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Note

Determination of ketobemidone in plasma by high-performance liquid chromatography with electrochemical detection

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Ketobemidone, 1-[4-(3-hydroxyphenyl)-1-methyl-4-piperidyl]-1-propanone, is a narcotic analgesic used in the treatment of severe pain. It has been detected in urine by thin-layer chromatography (TLC) [1] and quantified in plasma and urine by gas chromatography—mass spectrometry (GC–MS) [2, 3]. However, TLC gives a poor quantitation and GC—MS is a relatively expensive technique not generally available in all clinical laboratories Since ketobemidone, like many other analgesics, is a phenolic substance, it is possible to oxidize it electrochemically The introduction of commercially available electrochemical detectors provides new possibilities for analysing these compounds in biological samples at low concentrations with a sensitivity equal to that obtained with GC—MS This paper reports a high-perforamnce liquid chromatographic (HPLC) method for quantification of ketobemidone in plasma with electrochemical detection

EXPERIMENTAL

Reagents

Ketobemidone hydrochloride was obtained from Lundbeck A/S (Copenhagen, Denmark) The internal standard, 3-(3-hydroxyphenyl)-N-*n*-propylpiperidine (3-PPP), was a gift from Drs A. Carlsson and L Gunne (Uppsala, Sweden) The drugs tested for interference (see Table II) were obtained from the respective manufacturers Acetonitrile, dichloromethane and 1-butanol were of LiChrosolv quality (Merck, Darmstadt, F R G.) Sodium dodecyl sulphate was of electrophoretic purity (Bio-Rad, Richmond, CA, U S A). The water used for the mobile phase was distilled over potassium

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permanganate at basic pH for eliminating traces of organic material. All other chemicals were of analytical grade (Merck).

Chromatography

The HPLC system consisted of a Constametric III pump (LDC, Riviera Beach, FL, U S A) equipped with a pulse dampener (Touzart-Matignon, France), a Rheodyne Model 7125 sample injector with a 50-μl sample loop, an LC-4A amperometric detector (Bioanalytical System, West Lafayette, IN, U.S.A.) equipped with a Model TL-5A glassy carbon working electrode, an Ag/AgCl reference electrode and a Servogor dual-pen recorder (Vienna, Austria). Chromatography was performed on a 150 X 3 9 mm I.D column packed with Nucleosil C_{18} (particle size 5 μm) The mobile phase consisted of 70% 0.11 mol/l acetate—0.044 mol/l citrate buffer (pH 5.2) and 30% acetonitrile—1 mmol/l sodium dodecyl sulphate containing 3 mg/ml EDTA The flow-rate was 1 0 ml/min The detector was set at a potential of +1.06 V For eliminating short-term fluctuations, the outlet of the column was passed through a vessel with water at room temperature

Extraction procedure

The extraction method was, with a few modifications, the same as that used by Edlund [4] for opiate extraction. To 1 0 ml of plasma or serum were added 100 μl of internal standard solution (100 ng/ml 3-PPP) and 1 ml of 1 *M* ammonium chloride buffer (pH 9 5) The solution was mixed and poured on to a glass extraction column packed with 1 g of Extrelut silica (Merck) After 10 min, elution was carried out with 5 ml of dichloromethane containing 5% 1-butanol The organic phase was extracted with 1 ml of 0.05 *M* sulphuric acid and the aqueous phase was separated by centrifugation and transferred into a new tube with a pasteur pipette The aqueous phase was made alkaline with 1 ml of ammonia buffer and extracted with 3 ml of the same solvent as above After centrifugation at 1500 *g* for 10 min, the aqueous phase was aspirated off and the organic phase was carefully transferred to another tube The organic phase was evaporated to dryness under a stream of nitrogen at 50°C and dissolved in 100 μl of the mobile phase. About 50 μl were injected into the liquid chromatograph. The concentration of ketobemidone was calculated from a standard calibration curve based on six known plasma samples

RESULTS AND DISCUSSION

Extraction

Ketobemidone is an ampholytic compound and the optimal pH for extraction is between 9 and 10 [2] The use of a solid extraction method in the first step avoids emulsions and gives a good recovery for morphine [4]. The recovery of ketobemidone from a water solution was found to be quantitative for the solid extraction step However, for plasma a third extraction step was necessary to obtain a sample clean enough for analysis. The total recovery of ketobemidone added to plasma was ca. 80%

