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RESEARCH ARTICLE

Development and Validation of Method for the Estimation of Prasugrel Base in Prasugrel Tablets by Powder X-Ray Diffractometer (PXRD)

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ABSTRACT:
A PXRD quantitative method is developed and validated for the estimation of Prasugrel Base in Prasugrel tablets 5mg and 10mg. The PXRD conditions for the Slow scan method developed for the estimation of Prasugrel base at 13.3°2□□□ with a radiation source of Copper $K_{\alpha 1}$ (λ=1.54060A°). The Angular range was 13.0 to 13.5°2□□□with the Voltage and Current set to 40KV and 40mA and a step size of 0.02° along with sample rotation of 30 rpm. The scan type is Locked coupled, continuous with a high sensitive Lynxeye detector. The developed method was validated for Specificity, Sensitivity (LOD and LOQ), Linearity, Precision (Method precision and intermediate precision), accuracy and Robustness. The PXRD method is Linear with a concentration range of 0.25% to 2.0% w/w. with correlation coefficient of 0.996. The recoveries ranged between 104.7% - 112.0% for LOQ levels and 83.1% - 102.1% for remaining levels. The Method was found to be specific, linear, sensitive, precise, accurate and robust for the estimation of Prasugrel base in Prasugrel tablets

KEYWORDS: Power X-ray Diffactometer, Prasugrel base, Prasugrel Tablets.

1. INTRODUCTION:
Acute or delayed CINV is an unpleasant side effect experienced by over 80% of patients undergoing initial and repeated highly emetogenic cancer chemotherapy [1], for example cisplatin. As a result of this, toward the end of the decade, initial research was conducted to try to develop a drug that eases the severity and decreases the likelihood of CINV [2], and after several years of research Merck and Co. successfully developed a drug known as Emend. The active substance of Emend is Aprepitant, which is effective in helping to prevent CINV because it antagonizes the NK1 receptor [3]. This receptor is located at the brain stem nuclei of the dorsal vagal complex and is a crucial part of the regulation of vomiting. This is due to the receptor binding with substance P, a peptide neurotransmitter [4].

Aprepitant is chemically designatedas5-([(2R,3S)-2-((R)-1-[3,5-bis(trifluoromethyl) phenyl]ethoxy)-3-(4-fluorophenyl)morpholino]methyl)-1H-1,2,4-triazol-3(2H)-one with molecular weight of around 534.53 {Figure 1}. It is an off-white crystalline solid. It has very limited solubility in water. It does have a reasonably high solubility in non-polar molecules such as oils. This would, therefore, suggest that aprepitant as a whole, despite having components that are polar, is a non-polar substance. In his study, developing and validating [5] an Analytical method for Estimating Aprepitant Form-II [6] in Aprepitant Drug Substance (mixture of Aprepitant Form-I and Form-II) by Powder X-ray Diffractometer (PXRD) was discussed.

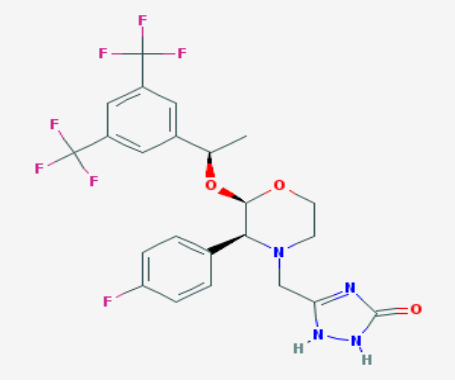

Figure 1:Structural formula

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Molecular formula: $C_{23}H_{21}F_7N_4O_3$
Molecular mass : 534.427 g/mol

2. Experimental:

2.1. Samples:

The polymorphic forms of Aprepitant Form-I and Form-II [6] and Aprepitant API (Mixture of Form-I and Form-II) was gifted by a reputed API manufacturing unit.

2.2 Details of physical properties and preparation of Aprepitant polymorphs (Form-I and Form-II):

The process for the preparation of crystalline Form-I of aprepitant involves crystallizing aprepitant from ethanol,2-propanol, acetonitrile and isopropylacetate. Alternatively Form-I can be prepared by heating a sample of aprepitant Form-II to a temperature range of 215º to 230ºC and cooling to ambient conditions.

