Method no.:	1016
Version:	1.0
Target concentration:	0.5 ppm (2.05 mg/m^3) (TWA)
Procedure:	Active samples are collected by drawing workplace air through specially dried silica gel tubes with personal sampling pumps. Samples are extracted with 95:5 ethyl alcohol:water and analyzed by gas chromatography using a flame ionization detector (GC-FID).
Recommended sampling time and sampling rate:	200 min at 50 mL/min (10.0 L) (TWA); 15 min at 0.2 L/min (3 L) (short term) 180 min at 50 mL/min (9.0 L) (TWA); 15 min at 0.2 L/min (3 L) (short term) if sampling for acetoin and diacetyl along with 2,3-pentanedione
Reliable quantitation limit:	9.3 ppb (38 µg/m^3)
Standard error of estimate at the target concentration:	10.1%
Special requirements:	Protect samplers from the light exposure during sampling, shipping, and analysis. Samples should be kept cold and shipped cold to the lab as soon as possible after sampling, preferably by overnight or express shipping. Samples should be analyzed within 17 days of sampling.
Status of method:	Fully validated method. This method has been subjected to the established validation procedures of the Methods Development Team.

July 2010

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1. General Discussion

For assistance with accessibility problems in using figures and illustrations presented in this method, please contact Salt Lake Technical Center (SLTC) 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

OSHA is concerned about workplace exposure to 2,3-pentanedione because it is a butter flavoring agent that is sometimes substituted for diacetyl.[1] 2,3-Pentanedione is chemically similar to diacetyl and may have similar toxicological properties.[2] This work was performed because OSHA has no sampling and analytical method for 2,3-pentanedione and none was found in a literature review.

One of the main objectives of this work was to enable OSHA CSHOs to monitor workplace exposure to diacetyl, acetoin and 2,3-pentanedione simultaneously on the same sample. Because of the similarities of the chemicals, it was decided to validate existing sampling and analytical methodology specified in OSHA Method 1013[3] for 2,3-pentanedione. That method requires sampling with two commercially available silica gel tubes connected in series. This method specifies a different GC column than specified in Method 1013 in order to optimize the analytical separation. The reliable quatitation limits for acetoin and diacetyl cited in OSHA Method 1013 were confirmed with the GC column used in this validation.

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

2,3-Pentanedione is moderately toxic by ingestion, a skin irritant, and can cause eye and respiratory tract irritation.[4] The oral LD_{50} in rats is 3000 mg/kg. The skin irritation test in rabbits showed moderate irritation for an exposure of 500 mg/24h. Studies exposing rats to 118, 241, 318, or 354 ppm 2,3-pentanedione for 6 hours showed epithelial changes in the airways which increased with increasing air concentrations with necrosuppurative tracheitis in the rats exposed to 354 ppm.[5] This epithelial cell damage was found to progress post-exposure in rats sacrificed a day later. These epithelial changes included degeneration, apoptosis, necrosis, and neutrophilic inflammation.

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1 News Watch, Diacetyl. *The Synergist.* **March 2010**. American Industrial Hygiene Association Web site. http://www.aihasynergist-digital.org/aihasynergist/201003#pg39 (accessed August 2010).

2 Hubbs, A.F.; Mosely, A.E.; Goldsmith, W.T.; Jackson, M.C.; Kashon, M.L.; Battelli, L.A.; Schwegler-Berry, D.; Goravanahally, M.P.; Frazer, D.; Fedan, J.S.; Kreiss, K.; and Castranova, V. Airway Epithelial Toxicity of the Flavoring Agent, 2,3-Pentanedione. *Toxicologist* [CD-ROM] **2010**, *114*, 319.

3 Simmons, M., Hendricks, W. Acetoin and Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. https://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed December 2009).

4 *Sax's Dangerous Properties of Industrial Materials*, 10th ed.; Vol. 3, Lewis, R.J. Ed.; John Wiley & Sons; New York, 2000, p 2843.

5 Hubbs, A.F.; Mosely, A.E.; Goldsmith, W.T.; Jackson, M.C.; Kashon, M.L.; Battelli, L.A.; Schwegler-Berry, D.; Goravanahally, M.P.; Frazer, D.; Fedan, J.S.; Kreiss, K.; and Castranova, V. Airway Epithelial Toxicity of the Flavoring Agent, 2,3-Pentanedione. *Toxicologist* [CD-ROM] **2010**, *114*, 319.

1.1.3 Workplace exposure

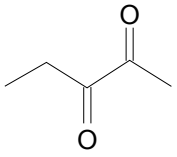
2,3-Pentanedione is a natural flavorant and odorant that is also synthesized for use in odor and flavor manufacturing.[6] It is used to give products a buttery, nutty, cheesy, fruity, toasted, chocolate, or caramel taste. It also gives products a buttery, fruity, and caramel odor. There can be as much as 58 ppm in food flavorings, and up to 0.08% in fragrances.

