Acetoin
Diacetyl

Method no.:	1012
Control no.:	T-1012-FV-01-0811-M
Target concentration:	0.05 ppm (TWA) (0.18 mg/m^3) acetoin 0.05 ppm (TWA) (0.18 mg/m^3) diacetyl
OSHA PEL:	none acetoin none diacetyl
ACGIH TLV:	none acetoin none diacetyl
Procedure:	Samples are collected by drawing workplace air through two tubes containing specially cleaned and dried silica gel connected in series. Samples are extracted and derivatized with a solution of 95:5 ethyl alcohol:water containing 2 mg/mL of *O*-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) and analyzed by gas chromatography using an electron capture detector (GC-ECD).
Recommended sampling time and sampling rate:	180 min at 0.05 L/min (9.0 L) (TWA) 15 min at 0.2 L/min (3.0 L) (short term)
Reliable quantitation limit:	1.49 ppb (5.37 µg/m^3) acetoin 1.30 ppb (4.57 µg/m^3) diacetyl
Standard error of estimate at the target concentration:	5.06% acetoin 5.11% diacetyl
Special requirements:	Protect samplers from the light during and after sampling with aluminum foil or opaque tape.
Status of method:	Evaluated method. This method has been subjected to the established evaluation procedures of the OSHA Salt Lake Technical Center 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 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

On September 24, 2007 OSHA issued a Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl[1] in which diacetyl was identified as an indicator compound for hazardous exposures found at plants packaging microwave popcorn. This was based on Health Hazard Evaluations performed by NIOSH which found the occurrence of severe lung disease in some employees at microwave popcorn packaging plants and flavorings manufacturing facilities. In three NIOSH Health Hazard Evaluation reports, acetoin and diacetyl are listed as major constituents of butter flavoring and they were used as indicators of exposure to butter flavoring vapors.[2,3,4]

OSHA has a partially validated method for diacetyl, PV2118, which recommends the use of two standard sized silica gel tubes in series to collect diacetyl at 0.05 L/min for 1 hour.[5] There were three reasons a new method was needed: 1) the reliable quantitation limit of PV2118 is 0.28 ppm which is higher than the target concentration of 0.05 ppm for this method; 2) a new medium was needed to enable the industrial hygienist to sample for a longer sampling time and take fewer samples; and 3) to allow acetoin and diacetyl to be sampled and analyzed together. The new medium used in this method is a tube packed with specially cleaned and dried silica gel (600 mg) with a glass wool plug and a glass fiber filter in front of the dried silica gel bed (this medium is referred to as dried silica gel in this method). It was necessary to specially dry the silica gel to obtain a higher capacity because of the amount of water already present on the silica gel in the currently commercially available tubes. The dried silica gel tube can be used to sample diacetyl for up to 1.5 times longer than the currently available silica gel tube. There was not a capacity problem with acetoin. 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 glass fiber filter in the tube serves only to trap these materials before they enter the silica gel bed. Retention studies using a powder containing acetoin and diacetyl showed that the acetoin and diacetyl can be stripped off the powder and collected on the silica gel, especially when sampling high humidity air. (Section 4.9)

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1 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 3/17/2008).

2 HETA 2001-0474-2943 American Pop Corn Company, 2004. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed 3/15/2008).

3 HETA 2002-0408-2915 Agrilink Foods Popcorn Plant, 2003. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2002-0408-2915.pdf (accessed 3/15/2008).

4 HETA 2003-0112-2949 ConAgra Snack Foods, 2004. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2003-0112-2949.pdf (accessed 3/15/2008).

5 Shah, Y. C. OSHA 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 3/17/2008).

To obtain adequate sensitivity for this method, it was necessary to derivatize the acetoin and diacetyl. 2,4-Dinitrophenyl hydrazine (DNPH) was the first derivatizing agent tried, but DNPH can react with both ketone and α-hydroxy ketones[6], and while it initially formed unique derivatives of acetoin and diacetyl by reacting with the first ketone group, it eventually reacted also with the alcohol group on acetoin and the second ketone group on diacetyl, forming the same derivative. In EPA Method 556.1 *O*-pentafluorobenzyl hydroxylamine hydrochloride (PFBHA) was used to derivatize ketone and aldehyde groups.[7] Unique derivatives of acetoin and diacetyl are formed by reacting them with PFBHA. The first ketone group on diacetyl reacts within four hours with PFBHA, but the second ketone group takes 36 hours to reach completion. Acetoin reacts within 3 hours. In this method, samples are extracted and derivatized in an extraction solution containing PFBHA. This is accomplished by first rotating the samples for 60 min and then allowing the samples to stand at room temperature for an additional 36 hours for the derivatization reaction to reach completion.

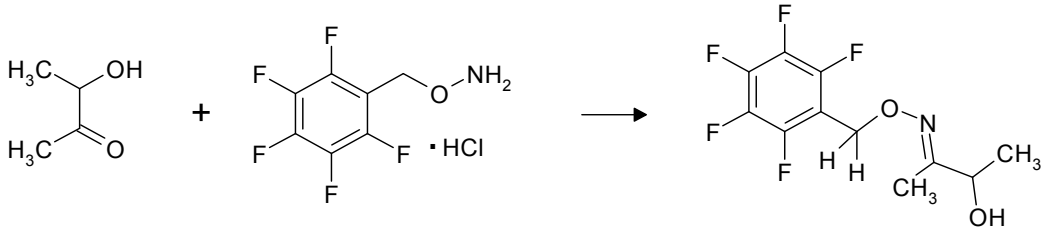

Figure 1.1.1.1. The reaction of acetoin with PFBHA to form the acetoin-PFBHA derivative.

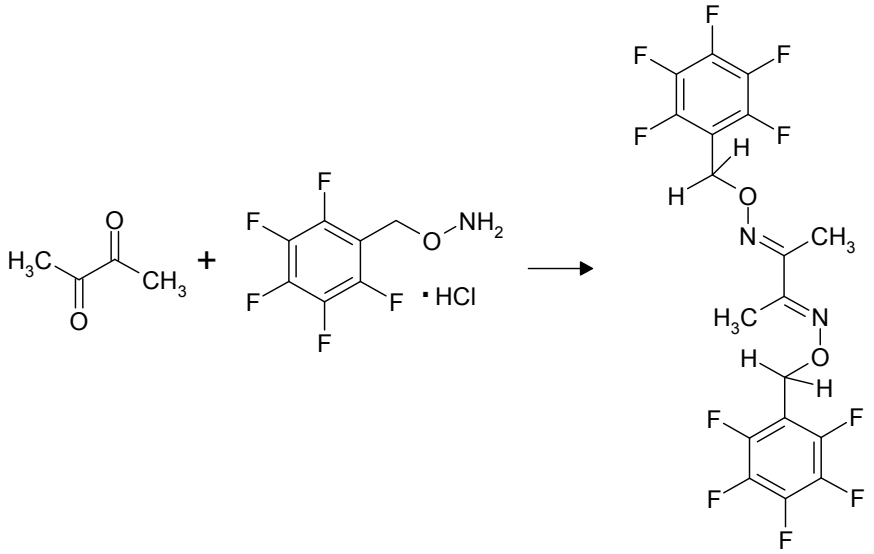

Figure 1.1.1.2. The reaction of diacetyl with PFBHA to form the diacetyl-PFBHA derivative.

This method is designed for low air concentrations of acetoin, diacetyl, and potential interferences. If high exposures are anticipated, use OSHA Method 1013[8] or increase

[6] Smith, M., March, J.; *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure,* 5th ed.; John Wiley & Sons Inc.: New York, 2001, p 1193.

[7] EPA Method 556.1 Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography, 1999. U.S. Environmental Protection Agency Web site. http://www.epa.gov/safewater/methods/pdfs/methods/met556_1.pdf (accessed 3/17/2008).

[8] Simmons, M., Hendricks, W. Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

the amount of PFBHA in the extraction solution to ensure complete derivatization. Samples extracted by OSHA Method 1013 can be derivatized and analyzed by this method to detect lower concentrations.

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

NIOSH Health Hazard Evaluations (HHE) of microwave popcorn manufacturing plants found fixed airway obstruction, in some cases, consistent with bronchiolitis obliterans in some employees.[9] Acetoin, diacetyl, acetic acid, acetaldehyde, and 2-nonanone were amongst the chemicals found by NIOSH in several popcorn manufacturing plants.[10] Diacetyl was found to be present in all workplaces where the bronchiolitis obliterans was observed, and acetoin was found in some of the workplaces. Animal toxicology studies were performed by NIOSH with diacetyl, or butter flavorings containing diacetyl. Respiratory tract damage, including necrosis of the nasal and tracheal epithelium, and death were reported in rodents exposed to diacetyl, and butter flavorings containing diacetyl, at an air concentration of approximately 200 ppm of diacetyl for 6 hours. Mice exposed to 200 and 400 ppm diacetyl via inhalation for 6 hours per day over 5 days had the following health effects: death, acute necrotizing rhinitis, and erosive or necrotizing laryngitis. Mice exposed to 200 and 400 milligrams per kilogram (mg/kg) diacetyl via oropharyngeal aspiration for 6 hours per day over 5 days had bronchiolar fibrosis and death. Rats exposed to butter flavoring vapors containing 300 ppm diacetyl for 6 hours had epithelial injury in the nasal passages and pulmonary airways.[11]

1.1.3 Workplace exposure

Workers are exposed to acetoin and diacetyl in various manufacturing processes. Acetoin and diacetyl are natural flavorings that are also synthesized for use in odor and flavor manufacturing.[12,13] Acetoin and diacetyl are found in tobacco smoke, vapors from garbage, vapors from liquid and solid animal wastes, exhaust emissions from petroleum based fuels, vapors from moldy buildings, charcoal production, vapors from latex-polyurethane backed carpet, and as chemical reagents and in chemical reactions.[14] Diacetyl is also used as an anti-microbial preservative, modifier of radiation responses for chemical and biological systems, and as a photoinitializer in polymerization of plastics.

Occupational exposure to acetoin and diacetyl in microwave popcorn manufacturing has been studied since the first reported case of severe obstructive lung disease in 2000.[15] NIOSH identified acetoin and diacetyl as useful indicator compounds that can

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[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 3/17/2008).

[10] Flavorings-Related Lung Disease: Health Hazard Evaluations. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/topics/flavorings/hhe-eval.html (accessed 3/17/2008).

[11] 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 3/17/2008).

[12] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 11.

[13] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 411.

[14] Chemical Information Review Document for Artificial Butter Flavoring and Constituents Diacetyl (CAS No. 431-03-8) and Acetoin (CAS No. 513-86-0), 2007. Department of Health and Human Services, National Toxicology Program Web site. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumpdf/ Artificial_butter_flavoring.pdf (accessed 3/17/2008).

[15] HETA 2000-0401-2991 Gilster-Mary Lee Corporation, 2000. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www2a.cdc.gov/hhe/select.asp?PjtName=40422&bFlag=1&ID=1 (accessed 3/17/2008).

be used to represent exposure to butter flavorings. Areas of concern were the flavor production rooms, mixing/blending rooms, packaging/production rooms, rooms where the mixing tanks were located, maintenance and cleaning operations, and quality control labs.[16]

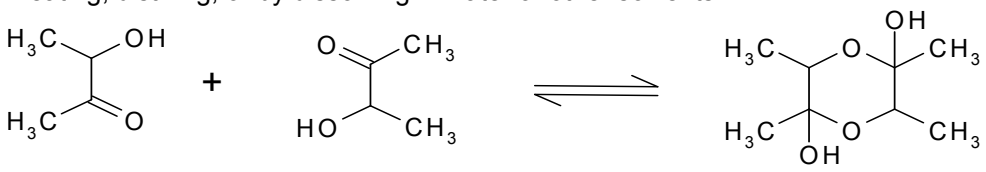
Acetoin is used as an aroma carrier and as a flavor ingredient to impart a creamy taste in fragrances and flavorings.[17] Acetoin annual use in food and flavors manufacturing in 2004 was 34,000 pounds. Acetoin is used as a flavor ingredient for butter, milk, yogurt, and strawberry flavors. The FDA maximum allowable concentration for acetoin in beverages is 5 ppm, and in food is 50 ppm. Acetoin is naturally found in fresh apple, cooked apple, leek, cooked leek, corn, honey, cocoa, butter, roasted coffee, cheeses, yogurt, milk, wines, beer, fermented tea, scallops, crowberry, quince, and other sources. Acetoin is used in manufacturing alcoholic beverages, baked goods, breakfast cereals, cheese, chewing gum, condiments and relishes, confections and frostings, fats and oils, frozen dairy products, fruit juices, gelatins and puddings, gravies and mixes, hard candy, imitation dairy products, meat products, milk products, nonalcoholic beverages, grains, reconstituted vegetables, seasonings and flavorings, snack foods, soft candy, soups, and sweet sauce.

