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Quantitative gas chromatographic determination of clofibrinic acid in plasma

Clofibrate (Atromid-S), or 2-(4-chlorophenoxy)-2-methylpropionic acid ethyl ester, is used chronically to lower the cholesterol and triglyceride contents of human blood plasma (Oliver, 1971; Havel & Kane, 1973) and is rapidly and quantitatively hydrolysed *in vivo* to its corresponding acid. To enable an evaluation of therapeutic plasma concentrations and pharmacokinetics of clofibrinic acid it was necessary to develop a selective method for its quantitative determination in blood plasma. In previous methods (Tolman, Tepperman & Tepperman, 1970; Öster & Örtengren, personal communication) ultraviolet measurements were made in which interference from metabolites could not be excluded.

Reagents. The internal standard, 2-(4-chloro-3-methylphenoxy)-2-methylpropionic acid was synthesized at the Department of Organic Chemistry, Astra Research and Development Laboratories.

Internal standard solution: 9 μg ml^{-1} toluene. Diazomethane was prepared from nitrosomethylurea (Arndt, 1943).

Chromatography. A Varian 1200 gas chromatograph equipped with a flame ionization detector was used. The column was a 6 ft 3 mm i.d. silanized glass column packed with OV-17 (3% on Gas-Chrom Q; 80/100 mesh). Temperatures were 131° in the column, 232° in the injector and 240° in the detector. The nitrogen flow rate was 40 ml min^{-1} and the hydrogen and oxygen flow rates 20 and 200 ml min^{-1} respectively. The retention times were 4·3 min for clofibrinic acid methyl ester and 6·7 min for the internal standard methyl ester.

Partition coefficients. Partition coefficients were determined according to the principles described by Persson & Schill (1966) using tritium labelled clofibrinic acid (24 μCi mg^{-1}). The partition coefficients for methylene chloride and toluene were determined with equal volumes of organic phase and phosphate buffers ($\mu = 0{\cdot}1$) with pH ranging from 5·2 to 6·3. The results show that for clofibrinic acid the log $k_{d(HA)} \times K^1_{HA}$ value in methylene chloride was $-5{\cdot}5$ and in toluene 5·0. It could thus be concluded that log $k_{d(HA)}$ is 1·6 for methylene chloride and 1·1 for toluene assuming a pK^1_{HA} of 3·9 (estimated by potentiometric titration of the sodium salt of clofibrinic acid).

Procedure. To blood plasma (200 μl) is added H_3PO_4 2M (50 μl) and internal standard solution (0·50 ml). After shaking (10 min) the organic phase is transferred to a 3 ml tapered glass tube and the plasma is re-extracted with 0·5 ml toluene. Na_2HPO_4 0·5M (0·5 ml) is added to the combined organic phases and the mixture

shaken for 10 min. The organic phase is removed and the aqueous phase is made acidic with H_3PO_4 5M (50 μl). CH_2Cl_2 (100 μl) is added and the mixture is extracted on a whirl-mixer for 30 s. After removal of the aqueous phase, diazomethane in ether (50 μl) is added and the solution is evaporated under nitrogen to about 1/5 of its volume. One μl is analysed by gas chromatography.

Standard deviation. The standard deviation was determined by subjecting 5 plasma samples at each of three different concentrations (2·5, 10·1 and 50·5 μg ml^{-1}) to the procedure described and for these the standard deviation % was respectively 4·8, 4·8, 4·9.

The values of the partition coefficients for clofibrinic acid indicate that a single extraction is not sufficient to achieve a quantitative extraction as acid into toluene. However, a second extraction will increase the recovery to the 98% level.

The re-extraction to phosphate buffer was carried out to remove non-protolytic lipophilic compounds and to achieve a selective extraction from acids with higher $k_{d(HA)}$. Since the volume of the aqueous phase is kept low, the loss of clofibrinic acid in the final extraction step can be limited to 10%. The recovery of the internal standard, however, is quantitative due to a higher log $k_{d(HA)}$ according to the studies by Gustavii (1967).

Blank plasma samples show no peaks interfering with the clofibrinic acid or internal standard methyl ester peaks.

The method is limited to determination of plasma concentrations down to 1 μg ml^{-1} due to the concentration chosen for the internal standard.

Plasma concentrations of clofibrinic acid during chronic treatment are reported to be *ca* 80 μg ml^{-1}.

The sensitivity of the method could, if necessary, be increased by using a lower concentration of the internal standard and/or by making a further evaporation of the final solution before subjecting it to gas chromatographic analysis.

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During the preparation of this paper, Knüchel & Ochs reported a method for the quantitative determination of *p*-chlorophenoxyisobutyric acid in plasma [*Arzneimittel-Forsch.* (Drug Res.), **24**, 576–578 (1974)]. When determining fatty acid methyl esters they found an interfering peak which originated from clofibrate and could be quantitatively estimated. The present method, however, has the advantage of including a selective extraction and no peaks appear after the internal standard in the gas chromatograms.

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