THERMOANALYTICAL AND SPECTROSCOPIC STUDY ON METHOTREXATE – ACTIVE SUBSTANCE AND TABLET

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TG/DTG and Heat Flow (HF) data were used to determine the thermal parameters of methotrexate - active substance and tablets. A tablet with 2.5 mg active substance was analysed. The TG curves of MTX active substance and tablet displayed three and five thermal decomposition processes, respectively. Analysis of the HF data showed some interactions between methotrexate active substance and the excipients of tablets, suggested by alterations in the melting point of methotrexate and the modification of enthalpies of melting value (ΔH), but these interactions are not confirmed by the FTIR analysis.

1. Introduction

Methotrexate((2*S*)-2-[(4-{[(2,4-diaminopteridin-6-yl)methyl](methyl)amino}benzoyl)amino]pentanedioic acid) (MTX) is an antineoplastic agent used in the therapy of numerous types of cancers [1], especially breast cancer [2], as well in the treatment of some autoimmune disorders like rheumatoid arthritis [3]. Molecular structure of MTX is presented in Figure 1:
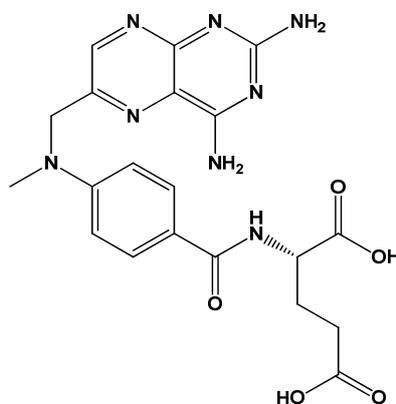

Fig.1. Molecular structure of MTX

Several studies have shown that coadministration of misoprostol with MTX is highly efficient as abortifacient in early stage of pregnancies, but as well in the therapy of psoriasis, dermatomyositis, Wegener's granulomatosis and Chron's disease [4]. MTX is an antifolate drug

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which is a potent inhibitor of dihydrofolate reductase (DHFR), an enzyme responsible for maintaining the cellular pool of reduced folates [5]. Inhibition of DHFR leads to partial depletion of the tetrahydrofolate cofactors, required for the synthesis of thymidylate and purine precursors for DNA synthesis [4,6]. Regarding the administration of MTX, it can be taken *per os* or by injection (intravenously, intramuscular, subcutaneous or intrathecal) [4].

Thermal methods of analysis (TG/DTG/HF) are important tools which can be used in physic-chemical characterization of pharmaceuticals, revealing important information about thermal stability and decomposition [7-10], polymorphism and polymorphic transitions, solid-state transformations, compatibility with excipients [11-14] and evolved gas analysis using hyphenated TG-FTIR technique [15-16].

The motivation of the present paper is that, according to our knowledge, a study regarding the solid-state characterization of MTX and tablets containing MTX as active substance was not reported earlier.

2. Materials and methods

The active substance, methotrexate trihydrate, was obtained from Calbiochem, Japan (lot No. D00135520) and has an analytical purity (HPLC purity 99.7%). The tablets are commercial' products, containing different excipients, like: lactose monohydrate, corn and potato starch, microcrystalline cellulose, magnesium stearate, colloidal silicon dioxide anhydrous, etc. Each tablet contains either 2.5mg of the active ingredient.

Thermal analysis was completed using a simultaneous TG/DTA instrument from Perkin-Elmer DIAMOND. The experiments were carried out using aluminium crucibles with approximately 7-8 mg of the sample. For determination of the heat effects the DTA curves (in μV) were changed with the Heat flow curves (in mW), so that the peak area corresponds to an energy in $J \cdot g^{-1}$ or $kJ \cdot mol^{-1}$. The experiments were completed in an air atmosphere at a flow rate of 100 $mL \cdot min^{-1}$ and they were performed under non-isothermal conditions by increasing temperature from ambient up to 500 °C, at a heating rate β= 10 $°C \cdot min^{-1}$.

