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Short communication

Assessment of the solid-state composition of an active salicylanilide compound by FT-Raman spectroscopy

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Abstract

A biologically active salicylanilide compound currently appears in three known solid-state forms: polymorph I (Pol I), polymorph II (Pol II) and the amorphous form (Amorph). The obtained FT-Raman spectra revealed several regions of interest (ROIs) qualitatively distinguishing the different forms, allowing samples with an unknown polymorphic composition to be quantitatively analysed by FT-Raman spectroscopy. The Markov-transformed peak areas of the Raman-bands in the ROIs from the samples were determined and compared with the transformed peak areas obtained for the reference solid-state forms. A constrainted linear regression model estimated the contribution of each reference to the different samples. The applicability of this approach was demonstrated by analysing commercially available batches.

Keywords: FT-Raman; Quantification; Solid-state polymorphs; Salicylanilide active substance

1. Introduction

The solid-state characterisation of biologically active compounds as sole ingredients as well as formulated in solid or semi-solid finished products is an increasingly important aspect in the development of biologically active substances like pharmaceuticals or biocides [1]. Different crystalline forms, *i.e.* polymorphs (different unsolvated crystal forms) and solvates (encompassing hydrates and solvated forms), as well as the amorphous form (non-crystalline solid) are often possible. The active moiety of these different solid-state forms have the same chemical composition, but due to the different internal crystal-structure, they will usually possess differences in their physico-chemical properties such as dissolution rate, density, melting point and even colour [2]. These differences may have an important effect on the absorption of the biologically active compound from its solid formulation (*in vivo* relevance), but also on the processing of the active ingredient into the finished product and on the stability characteristics (quality relevance). As a consequence, in the approval of a new drug or biocide, regulators are currently not only focussing on chemical and microbiological aspects, but also on the solid state characterisation of the active substance as such as for biological, manufacturing and stability reasons. The increase in the number of sparingly water-soluble compounds together with the appearance of generic and counterfeit products has fuelled this interest.

Various analytical methods are currently being used to characterise the solid-state form of these compounds [3]. Single-crystal and powder X-ray diffractometry provides molecular and crystalline structural information [4,5]. Spectral (*i.e.* infra-red [IR], Raman, solid-state nuclear magnetic resonance spectroscopy [SSNMR]) and thermal (*i.e.* differential scanning calorimetry [DSC], thermogravimetric analysis [TGA] and hot-stage microscopy) methods are used for further characterisation and routine quality-consistency purposes

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[6–9]. Raman spectroscopy has been shown to be a valuable technique in the identification of the polymorphic form of bulk pharmaceutical materials. Moreover, the quantification of binary systems, consisting of two solid-state forms, of drugs by Raman spectroscopy has been reported previously, *e.g.* fluconazole [10] and an unidentified compound H [11], and still is used frequently [12–15]. However, the analysis of multiple forms and/or its possible use to assess new solid-state forms has been up till now more rarely studied, *e.g.* a benzimidazole drug [16] or chloramphenical palmitate [17]. Uni-variate, using most often the ratio of two specific Raman band areas as response factor [12,17], as well as multi-variate approaches using more complete spectral data and including principal component analyses, linear discriminant analyses, classical and partial least squares [13] have been used in the quantification. While simple and controllable system investigations as required for QC purposes can use an appropriate uni-variate quantification, more complex systems like biological materials or conversion mechanistic studies will benefit from the multi-variate approaches [18–20].

Salicylanilide compounds exhibit a diverse bioactivity spectrum, including antimicrobial, antitumor, molluscicidal and taeniacidal functions, and are used in pharmaceutical drugs (*e.g.* closantel) and pesticides. Up till now, however, nothing has been reported related to the solid-state assessment of salicylanilide compounds.

The objective of the present study was to characterise the solid-state polymorphic composition of a biologically active salicylanilide by FT-Raman spectroscopy. Raman spectra of the three available polymorphic references were recorded and compared with the spectra of different samples obtained from commercially available batches of different origin. The regions of interest (ROI) were identified. A non-linear regression method estimated the goodness-of-fit for the model and the contribution of each available polymorphic form to the samples.

2. Materials and methods

2.1. Materials

A biologically active salicylanilide compound (*N*-[5-chloro-4-[(4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide) was investigated in this study. Three solid-state polymorphic forms have been identified and certified by DSC and IR by the originator (JRF): polymorph I (Pol I), polymorph II (Pol II) and the amorphous form (Amorph). Reference standards of each of the three known solid-state polymorphic forms of the salicylanilide compound, considered 100% pure polymorph in this assessment, were obtained from Janssen Research Foundation (JRF). In addition, three samples from commercially available batches of the salicylanilide compound with unknown polymorphic composition were worldwide obtained from different sources.

