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RP- HPLC Estimation of Ceftriaxone Sodium in Pharmaceuticals

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Abstract

This work aimed to determineof Ceftriaxone sodium (CFT) through the development and verification of a accurate and rapid isocratic of RP-HPLC method using a C18 as stationary phase column. The mobile phase was described as a mixture of 80:20 (5.8 - 8) Buffer:methanol. The optimum conditions for estimation were; column temperature 30 °C and flow rate of 0.7 mL/min. by using the UV-detection at a 241nm wavelength. The analysis data showed that the determination coefficient, R2 was 0.9985, detectioin limit of 4.3 x 10−6 µg/mL and limit of quantification of 1.4 x 10−5 µg/mL. This developed method was successfully examined for the estimate of ceftriaxone sodium in their pure form and in numeral commercial pharmaceuticals preparations with little interference of additives.

Keywords: Ceftriaxone Sodium, Analysis, HPLC.

1. Introduction

Ceftriaxone sodium (CFT) is third-generation cephalosporin; the molecular formula is $C_{18}H_{18}N_8O_7S_3$[1-3]. Chemically (6R, 7R)-7- {[(2Z) -2- (2-amino-1,3 – thiazol-4-yl) -2-(methoxyimino) acetyl] amino}-3-{[(2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)thio] methyl}-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, scheme I [4, 5].

The CFT is resulting from a fermentation product, this group of unsafe importance because they are able to overcome the blood-brain barrier. However, it has activity broad spectrum next to Gram-negative and Gram-positive bacteria. Ceftriaxone sodium injection is an antibacterial use to treat diseases, for example, lower respiratory tract infections, skin structure infections, urinary tract infections, pelvic inflammatory disease, bone and joint infections also meningitis [6, 7].

More papers were published in articles aimed at expanding and demonstrating HPLC and LC / MS resistors for similarity and evaluation of this active ingredient in pharmaceuticals [8-10]. The essential examination of the work of organic modifiers on selectivity, determination, and the temperature is an effect on chromatographic behaviour[11,12]. HPLC has also been developed through the stationary phases that have been improved through the 30-alkyl carbon atoms chains [13- 15]. A number of techniques have been described the determination of ceftriaxone in literature such as HPLC-UV [16- 18], HPLC-MS [19, 20], UPLC-MS/MS [21, 22], spectrophotometry [23-25], spectrofluorometric [26] and infrared [27].The technique used by HPLC to identify more drugs in standard solution, pharmaceutical preparation[28-31] and biological samples[32- 34].

This study aimed to developing a simple and rapid RP-HPLC for the estimation of CFT for quality control propose.

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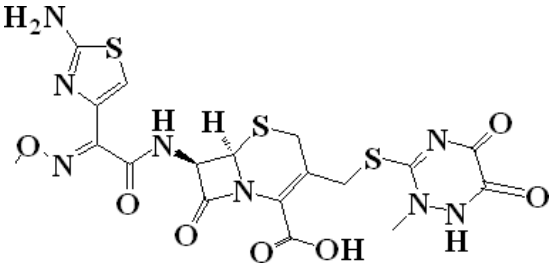

Scheme 1: Structure of Ceftriaxone Sodium

2. Materials and Methods

2.1. Apparatus

The HPLC system, which was used in this work, is Shimadzu, Kyoto, Japan with RP-sunfire^{TM} C18 (3.5 µm ×4.6× 100 mm) column. Glass bottles were used as solvent reservoirs with capacity of 1000 mL. The mobile phase was passed through a fine mesh stainless steel filter of 0.45 μm pore size fitted at the end of PTFE tube so as to avoid several strange particles to go over the column, which may eliminate the pump as well as reduce the lifetime of the column. The HPLC system is equipped with Rheodyne injection valve for manual sample injection. The injector could be supplied with a 10 or 20 μL internal capacity sample loop. Forced air circulation column oven type CTO-20A used regulated temperature from 10 to 85 °C. The on-line degasser unit, DGU-20A5 in HPLC system was used for quick degassing the HPLC mobile phases. The 0.4 ml internal volume capacity is small. The SPD-20A is a UV-VIS detector that suggests excellentsensitivity and stability, at wavelength ranged from 190 to 700 nm and slit width of 0.8 nm with wavelength accuracy of 1.0 nm and 12 μL flow cell and dual-wavelength measurement.

2.2. Chemicals (Preparation stock solution of ceftriaxone sodium)

The CFT standard and the rest of the reagents (methanol and contents of buffer solutions) were supplied by Sigma- Aldrich. 100 µg/mL of standard CFT was prepared by dissolved 0.1g of it in 1000 mL of Water then other diluted solution prepared.

