

Gas Chromatography/  
Mass Spectrometry**Author**

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## The Determination of Low Levels of Benzene, Toluene, Ethylbenzene, Xylenes and Styrene in Olive Oil Using a Turbomatrix HS and a Clarus SQ 8 GC/MS

### Introduction

Levels of benzene, toluene, ethylbenzene, xylenes and styrene (BTEXS) are a concern in olive oil. These compounds find their way into olive trees and hence into the olives and olive oil mainly as a result of emissions from vehicles, bonfires, and paints into ambient air near the orchards.

Various methods have been developed to detect and quantify these compounds down to levels of 5 ng/g (5 ppb w/w). This application note describes an easy to perform method using PerkinElmer® Clarus® SQ 8 GC/MS with a TurboMatrix™ 110 headspace sampler to achieve detection limits below 0.5 ng/g.

## Method

The experimental conditions for this analysis are given in Tables 1 to 4.

**Table 1. GC Conditions.**

Gas Chromatograph	Clarus 680
Column	30 m x 0.25 mm x 1.0 $\mu$ m Elite-Wax
Oven	35 °C for 1 min, then 10 °C/min to 130 °C
Injector	Programmable Split Splitless (PSS), 180 °C, Split OFF
Carrier Gas	Helium at 1.0 mL/min constant flow (7.2 psig initial pressure), HS Mode ON

**Table 2. HS Conditions.**

Headspace System	TurboMatrix 110 HS Trap in standard HS mode (trap port capped).
Vial Equilibration	90 °C for 20 minutes
Needle	130 °C
Transfer Line	140 °C, long, 0.150 mm i.d. fused silica (chosen to facilitate rapid conversion to HS trap operation for other applications)
Carrier Gas	Helium at 35 psig
Injection Time	0.15 min

**Table 3. MS Conditions.**

Mass Spectrometer	Clarus SQ 8 MS, Large Turbo Pump
Scan Range	35 to 350 Daltons
Electron Energy	70eV
Scan/Dwell Time	0.1 s
Interscan/Interchannel Delay	0.02 s
Source Temp	200 °C
Inlet Line Temp	200 °C
Multiplier	1400V

**Table 4. Sample Details.**

Sample	10.00 $\pm$ 0.01 g of olive oil weighed directly into vial
Vial	Standard 22-mL vial with aluminum crimped cap with PTFE lined silicon septum

## Calibration solutions

1 mL of each BTEXS component was added to a 100-mL volumetric flask and diluted to volume with methanol. 1 mL of this stock solution was further diluted to 100 mL with methanol to produce the working solution used to fortify 'clean' olive oil for calibration purposes. The w/v concentration of each analyte in each of these two solutions is given in Table 5.

**Table 5. BTEXS concentrations in calibration solutions.**

Component	Stock Solution ( $\mu$ g/ $\mu$ L)	Working Solution (ng/ $\mu$ L)
Benzene	8.77	87.7
Toluene	8.70	87.0
Ethylbenzene	8.67	86.7
p-Xylene	8.80	88.0
m-Xylene	8.64	86.4
o-Xylene	8.80	88.0
Styrene	9.06	90.6
Methanol	Balance	Balance

## Experimental

### Method Optimization

Figure 1 shows a total ion chromatogram (TIC) obtained from an empty vial into which 2  $\mu$ L of the working mixture of the BTEXS components in methanol was injected and fully evaporated. The conditions given in Tables 1 to 3 were applied.

Excellent peak shape is apparent and a full baseline separation of all components has been achieved. Meta-xylene and para-xylene are easily separated on this highly polar chromatographic column. A solvent delay of 4.6 minutes eliminates the appearance of the methanol solvent peak in this chromatogram.

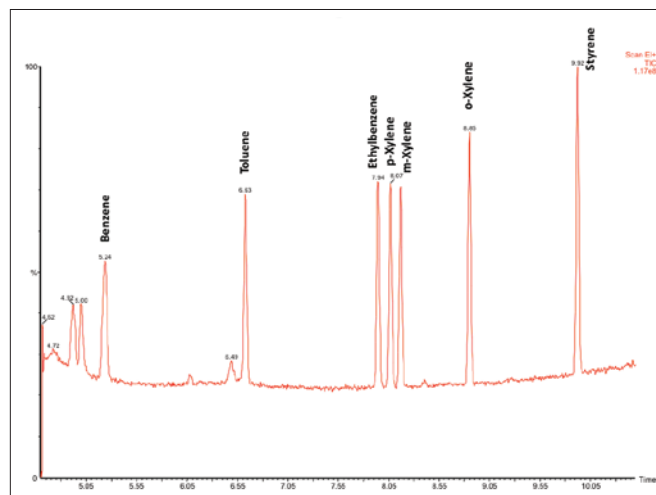


Figure 1. Chromatogram of 2  $\mu$ L of working calibration solution added to an empty 22-mL HS vial.

