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APPLICATION OF HYDROPHILIC INTERACTION CHROMATOGRAPHY METHODS FOR PREDNISOLONE ACETATE DETERMINATION IN THEIR PURE AND TABLET, SYRUP DOSAGE FORMS

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Abstract

Hydrophilic stationary phases (ZIC1 and ZIC5) were investigated for chromatographic prednisolone acetate separation. The eluent's retention behavior in various sodium buffer-containing acetonitriles and pH of the prednisolone acetate was reported. The separation mechanism leads to a mode for prednisolone acetate according to hydrophobic interactions. The calibrating graphs were produced for two exchangers and linear range (0.02-1.5 µgmL^{-1}), RSD% (0.56± 0.08 and 0.9± 0.09), LOD (0.038 and 0.019µg mL^{-1}), LOQ (0.133 and 0.066 µg mL^{-1}), respectively. For pharmaceutical samples, the methods proposed have been effective. And the results of the proposed methods are compared with comparison method and their precision and accuracy are comparable.

Key words: Prednisolone acetate, Hydrophilic stationary phases, hydrophobic interaction, pharmaceutical samples.

Introduction

Prednisolone (PNSA-Fig. 1) is a glucocorticoid and several variations of these drugs are available on the prescription market for ophthalmonic use in the topical treatment of allergic and inflammatory conditions of the skin (Hoffman *et al*., 2016). It is generally used to treat a number of autoimmune and inflammatory diseases such as multiple asthma sclerotics, rheumatoid arthritis and autoimmune hepatitis. Often known as arthritic disease-modifying medicines, PNSA has an anti-inflammatory effect on cytokines and cell adherence molecules by inhibiting gene transcription (Heyneman, 1995; Paulus and Bulpitt, 1993; DiPiro *et al*., 2014; Bagge and Brooks, 1995). Hydrophilic interaction chromatography (HILIC) in conditions where the organic solvents are highly concentrated in hydrophilic compounds is the rapidly increasing alternative to RPLC. The selectivity observed is NPLC similar. Alpert (Alpert, 1990) was the first to suggest a method for HILIC separating a water-enhanced layer of the surface into a mobile phase which is primarily organic. The approach of HILIC to a number of previously daunting separation issues is actually highly appealing. HILIC-technology has successfully studied drugs (Seubert and Saad Rasheed, 2017; Abbas and Rasheed, 2018; Abbas and Rasheed), dansyl amino acids (Rasheed *et al*., 2016), inorganic anions (S. Rasheed and Seubert, 2016), carboxylic acid (Al-Phalahy *et al*., 2016), sugar (Palmer, 1975), saccharides (Linden and Lawhead, 1975) and 2-deoxyuridine (Ashraf Saad Rasheed and Rashid, 2020). Despite the numerous PNSA separation works in HPLC (Patel *et al*., 2012; Razzaq *et al*., 2012; Owen *et al*., 2005; Nicolay *et al*., 2011; Parekh Akshita S. and Joshi, 2016; Bhusnure *et al*.,), no work has been carried out on the retention properties of PNSA in HILIC mode. In addition, the influence of the ZIC- HILIC column chain length on PNSA retention behavior was not previously observed. Better awareness of HILIC retention mechanisms extends the range of possible applications. The goal is ultimately to incorporate simple methods for the evaluation of PNSA in tablets and syrup formulations.

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Materials and Methods

The Merck Hitachi HPLC System, including a gradient pump of L-6200 and a UV-visible L-4200, is used with a 20 µL injection loop. PH tests were performed on the pH 740 (WTW). The Photo Data Workstation software of N2000 has been used to control my chromatography and analyze the data. The PNSA detection was carried out in ultraviolet region with

wavelengths of 275 nm. On the PS / DVB with PEEK columns, a grafted sulphobetaine monomer (100 mm × 4 mm I.D) was used to create the ZIC1 and ZIC5 exchangers for PNSA separation (Seubert and Saad Rasheed, 2017; Rasheed *et al*., 2016; S. Rasheed and Seubert, 2016; Al-Phalahy *et al*., 2016). The detailed process of the grafting reaction was described by (Raskop *et al*., 2007). Purely pursued PNSA from Sigma. Acetic acid was obtained for BDH. Sodium acetate (NaOAc) was obtained from Fluka.

