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# PHYSICAL-CHEMICAL CHARACTERIZATION OF REFERENCE DRUGS FOR THE TREATMENT OF TUBERCULOSIS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) AND THERMAL ANALYSIS (TG/DTG & DSC)

## *Caracterização Físico-Química de Medicamentos de Referência para o Tratamento da Tuberculose usando Espectroscopia de Infravermelho com Transformada de Fourier (FTIR) e Análise Térmica (TG/DTG & DSC)*

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**ABSTRACT**

Tuberculosis, a global public health problem, is an infectious disease caused by the bacteria *Mycobacterium tuberculosis*. Treatment for uncomplicated tuberculosis takes at least six months and, in most cases, treatment is with two first-line antibiotics: rifampicin and isoniazid. In cases of active tuberculosis, the number of bacteria is very high and, therefore, the immune system is not able to fight the infection alone, being necessary to use a combination of several antibiotics for more than six months. The most used drugs are: isoniazid; rifampicin; ethambutol and pyrazinamide. The objective of this work is the physicochemical characterization by FTIR (Fourier Transform Infrared Spectroscopy), TG (Thermogravimetry) / DTG (Derivative

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Thermogravimetry) and DSC (Differential Scanning Calorimetry) of the most used antibiotics that are rifampicin, isoniazid and ethambutol, which are produced by Farmanguinhos/FIOCRUZ/Brazil. The FTIR, TG and DSC techniques are promising tools for quality control of active principles in drug formulation for the pharmaceutical industry. Furthermore, with the results observed, greater care must be taken in the formulation of rifampicin with excipients, since this drug has polymorphism, while the others (isoniazid and ethambutol) used did not show such phenomenon.

**Keywords**: *Mycobacterium tuberculosis*. Rifampicin. Isoniazid. Ethambutol. Thermogravimetry. Differential Scanning Calorimetry. Attenuated Total Reflectance.

**RESUMO**

A tuberculose, um problema de saúde pública global, é uma doença infecciosa causada pela bactéria *Mycobacterium tuberculosis*. O tratamento para tuberculose sem complicações leva, no mínimo, seis meses e, na maior parte dos casos, o tratamento é feito com dois antibióticos de primeira linha: rifampicina e isoniazida. Nos casos de tuberculose ativa, o número de bactérias é muito elevado e, por isso, o sistema imune não é capaz de combater a infecção sozinho, sendo necessário utilizar uma combinação de vários antibióticos por mais de seis meses. Os remédios mais utilizados são: isoniazida; rifampicina; etambutol e pirazinamida. O objetivo deste trabalho consiste na caracterização físico-química por FTIR (Espectroscopia no Infravermelho com Transformada de Fourier), TG (Termogravimetria) / DTG (Derivada Termogravimétrica) e DSC (Calorimetria Exploratória Diferencial) dos antibióticos mais usados que são a rifampicina, isoniazida e etambutbol que são produzidos pela Farmanguinhos/FIOCRUZ/Brasil. As técnicas de FTIR, TG e DSC são ferramentas promissoras no controle de qualidade de princípios ativos na formulação de medicamentos para a indústria farmacêutica. Além disso, com os resultados observados, maiores cuidados devem ser tomados na formulação da rifampicina com

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excipientes, visto que tal fármaco possui polimorfismo, enquanto os demais utilizados não apresentaram tal fenômeno.

**Palavras-chave**: *Mycobacterium tuberculosis.* Rifampicina. Isoniazida. Etambutol. Termogravimetria. Calorimetria Exploratória Diferencial. Refletância Total Atenuada.

## INTRODUCTION

Tuberculosis (TB) is an infectious disease that mainly affects the lungs, but it can also affect organs such as bones, kidneys and meninges (membranes that surround the brain). It is caused by *Mycobacterium tuberculosis* or Bacillus of Koch (BK), but other species of microbacteria can also cause it such as *Mycobacterium bovis*, *Mycobacterium africanum* and *Mycobacterium microti* [1, 2, 3]. Tuberculosis remains an epidemic in much of the world, causing the death of nearly 1.5 million people each year, mainly in developing countries (Asian continent with 55% of cases, namely India, China, Indonesia and Nigeria) (2019). Brazil ranks 18th among the 22 countries responsible for 82% of the total number of tuberculosis cases in the world. Although it is a disease that can be prevented, treated and cured, it still kills around 4,400 people every year in Brazil (2020) [4, 5, 6].

