Experiment 7: Assay of Isonicotinic acid hydrazide

Isoniazid tablets (Isoniazid compressi)

**Category.** Antituberculosis drug.

**Additional information.** Strength in the current WHO Model list of essential medicines: 100 - 300mg. **Requirements**

Isoniazid tablets contain not less than **90.0%** and not more than **110.0%** of the amount of C6H7N3O stated on the label. **Identity tests**

* Either test A alone or tests B and C may be applied.
	1. To a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid add 10ml of ethanol (~750g/l) TS and shake for 15 minutes. Centrifuge and decant the supernatant liquid. Extract the remaining liquid with two further 10-mL quantities of ethanol (~750g/l) TS and evaporate the combined extracts to dryness. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from isoniazid RS or with the *reference spectrum* of isoniazid.
	2. To a quantity of the powdered tablets equivalent to about 0.1g of Isoniazid add 2.0ml of water, shake, and filter. Then add a mixture composed of 1.0ml of silver nitrate (40g/l) TS and 1.0ml of ammonia (~100g/l) TS; bubbles of nitrogen evolve, the mixture turns from yellow to black and a metallic silver mirror appears on the sides of the test- tube.
	3. To a quantity of the powdered tablets equivalent to about 1mg of Isoniazid add 50ml of ethanol (~750g/l) TS, shake, and filter. To 5ml of the filtrate add 0.1g of sodium tetraborate R and 5ml of 1-chloro-2,4 dinitrobenzene/ethanol TS, evaporate to dryness on a water-bath, and continue heating for a further 10 minutes. To the residue add 10ml of methanol R and mix; a reddish violet colour is produced.

**Assay.** Weigh and powder 20 tablets. Dissolve a quantity of the powdered tablets equivalent to about 0.4g of Isoniazid as completely as possible in water, filter, and wash the residue with sufficient water to produce 250ml. Place 50ml of the resulting solution in a titration vessel, add 50ml of water, 20ml of hydrochloric acid (~250g/l) TS, and 0.2g of potassium bromide R, and titrate with potassium bromate (0.0167mol/l) VS as described under 2.7 Nitrite titration. Each mL of potassium bromate (0.0167mol/l) VS is equivalent to 3.429mg of C6H7N3O.