Method number:	PV2118
Target concentration:	25 ppm (88 mg/m^3)
Procedure:	Samples are collected by drawing a known volume of air through two silica gel sampling tubes connected in series. Samples are extracted with ethyl alcohol:water (95:5) and analyzed by GC using a flame ionization detector (FID).
Recommended sampling time and sampling rate:	60 min at 0.05 L/min (3 L)
Reliable quantitation limit:	0.28 ppm (1.00 mg/m^3)
Special requirements:	Samples are collected on two silica gel tubes in series. The second tube is used as a backup for the first tube. Samples should be protected from the light after sampling.
Status of method:	Partially evaluated method. This method has been subjected to established evaluation procedures of the Method Development Team and is presented for information and trial use.

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1. General Discussion

1.1 Background

1.1.1 History

The purpose of this evaluation was to develop a sampling procedure for diacetyl that gave a better storage stability than did the NIOSH Method 2557, which used SKC Anasorb CMS as the sampling media[1]. The NIOSH method requires that the samples be refrigerated immediately after sampling, and the analysis be performed within 7 days. A more stable sampling media was desired for OSHA samples. The following media were tested at SLTC but all gave poor storage stability: coconut shell charcoal Lot 2000, 4-*tert*-butylcatechol coated charcoal, XAD-7, and OVS-7. Silica gel tubes (150mg/75 mg) were tried next and had an average storage recovery of 94.9% for samples stored at room temperature for 14 days. A sampling train of two silica gel tubes in series was necessary because a significant amount of the diacetyl was found on the smaller, backup section of the first tube in the retention study. A second tube in series insures that all of the sample will be collected on the sampling train. The desorbing solvent of 95:5 ethyl alcohol:water with 0.25 µL/mL *p*-cymene internal standard gave an average recovery of 99.1% over the concentration range of 26.5 to 529 µg of diacetyl.

1.1.2 Toxic Effects[2,3] (This section is for information only and should not be taken as the basis of OSHA policy.)

Diacetyl is a skin, eye, mucous membrane, and respiratory irritant, which is harmful in large quantities if ingested. The FDA has approved the use of diacetyl as an artificial flavoring agent, because it is naturally occurring in butter, beer, coffee, vinegar, and other food products. NIOSH issued a Health Hazard Alert July 2002 on the worker exposures at a popcorn plant in Missouri.[4] The workers developed a rare lung disease called bronchiolitis obliterans. It was severe enough to require lung transplants, after exposure to heated artficially butter flavored soybean oil. The main volatile organic found in the workplace atmospheres was diacetyl, the butter flavoring agent. This was chosen as a marker compound for exposures. The other compounds found in the workplace at lower concentrations were acetoin and nonanone. The workers at the coating plant had exposures over 100 ppm diacetyl and exhibited marked lung function impairment. Workers in the mixing area were exposed to 18 ppm and showed lesser lung effects. Workers in the packing area were exposed to 1.8 ppm and had little or no lung effects.

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[1] NIOSH Method 2557, http://www.cdc.gov/niosh, (accessed July 2002).

[2] O'Neil, M., *The Merck Index,* 13th ed., Merck & Co. Inc.: Whitehouse Station, NJ, 2001, p 522.

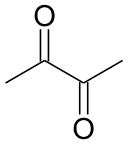
[3] Lewis, R., *Sax's Dangerous Properties of Industrial Materials*, 10th ed., Vol. 2, John Wiley & Sons, New York, 2000, p 595.

[4] NIOSH, *NIOSH Evaluates Worker Exposures at a Popcorn Plant in Missouri*, Cincinnatti OH, HHE 2002-128.

