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FT-IR and Raman spectroscopic methods for identification and quantitation of orthorhombic and monoclinic paracetamol in powder mixes

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Abstract

FT-IR and Raman spectroscopic methods are suggested for identification of orthorhombic (form **II**) and monoclinic (form **I**) paracetamol and for their quantitative determination in mixes. The intensity ratio of the 836 cm^{-1} FT-IR band (attributed to the presence of both forms) to the 806 cm^{-1} monoclinic band plotted against the inverse monoclinic molar fraction (X) yields a straight line: $I_{836}/I_{806} = 0.515/X + 0.700$, $r = 0.9965$ for eight calibration points on the regression line. Similarly, the area under the 454 cm^{-1} band in FT-Raman spectra (which is attributed to both forms) over the area under the 465 cm^{-1} band of monoclinic form is inversely related to its molar fraction (X): $A_{454}/A_{465} = 0.482/X - 0.324$, $r = 0.9954$ for eight calibration points. Precision (RSD%) was $< 5\%$ for both methods. Linear regression analysis between content and intensity of characteristic XRD reflections for four different samples gave $r = 0.9964$ at 4.62 Å and $r = 0.9894$ at 3.70 Å, for form **II**. For the content of form **I**, $r = 0.9596$ at 3.37 Å. The limit of detection for monoclinic form was estimated to be 0.012 mole fraction for both methods.

Keywords: Identification; Quantitation; Paracetamol polymorphs; FT-IR and Raman spectroscopy; X-ray diffractometry

1. Introduction

There is an ever-growing interest for fast, simple and reliable analytical methods for the determination of active pharmaceutical substances in the solid state. Such methods become more attractive when two substances co-exist in the same commercial dosage form or two polymorphs of the same compound are present in mixture. IR spectroscopy is a widely recommended method, wherein the spectrum of the test compound is compared with that obtained concomitantly of the reference standard. Also, X-ray diffractometry (XRD) is a powerful technique for the identification of crystalline solid phases [1,2]. It is unique, because combines absolute specificity with high degree of accuracy. Despite these attributes, the XRD method finds very limited application for the evaluation of drug product quality. Recently FT-Raman spectroscopy has been suggested as a

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capable method for identifying polymorphs and testing crystallinity of substances [3–6].

For paracetamol (acetaminophen, 4′-hydroxy acetanilide) two polymorphs are fully described in the literature: the monoclinic, form **I**, and the orthorhombic, form **II** [7–10]. A third form has been reported, but is very unstable [9,10]. The monoclinic form is thermodynamically stable and is used commercially, but it lacks slip planes and therefore it is not suitable for direct compression into tablets. Orthorhombic paracetamol is characterized by well-developed slip planes in its crystal structure and undergoes plastic deformation making it suitable for tabletting by direct compression

