

## Development and validation of RP-HPLC method for estimation of Torsemide and Spironolactone in bulk and pharmaceutical dosage forms: a quality by design approach

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**Abstract:** In a Quality by Design (QbD) approach, the impact and interaction of critical variables are understood and optimized using Design of Experiment (DoE). DoE includes statistical multivariate analysis and modeling of data for continuous improvement of the method. In the present research work, 24 full factorial designs were applied to optimize and select appropriate chromatographic conditions for the RP-HPLC method for the development of Torsemide (TOR) and Spironolactone (SPI) in synthetic mixtures. The drug was analyzed using a LiChrospher® C 18 (5µm, 250×4 mm) and a mobile phase of acetonitrile: buffer (57.4:42.6 v/v) at a flow rate of 1 mL/min, with TOR showing considerable absorbance at 290 nm and SPI showing significant absorbance at 238 nm. For TOR and SPI, the technique was shown to be linear over the concentration range of 2–12 µg/mL and 10–60 µg/mL, respectively. LOD and LOQ values for TOR were 0.10 g/mL and 0.32 g/mL, respectively, whereas those for SPI were 0.75 µg/mL and 2.29 µg/mL. The correlation coefficient (R<sup>2</sup>) was found to be 0.9973 for TOR and 0.9976 for SPI. The recovery study for accuracy was found in the range of 97.55%–99.05% and 94.82%–100% for TOR and SPI, respectively.

**Keywords:** Torsemide; spironolactone; RP-HPLC; validation; QbD; Measurement uncertainty. © 2023 ACG Publications. All rights reserved.

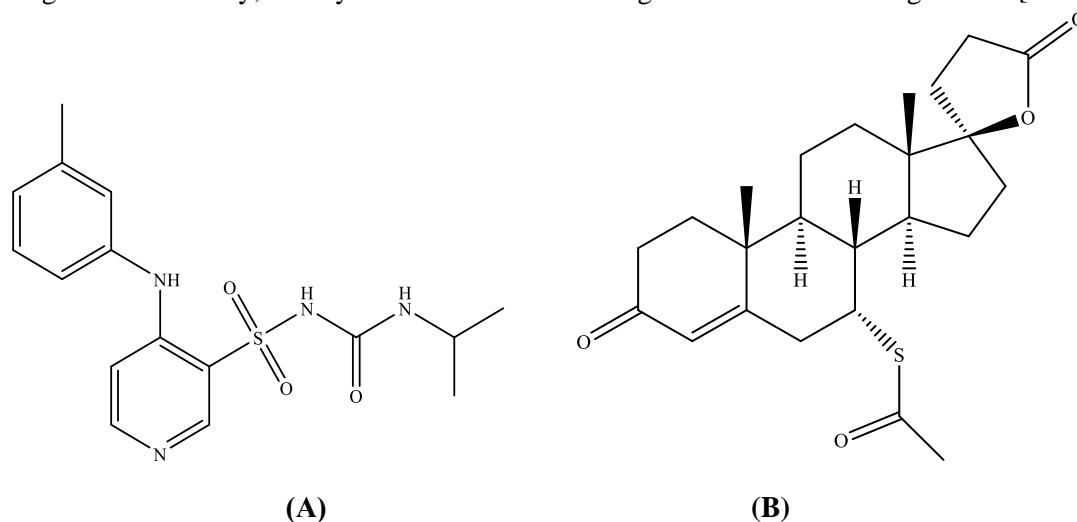
### 1. Introduction

In pharmaceutical research, QbD is a modern approach for assessment of product quality, and QbD is a cutting-edge strategy for monitoring product quality and fostering ongoing advancement in the pharmaceutical industry. Companies receiving warning letters from the United States Food and Drug Administration (USFDA) [1–4] said that quality issues were a primary focus of their research efforts. QbD specialist Joseph M. Juran has integrated the concept of conventional statistical practice into contemporary quality management. [3] Quality cannot be tested in products; therefore, quality should be built into design, as stated in the International Council for Harmonization (ICH) Q8 standard. According to ICHQ8, Quality by Design (QbD) is a methodical strategy for developing pharmaceuticals that places an emphasis on prioritizing product knowledge, process control, and quality risk management. When it comes to the development of products, Analytical Quality by Design (AQbD) is the standard method.

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Due to its importance in both the initial stages of drug creation and in final quality assurance [7–10], analytical method development is a pivotal factor in the pharmaceutical product lifecycle. The industry-standard procedure for developing new analytical methods involves slowly and carefully changing One-Factor-at-a-Time (OFAT) [11, 12]. As AQbD was less explored by researchers and it was believed that the results obtained by the application of AQbD were inaccurate and misleading, it has different components such as the Analytical Target Profile (ATP), risk management, screening design, optimization method, Design of Experiment (DoE), design space, validation, continuous method monitoring, etc. This has led researchers to focus on improving analytical methods by employing QbD and DoE [13, 14]. Similar to how QbD aids in product development, the process of creating and validating an analytical method using QbD is useful in the creation of high-quality analytical data for the pharmaceutical industry. DoE is the part of the AQbD concept that aims to evidence the interaction between factors affecting process development and the output of the process and to find the best-fit region that fulfills the target requirement for Critical Method Attributes (CMA) [15].

Torsemide (TOR) is chemically N- [(isopropyl amino) carbonyl]. -4-[(3methylphenyl) amino] pyridine-3-sulfonamide (Figure 1A). Torsemide, a loop diuretic, works within the lumen of the thick ascending portion of the loop of Henle, where it inhibits the Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> carrier system. Chemically, Spironolactone (SPI) is S- [7R, 8R, 9S, 10R, 13S, 14S, 17R]-10,13-Dimethyl-3,5'-dioxospiro [2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthrene-17,2'-oxalone]. -7yl] ethanethioate (Figure 1B). It binds to this mineralocorticoid receptor (MR), blocking the actions of aldosterone on gene expression. A safe, accurate, and reliable procedure has to be incorporated to detect the concentration of these drugs simultaneously, as they are available in both single and combined dosage forms [16–20].



**Figure 1.** Chemical structures of torsemide (A) and spironolactone (B)

A review of the literature revealed that various methods, including spectrophotometry [21], HPLC [22–23], HPTLC [24–25], and LC-ESI-MS [26], have been established for measuring TOR in biological fluids and pharmaceutical dosage forms, or in both mixed and single dosage forms. A review of the literature revealed that various methods, including spectrophotometric [27], HPLC [28–32], HPTLC [33–34], HPLC-APCI-MS [35], and UPLC [36], have been developed for measuring SPI in biological fluids and pharmaceutical dosage forms, or in both mixed and single dosage forms with other drugs. Additionally, a confirmed method for TOR and SPI and their tablet, which was available on the market, i.e., Dytor Plus 10, was selected for the study.

In order to determine the statistical significance of the difference between the measurement and a relevant reference value, it is necessary to estimate the uncertainty associated with the measurement. The uncertainty in each individual stage of an analytical technique is due in part to the uncertainty in the measurements used. So, it's important to identify the causes and forms of uncertainty in each of these procedures. In order to calculate an estimation of the HPLC measurement uncertainty, the EURACHEM

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[37, 38] guideline was applied. The variability of each individual measurement was accounted for either by calculating the standard deviation of replicates or by analyzing the data from the calibration certificate.

The purpose of this research is to develop and verify a quality-by-design-based RP-HPLC method for simultaneous determination of TOR and SPI in pharmaceutical dosage form. The purpose of this research was to develop a sensitive, specific, and reliable HPLC test method for measuring TOR and SPI in both bulk and tablet dosage forms. Since the FDA first instituted quality by design, it has been an essential concept for healthcare organizations (USFDA). The focus of this work is on generating the design space for simultaneous estimation of TOR and SPI in bulk and tablet dosage form, as well as developing and validating a stability indicating HPLC method.

## 2. Experimental

### 2.1. Materials and Methods

Equipment used in the experiment: Ultra Sonicator Quantrex 140 was used for sonication. Chromatographic separation was performed using the HPLC: Ultimate 3000 ThermoScientific with a P2230 plus HPLC pump and UV-2230 plus UV-visible detector. Shimadzu Aux 220 was the analytical balance that was used to weigh the standard and sample. Torsemide (98.91% purity) and Spironolactone (99.11% purity) were obtained and gifted by Cipla Pharmaceuticals Ltd., Solan, India. The reagents and solvents, acetonitrile (99.9%), methanol (99.9%), and water (99.9%), were of HPLC grade and obtained from Sigma Aldrich, India.

### 2.2. Chromatographic Conditions

The analysis was performed using the LiChrospher® C<sup>18</sup> (5µm, 250×4 mm) column using a mobile phase made up of acetonitrile: buffer (57.4:42.6% v/v) was used at a flow rate of 1 mL/min. Using UV detection at 290 and 230 nm, respectively, TOR and SPI at room temperature, the eluent was observed. It used a 20-µL injection volume. There were 15 minutes in the runtime. Before usage, a Whatman filter paper no. 41 was used to filter the mobile phase.

### 2.3. Preparation of the Mobile Phase

3.9 g of ammonium acetate was weighed accurately by using analytical balance and it was transferred to a 1 L of beaker, and 1 mL of triethylamine was added on it. Then it was diluted to 1 L by using distilled water. The specific pH was adjusted (see Table 1) by using glacial acetic acid.

