Analytical Method Development And Validation Of Fosaprepitant Dimeglumine By Rp-Hplc & Uv Spectroscopy

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Abstract

Fosaprepitant is an NK-1 receptor antagonist that is used to treat nausea and vomiting caused by chemotherapy. In the present study, the analytical methods were developed and validated for Fosaprepitant Dimeglumine in bulk Drug. The study was performed in two parts. UV method was developed and validated for the Fosaprepitant Dimeglumine drug in bulk. In the second part, the HPLC method was developed and validated for the determination of Fosaprepitant Dimeglumine Drug in bulk. The UV method was performed on a UV-visible double beam spectrophotometer (Lab IndiaT60 Model UV – 1800-240V, Wavelength Range 190-1100nm, Band Width 2 nm,1 cm Matched Quartz Cells, UV WIN Version 5 Software).

Chromatography was performed using Thermo Fisher Scientific with Detector and Pump (Thermo Model UV 1000) & (Thermo, Model P4000) using Software (ChromQuest, Version 4.1)The Buffer was prepared by ACN (40:60) HPLC Grade (0.1 M KH2PO4 solution prepared, pH of this solution was adjusted to3.10 $\pm$ 0.10 with 1% phosphoric acid (Filtered this solution through 0.45 µ filter.) The analysis was carried out on C-8, 150 x 4.6 mm, 5µ C18, 250 x 4.6mm, 5µ Column having stationary phase C-8 Luna internal diameter filled with octylsilane chemically bonded to porous silica particles of 5 µm diameters]. LOD and LOQ of Fosaprepitant Dimeglumine by UV method was found 7.3and 22.2 respectively and by HPLC method LOD and LOQ was found to be 7.4 and 22.3 respectively. We have successfully developed analytical methods namely UV and HPLC for Fosaprepitant Dimeglumine Drug in bulk form. These methods were validated and found to be simple, sensitive, accurate and precise.

Keywords: *Fosaprepitant Dimeglumine, UV method, HPLC method*

INTRODUCTION

Fosaprepitant is an NK-1 receptor antagonist that is used to treat nausea and vomiting caused by chemotherapy (CINV). It's the aprepitant's N-phosphoryl derivative [1]. To exert its antiemetic effects, the prodrug fosaprepitant is transformed into the active aprepitant. 1-Deoxyfosaprepitant dimeglumine is the chemical name for fosaprepitant dimeglumine. [3-[[-1-(methylamino)-D-glucitol (2 R ,3 S) [-2] (1 R) -1-[3,5-bis(trifluoromethyl)phenyl]ethoxy] -1-[3,5-bis(trifluoromethyl)phenyl]ethoxy] -3-(4-fluorophenyl)-4-morpholinyl]methyl] phosphonate (2:1) -2,5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl (salt). C 23 H 22 F 7 N 4 O 6 P is its empirical formula (C 7 H 17 NO 5) with a molecular weight of 1004.83; fosaprepitant dimeglumine is a white to off-white amorphous powder. It dissolves easily in water [2]. The US Food and Drug Administration (USFDA) has approved fosaprepitant dimeglumine (Emend), the first single-dose intravenous NK1 receptor antagonist, for the treatment of nausea and vomiting associated with mildly and extremely emetogenic chemotherapy [3]. It is a prodrug of aprepitant (APT), which converts to APT in the body. It has a unique method of action and is a selective high-affinity antagonist at the human substance Neurokinin-1 (NK1) receptors. Furthermore, the (NK1) receptor is 3,000 times more selective than any other enzyme, transporter, ion channel, or receptor site [4]. Fosaprepitant dimeglumine was approved as a substitute dose (115 mg i.v.) for the oral medication aprepitant in January 2008. (125 mg on day 1 and 80 mg daily p.o. on days 2 and 3 of the 3-day regimen). The area under the plasma concentration-time curve (AUC) of the 115-mg fosaprepitant dose delivered (1 mg/ml i.v.) over 15 minutes was found to be equal to the 125-mg oral aprepitant dose of day1 [5]

