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Short Communication

Rapid and sensitive method for the determination of albendazole and albendazole sulphoxide in biological fluids

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ABSTRACT

A sensitive and selective reversed-phase high-performance liquid chromatographic method for the determination of albendazole and its active metabolite albendazole sulphoxide in plasma has been developed. It involves single-step extraction of plasma with dichloromethane, evaporation of the solvent and chromatography on a μBondapak phenyl column with a mobile phase of water containing 1% (v/v) triethylamine–methanol–acetonitrile (70:10:20, v/v) at pH 3.1. Run time is 12 min. The assay satisfies all of the criteria required for use in clinical pharmacokinetic studies and possesses important advantages, notably speed and expense, over current methods.

INTRODUCTION

Albendazole (ABZ) is a broad-spectrum anthelmintic used against intestinal helminth infections in an attempt to lower worm burdens and reduce morbidity. ABZ is virtually insoluble in water and consequently is poorly absorbed from the

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gastrointestinal tract. This property is ideal for its use against the geohelminths but is a major disadvantage if it is to be used in the treatment of systemic helminthiasis such as echinococcosis and onchocerciasis. The low systemic availability of ABZ is due in part to poor gastrointestinal absorption, but also to extensive metabolism, to albendazole sulphoxide, ABS [1]. Indeed, ABZ is undetectable in plasma after oral administration. A number of published methods exist for the estimation of ABZ and ABS. Bogan and Marriner [2] developed a high-performance liquid chromatographic (HPLC) method to detect ABZ in plasma and gastrointestinal fluids but this required a large sample volume (4 ml) and repetitive exxtractions. Meulemans *et al.* [3] reported a method for the determination of ABZ and ABS in various human organs and fluids but this used different separation procedures for each analyte. A more recent procedure was described by Hurtado *et al.* [4] which possessed acceptable limits of sensitivity but required solid-phase extraction thus increasing expense. We report an HPLC method for the determination of ABZ and ABS in human plasma which is appropriately sensitive, selective, rapid and inexpensive.

EXPERIMENTAL

ABZ and ABS were gifts from Smith Kline Beecham (Welwyn Garden City, U.K.). Proguanil hydrochloride (PG), the internal standard, was obtained from ICI Pharmaceuticals (Alderley Park, U.K.). Solvents of either HPLC or analytical grade were supplied either by Fisons (Loughborough, U.K.) or BDH (Poole, U.K.).

Sample preparation

The extraction was performed in glass culture tubes (15 ml capacity) pretreated with dichlorodimethylsilane in 5% (v/v) toluene in order to minimise drug adsorption. To samples of plasma (0.2–1.0 ml) containing the internal standard (proguanil hydrochloride 150 ng) dissolved in distilled water was added acetonitrile (0.2–1.0 ml) to precipitate plasma proteins.

Following vortex-mixing (5 s) the samples were centrifuged (1000 *g* for 10 min) before being transferred to clean culture tubes to which distilled water (1.0 ml) was added. This mixture was extracted with dichloromethane (10 ml) by vortex-mixing (10 s). After further centrifugation (1000 *g* for 10 min) the aqueous phase was discarded and the organic layer transferred to a clean tube and evaporated to dryness under nitrogen at 37°C. Samples were reconstituted with mobile phase (0.1 ml) and an aliquot (0.01–0.05 ml) was used for chromatography.

Chromatography

A Spectra Physics chromatographic system was used. This consisted of an Isochrom solvent delivery system with a Rheodyne valve injector, a Spectra 100 variable-wavelength UV absorbance detector operating at 254 nm and an SP

4270 computing integrator. Chromatography was obtained using a prepacked cartridge column (μBondapak phenyl, 10 μm particle size; 10 cm × 0.8 cm I.D., Millipore-Waters Harrow, U.K.) fitted with a CN Guard-Pak (10 μm particle size) guard column system. Two mobile phases, running at ambient temperature, were evaluated: first, water–methanol–acetonitrile (36:46:18, v/v) containing sodium octanesulphonate ($5 \cdot 10^{-3}$ *M*) as an ion-pairing agent, buffered to pH 4.0 with orthophosphoric acid and flowing at 1.5 ml min^{-1}; second, water containing 1% triethylamine (v/v)–methanol–acetonitrile (70:10:20, v/v) buffered to pH 3.1 with orthophosphoric acid and flowing at 2.5 ml min^{-1}.

