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RESEARCH ARTICLE

Development and Validation of Method for the Estimation of Tacrolimus Crystalline API in Tacrolimus Extended Release Capsules 0.5mg, 1mg and 5mg by Power X-ray diffractometer (PXRD)

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ABSTRACT:
A PXRD quantitative method is developed[1] for the Quantitation of % Tacrolimus crystalline in Tacrolimus Extended Release capsules using PXRD. The PXRD conditions for the Slow scan method developed for the estimation of % Tacrolimus crystalline, with a radiation source of Copper $K_{\alpha 1}$ (λ = 1.54060A°). The Angular range was 9.65 to 10.80° 2θ, with the Voltage and Current set to 40kV and 40mA and a step size of 0.02° along with sample rotation of 15rpm. The scan type is Locked coupled, continuous with a high sensitive Lynxeye XET detector. The developed method[2] was validated[3] for Specificity, Sensitivity (LOD and LOQ), Linearity, Accuracy and Robustness. The PXRD method is Linear with a concentration range of 0.2% to 1.0% with correlation coefficient of 0.996. The recoveries ranged between 83% - 110% for 0.2%, 0.6% and 1.0% levels. The XRD Method was found to be specific, linear, sensitive, accurate and robust for the estimation of % Tacrolimus Crystalline in Tacrolimus Extended Release capsules 0.5mg, 1mg and 5mg.

KEYWORDS: Power X-Ray diffractometer (PXRD), Tacrolimus Crystalline, Tacrolimus Extended release capsules.

INTRODUCTION:
Tacrolimus introduction as a therapeutic agent in 1993 unveiled a new era in the prevention of rejection following organ transplantation. Tacrolimus (FK506), as a macrolide derivative, was the first in a new class of immunosuppressant[4] drugs. Fujisawa Pharmaceutical company discovered this molecule initially in 1984 by utilizing a mixed lymphocyte reaction as a screening system for compounds isolated from the broth of *Streptomyces tsukubaensis*, a bacterium found in the soil at the base of Mount Tsukuba. The company has given this compound the designation of FK506, however the generic name of tacrolimus is an acronym based on the following basis: t for Mount Tsukuba, acrol for macrolide, and imus for immunosuppressant.

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Tacrolimus (FK506), a macrolide antibiotic, is frequently used in solid organ transplantation, it is a strong immunosuppressive agent that produces its pharmacological effect through the inhibition of calcineurin, an intracellular protein required for the production of interleukin by T-cells and consequently, inhibits proliferation and maturation of T-lymphocytes. It is used to prevent allograft rejection including liver, kidney[5] or heart as a monotherapy or in combination with azathioprine or mycophenolate mofetil and is approved by the regulatory authorities like USFDA. It also belongs to narrow therapeutic index drug class which often requires close therapeutic drug monitoring in order to prevent drug toxicity[6] or therapeutic failure.

Samples:
The polymorphic forms of Tacrolimus Crystalline API, Tacrolimus Placebo and Tacrolimus Extended Release capsule was gifted by a reputed Formulation unit.

Formulation Strategies of Poorly Soluble Drugs:
Since tacrolimus is a poorly soluble[7][8] drug in aqueous media, special techniques are needed to ensures that it dissolves sufficiently in the aqueous gastrointestinal environment for enhanced bioavailability[8]. The poor solubility of drug substance creates many problems in drug research and development. The dissolution rate of a drug is determined by its aqueous solubility. The low bioavailability of Oral solid dosage drugs results from the limited dissolution rate, generally have dissolution-limited absorption. Moreover, a number of approaches have been found in the available literature, to increase the dissolution of poorly soluble drugs.

The most commonly manufacturing process for preparing amorphous solid dispersions are spray drying, solvent evaporation and hot melt extrusion. Spray drying process includes atomizing a solution of API and carrier, in a common organic solvent or mixture of solvents, using a nozzle for the rapid solvent evaporation (order of magnitude is milliseconds).

The resultant dry powdered solid dispersion often needs some post drying to have acceptable residual solvent levels. The very fast solvent evaporation process contributes to the amorphous nature of the solid dispersion.

