INTERNATIONAL COUNCIL OF BEVERAGES ASSOCIATIONS
WASHINGTON, DC · BRUSSELS · TOKYO

Guide de bonnes pratiques pour diminuer le risque de formation de benzène dans les boissons

Adopté par le Conseil ICBA du 29 avril 2006

1 ère MISE À JOUR - 22 JUIN 2006

Traduction : SNBR (Syndicat National des Boissons Rafraichissantes)

Secrétariat 2005-2006
UNESDA - Union of European Beverages Associations
Boulevard Saint Michel 77-79, B-1040 Br,uxelles Belgique
Tél : 32 2 743 40 50 Fax: 32 2 732 51 02 · E-mail : icba@agep.be
www.icba-net.org

Guide de bonnes pratiques pour diminuer le risque de formation de benzène dans les boissons

TABLE DES MATIERES

INTERNATIONAL COUNCIL OF BEVERAGES ASSOCIATIONS
WASHINGTON, DC · BRUXELLES · TOKYO

Guide de bonnes pratiques pour diminuer le risque de formation de benzène dans les boissons

1. Introduction

L'International Council of Beverages Associations (ICBA) est une organisation non gouvernementale représentant les intérêts de tous les acteurs de l'industrie des boissons à l'échelle mondiale. Les membres de l'ICBA produisent, distribuent et commercialisent toute une gamme de boissons non alcoolisées, y compris des boissons gazeuses et non gazeuses telles que les boissons aux fruits, les eaux embouteillées ainsi que les cafés et thés prêts à boire.

2. Historique

En 1990-1991, le secteur des boissons non alcoolisées a pris connaissance que des niveaux élevés de benzène pouvaient être présents dans certaines boissons sous certaines conditions. En partenariat avec la FDA américaine, le secteur a découvert que lorsque l'acide ascorbique (la vitamine C) était utilisé en association avec le benzoate de sodium (un conservateur), une formation de benzène pouvait intervenir. Cette formation se trouvait augmentée lorsque la boisson était stockée pendant de longues périodes à des températures élevées.

Bien que les niveaux et la fréquence à laquelle se formait le benzène n'ont pas été considérés comme posant un risque de santé publique, le secteur avait pris immédiatement l'initiative de reformuler les produits concernés afin de minimiser tout risque de formation tout en poursuivant d'assurer l'intégrité microbiologique.

Pour les boissons et les autres denrées alimentaires, les autorités de contrôle par exemple la FSANZ pour l'Australie et la Nouvelle Zélande, Le JRC pour l'UE, le Canada, la FSA au Royaume-Uni et la FDA aux Etats-Unis suivent une approche d'ensemble de surveillance et d'évaluation de l'exposition pour évaluer les risques. La dernière étude, intitulée *Composés organiques volatiles présents dans les aliments : Etude sur cinq années* était dirigée par la FDA et publiée dans le Journal of Agriculture and Food Chemistry en 2003. Cette étude a révélé que le benzène avait été décelé dans *tous* les aliments testés, fruits et légumes y compris, à l'exception du fromage et de la glace à la vanille américains. Ces niveaux s'échelonnaient de 1 à 190 ppb (microgrammes par kilo). La FDA a conclu que les données recueillies au cours de l'étude indiquaient que l'approvisionnement alimentaire aux USA est comparativement plus sûr et que bien qu'une exposition orale existe face aux composés organiques volatiles tels que le benzène, l'exposition est en réalité bien plus élevée par inhalation. En fait, selon un article paru dans Food Chemical News du 27 février 2006, une publication officielle du Centre de Science Alimentaire et de Nutrition Appliquée de la FDA, il a été établi que l'ensemble des produits alimentaires était responsable pour seulement 5% de l'exposition totale au benzène. D'autres études ayant abouti à la même conclusion regroupent les entités suivantes : Surveillance alimentaire MAAF - Royaume-Uni No 58 – Benzène et autres hydrocarbures aromatiques dans les rations alimentaires moyennes au Royaume-Uni – Mars 1995; Centre de recherche commune UE, HEXPOC, 2005 – Caractérisation de l'exposition humaine aux substances chimiques ; quantification des voies d'exposition ; Canada : B.D. Page et autres – Journal de l'AOAC Intl., 1992, 75, (2) 334-340.

Malgré cela, le secteur a suivi une approche responsable pour prévenir/minimiser la présence du benzène dans les boissons. Aujourd'hui, alors que le secteur des boissons poursuit sa croissance et son expansion, L'ICBA (International Council of Beverages Associations) renouvelle son engagement pour apporter des conseils en matière de prévention/diminution de la formation de benzène. Ce document d'orientation sera mis à la disposition de *toutes* les sociétés du secteur des boissons dans le monde, qu'elles soient ou non membres affiliés de l'ICBA. Ainsi, l'ICBA va s'engager dans un effort concerté, par le biais de ses Associations-membres, à fournir ces informations à toutes les entreprises productrices de boissons.

3. Facteurs déclencheurs et modérateurs de la formation de benzène dans les boissons

3.1 Facteurs déclencheurs pouvant conduire à la formation de benzène dans les boissons

* *Dominante principale :* **La formation de benzène peut se produire en parties par milliard (microgrammes par kilo) dans certaines formulations de boissons contenant du benzoate de sodium ou du benzoate de potassium associé à de l'acide ascorbique.(1) Les quantités augmentent avec la chaleur et/ou la lumière, la chaleur étant le facteur prédominant.**

***** Des études ont indiqué que l'acide érythorbique – lorsqu'il est autorisé – peut conduire à la formation de benzène selon, quasiment, le même processus que l'acide ascorbique.

* La formation de benzène peut également intervenir lorsque du jus de fruits et d'autres ingrédients qui contiennent naturellement ou non de l'acide benzoïque et de l'acide ascorbique sont utilisés dans les formulations des boissons.

---

[1] L.K. Gardner et G.D. Lawrence, J. Agric. Fd. Chem. 1993, 41 (5), 693-695

3.2 Facteurs modérateurs qui peuvent atténuer la formation de benzène dans les boissons contenant de l'acide benzoïque et de l'acide ascorbique

* Les ingrédients, tels que les glucides simples (sucre, sirop de sucre, de glucose riche en fructose et glucose cristallisé) et l'acide éthylène-diamine-tétraacétique de calcium disodium (EDTA) – s'il est autorisé – ou le polyphosphate (ou hexamétaphosphate) de sodium, peuvent atténuer la formation de benzène.

* Il est prouvé que les glucides simples peuvent retarder la réaction, car le phénomène semble davantage perceptible dans les boissons light, toutefois, plus le produit est présent longtemps sur le marché (rayons-étalages), plus grand est le risque de voir la formation de benzène, si ses précurseurs sont présents.

* Des faits indiquent également que l'EDTA – lorsqu'il est autorisé – peut atténuer la réaction, peut-être en complexant des ions métalliques qui pourraient agir comme des catalyseurs. Cette réaction peut être perturbée dans des produits contenant du calcium ou d'autres minéraux – notamment lorsqu'ils sont utilisés en enrichissement – car ils peuvent interférer dans le processus.