Crystalline Form-I of aprepitant is reported to have superior properties over other forms of aprepitant, i.e., Form-II [6], in that this form demonstrates superior thermodynamic stability [6] and is non-hygroscopic when compared with other crystalline forms of aprepitant.

Further, crystalline Form-I of aprepitant has been shown to have a lower solubility (0.9±0.1 mg/ml) when compared with Form-II (1.3±0.2 mg/ml) in a 2:1 v/v methanol/water mixture at 0 ºC

Different morphological forms of the same compound may exhibit significantly different properties such as for example enhanced thermodynamic stability [6] or improved dissolution characteristics among other properties. The discovery of such novel forms and processes to make these forms of interest to the pharmaceutical formulation scientist as these improved properties could help in developing pharmaceutical dosage forms with improved stability or handling characteristics.

It is thus imperative that the pharmaceutical scientist be assured of a single polymorphic form substantially free from other polymorphic forms or that a mixture of different polymorphic forms in specified ratios are used in the preparation of a pharmaceutical formulation [6].

Mixture of polymorphic forms can provide a viable alternative to the pharmaceutical formulation scientist in the development of a formulation of aprepitant with improved properties. Nevertheless, mixture of polymorphic forms of aprepitant and processes for their preparation are desirable.

3. Instrumentation and Conditions:

Powder X-ray Diffractometer equipped with a $\theta/2\theta$ goniometer using Cu-anode radiation source, automatic divergence slit and Lynxeye detector. Data was collected at a tube voltage of 40 KV and a tube current of 30 mA, at a scan step of 0.03ºin the angular range of 2θ of 2–50º for normal scan and tube voltage of 40 KV and a tube current of 40 mA, at a scan step of 0.005º in the angular range of 2θ of 11.0 to 13.0º for slow scan. The instrument was calibrated by using Corundum (NIST standard SRM 1976) for checking the angular position, line intensity and FWHM.

The following table lists the instruments that were used in this study.

1. Powder X-ray Diffractometer, Make: Bruker, Model: D8-Advance
2. Analytical Microbalance; Make: Mettler Toledo, Model: MX5
3. Analytical Balance; Make: Mettler Toledo, Model: XS205

4. Instrumental parameters for Slow scan (13.0 – 13.5) °2θ

Radiation	Cu Kα1 (λ = 1.54060A°)
Detector	LynxEye
Voltage (kV), Current (mA)	40, 40
K beta Filter	Nickel
Scan Type	Locked coupled, Continuous
Angular range (°2θ)	11.0 to 13.0
Step Size (°)	0.005
Time per Step (Seconds)	2
Rotation per minute (RPM)	30
Motorized Divergence slit	0.3°

5. Preparation of Spike sample and Test sample:

5.1 Preparation of Test sample:

Grind about 500mg of the Aprepitant test sample to a fine powder using mortar and pestle made of Agate for normal holder (PMMA:25mm). Fill the ground powder in the sample holder and smooth the surface free from crack and crevices.

5.2 Preparation of spiked sample:

Weigh accurately Aprepitant Form-II and Aprepitant Form-I to get the required composition. Transfer the weighed Aprepitant Form-II into the mortar and add Aprepitant Form I to this powder. Mix geometrically the Aprepitant Form-I and Form-II in mortar with the help of spatula and grind the mixture in mortar with a pestle. Fill the ground powder in the sample holder and smooth the surface free from cracks and crevices.

5.3. Validation of Method:

This method was validated according to International Conference on Harmonization (ICH) guidelines [5] for Validation of analytical procedures.

5.4. System suitability:

System suitability [5] was assessed by scanning NIST traceable Corundum standard for assessing parameters like the line position, line intensity and FWHM (resolution).

5.5.. Specificity:
Specificity is the ability of analytical method to assess unequivocally the analyte in the presence of component that may be expected to be present in the drug substance or drug product [5]. Specificity is carried out to identify and demonstrate a characteristic peak of a pure polymorph, which can be used to Quantify and absence or presence of which in the main polymorph can be proved.

Specificity was demonstrated by scanning the Polymorphs

Form-I and Form-II in both Normal and Slow scan method{Figure 2}.

