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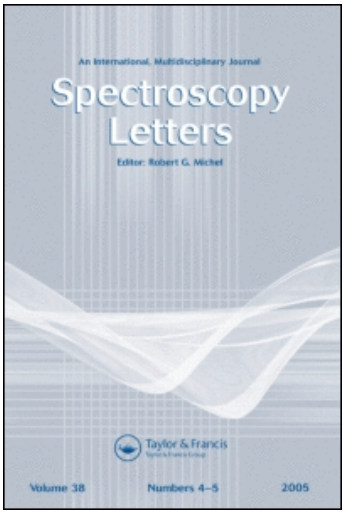

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ANALYSIS OF MEBENDAZOLE POLYMORPHS BY FOURIER TRANSFORM INFRARED SPECTROMETRY USING CHEMOMETRIC METHODS

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ANALYSIS OF MEBENDAZOLE POLYMORPHS BY FOURIER TRANSFORM INFRARED SPECTROMETRY USING CHEMOMETRIC METHODS

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ABSTRACT

A Fourier transform infrared (FT-IR) spectrometric method diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was developed for the rapid, direct measurement of mebendazole in drugs. Conventional KBr-spectra and

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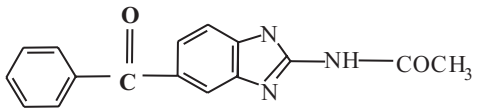
DRIFTS-spectra were compared for best determination of active substance in drug formulations. Two chemometric approaches, partial least-squares (PLS2) and principal component regression (PCR+) methods were used in data procesing. The best results were obtained with the PLS2 method.

Key Words: 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[4]. In principle, the different polymorphic forms of a compound have different energies and this could affect their bioavailability[5]. Sometimes there are more than two forms. Mebendazole used in clinical and veterinary treatment exists in three polymorphic forms (A, B and C, respectively), all of them being in accord with USP specifications[6].

The pharmaceutical importance of polymorphism was reviewed by Haleblian and McCrone[7]. 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 differential 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 which include the

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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 information 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[8,9]. 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[10].

The partial least squares (Projection to Latent Structures, PLS) regression method was developed by Wold[11] in 1966. There is a substantial amount of literature devoted to the theoretical elucidation of properties of the PLS algorithm. A good introduction to the method is given by Geladi and Kowalski[12].

PLS seeks to express the variance in the property information by correlating it with the spectral information (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 treats all properties individually, if there is non-linearity which differs from property to property, it would be expected that PLS1 would build better models than PLS2, which is trying to compensate for the non-linearities of all properties simultaneously[13]. 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 information from complex spectra containing overlapping absorption peaks, interference effects from diffuse reflectance and light 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 ($N_{objects} \times K_{wavelengths}$) and a set of dependent variables, the **Y** matrix ($N_{objects} \times M_{measurements}$). The resulting model has the form:

$$\mathbf{Y} = \mathbf{X} \cdot \mathbf{B} + \mathbf{E} \tag{1}$$

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

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The purpose of the present study is to use FT-IR spectrometry to investigate the structural features of the various polymorphic forms exhibited by mebendazole. The main objective of this work was to develop a chemometric procedure for the fast and accurate determination of different polymorphic forms of mebendazole 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 spectral resolution 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 Ltd., Beaconsfield, Bucks, UK). The commercial software used to generate analysis for the principal component analysis was QUANT+ expert Version 4.10 (Perkin Elmer Ltd.).

Mebendazole (R 17635), reference polymorphic forms A, B and C (lot V890 - 359, V890 - 353 and V890 - 386, respectively) and Vermox® tablets (containing 100 mg 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 Ltd. 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 10 s. 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[14] 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}). This way we obtained two sets of spectra for each

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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 mebendazole.

