10.37021/ijper.v5i1.03

RESEARCH ARTICLE

Analytical Method Development and Validation of Ubrogepant and their Degradation

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ABSTRACT

An accurate, rapid economical and straight forward, reliable new analytical method was developed and validated for the quantification of Ubrogepant and their degradation studies by using RP-HPLC.In the proposed method efficient chromatographic separation was achieved by using Ammonium aecetate buffer with pH adjusted to 5.0 with dilute Orthophosphoric acid solution and methanol (30:70 v/v) as a mobile phase with a flow of 1 ml/min and Water and Acetonitrile(30:70) is used as a diluent, the wave length was observed at 280 nm with isocratic mode at ambient temperature and run time was approximately 10 min and the retention time (Rt) of Ubrogepant was observed as 4.570min.

The method validated as per ICH guidelines. System suitability parameters were studied by injecting six standard solutions of Ubrogepant and results were well under acceptance criteria. Linearity study was administered between 25% to 150% levels, Regression coefficient value was observed as 0.999. LOD and LOQ were observed as 0.02 ug/ml and 0.2 ug/ml, respectively. Precision was found to be 0.54 for repeatability and 0.33 for intermediate precision. Recovery of the drug was found to be 100.86% indicates that the recovery is in the acceptable limit. Stress conditions of degradation in Acidic, Alkaline, Peroxide and Thermal were studied for Ubrogepant and validation results were found to be satisfactory and the proposed method was suitable for regular analysis and quality control of pharmaceutical preparations.

Keywords: Ubrogepant, validation, RP-HPLC, Stress studies.

How to cite this article: Balasekhara Reddy Ch., Kumari M.V., Eswaramma P., Analytical Method Development and Validation of Ubrogepant and their Degradation Studies in Bulk and Pharmaceutical Dosage form by RP-HPLC. Int. J. Pharm. Edu. Res. 2023;5(1):13-19.

Source of support: Nil

Conflict of interest: None

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INTRODUCTION

Ubrogepant is an oral CGRP(Calcitonin gene-related peptide) receptor and it is indicated for the acute migraine headaches with or without aura in adults.[5] Several oral small molecule CGRP receptor antagonists belonging to class of medications referred as gepants have been investigated for migraines but ubrogepant and rimegepant remain in clinical development.[3,4] Triptans such as sumatriptan and almotriptan, CGRP antagonists present several advantages.[1] Several parenteral monoclonal antibodies acting against the CGRP pathway (e.g. erenumab, fremanezumab, galcanezumab) have also been approved in recent years.[3] The development of oral gepants, including ubrogepant, may therefore constitute a significant advance in migraine headache treatment. Some side effects of Ubrogepant are nausea, Drowsiness, rash,itching,swelling of the face,tongue or throat,severe dizziness.

Structure of Ubrogepant

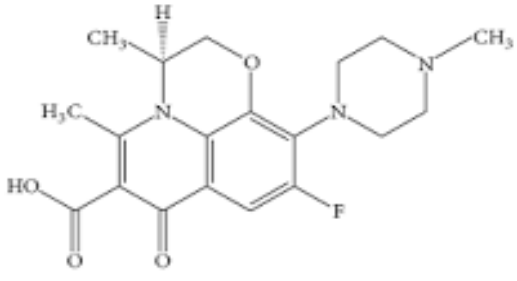

Figure 1: Chemical structure of Ubrogepant

MATERIALS AND METHODS

Instrumentation

The details of the HPLC instrument used in the development and validation are Waters Company, alliance model and e2695 model number with quaternary pump, PDA detector of 2996 is used. The chromatographic software used was Empower version 2.0.

Chemicals and reagents

Ubrogepant was procured from Aurobindo Pharma Pvt Ltd., Hyderabad. Commercial Pharmaceutical preparations which were claimed to contain 50 mg of Ubrogepant tablets was used in analysis.

