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Cloud Point Pre-concentration with Spectrophotometric Detection for Determination of Oxymetazoline in Pharmaceutical Formulations

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Abstract

Simple and sensitive method was proposed for enrichment and determination of Oxymetazoline (OMZ) in its pharmaceutical matrix through Cloud Point Extraction (CPE) technique, the method depends on forming misciles by using non-ionic surfactant triton-x114 to enrichment colored azo dye product that results from diazotization-coupling reaction of the OMZ drug with diazotized metoclopramide (also drug) in alkaline medium and subsequently detected spectrophotometrically at 510 nm. The optimal reaction and pre-concentration (such as PH, surfactant concentration, temperature and centrifugations time) conditions optimized, under the optimized conditions, the analytical characteristics were obtained, linearity was obeyed in the range of 0.05-17.0 $\mu g.ml^{-1}$ the limit of detection and the limit of quantification calculated to be 0.047 and 0.15 $\mu g.ml^{-1}$, respectively and enrichment factor was 20. The proposed method was successfully applied to the determination of Oxymetazoline (OMZ) in commercial nasals.

Keywords: Oxymetazoline • Cloud point extraction • CPE-spectrophotometric • Pharmaceutical formulations

Introduction

Oxymetazoline non selective adrenergic drug which has been used as a typical decongestant through promote constriction of the nasal mucosa by constricting blood vessels (veins and arteries) in nasal tissues [1]. Several methods have been studied in the literatures for determination of OXZ drug in the pharmaceutical formulations such as High-Performance Liquid Chromatography (HPLC) [2-4], RP-HPLC [5], ion-selective electrode [6-8], modified carbon paste ion selective electrode [9], fluorimetry [10], UV and derivatives spectrophotometric [11] and visible spectrophotometric [12-16], all these methods are less sensitivity and used many organic solvents with expensive materials needed. Cloud Point Extraction (CPE) was considered a green analytical separation and pre-concentration technique based on the formation of micelles by surfactant at specific condition when surfactant concentration at or above critical micelle concentration formed, there are many applications of cloud point extraction for the pre-concentration and separation of organic substance, pharmaceuticals and metal ions [17,18]. Because of its availability and economic advantages spectrophotometric method is more favorable method for detection, to our knowledge, there is no work reported in the literatures related to the determination of oxymetazoline in any matrices using CPE- spectrophotometric procedure thus, the present work is an attempt to establish a new method for the pre-concentration of OMZ drug using diazotization-coupling reaction employing CPE followed their detection by spectrophotometer at 510 nm.

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Experimental Procedure

Apparatus

All UV–Visible spectra and absorbance measurements were performed using a double beam spectrophotometer Shimadzu model UV- visible 260 digital recording (Tokyo-Japan) equipped with a 50 µL quartz cell with 1cm path length. A thermostatic water bath expert (England) was used for CPE experiments. A Hettich, EBA 21 model centrifuge with 20 mL calibrated centrifuge tubes were used to achieve the separation process.

Reagents and Solutions

Metoclopramide (99.9% m/m) and oxymetazoline (99.9% m/m) was obtained from state company for Drug Industries and Medical Appliance, SDI, Samara-Iraq, NaOH (99.9%m/m), $NaNO_2$ (99.9% m/m) and HCl (36%m/m) were purchased from BDH (England). All reagents used in this work were of analytical reagent grade.

- **Solution of oxymetazoline (OMZ) (500 µg. mL^{-1}):** A stock standard solution of OMZ was prepared by dissolving 50 mg of pure OMZ in water, and made up to volume in a 100 mL calibrated flask with the same solvent.
- **Diazotized Metoclopramide (DMCP) (2 × 10^{-3}M) reagent solution:** 0.0708 g amount of pure metoclopramide was dissolved in an amount of distilled water in a 100 mL volumetric flask, 3 mL of hydrochloric acid (1 M) was add and was placed on ice bath, then a 0.0134 g of sodium nitrite was added, after 1 minute the volume was completed to mark with distilled water and used as stock solution.
- **Hydrochloric acid (1M):** Was prepared by diluting 8.6 mL of concentrated hydrochloric acid (11.64 M) with distilled water in 100 mL volumetric flask.
- **Sodium hydroxide (1M):** A 4 g amount of NaOH was dissolved in a 100 mL volumetric flask with distilled water. 0.1M of sodium hydroxide was prepared by dilution with distilled water.
- **Triton X-114 (4% v/v):** This solution was prepared by dissolving 4 mL of Triton X-114 (purity > 99.9%, Fluka) in distilled water and diluted to mark in 100 mL volumetric flask with the same solvent.

