*Research Article*

# Impact of Storage Conditions on a New Child-Friendly Dispersible Tablet for Treating Tuberculosis in Pediatrics

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## Abstract

*Background:* In 2020 the composition and procedure to elaborate a new formulation containing Isoniazid, Pyrazinamide and Rifampicin to treat tuberculosis in pediatric patients was published. The temperature and relative humidity in Tuberculosis-endemic countries are high, >30 ºC and >70% respectively and thus these meteorological conditions required a new dosage form. The objective of this work is to register changes in tablet quality and stability over time when exposed to different storage conditions according to ICH.

*Methods:* Tablets were subjected to accelerated, long term and low relative humidity conditions. The effect of light was also tested. Quality was measured by evaluating weight changes tensile strength, disintegration time, and drug content. Hydrazine formation was also evaluated as it is considered a mutagenic degradation product.

*Results:* Tablets stored at low relative humidity showed the best stability. There was no statistically significant difference between tablets exposed to or protected from light. Moreover, the formation of Hydrazine was not detected during stability studies.

*Conclusion:* This new dosage form for treating Tuberculosis is stable and able to maintain its quality when appropriate storage conditions are used.

## Introduction

At present there is no commercially available child-friendly medicine for treating Tuberculosis (TB) in pediatric patients. In 2019, 168,000 children died of TB worldwide.[1,2] In 2020 and for the first time, a new child-friendly dispersible tablet of Isoniazid (INH), Pyrazinamide (PZA) and Rifampicin (RFP) for treating TB in pediatric patients was published by our research group and the production procedure was available to be used by pharmaceutical companies. Compendial requirements in terms of friability, disintegration time, and content uniformity were met by this new formulation and constitutes a way of treating TB in pediatrics. In addition, it can be categorized as the first child-friendly medicine developed to increase patient acceptability and with the lowest amount and number of accepted excipients.[3,4]

However, the literature has described the interaction between two of the three active pharmaceutical ingredients (APIs), INH and RFP, which produce a reduction in drug content and the formation of Hydrazine (HYD), a degradation product that is considered mutagenic by the International Conference of Harmonization (ICH).[5-8]

Few studies were carried out regarding the stability of this APIs in commercially available solid dosage forms for adults. In addition, significant changes regarding physical attributes (weight, tensile strength (TS), and disintegration time) and drug content have been detected during these stability studies.[7-10]

Usually, temperature and relative humidity (RH) in TB-endemic areas (South Africa, Philippines, Thailand, Indonesia and etc.) are high, $> 30$ºC and $> 70\%$.[11] These meteorological conditions produce significant changes in drug content, hardness, disintegration time and dissolution profile during storage, unless packed adequately, and will have a marked impact on treatment efficacy and safety.[7,9,10,12-16] With this in mind, it is essential to ensure the stability of this new dosage form during storage in extreme conditions.

The objective of this work is to comprehend the way in which the quality of the dosage form changes over time depending on different storage conditions (temperature, RH and light) following the recommendations of the ICH guideline regarding the stability of new dosage forms. The quality will be measured evaluating changes in tablet weight, TS, disintegration time, INH, PZA and RFP content. In addition, HYD formation will be evaluated.

## Materials and Methods
### Materials
The APIs used to develop a fixed dose combination (FDC)

tablet were INH (Acofarma®), PZA (Sigma-Aldrich®), and RFP (Fagron®). The following excipients were used: AcDiSol® (Croscarmellose Sodium, FMC Corp., Philadelphia, PA), Avicel® PH102, (Microcrystalline Cellulose, FMC Corp., Philadelphia, PA), Explosol® (Sodium Starch Glycolate, Blanver, Taboao da Serra, Spain), CompactCel® (Isomalt, sucralose, betadex, carboxymethylcellulose sodium, Biogrund GmbH, Hunstetten, Germany), Luzenac® (talc, Imerys Talc, Paris, France) and CabOSil® (fumed silica, Cabot CorporaFon, Boston, MA).

