



## RESEARCH ARTICLE

# Development of a validated analytical method for the estimation of sodium fusidate in pharmaceutical dosage form by RP-HPLC

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### ABSTRACT

**Introduction:** The determination of sodium fusidate in pharmaceutical dose form was accomplished using a straightforward reverse-phase (RP) high-pressure liquid chromatography (HPLC) approach. The separation through chromatography was accomplished by isocratic elution, on Waters e2695 HPLC system with Empower-3 software and 2998 module Photo Diode Array detectors configured with a pump that delivers quaternary solvents, an automated sample injector, and a column thermostat. A validated RP-HPLC method that is reproducible, rapid, simple, precise, rugged, economical, and accurate has been developed. **Materials and Methods:** Separation succeeded using a Thermo Scientific Hypersil ODS (C18) Column (150 × 4.6 mm i.d. and 5 μm particle size), and methanol: 1% V/V glacial acetic acid: acetonitrile used as mobile phase in ratio of 10:30:60% v/v/v, respectively. Ultraviolet detector was used to detect the separation at a wavelength of 235 nm with 1.1 mL/min of flow rate and volume of injection of 20 μL was maintained during the elution with 10-min run time. **Results:** It was estimated that the retention time was 5.597 min with a correlation coefficient of 0.999, the anticipated separation was found to be linear in the concentration range of 25–150 mcg/mL and it was found out that according to ICH regulations, every validation parameter fulfilled the acceptance criteria. **Conclusion:** Validation of the chromatographic method was done for linearity, specificity, accuracy, precision, robustness, system suitability, limit of detection, and limit of quantification study that it can be utilized for sodium fusidate routine analysis in accordance with ICH recommendations.

**KEY WORDS:** Isocratic elution, Method validation, Reverse-phase high-pressure liquid chromatography, Sodium fusidate

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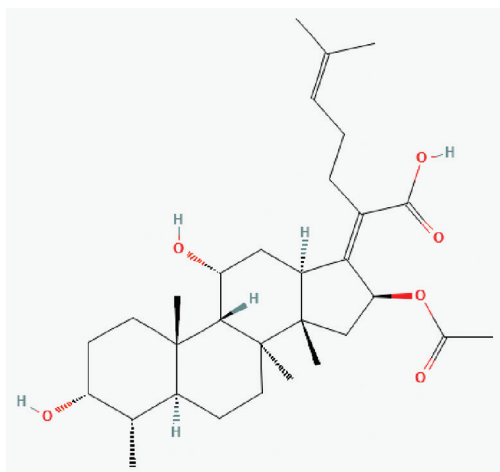
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## INTRODUCTION

Fusidic acid (FA) or the sodium salt of FA, that is, sodium fusidate (SF), an oral antistaphylococcal antibiotic with the chemical formula (3 $\alpha$ ,4 $\alpha$ ,8 $\alpha$ ,9 $\beta$ ,11 $\alpha$ ,13 $\alpha$ ,14 $\beta$ ,16 $\beta$ ,17Z)-16-Acetyloxy-3,11-dihydroxy-29-nordammara-17(20),24-dien-21-oic acid,<sup>[1]</sup> as shown in Figure 1, possesses bacteriostatic as well as bactericidal action at higher dose.<sup>[2]</sup> It belongs to the member of fusidane group having steroidal moiety but no corticosteroid-like actions.<sup>[3]</sup> FA was first identified in fungi, *Fusidium coccineum* in 1960,<sup>[4]</sup> exert antimicrobial action and specifically target Gram-positive bacteria like *Staphylococcus* spp., especially the Methicillin-resistant *Staphylococcus aureus* and some anaerobic strains of *Moraxella catarrhalis*, *Bordetella pertussis*, *Neisseria* spp., etc. It is also used to treat mild-to-moderate skin infections such as impetigo, erythrasma, folliculitis, abscesses, furunculosis, boils, atopic dermatitis, and infected traumatic wounds.<sup>[3]</sup> FA interacts with elongation factor G (EF-G) to inhibit bacterial protein synthesis that facilitates the peptide bond formation during protein synthesis followed by the translocation of amino acids to the ribosome. In conjunction with inactive or active guanosine triphosphate (GTP), when FA binds to EF-G in the ribosome, EF-G's GTPase activity is inhibited, inhibiting further elongation and thus showing antimicrobial effect. The drug has a weak acidic pKa of 5.7 and at a healthy pH of 7.4 is primarily ionized in plasma and tissue. It is a white powder with mild hygroscopic effect, insoluble in ether, water, and hexane, and readily soluble in solvent such as acetone, ethanol, chloroform, dioxane, pyridine, and acetonitrile.<sup>[5]</sup>

