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Gas chromatographic-mass spectrometric identification and determination of residual by-products in clofibrate preparations

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Three products of clofibrate (ethyl-2-(4-chlorphenoxy)-2-methylpropionate), which are used in the treatment of hyperlipidemia, can be purchased in Sweden. In a clinical study, the effects of two clofibrate products on plasma lipid-concentration were compared (Olsson Orö & Rössner, 1974). The results revealed that the two products had the same effect against hyperlipidemia and no difference in the frequency of side-effects could be noticed. In an earlier study at this laboratory the three clofibrate preparations were analysed by gas chromatography (Mårde & Ryhage, 1975). The total amount of impurities in each product did not exceed the maximum accepted concentration of 1·5 ‰ (Pharmacopoea Nordica, 1973). Impurities in clofibrate have recently been studied by gas chromatography-mass spectrometry (Diding, Sandström & others, 1976). Three main impurities were found, the methyl ester analogue of clofibrate its deschloro analogue and the dichloro analogue.

We have used gas chromatography-mass spectrometry (g.c.-m.s.) for identifying other impurities in clofibrate preparations.

Samples of three drug preparations (I, II and III) from three manufacturers were obtained by dissolving 0·5 ml of capsule-content in 0·5 ml chloroform. 5 μl of the sample was injected into the combined gas chromatograph-mass spectrometer LKB 2091. The g.c.-column used was a 3 % SE-30 glass column 2·7 m × 2 mm (i.d.). A constant temperature of 160° for the first 8 min, followed by temperature programming at a rate of 10° min^{-1} was used. The carrier gas flow rate was 25 ml helium min^{-1}. The mass spectra were obtained with a constant accelerating voltage of 3·5 kV, an electron energy of 70 eV and an ionizing current of 100 μA. The g.c.-m.s. instruments was connected to the LKB 2130 data system and repetitive scanning of the mass range 10 to 500 in 2 s was used (Hedfjäll & Ryhage, 1975).

One of the analysed samples (I) is shown in Fig. 1, which represents a gas chromatogram obtained by using a flame ionization detector and isothermal operation of the column in accordance with instructions for analysis of clofibrate preparations (Mårde & Ryhage, 1975; Pharmacopoea Nordica, 1973). By using this standard method in drug purity control, impurities having a longer retention time than clofibrate itself will not be detected and due to the high concentration of clofibrate, neither will impurities having the same retention time be detected. Identification of the different components is not required.

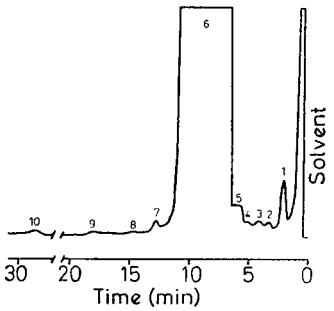


FIG. 1. Gas chromatographic analysis of clofibrate-preparation I where peak 6 is clofibrate.

The three samples were studied by g.c.-m.s. and Fig. 2 shows the total ion current (TIC) diagram of the same sample as shown in Fig. 1 where the corresponding

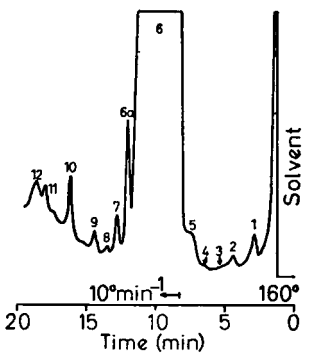


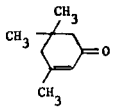
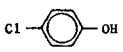
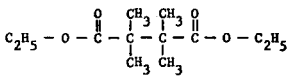
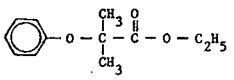
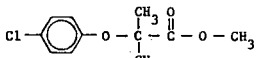
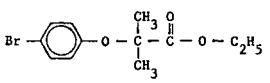
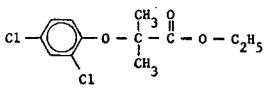
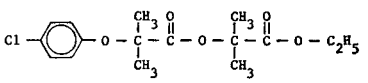
FIG. 2. Gas chromatographic-mass spectrometric analysis of clofibrate-preparation I.

