Comparison of BTEXS in Olive Oils by Static and Dynamic HT3 Headspace

Application Note

Abstract

The health benefits of consumption of olive oils as part of a healthy diet reaches back to the mid 1950's. In the late 1990's, interest in characterizing other components of olive oils included determining the concentration of benzene, toluene, ethylbenzene, xylenes and styrene (BTEXS). Some of these compounds are naturally occurring, while others may be introduced through the processing of olive oil.

The method suggested for the determination of BTEXS in olive oil is static headspace. This application note will reproduce some of the static parameters found in this suggested method with the static features of the Teledyne Tekmar HT3.

This application note will also demonstrate the versatility of the static and the dynamic capabilities of the HT3. These features allow laboratories the capability to use both static and dynamic headspace methods to characterize the volatile flavor components of olive oils and accurately quantify low level compounds like BTEXS.

Introduction

Olive oil has been part of the Mediterranean culture for thousands of years. Ancel Keys of the University of Minnesota was intrigued by the low incidence of coronary heart disease in the adult population of Naples Italy in 1952. His research has led in part to the current interest in the Mediterranean diet. This diet includes using olive oil, an oil low in saturated fats typically found in animal fats, palm, and coconut oil, and also a rich source of monounsaturated fats. Since then, other healthy uses for olive oil, such as salad dressing, have been discovered.[1]

Because of the natural origin of olive oil, recent methods have been used to determine the amounts of benzene, toluene, ethylbenzene, xylenes and styrene (BTEXS) present.[2,3] These methods recommend static headspace techniques to extract the BTEXS from various olive oils. These methods use a variety of techniques including differing sample sizes, GC/MS with selected ion monitoring (SIM), and stirring the sealed sample with Teflon coated stir bars to achieve the lowest detectable levels of these compounds.

This application note will explore the ability of the static and dynamic methods of the Teledyne Tekmar HT3 to detect BTEXS in various olive oils. The static method is utilized to characterize volatile flavor compounds responsible for the flavor and aroma of olive oils. The dynamic method is utilized for the concentration of low concentration of volatile organic compounds, like BTEXS, and to enhance the signal to noise (s/n) ratio for lower detection levels. The HT3 allows both methods to be performed in the same sequence.

Seven olive oil samples were obtained from a local market. Three of these were from the same manufacturer and consisted of a light flavor, pure, and extra virgin olive oil. The four remaining olive oils were all extra virgin olive oils from different manufacturers, including one labeled as organic. Six of the olive oils were packaged in glass containers. One was packaged in a plastic container. The labels of all of the olive oil indicated countries of origin.

Sales/Support: 800-874-2004 · **Main:** 513-229-7000
4736 Socialville Foster Rd., Mason, OH 45040
www.teledynetekmar.com

Experimental-Instrument Conditions

The HT3 static/dynamic headspace instrument was connected to a Thermo Scientific Focus GC/DSQII mass spectrometer with a Phenomenex Zebron ZB-624 30m x 0.32mm x 1.8µm column. A Vocarb 3000 trap (Supelco K trap) was used for the dynamic headspace analysis. Table 1 displays the HT3 static and dynamic headspace instrument conditions, while Table 2 displays the GC/MS parameters.