Treatment for uncomplicated tuberculosis takes at least six months and, in most cases, treatment is with two first-line antibiotics: rifampicin and isoniazid. In cases of active tuberculosis, the number of bacteria is very high and, therefore, the immune system is not able to fight the infection alone, being necessary to use a combination of several antibiotics for more than six months. The most used drugs are isoniazid, rifampicina and ethambutol. Side effects in the treatment of this disease are rare, however, as different antibiotics are used for a long time, it is possible the appearance of secondary effects such as: nausea, vomiting, frequent diarrhea; loss of appetite; yellowish skin; dark urine and even fever above 38ºC [7].

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Rifampicin is the only drug, among those used in the treatment of tuberculosis, that is insoluble in water, which allows for possible problems of dissolution and consequently bioavailability. Furthermore, formulations with this drug are subject to physicochemical instability and the presence of polymorphs [8, 9]. For this, it is interesting to evaluate rifampicin, ethambutol and isoniazid by characterization techniques, such as FTIR and Thermal Analysis, techniques not observed in other articles linked to studies of these drugs. Rifampicin is the only drug of this combination insoluble in water, causing dissolution problems and consequently bioavailability. Furthermore, formulations with this drug are subject to physical chemical instability and the presence of polymorphs [10, 11, 12].

Infrared spectrometry has been widely used in organic and analytical chemistry for many years and by comparing the energy values of IR radiation it is possible to identify the molecules or types of molecules present in the samples [13].

With the evolution of the IR spectrometer using the mathematical treatment method, the Fourier transform – FT, spectroscopy in the IR region has become a valuable tool for studies of biological systems [14].

The Fourier transform method is fast and sensitive and by coupling accessories such as ATR (attenuated total reflectance) it is possible to study the surface layers of the sample, allowing a non-destructive analysis of the surface. This accessory simplifies FTIR analysis of pastes, gels, semi-solids, powders and films. The horizontal surface of the sample allows to easily collect the infrared spectrum [15].

Thermal Analysis is defined as a set of techniques that make it possible to measure changes in a material's physical or chemical property as a function of temperature or time, under a controlled temperature schedule [16].

Thermogravimetry (TG) is a widely used technique to characterize the degradation profile of materials. Exposure to high temperature can sometimes change the chemical structure and therefore the physical properties of materials. Therefore, the thermal degradation curve, under non-isothermal conditions, shows the resistance

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profile or thermal stability that the material presents when subjected to a temperature scan [16].

Differential Scanning Calorimetry (DSC) can be defined as a technique that measures temperatures and heat flux associated with material transitions as a function of temperature and time. Such measurements provide qualitative and quantitative information about physical and chemical changes involving endothermic processes (heat absorption), exothermic processes (heat evolution) or changes in heat capacity [16].

The aim of this study was to use the techniques mentioned above for the physicochemical characterization of reference drugs (rifampicin, isoniazid and ethambutol) in the fight against tuberculosis in the Brazilian health system.

## MATERIAL AND METHODS

The reference drugs (isoniazid, rifampicina and ethambutol) were purchased from *Farmanguinhos/FIOCRUZ/Brazil*.

FTIR was performed on a *Perkin Elmer* spectrophotometer, model Spectrum One, with a KBr beam splitter, equipped with ATR accessory, ZnSe cell, (four reflections) was used. All spectra were recorded within the range 4000-600 $cm^{-1}$ at 4 $cm^{-1}$ resolution, after 128 scans. The reference spectrum of pure water was subtracted from each spectrum obtained during the titration process. To reduce the atmosphere effects, the sample detector was purged with $N_2$ before the background was collected and during each titration. All spectra were collected in duplicate and all experiments were carried out at room temperature at 298±1ºC. The ZnSe crystal was rinsed with distilled water and dried with soft paper after the titration [15].

Measurements of mass variations as a function of temperature are observed in Thermogravimetry (TG) that were performed in a thermogravimetric analyzer, model Q500, *TA Instruments*. The samples were heated at a rate of 10 ºC.$min^{-1}$ from 25 to

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700ºC under nitrogen atmosphere. Additionally, the thermal properties (endo/exothermic transitions) of the drugs were measured with a Differential Scanning Calorimeter (DSC), model Q1000, *TA Instruments*. The samples (about 10 mg) were sealed in aluminium pans and heated at a rate of 10 ºC.min$^{-1}$ from -10 to 200ºC under nitrogen atmosphere [16, 17].

