The Determination of Benzene, Toluene, Ethyl Benzene, Xylenes and Styrene in Olive Oil using Headspace Extraction and Gas-Chromatography/Mass Spectrometry.

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1 Introduction

Levels of benzene, toluene, ethylbenzene, xylenes and styrene (BTEXS) find their way into olive trees and hence into the olives and olive oil mainly as a result of the presence of vehicle exhaust in ambient air. Although there is widespread concern about the presence of these carcinogenic compounds in olive oil, no definitive methods or limits have yet been prescribed.
Various methods have been developed to detect and quantify these compounds down to levels of 5ng/g (5 ppb w/w). In this work, we have developed a simple method to determine these components in olive oil using headspace (HS) extraction and gas chromatography/mass spectrometry (GC/MS).

2 Choice of headspace sampling

Olive oil is a viscous sample that is not suited for direct injection into a GC.

Some form of extraction technique is required to separate the BTEXS from the sample matrix prior to introduction into the GC. Multiple steps for sample preparation introduce possible contamination and increase error and variation to the final result. The choice of headspace sampling offers a simple alternative with minimal preparation.

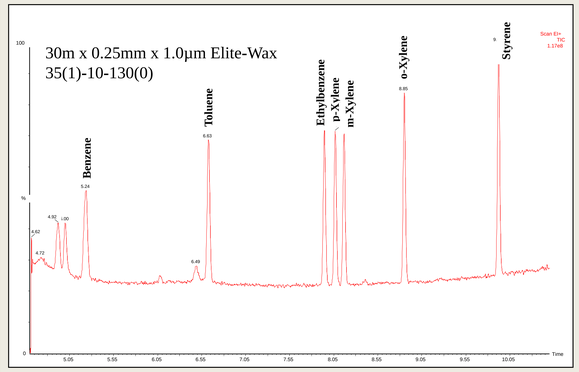
10g of sample are placed in a vial which is then sealed with a cap and maintained at 90°C for 20 minutes. The volatile components (including BTEXS) will partition into the vapor phase.

3 Chromatography

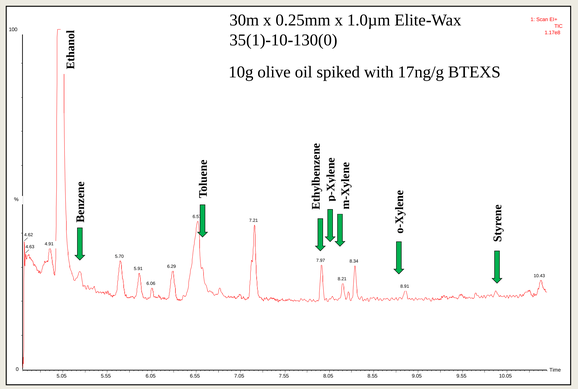
Mass Spectrometer Clarus SQ 8 MS, Large Turbo Pump	
Scan Range	35 to 350 Daltons
Electron Energy	70eV
Scan/Dwell Time	0.1s
Interscan/ Interchannel Delay	0.02s
Source Temp	200 °C
Inlet Line Temp	200 °C
Multiplier	1400V

Clarus 680 Gas Chromatograph	
Programmable split/splitless injector	180 °C splitless
Carrier gas	Helium at 1.0 ml/min constant flow (7.2 psig initial pressure)

Turbomatrix 110 HS trap in standard HS mode	
Needle	130 °C
Transfer line 0.150 mm id fused silica	140 °C
Carrier gas	Helium at 35 psig
Injection time	0.15 min



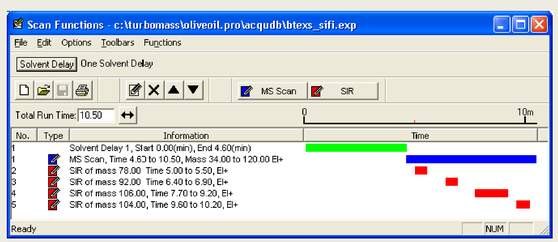
~0.6ng from 2µl of standard evaporated in a 22ml vial



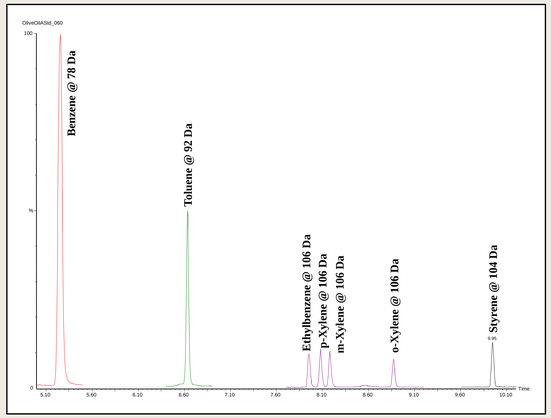
Matrix effect showing low partition of analytes form the olive oil to the headspace

The above chromatogram (with the same scaling as the 0.6ng standard) was run under the same analytical conditions with 2 µL of a 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).

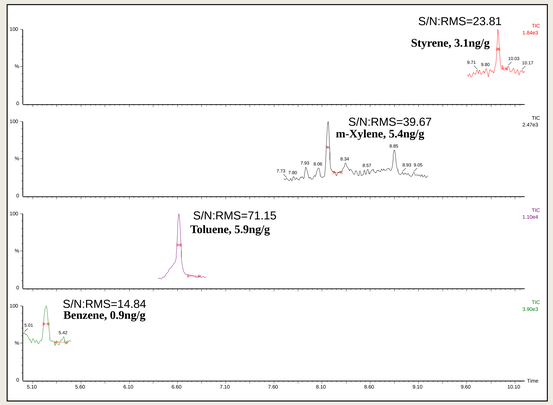
4 Selective ion monitoring



Selective ion monitoring on the SQ8 for improved detection



SIM of Olive oil spiked with 17ng/g BTEXS

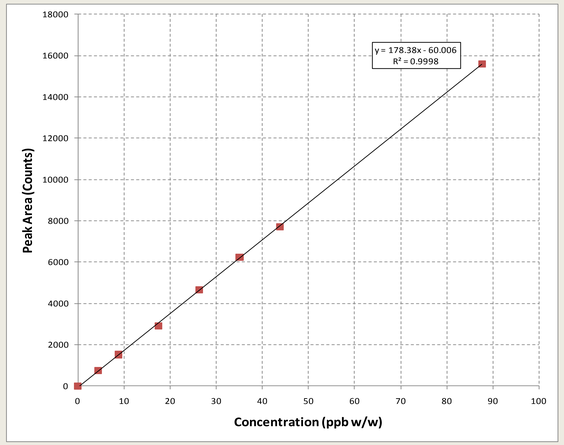


Chromatography of low levels of analytes with corresponding signal to noise

5 Results

Compound	RSD (% at 45ng/g, n=10)	r^2 (from 4.5 to 90ng/g)	Limit of Detection (ng/g)
Benzene	1.7	0.9998	0.1
Toluene	3.8	0.9986	0.2
Ethylbenzene	2.3	0.9995	0.3
p-Xylene	3.5	0.9997	0.3
m-Xylene	3.7	0.9998	0.3
o-Xylene	3.3	0.9995	0.3
Styrene	3.5	0.9997	0.3

Calibration data and detection limits for the selected analytes



Calibration curve for benzene

Sample Source	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

BTEXS results for selected olive oils

6 Summary

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.