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Solid State Analysis of Pharmaceutical Substances:

Methods of Polymorph Analysis in the Pharmaceutical Industry

Theses

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1. Introduction

As both original and generic companies realized the economic potential of new polymorphic forms of known active pharmaceutical ingredients (APIs), which may become the subject of patented intellectual property, the pharmaceutical relevance of polymorphism has got particular attention. In fact the originator can prolong the duration of his chemical patent protection by patenting new solid forms of the compound. At the same time generics can enter the market with their own different solid forms before the expiration of the originator's patent protection without infringement. Unfortunately applicants are not interested in revealing their knowledge how to differentiate and how to produce polymorphs, therefore the industrial property right background of generic APIs is usually really ambiguous both from legal and scientific points of view.

Regulatory authorities exert their influence by requirements assuring the quality of pharmaceutical products in vide variety of circumstances. Manufacturers have to justify not only chemical but also physical stability of their products during the formulation and shelf life, i.e. that the active substance does not suffer polymorph transition. If it is not verified that bioavailability of the product is not affected by polymorphism, which is relatively often the case, this regulation tasks quality assurance considerably.

It is understandable therefore why pharmaceutical companies invest more and more in API polymorphism research and development, why high-throughput crystallization experiments for producing new solid forms (polymorph screening) is particularly actual, why new techniques and instruments for solid state characterization appear, as well as why crystallization and solid state qualitative and quantitative analysis of drug substances becomes an increasingly popular discipline, as it is demonstrated by constantly growing number of patent applications and scientific papers dealing with this subject.

2. Aims of the research and the thesis

My task during the research was to discover new solid forms of various active pharmaceutical substances, to characterize these forms with suitable analytical techniques and to unequivocally differentiate them from each other, furthermore, to reveal their relative physical stability relationships, as well as to develop analytical methods for their quantitative determination in binary polymorphic mixtures. As these studies have usually concerned APIs of products currently under original or generic development, intellectual property interests of Gedeon Richter Plc. made it necessary that my thesis be confined to the results of the investigations of substances known in the open literature.

Polymorph analysis of organic substances is a complex and wide-ranging, multidisciplinary field. The problems can not always be solved by using single analytical techniques; the task rather necessitates adopting a complex analytical approach. According to this, the essential aim of the thesis is to reveal the potentials and limitations of the applied techniques through the solution of solid state analytical problems arisen with selected model compounds. At the same time, the thesis also would like to offer some guidance for performing similar investigations, as thorough and comprehensive literature is not available in this field in Hungarian language.

Though one of the goals of my studies was to solve the solid state analytical problems of particular investigated substances, polymorphic systems; the main purpose behind discussing the results, however, was to work out analytical methods which can be utilized in industrial development. I have pursued to meet the analytical challenges in connection of industrial legal situations which often raise not clearly defined questions regarding the differentiation of polymorphic forms and quantitative determination of their composition in mixtures. Increasing the potential of the analysis results in significant economical consequences for the manufacturer.

The thesis deals with three main aspects of polymorph analysis and the three main chapters are divided according to this. The purpose of the first main chapter is the discussion of the *solid state analytical characterization* of different phases of the compounds. Through some non-trivial examples, I have tried to ascertain potential advantages that can be obtained from the complex analytical approach compared to the conventional use of analytical techniques. In the case of polymorphic system of famotidine, beyond the analytical aspects, I also intended to analyse thermodynamic relations and kinetic behaviour. The studies discussed in the second chapter aimed to clarify *polymorph stability relationships*, considering the potential of thermal analysis including the modulated DSC method, the applicability of variable-temperature infrared and Raman spectroscopy, as well as X-ray powder diffraction. During the investigation of

polymorphic systems of prednisolone and losartan potassium, the main goal was to explore the potentials and limitations of these techniques; furthermore, to explain the relation between mechanical activation and polymorph transitions of bicalutamide. The third chapter discussing *quantitative polymorph analysis* aimed at the development of suitable analytical methods for the quantitation of polymorphic mixtures. The potential of quantitative determination by X-ray powder diffraction and Raman spectroscopy, as well as the performance of univariate and multivariate data processing methods were investigated for famotidine polymorphs. Quantification of low levels of polymorphic impurity in the case of clopidogrel bisulphate – where the utilization of univariate data analysis is insufficient – was intended to be performed by chemometrics. To achieve these goals, it proved necessary to develop a unique sampling procedure which makes Raman spectroscopy more effective in quantitative solid state analysis.