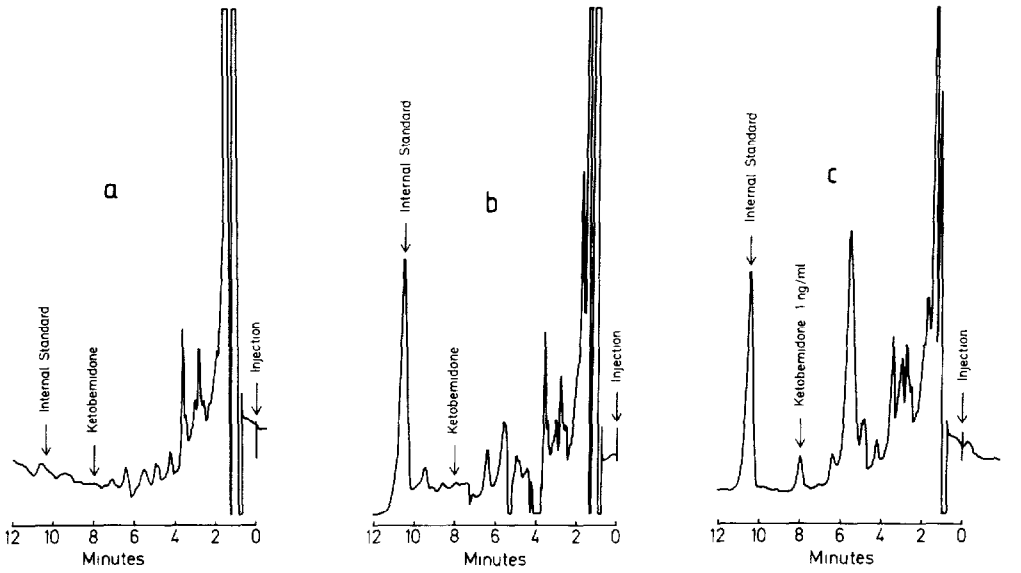

Fig. 1 Chromatograms of plasma extracts of blank plasma (a), plasma containing only the internal standard (b) and plasma containing ketobemidone (1 ng/ml) and the internal standard (c)

Chromatography

The use of reversed-phase ion-pair HPLC has been described earlier for separation of related opium alkaloids [5—7] The use of a combination of a high concentration of acetonitrile and sodium dodecyl sulphate as the ion-pair reagent provides a good separation of ketobemidone from both endogenous compounds and the internal standard (Fig. 1). A relatively high potential (+1.06 V) was needed for the oxidation of ketobemidone. This is in good agreement with oxidation potentials used in analysis of other analgesics with a non-substituted phenolic group [8].

Standard curves and sensitivity

The plasma standard curves for ketobemidone were linear, both in the range 1—500 ng/ml (r = 0 99) and 1—50 ng/ml (r = 0.99). The sensitivity was ca. 0.5 ng/ml, which is the same as that obtained with GC—MS [2]

HPLC compared to GC—MS

Twelve patient samples were analysed with both HPLC and GC—MS The results (Fig. 2) show a good agreement between the two methods.

Precision

Ketobemidone was added to plasma in known amounts and the precision of the method was tested. Ten samples containing 1 ng/ml ketobemidone and ten samples containing 5 ng/ml ketobemidone were analysed. The results (Table I) show a good precision at both levels for the method.

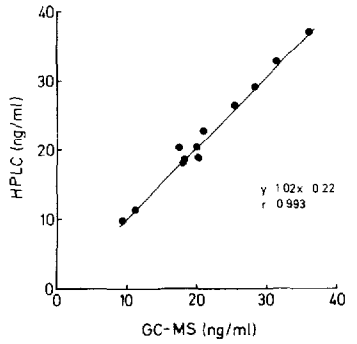

Fig 2 Comparison between plasma samples from one patient obtained at different times analysed with HPLC or GC—MS

TABLE I

PRECISION OF THE METHOD

Ten samples were analysed with a known amount of ketobemidone added to drug-free plasma

Concentration added (ng/ml)	Concentration found (mean ± S D) (ng/ml)	Coefficient of variation (%)	Relative variance (%)
1	1 2 ± 0 1	0 1	11 0
5	5 1 ± 0 3	0 1	5 2

TABLE II

RETENTION TIMES FOR VARIOUS ANALGESICS

Drug	Retention time (min)
Ketobemidone	7 70
3-PPP (internal standard)	10 30
Salicylic acid	0 72
Acetylsalicylic acid	0 80
Acetaminophen (paracetamol)	1 52
Phenazone	2 02
Morphine	2 60
Codeine	4 18
Naproxen	5 25
Pentazocine	5 83
Buprenorfin	21 38
Pethidine (meperidine)	24 40
Methadone	Not detected
Dextropropoxyphene	Not detected
N,N-Dimethyl-3,3-diphenyl-1-methylallylamine*	Not detected

*The spasmolytic compound included in the ketobemidone product Ketogin

Interferences

Other analgesics and N,N-dimethyl-3,3-diphenyl-1-methylallylamine, the spasmolytic compound included in the ketobemidone product Ketogin®, were tested for possible interference. None of the compounds were found to interfere (Table II).

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