CPE and determination procedure

An aliquot of OMZ drug standard or sample solution was transferred to 10 mL volumetric flask in the total concentration range for calibration graph (0.05-17 µg.mL^{-1}), 2 mL of DMCP (2 × 10^{-3}M) reagent, 1 mL NaOH (0.1 M), and 3 mL of triton-x114 (4%) were added, mixed and diluted to the mark with distilled water (as showed in (Figure 1), then transferred to 10 mL centrifuging tube and kept in thermostatic water bath for 5 min at 40°C. separation of aqueous and rich- surfactant phases was accelerated by centrifugation at 3500 rpm for 10 min, after that the mixture was cooled in an ice bath for 1 min to increase the viscosity of the surfactant-rich phase and aqueous phase was decanted by inverting the tube. The surfactant-rich phase was dissolved with 2 mL of ethanol and the absorbance of the colored product measured at 510 nm.

Sample's preparation of pharmaceutical for OMZ determination

Nazordin drops, (200 µg.m^{-1}): Provided from the State Company for Drug Industries and medical appliances (SDI), Sammara-Iraq.

Two containers of drug (each contains 10 mL of 0.05% OMZ) are mixed, then 20 mL of the above solution was diluted with distilled water to 50 mL in a volumetric flask to prepare a solution of 200 µg.mL^{-1} OMCl.

Blocked Nose Relief, (200 µg.mL^{-1}): Provided from Gal pharm company-UK. Two containers of drug (each contains 15 mL of 0.05% OMZ) are mixed, then 20 mL of the above solution was diluted with distilled water to 50 mL in a volumetric flask to prepare a solution of 200 µg.mL^{-1} OMCl.

Results and Discussion

Diazotization-coupling reaction between diazotized reagent (DMCP) and OMZ drug in alkaline medium was performed to form pink colored azo-dye (Figure 2), which observed maximum absorption at 510 nm wave lengths against the blank as obtained in Figure 3. For increasing the sensitivity and selectivity for the determination of OMZ in the pharmaceuticals, pre-concentration for OMZ was done using CPE technique, which involves adding for non-ionic surfactant Triton-x114 and attained to its cloud point in the thermostatic water bath and then phases separation. Factors affecting in the reaction sensitivity and extraction efficiency were studied as followed.

Effect of alkaline medium type

Mechanism of coupling reaction for phenolic drug (OMZ) and diazotized reagent needed alkaline medium to be done, thus type of alkaline medium must be studied using varies types as bases such as (NH_4OH, Na_2CO_3, NaOH and CH_3COONa) with follow the determination procedure as above, as shown in Figure 4 the results obtained using NaOH (0.1M) gave higher absorbance than other bases because of strong base made suitable medium for reaction, therefore, it was used in furthered experiments.

Effect of the volume of NaOH

pH is the most important parameter that affect the reaction complement and extraction efficiency because the reaction occurred in a narrow range of slight basic medium, also pH influence on the missiles formation and extraction performance, the pH effect was studied by varying in the volume of NaOH (0.1 M) added during determination procedure and other condition constant, as shown in Figure 5 was observed at 3 mL. Maximum extraction recovery percentage and absorption obtained, and when added more volumes of NaOH the absorbance of colored product decreased due to side reaction occurred with reagent molecules in high basic medium, thus, 3 mL of NaOH was chosen as optimized factor for determination of the OMZ drug in the work.

