Research Article

Analytical QbD Approach for Development and Validation of RP-High Performance Liquid Chromatography Method for Determination of Tofisopam in Pure Form and Tablets

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ABSTRACT

A novel, simple, optimized reversed-phase chromatography method for assay of Tofisopam in pure and tablet form is developed. The experimental trial was by Box Behnken design using the Design Expert® software 10 version. The attributes selected were peak symmetry, number of theoretical, and peak purity. The predicted data satisfied with actual experimental data. The optimized chromatographic conditions required a quaternary pump with a mobile phase of Water: Acetonitrile 25:75 v/v at 1 mL/min, oven temperature at 25°C at 310 nm using C_{18}(250 × 4.6 mm Id, 5μm) column and PDA detector with a run time of 5 min. The method was validated for linearity, precision, accuracy, and specificity. The method produced a linear response over a concentration range of 4–24 ppm with an overall average accuracy of 99.98%. The method was robust, reproducible, and specific with respect to the retention time of tofisopam.

INTRODUCTION

Tofisopam is a antianxiety[1] agent chemically known as 1-(3,4-Dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-4-methyl-5H-2,3-benzodiazepine Fig. 1. It is a white, pale yellowish-white crystalline powder, practically insoluble in water, sparingly soluble in ethanol, freely soluble in methanol, and acetonitrile with melting range 155-159°C.[2]

There are analytical methods for the estimation of tofisopam with spectrophotometric and spectrofluorimetic[3] chromatographic,[4-6] enantiomeric separation,[7,8] gas-liquid chromatography (GLC),[9] and super-critical chromatography[10] method. All these methods require a large sample volume (1-5 mL) and time-consuming sample clean up procedure. Literature reveals no RP-High
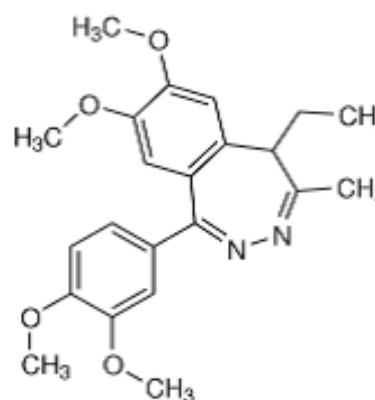

Fig. 1: Structure of tofisopam

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performance liquid chromatography method developed by quality by design for determination of tofisopam in pure form and tablet. In view of these points, an attempt was made to develop a simple, precise, accurate, and validated method for the determination of tofisopam in pure form and tablet by analytical quality by design approach.

Materials and Methods

Chemicals and Reagents

The TF was gifted from Ajanta Pharmaceutical, Mumbai India. The commercial dose formulation containing 50 mg TF, Nextril tablet, was procured from the local market. All the reagents and chemicals like acetonitrile, water, methanol were of HPLC grade.

Instrumentation

The determination of TF was done by using HPLC (JASCO LC system-1200 series), connected to the autosampler, column oven, and wavelength detector. It consists of a quaternary pump, a Rheodhyne injector with a 20 μL loop, and a photodiode array detector. C_{18} (250 × 4.6 mm Id, 5μm) column was used for separation.

Procedure for Standard Stock Solution

The standard stock solution was prepared by accurately weighing 100 mg of TF in 100 mL volumetric flask and diluted up to mark with acetonitrile, which gives a concentration of 1000 μg/mL of solution. From the above standard stock solution, an aliquot of 1 mL was transferred into 10 mL volumetric flask, and the volume was made up to the mark with acetonitrile, which gives a concentration of 100 μg/mL of solution (sub-stock solution).

Preparation of Solution and Selection of Wavelength

From the sub-stock solution (100 μg/mL), aliquot of 1 mL were transferred to 10 mL of volumetric flask and diluted with acetonitrile, to form a solution of 10 μg/mL and it was scanned in the range of 400-200 nm, and the absorbance maximum was noted at 310 nm (Fig. 2).

Optimization of Mobile Phase

A variety of solvents with different compositions were screened to find out the ideal mobile phase (Table 1).

