Title: Chemometric study of the excipients' influence on polymorphic-behavior. Mefenamic acid as case of study

Authors: Marina Antonio, Natalia L. Calvo, Rubén M. Maggio

PII: S0731-7085(18)32824-3
DOI: https://doi.org/10.1016/j.jpba.2019.03.021
Reference: PBA 12534

To appear in: *Journal of Pharmaceutical and Biomedical Analysis*

Received date: 20 December 2018
Revised date: 12 March 2019
Accepted date: 13 March 2019

Please cite this article as: Antonio M, Calvo NL, Maggio RM, Chemometric study of the excipients' influence on polymorphic-behavior. Mefenamic acid as case of study, *Journal of Pharmaceutical and Biomedical Analysis* (2019), https://doi.org/10.1016/j.jpba.2019.03.021

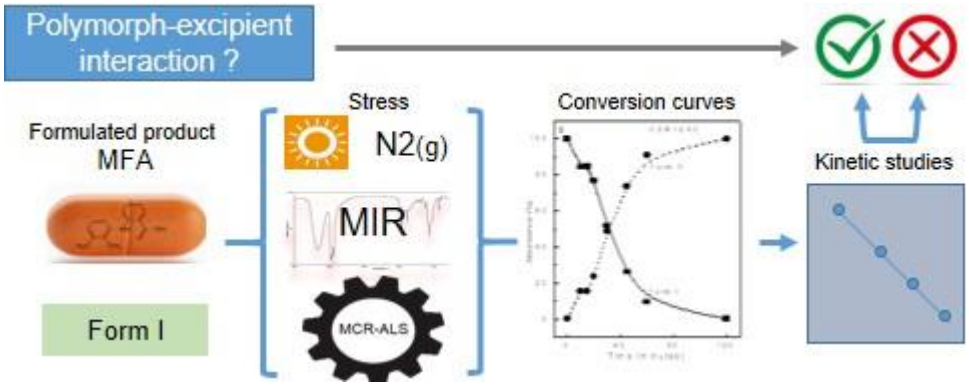
Chemometric study of the excipients' influence on polymorphic-behavior. Mefenamic acid as case of study

Marina Antonio, Natalia L. Calvo and Rubén M. Maggio*

Área de Análisis de Medicamentos, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario and Instituto de Química Rosario (IQUIR, CONICET-UNR), Suipacha 531, Rosario S2002LRK, Argentina

* Corresponding author. Tel.: +54-341-4370477, ext. 119; *E-mail address*: maggio@iquir-conicet.gov.ar (Rubén M. Maggio);

Graphical abstract



Highlights

- Influence of API-excipients interaction of polymorphism
- Polymorph thermal transformation of monitoring by MIR/MCR-ALS approach
- MIR spectroscopy applied to the study of Mefenamic acid polymorphism

Abstract

The assessment of polymorphism is a problematical issue for regulatory agencies, because variations among crystalline forms of active pharmaceutical ingredient (API) can lead to changes in the efficacy and safety of formulated product. Such conversions are very hard to be detected, thus, the development of techniques for the identification, characterization and quantification of polymorphs results essential in all stages of the manufacturing process.

The presence of excipients in formulated products may change the crystal stability of an API, by catalyzing a polymorphic transformation or stabilizing the less stable form. As paradox, all suitable analytical techniques (spectroscopies, thermal analysis, NMR and DRX, and others) for polymorphic analysis are affected by excipients. A deep understanding of the polymorphism-excipient relationship is in full accordance with Quality by Design (QbD) paradigm, the systematic approach focused in quality building into a product based in the full understanding of the products and process.

In this work, a novel approach based on thermal stress, MIR monitoring, multivariate curve resolution with alternating least squares (MCR-ALS) and kinetic analysis was developed and applied to monitor polymorphism behavior of model API in formulated products.

Commercial tablets, physical mixtures and commercial API, were processed and analyzed under the proposed approach. Commercial tablets of MFA revealed a fast conversion to Form II, contrasting to the behavior of the pure API. Physical mixtures showed similar behavior to commercial tablets, thus reduction in transformation times was related to MFA-excipients physical interaction, even at surface level. Calorimetric studies support the conclusion obtained.

The developed approach could be extended to others APIs and other stress sources (humidity, solvents, mechanical forces and its combinations), being a valuable tool for QbD environment.

Abbreviations: API, active pharmaceutical ingredient; ATR, attenuated total reflectance accessory; BCS, biopharmaceutics classification system; COM, commercial bulk product; EXC, excipients; COMEX, physical mixtures of COM and EXC; DSC, differential scanning calorimetry; MCR-ALS, multivariate curve resolution with alternating least squares; MFA, Mefenamic Acid; MIR, middle infrared spectroscopy; NIR, near infrared spectroscopy; PAT, Process Analytical Technology; QbD, Quality by Design; TAB1, tablets sectioned longitudinally; TAB2, tablets reduced to powder.

Keywords: crystal polymorphism, mefenamic acid, excipient compatibility, MIR, chemometrics

1. Introduction

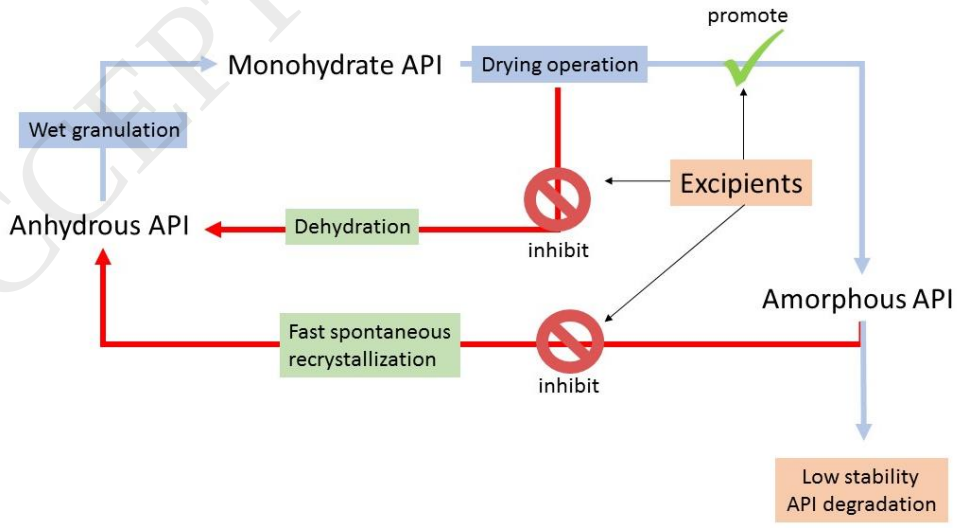
The polymorphism of active pharmaceutical ingredients (APIs) is a major concern for pharmaceutical industry due to its influence on physical and chemical properties of pharmaceutical powders [1]. Changes in crystalline form can also lead to variations in efficacy and safety of formulated product due to polymorphic systems with intrinsically different lattice energies manifest dissimilar enthalpies of fusion and solubilities [2].