### Preparation of tablets

Research Tablet Press (Korsch, Germany) was used to produce tablets of 900 mg (50 mg INH, 150 mg PZA, 75 mg RFP, 5% AcDiSol®, 45% Avicel®, 9% Explosol®, 7% CompactCel®, 1% CabOSil® and 2.5% Luzenac®) This Press is an instrumented eccentric tablet machine XP1, that use 15-mm flat-faced bisect (FFBP) and 12-mm flat-faced with beveled edge (FFBE) punches.[4] PharmaReseach® (Korsch, Germany) controlled the compression force and press speed.

### Stability studies

Tablets were placed under accelerated (40 ± 2 °C/75 ± 5% RH) and long term conditions (30 ± 2 °C/65 ± 5% RH) in a climate chamber (Memmert ICH110L, Spain) to evaluate the consequences of temperature and RH. ICH recommends conducting accelerated studies in order to look for significant changes during a 6 month testing period. A significant change is described as a decrease of 5% of their initial value.[17,18]

In addition, the influence of light and the stability of the dispersible tablets were also tested as indicated ICH (30 ± 2 °C/30 ± 5% RH) (Memmert ICH110L, Spain).[17,18] Three fluorescent lights with a cold white light (standard illuminant D65, 6.500K) were selected according to the ICH guidelines.[17] In addition, tablets were packaged in a Personalized System of Dosification (SPD) venalink system for photosensitive APIs and the results were compared with tablets without protection.

Tablets were stored under low RH conditions (25 ± 2 °C/11 ± 5% RH) to observe changes regarding physical and chemical properties. The color and shape of the tablets as well as weight (mg), TS (N/cm²), disintegration time (min) and API content (% declared value) were checked, in each sampling time, by triplicate (n=3). In addition, HYD content was analyzed at the end of the study for each condition tested.

### Statistical analysis

Regression analysis and analysis of variance (ANOVA) were performed to check the correlation between every property tested and time. In addition, a Student's t-test and F-test of equality of variances were carried out to control the influence of light. The level of significance of all tests were performed at 5% ($\alpha = 0.05$).

### Quantification method

A reversed phase Ultra Performance Liquid Chromatography (UPLC) was used to analyze all APIs. The chromatograph is an Acquity UPLC® H-Class System (Waters Corporation, Milford, MA) that uses the Astra 6.0.1 acquisition software (Chromatographic Manager, Waters Corporation, Milford, MA). This system uses a method already validated and published which is able to detect and quantify the INH, RFP and PZA content.[4,19]

HYD content was analyzed following the European Pharmacopeia (Ph. Eur.) method designed for High Performance Liquid Chromatography (HPLC).[20] This method was adapted to be used in an Acquity UPLC® H-Class System (Waters Corporation, Milford, MA). A XSelect™ CSH™ C18 (75 mm x 2.1 mm id, 2.5 mm) reversed phased column; a mobile phase with use a mixture of acetonitrile: water in proportion of 50:50 (v/v); a flow rate of 0.4 ml/min; a wavelength of 300 nm and an injection volume of 1 µl was used. In addition, a Mass Spectrometry (MS) detection was also used to confirm the retention time of HYD (Acquity Triple Quadrupole instrument (Waters Corporation, Milford, MA)). The mass spectrometer uses electrospray ionization in the positive and negative ion mode. The capillary and cone voltage used were 0.8 kV and 40 V respectively. The source and desolvation temperature was 600°C. Nitrogen was used as a desolvation gas.

HYD solutions with concentration from 3.07 to 15.40 ng/ml were used as standards for analytical method validation To confirm the linearity of the method, an ANOVA was carried out. The method precision (repeatability) was calculated after analyzing the same sample six times. System accuracy was determined as the percentage recovery by the analytical method of a known added amount of API (n=9). The detection and quantitation limits were also checked for each API.

In order to ensure that the method was able to detect and quantify the declared content of HYD in each tablet from a sample formed by a complex matrix (formed by non-soluble excipients mainly), every ingredient of the tablet was weighed and a precise amount of HYD was added.[21] This was dissolved in methanol (50 ml) and diluted with water up to 250 ml. Then, it was filtered using filter paper (110 mm) and 1 ml of this solution was extracted and mixed with 2.5 ml of a 0.02% (v/v) solution of benzaldehyde in water and then placed for 45 minutes in an ultrasonic bath to ensure the complete derivatization of HYD. Finally, the sample was diluted to be analyzed in the UPLC system. The amount of HYD was estimated and indicated as labeled content.

