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# SEPARATION AND QUANTITATIVE ANALYSIS OF CHLORPHENIRAMINE MALEATE BY THE APPLICATION OF THE ZIC-HILIC TECHNIQUE IN BULK AND MEDICINE FORMULATIONS

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## Abstract

For chromatographic chlorpheniramine maleate separation, hydrophilic stationary phases (ZIC1 and ZIC5) have been investigated. The retention activity of the eluent in different acetonitrile containing sodium acetate buffer amounts and pH was investigated for chlorpheniramine maleate. According to hydrophobic and cation interactions, the separation mechanism leads to a mixed-mode for the chlorpheniramine maleate. The calibrating graphs were produced for two exchangers and linear range (0.03-3 µg $mL^{-1}$), RSD percent (0.37 ± 0.10 and 0.57 ± 0.18), LOD (0.015 and 0.023 µg mL-1), LOQ (0.052 and 0.080 µg $mL^{-1}$), respectively. For pharmaceutical samples, the methods proposed have been effective. And the results of the proposed methods are compared with the comparison method and their precision and accuracy are comparable.

***Key words:*** Chlorpheniramine, HILIC, ion exchange interaction, mixed-mode.

## Introduction

The rapidly growing alternative to RPLC is hydrophilic interaction chromatography (HILIC), in conditions with a high concentration of organic solvents in hydrophilic compounds. The observed selectivity is similar to NPLC. Alpert (Alpert, 1990) was the first one to suggest a HILIC separation mechanism that would partition a water-enhanced surface layer into a mostly organic mobile phase. HILIC is currently strongly attracted by its addressing several previously daunting separation problems. The analysis of pharmaceuticals (Seubert and Saad Rasheed, 2017; Abbas and Rasheed, 2018; Abbas and Rasheed), dansyl amino acids (Al-Phalahy *et al*., 2016), inorganic anions (S Rasheed and Seubert, 2016), carboxylic acid (Rasheed *et al*., 2016), sugar (Palmer, 1975), saccharides (Linden and Lawhead, 1975) and 2-deoxyuridine (Ashraf Saad Rasheed and Rashid, 2020) by HILIC-technology has been successfully carried out successfully. The $H^{-1}$ receptor blocker is chlorpheniramine maleate (CPM-Fig. 1).

The antihistamine used to soft cough, hay fever or common cold symptoms is the chlorpheniramine maleate (Fig. 1). These include itching, watery eyes, itchy eyes, skin/nose, cough and runny nose and sneezing (O'Neil, 2013; Pharmacopoeia, 2008). No work has been carried out on retention characteristics of CPM in HILIC mode despite the availability of numerous CPM separation works in HPLC (Sanchaniya *et al*., 2013; Acheampong *et al*., 2016; Magesh and Dhanaraju, 2017; Heydari, 2008; Vignaduzzo and Kaufman, 2013; Maham *et al*., 2014; Şenyuva and Özden, 2002). Furthermore, the effect on CPM retention behavior of the ZIC-HILIC columns chain length has not been studied before. The improvement in understanding of HILIC separation retention mechanisms expands the range of applications possible. The ultimate aim is to implement simple methods in pure, tablets, ampoule and syrup formulations for evaluating CPM.

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## Materials and Methods

A 20 µL injection loop has been used with an HPLC system from Merck Hitachi, which includes a gradient pump L-6200 and an ultraviolet-visible L-4200. pH tests on the pH 740 (WTW) were carried out. To control my chromatography and analysis the data the Photo Data Workstation software of N2000 was used. The ultraviolet region at a wavelength of 275 nm was used for detecting CPM. The ZIC1 and ZIC5 exchangers used for the CPM separation were developed on the PS / DVB with PEEK

columns by means of a grafted sulfobetaine monomer (100 mm × 4 mm I.D) (Seubert and Saad Rasheed, 2017; Rasheed *et al*., 2016; S. Rasheed and Seubert, 2016; Al-Phalahy *et al*., 2016).

