RESEARCH ARTICLE

A Study of Retention Behavior and Method Development of Salbutamol Sulfate in Hydrophilic Interaction Chromatography

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ABSTRACT

For chromatographical separation and estimation of salbutamol sulfate, zwitterionic stationary phases with large capacity were obtained by zwitter-molecules attached to a polystyrene-divinylbenzene (PS/DVB) particle. Salbutamol sulfate retention activity was studied with eluent at various pH mobile phase, mobile phase concentrations, and acetonitrile (ACN) percentage. The methods of separation are based on separating the salbutamol sulfate into hydrophobic and cations interactions. Linearity of 0.01 to 0.9 μg.mL^{-1} for two columns was developed with direct calibration curves, %RSD percent (0.48 ± 0.12 and 0.49 ± 0.22), LoD (0.058 and 0.04 μg.mL^{-1}), and LoQ (0.203 and 0.14 μg.mL^{-1}) were created, respectively.

Keywords: Cation interaction, Hydrophobic, Salbutamol sulfate, ZIC-HILIC.

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INTRODUCTION

Salbutamol sulfate (SBS) (Figure 1) is a 2-adrenergic receptor agonist, which is used for bronchospasm relief in conditions, such as, asthma and chronic pulmonary obstructive diseases. SBS includes a nucleus of phenylethanolamines that has a group of channels called receptors. The inhaled route is usually given to the bronchial smooth muscle for a direct effect. It may also be administered orally or intravenously. While SBS is well absorbed, its systemic bioavailability in the gastrointestinal and liver tract is only 50 percent due to extensive pre-systemic metabolism.[1-3]

The phenomenon of ion chromatography (IC), used by stationary zwitterionic phases for anion and cation separations, is zwitterionic ion chromatography (ZIC). In a single molecule during the fixed process the definition combines anionic and cationic feature groups to boost or make ion-exchange selectivity.[4,5] Nevertheless, there is no literary research in this field of estimating and separating SBS using ZIC-hydrophilic (ZIC-HILIC) techniques, despite a number of studies for the separation and determination of SBS in High-performance liquid chromatography (HPLC).[6-9] In addition, the effect of the ZIC-HILIC column's chain longitude on SBS retention behavior was not previously studied. In a recent study, Rasheed and coworkers[10-12] studied the effect of chain length on HILIC columns and its effect on ranitidine and famotidine behaviors. They found that the longer the length of the chain, the greater the interaction between the ranitidine and famotidine with the HILIC column, and therefore, the longer the retention time. The ultimate goal is to implement a straightforward method of measuring SBS in pure and medicinal samples.

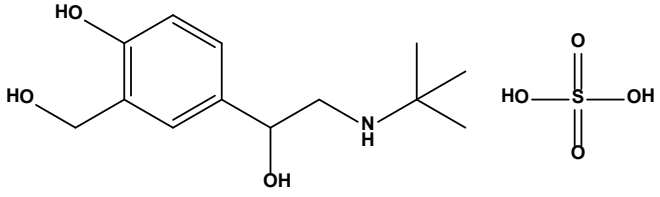

Figure 1: Structure of SBS

MATERIALS AND METHODS

The 40 μL injection loop was used as a Merck-Hitachi L-6200 gradient pump and a UVD L-4200 system. To monitor the chromatography and analyze the data, the N2000 photographic results workstation program has been used. Using ultraviolet regions with a wavelength of 275 nm, the SBS detection was carried out. The stationary phases used for the separation of SBS were performed onto the PS/DVB, using PEEK columns (100 × 4 mm ID) by means of grafted sulfobetaine monomers.[13-16] HPLC grade of ACN was purchased from Aldrich (total 99.93 percent). The capacity of the ZIC1 and ZIC5 columns are 432 and 488 μeq g^{-1},[10] respectively. Ten tablets were crushed for each of the samples and respectively, 2 and 4 mg SBS of water were dissolved, and dissolved with water to a 100 mL volumetric flask and diluted to a label. The equivalent of approximately 2 mg of syrup SBS has water dissolved and transferred to a 100 mL volumetric

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flask, diluted in a watermark. Afterward, millipore (0.45 µm) filtered the solution.