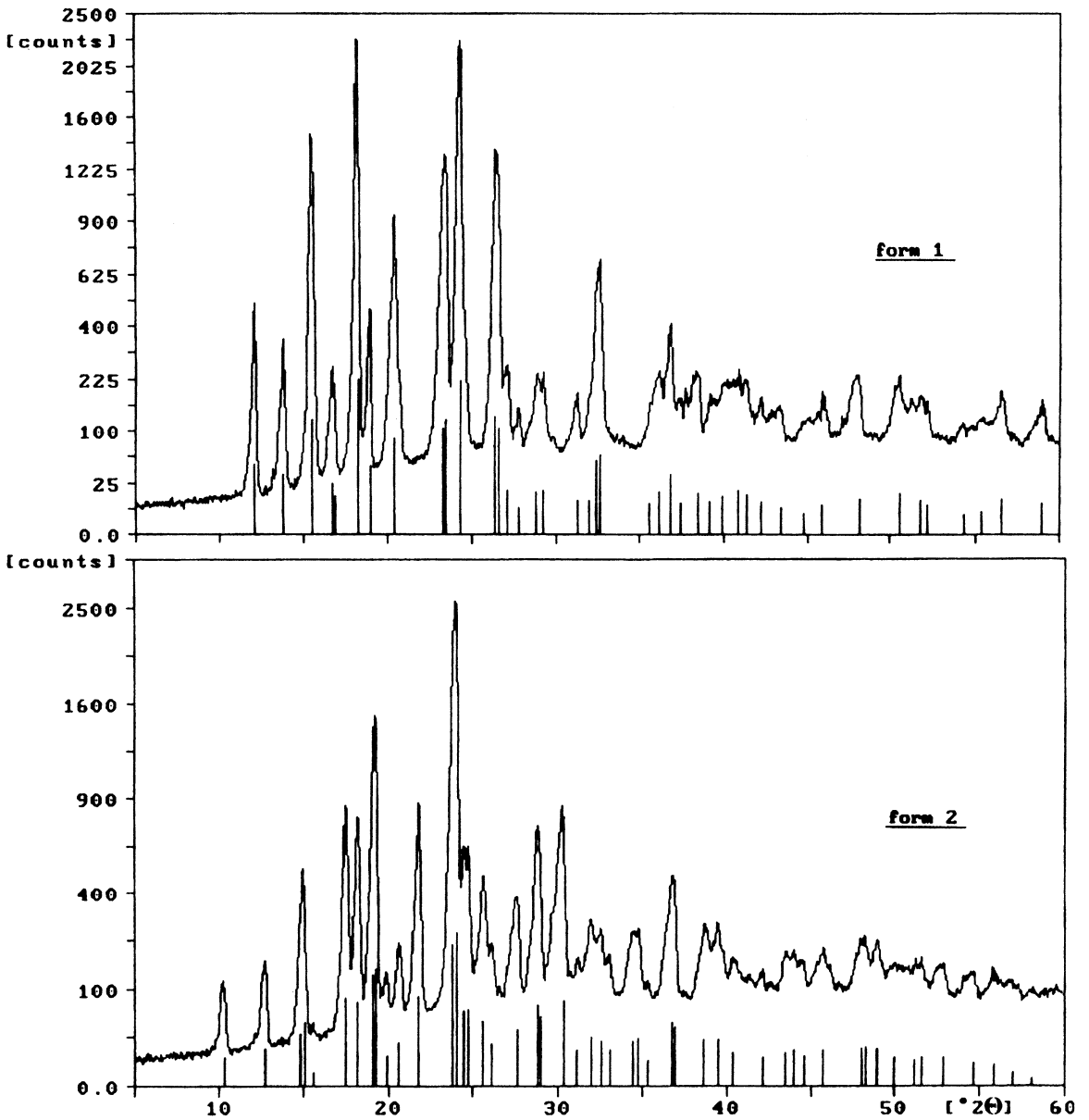

Fig. 1. Experimental XRD patterns for the characterization of the reference monoclinic and orthorhombic samples (form **I** and **II**).

[11,12]. Experimental preparation of orthorhombic paracetamol by crystallization from solution has been reported recently in a laboratory scale by seeding [12]. This requires a quick identification of orthorhombic form because it is used as seeding material and is obtained by irreproducible crystallization from melt [9]. Therefore, a simple, fast and reliable identification and quantification method for the orthorhombic paracetamol in powder mixes with monoclinic was thought of interest.

The objective of the present work was the development of an FT-IR spectroscopic method because thermal behavior of form **II** precludes the use of differential scanning calorimetry (DSC) as a reliable method for its quality control [12,13]. XRD and FT-Raman spectroscopy were used to validate the method developed.

2. Materials and methods

2.1. Materials

Monoclinic paracetamol of pharmaceutical grade, in microcrystalline powder form, was used. Boehringer Ingelheim, Athens, Greece, kindly offered it. KBr and ethanol were of analytical grade.

2.2. Preparation of orthorhombic paracetamol (form *II*)

Orthorhombic paracetamol (form **II**) was prepared using monoclinic raw material (form **I**) according to Nichols and Frampton (1998), from ethanolic solution by seeding [12]. Monoclinic raw material and orthorhombic paracetamol were characterized by X-ray powder diffractometry (XRD) and used as reference samples. Mixtures of form **I** and **II** were prepared in different molar fractions and ground gently with an agate pestle and mortar. Each fraction was examined by FT-IR and FT-Raman spectroscopy and by XRD.