Teledyne Tekmar HT3 Parameters Olive Oil Samples			
Variable	**Static Value**	**Dynamic Variable**	**Dynamic Value**
Constant Heat Time	Off	Quantitative Trap	K
GC Cycle Time	30.00 min		
Valve Oven Temp	140°C	Valve Oven Temp	170°C
Transfer Line Temp	140°C	Transfer Line Temp	180°C
Standby Flow Rate	100mL/min	Standby Flow Rate	50mL/min
		Trap Standby Temp	30°C
		Trap Sweep Temp	0°C
Platen/Sample Temp	90°C	Platen/Sample Temp	90°C
Platen Temp Equil Time	1.00 min		
Sample Equil Time	20.00 min	Sample Preheat Time	10.00 min
Mixer	On	Preheat MIxer	Off
Mixing Level	Level 5	Preheat Mixing Level	Level 5
Mixing Time	10.00 min	Preheat Mixing Time	2.00 min
Mixer Stabilize Time	5.00 min	Preheat Mixer Stabilize Time	0.50 min
Pressurize	8 psig	Sweep Flow Rate	75mL/min
Pressurize Time	2.00 min	Sweep Flow Time	10.00 min
Pressurize Equil Time	0.20 min	Dry Purge Time	1.00 min
Loop Fill Pressure	5 psig	Dry Purge Flow	50mL/min
Loop Fill Time	2.00 min	Dry Purge Temp	25°C
Inject Time	0.50 min	Desorb Preheat	245°C
		Desorb Temp	250°C
		Desorb Time	2.00 min
		Trap Bake Temp	260°C
		Trap Bake Time	15.00 min
		Trap Bake Flow	200mL/min

Table 1: Static and Dynamic HT3 Headspace Conditions

Thermo Focus GC / DSQII Parameters	
Column	Phenomenex® Zebron ZB-624, 30m x 0.32mm ID, 1.8µm Film thickness; Constant Flow 1.5mL/min
Oven Program	35°C for 2 min, 10°C/min to 130°C, 20°C/min to 240°C hold for 2 min, run time 19 min
Inlet:	Temperature 200°C, Split Flow 15mL/min Helium Carrier Gas, Constant Flow 1.5mL/min
MS Transfer Line	Temperature 230°C
MS Parameters	Source Temp 230°C, Start time 0.5 min, Scan Range 25.0 *m/z* to 350.0*m/z,* Scan Rate 1492.11, Chrom Filter Width 2.5 secs

Table 2: Thermo Focus GC / DSQII MS Parameters

Sales/Support: 800-874-2004 · **Main:** 513-229-7000
4736 Socialville Foster Rd., Mason, OH 45040
www.teledynetekmar.com

Standard Sample Preparation

Seven different olive oil samples were obtained from a local market. Three of these, identified as S1, were from the same manufacturer and consisted of a light flavor, pure, and extra virgin olive oil. The four remaining olive oils were all extra virgin olive oils from different manufacturers, including one labeled as organic. Six of the olive oils were packaged in glass containers. One, labeled S2, was packaged in a plastic container. The labels of all of the olive oil indicated countries of origin. Table 3 lists the label ingredients.

Supplier	Package	Grade	Olive Oil Origin	Package Origin
S1	Clear Glass	Lighter Flavor	Italy	Italy
S1	Clear Glass	Pure	Italy, Spain	Italy
S1	Clear Glass	Extra Virgin	Spain, Tunisia	Italy
S2	Clear PET Plastic	Extra Virgin	Spain, Tunisia	
S3	Green Glass	Extra Virgin	Italy	
S4	Clear Glass	Extra Virgin	Italy, Spain, Greece, Tunisia	
S5	Dark Green Glass	Organic Extra Virgin	Italy	

Table 3: Label Statements of the Seven Locally Available Olive Oils Indicating the Package Container, Olive Oil Grade, and the Source Country of the Oil.

The samples for the static headspace analysis were prepared by placing 10g of each olive oil into separate 22mL headspace vials. The samples for the dynamic headspace analysis were prepared by placing 2g of each olive oil into separate 22mL headspace vials. All headspace vials were sealed with Teflon lined septa and capped and sealed with aluminum crimp caps.

An environmental standard containing benzene, toluene, ethylbenzene, o-, m-, and p-xylene and styrene was spiked into one of the olive oil samples to determine the retention times of these compounds. 1µL of a 2,000µg/mL solution was added to 10g of olive oil in a 22mL headspace vial.

All of the static and dynamic olive oil headspace vials were analyzed in a single sequence on the HT3 and the Thermo GC/MS system with the parameters listed in Tables 1 and 2.

