Original Article

Development and validated spectrophotometric method for the determination of Ceftriaxone in pharmaceutical preparations

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Abstract

The study involves the development of a simple, rapid, selective, and low-cost spectrophotometric method for the determination of ceftriaxone as pure and in its pharmaceutical preparations. The ceftriaxone (CF) was diazotized by sodium nitrite NaNO2 solution in the presence of hydrochloride acid HCl in the ice bath at range of 0 to 5 ºC to form diazonium salt, and then coupled with 4,5-diphenylimidazole reagent (4,5-DPI) in a basic solution of sodium hydroxide solution to form a purple color water–soluble azo dye as a result, that has a maximum absorption λmax at 550 nm opposite reagent blank, in the optimum conditions. The linearity of the process obeyed Beers law in the concentration range of 10 to 100 µg/mL at a correlation coefficient R2 of 0.9973. The molar absorptivity was 3772.08 L/mol.cm. The detection limit and quantification limits were 4.9425 and 16.475 µg/mL, respectively. Relative error and precision were determined to be 2.8% and 1.44%, respectively. The resulting stoichiometry of the AZO dye (drug: reagent) formed was found 1:1.

Keywords: Ceftriaxone (CF),4,5-diphenylimidazole, azo dye, validated, spectrophotometric.

1- INTRODUCTION

The majority of antibiotics are natural or semi-natural in which glass β-lactams are the first glass of these natural product derivatives utilized to treat bacterial infections.(1) Cephalosporin has been utilized as anti-infective medicine since 1970 and is among the most effective and popular ones, and is a derivation of 7-aminoceph-alospric acid and is closely related to penicillin in structure, which has broad spectrum activity against gram-negative and gram positives bacteria.(2-5) Ceftriaxone is chemically known as (6R,7R)-7-[(2Z)-(2-aminothiazol-4-yl) (methoxyimino)acetyl]amino]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-ioxo-1,2,4-triazin-3-yl)thio]-methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid as Figure 1.(6)

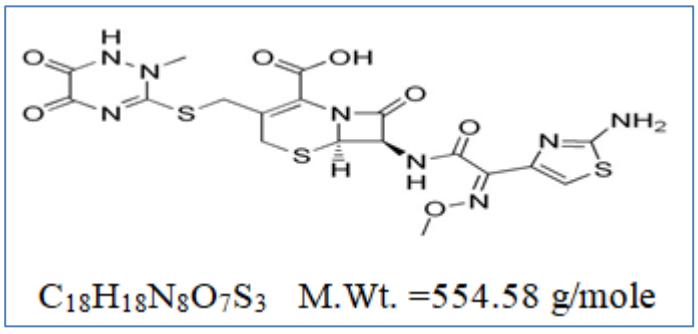

Figure 1. Chemical Structure of ceftriaxone

Cephalosporin antibiotic is a third-generation.(7) closely related to penicillin's in, It has broad spectrum activity against Gram-negative and Gram-positive bacteria,(8-10) and it is often used (in combination with macrolide and/or aminoglycoside

antibiotics) for the treatment of community-acquired pneumonia.(11-13) Ceftriaxone was estimated by several techniques such as spectrophotometric methods,(14-16) RP-HPLC technique in the presence of statin drugs in formation and human serum, (17) chromatographic separation using XTerra,(18)derivatization with p-dimethyl amino benzaldehyde.(19) It is also used as a routine prophylactic antibiotic for patients undergoing orthopedic surgery.(20) In the present study, the synthesizes of new azo dye color by chemical reaction between diazotized ceftriaxone with 4,5-diphenylimidazole in alkaline media and utilized for the spectrophotometric determination of ceftriaxone in pure form and pharmaceutical preparations.

2- Experimental

2.1. Apparatus

Spectrophotometric measurements were achieved, Shimadzu, Japan, UV-1800 PC double-beam, the UV-Probe software 2.21 is installed on the device that is connected to the computer with 1cm quartz cuvette. A Sensitive balance of type Mettler Toledo, Switzerland, with four decimal places. Julabo F12, a heating–cooling water path made in Germany.

2.2. Chemicals and reagent.

All chemical compounds that were used without further purification, that have high purity greater than 99.99%, Ceftriaxone (SDI) was obtained by a gift from Samara drugs company Iraq (SDI), The reagent is 4,5-diphenylimidazole from (Merck), absolute ethanol, hydrochloride acid, sodium hydroxide, and sulfamic acid from (sigma, Germany), and sodium nitrite from (LOBA Chemie, India).