The evolved gas analysis (EGA) was carried out by a coupled TG/FTIR technique, using a Perkin Elmer SPECTRUM 100 device with an IR gas chamber connected by a transfer line to the exit of the DIAMOND furnace. The air flow of 100 $mL \cdot min^{-1}$ and a heating rate of 20 $°C \cdot min^{-1}$ were used. The FTIR spectra were processed by the Sadtler Gas Vapor Library. The highest flow rate of 100 $mL \cdot min^{-1}$ app ears to provide a minimum transfer time and was used in all subsequent measurements. In addition to the flow rate, the instrumental design and coupling system has been optimized to reduce the effect of transfer time.

3. Results and discussion

a) Thermal behaviour

The thermal behaviour of these samples was investigated in air atmosphere by thermal analysis (TG, DTG, HF) in order to evidence the modifications during heating and also the thermodynamic effects that accompany them.

Thermal decomposition of the two studied samples (active substance MTX_{AS} and tablets MTX_{TAB}) during heating at β=10 $°C \cdot min^{-1}$ in an air atmosphere occurs in four and five thermal decomposition processes, respectively.

For the active substance, the first two events start at 34 °C with the mass loss of 4% and DTG_{peak}=39 °C, respectively at 71 °C with the mass loss of 7% and DTG_{peak}=91 °C as presented in Fig.2. The experimental values for the mass loss are in good agreement with the total theoretical percentage of the water loss ($\Delta m_{theoret}$= 10.62%). The mass loss value for one mole of water is 3.54%. After the removal of the hydration' water, the anhydrous methotrexate is formed and it shows a good thermal stability in 127-194 °C temperature range. The thermal decomposition of active substance, anhydrous form, takes place in 225-320 °C temperature range with a mass loss Δm= 40%. The decomposition residue is 44.04 % from initial mass.
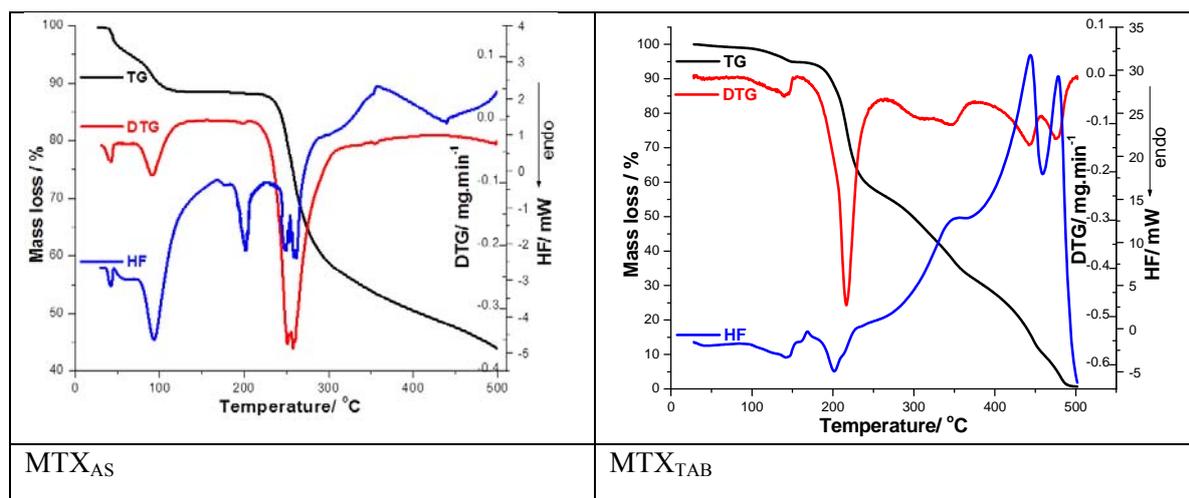
| MTX$_{AS}$ | MTX$_{TAB}$ |

Fig. 2. The thermoanalytical curves TG/DTG/HF obtained in air at
β=10 °C·min^{-1} for the analysed samples.