Figure 2 shows a chromatogram (with the same scaling as Figure 1) run under the same analytical conditions of 2  $\mu\text{L}$  working calibration mixture mixed into a 10 g sample of 'clean' olive oil. The analyte peaks are either close to the background noise level or are obscured by other components. The effective concentration of each analyte in the oil is approximately 17 ng/g (or ppb w/w). We need to see levels below 5 ng/g with this analysis and so it is clear that this will be a challenge with the method used to produce this chromatogram. The BTEXS compounds obviously have an affinity for the olive oil and so the partition coefficients are not favorable to the headspace phase – only a very small fraction of these will make it into the headspace.

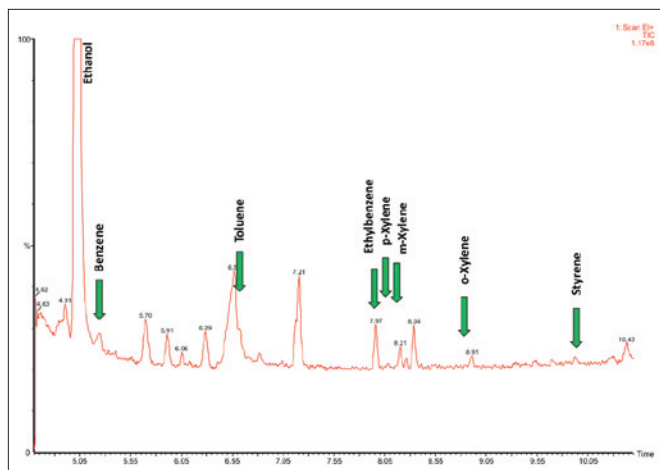


Figure 2. Chromatogram of 17 ng/g BTEXS in 10 g olive oil in a 22-mL HS vial with expected analyte retention times annotated.

By using the MS single-ion recording (SIR) mode of operation, the detector sensitivity and selectivity is significantly enhanced as shown in Figure 3. This chromatography was produced using the same chromatographic conditions as for Figure 2 but with the mixed single ion/full ion (SIFI) regime given in Figure 4 applied.

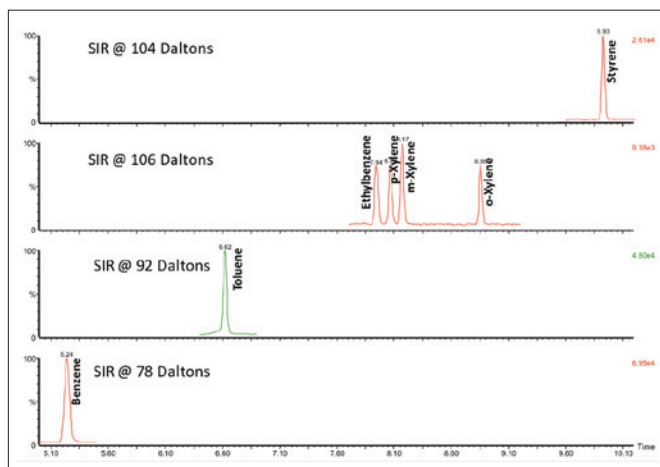


Figure 3. Chromatogram of 17 ng/g BTEXS in 10 g olive oil using SIFI settings given in Figure 4.

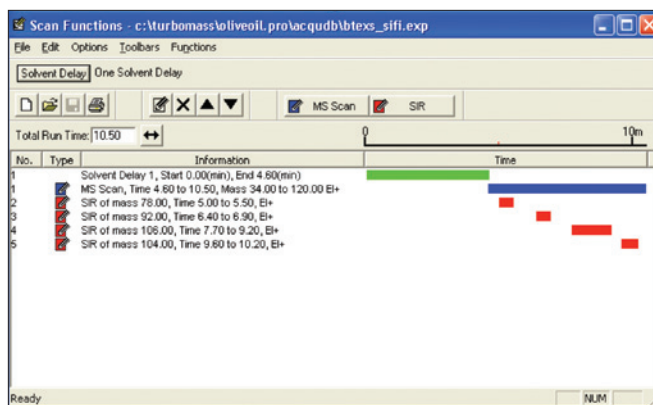


Figure 4. SIFI™ settings used to produce the chromatography shown in Figure 3.

### Linearity

A series of calibration mixtures was prepared by adding volumes of the working solution to clean olive oil as listed in Table 6. Note – this is often referred to as “method of standard addition”.