4. Recommandations ICBA importantes destinées aux producteurs de boissons pour prévenir/minimiser la présence de benzène

En prenant en compte les facteurs déclencheurs et modérateurs de la formation de benzène dans les boissons, comme précisé ci-dessus (section 3), l'ICBA recommande les points suivants :

- ✓ ***RECOMMANDATION 1 : CONTROLE***
  Toutes les sociétés du secteur des boissons **réexaminent leurs produits déjà existants ainsi que les nouvelles formulations** en prenant en considération les informations fournies plus haut relatives aux procédures permettant de prévenir / minimiser la formation de benzène.

- ✓ ***RECOMMENDATION 2 : TESTS***
  Toutes les sociétés du secteur des boissons **réalisent des analyses d' échantillons des produits concernés** pour déceler la présence éventuelle de benzène par des tests probatoires de stockage accélérés *(pour plus de détails sur les phases de tests, voir la section 5).*

- ✓ ***RECOMMENDATION 3 : REFORMULATION***
  Les entreprises du secteur des boissons **reformulent tous les produits concernés** dans lesquels le benzène peut être présent pour prévenir/minimiser au mieux sa formation.

- ✓ ***RECOMMENDATION 4 : SURVEILLANCE POST-LANCEMENT***
  Dans le processus d'échantillonnage des produits commercialisés, ou toute autre procédure appropriée, les entreprises doivent **confirmer que toutes les nouvelles formulations ou reformulations répondent aux exigences de ce document.**

5. Documents d'orientation : Tests pour vérifier la présence éventuelle de benzène dans les boissons

5.1 Tests accélérés

Des tests en accéléré doivent être conduits pour les formulations produits contenant de l'acide benzoïque – y compris le benzoate ajouté – et de l'acide ascorbique. Les conditions de tests spécifiques peuvent varier d'un producteur à un autre mais elles doivent regrouper les facteurs temps et température qui doivent reprendre les conditions normales de distribution que le produit connaîtra. Comme point de départ, les producteurs peuvent envisager de soumettre les produits à des températures d'un minimum de 40-60 degrés C pendant 24 heures ou plus longtemps en fonction de la formulation ; par exemple certaines formulations nécessitent une exposition test en accéléré de 14 jours pour évaluer les risques de réaction.

5.2 Procédures analytiques

Des procédures analytiques fiables pour le benzène devraient être validées grâce à des tests de performance appropriés ou des laboratoires externes accrédités, capables de déterminer au moins 5 ppb (microgrammes/kg) de benzène dans les boissons. (Voir en annexe les exemples de méthodes d'analyse).

6. Document d'orientation : Stratégies de contrôle des formulations

Comme noté précédemment, les principaux facteurs de formation du benzène dans les boissons sont généralement constitués d'une combinaison de la présence d'acide benzoïque, d'acide ascorbique, de la chaleur et du temps. Cependant, d'autres points de contrôles (CP) que les producteurs de boissons doivent considérer lors de la formulation d'un produit peuvent par exemple être les suivants :

♦ Eaux embouteillées
? doivent répondre aux exigences réglementaires locales, y compris sur les limites fixées pour le benzène, dans l'eau potable. *De plus, voir la section 'Métaux de transition'*
CP – Vérifier la présence de benzène dans l'eau.

♦ Les sucres (glucides simples)
? apparaissent comme des ralentisseurs de la formation de benzène, mais ne l'empêchent pas totalement.

♦ Jus de fruits
? peuvent être livrés avec du benzoate – lorsqu'il est autorisé - et/ou contenir une source naturelle d'acide benzoïque.
CP – revoir les spécifications avec le fournisseur pour éliminer le benzoate ou en réduire la teneur.

? peut être une source d'acide ascorbique (additif ou naturellement présent)
CP – analyser pour déceler la présence d'ascorbate ou obtenir les teneurs auprès du fournisseur.

♦ Edulcorants intenses
? les produits light présentent les risques les plus élevés de formation de benzène si les précurseurs sont présents.

♦ Gaz carbonique
? assurer la conformité avec les exigences réglementaires locales ou la norme de la International Society of Beverage Technologists (ISBT) de 20 ppb (microgrammes/kg) maximum de benzène.
CP – spécifications du fournisseur et analyses avec vérifications.

♦ Acides
? A un pH faible, l'acide ascorbique et/ou l'acide érythorbique, en association avec de l'acide benzoïque, conduit à un risque plus élevé de formation de benzène.

- Arômes/
  ? Arômes, agents émulsifiants et épaississants peuvent contenir des conservateurs et des antioxygènes.
  CP – revoir les spécifications avec le fournisseur pour éliminer le benzoate ou en réduire la teneur.

  ? Le benzaldéhyde et l'acide ascorbique peuvent également former du benzène. CP – vérifier la présence de benzaldéhyde.

- Colorants
  ? peuvent contenir de l'ascorbate comme antioxygène pour éviter la décoloration.
  CP – vérifier avec les fournisseurs et changer les spécifications si nécessaire.

- Conservateurs
  Tous les sites de fabrication de boissons sont soumis à des conditions d'hygiène strictes, conformément aux principes HACCP.

  ? Prendre en considération l'utilisation de mélanges de sorbate et de benzoate, s'il existe une nécessité technologique (stabilité microbiologique ou solubilité du sorbate).
  CP – Etudier la possibilité de retirer/réduire ou remplacer le benzoate par le sorbate ou d'autres méthodes de conservation. Noter que le sorbate peut précipiter dans des sirops destinés aux distributeurs automatiques (fontaines)

  ? Produits à diluer : (Squash, sirops de fruits – habituellement 5 fois concentrés, dilués avant consommation) nécessitent d'être protégés par des conservateurs parce qu'ils sont fréquemment ouverts au cours de leur longue durée d'utilisation.

  CP – Etudier la possibilité de retirer/réduire ou remplacer le benzoate par le sorbate ou par d'autres systèmes de conservation.
  Noter que le sorbate doit être utilisé avec soin pour éviter qu'il précipite. Etudier la possibilité d'utiliser des produits alternatifs à l'acide ascorbique s'il est présent.

- Antioxygènes
  ? Prendre en considération l'utilisation d'ascorbate pour l'ensemble de la formule, particulièrement si les jus d'agrumes ou d'autres vecteurs naturels d'acide ascorbique sont présents.
  CP – Retirer/réduire/remplacer l'ascorbate lorsque c'est possible si une source d'acide benzoïque est présente.

- Lumière
  ? Les rayons UV peuvent induire la formation de radicaux libres dans les produits.
  CP – Revoir les conditions de stockage et de présentation en rayon ainsi que les instructions portées sur les étiquettes.

- Température
  ? Elle accélère la formation de benzène si les précurseurs sont présents.
  CP – Revoir les conditions de stockage et la DLUO ainsi que les instructions portées sur les étiquettes.

