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# GAS–LIQUID RADIOCHROMATOGRAPHY OF $^{14}$C-CONTAINING COMPOUNDS WITH COMBUSTION AND COLLECTION OF THE RESULTING [$^{14}$C]CARBON DIOXIDE IN HYAMINE

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## SUMMARY

A new approach is reported for the gas–liquid radiochromatography of $^{14}$C-containing compounds. The column effluent is split between a mass detector and a combustion system, and the [$^{14}$C]carbon dioxide arising from the individual chromatographic components is trapped in vials containing Hyamine. Each vial is then subjected to conventional radioassay by liquid scintillation counting. Applications of this approach to biosynthesis and drug metabolism problems are presented.

## INTRODUCTION

Gas–liquid chromatographic techniques are frequently employed in biosynthesis and drug metabolism studies. As the precursor or substrate is usually labeled with a radioactive atom (*e.g.*, $^{14}$C), it is imperative to ascertain which of the components or peaks in the chromatogram resulting from analysis of a radioactive isolate contains the label. Methods for combined gas–liquid chromatography (GLC) and radioassay have been reviewed recently[1,2]. We now wish to report on a new technique in gas–liquid radiochromatography of $^{14}$C-containing compounds—the combination of column effluent combustion with collection and counting in Hyamine of the resulting $^{14}CO_2$.

## EXPERIMENTAL

A Barber-Colman Model 5000 gas chromatograph was employed in these studies. The columns were glass U-tubes, 6 ft. × 4 mm I.D. Acid-washed and silanized Gas-Chrom P (80–100 mesh) was employed as the support. The carrier gas was nitrogen, 75 ml/min. A splitter allowed 25% of the column effluent to go to a flame detector, and the other 75% to pass through an 8 × $^3/_4$ in. quartz tube containing copper oxide (wire) and steel wool maintained at 800° (the oven is available from Nuclear-Chicago). The combustion products then pass through a magnesium

perchlorate water trap. A series of three-way solenoid valves (Skinner Precision Industries, New Britain, Conn.; No. B3DA9150) was connected with $^1/_{16}$ in. monel metal tubing so that the gas would flow through all to vent when none were energized, or would be diverted by means of a manually selected switching system to a given valve. Each valve is fitted with a vented PTFE screw cap support which can accept a standard 20 ml glass scintillation counting vial in such a way that the gas may be bubbled into 10 ml of trapping solution (the gas is led below the surface of the solution by a short length of PTFE tubing). The trapping solution consists of 10 ml of 3:1.8 v/v scintillation solution [4 g Omnifluor (New England Nuclear Corp.)/l toluene] and hydroxide of Hyamine 10-X (New England Nuclear Corp.). Up to twelve valves can be activated in succession as desired; when a particular valve is not energized it is bypassed and the gas passes to the next valve.

## RESULTS AND DISCUSSION

One procedure in gas–liquid radiochromatography is to follow the separation by a mass detector and collect or trap the components (mini-scale preparative GLC) and subject them to radioactivity determination in a liquid scintillation counter. This approach permits long term counting of samples containing low levels of radioactivity, but requires quantitative trapping of the components. Radioactivity in effluents can be monitored continuously by flow-through detectors, but problems may arise from condensation or adsorption of compounds inside the detector[1]. Superior results are often obtained if the column effluent is burned prior to delivery to the detector, and KARMEN[1] has stated that "there are few applications for which the radioactivity ought to be analyzed without combustion". With respect to the determination of $^{14}C$, conversion to $CO_2$ precludes problems (*e.g.*, aerosol formation, condensation between the column and the collection device) often associated with collecting compounds directly. SWELL[3] has successfully employed combustion (heated copper oxide) to $CO_2$ with a flow-through proportional counter for the simultaneous mass and radioactivity analysis of $^{14}C$-labeled sterols. Continuous detection or monitoring possesses an inherent disadvantage, however—the residence time in the detector of the $CO_2$ from a given component is limited, and thus significantly more radioactivity is required for detection by this means than by the "collect and count" approach. Further, this latter method is more amenable to quantitative radioassay.

In an effort to combine the advantages of (a) combustion to $CO_2$ and (b) collection followed by counting, we have devised an approach employing quantitative trapping of the $CO_2$ in Hyamine. The first report on the direct collection of $CO_2$ in this organic base followed by radioactivity determination in a liquid scintillation counter appears to be that of FREDRICKSON AND ONO[4] on the assay of expired air for $^{14}CO_2$.