Thus, polymorphism results in APIs that exhibit differential properties such as apparent solubility, dissolution rate, chemical stability and processability, which may influence behavior

of pharmaceutical form and its bioavailability [3,4]. Hence, it is crucial to identify the different polymorphs, to determine their stability, and to assess the effect of processing conditions on polymorphic conversions [5].

Thermal or mechanical energy given from drying, milling, or compaction may accelerate the transition to the stable polymorph of the API. Generally, solid-state phase transitions are influenced by crystalline defects, impurities, particle size, APIs distribution, excipients, and environment factors such temperature, pressure and relative humidity (RH) [6]. In a Quality by design (QbD) environment, determination of physical or chemical interactions between APIs and excipients is crucial [7,8], in order to build the understanding of the product and process along a knowledge of the risks involved in manufacturing and how best to mitigate those risks.

The process of polymorph transformation can be inhibited or catalyzed by components of the formulate product. Excipients may facilitate conversion to the amorphous drug, which may subsequently compromise chemical stability [9,10], such is the case of immediate release tablets containing a prototype API [11]. Although, the API demonstrate to be chemically stable and compatible with the excipients; a potency loss was observed over accelerate stability tests (6 months, 40ºC/75RH) due to rapid API degradation. Authors revealed that during wet granulation anhydrous API converted to the monohydrate API and, after the drying operations in the presence of excipients, the monohydrate transformed to the amorphous form. The excipients inhibited the fast re-crystallization of amorphous API, resulting in an increased its content. The amorphous form was responsible for the poor stability of the wet granulated formulation (Scheme 1).



Scheme 1. Influence of excipients on the chemical stability of immediate release tablets containing a prototype API [11].

Only a few analytical techniques are able to detect polymorphic/pseudo-polymorphic transformations as descripted above [11]. In such scenario, the quantitative analysis is even more difficult to perform and it gets worse when the excipients interferer [6].

Calorimetric methods and vibrational spectroscopies are the more widespread techniques for polymorphs characterization; however, solid state nuclear magnetic resonance (ssNMR) and powder X-ray diffraction (pXRD) remain as gold standard [12]. Nevertheless, pXRD and ssNMR, are of difficult implementation in routinely quality control due to the high cost of the equipment. Additionally, pXRD had two major issues, the need of high theory knowledge of system for Rietveld calculations [13] and crystalline excipients may affect diffractogram acquisition.

Differential thermal gravimetry (DTG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC), are able to sense transformations (fusions, conversions, recrystallization, desolvation and others). Nevertheless, they do not provide information about identity of species involved such conversions, instead this transformations are "the key features" of the polymorphs [14]. The thermosgrams may be interfered by the excipient transformation (i.e melting point) or excipient-API interactions (ie. in the cases of eutectic mixtures).

Vibrational spectroscopies [Raman, near (NIR) and middle infrared (MIR) spectroscopies], are widespread techniques used for the polymorphism analysis, since they are sensitive to minor variations in conformation of organic molecules, such those involved in lattice structure changes [15]. However, the high number of signals, sometimes poorly resolved, present in their spectra make impossible quantitative analysis of polymorph mixtures using naked eye. Furthermore, the presence of excipients does not allow to detect any polymorph change even at qualitative level. Nevertheless, these methodologies do not require solvent, gases or another consumable, operate fast and without sample destruction, making them the first choice for routinely quality control and even as process analytical technology (PAT) tool.

In such way, several groups developed approaches to identify or quantitate polymorphs in drug substance and drug products using vibrational spectroscopies aided by chemometrics. Raman spectroscopy was coupled to PLS algorithm to analyze the main polymorph in albendazole bulk drug [16]. On the other hand, our group applied MIR spectroscopy on

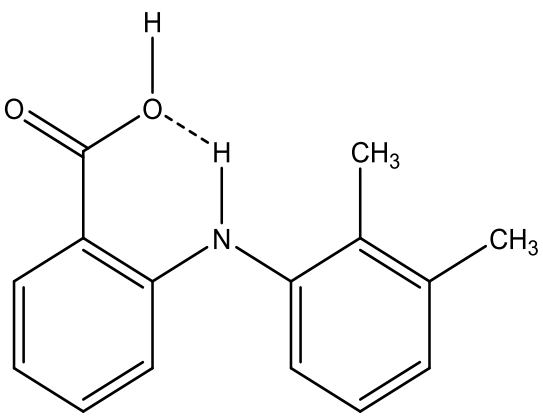
mebendazole and cimetidine tablets to polymorph assignment. There, PCA allow data compression and visualization, and further statistical analysis using Mahalanobis distance [17,18]. Additionally, NIR spectroscopy was the only vibrational spectroscopy able to successfully predict polymorphic content in commercial tablets of Mefenamic acid, using the obtained polymorphic content to predict the solubility performance [19].

Dynamic process, such a polymorphic (and pseudo polymorphic) inter-conversion were also studied using chemometrics and vibrational spectroscopies. This is the case of a monohydrate of cimetidine in which become selectively to form A under thermal treatment. Such transformation was unveiled analyzing MIR data by multivariate curve resolution with alternating least squares (MCR-ALS) algorithm, since it has no resolution using thermal analysis (DSC or DTG) [20]. In the same way, the inter-conversion of two crystalline forms of nimodipine was resolved by MCR-ALS and MIR spectroscopy [21]. However, the last approaches were only applied to drug substance and not to formulated products.

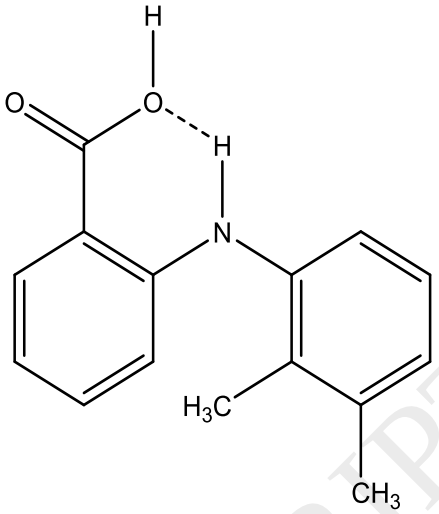
Hence, the scope of this work is to reveal the influence of excipient mixture in a plausible polymorphic transformation. A novel approach based on thermal stress, MIR monitoring, MCR-ALS and kinetic analysis was developed for polymorphism monitoring in formulated products. The present work deals with monitoring of Mefenamic Acid (MFA) polymorphism in commercial tablets.

MFA is a potent inhibitor of prostaglandin synthesis closely related to inflammatory processes [22]. MFA polymorphic forms show enantiotropic relationship, a conversion of Form I to Form II (**Figure 1**) occurs above 180 °C [23]. Hence, conversion to Form II (metastable form) may compromise stability of the pharmaceutical product and may alter the safety and efficacy of the API.

