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Mebendazole crystal forms in tablet formulations. An ATR-FTIR/chemometrics approach to polymorph assignment

Natalia L. Calvo, Teodoro S. Kaufman*, Rubén M. Maggio*

Pharmaceutical Analysis, Department of Organic Chemistry, School of Pharmaceutical and Biochemical Sciences, National University of Rosario and Institute of Chemistry of Rosario (IQUIR, CONICET-UNR), Suipacha 531, Rosario S2002LRK, Argentina

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ABSTRACT

Structural polymorphism of active pharmaceutical ingredients (API) is a relevant concern for the modern pharmaceutical industry, since different polymorphic forms may display dissimilar properties, critically affecting the performance of the corresponding drug products. Mebendazole (MEB) is a widely used broad spectrum anthelmintic drug of the benzimidazole class, which exhibits structural polymorphism (Forms A–C). Form C, which displays the best pharmaceutical profile, is the recommended one for clinical use. The polymorphs of MEB were prepared and characterized by spectroscopic, calorimetric and microscopic means. The polymorphs were employed to develop a suitable chemometrics-assisted sample display model based on the first two principal components of their ATR-FTIR spectra in the 4000–600 cm^{-1} region. The model was internally and externally validated employing the leave-one-out procedure and an external validation set, respectively. Its suitability for revealing the polymorphic identity of MEB in tablets was successfully assessed analyzing commercial tablets under different physical forms (whole, powdered, dried, sieved and aged). It was concluded that the ATR-FTIR/PCA (principal component analysis) association is a fast, efficient and non-destructive technique for assigning the solid-state forms of MEB in its drug products, with minimum sample pre-treatment.

1. Introduction

Helminthic infestations have an enormous health and economic impact, of worldwide dimension; therefore, their management is a relevant public health issue. An important part of the world's human population, which is estimated in more than 3 billion people [1], is infected with roundworms, whipworms or hookworms [2], being the size of this combined disease burden comparable to those of tuberculosis and malaria [3].

Mebendazole (MEB) is methyl 5-benzoyl-2-benzimidazole carbamate, a synthetic unsymmetrically substituted benzimidazole (Fig. 1) with a broad-spectrum anthelmintic activity, which is orally effective against a number of cestodes and nematodes, being widely used in large scale deworming programs [4]. MEB acts by interfering with carbohydrate metabolism and inhibiting polymerization of microtubules. In recent times, the drug was shown to display promising antitumor activity, especially in cases of colon cancer, medulloblastoma and glioblastoma [5]. The high efficacy, ease of administration, broad spectrum and low cost of MEB granted its inclusion in the World Health Organization (WHO) essential drugs list.

MEB belongs to class II of the biopharmaceutics classification systems (BCS); therefore, not surprisingly, its absorption from the gastrointestinal tract is at best only 5–10%, but it is increased when taken with fatty food. The drug exhibits amino-imino tautomerism, as exemplified with MEB-1, MEB-2 and MEB-3 (Fig. 1) and three polymorphs (A–C).

The polymorphs have different thermodynamic stability (A > C > B) and very low solubility in water (Form A: 9.84 ± 0.05; Form B: 71.3 ± 0.5; Form C: 35.4 ± 0.5 mg l^{-1}) [6,7] and 0.1 M HCl (Form A: 20 ± 5; Form B: 70 ± 4; Form C: 40 ± 3 mg l^{-1}) [8]. Therefore, some derivatives, such as the hydrochloride salt [9], and crystal engineering [10] alternatives have been recently proposed to promote polymorph conversion or improve drug solubility and bioavailability.

Crystal polymorphism is a property of the solid state by which, as a result from at least two different molecular arrangements, a compound exhibits different solid crystalline phases. The polymorphs usually display variation in their mechanical and physical properties, including melting point, solubility, stability, and disso-

* Corresponding authors. Fax: +54 341 4370477x112.
E-mail addresses: kaufman@iquir-conicet.gov.ar (T.S. Kaufman), maggio@iquir-conicet.gov.ar (R.M. Maggio).

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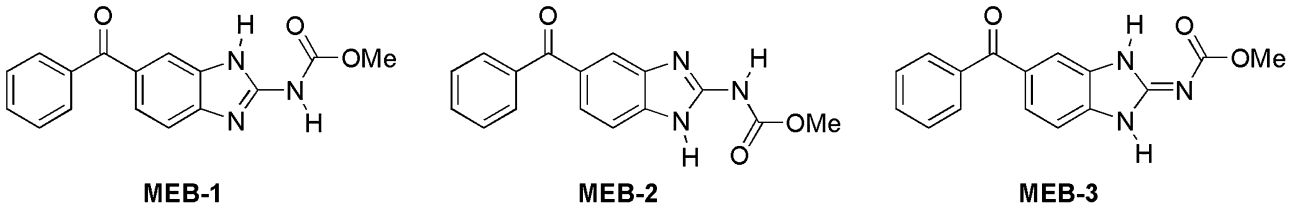

Fig. 1. Chemical structures of the amino-imino tautomers of MEB.

lution rate, as well as crystal habit, lattice energy, intermolecular interactions, particle density and thermodynamic activity [11].

These differences may affect not only the production process, modifying manufacturing reproducibility, but also product performance, influencing drug dissolution, its absorption and bioavailability [12].

