Cloud Point Extraction as an Environmentally Friendly Technique for Estimating of Meperidine and Morphine in Pharmaceuticals

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Abstract: The investigation's objective is to develop a new spectrophotometric method for determining Meperidine [MPD] and Morphine [MOP] which involves the reaction of bromothymol blue [BTB] as a reagent with Cloud Point Extraction [CPE] in the presence of the nonionic surfactant, Triton X-114. The method has been shown to be respectably accurate in our previous studies, but has a limited sensitivity and the excipient interferences affect the selectivity. In the present investigation, CPE was employed as a preconcentration step to circumvent these issues and thus bypassed these issues allowing the extraction of the colored product into the surfactant-enriched phase. The method was optimized with respect to the type, concentration, equilibrium temperature, and incubation time of the surfactant. The results had an impressive improvement in sensitivity and high-quality precision with a detection wavelength of [512 and 550] nm, mean recoveries of 99.38% for meperidine and 99.79% for morphine, and very low relative standard deviations. The technique was successfully used on medicinal preparations of the two drugs without the necessity of additional complex procedures. Compared with the other method alone, the combined approach provided superior sensitivity, selectivity, and enviro-nmental compatibility, establishing CPE as a simple, cost-effective, and green alternative for the analysis of Meperidine and Morphine in pure and pharmaceutical forms.

1 INTRODUCTION

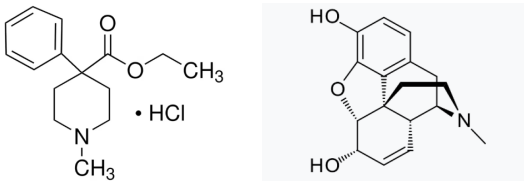
Determination of pharmaceutical substances in biological matrix is crucial in various fields of medicine and pharmacy, e.g., in pharmacokinetic studies, development of new drugs or in therapeutic drug monitoring [1]. Patients frequently take more than one medication at a time, which may interfere in their individual measurements [2]. There are also large interindividual differences between humans; therefore, the concentration of drug might significantly differ between different individuals. The therapeutic effect of drug depends on its concentration in human plasma [3], [4]. Another problem is the occurrence of relatively low concentrations of drugs in human fluids and tissues [5], which should be preconcentrated before analysis [6]. The cloud point extraction [CPE] approach is a green and fast extraction method based on the preconcentration of small amounts of various chemical and inorganic substances using micelle systems [7]. When surfactant molecules in aqueous solutions form micelles at a certain temperature known as "cloud point temperature [8]," the solution turns turbid, and then turbid solution separates into an aqueous and surfactants rich phase above this temperature [9]. The CPE method has various advantages [10] over otherextraction techniques, including high efficiency [11], sensitivity [12], and enrichment factor [13], as well as the use of safe aqueous medium rather than hazardous organic solvents [14], [15].

Opiates are the drugs of choice for short-term treatment of post-surgical and traumatic pain as well as for long-term treatment of severe pain in cancer patients. Meperidine [MPD] [1-Methyl-4-phenylpiperidine-4-carboxylic acid ethyl ester hydrochloride, Pethidine hydrochloride] (Fig. 1a) is a synthetic opioid that belongs to the phenylpiperidine class and is a weak mu receptor agonist [16]. Meperidine is an opioid analgesic used for moderate to severe acute non-chronic pain in patients who have not responded to alternative treatments or who have experienced side effects from them. Constipation,

stomach upset, and mood swings are among its most common side effects [17].

Morphine is the most potent painkiller, it is the reference by which analgesics are assessed, Morphine [MOF] [5α, 6α-didehydro-4, 5-epoxy-17-methylmorphinan- 3, 6-diol] (Fig. 1b) is a therapeutic drug that is normally used for pain control and also abused as an illicit drug [18]. It is recommended by the World Health Organization [WHO] for the relief of moderate cancer-related pains [19]. However, it has lethal effect when abused. As, heroin is hydrolyzed into morphine by an organism; therefore, the determination of the morphine content of biological samples is helpful for clinical and forensic purposes.1,2 To avoid overdose-induced toxicity, it is necessary to accurately monitor the concentrations of morphine in a patient's blood or urine [20].