## RESULTS AND DISCUSSION

The IR spectra of the ethambutol, isoniazid and rifampicin (reference drugs) are shown in Figures 1, 2 and 3, respectively.

The bands observed in each graph corroborate the structural transitions of each drug, intensifying the pertinent characteristics of each structure, intensifying the existence of a possible polymorphism in the structure of rifampicin [18].

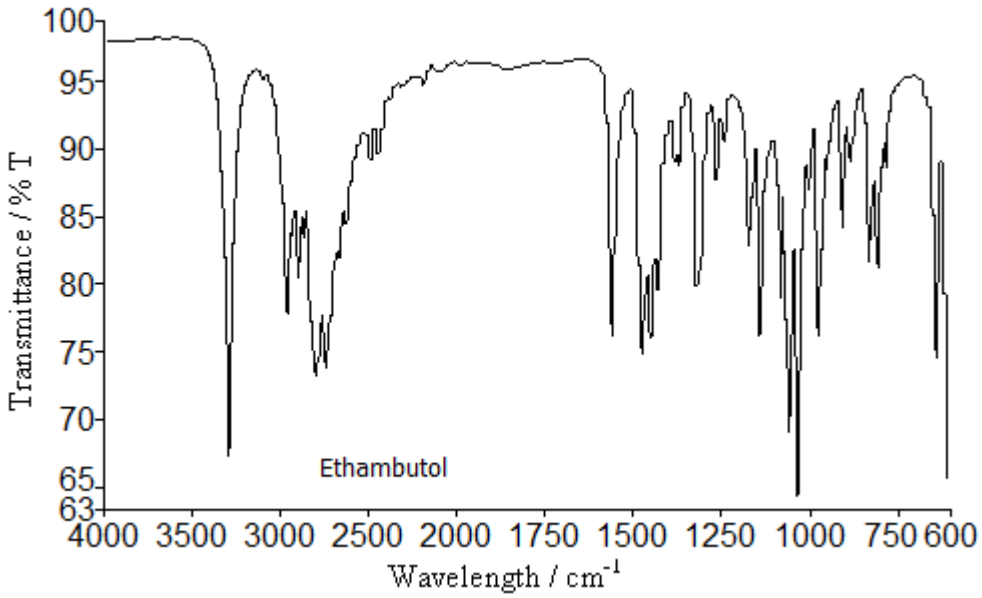


Figure 1: FTIR-ATR spectra of Ethambutol.

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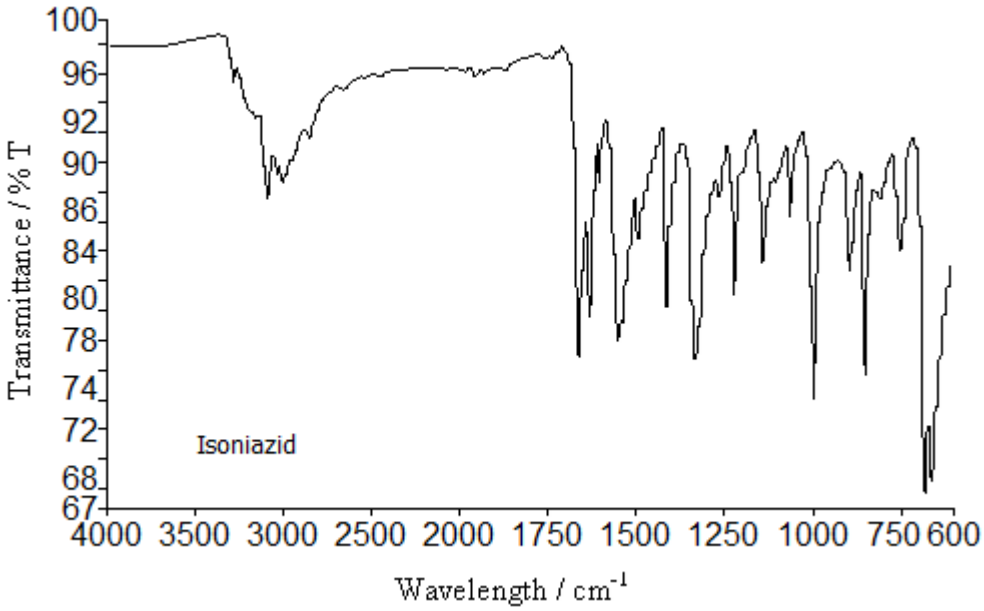


Figure 2: FTIR-ATR spectra of Isoniazid.

