

CHROM. 3555

Gas-liquid chromatography of iodoamino acids and iodinated contrast media as their trimethylsilyl derivatives

Recently, the successful use of gas chromatography in the analysis of thyroid hormones has been reported^{1,2}. By forming the dipivalyl methyl esters of the iodoamino acids and utilizing electron capture detection, JAAKONMÄKI AND STOFFER³ achieved the separation and quantitation of these compounds in the nanogram range. In this communication the gas chromatography of the 4 principal iodoamino acids as their trimethylsilyl (TMS) derivatives is described. Furthermore, the gas chromatographic behaviour of a number of iodinated radiographic contrast media, known to interfere with the chemical determination of these compounds, was studied.

Experimental

Materials. The iodoamino acids 3-monoiodotyrosine (MIT), 3,5-diiiodotyrosine (DIT), 3,3',5'-triiodothyronine (T_3) and thyroxine (T_4) were obtained commercially. The iodinated radiopaques Telepaque, Hypaque, Bilopaque, Urokon, Conray, Miokon, Orabilix and Teridax were generously supplied as pure substances by respectively Mallinckrodt (St. Louis, Mo.) and Sterling-Winthrop Research Institute (Rensselaer, N.Y.). A solution of the samples in methanol, singly or as a mixture, was prepared containing 1 mg/ml of each compound.

Preparations of TMS derivatives. 100 μ l of the methanol solution (or appropriate dilutions) was evaporated to dryness under nitrogen at room temperature. The residue was treated with 100 μ l of bis(trimethylsilyl)-acetamide containing 5% trimethylchlorosilane, and heated for 20 min at 50° in a closed vial. Aliquots of 1-3 μ l were taken for gas chromatography.

Apparatus. Gas chromatography was carried out employing a 2 mm \times 5 ft. glass column packed with 3% OV-1 on 100-120 mesh Gas Chrom Q and using a Beckman GC-4 with hydrogen flame and electron capture detection. Helium was used as carrier gas at a flow rate of 70 ml/min. The unit was run isothermally or with temperature programming from 210-220°.

Results and discussion

In Figs. 1-A and 1-B the separation of 0.5 μ g each of monoiodotyrosine and diiodotyrosine at 210° and 1.5 μ g each of triiodothyronine and thyroxine at 285° is shown. Flame ionization was used as a means of detection. The peaks, corresponding to retention times obtained with preparations of the individual compounds, were symmetrical and no evidence of decomposition during the gas chromatographic separation was obtained. The limits of detection, employing the hydrogen flame detector, were in the submicrogram range (diiiodotyrosine: 100 ng and triiodothyronine: 300 ng).

A higher degree of sensitivity was obtained using electron capture detection. Fig. 1-C illustrates the injection of 2 ng MIT and 6 ng DIT. With respect to the electron capture detector, the limit of detection for monoiodotyrosine, diiodotyrosine, triiodothyronine and thyroxine was found to be 0.3 ng, 1 ng, 5 ng, and 30 ng respectively.

Interest in the future adoption of this technique for the estimation of iodinated amino acids in serum prompted us to test the gas chromatographic behaviour of a number of iodinated compounds used in contrast radiography and interfering with the chemical determination of thyroid hormones^{4,5}. Fig. 2 shows the separation of these compounds from mono- and diiodotyrosine. The contrast media, eluted from the column at much lower temperatures than the thyroid hormones, did not interfere with the separation of the hormones carried out at 285°.

The sensitivity and specificity of the gas chromatographic technique appear to be very promising and should permit the quantitation of thyroid hormones in serum. Preliminary attempts to isolate the hormones from serum prior to derivatization,

