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Biomedical Applications

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Note

The gas—liquid chromatographic analysis of trimethoprim in plasma and urine

GRAHAM LAND, KAREN DEAN and ALAN BYE

Department of Clinical Pharmacology, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent (Great Britain)

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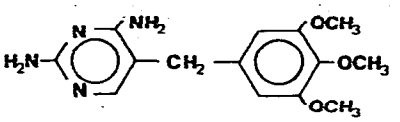
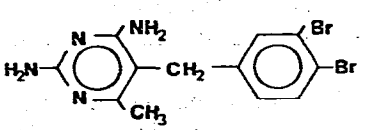
Trimethoprim (I) has been used in combination [1, 2] with various sulphonamides as a broad-spectrum antibacterial agent. Assays based on microbiological [3], differential pulse polarography [4], spectrofluorimetric [5—7] and gas chromatographic [8] methods have been reported. The assay from plasma and urine described here employs GC with nitrogen-selective detection, and obviates derivatisation.



I–TRIMETHOPRIM



II–BW 214U

EXPERIMENTAL

Materials

Trimethoprim (TMP; 2,4-diamino-5-(3′,4′,5′-trimethoxybenzyl)-pyrimidine) and 2,4-diamino-5-(3′4′-dibromophenyl)-6-methyl pyrimidine (BW51-214) (II) were obtained from the Wellcome Research Labs. (Beckenham, Great Britain). Chloroform of AnalaR grade (BHD, Poole, Great Britain), isopropanol (Rathburn Chemicals, Walkerburn, Great Britain) and freshly deionised water were also used.

Glassware

Soveril 20-ml stoppered test tubes (type 611-03; V.A. Howe, London, Great Britain) were used for the extraction and Quickfit BC24/C14T (Q and Q) tapered centrifuge tubes were used in the drying down stage. Micro-vials (Hewlett-Packard, Winnersh, Great Britain) were used for the gas chromatograph auto-sampler. All glassware was hydrochloric acid-washed before use.

Gas chromatograph

A Perkin-Elmer (Beaconsfield, Great Britain) F-30 gas chromatograph equipped with a nitrogen—phosphorous detector (Part No. 3006—7178) was used. The injection port was modified to take a Hewlett-Packard 7670A/002 auto-sampler.

Method

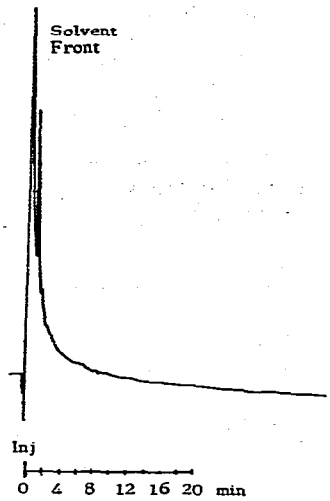
Standard solutions of TMP in plasma or urine were prepared by adding the appropriate volume from a 1 mg/ml stock solution in water containing HCl (pH 4.5).

Plasma or urine samples (0.5 ml) containing TMP were placed in 20-ml extraction tubes to which were added 20 μl of a 100 μg/ml solution of BW51-214 in isopropanol, 4 ml of 0.1 mol/l sodium carbonate and 10 ml of chloroform. The test tubes were then stoppered and mixed for 30 min along their long axes at 25 oscillations per min. The liquid phases were then separated by centrifugation at 100 *g* for 20 min, the aqueous layer was removed by suction and discarded. An 8-ml aliquot from the remaining organic layer (chloroform) was then transferred to the tapered centrifuge tubes. The chloroform was evaporated by heating the tubes at 60° under a stream of nitrogen. The residue was then taken up in 100 μl of isopropanol and transferred to a microvial followed by two further 50-μl washes of isopropanol. The vial was capped for subsequent GC analysis the same day.

Gas—liquid chromatographic conditions

A 1.8 m × 4 mm I.D. glass column was hand packed with 10% Poly S-179 on Chromosorb W HP, 80—100 mesh (Field Instruments, Richmond, Great Britain) and conditioned at 350° with a 30 ml/min helium carrier-gas flow for 24 h before use.