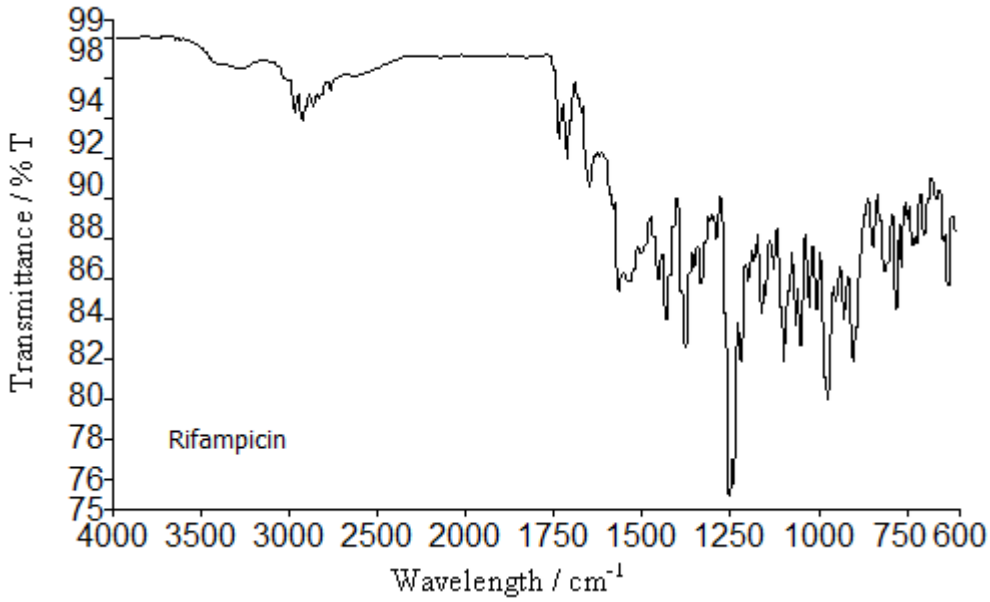


Figure 3: FTIR-ATR spectra of Rifampicin.

In Figure 4 showed that ethambutol had only a single stage of decomposition with maximum decomposition rate at 280ºC and final residue of 0.5%. In Figure 5,

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isoniazid showed two stages of decomposition with maximum decomposition rates at 243ºC and 293ºC and final residue of 0.1%.

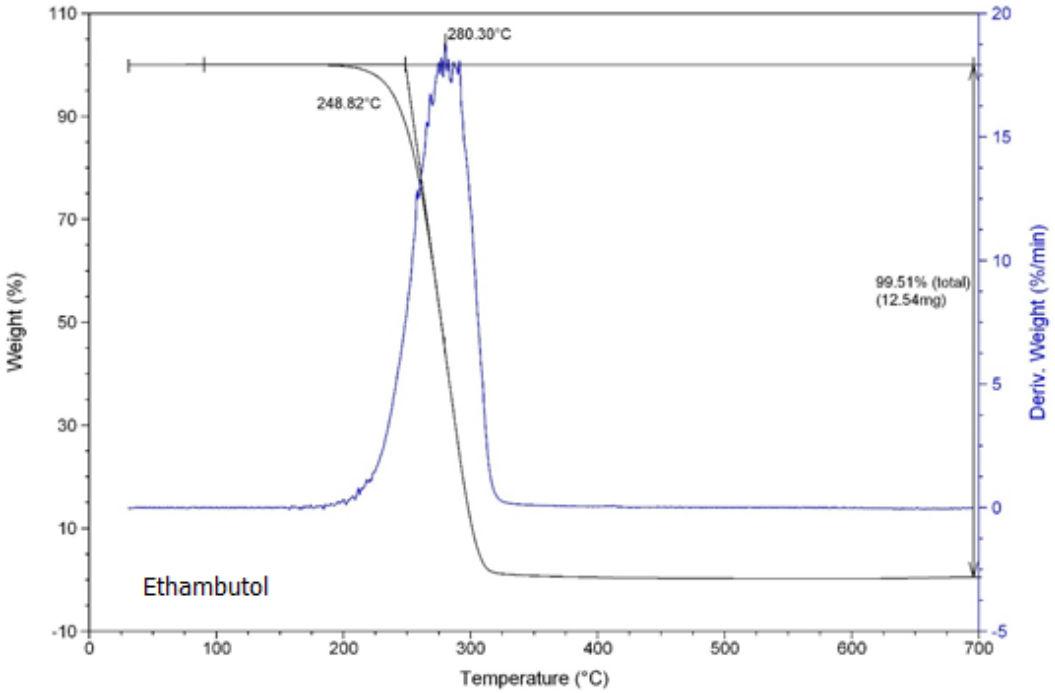


Figure 4: TG/DTG curves of Ethambutol.

