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A HYDROPHILIC INTERACTION CHROMATOGRAPHY ASSAY FOR QUANTIFICATION OF CEFTRIAXONE CONCENTRATIONS IN PHARMACEUTICAL INJECTION FORMS

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Abstract

For the determination of ceftriaxone in pharmaceutical injection forms the hydrophilic interaction chromatography method has been developed and validated. The separation was carried out using the HALO ® HILIC 2.7 column (100 mm-2.1 mm I.D) with buffer acetate (30 mM-pH 5.5) and acetonitrile as eluent at a rate of 15:85 v/v at a flow rate of 0.5 mL/min and wavelength of 254 nm. The method for ceftriaxone was found to be linear in the range of 100-2500 ng mL^{-1} (R2 = 0.9988). The intra-day percentage RSD was 0.7 and inter-day precision was 0.8. The method showed good, consistent ceftriaxone recoveries (98.33-99.33%). The method for determining ceftriaxone in pharmaceutical injections was found to be specific, accurate, precise and linear.

Key words: Ceftriaxone, hydrophilic interaction chromatography, hydrophilicity, lactam pharmaceutical

Introduction

Ceftriaxone (Fig. 1 CRO) is a cephalosporin third generation with a wide spectrum of in vivo and *in vitro* action, including penicillin resistant pneumococci and anaerobic bacteria, against aerobic gram-negative and gram-positive micro-organisms (Fischer *et al.*, 2010, Organization, 2019, Pharmacopoeia, 2015). The half life is 8-10 hours compared to other cephalosporins of third generation, which permits administration once daily (Capri and Dellamano, 1993). Several surveys have seen the antibiotic to be parentally active with similar antibacterial range and with resistance to β-lactamase from other third-generation parenteral cerebrosporins (Rodrýµguez *et al.*, 2000). For the determination of CRO there are several analytical methods such as HPLC (Shrestha *et al.*, 2013, Shrivastava *et al.*, 2009, Kale *et al.*, 2011, Wongchang *et al.*, 2019), TLC (Joshi *et al.*, 2009) and spectrophotometry (Rind *et al.*, 2008, Morelli, 1994).

The chromatography of hydrophilic interactions (HILIC) combines a polar phase with a mobile phase with a large water volume and a lower polar solvent increase. Typical divisions are made with water buffers between 5 and 40 percent; the procedure also coincides with gradient elution. Alpert (Alpert, 1990) coined the word HILIC in 1990, describing its guidelines and some important applications. Consequently recently, the estimates of dansyl-amino acids, inorganic ions, nucleosides, carboxylic Acids and pharmaceuticals have started to increase dramatically with HILIC technology innovation (Yaqout Abd Al-Hakeem Hamed and Rasheed, 2020, Ashraf Saad Rasheed and Rashid, 2020, Ashraf Saad Rasheed *et al.*, 2019, Abbas and Rasheed, 2018, Seubert and Saad Rasheed, 2017, Rasheed *et al.*, 2017, S Rasheed and Seubert, 2016, Al-Phalahy and Rasheed, 2016, Al-Phalahy *et al.*, 2016, Abbas and Rasheed, 2017a, Abbas and Rasheed, 2017b). The objective of this study was to develop a simple, sensitive, reliable, flexible, quick, and time-saving dosage model of pharmaceutical injections.

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Materials and Methods

Chemicals and reagents:

Millipore filters (0.45 µm) were used to purify the solutions. As far as chemicals are concerned, obtained from Sigma-Aldrich as follows: acetic acid, sodium

acetate, Acetonitrile (ACN), and ceftriaxone. 0.05 µs/cm of Millipore water conductivity has been used (System-US Millipores). The use of a group of pharmaceutical preparations for ceftriaxone available in pharmacies, ceftriaxone (1000 mg) IM-LDP-spain, ceftriaxone (1000 mg) IM/IV-SANAVITA-Germany and ceftriaxone (1000 mg) Mesporin-IM-acino- Switzerland.

Preparation of stock solution for ceftriaxone:

To be dissolved accurately with ceftriaxone (1 mg) in 100-ml mobile phase, the stock solution of ceftriaxone (10000 $ng.ml^{-1}$) was prepared. The solution was filtered with 0.22 µm.