Preparation of Dilute Phosphoric Acid solution

5mL of Orthophosphoric Acid (85%) was transferred and diluted to 100ml volumetric flask make up with water.

Preparation of Mobile Phase

PH 5.0 Buffer preparation

Add about 1.90gm of Ammonium aecetate into 1liter of water Adjust the pH to 5.0 with dilute orthophospharic acid solution.

Mobile Phase

Mix Buffer and Methanol in the ratio of 30:70(v/v) respectively.

Diluent

Mixture of Water and Acetonitrile in the ratio of 30:70(v/v) respectively.

Preparation of Standard Solution

Standard Stock Preparation

Weigh accurately 54mg of Ubrogepant working standard was transferred into a 50mL volumetric flask. 35mL of diluent was added, sonicated to dissolve and upto volume with diluent.

Standard Preparation

4ml of standard stock solution was pipette into a 50 mL volumetric flask and the volume was made upto mark with diluent. Filter about 5ml through 0.45µm pall pharma lab nylon 66 membrane filter or 0.45µ Durapore PVDF hydrophilic membrane filter.

Sample Preparation

Weigh and transfer 10Tablets of Ubrogepant into a 500 ml volumetric flask and add about 50ml of water and sonicate the solution to disperse the coating layer for about 5minutes at room temperature, add about 300ml of diluents and sonicate for 45minutes at room temperature and shake for 5minutes and make up the volume with diluents and mix well. Centrifuge the solution at 10000 rpm for about 10 minutes. Further dilute 7ml of clear transparent solution to 50ml with diluents and mix well.Filter the solution through 0.45 µ membrane filter (whattman PVDF or whattman Nylon).

CHROMATOGRAPHIC CONDITIONS

Chromatographic separation for Ubrogepant was administered in isocratic mode at ambient temperature employing a Primesil C18 (150mm x 4.6 mm, 5µm) as a column and the combination of Ammonium acetate and Methanol in ratio of (30:70) V/V used as a mobile phase and Water: Acetonitrile (30:70) used as a diluent with a flow 1 ml/min ,run time was 10min,the volume of injeciton was 20 µl and eluent was observed at 280 nm, these chromatographic conditions are used for the ubrogpant optimized method.The Retention time of Ubrogepant was found to be 4.570min.Assay for Marketed formulation for Ubrogepant was shown in the Table 1.

RESULTS AND DISCUSSION

Table-1: Assay calculation for Marketed formulation

S. No	Formulation (Tablet)	Sample peak area	Standard peak area	Labelled amount (mg/Tab)	Amount Found	%Assay
1	Ubrogepant (Ubrelvy)	2416788	2402744	50mg/ Tablet	50.6mg	101.2%

VALIDATION OF THE PROPOSED METHOD

The developed Method was validated in accordance with the ICH guidelines for the parameters like linearity, precision, accuracy, robustness, ruggedness, forced degradation and stability studies for Ubrogepant.

System Precision and System Suitability

The standard solution was prepared by using Ubrogepant working standard as per test method and injected five times into the HPLC system. The system suitability parameters were evaluated and found to be within the limits.

The RSD for peak areas from five replicate injections of Ubrogepant was found to be .3%. The results were summarized in Table 2-3 and the chromatogram for system suitability shown in the (Figure 2).

Table 2: System suitability data for Ubrogepant

S.No	System Suitability Parameter	Observed value	Acceptance criteria
1	The % RSD of peak areas of Ubrogepant	0.3	NMT 2.0
2	The Tailing factor for Ubrogepant peak in standard solution	1.8	NMT 2.0
3	US plate count	6853	NLT 3000

Table 3: System precision data for Ubrogepant

Injection Number	Ubrogepant Peak Area	Acceptance Criteria
01	2387691	The % relative standard deviation of peak areas of Ubrogepant should not be more than 2.0
02	2401134	
03	2403960	
04	2395224	
05	2395224	
Mean	2396646.6	
%RSD	0.2	

