Egypt. J. Chem. **Vol. 66,** No. 5 pp. 385 - 392 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Spectrophotometric estimation of Ceftazidime via batch Method and Flow injection analysis

Huda abdulkarim,[a] Mohammed Jasim M Hassan[b]

[a]*Department of Chemistry, College of Science, Mustansiriyah University Baghdad, IRAQ*

Abstract

Simple, rapid, and sensitive spectrophotometric technique has been developed for the estimation of ceftazidime (CFT) in pure form and pharmaceutical preparation. The first technique depends on the conversion of NH_2 in ceftazidime to diazanium salt of ceftazidime and coupled with the 1-Hydroxy-2- naphthalicacid reagent in alkaline medium with a molar absorptivity of 1.01115×10^4 $L.mol^{-1}.cm^{-1}$ and a detection limit of 0.63978µg/ml.The violet-colored product showed up at ƛmax 574nm and followed Beer's law over a concentration range of 3-40µg/ml. The diazotization of the investigated drug (ceftazidime) and its interaction with 1-HNA was investigated via a newly advanced flow injection (FLA) technique based on the detection of dye–azo salt absorption at ƛmax 574 nm. The concentrations ranged from 5 to 50 µg/ml. By comparing the proposed methods to previous studies, it was determined that they were compatible with appreciating ceftazidime in pharmaceuticals.

Keyword: Ceftazidime , Spectrophotometric , Flow injection , Diazotization , 1-Hydroxy 2-naphthalic acid .

1. Introduction

An azo coupling is an organic reaction that produces an azo compound by combining a diazonium compound with another aromatic compound. The electrophile in this electrophilic aromatic substitution reaction is the aryldiazonium cation, while the nucleophile is the activated arene. [1] The diazonium compound is usually aromatic as well. Diazotization is a type of diazotization. Diazotization is the procedure of transfiguring primary aromatic amines into their diazonium salts. Diazonium salts are vital synthetic intermediates that can be used in azo dye coupling reactions and electrophilic substitution reactions to introduce functional groups. Theapplication of these reactionsare duo to extended conjugated systemsthereforeromatic azo compounds have a bright color. Many of them are dyes. [2] Methyl red and pigment red 170 are two important azo dyes. This reaction is also applied in azo printing [3] Azo coupling is also used to produce prontosil and other sulfamedications. This method has many uses, such as: study of the reaction mechanics [4] and used in the spectroscopic estimation of drugs [5-14]; determination of vitamins [15]; and the study of the stability of the composition of some organic compounds [16]. Ceftazidime is a third-generation antibiotic with a molecular formula of 546.58g/mol [17], Fig (1)

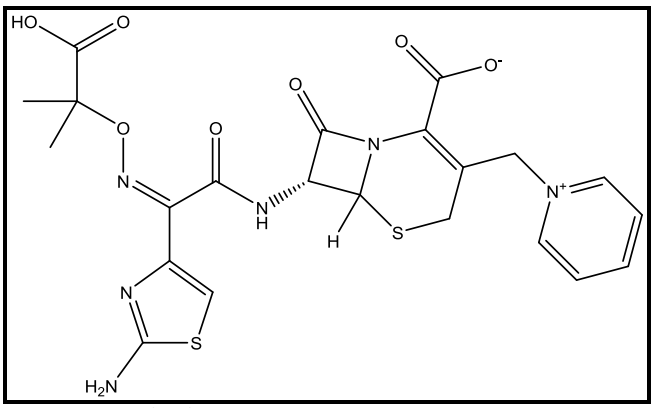

Fig1: Structure of Ceftazidime

It has many uses in the medical field, such as treating infections of the blood, bones, and central nervous system. It has a role in managing P. aruginosa infection [18].Ceftazidime is effective in the treatment of moderate and severe COVID-19 patients[19], and urinary tract infection[20]. Several studies have estimated ceftazidime in various methods, such as: RP-UPLC [21], HPLC [22],Electrochemiluminescence [23] Electro-oxidation [24] Quantification of CFT in human plasma[25], etc,, These approaches offer various benefits, including low price,

*Corresponding author: (Huda abdulkarim).
Receive Date: 10 July 2022, Revise Date: 31 May 2023, Accept Date: 29 August 2022
DOI: 10.21608/ejchem.2022.149900.6489

sensitivity, exactness, speediness, low toxicity, procedural uncomplicatedness, and environmental friendliness. They have been used to determine the active ingredient in medications and have proven to be successful.

