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Thermo Gravimetric and Mass Spectrometric Study of Ionized Rifampicin and their Fixed Dose Combination Antituberculosis Drugs

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Abstract: Thermogravimetry and differential thermal analysis in combination with FTIR, elemental analysis and mass spectrometry were used to investigate the presence of mutual interactions in 1:2 w/w % solid state fixed dose combination of Ionized and Rifampicin and responses compared with those of pure drugs. The TG/DTA studies were carried out in temperature range ambient to 650˚C, Mass spectra were recorded for the mixture and compared with those of the pure drugs. Ionized was found to undergo two stage weight losses where as Rifampicin showed extensive decomposition in both inert and air atmospheres. The drugs in mixture behave completely different and show multiple decomposition patterns on heating. DTA curves revealed the presence of internal oxidation in the Rifampicin affecting its stability, its weight loss pattern and that of the Ionized present in the mixture. Mass spectra of pure drugs and the mixture recorded under various conditions confirm the presence of mutual interactions of drugs in mixture and suggest that in N_2 atmosphere a new compound having definite composition is formed in the temperature range 320 to 450˚C as revealed from elemental analysis and mass spectral data.

Keywords: Antituberculosis drugs, Isoniazid, Rifampicin, TG/DTA, Mass spectrometry.

1. INTRODUCTION

Tuberculosis (TB) is a public health problem of global dimensions. The disease has high prevalence in Pakistan whereby country witnesses' emergence of over four hundred thousand cases every year of which, about fifty percent happen to be smear positive. The disease has placed Pakistan at fifth number among countries having high burden of diseases. TB has also been declared global emergency by World Health Organization therefore Pakistan has developed national TB control program incorporating WHO guidelines and other international standards (National Guidelines, 2015). One of the key points of program includes treatment protocol consisting of administration of front line antituberculosis drugs Isoniazid (INH), Rifampicin, (RIF), Pyrazinamide (PA) and Ethambutol (EB) base and its dihydrochloride as binary mixtures of fixed dose combinations (FDC) or in the form or single drugs to tuberculosis patients. Being potentially important, these drugs are increasingly relied upon with special emphasis on FDCs due to their specific roles in the enhancement of patient satisfaction (Blomberg, *et al*. 2001). However the use of FDCs has been found to be problematic in terms of bioavailability of active ingredients, inter drug and drug excipient interactions giving rise to compositional issues and changes in their thermodynamic characteristics necessary for bioavailability. Isoniazid (INH) Rifampicin (RIF) mixture is one such potential combination suffering from the issue of inadequate bioavailability of rifampicin from FDC hindering TB therapy severely. (Singh and Mariappan 2001, Singh *et al*. 2000 and & Laing *et al*. 1999)**.** To address the issue number of studies has been carried out, Agarwal *et al*. (2004) used thermogravimetry (TG), particle size analysis and other techniques to characterize the drug in solid state and concluded that rifampicin in mixture has poor solubility due to presence of its hydrogen bonded polymorphs causing decrease in its bioavailability. Freire *et al*. (2009) and Lavor *et al*. (2014) studied inter drug and drug excipient interactions using TG and other techniques and reported mixed findings. Other factors such as falsification and sub standard nature of anti tuberculosis drugs available in market have also been implicated in the poor bioavailability of the rifampicin in FDC **(**Bate *et al*. 2013). Despite the use of different approaches (Satish and Sevukarajan 2009, Silva *et al*. 2014 and Henwood, *et al*. 2000), the problem continues to be a challenge in TB therapy therefore it is necessary to further investigate the potential hindrances in terms of molecular interactions leading to formations of new products within the combinations under different thermodynamic and biological conditions. The techniques of thermal analysis including TG, DTA and DSC coupled with spectroscopy are capable of detecting such changes and routinely used in drug compatibility

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studies (Caron, 2002). None of the studies reported so far have used high temperature approach to understand the problem therefore present work was planned to investigate the behavior of FDCs at high temperatures using the technique of TG and mass spectrometry. The results of the approach might prove helpful in further understanding of the inter drug and drug excipient interactions.