Crystalline solids have less energy than amorphous ones; hence higher solubility is expected from the amorphous form compared to that of the corresponding crystalline state. The differences of the solubility between amorphous form and crystalline form have been reported to be between 1 and 1000- fold. The improvement in the saturated solubility of amorphous drug may lead to a significant enhancement of oral bioavailability. Stable amorphous formulation can be obtained by solid dispersion techniques. The transformation to crystalline form would results in a reduction of oral bioavailability of the incorporated drugs. Since many tacrolimus formulations are solid dispersions.

EXPERIMENTAL:

Instrumentation and Conditions:
Powder X-ray Diffractometer equipped with a θ/2θ configuration using Cu-anode radiation source, programmable divergence slit and Lynxeye XET detector. Data was collected at a voltage of 40kV and a current of 30mA, at a scan step of 0.03º in the angular range of 2θ of 2–50º for normal scan and voltage of 40kV and a current of 40mA, at a scan step of 0.02º in the angular range of 2θ of 9.65º to 10.80º for slow scan. The instrument was calibrated by using Corundum (NIST standard SRM1976) 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

Trial-1: For Specificity check
Instrumental parameters for Normal scan from (2-50)° 2θ

Radiation	Cu $K_{\alpha1}$ (λ = 1.54A°)
Detector	Lynx Eye X_ET
Voltage (kV), Current (mA)	40, 30
K beta Filter	Nickel
Scan Type	Locked coupled, Continuous
Angular range (° 2θ)	2.0 to 50.0
Step Size (°)	0.03
Time per Step (Seconds)	0.2
Rotation per minute (RPM)	15
Total time	5 min.

Sample Preparation (As such):
To check the regular pattern of Tacrolimus API, Tacrolimus ER Capsules placebo and Tacrolimus ER capsules were placed with required quantity of the samples in into a round cavity of a PMMA holder. Smoothen the surface to free from cracks and Crevices.

Sample preparation (After grinding):
To check the effect of grinding on Tacrolimus API, Tacrolimus ER capsules placebo and Tacrolimus ER capsules were ground Individually and placed with the required quantity of the samples Into a round cavity of PMMA. holder. Smoothen the surface free from cracks and crevices.

Sample Preparation (After 24 hr exposing)
To check the effect of exposing on Tacrolimus API, Tacrolimus ER capsules placebo and Tacrolimus ER capsules samples which were performed initially as such were kept at room Temperature for 24 hr.

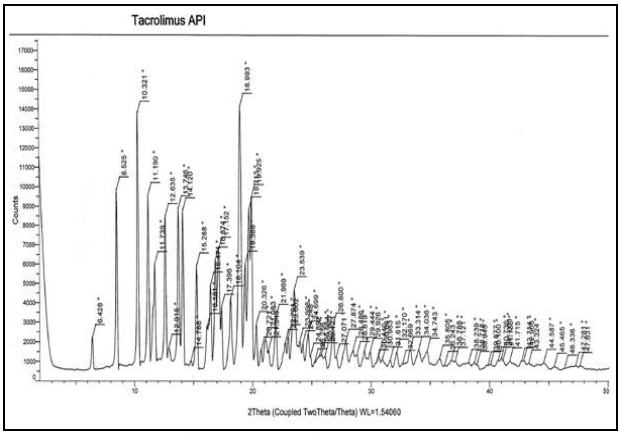


Figure 1: Typical diffractogram of Tacrolimus API

Results Observed:

- Each three sample patterns are Specific to each other.
- There is no impact on grinding the sample. The diffractogram pattern of each samples after exposing at Room temperature for 24 hrs are matching with its initial as such samples.
- Tacrolimus API is showing distinct peaks at 6.4°, 8.5°, 10.3° and 11.2° 2θ. Out of four peaks the peak with 10.3° is having highest intensity, so 10.3° 2θ peak is selected as a distinct peak for further development.