2.3. FT-IR and Raman spectroscopy

FT-IR spectra were obtained employing a Perkin–Elmer FT-IR 1605 Spectrophotometer with a DTGS detector. Samples were scanned as KBr disks (1% w/w); resolution: 4 cm^{-1}; OPD velocity: 0.3 cm s^{-1}. The data region was 4000–500 cm^{-1}, operation in auto mode and the number of scans per spectrum 16. Spectra were obtained in transmission and absorption mode for the identification and the quantitative determination, respectively. Two milligrams of sample was mixed with 200 mg KBr and ground gently with an agate pestle and mortar under an infrared lamp and afterwards was pressed into a 13-mm diameter disk by applying 10 tons pressure for 2 min.

Raman spectra were recorded using a FRA-106/S FT-Raman spectrometer (Bruker, Karlsruhe, Germany) using a Nd:YAG laser (1064 nm). A reference source (He–Ne laser) was used to measure the instrumental response and for checking of the interferometer. Filters were employed to remove the Rayleigh line and the optical output of the He–Ne laser. The scattered light was collected at an angle of 180° (backscattering). The system was equipped with a LN_2 cooled Ge detector (D 418). The power of the incident laser beam was about 150 mW at the sample's surface. Typical spectral resolution was 1 cm^{-1}. The system was interfaced with a computer and 'Peak fit' v4.0 software (Jandel Scientific) was employed for Raman band de-convolution. Specimen preparation for the Raman spectroscopy was similar to that for XRD. Powder samples were lightly packed into an aluminium holder prior to analysis.

2.4. X-ray powder diffractometry (XRD)

XRD patterns were recorded at room temperature (25 °C) for the characterization of monoclinic and orthorhombic paracetamol on a Philips PW 1710 diffractometer (Cu anode operated at 40 kV and 25 mA). The samples were scanned from 5 to 60° two theta at a rate of 1.5° min^{-1}. For the evaluation of the FT-IR method or for quantitative analyses, the XRD patterns were recorded on a Philips PW 1730/10 diffractometer (Ni filtered Cu–Kα, voltage 35 kV, current 25 mA, 1.2° min^{-1}) from 3 to 63° two theta. Form **I** and form **II** paracetamol and their mixtures were prepared

