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Hydrophilic Interaction Chromatography with Sulfobetaine Zwitterionic Polymer-Bonded Stationary Phases for the Simultaneous Quantification of Atorvastatin and Rosuvastatin Pharmaceuticals in Bulk and Dosage Forms

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Summary: A rapid method for simultaneous determination of two statin drugs based on zwitterionic chromatography (ZIC) had been developed and validated. The development method included examining the effects of chromatographic conditions, including the percentage of organic modifier, pH values, ionic strength of the acetate buffer, and the predominant retention mechanism's experimental determination. Separation developed by two zwitterionic stationary phases (100 mm × 4.6 mm I.D., 3.5μ). The influence of various spacer lengths was being used as an examination tool on atorvastatin and rosuvastatin retention behaviours. Two zwitterionic stationary phases and a mobile phase consisting of acetonitrile and acetate (pH = 4.75, 40 mM) in a ratio of 80:20 V/V were used to achieve optimum chromatographic conditions. The methods had been validated for linearity, accuracy, and precision. This validation shows that the ZIC-HILIC methods proposed were sufficient for quantification analysis of atorvastatin and rosuvastatin. Quantifications were achieved with U.V. detection at 240 nm over the concentration range of 0.1–7.0 μg mL^{-1} for atorvastatin and rosuvastatin, respectively.

Keywords: Atorvastatin, Rosuvastatin, Statin drugs, Dosage form**,** ZIC-HILIC.

Introduction

Cholesterol is essential to the functioning of the body regularly. Conversely, it is also known to increase the risk of atherosclerosis. Plaques can constrict arteries, causing a stoppage of flow or burst and causing clot buildup. These blockages can lead to angina, heart attack, and even a stroke [1]. The statin treatments can be administered at dinner or before going to sleep. Generally speaking, results can be observed after four to six weeks of use. A group of drugs in this category typically have little to no side effects [2]. Ring structures of statins vary, and changes in the composition of the statins influence their pharmacological properties. Some studies classify the various statins into the water-soluble and the water-insoluble categories.

Simvastatin is a statin for low cholesterol (type 1). Compared to HMG-like statins, it is generally stated that the more significant, synthetic classes tend to refer to as type 2 statins. Simultaneously, everybody seems to think of statins as belonging to one category or the other. Two different statins merit their distinct names: atorvastatin and rosuvastatin (Fig. 1) [3]. Rosuvastatin (ROSU) is a statin drug in the United States that Astra-Zeneca first synthesized and received approval in 2003 [4]. Mevalonique coenzyme A also targets the following enzyme: 3-hydroxy-3-methylglutaryl-coenzyme (HMG-CoA) reductase, which catalyzes the breakdown of HMG-CoA to mevalonate. The hydroxymethyl-glyceramino-glutylation of cholesterol is a rate-limiting step in cholesterol biosynthesis [5]. At nearly every single stage of cholesterol biosynthesis, atorvastatin is a selective, competitive inhibitor in cholesterol synthesis's three-carbon (mevalonate) coenzyme (HMG-CoA). That is involved in the total synthesis of HMG-CoA (a precursor of sterols, including cholesterol). Cholesterol decreased in the cell membranes causes an increase in the LDL receptor's density on the liver's surface, which causes an increase in the removal of LDL (low-density lipoprotein) from the blood [6].

Atorvastatin calcium (ATOR) has an anti-inflammatory effect and helps prevent the development of atherosclerotic plaque accumulation. The medication inhibits vascular smooth muscle cell proliferation, which is a significant pathologic factor in the development of atherosclerosis Atvastatin has the effect of restricting further development of atherosclerosis and vascular stenosis [7, 8]. ATOR is used to lower total cholesterol, LDL, and apolipoprotein B levels in primary hypercholesterolemia. In addition to that, a booster to improve HDL levels in patients with mixed dyslipidemia patients with elevated serum T.G. levels and those who fail to respond to diet treatment can also benefit from drug treatment. Since it is used in those with homozygous familial hypercholesterolemia, a disease in which both the father and the mother have the same abnormal, elevated levels of LDL-cholesterol and total cholesterol (e.g., LDL apheresis) [9].