2,3-Pentanedione is used as a solvent for cellulose acetate, paints, inks, lacquers, as a starting material for dyes, pesticides and pharmaceuticals, and as a photoinitializer for photo-reactive dyes.[7]

1.1.4 Physical properties and other descriptive information[8,9,10]

synonyms:	acetyl propanal; acetyl propionyl; β,γ-dioxopentane; beta, gamma-dioxopentane; 2,3-pentadione		
IMIS[11]:	P110	CAS number:	600-14-6
boiling point:	110-112 °C (230-234 °F)	melting point:	-52 °C (-62 °F)
density:	0.957 g/mL @ 25 °C	molecular weight:	100.12
flash point:	19 °C (66 °F) (open cup)	molecular formula:	$C_5H_8O_2$
appearance:	yellow to yellow-green liquid	lower explosive limit:	1.8% (by volume)
autoignition temperature:	265 °C (509 °F)		
solubility:	66.7 g/L water; miscible with alcohol, fixed oils, propylene glycol		
odor:	butter-like in dilute concentration, quinone-like in high concentration		
reactive hazards:	light sensitive (Section 4.9); vapors are highly flammable and may ignite when pouring or pumping due to static electricity		

structural formula of 2,3-pentanedione



This method was validated according to the OSHA SLTC "Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[12]. 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. Air concentrations in ppm are referenced to 25 °C and 760 mmHg (101.3 kPa).

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[6] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 1495.

[7] 2,3-Pentanedione, Chemicalland21 Website. http://chemicalland21.com/lifescience/foco/2,3-PENTANEDIONE.htm (accessed February 2010).

[8] *Sax's Dangerous Properties of Industrial Materials*, 10th ed.; Vol. 3, Lewis, R.J.; John Wiley & Sons; New York, 2000, p 2843.

[9] Lewis, R. J. Sr., Ed. *Hawley's Condensed Chemical Dictionary*, 14th ed.; Van Nostrand Reinhold Co.: New York, 2001, p 14.

[10] 3-Pentanedione(600-14-6) Chemical Book Web site. http://www.chemicalbook.com/ProductMSDSDetailCB6166470_EN.htm (accessed 1/27/2010).

[11] 2,3-Pentanedione (OSHA Chemical Sampling Information), 2010. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_260240.html, (accessed 1/5/2010).

[12] Eide, M.; Hendricks, W.; Simmons, M. Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 2010. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.html (accessed January 2010).

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 with 110-cm × 7-mm o.d. glass sampling tubes packed with a single section (600 mg) of specially cleaned and dried silica gel. The section is held in place with glass wool and with a glass fiber filter in the front and glass wool at the back. A sampling train is prepared by placing two tubes in series. For this validation, commercially prepared sampling tubes were purchased from SKC, Inc. The two tubes are identical, but SKC labels the tubes as "Part A" which is the front tube and as "Part B" which is the back tube (Catalog no. 226-183, lot no. 6148).

Use an opaque tube holder, such as SKC, Inc. Tube Cover D (cat. no. 224-29D) to cover the sampling train during sampling. If the tube holder is not opaque, wrap the sampler with aluminum foil. Light can decompose collected 2,3-pentanedione.

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

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, break off both 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 and to minimize the potential of glass shards entering the foodstuffs. All tubes should be from the same lot.

A sampling train is prepared by attaching a Part A tube in front of and in series with a Part B tube, with both glass fiber filters facing forward.

The Part B tube in the sampling train is used as a back-up and is positioned nearest the sampling pump. Attach the tube holder (with the adsorbent tube sampling train) to the sampling pump so that the sampling train 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 the air to be sampled directly into the inlet of the tube holder. The air being sampled is not to be passed through any hose or tubing before entering the sampling tube.

Sample for up to 200 min at 50 mL/min (10 L) to collect TWA (long-term) samples. If acetoin and/or diacetyl are anticipated to be present, sample for up to 180 min at 50 mL/min (9 L) to collect TWA (long-term) samples.

Sample for 15 min at 0.2 L/min (3 L) to collect short-term samples.

After sampling for the appropriate time, remove the sampling train, separate the tubes, and cap each tube with plastic end caps. Separately wrap each tube in aluminum foil and seal each tube 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 volumes (L), sampling time (min), and sampling rate (mL/min) for each sample, along with any potential interferences on the Form OSHA-91A.

Submit the samples to the laboratory for analysis as soon as possible after sampling, preferably by overnight or express shipping. If delay is unavoidable, store the samples in a refrigerator. Ship samples cold to laboratory, such as shipping with frozen plastic ice packs in a cooler.

Ship any bulk samples separate from the air samples.

3. Analytical Procedure

Adhere to the rules set down in your laboratory's Chemical Hygiene Plan[13] (for instance OSHA SLTC adheres to: "The OSHA SLTC Chemical Hygiene Plan"). Avoid skin contact and inhalation of all chemicals and review all MSDSs before beginning this analytical procedure. Follow all applicable quality assurance practices established in your internal quality system (for instance OSHA SLTC follows: "The OSHA SLTC Quality Assurance Manual").

3.1 Apparatus

Gas chromatograph equipped with an FID. An Agilent 6890 GC System equipped with a Chemstation, an automatic sample injector, and an Agilent tapered, deactivated, split, low pressure drop injection port liner with glass wool (catalog no. 5183-4647) was used in this validation.

A GC column capable of separating 2,3-pentanedione from the extraction solvent, potential interferences, and internal standard. A DB-1 60-m × 0.32-mm i.d. (5-µm df) capillary column was used in this validation.

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

Amber glass vials with PTFE-lined caps. Two and 4-mL vials were used in this validation.

A dispenser capable of delivering 2.0 mL of extraction solvent to prepare standards and samples. If a dispenser is not available, 2.0-mL volumetric pipettes can be used.

