Acetoin
Diacetyl

Method no.:	1013
Control no.:	T-1013-FV-01-0809-M
Target concentration:	0.5 ppm (1.80 mg/m^3) acetoin 0.5 ppm (1.76 mg/m^3) diacetyl
OSHA PEL:	none for acetoin none for diacetyl
ACGIH TLV:	none for acetoin none for diacetyl
Procedure:	Samples are collected by drawing workplace air through two sampling tubes, containing specially dried and cleaned silica gel, connected in series. Samples are extracted with ethyl alcohol:water (95:5) and analyzed by gas chromatography (GC) using a flame ionization detector (FID).
Recommended sampling time and sampling rate:	180 min at 0.05 L/min (9 L) (TWA) 15 min at 0.2 L/min (3 L) (short term)
Reliable quantitation limit:	0.011 ppm (0.039 mg/m^3) acetoin 0.012 ppm (0.041 mg/m^3) diacetyl
Standard error of estimate at the target concentration:	5.7% acetoin 5.2% diacetyl
Special requirement:	Protect samples from light exposure during sampling, shipping and analysis.
Status of method:	Evaluated method. This method has been subjected to the established evaluation 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

In 2003 OSHA issued Method PV2118[1] for sampling and analysis of diacetyl using two silica gel sorbent tubes (150/75 mg) in series. PV2118 has a recommended sampling volume of 3 L and a reliable quantitation limit of 3 µg (0.28 ppm). In 2003 NIOSH issued Method 2557[2] for diacetyl and Method 2558[3] for acetoin. Both methods use Anasorb CMS sorbent (150/75 mg) tubes, can sample up to 10 L of air and have a limit of detection for acetoin of 1 µg and 0.6 µg for diacetyl. These two methods use slightly different acetone/methanol extraction solvents and were not optimized for simultaneous analysis of both analytes. In 2008 a note was placed on NIOSH Method 2557 indicating that high humidity is a sampling interference that results in underestimation of the true concentration.

In September of 2007, OSHA published a Hazard Communication Guidance Document[4] and a Safety and Health Information Bulletin on Respiratory Disease among Employees in Microwave Popcorn Processing Plants[5] for diacetyl. Due to the increasing concern of workplace exposure to diacetyl, two new sampling and analytical methods were validated that permitted longer sampling times and had lower quantitation limits than PV2118. The new methods were also validated for acetoin because it has been found in facilities in which diacetyl was in use.

This procedure, Method 1013, was streamlined for monitoring low ppm levels, and Method 1012[6] was optimized for ppb levels. Both methods use two 600 mg silica gel sorbent tubes in series. Both methods have a recommended sampling time of 3 hours (9 L) and both use the same solvent for sample extraction. However, in Method 1012, acetoin and diacetyl are derivatized using *O*-pentafluorobenzyl hydroxylamine hydrochloride. This derivatization results in a reliable quantitation limit approximately 10 times less than Method 1013. The disadvantage of derivatizing acetoin and diacetyl is that the derivatization step requires 36 hours; whereas, with this method sample preparation can be performed in 1 hour. Also, samples extracted and analyzed according to this procedure can then be derivatized and analyzed using Method 1012, if needed.

The silica gel used in the sampler for this method, and for Method 1012, has been specially cleaned and dried as described in Appendix A. It was found that sampler

[1] Shah, Y. C. Diacetyl (OSHA Method PV2118), 2003. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/partial/t-pv2118/t-pv2118.html (accessed July 2008).
[2] Pendergrass, S. M. Diacetyl (NIOSH Method 2557), 2003. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/nmam/pdfs/2557.pdf (accessed July 2008).
[3] Pendergrass, S. M. Acetoin (NIOSH Method 2558), 2003. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web Site. http://www.cdc.gov/niosh/nmam/pdfs/2558.pdf (accessed July 2008).
[4] Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed July 2008).
[5] Respiratory Disease Among Employees in Microwave Popcorn Processing Plants, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/shib/shib092107.html (accessed July 2008).
[6] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

capacity for diacetyl was not based on analyte concentration but limited by the amount of water remaining on the silica gel after cleanup and on the amount of water collected during sampling. In other words, the silica gel tube acts as a chromatography column and water elutes the collected diacetyl. By removing as much water as possible from the silica gel prior to sampling, the sampling volume for diacetyl can be increased because the time required to saturate the silica gel during sampling increases. Diacetyl was also found to gradually migrate within the sampling tube during storage resulting in the need to use a second tube in series during sampling in order to detect breakthrough. Acetoin has no capacity or migration issues on silica gel at the recommended sampling volume.

The powder and liquid formulated forms of acetoin and diacetyl may contain oily compounds and other base materials such as maltodrextin. These materials could affect the extraction of acetoin and diacetyl from the silica gel. The sampler contains a front glass wool plug followed by a glass fiber filter that serves only to trap any of these materials before they enter the silica gel bed. Retention studies using a powder containing acetoin and diacetyl showed the acetoin and diacetyl can be stripped off the powder and collected on the silica gel. These studies demonstrate that the glass fiber filter is not an efficient collector for diacetyl and acetoin, and will not normally be analyzed (see OSHA Method 1012[7], Section 4.9).

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

Exposure to acetoin may result in skin, eyes, nose and throat irritation.[8]

Exposure to diacetyl "liquid or vapors can cause irritation to the skin, eyes, nose, and throat". "Animals exposed to diacetyl experienced damage to the nose and upper airways, including severe damage to cells lining the respiratory tract" and "NIOSH has reported that employees exposed to butter flavorings containing diacetyl are at risk of developing occupational lung diseases".[9]

Diacetyl, and to some extent acetoin, may be responsible for the occurrence of a rare and potentially fatal lung disease, bronchiolitis obliterans, among workers in microwave popcorn manufacturing plants and flavor manufacturing plants.[10] Symptoms of bronchiolitis obliterans include cough, shortness of breath with exertion, and spirometry test results showing fixed airways obstruction.[11]

Acetoin and diacetyl are used in the production of powdered flavorings.[12] These powdered flavorings may provide a means to deliver the substances deep into the lungs of exposed workers, however, the significance of this form of exposure is presently unknown.[13]

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[7] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

[8] Acetyl Methyl Carbinol (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_217010.html (accessed July 2008).

[9] Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed July 2008).

[10] van Rooy, F.; et al. Bronchiolitis Obliterans Syndrome in Chemical Workers Producing Diacetyl for Food Flavoring. *Am. J. Crit. Care Med*. **2007**, 176 (5), 498-504.

[11] Kanwal, R. Bronchiolitis obliterans in workers exposed to flavoring chemicals. *Curr Opin Pulm Med*. **2008**, 14 (2), 141-6.

[12] Kanwal, R.; Kullman, G. Report on Severe Fixed Obstructive Lung Disease in Workers at a Flavoring Manufacturing Plant Health Hazard Evaluation Report #2006-0303-3043, 2007. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2006-0303-3043.pdf (accessed July 2008) pp 11-13.

[13] Boylstein, R. J.; et al. Diacetyl Emissions and Airborne Dust from Butter Flavorings Used in Microwave Popcorn Production. *J. Occup. Environ. Hyg*. **2006**, 3 (10), 530-535.

1.1.3 Workplace exposure

Acetoin has a somewhat creamy taste and a woody yogurt odor. It is used as an ingredient in yogurt, butter, milk and strawberry flavors. It occurs naturally in foods such as wines, chesses, fruits and vegetables.[14] Occupational exposures can occur by inhalation or skin contact in locations where it is produced, used as a food additive, or used to produce flavorings or aromas.

Diacetyl has a strong butter odor in dilute form and a chlorine-quinone odor when concentrated. It is used as an ingredient to produce a butter flavor in many foods and beverages. It occurs naturally in alcoholic and nonalcoholic beverages, dairy products, fruits, plants, vegetables, meats, and natural aromas.[15] Like acetoin, occupational exposures to diacetyl can occur by inhalation or skin contact in locations where it is produced, used as a food additive, or used to produce flavorings or aromas.

