Journal of Chromatography, 269 (1983) 108–113
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,070

Note

Quantitative determination of allyl chloride (3-chloropropene) in rat blood by electron-capture gas chromatography and gas chromatography–mass spectrometry with selective ion monitoring

BRUCE E. KROPSCOTT*, PAT E. KASTL and EMILE A. HERMANN
Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical U.S.A., Midland, MI 48640 (U.S.A.)
(Received June 14th, 1983)

Allyl chloride (3-chloropropene, $CH_2=CHCH_2Cl$) is a volatile and highly flammable liquid which is used as a chemical intermediate in the manufacture of monomers, such as epichlorohydrin, glycerol dichlorohydrins, and allyl alcohols. The present time-weighted average (TWA) exposure limit for up to a 10-h workday in a 40-h workweek is 1.0 ppm with a ceiling concentration of 3 ppm for any 15-min sampling period[1].

An analytical method was required to determine low levels of allyl chloride in blood to assess the pharmacokinetic parameters of allyl chloride in rats. The method would require a rapid extraction followed by a selective and sensitive mode of detection. Previous analytical methods for allyl chloride have centered around occupational and environmental monitoring of air samples[2–4]. None of these previous methods was suitable for this application. An electron-capture gas chromatographic method (GC–ECD) was developed to determine at concentrations of allyl chloride in blood as low as 24 ng/ml, which was the minimum limit of detection required for the pharmacokinetic study.

Allyl chloride in rat blood can also be quantitated by selective ion monitoring utilizing either chemical ionization (CI) or electron impact (EI) ionization gas chromatography–mass spectrometry (GC–MS). The ions monitored were 77,79 (m/z) for CI and 76,78 (m/z) for EI. The limit of detection was *ca.* 500 ng/ml using either GC–MS procedure.

MATERIALS AND METHODS

Materials

Allyl chloride was supplied by Dow Chemical (Midland, MI, U.S.A.). Distilled UV hexane was obtained from Burdick & Jackson (Muskegon, MI, U.S.A.). Adult male Fischer 344 rats were purchased from the Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). Following cervical dislocation, fresh blood was withdrawn from the Fischer rats via open chest heart puncture into a syringe containing heparin.

TABLE I

GC–MS PARAMETERS FOR EI AND CI

Parameters	*EI*	*CI*
Ions monitored (*m/z*)	76, 78	77, 79
Carrier/reagent gas	Helium	Methane
Flow-rate	25 ml/min	–
Source pressure	–	500 μmHg
Injector temperature	150°C	150°C
Column temperature	85°C	85°C
Separator/transfer line temperature	270°C	250°C
Ionization energy	70 eV	100 eV
Preamplifier gain	10^{-8} A/V	10^{-8} A/V

Instrumentation

A Varian 3700 gas chromatograph equipped with a ^{63}Ni electron-capture detector and CDS-111 integrator was used for the quantitative analysis of the samples. A glass column (3.5 m × 2 mm I.D.) packed with 20% Carbowax 20M on 80–100 mesh Chromosorb W AW (Supelco, Bellefonte, PA, U.S.A.) was used to obtain separation. The column was conditioned overnight at 200°C with a nitrogen flow-rate of 15 ml/min. During the analysis the column was maintained at 80°C with a nitrogen flow-rate of 15 ml/min. The injection port and detector temperatures were maintained at 150°C and 250°C, respectively.

The gas chromatograph–mass spectrometers for EI and CI were Finnigan Models 3000 and 3200, respectively. Each mass spectrometer was equipped with a Model 6010 data handling system. The chromatographic columns were as described above. The GC–MS parameters are listed in Table I.

