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DEVELOPMENT, VALIDATION, AND PHARMACEUTICAL DOSAGE FORMS APPLICATION OF HYDROPHILIC INTERACTION CHROMATOGRAPHY ASSAY FOR THE QUANTIFICATION OF THEOPHYLLINE

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Abstract

The present work establishes and validates HILIC strategies simple, accurate, exact and precise in pure form and in pharmaceutical dosage for separating and determining theophylline. These methods are developed on HILIC theophylline separation in columns ZIC_2 and ZIC_3. The eluent was prepared by mixing buffer (20% sodium acetate-40 mM, pH 5.5), 80% acetonitrile. The flow rate is 0.8 mL/min, with gradient elution and UV detection at 270 nm. In the ZIC_2 and ZIC_3 columns of theophylline determining, the concentration range was 0.01-4µg.ml^{-1}. The lower limit of detection and quantification for theophylline were determined as 0.130, 0.190 µg.ml^{-1} and accuracy were 99.70%, 99.58% on ZIC_2 and ZIC_3, respectively. The HILIC methods developed and validated and statistical analysis indicate that the methods for estimating theophylline can be replicated and chosen.

Key words*:* Theophylline, pharmaceutical dosage forms, hydrophilic interaction, mix-mode interaction.

Introduction

Theophylline, a methylxanthine, is a muscle stimulant that increases the contractibility of the heart muscle and increases the endurance and strength of the respiratory muscles, including the muscles and the diaphragm theophylline has been used to treat respiratory diseases such as asthma and chronic lung obstructive disease. Moreover to bronchodilator and anti-inflammatory features, theophylline (Fig. 1) serves as an antitussive drug (Nishii *et al.*, 2008, Jagers *et al.*, 2009). Although theophylline has been studied in recent years using many techniques such as high-performance liquid chromatography (HPLC) (Abdallah, 2014, Al-Jenoobi *et al.*, 2015, Bispo *et al.*, 2002, Charehsaz *et al.*, 2014, Fernandes *et al.*, 2017, Kanakal *et al.*, 2014, Lo Coco *et al.*, 2007, Oellig *et al.*, 2018, Srdjenovic *et al.*, 2008, Thomas *et al.*, 2004). But there is no research to investigate this drug using hydrophilic interaction liquid chromatography (HILIC) technique, so this will be the first of the goal of this study is the use of (HILIC) technique to analyze theophylline. Considering the problems caused by the separation of high polar and hydrophilic compounds in reverse-phase liquid chromatography (RPLC) in addition to the problem of the mobile phase (MP) which is expensive and dangerous. To solve these problems, the HILIC technique was used because of its many benefits in solving the problems resulting from the use of highly polar and hydrophilic compounds. Where in the HILIC technique a polar stationary phase (SPs) is used to retain polar compounds and a mobile phase (MP) which is a combination of organic solvents it is mostly used acetonitrile (ACN) and water (Buszewski and Noga, 2012, Hemström and Irgum, 2006). Thus recently, HILIC technology has begun to grow significantly in the estimation of drugs (Yaqout Abd Al-Hakeem Hamed and Rasheed, 2020, Ashraf Saad Rasheed and Rashid, 2020, Ashraf Saad Rasheed *et al.*, 2019, Abbas and Rasheed, 2018a, Seubert and Saad

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Rasheed, 2017, Rasheed *et al.*, 2017, Abbas and Rasheed, 2017a, Abbas and Rasheed, 2017b, S Rasheed and Seubert, 2016, Al-Phalahy and Rasheed, 2016, Al-Phalahy *et al.*, 2016, AL-Ayash *et al.*, 2008).

One of the challenges in this study is the knowledge of mechanisms separation of theophylline in the columns of HILIC for the first time and there is no prior study to know the mechanisms of separation of this drug in these columns, so this challenge will be the second goal of the objectives of this study. In order to complete the vision and to be clear, it is necessary to study the effect of the length of the chain in the columns of HILIC in the separation of this drug and this study has not been proving before so that the third objective of this research is to be achieved. In a recent study (Abbas and Rasheed, 2018b), it has been studied the effect of the chain length on the behavior of ranitidine, and they found that when increasing the methyl group, the retention time of drug retention increases. The end goal is to present a new method for determining the theophylline in pharmaceutical dosage forms.

Material and Method

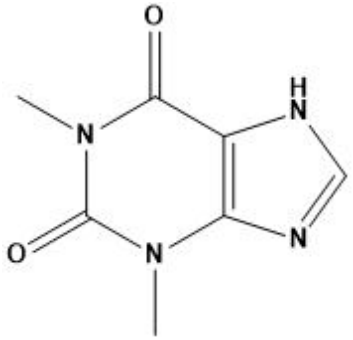

Fig.1: Structure formulae of theophylline.

