Extemporaneous Isoniazid Mixture: Stability Implications

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ABSTRACT

Background: Isoniazid mixtures are compounded in Australia using commercially available isoniazid tablets.

Aim: To determine the stability of isoniazid 10 mg/mL mixture compounded from commercially available isoniazid tablets.

Method: The stability of the compounded isoniazid mixture stored at a range of temperatures (4–60 °C) was assessed with high performance liquid chromatography. Differential scanning calorimetry was used to investigate the compatibility of isoniazid with the excipient, lactose.

Results: The compounded isoniazid mixture exhibited significant degradation ($\geq 10\%$ after 3 days at 4 and 25 °C), whereas the control (using isoniazid powder) retained desired stability ($> 90\%$ at 30 days) under identical conditions. A replicate control formulation, spiked with lactose, produced statistically similar degradation profiles to that of the compounded isoniazid mixture ($p > 0.05$), indicating lactose to be responsible for the degradation of isoniazid. The thermoanalytical studies demonstrated an incompatibility between isoniazid and lactose (broadening and shifting of melting endotherm of isoniazid at 171.46 °C).

Conclusion: The British Pharmaceutical Codex specifies the use of isoniazid powder for compounding isoniazid mixture. This study highlights the importance for stability evaluations on all modified extemporaneous preparations in order to ensure that quality pharmaceuticals are delivered to patients.

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INTRODUCTION

Isoniazid (isonicotinic acid hydrazide) is an antimicrobial used for the treatment of tuberculosis in Australia.¹ An isoniazid mixture is a useful formulation for children and in situations where tablets are unsuitable. Due to the unavailability of commercial isoniazid liquid formulations, a mixture is commonly prepared in pharmacy practice from tablets, more conveniently acquired than the raw material (powder). The British Pharmaceutical Codex recommends isoniazid powder for compounding isoniazid mixture.² Although the incompatibility of isoniazid and lactose is well documented, the compounded isoniazid mixture (from commercially available tablets) has been prepared without considering the possible presence of lactose as an excipient in the tablets.²

Isoniazid is susceptible to hydrolysis and oxidation and interacts with excipients, particularly reducing sugars, to form hydrazones.^{2,3} The hydrazone formed by the reaction of isoniazid with lactose (pH 1.0–6.0) is 1-isonicotinoyl-2-lactosylhydrazine.^{2,3} There are also reported incompatibilities between lactose and other drugs containing a primary or secondary amine functional group, as is the case with isoniazid.⁴

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Differential scanning calorimetry is a powerful tool in preformulation and stability studies for the rapid evaluation of the compatibility of active ingredients and excipients used in solid dosage forms.⁵⁻⁸ It is possible to derive information about potential physicochemical incompatibilities between active ingredients and tablet excipients. Differential scanning calorimetry can distinguish between excipients that are unlikely to cause problems and those that may cause problems in the formulation. It is valuable for its sensitivity and rapid response to identify incompatibilities in a very short time.⁶ However, to substantiate differential scanning calorimetry findings, other more direct and conclusive techniques have to be used.⁴

The aim of this study was to determine the stability of isoniazid 10 mg/mL mixture compounded from commercially available isoniazid tablets.

METHOD

Isoniazid 10 mg/mL mixtures were compounded according to the following formula: 20 x 100 mg tablets (Isoniazid BP, Fawns & McAllan Pty Ltd), 0.5 g citric acid, 2.4 g sodium citrate, 80 mL glycerol, 2 mL Compound Hydroxybenzoate Solution APF, purified water BP to 200 mL (chemicals from David Craig Galenicals). The control mixtures were compounded in a similar manner using isoniazid powder (Sigma Chemicals). A replicate control isoniazid mixture spiked with lactose (David Craig Galenicals) was also prepared. All samples were inspected for uniformity and colour prior to storage. Sample bottles (in duplicate) were stored, protected from light at 4 ± 1 °C, 25 ± 1 °C, 40 ± 1 °C, 50 ± 1 °C and 60 ± 1 °C. At the time of sampling, samples were shaken to allow for uniform distribution of the ingredients and 0.5 mL of sample and control solutions were analysed daily in triplicate for the isoniazid concentration from days 0 to 7.