3. Experimental

X-ray Powder Diffraction

PANalytical X'Pert Pro MPD

For experiments in transmission geometry: parafocusing Göbel mirror

For variable-temperature experiments:

HTK-2000 chamber with TCU-2000/20 controlling unit

TTK-450 chamber with TCU-100 controlling unit

Infrared Spectroscopy

Thermo Nicolet 6700 FT-IR spectrometer

Thermo Nicolet Continuum FT-IR microscope

For variable-temperature experiments:

Linkam FTIR 600 hot-stage with Linkam TMS 94 controller

Raman Spectroscopy

Thermo Nicolet 9650-NXR FT-Raman spectrometer and MicroStage™

For variable-temperature experiments:

Linkam FTIR 600 hot-stage with Linkam TMS 94 controller

Thermogravimetric Analysis

TA Instruments TGA Q5000 IR

Differential Scanning Calorimetry

TA Instruments DSC Q10 and TA Instruments DSC Q1000 with RCS cooling unit

Optical Microscopy

Leitz Laborlux S polarized light microscope and Olympus Camedia C-3000ZOOM camera

For variable-temperature experiments:

Linkam FTIR 600 hot-stage with Linkam TMS 94 controller

Multivariate Data Analysis:

Thermo Nicolet TQ Analyst 7.2 software

4. Theses

4.1. I have investigated the practical relevance of complex analytical approach through the analysis of zaleplon samples obtained from various crystallization experiments. Low limits of detection by X-ray powder diffraction for various solid phases were only exceeded by microscopic techniques. Anhydrate, hydrate and other solvate forms of the substance were easily differentiated by the joint application of thermal analysis and vibrational spectroscopy. I have unequivocally identified two phases of zaleplon by visible light and infrared microscopic examinations in their mixture.

4.2. Through the thorough characterization of two enantiotropically related polymorphic forms of aripiprazole (here named α and β form), I have ascertained that α form transforms to β above 130 °C; the reverse process is, however, kinetically hindered. The β form does not suffer polymorph transition at room temperature until mechanical action does not affect it. I have also found that phase transition occurs unusually easily, even on slight compression; thus analytical characteristics of the β form can only be measured with particular care. I have developed a procedure involving the treatment of the sample at elevated temperature after the sample preparation, which turned out to be a proper solution in the case of both X-ray powder diffraction and vibrational spectroscopy.

4.3. Polymorphic mixtures of famotidine were not identified in pharmaceutical formulations before. Investigating tablets of competitor companies I have found that both X-ray powder diffraction in transmission mode and Raman spectroscopy are suitable to detect about 10 % of form A in polymorphic mixtures with form B. In spite of the fact that the active ingredient constitutes only 10 % of the tablet weight, Raman spectroscopic investigation provided results within several minutes. Although X-ray powder diffraction measurement requires much more time (ca. 20 hours), it is also suitable for estimating the polymorph composition of the API in formulations.

4.4. Disproving previous assumptions, I have revealed by X-ray powder diffraction, as well as by infrared and Raman spectroscopy that metastable form B of famotidine does not suffer polymorph transition either during micronization or under high static pressure. It becomes mechanically activated on these effects instead, which is then results in polymorph transformation to the thermodynamically stable form A at elevated temperature. The effect of extended grinding is similar; it renders, however, the metastable form partially amorphous, and

the transition to the stable form A may also occur in extreme cases. If the sample of form B contains some amount of form A, grinding can significantly increase its percentage. I have demonstrated that the explicit sensitivity of famotidine to thermally stimulated chemical degradation may lead to misinterpretation of the mechanism of mechanical activation. Local overheating during extended grinding also results in chemical decomposition leading not only to melting point depression but also influencing the polymorph transition at elevated temperature. It is an important methodological consequence of these findings that solid state analytical characterization of famotidin by DSC has to be performed cautiously, and the results should be confirmed by – at least one – other analytical technique [1, 2].

4.5. In spite of that dehydration process of prednisolone sesquihydrate has already been investigated, it was unknown in the literature that the transition of the hydrate to form I at elevated temperature takes place via intermediate metastable anhydrate and amorphous forms. I have shown this by variable-temperature infrared and Raman spectroscopy as well as hot-stage X-ray powder diffraction. I have revealed the relative stability of the amorphous phase obtained this way, which was also unknown previously.