- Métaux de transition
  ? Des traces d’ions métalliques, tels que le cuivre et le fer, peuvent agir comme des catalyseurs de la formation du benzène dans les boissons en présence d’acide benzoïque et d’acide ascorbique. Les sources de métaux de transition peuvent inclure l’eau de fabrication, les édulcorants ou d’autres ingrédients.
  PC- Des composés chélatés tels que l’EDTA (lorsqu’il est autorisé) ou les polyphosphates de sodium peuvent aider à atténuer la formation de benzène. Un enrichissement en calcium ou en d’autres minéraux, peut diminuer cet effet.

ANNEXE : EXEMPLES DE METHODES D'ANALYSES

NB: Les méthodes d'analyses suivantes sont données comme exemples destinés aux entreprises lorsque cela est applicable (Version anglaise uniquement).

ANNEX: EXAMPLES OF METHODS OF ANALYSIS

NB: The following methods of analysis are non-binding suggestions and examples for use by companies as appropriate.

1. Determination of Benzene in Carbonated and Non-Carbonated Beverages – Gas Chromatography Method using Headspace

2. Purge/Trap GCMS Quantitation of Trace Level Benzene in Carbonated Soft Drinks and Juice Products

1. Determination of benzene in carbonated and non-carbonated beverages – gas chromatography method using headspace

1. SCOPE AND PURPOSE

This method specifies a gas chromatography method using headspace for the determination of benzene in carbonated and non-carbonated beverages.

2. RESPONSIBILITIES

2.1. Trained lab associates are responsible for preparing the reagents and for conducting the analyses. They are also responsible for recording the results of the samples and check samples properly in the proper raw data forms. They need to evaluate the obtained results critically and apply the means necessary to control themselves by calibrations and checks.

2.2. The Laboratory Operation Manager is responsible for the effectiveness of the procedure. He/she also provides expertise assistance in evaluating methods and results.

2.3. The Laboratory Manager is responsible for making sure the laboratory has the necessary resources(equipment and personnel) to perform the tests, checking and calibration activities properly.

3. PRINCIPLE

The sample is being heated in a closed headspace vial, in order to obtain equilibrium between the concentration of the benzene in the headspace above the sample and the concentration in the sample. Prior to analysis, carbonated samples are being treated with sodium hydroxide in order to neutralize the carbon dioxide. In order to increase the headspace efficiency of benzene, a matrix modifier (sodium chloride) is being added to the vial. After establishment of the equilibrium, an aliquot of the gaseous phase is injected on a gas chromatograph. In order to obtain higher sensitivity the benzene is trapped on a cold trap prior to injection on the capillary column. The components are detected by mass spectrometry detector (MS) in full scan mode. Identification is based on retention time and library mass spectra comparison. Quantification is based on the intensity of the main ion using a four-point calibration and the internal standard method.

4. EQUIPMENT AND GLASSWARE

4.1. Appropriate laboratory equipment
4.2. Volumetric flasks with glass stoppers
4.3. 20 ml headspace vials with magnetic crimp caps
4.4. Finnigan Trace GC- DSQ- mass spectrometer with Combipal auto sampler with headspace
configuration (GC210 or 212)
4.5. Capillary column: Restek RTX-1 60m * 1µm df * 0.25 mm ID
4.6. Ultra pure water generator: Millipore MilliQ (PW202 or 203)
4.7. Graduated Hamilton syringes (10, 25 and 50 µl)
4.8. Hamilton digital syringe 25 µl
4.9. Analytical balance (AB201, 202 or 203)

5. REAGENTS AND SOLUTIONS

5.1. REAGENTS

5.1.1. Ultra pure water cooled at 4 °C
5.1.2. Benzene (CHBEN92)
5.1.3. Benzene-d6 (CHBEN91)
5.1.4. Methanol, purge and trap grade (CHMET05)
5.1.5. Sodium hydroxide (CHSOH04)
5.1.6. Sodium chloride (CHSOC05)

5.2. SOLUTIONS

Only use volumetric flasks with glass stoppers (4.2)

5.2.1. Benzene stock solution 1000 mg/l (solution code SL-045-01)

Weigh a dry, empty volumetric flask of 50 ml with stopper to an accuracy of 0.1 mg. (**m1**) on the
analytical balance (AB201). Place 25 ml methanol (5.1.4) in the volumetric flask, place the flask on the analytical balance and tare. Using a pasteur pipette, add +/- 50 mg of benzene (5.1.2) and determine the exact weight. (**m2**). Make sure the benzene is directly added to the methanol (no contact with the inside surface). Make up to the mark with methanol.
Determine the weight of the flask with the solution with stopper to an accuracy of 0.1 mg (**m3**).
The exact concentration of the working standard is calculated as follows:
$C1 = (m2/ ((m3-m1)/0.7915)) * 1000$ (mg/l)
Where
$C1$ is the concentration of the benzene stock solution (mg/l)
$m1$ is the mass of the empty flask (g)
$m2$ is the mass of the benzene (mg)
$m3$ is the mass of the full flask (g)

0.7915 is the density of methanol (20°C)
This solution is stable for three months if stored at -18 °C in the dark. Store the solution in an amber glass

5.2.2. Benzene control stock solution 1000 mg/l (solution code SL-045-02)

Prepare the independent benzene control stock solution 1000 mg/l using the same procedure as
described in 5.2.1. This solution is stable for three months if stored at -18 °C in the dark. Store the solution in an amber glass flask.

5.2.3. Benzene working spike solution 2 ppm (solution code SL-045-03)

Weigh a dry empty volumetric flask of 50 ml with stopper to an accuracy of 0.1 mg (**m4**). Place +/- 45 ml of methanol in the volumetric flask. Using a 0.25 ml syringe add 0.1 ml of the benzene stock solution 1000 mg/l (5.2.1). Record the weight of the added solution (**m5**). Make up to the mark with methanol and determine the weight of the full flask with stopper. (**m6**).
The exact concentration of the working spike solution is calculated as follows:
C2 = ((m5)/(m6-m4)) * C1 (mg/l)
Where
C2 is the concentration of the benzene working spike solution
m4 is the mass of the empty flask (g)
m5 is the mass of the added benzene control stock solution 1000 mg/l (mg)
m6 is the mass of the full flask (g)
C1 is the concentration of the benzene stock solution 1000 mg/l (5.2.1)
This solution is stable for one month if stored at -18 °C in the dark. Store the solution in an amber glass flask.

5.2.4. Benzene control spike solution 2 ppm (solution code SL-045-04)

Weigh a dry empty volumetric flask of 50 ml with stopper to an accuracy of 0.1 mg (**m7**). Place +/- 45 ml of methanol in the volumetric flask. Using a 0.25 ml syringe add 0.1 ml of the benzene control stock solution 1000 mg/l (5.2.2). Record the weight of the added solution (**m8**). Make up to the mark with methanol and determine the weight of the full flask with stopper. (**m9**).
The exact concentration of the working spike solution is calculated as follows:
C3 = ((m8)/(m9-m7)) * Ccontr (mg/l)
Where
C3 is the concentration of the benzene control spike solution
m7 is the mass of the empty flask (g)
m8 is the mass of the added benzene control stock solution 1000 mg/l (mg)
m9 is the mass of the full flask (g)
Ccontr is the concentration of the benzene control stock solution 1000 mg/l 5.2.2.
This solution is stable for one month if stored at -18 °C in the dark. Store the solution in an amber glass flask.