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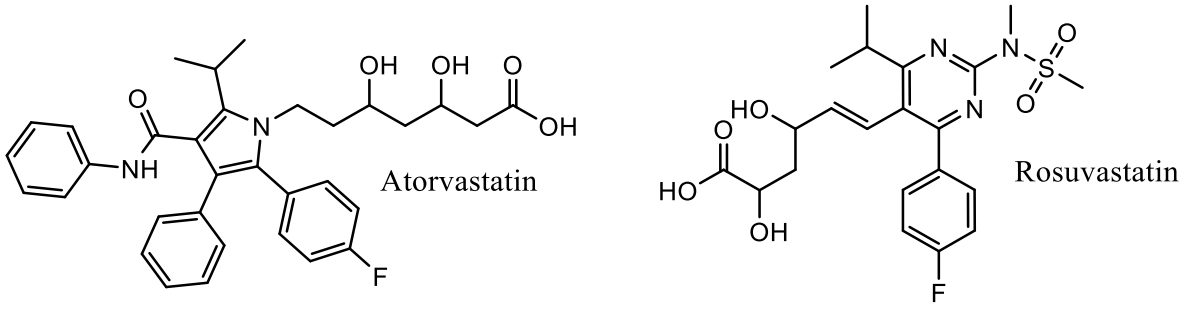

Fig. 1. Chemical structures of ATOR and ROSU.

Various chromatographic methods are mentioned in the literature to determine ATOR and ROSU in pharmaceutical preparations and biological samples [10-17]. Few reports separate ATOR by HILIC stationary phases [18, 19]. The objective of this study was to develop a hydrophilic interaction chromatography (HILIC) method for simple ATOR and ROSU routine analysis. Two stationary PS/DVB-linked sulfobetaine monomers, the various partition, ion exchange, and hydrophobic mechanisms employed, conducted exhaustive studies to investigate ATOR and ROSU retention.

HILIC can separate polar and non-polar pharmaceutical compounds using a mobile phase rich in organic solvents [20-27]. Under HILIC conditions, the separation mechanism is mainly based on the hydrophilic partitioning of analytes between a buffer layer formed on the stationary and organic solvent-rich mobile phases. HILIC stationary phases can be categorised as neutral, positively charged, and negatively charged. The influence of spacer length between charges between its ZIC-HILIC columns on the separation of pharmaceutical, nucleosides and carboxylic acids [20, 23, 27-31] found that the longer the chain between charges between its ZIC-HILIC columns, the greater the interaction between the analytes and stationary phases. It found that the lengthier spacer between charges gave more excellent retention between solutes and stationary phases. Currently, the study of the influence of spacer length for the estimation of ATOR and ROSU has not been established before, so the second objective investigation would be to determine that.

Experimental

Apparatus

Chromatographic measurements were made on an 844 UV/VIS compact I.C. (Metrohm AG, Herisau, Switzerland) model with a 5µ sample loop. The mobile phase was degassed using degasser model 3493 (Kontron Instruments, Germany). The I.C. Net 2.3 software (Metrohm AG, Herisau, Switzerland) was used to monitor the chromatogram and analyze the data.

Chemicals and reagents

Atorvastatin calcium trihydrate (ATOR) and rosuvastatin calcium (ROSU) were purchased from Sigma-Aldrich (Darmstadt, Germany). Acetonitrile (MeCN-HPLC grade), sodium acetate (NaOAc) and acetic acid (HOAc) were of analytical reagent grade supplied by Sigma-Aldrich (Darmstadt, Germany). All reagent solutions were prepared with Millipore water's 0.1 µs/cm conductivity (Millipore system-USA). The pharmaceutical dosage forms of atorvastatin (atorvastatin-20 mg, Lipitor-20 mg and Siros-10 mg) were purchased from Accord (England), Pfizer (USA) and PHARMA development (France), respectively. As for rosuvastatin, pharmaceutical samples (CRESTOR-10 mg, ROSALUS-10 mg, Lodal-10 mg and Rosuvastatin Denk-10 mg were purchased from Astra Zeneca (India), HARMA development (France) and Denk Pharma (Germany), respectively.

Standard solutions

The stock solutions ATOR and ROUS were prepared by dissolving an accurate weighted ATOR and ROUS amount (10 mg) in 100 mL of the mobile phase, which resulted in a dilution of 10 mL of the solution in the mobile phase into 100 mL to create the stock solution ATOR and ROUS (10 µg mL^{-1}). The obtained yield was dissolved and filtered via Millex® Syringe filter (0.45 µm-Merck-Germany). In the range, 0.1–7.0 µg mL^{-1} for ATOR and ROUS calibration curves were developed, indicating peak area ratios of ATOR and ROUS versus ATOR and ROUS concentrations.

