Analysis of Terbuthylazine in Olive Oil by LC/Ion Trap MS and LC/Time-of-Flight MS

Application

Food safety

Authors

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Abstract

This application note describes the use of liquid chromatography/ion trap mass spectrometry (LC/ITMS) and liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS) for the identification and quantitation of terbuthylazine in olive oil samples. The method includes a sample treatment step based on a preliminary liquid-liquid extraction, followed by matrix solid-phase dispersion (MSPD) using an aminopropyl-bonded silica as a sorbent material. A final clean-up step is performed with Florisil using acetonitrile as an eluting solvent. The analysis by ion trap was achieved in MS/MS mode, monitoring the characteristic fragment ion at *m/z* 174. The identification by LC/TOFMS was accomplished with the accurate mass (and the subsequently generated empirical formula) of the protonated molecule ($[M+H]^+$ *m/z* 230), along with the accurate mass of the fragment ion and the characteristic chlorine isotope cluster present in terbuthylazine. The accuracy typically obtained was routinely better than 2 ppm. The method sensitivity, linearity, precision, accuracy, matrix effects, and limit of detection were also studied; they supported the potential of LC/TOFMS and LC/ITMS for the routine quantitative analyses of terbuthylazine in olive oil.

Introduction

Olive oil is one of the most-used food products in Mediterranean countries. The positive effects of olive oil on health have prompted a demand for this product world-wide. "Virgin" olive oil is obtained from the fruit of the olive tree (*Olea Europaea*) exclusively by mechanical and physical processes without any further treatment, which may alter the olive oil quality. The most extensively applied agrochemicals in olive plantations of Mediterranean countries (Greece, Spain, and Italy) are herbicides and insecticides. Although herbicides are mainly applied to soils, some residues can persist to the harvest stage, thus contaminating the olives picked up from soil. This can result in the presence of trace amounts of these pesticides in olive oil. Consequently, both the European Union and the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) of the United Nations have established maximum pesticide residue levels in olives and olive oil. Currently, various olive oil pesticide residue regulatory programs are being carried out to update and to establish new and more stringent regulations concerning the maximum residue levels in these commodities [1]. This fact has fostered the development of more powerful analytical tools in order to provide enough sensitivity and selectivity to meet these requirements in food samples such as edible oils, which have a complex matrix due to the high fat content of the extracts obtained after the sample treatment step.

Many multiresidue procedures employing different clean-up techniques and a variety of detection methods are reported on the determination of pesticide residues in olive oil. The most commonly used methodology is based on GC after a comprehensive clean-up step, in most cases based on liquid-liquid partitioning or gel permeation chromatography to separate the low molecular mass pesticides from the higher molecular mass fat constituents of the oil, such as triglycerides [2, 3]. The preparation of oil samples for the determination of pesticides by GC requires the complete removal of the high-molecular-mass fat from the sample to maintain the chromatographic system in working order. Recently, a multiresidue method for the determination of triazines and organophosphorous pesticides using MSPD followed by GC/MS and ion trap MS techniques was reported [4]. In this work, we further explore the capabilities of LC/ITMS and LC/TOFMS techniques for the identification of terbuthylazine, one of the most common pesticides found in olive oil.

Experimental Methods

Olive Oil Extraction

MSPD was used for the extraction of terbuthylazine from olive oil after a preliminary liquid-liquid extraction with organic solvents.

1. The liquid-liquid extraction:
    - An aliquot of approximately 5 g (ca. 5.5 mL) of olive oil sample was mixed with 15 mL of petroleum ether, saturated with acetonitrile, in a 100-mL separatory funnel, in which a two-step liquid-liquid extraction was performed.
    - A solution of 25 mL of acetonitrile, saturated with petroleum ether, was added to the olive oil mix obtained previously. The funnel was shaken vigorously for 3 minutes, and the acetonitrile phase was separated from the petroleum ether phase.
    - Another 10 mL of acetonitrile saturated with petroleum ether was added to the petroleum ether extract. The mixture was shaken again for 3 minutes and the acetonitrile phase was collected and added to the previous one.
    - Finally, a 7-mL aliquot of the acetonitrile extract was transferred to a 10-mL glass test tube. The extract was then carefully evaporated down to a final volume of about 2 mL. This remaining extract was transferred to a glass mortar to be subjected to matrix solid-phase dispersion.
2. The MSPD:
    - The extract obtained in the liquid-liquid extraction step was homogenized with 2 g of aminopropyl-bonded sorbent (Bondesil-NH_2, 40-m particle size, Varian Inc.) until a fine powder was obtained.
    - The mixture was transferred to a commercially available minicolumn containing 2 g of Florisil (12-mL Bond-Elut Varian minicolumn, Varian Inc.). This minicolumn was connected to a vacuum system for solid phase extraction adjusting the flow to 3 mL/min.
    - An elution step was carried out with 2×5 mL of acetonitrile. The final extract was evaporated until near dryness, then dissolved with 1:1 acetonitrile:water.
    - Prior to LC/ITMS and LC/TOFMS analysis, the extract was filtered through a 0.45-m PTFE filter (Millex FG, Millipore, Milford, MA, USA).

