Method no.:	PV2144
Control no.:	T-PV2144-01-0612-M
Target concentration:	1 mg/m^3
Procedure:	Samples are collected by drawing known volumes of air through closed-face 37-mm polystyrene cassettes containing glass fiber filters. Samples are extracted with 2 mL of 20 mM methanesulfonic acid in deionized water and analyzed by liquid chromatography using an ultraviolet detector (LC/UV).
Recommended sampling time and sampling rate:	120 min at 1.0 L/min (120 L)
Reliable quantitation limit:	2.3 µg/m^3
Special requirements:	Samples should be refrigerated upon receipt at SLTC.
Status of method:	Partially evaluated method. This method has been subjected to established evaluation procedures of the Methods Development Team and is presented for information and trial use.

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1. General Discussion

For problems with accessibility in using figures and illustrations in this method, please contact OSHA Salt Lake Technical Center at (801) 233-4900. These procedures were designed and tested for internal use by OSHA personnel. Mention of any company name or commercial product does not constitute endorsement by OSHA.

1.1 Background

1.1.1 History

Air samples collected using glass fiber filters (GFF) were received at OSHA SLTC with requested analysis for scopolamine methyl nitrate. This partially-validated work was performed because SLTC had no sampling and analytical method for scopolamine methyl nitrate. The analysis by liquid chromatography using a UV detector at 217 nm was tried and found to be effective. Scopolamine methyl nitrate is a salt that disassociates in most solvents in which it is soluble. The nitrate group has a response at 217 nm, resulting in two peaks on the chromatogram, one for the nitrate and the other for the methylscopolamine. Because nitrate commonly appears in the workplace environment, and is a contaminant on GFFs, the peak for methylscopolamine was used for quantitation. Extraction and retention studies showed good recoveries. The 15 day storage study showed an average recovery of 93.6% for refrigerated samples and 86.4% for ambient samples.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)[1]

Scopolamine methyl nitrate is a drug used as an anticholinergic agent to treat motion sickness, as an adjunct in treating duodenal and gastric ulcers, and to treat infantile colic. Adverse effects include dry mouth, difficulty in swallowing and speaking, thirst, dilated pupils, loss of focus, sensitivity to light, skin flushing and dryness, temporary slowing of heart rate followed by rapid heart rate with palpitations and irregularities in rhythm, chest pain, and dizziness. Overdose may result in rapid breathing, high fever, restlessness, confusion, paranoia, psycosis, hallucinations, delirium, seizures, and rash on the torso and face. Severe overdose depresses the central nervous system causing lack of coordination, drowsiness, stupor, unconsciousness, coma, stoppage of circulation followed by death. The Canada-Saskatchewan Occupational Health and Safety Regulations for scopolamine methyl nitrate are a 3 mg/m^3 TWA and a 6 mg/m^3 STEL.

The normal adult dosage for scopolamine methyl nitrate is 1.25 mg.[2]

1.1.3 Workplace exposure[3]

Workers are exposed to scopolamine methyl nitrate in compounding and packaging pharmaceutical formulations of the drug.

1.1.4 Physical properties and other descriptive information[4]

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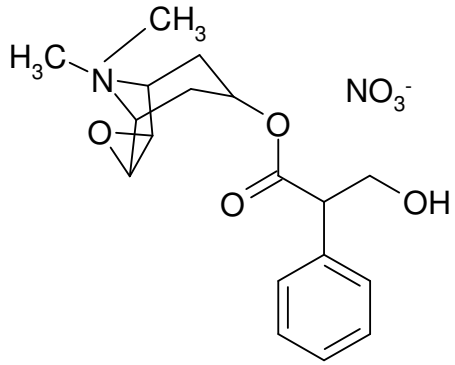
1 Material Safety Data Sheet: hyoscine methonitrate, Chemwatch, Victoria, Australia, (accessed 9/17/06).

