



Utilizing hot-stage polarized microscopy and ATR-FTIR for ramipril co-crystal screening, supported by principal component analysis and cluster analysis

[Utilización de microscopía polarizada en caliente y ATR-FTIR para el cribado de co-cristales de ramipril, con el apoyo del análisis de componentes principales y el análisis de conglomerados]

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Abstract

Context: Co-crystal formation, a method for enhancing the physicochemical properties of active pharmaceutical ingredients (APIs), has gained traction in pharmaceutical research. However, the current landscape lacks comprehensive and dependable co-crystal screening methods.

Aims: To implement and assess a comprehensive methodology for co-crystal screening. This methodology combines hot-stage polarized microscopy (HSPM) and attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy, along with principal component analysis (PCA) and cluster analysis (CA).

Methods: Three binary compounds containing ramipril, an API that had not previously been co-crystallized, and three pharmaceutical cofomers were investigated. The Kofler mixed fusion method was initially employed for initial co-crystal system identification. Subsequently, potential co-crystals were produced in a solid state via a procedure involving the gradual evaporation of the solvent. PCA and CA were applied to ATR-FTIR spectral data to identify patterns indicative of co-crystal formation.

Results: Our analysis revealed characteristic ATR-FTIR bands indicative of the formation of hydrogen bonds between ramipril and its cofomers, signifying the successful formation of co-crystals. Differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) measurements confirmed these findings. Experiments revealed two potential co-crystals, ramipril-vanillin, and ramipril-anthranilic acid. The study discusses the intricate HSPM images, spectra, thermogram of DSC, and X-ray diffraction properties of these systems in depth.

Conclusions: Our findings validate the proposed methodology as a prospective tool for co-crystal screening, as ramipril co-crystals were successfully identified and characterized. This integrated method simplifies co-crystal screening and has the potential to substantially advance pharmaceutical research.

Keywords: anthranilic acid; crystallization; isonicotinamide; multivariate analysis; vanillin; X-ray diffraction.

Resumen

Contexto: La formación de cocristales, un método para mejorar las propiedades fisicoquímicas de los principios activos farmacéuticos (API), ha ganado terreno en la investigación farmacéutica. Sin embargo, el panorama actual carece de métodos de cribado de cocristales completos y fiables.

Objetivos: Implementar y evaluar una metodología integral para el cribado de cocristales. Esta metodología combina la microscopía polarizada de fase caliente (HSPM) y la espectroscopia infrarroja de transformada de Fourier de reflexión total atenuada (ATR-FTIR), junto con el análisis de componentes principales (PCA) y el análisis de conglomerados (CA).

Métodos: Se investigaron tres compuestos binarios que contenían ramipril, un API que no se había co-cristalizado previamente, y tres cofomadores farmacéuticos. Inicialmente se empleó el método de fusión mixta de Kofler para la identificación inicial del sistema de cocristales. Posteriormente, se produjeron posibles cocristales en estado sólido mediante un procedimiento que implicaba la evaporación gradual del disolvente. Se aplicaron PCA y CA a los datos espectrales ATR-FTIR para identificar patrones indicativos de la formación de cocristales.

Resultados: Nuestro análisis reveló bandas ATR-FTIR características indicativas de la formación de enlaces de hidrógeno entre el ramipril y sus cofomadores, lo que significa la formación satisfactoria de cocristales. Las mediciones de calorimetría diferencial de barrido (DSC) y difracción de rayos X en polvo (PXRD) confirmaron estos hallazgos. Los experimentos revelaron dos posibles cocristales, ramipril-vainillina y ramipril-ácido antranílico. El estudio analiza en profundidad las intrincadas imágenes HSPM, los espectros, el termograma de DSC y las propiedades de difracción de rayos X de estos sistemas.

Conclusiones: Nuestros resultados validan la metodología propuesta como una herramienta prospectiva para el cribado de co-cristales, ya que los co-cristales de ramipril fueron identificados y caracterizados con éxito. Este método integrado simplifica el cribado de cocristales y tiene el potencial de hacer avanzar sustancialmente la investigación farmacéutica.

Palabras Clave: ácido antranílico; análisis multivariante; cristalización; difracción de rayos X; isonicotinamida; vainillina.

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INTRODUCTION

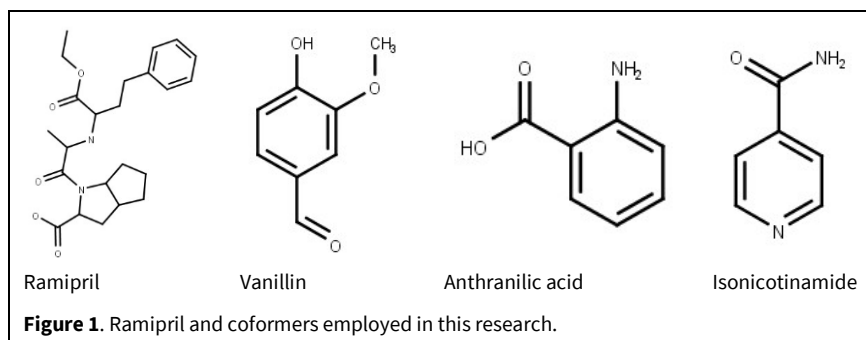
Owing to their physicochemical characteristics, active pharmaceutical ingredients (APIs) may present a challenge when seeking therapeutical results. There is the potential for an API to have limited or no therapeutic application due to insolubility in water, poor dissolution, or instability. Consequently, research into the improvement of API properties is crucial. Countless scientific studies have shown that modifying the crystal form of APIs can improve their physicochemical properties (Budziak-Wieczorek and Maciołek, 2021). The most common ways to accomplish this include salt formation, amorphization, and novel polymorphism (Cerrea Vioglio et al., 2017). In the past decade, co-crystals have attracted attention due to their capacity to improve essential API properties (Karimi-Jafari et al., 2018). The increased excitement about co-crystallization is expressed in the publication of three documents on the subject by the Food and Drug Administration (FDA) over five years (Center of Drug Evaluation and Research, 2018; FDA, 2016) and the global debate among researchers about co-crystal classification (Aitipamula et al., 2012).