Mebendazole is official in the European [13], Indian [14] and American [15] Pharmacopoeias, among others, where the bulk drug and its oral formulations are assayed by potentiometric titration, UV-spectrophotometry or HPLC. Except perhaps for the IR identification tests for the bulk drug described in these codices and possibly the dissolution test for its tablets, described in the USP, none of these methods takes into account drug polymorphism.

On the contrary, however, the 5th edition of the International Pharmacopoeia clearly restricts the suitable polymorphic form of MEB to Form C in the MEB chewing tablets, emphasizing that the process of formulating, manufacturing, and packaging should be designed and controlled so as to minimize the conversion of Form C into polymorph A [16].

The structural polymorphism of MEB has been extensively investigated by X-ray diffraction [6,17]. This allowed the elucidation of the structures of Forms A [18] and C [19], which have been shown to correspond to amino-imino tautomers MEB-1 and MEB-2, respectively (Fig. 1). In addition, variable temperature X-ray powder diffraction was employed to study the transformation of Form C into the most stable Form A [20], and the Rietveld method has been used for analysis of MEB polymorphism in commercial tablets of the drug [21].

Vibrational spectroscopic methods, which are highly sensitive means to detect crystalline forms, especially when combined with chemometric tools, have also found a wide use in detection and quantitation of MEB polymorphs. The group of Aboul-Enein found that "the IR region of the spectrum is not useful in differentiating the polymorphs of mebendazole" because "the differences are minor, and distinguishable peaks are not easily available for characterization", when spectra were taken from the samples prepared as KBr disks. Therefore, they developed diffuse reflectance (DRIFTS)-based methods and reported the use of partial least squares (PLS) and principal component regression to quantitate MEB based on subtle spectral differences, assigning the polymorphic identity by rough comparison between spectra of the sample and those of standards of the polymorphs [22].

More recently, second derivative DRIFTS [23] and attenuated total reflection (ATR) [7] data have been combined with artificial neural networks to quantitate Forms A and C of MEB. On the other hand the ability of Raman spectroscopy [24] and the near infrared-PLS combination [25], to identify the crystal forms of MEB have also been examined. In some of these alternatives the excipients were not taken into account or removed prior to the determination, whereas in others polymorph B was excluded from the analysis.

The low solubility of MEB and the dissimilar rate of dissolution of its polymorphs [8] have resulted in different therapeutic outcomes. The very poor solubility of Form A, which causes lack of efficacy, strongly discourages its use [26], whereas the most soluble and least stable polymorph B has shown to be more toxic during both, anthelmintic and anticancer tests [27,28]. On the other hand, Form C, which is more soluble than Form A and exhibits the best dissolution profile, is the polymorph of choice for therapeutic use in human and veterinary medicine [26,29].

However, despite it was shown that Form C is stable enough to compression [30], it has been demonstrated that tablets prepared with this polymorph were transformed into products containing Form A under the effects of moisture and heat [1]. Due to the same phenomenon, suspensions of Form C may also contain part of their active principle converted to Form A [7]. These findings suggest that processing operations and storage conditions entail the potential risk of polymorph conversion. Aware of this problem, several groups have proposed discriminating dissolution tests to differentiate among the polymorphic forms of MEB [31,32].

Furthermore, the polymorphic composition of the bulk drug and formulations marketed in different countries (South Africa, Brazil, Germany) has been analyzed [21,30,33–35]. These studies revealed that all three polymorphic forms of MEB are found in the market. Their conclusions also indicate that too often the dosage forms contain polymorphic mixtures or the wrong crystal form, also suggesting that the use of only one simple analytical technique might not clearly identify the polymorphic form [35]. Hence, the determination of the main polymorph in drug products containing MEB is nowadays of paramount importance.

We have developed chemometrics-assisted ATR/FTIR solid-state studies on cimetidine [36,37] and a chemometrics-based method to differentiate polymorphs through dissolution tests [38]. In pursuit of our interest in the development of chemometrics tools to assess relevant characteristics of the solid state of drugs and drug products, herein we report a fast, simple and non-destructive chemometrics-assisted ATR-FTIR method useful for polymorph assignment in pharmaceutical formulation samples of MEB.

2. Materials and methods

2.1. Instrumentation

The FTIR spectra were acquired in a Shimadzu Prestige 21 spectrometer (Shimadzu Corp., Kyoto, Japan). The ATR experiments were carried out with a diamond-based ATR accessory (GladiATR, Pike Technologies, Madison, USA), fitted with a Pike temperature control unit.

The calorimetric determinations were performed in a Shimadzu model 60 differential scanning calorimeter (Shimadzu Corp., Kyoto, Japan), operating under a constant flow of nitrogen (30 ml min^{-1}). The sample powders (2 mg) were placed in aluminum pans, and heated at a rate of 5 °C min^{-1} between 30 °C and 300 °C. An empty pan was used as a reference.

Digital optical microscopy of the polymorphs was performed with a Correct microscope (Seiwa Optical, Tokyo, Japan), fitted with different objective lenses (10× and 40×) and a 5.0 Megapixels [resolution 2592 × 1944 (H × V)] Beion CMOS digital camera (Shanghai Beion Medical Technology Co., Ltd., Shangai, China).

