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Gas chromatography of the nitrate esters of glycerol, isosorbide and isomannide

The organic nitrate glyceryl trinitrate and its nitrated metabolites have been studied by gas chromatography (GC), with a cathaeometer[1,2], and by flame ionization detection (FID)[3] and electron capture detection (ECD)[4]; a method for the detection and separation of the individual nitrates in a mixture of glyceryl trinitrate with its lower esters has been described[3], but the sensitivity for glyceryl trinitrate was inadequate and for the lower nitrates it was necessary to convert them to derivatives. For isosorbide dinitrate, only the GC detection of the unchanged product has been studied[5,6].

In view of the current interest in the metabolic fate of the organic nitrates used in human therapeutics, we developed a GC procedure for assay of mixtures of glyceryl trinitrate with its nitrated metabolites, and of mixtures of isosorbide dinitrate and its two mononitrates. We also studied isomannide dinitrate (a stereoisomer of isosorbide dinitrate) and its mononitrate.

Experimental

Nitrates. Glyceryl trinitrate (nitroglycerin, GTN) was available as a commercial 1% solution in ethyl alcohol. Glyceryl 1,3-dinitrate (GDN 1-3), glyceryl 1,2-dinitrate (GDN 1-2) and glyceryl 1-mononitrate (GMN) were prepared and purified as described previously[7].

Isosorbide dinitrate (ISDN) was available as a powder; isomannide dinitrate (IMDN) was prepared by the method of JACKSON AND HAYWARD[8]. Isosorbide 5-mononitrate (5-ISM), isosorbide 2-mononitrate (2-ISM) and isomannide mononitrate (IMMN), the only possible mononitrate of IMDN, were obtained by acid-catalyzed hydrolysis of the parent products, purified and identified by different procedures (to be published elsewhere). All substances were injected in 1–2 μl of ethyl acetate.

Apparatus. The chromatograph was a Packard, Series 7400, dual-column gas chromatograph equipped with a flame ionization detector and a tritium (150 mCi) electron capture detector.

Operating conditions. Columns were made of glass, 6 ft. × 2 mm I.D., containing Gas-Chrom Q (60–80 mesh) (Supelco), packed with either 3% of XE-60 or 3.5% of QF-1 (both from Applied Science Laboratories). Both columns were operated isothermally, the QF-1 at 110° and the XE-60 at 150° for FID, and the QF-1 at 120° and the XE-60 at 150° for ECD. The detector temperature was 200° and the injection port temperature 160°; the low temperature was necessary because nitroglycerin decomposed at temperatures above 200° (see also ref. 9).

The nitrogen carrier gas flow-rate was 25 ml/min. For FID the hydrogen flow-rate was 18 ml/min and the air flow-rate 370 ml/min. The electrometer range was $3 \cdot 10^{-11}$ A for FID and $1 \cdot 10^{-10}$ A for ECD.

Calculations. Relative retention was calculated for each product as a function of the retention time of isosorbide dinitrate (ISDN) injected simultaneously; the retention time of ISDN was taken as unity.

Results and discussion

The retention times for the different nitrates are given in Table I for the two

TABLE I

RELATIVE RETENTIONS OF DIFFERENT ORGANIC NITRATES

The results obtained with flame ionization detection, calculated as a function of the retention time of isosorbide dinitrate (=1) injected simultaneously, are given for two different columns, XE-60 and QF-1. For experimental details, see text.

Nitrate	*Relative retention*	
	XE-60	*QF-1*
Glyceryl trinitrate	0.548	0.415
Glyceryl 1,3-dinitrate	0.641	0.198
Glyceryl 1,2-dinitrate	0.560	0.184
Glyceryl 1-mononitrate	0.237	0.075
Isosorbide dinitrate	1	1
Isosorbide 5-mononitrate	1.024	0.420
Isosorbide 2-mononitrate	0.269	0.208
Isomannide dinitrate	1.58	1.42
Isomannide mononitrate	0.500	0.360