Effect of DMCP reagent concentration

The effect of Diazotized Metoclopramide (DMCP) reagent concentration was investigated using different volume (0.5-6 mL) of DMCP while other condition constant, the results obtained are shown in Figure 6, the absorbance and extraction recovery increased with increasing the volume of reagent, and

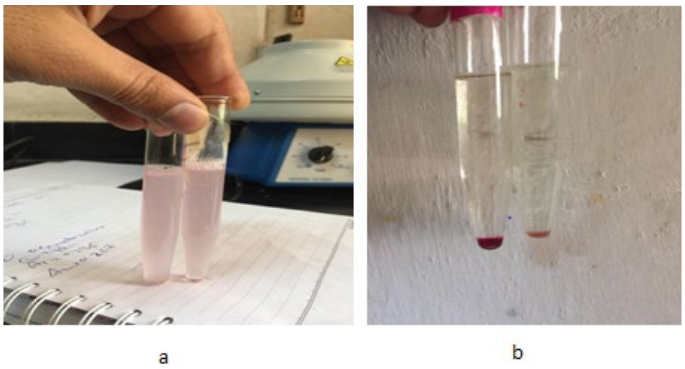

Figure 1. Determination procedure steps, **a**-Before incubation; **b**-After centrifugation.

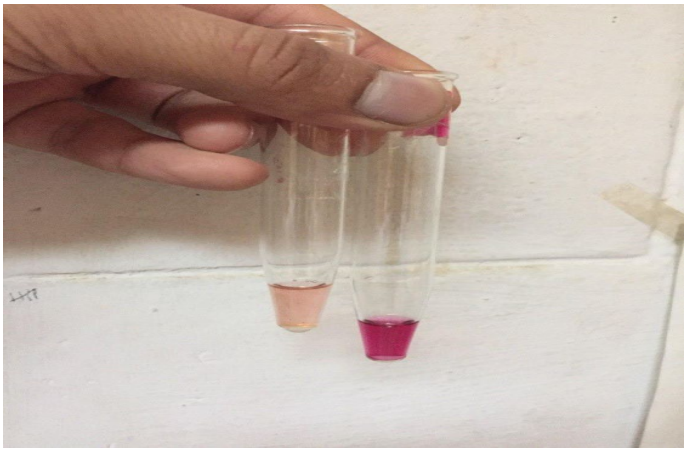

Figure 2. Primary investigation of colored reaction.

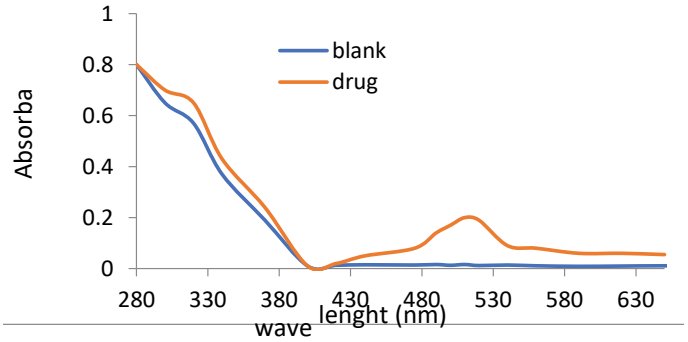

Figure 3. Absorption spectra of 20 µg/mL OMZ measured against reagent blank without CPE, and the reagent blank measured against distilled water.

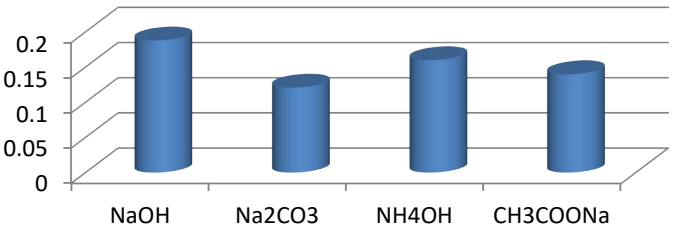

Figure 4. Effect of alkaline medium type.

reached maximum at 3 mL, then decreased immediately when more volumes of reagent were added, therefore, a volume 3 mL of (2 × 10^{-3}) M DMCP was chosen as optimal for complete coupling reaction.