1.1.3 Workplace exposure[5,6]

Most exposures to diacetyl occur in the food industry. Diacetyl is a naturally occuring chemical in bay and other oils, beer, butter, coffee, vinegar, and other food products. It is an artificial flavoring which adds the flavor of butter, cream or creaminess, and butterscotch. Preliminary studies indicate diacetyl used for taste and smell of butter may be responsible for the incident. Presently some 138 plants manufacturing butter flavor popcorn employing some 3400 employee may be at risk of contacting lung related illness. NIOSH found that heated artificial butter flavor soybean oil caused lung disease in many health hazard surveys of popcorn plants and bakeries.[7]

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

CAS number:	431-03-8	IMIS:	D740[10]
RTECS number:	EK2625000	molecular weight:	86.09
melting point:	-3 °C	boiling point:	88 °C
appearance:	green-yellow liquid	molecular formula:	$C_9H_{10}O_2$
odor:	characteristic buttery	flash point:	6 °C
autoignition temperature:	365 °C	density(g/mL):	0.99
solubility:	ether; alcohol; acetone; DMSO		
synonyms:	2,3-butanedione; 2,3 - diketobutane; dimethyl diketone; dimethylglyoxal		
structure:			

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis"[11]. 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 listed in ppm are referenced to 25°C and 101.3 kPa (760 mmHg).

1.2 Detection Limit of the Overall procedure (DLOP) and Reliable Quantitation Limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equal descending increments of analyte, such that the highest sampler loading was 3.7 µg diacetyl. This is the amount spiked on a sampler that would produce a peak approximately 3 times the response for a sample

[5] O'Neil, M., *The Merck Index,* 13th ed., Merck & Co. Inc.: Whitehouse Station, NJ, 2001, p 522.

[6] Lewis, R., *Sax's Dangerous Properties of Industrial Materials*, 10th ed., Vol. 2, John Wiley & Sons, New York, 2000, p 595.

[7] NIOSH HHS, www.cdc.gov/niosh accessed 01/20/03.

[8] O'Neil, M., *The Merck Index,* 13th ed., Merck & Co. Inc.: Whitehouse Station, NJ, 2001, p 522.

[9] Lewis, R., *Sax's Dangerous Properties of Industrial Materials*, 10th ed., Vol. 2, John Wiley & Sons, New York, 2000, p 595.

[10] OSHA Chemical Sampling Guide, http//www.osha.gov.

[11] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. *Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis* ; OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 1999.

blank. These spiked samplers were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (standard error of estimate and slope) for the calculation of the DLOP. The slope was 13.89 and the SEE was 41.82. The RQL is considered the lower limit for precise quantitative measurements.

Table 1.2
Detection Limit of the Overall Procedure for Diacetyl

mass per sample (µg)	area counts (µV-s)
0.00	293
1.3	504
1.58	631
1.85	658
2.11	676
2.38	700
2.64	734
2.90	759
3.17	788
3.43	816
3.70	838

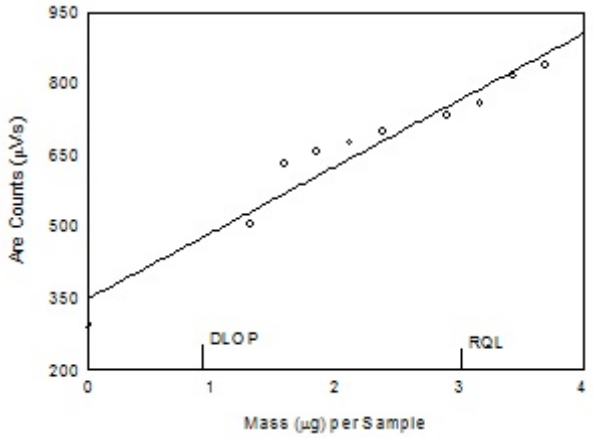


Figure 1.2.1. Plot of data to determine the DLOP/RQL for diacetyl. (Y = 139X +349)

RQL is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The DLOP and RQL were 0.902µg and 3.01 µg respectively

Below is chromatogram of the RQL level.