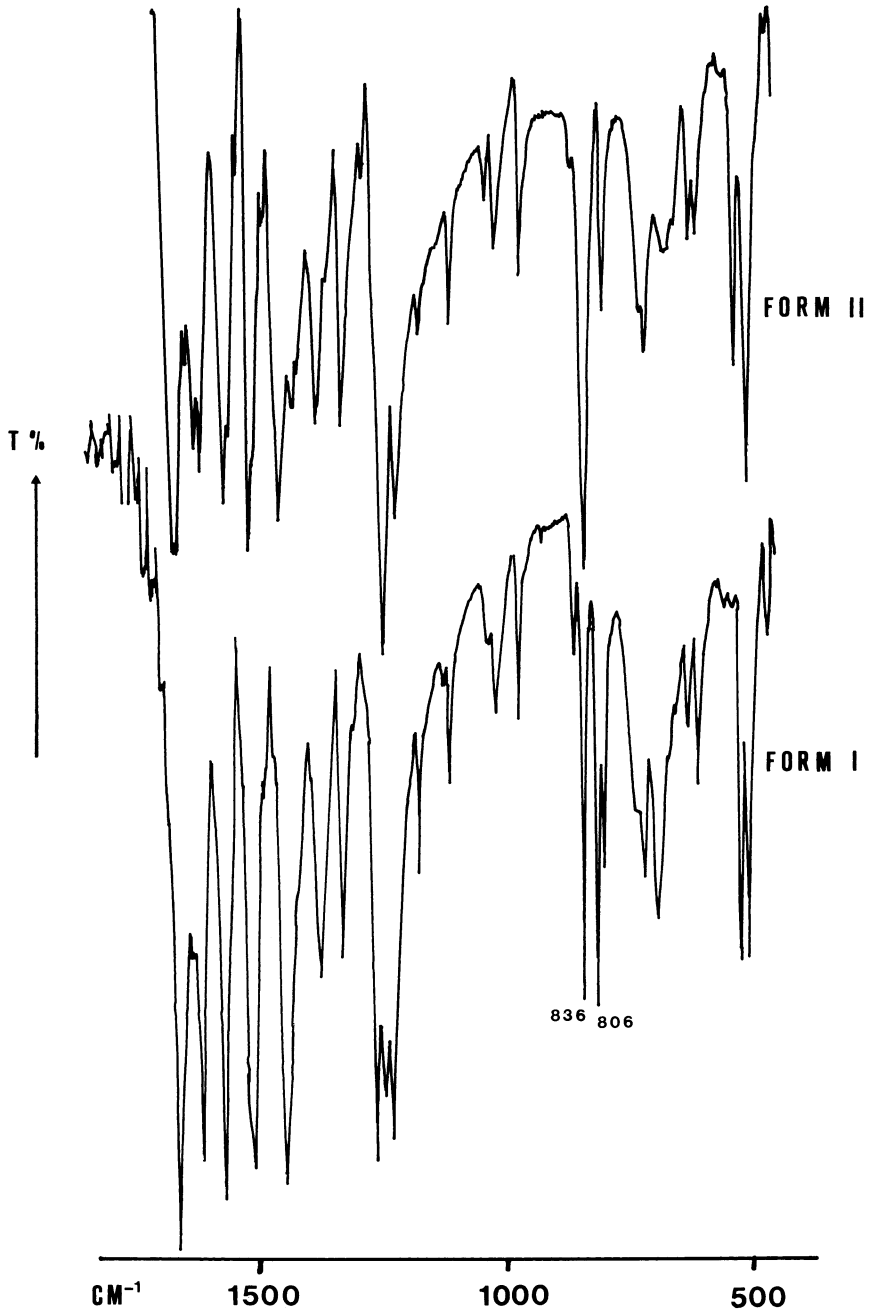

Fig. 2. Partial FT-IR spectra of monoclinic and orthorhombic paracetamol reference samples (form **I** and **II**).

Table 1
Experimental XRD data[a] for the reference samples of monoclinic and orthorhombic paracetamol

Monoclinic (form **I**)		Orthorhombic (form **II**)	
d-spacing (Å)	% Relative intensity	d-spacing (Å)	% Relative intensity
7.32	20.4	6.93	5.9
6.43	15.2	6.01	11.1
–	–	5.91	16.7
5.72	55.3	5.07	33.1
5.31	10.8	4.89	29.9
5.26	6.1	4.65	52.0
4.88	100.0	4.62	58.6
4.69	19.6	4.32	7.5
4.35	39.3	4.07	34.0
–	–	3.73	85.3
3.82	47.4	3.70	100.0
3.78	54.7	3.63	23.3
3.65	97.5	3.59	25.1
–	–	3.47	17.9
–	–	3.41	7.1
3.37	57.5	3.22	13.4
3.35	47.1	3.10	27.8
3.28	7.9	3.08	20.3
3.09	7.6	2.95	30.4
3.05	8.3	2.87	5.0
2.76	22.5	2.80	9.6
2.74	26.7	2.75	8.4
–	–	2.71	5.3
2.48	7.5	2.60	8.0
–	–	2.58	9.4
2.43	14.9	2.44	17.4
–	–	2.43	14.3
2.33	7.1	2.33	8.8

[a] Only lines with relative intensities >5% are considered.

as flat plate specimens using aluminium holders. For mixtures, the traces of form **I** and/or **II** were detected and the intensities of the characteristic reflections gave the ratio of the two forms, known that the sensitivity threshold of detection is 2% [9,14,15].

