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Short communication

Determination of the relative amounts of three crystal forms of a benzimidazole drug in complex finished formulations by FT-Raman spectroscopy

B. De Spiegeleer [a,*], D. Seghers [b], R. Wieme [c], J. Schaubroeck [d], F. Verpoort [c], G. Slegers [a], L. Van Vooren [b]

[a] *Department of Pharmaceutical Analysis, Laboratories of Radio pharmacy and Drug Analysis, Ghent University, Harelbekestraat 72, B-9000 Gent, Belgium*
[b] *Health Engineering and Assurance (HEA), Keizer Karelstraat 175, B-9000 Gent, Belgium*
[c] *Department of Inorganic Chemistry, Ghent University, Krijgslaan 281 (S3), B-9000 Gent, Belgium*
[d] *Hogeschool Gent, CTL-BME, Schoonmeersstraat 52, B-9000 Gent, Belgium.*

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Abstract

A 5% (m/m) premix for animal use was quantitatively characterized for the polymorph composition of its benzimidazole drug substance. Raman spectra of reference samples (pure polymorphs A, B and C in lactose at a concentration of 5%, m/m) were compared with the spectra of benzimidazole samples with a known polymorph composition and with the spectra of uncharacterized premixes. The raw intensities of 78 selected wavenumbers were vector-normalized and application of stepwise linear regression models estimated the relative quantities of the benzimidazole-drug polymorphs A, B and C in the different samples. Modelling results of the samples with known polymorph composition were in compliance with the expected concentrations, validating the proposed methodology. The benzimidazole drug substance in the premixes was predominantly polymorph B. Although statistically not significant, some traces of polymorph A could not be ruled out. Similar analyses were performed to evaluate the solid-state stability of the benzimidazole drug substance in another drug formulation, i.e. a suspension-emulsion. Suspension-emulsions originally determined as containing polymorph B benzimidazole drug substance were stored for 12 months at 25 °C/60%RH. FT-Raman spectroscopy revealed that no polymorph transformations occurred during this storage.
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1. Introduction

Polymorphism describes different crystal-packing arrangements of the same molecular species in its solid state, while the pseudo-polymorphs are the solvated and amorphous solid-state forms. Polymorphism is especially important for pharmaceutical substances as it may affect, i.a. their chemical stability, their physico-chemical behaviour in pharmaceutical formulations, their dissolution rate and their bio-availability affecting safety and efficacy. As a consequence, in the approval of a solid drug, regulators are currently not only focusing on chemical and microbiological aspects, but also on the solid-state characterization of the drug substance as such, as well as after incorporation into a finished drug product for biological, manufacturing and stability reasons. In addition, by investigating the possible polymorph modifications of a solid drug substance, costly repercussions through inappropriate and unexpected appearance of new forms can be avoided [1].

Various analytical methods are currently being used to verify solid-state structural differences and hence to confirm the

* Corresponding author. Tel.: +32 9 264 80 63; fax: +32 9 264 81 93.
E-mail address: bart.despiegeleer@ugent.be (B. De Spiegeleer).

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presence of polymorphism. The most common technique for providing structural information is single-crystal X-ray crystallography. Unfortunately, this method can often not be used due to the requirement of high-quality single crystals. Consequently, other techniques have been developed like powder X-ray diffraction (XRD), calorimetry, e.g. differential scanning calorimetry (DSC), solid-state NMR and Fourier transformed infrared (FT-IR) spectroscopy [2,3].

The phenomenon of Raman scattering was first described several decades ago [4]. Raman spectra are generated when a laser beam is scattered by a sample: the obtained vibration spectrum not only provides chemical information on the sample, as further quantitatively applied in chemical determinations (e.g. [5]), but reflects also the crystalline phase, and it was suggested that Raman spectroscopy could be a viable method for characterising the (pseudo-)polymorphs of solid drug substances [6,7]. Some recent overview articles summarize the current status of Raman spectroscopy with emphasis on the pharmaceutical applications [8–12]: most of the Raman research has however focused on the chemical and solid-state characterization of the pure chemical compounds, e.g. [13–16], and only rarely on the quantification of the solid-states of the drug substances formulated in a finished drug product, e.g. [17–19]. Raman spectroscopy has the advantage that it requires minimal sample preparation, thus reducing the risk of solid-state conversions during sample preparation. Additionally, it allows measurements in aqueous systems, making this technique very convenient for the characterization of semi-solid pharmaceutical preparations.

