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Determination of dextromoramide in plasma and whole blood using high-performance liquid chromatography with ultraviolet absorbance detection

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ABSTRACT

Dextromoramide was determined in plasma and whole blood after solid-phase isolation by high-performance liquid chromatography using dextropropoxyphene as internal standard and ultraviolet detection at 215 nm. Owing to its good selectivity, sensitivity and reproducibility, the technique is available for forensic toxicology purposes as well as for clinical pharmacology. The concentrations of dextromoramide were determined in three cancer patients receiving intravenous treatment with one to three 5-mg daily doses. On the fourth day the plasma level was 13.85 ± 3.27 ng ml^{-1} just before the first daily dose and 84.28 ± 12.60 ng ml^{-1} 30 min after dosing. The whole blood concentration, determined in one of the patients, was undetectable just before the dose and was 76 ng ml^{-1} 30 min after dosing.

INTRODUCTION

Dextromoramide, 2,2-diphenyl-3-methyl-4-morpholinobutyrylpyrrolidine (Fig. 1), is a synthetic narcotic analgesic agent (Palfium, Delalande Laboratories). It was prepared by Janssen [1] in 1956 as a drug showing low dependence and greater potency than morphine. However, prolonged therapy results in a tolerance phenomenon and leads to addiction. When misused it may induce a toxic overdosage reaction, as may occur in accidental and suicidal poisoning [2,3].

Some pharmacokinetic parameters of dextromoramide were determined by Pagani *et al.* [4], but the blood concentration–clinical activity relation was not established. In the same study on nine patients, the analgesic action of dextromoramide was generally observed 0.5 h after oral administration. The concentration of the drug in the cerebrospinal fluid measured at 1 h (corre-

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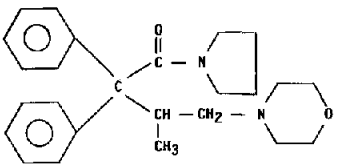
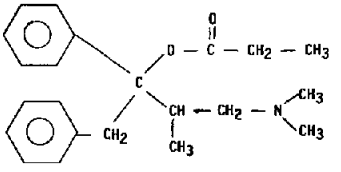


Dextromoramide (Mol. Weight : 392.5)



Dextropropoxyphene (I.S.) (Mol. Weight : 339.5)

Fig. 1. Structures of dextromoramide and dextropropoxyphene (internal standard).

sponding to the peak time in plasma) was not detectable (below the limit of detection, 2 ng ml^{-1}) in all patients, whereas the plasma concentration at 1.9 ± 0.6 h was found to be 106 ± 13 ng ml^{-1}. These findings led to two hypotheses: (1) detectable levels of the drug appear in the cerebrospinal fluid at a later stage and (2) active metabolites that can contribute to the pharmacological effect are produced [4].

The metabolism of dextromoramide is not clear. Temple and Oelschläger [5], working on the forensic detection of the drug, found no unchanged compound in the urine of any animals or human volunteers after dextromoramide bitartrate administration, indicating that the drug is rapidly and completely metabolized. Moreover an N-oxide metabolite, which would be most suitable for their forensic purpose, was also not detected. According to the structure of dextromoramide and the known metabolic processes, Caddy *et al.* [6] suggested that 2′-hydroxydextromoramide is a major metabolite of the drug.

Up until now, no information has been available about the quantitation or the pharmacological activity of any metabolite. But dose–blood level–fatal toxicity relations have been reported for the unchanged compound [3,7].

Several methods using ultraviolet spectrometry [7] or chromatography [gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), thin-layer chromatography (TLC)] [4,7–10] have been described for detecting and even determining dextromoramide in biological samples. A few methods using high-performance liquid chromatography (HPLC) have been published [7,11], but none of these is effective on whole blood.

The present study was undertaken to develop an HPLC procedure for the determination of dextromoramide in both plasma and whole blood after solid-phase isolation using a photodiode-array detector. Owing to lack of information and standard dextromoramide metabolites, our study was limited to the unchanged drug.

