

**STUDIES ON THE PHYSICO-CHEMICAL CHARACTERIZATION AND
OPTIMIZATION OF THE BIOPHARMACEUTICAL PROFILE OF SOME
COMPOUNDS WITH SPECIFIC BIOACTIVITY**

PhD Thesis – Abstract

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A pharmaceutical dosage form consists of one or more active pharmaceutical ingredients and numerous auxiliary substances (excipients). These excipients present multiple roles during the preformulation stage (binder, disintegrant, sweetener, colouring agent, etc.) and can influence the release profile of the active pharmaceutical ingredient, for example film-forming excipients used in the development of sustained-release dosage forms. The continuously increasing demand for medicines, both original and generic, represents a challenge for the pharmaceutical industry, putting pressure on the need to improve the efficiency of technological processes and production costs. This context emphasizes the need for a multidisciplinary approach in the development of new pharmaceutical formulations, as well as optimizing the existing ones. A major role in achieving these objectives falls to chemical engineering, by introducing analytical methods that aim to identify the problems from the preformulation stage of the pharmaceutical formulation, such as degradation of the molecular structure of the active pharmaceutical ingredient due to the existence of chemical incompatibilities between it and excipients or an inadequate thermoanalytical profile for the chosen technological process. The literature does not provide sufficient details regarding these preformulation aspects, representing a major gap in the field. In this context, in this PhD thesis I aimed to contribute to existing literature by investigating compounds belonging to the class of steroid hormones, subclass of progestogens and estrogens. The choice of these molecules is justified by their low therapeutic doses, which represent up to 10% of a tablet's mass, the rest consisting of excipients, this mass difference significantly increasing the risk of the occurrence of chemical interactions which can lead to a decrease in the concentration of the active pharmaceutical ingredient and a therapeutic failure.

Thus, my doctoral research project has pursued the achievement of two major objectives:

1. The determination of the thermal stability of compounds with steroid structure, and the application of the kinetic analysis methods to obtain information regarding the degradation mechanism occurring under non-isothermal conditions
2. Carrying out compatibility studies of hormonal compounds with a series of pharmaceutical excipients used in the preparation of solid pharmaceutical formulations

1st Objective: The determination of the thermal stability of compounds with steroid structure, and the application of the kinetic analysis methods to obtain information regarding the degradation mechanism occurring under non-isothermal conditions

To achieve this objective, the thermal stability profiles of two compounds from the progestogen subclass (levonorgestrel and gestrinone) and two compounds from the estrogen subclass (estradiol and estriol) were determined.

A) Kinetic analysis of the thermal decomposition of two endogenous estrogens with similar structure [1]

The first step of this study was represented by obtaining the FTIR spectrum to confirm the identity and purity of the two compounds. All absorption bands characteristic of estradiol hemihydrate and estriol could be found on the spectrum.

The thermogravimetric curves (TG and DTG) were recorded at five different heating rates ($\beta = 2, 4, 6, 8$ și $10\text{ }^{\circ}\text{C min}^{-1}$) and revealed a high thermal stability for the two endogenous estrogens analyzed. The analysis of the thermogravimetric curve obtained at $10\text{ }^{\circ}\text{C min}^{-1}$ indicated a thermal degradation of estradiol hemihydrate in 3 steps, the first corresponding to dehydration, and the second to the anhydrous estradiol, demonstrating its thermal stability up to $191\text{ }^{\circ}\text{C}$. The DSC curve indicated the presence of an endothermic process at $180\text{ }^{\circ}\text{C}$, corresponding to the melting point of the analyzed compound. In the case of estriol ($\beta = 10\text{ }^{\circ}\text{C min}^{-1}$), the results of the thermal analysis indicate a stability up to $251\text{ }^{\circ}\text{C}$ and a degradation process consisting of 2 steps. The endothermic process observed on the DSC curve at $287\text{ }^{\circ}\text{C}$ corresponds to the melting point of estriol.

The kinetic analysis was applied to the first degradation process observed on the DTG curves corresponding to the active pharmaceutical ingredient. The kinetic methods applied were: the preliminary ASTM E698 method, followed by the application of isoconversional methods: an integral method (Flynn-Wall-Ozawa) and a differential one (Friedman). To obtain a detailed description of the physicochemical nature of the processes that occur during degradation, the non-parametric kinetic method (NPK) was also applied.

In the case of anhydrous estradiol, the results of the kinetic analysis (Flynn-Wall-Ozawa and Friedman) indicated that the degradation occurs in a single step, with most of the activation energy values being within $\pm 10\%$ range. The results of NPK analysis indicated the presence of both chemical transformations and physical processes.

