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Original Article

A ROBUST METHOD FOR SIMULTANEOUS QUANTITATIVE DETERMINATION OF EPLERENONE POLYMORPHS IN TABLET FORMULATION BY X-RAY POWDER DIFFRACTION

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ABSTRACT

Objective: Eplerenone (EPL) is a selective aldosterone blocker used for the treatment of cardiovascular disease. It is known from the literature that the Polymorphs of EPL, form-H and form-L exist thermodynamically enantiotropic in relation, and encompass noticeably different dissolution rates in aqueous media. In view of increasing regulatory concern and bio-pharmaceutical performance, having an accurate method to quantitatively evaluate the solid phase(s) of the drug substance in drug product is reasonably vital. The aim of this work is to develop and validate an accurate, precise, rapid and robust method for simultaneous quantitative determination of EPL polymorphs in tablets formulation by X-Ray Powder Diffraction.

Methods: Geometric mixtures were prepared by taking varying amounts of form-H and from-L, while keeping placebo amount fixed. These spiked samples were scanned using X-Ray Powder Diffractometer and the method was validated. Robustness of the method was tested against intensity variation, scan time variation and specimen holder type.

Results: Validation results of the method demonstrated acceptable correlation (R^2 0.9991) between actual and predicted values, acceptable accuracy (recovery from 88% to 102%) and precision with an RSD less than 1.0%. Additionally, the method was tested for robustness, and found to be very robust to the effects caused by the plausible variations in X-ray intensity, scan time and sample quantity.

Conclusion: The method can readily be used by any quality control laboratory as a control measure for Eplerenone polymorphs composition in tablets so as to ensure the quality and efficacy.

Keywords: X-Ray Powder Diffraction, Eplerenone, Polymorph, Formulation, Quantitative.

INTRODUCTION

Polymorphs are different crystal forms of same chemical entity. The differences in physico-chemical properties like dissolution rate, melting point, packing etc. [1] of the polymorphs have considerable impact on drug product stability (physical and chemical) and Bio-Pharmaceutical performance [2]. The Phenomenon of Polymorphism and its Impact are well recognized in the pharmaceutical industry. Many recent studies in solid state characterization and related areas lead to increased awareness and raising concern [3, 4]. As a consequence regulatory bodies like FDA provides guidance which illustrates how to select suitable drug polymorph or the alternatives for the product development, monitoring its stability and to have control, so as to ensure quality maintained [5, 6].

Burger and Ramberger [7] discussed four fundamental rules to understand thermodynamic relationship (Enantiotropic or Monotropic) between polymorphs using Energy/Temperature phase diagrams. The rules are (1) Heat of Transition rule (HTR), (2) Heat of Fusion rule (HFR), (3) Density Rule (DR) and (4) Infrared rule. However, HTR is most commonly used to explain an Enantiotropic relation, according to this rule, one polymorphic form is thermodynamically more stable than other over a particular temperature range only and the other polymorph is thermodynamically more stable at a different temperature range [8, 9].

Eplerenone [Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3oxo-, γ-lactone, methyl ester, (7α,11α,17α)-] with a trade name Inspra, is a selective aldosterone blocker used for the treatment of cardiovascular mortality and hospitalization for heart failure[9]. Its empirical formula is $C_{24}H_{30}O_6$ and it has a molecular weight of 414.50. The structural formula of Eplerenone is represented in Figure 1. It is known from the literature [10], a patent document, that the Eplerenone has two polymorphs namely form-H and form-L.

The forms are enantiotropically related to each other with a transition temperature at about 105°C. Form-I is thermodynamically more stable below transition temperature and Form-II is thermodynamically more stable above transition temperature. Different techniques have been explored for the physical characterization of pharmaceutical solids like X-Ray Powder Diffraction (XRPD) [11,12], Thermal analysis [13-16], Spectroscopic Methods [17,18], and Microscopy etc. Among these, XRPD is most widely accepted method due to the specificity of diffraction patterns to a particular crystal phase and the fundamental direct relationship between intensity of peak in a powder diffraction pattern and the quantity of particular crystal phase present in a mixture [19].

