History of Medicine, 2023, 9(1): 1233–1241
DOI: 10.17720/2409-5834.v9.1.2023.147

Spectrophotometric assessment of Amino drugs employing flow injection analysis and cloud point extraction techniques

Dalia M. Jamil[1*], Nisreen K. Abood[2]

[1] Department of Chemistry, College of Science, Al_Nahrain University, Baghdad, Iraq.
[2] Department of Chemistry, College of Science, Mustansyria University, Baghdad, Iraq.

*Corresponding author: Dalia M. Jamil

Received: 20 January 2023 **Accepted:** 15 April 2023

Citation: Jamil DM, Abood NK (2023) Spectrophotometric assessment of Amino drugs employing flow injection analysis and cloud point extraction techniques. History of Medicine 9(1): 1233−1241. https://doi.org/10.17720/2409-5834.v9.1.2023.147

Abstract

The first method on the oxidative coupling Cefotaxime reaction with 2,4- dinitrophenyl hydrazine and Sodium hydroxide to produce a soluble Reddish- Orange dye product was devised to estimate the Cefotaxime in bulk and pharmaceutical preparation. The concentration range is between 1 and 50 g/mL. Beer's law is met by the product in terms. With a molar absorptivity of 6.6Ч104L.mol-1.cm-1 and a correlation coefficient of 0.9995, Sandell's sensitivity is 6.85Ч10Їi g.cm-2 at wave length 615 nm. A second technique for determining a trace amount in an aqueous solution product is called cloud point extraction (CPE), and it is based on measurements made with a UV-visible spectrophotometer at a wavelength of 610 nm. The correlation coefficient was 0.9995 and the Beer's law concentration range was 0.25−6 g/mL The molar absorptivity was 1.4Ч104 L.mol-1.cm-1. Pre-concentration 25, detection of limit 0.021 µg/mL. The distribution coefficient (D) was 298.97, and enrichment factor was 18.59. The last method is flow injection analysis, which is straightforward to estimate. The Beer's law concentration range was 2-150 µg / mL, Sandell's sensitivity is 0.11 µg.cm-2 at 605 nm, with a molar absorptivity of 4Ч103 L.mol-1.cm-1 and correlation coefficient was 0.9995, it is successfully used for drug estimation. Qualities in a straightforward situation or pharmacological formulations.

Key words
Cefotaxime, Batch method, Cloud point extraction, Flow injection analysis.

Cefotaxime is a member of the -lactam family of third-generation cephalosporin antibiotics. Cefotaxime is widely prescribed due to its broad antimicrobial range and high tissue penetration. Serious infections are prevalent in critically sick children, including Meningitis and severe, unrelated sepsis. Even with its frequent usage in children with serious illnesses, pharmacokinetic (PK) evidence for proper dose in this particular population a rarity [1].

The IUPAC designation for Cfm is sodium salt of cefotaxime (6R,7R) -3[(acetyloxy)methyl] -7-[[(2Z)-2-(2-aminothiazol4-yl)-2-(methoxyimino)acetyl]amino] 8-oxo-5-thia1-azabicyclo[4.2.0] Oct-2-ene-2-carboxylate is used [1]. Cfm is an antibiotic from the third generation of cephalosporins. As depicted in Figures 1, Cfm contains the -lactam core ring of cephalosporins [2]. The third generation of cephalosporin antibiotics includes cefotaxime Cfm. It is widely utilized in medically recommended antibiotic medications as an anti-infection for both gram-positive and gram-negative pathogens. The goal of the research was to create an HPLC method of Cfm analysis that was extremely linear, repeatable, robust, rugged, selective, quick, and cost-effective to employ. A BDS column

(150 mm x 4.0 mm x 5 m) in reversed phase is used in the chromatographic process [3].

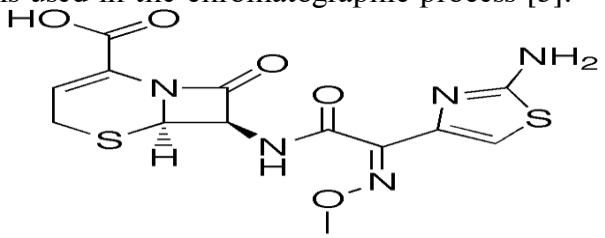


Figure 1: Structure of Cefotaxime

Different analytical techniques are used to determine cefotaxim. These techniques include HPLC and spectrophotometry. [4-6]. The coupling reaction between diazotized cefotaxime and 3,5-dimethyl phenol (3,5-DMPH) in basic medium to form light orange, water soluble dye, that is stable and has a maximum absorbance at 497nm [7]. For the determination of sodium cefotaxime in both pure form and injections, flow-injecton spectrophotometric techniques (FIA) were developed. These procedures relied on a diazotization and coupling reaction between thymol and diazotized sodium cefotaxime in an alkaline solution to create a strong red water-soluble dye that is stable and has a maximum absorption at 522 nm [8]. Typically, both humans and animals are treated with antibiotics known as β-lactams to prevent illness. Due to the antibiotics' environmental discharge, there are substantial issues [9].

