Analysis Improvement of Drugs Using Modern Methods

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Abstract

A Fourier transform infrared (FT-IR) spectrometric method (DRIFTS) was developed for the rapid, direct measurement of mebendazole in drugs. Conventional KBr-spectra and DRIFTS-spectra were compared for best determination of active substance in drug preparations. Two chemometric approaches, partial least-squares (PLS2) and principal component regression (PCR+) methods were used in data procesing. The best results were obtained with PLS2 method.

Keywords: fourier transform infrared spectrometry, polymorphic analysis, mebendazole drug analysis, chemometric analysis.

Introduction

Mebendazole, (Meben) { methyl - (5-benzoyl-1H-benzimidazol-2yl) carbamate} is a synthetic broad – anthelmintic drug, active against nematodes and cetodes[1,2], being indicated in the treatment of single or mixed infestation by *Enterobius vermicularis, Trichuris trichiura, Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strogyloides stercoralis, Taenia app.* in human and veterinary therapeutics[3].
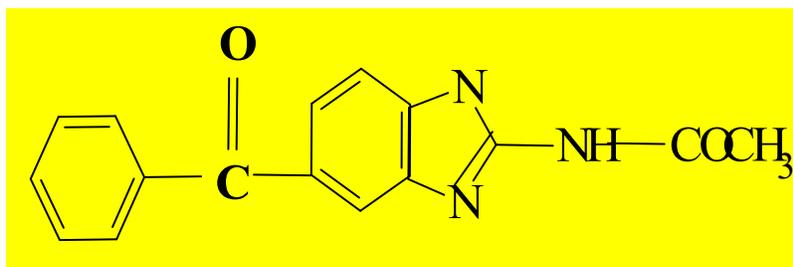
Polymorphism is a technical term used both in biology and crystallography, describing the fact that a natural phenomenon may occur in two or more different forms. Polymorphs are the same chemicals with different molecular arrangements within the crystal lattice. In principle, the different polymorphic forms of a compound have different energies and this could affect their bioavailability. Sometimes there are more than two forms. Meben used in clinical and veterinary treatment is composed of three polymorphic forms (A, B and C, respectively), all of them being in accord with USP specifications[4].

The pharmaceutical importance of polymorphism was reviewed by Haleblian and McCrone[5]. If the molecular interactions in the crystal lattice are fairly strong, arising from a phenomenon such as hydrogen bonding, differences in polymorphic forms can be differentiated with techniques such as FTIR, Raman spectroscopy or different scanning calorimetry (DSC) as well as the usually definitive techniques of X-ray powder diffraction pattern and single crystal X-ray analysis. In most pharmacopoeias there are only three tests that are carried out in the solid phase. They are the determination of melting point, the recording of IR spectrum and the test for loss on drying.

Determination of the major component in drugs with infrared (IR) spectrometry involves utilization of an enormous amount of spectroscopic informations about a sample. Chemometric methods, such as principal component regression (PCR+, Improved Principal Component Regression) and partial least-squares (PLS2, Multicomponent Partial Least Squares) analysis are commonly used to extract the specific information relevant to the analyte of interest from the full spectrum[6,7]. These two techniques yields more accurate calibration models compared with multiple linear regression (MLR) where a restricted set of absorption bands is used in the calibration[8].

The partial least squares (Projection to Latent Structures, PLS) regression method was developed by Wold[9] in 1966. There is a substantial amount of literature devoted to the theoretical elucidation of properties of PLS algorithm. A good introduction to the method is given by Geladi and Kowalski[10].

PLS seeks to express the variance in the property information by correlating it with the spectral imformation (compare with PCR+, which, in the PCA stage – Principal Component Analysis – only seeks to account for variation in the spectral data and then in the MLR stage correlates this with the property data). This means that if there is a high degree of correlation between the properties, it is more efficient to use the PCR+ or PLS2 algorithms. Because PLS1 trats all properties individually, if there is non-linearity differs from property to property, it would be expected that PLS1 would build better models than PLS2, which is trying to cater for the non-linearities for all properties simultaneously[11]. However, if there is a significant amount of noise associated with property values, PLS2 may be expected to perform better than PLS1 because the noise in the properties form PLS2 will be averaged out when determining the PLS factors.