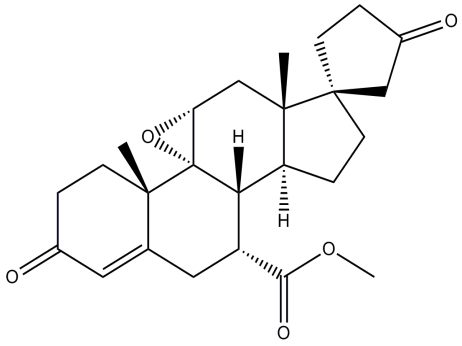

Fig. 1: Molecular structure of Eplerenone

However, various other factors affect this relationship, such are homogeneity of the phase distribution in the powder, preferred orientation, Instrumental and operational errors [20-22]. There are many users in the pharmaceutical industry still use conventional single peak based measurements using linearity of the response with that of the phase quantity. Accuracy and precision of these single peak based methods are often affected by inhomogeneous distribution of the phase in the powder, X-Ray tube intensity decay over a period of time, specimen preparation procedure and sample quantity variations [23]. The challenge lies in developing a reliable method for quantitative

analysis of polymorphs in a complex case like formulations where the drug substance is present in a diluted state in matrix of several inactive ingredients. The XRPD method transfer becomes more difficult because different laboratories may contain XRPD machines with different make, model, specimen holder types, procedures and operational conditions. The current study uses Intensity Ratio (RIR) method, wherein area ratio of the two polymorphs is considered for the calculation quantitative weight fractions of respective polymorphs, the approach can overcome some limitations of conventional single peak analysis and the same is demonstrated.

Mathematical

A simplified equation for Intensity of powder diffraction peak(s) in Bragg–Brentano geometry is given below [24].

$$I_H = \frac{C_H W_H}{\rho_H \mu_H} \text{ (Equation-1)}$$

Where, I_H = The Intensity of diffraction peak of Form-H, C_{iH} = A constant for reflection i of Form-H, W_H = Weight fraction of Form-H, ρ_H = Density of Form-H and μ_H = Mass absorption coefficient of Form-H.

Since C_H and μ_H are constant for a particular crystal phase, the above equation can be written down as

$$I_H \alpha \frac{W_H}{\rho_H} \text{ (Equation-2)}$$

The equation also can be written as

$$\frac{I_H}{I_L} \alpha \frac{W_H \rho_L}{W_L \rho_H} \text{(Equation-3)}$$

Where, I_L, W_L and ρ_L are intensity of diffraction peak, weight fraction, and density of Form-L, respectively.

An assumption is made that the difference in densities of both polymorphs of Eplerenone in the formulation is negligible, and thus the equation (3) becomes

$$\frac{I_H}{I_L} \alpha K \frac{W_H}{W_L} \text{(Equation-4)}$$

Where, K is a constant, henceforth called as response factor (RF), the value can be determined by taking equal quantities of both forms. Thus determined K can be used for the calculation of weight percentage of each polymorph by using following equation that is deduced for equation (4).

$$\text{Fraction of Form} - H = \frac{1}{[1+K(i_L/i_H)]} \text{ (Equation-5)}$$

$$\text{And, Fraction of Form} - L = \frac{1}{[1+K(i_H/i_L)]} \text{(Equation-6)}$$

Where, i_H and i_L are the intensities of the peaks of Form-H and Form-L respectively in the diffraction patterns of a given sample.

MATERIALS AND METHODS

Spiked Standards Preparation

Eplerenone form-H and form-L are used as obtained. Placebo was prepared by physically mixing inactive ingredients as per the formulation. Average weight of Eplerenone Tablets of 25 mg strength is about 150 mg. In view of this, each spiked concentration was maintained to have the same Eplerenone versus placebo composition. However, quantity of form-H and form-L are varied by keeping total weight of Eplerenone constant. Prior to weighing both forms and Placebo (Physical mix of all constituent excipients quantitatively as present in Eplerenone Tablets formulation) are crushed into fine powder with the help of pestle and mortar and with gentle force to reduce particle size differences.

The three components are accurately weighed as per desired concentration levels and transferred into a vial, mixed well by shaking, later mixed in a mortar with pestle and packed into specimen holder. The actual weights of each component and the composition of the final spiked standard are given in Table 1.

X-Ray Powder Diffraction

Bruker D8 ADVANCE X-Ray Diffractometer (Bruker AXS, Germany) with Lynxeye detector and Copper anode (wavelength 1.51 Å) has been used to generate diffraction patterns. The generator was operated at 40 kV and 40 mA (1.6 kW power).