The measurement of cefotaxime-dose Na's and pure forms quantitatively. The diazotization of cefotaxime-Na and subsequent coupling with 8-hydroxyquinoline in an alkaline medium formed the basis of the spectrophotometric approach. Maximum absorption of the resultant azo dye was seen at 551 nm [10]. There are numerous analytical techniques for the determination of cefotaxime sodium, including HPLC [11, 12], LC-MS [13], HPTLC [14], electrochemical [15], chemiluminescence [16], cyclic voltammetry and adsorptive stripping differential pulse voltammetry [17], flow injection analysis [18], infrared spectroscopy [19], capillary zone electrophoresis [20], spectrophotometric-kinetic [21], The literature reports the use of extraction-flotation technology [22] and turbidimetric flow injection [23].

We demonstrate the ability of the aforementioned medications to transform Ag+ ions into silver nanoparticles (Ag-NPs) when PVP is present as a stabilizing agent, leading to extremely potent surface plasmon resonance. Ag-NP peak (max. = 410−430 nm) [24].

Instrumentation

Single-beam Spectrophotometric UV Visible 295 (Lasany-India) Quartz spectroscopic cells in sizes of 1 cm and 0.5 cm, along with a thermostatic and ultrasonic device Elma's water for bathing Additionally, Hans Schmidbauer Gmbh. Co.KG is used in conjunction with sample extraction. Manifold for two-channel flow injection as shown in figure2.

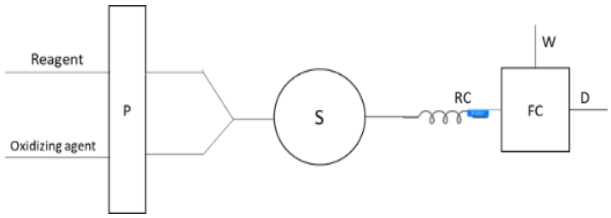


Figure 2: Scheme of the employed flow system, P: peristaltic pump, R.C: reaction coil, S: sample injection, W: waste, FC: flow cell

Chemicals and Reagents

Cefotaxime can be provided from Samarra Drag Iraq (SDI) as purity 99.7%, which were of analytical purity. Cefotaxime was delivered to the quality control laboratory. (The general company for the manufacture of medicines and medical supplies Spain, Ukraine).

Standard solution preparation and Standard Reagents Pharmaceutical

Cefotaxime solutions from the Spain and Ukraine 10mL containers were carefully weighed, and the average weight was taken from each tablet. That prepares 100 mL of D.W. with 1000 ppm from each tablet.

Reagents

Stander solutions of 1000 ppm by dissolving 0.1 mL of pure drug in water and adding volumetrically to the mark, Cefotaxime was created. 0.1 g of 2,4 - di nitro phenyl hydrazine (1000 g/mL) should be dissolved in 100 mL of distilled water to make a standard solution, which should then be diluted to the

appropriate concentration. Preparation 5% w/v Na_2SO_4, 10% CTAB, 10% Triton X-114, and 4% sodium hydroxide and 0.3644g in 100 ml 0.1g in 100ml Sodium sulfate.

Making a standard solution and the necessary Reagents Standard

The solutions of pharmaceutical Cefotaxim supplied from the Spain, and Ukrane Vial (1g) were carefully weighed; the average weight was extracted from the individual powder. That prepares
1000ppm from all powder in 100 mL D.W.A general

Basic Technique Oxidative Coupling

The best procedure to prepare oxidative coupling reaction of Cefotaxime by adding (1mL 2,4-di- nitro phenyl hydrazine) to the Oxidizing agent (1000ppm, 1mL) potassium ferric cyanide to form the Reddish- Orange solution that give absorbance at λ max 615nm.

Extraction of cloud points: a general process (CPE)

Various ranging from 0.25 to 6 µg/ mL with dye 2mL with 1mL of triton x-114, 1mL CTBA and 2mL of Na2SO4 with complete 12.5 mL distilled water. Test tube placed in the heating until the formulation cloudy and separation two phases is content then transfer in the centrifuge for 2 min until 4000m2/sec. then put in the ice water to produce the clear cloud. Add 0.5 mL ethanol and shack the tube and measured the spectrum to determine the wavelength.1000 g.m-1 stander solutions Cefotaxim was made by dissolving 0. 1 g of pure medication dissolved in water and filling volumetrically to the mark. 100 mL flask with distilled water a standard solution of by dissolving 2,4- di nitro phenyl hydrazine (1000 g/mL) 2,4- di nitro phenyl hydrazine, 0.1 g in distilled water in a 100ml volumetric flask, dilute to the desired concentration. Preparation 4%, sodium hydroxide, 10% Triton X-114, 5% w/v Na_2SO_4, and 10% CTAB (cetyle trimethy ammonium bromide) 0.3644g in 100ml D.W.

