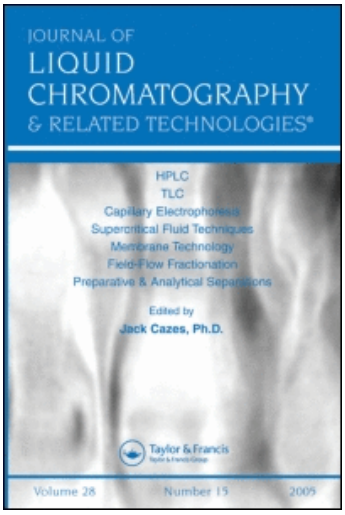

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LIQUID CHROMATOGRAPHIC ASSAY FOR DEXTROMORAMIDE IN HUMAN PLASMA

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ABSTRACT

Dextromoramide is a narcotic analgesic drug which has been said to be clinically useful where rapid onset and short duration of action is required. The present communication describes a modification of previous high performance liquid chromatographic methods for determining plasma dextromoramide concentrations. The method described is sensitive, accurate and precise, with intra-assay CV's of 4.1%, 4.1% and 4.2% and between-assay CV's of 2.9%, 2.4% and 3.9% at concentrations of 10, 100 and 1000 μg/L, respectively. It has a limit of quantitation of 5 μg/L with a chromatographic run time of 8 min. Pharmacokinetic studies in 2 patients given 5mg of dextromoramide intravenously are presented as applications of this method. These studies showed a bi-exponential decay of dextromoramide in plasma over 24h with terminal half-lives of 3.7 and 23.5h which resulted from variability in plasma dextromoramide clearance and distribution volumes.

INTRODUCTION

Dextromoramide tartrate [Palfium®, (+)-1-(3-methyl-4-(4-morpholinyl)-1-oxo-2,2-diphenylbutyl)pyrrolidine] is an older narcotic

analgesic drug [1] described as having a potency equal to or greater than morphine [1,2]. It has a rapid onset of action with a duration similar to morphine [2]. It is eliminated primarily by hepatic metabolism to 2'-hydroxy-dextromoramide metabolite [3,4]. Tolerance and addiction properties have also been described [5]. Hence the clinical application of this drug in this institution has typically been restricted to short-term usage, such as in "break-through" pain in cancer patients treated with morphine, in post-operative pain, etc.

Previous methods for the determination of dextromoramide in plasma have been primarily directed at either the drug-abuse situation [6], or forensic testing [7, 8] or pharmaceutical product testing [FH Faulding & Co Ltd, personal communication], limited clinical pharmacology testing [7], or animal doping analysis [9]. Hence, these methods were not considered completely appropriate for pharmacokinetic studies where greater analytical sensitivity, as well as appropriate precision and accuracy, may be required.

This communication reports a refinement of the previous methods to improve analytical sensitivity, as well as reliability for kinetic studies. By way of example, an application of this method is presented in 2 patient studies where 5 mg of dextromoramide was administered intravenously and blood sampled over the ensuing 24 h.

MATERIALS & METHODS

Stock Solutions

A 1.0 g/L dextromoramide (base concentration) stock solution was prepared by dissolving dextromoramide tartrate (F.H. Faulding & Co Ltd, Salisbury, South Australia) in glass distilled water. This solution was serially diluted to give concentrations of 100, 10 and 1.0 mg/L.

Desipramine HCl (internal standard, Geigy Pharmaceuticals, Lane Cove, NSW, Australia) was prepared in a similar manner to give 1.0 mg/L. The sodium chloride, sodium hydroxide and hydrochloric acid were Univar grade (Ajax Chemicals, Auburn, New South Wales, Australia), and orthophosphoric acid was Analar grade (BDH, Kilsyth, Victoria, Australia).

Plasma Extraction

Each assay was calibrated by adding appropriate volumes of the dextromoramide stock solutions to a series of 1.0 ml dextromoramide-free heparinized plasma aliquots to give final concentrations of 0, 100, 250, 500, 1000, 2000 µg/L. These 1.0 ml calibration standards, as well as quality control samples (described below) and patient specimens were spiked with; 200 µl of 1.0 mg/L of the internal standard, 200 µl of sodium hydroxide (1.0 mol/L) and, following a brief vortex mixing, 8 ml of extracting solvent, 95:5 mixture of cyclohexane (BDH, Hypersolv grade) and iso amyl-alcohol (BDH, Analar grade) in 15 ml disposable borosilicate glass screw-capped tubes. This mixture was shaken by vortex mixing for 1 min, followed by centrifugation at 1000 xg for 10 min. The organic layer was transferred to a 15 ml conical borosilicate glass tube containing 150 µl of 0.05 mol/L HCl. The mixture was again vortex mixed for 60 sec and centrifuged at 1000 xg for 10 min. The aqueous phase was transferred to an autosampler tube and 50 µl injected for chromatographic separation.