### 2.4. Preparation of Stock solution of TOR and SPI

Accurately weighed 25 mg of TOR and SPI were added to separate 25 mL volumetric flasks, and the remaining volume was diluted with mobile phase and the final concentrations were obtained as 1000 µg/mL for each standard. 1 mL of the final solutions were diluted to 10 mL in volumetric flasks using the mobile phase. The concentration of the solution was obtained as 100 µg/mL for each standard.

### 2.5. Determination of Wavelength

In the region of 200–400 nm, stock solutions of TOR (100 µg/mL) and SPI (100 µg/mL) in the mobile phase were scanned individually against a blank acetonitrile: ammonium acetate buffer. TOR exhibited significant absorbance at 290 nm, and SPI exhibited significant absorbance at 238 nm. Hence, the wavelengths were selected for the present study.

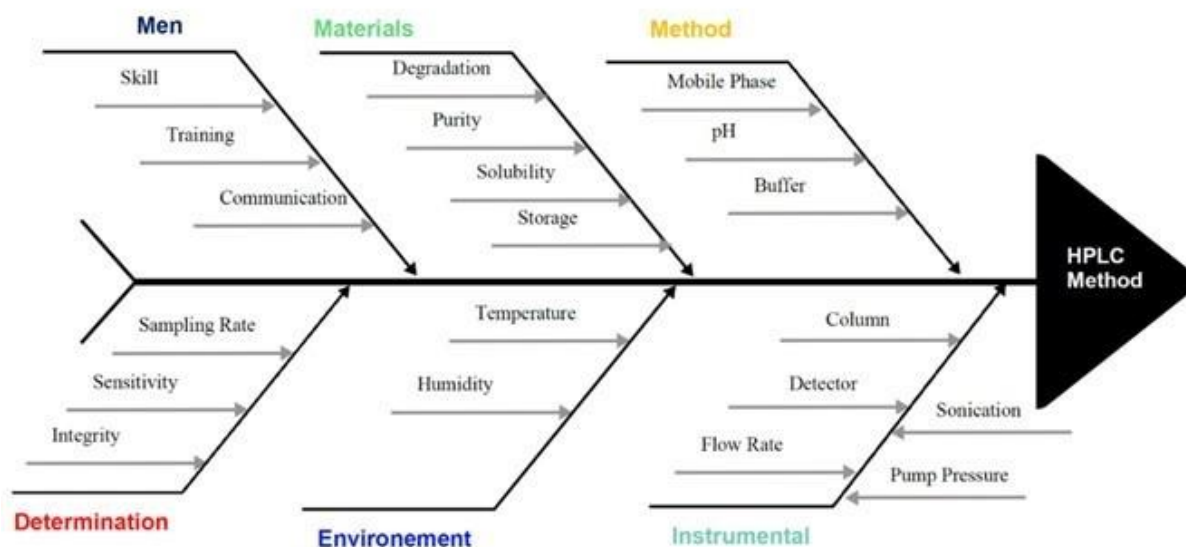
### 2.6. Preparation of Calibration Curve

The standard stock solution's aliquots (0.2, 0.4, 0.6, 0.8, 1, and 1.2 mL) were transferred to a series of 10-mL volumetric flasks and diluted with mobile phase to get 2, 4, 6, 8, 10, and 12  $\mu\text{g/mL}$  TOR respectively, until the desired concentration was reached.

The standard stock solution's aliquots (1, 2, 3, 4, 5, and 6 mL) were transferred to a series of 10-mL volumetric flasks and diluted with mobile phase to get 10, 20, 30, 40, 50, and 60  $\mu\text{g/mL}$  SPI respectively, until the desired concentration was reached.

### 2.7. Analysis of Synthetic Mixture

To produce a concentration of 100  $\mu\text{g/mL}$  TOR and SPI, 20 tablets (Dytor Plus 10) was crushed and average weight of crushed powder (1 tablet) which is equivalent to 10 mg of TOR and 50 mg of SPI was added to a 100mL volumetric flask and diluted up to the mark with mobile phase to obtain stock solution (100  $\mu\text{g/mL}$  of TOR and 500  $\mu\text{g/mL}$  of SPI). From this, 0.6 mL was taken out and diluted to a volume of 10 mL to produce TOR and SPI concentrations of 6  $\mu\text{g/mL}$  and 30  $\mu\text{g/mL}$ , respectively.



**Figure 2.** Fishbone diagram indicating analytical method development variables

### 2.8. QbD and DoE Approach

For the development of an RP-HPLC technique, the QbD technique was used to choose and optimize the chromatographic conditions. AQbD involves picking, evaluating, and improving different steps for sample preparation, chromatographic separation, detection, and drug quantification. It does this by using a number of Quality Risk Assessment (QRA) and management methods. To identify the best-fitting Critical Method Parameters (CMPs) and Critical Material Attributes (CMAs), the Ishikawa/fishbone (Causes and Effects) approach and DoE were used. The effective variable for method development was first identified using the fishbone diagram illustrated in Figure 2. After defining and identifying effective variables, the DoE approach was applied to optimize and understand the relationship between independent variables and dependent variables. A full factorial design was used to optimize the chromatographic conditions of the developed analytical method. For optimization, four independent variables and two dependent variables were selected. pH of the mobile phase (A), composition of the mobile phase (B), mobile phase flow rate (C), and temperature (D) were selected as independent variables,

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while retention time (RT) for TOR (Y1) and SPI (Y2) were selected as dependent variables. The experimental runs, levels, and values of the independent factors are indicated in Table 1.

**Table 1.** Experimental Runs to optimize chromatographic condition of HPLC

Experimental Runs	Independent Factor Coded Value			
	A	B	C	D
1	-1	-1	-1	-1
2	-1	-1	+1	-1
3	-1	-1	-1	+1
4	-1	-1	+1	+1
5	-1	+1	-1	-1
6	-1	+1	+1	-1
7	-1	+1	-1	+1
8	-1	+1	+1	+1
9	+1	-1	-1	-1
10	+1	-1	+1	-1
11	+1	-1	-1	+1
12	+1	-1	+1	+1
13	+1	+1	-1	-1
14	+1	+1	+1	-1
15	+1	+1	-1	+1
16	+1	+1	+1	+1

Levels of Independent Factors			
Independent Factor Name	Unit	-1	+1
A: pH of the mobile phase	-	4	5
B: Composition of mobile phase (Acetonitrile: Ammonium acetate Buffer)	%	40:60	60:40
C: Flow rate of mobile phase	mL/min	0.8	1.0
D: Temperature	°C	30	50

### 2.9. Method Validation

The method validation parameters of the method were determined as accuracy, linearity, stability, precision, specificity, limit of quantitation (LoQ), limit of detection (LoD), and robustness as described in ICH guidelines Q2 (R1) [36–40].

#### 2.9.1. Linearity

The linear concentration ranges were applied as of 2–12 µg/mL for TOR and 10–60 µg/mL for SPI. The calibration curve was constructed by plotting the graph of peak area vs. concentration (see Table 8).

#### 2.9.2. Precision and Repeatability

The instrument's precision was tested by scanning and measuring the absorbance of solutions ( $n = 6$ ) for TOR (4 µg/mL) and SPI (20 µg/mL) without modifying the parameter of the proposed method's parameter. The results were represented as a relative standard deviation (% RSD).

### 2.9.3. Intermediate Precision

The proposed method was tested for intra-day and inter-day precision by analyzing the corresponding responses three times on the same day and three times on different days for three different concentrations [41–43] over the calibration range of 2, 4, and 8 µg/mL and SPI (10, 20, and 30 µg/mL). The results were represented as a relative standard deviation (% RSD).

### 2.9.4. Accuracy

The accuracy of the method was determined by calculating the recovery of TOR and SPI by the standard addition method [44–46]. Known amounts of standard solutions of the percent recovery of the sample were calculated when TOR and SPI were added at 50, 100, and 150% levels to prequantified sample solutions of TOR and SPI (4 µg/mL and 20 µg/mL).

### 2.9.5. Limit of quantification and Limit of detection

LOD and LOQ were calculated using the standard deviation of response ( $\sigma$ ) and slope (S) of the calibration curve [44–47].

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where,  $\sigma$  = the response's standard deviation

S = slope of the calibration curve

### 2.9.6. Robustness

The analysis of the TOR and SPI sample data using purposeful modifications of the technique parameters allowed for the study of robustness. The change in the response of TOR and SPI was observed. The robustness of the method was investigated by altering the flow rate by 0.2 mL/min [48–54]. The change in the response of TOR and SPI was recorded and compared with the original one.

## 2.10. Studies on Forced Degradation

Stress testing must be done to clarify the inherent stability properties of the active chemical, according to the ICH guideline entitled Stability Testing of New Pharmacological Substances and Products. TOR and SPI were subjected to forced deterioration using 0.5 N HCl, 0.5 N NaOH, 3% H<sub>2</sub>O<sub>2</sub>, photolytic degradation, and water degradation.