TABLE II

PERCENTAGE RECOVERY OF ALLYL CHLORIDE FROM RAT BLOOD BY GC–ECD

Targeted concentration of allyl chloride rat blood (ng/ml)	*Dilution**	*Recovery of allyl chloride in rat blood*** (%)*			*Mean recovery (%)*	*Standard deviation*	*Coefficient of variation*
		1	*2*	*3*			
$2.35 \cdot 10^1$	–	91.1	95.3	93.2	93.2	2.1	2.3
$4.69 \cdot 10^1$	–	97.0	93.6	92.3	94.3	2.4	2.5
$9.35 \cdot 10^1$	–	92.2	92.1	95.2	93.2	1.8	1.9
$1.17 \cdot 10^2$	–	100.7	99.0	90.9	96.9	5.2	5.4
$4.69 \cdot 10^2$	–	91.0	96.2	91.1	92.8	3.0	3.2
$1.17 \cdot 10^3$	1/5	88.7	90.0	97.5	92.1	4.8	5.2
$1.05 \cdot 10^4$	1/41	83.2	92.9	93.5	89.9	5.8	6.5
$2.11 \cdot 10^4$	1/41	90.1	89.8	89.1	89.7	0.5	0.6
Overall					92.8	3.6***	3.9§

* Samples above $4.69 \cdot 10^2$ ng/ml were diluted into the linear range.

** Each data point is the mean of two determinations.

*** Average standard deviation (σ) = $[\Sigma(\sigma^2)/n]^{1/2}$.

§ Average coefficient of variation (C.V.) = $[\Sigma(\text{C.V.}^2)/n]^{1/2}$.