2 Arky, R., *Physicians' Desk Reference*, 51st ed., Medical Economics Company: Montvale NJ, 1997, p. 972.

3 Lewis, R., *Hawley's Condensed Chemical Dictionary,* 14th ed., John Wiley & Sons Inc.: New York, 2001, p. 58.

4 MSDS (-)scopolamine methyl nitrate, http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/S2250 (accessed 8/28/2006)

synonyms:	hyoscine methonitrate; hyoscine methyl nitrate; methylscopolamine nitrate; methscopolamine nitrate
IMIS[5]:	S150
CAS number:	6106-46-3
molecular weight:	380.39
appearance:	white to cream solid
molecular formula:	$C_{17}H_{21}NO_4 \bullet CH_3NO_3$ or $C_{18}H_{24}N_2O_7$
odor:	faint amine
solubility:	soluble in water, alcohol, and acetonitrile
structural formula:	



This method was evaluated according to the OSHA SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[6]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters.

1.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentration, based on the recommended sampling parameters. Ten samplers were spiked with equally descending increments of scopolamine methyl nitrate, such that the highest sampler loading was 1 µg. This is the amount spiked on a sampler that would produce a peak approximately 10 times the response for a sample blank. These spiked samplers were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (standard error of estimate (SEE) and slope) for the calculation of the DLOP. The slope was 6729 and the SEE was 180.6. The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The DLOP and RQL were 0.08 µg (0.66 µg/m^3) and 0.27 µg (2.3 µg/m^3), respectively. The recovery at the RQL was 96.5%.

[5] OSHA Chemical Sampling Information. http://www.osha.gov/dts/chemicalsampling/toc/toc_chemsamp.html (accessed 8/28/2006).

[6] Burright, D.; Chan, Y.; Eide, M.; Elskamp, W.; Rose, M.; *Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis.* http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/06), OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 1999.

Table 1.2.1
Detection Limit of the Overall Procedure
for Scopolamine Methyl Nitrate

mass per sample (μg)	area counts (μV•s)
0.0	0
0.1	615
0.2	1157
0.3	1779
0.4	2474
0.5	3076
0.6	3621
0.7	4322
0.8	5091
0.9	6102
1.0	6807

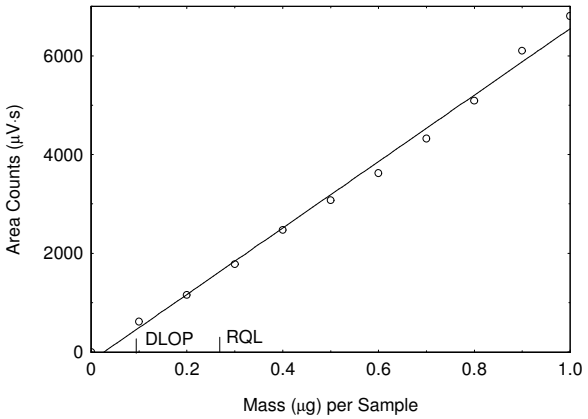


Figure 1.2.1. Plot of data to determine the DLOP/RQL for scopolamine methyl nitrate. (y = 6729x - 179: SEE = 180.6)

Below is a chromatogram of scopolamine methyl nitrate at the RQL of 0.27 μg/sample. The recovery at the RQL was 96.5%.

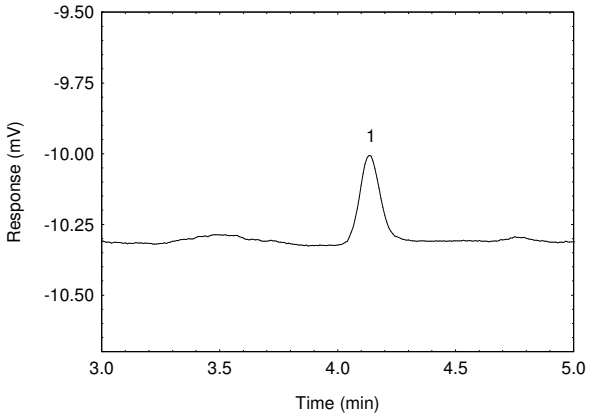


Figure 1.2.2. Chromatogram of the scopolamine methyl nitrate analytical standard near the RQL. (Key: 1 = methylscopolamine)

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate.

Samples are collected with 37-mm polystyrene cassettes containing glass fiber filters. For this evaluation, glass fiber filters were purchased from Pall Corporation, Ann Arbor, MI. (Catalog no. 61652, lot 53471).

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, remove both end plugs from the cassette. All glass fiber filters should be from the same lot.

Attach the cassette to the sampling pump so that it is in an approximately vertical position with the inlet facing down during sampling near the worker's breathing zone. Position the sampling pump, cassette, and tubing so it does not impede work performance or safety.