Table 6. Calibration mixture preparation.

Olive Oil (g)	Working Solution ( $\mu\text{L}$ )	Nominal Concentration (ng/g)
10.00	0	0
10.00	0.5	4.4
10.00	1.0	8.8
10.00	2.0	17.6
10.00	3.0	26.3
10.00	4.0	35.1
10.00	5.0	43.9
10.00	10.0	87.8

These mixtures were chromatographed using the conditions given in Tables 1 to 3. The analyte peak areas were obtained from the SIR traces. The clean olive oil was an off-the shelf product found to have low levels of BTEXS. The analyte peak areas found in this oil were subtracted from the calibration mixture responses, which were then used to prepare linear calibration profiles.

Figures 5 and 6 show calibration plots for the first and last eluting analytes, benzene and styrene, and Table 7 shows the least squares fit for each analyte. The linearity is excellent across this low concentration range especially for a complex sample matrix like olive oil.

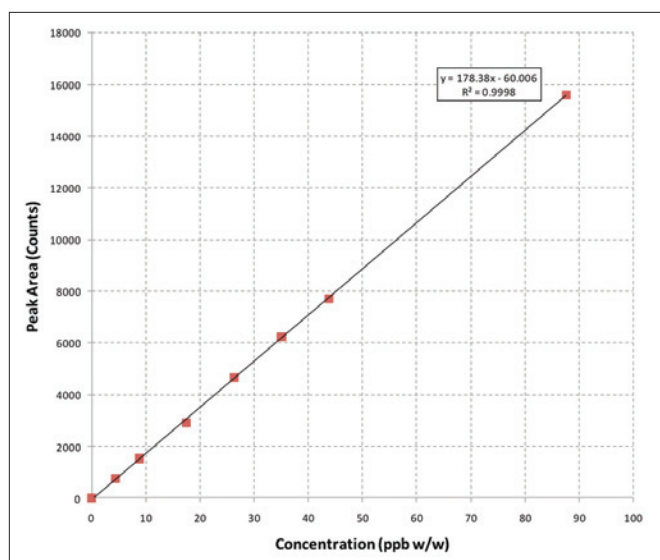


Figure 5. Calibration plot for benzene.

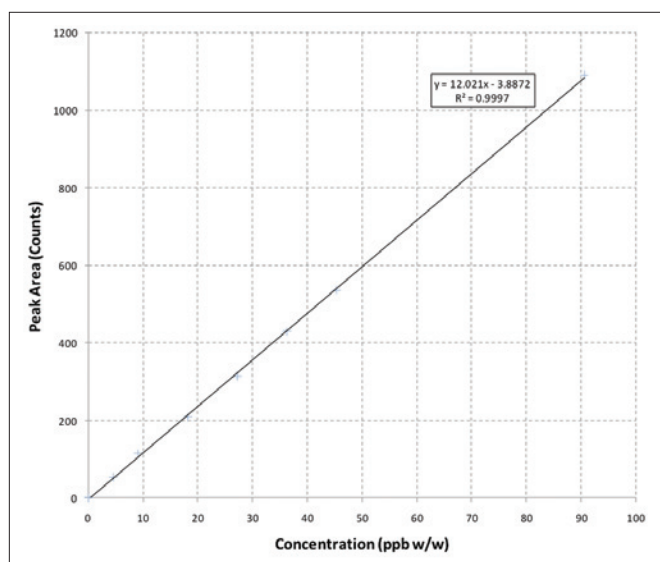


Figure 6. Calibration plot for styrene.

## Quantitative Precision

Ten samples of the clean olive oil were fortified with 5  $\mu$ L of the working solution. Each was analyzed using the conditions given in Tables 1 to 3 and the amount of each analyte was determined using the calibration data from Table 7. The results are given in Table 8. An overall precision of 1.69 to 3.76% relative standard deviation is a very good result from this complex matrix.

## Detection Limits

Figure 7 shows chromatography of a low-level sample. The calculated signal to noise ratios were used to predict the analytical detection limits shown in Table 9 based on a 2:1 ratio. These limits are over an order of magnitude below that of the 5ng/g requirement.