Chromatographic Conditions

The mobile phase was consists of acetonitrile: water (75:25), and it was filtered, degassed, and sonicated prior to use. A C_{18} (250 × 4.6 mm Id, 5 μm) column was used as a stationary phase. The column temperature was kept at 25°C with a thermostatically controlled column oven. The flow rate was maintained at 1.0 mL/min. The detection was carried out at 310 nm wavelength PDA detector, and the injection volume was 20 μL.

Experimental Design

The experimental design was constructed using design-expert software version 10 for the study of different variables (oven temperature, percentage of the organic phase, and flow rate) and to verify method performances. The levels of these variables are as given in Table 2. The retention time, peak symmetry, peak purity, and several theoretical plates were used as a response in experimental design as controlling response, which is expected to affect and control method responses. A 3^3 factorial design consisting of three factors at three levels was considered for the experimental plan. Initially and after confirming that the process is a non-linear, Box-Behnken design was used. The experimental observations along with Design (DOE) plan are shown in Table 3.

Evaluation of Experimental Results and Selection of Final Method Conditions

The different method conditions (13 runs) obtained from experimental design was run and evaluated for retention time, peak symmetry, number of theoretical plates, and peak purity. Response surface Box-Behnken statistical screening design was used to optimize and evaluate main effects, interaction effects, and quadratic effects. A 3-factor, 3-level design used is suitable for exploring quadratic response surfaces with Design Expert® (Version 10.0, Stat-Ease Inc).
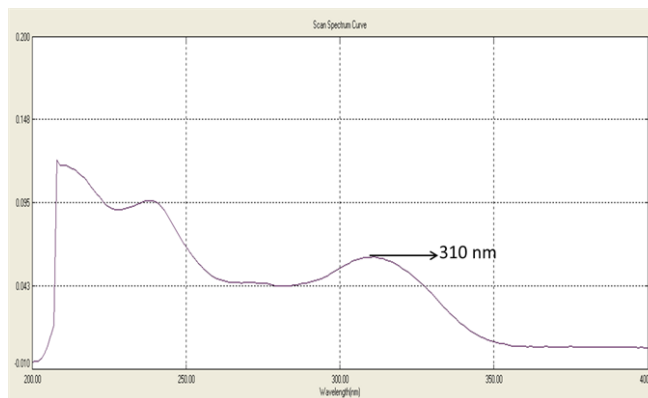
Fig. 2: UV spectrum of tofisopam

Table 1: List of mobile phase compositions screened

Sr. No.	Mobile phase	Ratio (v/v)	Remark
1	Methanol : Water	90:10	Peak tailing was observed
2	Methanol : Water	80:20	Peak fronting
3	Methanol : Water	70:30	Peak was not proper
4	ACN : Water	90:10	Peak was not resolved
5	ACN : Water	75:25	Peak was symmetric

Table 2: Factors and levels of independent variables

Factors	Level 1	Level 2	Level 3
Percentage of the organic phase (in percentage)	20	22.5	25
Column temperature (degree Celsius)	25	27.5	30
Flow rate (mL/min)	0.9	1	1.1

Table 3: BoxBehnken Design Plan and Responses

Std	Run	Factor 1 A: Oven temperature	Factor 2 B: Organic concentration	Factor 3 C: Flow rate	Response 1 NTP	Response 2 Peak symmetry	Response 3 Rt	Response 4 Peak purity
7	2	25	22.5	1.1	6271	0.996	3.923	99.19
5	3	25	22.5	0.9	6275	1.027	4.753	99.55
3	12	25	25	1	6445	0.998	4.46	98.89
1	13	25	20	1	6027	1.045	4.14	98.37
11	1	27.5	20	1.1	6928	1.081	3.737	99.11
12	4	27.5	25	1.1	6724	1.092	3.95	98.08
13	6	27.5	22.5	1	6632	1.036	4.227	99.22
9	8	27.5	20	0.9	6456	1.106	4.543	99.14
10	11	27.5	25	0.9	6315	1.09	4.917	99.15
2	5	30	20	1	6512	1.103	4.08	99.49
6	7	30	22.5	0.9	6676	1.108	4.687	99.11
4	9	30	25	1	6216	1.064	4.443	98.09
8	10	30	22.5	1.1	6128	1.094	3.867	98.97

Method Validation

Method validation is an analytical process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirement for the intended analytical application.