Trial-2: For Area observation and peak detection at distinct Peak

Instrumental parameters for Slow scan (9.65–10.80)° 2θ

Radiation	Cu $K_{\alpha1}$ (λ = 1.54A°)
Detector	Lynx Eye X_ET
Voltage (kV), Current (mA)	40, 40
K beta Filter	Nickel
Scan Type	Locked coupled, Continuous
Angular range (° 2θ)	9.65 to 10.80
Step Size (°)	0.02
Time per Step (Seconds)	18
Rotation per minute (RPM)	15
Total time	1hr

0.5% Tacrolimus API in Tacrolimus ER capsules placebo blend:

Weighed 2.592mg of Tacrolimus API placed it into a mortar, to this added 497.52mg of Tacrolimus ER capsules placebo, mixed well by using geometrical mixing until it gets homogeneous mixture.

0.2% Tacrolimus API in Tacrolimus ER capsules placebo blend:

Weighed 2.015mg of Tacrolimus API placed it into a mortar, to this added 999.22mg of Tacrolimus ER capsules placebo, mixed well by using geometrical mixing until it gets homogeneous mixture. Divided the sample to two equal half and placed it into two different holders as duplicate sample preparations.

Results Observed:

S. No.	Preparation	Wt. of API (in mg)	Wt. of Placebo (in mg)	Total wt. (in mg)	% concentration	Area observed (in cps x deg)
1	-	2.592	497.57	500.162	0.52	0.2423
2	Preparation-1	2.015	999.22	1001.235	0.20	0.08896
3	Preparation-2				0.20	0.09298

Trial-3: For Intensity and LOD check:

Instrumental parameters for Slow scan from (9.65 – 10.80)° 2θ

Radiation	Cu $K_{\alpha1}$ (λ = 1.54A°)
Detector	Lynx Eye X_ET
Voltage (kV), Current (mA)	40, 40
K beta Filter	Nickel
Scan Type	Locked coupled, Continuous
Angular range (° 2θ)	9.65 to 10.80
Step Size (°)	0.02
Time per Step (Seconds)	36
Rotation per minute (RPM)	15
Total time	2hrs

0.2% Tacrolimus API in Tacrolimus ER capsules placebo blend:

Weighed 2.070mg of Tacrolimus API placed it into a mortar, to this added 998.36mg of Tacrolimus ER capsules placebo, mixed well by using geometrical mixing until it gets homogeneous mixture. Divided the sample to two equal half and placed it into two different holders as duplicate sample preparations.

0.1% Tacrolimus API in Tacrolimus ER capsules placebo blend:

Weighed 500.10mg of 0.2% Tacrolimus API placed it into a mortar, to this added 500.23mg of Tacrolimus ER capsules placebo, mixed well by using geometrical mixing until it gets homogeneous mixture.

Results Observed:

S. No.	Preparation	Wt. of API (in mg)	Wt. of Placebo (in mg)	Total wt. (in mg)	% concentration	Area observed (in cps x deg)
1	Preparation-1	2.070	998.36	1000.43	0.207	0.1105
2	Preparation-2				0.207	0.07936
3	-	-	-	1000.33	0.10	0.04646

To increase intensities and to check the LOD, LOQ values same 0.2%, 0.1% preparation and additional 0.05% Spiked sample were scanned with increasing run time of the sample as 3 hr run time.

Trial-4: LOD and LOQ check

Instrumental parameters for Slow scan from (9.65 – 10.80)° 2θ

Radiation	Cu $K_{\alpha1}$ (λ = 1.54A°)
Detector	Lynx Eye X_ET
Voltage (kV), Current (mA)	40, 40
K beta Filter	Nickel
Scan Type	Locked coupled, Continuous
Angular range (° 2θ)	9.65 to 10.80
Step Size (°)	0.02
Time per Step (Seconds)	54
Rotation per minute (RPM)	15
Total time	3 hrs

0.05% Tacrolimus API in Tacrolimus ER capsules placebo blend:

Weighed 500.15mg of 0.1% Tacrolimus API placed it into a mortar, to this added 500.52mg of Tacrolimus ER capsules placebo, mixed well by using geometrical mixing until it gets homogeneous mixture.