Recently, occupational exposure to butter flavorings in the production of microwave popcorn and in other industries has received much publicity. NIOSH has identified acetoin and diacetyl as useful indicator compounds that can be used to represent exposure to butter flavorings.[16] Areas of special concern include flavor production rooms, areas where mixing/blending operations occur, packing/packaging operations, areas where flavors are handled openly, rooms where mixing tanks are located, quality control laboratories, and maintenance and cleaning operations.[17, 18]

1.1.4 Physical properties and other descriptive information

Acetoin[19, 20]

Acetoin occurs as the liquid monomer and the solid dimer. The monomer can be formed from the dimer by dissolving in water or other solvents.

synonyms:	acetyl methyl carbinol; 2,3-butanolone; dimethylketol; *γ*-hydroxy-*β*-oxobutane; 1-hydroxyethyl methyl ketone
IMIS[21]:	A624
CAS number:	513-86-0 (monomer)
boiling point:	148 ºC (298 ºF) @ 760 mmHg (monomer)
melting point:	15 ºC (59 ºF) (monomer); 91 ºC (196 ºF) (dimer)
density:	1.005 (g/mL@ 25 ºC) (monomer)
molecular weight:	88.11 (monomer)
flash point:	46.7 ºC (116 ºF) (closed cup) (monomer)
appearance:	Pale yellow to colorless as liquid or solid
molecular formula:	$C_4H_8O_2$ (monomer); $C_8H_{16}O_4$ (dimer)

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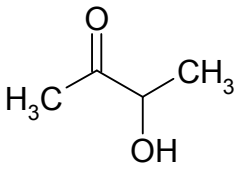
[14] Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005; pp 11-12.
[15] Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005; pp 411-412.
[16] Kanwal, R.; Boylstein, R. J.; Piacitelli, C. NIOSH Health Hazard Evaluation Report #2001-0474-2943, 2004. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed July 2008) pp 8-9.
[17] Kanwal, R. Bronchiolitis obliterans in workers exposed to flavoring chemicals. *Curr Opin Pulm Med*. **2008**, 14 (2), 141-6.
[18] Kreiss, K. Flavoring-related bronchiolitis obliterans. *Curr Opin Allergy Clin Immunol* **2007**, 7 (2), 162-167.
[19] *The Merck Index*; 12th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 1996; p 12.
[20] Material Safety Data Sheet: Acetoin, 2008. The Good Scents Company Web site. http://www.thegoodscentscompany.com/msds/md102388.html (accessed July 2008).
[21] Acetyl Methyl Carbinol (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_217010.html (accessed June 2008).

solubility:	miscible with water and alcohol; sparingly soluble in ether and petroleum ether

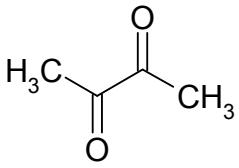
structural formula:



Diacetyl[22,23]

synonyms:	biacetyl; 2,3-butanedione; 2,3-butadione; 2,3-diketobutane; dimethyl diketone; dimethylglyoxal; glyoxal, dimethyl-; 2,3-diketobutane
IMIS[24]:	D740
CAS number:	431-03-8
boiling point:	88 ºC (190 ºF)
melting point:	3-4 ºC (37.4-39.2 ºF)
density:	0.99 (g/mL@ 15/15)
molecular weight:	86.09
vapor pressure:	7 kPa @ 20 ºC
flash point:	26.7 ºC (80 ºF) (closed cup)
appearance:	yellow to yellow-green liquid
vapor density:	3 (air = 1)
molecular formula:	$C_4H_6O_2$
odor:	quinone odor in higher concentrations, butter in lower concentrations
solubility:	4 parts water; miscible with alcohol, ether
autoignition temperature:	285 ºC (545 ºF)

structural formula:



[22] *The Merck Index*; 12th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 1996; p 503.
[23] Material Safety Data Sheet: Diacetyl, 2007. Chemwatch; Victoria, Australia (accessed March 2008).
[24] Diacetyl (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_231710.html (accessed 2008).

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[25]. 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 101.3 kPa (760 mmHg).

1.2 Limit defining parameters

1.2.1 Detection limit of the analytical procedure

The detection limit of the analytical procedure is 0.017 ng for acetoin and 0.033 ng for diacetyl. These are the amount of analytes that will give a detector response that is significantly different from the response of a calibration blank. (Section 4.1)

1.2.2 Detection limit of the overall procedure

The detection limit of the overall procedure for acetoin is 0.10 µg per sample (0.0031 ppm or 0.011 mg/m^3) and 0.11 µg per sample for diacetyl (0.0034 ppm or 0.012 mg/m^3). These are the amounts spiked onto the sampler that will give a detector response that is significantly different from the response of a sampler blank. (Section 4.2)

1.2.3 Reliable quantitation limit

The reliable quantitation limit for acetoin is 0.35 µg per sample (0.011 ppm or 0.039 mg/m^3 for a TWA sample) and 0.37 µg per sample for diacetyl (0.012 ppm or 0.041 mg/m^3 for a TWA sample). These are the amounts spiked onto the sampler that will give a detector response that is considered the lower limit for precise quantitative measurements. (Section 4.2)

1.2.4 Instrument calibration

The standard error of estimate is 0.42 µg for acetoin over the range of 3.73 µg to 31.0 µg. The standard error of estimate is 0.82 µg for diacetyl over the range of 3.58 µg to 29.9 µg. These ranges correspond to approximately 0.25 to 2 times the target concentration. (Section 4.3)

1.2.5 Precision

The precision of the overall procedure at the 95% confidence level for the ambient temperature 18-day storage test (at the target concentration) is ±11.2% for acetoin and ±10.1% for diacetyl. These include an additional 5% for sampling pump variability. (Section 4.4)

1.2.6 Recovery

The recovery from samples used in a 18-day storage test remained above 88.5% for acetoin and 102.7% for diacetyl when the samples were stored at ambient temperature. (Section 4.5)

[25] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed November 2007).

1.2.7 Reproducibility

Six samples collected from a controlled test atmosphere were submitted for analysis by the OSHA Salt Lake Technical Center. The samples were analyzed according to a draft copy of this procedure after 20 days of storage at refrigerated temperature. No individual sample result deviated from its theoretical value by more than the precision reported in Section 1.2.5. (Section 4.6)

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

Sampler: glass tube with both ends flame sealed, 110-mm × 7-mm i.d., containing a glass fiber filter and 1 section of 20/40 mesh silica gel. From front to back, the sampling tube consists of a silane-treated glass wool plug, a glass fiber filter to collect particulate, 600 mg of silica gel and a second plug of silane-treated glass wool. The silica gel should be cleaned and dried as described in Appendix A. Sampling tubes are available for purchase through SKC, Inc. (cat. no. 226-183).

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

Use aluminum foil or a tube cover, such as SKC, Inc Tube Cover D (cat. no. 224-29D), to protect samples from light.

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, break the ends off of two flame-sealed glass tubes to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking ends. Use a tube holder to minimize the hazard of broken glass and to protect samplers from light exposure during sampling. All tubes should be from the same lot.

Connect the two silica gel sampling tubes in series, using the least amount of flexible tubing as possible between the sampling tubes, and then connect to a sampling pump with flexible tubing. The filter in the silica gel tubes should be positioned away from the sampling pump. The tube closer to the pump is used as a backup. Use a tube cover or wrap sampling tubes in aluminum foil to insure that both sampling tubes are protected from light exposure. Place the sampling tubes in a vertical position with the inlet in the breathing zone and position the sampling pump and tubing so they do not impede work performance or safety.

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

After sampling for the appropriate time, disconnect the tubes from the pump tubing and seal each tube with plastic end caps. Separately wrap each tube in aluminum foil and seal end-to end with a Form OSHA-21.

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 (L), sampling time (min) and sampling rate (L/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. If a delay is unavoidable, store the samples in a refrigerator. Ship any bulk samples separate from the air samples.

2.4 Sampler capacity (Section 4.7)

The sampling capacity of the front tube was tested by sampling a dynamically generated test atmosphere of acetoin (3.58 mg/m^3 or 0.99 ppm) and diacetyl (3.55 mg/m^3 or 1.01 ppm) with an average relative humidity of 40% at 34 °C (absolute humidity of 14.8 mg/L H_2O). The samples were collected at a sampling rate of approximately 0.05 L/min for 270 min. The 5% breakthrough sampling time was determined to be 248 min for diacetyl. No breakthrough was observed for acetoin. (Note: In order to volatilize acetoin the test atmosphere generation conditions were modified slightly for this method evaluation as described in the second paragraph of Section 4.11.)