The Heat Flow (HF) curve of methotrexate presents four sharp endothermic events. First two processes are accompanied by a mass loss and occur up to T= 132.5 °C, when appears a horizontal landing which highlights a constant mass of the sample. At 196 °C (T_{onset}=192 °C; ΔH_{fusion}=32.069 J·mol^{-1}) a new endothermic peak appears, indicating the melting and corresponding to the values from the literature (195 °C) [17]. Heat Flow technique confirmed the purity of the methotrexate AS and demonstrated the MTX melting followed by the decomposition. This does not occur with the MTX$_{TAB}$ sample which has a more complex thermal behaviour due to the presence of excipients.

The thermogravimetric profile of MTX tablets presents five thermal decomposition stages. The TG curve of the tablet shows that the formulation is thermally stable up to 53 °C which corresponds well to the initial decomposition temperature of the methotrexate trihydrate when takes place the loss of three water molecules. This process is continuous and is followed by the decomposition of lactose monohydrate which is one of the excipient. All the others excipients are thermally stable up to: starch 278 °C; microcrystalline cellulose 285 °C; magnesium stearate 290 °C. Silicon dioxide is thermally stable to significantly higher temperatures. The HF curve for lactose monohydrate shows two endothermic peaks. The first peak with a maximum at 143 °C corresponds to water loss and the second one with maximum at 203 °C is attributed to the fusion of this excipient [18], but MTX-AS also melts in this temperature range. In the case of the tablet, the melting process of the active substance and lactose monohydrate is accompanied by simultaneous thermal decomposition.

The similar thermal behaviour of MTX$_{AS}$ and MTX$_{TAB}$ denies the occurrence of any chemical interaction between MTX and the excipients upon tablet making as well as during its storage which is supported by the results of the FTIR analysis of tablets (Fig. 3) as well, at temperature lower than the one corresponding to the melting point of the active substance.

The thermal analysis results of the both samples, determined in non-isothermal conditions, are summarized in Table 1.

Table 1. Thermoanalytical data of the samples, at β=10 °C·min⁻¹.

Sample	Process	T_i/°C	T_f/°C	$T_{\text{max DTG}}$/°C	$T_{\text{max HF}}$/°C	Δm/%
MTX$_{AS}$	I	34	46	39	43	5
	II	71	127	91	93	7
	III	192	224	-	196	0
	IV	225	320	258	261	40
MTX$_{TAB}$	I	53	148	141	142	5
	II	148	261	217	201	38.6
	III	261	375	344	348	24.6
	IV	375	458	441	444	21.2
	V	458	490	476	479	9.5

