Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 572–576

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Rietveld refinement in the routine quantitative analysis of famotidine polymorphs

Zoltán Német [a,*], István Sajó [b], Ádám Demeter [a]

[a] Drug Polymorphism Research Division, Gedeon Richter Plc., P.O. Box 27, H-1475 Budapest, Hungary
[b] Department of X-ray Diffraction, Chemical Research Center of Hungarian Academy of Sciences, H-1525 Budapest, P.O. Box 17, Hungary

ARTICLE INFO

Article history:
Received 17 July 2009
Received in revised form
11 September 2009
Accepted 14 September 2009
Available online 22 September 2009

Keywords:
Famotidine
X-ray powder diffraction
Quantitative analysis
Rietveld refinement
Transmission

ABSTRACT

Accurate, precise and reliable X-ray powder diffraction method was developed for the quantitative determination of famotidine polymorphic forms in their binary mixtures, which slightly outperforms the previously established Raman method. The study highlights the advantage of focused beam transmission geometry in diminishing the effect of preferred orientation in general, and the straightforward transmission foil sample preparation technique in facilitating high-throughput measurements in particular. This combination can provide good quality data for Rietveld refinement which assures more reliable quantitative results than utilizing intensity ratios of selected single reflections. After careful adjustment of profile parameters, simple routine application of the method was achieved.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The importance of quantitative solid state analysis in the pharmaceutical industry is constantly growing [1]. There is an ever-increasing demand for the quality assurance to justify the polymorphic purity of active ingredients [2,3]; while intellectual property considerations often specify the limits of non-infringing compositions [4,5].

Famotidine, a widely used histamine H_2-receptor antagonist, has two polymorphic forms: the thermodynamically stable form A and the kinetically favoured form B [6]. Previous experiences with the quantitative analysis of famotidine polymorphs are detailed in our recent study [7]. Those results suggested the superiority of Raman spectroscopy over X-ray powder diffraction (XRPD). The poorer performance of the latter was ascribed to preferred orientation effects.

Transmission geometry in XRPD, with sample filled in a capillary, is usually efficient in decreasing preferred orientation. Filling capillary does not require forcing the crystals onto a certain reflection plane, and the orientation of crystallites in the active volume will exhibit more random distribution. In this case, however, the sample preparation and capillary alignment is relatively tedious. A promising alternative is to use X-ray transparent foils enclosing a thin layer of the sample. In this case one takes out *ca.* 10–20 mg of sample from the bulk, puts it on a foil, and then encloses it with another piece of foil. The specimen can safely be stored; and in addition to quick sample preparation, it also permits the automatization of series measurements by utilizing sample changer.

Our aim was to improve the previously established XRPD method for the quantitative determination of famotidine polymorphs, and to develop a Rietveld refinement method which can be simply utilized in the routine phase analysis of famotidine mixtures.

While the Rietveld technique was initially developed for the refinement of crystal structure, it proved very efficient also in quantitative phase analysis, as the Rietveld scale factor of a phase relates to its relative amount in a multiphase mixture. The improved performance of the method compared to conventional single peak methods derives from the fact that the whole diffraction pattern contributes to the analysis, thus the impact of peak overlap and sample related effects (as preferred orientation) is minimized. Using physical constants from crystal structure data for calculating reflection intensities eliminates the errors associated with intensity measurements and calibration procedures. Detailed description of quantitative Rietveld methods [8] and practical guidance to its use in general [9] can be found in the literature. The method has been successfully used for quantitative applications of pharmaceutical solids [10,11].

* Corresponding author. Tel.: +36 1 431 4991.
E-mail address: z.nemet@richter.hu (Z. Német).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.09.017

2. Materials and methods

2.1. Materials

The preparation of pure polymorphic forms, calibration mixtures and samples of unknown composition was described previously [7]. In order to decrease the particle size allowing sufficient particle statistics, every sample measured was previously lightly ground for 3 min in an agate mortar with a pestle. It has already been shown that such grinding does not cause polymorph transition or amorphization [7].

2.2. X-ray powder diffraction

Diffraction patterns were measured on PANalytical X'Pert PRO MPD diffractometer using Cu Kα radiation with 40 kV accelerating voltage and 40 mA anode current at a scanning rate of 0.005–0.010° $2\theta\,min^{-1}$ with 0.013° 2θ step size in transmission mode, spinning the sample holder by $1\,s^{-1}$. PIXcel detector (0.75° active length) and focusing mirror (1/2° entrance slit) was used with 0.04° Soller slits. About 20 mg of the sample was enclosed between two mylar foils. The majority of the mixtures and unknowns were measured multiple times (different portion of the sample was repacked and measured). Data were collected by PANalytical Data Collector software, version 2.2.