The particle size of the samples was standardized by sieving, employing a Zonytest EJR 2000 fine mesh vertical vibratory siev-

Table 1
Selected FTIR spectral data of the different forms of MEB.

Form	Characteristic absorption peaks (cm^{-1})					
	ν_{NH} Carbamate	ν_{CO} Carbamate	ν_{CO} Benzoyl	δ_{NH} In plane	δ_{CH}[a]	ν_{NC} Benzimidazole
A	3368	1730	1633	1528	1420–1465	–
B	3339	1697	1643	1524	1400–1465	1379
C	3400	1715	1643	1520	1410–1473	1371

[a] Forms A and B exhibit three peaks in this region, whereas form C displays four peaks.

ing tower (Rey & Ronzoni, Buenos Aires, Argentina), operating at 120 rpm. In all cases, the 50–100 mesh fractions (300–150 μm) were collected.

Mixing of powders was performed employing either a stainless steel mini pharmaceutical powder V-blender, or a Z-mixer, coupled to a Precytec model AT-15D rotary stirrer (Precytec, Buenos Aires, Argentina) fitted with an Adleepower MS2-IPM electronic variable speed control (ADLEE Powertronic., Ltd., Shenkang, Taiwan).

2.2. Chemicals

Pharmaceutical grade (USP 24) mebendazole (Form A) and excipients were employed. The chemicals were acquired to Saporiti, Prest and Parafarm (Buenos Aires, Argentina). MEB bulk drug was kept in a desiccator, protected from light throughout the experiments. Double distilled water was used during the experiments. All other chemicals used were of analytical grade.

The commercial mebendazole tablets (average weight = 349.7 mg) were acquired at a local drugstore. According to the label, each tablet contains MEB (200 mg) microcrystalline cellulose (114.5 mg) sodium croscarmellose (6.0 mg), polyvinylpyrrolidone (15.0 mg), sodium saccharine (0.5 mg), talc (8.0 mg), sodium lauryl sulfate (1.0 mg), colloidal silicon dioxide (0.7 mg) and magnesium stearate (4.0 mg).

2.3. Preparation of the polymorphs of mebendazole

The Forms A–C were obtained as follows: Form A was the commercial starting product. Form B was prepared by dissolution of the drug (100 mg) in refluxing chloroform (200 ml), followed by slow cooling and recovery of the crystals after solvent evaporation under reduced pressure in a Büchi R-114 rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) [23,39]. Several batches were combined in order to obtain enough amount of the compound for the experiments.

On the other hand, in order to obtain Form C, a magnetically stirred solution of the drug (1000 mg) in formic acid (50 ml) was treated with double distilled water (200 ml) at room temperature. The solids were filtered under reduced pressure, and the remaining solvent was stripped off from the residue under vacuum. The different forms were kept in a desiccator during the experiments.

2.4. Preparation of the samples containing polymorphs of mebendazole

2.4.1. Mixtures of mebendazole Forms A– C

Binary and ternary blends containing the polymorphs of MEB were prepared by mechanically mixing, in a Z-mixer/blender, accurately weighed amounts of the previously sieved pure forms of the drug.

2.4.2. Blend of excipients

Aliquots of sieved powders of the different excipients were accurately weighed [microcrystalline cellulose (2290 mg), sodium croscarmellose (120 mg), polyvinylpyrrolidone (300 mg), sodium saccharine (10 mg), talc (160 mg), sodium lauryl sulfate (20 mg), colloidal silicon dioxide (14 mg) and magnesium stearate (80 mg)], added to the V-mixer and mixed for 30 min. at 30 rpm.

2.4.3. Mixtures of excipients and polymorphs

Accurately weighed amounts of the pure polymorphs or their mixtures were sieved and blended, in the proportion 100:75, with accurately weighed aliquots of the blend of excipients, employing a Z-mixer. Stirring was performed for 30 min. at 40 rpm.

2.4.4. Commercial tablets

The commercial MEB tablets (12 units) were carefully crushed and reduced to fine powder, which was sieved. The 50–100 mesh fraction was employed for the experiments.

2.5. FTIR determinations

Data acquisition (20 scans per spectrum at a resolution of 4 cm^{-1} over a wavenumber range of 4000–600 cm^{-1}) was performed by the ATR technique at 30 °C on 20 mg samples. Each sample was processed in quadruplicate. The spectra were saved in .txt format for their further processing and analysis.

2.6. Chemometrics and graphics software

The computing routines involving spectral data manipulation and the PCA (principal component analysis) method were run in Matlab R2010a (Mathworks, Natick, USA). Mixture designs were performed with Design Expert v.7.0 (Stat-Ease, Inc., Minneapolis, USA). Statistical data analyses and graphics construction were carried out with Origin 8.5 (OriginLab Co., Northampton, USA).

3. Results and discussion

Samples of the polymorphs A–C of MEB were prepared and characterized employing spectroscopic (ATR/FTIR), calorimetric (DSC) and microscopic (optical microscopy) techniques. Form B was obtained from a chloroform solution, whereas Form C was accessed by addition of water to a formic acid solution of the drug.

3.1. Characterization of the solid forms

3.1.1. FTIR-ATR spectroscopy

For characterization purposes, the operating temperature, particle size, amount of sample and the pressure exerted on the sample during the measurements were standardized. Under these conditions, the singularities of each spectrum were clearly observed.