Diacetyl is used as a fragrance and flavor ingredient to give products a buttery or creamy odor and flavor.[18] Diacetyl annual use in food and flavor manufacturing in 2004 was 153,500 pounds. The FDA maximum allowable concentration for diacetyl in beverages is 5 ppm, and in food is 50 ppm. Diacetyl naturally occurs in butter, milk products, yogurt, grains, meat, wines, beer, oils of pine, oil of angelica, oils of lavender and other flowers, many flowers, raspberries, strawberries, citrus, ligonberry, guava, cabbage, peas, tomato, vinegar, cheeses, chicken, beef, mutton, pork, cognac, whiskies, tea, and coffee. Diacetyl is used in manufacturing as a flavoring in alcoholic beverages, baked goods, cheese, chewing gum, fats and oils, frozen dairy products, gelatins and puddings, gravies, hard candy, soft candy, imitation dairy, meat products, milk products, nonalcoholic beverages, and snack foods.

1.1.4 Physical properties and other descriptive information

acetoin[19,20,21]

Acetoin is found as the liquid monomer and the solid dimer. The pure monomer forms the dimer at room temperature. The monomer can be formed from the dimer by heating, distilling, or by dissolving in water or other solvents.



[16] HETA 2001-0474-2943 American Pop Corn Company, 2001. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www2a.cdc.gov/hhe/select.asp?PjtName=36271&bFlag=0&ID=2 (accesed 3/17/2008).

[17] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 11.

[18] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 411.

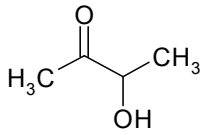
[19] Budavari, S., Ed; *The Merck Index*, 13th ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 2001; p 68.

[20] Material Safety Data Sheet: Acetoin, Chemwatch, Victoria, Australia (accesed 3/17/08).

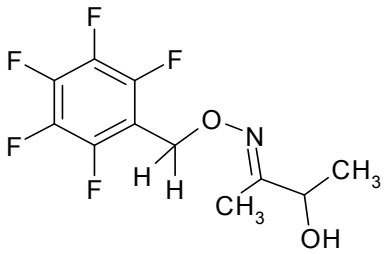
[21] Acetoin MSDS. SigmaAldrich Web site. http://www.sigmaaldrich.com/catalog/search/ProductDetail/ALDRICH/A17951 (accessed 3/17/2008).

synonyms:	acetyl methyl carbinol; 2,3-butanolone; 2-butanone, 3-hydoxy-; 2-butanol-3-one; dimethylketol; γ-hydroxy-β-oxobutane; 3-hydroxybutan-2-one; 3-hydroxy-2-butanone; 1-hydroxyethyl methyl ketone; methyl acetyl carbinol
IMIS[22]:	A624
CAS number:	513-86-0 (monomer); 23147-57-1 (dimer)[23]
boiling point:	148 ºC (298 ºF) (monomer)
melting point:	15 ºC (59 ºF) (monomer); 90 ºC (194 ºF) (dimer)
density:	1.005 g/mL @ 20/20 (monomer)
molecular weight:	88.11 (monomer)
flash point:	50.6 ºC (123 ºF) (closed cup) (monomer)
autoignition temperature:	370 ºC (773.8 ºF)
appearance:	clear to light yellow liquid (monomer); light cream to light yellow crystals (dimer)
vapor density:	>1 (air = 1)
molecular formula:	$C_4H_8O_2$ (monomer); $C_8H_{16}O_4$ (dimer)
odor:	pleasant buttery odor
solubility:	soluble in water; miscible with alcohol; sparingly soluble in ether and petroleum ether
reactive hazards:	acetoin is light sensitive [24] (Section 4.9)

structural formula:
(monomer)



structural formula:
(acetoin-PFBHA derivative)



22 Acetoin (OSHA 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 3/17/2008).

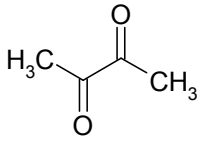
23 CID: 90884 Acetyl Methyl Carbinol Dimer, 2008. Department of Health and Human Services, National Institutes of Health, National Center for Biotechnology Information. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=90884&loc=ec_rcs (accessed 3/17/2008).

24 Material Safety Data Sheet: Acetoin, 2008. The Good Scents Company Web site. http://www.thegoodscentscompany.com/msds/md102388.html (accessed 3/17/2008).

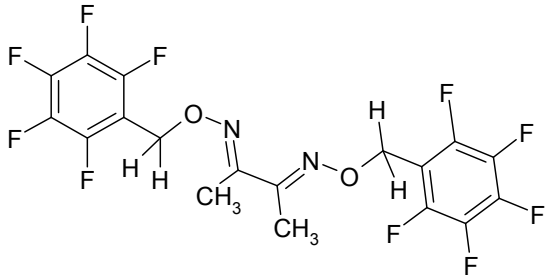
diacetyl[25,26,27,28]

synonyms:	biacetyl; 2,3-butanedione; 2,3-butadione; 2,3-diketobutane; dimethyldiketone; dimethylglyoxal; glyoxal, dimethyl-;
IMIS[29]:	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:	butter in lower concentrations, quinone odor or chlorine-like odor in higher concentrations
solubility:	4 parts water; miscible with alcohol, ether
reactive hazards:	diacetyl is light sensitive (Section 4.9); vapors may ignite when pouring or pumping due to static electricity
autoignition temperature:	285 ºC (545 ºF)

structural formula:



structural formula:
(diacetyl-PFBHA derivative)



[25] *The Merck Index*, 13th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 2001; p 522.

[26] Material Safety Data Sheet: Diacetyl, Chemwatch, Victoria, Australia (accessed 3/17/2008).

[27] Material Safety Data Sheet: 2,3-Butanedione, 2007. Fisher Scientific Web site. https://fscimage.fishersci.com/msds/03275.htm (accessed 3/17/2008).

[28] Material Safety Data Sheet: 2,3-Butanedione, 2007. Chem Service Inc Web site. http://www.chemservice.com/msds/msds_detail.asp?catnum=O-816 (accessed 3/17/2008).

[29] Diacetyl (OSHA 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 3/17/2008).

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[30]. 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 0.17 pg for acetoin and 0.11 pg for diacetyl. These are the amounts of analyte that will give a detector response that is significantly different from the response of a reagent blank. (Section 4.1)

1.2.2 Detection limit of the overall procedure

The detection limit of the overall procedure is 14.5 ng (0.447 ppb or 1.61 µg/m^3) for acetoin and 12.3 ng (0.389 ppb or 1.37 µg/m^3) for diacetyl. These are the amounts of analyte spiked on the sampler that will give detector responses that are significantly different from the responses of the respective sampler blanks. (Section 4.2)

1.2.3 Reliable quantitation limit

The reliable quantitation limit is 48.4 ng (1.49 ppb or 5.37 µg/m^3) for acetoin and 41.1 ng (1.30 ppb or 4.57 µg/m^3) for diacetyl. These are the amounts of analyte spiked on the samplers that will give detector responses that are considered the lower limits for precise quantitative measurements. (Section 4.2)

1.2.4 Instrument calibration

The standard error of estimate is 0.019 µg/sample for acetoin over the range of 0.41 to 3.28 µg/sample. The standard error of estimate is 0.052 µg/sample for diacetyl over the range of 0.40 to 3.16 µg/sample. This range corresponds to 0.25 to 2 times the TWA 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 from dried silica gel tubes was ±9.9% for acetoin and ±10.0% for diacetyl. These each include an additional 5% for sampling pump variability. (Section 4.4)

1.2.6 Recovery

The recoveries of acetoin and diacetyl from samples used in the 18-day storage test remained above 98.4% for acetoin and 98.0% for diacetyl when the samples were stored at 23 °C. (Section 4.5)

[30] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M.; 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/index.html (accessed 3/15/2008).

1.2.7 Reproducibility

Six samples were collected from a controlled test atmosphere and submitted for analysis by the OSHA Salt Lake Technical Center. The samples were analyzed according to a draft copy of this procedure after being stored at 4 ºC for 20 days and at -12 °C for an additional 19 days. 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

Samples are collected with two tubes in series. The tubes consist of 110-cm × 7-mm o.d. glass sampling tubes packed with one section (600 mg) of specially cleaned and dried silica gel. From the front to back, the sampler consists of a silane-treated glass wool plug, glass fiber filter, 600 mg specially cleaned silica gel, and a second silane-treated glass wool plug. The silica gel should be cleaned and dried as described in Appendix A of OSHA Method 1013.[31] The tubes used in this evaluation were labeled front and back tube. The front tube is connected to the back tube with a piece of tubing to form the sampling train. For this evaluation commercially prepared sampling tubes containing the speacially dried silica gel were purchased from SKC, Inc. (Catalog no. 226-183, lot no. CPM112907-001).

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, opaque tape, or a tube holder, such as SKC, Inc. Cover D (catalog no. 244-29D), to protect samples from light.

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 the tube. Use tube holders to minimize the hazard of broken glass and to protect tubes from light exposure during sampling. All tubes should be from the same lot.

A sampling train is created by attaching two tubes in series with a small section of tubing so that the front opening of the back tube is close to the back opening of the front tube. The front of each tube contains glass wool followed by a glass fiber filter, and the back of the tube contains only the glass wool.

The back tube is used as a back-up and is positioned nearest the sampling pump. Attach the tube holder to the sampling pump so that the adsorbent tube is in an approximately vertical position with the inlet in the breathing zone. Position the sampling pump, tube holder, and tubing so they do not impede work performance or safety. Use a tube holder or wrap the tubes

[31] Simmons, M., Hendricks, W. Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

in aluminum foil to insure that both sampling tubes are protected from light exposure. Light will decompose the acetoin and diacetyl.

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

After sampling for the appropriate time, remove the sampling train, separate the tubes, and seal each tube with plastic end caps. Wrap each tube in aluminum foil or opaque tape, and then seal each sample end-to-end with a Form OSHA-21 seal 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 (liters), sampling time (minutes), 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. As a precaution, store the samples at refrigerator temperature if a delay in shipment is unavoidable. Ship any bulk samples separate from the air samples.

2.4 Sampler capacity (Section 4.7)

The sampling capacity was determined using test atmospheres containing the analytes. The concentrations of the test atmospheres were: 0.101 ppm (0.365 mg/m^3) acetoin, and 0.101 ppm (0.355 mg/m^3) diacetyl with an average relative humidity (RH) of 80% at 23 ºC. The samples were collected at 0.05 L/min. The 5% breakthrough air volumes were determined to be 12.1 L for diacetyl and greater than 24 L for acetoin.

There was no acetoin or diacetyl on the back-up tube when a 15 min sample was taken at 0.2 L/min. The 5% breakthrough air volumes for a flow rate of 0.2 L/min were determined to be 11.98 L for diacetyl and greater than 13 L for acetoin.

2.5 Extraction efficiency (Section 4.8)

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

The mean extraction efficiencies from dry silica gel over the range of RQL to 2 times the target concentration were: 102.0% (0.022 to 3.28 µg/sample) for acetoin and 97.6% (0.01 to 3.16 µg/sample) for diacetyl. The extraction efficiency was not affected by the presence of water.

