OPTIMIZATION OF HYDROPHILIC INTERACTION CHROMATOGRAPHY METHOD FOR DETERMINATION OF CEFIXIME IN SOME PHARMACEUTICAL PREPARATIONS USING HPLC COUPLED WITH UV DETECTION

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Abstract

This work explains the procedure for determining cefixime in pharmaceutical dosage types with hydrophilic interaction chromatography method. The proposed HILIC method was developed by using Merck Hitachi system equipped with UV-visible detector and chromatographic separation was carried out on Halo®HILIC 2.7 column (2.1 × 100 mm) column at a flow rate of 0.5 mL/min. The mobile phase consisted of buffer acetate (45 mM-pH 5.5) and acetonitrile at a rate of 20:80 v/v and cefixime was scanned using UV detector at 280 nm. The cefixime method has been found to be linear between 30-7000 ppb (R² = 0.9998). The method has demonstrated strong, reliable cefixime (98.50-101.25%) recoveries. The developed method was successfully applied to the determination of cefixime in pharmaceutical preparations.

Key words: cefixime, pharmaceutical preparations, hydrophilicity, cephalosporine.

Introduction

Hydrophilic interaction chromatography (HILIC) in conditions of high concentration of organic solvents in hydrophilic compounds is the rapidly emerging alternative to RPLC. The range observed resembles the NPLC. In 1990 (Alpert, 1990), in defining its guidelines and some important implementations Alpert had coined the term HILIC. As a result, in recent years the HILIC technology breakthrough has significantly increased levels of pharmaceutical drugs, inorganic ions, carboxylic acids, nucleosides and dansyl-amino acids (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; Abdulla and Rasheed, 2020; Karabat et al., 2020; Ali and Rasheed, 2020b; Ali and Rasheed, 2020a). Over the years, antibiotics have been organic compounds which are toxic in one micro organism, Beta-lactam antibiotics are the most widely used antibiotics such as penicillin types, cephalosporine, monobactam, carbapenems and inhibitors of lactamase. The term “antibiotics” was derived from “antibiotics” which means “contra-life.” The lactamic rings of both cephalosporins and penicillins are the same structure (Stolker and Brinkman, 2005). Cefixime (CFM- Fig. 1) is an essential and active component of cephalosporin of the third generation.

The antibacteria of CFM are caused by mucopeptide synthesis inhibition in the cell wall of the bacterium. In the presence of β-lactamase enzymes, CFM is highly stable. CFM shall be used to treat uncomplicated

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Fig. 1: Chemical structure of Cefixime.
infections of the urinary tract caused by Escherichia coli and Proteus mirabilis, Haemophilus influenzae otitis media, Pharyngitis and S. tonsillitis. Neisseria gonorrhoeae induces pyogenes and uncomplicated gonorrhoea (Goodman, 1996; Sweetman, 2009). CFM is an antibiotic that destroys bacteria by preventing their own protective cover that is necessary for survival in the human body (Fischer et al., 2010; Organization, 2019; Pharmacopoeia, 2015). Different methods for individual estimates in pharmaceutical dosage with CFM in the literature have been published using voltametry (Jain et al., 2010), spectrophotometry (Thakkar and Mashru, 2012; Wani and Patil, 2017; Dey et al., 2012; Nayon et al., 2013), flow injection spectrophotometry (Khan et al., 2011), HPLC (Talebpour et al., 2013; Dhoka et al., 2010; Kathiravan et al., 2010; Wankhede et al., 2010). This study aimed at developing a simple, responsive, effective, versatile, speedy and time-saving pharmaceutical injections dosage model.

Materials and Methods

Instrumentation

A flow rate of 0.5 ml / min was performed in UV regions with a wavelength of 280 nm for CFM detection. A 20μL injection loop is provided with the gradient L-6200 for Merck Hitachi HPLC and UV-visible L-4200. For pH 740 (WTW), the pH tests are carried out. With the photographic software from the N2000 workstation, the chromatogram can be measured. For CFM separation was used Halo®HILIC 2.7 column (2.1 × 100 mm) from Advanced material.

Reagents

As to chemicals, acetic acid, sodium acetate, acetonitrile (ACN) and CFM are the following products from Sigma-Aldrich. Usage of the pharmaceuticals group CFM as applications, CFM (400 mg)-LDP-Spain, CFM (400 mg)-Pharma-international-Jordan and CFM (400 mg)-Bravn-India. In the process of solution purification, Millipore filters (0.45 μm) have been used. A conductivity of 0.05 μs / cm Millipore (System-US Millipores) was used.

Preparation of stock solution and pharmaceutical samples for CFM

The stock solution of CFM (10000 ppb) was prepared for precise dissolution with CFM (1 mg) in a 100-ml mobile phase. The solution has been filtered by Millipore filter 0.45 μm. Ten vials have been stored and up to 1 mg CFM is dissolved in a 100 ml mobile phase volumetric flask and diluted with mobile phase to indicate for each of the commercial firms. Then the solution was purified by Millipore (0.45 μm) filters. The stock solution has then been diluted and other standard solutions have been developed.

