A METHOD FOR THE GAS CHROMATOGRAPHIC ANALYSIS OF INHALATION ANAESTHETICS IN WHOLE BLOOD BY DIRECT INJECTION INTO A SIMPLE PRECOLUMN DEVICE

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SUMMARY

A simple heated precolumn device constructed from standard gas chromatographic components is described and evaluated. The device permits the analysis of concentrations of anaesthetic agents by direct injection of whole blood containing a suitable internal standard. A method is described for the analysis of halothane, chloroform, trichloroethylene, and methoxyflurane. The procedure is rapid and of acceptable accuracy.

A number of methods have been published in recent years for the gas chromatographic analyses of volatile anaesthetics in blood using solvent extraction techniques (Atallah and Geddes, 1972; Douglas, Hill and Wood, 1970; Wortley et al., 1968; Allott, Stewart and Mapleson, 1971; Wolfson, Ciccarelli and Siker, 1966). While accurate results may be obtained, extraction techniques are time-consuming. Equilibration techniques (Fink and Morikawa, 1970; Yamamura et al., 1966) provide an alternative method for the analysis of blood specimens. Although head-space analysis does allow the injection of “clean” samples onto the chromatograph column, the technique is time-consuming, since full equilibration requires up to 30 min. Direct injection of blood into the gas chromatograph (Lowe, 1964), although rapid, produces significant variations in peak heights, whilst accumulation of dried blood at the head of the column is a disadvantage. The use of a simple precolumn coupled to an isolating valve avoids the variance accompanying a manual injection technique, while the use of easily changeable glass liners reduces the problem of accumulation of blood residue. This paper presents the construction and evaluation of such a device for the determination of inhalation anaesthetics in whole blood.

MATERIALS AND METHODS

Halothane and trichloroethylene were obtained from Imperial Chemical Industries Ltd, Macclesfield, Cheshire, and methoxyflurane from Abbott Laboratories Ltd, Queenborough, Kent. Chloroform (British Drug Houses Ltd, Poole, Dorset) was redistilled before use.

Gas chromatography

Gas chromatography (GC) was performed using a Pye Series 104 gas chromatograph equipped with a heated-head flame ionization detector (FID) and a gas-sampling valve (Pye-Unicam Ltd, Cambridge). The FID was maintained at 200°C and was operated with a hydrogen flow rate of 40 ml/min and an air flow rate of 500 ml/min. Analyses were made using a coiled glass column (275 cm long × 0.4 cm i.d.) packed with 15% FFAP (free fatty acid phase; Phase Separations Ltd, Flintshire) coated on acid-washed silanized diatomite “C” 80–100 mesh (Pye-Unicam Ltd, Cambridge). The glass column and support material were deactivated with dimethyldichlorosilane (B.D.H. Ltd, Poole) according to the method of Eik-Nes and Horning (1968), and the packed column was conditioned for 24 hr before use. The carrier-gas (“white spot” nitrogen, British Oxygen Company Ltd, Worsley) flow rate was 50 ml/min. The column oven temperature was maintained at 100°C during halothane and chloroform analyses and at 120°C for trichloroethylene and methoxyflurane analyses.

Heated precolumn

The heated precolumn device (fig. 1) is constructed from standard chromatographic components (Pye-Unicam Ltd, Cambridge). Coupling body (D) and nut with olive (E) are used to connect the various lengths of stainless steel tubing, (F) (1.47 mm o.d. and 0.73 mm i.d.). The glass to metal seal (C) is connected to glass tubing (4 mm i.d.), into which are inserted thin-walled glass liners (2 mm i.d.). The tubing passes through an injection heater assembly (B) which is held firmly in place by a spring clip (G). These
components are enclosed in a simple metal box which couples directly into the gas-sampling valve (fig. 2). The incorporation of the injection septum holder (A) with its compression “O” ring seal enables rapid and convenient changing of the glass liners.

The injection heater assembly (B) will give non-uniform heating along the length of the central glass tube (fig. 3). The heater was adjusted, therefore, to give a temperature of 100–120°C in the region where subsequent standards and blood samples were deposited upon injection.

The optimum time for vaporization within the preheater before injection onto the column was determined. With the valve in the “fill” position, standard solutions were injected through the septum and heated for 30 sec, 60 sec, and 90 sec before turning the valve to the “inject” position, and flushing the volatiles onto the GC column. A series of 10 injections was made for each preheating time, and the coefficients of variation for the peak heights obtained were 3.63, 1.27 and 1.81 respectively. A preheating time of 60 sec was subsequently used throughout the evaluation.

**Blood sampling procedure**

Whole blood was aspirated directly into glass syringes (2 ml) pretreated with heparin (100 μlitre), and the syringes were sealed with metal luer tapered caps. Samples could be stored at 4°C for 24 hr without apparent loss of halothane. The sampled blood (1 ml) was transferred via a glass tuberculin syringe from the storage syringe into a second chilled glass syringe (2 ml) containing a glass bead. The chloroform internal standard (100 μlitre) was added to the blood, excess air was ejected carefully, and the syringe was capped, shaken vigorously and stored in ice. Analyses were performed by injecting 1-μlitre samples.

**Preparation and storage of internal standard**

The method was essentially the same regardless of the internal standard used, and only that for chloroform is described below. A chloroform solution
(750 mg/100 ml water) was made up at 4°C by stirring for 4 hr with a glass-covered magnetic stirrer to effect full homogeneity. The solution was stored at 4°C in brown glass test tubes (5 ml) with ground glass stoppers. The brown glass was used to reduce photochemical decomposition of the chloroform. Care was taken to ensure that no air remained above the liquid and a clean glass bead was inserted to facilitate mixing in the absence of this head-space.