Extracted samples remain stable for at least 24 h.

2.6 Recommended sampling time and sampling rate

Sample with dried silica gel tubes for up to 180 min at 0.05 L/min (9 L) to collect TWA (long-term) samples, and for 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 limits for dried silica gel tubes for a 15 min sample taken at 0.2 L/min are 0.0044 ppm (0.016 mg/m^3) for acetoin and 0.0042 ppm (0.015 mg/m^3) for diacetyl.

2.7 Interferences, sampling (Section 4.9)

Retention efficiency

The mean retention efficiency was 96.7% for acetoin and 96.9% for diacetyl when dried silica gel tubes containing 0.819 µg of acetoin and 0.808 µg of diacetyl were allowed to sample 6.75 L of contaminant-free air having an average relative humidity of 80% at 23 ºC. (Section 4.9)

Low humidity

The ability of dried silica gel tubes to collect the analytes from a relatively dry atmosphere was determined by sampling an atmosphere containing two times the target concentration and at an average relative humidity of 20% RH at 23 ºC. The mean recoveries (% of theoretical) were 98.7% for acetoin and 98.5% for diacetyl. (Section 4.9)

Low concentration

The ability of dried silica gel tubes to collect the analytes at low concentrations was tested by sampling an atmosphere at 0.1 times the target concentration with at an average relative humidity of 80% RH at 23 ºC. The mean recoveries (% of theoretical) were 99.0% for acetoin and 98.4% for diacetyl. (Section 4.9)

Sampling interference

The ability of dried silica gel tubes to collect the analyte when other potential interferences are present was tested under two separate series of tests. The first test was an atmosphere similar to ones found at some popcorn manufacturing plants consisting of acetoin and diacetyl at the target concentration with an interference mixture of acetaldehyde, acetic acid, and methyl ethyl ketone at an average humidity of 80% at 23 ºC. All three of these interferences can react with PFBHA. The concentrations of the analytes in this test atmosphere were: 0.051 ppm (0.184 mg/m^3) acetoin and 0.051 ppm (0.180 mg/m^3) diacetyl, 1.01 ppm (1.82 mg/m^3) acetaldehyde, 1.05 ppm (2.58 mg/m^3) acetic acid, and 1.02 ppm (3.01 mg/m^3) methyl ethyl ketone. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The mean recoveries (% of theoretical) were: acetoin 97.9% and diacetyl 98.2%.

The second series of tests was with acetoin and diacetyl at the target concentration and each of the interferences listed above individually at their PEL concentration following the guidelines in SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[32]. The concentrations of these interferences are much higher than would normally be expected in a food or flavoring manufacturing workplace. The PFBHA extraction solution needed to be modified to 18 mg/mL PFBHA (72.1 µmoles/mL) to insure that there was enough PFBHA to derivatize all the analytes. These interferences and acetoin react fully within 4 hours of extraction, but the diacetyl requires 36 hours to fully react. These three test atmospheres each contained the one of the following concentrations of interference: 190 ppm (350 mg/m^3) acetaldehyde, 9.49 ppm (23.3 mg/m^3) acetic acid, or 190 ppm (560 mg/m^3) methyl ethyl ketone. These three compounds were chosen because they can collect onto the dried silica gel tubes and can react with the PFBHA. For each test, three sampling trains had contaminated air (air containing the analytes and an interference) drawn through them at 0.05 L/min for 180 min for each test. All of the samples were immediately analyzed. The average recoveries (% of theoretical) with 190 ppm acetaldehyde were 97.8% for acetoin and 95.5% for diacetyl. The average recoveries (% of theoretical) with 9.49 ppm acetic acid were 97.3% for acetoin and

[32] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. 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/index.html (accessed 3/15/2008).

98.2% for diacetyl. The average recoveries (% of theoretical) with 200 ppm methyl ethyl ketone were 98.4% for acetoin and 97.6% for diacetyl. These interferences were not a sampling interference, but under normal sample analysis, these levels of interferences would be analytical interferences. (Section 4.9)

Light

Acetoin and diacetyl are light-sensitive. The interference of light during sampling was tested using three foil-wrapped sampling trains and three uncovered sampling trains. An atmosphere containing twice the target concentration at an average relative humidity of 78% at 23ºC was sampled for 180 min at 0.05 L/min, and the samples were extracted that day. The average recovery for acetoin of the foil-wrapped samplers was 98.5% and the uncovered samplers had an average recovery of 93.9%. The average recovery for diacetyl of the foil-wrapped samplers was 98.9% and the uncovered samplers had an average recovery of 94.3%. An additional three sampling trains were collected at the same time, and were protected from the light by aluminum foil. After collection, these samplers had the foil removed and were placed on the counter at ambient temperature under room light. These samples were analyzed 24 h after sampling during which they were exposed to the room light for 14 of the 24 h. The average recoveries were 81.3% for acetoin and 80.0% for diacetyl. Light is a significant interference; therefore, both tubes in the sampling train need to be covered by aluminum foil or opaque tape during and after sampling. (Section 4.9)

Powder form

The powder form of acetoin and diacetyl tested consisted of starch coated with acetoin and diacetyl. Three tests were performed on this powder. The first consisted of a sampling train of a pre-weighed PVC filter in a conical cassette in series with two dried silica gel tubes. The two dried silica gel tubes were used to collect any vapors of acetoin and diacetyl which would strip off from the powder. Known amounts of the powder were placed onto the PVC filter, and 9 L of air at an average relative humidity of 78% at 22 °C were pulled through the sampling trains at 0.05 L/min. The recovery of acetoin and diacetyl on the pre-weighed PVC filters was 0% to 1.9% for acetoin and 0% to 2.3% for diacetyl. The recovery on the dried silica gel tubes was 96.6% for acetoin and 97.8% for diacetyl. The acetoin and diacetyl recoveries were calculated from the percentages obtained from analysis of the powder and the amounts of powder weighed out. The second and third tests consisted of a sampling train of two dried silica gel tubes in series, with the powder spiked on the front glass wool of the front tube. The two tests had 9 L of air drawn through the sampling trains at 0.05 L/min, the first test used air at an average relative humidity of 20% at 22 °C, and the other test used air at an average relative humidity of 78% at 22 °C. At 20% RH most of the acetoin and diacetyl were found on the front glass wool and glass fiber filter, but at 78% RH most of the acetoin and diacetyl were found on the dried silica gel beds. These tubes can collect particulates, but cannot be used as a particulate sampler at 0.05 L/min. (Section 4.9)

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan[33]. Avoid skin contact and inhalation of all chemicals and review all MSDSs before beginning this analytical procedure.

3.1 Apparatus

Gas chromatograph equipped with an electron capture detector. An Agilent Model 6890 GC equipped with a Chemstation, an automatic sample injector, and a μ-electron capture detector (μECD) was used in this evaluation.

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

A GC column capable of separating the PFBHA derivatives of acetoin and diacetyl from the PFBHA extraction solution, potential interferences, and internal standard. A 30-m × 0.32-mm i.d. fused silica capillary column (DB-5 0.25-µm df) (Agilent Technologies, Santa Clara CA) was used in this evaluation.

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

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

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

Class A volumetric flasks of appropriate sizes such as 10-mL and other convenient sizes for preparing standards.

Calibrated 10-µL syringe for preparing standards.

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Micro-analytical balance capable of weighing at least 0.001 mg. An Ohaus Galaxy 160D was used in this evaluation.

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

3.2 Reagents

Acetoin, [CAS no. 513-86-0], reagent grade or better. Acetoin used in this evaluation was 99+% (lot no. 05025DH) purchased from Sigma-Aldrich (Milwaukee, WI).

Diacetyl, [CAS no. 431-03-8], reagent grade or better. Diacetyl used in this evaluation was 97% (lot no. 10815TD) purchased from Sigma-Aldrich (Milwaukee, WI).

Ethyl alcohol, [CAS no. 64-17-5], 95% v/v (190 proof) A.C.S. Spectrophotometric grade. Ethyl alcohol used in this evaluation was 95% (lot no. B0513970) purchased from Acros (Morris Plains, NJ).

O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride, [CAS no. 57981-02-9] (PFBHA), reagent grade or better. PFBHA used in this evaluation was 99+% (lot no. 1242759 54706063) purchased from Fluka, a subsidiary of Sigma-Aldrich (Milwaukee, WI).

4-Bromobenzylbromide, [CAS no. 589-15-1], reagent grade or better. 4-Bromobenzylbromide used in this evaluation was 98% (lot no. A0251708) purchased from Acros (Morris Plains, NJ).

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

The PFBHA extraction solution used for this evaluation consisted of 20 µg/mL 4-bromobenzylbromide in the 95:5 ethyl alcohol:water with 2 mg/mL PFBHA. The 4-bromobenzylbromide was added to 95:5 ethyl alcohol:water as an internal standard. Other internal standards can be used provided they are fully tested. Store this solution in a tightly sealed container in a refrigerator that does not contain solutions of aldehydes, acids, or ketones. This solution can absorb formaldehyde, other aldehydes, ketones, and acids out of the air. These compounds will react with the PFBHA, decreasing the amount available to react with acetoin or diacetyl. This solution can be stored in the refrigerator for 1 week.

3.3 Standard preparation

Prepare stock solution of acetoin and diacetyl in water. Acetoin is usually sold as the dimer, which will disassociate in water to the monomer as the solid dimer dissolves. This stock solution will remain stable for four weeks if stored in an amber bottle in the refrigerator.[34]

Freshly prepare analytical standards from the stock solutions for each analysis. These analytical standards are prepared for each of the analytes by injection of microliter amounts of a stock solution into 2-mL volumetric flasks and diluting with the PFBHA extraction solution over a concentration range of 0.02 to 6 µg/sample. For example: a target concentration standard of 1.60 µg/sample acetoin and 1.56 µg/sample diacetyl was prepared by injecting 16 µL of a stock solution containing 0.10 µg/mL acetoin and 0.10 µL/mL (0.0975 µg/mL) diacetyl in water into a 2-mL volumetric flask containing about 1.75 mL of PFBHA extraction solution and then diluting to the mark with PFBHA extraction solution (this is equivalent to 0.80 µg/mL acetoin or 0.049 ppm based on a 2-mL extraction and 9 L air volume, and 0.78 µg/mL diacetyl or 0.049 ppm based on a 2-mL extraction and 9 L air volume). Standards must be allowed to react with the PFBHA at room temperature for 36 hours.

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 PFBHA extraction solution and reanalyze the diluted samples.

3.4 Sample preparation

Remove the plastic end caps from the sample tube and carefully transfer the section of the adsorbent from each tube into separate 4-mL amber vials. Normally the front glass wool plug and glass fiber filter are discarded. If the industrial hygienist requests the analysis, the front glass wool plug and the glass fiber filter should be placed into a separate 4-mL amber vial. Discard the glass tubes and back glass wool plugs.

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

Place the samples on a mechanical rotator and rotate at approximately 40 rpm for 60 min. Do not use a shaker to extract samples, as the recoveries will be lower.

Allow the samples to stand at room temperature for an additional 36 hours for the derivatization reaction to reach completion.

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

If more sensitivity is desired for samples prepared by OSHA Method 1013[35], they can be derivatized by the PFBHA solution and analyzed by GC-ECD. The samples in OSHA 1013 are extracted with 2 mL 95:5 ethyl alcohol:water. The samples can be derivatized by the following procedure: add 0.5-mL of sample and 0.5-mL of PFBHA extraction solution into a labeled 2-mL vial, and react for 36 hours, and then analyze by GC-ECD following the analytical conditions in this method. Standards prepared by OSHA Method 1013 are derivatized following the same procedure. The RQL will be a factor of 2 higher due to this dilution of the samples.

