



Cyclohexane

Method number:	1022
Version:	1.0
Target concentration:	300 ppm
OSHA PEL:	300 ppm (1,050 mg/m ³) TWA general, construction, and shipyard
NIOSH REL:	300 ppm (1,050 mg/m ³) TWA
ACGIH TLV:	100 ppm (350 mg/m ³) TWA
Procedure:	Active samples are collected by drawing workplace air through charcoal tubes with personal sampling pumps. Diffusive samples are collected by exposing passive samplers to workplace air. Samples are extracted with 99/1 (v/v) carbon disulfide/ <i>N,N</i> -dimethylformamide and analyzed by gas chromatography (GC) using a flame ionization detector (FID).
Recommended sampling time:	
SKC 226-01 CSC Tube	190 min due to breakthrough
SKC 575-002 Passive Sampler	240 min
Sampling rates:	
SKC 226-01 CSC Tube	50 mL/min (9.5 L)
SKC 575-002 Passive Sampler	15.2 mL/min (3.65 L) at 760 mmHg and 25 °C
Reliable quantitation limit TWA:	
SKC 226-01 CSC Tube	0.043 ppm (0.149 mg/m ³)
SKC 575-002 Passive Sampler	0.416 ppm (1.43 mg/m ³)
Standard error of estimate at the target concentration:	
SKC 226-01 CSC Tube	5.58%
SKC 575-002 Passive Sampler*	9.68%
	*For diffusive samplers when sampling site atmospheric pressure and temperature are known. When either or both of these values are unknown, see Section 4.5 for applicable standard errors of estimate.
Special requirements:	Refrigerated storage of collected samples is recommended but not required. When using a SKC 575-002 Passive Sampler, report the temperature and uncorrected sampling site atmospheric pressure.
Status of method:	Fully validated method. This method has been subjected to the established validation procedures of the Methods Development Team.

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1. General Discussion

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

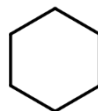
1.1 Background

1.1.1 History

OSHA has routinely collected and analyzed samples for cyclohexane on Anasorb CSC, Coconut Charcoal, SKC 226-01 sorbent tubes (SKC 226-01 CSC Tube) using NIOSH 1500.¹ OSHA 1022 makes changes to the flow rate, collection time, extraction solvent, and analytical conditions. Anasorb 747, SKC VOC Chek 575-002 passive badges (SKC 575-002 Passive Sampler) were also added as a sampling option.

1.1.2 Physical properties and descriptive information²

analyte:	cyclohexane
synonyms:	hexahydrobenzene; hexamethylene; hexanaphthene
solubility:	57 grams in 100 mL methanol at 20 °C; miscible with ethanol, ethyl ether, acetone, benzene, carbon tetrachloride
IMIS number:	0810
CAS number:	110-82-7
molecular weight:	84.16
density:	0.7781 g/mL
boiling point:	80.7 °C
melting point:	6.47 °C
formula:	C ₆ H ₁₂
appearance:	liquid
odor:	solvent
structure:	



¹ Pendegrass, S.; May, L. Hydrocarbons, BP 36 °C-216 °C (NIOSH Method 1500, Issue 3), 2003. Center for Disease Control, The National Institute for Occupational Safety and Health (NIOSH) Web site. <http://www.cdc.gov/niosh/docs/2003-154/pdfs/1500.pdf> (accessed March 2018).

² O'Neil, M.; Heckelman, P.; Koch, C.; Roman, K. *The Merck Index*; 14th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 2006; p 456.

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 a manner that will not interfere with work performance or safety.

2.1 Apparatus

2.1.1 SKC 226-01 CSC Tube

Collect active samples with a 6-mm x 70-mm glass sampling tubes packed with two sections of Anasorb CSC. The front section contains 100 mg and the back section contains 50 mg of Anasorb CSC 20/40 mesh. The sections are held in place and separated with foam and glass wool plugs. For this validation, commercially prepared tubes were purchased from SKC, Inc. catalog no. 226-01.

A sampling tube holder is required to protect the worker from the sharp end of the glass sampling tube.

Collect samples using a personal sampling pump calibrated to within $\pm 5\%$ of the recommended flow rate with the sampling device in-line.

2.1.2 SKC 575-002 Passive Sampler

Collect passive samples with a 575-002 Passive Sampler. These samplers contain 500 mg of Anasorb 747. For this validation, commercially available samplers were purchased from SKC, Inc. catalog no. 575-002.

2.1.3 A thermometer and barometer are required to determine the sampling site air temperature and atmospheric pressure.

2.2 Reagents

None required.

2.3 Technique

2.3.1 SKC 226-01 CSC Tube

Immediately before sampling, break off the ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking ends. Use sampling tube holders to minimize the hazard to the worker from the broken ends of the tubes. All tubes should be from the same lot.

The smaller section of adsorbent in the tube is used as a back-up and is positioned nearest the sampling pump. Attach the tube holder (with the adsorbent tube) to the sampling pump so that the adsorbent tube is in an approximately vertical position with the inlet facing down in the worker's breathing zone during sampling. Position the sampling pump, tube holder, and tubing so they do not impede work performance or safety.

Draw air directly into the inlet of the sampling tube holder. The air being sampled should not pass through any hose or tubing before entering the sampling tube.

Sample for up to 190 min at 50 mL/min (9.5 L) when using Anasorb CSC, Coconut Charcoal to collect TWA (long-term) samples.

After sampling for the appropriate time, remove the adsorbent tube and seal it with plastic end caps. Seal each sample end-to-end with a Form OSHA-21 as soon as possible.

Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

Record sample air volume (liters), sampling time (min) and sampling rate (mL/min) for each sample, along with any potential interference on the Form OSHA-91A.

Submit the samples to the laboratory for analysis as soon as possible after sampling. If delay is unavoidable, store the samples in a refrigerator as a precaution.

Ship any bulk sample(s) separate from the air samples.

2.3.2 SKC 575-002 Passive Sampler (In general, follow the manufacturer's instructions.)

Remove the sampler from its air-tight package.

Record the start time on the Form OSHA-91A. Remove the cover when ready to begin sampling. CAUTION - The sampler immediately begins to sample when the cover is removed. Keep the press-on cover in the package for later use.

Attach the sampler to the worker near his/her breathing zone with the perforations in the sampler facing forward. Assure that the area directly in front of the sampler is unobstructed throughout the sampling period.

Sample for 15 min to 240 min when using SKC 575-002 Passive Samplers to collect samples. The sampling rate for cyclohexane using SKC 575-002 Passive Sampler is 15.2 mL/min at 760 mmHg and 25 °C.

At the end of the sampling period, immediately detach the sampler from the worker and replace the cover over the front of the sampler to cover the perforations. Record the stop time on the Form OSHA-91A. Return each sampler to its package, close the package, and seal it with a Form OSHA-21.

Prepare a blank in a low background area by removing an unused sampler from its package. Then remove the cover. Immediately replace the cover onto the sampler. Return the blank sampler to its package, close the package, and seal it with a Form OSHA-21.

Verify that the sampling times are properly recorded on the Form OSHA-91A for each sample. Also, identify blank samples on this form.

Record the ambient temperature and atmospheric pressure at the sampling site on the Form OSHA-91A.

List any compounds that could be considered potential interferences, especially solvents that are being used in the sampling area.

Submit the samplers to the laboratory for analysis as soon as possible. If delay is unavoidable, store the samples in a refrigerator as a precaution.

Ship any bulk sample(s) in a container separate from the air samples.

3. Analytical Procedure

3.1 Apparatus

Gas chromatograph equipped with an FID. An Agilent 7890 Series GC System was used in this validation.

GC injection port liner. Restek Sky 4.0-mm ID Low Pressure Drop Precision Inlet Liner w/wool (Restek catalog no. 23309.1, or equivalent) was used in this validation.

