Journal of Chromatography, 307 (1984) 426—431
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2026

Note

Quantification of antimalarial drugs

II. Simultaneous measurement of dapsone, monoacetyldapsone and pyrimethamine in human plasma

MICHAEL EDSTEIN

Army Malaria Research Unit, MILPO, Ingleburn, Sydney, NSW 2174 (Australia)

(Received October 26th, 1983)

Maloprim® is widely used as a prophylactic agent against malaria, particularly in areas where chloroquine-resistant strains of *Plasmodium falciparum* are prevalent [1]. Each tablet of Maloprim contains 100 mg of dapsone (4,4′-diaminodiphenyl sulphone, DDS) and 12.5 mg of pyrimethamine [2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine, PYR]. DDS and PYR act at sequential steps in malaria parasite folate metabolism, resulting in a synergistic inhibition.

Analytical methods capable of simultaneously measuring the components of Maloprim and a major metabolite of DDS, monoacetyldapsone (4-acetamido-4′-aminodiphenyl sulphone, MADDS) are based on chromatographic methods. Jones and Ovenell [2] developed a high-performance liquid chromatographic (HPLC) method which was difficult to standardize because of the presence of volatile diisopropyl ether and ammonium hydroxide in the mobile phase. Ahmad and Rogers [3] reported a pharmacokinetic study using thin-layer chromatography with detection limits of 20 ng/ml for DDS and MADDS and 15 ng/ml for PYR, but the method required 3 h equilibration of glass tanks containing mobile phase before analysis was undertaken. A simple, reproducible and sensitive method for the estimation of Maloprim is required for measuring ng/ml concentrations of the drug combination found in plasma after a prophylactic dose (i.e. one tablet per week).

The purpose of the present work was to develop a rapid, selective and sensitive method for the simultaneous quantification of DDS, MADDS and PYR in human plasma using HPLC. The described method is similar to an HPLC procedure developed by the author [4] for the analysis of the antimalarial drug Fansidar® (sulphadoxine and PYR). Reversed-phase ion-pair

chromatography was used with pentane sulphonic acid as the counter-ion and with quinine as the internal standard.

MATERIALS AND METHODS

Chemicals and standards

HPLC grade acetonitrile, methanol and 1-pentane sulphonic acid (Waters Assoc., Australia) were used. Sodium hydroxide and ethylene dichloride were of analytical quality.

Reference compounds were donated as follows: DDS by Imperial Chemical Industries (Australia), MADDS by Parke Davis (Warner-Lambert, Ann Arbor, MI, U.S.A.), and PYR by Wellcome (Australia). A stock standard solution of DDS, MADDS and PYR was prepared containing 500 μg base of each compound per ml of methanol. Intermediate and working standard solutions were prepared by diluting the stock standard solution with water. A stock standard solution of quinine dihydrochloride was prepared containing 500 μg base per ml of water. All standard solutions were stored at 4°C in amber glass bottles.

Instrumentation and chromatographic conditions

The liquid chromatographic system comprised a Pye Unicam LC-XPD pump, a Model 710B sample programmer WISP (Waters Assoc.), a Pye Unicam DP88 integrator and a Waters M440 UV absorbance detector operated at 254 nm at a sensitivity of 0.005 a.u.f.s. The column was a 30 cm × 3.9 mm I.D., 10-μm particle size, μBondapak C_{18} column (Waters Assoc.).

The mobile phase consisted of methanol—acetonitrile—water (25:15:60, v/v) containing 0.005 *M* pentane sulphonic acid (pH 3.40). The flow-rate was 1.5 ml/min (approximately 155 bar) and the system was operated at ambient temperature. The mobile phase was filtered (FHUP04700 Millipore) prior to use and was purged with helium (50 ml/min) during analysis.

Extraction procedure

To a plasma sample (1 ml) in a 15-ml glass culture tube (Teflon-lined screw cap) were added 25 μl of quinine solution (125-ng base per 25 μl), 150 μl of 2 *M* sodium hydroxide and 6 ml of ethylene dichloride. The tube was agitated for 10 min on a Dymax shaker (100—120 strokes/min). After centrifugation at 1000 *g* for 5 min, the aqueous phase was removed and the organic phase was transferred to a clean glass tube. The organic phase was evaporated to dryness at 60°C using a gentle stream of air. The residue was dissolved in 100 μl of mobile phase and an aliquot (40 μl) injected. Analysis was done in duplicate.

To minimise adsorption of the compounds onto glass surfaces, glassware used in extraction was silanised using 0.2% Aquasil (Pierce, Rockford, IL, U.S.A.).