### 2.10.1. Stress Degradation by Hydrolysis Under Acidic Media

For the stress degradation study, 25 mg SPI and TOR accurately weighed and added to 25 mL volumetric flask, added 1 mL of 0.5 N HCl, and kept for 24 hours. After 24 hours. Neutralize it with 0.5 N NaOH and make up the mobile phase up to 25 mL (1000 µg/mL). Withdraw 2.5 mL of this solution and dilute it with the mobile phase in 25 mL (100 µg/mL). Measure the peak area and find out the percent degradation.

### 2.10.2. Stress Degradation by Hydrolysis Under Alkaline Media

25 mg SPI and TOR accurately weighed and added to 25 mL volumetric flask, added 1 mL of 0.5 N NaOH, and kept it for 24 hours. After 24 hours. Neutralize it with 0.5 N HCl and make up the mobile phase up to 25 mL (1000 µg/mL). Withdraw 2.5 mL of this solution and dilute it with the mobile phase in 25 mL (100 µg/mL). Measure the peak area and find out the percent degradation.

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### 2.10.3. Oxidative Degradation

25 mg SPI and TOR accurately weighed and added to 25 mL volumetric flask, and added 1 mL of 3% H<sub>2</sub>O<sub>2</sub>. kept it for 24 hours. After 24 hours. This solution is made up of buffer and ACN up to 25 mL (1000 µg/mL). Withdraw 2.5 mL of this solution and dilute it with the mobile phase in 25 mL (100 µg/mL). Measure the peak area and find out the percent degradation.

### 2.10.4. Photolytic Degradation

25 mg SPI and TOR accurately weighed and added to 25 mL volumetric flask and put this solution in UV light and kept for 24 hrs. After 24 hrs. This solution made up with buffer/ACN up to 25 mL (1000µg/mL). Withdraw 2.5 mL of this solution and diluted with mobile phase in 25 mL (100µg/mL). Measure the peak area and find out % degradation.

### 2.10.5. Degradation by Water

25 mg SPI and TOR accurately weighed and added to 25 mL volumetric flask, added 1 mL of water, and kept it for 24 hours. After 24 hours. This solution is made up of buffer and ACN up to 25 mL (1000 µg/mL). Withdraw 2.5 mL of this solution and dilute it with the mobile phase in 25 mL (100 µg/mL). Measure the peak area and find out the percent degradation.

### 2.11. Measurement Uncertainty (MU)

Amount of sample mass ( $U_{Ms}$ ), mass of standard compounds ( $U_{Sm}$ ), volume ( $U_V$ ), purity of the standards ( $U_P$ ), calibration curve ( $U_C$ ), stock solution preparation ( $U_{Sprep}$ ), and repeatability ( $U_{Mr}$ ) were selected as parameters for the estimation of uncertainty budget of the applied method for both measurand. Thus, uncertainty budget of the method was estimated following equations as described in EURACEM CITAC Guides and published papers.

$$u_{Combined} = \chi \times \sqrt{U_{Ms}^2 + U_{Sm}^2 + U_V^2 + U_P^2 + U_C^2 + U_{Sprep}^2 + U_{Mr}^2} \quad (\text{Eq.1})$$

$$U = k \times u_{Combined} \quad (\text{Eq.2})$$

Where,  $U$  is the expanded uncertainty, it is a dimension representing the range of measurement results within which one can have some degree of confidence in the result and,  $k$  stands for the typical coverage factor, which is 2.

## 3. Results and discussion

### 3.1. Selection of Mobile Phase

Based on the literature review, solubility, and pKa of drugs, the C18 column was selected for the study. The mobile phase was allowed to saturate the column. To obtain the optimum separation of TOR and SPI, multiple mobile phases made up of methanol, acetonitrile, water, and ammonium acetate buffer were attempted in varied compositions at varying flow rates. The detection wavelength was set at 290 and 238 nm, which produced noticeably better detector responses for drugs. In terms of resolution and peak shape, the mixture of acetonitrile and ammonium acetate buffer (57.4:42.6% V/V) at 1.0 mL/min of flow rate at 30 °C outperformed the other mixes.

### 3.1. Optimization of Chromatographic Conditions for HPLC method

For optimization of effective variables, a 2<sup>4</sup> full factorial design was applied, and experimental runs were carried out. The results of various experimental runs are presented in Table 2.

**Table 2.** Results of Experimental Runs as per DoE

Sr. No.	pH	Composition of Mobile phase (Acetonitrile: Ammonium acetate Buffer)(%v/v)	Flow rate (mL/min)	Temp. (°C)	RT for TOR (min)	RT for SPI (min)
1	4	40:60	0.8	30	3.717	8.335
2	4	40:60	1.0	30	2.988	6.705
3	4	40:60	0.8	50	3.685	7.292
4	4	40:60	1.0	50	2.927	5.802
5	4	60:40	0.8	30	5.687	14.207
6	4	60:40	1.0	30	4.567	8.610
7	4	60:40	0.8	50	5.583	12.763
8	4	60:40	1.0	50	4.480	13.295
9	5	40:60	0.8	30	3.412	8.400
10	5	40:60	1.0	30	2.737	6.725
11	5	40:60	0.8	50	3.227	7.212
12	5	40:60	1.0	50	2.587	5.767
13	5	60:40	0.8	30	5.458	34.217
14	5	60:40	1.0	30	4.348	27.055
15	5	60:40	0.8	50	5.278	26.553
16	5	60:40	1.0	50	4.182	21.050

Outcome of Dependent Variable						
Dependent Variable	Name of Variable	Analysis	Minimum RT	Maximum RT	Mean	Standard Deviation (SD)
Y <sub>1</sub>	RT for TOR	Factorial	2.587	5.687	4.054	1.020
Y <sub>2</sub>	RT for SPI	Factorial	5.767	34.217	13.374	8.685

#### 3.1.1. Optimization of Retention time (RT) of TOR (Y<sub>1</sub>)

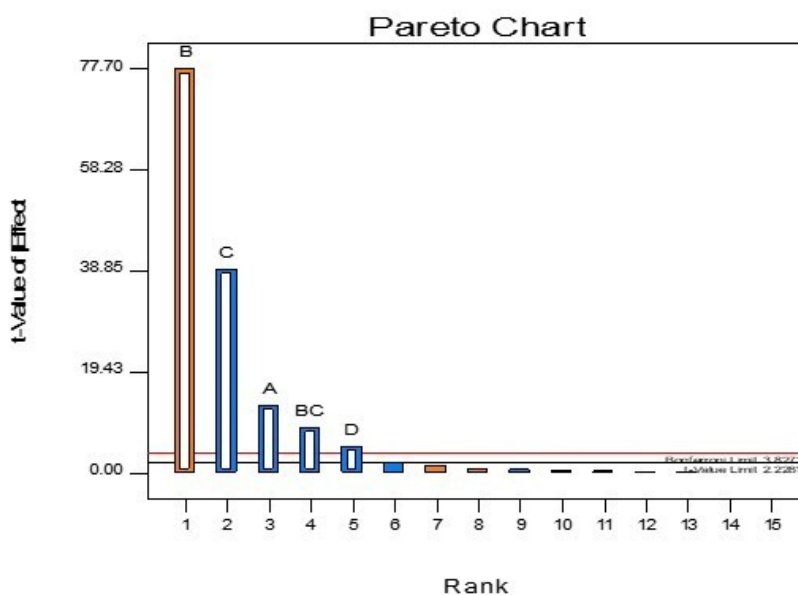
The effect of the factors and the factor interactions on the retention behavior of TOR was evaluated, and the model was developed using the significant effects and interactions. Figure 3 represents a paretochart showing the effect and interaction of independent variables on the RT of TOR. It also indicated that composition (B) showed the highest influence and temperature (D) showed the lowest influence on the RT of TOR.

Positive coefficients of factor B indicated its positive effect on the RT of TOR. The negative coefficients of A, C, D, and BC factor interactions indicated their negative effect on the RT of TOR. The MLRA equation for statistical modeling was described under:

$$RT\ TOR\ (Y_1) = + 4.05 - 0.15*A + 0.89*B - 0.45*C - 0.060*D - 0.10*BC$$



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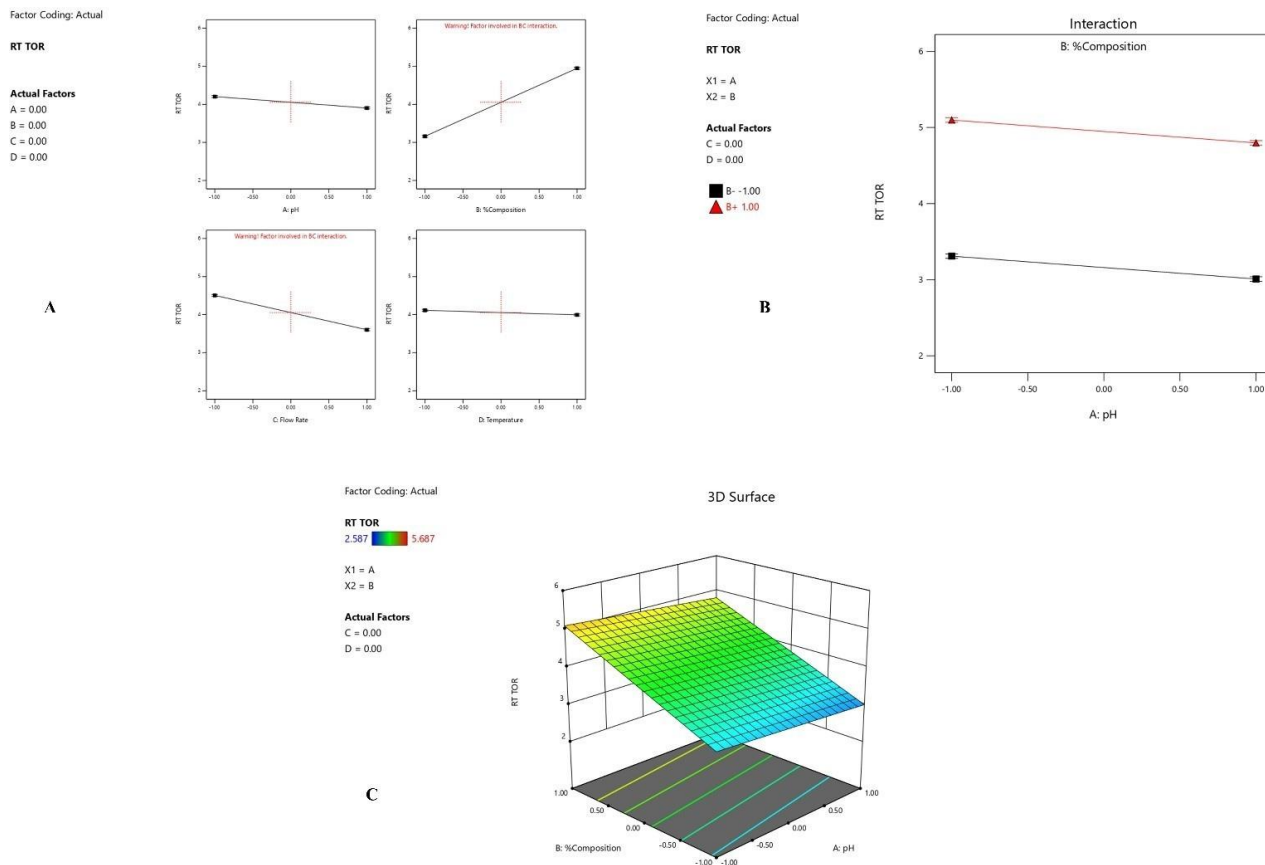
**Figure 3.** Effects and interaction of variables on RT of TOR

The model's F-value of 1571.50 demonstrates model significance. A "model F-value" this big might happen owing to noise just 0.01% of the time. When "Prob > F" is less than 0.0500, model terms are considered significant. A, B, C, D, and BC are important model terms in this case. Model terms are not significant if the value is higher than 0.1000. Model reduction may enhance your model if it has a lot of unnecessary words (except those needed to maintain hierarchy). The "Pred R-Squared" of 0.9967 and the "Adj R-Squared" of 0.9981 are reasonably in agreement. The signal-to-noise ratio is measured using "Adeq Precision". An ideal ratio is larger than 4, and the ratio of 110.469 shows a strong indication. To move about the design space, utilize this model. A result of the ANOVA was presented in Table 3.

**Table 3.** ANOVA for selected factorial model analysis of variance table  
[Partial sum of squares - Type III]

Source	Sum of Square	DF	Mean Square	F value	P value Prob>F
Model	16.64	5	3.33	1571.50	<0.0001
(A) Ph	1	0.36	170.71	<0.0001	
(B)%composition	12.79	1	12.79	6037.93	<0.0001
(C)Flow rate	3.27	1	3.27	1543.23	<0.0001
(D)Temperature	0.058	1	0.058	27.48	0.0004
BC 0.17	1	0.17	78.13	<0.0001	
Residual	0.021	10	2118E-003		
Cor total	16.66	15			

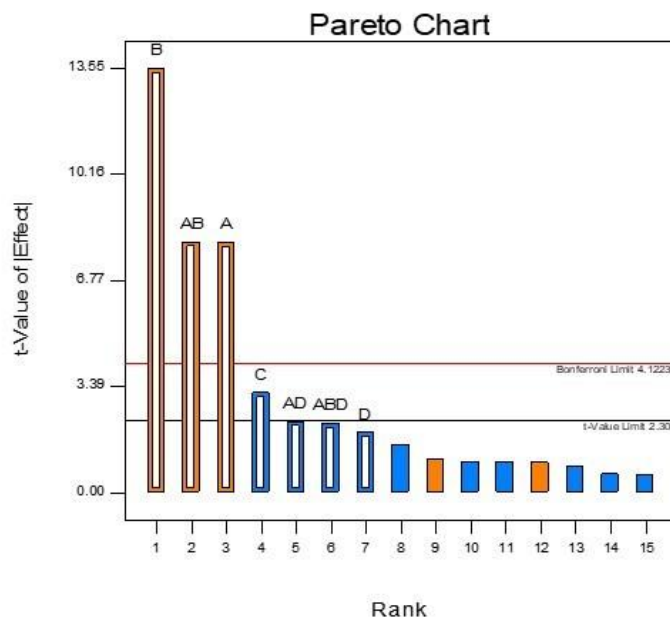
Figure 4 represents the one factor and interaction effect of the selected variable on the RT of TOR. It indicated that an increase in flow rate and pH in the mobile phase decreases the RT of TOR linearly. Figure 4A showed a change in composition in the mobile phase that linearly increased the RT of TOR, whereas temperature showed the least effect on the RT of TOR, indicating a negative effect of temperature on RT. Figures 4B and 4C indicate an interaction between composition and flow rate, and hence the effect of factors B and C was nonlinear; they indicated that an increase in B increased the RT of TOR and an increase in C decreased the RT of TOR.



**Figure 4.** Effect of Variables on RT of TOR (A) one factor effect (B) interaction effect of variables (C) 3D response surface plot

3.1.2. Retention time (RT) of SPI ( $Y_2$ ):

The effect of the factors and the factor interactions on the retention time behavior of SPI was evaluated, and the model was developed using the significant effects and interactions.



**Figure 5.** Effects and interactions of independent variables on RT of SPI

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Figure 5 represents a paretochart showing the effect and interaction of independent variables on the RT of SPI. It also indicated that composition (B) showed the highest influence and temperature (D) showed the lowest influence on the RT of SPI.

Positive coefficients of factors B, A, and AB indicated their positive effect on the RT of SPI. Negative coefficients of C, factor, and CD, AD, and ABD factor interactions indicated their negative effect on the RT of SPI. The MLRA equation for statistical modeling was described under:

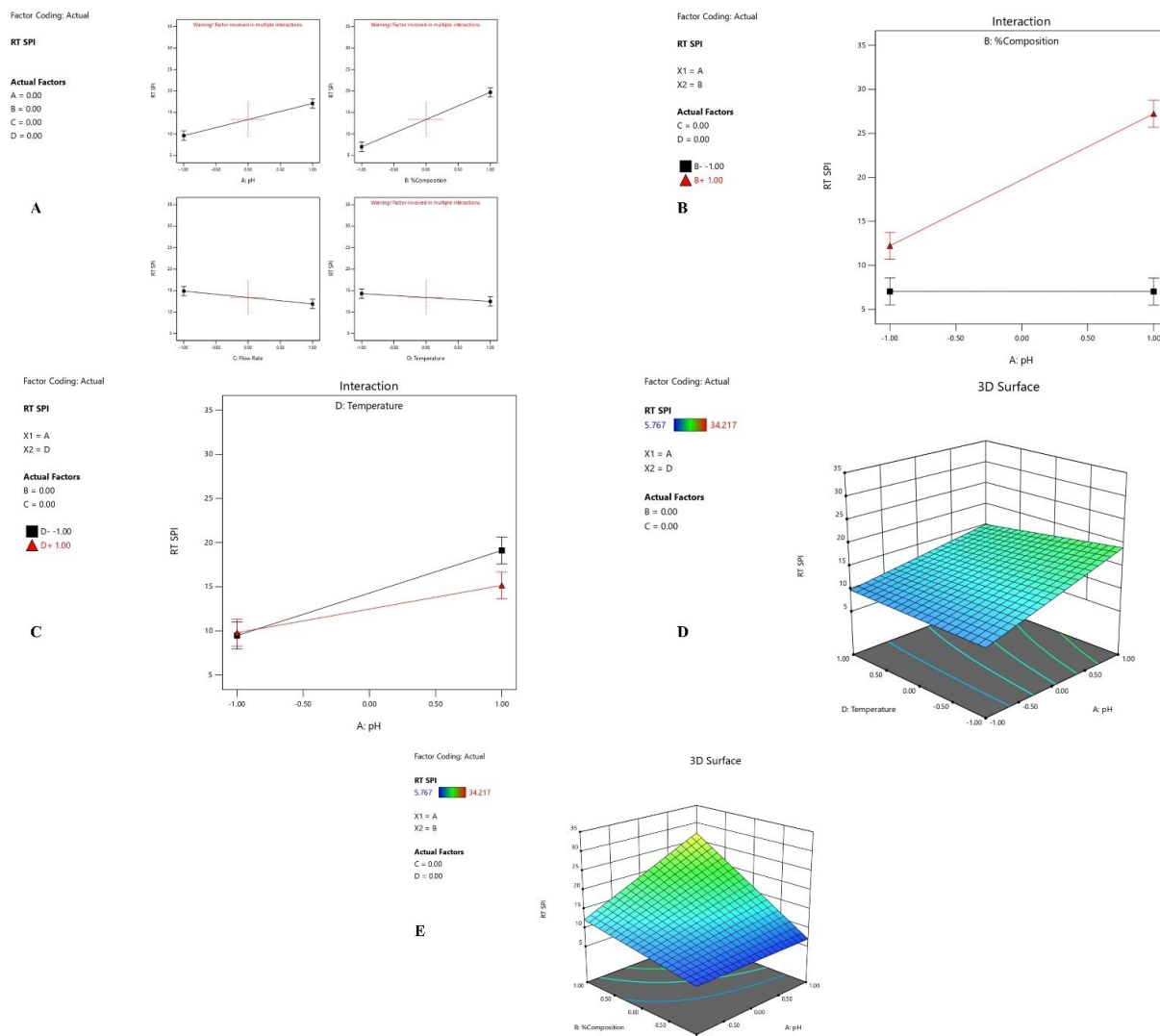
$$\text{RT SPI (Y}_2\text{)} = +13.37 + 3.75*A + 6.34*B - 1.50*C - 0.91*D + 3.75*A*B - 1.07*A*D - 1.04*A*B*D$$