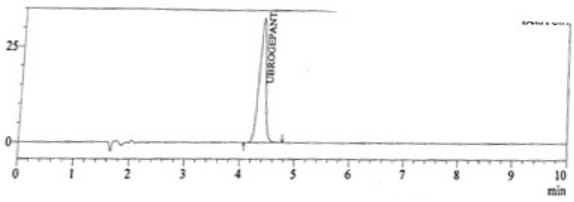

Figure 2: Standard Chromatogram for System suitability

Specificity

Specificity was the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these include impurities, degrades, matrix etc. Placebo interference was checked for one strength in duplicate, equivalent to about the weight of placebo as per the test method. It was observed that there is no interference at retention time of Ubrogepant peak and the results summarized in Table 4 and the chromatograms are shown in the figure (3-5).

Table 4: Specificity study

Name of the solution	Retention time
Blank	No peak
Placebo	No peak
Ubrogepant	4.4min

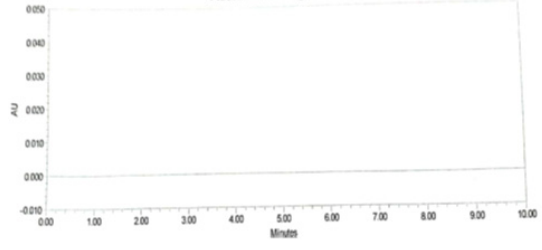

Figure 3: Chromatogram of blank

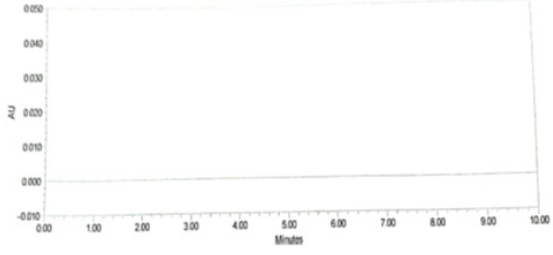

Figure 4: Chromatogram of Placebo

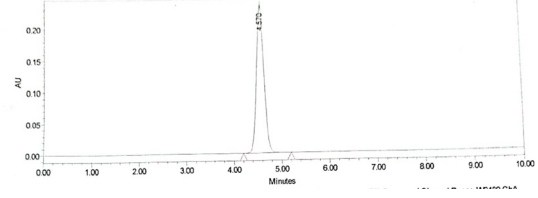

Figure 5: Chromatogram of standard

Forced degradation

Forced degradation studies were performed to determine the stability of Ubrogepant in different stress conditions. Forced degradation conditions such as acidic, basic, peroxide, hydrolysis, reduction and thermal stress were studied in 0.1 N to 1 N concentration levels. The discoveries of such conditions were based on trial and error shown in the Table 5

Table 5: Results of forced degradation studies

S. No.	Stress conditions (1N concentration)	%Assay	Degraded sample area	Peak purity angle	Peak purity Threshold	% degradation
1	Control	100.1	2865196	0.329	5.134	-0.10
2	Acid degradation	67	1918506	0.394	5.134	33.1
3	Alkali degradation	68.6	1964105	0.377	5.141	31.4
4	Peroxide degradation	66.6	1906598	1.964	5.126	33.4
5	Thermal degradation	66.7	1909856	1.348	5.126	33.3

Acid degradation

Weigh 10 Tablets and transfer weight equivalent to 50mg of Ubrogepant transfer to 500ml volumetric flask and add 75ml of diluents and sonicate for 45minutes further add 5ml of 5N HCL heat for 1hr at 850C then cool to room temperature and neutralized the above solution with 5ml 5N NaoH and make up to 100ml with diluent. Take 7ml of above solution and make upto 50 ml with diluents. For Acid degradation study there was no interference with the peak of Ubrogepant.