Due to the potentially desirable features, the construction of novel co-crystals has become a crucial research subject. As a result, screening methods for the search of co-crystals are undergoing comprehensive growth (Luu et al., 2013), with spectroscopic methods being the most commonly suggested instruments. Fourier-transform infrared (FTIR) spectroscopy is one of the spectroscopic techniques that can be used for this purpose (Du et al., 2016). We selected infrared spectroscopy as the primary analytical technique because it is generally less expensive and more accessible than X-ray diffraction approaches (Issa et al., 2012). Generally, screening procedures may be classified as solid-state or liquid-state (Lin et al., 2013). In both cases, FTIR is a valuable technique for verifying co-crystal formation. It allows the recognition of co-crystals and can serve as an insightful source of knowledge about the structures obtained (Wu et al., 2011). The formation of hydrogen bonds between the API and the coformer alters the spectrum of the co-crystal in addition to the spectra of the initial components (Rahman et al., 2011). Nevertheless, FTIR spectroscopy has limitations in identifying co-crystals due to the interference of API and coformer patterns in the co-crystal spectrum and the potential absence or introduction of FTIR bands, shifts in band intensities, and broadening. To improve co-crystal identification accuracy, additional methods should be used alongside FTIR spectroscopy (Saganowska and Wesolowski, 2017).

Lately, chemometric applications have obtained the most attention as a confirmatory instrument for co-crystal formation (Saganowska and Wesolowski, 2017). Principal component analysis (PCA) and cluster analysis (CA) have received the most coverage among multivariate approaches (Garbacz and Wesolowski, 2018). PCA calculations are used to visualize the co-crystal formation based on data obtained from the FTIR spectra (Sarraguça et al., 2016). PCA identifies critical stages in the operation, which can result in better parameter regulation during co-crystallization. FTIR spectroscopy in conjunction with PCA was also found to be beneficial for observing co-crystallization pathways when processes involving the exact mechanism of co-crystallization were carried out in various ways (Chun et al., 2014). Additionally, PCA can distinguish polymorphic types of carbamazepine and saccharin from co-crystals using both powder X-ray diffraction (PXRD) and FTIR results (Caliandro et al., 2013).

Hot-stage polarized microscopy (HSPM) is a mix of thermal analysis techniques with the most advanced imaging technology, such as digital cameras and optical microscopes, capable of assessing sample changes through recorded data (photos or videos) produced during thermal studies (Kumar et al., 2020). Due to its capacity to detect the probability of co-crystal formation between API and prospective cofomers, HSPM has recently gained widespread attention as a technique for co-crystal screening. Co-crystal screening with HSPM becomes significantly faster and avoids the requirement for co-crystal samples obtained from time-consuming and arduous conventional techniques (e.g., solution-based method) (Berry et al., 2008).

This paper proposes an enhanced methodology for co-crystal screening employing HSPM and FTIR instruments in conjunction with chemometric analysis. Ramipril was employed as a model substance since it is poorly water soluble and belongs to the biopharmaceutical classification system class II (Indra et al., 2020). Despite various attempts to enhance the aqueous solubility of ramipril, the formation of a co-crystal form remains elusive. The intricate molecular flexibility and conformational complexity of ramipril provide multiple potential sites for intermolecular interactions with the coformer, thereby resulting in the formation of several co-crystals or none at all (Karagianni et al., 2018). Curiously, the Cambridge Crystallographic Data Centre (CCDC) database only has one crystallographic structure of the salt form of ramipril. Therefore, this work aimed to demonstrate to what extent HSPM and FTIR, supplemented by chemometric analysis, can be employed as proper techniques to



screen for the co-crystallization of ramipril. HSPM was applied based on Kofler mixed fusion, to determine if a co-crystal phase occurs. Chemometric analyses, such as PCA and CA, were applied to the FTIR spectra to precisely identify co-crystal formation between ramipril and cofomer (Fig. 1). In addition to being validated, the novel co-crystal form was identified using DSC and PXRD.

MATERIAL AND METHODS

Chemicals

Ramipril was purchased from Tokyo Chemical Industry. Vanillin, anthranilic acid, and isonicotinamide were supplied by Wako Pure Chemical Industries Ltd. Early studies of these compounds revealed that ramipril existed in the anhydrous form. In contrast, vanillin, anthranilic acid, and isonicotinamide were confirmed to be polymorphs of form I. Analytical grade solvents were obtained from Tokyo Chemical Industry and utilized without purification.

Hot-stage polarized microscopy (HSPM)

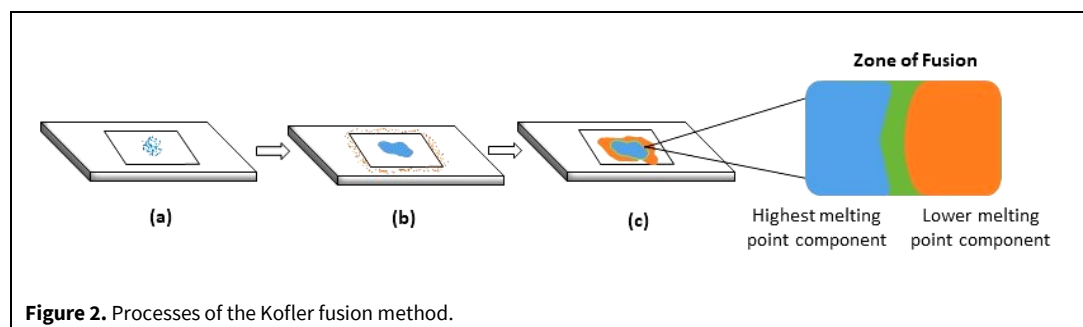
The experimental procedures were carried out utilizing an NPL-107A polarizing microscope (All-Pro, China) that was fitted with a D-line automation hot stage (Heater Analyzer V.02, Indonesia). Photomicrographs were obtained through the use of a CCD cam-

era (ToupTek Photonics, China). Contact thermal microscopy (NPL-107, Biobase, China) was conducted by gradually heating from ambient temperature utilizing a heating rate of 5°C/min and ceased upon the complete melting of all the material.

Positioned in the center of the glass slide and shielded from view by a cover glass (Fig. 2a), the component with the higher melting temperature was allowed to solidify after melting. A second component with a lower melting temperature was placed on the edge of the cover glass and melted (Fig. 2b). Under the cover glass, the capillary force caused the molten liquid to contact the solid component. When all the material had recrystallized, the "fusion zone" was observed (Fig. 2c).