Linearity

The linearity of TF was assessed in the range of (4–24 µg/mL) in terms of slope, intercept, and correlation co-efficient values.

Precision

Precision study was calculated by interday and intraday studies. Both precisions were determined by analyzing 8, 12, 16 µg/mL concentrations daily for 3 days and %RSD were calculated.

Accuracy

Accuracy of the method was confirmed by a recovery study from marketed formulation at 3-level of standard addition. Percentage recovery of TF was found out.

Specificity

Specificity was determined by checking the interference of any possible degradation products. Both the standard and test sample solutions were scanned separately in the range of 200–400 nm.

Ruggedness

It was performed by analyzing three dilutions using different analysts on a different day. The overall % RSD was calculated.

Robustness

The three dilutions were analyzed under different conditions by changing the wavelength and mobile phase composition. The percentage RSD of the assay was calculated.

Stability in Analytical Solution

Prepared sample as per test method and injected in duplicate into HPLC at initial and at different time intervals up to 24 hours. The percentage assay at different time intervals was determined.

Assay

For the assay of TF, twenty tablets were accurately weighed and triturated to make a fine powder. Quantity equivalent to 50 mg was calculated and transferred into 50 mL volumetric flask, diluted with acetonitrile and sonicated for 15 minutes. From the above stock solution, 1 mL was transferred into 10 mL volumetric flask and diluted with acetonitrile, which gives a concentration of 100 µg/mL of solution. From the above solution, an aliquot of 1 mL was transferred into 10 mL of volumetric flask and diluted with acetonitrile, which givesa concentration of 10 µg/mL of solution. The 20 µL of above dilution was injected for HPLC analysis.

RESULTS AND DISCUSSION

The proposed work was planned for the development and evaluation of the RP-HPLC method for the estimation of TF in bulk and pharmaceutical formulation. Accordingly, the initial solvent was selected for TF, and acetonitrile showed good solubility for it. Further, the chromatographic

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conditions were analyzed and parameters were finalized, as shown in Table 4. TF showed a sharp peak in mobile phase Acetonitrile: water (75:25) with retention time 4.46 minutes, as shown in Fig. 3.

QbD Implementation

Design Expert® (Version 10.0, Stat-Ease Inc) software was used for the statistical analysis of the experimental observations and the analysis was conducted by using Box-Behnken design. The inputs given to software are given in Tables 5 a, b.

Inputs were given to the software and design was run, which provided selected 13 runs to be performed. After completing 13 runs, the results in terms of responses like retention time, peak symmetry, number of theoretical plates and peak purity were entered in software to give a statistical evaluation in terms of degrees of freedom (Tables 6 and 7) along with 3D surface plot of desirability as shown in Figs. 4-8.
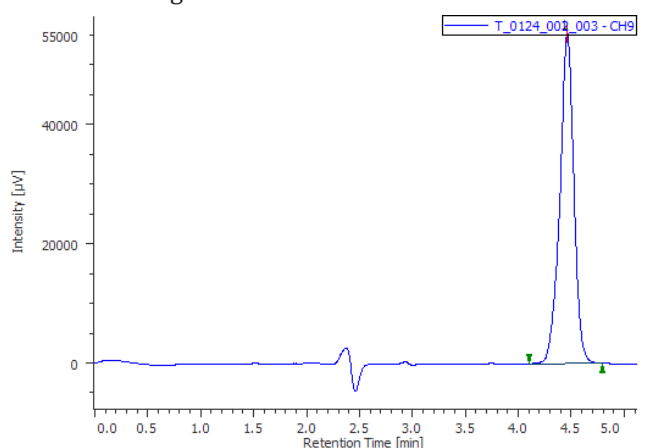

Fig. 3: Chromatogram of TF using ACN: Water (75:25)

Table 4: Chromatographic conditions

Parameters	Results
Column	C_{18} (250 X 4.6 mm Id, 5µm) column
Flow rate	1.0 mL/minute.
Detector	PDA
Detector wavelength	310 nm
Injection volume	20 µL
Retention time	4.46 min
Mobile phase	Acetonitrile: water (75:25)

Method Validation

Linearity

The TF showed linearity between concentration range of 4–24 ppm and had a good correlation coefficient of 0.999 with slope 49297 and y-intercept 24739 for the graph of concentration against area Fig. 9.