RESULTS AND DISCUSSION

Separation of SBS

In order to study their retention mechanism in HILIC mode, SBS was selected as a pharmaceutical test by applying the NaOAc mobile step with specific ACN content to ZIC-HILIC. Figures 2 and 3 display the chromatograms. At 85 percent ACN and 40 mm (pH 3.5) of NaOAc, the chromatogram was obtained.

It is clear from Figures 2 and 3 that the interaction between SBS and the column increases by increasing the length of the chain, as is evident with the use of ZIC5, thus, increasing the SBS retention time. This is due to the increase in the methyl groups between charges in the ZIC-column. The systemic variability of the contents of the ACN is increasing in mobile phase compounds between 60 and 95%; the concentration of eluent between 20 and 100 mm, with a pH between 3 and 5.5, to obtain an understanding of the separation characteristics of each stationary stage and thus, of the mechanism for separation. As a reference point for ion retention behavior, an anion exchange column was used.

ACN Content Effect on SBS Retention

With that ACN content in ZIC-HILIC mode, retention for pharmaceutical separations has increased or decreased. Therefore, two hydrophobic [Reversed-phase (RP)] and hydrophilic (HILIC)] activities display the pharmaceuticals with a decreased water content of the eluent. The explanation for this functional disparity is due to the medication hydrophilicity. For stationary phases of ZIC1 and ZIC5, SBS demonstrates hydrophobic activity (Figures 4 and 5). This is because of the SBS log P_{ow} (0.34).[17,18]

Eluent Concentration Effect on SBS Retention

In general, retention increased in the ZIC-HILIC mode with increasing eluent concentration, leading to intramolecular ion pairs to deactivation. Therefore, the linearization of the stationary phase functional groups, while ACN is present, is improved.[14] The pharmaceutical retention decreased or increased with an increasing buffer concentration in stationary ZIC-HILIC phases. The explanation for this is the exchange of cations and anions.[15]