Alkaline Degradation

Weigh 10Tablets and transfer weight equivalent to 50mg of Ubrogepant transfer to 500ml volumetric flask and add 50ml of water and sonicate for 5minutes further add 300ml diluents and add 5ml of 5N NaoH heat for 1hr at 850C then cool to room temperature and neutralized the above solution with 5ml 5N HCL and make up to 500ml with diluent. Take 7ml of above solution and make upto 50 ml with diluents. For base degradation study there was no interference with the peak of Ubrogepant.

Peroxide Degradation

Weigh 10 Tablets and transfer weight equivalent to 50mg of Ubrogepant transfer to 500ml volumetric flask and add 50ml of water and sonicate for 5minutes further add 300ml diluents and sonicate for 30min further add 5ml of 30%H2O2 heat for 60min at 850C then cool to room temperature and neutralized the above solution with 5ml1N HCL and make up to 500ml with diluent. Take 7ml of above solution and make upto 50 ml with diluents. For peroxide degradation study there was no interference with the peak of Ubrogepant.

Thermal Degradation

Weigh 10 Tablets and the powder exposed to heat at 1050 C for 48 hrs transfer weight equivalent to 50mg of Ubrogepant transfer to 500ml volumetric flask and add 50ml of water and sonicate for 5minutes further add 300ml diluents, then make up the solution upto 500 ml with diluent. From the stock solution take 7ml and make up to 50ml with diluent. For Thermal degradation study there was no interference with the peak of Ubrogepant.

Linearity

A series of solutions were prepared by using Ubrogepant working standard at concentration levels from 25%to150% of test concentration and each solution was injected into HPLC.

Procedure

A graph was plotted to standard peak area obtained versus "Actual concentration of standard" in linearity of the detector response section. The correlation coefficient was found to be 0.999. From the above study it was established that the detector linearity is from 25% to 150% of the target assay concentration. A calibration curve was plotted for concentration v/s peak area and the results of linearity was discussed. The results of linearity was shown in the Table 6 and (figure 6).

Table 6: Linearity of Ubrogepant

S.No	Spike level	Standard concentration(ppm)	Area
1	25%	20.117	614897
2	50%	40.235	1227173
3	80%	60.352	1824163
4	100%	80.470	2416788
5	120%	100.587	3028153
6	150%	120.705	3600446
Correlation coefficient			0.9999
Slope (m)			29715
Intercept (b)			26288
Bias for 100% response			1.09

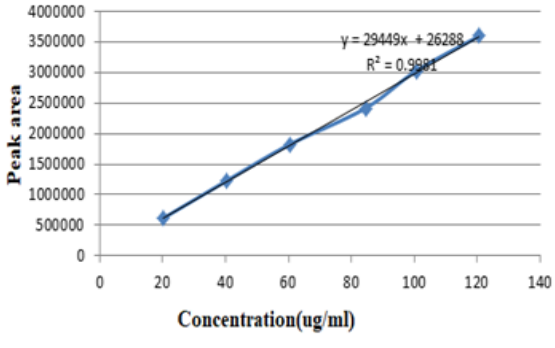

Figure 6: Linearity plot of Ubrogepant

Accuracy

Recovery

A study of accuracy was conducted drug assay was performed in six times for lower and higher levels and triplicate for remaining levels by adding Ubrogepant drug substance with the equivalent amount of placebo at 50%,100%and 150% of the targeted assay concentration into each volumetric flask.

The average % recovery was found to be wit in the limits .The % relative standard deviation of recovery of Ubrogepant at 50% and 150% level was found to be within the limits .

The results were summarized in Table 7. The Chromatograms were represented in the (Figure 7-9).

