UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE

CENTRO DE CIÊNCIAS DA SAÚDE

CURSO DE GRADUAÇÃO EM FARMÁCIA

INGRID DA SILVA ALBUQUERQUE

SELECTION OF SUITABLE POLYMER AND OBTAINING NEW AMORPHOUS SOLID DISPERSIONS WITH FERULIC ACID AND SOLUPLUS®

NATAL, RN

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Trabalho de Conclusão de Curso apresentado ao Curso de Graduação em Farmácia da Universidade Federal do Rio Grande do Norte, como requisito parcial para obtenção do título de Bacharel em Farmácia.

Orientador: Prof. Dr. Ádley Antonini Neves de Lima
Co-Orientadora: Fernanda Ílary Costa Duarte

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“Tudo posso naquele que me fortalece”

Filipenses 4:13

RESUMO

Ácido ferúlico (FA) é uma substância com atividades promissoras, entretanto, sua solubilidade aquosa limitada prejudica a obtenção de novas formulações. Diversas estratégias tecnológicas têm sido desenvolvidas para incrementar a solubilidade de fármacos, dentre essas tem-se as dispersões sólidas amorfas (ASD). Assim, o objetivo do presente estudo foi desenvolver uma ASD contendo FA utilizando diferentes métodos de obtenção. O Soluplus® foi selecionado por meio de estudo de solubilidade com o FA, a partir disto foram obtidas ASD com ácido ferúlico por mistura física (PM), malaxagem (KND) e rota evaporação (ER). As dispersões sólidas foram quantificadas por espectrofotometria UV/Vis e caracterizadas físico quimicamente por difração de Raio X (XRD), espectroscopia de infravermelho com transformada de Fourier (FTIR), microscopia eletrônica de varredura (SEM) e calorimetria exploratória diferencial (DSC). Os resultados demonstraram a formação de dispersão solida amorfa através das técnicas utilizadas, sendo que KND e ER demonstraram melhores resultados com significativas reduções das intensidades das reflexões cristalinas pelo XRD, como também, na intensidade das bandas no FTIR e a ausência do pico de fusão característico do FA no DSC das amostras. Dessa maneira, pode-se concluir que foram obtidas dispersões sólidas amorfas com ácido ferúlico que serão investigadas posteriormente quanto à dissolução e atividade antioxidante *in vitro*.

Palavras-chave: Ácido Ferúlico, Dispersões Sólidas Amorfas, Soluplus®

ABSTRACT

Ferulic acid (FA) is a substance with promising activities, however, its limited aqueous solubility impairs the obtaining of new formulations. Several technological strategies have been developed to increase the solubility of drugs, among which there are amorphous solid dispersions (ASD). Thus, the aim of the present study was to develop an ASD containing FA using different obtaining methods. Soluplus® was selected by solubility study with FA, from this were obtained ASD with ferulic acid by physical mixture (PM), kneading (KND) and evaporation rotary (ER). The solid dispersions were quantified by UV/Vis spectrophotometry and characterized physical-chemically by X-ray diffraction (XRD), Fourier transformed infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and differential exploratory calorimetry (DSC). The results demonstrated the formation of amorphous solid dispersion through the techniques used, and KND and ER showed better results with significant reductions in the intensities of crystalline reflections by the XRD, as well as in the intensity of the bands in the FTIR and the absence of the characteristic fusion peak of FA in the DSC of the samples. Thus, it can be concluded that solid dispersions with ferulic acid were obtained, which will be investigated later for dissolution and antioxidant activity *in vitro*.

Keywords: Ferulic Acid, Amorphous Solid Dispersions, Soluplus®

LIST OF FIGURES

LIST OF TABLES

SUMMARY

1. Introduction

In recent times, several Active Pharmaceutical Ingredients (APIs) have been developed, however, the vast majority of these new products have low solubility in water, which is an obstacle in the development of new formulations, especially solid ones for oral administration, such as tablets and capsules. These formulations are usually more stable, with greater ease in their production, have a correct dose and are simpler for administration, as well as have greater adherence when compared to the others[1].

It is known that the solubility of APIs is directly correlated with their bioavailability, for this the Biopharmaceutical Classification System (BCS) establishes an order of classification of drugs according to their absorption. Compounds with low aqueous solubility are inserted in Class II (high permeability, low solubility) or Class IV (low permeability, low solubility)[2]. Studies reveal that about 75% of the compounds under development fall into the Class II and Class IV groups, in addition, of the existing drugs approximately 40% also have low aqueous solubility, so this property is configured as an obstacle in the promotion of new pharmaceutical products[3,4]. Therefore, in search of improvements and new formulations, scientists and industries are in constant search for new technologies aimed at overcoming these challenges, for example there is the use of salts, development of cocrystals, complexation with cyclodextrins, polymorphs, solid dispersions and particle size reduction[5].

Amorphous solid dispersions (ASD) are currently established as a platform technology for the formulation of few soluble drugs. The solid dispersion system (SD) consists of a hydrophobic drug dispersed in a hydrophilic vehicle, which will classify SD in first class (sugars and urea), second class (synthetic or natural polymers) and third class (surfactants, mixtures surfactants or polymer mixture)[6]. In addition, the main objective of the ASD is to minimize the packaging energy of the crystal, interrupting its crystalline structure, resulting in an amorphous material that together with the polymer network corroborates the increase in solubility, consequently, the dissolution rate, since hydrophilic transporters can reduce agglomeration and promote a release of the drug in a state of supersaturation, as a consequence increases absorption and improved bioavailability[7]. This generation of a supersaturated state and inhibition of precipitation has been referred to as the “spring and parachute” effect, and this effect is dependent on the type and molecular weight of the polymer[8].

Ferulic acid (FA) or chemically known as 4-hydroxy-3-metoxicinamic acid (**Figure 1**) is found in abundance in the plant kingdom which has a low toxicity, can be found in whole grains, spinach, grapes, cereal seeds such as wheat, oats and rye, in addition, corn and wheat bran, artichoke and beetroot[9,10]. This phenolic compound through its structural portions of its molecule is able to combat reactive oxygen and nitrogen species, as well as promote a down regulation in the responses of the pathways of cell death, such as activating the synthesis of proteins that improve cellular stress[11]. Therefore, the main therapeutic activities originated from FA are due to its antioxidant and anti-inflammatory properties, but also has biological effects such as antibacterial, antifungal, antidiabetic, anti-aging, radioprotection a and anticarcinogenic[12]. Also, in this context, it is known that FA is absorbed by the small intestine, is excreted by the renal route and that for pharmacological efficacy will depend on physiological concentrations as well as pharmacokinetic activities, such as absorption[13].