Results

The Total Ion Chromatograms were used to quantitate the BTEXS in the samples using their primary quantitation ions typically used in environmental analysis. The primary quantitation ions and their secondary confirmation ions for the compounds are listed in Table 4.

Compound	Retention Time		Primary Quantitation Ion	Secondary Confirmation Ion
	Static	Dynamic	*m/z*	*m/z*
Benzene	6.20	6.37	78	77, 51
Toluene	8.42	8.54	91	92, 65
Ethylbenzene	10.34	10.42	91	106, 105
m-, p-Xylene	10.49	10.57	106	91, 105
o-Xylene	11.03	11.10	106	91, 105
Styrene	11.05	11.14	104	103, 78

Table 4: Primary Quantitation Ions and Secondary Confirmation Ions for the BTEXS Compounds.

The peak areas for the BTEXS were tabulated for the static and dynamic headspace methods. The peak areas were normalized to the largest peak area of the 7 olive oil samples for each headspace method. The normalized peak area data is presented in Table 5.

Sales/Support: 800-874-2004 · **Main:** 513-229-7000
4736 Socialville Foster Rd., Mason, OH 45040
www.teledynetekmar.com

Compound	Method	S1 Light	S1 Pure	S1 Ex Vir	S2	S3	S4	S5
Benzene	Static	100.0	35.8	13.5	14.4	18.3	12.8	15.7
	Dynamic	100.0	67.4	51.7	57.7	77.7	42.4	73.5
Toluene	Static	11.6	25.7	68.6	37.0	100.0	48.2	28.4
	Dynamic	6.9	22.5	65.8	42.2	100.0	48.0	32.0
Ethylbenzene	Static	0.0	34.0	36.5	23.0	100.0	50.1	42.1
	Dynamic	7.0	43.2	45.3	34.9	100.0	57.4	19.6
m,- p-Xylene	Static	1.8	21.0	46.4	32.5	100.0	55.7	14.1
	Dynamic	4.0	29.6	46.9	32.9	100.0	57.4	18.3
o-Xylene	Static	0.0	26.0	45.2	29.8	100.0	54.4	15.8
	Dynamic	1.7	31.0	41.2	29.1	100.0	52.0	20.3
Styrene	Static	0.0	4.4	100.0	17.1	15.3	51.8	10.7
	Dynamic	0.8	11.0	100.0	19.2	16.8	54.2	14.8

Table 5: Normalized Peak Area Data for BTEXS Observed in Seven Different Olive Oil Samples by Static and Dynamic Headspace Methods.

The BTEXS ions of 78*m/z*, 91*m/z*, 104*m/z*, and 106*m/z* were summed by the mass spectrometer software to create a summed ion chromatogram (SIC). The SIC of the static and dynamic headspace methods were compared for each olive oil sample. Figures 3 through 9 are these comparisons. The dynamic headspace method was at least 100 times more sensitive for these compounds in olive oil than the static headspace method.

