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IN SITU CHARACTERIZATION OF POLYMORPHIC FORMS
The potential of Raman techniques

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Abstract

The use of hot-stage Raman microscopy – the direct coupling of Raman spectroscopy and thermomicroscopy – is demonstrated for the drug substances paracetamol and lufenuron.

Paracetamol is a well-known analgesic and antipyretic drug, for which three polymorphic forms are currently known. Lufenuron is a benzoylphenyl urea derivative that has been classified as a chitin synthesis inhibitor. It is indicated for the use in pets for the prevention and control of flea population and used in crop protection for the control of Lepidoptera, Western Flower thrips and rust mites. It is the first time that the polymorphism of lufenuron is addressed. All known modifications of paracetamol and lufenuron were produced and identified by hot-stage Raman microscopy. A close correlation of thermal and spectroscopic information was achieved by this combination of techniques.

For lufenuron a series of new polymorphic forms were found and characterized. Raman spectroscopy allowed to identify the thermodynamic stable form A as the one which is marketed in tablets.

Keywords: hot-stage Raman microscopy, lufenuron CAS RN [103055-07-8], paracetamol CAS RN [103-90-2], polymorphism

Introduction

Organic molecules often crystallize in various solid forms. These polymorphs are identical chemical entities with different crystal structures. Polymorphs have different physical properties – i.e. solubility, dissolution rate, stability or bioavailability. Therefore, it is important to identify and control the polymorphic form and polymorphic purity at all stages of a drug product: starting in research, in development and in production by in process and quality control.

Different strategies for a systematic study of polymorphism can be applied. They usually involve a combination of different techniques. DSC, DTA, thermomicros-

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copy, X-ray diffraction, solution calorimetry, solid state NMR, IR and Raman spectroscopy are among other techniques applied in the characterization and quantification of polymorphs [1, 2].

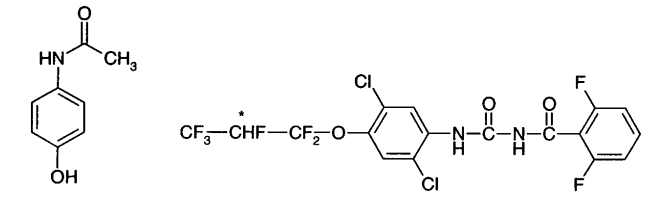
Due to major instrumental advances [3, 4] Raman spectroscopy has been introduced in the past few years for applications in process and quality control [5, 6] as well as for the identification of very small masses of materials in the range of micrograms [7, 8]. The ability of Raman spectroscopy to discriminate between polymorphs has been demonstrated by several authors [9–16]. Different crystal structures usually show intensity and frequency changes in the Raman spectrum, which can be evaluated qualitatively or quantitatively by linear regression of chosen peaks or factor analysis techniques [17]. Unlike X-ray diffraction, Raman techniques can be applied to pure solids or dosage forms without sample preparation.

Thermomicroscopy enables direct observation of a sample during heating and cooling or under isothermal conditions. Such investigations provide reliable first-hand evidence about the processes occurring. DSC or thermomicroscopy are often the first step in polymorphism studies [18]. Auer *et al*. [19] showed that crystal films can be measured by Raman microscopy. We went one step further and directly coupled the Raman microscope with a hot stage. This combination allows a direct correlation between the visual observations from the thermomicroscope and the Raman spectra.

Thus, vibrational spectra can be obtained at a very early stage of the investigation of polymorphs when only small amounts of material are available. It is also possible to measure amorphous and metastable forms, which are not produced at room temperature and which may be already recrystallized or transformed during the cooling cycle. In such a case any off-line characterization is impossible.

The spectral information obtained by hot-stage Raman microscopy can be used to identify polymorphic forms throughout the development of a drug substance. The versatility of Raman spectroscopy allows the identification of polymorphs during production processes (crystallization, drying, milling, granulation) or within dosage forms (tablets, capsules, suspensions), even in the presence of excipients.

The application of hot-stage Raman microscopy and the identification of polymorphic forms in tablets is first illustrated by the example of paracetamol (4-acetamino-phenol, Scheme 1), a well-known analgesic and antipyretic drug, for which three polymorphic forms are currently known [20]. As a second example the polymorphic behaviour of lufenuron, a benzoylphenyl-urea derivative (Scheme 1), is investigated by the use of hot-stage Raman microscopy. Lufenuron is classified as a chitin synthesis inhibitor and indicated for use in pets for the prevention and control of flea populations. It is available under the tradename Program® as tablets for dogs



Paracetamol Lufenuron

Scheme 1 Molecular structure of paracetamol and lufenuron

and suspension for cats for monthly oral administration. For cats a six-month injectable suspension is also marketed. Novartis Crop Protection is marketing lufenuron as emulsifiable concentrate under the tradenames Match®, Sobra® and Lufox® (in combination with fenoxycarb), for the control of Lepidoptera, Western Flower thrips and rust mites in maize, cotton, citrus, soy, vegetables, ornamental plants, grapes, pome and potatoes.

Experimental

Paracetamol

Paracetamol (4-acetamino-phenol) was used as purchased (Fluka, No. 00370, 98% purity).

Synthesis of lufenuron racemate

Crude lufenuron is synthesized in two standard reaction steps. 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-aniline is phosgenated (step 1). After concentration by distillation, the resulting isocyanate is reacted with 2,6-difluorobenzamide to the crude lufenuron, which crystallizes after cooling (step 2). The crude product is purified (min. 98% purity) by recrystallization from isopropanol and milled to the final product, lufenuron 'very fine', using a jet air type mill [21].

Preparation of the lufenuron enantiomers

Preparative HPLC was performed with a Shimadzu modular liquid chromatograph (Burckard Instrumente, Zürich, Switzerland) composed of an LC-8A pump and a multiwavelength UV-VIS detector model SPD-10A. The UV signal was recorded and processed by an Epson microcomputer, using the Class LC-10 chromatographic software (Shimadzu, Burckard Instrumente, Switzerland).