The phenomena of crystalline conversion of MFA in formulated product were studied analyzing physical mixtures and commercial tablets to the influence of the excipients/formulation process. The application of MIR spectroscopy coupled to MCR-ALS result in a new alternative in the monitoring of drug-excipient interactions that can be very useful in development stages of new solid pharmaceutical forms following, the guidelines QbD. As far we know, no approaches have been previously reported in the literature that monitoring the influence of excipients in the polymorphic conversion process using MIR/MCR-ALS approach.



Form I



Form II

Figure 1. Conformational changes in MFA between Form I and Form II.

2. Materials and methods

2.1. Instrumentation

Calorimetric determinations were performed in a Shimadzu 60 differential scanning calorimeter (Shimadzu Corp., Kyoto, Japan), operating under a Nitrogen atmosphere at a constant flow of 50 mL by min^{-1}. The samples (~5 mg) were placed in closed aluminum pans with a pinhole to equilibrate pressures, and heated at a rate of 5 C min^{-1} between 30 and 300 ºC. An empty aluminum pan was used as a reference.

MIR spectra were acquired in a Shimadzu Prestige 21 spectrophotometer (Shimadzu Corp., Kyoto, Japan) in 3800–600 cm^{-1} range with a resolution of 4 cm^{-1}. Attenuated total reflectance (ATR) experiments were carried out with a diamond-based ATR accessory (GladiATR, Pike Technologies, Madison, USA). The samples were measured by triplicate.

The particle size of the solids samples was standardized by sieving, employing a Zonytest EJR 2000 fine mesh vertical vibratory sieving tower (Rey & Ronzoni, Buenos Aires, Argentina), operating at 1200 rpm. In all cases, the 100–140 mesh fractions were collected.

The physical mixtures of solids were homogenized using a Z-mixer powered by rotatory platform with an electronic control of speed Precytec AT-15D, at 30 rpm.

The thermal treatment of the samples was carried out with a hotplate stirrer Talboys-all (Troemner, Thorofare, USA) digital controlled and fitted with an RTD temperature probe immersed in the silicone oil bath.

2.2. Chemicals

The MFA pharmaceutical grade (COM) was gently donate by Laboratorios ELEA. Excipients used (methylcellulose, cornstarch, silicon dioxide, microcrystalline cellulose, sodium croscarmellose, sodium lauryl sulfate and magnesium stearate) were of pharmaceutical grade and were acquired from "Droguería Saporiti" (Buenos Aires, Argentina). Commercial tablets containing 500 mg of MFA (average total weight: 713.91 mg) were purchased from a local pharmacy.

All other chemicals were of analytical grade and were used as received. During the experiments, API and its forms were kept in a desiccator and protected from light.

2.3. MFA pure forms

MFA pure forms (Form I and Form II) used as references were prepared according to recently reported procedures [19]. Identity of purity of the forms were confirmed by optical microscopy, MIR and NIR spectroscopy, melting point determination and DSC.

2.4. Sample preparation

2.4.1. MFA drug substance

Before experiments and analysis, COM samples were previously sieved, collecting the fractions comprised between 100-140 mesh, in order to homogenize the particle size. Then, obtained fractions were mechanical mixed.

2.4.2. Physical mixtures

Physical mixtures (COMEX) containing COM and excipient matrix (EXC) in proportions equivalent to commercial product were prepared to simulate the tablet environment.

EXC was prepared by weighting and mixing the following components: methylcellulose (9.55 g), corn starch (95.53 g), silicon dioxide (2.00 g), microcrystalline cellulose (96.61 g), sodium croscarmellose (3.52 g), sodium lauryl sulfate (2.89 g) and magnesium stearate (2.00 g). Particle size of excipients was previously homogenized by sieving, collecting the fractions comprised between 100-140 mesh. Subsequently, the mechanical mixing of the components was carried out.

2.4.3. Commercial samples

Commercial samples were divided in two sets, in set 1 (TAB1) tablets were longitudinally sectioned to maintain the original distribution of the components in the pharmaceutical form during the test. In set 2 (TAB2), tablets were gently reduced to powder and sieved to obtain homogeneous samples, 100-140 mesh fractions were finally used for the analysis.

2.5. Thermal treatment

For each sample, six aliquots of approximately 250 mg were placed in hermetical glass tubes, under a N_2 atmosphere, in order to avoid oxidation process during heating. The tubes were heated at a constant temperature (160 ºC).

Samples of COM were analyzed at the following times: 0, 30, 60, 120, 180, 240, 300, 360, 420, 480, 540, 600, 1800, 3600 and 5040 minutes (83 h). COMEX, TAB1 and TAB2 were sampled at nine pre-established times (0, 5, 10, 15, 20, 30, 45, 60 and 120 minutes).

2.6. *Chemometrics and graphics software*

The computer routines involving spectral data manipulation and the MCR-ALS algorithms were run in Matlab R2010a (Mathworks, Natick, USA). MCR-ALS Toolbox 1.0 was employed as interface for als2004 routine, all routines were available at https://mcrals.wordpress.com/download/.

MCR-ALS is based on the assumption of raw data matrix (D) from could be deconvoluted following the Lambert-Beer's law. MCR-ALS is able to obtain the spectral (S) and concentration (C) contributions of the pure species of involved in process from raw spectral data obtained through time; $D = C \cdot S^T + E$, where E is the matrix of error associated to model fitting or the instrumental noise.

MCR-ALS solves the equation, employing an alternating least squares algorithm which iteratively C and S^T matrices, which optimally fit the experimental D. This optimization is carried out for a number of components established *a priori*, and using initial estimates of them.

Graphics and statistical data analyses were performed using OriginPro 8 SR0 (Originlab Corporation, Northhampton, USA).

3. Results and discussion

3.1. Characterization of MFA pure polymorphs and commercial form

The polymorphs of MFA (Form I and Form II) were obtained as previously reported [19] and unequivocally characterized by optical microscopy (Figure S1), MIR and NIR spectroscopy

(Figure S2), melting point determination and DSC. The obtained results were in full agreement with the literature [24, 25, 26].

The samples of the pure forms of MFA were characterized using DSC determinations and observations are detailed below. COM and Form I exhibited similar behavior (a transformation followed by a fusion) where it showed two endothermic peaks at 175 and 238 ºC, corresponding to Form I-Form II transition and Form II melting point, respectively. Form II showed only one endothermic peak corresponding to its melting point at 233 ºC. The results obtained were in agree with literature [19,27].

MIR spectra of MFA polymorphs (Figure 2) was divided in 3 main regions for its further analysis, 3500-1800 cm^{-1}, 1800-1500 cm^{-1} and fingerprint region (1500-750 cm^{-1}). MFA presented bands related to NH stretch at 3311 and 3347 $cm^{-1,}$ for in Form I and Form II respectively, in agree with previously published data [28].

