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Short communication

High-speed capillary gas chromatography for determination of inhalation anesthetics

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Abstract

To increase sample throughput for GC analysis of inhalation anaesthetics without affecting the separation of nitrous oxide (N_2O) and halogenated anaesthetics (sevoflurane, isoflurane and halothane), we explored the effectiveness of a tailor-shortened (12 m) PlotQ capillary column and developed a high-speed version of a previously reported GC technique (involving chromatographic separation of analytes using a GC–MS system, coupled with a selected ion monitoring (SIM) method to increase sensitivity). Efficient separation and repeatable results were achieved at a reduced runtime of ~7 min (versus 18 min with the original method) at a carrier gas flow of 1.5 ml/min. This approach should more than double the previous throughput.

Keywords: High speed GC; GC–MS; Inhalation anesthetics; Carrier gas flow

1. Introduction

Ever since the introduction of capillary GC columns by Golay in 1958 [1], speed of analysis has been a key objective for chromatographers. Higher sample throughput obtained with increased GC separation can improve laboratory efficiency [2] and significantly reduce costs. However, conventional GC methods for mixtures of medium complexity (<30 components) involve long columns, slow programmed temperature rates, and consequently high time-related costs [3].

Approaches to improve the speed of analysis include decreased length and internal diameter of columns (e.g. 0.10 mm), thinner stationary phase film thickness, increased carrier gas linear velocities, and faster oven temperature programming rates. Alternatively, carrier gas flow-modulation [4] and direct resistive heating of the capillary column [5] have been proposed to obtain high-speed separation. However, changes of analysis parameters often lead to loss of peak resolution.

In our inhalation anaesthetics laboratory, we routinely analyze a large number of biological and environmental samples utilizing an internally developed, validated single-run GC method for rapid and quantitative screening of commonly used inhalational anesthetics [6]. The aim of this work was to develop a high-speed version of this GC technique with reduced runtime without any loss of resolution. This could provide a valuable way of increasing sample throughput for screening and analysis of inhalation anaesthetics (both N_2O and halogenated anesthetics).

2. Experimental

2.1. Instrumentation

Chromatographic separation of analytes was performed using a conventional GC–MS quadrupolar system (GCD; Agilent Technologies) with electronic pressure control coupled with a static headspace sampler system (HP 7694; Agilent Technologies, Little Falls, DE, USA) equipped with a fixed-volume (1 ml) loop.

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2.2. GC conditions

The GC injector was operated with the usual split ratio (1:20) at 200 °C. A conventional capillary PoraplotQ (0.25 mm i.d., 8 μm thickness) GC column (Varian, Walnut Creek, CA, USA) was hand-shortened to 12 m and installed in the GC oven. The carrier gas (helium) was set at different flows (1–2 ml/min). The GC oven was held at different temperatures (35–40 °C), and ramped at 40–70 °C/min to final temperatures 120° or 160 °C. MS interface temperature was set to 280 °C.

2.3. Preparation of standards

After settings of best GC separation conditions, known amounts of N_2O (0.36–3.6–36.0 μg), sevoflurane (0.06–0.6–6.0 μg), isoflurane (0.06–0.61–6.1 μg) and halothane (0.07–0.7–7.5 μg) were spiked into 22 ml presealed headspace vials from a primary certified source of N_2O (20,000 ppm; SIAD, Bergamo, Italy) and from primary in carbon disulfide stock solutions, respectively. After conditioning at 41 °C for 60 min, 1 ml of mixture air from each vial was injected in the chromatographic system.

3. Results and discussion

Under standard operating conditions with 0.25 mm i.d. PlotQ columns 25 m long, chromatographic separation of these analytes is performed in 18 min [6]. To reduce the runtime, we initially thought of using a column with an internal diameter <0.25 mm. However, no such PlotQ column turned out to be commercially available. Use of short columns has been proposed to speed up GC separation [7]. We therefore performed a series of tests to investigate how we could reduce runtime by modifying column length and optimizing the other parameters (initial oven temperature, temperature ramping rate, and carrier gas flow).