5.6. Linearity:
The Linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte (Polymorphic impurity) in samples within a given range. Performed the linearity with Aprepitant Form-II in the range of 5% to 90% level. Recorded the area response for each level and calculate slope, intercept and Correlation coefficient [5]. Plotted a graph of Aprepitant concentration on X-axis and Area responses on Y-axis. The correlation coefficient is more than 0.995.

5.7. Precision of Method:
Precision was measured in accordance with ICH recommendations [5]. The precision study was carried out by scanning six different spiked sample, 5% Aprepitant Form-II in Form-I. The % RSD for the area responses is calculated and are found well below 5.0

5.8 Accuracy:
The accuracy of an analytical method is the closeness of test observations obtained by that method to the true value (Standard value) [5]. Accuracy was performed by spiking known quantities of Aprepitant form-II at 10% and 60% level. Analyzed these samples in triplicate for each level. Calculated the % recovery.

5.9. Robustness:
Robustness of the analytical method was proved by varying the Sample rotation and time per step by 5%. RPM was varied [5] from 30rpm to 28.5rpm and 31.5rpm, Time per step was varied from 2.0s to 1.9s and 2.1s. The standard preparation (5% Aprepitant Form-II in Form-I) was scanned for six replicates and calculated the %RSD for the area responses and are found well below 5.0.

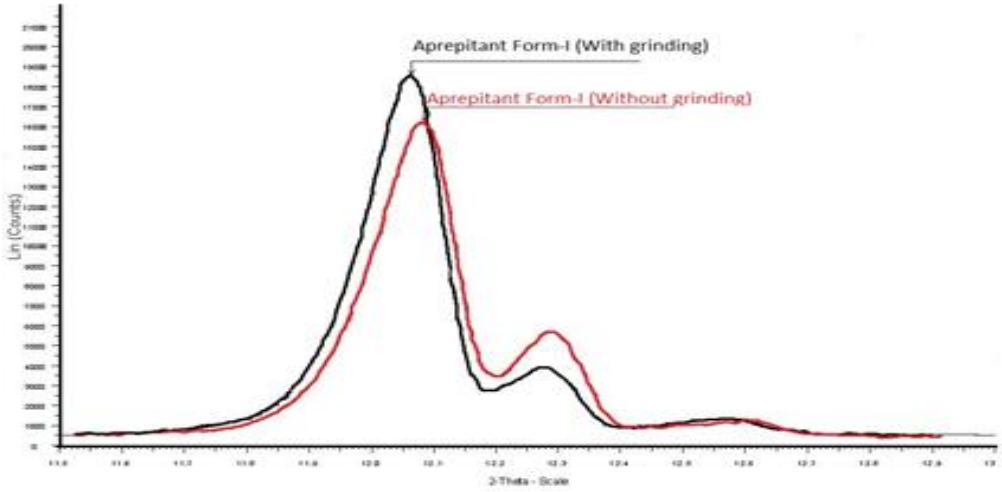
6. RESULTS AND DISCUSSION:

6.1 Method Development and Optimization:
The parameters like angular range, step size and time per step were studied as a part of method development and based on the outcome of the final parameters the method validation activity was initiated.

6.2 Method Validation:

6.2.1 System suitability:
System suitability [5] was assessed by scanning NIST traceable Corundum standard for assessing parameters like the line position (35.149°), line intensity (± 10% of the previous intensity counts) and FWHM (resolution) (<0.06°) were well within the acceptance criteria.

6.2.2 Specificity:
The Aprepitant Form-I and Form-II were scanned in both Normal and Slow scan methods {Figure 2} and based on the diffractograms obtained it can be concluded that no interference observed at 12.6°2□□in Form-I, which is the peak of interest of Aprepitant Form-II [5].