3. Results and discussion

The experimentally obtained XRD patterns for the characterization of the reference monoclinic and orthorhombic samples (form **I** and **II**) are presented in Fig. 1, and show that have different crystal structures. In Table 1 are given the experimental peaks. Those that are characteristic for each form, are not closely spaced to others and correspond to relatively high intensity were selected (3.70, 4.62 and 3.37 Å) for quantitative

Table 2
Experimental data of quantitative XRD analysis of the reference samples and their mixtures

Orthorhombic mole fraction	XRD peak intensity [counts] at d-spacing		
	4.62 Å	3.70 Å	3.37 Å
1.000	7496	12 566	–
0.750	5930	9678	1798
0.500	4112	7652	2388
0.250	1834	3404	4098
0.000	–	–	6930

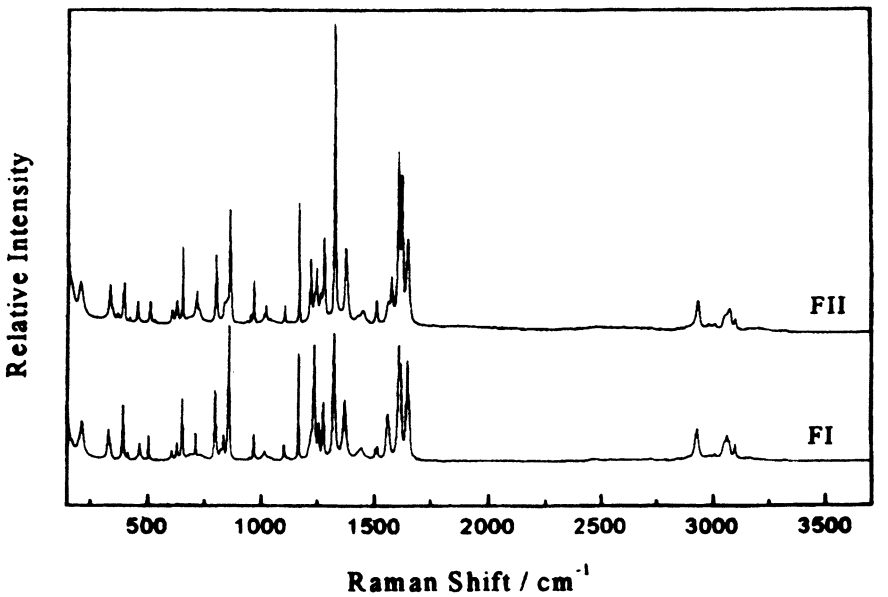

Fig. 3. FT-Raman spectra of monoclinic and orthorhombic paracetamol reference samples (form **I** and **II**).

determination of the two forms in mixtures. Intensities for the above peaks of the reference samples and of their mixtures in different mole fraction are listed in Table 2. Linear regression analysis between content and intensity gave for form **II** correlation coefficient $r = 0.9964$ at 4.62 Å and $r = 0.9894$, at 3.70 Å. For form **I** the value of r was 0.9596 at 3.37 Å.

The IR spectra of reference samples (form **I** and **II**) are shown in Fig. 2. Noticeable differences are assigned for the C–N amide stretching peaks (1566, 1507 and 1560, 1513 cm^{-1}) and the C–C aromatic stretching peaks (1614, 1507, 1442 and 1626, 1513, 1453 cm^{-1}). But, the most distinct difference between the two spectra is the presence of extra absorption peaks. They should correspond to inter- or intra-molecular interactions. For the monoclinic form such peaks are at 806, 682 and 1228 cm^{-1}. In addition, other absorption peaks vary in intensity. Ratios of characteristic peaks (at 806 cm^{-1}) with others present in both spectra (at 836 cm^{-1}) are considered for quantification of the two forms in mixture.

Repeated IR spectroscopic analysis on three replicate samples for each mole fraction was applied and the results of mean peak ratio together with between assay precision data (RSD%) are presented in Table 3. Linear regression analysis between content and the peak ratio data gave a straight-line plot $y = 0.52\ (\pm 0.02)X^{-1} + 0.70(\pm 0.08)$ with confidence intervals 0.56 to 0.48 and 0.89 to 0.51 for slope and intercept, respectively. Therefore, the linear relationship is:

$$I_{836}/I_{806} = 0.515/X + 0.700 \quad (1)$$

where X is the mole fraction of monoclinic form and $r = 0.9965$ for eight calibration points on the regression line. Using the criterion of minimum delectability as three times the system noise, the limit of detection for monoclinic form was estimated to be 0.012 mole fraction.

