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Separation and determination of simvastatin on ZIC-HILIC stationary phases by hydrophilic interaction chromatography in pharmaceutical material products

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ABSTRACT

The hydrophilic interaction chromatography (HILIC) technique outperforms other methods in terms of precision and sensitivity. The development and optimization of a HILIC method for validating a separation method for simvastatin estimation in pharmaceutical formulations necessitated determining the optimal mobile phase, buffer concentration, and pH value. The proposed HILIC methods using two columns demonstrated high precision (RSD less than 1%) and a robust linear relationship between the calibration graph and simvastatin concentration ranges of 0.05–4 μg mL^{-1} with a coefficient of determination (R^2) of 0.9996 and 0.9994 for simvastatin. The HILIC methods provided excellent validated LOD (0.023 and 0.015 μg mL^{-1}) and LOQ (0.069–0.045 μg mL^{-1}) values for simvastatin. The HILIC approach was successfully used to estimate simvastatin in commercial pharmaceutical tablets, resulting in an excellent simvastatin recovery > 99%. Using statistical tests, the findings of the HILIC method were compared to the British pharmacopoeia protocol for simvastatin drug. There was no difference in accuracy between the methods.

Selection and peer-review under responsibility of the scientific committee of the Indo-UK International Virtual Conference on Advanced Nanomaterials for Energy and Environmental Applications (ICANEE-2020)

1. Introduction

Hyperlipidemia, identified as an elevated lipid concentration in the blood, is a risk factor for cardiovascular disease when combined with other risk factors such as hyperglycemia, obesity, high blood pressure, and defective fibrinolysis. Additionally, it can play a role in the development of atherosclerosis in people with diabetes mellitus [1-3]. Statins in conjunction with fibrates have been shown to improve the lipoprotein profile in patients with combined hyperlipidemia, and this is widely known, with the same safety profile as single statins [4]. Simvastatin (Fig. 1), a methylated derivative of lovastatin, has become the most commonly prescribed medication worldwide for the treatment of hypercholesterolemia, a significant risk factor for the development of atherosclerosis [5].

Simvastatin in standard form, pharmaceutical formulations, and plasma is determined using a variety of HPLC techniques [6-11]. However, a review of the literature from the last few years revealed that no studies have been conducted to determine simvastatin using hydrophilic interaction chromatography (HILIC). As a result, our study's first objective is to separate and estimate simvastatin using ZIC-HILIC columns. HILIC is a particularly well-suited technique for determining polar compounds, such as pharmaceuticals [12-17], carboxylic Acids [18], amino acids [19], and biopharmaceuticals [20-22].These substances exhibit insufficient retention in reversed-phase liquid chromatography. Although both normal-phase liquid chromatography and ion-exchange chromatography are capable of separating polar analytes, polar compounds are often insoluble in organic normal-phase liquid chromatography mobile phases, and ion-exchange chromatography is applicable only to ionic compounds. HILIC, like normal-

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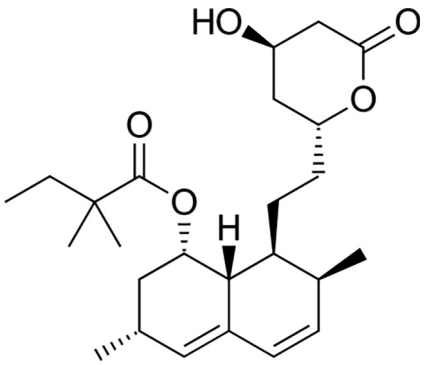

Fig. 1. Chemical structure of simvastatin.

phase chromatography, utilizes conventional polar stationary phases. Nonetheless, mobile phases are comparable to those used in reversed-phase chromatography. However, as several recent reviews have noted [23-26], retention mechanisms in HILIC are complex and are currently being investigated. Even the electrolyte's composition has an effect on retention and selectivity [27]. The second objective of the analysis was to determine the effect of the ZIC-HILIC columns chain length on simvastatin retention behavior, which had not been observed previously. Increased understanding of HILIC retention mechanisms broadens the application possibilities. The ultimate aim is to integrate straightforward methods for simvastatin evaluation into pharmaceutical products

2. Experimental work

2.1. Chemicals

Simvastatin was used as a standard, and acetonitrile (ACN), sodium acetate, and acetic acid were purchased from Sigma-Aldrich. Pharmaceutical tablets (20 and 10 mg) were collected from a pharmacy and distributed to various companies (Pharma International-Jordan, Crescent Pharma-UK, and Mylan-New Zealand).