In the current study, our goal is to develop a spectrophotometric method devise for the MPD and MOF, drugs examination, the two drugs were extracted and subsequently quantified using the safe and affordable reagent Bromothymol blue in an acidic medium. The color product was extracted, dissolved, and spectrophotometrically quantified using the CPE procedure.



a) b)

Figure 1: a) Meperidene [16], b) Morphine [18].

2 MATERIALS AND METHODS

A Shimadzu UV/Vis-spectrophotometer [1260/Japan] supplied with matching quartz cells [50 µL] was used to measure the absorbance of each sample as well as its absorption spectra.

For the CPE process, a centrifuge [Hettich, EBA 21] equipped with standardised centrifuge tubes [50 mL] were utilized to separate the two phases.

In addition, a thermostatic water bath [England] was used to demonstrate different ranges of temperature.

The materials used in the current study were all of an analytical grade. Meperidene and Morphine [purity 99.9%] were provided from the state company for drug industries [Egypt/Pha]. The commercial pharmaceutical applications containing Meperidine [injection and tablet, sun pharmaceutical, India] Morphine [injection, syrup and tablet, Egypt pharma Co] were obtained from local pharmacies.

2.1 Meperidine and Morphine Solution [1000 µg/mL]

In 100 mL volumetric flask, stock solution of drug was prepared by weighing 0.1 g of standard Meperidene and Morphine, dissolving in distilled water, and completed the flask to the mark with the same solvent.

2.2 Hydrochloric Acid [0.5M]

Prepared by transferring 4.2 mL of concentrated HCl [36.4% w/w] to 100 mL volumetric flask, and diluted with distilled water, and then standardized.

2.3 Ammonium hydroxide [1M]

Prepared by transferring 5.34 mL of concentrated ammonium hydroxide [18.69M w/w] to 100 mL volumetric flask, and diluted with distilled water.

2.4 Bromothymol blue [100µg.mL-1]

In 100 mL volumetric flask, stock solution of Bromothymol blue was prepared by weighing 10 mg of standard BTB, dissolving in distilled water, and completed the flask to the mark with the same solvent.

2.5 Triton X-114 [10% v/v]

Prepared by diluted 10 mL of Triton X-114 [99.9%, Fluka] with 100 mL of distilled water.

2.6 Preparation Solutions of Meperidene[MPD] and Morphine[MRN]

The contents of ten tablet covering active ingredient were weighed and the powder was mixed. An exactly weighed portion of the powder equivalent to 50 mg of MPD and MRN was dissolved in 30 mL of distilled water, and then filtered into a 50 mL volumetric flask. The residue was washed with distilled water and diluted to the mark with the same solvent to obtain 1000 µg/mL of the drugs.

2.7 Procedure Cloud Point Extraction Method

Into 10 mL calibrated flasks, 1ml of [5µg/ml] of MPD and MRN were reacted in the presence of 1ml hydrochloric acid [0.5M] a color reaction occurred with BTB after adding surfactant Triton X-114 and heating the mixture in in thermostatic water bath at 60°C For 10 minutes. The Supernatant was Separated from precipitate using centrifuge for 10 minutes [2500 rpm]. The Solution Components were cooled to increase viscosity. Then, two distinct layers were clearly separated. A small quantity of the surfactant - rich phase was extracted using ethanol and complete volume to 10ml with distilled water.

3 RESULTS AND DISCUSSION

3.1 Absorption Spectra

In a series of preliminary attempts to ensure the formation of the complex between the drugs and BTB at concentration of $3x10^{-5}$ M in a acidic medium, an yellow water soluble complex were formed with a maximum absorbance at 512nm, 550nm to meperidine and morphine (Fig. 2 and 3) respectively when measured against reagent blank which has negligible absorption at this wavelength. Thereafter, the absorption spectrum of the product color between 190-900 nm was recorded after obtaining the optimal conditions listed below by applying the general CPE procedure.

