Research Article

Sampling and Analytical Method for Alpha-Dicarbonyl Flavoring Compounds via Derivatization with o-Phenylenediamine and Analysis Using GC-NPD

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A novel methodology is described for the sampling and analysis of diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanediione. These analytes were collected on o-phenylenediamine-treated silica gel tubes and quantitatively recovered as the corresponding quinoxaline derivatives. After derivatization, the sorbent was desorbed in 3 mL of ethanol solvent and analyzed using gas chromatography/nitrogen-phosphorous detection (GC/NPD). The limits of detection (LOD) achieved for each analyte were determined to be in the range of 5–10 nanograms/sample. Evaluation of the on-tube derivatization procedure indicated that it is unaffected by humidities ranging from 20% to 80% and that the derivatization procedure was quantitative for analyte concentrations ranging from 0.1 μg to approximately 500 μg per sample. Storage stability studies indicated that the derivatives were stable for 30 days when stored at both ambient and refrigerated temperatures. Additional studies showed that the quinoxaline derivatives were quantitatively recovered when sampling up to a total volume of 72 L at a sampling rate of 50 cc/min. This method will be important to evaluate and monitor worker exposures in the food and flavoring industry. Samples can be collected over an 8-hour shift with up to 288 L total volume collected regardless of time, sampling rate, and/or the effects of humidity.

1. Introduction

In August 2000, the National Institute for Occupational Safety and Health (NIOSH) received a request for technical assistance (HETA # 00-0401) in an investigation of severe obstructive lung disease (bronchiolitis obliterans) in former workers of a microwave popcorn plant in Missouri [1]. NIOSH was asked to investigate a cluster of past and present employees experiencing severe respiratory symptoms after working in microwave popcorn processing facilities over a period of 3 months to 3 years [2]. A NIOSH medical and environmental survey at the plant in November 2000 demonstrated a strong exposure-response relationship between quantities of estimated cumulative exposure to diacetyl (a volatile butter flavoring chemical contaminating the air in the plant) and the frequency of airway obstruction on spirometry tests [1].

NIOSH method 2557, an air sampling method that uses Anasorb Carbon Molecular Sieve (CMS) sorbent tubes, was developed based on an urgent need for a method to collect and quantitate exposures and evaluate subsequent engineering control effectiveness [3]. This method was used extensively in the field for a number of years. Subsequent field evaluation work suggested a tendency of NIOSH method 2557 to underestimate the true concentration of diacetyl in air [4]. Additional laboratory studies identified that this method had reduced recoveries when samples were collected in moderate-to-high humidity environments. A NIOSH laboratory-based study and a chamber study with generated atmospheres established a correction method for previously
collected data with the initial NIOSH method [5]. Concur-
rently, the Occupational Safety and Health Administration
(OSHA) developed method PV2118 that collected diacetyl
on a silica gel sorbent. While the method exhibited good
storage stability for diacetyl, it had limitations in sampling
time/volume because of the collection of water during air
sampling [6].

In an effort to address the humidity concerns encoun-
tered by the NIOSH and OSHA methods, OSHA developed
another method for the collection and analysis of diacetyl
on specially dried silica gel tubes (2 tubes in series) [7].
By using the dried silica gel tubes in series, this OSHA
method addressed migration issues encountered when a
single silica gel tube was used. All of these methods utilize
gas chromatography equipped with flame ionization detection
(GC/FID) for sample analyses.

In 2011, NIOSH published a draft criteria document
titled “Criteria for a Recommended Standard: Exposure
to Diacetyl and 2,3-Pentanedione” that contained a draft
NIOSH Recommended Exposure Limit (REL) of 5 ppm,
to Diacetyl and 2,3-Pentanedione” that contained a draft
NIOSH Recommended Exposure Limit (REL) of 5 ppm,
to Diacetyl (8). The criteria document recom-
mended OSHA method 1012 for sampling diacetyl expo-
8hr-TWA for diacetyl [8]. The criteria document recom-
mended OSHA method 1012 for sampling diacetyl expo-
sures. This method utilizes o-(2,3,4,5,6-pentafluorobenzyl)
hydroxylamine hydrochloride (PFBHA) to derivatize diacetyl
followed by analysis using gas chromatography with electron
capture detection (GC-ECD) [9]. OSHA method 1012 has
limitations in sampling time and capacity due to the potential
collection of water during air sampling, as well as an extended
derivatization time up to 36 hours.

To address the limitations in diacetyl sampling, a research
protocol was designed based upon the derivatization of
diacetyl (which was subsequently applied to analogous alpha-
dicarbonyl compounds) with o-phenylenediamine (o-PDA).
Several research groups have documented the conversion
of alpha-dicarbonyl compounds into the corresponding
quinoxalines using o-PDA [10–12].

Therefore, the focus of this research project was to
develop a method for the collection, derivatization, and
stabilization of diacetyl and the other alpha-dicarbonyl
compounds (2,3-pentanedione, 2,3-hexanedione, and 2,3-
heptanediene) as quinoxaline derivatives.

2. Methods

2.1. Apparatus. Gas chromatographic (GC) analyses were
conducted using a Hewlett Packard Model 5890 Series II
GC with a nitrogen/phosphorus detector (NPD) (Agilent
Tech., Avondale, PA) equipped with a 30 m RTX-5 fused silica
capillary column (0.25 mm ID, 1 μm film) (Restek Corp.,
Bellefonte, PA).