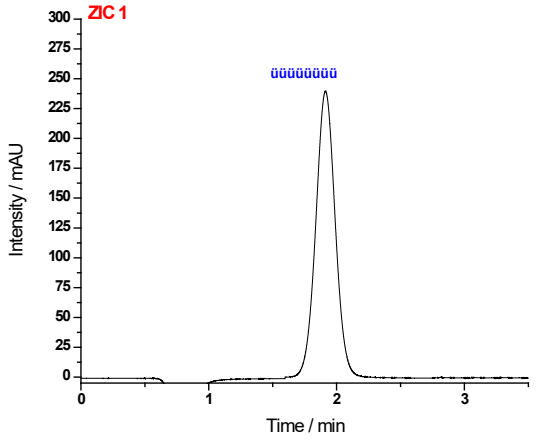

Figure 2: Chromatograms for the separations of SBS in ZIC1 column

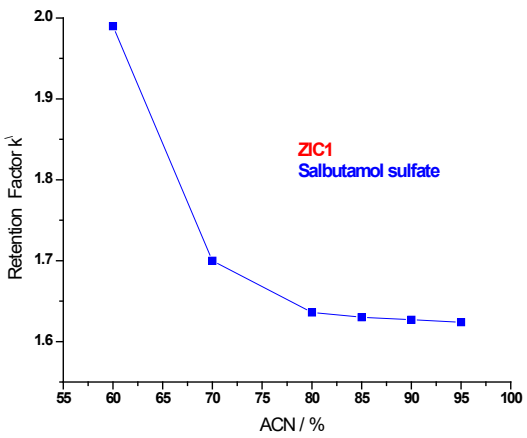

Figure 4: ACN content impact on SBS retention in ZIC1 column

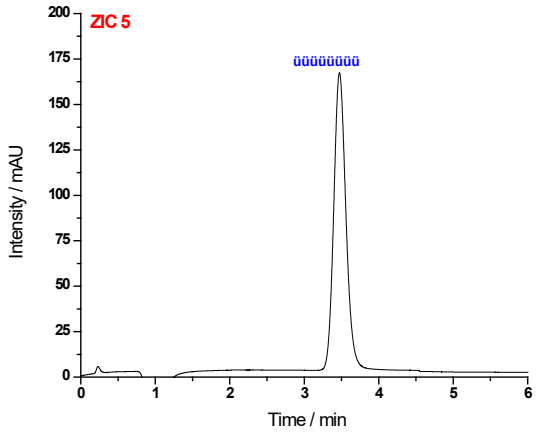

Figure 3: Chromatograms for the separations of SBS in ZIC5 column

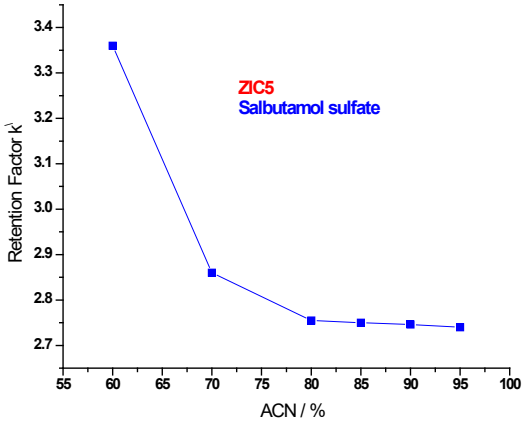

Figure 5: ACN content impact on SBS retention in ZIC5 column

The retention factor of SBS decreases with the rise in the NaOAc buffer from 20 to 100 mm, with a pH of 3.5 and ACN of 85%, as shown in Figures 6 and 7. From Figures 6 and 7, it appears that this slope was determined on standard ion exchange columns,[19] which are similar to the slopes 0.0746 and -0.0643, were obtained. What is the true mechanism of separation now? When the buffer concentration increases, retention decreases, SBS has another picture, and we believe that is because of two factors, *viz.*, SBS hydrophilicity and column core materials, and the Pka (9.65) value and the SBS's isoelectric points (9.40). The SBS will consequently be in cationic form. Then, the interaction between the SBS and the HILIC columns was based on cation exchange.

Eluent pH Effect on SBS Retention

In order to complete the SBS separation mechanism principle, the eluent pH must be varied. Retention of SBS was decreased by increasing the eluent pH from 3 to 5.5, while retaining the sodium acetate concentration at 40 mm and 85% of ACN, as Figures 8 and 9 indicate. Due to the deprotonation of the amino group in SBS, SBS with the isoelectric 9.65 has reduced retention in the stationary phases ZIC1 and ZIC5.

Calibration Curve

A calibration curves SBS defining by area plotting (mV × sec) against the amount (μg.mL^{-1}) of SBS, indicates the range of ZIC1 and ZIC5 stationary phases (Figure 10) (0.01–0.9 μg.mL^{-1}).