2.3. Preparation Buffer solution

The buffer solution was prepared in a 100 mL volumetric flask by dissolving A) 0.323 g of KH_2PO_4 in 100 mL of distilling water and B) 0.4564 g of K_2HPO_4 in 100 mL of distilling water, mix the proportions indicated of solutions (A) and (B) and adjust the final voulum to 200 ml with D.W.to achieved a pH ranged between 5.8-8.[35]

2.4. Determination analysis conditions and mobile phase

The CFT identification was performed using a mobile phase consist of (80:20 v/v) methanol and buffer de-ionized water. The determination was donned with a 0.7 mL/min. flow rate at 30°C and injection volume of 20µL. The isocrant process is used from the flow mode and is operated by the LC program. The detection is controlled at multiple wavelengths. Peak area and peak height were recorded and used in this study.

3. Results and Discussions

This search summarizes to development a chromatographic mode for the separation and estimation of ceftriaxone sodium in the pure dosage form and in different commercial shapes. Changes the HPLC parameters to reach perfect conditions of mobile phase (buffer: methanol), flow rate and ambient temperature by study improvement the effects.

3.1. Effect of mobile phase (buffer: methanol):

Used RP-sun fireTMC18 (3.5µm×4.6×100mm) column was kept at30ºC for all circumstances of organic change to determination ceftriaxone sodium. The buffer is Dipotassium hydrogenphosphate. Extensively used, by ethanol in the chemical-pharmaceutical analysis by HPLC. Ethanol has fewer ecological influence and inferior danger to the health of the expert disproportion to classical solvents methanol[36]. That significant changes in the work environment to optimize the working conditions for isocratic RP-HPLC to improving determination method a number of parameters such as flow rate and proportion organic phase (buffer: methanol).

3.1.1. Flow rate

To decrease the retention time and improve the symmetric, sharp and good peak must adjust the flow rate of mobile phase by used deference of them, such as (0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0) mL/ min. The flow rate of 0.7 mL/ min. was chosen as the best one, Figure-1.

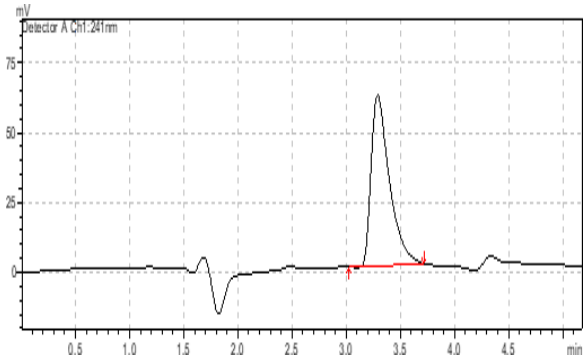

Fig. 1: CFT chromatogram at Flow rate of 0.7 ml/min buffer: methanol at 80:20%, at 30 °C.

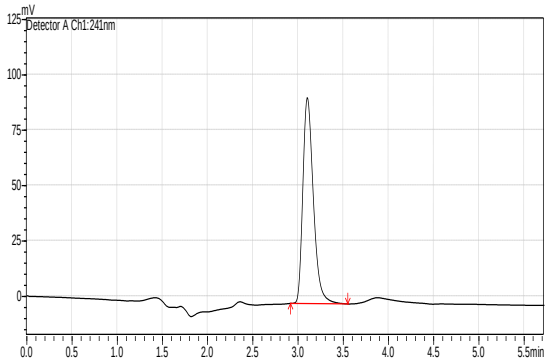

Fig.3: CFT chromatogram at temperature of 30 °C., buffer: methanol 80:20% and flow rate of 0.7 ml/min.

3.1.2. Uses organic modifier

The used mixture of buffer and methanol with various ratios (85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55,45 and 50:50) % to inject as organic modifier which is considered an isocratic elution. The wavelength detection was 241 nm at 0.7 ml/min flow rate and 30 °C, Figure 2. The optimum of buffer and methanol 80:20% because the retention time decrease from 7.2 min. in 85:15% buffer and methanol to 4.69 min. and give sharp, symmetrical peaks and good sensitivity that give short analysis time and be contingent on possessions of the analyzed materials.

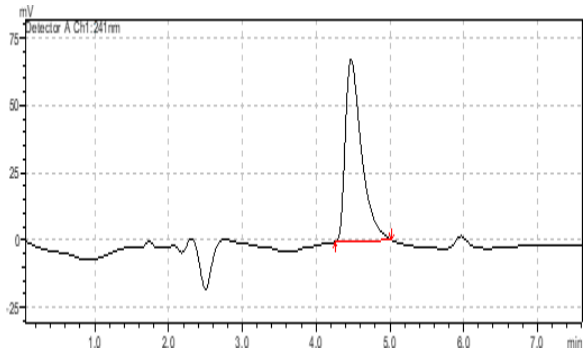

Fig. 2: CFT chromatogram at organic modifier buffer with methanol 80:20%, at flow rate of 0.7 ml/min and 30 °C.