GC column capable of separating cyclohexane from the extraction solvent, potential interferences, and internal standard. An Agilent J&W DB-624 20-m × 0.18-mm i.d. (film thickness 1.0- μ m) capillary column was used in this validation.

Electronic integrator or other suitable means of measuring GC detector response. A Waters Empower 3 Data System was used in this validation.

Glass vials (clear or amber) with PTFE-lined crimp caps. In this validation clear 2.0-mL vials were used.

Glass vials (clear or amber) with PTFE-lined screw caps. In this validation amber 4.0-mL vials were used.

A dispenser capable of delivering 1.0 mL of extraction solvent to prepare standards and samples. If a dispenser is not available, 1.0-mL volumetric pipettes can be used.

Class A volumetric flasks of convenient sizes for standard preparation. In this validation 1-mL, 2-mL, and 5-mL flasks were used.

Calibrated microliter syringes of convenient sizes for standard preparation. An SGE 25- μ L syringe was used in this validation.

Mechanical shaker. An Eberbach Model 6000 shaker was used in this validation.

Mechanical rotator. A Thermo Scientific Labquake Shaker Rotisserie Model 415110 was used in this validation.

3.2 Reagents

Carbon disulfide (CS₂) low benzene, [CAS no.75-15-0], reagent grade or better.

Cyclohexane. [CAS no.110-82-7], analytical grade or better.

N,N-Dimethylformamide (DMF), [CAS no. 68-12-2], reagent grade or better.

n-Hexylbenzene, [CAS no.1077-16-3], reagent grade or better.

Extraction solvent. The extraction solvent used in this method consists of 0.25 μ L/mL *n*-hexylbenzene in 99:1 (v/v) CS₂:DMF. The *n*-hexylbenzene was added as an internal standard (ISTD). The extraction efficiency is affected by the extraction solvent, the internal standard, the sampling medium, and the technique used to extract the samples. Reagents and other

techniques than those described in this method can be used provided they are tested as specified in the validation guidelines.³

3.3 Standard preparation

Prepare working analytical standards by injecting microliter amounts of the neat chemical into various volumetric flasks that are $\frac{3}{4}$ full of the extraction solvent and then fill to the mark on the flask with extraction solvent. For example, to prepare a target level standard based on active sampling at the recommended sampling rate and time, inject $25.00 \pm 0.25 \mu\text{L}$ of neat cyclohexane into a flask that is then filled up to $2.000 \pm 0.015 \text{ mL}$ of extraction solvent.

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 analyze the diluted samples.

3.4 Sample preparation

3.4.1 SKC 226-01 CSC Tube

Remove the plastic end caps from the sample tube and carefully transfer each section of the adsorbent to separate 2-mL vials. Discard the glass tube, foam, and glass wool plugs.

Add 1.0 mL of extraction solution to each vial and immediately seal the vials with PTFE-lined caps.

Extract the samples for 30 min by shaking.

3.4.2 SKC 575-002 Passive Sampler (In general, follow the manufacturer's instructions.)

Remove the plug and foam insert. Carefully transfer media to a 4-mL screw-cap vial.

Slowly add 2.0 mL of extraction solvent and cap the vial.

Mount the vials on the rotator rack and rotate the samplers for 30 min.

Transfer the solvent extract to a 2-mL vial.

³ Eide, M.; Simmons, M.; Hendricks, W. Validation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis, 2010. United States Department of Labor, Occupational Safety & Health Administration Web site. <http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf> (accessed March 2018).

3.5 Analysis

3.5.1 Analytical conditions

GC conditions

oven temperature: 40 °C (hold 2.25 min), ramp to 90 °C at 7 °C/min (hold 0 min), ramp to 225 °C at 25 °C/min (hold 0.2 min)
injection conditions: 250 °C, 1.0 µL (150 to 1 split)
run time: 15 min
column: Agilent J&W DB-624 capillary column, 20-m x 0.18-mm i.d, $d_f = 1.0\text{-}\mu\text{m}$, or equivalent
column mode: constant pressure (18 psi)
initial column gas flow: 1.5 mL/min (hydrogen)
septum purge: 3.0 mL/min (hydrogen)
inlet liner: Restek Sky 4.0-mm ID Low Pressure Drop Precision Inlet Liner w/wool (Restek catalog no. 23309.1, or equivalent)
retention times: 1.65 min (CS₂)
3.46 min (cyclohexane)
8.54 min (DMF)
14.1 min (n-hexylbenzene ISTD)

FID conditions

detector temperature: 300 °C
hydrogen flow: 30 mL/min
air flow: 400 mL/min
nitrogen make up flow: 25 mL/min

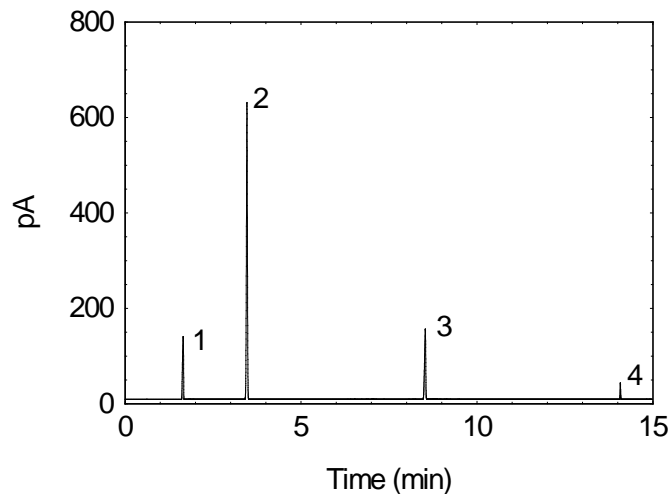


Figure 3.5.1. Chromatogram obtained at the target concentration for samples collected on SKC 226-01 CSC Tube with the recommended analytical conditions (1: CS₂; 2: cyclohexane; 3: DMF; 4: n-hexylbenzene (ISTD)).

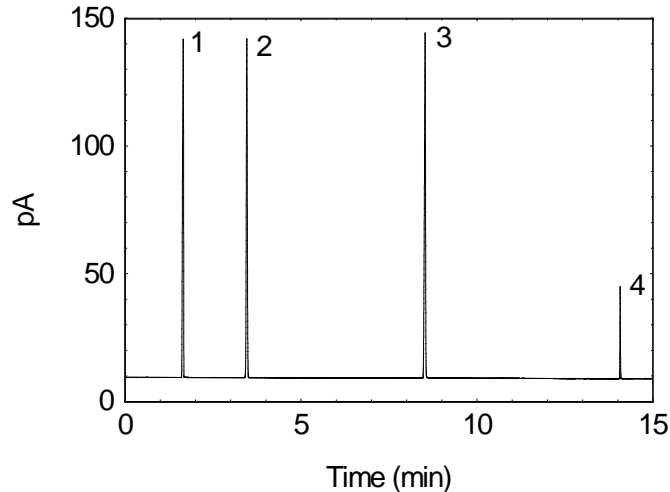


Figure 3.5.2. Chromatogram obtained at the target concentration for samples collected on SKC 575-002 Passive Sampler with the recommended analytical conditions (1: CS₂; 2: cyclohexane; 3: DMF; 4: n-hexylbenzene (ISTD)).

3.5.2 An internal standard (ISTD) calibration method is used. A linear calibration curve can be constructed by plotting ISTD-corrected response of standard injections versus micrograms of analyte per sample. When adding reporting limit standards to the calibration, the curves can be weighted to reduce bias at the reporting limit. Bracket the samples with freshly prepared analytical standards over a range of concentrations.