* Correspondence.

peaks have been given the same numbers. Samples from the other manufacturers give a somewhat different TIC diagram. Additional studies of these drug preparations emphasize the importance of optimal column conditions for the separation of impurities from clofibrate. Table 1 illustrates the proposed structures of the detected compounds in the order of increasing retention times and in which samples they were found. The first component in this Table was found only in one sample (II) close to the solvent peak. The spectrum of this component was compared and found to be identical to the AWRE reference spectrum TRC 0094. The mass spectrum of component 1 is identical to the AWRE reference spectrum DOW 0529. The mass spectrum of component 2 shows a base peak at *m/e* 116. This peak is probably caused by the relatively easy cleavage of the 2,3-bond and by a rearrangement of one hydrogen atom from the lost part of the molecule. It is known that methyl esters of saturated aliphatic compounds with methyl and ethyl groups at carbon 2 show a peak with rearrangement of one hydrogen atom from the lost part of the molecule (Ryhage & Stenhagen, 1960). Other characteristic peaks were found at *m/e* 157 and 185. If these ions belong to the lost neutral fragment of *m/e* 73 and 45 respectively (typically for dibasic acids) the molecular ion should be M = 230, but no molecular ion could be seen. However mass spectra or methyl esters of saturated dibasic acids usually show very small or absent molecular ions (Ryhage & Stenhagen, 1959). The component 2 would then correspond to a proposed formula of ethyl 2,2,3,3-tetramethylbutane-1,4-dioate. The mass spectra of small amounts of components 3, 4, 8, 9, 11 and 12 could not be identified with certainty.

The mass spectra of a component with a parent ion at *m/e* 208 (found in sample II only) and components 5 and 7 have been identified as the deschloro, methyl ester and dichloro analogues of clofibrate respectively. These impurities were also found by Diding & others (1976), who compared the mass spectra of the impurities with spectra of reference compounds. The peak 6A in Fig. 2 could not be seen in Fig. 1 nor in the other two samples II and III which also were analysed in accor-

Table 1. *Impurities detected in samples I, II, III of clofibrate and the proposed structure of the detected compounds with the peak No. in the g.c. diagram of sample 1.*

Mol wt	I	II	III	Structure	No.
138		X			
128	X	X	X		1
230	X		X		2
208		X			
228	X				5
286	X	X	X		6A
276	X	X	X		7
328	X	X	X		10

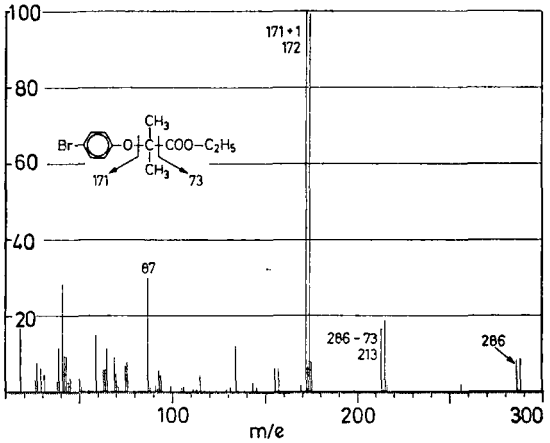


FIG. 3. Mass spectrum of component 6A with proposed structure.

