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Note

Mass fragmentographic method for the determination of ketobemidone in plasma

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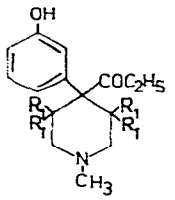
Ketobemidone (Fig. 1), a narcotic analgesic drug, has been in clinical use for more than 25 years in treatment of severe pain[1]. It has been detected in urine using thin-layer chromatography[2,3]. In spite of its widespread use, no reliable method has been described for the quantitative determination of the drug in biological samples. Ketobemidone has been included as internal standard in the determination of pentazocine by electron capture gas chromatography (GC–ECD) after extractive alkylation[4]. However, this method has a tedious work-up procedure and was also disturbed by adsorption losses of the amines in the gas chromatographic system.



Ketobemidone $R_1 = H$

[$^2H_4$]Ketobemidone $R_1 = {}^2H$

Fig. 1. Structure of ketobemidone and [$^2H_4$]ketobemidone.

Morphine, also an aminophenol, has been determined by gas chromatography with flame ionization detection[5,6] or by mass fragmentography[7] after silylation. A sensitive method using GC–ECD after pentafluoropropionylation has also been reported[8].

In the present method, acetylation of ketobemidone is preferred after selective purification of the plasma extract. Determination was made by mass fragmentography.

EXPERIMENTAL

Reagents and chemicals

Ketobemidone, as the chloride salt, was supplied by A/G Lundbeck, Copenhagen,

Denmark. [$^2H_4$] Ketobemidone was synthesized as described previously[12] and the chloride salt was diluted to 490 ng/ml with water. Acetic anhydride and triethylamine were purchased from E. Merck, Darmstadt, G.F.R., and were used in 1:1 proportions in the derivatization of ketobemidone.

The organic solvents used, dichloromethane, 2-butanol, chloroform and toluene, were obtained from E. Merck and used without further purification. Phosphate and carbonate buffers, $\mu = 0.1$, were used. In the analysis of plasma samples a 1 *M* carbonate buffer, pH = 9.6, was used.

Gas chromatography–mass spectrometry (GC–MS)

An LKB 2091 gas chromatograph–mass spectrometer was used and operated at an ionization energy of 35 eV. The sample was introduced by the solventless injection technique with a moving needle and the separation performed at 235° in an capillary column (18 m × 0.3 mm I.D.) filled with SP 1000 (Ultrasep®; OY Separation Research, Turku, Finland). The injector temperature was 245°. Helium was used as carrier gas with a flow-rate of 1.5 ml/min. The mass spectrometer was adjusted to record the ions $m/e = 289$ (M^+) for O-acetylketobemidone (Fig. 2) and $m/e = 293$ (M^+) for the internal standard.

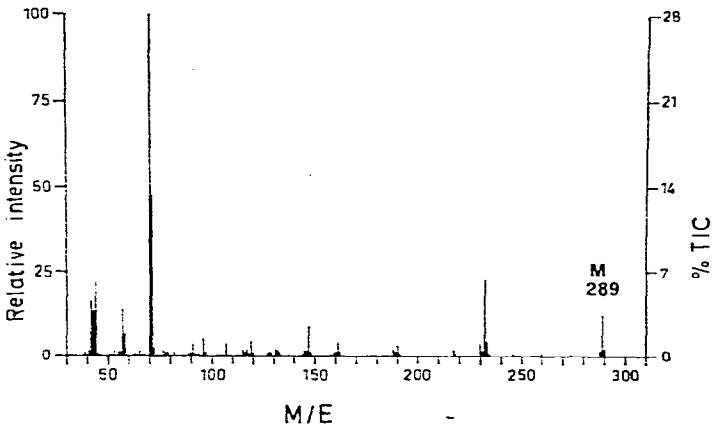


Fig. 2. 70 eV mass spectrum of O-acetylketobemidone.

Evaluation of extraction and acetylation conditions of ketobemidone

The partition studies were done using equal volumes of water-shaken organic solvent and aqueous phosphate or carbonate buffers ($\mu = 0.1$) and an equilibrium time of 30 min. After separation of the phases the concentration of ketobemidone in the organic phase was determined by GC with flame ionization detection.

The acetylation was carried out by addition of acetic anhydride and in some cases equal parts of acetic anhydride and triethylamine to a known amount of ketobemidone (200 ng). The reaction was quenched at different time intervals with aqueous 1 *M* carbonate buffers, pH = 9.6. The evaluation was done by mass fragmentography using an internal standard, added after removal of the aqueous phase.