We started by tailoring the column length to 12 m. We initially applied the conditions used for the 25 m column (starting temperature 40 °C; ramping rate, 40 °C/min; final temperature, 160 °C for 5 min; carrier gas flow, 1.0 ml/min). However, under these conditions, there was a negative peak in correspondence with the sevoflurane retention time. This problem was overcome by increasing the temperature ramp rate to 70 °C/min, with a final temperature of 120 °C being held for 5 min, the starting oven temperature being set to 35 °C for 1 min in order to maintain effective separation of N_2O from CO_2. Furthermore, the carrier gas flow was increased to 1.5 ml/min (foreline pressure, 95 m Torr) with no negative effect on peak resolution or increase of speed (whereas at a flow rate of 2 ml/min, the N_2O and CO_2 peaks partially overlapped and the foreline pressure rose to 120 mTorr). Under the conditions described above (and in Table 1), we were able to obtain complete resolution of four different anaesthetic agents in approximately 7 min (Fig. 1). Three selected ion monitoring (SIM) windows with a total of six ions were used to provide selectivity and sensitivity,

Table 1
Description of GC parameters of the high-speed method for biological and environmental monitoring of inhalation anaesthetics

Injection split ratio: 20:1
Injector temperature: 200 °C
MS interface temperature: 280 °C
Oven temperature: 35 °C (1 min) then ramp to 120 °C at 70 °C/min.
Runtime: 7.21 min

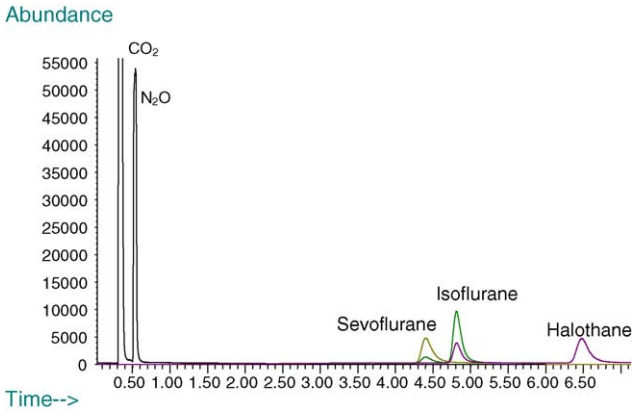

Fig. 1. Reliable and sensitive high speed GC separation of inhalation anesthetics with a short PoraplotQ column. Chromatogram of total ion current showing the four quantifier ions used: $m/z = 30$ (N_2O, 89 ppm), $m/z = 131$ (sevoflurane, 3.3 ppm), $m/z = 51$ (isoflurane, 3.6 ppm) and $m/z = 117$ (halothane, 4.2 ppm).

Table 2
Description of MS-SIM conditions of the high-speed method for biological and environmental monitoring of inhalation anaesthetics

Analyte	Quantifier ion (m/z)	Qualifier ion (m/z)	Dwell time (ms)
N_2O	30	44	30
Sevoflurane	131	51	60
Isoflurane	51	117	60
Halothane	117	198	60

Table 3
Linear range, correlation coefficient (R^2) and precision (determined at intermediate concentrations: N_2O, 89 ppm; sevoflurane, 3.3 ppm; isoflurane, 3.6 ppm; halothane, 4.2 ppm) for each volatile anaesthetic sampled by static headspace sampler and analyzed by GC–MS

Analyte	Range (ppm)	R^2	Intra-day precision (RSD $n = 5$)	Inter-day precision (RSD 3 days)
N_2O	8.90–889.60	0.9999	3.2	13.5
Sevoflurane	0.33–33.21	0.9997	9.1	18.8
Isoflurane	0.36–35.91	0.9997	8.0	16.6
Halothane	0.42–42.16	0.9995	9.4	16.1