Raskop *et al*., (Raskop *et al*., 2007) have identified the detailed process of the grafting reaction. Sigma purchased CPM. BDH was bought with acetic acid. Fluka was acquired with sodium acetate (NaOAc). HPLC grade (to 99.93 percent) of acetonitrile (ACN) was

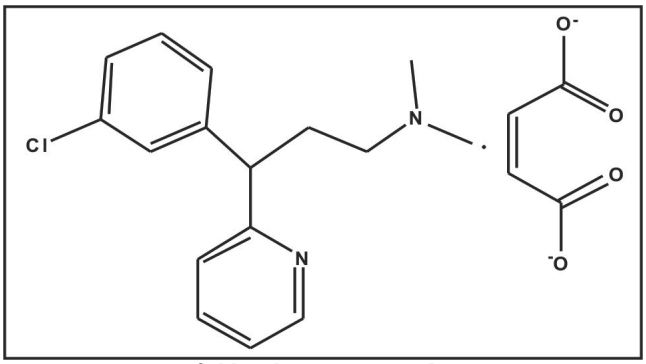


**Fig. 1:** Structure of chlorpheniramine maleate (CPM).

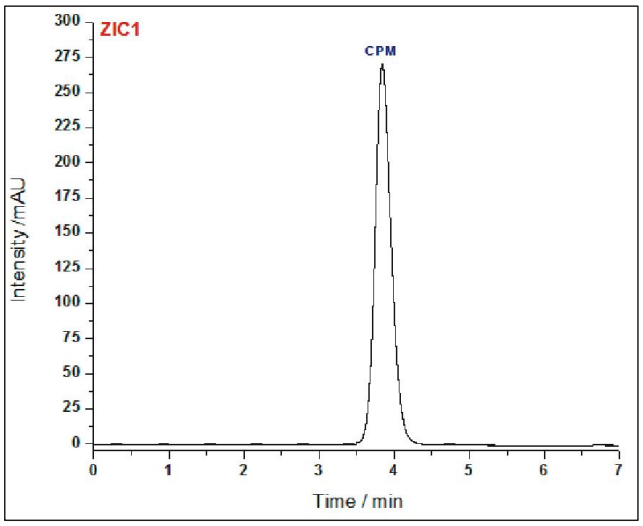


**Fig. 2:** Chromatogram for the separations of chlorpheniramine maleate (CPM) in the ZIC1 column.

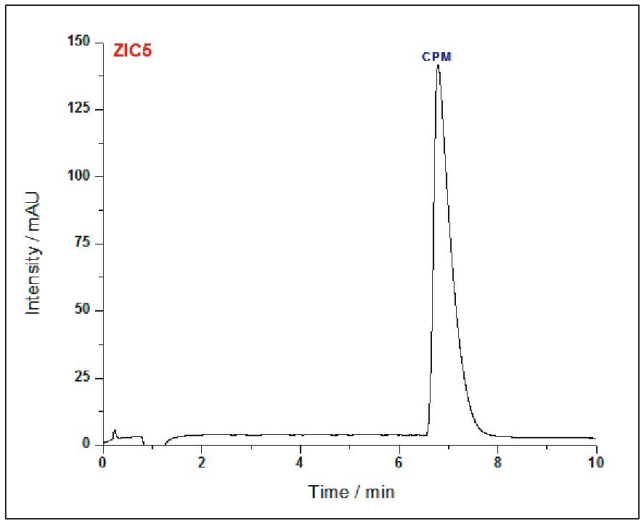


**Fig. 3:** Chromatogram for the separations of chlorpheniramine maleate (CPM) in the ZIC 5 column.

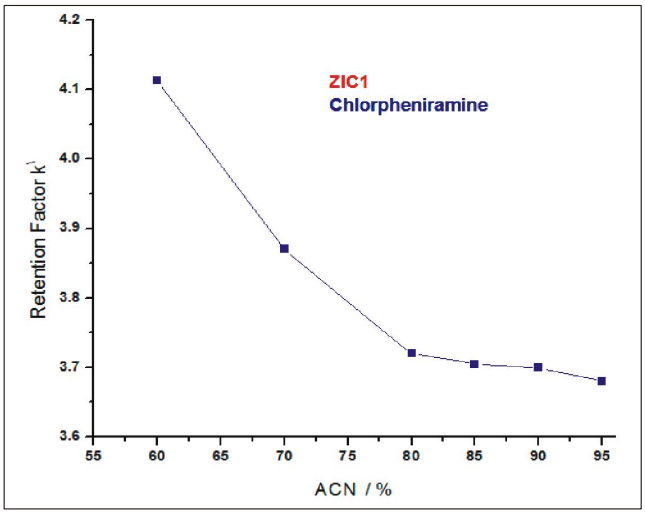


**Fig. 4:** ACN content impact on CPM retention in ZIC1 column.

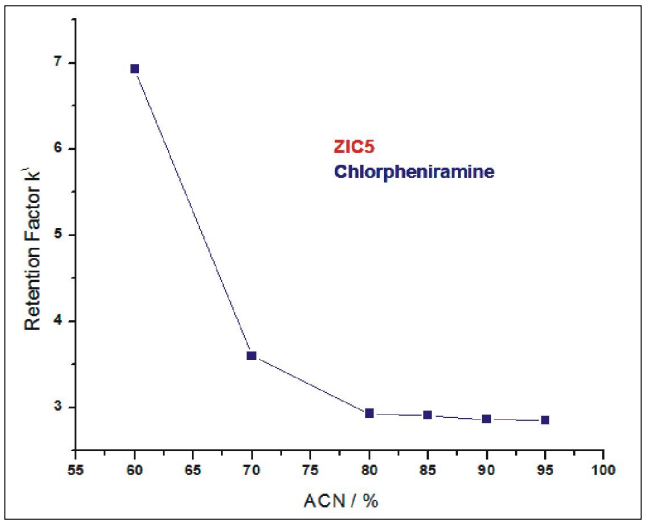


**Fig. 5:** ACN content impact on CPM retention in ZIC5 column.

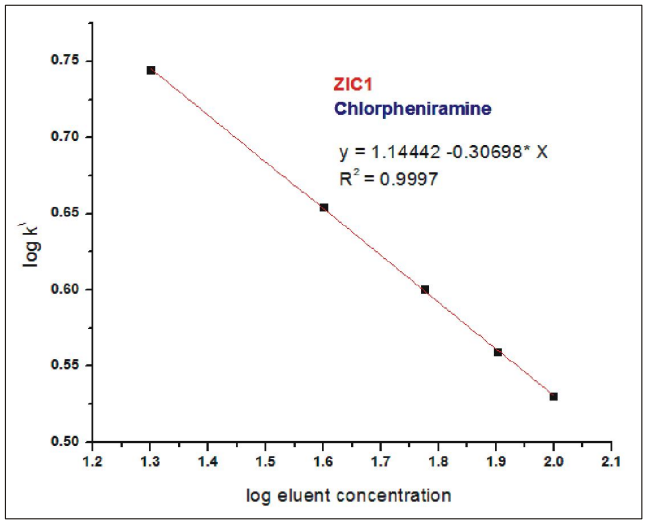


**Fig. 6:** Eluent concentration impact on CPM retention in ZIC1 column.

acquired by Aldrich. The capacity 432 and 488 µeq $g^{-1}$ (Abbas and Rasheed, 2018) respectively are accessible to ZIC1 and ZIC5 exchangers. For each sample, ten tablets were crushed and 4 mg CPM water was dissolved and dissolved into a 100 mL volumetric flask with water and diluted to a mark. The equivalent of approximately 10 mg of syrup CPM has water dissolved and transferred to a 100 mL volumetric flask, diluted in a watermark. The commercial company collected ten ampules, transferred 10 mg CPM into 100 mL and water diluted into the mark. Afterward, millipore (0.45 µm) filtered the solution.

**Table 1:** Evidence of performance analysis.

| Parameter | ZIC1 column | ZIC5 column |
|---|---|---|
| Linearity (µg.ml$^{-1}$) | 0.03-3.00 | 0.03-3.00 |
| Regression equation | y = 1033.13 + 2221.83*x | y = 2133.13 + 2221.83*x |
| $r^2$ | 0.9998 | 0.9998 |
| LOD (µg.mL$^{-1}$) | 0.015 | 0.023 |
| LOQ (µg.mL$^{-1}$) | 0.052 | 0.080 |