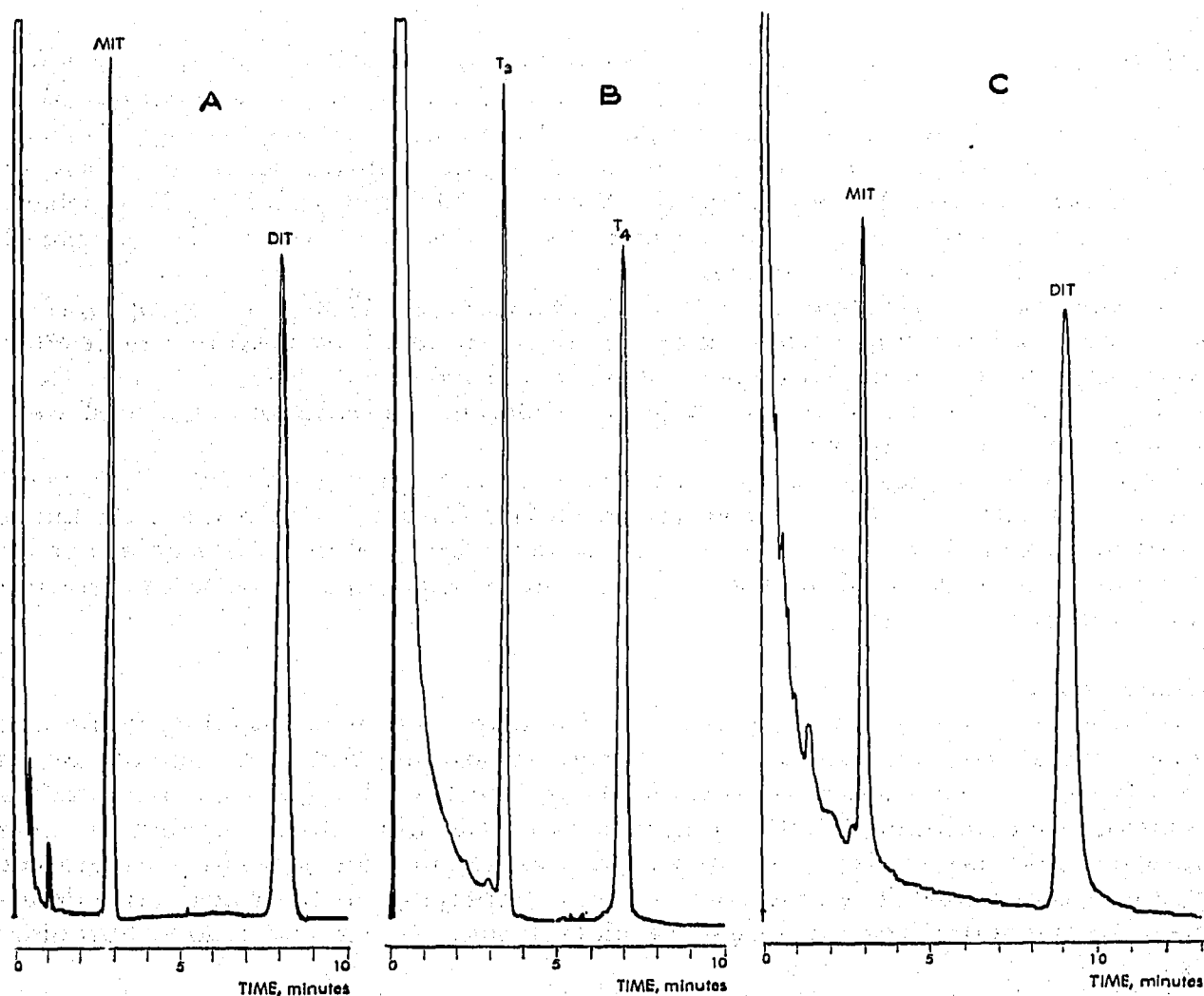


Fig. 1. A and B. Gas chromatographic separation and hydrogen flame detection of monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T_3) and thyroxine (T_4) as their trimethylsilyl derivatives. C. Gas chromatogram showing injection of 2 ng monoiodotyrosine (MIT) and 6 ng diiodotyrosine (DIT) as their trimethylsilyl derivatives. Electron capture detection.