### Surface area and pore distribution determination

To calculate the surface area, pore volume and pore size of 12- and 15-mm tablet at accelerated conditions, nitrogen adsorption/desorption isotherms were used at 77 K (ASAP 2020, Micromeritics Instrument Co.). To calculate the surface area and pore distribution Brunauer-Emmett-Teller (BET) and Barrett, Joyner, and Halenda (BJH)

methods were used respectively.[22-24]

## Results and Discussion

The ANOVA confirmed the linearity of the method for HYD quantification, through rejection of the null hypothesis of deviation from linearity for a significant level of 0.05 ($\alpha = 0.05$). With a 5.06% of coefficient of variation of the method, the equation of the linear regression was: Area ($\mu V \cdot sec^{-1}$) = 98.280 × C (ng/ml); $r^2 = 0.995$. It is precise (0.06%), accurate (101.30%), and has a detection and quantification limit of 1.44 and 4.37 ng/ml, respectively. The average extraction yield for HYD was 100.1% of the declared content.

Figure 1 shows the UV and mass spectrum obtained by the UPLC-UV/MS method for a pure pattern and a sample of HYD.

### Stability studies

Results for physical attributes and drug content are shown in Tables 1 and 2, respectively.
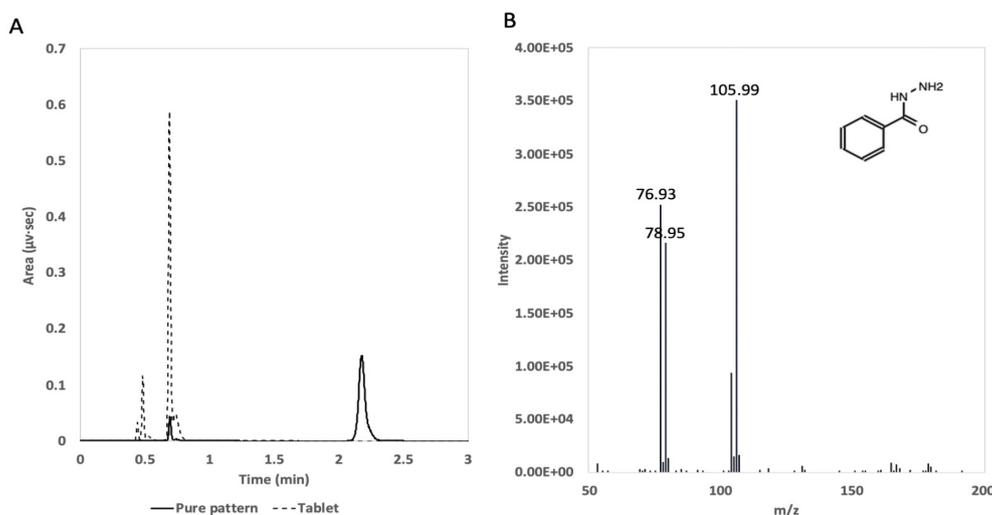


**Figure 1.** A. HYD pure pattern chromatographic peak as standard of 10 ng/ml (continuous bold line) and HYD extracted from a tablet after 6 months of storage at accelerated conditions (discontinuous line). B. Mass spectrum (B) for a pure pattern of HYD derivatized with benzaldehyde extracted at 2.18 min of retention time (Molecular weight: 136 g/mol).[22]

**Table 1.** Results for physical properties of tablets stored at accelerated condition (AC), long term condition (LC), low RH (LRH) and photostability condition (L, light and PL, protected from light). n =3.