3.1.3. Temperature

The temperature is working on improve the retention time in RP-HPLC of the determination ceftriaxone by decrease the mobile phase viscosity and efficiency of the column and make on decrease back pressure which in turn on lowest the interface with the stationary phase[37]. Different temperatures have used at range of 25 –55 ºC. Figure-3 is shown the perfect temperature at 50 ºC because that increase efficiency, a good shape and fast determination

3.1.4. Effectiveness

Estimation of all analytical statistical data by using the line regression equation for the calibration curve, which represents the relationship between concentrations, µg/mL vs peak areas. The calibration curve for a series of standard solutions was within a range of 2.0 to12.0 µg/mL. Figure-4 shows that. Moreover, estimate the accuracy and precision for this method to determination ceftriaxone sodium by examining five duplicate three different concentrations. Calculate the precision by knowing the RSD%., the accuracy by know the recovery, Table-1. There are no overlaps in the estimate of ceftriaxone sodium in this method because the drug is free of additives.

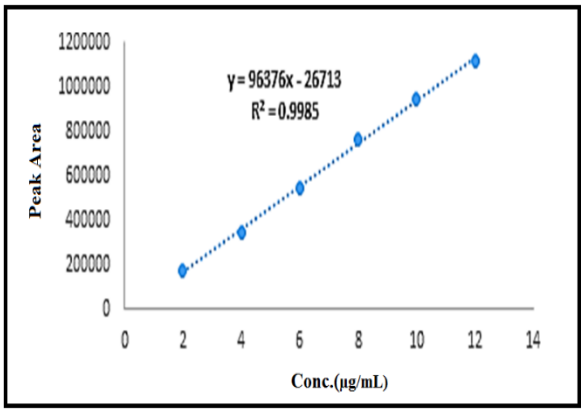

Fig. 4: Calibration graphs of CFT at concentration range between 2-12 µg/mL vs. peak area

Table 1: The analytical values achieved using the HPLC technique with 10 µg/mL of CFT, 80:20% buffer: methanol, 0.7 ml/min flow rate at 30 0C

Parameters	**Result**
Dynamic range, µg/mL	2.0 –12.0
Slope	96376
Intercept	26713
Determination coefficient R2	0.9985
Correlation coefficient (r)	0.9992
LOD, Limit of detection, µg/mL,	4.36 *10−6
LOQ, Limit of quantification, µg/mL,	1.44 *10−5
Recovery%	99.57
RSD%	2.15
Regression equation	y = 96376 X -26713

3.1.5. Analysis of ceftriaxone sodium in pharmaceutical formulation:

The current work method has been applied on some of the pharmaceuticalformulations for ceftriaxone sodium of various commercial source (Malaysia, Saudi and United State Emirate) containing 1.0 gm from the active ingredient. The result was shown in Table-2.

Table 2. Accuracy and precision of the 10 µg/mL of CFT, 80:20% buffers: methanol at flow rate of 0.7 ml/min at 30°C.

Pharmaceuticals of ceftriaxone sodium	Amount of Drug µg/mL Taken	Found	Relative Error %	Recovery %
Malaysia	6.0	6.034	0.567	100.567
	10.0	10.424	4.240	104.240
Jordan	6.0	6.552	9.200	109.200
	10.0	10.620	6.200	106.200
United State Emirate	6.0	5.977	-0.380	99.617
	10.0	10.161	1.610	101.610

Table 1. Developed method compared with other methods for determination of ceftriaxone.

Type of technique	Type of sample	Linear range µg L^{-1}	LOD µg L^{-1}	Recovery %	RSD%	N	Ref.
HPLC–UV	Hospitalised sewage	5.0 - 600	2.0	152.38	5.2	5	(38)
HPLC–MS/MS	human plasma	3.0-300	------	87.35	8.55	5	(5)
HPLC	Sterile powder for Injection	20-150	------	99.42	0.59	5	(39)
HPLC	Human urine	0.24-250	0.05	97.73 - 100.7	2.5	6	(40)
RP-HPLC	Pharmaceutical Formulations	2.5-25	0.51 - 1.54	>98.1	< 2.0	6	(41)

4. **Conclusion**

In the current work, RP-HPLC is a simple and fast way. This method was developed to estimate sodium ceftriaxone in the preparation of pure pharmaceuticals. This method was validated comprehensively in provisos of linearity, accuracy, selectivity and accuracy in injecting pharmaceutical samples. Interventions are not present in this method due to the injection of drugs without additives.

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6. **References**

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