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RESEARCH PAPER

An Explanation for the Physical Instability of a Marketed Fixed Dose Combination (FDC) Formulation Containing Isoniazid and Ethambutol and Proposed Solutions

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ABSTRACT

An investigation was carried out to explore the possible reason for the physical instability of a marketed strip packaged anti-TB fixed dose combination (FDC) tablet containing 300 mg of isoniazid (H) and 800 mg of ethambutol hydrochloride (E). The instability was in the form of distribution of white powder inside the strip pockets. High-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS-MS) studies confirmed that both H and E were present in the powder. The same was also confirmed through Fourier-transform infrared (FTIR) spectroscopy, which also indicated absence of interaction between the two drugs. No sublimation of the drugs was observed up to 110°C, indicating that the observed instability was not due to this reason. Subsequently, attention was paid to the possibility of moisture gain by the tablets through defective packaging (which was established) due to hygroscopicity of E. To understand the phenomenon further, pure drugs and their mixtures were stored under accelerated conditions of temperature and humidity [40°C/75% relative humidity (RH)] and both increase in weight and physical changes were recorded periodically. The mixtures gained moisture at a higher rate than pure E and those with higher content of E became liquid, which on withdrawal from the chambers, became crystallized. The drug mixture containing H:E at a ratio of 30:70 w/w, which was similar to the ratio of the drugs in the tablets (27:73 w/w), crystallized fastest, indicating formation of a rapid crystallizing saturated system at this ratio of the drugs. It is postulated that the problem of instability arises because of the formation of a saturated layer of drugs upon moisture gain through the defective packaging material and drying of this layer with time. The study suggests that barrier

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packaging free from defects and alternatively (or in combination) film coating of the tablets with water-resistant polymers are essential for this formulation.

Key Words: Isoniazid; Ethambutol; FDC; Instability; Formulation.

INTRODUCTION

In our laboratory, we are working on the behavior of moisture gain by drugs, excipients, and packaged products in the absence and presence of light.[1–3] During one such investigation on marketed antituberculosis drug formulations,[2] a tablet containing isoniazid (H) and ethambutol (E) was found to gain moisture when exposed to accelerated stability test conditions of 40°C/75% relative humidity (RH),[4] despite the fact that it was packed in an apparently impervious strip. An investigation revealed that there were pinholes in the strip material that allowed moisture to ingress, under the driving force of hygroscopicity of E.[2] A photograph of a pinhole is shown in Fig. 1.

The same formulation showed another type of instability, when strip pockets were opened to remove the tablets for moisture gain studies. A fine layer of powder was distributed inside the pockets, including the surface of the tablets. The photograph in Fig. 2 shows the interior of an open strip with a layer of powder. Unfortunately, the product was just 4 months old at the time of purchase, and it had a total expiry of 5 years (Date of purchase: April 2002; Manufacturing date: December 2001; Expiry date: November 2006). Generally, the problem was found in each pocket, however, variation was seen in the extent of the distributed powder.

Therefore, an investigation was initiated to find out the composition of the white powder layer and to explore the reason behind the emergence of this instability. The details of the study and the possible solutions to the problem are reported in this communication.

MATERIALS

The formulation containing 300 mg of H and 800 mg of E in a strip package was purchased from the local market. Pure H and E were gift samples from Panacea Biotec Ltd., Lalru, India. Acetonitrile and methanol [high-performance liquid chromatography (HPLC) grade] were purchased from J.T. Baker (Mexico City, Mexico) and Mallinckrodt Baker Inc. (Paris, KY), respectively. All other chemicals were of analytical reagent grade. Ultra-pure water was obtained from an ELGA water purification unit (Elga Ltd., Bucks, England).

METHODS

Analysis of Powder for H and E

The powder was collected from inside the strip pockets and tested for the presence of H and E using HPLC and liquid chromatography-mass spectrometry (LC-MS-MS), respectively. The HPLC system consisted of a DGU-14A degasser module, SIL-10ADVP auto injector, LC-10ATVP pump, CTO-10ASVP column oven, SPD-10AVP UV-visible dual wavelength detector, and an SCL-10AVP system controller; data were acquired and processed using CLASS-VP software (all from Shimadzu, Kyoto, Japan). The chromatographic separations were carried out on a Zorbax XDB C-18 (250×4.6 mm, particle size 5 μ) column (Agilent Technologies, Wilmington, DE). The presence of H was checked by comparison with the retention time of the standard using a validated HPLC method, previously reported by us.[5] The presence of E was determined by LC-MS-MS using positive atmospheric