A stability indicating high performance liquid chromatography (HPLC) assay was developed and validated (linearity, accuracy, precision, specificity) to quantify isoniazid in the presence of its degradants and formulation excipients. The HPLC system consisted of a ProStar 240 Solvent Delivery Module, ProStar 210 AutoSampler, ProStar 330 Photodiode Array Detector and Varian Star Chromatography Workstation software. A Varian C18 (5 mm, 150 x 4.60 mm) reverse-phase column with sodium acetate (Sigma Chemicals) buffer (pH 3.6): methanol (Sigma Chemicals) (50:50) as mobile phase and a detection wavelength of 260 nm was used. The flow rate was 1 ± 0.1 mL/min and the injection volume was 50 mL.

Binary mixtures of isoniazid and lactose were prepared by mechanical shaking to produce isoniazid:lactose mixtures in the ratios of 90:10, 80:20 and 70:30. Samples (5–10 mg) of isoniazid and lactose individually, as well as the respective isoniazid:lactose mixtures, were weighed and sealed in 40 mL aluminium crucibles with a pierced aluminium lid. Differential scanning calorimetry thermograms were obtained using a Mettler-Toledo STAR system and the DSC 822/700/1089 module (calibrated with indium). A constant heating rate of 10 °C/min was applied over a temperature range of 100–200 °C under dynamic nitrogen atmosphere (50 mL/min).

Standard error of means and percentage relative standard deviations were determined for representation of accuracy in the measurement. Statistical Package for the Social Sciences was used for ANOVA analysis to determine the level of significance (p < 0.05) of results obtained. The time taken for the concentration of isoniazid to be reduced to 90% of the initial concentration was determined using a linear regression analysis.

RESULTS

A retention time of 1.5 ± 0.1 minutes was obtained and confirmed for isoniazid (method validated). Accuracy expressed as a per cent recovery of isoniazid was 99%, while precision was expressed as per cent coefficient of variation of the method which was 2.9% (n = 6). Linearity was confirmed over the concentration range used and was r² = 0.9998. Isoniazid peak purity was determined through spectral library comparison and peak purity determinations of the respective samples and standard solutions. The absence of co-eluting degradants and excipients was verified using a photodiode array detector; spectral similarities of above 0.999 for the pure and sample isoniazid peaks were achieved. Concentrations of samples were determined from respective peak areas in relation to constructed standard curves and then converted to a percentage of the initial isoniazid concentration. The expiration date was based on the time at which the 95% lower confidence interval intersects the line for 10% decomposition of isoniazid.

An increase in temperature did not result in a corresponding increase in the degradation of the compounded isoniazid mixture, with ≥ 10% degradation occurring at all temperatures after three days.

Table 1 shows the percentage of isoniazid remaining in the mixtures stored at room temperature. The isoniazid mixture prepared from tablets exhibited significant degradation (71% remaining after 7 days). The control mixture, prepared from isoniazid powder, retained desired stability (over 90% remaining), substantiating the British Pharmaceutical Codex claim for the stability of this formulation. The replicate control mixture, spiked with lactose, produced statistically similar degradation profiles to that of the mixture made from isoniazid tablets (p > 0.05).

Table 1. Stability of isoniazid mixtures at room temperature				
Isoniazid mixture (10 mg/mL)	Isoniazid remaining (%)*			
	Day 0	Day 3	Day 5	Day 7
Prepared from tablets	100	78	73	71
Prepared from powder + lactose powder	100	84	76	65
Prepared from powder	100	100	100	100

*values reported as the mean (n = 6); variability around the mean < 5% relative standard deviation

The thermal behaviour of lactose shows the water content of lactose monohydrate to evolve at temperatures up to 160 °C (an endothermic event corresponding to the dehydration reaction at 148 °C), a small exothermic event due to crystalline transition in the range 171–181 °C, and an endothermic peak corresponding to the melting point at 217.9 °C followed by thermal decomposition.^{5,8,9}

The differential scanning calorimetry curves of isoniazid and the isoniazid:lactose mixtures showed a broadening and shifting of the isoniazid melting endotherm from 171.46 °C for pure isoniazid to 165.39–169.17 °C demonstrating a concentration-dependant incompatibility

between isoniazid and lactose. A smaller endothermic event (142–148 °C) was observed in the isoniazid:lactose mixtures, corresponding to the dehydration reaction of lactose.