General flow injection technique

A 100µL of CFM pure or pharmaceutical was injected into system carrier, the manifold of this reaction consist from three channels. The first channel carry the reagent ($4.5Ч10^{-3}$) M 2,4-dinitrophenyl hydrazine, second channel carry the oxidizing agent ($4.6Ч10^{-3}$) Sodium Iodate hydrate, by using T-shaped, then injected the drug. The reaction mixed in reaction coil (50) cm, the absorbance of the produce Reddish- Orange at λmax 615nm.

Result and Discussion

An oxidative coupling reagent (2, 4- di nitro phenyl hydrazine) and an oxidizing agent are used to produce the Reddish- Orange color with a wavelength of 615 nm. The idea behind this technique is to increase CFM' sensitivity. The oxidative coupling spectra are showed in Figure 3.

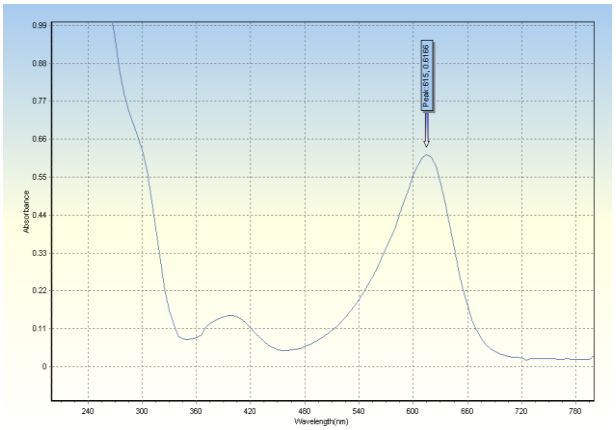


Figure 3: Absorption spectrum for 100 µg/mL CFM with the reagent against the reagent blank under optimum Conditions

The best oxidative medication pairing was studied.

Numerous factors, including the order of addition, have been studied in relation to colored dye absorbance. Due to the high absorption at this wave length, the addition of reagent + oxidizing agent + base + Drug has the best effects. The best absorption occurs in base media, as indicated in figure 4, which also influences the character of the media. And the coupling reagent's impact Volume, the ideal reagent volume in 1 mL is depicted in figure 5. The best volume of the reagent was 1 mL at the conclusion of the oxidizing agent's impact in base medium (1.5) mL, as illustrated in figure 6. Effect of Temperature; ideal temperature is 25^0C. Effect of Time, because the compound is broken down after 60 minutes and cannot react, the best time is 20

min. and measured right away.