Sample preparation

Binary physical mixtures of ramipril and cofomer, vanillin, anthranilic acid or isonicotinamide, were prepared by gently combining ramipril and cofomer in an agate mortar for 5 minutes. Ramipril co-crystals were prepared according to the slow solvent evaporation method, with the ramipril and cofomer being mixed in a stoichiometric 1:1 molar ratio and dissolved in dichloromethane at an ambient temperature. The solution evaporated slowly, and the sample appeared as a white needle form after 2-3 days.



ATR-FTIR analysis

The infrared FTIR spectra were recorded using an Agilent (Agilent Technologies, United State) Cary-630 spectrometer equipped with an Attenuated Total Reflectance (ATR) accessory with a ZnSe crystal. The measurement was made at ambient temperature, pressing samples against a high-refractive index prism. The FTIR spectra were obtained for all starting materials (ramipril, cofomers) and co-crystals, and the physical mixture was also analyzed for comparison. Spectra were obtained with Microlab (Microlab Expert, Agilent, Unites State) PC software in the 4000-650 cm^{-1} spectral range with a resolution of 4 cm^{-1} , and background spectra were taken with an average of 16 curves.

Differential scanning calorimetry (DSC)

The thermal characteristics of the samples were assessed using a Shimadzu DSC-60Plus, which was calibrated for temperature and cell constants with indium. Analysis was carried out on 5-7 mg of each sample, secured in an aluminum pan. The temperature range for analysis was set between 40 and 250°C, with a heating increment of 10°C per minute. Throughout the process, the samples were consistently flushed with nitrogen at a rate of 10 mL/min.

Powder X-ray diffraction (PXRD) analysis

The diffraction pattern was determined by PXRD (Rigaku, Tokyo, Japan) using Cu-K α radiation (1.54184 Å) with a tube voltage of 45 kV and a current of 200 mA in the 2 θ angle range of 5-40°. The sample was gently ground, placed on thin polyester film (Mylar, USA), and measured at room temperature with a scanning speed of 2°/min.

Data analysis

The spectra were investigated using PCA and CA to compare the spectra of API and cofomers as physical mixtures and as potential co-crystals at 1:1. The FTIR spectra were recorded in triplicate and served as the input matrices for the PCA and CA calculations. The number of rows in matrices including ramipril, physical mixtures, and co-crystal prepared in 1:1 molar ratio was constant at a figure of seven. The variables used in both analyses were transmittance values obtained from FTIR spectra, measured at an interval of 3.86 cm^{-1} within a spectral range of 653.49 to 3601.47 cm^{-1} . Prior to PCA, the FTIR spectra were pre-processed with a standard normal variate (SNV) algorithm using Spectragryph Ver. 1.2.14 (Friedrich Menges, Germany) to account for light scattering-induced noise (Lee et al., 2017). The SNV correction was carried out using the equation [1].

$$x_{corr} = \frac{x_{org} - \alpha_0}{\alpha_1} \quad [1]$$

where x_{corr} is the corrected value, x_{org} is the original value, α_0 is the average value of the sample spectrum to be corrected, and α_1 is the standard deviation of the sample spectrum (Rinnan et al., 2009).

The PCA and CA models were estimated using Minitab 21 (Minitab Inc, State College, Pennsylvania, USA). In the case of principal component analysis (PCA), the covariance matrices were utilized to calculate the principal components (PCs) and the data were centered accordingly. The results of the PCA calculations were displayed as score scatter plots, which depicted the distribution of objects along the first two PCs. Cluster analysis (CA) calculations were performed using the Euclidean distance to measure the dissimilarity between pairs of objects, and Ward's linkage criterion was employed to merge objects or clusters of objects. The resulting classification of objects was visually presented in the form of a tree diagram.

