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Determination of herbicide residues in olive oil by gas chromatography–tandem mass spectrometry

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Abstract

This paper reports a method for the analysis in olive oil of multiresidues of the herbicides of low-medium polarity most widely used by Andalusian olive growers. The method, which uses gas chromatography/tandem mass spectrometry (GC–MS/MS), was developed within the framework of Project CAO00-005, which spanned the period from 2000 to 2004. The results obtained for more than 3000 samples of virgin olive oil and organic olive oil analyzed over such a period are reported. Samples were extracted with an acetonitrile/*n*-hexane mixture and cleaned up by passage through Florisil columns prior to analysis. A linear determination range for the herbicides from 1 to 500 μg kg^{-1} and a correlation coefficient better than 0.996 were achieved. The reproducibility, as relative standard deviation, was quite acceptable (8–11%), and so were herbicide recoveries (90–102%). The proposed method has been transferred to both public and private laboratories in the Andalusian region.

Keywords: Herbicides; Olive oil; Gas chromatography; Tandem mass spectrometry; Triazines; Diuron; Norflurazone; Diflufenican; Oxyfluorfen

1. Introduction

Crop protection products in general and herbicides in particular are essential for the subsistence of modern agriculture. However, the use of these substances poses potential hazards for humans, animals and the environment, and has thus aroused a strong interest in identifying their presence in food, drinking water and soils. The triazines simazine and terbuthylazine, and diuron, are among the herbicides most widely used by olive growers in the Spanish region of Andalusia. The low detection levels set by regulatory bodies and the complex nature of olive oils potentially containing herbicides have so far precluded the development of a straightforward, robust, sensitive, selective, accurate method for their routine screening in such an intricate type of matrix as regards identification and quantitation.

Abbreviations: GC–MS/MS, gas chromatography-double fragmentation mass spectrometry; MRL, maximum residue level (or limit); GPC, gel permeation chromatography; GC-NPD, gas chromatography–nitrogen–phosphorus detection; GC–MS, gas chromatography–mass spectrometry; LC–LC-UV, coupled-column liquid chromatography with UV detection; HPLC-DAD, high-performance liquid chromatography-diode array detection; HPLC–APcI–MS, high-performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry; HPLC–ES–MS, high-performance liquid chromatography–electrospray ionization–mass spectrometry; K_{ow}, octanol–water partition coefficient; LD_{50}, lethal dose; 50% or median lethal dose; GC–ECD, gas chromatography–electron capture detection; RSD_r, relative standard deviation for repeatability; RSD_R, relative standard deviation for reproducibility; Rec, recovery; LOQ, limit of quantitation.

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The US Environmental Protection Agency (EPA) recommends the use of analytical methods where identification is confirmed by mass spectrometry (MS) for this purpose. The US Food and Drug Administration endorses the use of this technique with three ions for confirmatory purposes (Makovi & Mahon, 1999). Moreover, selected-reaction monitoring with triple quadrupole instruments (Dagnac, Bristeau, Jeannot, Mouvet, & Baran, 2005; Gonçalves, Carvalho, Azenha, & Alpendurada, 2006; Sauret-Szczepanski, Mirabel, & Wortham, 2006) or ion-trap equipment has become the preferred tool for quantitation in this context. Because a full product spectrum can be recorded, the ion(s) to be used for quantitation can be chosen before or after acquisition and changed without the need for further analyses. In addition, full-scan product spectra can be used to exclude false positives.

Olive oil, which is a major ingredient of Mediterranean diet, is said to have significant protective effects against some types of cancer and coronary heart disease (Roche, Gibney, Kafatos, Zampelas, & Williams, 2000). This property has been ascribed to the presence of phenols in addition to squalene or oleic acid. The European Union produces 80% of the global output of olive oil (more than 2 million tonnes each year), with Spain as the leading country (34% of the output). Spanish olive groves are located mainly in Andalusia, a strongly agriculture-dependent region where the competent council is continuously striving to preserve the high-quality features of the oil.

There is currently no specific legislation in Spain as regards maximum residue limits (MRLs) for herbicides in olive oil, but only in olives. However, levels five times higher than those set for olives are commonly accepted for oil. With this rule of thumb, the maximum acceptable contamination levels for some of the more commonly used pesticides would be 500 $\mu g\ kg^{-1}$ for atrazine and simazine, 250 $\mu g\ kg^{-1}$ for terbuthylazine, 100 $\mu g\ kg^{-1}$ for oxyfluorfen and diflufenican, 250 $\mu g\ kg^{-1}$ for norflurazone and 1000 $\mu g\ kg^{-1}$ for diuron (see Table 1).

However, the presence of pesticide residues at low or even undetectable levels can be expected to become a major quality criterion for rejection of olive oil in the future. This has raised the need to develop new methods capable of detecting herbicides at levels below those accepted by current regulations. Until such methods are developed, some producers are offering organic olive oil, which is produced from olives treated with no pesticide or man-made fertilizer. The new methods should rely on sensitive enough techniques to allow herbicides to be detected at very low levels in order to avoid fraud and/or alert farm owners of the presence of contamination in nearby holdings.

Spanish Ministry of Agriculture banned the use of simazine on olives in 2003. This has fostered the use of terbuthylazine as a substitute pesticide.