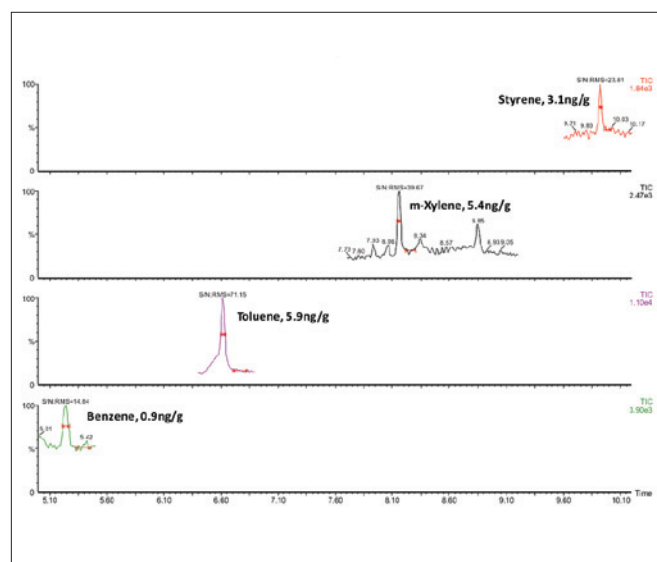


Figure 7. Chromatography of a sample containing low-levels of BTEXS with annotated signal to noise values.

Table 7. Least squares linear fit to calibration data.

Statistic	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
Slope	178.38	51.465	10.07	11.568	10.708	8.4239	12.021
Intercept	-60.006	-1.6527	-5.6768	-6.7959	-1.1014	-6.7186	-3.8872
r <sup>2</sup>	0.9998	0.9986	0.9995	0.9997	0.9998	0.9995	0.9997

**Table 8. Quantitative precision.**

Run #	Concentration in Spiked Sample (ng/g)						
	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
1	42.84	48.01	43.17	41.05	44.09	43.53	43.83
2	42.60	46.35	44.46	42.95	46.24	45.43	45.16
3	44.27	47.42	45.45	44.85	49.32	46.98	48.32
4	43.30	47.17	44.85	42.51	46.98	45.55	45.66
5	42.87	45.44	43.56	40.09	44.65	44.25	45.16
6	42.40	43.83	43.66	40.27	44.18	42.46	42.75
7	42.90	49.37	44.56	41.91	45.49	44.01	45.25
8	43.30	45.03	44.85	42.08	45.95	44.13	44.66
9	41.91	44.18	43.37	40.35	44.37	43.65	44.33
10	41.77	46.41	42.17	41.30	44.18	42.23	42.92
Mean	42.82	46.32	44.01	41.74	45.54	44.22	44.81
RSD%	1.69	3.76	2.25	3.51	3.66	3.26	3.53

**Table 9. Predicted limits of detection.**

Compound	Predicted Limit of Detection (ng/g)
Benzene	0.12
Toluene	0.16
Ethylbenzene	0.26*
p-Xylene	0.26*
m-Xylene	0.26
o-Xylene	0.26*
Styrene	0.26

\* Peaks too small to quantify and so are based on value for m-Xylene.

### Sample Analysis

Seven different branded bottles of olive oil were purchased from a local supermarket and analyzed using this method. The results are given in Table 10. The determined concentrations are well within the range of this method.

**Table 10. Results from analysis of supermarket samples.**

Sample Source(s)	Concentration in Sample (ng/g)						
	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
California	0.89	5.86	1.66	1.45	5.24	3.77	3.07
Italy, Greece, Spain, Tunisia	2.86	27.55	6.12	5.86	16.73	8.75	41.34
Italy, Spain, Greece, Tunisia	3.07	24.22	13.47	7.85	23.64	13.97	39.59
Italy, Spain, Tunisia, Turkey, Argentina	2.99	17.03	3.74	3.44	9.35	6.14	40.09
Spain, Argentina	2.43	34.99	7.22	7.42	18.97	10.65	126.11
Italy, Spain, Greece, Tunisia, Morocco, Syria, Turkey	4.09	35.71	19.13	17.10	59.31	28.10	61.05
Italy, Greece, Spain, Tunisia	1.25	2.79	ND	1.80	3.74	3.17	7.39

## Conclusions

This method uses the new Clarus SQ 8 GC/MS to great effect. Sample preparation is extremely easy – 10 g of olive oil is weighed into a standard headspace vial and then sealed with a crimped cap. The analysis is fully automated and takes just 10.5 minutes for the chromatography and an additional 3.5 minutes for cool-down and equilibration between analyses.

Sub-ppb levels are possible using standard headspace sampling of light aromatics in a complex natural oil matrix without the need for vapor pre-concentration (for example with an HS Trap). Excellent quantitative performance has been demonstrated and the system is easily able to see low concentrations of these compounds in olive oil bought from a local supermarket.

## PerkinElmer Accessories and Consumables for this application:

Item Description	Part No.
Elite Wax	N9316485
Injector Port Septa 6pk	N6101748
Ferrules	09920104
H/S Vials/Caps/Septa	N9303992
Marathon Filament	N6470012
Ergo Crimper	N6621037

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