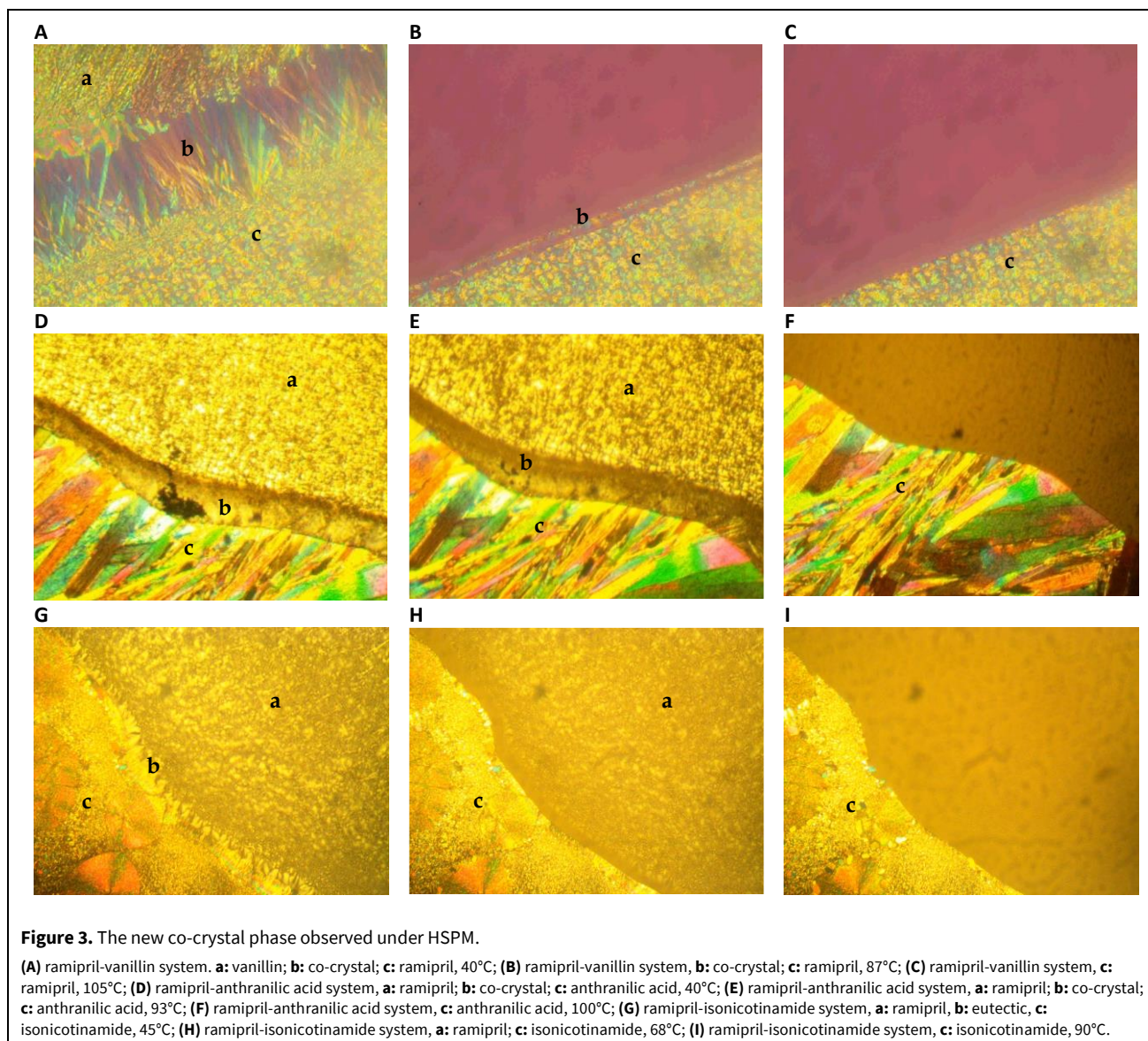
RESULTS AND DISCUSSION

HSPM analysis

Two of the three cofomers were identified as potential ramipril co-crystal systems using the Kofler mixed fusion method (Fig. 3). These cofomers, vanillin, and anthranilic acid, each displayed two eutectic points during heating analysis with HSPM. The fusion region between a high melting point (ramipril) and low melting point (vanillin), which may represent the formation of a new co-crystalline phase, is clearly observed to form needle crystals in Fig. 3B (section b) at 87°C, followed by the loss of one strip (co-crystal) at 105°C. Co-crystal formation for the ramipril-anthranilic acid system is observed in Fig. 3E (section b) at 93°C, followed by the loss of the middle region (co-crystal - section b) at 100°C. In contrast, the ramipril-isonicotinamide system does not exhibit a strip line between ramipril and isonicotinamide (fusion zone) during the heating process with HSPM; therefore, no new co-crystalline phase is generated in the ramipril-isonicotinamide system.

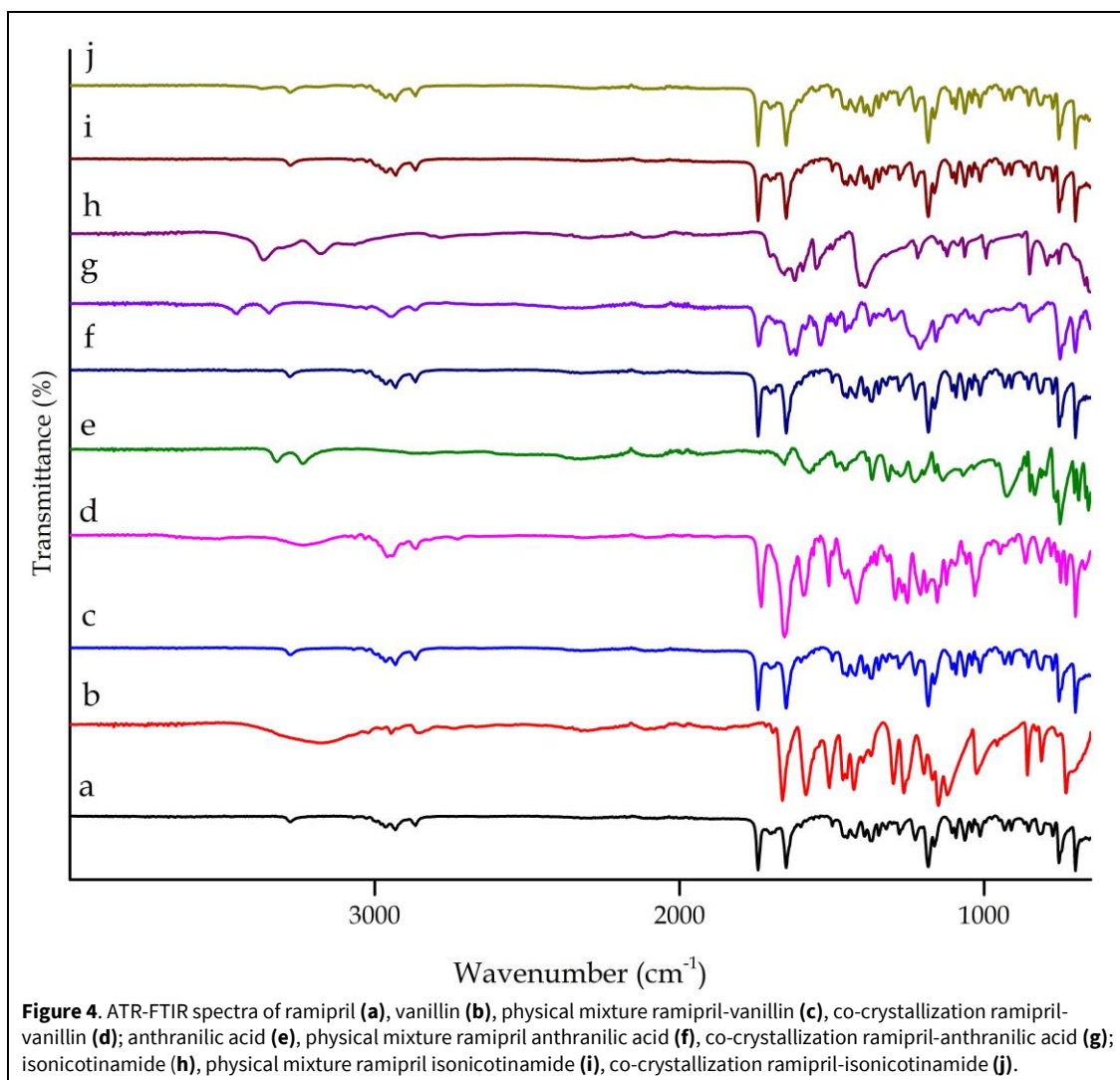
Interpretation of ATR-FTIR spectrum

The aim of the study was attained by recording the FTIR spectra of ramipril, cofomers, a 1:1 physical mixture, and co-crystallized samples. Potential co-crystals were confirmed by comparing the spectrum of the parent compound, the physical mixture, and potential co-crystals. In the next step, PCA and CA calculations were performed to extract detailed information from the FTIR spectroscopy data.