Using the MSPD method, recoveries for terbuthylazine from olive oil samples were higher than 96% with a 6% relative standard deviation (RSD) (n = 5).

Agilent 1100 Series LC/MSD TOF Methods

LC conditions

LC Pumps were Agilent 1100 binary pumps

Injection volume:	50 µL with standard Agilent 1100 ALS
Column:	ZORBAX Eclipse XDB-C8, 4.6 mm × 150 mm, 5 µm, p/n 993967-906
Mobile phases:	A = acetonitrile and B = 0.1% formic acid in water
Gradient:	5 minutes isocratic at 10% A, followed by a linear gradient to 100% A in 25 minutes at a flow rate of 0.6 mL/min

MS conditions

LC/MSD TOF

Source:	Positive ESI (electrospray ionization)
Capillary:	4000 V
Nebulizer:	40 psig
Drying gas:	9 L/min
Gas temp:	300 °C
Fragmentor:	190 V
Skimmer:	60 V
Oct DC1:	37.5 V
Oct RF V:	250 V
Reference masses:	*m/z* 121.0509 and 922.0098
Resolution:	9500 ± 500 @ *m/z* 922.0098
Mass range:	50–1000 *m/z*
Reference A sprayer 2:	Constant flow during the run

Agilent 1100 Series LC/MSD Trap Methods

LC/MSD Trap

Methods identical to LC/MSD TOF for direct comparison of peaks.

LC Pumps:	HP 1100
Inject vol:	50 µL
Column:	ZORBAX Eclipse XDB-C-8, 4.6 mm × 150 mm, 5 µm, p/n 993967-906
Mobile phases:	A = acetonitrile (ACN) B = 0.1% formic acid in water
Gradient:	10% A, isocratic, for 5 minutes followed by linear gradient to 100% A in 25 min at a flow rate of 0.6 mL/min

LC/MSD Trap

Positive ESI:	Capillary 3200 V
Nebulizer:	40 psig
Drying gas:	9 L/min, gas temp 300 °C
Capillary exit:	70 V
Skimmer:	60 V
Trap accumulation:	50,000 counts with maximum accumulation time of 200 ms

Results and Discussion

Identification of Terbuthylazine by LC/ITMS and LC/TOFMS

Olive oil is one of the most difficult food matrices due to the presence of numerous interferences that show up in full-scan mode. For this reason, the ion trap method was optimized in MS/MS mode by isolating the precursor ion at *m/z* 230, which corresponds to $[M+H]^+$. An isolation mass window of *m/z* 2 and optimized fragmentation amplitude was used in order to enhance the signal-to-noise (S/N) ratio. An amplitude voltage of 0.8 V was used to obtain good fragmentation for terbuthylazine, which fragments by losing the terbutyl group (–56) forming the *m/z* 174 fragment ion.

Figure 1 shows the analysis of an olive oil sample (spiking level 0.025 mg/kg) by ion trap MS/MS. The extracted ion chromatogram (EIC) for *m/z* 174, the main fragment of terbuthylazine, as well as its mass spectrum is shown. The fragmentation occurs at the C–N bond via the cleavage of the terbutyl group. This represents a characteristic fragmentation for this analyte, allowing for the structural confirmation of terbuthylazine in a relative complex matrix such as olive oil.

