Comparative Study of Teophylline - Active Substance and Commercial Drug - Using the Physico-chemical Methods

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In this paper, in order to investigate the possible interactions between theophylline – active substance with excipients we used the physico-chemical methodes: differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM). Theophylline is a methylxanthine drug used in therapy for respiratory diseases. Excipients are important components of pharmaceutical formulations and they can take an active part in the improvement of the characteristics of the pharmaceutical formulations. Analysing the curves of differential scanning calorimetry of the pure theophylline and tablets we observed the absence of incompatibility between theophylline and excipients used. XRPD patterns and FTIR spectra sustained these results, they did not show evidence in interactions in the solid state between bioactive substance and excipients. Based on the results supplied by DSC, XRPD and FTIR, were found that the excipients used were compatible with theophylline so they can be used in formulation of the tablets.

Keywords: theophylline, differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), scanning electron microscopy (SEM)

Theophylline belongs to peripheral and cerebral vasodilating agents class of drugs. Pharmaceuticals of this therapeutical class are used in the tratament the symptomatic treatment of peripherals ischemic diseases caused by some inflammatory processes, athero-sclerosis or which may occur as a result of vascular smooth muscle spasms.

From the methylxanthines compounds theophylline is used for general treatment of asthma due to its immuno-modulatory and also anti-inflammatory properties. General mechanism by which theophylline exerts its effects consist in phosphodiesterase inhibition coupled with adenosine antagonism, producing in this way bronhodilation, which is conducting to smooth muscle relaxation [1-3].

Theophylline is a bronchodilator drug used to control the symptoms of asthma, which is not supplied as an inhaler. Because theophylline can control the asthma attack represent a long time drug supplied oral and also intravenous.

Natural theophylline was founded in several plants where presents a weak diuretic properties and also act as a stimulant of central nervous system. The diuretic effect is decreasing in to the series: theophylline / theobromine / caffeine, also was observed that the stimulating effect is increasing in series, so that the most important product for industrial application is theophylline due to its diuretic effect [4].

The theophylline, or theophyllinum as it appears in FR X, has the molecular formula $C_7H_8N_4O_2$, and the chemical structure is (fig. 1).

Its denomination is: 3, 7-dihydro-1, 3-dimethyl-1H purine-2, 6-dione (1, 3-dimethyl-xanthine).

Taking into account the legal requirements all drugs must fulfill certain specifications. Any new pharmaceutically formulation is approved only if the drug can guarantees well defined levels of safety, efficiency and stability. Almost all the time, the stability level can be difficult to achieve because the active drug substance and also the excipients use in drug formulation may suffer some degradation and also can interact with each other [5,6].

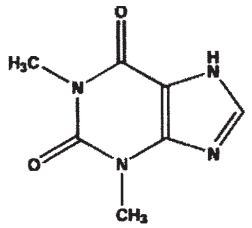

Fig. 1. Chemical structure of theophylline

The formulation represents the process by which one or more active substances are associated with different auxiliary substances in order to produce an commercial product with suitable dosage for human administration [5-8].

Any commercial drug must be able to provide the correct dosage of therapeutically active substance which is bioavailable, stable and accepted by patient.

By formulation can be determined the qualitative and quantitative drug composition, all components included in pharmaceutical formula that will be produced, the exact quantities of each component (active substance or excipients) used in drug production.

Experimental part

Materials and methods

Theophylline was used without further purification and it was producted in China\. Theophylline drug was used as product from Romania.

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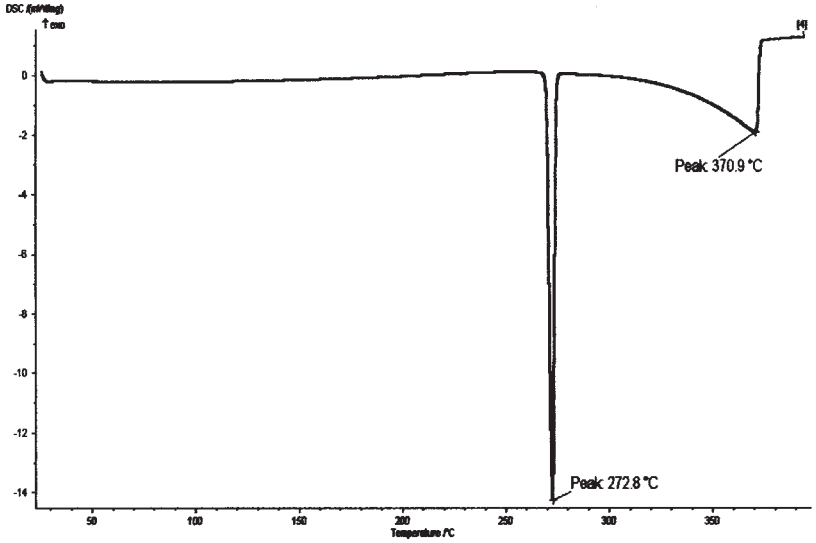