The objective of this study was to characterize and to quantify the polymorph composition of a benzimidazole drug substance in commercial premixes at a concentration of 5% (m/m). Raman spectra of reference samples (pure polymorphs A, B and C in lactose at a concentration of 5%, m/m) were compared with the spectra of the unknown premixes. Additionally, similar analyses were performed to evaluate the solid-state stability of the same benzimidazole drug substance but in another formulation, being a three-phase suspension-emulsion.

2. Materials and methods

2.1. Materials

The benzimidazole drug substance (5-*p*-fluorobenzoyl-2-benzimidazolecarbamic acid, methyl ester) is an anthelminticum, commercially available under different solid formulations such as tablets and feed premixes. Like most benzimidazoles, this drug substance displays also polymorphism. So far, three polymorphic forms have been identified: polymorph A (Pol A), polymorph B (Pol B) and polymorph C (Pol C). Four (4) commercial benzimidazole premixes were obtained from different sources with an unknown polymorphic composition. All premixes contained 5% (m/m) benzimidazole drug substance. Moreover, the composition of the carrier or filler of the commercial samples was unknown, and was different between the commercial premixes. Therefore, lactose (lactose monohydrate 80 mesh, Certa) was chosen as the model carrier for the preparation of the analytical reference samples.

The premixes were analyzed twice, on two different days. The applied codes were MIXid, with i = sample number and d = day number, e.g. MIX31 represents premix sample 3 on day 1. In addition, pure reference batches of all three solid-state forms of the benzimidazole drug substance were obtained from a drug substance manufacturer (Janssen Pharmaceutica NV). Powder mixtures of the pure polymorphic forms of the benzimidazole drug substance and lactose were prepared in different combinations. The lactose:benzimidazole drug substance ratio was always 95%:5% (m/m), corresponding with the benzimidazole drug substance concentration in the commercial premixes. An overview of the prepared reference samples is given in Table 1. In addition, the pure benzimidazole drug substance solid-state references were also analyzed and were coded as REFA, REFB and REFC.

The interest for drugs formulated in a suspension-emulsion, i.e. the active drug substance suspended in oil droplets which are emulsified in an aqueous matrix,

Table 1
Overview of the reference samples (lactose + benzimidazole drug substance 95 + 5%, m/m)

No.	Sample code	Sample description	Relative solid-state polymorphic content (%)		
			Polymorph A	Polymorph B	Polymorph C
1	LAC0	Pure lactose	0	0	0
2	LAC31 (LAC A)	Lactose/(100% A)	100	0	0
3	LAC32 (LAC B)	Lactose/(100% B)	0	100	0
4	LAC33 (LAC C)	Lactose/(100% C)	0	0	100
5	LAC41	Lactose/(50% A 50% B)	50	50	0
6	LAC42	Lactose/(50% B 50% C)	0	50	50
7	LAC43	Lactose/(25% A 50% B 25% C)	25	50	25
8	LAC51	Lactose/(30% A 70% B)	30	70	0
9	LAC52	Lactose/(70% B 30% C)	0	70	30
10	LAC53	Lactose/(15% A 70% B 15% C)	15	70	15
11	LAC61	Lactose/(10% A 90% B)	10	90	0
12	LAC62	Lactose/(90% B 10% C)	0	90	10
13	LAC63	Lactose/(5% A 90% B 5% C)	5	90	5

is increasing, with applications in the pharmaceutical, phyto-pharmaceutical, cosmetic and nutrient field. In this study, FT-Raman spectroscopy was used to evaluate the solid-state stability of the benzimidazole drug substance in a suspension-emulsion. Therefore, FT-Raman spectra were recorded from suspension-emulsion reference samples (pure polymorphs A, B and C in placebo suspension-emulsion SE at a concentration of 5% (m/m); coded SEA, SEB and SEC) and compared with the spectra from three suspension-emulsion samples that were stored for 12 months in stability rooms under well-defined ICH-storage conditions of 25 °C/60%RH (coded SE1, SE2 and SE3). The original suspension-emulsions before storage at the start of the stability investigation were characterized as containing only polymorph B.