3.2 Optimization of Experimental Conditions

The effect of various important factors that impact the efficiency of the cloud point extraction procedure was carried via a follow-up series of experiments using classical optimization which suggests observing the effect of one factor at a time on the instrumental response while other factors are maintained at constant level. Thus, a satisfactory sensitivity of the measurement can be achieved. In this regard, the factors such as, HCl concentration, BTB concentration and Triton X-114 amount, incubation time was studied.

3.2.1 Effect of Acid Type

This study was conducted by using a series experiments. Different acid solution [CH_3COOH, HNO_3, H_3PO_4, H_2SO_4, HCI] were prepared at concentration 0.5M. A constant amount 1mL of each acid added to [5µg/ml] of drugs [Meperidine and Morphine], 2mL of BTB, 2ml of Triton X-114 were added and complete the volume to mark by distilled water. The mixture heating in water-bath at 60 °C for 10 minutes. Then transfer the solution components into test tube and place in a centrifuge at 3600 rpm for 20 minutes. The phase rich in Surfactant and the drug to be extracted is separated. Then the absorbance is measured at 512nm, 550nm, to meperidine and morphine respectively.

The result show in Figures 4 and 5. The figures show the best acid that give highest absorbance HCl [0.550, 0.690, to meperidine and morphine respectively. Therefore it is used in subsequent experiments.

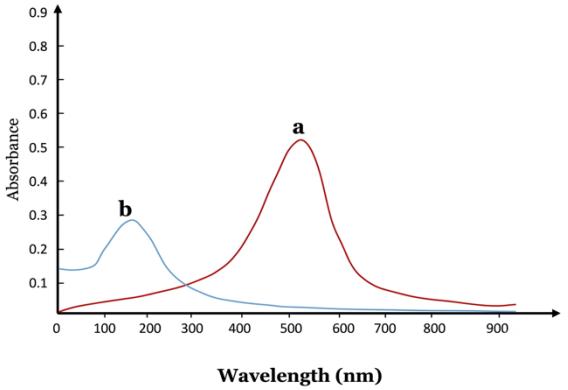


Figure 2: a) The absorption Spectra of Meperidine with Bromothymol Blue Versus reagent blank at [512] nm b) reagent blank Versus distilled water.

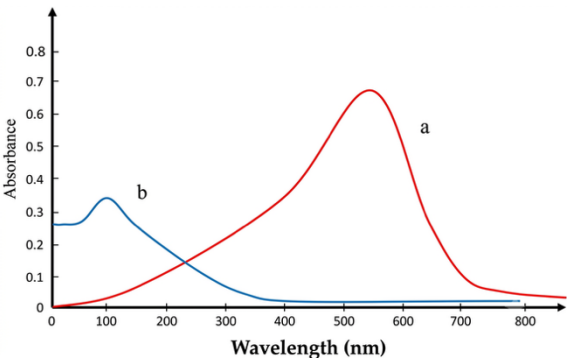


Figure 3: a) The absorption Spectra of morphine with BTB Versus reagent blank at [550] nm, b) Reagent blank versus water.

3.2.2 Effect of pH

This study was conducted by using a series experiments. Adding increasing amount of hydrochloric acid to [5µg /mL] of drugs, 2mL of

BTB, 2mL of Triton X-114 and completed the volume by distilled water. The mixtures of each drug placed in a water bath at 60Cº for 10 minutes. then the Cloud point extraction was applied.

The Figure 6 show the highest absorbance of Meperidine 0.56 by adding 1.2 mL of HCl at pH 4.4. Also the highest absorbance of Morphine is 0.690 by adding 0.8 ml of HCl at pH 5 as shown in Figure 7.

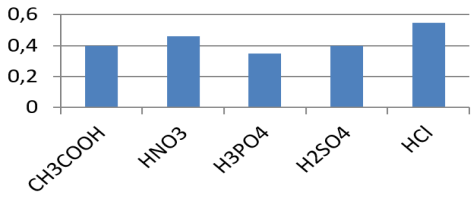


Figure 4: Effect of acid type of absorbance Meperidine drug.

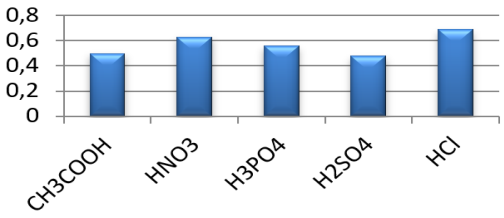


Figure 5: Effect of acid type to absorbance of Morphine drug.