Pharmaceutical samples preparation

Fifteen tablets were weighed and finely powdered. The pharmaceutical samples equivalent to 10-20 mg of ATOR pharmaceutical samples with 10

mg of ROUS pharmaceutical samples were dissolved in the mobile phase and transferred into a 100 mL volumetric flask. After that, the mixture solutions were subjected to sonication for 15 min using an ultrasonic water bath (Fisherbrand-CPXH, USA) and filtered via 0.45 µm Millex® Syringe filter. The final concentrations of ATOR pharmaceutical samples were 100 and 200 µg mL^{-1}, and for ROUS pharmaceutical samples, 100 µg mL^{-1}.

Chromatographic procedure

Simultaneous separation of ATOR and ROUS was performed on the stationary phases (ZIC-1 and ZIC-4, 100 mm × 4.6 mm I.D.) were home-made according to reference [32]. Under gradient conditions, use a mixture of MeCN and acetate buffer (40 mM, pH 4.75; 50) as mobile phase with a flow rate of 0.5 ml/min, at 25 °C. The injection volume was 5µl. In stationary phases, the numbers 1 and 4 refer to methylene groups between the charged groups in sulfobetaine monomers. The ZIC-1 and ZIC-4 columns have capacity 432 and 488 µeq g^{-1}, respectively [32]. Detection was performed at 240 nm for ATOR and ROUS. The anion exchange column was produced using a grafting reaction with a capacity of 48 µeq g^{-1} utilising a column of PEEK (100 mm x 4 mm I.D.) [33].

Validation

Validation investigations were performed using the criteria outlined in the ICH guidelines [34]. The study parameters, namely, linearity, accuracy, precision, specificity, and sensitivity of the method developed, were examined.

Results and Discussion

ZIC-HILIC methods development

In ZIC-HILIC mode, the mobile phase with high organic solvent is used [35]. The separation mechanism in ZIC-HILIC is more complicated, and partition interaction Can take place alongside hydrophobic and ion exchange [29, 35, 37, 38]. The separation mechanism in ZIC-HILIC is not persistent, and it can be varied from one to another, fundamentally when different solutes and stationary phases are applied [35, 38]. In this work, the chromatographic conditions were optimized to simultaneously determine ATOR and ROUS, e.g., the organic modifier content, buffer concentration, and pH value. Furthermore, the separation mechanism of the ATOR and ROUS was investigated.

Influence of the organic modifier content

In HILIC mode, the mobile phase most typically contains at least 5-10% of the aqueous phase, representing the stronger eluent [35]. The influence of MeCN content was investigated in the range from 60-95% while keeping acetate buffer (NaOAc/ HOAc) constant at 40 mM and the pH at 4.75 in the range from 5-40 %. Increasing the MeCN content in the mobile phase reduced ATOR and ROUS retention (Fig. 2). The retention mechanism had demonstrated hydrophobic interaction (RP) for ATOR and ROUS. The reason for this behaviour is due to the partition coefficient (log P_{ow}) values of ATOR and ROUS (4.9 and 1.23), respectively [39].