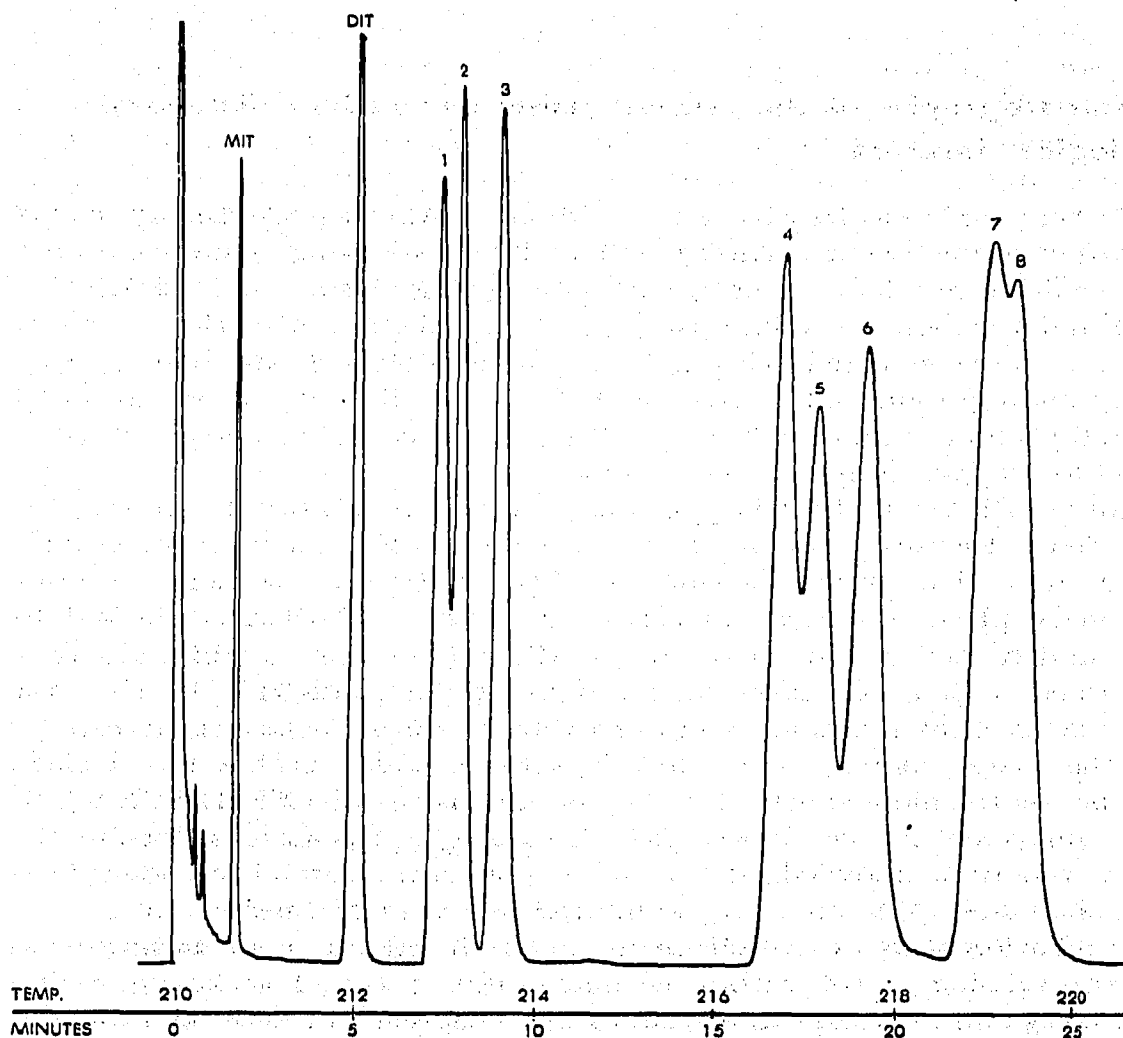


Fig. 2. Separation of monoiodotyrosine (MIT), diiodotyrosine (DIT), Urokon (1), Telepaque (2), Teridax (3), Bilopaque (4), Orabilix (5), Hypaque (6), Miokon (7) and Conray (8) as their trimethylsilyl derivatives. Separation was carried out by temperature programming from 210–220° with a temperature rise of 2°/5 min. Hydrogen flame detection.

using either cation or exchange resins^{4,5}, did not yield extracts suitable for trimethylsilylation of the hormones. Other approaches to the problem of serum processing require exploration before this technique becomes useful for assay of thyroid hormones in blood.

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