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Method Development for the Determination of Diacetyl and Acetoin at a Microwave Popcorn Plant

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Separate sampling and analytical methods for the determination of diacetyl and acetoin have been developed to assess workplace exposures at a popcorn processing facility have been described. Diacetyl (NMAM 2557) is efficiently recovered from an Anasorb CMS sampler tube when the composition of methanol in the desorption solvent is 1%, and acetoin (NMAM 2558) is efficiently recovered when the concentration of methanol is increased to 5%. Desorption efficiencies for diacetyl and acetoin were acceptable, 89.9% (RSD = 0.018) and 94.9% (RSD = 0.019), respectively. Recoveries for nonanone, methyl ethyl ketone, and ethyl acetate were not optimized because they were present in very low concentrations in the popcorn processing facility and not considered to be major occupational health hazards. Samples were collected on Anasorb CMS solid sorbent tubes. All analytes were separated using a 30-m Stabilwax-DA fused silica capillary column, followed by analysis using gas chromatography with flame ionization detection. These methods were acceptable for monitoring and identifying exposures to diacetyl and acetoin present in the butter flavoring mixture used at popcorn processing facilities. For example, in the initial site visit the method was used to determine that maximum workers exposures to diacetyl (462.6 mg/m^3), acetoin (59.1 mg/m^3), and nonanone (0.45 mg/m^3) occurred as the butter flavoring was added to the mixing kettle (*1*). When protective measures were recommended by NIOSH personnel and implemented by the popcorn processing facility, the methods were then used to determine the effectiveness of these changes, which showed that diacetyl and acetoin concentrations had been reduced significantly to 0.97 and 2.3 mg/m^3, respectively, while the concentration of nonanone fell to levels below the detection limit (LOD).

Introduction

In November 2000, National Institute for Occupational Safety and Health (NIOSH) officials received a Health Hazard Technical Assistance Request (HETA # 00-0401) to investigate a cluster of past and present employees experiencing severe breathing difficulties after working in a microwave popcorn processing plant. Initial studies indicated that workers at the popcorn plant, when compared to other Americans of the same age and smoking habits, were twice as likely to have severe respiratory problems and in some instances rashes so severe that skin peeled off (*1*). Initial evaluation of the workplace by NIOSH industrial hygienists investigating these symptoms suggested that the lung disease afflicting the workers was related to inhaling high concentrations of the butter flavoring used in the popcorn plant (*2*). Additionally, employees experiencing the most severe lung illness had work histories in the packaging and/or mixing area where the flavorings are directly added to a heated tank.

On the basis of their initial evaluation, Kullman et al. suspected that the artificial butter flavoring mixture added to the popcorn may be the cause of the respiratory problems affecting the workers at the microwave popcorn plant. The butter flavoring mixture, a yellow substance with a thick consistency, contains many organic compounds, including diacetyl and acetoin, which are found naturally in other buttery foods such as beer, butter, and cheese (*3*). Nonanone, another organic compound present in the flavoring mixture, was initially included in the health hazard evaluation (HETA) study. Diacetyl (2,3-butanedione) is a yellowish liquid with a butter-like odor (*3*). Due to its chemical structure (a dione) and possible equilibrium with 1,3-butanediene-2,3-diol in solution (keto−enol tautomerism) (*4*), diacetyl can be reactive and sensitive toward both reducing and oxidizing agents. While diacetyl is Food and Drug Administration approved as a food additive, it is highly toxic by inhalation and is listed as a possible animal carcinogen (*5*). Acetoin (3-hydroxy-2-butanone) is a liquid that over time converts to a whitish solid dimer upon exposure to air and moisture (*6*). Acetoin, a reduction product of diacetyl, is commonly found in food products containing diacetyl and is responsible for the “bitter beer” phenomenon often found in stale beer (*3*). Acetoin is a respiratory irritant that emits an earthy, musty odor.

Nonanone (methyl heptyl ketone) and 2-butanone (methyl ethyl ketone), organic ketones present as minor constituents of the artificial butter flavoring, are clear liquids with slight odors. They are classified as an eye, skin, and respiratory irritants.(*7*−*8*)

Because the compounds noted previously are defined as respiratory irritants or as toxic by inhalation (diacetyl) and have been determined to be components of the artifical butter flavoring mixture, 30 cases of severe respiratory illness in workers, ranging from 3 months to 3 years in duration, were identified and investigated at the initial popcorn processing plant (*1*). This cluster, coupled with the possible links with cases of lung illness discovered at other food processing plants that use artificial butter flavoring (*9*), required the development of sampling and analytical methods for diacetyl and acetoin to facilitate exposure assessment and subsequent engineering control effectiveness.