The acquired FTIR spectra of the pure forms (Fig. 2a) were analyzed and their most significant bands are detailed in Table 1. The results were in full agreement with the literature [7,22,23,31].

3.1.2. Thermal analysis

Fig. 2b displays the DSC sweeps of the polymorphs. Form A exhibited an endothermic transition peak at 233.6 °C with an onset temperature of 184.5 °C, associated with the melting process.

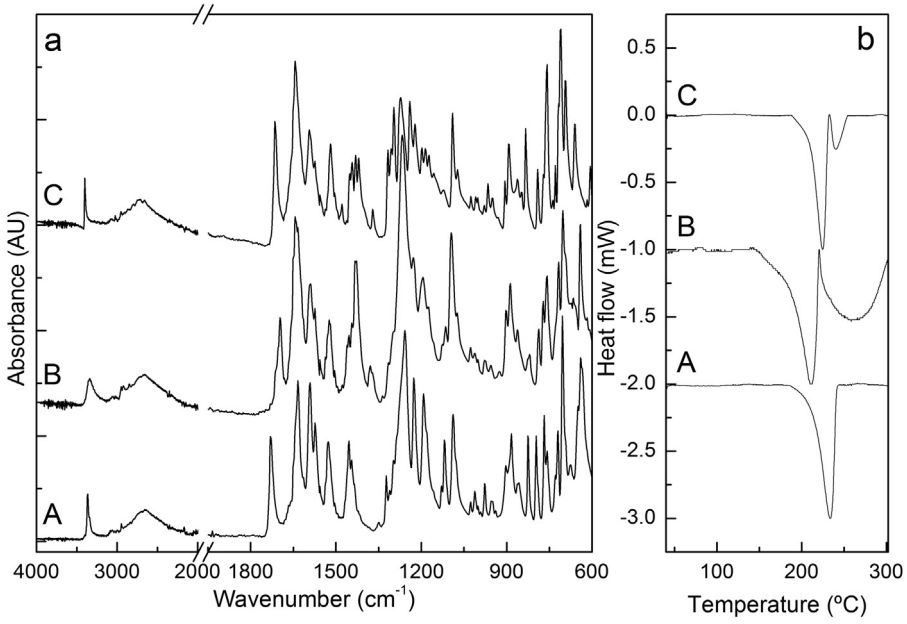

Fig. 2. (a) FTIR spectra of the different forms of MEB. (b) DSC thermograms of the different crystal forms of MEB. Letters A–C refer to polymorphs A–C, respectively).

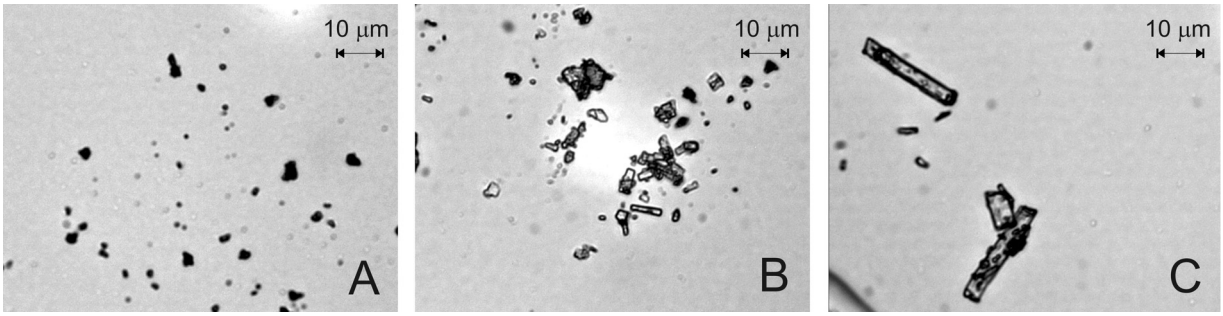

Fig. 3. Digital optical microscopic images of Forms A, B and C of MEB.

On the other hand, Form B showed two peaks without shoulders at 211.1 °C and 258.3 °C; the first one is related to its transformation into Form A, whereas the second responds to the complete melting of the product. Finally, Form C displayed a major peak at 224.6 °C and a minor peak at 240.3 °C; they have the same meaning as those observed for Form B.

3.1.3. Optical microscopy

The digital optical microscopy, performed with a 40× objective, revealed the crystalline habits of the polymorphs (Fig. 3). Form A was observed as very tiny conglomerates, which hindered appreciation of their shape. On the other hand, polymorph B exhibited small irregularly-shaped crystals, whereas Form C was seen as needles. The morphology of the three types of crystals was in full agreement with the literature [40].

3.2. ATR-FTIR/chemometric method for assignment of the polymorphs

3.2.1. Method development

A series of 19 independent samples containing the polymorphic forms MEB and the excipients found in its drug formulation was prepared according to a ternary mixture of simplex lattice design, as shown in Fig. 4, where each edge of the triangle corresponds to a binary mixture, and the vertices of the triangle correspond to the formulations with pure components. The possible ternary mixtures are located inside the triangle.

