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Quality by Design Approach for Development and Validation of Stability Indicating RP-HPLC Method for Fosaprepitant Dimeglumine

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Quality by design approach has been used to develop simple, rapid, sensitive gradient RP-HPLC stability indicating method for fosaprepitant dimeglumine and its related impurities. The chromatographic method has been developed by using symmetry shield RP-18 (250 mm × 4.6 mm; 5 µm) column maintained at column temperature of 20 ºC. The mobile phase-A consisted of water and acetonitrile (800:200, v/v), added 2 mL of orthophosphoric acid and 0.17 g of tetrabutylammonium hydrogen sulphate. The mobile phase-B consisted of water and acetonitrile (200:800, v/v), added 2 mL of orthophosphoric acid and 0.17 g of tetrabutylammonium hydrogen sulphate. Gradient program was executed as time (min)/% MP-A: 0/80, 3/80, 12/40, 20/20, 24/20, 25/80, and 30/80. The UV detection was carried out at wavelength 210 nm and 20 µL of sample was injected. Sample cooler was maintained at 5 ºC. Stability of fosaprepitant dimeglumine sample was investigated in different stress condition as acid, base, oxidation, thermal, humidity and photolytic. The method was developed in two phases, screening and optimization. During the screening phase, the most suitable stationary phase, organic modifier, and solvent were identified based on the behaviour of each stationary phase with fosaprepitant dimeglumine and its impurities using each buffer and solvent. Total 18 experiments were performed to find out the best experimental condition. The optimization was done for secondary influential parameters like column temperature, gradient program, using six experiments to examine multifactorial effects of system suitability parameters and generated design space representing the robust region. A verification experiment was performed within the working design space and the model was accurate. Drug showed unstable behaviour under acid, base, oxidation, thermal, and humidity conditions. Apripetant was found as major degradation impurity. The method was validated as per ICH guideline for specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity, accuracy, precision, ruggedness and robustness. Correlation coefficient is about 0.999 for all impurities, recovery is between 90% to 103% at all level. LOD value of each impurity is less than 0.01% w/w. DOE statistically based experimental designs proved to be an important approach in optimizing selectivity-controlling parameters for the organic impurities determination in FD API. The method was found to be specific, linear, accurate, precise and robust. The peak purity test results confirmed that the fosaprepitant dimeglumine peak was homogenous in all stress samples and the mass balance was found to be more than 99%, thus proving the stability indicating power of the method. Present method is found to be suitable for routine analysis in quality control laboratory.

Keywords: Quality by Design, Fosaprepitant dimeglumine, Monobenzyl impurity, Aprepitant, HPLC.

INTRODUCTION

Fosaprepitant dimeglumine, chemical name as 1-doexy-1-(methylamino)-D-glucitol [3-[[2R,3S)-2-[(1R)-1-[3,5-*bis*-(trifluoromethyl)phenyl]ethoxy]-3-(4-flurophenyl)-4-morpholinyl]methyl]-2,5-dihydro-5-oxo-1*H*-1,2,4-triazol-1-yl]phosphonate (2:1) (salt), with molecular weight 1004.83 and m.f. $C_{23}H_{21}F_7N_4O_6P{\cdot}2(C_7H_{17}NO_5)$ is from class of anti-emetic therapeutic category, an intravenous neurokinin-1 antagonist used for prevention of chemotherapy induced nausea and vomiting [1,2]. It is pro-drug of aprepitant (APT) (Fig. 1) and converts as APT in body, which has unique mode of action and selective high affinity antagonist at the human substance Neurokinin-1 (NK1) receptors [3,4]. Moreover, it is 3,000-fold selective for the (NK1) receptor over the other enzyme, transporter, ion channel and receptor site [5-7]. Fosaprepitant

dimeglumine can be substituted for oral aprepitant in day 1 of a 3-day regimen. From the study, it is proved that a single day fosaprepitant dimeglumine regimen is also bioequivalent to the 3-day APT regimen. This could significantly beneficial for patients in future in chemotherapy [8]. It is available as lyophilized powder in single dose vial for reconstitution and each vial is having 150 mg of equivalent fosaprepitant. Studies show that < 0.0002 mg/mL of aprepitant (APT) forms in a fosaprepitant dimegl-umine sample preparation that stored for the period of 19 h at 5 ºC [9].