2.2. High-performance liquid chromatography apparatus

Merck-Hitachi fitted the chromatographic system with a Model L-6200 flow pump, a Model L-4200 UV/Vis detector, and a N2000 Photographic Data Workstation Module Integrator. Chromatographic separation was carried out using an on homemade stationary phases (ZIC-1 and ZIC-4, 100 mm 4.6 mm I.D.) were homemade according to reference [28]. At 25 °C, using a gradient of acetonitrile and acetate buffer (40 mM, pH 4.75), as mobile phase and a flow rate of 0.5 mL/min. 5 μL of injection fluid was used. In stationary phases, the numbers 1 and 4 apply to the methylene groups between the charged groups in sulfobetaine monomers. Simvastatin was detected at 240 nm.

2.3. Preparation of standard solutions

Simvastatin (500 μg mL^{-1}) was prepared as a stock solution by dissolving 0.05 g in 100 mL of the mobile phase. The working standard solutions were prepared by diluting the stock solution to the desired concentration with the mobile phase. A working solution (10 μg mL^{-1}) of simvastatin was prepared in a brown flask using millipore water from the stock solution. Calibration requirements were prepared immediately after this working solution was prepared. Simvastatin calibration standards of 0.05, 0.1, 0.5, 1, 2, 3 and 4 μg mL^{-1} were used.

2.4. Preparation of simvastatin pharmaceutical products

Ten simvastatin tablets containing 10 and 20 mg of commercially available medication were weighed. We measured the average weight and powdered it. After vigorously shaking and pouring an equal amount of 10 and 20 mg into a 100 mL volumetric flask. For waste, simvastatin was dissolved in 50 mL of mobile phase. The residue has been washed with mobile phase, and the amount of mobile phase added has been steadily increased to 100 mL. The solution was then purified using 0.22 μm Millex® Syringe filters.

2.5. ZIC-HILIC method development

The sensitive and simple ZIC-HILIC method employs two stationary phases (ZIC-1 and ZIC-4, 100 mm 4.6 mm I.D.) and a flow rate of 0.5 mL/min. The established and validated mobile phase consisted of acetate buffer solution and acetonitrile to determine simvastatin. Significant factors were optimized in order to achieve an effective ZIC-HILIC system with high resolution and efficient separation. Along with simvastatin retention behavior, various acetonitrile amounts, buffer concentrations, and pH buffer were examined. The detection was carried out at a wavelength of 240 nm. Additionally, the conditions were modified to facilitate the creation of calibration curves for simvastatin estimation.

3. Results and discussion

3.1. Separation of simvastatin

Separation is a preliminary stage in the production and quantitative estimation of the ZIC-HILIC method. The primary goal of this project was to choose a straightforward method that ensures simvastatin is properly isolated. Simvastatin was chosen as a test pharmaceutical for a study on their retention mechanism in ZIC-HILIC mode using a mobile acetate buffer step with varying ACN content on two stationary phases (ZIC-1 and ZIC-4). The chromatograms are shown in Fig. 2. Chromatograms were prepared in acetate buffer containing 80% ACN and 40 mM (pH 4.75).