2.3. Data analysis

Univariate data analysis was performed by PANalytical Data Viewer, version 1.2c and Microsoft Excel. Rietveld refinement was carried out using Fullprof program [12] from the published single crystal structure data [13]. The results of different data processing procedures were compared using linear correlation coefficients (r) and root-mean-squared error of prediction (RMSEP). The latter is defined by the following equation: $\mathrm{RMSEP} = \sqrt{\sum_{i=1}^{n}(y_i - Y_i)^2/n}$, where y_i, Y_i and n are the calculated value, the theoretical value (neglecting the possibility of inaccurate mixture preparation, this was considered equal to the nominal value) and the number of measurements, respectively.

3. Results and discussion

3.1. Univariate data analysis

Transmission measurements from foil sample preparations proved highly efficient in decreasing preferred orientation. Fig. 1 shows the comparison of diffractograms measured in reflection and transmission to those calculated from the single crystal structure. Transmission patterns show very good match to the calculated patterns, while the reflection patterns have significant relative intensity distortions.

It was previously ascertained [7] that the reflections at 11.6° 2θ, i.e. (0 0 2) reflection for form B, as well as 14.4° 2θ, and 18.7° 2θ, i.e. (1 1 0) and (1 1 2) reflection for form A, respectively are the most appropriate for phase analysis. These were selected for the univariate quantification also in transmission. Peak heights and integrated peak intensities gave substantially the same results, thus the former was used in the followings because of its simplicity.

It is evident that neither the absolute amount of the sample between the two foils, nor the actual sample thickness can be controlled in measurements applied. The use of internal standard was avoided, because of its additional labour with sample mixing. According to Chung's matrix-flushing method, however, there is no need for internal standard in the quantitative analysis of binary mixtures, providing that the occurrence of a third phase, typically

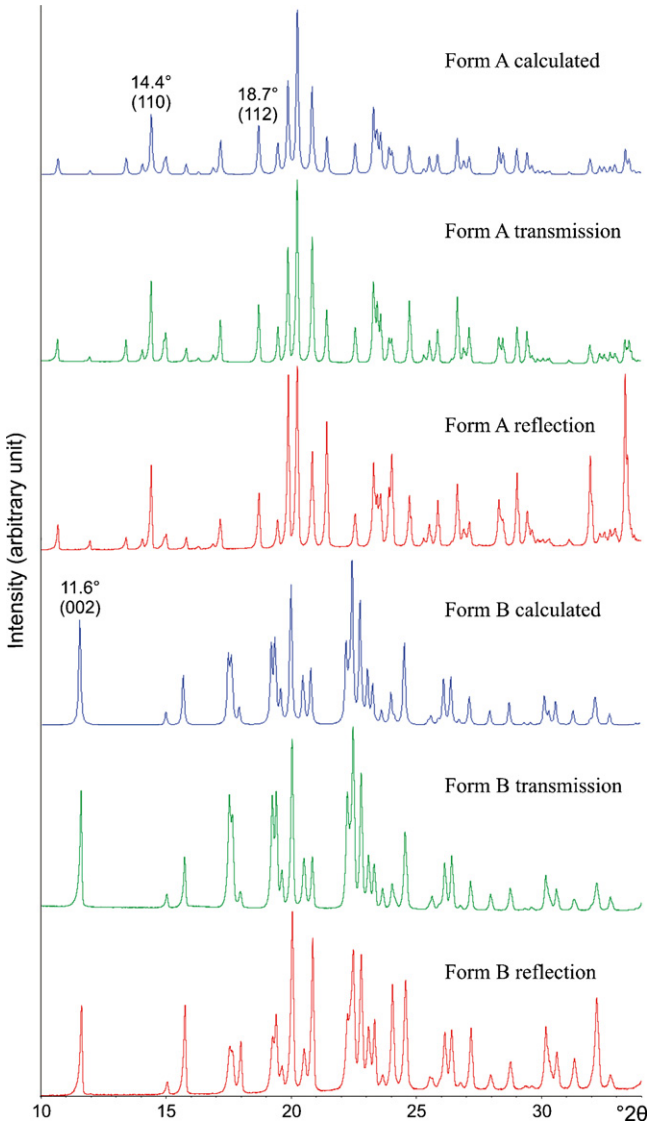

Fig. 1. X-ray powder diffraction patterns of famotidine form A calculated, measured in transmission and reflection, as well as form B calculated, measured in transmission and reflection, from top to bottom, respectively.