Instrumentation and Operating Conditions

The Merck-Hitachi HPLC system was used with a L-6200 gradient pump and a 20 µL injection loop L-4200 UV visible detector. Flow rate (0.8 mL/min), temperature (45°C) and eluent ACN / (NaOAc / HAc) buffer. The N2000 Photographic Data Workstation software has been used for chromatographic control and data analysis. Two columns (ZIC2 and ZIC3) have been used for the analysis of theophylline. Theophylline was detected using the UV at a wavelength of 270 nm.

Chemicals and Stock Solutions

Theophylline was ordered from Sigma and acetic acid (HAc) was ordered from BDH. Sodium acetate (NaOAc) has been ordered from Fluka. Acetonitrile (ACN) HPLC grade (= 99.93%) was ordered from Sigma-Aldrich. Theophylline solutions have been prepared by dissolving the standard in acetonitrile (ACN). Less concentrated solutions were prepared as required by dilution with acetonitrile.

Preparation of Samples

The content of 15 tablets of the theophylline dosage forms (both contain 120 and 200 mg of theophylline) was mixed together, and then 0.2 and 0.12 gm were diluted to 1000 mL. Solutions were then prepared by diluting 10 mL to 100 mL with water then the solution was filtered using a millipore filter (0.45 µm).

Results and Discussion

Theophylline was selected as a pharmaceutical test in an analysis of its retention mechanism in the HILIC mode with a mobile NaAc / HAc buffer phase with different levels of ACN on the two columns of the ZIC HILIC. (Fig. 2) and (Fig. 3) show chromatograms. For

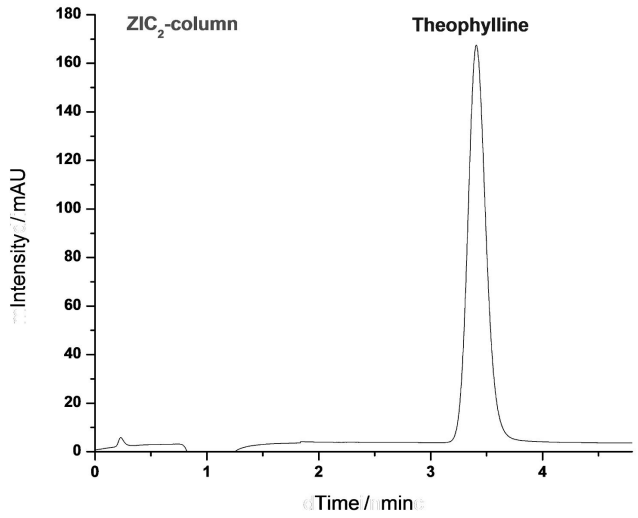

Fig. 2: Separation of theophylline using ZIC_2-column.

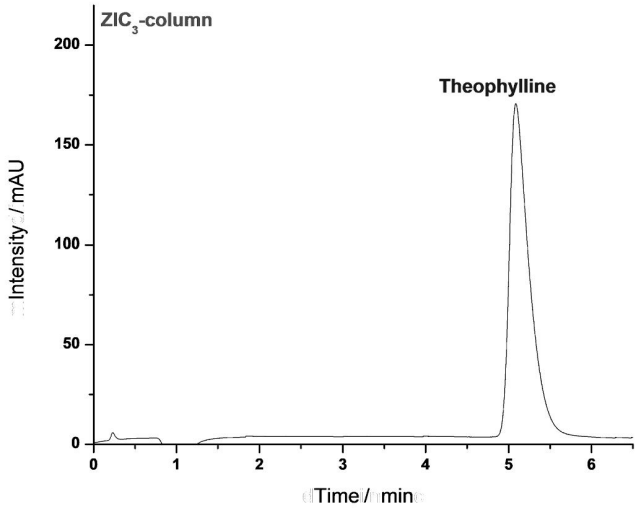

Fig. 3: Separation of theophylline using ZIC_3-column.

NaOAc / HAc buffer 80% of ACN and 40 mM (pH 5.5) were obtained for the chromatograms. The composition of the mobile phases is systemically adjusted by changing the amount of ACN from 60% to 95% (v/v); the concentration of eluent from 20 to 100 mM and pH (3.5 to 5.5).