2. Experment:

2.1.1.Equipment;The single beam advanced microprocessor UV-Vis spectrophotometerLI-295 (Lasany®-India) .was used to perform all spectroscopic measurements with a 10 cm quartz cell. For the flow injection design, a three-channel manifold was used. Peristaltic pump with a polyvinyl chloride tube (0.8mm) internal diameter (AlLITEA, C4, Sweden)

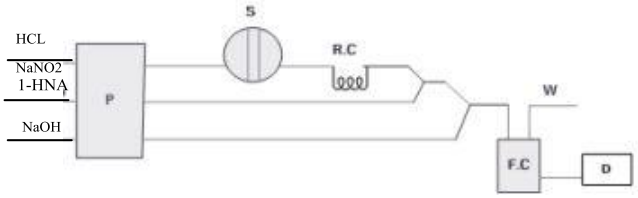

Fig 2. :Diagram of the Applied Flow System
P. stands for peristaltic pump, R.C stands for reaction coil, S stands for sample injection, W stands for waste, and FC stands for flow control.

2.1.2. Chemicals: All of the reagents were of analytical quality**, It** was purchased from various companies. The drug CFT was obtained from three different commercial sources ; , (TOTTIZIM made in ITALY),(YENIZIDIME –Turkey – Ankara) and (Ceftazidime Roth Wiesbaden – Germany) . Stock solutions (1000 µg/ml) of Ceftazidime (LaboratoriosTorlan Barcelona, Spain) was made by dissolving 0.1 g of the drug in distilled water and diluting to the desired concentration in a 100 ml volumetric flask. The reagent 1-Hydroxy-2-naphthalic acid (Sigma Aldrich company) (2.710-3M) was prepared by dissolving 0.1 g of 1-HNA in distilled water with 1 ml of base 6.2505 M NaOH to aid dissolution and diluting to the mark in a 50 ml conicalflask. The other chemicals; HCl (ADR) 1:1 11.9 M , 25% w/vsodiumhydroxideNaOH (BDH) , (6.2505M), 1% sodium nitrate $NaNO_2$ (BDH) were prepared by dissolving in distilled water (D.W).

2.2. General Procedure of Diazotizationmethod:

The good method for preparing Azo coupling was developed by adding 1 ml of 1000 µg/ml ceftazidime to a 20 ml conical flask submerged in an ice bath at 0–5℃, then adding 1 ml of (1:1) HCl and gradually 1 ml of 1 percent sodium nitrite, waiting for 10 minutes, then adding 1 ml of reagent 2-HNA and 1 ml of 25 percent NaOH. The azo-dye produced is violet in color and has a maximum absorbance of ƛ max 574nm.

2.3.General Procedure of Flow injection:

Using a three-channel peristaltic pump, the first of which contains a mixture of HCI and $NaNO_2$, the second of which contains the reagents (1-Hydroxy-2-naphthalic acid), and the product is passed on to the injector, which contains the drug, which is passed on to the reaction coil (100cm), where the product is treated with the base sodiumhydroxide 5%, and then to a UV-Visible device for measuring absorption at λ max 574nm .

3. Result and Discussion:

3.1. Absorption spectra

Figures (3) show the spectrum product of 50µg/ml solution of CFT -1-HNA against blank solution . The spectra show that the ƛmax574nm .

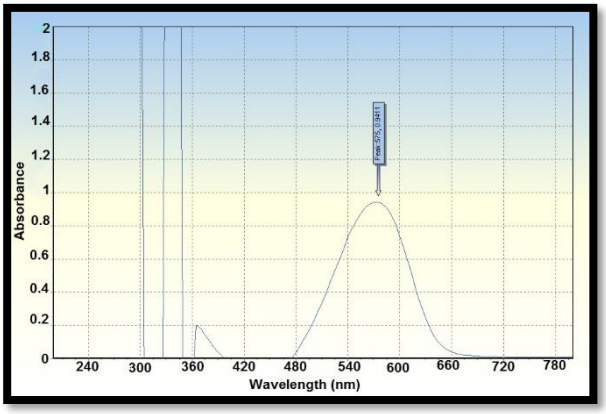

Fig .3 : Absorption spectrum for 50 µg/mL of CFT-1- HNA against reagent blank.

3.2.Part 1: Study of optimization reaction of diazozation:

To determine the best circumstances in the CFT estimate, the impacts of different variables on absorption intensity were investigated. The CFT was tested at a concentration of 50 µg/ml in a 20 ml conical flask. The effect of acid type was investigated, and some acids(HCI, HNO_3 , H_2SO_3 and H_3PO_4) were diluted (1:1) to concentrations of (11.9, 14.2, 18.35, and 16.5 M) and tested in a diazotization-coupling reactionasshownin Table 1. When HCl was applied, the maximum absorption was achieved. The diazotization-coupling reaction was studied with volumetric effect. In the diazotization process, hydrochloric acid was investigated by testing different volumes (0.5–2.00 ml) of M HCl.It was discovered that 1 ml offered the best absorption intensity, see figure4.