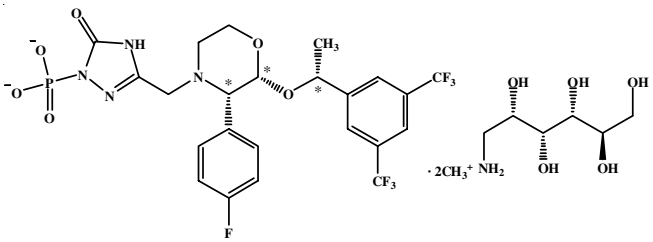

Fig. 1. Chemical structure of fosaprepitant dimeglumine

Initial literature search shows that only one RP-HPLC method was available for quantification of impurities in fosaprepitant dimeglumine drug substances [10], which consist of long run time of 45 min and do not represent life cycle management. Simultaneous determination of aprepitant and fosaprepitant dimeglumine in plasma by HPLC was reported by Xu *et al.* [11]. Preliminary experiments for optimization were performed as per the available procedure in literature, but was not suitable in terms of peaks resolution and shape for both monobenzyl impurity and aprepitant. As no other detailed RP HPLC method were reported, we initiated the development as per the general concept of the HPLC method [12,13].

In present study, fosaprepitant dimeglumine, aprepitant, monobenzyl impurity and dibenzyl fosaprepitant (Fig. 2), were considered for analysis. For the any active pharmaceutical ingredient (API), single maximum unknown impurity should be control below 0.1% level. Known impurity can be decided based up on its toxicology data and biological safety. For any impurity reporting threshold should be 0.05%, Identification threshold should be 0.10% and qualification threshold should be 0.15%, these values vary based on daily intake of the drug [14]. For aprepitant specification can be higher as fosaprepitant dimeglumine is prodrug of aprepitant. This developed method is capable of separating all related impurities with good resolution and the method has been validated as per ICH guidelines [15]. This procedure can be used for regular analysis of all the process related and stress generated impurity in stability and forced degradation in the fosaprepitant dimeglumine. Moreover, validated stability indicating method should be applied in the stability study [16].

EXPERIMENTAL

HPLC grade water, acetonitrile HPLC grade, tetrabutylammonium hydrogen sulphate (TBAHS) and ortho-phosphoric acid purchased from Merck India Limited. Working standards of fosaprepitant dimeglumine, impurities and test samples obtained as donation for research purpose. High purity deionized

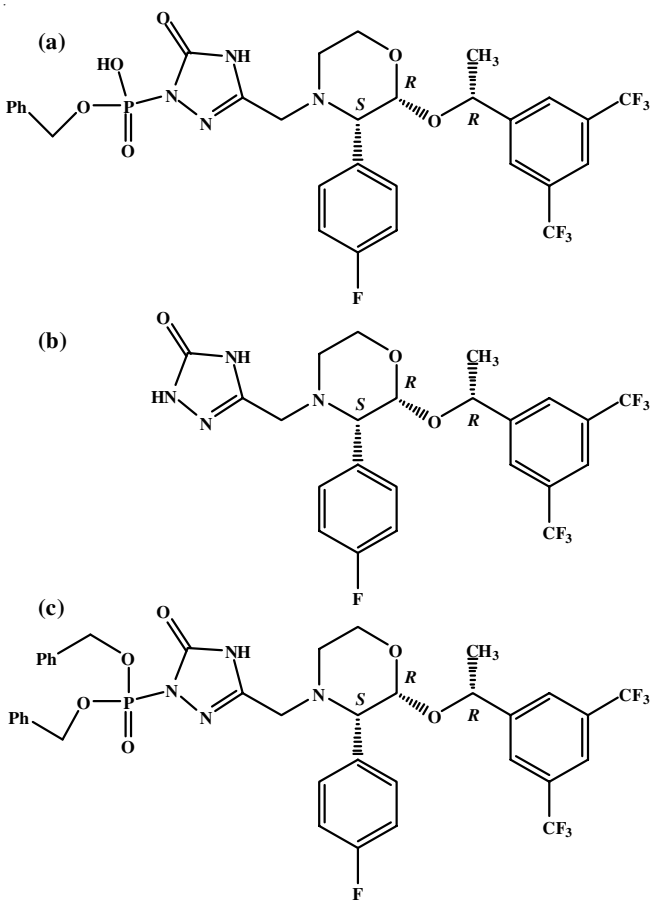

Fig. 2. Structure of (a) monobenzyl impurity, (b) aprepitant, (c) dibenzyl fosaprepitant

water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system.

Instrumentation: HPLC system Waters 2489 with UV/visible detection, auto-sampler HPLC system USA, consisting of empower software build 2154 SPs Installed, quaternary system with pump model 270782 and PDA detector were employed for analysis. Chromatographic data was acquired using Empower software.

Chromatographic conditions: Symmetry shield RP-18 (250 mm × 4.6 mm; 5 µm) (Thermo, USA) column was used as a stationary phase. Gradient elution at a flow rate of 1.2mL/min was employed for the separation, column temperature kept 20 ºC. Gradient was mixture of mobile phase-A (MP-A) and mobile phase-B (MP-B). The mobile phase-A consisted of water and acetonitrile (800:200, v/v), added 2 mL of orthophosphoric acid and 0.17 g of TBAHS. The mobile phase-B consisted of water and acetonitrile (200:800, v/v), added 2 mL of ortho-phosphoric acid and 0.17 g g of TBAHS. Gradient program was executed as time (min)/% MP-A: 0/80, 3/80, 12/40, 20/20, 24/20, 25/80, and 30/80. The UV detection was carried out at wavelength 210 nm and 20 µL of sample was injected. Sample cooler was maintained at 5 ºC.