3.6 Interferences (analytical)

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

3.6.2 When necessary, the identity of an analyte peak can be confirmed with additional analytical data or procedures (Section 4.10).

3.7 Calculations

3.7.1 SKC 226-01 CSC Tube

Obtain the micrograms per sample (*M*) for each analyte. The back section is analyzed primarily to determine the extent of sampler saturation. If any analyte is found on the back section, it is added to the amount on the front section. If more than 20% of the total amount is found on the back section, report that the sampler may have been saturated on the Form OSHA-91B. 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.

$$M = \frac{M_{obs} - M_{blk}}{E_E}$$

where *M* is micrograms per sample blank corrected and extraction corrected
M_{obs} is micrograms per sample
M_{blk} is micrograms per sample blank
E_E is extraction efficiency, in decimal form

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

where C_M is concn by weight in air (mg/m^3)
 M is the total blank and extraction corrected microgram per sample
 V is liters of air sampled

$$C_V = \frac{C_M V_M}{M_r}$$

where C_V is concn by volume (ppm)
 C_M is concn by weight in air (mg/m^3)
 V_M is 24.46 (molar volume at NTP)
 M_r is molecular weight 84.16

3.7.2 SKC 575-002 Passive Sampler

Obtain the micrograms per sample (M) for each analyte as in section 3.7.1. The air concentration is calculated using the following formulas.

$$R_{SS} = R_{NTP} \left(\frac{T_{SS}}{T_{NTP}} \right)^{\frac{3}{2}} \left(\frac{P_{NTP}}{P_{SS}} \right)$$

where R_{SS} is the sampling rate at the sampling site (mL/min)
 R_{NTP} is the sampling rate at NTP (mL/min)
 T_{SS} is the temp at the sampling site (K)
 T_{NTP} is 298.2 K
 P_{SS} is the sampling site pressure (mmHg)
 P_{NTP} is 760 mmHg

$$C_M = \frac{M 1000}{R_{SS} t}$$

where C_M is concn by weight in air (mg/m^3)
 M is the total blank and extraction corrected microgram per sample
 R_{SS} is the sampling rate at the sampling site (mL/min)
 t is the sampling time (min)

$$C_V = \frac{C_M V_M}{M_r}$$

where C_V is concn by volume (ppm)
 V_M is 24.46 (molar volume at NTP)
 C_M is concentration by weight (mg/m^3)
 M_r is molecular weight 84.16

If the sampling site temperature is not provided, assume that it is 22.2 °C. If the sampling site atmospheric pressure is not given, calculate an approximate value based on the sampling site elevation from the following equation.

$$P_{SS} = AE^2 - BE + 760$$

P_{SS} is the approximate atmospheric pressure (mmHg)
 E is the sampling site elevation (ft)
 A is $3.768 \times 10^{-7} \text{ mmHg}/\text{ft}^2$
 B is 0.02741 mmHg/ft

4. Method Validation

General instruction for the laboratory validation of OSHA sampling and analytical methods that employ chromatographic analysis is presented in "Validation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"³. These Guidelines detail required validation tests, show examples of statistical calculations, list validation acceptance criteria, and define analytical parameters. Air concentrations listed in ppm are referenced to 25 °C and 760 mmHg (101.3 kPa).

4.1 Detection limit of the analytical procedure (DLAP)

The DLAP is measured as the mass of analyte introduced onto the chromatographic column. Ten analytical standards were prepared separately with equally descending increments of cyclohexane with the highest standard containing 9.34 µg/mL. This is the concentration that would produce a peak approximately 10 times the response of a reagent blank at or near the chromatographic retention time of the analyte. These standards and the reagent blank were analyzed with the recommended analytical parameters (1-µL injection with a 150:1 split). The data obtained were used to determine the required parameters (standard error of estimate and slope) for the calculation of the DLAP. Values of 0.019 and 0.023 were obtained for the slope and standard error of estimate, respectively. The DLAP was calculated to be 3.63 pg.

Table 4.1
Detection Limit of the Analytical Procedure
for Cyclohexane

concentration (µg/mL)	mass on column (pg)	area counts (µV•s)
0	0	0
0.934	6.23	0.138
1.87	12.5	0.273
2.80	18.7	0.430
3.73	24.9	0.510
4.67	31.1	0.608
5.60	37.3	0.754
6.54	43.6	0.870
7.47	49.8	0.958
8.40	56.0	1.07
9.34	62.3	1.22

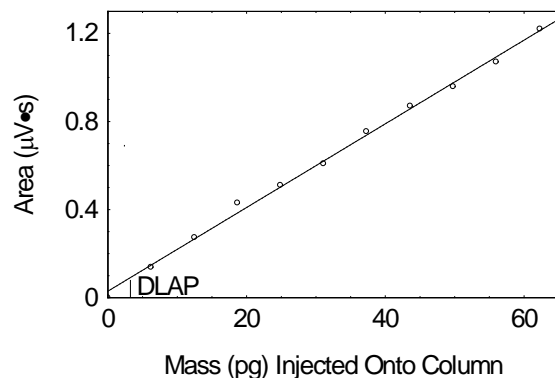


Figure 4.1. Plot of data to determine the DLAP for cyclohexane ($y = 0.019x + 0.030$).

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

4.2.1 SKC 226-01 CSC Tube

The DLOP is measured as mass per sample and expressed as equivalent air concentrations based on the recommended sampling parameters. Ten SKC 226-01 CSC Tube samplers were spiked with equally descending increments of analyte such that the highest sampler loading for cyclohexane was 9.34 µg/sample. This is the amount spiked on a sampler that would produce a peak approximately 10 times the response of a sample blank at or near the chromatographic retention time of the analyte. These spiked samplers and the sample blank were analyzed with the specified analytical parameters, and the data obtained were used to calculate the required parameters (standard error of estimate and the slope) for the calculation of the DLOP. Values of 0.134 and 0.019 were obtained for the slope and standard error of estimate, respectively. The DLOP was calculated to be 0.425 µg/sample (0.013 ppm or 0.045 mg/m³).

Table 4.2.1
Detection Limit of the Overall Procedure
for Cyclohexane on SKC 226-01 CSC Tube

mass per sample (μg)	area counts ($\mu\text{V}\cdot\text{s}$)
0	0
0.934	0.107
1.87	0.295
2.80	0.401
3.73	0.502
4.67	0.635
5.60	0.774
6.54	0.907
7.47	1.02
8.40	1.12
9.34	1.26

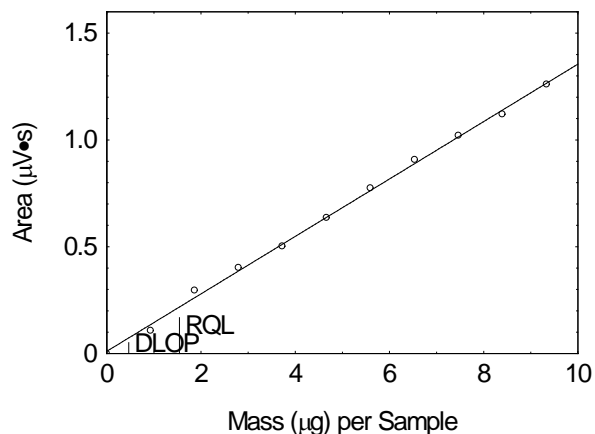


Figure 4.2.1.1. Plot of data to determine the DLOP for cyclohexane on SKC 226-01 CSC Tubes ($y = 0.134x + 0.011$).

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters that were obtained for the calculation of DLOP providing 75% to 125% of the analyte is recovered. The RQL is $1.42 \mu\text{g}/\text{sample}$ (0.043 ppm or $0.149 \text{ mg}/\text{m}^3$). Recovery at this concentration is 113%.