Table 7: Results of accuracy of Ubrogepant

Sample no	*Spiking level at about (in %)*	*Amount of Ubrogepant added (ppm)*	*Amount of Ubrogepant Recovered (ppm)*	*% Recovery*	*%Mean Recovery*
1	50%	23.21	24.03	100.4	100.4
	50%	23.88	24.12	101.03	
	50%	23.69	24.06	99.9	
2	100%	47.02	47.59	100.8	100.86
	100%	47.09	47.65	101.20	
	100%	47.03	47.64	100.6	
3	150%	69.71	70.18	100.9	100.6
	150%	69.81	70.21	100.57	
	150s%	69.79	70.19	100.4	

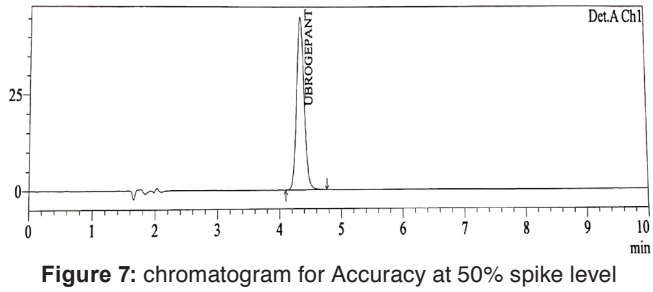

Figure 7: chromatogram for Accuracy at 50% spike level

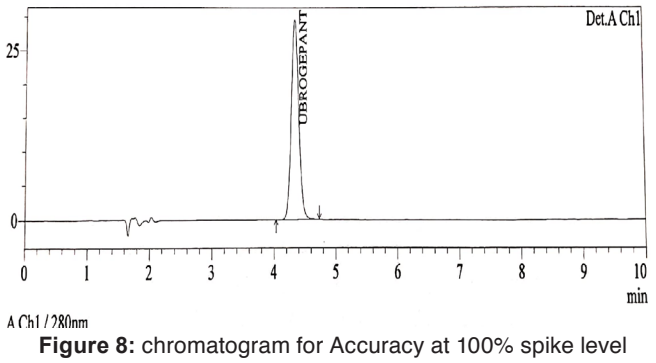

Figure 8: chromatogram for Accuracy at 100% spike level

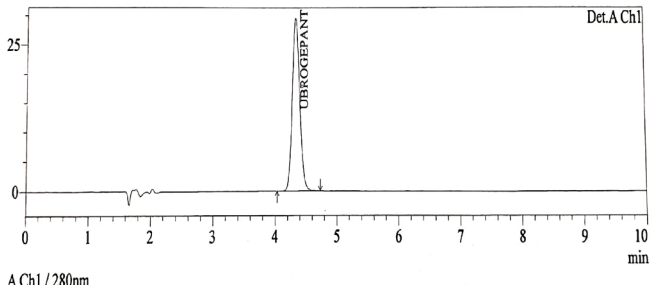

Figure 9: Chromatogram for Accuracy at 150% spike level

Precision

Precision is the degree of repeatability of an analytical method under an operation conditions. Precision is of three types.

- System precision
- Method precision
- Intermediate precision

By using the standard solution system precision is checked to know that the analytical system is working properly. In system precision response of the drug and % RSD should be measured. By using the single batch homogeneous sample method precision was analyzed 6 times. This indicates whether a method is giving constant results for a single batch. In this analyze the sample six times and measure the % RSD.The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 80ppm of Ubrogepant.

Method precision

Precision of the test method was determined by injecting six samples prepared by spiking Ubrogepant raw material with the equivalent amount of placebo or on the tablets. The results were given in Table 8.

Table 8: Repeatability data for Ubrogepant

Sample Name	Peak Retention Time (Rt)	Day1
Sample-1	4.220	100.3
Sample-2	4.122	99.0
Sample-3	4.425	100.3
Sample-4	4.126	99.7
Sample-5	4.128	99.2
Sample-6	4.230	99.8
Average	4.2085	99.72
SD		0.5384
%RSD		0.54

Intermediate precision

Intermediate Precision of assay method was conducted on Ubrogepant tablets using two different systems by different analysts using the different columns and analyzed under Day1 and Day2 similar conditions as per the test method. The results were summarized in Table 9.