Air being sampled should not pass through any hose or tubing before entering the cassette.

After sampling for the appropriate time, remove the sample, and replace both end plugs. Wrap each sample end-to-end with a Form OSHA-21 seal.

Submit at least one blank sample with each set of samples, making sure that it is from the same lot as the filters used for sampling. Handle the blank sampler in the same manner as the other samples except draw no air through it.

Record sample volumes (in liters of air) for each sample and any potential interferences.

Submit the samples to the laboratory for analysis as soon as possible after sampling. If delay is unavoidable, store the samples at refrigerator temperature. Ship any bulk samples separate from the air samples.

2.4 Extraction efficiency

The extraction efficiency was determined by spiking 24 GFFs with scopolamine methyl nitrate at 0.1 to 2 times the target concentration. These samples were stored overnight at ambient temperature and then extracted for 30 minutes using a lab shaker, and analyzed. The wet extraction efficiency was determined at 1 x the target concentration by spiking the analyte onto GFF that had 120-L humid air (80% RH at 23 ºC) drawn through them. The mean extraction efficiency over the studied range was 99.6%. The wet recoveries were similar to the mean recoveries.

Table 2.4
Extraction Efficiency (%) of Scopolamine Methyl Nitrate

level		sample number				mean
× target concn	µg per sample	1	2	3	4	
0.1	12	100.2	100.1	98.4	98.3	99.3
0.25	30	102.2	99.0	98.7	101.3	100.3
0.5	60	97.0	99.4	100.3	101.2	99.5
1.0	120	98.9	101.3	97.6	99.8	99.4
1.5	180	101.7	100.3	97.5	98.4	99.5
2.0	240	98.5	101.0	97.6	101.8	99.7
1.0 (wet)	120	101.2	98.5	99.5	100.7	100.0

2.5 Retention efficiency

Six GFFs were spiked with 240 µg (2.0 mg/m^3 based on 120-L air volume) of scopolamine methyl nitrate. These filters were each placed in a separate 37-mm polystyrene cassette. This cassette containing the spiked filter was placed in a sampling train with a second cassette containing an unspiked GFF. These sampling trains each had 120-L humid air (78% relative humidity at 23°C) pulled through them at 1.0 L/min. The samples were extracted and analyzed. The mean recovery was 99.3%. There was no analyte found on the filters of the back-up cassette of any of the sampling trains.

Table 2.5
Retention Efficiency (%) of Scopolamine Methyl Nitrate

section	sample number						mean
	1	2	3	4	5	6	
spiked GFF	98.3	97.6	100.6	99.6	100.0	99.7	99.3
back-up cassette	0.0	0.0	0.0	0.0	0.0	0.0	0.0
total	98.3	97.6	100.6	99.6	100.0	99.7	99.3

2.6 Sample storage

Fifteen GFFs were each spiked with 120 µg (1.0 mg/m^3) of scopolamine methyl nitrate. Each of these filters was assembled into a 37-mm cassette. These cassettes had 120 L of air (80% relative humidity at 23°C) drawn through them at 1.0 L/min. Three samples were analyzed immediately, and the rest were sealed. Six were stored at room temperature (23°C), while the other six were stored at refrigerated temperature (4°C). Three samples stored at room temperature and three samples stored at refrigerated temperature were analyzed after 8 days and the remaining six after 15 days. The amounts recovered indicate samples should be stored at refrigerated temperature until analyzed.

Table 2.6
Storage Test for Scopolamine Methyl Nitrate

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	97.6	98.8	100.8			
8	89.3	90.6	94.2	97.3	94.2	97.1
15	88.5	84.5	86.1	93.9	94.8	92.1

2.7 Recommended air volume and sampling rate

Based on the data collected in this evaluation, 120-L air samples should be collected at a sampling rate of 1 L/min for 120 minutes.

2.8 Interferences (sampling)

There are no known compounds which will severely interfere with the collection of scopolamine methyl nitrate.

Suspected interferences should be reported to the laboratory with submitted samples.

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan. Avoid skin contact and inhalation of all chemicals and review all appropriate MSDSs.