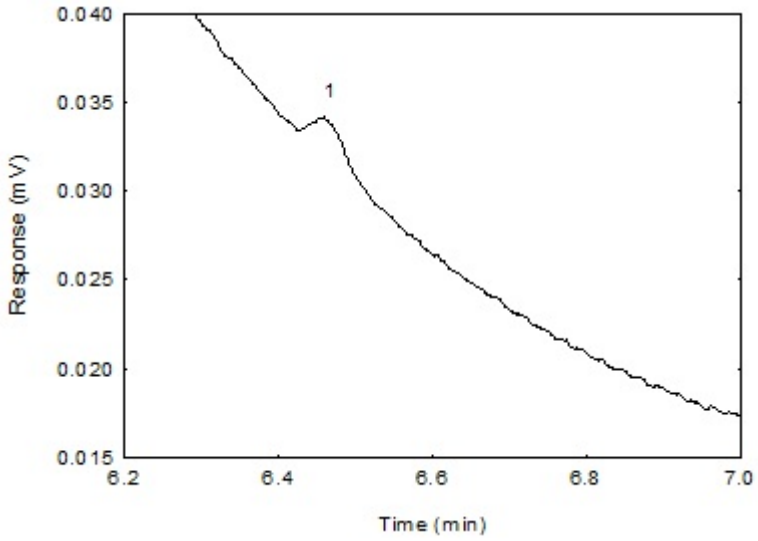


Figure 1.2.2. Chromatogram of the diacetyl standard near RQL (key: (1) diacetyl).

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

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

2.1.2 Silica gel tubes: glass tube with both ends flame sealed, 70 mm × 6-mm i.d. containing 2 sections of 20/40 mesh silica gel separated by a 2-mm portion of urethane foam. The adsorbing section contains 150 mg of silica gel, the backup section 75 mg. A 3-mm portion of urethane foam is placed between the outlet end of the tube and the backup section. A plug of silane-treated glass wool is placed in front of the front section(SKC No. 226-10) tubes or equivalent was used in this evaluation.

2.2 Reagents

None required.

2.3 Technique

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

2.3.2 Connect two tubes in series to the sampling pump with flexible tubing. The smaller sections of the silica gel tubes should be positioned nearer the sampling pump. The tube closer to the pump is used as a backup. A minimum amount of tubing is used to connect the two sampling tubes together. Position the sampling pump, tube holder and tubing so they do not impede work performance or safety.

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

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

2.3.5 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.

2.3.6 Record sample air volumes (liters), sampling time (minutes) and sampling rate (mL/min) for each sample, along with any potential interferences on the OSHA-91A form.

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

2.4 Extraction efficiency

The extraction efficiency was determined by liquid-spiking silica gel tubes with diacetyl at 0.1 to 2 times the target concentration. These samples were stored overnight at ambient temperature and then extracted for 30 minutes with occasional shaking and analyzed. The mean extraction efficiency over the studied range was 99.1%. The wet extraction efficiency was determined at the target concentration by liquid spiking the analyte on the front, larger, section of the first silica gel tube of the sampling train of two silica gel tubes in series, and drawing 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 °C) through them. The mean recovery for the wet samples was 100.2 %

Table 2.4
Extraction Efficiency (%) of Diacetyl

level		sample number					
× target concn	µg per sample	1	2	3	4	5	mean
0.1	26.5	105.0	105.0	105.8	100.3	100.8	103.4
0.25	66.5	110.4	98.6	100.5	97.7	100	101.4
0.5	133	91.0	90.8	90.8	90.6	95.1	91.7
1.0	265	98.8	100.3	99.1	98.9	99.6	99.3
2.0	529	100.8	101.3	99.2	98.7	99.6	99.9
1.0 (wet)	265	104.2	101.6	99.6	93.3	102.2	100.2

2.5 Retention efficiency

Six silica gel tubes were spiked with 0.265 mg (25.ppm) of diacetyl and allowed to equilibrate for 6 h at room temperature in a drawer.. The spiked tubes were placed in series with a second unspiked silica gel tube and had 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 °C) pulled through them at 0.05 L/min. The samples were extracted and analyzed. The mean retention recovery was 94.3%. There was no analyte found on the backup section of any of the tubes.