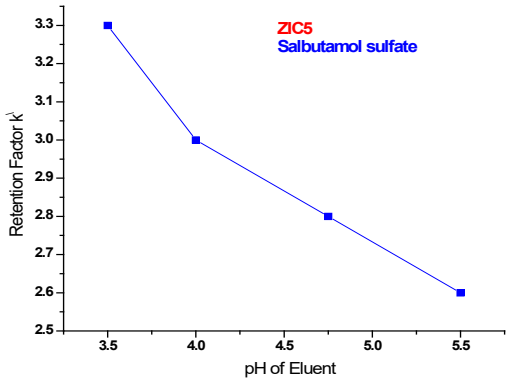

Figure 8: Eluent pH impact on SBS retention in ZIC1 column

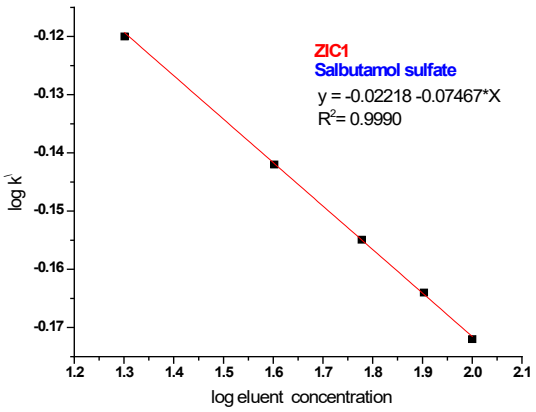

Figure 6: Eluent concentration impact on SBS retention in ZIC1 column

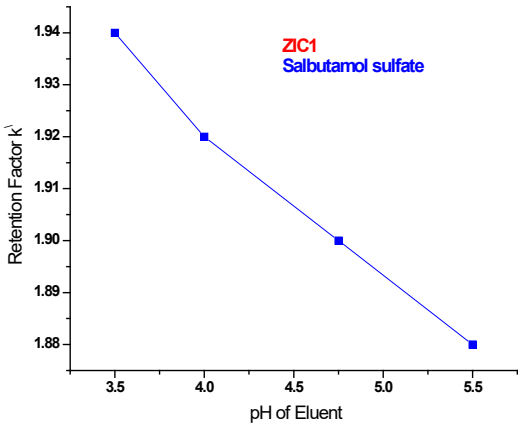

Figure 9: Eluent pH impact on SBS retention in ZIC5 column

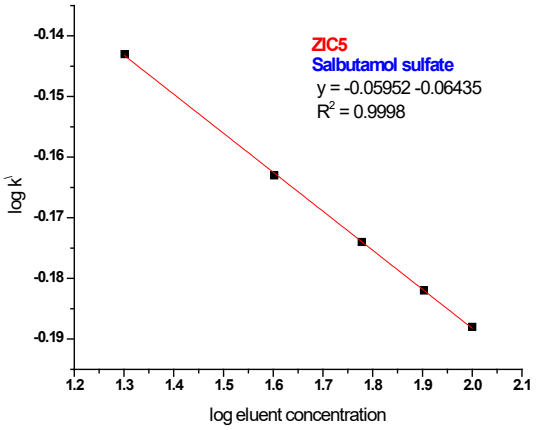

Figure 7: Eluent concentration impact on SBS retention in ZIC5 column

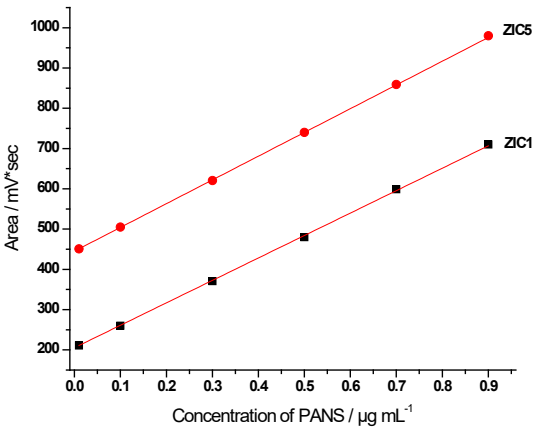

Figure 10: Calibration graph for SBS using ZIC1 and ZIC5 columns

Table 1: Performance analytics results

Parameter	*ZIC1 column*	*ZIC5 column*
Linearity[a] (μg.mL^{-1})	0.01–0.9	0.01–0.9
Regression[a] equation[a]	y = 205.42 + 557.17 × x	y = 445.21 + 589.55 × x
r^2	0.9981	0.999
LoD (μg.mL^{-1})	0.058	0.04
LoQ (μg.mL^{-1})	0.203	0.14