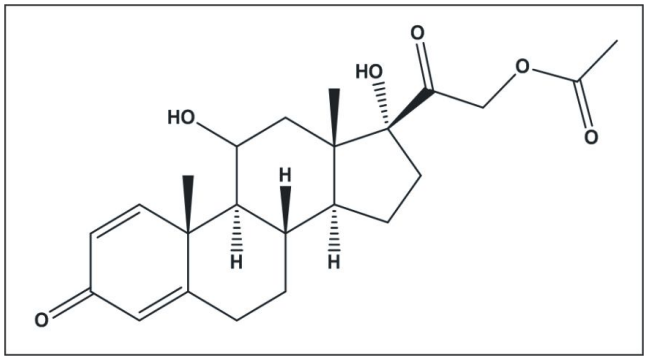

Fig. 1: Structure of prednisolone acetate (PNSA).

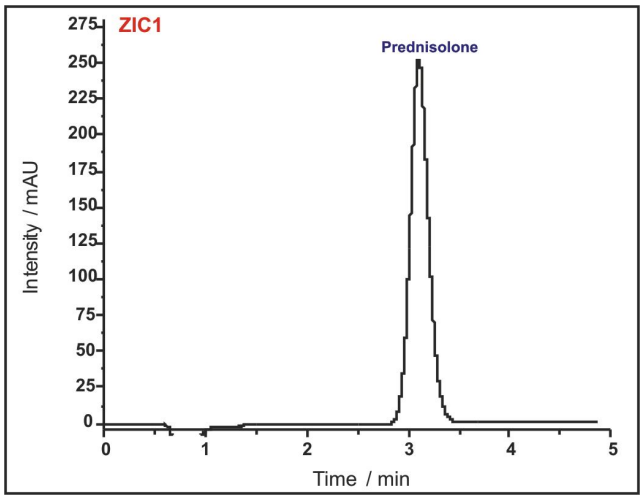

Fig. 2: Chromatogram for the separations of prednisolone acetate (PNSA) in ZIC1 column.

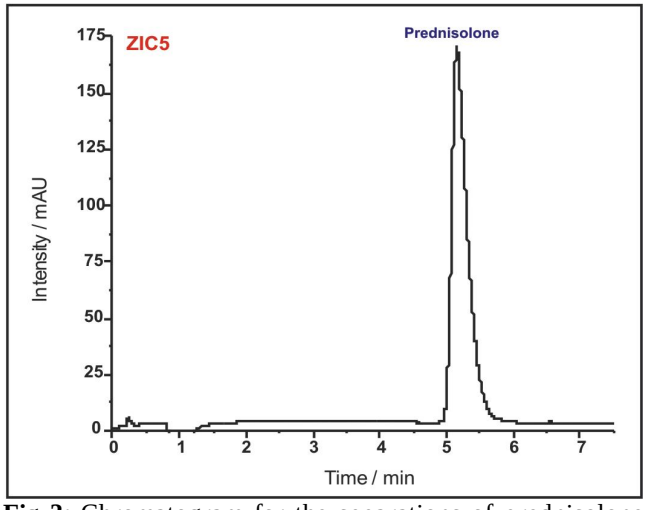

Fig. 3: Chromatogram for the separations of prednisolone acetate (PNSA) in ZIC 5 column.