Standard solution: Accurately weighed and transferred about 50 mg of fosaprepitant dimeglumine working standard in 50 mL volumetric flask, containing 25 mL of acetonitrile: water in the ratio of 1:1 (diluent) and flask was sonicated to

obtained clear solution. Further, it was diluted to made up to 50 mL with diluent.

Sample solution: Accurately weighed and transferred about 50 mg of fosaprepitant dimeglumine sample in 50 mL volumetric flask, containing 25 mL of diluent and flask was sonicated to obtained clear solution. Further, it was diluted to made up to 50 mL with diluent.

System suitability: Prepared fosaprepitant dimeglumine sample at 1.0 mg/mL and spiked the related impurities at 0.1% w/w.

Method validation: This method was validated for linearity, precision, intermediate precision, accuracy and robustness. The specificity of the method was determined by injecting the system suitability solution, impurity mixture, standard solution and degradation solution. This ensure that method is capable of separating all impurity and active pharmaceutical ingredients without any interference.

System suitability: The solution containing mixture of fosaprepitant dimeglumine and related impurities was injected for resolution and standard solution in replicates to check the system suitability criteria. The criterion includes the following parameters like percentage RSD, resolution, and tailing factor. The criterion is as follows: resolution should be greater than 1.5, RSD value should not exceed 2%, the tailing factor should be in between 0.7-1.5.

Linearity: Mixture of five solutions of fosaprepitant dimeglumine, aprepitant, monobenzyl impurity and dibenzyl fosaprepitant were prepared for the linearity in the range of 0.00015-0.0015 mg/mL level. Each solution was injected in three replicates and linear was calculated from calibration curve. The limit of detection (LOD) of impurities were determined from the regression data of calibration curve by using formula as LOD = 3.3 (SD)/S, where SD is the average residual standard deviation and S is slope of the calibration curve. Limit of quantitation (LOQ) was calculated using the formula LOQ = 10(SD)/S.

Accuracy: Known amount of the impurities were spiked from LOQ to 150% of specification in fosaprepitant dimeglumine and calculated against the impurity standard solution. The accuracy results were reported as percentage recovery with difference in results from actual concentration and recovered concentration. Acceptance criteria of the result was kept as mean recovery should be in the range of 85% to 115% and %RSD should be not more than 5.

Precision: Precision was checked by carrying out six spiked determination of related impurities in fosaprepitant dimeglumine at specification level and calculated results against impurity standard solution. %RSD criteria was used to evaluate the study.

Robustness: The flow rate of mobile phase was changed (± 0.2 mL/min) from 1.2 mL/min to 1.0 mL/min and 1.4 mL/min. The organic strength varied by ±2% units of minor components. System suitability solution was injected in six replicates for each change. Ensured that system suitability parameter should pass for each study.

Specificity: The forced degradation study of the method was carried out for aqueous hydrolysis, acid hydrolysis with 1.0 N HCl, oxidative degradation with 3% peroxide, base hydrolysis with 1.0 N NaOH, thermal degradation at 60 ºC for 3 days. Photo-stability was conducted with the sample exposed to UV and sunlight. Sample was kept with covered and uncovered with aluminium foil. In aqueous degradation, 100 mg of sample was diluted to 10 mL and refluxed at 60 ºC for 7 h. In acid hydrolysis, 100 mg of sample was dissolved in 10 mL of 1 N HCl and sample was refluxed at 60 ºC for 1 day. Before the analysis, sample solution was neutralized with 1 N of NaOH solution and appropriate dilution was given to achieve the test concentration. Oxidative degradation was carried out by using 3% of H_2O_2, about 100 mg of sample was dissolved in 10 mL of 3% of H_2O_2 and made up to the mark with diluent. The solution was analyzed after appropriate dilution with mobile phase. For base hydrolysis, 100 mg of NaOH and the sample was the refluxed at 60 ºC for 1 day. Before analysis, the sample solution was neutralized with 1.0 N acetic acid, then it was diluted to get the test concentration.

In thermal degradation, 1 g of the sample was spread as a uniform thin layer in petri dish and then kept in the oven at 60 ºC for 3 days. Finally, sample was diluted to get final test concentration. In photostability study, 1 g of the sample was spread as a uniform thin layer in petri dish and kept under UV and sunlight for 7 h. Finally, sample was diluted to get final test concentration. In humidity, 1 g of sample was kept under 85º/85% RH in humidity chamber for 1 day, then sample solution was prepared and diluted to get final test concentration.