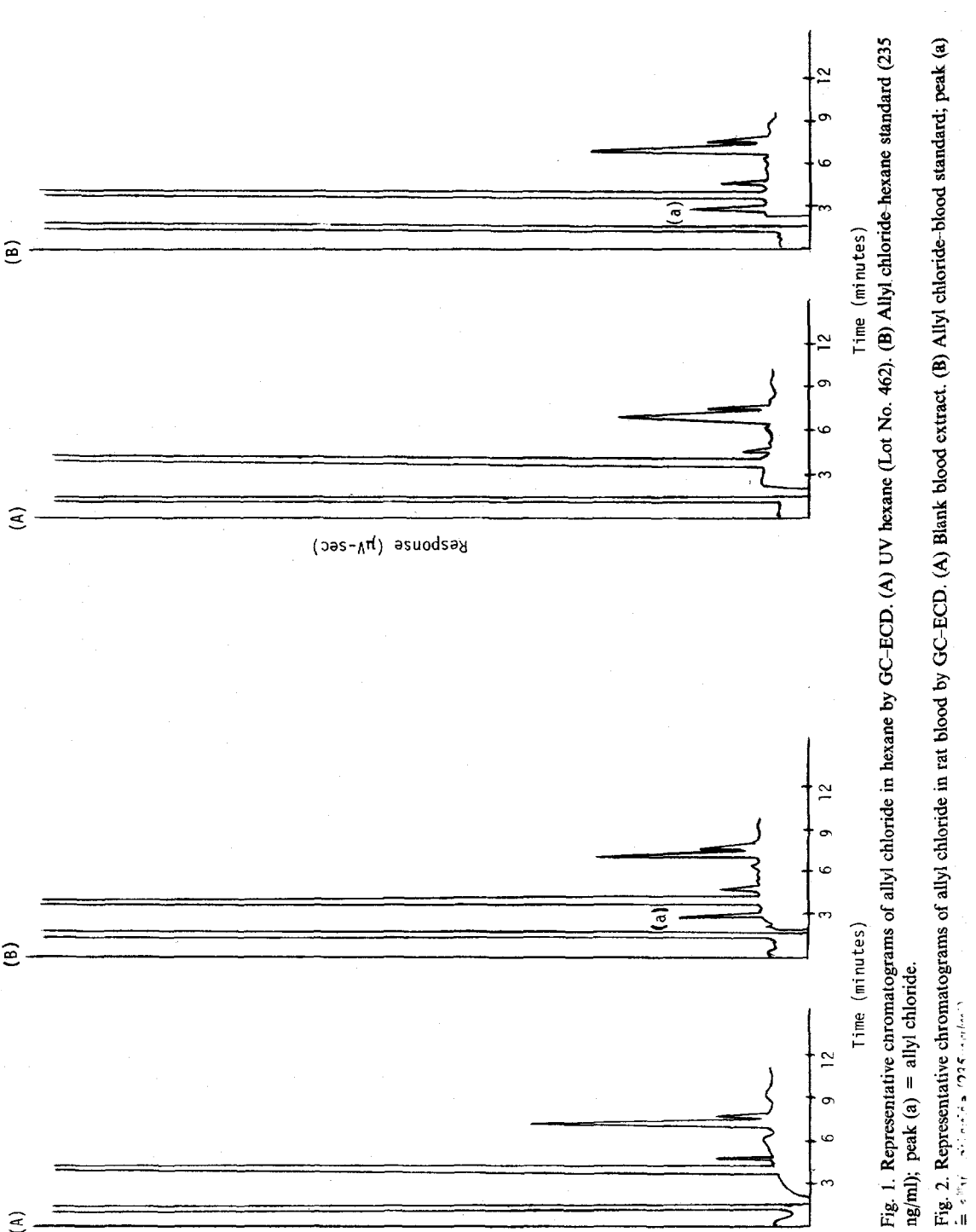

Fig. 1. Representative chromatograms of allyl chloride in hexane by GC–ECD. (A) UV hexane (Lot No. 462). (B) Allyl chloride–hexane standard (235 ng/ml); peak (a) = allyl chloride.

Fig. 2. Representative chromatograms of allyl chloride in rat blood by GC–ECD. (A) Blank blood extract. (B) Allyl chloride–blood standard; peak (a) = allyl chloride (235 ng/ml).

Extraction method

The blood standards were extracted with 200 μl of hexane, vigorously vortexted for 1 min and then centrifuged for 1 min. Then 2 μl of the organic supernatant were injected directly into the chromatograph. All blood standards with an allyl chloride concentration above 469 μl/ml were diluted into the linear range of the electron-capture detector.

Standards

A standard stock solution was prepared by adding an accurately measured amount of allyl chloride to a known volume of hexane. Allyl chloride–hexane standards were prepared by serial dilutions of the stock solution into a concentration range of 18.8–469 ng/ml.

The allyl chloride spiking solutions were prepared by adding an accurately measured volume of allyl chloride to a known volume of 95% ethanol and then serially diluting with a known volume of 95% ethanol to yield solutions of 938 ng/ml to 844 μg/ml.

The allyl chloride blood standards were prepared by adding 5 μl of an appropriate spiking solution to an accurately weighed sample (*ca.* 200 mg) of fresh blood. After spiking, the standards were shaken by hand for *ca.* 15 sec to ensure mixing.

