

# Comparison of WCOT and PLOT Columns for the GC/MS Analysis of Benzene in Soft Drinks

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## Overview

The FDA method for the quantitation of benzene in soft drinks allows for the use of two approaches:

1. A capillary 6% cyanopropylphenyl polysiloxane (polar) wall coated open tubular (WCOT) column with cryogenic trapping
2. A Porous Layer Open Tubular (PLOT) column fitted with headspace sampling.

We have evaluated the Thermo Scientific equivalent columns specified in the FDA method and compared these for their suitability in the quantification of benzene at low concentrations.

## Introduction

The presence of benzene in soft drinks is a result of the reaction between sodium benzoate and ascorbic acid (vitamin C) under certain conditions of heat and light. Sodium benzoate is added as a preservative to prevent mould growing in the drinks and vitamin C may be used as an antioxidant or may be naturally present.

Benzene is widely known as a human carcinogen, therefore many regulatory bodies such as FDA, WHO, EPA and EU have established limits for benzene levels in food and drinking water. It is therefore necessary to have robust, reproducible and simple methods for the determination of benzene in soft drinks.

The work presented in this poster uses GC/MS with headspace sampling to analyse benzene in soft drinks. The work is based on the FDA method [1] for determination of benzene in soft drinks and other beverages. The FDA method uses cryogenic focussing at -50 °C prior to headspace GC/MS analysis. However, cryogenic focussing was not applied in this case but direct headspace analysis was performed.

The performance of the method on a 6%cyanopropylphenyl polysiloxane WCOT (Wall Coated Open Tubular) column is compared with that of a PLOT (Porous Layer Open Tubular) column. Traditional PLOT columns suffer from poor particle stability and very often these are released from the column causing blockages and flow irreproducibility. Thermo Scientific TracePLOT columns use a proprietary particle adsorption process which minimises particle release and therefore improves chromatography. However, to ensure no particles entered the detector a particle trap was used between the analytical column and the MS detector.

Benzene in a carbonated energy drink is quantified by isotope dilution using benzene-*d*<sub>6</sub>. A six-point calibration curve shows good method linearity on both types columns. In this study it was also confirmed that benzene was not formed during analysis.

## Methods

### Sample preparation

A non-branded carbonated energy drink which contained ascorbic acid and sodium benzoate, was obtained from a local supermarket. Calibration standards were prepared by weighing 10g of water into a headspace vial and adding benzene to obtain concentrations of 0.5, 1.0, 2.5, 5.0, 10.0 and 20ng/g. To each calibration standard 10ng/g of internal standard benzene-*d*<sub>6</sub> was added. To determine the benzene in the carbonated soft drink, 10g of the sample was weighed into the headspace vial to which 10ng/g of internal standard was added.

**Thermo Scientific Columns:** TRACETM TR-V1 30m x 0.25mm x 1.4 μm  
TracePLOT™ TG-BOND Q, 30 m x 0.32mm x 10 μm  
TracePLOT TG-BOND Q, 15 m x 0.32mm x 10 μm  
Particle Trap: 2.5 m x 0.32 mm

The analytical column and the particle trap were connected via a glass fitting. This process takes place under high temperature and pressure.

### GC/MS Conditions:

Thermo Scientific TriPlus Headspace Autosampler:

Sample Volume: 1 mL  
Sample analysis time: 30 min  
Agitator temperature: 60 °C  
Incubation time: 15 min  
Agitator shake: On 15s, Off 15s  
Syringe temperature: 100 °C  
Post injection flush: 30s

Thermo Scientific TRACE GC Ultra:

Oven Program: 40 °C, 7.5 °C/min, 210 °C (7.33 min)  
Equilibration time: 0.5 min.  
Injector: 200 °C, Split  
Split ratio: 10  
Column Flow:  
1.0 mL/min, constant flow for TRACE TR-V1 column  
1.7 mL/min, constant flow for TracePLOT TG-Bond Q15 m column  
1.2 mL/min, constant flow for TracePLOT TG-Bond Q 30 m column  
Transfer line temperature: 230 °C

Thermo Scientific DSQII MS:

Source temperature: 200 °C  
Ion volume: Closed EI  
Emission current: 50 μA  
Detector gain: 3 × 10e5 (1200V)  
Electron energy: -70 V  
Filament delay: 5.5 min  
Scan parameters: SIM: Benzene: m/z 51, 77 and 78  
Benzene-*d*<sub>6</sub>: m/z 52, 82 and 84  
Dwell time: 100 ms.