Baseline separation and optimal resolution of diacetyl,
2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanediene
from the excess derivatizing reagent were achieved using the
following parameters. The GC oven temperature program
was ramped up from 50 °C (held 1 min) to 200 °C (10 °C/min)
and held for 2 min. The injection port temperature was set at
240 °C, the detector temperature at 300 °C, and the carrier gas
(helium) to a flow rate of 1.36 mL/min. The injection solvent
was ethanol, which was also used as the method desorption
solvent. A splitless GC injection port liner was used and 1 μL
aliquot was injected.

2.2. Reagents. Diacetyl (97%, CAS # 431-03-8), o-PDA
(99.5%, CAS # 95-54-5), 2,3-dimethylquinoxaline (97%, CAS
# 2379-55-7), 2,3-pentanedione (97%, CAS # 600-14-6), 2,3-
hexanedione (≥93%, CAS # 3848-24-6), 2,3-heptanediene
(≥97%, CAS # 96-04-8), and ethanol (99.5%, CAS # 64-
17-5) were purchased from Sigma-Aldrich Chemical Co.
(Milwaukee, WI).

Commercially available silica gel sorbent tubes (SKC
# 226-183) and specially prepared o-PDA-treated silica gel
sorbent tubes (SKC # CPM021109-001) were obtained from
SKC, Inc. (Eighty Four, PA). The commercially available
silica gel sorbent tubes contain two sections of silica gel
(600 mg front section and 600 mg back section). The o-
PDA- (nominally 0.1% by weight) treated silica gel sorbent
tubes contain two sections of treated silica gel (520 mg front
section, a PUF separator, and 260 mg back section). Ethanol
(99.5%, CAS # 64-17-5) was used as the solvent for all spiking
solutions and as the eluting solvent. For all sorbent tubes,
the front section (A) and back section (B) were desorbed
separately in 3 mL of ethanol in autosampler vials (sealed)
and placed on a shaker for 90 minutes to facilitate desorption.
Analyte spikes, depending on the study, were placed on the
front section of the sorbent tube, or onto the initial glass wool
plug, or from generated aerosols. For each concentration level
evaluated, six samples (N = 6) were prepared. A Teflon
magnetic stir bar (12.7 mm x 7.9 mm, VWR, Inc.) was placed
in each vial. After the desorption period, a portion (1 mL)
of each sample was transferred to 2 mL autosampler vials for
analysis using GC-NPD (1 μL injection).

2.3. Procedures. In order to address the identified limitations
of current methods, a number of laboratory evaluations
were conducted: (a) determination of LOD and Limit of
Quantitation (LOQ), (b) determination of the efficacy of
the post-sampling derivatization of diacetyl collected on large
untreated silica gel tubes, (c) determination of diacetyl, 2,3-
pentanedione, and 2,3-hexanediene recovery from o-PDA-
coated silica gel sorbent, (d) determination of the effects of
high humidity on the derivatization process, (e) determina-
tion of the maximum collection capacity of the coated silica
gel sorbent, and (f) determination of analyte storage stability.

2.3.1. LOD/LOQ Determination. Using GC-NPD, eight stan-
dards (analyzed in duplicate) were prepared and derivatized
on-tube ranging from 2.65 ng/mL to 662.5 ng/mL for diacetyl,
from 10 ng/mL to 100.7 ng/mL for 2,3-pentanedione, and
from 5 ng/mL to 201.6 ng/mL for 2,3-hexanediene.

For LOD and LOQ determination, analytical standards
were prepared by serial dilution for diacetyl, 2-pentanediene,
and 2,3-hexanediene solutions and 1 μL aliquots were spiked
directly onto the sorbent tube. After equilibration, the sorbent
sections were desorbed in 2 mL of ethanol for 60 minutes [13].

2.3.2. Recovery Study (Untreated Silica Gel Sorbent Tubes).
Initially, untreated silica gel sorbent tubes were prepared for
the desorption efficiency (DE) studies after the determination of the method LOD/LOQ using GC-NPD.

Spikes were prepared at the following levels: 0.0955 µg, 9.55 µg, 95.5 µg, 239 µg, and 478 µg.

2.3.3. Recovery Study (o-PDA-Treated Silica Gel Sorbent Tubes). For the initial desorption efficiency study, where the custom-made and o-PDA-treated silica gel tubes (SKC # CPM021I09-001) containing 0.1% o-PDA by weight were used, the desorption efficiencies for diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione were evaluated. Spikes were prepared ranging from approximately 0.1 µg to 500 µg (10 to 100 µg for 2,3-heptanedione) and are listed in Table 1. The ensuing sample preparation and analyses were the same as described in the previous section.

2.3.4. Low-Level Recovery Studies. To further define the lower sample recovery limits, a low-level recovery study (0.1 to 1 µg) was conducted for each analyte. Using the custom-made, unwashed, and dried o-PDA-treated silica gel tubes (SKC # CPM021I09-001), diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione were evaluated.