RESULTS AND DISCUSSION

Quality by Design (QbD) approach for method development: As per the recent communication from FDA on Quality by Design (QbD) approach for method development, initiated the development of method by using QbD approach [17]. In this approach, development was carried out in two phases.

Screening (phase-1): In the screening process, different experiments were performed by using different column, mobile phases and solvent composition. A total of 18 experiments were performed (3 columns × 3 buffers × 2 solvent) as shown in Table-1. Three different columns were tried to understand the behaviour of each stationary phase with fosaprepitant dimeglumine and its impurities using each buffer and solvent. Total 18 experiments were performed to find out the best experimental conditions. The effects of change in resolution were investigated thoroughly by conducting different experiments. The type of reverse phase ion pair chromatography was used for the separ-

TABLE-1
PHASE-1 SCREENING EXPERIMENT

Columns	Buffers	Solvent
ACE C-18 AR (250 mm × 4.6mm × 5 µ)	KH_2PO_4 and octane sulphonic acid/ TBAHS	Methanol and acetonitrile
Symmetry shield C-18 (250 mm × 4.6 × 5 µ)	Ammonium acetate and octane sulphonic acid/TBAHS	Methanol and acetonitrile
Zorbax SB C-18 (250 mm × 4.6 mm × 5 µ)	Orthophosphoric acid and octane sulphonic acid/TBAHS	Methanol and acetonitrile

ation of ionic and acidic-basic compound. It was concluded that symmetry shield C18 column with mobile phase A as water: acetonitrile (80:20, v/v) with orthophosphoric acid and TBAHS and mobile phase B as acetonitrile:water (80:20, v/v) with orthophosphoric acid and TBAHS was found to be more suitable in terms of peak shape, tailing and resolution. Hence this condition was selected for optimization (phase-2).

Optimization (phase-2): From screening process (phase-1), best selected condition was further evaluated for final method optimization. In this study, different gradient programs with change in temperature were executed by performing six experiments: three gradient programs with two different temperature. From these experiments best chromatographic condition was selected and sample was subjected to specificity, method validation, solution stability to prove the method is capable for its extended use. This developed method was having sharp narrow peak shape with more than 1.5 resolution, tailing about 1.0 and relative retention time of aprepitant 1.26, monobenzyl impurity 1.38, dibenzyl fosaprepitant 1.53, respectively. Elution order was found as fosaprepitant dimeglumine (RT = 12.62 min), aprepitant (RT = 15.86 min), monobenzyl impurity (RT = 17.43 min) and dibenzyl fosaprepitant (RT =19.26 min) (Fig. 3), sample analysis was also performed (Fig. 4) before degradation and validation to know amount of impurities present.

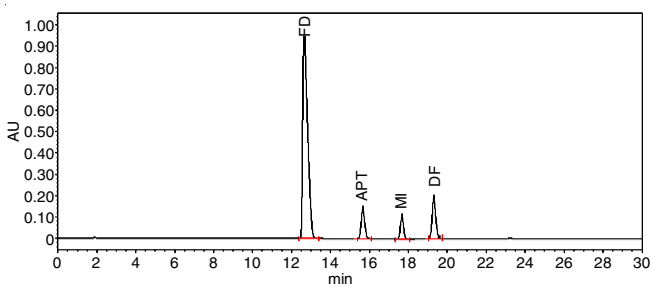

Fig. 3. System suitability chromatogram of fosaprepitant dimeglumine

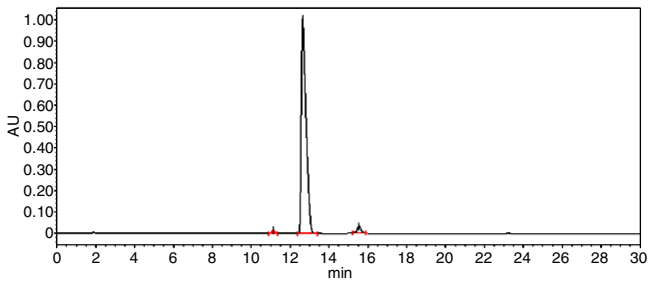

Fig. 4. Sample chromatogram of fosaprepitant dimeglumine

Design space (DS) generation with DOE and verification of model: The study was conducted with 18 different experiments, after processing all the optimization experiments using empower software, all the system suitability results were transferred into the Design Expert modeling Software. Generally, the retention time ratio of peaks in the chromatogram used for accurate quantitative analysis. The effect of these parameters was studied simultaneously and made few observations from the contour plot shown in Figs. 5 and 6 as design space. Based on the colour code, the working region can easily be