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

Calibrated syringe - 25-µL and other convenient sizes for preparing standards.

.

Rotator. A Fisher Roto Rack was used to extract the samples in this validation.

3.2 Reagents

DI water, 18.0 MΩ-cm. A Barnstead NanoPure Diamond system was used to purify the water in this validation.

Ethyl Alcohol, [CAS no. 64-17-5]. The ethyl alcohol:water solution used in this validation was 95% v/v (190 proof) A.C.S. spectrophotometric grade (lot no. B0513920) purchased from Acros Organics (Morris Plains, NJ). Do not use absolute alcohol or denatured alcohol in this method.

[13] Occupational Exposure to Hazardous Chemicals in Laboratories. *Code of Federal Regulations*, Part 1910.1450, Title 29, 2003.

2,3-Pentanedione [CAS no. 600-14-6]. The 2,3-pentanedione used in this validation was 97% (lot no. 29598LJ) purchased from Aldrich (Milwaukee, WI).

3-Pentanone [CAS no. 96-22-0]. The 3-pentanone used in this validation was 99+% (lot no. HR 00231KF) purchased from Aldrich (Milwaukee, WI).

The extraction solvent used for this validation consisted of 0.007 µL/mL 3-pentanone in 95% v/v ethyl alcohol/water. The 3-pentanone was added to the ethyl alcohol as an internal standard (ISTD).

3.3 Standard preparation
(Note: Store all standards in amber glass bottles and vials)

Prepare concentrated stock standards in water at 1.021 mg/mL (1.021 µg/µL) by injecting 11 µL of neat 2,3-pentanedione into water in a 10-mL volumetric flask and diluting to the mark. This stock standard will remain stable for two weeks if stored in an amber bottle in the refrigerator. When using refrigerated stock standards, be sure to allow the standards to warm to room temperature and then shake them vigorously before use. Prepare analytical standards by injecting microliter amounts of concentrated stock standards into 2-mL volumetric flasks containing about 1.75 mL of extraction solvent and then diluting with extraction solvent over a concentration range of 0.1 to 20 µg/mL (0.2 to 40 µg/2 mL). For example: a target concentration standard of 20.4 µg/sample was prepared by injecting 20 µL of the stock standard into a 2-mL flask containing about 1.75 mL of extraction solvent and then diluting to the mark with extraction solvent (10.2 µg/mL or 0.5 ppm based on a 2-mL extraction volume per sample and 10 L air volumes).

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
(Note: prepare all samples in amber glass vials)

Remove the plastic end caps from the front sample tube and carefully transfer the silica gel to a labeled 4-mL amber glass vial. The sampling tube and the back of the glass fiber filter should be carefully inspected to ensure that all the silica gel is transferred into the 4-mL vial. Remove the plastic end caps from the backup tube and carefully transfer the silica gel to a second labeled 4-mL amber glass vial. If the industrial hygienist requests analysis of the front glass fiber filter, which is not normally analyzed, place the front glass wool plug and filter from the front tube into a third 4-mL vial. If analysis of filter is not requested then discard the front glass wool plug and filter. Discard the glass tubes and back glass wool plugs and back glass fiber filter.

Add 2.0 mL of extraction solvent to each vial and immediately seal the vials with PTFE-lined caps.

Immediately place the vials on a rotator for 60 min. Transfer the sample into autosampler vials for analysis.

3.5 Analysis

3.5.1 Gas chromatographic conditions (these conditions are different from OSHA Method 1013 to obtain better separation of the 2,3-pentanedione peak from the 3-pentanone internal standard peak).

GC conditions

oven temperature:	initial 60 °C, hold 4 min, program at 10 °C/min to 150 °C, hold 5 min, 20 °C/min to 200 °C hold 1 min
injector temperature:	240 ºC
detector temperature:	250 ºC
run time:	total time is 21.5 min, data is collected for 15 min, the excess time is to clear the column
column:	60-m × 0.32-mm i.d. DB-1 capillary column (df = 5-µm)
column mode:	constant pressure
initial column gas flow:	1.8 mL/min (hydrogen)
column pressure:	9.4 psi
injection size:	1.0 µL (2:1 split)
inlet liner:	Agilent 5183-4647 or equivalent
retention times:	13.2 min 2,3-pentanedione 13.5 min 3-pentanone

FID conditions:

hydrogen flow:	40 mL/min
air flow:	450 mL/min
nitrogen makeup flow:	40 mL/min

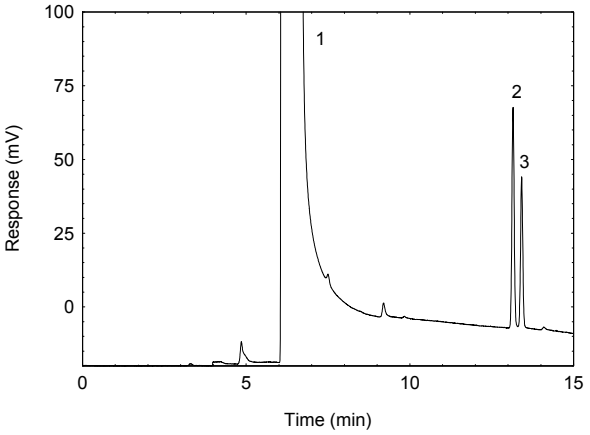

Figure 3.5.1.1. A chromatogram of 20.4 µg/sample 2,3-pentanedione. (Key: 1) ethyl alcohol; 2) 2,3-pentanedione; and 3) 3-pentanone.)