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[34] Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

[35] Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

3.5 Analysis

3.5.1 Analytical conditions:

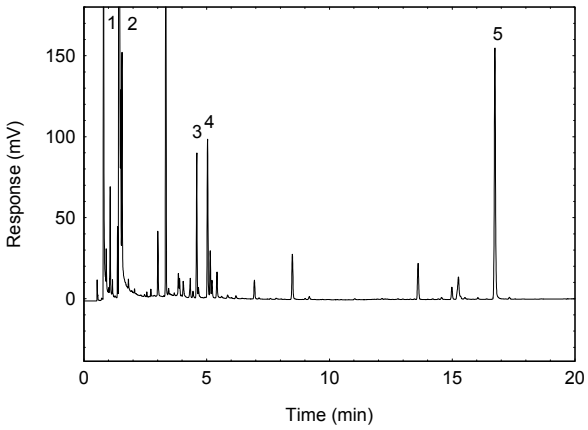

Figure 3.5.1. A chromatogram of the PFBHA derivatives of 1.60 µg/sample acetoin and 1.56 µg/sample diacetyl in the extraction solution. (Key: (1) ethyl alcohol; (2) PFBHA; (3) 4-bromobenzylbromide (ISTD); (4) acetoin-PFBHA; and (5) diacetyl-PFBHA; all other peaks are from PFBHA and its breakdown products)

GC conditions:

column:	initial 100 ºC, hold 1 min, program at 5 ºC/min to 200 ºC, hold 0 min
injector:	250 ºC
detector:	250 ºC
run time:	20 min
column gas flow:	3.0 mL/min (hydrogen)
column mode:	constant pressure
column pressure:	6.8 psi
injection size:	1.0 µL (40:1 split)
column:	30-m × 0.32-mm i.d. capillary column (DB-5 df = 0.25 µm)
retention times:	0.85 min ethyl alcohol 1.44 min PFBHA 4.60 min 4-bromobenzylbromide 5.04 min acetoin-PFBHA 16.75 min diacetyl-PFBHA

ECD conditions:

makeup flow:	40 mL/min (nitrogen)

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

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

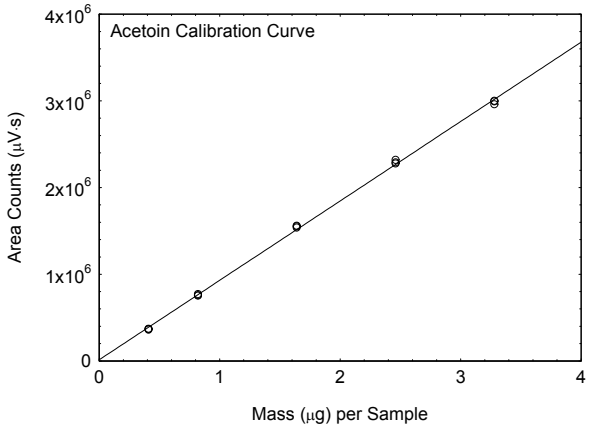

Figure 3.5.2.1. Calibration curve for acetoin. (y = 9.16E5x + 1.44E4)

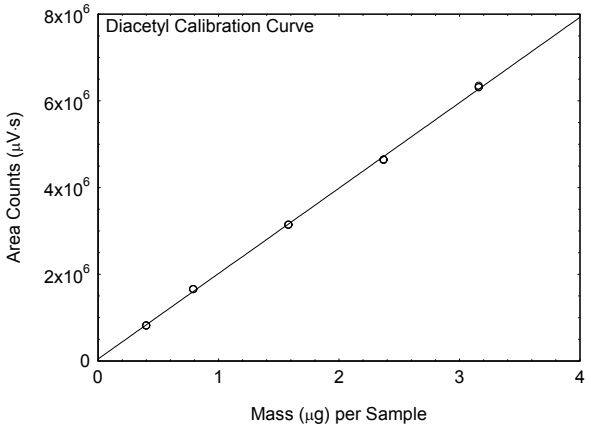

Figure 3.5.2.2. Calibration curve for diacetyl. (y = 1.97E6x + 4.59E4)

3.6 Interferences (analytical)

Any compound that produces a GC-ECD response and has a similar retention time as the analyte 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.

3.7 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms of analyte per sample, uncorrected for extraction efficiency. The front amount found is then corrected by subtracting the total amount (if any) found on the front blank. The back amount found is then corrected by subtracting the total amount (if any) found on the back blank. The amount found on the back dried silica gel tube is added to the front tube for the total loading on each sample. The back-up tube is analyzed separately to determine the extent of analyte saturation to determine if breakthrough occurred. Even though the analytes are analyzed as the PFBHA derivatives and the calibration and results are as the amount of analyte. The air concentration is calculated using the following formulas.

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

where M is total micrograms per sample
M_{front} is micrograms found on front tube
M_{back} is micrograms found on back tube
$M_{\text{front blank}}$ is micrograms found on front blank tube
M_{blank} is micrograms found on back blank tube

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

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

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

where C_V is concentration by volume (ppm)
V_M is 24.46 (molar volume at NTP)
C_M is concentration by weight (mg/m^3)
M_r is molecular weight of analyte
(acetoin = 88.11 and diacetyl = 86.09

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".[36] 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 the mass of analyte introduced onto the chromatographic column. Ten analytical standards were prepared with equally descending increments with the highest standard containing 97.9 ng/mL acetoin, and for diacetyl the highest standard was 95.5 ng/mL.

[36] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. 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/index.html (accessed 3/15/2008).

These are the concentrations that would produce peaks at least 10 times the response of a reagent blank near the elution time of the analyte. These standards, and the reagent blank were analyzed with the recommended analytical parameters (1-µL injection with a 40:1 split), and the data obtained were used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLAP. For acetoin, the slope and standard error of estimate, respectively, were 3818 and 219. For diacetyl, the slope and standard error of estimate, respectively, were 9595 and 366.

Table 4.1.1
Detection Limit of the Analytical Procedure for Acetoin

concentration (ng/mL)	mass on column (pg)	area counts (µV•s)
0	0	0
9.79	0.245	863
19.6	0.490	1679
29.4	0.735	2588
39.2	0.980	3443
49.0	1.23	4167
58.7	1.47	5301
68.5	1.71	6084
78.3	1.96	7465
88.1	2.20	8098
97.9	2.45	9529

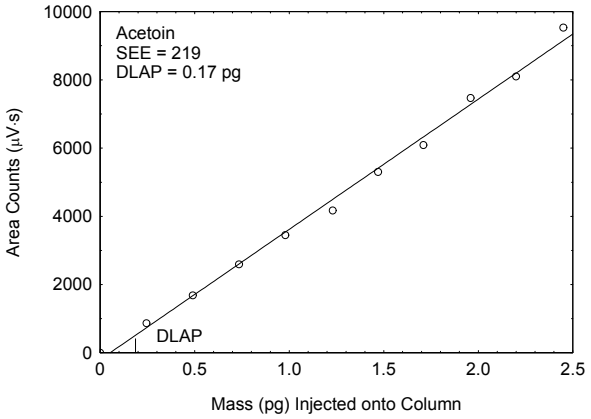

Figure 4.1.1. Plot of data to determine the DLAP for acetoin. (y = 3818x - 202)

Table 4.1.2
Detection Limit of the Analytical Procedure for Diacetyl

concentration (ng/mL)	mass on column (pg)	area counts (µV•s)
0	0	0
9.55	0.238	2824
19.1	0.478	5099
28.7	0.718	7020
38.2	0.955	9587
47.8	1.20	11701
57.3	1.43	13790
66.9	1.67	15745
76.4	1.91	18523
86.0	2.15	20511
95.5	2.39	23882

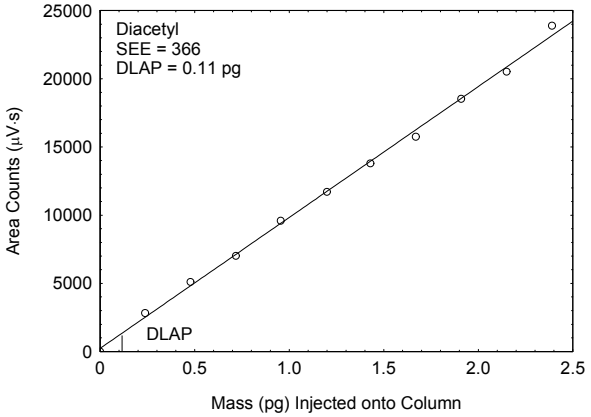

Figure 4.1.2. Plot of data to determine the DLAP for diacetyl. (y = 9595x + 238)

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

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. These spiked samplers and the sample blank were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For acetoin, the slope and standard error of estimate, respectively, were 46.9 and 227. For diacetyl, the slope and

standard error of estimate, respectively, were 121 and 497. For acetoin, the DLOP was 14.5 ng and the RQL was 48.4 ng. For diacetyl, the DLOP was 12.3 ng and the RQL was 41.1 ng.

Table 4.2.1
Detection Limit of the Overall Procedure for Acetoin

mass per sample (ng)	area counts (μV•s)
0	0
19.6	866
39.2	1901
58.7	2927
78.3	3421
97.9	4158
117	5543
137	6002
157	7399
176	8221
196	9373

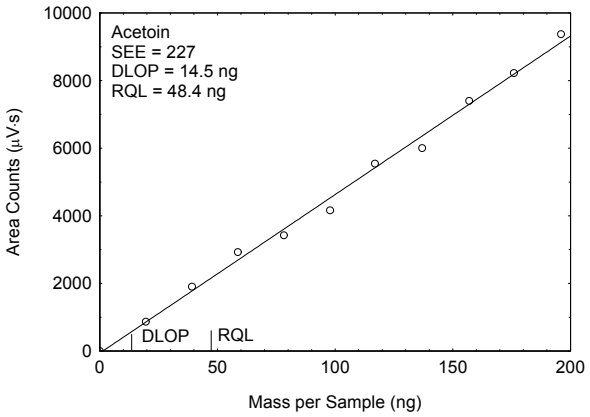

Figure 4.2.1. Plot of data to determine the DLOP/RQL for acetoin. (y = 46.9x - 63.1)

Table 4.2.2
Detection Limit of the Overall Procedure for Diacetyl

mass per sample (ng)	area counts (μV•s)
0.0	0
19.1	2758
38.2	5554
57.4	7690
76.4	10101
95.5	11743
115	13988
134	15701
153	18651
172	21621
191	23995

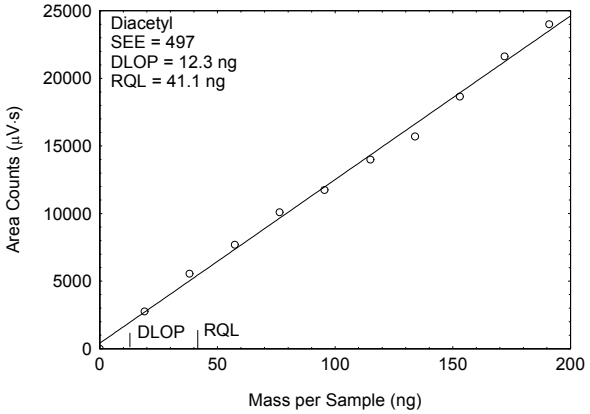

Figure 4.2.2. Plot of data to determine the DLOP/RQL for diacetyl. (y = 121x + 407)

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 RQLs are listed in Table 4.2.3.