Table 2: Accuracy and precision of the methods suggested

	Same-day analysis n = 5				*Day-to-day analysis n = 5*			
				ZIC1 column				
SBS taken (μg.mL^{-1})	SBS found (μg.mL^{-1})	% Rec.	% $E_{rel.}$	% RSD	SBS found (μg.mL^{-1})	% Rec.	% $E_{rel.}$	% RSD
0.3	0.294	99	-1	0.52	0.302	100.66	0.66	0.88
0.6	0.603	100.5	0.5	0.61	0.594	99	-1	0.73
0.8	0.801	100.12	0.12	0.31	0.795	99.37	-0.63	0.52
				ZIC5 column				
0.3	0.305	101.66	1.66	0.46	0.304	99	-1	0.55
0.6	0.598	99.66	-0.34	0.78	0.601	99.6	-0.4	0.65
0.8	0.797	99.62	-0.38	0.23	0.802	100.25	0.25	0.41

Table 3: Appliance in tablets and syrups medical samples of two proposed methods for the determination of SBS

Name of drug	*Company*	*Started conc. (mg)*	*Get it (mg)*	*% Rec.*	*% RSD n = 5*	*% $E_{rel.}$*
			ZIC1 column			
Salbutol-syrup	Awamedica-Iraq	2	1.97	98.5	1.23	-1.5
		2	1.95	97.5	1.36	-2.5
Butadin-syrup	SDI-Iraq	4	4.03	100.75	0.55	0.75
Ventolin-tablet	GSK-Turkey	2	2.02	101	0.62	1
Butalin-tablet	Julphar-UAE	2	2.03	101.5	0.83	1.5
Butadin-tablet	SDI-Iraq			*ZIC5 column*		
		2	1.99	101	0.98	1
		2	1.98	101.5	1.11	1.5
		4	3.98	99.5	0.71	-0.5
		2	1.97	98.5	0.78	-1.5
		2	2.02	101	1.26	1

Table 4: Comparison of the proposed methods ZIC1 and ZIC5 with the comparison method[20] for SBS analysis by investigating t- and F-statistical tests

Name of drug	*ZIC1 method*	*ZIC5 method*	*Comparison method[20]*	*t test (theor.)*	*F test (theor.)*
Salbutol-syrup	98.5	101	99.85	0.8228* (2.306)	2.8491* (6.39)
Butadin-syrup	97.5	101.5	99.5	0.7499** (2.306)	1.4712** (6.39)
Ventolin-tablet	100.75	99.5	101.55	-	-
Butalin-tablet	101	98.5	98.85	-	-
Butadin-tablet	101.5	101	100.55	-	-

*For ZIC1 proposed method; **For ZIC5 proposed method

Review of Statistic Data of SBS

The corresponding calibration charts have been built to specifically assess SBS under HILIC conditions, and statistical findings are shown in Table 1. On the same day, accuracy and day by day, recovery percent, and relative standard deviation (RSD) percent were analyzed and measured. The

small relative standard deviations and the high recovery values indicate that the methods suggested are successful (Table 2).

SBS in Medical Samples Determination

In five of the pharmaceutical formulations, the proposed methods were successfully used in evaluating SBS; the results are described 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.[20] 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 SBS determination in syrups and tablets.

CONCLUSION

The present study involves the development of HILIC techniques in pharmaceutical samples to evaluate SBS. A flexible separation tool with the advantage of enabling at least two distinct retention modes by varying conditions was used for the HILIC stationary phases, with one and five methylene groups between the charged groups. This paper illustrates how SBS deals with columns ZIC1 and ZIC5. The column ZIC5 with SBS has been found to be maintained longer. The explanation may be because the ZIC5 column is geometrically aligned. The observational data indicates that the retention process is both hydrophobic and cation exchange behavior. In pharmaceutical samples, the methods developed were successfully employed.

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