1.25 g of racemic lufenuron was dissolved in 40 ml of ethanol. The obtained solution was diluted with 150 ml of heptane/isopropanol 97/3 (v/v) and this mixture was injected via the pump on a 5 cm (*i.d.*) by 50 cm Chiralpak AD column (Daicel Chemical Industries, Japan). The chromatography was achieved at room temperature at a flow-rate of 100 ml min^{-1} and UV detection was performed at 210 nm. The mobile phase consisted of a mixture of heptane/isopropanol 97/3 (v/v). Under the above chromatographic conditions, 3 fractions were collected; the fraction isolated between 50 and 67 min contained the (–) enantiomer (*ee*>99.8%), and the fraction collected between 76 and 110 min contained the (+) enantiomer (*ee*>99.8%). The fraction collected between 67 and 85 min contained a mixture of the enantiomers. The separation of a second portion of racemic lufenuron using the same mass as above was performed analogously. The mixed fractions (67–85 min) of both chromatographic experiments were added together and evaporated. The residue was reinjected for separation of the enantiomers. After the successive injections and evaporation of the collected fractions, a total of 1.73 g of crude (–) enantiomer and 1.68 g of crude (+) enantiomer were isolated. The optical purity of the isolated enantiomers was

determined by analytical chromatography on a Chiralpak AD column (0.46×25 cm); mobile phase, hexane/isopropanol 96/4 (v/v); 1 ml min^{-1}; separation factor, 1.31.

Application test of the single enantiomers

The relative efficacy of the single enantiomers against the cat flea (*Ctenocephalides felis*) was compared in an artificial membrane feeding system [22]. There was no statistically significant difference in activity between the enantiomers although, at all concentrations, one enantiomer produced slightly higher mortalities. The results indicate that the activity is independent of the spatial configuration of the lufenuron molecules.

Materials

Acetone, dichloromethane, toluene, cyclohexane and isopropanol (all of spectroscopic grade) were purchased from Merck. Ethanol 96% (spectroscopic grade), and N-methyl-2-pyrrolidinone (purum) were purchased from Fluka. Water was distilled.

Hot-stage Raman microscopy

For the hot-stage Raman microscopy a Kaiser Holoprobe 5000 Raman spectrometer coupled via mono-mode optical fiber to an Olympus BX 60 microscope was used. A stabilized diode laser (wavelength 785 nm) served as the excitation source. The measurements were carried out with a 50× long-working-distance objective (*NA* value of 0.5). The spatial resolution was estimated to be 4 μm. The Raman shifts between 50 and 3500 cm^{-1} were recorded with an acquisition time of 1 min/spectrum. A Mettler FP82 hot stage was mounted on the microscope stage for temperature control. The crystal films of paracetamol were prepared on standard microscope slides, for lufenuron cavity slides were used.

FT-Raman spectroscopy

Measurements were carried out on a Bruker RFS 100 FT-Raman system with a near infrared Nd:YAG laser operating at 1064 nm and a liquid nitrogen-cooled germanium detector. For the paracetamol tablets, 64 scans were accumulated with a resolution of 2 cm^{-1}. No sample preparation was required. The lufenuron tablets (Program®) were broken in half and the cut was measured with 250 scans and a resolution of 4 cm^{-1}. The five lufenuron modifications A–E were measured as powders in an aluminum holder with 64 scans and a resolution of 2 cm^{-1}.

X-ray powder diffraction

All the measurements were made with Cu radiation. Crystal modifications were characterized by the Guinier technique (Guinier FR 552 camera by Enraf-Nonius), data evaluation was performed with line scanner LS-18/Scanpi software. Experiments at elevated temperatures were performed under nitrogen atmosphere on an X'Pert powder diffractometer (Philips) equipped with a TTK chamber (Anton Paar).

DSC

A Perkin Elmer Series 7 thermal analysis system was used for differential scanning calorimetry. DSC scans were obtained using sealed aluminum pans at a heating/cooling rate of 10 K min^{-1} on samples of 2 to 11 mg. In order to measure the melting points of the single enantiomers of lufenuron a heating rate of 80 K min^{-1} was used.

To generate the amorphous form of lufenuron, a sample of 5.5 mg was heated to 190°C and rapidly cooled in liquid nitrogen. The glass transition temperature was obtained by reheating the sample using a heating rate of 20 K min^{-1}.

To examine the thermal stability of lufenuron, samples of 4–5 mg (in sealed aluminum pans) were heated at a rate of 10 K min^{-1} to temperatures between 190 and 220°C. After cooling, the pans were opened and the sample dissolved in ethanol and measured using HPLC.

TG-FTIR

Experiments were performed using the commercially available Netzsch thermomicrobalance TG 209, coupled to a Bruker FTIR spectrometer IFS 28. The dynamic measurements were carried out in a N_2 atmosphere using a heating rate of 10 K min^{-1} with sample weights of 4 to 6 mg. The FTIR spectra were recorded in the wavenumber range 4500 to 600 cm^{-1} and 20 spectra coadded every 7 s.

Density

The density was determined using a Micromeritics AccuPyc 1330 pycnometer (cell volume 1 cm^3) at 25°C.

Optical rotation

The samples were dissolved in acetone (50 to 52 mg in 2.0 ml) and the optical rotation was measured using a Perkin Elmer polarimeter (type 341) at 20°C.

HPLC

Lufenuron purity and degradation products were assessed on a reversed phase spectra-physics liquid chromatograph. The stationary phase consisted of a Merck Lichrochart 125-4 (Lichrospher 100 RP-18 (5 μm)) and the mobile phase of a mixture of 70% acetonitrile and 30% bidistilled water. With a flow of 1 ml min^{-1}, 20 μl injection volume and UV detection at 220 nm lufenuron elutes at around 5.2 min. Drug samples were prepared by dissolving the material in UV-grade ethanol at a concentration of 0.32–0.37 mg ml^{-1}.

Solubility

Solubilities were measured in isopropanol. 20–30 mg crystalline drug substance were placed into a screwcap vial and 0.5 ml solvent was added. The vials were

placed in a thermostated (23±1°C) bath and agitated by micro magnetic stirrers. After 0.5, 1 and 3 h the solid was separated from the solution by centrifugation through a Millipore 0.22 μm Durapore centrifuge filter. The recovered solids were then examined by FT-Raman spectroscopy. The concentration of dissolved substance was determined by UV-VIS spectroscopy in 1 mm quartz cuvettes after 50 fold dilution of the sample (lufenuron: ε_{255nm}=17260 M^{-1} cm^{-1}).

In slurries, the form C transforms to form A efficiently, therefore, the solubility of the metastable form was estimated by different means. Firstly, after mixing in isopropanol, aliquots were taken every minute from the sample and the concentration in the supernatant was determined. Secondly, the lufenuron depletion in the solution was followed by time-resolved UV-VIS spectroscopy in a methylenechloride/cyclohexane (1/10) mixture. Thirdly, the crystalline almost dissolves completely if 0.5 ml isopropanol is mixed with 20 mg of form C. All experiments indicate that form C is more soluble than form A by a factor between 1.5–2.5.

Recrystallization experiments

1.0 g lufenuron in the crystal modification A was slurried in 2 to 12 ml of solvent (acetone, ethanol 96%, dichloromethane, toluene, N-methyl-2-pyrrolidinone) and heated until a clear solution was achieved (max. 89°C). The solution was filtered into a suction bottle (glass filter, pore size 10–20 μm) at about 20°C. After recrystallization the crystals formed were dried at room temperature and immediately examined by FT-Raman spectroscopy.