The second region (1800-1500 cm^{-1}) showed the signals associated with carbonyl and benzene ring stretching vibrations. The carbonyl stretch is observed at 1643 cm^{-1} for both polymorphs, while the bands at 1593, 1570 and 1508 cm^{-1} in Form I and 1564 cm^{-1} in Form II were attributed to benzene-ring stretching and in-plane NH deformation. Finally, examination of the fingerprint region revealed vibrations associated with in-plane CH or ring deformation, represented by the bands in 1450-1200 cm^{-1} region. The out-of-plane CH deformations were found in 960-800 cm^{-1} range and 885-820 cm^{-1}, for Form I and Form II respectively. The bands at 752-744 cm^{-1} (Form I) and 742 cm^{-1} (Form II) were assigned to ring deformation coupled with CO_2 wagging. COM showed the same peak position and intensity of Form I in MIR.

The observations listed above for MIR and DSC, together complementary analysis (see supplementary section for NIR and Microscopy analysis), allows to conclude that crystalline structure present in COM correspond to Form I.

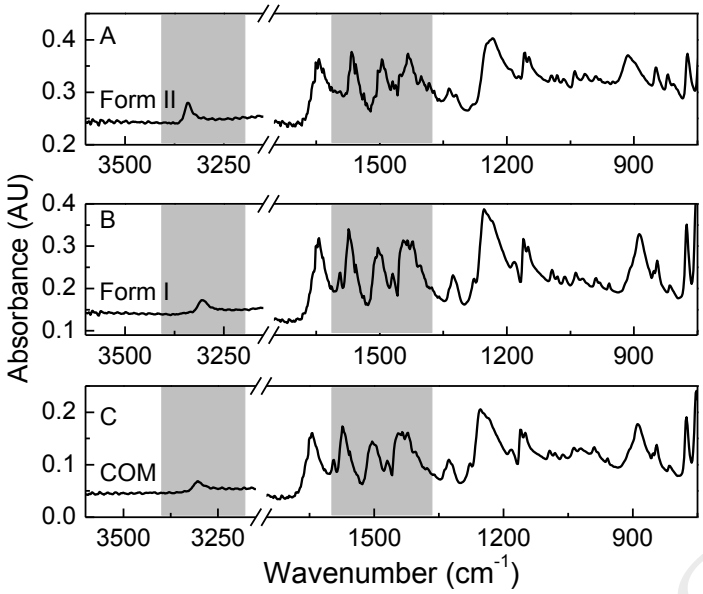


Figure 2. MIR Spectra for MFA polymorphs, Form II (A), Form I (B) and COM (C).

3.2. Monitoring polymorphism behavior of MFA in formulated products

In order to reveal the influence of excipient mixture in a plausible polymorphic transformation, several samples of MFA were stressed and monitored using MIR spectroscopy.

As first step, the polymorphic transformation of COM submitted to thermal treatment (Section 2.5) was assessed in order to take into account behavior of pure MFA under experimental conditions. The thus obtained aliquots were measured in the range 3800–600 cm^{-1} using an ATR accessory. Figure 3 shows the evolution of MIR spectra obtained for the COM samples submitted to treatment. A double peak was observed in the region between 3282 and 3360 cm^{-1} after 6 hours of heating, which corresponds to the displacement of the stretch band of the -NH group, due to the conversion from Form I to II. Other changes in could be visualized among 1593-1492 due to benzene ring stretching, 1469-1402 for C-H or ring deformation, 1327-1230 for Anti-symmetric CH3 and CH3' stretching, 958-819 for Out-of-plane CH deformation and at 775-742 for ring deformation and CO2 wagging (Table S1). The maximum polymorphic transformation was observed at 84 hours, but not complete transformation was observed into experiment time.

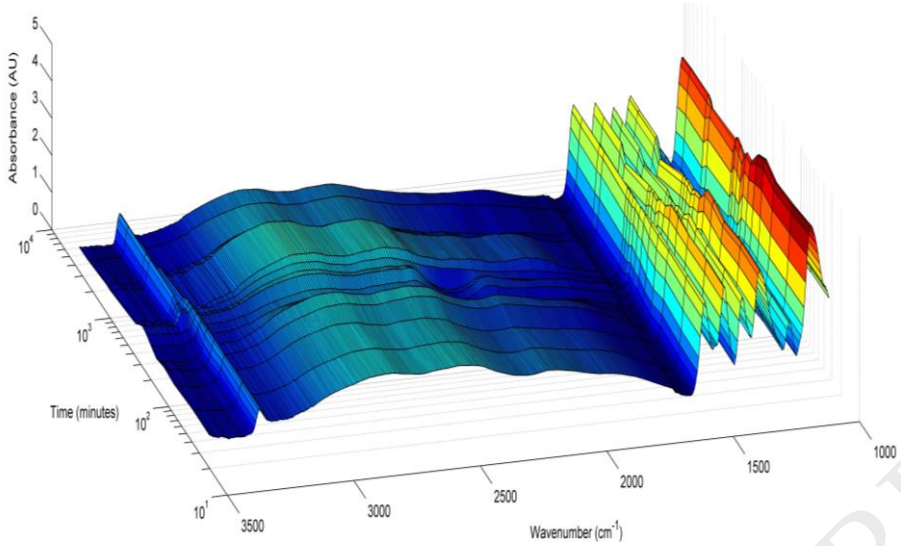


Figure 3. Surface image of the MIR spectra obtained during the thermal treatment of COM.

In order to determine the possible influence of the excipients in the conversion of Form I to Form II, the physical mixtures (COMEX) containing COM and excipient matrix and commercial tablets (TAB1 and TAB2), were analyzed following the same strategy.

Figure 4 shows the overall phenomena of conversion in COMEX, TAB1 and TAB2 samples, qualitative changes can be visualized using naked eye, for example stretch band of the -NH (from 3282 to 3360 cm^{-1}). However, excipients, especially carbohydrates [29] that show MIR absorption on the entire spectral range, interfere quantitative analysis. Thus chemometrics treatment of spectroscopic data arise as a plausible solution to obtain concentration profiles, kinetic curves, and pure spectra of polymorph. MCR-ALS emerges as the best choice, since it is especially suited to deal with time-evolving phenomena. MCR-ALS, is able to obtain, the spectral and concentration contributions of the pure species of involved in process from raw spectral data obtained through time. MCR-ALS is based on the assumption of raw data matrix from could be deconvoluted following the Lambert-Beer's law.

Additionally, MCR-ALS is a widespread chemometrics tool available as validated toolbox for R (https://cran.r-project.org/web/packages/ALS/index.html) and Matlab (https://mcrals.wordpress.com/download/), and it has a version in Unscrambler Software (https://www.camo.com/resources/multivariate-curve-resolution.html), especially suited to work into industry environment. Moreover, MCR is proposed as PAT tool to monitoring different unitary operations in pharmaceutics production coupled to Raman, MIR, NIR and UV spectroscopy [30]. These PAT tools could be combined with QbD to allow process control and

increase the guarantee that product quality is achieved consistently, and that product is manufactured with efficiency.