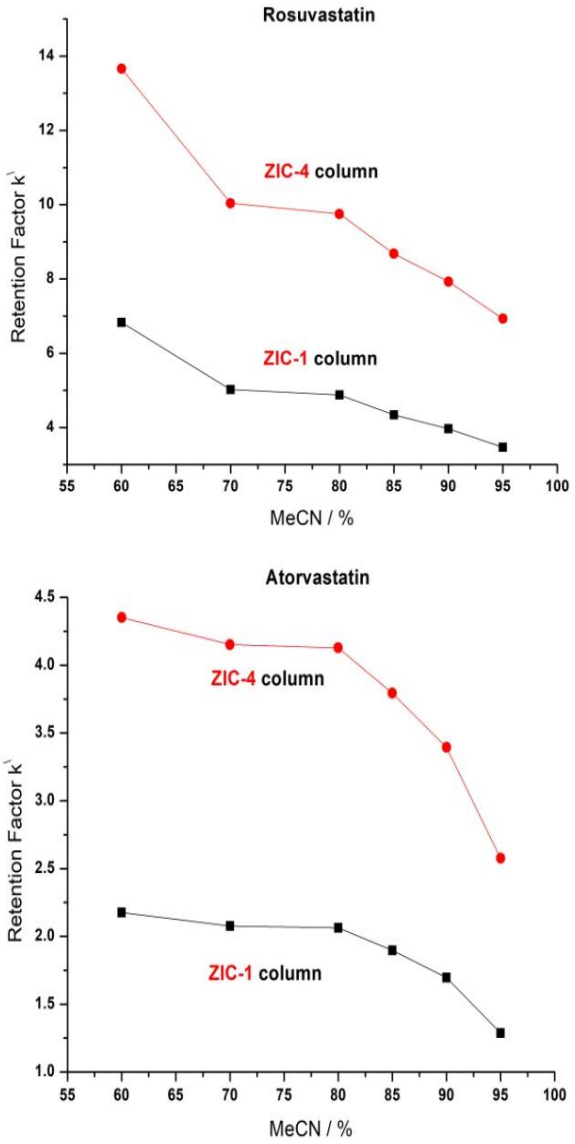

Fig. 2: Plot retention factor (k') vs. acetonitrile percentage (MeCN%) in mobile phase.

Influence of the pH buffer

The buffer pH value effect was investigated using different pH values (3.0, 3.50, 4.00, 4.50, 4.75 and 5.50) of the acetate buffer (40 mM) in the mobile phase. By increasing the buffer pH value, ATOR and ROUS retention time were reduced (Fig. 3). The pKa values range from 4.31 and 4 of ATOR and ROUS, respectively [39]. This is likely to be due to the increase in the ionization state of the ATOR and ROUS. That led to enhanced electrostatic repulsion repulsions between the negatively charged ATOR and ROUS and the zwitterionic sulfobetaine stationary phase.

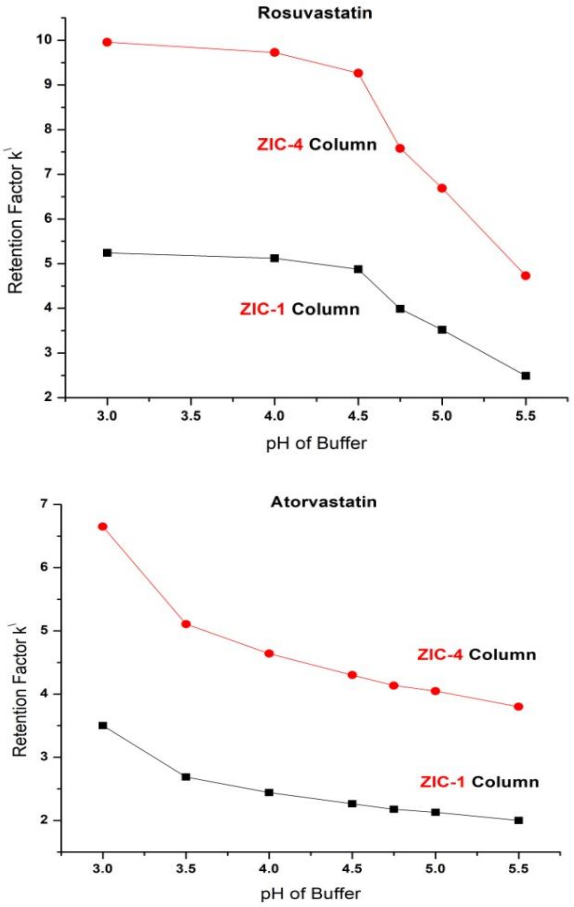

Fig. 3: Plot retention factor (k') vs variation pH of the buffer.

Influence of the ionic strength of the buffer

The buffer ionic strength buffer used in the mobile phase has a considerable influence on retention in ZIC-HILIC mode of polar and non-polar compounds because of its effect on the stationary phases degree of ionization the solute and the polarity the mobile phase [40, 41]. The influence of buffer ionic strength on retention factor was investigated using concentrations range (10-80 mM) of acetate buffer (pH 4.75) at constant MeCN percentage (80%) in the mobile phase. ATOR and ROUS retention factor has been reduced by increasing the acetate buffer concentrated (Fig. 4). There is a question about an exact separation mechanism. ATOR and ROUS take a different approach to decreasing retention; hence, we advise two factors. The first factor is ATOR and ROUS hydrophilicity values, and the second factor is the core material (PS/DVB) for the stationary phases. The predicted negative slopes (Fig. 5) were demonstrated by a similarly built anion exchanger using trimethylalkylammonium and the identical grafting procedure. From Fig. 4, the slopes obtained from ZIC1 and ZIC4 columns, such as an ion-exchange column slope, seem to have been measured [43]. Subsequently, ATOR and ROUS separation depended on anion exchange with ZIC-1 and ZIC-4 stationary phases [21].

