

Determination of Ethylene Oxide in Spices Using Headspace Gas Chromatography

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A method was developed for determining the residues of ethylene oxide (ETO) in dry spices. These residues result from commercial fumigation practices to control insect pests. The analytical method makes use of a headspace gas chromatograph, equipped with a porous layer open tubular (PLOT) megabore column, and sealed 22 mL headspace vials containing 1 g aliquots of the spices. The spice samples, containing added 1-octanol, are thermostated for 20 min at a temperature of 60 °C prior to injection of microliter aliquots of the equilibrated headspace. By holding the PLOT column at 70 °C, ETO will elute in about 8 min, after which time the column is heated at 30 °C/min to 220 °C, at which the temperature is held for 5 min to clear the column for the next injection. On the basis of spice matrix standards, recoveries of ETO from a variety of different spices fell in the range 75–130% (av = 100%, n = 120) for spiking levels covering the range 1–1000 ppm. The practical limit of detection was 1 ppm. By using an automated headspace instrument, it is possible to analyze over 60 samples in a 24-h period.

Keywords: *Ethylene oxide; spices; headspace analysis*

INTRODUCTION

Ethylene oxide (ETO) is a low molecular weight, highly volatile compound (178 kPa at 25 °C) that is primarily used as an intermediate in the production of various chemicals (about 99% of total use). Because ETO is a broad-spectrum pesticide/microbicide, it is also used as a fumigant for foodstuffs and textiles and as a sterilant for surgical instruments (about 1% of total use). Although its use as a fumigant is low, this use has a potentially direct impact on human health because of the possibility of residues remaining in foodstuffs and because of the fact that ETO has been classified as a suspect human carcinogen [A2 carcinogen (ACGIH, 1991)]. Furthermore, while ETO dissipates rapidly from fumigated commodities (days–weeks), it will react with chloride and bromide ions and with water to produce 2-chloroethanol, 2-bromoethanol, 1,2-ethanediol, and 2,2'-oxybis(ethanol), all of which persist for longer periods of time (weeks–months) (Rourke and Darbishire, 1965; Scudamore and Heuser, 1971; van Rillaer and Beernaert, 1982; Toet, 1984; Aitkenhead and Vidnes, 1988). These compounds, produced rapidly under ambient conditions, could also be of concern if not allowed to dissipate to levels below which there would not be any observable chronic effects.

The concern with ETO and related residues in food commodities is partly related to the dramatic increase in spice consumption in the United States. For example, from 1978 to 1992, spice consumption increased from about 204 million kilograms to about 386 million kilograms (worth about \$600 million). This increase was due partly to increased demand for ethnic foods and the declining use of salt as a seasoning in foods.

We describe here a method for determining ETO residues in dry spices that have been commercially fumigated to control microbe and insect pests. The

method is based on headspace gas chromatography, which made use of the equilibrium between the volatile ETO in the solid spices and the surrounding gas phase in a sealed vessel. The method was developed to monitor ETO residues after fumigation to determine when the spice commodities could be released to the food industry. Data were also generated using this method in fulfillment of U.S. EPA requirements for re-registration of ETO.

MATERIALS AND METHODS

Materials. The following 15 control spices, used to develop the method, were supplied by Baltimore Quality Assurance, Inc. (Baltimore, MD), via the American Spice Trade Association (Washington, DC): ginger, oregano, celery, turmeric, nutmeg, cassia, basil, sage, red pepper, fennel, sesame seed, caraway, cumin, black pepper, and coriander. When received, these spices were stored at –20 °C to minimize losses of volatile compounds normally found in spices. The ETO was obtained in a lecture bottle from Matheson (Secaucus, NJ), and the 1-octanol (99%) was supplied by Aldrich Chemical Co. (Milwaukee, WI) and used as received. The 22-mL glass headspace vials (with their caps and Teflon-lined septa) used to contain the spiked spice samples were obtained from Perkin-Elmer Corp. (Norwalk, CT).

Methods. Standards in 1-octanol were prepared gravimetrically and volumetrically from chilled liquid ETO and 1-octanol using chilled glassware. A primary standard (10 µg/µL) was prepared by weighing 1 g of chilled liquid ETO into a 100-mL volumetric flask that contained chilled 1-octanol. Diluted standards (0.005, 0.010, 0.030, 0.050, 0.100, 0.500, and 5.000 µg/µL) for headspace gas chromatography (HS-GC) were prepared by using chilled pipets to transfer aliquots of the primary standard to volumetric flasks containing chilled 1-octanol. All standards were stored at 4 °C.