ATR-FTIR spectroscopy was applied to confirm the formation of co-crystals due to its ability to detect vibrational shifts of functional groups, indicating interactions within distinct molecules. Fig. 4 presents the spectra of ramipril, vanillin, anthranilic acid, their physical mixtures, and potential co-crystals. Ramipril (Fig. 4A) revealed a high peak at 3278 cm^{-1} due to NH vibration; -OH and aromatic -CH stretching induced the peaks at 2932 cm^{-1} and 2867 cm^{-1} , respectively; high peaks at 1742 cm^{-1} and 1650 cm^{-1} were due to -C=O stretching of the ester and acid groups, respectively, while a minor peak at 1701 cm^{-1} was due to the carbonyl (C=O) stretching vibration of the amide group; CH bending of aromatic bending can be attributed to other peaks at 1499 cm^{-1} and 1450 cm^{-1} . The FTIR spectrum of vanillin (Fig. 4B) indicates characteristic stretching with a broadband, and the

bending vibration of the -OH group was identified at 3154 cm^{-1} and 1263 cm^{-1} (Fatoni et al., 2018); the stretching vibration of -CH was detected at 2860–3022 cm^{-1} with weak band absorption; the sharp peaks at 1662 cm^{-1} and 632 cm^{-1} relate to the stretching and bending vibrations of the aldehyde group (C=O); and the peak at 1150 cm^{-1} shows the presence of ether groups in the vanillin. The FTIR spectra of anthranilic acid (Fig. 4E) show two peaks at 3321 cm^{-1} and 3235 cm^{-1} , characteristic of amino groups, and a single peak around 1656 cm^{-1} attributed to carboxylic groups (Levy et al., 2003). For isonicotinamide (Fig. 4H), the bands observed at 3368 cm^{-1} and 3186 cm^{-1} were attributed to the -NH₂ stretching vibration, and carbonyl group (C=O) vibration was identified at 1668 cm^{-1} (Wisudyaningsih et al., 2019).



The results of the FTIR investigation revealed that the spectra of the three mixtures (Fig. 4c, f, i) shows only superimposition of the characteristic bands of the original compounds. Hence, it can be concluded that co-crystallization is not possible in these ground mixtures. Detailed inspection of the spectra of the mixtures revealed that the bands characteristic of the original components occurs in the same spectra of the physical mixtures. The lack of change in FTIR spectral positions suggests that the grinding of the initial components did not cause any change in chemical structure and that they still exist as a physical mixture. On the other hand, the spectra of the potential co-crystals revealed a shift in the characteristic bands of ramipril and coformer to lower or higher wavenumbers (Table 1). Bands of ramipril at 1650 cm^{-1} and 1740 cm^{-1} shifted to 1655 cm^{-1} and 1732 cm^{-1} , respectively, in the ramipril-vanillin system (Fig. 4d), while a band of vanillin at 1585 cm^{-1} shifted to 1592 cm^{-1} . The band shift in the FTIR spectra of co-crystals compared to the starting materials indicates changes in the chemical environment and intermolecular interactions,

providing evidence for the formation of the co-crystals.

Moreover, the disappearance of a characteristic band of ramipril at 3278 cm^{-1} and the broad peak at 3229 cm^{-1} was also detected in the ramipril-vanillin system. Shifted bands were also detected in the FTIR spectrum of ramipril-anthranilic acid (Fig. 4g). Shifts in the spectral position of the bands assigned to the amino and carbonyl groups of ramipril and the carboxylic group of anthranilic acid confirm the formation of hydrogen bonds between free hydrogen donors and acceptors of both components, confirming co-crystal formation (Manin et al., 2018). Meanwhile, the spectrum of the co-crystal ramipril-isonicotinamide was similar to that of the physical mixture (Fig. 4i, j), indicating that co-crystallization did not occur.

These findings were further validated using the chemometric methods PCA and CA. The PCA analysis allows to visualize the similarities and differences in the FTIR spectra of the physical mixture, ramipril, and potential co-crystals (Ouiyangkul et al., 2020). If

the physical mixture, ramipril, and potential co-crystals are located in the same PCA space along the PC1 or PC2 axis, it indicates the absence of a co-crystallization process, and as a result, there is no significant FTIR spectral difference. However, if the physical mixture, ramipril, and co-crystals are localized in different PCA spaces along the PC1 and PC2 axes, it indicates the formation of co-crystals, and this can be confirmed by the significant differences in the FTIR spectra. Therefore, the localization of these components in different regions of the PCA space is a critical factor in the determination of co-crystal formation.

The results of the PCA calculations for the studied samples are shown in Fig. 5. An in-depth analysis of the PCA plots revealed that all the physical mixture, the potential co-crystal of ramipril-isonicotinamide, and ramipril (Fig. 5a, b, c, e, g) were clustered on the right side of the plot. PC1 (x-axis) was unable to distinguish between the physical mixtures (Fig. 5a) and the samples for ramipril-isonicotinamide (Fig. 5b), which are quite distinct from the other potential co-crystals. This could be due to the FTIR spectral similarity between the potential co-crystal of ramipril-

