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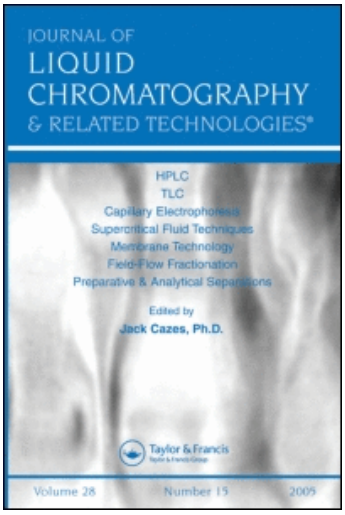

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A MODIFIED HPLC METHOD FOR RAPID DETECTION OF MOCLOBEMIDE AND ITS *N*-OXIDE METABOLITE IN HUMAN URINE

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ABSTRACT

A rapid and simple high performance liquid chromatographic (HPLC) method to measure moclobemide and its *N*-oxide metabolite (Ro12-5637) in human urine has been developed by modifying an existing HPLC technique. Sample preparation involves mixing urine with water and internal standard (Ro11-9900) followed by centrifugation. Separation of the analytes in the supernatant was achieved using a reversed-phase Spherisorb C6 column (Phenomenex, 5μm, 250 × 4.6 mm i.d.) eluted with methanol-triethylamine-phosphate buffer (pH7.0). Moclobemide and Ro12-5637 were detected by UV absorption at 240 nm.

The limits of quantification for moclobemide and Ro12-5637 were 0.5 and 2.0 μg/mL, respectively. This method has been used to measure the concentrations of moclobemide and Ro12-5637 in urine samples from healthy Caucasians administered a 300 mg oral dose of moclobemide.

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INTRODUCTION

Moclobemide is a selective and reversible inhibitor of monoamine oxidase A (MAO-A) that is commonly prescribed for the treatment of depression.[1] The initial pathways of metabolism in humans are *N*- and *C*-oxidation of the morpholine ring to form the two major metabolites in plasma, Ro12-5637 (Figure 1) and Ro12-8095, respectively.[1] The *C*-oxidation pathway cosegregates with the cytochrome P450 2C19 (CYP2C19) or S-mephenytoin genetic polymorphism in drug metabolism.[2]

The major plasma metabolite, Ro12-8095, undergoes further extensive oxidation including ring opening with less than 1% of the oral dose of moclobemide being recovered in the urine as this metabolite.[1] In contrast, Ro12-5637 undergoes very little further metabolism and poor metabolisers of S-mephenytoin excrete more of this metabolite in urine than extensive or normal metabolisers.[1,2]

A single high performance liquid chromatographic (HPLC) method to measure moclobemide and its *N*-oxide metabolite, Ro12-5637, in urine has been found in the literature.[3] The reversed-phase technique uses solid phase extraction Extrelut 1 glass columns packed with keiselguhr (natural diatomaceous earth) to extract moclobemide and Ro12-5637 from urine. The cost of these columns is high.

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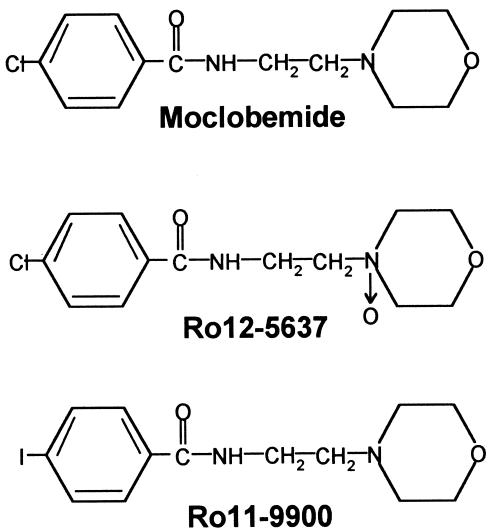

Figure 1. Chemical structures of moclobemide, the *N*-oxide metabolite, Ro12-5637, and internal standard (Ro11-9900).