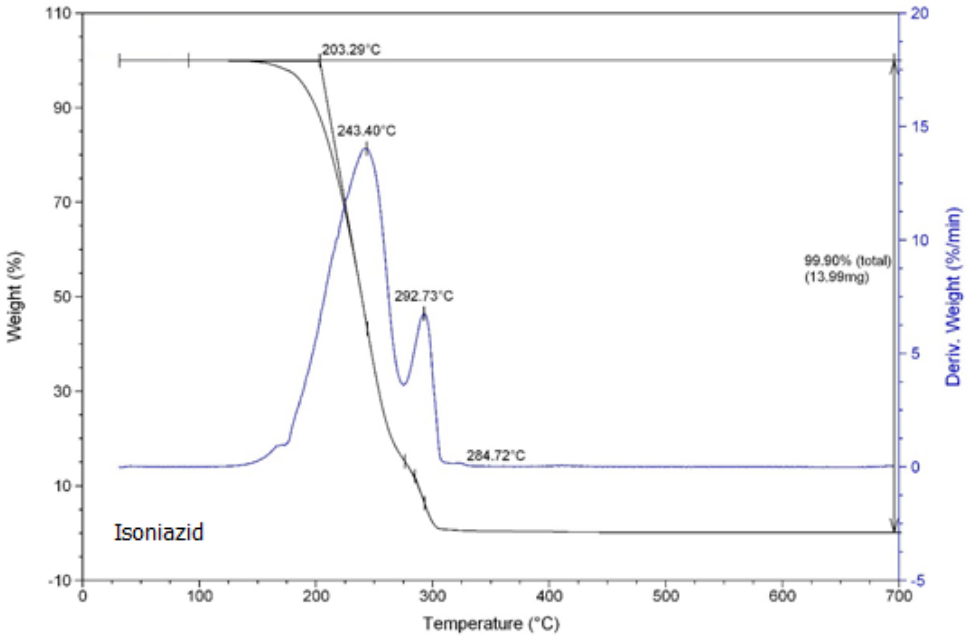


Figure 5: TG/DTG curves of Isoniazid.

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However, in Figure 6, rifampicina due to its polymorphism, had the highest number of decomposition stages in relation to the other drugs, that is, three decomposition stages with maximum decomposition rates at 211ºC, 251ºC and 335ºC, respectively, and a residue value of 37 % relatively high. Some authors observed that only at 900ºC the residue of rifampicin was 0.5%, therefore such residual value observed at 700ºC suggests the partial formation of elemental carbon during the last two stages of decomposition [19].