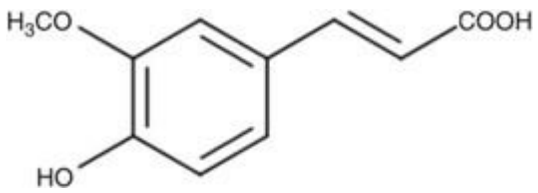

Figure 1. Chemical structure of ferulic acid

Despite the diversity of biological properties and activities, FA has limited therapeutic application because it presents low aqueous solubility, 6.63 $mg.dL^{-1}$ at pH 7.2, as well as demonstrating instability of the molecule which can decompose in inactive products, induced by light and/or oxidation[14]. Although the chemical structure presents polar groups, the presence of the phenolic group may compromise the solubility in water of this compound, considering that even being able to promote hydrogen bonds with water, there is a high number of carbons in the chain corroborating to reduce solubility, in addition, the presence of the ether group also contributes to this molecule property[15]. Therefore, some technological methods have already been developed in order to improve these limiting characteristics, such as inclusion complexes with cyclodextrins and cocrystals[16].

Given the need to optimize the therapeutic potential of ferulic acid, in order to preserve its biological activities and increase its bioavailability, it becomes relevant the use of obtains capable of stabilizing it. In this context, the present study aimed to develop

amorphous solid dispersions elaborated by kneading method and evaporation rotary, to characterize the physicochemical properties of these systems.

2. Materials and methods

2.1 Materials

Ferulic acid was purchased from FAGRON® (Lot: 18F23-BO17-0348) and the polymers used were hydroxypropylmethylcellulose (HPMC), polyvinyl caprolactam-acetate polyvinyl-polyethylene glycol (Soloplus®), vinyl copolymer pyrrolrolidone-vinyl acetate (Kollidon® VA64), polyvinylpyrrolidone (PVP K30) and polyethylene glycol 6000 (PEG 6000); All solvents used are analytical grade.

2.2 Methods

2.2.1 Phase solubility diagram with polymers

10 mL of aqueous solution (using Milli-Q water) of the polymers was added at increasing concentrations (0%; 0.1%; 0.2%; 0.4%; 0.6%; 0.8%; 1.0%). Specifically for the HPMC due to its lower solubility in water, the concentrations tested were 0%; 0.1%; 0.2%; 0.4%.

The solutions were under agitation for 24 hrs, protected from light and after this procedure they were filtered and analyzed in UHPLC-DAD, using a previously validated method[17].

2.2.1.1 Gibbs Free Energy Determination

Gibbs free energy values were used to calculate the spontaneity of the drug solubilization process in aqueous solutions.

From the equation:

$$\Delta G = -2.303RT.\ log\frac{Sc}{So}$$ (Equation 1)

Where:

R: Universal constant of perfect gases (8.314472 $J.K^{-1}.mol^{-1}$);

T : Temperature in Kelvin;

Sc: FA solubility at a given carrier concentration and;

So: concentration of FA in the absence of polymer.

2.2.2 Preparation of solid dispersions

2.2.2.1 Phisycal mixture (PM)

The FA and the respective polymer sat in the solubility study were weighed in proportion (1:1 w/w) and homogenized with pestle and mortar. Subsequently stored in a dissector and protected from light.

2.2.2.2 Kneading method (KND)

The FA and the respective polymer scans selected in the solubility study were weighed in proportion (1:1 w/w) and homogenized with pestle and mortar, then 10% of the total weight volume was added to the ethanol solution: water (50:50 v/v) until the formation of a moist mass, as paste. It was taken to the greenhouse for approximately 24h at 50°C ± 2°C. Subsequently stored in a dissector and protected from light.

2.2.2.3 Evaporation Rotary method (ER)

The FA and its polymers were weighed in proportion (1:1 p/w) and dissolved in an ethanol:water solution (50:50 v/v) followed by vacuum evaporation at 45°C ± 1°C using the 150rpm rotaevaporator until complete drying. Subsequently stored in a dissector and protected from light.

2.2.3 Yield of solid dispersions

The yield of the solid dispersions obtained was calculated by means of the ratio between the final mass after preparation, by the initial mass of FA plus polymer, depending on the

Following equation:

$$Yield(\%) = \frac{final\ mass\ (SD)}{initial\ mass\ (FA+polymer)}\ x\ 100 \text{ (Equation 2).}$$

2.2.4 Quantification of solid dispersions

The quantification of FA present in the solid dispersions (mg/g) developed was performed by spectrophotometry in the ultraviolet region, in evolution 300 UV-Vis spectrophotometer with wavelength analysis at 311 nm, by previously validated method,

using quartz buckets.

Solutions were prepared with solid dispersions in mass equivalent to 5mg of ferulic acid, being diluted in Milli-Q water, obtaining a theoretical concentration of 100µg/mL. This material was sonicated for 30 min and after proper dilution the UV reading was performed, and quantification was performed in triplicate, in relation to the previously validated method. All the solutions were protected from light.

2.2.5 Incorporation efficiency (IE)

Considering the results obtained by the quantification of ferulic acid, equation 3 was used to obtain the efficiency of incorporation based on the masses of initially added FA and the value of this, present in the solid dispersions obtained[18].

$$IE = \frac{FA\ mass\ in\ solid\ dispersions}{initial\ mass\ of\ FA} X\ 100 \text{ (Equation 3).}$$

2.2.6 Validation of the analytical method for quantification of ferulic acid in solid dispersions

The validation of the analytical method was performed according to the criteria of the Resolution of the Collegiate Board (RDC) no. 166 of July 25, 2017 of the Agência Nacional de Vigilância Sanitária (ANVISA)[19].

The parameters analyzed were spectral scanning, selectivity, linearity, intermediate precision and repeatability, accuracy, detection limit and limit of quantification and the tests were performed on Spectrophotometer Evolution 300 UV-Vis, using quartz buckets with 1cm of optical path and wavelength at 311 nm.

2.2.6.1 Stock Solution Preparation

The standard solution containing FA was prepared by solubilizing 10 mg of FA in 100mL of Milli-Q water, obtaining a concentration of 10 µg/mL and for other analyses at different concentrations, the same solvent was used for dilution. These solutions were protected from light for the achievement of other analyses.

2.2.6.2 Spectral Scan

To perform the scan, a pure FA solution was prepared at a concentration of

20µg/mL and a reading of 200 to 600 nm was performed, analyzing the peak of highest absorption.

2.2.6.3 Selectivity

To perform the selectivity, a pure FA solution was prepared at a concentration of 20µg/mL and another solution containing Soluplus® at 200 µg/mL, and dilutions were performed necessary to read and obtain the spectral graph.

2.2.6.4 Linearity

For linearity study, three analytical curves were elaborated with seven concentration levels (2,4,5,6,8,10,12 µg/mL), starting from a stock solution of 100 µg/mL. These concentrations followed an interval of 80% to 120% of the test concentration (5µg/mL), with three replicates for each concentration level. The equation of the line was determined by the mean of the three analytical curves, plotted the values of the concentration as a function of absorbance, thus obtaining the angular coefficient of the line, point of intersection and correlation coefficient (R2), having as acceptance criterion R2 greater than or equal 0.99.