2. EXPERIMENTAL PROCEDURE

Pure samples of Ionized, Rifampicin and Ionized/ Rifampicin FDC (1:2 w/w) were donated by Novartis Pharma Ltd Jamshoro Pakistan. The purity of the individual drugs was checked by recording FTIR spectra and melting point determination. Mass Spectra were recorded on Jeol JMS-HX – 110 Mass Spectrometer at HEJ Research Institute of Chemistry University of Karachi. Thermogravimetric and differential thermal analysis studies were conducted on Perkin-Elmer Diamond TG/DTA analyzer and the equipment calibrated with high purity indium (M.P: 156.6˚C) at regular intervals during the course of study. The curves were recorded in high purity nitrogen and air flowing around the sample at 100cc min^{-1} in the temperature range 30 to 650˚C and optimized heating rate of 10˚C min^{-1}. For Mass spectral analysis of decomposed products the method of Wesołowski and Konarski (1995) was followed with the exception that mass of the samples used was equal to or less than 100mg.

3. RESULTS AND DISCUSSION

Thermogravimetry (TG) and DTA curves of pure INH, RIF and INH/RIF FDC recorded in nitrogen and air atmospheres are shown in **(Fig-1, 2 and 3)**, respectively. The temperatures and magnitudes of weight losses and corresponding ΔH values are listed in **(Tables-1 and 2)**.

Table 1 Thermogravimetry characteristics of Antituberculosis Drugs ionized Rifampicin Fixed dose combination mixture

sample	Temperature range ˚C		weight loss %	
	N_2	Air	N_2	Air
INH	143 – 234	150 – 307	85.68%	83.43%
	234 – 305	307 – 343	14.31%	16.45%
RIF	280 – 218	189 -243	10.05%	12.71%
	218 – 263	243 – 414	15.80%	26.27%
	263 – 530	414 – 647	70.68%	62.71%
FDC	145 – 179	163 – 213	4.79%	13.69%
	182 – 270	215 – 259	63.42%	35.75%
	270 – 536	259 – 618	27.28%	33.93%

Table 2 Characteristics of DTA Antituberculosis Drugs ionized, Rifampicin and Fixed dose combination mixture and respective enthalpy changes

sample	Temperature range ˚C		Transition ± ΔH J/g Enthalpy	
	N_2	Air	N_2	Air
INH	164 – 192	174 – 199	+76.219	+25.979
	211 – 289	232 – 291	+65.144	-22.043
	302 – 318	298 – 318	+1.954	-2.624
	-	331 – 347	-	-1.093
	-	396 – 410	-	+1.530
RIF	184 – 194	222 – 234	+1.534	-1.276
	256 – 300	272 – 309	+7.163	-7.659
	300 – 529	402 – 636	-63.800	-625.30
FDC	147 – 163	138 – 160	-6.187	+3.337
	163 – 177	161 – 186	+17.302	+26.701
	222 – 566	195 – 232	-595.606	-4.353

Table 3 Elemental Analysis of the product formed due to interactions of Antituberculosis Drugs ionized and Rifampicin in Fixed dose combination mixture.

Element	% C	% H	% N	% O
%	69.46	12.25	11.41	6.50

TG curves show that the INH is fairly volatile in nitrogen atmosphere and undergoes 85% first stage weight loss between 143 to 234˚C and 14% second stage weight loss between 234 to 305˚C. The FTIR and Mass spectral analysis of the first stage weight loss showed non decomposed volatilization of INH where as during second stage, it formed ethylenediamine. This was confirmed from the mass spectroscopic and FTIR