2.5 Extraction efficiency (Section 4.8)

It is the responsibility of each analytical laboratory to determine the extraction efficiency because the adsorbent material, reagents and laboratory techniques may be different than those listed in this evaluation and influence the results.

The mean extraction efficiency for acetoin from dry silica gel over the range of RQL to 2 times the target concentration (0.33 to 31.0 µg per sample) was 92.9%. The extraction efficiency was not affected by the presence of water.

The mean extraction efficiency for diacetyl from dry silica gel over the range of RQL to 2 times the target concentration (0.38 to 29.9 µg per sample) was 99.6%. The extraction efficiency was not affected by the presence of water.

Extracted samples remain stable for at least 72 hr.

2.6 Recommended sampling time and sampling rate

Sample for up to 180 min at 0.05 L/min (9 L) to collect TWA (long-term) samples.

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

When short-term samples are collected, the air concentration equivalent to the reliable quantitation limit becomes larger. For example, the reliable quantitation limit is 0.032 ppm (0.12 mg/m^3) for acetoin and 0.035 ppm (0.12 mg/m^3) for diacetyl when 3 L are collected.

2.7 Interferences, sampling (Section 4.9)

Retention efficiency

The retention efficiency for all samples was 100.6% of theoretical for acetoin and 96.6% for diacetyl, when samplers containing approximately 8.3 µg of acetoin and 8.1 µg of diacetyl were allowed to sample 6.75 L of contaminant-free air having an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min.

Low humidity

The collection efficiency for all samples was 100.7% of theoretical for acetoin and 101.5% for diacetyl, when the samplers were used to sample a test atmosphere containing two times the target concentration having an average relative humidity of 8% at 33 °C (absolute humidity of 2.82 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min for 180 min.

Low concentration

The collection efficiency for all samples was 91.8% of theoretical for acetoin and 95.6% for diacetyl, when the samplers were used to sample a test atmosphere containing approximately 0.1 times the target concentration having an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min for 180 min.

The collection efficiency for all samples when taking short term samples was 106% of theoretical for acetoin and 90.6% for diacetyl, when the samplers were used to sample a test atmosphere containing approximately 0.1 times the target concentration having an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). Samples were collected at a sampling rate of 0.2 L/min for 15 min.

Sampling interference

The collection efficiency for all samples was 95.5% of theoretical for acetoin and 101.8% for diacetyl, when the sampler was used to sample a test atmosphere containing approximately one times the target concentration of acetoin and diacetyl and 2.59 mg/m^3 of 2-nonanone and 1.88 mg/m^3 of 2,3-pentanedione. The test atmosphere had an average relative humidity of 38% at 34 °C (absolute humidity of 14.1 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min for 181 min.

Sampler exposure to light, particularly sunlight, during sampling will result in degradation of both acetoin and diacetyl. The recovery for all samples was 67.0% of theoretical for acetoin and 6.43% for diacetyl, when the sampler was used to sample a test atmosphere containing approximately one times the target concentration of acetoin and diacetyl and then exposed to 3 h of direct sunlight (samples were covered during sampling). The test atmosphere had an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L H_2O). Samples were collected at a sampling rate of 0.05 L/min for 180 min. See Section 4.9 for data on other light tests performed.

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan[26]. Avoid skin contact and inhalation of all chemicals and review all appropriate MSDSs.

3.1 Apparatus

A gas chromatograph equipped with an FID. For this evaluation an Agilent Technologies 6890 Plus Gas Chromatograph equipped with a 7683 Automatic Sampler and an Agilent tapered, deactivated, split, low pressure drop liner with glass wool (catalog no. 5183-4647).

A GC column capable of separating acetoin and diacetyl from the desorption solvent, internal standard and any potential interferences. A Restek 60-m × 0.32-mm i.d. Rt_x-Volatiles (1.5-µm df) capillary column was used in this evaluation.

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

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

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

Amber glass vials with PTFE-lined caps. For this evaluation 2 and 4-mL vials were used.

Calibrated 10-µL and 25-µL syringes for preparing standards.

Water purifier. A Barnstead NANOpure Diamond system was used to produce 18.0 MΩ-cm DI water in this evaluation.

Water bath. A Precision Scientific (5 – 100 °C range) water bath was used in this evaluation.

A mechanical rotator. A Fisher Roto-Rack was used in this evaluation.

Class A 1-L volumetric flasks.

Class A 1-mL and 5-mL volumetric pipets.

3.2 Reagents and Standards

Acetoin ($C_4H_8O_2$), [CAS no. 513-86-0]. The acetoin (lot no. 05025DH) used in this evaluation was purchased from Sigma Aldrich (Milwaukee, WI).

Diacetyl ($C_4H_6O_2$), [CAS no. 431-03-8]. The diacetyl used in this evaluation was 97+% (lot no. 17823LD) purchased from Sigma Aldrich (Milwaukee, WI).

DI water, 18.0 MΩ-cm.

Ethyl Alcohol [CAS no. 64-17-5]. The ethyl alcohol used in this evaluation was 95% v/v (190 proof) A.C.S. spectrophotometric grade (lot no. B0513920) purchased from Acros Organics (Morris Plains, NJ).

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

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

3.3 Standard preparation

Prepare a concentrated stock standard of acetoin and diacetyl in 18.0 MΩ-cm DI water and store in an amber vial or bottle. (**Note**: Acetoin is usually obtained as the solid dimmer and will convert back to the monomer when dissolved in water.) Acetoin will slowly dissolve in water, however, this process can be accelerated by placing the solution in a 60 ºC water bath for 10 min. Refrigerate the stock standard when not in use and remake once a month.

Prepare working analytical standards by injecting microliter amounts of the concentrated stock standard into amber 4-mL vials containing 2 mL of the extraction solvent delivered by the same dispenser used to extract samples. For example, to prepare a target level standard (16.25 µg/sample acetoin and 15.86 µg/sample diacetyl) , inject 13 µL of a stock standard containing 1.25 µg/µL acetoin and 1.22 µg/µL diacetyl into 2-mL of extraction solvent. Transfer working standards to 2-mL amber glass autosampler vials.

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

3.4 Sample preparation

Remove the plastic end caps from the front sample tube and carefully transfer the silica gel to a 4-mL amber glass vial. The sampling tube and the back of the glass fiber filter should be carefully inspected to insure 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 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 solution to each vial and immediately seal with PTFE-lined caps.

Note: The use of an extraction solution or internal standard other than that specified in Section 3.2 should not be used unless a full extraction efficiency study is performed using both dry and wet media as described in Section 4.8.

Place the 4-mL vials on a mechanical rotator and rotate at approximately 40 rpm for 60 min.

Transfer the extraction solution in each 4-mL vial to a 2-mL amber glass autosampler vial and seal with a PTFE-lined cap.

Analyze samples for acetoin and diacetyl as described in Section 3.5.

Note: If after analysis lower detection limits are needed samples can be derivatized and analyzed according to Section 3.4 of OSHA Method 1012[27].

3.5 Analysis

3.5.1 Analytical conditions

GC conditions

column temperature:	Initial 60 °C, hold 4 min; ramp at 15 °C/min to 135 °C, hold 0 min; ramp at 60 °C/min to 250 °C, hold 4 min
zone temperatures:	240 °C (injector); 250 °C (detector)
run time:	14.75 min
column mode:	constant pressure
column pressure:	14 psi
initial column gas flow:	3.3 mL/min (hydrogen)
injection size:	1.0 µL (2:1 split)

[27] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

column: Restek 60-m × 0.32-mm i.d. capillary Rt_x-Volatiles (df = 1.5-µm) or equivalent

inlet liner: Agilent 5183-4647 or equivalent

retention times: 5.2 min (diacetyl)
8.1 min (acetoin)
7.5 min (ISTD)

FID conditions

hydrogen flow: 40 mL/min

air flow: 450 mL/min

nitrogen makeup flow: 45 mL/min

Figure 3.5.1. Chromatogram obtained at target concentrations with recommended conditions.

3.5.2 Calibration

An internal standard 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.

