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Development of a validated hydrophilic interaction chromatography method for the determination of cefotaxime in pharmaceutical preparations

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Abstract

Cefotaxime is considered as one of the semi-synthetic, 3rd generation, cephalosporin antibiotics that have been utilized for treating a lot of infections resulting from different organisms. The technique of hydrophilic chromatography interaction (HILIC) has increased the accuracy and sensitivity concerning other methods, particularly spectrophotometer. The objective of this article was to introduce a simple method for the estimation of cefotaxime in pure and pharmaceutical injection forms and study the separation mechanism of cefotaxime. HILIC mode achieved excellent separation under chromatographic conditions on a HALO ® HILIC 2.7 column (100 mm x 2.1 mm I.D.) at 35 ° C with the following conditions: 10:90% acetate buffer (pH 5.5-40 mM): acetonitrile as eluent and wavelength of detection 254 nm. The proposed HILIC method exhibited high precision (RSD% < 0.5%), concentration ranges of 100-5500 ppb, and the lowest detection limit was 6.8167 ppb with a coefficient of determination of 0.9998 for cefotaxime. The findings of the method were used statistical tests compared to the British Pharmacopoeia protocol, which did not show any difference in accuracy among the methods

Keywords: Cefotaxime, HILIC, Injection forms, UV-detection, Beta-lactam, antibiotics

Introduction

Cefotaxime (Fig. 1-CTX) is a cephalosporin of the third generation. It has the same bactericidal effect as cefamandole, but it has a broader variety of activities, which has broad clinical applications to treat respiratory, gynecological, skin, bone and articulation infections, urinary tract, sperm [1]. The majorities of beta-lactamase are very stable in hydrolyses and have more activity against Gram-negative bacteria than the first or second generation of cephalosporin. While cefotaxime is commonly thought to have a slightly lower activity against Grampositive bacteria than cephalosporines of the first generation, many streptococci are extremely sensitive [2-5]. Many studies have shown that this is a parentally active antibiotic with a similar antibacterial range and β-lactamase resistance to other parenteral cerebrospinal fluid of the third generation [6]. Several methods have been applied both in the fields of pharmacy and biological fluids to determination CTX: polarographic [7], voltammetric [8-11], RP-HPLC [12-16], spectrophotometry [17, 18], chemiluminescence [19, 20], capillary electrophoresis [21, 22].

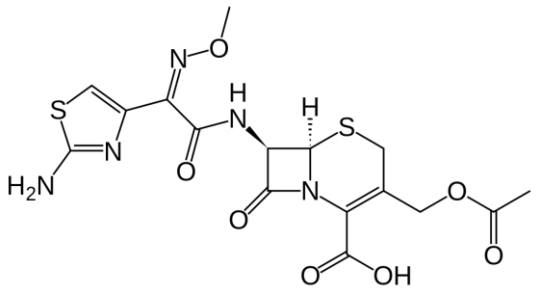

Fig. 1. Chemical structure of cefotaxime

The chromatography of hydrophilic interactions (HILIC) is a liquid chromatography (LC) strategy that combines a polar, stationary phase, and a mobile phase with significant water content, combined with an increase of a lower polar solvent. Separations are typically performed with 5–40% water; the procedure is also consistent with gradient elution. In 1990, Alpert coined the term HILIC, which explains its

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principles and certain important applications [23]. Hydrophilic and polar compounds, as compared to hydrophobic neutral compounds, are retained preferentially in HILIC the contrary of RP-HPLC. Thus recently, HILIC technology has thus recently begun to increase dramatically in the estimation of drugs, nucleosides, carboxylic acids, inorganic ions, and amino acids by Rasheed and its co-workers [24-34].