The study effect of acetonitrile content

Acetonitrile was used to analyze the effect of organic solvent content on the retention of theophylline in the mobile phase. The study was conducted with sodium acetate (NaOAc) at (pH 5.5, 40 mM), referring to the mobile phases aqueous component. It is noted that the evident pH of the mobile phase containing a high percentage of organic solvent is different from the aqueous component's value. As the pH of the mobile phase plays a very important role in the preservation of the materials to be analyzed, and this is due to its effect on the ionization of both the analyte and its effect on the portable groups in the stationary phases. Theophylline demonstrated conventional hydrophilic behavior with increased retention times and an increase in the mobile phase of acetonitrile percentage (Fig. 4 and Fig. 5). The relation between ZIC_2 and ZIC_3 columns behavior. The justification for this hydrophilic interaction is its hydrophilicity value -0.769 of theophylline .

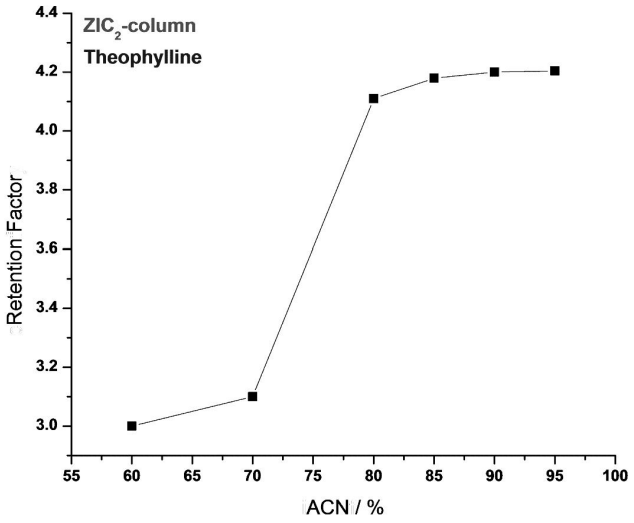

Fig. 4: The study effect of acetonitrile content of theophylline using ZIC_2-column.

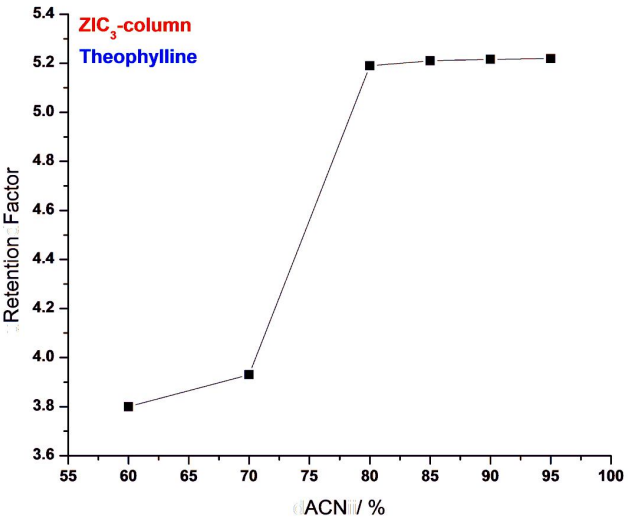

Fig. 5: The study effect of acetonitrile content of theophylline using ZIC_3-column.

Study the effect of eluent concentration on theophylline retention

In order to control interactions between theophylline and HILIC exchangers, salts are usually added to the eluent. The effect of the NaOAc / HAc buffer concentration on the retention activity of theophylline in

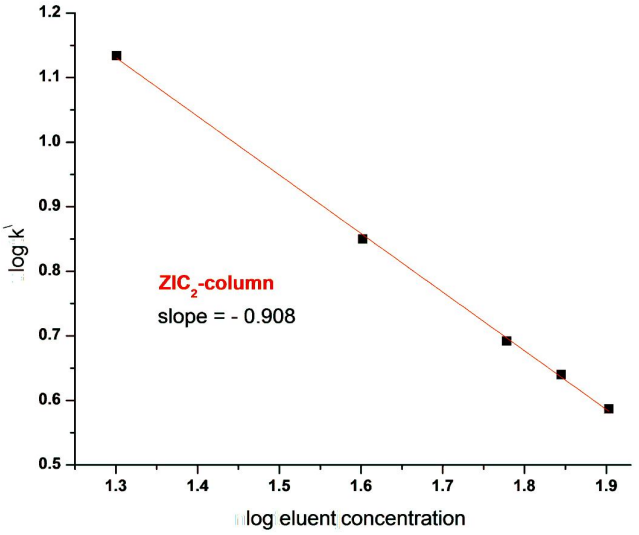

Fig. 6: The study effect of the eluent concentration of theophylline using ZIC_2-column.