Calibration

Calibration curves were prepared by analysing 1.0-ml plasma samples spiked with known amounts of the compounds. The concentration range studied was

0.045—1.515 μg/ml for each compound. Peak area ratios of DDS, MADDS and PYR peaks to the internal standard peak were plotted against concentrations. Calibration standards were run on each day of analysis.

Recovery and reproducibility of the method

DDS, MADDS and PYR recovery was determined by comparing peak areas of each compound extracted from spiked plasma (range 0.023—0.909 μg/ml) with areas obtained by direct injection of pure compounds. Within-day reproducibility was determined for each compound over the range of 0.023—0.909 μg/ml of plasma, and day-to-day reproducibility was determined by assaying plasma standards over the range of 0.045—0.909 μg/ml for each compound on six occasions.

Stability of DDS, MADDS and PYR

The stability of each compound was determined by storing plasma standards and working standard solutions for six months at −15° C and 4° C, respectively. Concentrations were measured periodically using the described HPLC method.

RESULTS AND DISCUSSION

The described HPLC method uses the same instrumentation and chromatographic conditions as the simultaneous quantification of the components of Fansidar [4]. The major difference between the two methods is that Fansidar components are extracted under acidic conditions whereas Maloprim components are extracted under alkaline conditions. Pentane sulphonic acid was an essential component of the mobile phase for the separation and elution of quinine and PYR.

The separation of DDS, MADDS, quinine, and PYR extracted from plasma is shown in Fig. 1a. Retention times for DDS, MADDS, quinine and PYR were 3.4, 4.2, 7.1 and 9.3 min, respectively. Endogenous substances in plasma were found not to interfere with the assay (Fig. 1b). A typical chromatogram of the extract of a plasma sample obtained from a volunteer following one tablet of Maloprim is shown in Fig. 1c.

Calibration curves for the three compounds were linear, with correlation coefficients of 0.997 or better. Extraction recoveries were good with mean values of 96%, 94% and 93% for DDS, MADDS and PYR, respectively (Table I). Recovery of the weak base PYR was approximately 7.5% greater using alkaline extraction compared with the acidic conditions used in the analysis of Fansidar [4]. Within-day coefficients of variation averaged 7.8% for DDS, 6.2% for MADDS and 7.2% for PYR, and the day-to-day coefficient of variation averaged 8.5% for DDS, 6.5% for MADDS and 6.2% for PYR (Table II).

Jones and Ovenell [2], using HPLC, reported that metoprine [2, 4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine], a compound closely related to PYR, was a good internal standard. Metoprine and 4-nitro-4′-aminodiphenyl sulphone, an analogue of DDS, were investigated for suitability as internal standards under the described conditions. Metoprine and 4-nitro-4′-aminodiphenyl sulphone were found to coelute with PYR and an endogenous substance in plasma, respectively. As an alternative to using compounds of similar

