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Note

High-performance liquid chromatographic method for the direct determination of the volatile anaesthetics halothane, isoflurane and enflurane in water and in physiological buffer solutions

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The effects of anaesthetic agents on *in vitro* cell preparations and isolated perfused organ preparations have been widely studied. The determination of the concentration of dissolved anaesthetic agents is critical when observations of the effects of these agents on cells or organs are to be made. Several methods have been utilized to administer these volatile agents to the *in vitro* preparations, *e.g.*, bubbling of the agents in the gaseous phase through the solutions or the addition of a saturated aqueous solution of the anaesthetic agents to the preparation [1,2]. It is also important to monitor the concentrations of the anaesthetics at various stages in the experiments because of the relatively rapid evaporation of these volatile substances from the warm (37°C) experimental solutions. Gas chromatography (GC) is widely utilized for the determination of the concentrations of anaesthetics [3,4]. However, it requires the extraction of the samples into an organic solvent, which is both time consuming and expensive.

We describe here a simple high-performance liquid chromatographic (HPLC) method for the direct determination of the commonly used volatile anaesthetics halothane, isoflurane and enflurane. The method makes use of the fact that halothane and to a lesser extent isoflurane and enflurane, in addition to the possession of an absorbtion band in the infrared spectrum, also absorb ultraviolet radiation [1,5,6].

EXPERIMENTAL

Chemicals

Halothane (2-bromo-2-chloro-1,1,1,-trifluoroethane) was obtained from Maybaker (Johannesburg, South Africa). Isoflurane (Forane, 1-chloro-2,2,2-trifluoroethyldifluoromethyl ether) and enflurane (Enthrane, 2-chloro-1,1,2-trifluoroethyldifluoromethyl ether) were obtained from Abott Laboratories (Johannesburg, South Africa). Toluene of analytical-reagent grade was purchased from BDH (Poole, U.K.)

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and methanol of HPLC grade from Merck (Darmstadt, F.R.G.). The water used was glass distilled.

Apparatus

The chromatographic system consisted of Waters M45 HPLC pump with a variable-wavelenght Pye Unicam PU4020 UV detector (Philips). The UV detector was operated at 0.005 a.u.f.s. The samples were injected (injection volume 20 μl) using a Rheodyne 7125 loop injector onto the column (Nova-Pak C_{18}, Radial Compression Module, 10 cm × 8 mm I.D., 4 μm spherical particles (Waters Assoc., Milford, MA, U.S.A.) equipped with an RCSS Guard-Pak μBondapak C_{18} precolumn cartridge (Waters Assoc.). The chromatograms were recorded and processed by a Spectra-Physics Chrom Jet SP 4400 recording integrator. The UV spectra of the investigated volatile anaesthetics dissolved in the mobile phase were measured using a Pye Unicam PU 8800 UV–VIS scanning spectrophotometer (Philips).

Chromatographic conditions

The eluent was methanol–water (50:50, v/v). The flow-rate was 3.5 ml/min. The temperature was ambient and the detector wavelength was set at 210 nm for halothane and 203 nm for isoflurane and enflurane determinations. The mobile phase was degassed and filtered under vacuum through 0.5-μm Millipore FH filters before use.

Procedure

For the preparation of the calibration graph various amounts of volatile anaesthetics were dissolved in methanol and spiked with phosphate buffer in order to obtain the desired final concentrations in buffer (1 μmol/l–1 mmol/l of halothane and 0.2–20 mmol/l of isoflurane and enflurane). After mixing in a tightly closed glass tube, 50 μl of spiked buffer were added to 50 μl of internal standard mixture (0.05 mmol/l toluene in methanol). A 20-μl volume of the final sample was injected onto the column.

RESULTS AND DISCUSSION

The UV spectra (Fig. 1A) show that the anaesthetic agents dissolved in methanol–water (50:50, v/v) as the mobile phase exhibited maximum absorbance of halothane at 210 nm and of both isoflurane and enflurane at 203 nm. It must be stressed, however, that when the absorptions of all three anaesthetics were compared at the same molar concentrations in the mobile phase, the absorption of halothane was at least 200 times greater than that of either enflurane and isoflurane. Under the chosen chromatographic conditions we were able to separate and detect at 203 nm all three anaesthetics in one sample (Fig. 1 B and C). In an experimental situation, for which the proposed method is intended, all three agents would not be present in the same sample. We therefore used toluene as an internal standard for the three different anaesthetics.

