Journal of Chromatography, 387 (1987) 505–508
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 163

Note

Simple and highly sensitive technique for radio-gas chromatography

C. R. LEE* and H. ESNAUD
Laboratoires d'Études et de Recherches Synthélabo (L.E.R.S.), 31, ave. Paul Vaillant Couturier, F-92220 Bagneux (France)
and
R. J. POLLITT
Regional Neonatal Screening Laboratory, Middlewood Hospital, Sheffield S6 1TP, S. Yorks (U.K.)
(First received August 12th, 1986; revised manuscript received October 17th, 1986)

Radio-gas chromatography is useful in the biomedical sciences whenever simpler methods such as thin-layer chromatography lack the necessary resolving power, and provided suitable volatile derivatives can be formed. Unfortunately, commercial on-line radioactivity detectors are relatively expensive, and their sensitivity is inherently limited by the time available for counting. In these instruments, the eluate is oxidised in a cupric oxide furnace, and the gases are counted (after appropriate scrubbing) in a proportional counter.

The conventional flame ionisation detector is an equally efficient combustion device, but for on-line counting large amounts of water must be trapped, and a special large-volume ionisation chamber is required to accommodate the high flow-rate[1]. The exhaust gases from a flame ionisation detector can be trapped quantitatively into scintillation fluid using a special countercurrent flow apparatus connected to a fraction collector[2]. Even more simply, radioactive carbon dioxide can be collected quantitatively by bubbling the gases through a vial of an alkaline scintillant, using a Pasteur pipette connected to the detector with a piece of rubber tubing (unpublished observation). Unfortunately, with some types of instrument, the detector outlet is directly above the jet, and falling droplets of condensed water vapour (which are not otherwise troublesome) tend to put out the flame.

It is thus desirable to heat the transfer line, and we show that when this is done, excellent results may be obtained for tritium as well as for carbon-14.

MATERIALS AND METHODS

The transfer line was made using materials to hand, and it took about 1 h to construct. A 1-m length of PTFE tubing (1/8 in. O.D., 1/16 in. I.D.) was wrapped to within 1 cm of each end with stainless-steel wire (0.25 mm diameter), at about 1 turn/cm. The wire was held down at the ends with heat-resistant adhesive tape (Prolabo), and then the tube was pushed into a length of silicone tubing. Leads to the heater wire were attached using screw connectors, and the assembly was secured with more adhesive tape. A thermocouple poked into the PTFE tube registered 120°C

0021-9673/87/$03.50

with 1A at 35 V applied from a variable power supply. Safety note: use of an auto-wound variable transformer would be dangerous.

The gas chromatograph was a Hewlett-Packard Model 5700, fitted with a flame/nitrogen–phosphorus detector. This detector is of an air-tight design, and the only opening is through the collector electrode, which also serves as the exhaust chimney. Any detector which can be rendered air-tight should be suitable[2,3], and dead volumes can be neglected in view of the high flow-rates (> 300 ml/min at S.T.P.).

One end of the transfer line was held over the detector using a laboratory clamp, and connected to it with a piece of thick-walled silicone tubing. The other end was trimmed to within a few millimeters of the heater winding, and attached to a 20-μl disposable glass pipette (Corning; 13 cm × 0.5 mm I.D.) by means of two very short pieces of silicone tubing telescoped together. This connection must be short, and close to the end of the heater winding, in order to avoid condensation.

Evaluation

A glass column (2 m × 2.5 mm I.D.) packed with 10% OV-17 on Gas-Chrom Q, was used at a flow-rate of 30 ml helium. The flame ionisation detector was operated at the usual flow-rates of 30 ml/min hydrogen and 300 ml/min air. [^{3}H]Phenylethylamine (20 nCi), mixed with unlabelled carrier (50 μg), was acetylated in aqueous solution[4] and taken up in propyl acetate (50 μl).