5.2.5. Internal standard stock solution 1000 ppm (solution code SL-045-05)

Weigh a dry, empty volumetric flask of 50 ml with stopper to an accuracy of 0.1 mg. (**m10**) on the analytical balance. Place 25 ml methanol (5.1.5) in the volumetric flask. Weigh ± 50 mg of benzene-d6 (5.1.3) into the flask and note the exact weight (**m11**). Make up to the mark with methanol and close. Make sure the benzene is directly added to the methanol (no contact with the inside surface).
Determine the weight of the flask with the solution with stopper to an accuracy of 0.1 mg (**m12**).
The exact concentration for each internal standard is calculated as follows:
$C4 = (m10/((m11-m9)/0.7915))*1000$ (mg/l)
Where
C4 is the concentration of the internal standard stock solution
m9 is the mass of the empty volumetric flask with stopper (g)
m11 is the mass of the volumetric flask with solution and stopper (g)
m10 is the mass of the internal standard (mg)
0.7915 is the density of methanol (20°C)
This solution is stable for six months if stored cooled in the dark. Store the solution in an amber glass flask at – 18 °C in the dark.

5.2.6. Internal standard spike solution 1 ppm (solution code SL-045-06)

Place ± 40 ml of methanol (5.1.5) into a 50 ml volumetric flask. Inject 50 µl of the internal standard stock solution (5.2.5) using a 0.25 ml syringe under the surface of the methanol. Make up to the mark with methanol. This solution is stable for one month if stored cooled in the dark. Store the solution in an amber glass flask at – 18 °C in the dark.

5.2.7. VOC calibration standards (0.4 – 4 ppb) and control standard 1 ppb

All the calibration standards have to be prepared with cooled ultra pure water (5.1.1)
Prepare the calibration standards as follows:

5.2.7.1. 0.4 ppb: Weigh +/- 3.5 gr of NaCl (5.1.6) into a 20 ml headspace vial. Pipette 10 ml of
cooled ultra pure water. Add 10 µl of the internal standard spike solution (5.2.6) using a 25 µl digital syringe (4.8). Add 2 µl of benzene working spike solution 2 ppm (5.2.3) using a 10 µl syringe. Close the vial immediately with a magnetic crimp cap.

5.2.7.2. 1 ppb: Weigh +/- 3.5 gr of NaCl (5.1.6) into a 20 ml headspace vial. Pipette 10 ml of
cooled ultra pure water. Add 10 µl of the internal standard spike solution (5.2.6) using a 25 µl digital syringe (4.8). Add 5 µl of benzene working spike solution 2 ppm (5.2.3) using a 25 µl syringe. Close the vial immediately with a magnetic crimp cap.

5.2.7.3. 2 ppb: Weigh +/- 3.5 gr of NaCl (5.1.6) into a 20 ml headspace vial. Pipette 10 ml of
cooled ultra pure water. Add 10 µl of the internal standard spike solution (5.2.6)
using a 25 µl digital syringe (4.8). Add 10 µl of benzene working spike solution 2
ppm (5.2.3) using a 25 µl syringe. Close the vial immediately with a magnetic crimp cap.

5.2.7.4. 4 ppb: Weigh +/- 3.5 gr of NaCl (5.1.6) into a 20 ml headspace vial. Pipette 10 ml of
cooled ultra pure water. Add 10 µl of the internal standard spike solution (5.2.6) using a 25 µl digital syringe (4.8). Add 20 µl of benzene working spike solution 2 ppm (5.2.3) using a 25 µl syringe. Close the vial immediately with a magnetic crimp cap.

5.2.7.5. Contr 1 ppb: Weigh +/- 3.5 gr of NaCl (5.1.6) into a 20 ml headspace vial. Pipette 10 ml of cooled ultra pure water. Add 10 µl of the internal standard spike solution (5.2.6)
using a 25 µl digital syringe (4.8). Add 5 µl of benzene control spike solution 2 ppm (5.2.4) using a 25 µl syringe. Close the vial immediately with a magnetic crimp cap.

5.2.8. Sodium hydroxide 30 %

Weigh +/- 60 g of sodium hydroxide (5.1.5) into a clean glass beaker and add 200 ml of ultrapure water.
Dissolve the sodium hydroxide and allow cooling to room temperature.

6. SAMPLE STORAGE

Samples have to be stored at 4 °C in the dark.

7. PROCEDURE

7.1. Pretreatment of samples

For carbonated samples add 1 ml of sodium hydroxide 30% to a 40 ml vial and fill the vial to the top with the sample. Close the vial with a PTFE septum containing screwcap. Non carbonated samples are not pretreated.

7.2 Sample and blank preparation

Weigh +/- 3.5 g NaCl (5.1.6) into a 20 ml headspace vial. Pipette 10 ml of the sample into the 20 ml headspace vial (4.3). Add 10 µl of the internal standard spike solution (5.2.6) using the digital syringe of 25 µl. The vial is closed with a magnetic crimp cap with a silicon/PTFE septum.
For blank preparation, 10 ml of ultrapure water is treated the same way as a sample.

7.2. Preparing the headspace auto sampler.

Place the vials on the tray of the Combipal headspace auto sampler. The vials have to be analyzed in the following order:
blank - calibration standards - blank – control standard 1 ppb – samples – control standard 1 ppb
After every 10 samples a control standard of 1 ppb has to be analysed. Indicate in the Cycle Composer software which vials have to be analysed and start the autosampler. Start the cryo-cooling trap once the first thermostatization time has almost expired.

7.3. Excalibur software

Fill in the sample table according to the samples to be analysed.
Mark the blanks and samples as unknown and the calibration standards as standard. Use appropriate filenames to save the data. Use the sample name field to describe the sample clearly. Select the correct method for the gas chromatograph. The parameters for the gas chromatograph are described in appendix 2. Save and start the sample table.

7.4. Calibration

7.5.1. Calibration Frequency

A full calibration curve has to be analyzed at least once a week. Each day a calibration standard of 1 ppb has to be analyzed.

7.5.2. Calibration type

A linear calibration curve using internal standard is used for the calculation.
$Y=aX+b$ where
Y= relative response factor calculated by A_s/A_i
Where A_s = area benzene in the sample
A_i = area internal standard in the sample
X= concentration of benzene in the sample
a= slope of the linear regression curve (dY/dX)
b= intercept

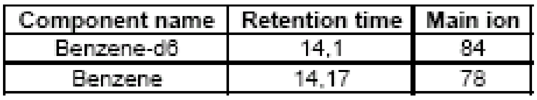
8. EXPRESSION OF RESULTS

8.1. Identification and quantification

The identification of the components is based on their retention times and mass spectra library
comparison. Table 1 contains the indicative retention times of the components.
These retention times slightly vary due to aging of the column and are to be corrected.
The quantification of the components is being done automatically by the software using linear regression with internal standard compensation. For carbonated samples, a

correction factor of 1,025 is used to calculate the final result (takes into account the addition of NaOH).
Subtracting blank values from the sample results is not permitted.