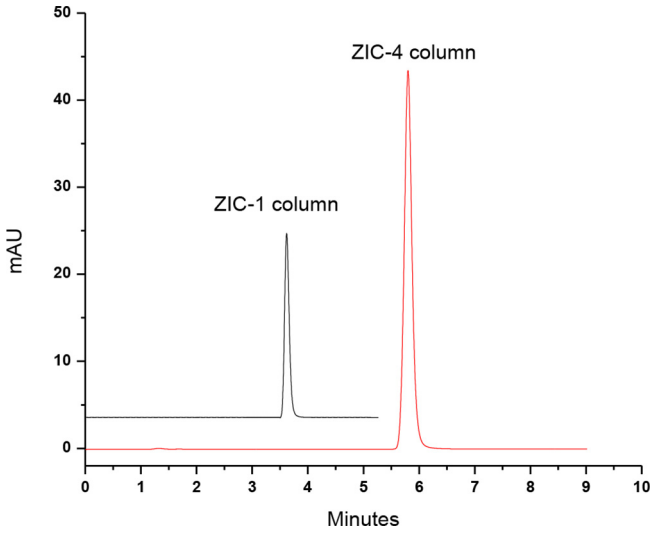

Fig. 2. Chromatograms of the simvastatin using ZIC-1 and ZIC-4 columns.

3.2. The impact of ACN content on simvastatin retention

The mobile phase compositions are modified systemically by varying the ACN content from 60% to 95% (v/v) while maintaining a constant buffer concentration of 40 mM at pH 4.75 (Fig. 3). Simvastatin, a pharmaceutical, exhibits a hydrophobic relationship with rising ACN content in the mobile process. Consequently, the simvastatin's retention time decreases by increasing the ACN content. Simvastatin's hydrophilicity is responsible for this behavior. The log P_{ow} demonstrates the simvastatin's worth. This is discussed in greater detail in log P_{ow} simvastatin (4.46) [29].

3.3. The impact of buffer pH on simvastatin retention

The buffer pH was varied between 3.5 and 5.5 while maintaining a steady buffer concentration of 40 mM at 80 percent ACN content (Fig. 4). Simvastatin retention factor decreased slightly. The simvastatin shows a very slight decrease in pH interaction thanks to the simvastatin's unchanged (neutral) charging.

3.4. Impact of buffer concentration on simvastatin retention

At the conclusion of the optimization conditions, the buffer concentration was increased from 10 to 80 mM with a constant ACN content of 80% at pH 4.75, and thus no substantially altered pH values were observed (Fig. 5).

3.5. Calibration graph

Simvastatin calibration graphs were created under optimal conditions (mobile phase: (40 mM acetate buffer, pH 4.75, 80% acetonitrile), 240 nm UV detection, 5 μL injection volume, flow rate 0.5 mL/min, and 25 °C temperature) by plotting area versus simvastatin concentrations and displaying the 0.05–4 μg mL^{-1} ranges, respectively (Fig. 6).

3.6. Analyzing statistical data

The direct calibration curves and statistical results for simvastatin determination in HILIC mode are shown in Table 1. The method has been validated by the International Conference on Harmonization (ICH) [30] for two concentrations covering the range. Each concentration was replicated (n = 4), and calibration

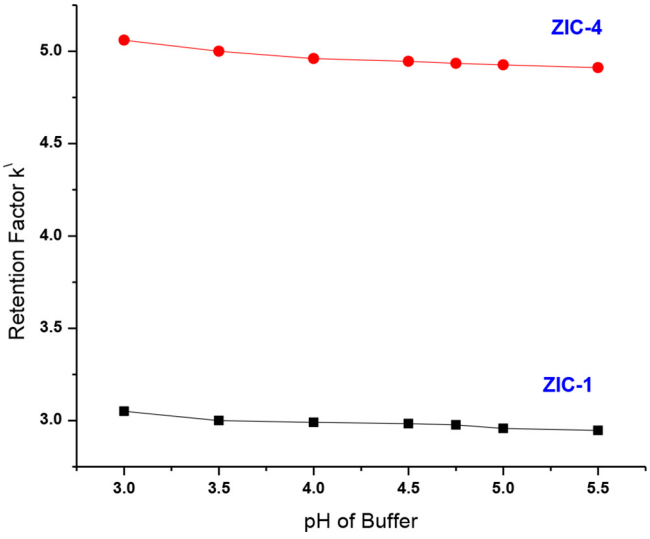

Fig. 4. The impact of buffer pH on simvastatin retention.