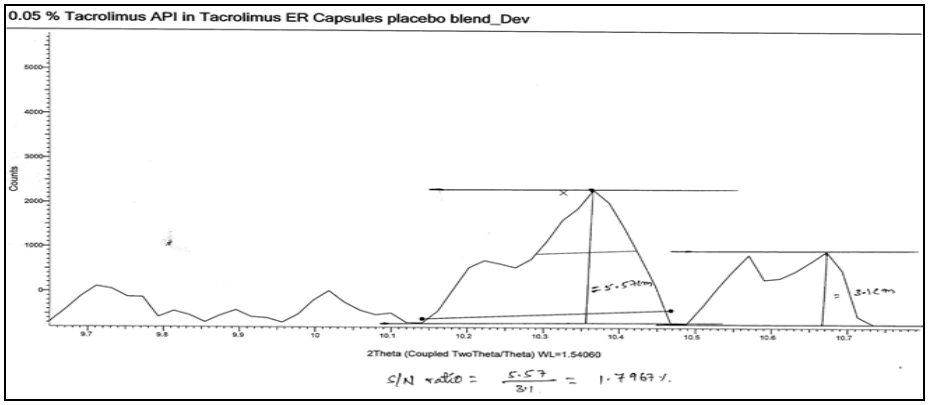


Figure 2: Typical diffractogram of 0.05% Tacrolimus API in Placebo blend – 3hrs

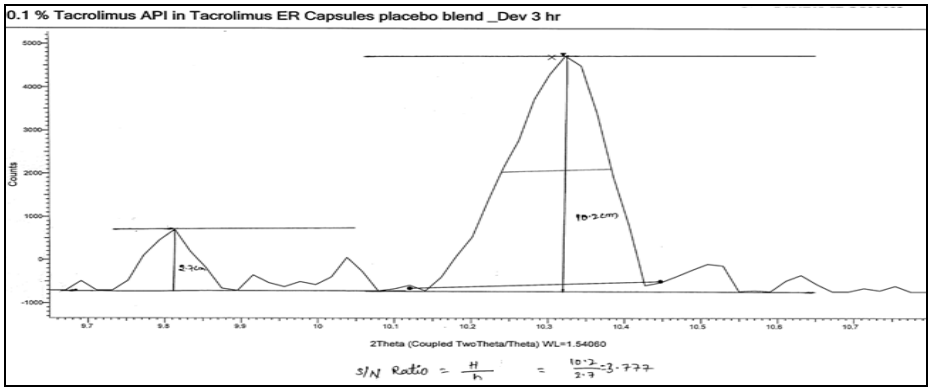


Figure 3: Typical diffractogram of 0.1% Tacrolimus API in Placebo blend – 3hrs

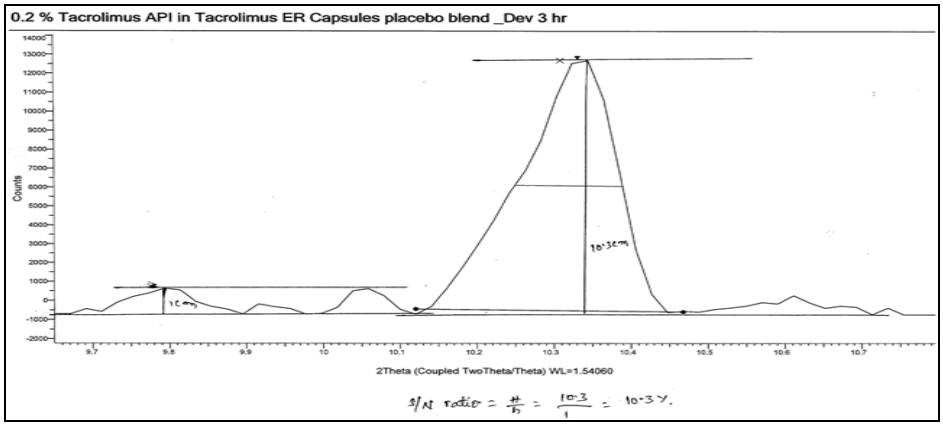


Figure 4: Typical diffractogram of 0.2% Tacrolimus API in Placebo blend – 3hrs

Results Observed:

Table 1: Results for LOD and LOQ

S. No.	Wt. of API (in mg)	Wt. of Placebo (in mg)	Total wt. (in mg)	% Concentration	Area observed (in cps x deg)	S/N ratio (Signal height/ Noise height)
1	2.070	998.36	1000.43	0.207	0.1865	10.3/1 = 10.3
2	500.10 (0.2%)	500.23	1000.33	0.10	0.07279	10.2/2.7 = 3.8
3	500.15 (0.1%)	500.52	1000.67	0.05	0.04244	5.57/3.1 = 1.8

The result observed indicating that the LOD is 0.1% and LOQ is 0.2%. Hence the method is finalized with 3 hrs run time. To check the concentration levels with respect to area observed Linearity is performed.

Final Method:

Instrumental parameters for Slow scan from (9.65 – 10.80)° 2θ

Radiation	Cu $K_{\alpha 1}$ (λ = 1.54A°)
Detector	Lynx Eye X_ET
Voltage (kV), Current (mA)	40, 40
K beta Filter	Nickel
Scan Type	Locked coupled, Continuous
Angular range (° 2θ)	9.65 to 10.80
Step Size (°)	0.02
Time per Step (Seconds)	54
Rotation per minute (RPM)	15
Total time	3 hrs

Specificity:

Analyzed Tacrolimus API and Tacrolimus ER capsule placebo samples as per the final method.

S. No.	Name of the product	No. of scans
1	Tacrolimus API	01
2	Tacrolimus ER capsule placebo	01

Results Observed:

S. No.	Name of the product	Area observed for peak at 10.3°2θ (in cps x deg)
1	Tacrolimus API	144.8
2	Tacrolimus ER capsule placebo	-

Limit of Detection (LOD):

Weighed 500.52mg of 0.2% Tacrolimus API in placebo and to this added 500.23mg of Tacrolimus placebo. Mixed well by using geometrical mixing.

Result Observed:

S/N ratio = 3.8

Limit of Quantification (LOQ):

Weighed 2.070mg of Tacrolimus crystalline API to this added 998.36mg of Tacrolimus placebo. Mixed well by using geometrical mixing.

Result Observed:

S/N ratio = 10.3

Precision at LOQ (0.2% Tacrolimus API in placebo):

Weighed about 2.0mg of Tacrolimus crystalline API to this added about 998mg of Tacrolimus placebo individually six times. Mixed well by using geometrical mixing and prepared six different samples.

Table 2: Results for Precision at LOQ

S. No.	Preparation	Wt. of API (in mg)	Wt. of Placebo (in mg)	Total weight (in mg)	% Concentration	Area observed (in cps x deg)
1	Preparation-1	2.033	998.20	1000.233	0.203	0.1824
2	Preparation-2	2.074	998.38	1000.454	0.207	0.1765
3	Preparation-3	2.079	998.55	1000.629	0.208	0.1584
4	Preparation-4	2.066	998.25	1000.316	0.207	0.1705
5	Preparation-5	2.056	998.37	1000.426	0.206	0.1605
6	Preparation-6	2.056	998.13	1000.186	0.206	0.2061
Avg.						0.178
St. dev.						0.017
% RSD						9.9

Linearity:

At 0.2% level:

Weighed about 2.022mg of Tacrolimus crystalline API to this added about 998.46mg of Tacrolimus Placebo. Mixed well by using geometrical mixing.

At 0.4% level:

Weighed about 2.08mg of Tacrolimus crystalline API to this added about 498.48mg of Tacrolimus Placebo. Mixed well by using geometrical mixing.

At 0.6% level:

Weighed about 3.037mg of Tacrolimus crystalline API to this added about 497.07mg of Tacrolimus Placebo. Mixed well by using geometrical mixing.