2.2. FT-Raman spectroscopy

A Bruker FT spectrometer Equinox 55S provided with a Raman modula FRA 106 with a cooled (77 K) germanium detector D418-T was used to perform the Raman spectroscopy. The samples, placed in glass capillary tubes, were excited by a 1064 nm beam from a Nd:YAG laser. The laser power was adjusted to 100 mW and distributed over the surface of the sample. All samples were placed in the focus point of the beam using a movable sample holder, giving a probed area of approximately 1 mm^2. The scattered light was collected at an angle of 0° and the spectral resolution was 2 cm^{-1}. Multiple scans of each sample were taken ($n = 100$) in order to obtain the final spectra.

2.3. Data processing

Data processing was performed with the statistical package SPSS, Version 11. The applied model is a linear regression model with one additional constraint (sum of coefficients equals 1). The practical implementation of this constrainted linear regression model was realised with the non-linear regression procedure of SPSS, since 'constrainted' models are only implemented in the non-linear module of the package. Although the iterative calculations will be done differently, the final 'least-squares' linear parameter estimates are expected to be identical as compared to results obtained with a dedicated constrainted linear regression software (*i.e.* linear programming).

Seven regions of interest were selected based upon their discriminative capacity towards the different polymorphs of interest. The raw intensities of the ROI, for both the reference standards and drug substance samples were first transformed into a Markov vector, *i.e.* a vector of non-negative reals that sum to unity and thus is corresponding to a probability distribution, according to the following formula:

$$A^{\nu'} = \frac{A^{\nu}}{\sum_{i=1}^{n} A^{\nu}}$$

where A^{ν} is the Raman peak area, $A^{\nu'}$ the transformed Raman peak area and n is the number of ROI-bands. Subsequently, the transformed values obtained for the commercially available drug substance samples were compared with the similarly transformed values obtained for the reference standards in following mathematical linear model:

$$Y_i^{\nu'} = B_1 A_i^{\nu'}(\text{Amorph}) + B_2 A_i^{\nu'}(\text{Pol I}) + B_3\ A_i^{\nu'}(\text{Pol II})$$
$$\text{or in matrix-notation}: \ \boldsymbol{Y}^{\nu'} = \boldsymbol{A}^{\nu'} \cdot \boldsymbol{B}$$

where i is an index from 1 to n ($n = 7$, being the number of ROI-bands), $Y_i^{\nu'}$ the transformed Raman peak area of the commercial sample, $A_i^{\nu'}$ the Markov-transformed Raman peak area of the reference batches and B_1, B_2 and B_3 are the parameters giving the relative importance of each reference batch in the spectra of the commercial sample. Following constraints were incorporated into the model: $\Sigma(B_1 + B_2 + B_3) = 1$; boundary values of the parameters B_1, B_2 and B_3 are between 0 and 1. This model was used to obtain the best estimate for the **B**-vector for each commercial sample.

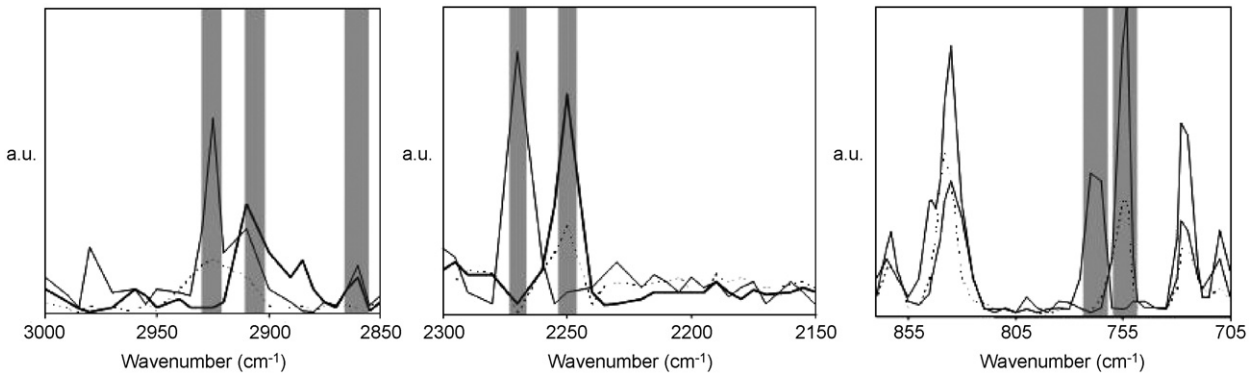

Fig. 1. FT-Raman spectra of the three reference batches, showing the seven ROIs. Polymorph I (solid line), polymorph II (bold line) and amorphous (dotted line).

Table 1
Raman peak areas of ROI

ROI	Wavenumber (cm^{-1})	Reference			Sample		
		Amorph	Pol I	Pol II	Batch 1	Batch 2	Batch 3
A1	746–763	0.223164	0.403737	0	0	0.076207	0.016262
A2	760–780	0	0	0.304617	0.239964	0.099913	0.05212
A3	2240–2260	0.06438	0	0.173919	0.171343	0.078908	0.085025
A4	2260–2277	0	0.13653	0	0	0.019914	0.009434
A5	2852–2875	0	0.030406	0.032275	0.037428	0.051985	0.026305
A6	2895–2920	0	0.04238	0.161282	0.153244	0.070438	0.128282
A7	2920–2937	0.030624	0.138908	0	0	0.007823	0