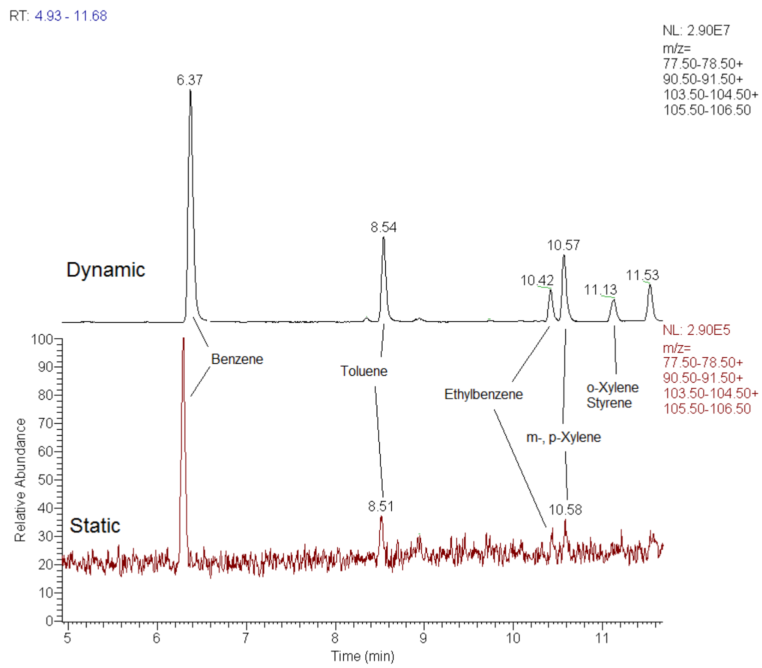

Figure 3: Comparison of the Static and Dynamic Summed Ion Chromatograms of the BTEXS Ions for Olive Oil Sample S1 Lighter Flavor. Note the 100 times difference in the scale factor (NL Dynamic 2.90e7, NL Static 2.90e5)

Sales/Support: 800-874-2004 · **Main:** 513-229-7000
4736 Socialville Foster Rd., Mason, OH 45040
www.teledynetekmar.com

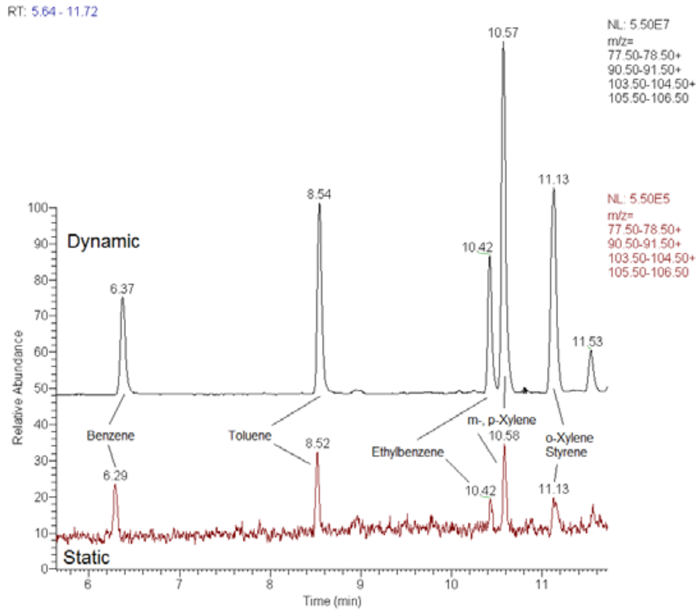

Figure 4: Comparison of the Static and Dynamic Summed Ion Chromatograms of the BTEXS Ions for Olive Oil Sample S1 Pure. Note the 100 times difference in the scale factor. (NL Dynamic 5.5e7, NL Static 5.50e5)