The method is often used in spectroscopy to extract informations from complex spectra containing overlapping absorption peaks, interference effects from diffuse lightly scatter, and noise from the hardware used to collect the data. When applied to spectra, the aim of chemometric methods analysis is to find a mathematical relationship between a set of independent variables, the **X** matrix ($N_{objects}$ x $K_{wavelengths}$) and a set of dependent variables, the **Y** matrix ($N_{objects}$ x $M_{measurements}$). The resulting model has the form :

$$Y = X \cdot B + E \qquad (1)$$

where **B** is the matrix of regression coefficients obtained from PLS analysis, and **E** is the matrix of residuals.

The purpose of the present study is to use FT-IR spectrometry to investigate the structural features of the various polymorphic forms exhibited by Meben. The main objective of this work was to develop a chemometric procedure for the fast and accurate determination of different polymorphic forms of MEB in commercial pharmaceutical formulations, VERMOX, using PCR+ and/or PLS2 approaches for calibration and quantification, reducing the sample pre-treatment and providing the direct IR determination by using a simple matrix calibration including only four standards (MebenA, MebenB, MebenC and MebenABC(1:1:1), respectively). Data acquisition parameters, such as resolutions of 4 and 8 cm^{-1}, and calibration methods, such as PCR+ , PLS1 and PLS2, were compared and recommandations on the best options for mebendazole analysis were made.

Experimental

Data acquisition was performed using a Spectrum1000 FT-IR spectrometer equipped with Spectrum for Windows version 2.00 (Perkin Elmer Co., Beaconsfield, Bucks, UK). The commercial software used to generate analysis for the principal component analysis was QUANT+ expert Version 4.10 (Perkin Elmer Co.).

Mebendazole substances (R 17635) - reference polymorphic forms A, B and C (lot V890 - 359, V890 - 353 and V890 – 386, respectively) and a tablet VERMOX® (containing 100mg mebendazole per tablet) (batch no. 96K22/050) were supplied by JANSSEN Pharmaceutica Biotech. N.V. (Belgium).

Microdiffuse reflectance cup and a DRIFTS accessory made by Perkin-Elmer Co, were used. DRIFTS samples were prepared by mixing 30 mg of the bulk drug with 270 mg of spectral-grade potassium bromide in a dental amalgator (WIG-L-BUG) for 10s. After filling of the cup with a sample-KBr blend, excess material was removed by placement of a microscope slide (with frosted face towards the powder) to minimize the specular reflectance component[12] in the diffuse reflectance spectrum. For each sample, 64 scans were collected employing a TGS detector.

Conventional fused KBr disk spectra were recorded with a DTGS detector from samples prepared by compressing a 0.3% mixture of standard substance with spectral grade KBr.

DRIFTS and FTIR spectra were recorded with different resolutions. The spectra were scanned between 4000 and 400 cm^{-1}, by averaging 64 scans for each spectrum with a resolution of 4 cm^{-1} (data point resolution/interval 1 cm^{-1}) and with a resolution of 8 cm^{-1} (data point resolution/interval 2 cm^{-1}). In this way we have obtained two sets of spectra for each sample. The background spectra were obtained for each experimental conditions.