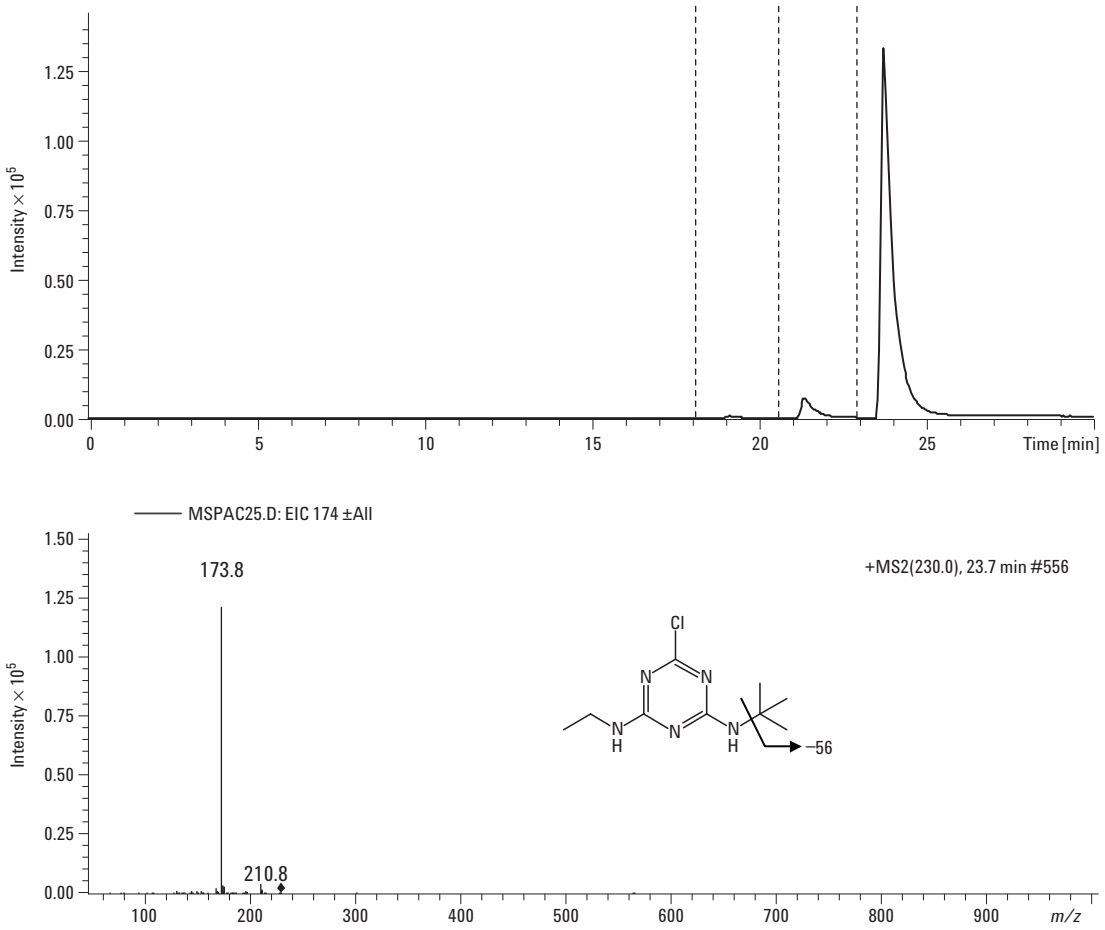

Figure 1. Ion Trap MS/MS chromatogram corresponding to the analysis of a spiked olive oil sample with terbuthylazine (0.025 mg/kg). The EIC for *m/z* 174 and its corresponding spectrum are shown.

LC/TOFMS analyses were optimized in terms of fragmentation. The in-source collisionally induced dissociation (CID) fragmentation is greatly enhanced at high fragmentor voltages. This provides highly valuable structural information since the accurate mass of the characteristic fragment ion can be used along with that of the molecular ion for confirmation purposes. The relative abundances for both the main fragment and the protonated molecule of terbuthylazine are summarized in Table 1 at three different voltages: 160 V (low), 190 V (medium), and 230 V (high). In order to obtain sufficient sensitivity for quantitative purposes (using the protonated molecule) and additional qualitative spectral information provided by the fragment ions generated by in-source fragmentation, a value of 190 V was chosen for further analyses. The accurate masses for both the main fragment and the protonated molecule of terbuthylazine in a spiked olive oil matrix are shown in Table 2.