Recovery

Following accurate injections of 4.0 μl of the [^{3}H]phenylethylamine derivative, gases from the transfer line were bubbled into 10-ml aliquots of Instagel (Packard) in a series of standard polyethylene counting vials. Effluent corresponding to the labelled compound was collected in a single vial, and the others were used to check for background. For comparison, six control aliquots of 4.0 μl of the injected solution were taken using the same syringe, and counted in 10 ml of scintillant.

Elution profile

Following an injection (1 μl; 0.4 nCi) of the labelled compound, the transfer line was passed rapidly from vial to vial at 5-s intervals, the times being marked on the recorder trace. Counts per fraction were plotted without correcting for losses during transfer (*ca.* 0.5 s).

Whenever sampling was not being performed, the glass capillary was immersed in a beaker of water, which was changed frequently.

Scintillation counting

A Searle MK III counter was used, with external quench compensation.

RESULTS AND DISCUSSION

Recovery of tritium from acetylated [^{3}H]phenylethylamine was 97.4 ± 4.7%. (The control samples gave 14322 ± 52 dpm; samples collected from the flame ionisation detector gave 13945 ± 656 dpm.) In this experiment, baseline fractions contained negligible amounts of radioactivity, and correction for background counts was

not made. Besides indicating quantitative recovery, this result shows that the derivatisation had eliminated any radioactive impurities from the phenylethylamine.

The peak profiles (Fig. 1) indicate that, as in the well-known case of deuterium labelling, the retention time of the tritiated compound was shorter than that of the carrier. There was no evidence of peak broadening. We have not attempted to use capillary columns because quantitative injection is more difficult, and their replacement in case of contamination would be expensive; high baseline count levels occur frequently with this technique (for example, see Fig. 1).

It would be surprising if such a straightforward technique had not been described before, but we have not found any reference to it. Belham and Neal[3] collected tritium from a flame ionisation detector by cooling a section of the transfer line and allowing the flow of gases to drive the condensed water vapour into a scintillation vial. Recoveries were not quantitative (although this may depend on the compounds used for evaluation) and the conditions under which condensation occurs may be critical if peak tailing is to be avoided. In fact, the only critical point in constructing our interface was the probe at the end of the transfer line. Pipettes wider than that specified provoked visible condensation of water vapour, and this led to unacceptable tailing for tritiated compounds. Dipping the end of the transfer line into the scintillant did not perturb the detector, even when recording at high sensitivity. The flow resistance of the capillary pipette, which gave a pressure in the transfer line of 35 cm of water, presumably buffers the detector against changes in hydrostatic back-pressure.

In conclusion, lack of special equipment should not prevent the occasional use for radio-gas chromatography of any gas chromatograph fitted with a flame ionisation detector. The straightforward method described here could readily be automated using an automatic sampling device. For prolonged or intensive use, however, it would be desirable to include a means of trapping the gases whenever sampling is not being performed.

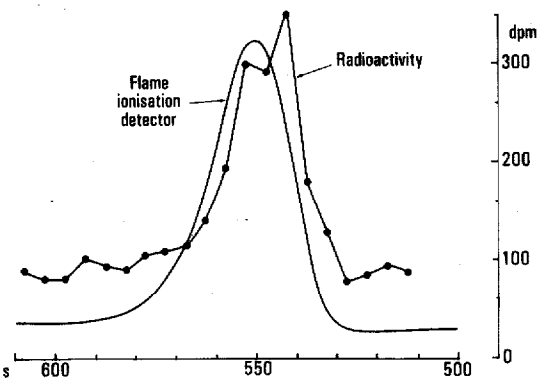

Fig. 1. Elution profile of the acetyl derivative of 0.4 nCi [^{3}H]phenylethylamine, in the presence of 1 μg of unlabelled carrier.

REFERENCES

1 H. Fock, *Chromatographia*, 9 (1976) 99.
2 C. M. Wels, *J. Chromatogr.*, 142 (1977) 459.
3 J. E. Belham and G. E. Neal, *Anal. Biochem.*, 45 (1972) 6.
4 D. G. Calverley, G. B. Baker, R. T. Coutts and W. G. Dewhurst, *Biochem. Pharmacol.*, 30 (1981) 861.