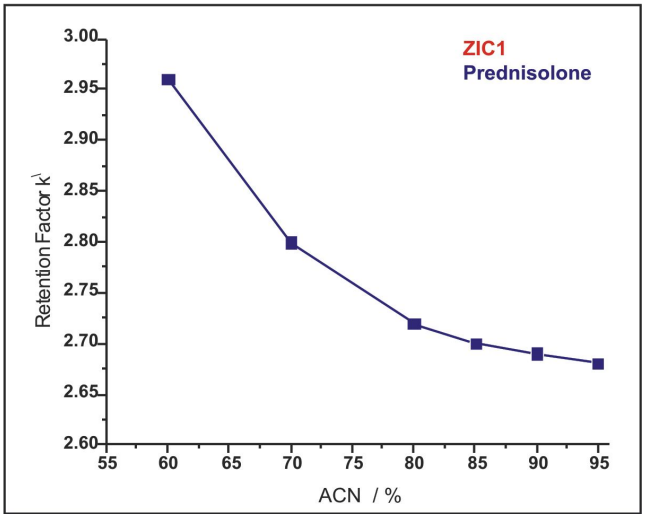

Fig. 4: ACN content impact on PNSA retention in ZIC1 column.

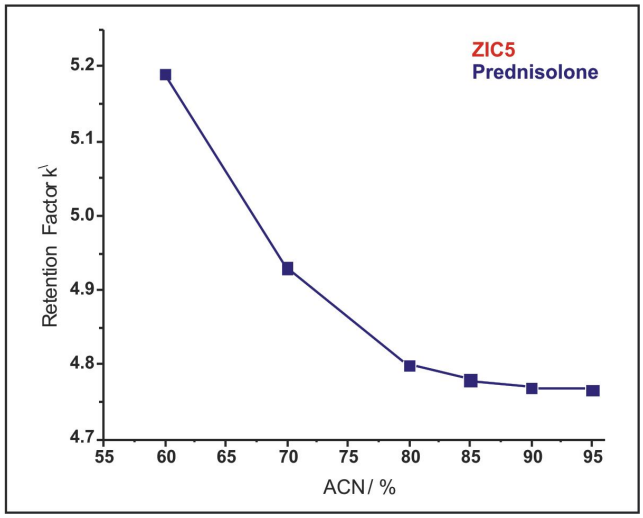

Fig. 5: ACN content impact on PNSA retention in ZIC5 column.

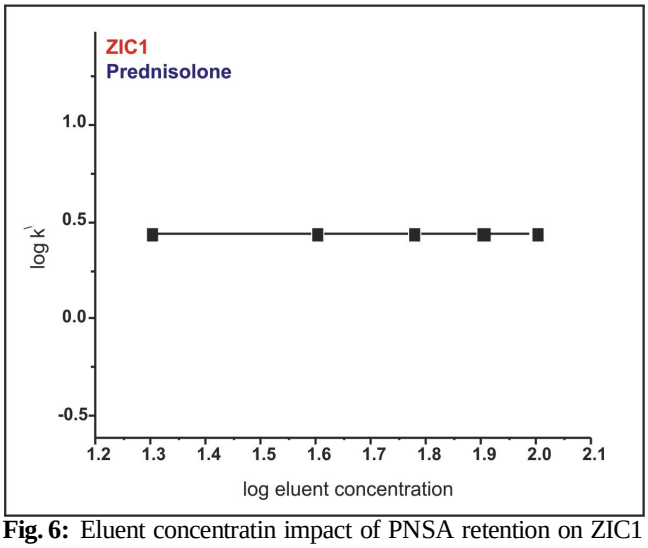

Fig. 6: Eluent concentratin impact of PNSA retention on ZIC1 Column.

Aldrich was given the grade of HPLC (to 99.93%) of acetonitrile (ACN). For exchangers ZIC1 and ZIC5, the capacities 432 and 488 µeq g^{-1} (Abbas and Rasheed, 2018) are available. Ten tablets have been crushed and 5 mg PNSA water dissolved into a 100 ml volumetric water flask for each sample diluted to a mark. Approximately 15 mg of PNSA syrup has been dissolved in water and transferred to a diluted flask of 100 mL. The solution was subsequently filtered out by millipore (0.45 µm).