The set was divided into two groups. The first one, corresponding to the training samples, was used for model development, while

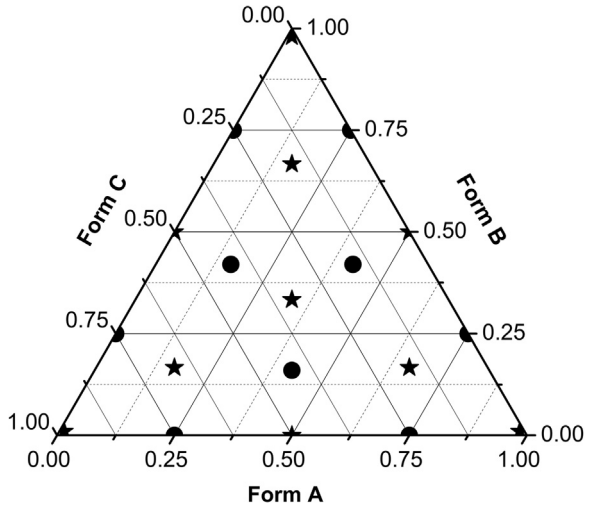

Fig. 4. Ternary plot showing the partitioning of input data into training (★) and validation (●) sets.

Table 2
Composition of the sets of training and validation samples.

Training set					Validation set				
Sample	Polymorphic form (%)			Excipients	Sample	Polymorphic form (%)			Excipients
No.	*A*	*B*	*C*	(%)	No.	*A*	*B*	*C*	(%)
1	57.7	–	–	42.3	11	9.2	23.7	24.1	43.0
2	38.1	9.6	9.7	42.6	12	23.9	8.9	24.0	43.2
3	–	57.0	–	43.0	13	23.9	23.6	9.1	43.4
4	9.7	9.8	37.8	42.7	14	42.2	14.3	–	43.4
5	27.8	–	27.8	44.3	15	14.6	41.5	–	43.9
6	–	–	56.3	43.7	16	–	41.8	15.4	42.8
7	18.8	18.9	19.8	42.6	17	–	14.5	42.7	42.7
8	19.1	19.6	19.1	42.1	18	15.2	–	42.2	42.6
9	28.2	27.8	–	44.0	19	42.6	–	14.4	43.0
10	9.5	37.5	9.9	43.1					

the second was employed for validation purposes. The composition of each sample is detailed in Table 2.

Being a chemical system with relatively low variability and high reproducibility, it was considered that a lot of 10 samples sufficed for method development. Therefore, the validation group consisted of the remaining 9 samples.

In order to avoid any possible polymorphic conversion, all spectra were taken shortly after the preparation of the mixtures. In addition, no conversion related to the pressure within the ATR accessory was observed. Analysis of each sample was performed in quadruplicate, in order to better expose experimental errors.

Fig. 5a displays the 40 FTIR spectra of the training set; each spectrum contains 1764 sensors. In order to obtain the distinguishing molecular information about the polymorphs of MEB, the baselines of the spectra were corrected with reference to the 4000–3684 cm^{-1} region, where the samples have no absorption, and then mean-centered before being processed, as shown in Fig. 5b.

Principal component analysis (PCA) is a data dimensional reduction technique, which enables access to the most relevant system information through a limited number of variables. Therefore, the singular value decomposition (SVD) algorithm was employed to obtain the principal components (PCs) from the resulting 40 × 1764 input matrix.

Inspection of a scree plot (Fig. 5c) revealed that the first 6 PCs described 99.4% of the data set variation, whereas the cumulative variance explained by the first two and three PCs amounted to 94.1% and 97.5% of the system variance, respectively (Fig. 5d).

A systematic investigation was carried out to find the proper combination of PCs that would provide the most suitable conditions for the assigning the polymorphic form of the training samples. The results (Fig. 6) suggested that, as expected, PC1 and PC2 were able to provide the best discrimination among the samples containing the different polymorphs.

An amplified view of the distribution of the classified samples is shown in Fig. 7a. It can be observed that only sample 8 (a 1:1 mixture of Forms A and B) was less resolved than expected, being projected very close to the space containing the 1:1:1 mixture of the three polymorphs (sample 7).

In an attempt to improve the quality of class separation, variable selection was explored, but no significant improvements were observed. In addition, a ternary model based on the first three components was build, the visual inspection of which suggested that sample 8 appeared to display a better resolution; however, use of