Experimental Section

Apparatus. Gas chromatographic analysis was performed using an Agilent 6890 series gas chromatograph (GC) equipped with a flame ionization detector (FID) and an autosampler (Agilent Tech., Avondale, Pa). A 30-m Stabilwax-DA fused silica capillary column (0.32 mm i.d., 1-μm film) (Restek Corporation, Bellefonte, Pa) was used.

Baseline separation and optimal resolution of diacetyl and acetoin (as well as nonanone and methyl ethyl ketone) was achieved using the following gas chromatographic parameters: A temperature program ramped from 35 (hold 3 min) to 200 °C (6 °C/min) and held for 5 min. The injection port temperature was set at 225 °C, the detector temperature at 250 °C, and the carrier gas (helium) to a flow rate of 2.8

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mL/min. The sample injection volume was 1 μL, splitless mode. A 4-mm base-deactivated gooseneck inlet liner containing FS wool (Restek Corp., Bellefonte, PA) was required because of the reactivity of the analytes. The desorption solution for diacetyl was an acetone/methanol mixture (99:1) and for acetoin an acetone/methanol mixture (95:5).

Reagents. Diacetyl (97%), acetoin (crystalline dimer), nonanone (99+%), and methyl ethyl ketone (99.5+%) standards were purchased from Aldrich Chemical Co. (Milwaukee, WI). High-performance liquid chromatograph-grade methanol and acetone (Burdick and Jackson, Muskegon, MI) were used as the desorption solvents in varying ratios.

Commercially available Anasorb CMS solid sorbent sampling tubes were obtained from SKC, Inc. (Eighty Four, Pa). The CMS sampling tube (SKC # 226−121) contains two sections of carbon molecular sieve resin (150 mg front section, a polyurethane foam [PUF] separator, and a 75 mg back section).

Procedure. Solid sorbent tubes were prepared for the desorption efficiency study (DE) at four levels ($N = 6$). The front sections of the Anasorb CMS solid sorbent tubes were spiked with 20-, 10-, 5-, and 2-μL aliquots of diacetyl and acetoin. The range of study was 47−470 μg for diacetyl and 38−378 μg for acetoin in acetone/methanol (99:1) solution. The tubes were capped and allowed to stand overnight at room temperature. The front sections of each sample tube were placed in separate amber crimp-capped autosampler vials, extracted with 1 mL of acetone/methanol (99:1), and placed on a rotary mixer for 1.5 h (NMAM 2557). For the optimized DE recovery of acetoin (NMAM 2558), the sorbent tubes were extracted with acetone/methanol (95:5) and placed on a rotary mixer for 1 h. The backup sections of the CMS tubes were extracted separately to check for analyte migration. The PUF plug separating the two sections of the CMS tube was discarded.

Eighteen Anasorb CMS solid sorbent tubes were spiked with diacetyl (196.2 μg) and acetoin (200.0 μg). The samplers were capped and stored at ambient temperature for 7 days in the dark. After 7 days, six samples were desorbed and analyzed. The remaining 12 samplers were stored at 5 °C in the dark for 14 and 30 days, respectively (*10*).

Results

The initial phase of the method development focused on the selection of a suitable capillary column for the baseline separation of all the analytes. A number of capillary columns (Rtx-35, Rtx-200, Stabilwax, and Stabilwax-DA) were evaluated for resolution, retention, stability, and inertness. The Stabilwax-DA capillary column exhibited the best combination of these characteristics: good separation and resolution of all analytes, inertness to diacetyl and acetoin, and reasonable retention times. Separation was achieved using the following temperature program: 35 (hold 3 min) to 200 °C (6 °C/min); injection port = 225 °C; detector = 250 °C, see Figure 1.