Precision

Results of precision are shown in Table 8, and it shows a standard deviation of less than 2, which indicates that the proposed method is precise.

Accuracy

Results of accuracy are shown in Table 9. The percentage recovery was 99.98 and also showed a standard deviation << 2, which states that the method is accurate.

Specificity

It was tested for the interference of any degraded substance, and as shown in the chromatogram (Fig. 10),
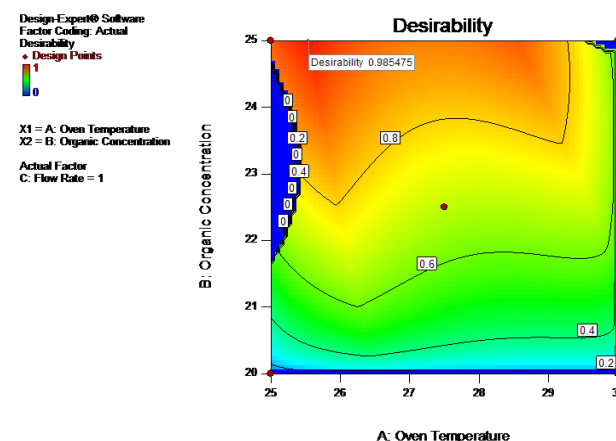

Fig. 4: Contour plot of desirability for obtaining optimized HPLC conditions

Table 5 (a): Design summary for optimization by using Box-Behnken design

Study type	Response surface
Design type	Box-Behnken
Design model	Quadratic
Runs	13

Table 5 (b): Design summary for optimization by using Box-Behnken design

Factor	Name	Units	Type	Subtype	Minimum	Maximum
A	Oven temperature	Degree Celcius	Numeric	Continuous	25	30
B	Organic concentration	%	Numeric	Continuous	20	25
C	Flow rate	mL/min	Numeric	Continuous	0.9	1.1

Table 6: Evaluation of degrees of freedom

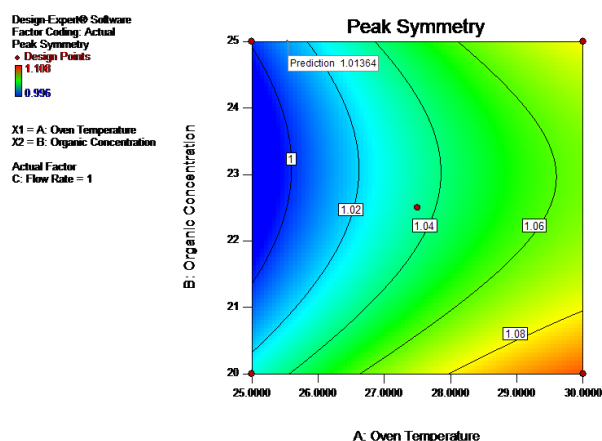| Response	Name	Obs	Analysis	Minimum	Maximum	Ratio	Model
R1	NTP	13	Polynomial	6027	6928	1.14949	Quadratic
R2	Peak Symmetry	13	Polynomial	0.996	1.108	1.11245	Quadratic
R3	Rt	13	Polynomial	3.737	4.917	1.31576	Linear
R4	Peak purity	13	Polynomial	98.08	99.55	1.01499	Quadratic