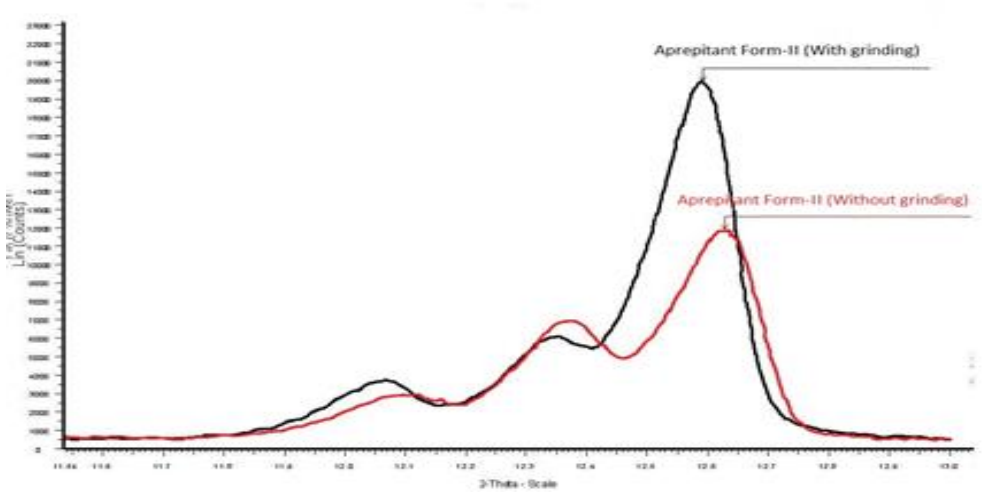


Figure 2: Overlay of Aprepitant Form-I and Form-II, both with and without grinding

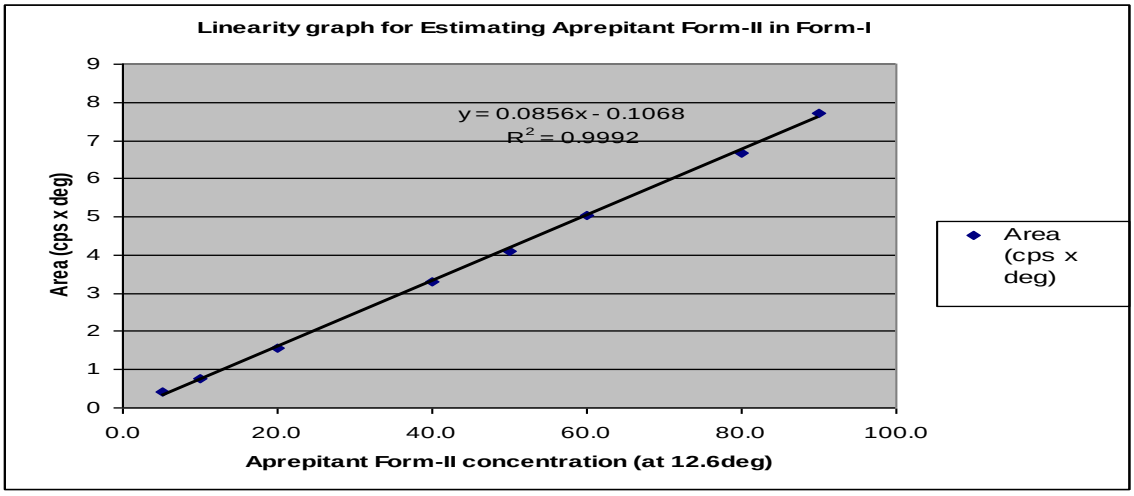


Figure 3: Linearity of Aprepitant Form-II in Form-I

6.2.3 Linearity:

The linearity with Aprepitant Form-II in the range of 5 % to 20 % level. Recorded the area response for each level and calculate slope, intercept and Correlation coefficient. Plotted a graph Aprepitant Form-II concentration on X-axis and Area responses on Y-axis [5]. The correlation coeffiecient (R^2) is 0.999 {Table 1, Figure 3}.