3.1 Apparatus

A liquid chromatograph equipped with a UV detector. A Waters 600 Controller and Pump, with a Waters 2487 Dual λ Absorbance Detector, and a Waters 717 plus Autosampler was used for this evaluation. An Eclipse XDB-C18 3.5µm 150 × 4.6-mm column (Agilent Wilmington, DE) was used in this evaluation.

An electronic integrator or other suitable means of measuring LC detector response. A Waters Empower 2 Data System was used in this evaluation.

Glass vials with PTFE-lined caps. In this evaluation 4-mL vials were used.

A dispenser capable of delivering 3.0 mL of extracting solvent to prepare standards and samples. If a dispenser is not available, a 3.0-mL volumetric pipet can be used.

Class A volumetric flasks - 10-mL and other convenient sizes for preparing standards.

Class A volumetric pipets for making analytical standards.

Calibrated 10-µL syringe for preparing standards.

Micro-analytical balance capable of weighing to at least 0.01 mg. An Ohaus Galaxy 160D balance was used in this evaluation.

A mechanical shaker. An Eberbach mechanical shaker was used in this evaluation.

Optional: Centrifuge for spinning down the particles of the glass fiber filters in samples. An International Equipment Company Centra CL3 centrifuge was used in this method.

3.2 Reagents

Scopolamine methyl nitrate [CAS no. 6106-46-3], reagent grade. Sigma-Aldrich 99%+ (lot no. 055K1425) was used in this evaluation.

Methanesulfonic acid [CAS no. 75-75-2], reagent grade. Acros 99% (lot no. A016643201) was used in this evaluation.

Acetonitrile [CAS no. 75-05-8], HPLC grade. Sigma-Aldrich 99%+ (lot no. 08995CE) was used in this evaluation.

DI water, 18 MΩ-cm. A Barnstead NanoPure Diamond system was used to purify the water for this evaluation.

The extraction solvent solution was 20 mM methanesulfonic acid in DI water. This was prepared by placing 1.3 mL of methanesulfonic acid in a 1.0 liter flask and bringing it up to the mark with DI water.

The mobile phase solution was 80:20 of 10 mM methanesulfonic acid:acetonitrile. Prepare the eluent by placing 400 mL of 20 mM methanesulfonic acid and 400 mL of DI water into a 1-L volumetric cylinder, and then add acetonitrile up to the 1-L mark. Transfer the solution to the mobile phase container of the LC. Degas the eluent before use. The eluent was degassed with house vacuum and an ultrasonic bath in this evaluation.

3.3 Standard preparation

Prepare stock standards by weighing out known amounts of scopolamine methyl nitrate into volumetric flasks and diluting with the extraction solvent. Dilutions of the stock standard are made with the extraction solvent to cover the range of 0.1 to 250 µg/sample.

Bracket sample concentrations with standard concentrations. If, upon analysis, sample concentrations fall outside the range of prepared standards, prepare and analyze additional standards to confirm instrument response, or dilute high samples with extraction solvent and reanalyze the diluted samples.

3.4 Sample preparation

Remove the GFF from the cassette and carefully transfer each filter to a separate labeled 4-mL vial. Wipe the interior walls of the cassette with a GFF wetted with a drop of DI water and place it into a separate labeled 4-mL vial. Prepare and analyze a blank wipe sample.

Add 3.0 mL of extraction solvent to each vial using the same dispenser as used for preparation of standards.

Immediately seal the vials with PTFE-lined caps, and shake the vials on a shaker for 30 minutes. Allow the vials to settle for 3 hours or spin them down on a centrifuge for 5 min at 2500 rpm.

3.5 Analysis

Liquid chromatography conditions:

run time:	7 min
injection size:	10 µL
mobile phase:	1 mL/min of 80% 10 mM methanesulfonic acid/ 20% acetonitrile (v/v)
detector wavelength:	217 nm
output range:	2 AUFS
column:	Eclipse XDB-C18 3.5 µm, 150 × 4.6 mm
retention times:	nitrate (1.5 min); methylscopolamine (4.1 min)