Preparation of pharmaceutical samples

For each of the three commercial firms, thirteen vials were stored and approximately 1 mg ceftriaxone was dissolved into a 100 ml volumetric flask with a sufficient size of mobile phase and diluted with mobile phase to mark. The solution was then filtered with Millipore filters (0.45 µm). Then the stock solution was diluted and other standard solutions were generated.

Chromatographic condition and instrumentation

Ceftriaxone detection was carried out at a flow rate of 0.5 ml / min in UV regions with a wavelength of 254 nm. For Merck Hitachi HPLC and the UV-visible L-4200, a 20µL injection loop is supplied with the gradient L-6200 pump. The pH reviews were performed with the pH 740 (WTW). The chromatogram can be measured with the photographic software from the N2000 workstation. Advanced materials technology (100 mm-2.1 mm I.D) is available for the HALO ® HILIC 2.7 column for ceftriaxone separation.

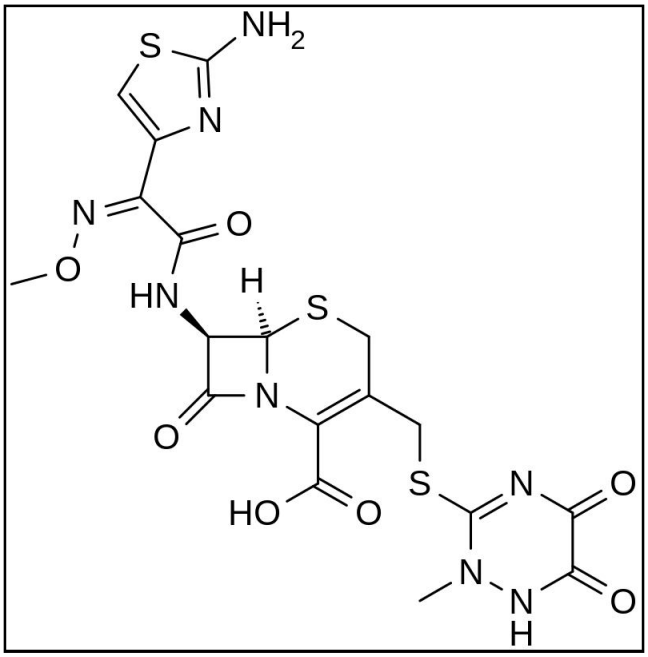

Fig. 1: Chemical structure of ceftriaxone.

Results and Discussion

Ceftriaxone Separation Optimization

HILIC retention mechanism was checked by the HALO column by using the mobile phase mix ACN with acetate buffer. A chromatogram Fig. 2 was obtained at 85% ACN and 30 mM (pH 5.5) acetate buffer. In mobile phase compounds the systemic variation of the contents of ACN increases from 50%-95%, the eluent concentration varies from 10-80 mM with pH between 3-5.5.

The effect on the retention of ceftriaxone of the ACN content

ACN effect on ceftriaxone retention was noted at 5.5 pH 30 mM acetate buffer. Ceftriaxone HILIC activity appears to increase the eluent ACN ratio from 50% to 95%. This is because it has ceftriaxone hydrophilicity; the HILIC behavior in ceftriaxone is shown in HALO column Fig . 3, because of the ceftriaxone log P_{OW} (-1.87).

The influence of the buffer concentration on retention of ceftriaxone

In 10-80 mM (pH 5.5) at 85 percent ACN in the eluent, the effect of the acetate buffer on the elucent retention behavior of ceftriaxone was recorded. The results appear in Fig. 4. Increase buffer levels in the acetate eluent increase the retention factor of ceftriaxones in the column. It is due to the hydrophilicity of ceftriaxone. The stationary process of the HILIC material is closely related.

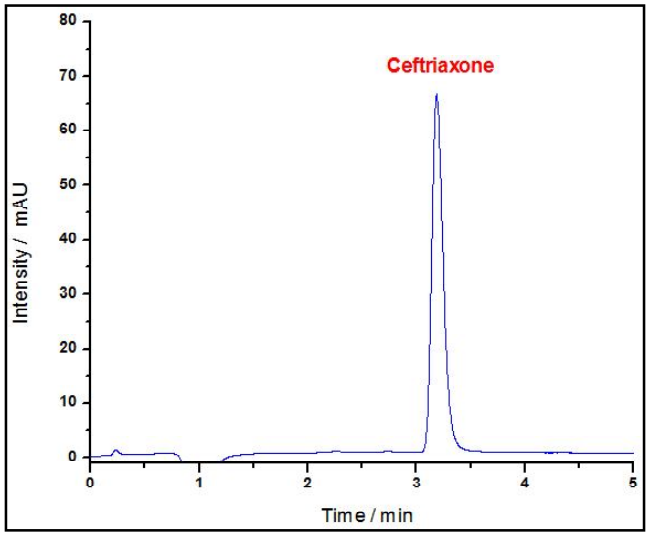

Fig. 2: Chromatogram of ceftriaxone using the HALO column.