Results and Discussion

Figures 1 and **2** present comparatively the FTIR and respectively DRIFTS spectra of the three polymorphic forms of meben.
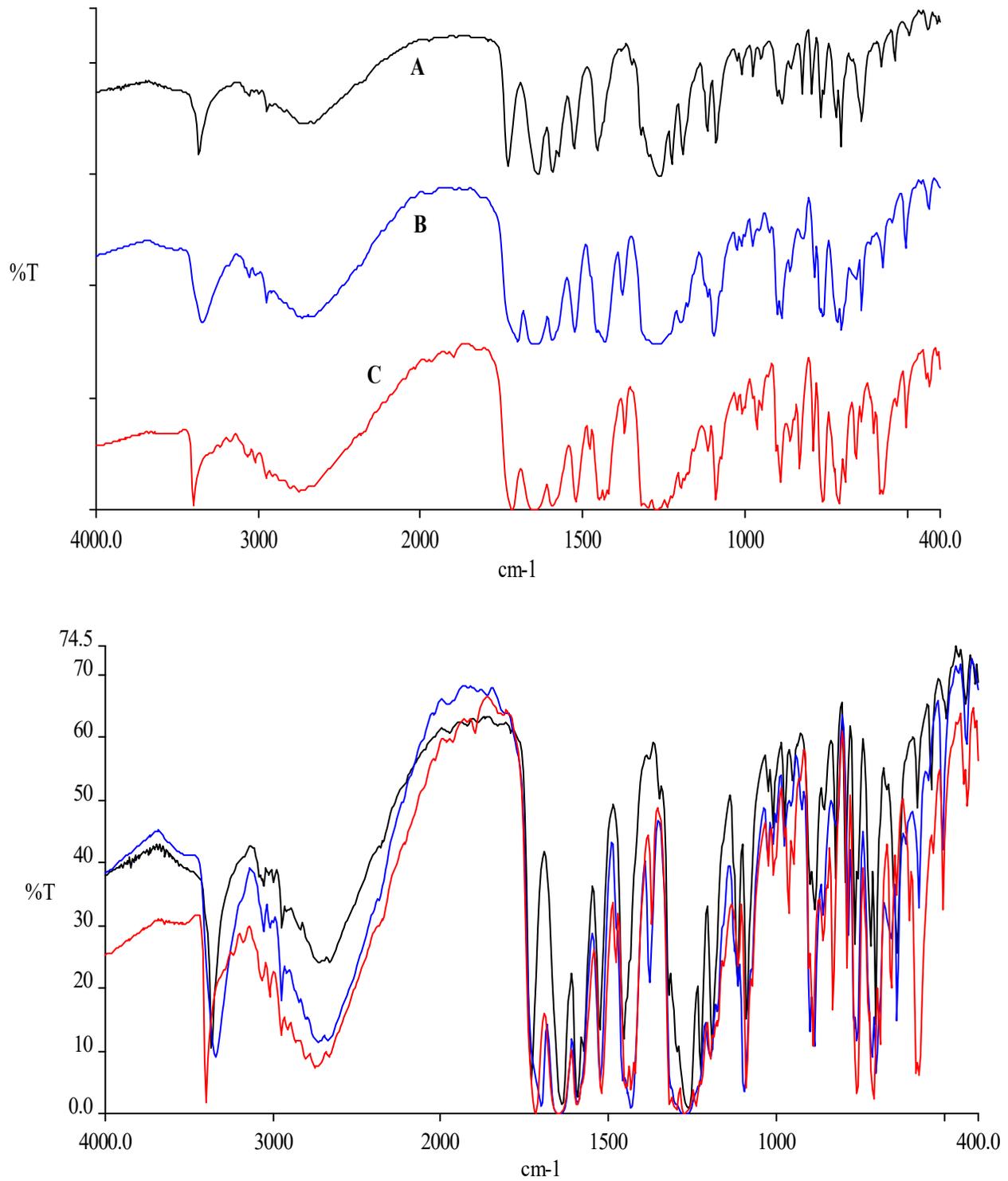