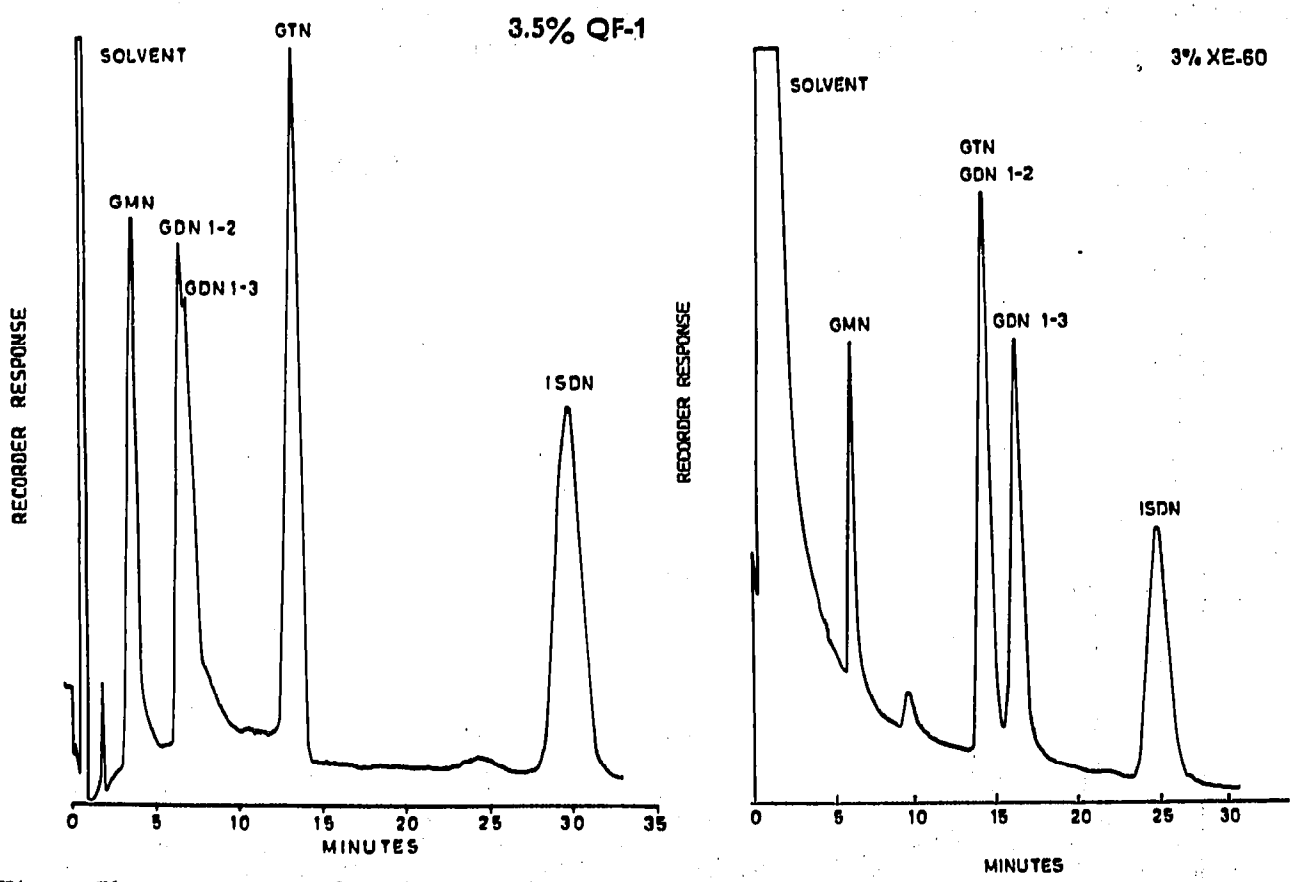

Fig. 1. Chromatograms of a mixture of glyceryl trinitrate and its lower esters. (a) Using a column with 3.5% of QF-1 at 120°, with electron capture detection. GMN = glyceryl 1-mononitrate, 20 ng; GDN 1-2 = glyceryl 1,2-dinitrate, 10 ng; GDN 1-3 = glyceryl 1,3-dinitrate, 10 ng; GTN = glyceryl trinitrate, 7 ng; ISDN = isosorbide dinitrate, 10 ng, injected as standard. (b) Using a column with 3% of XE-60 at 150°, with flame ionization detection. GMN = glyceryl 1-mononitrate, 1 μg; GTN = glyceryl trinitrate, 5 μg; GDN 1-2 = glyceryl 1,2-dinitrate, 1.5 μg; GDN 1-3 = glyceryl 1,3-dinitrate, 1.5 μg; ISDN = isosorbide dinitrate, 2 μg, injected as standard.

columns used; relative retention as a function of the retention time of isosorbide dinitrate is given.

For a mixture of glyceryl trinitrate and its partially denitrated metabolites, satisfactory separation can be obtained only by using both columns; the parent product is well separated from its lower nitrates on QF-1; the dinitrates can be separated from each other on XE-60, but on this column GTN and GDN 1-2 overlap. This is illustrated in Figs. 1a and 1b; it should be noted that, as the example in Fig. 1a is obtained with ECD at a column temperature of 120°, the retention times are slightly different from the data in the Table I, which were obtained with FID at 110°.

It can also be seen that, with FID, incomplete separation of the mononitrates from the solvent peak would make quantitative observation difficult; lowering of the column temperature improves the separation. With ECD, however, this problem does not occur owing to the much reduced width of the solvent peak.

Fig. 2 shows the good separation of the different nitrates in a mixture of isosorbide dinitrate, isomannide dinitrate and their mononitrates on the QF-1 column. For this mixture, the XE-60 column is less satisfactory, as 5-ISM overlaps with isosorbide dinitrate.