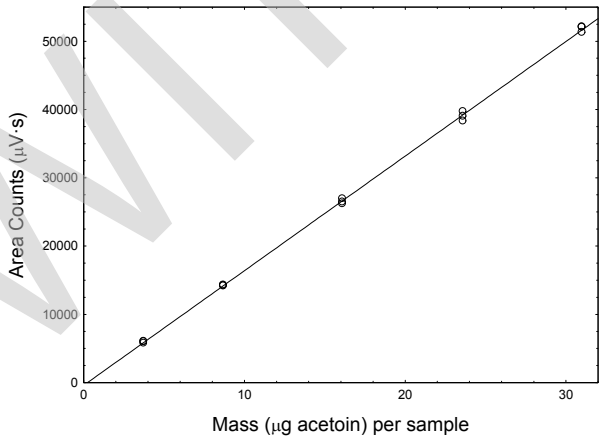


Figure 3.5.2.1. Calibration curve of acetoin. (Y = 1678X – 389)

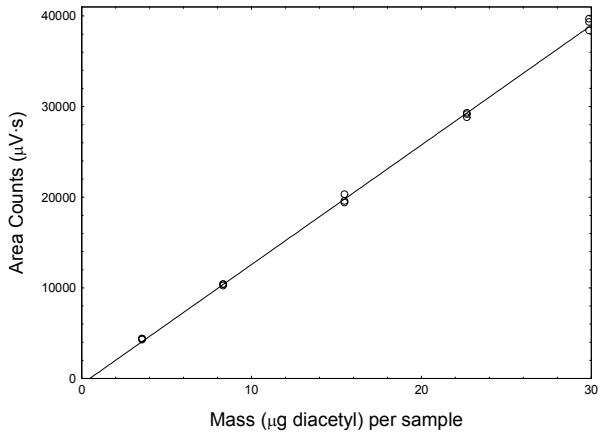


Figure 3.5.2.2. Calibration curve of diacetyl. (Y = 1320X – 625)

3.6 Interferences (analytical)

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

3.6.2 When necessary, the identity of an analyte peak may be confirmed with additional analytical data (Section 4.12).

3.7 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. The back 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. 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.

Total micrograms per sample of analyte is

$$M = [M_{front} - M_{blank}] + [M_{back} - M_{blank}]$$

where

M is total µg per sample
M_{front} is total µg found on front tube
M_{back} is total µg found on back tube
M_{blank} is total µg found on blank tube

Concentration by weight of analyte (mg/m^3) is

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

where

C_M is concentration by weight (mg/m^3)
M is total µg per sample
E_E is extraction efficiency in decimal form
V is L of air sampled

Concentration by volume of analyte (ppm) is

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

where

C_V is concentration by volume (ppm)
C_M is concentration by weight (mg/m^3)
V_M is molar volume at NTP (24.46 L/mole)
M_r is molecular weight (88.1 for acetoin, 86.09 for diacetyl)

4. Backup data

General background information about the determination of detection limits and precision of the overall procedure is found in the “Evaluation Guidelines for Air Sampling Methods Utilizing

Chromatography Analysis"[28]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria.

4.1 Detection limit of the analytical procedure (DLAP)

The DLAP is measured as mass of analyte introduced onto the chromatographic column. Ten analytical standards were prepared with equally descending increments with the highest standard containing 1.10 µg/sample acetoin and 1.05 µg/sample diacetyl. This is the concentration that would produce a peak approximately 10 times the response of a calibration blank. These standards, and the calibration blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 spit), and the data obtained were used to determine the required parameters (standard error of estimate and slope) for the calculation of the DLAP. For acetoin values of 5171 and 30 were obtained for the slope and standard error of estimate respectively. The DLAP for acetoin was calculated to be 0.017 ng acetoin.

Table 4.1.1
Detection Limit of the Analytical Procedure for Acetoin

concentration (µg/sample)	mass on column (ng)	area counts (µV·S)
0.000	0.000	0
0.110	0.028	157
0.220	0.055	224
0.330	0.083	386
0.440	0.110	515
0.550	0.138	738
0.660	0.165	818
0.770	0.193	998
0.880	0.220	1117
0.990	0.248	1248
1.100	0.275	1414

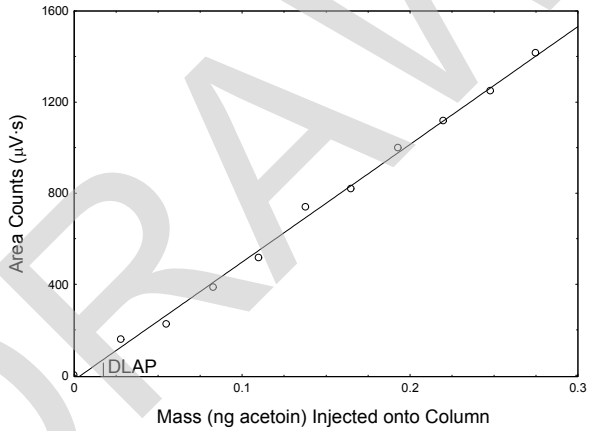


Figure 4.1.1. Plot of data to determine the DLAP for acetoin. (Y = 5171X – 19.9)

For diacetyl values of 4325 and 47 were obtained for the slope and standard error of estimate respectively. The DLAP for diacetyl was calculated to be 0.033 ng diacetyl.

Table 4.1.2
Detection Limit of the Analytical Procedure for Diacetyl

concentration (µg/sample)	mass on column (ng)	area counts (µV·S)
0.000	0.000	0
0.191	0.048	155
0.287	0.072	201
0.382	0.096	350
0.478	0.120	417
0.573	0.143	590
0.669	0.167	615
0.764	0.191	706
0.860	0.215	877
0.955	0.239	1043
1.051	0.263	1089

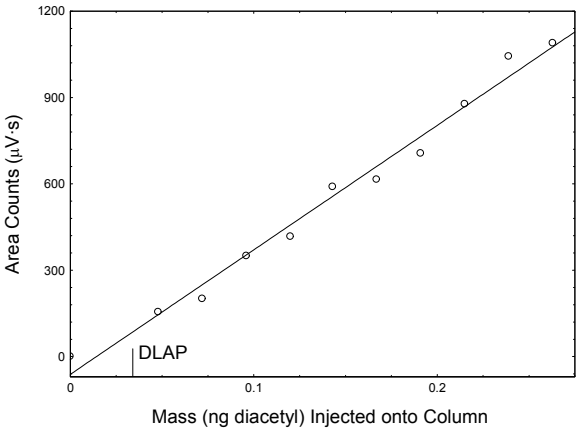


Figure 4.1.2. Plot of data to determine the DLAP for diacetyl. (Y = 4325X - 62)

[28] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed November 2007).

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 acetoin and diacetyl, such that the highest sampler loading was equivalent to 1.10 µg of acetoin per sample and 0.96 µg of diacetyl per sample. This is the amount spiked on a sampler that would produce a peak approximately 10 times the response of a calibration blank. These spiked samplers, and the sample blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 spit), and the data obtained were used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For acetoin values of 1029 and 36 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.10 µg acetoin per sample (0.0031 ppm or 0.011 mg/m^3 for a TWA sample).

Table 4.2.1
Detection Limit of the Overall Procedure for Acetoin

mass per sample (µg/sample)	area counts (µV·s)
0.000	0
0.110	119
0.220	226
0.330	316
0.440	432
0.550	517
0.660	605
0.770	771
0.880	848
0.990	1057
1.100	1150

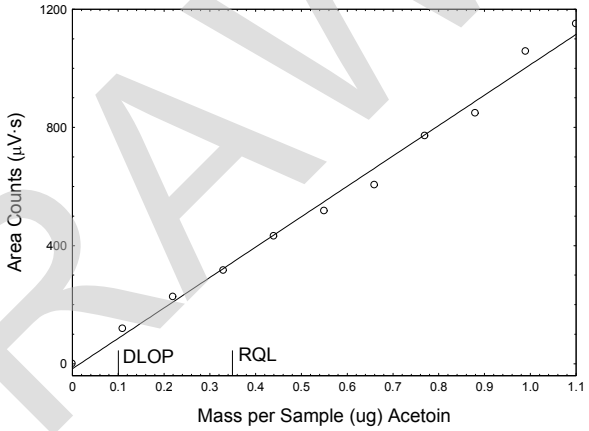


Figure 4.2.1. Plot of data to determine the DLOP/RQL for acetoin. (Y = 1029X – 16.8)

For diacetyl values of 1241 and 46 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.11 µg diacetyl per sample (0.0034 ppm or 0.012 mg/m^3 for a TWA sample).