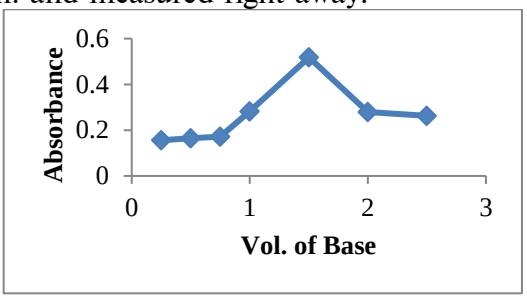


Figure 4: Effect the base Volume

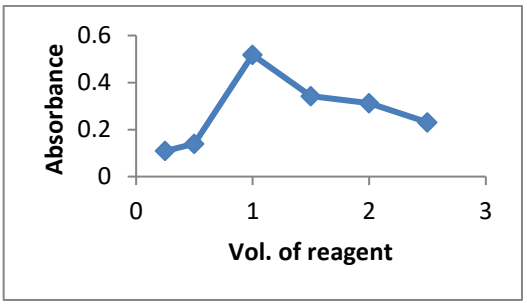


Figure 5: Effect the reagent Volume

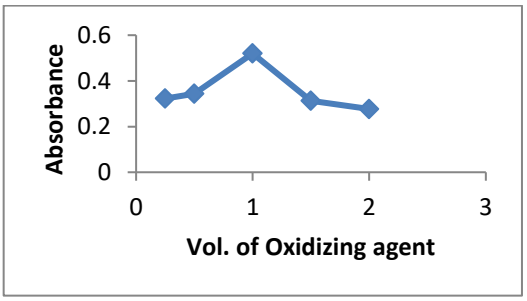


Figure6: Effect Oxidizing Volume of agent

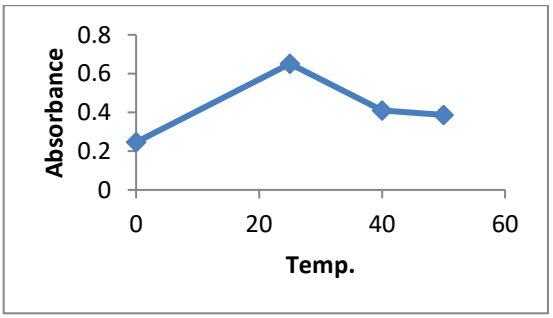


Figure 7: Effect the Temperature

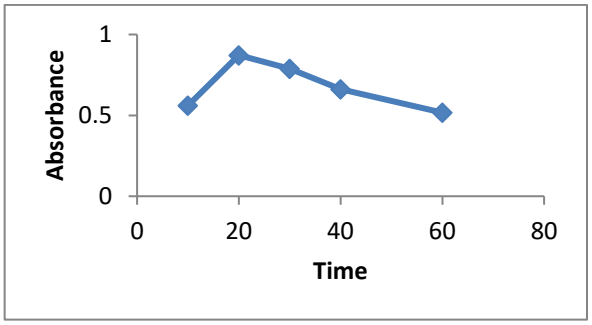


Figure8: Effect of Time

To determination the reaction of CFm with 4-AAP,mole ratio method was used to indicates that the formed dye that have composition ratio 1:1 as shown in figure 9.

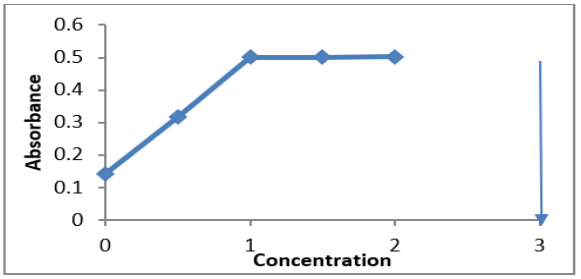
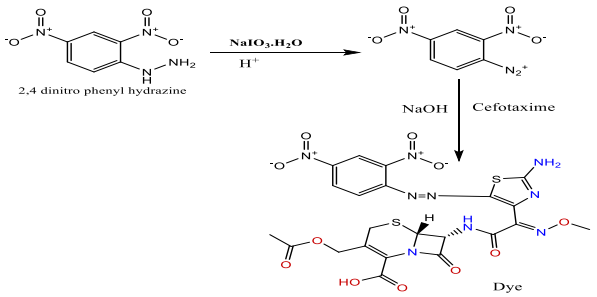


Figure 9: Mole ratio of CFM and 2,4- di nitro phenyl hydrazine

Thus, the creation of dye colored in accordance with the medication and reagent reaction in the current oxidizing agent shown in figure 10.



After the optimization of the CFm oxidizing coupling, the calibration graph was made using different CFO concentrations (1−50 g/mL), and the correlation coefficient (R), slope (a), and intercept (P) of the linear regression equation were determined as shown in figure 11.

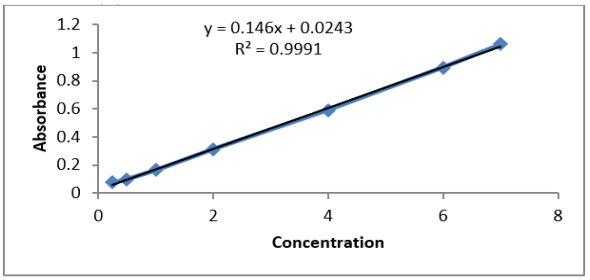


Figure 11: Calibration Curve of the Oxidative Coupling of Cefotaxime

Cloud Point for Cefotaxime Oxidative Coupling

Triton X-114 (0-2.5) mL amounts were examined to determine their effects. When it comes to enhancing your income, TritonX-114 up to 0.5 mL boosted the process's absorption while decreasing it at higher doses. In this instance, 1 mL of TritonX-114 was selected, as seen in figure 12. The nonionic of the CMC of

the surfactant decreased as the temperature increased, showing how temperature affects the effectiveness of CFM extraction. Figures 13 and 14 illustrate the results of a study using various CTAB and salt volumes.