From Figs. 1 and 2 it can be seen that the GC procedure used allows very small amounts of the different organic nitrates tested to be detected. Table II gives the minimum amounts that can be determined using FID and ECD; as the sensitivity

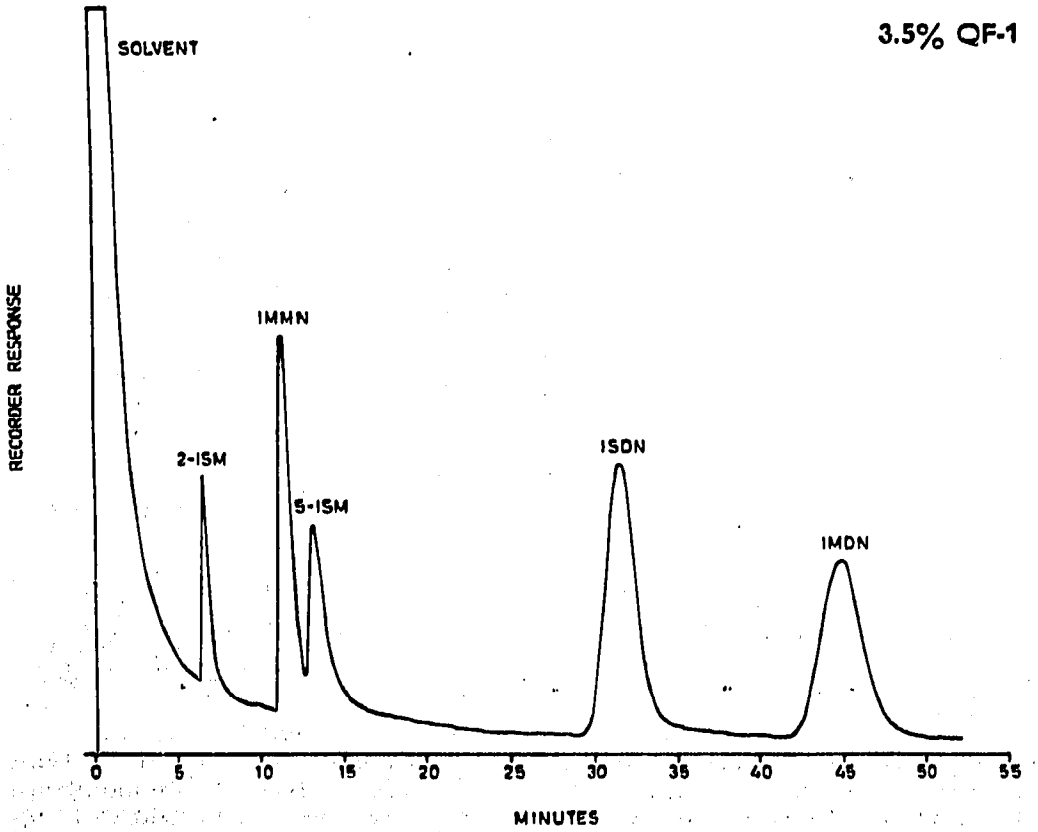

Fig. 2. Chromatogram of a mixture of isosorbide dinitrate, isomannide dinitrate and their lower esters on column with 3.5% of QF-1 at 110°, with flame ionization detection. 2-ISM = isosorbide 2-mononitrate, 5 μg; IMMN = isomannide mononitrate, 1 μg; 5-ISM = isosorbide 5-mononitrate, 1 μg; ISDN = isosorbide dinitrate, 2 μg; IMDN = isomannide dinitrate, 2 μg.

TABLE II

MINIMUM AMOUNTS OF DIFFERENT NITRATES, THAT CAN BE DETERMINED

The data given are average values, as the sensitivity shows day-to-day variations. The electrometer range is $3 \cdot 10^{-11}$ A with flame ionization detection and $1 \cdot 10^{-10}$ A with electron capture detection.

Nitrate	*With flame ionization detection (μg)*	*With electron capture detection (ng)*
Glyceryl trinitrate	5	1
Glyceryl 1,3-dinitrate	1	10
Glyceryl 1,2-dinitrate	1	10
Glyceryl 1-mononitrate	1	10
Isosorbide dinitrate	0.5	8
Isosorbide 5-mononitrate	1	2
Isosorbide 2-mononitrate	0.5	2
Isomannide dinitrate	1	10
Isomannide mononitrate	0.5	10

shows day-to-day variations, especially for ECD, only approximate values can be given. Although Table II gives the minimum amounts that can be determined, smaller amounts can easily be detected. These data were obtained with an electrometer range of $3 \cdot 10^{-11}$ A for FID and $1 \cdot 10^{-10}$ A for ECD; this means that maximum sensitivity is achieved as beyond this range the width of the solvent peak becomes too large for FID; for ECD, baseline variations also become important. It can be seen from Table II that whereas FID is only slightly superior to densitometry on thin-layer chromatograms[10], ECD gives the possibility of easily detecting less than 10 ng of each of the nitrates used. For glyceryl trinitrate, for instance, the ECD method is very interesting; the high sensitivity for this product is undoubtedly due to the electron-absorbing properties of the nitrate groups.

We are currently applying the method described for assay of the organic nitrates in biological material.

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