Table 4.2.3
Reliable Quantitation Limits

analyte	ng	ppb	μg/m^3	E_E
acetoin	48.4	1.49	5.37	102.3
diacetyl	41.1	1.30	4.57	97.3

E_E = extraction efficiency

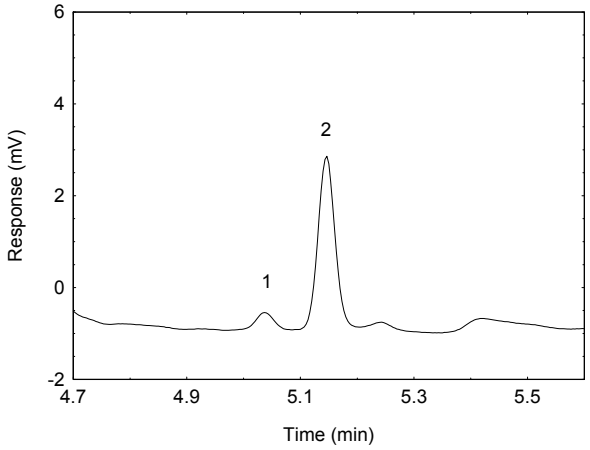

Figure 4.2.3. A chromatogram of the RQL of acetoin. (Key: (1) acetoin-PFBHA, (2) interference)

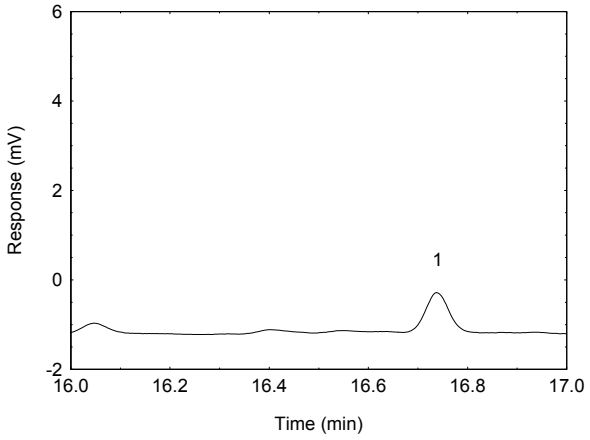

Figure 4.2.4. A chromatogram of the RQL of diacetyl. (Key: (1) diacetyl-PFHBA)

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 0.25 to 2 times the TWA target concentration. Calibration curves were constructed and shown in Section 3.5.2 from the three injections each of five standards. The standard errors of estimates were 0.019 µg for acetoin and 0.052 µg for diacetyl.

Table 4.3.1
Instrument Calibration for Acetoin

standard concn (µg/sample)	area counts (µV·s)		
0.41	367186	360667	370276
0.82	759141	752935	771533
1.64	1550965	1559979	1538639
2.46	2318162	2277568	2290341
3.28	2993893	2999180	2959244

Table 4.3.2
Instrument Calibration for Diacetyl

standard concn (µg/sample)	area counts (µV·s)		
0.40	818644	817236	817895
0.79	1658619	1654024	1658622
1.58	3140780	3142807	3140857
2.37	4604360	4645231	4644018
3.16	6349382	6315236	6309791

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. The precisions of the overall procedure were obtained from the ambient temperature 18 day storage tests were ±9.9% for acetoin and ±10.0% for diacetyl.

4.5 Storage test

Storage samples for acetoin and diacetyl were prepared using dried silica gel tubes from controlled test atmospheres using the recommended sampling conditions. The concentrations were 0.051 ppm (0.184 mg/m^3) acetoin and 0.050 ppm (0.180 mg/m^3) diacetyl at an average relative humidity of 80% at 23 ºC. 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 ºC) and the other fifteen were stored in a closed drawer at ambient temperature (about 23 ºC). At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. Recoveries are not corrected for extraction efficiency.

Table 4.5.1
Storage Test for Acetoin at 80% RH

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.4	98.5	101.1			
4	99.1	100.3	98.9	100.1	100.4	98.6
7	99.5	99.1	98.6	98.9	99.7	100.8
10	100.5	98.8	99.4	98.5	100.1	99.9
14	97.9	99.3	98.3	99.9	99.3	98.6
18	98.5	99.3	97.6	99.8	98.3	99.1

Table 4.5.2
Storage Test for Diacetyl at 80% RH

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.2	100.4	98.2			
4	99.3	100.1	98.1	99.4	100.1	97.3
7	99.8	98.7	97.2	100.3	99.3	97.1
10	97.3	99.8	98.9	97.5	100.0	99.8
14	99.7	99.1	97.6	99.7	98.9	96.6
18	98.7	97.7	96.8	98.6	97.7	96.5

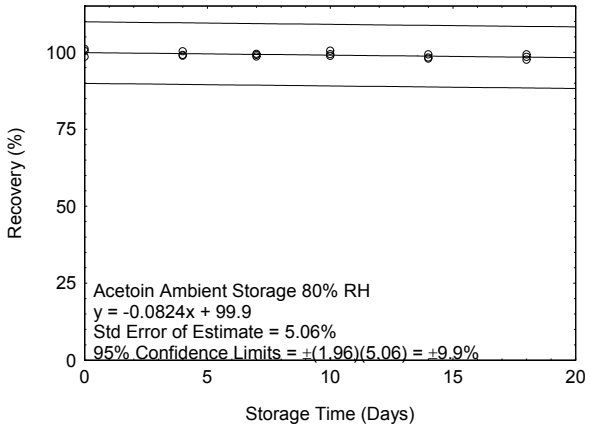

Figure 4.5.1. Ambient storage test for acetoin at 80% RH.

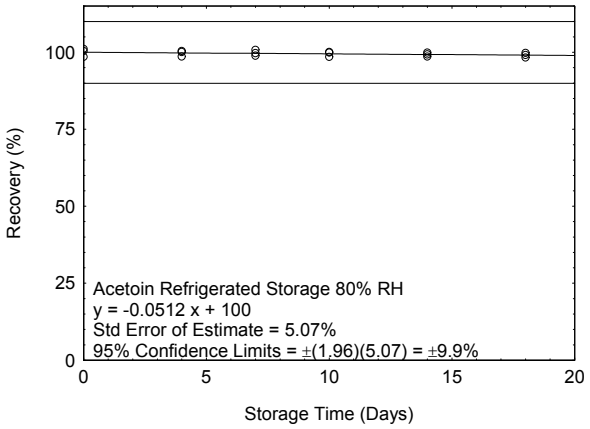

Figure 4.5.2. Refrigerated storage test for acetoin at 80% RH.

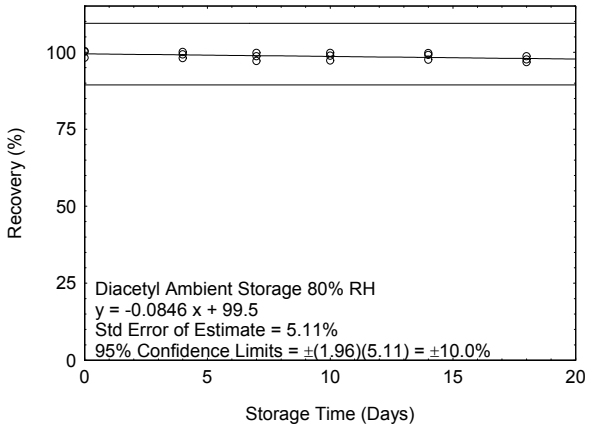

Figure 4.5.3. Ambient storage test for diacetyl at 80% RH.

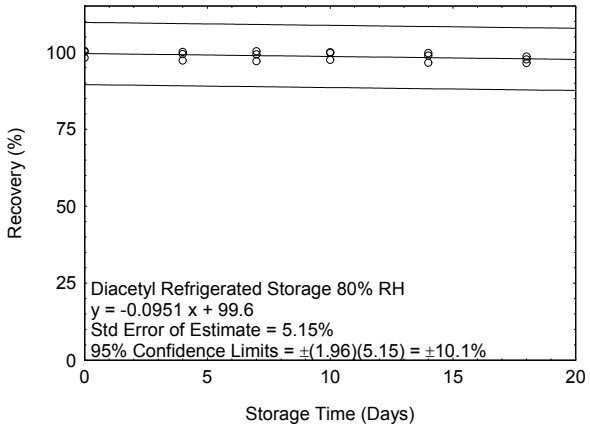

Figure 4.5.4. Refrigerated storage test for diacetyl at 80% RH.

Storage studies were also performed using tubes packed with 400/200 mg sections of dried silica gel, at an average relative humidity of 22% RH at 23 °C to determine the effects of low humidity on storage and on migration. The concentrations were 0.051 ppm (0.184 mg/m^3) acetoin and 0.050 ppm (0.180 mg/m^3) diacetyl. Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. 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 22% RH ambient and refrigerated storage samples showed no migration for acetoin or diacetyl. Recoveries are not corrected for extraction efficiency.

Table 4.5.3
Storage Test for Acetoin at 22% RH

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.2	99.8	97.9			
4	99.9	97.4	98.4	100.1	97.4	99.6
7	98.2	100.5	96.9	99.7	98.8	97.5
10	99.9	97.7	97.1	99.4	97.7	100.3
14	98.9	99.4	96.8	98.2	99.9	96.9
17	99.2	97.3	95.7	96.2	98.7	99.3

Table 4.5.4
Storage Test for Diacetyl at 22% RH

time (days)	ambient storage recovery (%)			refrigerated storage recovery (%)		
0	100.4	97.1	98.5			
4	99.9	98.2	97.0	99.5	100.1	97.3
7	99.6	98.8	97.1	99.9	98.7	97.4
10	99.9	98.1	96.9	99.8	98.9	97.0
14	99.7	96.5	98.4	99.5	98.0	96.8
17	99.0	98.0	95.7	98.1	99.3	96.3

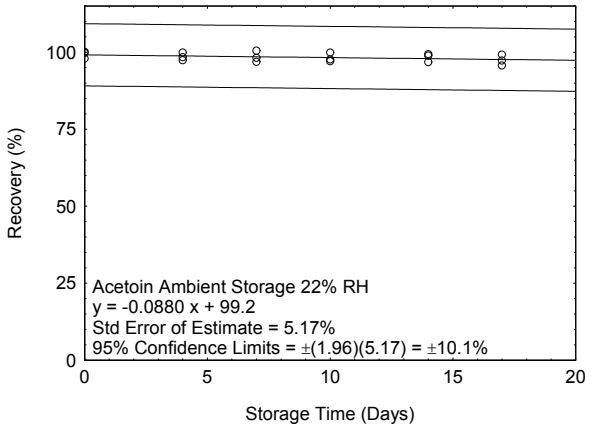

Figure 4.5.5. Ambient storage test for acetoin at 22% RH.

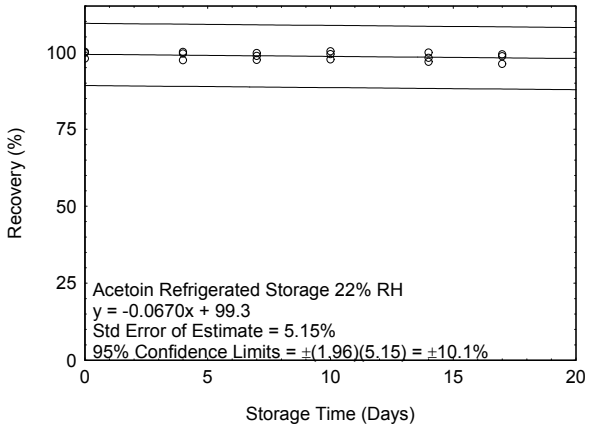

Figure 4.5.6. Refrigerated storage test for acetoin at 22% RH.

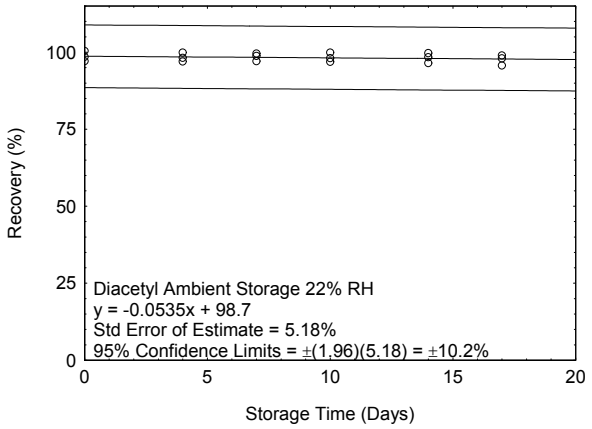

Figure 4.5.7. Ambient storage test for diacetyl at 22% RH.