Table 1 :The effect Acid type on absorbance single of CFT

Type of Acid	Absorbance CFT -1-HNA at 574 nm
HCl	0.509
H_2SO_4	0.114
HNO_3	0.125
H_3PO_4	0.242

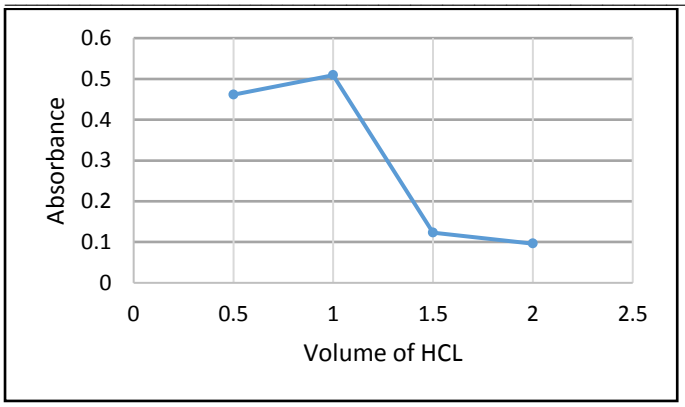

Fig 3 :The effect of HCl (1:1) volume

The influence of volume sodiumnitrite was investigated by testing several volumes (0.5 -2.00ml) of (1 % $NaNO_2$) in the Azo – dye process, and it was discovered that 0.5 ml offered best absorbance,see figure4.

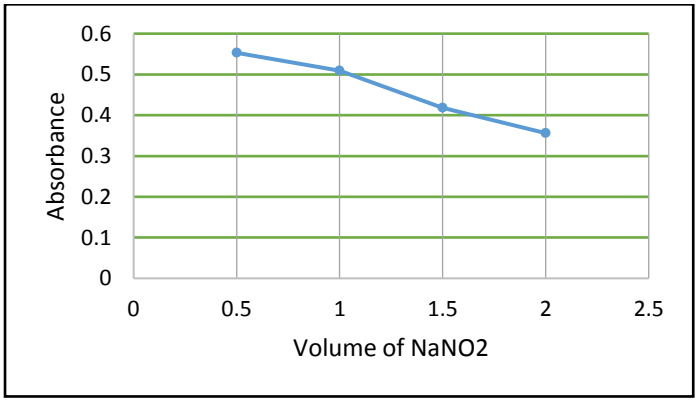

Fig 4 : The effect of $NaNO_2$ volume

Different waiting intervals were attempted after adding $NaNO_2$ to the mixture of the reaction (5-30 minutes). As illustrated in Figure 5 , a waiting time of minutes was the ideal time for obtaining the highest absorption intensity.

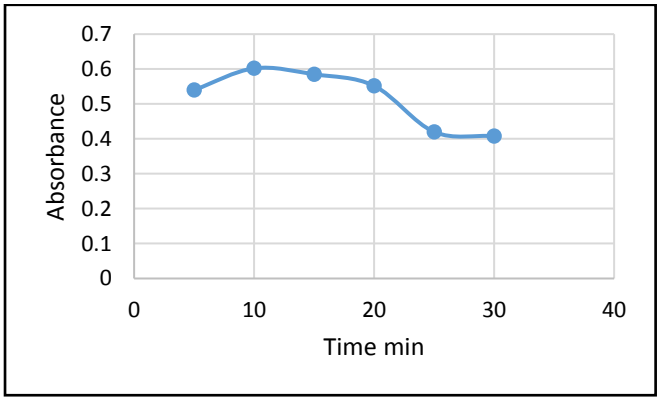

Fig 5 :The effect of time after the addition of $NaNO_2$

The influence of three different bases (KOH, NaOH, and Na_2CO_3) on the diazotization- coupling reaction was investigated. In this reaction, it was discovered that NaOH has the maximum absorbance. As a result, the influence of varied quantities of 6.2505 M NaOH (0.5 -2.00 ml) on absorption was investigatedas demonstrated in figure 6. 1 ml was the ideal volume for obtaining the maximum absorption.

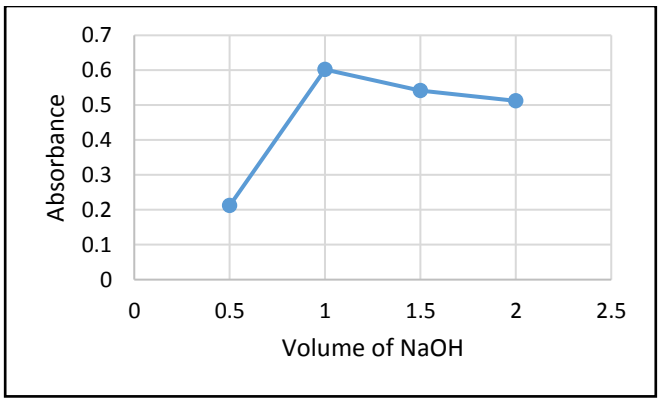

Fig 6 : The effect of 25 %sodium hydroxide .