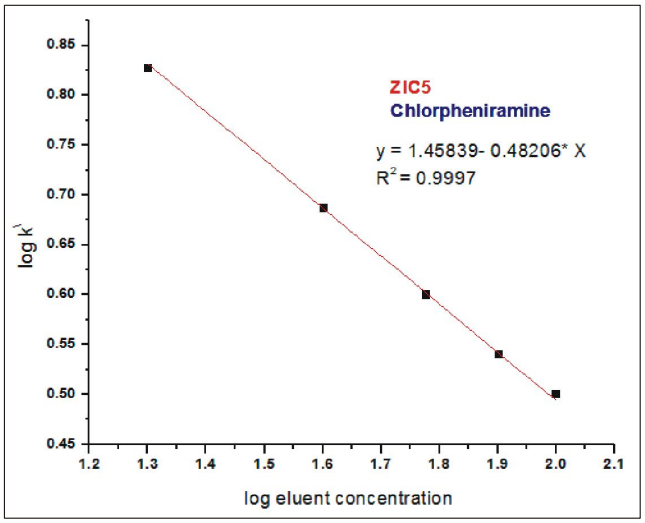


**Fig. 7:** Eluent concentration impact on CPM retention in ZIC5 column.

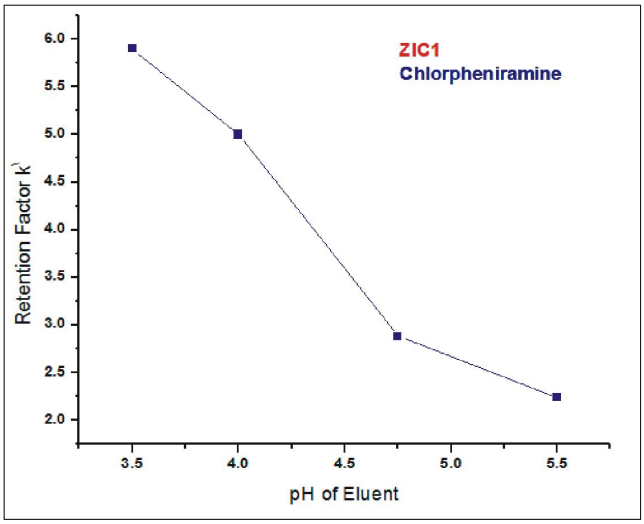


**Fig. 8:** Eluent pH impact on CPM retention in ZIC1 column.

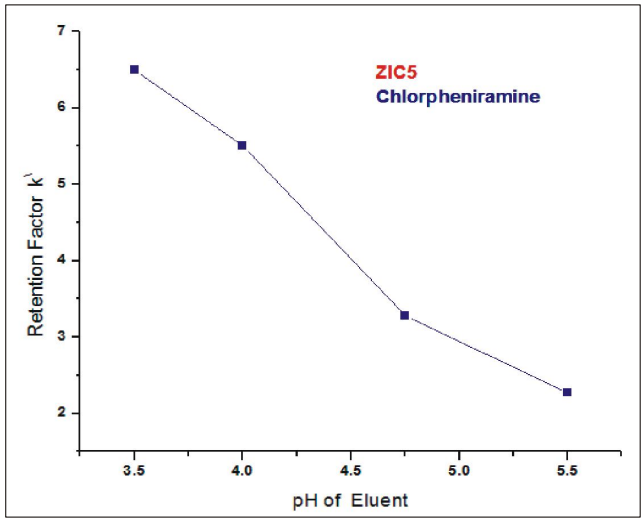


**Fig. 9:** Eluent pH impact on CPM retention in ZIC5 column.

## Results and Discussion

### Separation of CPM

Separation of HILIC-mode CPM was tested by the application of mobile sodium acetate in the exchangers ZIC1 and ZIC5 with varying ACN amounts. Fig. 2 and 3 display the chromatogram. The 40 mm (pH 3.5) sodium acetate and 85% ACN chromatogram were accomplished.

The interaction between the CPM and the column is evident in fig. 2 and 3 by increasing the chain length, as is evident from ZIC5, thus increasing the retention time of CPM. This is because the methyl group in the ZIC column increases between charges. In mobile phase compounds, a systemic variation of ACN content rises between 60 and 95%; the eluent concentration between