amorphous, is unlikely [14]. In the famotidine case this is true. The corrected intensity ratio of the two characteristic reflections is given by Eq. (1):

$$r = \frac{I_a}{I_a + I_b(I_{a0}/I_{b0})} \quad (1)$$

where I_a and I_b are the intensities of specific reflections of form A and form B in the mixture, while I_{a0} and I_{b0} are the corresponding intensities of pure forms, respectively. The reference intensity ratio, I_{a0}/I_{b0} can easily be obtained by the measurement of a mixture containing both polymorphs in the same amount (i.e. the 50% mixture). This intensity ratio, r, should be equal to the weight fraction of one polymorph, in this case form A (w_A).

The reference intensity ratios, determined from triplicate measurement of the 50 wt.% mixture, are 1.314 (0.074) and 0.709 (0.014) for (1 1 0) and (1 1 2) reflections, respectively (with standard deviations in parentheses). The composition of calibration samples were calculated by using these ratios, and were plotted against their actual (nominal) composition (Fig. 2). The correlation is very good, the slope is nearly equal to unity, and the intercept is practically zero, irrespective of whether (1 1 0), or (1 1 2) reflection's intensity,

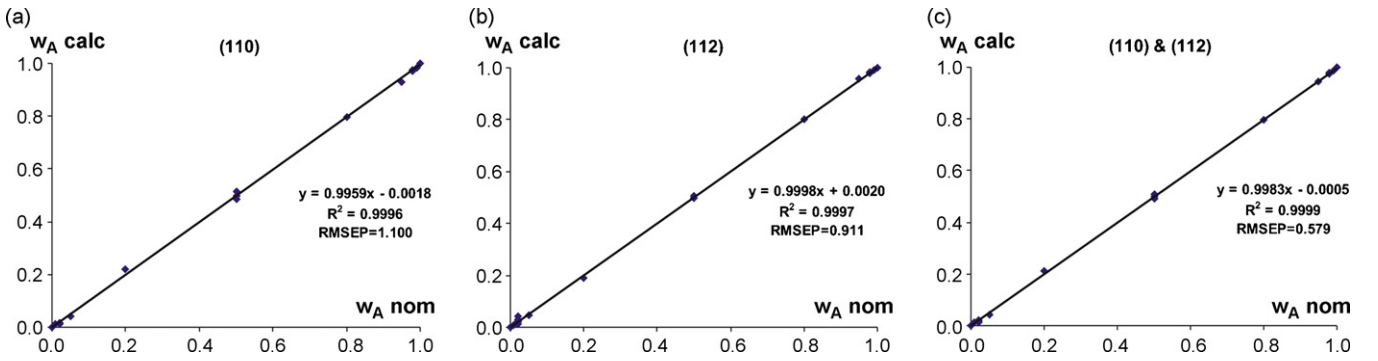

Fig. 2. Calibration correlation for famotidine transmission XRPD data using the intensities of specific reflections: (a) (1 1 0) form A reflection, (b) (1 1 2) form A reflection, and (c) the sum of both; (0 0 2) reflection is used for form B in every case.

or the sum of these were used for form A. This means that calibration is actually unnecessary; the composition can be calculated solely from I_a and I_b using Eq. (1).

Although all three evaluation methods seem to be precise and unbiased, the sum of intensities possesses the smallest RMSEP. Note that the intercept of the correlation line is a little bit smaller and larger than zero for (1 1 0) and (1 1 2) reflections, respectively, while it is practically zero for the sum of the intensities of these two reflections. This negligible bias is certainly caused by the remaining preferred orientation. Table 1 shows the limits of detection (LOD) and quantitation (LOQ) for these three evaluations, which were calculated by multiplying 3.3 and 10 the standard deviation of 2 wt.% mixtures (results of five independent measurements), respectively (2 wt.% mixtures were chosen because the concentration of their minor component is presumably around the LOD). Detection limit for form B is around 1 wt.%, which is rather low from a quantitative method also working in the range of high concentrations. Detection and quantitation limits of form A are similar; however, (1 1 2) reflection gives significantly higher LOD and LOQ. This is also the result of orientation of form A crystals relating to this lattice plane, causing relatively high intensity variation.