Results and Discussion

Separation of CFM

Through using the ACN mobile Phase Mix with

![Fig. 2: Chromatogram of CFM.](image)

![Fig. 3: The effect of acetonitrile proportion.](image)

![Fig. 4: The effect of buffer strength.](image)
Table 1: The calibration results for the CFM curve are monitored by the halo exchanger.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HILIC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (ppb)</td>
<td>30-7000</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = 1114.40 + 2.39 \times x$</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9998</td>
</tr>
<tr>
<td>LOD (ppb)</td>
<td>22.78</td>
</tr>
<tr>
<td>LOQ (ppb)</td>
<td>69.03</td>
</tr>
</tbody>
</table>

The Influence of the acetonitrile

At 5.5 pH, 45 mM acetonitrile buffer, acetonitrile effect on CFM retention was noted. The eluent acetonitrile ratio seems to be increased by 50 to 95% by CFM HILIC action. The HILIC behavior in CFM shows in the HALO column, (Fig. 3), due to the CFM log Pow (-1.28). This is because of the hydrophilicity of CFM.

The Influence of buffer strength

The effect on the eluent retention behavior of CFM was reported with 10-80 mM (pH 5.5) at 80% acetonitrile in the eluent. The findings are given in fig. 4. Retaining the CFM retention in the column raises the buffer level in the acetate eluent. The hydrophilicity of CFM is responsible.

The Influence of mobile phase pH

In order to further improve the composition of the mobile process, an eluent pH change is required. If the CFM is to be separated in HILIC mode, mobile phase pH must be changed. The pH was increased from 3 to 5.5 with a constant buffer level of 45 mM and 80% by ACN. As seen in fig. 5, the retention of CFM declines. This is because the CFM hydroxyl group is deprotonated. This reflects the anticipated knowledge of CFM physicochemical. The CFM is 2.54 and the pKa is 2.78. Consequently, the CFM is anionic.

Calibration graph

The calibration chart shows the CFM (30-7000 ppb), determined via a plot area versus the CFM concentration. The calibration chart shows the CFM concentration.

Statistical analysis

In the corresponding calibration curve detailed CFM

Table 2: On the same day as for various days CFM methodological performance.

<table>
<thead>
<tr>
<th>CFM Taken</th>
<th>Same-Day Analysis=6</th>
<th>Day-to-Day Analysis=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ppb)</td>
<td>CFM Found (ppb)</td>
<td>% Rec.</td>
</tr>
<tr>
<td>900</td>
<td>893</td>
<td>99.22</td>
</tr>
<tr>
<td>2000</td>
<td>1990</td>
<td>99.50</td>
</tr>
<tr>
<td>5000</td>
<td>4990</td>
<td>99.80</td>
</tr>
</tbody>
</table>

Table 3: Experimental findings for the pharmaceutical application CFM evaluation.

<table>
<thead>
<tr>
<th>Name of application</th>
<th>Company</th>
<th>Present (mg)</th>
<th>Get it (mg)</th>
<th>%Rec.</th>
<th>%RSDn=6</th>
<th>% Erel.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cefixime</td>
<td>LDP-spain</td>
<td>400</td>
<td>396</td>
<td>99.00</td>
<td>0.98</td>
<td>-1.00</td>
</tr>
<tr>
<td>cefixime</td>
<td>Pharma-international-Jordan</td>
<td>400</td>
<td>405</td>
<td>101.25</td>
<td>0.67</td>
<td>1.25</td>
</tr>
<tr>
<td>cefixime</td>
<td>Bravn-India</td>
<td>400</td>
<td>394</td>
<td>98.50</td>
<td>0.75</td>
<td>-1.50</td>
</tr>
</tbody>
</table>
analyses under HILIC conditions and table 1 statistics were used. The exactness, precision and consistency (RSD% and Rec.%) were measured the same day and on different days. Table 2 indicates the success of the suggested approach by the relatively low pre-default values and high recovery value.

**CFM determination in pharmaceutical preparations**

The proposed method has been used successfully in the evaluation of CFM in three of the pharmaceutical samples, the findings listed in table 3.

These findings are contrasted with those of the British Pharmacopeia (Pharmacopoeia, 2009) to determine the skill and efficiency of the HILIC method. As statistical analyses were used for two methods, the F-test t-test ratio and the F-test variance ratio (Table 4), which were 95% confident. The values of t and F were not theoretical, so the exactness of the CFM determination for three pharmaceutical types did not differ significantly from both approaches.

**Conclusion**

A simple new method was introduced in the ongoing study, which uses HILIC exchanger to separate and estimate CFM. Finding that the HILIC method was quick, linear, exact, sensitive, stability indicative and applicable for determining CFM, according to ICH international Guidelines. CFM’s LOD and LOQ were thus obtained between 22.78-69.03 ppb and the findings showed that CFM was more selectivity and more sensitivity. In the stationary HALO, hydrophilic conduct with CFM is shown. In pharmaceutical preparations, the techniques have been successfully developed.

**References**