One-gram aliquots of the dry spices were added to the headspace vials, which were then chilled in water ice for 15 min. After that time, 100 µL of either pure 1-octanol or the ETO standards in 1-octanol was added to each spice sample (ETO concentration ranges in the spice samples were 1–50 and 50–1000 ppm), each vial was sealed with a crimped cap containing the Teflon-lined septum, and each vial was tumbled for about 1 min to mix the sample. The sealed vials were

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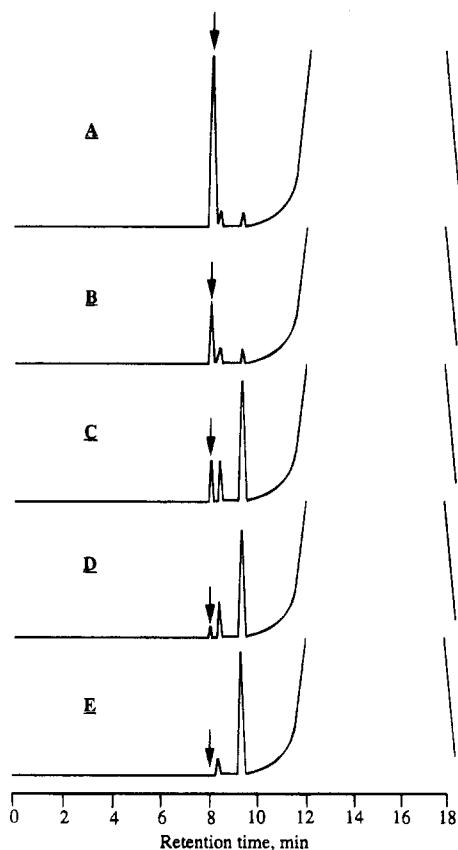


Figure 1. Headspace gas chromatograms of pure ethylene oxide: 3 μg (A); 1 μg (B); 3 μg in spice matrix (C); 1 μg in spice matrix (D); and spice matrix alone (E).

placed in a Model HS-100 autosampler (Perkin-Elmer) and thermostated for 20 min at 60 °C. After this time, each vial was pressurized to 138 kPa (gauge) by the instrument, and then the equilibrated vapor was automatically sampled for 0.02 min and injected onto a 30 m \times 0.53 mm (i.d.) GSQ (divinylbenzene homopolymer) porous layer open tubular (PLOT) column (J&W Scientific, Folsom, CA) contained in a Model Sigma 2000 gas chromatograph (Perkin-Elmer) for analysis using a flame ionization detector. The column head pressure was maintained at 69 kPa (gauge), which gave a helium flow rate (at 70 °C) of 5 mL/min. Initial temperature was 70 °C (8.2 min), followed by a program rate of 30 °C/min to 220 °C, at which the column was held for 5 min. The absolute recovery of ETO from the spiked spices was determined by comparing their GC responses to those of standards prepared by transferring microliter aliquots of ETO dissolved in 1-octanol to empty headspace vials. Relative recovery of ETO in spices was determined by comparing GC responses with those of "matrix" standards, which were derived from spices spiked with ETO (Figure 1).

RESULTS AND DISCUSSION

Many published techniques for the determination of ETO employ solvent extraction of the ETO-containing media (e.g., air samples, plastics) followed by either direct GC analysis of the extract or GC analysis after chemical conversion of ETO (Ball, 1984; Esposito et al., 1984; Puskar et al., 1988). Alternative methods have employed headspace techniques, in which the ETO-impregnated material (e.g., surfactants, plastics) was heated and the equilibrated headspace was sampled for analysis using gastight syringes (Kaye and Nevell, 1985) and automated, coupled headspace gas chromatography (HS-GC) (Dahlgran and Shingleton, 1986). We chose HS-GC as the underlying technique for determining ETO residues in spices because HS-GC minimizes or