At 0.8% level

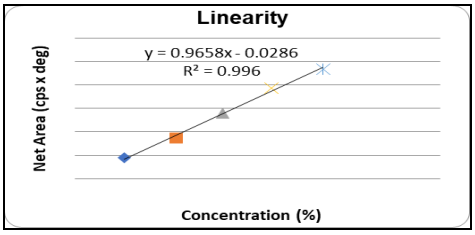
Weighed about 4.036mg of Tacrolimus crystalline API to this added about 496.16mg of Tacrolimus Placebo. Mixed well by using geometrical mixing.

At 1.0% level

Weighed about 5.091mg of Tacrolimus crystalline API to this added about 495.40mg of Tacrolimus Placebo. Mixed well by using geometrical mixing.

Table 3: Results for Linearity

S. No.	Preparation	Wt. of API (in mg)	Wt. of Placebo (in mg)	Total weight (in mg)	% Obtained Concentration	Area observed (in cps x deg)
1	0.2% (Linearity-1)	2.022	998.46	1000.482	0.202	0.1763
2	0.4% (Linearity-2)	2.080	498.48	500.560	0.416	0.3490
3	0.6% (Linearity-3)	3.037	497.07	500.107	0.607	0.5607
4	0.8% (Linearity-4)	4.036	496.19	500.226	0.807	0.7763
5	1.0% (Linearity-5)	5.091	495.40	500.491	1.017	0.9396



Method Precision at 1% Spiked level (1% Tacrolimus API in placebo):

Weighed about 5.091 mg of Tacrolimus crystalline API to this added about 495.40 mg of Tacrolimus Placebo. Mixed well by using geometrical mixing and prepared six different samples.

S. No.	Preparation	Wt. of API (in mg)	Wt. of Placebo (in mg)	Total weight (in mg)	% Concentration	Area observed (in cps x deg)
1	Preparation-1	5.049	495.38	500.429	1.009	0.9097
2	Preparation-2	5.069	495.44	500.509	1.013	0.8970
3	Preparation-3	5.061	495.05	500.111	1.012	0.9238
4	Preparation-4	5.015	495.49	500.505	1.002	0.9338
5	Preparation-5	5.078	495.70	500.778	1.014	0.9143
6	Preparation-6	5.060	495.20	500.260	1.011	0.9242
Avg.						0.9180
Stdev.						0.0143
% RSD						1.6

Accuracy at 0.2%, 0.6% and 1.0% level

Accuracy at 0.2% (LOQ level):

Weighed about 2.0mg of Tacrolimus crystalline API to this added about 998.0mg of Tacrolimus Placebo. Mixed well by using geometrical mixing and prepared three different samples.

Accuracy at 0.6% (LOQ level):

Weighed about 3.0mg of Tacrolimus crystalline API to this added about 497.0mg of Tacrolimus Placebo. Mixed well by using geometrical mixing and prepared three different samples.

Accuracy at 1.0% (LOQ level):

Weighed about 5.0mg of Tacrolimus crystalline API to this added about 495.0mg of Tacrolimus Placebo. Mixed well by using geometrical mixing and prepared three different samples.

Table 4: Results for Accuracy

S. No.	Preparation	Actual concentration (%)	Wt. of API (in mg)	Wt. of Placebo (in mg)	Total weight (in mg)	Area observed (in cps x deg)
1	Preparation-1	0.2	2.101	998.51	1000.611	0.210
2	Preparation-2		2.120	998.28	1000.400	0.212
3	Preparation-3		2.155	998.33	1000.485	0.215
4	Preparation-1	0.6	3.056	497.38	500.436	0.611
5	Preparation-2		3.080	497.52	500.600	0.615
6	Preparation-3		3.100	497.70	500.800	0.619
7	Preparation-1	1.0	5.076	495.59	500.666	1.014
8	Preparation-2		5.070	495.75	500.820	1.012
9	Preparation-3		5.041	495.82	500.860	1.006

S. No.	Preparation	Spiked concentration (%)	Actual concentration (%)	Area observed (in cps x deg)	% Recovery
1	Preparation-1	0.2	0.210	0.1572	83
2	Preparation-2		0.212	0.1693	91
3	Preparation-3		0.215	0.1635	87
4	Preparation-1	0.6	0.611	0.5712	107
5	Preparation-2		0.615	0.5344	100
6	Preparation-3		0.619	0.5686	107
7	Preparation-1	1.0	1.014	0.9002	99
8	Preparation-2		1.012	1.006	110
9	Preparation-3		1.006	0.8286	91

Robustness:

Small variations to final results by changing routine analytical parameters were studied with the robustness study. In order to establish the robustness study, the robustness is performed with changes in the generator settings and RPM. For this analysis Reference area is taken from Accuracy preparation-1as same sample performed for Robustness analysis.