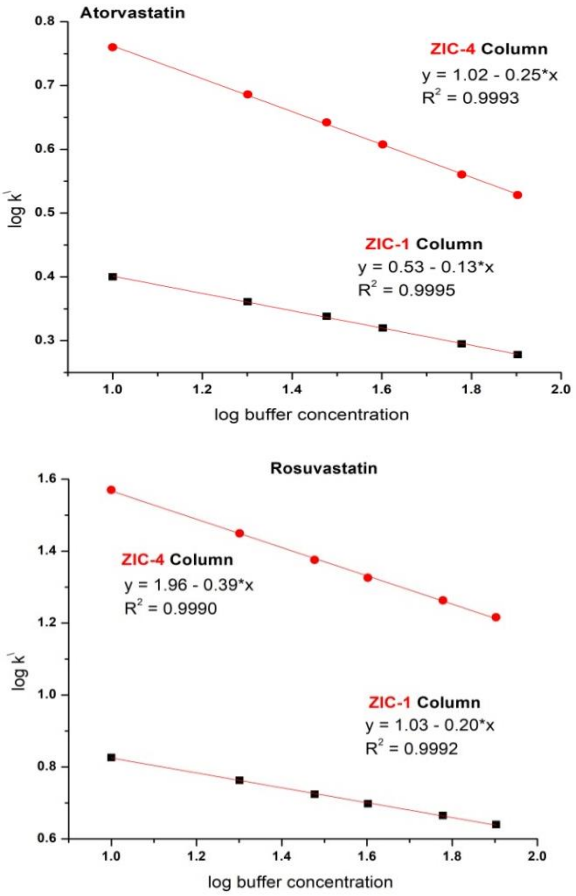

Fig. 4: Plot retention factor (k') vs variation buffer ionic strength.

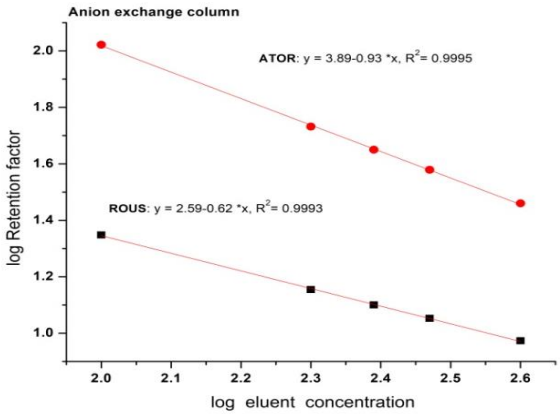

Fig. (5): Effect of eluent strength on the ATOR and ROUS using the anion exchange column.

Optimization of the ZIC-HILIC methods for separation ATOR and ROUS

The buffer concentration, pH buffer and MeCN content will be optimized. The optimum condition for separation of ATOR and ROUS was 80% MeCN and acetate buffer (40 mM-pH 4.75). The separation ATOR and ROUS chromatograms as shown in Fig. 6. The retention of ATOR and ROUS demonstrates the highest retention in ZIC-4 stationary phase compared to ZIC-1 stationary phase (Fig. 6). The unavoidable explanation for this is the methylene groups in ZIC stationary phases between charged groups [21, 23]. In the ZIC-4 exchanger, the strongest ATOR and ROUS retention tend to be the sulfobetaine groups' geometrical arrangement. Such interactions occur because the sulfobetaine chains have different flexibilities that can form intra- and intermolecular ion pairs. Therefore, the spacers between the charges of stationary phases should impact pharmaceutical retention.

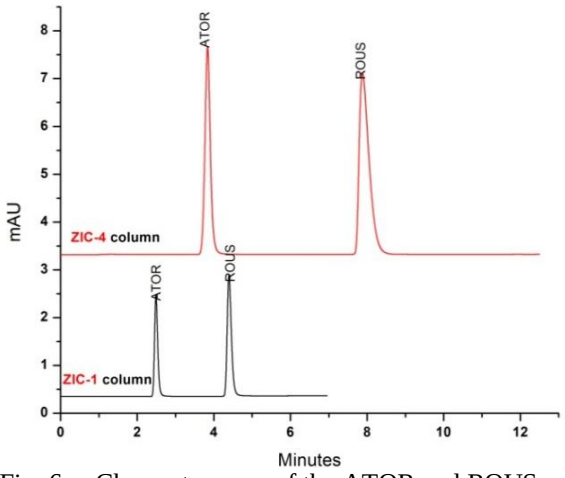

Fig. 6: Chromatograms of the ATOR and ROUS using ZIC-1 and ZIC-4 stationary phases.