Literature review described numerous for the assessment of FA alone or in combination with other medications in pharmaceutical dose forms, analytical methods have previously been devised, plasma samples, etc. Spectrophotometry is one of the most frequently employed analytical technique for determining substances.<sup>[5]</sup> First derivative ultraviolet spectrophotometry was used to



**Figure 1:** Chemical structure of fusidic acid

evaluate the number of capsules and creams containing SF and FA.<sup>[6]</sup> To recognize these two medications in pills and liquids, different spectrophotometric research was developed.<sup>[1]</sup> This study uses identification or colorimetric processes that take place in the visible absorption range. In addition, the regulation of FA biosynthesis, separation, and purification has been achieved using spectrophotometric absorption in the visible range.<sup>[7]</sup> The real-time assessment of butyl hydroxyanisole, FA, and m,p-hydroxybenzoates in topical preparations, thin-layer chromatography with densitometric detection, has demonstrated the effectiveness of some products, such as ointments.<sup>[8-12,17]</sup> High-pressure liquid chromatography (HPLC) has been used in both blood samples and dosage forms as one of the widely used liquid chromatographic techniques; analysis of FA is well-documented in the literature. The study of FA complexes including – and – cyclodextrins has also been reported to employ nuclear magnetic resonance methods.<sup>[9-15]</sup>

Based on the literature review, it has been found out that while there are many analytical methods for the quantification of FA, the demand for a well-established stability-indicating approach for FA estimation in dosage forms is still unfulfilled as the reported methods faced one or more practical difficulties related to longer retention time and complex mobile phase compositions. For the simultaneous measurement of FA in pharmaceutical dose form, it was crucial to develop a selective, single run reverse phase (RP)-HPLC approach that would save both time and resources in comparison to the limited methods that already existed. The present study's objective is to create a RP HPLC method for the scrutiny of FA that is reproducible, rapid, accurate, reliable, simple, and sensitive with isocratic elution in a single run with good recovery and precision. In accordance with ICH requirements, the method was also validated.

## MATERIALS AND METHODS

### Reagent and chemicals

In this study, various gifted laboratory batch samples of Fucidin (250 mg) tablets were provided by GNH India Pharmaceutical limited (Mumbai, India). Reference standard (99.92%) of sodium fusidate was purchased from Joshi Agrochem Pharma Pvt Ltd., (Mumbai, India). The solvents, that is, acetonitrile and methanol of HPLC grade were employed in this study and gotten from Qualigens Pharma Private Limited, (Mumbai, India). Whereas, ammonium acetate and glacial acetic acid (analytical grade) were procured from Sisco Research Laboratories Pvt., Ltd. (Hyderabad, India).

### Instrumentation

The study was conducted using a Waters e2695 HPLC system with Empower-3 software and 2998 module Photo

Diode Array detectors configured with a pump that delivers quaternary solvents, an automated sample injector, and a column thermostat. Other instruments such as digital Ph meter (Mettler Toledo), electronic analytical balance (Mettler Toledo), ultrasonic bath (Analab), and hot air oven (Sigma scientific Products) were also incorporated.

### Chromatographic conditions

For analytical separation and quantitation, the HPLC column used was Thermo Scientific Hypersil ODS (C18) Column (Length 150 mm, 4.6 mm ID, 5  $\mu$ m particle size) and a suitable guard-column to protect the analytical column from strongly bonded material. The mobile phase comprises methanol: 1% V/V glacial acetic acid: acetonitrile in the ratio of (10:30:60% v/v/v) and the pH was adjusted to  $3.5 \pm 0.05$  with ammonium acetate. After being sonicated for 10 min for degassing, a 0.45-micron membrane filter was used to filter the mobile phase. Eluents were observed at 235 nm while the mobile phase was kept at a consistent flow rate at 1.1 mL/min for isocratic elution. The volume of the injection was 20  $\mu$ L. All determinations were carried out 10 min of operation at 30°C. Table 1 displays the ideal chromatographic conditions.