Quality control was assessed in each run by analysing aliquots from 3 separate plasma pools which had been previously spiked from a separate dextromoramide stock solution to give concentrations of 10, 100 and 1000 µg/L. Patient blood samples were drawn into lithium heparin blood collection tubes as described below and the plasma fraction separated by centrifugation at 1000 xg for 10 min. Specimens were stored at -20°C prior to analysis.

Chromatography

The mobile phase consisted of a 40:60 (v:v) mixture of acetonitrile (BDH Hypersolv, Far UV grade) and NaCl (0.1 g/L) with H_3PO_4 (0.114 ml/L) (pH=2.9) in glass distilled water. This mixture was filtered (0.2 µm, Millipore, part number GVWP-04700) under vacuum before use, degassed continuously with helium and pumped (model P4000, Spectra Physics Analytical, San Jose, California, USA) at 2.0 ml/min via an autosampler (Spectra Physics, model AS3000) through a 10 µm phenyl column (30 cm x 3.9 mm, part WAT27198, Millipore/Waters, Milford, MA, USA) maintained at 50°C. Compounds separated by this system were quantified by UV detection (Spectra Physics, model UV2000) at a wavelength of 215 nm and range of 0.05 AUFS. The output to a dual-pen chart recorder was plotted at both 10 and 100 mV at a chart speed of 0.25 cm/min. The retention time of the internal standard and dextromoramide peaks were 5.25 and 7.5 min, respectively, with baseline separation. Samples were quantified using the peak height ratio of dextromoramide to internal standard.

Statistical Considerations

The method was validated within a single run by assaying 6 replicates at 10, 100 and 1000 µg/L. Between-run performance was assessed by considering the run-to-run (n=5) reproducibility of these 3 controls, ie., at 10, 100 and 1000 µg/L. The performance of each assay run was controlled by reviewing both the coefficient of variation (CV%) of the concentration-corrected peak height ratio for each calibration standard (ie., the apparent slope of the calibration curve indicated by each calibration standard), as well as by the concentration derived from the calibration curve for each of the 3 quality control samples. Runs were accepted when the CV% of the concentration-corrected calibration standard values was <10% and each of the controls within 10% of their respective target concentrations.

The limit of quantitation was determined by guaging that dextromoramide concentration which could be measured with a CV% of <15%. For samples at this very low concentration the back extraction into HCl was amended to 100 µl and range on the detector attenuated appropriately.

Patient Studies

Ethics Committee support was obtained to invite patients, admitted for various medical conditions, who required narcotic analgesia (other than dextromoramide) as part of their therapy. Inclusion criteria were; age >18 and <80 years, renal or hepatic function less than 50% above upper limit of normal, no known dextromoramide allergy, ability to understand and complete standard consent criteria, adequate venous access, no evidence of anaemia, and where survival was likely to exceed one week . On the day of the study, other analgesic medication was with-held if ethically acceptable and, following the insertion of an indwelling venous catheter, the 5 mg dose of dextromoramide was administered over 5 min into an arm vein. Blood samples were drawn into lithium heparin blood collection tubes, pre-dose, and at 5, 10, 15, 20, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 8 and 24 h post-dose. Samples were then held on ice for up to 30 min before separating the plasma fraction by centrifugation at 1000 xg for 10 min. This plasma was stored at -20°C until assayed.

RESULTS & DISCUSSION

Table 1 shows the accuracy and precision of the method described and suggests that acceptable data were observed both within and between analytical runs for pharmacokinetic purposes. Within-run CV%'s ranged from 4.1 to 4.2%, and between-run ranged from 2.4 to 3.9%. Figure 1 shows a range of chromatograms, including a dextromoramide-spiked calibration standard extract, a

TABLE 1.

Precision and Accuracy Data of the Method Described in the Text.

Dextromoramide concentration	n	Measured concentration (mean ± SD)	Accuracy (%)	C.V. (%)
With-in run:				
10 µg/L	6	10.33 ± 0.42	+3.3	4.1
100 µg/L	6	106.6 ± 4.46	+6.6	4.2
1000 µg/L	6	1063 ± 44.7	+6.3	4.2
Between-run:				
10 µg/L	5	10.4 ± 0.29	+3.6	2.9
100 µg/L	5	102.1 ± 2.42	+2.1	2.4
1000 µg/L	5	979.8 ± 38.0	-2.1	3.9