| Tablet size | | 12-mm | | | 15-mm | | |
|---|---|---|---|---|---|---|---|
| Time (months) | Condition | Weight (mg) | TS (N/cm²) | Disintegration time (seconds) | Weight (mg) | TS (N/cm²) | Disintegration time (seconds) |
| 0 | AC | 458.3 ± 2.1 | 148.9 ± 6.8 | 85.7 ± 9.7 | 897.7 ± 5.7 | 163.4 ± 1.4 | 162.7 ± 301 |
| | LC | 458.3 ± 2.1 | 148.9 ± 6.8 | 85.7 ± 9.7 | 897.7 ± 5.7 | 163.4 ± 1.4 | 162.7 ± 3.1 |
| | LRH | 436.0 ± 1.8 | 132.4 ± 0.2 | 80.0 ± 0.7 | 857.7 ± 2.0 | 155.7 ± 4.4 | 143.0 ± 1.2 |
| | L | 447.0 ± 1.0 | 141.0 ± 3.2 | 90.7 ± 9.3 | 885.3 ± 22.6 | 166.6 ± 10.5 | 149.7 ± 16.6 |
| | PL | 447.0 ± 1.0 | 141.0 ± 3.2 | 90.7 ± 9.3 | 885.3 ± 22.6 | 166.6 ± 10.5 | 149.7 ± 16.6 |
| 1.5 | AC | 470.7 ± 5.0 | 49.0 ± 2.8 | 43.3 ± 5.7 | 930.7 ± 6.0 | 58.1 ± 4.3 | 56.5 ± 9.5 |
| | LC | - | - | - | - | - | - |
| | LRH | 437.3 ± 2.9 | 134.0 ± 4.2 | 73.30 ± 0.0 | 855.6 ± 6.4 | 156.1 ± 13.2 | 140.0 ±26.5 |
| | L | 441.7 ± 0.6 | 137.6 ± 5.5 | 73.7 ± 10.0 | 875.3 ± 14.8 | 153.5 ± 12.7 | 166.3 ± 18.9 |
| | PL | 443.0 ± 2.0 | 130.9 ± 6.3 | 78.7 ± 2.1 | 886.7 ± 5.1 | 158.5 ± 2.6 | 128.3 ± 26.6 |
| 3 | AC | 472.0 ± 4.6 | 39.3 ± 4.3 | 56.7 ± 5.7 | 921.0 ± 10.6 | 46.9 ± 1.9 | 54.3 ± 4.7 |
| | LC | 459.0 ± 3.6 | 88.2 ± 2.3 | 50.3 ± 13.0 | 909.6 ± 6.0 | 92.9 ± 5.5 | 62.0 ± 3.0 |
| | LRH | 431.0 ± 1.0 | 138.2 ± 2.9 | 68.7 ± 2.3 | 858.6 ± 5.7 | 156.7 ± 13.9 | 129.3 ± 8.1 |
| | L | 442.3 ± 0.6 | 95.9 ± 2.0 | 68.7 ± 3.7 | 879.3 ± 3.2 | 154.5 ± 6.7 | 91.6 ± 4.7 |
| | PL | 443.0 ± 2.0 | 129.4 ± 1.8 | 63.3 ± 1.5 | 887.3 ± 7.2 | 155.9 ± 3.3 | 131.0 ± 3.6 |
| 4.5 | AC | 465.3 ± 7.6 | 39.1 ± 2.3 | 40.0 ± 6.0 | 919.7 ± 1.2 | 44.6 ± 1.9 | 72.7 ± 15.0 |
| | LC | - | - | - | - | - | - |
| | LRH | 434 ± 6.2 | 138.6 ± 2.5 | 102.7 ± 6.0 | 850.3 ± 2.5 | 154.1 ± 2.8 | 148.3 ± 19.4 |
| | L | 439.0± 1.7 | 131.2 ± 3.5 | 52.0 ± 1.0 | 879.0 ± 8.9 | 157.2 ± 8.6 | 106.3 ± 9.1 |
| | PL | 441.0 ± 2.0 | 131.5 ± 7.2 | 53.0 ± 2.6 | 886.3 ± 5.7 | 154.8 ± 6.2 | 108.0 ± 8.7 |