Determination of plasma ketobemidone

To a 2.0-ml plasma sample, 0.1 ml of internal standard solution was added together with 1.0 ml of 1 *M* carbonate buffer, pH = 9.6, and 6.0 ml of a mixture of toluene and 2-butanol (9:1). This mixture was shaken for 15 min and centrifuged at 500 *g* for 10 min. The organic phase was transferred to a tube containing 1.0 ml of 0.05 *M* sulphuric acid. The mixture was shaken for 10 min and centrifuged. The organic phase was discarded and 1.0 ml of 1 *M* carbonate buffer, pH = 9.6, and 5.0 ml of dichloromethane–2-butanol (9:1) were added. The mixture was shaken for 10 min and centrifuged. The organic phase was transferred to another tube and the solvent was evaporated to dryness at 50° in a stream of nitrogen. Acetic anhydride and triethylamine in equal parts (75 μl) were added to the residue. After 10 min at 50°, excess of reagent was evaporated and the residue dissolved in 50 μl of chloroform. A 2-μl aliquot was taken for analysis by mass fragmentography.

A standard curve (2–200 ng/ml) was prepared (Fig. 3) by adding known amounts of ketobemidone to plasma and analysing the solutions according to the above procedure.

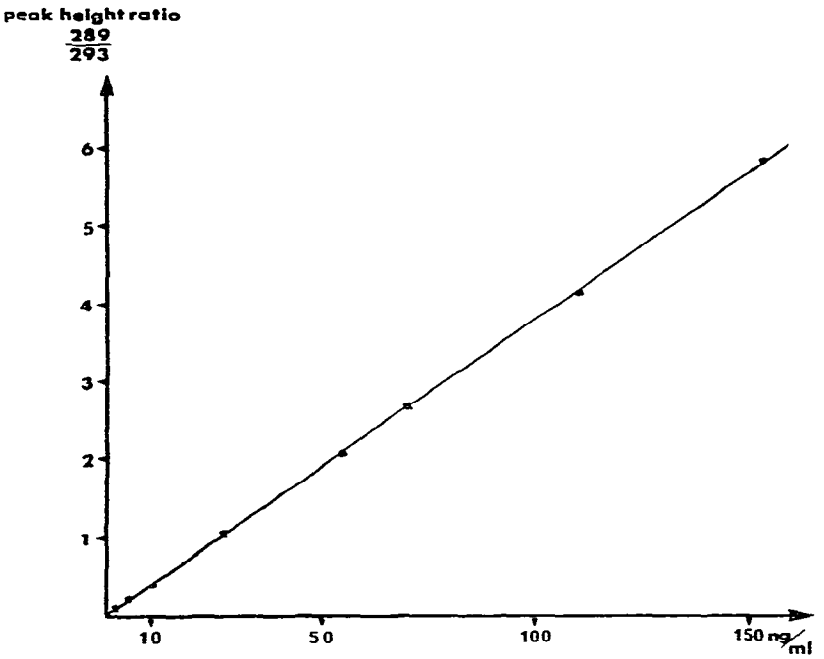


Fig. 3. Standard curve for ketobemidone with [$^2H_4$]ketobemidone as internal standard. Peak height ratio of ketobemidone to [$^2H_4$]ketobemidone is plotted *versus* the concentration of ketobemidone in plasma.

RESULTS AND DISCUSSION

Extraction conditions

Ketobemidone has both a basic amino group and an acidic phenolic group.

This means that careful studies on the extraction conditions should be undertaken for choice of optimal pH and extraction medium.

Fig. 4 shows the partition ratio log D of ketobemidone in the pH range 6–12 using three organic solvents. About 50% of ketobemidone was extracted with toluene from the aqueous phase at pH 9–10. A higher degree of extraction (80%) was achieved with dichloromethane as organic solvent.

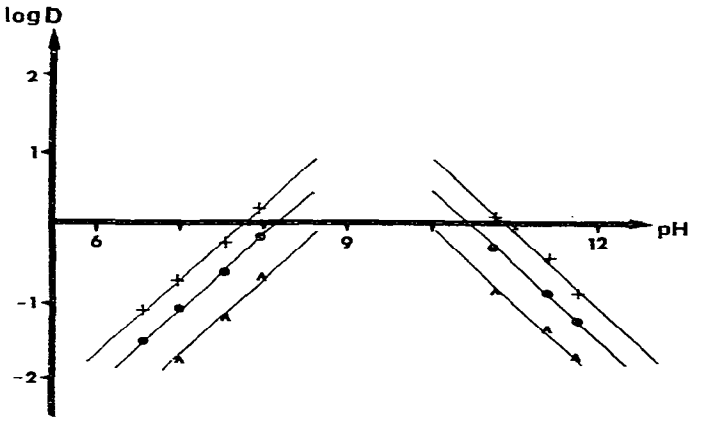


Fig. 4. Distribution of ketobemidone between different organic solvents and aqueous solution at various pH values. Solvents: +, dichloromethane; ∧, toluene; ●, toluene–2-butanol (9:1).