2.2.6.5 Precision

This parameter was evaluated from stock solutions at 100 µg/mL, performing determinations of three levels, being a high (12 µg/mL), medium (5 µg/mL) and low (2 µg/mL). The precisions were determined by the RSD (relative standard deviation) of the actual concentrations found and by the statistical test ANOVA.

2.2.6.5.1 Repeatability accuracy

The determination of accuracy by repeatability was performed from three stock solutions (100 µg/mL) analyzing in triplicate the three concentrations being one high (12 µg/mL), medium (5 µg/mL) and low (2 µg/mL), totaling nine determinations. This analysis occurred by the same analyst and equipment.

2.2.6.5.2 Intermediate accuracy

For this criterion, the agreement between the results obtained in the same laboratory under the same conditions was evaluated, however, being performed by

analysts and on different days. In total, nine determinations of three concentrations were made, one high (12 μg/mL), medium (5 μg/mL) and low (2 μg/mL) from three stock solutions of 100 μg/mL.

2.2.6.6 Accuracy

The accuracy of the method was verified from nine determinations within the linear range, three concentrations with three replicates each. Therefore, low concentration solutions (2 μg/mL) were analyzed. average (5 μg/mL) and high (12 μg/mL), according to the linearity limits defined above.

Accuracy was expressed by the ratio between the average concentration determined and the theoretical concentration, according to the formula below:

$A\,(\%) = \frac{CME}{CMT}\ X\ 100$ (Equation 4)

Where:

A (%): accuracy in percentage.

CME: experimental mean concentration.

CMT: theoretical mean concentration.

2.2.6.7 Detection and quantification limit

The detection limit (DL) and quantification limit (QL) were calculated based on the relationship between the standard deviation of the analytical curve and its inclination, using the multiplier factors according to RDC n°166, according to Equations 5 and 6.

$$DL = \frac{3{,}3\ x\ SD}{CI}$$ (Equation 5)

$$QL = \frac{10\ x\ SD}{CI}$$ (Equation 6)

Where:

CI: curve slope;

SD: standard deviation.

2.2.7 Characterization of solid dispersions

2.2.7.1 X-ray diffraction (XRD)

The crystalline characterization and crystalline profile of the dispersions were obtained in a Bruker diffratometer, model D2 Phaser, using CuKα radiation (λ=1.54Å) with a Ni filter, in the variation of 10-70° (2θ), with a step of 0.02º, current of 10 mA, voltage of 30kV, using a Lynxeye detector.

2.2.7.2 Fourier transformed infrared spectroscopy (FTIR)

The isolated components and dispersions obtained were characterized by FTIR, using shimadzu spectrometer, model IR Prestige-21. The spectra were obtained with spectral resolution of 4 cm^{-1} and 20 scans in the region of 400 to 4000 cm^{-1}.

2.2.7.3 Scanning Electronic Microscopy (SEM)

The samples were metallized with gold and then mounted on aluminum stubs using double-sided tape. Subsequently, they were analyzed by high-resolution scanning electron microscopy. Secondary electronic images were acquired with an acceleration voltage between 5 and 10 kV. Images of retrodiffusion electrons being performed with an acceleration voltage of 15 kV.

2.2.7.4 Differential Exploratory Calorimetry (DSC)

Differential Exploratory Calorimetry (DSC) was performed in Shimadzu® Model DSC-50, weighing approximately 2.0 mg of the sample in hermetically sealed aluminum crucible, under nitrogen atmosphere, purge gas flow of 100 $mL.min^{-1}$, heating ratio of 10° $C.min^{-1}$ with final temperature of 250°C. The analysis of the curves obtained was performed using TA Analysis software.

3. Results and discussion

3.1 Study of ferulic acid solubility in the presence of polymers

The objective of this analysis is to observe the influence of polymers on the solubility of ferulic acid according to the increase in the concentration of carriers in the solutions. These results can be analyzed according to the figure obtained through studies conducted in UHPLC, in which it is important to verify the increasing linearity of the curves that each polymer presented according to the concentration used and the percentage of solubilization for ferulic acid in solution.

According to figure 2 it can be perceived that the copolymer Soluplus® was the best polymer increasing the solubility of FA from 15.40 to 36.45 µg/mL at the concentration of 1%, corroborating the result of Shamma and Basha[20], which obtained better solubility of Carvedilol in the presence of Soluplus®. It is also worth emphasizing the importance of evaluating Gibbs free energy on these prepared solutions, correlating spontaneity in the solubilization process. In general, it is known that when $\Delta G° < 0$, the greater the increase in solubility by polymers according to the increase of their concentrations, therefore, the more negative the result, the better the solubilizer effect of the carriers. According ly, it can be seen that Soluplus® also presented the most negative Gibbs free energy at the concentration of 1%, and that it was proportional to the increase in concentration, as shown in Table 1.

Soluplus® is a polyvinyl caprolactam copolymer - polyvinyl acetate - polyethylene glycol, classified as a member of the fourth generation of carriers for SDs, which aims to achieve the highest degree of increase in the dissolution of drugs with low aqueous solubility. Soluplus® is a polymeric solubilizer with amphiphilic structure, with a large number of groups with hydroxyls present, making it a good carrier[21,22].

Therefore, according to this result and the characteristics of Soluplus®, in order to develop a good solid dispersion, this polymer was chosen for the development of the formulations, as well as the characterizations performed in this work.