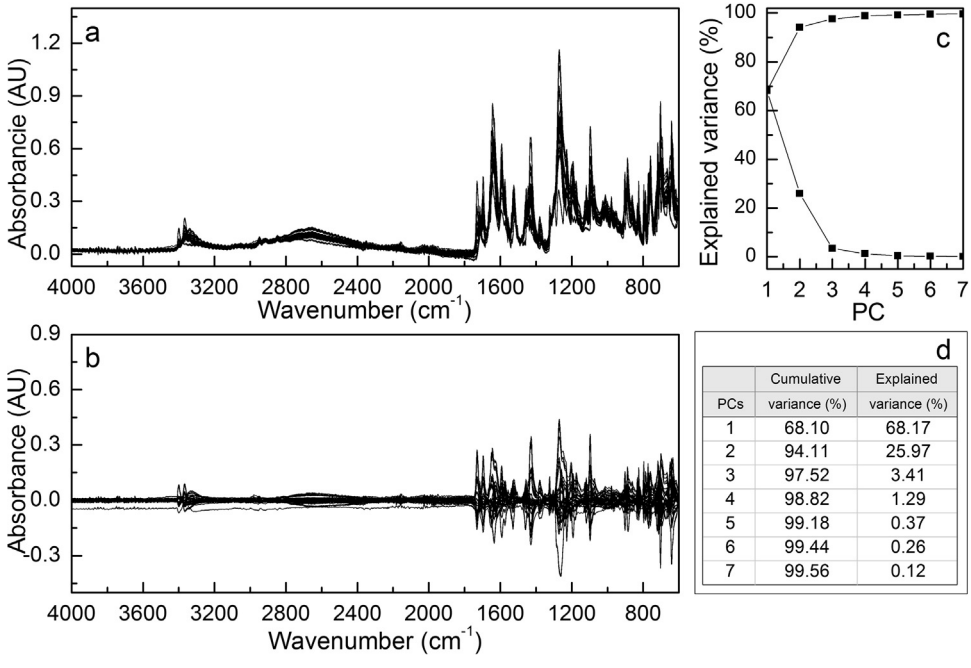

Fig. 5. Raw data matrices (40 × 1764); (a) mean-centered data matrix of the training set; (b) mean-centered data matrix of the validation set; (c) and (d) accumulated and explained variance (scree plot) of the PC models as a function of the number of PCs.

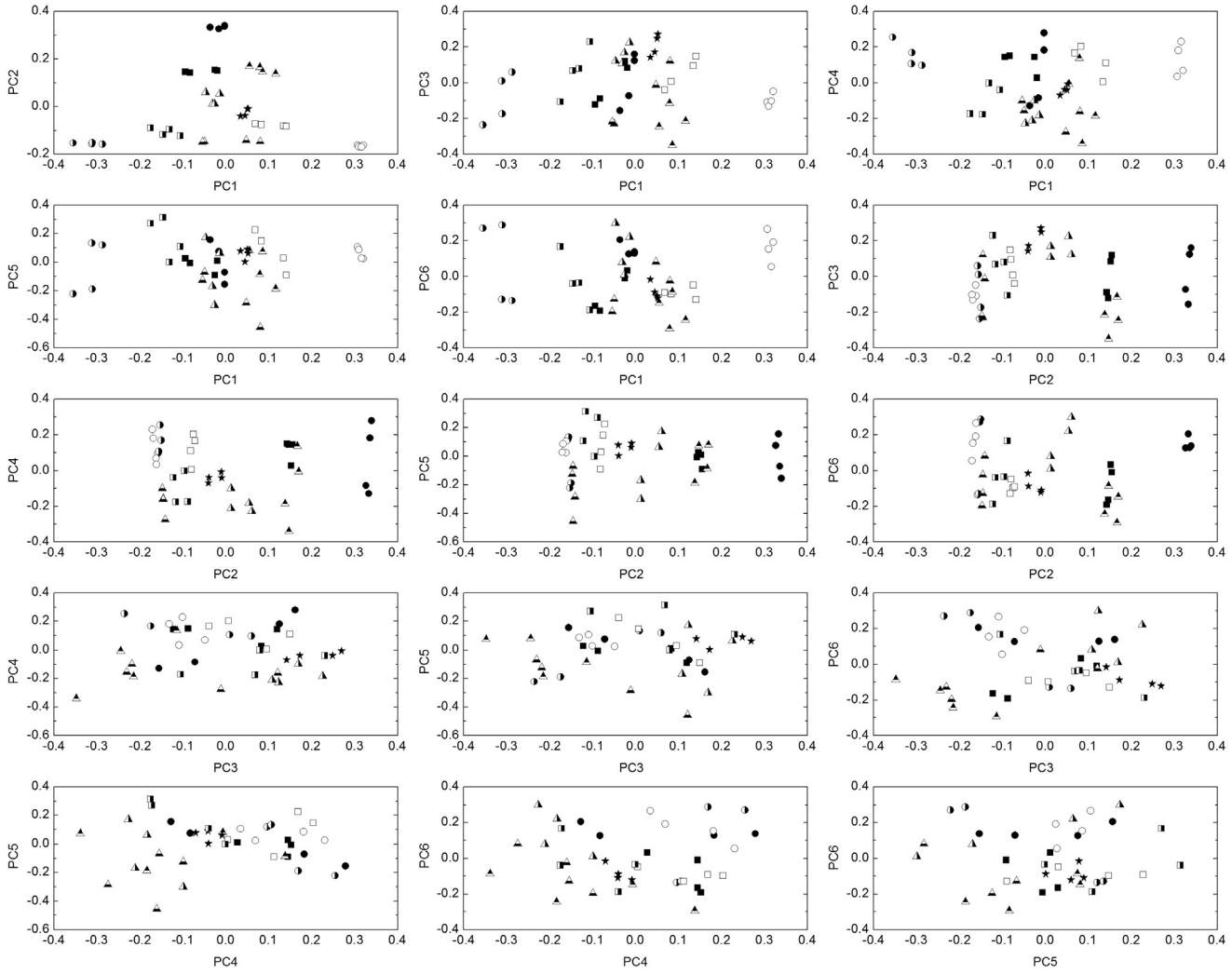

Fig. 6. Plots of the PC loadings, in binary combinations for the first 6 PCs. Grouping of Forms A (●), B (◑) and C (○); their 1:1 binary mixtures [B:C (▲), A:C (▲) and A:B (▲)] and the ternary mixtures [A:B:C (4:1:1, ■; 1:4:1, ◨; 1:1:4, □; and 1:1:1, ★)].

the latter was discarded after performing the validation experiments.