2.3.5. Studies of the Effect of Humidity on Recovery. To evaluate the effects of relative humidity on sample collection and recovery, the treated sorbent tubes were placed on an air sampling manifold (Miller-Nelson Flow Temperature Humidity Control System, Model HCS-401) and the flow rate of the manifold was adjusted to 50 cc/min. Each tube was then spiked with a solution containing diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione at multiple levels ranging from 0.1 µg to 500 µg (100 µg for 2,3-heptanedione since it was a minor component in all samples). The tubes were allowed to draw laboratory air for two minutes (50 cc/min) to volatilize the analytes of interest before being connected to a Miller-Nelson atmosphere generator. Humidity-controlled air (20%, 50%, and 80%) was sampled for 240 minutes resulting in a total volume of 12 L. The tubes were then refrigerated overnight. To determine whether breakthrough or migration had occurred during sampling, the sorbent from the individual sections of the tubes was removed and placed into individual 4 mL amber colored desorption vials required to prevent UV degradation of samples.

2.3.6. Capacity Studies. The initial collection capacity study was conducted to evaluate the effects of relative humidity on recovery. The custom-made o-PDA-treated silica gel tubes were placed on the air sampling manifold and the flow rate of the manifold was adjusted to 50 cc/min. Each tube was then spiked with a mixture containing diacetyl, 2,3-pentanedione, and 2,3-hexanedione at concentrations of 1 µg and 500 µg. Spikes were made on the glass wool preceding the sorbent and humid air was pulled through the tubes.

The tubes were allowed to draw laboratory air for two minutes to volatilize the analytes before being connected to an atmosphere generator to produce the humidity-controlled air (20% and 80%). Humidity-controlled air was sampled for total volumes ranging from 3 L to 24 L (60 to 480 minutes). Sample preparation and analyses were conducted under the parameters previously described.

In an effort to evaluate the effect of an increased sampling rate (200 cc/min) and maximize sampling volumes collected, a more in-depth capacity study was conducted. In this study, 2,3-heptanedione was added as an analyte due to its continued presence as a minor component in alpha-dicarbonyl based flavoring compounds. The custom-made o-PDA-treated silica gel tubes were placed on the air sampling manifold and the flow rate of the manifold was adjusted to 200 cc/min. Each tube was then spiked on glass wool at the front section with a solution of diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione at concentrations of 1 µg and 500 µg. Spikes were made on glass wool preceding the sorbent and humid air was pulled through the tubes.

The tubes were allowed to draw laboratory air for two minutes to volatilize the analytes before being connected to an atmosphere generator to produce the humidity-controlled air (20%, 50%, and 80%). Humidity-controlled air was sampled for total volumes ranging from 96 L to 288 L (480 to 1440 minutes). Tubes were collected from each volume sampled and placed in refrigerated storage overnight. Sample preparation and analyses were conducted under the parameters previously described.

2.3.7. Storage Stability Studies. To evaluate sample stability [13], custom-made o-PDA-treated silica gel tubes were spiked with 0.6 µg each of diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione as shown in Table 2. Six sorbent tubes were analyzed after 1, 7, 14, and 30 days. Separate sets of samples were analyzed after storage under ambient and refrigerated storage conditions. Sample preparation and analyses were conducted under the parameters previously described.
Table 2: Standard stock spiking solution preparation and spiking volumes for stability studies.

| Analyte               | Amount neat analyte spike (μL) | Final volume (mL) | Final concentration (μg/mL) | Volume spiked (μL) | Amount spiked (μg) |
|-----------------------|---------------------------------|-------------------|-----------------------------|--------------------|-------------------|
| Diacetyl              | 1                               | 10                | 98.5                        | 6                  | 0.591             |
| 2,3-Pentanedione       | 1                               | 10                | 95.9                        | 6                  | 0.575             |
| 2,3-Hexanedione (90%)  | 1                               | 10                | 84.0                        | 6                  | 0.504             |
| 2,3-Heptanedione       | 1                               | 10                | 92.0                        | 6                  | 0.552             |

Table 3: Limit of detection (LOD) and Limit of Quantitation (LOQ) for alpha-dicarbonyl compounds.

| Analyte               | LOD \(^{14}\) | LOQ \(^{14}\) |
|-----------------------|----------------|---------------|
| Diacetyl              | 7 ng/mL        | 23 ng/mL      |
| 2,3-Pentanedione       | 17 ng/mL       | 58 ng/mL      |
| 2,3-Hexanedione        | 5 ng/mL        | 15 ng/mL      |

Table 4: Diacetyl recovery after extraction of spiked sorbent with o-PDA solution.

| Spike level (μg) | Average recovery (%) | RSD   |
|------------------|----------------------|-------|
| 0.096            | 56.3                 | 0.094 |
| 9.55             | 96.4                 | 0.019 |
| 95.5             | 93.0                 | 0.032 |
| 239.0            | 80.1                 | 0.017 |
| 478.0            | 104.3                | 0.033 |

Table 5: Recovery results for the on-tube derivatization of diacetyl, 2,3-pentanedione, and 2,3-hexanendione.

| Analyte               | Spike level (μg) | Average recovery (%) | RSD   |
|-----------------------|------------------|----------------------|-------|
| Diacetyl              | 0.096            | 870                  | 0.106 |
|                      | 9.55             | 99.6                 | 0.037 |
|                      | 95.5             | 91.4                 | 0.099 |
|                      | 239.0            | 104.0                | 0.080 |
|                      | 478.0            | 103.0                | 0.077 |
| 2,3-Pentanedione      | 0.96*            | —                    | —     |
|                      | 9.59             | 106.5                | 0.045 |
|                      | 95.9             | 96.1                 | 0.043 |
|                      | 240.0            | 92.3                 | 0.064 |
|                      | 480.0            | 87.3                 | 0.041 |
| 2,3-Hexanedione       | 0.117            | 69.7                 | 0.212 |
|                      | 11.7             | 73.5                 | 0.032 |
|                      | 117.0            | 69.6                 | 0.032 |
|                      | 292.0            | 66.8                 | 0.040 |
|                      | 584.0            | 62.5                 | 0.028 |

* Data unavailable due to sample loss during analytical preparation.