2.3. Standard solutions

Ceftriaxone stock standard solution 500 μg/mL, This standard solution is prepared by dissolved 0.010 g of ceftriaxone in 5mL of absolute ethanol, then completing 20 mL of the volumetric flask with distilled water. 4,5-diphenylimidazole reagent solution 0.001 mole/L. This reagent solution was prepared by dissolving 0.022 g of 4,5-diphenylimidazole in 10mL of absolute ethanol and completing the volume with distilled water to 100 mL. Sodium nitrite solution 0.5 mole/L, This solution was prepared by dissolving 1.725 g sodium nitrite $NaNO_2$ in 100 mL distilled water. Hydrochloric acid solution 1.0 mole/L, this solution was prepared by diluting 8.8 mL of concentrated hydrochloric acid HCl to 100 mL of distilled water. Sodium hydroxide solution 1.0 mole/L was prepared by waiting for 4.0 g sodium hydroxide NaOH and dissolved in 100 mL distilled water to make this solution. Sulfamic acid solution 0.2mol/L, This solution was prepared by dissolving 1.940 g of sulfamic acid in 100 mL distilled water.

3- Results and Discussion

Many factors can influence the selectivity, accuracy, and precision of this spectrophotometric determination of ceftriaxone drug in the current study. These elements include the following:

3.1.1. Effect of HCl volume

Effect of HCl solution on the diazotization of ceftriaxone by using 1.0 mole/L HCl solution at different volumes at range of 0.25 to 2.0 mL, It was investigated for creating the diazonium salt. The 0.5 mL solution of 1.00 mole/L HCl produced the greatest results, and the results were as in Figure 2.

3.1.2. Effect of NaOH volume

Various volumes of 1.0 mole/L NaOH solution at range of 0.25 to 2.0 mL. were investigated. The 1.25 mL that produces the best result is used to form the azo dye compound, and the results were as in Figure 2.

3.1.3. Effect of sodium nitrite volume

Effects of sodium nitrite were studied by altering the volumes of a 0.50 mole/L NaNO2 solution at range of 0.25 to 2.0 mL. The greatest absorbance was found to be achieved by adding 0.75 mL of NaNO2 solution and waiting 10 minutes, and the results were as in Figure 2.

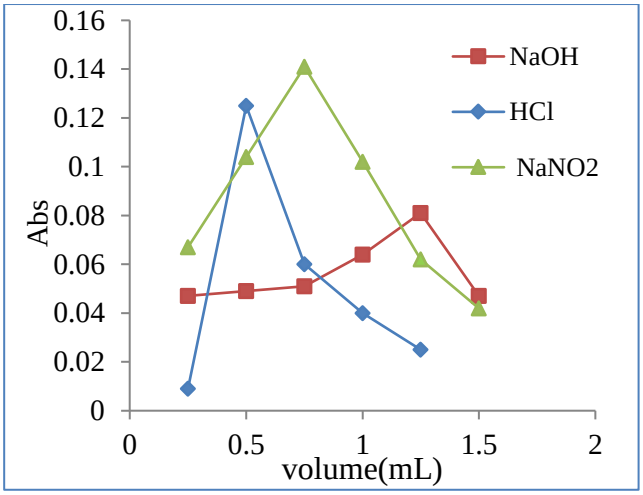

Figure 2. Volume effect of NaOH, HCl, and NaNO2 on complex formation.

3.1.4. Effect of sulfamic acid volume

Various volumes of (0.20 mole/L) sulfamic acid ranging from 0.25 to 1.50 mL , have been studied. The best outcomes come from 0.75 mL of sulfamic acid, and the results were as in Figure 3.

3.1.5. Effect of reagent volume

Effect of (4,5-DPI) reagent volume: The effect of reagent volume was ascertained by altering the volumes of 0.001 mole/L of 4,5-diphenylimidizole solution at range of 0.25 to 2.00 mL. The results show that 0.75 mL of reagent is enough to produce the highest absorbs, and the results were as in Figure 3.