Table 4.2.2
Detection Limit of the Overall Procedure for Diacetyl

mass per sample (µg/sample)	area counts (µV·s)
0.000	0
0.096	118
0.191	214
0.287	357
0.382	515
0.478	623
0.573	744
0.669	916
0.764	864
0.860	1043
0.955	1208

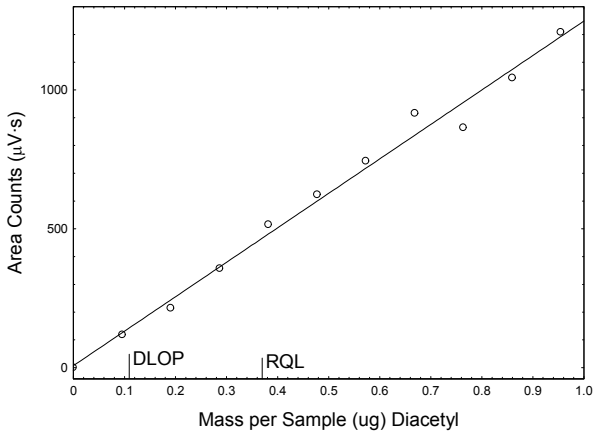


Figure 4.2.2. Plot of data to determine the DLOP/RQL for diacetyl. (Y = 1241X – 7.3)

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 acetoin is 0.35 µg per sample (0.011 ppm or 0.039 mg/m^3 for a TWA sample). Recovery at this concentration is 102%. The RQL for diacetyl is 0.37 µg per sample (0.012 ppm or 0.041 mg/m^3 for a TWA sample). Recovery at this concentration is 93.5%.

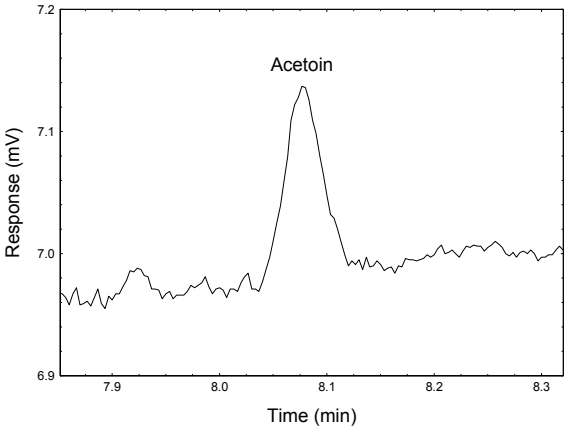


Figure 4.2.3. Chromatogram of acetoin at the RQL.

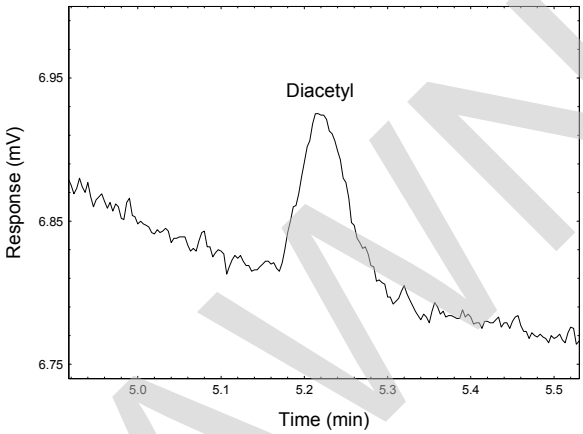


Figure 4.2.4. Chromatogram of diacetyl at the RQL.

4.3 Instrument calibration

The standard error of estimate was determined from the linear regression of data points from standards over a range that covers approximately 0.25 to 2 times the target concentration. Calibration curves for acetoin and diacetyl were constructed and are shown in Section 3.5.2 from the three injections of five standards. The standard error of estimate is 0.42 µg/sample for acetoin and 0.82 µg/sample for diacetyl.

Table 4.3.1
Acetoin Instrument Calibration

standard concn (µg/sample)	area counts (µV·s)		
3.73	5782	6047	6004
8.69	14230	14168	14323
16.1	26940	26458	26198
23.6	38318	39021	39714
31.0	52053	51292	52127

Table 4.3.2
Diacetyl Instrument Calibration

standard concn (µg/sample)	area counts (µV·s)		
3.58	4242	4347	4352
8.36	10205	10350	10373
15.5	20275	19361	19540
22.7	28772	29121	29255
29.9	39287	38363	39653

4.4 Precision (overall procedure)

The precision 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). In Section 4.5, 95% confidence intervals are drawn about their respective regression lines in the storage graph figures. For acetoin the precision of the overall procedure of ±11.2% was obtained from the standard error of estimate of 5.73% in Figure 4.5.1. For diacetyl the precision of the overall procedure of ±10.1% was obtained from the standard error of estimate of 5.15% in Figure 4.5.3. The precision includes an additional 5% for sampling error.

4.5 Storage test

Storage samples for acetoin and diacetyl were prepared by collecting samples from a controlled test atmosphere using the recommended sampling conditions. The concentration of acetoin and diacetyl were at the target concentration with an average relative humidity of 41% at 34 °C (absolute humidity of 15.2 mg/L H_2O). Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. Fifteen of the samples were stored at reduced temperature (3 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 21 °C). At 3-4 day intervals, three samples were selected from each of the two storage sets and analyzed. Sample results were not corrected for extraction efficiency.

Table 4.5.1
Storage Test for Acetoin

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	86.9	87.7	89.8	86.9	87.7	89.8
4	83.1	92.0	88.3	88.0	86.1	87.4
7	91.8	85.1	90.4	95.3	90.0	94.0
11	89.1	92.3	90.9	90.6	91.4	92.1
14	90.9	88.5	91.5	90.7	88.5	91.9
18	86.5	85.5	86.1	91.7	87.6	89.9

Table 4.5.2
Storage Test for Diacetyl

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.5	99.9	100.7	100.5	99.9	100.7
4	98.6	100.9	100.3	97.4	96.2	98.7
7	102.6	100.9	101.2	101.5	98.8	100.9
11	102.7	104.8	101.6	101.9	101.9	102.4
14	101.9	101.0	102.7	100.2	98.8	103.2
18	101.1	103.8	101.9	100.7	98.4	102.8

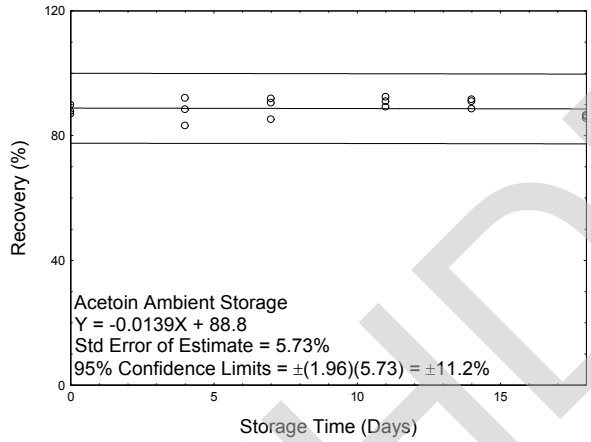


Figure 4.5.1. Ambient storage test for acetoin.

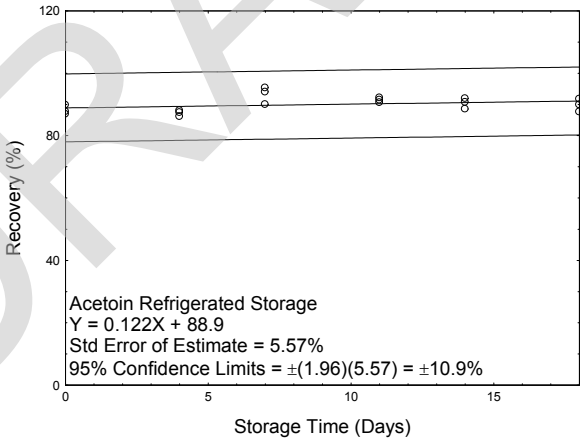


Figure 4.5.2. Refrigerated storage test for acetoin.