Results and Discussion

Separation of PNSA

The application of mobile sodium acetate to the switches ZIC1 and ZIC5 with different amounts of ACN was tested for HILIC-mode PNSA separation. The chromatogram is shown in fig. 2 and 3. The acetate was 40 mm (pH 3.5) in sodium and the chromatogram in 85% ACN.

Table 1: Evidence of performance analysis.

Parameter	ZIC1 column	ZIC5 column
Linearity (µg.ml^{-1})	0.02-1.5	0.02-1.5
Regression equation	y = 1115.71 + 517.35*x	y = 224.47 + 972.24*x
r^2	0.9994	0.9991
LOD (µg.mL^{-1})	0.038	0.019
LOQ (µg.mL^{-1})	0.133	0.066

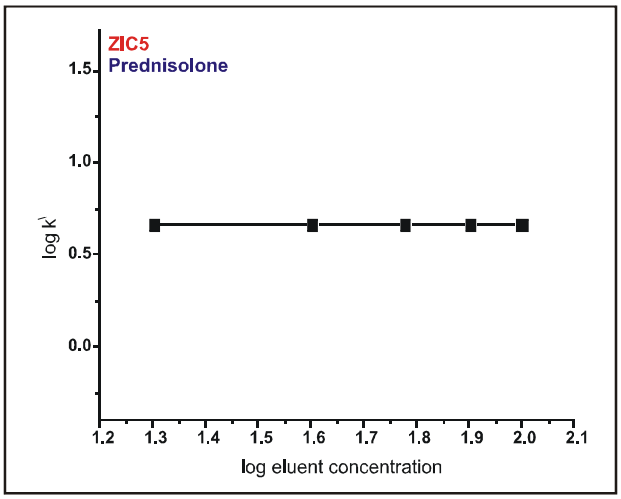

Fig. 7: Eluent concentration impact on PNSA retention in ZIC5 column.

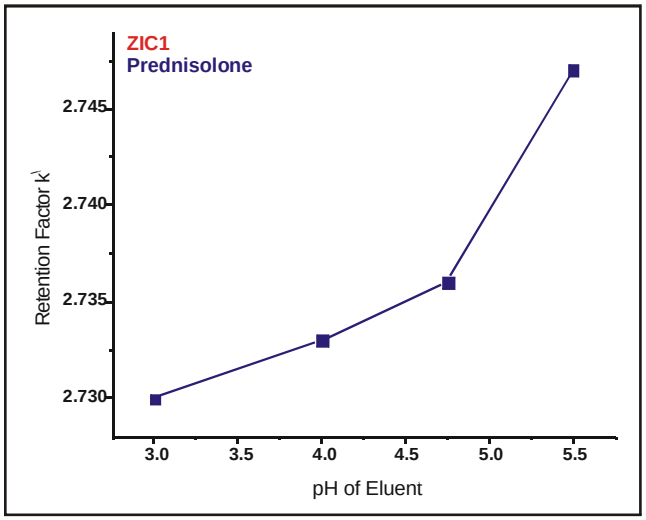

Fig. 8: Eluent pH impact on PNSA retention in ZIC1 column.

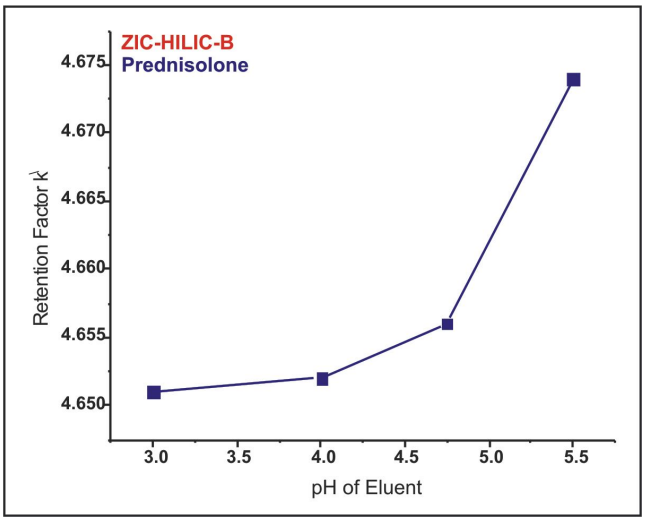

Fig. 9: Eluent pH impact on PNSA retention in ZIC5 column.