Fig.2. Differential scanning calorimetry (DSC) of theophylline – active substance

Differential scanning calorimetry (DSC)

For the DSC analysis we used an apparatus NETZSCH-STA 449C, heating rate being 10°K/min. The samples were placed in platinum crucibles under dynamic nitrogen atmosphere (20 mL·min^{-1}).

X-ray powder diffraction (XRPD)

X-ray powder diffraction pattern was obtained using a Philips X-pert Pro X-Ray powder diffractometer. The X-radiation used has the specific length of 1.5406A. X-ray diffraction was performed using a tube voltage of 40 kV and a tube current of 30 mA. The X-ray intensity was measured over a 2θ diffraction angle from 0 to 30°.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded using an FTIR –PRESTIGE 21 Shimadzu spectrometer using the classical KBr technique, in the spectral range of 4000 - 400 cm^{-1}. All FTIR spectra were recorded using a resolution of 2 cm^{-1} using the well known KBr pellet technique.

Scanning electron microscopy (SEM)

A scanning electron microscopy using Inspect S PANalytical model coupled with the energy dispersive X-ray detector (EDX) was used to characterize the external surface of the pure theophylline and also commercial product supported on carbon tape.

Results and disscutions

DSC of theophylline –active substance and theophylline drug

Analyzing the DSC image presented in figure 2 can be observed that the theophylline (active substance) presents a high stability, his thermal decomposition is starting when the temperature is over 300°C. From the DSC data presented in figure 2 can be observed the apparition of two peaks: first of them at 272.8°C and the second one at 370.9°C. By correlating this observation with data presented in scientific papers can be concluded that the first peak can be attributed to theophylline melting process (pure theophylline is melted starting from 268°C) [9, 10], and the second peak can be associated with theophylline thermal decomposition.

In case of the commercially product (drug), from the DSC curve presented in figure 3 can be observed the presence of three different peaks. First peak is observed at 134.7°C, and can be associated with a physical modification of excipients used in commercially drug formulation, or can be associated with some water losses from the excipients. The other two peaks correspond with the peaks observed in case of pure theophylline.

In figure 4 is presented the FTIR spectra recorded for pure theophylline. By analyzing the data presented in this spectrum can be observed the presence of some bands associated with planar deformation vibration at wave numbers under 841 cm^{-1} and also at wave number 977 cm^{-1}. These bands present an average intensity. Simultaneously, two intense vibrations can be observed at 741 and 507 cm^{-1}, vibrations associated with planar