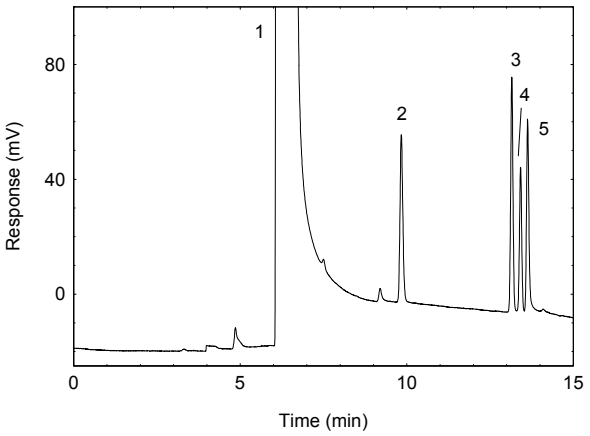

Figure 3.5.1.2. A chromatogram of 20.4 µg/sample 2,3-pentanedione, 15.8 µg/sample acetoin, and 15.6 µg/sample diacetyl. (Key: 1) ethyl alcohol; 2) diacetyl; 3) 2,3-pentanedione; 4) 3-pentanone; and 5) acetoin.)

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

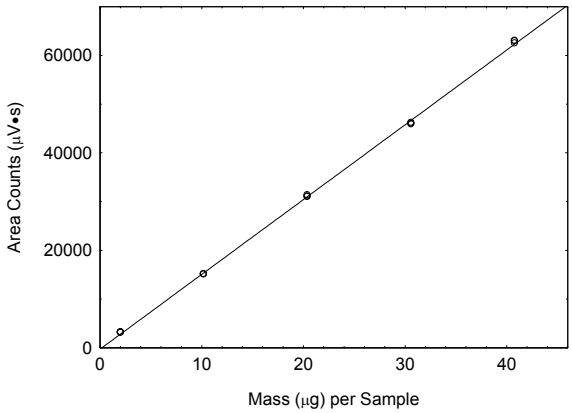

Figure 3.5.2.1. Calibration curve for 2,3-pentanedione.
(y = 1535x - 295)

3.6 Interferences (analytical)

3.6.1 Any compound that produces a GC response and has a similar retention time as the analyte or internal standard 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 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

The amount of analyte per sample is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. The second tube is analyzed primarily to determine the extent of sampler saturation. If any analyte is found on the back tube, it is added to the amount on the front tube. If more than 20% of the total amount is found on the back tube, 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.

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

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

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

where C_V is concn by volume (ppm)
C_M is concn by weight (mg/m^3)
V_M is 24.46 (molar volume at NTP)
M_r is molecular weight of analyte
(2,3-pentanedione = 100.12)

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

Chromatography Analysis"[14]. 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 mass of analyte introduced into the chromatographic column. Ten analytical standards were spiked with equally descending increments of analyte. The highest amount is the amount spiked on the sampler that would produce a peak approximately 10 times the response of a reagent blank at or near the retention time of the analyte. The standards and the reagent blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 split). The data obtained were used to determine the required parameters (standard error of estimate and slope) for the calculation of the DLAP. The slope and standard error of estimate, respectively, were 6.62 and 62.2. The DLAP was calculated to be 28 pg.

Table 4.1
Detection Limit of the Analytical Procedure

concn (ng/mL)	mass on column (pg)	area counts (µV•s)
0	0	0
51	26	160
102	51	367
153	77	556
204	102	618
255	128	883
306	153	949
357	179	1084
408	204	1281
460	230	1547
511	256	1784

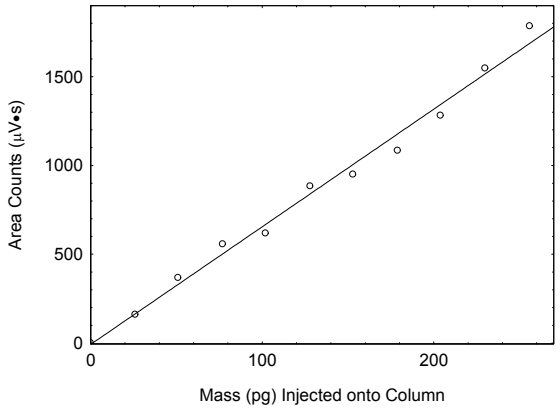

Figure 4.1. Plot of data to determine the DLAP (y = 6.62x - 7.12).

4.2 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 concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equally descending increments of analyte. The highest amount is the amount spiked on the sampler that would produce a peak approximately 10 times the response of a sample blank at or near the retention time of the analyte. The spiked samplers, and the sample blank were analyzed with the recommended analytical parameters, and the data obtained used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For 2,3-pentanedione values of 1597 and 61.2 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.11 µg (2.7 ppb or 11 µg/m^3).

[14] Eide, M.; Hendricks, W.; Simmons, M. *Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis.* https://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf, OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 2010 (accessed January 2010).