### Preparation of stock and working standard solution

Typical stock solution for the drug having concentration 1000  $\mu$ g/mL were prepared by accurately weighing and dissolving 100 mg of pure sodium fusidate in mobile phase (upto  $\frac{3}{4}$ <sup>th</sup> volume) in a very clean, 100 mL volumetric flask, ultrasonicated until completely dissolved followed by adding mobile phase to bring the total volume up to 100 mL. A second dilution was created by withdrawing 1 mL of the standard stock solution and to obtain working standard solutions of 100 g/mL, the volume is increased to the required level with mobile phase and transferred into 10-mL volumetric flasks. These solutions are then refrigerated. The solution was run through a 0.45 mm diameter membrane filter before being poured into vials for chromatographic analysis.

**Table 1: Optimized chromatographic conditions for sodium fusidate**

Parameters	Conditions
Stationary phase (column)	C18 (15 cm $\times$ 4.6 mm, 5 $\mu$ m)
Mobile phase	Methanol: Glacial acetic acid: Acetonitrile (10:30:60% v/v), pH adjusted to $3.5 \pm 0.05$
Flow rate (mL/min)	1.1
Run time (min)	10
Temperature of column (°C)	30
Volume of injection loop ( $\mu$ L)	20
Detection wavelength (nm)	235
Retention time (min)	5.597

### Preparation of calibration curve

A 10 mL volumetric flask was filled with aliquots of the standard stock solutions, which were then diluted with mobile phase to the appropriate concentration. Thus, the final drug concentrations ranged from 25 g/mL to 150 g/mL. The produced solutions were filtered through a 0.45 membrane filter, and 20 L triplicate injections were made for each dilution before being analyzed by a chromatograph under the previously mentioned conditions. Peak regions were reported after the medication was evaluated. The peak area on the Y-axis was plotted against the corresponding concentration of the drug on the X-axis to create the calibration curve for sodium fusidate. Using its coefficient of determination ( $R^2$ ), the calibration curve was assessed.

### Preparation of sample solution

Twenty tablets were carefully and neatly pulverized into weight. Once carefully weighed, in a volumetric flask with a 100 mL capacity, a portion of tablet powder containing 100 mg of sodium fusidate was added. To the volumetric flask, sufficient quantity of mobile phase was then added, and it was ultrasonically shaken for 10–15 min. The volume was then properly diluted and made up to the required level. To ensure there were no particles present, a 0.45 filter was then used to filter the solution. After that, 10 mL of mobile phase was added to the volumetric flask to create a solution with a concentration of 100 g/mL by adding 1 mL of the filtrate.

### Method validation

The method was validated in accordance with the ICH guideline Q2 (R1) and includes studies on linearity, specificity, accuracy, precision, robustness, system appropriateness, limit of detection (LOD), and limit of quantification (LOQ).

### Linearity and range

A method is considered linear if the results of the test are directly proportional to the amount of the analyte in the sample. Five different concentrations generated in the range of 25 mcg/mL to 150 mcg/mL, respectively, were used to inject triplicates ( $n = 3$ ) of the standard dilution to determine the linearity range. The calibration curve was plotted with the peak area of the analyte versus the analyte concentration, and the linear correlation was assessed using the least square approach with the aid of the Microsoft Excel® program to confirm the linearity of the analytical technique being developed.

### Accuracy

The degree of agreement between actual data and the mean analytical value is what is referred to as an analytical

method's accuracy. Recovery studies using the usual addition method were used to determine the approach's accuracy. By adding a known quantity of sodium fusidate standard to a prequantified sample solution, the accuracy was assessed by injecting the sodium fusidate 3 times at concentrations that were 50%, 100%, and 150% of the active ingredient, respectively. For each concentration, the relative standard deviation (%RSD) and percentage recovery were calculated.

### Precision

When a procedure is used repeatedly on numerous homogenous sample samples, its precision is measured by how well individual test findings agree with one another. The %RSD values obtained were used to evaluate the analytical method's precision value.

The intraday variability was measured using the same methods on three distinct days, whereas the interday variability was calculated using three injections of the standard solution at three different concentrations (50%, 100%, and 150%, respectively).

### Specificity

The established method's specificity refers to its ability to identify the analyte even when other components could be anticipated to exist. These usually involve degradants, matrix, and impurities.