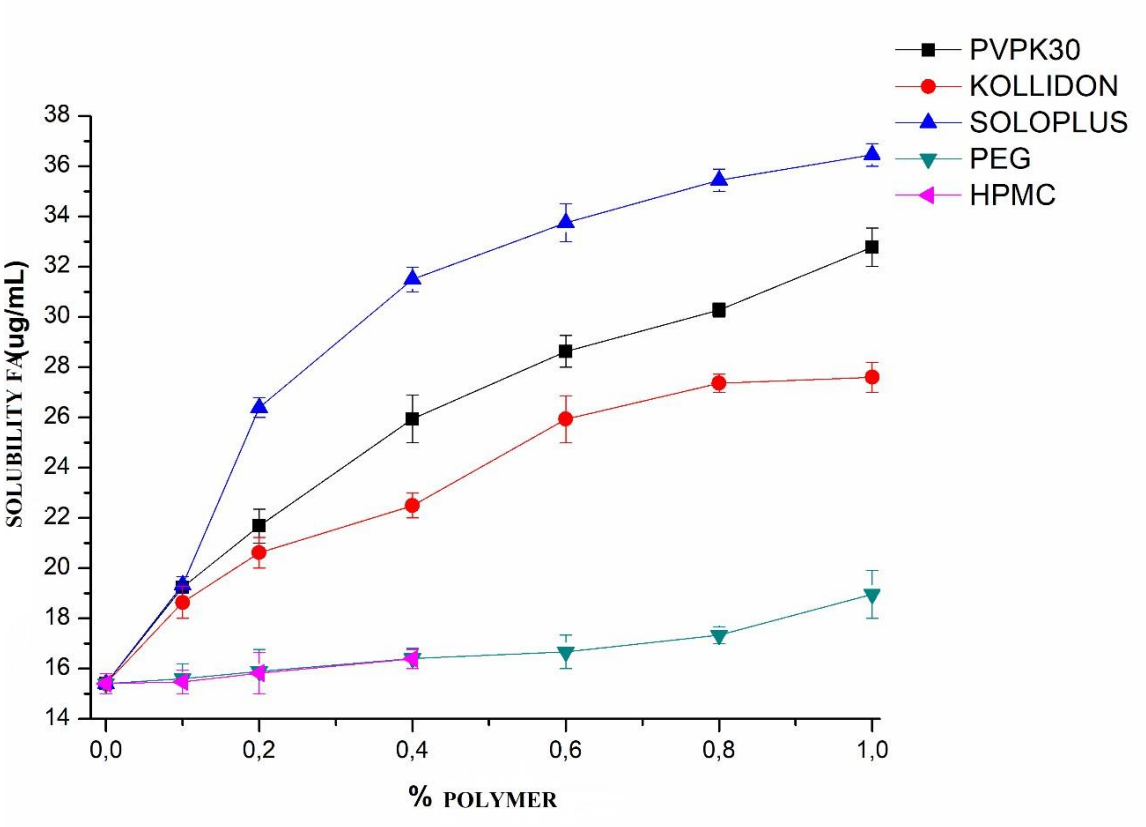

Figure 2. Curves of the FA solubility diagram with polymers performed in triplicate

CONCENTRATION (%)	PVPK30	KOLLIDON	SOLUPLUS	PEG	HPMC
0.1	-686.37	-590.78	-701.77	-39.96	14.50
0.2	-827.07	-590.78	-701.77	-39.96	14.50
0.4	-1208.97	-893.52	-1610.44	-98.87	86.17
0.6	-1280.2	-1149.66	-2106.6	-199.06	
0.8	-1371.41	-1560.53	-2298.09	-248.98	
1.0	-1500.18	-1712.43	-2432.47	-370.83	

Table 1. Gibbs free energy for each polymer concentration

3.2 Validation of the analytical method by UV/VIS spectrophotometry

3.2.1 Spectral scanning

To start the validation protocol, the scan was performed in order to identify the spectral profile of FA in the UV-Vis region. Thus, it was found that the maximum absorption occurs at a wavelength of 311 nm (Figure 3).

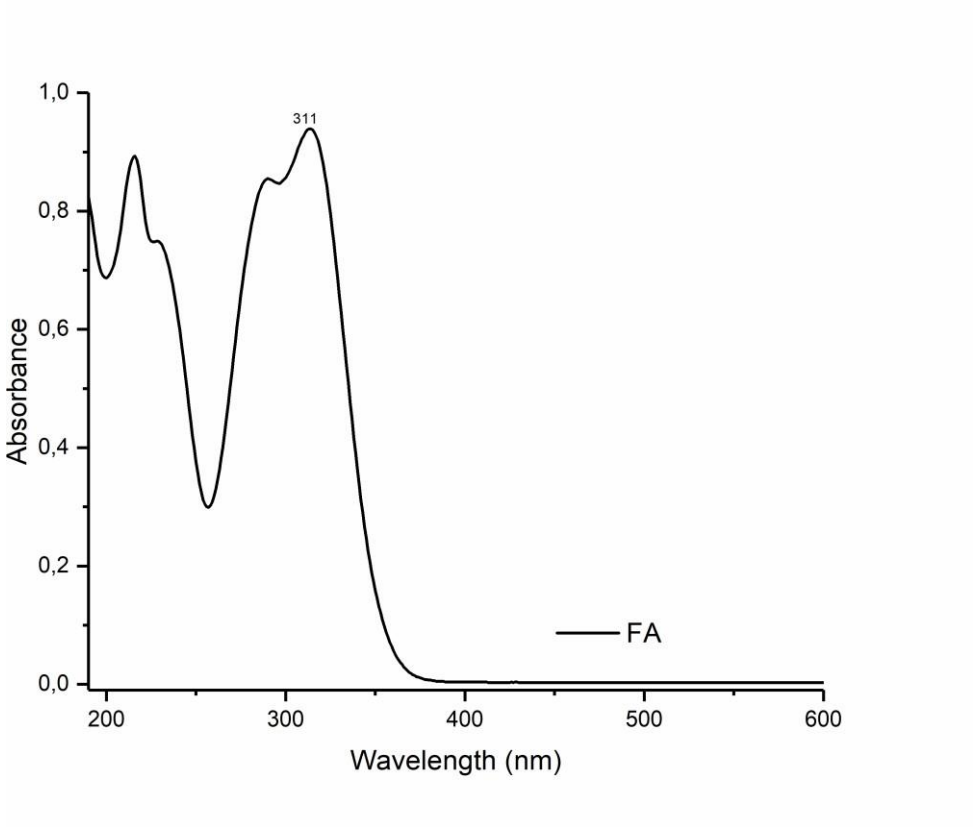

Figure 3. Absorption spectrum of ferulic acid in the UV/Vis region

3.2.2 Selectivity

The spectra obtained by the FA solution between the length of 200 to 600 nm and for a solution composed only for Soluplus® present in the solid dispersion, were compiled and are represented in Figure 4.

Depending on the spectra obtained, it can be affirmed that the validation method is adequate in terms of selectivity, considering that there are no absorption peaks for Soluplus® at the same wavelength characteristic of FA, thus there is no interference of the polymer to the drug.