Table 4.2
Detection Limit of the Overall Procedure

mass per sample (µg)	area counts (µV•s)
0.00	0
0.10	153
0.20	349
0.31	545
0.41	599
0.51	848
0.61	930
0.71	1063
0.82	1217
0.92	1485
1.02	1731

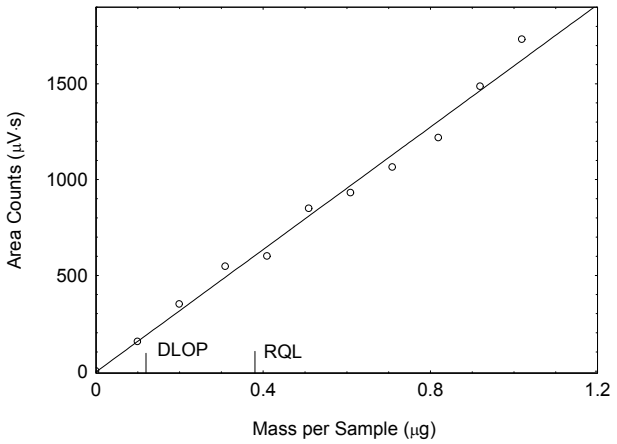

Figure 4.2.1. Plot of data to determine the DLOP/RQL (y = 1597x – 3.74).

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 RQL for 2,3-pentanedione is 0.38 µg per sample (9.3 ppb or 38 µg/m^3 for a TWA sample). Recovery at this concentration is 97.9%.

When short-term samples are collected, the air concentration equivalent to the reliable quantitation limit becomes larger. For example, the reliable quantitation limit for the recommended sampler is 31 ppb (127 µg/m^3) when 3 L is sampled.

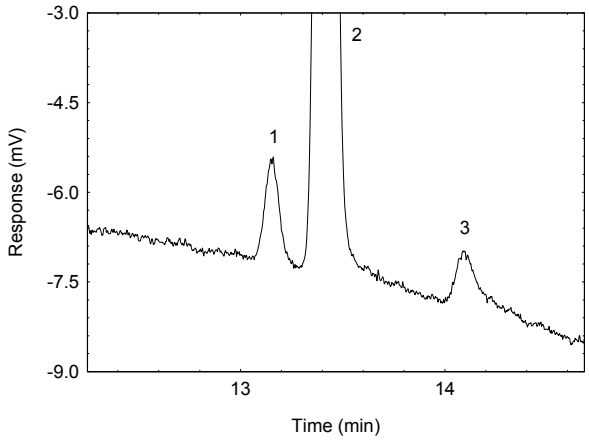

Figure 4.2.2. A chromatogram of the RQL of 2,3-pentanedione. (Key: 1) 2,3-pentanedione; 2) 3-pentanone; and 3) interferant.)

4.3 Precision of the analytical method

The precision of the analytical method was measured as the mass equivalent to the standard error of estimate determined from the linear regression of data points from standards over a range that covers 0.1 to 2 times the TWA target concentration for the sampler. A calibration curve was constructed and shown in Section 3.5.2 from the three injections each of five standards. The standard error of estimate was 0.49 µg.

Table 4.3
Instrument Calibration

x target concn (µg/sample)	0.1 x 2.04	0.5 x 10.2	1.0 x 20.4	1.5 x 30.6	2.0 x 40.8
area counts (µV·s)	3175	15104	31328	46035	62945
	3189	15132	30963	45869	63015
	3091	15094	31087	46183	62497

4.4 Storage stability test

Storage samples for 2,3-pentanedione were prepared by sampling a dynamically generated controlled test atmosphere using the recommended sampling parameters. The concentration of 2,3-pentanedione in the test atmosphere was 0.501 ppm (2.05 mg/m^3) and the relative humidity was 80% at 23 °C. Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. Fifteen of the tubes were stored at reduced temperature (4 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 23 °C). At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. Sample results are not corrected for extraction efficiency. Results for the ambient storage test decreased by more than 10% which is a significant uncorrectable bias that must be avoided, therefore, samples should be stored in a refrigerator until analyzed, and analysis should be completed within two weeks of sampling. Recovery is determined from the regression line and the maximum change allowed by OSHA methods development guidelines is ±10%.

Table 4.4
Storage Test for 2,3-Pentanedione

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	95.1	96.7	97.7	95.1	96.7	97.7
4	94.4	93.2	95.4	96.6	97.5	95.9
7	91.2	93.0	94.4	97.1	94.8	96.2
10	87.8	86.9	91.4	92.5	94.3	93.0
14	85.1	84.3	86.6	90.8	92.5	93.2
17	82.4	85.0	83.9	91.4	89.5	92.7

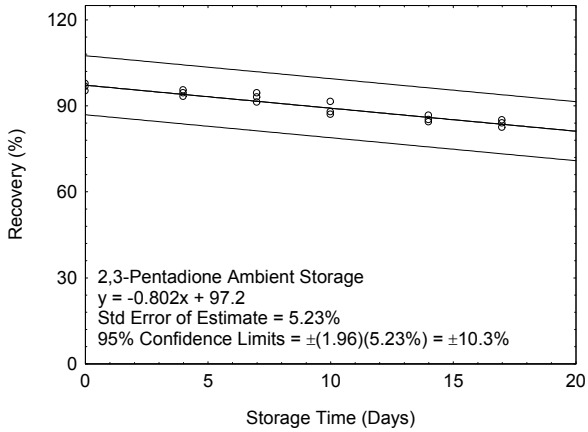

Figure 4.4.1. Ambient storage test for 2,3-pentanedione.