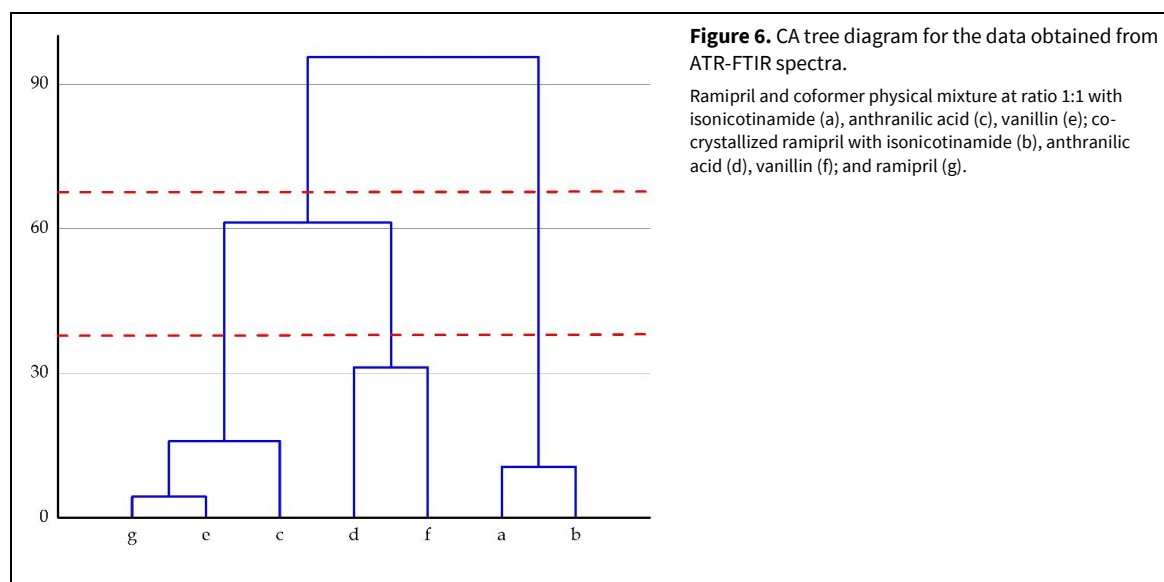
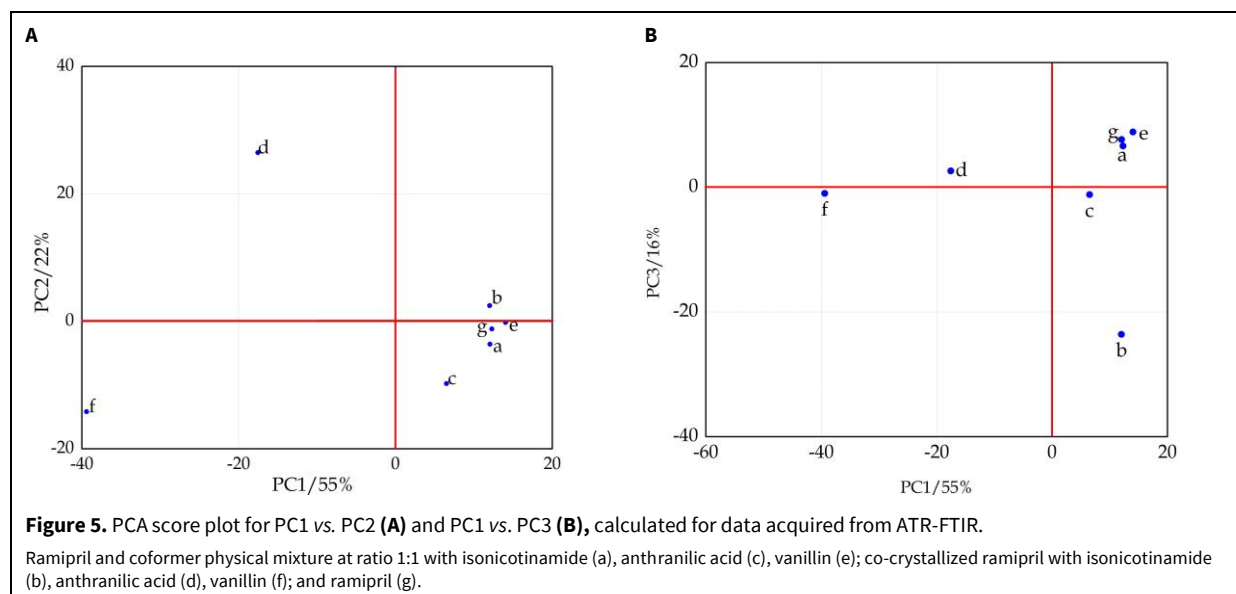
isonicotinamide and the physical mixture, which would rule out the formation of co-crystals of ramipril-isonicotinamide. The potential co-crystals of ramipril-anthranilic acid and ramipril-vanillin (Fig. 5d, f) form a cluster on the left, distinct from their physical mixtures. PC2 and PC3, as a y-axis, had a positive correlation with the ramipril-anthranilic co-crystal (Fig. 5d) and a negative correlation with the ramipril-vanillin co-crystal (Fig. 5f).

The clustering of the physical mixture and potential co-crystal ramipril-isonicotinamide (Fig. 6a, b) was less than 30% distance, indicating that the similarity of the sample spectra made co-crystal formation of ramipril-isonicotinamide not attainable. Therefore, these samples were grouped in the same cluster.

The next cluster shows a group consisting of ramipril (Fig. 6g) with the physical mixture of ramipril-anthranilic acid and ramipril-vanillin (Fig. 6c, e). The possibility of co-crystallization was verified, as ramipril-anthranilic acid and ramipril-vanillin (Fig. 6d, f) formed clusters of greater than 30% distance and separated from the clusters of the physical mixture, indicating that co-crystallization had occurred.

Table 1. Vibrational peaks of ramipril, coformer and potential co-crystal.