3.2.3 Effect of BTB Volume

Different volumes of BTB reagent were added to [5µg/mL] of meperidine and morphine, with Standard volumes of hydrochloric acid to each drugs and 2mL of Triton X-114. The mixture heated to 60 Cº. After which the cloud Point extraction applied. Figure 8 show the best volume of BTB to obtained the highest absorbance [1mL, 1.5mL] to meperidine and morphine respectively. Therefore it is depended in subsequent Studies.

3.2.4 Effect of Surfactant Type

To find the best surfactant that gives the highest sensitivity. Different type of surfactant were added [Triton X-114, TritonX-100 - Tween 80 - Tween 20], SDS and [TAP] to [5µg/ml] of Meperidine and morphine, with standard volumes of hydrochloric acid and BTB reagent. The mixture heated at 60Cº to 10 mints. After this the cloud Point extraction applied. The absorbance measured at 512 nm and 550 nm to meperidine and Morphine respectively. The result show at Table 1, the best surfactant Triton X-114 that gave highest absorbance.

3.2.5 Effect of Triton X-114 Volume

This study was conducted by using different Volume of Triton X-114 [0.5-3]mL to [5 µg/mL] of Meperidine and morphine with standard volume of hydrochloric acid and BTB reagent. The mixture heated at 60 ºC to 10 min., and cloud Point extraction was applied. The results show at Table 2. The best Volume of surfactant Triton X-114 2mL that gave highest absorbance at 512 nm and 550 nm to meperidine and morphine respectively.

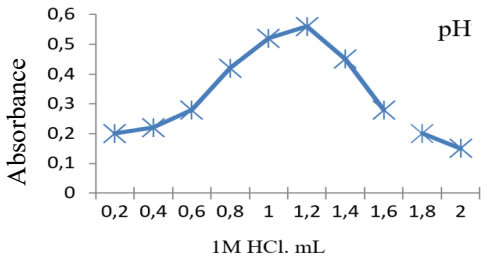


Figure 6: Effect of pH to Absorbance of Meperidene drug.

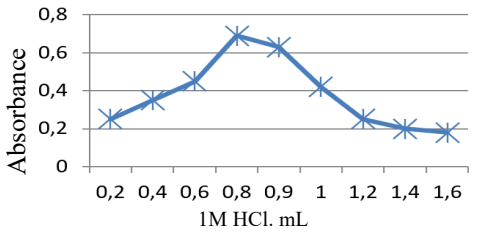


Figure 7: Effect of pH to Absorbance of Morphine.

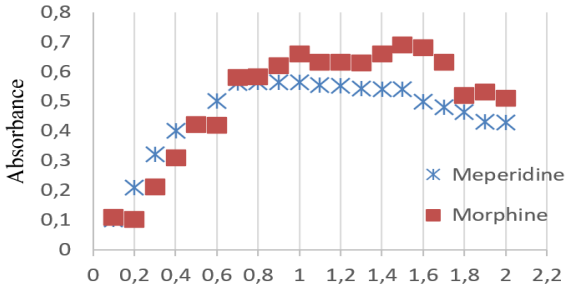


Figure 8: Effect of BTB Volume to obtain highest Absorbance.

3.2.6 Effect of Buffer Solution

This study was conducted by using many experiments. Different type of buffer Solution added to [5µg/mL] of Meperidine and morphine, with standard Volumes of hydrochloric acid, BTB reagent and Triton X-114, The mixture heated at 60Cº to 10 minutes. The Cloud point extraction applied and the absorbance measured at 512nm and 550nm to

meperidine and morphine respectively. The results show at Table 3. Which clearly show the best buffer for drugs [meperidine and morphine] is carbonate [Na_2CO_3 + $NaHCO_3$], therefore depend in subsequent experiments.

Table 1: Effect of Surfactant type to color Products drugs.