The detector was used in the nitrogen mode under the following conditions: hydrogen (3.8 ml/min); air (105 ml/min); heating position (680 in the NP-mode); carrier gas (helium, 45 ml/min); manifold, oven and injection port (350°, 330° and 350° respectively). Chromatography was good (see Figs. 1 and 2) with BW51-214 having a retention time of 6.3 min and TMP having a retention time of 8.5 min. No interfering peaks were seen in human plasma or urine, even from the sulphonamides commonly administered in conjunction with TMP, namely sulphamethoxazole and sulphadiazine. A computer-based data system (Hewlett-Packard Model 3352) was used to calculate peak areas and their ratios, and also it controlled the operation of the automatic liquid sampler.



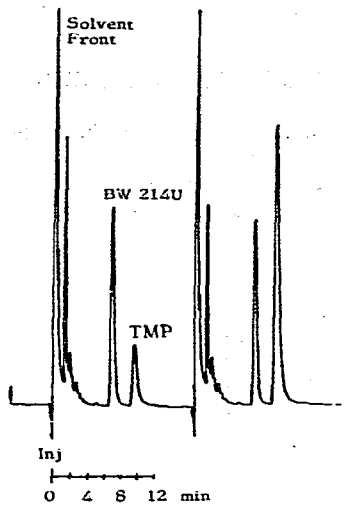


Fig. 1. Chromatogram produced from a urine "blank" sample analysed by this procedure.

Fig. 2. Chromatograms produced by plasma samples containing 5 μg/ml and 20 μg/ml TMP, respectively, with internal standard.

RESULTS

Calculation of results

A known mass of internal standard (2 μg of BW51-214) was added to a range of TMP standard solutions. A calibration curve was constructed with the concentration of TMP on the abcissa and the ratio of peak area TMP to peak area BW51-214 on the ordinate. Since the same mass of internal standard was added to the unknown samples the unknown concentrations can be determined from the calibration curve.

Validation

The method was tested by analysing samples of plasma to which quantities of TMP unknown to the analyst had been added. Six determinations were carried out on each sample and the precision is shown in Table I.

Applications

An example of the plasma concentration curve in a healthy female volunteer after taking 200 mg TMP by mouth is shown in Fig. 3.

Linearity, sensitivity and recovery

The detector gave a linear response from 2 to 400 ng of TMP injected on column. The lower limit of sensitivity being 1 ng under the standard conditions. Under the operating conditions described the method is capable of routinely detecting down to 0.1 μg/ml of TMP in plasma or urine. Total recovery was 78% because of extraction and transfer losses.

TABLE I

ANALYSIS OF TRIMETHOPRIM ADDED TO PLASMA

Each result is the mean of six determinations

Trimethoprin conc. (μg/ml)		Standard deviation (μg/ml)	Sample coefficient of variation (%)	Deviation from theory (%)
Added	Assayed			
0.85	0.85	0.11	12.9	0
2.13	2.14	0.08	3.74	+0.47
3.25	3.21	0.31	9.66	−1.23
4.33	4.47	0.27	6.04	+3.23
5.63	5.89	0.28	4.75	+4.62
7.50	7.73	0.40	5.17	+3.07

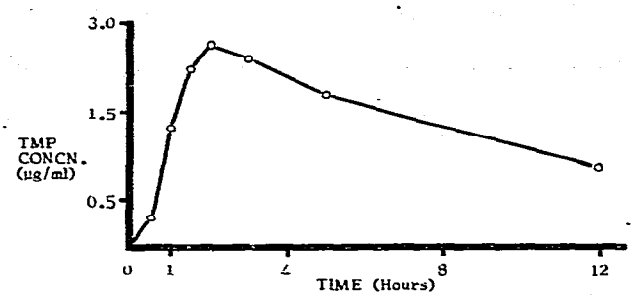


Fig. 3. A typical plasma profile from a healthy female after 200 mg of TMP was given orally.

DISCUSSION

The described method was found to be simple and specific for unchanged TMP and also extremely sensitive, 1 ng of TMP injected on column being readily detected. Several precautions were necessary to ensure reproducible results. Firstly, some TMP adsorption occurs to glass, which causes carry-over from the glass injection syringe of the auto-sampler. The use of a syringe wash in chloroform between injections overcame the problem. Secondly, sensitivity could be increased by increasing the bead temperature of the detector, however this was detrimental to reproducibility. Finally, the use of an internal standard reduces error from transfer losses.

The method is in routine use for human pharmacokinetic and bioavailability studies.

ACKNOWLEDGEMENTS

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