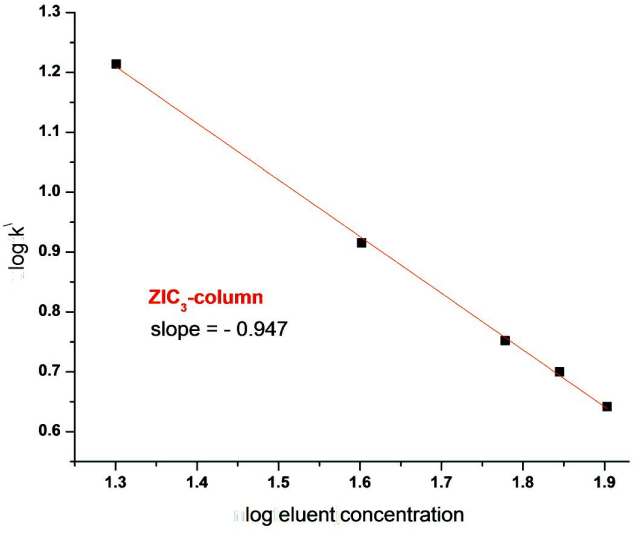

Fig. 7: The study effect of the eluent concentration of theophylline using ZIC_3-column.

the eluent was examined at a concentration of 20-100 mM (pH 5.5) at 80% ACN in the eluent. The findings are shown in (Fig. 6 and Fig. 7). Usually in HILIC mode, when the salt concentration in the mobile phase increases, the retention time for the analytical compounds increases. Analytical results showed that this effect was studied otherwise, as when the salt concentration increased, the theophylline retention time decreased. Consequently, the reason for this is due to the nature of the columns used. The slope values (-0.908 and -0.947) obtained from Figures 6 and 7 indicate similarity with the slope values from the traditional ion-exchange chromatography (Haddad and Jackson, 1990).

The study effect of eluent pH on the retention of theophylline

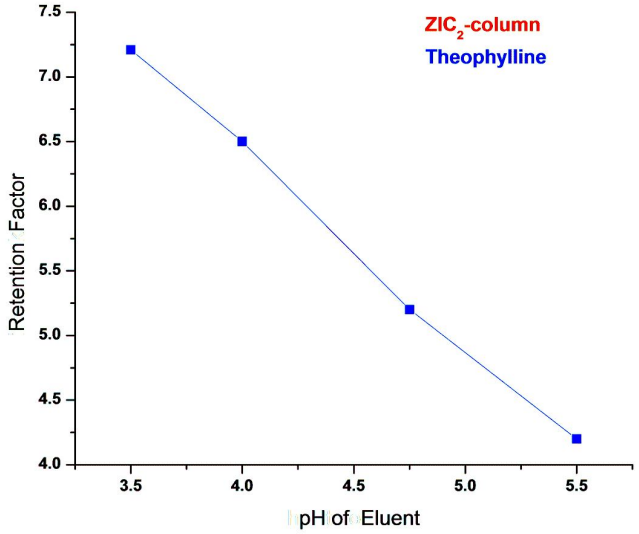

Fig. 8: The study effect of the eluent pH of theophylline using ZIC_2-column.

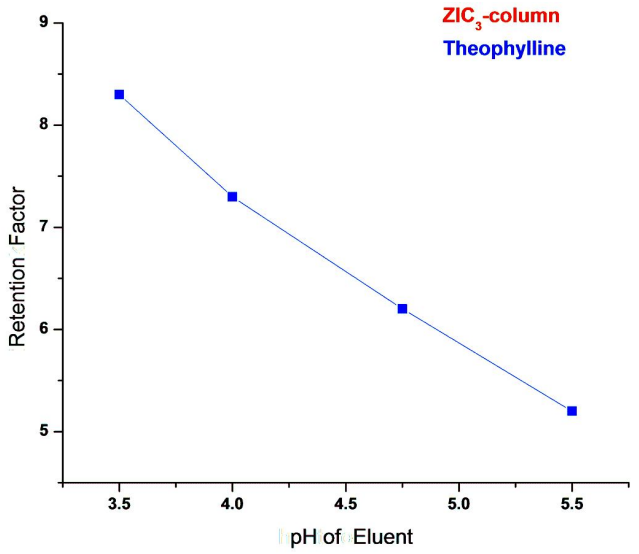

Fig. 9: The study effect of the eluent pH of theophylline using ZIC_3-column.

An improvement in eluent pH is the next enhancement of the eluent composition that can be introduced. To complete the separation of the theophylline in HILIC mode, the eluent pH must be modified. The pH was increased from 3 to 5.5 with a steady buffer concentration of 40 mM and 80% ACN. The theophylline retention factor was decreased with increased pH range, as indicated in (Fig. 8 and Fig. 9). This is because of the amino group deprotonation in theophylline. The physicochemical details to be expected for theophylline were taken into account. The isoelectric point of theophylline is 3.52.