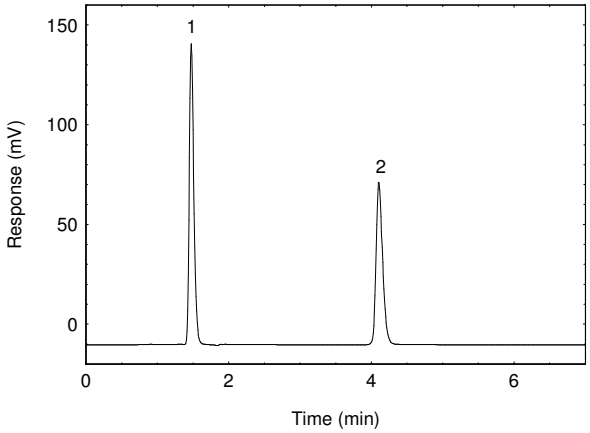


Figure 3.5.1. A chromatogram of 50 µg/mL scopolamine methyl nitrate. [Key: 1 = nitrate, 2 = methylscopolamine]

Peak areas are measured by an integrator or other suitable means.

An external standard (ESTD) calibration method is used. A calibration curve can be constructed by plotting response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over the range of concentrations.

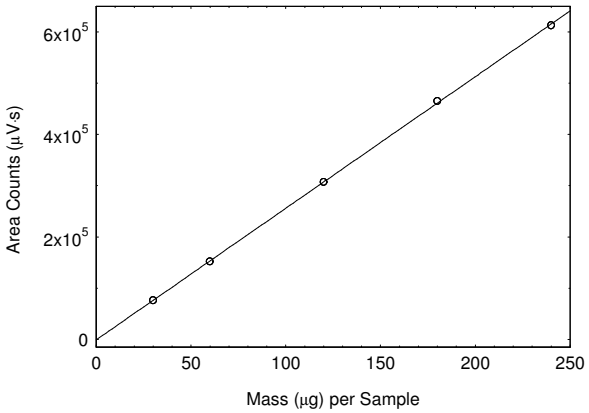


Figure 3.5.2 Calibration curve for scopolamine methyl nitrate as methylscopolamine. (y = 2567x – 846)

The standard error of estimate was determined from the linear regression of data points from standards over a range that covers 0.25 to 2 times the TWA target concentration. Calibration curves were constructed and shown in Section 3.5 from the three injections each of five standards. The standard error of estimate of the calibration curve is 1.21 µg/sample for methylscopolamine.

Table 3.5.1
Instrument Calibration for Scoplamine Methyl Nitrate (as Methylscopolamine)

standard concn (µg/sample)	x OSHA PEL	area counts (µV·s)		
30	0.25	76224	76134	76203
60	0.5	152021	151982	152123
120	1.0	307132	307002	306982
180	1.5	465062	465132	465223
240	2.0	612808	612789	612923

3.6 Interferences (analytical)

Any compound that produces a LC response and has a similar retention time as the analyte is a potential interference. If potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

When necessary, the identity or purity of an analyte peak can be confirmed by GC-mass spectrometry.

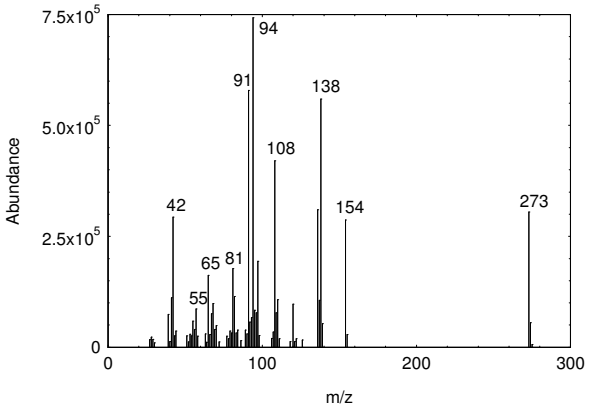


Figure 3.6.1. Mass spectrum of methylscopolamine.

3.7 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

$$M = [(\mu_S - \mu_B) + (\mu_C - \mu_{BW})]$$

where M is micrograms per sample
μ_S is µg/sample analyte in sample
μ_C is µg/sample analyte in cassette wall wipe
μ_B is µg/sample analyte in blank
μ_{BW} is µg/sample analyte in blank cassette wall wipe

$$C_M = \frac{M}{VE_E}$$

where C_M is concentration by weight (mg/m^3)
M is micrograms per sample
V is liters of air sampled
E_E is extraction efficiency, in decimal form

4. Recommendations for Further Study

Collection, reproducibility, and other detection limit studies need to be performed to make this a fully validated method.