Table 9: Intermediate precision data of Ubrogepant

Sample Name	Peak retention time (Rt)	Day1	Day2
Sample-1	7.220	100.3	99.4
Sample-2	7.122	99.0	97.15
Sample-3	7.125	100.3	101.65
Sample-4	7.126	99.7	99.21
Sample-5	7.128	99.2	99.52
Sample-6	7.230	99.8	97.57
Average		99.72	99.8
%RSD		0.54	0.33

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ of the drug was calculated by using following equation as per ICH guidelines. The LOD and LOQ were evaluated by serial dilution of Ubrogepant stock solution in order to determine s/n ratio 3:1 for LOD and 10:1 for LOQ. The concentration of LOD and LOQ for Ubrogepant were listed in Table 10.

LOD = 3.3 σ/s and LOQ = 10 σ/s

Table 10: Sensitivity parameter values

Name of the drug	LOD (µg/ml)	LOQ (µg/ml)
Ubrogepant	0.02	0.2

RUGGEDNESS

Analyst to analyst/ System to system/ Column to column variability

Ruggedness of assay method was conducted on Ubrogepant using two different systems by different analysts using the different columns and analyzed under similar conditions as per the test method. Comparison of the results obtained on two systems showed that the assay of tablets method is rugged for system-to-system variability and column to column variability and analyst to analyst variability. The results of Ruggedness are summarized in the Table 11.

Table 11: Ruggedness results for (Analyst-1, Analyst-2)

S.No.	System suitability	Observed value		Acceptance criteria
		Analyst-1	Analyst-2	
1	%RSD for Ubrogepant in standard solution	0.3	0.2	NMT 2.0%
2	The Tailing factor for Ubrogepant peak in standard solution	1.9	1.8	NMT 2.0

SOLUTION STABILITY

Bench top stability and Refrigerator stability

Standard and test solutions were prepared in duplicate by using Mycophenolate mofetil working standard and

Ubrogepant tablets. By keeping the preparations on bench top and refrigerator these were injected at initial, after 1st day, 2nd day and 7th day. If the bench top stability fails at 24hrs then the study was conducted on hourly basis. Similarity factor for standard solution was calculated.

The % difference in % Assay results from initial to 1st, 2nd and 7th day was calculated. For Ubrogepant bench top stability at 24hr has got failed so hourly analysis is carried for each hour solution stability has carried out for each hour and for about 21hours.

Bench top stability of mobile phase

Five replicate injections of standard preparation were injected into HPLC system using mobile phase stored on bench top for day 1 day 2 and day 7. %RSD was calculated and system suitability was evaluated. Assay of test preparation was performed in duplicate and results for individual preparations were calculated. The average value for two preparations was reported. The difference in assay results for each day against initial was determined. If mobile phase was found to be not stable for 1 day, then the study at short intervals was conducted. The results of solution stability data was summarized in the Tables 12,13.

Robustness

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method. The result of the Robustness study of the developed assay method was established in Table 14 and 15.

Table 12: Bench Top Stability Include Similarity Factor

Time in Hours	% Ubrogepant in bench top sample preparation		
	Area	%Difference from initial	%Absolute difference from initial
Initial	2382226	NA	NA
After 2 HR	2391481	-0.39	0.39
After 4HR	23929048	-0.41	0.41
After 6HR	2397620	--0.65	0.65
After 8HR	2399005	-0.70	0.70
After 10HR	2399239	-0.71	0.71
After 12HR	2397745	-0.65	0.65
After 14HR	2391948	-0.41	0.41
After 16HR	2395056	-0.54	0.54
After 18HR	2394193	-0.50	0.50
After 20HR	2399669	-0.73	0.73
After 24HR	2402744	-0.86	0.86
After 32HR	2418175	-1.51	1.51
After 36HR	2420572	-1.61	1.61
After 40HR	2430585	-2.03	2.03
After 44HR	2440774	-2.46	2.46