4.6. I have observed a previously unknown, reversible solid phase transition by thermal analysis in the case of losartan potassium. I have shown by temperature-modulated DSC that this transition can be clearly distinguished from the enantiotropic transformation of the substance appearing at higher temperature; thus confirming a recently appeared conception that the methodology is suitable for differentiating between kinetically reversible and irreversible solid state transitions. In turn using variable-temperature X-ray powder diffraction, as well as infrared and Raman spectroscopy, I have ascertained that there is no measurable structural change during this reversible transition. There is only a slight discontinuity in the change of lattice parameters, which also influences the displacement of vibrational frequencies. I have shown the limitations of variable-temperature characterization techniques in investigating high temperature transitions of thermally labile substances, which was clear in the case of the polymorph transition of losartan potassium form I to form II. In this case hot-stage FT-IR microscopy turned out to be the best analytical method.

4.7. Crystallization of the amorphous phase of bicalutamide was not investigated in detail before. I have shown that amorphous bicalutamide is particularly sensitive to mechanical activation. I have ascertained that amorphous form crystallizes to form II only if it was not

subjected to mechanical action previously. If the quenched melt is ground or even scratched with a pin prior to the heating program, form I also crystallizes. I have revealed by hot-stage X-ray powder diffraction investigations that crystallization of kinetically favoured form II and thermodynamically stable form I take place concomitantly above 100 °C. I have ascertained that mechanical activation results in both partial amorphisation of the metastable form and nucleation of the stable form in the amorphous phase. In turn I have shown that form II can transform to form I at sufficiently high temperature within sufficiently long time without mechanical activation. The rate of transformation is determined by the extent of physical contact between solid phases [3].

4.8. Only the DSC method had been previously available for the quantitative determination of famotidine polymorphic mixtures. However, I have shown that DSC provides biased results depending on the state of the sample prior to measurement. I have developed X-ray powder diffraction method for the quantitative determination of relative ratio of form A and form B. I have shown that both the calibration mixtures and the analyte require grinding before the measurement in order to get accurate results independent of secondary properties of the sample (particle size, crystal habit). I have established that multivariate data analysis does not provide substantial benefit compared to univariate data analysis provided that characteristic reflections are properly selected for the latter. Quantitation limit of the method is about 6 % [4].

4.9. I have developed Raman spectroscopic method for the quantitative determination of famotidine polymorphic mixtures. This is not only faster than X-ray powder diffraction method but also outperforms it in limit of detection, precision and robustness. Although multivariate data processing has halved the detection limit of form A, assessing composition of validation and ground samples proved that this is only an apparent improvement. Both univariate and multivariate methods are suitable for the determination of polymorphic impurity down to 3 %, i.e. in this case chemometric approach has no real benefit. Furthermore the developed quantitative analytical method proved to be robust in relation to particle size change, which is usually not valid for similar assays known from the literature [4].

4.10. An X-ray powder diffraction method was only available for the quantitative determination of low levels of form II of clopidogrel bisulphate in polymorphic mixtures with the thermodynamically less stable form I. I have shown that both infrared and Raman spectroscopy are applicable for this purpose by chemometrics, even though form II below 15 % has no

visually detectable spectral signs. Utilizing various data processing methods I have ascertained that, if the quality of the data is high (spectra with good resolution and signal-to-noise ratio collected from calibration samples sufficiently homogenized on the analysis scale), different algorithms (CLS, PCR and PLS) work equally well. However, models can be improved by selecting spectral regions most sensitive for concentration variation. Using appropriate range selection and preprocessing procedures both methods provide 1 % detection and 2-3 % quantitation limit [5].

4.11. As the excitation laser beam is readily focused on very small spot size, sub-sampling is a common problem of quantitative Raman spectroscopy. In order to eliminate this, I have developed a unique sampling procedure, in which spectra are collected from a home-made sampling accessory permitting simultaneous placement of 12 different samples. Filling the holes of this plate can easily be carried out by using a sample filling accessory; and this results in smooth and equally compact sample surface. The accessory is effective in utilizing possibilities of the instrument and its data collection software. It allows programming measurements from different samples with an arbitrarily chosen number of sample positions wherefrom collected spectra are averaged. For measuring substances not sensitive to the heating effect of the laser beam the procedure may be more efficient than the alternative method using rotating sample holder [5].

5. Importance and utilization of the results

Examples presented in the thesis are related to problems and solutions in the real practice of pharmaceutical industrial polymorph analysis. Thus, the majority of the results have already been utilized.

Mechanical activation is not uncommon among pharmaceutical solids; the case of aripipirazole polymorphs and amorphous bicalutamide, however, due to its unusual sensitivity, may be the subject of further investigations in this field.