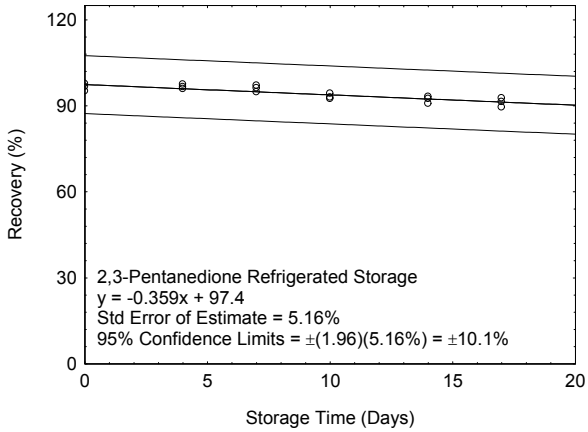

Figure 4.4.2. Refrigerated storage test for 2,3-pentanedione.

4.5 Precision (overall procedure)

The precision of the overall procedure at the 95% confidence level is obtained by multiplying the 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 Two dried silica gel tubes in series (SKC 226-183)

The precision at the 95% confidence for the refrigerated temperature (4 °C) 17-day storage test was ± 10.1%. It contains an additional 5% for sampling pump error.

4.5.2 Recovery

The recovery of 2,3-pentanedione from samples used in a 17-day storage test remained above 91.3% when samples were stored at 4 °C.

4.6 Reproducibility

Six samples were prepared by sampling a dynamically generated controlled test atmosphere similar to that used in the collection of the storage samples. The concentrations of 2,3-pentanedione in the test atmosphere was 0.501 ppm (2.05 mg/m^3) at 78% relative humidity and 23 °C. The samples were submitted to the OSHA Salt Lake Technical Center for analysis. The samples were analyzed after being stored at 4 °C for 4 days. Sample results were corrected for extraction efficiency. No sample result had a deviation greater than the precision of the overall procedure determined in Section 4.4.

Table 4.6
Reproducibility Data

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
20.5	20.0	97.6	-2.4
20.4	19.4	95.1	-4.9
21.0	19.8	94.3	-5.7
20.5	19.9	97.1	-2.9
21.0	20.3	96.7	-3.3
23.0	22.5	97.8	-2.2

4.7 Sampler capacity

The sampling capacity of the front tube of the recommended air sampler (two dried silica gel tubes in series) was tested by sampling a dynamically generated controlled test atmosphere containing 2,3-pentanedione at two times the target concentration (1.01 ppm or 4.10 mg/m^3) and 80% relative humidity at 23 °C. The samples were collected at 50 mL/min. The second tube in the sampling train was changed at 3 h then at 0.25 h intervals for the rest of the sampling. The presence of analyte on the second tube was defined as breakthrough. The percentage of the amount found on the second tube in relation to the concentration of the test atmosphere was defined as % breakthrough. The % breakthrough was plotted versus the air volume sampled to determine breakthrough air volumes. Breakthrough is considered to have occurred when the effluent from the active sampler contains a concentration of analyte that is 5% of the upstream concentration. The 5% breakthrough air volume for 2,3-pentanedione was 12.5 L. The recommended air volume is 80% of the breakthrough air volume which is 10 L (200 min sampled at 50 mL/min).

Table 4.7
Breakthrough of 2,3-Pentanedione From Front Sampling Tube of Recommended Air Sampler

test no.	air vol (L)	sampling time (min)	downstream concn mg/m^3	break-through (%)
1	9.27	180	0	0.0
	10.8	210	0	0.0
	11.6	225	0	0.0
	12.4	240	0.19	4.63
	13.2	255	2.32	56.6
2	9.06	180	0	0.0
	10.6	210	0	0.0
	11.3	225	0	0.0
	12.1	240	0.22	5.36
	12.8	255	0.67	16.3
3	8.69	180	0	0.0
	10.1	210	0	0.0
	10.9	225	0	0.0
	11.6	240	0	0.0
	12.3	255	0.18	4.59
	13.0	270	1.29	31.5

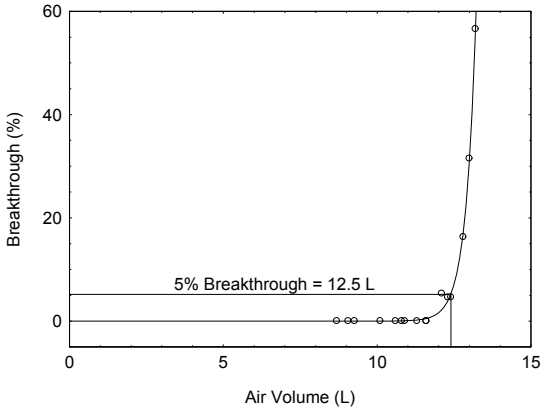

Figure 4.7. Five percent breakthrough air volume for 2,3-pentanedione.

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 guidelines.[15]

Extraction efficiency

The extraction efficiency of 2,3-pentanedione was determined by liquid-spiking four front sampling tubes of the recommended air sampler 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 97.6%. 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 sampling humid air (78% RH at 23 °C) for 200 min at 50 mL/min. The data obtained are shown in Table 4.8.1.