In Previous studies research was to develop and validate a robust HPLC method for quantification related impurities in Fosaprepitant which are the most commonly used antibiotics in intensive care units. 3.4g of K2HPO4 in 1000 ml of water was used as a buffer solution. Mobile phase A (buffer and acetonitrile 80:20 v/v) and mobile phase B (Acetonitrile and acetonitrile 80:20 v/v) were used as mobile phases. Zorbax Eclipse XDB 250x4.6mm, 5µ column; Flow rate 1.2ml/min, 30°C column temperature, 210 nm were used as chromatographic conditions. Gradient program at 0 min 30% mobile phase B, at 15 min 60%, at 17 min 65%, at 18 min 30%, at 25 min 30%; 7. Diluent: water and acetonitrile 50:50 v/v were applied. Method validation was performed as per ICH Q2 guidance with precision, linearity, accuracy, ruggedness, robustness and specificity [6].

As no other detailed RP HPLC and UV methods were reported, we initiated the development as per the general concept of the HPLC and UV method [7],[8]. In the present study, the analytical methods were developed and validated for Fosaprepitant Dimeglumine in bulk Drug. The study was performed in two parts; UV method was developed and validated for the Fosaprepitant Dimeglumine drug in bulk. In the second part, the HPLC method was developed and validated for the determination of Fosaprepitant Dimeglumine Drug in bulk. A literature survey revealed that reports on analytical methods and validation methods such as UV-Visible, and HPTLC for

the determination of Fosaprepitant Dimeglumine very few analytical methods are reported [3],[9]-[14].

MATERIAL AND METHOD

Material and chemicals

HPLC water (molychem), Acetonitrile (Rankem), Ortho Phosphoric acid SQ (Qualigens), Potassium dihydrogen phosphate AR purchased from Merck India Ltd. Working standards of fosaprepitant dimeglumine, impurities and test samples were obtained as donations for research purposes.

Instrumentation and Chromatographic conditions:

The UV method was performed on a UV-visible double beam spectrophotometer (LABINDIAT60 Model UV – 1800-240V, Wavelength Range 190-1100nm, Band Width 2 nm, 1 cm Matched Quartz Cells, UV WIN Version 5 Software)

Chromatography was performed using Thermo Fisher Scientific with Detector and Pump (Thermo Model UV 1000) & (Thermo, Model P4000) using Software (ChromQuest, Version 4.1)The Buffer was prepared by ACN (40:60) HPLC Grade (0.1 M KH_2PO_4 pH of this solution was adjusted to 3.10 $\pm$ 0.10 with 1% phosphoric acid solution was filtered through 0.45 µ filter.

The analysis was carried out on C-8, 150 x 4.6 mm, 5µ C18, 250 x 4.6mm, 5µ Column having stationary phase C-8 Luna internal diameter filled with octylsilane chemically bonded to porous silica particles of 5 µm diameters.

UV Spectroscopic method for Fosaprepitant Dimeglumine

Selection of solvents –

The solvent was selected based on the solubility of API. Acetonitrile & Buffer solvent selected for the spectroscopic study.

Selection of analytical wavelength

Using appropriate dilution of standard stock solutions were scanned in UV range 200- 400 to get λmax. The wavelength selected should be such that at the wavelength the absorption of the component should be as large as possible. So the wavelength chosen was 264 nm for Fosaprepitant Dimeglumine.

Preparation of standard solution

10 mg of Fosaprepitant API was weighed and transferred into a 10ml volumetric flask. Then the drug was dissolved in 10ml Diluents (Acetonitrile and Buffer) (60:40 v/v) to obtain a concentration of 1000µg/ml.

Dilutions

Serial dilutions of Fosaprepitant Dimeglumine were prepared using 0.8, 1, 1.2, 1.4, and 1.6, 1.8ml up to 10mlwith diluents which gave the concentration of 80, 100, 120, 140, 160, and 180µg/ml respectively.