**Table 1.** Continued.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | AC | 467.7 ± 5.0 | 40.5 ± 6.6 | 43.0 ± 0.1 | 930.7 ± 4.7 | 42.6 ± 0.9 | 51.0 ± 2.6 |
| | LC | 467 ± 7.2 | 81.7 ± 3.2 | 34.7 ± 1.5 | 905.0 ± 7.2 | 82.4 ± 3.2 | 43.7 ± 0.6 |
| 6 | LRH | 437.7 ± 5.5 | 154.5 ± 14.9 | 99.7 ± 17.6 | 866.7 ± 18 | 158.1 ± 11 | 140.0 ± 5 |
| | L | 441.7 ± 0.6 | 129.6 ± 3.4 | 42.0 ± 0.0 | 879.3 ± 4.5 | 153.4 ± 4.8 | 96.0 ± 21.8 |
| | PL | 442.0 ± 0.0 | 122.1 ± 4.2 | 43.0 ± 0.1 | 882.3 ± 8.7 | 142.0 ± 3.9 | 95.3 ± 18.0 |
| 9 | LC | 460 ± 9.0 | 79.9 ± 4.4 | 35.0 ± 5.0 | 894.3 ± 3.2 | 78.7 ± 2.0 | 47.3 ± 1.2 |
| 12 | LC | 457.3 ± 3.0 | 85.4 ± 2.6 | 30.7 ± 2.1 | 911.7 ± 4.0 | 93.6 ± 6.2 | 49.3 ± 2.1 |

**Table 2.** Results for drug content, expressed as % of declared value (DV), of tablets stored at accelerated condition (AC), long term condition (LC), low RH (LRH) and photostability condition (L, light and PL, protected from light). n =3.

| Tablet size | | 12-mm | | | 15-mm | | |
|---|---|---|---|---|---|---|---|
| Time (months) | Condition | INH (% DV) | PZA (% DV) | RFP (% DV) | INH (% DV) | PZA (% DV) | RFP (% DV) |
| | AC | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |
| | LC | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |
| 0 | LRH | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |
| | L | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |
| | PL | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |
| | AC | 98.3 ± 6.2 | 101.5 ± 1.2 | 92.9 ± 3.1 | 98.3 ± 6.2 | 101.5 ± 1.2 | 92.9 ± 3.1 |
| | LC | - | - | - | - | - | - |
| 1.5 | LRH | 100.0 ± 3.5 | 99.0 ± 0.6 | 102.6 ± 0.9 | 98.0 ± 1.7 | 96.1 ± 1.3 | 99.3 ± 2.5 |
| | L | 93.6 ± 0.6 | 97.6 ± 3.3 | 96.5 ± 2.0 | 95.0 ± 6.6 | 100.8 ± 2.5 | 100.9 ± 2.8 |
| | PL | 88.0 ± 2.2 | 97.1 ± 3.4 | 93.4 ± 3.3 | 94.3 ± 2.6 | 103.4 ± 4.1 | 102.2 ± 0.7 |
| | AC | 86.6 ± 3.2 | 99.8 ± 2.4 | 101.8 ± 4.0 | 88.8 ± 7.0 | 95.3 ± 2.0 | 95.0 ± 2.7 |
| | LC | 103.0 ± 4.1 | 99.9 ± 0.5 | 97.7 ± 0.3 | 95.7 ± 6.6 | 95.7 ± 0.1 | 98.1 ± 2.9 |
| 3 | LRH | 99.8 ± 4.3 | 101.0 ± 0.4 | 103.0 ± 0.9 | 99.0 ± 7.6 | 99.3 ± 1.8 | 103.0 ± 2.8 |
| | L | 94.2 ± 0.8 | 93.5 ± 3.2 | 93.4 ± 2.1 | 91.6 ± 4.7 | 98.5 ± 2.0 | 93.8 ± 7.1 |
| | PL | 93.7 ± 1.7 | 93.5 ± 2.2 | 95.4 ± 2.4 | 96.9 ± 6.5 | 100.1 ± 3.4 | 100.2 ± 5.2 |
| | AC | 78.6 ± 3.9 | 96.0 ± 3.1 | 93.0 ± 3.8 | 91.1 ± 7.8 | 100.1 ± 3.1 | 95.5 ± 1.2 |
| | LC | - | - | - | - | - | - |
| 4.5 | LRH | 101.4 ± 2.3 | 98.2 ± 0.8 | 98.9 ± 1.5 | 99.0 ± 1.3 | 96.2 ± 2.9 | 104.7 ± 3.9 |
| | L | 86.4 ± 1.7 | 95.5.2 ± 1.4 | 90.2 ± 0.9 | 98.3 ± 3.9 | 99.2 ± 3.3 | 99.9 ± 4.4 |
| | PL | 88.0 ± 2.2 | 97.1 ± 3.4 | 93.4 ± 3.3 | 96.1 ± 6.3 | 98.6 ± 4.4 | 102.0 ± 5.7 |
| | AC | 79.1 ± 1.3 | 95.5 ± 2.7 | 88.3 ± 1.3 | 91.0 ± 4.7 | 97.5 ± 2.1 | 90.0 ± 2.0 |
| | LC | 93.2 ± 8.7 | 96.0 ± 3.1 | 91.8 ± 4.3 | 90.2 ± 2.5 | 96.4 ± 1.8 | 88.9 ± 0.8 |
| 6 | LRH | 105.0 ± 2.0 | 101.0 ± 3.5 | 102.6 ± 0.4 | 104.1 ± 1.0 | 98.9 ± 3.7 | 99.8 ± 4.5 |
| | L | 89.4 ± 1.4 | 92.7 ± 4.8 | 86.9 ± 0.7 | 98.0 ± 3.7 | 100.7 ± 2.5 | 101.5 ± 0.4 |
| | PL | 87.0 ± 2.4 | 95.0 ± 2.7 | 88.2 ± 1.6 | 95.6 ± 3.6 | 100.0 ± 1.7 | 102.2 ± 4.6 |
| 9 | LC | 85.6 ± 2.2 | 95.1 ± 2.1 | 95.0 ± 4.5 | 90.8 ± 5.8 | 95.4 ± 0.4 | 94.8 ± 3.2 |
| 12 | LC | 91.7 ± 3.3 | 94.3 ± 1.4 | 94.5 ± 2.9 | 96.1 ± 4.5 | 93.0 ± 1.4 | 95.6 ± 2.8 |