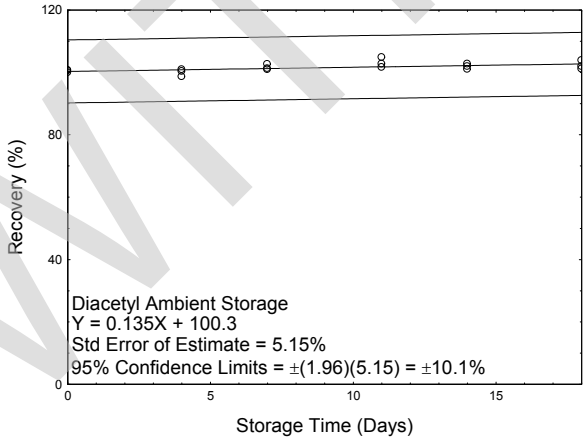


Figure 4.5.3. Ambient storage test for diacetyl.

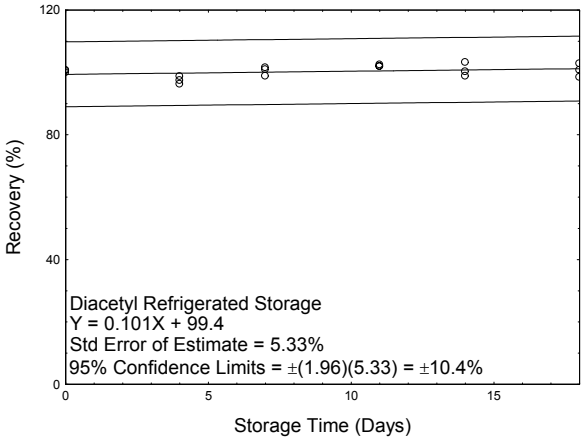


Figure 4.5.4. Refrigerated storage test for diacetyl.

4.6 Reproducibility

Six samples were prepared by collecting them from a controlled test atmosphere similar to that which was used in the collection of the storage samples. The samples were submitted to the OSHA Salt Lake Technical Center for analysis along with a draft copy of this method. The samples were analyzed after being stored for 20 days at refrigerated temperature (about 3 °C). Sample results were corrected for extraction efficiency. No sample result for acetoin and diacetyl had a deviation greater than the precision of the overall procedure determined in Section 4.4.

Table 4.6.1
Reproducibility Data for Acetoin

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
16.3	17.3	106.1	6.1
16.4	15.8	96.3	-3.7
16.1	16.8	104.3	4.3
15.8	15.2	96.2	-3.8
16.1	15.7	97.5	-2.5
16.6	16.0	96.4	-3.6

Table 4.6.2
Reproducibility Data for Diacetyl

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
15.9	16.6	104.4	4.4
15.9	16.3	102.5	2.5
15.7	16.5	105.1	5.1
15.4	15.8	102.6	2.6
15.7	16.0	101.9	1.9
16.2	16.6	102.5	2.5

4.7 Sampler capacity

The sampling capacity of the front tube was tested by sampling from a dynamically generated test atmosphere at 2 times the target concentration of acetoin (3.58 mg/m^3 or 0.99 ppm) and diacetyl (3.55 mg/m^3 or 1.01 ppm) with an average relative humidity of 40% at 34 °C (absolute humidity of 14.8 mg/L H_2O). The samples were collected at a sampling rate of 0.05 L/min. Backup tubes were placed in-line behind the front tube and were changed regularly after the initial collection of 225 min. Breakthrough for diacetyl was observed after sampling 12.4 L. No breakthrough was observed for acetoin even after sampling for 265 min. The recommended sampling time is 3 h.

Table 4.7
Breakthrough of Diacetyl

test no.	air vol (L)	sampling time (min)	downstream concn (mg/m^3)	breakthrough (%)
1	11.1	225	0.00	0.00
	11.8	240	0.00	0.00
	12.1	245	0.00	0.00
	12.3	250	0.00	0.00
	12.6	255	0.06	1.55
	12.8	260	0.24	6.68
	13.0	265	0.61	17.2
2	12.0	225	0.00	0.00
	12.7	240	0.22	6.32
	13.0	245	0.49	13.8
	13.3	250	0.90	25.3
	13.5	255	1.36	38.3
	13.8	260	1.86	52.3
	14.1	265	2.05	57.7
3	11.6	225	0.00	0.00
	12.4	240	0.25	7.04
	12.6	245	0.66	18.7
	12.9	250	1.32	37.0
	13.1	255	1.96	55.1
	13.4	260	2.36	66.5
	13.6	265	2.96	75.8

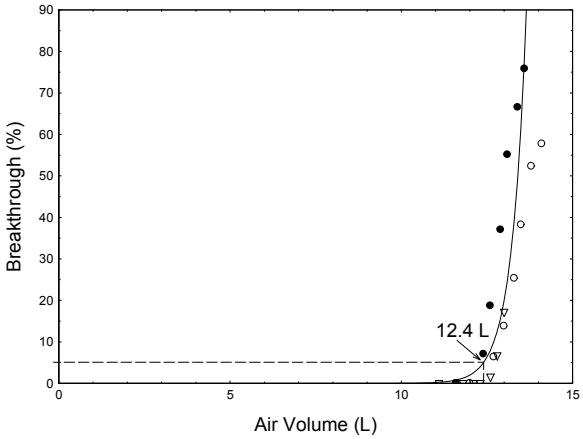


Figure 4.7. Five percent breakthrough air volume for diacetyl.

4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is dependent on the extraction solvent as well as the internal standard. Other extraction solvents or internal standards may be used provided that the new extraction solution or internal standard is tested. The new extraction solvent or internal standard should be tested as described below.

Extraction efficiency

The extraction efficiency of acetion and diacetyl was determined by liquid spiking four samplers, at each concentration level, with the analytes from the RQL to 2 times the target concentrations. These samples were stored overnight at ambient temperature and then analyzed. The mean extraction efficiency over the working range of the RQL to 2 times the target concentration is 92.9% for acetoin. The extraction efficiency for the wet samplers was not included in the overall mean because it would bias the results.

Table 4.8.1
Extraction Efficiency (%) of Acetoin

level		sample number				
x target concn	µg acetoin per sample	1	2	3	4	mean
RQL	0.33	94.0	96.5	97.4	96.7	96.2
0.25	3.73	90.5	87.8	90.1	90.5	89.7
0.5	8.69	90.2	92.4	94.6	95.6	93.2
1.0	16.2	93.2	93.7	91.9	92.6	92.8
1.5	23.6	92.3	93.6	93.5	92.0	92.8
2.0	31.0	92.7	93.8	92.7	92.5	92.9
1.0 (wet)	16.2	96.8	94.5	95.3	95.0	95.4

The mean extraction efficiency over the working range of the RQL to 2 times the target concentration is 99.6% for diacetyl. The extraction efficiency for the wet samplers was not included in the overall mean because it would bias the results.

Table 4.8.2
Extraction Efficiency (%) of Diacetyl

level		sample number				
x target concn	µg diacetyl per sample	1	2	3	4	mean
RQL	0.38	94.1	97.5	101.2	89.9	95.7
0.25	3.58	96.8	97.9	99.3	98.4	98.1
0.5	8.36	101.8	100.4	101.9	101.6	101.4
1.0	15.5	98.0	101.4	100.2	101.7	100.3
1.5	22.7	100.9	102.2	101.4	100.5	101.2
2.0	29.9	100.9	101.2	100.7	100.4	100.8
1.0 (wet)	15.5	97.8	97.3	97.2	99.7	98.0

Stability of extracted samples

The stability of extracted samples was investigated by reanalyzing the target concentration samples 24 h and 72 h after initial analysis. After each analysis was performed, two vials were recapped with new septa while the remaining two retained their punctured septa. The samples were reanalyzed with fresh standards. Samples were stored at ambient temperature and each septum was punctured 4 times for each analysis.

The average percent change for acetoin samples after 24 h was +0.5% for samples that were resealed with new septa and +0.5% for those that retained their punctured septa. The test was performed at room temperature (about 21 °C).