To observe the chemical and/or physical interactions between the drug and the excipients in tablets, UATR technique was used and revealed that FTIR spectroscopy coupled to the thermoanalytical methods are complementary tools in drug analysis and is appropriate for this study. The FTIR spectra of methotrexate, active substance and tablet, are presented in Fig.3.
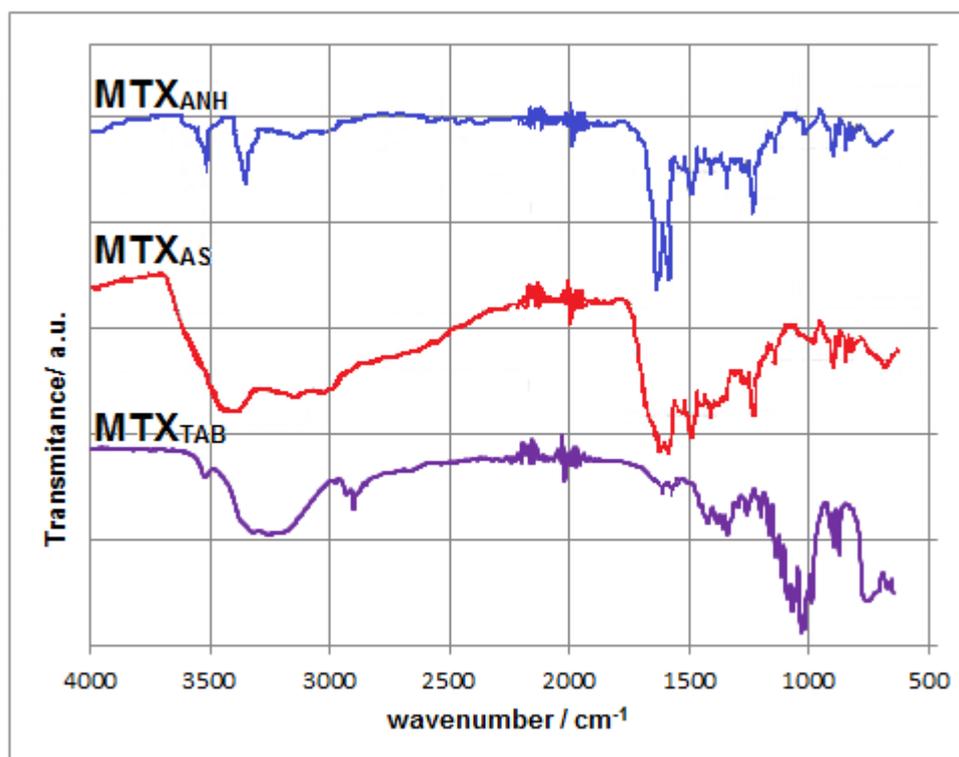

Fig. 3. FTIR spectra of methotrexate – active substance (trihydrate and anhydrous) and tablet

b) UATR-FTIR analysis of MTX$_{AS}$, MTX$_{ANH}$ and MTX$_{TAB}$

The UATR-FTIR spectrum of pure methotrexate – active substance (MTX$_{AS}$) shows characteristic absorptions band as a broad signal at 3450 cm⁻¹ (O–H stretching from carboxyl groups superposed with the O-H stretching from crystallization water), at 3080 cm⁻¹ (primary amine N–H stretching), at 1670-1600 cm⁻¹ assigned to C=O stretching (–C=O stretching from carboxylic group and C=O stretching from amidic group, so the C=O band is splited into a doublet in the MTX$_{AS}$ sample). The bands corresponding to N-H bending from amidic group appear in the 1550–1500 cm⁻¹ spectral range, partly overlapping with the aromatic –C=C stretching. Another prominent bands, such as 1400-1200 cm⁻¹ correspond to –C–O stretching from carboxylic group,

930 cm^{-1} to O–H bending out of plane and 820 cm^{-1} to C–H - 2-adjacent hydrogens on an aromatic ring, *para* substitution. All the bands identified in the FTIR spectrum are in good agreement with the molecular structure of MTX and confirm its purity.

The UATR-FTIR analysis of the anhydrous methotrexate (MTX$_{ANH}$) was carried out on the anhydrous sample. The anhydrous sample was obtained by heating hydrate MTX at 130 °C, than allowed to cool down at 25 °C in anhydrous inert atmosphere. A similar UATR-FTIR spectrum was obtained, with the difference that the bands corresponding to hydration water were no longer present. According to this, it can be noticed the disappearance of the broad signal from 3500-3000 cm^{-1}, as well the sharpening of the signals corresponding to O–H stretching from carboxyl groups and the ones corresponding to N–H stretching. A sharpening is also observed in the case of C=O bands, due to the facts that no more overlapping with the bands of water around 1640 cm^{-1} takes place. The similarity of the two FTIR spectra (MTX$_{ANH}$ and MTX$_{AS}$) leads to the conclusion that no polymorphic transitions occur in this temperature range.