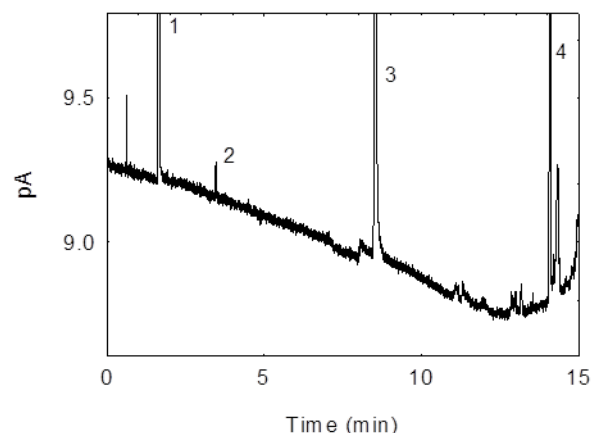


Figure 4.2.1.2. Chromatogram of the RQL for SKC 226-01 CSC Tubes (1: CS_2 ; 2: cyclohexane; 3: DMF; 4: n-hexylbenzene (ISTD)).

4.2.2 SKC 575-002 Passive Sampler

The DLOP is measured as mass per sample and expressed as equivalent air concentrations based on the recommended sampling parameters. Ten SKC 575-002 Passive Samplers were spiked with equally descending increments of analyte such that the highest sampler loading for cyclohexane was $10.3 \mu\text{g}/\text{sample}$. This is the amount spiked on a sampler that would produce a peak approximately 10 times the response of a sample blank at or near the chromatographic retention time of the analyte. These spiked samplers and the sample blank were analyzed with the recommended analytical parameters, and the data obtained were used to calculate the required parameters (standard error of estimate and the slope) for the calculation of the DLOP. Values for cyclohexane of 0.073 and 0.038 were obtained for the slope and standard error of estimate, respectively. The DLOP was calculated to be $1.56 \mu\text{g}/\text{sample}$ (0.124 ppm or $0.428 \text{ mg}/\text{m}^3$).

Table 4.2.2
Detection Limit of the Overall Procedure
for Cyclohexane
on SKC 575-002 Passive Sampler

mass per sample (μg)	area counts ($\mu\text{V}\cdot\text{s}$)
0	0
1.87	0.151
2.80	0.266
3.73	0.237
4.67	0.394
5.60	0.464
6.54	0.540
7.47	0.579
8.40	0.677
9.30	0.685
10.3	0.728

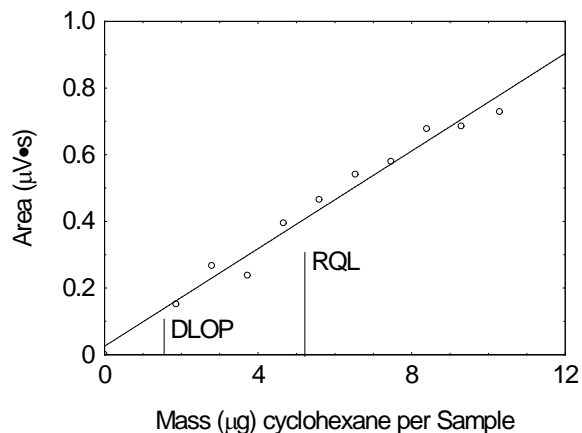


Figure 4.2.2.1. Plot of data to determine the DLOP for cyclohexane on SKC 226-01 Anasorb CSC ($y = 0.073x + 0.025$).

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters that were obtained for the calculation of DLOP providing 75% to 125% of the analyte is recovered. The RQL for cyclohexane is 5.21 $\mu\text{g}/\text{sample}$ (0.416 ppm or 1.43 mg/m^3). Recovery at this concentration is 107%.

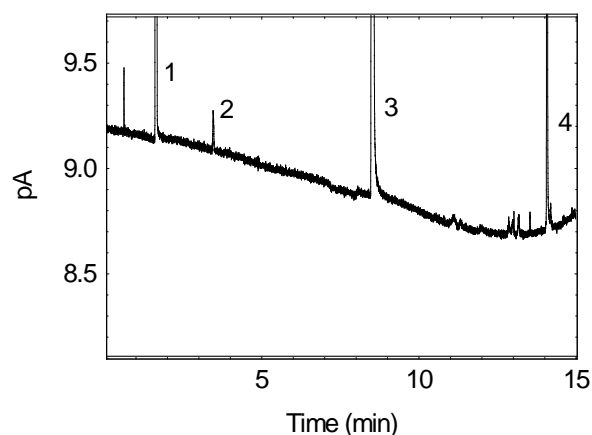


Figure 4.2.2.2. Chromatogram of the RQL for SKC 575-002 Passive Sampler (1:CS₂; 2: cyclohexane; 3: DMF; 4: n-hexylbenzene (ISTD)).

4.3 Precision of the analytical method

The precision of the analytical method, measured as the mass equivalent to the standard error of estimate, is determined from the linear regression of data points from standards over a range that covers 0.02 to 2 times the target concentration for the active sampler. This range was selected to cover both active and passive samples in one calibration. Calibration curves were constructed from three injections of five standards and weighted $1/x^2$. The standard error of estimate is 3.51 μg .

Table 4.3.1
Cyclohexane Instrument Calibration Areas

\times target concn ($\mu\text{g}/\text{sample}$)	0.02x	0.5x	1.0x	1.5x	2.0x
area counts ($\mu\text{V}\cdot\text{s}$)	217.9	4863	9726	14395	19453
peak	27.48	29.41	611.5	28.95	1216
ISTD	29.41	27.48	28.95	1216	28.62
peak	27.48	29.52	609.7	28.74	1228
ISTD	29.52	27.48	28.74	1228	28.93
peak	27.69	29.57	612.5	28.94	1226
ISTD	29.57	27.69	28.94	1226	28.99
peak	1807	1816	1826	1807	1807
ISTD	1807	1816	1826	1807	1807
peak	2436	2432	2468	2436	2436
ISTD	2436	2432	2468	2436	2436

Table 4.3.2
Instrument Calibration Area Ratio Cyclohexane

x target concn ($\mu\text{g}/\text{sample}$)	0.02x	0.5x	1.0x	1.5x	2.0x
217.9	4863	9726	14395	19453	
peak/ISTD	0.9344	21.12	42.49	62.53	85.68
	0.9309	21.21	42.45	62.11	85.36
	0.9364	21.16	42.29	61.90	84.84

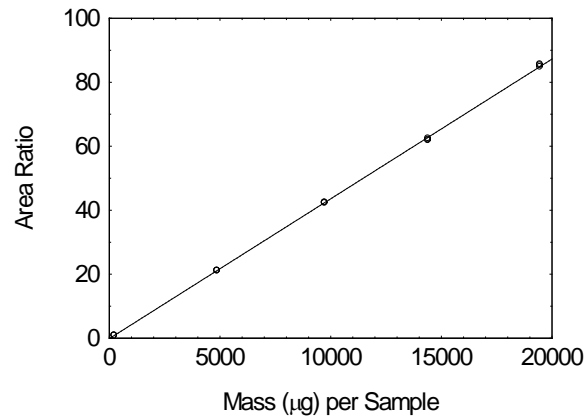


Figure 4.3. Weighted ($1/x^2$) calibration curve for cyclohexane ($y = 0.0044x - 0.015$).