This paper describes a modification of this technique[3] for the measurement of moclobemide and its *N*-oxide metabolite in urine that does not require sample extraction and is, therefore, simpler and allows for more rapid sample throughput than the existing method.[3] This technique has been applied to measure the concentrations of moclobemide and Ro12-5637 in urine samples from a moclobemide pharmacokinetic study in healthy Caucasians of known *CYP2C19* genotype.[4]

EXPERIMENTAL

Chemicals and Reagents

Moclobemide (MW 268.75), the metabolites, Ro12-5637 (MW 284.7) and Ro12-8095 (MW 282.7), and the internal standard, Ro11-9900 (internal standard, MW 360.19), were gifts from Roche (Sydney, NSW, Australia). Ro16-3177 was a gift from Dr P. Hackett (Perth, WA, Australia). Methanol and triethylamine were HPLC grade and purchased from BDH (Kilsyth, NSW, Australia).

Potassium phosphate salts and 85% orthophosphoric acid were analytical grade and purchased from Ajax Chemicals (Auburn, NSW, Australia).

All solutions and buffers were prepared in deionised water (MilliQ, Millipore, Bedford, MA, USA).

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Liquid Conditions

The mobile phase, which consisted of methanol-triethylamine-33.5 mM potassium dihydrogen orthophosphate (40:0.1:59.9, v/v/v) adjusted to pH 7.0 using orthophosphoric acid, was pumped through a C18 guard column (5 μm, 2 mm x 2 cm i.d.) (Activon, Selby-Biolab, Sydney, NSW, Australia) and a C6 column (5 μm, 250 × 4.6 mm i.d.) (Spherisorb, Phenomenex, CA, USA) by an LC-10AT pump (Shimadzu Scientific Instruments (Oceania) Pty Ltd, Rydalmere, NSW, Australia) at a flow rate of 1.0 mL/min, which resulted in a back pressure of 130 kg/cm^2.

Samples were injected using an SIL-10AXL Autoinjector (Shimadzu Scientific Instruments Pty Ltd) and analytes were detected using an SPD-10A UV-Vis detector (Shimadzu Scientific Instruments Pty Ltd) set at 240 nm. After 60 samples were injected, the column was washed with 30 mL of methanol-water (25:75, v/v) followed by 30 mL of 100% methanol.

Calibration standards of moclobemide and Ro12-5637 over the concentration range of 0.5–20 and 2–100 μg/mL, respectively, were prepared in drug-free urine and stored at $-20^{\circ}C$ until used.

Sample Preparation

In a 1.7 mL centrifuge tube (Edwards Instrument Co, Narellan, NSW, Australia), 0.4 mL urine was diluted with 0.3 mL of deionised water and 0.1 mL of an aqueous 145 μg/mL Ro11-9900 solution. Samples were vortexed and centrifuged for 10 min at 6800 g and 40 μL of the supernatant was injected onto the column. All samples were prepared in duplicate.

Reproducibility, Accuracy, and Specificity

To determine the reproducibility of the technique, three quality control samples were analysed in duplicate on five separate occasions to assess between-day reproducibility, and six of each sample on a single occasion to assess within-day reproducibility. These quality control samples were 12 h urine samples collected from three individuals following the ingestion of 300 mg of moclobemide.

To assess the accuracy of the assay, moclobemide and Ro12-5637 were added to drug-free urine. The final concentrations of Ro12-5637 and moclobemide ranged from 0 to 100 and 0 to 20 μg/mL, respectively. The relationship between the measured and actual concentrations was assessed using linear regression analysis.

Drug-free urine samples from 24 individuals were tested for potential interference from endogenous peaks at the same retention times as moclobemide, Ro12-5637, and internal standard. Moclobemide metabolites, Ro12-8095 and Ro16-3177, were tested for their ability to interfere with the detection of moclobemide and Ro12-5637.

