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Note

Determination of ketoprofen in plasma by extractive methylation and electron-capture gas chromatography

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Ketoprofen (2-(3-benzoylphenyl)propionic acid)[1] is one of several recently introduced anti-inflammatory agents based on a phenylpropionic acid structure. Previously described methods for the analysis of ketoprofen are based on colorimetry[2], polarography[2], high-performance liquid chromatography (HPLC)[3,4], gas–liquid chromatography (GLC)[2,3,5] and thin-layer chromatography combined with GLC[6] or with UV spectrophotometry[7]. Except for the recently described HPLC method[4], these techniques are complicated or lack sensitivity.

In 1971, Ehrsson[8] introduced extractive alkylation for the derivatization of acidic compounds prior to quantification by GLC. Thanks to its simplicity, this technique has been applied to bioanalysis of a great number of drugs[9–14]. In the present study, this derivatization procedure, combined with GLC with electron-capture detection, has been utilized for the determination of ketoprofen in plasma.

EXPERIMENTAL

Reagents and chemicals

Ketoprofen and the internal standard 2-(4-benzoylphenyl)butyric acid (19,115 R.P.) were supplied by Bayer (Leverkusen, G.F.R.) and Rhône-Poulenc (Paris, France), respectively. Stock solutions were prepared in 0.1 *M* sodium phosphate buffer (pH 7.3).

A 0.5 *M* solution of tetrabutylammonium hydrogen sulphate (Labkemi, Gothenburg, Sweden) was neutralized with 1 *M* sodium hydroxide. After washing with equal volumes of methylene chloride (five times) and *n*-heptane (twice), the solution was diluted to 0.2 *M* with 66 m*M* sodium phosphate buffer (pH 7.3, μ = 0.1).

Methylene chloride (6044; Merck, Darmstadt, G.F.R.), iodomethane (6064; Merck), toluene (8325; Merck) and silver sulphate (1509; Merck) were used without further purification.

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Instrumentation

Gas chromatography. A Varian 3700 equipped with a ^{63}Ni electron-capture detector was used. The glass column (150 × 0.2 cm I.D.) was packed with 3% OV-17 on Gas-Chrom Q, 80–100 mesh. The oven temperature was set at 225°. The injector and detector temperatures were kept at 250° and 240°, respectively. Nitrogen (30 ml/min) was used as carrier gas.

Mass spectrometry (MS). Mass spectra were obtained on a JEOL JMS D-300 instrument, which was linked via a glass jet separator to an ANTEC gas chromatograph equipped with a glass column (100 × 0.2 cm I.D.) packed with 3% OV-1 on Gas Chrom Q, 80–100 mesh. The flow-rate of the helium carrier gas was 50 ml/min, and the temperature of the column oven was set at 230°. MS conditions were: source 200°; separator 200°; ionization energy 70 eV; ionization current 300 μA. Chemical ionization spectra were run under the same conditions except that the ionization energy was 200 eV. Isobutane was used as a reactant gas. The source pressure was approximately 30 Pa.

Methods

The reaction time was determined by mixing plasma samples (400 μl), containing 31.2 μM ketoprofen and 58.4 μM internal standard, with 0.4 ml of tetrabutylammonium hydroxide (0.2 *M*) and 3.0 ml of 1.1 *M* iodomethane in methylene chloride at room temperature. At different times (5, 10, 20, 35, 50, 75, 90, 120 and 180 min), aliquots of 500 μl were withdrawn and mixed with 0.5 ml phosphoric acid (0.1 *M*) in order to quench the reaction.

Aliquots of the organic phase (300 μl) were separated from the aqueous solution and evaporated to dryness. The residue was then suspended in 300 μl of toluene and the solution washed with 1 ml of aqueous saturated silver sulphate for 10 min. A 2-μl volume of the toluene phase was injected into the gas chromatograph.