Calibration

Standard curves were generated by the addition of known quantities of ABZ and ABS (0–1 μg) to a fixed amount of the internal standard (PG; 250 ng) in drug-free plasma. The efficiency of extraction was determined by comparison of peak areas of extracted samples containing known quantities of each analyte with the peak areas obtained after injection of stock solutions containing the same quantities of drug. Samples were analysed as described above and the peak-area ratio of internal standard to either ABZ or ABS was plotted against the corresponding mass of ABZ of ABS. Peak-area ratios of unknowns were treated similarly and concentrations determined from the standard curves.

Assay specifications

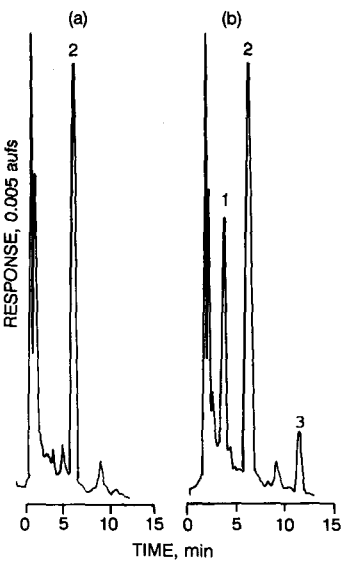
Inter- and intra-assay coefficients of variation (*i.e.* assay precision) were determined by analysis of samples of drug-free plasma to which had been added ABZ and ABS to produce final concentrations of 50 μg l^{-1} ($n = 6$) and 250 μg l^{-1} ($n = 6$) for ABZ and 20 μg l^{-1} ($n = 5$), 50 μg l^{-1} ($n = 6$) and 250 μg l^{-1} ($n = 6$) for ABS.

Clinical study

Male onchocerciasis patients, aged 20–54 years and weight range 45.5–75.5 kg under treatment at the Onchocerciasis Chemotherapy Research Centre (Hohoe, Ghana) received either 800 mg ($n = 18$) or 1200 mg ($n = 14$) ABZ as a single oral dose for three days given with a simple, fatty breakfast (43.1 g fat). Blood (10 ml) was removed by venepuncture prior to dosage and at 4, 8, 24, 48 and 72 h thereafter. Following centrifugation (1000 *g* for 15 min) the plasma was removed and frozen (−20°C) prior to transport to Liverpool for analysis.

RESULTS AND DISCUSSION

Optimal recovery of ABZ (97%) and ABS (75%) was achieved with dichloromethane. Other solvents [hexane, ethyl acetate, ethyl acetate–hexane (9:1, v/v)] were proportionately less efficient although all gave recoveries of ABZ in excess of 70%. However, none extracted ABS with an efficiency of > 70%. Optimal



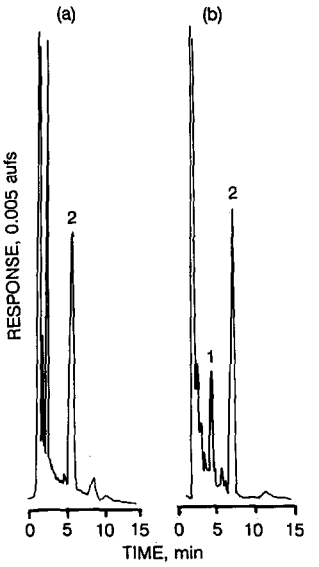


Fig. 1. Chromatograms of extracts of (a) drug-free plasma containing the internal standard, PG (150 ng), and (b) 0.3 ml of plasma containing 50 ng ABZ, 50 ng ABS and 150 ng PG. Peaks: 1 = albendazole sulphoxide; 2 = proguanil; 3 = albendazole.