The proportion of hydrophobic micelles increased with temperature. because Triton has increased in the Surfactant Process Due to the extraction capacity and X-114 spacing Dehydration of the external micelle layer. Because of the viscosity, CFM absorption reduced after 40 ^{0}C whereas it rose at 40^{0}C. Rising extraction the aqueous and rich phases of the surface must be in equilibrium before extraction via the cloud point may take place. Higher micelle concentration in the material. The amount of heat that had collected in the solution during this period allowed Micelles to lose water molecules and form a small amount of hydrophobic material. Time to incubate (10–60 minutes) Viscosity is important because it traps dye quickly, and temperature It was agreed to cook the food for 20 minutes at a temperature of 40 °C. Figures 15 and 16 show the results, respectively.

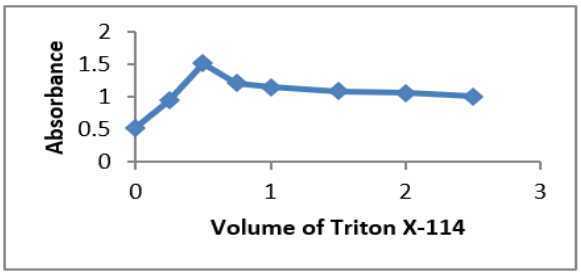


Figure 12: effect Triton- x114 Volume

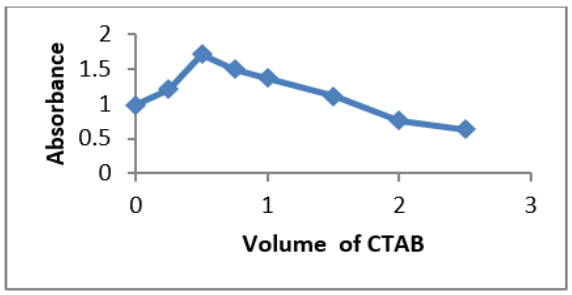


Figure 13: Effect the Volume CTAB

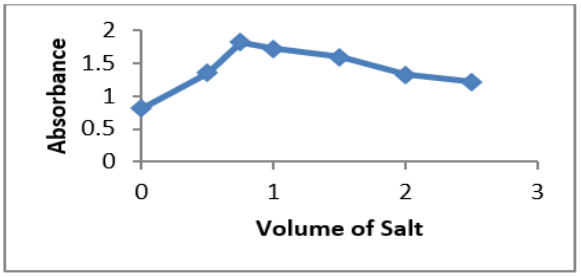


Figure 14: Effect the Volume Salt

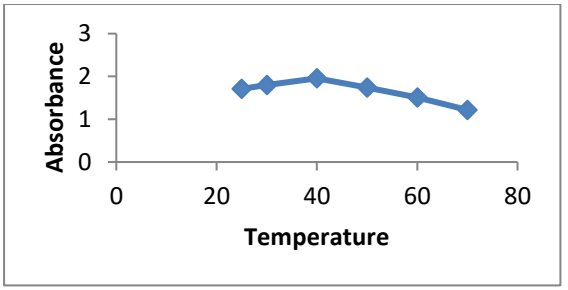


Figure 15: Effect Temperature

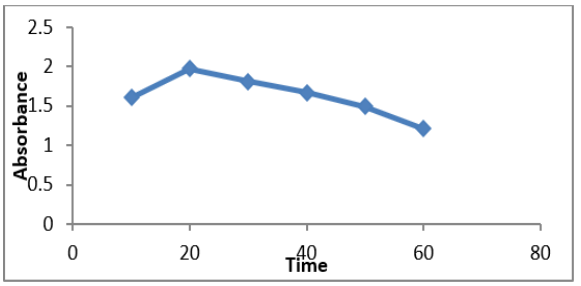


Figure 16: Effect Time incubation

After making a calibration graph with different CFM concentrations (0.25-6)g/mL, the optimal cloud point extraction of CFM, the slope (a), and the intercept (P) were computed, as shown in figure 17.

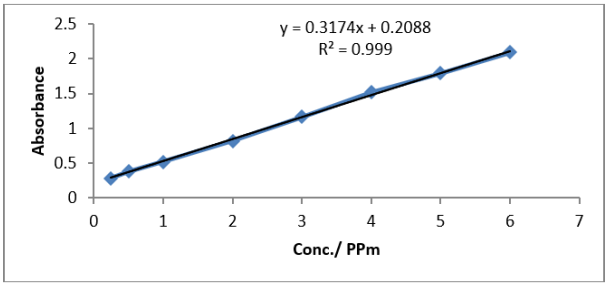


Figure 17: Calibration Curve of the Cloud Point extraction of Cefotaxime

Optimum reaction conditions of flow injection analysis technique

Different parameter chemical and physical study such as concentration of reagent, concentration of oxidizing agent, reaction coil and flow rate , the results show in figure,18,19,20,21 and 22 respectively.