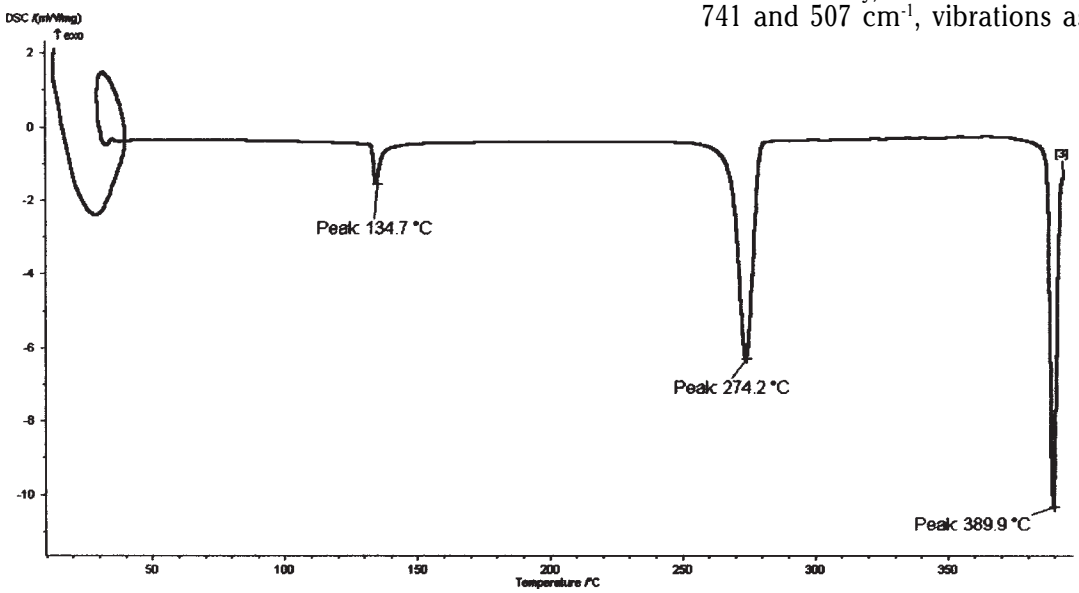

Fig.3. Differential scanning calorimetry (DSC) of theophylline – drug

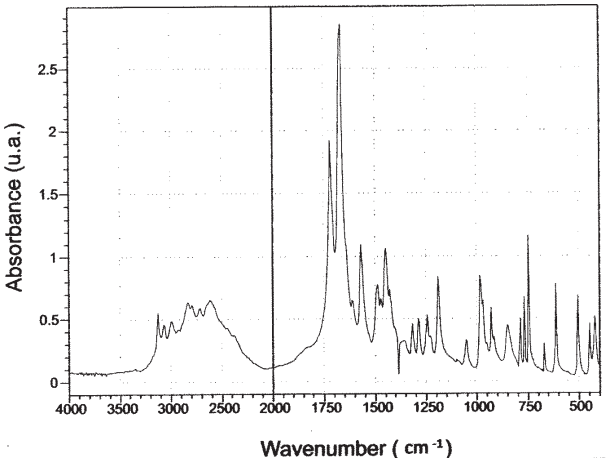

Fig.4. FTIR spectra of the theophylline – active substance

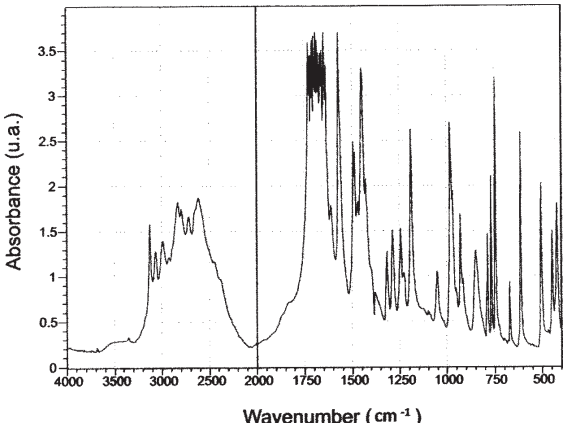

Fig.5. FTIR spectra of the theophylline commercial product

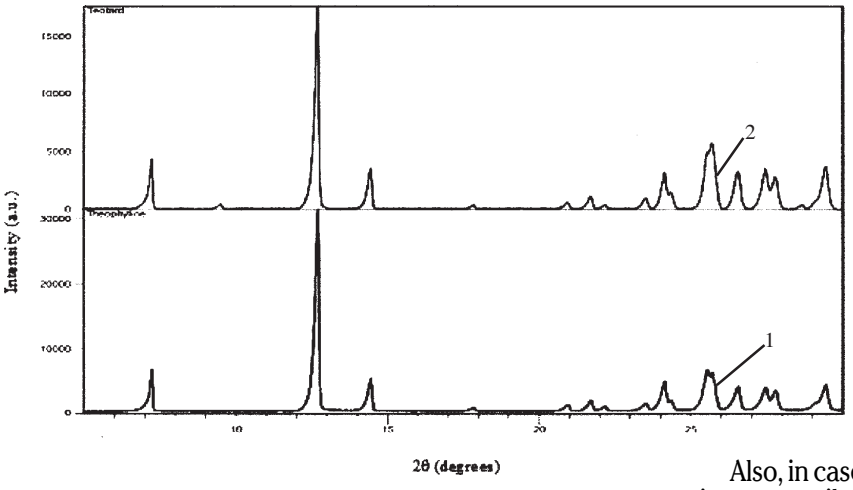

Fig.6. X-ray powder pattern diffraction for theophylline: active substance (1) and commercial product (2)

deformation of O=C-C link and with planar asymmetric deformation of C-N-C link.

Vibrations associated with N-H link stretching are more sharpened and have a lower intensity in comparison with the vibration observed in case of O-H link [11-13]. Theophylline is a dimethyl xanthines in which the hydrogen atom linked direct at nitrogen atoms are replaced by methyl groups in 1 and 3 position. By analyzing the vibration observed at 2993 cm^{-1}, which is a sharpened vibrational band, can associate this vibration with the stretching vibration of N-H link, due to the imidazole ring.