Validation of the methods

ATOR and ROUS calibration graphs (Fig. 7) were formed by plotting the peak area opposite the ATOR and ROUS concentration using two ZIC-1 and ZIC-4 stationary phases. To ICH [34], the methods criteria were evaluated regarding their linearity, precision, accuracy, repeatability, and specificity. The validation results (Table-1 and 2) show that the methods are specific, linear, precise and exact.

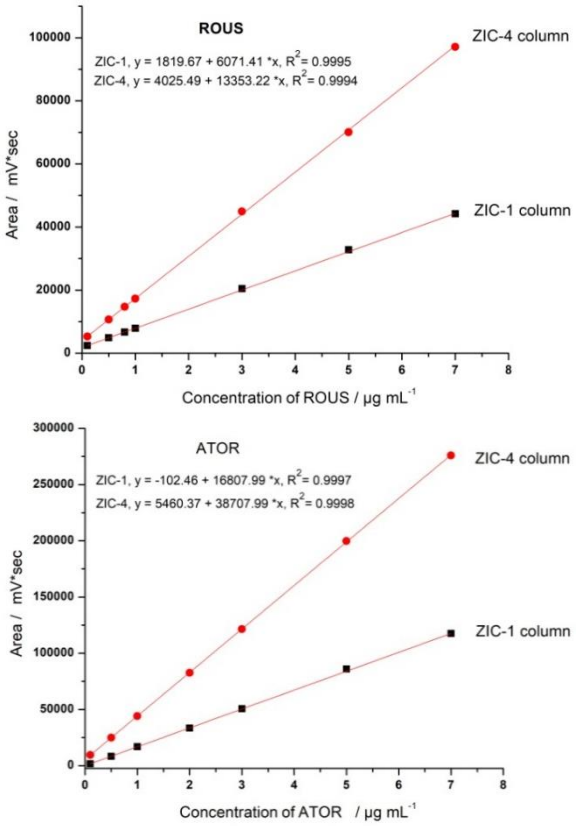

Fig. 7: Calibration graphs for ATOR and ROUS using ZIC-1 and ZIC-4 stationary phases.

Determination of ATOR and ROUS in pharmaceutical dosage forms

The proposed methods were successfully used to determine ATOR and ROUS in three and four pharmaceutical dosage types, respectively; the findings are summarized in Table-3. To test the ZIC-1 and ZIC-4 methods competence and performance, compared these results to those obtained using the standard method [44]. Statistical tests were conducted using the t-test and variance ratio F-test (Table 4), each with a 95% confidence limit. The determined t and F values did not surpass the theoretical values, indicating that neither method substantially differs in the precision of ATOR and ROUS determination in pharmaceutical dosage types.

Table-1: Validation parameters to evaluate the ZIC-HILIC proposed methods.

	Stationary phase			
	ZIC-1	ZIC-4	ZIC-1	ZIC-4
Parameter	**ATOR**		**ROUS**	
Concentration range (µg mL^{-1})	**0.1-7.0**	**0.1-7.0**	**0.1-7.0**	**0.1-7.0**
Coefficient of determination (R^2)	**0.9997**	**0.9998**	**0.9994**	**0.9995**
Limit of detection (LOD) (µg mL^{-1})	**0.0094**	**0.0081**	**0.0020**	**0.0013**
Limit of quantification (LOQ) (µg mL^{-1})	**0.0284**	**0.0245**	**0.0060**	**0.0039**