4.4 Storage stability test

4.4.1 SKC 226-01 CSC Tube

Storage samples for cyclohexane were prepared by sampling a dynamically generated controlled test atmosphere using the recommended sampling parameters. The concentration for ambient storage of cyclohexane in the test atmosphere was the target concentration (305 ppm or $1048 \text{ mg}/\text{m}^3$) and the relative humidity was 69.2% at 25.2 °C. The concentration for refrigerated storage of cyclohexane in the test atmosphere was the target concentration (304 ppm or $1047 \text{ mg}/\text{m}^3$) and the relative humidity was 69.2% at 25.2 °C. Eighteen storage samples were prepared for each test. Three samples were analyzed on the day of generation. Fifteen samples were stored at reduced temperature (4°C) and the other fifteen were stored in a closed drawer at ambient temperature (about 22°C). At 3-4 day intervals, three samples were selected from each of the two storage sets and analyzed. Sample results are not corrected for extraction efficiency. A slight migration of cyclohexane to the B section of the tube was observed by day 19. This migration was not observed on the refrigerated samples.

Table 4.4.1
Storage Test for Cyclohexane
on SKC 226-01 CSC Tubes

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	101.6	102.6	101.2	105.3	100.9	101.7
3	105.2	101.1	100.4	100.4	100.3	101.7
7	103.8	101.3	102.7	101.4	101.7	102.0
10	105.7	101.5	102.0	102.3	103.5	102.0
14	101.7	101.6	93.90	103.0	101.9	101.9
17	97.52	100.6	100.8	101.7	101.5	101.6

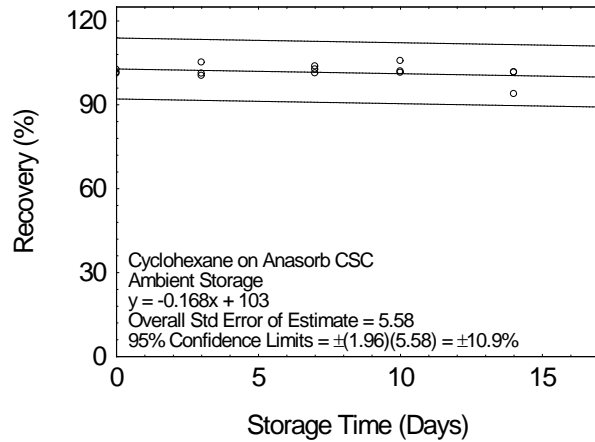


Figure 4.4.1.1. Ambient storage test for cyclohexane on SKC 226-01 CSC Tubes.

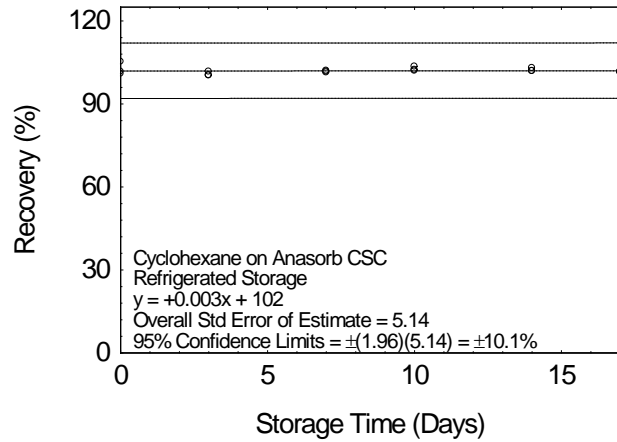


Figure 4.4.1.2. Refrigerated storage test for cyclohexane SKC 226-01 CSC Tubes.

4.4.2 SKC 575-002 Passive Sampler

Storage samples for cyclohexane were prepared by sampling a dynamically generated controlled test atmosphere using the recommended sampling parameters. The concentration for ambient storage of cyclohexane in the test atmosphere was the target concentration (305 ppm or 1048 mg/m³) and the relative humidity was 69.2% at 25.2 °C. The concentration for refrigerated storage of cyclohexane in the test atmosphere was the target concentration (304 ppm or 1047 mg/m³) and the relative humidity was 69.2% at 25.2 °C. Eighteen storage samples were prepared for each test. Three samples were analyzed on the day of generation. Fifteen samples were stored at reduced temperature (4 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 22 °C). At 3-4 day interval, three samples were selected from each of the two storage sets and analyzed. Sample results are not corrected for extraction efficiency.

Table 4.4.2
Storage Test for Cyclohexane
on SKC 575-002 Passive Sampler

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
	0	94.37	93.97	99.15	108.4	106.8
3	100.4	97.22	101.3	103.0	107.0	104.3
7	101.8	102.7	106.6	89.88	101.2	102.6
10	112.7	102.2	104.8	103.8	100.1	100.8
14	107.8	100.8	104.1	105.1	105.2	105.2
17	102.6	106.0	96.96	100.3	101.5	98.83

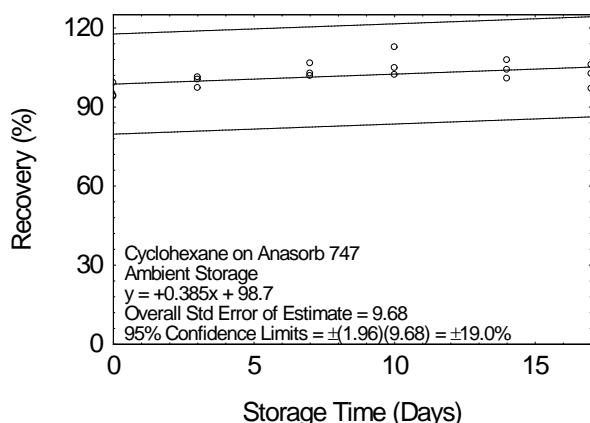


Figure 4.4.2.1. Ambient storage test for cyclohexane on SKC 575-002 Anasorb 747.

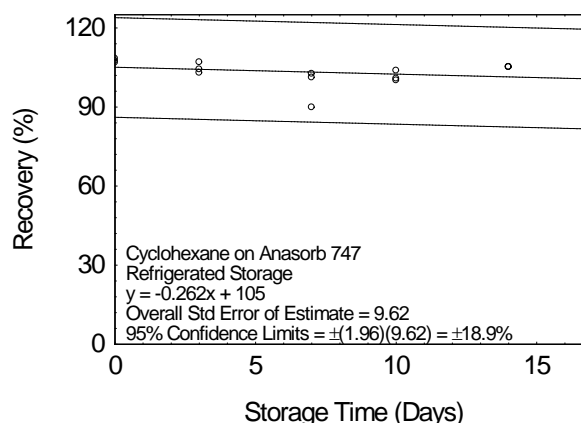


Figure 4.4.2.2. Refrigerated storage test for cyclohexane on SKC 575-002 Anasorb 747.

4.5 Precision (overall procedure)

The precision of the overall procedure at the 95% confidence level is obtained by multiplying the overall standard error of estimate by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). Ninety-five percent confidence intervals are drawn about the regression lines in the storage stability figures shown in Section 4.4.

4.5.1 SKC 226-01 CSC Tube

The precision of the overall procedure at the 95% confidence level for the ambient 17-day storage test (at the target concentration) for cyclohexane on SKC 226-01 CSC Tubes is $\pm 10.9\%$. It was obtained from the overall standard error of estimate value of 5.58% for the data shown in Figure 4.4.1. It contains an additional 5% for sampling pump error.

4.5.2 SKC 575-002 Passive Sampler

The precision values of the overall procedure at the 95% confidence level for the ambient temperature 17-day storage test at the target concentration for SKC 575-002 Passive Sampler are given in Table 4.5.2. They each include an additional $\pm 8.7\%$ for sampling rate variation^{4,5}. There are different values given, depending on whether both, either, or neither temperature (*T*) or atmospheric pressure (*P*) are known at the sampling site. If the sampling site temperature is unknown, it is assumed to be $22.2 \pm 15^\circ\text{C}$ ($72 \pm 27^\circ\text{F}$) and a variability of $\pm 7.7\%$ is included. If the atmospheric pressure is not known, it is estimated from the sampling site elevation and a variability of $\pm 3\%$ is included.

known condition	error (%)	precision ($\pm\%$)
both <i>T</i> & <i>P</i>	9.68	19.0
only <i>T</i>	10.1	19.8
only <i>P</i>	12.4	24.3
neither <i>T</i> nor <i>P</i>	12.7	24.9

⁴ Hendricks, W. Development of a Protocol for Laboratory Testing of Diffusive Samplers, 1996. United States Department of Labor, Occupational Safety & Health Administration. <http://osha.gov/dts/sltc/methods/studies/3movm/3movm.html> (accessed March 2018).

