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Note

New gas–liquid chromatographic method for biologically important indoleamines

R. PORTA, C. ESPOSITO, M. CAMARDELLA and G. DELLA PIETRA*

Department of Biochemistry, Ist Medical School, University of Naples, via Costantinopoli 16, 80138 Naples (Italy)

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The presence of enzyme systems producing N- and O-methylated indoleamines in various tissues has prompted the development of procedures aimed at the identification and quantitative estimation of trace amounts of these compounds. Gas–liquid chromatography (GLC) has received increased attention, owing to its high resolving power and sensitivity. In the GLC methods reported the indoleamines were trimethylsilylated[1] in the phenolic group, and or heptafluorobutyrylated[2], trifluoroacetylated[3] or pentafluoropropriony lated[4] in the aminic group.

In connection with our studies on indoleamine-N-methyltransferase[5], we have developed a procedure for determining tryptamine, N-methyltryptamine, 5-methoxytryptamine and 5-hydroxytryptamine simultaneously by means of flame ionization detection, without derivatization and in a total analysis time of 13 min.

EXPERIMENTAL

Materials

Tryptamine hydrochloride, N-methyltryptamine, 5-hydroxytryptamine oxalate salt and 5-methoxytryptamine hydrochloride were purchased from Sigma, St. Louis, Mo., U.S.A. Other solvents and reagents were obtained from commercial sources.

Apparatus

A Varian Model 3700 gas chromatograph equipped with a hydrogen flame detector and a linear temperature programmer was used. The GLC column (50 cm × 3 mm I.D.) was packed with 5% OV-101 on 100–120 mesh Chromosorb G HP. The injector block was maintained at 200° and the flame ionization detector at 230°. Nitrogen was used as the carrier gas at a constant flow-rate of 20 ml/min. Quantitation of peak area was performed by the peak height × width at half height method.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram obtained from 1 μg of a mixture of pure

* To whom correspondence should be addressed.

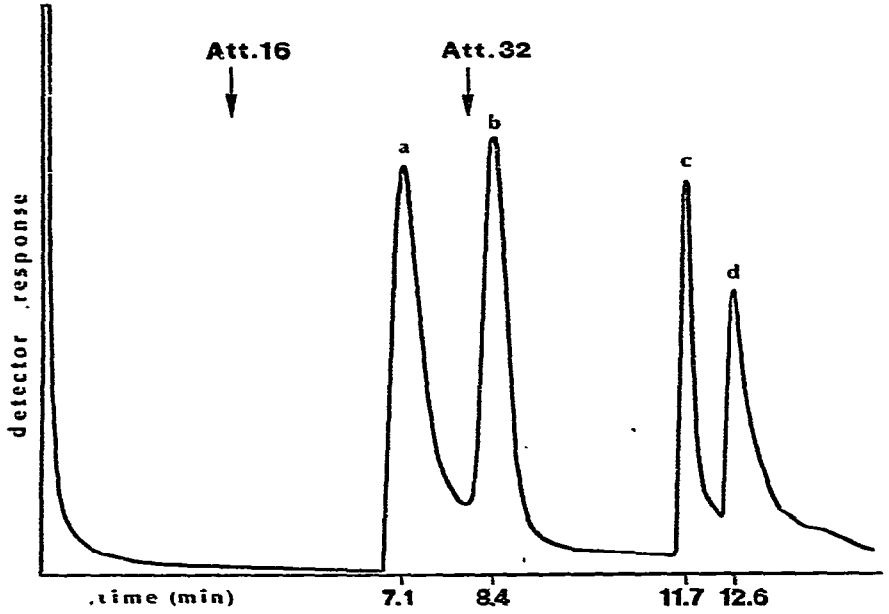

Fig. 1. Gas chromatogram of 1 μg of each indoleamine shown in Table I. Changes in instrument attenuation (Att.) are indicated by vertical arrows.

indoleamines injected in 1 μl of ethyl acetate. In the analysis the oven was maintained at 140° for 7 min, and temperature programming was carried out from 140° to 230° at 15° per min. Under these conditions the peaks are symmetrical and well resolved, thus allowing their complete integration. Fig. 2 shows the standard calibration curves for the indoleamines analysed. Response, as measured by peak area multiplied by attenuation in the gas–liquid chromatogram of indoleamines, was found to be linear,

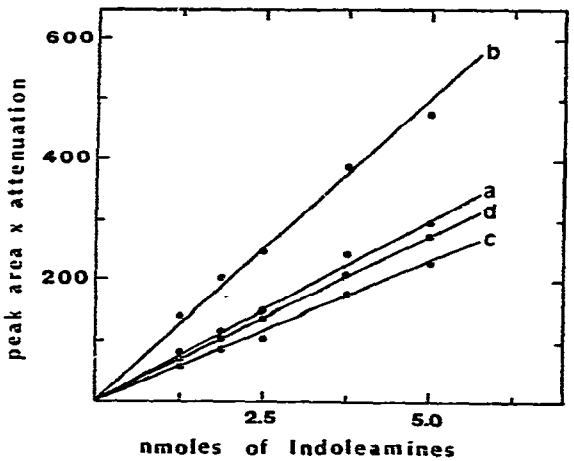

Fig. 2. Linearity plot. The y-axis is the GLC chromatogram peak area multiplied by the instrument attenuation; the x-axis is the concentration of indoleamines in nmoles.

while the emergence times were reproducible to 0.1 min or better. The lower detection limit, for the identification and quantitation was 5 ng. The retention data are given in Table I.

TABLE I

RETENTION DATA FOR STANDARDS

Compound	*Emergence time (min)*	*Peak area × attenuation* (cm^2)*
(a) Tryptamine	7.1	294
(b) N-Methyltryptamine	8.4	458
(c) 5-Methoxytryptamine	11.7	226
(d) 5-Hydroxytryptamine	12.6	274

* Data correspond to 1 μg of indoleamine.

This technique is very selective and sensitive for the identification of products of reactions catalysed by hydroxyindole-O-methyltransferase and indoleamine-N-methyltransferase, using 5-hydroxytryptamine and tryptamine respectively as substrates. It is not necessary to use derivatization for the identification and quantitation of 5-methoxytryptamine and N-methyltryptamine. We are presently examining the indoleamine content in various tissues from different animal species. These results will be reported elsewhere.

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