Table 4.8.3
24 Hour Stability of Extracted Samples for Acetoin

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
93.2	93.1	-0.1	91.9	92.9	+1.0
93.7	94.7	+1.0	92.6	92.5	-0.1
	(mean)			(mean)	
93.4	93.9	+0.5	92.2	92.7	+0.5

The average percent change for acetoin samples after 72 h was -1.8% for samples that were resealed with new septa and -0.9% for those that retained their punctured septa.

Table 4.8.4
72 Hour Stability of Extracted Samples for Acetoin

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
93.2	91.5	-1.7	91.9	91.3	-0.6
93.7	91.8	-1.9	92.6	91.3	-1.3
	(mean)			(mean)	
93.4	91.6	-1.8	92.2	91.3	-0.9

The average percent change for diacetyl after 24 h was +0.4% for samples that were resealed with new septa and -1.4% for those that retained their punctured septa. The test was performed at room temperature (about 21 °C).

Table 4.8.5
24 Hour Stability of Extracted Samples for Diacetyl

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
98.0	99.0	+1.0	100.2	99.5	-0.7
101.4	101.2	-0.2	101.7	99.7	-2.0
	(mean)			(mean)	
99.7	100.1	+0.4	101.0	99.6	-1.4

The average percent change for diacetyl samples after 72 h was +1.0% for samples that were resealed with new septa and -0.8% for those that retained their punctured septa.

Table 4.8.6
72 Hour Stability of Extracted Samples for Diacetyl

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
98.0	99.8	+1.8	100.2	100.7	+0.5
101.4	101.5	+0.1	101.7	99.7	-2.0
	(mean)			(mean)	
99.7	100.6	+1.0	101.0	100.2	-0.8

4.9 Interferences (sampling)

Retention

The ability of the sampler to retain acetoin and diacetyl was tested by sampling from a dynamically generated test atmosphere of acetoin (3.67 mg/m^3 or 1.02 ppm) and diacetyl (3.58 mg/m^3 or 1.02 ppm) with an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L H_2O). Six samplers had contaminated air drawn through them at 0.05 L/min for 45 min. Sampling was discontinued and three samples set aside (first set). The generation system was flushed with contaminant-free air. Sampling resumed with the other three samples having contaminant-free air drawn through them at 0.05 L/min for 135 min and then all six samplers were analyzed. The mean of the samples in the second set had retained 100.6% for acetoin and 96.6% for diacetyl of the mean collected by the first three samples.

Table 4.9.1
Retention Efficiency (%) of Acetoin

set no.	1	2	3	mean
first	93.6	92.5	99.8	95.3
second	94.0	95.6	98.1	95.9
second/first				100.6

Table 4.9.2
Retention Efficiency (%) of Diacetyl

set no.	1	2	3	mean
first	108.0	103.0	108.5	106.5
second	102.4	102.3	103.9	102.9
second/first				96.6

Low humidity

The ability of the sampler to collect acetoin and diacetyl from a relatively dry atmosphere was tested by sampling from a dynamically generated test atmosphere of acetoin (4.06 mg/m^3 or 1.13 ppm) and diacetyl (4.03 mg/m^3 or 1.14 ppm) with an average relative humidity of 8% at 33 °C (absolute humidity of 2.82 mg/L H_2O). Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The samplers collected 103.0%, 96.9% and 102.2% of theoretical for acetoin and 96.7%, 106.6% and 101.2% of theoretical for diacetyl.

Low concentration

The ability of the sampler to collect acetoin and diacetyl at low concentrations was tested by sampling from a dynamically generated test atmosphere of 0.1 times the target concentration of acetion (0.185 mg/m^3 or 0.0515 ppm) and diacetyl (0.175 mg/m^3 or 0.0497 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The samplers collected 93.9%, 91.5% and 89.9% of theoretical for acetoin and 92.8%, 97.4% and 96.7% of theoretical for diacetyl.

The ability of the sampler to collect acetoin and diacetyl at low concentrations when taking short term samples was tested by sampling from a dynamically generated test atmosphere of 0.1 times the target concentration of acetion (0.185 mg/m^3 or 0.0514 ppm) and diacetyl (0.175 mg/m^3 or 0.0497 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). Three samplers had contaminated air drawn through them at 0.2 L/min for 15 min. All of the samples were immediately analyzed. The samplers collected 103.8%, 104.1% and 110.0% of theoretical for acetoin and 88.1%, 89.2% and 94.4% of theoretical for diacetyl.

Interferences

The ability of the sampler to collect acetoin and diacetyl was tested when other potential interferences are present by sampling an atmosphere containing 1.63 mg/m^3 (0.45 ppm) of acetoin, 1.56 mg/m^3 (0.44 ppm) of diacetyl, 2.59 mg/m^3 (0.44 ppm) of 2-nonanone and 1.88 mg/m^3 (0.44 ppm) of 2,3-pentanedione with an average relative humidity of 38% at 34 °C

(absolute humidity of 14.1 mg/L H_2O). Three samplers had contaminated air drawn through them at 0.05 L/min for 181 min. All of the samples were immediately analyzed. The samplers collected 93.2%, 96.5% and 96.8% of theoretical for acetoin and 100.6%, 100.6% and 104.1% of theoretical for diacetyl. Selection of 2-nonanone as a potential interference was based on its common use in butter flavorings used in microwave popcorn manufacturing facilities[29]. 2,3-Pentanedione was selected because it has been suggested as a possible replacement for diacetyl. (Note: The GC retention time of 2-nonanone was 14.4 min and 7.4 min for 2,3-pentanedione. For this test the GC column temperature program was slightly changed to Initial 60 °C, hold 4 min; ramp at 15 °C/min to 225 °C, hold 0 min; ramp at 60 °C/min to 250 °C, hold 4 min to allow for the elution of 2-nonanone.)

Light

The possibility of light degradation was tested for both acetoin and diacetyl on the sampling medium and in the extraction solution. For the sample medium test 12 samples were collected by sampling from a dynamically generated test atmosphere of acetoin (1.92 mg/m^3 or 0.53 ppm) and diacetyl (1.87 mg/m^3 or 0.53 ppm) with an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L H_2O). The samples were collected at a sampling rate of 0.05 L/min for 3 hours. Nine of the samples were covered with aluminum foil during sampling and three were not covered. The three samples not covered and three of the covered samples were immediately analyzed after sampling. Three of the covered samples were placed under a fluorescent lamp for 24 h and the reaming three were placed outside in direct sunlight for three hours before analyzing. The samples covered during sampling and immediately analyzed after sampling had mean recoveries of 94.4% of theoretical for acetoin and 96.6% for diacetyl. The samples not covered during sampling and immediately analyzed after sampling had mean recoveries of 94.1% of theoretical for acetoin and 96.2% for diacetyl. The samples covered during sampling and then exposed to fluorescent light for 24 h before analysis had mean recoveries of 88.7% of theoretical for acetoin and 86.8% for diacetyl. The samples covered during sampling and then exposed to sunlight for 3 h before analysis had mean recoveries of 67.0% of theoretical for acetoin and 6.43% for diacetyl. This data clearly indicates that the sampler should be protected from exposure to light.

Table 4.9.3
Sampler Light Exposure Test for Acetoin

	sample number			
type of sampler light exposure	1	2	3	mean
no light exposure	94.0	97.3	92.0	94.4
3h ambient light exposure during sampling	95.0	91.3	96.0	94.1
24h direct fluorescent light exposure after sampling, none during sampling	92.5	86.4	87.1	88.7
3h direct sunlight exposure after sampling, none during sampling	79.7	63.5	63.7	67.0

Table 4.9.4
Sampler Light Exposure Test for Diacetyl

	sample number			
type of sampler light exposure	1	2	3	mean
no light exposure	95.4	97.6	96.8	96.6
3h ambient light exposure during sampling	98.0	94.9	95.8	96.2
24h direct fluorescent light exposure after sampling, none during sampling	88.4	86.1	86.0	86.8
3h direct sunlight exposure after sampling, none during sampling	5.68	7.08	6.52	6.43

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

[29] Kanwal, R.; Boylstein, R. J.; Piacitelli, C. NIOSH Health Hazard Evaluation Report #2001-0474-2943, 2004. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed July 2008) p 46.