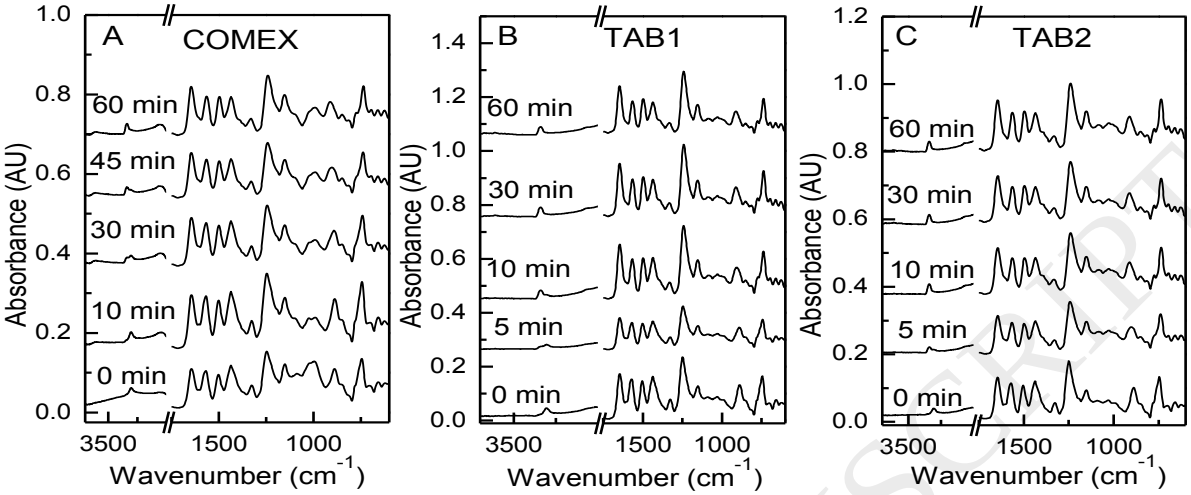


Figure 4. Selected MIR spectra obtained during the thermal treatment of COMEX (A), TAB1 (B) and TAB2 (C).

Thus, MIR data obtained for the experiments (COMEX, TAB1 and TAB2) were arranged as matrices, time (0, 5, 10, 15, 20, 30, 45, 60 and 120 minutes) × wavenumber (3600-600 cm^{-1}), and analyzed using the MCR-ALS algorithm. COM samples were also analyzed in the same way in order to obtain comparable data for further analysis.

Therefore, the algorithm was initialized selecting the spectra of Form I, Form II and excipient matrix. In order to confer physical sense to the results, data were analyzed using "non-negativity" restriction in spectral mode and "non-negativity" and "unimodality" restrictions for the concentrations. The “closure condition” of the concentration values was applied among Form I and Form II of MFA (indicating that the sum of the abundance of species studied is equal to 100%). This restriction was applied after assuming that no decomposition takes place during the process being studied and that, therefore, there is no change in the total number of moles of MFA along the whole operation.

The so obtained “pure” spectra were used to establish the identity of the species involved in the process. A comparison between the reference spectra of Form I and Form II and “pure” spectra provided by the MCR-ALS algorithm for COM, COMEX and commercial tablets (TAB1 and TAB2) is shown in Figure 5.

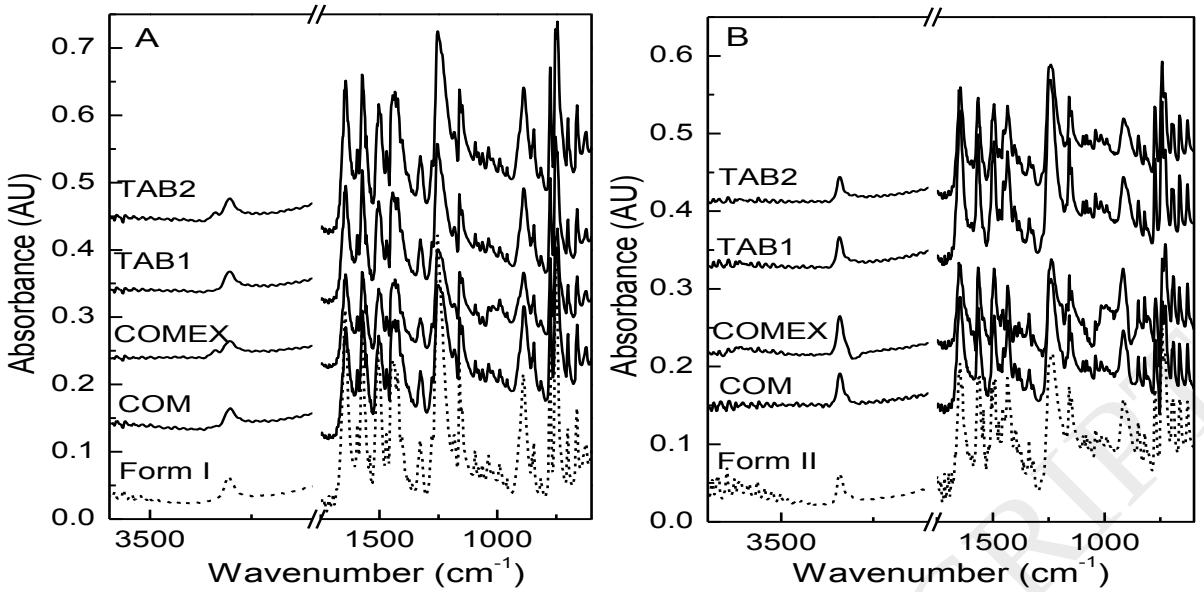


Figure 5. Comparison between reference spectra (⋯) and MCR-ALS spectra (-) involved in polymorphic conversion of MFA in COM, COMEX, TAB1 and TAB2 for Form I **(A)** and Form II **(B)**.

Figure 6A shows the concentration profiles of Form I and Form II obtained from COM, as expected no intensity was found in the excipient vector form COM samples. At 7 h after heating, abundance of Form I was reduced at 50%, reaching 20% at 83 h after beginning of the process.