Volume .

A 1-HNA reagent solution with a mole ratio equal to the CFT was created to investigate the impact of reagent volume and the nature of the coloreddye. As a result, the approach used in this study is the same as the mole ratio method, which may be used to determine the drug-to-reagent ratio. With l ml (1.77×10^{-3} M) of Ceftazidime , Many volumes) of (2.7×10^{-4} M) 1-HNA (0.5 - 1 ml) were investigated. At 0.5 ml of reagent, the optimum absorption was attained, and afterward, the absorption had almost reached a plateau. The colored dye is produced in a 1:1 ratio. Fig 7.

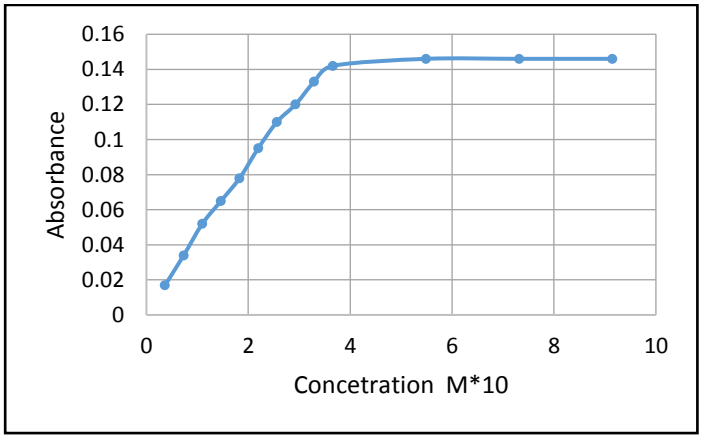

Fig 7 : The mole-ratio plot for batch method of ceftazidime to 1-HNA .

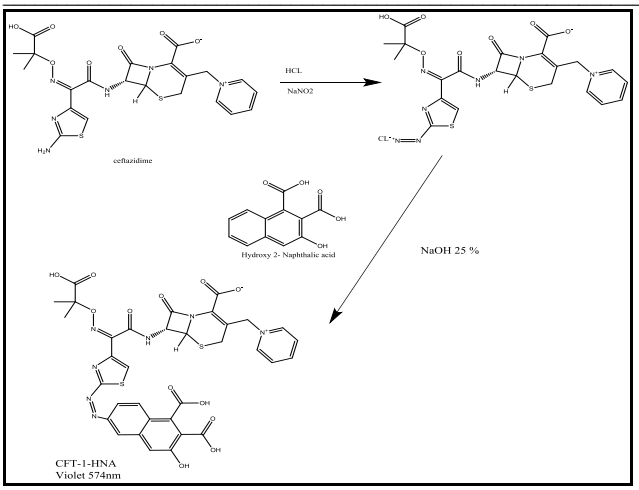

Fig 8 : The suggested mechanism for CFT – 1-HNA .

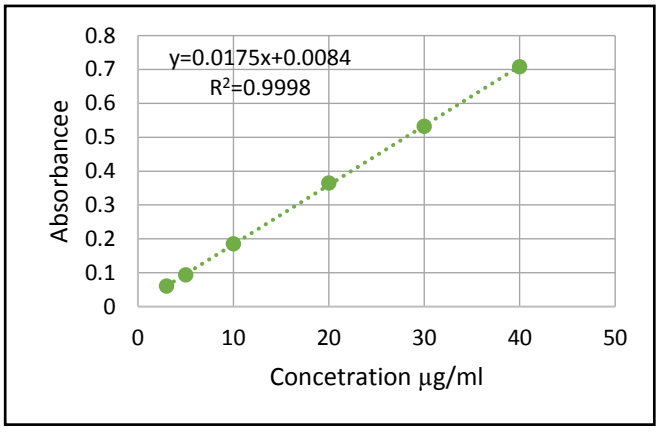

Fig 9 : Calibration curve if CFT -1- HNA .

3.2.1.Analytical data :Under the optimized experimental conditions, Calibration curve was obtained for CFT concentration ranging from 3 to 40 µg/ml. The analytical curves are presented in Figure 10, and the linearity, regression equation,Sandell's sensitivity, LOD and LOQ , and other analytical are presented in Table 2.

Table 2 .characteristic parameters of proposed diazonum salt methods.