HPLC method for Fosaprepitant Dimeglumine

Selection of mobile phase

Different combinations of Acetonitrile, water, and buffers were used based on polarity. The solvent system was optimized to provide a good performance of the assay. Finally, mobile phase which contains 0.1 M KH2PO4 solution was prepared and pH adjusted with 1% Phosphoric acid buffer: Acetonitrile HPLC Grade (40:60) was selected for further analysis.

Selection of detection wavelength

The detection wavelength was measured by running the 180µg/ml solution of Fosaprepitant Dimeglumine in the mobile phase, and the wavelength of maximum absorption was selected as 264nm.

Preparation of Mobile phase

The mobile phase was prepared by mixing 40 ml of buffer and 60 ml of Acetonitrile. This mobile phase was properly mixed and then filtered through 0.45µ Whatmann filter paper.

Preparation of standard solution

An accurately weighed quantity of Fosaprepitant Dimeglumine (100 mg) was transferred into a 100 ml volumetric flask; the drug was dissolved in a sufficient quantity of mobile phase (40:60). The volume was made up to the mark with the mobile phase to obtain a concentration of 1000 µg/ml. Further dilutions were prepared from this stock solution.

Validation of analytical Methods –

I) UV Method

a) Linearity-

For quantitative analysis of Fosaprepitant Dimeglumine, the calibration curve was plotted for each concentration range.

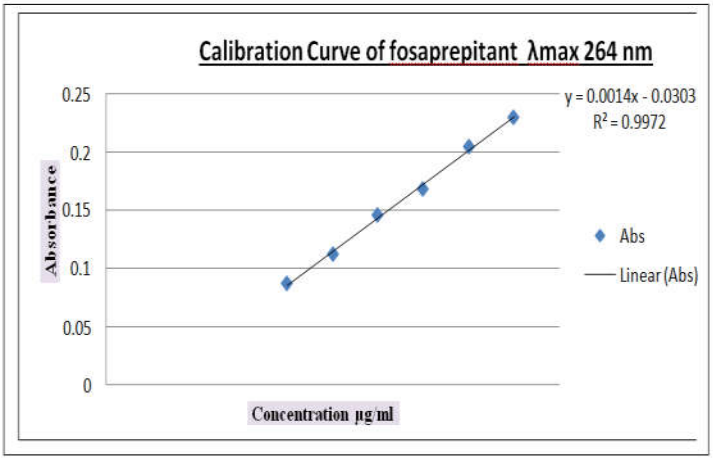

Fig 1 – calibration curve for Fosaprepitant Dimeglumine by UV

b) Limit of detection (LOD) and limit of quantification (LOQ) –

The LOD and LOQ of Fosaprepitant by the proposed method were determined using calibration standards. The LOD and LOQ values were calculated as 3.3 SD/D and 10 SD/D respectively. Where D is the slope of the calibration curve and SD is the standard deviation.

c) Precision

The reproducibility of the proposed method was determined by performing analysis at different time intervals (3-hour intervals) on the same day (intra-day precision) and on three different days (inter-day precision)

d) Robustness –

The robustness study was carried out by determining the effect of small variations in wavelength

II) RP-HPLC Method

a) Linearity

For quantitative analysis, the calibration curve was plotted for each concentration range.

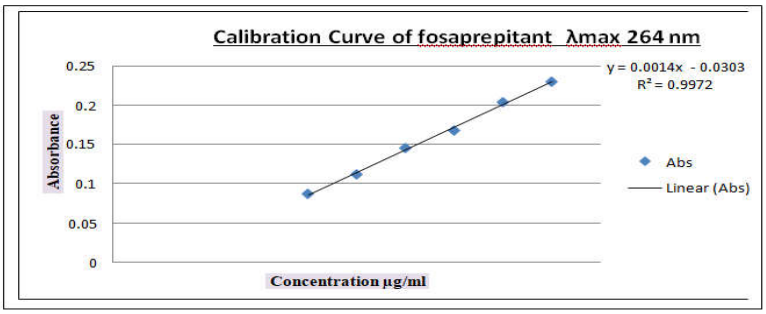

Fig 2 – Calibration curve of Fosaprepitant Dimeglumine

b) Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ by the proposed method were determined using calibration standards. LOD and LOQ values were calculated as 3.3SD/D and 10 SD/D respectively, where D is the slope of the calibration curve and SD is the standard deviation.

c) Precision

The reproducibility of the proposed method was determined by performing at a different time intervals (3-hour intervals) on the same day (intra-day precision) and three different days (inter-day precision)

d) Robustness

The robustness study was carried out by determining the effect of small variation in wavelength and flow rate.

e) System suitability

System suitability was done to verify the repeatability of the HPLC method. Theoretical plate, repeatability of retention time and peak area were determined and compared.