Table 1: Indicative retention times of the components to be quantified



Component name	Retention time	Main ion
Benzene-d6	14,1	84
Benzene	14,17	78

8.2. Reporting of results

Results are expressed as µg/l . Results below 0.5 µg/l are to be reported as '<0.5 µg/l'.

9. REFERENCES

- EPA method 5021A. Volatile organic compounds in various sample matrices using equilibrium headspace analysis.
- EPA method 8260B Volatile organic compounds by gas chromatography/mass spectrometry.
- EPA method 524.2 Measurement of purgeable organic compounds in water by capillary column gas chromatography/mass spectrometry.

Appendix 1: Combipal headspace parameters

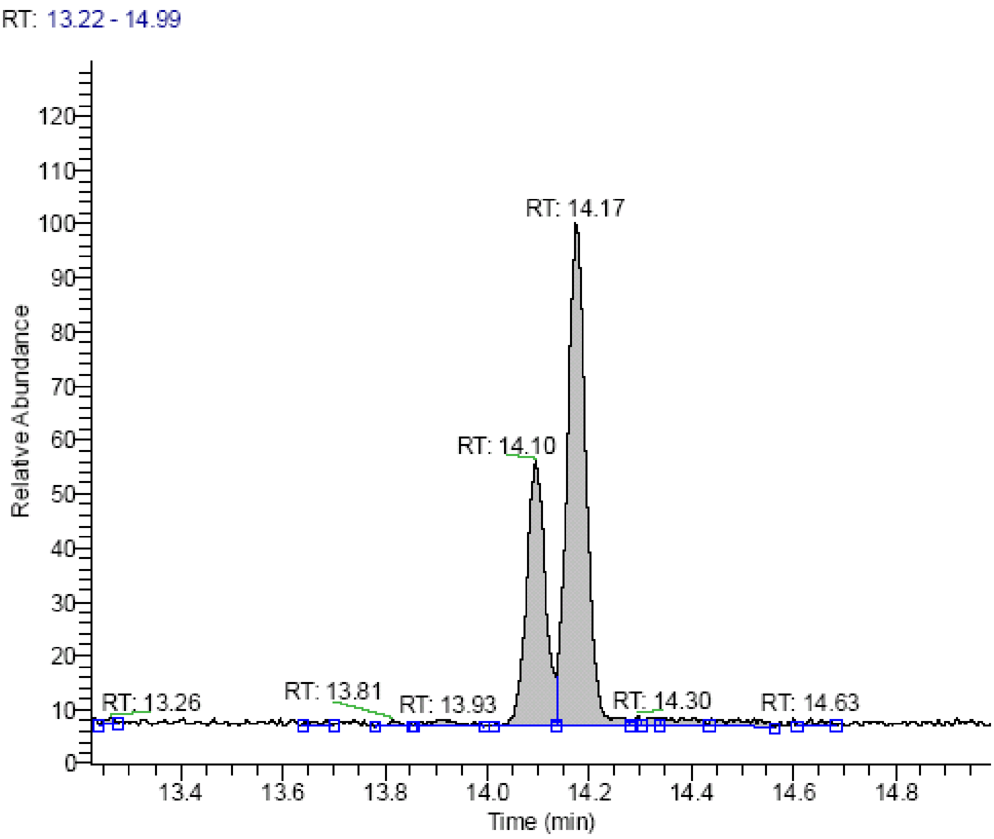
Method name: VOC-25min-benz
Incubation temperature: 60 °C
Incubation time: 25 min
Syringe temperature: 90 °C
Agitator speed: 500 rpm
Agitator on time: 2 sec
Agitator off time: 5 sec
Fill speed: 250 µl/sec
Injection speed: 15µl/sec
GC runtime: 30 min

Appendix 2: GC/MS parameters

Method name: VOC-20-benzene
MS parameters:
Acquisition time: 16 min
Source temperature : 280 °C
Scan mode : Full scal
Scan rate : 750 amu/s
Scan range : 40-250 amu

GC parameters:
Oven method:
Initial temp: 40 °C
Initial time: 5 min
Rate 1: 5 °C/min
Temp 1: 90 °C
Hold time 1: 0 min
Rate 2: 80 °C/min
Temp 2: 300 °C
Hold time 2: 5 min
Injection method:
Injection temp: 230 °C
Mode: Splitless
Splitless time: 6 min
Split flow: 25 ml/min
Flow method:
Mode: Constant flow
Flow: 1 ml/min

Appendix 3: Example chromatogram



2. Purge/Trap GCMS Quantitation of Trace Level Benzene in Carbonated Soft Drinks and Juice Products

Summary:

A. Instrument Operation Parameters

Three purge and trap GC/MS instruments were used for the analyses:

1. Agilent 6890/5973 #1 GC/MS, Tekmar Velocity purge and trap concentrator and Tekmar Solatek purge and trap autosampler.
2 Agilent 6890/5973 #2 GC/MS, EST Encon purge and trap concentrator and EST Archon 5100 purge and trap autosampler.
3 Agilent 6890/5973 #3 GC/MS, Tekmar Velocity purge and trap concentrator and Archon (OI 4552) autosampler.

1. 6890/5973 #1 A 30 m x 0.25 mm x 0.25 µm HP-5MS column (Agilent 19091S-433) was held at 45 ºC for 2 minutes, than increased at 10 ºC/minute to 65 ºC, increased at 25 ºC to 250 ºC and held at 250 ºC for 5 minutes. The injection port was set at 225 ºC with a 50:1 split ratio and a 4 mm ID split/splitless liner. Carrier gas regulated by the 6890's EPC is supplied to the Velocity. It is returned to the 6890 via the heated transfer line which is plumbed into the GC inlet.

The column was operated in the constant pressure mode at 12.33 psig with a nominal flow rate of 0.8 mL/minute. The gas saver was set to 20 mL/minute after 2 minutes run time. The column terminated in an Agilent microfluidics splitter which diverted approximately 20% of the column effluent to an Agilent µ-ECD. The ECD data was not collected for this analysis. The splitter had a 3.8 psig make up gas supply, making the column output 3.8 psig above ambient pressure.

The MSD transfer line was set at 280 ºC, the MS Quad was at 150 ºC and the MS Source was at 230 ºC. The electron multiplier offset was 106 volts and the multiplier voltage was 1694 volts. A tune file of ATUNE was used. Selected-Ion-Monitoring was employed for the analysis. Ions monitored were m/z 77, 78 for benzene and 82, 84 for d^6-benzene internal standard in low resolution mode and the dwell time for these ions was 100 ms.

Benzene, target ion m/z 78, qualifier ion 77, and d^6-benzene, target ion m/z 84, qualifier ion 82, were monitored from 3.23 to 3.65 minutes. Retention times are benzene, 3.45 minutes, and d^6-benzene, 3.43 minutes.

A Tekmar Velocity XPT purge and trap concentrator with the Solatek autosampler was used in the soils mode. In this mode samples are purged in the 40-mL sample vial (I-

Chem Certified 200 series, Fisher Scientific 05-719-102) by the autosampler. Purge gas is carried to the head of the concentrator trap via a heated SilcoSteel transfer line.