Table 1. Effect of the Fragmentor Voltage on CID Fragmentation for LC/TOFMS

***m/z* ion**	**Relative abundance**		
	160 V	**190 V**	**230 V**
230 $[M+H]^+$	100	100	20
174 $[M+H–C_4H_8]^+$	5	30	100

Table 2. LC/TOFMS Accurate Mass Measurements for Terbuthylazine and its Main Fragment Ion in Olive Oil Matrix-Matched Standard (Fragmentor Voltage 190 V). Spiking Level: 0.025 mg/Kg

Elemental composition	Ion	Theoretical mass	Measured mass	Error mDa	Error ppm
$C_9H_{16}N_5{}^{35}Cl$	$[M+H]^+$	230.1167	230.1168	0.1	0.4
$C_9H_{16}N_5{}^{37}Cl$		232.1137	232.1134	–0.3	–1.5
$C_5H_9N_5{}^{35}Cl$	$[M+H–C_4H_8]^+$	174.0541	174.0542	0.1	0.6
$C_5H_9N_5{}^{37}Cl$		176.0511	176.0511	–0.05	–0.3

Along with the accurate mass of the protonated molecule and the information provided by the fragments obtained with an optimized in-source fragmentation, terbuthylazine presents another feature which enables its identification; the presence of a chlorine atom. The accurate mass pattern of the ^{37}Cl isotope signal confirms that the peak unequivocally contains only one chlorine atom (Figure 2). Therefore, the accurate mass of each ion as well as the presence of the chlorine signal, together with the characteristic retention time represent sufficient information to unequivocally identify and confirm this specie in such complex matrices.

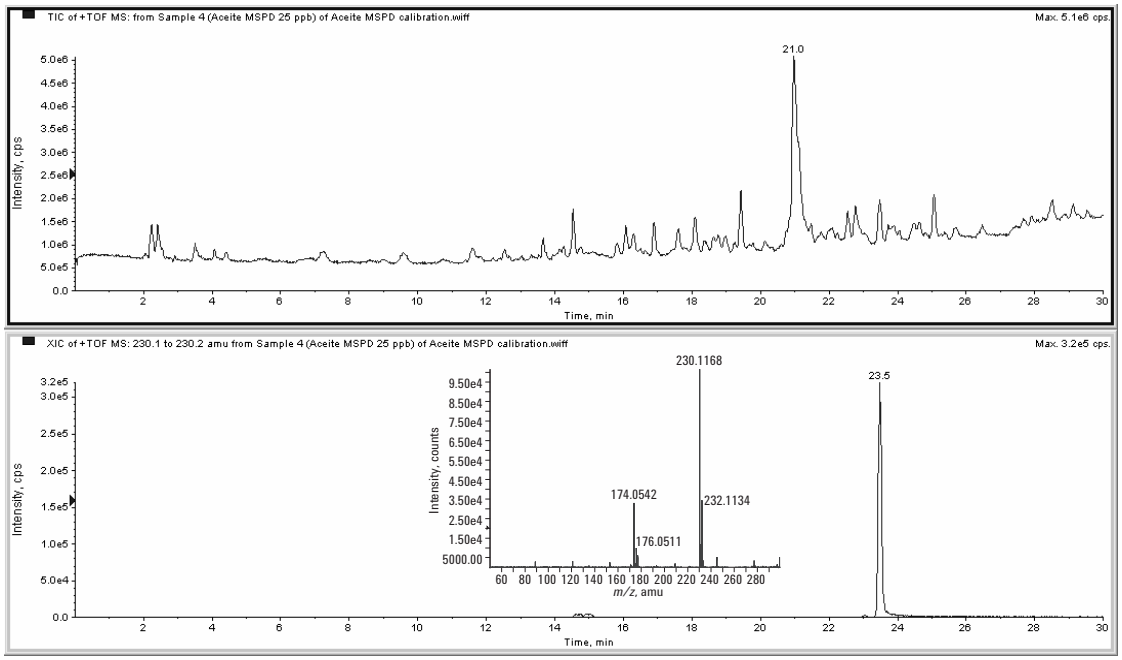

Figure 2. LC/TOFMS total ion chromatogram (TIC) corresponding to the analysis of a spiked olive oil sample with terbuthylazine (0.025 mg/kg). The EIC for *m/z* 230 and its corresponding spectrum are also shown.