In order to acquire a better understanding of the behavior of the chosen sample display system (PC2 vs PC1), the first two loadings (L1 and L2, respectively) were examined (Fig. 8) taking into account the sign of each contribution and its assignment to a specific polymorph. This study revealed that all three forms of MEB have influence on both loadings, indicating that a classification

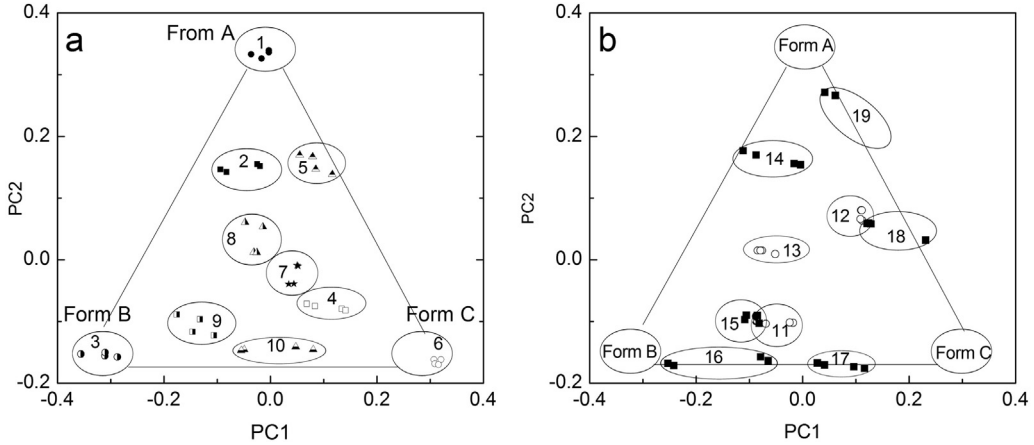

Fig. 7. (a) Training samples. Two principal components model (PC2 vs PC1). (b) Classification of the validation samples employing the two PCs model. Samples 11–13 are ternary mixtures, whereas samples 14–19 are binary polymorphic mixtures. Lines, ellipses and sample numbers are included as visual aids.

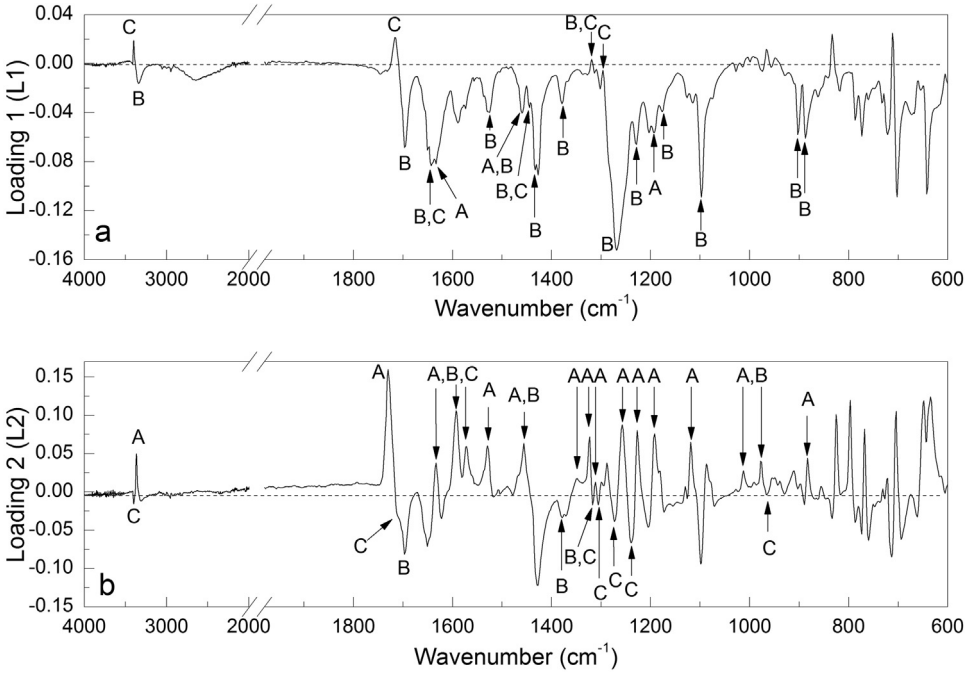

Fig. 8. Loadings of the first (a) and second (b) principal components of the samples of the training set, and their form (A, B or C) assignment.