The carbonyl group present in the theophylline molecule present a strong absorption band in region of 1850 – 1550 cm^{-1}, band associated with the link C=O stretching. This characteristic band can be easily associated with the vibration of C=O link due to his position in spectrum and also due to his high intensity [14]. In case of aromatic cetons, such as in case of theophylline, the bands observed at 1719 and 1669 cm^{-1} are associated with the symmetric and also asymmetric stretching vibrations of C=O link.

The vibration associated with the C-N link due to the methyl group grafted on the nitrogen atom can be observed at approximately 1151 cm^{-1}, in case of pyridinium N-methylated ion. Bands presented at 1236 and 1286 cm^{-1} can be associated with the stretching vibration of C-N link presented in the pyrimidine ring.

The imidazoles present several bands with variable intensity between 1660 and 1450 cm^{-1}, associated with the stretching vibrations of C=N and C=C links [15]. Pure theophylline present a strong absorption band at 1600 cm^{-1}, band associated with the symmetrical stretching of C=N link, in case of ours product, this band was observed at 1570 cm^{-1}.

Also, in case of pure theophylline, can be observed some important vibration around 2609 cm^{-1} and also around 3429 cm^{-1}; first line correspond to the 5 atoms ring stretching vibration and the second one is corresponding to the 6 atoms ring stretching vibration.

In figure 5 is presented the FTIR spectra recorded in case of commercial theophylline. From this spectra can be observed presence of vibrational band associated with the planar deformation of N=C-H and C-N-H links at 980 cm^{-1} and under 928 cm^{-1}. These bands present a higher intensity, higher than the intensity observed in case of pure theophylline. Also, a similar comportment can be observed in case of the vibrations associated with the planar deformation of O=C-C, and with the asymmetric planar deformation of C-N-C. In both cases, the vibration appears at the same wave number, but the intensity is increased in case of commercial product.

Vibrational band associated with the stretching of N-H link, due to imidazole ring appear at 2994 cm^{-1} and has also a sharpen form. Also, in case of commercial product, vibrational bands observed at 1711 and 1672 cm^{-1} are associated with the symmetrical and asymmetrical vibrations of C=O link. Due to the presence of excipients, these vibrations are more intense and are shifted in comparison with the pure theophylline.

In case of commercial product, the band associated with symmetrical vibration of C=N link was observed at 1564 cm^{-1}. In this spectra are observed the vibrations associated with the stretching of 5 atoms ring at 2602 cm^{-1}, and also the one associated with 6 atoms ring stretching at 3429 cm^{-1}.

By comparing the spectra recorded in case of pure theophylline with the spectra recorded in case of commercial product we can conclude that between active

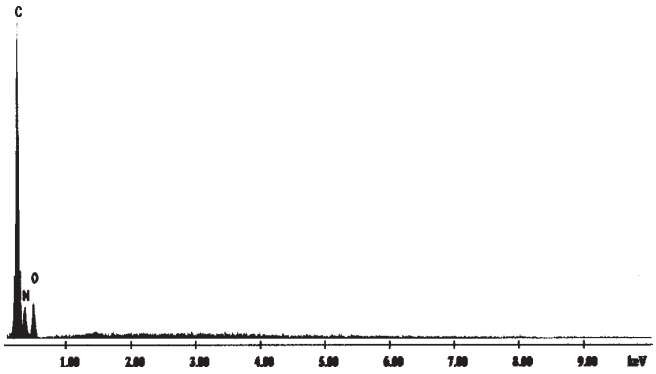

Fig.7. EDX spectrum (X-rays) of the elements on the theophylline analyzed micro area