The calibration graph was prepared with phosphate buffer spiked with the anaesthetics diluted with methanol in the ranges 1 μmol/l–1 mmol/l of halothane and 0.2–20 mmol/l of isoflurane and enflurane. Over these wide ranges the assay showed a

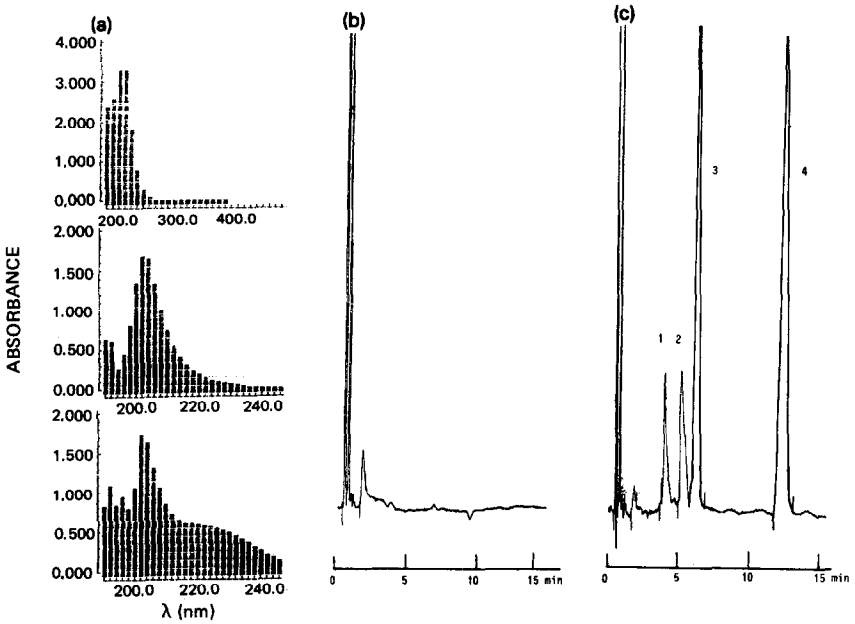

Fig. 1. (A) UV spectra for halothane, isoflurane and enflurane dissolved in mobile phase. The concentration of halothane in mobile phase was 200 times lower than those of isoflurane and enflurane. Chromatograms from injection of (B) blank phosphate buffer and (C) sample of phosphate buffer spiked with 0.1 mmol/l of halothane and 10 mmol/l of isoflurane and enflurane. UV detection at 203 nm. Peaks: 1 = enflurane; 2 = isoflurane; 3 = halothane; 4 = toluene (internal standard).

high degree of linearity on a line forced through the origin and the intra- and inter-assay relative standard deviations (R.S.D) were low (Table I). The sensitivity of the method is adequate for monitoring the concentrations of the anaesthetics in buffers through which the compounds are bubbled at levels as low as 0.5%. It was also found

TABLE I

CHARACTERISTIC PARAMETERS OF THE HPLC ASSAY OF VOLATILE ANAESTHETICS

Limit of detection = sample concentration when compound signal-to-noise ratio = 3, Correlation coefficient refers to the linear fitted calibration graph. Intra-assay R.S.D.: on each of three different days three samples each containing 0.1 mmol/l of halothane or 10 mmol/l of either isoflurane or enflurane were analysed; the R.S.D. of the three samples for each of the three days was calculated, the average of these R.S.D.s was taken and is reported as the intra-assay R.S.D. Inter-assay R.S.D. = the R.S.D. calculated collectively for all nine samples for each compound. Accuracy = the deviation of the average of the nine samples for each compound from the expected value of the concentration of the anaesthetic.

Parameter	Halothane	Isoflurane	Enflurane
Limit of detection (mmol/l)	0.001	0.2	0.2
Correlation coefficient	0.981	0.966	0.958
Intra-assay R.S.D. (%)	4.6	6.1	8.5
Inter-assay R.S.D. (%)	4.9	7.4	8.9
Accuracy (%)	5	6.5	10

that the anaesthetics could be determined directly in various types of buffers (phosphate-buffered saline, Krebs, Hanks) without disturbing the chromatographic conditions after direct injection of a buffer sample onto the column (not shown).

Many experiments of the effects of anaesthetic agents on isolated systems are performed in buffer solutions. The direct determination of an anaesthetic of interest in the buffer solution can be easily carried out without the use of an additional extraction procedure such as would be required with the use of a more sophisticated GC extraction technique.

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