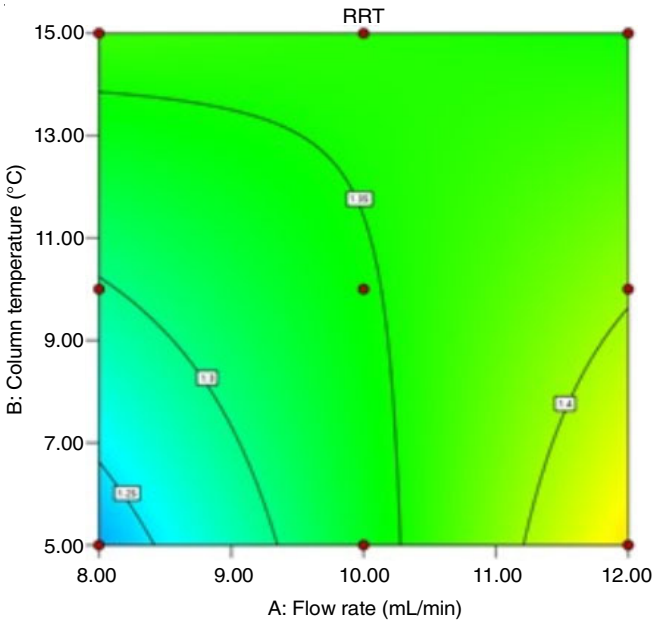

Fig. 5. Design space model for fosaprepitant dimeglumine impurities retention time ratio

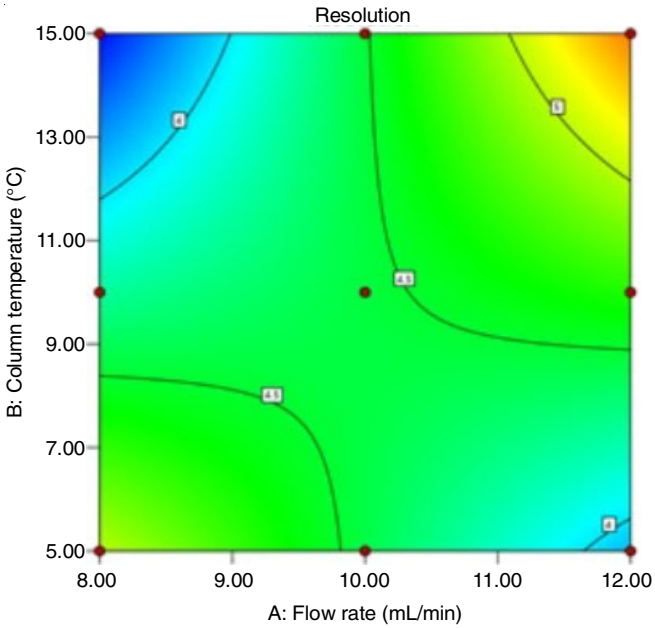

Fig. 6. Design space model for fosaprepitant dimeglumine impurities resolution

identified. Retention time maps represent the value of the critical fosaprepitant dimeglumine impurities RRT with warm "red" colours showing large values (1.6), "Yellow" colours showing desired values (1.2 to 1.4) and "blue" colours, less values (1.1). More DS map showed the RRT 1.2 to 1.4 with effect of flow, column temperature and solvent composition variation.

The resolution should not be less than 1.5 for any of the peak pairs in the chromatogram for accurate quantitative analysis. Therefore, the lowest resolution peak pair (aprepitant and monobenzyl impurity) was considered as critical peak pair and its resolution is the key separation interest of the method. Based on the color code, the working region can easily be

identified. Resolution maps represent the value of the critical resolution, with "warm bluish" region showing less resolution value (3.7), "bluish green" colours showing resolution values (4.0) and "green" colour region shoes highest resolution values (5.0). More DS map was showing 4 to 4.5 with effect of flow variation, column temperature variation and solvent composition variation and software predicted resolution value was 6.90. The effect of these parameters was studied simultaneously and applied on the separation of critical pair. The system suitability results were verified between Software predicted and original method, the accuracy of the results was found more than 90%.

Forced degradation: Fosaprepitant dimeglumine was subjected to different stress conditions as aqueous degradation, acid hydrolysis with 1 N HCl, oxidative degradation with 3% peroxide, base hydrolysis with 1 N NaOH, thermal degradation at 60 ºC at 3rd day. Photostability was conducted with the sample exposed to UV and visiblelight. Sample was kept with covered and uncovered with aluminum foil. Humidity study was carried out for 3 days at 80 ºC/85% RH.

During all above degradation conditions, peak purity of fosaprepitant dimeglumine was studied by using PDA and found that fosaprepitant dimeglumine peak was pure without any coelution based on purity angle and purity threshold values (Fig. 7). Aprepitant was found to be major degradant in above conditions and no other known impurities were formed. Major degradation was observed in acid, thermal and humidity condition and aprepitant was formed [18]. Forced degradation data is reported in Table-2.