Precipitation experiments

A method similar as the one for the recrystallization experiments was followed, but on filtering 500 ml of water or cyclohexane (3 to 5°C) were used in order to obtain precipitates which were then refiltered and washed.

Experiments on suspensions

In order to explore the behaviour of mixtures of the racemate and single enantiomers, we used the following procedure: in a first experiment, a mixture of 50 mg A(±), 25 mg E(–) and 25 mg E(+) in 1.0 ml isopropanol was stirred at 24°C for one day. The solvent was then evaporated at room temperature and the dried sample immediately examined by FT-Raman spectroscopy and X-ray. In a second experiment, a mixture of 51 mg A(±) and 51 mg E(–) was treated the same way.

Results and discussion

Paracetamol

Thermodynamic data

The results of the DSC measurements are shown in Fig. 1. The first heating cycle exhibits an endothermic peak at 169°C, due to the melting of the monoclinic form I.

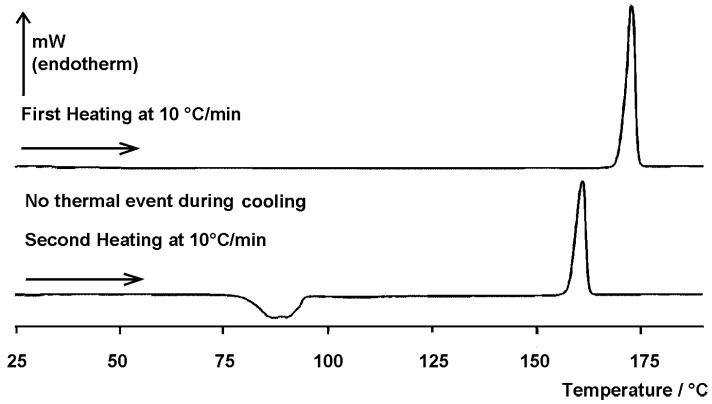


Fig. 1 DSC curves of paracetamol (heating/cooling rate: 10 K min^{-1})

Table 1 Thermodynamic data of the polymorphic forms of paracetamol

		Form I	Form II	Form III
T_{fus}/°C	[23]	168	155	$<T_{fus}$ II
	Present paper	169	157	
$\Delta_{fus}H$/kJ mol^{-1}	[23]	28.1	26.9	–
	Present paper	28.0	26.5	
Density/g cm^{-3}	[23]	1.293	1.336	–

Polymorphic transition		Transition temperature T_{trs} /°C	Transition enthalpy $\Delta_{trs}H$/kJ mol^{-1}
II→I	[23]	87 (DSC)[a]	+0.4
III→II	[23]		–1.2

[a] estimated thermodynamic transition point: <10°C [23]

The second heating cycle shows an exothermic process with an onset of 78°C corresponding to a recrystallization process. This is followed by an endothermic peak at 157°C, the temperature for the melting of the orthorhombic form II. The thermodynamic and kinetic data from these DSC measurements are summarized in Table 1. The polymorphic behaviour of paracetamol has been described by several authors [20, 23, 24]. Thermodynamic data are not available for the form III because this crystal modification can only be stabilized under certain conditions (i.e. between cover glass and slide [20]).

Hot-stage Raman microscopy

Figures 2–4 show the micrographs as well as the Raman spectra of the three modifications of paracetamol, which were obtained by hot-stage Raman microscopy by applying the following temperature program:

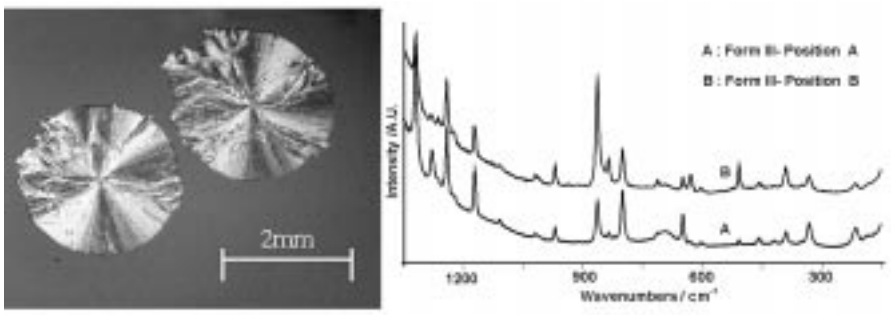


Fig. 2 Paracetamol: optical appearance and Raman spectra of the polymorphic form III. The spectra A and B were measured at different positions on the spherolites. The intensity differences between some bands of the two spectra are due to polarization effects

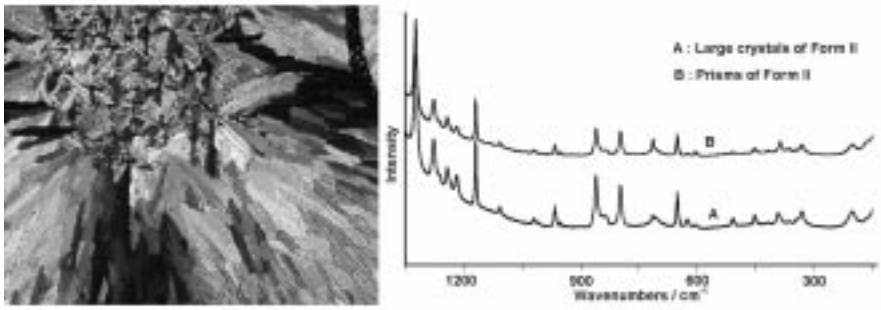


Fig. 3 Paracetamol: optical appearance and Raman spectra of the polymorphic form II. The prisms and the large crystals have similar Raman spectra

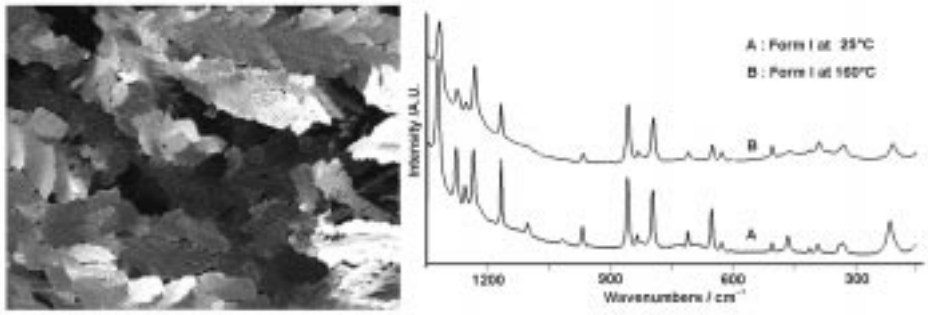


Fig. 4 Paracetamol: optical appearance and Raman spectra of the polymorphic form I. The spectra A and B were recorded at different temperatures and as a result some band frequencies have shifted

Paracetamol was melted at 170°C and cooled to 54°C. Spherolites appeared after 3 h isothermal hold and were analyzed as form III (Fig. 2). When heated at 4 K min^{-1}, the spherolites changed between 77 and 120°C into small prisms at the centers of the spherolites and large crystals at their peripheries. Both kinds of crystals have practi-

cally identical Raman spectra and melt between 157–159°C as form II (Fig. 3). At 160°C large crystal plates began to grow (heating rate 4 K min^{-1}), which melt at 170°C as form I (Fig. 4). The crystals of the modification I could also be cooled down to room temperature without a change of the modification.

