Rapid and Sensitive Method for the Determination of Propylene Oxide in Cigarette Mainstream Smoke by Gas Chromatography–Mass Spectrometry*

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Abstract

A rapid method using gas chromatography–mass spectrometry for the analysis of propylene oxide in cigarette mainstream smoke is reported. Validation data show the method, which requires a minimum of sample preparation, to be selective, sensitive, reliable, and robust. Propylene oxide is found in the University of Kentucky Reference Cigarettes 1R4F and 2R4F at concentrations of 0.93 and 0.65 µg/cigarette, respectively, with a quantitation limit of 0.135 µg/cigarette.

Introduction

Epoxides (oxiranes), such as ethylene oxide and propylene oxide (PO) (CAS 75-56-9), are three-ring systems with an oxygen atom in the ring. PO is a colorless, low-boiling liquid (boiling point of 34°C) with an ethereal odor, which is soluble in water and miscible with acetone, ether, benzene, and toluene. No natural source for PO has been reported (1). Most PO is used as a chemical intermediate in the production of polyurethane foams, coatings, and adhesives and in the synthesis of propylene glycol, which has widespread industrial applications and is used as a humectant in cigarettes (2). PO is also registered as a bactericide, fungicide, and insecticide and used as sterilization gas for medical equipment. The toxicological properties of PO are well documented and its genotoxic and carcinogenic properties were recently reviewed (3,4). PO is classified by the International Agency for Research on Cancer (IARC) as a 2B carcinogen (possibly carcinogenic to humans) (1). It is readily absorbed through the respiratory tract and metabolized via two major pathways: hydrolysis to propylene glycol and conjugation with glutathione (1). PO is considered to be a direct alkylating agent because the oxirane ring can easily be cleaved under generation of an electrophilic center. It introduces 2-hydroxypropyl groups by reaction with nucleophilic sites on DNA (5). Mutagenic activity of PO has been evaluated in several test systems, and the results showed PO to be weakly mutagenic (6,7).

There is little information available in the literature on PO in cigarette smoke. Kagan et al. (8) reported a PO concentration of 370 ng/g tobacco in smoke for two non-filter cigarettes and postulated that propylene glycol, which is used as a humectant in cigarette filler (2), is a precursor of PO in cigarette smoke. PO in cigarette smoke has also been measured by Gordon and Coleman (9), who found 0.89 µg/cigarette and 1.1 µg/cigarette for the University of Kentucky Reference Cigarettes 2R4F using two different experimental gas chromatographic (GC) setups and different smoking machines. Another important factor in investigating PO exposure in cigarette smokers is that cigarette smoke contains propylene (10), which can be oxidized in vivo by oxidation of the double bond via cytochrome P450-dependent monooxygenases yielding PO (11).

Two methods for the determination of PO in cigarette smoke have been outlined in conference presentations (8,9). However, these presentations gave limited information about the procedure and there is no analytical method available in the literature. Kagan et al. (8) presented a method based on the collection of smoke in wash bottles containing sodium ethanethiolate, which converts propylene oxide to 1-(ethylthio)-2-propanol, which is measured with atmospheric pressure chemical ionization (APCI)–liquid chromatography (LC)–mass spectrometry (MS). After the derivatization of 1-(ethylthio)-2-propanol with pentafluorobenzoyl chloride, its GC–MS detection in negative chemical ionization mode is also possible. PO in cigarette smoke has also been measured using a dual oven GC–MS system and the cigarette smoke was trapped on Tenax directly within the chromatographic system (9).

The purpose of our work was to determine PO in cigarette smoke using a fast and reliable method with a minimum of sample preparation. Keeping complex derivatization, extraction, and sample clean-up steps to a minimum was a primary concern because such steps are time consuming and may lead to losses of PO, thus increasing assay variability.

Experimental

Chemicals, solvents, and cigarettes

PO and PO-d_6 were purchased from Sigma-Aldrich (Deisenhofen, Germany) (99% and 98% purity, respectively), and toluene (SupraSolv) was purchased from Merck (Darmstadt, Germany).