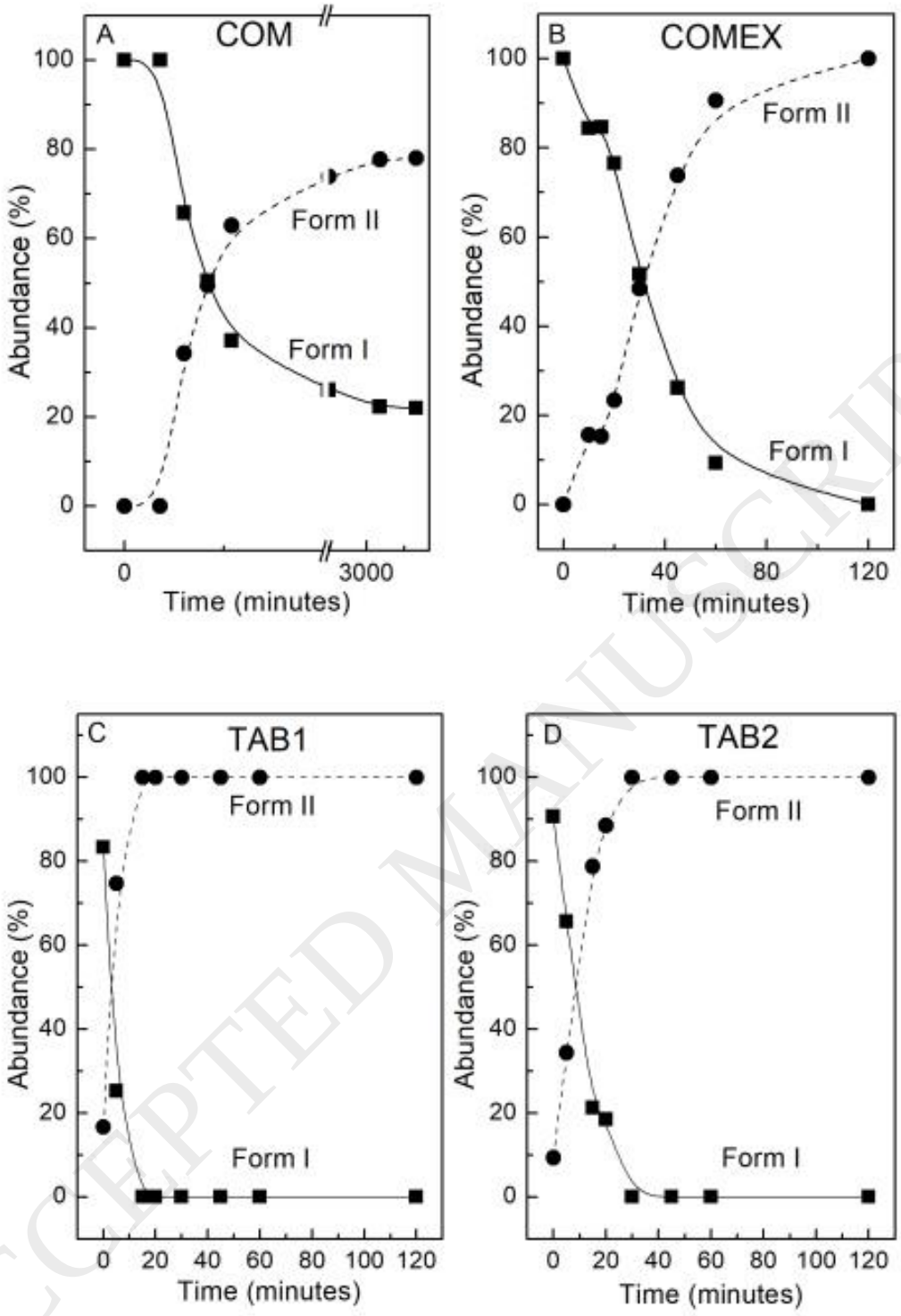


Figure 6. MCR-ALS profiles obtained for thermal processing of COM **(A)**, COMEX **(B)** TAB1 **(C)** and TAB2 samples **(D)**. Abundance of Form I (-■-) and Form II (-●-).

When COMEX samples were studied (Figure 6B), the conversion process results accelerated in comparison to COM by a reduction of induction time and an increase of conversion rate. The contact with the excipients does not introduce significative spectral signals

in pure polymorphs (Figure 5 A and B), but complete transformation to Form II was observed at 60 minutes. Excipient concentration profile and spectral vector showed no variation (Figure S3 and S4) along the experimented confirming no chemical interaction exists among MFA and excipients matrix.

When commercial tablets were analyzed (Figure 6 C and D) the transformation started within first 10 minutes, reaching a 50% of Form II at 15 minutes. The complete polymorphic transformation was observed after 30 minutes, showing a slight acceleration of transformation process, in comparison to COMEX, due to higher interactions of MFA and excipients gained during manufacture. As in the case of COMEX excipients concertation profile and spectral vector (see supplementary material, Figure S3 and S4).

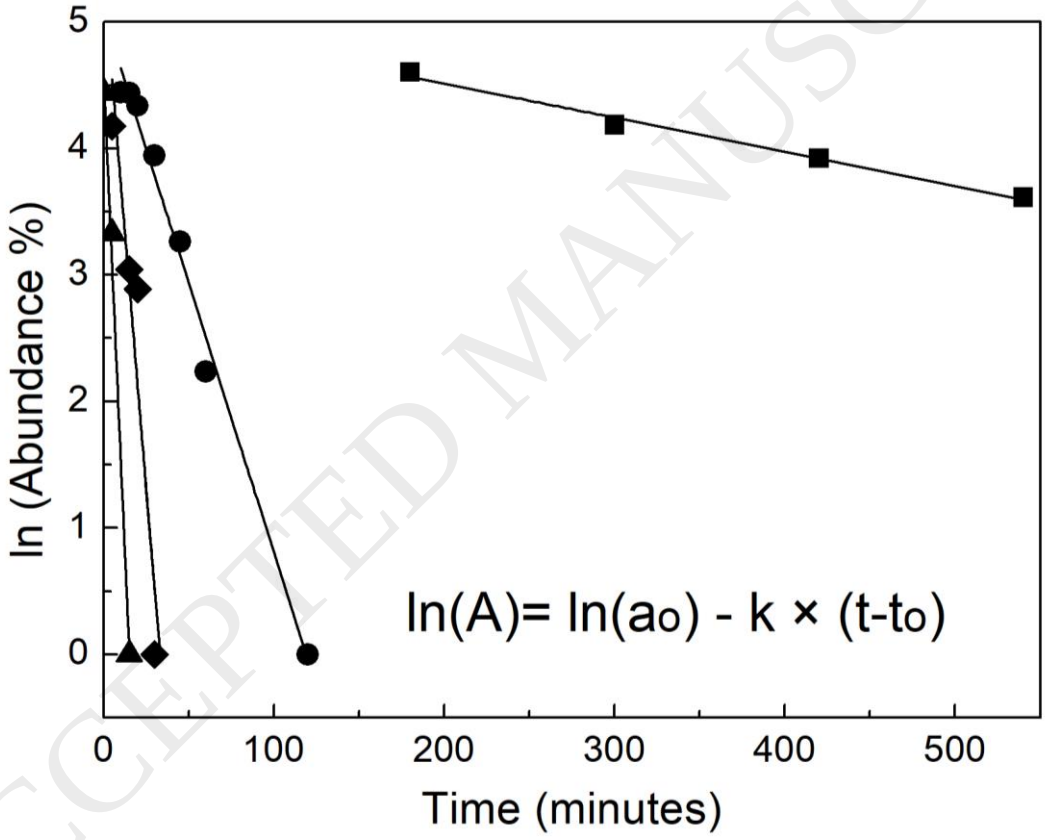


Figure 7. Decay curves for Form I abundance obtained during thermal treatment of COM (■), COMEX (●), TAB1 (▲) and TAB2 (♦).