In the case of estriol, the results of the kinetic analysis (Flynn-Wall-Ozawa and Friedman) demonstrated a complex degradation process, with a rapid increase of the values of the activation energy up to conversion degree of 0.25 and followed by a slight decrease until the end of the conversion. Those aspects suggest that a change in the degradation mechanism is possible. The NPK kinetic method indicated a degradation consisting of both chemical transformations and physical processes.

B) Kinetic analysis of the thermal decomposition of levonorgestrel [2]

In this study, the impact of excipients on the thermal stability of an active pharmaceutical ingredient was evaluated. The thermoanalytical profile of levonorgestrel was compared with that of an existing pharmaceutical formulation and with that of a pharmaceutical formulation enriched with the steroid hormone.

The results of the FTIR spectroscopic analysis confirmed the purity and identity of the active principle, as well as its presence within the pharmaceutical formulation and within the pharmaceutical formulation enriched with the active principle.

According to the results of thermal analysis (TG and DTG) levonorgestrel shows a thermal stability up to approximately 200 °C, and the endothermic process on the DSC curve (243 °C) corresponds to the melting point. In the case of the pharmaceutical formulation, the small amount of levonorgestrel (approximately 0.75% of the tablet weight) and the presence of excipients made it difficult to identify the degradation process corresponding to the active substance, hence the need for a pharmaceutical formulation enriched with it.

Kinetic analysis methods (ASTM E698, Flynn-Wall-Ozawa and Friedman) were applied to the first and second degradation processes of levonorgestrel, as well as to the corresponding processes observed for the modified pharmaceutical form.

The variation of the values of the activation energy values for the first process, obtained by applying the Flynn-Wall-Ozawa and Friedman isoconversional methods, indicated the presence of a complex decomposition process consisting of a reversible endothermic reaction, followed by an irreversible one. In the case of the enriched pharmaceutical form, a drastic change in the degradation mechanism is observed for conversion degrees values higher than 0.40. This aspect can be explained by the presence of competitive degradation processes of the excipients (α -lactose monohydrate presenting the main degradation process in this temperature range).

To verify the effect of lactose and other excipients on the degradation of levonorgestrel, kinetic analysis was also applied to the second degradation process of the two samples. This process presents a temperature range in which no degradation processes corresponding to α -lactose monohydrate occurs. The kinetic analysis of the second degradation process of levonorgestrel also indicated the presence of a complex degradation mechanism.

By comparing the average value of the activation energy of levonorgestrel with those of the enriched pharmaceutical formulation, it can be observed that the mixture requires an activation energy at least 30% higher for process 1, and 20% higher for process 2. These results indicate that the excipients determined an increase of the thermal stability of levonorgestrel.

C) Kinetic analysis of the thermal decomposition of gestrinone [3]

The thermoanalytical curves (TG and DTG) showed a thermal stability of gestrinone up to 178 °C and a three-step degradation. The first endothermic process observed on the DSC curve

(113 °C) corresponds to a solid-state phase transition, while the second endothermic process corresponds to the melting point of gestrinone (148 °C).

The application of kinetic analysis methods (Flynn-Wall-Ozawa, Kissinger-Akahira-Sunose and Friedman) on the thermal data corresponding to the first degradation process of gestrinone observed on the DTG curves recorded at 5 different heating rates, demonstrated the complex nature of the degradation process. For all three kinetic methods, a constant variation of the activation energy is observed up to the degree of conversion of approximately 0.80, where a rapid increase in the activation energy occurs, suggesting a modification of the decomposition mechanism. The NPK kinetic method showed a complex decomposition process consisting of 2 stages that occur simultaneously.

2nd Objective: Carrying out compatibility studies of hormonal compounds with a series of pharmaceutical excipients used in the preparation of solid pharmaceutical formulations

To achieve this objective, we determined the compatibility of three compounds from the progestin subclass (gestrinone, levonorgestrel and progesterone) with the excipients most frequently used in the preparation of solid pharmaceutical formulations. To determine the compatibility, binary mixtures containing the active principle and the excipient in a mass ratio of 1:1 (m/m) were prepared.