Fig. 2. Chromatograms of extracts of (a) a pre-dose plasma sample from a patient with onchocerciasis prior to treatment with ABZ and (b) a plasma sample from the same patient taken 4 h post treatment with ABZ (ABS concentration = 78 $\mu g\, l^{-1}$). Peaks: 1 = albendazole sulphoxide; 2 = proguanil.

chromatographic separation was achieved using a mobile phase of water–methanol–acetonitrile (70:10:20, v/v) at a flow-rate of 2.5 ml min^{-1}. Chromatograms of extracts of drug-free plasma and plasma to which ABZ and ABS (50 $\mu g\, l^{-1}$) had been added and of human plasma pre- and post-oral administration of albendazole are shown in Figs. 1 and 2. The plasma extract (0.3 ml) from the patient with onchocerciasis after treatment with albendazole showed two peaks at retention times of 4 min:6 s and 5 min:54 s, respectively, corresponding to 26 ng ABS (78 $\mu g\, l^{-1}$) and PG. ABZ, retention time 10 min:54 s (Fig. 1), was not detected. There was no chromatographic interference from any endogenous compounds or any of the benzimidazoles (or their metabolites) used in clinical practice. The intra- and inter-assay coefficients of variation (*i.e.* precision) of the analysis of spiked plasma samples were, respectively, 8.8 and 7.1% at 20 $\mu g\, l^{-1}$, 9.5 and 8.9% at 50 $\mu g\, l^{-1}$ and 6.7 and 5.9% at 250 $\mu g\, l^{-1}$ for ABS. The corresponding values for ABZ were 9.0 and 8.4% at 50 $\mu g\, l^{-1}$ and 9.4 and 7.4% at 250 $\mu g\, l^{-1}$.

Actual concentrations found in these samples were, with added concentrations in parentheses, 24 (20) $\mu g\, l^{-1}$, 49 (50) $\mu g\, l^{-1}$ and 257 (250) $\mu g\, l^{-1}$ for ABS and 55 (50) $\mu g\, l^{-1}$ and 268 (250) $\mu g\, l^{-1}$ for ABS. Calibration curves were linear in the range 0–800 $\mu g\, l^{-1}$ ABZ and ABS (r = 0.990). These observations demonstrate

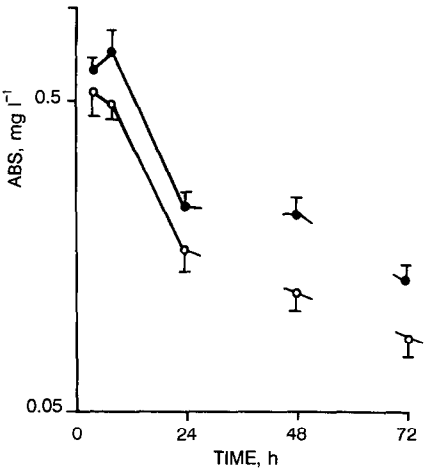


Fig. 3. Mean (± S.E.M.) plasma concentration of ABS following administration of 800 mg (○) or 1200 mg (●) ABZ to patients (800-mg dose, $n = 18$; 1200-mg dose, $n = 14$) with onchocerciasis.

the suitability of the method to measure ABS over the range of concentrations commonly achieved *in vivo* [1].

The assay was applied to a dose-finding study for ABZ in patients with onchocerciasis following either 800 or 1200 g ABZ given orally. ABZ was not detected in any sample, confirming the findings of earlier studies [4]. The mean plasma concentration–time profiles for ABS are shown in Fig. 3. Peak concentrations of ABS were attained within 4 h for the 800-mg dose and within 12 h for the 1200-mg dose. The area under the plasma concentration *versus* time curve ($AUC_{0-72\ h}$) was 14.3 mg h l^{-1} and 20.9 mg h l^{-1} for doses of 800 and 1200 mg, respectively, indicating approximate proportionality between dose and plasma concentrations of ABS.

In summary, we have reported an analytical method for the determination of ABS which satisfies all of the criteria required for an assay which is to be suitable for human pharmacokinetic studies and possesses important advantages, notably speed and inexpense, over currently published methods. The method has also been applied to the determination of ABZ and ABS in whole blood and tissue homogenates where similar standards of sensitivity and reproducibility were achieved.

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