Fig. 5: Contour plot of peak symmetry
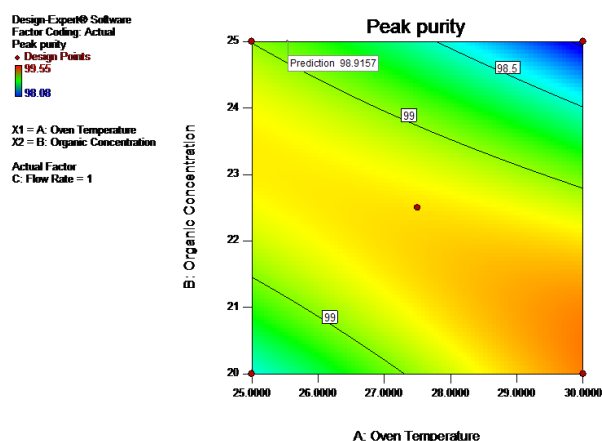

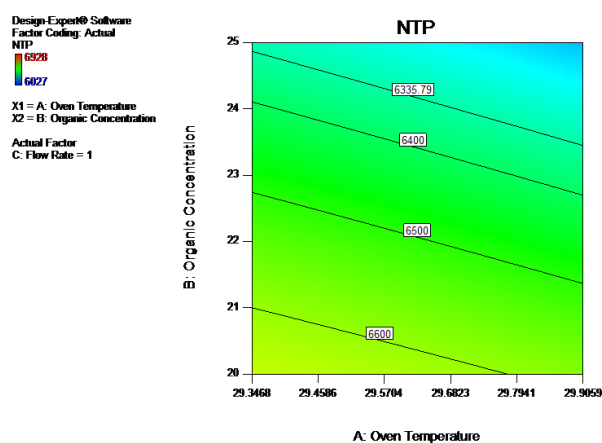**Fig. 6:** Contour plot of peak purity
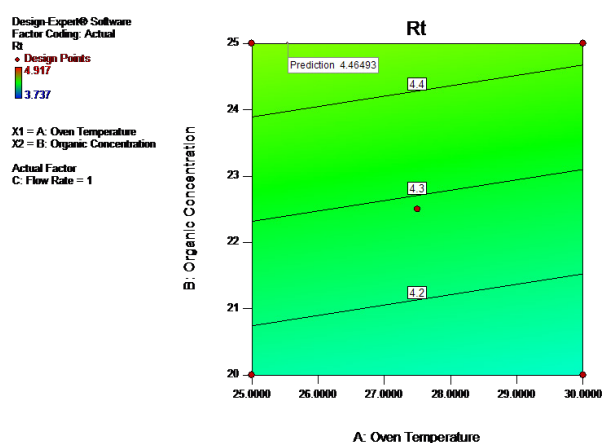

Fig. 7: Contour plot of NTP

Fig. 8: Contour plot of Rt

Table 7: Obtained solutions for optimized condition

Number	Oven temperature	Organic concentration	Flow rate	NTP	Peak symmetry	Desirability
1	25.000	24.952	1.031	6671.988	0.998	0.9854

Table 8: Precision studies

Concentration ppm	Area (n = 6)	Std Dev	% RSD	Mean RSD
8	415585.5	495.6518	0.119266	
12	606419.8	1321.209	0.21787	0.121064
16	797341.3	207.7592	0.026056	

Table 9: Accuracy studies

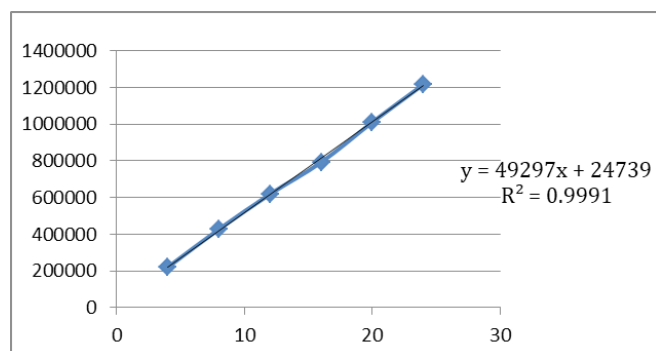| Sample	Formulation (µg/mL)	Pure drug (µg/mL)	% Recovery	Average	% RSD
S1: 80%	10	8	99.28		
S1: 80%	10	8	99.28	100.04	0.15
S1: 80%	10	8	99.28		
S2: 100%	10	10	99.18		
S2: 100%	10	10	99.03	99.63	0.23
S2: 100%	10	10	100.7		
S3: 120%	10	12	99.59		
S3: 120%	10	12	99.49	100.26	0.25
S3: 120%	10	12	101.72		
Overall average				99.98	
Overall SD				0.31	
Overall % RSD				0.31	

