Drug Metabolism

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Determination of anti-tuberculosis drugs in blood spots

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Objectives Tuberculosis affects a third of the world's population. Treatment failure resulting from non-compliance, incomplete drug supply or malabsorption (Shishoo et al 1999) can induce multi-drug resistant tuberculosis. Therapeutic drug monitoring (TDM) is not routinely undertaken. Dried blood on specimen collection cards does not require specific storage or transportation conditions. This study aimed to develop methods to quantify pyrazinamide (PZA) and isoniazid (INH), in blood spots (BS).

Methods PZA and nicotinamide (internal standard, IS) were extracted from BS with ammonium acetate pH4.0 (400 μL) and acetonitrile (ACN) (3 mL). PZA was also extracted from plasma by protein precipitation with ACN (1 mL). Both samples were centrifuged (3500 *g*, 7 min) and the supernatants reduced to dryness under a stream of nitrogen. PZA was derivatised with Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) + 10% v/v trichloromethylsiloxane (TMCS) and analysed by GC-MS on an OV-1 column (60 m × 0.25 mm i.d.) using a gradient temperature programme. Ionisation was by electron ionisation (70 eV): the MS was operated in SIM mode (*m/z* 179, 195, 266). The release of hydrazine (HYD) after spotting INH onto the cards was detected by the Silver Nitrate test (Sant, 1958). HYD was extracted from the cards using a small volume of water or phosphate buffer (1% w/v, p H5) and analysed by GC-MS after derivatisation with acetone or reaction with phthalimide (Gabriel Synthesis; Gibson & Bradshaw 1968).

Results The results from PZA (BS) method (Figure 1) show linearity over 10–60 μg/mL ($r^2 = 0.98$) and reproducibility: RSD of peak area ratios (PZA/IS) = 9.7%, n = 6. Recovery from BS is approximately 50% and from plasma approximately 76.24%, RSD = 8.3% (n = 3). INH appears to bind to the BS cards via an oxidation reaction involving HYD release. The presence of HYD was proven by the silver nitrate test. Quantification of HYD by GC-MS directly or indirectly was unsuccessful.

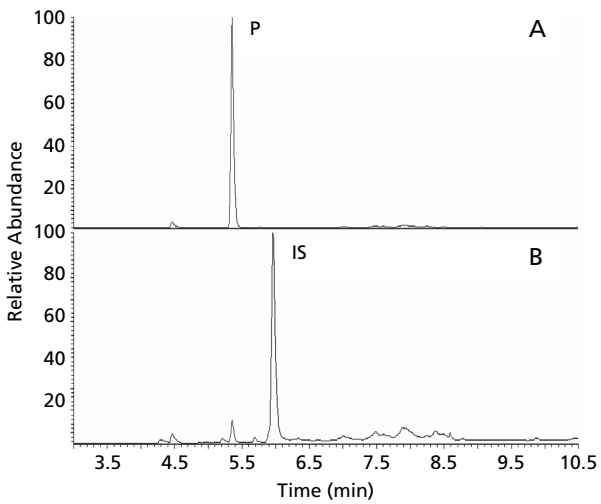

Figure 1 Chromatogram of blood spot spiked with 50 μg/mL PZA; (A) = Chromatogram with ions selected at m/z = 195, P = Pyrazinamide, $t_r = 5.36$ min; (B) = Chromatogram with ions selected at m/z = 179. IS = Nicotinamide, $t_R = 5.95$ min.

Conclusions A potential method for TDM of PZA has been developed. The method is reproducible and linear within the therapeutic C_{max} range for PZA. INH cannot be analysed on the cards due to irreversible binding to cellulose.

Gibson, Bradshaw (1968) *Angew. Chem. Int. Ed*., Engl. **7**: 919
Sant (1958) *Microchim. Acta* **46**: 169–170
Shishoo, et al (1999) *Int. J Pharm*. **190**: 109123

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Collagenase alone or in combination in the preparation of isolated hepatocytes? The effect on phase 1 and phase 2 biotransformations

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Objective To investigate the effect of digestion enzymes on metabolic biotransformations in suspensions of isolated rat hepatocytes. Isolated rat hepatocytes are a valuable whole cell *in vitro* tool for drug metabolism and toxicity studies. Several modifications of the original two-stage collagenase perfusion technique (Seglen 1972) have been reported for the preparation of hepatocytes. However, there are very limited published data on the influence of isolation procedures on phase 1 and phase 2 biotransformations in rat hepatocytes.

Methods Hepatocytes were isolated from male Sprague Dawley rats (180–220 g) using collagenase type II (C II) as described previously (Grant et al 2000). Modifications of this technique using collagenase A/trypsin inhibitor (C A/TI) and collagenase/dispase (C/D) were also investigated. The stereoselective hydroxylation of testosterone (100 μM incubated for 30 min), a well known phase 1 biotransformation, was used to evaluate cytochrome P450 (CYP P450) activity. Incubations were performed with a cell density of 1×10^6 viable cells/mL in Krebs Hepes buffer, pH 7.4, in rotating round bottomed flasks at 37 °C under an atmosphere of 5% CO_2/95% O_2. Following extraction with dichloromethane, testosterone and its metabolites (androstenedione, 6-β, 16-α, 2-α and 7-α hydroxytestosterone) were analysed using HPLC. The glutathione-conjugating capacity, a phase 2 pathway involved in detoxification, was investigated by incubating 50 μM 1-chloro-2,4-dinitrobenzene (CDNB) with intact fresh hepatocytes for 20 min. The activity of glutathione transferase (GST) was analysed in hepatocyte homogenates in the presence of 1 mM reduced glutathione (GSH). The concentration of the GSH conjugate was measured using the spectrophotometric absorbance at $\lambda = 340$ nm and an extinction coefficient of 9.8 mM^{-1}. Intracellular GSH levels were also measured during hepatocyte incubations.

Results The extent of total testosterone metabolism was significantly lower ($P < 0.05$) with C II (81.7 ± 3.3 nmol/10^6 cells) compared with C A/TI and C/D (96.6 ± 1.9 and 95.1 ± 2.1 nmol/10^6 cells, respectively) after 30 min. There were no significant differences in the formation of any of the individual testosterone metabolites analysed. In intact cells the glutathione-conjugating-capacity was significantly greater ($P < 0.05$) with C II (56.9 ± 5.9 nmol/10^6 cells) in comparison with C A/TI (31.6 ± 3.7 nmol/10^6 cells) after 20 min despite there being no significant difference in their initial intracellular GSH levels (8.3 ± 1.5 nmol/mg protein and 12.3 ± 2.0 nmol/mg protein respectively). However, in hepatocyte homogenate with an excess level of GSH supplied, no significant differences in GST activity were found.

Conclusions These findings suggest that the choice of digestion enzyme in the preparation of isolated rat hepatocytes may influence both phase 1 and phase 2 biotransformations in suspension.

Grant, M. H., et al (2000) *Hum. Exp. Toxicol*. **19**: 309–317
Seglen, P. (1972) *Biochim. Biophys. Acta* **264**: 398–410