Table-2: Precision and accuracy of the developed ZIC-HILIC proposed methods

Same-Day Analysis (n=5)				Day-to-Day Analysis (n=5)		
ZIC-1 stationary phase						
ATOR Added (µg mL^{-1})	**ATOR Obtained (µg mL^{-1})**	**Recovery %**	**RSD (%)**	**ATOR Obtained (µg mL^{-1})**	**Recovery (%)**	**RSD (%)**
1.00	**0.995**	**99.50**	**0.56**	**0.994**	**99.40**	**0.93**
2.00	**1.980**	**99.00**	**0.12**	**1.98**	**99.00**	**0.13**
ROUS Added (µg mL^{-1})	**ROUS Obtained (µg mL^{-1})**	**Recovery %**	**RSD (%)**	**ROUS Obtained (µg mL^{-1})**	**Recovery %**	**RSD (%)**
1.00	**1.01**	**101.00**	**0.63**	**1.00**	**100.00**	**0.74**
2.00	**2.02**	**101.00**	**0.78**	**2.01**	**100.50**	**0.84**
ZIC-4 stationary phase						
ATOR Added (µg mL^{-1})	**ATOR Obtained (µg mL^{-1})**	**Recovery %**	**RSD (%)**	**ATOR Obtained (µg mL^{-1})**	**Recovery (%)**	**RSD (%)**
1.00	**0.993**	**99.70**	**0.48**	**0.993**	**99.30**	**0.35**
2.00	**1.986**	**99.30**	**0.64**	**1.988**	**99.90**	**0.28**
ROUS Added (µg mL^{-1})	**ROUS Obtained (µg mL^{-1})**	**Recovery %**	**RSD (%)**	**ROUS Obtained (µg mL^{-1})**	**Recovery %**	**RSD (%)**
1.00	**1.00**	**100.00**	**0.55**	**1.00**	**100.00**	**0.91**
2.00	**2.01**	**100.5**	**0.65**	**2.01**	**100.5**	**0.14**

Table-3: Appliance in tablets medical samples of two proposed methods for the determination of ATOR and ROUS.

Trade Name	Started conc. (mg)	Get it (mg)	%Rec.	%RSD n=5
	ZIC-1 stationary phase			
ATOR	**20**	**19.91**	**99.55**	**0.32**
Atorvastatin				
Lipitor	**20**	**20.07**	**100.35**	**0.64**
Siros	**10**	**9.97**	**99.70**	**0.40**
ROUS				
Rosuvastatin Denk	**10**	**10.08**	**100.80**	**0.51**
CRESTOR	**10**	**9.99**	**99.80**	**1.16**
Lodal	**10**	**9.93**	**99.30**	**0.26**
	ZIC-4 stationary phase			
	20	**19.93**	**99.65**	**0.43**
	20	**20.04**	**100.20**	**0.56**
	10	**9.93**	**99.30**	**0.28**
	10	**10.12**	**101.20**	**0.34**
	10	**10.00**	**100.00**	**1.19**
	10	**9.95**	**99.50**	**0.66**

Table-4: The comparison of the proposed methods ZIC-1 and ZIC-4 with the standard method [44] for ATOR and ROUS Analysis by investigating t- and F-statistical tests.

Name of drug	ZIC-1 method	ZIC-4 method	Standard method	t-Test (theor.)	F-Test (theor.)
ATOR	**99.55**	**99.65**	**99.85**	**0.76701* (2.7764)**	**1.0038* (19.000)**
Atorvastatin					
Lipitor	**100.35**	**100.20**	**100.45**	**0.5086** (2.7764)**	**0.46670** (19.000)**
Siros	**99.70**	**99.30**	**99.63**		
ROUS	**100.80**	**101.20**	**99.95**	**0.6007^{*n} (2.7764)**	**3.9619* (19.000)**
Rosuvastatin Denk					
CRESTOR	**99.80**	**100.00**	**100.68**	**0.9818** (2.7764)**	**5.1845** (19.000)**
Lodal	**99.30**	**99.50**	**100.11**		

* For ZIC-1 proposed method
** For ZIC-4 proposed method

Conclusion

This article describes two ZIC-HILIC methods with UV-detection for the simultaneous determination of ATOR and ROUS in pharmaceutical preparations. The two ZIC-HILIC columns (ZIC-1 and ZIC-4) with various spacer lengths between the charged groups were used as flexible separation tools due to their ability to activate at mixed retention modes by varying the mobile phase conditions. It is worth noting that ATOR and ROUS show higher retention times and low detection and limit of quantification

with the ZIC-4 column than the ZIC-1 column. The geometrical alignment of the ZIC-4 column is probably responsible for this. The results obtained from the experimental data of the retention mechanism had demonstrated mixed-mode retention RP and anion exchange mechanism for ATOR and ROUS.

Disclosure statement

The authors reported no potential conflict of interest.

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