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Technical note

Measurement of physiological concentrations of dapsone and its monoacetyl metabolite: a miniaturised assay for liquid or filter paper-absorbed samples

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Abstract

A modification of existing HPLC assay methods is described for the measurement of dapsone and monoacetyldapsone in 50-μl samples of plasma and whole blood. This method, in particular the use of small sample volumes dried onto filter paper strips, is applicable to multi-sample clinical and pharmacokinetic studies in children with malaria, who are often anaemic, and where sample volume must be kept to a minimum. Basified samples were extracted into 5 ml of ethyl acetate–*tert.*-butylmethyl ether (1:1, v/v), chromatographed on a μBondapaK C_{18}, 10-μm column with water–acetonitrile–glacial acetic acid (81:17.5:5, v/v) containing 2 g/l 1-octanesulphonic acid as the mobile phase and detected at 274 nm.

Keywords: Dapsone; Monoacetyldapsone

1. Introduction

The determination of DDS and its metabolite monoacetyldapsone (MADDS) in biological fluids by HPLC has been described [1,2]. This note describes an improved assay technique, using similar methodology, but which allows the measurement of DDS and MADDS in 50-μl samples of either liquid or filter paper-absorbed plasma or whole blood.

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2. Experimental

2.1. Reagents

DDS and 1-octanesulphonic acid were obtained from Sigma (St. Louis MO, USA). MADDS was a gift from Dr. M.D. Coleman, University of Liverpool (Liverpool, UK). The internal standard, acetanilide was obtained from May and Baker (Dagenham, UK). Filter paper strips (Whatman grade 17) were obtained from Whatman (Kent, UK). Acetonitrile (HPLC grade), glacial acetic acid, ammonia, ethyl acetate and *tert.*-butylmethyl ether (all AnalaR grade) were obtained from BDH (Poole, UK). Working

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solutions were prepared in the mobile phase from methanolic stock solutions.

2.2. Sample extraction

To a 50-μl sample of liquid or filter paper-absorbed whole blood or plasma, 1 μg of I.S. was added. The filter paper-absorbed samples were air-dried for 30 min and the blots were cut into small pieces. In a 10-ml culture tube containing liquid or dried sample 200 μl of ammonia were added, and vortex-mixed for 5 s. After extraction, centrifugation and separation, the organic phase was evaporated to dryness at 37°C. The residue was reconstituted in 100 μl of mobile phase and 50-μl aliquots were injected.

2.3. Chromatography

This was performed using an isochrom delivery system (Spectrasystem P1000; Spectra-Physics) fitted to a Rheodyne valve injector (50-μl loop) and connected to a stainless-steel column (μBondapak C_{18}, 10 μm, 30 cm × 3.9 mm I.D.) preceded by a guard column (CN precolumn, RP-18 end-capped 5 μm, 10 mm × 4.6 mm I.D.) (Waters, Milford, MA, USA). Column effluent was monitored using a variable-wavelength UV detector (Spectrasystem UV 1000; Spectra-Physics).

2.4. Recovery, standard curves and reproducibility

Recovery of DDS, MADDS and acetanilide (I.S.) was assessed by adding 0.1 μg of drug and metabolite and 1 μg of I.S. to each of five 50-μl aliquots of drug-free whole blood or plasma that were either dried onto filter paper strips or extracted as liquids. Recovery was defined as the ratio of the peak heights for DDS, MADDS and I.S. from the extract to those produced by direct injection of equivalent amounts of each compound. Standard curves were prepared by the addition of DDS and MADDS (0.1–5.0 μg/ml) to drug-free whole blood or plasma and extracted as above with the unknowns in each run. Intra- and inter-assay reproducibilities were assessed at 0.1 and 5.0 μg/ml (n = 6 in both cases). Inter-assay reproducibility was assessed weekly over one month.

3. Results

3.1. Chromatography

DDS, MADDS and I.S. were resolved to baseline, at DDS and MADDS concentrations of up to 5.0 μg/ml, with retention times of 6.0, 8.0 and 4.5 min, respectively (Fig. 1). There was no interference from other anti-malarials. The lowest detectable concentration of DDS and MADDS from a 50-μl sample was 15 and 10 ng/ml respectively, in both whole blood and plasma that was either dried or in liquid

Fig. 1. Chromatogram of a 50-μl extract of liquid plasma from a patient following oral administration of a dapsone/chlorproguanil combination. Peaks: X = Injection; 1 = I.S. (1 μg); 2 = DDS (1.22 μg/ml); 3 = MADDS (0.25 μg/ml). Comparable chromatograms were obtained from filter paper-absorbed samples.

Table 1
Recovery, reproducibility and standard curve correlation data obtained from spiked whole blood and plasma liquid and filter paper-absorbed samples

Matrix	Compound	Recovery (mean ± S.D.) (%)	Coefficient of variation (%)		Correlation coefficient (r^2)
			Intra-assay	Inter-assay	
Liquid					
Plasma	DDS	59 ± 4	2.4	4.5	0.989
	MADDS	99 ± 3	7.8	8.3	0.994
Whole blood	DDS	61 ± 3	2.2	4.3	0.985
	MADDS	103 ± 4	8.0	8.1	0.987
Filter-absorbed					
Plasma	DDS	60 ± 6	2.7	11.0	0.986
	MADDS	100 ± 7	9.8	8.6	0.997
Whole blood	DDS	61 ± 5	2.9	9.9	0.995
	MADDS	98 ± 5	9.6	8.4	0.992

form, which at 0.01 AUFS consistently produced a peak that was ≥ four times that of the baseline noise.

3.2. Recovery, standard curves and reproducibility

Assay recoveries, correlation coefficients (r^2) and reproducibilities for DDS and MADDS are shown in Table 1. The recovery of I.S. was 73 ± 8 in both liquid and filter paper-absorbed samples (n = 6).

4. Discussion

A simple, reliable method of sample collection and storage is an essential prerequisite for clinical drug studies in the tropics. Our assay, and its modification for filter paper-absorbed samples, facilities the collection, transportation and measurement of DDS and MADDS within the therapeutic range without detriment to specificity and sensitivity. The sensitivity of this method is higher than that reported with previous methods [1,2], since the same limit of detention is reported, but using a smaller sample volume, which is important in clinical studies in young children involving multiple sampling. It provides details on the processing of filter paper-absorbed samples and avoids double centrifugation. The new method has been used in the pharmacokinetic analysis of DDS and MADDS in a clinical trial of chlorproguanil plus dapsone as treatment for non-severe falciparum malaria in Kenyan children, monitoring of compliance and the measurement of unbound DDS concentrations in plasma water. This method may be applicable to field situations where fingerprick blood sampling is appropriate.

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References

[1] M. Edstein, J. Chromatogr., 307 (1984) 426–431.
[2] M.M. Lemnge, A. Ronn, H. Flachs and Ib. C. Bygbjerg, J. Chromatogr., 613 (1993) 340–346.