Because the FT-IR spectra of the three forms of mebendazole studied are very similar in the range 4000-400 cm^{-1}, DRIFTS spectra were collected. This method is generally regarded as the method of choice for examination of polymorphs[15,16]. Tables 1 and 2 highlight the differences observed in the FT-IR and DRIFTS spectra, respectively. These are noted as X, Y, Z which correspond to 4000-2000, 2000-1300 and 1300-700 cm^{-1} regions, respectively.

As the differences are minor, and not easily distinguishable peaks are available for characterization, the IR region of the spectrum is not useful in differentiating the polymorphs of mebendazole. This is in contrast with spectral information available in the DRIFTS method where distinct regions of differentiating characterizations were found. The situation found in this study illustrates once more the subtle nature of polymorphism. It is more

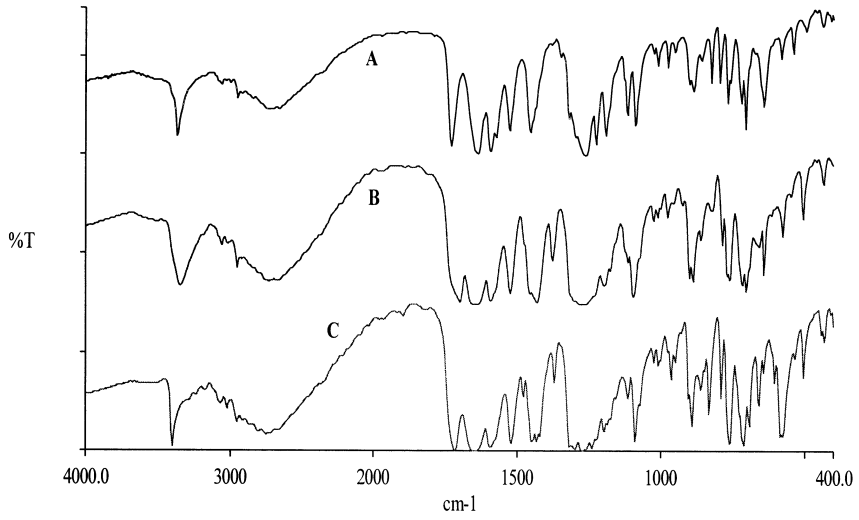

Figure 1. FT-IR transmittance spectra of Mebendazole A, B, C polymorphs in KBr-disks.

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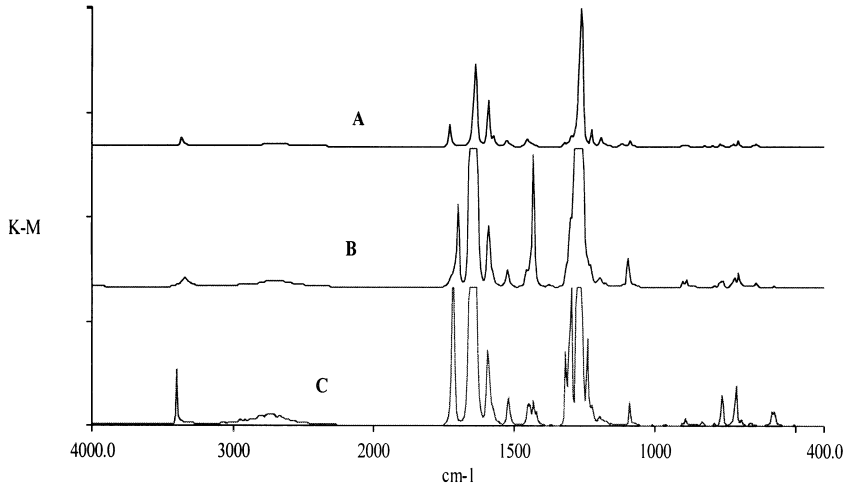

Figure 2. DRIFTS spectra of Mebendazole A, B, C polymorphs in KBr-blend.

often observed that both DRIFTS and FT-IR spectra contain regions in which the polymorph under study has useful characteristic differences.