Experimental

Chemicals and reagents

In purifying solutions, Millipore filters (0.22 µm) were used. From Sigma-Aldrich obtained cefotaxime, acetonitrile, and sodium acetate as far as the chemicals are concerned. 0.1 µs / cm (System-US Millipore) of Millipore water conductivity was used. Injection vials of three different commercial companies, Cefotaxime (1 gm)-LDP-Spain, Cefotaxime (500 mg)-SANAVITA-Germany and Cefotaxime (500 mg) - *PHIL* Inter *Pharma*.-Vietnam.

Preparation of stock solution for cefotaxime

For stock cefotaxime solutions (10000 ppb) to be precisely dissolved in a cefotaxime quantity (1 mg) in 100-mL of eluent were prepared to be provided. The findings were dissolved and more filtered with 0.22 µm during the mobile process.

Preparation of pharmaceutical injection forms

Ten vials were collected for each of the three commercial companies and approximately 1 mg CTX was dissolved in a sufficient size of eluent into 100 mL volumetric bottle and diluted with eluent to the mark. Afterward, Millipore filters (0.22 µm) filtered the solution. By subsequently diluting the stock solution, other standard solutions were made.

Chromatographic condition and instrumentation:

In UV regions with a wavelength of 254 nm, CTX detection was conducted at a flow rate of 0.5 mL/min. A 20µL injection loop is supplied with the L-6200 gradient pump for Merck Hitachi HPLC and the UV-visible L-4200. On the pH 740 (WTW) the pH reviews have been conducted. The photographic software from the N2000 workstation can be used to measure the chromatogram. The HALO®HILIC 2.7 column used for CTX separation is obtained from Advanced Materials Technology (100 mm x 2.1 mm I.D.).

Results and Discussion

Optimizing the separation of CTX

As a pharmaceutical model, CTX has been selected to test the HILIC retention mechanism using HALO®HILIC 2.7 column by applying the acetate buffer with ACN content as eluent. At 90% ACN and 40 mM (pH 5.5) of acetate buffer, the chromatogram was obtained (Fig. 2). The systemic variability of the contents of the ACN is increasing in mobile phase compounds between 50% and 95%; the concentration of eluent between 10 mM and 80 mM with a pH between 3 and 5.5

The impact of ACN content on the retention of CTX

The effect of eluent ACN on CTX retention at 5.5 pH 40 mM acetate buffer has been observed. HILIC behavior of CTX tends to increase in the proportion of eluent ACN from 50% to 95%. The reason for this behavior is CTX hydrophilicity; in HALO®HILIC 2.7 column, the HILIC behavior of CTX is shown (Fig. 3), which was due to the log P_{OW} of CTX (-1.49) [27].

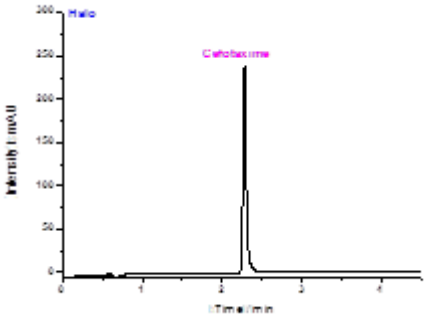

Fig. 2. Chromatogram of CTX using the HALO®HILIC 2.7 column.

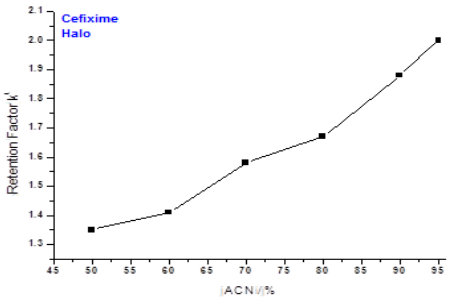

Fig. 3. Retention behavior of CTX as a function of ACN content

The impact of the buffer concentration on CTX retention

The effect of the acetate buffer on the eluent retention behavior of CTX has been reported in the 10-80 mM (pH 5.5) at 90% ACN in the eluent. The results are shown in (Fig. 4). Increasing buffer concentrations in the eluent of acetate increase the CTX retention factor in the column. The reason for CTX behavior is going to the hydrophilicity of CTX. This is closely connected with the stationary phase of the HILIC material