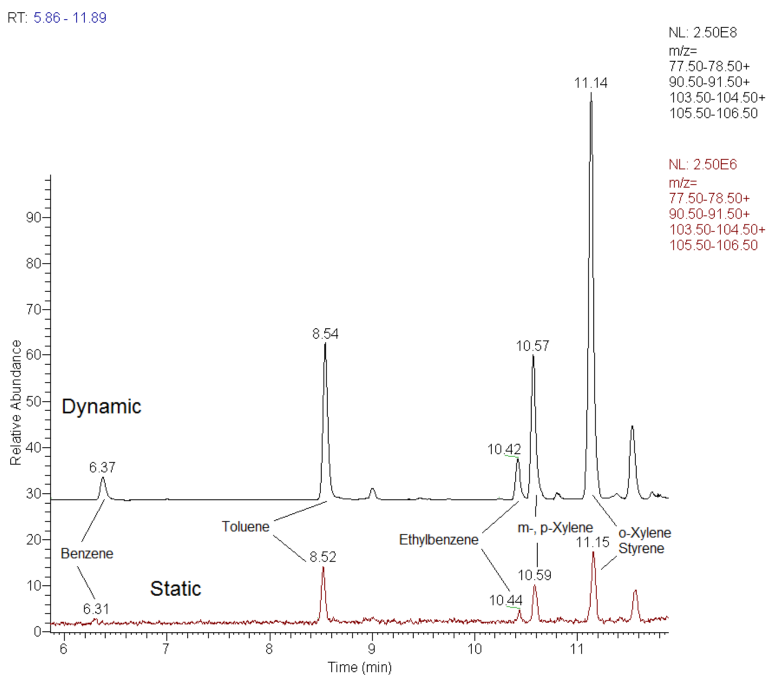

Figure 5: Comparison of the Static and Dynamic Summed Ion Chromatograms of the BTEXS Ions for Olive Oil Sample S1 Extra Virgin. Note the 100 times difference in the scale factor (NL Dynamic 2.50e8, NL Static 2.50e6)

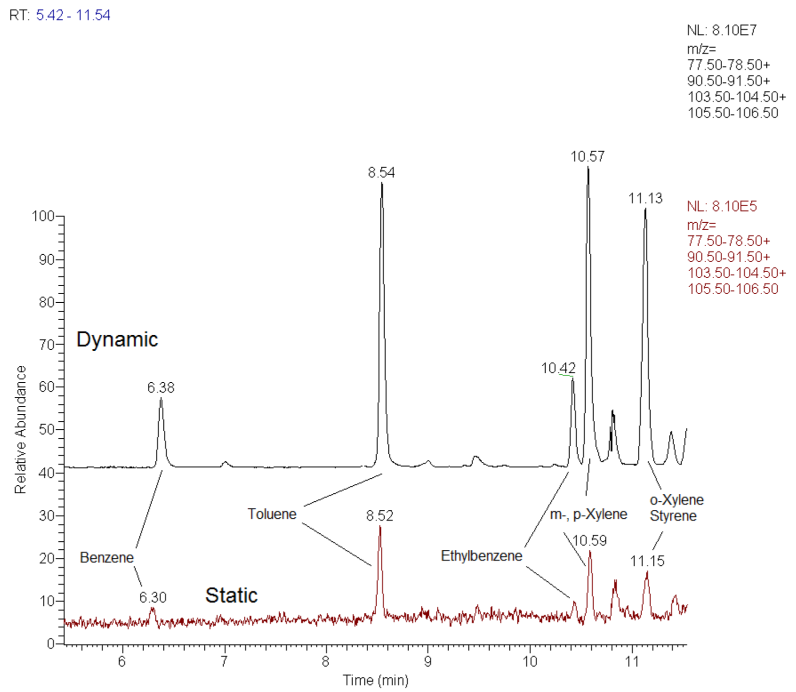

Figure 6: Comparison of the Static and Dynamic Summed Ion Chromatograms of the BTEXS Ions for Olive Oil Sample S2 Extra Virgin in PET Bottle. Note the 100 times difference in the scale factor (NL Dynamic 8.10e7, NL Static 8.10e5)