### Robustness

The ability of an analytical method to remain unaffected by deliberate but minute modifications to the technique parameters is known as resilience and provides insight into its dependability in normal circumstances by adjusting the chromatographic parameters, such as the flow rate, slightly, detector wavelength, and column temperature. The robustness of the procedure was evaluated. The %RSD was determined in this study.

### System suitability

The chromatographic system's appropriateness is confirmed by the repeatability and accuracy which are appropriate for routine analysis, making it an important step in chromatographic analysis. By injecting 6 times the usual sodium fusidate solution into the RP-HPLC apparatus, the study's goal was to identify the crucial characteristics, such as retention duration, peak area, and a tailing factor (TF).

### LOD and quantification

The term "LOD" refers to the lowest concentration that a technology is capable of detecting, although it is not necessarily required to quantify this concentration to an

exact quantity. The LOQ is the lowest concentration that might be accurately and precisely determined in a reliable manner.

The formulas  $LOD = 3.3 \sigma/S$  and  $LOQ = 10 \sigma/S$  were used to determine the limits of detection and quantification utilizing a technique based on the calibration plot's slope (S) and standard deviation ( $\sigma$ ).

## RESULTS AND DISCUSSION

### Method development and optimization of chromatographic separation

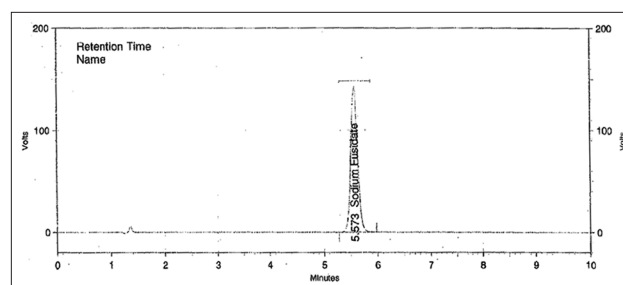
To adjust the separation of sodium fusidate in pharmaceutical dosage form, many chromatographic techniques were taken into consideration. According to the literature, RP C18 stationary phase with specifications of  $150 \times 4.6$  mm i.d. and  $5 \mu\text{m}$  particle size should be employed.

As a result, the Inertsil C18 column was used to develop a chromatographic separation process. To construct and develop a technique that would produce ideal separation, the mobile phase composition was carefully studied. After a number of trials, the mobile phase composition of methanol: 1% V/V glacial acetic acid: acetonitrile in the ratio of (10:30:60% v/v) obtained. Ammonium acetate was used to appropriately adjust pH to  $3.5 \pm 0.05$  to ensure and achieve desired separation. The elution was conducted in isocratic mode with a flow rate of 1.1 mL/min for a 10-min runtime, and the detector was set at 235 nm. It was discovered that sodium fusidate has a retention duration of 5.597 min, as depicted in Figure 2.

### Method validation

#### Linearity

After plotting the analytical calibration curve for sodium fusidate, it was discovered to be linear in the specified ranges (10–50 mcg/mL), indicating a correlation coefficient  $R^2$  of 0.999 (acceptance limit  $>0.98$ ). The intercept of the straight line was found to be 80001, and its slope was discovered to be 22356. Table 2 displays results, and Figure 3 shows the calibration curve.



**Figure 2:** Typical high-pressure liquid chromatography chromatogram of sodium fusidate



### Accuracy

The method's accuracy was determined by recovery study. According to mean recovery data, the results showed that the approach for identifying the analyzed pharmaceuticals had excellent accuracy. All experimental findings are within the acceptable accuracy range (98.0–102.0%). The % RSD results, which are 0.36%, 0.34%, and 0.08% at three different concentrations, are likewise within the acceptable limit of 2%. Table 3 presents results. In Figures 4-6, the accuracy at three levels was displayed.

### Precision

Greater than 50–150% the amount of the typical medicine, the approach was proven to be highly precise. In both instances, the % RSD readings were found to be <2%. According to Table 4, the % RSD ranges for intraday variability and interday variability, respectively, were 0.32–0.78% and 0.35–0.81%, respectively. This resulted in the conclusion that the procedure is precise enough

to identify the substance and that the variability was determined to be negligible.