RESULT AND DISCUSSION

The spectra showed better resolution and linearity of the calibration curve of Fosaprepitant Dimeglumine at 264nm. So the wavelength chosen was 264nm shown in the Fig 3.

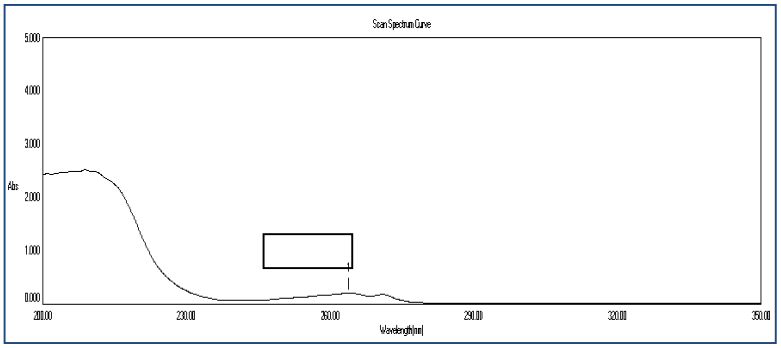

Fig 3 – spectra of Fosaprepitant showing λmax at 264nm.

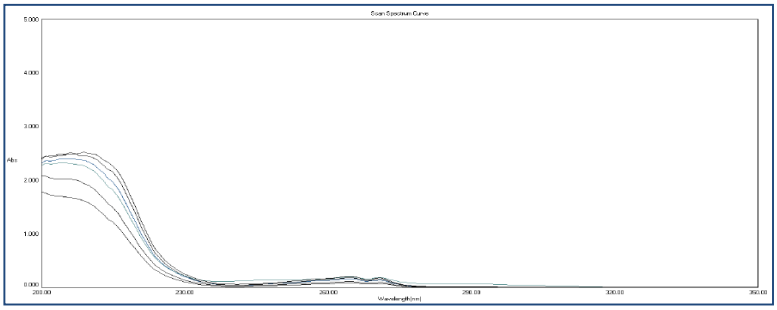

Fig 4 – Overlay spectra of Fosaprepitant Dimeglumine

Linearity

Linearity was determined for Fosaprepitant in the range of 80-180µg/ml. The regression equation for the calibration curve was found to be y = 0.0014x - 0.0303. And correlation coefficient was found to be R^2 = 0.9972. A linear relationship was observed between concentration and response to the drug. The absorbance was plotted against the corresponding concentration to obtain the calibration graphs which are shown in **table 1** and **Fig 1**.

Table 1– Calibration curve data for Fosaprepitant Dimeglumine Using UV

Conc. (µg/ml)	Abs. at 264nm
80	0.087
100	0.112
120	0.146

140	0.168
160	0.204
180	0.230

Precision

The repeatability of the method was determined by assaying Three standard solutions of Fosaprepitant Dimeglumine the reproducibility of the proposed method was determined by performing assay at different time intervals on the same day (intra-day precision) and three different days (inter-day precision) results of the intra-day and inter-day precision is expressed in %RSD. The result is shown in tables **2 and 3**

Table 2 – Intra-day precision by UV

Conc. (µg/ml)	Absorbance			Mean	RSD %	Intra-day precision
	Morning	Afternoon	Evening			
125	0.132	0.132	0.133	0.132	0.44%	**0.37%**
150	0.162	0.163	0.163	0.163	0.35%	
175	0.184	0.183	0.184	0.184	0.31%	
				0.160	0.37%	