Since reproducibility is high (RSD < 4.5%) and correlation coefficient (r) for IR peak ratio is close to one, it means that IR spectroscopy can be used not only for the identification, but also for the quantitative determination. Precision and accuracy data for the FT-IR spectroscopic determination of monoclinic paracetamol in mixes with orthorhombic form are given in Table 4.

Specimen preparation for the IR spectroscopy includes grinding of crystals with KBr while Raman spectroscopy gives information that comple-

Table 3
Calibration points on the regression line and reproducibility data of the FT-IR and Raman spectroscopic methods for determinations of paracetamol polymorphs in powder mixes ($n = 3$)

Mole fraction of monoclinic form		836/806 Peak height ratio in FT-IR spectra		A_{454}/A_{465} band area ratio in FT-Raman spectra	
X	X^{-1}	Mean	RSD (%)	Mean	RSD (%)
0.100	10.000	5.704	2.8	4.430	5.0
0.175	5.714	3.800	4.5	2.380	3.9
0.250	4.000	2.897	3.9	1.790	2.3
0.400	2.500	2.167	1.5	1.124	3.4
0.600	1.667	1.464	2.1	0.534	3.0
0.700	1.429	1.360	0.8	0.266	2.9
0.850	1.176	1.258	3.7	0.111	3.1
1.000	1.000	1.107	2.1	0.032	4.0

ments that of IR and involves specimen preparation similar to XRD (without grinding). A molecule absorbs IR radiation when the dipole moment changes during molecular vibration while the Raman effect is a scattering process and results from an induced dipole moment, dependent on a change in molecular polarisability during a vibration. Non-centrosymmetric molecules (like paracetamol) often exhibit important intensity differences between the Raman and IR bands. Also, a principal advantage of FT-Raman is that scattering is directly proportional to the concentration, whereas IR does not relate linearly to absorber concentration. This is important for quantitative analytical studies, although there are attendant problems in quantitative Raman spectroscopy with source stability and with refractive index [6,10]. Furthermore, the Raman spectrum of water is weaker than the IR. Therefore, effect of moist material frequently noted in the IR is minimised in the Raman spectrum. The principal disadvantage of Raman spectroscopy at present is that the technique is generally less widely

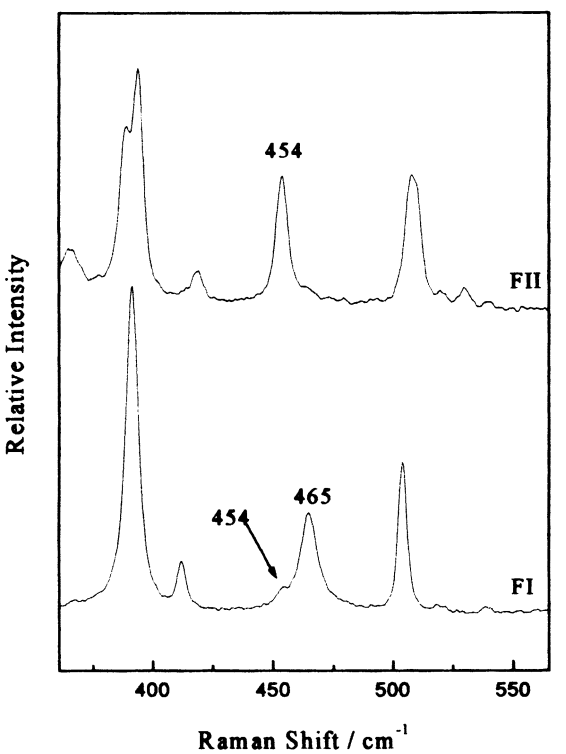

Fig. 4. Partial FT-Raman spectra of monoclinic and orthorhombic paracetamol reference samples (form **I** and **II**).