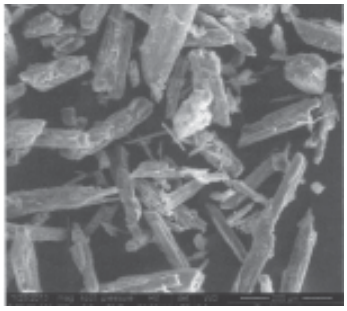

Fig.8. Theophylline granule surface aspect – 400 X

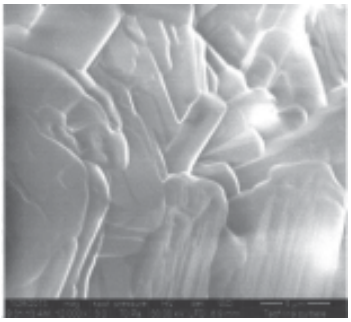

Fig.9. Theophylline granule surface aspect – 12000 X

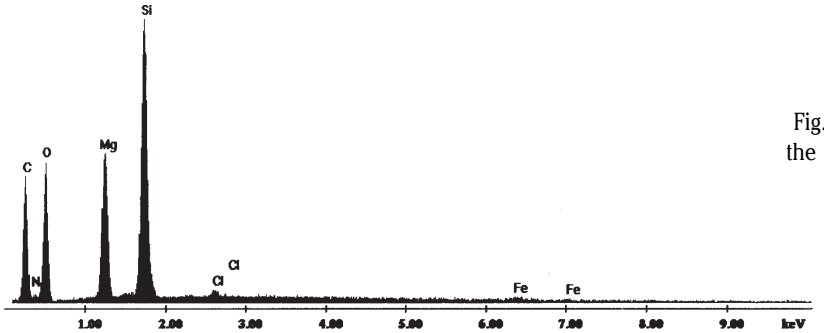

Fig.10. EDS spectrum (X-rays) of the elements on the theophylline drug analyzed micro area

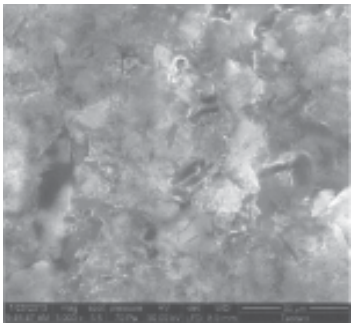

Fig.11. Theophylline- drug granule surface's aspect – magnitude 3000 X

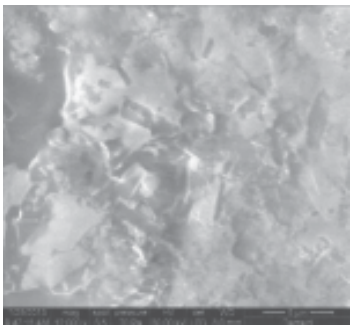

Fig.12. Theophylline drug granule surface's aspect – magnitude 12 000 X

compounds and excipients are not appearing the chemical interactions.

X Ray-diffraction of theophylline – active substances and theophylline commercial product

The XRD spectra recorded for pure theophylline and also for commercial product used in this study are presented in figure 6.

By analyzing the spectra presented in figure 6 can be observed that in case of pure theophylline are observed all the spectral lines presented in research papers dealing with theophylline [16, 17], which is confirming that the used theophylline was pure and well crystalline. From the spectra recorded in case of commercial product can be observed that all the spectral line have lower intensity, coupled with a small shift of all this lines at higher diffraction angles. Simultaneously, it was observed the presence of new lines into the diffraction spectra, peaks associated with presence of excipients in commercial product.

Based on that we can say that into the commercial product the possible interactions between active substance and excipients are not present. This observation is in concordance with the data obtained from DSC and FTIR spectra.

Analyzing the EDX spectra recorded for theophyline – active substance - presented in figure 7 can be observed that only the C, N and O were identified; based on that we can say that the analyzed theophyline has a high purity. This observation is in concordance with the data obtained from XRD spectra.

From SEM pictures presented in figures 8-9 it was observed the high crystallization of pure teophyline. Looking at the active substance at higher magnification was observed the high complexity of each teophyline

particle, correlated with a non-homogenous growth of crystallites.

EDX spectra recorded for commercial product shown that the excipients used in production are presented into the product.

From SEM pictures shown in figures 11-12, can be observed that the commercial product has the active substance particles embedded into the excipients mass. At higher magnification pictures can be observed that the production of commercial drugs the crystalline structure of active substance was not affected by production process of commercial product. The compact structure of commercial product can be associated with presence of different physical interactions between active substance and used excipients.

Conclusions

Theophylline (3, 7-dihydro-1, 3-dimethyl-1H purine-2, 6-dione is a methylxanthine drug used in therapy for respiratory diseases. Excipeints are very important components in drug formulation, they can influence positive or negative the characteristics of commercially drugs. Based on that it is really important to study all the possible interactions between active substance and all the excipeints used in drug formulation.

Analysing the curves of differential scanning calorimetry of the pure theophylline and tablets we observed the absence of incompatibility between theophylline and excipients used.

XRPD patterns and FTIR spectra sustained these results, they did not show evidence in interactions in the solid state between active substance and excipients used in the formulation.

Based on the results supplied by DSC, XRPD and FTIR, was found that the excipients used were compatible with theophylline so they can be used in formulation of the tablets.

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