3. Results

3.1. LOD/LOQ Determination. As previously described, eight standards (in duplicate) were analyzed using GC-NPD: diacetyl (2.65 to 662.5 ng/mL), 2,3-pentanedione (10 to 100.7 ng/mL), and 2,3-hexanedione (5 to 201.6 ng/mL). The instrumental LOD and LOQ were determined using calibration curves (diacetyl – slope = 904.66, intercept = 15.74, and \(R^2 = 0.9216\); 2,3-pentanedione – slope = 533.19, intercept = 29.8, and \(R^2 = 0.7918\); 2,3-hexanedione – slope = 426.30, intercept = 2.5, and \(R^2 = 0.9796\); and 2,3-heptanedione – slope = 113.28, intercept = 28.95, and \(R^2 = 0.9716\)). Results are listed in Table 3.

3.2. Recovery Study (Untreated Silica Gel Sorbent Tubes). On the basis of the initial recovery results achieved when diacetyl was spiked directly on untreated silica gel tubes and desorbed in a solution of 1 mg/mL of o-PDA, a full scale recovery study was evaluated. Desorption efficiency recoveries for diacetyl ranged from 56.3% (0.096 μg) to 104.3% (478.0 μg) with an average Relative Standard Deviation (RSD) of 0.039 are listed in Table 4.

3.3. Recovery Study (o-PDA-Treated Silica Gel Sorbent Tubes). The next phase in the method development process for the derivatization of diacetyl, 2,3-pentanedione, and 2,3-hexanedione was to determine the feasibility of collecting and derivatizing the analytes “on-tube” using o-PDA-coated silica gel sorbent tubes. The initial glass wool plugs were spiked with diacetyl. Ambient air, generated by a Miller-Nelson atmospheric generator, was drawn through the tubes at 0.05 L/min. Desorption efficiencies for the analytes’ derivatization with o-PDA are depicted in Table 5.

3.4. Low-Level Recovery Studies. As noted earlier in the Methods, after the successful recovery study at levels above 1 μg, a low-level recovery study was initiated. The recoveries for diacetyl ranged from 87.2% to 100.7% with an average RSD of 0.073; for 2,3-pentanedione ranged from 94.8% to 120.1% with an average RSD of 0.068; for 2,3-hexanedione ranged from 105.1% to 117.3% with an average RSD of 0.058; and for 2,3-heptanedione ranged from 83.4% to 90.6% with an average RSD of 0.071. Results are listed in Table 6.

3.5. Studies of the Effect of Humidity on Recovery. Due to the negative effects that humidity has on diacetyl recovery discovered during some of the more recent field sampling surveys conducted at food and flavoring sites using NIOSH method 2557 [5], the next progression in our method development effort was to evaluate the effect on sample collection of various levels of humidity when using the o-PDA-coated silica gel sorbent tubes. Spiking levels ranged from...
### Table 6: Low level recovery study for diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione.

| Analyte       | Spike level (µg) | Recovery (%) | RSD  |
|---------------|------------------|--------------|------|
| Diacetyl      | 0.099            | 87.2         | 0.139|
|               | 0.591            | 96.7         | 0.046|
|               | 0.985            | 100.7        | 0.036|
| 2,3-Pentanedione | 0.096          | 120.1        | 0.112|
|               | 0.575            | 94.8         | 0.051|
|               | 0.959            | 108.8        | 0.038|
| 2,3-Hexanedione | 0.084          | 105.1        | 0.043|
|               | 0.504            | 117.3        | 0.056|
|               | 0.840            | 105.1        | 0.043|
| 2,3-Heptanedione | 0.092          | 83.4         | 0.072|
|               | 0.552            | 90.5         | 0.071|
|               | 0.920            | 90.6         | 0.065|

approximately 0.1 µg to 500 µg. After sample collection for a period of 240 minutes and a total volume of 12 L, recoveries were determined for each derivatized analyte collected at relative humidities of 20%, 50%, and 80% (actual measured humidity). The results are listed in Table 7.

### 3.6. Capacity Studies.
In the initial collection capacity study, diacetyl, 2,3-pentanedione, and 2,3-hexanedione were sampled on the o-PDA-coated silica gel tubes at two levels (1 µg and 500 µg) for total air collection capacities ranging from 3 L (60 min) to 24 L (480 min). Sampling was conducted at relative humidities of 20% and 80% and the results are depicted in Tables 8 and 9.

In an effort to evaluate the effect of an increased sampling rate (200 cc/min) and maximize sampling volumes collected, a more in-depth capacity study was conducted. In this study, 2,3-heptanedione was added as an analyte due to its continued presence as a minor component (contaminant) in alpha-dicarbonyl based flavoring compounds. A more detailed depiction of the recovery data for each analyte is presented in Table 10 (20% RH), Table 11 (50% RH), and Table 12 (80% RH). Mean recovery (%) was calculated based on the average recovery of 3 samples evaluated at each volume sampled.