Consumables:

BTO 17mm Septa  
5mm ID Focus Liner, 105 mm long  
Liner Graphite seal  
2.5mL Headspace Syringe  
Graphite ferrules to fit 0.32 mm and 0.25mm ID columns  
Graphite/vespel 0.32 mm and 0.25mm ID ferrules for GC/MS interface  
20 mL clear crimp top vial  
Aluminium 20mm cap and Si/PTFE seal

## Results and Discussion

We compared the analysis of benzene in a carbonated soft drink using the two columns provided as alternatives in the FDA method.

### Capillary WCOT column

The FDA method allows for use of a polar capillary WCOT column with cryogenic focusing at -50°C. We evaluated a method in which the Thermo Scientific TRACE GC Ultra and DSQII fitted with headspace auto-sampler and a TRACE TR-V1 GC column demonstrated a detection limit of 1.54 ng/g (in matrix), good peak shape (Figure 1a) and good detector linearity without the need for cryogenic trapping.

### PLOT Column

The FDA also allows for use of PLOT column, thereby providing a legitimate alternative when cryogenic focusing is unavailable in a laboratory. When we tested this approach using a TracePLOT TG-BOND Q column, we observed a detection limit of 2.45 ng/g (in matrix) and good detector linearity (Figure 1b and 2a, Table 1). We also tested a column that was double the length stated in the FDA method (30 m) then the larger pressure drop across the column resulted in a lower pressure in the MS, which we attributed to an observed increase in the sensitivity (Figure 1c and Table 1).

Figure 1. SIM of Lower Standard 0.5 ng/g and of Benzene in Water

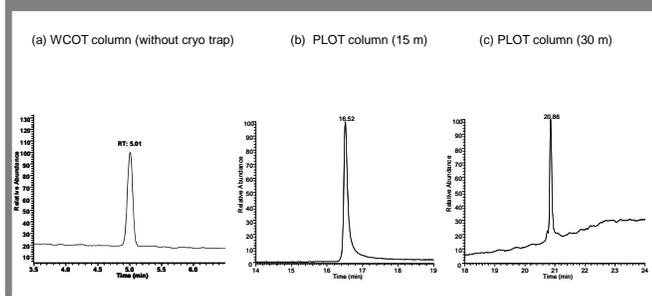


Figure 2. Calibration curve of benzene in water and TIC of 10ng/g standard using TracePLOT TG-BOND Q column (15 m).

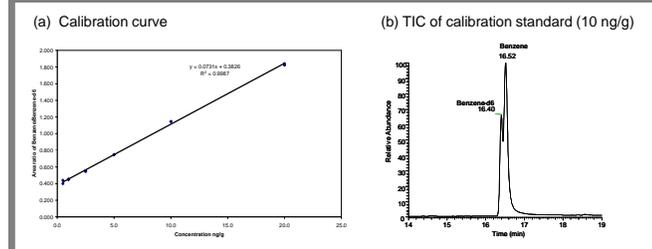


Table 1. Comparison of the calibration results of 3 methods used to quantify benzene in soft drinks

Column	Linearity R <sup>2</sup>		Limits of Detection		Limits of Determination	
	Water	Drink	Water ng/g	Drink ng/g	Water ng/g	Drink ng/g
TR-V1	1.000	0.998	0.16	1.54	0.52	5.15
TG-BOND Q 15m	0.999	0.994	0.81	2.45	2.69	8.18
TG-BOND Q 30m	0.999	0.997	0.78	1.77	2.60	5.91

## Conclusions

- It is possible to obtain good retention and sensitivity when quantifying benzene in soft drinks using the FDA polar capillary GC column, without the cryogenic focusing stipulated in the method.
- The PLOT GC columns provide good sensitivity when using the FDA method.
- Better sensitivity can be achieved with lower MS pressures, which are best achieved with a longer PLOT column than that stated in the FDA method.

## References

1. FDA Method, Determination of Benzene in Soft Drinks and Other Beverages. [www.cfsan.fda.gov/~dms/benzmeth.html](http://www.cfsan.fda.gov/~dms/benzmeth.html)

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PSCCS 1010 0910