The model F-value of 47.98 suggests that the model is significant. A "model F-value" this big might be caused by noise, but there is only a 0.01% chance that it would. When "Prob > F" is less than 0.0500, model terms are considered significant. A, B, C, and AB are important model terms in this case. Model terms are not significant if the value is higher than 0.1000. Model reduction may enhance your model if it has a lot of unnecessary words (except those needed to maintain hierarchy). The "Pred R-Squared" of 0.9069 is in reasonable agreement with the "Adj R-Squared" of 0.9564. Signal-to-noise ratio is measured using "Adeq Precision." A ratio of at least 4 is preferred. Your ratio of 20.489 suggests a strong enough signal. To move about the design space, utilize this model. The results of the ANOVA were presented in Table 4.

**Table 4.** ANOVA for selected factorial model Analysis of variance table  
(Partial sum of squares - Type III)

Source	Sum of Square	DF	Mean square	F value	P value Prob>F
Model	1178.85	7	168.41	47.98	<0.0001
(A) pH 224.79	1	224.79	64.05	<0.0001	
(B)%composition	644.02	1	644.02	183.49	<0.0001
(C)Flow rate	35.90	1	35.90	10.23	0.0126
(D)Temperature	13.18	1	13.18	3.76	0.0886
AB 225.21	1	225.21	64.17	<0.0001	
AD18.30	1	18.30	5.21	0.0518	
ABD17.45	1	17.45	4.97	0.0563	
Residual	28.08	8	3.51		
Cor total	1206.93	15			

Figure 6 represents the one factor and interaction effect of the selected variable on the RT of SPI. It indicated that the increase in composition and pH of the mobile phase increases the RT of SPI linearly. Figure 6A shows a change in flow rate and temperature increases; the RT of SPI was linearly increased. Figures 6B and 6C showed pH, temperature, and % composition interacted with each other, indicating a non-linear correlation of the variables. Figure 6D shows the effect of A and B on the RT of SPI; it indicates that an increase in A and B increases the RT of SPI. The effect of A and B was linear with positive coefficients. Figure 6E shows the effect of A and D on the RT of SPI; it indicates that an increase in A increased the RT of SPI and an increase in D decreased the RT of SPI. The effect of A and D was linear with opposite coefficients.



**Figure 6.** Effect of Variables on RT of SPI (A) one factor effect (B) interaction effect of variables (C) interaction effect of variables  $X_1$  and  $X_4$  (D) 3D response surface plot showing effect of  $X_1$  and  $X_4$  (E) 3D response surface plot showing effect of  $X_1$  and  $X_2$

### 3.2. Optimization of Chromatographic Condition

In order to determine optimized chromatographic conditions from the design, various constraints were applied to the design. In total, 4 independent factors and 2 dependent responses were screened based on their influences on the design space. The fixed values of factors A, B, C, and D were assigned. Only the RT of TOR and SPI was targeted as a constraint. The details of the constraints applied are shown in Table 5. The solution offered by the software is shown in Table 6.

**Table 5.** Optimization constraints for optimization of chromatographic condition

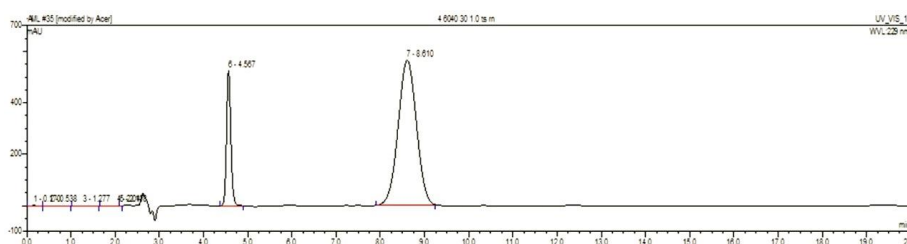
Sr. no	Name	Goal	Lower Limit	Upper Limit
1	pH	-1	-1	1
2	% Composition of mobile phase	1	-1	1
3	Flow Rate (mL/min)	1	-1	1
4	Temperature ( $^{\circ}$ C)	-1	-1	1
5	RT for Torsemide (TOR) (min)	3.5	2.587	5.687
6	RT for Spironolactone (SPI) (min)	7.0	5.767	34.217

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**Table 6.** Solutions offered by software as per optimization constraints

Sr. no	pH	% Composition of Mobile Phase	Flow Rate (mL/min)	Temperature (°C)	RT for Torsemide (min)	RT for Spironolactone (min)	Desirability
1	-1	0.99	1	-1	3.49	7.35	0.997
2	-1	0.38	1	-1	3.51	7.37	0.996
3	-0.99	-0.39	1	-1	3.5	7.39	0.996
4	-1	-0.37	1	-1	3.51	7.39	0.995
5	-0.98	-0.39	1	-1	3.5	7.42	0.995
6	-1	-0.38	0.99	-1	3.51	7.39	0.995
7	-1	-0.41	0.97	-1	3.5	7.37	0.994

The best chromatographic condition was found to be Solution 1 in our applications, which met all the requirements for both the independent and dependent constraints and had a high degree of attractiveness (0.997), which means it fit the constraints statistically well. For the purposes of further analytical method validation and to investigate forced degradation, the optimum chromatographic conditions were chosen. These included a mobile phase with a pH of 4.0, a mobile phase composition of 60% acetonitrile and 40% ammonium acetate buffer, a flow rate of 1 mL/min, and a temperature of 30°C. The chromatogram obtained from high-performance liquid chromatography (HPLC) using the optimal chromatographic conditions is shown in Figure 7. This demonstrates unequivocally that SPI and TOR are two separate targets. Thus, many criteria for validating the analytical method were further assessed under optimum chromatographic circumstances.

**Figure 7.** HPLC chromatogram for optimized chromatographic condition as per DoE

### 3.3. Analytical Method Validation

#### 3.3.1. System Suitability

The system suitability of the method was checked using determinations of capacity factor, repeatability, tailing factor, theoretical plates, and retention time of HPLC injections of standard TOR and SPI at 6 µg/mL and 30 µg/mL concentrations, respectively. Table 7 shows that observed values were compared with recommended values.

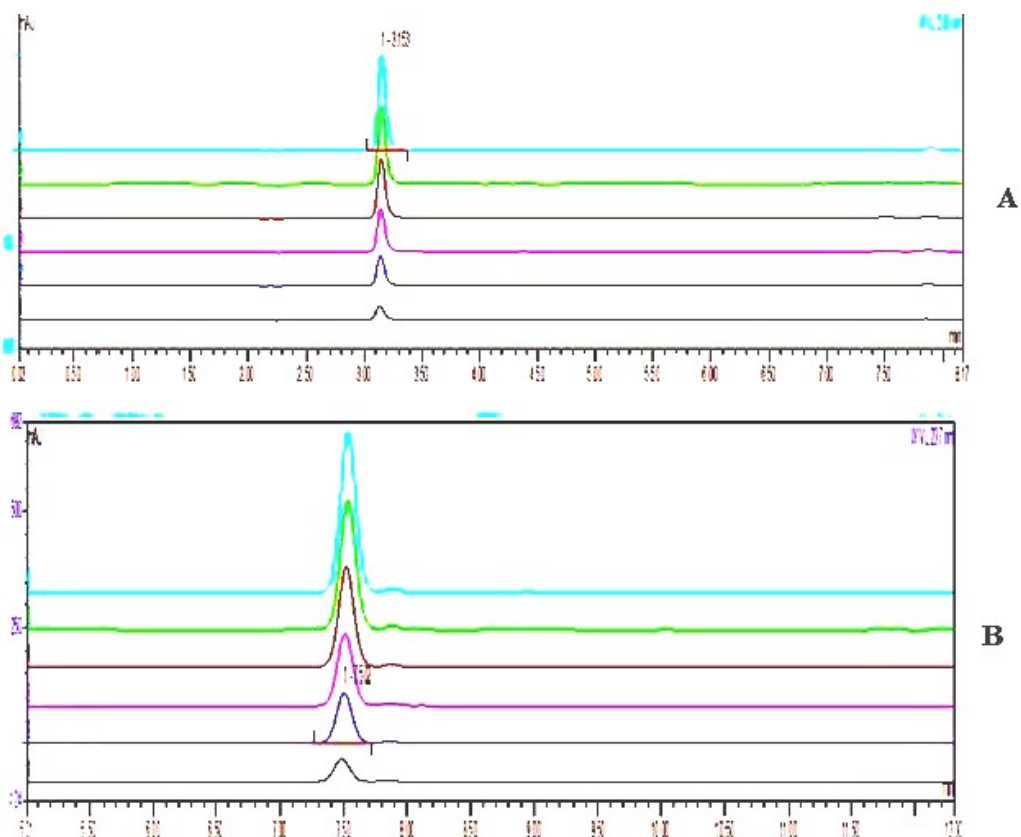
**Table 7.** System Suitability Test Parameters ( $n=4$ )