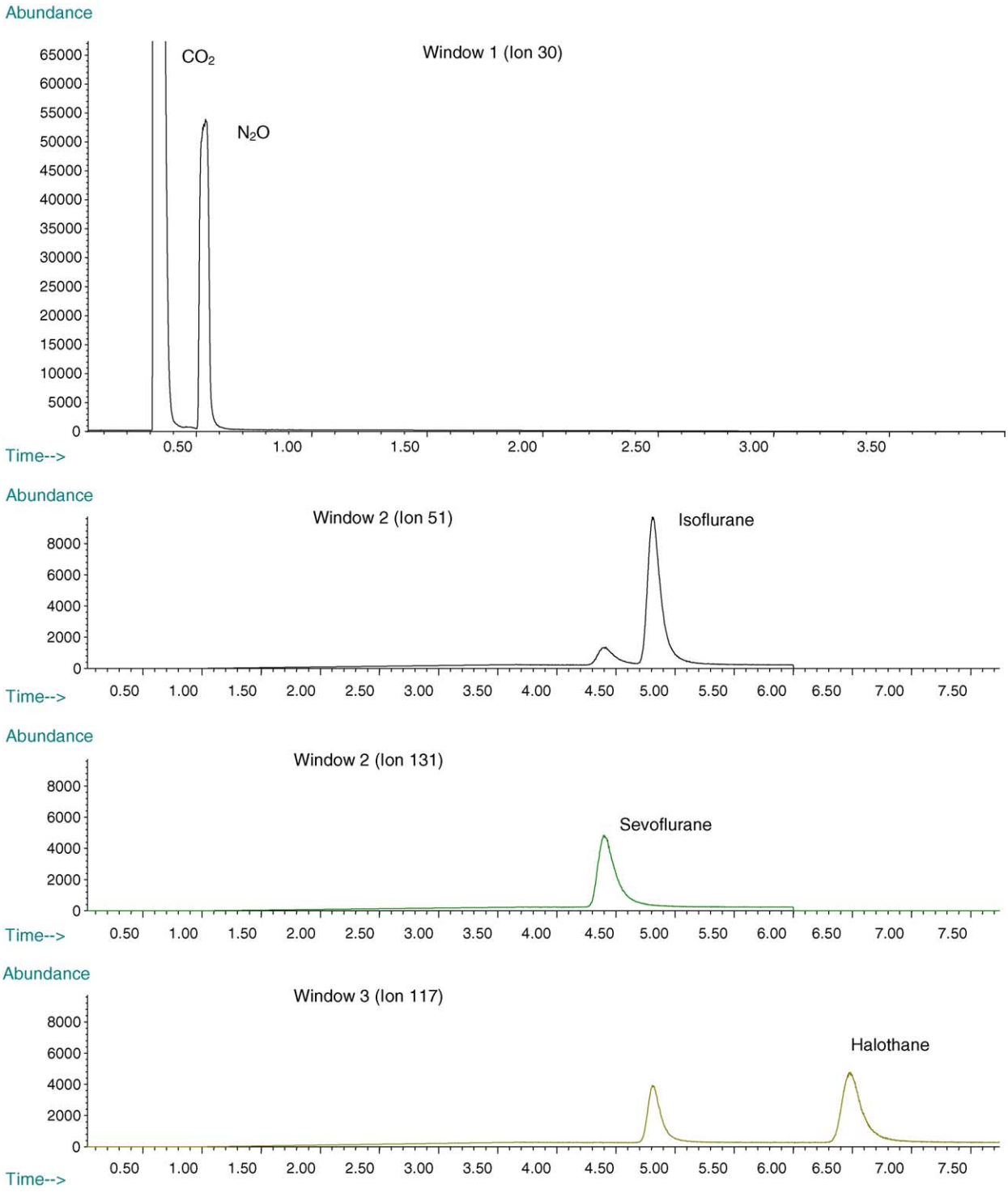

Fig. 2. Single-ion windows of an air-standard mixture containing spikes of N_2O (89 ppm), sevoflurane (3.3 ppm), isoflurane (3.6 ppm) and halothane (4.2 ppm) under the described high speed GC conditions.

as shown in the Table 2 and in the Fig. 2. Linearity and precision values are shown in Table 3. Four-point (three spiked plus blank vials) calibration plots showed good linearity in the range of airborne operative concentrations for each analyte. Precision was similar to that obtained with a conventional length PoraplotQ column (intra-day %RSD <5% for N_2O and <10% for each fluorinated anesthetic) [8]. The runtime of the analysis was reduced from 18 to approximately 7 min. Allowing for the oven's ∼9 min cool-down time (from 120 °C to the starting temperature of 35 °C) the described set

up provides a total GC cycle time of 16–18 min, more than doubling throughput with respect to our previously reported method [6].

In conclusion, the set up described using a shortened PoraplotQ column allows high speed analysis of volatile anaesthetics while maintaining the same sample capacity, resolution power and analytical linearity and repeatability of the original method. A similar approach could be explored with chiral GC phase for separation of racemic mixtures of fluorinated anesthetics [9].

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