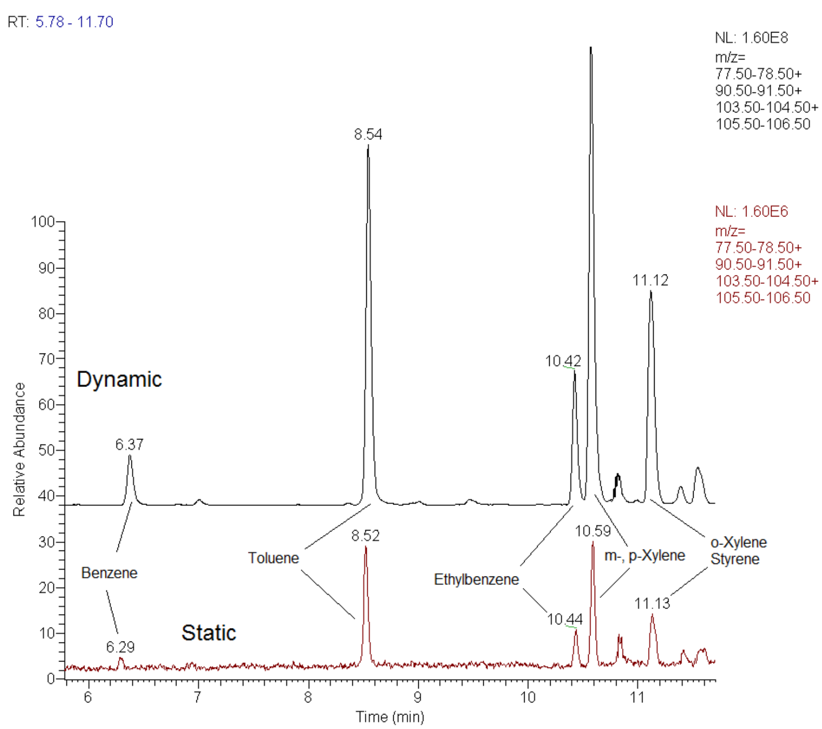

Figure 7: Comparison of the Static and Dynamic Summed Ion Chromatograms of the BTEXS Ions for Olive Oil Sample S3 Extra Virgin. Note the 100 times difference in the scale factor (NL Dynamic 1.60e8, NL Static 1.60e6). Note the differences between the static and the dynamic peak areas for the unidentified peaks between and after the xylenes and styrene peaks.

Sales/Support: 800-874-2004 · **Main:** 513-229-7000
4736 Socialville Foster Rd., Mason, OH 45040
www.teledynetekmar.com

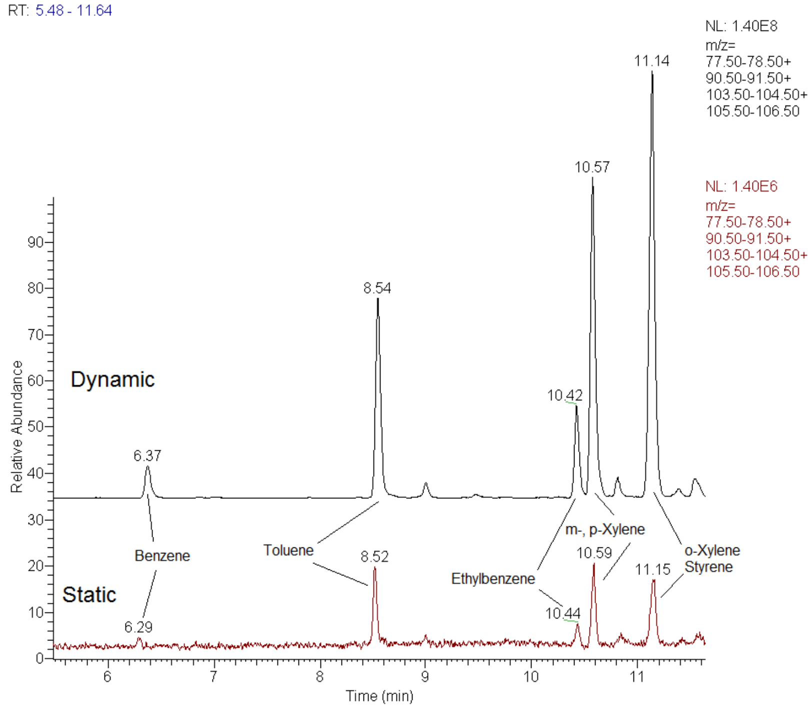

Figure 8: Comparison of the Static and Dynamic Summed Ion Chromatograms of the BTEXS Ions for Olive Oil Sample S3 Extra Virgin. Note the 100 times difference in the scale factor (NL Dynamic 1.40e8, NL Static 1.40e6). Note the differences between the static and the dynamic peak areas for the unidentified peaks between and after the xylenes and styrene peaks.