Sample	IR peaks
Ramipril	NH Vibration – 3278 cm ⁻¹ C=O stretching of the ester – 1742 cm ⁻¹ C=O acid group – 1650 cm ⁻¹
Vanillin	OH group – 3154 cm ⁻¹ C=O aldehyde – 1662 cm ⁻¹
Anthranilic acid	NH Vibration – 3235 cm ⁻¹ C-H – 1656 cm ⁻¹
Isonicotinamide	NH vibration – 3368 cm ⁻¹ C=O – 1668 cm ⁻¹
Ramipril – vanillin	OH group – 3228 cm ⁻¹ C=O stretching of the ester – 1732 cm ⁻¹ C=O acid group - 1655 cm ⁻¹
Ramipril – anthranilic acid	NH Vibration – 3346 cm ⁻¹ C=O stretching of the ester – 1739 cm ⁻¹ C=O acid group in ramipril – 1632 cm ⁻¹
Ramipril - isonicotinamide	NH Vibration – 3278 cm ⁻¹ C=O stretching of the ester – 1742 cm ⁻¹ C=O acid group – 1650 cm ⁻¹



DSC analysis

Fig. 7 illustrates the DSC curves of ramipril, vanillin, anthranilic acid, and potential co-crystals. Ramipril exhibited a melting endothermic peak at 114°C, while vanillin showed a melting endothermic peak at 84°C. The ramipril-vanillin co-crystal exhibited a single endothermic melting point at 88°C. A distinct co-crystal melting point, falling between those of the individual components, indicates their interaction to produce a new co-crystal phase. The DSC thermogram of anthranilic acid (Fig. 7B) shows a single endothermic peak at 149°C, due to its melting point. The DSC thermogram of the potential co-crystal ramipril-anthranilic acid shows an endothermic peak at 78°C. No other peaks appeared in this thermogram, which suggests that the drug has been completely co-crystallized. On the other hand, the DSC thermogram of isonicotinamide (Fig. 7C) shows an endothermic

peak at 155°C, confirming its melting point. The potential co-crystal of ramipril-isonicotinamide exhibits two endotherms; the first appears at 114°C and the second at 155°C, due to the melting points of ramipril and isonicotinamide, respectively. The DSC thermogram showed no new distinct melting endotherm, but rather an overlapping of the melting endotherms corresponding to ramipril and isonicotinamide separately. This observation excludes the formation of a co-crystal.

PXRD analysis

PXRD was used to identification patterns to validate co-crystal formation. The PXRD patterns of the co-crystallization products revealed distinct peaks that were not present in the ramipril or the representative coformers. As shown in Fig. 8, the PXRD of the ramipril starting material showed characteristic

diffraction peaks at 7.5, 7.9, 16.0, and 17.8°, consistent with the literature data (Kelleher et al., 2018). The PXRD of vanillin (Fig. 8A) had the most significant diffraction peak at 13.3°, as previously reported (Ouyang et al., 2022). The diffraction pattern of anthranilic acid (Fig. 8B) presented distinctive peaks at 10.6, 14.3, 18.6, and 24.2°, similar to that reported in the literature (Gong et al., 2021). The PXRD of isonicotinamide (Fig. 8C) showed distinctive peaks at 17.8, 20.8, 23.3, and 25.8°, similar to a previous study (Castiñeiras et al., 2022). The ramipril-vanillin exhibited new diffraction peaks at 6.1, 8.8, 15.3, and 18.4°

(Fig. 8A). For ramipril-anthranilic acid, some new peaks were observed at 6.5, 8.2, 11.8, and 17.5° (Fig. 8B). These ramipril-vanillin and ramipril-anthranilic acid co-crystals showed different diffraction peaks from either of the starting materials, indicating the formation of the new crystallographic structure of these co-crystals. However, the ramipril-isonicotinamide diffraction pattern did not show any new peaks (Fig. 8C), and the peaks of the starting material were still observed, indicating that co-crystal formation did not occur.

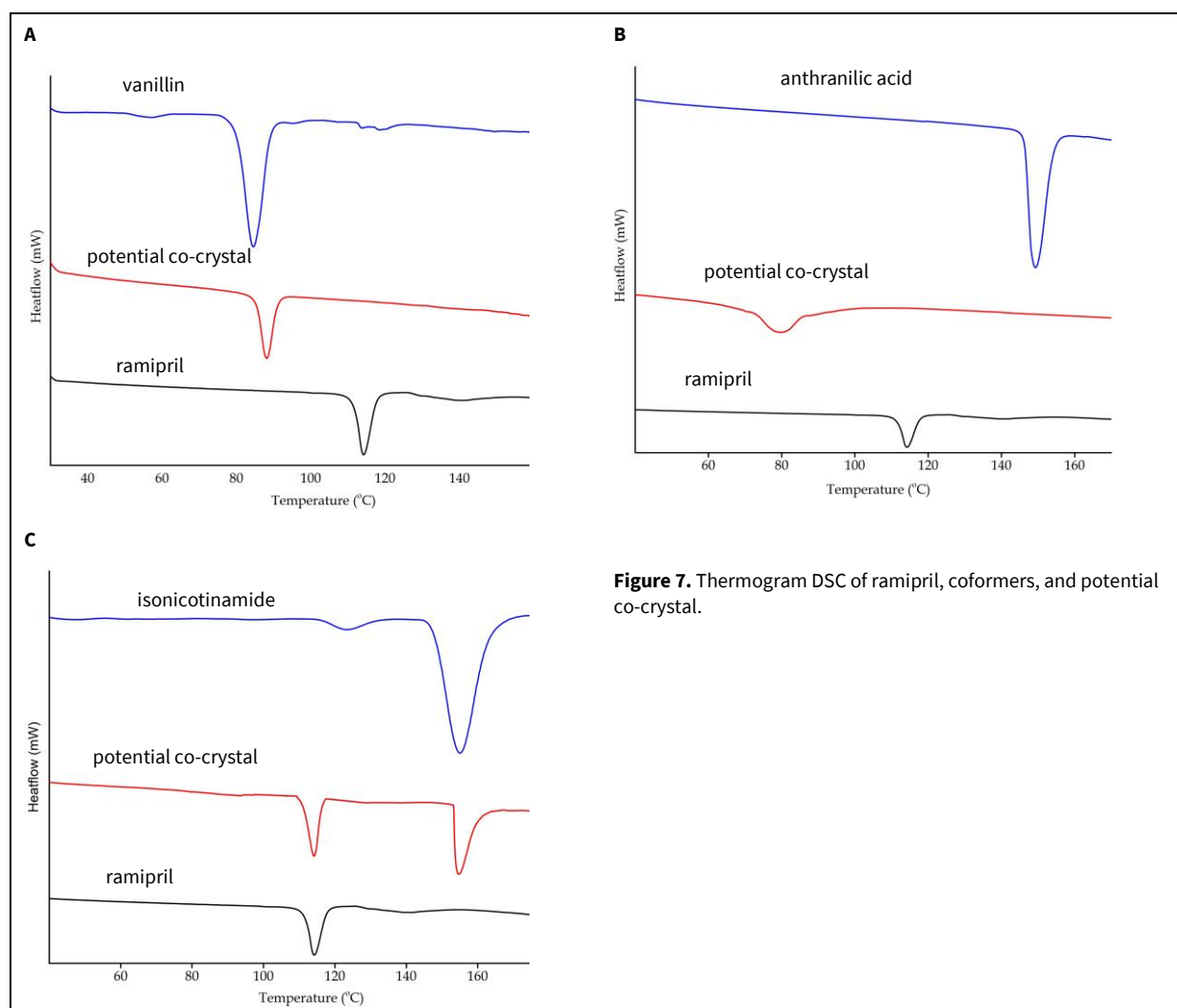


Figure 7. Thermogram DSC of ramipril, cofomers, and potential co-crystal.