Calibration graph

The theophylline calibration graphs are created by plotting the concentrations of theophylline inversion to the peak area and showing the concentration range (0.01-4 µg mL^{-1}) of the two stationary phases (Fig. 10).

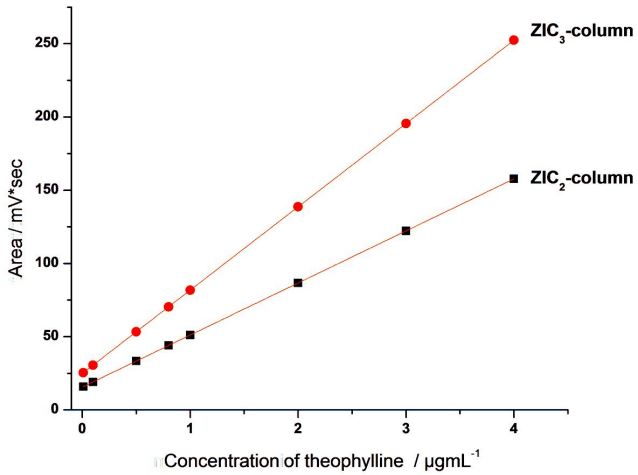

Figure 10: The calibration graph of theophylline using ZIC_2 and ZIC_3 columns.

Statistical analysis

The HILIC mode is used to calibrate theophylline graph statistical results in table 1. Table 2 shows the precision and accuracy of the % RSD and %recovery on the same day and on different days for two stationary phases. The standard deviation is relatively small and the high recovery value indicates the accuracy of the two suggest methods.

Determination of theophylline in two industrial pharmaceutical dosage forms

Two forms of theophylline pharmaceutical dosage were examined and gave better accuracy and precision. Two kinds of tablets were also successfully implemented for the proposed methods with columns ZIC_2 and ZIC_3 and the findings are presented in table 3.

Table 1: Details of the performance analysis.

Parameter	ZIC_2	ZIC_3
Linearity (µg.ml^{-1})	0.01-4.00	0.01-4.00
Regression equation	y=15.55+35.55*x	y=24.88+56.88*x
r^2	0.9993	0.9995
LOD (µg.mL^{-1})	0.130	0.190
LOQ (µg.mL^{-1})	0.455	0.665

Table 2: %RSD and Recovery% of the ZIC_2 and ZIC_3 methods.

intra-day variability n=3					inter-day variability n=3			
ZIC_2 approach								
Theop. Taken µg.mL^{-1}	**Theop. Found µg.mL^{-1}**	**%Rec.**	**% $E_{rel.}$**	**%RSD**	**Theop Found µg.mL^{-1}**	**%Rec.**	**% $E_{rel.}$**	**%RSD**
3.00	**2.96**	98.66	-1.34	0.32	**2.99**	99.66	-0.34	0.43
4.00	**4.03**	100.75	0.75	0.21	**4.05**	101.25	0.25	0.28
ZIC_3 approach								
3.00	**3.02**	100.66	0.66	0.55	**3.04**	101.33	0.33	0.62
4.00	**3.94**	98.50	-1.50	0.58	**3.96**	99.00	-1.00	0.71

Table 3: Results of the experiments in two industrial pharmaceutical dosage forms for the determination of theophylline.

Name of drug	Company	Started conc. (µgmL^{-1})	Get it (µgmL^{-1})	%Rec.	%RSD n=3	% $E_{rel.}$
		ZIC_2				
ASMASAM-Tablet	SDI-Iraq	0.50	0.493	98.60	0.73	-1.40
		0.50	0.503	100.60	0.62	0.60
Theophylline-Tablet	Darou pakhsh-Iran	**ZIC_3**				
		0.50	0.496	99.20	0.89	-0.8
		0.50	0.505	101.00	0.76	1.00

Conclusion

In these study HILIC methods to analyze theophylline in pharmaceutical dosage forms. Two HILIC stationary phases with the same core materials, the same PVC / DVB spacers, and near capability will analyze the impact of interchange chain length on the separation of theophylline. The ZIC_3 stationary phase with long chains is interacting more with theophylline compared to ZIC_2 stationary phase. Two explanations are given, the first being the groups of methylene between the ionic site groups in the stationary phase (ZIC_2 and ZIC_3). The second explanatory proof that the core material (backbone) of stationary phases, which that the PS / DVB as a backbone in ZIC_2 and ZIC_3. Theophylline shows hydrophilic and ion-exchange mixed-mode retention with HILIC columns.

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