### 3.7. Storage Stability Recoveries.
Evaluation of the ambient and refrigerated storage stability recovery results for diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione indicates that the derivatized analytes were stable for up to 30 days at the 0.6 µg spiking levels. The average storage stability results evaluated for each analyte at 1, 7, 14, and 30 days are reported in Table 13.

### 4. Discussion and Conclusions
A method for alpha-dicarbonyl flavoring compounds has been developed using derivatization with o-PDA. This method has several advantages when compared to other

### Table 7: Effects of varying humidity levels on analyte recovery.

| Analyte       | Level (µg) | Relative humidity (%) | Mean recovery (%) | RSD  |
|---------------|-----------|-----------------------|-------------------|------|
| Diacetyl      | 0.118     | 16.7                  | 86.4              | 0.130|
|               | 0.118     | 58.0                  | 101.0             | 0.146|
|               | 0.640     | 22.6                  | 97.7              | 0.040|
|               | 0.640     | 58.0                  | 111.0             | 0.098|
|               | 0.640     | 80.4                  | 79.3              | 0.076|
|               | 0.938     | 20.7                  | 92.4              | 0.032|
|               | 0.938     | 58.0                  | 115.0             | 0.121|
|               | 0.938     | 78.5                  | 97.8              | 0.110|
|               | 9.85      | 17.9                  | 107.6             | 0.035|
|               | 9.85      | 52.0                  | 100.1             | 0.076|
|               | 9.85      | 79.6                  | 99.6              | 0.028|
|               | 98.5      | 17.9                  | 111.0             | 0.019|
|               | 98.5      | 52.0                  | 100.1             | 0.076|
|               | 98.5      | 49.6                  | 99.6              | 0.028|
|               | 246.25    | 17.9                  | 105.0             | 0.074|
|               | 246.25    | 51.1                  | 101.2             | 0.087|
|               | 246.25    | 80.6                  | 88.9              | 0.042|
|               | 492.5     | 17.9                  | 102.7             | 0.049|
|               | 492.5     | 50.0                  | 108.9             | 0.037|
|               | 492.5     | 81.0                  | 99.5              | 0.022|
| 2,3-Pentanedione | 0.115     | 16.7                  | 63.9              | 0.133|
|               | 0.115     | 58.0                  | 98.7              | 0.112|
|               | 0.115     | 80.1                  | 73.9              | 0.123|
|               | 0.622     | 22.6                  | 88.4              | 0.031|
|               | 0.622     | 58.0                  | 85.6              | 0.080|
|               | 0.622     | 80.4                  | 76.7              | 0.074|
|               | 0.909     | 20.7                  | 84.9              | 0.026|
|               | 0.909     | 58.0                  | 102.0             | 0.142|
|               | 0.909     | 78.5                  | 98.8              | 0.137|
|               | 9.59      | 21.1                  | 94.8              | 0.072|
|               | 9.59      | 51.3                  | 85.8              | 0.071|
|               | 9.59      | 80.8                  | 64.1              | 0.107|
|               | 95.9      | 21.1                  | 102.7             | 0.085|
|               | 95.9      | 51.4                  | 100.0             | 0.102|
|               | 95.9      | 79.7                  | 46.2              | 0.060|
|               | 239.75    | 21.1                  | 104.9             | 0.051|
|               | 239.75    | 50.7                  | 99.1              | 0.119|
|               | 239.75    | 80.6                  | 73.9              | 0.110|
|               | 479.5     | 21.1                  | 90.2              | 0.084|
|               | 479.5     | 50.4                  | 87.1              | 0.125|
|               | 479.5     | 79.9                  | 80.3              | 0.112|
Table 7: Continued.

| Analyte          | Level (µg) | Relative humidity (%) | Mean recovery (%) | RSD  |
|------------------|------------|-----------------------|-------------------|------|
| 2,3-Hexanedione  | 0.101      | 16.7                  | 120.0             | 0.195|
|                  | 0.101      | 58.0                  | 114.3             | 0.085|
|                  | 0.101      | 80.1                  | 49.9              | 0.062|
|                  | 0.546      | 22.6                  | 108.0             | 0.038|
|                  | 0.546      | 58.0                  | 109.0             | 0.060|
|                  | 0.546      | 80.4                  | 82.4              | 0.064|
|                  | 0.799      | 20.7                  | 103.8             | 0.034|
|                  | 0.799      | 58.0                  | 124.0             | 0.206|
|                  | 0.799      | 75.8                  | 90.8              | 0.107|
|                  | 11.68      | 21.1                  | 67.8              | 0.055|
|                  | 11.68      | 51.3                  | 60.4              | 0.056|
|                  | 11.68      | 80.8                  | 45.4              | 0.078|
|                  | 116.75     | 21.1                  | 66.4              | 0.051|
|                  | 116.75     | 51.4                  | 68.6              | 0.060|
|                  | 116.75     | 79.7                  | 31.1              | 0.039|
|                  | 291.88     | 21.1                  | 66.8              | 0.028|
|                  | 291.88     | 50.7                  | 72.1              | 0.107|
|                  | 291.88     | 80.6                  | 40.4              | 0.080|
|                  | 583.75     | 21.1                  | 59.6              | 0.057|
|                  | 583.75     | 50.4                  | 52.5              | 0.079|
|                  | 583.75     | 79.9                  | 53.7              | 0.088|
|                  | 0.110      | 16.7                  | 90.3              | 0.180|
|                  | 0.110      | 58.0                  | 69.8              | 0.142|
|                  | 0.110      | 80.1                  | 75.9              | 0.062|
|                  | 0.598      | 22.6                  | 81.0              | 0.034|
|                  | 0.598      | 58.0                  | 88.7              | 0.066|
|                  | 0.598      | 80.4                  | 78.4              | 0.061|
|                  | 0.874      | 20.7                  | 75.3              | 0.067|
|                  | 0.874      | 58.0                  | 108.0             | 0.195|
|                  | 0.874      | 78.5                  | 107.0             | 0.111|
|                  | 9.20       | 21.5                  | 84.9              | 0.099|
|                  | 9.20       | 47.3                  | 84.5              | 0.053|
|                  | 9.20       | 80.3                  | 81.9              | 0.077|
|                  | 9.20       | 21.5                  | 103.1             | 0.044|
|                  | 9.20       | 52.7                  | 92.3              | 0.030|
|                  | 9.20       | 83.8                  | 90.2              | 0.031|