Table 13: bench top stability include similarity factor

Time in Hours	% Ubrogepant in bench top standard preparation		
	Area	%Difference from initial	%Absolute difference from initial
Initial	2387691	NA	NA
After 3 HR	2401134	-0.56	0.56
After 5HR	2403960	-0.68	0.68
After 7HR	2395224	-0.32	0.32
After 9HR	2394614	-0.29	0.29
After 11HR	2396400	-0.36	0.36
After 13HR	2395289	-0.32	0.32
After 15HR	2400939	-0.55	0.55
After 17HR	2409709	-0.92	0.92
After 19HR	2408160	-0.86	0.86
After 21HR	2410938	-0.97	0.97
After 25HR	2419122	-1.32	1.32
After 33HR	2425680	-1.59	1.59
After 37HR	2429795	-1.76	1.76
After 41HR	2450082	-2.61	2.61
After 45HR	2461059	-3.07	3.07

Table 14: Results of Filter validation

	Standard solution		Sample solution	
S.no	Area	%Difference	Area	%Difference
1.Unfiltered	2411398	NA	2397849	NA
2.PVDF Filtered	2410003	0.06	2383043	0.41
3.Nylon Filtered	2401049	0.43	2402035	0.36

Table 15: Robustness studies for Ubrogepant

S.no	CONDITION	%ASSAY	%RSD
1	Flow rate (1.2ml/min)	99.2	0.38
2	Flow rate(1ml/min)	100.2	0.38
3	Flow rate(0.8ml/min)	100.7	0.11
4	Organic phase composition (40:60)	100.4	0.75
5	Organic phase composition (30:70)	100.2	0.38
6	Organic phase composition (20:80)	100.7	1.56

Filter Validation

Standard solution of Ubrogepant tablets were prepared as per the method. These solutions were subjected to centrifugation, filtration through 0.45µ Millipore PVDF and Nylon filters. The centrifuge /unfiltered and filtered standard/samples were analyzed as per methodology.

Flow rate was varied at 0.8 ml/min to 1.2 ml/min

Standard solution (20 ppm of Ubrogepant) was prepared and analyzed using the varied flow rates along with the method flow rate. On evaluation of the above results, it can be finalized that the change in the flow rate affected the method significantly. Hence it shows that the technique is robust even by change in the flow rate is ±2.

Variation of organic phase ratio

Standard solution (20ppm of Ubrogepant) was prepared and analyzed using the varied mobile phase ratio. On evaluation of the above results it can be finalized that the change in the mobile phase ratio affected the method significantly. Hence it shows that the method is robust even by change in the mobile phase is ±10%.

CONCLUSION

This method describes the quantification of Ubrogepant in all categories as per ICH guidelines. The developed method was found to be accurate, precise, linear and reliable. The advantage lies within the simplicity of sample preparation and therefore the the less costly reagents were used. The proposed HPLC conditions ensure sufficient resolution and therefore the precise quantification of the compounds

The author developed a new stability indicating RP-HPLC method using Primesil C18 (150mm x 4.6 mm, 5µm. column with mobile phase Mix Ammonium acetate and Methanol in ratio of (30:70) V/V and the Ammonium acetate buffer adjusted with OPA to pH 5.0 in the ratio of (50:50) and run in isocratic mode. Flow rate was 1.0ml/min, with injection volume 20ul detection done by using PDA detector at 280nm.The runtime was 10min and the Retention time was 4.50 min which enables rapid quantitization of many samples in routine and quality control analysis of Tablet formulation.

The method was validated according to ICH guidelines by using various validation parameters like linearity, accuracy, precision, specificity, robustness and solution stability.

From the specificity study it was concluded that the developed technique was specific and the degradation studies under various conditions concluded that the degradation was observed in sufficient quantity and it was the stability indicating method. Statistical analysis of the experimental results indicates that the precision and reproducibility data are satisfactory. The developed chromatographic method was often effectively applied for routine analysis in drug research.

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