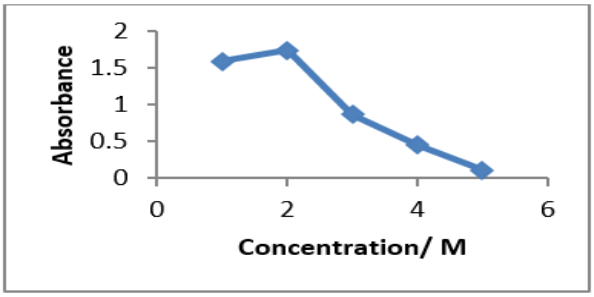


Figure 18: different Reagent Concentration

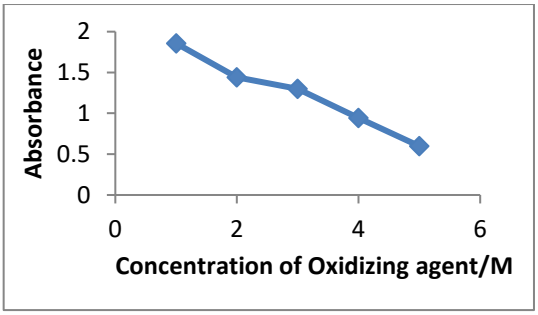


Figure 19: different concentration of oxidizing agent

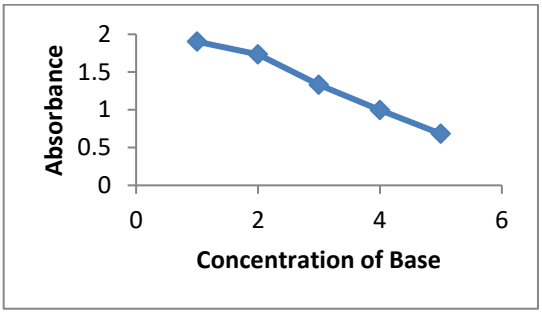


Figure 20: different concentration of base

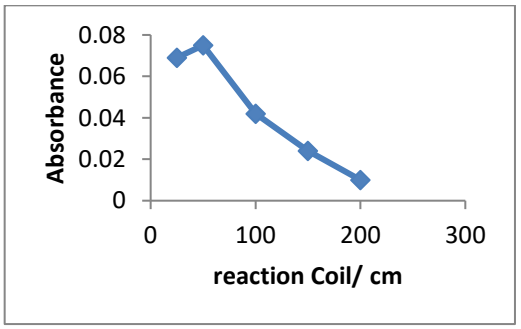


Figure 21: Change in reaction coil

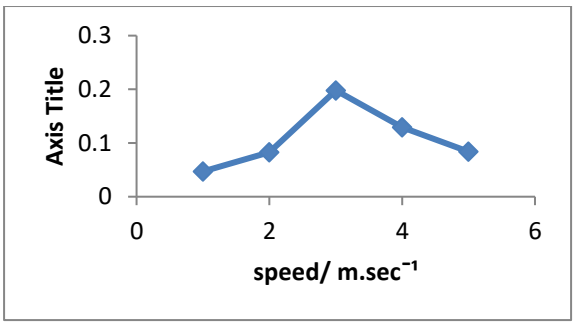


Figure22: Effect the rate

After performing an optimal flow injection study of CFM, creating a calibration graph for CFM at various concentrations (2-150 g/mL), and calculating the correlation coefficient (R), slope (a), and intercept (P) of the linear regression equation, the results are displayed in figure 20. The regression equation's characteristic parameter for the proposed oxidative coupling CPE and FIA methods is provided in Table 1.

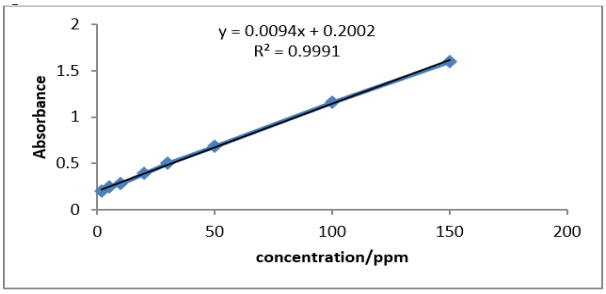


Figure 20: Calibration Curve of the Flow Injection of Cefotaxime

Table 1. Characteristic parameter for the regression equation of the proposed oxidative coupling CPE and FIA methods.