EXPERIMENTAL

Reagents and glassware

All reagents were of analytical grade. Ammonium acetate (RPE = Reagent Puro Erba), methanol (RS = Reagente Speciale), acetonitrile (RS for HPLC), diethyl ether (RS), methylene chloride (RS) and Normex buffer solution pH 11 were from Carlo Erba (Milan, Italy). Acetic acid (100%) for HPLC was from Prolabo (Paris, France). Extrelut 3-ml cartridges were from Merck (Nogent sur Marne, France). All glassware was washed with a 3% RBS 25 biodegradable alkaline solution from Biolyon (Dardilly, France), which contains a mixture of anionic and non-ionic detergents, and then rinsed with distilled water and dried before use.

Standard

Dextromoramide bitartrate and dextropropoxyphene hydrochloride (internal standard) were kindly supplied by Delalande and Houdé Laboratories, respectively. Stock solutions were made in methanol at a concentration of 1 mg ml^{-1} (expressed as dextromoramide and dextropropoxyphene) and stored at 4°C. The quality remained good for at least one month. Stock solutions were diluted to 0.1, 0.01 and 0.001 mg ml^{-1} with methanol before use.

Procedure

A 2-ml aliquot of the sample (whole blood or plasma) was pipetted into a 5-ml glass tube. A 20-μl aliquot of dextropropoxyphene (internal

standard) in methanol (0.1 μg μl^{-1}) and 1 ml of Normex buffer solution pH 11 were added. After vortex-mixing for 20 s, the mixture was passed onto a 3-ml Extrelut cartridge. Elution was then carried out with a diethyl ether–methylene chloride mixture (70:30, v/v). The eluate was collected in a 20-ml evaporation glass tube containing 100 μl of 0.01 *M* hydrochloric acid, and evaporated under a stream of nitrogen in a 40°C water bath. The residual acid solution was washed by vortex-mixing with 3 ml of diethyl ether for 20 s on a whirlimixer and centrifuged for 5 min at 2800 *g*. The ether layer was discarded. Then 40 μl of the acid extract were injected into the chromatograph.

Apparatus and chromatographic parameters

Chromatographic analysis was performed on a Waters system consisting of an M590 pump, a WISP 712 automatic sample injection module, a μBondapak C_{18} column (30 cm × 3.9 mm I.D., particle size 10 μm, ambient temperature) connected to a μBondapak C_{18} T.M. guard-pak column (5 mm × 6 mm I.D., two filters, one at either end, held the packing in place and provided a 2-μm filtering capability), a UV–VIS M990 photodiode-array detector, which permits scanning chromatographic and spectral data, and an M380 control station.

The mobile phase consisted of 75 ml of acetonitrile and 25 ml of a mixture of 0.01 *M* ammonium acetate and acetic acid (100:0.1, v/v), at a flow-rate of 1.5 ml min^{-1}.

The effluent was monitored at 215 nm and the wavelengths were scanned between 200 and 350 nm.

Calculation

The ratio between the peak height of the drug and that of the internal standard (I.S.) was calculated and plotted against the concentration of the drug tested after analysis of blank samples, spiked, respectively, with increasing concentrations (20, 50, 100, 500 and 1000 ng ml^{-1}) of dextromoramide and a constant amount of internal standard (2 μg of dextropropoxyphene). Within this concentration range, the relations were linear. The equations of the curves and their correlation coefficients were $y = 0.00355x - 0.0022$ ($r = 0.998$) for whole blood and $y = 0.00385x + 0.0065$ ($r = 0.999$) for the plasma, where y is the ratio of dextromoramide to internal standard and x is the quantity of spiked dextromoramide. For concentrations higher than 1500 ng ml^{-1}, the analyzed sample must be diluted.

RESULTS AND DISCUSSION

Under the conditions described above, the capacity factor (k') of the analyzed compounds was 2.28 for dextromoramide and 4.12 for dextropropoxyphene.