RESULTS AND DISCUSSION

The mean percent recovery by GC–ECD for the eight allyl chloride blood

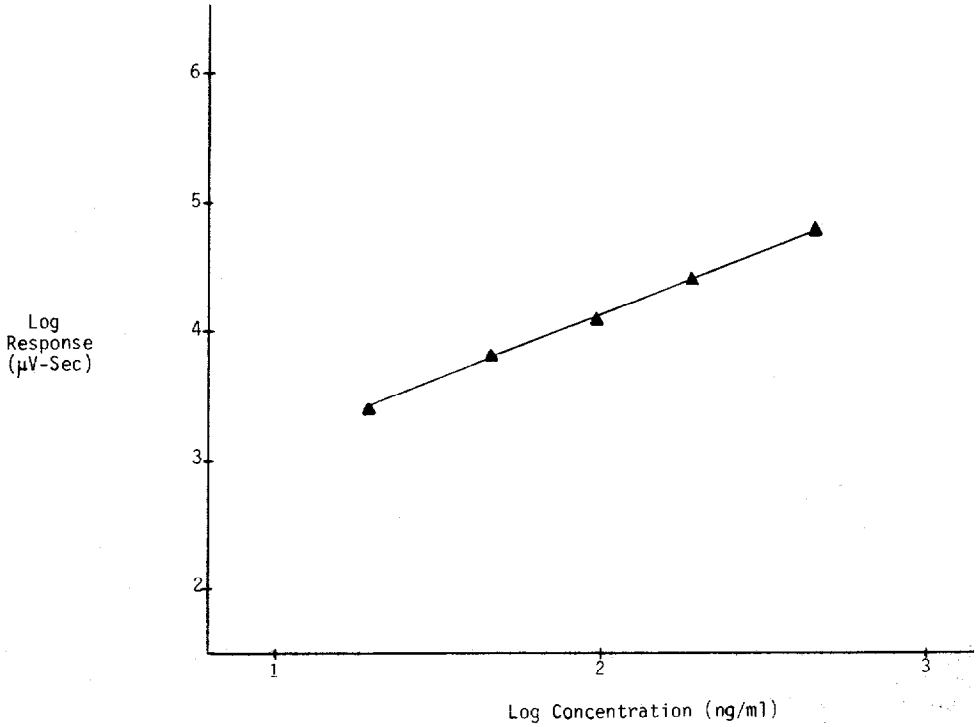

Fig. 3. Log/log plot of detector response *versus* allyl chloride concentration in hexane by GC–ECD.