Collection in Hyamine of the $CO_2$ resulting from combustion and counting of the resulting solution is an alternative approach to continuous monitoring of the combustion products by a proportional counter. Briefly, the column effluent is split with the smaller fraction going to a flame ionization detector to allow mass detection, and the larger portion passing through a combustion system. The effluent containing the combustion products is led to a series of collection ports consisting of solenoid

valves (each of which can be activated electrically by a manually-operated switching system) and vials containing a trapping solution. Following an appropriate collection period with a given collection port the gas flow is diverted to a second port, and this sequence repeated, if necessary. The vials are then unscrewed, additional scintillation solution (10 ml) added, and the contents analyzed for radioactivity in a liquid scintillation counter. The presence of Hyamine in the samples reduces the counting efficiency (by ~ 10%), and a quenching factor must be determined by use of standards.

Trapping of the $CO_2$ is quantitative at carrier gas flow rates up to 75 ml/min as proven by use of $^{14}C$-containing standards. A sample of [*n*-$^{14}C$]octadecane containing 1500 c.p.m. was applied to the column, and three fractions were taken during the elution of the compound, as monitored by the flame ionization detector (Fig. 1). A

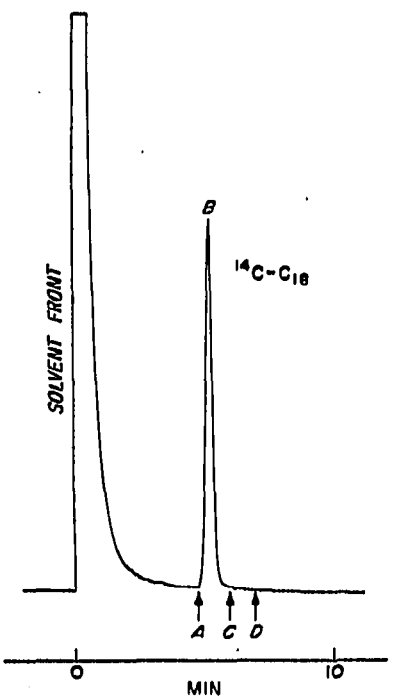


Fig. 1. Gas–liquid chromatogram resulting from analysis of 2 μg (1500 c.p.m.) of [*n*-$^{14}C$]octadecane; column (5% F-60) temperature 190°. The effluent was split between the flame detector and combustion system as described in the EXPERIMENTAL section. Three fractions of the resulting $CO_2$ (A–B, B–C, C–D) were collected and assayed for radioactivity (see text below).

recovery of 98% was observed (allowing for the split). Because the mass detector is closer to the column than are the collection vials, there is a time lag (~ 10 sec, depending upon which collection port is employed) between the response of the flame ionization detector to a component and the collection of the $CO_2$ arising from that component. This lag must be taken into consideration when $CO_2$ is collected, especially with components not well separated by the column. During the period A–B 29% of the total recovered radioactivity was trapped in the first collection vial; 66% was collected in a second vial during the period B–C; and 3% trapped in a third vial during the period C–D. When more than three cuts are taken across a peak the recovery decreases in proportion to the number of fractions collected, presumably

because of non-collection of $CO_2$ remaining in the connecting lines. Use of a purge gas should improve this situation.

We have found that samples containing only several hundred c.p.m. of $^{14}C$ can be chromatographed, combusted to $CO_2$, collected and counted satisfactorily. Success with microgram or larger samples containing little radioactivity (low specific activity) is dependent primarily upon quantitative combustion and collection of the [$^{14}C$]carbon dioxide and upon counting statistics. With submicrogram amounts of mass containing more than sufficient radioactivity for radioassay (high specific activity) the sample may be partially lost on the column (this is especially true for polar compounds). For this reason use of derivatization and/or a carrier may be advisable when a sample of polar material containing very little mass is analyzed. An application of this gas–liquid radiochromatographic approach to characterizing the product of an *in vitro* enzymatic reaction is illustrated in Fig. 2. N-methyltryptamine was incubated with S-adenosylmethionine-[$^{14}C$]methyl in the presence of indoleamine-N-methyltransferase (isolated from human lung)[5]. The product of this enzymatic reaction was assumed to be N,N-[$^{14}C$]dimethyltryptamine, and it was necessary to prove that the radio-