I have demonstrated the power of variable-temperature infrared and Raman spectroscopy, as well as X-ray powder diffraction in the investigation of polymorphic and solvatomorphic transitions through many examples. As there is relatively little knowledge about the relative benefit of these methods, I have tried to reveal this. The thoroughly investigated variable-temperature techniques are suitable for the investigation of polymorphism of pharmaceutical materials, particularly those of melting with decomposition or having reversible transitions, because thermal analysis is ineffective in the characterization of their *in situ* formed new phases. My research has greatly promoted that these investigations are now performed routinely searching for new solid forms of new chemical entities in Gedeon Richter Plc. Designing experiments and drawing conclusions in studies of similar polymorphic systems can be accomplished more efficiently based on the experience discussed in the thesis.

The literature of quantitative polymorph analysis is constantly growing regarding the application of both vibrational spectroscopy and X-ray powder diffraction. The majority of scientific discussions, however, do not pay sufficient attention to the industrial utilization of developed methods. Experiences discussed in the thesis have aimed at communicating this kind of knowledge, as well as at comparing the applicability of univariate and multivariate data processing methods. The results may provide useful information for sample preparation, analytical measurement and data processing in the quantitative analysis of different polymorph systems.

The presented examples try to turn the attention to the fact that pharmaceutical polymorph research needs complex analytical approach. One has to know the potential of analytical techniques and relationships of obtainable results, in order to understand the behaviour of various polymorph systems in detail. Handling this task with the essential infrastructure has strategic importance in industrial practice.

6. Publications

1. Német, Z.; Hegedűs, B.; Szántay, Cs. Jr.; Sztatisz, J.; Pokol, G., Pressurization effects on the polymorphic forms of famotidine. *Thermochim. Acta* **2005,** 430, 35-41.

Impact factor: 1.562 Number of independent citations: 6

2. Demeter, Á.; Német, Z.; Varga, Z., Hatóanyagok mikronizálása. In *Kristályosítástól a tablettázásig*, Farkas, B.; Révész, P., Eds. Universitas: Szeged, 2007.

3. Német, Z.; Sztatisz, J.; Demeter, Á., Polymorph transitions of bicalutamide: a remarkable example of mechanical activation. *J. Pharm. Sci.* **2008,** 97, 3222-3232.

Impact factor: 2.942 Number of independent citations: 0

4. Német, Z.; Csonka Kis, G.; Pokol, G.; Demeter, Á., Quantitative determination of famotidine polymorphs: X-ray powder diffractometric and Raman spectrometric study. *J. Pharm. Biomed. Anal.* **2009**, 49, 338-346.

Impact factor: 2.761 Number of independent citations: 0

5. Német, Z.; Demeter, Á.; Pokol, G., Quantifying low levels of polymorphic impurity in clopidogrel bisulphate by vibrational spectroscopy and chemometrics. *J. Pharm. Biomed. Anal.* **2009**, 49, 18-25.

Impact factor: 2.761 Number of independent citations: 0

7. Oral presentations

Német, Z.; Demeter, Á.; Hegedűs, B.; Varga, Z.; Szántay, Cs. Jr.
Gyógyszerhatóanyag-polimorfia komplex analitikai megközelítésben
MKE Ifjú Analitikusok Előadói Ülése, Budapest, November 25, 2003.

Német, Z.; Hegedűs, B.; Sztatisz, J.
Csapdák a morfológiai homogenitás vizsgálata körül
Analitikai Vegyészkonferencia, Balatonföldvár, June 30–July 2, 2004.

Német, Z.; Körtvélyesi, Zs.; Demeter, Á.
A modulált DSC módszer és alkalmazása a gyógyszeripari kutatásban
MTA Termoanalitikai Munkabizottságának ülése, Budapest, February 20, 2007.

Német, Z.; Sztatisz, J.; Demeter, Á.
A bikalutamid polimorf módosulatváltozásainak vizsgálata
Centenáriumi Vegyészkonferencia, Sopron, May 29–June 1, 2007.

Német, Z.
A fűthető tárgyasztal új lehetőségei a polimorf átalakulások vizsgálatában
MKE Kristályosítási és Gyógyszerformulálási Szakcsoportjának ülése, Budapest, February 21, 2008.

Német, Z.
Polimorf keverékek mennyiségi analízise
XXXV. Gyógyszeranalitikai Továbbképző Kollokvium, Sopron, April 24–26, 2008.

Német, Z.; Demeter, Á.
Gyógyszerhatóanyagok új szilárd formáinak előállítása szilárdfázisú vizsgálatokkal
Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium ’2008, Zalakaros, September 29–30, 2008.