Table 3– Inter-day precision by UV

Conc. (µg/ml)	Absorbance			Mean	RSD %	Inter-day precision
	Morning	Afternoon	Evening			
125	0.132	0.133	0.134	0.133	0.75%	**0.55%**
150	0.162	0.162	0.163	0.162	0.36%	
175	0.184	0.185	0.183	0.184	0.54%	
				0.159	0.55%	

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

The LOD and LOQ of Fosaprepitant Dimeglumine by the proposed methods were determined using the slope of the line and standard deviation obtained from Linearity studies. Values for LOQ and LOD are shown in **table 4.**

LOD = 3.3(SD/S)

Where, SD= Standard Deviation of Y-intercept,

S= Slope of Curve

LOQ = 10(SD/S)

Where, SD= Standard Deviation of Y-intercept,

S= Slope of Curve

Table 4– LOD and LOQ of Fosaprepitant Dimeglumine

LOD (µg/ml)	LOQ (µg/ml)
7.3	22.2

Robustness

Robustness is taken by changing the temperature of the drug & Concentration of diluents and the result are shown in **Table 5**

Table 5 – Robustness of the method

Parameters	Absorbance
Temperature	
Room Temperature	0.108
8ºC	0.120
Concentration	
54:46v/v (ACN:Buffer)	0.129
66:34v/v(ACN:Buffer)	0.147

II) HPLC Method Development and Validation

Chromatographic condition

RP-HPLC method was developed for Fosaprepitant Dimeglumine, which can be conveniently employed for routine Quality Control in pharmaceutical dosage forms. The chromatographic conditions were optimized to provide a good performance of the assay. A different combination of Water, Buffer, Acetonitrile, and buffer was based on its polarity. Finally, Mobile phases containing Buffer: Acetonitrile {0.1 M KH2PO4 solution prepared, pH of this solution was adjusted to 3.10 ± 0.10 with 1% phosphoric acid.} (40:60) was selected for further analysis. This chromatographic condition gave a retention time for Fosaprepitant Dimeglumine found to be 3.358 min with a flow rate of 0.6ml/min.

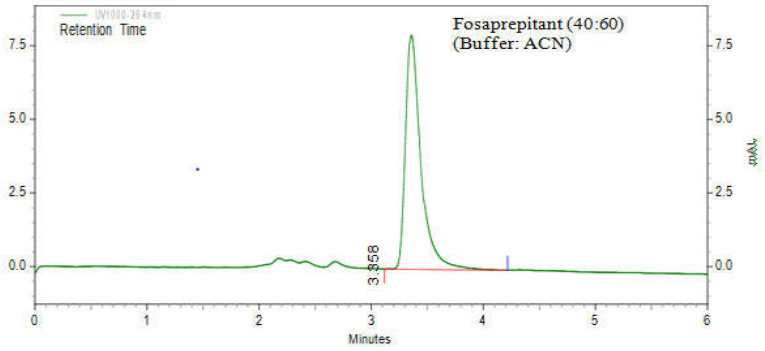

Fig 5– HPLC chromatogram of Fosaprepitant Dimeglumine

Sample	Retention Time	Area	Area per cent	Theoretical Plate	Asymmetry
Fosaprepitant	3.358	76529	100.000	3346	1.3

Linearity

A set of six solutions of Fosaprepitant at concentrations ranging from 80-180 µg/ml were prepared. Each sample was analyzed in triplicate; the calibration curve was constructed by plotting the peak area against concentration using linear regression analysis. The correlation coefficient was found to be 0.9972. Which are shown in **table 6** and **fig2**

Table 6 – Linearity data for Fosaprepitant Dimeglumine

Concentration µg/ml	Area
80	5777 6

100	72257
120	84410
140	100051
160	115786
180	125884

Precision

The intra-day precision of the developed HPLC method was obtained as %RSD values were for Fosaprepitant Dimeglumine. The inter-day precision was also determined by assaying the Area in triplicate per day for consecutive 3 days, which was found to be as shown in the table. 7 And 8.