The UATR-FTIR analysis of the tablet (MTX$_{TAB}$) is not offering relevant spectroscopic information due to the fact that the pharmaceutical formulation contains a low quantity of MTX comparing to the ones of excipients. Mainly, the FTIR spectrum consists of superposing of characteristic bands of used excipients. Due to superposing and to the fact that excipients are in a higher concentration than MTX in the tablet, the characteristic bands of the active substance are significantly decreased, making them almost indistinguishable in the spectrum. Although a band around 3500 cm^{-1} can be assigned to O–H stretching of carboxyl groups and two weak bands are still appearing in the spectrum of MTX$_{TAB}$, namely the ones corresponding to C=O stretching.

c) UATR-FTIR analysis of remaining chars (MTX$_{AS}$ and MTX$_{TAB}$)

The analysis of the remaining chars from both MTX$_{AS}$ and MTX$_{TAB}$ was realised on the solid sample after thermal treatment at 500 °C (Fig.4). A surprisingly similarity of the two spectra can be observed and can be explained by the fact that at 500 °C the molecular structure of MTX molecule isn't completely destroyed. The similarity of the composition of the two remaining chars can be explained by the fact that the used excipients (lactose, starch, cellulose and magnesium stearate) present a lower thermal stability than the one of MTX molecule, so their degradation occurs at temperatures lower than 500 °C, as previously stated. For MTX$_{TAB}$ char, a distinctive band appears at 1110 cm^{-1} and can be associated to Si–O stretching of silica [19], being known that SiO$_2$ is thermally stable up to 750°C [20].
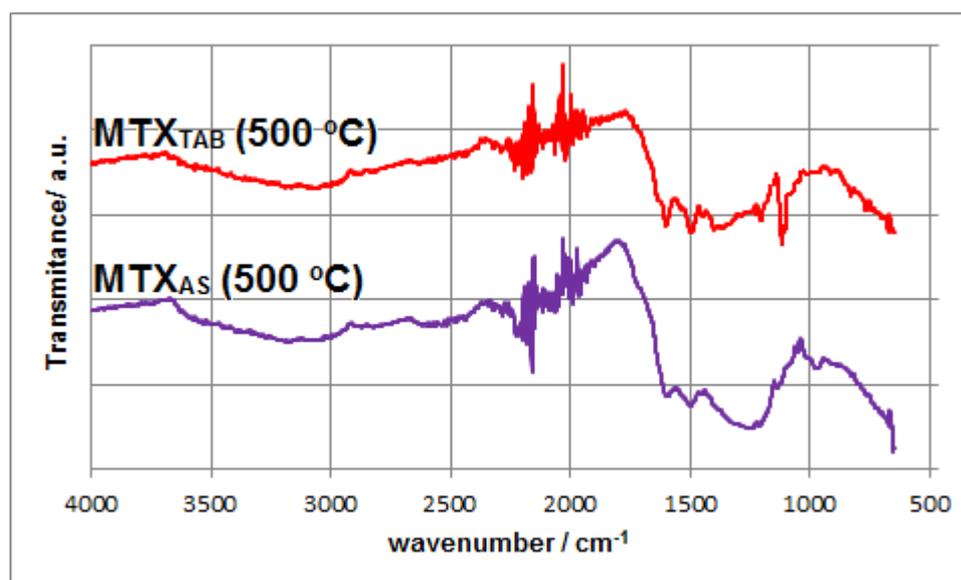

Fig.4. Comparative analysis of remaining chars

4. Conclusions

Thermal decomposition of MTX active substance and a corresponding pharmaceutical formulation (2.5 mg per tablet) during heating at $\beta=10$ °C·min^{-1} in air atmosphere occurs in four and five thermal decomposition processes, respectively. TG curve of the tablet reveals the same decomposition profile with the one of the MTX active substance. The differences appear because the excipient's presence but which not changes the drug behaviour in the mixture. All the results obtained from thermal analysis were corroborated with the data obtained by a spectroscopic technique, namely UATR-FTIR and a good agreement was found. The study revealed that MTX molecule is stable up to 127 °C as hydrate, than as anhydrous substance up to 194 °C. Also, it was confirmed that no interactions occur in tablet up to the melting point of active substance (196 °C).

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