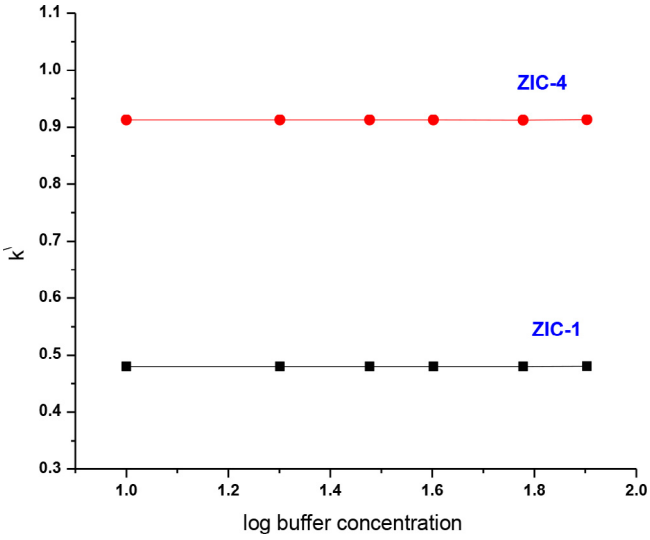

Fig. 5. Impact of buffer concentration on simvastatin retention.

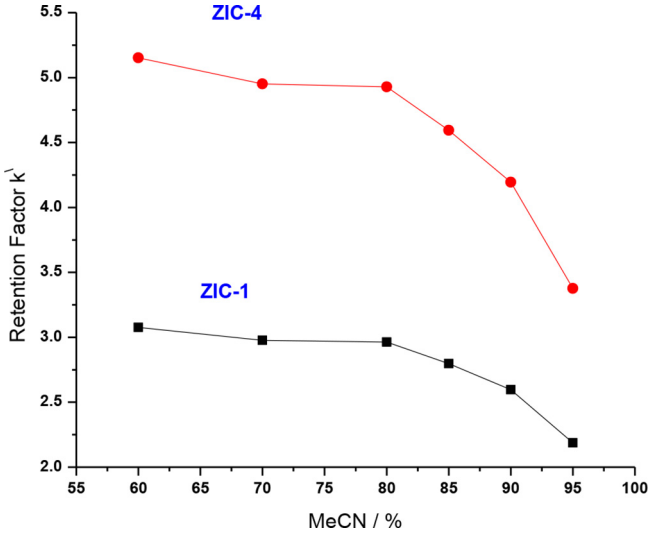

Fig. 3. The impact of ACN content on simvastatin retention.

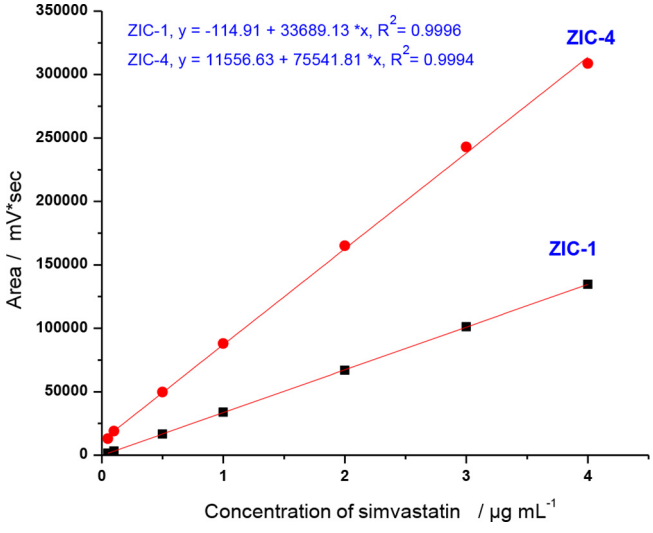

Fig. 6. Calibration graphs for simvastatin using ZIC-1 and ZIC-4 columns.