Limit of detection (LOD) and limit of quantitation (LOQ): Initially 0.1% impurity blend was prepared and then further diluted to achieve desired signal to noise value. Precision study at LOQ level was performed by injecting LOQ level solution in fosaprepitant dimeglumine sample and calculated the percentage RSD. For LOD signal/noise should be not less than 3, and for LOQ Signal/Noise should be not less than 10 (Fig. 8). Concentration of each impurity at the LOD and LOQ level is reported in mg/mL with respect to the test concentration (mg/mL) (Table-3).

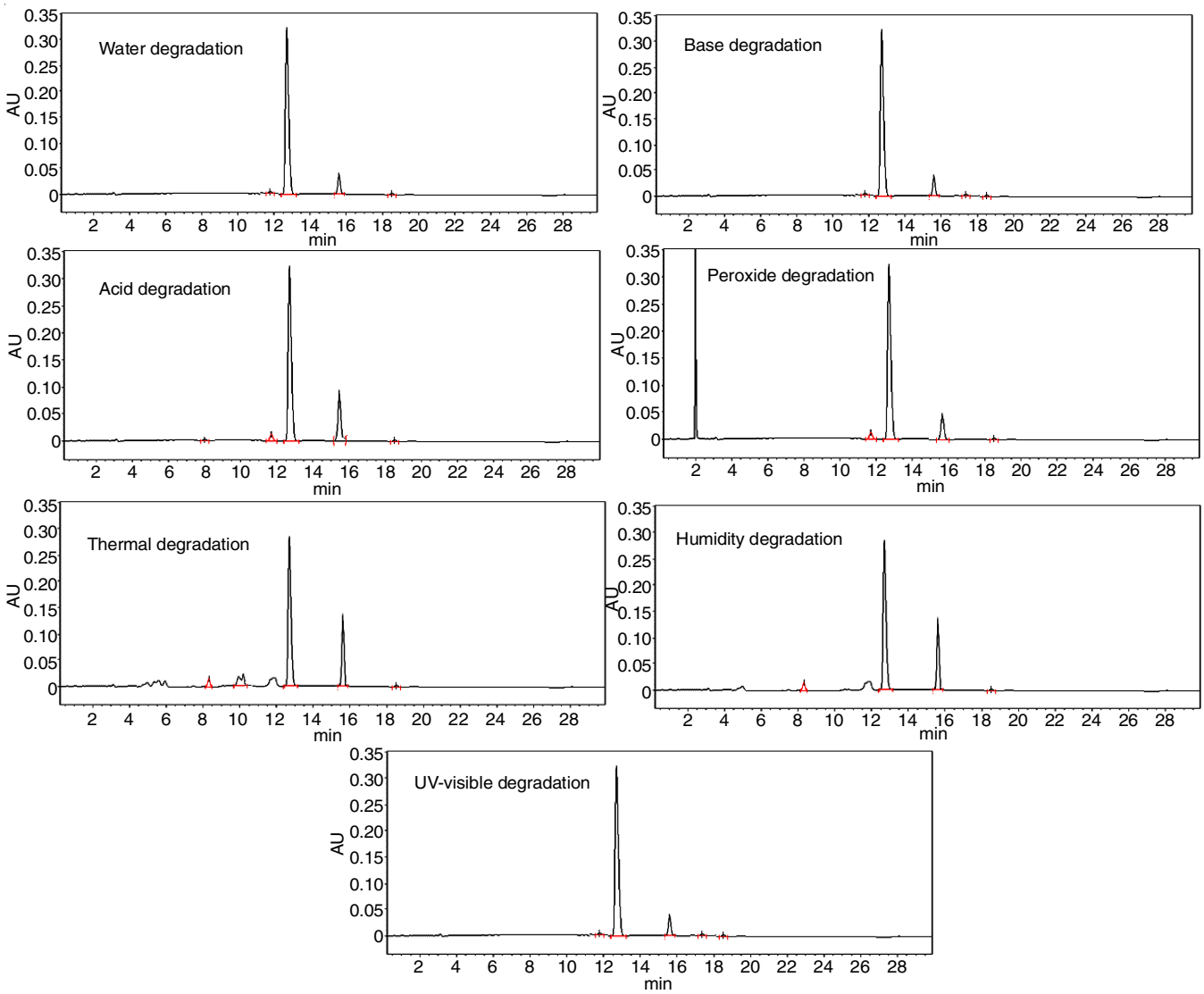

Fig. 7. Forced degradation chromatogram (a) aqueous hydrolysis, (b) base hydrolysis, (c) acid hydrolysis, (d) peroxide, (e) thermal degradation, (f) humidity degradation, (g) UV-visibility degradation