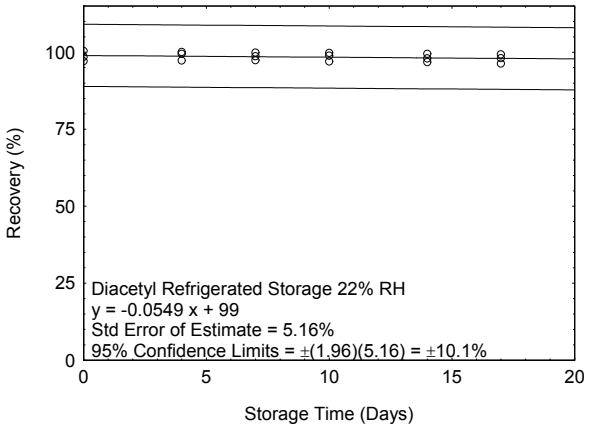

Figure 4.5.8. Refrigerated storage test for diacetyl at 22% RH.

At the beginning of this method, the SKC 226-183 tubes were available as a 400/200 mg tube. Migration studies showed that it would be necessary to use two tubes in series, so subsequent tubes were packed as a single 600 mg tube. A 600 mg section makes it easier for the analyst to prepare the samples for extraction. Migration occurs when the analyte equilibrates between the two sections of the tube after collection. There is more migration with higher humidities, due to the higher amounts of water collected. Using 400/200 mg dried silica gel tubes, at 80% RH acetoin showed no migration but the diacetyl refrigerated samples at day 18 showed a 4.5% migration and ambient showed 15.2% migration. Based on these results, a single 400/200 mg dried silica gel tube should not be used for sampling.

Table 4.5.5
Migration of Diacetyl on 400/200 mg Dried Silica Gel Tube
Sampled at 0.05 L/min for 180 min from 0.05 ppm Atmosphere

day	ambient		refrigerated	
	400 mg % of total found	200 mg % of total found	400 mg % of total found	200 mg % of total found
4	96.1	3.2	99.4	0.0
	96.0	4.1	100.1	0.0
	94.4	3.7	97.3	0.0
7	93.4	5.4	100.3	0.0
	92.9	5.8	99.3	0.0
	91.5	5.7	97.1	0.0
10	89.1	8.2	97.5	0.0
	91.3	8.5	100.0	0.0
	90.9	8.0	99.8	0.0
14	88.0	11.7	97.9	1.8
	87.8	11.3	97.3	1.6
	86.0	11.6	95.7	0.9
18	81.2	17.5	86.9	4.2
	82.7	15.0	85.5	4.5
	83.7	13.1	83.2	4.8

4.6 Reproducibility

Six samples were prepared from a controlled test atmosphere at the target concentration at an average relative humidity of 78% at 23 °C. 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 at 4 °C for 20 days and at -12 °C for an additional 19 days. Sample results were corrected for extraction efficiency. No sample result for acetoin or 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 (%)
1.62	1.59	98.1	-1.9
1.65	1.53	92.7	-7.3
1.67	1.54	92.2	-7.8
1.66	1.56	94.0	-6.0
1.69	1.64	97.0	-3.0
1.64	1.51	92.1	-7.9

Table 4.6.2
Reproducibility Data for Diacetyl

theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)
1.62	1.53	94.4	-5.6
1.64	1.48	90.2	-9.8
1.60	1.49	92.5	-7.5
1.61	1.50	93.2	-6.8
1.66	1.53	92.2	-7.8
1.62	1.50	92.6	-7.4

Samples that are prepared and analyzed by OSHA Method 1013[37] can be derivatized and re-analyzed by this method to detect lower levels. The following samples were prepared from a controlled test atmosphere at 0.51 ppm (0.184 mg/m^3) acetoin and 0.50 ppm (0.180 mg/m^3) diacetyl at 74% RH and 24 °C. They were submitted for analysis by OSHA Method 1013 and then reanalysis by OSHA Method 1012. The average acetoin recovery of samples analyzed by OSHA Method 1013 was 99.3% and by OSHA Method 1012 was 97.1%. The average diacetyl recovery of samples analyzed by OSHA Method 1013 was 98.9% and by OSHA Method 1012 was 96.6%.

[37] Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

Table 4.6.3
Samples for Acetoin Analyzed by OSHA Method 1013 and Then by OSHA Method 1012

	OSHA Method 1013 GC-FID			OSHA Method 1012 GC-ECD		
theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)	recovered (µg/sample)	recovery (%)	deviation (%)
16.5	16.4	99.4	-0.6	16.2	98.2	-1.8
16.4	16.2	98.8	-1.2	16.0	97.6	-2.4
16.6	16.3	98.2	-1.8	16.1	97.0	-3.0
15.9	16.1	101.3	+1.3	15.6	98.1	-1.9
16.5	16.1	97.6	-2.4	15.8	95.8	-4.2
16.3	16.4	100.6	+0.6	15.6	95.7	-4.3

Table 4.6.4
Samples for Diacetyl Analyzed by OSHA Method 1013 and Then by OSHA Method 1012

	OSHA Method 1013 GC-FID			OSHA Method 1012 GC-ECD		
theoretical (µg/sample)	recovered (µg/sample)	recovery (%)	deviation (%)	recovered (µg/sample)	recovery (%)	deviation (%)
16.0	15.9	99.4	-0.6	15.6	97.5	-1.8
15.7	15.4	98.1	-1.9	15.1	96.2	-2.4
15.8	15.5	98.1	-1.9	15.1	95.6	-3.0
15.6	15.8	101.3	+1.3	15.2	97.4	-1.9
15.7	15.2	96.8	-3.2	15.0	95.5	-4.2
15.9	15.8	99.4	-0.6	15.5	97.5	-4.3

4.7 Sampler capacity

The sampling capacity of the front tube of two dried silica gel tubes in series was tested by sampling from a dynamically generated test atmosphere with an average relative humidity of 81% at 23ºC at concentrations of 0.101 ppm (0.365 mg/m^3) acetoin, and 0.101 ppm (0.355 mg/m^3) diacetyl. The second tube in the sampling train was changed at 1 h intervals for the first 3 hours then at 0.5 hour intervals for the rest of the sampling. The dried silica gel tube sampling trains were used to sample at approximately 0.05 L/min (each air volume listed below uses that specific tube's flow rate). The presence of analyte on the second tube was defined as breakthrough. The percentage of the amount found on the second tube of the total concentration is the % breakthrough. The % breakthrough was plotted versus the air volume sampled to determine the 5% breakthrough air volumes. The 5% breakthrough air volume for diacetyl was 12.1 L. The recommended air volume is 80% of the breakthrough air volume which is 9.68 L. Acetoin had no breakthrough after samples were collected for up to 8 hours.

Table 4.7.1
Capacity Test for Diacetyl on Dried Silica Gel Tubes at 0.101 ppm

sampling train 1		sampling train 2		sampling train 3	
air volume	% BT	air volume	% BT	air volume	% BT
2.71	0.0	2.80	0.0	2.78	0.0
5.51	0.0	5.69	0.0	5.67	0.0
8.36	0.0	8.64	0.0	8.60	0.0
9.69	0.0	10.0	0.0	9.97	0.0
12.0	5.2	12.6	27.8	12.5	20.3

%BT = % breakthrough

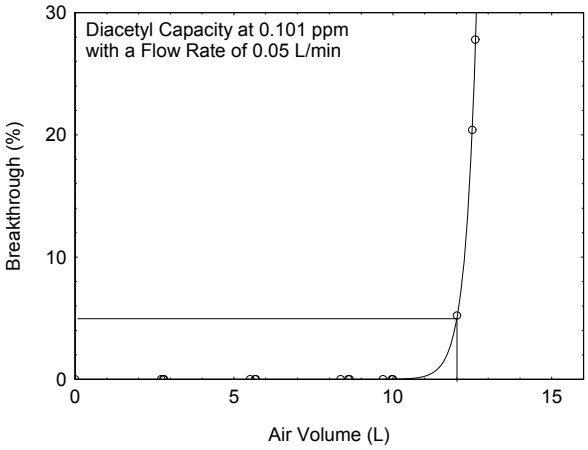

Figure 4.7.1. Five percent breakthrough test for diacetyl from a 0.101 ppm atmosphere, with a flow rate of 0.05 L/min.

A capability of collection at higher flow rates with a 15 minute short term sample was tested for breakthrough. A test atmosphere was dynamically generated with an average relative humidity of 79% at 23 ºC at concentrations of 0.101 ppm (0.365 mg/m^3) acetoin and 0.101 ppm (0.355 mg/m^3) diacetyl. A sampling train consisting of two dried silica gel tubes (400/200 mg) in series was used to test the capacity. Three sampling trains at each flow rate of 0.1 L/min or 0.2 L/min were tested. There was no acetoin or diacetyl on the second tube of any of the sampling trains. Since the short term sampling may be a time of higher exposure, two higher concentrations were also tested. The first was 0.541 ppm (1.95 mg/m^3) acetoin and 0.506 ppm (1.78 mg/m^3) diacetyl and a relative humidity of 79% at 23 ºC. The second was 23.2 ppm (83.5 mg/m^3) acetoin and 22.4 ppm (78.8 mg/m^3) diacetyl at an average relative humidity of 79% at 23 ºC. In all of these tests there was no acetoin or diacetyl on the back-up tube of the sampling train.

Table 4.7.2
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 0.101 ppm Acetoin and 0.101 ppm Diacetyl

	acetoin		diacetyl	
flow rate (L/min)	front tube (%)	back tube (%)	front tube (%)	back tube (%)
0.1	98.6	0.0	99.4	0.0
0.1	99.4	0.0	98.7	0.0
0.1	99.9	0.0	99.1	0.0
0.2	99.2	0.0	99.5	0.0
0.2	98.5	0.0	98.4	0.0
0.2	97.7	0.0	99.8	0.0

Table 4.7.3
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 0.541 ppm Acetoin and 0.506 ppm Diacetyl

	acetoin		diacetyl	
flow rate (L/min)	front tube (%)	back tube (%)	front tube (%)	back tube (%)
0.1	99.7	0.0	99.9	0.0
0.1	99.0	0.0	98.4	0.0
0.1	98.8	0.0	97.9	0.0
0.2	99.3	0.0	99.4	0.0
0.2	97.9	0.0	98.9	0.0
0.2	99.5	0.0	99.0	0.0

Table 4.7.4
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 23.2 ppm Acetoin and 22.4 ppm Diacetyl

	acetoin		diacetyl	
flow rate (L/min)	front tube (%)	back tube (%)	front tube (%)	back tube (%)
0.1	98.6	0.0	99.6	0.0
0.1	99.4	0.0	98.7	0.0
0.1	99.0	0.0	97.3	0.0
0.2	99.9	0.0	99.6	0.0
0.2	97.5	0.0	99.0	0.0
0.2	98.1	0.0	97.8	0.0

A capacity test at 0.2 L/min was performed at two test air concentrations, 0.101 ppm (0.365 mg/m^3) acetoin and 0.101 ppm (0.355 mg/m^3) diacetyl at an average relative humidity of 78% air at 22 °C; and 23.2 ppm (83.5 mg/m^3) acetoin and 22.4 ppm (78.8 mg/m^3) diacetyl at relative humidity of 77% at 22 ºC. There was no acetoin on the back-up tube after 13.9 L was sampled. The 5% breakthrough air volume for diacetyl with 0.101 ppm atmosphere was 11.98 L, and with a 22.4 ppm atmosphere was 11.64 L.