methods [3, 6, 7] for alpha-dicarbonyl flavoring compounds, such as improved sensitivity (instrumental LODs of 5–17 ng/sample), use of a single sampling tube amenable to on-tube derivatization of the analytes of interest, longer sampling times, variable sampling rates, and greater sampling capacity (up to 288 L with low-to-moderate humidity). Chromatographic separation of the alpha-dicarbonyl derivatives was good and the overall recovery of the analytes of interest down to the 0.1 µg level was acceptable.

Diacetyl recoveries on untreated silica gel tubes following by desorption in ethanol containing the o-PDA derivatizing agent were acceptable at all spiking levels except the lowest (0.096 ng).

Recoveries of diacetyl and 2,3-pentanedione from the silica gel tubes coated with o-phenylenediamine were very good while the recoveries for 2,3-hexanedione were approximately 20% lower. Lower recoveries of 2,3-hexanedione and 2,3-heptanedione may be the result of the increasing hydrocarbon nature of these compounds and/or the fact that they possibly require an increased derivatization period. In addition, when larger amounts of the analyte were evaluated, some lower recoveries were found. This may be the result of incomplete derivatization and the need for a greater concentration of the derivatizing reagent on the sorbent.

Table 8: Determination of air sampling capacity of o-PDA-treated silica gel tubes at 20% relative humidity at 2 concentration levels.

| Analyte          | Level (µg) | Sampling volume (L) | Mean recovery (%) | RSD  |
|------------------|------------|---------------------|-------------------|------|
| Diacetyl         | 0.985      | 3                   | 85.5              | 0.016|
|                  | 0.985      | 6                   | 99.9              | 0.059|
|                  | 0.985      | 12                  | 91.1              | 0.058|
|                  | 0.985      | 18                  | 84.0              | 0.020|
|                  | 490.0      | 3                   | 102.9             | 0.131|
|                  | 490.0      | 6                   | 104.6             | 0.052|
|                  | 490.0      | 12                  | 99.0              | 0.014|
|                  | 490.0      | 18                  | 91.8              | 0.019|
|                  | 490.0      | 24                  | 100.4             | 0.058|
| 2,3-Pentanedione | 0.959      | 3                   | 105.2             | 0.056|
|                  | 0.959      | 6                   | 103.3             | 0.085|
|                  | 0.959      | 12                  | 105.8             | 0.053|
|                  | 0.959      | 18                  | 85.8              | 0.016|
|                  | 480.0      | 3                   | 106.7             | 0.098|
|                  | 480.0      | 6                   | 96.3              | 0.038|
|                  | 480.0      | 12                  | 97.0              | 0.045|
|                  | 480.0      | 18                  | 116.6             | 0.077|
|                  | 480.0      | 24                  | 102.3             | 0.039|
| 2,3-Hexanedione  | 0.841      | 3                   | 72.7              | 0.087|
|                  | 0.841      | 6                   | 91.8              | 0.077|
|                  | 0.841      | 12                  | 110.8             | 0.109|
|                  | 0.841      | 18                  | 85.0              | 0.016|
|                  | 420.0      | 3                   | 92.6              | 0.114|
|                  | 420.0      | 6                   | 89.6              | 0.036|
|                  | 420.0      | 12                  | 86.4              | 0.089|
|                  | 420.0      | 18                  | 90.8              | 0.059|
|                  | 420.0      | 24                  | 108.5             | 0.103|
Table 9: Determination of air sampling capacity of o-PDA-treated silica gel tubes at 80% relative humidity at 2 concentration levels.