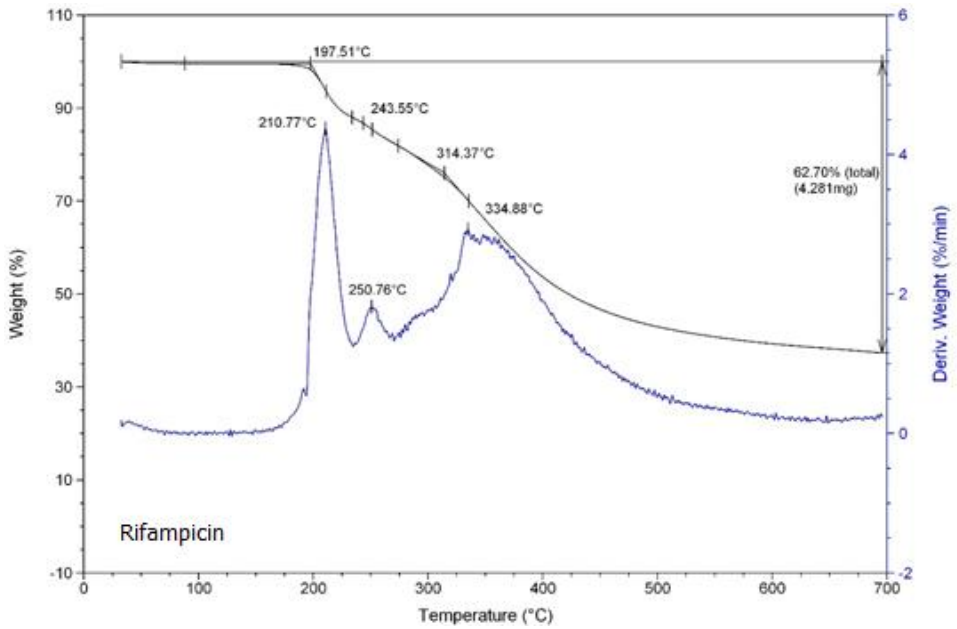


Figure 6: TG/DTG curves of Rifampicin.

Regarding the DSC curves, ethambutol (Figure 7) has a single endothermic event at 75ºC (in the three heatings). Isoniazid showed no transition in the studied range (Figure 8). In Figure 9, Rifampicin presented an endothermic event at 77ºC in the first heating, but in the second and third heating it observed - the non-existence of the first event at 77ºC, but rather a slight endothermic event around 160ºC, which corroborates the fact that this drug presents polymorphism [20].

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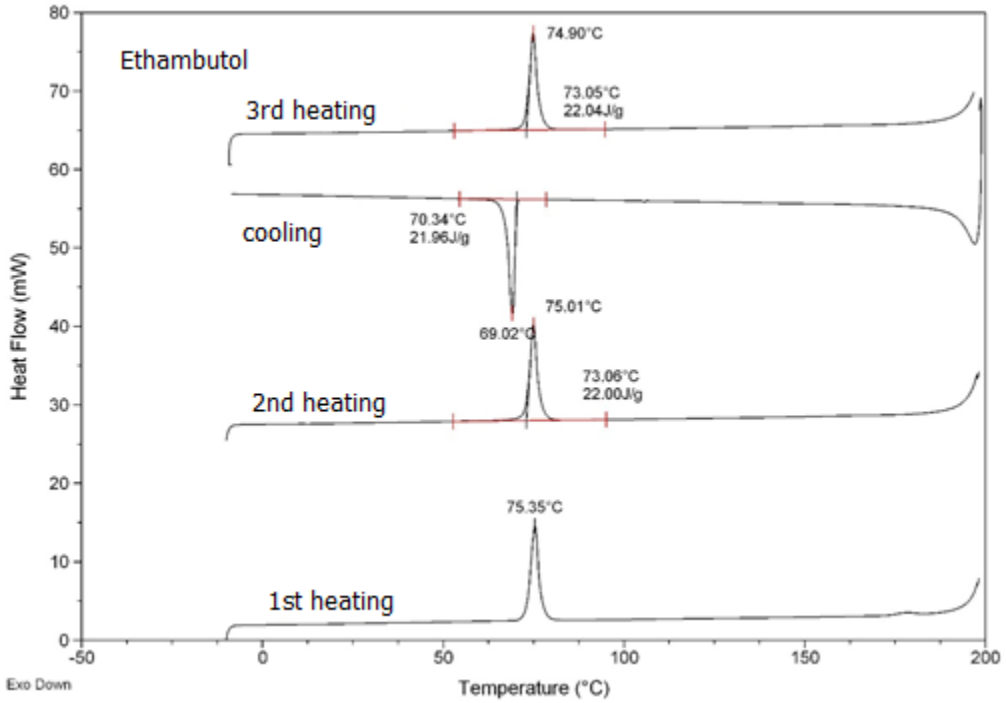


Figure 7: DSC curves of Ethambutol.