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The test cigarettes were the University of Kentucky Reference Cigarettes 1R4F and 2R4F, which are part of a series of reference cigarettes developed for research purposes in a joint effort by the National Cancer Institute of the National Institute of Health, the Agriculture Research Service of the United States Department of Agriculture, and the University of Kentucky Tobacco and Health Research Institute. In general, standard blended reference cigarettes are representative for conventional commercial cigarettes of similar total particulate matter (TPM) yield (12). The reference cigarettes are 84 mm in length, 25 mm in circumference, with a butt length of 35 mm. TPM per cigarette is 10.8 mg for the 1R4F and 11.7 mg for the 2R4F with 0.8 mg nicotine per cigarette for the 1R4F and 0.85 mg nicotine per cigarette for the 2R4F (13,14). They were provided by Philip Morris USA (Richmond, VA). The cigarettes were stored at –16°C ± 2°C and conditioned at 22°C ± 1°C with a relative humidity of 60% ± 2% and an atmospheric pressure of 96 kPa ± 10 kPa for at least 8 days according to ISO standard 3402 (15). Stock solutions of PO and PO-d_6 were prepared in toluene at concentrations of 4.5 mg/mL each. From these stock solutions, standard solutions were prepared for an internal standard calibration. A second stock solution of 3.76 mg/mL was prepared for quality control samples of PO.

Sample collection

Mainstream smoke was generated in basic conformity with International Organization for Standardization 3308 (16). Ten cigarettes were smoked on a 20-port rotary smoking machine (Borgwaldt, Hamburg, Germany) with a 35-mL puff volume (± 0.5 mL) and a 2.0-s puff duration (± 0.1 s) every minute. Cigarette mainstream smoke collection was adapted from a published method (17), and PO was collected in three wash bottles connected in series (Figure 1). A glass fiber filter (Pall, East Hills, New York) was placed in front of the wash bottles to remove the particle phase from the smoke. The first wash bottle contained approximately 6 g glass beads (3-mm diameter) and 7 mL toluene. The second and the third wash bottles each contained approximately 5 g glass beads and 6 mL toluene. Prior to sampling, the wash bottles were cooled to –78°C with a mixture of isopropanol and dry ice. After sampling was completed, the wash bottles were brought to room temperature and the contents funneled into a volumetric flask. The wash bottles were rinsed with toluene, and the volumetric flask was filled to the 25-mL mark. One mL of the sample was transferred to an autosampler vial and the internal standard PO-d_6 was added to the sample. The sample was injected directly into the GC–MS.

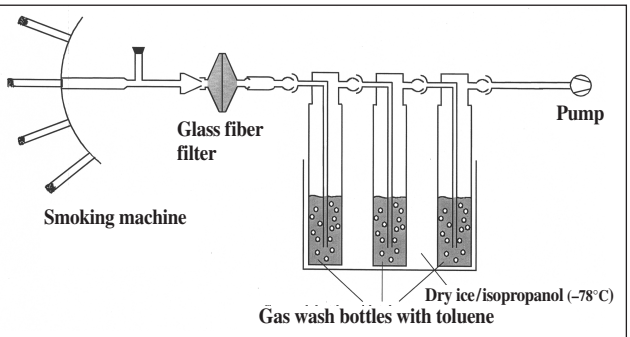

Figure 1. Smoke collection.

GC–MS

An Agilent Technologies, Inc. (Palo Alto, CA) 6890 GC equipped with a CTC Analytics (Zwingen, Switzerland) GC PAL autosampler was coupled to an Agilent 5973 mass selective detector. The GC worked with helium in splitless mode, the front inlet pressure was maintained at 70 kPa, and the purge flow was set to 50 mL/min, resulting in a total flow of 54 mL/min. The GC oven was started at 60°C and held for 1 min, then heated at 30°C/min to a final temperature of 300°C and held for 10 min (total GC run time 19 min). Injection volume was 1 µL. The GC was equipped with an Agilent splitless liner without glass wool and was fitted with a 25-m Varian (Palo Alto, CA) CP-PoraBond Q capillary column with 0.25-mm i.d. and 3-µm film thickness. For data analysis the chromatographic peak areas were determined automatically by the Agilent ChemStation Integrator program in the Agilent Enhanced ChemStation software (version D.00.00.38).

The MS was operated in electron impact (70eV) mode. The MS transfer line temperature was set to 230°C and the MS quadrupole and source heaters were maintained at 120°C and 220°C, respectively.