On the basis of the preliminary recovery results achieved for diacetyl collected on Anasorb CMS solid sorbent tubes and desorbed with acetone/methanol (99:1), a full-scale recovery study (four levels, $N = 6$) was conducted. Desorption efficiency recoveries for diacetyl ranged from 92% (47 μg) to 87% (470 μg) with a RSD of 0.018. The recoveries for acetoin ranged from 62.7% (5 μg) to 83.1% (250 μg). Desorption of acetoin with acetone/methanol (99:1) (NMAM 2557) was originally evaluated because both diacetyl and acetoin would be collected simultaneously on Anasorb CMS sorbent tubes during the collection of field samples. However, when the separately determined sampling and analytical parameters optimized for acetoin (NMAM 2558) were applied to the recovery study, the recoveries ranged from 94.2% (56.6 μg) to 95.1% (378.0 μg) with a RSD of 0.019. While nonanone and methyl ethyl ketone recoveries were <50% when either NMAM 2557 or NMAM 2558 were applied, no full-scale recovery studies were conducted because these analytes were not considered high-risk exposures. The results for these compounds were reported as estimates as recoveries below 75% are considered unacceptable for reporting as quantitative data (*10*).

The final phase of the method development was a 30-day storage stability study. Diacetyl samples, fortified at the 196.2 μg level, were analyzed after storage for 7 days at ambient temperature in the dark. The average recovery was 101.6% (RSD = 0.015) when corrected based on averaged analyte recovery after 1 day at the 196.2 μg level. A 7-day storage stability study conducted earlier in the method development procedure (during light/dark stability experiments) yielded an average recovery of 97.1% (RSD = 0.033) when samples were fortified at the 19.6 μg level and stored at ambient temperature (*10*). After 14 days (7 days at ambient temperature, 7 days at 5 °C), the storage stability recovery for diacetyl dropped off significantly to 82.8% (RSD = 0.010) (*10*). Because the difference between the 7 day recovery and 14 day recoveries is greater than 10%, diacetyl can only be considered stable for 7 days (*10*).

Acetoin samples, fortified at the 160.5 μg level, were analyzed after storage for 7 days at ambient temperature in the dark (*10*). The average recovery was 93.8% (RSD = 0.010) when corrected based upon the averaged recovery after 1 day at the 160.5 μg level. As was the case with diacetyl, an earlier 7 day storage stability study yielded an average recovery of 79.9% (RSD = 0.070). However, at the time, the samples were analyzed under the analytical parameters optimized for diacetyl. After 14 days (7 days at ambient temperature, 7 days at 5 °C), the recovery for acetoin was 86.5% (RSD = 0.016) and after 30 days was 83.6% (RSD = 0.021) (*10*), respectively. Diacetyl and acetoin, when sampled onto the Anasorb CMS solid sorbent tubes, were stable for 7 and 30 days, respectively. The storage stability for diacetyl possibly could be extended by immediate storage at 5 °C after sampling.

Discussion

Laboratory Experiments. A number of problems were encountered in the method development process. These included the thermal instability of diacetyl in the GC injection port and its slow degradation in the presence of light and air, as well as the slow conversion of acetoin to diacetyl in the presence of methanol and light. To prevent these types of degradation, deactivated glass liners were installed in the GC injection port while samples and standards were prepared in amber vials and stored in the dark.

To evaluate the effect of light on sample storage, two groups of samples were prepared and stored in the dark and light at ambient temperature. Analysis of these samples indicated that there was an average 20% higher recovery for the group stored in the dark. In samples where methanol (present in the desorption solvent) and light were present, acetoin is slowly converted to diacetyl (see Table 2). This phenomenon was noted during the latter stages of the storage stability study.

Another problem encountered was the varying solubilities of the analytes. Acetoin is only soluble in water, methanol, and acetone (limited). Initially, because of its universal application as a desorption solvent in gas chromatography, carbon disulfide was evaluated as the desorption solvent. However, diacetyl exhibited limited solubility and acetoin was slightly soluble at best.

Next, water, methanol, and water/methanol mixtures were considered, but methyl ethyl ketone, nonanone, and ethyl acetate have poor recoveries (generally <50%) from such polar solvents. In addition, diacetyl exhibited some reactivity