In the case of commercial tablets (Figure 6 C and D), the MCR-concentration profiles evidenced the initial composition is not pure Form I, but around 90%, showing a possible polymorphic change produced during the manufacturing process of the dosage form or its

storage. Both TAB1 and TAB2 exhibited comparable behavior, where the total transformation to Form II was achieved about 20 and 30 minutes for powdered (TAB1, Figure 6C), and sliced tablets (TAB2, Figure 6D), respectively.

In order to analyze the kinetic behavior of polymorphic conversion of MFA, kinetics curves of Form I decay were constructed (Figure 7), and the experimental equations were calculated for its analytical comparison (Table 1). As expected, COM showed the higher induction time t_o start the transformation and therefore the lowest value of k, in agreement with the observation listed above. The greater conversion rate observed in the commercial tablets (TAB1 and TAB2) in comparison with the COMEX (physical mixture) can be explained by the absence of induction times in the first ones and higher kinetics constants. This observation allows us to infer formulated products hold higher contact among MFA and excipients. This higher contact, maybe obtained during granulation process, maximizes the physical MFA-excipient interaction.

On the other hand, the faster transformation of TAB1 (the highest k=0.03018 min^{-1}) could be explained due to its small and homogeneous particle size in comparison to TAB2, which improve to heat transference sample-container.

Table 1. Equation parameters for first order exponential decay of Form I in thermal stressed samples.

$A= a_o \times e^{-k\,(t-to)}$	a_o	k	t_o (min)
COM	100	0.0027	180
COMEX	100	0.026	8
TAB1	90	0.3018	0
TAB2	90	0.0921	0

Finally, the thermal behavior of commercial tablets was also analyzed by DSC, in order to correlate the results obtained by MCR-ALS. The DSC thermograms are presented in Figure 8. As described in section 3.1, COM showed two endothermic peaks at 175 and 238 ºC, corresponding to the transition of Form I to Form II and the melting point of as Form II, respectively. TAB1 also showed two endothermic peaks similar to those evidenced by COM. However, a shift in first peak position, from 182 ºC to 165 ºC, was observed.

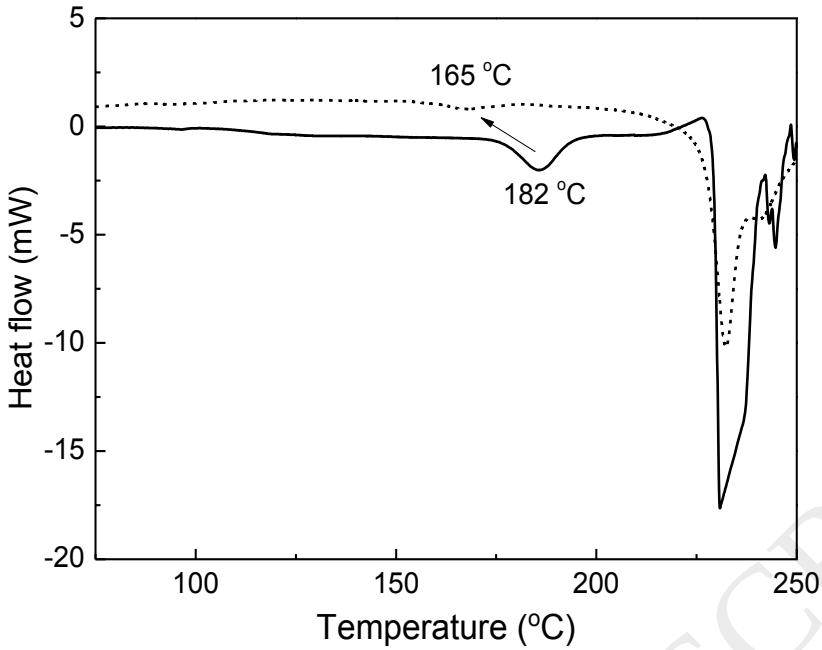


Figure 8. DSC thermograms obtained for COM (-) and TAB1 (--).

The heat for this transformation became almost imperceptible in comparison to the phenomena observed for COM. This change in the transition temperature and peak area suggests the presence of excipients in the formulated product interacts with MFA decreasing temperature and energy necessary to the crystal conversion.

It should be noted that although a possible interaction between API and excipients could be supposed from the changes evidenced in the DSC thermograms (shape or area of the peaks), only the use of MIR spectroscopy coupled to MCR-ALS provides qualitative and quantitative information of the phenomenon, evidencing about the nature of such interactions.

4. Conclusions

In the present work, a smart approach based on thermal stress, MIR monitoring, MCR-ALS algorithm and kinetic analysis was developed to explore API-excipients interactions which exhibit polymorphism. MFA as drug substance, physical mixtures and commercial tablets were thermally stressed and analyzed in order to unveil changes in the nature of enantiotropic conversion of API. The proposed MIR/MCR-ALS approach allowed to determine the influence of excipients in the conversion process (Form I to Form II), and the kinetic studies allowed to infer changes of the process and involved activation energies.

The MCR-ALS concentration profiles revealed commercial API (Form I) underwent a slow but progressive conversion to Form II (after 5 hours), for physical mixtures transformation

occurs in short time due to MFA-excipients physical interactions. TAB1 and TAB2 showed a very fast transformation of Form I to Form II, being TAB2 slightly faster, due to a better heat transference. Additionally, TAB1 and TAB2 MCR-analysis revealed a polymorphic change likely occurred during the manufacture or storage of tablets. Change in kinetics curves (induction time and kinetic constant) for COM, COMEX, TAB1 and TAB2 carried out from so obtained MCR-concentration profiles showed a marked fall in activation energy reinforcing the idea of API-excipient physical interaction.

Therefore, the developed approach, based on thermal stress, MIR/MCR-ALS resolution, the so obtained spectra analysis and kinetic calculations should be considered as a suitable strategy to reveal potentially incompatible excipients and unstable conditions from the point of view of polymorphism; thus improving QbD of a new product.

Acknowledgements

The authors gratefully acknowledge Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET, Project PIP 2011-0471) and Secretaría de Ciencia y Tecnología de la UNR (SECyT-UNR, Projects BIO300 and BIO498) for financial support. We would like to thank the staff from the English Department (Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario) for their assistance in the language correction of the manuscript. NLC thanks CONICET for its postdoctoral fellowship.