Addition of 1mL of each of surfactant	Absorbance of Meperidene at λmax 512nm	Absorbance of Morphine at λmax 550nm
Triton X-114	0.563	0.702
Triton X-100	0.345	0.605
Tween 80	0.235	0.510
Tween 20	0.351	0.431
SDS	0.421	0.321
CTAP	0.367	0.421

Table 2: Effect of Triton X-114 Volume on colored Products of drugs.

Volume of Triton X-100 [0.5-3] mL	Absorbance of Meperidene at 512nm	Absorbance of Morphine at 550nm
0.5	0.421	0.513
1	0.499	0.623
1.5	0.536	0.681
2	0.598	0.743
2.5	0.432	0.654
3	0.401	0.611

3.2.7 Effect the Volume of Buffer Solution

This study was conducted by using a series experiments. different volume [0.2- 2]mL of Carbonate buffer Solution. Were prepared. The results obtained were summarized in Table 4 which clearly show that the best volume of buffer solution with Meperidene is 0.6 mL and 1ml for morphine drug. as it gave the highest absorbance.

3.2.8 Effect of Centrifugation Time on Phase Separation

The effect of shaking time on the absorbance examined over range of 1 to 30 minutes. Using a centrifuge operated at 3500 rpm. It was observed that phase separation didn't occur until after 10 minutes of shaking, as the mixture remained heterogeneous prior to this duration, making measurement impossible under this conditions.

A shaking time of 10 minutes was adopted for all subsequent experiments. The result show in Figure 9.

Table 3: Effect of Buffer Solution to Absorbance of drugs.

Type of Buffer Solution	Absorbance at λmax	
	Meperidene	Morphine
NH_4Cl + NH_4OH	0.491	0.430
Sodium tetra borate + HCl	0.210	0.351
Na_2CO_3 + $NaHCO_3$	0.599	0.770
K_2HPo_4 + KH_2Po_4	0.324	0.251
without	0.510	0.680

Table 4: Effect the Volume of Carbonate buffer Solution to Absorbance of Meperidine and Morphine color Products.

Volume of Carbonate buffer Solution [mL]	Absorbance at λmax 512 of Meperidene	Absorbance at λmax 550 of Morphine
0.2	0.561	0.720
0.4	0.592	0.725
0.6	0.621	0.731
0.8	0.610	0.761
1	0.591	0.798
1.2	0.522	0.751
1.4	0.491	0.681
1.6	0.420	0.631
1.8	0.401	0.524
2	0.400	0.501

3.2.9 Effect of Temperature and Time on Absorbance and Stability of Complex

The effect of temperature and the Stability time of the drugs studied at temperature [20-70] ºC, Using constant quantities of the drugs and optimal amount of reagent, buffer solution and surfactant. The Formation of a turbid solution was observed. It was found that three complexes are formed with highest sensitivity after completing addition and remain stable for more than 55 minutes at a temperature of 60 ºC for Meperidene drug, more than 50 minutes at 60 ºC for morphine. However, it was found the absorbance decrease with increasing temperature above 60 ºC. Therefore a temperature 60 ºC was depended for subsequent studies Tables 5 and 6 show result obtained.

3.2.10 Effect of Addition Sequence

The study was conducted using a series of additions. It is observed from the result in Table 7. The best addition sequence for spectrophotometric solution is pattern [1], as it gave the highest absorbance to meperidine and morphine.

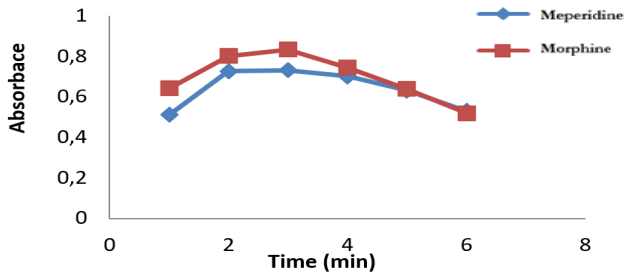


Figure 9: Effect of Shaking time in the centrifuge to the absorbance of (5µg/mL) of drugs color products.

Table 5: Effect of temperature and time on Absorbance and stability of Meperidine complex.