Table 7 –Intra-day precision by HPLC

Conc. (µg/ml)	Area			Mean	RSD %	Intra-day precision
	Morning	Afternoon	Evening			
125	87156	87225	87806	87395.7	0.41%	**1.64%**
150	110418	110834	110135	110462.3	0.32%	
175	121170	111999	119688	117619	4.18%	
				105159	1.64%	

Table 8 – Inter-day precision by HPLC

Conc. (µg/ml)	Area			Mean	RSD %	Inter-day precision
	Morning	Afternoon	Evening			
125	87156	87202	87350	82236	0.12%	**0.52%**
150	110418	109134	110048	109866.7	0.60%	

175	121170	119688	121597	120818.3	0.83%	
				105973.7	0.52%	

Acceptance Criteria

The %RSD for the six determinations shall be NMT 2%

Data Interpretation

From the above results, it is concluded that the method is precise.

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

The LOD and LOQ parameter was evaluated by using the slope of the line and standard deviation obtained from linearity studies. Values for LOD and LOQ are shown in **table9**

LOD = 3.3(SD/S)

Where, SD= Standard Deviation of Y intercept,

S= Slope of Curve

LOQ = 10(SD/S)

Where, SD= Standard Deviation of Y intercept,

S= Slope of Curve

Table 9 – LOD and LOQ of Fosaprepitant Dimeglumine

LOD µg/ml	LOQ µg/ml
7.4	22.3

Robustness

The robustness of the method was evaluated by deliberately varying the chromatographic condition of the method such as flow rate; wavelength and effect were seen on parameters like tailing factor and retention times. They showed adherence to these limits represented hence method was robust.

Table -10 Change in wavelength i.e. ± 2 nm

Parameter	Optimized	Changed	Area	RT	Asymmetry	Remark
Wavelength	264nm	262nm	72929	3.317	1.56	Robust
		266nm	72555	3.310	1.49	Robust

1. Change in Flow i.e. ± 0.1 ml/min 1. 0.5 ml/min2. 0.7 ml/min.

Table 11- Change in Flow i.e. ± 0.1

Parameter	Optimized	Changed	Area	RT	Asymmetry	Remark
Change in Flow rate	0.6 ml/min	0.5ml/min	72650	3.962	1.52	Robust
		0.7ml/min	73194	2.850	1.56	Robust

Data Interpretation

The method was found to be robust for Fosaprepitant Dimeglumine.By using deliberately changed Chromatographic conditions.

System suitability

Name	Area	Retention Time	Theoretical plates	Asymmetry
Fosaprepitant	76529	3.358	3346	1.3
Limit	**-------**	**----**	**nlt 1500**	**nmt 2.0**

CONCLUSION

Part – I for UV spectroscopic method

The UV Spectrophotometric method was developed for the quantitative determination of the Fosaprepitant Dimeglumine Drug in bulk form. No significant difference was found between the proposed Spectrophotometric methods. Therefore, developed methods can be recommended for routine and quality control analysis of Fosaprepitant Dimeglumine in commercial pharmaceutical products.

Part – II for HPLC method

HPLC technique has been regarded as the best among various instrumental ones, despite its heavy cost and maintenance problems. Hence the HPLC method was Developed and Validated for Fosaprepitant Dimeglumine drug in bulk. The developed HPLC technique is precise, specific and accurate.

From the above we can conclude that, in the present work, we have successfully developed analytical methods namely UV and HPLC for Fosaprepitant Dimeglumine Drug in bulk form. These methods were validated and found to be simple, sensitive, accurate and precise. Hence these methods can be used successfully for routine analysis of Pharmaceutical dosage forms of Fosaprepitant Dimeglumine. The

proposed methods will not replace the presently known methods available for the analysis of Fosaprepitant Dimeglumine. However, it can serve as an alternative for routine analysis.

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AUTHORS' CONTRİBUTİONS

1) Dr Rana Zainnuddin guided during entire research work and supervise the work; 2) Ajay Radheshyam Lonkar contributed to literature research, experimenting, analysis, interpretation of the data, statistics. 3) Syed Imran Jameel contributed to preparing the study text and writing original draft.

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