Sr. no	System Suitability Parameters	TOR	SPI
1	Tailing Factor	1.21	1.02
2	Theoretical Plates	10544	16247
3	Retention Time (Minutes)	3.148	7.518
4	Resolution		24.44

#### 3.3.2. Linearity

Both drugs were studied for linearity at six different concentration levels. The linearity of TOR and SPI was found in the range of 2–12 µg/mL and 10–60 µg/mL, respectively. Figure 8 shows peak areas generated with the relevant concentrations in µg/mL for TOR (A) and SPI (B). The correlation coefficient

value should not be less than 0.9900 over the working range for linearity of results. The correlation coefficients were found to be 0.9973 and 0.9976 for TOR and SPI (Table 8), respectively. Those data are within the acceptable limit, and hence, the method was found to be linear.



**Figure 8.** Linearity chromatograms for (A) TOR (B) SPI

**Table 8.** Results of linearity study of method for TOR and SPI

Linearity For TOR					
Sr. No.	Concentration (µg/mL)	Retention time (min)	Peak Area(mAU*min)	Linear Equation	Correlation Coefficient (r <sup>2</sup> )
1	2	3.132	1.2539	y = 0.6976x - 0.4575	0.9973
2	4	3.138	2.4884		
3	6	3.145	3.7106		
4	8	3.148	5.0131		
5	10	3.152	6.4572		
6	12	3.153	8.0264		
Linearity For SPI					
1	10	7.480	8.6875	y = 0.9098x - 1.1797	0.9976
2	20	7.488	15.8292		
3	30	7.508	26.9568		
4	40	7.518	34.5164		
5	50	7.532	43.9528		
6	60	7.533	53.2539		

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## 3.3.3. Accuracy

The standard addition of pharmaceuticals to the pre-analyzed sample at concentrations of 50%, 100%, and 150% were used for the evaluation of accuracy the proposed method herein. The recovery data for TOR and SPI were given in Table 9. The acceptable ranges of the data should be between 98% and 102% for recovery with a maximum RSD of 2.0%. Our results indicated pretty good %RSD for recoveries.

**Table 9.** Results of Recovery study

Drugs	Conc. Level (%)	Amount taken ( $\mu\text{g/mL}$ )	Amount added ( $\mu\text{g/mL}$ )	Total Amount ( $\mu\text{g/mL}$ )	%Recovery			Mean $\pm$ S. D	% RSD
					1	2	3		
TOR	50%	4	2	6	97.66	96.83	98.16	97.55 $\pm$ 0.67	0.6
	100%	4	4	8	97.25	98.26	97.52	97.67 $\pm$ 0.52	0.5
	150%	4	8	10	99.19	98.90	99.07	99.05 $\pm$ 0.15	0.1
SPI	50%	20	10	30	95.53	92.71	96.24	94.82 $\pm$ 1.86	1.9
	100%	20	20	40	97.00	96.88	97.40	97.09 $\pm$ 0.27	0.2
	150%	20	30	50	100.30	100.40	99.30	100.00 $\pm$ 0.60	0.6

## 3.3.4. Precision

The RSD should not be more than 2.0% [5] The RSD for TOR was determined as in the range of 0.43 to 0.64% for intra-day precision and 0.38 to 0.88% for inter-day precision, indicating that the method was precise. The RSD for SPI was determined to be in the range of 0.03 to 0.53% for intra-day precision and 0.20 to 1.07% for inter-day precision, indicating that the method was precise. A result of the precision study is presented in Table 10.

**Table 10.** Results of Precision study

Drug	Parameters	Intra-day precision concentration ( $\mu\text{g/mL}$ )			Inter-day precision concentration ( $\mu\text{g/mL}$ )		
		2	4	8	2	4	8
TOR	Mean	1.24	2.50	5.03	1.23	2.49	5.06
	S. D	0.009	0.01	0.026	0.008	0.009	0.044
	%RSD	0.64	0.43	0.51	0.68	0.38	0.88
SPI	Mean	<b>10</b>	<b>20</b>	<b>30</b>	<b>10</b>	<b>20</b>	<b>30</b>
	S. D	8.74	15.85	26.88	8.68	15.86	26.92
	%RSD	0.047	0.005	0.017	0.093	0.03	0.006
		0.53	0.03	0.06	1.07	0.20	0.22

## 3.3.5. Repeatability

The results of the repeatability ( $n = 6$ ) study of the developed method are shown in Table 11. The % RSD for repeatability of TOR and SPI was found to be 1.45 and 0.29 %, respectively. RSD was found to be less than 2% [5] which indicates the method had good repeatability.

**Table 11.** Repeatability study of developed method for TOR and SPI

Sr. No.	TOR (4µg/mL)	SPI (20µg/mL)
	Peak Area(mAU*min)	Peak Area(mAU*min)
1	2.4884	15.8292
2	2.5047	15.8568
3	2.5088	15.8950
4	2.5247	15.7598
5	2.5447	15.8051
6	2.5899	15.7958
<b>Mean</b>	2.52	15.82
<b>S. D</b>	0.0366	0.047
<b>%RSD</b>	1.45	0.29

### 3.3.6. Robustness

The robustness ( $n = 3$ ) data from a change in flow rate and mobile phase composition for TOR and SPI is shown in Table 12. The RSD of the method was found to be less than 2% and was within the acceptable limit.

**Table 12.** Results of robustness study

Drugs	Parameter Flow rate	Change 1	Change 2
		1.0 mL/min ( $n=3$ )	0.9 mL/min ( $n=3$ )
TOR	Area (conc. 4µg/mL)	2.492	2.501
	SD		0.0063
	% RSD		0.25
SPI	Area (conc.20 µg/mL)	15.859	15.863
	SD		0.0028
	% RSD		0.01
Drugs	Change in Mobile phase Composition	Buffer: ACN (40:60% v/v)	Buffer: ACN (42:58% v/v)
TOR	Area (conc.6µg/mL)	5.052	5.125
	S. D		0.0516
	% RSD		1.01
SPI	Area (conc.30µg/mL)	26.83	26.920
	S. D		0.0636
	% RSD		0.23

### 3.3.7. Limit of Detection (LoD) and Limit of Quantification (LoQ)

The LoD and LoQ for TOR were determined as 0.10 g/mL and 0.32 g/mL, respectively. For SPI, these values were found to be as 0.75 g/mL and 2.29 g/mL. The results were shown in Table 13.

**Table 13.** Results of LoD and LoQ for developed method

Sr. No.	Parameters	TOR	SPI
1	Mean of slope	0.683	0.909
2	Standard deviation of intercepts	0.022	0.209
3	LOD(µg/mL)	0.10	0.75
4	LOQ(µg/mL)	0.32	2.29



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## 3.4. Analysis of Tablet Dosage Form

The TOR and SPI in the form of tablets were successfully determined using the suggested RP-HPLC technique. The proportion of TOR and SPI was determined to be acceptable, and it was similar to the label claim. A result of the assay for tablet dosage form is presented in Table 14.

**Table 14.** Result of assay of tablet dosage form

Dytor Plus (10 mg TOR+ 50 mg SPI)	Parameters	TOR	SPI
		Concentration (6 µg/mL)	Concentration (30µg/mL)
	Mean ± S. D	101.02±0.86	102.22±0.69
	%RSD	0.85	0.68

## 3.5. Degradation Studies

The stability of TOR and SPI under different stress situations was studied by inducing their degradation. The research followed the ICH Q2R2 protocol. Control samples were used to double-check the results of the stability investigation. The outcomes of controlled examinations of sample and control degradation under different stresses are summarized in Table 15. Both TOR and SPI degrade noticeably under a variety of stresses. The results showed that in acidic and basic environments, TOR and SPI degraded more noticeably. The peak purity angle must be less than the peak purity threshold in order to meet ICH guidelines. As a result, the proposed method for analyzing TOR and SPI was unaffected by their degradation products. The stability of TOR and SPI in the pharmaceutical dose form was therefore determined using the proposed approach.