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Human Study

The Human Research Ethics Committee of Royal North Shore Hospital (Sydney, NSW, Australia) approved this study (HREC Protocol No. 9503.39M). Three healthy, unrelated Caucasian volunteers of known *CYP2C19* genotype[4] ingested a 300 mg dose of moclobemide (Aurorix®, Roche Products Pty Ltd, Sydney, NSW, Australia) (1116 μmol) with 200 mL of water. All urine voided for the next 48 h was collected in four 12 h samples. The urine volume was recorded and aliquots of urine were stored at −20°C pending analysis.

RESULTS AND DISCUSSION

Figure 2 shows a chromatogram of drug-free urine (A) and a 0–12 h urine sample that was collected after the ingestion of 300 mg of moclobemide by a

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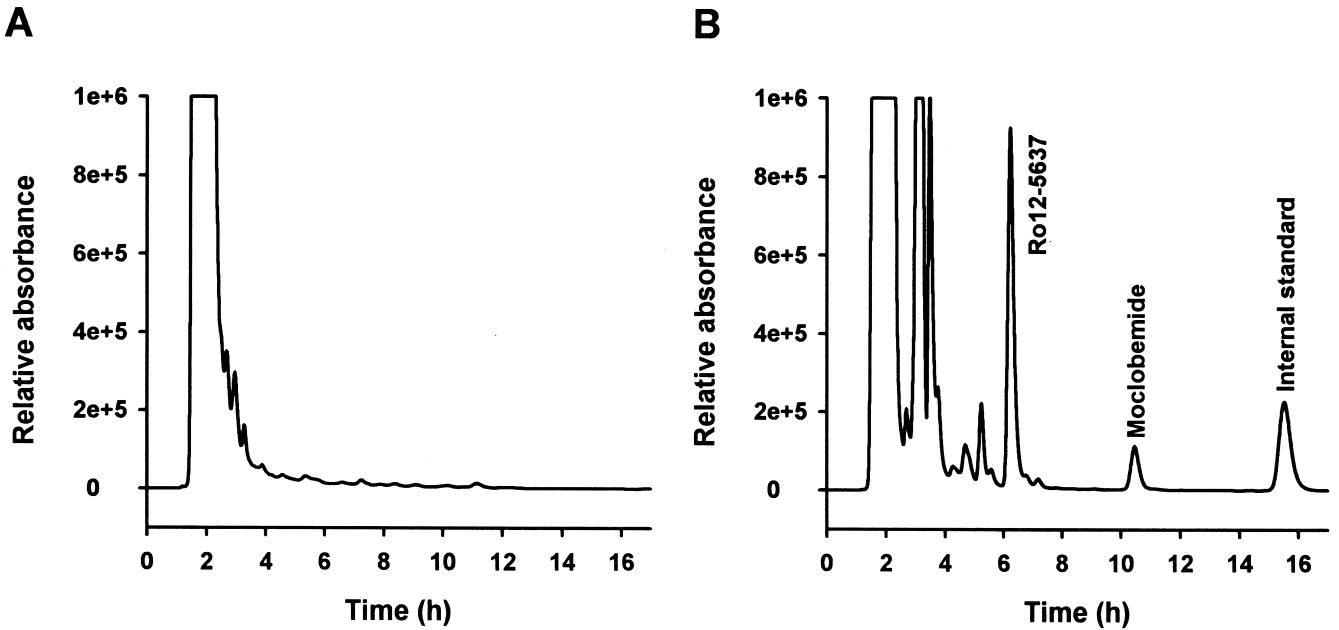

Figure 2. Chromatograms of (A) drug-free urine and a (B) 0–12 h urine sample from a healthy volunteer following a single 300 mg oral dose of moclobemide: [Ro12-5637] = 44.1 μg/mL and [moclobemide] = 7.4 μg/mL. The internal standard is Ro11-9900.

healthy Caucasian (B). Geschke et al.[3] used the UV wavelength of 240 nm for measuring moclobemide and metabolites in plasma and urine. This wavelength was selected for the present method because it corresponds to the maximal UV absorbance of both moclobemide and Ro12-5637. The C6 Spherisorb column employed by Geschke et al.[3] was also used for this technique.