Table 1
Simvastatin analytical values derived from the calibration graph.

Parameter	ZIC-1 column	ZIC-4 column
Linear range (μg mL^{-1})	0.05–4	0.05–4
Regression equation	y = -114.91 + 33689.13 *x	y = 11556.63 + 75541.81 *x
Coefficient of determination (r^2)	0.9996	0.9994
LOD (μg mL^{-1})	0.023	0.015
LOQ (μg mL^{-1})	0.069	0.045

samples from seven consecutive days were analyzed. The precision and accuracy of RSD and recovery measurements were determined in one run (intra-day) and between runs (inter-day) (Table 2).

3.7. Determination of simvastatin in pharmaceutical products

Three pharmaceutical products containing the target drug at a specified concentration of 10 and 20 mg per unit have been successfully used to determine simvastatin. Table 3 summarizes the collected data. The HILIC-mode data (Table 3) were compared to the standard method [31] using the 95 percent confidence student *t* test and the variance F test. The estimated t and F values (Tables 4) did not surpass the theoretical values, indicating that the accuracy and precision of the process used to determine simvastatin in pharmaceutical products were not significantly different.

4. Conclusions

For quantitative estimation of simvastatin in dosage tablets, the proposed method was validated for precision, accuracy, specificity, and reproducibility. Chromatographic conditions were investigated in order to obtain a high degree of separation efficiency. Simvastatin have a retention time of approximately 3 and 6 min in two columns, respectively. The proposed HILIC-HPLC approach was used to accurately and precisely determine simvastatin in pharmaceutical formulations. As a result, the proposed HILIC-HPLC

Table 2
The precision and accuracy of the proposed methods for simvastatin determination.

	intra-day n = 7			inter-day n = 7		
ZIC-1 column						
Present (μg mL^{-1})	Found (μg mL^{-1})	%Rec.	%RSD	Found (μg mL^{-1})	% Rec.	%RSD
1	0.991	99.10	0.55	0.991	99.10	0.59
3	2.990	99.66	0.44	2.992	99.73	0.65
ZIC-4 column						
1	1.010	101.00	0.36	1.020	102.00	0.47
3	2.995	99.83	0.42	2.995	99.83	0.68

Table 3
Implementation of the proposed methods for simvastatin determination in pharmaceutical preparations.

Name ofpharmaceutical	Manufacturer	Present conc. (mg)	Found direct calb. (mg)	Recovery %	RSD % n = 5
ZIC-1 column					
Simvastatin	Crescent Pharma-UK	20	20.05	100.25	0.36
Simvastatin	Pharma International-Jordan	20	19.90	99.50	0.56
Simvastatin	Mylan-New Zealand	10	9.94	99.40	0.50
ZIC-4 column					
		20	20.07	100.35	0.55
		20	19.93	99.65	0.43
		10	9.97	99.70	0.27

Table 4
The *t*-test and F-statistical tests were used to compare the proposed ZIC-HILIC methods to traditional method [31] for simvastatin calculation.

Manufacturer	Proposed method Recovery %	Standard method Recovery%	t_{cal}	F_{cal}
	ZIC-1 column			
Crescent Pharma-UK	100.25	99.34	0.8362	0.6709
Pharma International-Jordan	99.50	100.44		
Mylan-New Zealand	99.40	99.65		
	ZIC-4 column			
	100.35		0.8320	0.4740
	99.65			
	99.70		$^{*}t_{tab}$ = 2.7764	$^{**}F_{tab}$ = 19.000

approach can be used to determination simvastatin in pharmaceutical dosage forms on a routine analysis.

CRediT authorship contribution statement

Ali Mohammed Mahir Fahad: Writing – original draft, Supervision. **Ashraf Saad Rasheed:** Conceptualization, Methodology, Visualization. **Hameed Hussien Ali:** Validation, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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