Table 1: Varying amounts of form-H, form-L and Placebo weighed

S. No.	Weight of form-H taken (mg)	Weight of form-L Taken (mg)	Weight of Placebo Taken (mg)	Actual % of form-H in Total Drug in the Formulation	Actual % of form-L in Total Drug in the Formulation
1	0.00	35.10	87.41	0.0	100.0
2	3.51	31.60	87.17	10.0	90.0
3	6.83	28.13	87.34	19.5	80.5
4	10.50	24.74	87.27	29.8	70.2
5	17.61	17.42	87.13	50.3	49.7

The samples were scanned from 9.5 to 12.5° 2θ with a step size of 0.01° 2θ and with a scan speed of 0.1° per minute. Programmable slits (automatic 20 mm) were used on both incident and divergent side. Sample spinning was enabled with a speed of 0.5 revolutions per second during measurement. All the spiked standards were analyzed according to these parameters. However, Individual pure forms and Placebo were scanned for Identification from 3 to 45° 2θ with a scan speed of about 5° per minute and by keeping remaining parameters same. The methods were created using XRD Wizard software, XRD Commander Software was used to run the measurements and Eva software is used for data evaluation.

Robustness

20% form-H spiked standard prepared and is considered for this test. And the following variations are studied.

X-Ray Intensity change

The 20% form-H spiked standard measured three times by operating X-ray generator power 1.8 (45 kV and 40 mA) and 1.2 kW (40 kV and 30 mA) by keeping remaining parameters same.

Scan time (Time per step) change

The 20% form-H spiked standard measured three times by scanning for 20 min (time / step 12 sec) and 40 min (time / step 18 sec) respectively by keeping remaining parameters same.

Specimen Quantity change

The 20% form-H spiked standard measured three times by analyzing on Silicon low back ground (8 x 0.5 mm, takes about 120 mg of powder) specimen holder and PMMA (8 x 0.5 mm, takes about 500 mg of powder) specimen holder.

RESULTS AND DISCUSSION

Identification of polymorphs by XRPD

The characteristic XRPD profiles of two polymorphs are compared (Figure 2) with placebo's. Characteristic Peaks of form-H and form-L at about 12.1 and 10.1° 2θ respectively are chosen for quantitative measurements. As a result the selected range for quantitative measurements is from 9.5 to 12.5° 2θ.

Relative Response factor (K) determination

Average *K* value is calculated by taking Area under the peak of each form-H and form-L from diffraction pattern of the spiked standard (Serial No. 5 in Table 1) with equal concentration of both polymorphs of three consecutive measurements and respective weights by substituting in equation (4). The calculated average *K* value is 0.714. Table 2 comprises area values of three measurements.

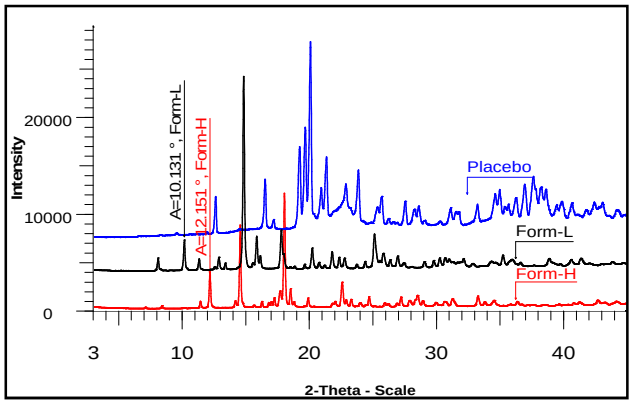

Fig. 2: Overlaid x-ray diffractogram of from-H and form-L with placebo, which enables the selection of characteristic peaks for the purpose of quantification.

Accuracy, Precision and Regression

Amount (% weight / weight) of each polymorph in the total Eplerenone calculated for all the spiked standard using equation (4) and (5). Each measurement was carried out in triplicates. Actual Vs average calculated values are compared to see check the regression and % recovery calculated. % RSD of the three calculated consecutive measurements of each standard was calculated.

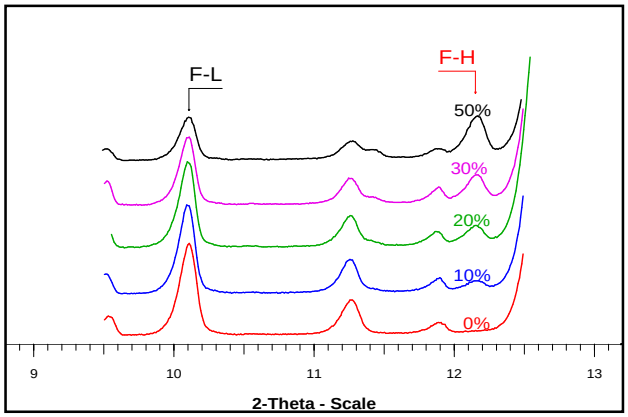

Fig. 3: Overlaid diffractogram representing linear change in the area under peak (at about 10.1° 2θ, for from-L and at about 12.2° 2θ, for form-H), with respective to the concentration.