TABLE-3
SUMMERY OF VALIDATION PARAMETER

Parameter	FD	APT	MI	DF
LOD (mg/mL)	0.00007	0.00006	0.00005	0.00005
LOQ (mg/mL)	0.00013	0.00016	0.00015	0.00015
Linearity range (mg/mL)	0.00015-0.0015	0.00015-0.0015	0.00015-0.0015	0.00015-0.0015
Correlation coefficient	0.9999	0.9992	0.9996	0.9999
Recovery (%)	NA	98.6	99.1	97.3
Precision (%RSD)	NA	2.8	1.3	2.7
Intermediate precision	NA	0.8	1.1	3.2
Robustness	Robust	Robust	Robust	Robust
Resolution	NA	5.8	4.5	4.3
Tailing factor	1.11	0.97	0.98	1.21

TABLE-2
FORCED DEGRADATION DATA FOR
FD AT DIFFERENT STRESS CONDITIONS

Duration	APT	MI	DF	SMUI	Total impurities
Test sample	0.07	ND	ND	0.06	0.13
Aqueous degradation	4.75	ND	ND	0.06	4.81
Acid hydrolysis	10.21	ND	ND	0.05	10.26
Peroxide degradation	5.45	ND	ND	0.11	5.56
Base hydrolysis	5.11	ND	ND	0.09	5.20
Thermal degradation	19.71	ND	ND	0.11	19.82
UV-Visibility light	3.65	ND	ND	0.06	3.71
Humidity study	22.5	ND	ND	0.17	22.67

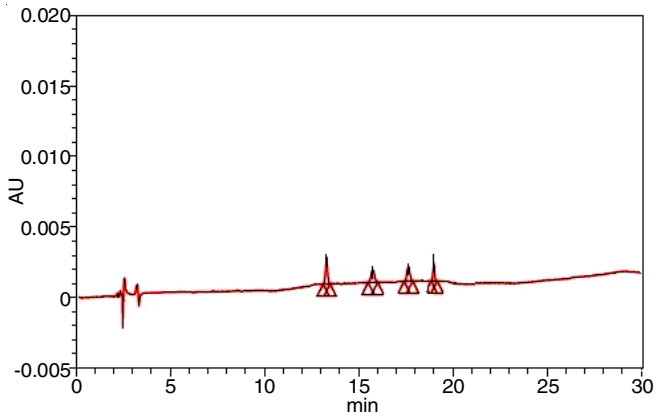

Fig. 8. LOQ chromatogram for FD, APT, MI, DF

Linearity: Linearity study was performed by injecting the series of dilution from LOQ concentration to 150% of the specification level and calculated the statistical values like slope, correlation coefficient, intercept from the plot drawn for concentration versus area. From the data obtained (Table-3), it was proved that complete linear response at all concentration level for all impurities.

Accuracy: The accuracy study was performed from the LOQ level, 50% level, 100% level and to 150 % level by spiking the impurities at each level in triplicate and results were expressed in terms of percentage recoveries of aprepitant, monobenzyl impurity and dibenzyl fosaprepitant in the presence of fosaprepitant dimeglumine sample solution. The mean recovery data (Table-3) of all the impurities were found to be within the range 85 to 115 % from the accepted value as per ICH Q2R1 [19].

Precision: The precision and intermediate precision was performed as per the ICH Q2R1 [19]. Batch analysis was performed for the initial results as shown in Table-4. The precision and intermediate precision were performed by spiking the impurities at specification level in six replicates and the results are shown in Table-3. The results were found to be well within the acceptance limit of the impurity precision limit (RSD < 10%), this proved the method was precise for the analysis.

TABLE-4
DATE FOR THE BATCH ANALYSIS
FOR VALIDATION COMPARISON

Impurity name	Precision	Intermediate precision	Acceptance criteria
APT	0.04	0.05	Not > 0.30%
MI	ND	ND	Not > 0.15%
DF	ND	ND	Not > 0.15%
Single maximum unknown	0.04	0.04	Not > 0.10%
Total impurities (%)	0.12	0.11	Not > 1.0%

Stability sample evaluation: As the method was validated; stability study of fosaprepitant dimeglumine solid sample was performed for determination of the impurity content for 10 days, 20 days and 30 days at -20 ºC and 2-8 ºC. The results are presented in Tables 5 and 6. The results were well within the limits and complies the label claim very well reflecting the reproducibility of the proposed method.