Analytical Performance

The analytical performance of the proposed methods was studied in order to explore the ability to carry out quantitative analyses of terbuthylazine in these complex matrices with a high content of fat. The calibration was carried out using matrix-matched standards prepared by the extraction method based on MSPD described in the experimental section. Linearity was evaluated by analyzing solutions of matrix-matched standards at seven different concentration levels in the range 0.005–0.5 mg/kg. Using ion trap the quantitation was performed in MS/MS mode of the *m/z* 230 ion. Using LC/TOFMS the quantitation was carried out using the peak area from the EIC of the protonated molecule with a mass window of 0.1 Da. As an example, Figure 3 shows the linear calibration curve obtained by LC/TOFMS for terbuthylazine in an olive oil matrix compared to the curve obtained in pure solvent.

The limits of detection (LOD) were estimated from the injection of matrix-matched standard solutions with concentration levels giving a S/N ratio of about 3. The results obtained are shown in Table 3. The LOD obtained are remarkable since they are far below the maximum residue level regulations established for these pesticides. In this sense, LC/TOFMS analyses benefits of the use of narrow mass windows for quantitation purposes, which results in enhanced S/N ratio, thus providing lower detection limits. This fact illustrates the analytical potential of the proposed method based on MSPD and LC/TOFMS for the analyses of pesticides in complex matrices with high content of fat.

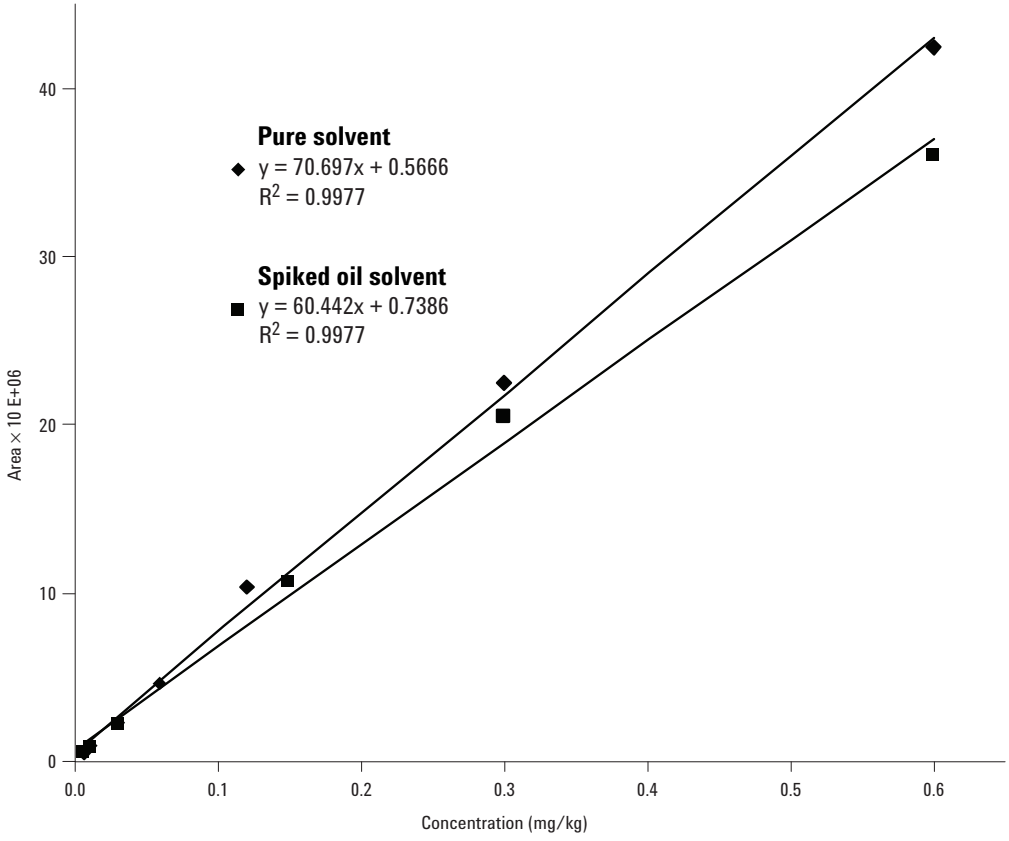

Figure 3. Calibration plot obtained from spiked olive oil samples versus solvent based samples by LC/TOFMS.