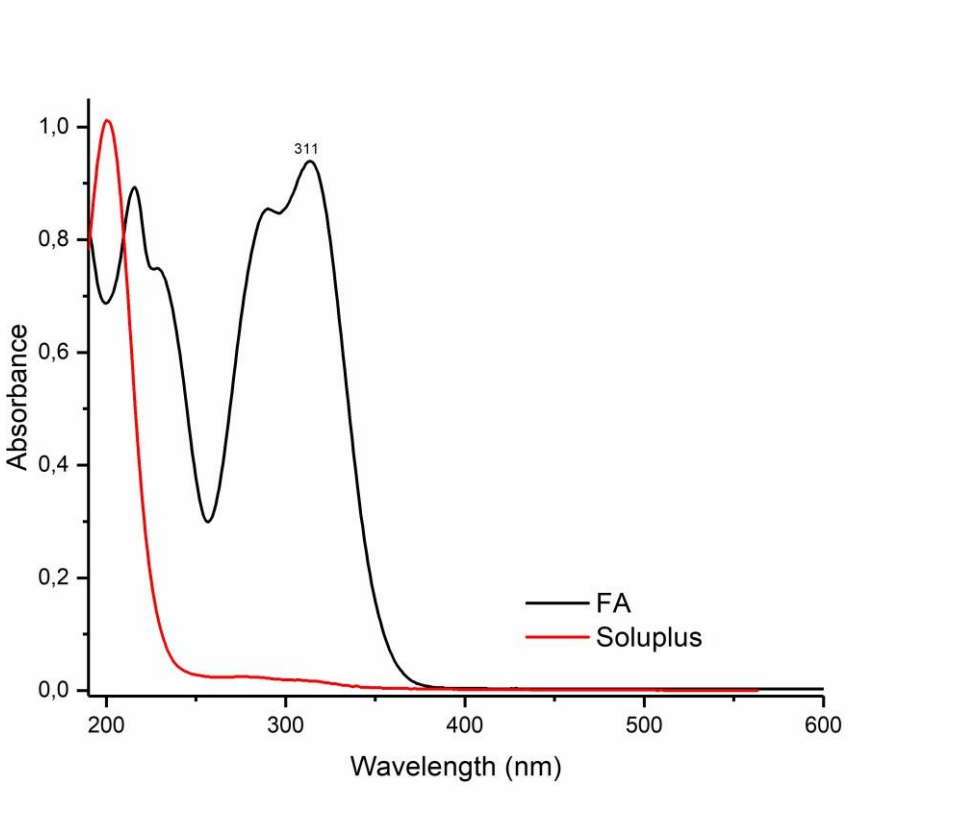

Figure 4. Spectrum in the UV region of ferulic acid and Soluplus®

3.2.3 Linearity

The analytical curve was constructed according to the resolution recommendations, with seven concentration points, in the content range of 80 to 120%. The means of absorbance values and RSD (%) for each concentration are in Table 2.

From these values, the mean analytical curve was obtained for the quantification of FA, the equation of the line and the value of the r found, as can be observed in Figure 5. Therefore, in line with the results of the analytical curve obtained in triplicate, there is an average correlation coefficient (r) value equal to 0.9994, which indicates a significant linear regression in accordance with RDC n°166.

Concentration (µg/mL)	2	4	5	6	8	10	12
Absorbance	0.1550	0.3221	0.4008	0.4842	0.6418	0.8149	0.9907
	0.1561	0.3124	0.3878	0.4742	0.6311	0.8185	0.9585
	0.1611	0.3278	0.4036	0.5167	0.6226	0.7965	0.9723
Average	0.1574	0.3208	0.3974	0.4917	0.6318	0.8099	0.9738
RSD (%)	1.6865	1.9819	1.7322	3.6898	1.2432	1.1895	1.3544

Table 2. Absorbance values obtained in the construction of the analytical curve of FA

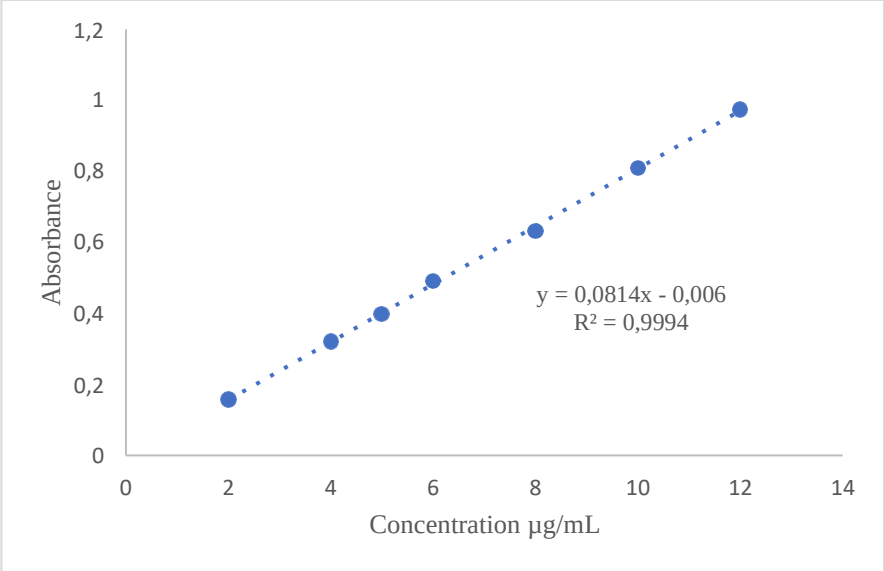

Figure 5. FA analytical curve at concentrations from 2 to 12 µg/mL obtained by UV/Vis spectrophotometer.

3.2.4 Precision

The values for the mean of the actual concentration and the relative standard deviation (RSD) of this concentration for repeatability and intraday precision are arranged in tables 3 and 4, respectively. For the evaluation of this parameter, a maximum value of 5% of RSD was determined, and it was observed, therefore, that the method was accurate and was not influenced by the period that was performed or by the change of analyst.

Theoretical Concentration (µg/mL)	Average Real Concentration (µg/mL)	RSD (%)
2	2.007	1.624
5	4.955	1.706
12	12.037	1.346

Table 3. Values obtained for repeatability accuracy

Analyst	Theoretical Concentration (µg/mL)	Average Real Concentration (µg/mL)	RSD (%)
Analyst 1	2	1.980	1.228
	5	4.975	1.734
	12	12.243	1.045
Analyst 2	2	2.135	1.686
	5	5.057	1.938
	12	12.118	1.407

Table 4. Values obtained for intra-day accuracy

3.2.5 Accuracy

Through the analysis of accuracy, it is possible to prove the proximity of the results of the method, with regard to the result admitted as true, that is, according to the actual concentrations. For this analysis, three different concentrations were admitted, high, medium and low, determining the concentration of each one. Thus, it was obtained values of accuracies between 99.11 and 100.36% (Table 5) and these in agreement with the true values and within the acceptable range that is 95-105% as established as a criterion for this parameter.

Theoretical Concentration (µg/mL)	Average Real Concentration (µg/mL)	Accuracy (%)
2	2.007	100.368
5	4.955	99.115
12	12.037	100.310

Table 5. Accuracy values at three levels of FA concentration.

3.2.6 Detection and Quantification Limits

With the data obtained by the calibration curves, the limits of detection and quantification were calculated using equations 5 and 6, respectively. The resulting values were 0.4112 µg/mL for DL and 1.2461 µg/mL for QL.

3.3 Quantification of solid dispersions

The ferulic acid content in solid dispersions, yield and incorporation efficiency by the applied method are shown in Table 6. From the values obtained, it can be denoted that the ferulic acid content was satisfactory through the two techniques performed, as well as the incorporation efficiency had similar values. However, there was a highlight in the performance for the kneading technique, which allowed a considerable value if considered with the evaporation rotary.