**Table 2:** Recovery of the methods suggested.

| Same-Day Analysis n=5 | | | | | Day-to-Day Analysis n=5 | | | |
|---|---|---|---|---|---|---|---|---|
| **ZIC1 column** | | | | | | | | |
| **CPM Taken µg.mL$^{-1}$** | **CPM Found µg.mL$^{-1}$** | **% Rec.** | **% $E_{rel}$.** | **% RSD** | **CPM Found µg.mL$^{-1}$** | **% Rec.** | **% $E_{rel}$.** | **% RSD** |
| **0.90** | **0.902** | 100.22 | 0.22 | 0.43 | **0.904** | 100.44 | 0.44 | 0.29 |
| **1.00** | **0.982** | 98.20 | -1.80 | 0.46 | **0.983** | 98.30 | -1.70 | 0.50 |
| **1.50** | **1.491** | 99.40 | -0.60 | 0.22 | **1.495** | 99.66 | -0.34 | 0.33 |
| **ZIC5 column** | | | | | | | | |
| **0.90** | **0.892** | 99.11 | -0.89 | 0.78 | **0.901** | 100.11 | 0.11 | 0.91 |
| **1.00** | **1.015** | 101.50 | 1.50 | 0.61 | **1.012** | 101.20 | 1.20 | 0.82 |
| **1.50** | **1.502** | 100.13 | 0.13 | 0.34 | **1.508** | 100.53 | 0.53 | 0.47 |

**Table 3:** Appliance for two proposed methods to assess CPM in tablets, ampoule and syrups pharmaceutical samples.

| Name of drug | Company | Started conc. (mg) | Get it (mg) | %Rec. | %RSD n=5 | % $E_{rel.}$ |
|---|---|---|---|---|---|---|
| **ZIC1 column** | | | | | | |
| HISTADIN-Tablet | SDI-Iraq | 4.00 | 3.95 | 98.75 | 1.18 | - 1.25 |
| HISTOFEN-Tablet | Al-Kindi-Iraq | 4.00 | 3.94 | 98.50 | 0.77 | - 1.50 |
| CHLOROHISTOL-Tablet | Julphar-UAE | 4.00 | 4.02 | 100.50 | 1.06 | 0.50 |
| RELIEF-Tablet | LABORATE-India | 4.00 | 3.98 | 99.50 | 0.52 | - 0.50 |
| CHLOROHISTOL-Ampoule | Julphar-UAE | 10.00 | 10.05 | 100.50 | 0.61 | 0.50 |
| ALLERMINE- Syrup | SDI-Iraq | 10.00 | 10.03 | 100.30 | 0.55 | 0.30 |
| **ZIC5 column** | | | | | | |
| | | 4.00 | 3.96 | 99.00 | 1.21 | -1.00 |
| | | 4.00 | 3.94 | 98.50 | 0.84 | -1.50 |
| | | 4.00 | 4.03 | 100.75 | 1.05 | 0.75 |
| | | 4.00 | 4.01 | 100.25 | 0.77 | 0.25 |
| | | 10.00 | 9.99 | 99.90 | 0.42 | -0.10 |
| | | 10.00 | 10.02 | 100.20 | 0.67 | 0.20 |

20 and 100 mM, at pH levels of 3 to 5.5, ensures an appreciation for each exchangers separating characteristics and hence the mechanism for separation.

### ACN content effect on CPM retention

The retention of drug separations has increased or decreased with this ACN content in ZIC-HILIC mode. Furthermore, the pharmaceuticals with a lower water content of eluent are identified in two hydrophobic (RP) and hydrophilic (HILIC) activities. This functional difference is explained by the hydrophilicity of the drug. CPM shows hydrophobic behavior (Fig. 4 and 5) in exchangers ZIC1 and ZIC5. The CPM log $P_{ow}$ (3.58) (2019a, 2019b) is the result.

### Eluent concentration effect on CPM retention

In the ZIC-HILIC mode, retention was generally increased with increased eluent levels, which contributed to the intramolecular ion pairs being deactivated. Thus, when ACN is present, the linearization of functional phase groups is improved (Rasheed *et al*., 2016). The retention of drugs in steady ZIC-HILIC phases has decreased or increased with an increased buffer level. The exchange of cations and anions (S. Rasheed and Seubert, 2016) is an explanation of this. With the NaOAc buffer rising from 20 to 100 mM and a pH of 3.5 and ACN up to 85% as shown in fig. 6 and 7, the CPM retention factor decreases. In fig. 6 and 7, this slope seems to be calculated on ion-exchange standard columns (Haddad and Jackson, 1990) close to 0.3069 and -0.4820 slopes. So, what is the real separation mechanism? When retention decreases, CPM gets a different image when the buffer concentration increases and that is because of two factors. The exchanger core materials and CPM. The CPM is thus cationic in the values of Pka (3.57-9.47) in CPM. Then the cation exchange was based on the interaction between CPM and HILIC columns.