⁵ Hendricks, W. Determination of the Sampling Rate Variation for SKC 575 Series Passive Samplers, 1998. United States Department of Labor, Occupational Safety & Health Administration. <http://osha.gov/dts/sltc/methods/studies/skc575/skc575.html> (accessed March 2018).

4.5.3 Recovery

The recovery of cyclohexane from samples used in a 17-day ambient storage test remained above 100.1% for SKC 226-01 CSC Tubes and above 105.2% for SKC 575-002 Passive Samplers. These results are not corrected for extraction efficiency.

4.6 Reproducibility

Six samples were prepared for each type of samplers by sampling a dynamically generated controlled test atmosphere similar to that used in the collection of the storage samples. The concentration of cyclohexane in the test atmosphere was the target concentration (304.9 ppm or 1049 mg/m³) and the relative humidity was 67.64% at 25.0 °C. The samples were submitted to the OSHA Salt Lake Technical Center for analysis using the procedures described in this method. SKC 226-01 CSC Tube and SKC 575-002 Passive Sampler samples were stored for 19 days at 4 °C before analysis. Sample results were corrected for extraction efficiency. No sample result for cyclohexane had a deviation greater than the precision of the overall procedure determined in Section 4.5. Theoretical concentrations for SKC 575-002 Passive Samplers were determined using the sampling rate determined in Section 4.7 converted to its equivalent sampling rate at sampling site temperature and atmospheric pressure.

Table 4.6.1
Reproducibility Data for Cyclohexane
Collected on SKC 226-01 CSC Tubes

theoretical (ppm)	recovered (ppm)	recovery (%)	deviation (%)
304.9	313.7	102.9	+2.89
304.9	305.2	100.1	+0.10
304.9	328.6	107.8	+7.77
304.9	309.9	101.6	+1.64
304.9	299.9	98.36	-1.64
304.9	309.2	101.4	+1.41

Table 4.6.2
Reproducibility Data for Cyclohexane
Collected on SKC 575-002 Passive Sampler

theoretical (ppm)	recovered (ppm)	recovery (%)	deviation (%)
304.9	310.0	101.7	+1.67
304.9	300.0	98.39	-1.61
304.9	320.4	105.1	+5.08
304.9	307.7	100.9	+0.92
304.9	292.9	96.06	-3.94
304.9	308.2	101.1	+1.08

4.7 Sampler capacity and sampling rate for SKC 226-01 CSC Tubes and SKC 575-002 Anasorb 747

4.7.1 SKC 226-01 CSC Tubes

The sampling capacity of the front section of a coconut-shell charcoal tube was tested by sampling a dynamically generated controlled test atmosphere containing cyclohexane at two times the target concentration (613 ppm or 2108 mg/m³) with a 72.9% relative humidity at 23.5 °C. The samples were collected at 50 mL/min. A second tube was placed behind the first tube and changed at 150, 195, 210, 225, 240, and 300 minutes. Breakthrough exceeded 5% at 12.0 liters. Therefore, the recommended sampling time with a 20% safety margin is 190 minutes.

Table 4.7.1
Breakthrough of Cyclohexane
From Front Section of Anasorb CSC

test no.	air vol (L)	sampling time (min)	downstream concn (mg/m ³)	break-through (%)
1	7.05	150	0	0.00
	9.17	195	2.64	0.13
	9.87	210	11.3	0.54
	10.6	225	29.9	1.42
	11.3	240	65.6	3.11
	14.1	300	436	20.7
2	7.60	150	0	0.00
	9.88	195	0.847	0.04
	10.6	210	12.0	0.57
	11.4	225	51.6	2.45
	12.2	240	132	6.26
	15.2	300	560	26.6
3	7.39	150	0	0.00
	9.56	195	0	0.00
	10.3	210	2.60	0.12
	11.1	225	20.7	0.98
	11.8	240	78.5	3.72
	14.8	300	498	23.6

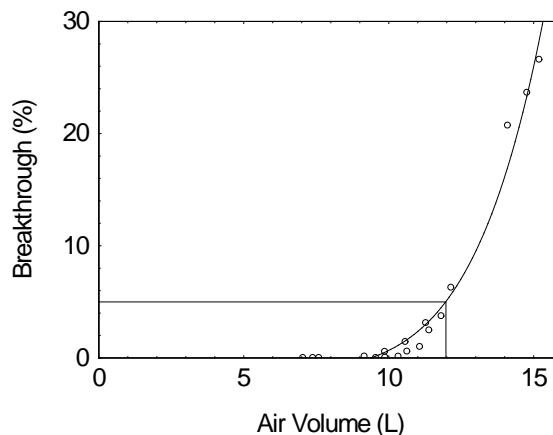


Figure 4.7.1. Five-percent breakthrough air volume for cyclohexane.

4.7.2 SKC 575-002 Passive Sampler

The sampling rate and sampler capacity for SKC 575-002 Anasorb 747 were determined by sampling a dynamically generated controlled test atmosphere for increasing time intervals. Sampler capacity is exceeded when the plotted sampling rate decreases rapidly as the sorbent bed becomes saturated. The concentration of the test atmosphere was two times the target concentration (613 ppm or 2110 mg/m³) at 71.4% relative humidity and 24.6 °C. The preliminary sampling rate was determined by averaging the nine values for the 0.5, 1 and 2 hour samples. Horizontal lines were placed 10% above and 10% below the preliminary sampling rate. The sampling rate for cyclohexane is 15.2 mL/min at 760 mmHg and 25 °C and represent the average of all values between the lines. The standard deviation and RSD is 0.397 mL/min and 2.61%. The data obtained are shown in Table 4.7.2 and Figure 4.7.2. Mass collected is corrected for extraction efficiency. The recommended sampling time is 4 hours.

Table 4.7.2
Determination of Sampling Rate and Time
for Cyclohexane

time (h)	sampling rate (mL/min)		
	first	second	third
0.083	15.1	14.3	14.6
0.167	15.5	15.3	14.8
0.5	15.4	15.7	14.9
1	14.9	14.9	15.9
2	15.4	15.4	15.3
3	15.3	15.3	15.2
4	15.2	15.5	15.4
6	15.0	15.1	15.2
8	15.0	15.2	14.9
10	15.8	15.6	16.3

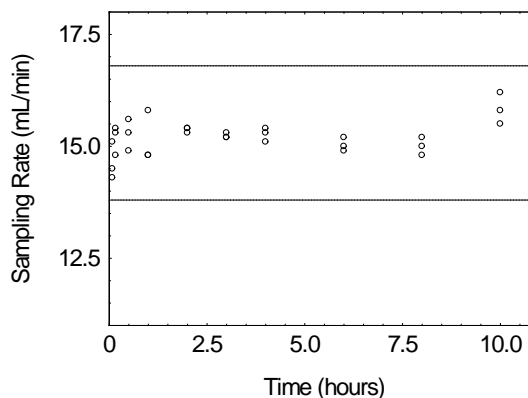


Figure 4.7.2. Plot of data to determine the sampling rate for cyclohexane.