Eluent pH impact on CTX retention

The next improved composition of the eluent can be applied with a change in eluent pH. To complete CTX separation in HILIC mode, the eluent pH must be changed. The pH improved from 3 to 5.5 at a steady buffer concentration of 40 mM and 90% ACN. As shown in Fig. 5 CTX retention factor decreases. This is because the hydroxyl group is deprotonated in CTX. This represents the physicochemical data of CTX that are predicted. The pKa value 2.73 and isoelectric point 3.16 of CTX. Hence the CTX is an anionic form

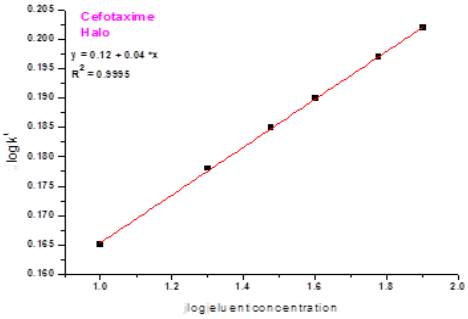

Fig. 4. Retention behavior of CTX as a function of buffer concentration

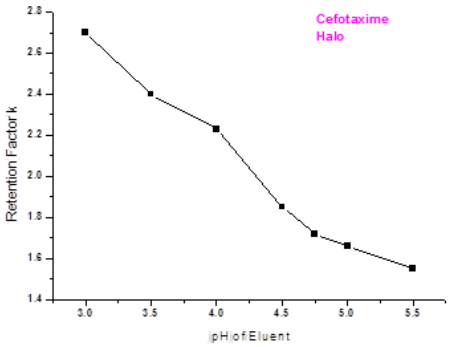

Fig. 5. Retention behavior of CTX as a function of eluent pH

Calibration graph

The standard curve of CTX is generated by plotting the CTX concentration against the peak area and showing concentration (100-5500 ppb) of the HALO®HILIC 2.7 column (Fig. 6).

Information about statistical data

The corresponding calibration curve used a thorough assessment of CTX under HILIC circumstances and record statistics in Table 1. Accuracy and precision were measured on the same day and different days and %RSD and %Rec. were determined. The relatively small defaults and high recuperation values indicate that the proposed method is successful (Table 2).

CTX determination in pharmaceutical injection forms

The proposed method was successfully used in the evaluation of CTX for three of the pharmaceutical injection forms; the results are outlined in Table 3.

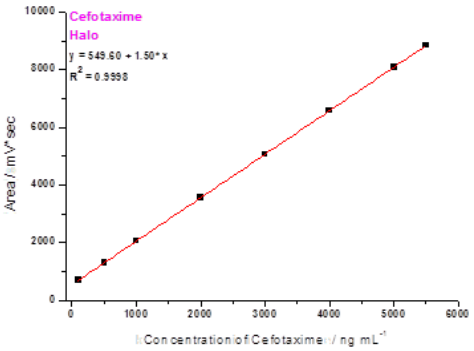

Fig. 6. Standard curve of CTX using the HALO® HILIC 2.7 column

Table 1. The verification of the results for the standard curve of CTX using the HALO®HILIC 2.7 column.

Parameter	**HILIC method**
Linearity (ppb)	100-5500
Regression equation	y = 549.60 + 1.50* x
R^2	0.9998
LOD (ppb)	6.8167
LOQ (ppb)	20.6567

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

	Same-Day Analysis n=5				**Day-to-Day Analysis n=5**			
CTX Taken (ppb)	**CTX Found (ppb)**	**Rec.%**	**Erel.%**	**RSD%**	**CTX Found (ppb)**	**Rec.%**	**Erel.%**	**RSD%**
500	493	98.60	- 1.40	0.42	495	99.00	- 1.00	0.56
1500	1495	99.66	-1.34	0.25	1493	99.53	-0.47	0.40
2500	2488	99.52	-0.48	0.27	2480	99.20	-0.80	0.40

Table 3. Appliance in pharmaceutical injection forms of the proposed method for the determination of CTX.