Eluent pH effect on retention of ceftriaxone

An eluent pH change is required to apply the next improvement in the composition of the eluent. The eluent pH must be changed if the ceftriaxone is to be separated in HILIC mode. At a constant buffer level of 30 mM and 85 percent of ACN, the pH was increased from 3 to 5.5. As Fig. 5 shows declines in ceftriaxone retention. This is due to the deprotonation of the hydroxyl group in ceftriaxone. This reflects the expected physicochemical knowledge of ceftriaxone. The pKa is 3.54 and ceftriaxone isoelectric is 2.91. The ceftriaxone is therefore anionic.

Table 1: The results for the calibration ceftriaxone curve are checked by the HALO column.

Parameter	HILIC method
Linearity[a] (ng ml^{-1})	100-2500
Regression equation	y = 433.01 + 2.00 * x
R^2	0.9988
LOD (ng ml^{-1})	12.35
LOQ (ng ml^{-1})	37.42

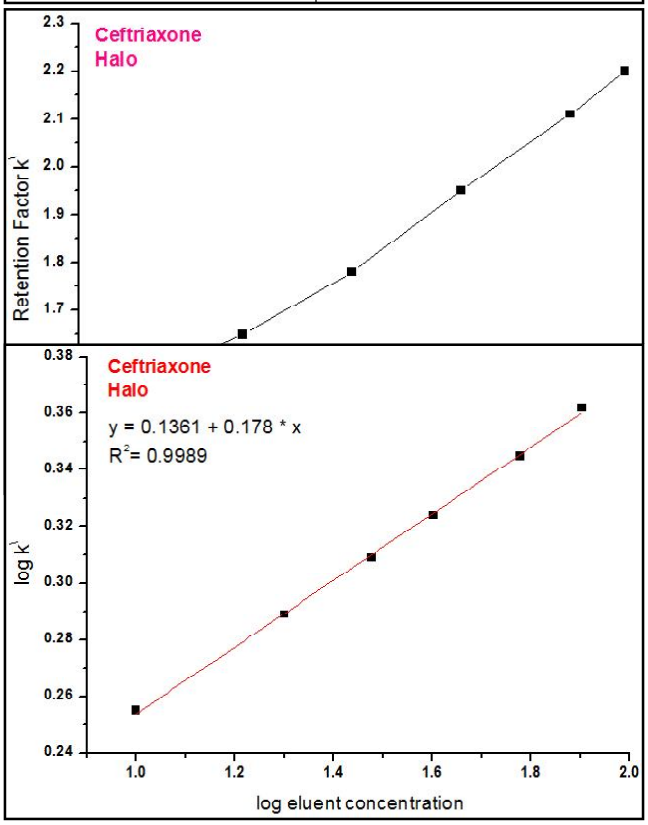

Fig. 4: The effect of buffer concentration.

Calibration graph

The calibration ceftriaxone curve is created by placing the ceftriaxone concentration against the peak area, showing the HALO column (100-2500 ng ml^{-1}).

Statistical data information

Detailed examinations of ceftriaxone under HILIC conditions and record statistics in table 1 were used in

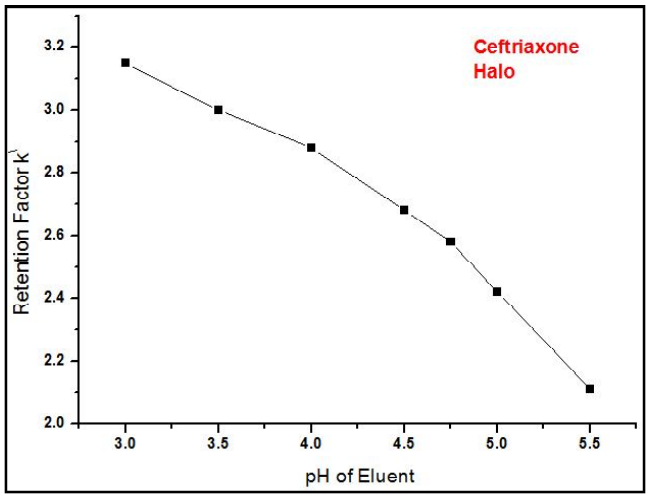

Fig. 5: The effect of eluent pH.