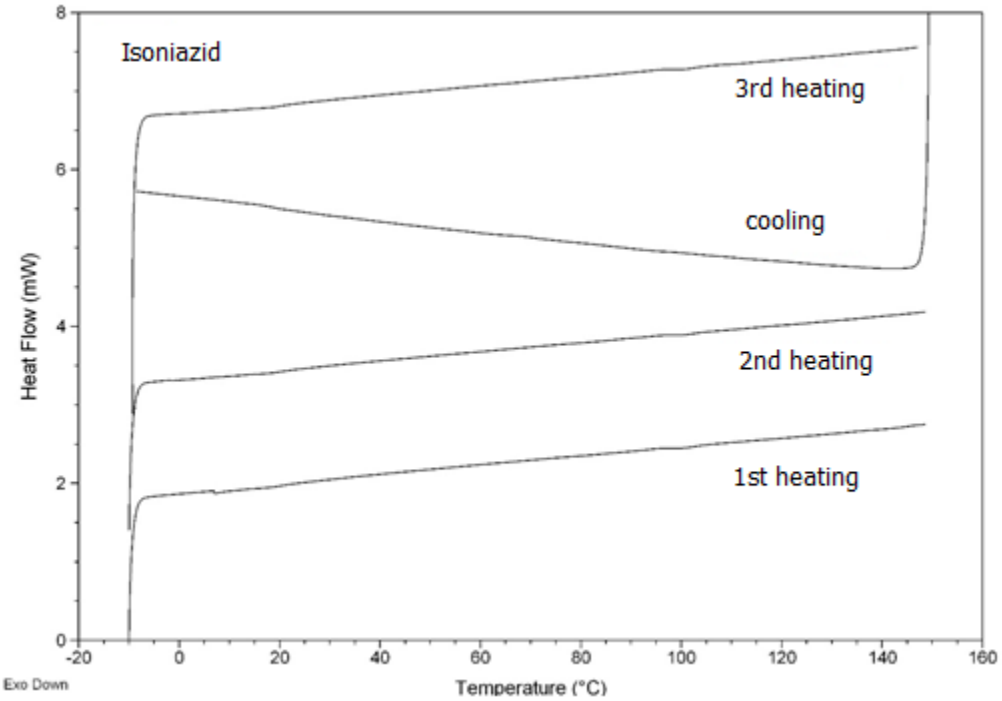


Figure 8: DSC curves of Isoniazid.

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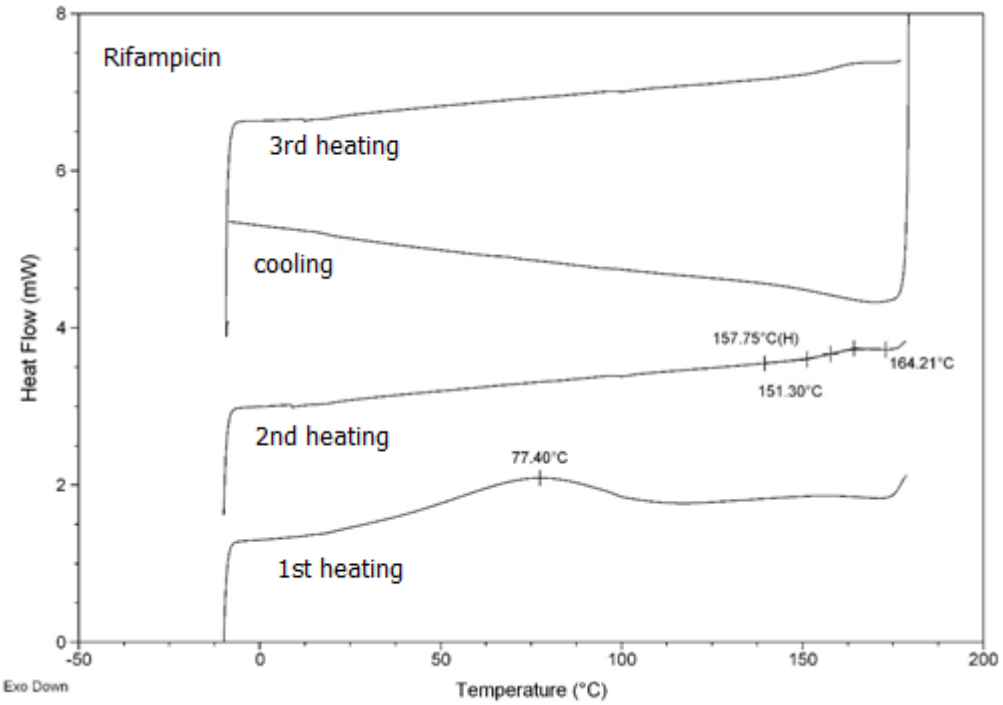


Figure 9: DSC curves of Rifampicin.

## CONCLUSIONS

The conclusions of the present work indicate that, firstly, Thermal Analysis combined with FTIR Spectroscopy is a promising tool in the quality control of active principles in the formulation of drugs for the pharmaceutical industry and, secondly, greater care must be taken in the formulation of rifampicin with excipients, due to its polymorphism. Therefore, the main objective of this work was achieved towards the physicochemical characterization of drugs and the observation of polymorphism in rifampicin.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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