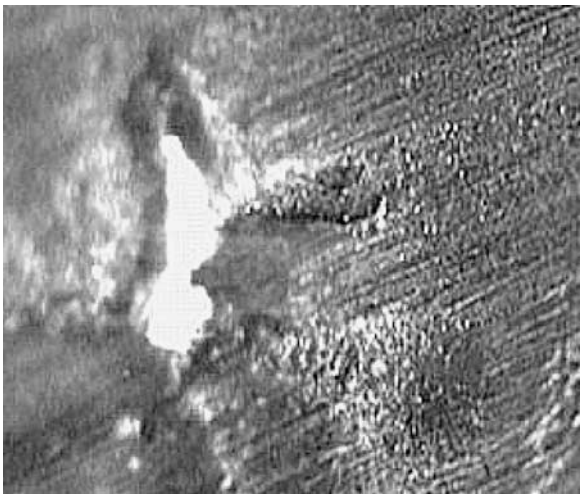

Figure 1. Microscopic view of a pinhole in the area of pocket containing the tablet (more details in Ref. 2). (*View this art in color at www.dekker.com.*)

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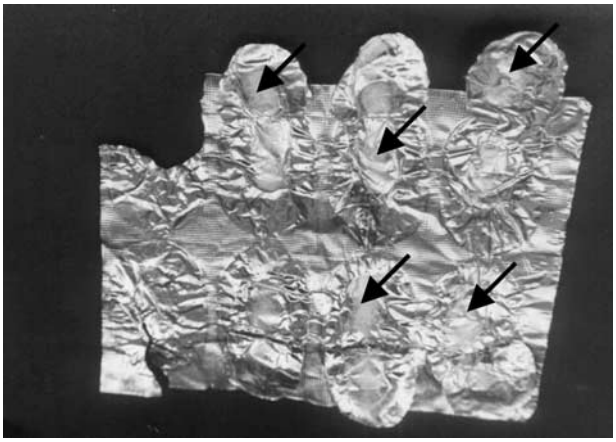

Figure 2. Photograph showing an open strip from where tablets containing H and E were removed. The arrows point to the fine sheet of powder distributed inside the pockets. (*View this art in color at www.dekker.com.*)

pressure chemical ionization selective reaction monitoring (APCI-SRM) of parent and fragment ions at m/z 205 and 115, respectively. These studies were carried out on an ion-trap equipment (LCQ, Finnigan Mat, San Jose, CA). The LC part consisted of a Spectrasystem P4000 pump, AS3000 autosampler, and an SCM1000 degasser (all equipment was from Thermo-separation products, San Jose, CA). The mobile phase consisted of methanol and water in a ratio of 80:20 at a flow rate of 1 mL/min.

Fourier-Transform Infrared (FTIR) Spectroscopy

The FTIR spectra were recorded in KBr using a Spectrum one spectrometer (Perkin Elmer, Huenenberg, Switzerland).

Sublimation Studies

To understand the observed instability, studies were initially focused on the sublimation property of H and E, which melt at 171°C and 202°C, respectively. The two drugs individually, their mixture in the same strength as present in the fixed dose combination (FDC) formulation, tablet powder, and an intact tablet were placed in five different 10-mL beakers. The beakers were covered with glass slides and heated on a solid aluminum thermostatic block (Thermolyne, Dubuque, Iowa) until 140°C.

Study of Moisture Gain Behavior and Time of Crystallization

Moisture gain studies were carried out on various mixtures of H:E ranging between 0:100 to 100:0 w/w.

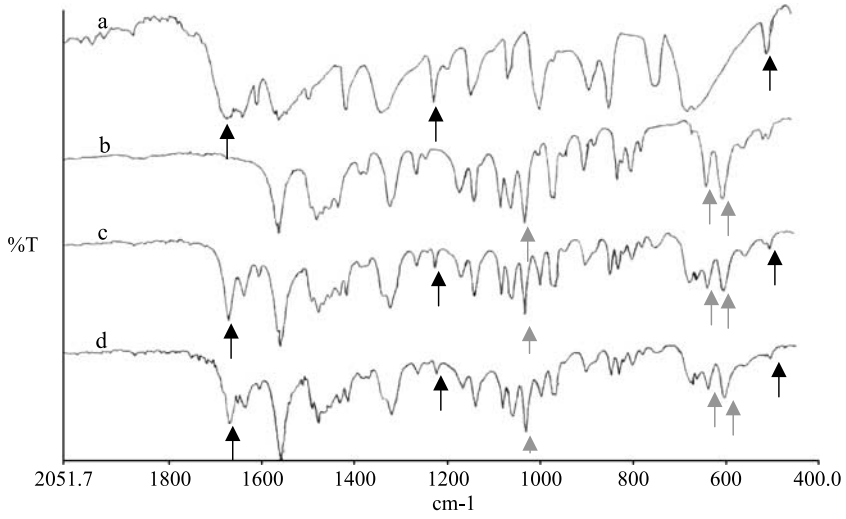

Figure 3. Finger-print region of FTIR spectra for pure H (a), E (b), the physical mixture of H and E at a ratio of 23:73 w/w (c), and the powder collected from within the strips (d). Arrow ↑ indicates a few selected peaks of H and arrow ↑ represents selected peaks of E. The presence of both types of peaks in spectra c and d confirms that both drugs were present in the powder from the strips. Above it, the spectra c and d are simply additive in nature, which suggests absence of interaction between the two drugs.