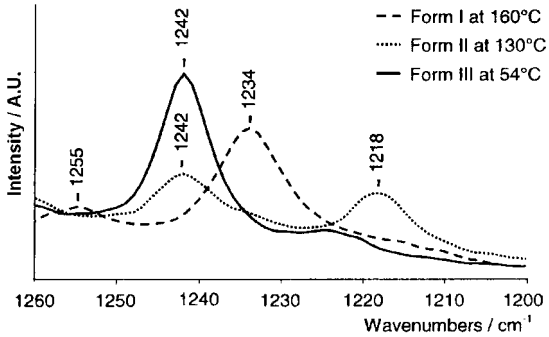


Fig. 5 Spectral region between 1200 and 1260 cm^{-1} showing the differences between the three modifications of paracetamol

Figure 5 reveals that the three modifications can be well discriminated by Raman spectroscopy in the spectral region between 1200 and 1260 cm^{-1}. In Table 2 characteristic Raman frequencies for discriminating the modifications are listed. These frequencies allow the assignment of the Raman spectra to a polymorphic form.

Table 2 Characteristic peak frequencies for differentiating the modifications of paracetamol

Raman shift/cm^{-1}	Form III/cm^{-1}	Form II/cm^{-1}	Form I/cm^{-1}
1200–1260	1241–1244	1242 & 1218–1219	1254–1258 & 1233–1238
855–865	863–865	860	857–859
790–810	799–801	797–798	795–798
450–470	454–458	451–452	460–466
200–220	212–216	200–201	207–215

A quantitative evaluation of the spectra should also be possible. In this case, the relative intensities of the bands have to be independent of the orientation of the crystals. To achieve this, it is favorable when the laser light which falls onto the sample does not exhibit a linear polarization (this can be realized by inserting a quarter wave plate into the light path). The effect of polarization can be well seen in Fig. 2, where the spectra were obtained at different positions of the spherolites. Because the orientation of the crystal axes within a crystal film changes at different positions, some band intensities are influenced. Polarization effects tend to be negligible when the measurement averages over many crystals [11, 25].

Raman spectra often show frequency shifts as a function of the sample temperature. Form I, which was measured at 160°C and at room temperature, showed consid-

erable temperature effects for some vibrations (Fig. 4), i.e. the frequency difference $\Delta\nu=\nu^{160^{\circ}C}-\nu^{25^{\circ}C}=-3$ cm^{-1} for the peak $\nu^{25^{\circ}C}$ =1234 cm^{-1}. Therefore certain frequency intervals given in Table 2 are rather large. Yet, it is still possible to identify the two spectra in Fig. 4 as belonging to the same modification, since a continuous frequency shift was observed when the sample was cooled down to room temperature.

Application to tablets

We measured paracetamol tablets sold by two different manufacturers. As shown in Fig. 6, the spectra are identical, even though different excipients (less than 20% by mass) were present. For the identification of the polymorphic form, the characteristic frequency regions of the tablets (Table 3) were compared with the characteristic frequencies obtained by hot-stage Raman microscopy. This comparison clearly showed that tablets A and B contain paracetamol in crystal form I.

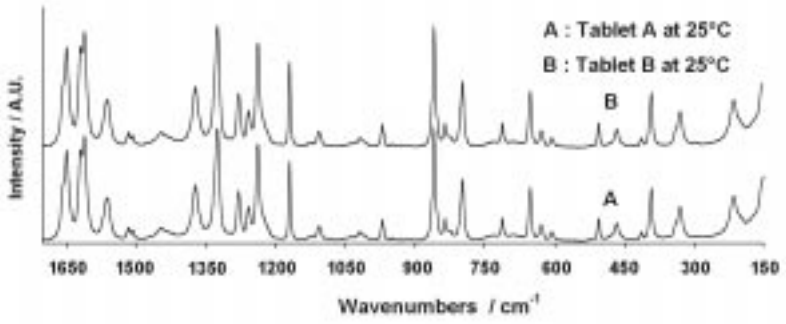


Fig. 6 FT-Raman spectra of paracetamol tablets from two different manufacturers

Table 3 The characteristic frequencies of the two tablets of paracetamol

Raman shift/cm^{-1}	Tablet A/cm^{-1}	Tablet B/cm^{-1}
1200–1260	1258 & 1237.5	1258 & 1237.5
855–865	859	859
790–810	798	798
450–470	466	466
200–220	215	215

Lufenuron

Lufenuron is a chiral compound (Scheme 1), which is marketed as a racemate. A first indication of its complex polymorphic behaviour was obtained by performing a DSC measurement of the active substance in its commercial form (Fig. 7). The first heating cycle shows an endothermic peak at 175°C. All subsequent heating cycles lead to a weak broad exothermic signal at about 100°C and a broad endothermic peak at about 150°C. It can be excluded that decomposition of lufenuron is the rea-

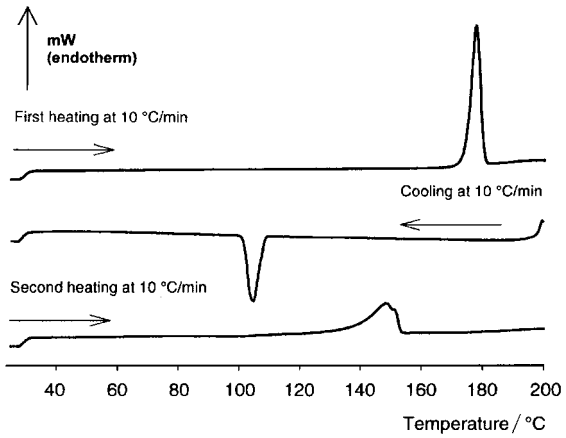


Fig. 7 DSC curves of lufenuron (heating/cooling rate 10 K min^{-1})

son for the lower melting peak during the second heating cycle, since LC measurements of thermally treated samples indicate that decomposition takes place only in the order of a few percents, even when the samples are heated up to 220°C.