analysis of trapped vapors and leftover residue (molecular ion M/e 60, FTIR ν_{NH} 3320cm^{-1} , ν_{CN} 1090cm^{-1}, δ_{NH} 1610cm^{-1}) which is in accordance with the findings reported by Wesolowski, and Konarski, (1995). The TG curve of INH in air retains same characteristics except 10 to 50˚C shift in thermal events to higher temperatures. This is indicative of the fair stability of the drug to oxidation at lower temperatures. The TG curve of rifampicin in N_2 on the other hand is characterized by multiple weight losses **(Table-1)** including major loss equal to 70% between 300 to 500˚C and thereafter another slow loss accompanied by decomposition. This is probably due to the polymorphic nature of the compound and the presence of enough oxygen as part of chemical constitution of the drug which may promote the internal oxidation initiating decomposition at fairly low temperatures (Agarwal, 2004 Byrn, *et al*. 2001). These effects became more significant when the drug was subjected to TG analysis in the air atmosphere. The TG curve of FDC mixture does not retain the major characteristics of any of the pure drugs and results in slow weight losses in the temperature range 150 to 530˚C **(Table-1)**. The process of internal oxidation in Rifampicin component seems to prevail at all temperatures and its decomposition products extensively interact with INH changing its weight loss pattern. The mass spectra recorded for samples withdrawn after heating up to 130˚C also indicated presence of un-decomposed INH, a feature which could not continue at high temperatures.

DTA Curves

DTA curves of pure drugs recorded in inert and air atmospheres are shown in **(Fig-2).** The DTA of INH in N_2 has characteristic melting endothermic event at 170˚C with ΔH values 76.219J g^{-1}. And another broad endothermic event appears around 280˚C due to decomposition. In air atmosphere the DTA curve of INH exhibits slight shifts in melting endotherm but the second peak around 300˚C becomes more pronounced as compared to one recorded in the N_2 atmosphere. The end stage small exothermic events reveal **e**xtensive reactions of oxygen with the drug. The DTA curve of Rifampicin on the other hand is an unsymmetrical broad exotherm composed of smaller shoulder peaks along its course. The onset temperature of curve and its overall shape indicated dominance of internal oxidation within the drug making difficult to precisely determine the magnitude of the enthalpy changes. In air atmosphere, the Rifampicin curve shows several minor poorly resolved thermal events indicative of extensive decomposition in the temperature range 222 to 309˚C and finally a fairly large exotherm peak at 530˚C with the enthalpy equal to 652.3J g^{-1}.

The DTA profile of FDC mixture in N_2 and air atmospheres shown in **(Fig-3)** is quite different from those of the pure drugs. In nitrogen the mixture gives rise to an endotherm around 166˚C (ΔH values 17.3J g^{-1}), while rest of the record is a broad exothermic peak. The drugs in FDC mixture seem to undergo mutual interactions resulting in the 90 degrees shift of thermal events to lower temperatures. The shape of the FDC exothermic peak in the range 250 to 520˚C is broad but fairly symmetrical as compared to pure Rifampicin showing smooth exothermic reaction indicative of the formation of some stable product of the drugs (ΔH 595.6J g^{-1}). In air FDC mixture shows all the endothermic events starting with a small peak at 153˚C followed by a peak at 160˚C due to melting of INH. The small endothermic effect prior to this peak indicates earlier interactions of the drugs in mixture which continues along the entire course of the curve forming a broad unsymmetrical trough characteristic of exothermic effect.

Mass Spectra

The mass spectra of the samples of pure drugs are shown in the **(Fig-4 and 5)** and the probable fragmentation pattern of Ionized is also shown in **(Fig-4)**. The mass spectrum of RIF **(Fig-5)** is fairly complex lacking molecular ion peak and poor reproducibility of M/e signals which made it very difficult to assign fragmentation pattern. It is interesting to note that the mass spectra of ionized samples with drawn at temperatures between 140 to 230˚C had identical pattern of fragments to those of INH pure drug but in the second stage weight loss between 240 to 300˚C it gave decomposition products of which ethylenediamine could be identified at M/e equal to 60.