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For our purpose, the supplied drugs were used without any grinding or treatment, as both were free flowing. The particle size of H and E ranged between 150–180 μ and 125–150 μ, respectively. The drugs were weighed in different proportions to a total quantity of 300 mg, transferred to 10-mL beakers, and mixed thoroughly using a spatula. The open beakers were charged to a stability chamber (KBF 720, WTB Binder, Tuttlingen, Germany) set at 40°C ± 1°C and 75% RH ± 2% RH. All the studies were done in triplicate. A precision analytical balance (AG 135, Mettler Toledo, Greifensee, Switzerland) was used for weighing the samples. The gain in weight was recorded until saturation. Afterwards, the samples were withdrawn from the chamber and kept at room temperature. Crystallization was observed to occur over this period. The time of crystallization varied, depending upon the ratio of the two drugs in the solution. This time was noted.

RESULTS AND DISCUSSION

Chemical Composition of Powder

The overlapping HPLC profiles of standard H and that of the powder collected from inside the strip pockets proved that H was present in the powder. Similarly, the presence of a peak in LC-MS-MS chromatogram relative to parent ion/fragment at m/z values of 205/115 for E confirmed that it was also a part of the powder seen in the strip pockets. This was also confirmed through FTIR studies. Figure 3 shows the fingerprint region of FTIR spectra for pure H (a), E (b), the physical mixture of H and E at the same ratio as contained in the tablets (c), and the powder collected from within the strips (d). Comparison of Figs. 3a and 3b with 3c shows that spectra for the physical mixture (c) was just the summation of the peaks of pure H and E, with no evidence of interaction, which should have resulted in the elimination of old and origin of new peaks.[6] Even the fingerprint region of spectrum d matched with spectrum c and had characteristic peaks of both H and E, thus confirming the presence of both these drugs in the powder in the strip pockets.

Sublimation as the Cause

On heating the beakers containing pure H, pure E, their mixture, tablet powder, and the intact tablet, there was no sublimation until ~110°C. Beyond this temperature, only slight haziness developed on the bottom surface of the glass covers in the beakers where H was present, viz., pure H, mixture of H and E, and the powdered tablet. Because no sublimation took place below 100°C, this was dropped as the possible reason for the observed instability.

Moisture Gain Behavior

Alternately, it was considered that the instability was linked to the hygroscopic nature of ethambutol[1] and moisture gain by the formulation through the pinholes in the packaging material (Fig. 1). The pinholes could be seen even by the naked eye when a laser pointer beam was focused from below the foil after removing the tablet from the pocket. Both the flaps covering each tablet were screened. On an average one or two pinholes could be seen in the foil portions covering each pocket. There was no problem with respect to the integrity of the seal of the packs.

To explore the phenomenon further, moisture gain studies were carried out on pure H and E and their mixtures under accelerated storage conditions. Figure 4 shows the profiles. The points along the curves represent a mean of three readings. At any point, the maximum standard deviation was < 0.2, with good reproducibility of the results. The figure shows a different behavior of E than all the other combinations of H and E. While pure E gained higher total moisture, the combinations of H:E between 80:20 to 10:90 w/w showed a higher rate of moisture gain differentially between 2 to 28 h, and lower total moisture.

The higher rate of moisture gain by combinations of H:E than E alone until 28 h was also observed visibly. Within 8 h, pure E became wet and turned liquid at around 20 h. The combinations of H:E in the ratios between 10:90 and 30:70 w/w became totally liquid much earlier, at 8 h, and continued in the same

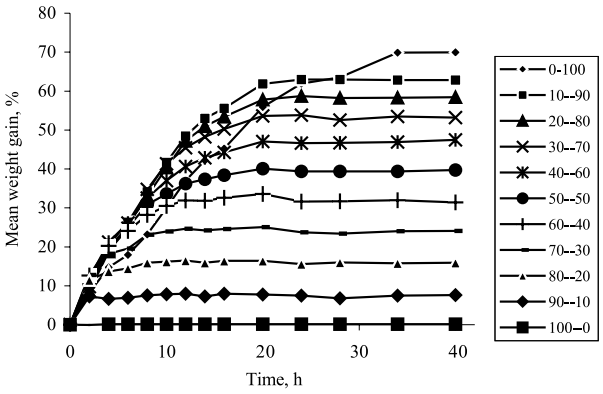

Figure 4. Weight gain by different compositions of H and E, subjected to accelerated conditions of temperature and humidity (40°C, 75% RH).