3. Results and discussion

3.1. Raman characterisation of the reference polymorphs

Raman spectra of the three polymorphic reference standards available were recorded. Significant and clear differences in the spectra were observed for the three reference polymorphs and consequently, seven different regions of interest were identified. The differences in the spectral region of 740–800 cm^{-1} (ROI A1–A2) is associated with C–H out-of-plane bending modes (Fig. 1). Within this region, Raman is very sensitive to the differences between Pol II and the two other polymorphs. The high frequency regions 2240–2280 cm^{-1} (ROI A3–A4) and 2850–2940 cm^{-1} (ROI A5–A7), characteristic for C–H stretching modes, shows distinct differences between the three polymorphs: Pol II as well as the amorphous form both shows a sharp band at 2250 cm^{-1}. This band is observed at 2270 cm^{-1} for Pol I. Between 2850 and 2940 cm^{-1}, three bands are observed for Pol I, two bands for Pol II and one band for the amorphous form.

For quantitative purposes, the peak areas of the seven ROI Raman-bands from the different reference polymorphs were used and are presented in Table 1.

3.2. Quantification of solid-state polymorphs in commercial drug substances

Next to the Raman-differentiation of the different solid-state forms of this drug, not yet previously reported, another objective was to quantitatively characterise the polymorphic form of samples from three commercially available drug batches by Raman spectroscopy. Although multi-variate techniques can be used, requiring chemometrical data evaluation of the full spectrum, we investigated the simpler uni-variate approach focussed on a

Table 2
Raman peak areas of ROI (after transformation)

ROI	Wavenumber (cm^{-1})	Reference			Sample		
		Amorph	Pol I	Pol II	Batch 1	Batch 2	Batch 3
A1	746–763	0.701403	0.536912	0	0	0.188078	0.051231
A2	760–780	0	0	0.453236	0.398625	0.246584	0.164195
A3	2240–2260	0.202346	0	0.258772	0.284633	0.194744	0.267856
A4	2260–2277	0	0.181565	0	0	0.049148	0.02972
A5	2852–2875	0	0.040436	0.048022	0.062175	0.128298	0.082869
A6	2895–2920	0	0.056359	0.23997	0.254567	0.17384	0.404129
A7	2920–2937	0.096251	0.184728	0	0	0.019307	0

Table 3
Quantitative polymorphic composition of samples

Sample	Amorphous (%)	Polymorph I (%)	Polymorph II (%)	R^2
Batch 1	2.5	0.0	97.5	0.978
Batch 2	7.7	27.9	64.4	0.719
Batch 3	0.0	19.8	80.2	0.311

few discriminating peaks only, which might be more suitable for routine investigations.

The peak areas of the seven ROI Raman-bands of the samples were determined under identical experimental conditions as the reference standards (Table 1). However, the measured intensities of the bands in the experimentally obtained Raman spectra are influenced by sample as well as by equipment characteristics [21]. Therefore, the peak areas of the ROI Raman-bands within each spectrum were first transformed into a Markov vector. The corresponding values are given in Table 2. Subsequently, the relationship between the Markov-transformed Raman intensities of the commercial samples and those of the reference standards was evaluated using linear modelling. The linear modelling procedure can be applied as the transformed Raman bands due to the different polymorphs are additive and linear with concentration [16]. The quantitative results for the three commercial batches obtained by this approach (contribution of each reference standard to the commercial sample spectra given by the parameters B_1, B_2 and B_3) are given in Table 3. All three commercial batches are qualitatively characterised as polymorph II. However, there is a significant quantitative difference in the sample spectra. Sample 1 is clearly polymorph II with no significant amounts of other forms. Sample 2 is predominantly polymorph II but with significant amounts of the polymorph I and amorphous material. As observed by the R^2, the model is appropriate for both batches as the majority (97.8% respectively 71.9%) of the observed variation could be explained by this model. However, the model is no longer appropriate for sample 3: while its results are predominantly polymorph II with significant amounts of polymorph I, the low R^2 value indicates that this sample may contain other forms or significant crystal impurities besides the three already known forms included in the applied model. As such, the goodness-of-fit is indicative that the sample may contain and/or consist of yet unknown forms.

Most of the previously described uni-variate quantifications are using only one wavenumber for each of the crystal forms and applying simple ratio's [22]. The Markov-transformed method here described employs the intensity of a selected set of several ROI-bands to characterise the different polymorphs. Consequently, a quantitative characterisation of multiple polymorphs, incl. amorphous form, was possible *via* a simpler procedure compared to the multi-variate approaches.

4. Conclusions

In the present study, different Raman spectra were observed for the polymorphs I and II and the amorphous form of an active salicylanilide compound, not yet investigated for its polymorphic composition. Several regions of interest were identified and the peak areas of these regions were determined. After a Markov-transformation, the contribution of each reference spectra to the spectra of the unknown samples could be determined by applying a constrained linear regression model. The obtained results indicate that qualitative and quantitative assessment of the solid-state composition of salicylanilide-compounds by Raman spectroscopy is possible and can be applied to the evaluation of commercially available generic samples.

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