One can conclude therefore, that the best simple "univariate" method for the quantitative phase analysis of famotidine mixtures is to take the intensity of (0 0 2) reflection for form B and the sum of (1 1 0) and (1 1 2) reflection intensities for form A, and calculate the composition of the analyte according to Eq. (1).

3.2. Rietveld refinement

Rietveld refinement was done using the Fullprof program suite [12]. During refinements the structural parameters (atomic coordinates and thermal coefficients) were kept fixed. In the first step, refinements were done on the diffraction patterns of famotidine pure polymorphs to determine profile, sample related and instrumental parameters. For this, unit cell coordinates, instrumental zero position, background, profile shape and asymmetry parameters, as well as overall B-factor and preferred orientation parameters were released. Stable and reliable background refinement was achieved using the 6 coefficient polynomial with 4 released coefficients. For the correction of preferred orientation the refinement of G1 coefficient of the modified March's function proved sufficient.

After reaching acceptable profile fit, the profile shape and asymmetry, as well as the modified March's function parameters were further refined on the diffraction pattern of the 50 wt.% polymorphic mixture. The obtained model was used for the quantification of other mixtures thereafter, refining only polynomial background function and preferred orientation. Keeping data collection and sample preparation parameters constant allowed fixing instrumental and sample related parameters at previously determined values. With only these few released parameters the convergence was always stable and quick. The resulting weight fraction values were used as phase concentrations. As the polymorphs exhibit identical mass absorption coefficients, no Brindley correction was necessary.

For the purpose of quantification the diffraction patterns were measured in the range of 3–60° 2θ with low scanning rate (150 s counting time) for *ca.* 3.2 h, to achieve good signal to noise ratio. The obtained Bragg *R*-values were 11.4 and 7.9 for pure forms, 10.4 and 9.4 for the 50 wt.% mixture, for form A and form B, respectively. Higher values were obtained for the minor components of mixtures, e.g. 26.7 and 18.2 for 5% form A and 5% form B, respectively. The relatively poor fit, however, does not seem to influence the quantitative result of the refinement. Data collection optimization revealed that only 3–40° 2θ range is necessary and scan speed can be increased to 0.01° 2θ min^{-1} (75 s counting time).

The obtained correlation between the calculated and the actual concentration of calibration mixtures is very good (Fig. 3a), RMSEP is around 0.6%. LOD and LOQ were calculated from the standard deviation of 2 wt.% mixtures, measured in triplicate. Table 2 shows that Rietveld refinement slightly outperforms the previously established Raman method, which proved unequivocally superior to the reflection XRPD method [7]. However, it seems that the former is a little bit biased in the low concentration range (less than 15 wt.%) of form B. Actually, this is the reason why the slope of the line in Fig. 3a slightly differs from unity.

In usual pharmaceutical applications, this bias can be neglected; however, needing very accurate quantification it has to be taken into account. In this case the result of the Rietveld refinement has to be adjusted to correct for this (1–2 wt.%) bias. Proper scaling factor was easily obtained by constructing a calibration line in the low concentration range of form B (Fig. 3b).

Table 1
Limits of detection and quantitation of famotidine polymorphs by univariate evaluations.

	(1 1 0)		(1 1 2)		(1 1 0)+(1 1 2)	
	Form A	Form B	Form A	Form B	Form A	Form B
Predicted concentration of 2% mixtures (wt.%)	1.4	2.5	2.6	1.8	1.8	2.2
LOD (wt.%)	0.8	1.4	3.8	0.5	1.2	0.9
LOQ (wt.%)	2.3	4.4	11.5	1.5	3.7	2.7