Table 4
Precision and accuracy data of the FT-IR spectroscopic method of monoclinic paracetamol determination in mixes with the orthorhombic form ($n = 5$)

Mole fraction of monoclinic form		RSD (%)	Bias (%)
Added	Found		
0.206	0.213	3.21	+3.40
0.282	0.289	2.02	+2.48
0.495	0.490	1.14	−1.01
0.661	0.651	1.72	−1.51

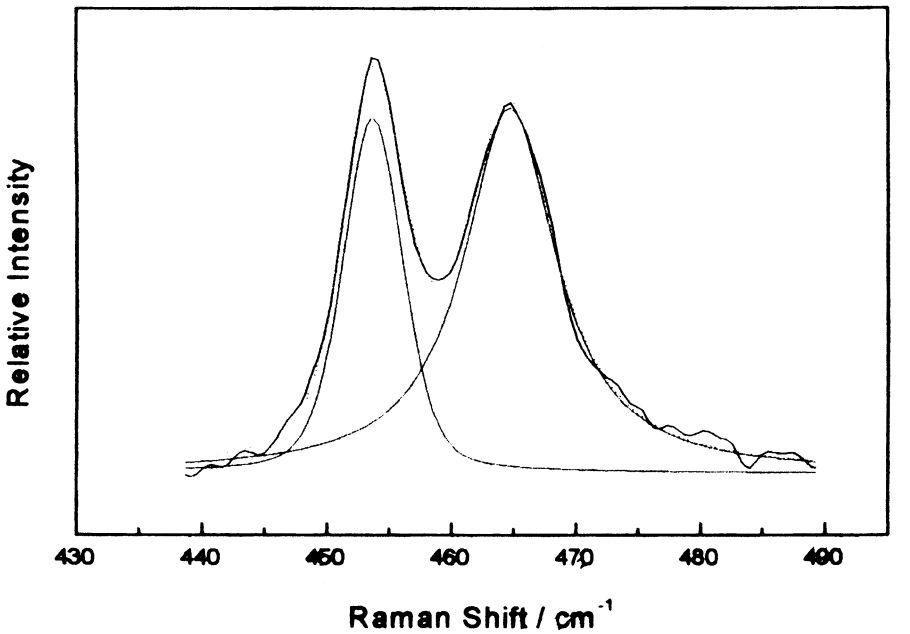

Fig. 5. De-convolution of band in the FT-Raman spectrum, for binary mixture of paracetamol polymorphs (orthorhombic mole fraction 0.4).

available than IR spectroscopy. For the above reasons FT-Raman spectroscopy was used to evaluate the FT-IR method besides XRD.

The Raman spectra of reference samples are shown in Fig. 3 and the characteristic frequencies (1244, 1220 and 454 for orthorhombic or 1258, 1238 and 465 for monoclinic) are in agreement with earlier reports [10]. For the quantitative determination, the 454 and 465 cm^{-1} bands were selected.

Representative partial Raman spectra in the range of 400–500 cm^{-1} are given in Fig. 4, for the two polymorphs of paracetamol. The 454 cm^{-1} bands are attributed to the presence of both forms and the 465 cm^{-1} bands to the monoclinic paracetamol. For the mixtures, complete separation (de-convolution) of the bands was achieved by employing the 'Peak Fit' statistical package (Fig. 5). The area under the curve ($A_{(\nu)}$) of the separated bands was used for the quantitative determination of the two forms in mixtures. Since the A_{454} corresponds to the total of paracetamol present in the mixture (monoclinic and orthorhombic) and the A_{465} to the monoclinic component, the ratio A_{454}/A_{465} should be directly proportional to the reciprocal mole fraction of monoclinic form ($1/X$).

Repeated Raman spectroscopic analysis on three replicate samples for each mole fraction was applied. The results of mean value for area ratio of the bands together with between assay precision data (RSD%) are presented in Table 3. Regression analysis of the data gave a straight-line calibration plot $y = 0.48\ (\pm 0.02)X^{-1} - 0.32\ (\pm 0.09)$ with confidence intervals 0.44 to 0.53 and -0.53 to -0.12 for slope and intercept, respectively. Therefore, the linear relationship is:

$$A_{454}/A_{465} = 0.482/X - 0.324,\ r = 0.9954 \text{ and } n = 8 \quad (2)$$

The validity of Eq. (2) is confirmed from the spectrum of the pure monoclinic form for which the ratio A_{454}/A_{465} is 0.032. The $A_{(\nu)}$ measured in the Raman spectrum depends on the intensity of the laser I_o, on a factor $K_{(\nu)}$ including the frequency dependent terms (such as overall spectrophotometer response, self absorption of the medium and molecular scattering of the Raman

active species), and on the mole fraction (X) of the active species:

$$A_{(\nu)} = I_o K_{(\nu)} X \quad (3)$$

Therefore,

$$A_{454}/A_{465} = [K^{I}_{(454)}X + K^{II}_{(454)}(1-X)]/K^{I}_{(465)}X \quad (4)$$