[15] Eide, M.; Hendricks, W.; Simmons, M. *Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis;* OSHA Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed 2/24/2010).

Table 4.8.1
Extraction Efficiency (%) of 2,3-Pentanedione

level		sample number				
× target concn	µg per sample	1	2	3	4	mean
0.1	2.05	98.2	97.1	96.6	98.8	97.7
0.25	5.12	97.2	98.1	95.4	96.1	96.7
0.5	10.3	98.4	95.9	97.4	97.6	97.3
1.0	20.5	96.6	96.0	97.3	98.5	97.1
1.5	30.8	98.5	98.1	98.9	96.8	98.1
2.0	40.1	97.4	99.3	99.0	98.4	98.5
RQL	0.4	98.4	96.5	97.7	99.0	97.9
1.0 (wet)	20.5	95.3	97.8	96.2	95.0	96.1

Stability of extracted samples

The stability of extracted samples was examined by reanalyzing the target concentration samples 24, 48, and 72 h after the initial analysis. After the original analysis was performed two vials were recapped with new septa which were replaced after each analysis. The remaining two vials retained their punctured septa throughout this test. All samples were allowed to stand in the autosampler tray at 22 °C. The samples were reanalyzed with freshly prepared standards. Diff is the difference between the initial analysis and the subsequent analysis. Each septum was punctured 5 times for each analysis. The data obtained are shown in Table 4.8.2.

Table 4.8.2
Stability of Extracted Samples for 2,3-Pentanedione

punctured septa replaced							punctured septa retained						
initial (%)	24 h (%)	diff (%)	48 h (%)	diff (%)	72 h (%)	diff (%)	initial (%)	24 h (%)	diff (%)	48 h (%)	diff (%)	72 h (%)	diff (%)
96.6	96.4	-0.2	96.0	-0.6	95.3	-1.3	97.3	98.7	+1.4	97.7	+0.4	95.0	-2.3
96.0	95.0	-1.0	94.8	-1.2	94.3	-1.7	98.5	97.3	-1.2	95.9	-2.6	96.4	-2.1
			(mean)							(mean)			
96.3	95.7	-0.6	95.4	-0.9	94.8	-1.5	97.9	98.0	+0.1	96.8	-1.1	95.7	-2.2

4.9 Sampling interferences

The tested sampling interferences had no significant effect on the ability of the recommended sampler to collect or retain 2,3-pentanedione when the samples were protected from exposure to light.

Retention

Retention was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (1 ppm or 4.1 mg/m^3) of 2,3-pentanedione at 80% relative humidity and 23 °C. The test atmosphere was sampled with the recommended sampler at 50 mL/min for 50 min. After 50 min sampling was discontinued and the samplers were separated into two sets of 3 samplers each. The generation system was flushed with contaminate-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 a set of three samples and contaminant-free air at 80% RH and 23 °C was

sampled at 50 mL/min for 150 min and then all six samplers were analyzed. The data obtained are shown in Tables 4.9.1.

Table 4.9.1
Retention of 2,3-Pentanedione

	recovery (%)			
set	1	2	3	mean
first	98.4	100.5	98.2	99.0
second	97.7	96.8	95.1	96.5
second/first				97.5

Low humidity

The effect of low humidity was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (1 ppm or 4.1 mg/m^3) of 2,3-pentanedione at 20% relative humidity and 23 °C. The test atmosphere was sampled with three of the recommended samplers at 50 mL/min for 200 min. All of the samples were immediately analyzed. Sample results were 98.8%, 99.1%, and 97.4% 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 (0.05 ppm or 0.205 mg/m^3) of 2,3-pentanedione at 80% relative humidity and 23 °C. The test atmosphere was sampled with three of the recommended samplers at 0.05 mL/min for 200 min. All of the samples were immediately analyzed. Sample results were 98.7%, 97.0%, and 95.8% of theoretical.

Chemical interference

The ability of the recommended sampler to collect 2,3-pentanedione was tested when other potential interferences are present by sampling an atmosphere containing 0.5 ppm (2.05 mg/m^3) 2,3-pentanedione at 80% relative humidity and 23 °C and two interferences whose concentrations were 0.51 ppm (1.82 mg/m^3) acetoin, and 0.51 ppm (1.78 mg/m^3) diacetyl. The test atmosphere was sampled with three of the recommended samplers at 50 mL/min for 200 min. All of the samples were immediately analyzed. Sample results for 2,3-pentanedione were 97.1%, 96.3%, and 95.5% of theoretical.

Light

2,3-pentanedione is light-sensitive. The interference of light during sampling was tested using nine foil-wrapped samplers and three un-wrapped samplers. An atmosphere containing 0.5 ppm (2.05 mg/m^3) 2,3-pentanedione at an average humidity of 80% at 23ºC was sampled for 200 minutes at 50 mL/min. The three foil-wrapped and three unwrapped samples were analyzed immediately and the average recovery for the foil wrapped was 98.2% and the un-wrapped sampler average recovery was 96.6%. Three of the foil-wrapped samplers had the foil removed after sampling and were exposed to fluorescent room lights for 24 h before analysis and had an average recovery of 90.3%. The last three foil-wrapped samplers had the foil removed and were exposed to 3 h of sunlight before analysis

Table 4.9.2
Effect of Light Exposure While Sampling

	sample number			
type of sampler light exposure	1	2	3	mean
no light exposure	97.5	98.0	99.1	98.2
200 min room light	95.1	96.8	97.9	96.6
24 h fluorescent	90.7	91.3	89.0	90.3
3 h sunlight	39.6	42.9	44.6	42.4

and had an average recovery of 42.4%. This data clearly indicates that the sampler should be protected from exposure to light.