The validation details are compiled in Table 3. Actual values of % form-H are plotted (Figure 3) against respective calculated average results, the R^2 value of the regression found to be 0.9991.

Robustness

The results from the sample analysis with various method parameters change like X-Ray Intensity (Figure 4), Time per step (Figure 5) and Sample quantities (Figure 6) are compiled Table 4. The amount (% weight / weight) of polymorph form-H in the total Eplerenone calculated for all the spiked standard using equation (4) and (5).

Table 2: Areas of form-H and form-L peaks for each of three preparations, along with respective weight fractions. The data is used for the calculation of relative response factor *k*.

Scan No.	Weight fraction of form-H	Weight fraction of form-L	Area under F-H peak (cps x degree)	Area under F-L peak (cps x degree)	Calculated K value
1	0.503	0.497	2.630	3.639	0.715
2	0.503	0.497	2.656	3.658	0.718
3	0.503	0.497	2.629	3.668	0.709
Average					0.714

Table 3: Accuracy and precision calculations - Relative standard deviation (RSD) was calculated for the three results at each concentration.

Actual % form-H (%wt/wt)	Scan number	Area under form-H peak	Calculated value of form-H (%)	% recovery	% RSD	Actual % form-L (%wt/wt)	Scan number	Area under form-L peak	Calculated value of form-L (%)	% recovery	% RSD
10.0	1	0.498	8.8	88	1.5	90.0	1	7.188	91.2	101	0.2
	2	0.520	9.0	90			2	7.330	91.0	101	
	3	0.522	9.1	91			3	7.290	90.9	101	
Average			9.0	90		*Average*			91.0	101	
19.5	1	1.074	17.8	91	0.8	80.5	1	6.954	82.2	102	0.2
	2	1.051	17.7	91			2	6.848	82.3	102	
	3	1.015	17.5	90			3	6.693	82.5	102	
Average			17.7	91		*Average*			82.3	102	
29.8	1	1.663	30.1	101	0.4	70.2	1	5.408	69.9	100	0.2
	2	1.686	30.3	102			2	5.420	69.7	99	
	3	1.653	30.3	102			3	5.329	69.7	99	
Average			30.2	101		*Average*			69.8	99	
50.3	1	2.660	50.6	101	0.3	49.7	1	3.643	49.4	99	0.4
	2	2.725	50.9	101			2	3.563	49.1	99	
	3	2.684	50.5	100			3	3.678	49.5	100	
Average			50.7	101		*Average*			49.3	99	

Table 4: Robustness calculations: 1) for variation in X-ray tube operational power, 2) Scan time and 3) Specimen holder type

Varied Parameter	Scan No.	Area under F-H peak	Area under F-L peak	Calculated % F-H (Wt/Wt)	Average	%RSD
Power 1.8 kW	1	1.256	7.330	19.4	19.5	1.0
	2	1.290	7.370	19.7		
	3	1.238	7.239	19.3		
Power 1.2 kW	1	0.804	4.620	19.6	19.5	0.5
	2	0.799	4.599	19.6		
	3	0.811	4.719	19.4		
Scan time 20 min (scan speed 0.15° / min)	1	1.072	6.329	19.2	19.2	0.9
	2	1.070	6.240	19.4		
	3	1.076	6.413	19.0		
Scan time 40 min (scan speed 0.075° / min)	1	1.096	6.478	19.2	19.2	0.0
	2	1.067	6.291	19.2		
	3	1.076	6.484	19.2		
Sample quantity about 120 mg	1	1.047	6.130	19.3	19.6	1.1
	2	1.138	6.504	19.7		
	3	1.086	6.208	19.7		
Sample quantity about 500 mg	1	1.150	6.745	19.3	19.2	0.2
	2	1.156	6.790	19.3		
	3	1.109	6.529	19.2		