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. With the exception of diacetyl in clear vials, acetoin and diacetyl did not degrade. This data clearly indicates that extracted samples should be protected from exposure to light. This data also indicates that acetoin and diacetyl are stable in the extraction solution for up to 9 days as long as they are stored in amber vials.

Table 4.9.5
Extracted Sample Light Exposure Test for Acetoin

	mean of 3 peak areas		
day	clear vials (ambient)	amber vials (ambient)	amber vials (refrigerated)
0	24226	23552	23485
1	24232	23642	23535
2	23693	23232	22932
3	23455	23376	23383
4	23765	23137	23050
7	24191	23973	23280
8	23734	22969	22684
9	24245	23740	23309

Table 4.9.6
Extracted Sample Light Exposure Test for Diacetyl

	mean of 3 peak areas		
day	clear vials (ambient)	amber vials (ambient)	amber vials (refrigerated)
0	20537	19789	19640
1	19037	19667	19716
2	17814	19301	19336
3	16289	19354	19723
4	15703	19026	19304
7	14603	19687	19577
8	13328	18509	19026
9	12408	19324	19606

The internal standard, 3-pentanone, was stable for up to 9 days in both the clear and ambient vials.

4.10 Diacetyl migration within sampling tubes

In the majority of solid sorbent sampling tubes used by OSHA the sampling bed and the backup bed of sorbent are placed in the same sampling tube. For diacetyl this was not possible due to the migration of diacetyl within the sampling tube during storage. To demonstrate migration fifteen tubes were packed with 600 mg of silica gel and a backup section of 200 mg silica gel separated with a glass wool plug. These fifteen tubes were used to collect samples from a dynamically generated test atmosphere of acetoin (3.35 mg/m^3 or 0.93 ppm) and diacetyl (3.17 mg/m^3 or 0.90 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L H_2O). The samples were collected at a sampling rate of 0.05 L/min for 3 hours. Three samples were analyzed on the day of generation and the other twelve were stored in a closed drawer at ambient temperature (about 21 °C). At 3-4 day intervals, three additional samples were analyzed. After 14 days up to 13.0% of diacetyl was found to have migrated from the front to the back section of the modified sampling tube. Acetoin did not migrate within the sampling tube.

Table 4.10
Ambient Storage Diacetyl Migration Test

time (days)	diacetyl found on backup section (%)		
0	0.00	0.00	0.00
4	3.07	0.54	0.82
7	8.30	4.59	4.94
11	5.81	11.2	7.69
14	9.63	11.9	13.0

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 dried and then humidified and regulated using a Miller Nelson Model 401 Flow-Temperature-Humidity Control System. A measured flow (typically 10 µL per min) of an acetoin and diacetyl water solution was pumped through a 0.53-mm uncoated fused silica capillary tube into the inlet manifold, using a Series D ISCO Syringe Pump with Controller, and mixed with dilution air (typically 100 liters per min) coming from the Miller Nelson Control System. The inlet manifold was heated by wrapping it in heat tape, regulated with a variable autotransformer, in order to insure vaporization of acetoin. The acetoin and diacetyl gas mixture then flowed continuously into the mixing chamber (76-cm × 15-cm) and then into the 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 an Omega Digital Thermo-hygrometer model RH411.

With the exception of low humidity tests OSHA normally generates test atmospheres at an average relative humidity of 80% at 22 °C resulting in an absolute humidity of 15.5 mg/L H_2O. Due to the use of heat tape on the inlet manifold, used as mentioned above to insure the vaporization of acetoin, the test atmosphere generation temperature for this evaluation was typically around 34 °C at the sampling chamber outlet, 37 °C in the middle of the sampling chamber, 45 °C at the sampling chamber inlet and 86 °C at the mixing chamber inlet. In order to maintain a humidity of 15.5 mg/L H_2O at 34 °C the relative absolute humidity was adjusted to approximately 41%.

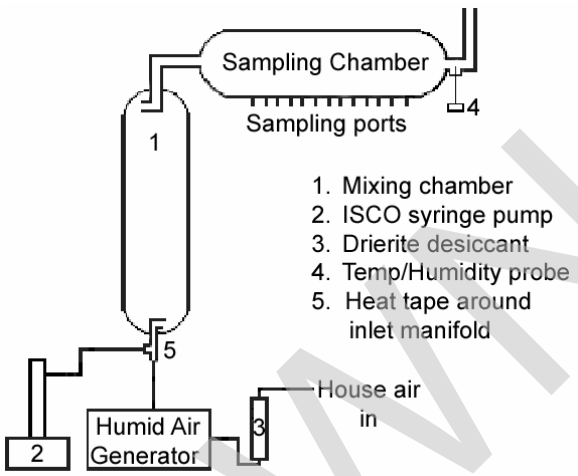


Figure 4.11. Diagram of apparatus used to generate test atmospheres.

4.12 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 spectra in Figure 4.12.1 and 4.12.2 are taken from the NIST spectral library.

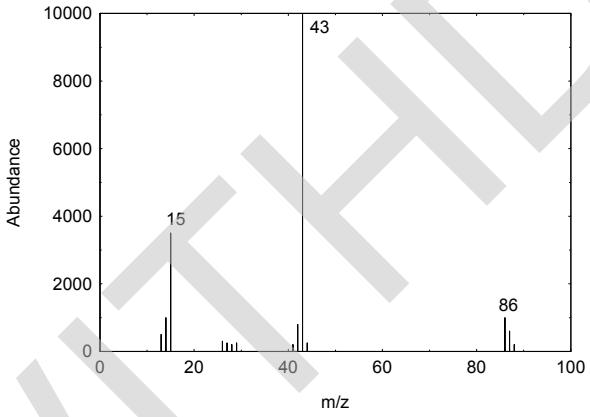


Figure 4.12.1. Mass spectrum of diacetyl.

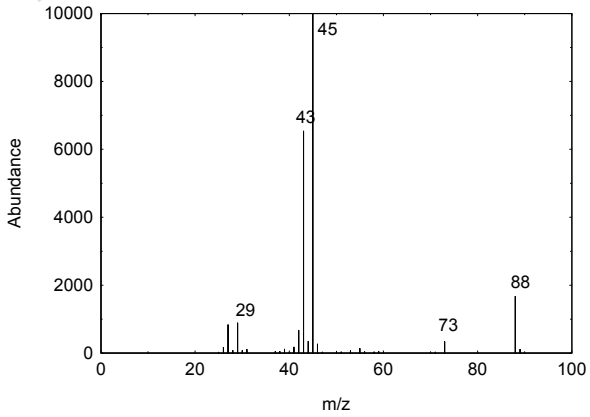


Figure 4.12.2. Mass spectrum of acetoin.

Appendix A

A.1 Silica gel preparation

For this evaluation sampling tubes were custom made by SKC, Inc. and are now available for purchase through SKC, Inc. (cat. no. 226-183).

Below are instructions on how the silica gel is prepared for the sampling tubes used in this evaluation.

A.1.1 Apparatus

Tube furnace and quartz process tube. A Lindberg model 55035 tube furnace and 1-inch diameter quartz process tube were used in this evaluation.

Nitrogen gas.

A.1.2 Silica Gel

Washed 20/40 mesh silica gel with 30 angstrom pore size (washed silica gel can be purchased from SKC, Inc.). A description of a washing procedure for silica gel can be found in the appendix of NIOSH 7903[30].

A.1.3 Preparation of silica gel

Insert a quartz wool plug in a 1-inch diameter quartz process tube, followed by 50 g of washed silica gel and a second quartz wool plug to hold the silica gel in place.

Place the process tube in a tube furnace and set the temperature to 180 °C. Continually purge the process tube with nitrogen at a rate of about 0.5 L/min. Allow the silica gel to dry in the tube furnace for 4 hours.

After 4 hours allow the process tube to cool while continuing to purge the tube with nitrogen. Once the silica gel is cool, remove one of the quartz wool plugs, and transfer silica gel into an airtight container.

[30] Cassinelli, M. E. Acids, Inorganic (NIOSH Method 7903), 1994. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web Site. http://www.cdc.gov/niosh/nmam/pdfs/7903.pdf (accessed July 2008).