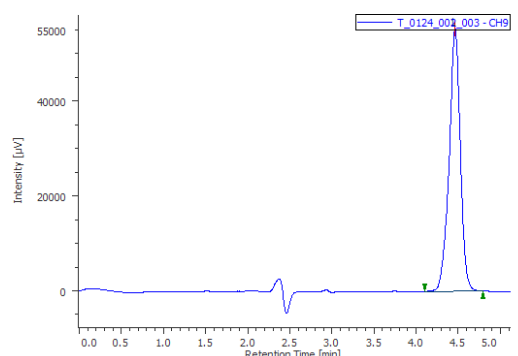**Fig. 9:** Linearity curve of transfer factor at 310 nm

Fig. 10: Chromatogram of transfer factor for Specificity

there is no interference as it showed a clear peak without any another peak.

Ruggedness

It showed an average percentage 99.089 which is within range and standard deviation 0.185 which is less than 2, indicating that the proposed method is satisfying criteria of ruggedness (Table 10).

Robustness

The results of robustness studies by varying mobile phase composition and wavelength are shown in (Table 11). RSD less than 2 indicated that the method is robust.

Solution Stability Studies

Table 12 shows the results for solution stability, and

Table 10: Ruggedness studies

Sr.No.	Analyst-1	Analyst-2
Sample -1	99.40	99.09
Sample -2	98.48	99.54
Sample -3	98.71	100.68
Sample -4	98.69	99.09
Sample -5	98.61	99.31
Sample -6	98.38	99.09
Average	98.71	99.46
SD	0.3593	0.621
%RSD	0.363	0.624
Overall average		99.089
Overall SD		0.185
Overall %RSD		0.186

Table 11: Robustness studies

Sample ID	Normal condition	Low λmax 309 nm	High λmax 311 nm	Low composition (74:24)	High composition (76:26)
Sample 1	99.40	98.6	98.8	100.3	99.4
Sample 2	98.48	99.7	99.2	99.1	99.0
Sample 3	98.71	98.5	100.9	99.7	99.5
Sample 4	98.69	-	-	-	-
Sample 5	98.61	-	-	-	-
Sample 6	98.38	-	-	-	-
Average	98.71	98.9	99.6	99.7	99.3
SD	0.3593	0.67	1.12	0.60	0.26
% RSD	0.363	0.67	1.12	0.60	0.27
Overall Average	-	98.9	99.1	99.1	99.0
Overall SD	-	0.35	0.69	0.52	0.28
Overall % RSD	-	0.36	0.69	0.53	0.28

Table 12: Solution Stability

Time Interval (hrs)	Area	% TF
Initial	514781	99.4
8	510254	98.48
16	511395	98.71
24	511287	98.69
Correlation (8)		0.98
Correlation (16)		0.987
Correlation (24)		0.986

Table 13: Results of estimation of TF in tablet dosage form by HPLC method

Tablet formulation	Label claim (mg/tab)	Label claim estimated (mg/tab)	Peak area	% RSD
Tofisopam	50	49.7 ± 0.0577	6672 ± 13.04	0.62

Table 14: System suitability

Parameters	Tailing factor	Theoretical plate	% RSD
Specificity	0.934	5895.66	0.200
Precision	1.009	6674.66	0.121
Linearity (range)	0.997	6157.33	0.67
Accuracy	1.02	6665.33	0.313
Solution stability 24 hours	0.934	5928.667	0.397
Ruggedness	0.925	5895.667	0.30
Robustness	0.93	5928	0.363

correlation was found to be less than 2,[11] indicating that TF is stable in acetonitrile: water (75:25).

Assay

Results of assay are shown in Table 13. The percentage of RSD was 0.62 which is within limits.

CONCLUSION

Tofisopam is an antianxiety drug. In this study, the concentration of organic phase, oven temperature, flow rate were considered as a qualitative variable that was controlled with three independent variables peak symmetry, retention time, and NTP. The experimental design suggested the robust MODR region for the TF HPLC method developed. All the validated parameters were within the acceptable criteria of ICH guidelines. Thus a simple, rapid, selective, precise, accurate, and robust HPLC method was developed for the estimation of TF in pure form and its tablet dosage form.

ACKNOWLEDGMENT

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