Effect of surfactant concentration

To improve efficient CPE extraction procedure, most affected parameter concentration of non-ionic surfactant was investigated within its concentration in the range (0.1-3.0 mL) of 4% of triton-x114 with measuring the absorbance

of rich surfactant phase, shown in Figure 7, the extraction recovery and absorbance increased with the increases surfactant volumes and reach maximum at 1 mL, and then still constant with slit decreasing when surfactant volume increased, therefore, 1 mL was selected to use in the further experiments.

Effect of temperature and time of heating

Temperature is necessary factor that aids surfactant attained its cloud point and form viscose rich-surfactant phase and time of incubation to reach equilibrium between two phases for separation procedure, temperature and time of heating effect were studied in the range of (10-80°C) and (2-15 min) respectively. The results shown in Figures 8 and 9 were observed when the temperature at 40°C and time at 5 min maximum absorbance obtained, thus, 40°C for 5 min were chosen as optimal factors.

Effect of separation time

For improving completed separation of two phases, rich-surfactant and aqueous phase, time of centrifugation was investigated in the range of (2-15 min) at 3500 rpm, and the results obtained were shown in Figure 10, indicate complete separation was done at 10 min, because of this time was sufficient for complete separation between aqueous phase and surfactant phase, thus, was selected as optimal parameter.

Analytical characteristics

Analytical features of the proposed method were calculated under the optimal condition by constriction of the calibration curve, all features were summarized in Table 1 such as concentration dynamic range, limit of detection and quantification limit, regression equation, correlation of determination, also the intercept, slope, and molar absorptivity as analytical statistical treatments [19] are calculated. These features (listed in Table 1) referred that this proposed method determines wide range of concentrations with lower detection limit, high sensitivity and high precision separation method. The features obtained from applied CPE were evaluated such as pre-concentration factor was 20 and improvement factor was calculated as the slope ratio of the calibration curves before and after extraction was 11.5 [20].

- Ratio of colored complex of OMZ concentration before and after the CPE method

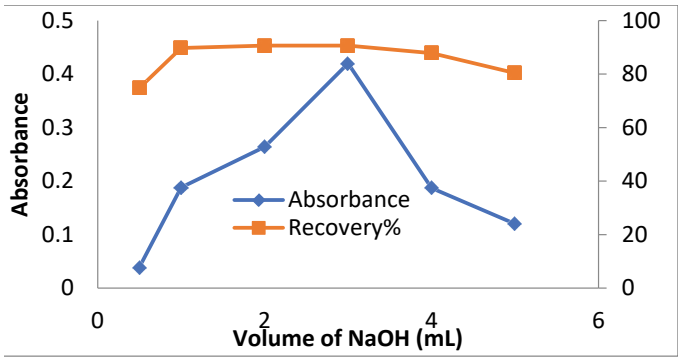

Figure 5. Effect of the volume of NaOH (0.1 M).

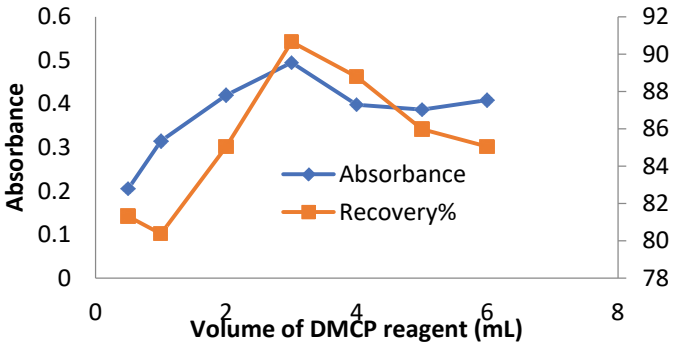

Figure 6. Effect of DMCP reagent concentration.