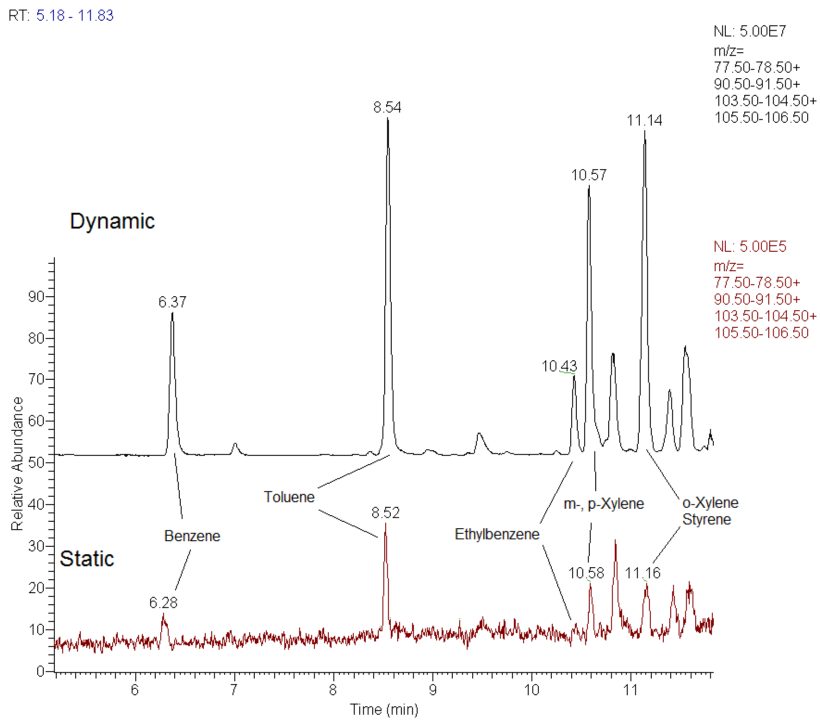

Figure 9: Comparison of the Static and Dynamic Summed Ion Chromatograms of the BTEXS Ions for Olive Oil Sample S3 Extra Virgin. Note the 100 times difference in the scale factor (NL Dynamic 5.00e7, NL Static 5.00e5). Note the differences between the static and the dynamic peak areas for the unidentified peaks between and after the xylenes and styrene peaks.

Sales/Support: 800-874-2004 · **Main:** 513-229-7000
4736 Socialville Foster Rd., Mason, OH 45040
www.teledynetekmar.com

Conclusions

Recent advances in characterization and enhanced detection limits have found trace amounts of BTEXS in samples of olive oils. One of the suggested methods for the determination of BTEXS in olive oils has been static headspace with GC/MS selection ion monitoring. The Teledyne Tekmar HT3 is a flexible instrument that performs both static and dynamic headspace analysis for a wide variety of sample matrices including olive oils.

Seven olive oil samples were concurrently tested with both the static and the dynamic modes of the HT3 for benzene, toluene, ethylbenzene, xylenes and styrene. The static method requires 10g of olive oil compared to 2g for the dynamic mode.

The data indicated similar results for all of these compounds, except benzene. The dynamic mode detected higher concentrations of benzene in six of the seven olive oil sample. The seventh olive oil sample was used to normalize the peak area data.

The dynamic method also demonstrated increased single to noise ratio for all of the compounds with 5 times less sample than the static method. The trap also allowed for the preliminary separation of the BTEXS compounds from other matrix effects from the olive oil.

The HT3 provides the olive oil industry the capability to perform both static and dynamic sampling of their products. This allows them to characterize olive oils for volatile flavor compounds in the static mode and then detect low levels of BTEXS in the dynamic mode.

References

1. http://www.international oliveoil.org/estaticos/view/244-dissemination-of-results
2. B. Gilbert-López, J Robles-Molina, J. F. García-Reyes, A. Molina-Díaz, Talanta 83 (2010) 391-399
3. F. Peña, S. Cárdenas, M. Gallego, M. Valcárcel, Journal of Chromatography A, 1052 (2004) 137-143

Sales/Support: 800-874-2004 · **Main:** 513-229-7000
4736 Socialville Foster Rd., Mason, OH 45040
www.teledynetekmar.com