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Determination of Benzene in Soft Drinks and Other Beverages by Isotope Dilution Headspace Gas Chromatography/Mass Spectrometry

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Determination of Benzene in Soft Drinks and Other Beverages by Isotope Dilution Headspace Gas Chromatography/Mass Spectrometry

1.0 Scope and Applicability

This method is for determination of benzene in carbonated and non-carbonated soft drinks and other beverages.

2.0 Definitions

3.0 Equipment and Supplies

3.1 Equipment

3.1.1 Agilent 6890 gas chromatograph equipped with cryogenic oven cooling.

3.1.2 Agilent 5973N mass selective detector, coupled to Agilent 6890 gas chromatograph.

3.1.3 Gerstel MPS 2 MultiPurpose autosampler, or equivalent, connected to Agilent 6890 gas chromatograph.

3.1.4 EASYpure RODI water purification system (Barnstead, Iowa), or equivalent.

3.1.5 Oven from VWR, or equivalent.

3.1.6 Balances.

3.2 Chemicals

3.2.1 Methanol (HPLC grade) from EM Science (Gibbstown, NJ).

3.2.2 Benzene (99.8 %) Sigma-Aldrich (St. Louis, MO).

3.2.3 Benzene-d_6 with isotopic purity of 99.95 atom % D from Sigma-Aldrich (St. Louis, MO).

3.2.4 Sodium sulfate (ACS reagent, >99.0%, granular) from Sigma-Aldrich (St. Louis, MO).

3.3 Materials

3.3.1 100 μL syringe for autosampler from Agilent Technologies.

3.3.2 5, 25, 50, 100, 200 μL syringes from Supelco

3.3.3 2 mL autosampler amber vials from Canadian Life Science.

3.3.4 40 mL amber vials from VWR.

3.3.5 DB-624 capillary column (30 m x 0.25 mm x 1.4 μm) from Agilent Technologies.

3.3.6 1 mL Eppendorf pipettor.

3.3.7 Pipettor tips for 1 mL Eppendorf pipettor.

3.3.8 UHP Helium from BOC.

3.3.9 Liquid CO_2 from BOC.

4.0 Health and Safety

4.1 Benzene is a known human carcinogen and, to prevent exposure, its solutions should be handled in a fume hood using appropriate precautions.

5.0 Responsibilities

6.0 Procedures

6.1 Preparation of Standard Solutions

6.1.1 Benzene stock #1

6.1.1.1 Add 20 mL methanol using a 20 mL volumetric pipette to a 20 mL amber vial, cap and weigh the vial.

6.1.1.2 Add 100 μL benzene through the septum to methanol, and weigh the vial to determine the exact amount of benzene added.

6.1.1.3 Cool the vial in the refrigerator, and then replace the septum.

6.1.1.4 Benzene concentration is 4437.8 μg/mL. Calculation must include the volume of benzene added.

6.1.2 Benzene-d6 stock #1

6.1.2.1 Add 20 mL methanol using a 20 mL volumetric pipette to a 20 mL amber vial, cap and weigh the vial.

6.1.2.2 Add 100 μL benzene-d6 through the septum to methanol, and weigh the vial to determine the exact amount of benzene-d6 added.