A Supelco type J, or BTEX, trap (Supelco 21064) was used. This is a 25 cm long 1/8" stainless steel tube packed with 7.7 cm of Carbopak C and 1.2 cm of Carbopak B. The analytical conditions for the purge and trap concentrator/autosampler are as follows. These analytical conditions are set up in Tekmar's Teklink software which operates the sampler and starts the GC/MS run when the desorption cycle begins.

Variable	Value	Variable	Value
Rinse Water Temp	90 ºC	Pre-Purge Flow	40 mL/min
Sample Cup Temp	40 ºC	Sample Heater	Off
Sample Needle Temp	50 ºC	Sample Preheat Time	1.00 min
Transfer Line Temp	150 ºC	Preheat Temp	40 ºC
Soil Valve Temp	125 ºC	Purge Temp	0 ºC (a default setting)
Sample Sweep Time	0.50 min	Purge Flow	40 mL/min
Needle Rinse Volume	15 mL	Dry Purge Time	0.50 min
Needle Sweep Time	1.00 min	Dry Purge Temp	20 ºC
Sample Preheat Time	1.00 min	Dry Purge Flow	50 mL/min
Preheat Stir	Off	GC Start	Start of Desorb
Preheat Stir Mode	Spin	Desorb Preheat Temp	250 ºC
Preheat Stir Speed	5	Desorb Drain	On
Purge Time	11.00 min	Desorb Time	2.00 min
Purge Stir	Off	Desorb Temp	250 ºC
Purge Stir Mode	Agitate	Desorb Flow	0 mL/min (a default setting)
Purge Stir Speed	5	Bake Time	4 min
Valve Oven Temp	150 ºC	Bake Temp	260 ºC
Transfer Line Temp	150 ºC	Dry Flow Bake Temp	300 ºC
Sample Mount Temp	90 ºC	Bake Flow	400 ml/min
Purge Ready Temp	35 ºC	Focus Temp	-150 ºC
Dry Flow Standby Temp	200 ºC	Inject Time	1.00 min
Standby Flow	20 mL/min	Inject Temp	180 ºC
Pre-Purge Time	0.50 min	Standby Temp	100 ºC

Table 1. Tekmar Velocity and Solatek Settings

2. 6890/5973 #2
A 60 m x 0.25 mm x 1.4 µm DB-624 column (Agilent 122-1364) was held at 40 ºC for 2 minutes, than increased at 10 ºC/minute to 180 ºC, than increased at 40 ºC/minute to 250 ºC, and held at 250 ºC for 2 minutes. The injection port was set at 200 ºC with a 40:1 split ratio. Carrier gas regulated by the 6890's EPC is supplied to the Encon. It is returned to the 6890 via the heated transfer line which is plumbed into the GC inlet.

Some problems were experienced with ghost peaks/sample carryover due to the length of the column. This instrument is configured for another analysis which requires that column. It is recommended to use no longer than a 30 m thin film column for benzene analysis. The column was operated in the constant flow mode at 16.65 psig initial pressure with a flow rate of 1.0 mL/minute. The gas saver was set to 20 mL/minute after 2 minutes run time.

The MSD transfer line was set at 280 ºC, the MS Quad was at 150 ºC and the MS Source was at 230 ºC. The electron multiplier offset was off and the multiplier voltage was 1670 volts. It used a tune file of ATUNE. Solvent delay was 10 minutes.

Selected-Ion-Monitoring was used for the analysis. Ions monitored were m/z 77, 78 for benzene and 84, 82 for d^6-benzene internal standard. The dwell time for these ions was 85 ms.

Benzene, Rt 11.3 minutes, was detected using target ion m/z 78, qualifier ion 77. d^6-benzene internal standard was detected using target ion 84 and qualifier ion 82. The mass spectrometer was operated with high resolution.

The Encon purge and trap concentrator with the Archon 5100 autosampler was used in the soils mode. Purge gas is carried to the head of the concentrator trap via a heated SilcoSteel transfer line. The Encon used a Type K, or Vocarb 3000 trap (PTS Catalog E70300-K03). It is a 25 cm long 1/8" stainless steel tube packed with 10 cm Carbopak B, 6 cm Carboxen 1000 and 1 cm Carboxen 1001.

Variable	Value	Variable	Value
Standby Flow	On	Drain	On
Bake Gas Bypass	On	Antifoam	Cont
Total GC Time	0 Minutes	Valve Oven	130 ºC
Transfer Line	130 ºC	MoRT Ready	40 ºC
MoRT Bake	260 ºC	Purge Ready	35 ºC
Purge Time	11 Minutes	Dry Purge Time	1 Minute
Purge Flow	40 mL/Minute	Desorb Flow	0 mL/Min (a default setting)
Preheat Temp	40 ºC	Preheat Time	0.5 Minute
Desorb Preheat	245 ºC	Desorb Temp	250 ºC

Desorb Time	2 Minute	Bake	260 ºC
Bake Time	10 Minutes		

Table 2. Encon and Archon Settings

3. 6890/5973 #3

A 20 m x 0.18 mm x 1 µm DB-624 column (Agilent 122-1324) was held at 40 ºC for 3 minutes, than increased at 15 ºC/minute to 210 ºC and held at 210 ºC for 0.33 minutes. The injection port was set at 210 ºC with a 50:1 split ratio. Carrier gas regulated by the 6890's EPC is supplied to the Encon. It is returned to the 6890 via the heated transfer line which is plumbed into the GC inlet.

The column was operated in the constant flow mode at 16.53 psig initial pressure with a flow rate of 0.8 mL/minute. The gas saver was off.

The MSD transfer line was set at 190 ºC, the MS Quad was at 150 ºC and the MS Source was at 230 ºC. The electron multiplier offset was off and the multiplier voltage was 1176 volts. It used a tune file of ATUNE. Solvent delay was 4.5 minutes.

Selected Ion Monitoring was used for the analysis. Ions monitored were m/z 77, 78 for benzene and 84, 82 for d^6-benzene internal standard. The MSD was set to low resolution and the dwell time for these ions was 85 ms.

Benzene, Rt 5.19 minutes, was detected and amount was quantitated using target ion m/z 78. d^6-benzene, Rt 5.16 minutes, was detected using target ion m/z 84.

The Velocity purge and trap concentrator with the Archon autosampler was used in the soil mode. Purge gas is carried to the head of the concentrator trap via a heated SilcoSteel transfer line. The Velocity used a Type K, or Vocarb 3000 trap. It is a 25 cm long 1/8" stainless steel tube packed with 10 cm Carbopak B, 6 cm Carboxen 1000 and 1 cm Carboxen 1001.