**Table 4:** The comparison of the proposed methods ZIC1 and ZIC5 with comparison method (Vignaduzzo and Kaufman, 2013) for CPM analysis by examining t- and F-statistical tests.

| Name of drug | ZIC1 method | ZIC5 method | Comparison (Vignaduzzo) and Kaufman method | t-Test (theor.) | F-Test (theor.) |
|---|---|---|---|---|---|
| HISTADIN-Tablet | 98.75 | 99.00 | 98.7 | 0.8592* (2.2281) | 1.1125* (5.0500) |
| HISTOFEN-Tablet | 98.50 | 98.50 | 100.80 | 0.6097** (2.2281) | 0.9961** (5.0500) |
| CHLOROHISTOL-Tablet | 100.50 | 100.75 | 99.50 | | |
| RELIEF-Tablet | 99.50 | 100.25 | 100.25 | | |
| CHLOROHISTOL-Ampoule | 100.50 | 99.90 | 98.90 | | |
| ALLERMINE- Syrup | 100.30 | 100.20 | 98.90 | | |
| ***For ZIC1 proposed method; **For ZIC5 proposed method** | | | | | |

### Eluent pH effect on CPM retention

The eluent pH must differ in order to complete the concept of the CPM separation process. The CPM retention was reduced, as shown in fig. 8 and 9, while the eluent pH was raised from 3 to 5.5 while sodium acetate rates were maintained at 40 mM and 85 percent of ACN. Because of the amino group deprotonation in the CPM, the retention of the CPM is decreased during the ZIC1 and ZIC5 stationary phases.

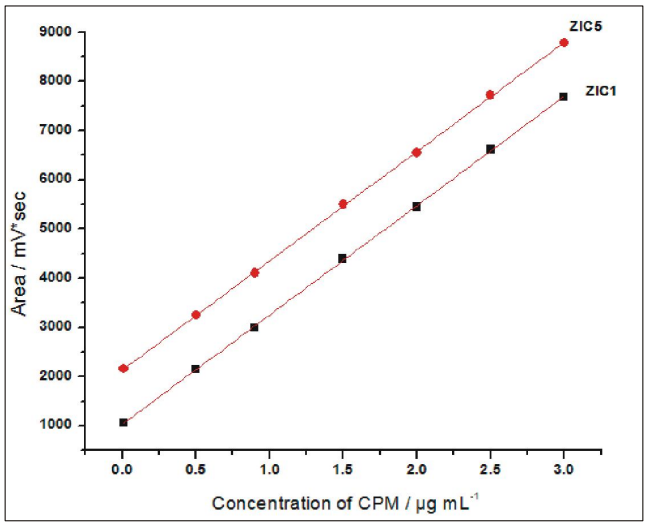


**Fig. 10:** Calibration graph for CPM using ZIC1 and ZIC5 columns.

### Calibration curve

The calibration graphs show the range concentration of CPM (0.03-3 µg $mL^{-1}$) of ZIC1 and ZIC5 exchangers (Fig. 10), established through a plotting area versus CPM concentration.

### CPM statistical data evaluation

A thorough assessment of the CPM under HILIC conditions was made using the corresponding calibration graphs and statistical results are reported in table 1. On the same day, accuracy was analyzed and daily recuperation percentage and RSD percent were calculated. The small relative defaults and the high recovery values indicate that the suggested methods are efficient (Table 2).

### CPM determination in medical samples

The proposed methods were successfully used in the CPM evaluation in six pharmaceutical formulations; the findings are listed in table 3.

In order to assess the competence and efficiency of the ZIC1/ZIC5 methods, these findings were compared with the results obtained by the comparison method (Vignaduzzo and Kaufman, 2013). Statistical analysis was performed based on the results of the two t-tests and F-test variance ratios (Table 4) (95%). The determined t and F values do not surpass the theoretical values, meaning that the accuracy of the CPM determination in syrups, ampoule and tablets does not vary significantly in both methods.

## Conclusion

This article cites the development of HILIC techniques for the evaluation of CPM in pharmaceutical samples. For HILIC Stationary Phases with one and five methylene groups between charged groups a flexible separation tool with the advantage of allowing at least two different retention modes under different conditions. This paper shows how CPM handles the ZIC1 and ZIC5 columns. It has been found that the ZIC5 column with CPM is longer preserved. This could be attributed to the geometric orientation of the column of ZIC5. The data show that both the hydrophobic and cation exchange actions are the retention mechanism. The methods developed have been used successfully in medicine samples.

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