Parameter	**CFT-1-HNA**
Color of product	**Violet**
λ max (nm)	**574**
Liner range (µg.ml-1)	**(3-40)**
Molar absorptivity, Ɛ (L.mol-1.cm-1)	**1.01115×10^4**
Regression Equation	**y=0.0175x+0.0084**
Sandell sensitivity, S (µg .cm-2)/0.001A.U	**0.5406**
Intercept (a)	**0.0084**
Slope (b) (L.mg-1.cm-1)	**0.0175**
Coefficient of determination % R^2	**99.98**
Correlation coefficient (r)	**0.9998**
Limit of detection (µg.mL-1)	**0.6398**
Limit of quantification (µg.mL-1)	**2.1326**
Standard error for regression line (Sy/x)	**0.2816**

3.2.2. Accuracy and precision

Under ideal conditions, the accuracy and precision of the proposed technique were investigated. Utilizing three different concentrations , as well as measuring absorbance for at least five measurements per concentration and measure t, F test for estimation of CFT in pharmaceutical preparations.

.

Table 4: Accuracy andPrecision of the Batch technique for CFT pure

Amount of CFT (µg/ml)		**E%**	**Recovery%**	**Average %**	**RSD% (n=5)**
Taken	Found				
5	4.83	-3.4	96.6	99.48	0.760
20	20.38	1.89	101.89		0.407
40	39.98	-0.58	99.9		0.4012

Table 5 : Application of the proposed Batch technique for the estimation of Ceftazidime in different companies of drugs by using the reagent 1-Hydroxy -2-naphthalic acid .

Type of drugs	Amount of drugs µg/ml		E%	Recovery%	Average%	Value		R.S.D%
	Taken	Found				t	F	(n=5)
Ceftazidime	5	4.77	-6	94				1.233
YENIZIM	20	20.32	1.6	101.6	98.47	0.0045	0.997	0.313
made in Turkey – Ankara	40	39.92	-0.2	99.9				0.185
Ceftazidime	5	4.71	-5.7	94.28				0.976
TOTTIZIM	20	20.26	1.3	101.3	98.40	0.0089	0.995	0.642
made in ITALY	40	39.85	-0.4	99.63				0.215
Ceftazidime	5	4.88	-2.4	97.6				1.420
ROTH madein	20	20.42	2.1	102.1	99.73	0.00201	1.008	0.643
Wiesbaden Germany	40	39.80	-0.5	99.5				0.369

3.3.Part 2 : Study of the optimum reaction conditions for the Flow injection analysis (FIA) method;

An FIA approach was developed using a batch method for spectrophotometric analysis of CFT. The CFT estimation manifold was created to allow for the control of various reaction conditions in order to amplifythe diazotization of CFT.Coupling with 1-Hydroxy-2-naphthalic acid in an alkaline medium produces an absorbance signal.

There are many conditions that affect the absorption density of colored solutions, including the effect of acid concentration, HCl where different concentrations (1.16 – 5.8) M of acid have been studied.It was found that 20 is the optimal concentration gives the highest absorption density. The impact of sodium nitrite $NaNO_2$ volume on absorbance was then investigated , with 20 ml providing the highest absorption density . In addition , the concentration of the reagent (1-HNA) has a significant effect on the naturalness of the violet solution formed, as several concentrations from (0.0694×10^{-3} to 0.832×10^{-3})M were tested for the reagent.It was found that 0.671×10^{-3}M was the best concentration of the reagent that gave the highest absorption intensity. Finally, the effect of the base concentration ofNaOH (5 – 25)% on the naturalness of the produced violet solution was investigated, as 5% the optimal base concentration affects absorbance,as shown in figure 10 – 13 .