eliminates sample preparation and cleanup procedures, such as solvent extraction, distillation, and column chromatography, common to other techniques. It also avoids overloading or contamination of the GC column with high-boiling or nonvolatile components. Furthermore, automated HS-GC allows for rapid turnaround time, high sample throughput, and good reproducibility. On the other hand, this technique depends on the equilibrium distribution of the volatile ETO between the solid spice sample and the surrounding gas phase in the sealed headspace vials. This means that depending on the volatility of the analyte and its interaction with the sample matrix, gas phase concentration of the analyte will vary. Also, this technique will work only with analytes that are thermally stable and can survive gas chromatography. However, automated HS-GC has been proven to be a versatile technique in other applications, such as the determination of vapor pressures of fuels and crude oils (Woodrow and Seiber, 1988), evaporative flux of pesticides from soil and water (Woodrow and Seiber, 1991), Henry's law constant (Schoene and Steinhanes, 1985; Sagebiel et al., 1992), and methyl bromide residues in air during and after fumigation of fields prior to planting (Woodrow et al., 1988; Majewski et al., 1995).

The scope of our study included the following tasks: (1) develop a method for preparing ETO standards in a suitable solvent, for spiking spice samples with these standards, and for analyzing the spiked samples using automated HS-GC; (2) perform a series of tests to evaluate the analytical method and instrumentation together using a variety of different spices to represent a wide range of spice matrix types; (3) spike these spices with ETO over a broad range of concentrations (e.g., 1–1000 ppm) that would represent most situations that might be encountered with fumigated spices; and (4) on the basis of the results of the series of tests (2), recommend a method for use in monitoring the dissipation of ETO from fumigated spices.

The boiling point of ETO was such (10.7 °C) that by keeping this material, 1-octanol, and associated glassware at water ice temperature, standard preparation was not a problem. Furthermore, standards of ETO in 1-octanol were stored at 4 °C for several weeks without measurable loss. Multiple injections of standard ETO gave instrument responses that varied to within 5%, day-to-day. The minimum detection limit was 0.5 ppm of ETO for 13 of the 15 spices (ETO could not be detected at 0.5 ppm in cassia and basil). Because of this, we adopted 1 ppm as a practical lower limit since this was met by all of the spices in this study. Furthermore, ~1 ppm of ETO in spices is an industry target concentration for determining when fumigated spices may be released to the food industry. If it becomes necessary to routinely reach 0.5 ppm, or less, this may be accomplished by increasing the headspace sample injection time, which would essentially increase the sample aliquot size. For example, the 0.02-min sampling time used in this study resulted in a sample aliquot of about 100 μL of equilibrated vapor, based on the sampling time and GC column flow rate (i.e., [0.02 min] \times [5 mL/min] = 0.1 mL). By increasing the vapor sample size at least an order of magnitude (to ~1 mL), it may be possible to decrease the practical lower limit from 1 ppm of ETO in spices to 0.1 ppm.

Thermostating the spices at 60 °C for 20 min gave adequate results. Longer times, and especially at

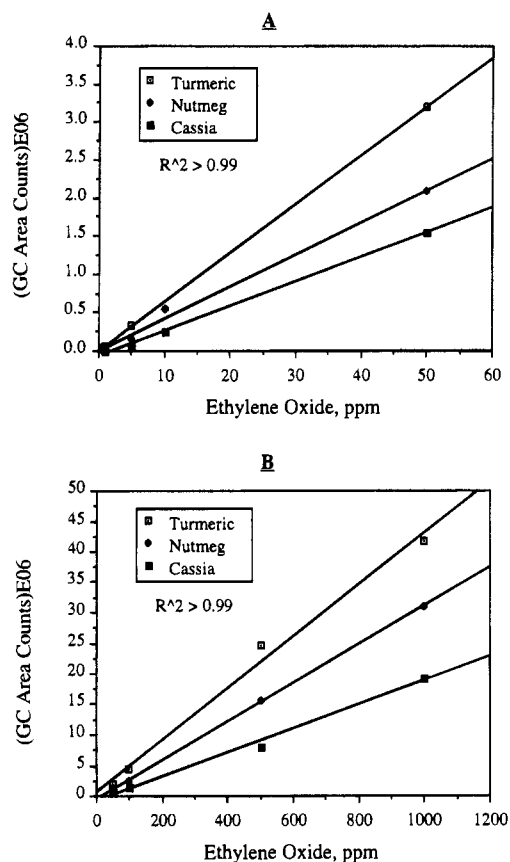


Figure 2. Matrix standard curves (A, 1–50 ppm; B, 50–1000 ppm) for three example spices.

elevated temperatures, resulted in greater interferences and some loss of ETO. Methanol, a potential interference that may be present in some spices, was not found in the spices tested. Acetaldehyde, derived from black and red peppers, eluted just prior to ETO on the GSQ PLOT column. When the retention time was adjusted to 8 min, ETO was resolved from potential interferences (Figure 1). Other potential interferences from the Teflon-lined butyl rubber septum/cap were kept at a minimum by thermostating the samples at 60 °C, since the septum/cap was designed to operate at temperatures below 100 °C.