6.1.2.3 Cool the vial in the refrigerator, and then replace the septum.

6.1.2.4 Benzene-d6 concentration is 4925.4 μg/mL. Calculation must include the volume of benzene-d6 added.

6.1.3 Benzene stock #2

6.1.3.1 Add 20 mL methanol using a 20 mL volumetric pipette to a 20 mL amber vial, cap the vial.

6.1.3.2 Add 200 μL benzene stock #1 (6.1.1) through the septum to methanol.

6.1.3.3 Cool the vial in the refrigerator, and then replace the septum.

6.1.3.4 Benzene concentration is 43.9 μg/mL. Calculation must include the volume of benzene stock #1 added.

6.1.4 Benzene-d6 stock #2

6.1.4.1 Add 20 mL methanol using a 20 mL volumetric pipette to a 20 mL amber vial, and cap the vial.

6.1.4.2 Add 200 μL benzene-d6 stock #1 (6.1.2) through the septum to methanol.

6.1.4.3 Cool the vial in the refrigerator, and then replace the septum.

6.1.4.4 Benzene-d6 concentration is 48.8 μg/mL. Calculation must include the volume of benzene-d6 stock #1 added.

6.1.5 Benzene stock #3

6.1.5.1 Add 20 mL methanol using a 20 mL volumetric pipette to a 20 mL amber vial, cap the vial.

6.1.5.2 Add 100 μL benzene stock #2 (6.1.3) through the septum to methanol.

6.1.5.3 Cool the vial in the refrigerator, and then replace the septum.

6.1.5.4 Benzene concentration is 0.219 μg/mL. Calculation must include the volume of benzene stock #2 added.

6.1.6 Benzene-d6 stock #3

6.1.6.1 Add 20 mL methanol using a 20 mL volumetric pipette to a 20 mL amber vial, and cap the vial.

6.1.6.2 Add 410 μL benzene-d6 stock #2 (6.1.4) through the septum to methanol.

6.1.6.3 Cool the vial in the refrigerator, and then replace the septum.

6.1.6.4 Benzene-d6 concentration is 0.98 μg/mL. Calculation must include the volume of benzene-d6 stock #2 added.

6.1.7 Benzene standard working solutions in methanol

6.1.7.1 Add 20 mL methanol using a 20 mL volumetric pipette to a 20 mL amber vial, and cap the vial.

6.1.7.2 Add 20 μL benzene-d6 stock #1 (6.1.2) through the septum to methanol.

6.1.7.3 Add 50 and 200 μL of benzene stock #2 for STD-1 and STD-2, or add 10, 25, 50, 100, and 200 μL of benzene stock #1 for STD-3, STD-4, STD-5, STD-6, STD-7, through the septum to methanol.

6.1.7.4 Cool the vial in the refrigerator, and then replace the septum.

6.1.7.5 Benzene-d6 concentration is 4.9 μg/mL, and benzene concentrations are 0.11, 0.43, 2.22, 5.53, 11.06, 22.06, and 43.9 μg/mL for STD-1 to STD-7, respectively. Calculation must include the volumes of benzene stock #1 or #2 and benzene-d6 stock #2 added.

6.1.8 Benzene standard working solutions in water

6.1.8.1 Weigh a 40 mL amber vial with cap.

6.1.8.2 Add deionized water to the 40 mL vial within 2 mm from the brim, and cap the vial.

6.1.8.3 Weigh the vial to determine the exact amount of water added using water density.

6.1.8.4 Add 40 μL of STD-1 to STD-7 benzene standard working solutions in methanol through the septum to water to generate STD-1 to STD-7 benzene standard working solutions in water with benzene-d6 at 4.8 μg/L and benzene at 0.107, 0.429, 2.197, 5.454, 11.007, 21.716, and 43.481 μg/L for STD-1 to STD-7, respectively.

6.1.8.5 Cool the vials in the refrigerator, and replace the septa.

6.2 Sample Preparation

6.2.1 Heat sodium sulfate in a beaker in the oven at 200°C for at least 24 hours.

6.2.2 Cool the samples to be analysed overnight in the refrigerator (0 to 4 ˚C).

6.2.3 Weigh 0.4 g sodium sulfate (pre-heated at 200 °C for at least 24 hrs) into a 2 mL autosampler vial.

6.2.4 Heat the vials containing 0.4 g Na_2SO_4 at 200°C for 2 hrs to remove any adsorption of benzene from lab air during the weighing process

6.2.5 Remove the vials from the oven, and cap them as soon as they can be handled comfortably.

6.2.6 Label the vials and then cool them in the refrigerator (0 to 4 °C).

6.2.7 Place the vials on metal tray and the samples in crushed ice.

6.2.8 Add 1 mL portion of the sample via the pipettor into the 2 mL pre-cooled (0 to 4°C) autosampler vial containing 0.4 g Na_2SO_4.