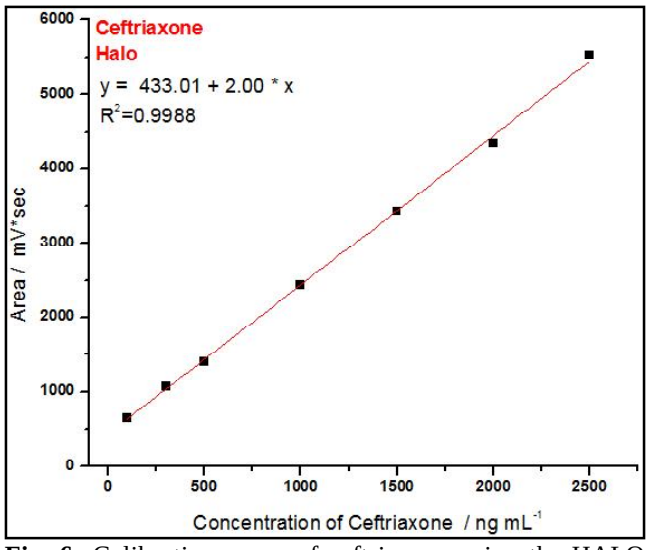

Fig. 6: Calibration curve of ceftriaxone using the HALO column.

Table 2: Methodological performance of ceftriaxone on the same day as on different days.

Same-Day Analysisn = 4					Day-to-Day Analysisn = 4			
CROTaken(ng ml^{-1})	CROFound(ng ml^{-1})	% Rec.	% Erel.	%RSD	CROFound(ng ml^{-1})	% Rec.	% Erel.	%RSD
300	295	98.33	- 1.67	0.83	292	97.33	- 2.67	0.80
10001500	9921490	99.2099.33	-0.80-0.67	0.720.70	9901493	99.0099.53	- 1.00-0.47	0.780.65

Table 3: Experimental results for evaluating the ceftriaxone in pharmaceutical appliance.

Name of drug	Company	Present (mg)	Get it (mg)	%Rec.	%RSD n=4	% $E_{rel.}$
ceftriaxone	LDP-spain	1000	984.55	98.45	0.76	-1.54
ceftriaxone	SANAVITA-Germany	1000	1008.44	100.84	0.88	0.84
ceftriaxone	Mesporin-acino- Switzerland.	1000	1000.88	100.08	0.38	0.08

Table 4: Compared the method proposed by t- and F-statistical tests with the compared method for ceftriaxone determination.

Name of drug	Halopr-ocedure	Comparison procedure	t-Test (theor.)	F-Test (theor.)
ceftriaxone-1000 mg	98.45	99.05	0.9561 (2.7764)	3.5660 (19.000)
ceftriaxone-1000 mg	100.84	99.85		
ceftriaxone-1000 mg	100.08	100.33		

the corresponding calibration curve. The accuracy, precision (RSD percent and Rec. percent) were calculated on the same day and on different days. The relatively low defaults and high recovery values show the effectiveness of the proposed method table 2.

Ceftriaxone determination in drug samples:

In the evaluation of ceftriaxone for three of the pharmaceutical samples, the proposed method was used with success; the findings are listed in table 3.

These results are compared to results obtained in the comparison to those achieved in British Pharmacopoeia procedure (Pharmacopoeia, 2009) in order to assess the expertise and efficiency of the HILIC method. The results for the two methods, t-test and variance ratio F-test table 4, which were 95% confident, were used as statistical analyzes. The determined values of t and F did not reach theoretical standards, meaning that the accuracy of the ceftriaxone determination in three pharmaceutical types is not substantially different from both approaches.

Conclusion

A method for estimating the amount of ceftriaxone in pharmaceutical samples has been identified in this paper. The proposed method was simple, quick and sensitive for ceftriaxone determination. To determine the low ppb ceftriaxone ranges, a hydrophilic chromatography interaction method was developed. Hydrophilic behavior with ceftriaxone is shown in the stationary HALO. It is because ceftriaxone log P_{OW} value is related to the Halo column. The methods developed were successfully used in pharmaceutical samples.

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