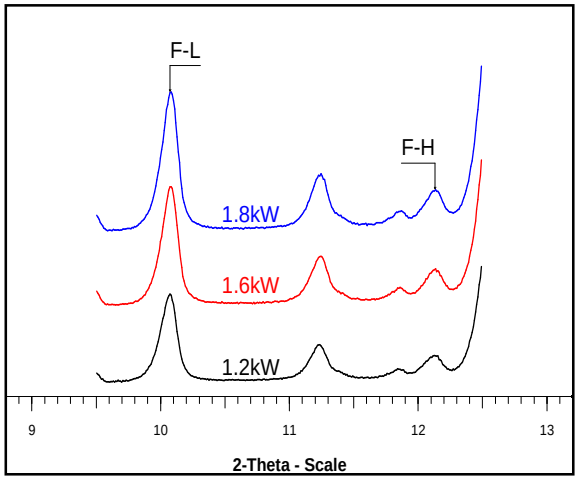

Fig. 4: Overlaid diffractogram shows increase in overall intensity of the diffraction peaks with increase in x-ray tube operational voltage, while maintaining constant ratio of the areas of the peaks (at about 10.1° 2θ, for from-L and at about 12.2° 2θ, for form-H).

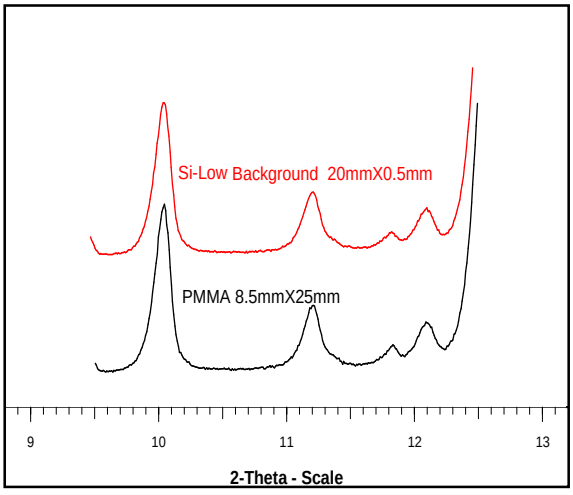

Fig. 5: Overlaid diffractogram of the sample analyzed in different type of specimen holders, representing constant ratio of the areas of the peaks (at about 10.1° 2θ, for from-L and at about 12.2° 2θ, for form-H).

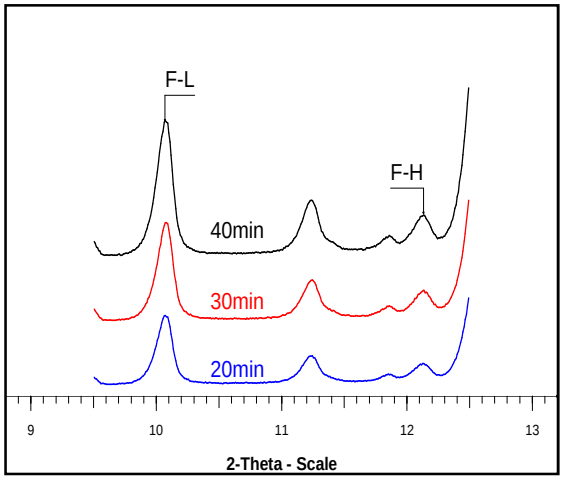

Fig. 6: Overlaid diffractogram shows increase in overall intensity of the diffraction peaks with increase in scan time, while maintaining constant ratio of the areas of the peaks (at about 10.1° 2θ, for from-L and at about 12.2° 2θ, for form-H).

CONCLUSIONS

An accurate, precise and robust method was developed and validated for the quantitative determination form-H and form-L of Eplerenone, simultaneously, in tablets. The scientific relevance for the need of the method was discussed. X-Ray Powder Diffraction in conjunction with intensity ratio method was used to achieve a robust method, overcoming the pitfalls of the conventional single peak analysis method and external standard method. Robustness towards X-Ray tube intensity variations, scan time variations and specimen quantity variations was demonstrated. The method can directly be adopted by any quality control laboratory without any much change; moreover, the method does not require use of any standard specimens during routine analysis, which is otherwise not possible in external standard method.

REFERENCES

1. Brittain HG, Bogdanowich SJ, Bugay DE, DeVincentis J, Lewen G, Newman AW. Physical characterization of pharmaceutical solids. Pharm. Res. 1991;8(8):963-973.
2. Ku MS. Use of the biopharmaceutical classification system in early drug development. The AAPS J.2008;10(1):208-212.
3. Chieng N, Rades T, Aaltonen J. An overview of recent studies on the analysis of pharmaceutical polymorphs. J. Pharm. Biomed. Anal. 2011;55(4):618-644.