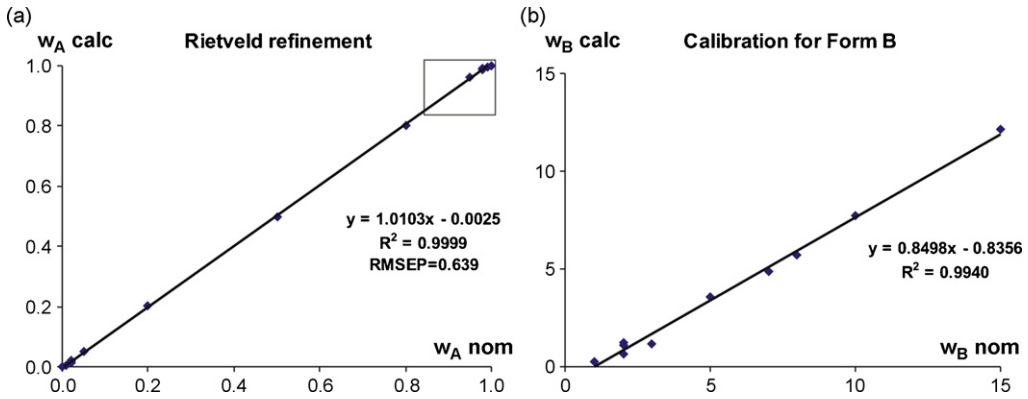

Fig. 3. Calibration correlation for famotidine transmission XRPD data by the Rietveld method: (a) in the whole concentration range and (b) in the low concentration range of form B.

Table 2
Limits of detection and quantitation of famotidine polymorphs by the Rietveld method and Raman spectroscopy.

	Rietveld XRPD		Raman univariate[a]		Raman multivariate[a]	
	Form A	Form B	Form A	Form B	Form A	Form B
Predicted concentration of 2% mixtures (wt.%)	1.7	1.0				
LOD (wt.%)	1.0	0.9	2.6	0.9	1.2	0.9
LOQ (wt.%)	3.1	2.8	7.8	2.6	3.7	2.8

[a] LOD and LOQ of the Raman method were determined from the standard deviation of 3% mixtures (see Ref. [7]).

It is worth to note that the univariate Raman method also failed to obtain accurate quantification in the whole concentration range with single calibration correlation; different calibration lines were used for the determination of low levels of form A and form B. Quantitative methods for solid state phase analysis working in the whole (say 1–99%) concentration range are relatively rare in the literature.

There are two other unsurpassable advantages of the quantitative Rietveld method. It utilizes the whole diffraction pattern and also corrects for moderate orientation effects, which assures the most reliable quantitation. As soon as proper Rietveld model is built, which requires a bit of expertise in this field, it can be easily utilized in routine industrial applications as a "push-one-button" method.

Except for the mentioned 1–2 wt.% bias in the low concentration range of form B, the method also does not need establishing calibration correlation, which eliminates the preparation of calibration mixtures. Only a few mixtures are required in order to check that refinement results in the correct phase composition.

3.3. Comparing univariate method and Rietveld refinement

As it has been already stressed [7], there is a need for testing the reliability of a quantitative method intended to be applied in routine industrial practice. Therefore, polymorphic mixtures of unknown composition were analyzed, and the quantitative results of the above evaluations were compared to the Raman spectroscopic method, described previously [7]. This may be thought of as a kind of robustness testing, as these samples had different crystal size, shape and perfection than those used for the preparation of any kind of calibration mixtures.

Table 3 shows that there is a good correlation between univariate transmission XRPD, Rietveld and Raman results; except Sample 2, the composition of which depends on the evaluation used. Form A crystals orient in the mixture in such a way that the intensity of (1 1 0) reflection is much smaller than it should be; thus even the intensity averaging method (Fig. 3c) estimates less form A than there actually is in the mixture. It is important to note that such pronounced preferred orientation may occur also in other crystallographic directions due to actual morphology of the sample crystallized under certain conditions, which implies that methods using single peak reflections lack robustness in this relation.

Table 3
Composition of unknown samples determined by univariate XRPD, as well as the Rietveld and Raman methods.

	wt.% of Form A[a]		
	(1 1 0)+(1 1 2)	Rietveld	Raman
Sample 1	3.0 (0.6)	3.3 (0.4)	2.9 (0.8)
Sample 2	56.6 (0.3)	65.4 (0.3)	66.6 (0.7)
Sample 3	88.6 (0.2)	89.5 (0.3)[b]	91.1 (0.7)
Sample 4	12.0 (0.6)	9.8 (0.6)	11.0 (0.6)
Sample 5	7.8 (0.6)	8.5 (0.4)	9.4 (0.6)
Sample 6	5.0 (0.9)	5.4 (0.6)	5.5 (0.8)

[a] Numbers are mean values from three measurements with standard deviations in parentheses
[b] The result is corrected for the bias observed in the low concentration range of Form B (see Fig. 3 and the text below that)

As it might be anticipated, the Rietveld method determines the polymorphic content of unknown samples correctly. There is a very good agreement between the results of Rietveld refinement and Raman methods; the former, however, seems to be more precise (providing results with smaller standard deviation).