Parameters	Oxidative coupling	Cloud point extraction	Flow injection analysis
λ_{max} nm	615	610	615
Color	Reddish-Orange	Deep Orange	Reddish-Orange
Regression equation	Y=0.146x+0.0243	Y=0.3174x+0.2088	Y=0.0094x+0.2002
Linearity range(µg/mL)	1-50	0.25-6	2-150
Correlation Coefficient (r)	0.9995	0.9995	0.9995
ε(L.mol^{-1}.cm^{-1})	6.6Ч10^{4}	1.4Ч10^{4}	4Ч10^{3}
Sandal' sensitivity (µg . cm^{-2})	6.85Ч10Ïi	3.15Ч10Ïi	0.11
Analytical sensitivity	0.366	0.47	0.019
Slope (b)	0.146027	0.3174371	0.009428
Intercept(a)	0.0243	0.2088	0.2002
Limit of detection(µg/mL)	0.023	0.021	0.35
Limit quantification(µg/mL)	0.07	0.063	1.06
Enrichment Factor (EF)	------	2.17	------
Pre-concentration factor (PF)	-------	25	------
Distribution coefficient(D)	------		------

Precision and accuracy

Examine the precision and accuracy of the suggested procedures, including Oxidative, cloud point, and flow injection, under ideal circumstances using various concentrations and measured absorbance for at least five readings per concentration. as determined by

RE (%), R (%), and RSD (%), as illustrated in Tables 2 and 3.

Table 2: Data the accuracy and precision of proposed methods for estimation of pure samples

Oxidative coupling						
Drug	Amount of drugs µg /ml		Relative Error %	Recovery %	Average Recovery%	RSD% (n=5)
	Taken	Found				
CFM	0.5 2 6	0.504 1.97 5.964	0.96 -1.5 -0.6	100.96 98.5 99.4	99.62	
Cloud point						
CFM	0.5 2 5	0.55 1.92 4.98	0.1 -4 -0.3	100.1 96 99.7	98.6	
Flow injection system						
CFM	5 20 50	4.98 20.4 51.26	-0.4 2 2.52	99.6 102 102.52	101.4	
LOD = 3.3ЧSDb/S, SDb= the standard deviation of intercepts of regression lines .						

Table 3: The accuracy and precision of proposed method for estimation of commercial pharmaceuticals

Oxidative coupling						
Type of Drugs	Amount of drugs µg /ml		Relative Error %	Recovery %	Average Recovery %	RSD% (n=5)
	Taken	Found				
CFM Spain10% w/v	0.5 2 6	0.49 2.01 6.03	-2 0.5 0.5	98 100.5 100.5	99.7	1.2 0.99 0.87
CFM Ukrane 10%w/v	5 2 6	0.49 2.05 5.9	-2 2.5 -2.2	98 102.5 97.8	99.4	0.48 1.32 0.98
Cloud point						
CFM Spain 10% w/v	0.5 2 5	0.495 1.997 4.96	-1 -0.15 -0.8 99.2	99 99.85	99.35	0.05 0.03 0.99
CFM Ukrane 10%w/v	0.5 2 5	0.493 1.995 5.04	-1.4 -0.25 0.8	98.6 99.75 100.8	99.72	0.12 0.98 0.76
Flow injection						
CFM Spain 10% w/v	5 20 50	5.04 19.32 49.7	0.8 -3.4 -0.6	100.8 96.6 99.4	98.93	1.3 0.06 0.01
CFM Ukrane 10%w/v	5 20 50	5.18 20.3 48.45	3.6 1.5 -3.1	103.6 101.5 96.9	100.7	0.78 0.01 0.08

E% stands for relative error found-taken/taken 100, Rec% for recovery, and RSD% for relative standard deviation in the average of five repetitions.

Conclusions

The CFM drug evaluation approaches in pharmaceutical readiness that are advised for use on actual samples have the advantages of being low cost, high sensitivity, streamlined, recurring, and reproducible CFM methods. The CFM component was isolated and pre-concentrated using the surfactant in medicinal preparations. A comparison of the approaches currently described for this procedure utilizing various instrumental techniques appears to be more sensitive and stable, easy, fast, quick, and inexpensive. The batch spectrophotometric approach for the determination of CFM medication was semi-automated using an FIA method. The suggested techniques were effectively used to estimate pure CFM and in drug dose.

References

Hartman SJF, Brъggemann RJ, Orriлns L et al. Pharmacokinetics and target attainment of antibiotics in critically Ill children: A systematic review of current literature. Clin Pharmacokinet 2020.

Nanda R, Bhagwat V, Potawale S, et al. Development and validation of a HPTLC method for simultaneous densitometric analysis of cefotaxime sodium and sulbactam sodium as the bulk drugs and in the pharmaceutical form. Pharm Res. 3: 1667-1669; 2010.

Mostafa F. Al-Hakkani; HPLC Analytical Method Validation for Determination of Cefotaxime in the Bulk and Finished Pharmaceutical Dosage Form; Sustaniable Chemical Engineering; Volume 1 Issue; 2020.