2.2. FT-Raman spectroscopy

A Bruker FT spectrometer Equinox 55S provided with a Raman modula FRA 106 with a cooled (77 K) germanium detector D418-T was used to perform the Raman spectroscopy. The samples, placed in glass capillary tubes, were excited by a 1064 nm beam from an Nd:YAG laser. The laser power was adjusted to 100 mW and distributed over the surface of the sample. All samples were placed in the focus point of the laser beam, using a movable sample holder giving a probed area of 1 mm^2. The scattered light was collected at an angle of 0° and the spectral resolution was 2 cm^{-1}. Multiple scans of each sample per measurement were taken ($n = 100$) in order to obtain the final spectra. Routinely, five measurements per sample (i.e. five randomly selected positions) were collected and mean values of the Raman intensities were calculated.

2.3. Data processing

Data processing was performed with the statistical package SPSS, version 9. For each individual measured spectrum, the raw intensities of the selected wavenumbers (78 wavenumbers were selected based upon their discriminative capacity towards the three polymorphs of interest) were vector-normalized with following formula:

$$I_i^{\mathrm{nor}} = \frac{I_i - \bar{I}}{\sqrt{\sum_{i=1}^{N}(I_i - \bar{I})^2}}$$

With $\bar{I}$ as the mean value of the intensities of a spectrum, I_i as the raw intensity at wave number i, i as wave number index (from 1 to 78).

Vector normalizations pulled down the middle of the spectrum to $I_i^{\mathrm{nor}} = 0$. To distinguish raw data and normalized data, the prefix 'N' was preceding the sample code for normalized data. Subsequently, the means of normalized intensities were calculated based on the replicate measurements per sample. Finally, the means of normalized data obtained for the pure polymorphs in lactose (indicated by MLAC31, MLAC32 and MLAC33) were used as independent variables in stepwise linear regression models. The individual normalized data of the created reference samples (NLAC41–NLAC63) and the commercial premixes with unknown composition (NMIX11–NMIX42) were used as dependent variables in the stepwise linear regression models. From the estimated regression coefficients for the factor variables MLAC31 (100% polymorph A), MLAC32 (100% polymorph B) and MLAC33 (100% polymorph C), the relative solid-state contents of the benzimidazole drug substance polymorphs A, B and C were determined.

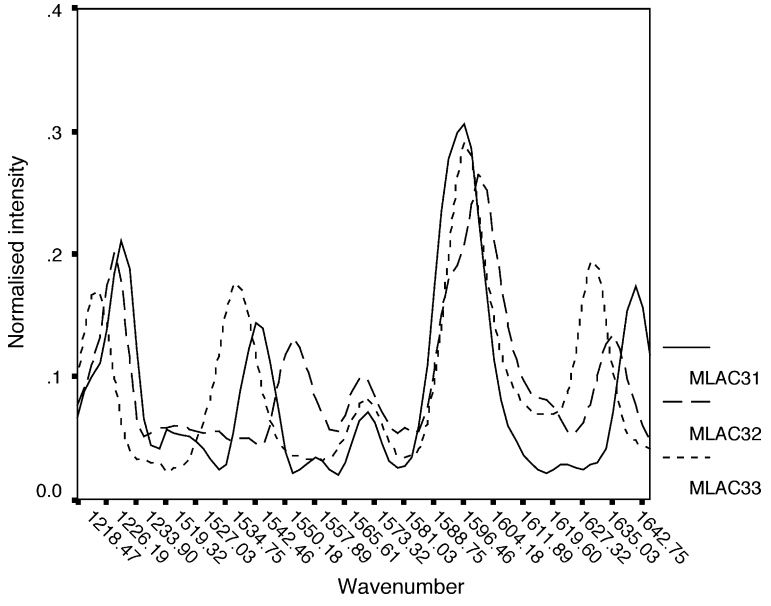

Fig. 1. Normalized spectra of reference samples containing 95% (m/m) lactose and 5% (m/m) of benzimidazole drug substance, polymorphs A (MLAC31), B (MLAC32) and C (MLAC33), respectively.