4. Conclusions

Quantitative XRPD method was developed for the determination of famotidine polymorphic forms in their binary mixtures. Both the platy crystals of form A and the acicular crystals of form B are prone to preferred orientation; transmission geometry is, however, highly efficient in diminishing orientation effects. Sample preparation between the two X-ray transparent foils is fast and simple; the measurement can also be automatized by using a sample changer.

Univariate data evaluation using the intensity of (0 0 2) and the sum of the intensities of (1 1 0) and (1 1 2) reflections for form B and form A, respectively, assures simple and relatively accurate determination. In some cases, however, it can provide biased results,

which means that the method is not sufficiently robust against variations in morphology and/or perfection of the crystals.

Quantitative determination was also accomplished by the Rietveld method, which utilizes the whole diffraction pattern, thus providing the most reliable results. The method requires crystal structure data for every phases of interest; however, no calibration standards are needed. After finding correct profile parameters and utilizing these appropriately, about 1 h of data acquisition is followed by the refinement needing only starting the algorithm fitting the polynomial background function and preferred orientation parameter in the modified March's function, and correcting for instrumental zero shift, if needed. Limits of quantitation are 3 wt.% for both the forms, and the method accurately measures the composition of arbitrary mixtures with the precision of about 0.5 wt.%. This is slightly better than that the previously established quantitative Raman method provides [7].

References

[1] G.A. Stephenson, R.A. Forbes, S.-M. Reutzel-Edens, Characterization of the solid state: quantitative issues, Adv. Drug Deliv. Rev. 48 (2001) 67–90.
[2] A.S. Raw, M.S. Furness, D.S. Gill, R.C. Adams, F.O. Holcombe Jr., L.X. Yu, Regulatory considerations of pharmaceutical solid polymorphism in Abbreviated New Drug Applications (ANDAs), Adv. Drug Deliv. Rev. 56 (2004) 397–414.
[3] Food and Drug Administration, Center for Drug Evaluation and Research, Guidance for Industry, ANDAs: Pharmaceutical Solid Polymorphism: Chemistry, Manufacturing, and Controls Information, 2007, http://www.fda.gov/cder/guidance/index.htm.
[4] J. Bernstein, Polymorphism of Molecular Crystals, Clarendon, Oxford, 2002, pp. 297–307.
[5] W. Cabri, P. Ghetti, G. Pozzi, M. Alpegiani, Polymorphisms and patent, market, and legal battles: cefdinir case study, Org. Process Res. Dev. 11 (2007) 64–72.
[6] B. Hegedűs, P. Bod, K. Harsányi, I. Péter, A. Kálmán, L. Párkányi, Comparison of the polymorphic modifications of famotidine, J. Pharm. Biomed. Anal. 7 (1989) 563–569.
[7] Z. Német, G. Csonka Kis, G. Pokol, Á. Demeter, Quantitative determination of famotidine polymorphs: X-ray powder diffractometric and Raman spectrometric study, J. Pharm. Biomed. Anal. 49 (2009) 338–346.
[8] I.C. Madsen, N.V.Y. Scarlett, Quantitative phase analysis, in: R.E. Dinnebier, S.J.L. Billinge (Eds.), Powder Diffraction: Theory and Practice, RCS Publishing, Cambridge, 2008, pp. 298–331.
[9] L.B. McCusker, R.B. Von Dreele, D.E. Cox, D. Louer, P. Scardi, Rietveld refinement guidelines, J. Appl. Crystallogr. 32 (1999) 36–50.
[10] S.S. Iyengar, N.V. Phadnis, R. Suryanarayanan, Quantitative analysis of complex pharmaceutical mixtures by the Rietveld method, Powder Diffr. 16 (2001) 20–24.
[11] S. Yamamura, R. Takahira, Y. Momose, Crystallization kinetics of amorphous griseofulvin by pattern fitting procedure using X-ray diffraction data, Pharm. Res. 24 (2007) 880–887.
[12] J. Rodrigues-Carvajal, Reference guide for the computer program Fullprof, Laboratoire Léon Brilluin CEA-CNRS, Saclay, France, 1996.
[13] G. Ferenczy, L. Párkányi, J.G. Ángyán, A. Kálmán, B. Hegedűs, Crystal and electronic structure of two modifications of famotidine. An experimental and theoretical study, J. Mol. Struct. (Theochem.) 503 (2000) 73–79.
[14] F.H. Chung, Quantitative interpretation of X-ray diffraction patterns of mixtures. I. Matrix-flushing method for quantitative multicomponent analysis, J. Appl. Crystallogr. 7 (1974) 519–525.