T ºC	Absorbance							
	10min	20min	25min	30min	40min	50min	55min	60min
40	0.297	0.452	0.581	0.593	0.621	0.634	0.641	0.691
50	0.329	0.421	0.429	0.431	0.598	0.652	0.742	0.731
60	0.421	0.431	0.452	0.597	0.652	0.793	0.812	0.710
70	0.592	0.541	0.421	0.310	0.290	0.211	0.121	0.121

Table 6: Effect of temperature and time on Absorbance and stability of Morphine complex.

T ºC	Absorbance							
	10min	20min	25min	30min	40min	50min	55min	60min
40	0.321	0.467	0.541	0.561	0.592	0.631	0.643	0.64
50	0.352	0.451	0.591	0.601	0.623	0.631	0.621	0.60
60	0.321	0.394	0.481	0.598	0.693	0.801	0.700	0.691
70	0.521	0.593	0.432	0.401	0.391	0.342	0.291	0.231

3.3 Calibration Curve and Working Method

Based on the optimal conditions obtained previously for meperidine and morphine two calibration curves were constructed as follows:

To a series of 10 ml volumetric flasks in creasing volumes of each of [MPD, MOF] were added separately. Then [1.2 and 0.8] mL HCI to [MPD, MOF], After that, 2mL of BTB reagent were added individually. [0.6 and 1] mL carbonate buffer solution added to [MPD and MOF] respective. The Volume made up to the mark with distilled water.

Table 7: Effect of addition effect.

No	Addition	Absorbance	
		Meperidine	Morphine
1	D + A + R + S + B	0.821	0.835
2	D + A + B + R + S	0.652	0.721
3	D + B + A + R + S	0.632	0.692
4	D + R + S + A + B	0.521	0.531
5	R + D + S + A + B	0.498	0.420
6	A + B + R + D + S	0.321	0.231

*D: drug A: acid R: reagent [BTB] S: Surfactant B :Buffer.

The Solutions were placed in water bath at 60 ºC. Following the working method CPE described.

The absorbances were measured against blank solutions at 512nm and 550nm [MPD and MOF] respectively.

Calibration curves were obtained that obey Beer's law range [0.1-20] and [0.2-25]µg/mL to [MPD and MOF] respectively. Figures 10 and 11 shows the calibration curves for drugs, which indicate that there in a negative deviation after upper quantification limits.

Table 8: Parameters to determination Meperidine and Morphine.

Parameters	Meperidine	Morphine
Linearity range [µg/mL]	[0.1-20]	[0.2-25]
Molar absorptivity [L/mol.cm]	3.214×10^{3}	2.567×10^{4}
LoD [µg/mL]	0.05	0.024
LoQ [µg/mL]	0.041	0.104
Intercept	0.0643	0.0729
Slope	0.0942	0.0971
Correlation coefficient	0.9952	0.9931

The value of correlation coefficients for Calibration curves were greater than 0.99, indicating they excellent linear Characteristics. The molar absorptivity. reached $3.214x10^3$ and $2.567\ x10^4$ L/mol.cm, for [MPD and MOP] respectively indicating that the method is sensitive. The detection limit 0.05 and 0.024 [µg/mL], and the quantification limit was 0.041 and 0.102 [µg/mL] respectively. Table 8 shows the result obtained.

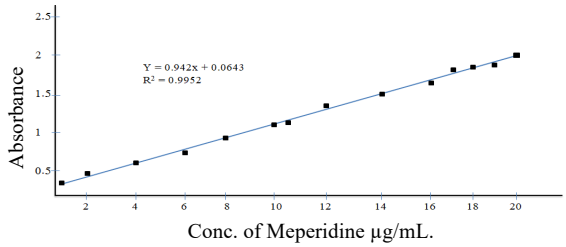


Figure 10: Calibration curve of Meperidine.

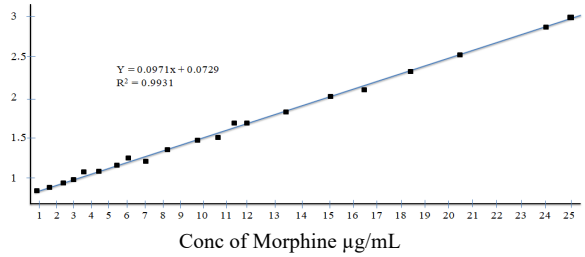


Figure 11: Calibration curve of Morphine.