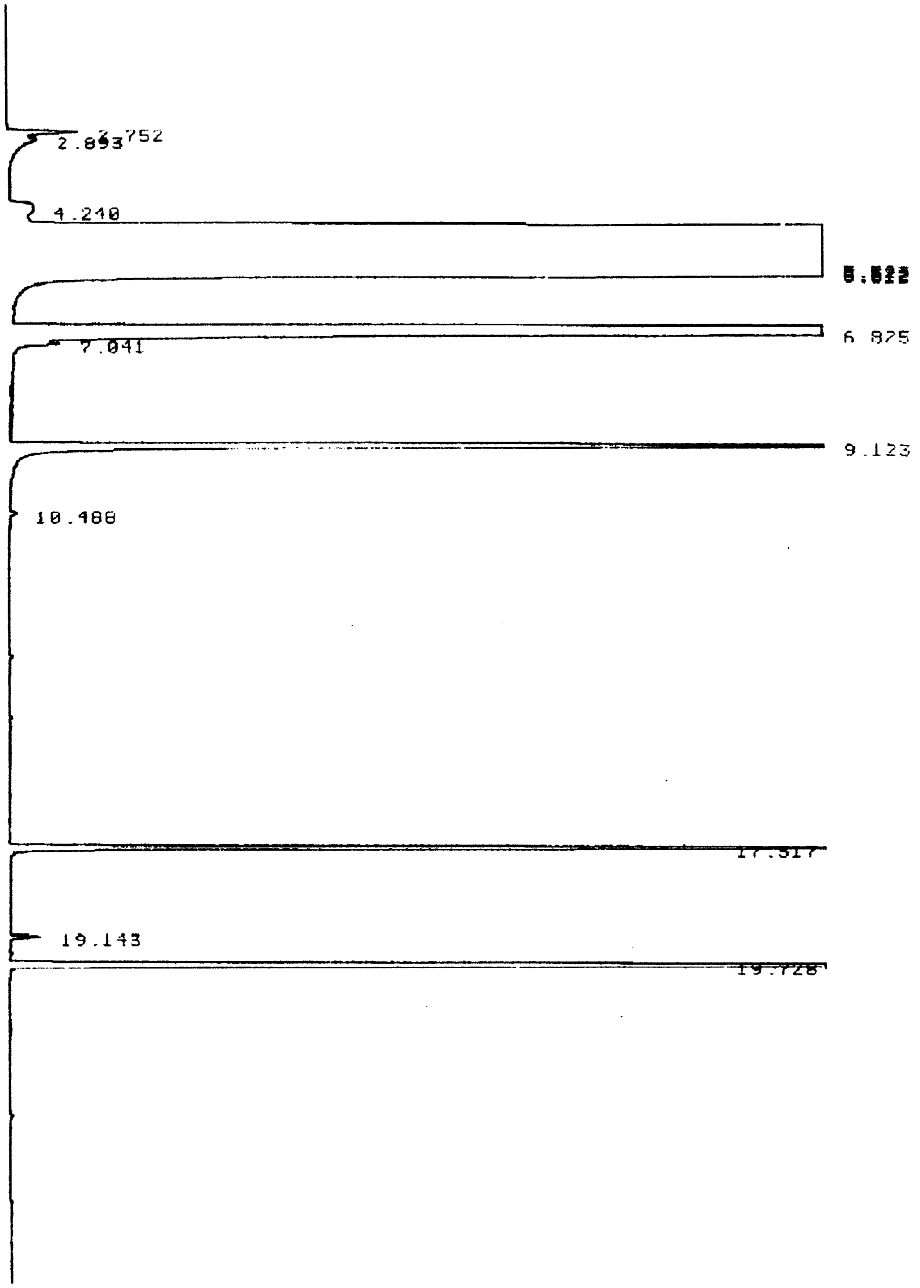

FIGURE 1. Analysis and separation of diacetyl (Rt = 9.12 min) and acetoin (Rt = 19.73) using a 30-m Stabilwax-DA fused silica capillary column.

TABLE 1. Conversion of Acetoin to Diacetyl during Acetoin Storage Stability Study

storage time (days)	acetoin (%)	diacetyl (%)
0	100	0
7	93.8	6−7
14	86.5	10−13
30	83.6	16−18

[a] Initial acetoin concentration was 160.5 μg/sample.

TABLE 2. Limits of Detection (LOD) and Limits of Quantitation (LOQ) for Diacetyl, Acetoin, and Nonanone[a]

analyte	LOD	LOQ	RSD
diacetyl	0.6	2.0	0.018
acetoin	1.0	3.0	0.008
nonanone	0.9	1.8	0.007

[a] Units are μg/sample.

or decomposition in these solvents and total baseline separation of all analytes was not possible due to the width of the methanol solvent peak. Also, while water is not normally a suitable solvent choice for capillary gas chromatography, it was considered because acetoin was only soluble in highly polar solvents. Acetone was also considered as a desorption solvent, but acetoin was only partially soluble, and analyte recoveries were marginally (approximately 75%) acceptable. Finally, a mixture of acetone/methanol (99:1) was evaluated, and this optimized the recovery of diacetyl. The recovery of acetoin was later optimized by increasing the methanol fraction in the desorption solvent to 5% and decreasing the desorption time of the samples to 1 h.