Table 1: Observations for Linearity study

Form-II Actual concentration (%) (at 12.6 deg 2theta)	Area (cps x deg)
5	0.422
10	0.760
20	1.562
40	3.284
50	4.091
60	5.036
80	6.674
90	7.72

6.2.4 Precision of Method:

The precision study was carried out by scanning six different spiked sample, 5% Aprepitant Form-II in Form-I [5]. The % RSD for the area responses is calculated and is found to be 3.4 {Table 2}

Table 2: Observations for Method precision

Method precision	
S.No.	**Area (cps x deg)**
1	0.470
2	0.516
3	0.499
4	0.512
5	0.504
6	0.512
Avg.	0.502
SD	0.0169
%RSD	3.37

6.2.5 Accuracy:

The accuracy of an analytical method is the closeness of test observations obtained by that method to the true value (Standard value). Accuracy was performed by

spiking known quantities of Aprepitant form-II at 10% and 60% level [5]. Analyzed these samples in triplicate for each level. Calculated the % recovery. The % Recovery for 10% Aprepitant Form-II in Form-I and 60% Aprepitant Form-II in Form-I is found to be 103 and 96 respectively {Table 3}. Based on the data obtained the Method developed is accurate to determine the Aprepitant Form-II content.

Table 3: Observations for recovery study
Results of Accuracy 10%

Pre-paration	Area (cps x deg)	Theoretical conc. (% w/w)	Measured (% w/w)	% Recovery
1	0.783	10.05	10.39	103.4
2	0.776	9.98	10.31	103.3
3	0.781	10.04	10.37	103.4
Average				103.4
%RSD				2

Results of Accuracy 60%

Pre-paration	Area (cps x deg)	Theoretical conc. (% w/w)	Measured (% w/w)	% Recovery
1	4.491	59.98	53.71	89.6
2	5.033	59.99	60.04	100.1
3	4.926	60.02	58.79	98.0
Average				95.9
%RSD				1

6.2.7 Robustness:

A study was conducted to know the effect of deliberate variation in sample rotation and time per step [5]. Robustness of the analytical method was proved by varying the Sample rotation and time per step by 5%. RPM was varied from 30rpm to 28rpm and 32rpm, Time per step was varied from 2.0s to 1.9s and 2.1s. The standard preparation (5% Aprepitant Form-II in Form-I) was scanned for six replicates. The % RSD for a variation of 5% in RPM (i.e.28rpm and 32rpm) and Time per step (1.9s and 2.1s) is 4.5, 4.4, 4.8 and 4.8 respectively {Table 4}. In all the cases the results were well within the acceptance criteria. From the above study the proposed method was found to be robust.

Table 4: Observations for Robustness study

	30 RPM ,Time per Step (2sec)	28 RPM	32 RPM	Time Per Step (1.9sec)	Time Per Step (2.1 sec)
	Observed Area (cps x deg)	**Observed Area (cps x deg)**	**Observed Area (cps x deg)**	**Observed Area (cps x deg)**	**Observed Area (cps x deg)**
1	0.47	0.47	0.47	0.47	0.47
2	0.516	0.516	0.516	0.516	0.516
3	0.499	0.499	0.499	0.499	0.499
4	0.512	0.525	0.531	0.53	0.531
5	0.504	0.526	0.521	0.529	0.536
6	0.512	0.531	0.526	0.533	0.526
Avg.	0.502	0.511	0.511	0.513	0.513
SD	0.0169	0.0231	0.0227	0.0245	0.0248
%RSD	**3.37**	**4.52**	**4.44**	**4.78**	**4.83**

7. CONCLUSION:

The PXRD method was developed and validated for the estimation of Aprepitant Form-II in Form-I. The quantitation peak of Aprepitant Form-II [2] peak is at 12.6° 2□□□□n which a small peak of Aprepitant Form-I is observed at 12.1° 2□□□Figure 2}□□The above method is found to be Linear from 5% to 90% Aprepitant Form-II in Form-I with a correlation coefficient [5] of 0.9992 {Table 1, Figure 3}. The above method is found to be Accurate with a % Recovery of 103 and 96 for 10% Aprepitant Form-II in Form-I and 60% Aprepitant Form-II in Form-I respectively {Table 3}.

The above method is found to be precise with a %RSD of 3.4 for six different preparations of 5% Aprepitant Form-II in Form-I {Table 2}. The above method is found to be Robust for a variation of 5% in RPM (i.e.28rpm and 32rpm) and Time per step (1.9s and 2.1s) with a % RSD of 4.5, 4.4, 4.8 and 4.8 respectively {Table 4}.The PXRD method developed for the quantitation peak of Aprepitant Form-II in Aprepitant Drug substance is found to be linear, Accurate, Precise and Rugged [5].

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