Standard curves derived from spiked spices (1–50 and 50–1000 ppm) were linear and had correlation coefficients (r^2) that were in general 0.99 or greater (see Figure 2 for three examples). Absolute recoveries of ETO from the test spices (compared to ETO in empty vials) fell in the range 3–91% (av = 40.6%, $n = 60$), for the 1–50 ppm spiking range, and in the range 12–99% (av = 46.8%, $n = 60$), for the 50–1000 ppm spiking range. However by using the matrix standard curves described above, relative recoveries for the spiking levels 1–50 and 50–1000 ppm fell in the ranges 90–130% (av = 102%, $n = 60$) and 75–120% (av = 99.0%, $n = 60$), respectively (Tables 1 and 2). We recommend using these two standard curves to characterize fumigated spices rather than one 1–1000 ppm broad-range curve, since the latter has the potential of giving erroneous results at or near the lower limit. The 50–1000 ppm curve would be used during the first few days or so after fumigation when concentrations are likely to be high (e.g., >50 ppm) and the second curve (1–50 ppm) when the concentration of ETO falls to the low parts per million range.

Table 1. Spice Samples (1 g) Spiked with 1–50 ppm of Ethylene Oxide (ETO) in 1-Octanol

spice	abs % ETO vaporized ^a	rel % ETO recovered ^b
ginger	34–62	99–110
oregano	21–43	100–110
celery	24–48	100
turmeric	37–70	99–130
nutmeg	19–48	99–120
cassia	4–23	90–110
basil	3–21	98–100
sage	14–50	93–100
red pepper	10–47	96–110
fennel	44–60	100–110
sesame seed	88–91	100
caraway	29–47	100
cumin	48–63	100–110
black pepper	43–64	100
coriander	44–54	99–120

^a Compared to standard ETO in headspace vials without spices.

^b Compared to standard ETO spiked to spices in headspace vials.

Table 2. Spice Samples (1 g) Spiked with 50–1000 ppm of Ethylene Oxide (ETO) in 1-Octanol

spice	abs % ETO vaporized ^a	rel % ETO recovered ^b
ginger	40–69	98–120
oregano	12–39	75–110
celery	29–40	99–100
turmeric	42–69	99–110
nutmeg	30–44	93–110
cassia	18–27	95–110
basil	22–28	90–110
sage	45–52	97–110
red pepper	31–41	96–110
fennel	40–62	90–100
sesame seed	38–99	84–100
caraway	35–56	92–100
cumin	37–59	94–100
black pepper	42–55	87–100
coriander	43–52	94–100

^a Compared to standard ETO in headspace vials without spices.

^b Compared to standard ETO spiked to spices in headspace vials.

CONCLUSIONS

HS-GC was used to quantitate ETO spiked to dry spices over the range 1–1000 ppm. Such a broad range of concentrations was selected for those situations that monitor the dissipation of ETO from fumigated spices. Spice matrix standards were used to determine residues in spices spiked with ETO since absolute recoveries by HS-GC were less than quantitative (av ~ 41–47%) and they varied with the matrix (3–99%). Recoveries of residues determined using matrix standards averaged 100% (75–130%; $n = 120$) over the entire 1–1000 ppm range.

Automated HS-GC gave good reproducibility and high sample throughput (i.e., over 60 samples can be processed in a 24-h period). However, sample sets should be limited to 40 samples each, since some ETO loss through the septum/cap seal may occur over time. The developed method has been used with success by McCormick & Co., Hunt Valley, MD, to monitor ETO in their fumigated spices. The results will be used to determine how long spices should be held to allow ETO to decline to ~1 ppm, or less, after fumigation. The method for ETO, as described here, will not allow determination of the metabolites 2-chloroethanol, 2-bromoethanol, and ethylene glycol because these chemicals are much less volatile than ETO. Analysis of these metabolites requires alternative approaches.

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