Solid dispersion	Ferulic acid content	Standard deviation	Yield (%)	IE (%)
KND	4.28 mg/10mg of SD	0.005	90.96%	85.6
ER	4.13 mg/10 mg of SD	0.018	63.89%	82.6

Table 6. FA content in solid dispersions and efficiency of incorporation (%)

3.4 Chemical physical characterization

3.4.1 X-ray diffraction (XRD)

X-ray diffraction is one of the techniques widely used for the development and analysis of pharmaceutical products, being possible from this the measurement of the crystallinity of a material, through the graph which presents the relationship between the intensity of crystalline reflections and the diffraction angle used. Therefore, crystalline materials present well-defined and intense reflections, while those that do not show any or low intensity are considered as amorphous substances[23]. In view of this importance, the behavior of isolated FA, the polymer used – Soluplus®, the physical mixture KND and ER method.

Based on the diffractogram obtained (Figure 6), it can be seen that FA shows a behavior of crystalline substance, with crystalline reflections of high intensity at 12.7º, 15.4º, 26.4º, 29.3º, 44.10° and 64.3 °, as well as several others of lower intensity at 10.5º, 17.2º, 24.5º, 31.3º, 35.9°, 39°, similar results were demonstrated in the study by Wang et al[10]. This crystallinity presented by FA corroborates the low aqueous solubility of the drug. With regard to Soluplus® copolymer the presence of a halo pattern, which is characteristic of amorphous powders, is denoted[24].

Although the crystalline reflections of FA have been slightly reduced in PM with Soluplus®, it is also observed that there is the presence of intense peaks characteristic of the drug, as in 12.8°, 15.4° and 26.4°. Unlike what occurs in SDs by KND and ER that there is a considerable reduction in the intensity of these peaks, as well as in the peaks at 44.10° and 64.3°, in addition, the disappearance of peaks in 17.2°, 21.1° and 24.5° which are lower. Thus, it is understood that the methods KND and ER applied for the development of SD promoted a greater process of amorphization of the drug and that there were no significant differences between the two systems obtained.

Unlike the result obtained by Moreira et al[25], in a development of SDs with Hecogenin and five polymers, this drug did not present a good interaction with Soluplus® which was obtained a material with similar reflections of the compound and without reducing the intensity considerably, presenting itself as the best polymer for this development - the HPMC, in contrast the present study demonstrates that Soluplus® was able to form solid dispersion with ferulic acid of the methods applied.

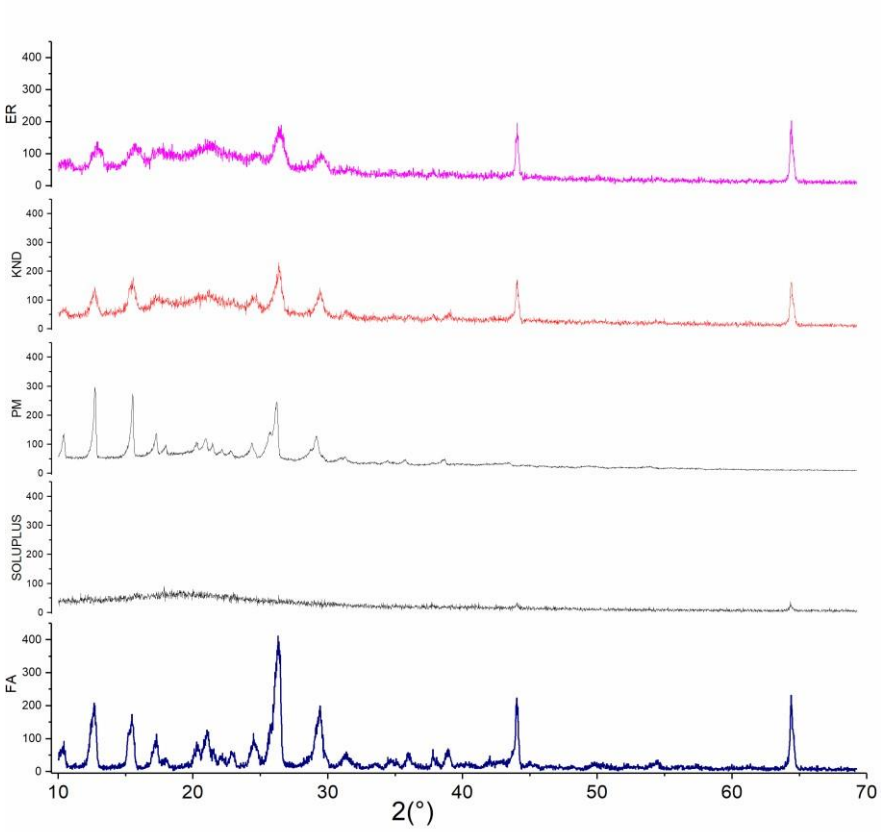

Figure 6. FA diffractograms, Soluplus®, PM, KND and ER methods.

3.4.2 Fourier transform infrared spectroscopy (FTIR)

Infrared spectroscopy is correlated with the absorption of electromagnetic energy by molecules and depending on the different chemical structures and concentration of each, these will confer different energy absorptions[26]. Using this method assumes that the mixing of two components at the molecular level should lead to changes, this are manifested in the spectrum in the shape changes, in the frequency and width of the bands through the interaction between drug and polymer[27].

Figure 7 shows the FTIR spectrum of FA, Soluplus®, physical mixture and solid dispersions obtained by KND and ER techniques. With regard to FA, a band in 3434 cm^{-1} corresponding to axial deformation of the hydroxyl group (O-H) and another band in 3016 cm^{-1} attributed to the asymmetric stretching of the C-H group can be observed in the most energetic region of the spectrum. In 1690 cm^{-1}, the characteristic band was observed to carbonyl and three intense bands were also exhibited in 1617,1510 and 1427 cm^{-1} corresponding to axial deformation C=C of the aromatic ring of FA. In addition,

bands were verified in 1269 cm^{-1} and 1205 cm^{-1}, respectively, corresponding to the asymmetric stretch C-O-C and stretch C–OH[18,28].