| Analyte       | Level (µg) | Sampling Volume (L) | Mean Recovery (%) | RSD   |
|---------------|------------|---------------------|-------------------|-------|
| Diacetyl      | 0.985      | 3                   | 78.1              | 0.016 |
|               | 0.985      | 6                   | 82.1              | 0.058 |
|               | 0.985      | 12                  | 95.2              | 0.097 |
|               | 0.985      | 18                  | 82.4              | 0.034 |
|               | 0.985      | 24                  | 103.6             | 0.028 |
|               | 490.0      | 3                   | 97.4              | 0.047 |
|               | 490.0      | 6                   | 88.5              | 0.074 |
|               | 490.0      | 12                  | 112.7             | 0.034 |
|               | 490.0      | 18                  | 100.6             | 0.018 |
|               | 490.0      | 24                  | 93.7              | 0.065 |
| 2,3-Pentanedione | 0.959      | 3                   | 100.1             | 0.030 |
|               | 0.959      | 6                   | 76.1              | 0.051 |
|               | 0.959      | 12                  | 101.2             | 0.068 |
|               | 0.959      | 18                  | 97.8              | 0.048 |
|               | 0.959      | 24                  | 81.1              | 0.139 |
|               | 480.0      | 3                   | 97.8              | 0.055 |
|               | 480.0      | 6                   | 92.3              | 0.085 |
|               | 480.0      | 12                  | 99.2              | 0.058 |
|               | 480.0      | 18                  | 87.1              | 0.010 |
|               | 480.0      | 24                  | 85.2              | 0.071 |
| 2,3-Hexanedione | 0.841      | 3                   | 100.7             | 0.018 |
|               | 0.841      | 6                   | 69.5              | 0.077 |
|               | 0.841      | 12                  | 98.3              | 0.056 |
|               | 0.841      | 18                  | 101.4             | 0.027 |
|               | 0.841      | 24                  | 80.4              | 0.141 |
|               | 420.0      | 3                   | 95.5              | 0.049 |
|               | 420.0      | 6                   | 92.5              | 0.086 |
|               | 420.0      | 12                  | 87.7              | 0.053 |
|               | 420.0      | 18                  | 94.7              | 0.048 |
|               | 420.0      | 24                  | 87.6              | 0.173 |

media at these higher levels and/or the fact that the higher concentrations evaluated may exceed the sampling capacity of the sorbent tubes. The recoveries for 2,3-hexanedione and 2,3-heptanedione are lower than what is normally considered acceptable [13]. While this method was developed for diacetyl and 2,3-pentanedione measurement to address humidity issues with existing methods, it can be used to determine the presence of larger chain alpha-dicarbonyl compounds that may be present as by-products.

Sample collection for diacetyl was unaffected by humidity ranging from 20% to 80%. For the other flavoring agents tested, high humidity reduced the recovery. While additional research studies are ongoing, it can be reasonably concluded that this method has achieved significant advancements in the sampling and quantitation of alpha-dicarbonyl flavoring compounds. Overall, recoveries were good for diacetyl when sampled in conditions of 80% humidity.

The resulting recovery for 2,3-pentanedione at 95.9 µg (46.2%) is abnormally low when compared to all other results and is most likely an aberration when compared to the results listed in Table 9 for 2,3-pentanedione. Recoveries for both 2,3-hexanedione and 2,3-heptanedione were lower than expected for those samples collected at 80% humidity in the extended air sampling capacity studies. These analytes are present as by-products or contaminants with either diacetyl or 2,3-pentanedione. This method can be used to detect these contaminants where other methods cannot.

In laboratory capacity studies, where diacetyl, 2,3-pentanedione, 2,3-hexanedione, and 2,3-heptanedione were collected at 20% relative humidity and with a sampling rate of 200 cc/min, the recoveries were good for diacetyl when sampled in conditions of 80% humidity.
Table 11: Determination of extended air sampling capacity of o-PDA-treated silica gel tubes at 50% relative humidity at two concentration levels using increased sampling rate (200 cc/min).

| Analyte          | Level (µg) | Sampling volume (L) | Time (min) | Mean recovery (%) | RSD  |
|------------------|-----------|---------------------|------------|------------------|------|
| Diacetyl         | 0.493     | 96                  | 480        | 102.0            | 0.015|
|                  | 0.493     | 144                 | 720        | 92.6             | 0.024|
|                  | 0.493     | 216                 | 960        | 102.0            | 0.018|
|                  | 0.493     | 288                 | 1440       | 87.0             | 0.026|
|                  | 98.5      | 96                  | 480        | 98.8             | 0.027|
|                  | 98.5      | 144                 | 720        | 106.0            | 0.016|
|                  | 98.5      | 216                 | 960        | 101.8            | 0.020|
|                  | 98.5      | 288                 | 1440       | 101.0            | 0.028|
| 2,3-Pentanedione  | 0.479     | 96                  | 480        | 92.7             | 0.010|
|                  | 0.479     | 144                 | 720        | 77.8             | 0.054|
|                  | 0.479     | 216                 | 960        | 98.5             | 0.158|
|                  | 0.479     | 288                 | 1440       | 88.5             | 0.009|
|                  | 95.7      | 96                  | 480        | 97.8             | 0.030|
|                  | 95.7      | 144                 | 720        | 100.0            | 0.019|
|                  | 95.7      | 216                 | 960        | 110.0            | 0.024|
|                  | 95.7      | 288                 | 1440       | 97.8             | 0.026|
| 2,3-Hexanedione  | 0.420     | 96                  | 480        | 93.7             | 0.024|
|                  | 0.420     | 144                 | 720        | 86.2             | 0.021|
|                  | 0.420     | 216                 | 960        | 87.9             | 0.020|
|                  | 0.420     | 288                 | 1440       | 71.4             | 0.018|
|                  | 84.1      | 96                  | 480        | 88.0             | 0.023|
|                  | 84.1      | 144                 | 720        | 102.0            | 0.023|
|                  | 84.1      | 216                 | 960        | 103.0            | 0.026|
|                  | 84.1      | 288                 | 1440       | 89.3             | 0.018|
| 2,3-Heptanedione  | 0.460     | 96                  | 480        | 103.0            | 0.019|
|                  | 0.460     | 144                 | 720        | 89.3             | 0.026|
|                  | 0.460     | 216                 | 960        | 97.8             | 0.031|
|                  | 0.460     | 288                 | 1440       | 98.5             | 0.041|
|                  | 92.0      | 96                  | 480        | 94.1             | 0.020|
|                  | 92.0      | 144                 | 720        | 99.8             | 0.017|
|                  | 92.0      | 216                 | 960        | 105.0            | 0.011|
|                  | 92.0      | 288                 | 1440       | 98.6             | 0.019|