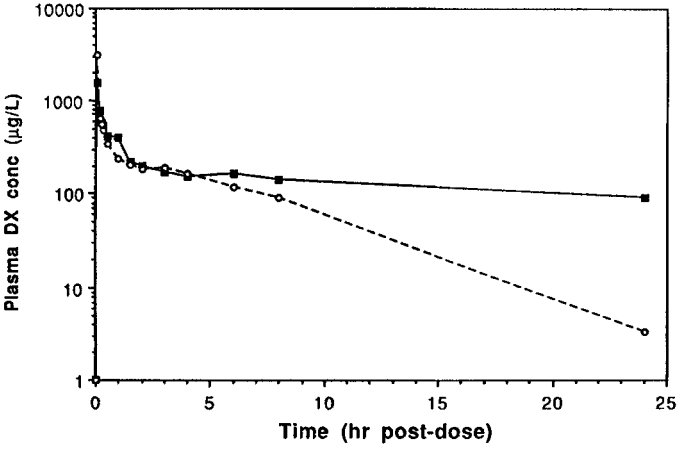

FIGURE 1. This shows chromatograms generated using the method described. Peaks A and B are desipramine (internal standard) and dextromoramide, respectively. The panels represented are; (a) a dextromoramide-spiked calibration standard, (b) dextromoramide-free plasma extract, (c) an extract from a dextromoramide-treated patient, and (d) a dextromoramide QC sample.

dextromoramide-free extract, a patient dextromoramide plasma extract, and a quality control sample plasma extract. The peak retention times were 5.25 and 7.5 min for the internal standard and dextromoramide, respectively, with a chromatographic run-time of approximately 8 min. In pilot studies from a range of other patients taking dextromoramide, as well as the present study patients, no chromatographic interferences have been noted from other concurrent medications (including; amiodarone, dexamethosone, digoxin, enalapril, flunitrazepam, haloperidol, indomethicin, lactulose, metoclopramide, metoprolol, morphine, nifedipine, paracetamol, propantheline bromide, prednisolone, omeprazole, oxycodone, ranitidine, simvastatin, warfarin). The advantage of testing the method using patient specimens was that it considers clinically relevant concentrations as well as potential interfering metabolites of these drugs.

The limit of quantitation for the method described was found to be 5μg/L with a CV of 14%, which represented adequate sensitivity for the proposed kinetic study. One could envisage extending this limit further, if required, by extracting a larger plasma sample volume (eg., 2 ml) and injecting more of the extract for HPLC separation, thereby potentially achieving a limit of around 1-2 μg/L.

Figure 2 presents the plasma dextromoramide concentrations observed in the 2 patient studies. These data, plotted on a semi-logarithmic scale, suggest a bimodal plasma dextromoramide profile following intravenous administration. The initial α-half-lives of 0.26 and 0.32 h were similar in these 2 patients; however, the terminal β-phases had apparent differences in slope, suggesting half-lives of 3.7 and 23.5 h, respectively. This latter difference appeared to result from variability in plasma clearances of 2.2 and 0.77 L/h and apparent distribution volumes of 11.8 and 26.1 L. These preliminary data, which will be pursued in further clinical studies, are consistent with the apparent variability in the trough dextromoramide concentration data of Rop and coworkers [7] who recorded undetectable whole blood

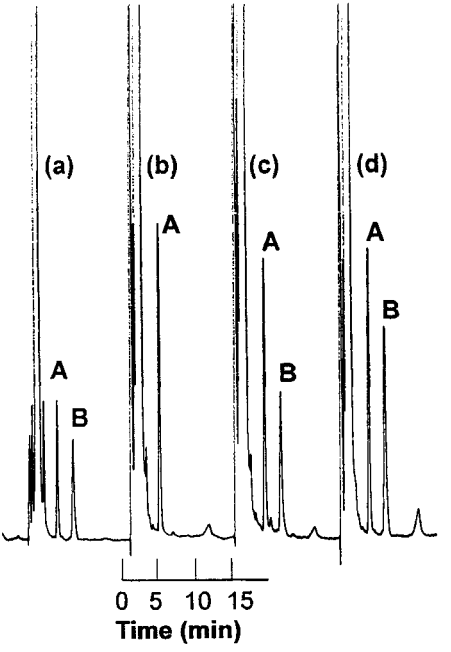

FIGURE 2. This shows the bi-phasic log-linear plasma dextromoramide concentration (μg/L) versus time profile for 2 patients given 5 mg of dextromoramide intravenously over 5 min.

concentrations of dextromoramide in one of 3 cancer patients on chronic intrvenous dextromoramide therapy and could potentially relate to a genetically determined biomodal distribution in metabolism. However, this latter issue remains speculative at this stage but will be persued further in subsequent clinical studies.

In summary, the analytical method presented provides a sensitive and reliable HPLC-UV method which has improved upon the previously described methods for the determination of dextromoramide in human plasma specimens.

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