Table 3. Analytical Parameters for the Analysis of Terbuthylazine in Olive Oil Samples by Ion trap MS/MS and LC/TOFMS

Method	Concentration range (mg/kg)	Linearity (R^2)	LOD (µg/kg)	RSD (%) n = 5
LC/ITMS	0.005–0.5	0.991	0.2	5.5
LC/TOFMS	0.005–0.5	0.995	1	2

Analysis of Olive Oil Samples

To evaluate the effectiveness of the proposed method, it was applied to the analysis of olive oil samples. An example is shown in Figure 4 where traces of terbuthylazine were found in an olive oil extract; the EIC for *m/z* 230 is also shown. This is a real example illustrating the usefulness of the routine accurate mass measurement capabilities of LC/TOFMS, especially when analyzing traces of pesticides in complex samples such as olive oil. In this sense, the selectivity of LC/TOFMS relies on the resolving power of the instrument on the *m/z* axis, enabling discrimination between the target species and an "isobaric" interference within 0.05 Da of the mass difference (using 230 *m/z*, as example). In the case of terbuthylazine, it is easily overcome using the isotopic chlorine signal or a characteristic fragment not affected by other interfering species.

Conclusion

LC/ITMS and LC/TOFMS can be used for the identification and quantitation of terbuthylazine in olive oil samples after performing several preliminary sample treatments. The analysis by ion trap was achieved in MS/MS mode, monitoring the characteristic fragment ion at *m/z* 174. The identification by LC/TOFMS was accomplished with the accurate mass (and the subsequently generated empirical formula) of the protonated molecule ($[M+H]^+$ *m/z* 230), along with the accurate mass of the fragment ion and the characteristic chlorine isotope cluster present in terbuthylazine. The accuracy typically obtained was routinely better than 2 ppm. The method sensitivity, linearity, precision, accuracy, matrix effects, and LOD were also studied and they supported the potential of LC/TOFMS and LC/ITMS for the routine quantitative analyses of terbuthylazine in olive oil.

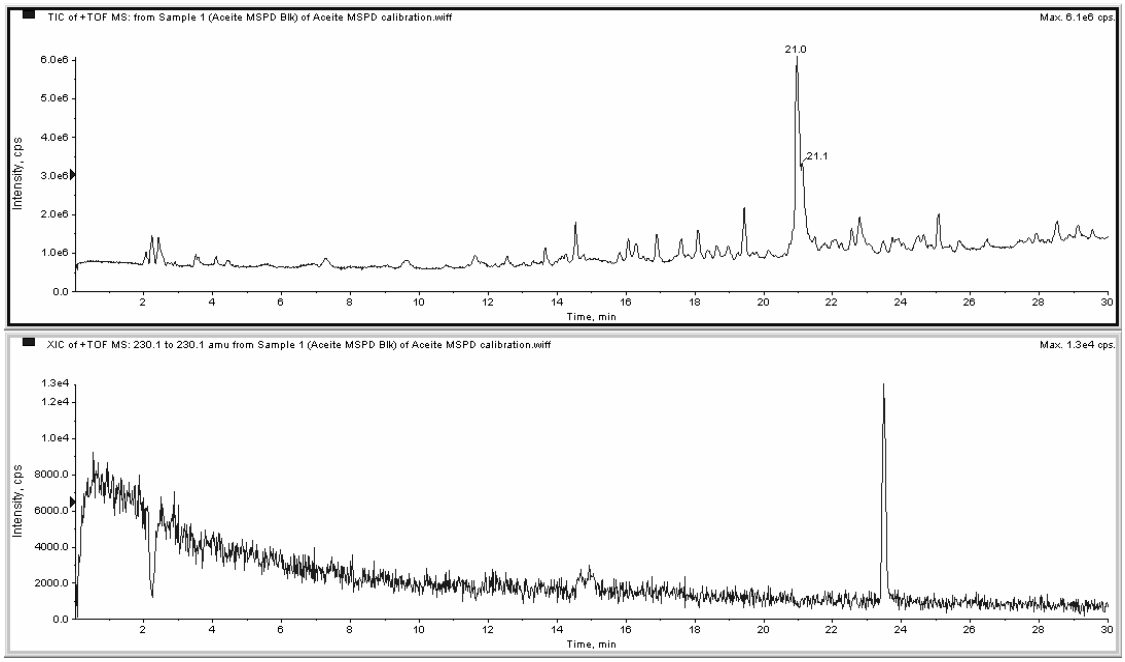

Figure 4. Upper: LC/TOFMS TIC of a "positive" for terbuthylazine in an olive oil sample. Lower: EIC of terbuthylazine using a 20 mDa mass window.

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