The average 30-day storage stability recovery results, almost quantitative in nature, are extremely good and acceptable for both the ambient and refrigerated samples of the derivatized diacetyl and 2,3-pentanedione, suggesting that temperature does not have either a positive or a negative effect on the derivatization and storage of the diacetyl samples. Since both the ambient and refrigerated samples had quantitative recoveries (>95%) and RSD values less than 1% [13], there is no difference between the two methods of storage.

Evaluation of both ambient and refrigerated storage stability recovery results for 2,3-hexanedione indicates that the derivatized analyte was stable for up to 30 days at the 0.6 µg...
Table 13: Storage stability conducted under ambient and refrigerated storage conditions.

| Day | Avg. recovery (ambient, %) | RSD | Avg. recovery (refrigerated, %) | RSD |
|-----|---------------------------|-----|---------------------------------|-----|
|     | Diacetyl                   |     |                                 |     |
| 1   | 96.1                      | 0.068 | 96.7                            | 0.046 |
| 7   | 93.4                      | 0.048 | 89.8                            | 0.039 |
| 14  | 98.6                      | 0.017 | 106.0                           | 0.065 |
| 30  | 103.0                     | 0.039 | 98.6                            | 0.047 |
|     | 2,3-Pentanedione           |     |                                 |     |
| 1   | 98.0                      | 0.049 | 94.8                            | 0.051 |
| 7   | 102.0                     | 0.045 | 100.0                           | 0.025 |
| 14  | 105.0                     | 0.091 | 97.1                            | 0.068 |
| 30  | 108.0                     | 0.065 | 96.8                            | 0.073 |
|     | 2,3-Hexanedione            |     |                                 |     |
| 1   | 109.8                     | 0.115 | 117.0                           | 0.056 |
| 7   | 93.9                      | 0.041 | 110.0                           | 0.054 |
| 14  | 87.0                      | 0.068 | 99.0                            | 0.040 |
| 30  | 81.4                      | 0.026 | 99.6                            | 0.053 |
|     | 2,3-Heptanedione           |     |                                 |     |
| 1   | 89.3                      | 0.109 | 90.5                            | 0.072 |
| 7   | 90.8                      | 0.055 | 84.9                            | 0.059 |
| 14  | 92.2                      | 0.053 | 83.6                            | 0.096 |
| 30  | 92.2                      | 0.021 | 97.0                            | 0.082 |

spiking levels. Analysis of the results indicates that there is improved storage stability (18% increase in average recovery) when the samples are refrigerated. This is especially true for the samples analyzed after 14 and 30 days. Evaluation of both ambient and refrigerated storage stability recovery results for 2,3-heptanedione indicates that the derivatized analyte was stable for 30 days. Comparison of the averaged recoveries for both the ambient and refrigerated samples of the derivatized 2,3-heptanedione revealed no differences based on storage temperature. Storage stability studies indicated that the compounds of interest, especially diacetyl and 2,3-pentanedione, as their quinoxaline derivatives, are stable at both ambient and refrigerated temperatures for 30 days. Separation of other alpha-dicarbonyls such as 2,3-hexanedione and 2,3-heptanedione can be achieved with this method and provide semiquantitative results. Overall, this method may be another useful tool for the evaluation and monitoring of workers exposed to airborne alpha-dicarbonyl food and flavoring compounds. Additional laboratory and field studies using the method are necessary to obtain full validation and publication in the NIOSH Manual of Analytical Methods (NMAM).

In summary, to date, all results suggest that this method provides the sensitivity needed for nanogram level sampling for alpha-dicarbonyl food and flavoring compounds (diacetyl and 2,3-pentanedione). Additionally, the method allows collection over a wide mass range and at relative humidities ranging from 20% to 80%, with acceptable recoveries achieved up to sampling volumes of 144 L and 288 L for 2,3-pentanedione and diacetyl, respectively. The sampling and analytical methodology has been unaffected by breakthrough when sampling at high flow rates (200 cc/minute) and high sample collection volumes (144 L), eliminating the need for a second sorbent tube in series with the backup section of the single sorbent tube collecting any sample that breaks through. Additionally, the on-tube derivatization eliminates humidity-related breakthrough of the alpha-dicarbonyl flavoring compounds by forming the stable quinoxaline derivatives.

Competing Interests

The authors declare that they have no competing interests.

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