The spectra of dextromoramide and dextropropoxyphene (I.S.) were characterized by a strong absorption below 220 nm, a minimum absorption at 250 nm and an irrelevant absorption over 250 nm. Even at levels up to 1 μg ml^{-1} the drugs would only exhibit a poor non-specific absorption in the 250–270 nm region. The detection wavelength was chosen to be 215 nm considering the ratio of peak height of dextromoramide to noise level in chromatogram.

Fig. 2 shows the chromatograms obtained with blank samples (plasma and whole blood) and samples from cancer patients treated with dextromoramide.

Recovery

Samples of 2 ml (blank whole blood or plasma) spiked with 20–560 ng ml^{-1} dextromoramide ($n = 6$ for each concentration) were extracted as described above. The internal standard was added to the eluate just before evaporating in the presence of 100 μl of 0.01 *M* hydrochloric acid (only for recovery studies). Peak-height ratios (drug/I.S.) of extract were compared with those obtained from injection of the residue of the methanolic solution (a mixture of 20–560 ng of drug and 2 μg of internal standard) after evaporating and dissolving in 100 μl of 0.01 *M* hydrochloric acid. The recoveries were between 77 and 80% (S.D. = 1–5) for whole blood and between 79 and 86% (S.D. = 1–4) for plasma (Table I).

Reproducibility

Reproducibility was tested on a pool of plasma and whole blood also containing 20–560 ng ml^{-1} dextromoramide. Within-day coefficients of var-