To test the possibility of light degradation on extracted samples nine analytical standards at the target concentration were prepared. Six of the standards were placed in 2-mL amber glass vials and three were placed in 2-mL clear glass vials. Three of the amber vials, along with the clear glass vials were stored on the autosampler tray during the entire test while the other three amber vials were stored in the refrigerator when not being analyzed. All nine standards were analyzed eight times over a 10 day period with none of the septa being replaced during the test. The standards in clear vials degraded significantly, but standards in amber vials did not degrade. This data clearly indicates that extracted samples should be protected from exposure to light. The internal standard, 3-pentanone was stable for up to 9 days in both the clear and ambient vials. The data obtained is shown in Table 4.9.3.

Table 4.9.3
Extracted Sample Light Exposure Test
of 2,3-Pentanedione

	mean of peak areas from 3 vials		
day	clear vials ambient	amber vials (ambient)	amber vials (refrigerated)
0	31456	31502	31435
1	29007	31003	31354
2	27183	30961	31269
3	25072	30839	31178
4	24193	30709	31073
7	22056	30423	30834
8	20502	30389	30805
9	19584	30355	30793

4.10 Qualitative analysis

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

The mass spectrum of 2,3-pentanedione shown in Figure 4.10 was obtained by analysis on an Agilent 7890A GC System with a 5975 Mass Selective Detector.

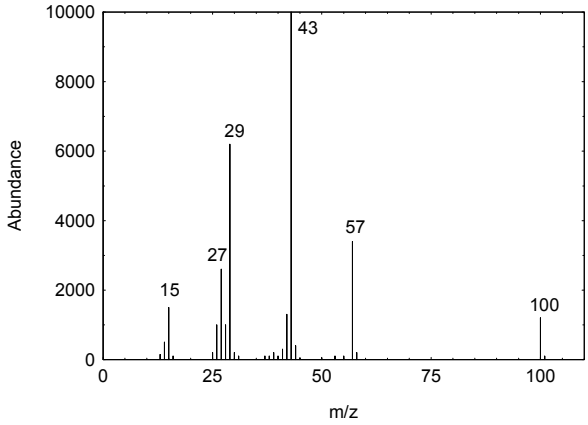

Figure 4.10. Mass spectrum of 2,3-pentanedione.

GC/MS conditions

oven temperature:	initial 35 °C, hold 5 min, program at 10 °C/min to 270 °C, hold 0 min
injector temperature:	240 °C
transfer line temperature:	250 °C
run time:	29 min
column gas flow:	1.0 mL/min (helium)
injection size:	0.5 µL (splitless)
column:	30-m × 0.25-mm i.d. DB-5 capillary column (df = 0.25 µm)

retention times: 3.8 min 2,3-pentanedione
4.2 min 3-pentanone

MS conditions

MS source temperature: 230 °C
MS quad temperature: 150 °C
Mass range: 12-250 amu

4.11 Generation of test atmospheres

The following apparatus was placed in a walk-in hood. The test atmospheres were generated by pumping low microliter volumes of a solution containing 2,3-pentanedione in water with an ISCO precision LC 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 the dilution air stream (Figure 4.11). The vapor generator consisted of a 15-cm length of 5-cm diameter glass tubing with a side port for introduction of the capillary tubing. The vapor generator was heated with a variable voltage controlled heating tape to evaporate the 2,3-pentanedione. The humidity, temperature, and volume of the dilution air were regulated by use of a Miller Nelson Flow-Temperature-Humidity controller. The test atmosphere passed into a glass mixing chamber (76-cm × 30-cm) from the vapor generator, and then into a glass exposure chamber (76-cm × 20-cm). Active samplers were attached to glass ports extending from the exposure chamber. The humidity and temperature were measured at the exit of the exposure chamber with an Omega Digital Thermo-hygrometer. The theoretical concentrations were calculated from the ISCO pump flow rate, the concentration of the 2,3-pentanedione solution, and the air flow volumes. The theoretical concentrations were used throughout this validation.

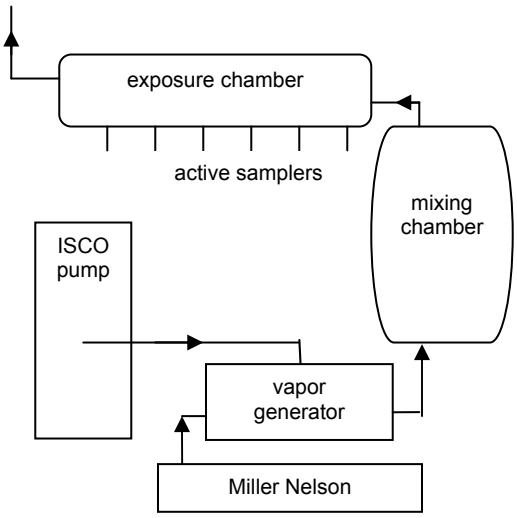

Figure 4.11. The test atmosphere generation and sampling apparatus.