The determination of ketoprofen in plasma was carried out as follows. 400 μl of the plasma sample, 100 μl of internal standard solution (217.9–21.8 μM) and 0.4 ml of 0.2 *M* tetrabutylammonium hydroxide solution were mixed (GFL rotator, 60 rpm) with 3 ml of 1.1 *M* iodomethane in methylene chloride in a 10-ml test tube for 70 min at room temperature. After centrifugation, *ca.* 2 ml of the organic phase was transferred to a new test tube and evaporated to dryness at 50°. The residue was suspended in 1 ml of toluene and washed with 3 ml of saturated aqueous silver sulphate solution for 10 min[15]. The organic phase was collected and 1–3 μl were injected into the gas chromatograph. A standard graph was prepared by treating known amounts of ketoprofen in plasma according to this procedure.

RESULTS AND DISCUSSION

Evaluation of reaction conditions

The methylation of ketoprofen was complete after *ca.* 20 min at room temperature. Probably owing to steric hindrance, the methylation of the internal standard proceeded at a lower rate, being complete after *ca.* 60 min. The methyl esters of ketoprofen and internal standard were stable for at least 3 h under the reaction con-

ditions. Consequently, a reaction time of 70 min is more than sufficient for securing complete alkylation and a constant ratio between peak heights.

The toluene solutions of the methyl esters could be stored for at least 24 h at 4° without affecting the chromatograms. A typical chromatogram is shown in Fig. 1.

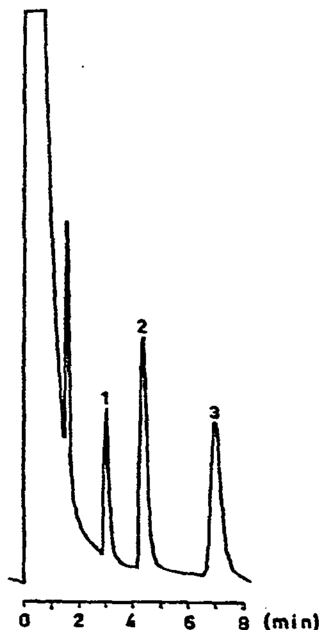

Fig. 1. Gas chromatogram from analysis of ketoprofen (31.2 μM) in plasma. Peaks: 1 = methyl ketoprofen; 2 = the methyl ester of internal standard; 3 = dibutyl phthalate.

Identification of the derivatives

The methyl esters of ketoprofen and the internal standard were identified by electron impact MS by continuously monitoring the molecular ion peaks (m/e 268 and 282, respectively). The typical benzoyl cation fragment (m/e 105) was found in both these spectra. To characterize the fractions further, chemical ionization spectra were recorded on a derivatized plasma extract. The expected peaks ($M + 1 = 269$ and $M + 1 = 283$, respectively) were found in spectra with the same retention times as those in the electron impact spectra.

The compound of the third peak was identified by electron impact MS as dibutyl phthalate giving a molecular ion peak at $m/e = 279$ ($M + 1$) and a characteristic base peak at $m/e = 149$.

Standard graph

A linear relationship between the peak height ratios and the ketoprofen concentration in the interval 0–50 μM was found ($r = 0.9997$, six points).

Electron-capture response

Benzophenones are known to exhibit a good electron-capture response[16]. This was also seen with ketoprofen as well as with the internal standard. The two com-

pounds displayed minimal detectable amounts[17] of $6 \cdot 10^{-16}$ mole/sec at a detector temperature of 240°.

Precision, sensitivity and selectivity

The precision of the method was tested by analysing eight times the same plasma sample with a known ketoprofen concentration (15.57 μM). A mean value equal to 15.50 $\pm$ 0.16 μM was found, which corresponds to a relative standard deviation of 1.0%.

Ketoprofen has been detected in plasma at concentrations down to 0.5 μM with this method.

The assay has been shown to be free from interference from such drugs as salicylates, ibuprofen, naproxene and chloroquine. However, probenecid interferes with the assay, yielding a peak at the same position as the methyl ester of ketoprofen.

CONCLUSIONS

Extractive methylation of ketoprofen and subsequent GLC with electron-capture detection is a simple, sensitive and selective technique with highly satisfactory precision for the determination of ketoprofen in plasma.

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