This method can be used to identify the polymorph present in Mebendazole pharmaceutical tablets such as Vermox®. As shown in Table 3, the major polymorph present in the pharmaceutical tablet is polymorph C when the principal absorption peaks of the spectra were compared with an authentic polymorph C mendazole standard. This is in agreement with the experimental data reported by Rodriguez-Caaseiro et al.[17] regarding the chemotheraphy and toxicity of mebendazole polymorphs in which they recommend the use of polymorph C in oral treatment.

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. The results were found to be very similar, as shown in Table 4.

Quantitative analysis of the component in pharmaceutical preparation by IR spectrometry is based upon the Lambert–Beer law. Therefore, the

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Table 1. Regions of Difference in IR Spectra (FT-IR) Obtained for Mebendazole 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		

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Table 2. Regions of Difference in IR Spectra (DRIFTS) Obtained for Mebendazole 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

Table 3. Regions of Difference in IR Spectra (DRIFTS) Obtained for the Vermox® Tablet in Comparison with Mebendazole C

	Principal Absorption Peaks/cm^{-1}										
	X	Y					Z				
Mebendazole 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

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: **4PCR+**, resolution of the data used in calibration using the PCR+ method; **8PLS2**, 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.

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conditions required for the Lambert–Beer law must be satisfied. The problems are the excipients and inactive 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 excepients such as microcrystalline cellulose, talc, sodium starch glycolate, etc. In this respect, the Fourier transform option is superior 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 of the direct determination of mebendazole polymorph in drug formulations. With the commercial software QUANT+, involving chemometric approaches, PCR+ and/or PLS2, the method proposed is simple, precise and fast.

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REFERENCES

1. Archer, J.D. J. Am. Med. Ass. **1974**, *230*, 1436.
2. Wolfe, M.S.; Wershing, J.M. J. Am. Med. Ass. **1974**, *230*, 408–411.
3. VERMOX, JANSSEN Pharmaceutica Biotech. N.V. (Belgium), medical presentation.
4. Brittain, H.G.; Grant, D.J.W. Effects of Polymorphism and Solid State Solvation on Solubility and Dissolution Rate. Chapter 7 In *Polymorphisim in Pharmaceutical Solids*; Brittain, H.G., Ed.; Marcel Dekker Inc.: New York, 1999; 321.
5. Brittain, H.G. In *Polymorphism in Pharmaceutical Solids*; Marcel Dekker Inc.: New York, 1999.
6. USP XXII – United States Pharmacopoeia, The United Pharmacopoeia Convention Inc., Rockville, MD, USA, **1990**, 809.
7. Haleblian, J.; McCrone, W. J. Pharm. Sci. **1969**, *58*, 911.

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8. Luinge, H.J.; Hop, E.; Lutz, E.T.; van Hemert, J.A.; de Jong, E.A. Anal. Chim. Acta **1993**, *284*, 419.
9. Van de Voort, F.R.; Sedman, J.; Emo, G.; Ismail, A.A. J. AOAC. Int. **1992**, *75*, 780.
10. Jong, E.A.M.; Kaper, J. Neth. Milk. Dairy J. **1996**, *50*, 35.
11. Wold, H. *Research Papers in Statistics*; David, F.N., Ed.; Wiley: New York, 1966.
12. Geladi, P.; Kowalski, B.R. Anal. Chim. Acta **1986**, *185*, 1.
13. Martens, H.; Naes, T. *Multivariate Calibration*; John Wiley & Sons: New York, 1989.
14. Messerschmidt, R.G. Appl. Spectrosc. **1985**, *39*, 737.
15. Roston, D.A.; Walters, M.C.; Rhinebarger, R.P.; Ferro, L.J. J. Pharm. Biomed. Anal. **1993**, *11*, 293.
16. Hartauer, K.J.; Miller, E.S.; Guillory, J.K. Int. J. Pharm. **1992**, *85*, 163.
17. Rodriguez-Caaseiro, F.; Criado-Forneleio, A.; Jimenez Gonzales, A.; Guzman, L.; Igual, A.; Perez, A.; Pujol, M. Chemotherapy **1987**, *33*, 266.

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