Journal of Chromatography, 398 (1987) 347–350
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 592

Note

Quantitative determination of 1,2-butylene oxide in whole rat blood by gas chromatography–mass spectrometry

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(First received November 5th, 1986; revised manuscript received April 3rd, 1987)

The alkylene oxides, such as ethylene, propylene and butylene oxides, are used extensively as chemical intermediates in the production of glycols, polyglycols, alkanolamines, glycol ethers and surfactants. The butylene oxides are highly flammable liquids that are available commercially as the single 1,2-isomer or as a mixture of 1,2- and 2,3-isomers. They are prepared commercially from butylene through the intermediate butylene chlorohydrin.[1]

The purpose of this study was to develop an analytical method to quantitate low levels of 1,2-butylene oxide in whole rat blood by gas chromatography–mass spectrometry with multiple ion detection (GC–MS-MID) in support of pharmacokinetic studies. No known analytical methodology existed for this material or related alkylene oxides in whole blood.

EXPERIMENTAL

Chemicals and solvents

1,2-Butylene oxide is a clear colorless liquid with the following physical and chemical properties: molecular formula, C_4H_8O; molecular weight, 72.1; specific gravity (25/25°C), 0.826; boiling point (°C at 760 mm Hg), 62.0–64.5; flash point (°C, closed cup), −22; explosive limits, 1.7–19.0% (v/v) in air.

1,2-Butylene oxide (lot No. 167) was supplied by Dow Chemical (Midland, MI, U.S.A.). No impurities greater than 0.1% were detected in the sample via GC, and the structure of this sample was confirmed by electron impact ionization GC–MS. The observed mass spectrum was consistent with that expected for butylene oxide. All solvents were Burdick and Jackson distilled-in-glass quality (Muskegon, MI, U.S.A.) except the 95% ethanol purchased from Union Carbide (Cincinnati, OH, U.S.A.).

Gas chromatography–mass spectrometry

The GC–MS determinations were accomplished on a Finnigan 3200 chemical ionization gas chromatograph–mass spectrometer (Finnigan MAT, San Jose, CA, U.S.A.) operating in the MID mode. The GC separation was achieved using a 3.2 m × 2 mm I.D. silylated glass column containing 15% Triton X-305 on 100–120 mesh Chromosorb W HP (Supelco, Bellefonte, PA, U.S.A.). The column temperature was 85°C isothermal; the column injection port temperature was 100°C and the transfer line temperature was 230°C. Methane was used as carrier/reactant gas at a flow-rate of 14 ml/min. Other pertinent MS operating parameters were: preamp, 10^{-8} A/V; electron energy, 105 eV; emission current, 1.02 mA; multiplier voltage, 1–1.2 kV; source temperature, 80°C; indicated pressure, $5 \cdot 10^{-4}$ Torr.

Butylene oxide was quantified with external standards by monitoring the M + 1 quasi-molecular ion, *m/z* 73.

Spiking solution preparation

Accurately measured volumes of 1,2-butylene oxide were added by syringe to ethanol yielding spiking solutions ranging in concentration from 837 μg/ml to 83.7 mg/ml. These butylene oxide–ethanol spiking solutions were used to fortify blood samples to determine extraction efficiency.

Spiked blood sample preparation

Accurately measured volumes (1 to 10 μl) of the butylene oxide spiking solutions were added to glass vials containing 250 or 500 mg of fresh whole rat blood. The spiked blood sample concentrations ranged from 0.335 μg butylene oxide/ml whole rat blood to 100 μg/ml. After spiking, the blood samples were briefly shaken by hand to insure mixing, then solvent extracted with 250 μl of hexane. The blood samples were agitated on a vortex mixer for 1 min and placed on dry ice for a minimum of 5 min prior to analysis. The dry ice chilling caused separation of the organic hexane layer from the aqueous blood layer. If necessary, the spiked blood samples were centrifuged for 3 min at 600 *g* rechilled to separate these layers. A 1-μl aliquot of the chilled organic layer was injected directly on-column. Fresh, whole rat blood was collected from healthy, adult male Fischer 344 rats (Charles River Breeding Laboratory, Kingston, NY, U.S.A.) via heart puncture with a syringe containing heparin.