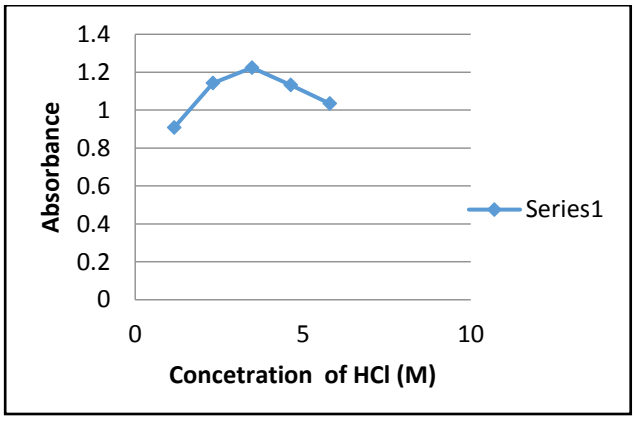

Fig 10 :The effect of HCl concentration

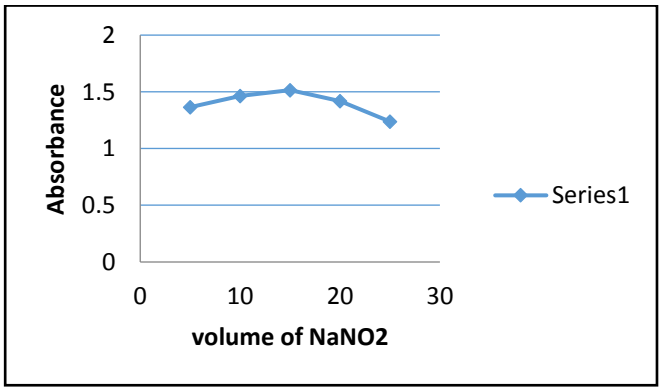

Fig 11 : The effect Influence of $NaNO_2$ volume

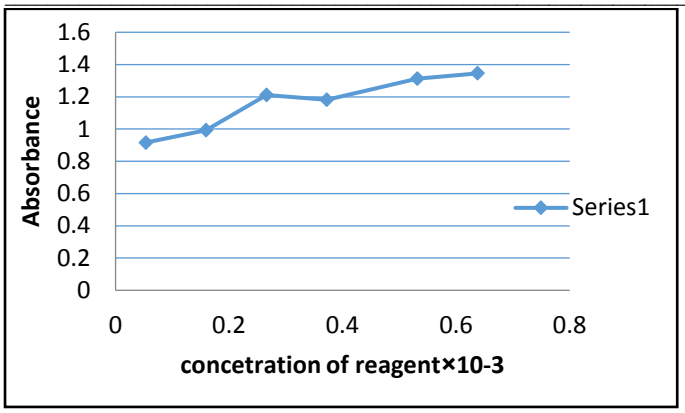

Fig 12 :The effect of reagent 1-HNA concentration.

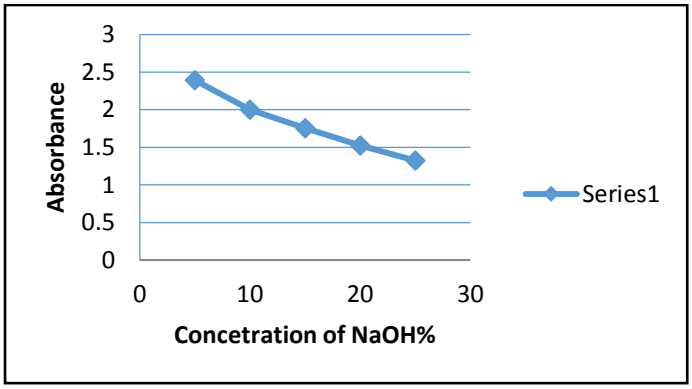

Fig 13 :The effect of %NaOH concentration

3.3.1. Analytical curve ; Under the optimized experimental conditions, Calibration curve was obtained for CFT concentration ranging from3 to 50 µg/ml. The analytical curves are presented in Figure 14 , and the linearity, regression equation,Sandell's sensitivity, LOD and LOQ.Other analytical are presented in Table3.

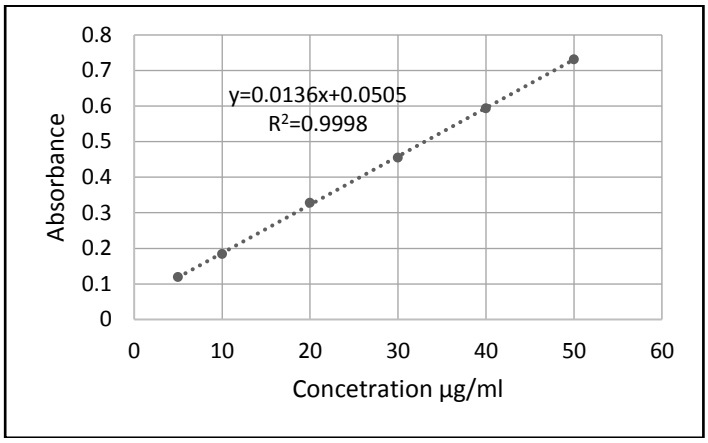

Fig 14 : Calibration curve of CFT -1-HNA

Table 6 .characteristic parameters of proposed FIA methods.

Parameter	CFT-1-HNA
Color of product	**Violet**
λ max (nm)	**574**
Liner range (µg.ml-1)	**(3-50)**
Molar absorptivity, Ɛ (L.mol-1.cm-1)	**1.00568×10^4**
Regression equation	**y=0.0136x+0.0505**
Sandell sensitivity, S (µg .cm-2)/0.001A.U	**0.5435**
Intercept (a)	**0.0505**
Slope (b) (L.mg-1.cm-1)	
Coefficient of determination % R^2	**99.98**
Correlation coefficient (r)	**0.9998**
Limit of detection (µg.mL-1)	**0.7895**
Limit of quantification (µg.mL-1)	**2.6318**
Standard error for regression line (Sy/x)	**0.3347**

3.3.2. Accuracy and Precision :

The accuracy and precision of the FIA-method were investigated when the prescribed process was used to estimate the five duplicates of the concentration of CFT at three concentration points of the drugs in their pure form and in pharmaceutical.

