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Short Communication

Determination of cyclohexanone in aqueous solutions stored in PVC bags by isotope dilution gas chromatography–mass spectrometry

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Introduction

Cyclohexanone is commonly used as a welding agent in assembling PVC biomedical devices. Because of its chemical properties (high boiling point, relatively low vapour pressure) it is difficult to evaporate the residual cyclohexanone in the welded objects. Determination of cyclohexanone in intravenous solutions stored in PVC bags has been reported [1, 2] as well as in the material of fabrication [2]. In the work concerning the quantitative determination of cyclohexanone in intravenous solutions, the methods used were gas chromatography (GC) with flame ionization detection [1] and liquid chromatography with UV-detection [2]. Apart from these studies, a number of methods based on GC, gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC), which enable the determination of cyclohexanone, have been published [3–14]. Most of these methods were designed for profiling metabolites in biological samples and are generally less accurate quantitatively (cf. refs 6, 8 and 9). Furthermore, none of these methods involved the use of isotopically labelled internal standards.

The objective of the present study was to establish a simple but highly accurate method for the determination of cyclohexanone in various aqueous solutions stored in PVC bags. The approach to meet this requirement, a method based on isotope dilution–capillary GC–MS with deuterium labelled cyclohexanone as internal standard was developed.

Experimental

Aqueous samples of 5 ml vol are taken, and to these 18.08 μg 2,2,6,6-$^2H_4$-1-cyclohexanone (MSD Isotopes, Montreal, Canada) dissolved in 0.1 ml saline, 0.9 g

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l^{-1}, is added as internal standard. After equilibration for 30 min, 1 ml hexane is added and the solution thoroughly mixed for 15 min and then centrifuged. Two hundred microlitres of the organic layer is transferred to a 300 μl vial (Chromacol, London, England) for injection into the GC–mass spectrometer. In the case of used continuous ambulatory peritoneal dialysis (CAPD) solutions, proteins are precipitated with 100 μl perchloric acid (70%) prior to assay. To verify the suitability of the method different brands of CAPD solutions containing different glucose concentrations were examined, as well as one used dialysis solution. Furthermore, one brand of intravenous solution at two different bag volumes was examined.

Analyses were performed on a Shimadzu QP-1000 GC–mass spectrometer (Shimadzu, Kyoto, Japan). The instrument was equipped with a Durabond-5 column, 15 m × 0.22 mm i.d. (J&W Scientific, Folsom, USA). The column, which was temperature programmed from 50°C (1 min) to 100°C, at 10°C min^{-1}, was interfaced directly to the ion source of the mass spectrometer. Injections were carried out in the split mode at a split ratio of 1:20. The transfer line and the ion source were kept at 250°C, and the instrument was operating in EI mode with an electron energy of 70 eV. The mass spectrometer was operated in the SIM mode, monitoring the molecular ion of cyclohexanone (*m/z* 98) and deuterium labelled cyclohexanone (*m/z* 102). The isotopic distribution of the internal standard at *m/z* 98 was 0.1% (defined as the ratio of the ion intensity at *m/z* 98 to *m/z* 102).

Results and Discussion

The above method for quantitative determination of cyclohexanone has been used in the concentration range of 0–5 mg l^{-1} (see Fig. 1). The lower level of detection, defined as injected amount (cyclohexanone dissolved in hexane) was 200 pg at a signal to noise ratio of 2:1 (split ratio 1:20).

The lower limit of detection of the method (spiked saline solution), using a 4-μl injection volume, was 30 μg l^{-1}. Thus, the devised method covers the concentration range of cyclohexanone generally found in dialysis and intravenous solutions, as shown in Table 1. The relative standard deviation (RSD) of the method for determination of cyclohexanone (2.71 mg l^{-1}) in a typical CAPD solution was 1.4% (n = 6).

The amount recovered at each extraction was calculated according to the theory of repeated gas extraction of solid samples proposed by Westendorf [15]. The amount recovered from the original sample is expressed by the following equation:

$$A_1 = T \times R, \quad (1)$$

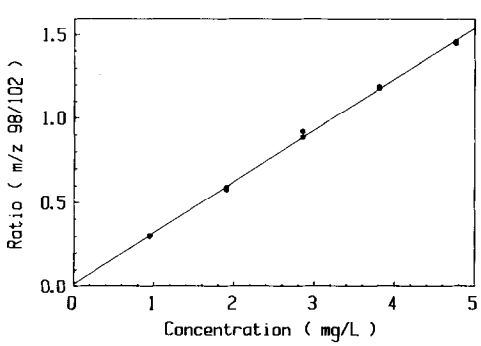

Figure 1
The ratio between peak areas at *m/z* 98 and *m/z* 102 in selected ion monitoring of different standard mixtures to which a fixed amount (i.e. 18.08 μg) of (2,2,6,6-$^2H_4$)cyclohexanone had been added. The correlation coefficient is 0.9994.