The polymorphism of a racemate needs consideration of the specific homochiral and heterochiral interactions between the enantiomers [26–29]. Originating from differences in the packing forces in the crystal lattice, a racemic species can exist as

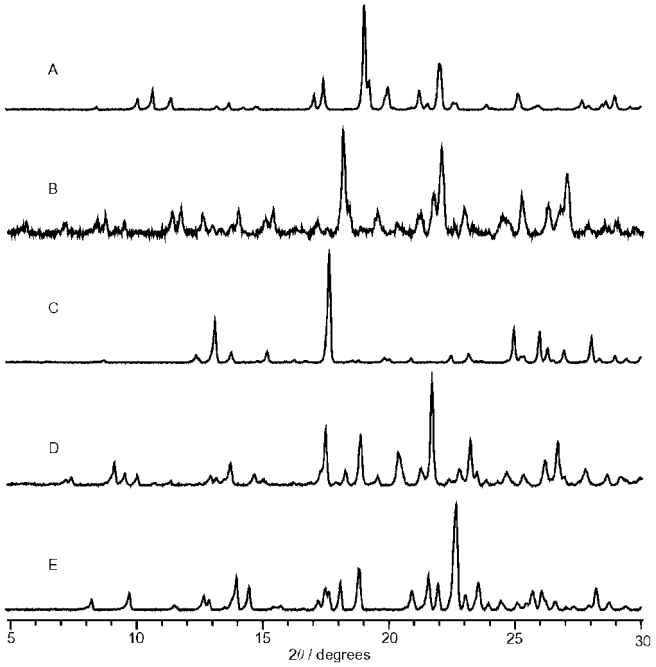


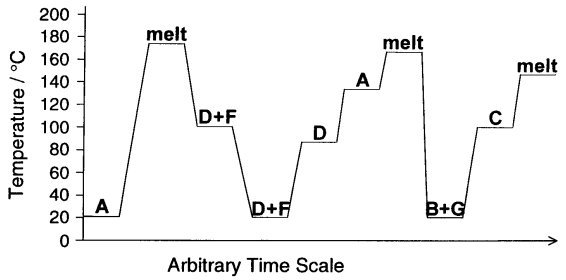
Fig. 8 X-ray diffraction patterns of the modifications A to E of lufenuron

a racemic compound, a racemic conglomerate or a pseudoracemate [29]. In a racemic mixture or conglomerate, each enantiomer has a greater affinity for molecules of its own kind than for those of the other enantiomer and the two enantiomers crystallize in separate phases. A well known example is the behaviour of sodium ammonium tartrate (observed by Pasteur in 1848). In a racemic compound, each enantiomer has a greater affinity for molecules of the opposite type than for its own kind and in this case a molecular complex is formed in the solid state as a so-called addition compound. In cases where there is little difference in the affinity between enantiomers of the same or the opposite configuration, the two enantiomers exist in a statistical distribution in the crystal. The solid phase of such a racemic modification shows nearly ideal mixing and forms a racemic solid solution or a so-called pseudoracemate.

In order to obtain a better insight into these interactions, the single enantiomers of lufenuron were prepared. Thermal analysis and recrystallization experiments were carried out on the racemate as well as on the single enantiomers. The new modifications found by these investigations were characterized by X-ray powder diffraction (Fig. 8 and Table 4) and by Raman spectroscopy (Figs 9 and 10). Some physical properties are collected in Table 5.

Thermal analysis and hot-stage Raman microscopy of the racemate

The DSC curve of the racemate shown in Fig. 7 suggests that the recrystallization of the melt ($T_{recryst}$=105°C) leads to a new form. Therefore, X-ray powder diffraction and Raman measurements of the original substance and of the recrystallized material were performed. Based on these measurements, three forms could be identified: modification A which is on market and two new modifications, forms B and D, which both recrystallize from the melt. Parallel to the DSC measurements, hot-stage Raman microscopy was performed. Scheme 2 shows the temperature program applied to the racemate. The *x*-axis can be considered as an arbitrary time axis representing the progress of the experiment. When not explicitly mentioned, the heating and cooling rates were 10 K min^{-1}. The same region of the sample was observed during a whole cooling/heating cycle in order to assign an observed melting or recrystallization process to a change in the Raman spectrum. Spectra were taken whenever



Scheme 2 Hot-stage Raman microscopy: temperature program applied to the racemate. The heating rate was 10 K min^{-1}. During the Raman measurements, the temperature was kept constant

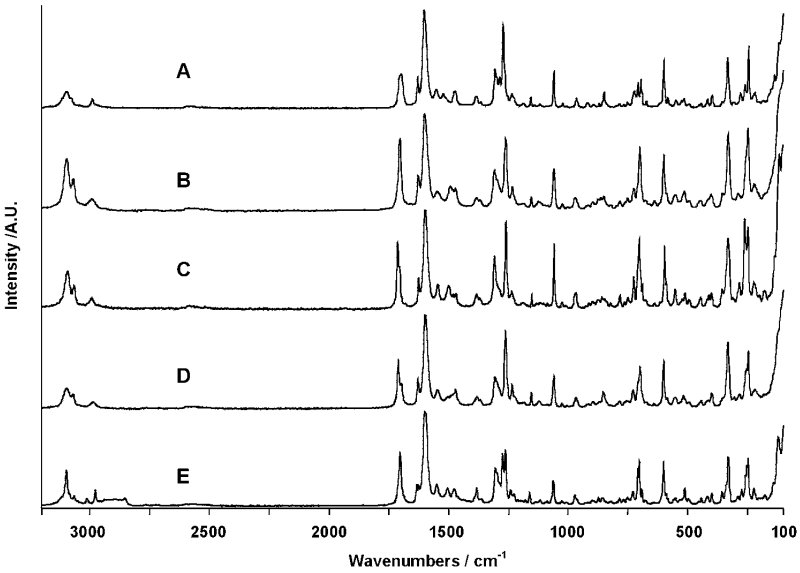


Fig. 9 FT-Raman spectra of the modifications A to E of lufenuron

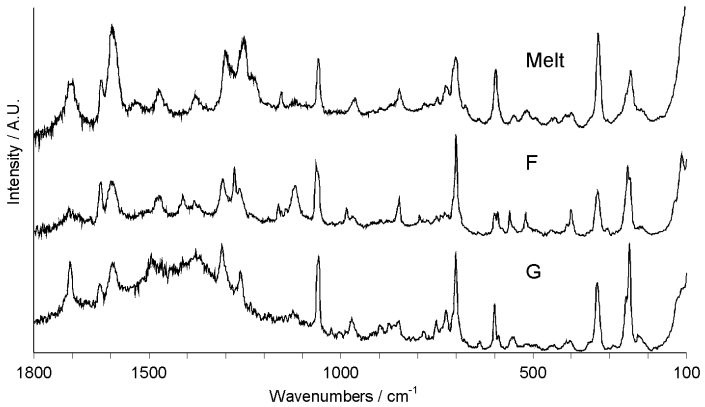


Fig. 10 Raman spectra of the melt and forms F and G of lufenuron

a change in the optical image was observed. The polymorphic forms as identified by Raman microscopy are indicated in Scheme 2.