4. Lara-Ochoa F, Espinosa-Pérez G. Crystals and patents. Cryst. Growth Des. 2007;7(7):1213-1215.
5. Raw AS, Furness MS, Gill DS, Adams RC, Holcombe Jr FO, Yu LX. Regulatory considerations of pharmaceutical solid polymorphism in Abbreviated New Drug Applications (ANDAs). Adv. Drug Deliv. Rev. 2004;56(3):397-414.
6. Byrn S, Pfeiffer R, Ganey M, Hoiberg C, Poochikian G. Pharmaceutical solids: a strategic approach to regulatory considerations. Pharm. Res. 1995;12(7):945-954.
7. Burger A, Ramberger R. On the polymorphism of pharmaceuticals and other molecular crystals. I. Microchim. Acta 1979;72(3-4):259-271.
8. Lohani S, Grant DJ. Thermodynamics of polymorphs. In: Hilfiker R, editor (s). Polymorphism in the pharmaceutical industry. Verlag GmbH: Weinheim WILEY-VCH Press; 2006. p.21-42.
9. Delyani JA, Rocha R, Cook CS, Tolbert DS, Levin S, Roniker B et al. Eplerenone: a selective aldosterone receptor antagonist (SARA). Cardiovasc. drug rev. 2001;19(3):185-200.
10. Barton K, Borchardt TB, Carlos MV, Desai S, Ferro LJ, Gaud HT, Scott G et al., European Patent 2002; EP 1177204.
11. Tiwari M, Chawla G, Bansal AK. Quantification of Olanzapine polymorphs using powder X-ray diffraction technique. J. Pharm. Biomed. Anal. 2007:43(3):865-872.
12. Uvarov V, Popov I. Development and metrological characterization of quantitative X-ray diffraction phase analysis for the mixtures of Clopidogrel bisulphate polymorphs. J. Pharm. Biomed. Anal. 2008;46(4):676-682.
13. Giron D. Applications of thermal analysis and coupled techniques in pharmaceutical industry. J. Therm. Anal. Calorim. 2002;68(2):335-357.
14. Giron D. Thermal analysis and calorimetric methods in the characterisation of polymorphs and solvates. Therm. Chim. Acta. 1995;248:1-59.
15. Kawakami K, Ida Y. Application of modulated-temperature DSC to the analysis of enantiotropically related polymorphic transitions. Therm. Chim. Acta. 2005;427(1):93-99.
16. Ravikiran A, Arthanareeswari M, Kamaraj P, Praveen C. Non-isothermal kinetics analysis of Dehydration of Ziprasidone Hydrochloride Monohydrate by Thermogravimetry. Indian J. Pharm. Sci. 2013;75(3):361-364.
17. Agatonovic-Kustrin S, Rades T, Wu V, Saville D, Tucker IG. (2001). Determination of polymorphic forms of ranitidine–HCl by DRIFTS and XRPD. J. Pharm. biomed. Anal. 2001;25(5):741-750.
18. Chieng N, Rehder S, Saville D, Rades T, Aaltonen J. Quantitative solid-state analysis of three solid forms of ranitidine hydrochloride in ternary mixtures using Raman spectroscopy and X-ray powder diffraction. J. Pharm. biomed. Anal. 2009;49(1):18-25.
19. Jenkins R, Snyder R. Introduction to X-ray powder diffractometry 2012; (Vol. 267). John Wiley & Sons.
20. Tian F, Zhang F, Sandler N, Gordon KC, Mc Goverin CM, Strachan CJ, et al. Influence of sample characteristics on quantification of Carbamazepine hydrate formation by X-ray powder diffraction and Raman spectroscopy. Eur. J. Pharm. Biopharm. 2007; 66(3):466-474.
21. Smith DK, Johnson GG, Scheible A, Wims AM, Johnson JL, Ullmann G. Quantitative X-ray powder diffraction method using the full diffraction pattern. Powder. Diffr. 1987;2(02):73-77.
22. Davidovich M, Gougoutas JZ, Scaringe RP, Vitéz I, Yin S. Detection of Polymorphism by Powder X-Ray Diffraction: Interference by Preferred Orientation. Am. Pharm. Rev. 2004;7:10-17.
23. Stephenson GA, Forbes RA, Reutzel-Edens SM. Characterization of the solid state: quantitative issues. Adv. Drug Deliver. Rev. 2001;48(1):67-90.
24. Suryanarayanan R, Herman CS. Quantitative analysis of the active ingredient in a multi-component tablet formulation by powder X-ray diffractometry. Int. J. Pharm. 1991;77(2):287-295.