Table 2: Recovery of the methods suggested.

	Same-Day Analysis n=5				Day-to-Day Analysis n=5			
ZIC1 column								
PNSA Taken µg.mL^{-1}	**PNSA Found µg.mL^{-1}**	**% Rec.**	**% E_{rel}.**	**% RSD**	**PNSA Found µg.mL^{-1}**	**% Rec.**	**% E_{rel}.**	**% RSD**
0.20	**0.198**	99.00	-1.00	0.66	**0.205**	102.50	2.50	0.72
0.50	**0.495**	99.00	-1.00	0.57	**0.501**	100.20	0.20	0.63
0.70	**0.702**	100.28	0.28	0.45	**0.703**	100.42	0.42	0.83
ZIC5 column								
0.20	**0.195**	97.50	-2.50	0.77	**0.198**	99.00	-1.00	0.80
0.50	**0.498**	99.60	-1.40	0.93	**0.498**	99.60	0.40	1.03
0.70	**0.703**	100.42	0.42	1.00	**0.701**	100.14	0.14	1.23

Fig. 2 and 3, display the interaction between PNSA and the column by increasing the length of the row, as is obvious from ZIC5, thereby increasing PNSA's retention time. The result is that in the ZIC column the methyl group increases among charges. The systemic variation of ACN content in mobile phase compounds increases between 60 and 95 percent; the eluent concentration between 20 and 100 mM is appreciated at pH levels of 3 to 5.5 for each separation and thereby for the separation mechanism.

ACN content effect on PNSA retention

Table 3: Appliance for two proposed methods to assess PNSA in tablets and syrups pharmaceutical samples.

Name of drug	Company	Started conc. (mg)	Get it (mg)	%Rec.	%RSD n=5	% $E_{rel.}$
ZIC1 column						
Prednabene-Tablet	PHARMA-France	5.00	5.03	100.60	0.55	0.60
PREDBISONE- Tablet	Mediphar-Lebanon	5.00	4.92	98.40	0.38	-1.60
Cortancyl-Tablet	SANOFI-France	5.00	4.97	99.40	0.78	-0.60
Prednicort- Syrup	Pioneer-Iraq	15.00	14.95	99.66	0.92	-0.44
ZIC5 column						
5.00			4.99	99.80	0.67	-0.20
5.00			4.95	99.00	0.42	-1.00
5.00			5.01	100.20	0.65	0.20
15.00			14.97	99.80	1.10	-0.20

With this ACN content in ZIC-HILIC mode the retention with drug separations has increased or decreased. In addition, two hydrophobic (RP) and hydrophilic (HILIC) processes classify pharmaceutical products of the low water content of eluent. The hydrophilicity of the drug explains this functional difference. In ZIC1 and ZIC5 exchangers PNSA shows a hydrophobic behavior (Fig. 4 and 5). This is the result of the PNSA log Pow (1.71) (2019a, 2019b).

Eluent concentration effect on PNSA retention

The retention in ZIC-HILIC was usually increased with higher elutent levels, which contributed to the deactivation of intramolecular ion pairs. Thus, the linearization of functional phase groups is enhanced when ACN is present (Rasheed *et al*., 2016). The retention has decreased or increased in the constant ZIC-HILIC modes and the buffer level has increased. This is demonstrated by the exchange of cations and anions (S Rasheed and Seubert, 2016). Significantly, the retention factors of PSNA are almost constant when the holding of ACN is 85% and pH is 3.5, whereas the NaOAc / HAc buffer is increased from 20 to 100mM. Thus, the uncharged nature of PSNA (Fig. 6 and 7) can be attributed. In order to better understand the retention behavior of compounds, the properties of analytes should be taken into account. The PSNA pka value (12.59). Consequently, the PSNA tested should be an uncharged molecule. Interestingly, the possibility of PNSA behaving like a neutral molecule has shown no PNSA effect on anion columns and cation and two ZIC columns. Since PNSA behavior is the same and only because of hydrophobic activity on two columns and persistence, electrostatic contact with functional groups in columns of ZICs cannot clarify PNSA behavior.