The experiment started from crystals of modification A, which were heated until they melted (at 175°C) and a Raman spectrum of the melt was taken (Fig. 10). Subsequently, the melt was cooled down until recrystallization was observed. The temperature was then kept constant at 100°C and within a small region (ca. 100 μm×100 μm) Raman spectra were measured at several positions. At some positions the spectra corresponded to the modification D. Other positions showed a new Raman spectrum which we tentatively assigned to a new modification F. At the ma-

Table 4 X-ray powder diffraction patterns of lufenuron forms A to E (vs: very strong, s: strong, m: medium, w: weak, vw: very weak); *d*-value error limits: ±0.2 Å for the *d*-value region 16 to 6 Å; ±0.05 Å for the *d*-value region 5.99 to 2.96 Å

Form A			Form B			Form C			Form D			Form E		
2Θ/ degree	*d*/ Å	Intensity	2Θ/ degree	*d*/ Å	Intensity	2Θ/ degree	*d*/ Å	Intensity	2Θ/ degree	*d*/ Å	Intensity	2Θ/ degree	*d*/ Å	Intensity
8.5	10.4	vw	5.7	15.5	w	8.8	10.1	vw	7.3	12.1	vw	8.3	10.6	w
10.1	8.7	m	7.3	12.2	w	12.4	7.1	m	7.5	11.7	w	9.8	9.0	m
10.7	8.2	m	8.5	10.4	w	13.2	6.7	s	9.2	9.6	m	11.6	7.6	vw
11.5	7.7	m	8.8	10.0	w	13.8	6.4	m	9.6	9.2	w	12.8	6.9	m
13.3	6.7	vw	9.6	9.2	w	14.9	5.95	vw	10.1	8.7	w	13.0	6.8	w
13.8	6.4	w	11.4	7.7	m	15.3	5.80	m	10.8	8.2	vw	14.1	6.3	s
14.4	6.2	vw	11.8	7.5	m	16.3	5.42	vw	11.4	7.7	vw	14.6	6.1	m
14.8	5.97	vw	12.6	7.0	m	16.8	5.28	vw	13.0	6.8	w	15.5	5.70	vw
17.1	5.17	m	13.0	6.8	vw	17.7	5.00	vs	13.3	6.7	w	15.8	5.59	vw
17.5	5.06	s	13.4	6.6	vw	18.6	4.76	vw	13.8	6.4	m	17.3	5.12	w
19.1	4.64	vs	13.8	6.4	vw	18.9	4.70	vw	14.7	6.0	w	17.6	5.04	m
19.3	4.59	m	14.1	6.3	m	19.9	4.45	w	15.1	5.85	vw	17.7	5.00	m
20.1	4.42	s	15.1	5.85	w	20.1	4.41	vw	17.4	5.10	w	18.2	4.87	m
21.3	4.17	m	15.4	5.74	m	21.0	4.24	w	17.6	5.05	s	18.9	4.68	s
21.6	4.11	w	17.2	5.15	w	22.5	3.94	m	18.3	4.83	w	21.0	4.22	m
22.1	4.02	vs	17.6	5.04	vw	23.2	3.82	m	18.9	4.68	s	21.7	4.09	s
22.6	3.92	w	18.2	4.87	vs	25.0	3.55	s	19.6	4.52	w	22.1	4.02	m

Table 4 Continued

Form A			Form B			Form C			Form D			Form E		
2Θ/ degree	*d*/ Å	Intensity	2Θ/ degree	*d*/ Å	Intensity	2Θ/ degree	*d*/ Å	Intensity	2Θ/ degree	*d*/ Å	Intensity	2Θ/ degree	*d*/ Å	Intensity
22.8	3.90	w	18.4	4.81	w	25.3	3.52	w	20.4	4.34	s	22.8	3.90	vs
24.0	3.71	w	19.6	4.54	m	25.4	3.50	w	21.3	4.16	m	23.2	3.84	w
25.2	3.53	m	20.3	4.37	w	26.1	3.42	s	21.8	4.08	vs	23.7	3.76	s
26.0	3.42	w	21.3	4.18	m	26.4	3.38	m	22.4	3.96	vw	24.1	3.69	w
27.7	3.21	w	21.8	4.08	s	26.6	3.35	vw	22.8	3.89	m	24.6	3.62	w
28.0	3.18	vw	22.1	4.02	vs	27.0	3.30	m	23.3	3.82	s	25.2	3.53	w
28.5	3.13	vw	22.6	3.93	w	28.1	3.17	s	23.5	3.78	w	25.6	3.48	vw
28.7	3.11	w	23.0	3.87	m	28.4	3.14	w	23.9	3.72	vw	25.8	3.45	m
29.0	3.07	m	24.5	3.63	m	29.1	3.07	w	24.7	3.60	m	26.2	3.40	m
29.6	3.01	vw	25.2	3.53	s	29.5	3.03	w	25.4	3.51	m	26.7	3.33	w
30.1	2.96	vw	26.3	3.39	m	30.1	2.97	w	26.2	3.40	m	27.2	3.28	vw
			26.8	3.33	m				26.7	3.33	s	27.5	3.25	vw
			27.0	3.30	s				27.0	3.31	vw	28.1	3.18	vw
			27.9	3.20	w				27.8	3.21	m	28.3	3.15	m
			28.5	3.13	w				28.7	3.11	w	28.9	3.09	w
			29.0	3.07	w				29.2	3.06	w	29.5	3.02	vw
									29.4	3.03	vw	30.2	2.96	w
									30.0	2.98	w			