The interesting feature of this study was formation and isolation of a new adducts resulting from heating of the FDC mixture in inert atmosphere. The product initially formed in the temperature range 320 to 450˚C in sample holder of TG analyser was again prepared in lab by heating of the solid FDC mixture (1:2 w/w) at 320 to 350˚C in the flowing nitrogen atmosphere. The product thus obtained was recrystalized from n- hexane and had melting point equal to 213˚C. it showed good solubility in ethanol, chloroform, lesser solubility in benzene and toluene but no solubility in water and acetone. The mass spectrum of the compound shown in **(Fig-6)** resulted in fair abundance of low molecular weight fragments identical to those of the INH (M/e, 44, 51, 78.1, 106.1) but none identical to mass spectrum of RIF except few peaks M/e 193.2 and 223.3 being equal to or nearest to those noted in mass spectrum of RIF. The elemental analysis of the compound for C, H, N, and Oxygen is given in **(Table-3)** and corresponds to molecular mass of 242.1 corresponding to empirical

formula $C_{14}H_{30}N_2O$ and the molecular ion peak M/e 242.2 present in its mass spectrum. This preliminary results shows that at high temperatures some type of chemical reaction occurs between the INH and RIF resulting in the formation of new product reasonably similar to INH. However this finding is inconclusive at this stage and needs to be further investigated.

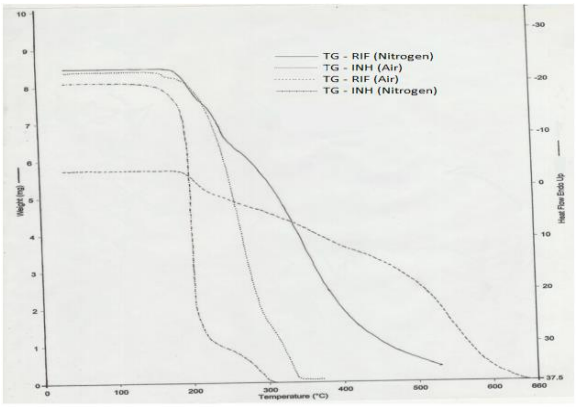

Fig 1 TG Curves of INH and RIF at 10˚C/min in N2 and air 100 cc/min

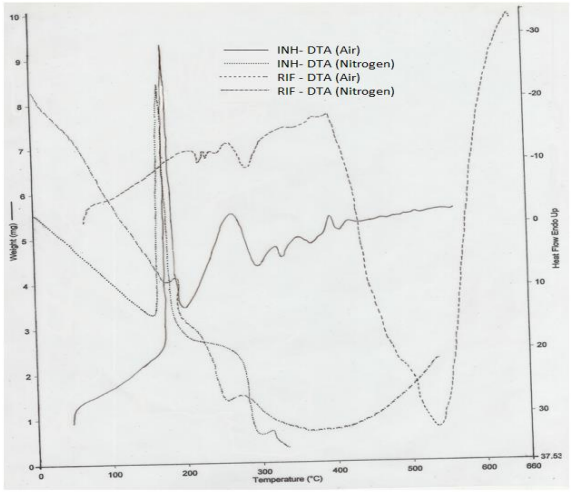

Fig 2 DTA Curves of INH and RIF at heating rate 10˚C/min in N_2 and air 100 cc/min

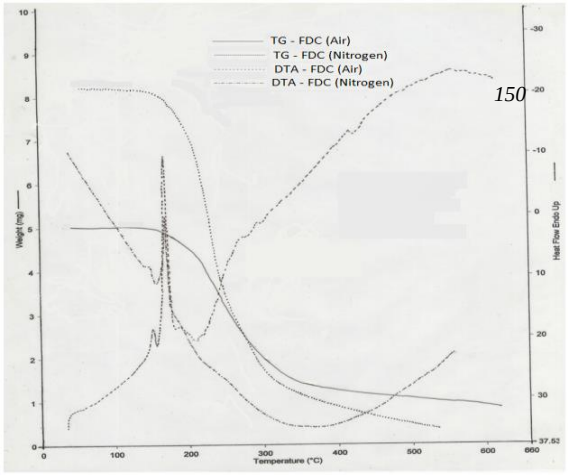

Fig 3 TG and DTA Curves of FDC mixture at heating rate 10˚C/min in N_2 and air 100 cc/min