Table 1

Brand	Glucose (mg ml^{-1})	Volume (ml)	Cyclohexanone (mg l^{-1})
Dialysis solutions			
A	15	2000	ND
A	23	2000	ND
A	42.5	2000	ND
B	25	2000	ND
C	40	2000	ND
D	15	1500	ND
E	38.6	2000	2.75
E	38.6	2000	1.83
E	22.7	2000	2.06
E	22.7	2000	2.71
E	13.6	2000	2.37
E bag 1			2.32
E bag 2	13.6*	2000	2.42
E bag 3			2.20
E bag 4			1.89
Used dialysis solution			
E	22.7	2000†	0.55
Intravenous solutions			
E, NaCl 9 mg ml^{-1}		500	6.05
E		50	9.24

ND, not detectable.
*Bags with the same batch number.
†Initial volume. Volume after use: 2550 ml.

where A_1 is the amount recovered at the first extraction, T is the total amount of analyte originally present in the sample, and R is the recovery factor. The amount recovered in the second extraction step, A_2, is consequently:

$$A_2 = (T - A_1)\, R. \tag{2}$$

Combination of equations (1) and (2) gives:

$$T = \frac{(A_1)^2}{A_1 - A_2}. \tag{3}$$

Since A_1 and A_2 are experimentally determined values generated by repeated extractions, equations (1) and (3) can be solved simultaneously to find values for T and R. Furthermore, the validity of the values obtained can be controlled by comparison of the experimentally determined A_3 value with that calculated A_{3c} [15]:

$$A_{3c} = (T - A_1 - A_2)\, R. \tag{4}$$

The extraction recovery factor of the method as determined by repeated extractions of spiked dialysis solution was 0.34 (25°C). Although, a single extraction step being far from complete, highly reliable quantitative data are generated due to the isotope-dilution technique. Furthermore, the use of hexane minimizes the extraction of more polar constituents which ensures longer column life and reduces the risk of peak interference.

Although no extensive hydrogen–deuterium exchange studies have been carried out, the precision of the method indicates that no significant exchange occurs. Furthermore, since the precision of the determination of cyclohexanone in used CAPD solutions is good (2.0%; $n = 6$), it was concluded, despite the presence of perchloric acid in this case, that no major exchange of hydrogen occurred.

In the dialysis where cyclohexanone is found the concentration varies between 1.83–2.75 mg l^{-1} for unused solutions, whereas in the one used solution examined, the concentration of cyclohexanone was 0.55 mg l^{-1}. The increase of cyclohexanone in the smaller volumes is probably due to an increase of the surface to volume ratio, i.e. the proportional contact area of the solution is higher for small volume samples.

As shown by Fig. 2, in the selected ion monitoring chromatogram a second peak is present. This compound was identified to be 2-ethylhexanol, by retention time and mass spectrum. 2-Ethylhexanol is a commonly found hydrolysis product of the PVC plasticizer, di(2-ethylhexyl)phthalate.

The present method provides a simple, selective and accurate method for determination of cyclohexanone in aqueous solutions as dialysis and intravenous solutions, with a sample throughput of the separation and detection step of <10 min. This combined with the simple work-up procedure renders the method suitable for quantitative determinations of a large number of samples.

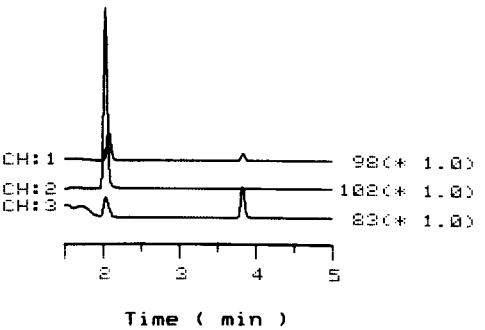

Figure 2
Reconstructed ion chromatogram of a used dialysis solution to which (2,2,6,6-$^2H_4$)cyclohexanone had been added. The peak at the retention time 3.8 min is due to 2-ethylhexanol, a hydrolysis product of the PVC plasticizer di(2-ethylhexyl)phthalate.

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