In order to maximise separation of moclobemide and Ro12-5637 from other moclobemide metabolites and endogenous peaks in urine, the mobile phase[3] was modified from acetonitrile-67 mM potassium dihydrogen orthophosphate (30:320, v/v) (pH 3.9) to methanol-triethylamine-33.5 mM potassium dihydrogen orthophosphate (40:0.1:59.9, v/v/v) (pH 7.0).

Changing the pH of the mobile phase changed the elution profile of moclobemide, Ro12-5637 and other metabolites, and moved the potentially interfering peaks into the void and, therefore, separated from the analytes of interest. Triethylamine was added to the mobile phase to improve the peak shape of moclobemide, and methanol replaced acetonitrile as the organic component of the mobile phase because it reduced the baseline noise. The resultant retention times of the metabolites, Ro16-3177, Ro12-5637, Ro12-8095, moclobemide, and the internal standard, Ro11-9900, were 5.1, 6.2, 7.1, 10.2, and 15.3 min, respectively.

Additional metabolite peaks were observed in the chromatograms of post-dose urine but were not identified. They did not interfere with the detection of moclobemide or Ro12-5637. The total run-time was 17 min. No interfering endogenous peaks were observed at the retention times of moclobemide, Ro12-5637 and internal standard in the 24 drug-free urine samples tested.

The within-day and between-day reproducibilities of moclobemide and Ro12-5637 concentrations, measured in 0–12 h urine from three healthy volunteers following the oral ingestion of 300 mg moclobemide, are presented in Table

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Table 1. Within-Day and Between-Day Reproducibilities of the Measurement of Moclobemide and Ro 12-5637 in 0- to 12-h Urine Samples from Three Healthy Volunteers After a 300-mg Oral Moclobemide Dose[a]

Analyte	Subject Number	Within-Day (n = 6)		Between-Day (n = 5)	
		Concentration (μg/mL)	CV (%)	Concentration (μg/mL)	CV (%)
Moclobemide	CA1	1.65 ± 0.02	1.1	1.80 ± 0.07	3.9
	CA13	7.40 ± 0.07	1.0	7.56 ± 0.13	1.8
	CA27	5.29 ± 0.04	0.8	4.86 ± 0.25	5.2
Ro 12-5637	CA1	45.4 ± 0.7	1.5	46.0 ± 1.5	3.2
	CA13	44.1 ± 0.5	1.2	43.7 ± 0.5	1.2
	CA27	87.6 ± 0.8	0.9	82.0 ± 3.3	4.0

[a]Data are presented as mean ± standard deviation.

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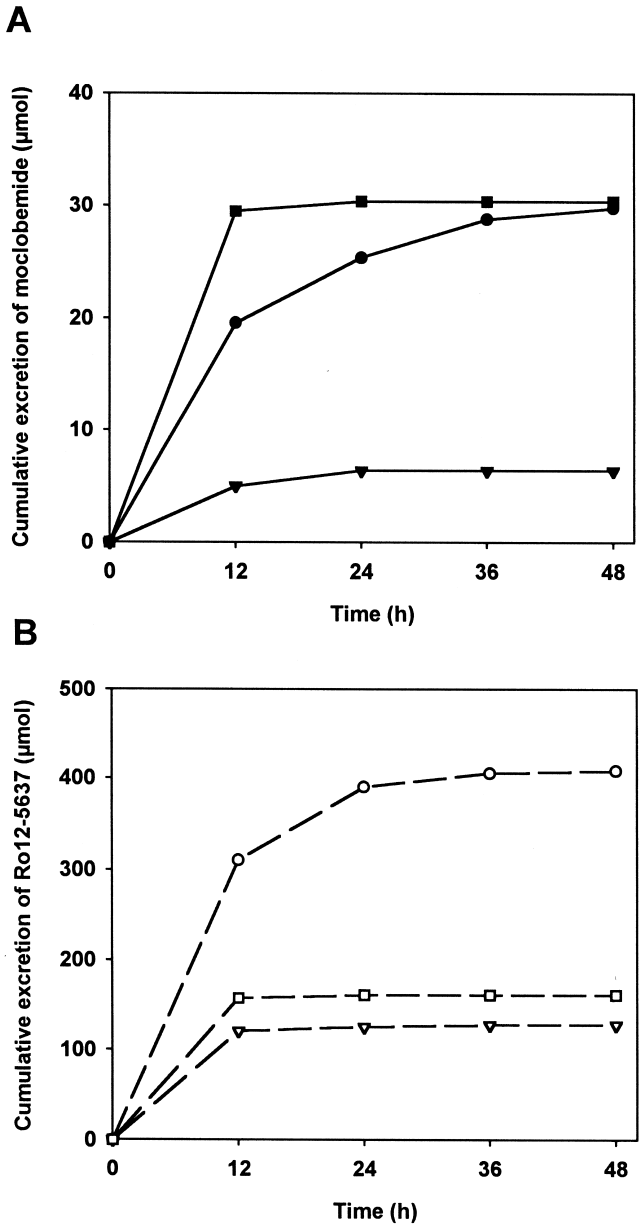