### Averages of three determinations

#### Specificity

The chromatogram showed no interference of peaks, proving that excipients employed in tablet formulation did not affect the estimated amount of the drug by the suggested method for determining sodium fusidate in pharmaceutical dosage forms. Thus, the technique is particular and summarized in Table 5.

#### Robustness

The robustness for determining sodium fusidate was carried out by intentional alteration of system suitability parameters such as the flow rate ( $\pm 0.2$  mL), wavelength ( $\pm 2$  nm), and column temperature ( $\pm 5^\circ\text{C}$ ). It was noted that the chromatograms did not show any significant variations, proving the robustness of the established RP-HPLC method. The results are displayed in Table 6.

#### System suitability

The most important variable in chromatographic analysis is system suitability. With a TF of not more than 1.01 in each peak and a retention period of 5.597 min for sodium fusidate, excellent peak symmetry was observed. 8746 theoretical plates were discovered. The outcomes attained with six replicate injections of the standard solution are summarized in Table 7.

#### LOD and LOQ

The value of LOD and LOQ value of sodium fusidate was 0.35 and 0.48  $\mu\text{g/mL}$ , respectively. These findings demonstrate the suggested method's excellent tendency to identify the drug's pharmaceutical dose form's lowest concentration at the same time.

**Table 2:** Linear regression equations generated from validation of sodium fusidate: Slope, intercept, and coefficient of determination

Concentration (mcg/mL)	Standard area
Concentration (mcg/mL)	Peak area
25	5640663
50	11281325
75	16921988
100	22562650
125	27603310
150	33843973
Correlation coefficient ( $R^2$ )	0.999
Intercept	80001
Slope	22356

**Table 3:** Accuracy observation table of sodium fusidate by RP-HPLC method

Recovery level	Amount of std. added (mg)	Amount recovered (mg)	Recovery%	Average recovery %	SD	RSD%
50%	57.5	57.26	99.59	99.50	0.36	0.36
	57.4	57.29	99.81			
	57.8	57.28	99.11			
100%	114.8	113.95	99.26	99.11	0.34	0.34
	114.7	113.95	99.35			
	115.4	113.93	98.73			
150%	167.1	165.59	99.10	99.02	0.08	0.08
	167.4	165.61	98.93			
	167.2	165.58	99.03			
			Overall average recovery %	99.21		
			Overall SD	0.33		
			Overall RSD%	0.34		

RP-HPLC: Reverse-phase high-pressure liquid chromatography, RSD%: Relative standard deviation, SD: Standard deviation