RESULTS AND DISCUSSION

Validation data

Quantification of butylene oxide in blood samples was performed via external standard calculations. Peak areas were determined by integration with electronic background subtraction. Individual recovery determinations of butylene oxide from the spiked blood samples ranged from 82.0 to 107.6%. The mean percent recovery for the concentration range of 0.335 to 100 μg butylene oxide/ml whole blood was 93.0 ± 4.2% (see Table I). The coefficient of variation for three individual determinations each at five concentrations ranged from 2.3% at 13.4 μg/ml to 10.4% at 0.335 μg/ml.

No peak(s) was observed in either the hexane or whole blood extracts which

TABLE I

RECOVERY OF 1,2-BUTYLENE OXIDE FROM WHOLE RAT BLOOD

Concentration (μg/ml) in whole rat blood	*Mean Recovery (%)**	*Standard deviation*	*Coefficient of variation*
0.335**	86.5	9.0	10.4
1.67	93.7	8.1	8.6
13.4	92.0	2.1	2.3
33.5	95.6	5.9	6.2
100	97.4	3.3	3.4
	Average, 93.0 ± 4.2%***		

* Each data point is the mean of 3 samples; 2 determinations per sample.
** 500 mg whole rat blood; all other blood samples were 250 mg.
*** Standard error of the mean.

would interfere with quantitative analysis of butylene oxide. Representative multiple ion chromatograms of hexane, a control (blank) blood sample extract, a solution standard and a spiked blood sample extract are presented in Fig. 1. The GC retention time of butylene oxide was approximately 1 min.

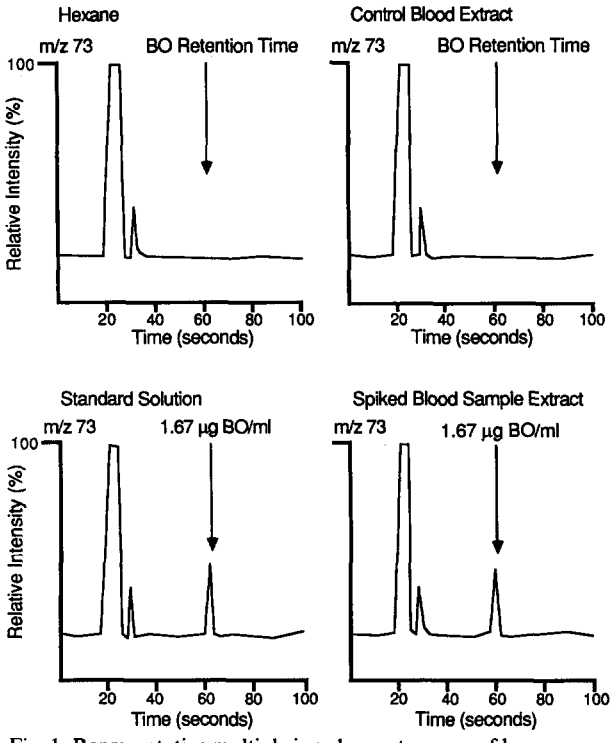

Fig. 1. Representative multiple ion chromatograms of hexane, a control (blank) blood extract, a standard solution and a spiked blood sample extract. BO = 1,2-Butylene oxide.

Sample collection and stability parameters were investigated to identify *a priori* the potential impact of conventional blood collection techniques on these parameters. During a pharmacokinetic study, blood is withdrawn by a syringe connected to an implanted venous cannula. Spiked blood samples were prepared in glass vials at two different concentrations representative of concentrations expected for rodents exposed to butylene oxide. These samples were then transferred with a syringe and venous cannula to duplicate the procedure used to collect blood samples from rodents.

After transferring the blood samples, each was prepared and analyzed as described earlier. The results indicated less than 5% of the butylene oxide was lost when blood was transferred via a syringe and venous cannula. The venous cannulas were rinsed with water and these rinses were analyzed for butylene oxide. The results showed no detectable butylene oxide retained by the cannulas. Additionally, spiked blood sample extracts were shown to be stable for up to 8 h when stored over dry ice.

The analytical method described is rapid, sensitive and specific for the quantitative determination of 1,2-butylene oxide in whole rat blood. The method requires as little as 250 μl of blood and can be applied to a concentration range of 0.335 to 100 μg butylene oxide/ml whole rat blood.

REFERENCE

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