A) Determination of the compatibility of gestrinone with pharmaceutical excipients [3]

In this study, the compatibility of gestrinone with two pharmaceutical excipients (starch and magnesium stearate) was determined. The determination of the compatibility at ambient temperature was performed using FTIR spectroscopic investigations, and with the help of thermal analysis methods (TG, DTG and DSC) the compatibility under thermal stress conditions was evaluated. The results of the FTIR analysis indicated the lack of any chemical interaction at ambient temperature for both excipients, while under thermal stress conditions the presence of chemical interactions between the components of the binary mixture were observed in both cases.

B) Determination of the compatibility of levonorgestrel with pharmaceutical excipients [4]

In the present study, the compatibility of levonorgestrel with eleven pharmaceutical excipients: colloidal silicon dioxide, aspartame, microcrystalline cellulose, sodium carboxymethylcellulose, calcium lactate pentahydrate, mannitol, lactose monohydrate, starch, polyvinylpyrrolidone, magnesium stearate and talc was determined.

The analytical methods used in this study were represented by FTIR and PXRD investigations to determine the compatibility of the active pharmaceutical ingredient with the excipient at ambient temperature, and thermal investigations (TG, DTG and DSC) to determine

the compatibility under thermal stress conditions. For a better interpretation of the results of the analytical methods applied to determine the compatibility at ambient temperature, the Pearson correlation coefficient was calculated for the significant regions of the components of the binary mixture from the spectrum or diffractogram obtained experimentally and those from a theoretical spectrum or diffractogram, built based on the experimental data of levonorgestrel and excipient.

The results of the FTIR analysis indicated the absence of chemical interactions between levonorgestrel and microcrystalline cellulose, sodium carboxymethylcellulose, lactose monohydrate, talc and mannitol, and the presence of chemical interactions in the case of the binary mixture containing magnesium stearate. In the case of colloidal silicon dioxide, aspartame, calcium lactate pentahydrate, polyvinylpyrrolidone and starch, the results of this analytical method did not show with certainty the presence or absence of chemical interactions at ambient temperature, additional investigations being necessary.

PXRD investigations confirmed the results of the FTIR analysis for the following excipients: microcrystalline cellulose, sodium carboxymethylcellulose, lactose monohydrate, talc, mannitol and magnesium stearate. In the case of the binary mixture formed between levonorgestrel and aspartame, this analytical method confirmed the presence of chemical interactions at ambient temperature. The results regarding the binary mixtures containing colloidal silicon dioxide, calcium lactate pentahydrate and starch indicated the absence of chemical interactions at ambient temperature. For the mixture formed with polyvinylpyrrolidone, the results did not confirm the presence or absence of chemical interactions, precautions being recommended if used due to the risk of chemical interactions.

Thermal analysis indicated the presence of chemical interactions under thermal stress conditions in binary mixtures containing: aspartame, magnesium stearate, polyvinylpyrrolidone, colloidal silicon dioxide, mannitol and starch.

C) Determination of the compatibility of progesterone with pharmaceutical excipients [5]

In the present study, the compatibility of progesterone with nine pharmaceutical excipients: calcium lactate pentahydrate, sodium carboxymethylcellulose, microcrystalline cellulose, avicel, sodium bicarbonate, methylcellulose (Methocel), mannitol, sodium lauryl sulfate and lactose monohydrate was determined.

The evaluation regarding the existence of chemical interactions at ambient temperature was carried out using FTIR and PXRD investigations, and in the case of subjecting the binary mixture to thermal stress conditions, thermal methods (TG, DTG and DSC) were used. For a better interpretation of the FTIR results, the Pearson correlation coefficient was determined between the regions in which the components of the binary mixture present significant absorption peaks, from the experimentally obtained spectrum and those from the theoretical spectrum built based on experimental data of the pure substances.

The results of FTIR analysis indicated the absence of chemical interactions between progesterone and mannitol, sodium lauryl sulfate, lactose monohydrate, avicel, microcrystalline cellulose and sodium bicarbonate. In the case of the binary mixtures formed by progesterone and calcium lactate pentahydrate, sodium carboxymethylcellulose and methylcellulose, further investigations are necessary to confirm the presence of chemical interactions.

The PXRD analysis results confirmed the FTIR results for the six excipients (mannitol, sodium lauryl sulfate, lactose monohydrate, avicel, microcrystalline cellulose, and sodium bicarbonate), and for calcium lactate pentahydrate, sodium carboxymethylcellulose, and methylcellulose, they indicated the absence of chemical interactions at ambient temperature.

Thermal analysis indicated the presence of chemical interactions between progesterone and methylcellulose, as well as the need to consider precautionary measures when using sodium lauryl sulfate and sodium bicarbonate not to exceed temperatures of 131 °C and 159 °C, respectively.

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