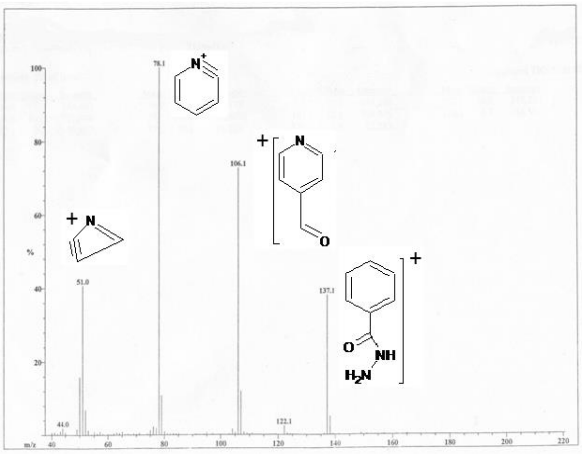

Fig 4 Mass spectrums of INH and its probable fragments

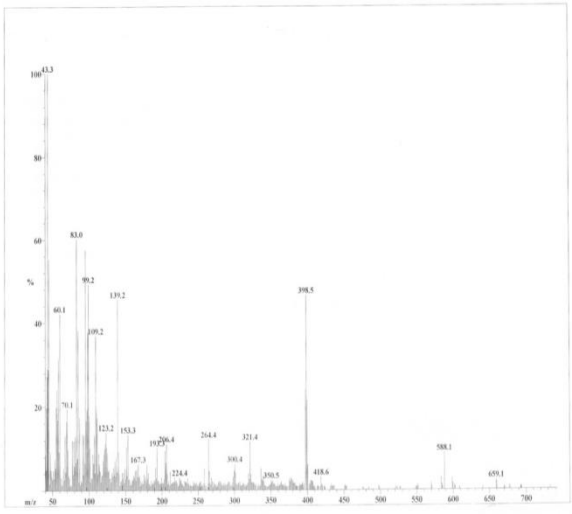

Fig 5 Mass spectrums of RIF

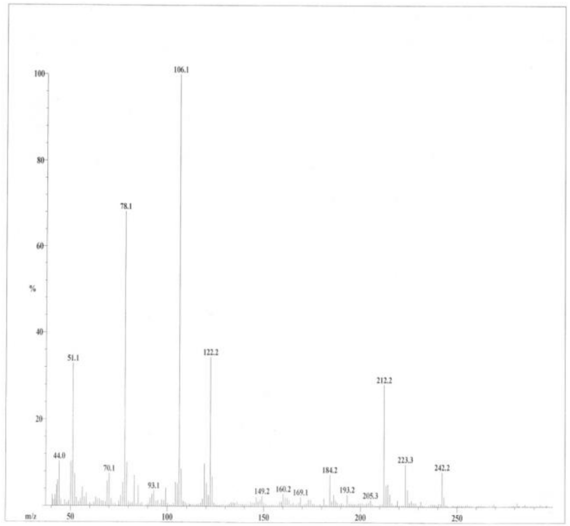

Fig 6 Mass spectrum of product formed from interaction of INH and RIF in FDC in temperature range 320 – 350˚C

4. CONCLUSIONS

The TG and DTA studies of the INH, RIF and their FDC mixture reveal several interesting feature in terms of stability, enthalpy changes intermolecular interactions in inert and oxidizing atmospheres. The Rifampicin is very sensitive to heat and atmospheric conditions, Ionized is fairly stable over wide range of temperatures but Rifampicin is less stable undergoing compositional changes at temperatures as low as 80˚C. In FDC mixtures the change induced in Rifampicin either by temperature or oxidation effects immediately extends to Ionized giving rise to formation of a new product. Presence of oxygen as part of the chemical constitution of drugs shows that Ionized has lesser amount of oxygen and less prone to oxidation but Rifampicin contains oxygen in significant amount rendering the drug to undergo internal oxidation at temperatures as low as room temperature.

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