**Table 15.** Results of forced degradation studies for TOR and SPI

Drug	Time (Hour)	Degradation Condition	Peak Area		% Degradation
			Control	Sample	
TOR	24	Acid degradation	90.5396	65.6106	27.5
SPI	24				
TOR	24	Alkali degradation	90.5396	60.6897	32.9
SPI	24			94.2349	73.7089
TOR	24	Oxidative stress	90.5396	71.7455	20.7
SPI	24			94.2349	74.4056
TOR	24	Photo degradation	90.5396	66.8133	26.2
SPI	24			94.2349	78.4527

## 3.6 Measurement Uncertainty

Even though validation is essential to ensuring proper interpretation and comparison of results, it is not sufficient on its own. Torsemide and Spironolactone's measurement uncertainty was evaluated for the applied RP-HPLC method. The measurement uncertainty budgets were estimated using the Guide to the Expression of Uncertainty in Measurement (GUM) and the EURACHEM Guide. Amount of sample mass ( $U_{Ms}$ ), mass of standard compounds ( $U_{Sm}$ ), volume ( $U_v$ ), purity of the standards ( $U_p$ ), calibration curve ( $U_c$ ), stock solution preparation ( $U_{Sprep}$ ), and repeatability ( $U_{Mr}$ ) were selected as parameters for the estimation of uncertainty budget of the applied method for both measurand. Table 16 shows the outcomes of individual uncertainty according to the literature, EURACHEM and GUM guides [55-60].

**Table 16.** Uncertainty for applied HPLC method

Source of Uncertainty	Name of Drug	
	Torsemide	Spirolactone
$u_{Ms}$	0.00067	0.00067
$u_{Sm}$	0.00066	0.00066
$u_v$	0.01897	0.01897
$u_p$	0.00315	0.00257
$u_c$	0.001521	0.005455
$u_{Sprep}$	0.00318	0.00321
$u_{Rep}$	0.036	0.047
$u_{combined}$	0.24 mg/6.00 mg	1.53 mg/30.00mg
U(expanded)	0.49 mg/6.00 mg	3.06 mg/ 30.00 mg

Table 16 shows that the uncertainty associated with sample and standard preparation among those volume measurements and the purity of TOR and SPI samples contributes the least to the overall uncertainty. The combined uncertainty is heavily influenced by the calibration procedure, stock solution preparation, and method repeatability. In the method, the uncertainty associated with the repeatability of measurement is typically the dominant part of the uncertainty budget.

#### 4. Conclusions

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed to determine the levels of Torsemide and Spirolactone, as well as to study their forced degradation. This was achieved by employing analytical Quality by Design (QbD) principles. The optimization of chromatographic conditions for the development of the analytical method was carried out using a  $2^4$  full factorial experimental design. The analysis of variance (ANOVA) was employed to investigate the statistical significance of the independent variables. Additionally, the outcomes were visualized through perturbation plots. The design of experiments offers valuable tools for optimizing variable parameters in the development of HPLC methods. The optimal chromatographic conditions were determined using Design of Experiments (DoE), with the following parameters: the pH of the mobile phase was set at 4.0, the composition of the mobile phase was 60% acetonitrile and 40% ammonium acetate buffer, the flow rate was maintained at 1 mL/min, and the temperature was set at 30°C. The method developed under optimal conditions was validated in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The specificity of the RP-HPLC method for the determination of TOR and SPI was demonstrated, as it was observed that no excipients or impurities caused any interference. The method under consideration was found to exhibit high levels of precision, accuracy, and robustness. The forced degradation study demonstrates the efficacy of the developed method in efficiently detecting degradants. The application of the AQbD (Analytical Quality by Design) approach during the process of method development has been determined to be an effective means of enhancing both the efficiency and quality of analytical method development. From the analysis of uncertainty budgets, we can draw the conclusion that sample and standard preparation contained just weights, measures of volume, and purities, so the uncertainty associated with them was minimal. A significant portion of the overall uncertainty in a technique under study is attributable to its repeatability component. In conclusion, the method developed in this study effectively determines the concentration of TOR and SPI in pharmaceutical dosage forms.

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### Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/journal-of-chemical-metrology>

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### References

- [1] J.N. Sangshetti, M. Deshpande, Z. Zaheer, D.B. Shinde and R. Arote (2017). Quality by design approach: regulatory need, *Arabian J. Chem.* **10**, S3412–S3425.
- [2] L.X. Yu, G. Amidon, M.A. Khan, S.W. Hoag, J. Polli, G.K. Raju and J. Woodcock (2014). Understanding pharmaceutical quality by design, *AAPS J.* **16**, 771–783.
- [3] J.M. Juran (1992). Juran on quality by design: the new steps for planning quality into goods and services. The Free Press, A division of Simon and Schuster, USA.
- [4] T. Tome, N. Žigart, Z. Časar and A. Obreza (2019). Development and optimization of liquid chromatography analytical methods by using AQbd principles: overview and recent advances, *Org. Process. Res. Dev.* **23**, 1784–1802.
- [5] ICH Expert Working Group (2009) ICH Harmonised tripartite Guideline. Pharmaceutical development Q8 (R2). 8, PP 1–28.
- [6] International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (2005). Validation of analytical procedures: text and methodology ICH Q2 (R1).
- [7] R. Peraman, K. Bhadraya, Y and Padmanabha Reddy (2015). Analytical quality by design: a tool for regulatory flexibility and robust analytics, *Int. J. Anal. Chem.* 1- 9. doi:10.1155/2015/868727
- [8] A. Dispas, H.T. Avohou, P. Lebrun, P. Hubert and C. Hubert (2018). Quality by design approach for the analysis of impurities in pharmaceutical drug products and drug substances, *Trend. Anal. Chem.* **101**, 24–33.
- [9] J.W. Manoel, G.B. Primieri, L.M. Bueno, N.R. Wingert, N.M. Volpato, C.V. Garcia, E.E. Scherman Schapoval and M. Steppe (2020). The application of quality by design in the development of the liquid chromatography method to determine empagliflozin in the presence of its organic impurities, *RSC Adv.* **10**, 7313–7320.
- [10] J. Djuris and Z. Djuric (2017). Modeling in the quality by design environment: regulatory requirements and recommendations for design space and control strategy appointment, *Int. J. Pharm.* **533**, 346–356.
- [11] L.X. Yu (2008). Pharmaceutical quality by design: product and process development, understanding and control, *Pharm. Res.* **25**, 781–791.
- [12] N. Desai and M. Potdar (2017). Introduction to quality by design for pharmaceuticals, PhermaMed plus Press.
- [13] Y. Abe and K. Emori (2022). Application of a statistical approach to the process development of futibatnib by employing quality-by-design principles part 2: development of design space for impurities using the response surface methodology, *Org. Process. Res. Dev.* **26**, 56–71.
- [14] L. Kovac, Z. Casar, T.T. Lusin and R. Roškar (2022). Development of an analytical method for determination of related substances and degradation products of cabotegravir using analytical quality by design principles, *ACS Omega* **7**, 8896–8905.