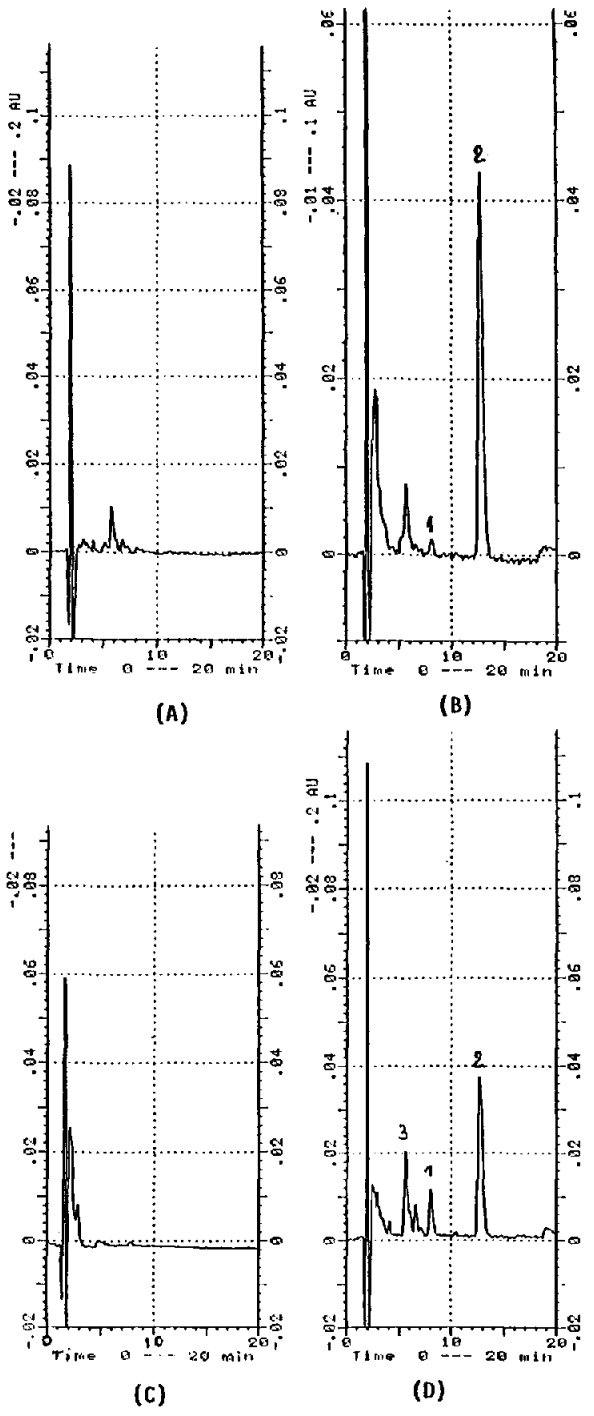


Fig. 2. (A) Chromatogram from blank plasma. (B) Chromatogram from the plasma of a patient treated with dextromoramide bitartrate (Palfium). Peaks: 1 = dextromoramide = (10 ng ml^{-1} = limit of determination); 2 = dextropropoxyphene (I.S.) (2 μg added). (C) Chromatogram from blank whole blood. (D) Chromatogram from whole blood of a patient treated with dextromoramide bitartrate (Palfium) and morphine sulfate. Peaks: 1 = dextromoramide (76 ng ml^{-1}); 2 = dextropropoxyphene (I.S., 2 μg added); 3 = morphine.

iation (C.V.) were between 1.96 and 9.63% for plasma and between 2.10 and 10.50% for whole blood (Table I). Day-to-day coefficients of variation were less than 11% for both plasma and whole blood over a period of one month.

Limit of determination

The limit of determination (signal-to-noise ratio = 2.5) was 10 ± 1 ng ml^{-1} (n = 10) for plasma and 14 ± 3 ng ml^{-1} (n = 10) for whole blood.

Selectivity

Chromatograms of blank plasma and blank whole blood extracts showed no background interferences from endogenous constitutents (Fig. 2A and C). Several drugs were tested for possible interference (Table II). Non-opiate analgesics, such as pentazocine, pethidine, dextropropoxyphene and methadone, were chromatographically resolved from dextromoramide. Therefore dextromoramide cannot be quantitated in samples which also contain dextropropoxyphene, which is used as internal standard. In such a case the use of methadone as internal standard is possible, because it gives the same response. Opiate analgesics, such as morphine (the biological indicator of heroin, monoacetylmorphine or codeine administration), codeine itself, ethylmorphine and buprenorphine, and their antagonists, naloxone and nalorphine, were also separated from dextromoramide. The possible interferences from metabolites of these analgesics (normorphine, norcodeine, norpethidine, nornaloxone, etc.) were not included in this study and thus must be discussed, although none of the unchanged drugs cited above interfered with dextromoramide. In the organism the unchanged drugs, as well as their metabolites, could also be conjugated. Maurer and Pfleger [10] have identified some of these in urine after hydrolysis. The present procedure (without hydrolysis) is limited to the measurement of the unconjugated drugs in blood, where the metabolites, generally present at low levels, are difficult to determine without hydrolysis, and thus do not interfere.

Non-narcotic analgesics, such as acetaminophen, acetylsalicylic acid and glafenine, and other drugs, such as lidocaine, bupivacaine and atro-

TABLE I

REPRODUCIBILITY AND RECOVERY OF DEXTROMORAMIDE

Added (ng ml^{-1})	Within-day ($n=10$)		Day-to-day ($n=8$)		Recovery (mean ± S.D., $n=6$) (%)
	Found (mean ± S.D.) (ng ml^{-1})	C.V. (%)	Found (mean ± S.D.) (ng ml^{-1})	C.V. (%)	
Plasma					
20	21.60 ± 2.08	9.63	20.70 ± 2.18	10.50	79 ± 4
30	29.72 + 1.20	4	31.40 ± 1.58	5.00	81 ± 3
60	59.66 ± 2.27	3.8	58.45 ± 2.77	4.70	80 ± 3
100	101.34 ± 3.17	3.13	101.05 ± 3.30	3.26	86 ± 2
280	284.60 ± 5.57	1.96	285 ± 6.60	2.30	85 ± 1
560	558.10 ± 16.62	2.97	560.5 ± 20.10	3.58	86 ± 1
Whole blood					
20	19.44 ± 2.04	10.50	19.86 ± 2.16	10.87	77 ± 5
30	29.42 ± 1.89	6.43	30.67 ± 2.30	7.47	77 ± 2
60	60.77 ± 2.30	3.80	61.38 ± 2.61	4.24	78 ± 5
100	99.93 ± 3.35	3.35	100.37 + 4.60	4.60	79 ± 2
280	285.00 ± 6.17	2.10	282.50 ± 7.60	2.70	78 ± 2
560	561.40 ± 15.82	2.80	562.50 ± 17.15	3.00	80 ± 1