TABLE-5
STABILITY ANALYSIS DATA FOR 10, 20 AND
30 DAYS OF THE BATCH-2 AT –20 °C

Duration	MI	APT	DF	SMUI	Total impurities
Initial	ND	0.06	ND	0.04	0.10
10 Days	ND	0.07	ND	0.04	0.11
20 Days	ND	0.08	ND	0.05	0.13
30 Days	ND	0.10	ND	0.05	0.15

TABLE-6
STABILITY ANALYSIS DATA FOR
30 DAYS OF THE BATCH-2 at 2-8 °C

Duration	MI	APT	DF	SMUI	Total impurities
Initial	ND	0.06	ND	0.04	0.10
10 Days	ND	0.08	ND	0.05	0.13
20 Days	ND	0.10	ND	0.05	0.15
30 Days	ND	0.12	ND	0.06	0.18

Conclusion

A novel, simple, specific, accurate, stability indicating RP-HPLC method has been developed for fosaprepitant dimeglumine and its related impurities by using QbD approach with modeling software Design Expert. The present method can separate all known, unknown impurities, suitable for detection and quantification of impurities. The method was validated as per ICH guideline and found to be simple, specific, accurate, precise, linear and robust. The proposed method would be helpful for routine analysis in quality control laboratories for the analysis of commercially available fosaprepitant dimeglumine drug substance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. S.J.P. van Belle and V. Cocquyt, *Expert Opin. Pharmacother.*, **9**, 3261 (2008); https://doi.org/10.1517/14656560802548463
2. F. Roila, J. Herrstedt, M. Aapro, R.J. Gralla, L.H. Einhorn, E. Ballatori, E. Bria, R.A. Clark-Snow, B.T. Espersen, P. Feyer, S.M. Grunberg, P.J. Hesketh, K. Jordan, M.G. Kris, E. Maranzano, A. Molassiotis, G. Morrow, I. Olver, B.L. Rapoport, C. Rittenberg, M. Saito, M. Tonato and D. Warr, *Ann. Oncol.*, **21**, 232 (2010); https://doi.org/10.1093/annonc/mdq194
3. R.M. Navari, *Expert Rev. Anticancer Ther.*, **8**, 1733 (2008); https://doi.org/10.1586/14737140.8.11.1733
4. N. Candelario and M.L.R. Lu, *Cancer Manage. Res.*, **2016**, 77 (2016); https://doi.org/10.2147/CMAR.S93620
5. P. Feyer and K. Jordan, *Ann. Oncol.*, **22**, 30 (2011); https://doi.org/10.1093/annonc/mdq600
6. L. Celio, F. Ricchini and F. De Braud, *Patient Prefer. Adherence*, **7**, 391 (2013); https://doi.org/10.2147/PPA.S31288
7. R.M. Navari, *Expert Rev. Anticancer Ther.*, **8**, 1733 (2008); https://doi.org/10.1586/14737140.8.11.1733
8. F.C. Gonzalez and W.K. Kraft, *Expert Opin. Drug Metab. Toxicol.*, **6**, 1277 (2010); https://doi.org/10.1517/17425255.2010.513970
9. P.J. Skrdla, A. Abrahim and Y. Wu, *J. Pharm. Biomed. Anal.*, **41**, 883 (2006); https://doi.org/10.1016/j.jpba.2006.02.005
10. G. Sen, K.R. Babu, N. Annapurna, N.A. Vekariya, V.J. Kumar, K.S.R. Pavan Kumar and H.K. Sharma, *Int. J. Pharm. Pharm. Res.*, **11**, 105 (2017).
11. M. Xu, J. Chu, T. Wu, W. Zheng, Z. Li, J. Zhang, S. Liu, D. Liang, A Method for Simultaneous Detection of Fosaprepitant and Aprepitant in Plasma, Faming Zhuanli Shenquing, Chinese Patent CN105974016A (2016).
12. H. Skoog, Nieman Instrumental Analysis, Harcourt College Publisher: San Diego, California, USA, edn 5, pp 725-746 (1998).
13. P.D. Sethi, High Performance Liquid Chromatography, Quantitative Analysis of Pharmaceutical Formulation, edn 1, CBS Publisher: New Delhi, India, pp 90-109 (2001).
14. International Conference on Harmonization, Food and Drug Administration, Impurities in the Drug Substance: Methodology (Q3B) (2005).
15. ICH, Stability Testing of New Drug Substances and Products (Q1AR2), International Conference on Harmonization, Food and Drug Administration, USA, February (2003).
16. ICH, Validation of Analytical Procedures: Methodology (Q2R1), International conference on Harmonization, Food and Drug Administration, USA, November (2005).
17. US Food and Drug Administration (FDA), Department of Health and Human Services, Pharmaceutical Quality for the 21st Century, A Risk-Based Approach Progress Report, May (2007).
18. A.S. Fayed, M.A. Shehata, A. Ashour, N.Y. Hassan and S.A. Weshahy, *J. Pharm. Biomed. Anal.*, **45**, 407 (2007); https://doi.org/10.1016/j.jpba.2007.06.028
19. ICH, Validation of Analytical Procedures: Methodology (Q2R1), International conference on Harmonization, Food and Drug Administration, USA, November (2005).