- [15] N.V.V.S.S. Raman, U.R. Mallu and H.R. Bapatu (2015). Analytical quality by design approach to test method development and validation in drug substance manufacturing, *J. Chem.* **2015**, 1-8. doi:10.1155/2015/435129
- [16] L. Brunton and R. H. Dandan (2006). Goodman and Gilman's the pharmacological basis of therapeutics, eleventh Edition, Mcgraw Hill Publishers, New York, PP. 736-766.
- [17] Sweetman SC (2009). Martindale the complete drug reference, Thirty-sixth edition, London, PP. 1400-1416.
- [18] United States Pharmacopoeia 30 National Formulary 25 (Asian edition, 2007), The official compendia of standards, PP.3593-3776.
- [19] Indian Pharmacopoeia (2007). Government of India, ministry of health and family welfare; Published by The Indian Pharmacopoeia commission, Ghaziabad, Volume III, PP. 2784-2785.
- [20] British Pharmacopoeia (2009). The Department of health, social services and public safety; Published by Stationary office on behalf of MHRA, London, Volume III, PP. 2928-2929.
- [21] J. Gupta, G. Kanojia, V. Yadav and S.R. Wakode (2010). Development and validation of a uv spectrophotometric method for the estimation of torsemide in bulk and in tablet dosage form, *J. Chem. Pharm. Res.* **2**, 513-517.
- [22] I. Khan, P. Loya and M. Saraf (2008). A simplified HPLC method for quantification of torsemide from human plasma and its application to a bioequivalence study, *Ind. J. Pharm. Sci.* **70**, 519-522.
- [23] A. Aragon, J. Navoni, C. Mcontartese, A. Villagra, C. Lopez and E. Lepori (2011). Quantitative analysis of torsemide in human plasma by high performance liquid chromatography with ultraviolet detection, *Rev. Mex. Patol. Clin.* **58**, 195-200.
- [24] R. Kakde, N. Chaudhary, A. Barsagade and D. Kale (2011). Stability-indicating HPTLC method for analysis of torsemide in pharmaceutical preparations, *Acta. Chromatogr.* **23**, 145-155.
- [25] R. Kakde, A. Barsagade, N. Chaudhary and D. Kale (2011), Stability-indicating hptlc method for analysis of ticlopidine in pharmaceutical preparations, *J. Plan. Chromatogr. - Modern TLC.* **24**, 145-149.
- [26] L. Zhang, R. Wang, Y. Tian and Z. Zhang (2016). Determination of torasemide in human plasma and its bioequivalence study by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry, *J. Pharm. Anal.* **6**, 95-102.
- [27] Z.M. Sayyed, S.A. Shinde, V.J. Chaware, B.P. Chaudhari, M. Zuber and M. Sayyed (2015). Development and validation of uv-spectrophotometric method for simultaneous estimation of spironolactone and hydrochlorothiazide in pharmaceutical formulation, *J. Pharm. Sci. Bio. Res.* **5**, 590-593.
- [28] V.R. Ram, P.N. Dave and H.S. Joshi (2012). Development and validation of a stability-indicating HPLC assay method for simultaneous determination of spironolactone and furosemide in tablet formulation, *J. Chromatogr. Sci.* **50**, 721-726.
- [29] E. Tekerek, M. Şukuroglu and O. Atay (2008). Quantitative determination of hydrochlorothiazide and spironolactone in tablets by spectrophotometric and HPLC methods, *Turk. J. Pharm. Sci.* **5**, 53-66.
- [30] J.M. Sandall, J.S. Millership, P.S. Collier and J.C. McElnay (2006). Development and validation of an HPLC method for the determination of spironolactone and its metabolites in paediatric plasma samples, *J. Chromatogr. B. Anal. Technol. Biomed. Life. Sci.* **839**, 36-44.
- [31] S. Smita, M.C. Sharma, Vipul Kohli and S.C. Chaturvedi (2010). Isocratic reverse phase HPLC estimation method of torsemide and spironolactone in pharmaceutical combined dosage form, *Optoel. Dev. Mater. Rapid Commun.* **4**, 234-237.
- [32] C. Nazareth, P. Reddy and B. Gurupadayya (2014). Development and validation of HPTLC method for simultaneous estimation of metolazone and spironolactone in bulk drug and pharmaceutical dosage form, *IOSR. J. Pharm.* **4**, 20-25.
- [33] M. A. Hinge and D. Patel (2022). Optimization of HPLC method using central composite design for estimation of torsemide and eplerenone in tablet dosage form, *Braz. J. Pharm. Sci.* **58**, 1-14.
- [34] Kher G, V Ram, M Kher and H Joshi (2012), Development and validation of a HPTLC method for simultaneous determination of furosemide and spironolactone in its tablet formulation, *Res. J. Pharm. Biol. Chem. Sci.* **4**, 365-377.
- [35] H. Dong, F. Xu, Z. Zhang, Y. Tian and Y. Chen (2006), Simultaneous determination of spironolactone and its active metabolite canrenone in human plasma by HPLC-APCI-MS, *J. Mass. Spectrom.* **41**, 477-486.
- [36] Y. Ismail, K. B. Chandrasekhar and V. Gunasekaran (2014). View of a new stability indicating UPLC spironolactone in bulk and in its pharmaceutical formulations, *Int. J. Pharm. Pharm. Sci.* **6**, 448-452.
- [37] ISO. ISO 98-3 guide to the expression of uncertainty in measurements, international organization for standardization: Geneva, Switzerland, 1995.
- [38] NIST. NIST guideline for evaluating and expressing the uncertainty of NIST measurement results; national institute of standards and technology: Gaithersburg, MD, USA, 1993.
- [39] M. Mabrouk, S. Hammad, F. Mansour and M. Amer (2016). development and validation of a reversed phase HPLC method for simultaneous determination of antidiabetic drugs alogliptin benzoate and pioglitazone Hcl, *Der. Phar. Sinic.* **7**, 32-40.

## Simultaneous determination of Aripiprazole and Escitalopram

- [40] C. Varaprasad, M. Asif and K. Ramakrishna (2015). RP-HPLC method for simultaneous estimation of metformin and linagliptin in tablet dosage form, *Rasayan J. Chem.* **8**, 426-432.
- [41] J. Rajbangshi, M. Alam, M. Hossain, M. Islam and A. Rouf (2018). Development and validation of a RP-HPLC method for quantitative analysis of linagliptin in bulk and dosage forms, *J. Pharm. Sci.* **17**, 175-182.
- [42] M. Attimarad, S. H. Nagaraja, B. Aldhubaib and A. Nair (2014). Simultaneous determination of metformin and three gliptins in pharmaceutical formulations using RP HPLC: application to stability studies on linagliptin tablet formulation, *Ind. J. Pharm. Edu. Res.* **48**, 45-53.
- [43] M. Sha'at, A. Spac, I. Stoleriu, A. Bujor, M. Cretan, M. Hartan, and L. Ochiuz (2022). Implementation of Qbd approach to the analytical method development and validation for the estimation of metformin hydrochloride in tablet dosage forms by HPLC, *Pharmaceutics* **14**, 1-22.
- [44] M.M. Annapurna, C. Mohapatro, and A. Narendra (2012). Stability-indicating liquid chromatographic method for the determination of letrozole in pharmaceutical formulations, *J. Pharm. Anal.* **2**, 298-305.
- [45] G. Srinubabu, C.A.I. Raju, N. Sarath, P.K. Kumar, and J.V.L.N.S. Rao (2007). Development and validation of a HPLC method for the determination of voriconazole in pharmaceutical formulation using an experimental design, *Talanta* **71**, 1424-1429.
- [46] Wankhede Sagar and Chitlange S (2009). Simultaneous reverse phase HPLC estimation of ofloxacin and satranidazole in tablet dosage form, *Int. J. Pharm. Tech. Res.* **1**, 1136-1138.
- [47] N. Sultana, M.S. Arayne and B. Iftikhar (2008). Simultaneous determination of atenolol, rosuvastatin, spironolactone, glibenclamide and naproxen sodium in pharmaceutical formulations and human plasma by RP-HPLC, *J. Chin. Chem. Soc.* **55**, 1022-1029.
- [48] P.B. Deshpande, S.V. Gandhi, N.V. Gaikwad and K. Skhandagle (2012). A simple and sensitive RP-HPLC method for simultaneous estimation of torsemide and spironolactone in combined tablet dosage form, *Acta. Chromatogr.* **24**, 15-22.
- [49] K. Takkis, R. Aro, L.T. Korgvee, H. Varendi, J. Lass, K. Herodes and K. Kipper (2017). Signal enhancement in the hplc-esi-ms/ms analysis of spironolactone and its metabolites using Hfip and nh 4 f as eluent additives, *Anal. Bioanal. Chem.* **409**, 3145-3151.
- [50] I. Baranowska, A. Wilczek and J. Baranowski (2010). Rapid UHPLC method for simultaneous determination of vancomycin, terbinafine, spironolactone, furosemide and their metabolites: application to human plasma and urine, *Anal. Sci.* **26**, 755-759.
- [51] R.R. Chavan, S.D. Bhinge, M.A. Bhutkar, D.S. Randive and V.R. Salunkhe (2021). Method development and validation of spectrophotometric and RP-HPLC methods for simultaneous estimation of spironolactone and furosemide in bulk and combined tablet dosage forms, *Anal. Sci. Technol.* **3**, 212-224.
- [52] D.I. Sora, Ş. Udrescu, F. Albu, V. David and A. Medvedovici (2010). Analytical issues in HPLC/MS/MS simultaneous assay of furosemide, spironolactone and canrenone in human plasma samples, *J. Pharm. Biomed. Anal.* **52**, 734-740.
- [53] K.S. Patel, A. Bendale, S.V. Luhar, and S.B. Narkhede (2016). Development and validation of RP-HPLC method for the simultaneous estimation of eplerenone and torsemide in pharmaceutical dosage form, *J. Pharm. Sci. Bio. Res.* **6**, 283-290.
- [54] R.B. Patel, B.R. Patel, J.G. Patel and V.S. Patel (2017). Stability Indicating RP-HPLC method development and validation for simultaneous estimation of eplerenone and torsemide in tablet dosage form, *World. J. Pharm. Pharm. Sci.* **6**, 1397-1406.
- [55] H. Kiziltas, A.C. Goren, Z. Bingöl, S.H. Alwasel and I. Gulcin (2021). Anticholinergic, antidiabetic and antioxidant activities of *Ferula orientalis* L. determination of its polyphenol contents by LC-HRMS, *Rec. Nat. Prod.* **15**, 513-528.
- [56] A.C. Gören, G. Bilsel and M. Bilsel (2007). Rapid and simultaneous determination of 25-OH-vitamin D 2 and D 3 in human serum by LC/MS/MS: Validation and uncertainty assessment, *J. Chem. Metrol.* **1**, 1-9
- [57] H. Kızıldaş, Z. Bingöl, A.C. Gören, S.M. Pinar, S.H. Alwasel and İ. Gülçin (2021). LC-HRMS profiling of phytochemicals, antidiabetic, anticholinergic and antioxidant activities of evaporated ethanol extract of *Astragalus brachycalyx* Fischer, *J. Chem. Metrol.* **15**, 135-151.
- [58] A. Kul (2022). Simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol by HPLC-PDA in pharmaceutical dosage forms, *J. Chem. Metrol.* **16**, 102-110.

- [59] B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The fitness for purpose of analytical methods – a laboratory guide to method validation and related topics, (2nd ed. 2014). ISBN 978-91-87461-59-0
- [60] Evaluation of measurement data — Guide to the expression of uncertainty in measurement (GUM). (2008). JCGM 100:2008 GUM 1995, BIPM. Paris.

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