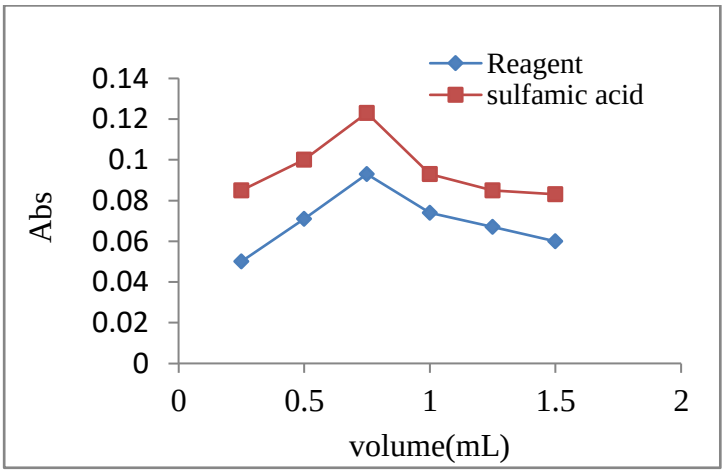

Figure 3. Volume effect of sulfamic acid and reagent on complex formation.

3.1.6. Effect of the temperature

The impact of temperature on absorption was study at a range of temperatures 0 to 50 0C. The results showed that the absorbance was quite stable over a temperature range of 0 to 30 0C. As the temperature rose, the absorbance value dropped, most likely as a result of azo dye dissociation, and the results were as in Figure 4.

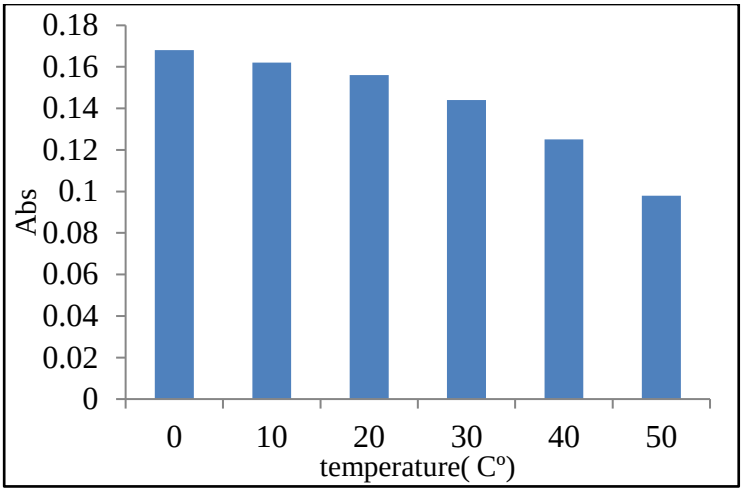

Figure 4. Temperature effect on complex formation.

3.1.7. Effect of the time for diazonium salt formation

The azo dyes give absorption high peaked at λmax 500 nm after 10 minutes and remained steady for up to an hour, per the findings. The results were as in Figure 5.

3.1.8. Effect of removal time of an excess nitrous acid

The best breakdown time for an excess of nitrous acid was obtained (1 minute), however varied times 1 to 5 minutes, were used to break down the excess of sulfamic acid solution. The results were as in Figure 5.

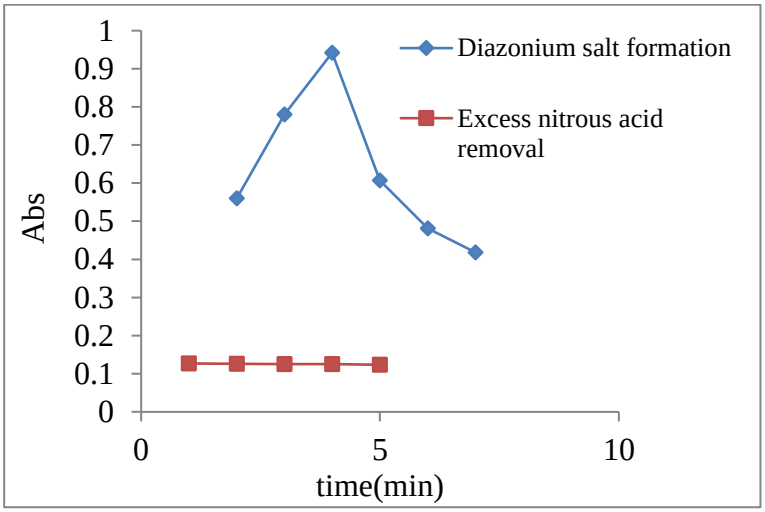

Figure 5. Effect of time diazonium salt formation and removal time of an excess nitrous acid