Eluent pH effect on PNSA retention

The eluent pH will differ so that the PNSA separation in the HILIC mode is completely indicative. As the pH eluent rose from 3 to 5.5, the retention time for PNSA improved with a 40 mM with ACN 85 percent retention factor as shown in fig. 8 and 9. PNSA retention factor increased slightly. The PNSA shows a very slight increase in pH interaction thanks to the unchanged (neutral) charging of PNSA.

Linearity of PNSA

A linear PNSA graphs showing the range from the plotting area to the PNSA concentration (0.02-1.5 μgmL^{-1}) of ZIC1 and ZIC5 columns (Fig. 10).

Assessment of statistical data

A thorough assessment of the PNSA 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).

Table 4: The comparison of the proposed methods ZIC1 and ZIC5 with comparison method (Ali *et al*., 2002) for PNSA analysis by examining t- and F-statistical tests.

Name of drug	ZIC1 method	ZIC5 method	Comparison (Ali *et al.*, 2002) method	t-Test (theor.)	F-Test (theor.)
Prednabene-Tablet	100.6	99.8	100.33	0.7569* -2.4469	1.2978* -9.2766
PREDBISONE- Tablet	98.4	99	99.32	0.9837** -2.4469	0.4018** -9.2766
Cortancyl-Tablet	99.4	100.2	98.78		
Prednicort- Syrup	99.66	99.8	100.41		
***For ZIC1 proposed method; **For ZIC5 proposed method**					

PNSA determination in medical samples

The proposed methods were

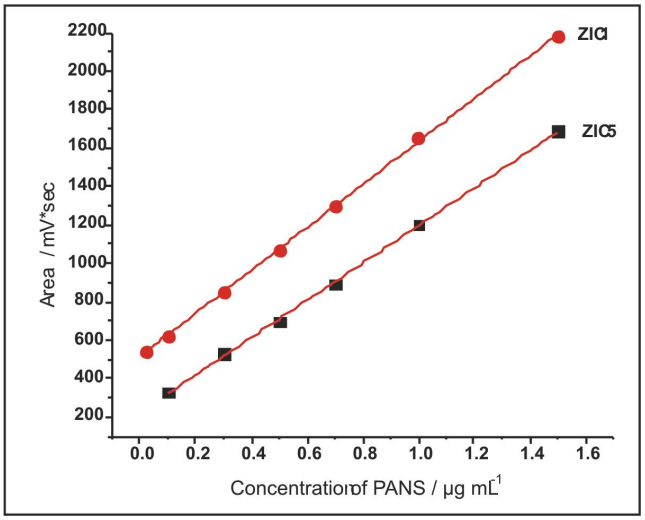

Fig. 10: Linearity graphs for PNSA using ZIC1 and ZIC5 columns.

successfully used in the PNSA evaluation in four 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 comparison method (Ali *et al*., 2002). 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 PNSA determination in syrups and tablets does not vary significantly in both methods.

Conclusion

This article refers to the application of HILIC methods in pharmaceutical samples for the assessment of PNSA. A versatile separational instrument allowing at least two different retention modes under different conditions for HILIC stationary phases, with one and five groups of methylene between loaded groups. This article shows how PNSA processes the columns ZIC1 and ZIC5. The ZIC5 column with PNSA has been found to be longer preserved. The geometric orientation of the ZIC5 column could be due to this. The data show that the hydrophobic interaction is the retention mechanism. The methods developed have been used successfully in medicine samples.

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