Table 7: Accuracy and Precision of FIA technique for CFT pure

Amount of CFT (µg/ml)		E%	Recovery%	Average %	RSD% (n=5)
Taken	Found				
10	9.82	-1.84	98.16	99.02	0.8593
30	29.74	-0.87	99.13		0.348
50	50.11	0.22	99.78		0.311

Table 8 : Application of the proposed FIA technique for the estimation of Ceftazidime in different companies of drugs by using the reagent 1-Hydroxy -2-naphthalic acid .

Type of drugs	Amount of drugs µg/ml		E%	Recovery%	Average %	Value		R.S.D% (n=5)
	Taken	Found				t	F	
Ceftazidime YENIZIM madeinTurkey –Ankara	10	9.89	-1.1	98.89	99.55	0.119	1.115	0.709
	30	29.82	-0.61	99.38				0.439
	50	50.18	0.37	100.3				0.155
Ceftazidime TOTTIZIM made in ITALY	10	9.74	-2.57	97.43	98.94	0.0049	0.991	0.991
	30	29.67	-1.10	98.89				0.423
	50	50.26	0.514	100.5				0.325
Ceftazidime ROTH madein Wiesbaden Germany	10	9.96	-0.37	99.63	99.98	0.0109	0.986	0.612
	30	29.88	-0.37	99.6				0.379
	50	50.33	0.66	100.6				0.314

4. Conclusion

This paper includes two simple, sensitive, techniques for determination of ceftazidime using the reagent 1-HNA which is cheaply available. The first method (AZO-dye process) includes the conversion of the ceftazidime into a colored dye measured by a UV- Vis spectrophotometer. The second technique involves a determination by flow injection . These techniques have been successfully used in the determination of ceftazidime in pharmaceuticals.

References

[1] Smith, Michael B.; March, Jerry Advanced Organic Chemistry: Reactions, Mechanisms, and Structure (6th ed.), New York: Wiley-Interscience, ISBN: 978-0-471-72091-1,(2007),

[2] Klaus Hunger; Peter Mischke; Wolfgang Rieper; RoderichRaue; Klaus Kunde; Aloys Engel . Ullmann's Encyclopedia of Industrial Chemistry. Weinheim ; Wiley-VCH(2005).

.

[3] Pai, Damodar M.; Melnyk, Andrew R.; Weiss, David S.; Hann, Richard; Crooks, Walter; Pennington, Keith S.; Lee, Francis C.; Jaeger, C. Wayne; Titterington. Imaging Technology, 2. Copying and Nonimpact Printing Processes. Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH, pp. 1–53(2017).

[4] Zhaoxiang Li, Xiaogang Mu, Bo Liu, Youzhi Zhang, XuanjunWang . Study on reaction mechanism of nitrogen dioxide detection by diazotization coupling reaction . International Journal of Frontiers in Engineering Technology ,3 (6) (2021).

[5] NisreenKaisAboodMohammed Jasim M Hassan1, Muneer A. AL-Da'amy2 .Determination of Cefixime Using Batch, Cloud PointExtraction and Flow Injection as New SpectrophotometricMethods . Al-Mustansiriyah Journal of Science,ISSN: 1814-635X (print), ISSN:2521-3520(online), (2021).

[6] Taif Th. Kzar ،Ashraf S. Rasheed b and Mohammed J. M. Hassan . Development of a validated hydrophilic interaction chromatographymethod for the determination of cefotaxime in pharmaceuticalpreparations . Egypt. J. Chem. Vol 64, No. 6 pp. 2967 – 2972 ,(2021).

[7] QasimMzban1Sarmad Bahjat2and Mohammed Jasim M. Hassan . DISPERSIVE LIQUID-LIQUID MICROEXTRACTION ANDSPECTROPHOTOMETRIC DETERMINATION OF CEFAZONE ANDPHENYLEPHRINE HYDROCHLORIDE IN THEIR PURE FORMS ANDPHARMACEUTICAL PREPARATIONS . Plant Archives Volume 20 No. 2, pp. 6771-6777,(2020) .

[8] Rana S Al-Saffar, Safaa A Zakaria, Nabeel S Othman .Spectrophotometric Determination of p-aminobenzoic acid via Diazotization and Coupling reaction . Research Journal of Pharmacy and Technology ,14 (10): 5313-5318,(2021).

[9] Abood, N.K., Hassana, M.J.M .Spectrophotometric determination of sulfadoxine drug use cloud point and flow

injection methods in pharmaceutical formulations. Egyptian Journal of Chemistry, 64(6): pp. 2913-2924,(2021).