In addition to diacetyl and acetoin, several other organic compounds were originally identified in the butter flavoring mixture. These included nonanone, methyl ethyl ketone, ethyl acetate, and acetic acid. Initial efforts focused on optimizing the sampling and analytical parameters for the analysis of diacetyl because of its high degree of toxicity by inhalation and to a lesser degree acetoin.

Because diacetyl and acetoin are similar in chemical structure to other members of the ketone family, Anasorb CSC (coconut shell charcoal) and Anasorb CMS (carbon molecular sieve) sorbent tubes were evaluated for analyte recovery. While recoveries using both Anasorb CSC and Anasorb CMS were similar, Anasorb CMS (carbon molecular sieve) is a synthetic charcoal that has a greater sampling capacity, is inert toward ketones, and is less affected by moisture than coconut shell charcoal. A sampling volume range of 1−10 L (used for the collection of related ketone analytes) for both diacetyl and acetoin was recommended to reduce the possibility of sample breakthrough and subsequent sample migration.

The solvent mixture of acetone/methanol (99:1), while optimized to maximize the recovery of diacetyl from Anasorb CMS sorbent tubes, yielded unacceptable recoveries of acetoin (<75%), nonanone (<50%), and methyl ethyl ketone (<50%). Because of the low acetoin recoveries using the acetone/methanol (99:1) desorption solvent, a separate analytical methodology was developed in order to optimize acetoin recovery from the Anasorb CMS sampling media.. Subsequent experiments indicated that by increasing the methanol fraction to 5%, the desorption efficiency recovery of acetoin from Anasorb CMS sorbent tubes was optimized to achieve acceptable results (>90%).

Initial recoveries for nonanone and methyl ethyl ketone were unacceptable (<50%) using either of the acetone/methanol solvent mixtures. Previous work had shown that acceptable recoveries for both methyl ethyl ketone and nonanone (>90%) could be achieved using carbon disulfide and carbon disulfide/2-propanol (98:2), respectively (*11*, *12*). However, because diacetyl and acetoin were the priority analytes of interest in this project, coupled with the fact that all analytes were collected simultaneously on the sampling media tubes, the low recoveries of methyl ethyl ketone and nonanone were not considered a major concern during this health hazard evaluation.

The limits of detection (LOD), limits of quantitation (LOQ), and relative standard deviation (RSD) values were determined for diacetyl, acetoin, and nonanone (see Table 2). The sampling and analysis of nonanone was requested in the initial health hazard evaluations but was de-emphasized in later studies. The LOD is defined as "the mass of an analyte that gives a signal three sigma above the mean blank signal, where sigma is the standard deviation of the blank signal," while the LOQ is defined as "the mass corresponding to the mean blank signal $+ 10\sigma_b$, or the mass above which recovery is greater than or equal 75% (*10*).

The low recovery (83.6%) for acetoin is attributed to the formation of diacetyl during storage. While there was no diacetyl present in the acetoin samples at day 0, the percentage of diacetyl (based on GC retention time) observed in the samples averaged 6−7% after 7 days, 10−13% after 14 days, and 16−18% after 30 days with corresponding losses noted in acetoin recovery. The overall precision for the results of the storage stability study was determined to be 1.6%.

Field Applications. Overall, sampling on Anasorb CMS solid sorbent tubes coupled with separation and analysis using gas chromatography provides acceptable methods for monitoring and identifying exposures to diacetyl and acetoin present in the butter flavoring mixture used at popcorn processing facilities. For example, in the initial site visit the method was used to determine that maximum workers exposures to diacetyl (462.6 mg/m^3), acetoin (59.1 mg/m^3), and nonanone (0.45 mg/m^3) occurred as the butter flavoring was added to the mixing kettle (*1*). When protective measures were recommended by NIOSH personnel and implemented by the popcorn processing facility, the methods were then used to determine the effectiveness of these changes, which showed that diacetyl and acetoin concentrations had been reduced significantly to 0.97 and 2.3 mg/m^3, respectively, while the concentration of nonanone fell to levels below the detection limit (LOD) (*1*). These results would serve to confirm the applicability of NMAM methods 2557 and 2558 as means for determining risks to worker health in popcorn processing facilities and as a means of monitoring and assessing the effectiveness of engineering safety procedures implemented.

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