Figure 3. Cumulative excretion of (A) moclobemide and (B) the *N*-oxide metabolite, Ro12-5637, in urine samples from three healthy Caucasians, CA1 (triangle), CA13 (square), and CA27 (circle), collected after ingesting a 300 mg oral dose of moclobemide.

1. The within- and between-day coefficients of variation (C.V.) were low for both moclobemide and Ro12-5637 (<5.1%). The standard curves for both analytes showed good linearity over the concentration ranges studied (Ro12-5637: r^2=0.998, C.V. 0.09%, n=5; moclobemide: r^2=1.000, C.V. 0.06%, n=5).

Typical slopes (n=5) for Ro12-5637 were 0.11 (C.V. 7.9%) and for moclobemide 0.09 (C.V. 9.6%). The accuracy of the measurement of Ro12-5637 and moclobemide was satisfactory. The relationships between the concentration of the analytes added and those measured (n=10) were $y=1.16x-1.09$ (r^2=0.999) for Ro12-5637 and $y=0.96x-0.23$ (r^2=0.999) for moclobemide.

The limits of detection for Ro12-5637 and moclobemide were 0.5 and 0.3 μg/mL, respectively, with signal to noise ratios of 4:1. The limits of quantification of this assay, 2.0 and 0.5 μg/mL, respectively, are higher than those of the previously published technique, 0.5 and 0.05 μg/mL, respectively.[3] However, the sensitivity of the present method was adequate for the measurement of moclobemide and Ro12-5637 in 0 to 12 h urine samples collected from Caucasians (n=11) following a single oral dose of 300 mg moclobemide (Figure 3).

The urinary concentrations of moclobemide and Ro12-5637 in these samples ranged from 1.3 to 7.4 and 23 to 88 μg/mL, respectively. Ro12-5637 could be measured up to 48 h in the urine of 2 individuals (including CA27) who were homozygous for mutant *CYP2C19* alleles (*CYP2C19*2/*2*) and poor metabolisers of the CYP2C19 substrate, proguanil.[4]

In summary, this paper describes a modification of an existing HPLC method for the measurement of Ro12-5637 and moclobemide in urine. This technique does not require sample extraction, is isocratic, and has a total runtime of 17 min. The method is simple and convenient and suitable for the rapid measurement of moclobemide and its *N*-oxide metabolite, Ro12-5637, in urine samples collected, following a single oral 300 mg dose of moclobemide.

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REFERENCES

1. Mayersohn, M.; Guentert, T.W. Clin. Pharmacokinet. **1995***, 29*, 292–332.
2. Gram, L.F.; Guentert, T.W.; Grange, S.; Vistisen, K.; Brosen, K. Clin. Pharmacol. Ther. **1995***, 57*, 670–677.
3. R.; Geschke, Korner, J.; Eggers, H. J. Chromatogr. **1987**, *420*, 111–120.

4. Hoskins, J.M.; Shenfield, G.M.; Gross, A.S. Br. J. Clin. Pharmacol. **1998**, *46*, 499–504.

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Accepted August 8, 2000

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