Results and Discussion

Method development

Initially, several different GC conditions and different GC columns were explored, which were not successful: the sensitivity was poor and the resulting PO and PO-d_6 peaks showed tailing and interference with matrix peaks. Success was achieved by using a porous layer open tubular column, which gave good peak shape and purity and sufficient separation of PO from other matrix peaks (Figure 2). PO was detected using the [M-CH_3]+ ion and PO-d_6 was detected using the [M]+ ion (*m/z* = 43 and 64) and quantitated from the resulting single ion chromatograms. Two possible qualifier ions [i.e., the [M]+ ion for PO (*m/z* = 58) and the [M-CD_3]+ for PO-d_6 (*m/z* = 46)] could not be used for confirmation because of matrix effects. Toluene, which is recommended as a solvent for PO for reference solutions in a publication from Gilbert et al. (18), was used for trapping the cigarette smoke at –78°C and for preparation of the calibration solutions. Other solvents that are useful trapping reagents at –78°C (e.g., methanol) were rejected because they may react with PO during sample collection or storage.

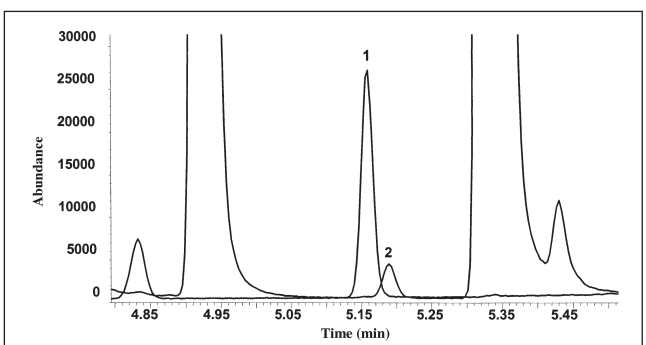

Figure 2. GC–MS–SIM chromatogram of a 1R4F sample for the ions at *m/z* = 64 for the internal standard PO-d_6 (1) and at *m/z* = 43 for PO (2).

Validation and analysis of cigarettes

A 14-point calibration curve for PO with a concentration range of 0.027 to 8.95 µg/mL was produced. The curve was linear with a correlation coefficient of r^2 = 1.000. The concentration at 0.054 µg/mL was found to be the quantitation limit with a signal-to-noise ratio of 10:1. Assuming that 10 cigarettes were smoked and the smoke trapped in 25 mL toluene, the quantitation limit would be 0.135 µg/cigarette. Back-calculated concentrations of the calibration standards were within 5.3% of the expected values. Trapping efficiency was excellent: PO was found in the first trap, but was below the quantitation limit in the second and third traps, and no PO was found on the glass fiber filter. Standard addition experiments were performed to verify the selectivity by adding four different concentrations of PO to the trapped smoke matrix from 10 1R4F cigarettes. The recovery was between 99% and 107% of the expected value. For determinations of samples from 10 1R4F cigarettes (N = 5), intraday precision was 7.7% and interday precision was 6.2%. Instrument precision was determined from 10 injections of a smoke sample from the 1R4F and had a relative standard deviation of 1.2%. The average yield of PO in 1R4F cigarettes was 0.93 µg/cigarette. with a relative standard deviation of 4.4% (N = 8). The average yield of PO in 2R4F cigarettes was 0.65 µg/cigarette with a relative standard deviation of 3.1% (N = 4).

Munch and Eichelberger (19), using Environmental Protection Agency method 524, reported PO to be unstable in aqueous solution. They could not determine PO because it decomposed too rapidly in water to create a valid calibration curve. In this study, we investigated whether there was a loss of PO concentration as a result of using toluene as a solvent for standard solutions and as a trapping solvent. PO in smoke trapped in toluene was measured immediately and again after 5 weeks storage at –78°C. The difference was only 0.8% (average of N = 3). A stock solution stored for 2 years at –78°C showed a loss in concentration of only 2.4%.

Conclusion

The GC–MS method we developed for the determination of propylene oxide in cigarette smoke requires little sample preparation and no derivatization, yet the validation data (accuracy, precision, quantitation limit, and trapping efficiency) are excellent. The method is therefore suitable for the routine analysis of PO in cigarette smoke.

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