Table 5 Physical properties of the polymorphic forms of lufenuron

	Form A(±)	Form B(±)	Form C(±)	Form D(±)	Form E(–)	Form E(+)
T_{fus} [a)]/°C	175	–	147[c)e)]	–	166[b)]	165[b)]
$\Delta_{fus}H$ [a)]/kJ mol^{-1}	33.3	–	22.3[c)e)]	–	–	–
Transformation into[a)]	–	C	A[d)]	A	A (–)	A (+)
T_{trs} [a)]/°C	–	66–92	92–121[d)]	94–112 112–124	133–153	127–152
$\Delta_{trs}H$ [a)]/kJ mol^{-1}	–	–3.3	–3.0[d)]	–1.7 +0.4	+4.1	+3.8
Mass loss[g)] 25 to 180°C/%	0.1	–	0.1	–	–	–
Solubility at 23°C/mg ml^{-1}	20	–	30–50[f)]	–	10	10
Density at 25°C/g cm^{-3}	1.68	–	1.69	–	1.68	1.65
$[\alpha]_{589\ nm}$ at 20°C/°	0.0	–	–0.1	–	–0.8	+0.7
$[\alpha]_{365\ nm}$ at 20°C/°	0.0	–	–0.3	–	–2.9	+2.5

a) DSC, heating rate 10 K min^{-1}; b) DSC, heating rate 80 K min^{-1}; c) Form C produced by melting of lufenuron Form A; d) Form C produced by precipitation of a solution of lufenuron; e) broad melting range; f) estimated value; g) TG-FTIR

jority of the positions (spatial resolution: ~4 μm) a mixture of both modifications was found. Cooling to room temperature did not alter the polymorphic form. When the sample was heated up again, the melting of some crystals was observed at 85°C. The temperature was therefore kept constant and the same positions which were measured at room temperature were measured again. It could be observed that the spectrum of modification F was no longer present. Further heating led to a partial melting and recrystallization process at 135°C. The Raman spectra indicated that modification A was formed, which melted upon further heating at 168°C. The melt was rapidly cooled down to room temperature. The first recrystallization was observed at ca. 100°C. At room temperature, Raman spectra were measured at several positions. Two different Raman spectra were found, one corresponding to modification B, the other showed differences in several spectral regions. Therefore, we tentatively assigned it to a new modification G. The sample was once more heated up until at about 100°C a transformation was observed. The Raman spectra indicated that a new form C was generated which melted upon further heating at 143°C.

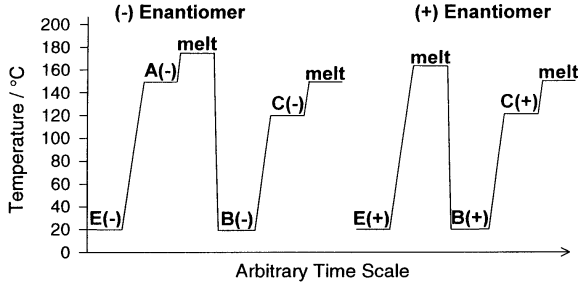
Recrystallization experiments of the racemate

Recrystallizations from a series of solvents (acetone, ethanol, dichloromethane, toluene, N-methyl-2-pyrrolidinone) were carried out, all leading to modification A. Form C can be obtained by two different precipitation experiments: precipitation of a solution of lufenuron in ethanol with water (5°C) and precipitation of a solution of

lufenuron in dichloromethane with cyclohexane (5°C) both lead to modification C. Thus, it was possible to produce form C in larger quantities (several grams). Precipitation of a solution of lufenuron in N-methyl-2-pyrrolidinone with water (5°C) led to modification A.

Investigation of the single enantiomers

Hot-stage Raman microscopy of the single enantiomers was performed in an analogous way as for the racemate. The procedure is illustrated in Scheme 3. First, the Raman spectra of the single enantiomers as obtained by preparative HPLC were measured. They indicated that the single enantiomers are present in a form, which has not yet been observed for the racemate. We will address this new modification as form E. Interestingly, the outcome of the first heating cycle was different for the two enantiomers: form E of the (+) enantiomer melted directly at 165°C. Form E of the (–) enantiomer showed a transformation into a new form at 150°C, which melted at 175°C. The Raman spectrum of this high temperature stable form was very similar to the one of form A of the racemate. We therefore address this modification as form A of the single enantiomers. When the melt was cooled down to room temperature, recrystallization was observed at about 100°C. The Raman spectrum, which was taken at room temperature, was very similar to the one of form B of the racemate. The sample was heated up again. At about 120°C a recrystallization was observed. The Raman spectrum corresponded again to a form, which was already observed for the racemate, namely form C. In order to discriminate the forms, which were observed for the single enantiomers from the ones of the racemate, we will specify them as A(+)/(–), B(+)/(–) and C(+)/(–). To emphasize that a form of the racemate is meant, we will add the sign (±), e.g. A(±).



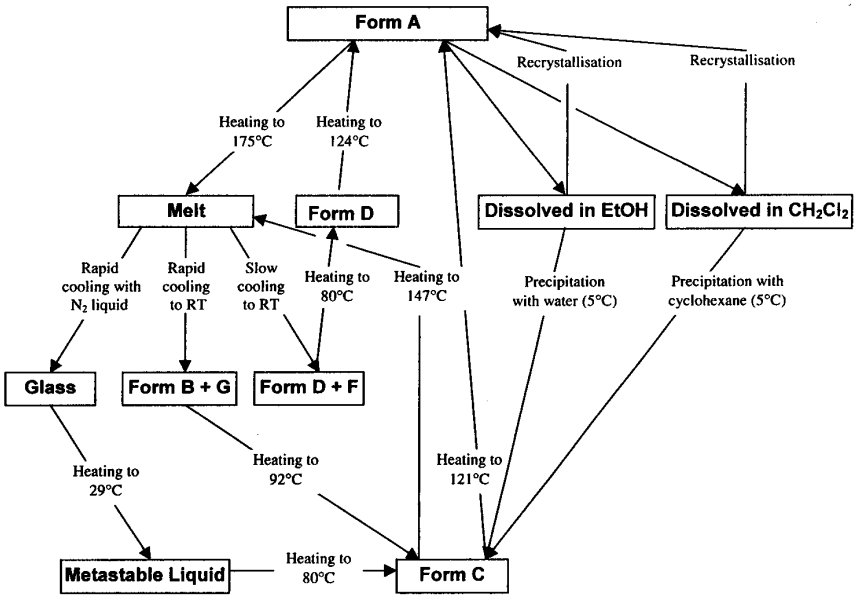
Scheme 3 Hot-stage Raman microscopy: temperature program applied to the single enantiomers. The heating rate was 10 K min^{-1}. During the Raman measurements, the temperature was kept constant

The direct melting of form E(+) at 165°C as well as the transformation of form E(–) into form A(–) could also be confirmed by other methods. The DSC curvess of the (+) and the (–) enantiomers, which were recorded at heating rates of 10 K min^{-1}, showed an endothermic signal between 130 and 150°C and a melting peak at 174°C. When the heating rate was increased to 80 K min^{-1}, the endothermic signal between 130 and 150°C increased and shifted to higher temperature (165°C),

the second peak remained at 174°C and decreased in intensity. The increase of the endothermic peak at 165°C is stronger for the (+) enantiomer than for the (–) enantiomer, most probably due to different amounts of impurities present in the (+) and (–) enantiomers. X-ray powder diffraction of the single enantiomers at elevated temperature (160°C) confirmed that modification E transforms into a form, which corresponds very closely to the one of A(±). This correspondence is not due to the rather unlikely racemization of the single enantiomers, which could have occurred at elevated temperatures: modification A(–) which is formed via transformation of E(–) shows the same optical rotation as the starting material.