TABLE II

CAPACITY FACTOR (k'), SELECTIVITY COEFFICIENT (α) AND SIGNAL INTENSITY OF TESTED DRUGS FOR POSSIBLE INTERFERENCE UNDER EXTRACTION AND HPLC CONDITIONS FOR DEXTROMORAMIDE

Drug (600 ng ml^{-1})	k'	α (relative to dextromoramide)	Response at 215 nm
Acetylsalicylic acid	0.04	0.017	None
Caffeine	0.10	0.044	Medium
Acetaminophen	0.22	0.096	Poor
Nalorphine	0.41	0.18	Good
Naloxone	0.43	0.19	Medium
Normorphine	0.60	0.26	Good
Lidocaine	0.82	0.36	Good
Morphine	1.10	0.48	Good
Norcodeine	1.20	0.53	Good
Glafenine	1.64	0.72	Medium
Bupivacaine	1.64	0.72	Good
Codeine	1.64	0.72	Good
Ethylmorphine	1.82	0.80	Good
Dextromoramide	2.28	1	Good
Atropine	2.73	1.20	Medium
Pentazocine	2.90	1.27	Medium
Buprenorphine	2.90	1.27	Good
Nordextropropoxyphene	3.32	1.45	Good
Pethidine	3.55	1.55	Good
Dextropropoxyphene	4.12	1.80	Good
Methadone	5.32	2.33	Good

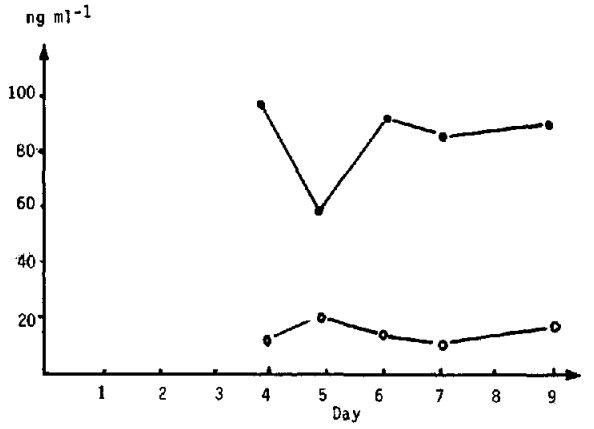


Fig. 3. Dextromoramide plasma concentrations in patient No. 2. (○) C_0; (●) C_1.

pine, that are often given together with dextromoramide in surgery, were also tested. No interferences were observed.

Application

Three cancer patients, two men and a woman, were treated intravenously for a period of at least four days with dextromoramide bitartrate (5 mg one to three times per day). Morphine sulfate (which does not interfere in the analysis) was also given *per os* in case another drug was needed. Between the fourth and ninth days blood samples were drawn from each patient just before the first dose, for determining the initial concentration (C_0) and the concentration 30 min after the same dosing (C_1). Dextromoramide was determined in both plasma and whole blood collected from patient No. 1. It was only measured in plasma from the patients Nos. 2 (Fig. 3) and 3.

The plasma concentrations of dextromoramide were 10 ng ml^{-1} (No. 1), 10–19 ng ml^{-1} (No. 2) and 16 ng ml^{-1} (No. 3) for C_0 (mean ± S.D., 13.85 ± 3.27 ng ml^{-1}), and 83 ng ml^{-1} (Nos. 1 and 3) and 56–98 ng ml^{-1} (No. 2) for C_1 (mean ± S.D., 84.28 ± 12.60 ng ml^{-1}), showing small inter-individual and intra-individual variations and a good dosage adjustment for each patient. The C_0 whole blood concentration in patient No. 1 was undetectable (below the limit of detection), whereas the C_1 concentration was found to be 76 ng ml^{-1}. According to the hematocrit (38%) measured before in patient No. 1, the erythrocyte/plasma ratio of dextromoramide concentration (ng ml^{-1}) could be estimated at 24.5/52.5 = 0.45.

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