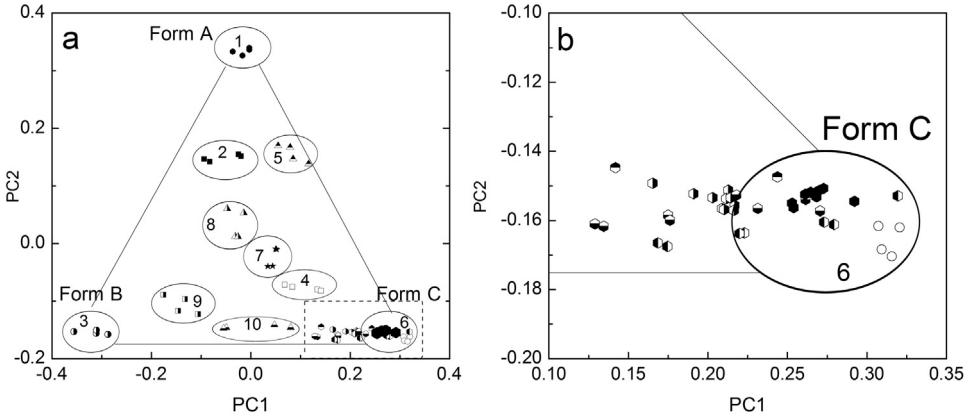

Fig. 9. (a) Classification of commercial samples of MEB employing the PC2 vs PC1 model. Samples including whole (◓) and finely crushed (◑) tablets, sieved powders (◒), dried (◐) and aged samples (●). (b) Amplified view of the classification of the commercial samples of MEB (dashed rectangle of Fig. 9a). The training samples containing Form C are marked with an empty circle (○). Lines and ellipses are included as visual aids.

based on these components would be sensitive to the three polymorphs used.

3.2.2. Validation of the chemometric method

In order to ensure the suitability of the model for the sought classification purposes, internal (cross-validation) and external validation stages were carried out. The cross-validation was performed using the leave-one-out strategy on the 40 spectra belonging to the 10 samples of the training set. One sample was removed at a time and then assigned to the nearest class, i.e., that displaying the minimum Euclidean distance from its group center.

The test revealed that the two PCs-based model was able to correctly classify 92.5% of the samples (37/40), whereas the three PCs-model exhibited a lower correct classification rate of 77.5% (31/40). These classification rates suggested that the latter model, despite being more complex, was unable to provide improved analytical information for polymorph assignment.

The external validation step was designed to evaluate the ability of the method to classify unknown samples containing binary and ternary mixtures of MEB polymorphs and the excipients found in the drug formulation. In this case, the 9 samples of the validation set, which contain binary and ternary combinations of the polymorphs and added excipients were pre-treated as those of the training set and classified using the developed PCA model, with the results presented in Fig. 7b.

It was observed that the ternary samples 11–13 were located within the triangle formed by the pure polymorphs, suggesting the presence of the three polymorphs MEB (ternary samples). Sample 11 is displayed at half way and over the B–C axis, being a 1:1 mixture of Forms B and C with a lower proportion of Form A. Meanwhile, sample 12 which contains a 1:1 mixture of Forms A and C, and a minor proportion of Form B was observed over the mid-point region of the A–C axis; on the other hand sample 13, containing a 1:1 mixture of Forms A and B and a minor proportion of Form C, was seen over the A-B axis.

Samples 14–19 are binary mixtures of the polymorphs. Specimens 14 and 19 are 3:1 mixtures of Form A with Forms B and C, which were classified in the vicinity of Form A, but on the direc-

tions of axes A–B and A–C, respectively. Analogously, samples 15 and 16, which are 3:1 mixtures with Form B with polymorphs C and A, respectively, were observed in the region near the pure Form B. Finally, samples 17 and 18 were classified closer than others to the pure Form C, being 3:1 binary mixtures of Form C with polymorphs B and A, respectively.

Since PCA is a linear modelling approach, the presence of non-linearities in the dataset may represent a potential risk. However, the proposed procedure is based on measuring the reflectance of the samples, which represents a weighted average of their individual components reflectance, and no signal saturation nor chemical interactions among the components of the mixtures were observed. In addition, the method's performance during the validation stage served to discard eventual non-linearities as a source of classification errors.

3.2.3. Application of the chemometric method

Finally, commercial products were evaluated, in the forms of whole tablets, finely crushed powders, sieved material and dried (in a desiccator) samples. As shown in Fig. 9, it was observed that regardless the kind of sample pre-treatment, the validated ATR-FTIR/PCA classification model positioned all the samples near the corner of Form C, enabling to conclude that the tablets contained mainly this polymorph.

Dispersion analysis of the scores from the different types of samples (whole, crushed, sieved and dried) allow to conclude that sample pre-treatment had no significant effect on the quality of the result. Furthermore, simply crushed samples exhibited comparatively lower dispersion than those being more homogeneous either in particle size or in moisture content (Fig. 9b).

Interestingly, aged samples (kept for 6 months in a desiccator) exhibited a small migration tendency towards the corner of group C. This could be evidencing an incipient, slow form interconversion during the storage lapse. It can be foreseen that this displacement has some potential as an indication of the effects of aging or stress conditions on commercial MEB tablets.

4. Conclusions

Mebendazole (MEB) is a WHO essential drug which has three polymorphic forms. Its drug products may therapeutically fail if prepared with the wrong form. Therefore, a new alternative was developed as a convenient means for assigning the polymorphic identity to samples of MEB in the presence of excipients. The method, which is non-destructive, requires only minor amounts of sample and no special sample preparation steps, is based on the use of ATR-FTIR spectroscopy coupled to PCA (ATR-FTIR/PCA), and involves the analysis of the first two principal components.

The suitability of the training model was assessed by cross-validation and external validation procedures, and the methodology was successfully applied to commercial tablets after performing different treatments (whole, pulverized, sieved powders, dried and aged). Therefore, the proposed approach should be considered as a fast and convenient strategy to assess the identity of the main polymorph of MEB contained in manufactured drug products.

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