4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is affected by the extraction solvent, the internal standard, the sampling medium, and the technique used to extract the samples. Other reagents and techniques than described in this method can be used provided they are tested as specified in the validation guidelines.³

4.8.1 SKC 226-01 CSC Tube

Extraction efficiency

The extraction efficiency of cyclohexane was determined by liquid-spiking four coconut-shell charcoal tubes at each concentration level. These samples were stored overnight at ambient temperature and then analyzed. The overall mean extraction efficiency over the working range of 0.1 to 2 times the target concentration was 101%. The extraction efficiency at the RQL was 94.08%. The presence of water had no significant effect on extraction efficiency. The extraction efficiencies for the RQL and for the wet samplers are not included in the overall mean. Wet media were prepared by drawing humid air (73.3% relative humidity at 24.8 °C) at 50 mL/min for 4 hours. The data obtained are shown in Table 4.8.1.

Table 4.8.1
Extraction Efficiency of Cyclohexane from 226-01 Anasorb CSC

<u>level</u>		<u>sample number</u>				
<u>x target concn</u>	<u>µg per sample</u>	1	2	3	4	mean
0.1	972.6	100.1	99.75	101.9	100.5	100.6
0.25	2490	100.7	100.9	99.41	100.3	100.3
0.5	4980	102.0	101.6	102.1	101.2	101.7
1.0	9726	100.8	100.8	102.2	100.7	101.1
1.5	14940	101.0	100.9	100.7	100.6	100.8
2.0	19453	101.1	101.5	101.4	101.4	101.4
RQL	1.56	102.2	82.70	107.3	84.12	94.08
1.0 (wet)	9726	103.8	105.2	104.9	105.7	104.9

4.8.2 SKC 575-002 Passive Sampler

Extraction efficiency

The extraction efficiency of cyclohexane was determined by liquid-spiking four SKC Anasorb 747 badges at each concentration level. These samples were stored overnight at ambient temperature and then analyzed. The overall mean extraction efficiency over the working range of 0.1 to 2 times the target concentration was 102.9%. The extraction efficiency at the RQL was 102.5%. The presence of water had no significant effect on extraction efficiency. The extraction efficiencies for the RQL and for the wet samplers are not included in the overall mean. Wet media were prepared by drawing humid air across the samplers (73.3% relative humidity at 24.8 °C) for 4 hours. The data obtained are shown in Table 4.8.2.

Table 4.8.2
Extraction Efficiency of Cyclohexane
from SKC 575-002 Anasorb 747

<u>level</u>		<u>sample number</u>				
\times target concn	μg per sample	1	2	3	4	mean
0.1	389.1	98.58	102.3	99.46	103.4	100.9
0.25	972.6	102.3	103.1	101.3	102.0	102.2
0.5	1945	103.6	104.7	103.9	104.0	104.1
1.0	3891	102.3	103.5	103.1	102.6	102.9
1.5	5836	102.7	105.0	103.7	104.6	104.0
2.0	7781	102.2	103.7	103.6	103.3	103.2
RQL	5.76	109.9	101.1	107.5	91.37	102.5
1.0 (wet)	3891	103.2	103.9	102.5	103.3	103.2

4.8.3 Stability of extracted samples

The stability of extracted samples was examined by reanalyzing the target concentration samples 24, 48, and 72 hours after the initial analysis. After the original analysis was performed two vials were recapped with new septa which were replaced after each reanalysis. The remaining two vials retained their punctured septa throughout the test. All samples were allowed to stand in the autosampler tray at 22.2 °C. The samples were reanalyzed with freshly prepared standards. The term “diff” refers to the difference between the initial analysis and the subsequent analysis. Each septum was punctured four times for each injection. The data obtained are shown in Tables 4.8.3.1-4.8.3.2.

Table 4.8.3.1
Stability of Extracted Samples on SKC 226-01 CSC Tube for Cyclohexane

<u>punctured septa replaced</u>							<u>punctured septa retained</u>						
initial (%)	24 h (%)	diff (%)	48 h (%)	diff (%)	72 h (%)	diff (%)	initial (%)	24 h (%)	diff (%)	48 h (%)	diff (%)	72 h (%)	diff (%)
96.96	100.2	3.2	99.06	2.1	100.8	3.8	101.0	103.8	2.8	101.2	0.2	100.0	-1.0
99.85	104.3	4.4	102.4	2.6	103.5	3.6	102.0	104.5	2.5	101.1	-0.9	99.57	-2.4
			mean							mean			
98.40	102.2	3.8	100.7	2.3	102.1	3.7	101.5	104.2	2.7	101.2	-0.3	99.80	-1.7

Table 4.8.3.2
Stability of Extracted Samples on SKC 575-002 Passive Sampler for Cyclohexane

<u>punctured septa replaced</u>							<u>punctured septa retained</u>						
initial (%)	24 h (%)	diff (%)	48 h (%)	diff (%)	72 h (%)	diff (%)	initial (%)	24 h (%)	diff (%)	48 h (%)	diff (%)	72 h (%)	diff (%)
107.4	107.2	-0.2	105.9	-1.6	106.3	-1.1	107.8	106.9	-0.9	104.2	-3.6	101.8	-6.0
108.5	108.2	-0.3	106.8	-1.7	107.3	-1.2	108.2	101.6	-6.6	103.7	-4.4	101.5	-6.7
			mean							mean			
107.9	107.7	-0.2	106.3	-1.6	106.8	-1.1	108.0	104.3	-3.8	104.0	-4.0	101.7	-6.3

4.9 Sampling interferences

The tested sampling interferences had no significant effect on the ability of SKC 226-01 CSC Tubes and 575-002 Anasorb 747 samplers to collect or retain cyclohexane.

4.9.1 SKC 226-01 CSC Tubes

Retention

Retention was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (609 ppm or 2095 mg/m³) of cyclohexane at 71.0% relative humidity and 24.7 °C. The test atmosphere was sampled with six SKC 226-01 CSC Tubes at 50 mL/min for 47.5 min. Sampling was discontinued and the samplers were separated into two sets of 3 samplers each.

Table 4.9.1
Retention of Cyclohexane
on 226-01 Anasorb CSC

set	Recovery (%)			mean
	1	2	3	
first	96.31	104.8	101.4	100.9
second	103.6	102.4	102.5	102.8
second/first				101.9

The generation system was flushed with contaminant-free air. Contaminant-free air is laboratory conditioned air at known relative humidity and temperature but without any added chemical except water. Sampling was resumed with the second set of three samples and contaminant-free air at 71.0% relative humidity and 24.7 °C at 50 mL/min for 142.5 min and then all six samplers were analyzed. The data obtained are shown in Table 4.9.1.

Low humidity

The effect of low humidity was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (611 ppm or 2103 mg/m³) at 17.3% relative humidity and 25.5 °C. The test atmosphere was sampled with three charcoal tubes at 50 mL/min for 190 min. All of the samples were immediately analyzed. Sample results were 95.80%, 98.51%, and 101.6% of theoretical.

Low concentration

The effect of low concentration was tested by sampling a dynamically generated controlled test atmosphere containing 0.1 times the target concentration (30.7 ppm or 105.8 mg/m³) at 69.0% relative humidity and 25.3 °C. The test atmosphere was sampled with three charcoal tubes at 50 mL/min for 240 min. All of the samples were immediately analyzed. Sample results were 108.8%, 103.6%, and 111.5% of theoretical.

Chemical interference

The effect of potential chemical sampling interference was tested by sampling a dynamically generated controlled test atmosphere containing one times the target concentration (305 ppm or 1049 mg/m³) at 70.5% relative humidity and 25.2 °C and toluene at it's target (198 ppm or 746.5 mg/m³). The test atmosphere was sampled with three charcoal tubes at 50 mL/min for 240 min. All of the samples were immediately analyzed. Sample results were 103.2%, 101.4%, and 101.1% of theoretical.