Table 2
Estimated relative benzimidazole drug substance polymorph A, B and C concentrations of the 5%-spiked lactose samples (mean ± S.E.M.)

Sample code	Sample description	Estimated polymorphic content (%)		
		Polymorph A	Polymorph B	Polymorph C
LAC41	Lactose/(50% A 50% B)	51 ± 13	53 ± 14	−3[a] ± 2
LAC42	Lactose/(50% B 50% C)	0 ± 0	40 ± 2	60 ± 2
LAC43	Lactose/(25% A 50% B 25% C)	31 ± 5	60 ± 5	9 ± 4
LAC51	Lactose/(30% A 70% B)	18 ± 1	82 ± 1	0 ± 0
LAC52	Lactose/(70% B 30% C)	0 ± 2	62 ± 7	38 ± 7
LAC53	Lactose/(15% A 70% B 15% C)	11 ± 3	82 ± 3	7 ± 3
LAC61	Lactose/(10% A 90% B)	7 ± 3	91 ± 3	2 ± 2
LAC62	Lactose/(90% B 10% C)	0 ± 0	95 ± 2	5 ± 2
LAC63	Lactose/(5% A 90% B 5% C)	3 ± 2	95 ± 4	2 ± 2

[a] Calculated negative value with however no physical interpretation, hence to be interpreted as zero.

3. Results and discussion

3.1. Evaluation of the pure polymorphs

First, the spectra of the pure benzimidazole-drug polymorphs (REFA, REFB and REFC) were used to select relevant wave number regions, discriminating the different polymorphic forms. The following discriminative wave number regions were selected: 1218–1240 and 1519–1645 cm^{-1}. In addition, the spectra of pure lactose were recorded to verify the absence of lactose spectral interferences. The absence of lactose (as model excipient) spectral interferences in the selected region was confirmed. Therefore, no further placebo corrections were necessary. Given that lactose is a main excipient used for premix formulations, this approach is also applicable for the tested premix samples. Subsequently, powder mixtures of the pure polymorphic forms and lactose were prepared (lactose:benzimidazole-drug ratio 95%:5%, m/m) to obtain the reference samples LAC31, LAC32 and LAC 33. A graphical visualization of the normalized spectra data of the reference samples is given in Fig. 1.

3.2. Quantification of polymorphs in spiked lactose samples

To quantify the different polymorphic forms within a sample, means of normalized data obtained for MLAC31, MLAC32 and MLAC33 were used as independent variables in stepwise linear regression models. The regression coefficients for these factors determined the relative abundance of benzimidazole-drug polymorphs A, B and C, respectively. To validate the quantification procedure, reference samples of lactose and the benzimidazole-drug (polymorphs A, B and C) in different combinations were prepared. The estimated relative polymorphic concentration in the reference samples