Table 2.5
Retention Efficiency (%) of Diacetyl

	sample number						
section	1	2	3	4	5	6	mean
front(a+b)	94.3	94.1	96.8	93.7	93.8	93.1	94.3
rear(a)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
rear(b)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
total	94.3	94.1	96.8	93.7	93.8	93.1	94.3

2.6 Sample storage

Nine silica gel tubes were spiked with 0.265 mg (25.ppm) of diacetyl and allowed to equilibrate for 6 h at room temperature in a drawer. The tubes were placed in series with a second unspiked silica gel tube and had 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2 °C) pulled through them at 0.05 L/min. Three samples were analyzed immediately, and the rest were sealed and stored at room temperature in a drawer. Three more were analyzed after 7 days of storage and the remaining three after 14 days of storage. The amounts recovered indicate good storage stability for the time period studied.

Table 2.6
Storage Test for Diacetyl (% Recovery)

	sample number			
time (days)	1	2	3	mean
0	99.4	96.9	96.2	97.5
7	94.5	97.7	95.0	95.7
14	97.2	92.8	94.8	94.9

2.7 Recommended air volume and sampling rate.

Based on the data collected in this evaluation, 3-L air samples should be collected at a sampling rate of 0.05 L/min for 60 minutes.

2.8 Interferences (sampling)

2.8.1 There are no known compounds that will severely interfere with the collection of diacetyl.

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

3. Analytical Procedure

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

3.1 Apparatus

3.1.1 A gas chromatograph equipped with an FID. For this evaluation, an Agilent 6890 Plus gas Chromatograph equipped with a 7683 Automatic Sampler was used.

3.1.2 A GC column capable of separating diacetyl from the desorption solvent, internal standard and any potential interferences. A 60-m × 0.32-mm i.d. capillary DBWAX with a 0.5-µm df (J&W Scientific) was used in the evaluation.

3.1.3 An electronic integrator or some other suitable means of measuring peak areas. A Waters Millennium[32] Data System was used in this evaluation.

3.1.4 Amber glass vials with poly(tetrafluoroethylene)-lined caps. For this evaluation 2-mL vials were used.

3.1.5 A dispenser capable of delivering 1.0 mL of desorbing solvent to prepare standards and samples. If a dispenser is not available, a 1.0-mL volumetric pipet may be used.

3.1.7 Volumetric flasks - 10-mL and other convenient sizes for preparing standards.

3.1.8 Calibrated 10-µL syringe for preparing standards.

3.2 Reagents

3.2.1 Diacetyl, Reagent grade. Aldrich 99% (lot 09122TS BO) was used in this evaluation.

3.2.2 Ethyl alcohol, USP grade 190 proof. Aaper (lot 98G23BB) was used for this evaluation.

3.2.3 *p*-Cymene, Reagent grade. Aldrich 99% (lot 306PZ) was used in this evaluation.

3.2.4 The extraction solvent was 0.25 µL/mL *p*-cymene in ethyl alcohol:water (95:5).

3.2.5 GC grade nitrogen, air, and hydrogen.

3.3 Standard preparation

3.3.1 Prepare working analytical standards by injecting micro liter amounts of diacetyl into volumetric flasks containing the extraction solvent. An analytical standard at a concentration of 0.530 mg/mL (5.3 µL/10 mL) is equivalent to 50 ppm based on a 3-L air volume. Stock standards were stored in amber vials at refrigerated temperature for stability.

3.3.2 Bracket sample concentrations with working standard concentrations. If sample concentrations are higher than the concentration range of prepared standards, prepare and analyze additional standards, at least as high a concentration as the highest sample, to ascertain the linearity of response, or dilute the sample with extracting solvent to obtain a

concentration within the existing standard range. The range of standards used in this study was from 0.00132 to 0.60 mg/mL.

3.4 Sample preparation

3.4.1 Remove the plastic end caps from the sample tubes and carefully transfer both adsorbent sections from front tube and each section of backup tube to separate labeled 2-mL amber glass vials. Discard the glass tube and glass wool plug.