Variable	Value	Variable	Value
Valve Oven Temp	150	Dry Purge Temp	40
Transfer Line Temp	150	Dry Purge Flow	200 mL/Min
Sample Mount Temp	90	GC Start	Start of Desorb
Purge Ready Temp	40	Desorb Preheat Temp	245
Dry Flow Standby Temp	175	Desorb Drain	ON
Standby Flow	0 mL/min	Desorb Time	2 min
Pre-Purge Time	0.5 min	Desorb Temp	250
Pre-Purge Flow	40 mL/min	Desorb Flow	0 mL/min (a default setting)
Sample Heater	OFF	Bake Time	2 min
Sample Preheat Time	1 min	Bake Temp	270
Preheat Temp	40	DryFlow Bake Temp	300
Purge Time	11 min	Bake Flow	400 mL/min
Purge Temp	0 (a default setting)	Focus Temp	-150
Purge Flow	40 mL/min	Inject Time	1 min
Dry Purge Time	1 min	Inject Temp	180
Sample Type	Soil	Sample Vol	5 mL
Dilution Factor	0	Rinse Vol	0 (a default setting)
# Rinses	0	Standard 1	YES
Standard 2	NO	Sample Preheat Stir	NO
Stir	NO	W. Stir Time	0 (a default setting)
W. Settle Time	0	Syringe Flushes	0 (a default setting)
Opr. Mode	Remote	Cycle Timer	0 (a default setting)
Aux Timer	0	Link To Method #	#0

Table 3. Velocity and Archon Settings

B. Instrument Calibration

Reagents and standards (d^6 benzene ISTD)

The calibration information is for GC/MS #1 with the Tekmar Velocity and Solatek.

0.9251 g of 5.023 mg/mL benzene in methanol was weighed into a 50 mL volumetric flask and filled to the mark with methanol (117,448 µg/L benzene). This solution was serially diluted 1:10 using 5 mL Class A glass pipettes and 50 mL volumetric flasks to make solutions of 11,745 µg/L benzene, 1,174 µg/L benzene and 117 µg/L benzene. Aliquots of these solutions were added to 10 mL of purged, deionized water in 40 mL EPA vials according to Table 5 to make calibration standards.

Hamilton 800 Series microliter syringes were used to add the calibration standards to the EPA vials containing water or sample. These syringes are listed in Table 4.

Syringe Volume	Model Number	Fisher Catalog Number
10 µ	801RNW	14-815-300
25 µL	802RNW	14-815-301
50 µL	805RNW	14-815-302
100 µL	810RNW	14-815-303

Table 4. Syringes Used for Addition of Standard Solutions to Vials

0.9712 g of 2.015 mg/mL d^6-benzene in methanol was weighed into a 50 mL volumetric flask and filled to the mark with methanol (49,460 µg/L d^6-benzene). This solution was diluted 1:5 with a 20 mL Class A glass pipet and a 100 mL volumetric flask to make a solution of 9892 µg/L d^6-benzene for use as internal standard. The purge and trap autosampler method was instructed to inject 5 µL of the internal standard to each EPA vial prior to purging.

This d^6-benzene solution was only used for GC/MS #1. The Solatek autosampler was able to inject 5 µL of internal standard solution. The other (Archon) autosamplers used for these analyses were capable of injecting 1 and 2 µL aliquots, respectively, of internal standard solution and required d^6-benzene solutions of different concentrations. The Archons did wash the ISTD into the sample with 10 mL of rinse water.

One of the standards reservoir vessels on the Solatek was filled with a portion of the internal standard solution. Prior to purging the sample, an injection valve loaded 5 µL of the d^6-benzene solution which was than added to the EPA vial with 10 mL of rinse water.

µL of 117 µg/L Benzene	Standard Conc µg/L	Cal Level	µL of 1,174 µg/L Benzene	Standard Conc µg/L	Cal Level	µL of 11,745 µg/L Benzene	Standard Conc µg/L	Cal Level
10	0.117	1	10	1.17	4	10	11.74	7
20	0.235	2	20	2.35	5			
40	0.470	3	40	4.70	6			
µL of 9892 µg/L d^{6}-Benzene								
5	4.90	All						

Table 5. Standards Preparation for 10 mL Calibration Standards and Samples

In addition to these calibration standards 5 and 50 µL of the 1,174 µg/L benzene solution were added to 10 mL aliquots of purged, deionized water. These are used to make calibration verification samples at 0.59 and 5.87 µg/L benzene.

Finally a check sample was prepared from an independent benzene source to verify that the instrument gave consistent data. A 100 µL aliquot of a solution containing 2000 µg/mL benzene in methanol was added to 100 mL of purged, deionized water.

A 5 µL aliquot of the 2000µg/L benzene solution was added to 10 mL purged, deionized water in an EPA vial giving 1.0 µg/L benzene. This check standard was treated as an unknown sample for calibration verification and to ensure that all the instruments used for the project gave consistent results.

The Agilent 6890/5973 #1 GC/MS, Tekmar Velocity purge and trap concentrator and Tekmar Solatek purge and trap autosampler was calibrated with these standards. The ChemStation was calibrated using a linear fit of response ratio to amount ratio. The ChemStation was “told” that the d^{6}-benzene internal standard concentration was 1.0 µg/L for all samples. This caused the Amount Ratio for each calibration level to display as being the same as the benzene concentration for that level.

A list of the purchased calibration standards is included. All are 1 mL of solution in a sealed ampoule.

1- Benzene standard: Supelco 40004, 5023 µg/mL benzene in methanol
2- d^{6}-benzene standard: Supelco 48940-U, 2015 µg/mL benzene in methanol
3- Check sample benzene standard: Restek 30249, 2000 µg/mL benzene

Instrument Calibration

Calibration and verification samples are made by adding microliter aliquots of the standards from Table 5 to 10 mL of purged, deionized water in 40 mL EPA vials. Internal standard solution aliquots of 5 µL are added by the Solatek. For other instruments the concentration of the d^6-benzene standard will have to be altered to accommodate the size of the aliquot of ISTD solution the autosampler is capable of injecting into the samples. Table 5 will still be applicable for dilution of the benzene calibration standards.

Table 6 shows the calibration data for the 6890/5973/Velocity/Solatek purge and trap GC/MS. Recall that the actual d^6-benzene concentration is 4.9 µg/L and the ChemStation was told it was 1 µg/L to adjust the amount ratio to equal the benzene concentration.

The first and last samples in the calibration runs were purged, deionized water used to check the background of the instrument. A typical sequence in these analyses starts with a water blank, a 5.87 µg/L benzene verification sample and than the unknown samples with either a 0.59 or a 5.87 µg/L verification sample between every 8 – 10 unknown samples. Figure 1 is the ChemStation calibration chart.
Following chart in Excel using the ChemStation data was created.