No HYD was detected in tablets regardless of storage condition or tablet size. Physical properties were adjusted to a polynomial model, and content values of APIs were adjusted to a zero kinetic order as it provided better adjustment.

### Accelerated condition

12- and 15-mm tablets increased in size, thickness and diameter by 0.05 cm. This is in accordance with the results found by Singh *et al.*[25]

According to data shown in Table 1, tablet weight remained within ± 5% of the initial value regardless of size. TS and disintegration time showed a statistically significant variation with time for both sizes (r > 0.90; p-value < 0.05). As can be seen in Table 1, dispersible tablets achieved a 33% of the initial TS value at 1.5 months of storage.

Accelerated studies were performed at the highest levels of temperature and HR. The absorption of humidity may be responsible for the weight increase and consequently, the reduction of TS and disintegration time. Disintegrants have a high moisture absorption capacity as their function is to uptake water, increase volume and break the tablet. In this case, sodium starch glycolate and cellulose derivates are classified as class II in the Hygroscopicity classification system; sodium starch glycolate is slightly higher hygroscopic than the cellulose derivates.[26-28] Fumed silica

is considered to be hygroscopic by some authors, while others classify it as class I (non-hygroscopic).[27] Changes regarding hardness are due to the incorporation of water molecules between interparticle and intermolecular bonds which make the tablet soft.[29,30] This procedure reduced hardness thus resulting in a reduction in TS due to the loss of bond-strength between solid particles.

API variation, expressed as declared value, for 12- and 15-mm are shown in Table 2. PZA is stable at this condition, % declared value (DV) is always greater than 95%, regardless of tablet size ($k = 0$; $r > 0.10$; p-value $> 0.05$). RFP shows a similar degradation in 12- and 15-mm tablets, its % DV decreases till reaching 90% at 6 months ($k \neq 0$; $r > 0.60$; p-value $< 0.05$). The most significant difference was found in INH, as its % DV decreases more in 12-mm ($79.0 \pm 1.3\%$) than 15-mm ($90 \pm 2.0\%$) tablets. In addition, in both cases there is a statistically significant time variation ($k \neq 0$; $r > 0.60$; p-value $< 0.05$).

In the literature regarding the stability of FDC of anti-TB for adults, the authors studied the same storage conditions for 3 months. According to these results, there is a large variability of degradation of APIs: 10 - 60% for INH, 40 - 75% for PZA and 17 - 60% for RFP after 3 months of storage.[7,9] Following data comparison, this new formulation seems to be more stable.

In Table 3 results for surface area and pore size are shown.