Transforming Eq. (4) to

$$A_{454}/A_{465} = [K^{I}_{(454)} - K^{II}_{(454)}]/K^{I}_{(465)} + [K^{II}_{(454)}/K^{I}_{(465)}]1/X \quad (5)$$

and taking into account the relation between A_{454}/A_{465} and $1/X$ (Eq. (1)), we can calculate the terms $[K^{I}_{(454)} - K^{II}_{(454)}]/K^{I}_{(465)} = -0.45 \pm 1.6 \times 10^{-2}$ and $[K^{II}_{(454)}/K^{I}_{(465)}] = 0.489 \pm 3 \times 10^{-3}$, from the experimental data. Furthermore, we can calculate the ratio $K^{I}_{(454)}/K^{I}_{(465)} = 0.04 \pm 1.6 \times 10^{-2}$, which is very close to the experimental value of $A_{454}/A_{465} = 0.032$ measured in the spectrum of the pure monoclinic form, confirming the validity of Eq. (2). The limit of detection for monoclinic form was estimated and was found to be 0.012 mole fraction, as for the FT-IR method.

4. Conclusion

The confirmation of the linearity in the Raman data together with the reproducibility, the detection limit and the high correlation coefficients for the regression equations render the suggested FT-IR and Raman methods suitable for identification and quantitative determination of orthorhombic and monoclinic paracetamol in a powder mixture. FT-IR requiring conventional laboratory instrumentation may be preferable for quick crystal form monitoring purposes.

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References

[1] The United States Pharmacopoeia XXIII revision. United States Pharmacopoeial convention, Rockville, MD, 1994, pp. 107, 241, 266, 1277, 1521.
[2] N.V. Phadnis, R.K. Cavatur, R. Suryanarayanan, J. Pharm. Biomed. Anal. 15 (1997) 929–943.
[3] F.W. Langkilde, J. Sjoblom, L. Tekenbergs-Hjelte, J. Mrak, J. Pharm. Biomed. Anal. 15 (1997) 687–696.
[4] W.P. Findlay, D.E. Bugay, J. Pharm. Biomed. Anal. 16 (1998) 921–930.
[5] L.S. Taylor, G. Zografi, Pharm. Res. 15 (1998) 755–761.
[6] L.S. Taylor, F.W. Langkilde, J. Pharm. Sci. 89 (2000) 1342–1353.
[7] M. Haisa, S. Kashino, H. Maeda, Acta Crystallogr. Sect. B 30 (1974) 2510–2513.
[8] M. Haisa, S. Kashino, R. Kawai, H. Maeda, Acta Crystallogr. Sect. B 32 (1976) 1283–1285.
[9] P. Di Martino, P. Conflant, M. Drache, J.-P. Huvenne, A.-M. Guyot-Hermann, J. Thermal Anal. 48 (1997) 447–458.
[10] M. Szelagiewicz, M. Marcolli, S. Cianferani, A.P. Hard, A. Vit, A. Burkhard, M. von Raumer, U.Ch. Hofmeier, A. Zilian, E. Francotte, R. Schenker, J. Therm. Anal. Cal. 57 (1999) 23–43.
[11] E. Joiris, P. Di Martino, C. Berneron, A.-M. Guyot-Hermann, J.-C. Guyot, Pharm. Res. 15 (1998) 1122–1130.
[12] G. Nichols, C.S. Frampton, J. Pharm. Sci. 87 (1998) 684–693.
[13] F.N. de Wet, J.J. Gerber, A.P. Lötter, J.G. van der Watt, T.G. Dekker, Drug Dev. Ind. Pharm. 24 (1998) 447–453.
[14] E. Nürnberg, A. Hopp, Pharm. Ind. 44 (1982) 1081–1084.
[15] R. Suryanarayanan, Powder Diffraction 5 (1990) 155–160.