CONCLUSION

A fast, green, and reliable method combining HSPM and FTIR has been developed here to analyze co-crystal formation. Two ramipril co-crystals with pharmaceutically acceptable cofomers, vanillin, and anthranilic acid, have been reported for the first time. The fusion zone for the binary phase could be formed in the HSPM profiles when a new phase (co-crystal) was generated. This study establishes that spectro-

scopic methods (ATR-FTIR) that accurately reflect chemical bonding between substances provide information about co-crystal formation.

CA and PCA can provide an adequate distinction between co-crystals and physical mixtures. This research indicates that the complex interpretation of data obtained by the CA and PCA inspection of FTIR spectra allows co-crystals and physical mixtures to be distinguished. The DSC curves and PXRD patterns

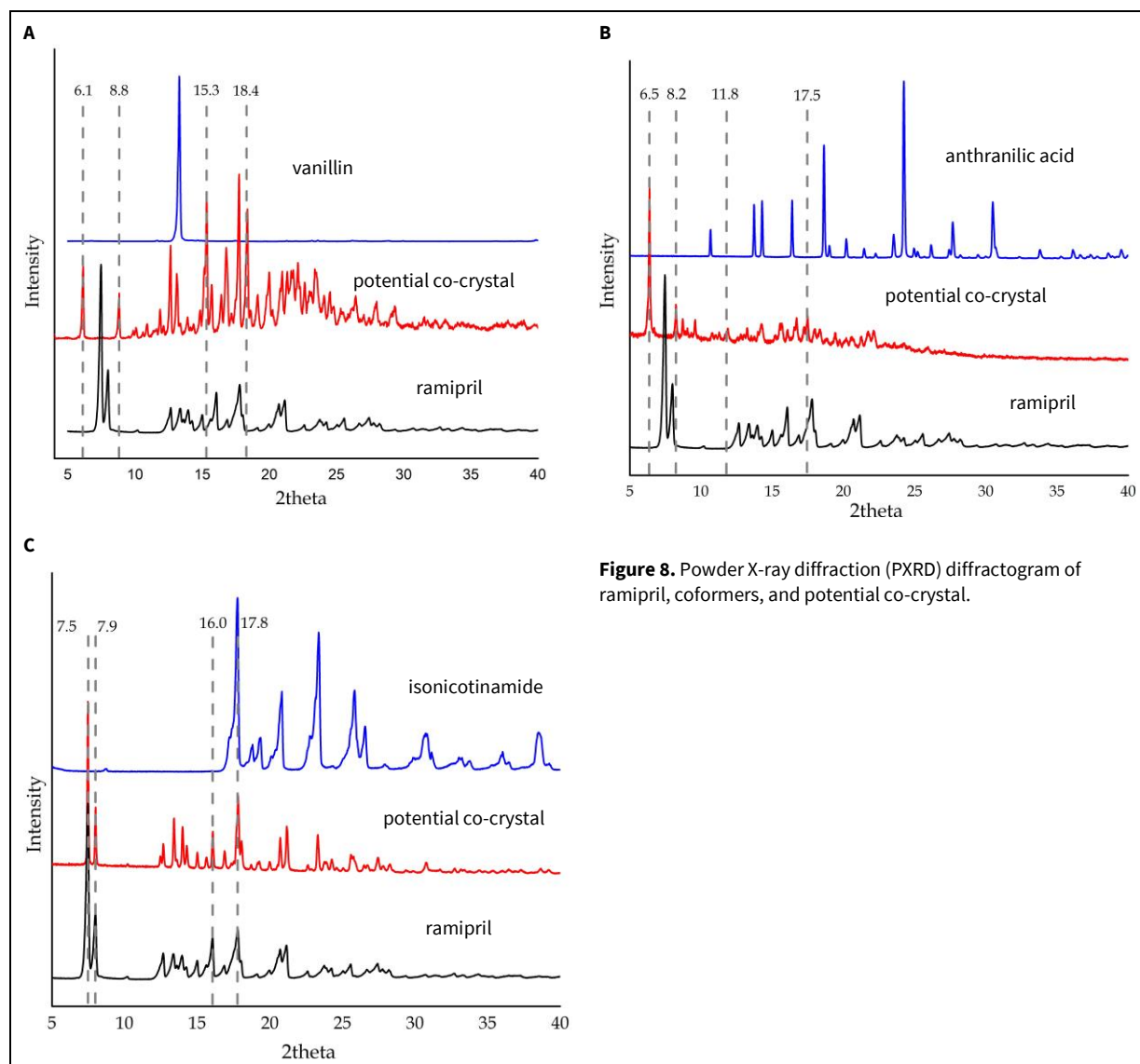


Figure 8. Powder X-ray diffraction (PXRD) diffractogram of ramipril, coformers, and potential co-crystal.

confirmed evidence of successful co-crystal formation for ramipril-vanillin and ramipril-anthranilic acid, as indicated by the appearance of unique endothermic melting points and distinct diffraction peaks. This result broadens the future applications of the combination of HSPM and FTIR in pharmaceutical co-crystal systems.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Indra I	Wikarsa S	Nugraha YP	Suendo V	Uekusa H	Soewandhi SN
Concepts or ideas	x					x
Design		x				x
Literature search			x			
Experimental studies	x				x	
Data acquisition		x	x	x	x	x
Data analysis	x		x		x	
Statistical analysis	x	x				
Manuscript preparation	x		x			x
Manuscript editing		x		x		
Manuscript review	x	x	x	x	x	x

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