Addition of a solvent with proton-donating properties, *e.g.*, an alcohol, further increased the extraction degree of ketobemidone from toluene, probably owing to adduct formation (*cf.* ref., 9). A mixture of toluene and 2-butanol was used as extraction medium for ketobemidone from plasma samples. This mixture will co-extract less endogenous material and is less prone to form emulsions than dichlorometane. The extraction of ketobemidone was found to be quantitative with toluene–2-butanol (9:1) using a stronger buffer e.g., 1 *M* carbonate buffer, pH = 9.6. The addition of 2-butanol will also prevent adsorption losses of ketobemidone to glass vessels in the work-up procedure.

To enhance the sensitivity of the method, the plasma extract was purified. From Fig. 4, it is obvious that the extraction of the compound into the acidic aqueous phase was quantitative.

Acetylation of ketobemidone

The perfluoroacyl, particularly the trifluoroacetyl, derivatives of phenols have been reported to be susceptible to hydrolysis[10]. Therefore, acetylation of ketobemidone was preferred. The acetyl derivative was prepared in the presence of triethylamine, the favourable effect of which has been emphasized[10]. A reaction time of 15 min was sufficient in the presence of the base, while more than 40 min were required when acetic anhydride was used alone (Fig. 5). The acetyl derivative was stable in the reaction medium for more than 2 h.

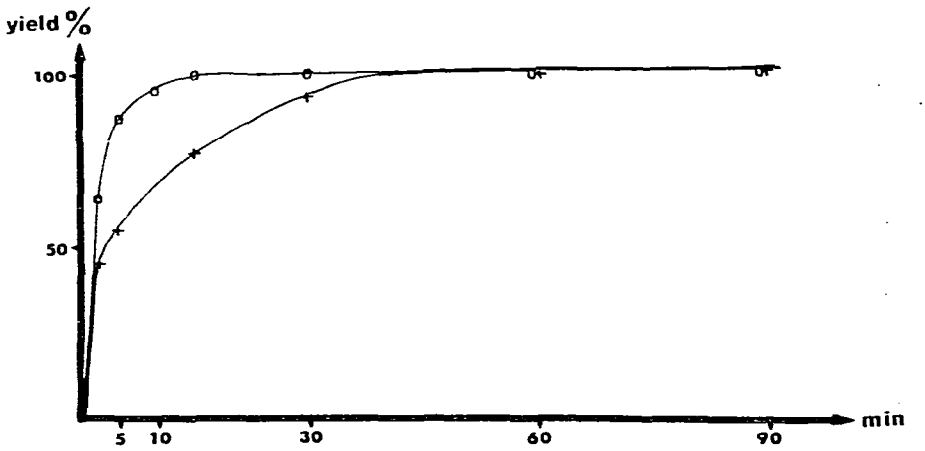


Fig. 5. Influence of triethylamine on acetylation rate. Reactants: ketobemidone, 200 ng; acetic anhydride, 35 μl. +, No triethylamine; ○, 35 μl triethylamine. Internal standard: acetylated *p*-hydroxypethidine in dichloromethane (200 ng/ml). Temperature: 50°. Sample: 0.5 ml carbonate buffer, pH 9.6 (μ = 1), and 1.00 ml of internal standard solution was added to the reaction mixture which was immediately shaken for 1 min; after centrifugation and evaporation, the residue was dissolved in 50 μl chloroform and a 2-μl aliquot was injected. Detector: mass fragmentograph.

Excess of reagent can be removed by aqueous buffer or by evaporation. To get a small final volume, evaporation of the reagent excess was used in this method. In chloroform, O-acetylketobemidone was found stable for several days.

Gas chromatographic and mass spectrometric conditions

In the early part of the study, O-acetylketobemidone was gas chromatographed on packed GC columns. Owing to the polar character of the amino grouping, some adsorption losses could be observed. To minimize adsorption and to enhance selectivity and sensitivity, separation on capillary columns was used in the analysis of low concentrations of ketobemidone.

By focusing the mass spectrometer on the molecular ions of both O-acetylketobemidone and the internal standard, high selectivity and high sensitivity were achieved.

Selectivity of the method

In the Scandinavian countries, ketobemidone is only available as a combination with an antispasmodic agent, N,N-dimethyl-3,3-diphenyl-1-methyl-allylamine (Ketogin®). Although the antispasmodic agent was extracted simultaneously in the method it did not interfere as it has a shorter retention time under the GC conditions used.

Preliminary results on the metabolism of ketobemidone show that the compound is to a large extent eliminated from the body as conjugated metabolites. Neither these metabolites nor an N-demethyl metabolite would cause interference in the method.

Sensitivity, recovery and precision

Ketobemidone could be detected down to 0.5 ng/ml in plasma and quantita-

tive determinations could be made to 2 ng/ml with acceptable precision. The relative standard deviation of the method at the 5 ng/ml level was 4.0% ($n = 10$). The absolute recovery of the whole method, using aliquots in all steps, was 91%.

The method is currently in use for pharmacokinetic studies on ketobemidone after intravenous and oral administration[11].

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