Additional thermoanalytical studies were performed with binary mixtures (90:10) of isoniazid and the excipients, glucose, sucrose, lactose and microcrystalline cellulose (MCC) respectively. A similar broadening and shifting of the isoniazid melting endotherm at 171.46 °C was seen for the sugars (glucose, sucrose, lactose). The isoniazid:MCC mixture showed no broadening and shifting of the characteristic endothermic event for isoniazid (171.09 °C). MCC has shown to be a comparatively inert excipient and compatible with many drugs.^{4-6,10-12} These results demonstrated that the incompatibility with lactose is not merely due to the accelerated nature of the test.

DISCUSSION

The British Pharmaceutical Codex formula for isoniazid mixture recommends isoniazid powder, however, due to ease of use and availability, isoniazid tablets have been used in practice. Isoniazid is unstable in the presence of sugars and degrades to hydrazine (a carcinogen) in liquid formulations at ambient temperatures.^{2,3,13} A stability indicating HPLC assay was able to quantify isoniazid in the presence of its degradants and formulation excipients. Degradation of isoniazid in the compounded mixture proved to be ≥ 10% after three days of storage at 4 and 25 °C, whereas the control mixture retained acceptable stability for up to 30 days suggesting an incompatibility with formulation excipients, also demonstrated in the solid state by the differential scanning calorimetry studies.

This study highlights the importance for stability evaluations on all modified compounded preparations so that quality pharmaceuticals are delivered to patients.

Competing interests: None declared.

References

1. Williams DA, Lemke TL. Foye's principles of medicinal chemistry. 5th ed. Philadelphia: Williams and Wilkins; 2002. p. 906-8.
2. Lund W. The pharmaceutical codex. 12th ed. London: The Pharmaceutical Press; 1994; 928-30.
3. Bailey LC, Abdou H. High-performance liquid chromatographic analysis of isoniazid and its dosage forms. J Pharm Sci 1977; 66: 564-7.
4. Venkataram S, Khohlokwane M, Wallis SH. Differential scanning calorimetry as a quick scanning technique for solid state stability studies. Drug Dev Ind Pharm 1995; 21: 847-55.
5. Ceschel GC, Badiello R, Ronchi C, Maffei P. Degradation of components in drug formulations: a comparison between HPLC and DSC methods. J Pharm Biomed Anal 2003; 32: 1067-72.
6. Mura P, Bettinetti GP, Faucci MT, Manderioli A, Parrini PL. Differential scanning calorimetry in compatibility testing of picotamide with pharmaceutical excipients. Thermochimica Acta 1998; 321: 59-65.
7. Martinez L, Uribarri E, Laguna A. Characterization and compatibility studies between policosanol, a new hypercholesterolemic drug, and tablet excipients using differential scanning calorimetry. Arch Pharm Pharm Med Chem 1999; 332: 439-41.
8. Araujo AAS, Storpirtis S, Mercuri LP, Carvalho FMS, Filho MdS, Matos JR. Thermal analysis of the antiretroviral zidovudine and evaluation of the compatibility with excipients used in solid dosage forms. Int J Pharm 2003; 260: 303-14.
9. Balestrieri F, Magri AD, Magri AL, Marini D, Sacchini A. Application of differential scanning calorimetry to the study of drug-excipient compatibility. Thermochimica Acta 1996; 285: 337-345.
10. Malan CEP, de Villiers MM, Lotter AP. Evaluation of compatibility of tablet excipients with albendazole and closantel using DSC and HPLC. Drug Dev Ind Pharm 1997; 23: 533-37.
11. Joshi BV, Patil VB, Pokharkar VB. Compatibility studies between carbamazepine and tablet excipients using thermal and non-thermal methods. Drug Dev Ind Pharm 2002; 28: 687-94.
12. Botha SA, Lotter AP. Compatibility study between naproxen and tablet excipients using differential scanning calorimetry. Drug Dev Ind Pharm 1990; 16: 673-83.
13. Carlin A, Gregory N, Simmons J. Stability of isoniazid in isoniazid syrup: formation of hydrazine. J Pharm Biomed Anal 1998; 17: 885-90.

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