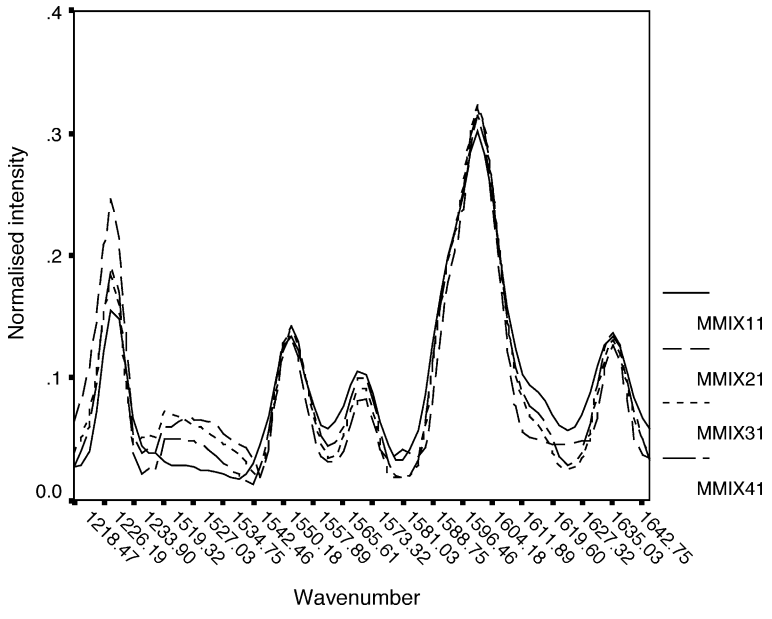

Fig. 2. Normalized spectra of premix samples MIX1, MIX2, MIX3 and MIX4 containing 95% (m/m) lactose and 5% (m/m) benzimidazole-drug.

is given in Table 2. The modelling results were generally in correspondence with the expected concentrations, confirming the validity of the approach followed. However, a high within-sample, or between-measurement variability in the reference samples was observed. This can be explained by the fact that each measurement is obtained on a small laser spot within the heterogeneous particulate matrix, so that focusing on either kind of the benzimidazole-drug polymorph particles cannot be ruled out. Nevertheless, this should be easily resolved by increasing the number of measurements per sample with expected decrease in variability. From the data overviewed in Table 2, the overall quantification limit for the different polymorphic forms in a 5% (m/m) benzimidazole-drug matrix was estimated around 5–10% of the 5% (m/m) drug, with minor quantities of false positives not to be ruled out (e.g. 2% polymorph C in LAC61, however statistically not significantly different from 0%).

3.3. Quantification of solid-state polymorph composition in premix samples

FT-Raman spectra were recorded from premix samples with an unknown polymorphic composition. A graphical visualization of the normalized spectra data of the premix samples is given in Fig. 2. Subsequently, a stepwise regression model determined the relative polymorphic concentration in the premix samples. The obtained results are described in Table 3. The polymorphic form of benzimidazole-drug in the premixes was predominantly polymorph B for all samples tested. Although statistically not significant, some traces of polymorph A in premixes 2 and 3 could not be ruled out. Low within-sample variability was observed in the premix samples. This can be explained by a more homogeneous and/or smaller particle distribution or the presence of a single polymorphic form. The latter was confirmed by the data.

Table 3
Estimated relative benzimidazole drug substance polymorph A, B and C concentrations of unknown premix-samples (mean ± S.E.)

Sample code	Estimated polymorphic content (%)		
	Polymorph A	Polymorph B	Polymorph C
MIX11	0 ± 0	100 ± 0	0 ± 0
MIX21	2 ± 2	98 ± 2	0 ± 0
MIX31	2 ± 1	99 ± 1	−1[a] ± 1
MIX41	0 ± 0	100 ± 0	0 ± 0
MIX12	0 ± 0	100 ± 0	0 ± 0
MIX22	3 ± 2	97 ± 2	0 ± 0
MIX32	0 ± 0	100 ± 0	0 ± 0
MIX42	0 ± 0	100 ± 0	0 ± 0

[a] Calculated negative value with however no physical interpretation, hence to be interpreted as zero.

3.4. Stability of solid-state polymorph in suspension-emulsion

FT-Raman spectra were obtained from reference suspension-emulsions, which are spiked placebo samples (SEA, SEB and SEC, Fig. 3) and from suspension-emulsions with an unknown polymorphic composition (SE1, SE2 and SE3, Fig. 4). The raw intensities of 78 selected wavenumbers were vector-normalized and stepwise liner regression models determined the relative abundance of polymorphs A, B and C in the suspension-emulsions which were stored for 12 months at 25 °C. The regression model identified only polymorph B in the stored samples. These data indicate that no polymorphic transformations occurred during storage of the pharmaceutical suspension-emulsion.