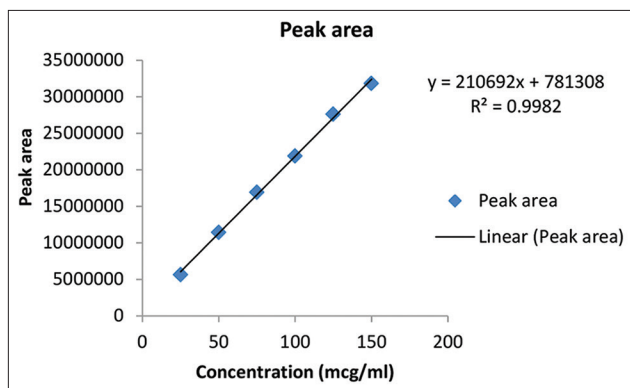


Figure 3: Calibration curve of sodium fusidate

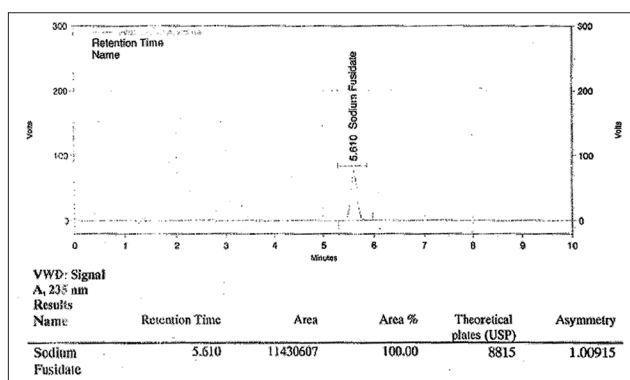


Figure 4: Accuracy at 50% level

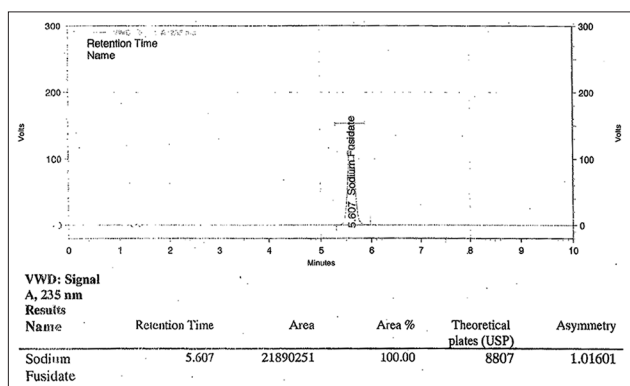


Figure 5: Accuracy at 100% level

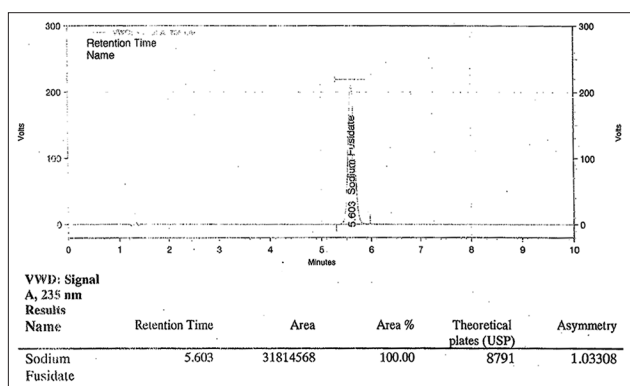


Figure 6: Accuracy at 150% level

Table 4: Intra- and interday precision for sodium fusidate in pharmaceutical dosage forms by the proposed RP-HPLC method

Concentration (mcg/mL)	Intraday variation		Interday variation	
	Area mean	%RSD	Area mean	%RSD
50	10946654	0.78	10906553	0.81
100	22502649	0.51	22492551	0.54
150	33853971	0.32	33783870	0.35

RP-HPLC: Reverse-phase high-pressure liquid chromatography, RSD%: Relative standard deviation

Table 5: Specificity of sodium fusidate for RP-HPLC method

S. No.	Name	Retention time
1.	Blank	----
2.	Placebo	-----
3.	Sodium fusidate in standard	5.580
4.	Sodium fusidate in test	5.597

RP-HPLC: Reverse-phase high-pressure liquid chromatography

Table 6: Robustness data of sodium fusidate for RP-HPLC method

Parameters	Variation	%RSD
Flow rate	0.9 mL	0.80
	1.3 mL	0.51
	1.7 mL	0.51
Detector wavelength	233 nm	0.09
	237 nm	0.10
Column temperature	25°C	0.99
	35°C	0.57

RP-HPLC: Reverse-phase high-pressure liquid chromatography, RSD%: Relative standard deviation

Table 7: System suitability of sodium fusidate for RP-HPLC method

Parameters	Values
Theoretical plates	8746
Retention time	5.597 min
Asymmetry	1.01
%RSD	0.24

RP-HPLC: Reverse-phase high-pressure liquid chromatography, RSD%: Relative standard deviation

## DISCUSSION

Since sodium fusidate is a brand-new drug, there are merely any methods available to determine its dosage in pharmaceutical form. Our present objective was to establish an innovative, suitable, and reliable method for the RP-HPLC evaluation of sodium fusidate in pharmaceutical dosage form. The newly created method

was validated in accordance with ICH recommendations for system linearity, accuracy, precision, robustness, system applicability, LOD, and LOQ criteria, and it was determined to be satisfactory. The percent recovery was discovered to be between 100 and 2, and the percent RSD value was below 2.

## CONCLUSION

For the determination of sodium fusidate from a pharmaceutical dosage form, the proposed approach was found to be simple, precise, accurate, and rapid. The method's validity was checked for a number of characteristics, including specificity, linearity, accuracy, precision, robustness, and system suitability; the values were all found to be within acceptable limits. ICH guidelines were followed in the method's validation. The approach has substantial advantages compared to previous reported data in terms of lesser retention time, selectivity, and accuracy. According to the validation study, the approach can be used for routine quality control and determination of sodium fusidate in pharmaceutical dosage form.

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## CONFLICTS OF INTEREST

No conflicts of Interest.

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