4.9.2 SKC 575-002 Passive Sampler

Reverse diffusion

Reverse diffusion was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (613 ppm or 2108 mg/m³) at 69.2% relative humidity and 25.5 °C. The test atmosphere was sampled with six SKC 575-002 Anasorb 747 Passive Samplers for 60 min. Sampling was discontinued and the samplers were separated into two sets of 3 samplers each. The generation system was

flushed with contaminant-free air. Contaminant-free air is laboratory conditioned air at known relative humidity and temperature but without any added chemical except water. Sampling was resumed with the second set of three samples and contaminant-free air at 61.4% relative humidity and 25.9 °C for 180 min and then all six samplers were analyzed. The data obtained are shown in Table 4.9.2. Reverse diffusion is not an issue as all analyte was retained.

Table 4.9.2
Reverse Diffusion of Cyclohexane
on 575-02 Anasorb 747

set	mass (µg)			mean
	1	2	3	
first	2387	2359	2406	2384
second	2442	2561	2444	2482
second/first				104.1

Low humidity

The effect of low humidity was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (611 ppm or 2103 mg/m³) at 17.3% relative humidity and 25.5 °C. The test atmosphere was sampled with three passive samplers for 240 min. All of the samples were immediately analyzed. Results were calculated using the average sampling rate determined in Section 4.7 converted to its equivalent sampling rate at sampling site temperature and pressure. The results were 98.93%, 100.2%, and 103.4% of theoretical.

Low concentration

The effect of low concentration was tested by sampling a dynamically generated controlled test atmosphere containing 0.1 times the target concentration (30.7 ppm or 105.8 mg/m³) at 72.2% relative humidity and 25.0 °C. The test atmosphere was sampled with three passive samplers for 240 min. All of the samples were immediately analyzed. Results were calculated using the average sampling rate determined in Section 4.7 converted to its equivalent sampling rate at sampling site temperature and pressure. The results were 111.4%, 108.7%, and 110.0% of theoretical.

Chemical sampling interference

The effect of potential chemical sampling interference was tested by sampling a dynamically generated controlled test atmosphere containing one times the target concentration (305 ppm or 1049 mg/m³) at 70.5% relative humidity and 25.2 °C and toluene at its target (198 ppm or 746.5 mg/m³). The test atmosphere was sampled with three 575-002 passive samplers for 240 min. All of the samples were immediately analyzed. Results were calculated using the average sampling rate determined in Section 4.7 converted to its equivalent sampling rate at sampling site temperature and pressure. The results were 101.8%, 103.0%, and 104.6% of theoretical.

4.10 Qualitative analysis

When necessary, the identity or purity of an analyte peak can be confirmed by GC/MS or by another analytical procedure. For the levels analyzed in this method the use of GC/MS is recommended. A total ion current (TIC) chromatograph and mass spectrum, obtained using the analytical conditions described below, are shown in Figure 4.10.1 and 4.10.2.

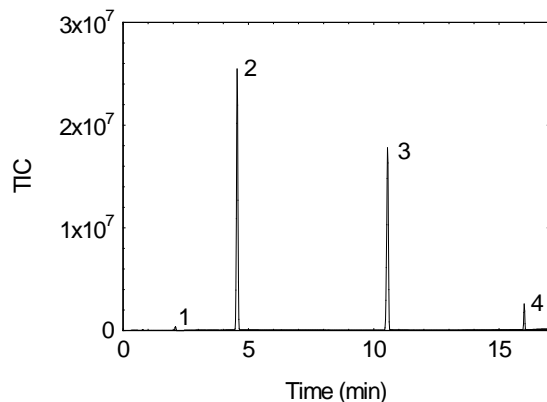


Figure 4.10.1. TIC obtained at the active target concentration with the recommended analytical conditions (1: CS₂; 2: cyclohexane; 3: DMF; 4: n-hexylbenzene (ISTD)).

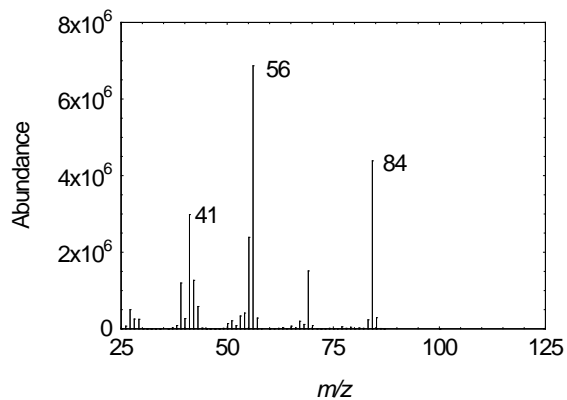


Figure 4.10.2. Mass spectrum of cyclohexane in the extraction solvent.

GC conditions

oven temperature:	40 °C (hold 3 min), ramp to 90 °C at 6 °C/min (hold 0 min), ramp to 225 °C at 25 °C/min (hold 0.27 min)
injection conditions:	250 °C, 1.0 µL (150 to 1 split)
run time:	17 min
column:	Agilent J&W DB-624, 20-m x 0.18-mm i.d., (1-µm df), (catalog no. 121-1324, or equivalent)
column mode:	constant flow (initial avg. velocity 44 cm/sec)
initial column gas flow:	1.0 mL/min (helium)
septum purge:	3.0 mL/min (helium)
inlet liner:	Restek Sky 4.0 mm ID Low Pressure Drop Precision Inlet Liner w/wool (Restek catalog no. 23309.1, or equivalent)
retention times:	2.09 min (CS ₂) 4.55 (cyclohexane) 10.8 min (DMF) 16.1 min (n-hexylbenzene ISTD)

MS conditions

mode:	Electron Ionization
timed events:	2.2 min MS off 2.6 min MS On
EMV mode:	gain factor 5
MS source:	250 °C
MS quadrupole:	150 °C
MSD transfer line:	300 °C
scan parameters:	m/z 25-400
threshold:	150

4.11 Generation of test atmospheres

A test atmosphere generator, as diagramed in Figure 4.11, was set up in a walk-in hood. House air was regulated using a Miller Nelson Model 401 Flow-Temperature-Humidity Control System. A measured flow of cyclohexane was introduced with an ISCO model 100DM syringe pump through a short length of 0.53-mm uncoated fused silica capillary tubing into a vapor generator where it was heated and evaporated into a measured flow of dilution air coming from the Miller Nelson control system. The cyclohexane and dilution air flowed into a mixing chamber (76-cm × 15-cm) and then into a sampling chamber (56-cm × 9.5-cm). Samples were collected through sampling ports on the sampling chamber. Temperature and humidity were measured near the exit of the sampling chamber using a Vaisala HUMICAP Hand-Held Humidity and Temperature Meter HM70.

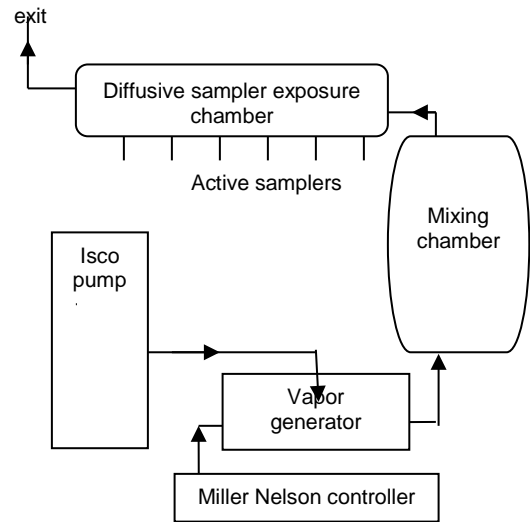


Figure 4.11. The test atmosphere generation and sample collection apparatus.