Discussion of the observed polymorphic forms

An overview of the most important modifications of the racemate and their occurrence is given in the transformation Scheme 4. Small changes of the experimental conditions strongly influence the crystallization of the different polymorphs: depending on the cooling rate, a glass, form B or form D is obtained from the melt. Since the cooling rate depends on the amount of material present, recrystallizations of larger quantities rather lead to form D, smaller quantities to form B. Form C, which can be obtained by precipitation from the solution or by heating of form B, melts in some cases directly at 147°C or transforms into form A upon heating. Interestingly, the transformation into form A was only observed when form C was produced by precipitation. One reason for this behaviour could be that the transformation into form A is induced by small amounts of solvent or crystal seeds of form A, which may still be present in the precipitated material. The melting temperature of



Scheme 4 Transformation scheme for lufenuron

form A decreased by 6 K upon the second heating on the hot-stage Raman microscope. One reason for this melting point reduction could be partial decomposition, since the sample was held for some time at elevated temperatures. The Raman spectra themselves showed no sign of a starting decomposition, it is however known from LC measurements that decomposition of the melt in the order of a few percents can occur.

Form A is the thermodynamically stable modification. Considerable stability was also found for the metastable form C. When this form is kept at room temperature, no transformation takes place over months. In slurries, form C transforms into form A within a short period of time. It exhibits a similar density and a larger solubility than form A. The DSC measurement showed that an exothermic transformation into form A occurs between 90 and 120°C. The modifications B, D, F, and G were not found to be stable enough to be readily prepared in larger quantities and were therefore not investigated further. The racemate could not be crystallized into form E. Even a suspension of 50% A(±), 25% E(–) and 25% E(+) in isopropanol (24°C) leads to 100% modification A(±) after 16 h.

The situation is more complex for the single enantiomers. Here, the most important modifications are form E, which was only found for the single enantiomers, and modification A(+)/(–). The DSC curves shows an endothermic transformation of form E into form A(+)/(–) between 130 and 150°C, indicating that form A(+)/(–) is the high temperature stable modification of the single enantiomers. Form E exhibits a similar density compared with form A of the racemate. The solubility with respect to the overall concentration of lufenuron is lower by a factor of two for form E compared to form A(±). When we relate the solubility of form E either to the (+) or the (–) enantiomer of the racemate, however, similar values for both forms can be found. Even for the highest achievable heating rate (160°C min^{-1}), the melting peaks of the forms E(+)/(–) and A(+)/(–) are both present in the DSC curves. Therefore, final conclusions regarding the relationship of the two forms – monotropic or enantiotropic – are not possible. Since the transformation of form E into form A(+)/(–) seems to be entropy driven, it is probable that at room temperature modification E is the thermodynamically stable form of the single enantiomers.

When a mixture of 75% (–) enantiomer and 25% (+) enantiomer is recrystallized, the crystallizate consists mainly of form A, a smaller quantity of form E, as well as little portions of further modifications. The DSC curves of this mixture (heating rate: 10 K min^{-1}) shows a broad endothermic signal between 100 and 140°C due to the transformation into form A and a melting peak at 174°C. We can therefore conclude that form A is the high-temperature stable modification of lufenuron irrespective of the ratio of (+) to (–) enantiomers present. Considering the very similar melting points (174–175°C) and X-ray diffraction patterns for all investigated (+) to (–) ratios, form A can be interpreted as a solid solution. This interpretation gains plausibility when we take a closer look at the chiral center of lufenuron as indicated in Scheme 1: since the two enantiomers differ only in the relative position of a hydrogen and a fluorine atom, which both exhibit comparable van der Waals radii, the two enantiomers should be able to replace each other without significant distortion of the

crystal lattice. To decide whether the racemic modification A forms a true or a pseudoracemate at room temperature, a single crystal X-ray study should be performed.

Application to tablets

Figure 11 shows the FT-Raman spectrum of a lufenuron tablet (II) and the corresponding placebo spectrum (I). It can be seen that in the tablet the peaks due to the active substance and to the excipients are of similar intensities. The comparison with the spectra of the known modifications indicates that in the tablet lufenuron is present in the stable form A. The assignment can be made directly or after spectral subtraction of the placebo spectrum.

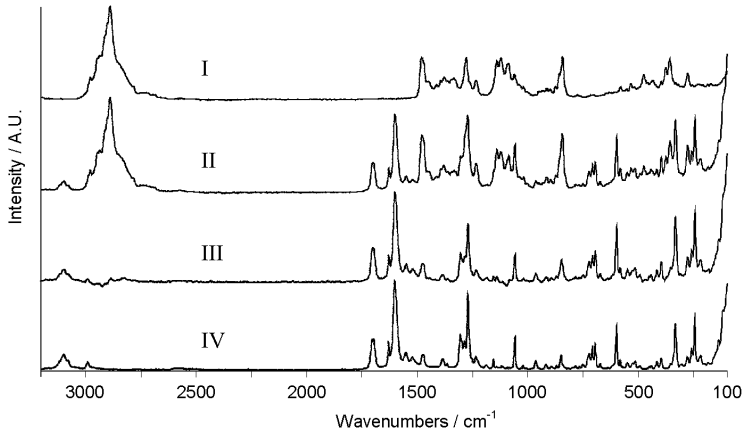


Fig. 11 FT-Raman spectra of a lufenuron tablet (II) and a placebo consisting of the excipients (I). Spectrum III was obtained by spectral subtraction of the placebo spectrum. Spectrum IV shows modification A of lufenuron

Conclusions

Hot-stage Raman microscopy proved to be a valuable tool for the investigation of the polymorphism of paracetamol and lufenuron. Whereas for paracetamol the temperature program to produce the different forms was already known from the literature, a very general temperature program had to be applied to lufenuron. In both cases the observation of all currently known solid state forms was possible. For paracetamol the identification of the different forms has been not straightforward due to temperature and polarization effects. However, the extent of these effects are dependent on any of the active substances themselves. Lufenuron spectra showed little dependences on these phenomena. For lufenuron a series of new crystal modifications were found. Raman spectroscopy allowed to identify the thermodynamic stable form A as the one which is available on the market in the form of tablets. To decide whether form A would be a true or a pseudoracemate, single crystal analysis should be performed.

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