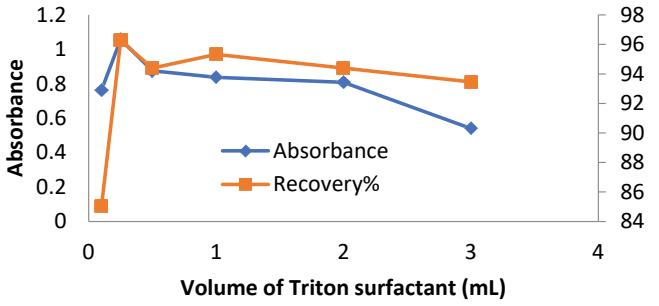

Figure 7. Effect of triton-x114 concentration.

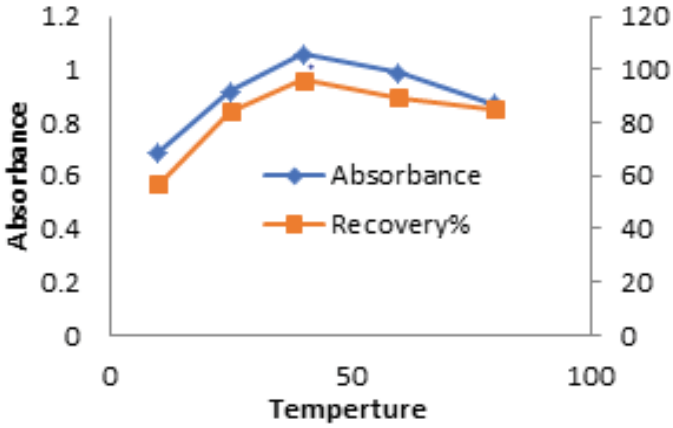

Figure 8. Effect of temperature.

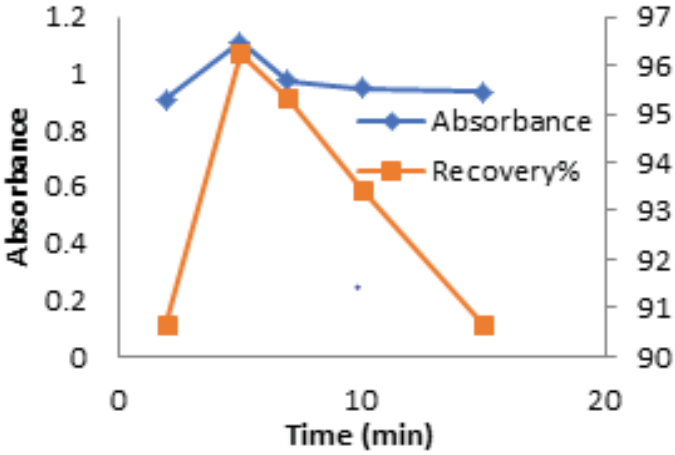

Figure 9. Effect of time of water bath.

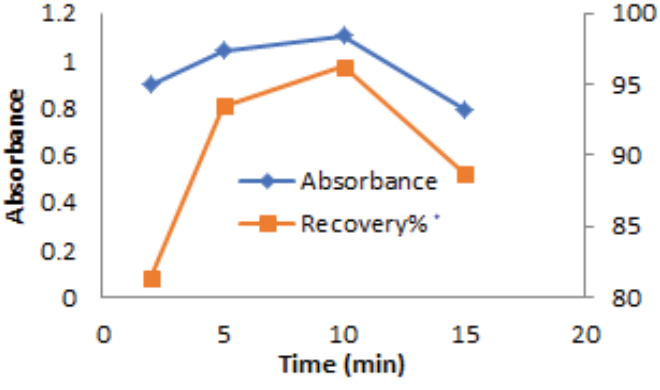

Figure 10. Effect of separation time.

Table 1. Analytical values of statistical treatments for the proposed method.

Parameters	Value
Regression equation	y = 0.104x+0.020
Correlation coefficient, r	0.9979
Linear range (µg/mL)	0.05-17
Limit of detection, LOD (µg/mL)	0.047
Limit of quantification, LOQ (µg/mL)	0.158
Average of recovery (%)	99.67
RSD (%)	4.46
Molar absorptivity (L/mol.cm)	2.7×10^{4}
Sandell′ s sensitivity, S (µg/cm^2)	9.6×10^{-3}
Slope, b (mL/µg)	0.104
Intercept, a	0.020
Precocentration factor*	20
Improvement factor **	11.5

*Ratio of colored complex of OMZ concentration before and after the CPE method.
**The slope ratio of the calibration curve of the CPE method to that calibration curve without enrichment.