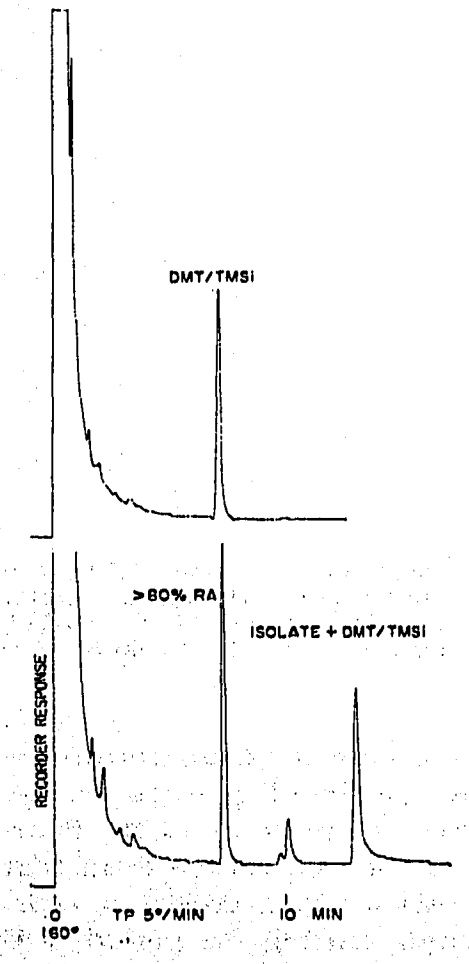


Fig. 2. Upper: gas–liquid chromatogram resulting from analysis of the TMSi derivative of authentic N,N-dimethyltryptamine. Lower: gas–liquid radiochromatogram resulting from analysis of a trimethylsilylated mixture of authentic N,N-dimethyltryptamine and the product of an *in vitro* enzymatic reaction (N-methyltryptamine plus S-adenosylmethionine-[$^{14}C$]methyl; see above text. Column (5% QF-1) temperature programmed from 160° to 210° at 5°/min.

activity present in the isolate indeed possessed the GLC behavior of N,N-dimethyltryptamine .As less than 100 ng of the radioactive product was available for analysis, 50 $\mu g$ "cold" N,N-dimethyltryptamine was added as carrier to an aliquot of the isolate, the mixture subjected to trimethylsilylation conditions to produce the less polar trimethylsilyl (TMSi) derivative, and a portion of this sample (1000 c.p.m.) analyzed by gas–liquid radiochromatography. Greater than 80% of the available radioactivity (based on the amount injected and the split ratio) was found to be associated with the TMSi derivative of N,N-dimethyltryptamine, both with F-60 and QF-1 (Fig. 2). two stationary phases possessing significantly different partitioning properties. Approximately 10% of the radioactivity could not be accounted for, possibly because of the presence of the N-oxide.

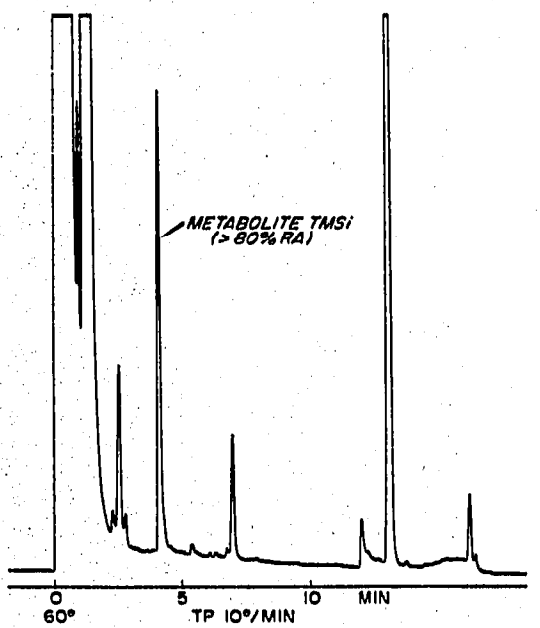


Fig. 3. Gas–liquid radiochromatogram resulting from analysis ot a metabolite fraction (see text below) which had been exposed to trimethylsilylation conditions. Column conditions same as for Fig. 1, except temperature programmed from 60° to 200° at 10°/min.

An example of the usefulness of the combustion–collection technique in a drug metabolism study is seen in Fig. 3. A non-volatile acidic metabolite isolated from the urine of a wether lamb fed a diet containing [$^{14}C$]bis(chloromethyl)sulfone[6] was treated with bistrimethylsilyltrifluoroacetamide in an attempt to form a volatile, neutral compound. Gas–liquid radiochromatography of an aliquot (900 c.p.m., 3 $\mu g$) of the derivatization mixture gave the chromatogram seen in Fig. 3, and $>80\%$ of the available injected radioactivity was found to be associated with the large peak as indicated. With the drug-related component identified, the sample was subjected to combined GLC–mass spectrometry, and the component of interest characterized as the TMSi ester of chloromethanesulfinic acid.

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## NOTE ADDED IN PROOF

A method in which the radioactive concentration of a gas is determined by oxidation to $CO_2$, which is trapped in a preweighed amount of ethanolamine in a counting vial, has appeared[7].

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