 0.58
Talc		3.99	99.86 ± 0.62
PVP		3.97	99.42 ± 1.23
Starch		3.95	98.79 ± 1.83
Mg stearate		3.96	99.02 ± 1.25
All excipients		3.99	99.75 ± 1.10

101%, indicating that these excipients had no significant effect on the HSP determination using batch and CPE methods. The results are summarized in Table 6.

Evaluation of Greenness for the CPE Method

The environmental sustainability of the proposed method was assessed using the AGREE metric. This freely available and widely accepted software tool evaluates analytical procedures based on the 12 principles of Green Analytical Chemistry (GAC). It operates using a math program that may be downloaded (https://mostwiedzy.pl/AGREE), which applies all 12 GAC presumptions. The center of the AGREE symbol represents the final rating, which is a fraction of one from 0 to 1. The color scheme displaying the results of the twelve AGREE symbol sections is the result-associated color in the center. The best approach yields a score of 1, using dark green [18]. As a result, the AGREE criteria are regarded as being user-friendly, thorough, simple to use, and extremely quick [19]. The graphics in Fig. 10 demonstrate the new technique's excellent greenness. In this study, the CPE method received an AGREE score of 0.59, showing good greenness with excellent green efficiency because it did not have waste or hazards (sections 7, 10, and 11). This method was safe and rapid (sections 4, 8, and 12). The evaluation confirms that the proposed CPE-based spectrophotometric method is efficient, accurate, and environmentally friendly, making it a promising tool for routine pharmaceutical analysis. AGREE software principles 1-(off-line analysis), 2-sample amount (10 mL), 3-device position (at-line), 4-number of steps of chemical analysis is 4, 5-the technique is manual and miniaturized sample preparation, 6-don't use any derivatization agents, 7-waste amount (2 mL), 8-samples throughout (6 samples/h), 9-energy of UV-vis detector (< 0.1 kWh per sample), 10-none of reagents are bio-based source, 11-use few milliliters of toxic reagent (PCA), 12-using 2 mL of ethanol as solvent (flammable material).

Fig 10. AGREE greenness evaluation of the CPE method

Table 7. Statistical comparison of the proposed methods with a conventional UV spectrophotometric method (t-test and F-test)

Pharmaceutical preparation	Batch		CPE		UV method	
	Rec. %	RSD %	Rec. %	RSD %	Rec. %	RSD %
Pure HSP	100.49	0.63	99.81	0.90	99.72	0.97
Hesperidin capsules 500 mg	100.10	0.54	98.88	0.90	101.27	0.88
t-Calculate (theoretical = 4.303)	0.100		0.968			
F-Calculate (theoretical = 161.4)	15.851		2.760			

The degree of freedom for F= n1+ n2 − 2 = 2, and for t = n1 − 1 = 1

Table 8. Comparison of the proposed method for HSP determination with previously reported spectrophotometric techniques

Methodology (description)	Conc. (µg/mL)	RSD%	LOD (µg/mL)	Comment	Ref.
Spectrophotometric methods	3–25	< 2	-	Low reproducibility	[20]
Spectrophotometric methods	3.50–50	< 2.0	0.164	Straightforward but simple, and affordable	[21]
UV-spectrophotometric	2–10	< 2	0.214	Simple, consistent, and accurate	[13]
Flow injection analysis (FIA)	5–100	1.5	1.09	Quick analysis, environmentally friendly, and cost-effective	[22]
Batch and CPE	3–40 and 1–8.5	0.75 and 1.17	0.409 and 0.023	Sensitive, low-cost, and inexpensive instruments	Proposed method

An effective comparison of the recovery values between the reported and recommended UV was carried out with a confidence level of 95%. To assess the effectiveness of the current method, the t-test and F-test values of the students were calculated. Table 7 indicates that these values are below the critical values. The comparison's findings indicated that there were no significant differences in the accuracy or precision of either the theoretical or estimated values of the approaches. Table 8 presents a comparison of the results with other validated spectrophotometric methods. The observations suggest that the proposed method offers greater reliability and cost-effectiveness. Additionally, it exhibits higher selectivity compared to UV spectrophotometric methods and has a broader range than colorimetric procedures and the new separation-spectrophotometric method for the HSP drug.

■ CONCLUSION

A spectrophotometric method utilizing batch and CPE has been developed to estimate HSP in supplement capsules and urine samples. This study presents a rapid and highly sensitive approach, adhering to green chemistry principles through the use of Triton X-114 as a non-ionic surfactant, which eliminates the dependency on hazardous organic solvents and offers a sustainable alternative to conventional extraction techniques. The methods were validated, exhibiting good precision, accuracy, and low detection limits. The diazotization and coupling process, employing PCA as a less hazardous and cost-effective coupling agent, further enhances the method's selectivity. With minimal sample preparation requirements, the proposed methods are highly suitable for routine application in pharmaceutical quality control laboratories. The successful application to pharmaceutical and biological matrices confirms its efficiency, eco-friendliness, and reliability as a tool for hesperidin analysis.

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■ CONFLICT OF INTEREST

On behalf of the author, the corresponding author states that there is no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Ali Mohammed Salman conducted the experiments, data analysis, and manuscript writing. Sadeem Subhi Abed conducted the study design, supervision, manuscript writing, and revisions. All authors read and agreed to the final version of this manuscript.

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