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3.2. Recommended Procedure and Calibration curve

In a series of 10 mL volumetric flasks, amounts of ceftriaxone solution equivalent to 10-100µg/mL was added to each flask, followed by 0.5 mL. of 1.0 mole/L hydrochloric acid solution and 0.75mL. of 0.50 mole/L sodium nitrite solution. After that, The flasks were allowed to stand in an ice bath from 0 to 5 0C for approximately 10 min. finally added to each flask a cold solution containing a 0.75mL of 0.001mol/L 4,5-diphenylimidazole in 1.25mL of 1.00 mole/L sodium hydroxide solution, left aside until appeared a purple azo dye color as a result of coupling between diazotized ceftriaxone with 4,5-diphenylimidazole, then added 0.75 ml of 0.10 mole/L of sulfamic acid to breakdown an excess amount of nitrous acid that formed. and complete the series 10mL. flask with distilled water. After leaving a resulting solution 15 min at 0 to 10 0C the absorbance of each solution was measured at λmax 550 nm using 1.00 cm cell against the blank solution which was prepared in the same mentioned method except for the Addition of the drug. Figure 6 and Table 1 show the calibration curve for the determination of ceftriaxone drug and the analytical values of statistical treatment of the calibration curve.

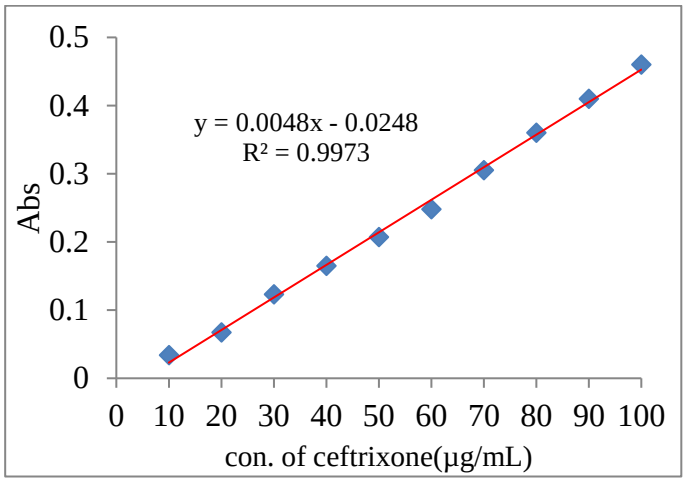

Figure 6. Calibration curve for the determination of ceftriaxone drug.

Table 1. Analytical values of statistical treatments of the calibration curve of the procedure developed to determine ceftriaxone.

Parameter	Value
Equation of regression	Y = 0.0048x - 0.0248
Slope	0.0048
correlation coefficient	0.9973
linear Range (µg \ mL)	10–100
molar absorptivity ($L.mol^{-1}.cm^{-1}$)	3772.08
limit of detection(LOD) (µg mL^{-1})	4. 9425
limit of quantification(LOQ) (µg mL^{-1})	16.475
sandal's sensitivity, S(µg cm^{-2})	0.208
Relative standard deviation (RSD%)	3.8
recovery (%)	100.84

3.3. Absorption spectra

Optimum conditions were obtained for forming a purple-colored azo dye, by the reaction between 4,5-diphenylimidazole in alkaline media with diazotized ceftriaxone. This colored azo exhibited an absorption maximum at λmax 550 nm against reagent blank as shown in Figure 7.