Table 4.7.5
Capacity Test for Diacetyl on Dried Silica Gel Tubes at a Flow Rate of 0.2 L/min and 0.101 ppm

sampling train 1		sampling train 2		sampling train 3	
air volume	% BT	air volume	% BT	air volume	% BT
5.98	0.0	5.95	0.0	6.03	0.0
7.97	0.0	7.94	0.0	8.04	0.0
9.97	0.0	9.92	0.0	10.05	0.0
10.96	0.7	10.91	0.0	11.06	1.4
11.96	5.4	11.90	3.4	12.06	8.8
12.95	26.4	12.90	22.7	13.07	35.1

%BT = % breakthrough

Table 4.7.6
Capacity Test for Diacetyl on Dried Silica Gel Tubes
at a Flow Rate of 0.2 L/min and 22.4 ppm

sampling train 1		sampling train 2		sampling train 3	
air volume	% BT	air volume	% BT	air volume	% BT
6.15	0.0	5.94	0.0	6.06	0.0
8.20	0.0	7.92	0.0	8.08	0.0
10.25	0.0	9.90	0.0	10.10	0.0
11.28	2.1	10.89	0.6	11.11	1.2
12.30	17.2	11.88	5.1	12.12	10.5
13.33	48.5	12.87	24.1	13.13	40.5

%BT = % breakthrough

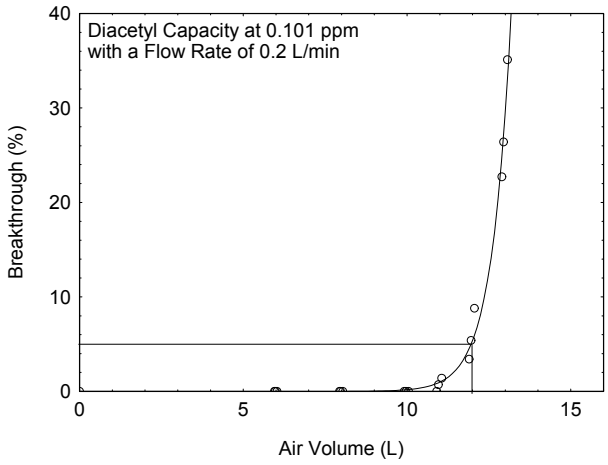

Figure 4.7.2. Five percent breakthrough test for diacetyl from a 0.101 ppm atmosphere, with a flow rate of 0.2 L/min.

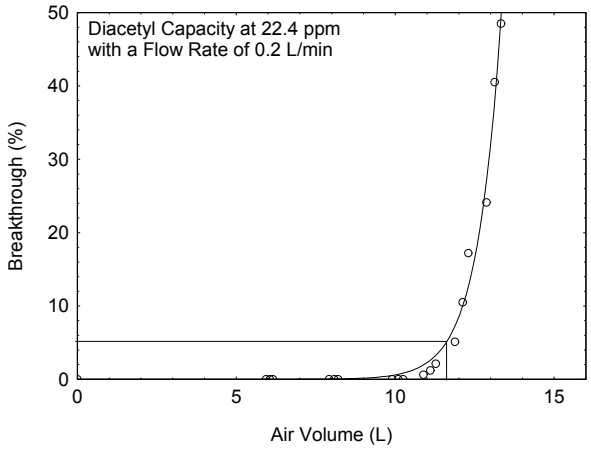

Figure 4.7.3. Five percent breakthrough test for diacetyl from a 22.4 ppm atmosphere, with a flow rate of 0.2 L/min.

4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is dependent on the extraction solvent as well as the internal standard. The extraction solvent used for this evaluation consisted of 95:5 ethyl alcohol:water with 2 mg/mL PFBHA and 20 µg/mL 4-bromobenzyl bromide. 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 efficiencies of acetoin and diacetyl were determined by liquid-spiking four dried silica gel tubes, at each concentration level, with the analyte from the RQL to 2 times the target concentration. These samples were stored overnight at ambient temperature and then analyzed. The samples need to be extracted on a rotator for 1 hour, and then allowed to set at room temperature for 36 hours. Do not use a shaker as recoveries will be much lower (Table 4.8.3). The mean extraction efficiency over the working range from the RQL to 2 times the target concentration is 102.0% for acetoin and 97.6% for diacetyl. The extraction efficiency for the wet samplers and samplers extracted on the shaker were not included in the overall mean because it would bias the results. The test of wet samplers was performed to determine if the amount of water that would collect under high humidity conditions at the recommended air volume would affect the extraction efficiency. Wet samplers were prepared by sampling humid

air having an average relative humidity of about 80% at 23 ºC for 180 minutes at 0.05 L/min and then liquid-spiking the sampler with the analyte. The dried silica gel tube (600 mg) collects 140 mg water at 78% RH and 23 °C when sampled for 9 L.

Table 4.8.1
Extraction Efficiency (%) of Acetoin

level		sample number				mean
× target concn	µg per sample	1	2	3	4	
RQL	0.022	104.2	102.1	101.2	101.6	102.3
0.25	0.41	103.7	102.3	102.1	100.8	102.2
0.5	0.82	100.7	102.4	101.1	100.9	101.3
1.0	1.64	102.3	100.5	103.3	103.5	102.4
1.5	2.46	102.6	103.1	100.6	100.8	101.8
2.0	3.28	103.0	103.3	101.6	100.4	102.1
1.0 (wet)	1.64	101.1	102.9	103.1	102.2	102.3

Table 4.8.2
Extraction Efficiency (%) of Diacetyl

level		sample number				mean
× target concn	µg per sample	1	2	3	4	
RQL	0.02	96.7	95.7	97.8	98.9	97.3
0.25	0.40	97.5	98.0	99.1	98.5	98.3
0.5	0.79	98.5	96.8	99.4	98.0	98.2
1.0	1.58	96.9	95.3	96.4	95.4	96.0
1.5	2.37	99.9	95.9	96.5	97.8	97.5
2.0	3.16	97.1	99.6	99.9	97.5	98.5
1.0 (wet)	1.58	98.1	96.6	95.8	97.1	96.9

Table 4.8.3
Extraction Efficiency (%) of Acetoin and Diacetyl at 1.0 x Target Concentration Using a Shaker

		sample number				
analyte	µg per sample	1	2	3	4	mean
acetoin	1.64	87.5	88.8	90.1	87.7	88.5
diacetyl	1.58	82.6	81.9	85.5	84.3	83.6

Stability of extracted samples

The stability of extracted samples was investigated by reanalyzing the target concentration samples 24 h after initial analysis. After the original analysis was performed, two autosampler vials were recapped with new septa while the remaining two retained their punctured septa. The samples were reanalyzed with fresh standards. The average percent change was +0.7% for acetoin and +1.6% for diacetyl when samples were resealed with new septa and -1.1% for acetoin and +0.3% for diacetyl when samples retained their punctured septa. Each septum was punctured 5 times for each analysis. The test was performed at room temperature.

Table 4.8.3
Stability of Extracted Samples for Acetoin

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
102.3	101.5	-0.8	103.3	101.9	-1.4
100.5	102.7	+2.2	103.5	102.7	-0.8
	(mean)			(mean)	
101.4	102.1	+0.7	103.4	102.3	-1.1

Table 4.8.4
Stability of Extracted Samples for Diacetyl

punctured septa replaced			punctured septa retained		
initial (%)	after one day (%)	difference (%)	initial (%)	after one day (%)	difference (%)
96.9	98.3	+1.4	96.4	95.1	-1.3
95.3	97.1	+1.8	95.4	97.3	+1.9
	(mean)			(mean)	
96.1	97.7	+1.6	95.9	96.2	+0.3

4.9 Interferences (sampling)

Retention

The ability of a dried silica gel tube to retain the analytes after they have been collected was tested by using a test atmosphere having an average relative humidity of 80% at 23 °C. The test atmosphere was dynamically generated at 0.101 ppm (0.364 mg/m^3) acetoin, and 0.102 ppm (0.359 mg/m^3) diacetyl. Six samplers had contaminated air drawn through them at 0.05 L/min for 45 min. Sampling was discontinued and three samples set aside. 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 recoveries for the samples in the second set divided by the first set were: 96.7% for acetoin, and 96.9% for diacetyl.

Table 4.9.1
Retention of Acetoin

	percent recovery			
set	1	2	3	mean
first	99.5	100.4	98.9	99.6
second	95.0	96.8	97.0	96.3
second/first				96.7

Table 4.9.2
Retention of Diacetyl

	percent recovery			
set	1	2	3	mean
first	100.2	99.9	98.1	99.4
second	96.3	97.4	95.3	96.3
second/first				96.9

Low humidity

The ability of dried silica gel tubes to collect the analytes from a relatively dry atmosphere was tested by using a test atmosphere having an average relative humidity of 20% at 23 °C. The test atmosphere was dynamically generated at 0.101 ppm (0.364 mg/m^3) acetoin and 0.102 ppm (0.359 mg/m^3) diacetyl. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The recoveries (% of theoretical) for acetoin were: 97.0%, 101.4%, and 97.8%; and for diacetyl were: 98.3%, 96.8%, and 100.3%.

Low concentration

The ability of dried silica gel tubes to collect the analytes from a low concentration atmosphere was tested by using a test atmosphere at 0.1 times the target concentration having an average relative humidity of 80% at 23 °C. The test atmosphere was dynamically generated at 0.0051 ppm (0.0184 mg/m^3) acetoin and 0.0051 ppm (0.0180 mg/m^3) diacetyl. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The recoveries (% of theoretical) for acetoin were: 99.8%, 99.9%, and 97.2%, and for diacetyl were: 97.3%, 98.1%, and 99.8%.

Sampling interference

The ability of dried silica gel tubes to collect the analytes from an atmosphere containing interferences was tested under two different sets of conditions. The first set of conditions was a test atmosphere of 0.051 ppm (0.0184 mg/m^3) acetoin and 0.051 ppm (0.0180 mg/m^3) diacetyl and an interference mixture of 1.01 ppm (1.82 mg/m^3) acetaldehyde, 1.05 ppm (2.58 mg/m^3) acetic acid, and 1.02 ppm (3.01 mg/m^3) methyl ethyl ketone at an average humidity of 80% at 23 °C. These lower concentrations were chosen for two reasons: they are similar to some of the concentrations found in plants manufacturing microwave popcorn, and all of these compounds will be derivatized by the PFBHA; therefore, there would be enough PFBHA in solution to derivatize all of the analytes that were collected (8.01 µmole/mL PFBHA). The recoveries (% of theoretical) of acetoin and diacetyl were: 95.4%, 98.5%, and 99.7% for acetoin and 95.8%, 98.9%, and 99.8% for diacetyl. There was no analyte on the backup tube of the two dried silica gel tubes in series for any of the tests.

The second series of tests was with acetoin and diacetyl at the target concentration and each of the interferences listed above individually at their PEL concentration following the guidelines in SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[38]. The concentrations of these interferences are much higher than would normally be expected in a food or flavoring manufacturing workplace. These three compounds were chosen as interferences because they collect on the dried silica gel tubes and react with the PFBHA. The extraction solution needed to be modified to 18 mg/mL PFBHA (72.1 µmoles/mL) to insure that there was enough PFBHA in solution to derivatize all the analytes. These three atmospheres each contained acetoin and diacetyl with one of the following concentrations of the interference mixture in it: 194 ppm (350 mg/m^3) acetaldehyde, 9.49 ppm (23.3 mg/m^3) acetic acid, or 190 ppm (560 mg/m^3) methyl ethyl ketone. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min for each test. All of the samples were immediately analyzed. The recoveries (% of theoretical) of acetoin and diacetyl with 190 ppm acetaldehyde were: 99.8%, 95.9%, and 97.7% for acetoin and 97.2%, 93.5%, and 95.7% for diacetyl. The recoveries (% of theoretical) of acetoin and diacetyl with 9.49 ppm acetic acid were: 95.3%, 97.7%, and 98.9% for acetoin and 95.5%, 99.3%, and 99.8% for diacetyl. The recoveries (% of theoretical) of acetoin and diacetyl with 190 ppm methyl ethyl ketone were: 96.7%, 98.7%, and 99.9% for acetoin and 95.8%, 97.8%, and 99.3% for diacetyl. There was no analyte found on the backup tube of the two dried silica gel tubes in series for any of the tests. These interferences were not a sampling interference, but under normal sample analysis, these levels of interferences would be an analytical interference.