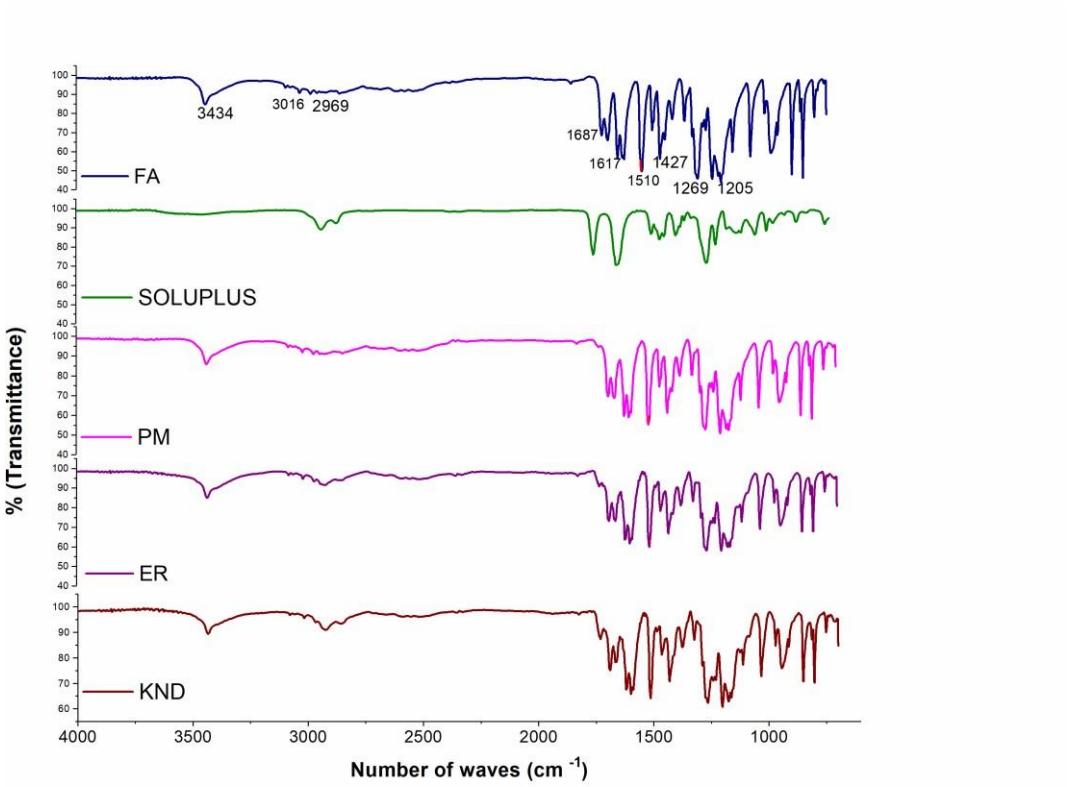

Figure 7. FTIR Spectra for FA, Soluplus®, PM, KND and ER methods

The spectra obtained for the physical mixture and the SDs developed, it can be denoted that the spectral profile of ferulic acid was maintained and Soluplus®, without the appearance of new bands, however, there are reductions in the intensity of certain bands, mainly in solid dispersions such as, in the 1730 cm^{-1} region which represents the carbonyl strain band of Soluplus®, in consideration of the 1617 cm^{-1} band present in the FA molecule by the vibration of the stretch in the aromatic nucleus, as well as in 1427 cm^{-1} and in the bands at 804 and 800 cm^{-1} there is a considerable reduction in the intensity of these peaks, especially in the SDs, which are characteristic of hydrogen atom adjacent to the phenyl group in the FA molecule, can infer that there is a greater interaction through hydrogen bonds in this region. In addition, it is important to highlight that there is enlargement and more intense reduction of the band in the region between 3000 and 2800 cm^{-1} in the spectra of the systems obtained by solid dispersion methods (KND and ER) and that in this region there is the band of 2930 cm^{-1} corresponding to the aromatic C-H

bond in Soluplus®, and it can be inferred that in this group there are significant interactions with FA and being more evident in the SD by ER.

From this analysis it can be understood that there is greater interaction of FA with the polymer matrix when the methods of obtaining solid dispersions by KND and ER methods are applied, considering that the reduction of band intensity was more visible when compared to the physical mixture.

3.4.3. Scanning electron microscopy (SEM)

Analyses of the photographs of pure ferulic acid, physical mixture and solid dispersions obtained by ER and KND, Figure 8A demonstrates the crystalline behavior of PA, as needle-like crystals, corroborating the result exposed by Wang et al[10]. This morphology is perceived by substances that present low aqueous solubility, due to its well organized and symmetrical structure of molecules which thus hinder the penetration of water. With regard to Soluplus® particles, according to the literature, morphology is presented with cluster-free aspects and is characterized more as an amorphous substance, besides presenting a rough aspect[29,25].

PM's photomicrograph (Figure 8B) demonstrates that it is already possible to obtain a good interaction between the drug and polymer through this technique, not being visualized the crystalline profile of the pure drug, this same characteristic is observed in the micrographs of the SDs obtained by the methods of KND and ER, in addition, is presented the formation of a polymer mesh in which it can be inferred that the FA is dispersed , thus demonstrating a good interaction between the two components for solid dispersion formation. This result corroborates those expressed by the XRD, in which through the physical mixture the reduction of crystalline reflexes was obtained, however, this reduction was more expressive for the solid dispersions systems developed by the methods of KND and ER, therefore, involving a greater process of amorphization of the drug that will corroborate with an increase in its solubility.