Surface area reduction is detected at 6 months of storage at 75% HR and 40ºC for both sizes. This could be related to the absorption of water in these storage conditions by disintegrants which produce the dissolution of the small particles, as described by Leeson and Mattocks.[31]

Before analyzing the samples in the instrument, they must be dry and therefore small particles which are dissolved in water molecules are lost. Hence, as particle numbers decrease the surface area also decreases. The reduction in surface area was 54% and 40.7% for 12- and 15-mm respectively, which could be explained due to the greater pore diameter of the smaller tablets. This alteration is caused by the different punches used, FFBP and FFBE.

This could explain why the declared value of INH decreases faster in the case of 12-mm tablets compared to the other tablets; these dispersible tablets absorbed more water due to the larger pore diameter.

**Table 3.** Results from the determination of surface area and pore size from tablets of 12-mm and 15-mm size.

| Time | Initial | 6 months of storage |
|---|---|---|
| **12-mm** | | |
| Surface Area (m²/g) | 2.34 | 1.09 |
| Volume of pores (cm³/g) | 0.026 | 0.014 |
| Average Pore diameter (Å) | 405.63 | 596.64 |
| **15-mm** | | |
| Surface Area (m²/g) | 2.87 | 1.70 |
| Volume of pores (cm³/g) | 0.027 | 0.019 |
| Average Pore diameter (Å) | 369.43 | 504.82 |

*Long term condition*

Tablets stored under long term conditions increased their dimensions by 0.02 cm. This increase was lower than the increase observed under accelerated conditions, 0.05 cm, due to the higher RH.

Tablet weight remains within ± 5% of initial value regardless of size. TS and disintegration time showed a statistically significant time variation for both sizes ($r > 0.85$; p-value $< 0.05$). As shown in Table 1, tablets achieved a TS value of 55% at three months of storage. The loss of hardness is lower compared to results obtained from accelerated studies due to the lower RH.

API variation over time is statistically significant for all APIs in the case of 12-mm tablets ($k \neq 0$; $r < 0.70$; p-value $< 0.05$). In these tablets 95% of declared content is achieved at 6 months of storage for INH and RFP and 9 months for PZA. In the case of 15-mm tablets there is no statistically significant variation of drug content over time, but this could be related to the low correlation coefficient ($k \neq 0$; $r < 0.40$; p-value $> 0.05$). As was observed in accelerated conditions, the reduction in INH content was higher in 12-mm tablet than in 15-mm tablet.

*Low RH*

Tablets in these conditions did not show any significant physical or chemical changes after six months of the study. This is probably due to the low RH.

In order to improve storage, an ideal packaging system which protects the APIs from moisture is required. For example, polyvinyl chloride films (PVC) laminated with high barrier plastics like polyvinylidene chloride (PVDC) which protect the dosage from moisture. Aluminum could also be added to improve protection or tubes and desiccant closures similar to those used to preserve effervescent tablets from moisture might be used.[30]

*Photostability condition*

The equality of variance was confirmed by F-test on both groups: exposed and protected from light. What is more, a t-test determined that there is no statistically significant difference between groups, regardless of the property studied. changing the tablet surface color did not produce a significant change in drug content as only affected the most external part of the surface, which was directly exposed to the light source. This concurs with the conclusions obtained by other authors during their research under similar conditions.[7,9]

**Conclusion**

According to the results obtained, the absorption of environmental humidity by the excipients was identified as the main reason for instability dispersible tablets used to treat TB in pediatric patients. On the other hand, tablets stored at low RH proved to be stable up to six months. HYD is the most important degradation product of INH due to its potential carcinogenic risk, but the formation of this product was not detected during stability studies.

This new dosage form for treating TB in children is effective (has the declared drug content), safe (HYD content remains inside ICH limits) and stable when it is stored in low RH conditions. Appropriate packaging (PVC, PVDC and aluminum) is fundamental to protect the APIs from moisture.

## Acknowledgments

## Author Contributions

Conceptualization: JBF and ASE; methodology: JBF and JSG; formal analysis: JSG; investigation: JSG, MS, ASE; data curation: ASE; writing—original draft preparation: JSG; writing—review and editing: JSG, MS, JBF and ASE; supervision: JBF and ASE; project administration: ASE: funding acquisition: ASE. All authors have read and agreed to the published version of the manuscript.

## Conflict of Interest

The authors report no conflicts of interest.

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