3.4.2 Add 1.0 mL of extraction solvent to each vial using the same dispenser as used for preparation of standards.

3.4.3 Immediately seal the vials with poly(tetrafluoroethylene)-lined caps.

3.4.4 Place vials on shaker and agitate for 60 minutes.

3.5 Analysis

3.5.1 Analytical conditions.

GC conditions

injector:	200°C
detector:	250°C
run time:	16 min
column gas flow:	2.5 mL/min (hydrogen)
septum purge:	1.9 mL/min (hydrogen)
injection size:	1.0 µL (10:1 split)
column:	60-m × 0.32-mm i.d. capillary DBWAX (0.5-µm df)
column temperatures:	50 °C for 6 min, 15 °C/min to 150 °C final time 3 min
retention times:	5.51 min ethyl alcohol, 6.48 min diacetyl, 12.46 min p-cymene

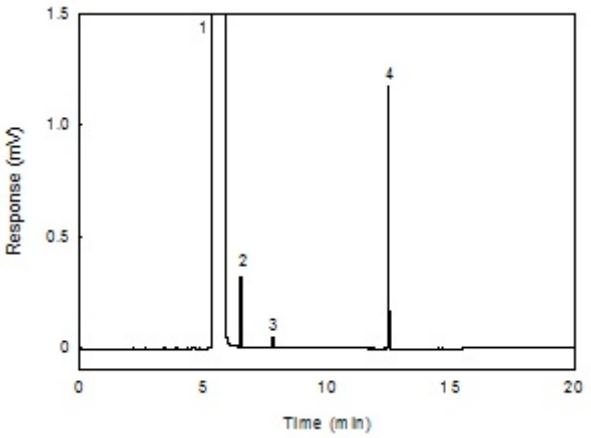


Figure 3.5.1. A chromatogram of 268 µg/ml diacetyl in 95:5 ethyl alcohol:water with 0.25 µl of p-cymene as internal standard. (Key: (1) ethyl alcohol, (2) diacetyl, (3) impurity, and (4) p-cymene).

FID conditions

hydrogen flow	30 mL/min
air flow:	400 mL/min
makeup flow:	25 mL/min (nitrogen)

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

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

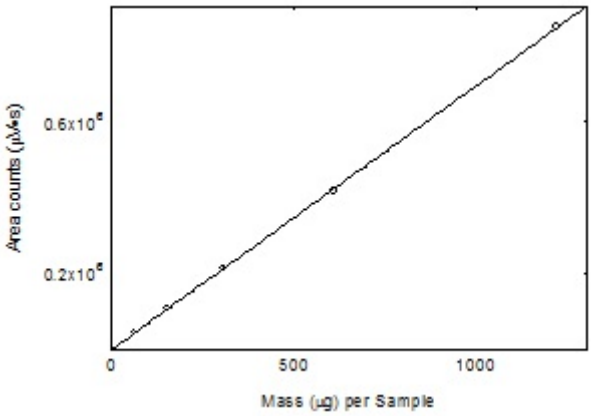


Figure 3.5.3 Calibration curve of diacetyl. (Y = 694 x - 336)

3.6 Interferences (analytical)

3.6.1 Any compound that produces a GC 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.

3.6.2 When necessary, the identity or purity of an analyte peak may be confirmed by mass spectrometry or by another analytical procedure. The mass spectrum in Figure 3.6.2 was from the NIST spectral library.

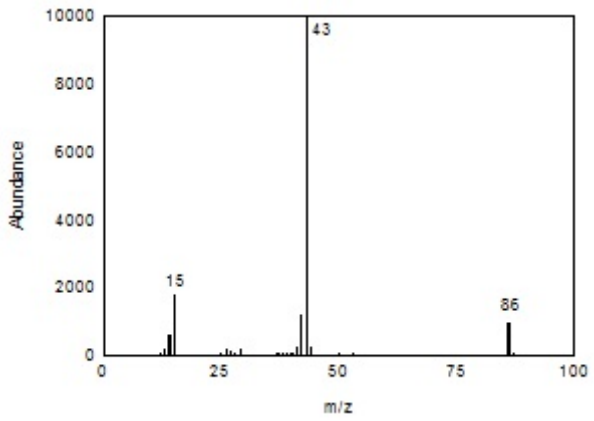


Figure 3.6.2. Mass spectrum of diacetyl.

3.6.3 Calculations

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

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

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 molar volume at 25 °C = 24.46
C_M is concentration by weight
M_r is molecular weight = 86.09

4. Recommendations for Further Study

Several other tests need to be performed to make this a validated method.