Table 2. The accuracy and precision of the proposed method.

Concentration, µg/mL		E (%)	Rec. (%)	RSD %
Present	Found			
2.00	1.98	-0.91	99.08	4.15
5.00	4.95	-0.80	99.19	3.80
10.00	10.07	0.73	100.73	5.43

Table 3. Applications of the proposed method in nasals samples.

Dosage form	Taken conc. (µg/mL)	Spiked conc. (µg/mL)	Found conc. (µg/mL)	Rec. (%) a	Mean	RSD	Mean RSD%
Nazordin nasal drops (0.05%,10ml) SDI-Iraq	2.00	-	2.09	104.17	102.96	5.34	3.56
		1	3.17	105.75		3.80	
		2	4.20	105.24		3.67	
	7.00	-	7.12	101.75		1.78	
Blocked Nose Relief Nasal drops (0.05%, 15 mL) Gal pharm-UK	2.00	-	2.00	100.23	100.81	3.11	2.86
		1	2.95	98.95		4.40	
		2	3.98	99.73		3.83	
	7.00	-	7.09	101.39		2.26	

Table 4. Comparison of the proposed method with other spectrophotometric methods.

Method (description)	Conc. (µg/mL)	Mean Rec.%	Mean RSD%	LOD (µg/mL)	Remarks	Ref.
Direct UV method at 320 nm, 1st and 2nd derivatives	Apr-22	99.86	0.54	0.24	Low detection limit and selectivity	11
Kinetic method based on oxidation with $KMnO_4$ in alkaline medium	0.5-10	99.67	0.92	0.29	Narrow conc. Range and less selective	12
OMZ reduce Fe^{+3} to Fe^{+2} and the form-colored complex with 1,10 phenanthroline	0.1-7.0	100.53	1.6	-	Narrow conc. range and less sensitive	16
Charge-transfer reaction between OMZ with DDQ at pH-9.79 and measured at 421 nm	0.4-4.0	99.48	1.47	0.0093	Narrow Conc. range and low sensitivity	13
Oxidation coupling reaction with 2,4-DNPH and KIO_4, then measured at 607nm	Jan-20	99.01	1.58	-	Lower sensitivity and high Temperature used	14

- The slope ratio of the calibration curve of the CPE method to that the calibration curve without enrichment.

For improvement accuracy and precision of the proposed method, three different concentrations were analyzed according to proposed procedure for five replicates. The results obtained summarized in Table 2, that observed small percentages of RSD% and relative recoveries indicated that the suggested method shows good precision and accuracy respectively.

Determination of OMZ in pharmaceutical forms

To estimate the analytical applicability of the suggested method, it was applied to determination of OMZ in the nasal's products using both direct and standard addition methods, five replicates determination for three different concentration were done for individual nasal product. The results are summarized in Table 3. That show the average percentages were 101.89%, 3.21% for recovery and RSD respectively, these results maintained the proposed method could be successfully applied to the determination of OMZ in the nasal products. Also, the results compared with other validated spectrophotometric methods reported recently in Table 4, we noted though remarks comment the suggested method was more reliable procedure with low cost, more selective than UV spectrophotometric methods and wider range than colorimetric procedures and new separation-spectrophotometric method for oxymetazoline drug.

Conclusion

A green, simple and high sensitive CPE-spectrophotometric method was proposed for determination of oxymetazoline drug in nasal drops matrices and non-ionic surfactant has been used for enrichment of OMZ drug, in comparison with previously reported methods of OMZ there is no extraction procedure received, thus, this suggested method considered a new reliable and high sensitive method with no toxic solvents used, its analytical features indicated that good reproducibility, wider dynamic range and lower detection limit, which successfully applied for estimation of OMZ drug in its pharmaceutical forms.

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