Name of drug	Company	Present (mg)	Get it (mg)	Rec.%	RSD% n=5	$E_{rel.}$%
Cefotaxime	LDP-Spain	1000	988	98.80	0.46	- 0.20
Cefotaxime	SANAVITA-Germany	500	495	99.60	0.33	- 0.40
Cefotaxime	*PHIL* Inter *Pharma*.-Vietnam	500	510	101.60	0.55	0.60

In order to assess the competence and efficiency of the HILIC method, these findings were compared with the results obtained by the compared with those obtained by the British Pharmacopoeia procedure [35]. Statistical analyses were performed with the findings of the two methods t-test and variance ratio F-test (Table 4), which were 95% confidence. The t and F values calculated did not exceed the theoretical values, which means that both methods do not differ significantly in the precision of the CTX determination in three pharmaceutical injection forms.

Table 4 The comparison of the proposed method with the standard method [35] for CTX analysis by investigating t- and F-statistical tests.

Name of drug	HILIC Method	standard Method [35]	t-Test (theor.)	F-Test (theor.)
Cefotaxime-1000 mg	98.80	99.65	0.8222 (2.7764)	0.9877 (19.000)
Cefotaxime-500 mg	99.60	98.3		
Cefotaxime-500 mg	101.60	101.2		

Conclusion

This paper has established a HILIC method to estimate the amount of CTX in three pharmaceutical injection forms. For CTX determination, the method proposed was simple, fast, and sufficiently sensitive. A HILIC method was developed to determine low ppb CTX ranges. The stationary HALO®HILIC shows HILIC behavior with CTX. This is due to the log P_{OW} value of CTX behaves with the HALO®HILIC column. In pharmaceutical samples, the methods developed were successfully employed

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تطوير واعتماد طريقة كروماتوغرافيا المحبة للماء لتقدير السيفوتاكسيم في المستحضرات الصيدلانية

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الخلاصة

يعتبر السيفوتاكسيم أحد المضادات الحيوية شبه الاصطناعية من الجيل الثالث واسعة الطيف من السيفالوسبورين والتي تم استخدامها لعلاج الكثير من الالتهابات الناتجة عن الكائنات الحية المختلفة. زادت تقنية الكروماتوغرافيا المحبة للماء (HILIC) من الدقة والحساسية فيما يتعلق بالطرق الأخرى ، وخاصة طرق الطيف الضوئي. كان الهدف من هذه المقالة هو أقتراح طريقة بسيطة لتقدير سيفوتاكسيم في المستحضرات الصيدلانية ودراسة آلية فصل سيفوتاكسيم. حققت طريقة HILIC فصلًا ممتازًا في ظل ظروف كروماتوجرافية على عمود HALO ® HILIC 2.7 (100 mm x 2.1 mm I.D.) عند 35 درجة مئوية مع الشروط التالية: 10: 90٪ عازلة أسيتات (درجة الحموضة 5.5–40 مم): أسيتونيتريل مثل شطف و الطول الموجي للكشف 254 نانومتر. أظهرت طريقة HILIC المقترحة دقة عالية (RSD٪ <0.5٪)، بالإضافة إلى العلاقة الخطية الجيدة لمنحني المعايرة عند التركيز من 5500–100 جزء في البليون، وكان الحد الأدنى للكشف 6.8167 جزء في البليون مع معامل تحديد 0.9998 لسيفوتاكسيم. تم استخدام نتائج الطريقة الاختبارات الإحصائية مقارنة ببروتوكول دستور الأدوية البريطاني ، والذي لم يظهر أي اختلاف في الدقة بين الطرق.