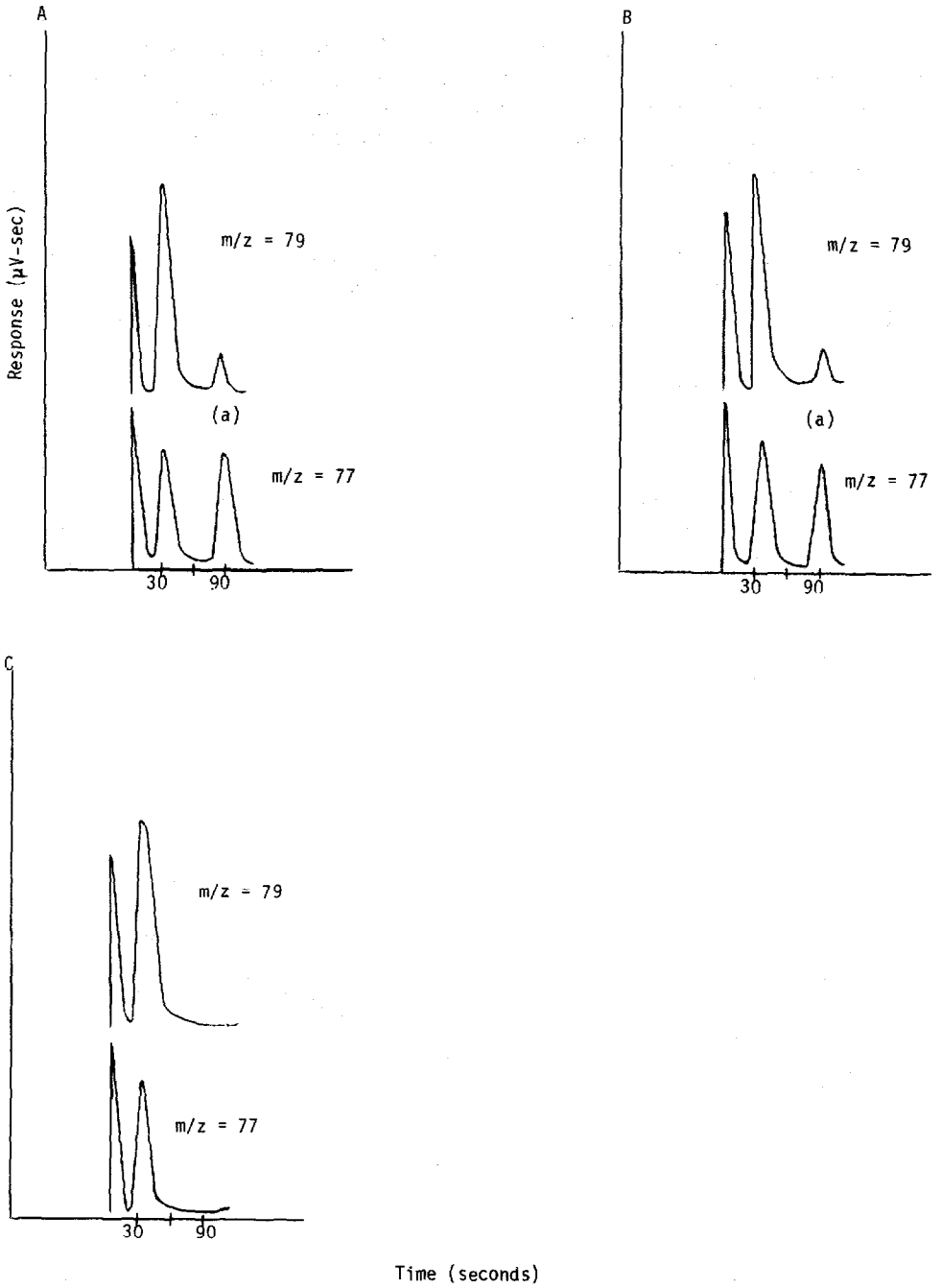

Fig. 4. Representative chromatograms from the Finnigan (CI) gas chromatograph–mass spectrometer (selective ion monitoring). (A) Allyl chloride–hexane standard (23.5 μg/ml); peak (a) = allyl chloride. (B) Allyl chloride–blood extract; peak (a) = allyl chloride. (C) UV hexane blank (Lot No. 385).

concentrations ranged from 89.7 to 96.9% (Table II). The overall percent recovery for the entire concentration range, 23.5 ng/ml to 21.1 μg/ml, was 92.8 ± 3.6%. The coefficient of variation for all eight blood concentrations ranged from 0.6 to 6.5%.

Representative GC-ECD chromatograms of a hexane blank and allyl chloride–hexane standard (235 ng/ml) are displayed in Fig. 1. The hexane should be analyzed, since interference peaks will vary from lot to lot and may produce peaks which could interfere with the allyl chloride analysis. Representative GC–ECD chromatograms of a blood extract and a blood standard (235 ng/ml) are displayed in Fig. 2. No peaks which would interfere with the allyl chloride analysis were noted in the hexane blank or blood extract blank. The GC–ECD retention time for allyl chloride was 3.0 min. The response was linear for a concentration range of 18.8 ng/ml to 469 ng/ml, as determined by the linear regression correlation coefficient of 0.9999 (Fig. 3). Allyl chloride could be observed at 9.4 ng/ml but the peak could not be reproducibly integrated.

The determination of allyl chloride in blood can be achieved by CI or EI GC–MS by selective ion monitoring. GC–MS enabled a shorter analysis time (2–3 min) than GC–ECD (10–15 min), which required more time for the remainder of the solvent peaks to elute from the column, but did not have the required sensitivity for the entire sample concentration range. The molecular ion (M) and associated chlorine isotope ion (M + 2) were monitored by EI. The pseudomolecular ions (M + 1 and M + 3) were monitored by CI. The recoveries were not determined for the GC–MS procedures but samples analyzed by both GC–ECD and GC–MS, gave comparable results. The limit of detection was *ca.* 500 ng/ml using the 1:1 blood–hexane ratio. Representative CI selective ion monitored chromatograms of an allyl chloride standard (23.5 μg/ml), an allyl chloride/blood extract, and a hexane blank are displayed in Fig. 4. No interfering peaks were observed in the hexane or blood blanks.

CONCLUSIONS

The method described in this paper is rapid, sensitive and specific for the determination of allyl chloride in rat blood for the concentration range 23.5 ng/ml to 21.1 μg/ml by GC–ECD. Allyl chloride blood concentrations above 469 ng/ml were diluted into the linear range of the electron capture detector, 18.8–469 ng/ml. Allyl chloride in rat blood can also be determined by selective ion monitoring using either CI or EI GC–MS. The limit of detection using GC–MS was *ca.* 500 ng/ml.

REFERENCES

1 NIOSH, Criteria for a recommended standard ... occupational exposure to allyl chloride, NIOSH 76-204, U.S. Department of Health, Education and Welfare, Washington, DC, 1976, p. 1.
2 K. J. Krost, E. D. Pellizzari, S. G. Walburn and S. A. Hubbard, *Anal. Chem.*, 54(4) (1982) 810.
3 F. H. Reid and W. R. Halpin, *Amer. Ind. Hyg. Assoc. J.*, 29 (1968) 390.
4 C. L. Fraust and E. R. Hermann, *Amer. Ind. Hyg. Assoc. J.*, 27 (1966) 68.