Hoang VD, Loan NT, Tho VT, Nguyen HM. U V spectrophotometric simultaneous determination of cefoperazone and sulbactam in pharmaceutical formulations by derivative, Fourier and wavelet transforms. Spectrochim Acta A Mol Biomol Spectrosc. 2014;121(1):704-714.

Sha OU, Liao YN, Zhou YN, Zhang SI, Weixng MA. Determination of cefoperazone sodium in pharmaceutical formulations by Fe3+-Phenanthroline spectrophotometry. Asian J Chem.;25(11):5965-5967; 2013.

Lalitha N, Puranik SB, Pai PNS, A stability indicating HPLC method for cefoperazone. Eurasian J Anal Chem.;4 (1):110- 118; 2009.

Sumayha Mohammed Abbas; Spectrophotometric Determination of Cefotaxime via Diazotization Reaction in Pure and Pharmaceutical Samples; Ibn Al-Haitham Jour. for Pure & Appl. Sci.; Vol. 30 (2) 2017.

Mouyed Q. Al-Abachi, Hind S. Al-Ward, Yassmin H. Mohammad; BATCH AND FLOW-INJECTION SPECTROPHOTOMETRIC DETERMINATION OF SODIUM CEFOTAXIME IN PHARMACEUTICAL PREPARATIONS; Iraqi Journal of Science, vol.53, No.2, pp 241-249; 2012.

Mengmeng Dou, JinWang, BoruGao, CeXu, FanYang; Mesoporous g-C3N4 photocatalyzes the degradation of amoxicillin and cefotaxime under visible light: Mechanism, degradation pathway, and DFT calculation; Chemical Engineering Journal; Volume 383; 123134; 1 March 2020.

LAMYA A. SARSAM1, SALIM A. MOHAMMED2 and SAHAR A. FATHE; Spectrophotometric and RP-HPLC Methods for Determining Cefotaxime Sodium in Pharmaceutical Preparations; AJ C SIAN OURNAL OF HEMISTRY https://doi.org/10.14233/ajchem. 22524; 2020.

N. K. Abood, M. J. M. Hassan, and M. A. Al-Da'amy, "Spectrophotometric determination methyldopa and salbutamol by oxidative coupling, cloud point and flow injection in pharmaceutical formulations," International Journal of Drug Delivery Technology, vol. 9, no. 2, pp. 182−192, Apr. 2019.

P. Guo, X. Li, J. Wang and A. You, J. Pharm. Biomed. Anal., 43, 1849 (2007); https://doi.org/10.1016/j.jpba.2006.12.009

C.H.L. Saranya, J.C. Thejaswini, B.M. Gurupadayya and B.Y.K. Sruthi, IOSR J. Pharm., 4, 12 (2014).

P.S. Jain, M.K. Patel, A.J. Chaudhari and S.J. Surana, Indian J. Pharm. Educ. Res., 46, 360 (2012)

N. K. Abood and M. J. M. Hassana, "Spectrophotometric Determination of Sulfadoxine Drug use Cloud point and Flow injection Methods in Pharmaceutical Formulations," Egypt J Chem, vol. 64, no. 6, pp. 2913−2924, Jun. 2021, doi: 10.21608/EJCHEM.2021.55297.3164.

J. Du and H. Li, Appl. Spectrosc., 64, 1154 (2010); https://doi.org/10.1366/000370210792973613

K. Nikolic, M. Aleksic, V. Kapetanovic and D. Agbaba, J. Serb. Chem. Soc., 80, 1035 (2015); https://doi.org/10.2298/JSC150129019N

M.Q. Al-Abachi, H.S. Al-Ward and Y.H. Mohammad, Iraqi J. Sci., 53, 241 (2012).

L.P. Consortti and H.R.N. Salgado, J. Pharm. Sci. Emerg. Drugs, 5, 1 (2017); https://doi.org/10.4172/2380-9477.1000118

R. Wang, Z.P. Jia, J.J. Fan, J. Ma, X. Hua, Q. Zhang and J. Wang, Pharmazie, 64, 156 (2009).
M.A. Omar, O.H. Abdelmageed and T.Z. Attia, Int. J. Anal. Chem., 5, 12 (2009).
N.S.T. Al-Awadie and M.H. Ibraheem, Int. J. Res. Pharm. Chem., 6, 891 (2016).
L.-L. Wu, Y. Zhang, W. Zhao and Q.-M. Li, J. Chin. Chem. Soc., 55, 550 (2008).
Hesham Salem, Ebtihal Samir; Determination of cefotaxime, cefoperazone, ceftazidime and cefadroxil using surface plasmon resonance band of silver nanoparticles; Brazilian Journal of Pharmaceutical Sciences http://dx.doi.org/;54(3):e17565; 2018.