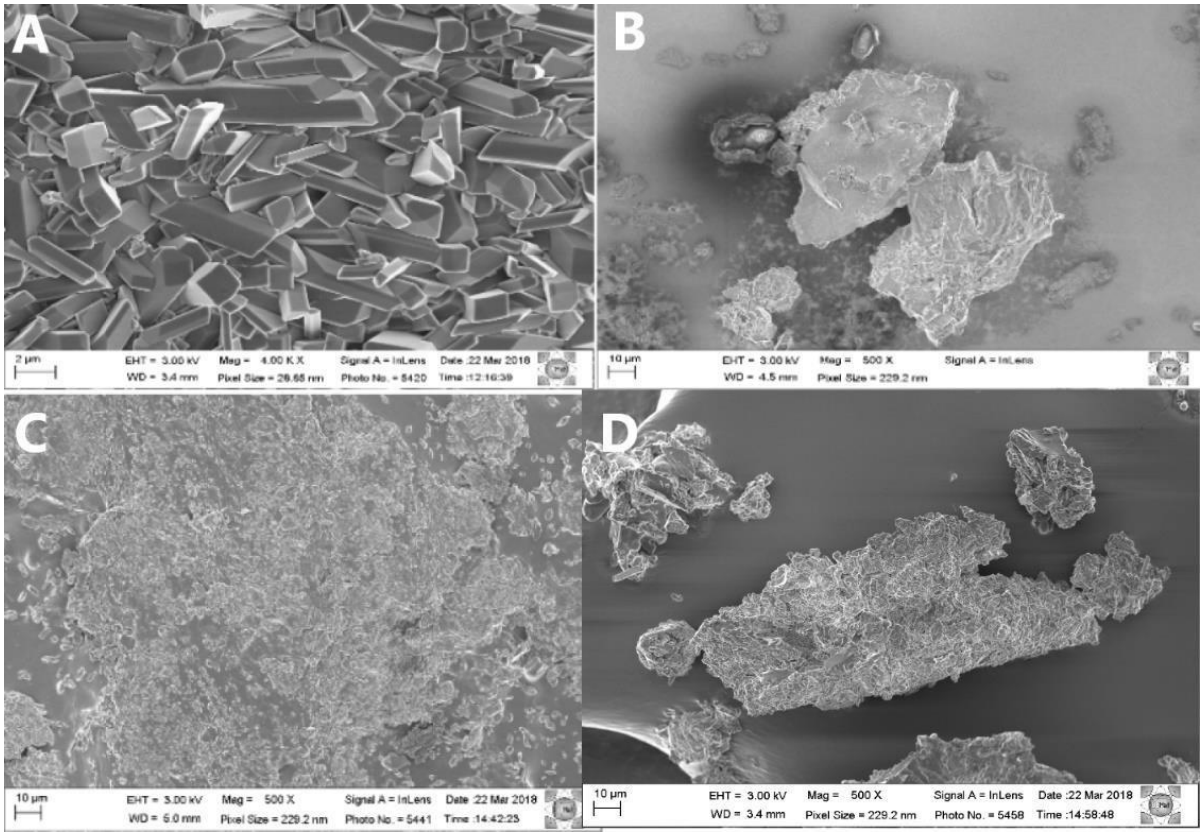

Figure 8. SEM of FA (A), PM (B), KND (C) and ER (D) with increase in 500x

3.4.4. Differential Exploratory Calorimetry

According to the calorimetric curve of FA (Figure 9), it is noted the presence of two important events, the first being related to the characteristic fusion event of crystalline substance, having as melting temperature (Tpeak) at 173.4 °C, with Tonset at 171.6 °C and Tendset at 175,84 °C and ∆H = -132.53 J/g, this result being consistent with that described in the literature by the Japanese Pharmacopoeia[30], which accepts melting temperatures for this drug between 173 and 176°C. In addition, the second endothermic event observed is related to the degradation of FA mainly related to the oxidation process, which has as Tonset 184,.9 °C and Tendset 233.,12°C, with peak temperature at 224.,78 °C. Similar results are presented by Bezerra et al[31].

Soluplus® presents a single endothermic event in the temperature range used, with Tonset at 47.,93 °C, Tendset at 109.,32 °C and Tpeak at 73.,5°C, which is related to copolymer dehydration, expressing equivalent result to the study developed by Prakash and Pravin[32].

With regard to the calorimetric curves for PM and SDs, an endothermic event with variation of enthalpy is observed indicating the possible fusion of the asset, however, in a subtle way due to the amorphization process resulting from the methodologies applied and interactions between the drug and polymer, similar findings are observed in the results exposed by the diffuser (Figure 9) and in the study developed by Zhang et al[33], in a study SD of Simvastatin and Soluplus®. For the physical mixture it is said that Tpeak occurs at 143 °C with enthalpy variation of -37.94 J/g; sd by KND method the Tpeak is at 158.53 °C and ΔH = - 26.48 J/g and for Tpeak at 160.81 °C and ΔH = -11.97 J/g, it is important to highlight that for the solid dispersion obtained by the ER method there is a variation of smaller enthalpy and it can be inferred that the solubilization of the drug in the polymer in this system occurred in a smaller way, understanding that there was a more representative interaction between the two components, corroborating this result with that obtained by the FTIR spectrum, which presents bands with lower intensities for this particular acquisition.

In view of this approach, it can be affirmed that the KND and ER techniques allowed a process of change from the crystalline state to the amorphous of FA, and it is not possible to observe the peaks of fusion of the asset in the systems obtained. This amorphation process corroborates the results of the XRD, as well as with studies developed by Alves et al[34] that developed solid dispersions by the same methodologies using PVP K30 and efavirenz, as well as the work developed by Yadav et al[35].

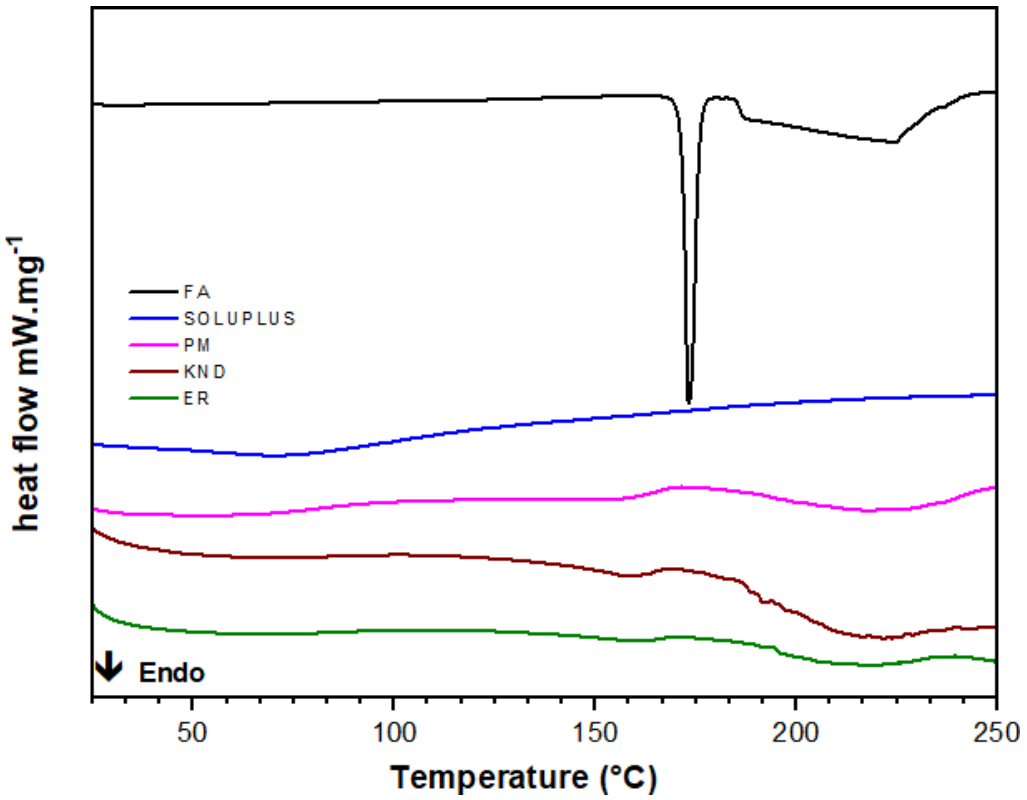

Figure 9. Calorimetric curves of FA, Soluplus®, PM, KND and ER methods

4. Conclusion

In the present study, the solubility diagram indicated that Soluplus® provided the best increase in the solubility of ferulic acid, demonstrating to be the best among all polymers tested. The validation of the methodology obtained was selective for quantification of FA, with proven precision and accuracy. In addition, the kneading technique and the evaporation rotary were effective, obtaining a satisfactory content FA of SD. The decrease in the crystallinity of the drug was confirmed through the chemical physical characterization, mainly by the results of the XRD and DSC, indicating the performance of solid amorphous dispersion, where the ER method presented a more expressive result, based on the FTIR and DSC, and this fact can be understood because for this method a greater amount of solvent is used to obtain, in which it can promote a greater and better interaction between the components producing , in this way, a more amorphous solid dispersion.

As future perspectives, additional research needs to be carried out, such as robustness, thermogravimetric analysis and the in vitro dissolution study. In moreover, the of in vitro antioxidant activity of solid dispersions.

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