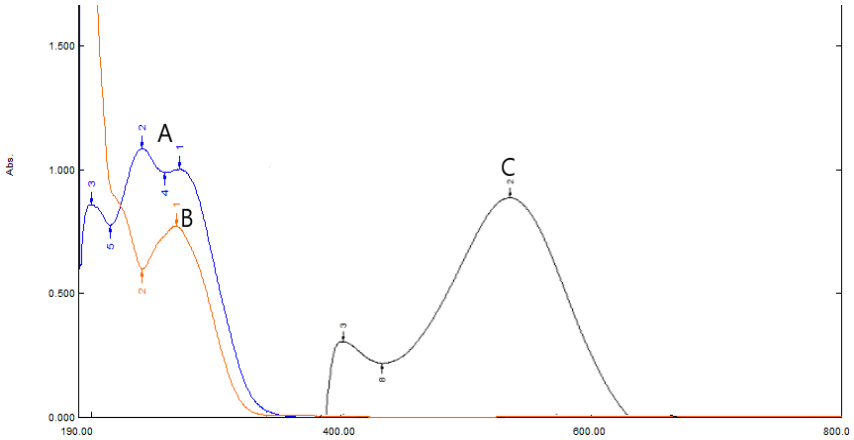

Figure 7. A absorption of Ceftriaxone drug λmax 220 nm, B absorption of 4,5-DPI λmax250nm and C absorption of conjugated complex of (drug + reagent).

3.4. Composition of AZO Dye

The stoichiometry of the product was investigated under established optimum conditions by applying the continuous variation(Job's method) (20), it was used to determine the stoichiometry of the diazotized ceftriaxone with the reagent 4,5-diphenylimidazole.The process was done in a series 5 ml. flask by adding increasing volumes of diazotized ceftriaxone (0.5,1,1.5,2,2.5,3,3.5,4,nad4.5) mL in each flask, and complete each with a sufficient volume of 4,5-diphenylimidazole reagent in alkaline media so that the total volume becomes 5mL , then the absorbance was measured at λmax. 550 nm against reagent blank. The obtained results indicate that the formation of azo dye (CF- 4,5- DPI) is 1:1 as in Figure 8.

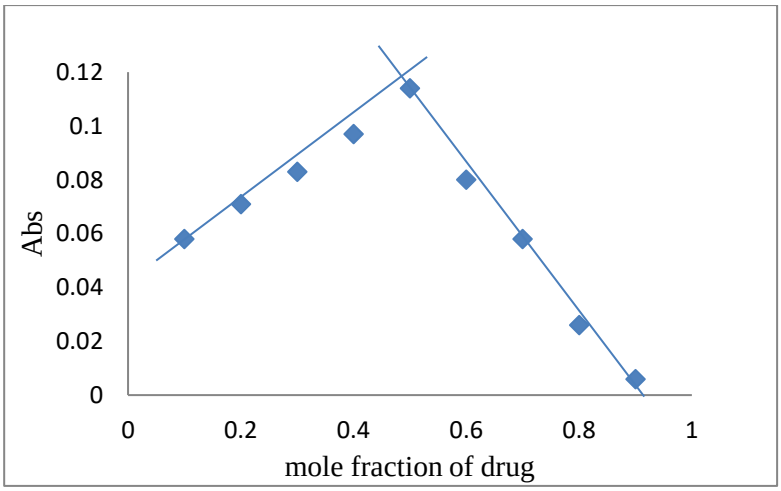

Figure 8. Job's method for the reaction between diazotized ceftriaxone with reagent 4,5-DPI.

3.5. Reaction mechanism

The estimated of reaction mechanism between diazotized ceftriaxone and 4,5-DPI reagent in alkaline media to formed azo dye colored compound according to previous studies.(20) The reaction mechanism can be proposed as shown in the Figure 9.