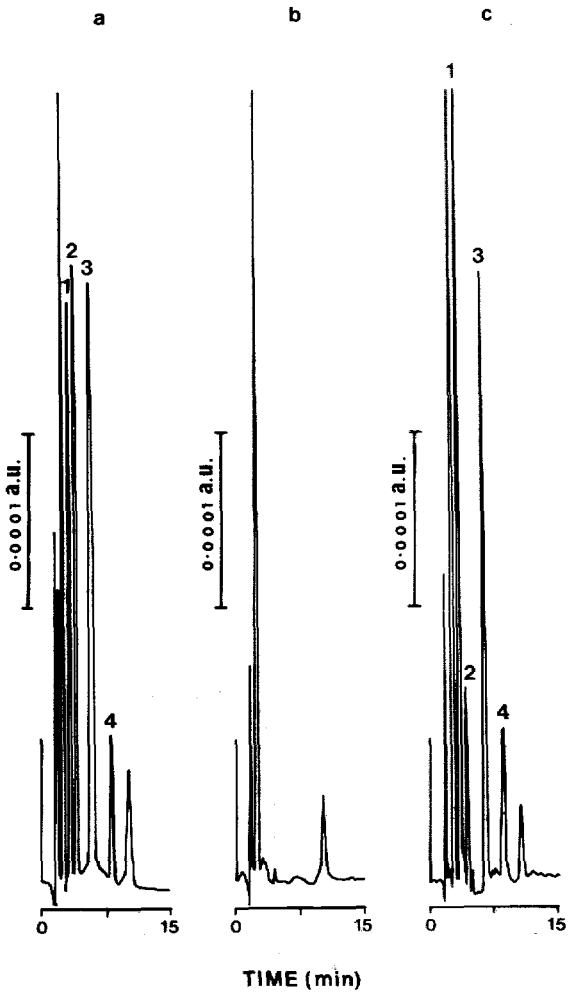

Fig. 1. Chromatograms of: (a) extracted spiked plasma sample containing 45 ng/ml each of DDS (1), MADDS (2) and PYR (4). Internal standard, quinine (3) = 125 ng base; (b) extracted drug-free plasma; and (c) extracted plasma sample obtained 72 h after administration of one tablet of Maloprim to a healthy volunteer (concentrations found in this sample were: DDS (1), 93 ng/ml; MADDS (2), 18 ng/ml; PYR (4), 40 ng/ml.

TABLE I

RECOVERY OF THE HPLC METHOD

Recovery (%) is expressed as mean % ± S.D. Number of observations = 5 per compound per concentration.

	Concentration (μg/ml)					
	0.023	0.045	0.182	0.413	0.909	Overall mean ± S.D.
DDS	94 ± 4.6	97 ± 5.0	95 ± 3.2	94 ± 2.3	98 ± 1.7	95.6 ± 1.8
MADDS	92 ± 3.2	98 ± 5.4	94 ± 0.9	92 ± 4.1	95 ± 1.1	94.2 ± 2.5
PYR	94 ± 4.7	97 ± 6.9	87 ± 3.1	92 ± 5.7	93 ± 6.1	92.6 ± 3.7

TABLE II

REPRODUCIBILITY OF THE HPLC METHOD: COEFFICIENTS OF VARIATION (%)

	Concentration (μg/ml)				
	0.023	0.182	0.909	1.515	Mean ± S.D.
Within-day (n = 5)*					
DDS	13.6	7.1	5.1	5.2	7.8 ± 4.0
MADDS	6.8	7.7	4.8	5.4	6.2 ± 1.3
PYR	6.9	8.2	7.6	6.2	7.2 ± 0.9

	Concentration (μg/ml)					
	0.045	0.091	0.182	0.413	0.909	Mean ± S.D.
Day-to-day (n = 6)*						
DDS	14.6	14.3	6.4	4.1	3.3	8.5 ± 5.5
MADDS	10.4	8.8	4.7	5.4	3.4	6.5 ± 2.9
PYR	12.6	6.7	3.5	4.4	3.8	6.2 ± 3.8

*n = number of observations per compound per concentration.

structure as internal standards, quinine was selected because it showed reproducible extraction, suitable retention and was well resolved from other peaks.

None of the following antimalarial drugs interfered with the determination of DDS, MADDS, quinine and PYR in plasma: chloroquine, mefloquine, primaquine and proguanil. Fansidar, because it contains PYR, does interfere with the analysis. No significant degradation of the three compounds was observed under the prescribed storage conditions.

The advantages of this new HPLC method over previously published methods [2, 3] are ease of sample preparation, a lower limit of detection and speed of analysis. The limit of detection for DDS and MADDS of 5 ng/ml is as sensitive as that reported by Jones and Ovenell [2], but the method is twice as sensitive for PYR determination with a detection limit of 5 ng/ml. The speed of the method was such that 50 samples could be analysed by one operator within 10 h.

Monitoring of antimalarial drug concentrations aids the assessment of possible drug resistance of the parasite. Lack of compliance with the recommended dosage regimen is a common cause of failure of malaria prophylaxis. The described HPLC method is used in our laboratory for routine monitoring of Maloprim levels and for pharmacokinetic studies.

CONCLUSION

In summary, a rapid, selective, and sensitive HPLC procedure for the simultaneous quantification of DDS, MADDS and PYR in plasma has been developed which is suitable for routine monitoring of Maloprim concentrations in man. The method is flexible in that by changing the extraction pH the principal components and the major acetylated metabolite of two widely used antimalarial drugs, Maloprim and Fansidar, can be quantified.

ACKNOWLEDGEMENT

I thank the Director General of Army Health Services, Australian Army, for approval to publish this paper.

REFERENCES

1 A.J. Spicer, Practitioner, 223 (1979) 521.
2 C.R. Jones and S.M. Ovenell, J. Chromatogr., 163 (1979) 179.
3 R.A. Ahmad and H.J. Rogers, Brit. J. Clin. Pharmacol., 10 (1980) 519.
4 M.D. Edstein, J. Chromatogr., 305 (1984) 502.