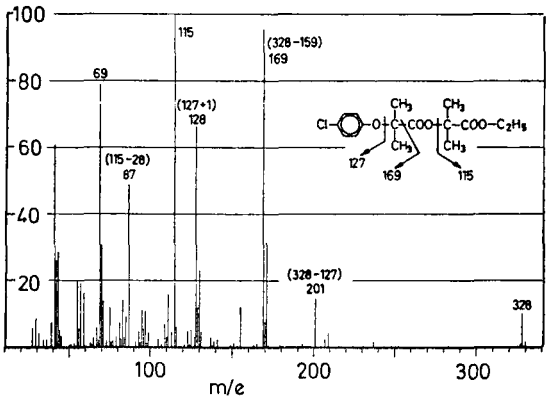


FIG. 4. Mass spectrum of component 10 with proposed structure.

dance with instructions for the standard gas chromatographic method (Pharmacopoea Nordica, 1973). The peak 6A is shown in Fig. 3 and is identified as a compound with a bromo substituent instead of a chloroatom as in clofibrate. Fig. 4 shows a mass spectrum of component 10 with a probable parent ion at *m/e* 328. If the area of peaks 6A, 11 and 12 had been added to the area of other by-products found in each product of clofibrate the differences in the amount would be much decreased. The major impurities in Fig. 1 were found at shorter retention times than clofibrate and the quantity of 4-chlorophenol, for instance is about 0·4‰ of the drug. This corresponds to an oral administration of 0·8 mg per day for a daily dosage of 2 g of clofibrate. The corresponding dosage for component 10 is about 0·6 mg per day. Due to the possible toxicity of different impurities in drugs the use of gas chromatography to identify these impurities is insufficient. Therefore it is expected that the g.c.-m.s. method will supplement the gas chromatographic method for such work which requires quantitative and qualitative analysis.

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Free energies of solution in water, of some androstanolone, nandrolone and testosterone esters

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The effects of the methylene group on the solubilities of homologues in various series have been the subject of numerous investigations (see Davis, Higuchi & Rytting, 1974). The evidence from these suggests that free energies of solution change by a constant increment for each additional methylene group, but the supporting results are mainly for volatile, liquid solutes, for which vapour pressures are readily measured. A similar approach to solid solutes is more difficult because they are rarely sufficiently volatile for vapour pressures to be determined, and because the energy required to liquify the solid, which does not always change uniformly as the series is ascended, must be considered. Thus, for example, James & Roberts (1968) showed that the solubilities in organic solvents of a range of testosterone esters changed irregularly as the series was ascended, and that the pattern took the same form as the ideal solubilities calculated from heats of fusion and melting points. Similar observations and conclusions have been made on solutions of alkyl *p*-aminobenzoates in n-hexane and in silicone oil (Yalkowski, Flynn & Slunick, 1972). In contrast solubilities in water do not follow the organic solubility pattern, e.g. solubilities of n-alkanols and n-fatty acids have been shown to decrease logarithmically with the numbers of methylene groups in the molecules (Robb, 1966) and a similar relationship has been observed with *p*-aminobenzoates (Yalkowski & others, 1972). The aqueous solubilities of numerous steroid esters have been determined in these laboratories over recent years, and it has invariably been found that while solubilities in organic solvents change irregularly as a homologous series is ascended, aqueous solubilities decrease logarithmically with a constant decrement for each additional methylene group. This information has been used to calculate some group contributions for aqueous solubilities of steroid esters.

Solubilities were obtained from the following sources: androstanolone esters (Bowen, James & Roberts, 1970; Ng, 1974), methyltestosterone esters (Roberts, 1969), nandrolone esters (Chaudry & James, 1974; Ng, 1974) and testosterone esters (Chaudry & James, 1974; James & Roberts, 1968; Ng, 1974). Ideal solubilities and heats of fusion of formate to valerate esters of testosterone were taken from James & Roberts (1968) and of the same esters of androstanolone and nandrolone from Ng (1974). The remainder were obtained from Chaudry (1972).

The concept of group contributions to physical properties assumes that a given substituent will add a constant, characteristic increment of free energy to that of the molecule to which it becomes attached. Group contributions to solubility are usually expressed in terms of ΔG^E, the free energy in excess of that required for ideal mixing, which can be calculated from equation (1). γ^∞ is the activity coefficient, relative to the pure liquid solute, at infinite dilution. The activity a_2 of a solid non-electrolyte in its saturated solution, is equal to its ideal solubility X_2^i, calculated from equation 2 (Hildebrand, Prausnitz & Scott, 1970).