Light

Diacetyl and acetoin are light-sensitive.[39,40,41,42] The interference of light during sampling was tested using three foil-wrapped sampling trains and three uncovered sampling trains. An

[38] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. 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/index.html (accessed 3/15/2008).

[39] Material Safety Data Sheet: Acetoin, http://www.thegoodscentscompany.com/msds/md102388.html (accessed 3/17/2008).

atmosphere containing twice the target concentration at an average humidity of 78% at 23 °C was sampled for 180 min at 0.05 L/min, and the samples were extracted that day.

Table 4.9.3
Light Interference During Sampling

	acetoin		diacetyl	
tube #	foil wrapped recovery (%)	uncovered recovery (%)	foil wrapped recovery (%)	uncovered recovery (%)
1	98.9	93.7	97.8	93.3
2	97.0	92.6	98.9	94.6
3	99.5	95.4	99.9	95.0
mean	98.5	93.9	98.9	94.3

An additional three sampling trains were collected at the same time, and were protected from the light by aluminum foil. After collection, these samplers had the foil removed and were placed on the counter at ambient temperature under room light. These samples were analyzed 24 h after sampling during which they were exposed to the room light for 14 of the 24 h, and the recoveries were 80.7%, 84.7%, and 78.5% for acetoin and 79.3%, 82.4%, and 78.4% for diacetyl.

Powder form

The powder form of acetoin and diacetyl tested consisted of starch coated with acetoin and diacetyl. Three tests were performed on this powder. The first consisted of a sampling train of a pre-weighed (tared) PVC filter in a conical cassette in series with two dried silica gel tubes. Two dried silica gel tubes were used to collect any vapors of acetoin and diacetyl which would be stripped off of the powder. Known amounts of the powder were placed onto the PVC filter, and 9 L of air at an average relative humidity of 78% RH and 22 °C were pulled through the sampling trains at 0.05 L/min. The recovery of acetoin and diacetyl on the pre-weighed PVC filters was 0% to 1.9% for acetoin and 0% to 2.3% for diacetyl, with larger amounts found on the PVC filters that were spiked with larger amounts of powder. Most of the acetoin and diacetyl was stripped from the starch and collected on the dried silica gel tubes. The average recovery found on the dried silica gel tubes was 96.6% for acetoin and 97.8% for diacetyl (Table 4.9.4). The acetoin and diacetyl theoretical weights were calculated from the percentages obtained from analysis of the powder and the amounts of the powder weighed out.

The second and third tests consisted of a sampling train of two dried silica gel tubes in series, with the powder spiked on the front glass wool of the front tube. The two tests had 9 L air drawn through the sampling trains at 0.05 L/min, the first test used air at an average relative humidity of 20% at 22 °C, and the other test used air at an average relative humidity of 78% at 22 °C. At 20% RH most of the acetoin and diacetyl were found on the front glass wool and glass fiber filter, but at 78% RH most of the acetoin and diacetyl were found on the dried silica gel beds. The sampling trains with 78% RH air drawn through them had the highest amounts of acetoin and diacetyl on the glass wool and filter on the tube spiked with the highest amount of powder, which may be due to the size of the clump of powder weighed out (Table 4.9.5 and 4.9.6).

[40] Material Safety Data Sheet: Diacetyl, Chemwatch, Victoria, Australia (accesed 3/17/2008).

[41] Material Safety Data Sheet: 2,3-Butanedione, https://fscimage.fishersci.com/msds/03275.htm (accessed 3/17/2008).

[42] Material Safety Data Sheet: 2,3-Butanedione, http://www.chemservice.com/msds/msds_detail.asp?catnum=O-816 (accessed 3/17/2008).

Table 4.9.4
% Recovery of Acetoin and Diacetyl from Powder on Tared PVC Filters in a Conical Cassette in Series with Dried Silica Gel Tubes with 78% RH Air Sampled

			acetoin					diacetyl				
amount of powder (µg)	powder weight found (µg)	theoretical weight (µg)	PVC filter (µg)	front tube (µg)	back tube (µg)	silica gel recovery (%)	theoretical weight (µg)	PVC filter (µg)	front tube (µg)	back tube (µg)	silica gel recovery (%)
1130	1082	18.1	0.0	18.0	0.0	99.4	29.4	0.0	28.0	0.0	95.2
2110	2021	33.8	0.6	32.1	0.0	95.0	54.9	1.0	53.1	0.0	96.7
2960	2856	47.4	0.9	46.3	0.0	97.7	77.0	1.8	75.9	0.0	98.6
2940	2809	47.0	0.3	45.0	0.0	95.7	76.4	0.8	75.7	0.0	99.1
1310	1265	21.0	0.2	20.5	0.0	97.6	34.1	0.6	34.0	0.0	99.7
1010	964	16.2	0.0	15.3	0.0	94.4	26.3	0.0	25.6	0.0	97.3

Table 4.9.5
% Recovery of Acetoin and Diacetyl from Powder Spiked on Dried Silica Gel Tubes with 20% RH Air Sampled

		acetoin					diacetyl					
amount of powder (µg)	theoretical weight (µg)	front glass wool and filter (µg)	front glass wool and filter recovery (%)	front tube (µg)	back tube (µg)	silica gel recovery (%)	theoretical weight (µg)	front glass wool and filter (µg)	front glass wool and filter recovery (%)	front tube (µg)	back tube (µg)	silica gel recovery (%)
1080	17.3	16.7	96.5	0.0	0.0	0.0	28.1	26.3	93.6	1.1	0.0	3.9
1240	19.8	19.5	98.5	0.0	0.0	0.0	32.2	30.1	93.5	1.5	0.0	4.7
1750	28.0	27.4	97.9	0.0	0.0	0.0	45.5	42.8	94.1	1.8	0.0	4.0
2080	33.3	32.1	96.4	0.0	0.0	0.0	54.1	50.2	92.8	2.3	0.0	4.3
2240	35.8	34.5	96.4	0.5	0.0	1.4	58.2	53.4	91.8	2.8	0.0	4.8
2380	38.1	36.7	96.3	0.7	0.0	1.8	61.9	55.8	90.1	3.6	0.0	5.8

Table 4.9.6
% Recovery of Acetoin and Diacetyl from Powder Spiked on Dried Silica Gel Tubes with 78% RH Air Sampled

		acetoin					diacetyl					
amount of powder (µg)	theoretical weight (µg)	front glass wool and filter (µg)	front glass wool and filter recovery (%)	front tube (µg)	back tube (µg)	silica gel recovery (%)	theoretical weight (µg)	front glass wool and filter (µg)	front glass wool and filter recovery (%)	front tube (µg)	back tube (µg)	silica gel recovery (%)
1220	19.5	0.0	0.0	19.1	0.0	97.9	31.7	0.0	0.0	30.9	0.0	97.5
1760	28.2	0.0	0.0	26.9	0.0	95.4	45.8	0.0	0.0	44.2	0.0	96.5
1070	17.1	0.0	0.0	16.9	0.0	98.8	27.8	0.0	0.0	27.5	0.0	98.9
1590	25.4	0.0	0.0	24.9	0.0	98.0	41.3	0.0	0.0	40.9	0.0	99.0
2030	32.5	0.0	0.0	32.4	0.0	99.7	52.8	0.0	0.0	52.5	0.0	99.4
5020	80.3	0.7	0.9	79.4	0.0	98.9	130.5	2.2	1.7	129.9	0.0	99.5

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 spectra of the acetoin-PFBHA and diacetyl-PFBHA derivative were determined by analyzing an analytical standard on an Agilent 6890 with a 5973 mass selective detector using a 30-m × 0.25-mm i.d. fused silica capillary column (DB-1-MS 0.25-µm df) capillary column at a temperature program of 50 °C, hold 2 min, program at 10 °C/min up to 180 °C hold 10 min, with injection port at 240 °C and mass spectrometer at 250 °C.

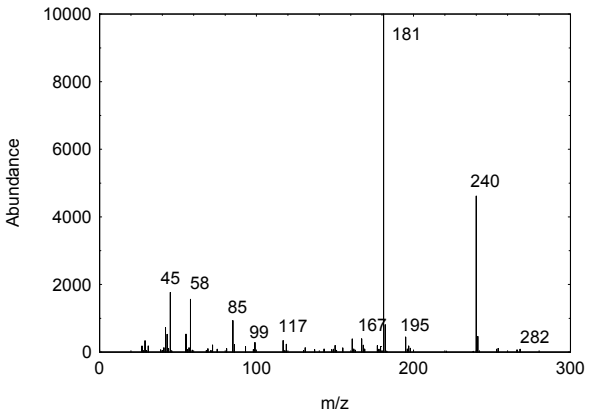

Figure 4.10.1. Mass spectrum of acetoin-PFBHA derivative.

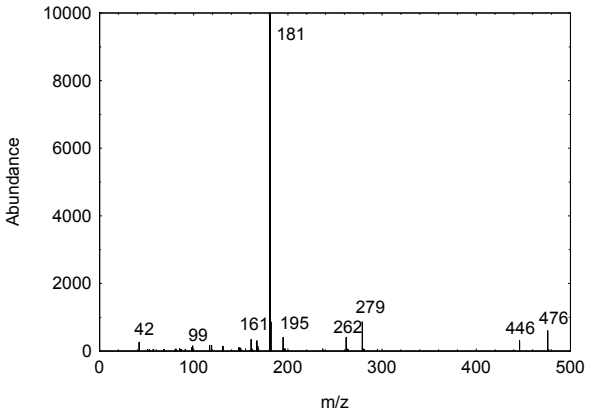

Figure 4.10.2. Mass spectrum of diacetyl-PFBHA derivative.

4.11 Generation of test atmospheres

The test atmosphere of acetoin and diacetyl was generated from a water solution.

The following apparatus was placed in a walk-in hood. The acetoin and diacetyl vapors were generated by pumping the solution, using the Isco 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 glass tube of the vapor generator was wrapped with heating tape to evaporate the chemicals. The humidity, temperature, and volume of the dilution stream of air were regulated by use of a Miller Nelson Flow-Temperature-Humidity controller. The test atmosphere passed into a glass mixing chamber (76-cm $\times$ 30-cm) from the vapor generator, and then into a glass exposure chamber (76-cm $\times$ 20-cm). Active samplers were attached to glass tubes extending from the exposure chamber. The humidity and temperature were measured at the exit of the exposure chamber with an Omega Digital Thermo-hygrometer.

Generation of test atmospheres required extra heating of the air stream to vaporize the acetoin. The temperature and humidity were measured after the air had exited the sampling chamber. The air stream cooled as it passed from the mixing chamber to the sampling chamber and then out the exit. While the air coming out of the exit was 23 °C and 80% RH, the temperature measured in the front of the sampling chamber was 30 °C and 54% RH, giving similar absolute humidities of 16.4 mg/L H_2O.

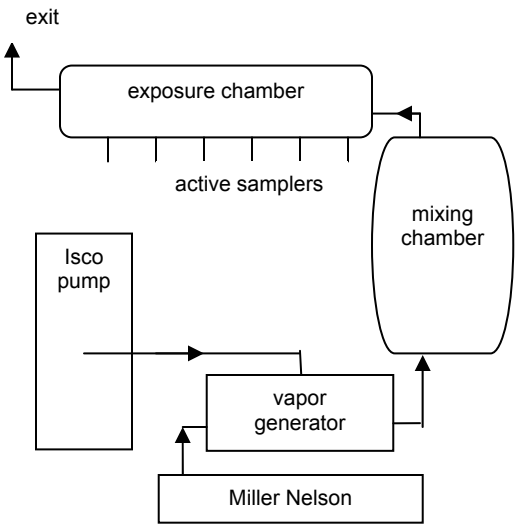

Figure 4.11. The test atmosphere generation and sampling apparatus.