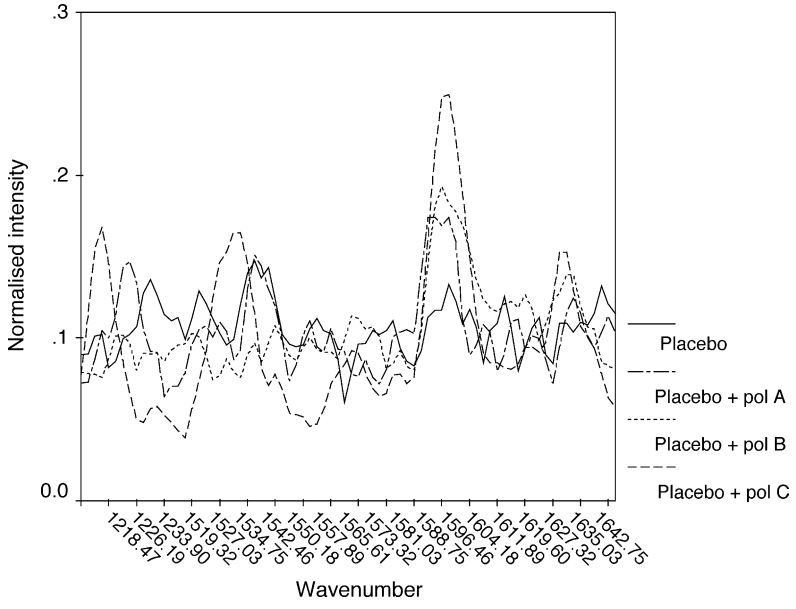

Fig. 3. Normalized spectra of suspension-emulsion samples SE, SEA, SEB and SEC.

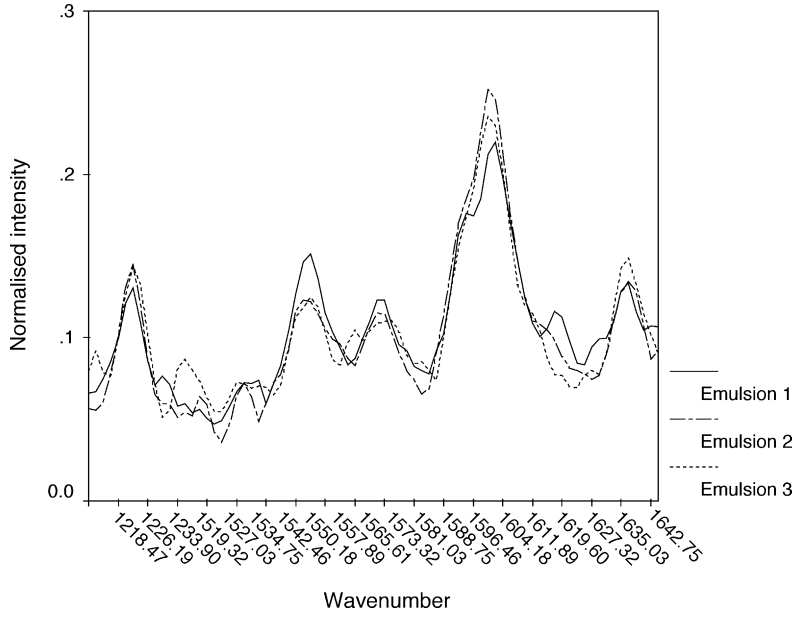

Fig. 4. Normalized spectra of stability suspension-emulsion samples SE1, SE2 and SE3.

4. Conclusions

Raman spectroscopy, coupled to an appropriate mathematical/statistical data-processing and modelling proved to be a useful and practical approach to identify and also to quantify benzimidazole-drug polymorphic forms in lactose-matrixes containing 5% (m/m) benzimidazole-drug. The quantification limit for benzimidazole-drug polymorphs A, B and C in premix samples was estimated between 5% and 10% of the total 5% (m/m) benzimidazole-drug present. In addition, the methodology was useful to confirm the polymorphic stability of benzimidazole-drug (polymorphic form B) in a suspension-emulsion. Overall, it is clear from the obtained results that quantitative assessment of the solid-sate polymorphic composition of benzimidazole drug substances in complex formulations by Raman spectroscopy is possible.

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