- Generator settings: 40 kV and 40mA
- Generator settings: 45 kV and 35 mA
- Rotation per minute : 10 rpm
- Rotation per minute : 20 rpm

.	Preparation	Wt. of API (in mg)	Wt. of Placebo (in mg)	Total weight (in mg)	% Concentration
1	Preparation-1	5.049	495.38	500.429	1.009

Table 5: Results for Robustness

S. No.	Condition	Spiked Concentration (cps x deg)	Average Area of Method Precision (cps x deg)	Observed Area in Robustness (cps x deg)	% Variation
1	At 40 kV and 40mA	1.009	0.91797	0.7997	12.88
2	At 45 kV and 35mA			0.8073	12.06
3	At 10 rpm			0.9238	0.64
4	At 20 rpm			0.9255	0.82

$$\% \text{ Variation} = \frac{\text{Observed area} - \text{actual area}}{\text{Actual area}} \times 100$$

Calculation for quantification of Tacrolimus crystalline API in Tacrolimus Extended Release capsules by XRD for sample:

Sample preparation:

Take sufficient quantity of powder sample from the capsule shell into the round cavity of the XRD sample holder. Smooth the surface of sample with the help of glass slide. Place the sample holder on the Auto sampler and perform the analysis using the diffraction conditions of the final method. Once the diffraction conditions are ready, then start the measurement using the DIFFRAC.SUITE commander software. Process the Raw data using the DIFFRAC EVA software.

$$\% \text{ of Tacrolimus Crystalline API} = \frac{\text{(Net Area Observed} - \text{Slope)}}{\text{Intercept}}$$

$$= \frac{\text{Net Area Observed} + 0.028}{0.965}$$

CONCLUSION:

Based on the above development result for the quantification of Tacrolimus API in Tacrolimus ER Capsules 0.5mg, 1mg and 5mg LOD is 0.1% and LOQ is 0.2% and the developed method is Specific, Precise, Linear, Accurate, Robust and Rugged.

REFERENCES:

1. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1), step 4 (2005).
2. Chinese Pharmacopoeia, The Third Method in Appendix of 2005 edition.
3. FDA, Guidance for Industry: Impurities in Drug Product, Draft Guidance, Center for Drug Evaluation and Research (CDER), 1998.
4. McCauley, Jerry (2004-05-19). "Long-Term Graft Survival In Kidney Transplant Recipients". Slide Set Series on Analyses of Immunosuppressive Therapies. Medscape. Retrieved 2006-06-06.
5. Naesens M, Kuypers DR, Sarwal M (February 2009). "Calcineurin inhibitor nephrotoxicity" (PDF). Clinical Journal of the American Society of Nephrology. 4 (2): 481–508. doi:10.2215/CJN.04800908. PMID 19218475.
6. Miwa Y, Isozaki T, Wakabayashi K, Odai T, Matsunawa M, Yajima N, Negishi M, Ide H, Kasama T, Adachi M, Hisayuki T, Takemura T (2008). "Tacrolimus-induced lung injury in a rheumatoid arthritis patient with interstitial pneumonitis". Modern Rheumatology.
7. Haberfeld, H, ed. (2015). Austria-Codex (in German). Vienna: Österreichischer Apothekerverlag. Prograf.
8. Dinnendahl, V; Fricke, U, eds. (2003). Arzneistoff-Profile (in German). 9 (18 ed.). Eschborn, Germany: Govi Pharmazeutischer Verlag. ISBN 978-3-7741-9846-3.