Sample Name	Benzene Area	Benzene Conc µg/L	d6 Benzene Area	d6 Benzene Area	Benzene Found µg/L
0 µg/L	10942	0.00	1	818157	0.0
0.12 µg/L	32807	0.12	1	817882	0.1
0.24 µg/L	57300	0.24	1	827709	0.3
0.47 µg/L	101968	0.47	1	828011	0.5
1.17 µg/L	221872	1.17	1	818283	1.2
2.35 µg/L	419894	2.35	1	818604	2.2
4.70 µg/L	906480	4.70	1	831742	4.8
11.74 µg/L	2192056	11.74	1	835169	11.7
0 µg/L	17214	0.00	1	825647	0.0
0.59 µg/L Check Sample	123141	0.59	1	833539	0.6
5.87 µg/L Check Sample	1153599	5.87	1	837021	6.1

Table 6. Calibration Data for 0.12 to 11.74 µg/L Benzene Standards

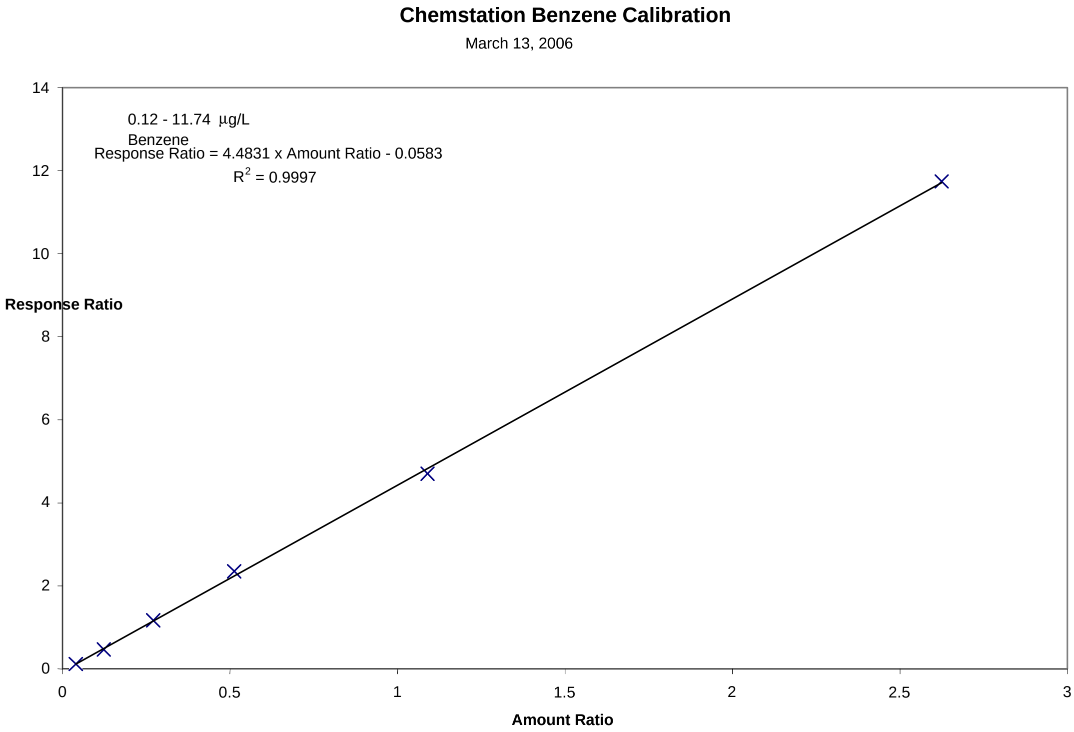


Figure 1. ChemStation calibration curve for 0.12 to 11.74 µg/L benzene standards

The manufacturer of the d^6-benzene standard solution claims 99.9 % purity. The 4.9 µg/L d^6-benzene internal standard could contain as much as 0.004 µg/L of benzene. In addition the mass spectrum of d^6-benzene in the NIST library has a small amount of m/z 78 and 77.

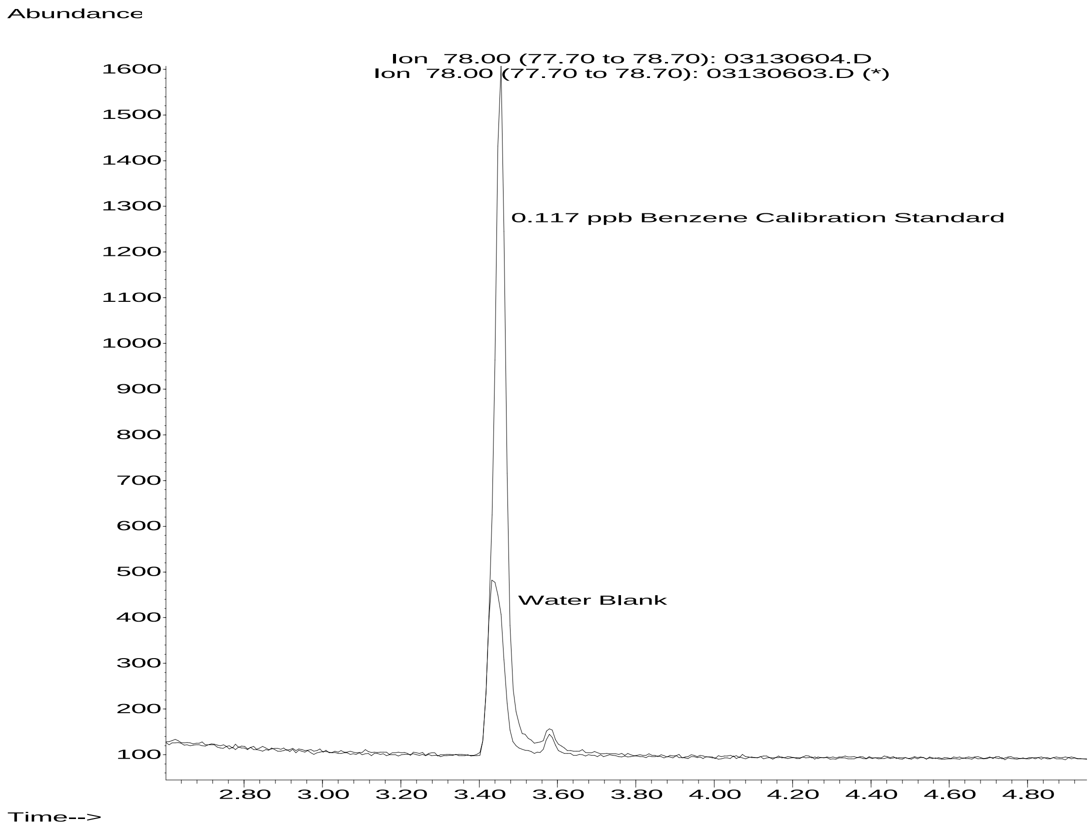


Figure 2. Superimposed ion chromatograms of blank run and 0.12 µg/L benzene

Sample Preparation
Ten mL sample aliquots (plus 10 mL rinse water) are purged directly in the 40-mL EPA vials. The samples are pipetted into the vials and the vials capped for analysis.

Carbonated beverages (CSDs) tend to experience CO_2 breakout when opened and during sampling by pipet. This is unavoidable and a certain (unknown) of loss of volatiles does occur. The CO_2 breakout can be minimized by cooling the CSDs in a refrigerator overnight or in bucket of ice for several hours before opening the container. Avoid agitating the container before opening. Pre-wetting the interior of the pipet with an aliquot of sample and disposing of it will help to minimize losses due to this bubbling. Fill and drain the pipet slowly to minimize CO_2 escape. Allow the sample to run down the interior wall of the 40-mL flask to reduce CO_2 and volatiles losses.

Remark:
Juices containing pulp and other high solids cannot be analyzed using this purge and trap method since they plug sample lines and valves.

These samples have to be analyzed by head-space – GC/MS.