[10] Fadhil, A.K., Hassan, M.J.M., Rasheed, A.S. A comparative review of methods for estimation of some antihypertensive drugs in pharmaceutical production . Egyptian Journal of Chemistry ,64(11): pp. 6301-6321,(2021).

[11] Kais, A.N., Hassan, M.J.M., Al-Daamy, M.A..Determination of phenolic drugs and their formulations via various analytical methods .Research Journal of Chemistry and Environment .23(Special Issue 1), pp. 188-199,(2019).

[12] methodsAbood, N.K., Hassan, M.J.M., Muneer, A.A.-D. New spectrophotometric determination cefdinir coupling with bisphenol A via various analytical . Journal of Global Pharma. Technology ,11(7):pp. 528-540,(2019).

[13] Narayana Chand Pasha, B . A simple spectrophotometric method for the determination of mosapride by diazotization method .Yanbu Journal of Engineering and Science, 1 (1): 68-74,(2021) .

[14] Mohammed Jasim M Hassan, WijdanShakirKhayoon, Shrouq Abdul-Fatah Hasssan . Batch and flow injection spectrophotometric methods for the determination of barbituric acid in aqueous samples via oxidative coupling with 4-aminoantipyrine . Karbala International Journal of Modern Science, 1 (3): 135-141(2015).

[15] Zakaria S Abdul-Aleem, Zakaria R Abdul-Aleem, Othman N Sabeeh .Spectrophotometric Determination of Vitamin B6 in Pure and Pharmaceutical Formulations with Diazotized Metoclopramide Hydrochloride .International Journal of Drug Delivery Technology, 11(3):787-792,(2021) .

[16] Ahmed Hussein Ali Mohsin, Hussein Al-Niemi, Mohammad Mahmoud .A thermodynamic study for the stability of some aromatic complexes formation derived from the reaction of 4-dimethyl amino benzaldehyde with diazotized dinitro aniline reagent.gyptian Journal of Chemistry ,65 (2): 1-2,(2022).

[17] Nibras Sabah Mohammed, Mohammed Jasim M Hassan, Amer Saleh Mahdi New .spectrophotometric method for determination of ceftazidime in pure form and pharmaceutical dosages . Al-Mustansiriyah Journal ofScience,30(3):47-52.(2019) .

[18] George L Daikos, ClóvisArns da Cunha, Gian Maria Rossolini, Gregory G Stone, Nathalie Baillon-Plot, Margaret Tawadrous, PaurusIrani . Review of ceftazidime-avibactam for the treatment of infections caused by Pseudomonas aeruginos .Antibiotics,10 (9): 1126,(2021).

[19] Ragaey A Eid, Marwa O Elgendy, Ahmed O El-Gendy, Sara O Elgendy, LassaadBelbahri, Ahmed M Sayed, Mostafa E Rateb . Efficacy of Ceftazidime and Cefepime in the Management of COVID-19 Patients: Single Center Report from Egypt .Antibiotics ,10 (11): 1278 ,(2021).

[20] Maria Di Pietrantonio, Lucia Brescini, Jennifer Candi, MorroniGianluca, .Ceftazidime–Avibactam for the Treatment of Multidrug-Resistant Pathogens: A Retrospective, Single Center Study . Antibiotics ,11 (3):321,(2022).

[21] Sridatla VVSSN Raju, S Venkat Rao, A Manikandan . Estimation of Ceftazidime and Avibactam in their Bulk and Formulations by a newly Developed and Validated of Stability Indicating RP-UPLC Method . Research Journal of Pharmacy and Technology, 14 (5): 2459-2463,(2021).

[22] Ranjot Kaur, Sumant Saini, Asha Patel, Teenu Sharma, Ripandeep Kaur, OP Katare, Bhupinder Singh.Developing a Validated HPLC Method for Quantification of Ceftazidime Employing Analytical Quality by Design and Monte Carlo Simulations . Journal of AOAC International , 104 (3): 620-632,(2021).

[23]HoseinAfshary, MandanaAmiri, AbolfazlBezaatpour, Michael Wark . Electrochemiluminescence Sensor Based on N-Doped Carbon Quantum Dots for Determination of Ceftazidime in Real Samples . Journal of The Electrochemical Society , 169(2) : 026523 (2022).

[24] PingzhouDuan, XiaoboJia, Jianing Lin, Rui Xia . Electro-oxidation of ceftazidime in real municipal wastewater using PbO2–Ce and SnO2–Sbelectrodes .influence of electrolyte and degradation pathway.Journal of Applied Electrochemistry,51 (2): 183-195(2021).

[25] Jens Martens-Lobenhoffer, Stefan Angermair, Stefanie M Bode-Böger . Quantification of ceftazidime/avibactam in human plasma and dried blood spots: Implications on stability and sample transport . Journal of Chromatography. B, 1193, 123164,(2022).