6.2.9 Spike with 5 µL of methanolic solution of benzene-d_6 directly into the sample and cap the vial with Teflon-lined silicone septum.

6.2.10 This sampling and spiking steps in 6.2.8 and 6.2.9 should be completed within 10-15 seconds.

6.2.11 Vortex the vial containing the sample for 1-2 mins to speed up dissolving Na_2SO_4 in the sample until saturation.

6.2.12 Weigh the vial to determine the exact amount of sample in the vial. Record the amount.

6.2.13 Samples should be equilibrated at room temperature for 3 hours before analysis.

6.3 Instrument Conditions

6.3.1 Flow rate of helium carrier gas: 1.5 mL/min.

6.3.2 Injector temperature: 150°C

6.3.3 Sample injection volume: 100 µL, through a 1.5 mm i.d. liner in splitless mode.

6.3.4 GC column: DB-624 capillary column (30 m x 0.25 mm x 1.4 µm).

6.3.5 GC oven temperature program: -5°C for 1 min., raised to 110°C at 10°C/min., then raised to 250°C at 40°C/min., and hold for 5 min.

6.3.6 Headspace sampling was conducted at room temperature (22-24˚C).

6.3.7 The syringe was flushed repeatedly with ambient air before and after an injection.

6.3.8 The mass selective detector was operated in selected ion monitoring (SIM) mode.

6.3.9 Three ions selected for benzene: 78 (quantification ion), 77 and 51.

6.3.10 Three ions for benzene-d_6: 84 (quantification ion), 82 and 52.

6.3.11 Dwell time was 50 ms for each ion.

6.3.12 The gas chromatograph/mass selective detector interface temperature: 280 °C.

6.3.13 Mass selective detector source temperature: 230°C

6.4 Calculations

6.4.1 Benzene concentration in samples was calculated by using an isotope dilution methodology with benzene-d_6 being the internal standard.

7.0 Record Keeping

8.0 Quality Control and Quality Assurance

8.1 For each sequence of analysis, 7 standard solutions in water should be analysed at the beginning. An air sample is analysed after the last standard to check any carry-over. A blank sample (de-ionized water with 0.4 g sodium sulfate) and a standard solution in water (one of the 7 standard solutions in water) are analysed after every 15 samples.

8.2 Linearity: The R^2 values for the calibration plots with benzene peak areas normalized to benzene-d_6 versus concentrations (0.1 to 40 µg/L) should be better than 0.999.

8.3 Repeatability: All samples should be analysed in triplicates, and should be re-analysed if the relative standard deviations of the results are greater than 10%.

8.4 Method Detection Limit: The method detection limit (MDL) was estimated using the U.S. EPA (Environmental Protection Agency) approach:

$$MDL = t_{(n-1,\ 1-\alpha=0.99)} \times SD$$

Where $t_{(n-1,\ 1-\alpha=0.99)}$ is the Student's t-value at 99% confidence level, and is 2.998 when eight replicates are used. SD is the standard deviation of n replicates.

Eight samples of deionized water blanks with 0.4 g sodium sulfate were analysed for benzene, and the average blank benzene level is 0.1 μg/L with standard deviation of 0.0054. Thus the MDL is estimated as 0.016 μg/L.

8.5 Recovery: The possible losses of benzene during sample preparation were evaluated by analysing deionized water samples spiked with benzene at three levels, 1, 5, and 10 μg/L, and the recoveries were 97.7±2.8%, 103.1±2.7%, and 100.4±2.9%, respectively.

9.0 Associated Documents

9.1 References

X.-L. Cao; V. Casey; S. Seaman; B. Tague; A. Becalski (2007) *Determination of benzene in soft drinks and other beverages by isotope dilution headspace gas chromatography/mass spectrometry*. *Journal of AOAC International*, Vol. 90, No. 2, 479-484.

X.-L. Cao; V. Casey (2007) *An improved method for determination of benzene in soft drinks at sub-ppb levels*. *Food Additives and Contaminants*, in press.