3.4 Recovery Percentage and Standard Deviation

The recover recovery percentage was calculated by using the final optimized method. In the which three concentration for each drugs. The result indicated that applying method is sensitive, accurate [average recovery [99.38 and 99.79] for MPD and MOP] respectively. Precise [RSD < 3]. The result show in Table 9.

3.5 Effect of Interference

The effect a number of excipients that inter into pharmaceutical preparations was Studied to ensure that the method is selective by adding quantities up to 200 times to a fixed quantity of drugs [ME and MO] [5µg/ml] and the CPE procedure was followed. The results in Table 10 indicate the selectivity of method due to the absence of interference from added excipients which reach up to 100 times.

3.6 Application of the Method on Pharmaceutical Preparations

3.6.1 Meperidine Drug

Different concentrations [5, 10, 15 µg/ml] were taken in a final volume of 10mL with distilled water. The concentration of Meperidine in one ampoule was determined by using the working method CPE. The results obtained in Table 11.

1) Injection of meperidine. The content of one ampoule of Meperidine injection containing 20 µg/ml of Meperidine was taken and diluted to 100mL with distilled water to obtain a concentration of 200µg/ml.
2) Meperidine Tablets. Ten tablets of meperidine [each tablet contains 10mg of Meperidine. They were ground well, and a quantity equivalent to one tablet was weighed, then dissolved in a suitable amount of solvent. The volume was completed to 100ml with distilled water to obtained a concentration of 100 µg/ml. From this concentration [5, 10, 15 µg/mL] were prepared in a final Volume of 10ml distilled Water to estimation the Concentration of Meperidine in one tablet. The results Show in Table 11.

3.6.2 Morphine Drug

The developed method was applied for the determination of morphine in different pharmaceutical formulations, including injection, syrup, and tablets, using appropriate sample preparation and dilution procedures as described:

1) Morphine injection. One ampoule of morphine injection containing 10 mg/mL of Morphine was taken and diluted to 100 mL with distilled water to obtained a concentration 100µg/mL. Different concentration [5, 10, 15] µg/ml were taken in a final volume of 10mL with distilled water. The results obtain show in Table 11.
2) b-Morphine Syrup. When 5ml of the syrup containing 10mg of Morphine diluted to 100ml with distilled water to obtain a concentration of 100µg/mL. A series concentration [5,10, 15] µg/mL were prepared, Then the concentration of morphine in the syrup was determined and the result were recorded in Table 11.
3) Morphine tablets. Ten tablets of morphine, each containing 10mg of morphine, were accurately weighed and ground. A quantity equivalent to one tablet was taken and dissolved in water. The resulting solution was

completed to 10 ml with distilled water to obtain 100 µg/ml. From this Solution [5, 10, 15] µg/mL were prepared. in a final volume of 10mL by dilution. The Concentration of Morphine in one tablet was determined and results were recorded in Table 11.

3.7 Evaluation of the Proposed Method

The reliability of the analytical method was assessed by comparing the results obtained using proposed method with those obtained from British pharmacopoeia method for the pure substance. This was done to determine the accuracy and applicability of the proposed analytical method for the drug Substance. [t-test and F-test] were applied to evaluate the extent of accuracy, applicability and reliability. The result show in Table 12. The results obtain indicates to absence of any significant between the proposed and the official standard methods.

This confirms that the proposed method is reliable and suitable for analytical application to pharmaceutical formulation.

3.8 Comparison of the Method

The proposed method using BTB reagent was compared with spectrophotometric method to estimation drugs. The results show in Table 13 indicate to the proposed method has higher sensitivity than other.

Table 9: The recovery percentage and standard deviation.

Drug	Amount added [µg/mL]	Recovery %	Average Recovery %	RSD
Meperidine	5, 10, 15	98.54, 100.16, 99.45	99.38	1.72, 0.093, 1.210
Morphine	5, 10, 15	101.4, 99.56, 98.43	99.79	1.234, 0.832, 1.670

Table 10: Effect of excipient.