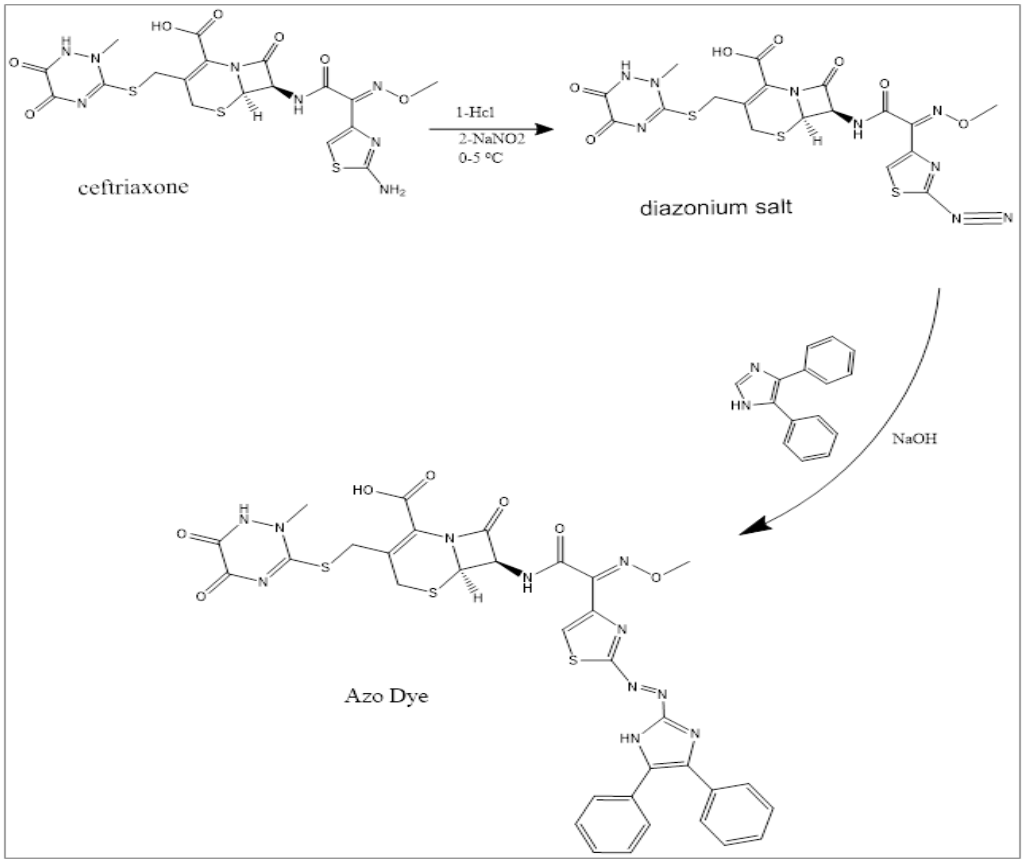

Figure 9. Proposed reaction between diazotized ceftriaxone and 4,5-diphenylimidazole reagent in alkaline media to formed azo dye.

3.6. comparison of the proposed method with the other techniques for determination of ceftriaxone

The comparison of ceftriaxone determination in this study with different techniques given in Table 2.

Table 2 comparison between the spectroscopic method for the determination of ceftriaxone using 4,5- DPI reagents and some of other techniques.

Technique	Reagent	Linearity range mg/ml	Reagent conc.	Ref.
Spectrophotometry	DMAB	2-50	0.05	21
UV-Spectrophotometry	--------	5-50	----------	22
RP-HPLC	------	2.5-25	----------	23
Spectrophotometry	2,5-DMP	3-50	0.002	24
Spectrophotometry	4-TBP	10-30	0.0025	24
Spectrophotometry	4,5-DPI	10-100	0.001	This work

3.6. Application of the method in the pharmaceutical preparations

Ceftriaxone was successfully quantified using the suggested method in its pharmaceutical forms. The findings in Table 3 imply that the assay solutions (Injection) were prepared with appropriate accuracy and precision and that the amount of ceftriaxone used in each type of assay was calculated based on the absorbance of the amount taken from the solution.

Table 3. Applications of the method for determination of ceftriaxone in pharmaceutical preparations.

Applications in pharmaceutical	Con. Taken (µg /ml)	Con. Found (µg /ml)	E %	Recovery %	R.S.D %
Ceftriaxone Injection (500 mg/2mL) (India)	50	48.1	3.8	96.2	1.68
	60	57.6	4	96	4.05
	70	71.1	1.5	101. 5	1.68
Ceftriaxone Injection (1g/2mL) (L.D.P) Spanish	50	47.9	4.2	95.2	1.98
	60	58.32	4.45	97.2	2.15
	70	69.5	1.25	98.75	2.25
Ceftriaxone Injection (1g/2mL) SANAVITA Germany	50	48.4	3.2	96.8	0.55
	60	60.24	0.4	100. 4	0.57
	70	69.1	1.2	98.8	1.25

4- Conclusion

The use of ceftriaxone medication aids in the development of studies that require analytical and bio-analytical quantification. The drug's characteristics, properties, and analytical methods are well defined. However, it is necessary to encourage and raise awareness about the need to develop and validate innovative analytical methods. The results obtained are consistent with the declared contents of dosage formulations. The statistical parameters confirm that the results are precise and accurate. This method has an advantage over the other reported methods due to its reliability, rapidity, simplicity, sensitivity, economical nature, good recovery, and precision.

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