**Calibration of the chromatograph for quantitative analysis**

Aqueous solutions of halothane, chloroform, trichloroethylene and methoxyflurane were prepared volumetrically and stored at 4°C in glass stoppered tubes. Triplicate injections of each standard were made into the preheater and the peak heights subsequently obtained were measured and used to prepare standard calibration curves. A relative detector response factor (Rf) obtained from this calibration procedure was then used to calculate the “unknown” anaesthetic concentration according to the following formula:

\[
\text{Concentration (U)} = \frac{(\text{Peak height (U)})}{(\text{Peak height (I)})} \times \frac{(\text{Concentration (I)})}{\text{Rf}}
\]

where U and I refer to the unknown and internal standard, respectively.

**Evaluation of methodology**

Initially, attention was directed to the reproducibility of the response of the instrument to prepared standards, and to the stability of the internal standard in storage. Subsequently, tonometry was used to obtain an assessment of the overall accuracy of the entire method.

To assess reproducibility, a number of injection sequences, each consisting of 10 injections, were performed. Prepared standards were injected first into the precolumn/chromatograph to examine the overall response and then directly onto the column to exclude any contribution by the precolumn to the variance obtained. These standards consisted of halothane and chloroform dispersed in three media: water, blood and ether. They were prepared volumetrically and stored in glass containers at 4°C.

The stability of the internal standard preparation was determined by a single experiment in which chloroform standards were compared, after 2 months' storage, with freshly made-up standards.

To obtain an overall evaluation of the whole method inclusive of sampling, preparation and addition of internal standard, precolumn and chromatograph performance, a tonometry experiment was performed. A rotating flask tonometer was perfused with humidified air at 37.4°C containing 4.9% carbon dioxide and approximately 1.0% halothane. This mixture was obtained by mixing metered streams of carbon dioxide and medical air (B.O.C., Ltd). It was then directed through a Fluotec vaporizer (Cyprane Ltd, Keighley, Yorks) and then through two scinttered glass humidifiers located in the tonometer water bath before passing along a lagged pipe to enter the tonometer. The gases leaving the tonometer were sampled regularly and their halothane content was estimated using an interferometer (Carl Zeiss, Jena, G.D.R.) calibrated for a diluent gas mixture containing 92.6% air, 4.9% carbon dioxide and 2.5% water vapour.

When the concentration of halothane in the effluent gases had stabilized at the correct value, freshly drawn heparinized human blood (20 ml) was introduced into the tonometer. After full equilibration (90 min) four samples were taken for analysis in triplicate by GC.

A number of runs were made to ensure that the whole procedure from sampling of blood to injection into the precolumn was less than 5 min. This objective was achieved without difficulty.

**RESULTS AND DISCUSSION**

The calibration procedure confirmed the linear response of the FID to increasing concentrations of halothane, chloroform, trichloroethylene and methoxyflurane as shown in figures 4 and 5 respectively. From the gradients of these lines the relative detector responses can be obtained. For halothane, using chloroform as the internal standard, this factor is 2.67, and for trichloroethylene using methoxyflurane as the internal standard it is 1.93. However, it is equally possible to reinterpret the gradients for the measurement of chloroform and methoxyflurane using halothane and trichloroethylene, respectively, as the standards.

In evaluating the reproducibility of the response of the instrument to prepared standards, the variation in peak height ratios for halothane and chloroform in water was found to be 2.0%. For halothane and chloroform in blood, however, the coefficients of variation for three sets of 10 injections varied between the limits of 2.0-4.0%. The best results were obtained by rotating the syringe body in a circular motion two or three times after injection of the blood. This minimized the streaking of the blood along the liner into colder regions (fig. 3). Also, the glass
liners required needed to be changed after about 15 injections. By comparison, direct "on-column" injection of the halothane and chloroform in water produced a coefficient of variation of 2.9%, and the corresponding figures for similar injections of halothane and chloroform in ether and in blood were 2.4% and 4.3% respectively. The failure to reduce significantly the variance with the ether standard suggests that incomplete mixing of the agents in the water was not an important factor. Although on-column injections reintroduce slight inconsistencies in injection technique, it was concluded that the unaltered variance associated with this method was a function of the column and detector system rather than the precolumn.

In the tonometry experiment, taking the triplicate means (four in number), the mean halothane concentration was 18.99 mg/100 ml (SEM 0.50 mg/100 ml). The mean halothane concentration of the gases leaving the tonometer was 1.12% (vol./vol.). The coefficient of variation of individual samples was less than 1.0%. At the temperature and pressure of the tonometer, this gas mixture contained 8.174 mg of halothane vapour/100 ml. Using these values, the blood-gas partition coefficient for halothane was calculated to be 2.32 (18.99/8.174). Assuming that the tonometered blood had a normal blood-gas partition coefficient (2.30) (Eger, 1974), the overall error of the whole method was 0.86%.

An incidental finding was an identical retention time for halothane both when the precolumn was used and when the vapour was introduced through a normal
gas sample loop. This raises the possibility of analysing anaesthetic agents in blood using a simple gas standard.

ACKNOWLEDGEMENTS

One of us (W.J.C.) would like to thank the Medical Research Council for financial assistance. We also thank Mr P. Brooks for helpful gas chromatographic discussion, and Mrs E. M. McCreery for secretarial work.

REFERENCES