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state until the end of the study. In combinations containing 40% and 50% w/w of H, the moisture gain was enough to dissolve the majority of the two drugs. As the ratio of H increased further, the extent of insolubility increased as less moisture was gained, due to the decrease in the content of E. The mixtures containing H between 60% and 90% w/w remained as wet masses. Pure H, being nonhygroscopic, did not take up any moisture.

It is postulated that H, which is freely water-soluble, takes away part of moisture gained by E for its solubilization. This forces E to gain more moisture from the environment, with the result that H:E combinations with ratios between 80:20 to 10:90 w/w gain moisture at a higher rate than even pure E. Eventually, equilibrium sets in and the total moisture gain corresponds to the ratio in which E is present in the system, which decreases as the content of H increases.

Crystallization Behavior at Room Temperature

An interesting phenomenon was observed when beakers placed in the chamber were brought out at room temperature after completion of the moisture gain studies. Crystallization occurred in the solutions containing H:E between 0:100 to 50:50 w/w. The time of crystallization varied, depending upon the ratio of the two drugs.

Figure 5 shows the plot of time for crystallization vs. composition of the two drugs in the mixture. Evidently, the H:E combination of 30:70 w/w took the least time for crystallization. In comparison, other combinations with less or more H, and even pure E took longer time. It indicated that the solution of H and E in a ratio of 30:70 was so saturated that it crystallized as soon as it was brought out of the chamber to room temperature. In the mixtures containing higher or lower content of E, the gained moisture was perhaps either more or less than sufficient to yield an instantly crystallizing saturated system.

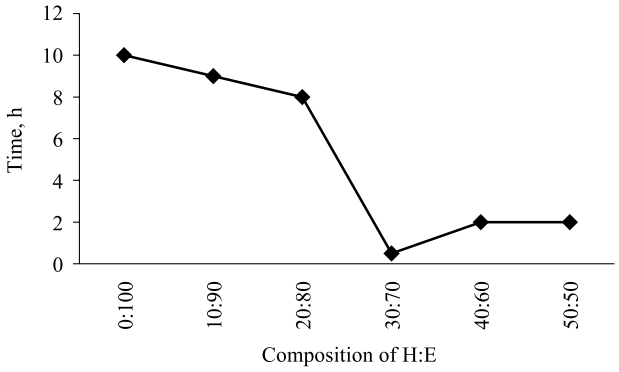

Figure 5. Crystallization time vs. composition of H:E.

Postulated Reason for the Instability Observed in Marketed Formulation

This H:E ratio of 30:70 is almost the same to ~27:73 w/w for the two drugs present in the marketed formulation. The present study amply shows that this ratio of the two drugs is critical with respect to crystallization of the systems containing H and E together. Thus, the observed instability of the marketed product can be explained in the following manner:

1. The moisture gets entry through the pinholes in the strip, driven by the hygroscopicity of E.
2. The gained moisture dissolves the water soluble H and E present on the surface of the tablets, resulting in a rapidly crystallizing saturated layer.
3. Because of the close contact of the tablets with the covering foil, part of this saturated layer gets transferred to the interior of the pocket. Drying occurs in due course, due to variation in the environmental conditions during transportation or storage of the product.

The Solution to the Instability Behavior

Because instability (Fig. 2) arises due to moisture gain from pinholes in the strip packaging material, the first solution to the problem lies in prevention of the entry of moisture by use of a resistant packaging material, free from all defects. In addition, as film coating of the tablets with moisture barrier polymers can effectively help in preventing moisture entry into the formulation, even if the package has some inherent defects, this is proposed as a complementary solution to the problem.

CONCLUSIONS

The present study provides an insight into the reason for physical instability of a marketed FDC formulation containing 300 mg of H and 800 mg of E. It suggests that the combination of drugs contained in the formulation and their relative ratio is so critical that

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even small moisture ingress can result in the transfer of drugs from the tablet to the inside surface of the strip pockets. The tentative solution lies in use of barrier packaging free from any defects, along with film coating of the tablets with moisture-resistant polymers.

The present study is a typical example of how proper preformulation studies, of the type carried out in this study, can help in the prediction of the occurrence of such instability problems in the marketed products. This may be true of formulations that contain combinations of hygroscopic and water-soluble nonhygroscopic drugs or excipients.

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