Excipient	Recovery %							
	5 µg/mL of Me				5 µg/mL of Mo			
	20	50	100	200	20	50	100	200
Glucose	100.10	101.02	98.58	100.54	98.31	100.40	101.32	101.55
Lactose	101.23	98.34	98.52	98.67	98.31	100.45	100.55	98.67
Acacia	98.71	101.32	100.46	100.32	98.73	99.14	101.32	101.73
Starch	99.37	98.51	101.32	100.42	100.51	100.46	100.78	100.11
NaCl	98.74	98.61	98.78	101.43	100.67	101.35	101.23	100.45
Arginine	98.74	98.55	98.32	98.43	101.41	101.92	100.56	100.31

Table 11: Application of on pharmaceutical preparations.

Pharmaceutical Preparation	Certified Value mg	Drug amount present µg/mL	Recovery %	Drug content fond [mg]	Average drug content found [mg]
Meperidine injection	20 mg	5, 10, 20	101.42, 98.34, 98.98	19.453, 20.210, 19.234	19.632
Meperidine Tablet	10 mg	5, 10, 20	100.42, 98.95, 101.32	9.721, 10.011, 9.542	9.757
Morphine injection	10 mg	5, 10, 20	101.34, 100.52, 99.41	9.832, 10.421, 9.210	9.821
Morphine Syrup	10 mg	5, 10, 20	98.43, 98.94, 100.59	9.421, 9.874, 9.520	9.605
Morphine Tablet	10 mg	5, 10, 20	98.52, 99.34, 101.52	9.324, 10.521, 10.320	10.055

Table 12: Comparison between the proposed method for determination of drugs in pharmaceutical preparations and the official standard method.

Pharmaceutical Preparation	% Average recovery		t - test	F-test	compound
	Present method	Standard method			
Injection	99.58	100.56	0.805	3.921	Mepereidine
Tablet	100.23		0.342	2.541	
Injection	100.42	99.87	0.256	4.671	Morphine
Syrup	99.32		0.752	3.521	
Tablet	100.52		0.325	2.673	

Table 13: Comparison of the method.

Analytical Parameters	Present method		Literature method	
	Me	Mo	Me	Mo
λ max	512nm	550nm	318nm	280nm
Linearity µg/mL	[0.1-20]	[0.2-25]	0.4-16	0.1-10
Temperature	60c°	60c°	40c°	-
Development time [min]	55min	50min	20min	15min
Molar absorptivity [$L.mol^{-1}.cm^{-1}$]	3.21×10^3	2.56×10^4	7.01×10^3	2.12×10^2
Average recovery %	99.38	99.79	100.56	99.87
RSD %	<1.90	<1.60	≤2.0	≤1.90

4 CONCLUSIONS

A new cloud point extraction (CPE) coupled with spectrophotometric method was successfully developed for the determination of meperidine (MPD) and morphine (MOP) using bromothymol blue (BTB) as a chromogenic reagent and Triton X-114 as a nonionic surfactant. The proposed method provides an effective preconcentration step through the extraction of the formed colored complexes into the surfactant-rich phase, resulting in enhanced sensitivity and improved selectivity compared with conventional spectrophotometric approaches.

The experimental parameters affecting the extraction efficiency, including surfactant type, surfactant concentration, temperature, incubation time, and other reaction conditions, were optimized to obtain maximum analytical response. Under the optimized conditions, the colored complexes showed maximum absorption at 512 nm for meperidine and 550 nm for morphine.

The developed method exhibited excellent analytical performance with high accuracy and precision. The average recoveries were 99.38% for meperidine and 99.79% for morphine, with low relative standard deviation values, confirming the reliability and reproducibility of the procedure. The method also demonstrated good selectivity, as common pharmaceutical excipients did not significantly interfere with drug determination.

The proposed CPE-spectrophotometric method was successfully applied for the analysis of meperidine and morphine in different pharmaceutical formulations without requiring complicated sample preparation procedures. The obtained results showed good agreement with the official method, confirming the accuracy and practical applicability of the developed approach.

Therefore, the proposed method can be considered a simple, sensitive, cost-effective, and environmentally friendly analytical procedure for routine determination of meperidine and morphine in pure substances and pharmaceutical preparations.

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