References

1. J. Haleblian, W. Mc Crone, Pharmaceutical applications of polymorphism, J. Pharm. Sci. 58 (1969) 911 – 929.
2. D. Singhal, W. Curatolo, Drug polymorphism and dosage form design: a practical perspective, Adv. Drug Del. Rev. 56 (2004) 335-347.
3. V. R. R. Cunha, C. M. S. Izumi, Mefenamic Acid Anti-Inflammatory Drug: Probing Its Polymorphs by Vibrational (IR and Raman) and Solid-State NMR Spectroscopies, The J. of Phys. Chem. 118 (2014) 4333-4344.
4. H. G. Brittain, Polymorphism in Pharmaceutical Solids, 2nd ed., Informa Healthcare, Inc., New York, USA, 2009.

5. R. Tinmanee, S. C. Larsen, K. R. Morris, L. E. Kirsch, Quantification of gabapentin polymorphs in gabapentin/excipient mixtures using solid state 13C NMR spectroscopy and X-ray powder diffraction, J. Pharm. Biomed. Anal. 146 (2017) 29-36.

6. G. G. Z. Zhang, D. Law, E. Schmitt, Phase transformation considerations during process development and manufacture of solid oral dosage forms, Adv. Drug Del. Rev. 56 (2004) 371-390.

7. B. Tita, A. Fulias, G. Bandur, Compatibility study between ketoprofen and pharmaceutical excipients used in solid dosage forms, J. Pharm. Biomed. Anal. 56 (2011) 221-227.

8. R. Chadha, S. Bhandari, Drug–excipient compatibility screening—Role of thermoanalytical and spectroscopic techniques, J. Pharm. Biomed. Anal. 87 (2014) 82-97.

9. K. Krūkle-Bērziņa, A. Actiņš, The effect of excipients on the stability and phase transition rate of xylazine hydrochloride and zopiclone, J. Pharm. Biomed. Anal. 107 (2015) 168-174.

10. R. K. Verma, S. Garg, Compatibility studies between isosorbide mononitrate and selected excipients used in the development of extended release formulations, J. Pharm. Biomed. Anal. 35 (2004) 449-458.

11. J. Wardrop, K. Engh, L. Faitsch, D. Law, C. Ling, Y. Qiu, Investigation of a process induced instability due to formation of amorphous ABT-232 in tablets, J. Pharm. Sci. 95 (2006) 2380-2392.

12. N. L. Calvo, R. M. Maggio, T. S. Kaufman. Chemometrics-assisted solid-state characterization of pharmaceutically relevant materials. Polymorphic substances. J. of Pharm. and Biomed. Anal., 147 (2018) 518–537.

13. Z. Német, I. Sajó, A. Demeter, Rietveld refinement in the routine quantitative analysis of famotidine polymorphs, J. Pharm. and Biomed. Anal. 51 (2010) 572-576.

14. P. Mura, M. T. Faucci, A. Manderioli, G. Bramanti, L. Ceccarelli, Compatibility study between ibuproxam and pharmaceutical excipients using differential scanning calorimetry, hot-stage microscopy and scanning electron microscopy, J. Pharm. Biomed. Anal. 18 (1998) 151-163.

15. D.Law, D.Zhou, Developing Solid Oral Dosage Forms, 2nd ed., Academic Press, (2017) 59-84.

16. N. L. Calvo, J. M. Arias, A. B. Altabef, R. M. Maggio, T. S. Kaufman , Determination of the main solid-state form of albendazole in bulk drug, employing Raman spectroscopy coupled to multivariate analysis, J. Pharm. Biomed. Anal. 129 (2016) 190–197.

17. N. L. Calvo, T. S. Kaufman, R. M. Maggio, Mebendazole crystal forms in tablet formulations. An ATR-FTIR/chemometrics approach to polymorph assignment, J. Pharm. Biomed. Anal. 122 (2016) 157–165.

18. N. L. Calvo, T. S. Kaufman, R. M. Maggio, A PCA-based chemometrics-assisted ATR-FTIR approach for the classification of polymorphs of cimetidine: Application to physical mixtures and tablets, J. Pharm. Biomed. Anal. 107 (2015) 419–425.

19. M. Antonio, R. M. Maggio, Assessment of mefenamic acid polymorphs in commercial tablets using chemometric coupled to MIR and NIR spectroscopies. Prediction of dissolution performance, J. Pharm. Biomed. Anal. 149 (2018) 603–611.

20. N. L. Calvo, R. M. Maggio, T. S. Kaufman, A dynamic thermal ATR-FTIR/chemometric approach to the analysis of polymorphic interconversions. Cimetidine as a model drug, J. Pharm. Biomed. Anal. 92 (2014) 90–97.

21. N. L. Calvo, N. M. Balzaretti, M. Antonio, T. S. Kaufman, R. M. Maggio, Chemometrics-assisted study of the interconversion between the crystalline forms of nimodipine, J. Pharm. Biomed.Anal. 158 (2018) 461–470.

22. M. A. Van Eijkeren, G. C. Christiaens, Effects of mefenamic acid on menstrual hemostasis in essential menorrhagia, American J. of Obstetrics and Gynecology 166 (1992) 1419-1428.

23. R. K. Gilpin, W Zhou, Infrared studies of the thermal conversion of mefenamic acid between polymorphic states, Vibr. Spectr. 37 (2005) 53–59.

24. A. Burger, R. Ramberger, Thermodynamische Beziehungen zwischen polymorphen. Modifikationen: Flufenaminsiure und Mefenaminsiure, Mikrochim. Acta (1980) 17–28.

25. T. Umeda, N. Ohnishi, T. Yokoyama, A kinetic study on the isothermal transition of polymorphic forms of tolbutamide and mefenamic acid in the solid state at high temperatures, Chem. Pharm. Bull. 33 (1985) 2073–2078.

26. S. Jabeen, T. Dines, S. Leharne, B. Chowdhry, Raman and IR spectroscopic studies of fenamates. Conformational differences in polymorphs of flufenamic acid, mefenamic acid and tolfenamic acid spectrochim, Acta A Mol. Biomol. Spectrosc. 96 (2012) 972–985.

27. M. Otsuka, J. Nishinawa, Quantitative Evaluation of Mefenamic Acid Polymorphs by Terahertz–Chemometrics, J. of Pharm. Sci. 99 (2010) 4048-4053.

28. R. Panchagnula, P. Sundaramurthy, O. Pillai, S. Agrawal, Y. A. Raj, Solid-state characterization of mefenamic acid, J. Pharm. Sci. 93 (2004) 1019-1029.

29. J. Chen, S. Sun, Q. Zhou, Rapid identification and quantification of carbohydrate excipients in Gardeniae Fructus formula granules by ATR-FTIR spectroscopy. Anal. Methods 47 (2016) 8329-8336.

30. J. Jaumot, B. Igne, C. A. Anderson, J. K. Drennen, A. de Juan. Blending process modeling and control by multivariate curve resolution. .Talanta. 15 (2013) 492-504.