Figure 1. FT-IR trasmittance spectra of Mebendazole A, B and C in KBr-disks.
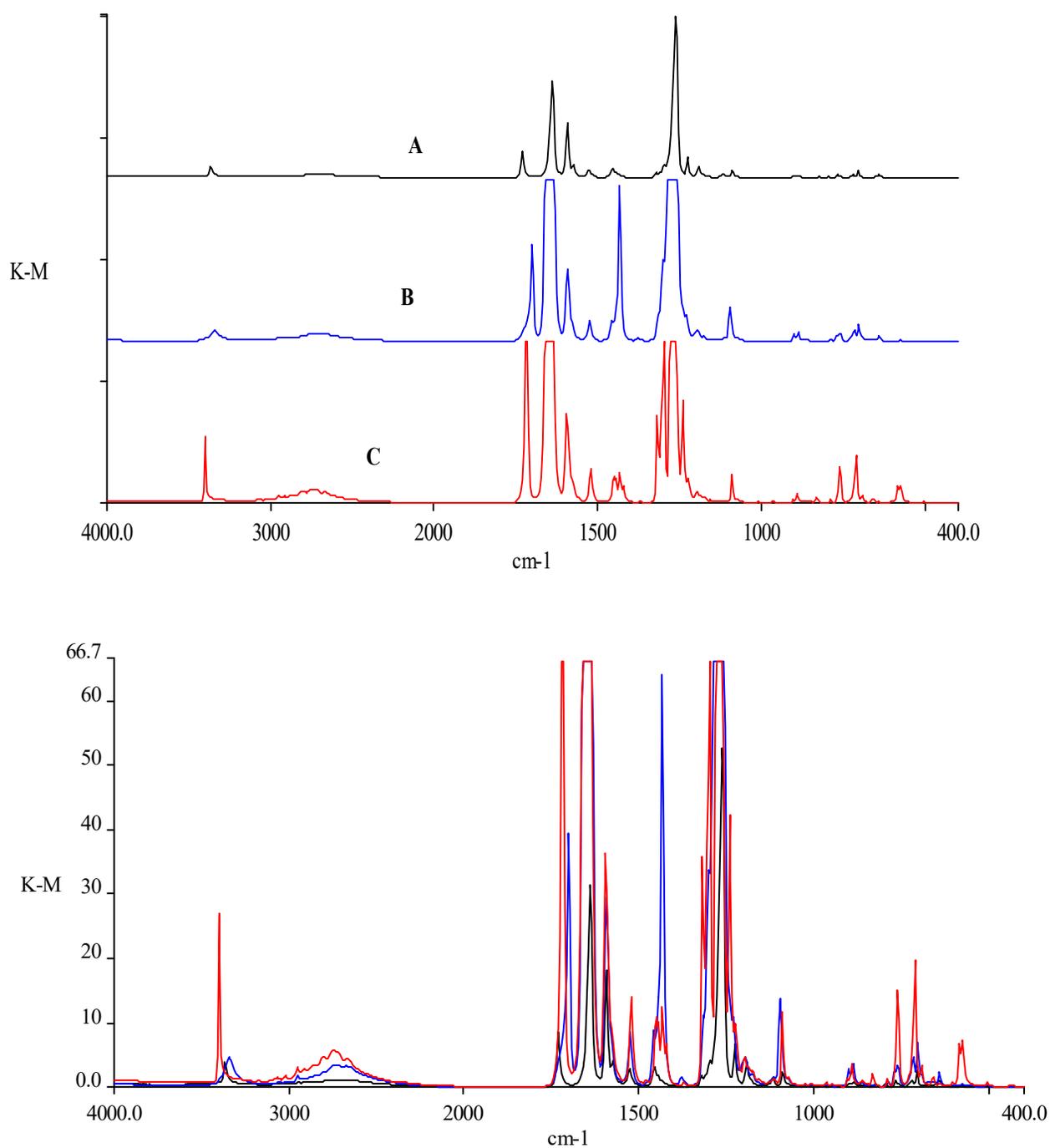
Figure 2. DRIFTS spectra of Mebendazole A, B and C in KBr-disks

NOTE In these figures with black line are represented Meben A form, with blue line Meben B, and with red Meben C respectively.

Because the FT-IR spectra of the three forms of meben studied are very similar in the range 4000 – 400 cm^{-1}, DRIFTS spectra were collected. This method is generally regarded as the one of choice for examination of polymorphs[13,14]. **Tables 1** and **2** highlights the differences observed in the FT-IR and DRIFTS spectra, respectively. These are noted as regions X, Y, Z which correspond to 4000-2000, 2000-1300 and 1300-700 cm^{-1}, respectively.

Table 1. Regions of difference in IR spectra (FT-IR) obtained for Meben polymorphs A, B and C crystals

	Principal absorption peaks / cm^{-1}		
	X	Y	Z
A	3369 3057 - 2948	1731 1638 1594 1528 -	- 1194 1118 1090 1013 903 861 792
B	3345 3057 - 2952	1700 1645 1594 1525 1379	1277 1199 1116 1099 1013 903 863 797
C	3404 3071 3024 2955	1718 1648 1596 1523 1372	1281 1198 1116 1092 1010 905 863 782

Table 2. Regions of difference in IR spectra (DRIFTS) obtained for Meben polymorphs A, B and C crystals

	Principal absorption peaks / cm^{-1}		
	X	Y	Z
A	-	- - 1638.5 1594 - -	- 1262 - - -
B	-	1700 1647.5 1594.5 - 1434	- 1272 1092 - -
C	3404	1718 1649.5 1596.5 1523 1434	1299 1273 1092 762 713

As the differences are minor, and no easily distinguishable peaks are available for characterization, the IR region of the spectrum is not useful in distinguishing the polymorphs of this compound. This is in contrast with spectral informations available in the DRIFTS method where distinct regions of characterizations were found. The situation found in this study, illustrates, once more, the subtle nature of polymorphism. It is more often observed that both DRIFTS and FT-IR spectra contain regions in which the polymorph under study has useful characteristics differences.

Taking into account that we do not know what form is presented in the pharmaceutical preparation, first of all we identify it due to the presence of peaks in the VERMOX spectra. We notice that the major component is Mebendazole C as can be seen from **Table 3**, where are presented the most important peaks of the spectra comparatively. This is in good agreement with the experimental data regarding the chemotherapy and toxicity in mice of Mebendazole[15],

Table 3. Regions of difference in IR spectra (DRIFTS) obtained for
Vermox drug comparatively with Meben C

	Principal absorption peaks / cm⁻¹		
	X	Y	Z
C	3404	1718 1649.5 1595.9 1523 1434	1299 1273 1092 762 713
Vermox	3404.5	1718 1649.5 1596.5 - 1434	1299 1277 1093 763 713

The data interval was expanded and parts of spectra were eliminated to reduce the size of the data matrix required by QUANT+ for calibration modelling. The calibrations of this study were carried out with the use of the 'expert' option.

Both PLS2 and PCR+ were carried out using a resolution of 4 cm⁻¹ and a resolution of 8 cm⁻¹. The first range used was 4000-400 cm⁻¹ while the second range was 3500-700 cm⁻¹. In both cases no blanks were first selected, but after calibration was performed, the computer select 19 and 16 ranges of blanks due to the thresholds. The number of data points used for analysis are 2377 and 1619, respectively.

Finally, the results are very similar, as can be seen in **Table 4**.

Table 4. Comparison of the Mebendazole determination in VERMOX

	Resolution 4 cm⁻¹		Resolution 8 cm⁻¹	
	4PLS2	4PCR+	8PLS2	8PCR+
n	2	1	2	1
MD	0.091	0.125	0.098	0.132
SDD	0.105	0.135	0.112	0.141
SEE	4.077	5.121	4.135	6.128
SEP	14.180	19.015	15.005	20.021
R	0.955	0.925	0.941	0.899

Abbreviations: **aPCR+,** resolution of the data used in calibration using the PCR+ method; **aPLS2**, resolution of the data used in calibration using the PLS2 method; **n** , number of PC or PLS factors used in the calibration model; **MD**, mean difference from the reference values; **SDD,** standard deviation of differences from the reference values; **SEE**, standard error of estimation ; **SEP**, standard error of prediction; **r**, correlation coefficient.

Quantitative analysis of the component in pharmaceutical preparation by IR spectrometry is based upon the Lambert – Beer law. Therefore, the conditions required for the Lambert – Beer law must be satisfied. The problems are the ingredients presented in the drug tablets. A common problem associated with all IR methods is that there are no specific or unique wavelengths of absorption for any of the components such as microcrystalline cellulose, talc, sodium starch glycolate, etc. In this respect, the Fourier transform option is superior to the filter option of spectrophotometers because the determination is not based on a single wavelength that is not unique for the analyte of interest.

Conclusions

It is clear that FT-IR spectrometry is capable for the direct determination of mebendazole in drug formulations, such as VERMOX. With the commercial software QUANT+, involving chemometric approaches, PCR+ and/or PLS2, the method proposed by us is simple, precise and not time consuming.

Acknowledgements

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