The number of methods available for determining triazines in water (Sabik & Jeannot, 2006) exceeds that of methods for their analysis in other types of sample matrix in combination (the sequence is water $\gg$ soil $>$ crops $\gg$ fats and oils). At the beginning of this research project, to the best of our knowledge, no such method existed, however, for the determination of triazines in olive oil, which is much more complex a matrix than is water. Furthermore, analytical methods for determining pesticides in fats and oils invariably require extraction of pesticide residues and fat from the matrix, followed by clean-up of the extract prior to chromatographic analysis. The most widely used technique in this context is liquid–liquid partitioning; however, other, more environmentally-friendly methods such as supercritical fluid extraction with carbon dioxide (Gonçalves et al., 2006; Hopper, 1999) are gradually gaining ground for this purpose. Extracts are usually cleaned up by passage through Florisil, alumina or silica columns; matrix solid-phase dispersion; solid-phase extraction or GPC (Albero, Sánchez-Brunete, Donoso, & Tadeo, 2004; Balinova, 1993, 1998; Sabik, Jeannot, & Rondeau, 2000). Finally, identification and determination are done with gas (GC-NPD (Gómez de Barreda et al., 1998; Sánchez-Brunete, Pérez, Miguel, & Tadeo, 1998), GC–MS (Frías, Rodríguez, Conde, & Pérez-Trujillo, 2003; Hernández, Hidalgo, Sancho, & López, 1998; Sánchez-Brunete et al., 1998)) or liquid chromatography (LC-LC-UV (Hernández et al., 1998; Hidalgo, Sancho, & Hernández, 1997; Martínez Galera, Martínez Vidal, Garrido Frenich, & Gil García, 1997), HPLC-DAD (Gómez de Barreda et al., 1998; Martínez Galera et al., 1997; Sabik et al.,

Table 1
Characteristics of the target herbicides

Compound	log K_{ow}[a]	Purity (%)	LD_{50} oral LD_{50} in rats ($mg\ kg^{-1}$)[b]	MRL ($\mu g\ kg^{-1}$) in olives[c]
Atrazine	2.6	99.2	3080	100
Propazine	2.9	99.5	>5000	Not regulated
Simazine	2.2	99.3	>5000	100
Terbuthylazine	2.8	99.0	2160	50
Diuron	2.7	99.4	3400	200
Norflurazone	2.3	98.6	9400	50
Bromophos (IS)	5.2	99.1	–	–
Oxyfluorfen	4.7	99.5	>5000	20
Diflufenican	4.9	99.5	>5000	20

[a] Data available at http://risk.lsd.ornl.gov/cgi-bin/tox/TOX_select?select=csf.
[b] Taken from EPA datasheets and reference Yokley (2003).
[c] MRLs as per Spanish Legislation (April 1st, 2002). As a rule of thumb, the limits accepted for olive oil are five times the MRLs in olives.

2000), HPLC-APcI-MS (Asperger, Efer, Koal, & Engewald, 2002; Thomas, 1998), HPLC-ES-MS (Huang, Mayer, Yokley, & Perez, 2006; Lacassie et al., 1999; Yokley, 2003)), which are used for pesticides in water mainly. An excellent review of the analytical methodologies available for determining triazines in water, soil, crops and biological fluids has been published (Yokley, 2003). In 2004 a multiresidue method for determining herbicides in olive oil was described (Sánchez, Vázquez, Andini, & Villén, 2004). Some other authors (Ballesteros, García Sánchez, & Ramos Martos, 2006) have recently used equipment and methods similar to ours, or even the same internal standard (methyl-bromophos). For obvious reasons, the results of our project have remained confidential until now; however, the method has been disclosed at dedicated meetings (Aramendía et al., 2002, 2005; Porras, 2005) and courses taught by SCAI (Central Service for the Support of Research) at the University of Córdoba.

The primary aim of this work was to develop a fast, efficient extraction, clean-up and detection method for the routine analysis of herbicide residues in olive oil. The idea was to develop a reliable, sensitive method which could detect such low herbicide concentrations as to conclude whether the herbicide was present or not. Obviously, undetection of the herbicides studied in the present manuscript does not imply that the oil can be termed as organic olive oil since, for instance, some other plaguicides could be present or man-made fertilizers could have been used. What follows is a description of the method and main results obtained by the authors over the period 2000–2004 within the framework of Project CAO00-005 (Improving the Quality of Olive Oil) with funding from the Andalusian regional government and FEDER.

2. Materials and methods

2.1. Chemicals

All herbicide standards were obtained from Riedel de Haën (Seelze, Germany). Their percent purity, logarithmic octanol–water partition coefficients ($\log K_{ow}$), oral toxicity to rats (LD_{50}, mg kg^{-1}) and maximum residue levels (MRLs) in olive oils are listed in Table 1.

All solvents (viz. acetone, acetonitrile, *n*-hexane, methanol, petroleum benzine and diethyl ether) were HPLC grade and supplied by Merck (Darmstadt, Germany).

Anhydrous sodium sulphate was purchased from Panreac (Montada i Reixac, Barcelona, Spain). Florisil polymer (mixed silica and magnesia, 0.150–0.250 mm for column chromatography, from Merck) and 10 cm 14/23 extraction tubes from Pobel (Madrid, Spain) were also used.

2.2. Standards

A stock solution of each studied herbicide at a 100 mg l^{-1} concentration was prepared in acetone. The herbicide solutions used to spike oil samples were made by diluting appropriate volumes of the stock solutions in order to obtain a herbicide concentration of 1–500 μg kg^{-1} for GC–MS/MS analyses and 40–2000 μg kg^{-1} for GC–ECD analyses. Quantitation was done by using blank matrix extracts as the GC response of many pesticides is known to be matrix-dependent (Erney, Gillespie, Gilvydis, & Poole, 1993; Hajslova et al., 1998). Bromophos-methyl, a compound structurally similar to the target herbicides, was used as internal standard in GC and GC–MS/MS analyses.

2.3. Matrices and real samples

Herbicide-free virgin olive oil samples obtained by various producers from three different olive varieties were used as matrices for spiked samples. The real samples studied were supplied by the SCAI-Mass Spectrometry Laboratory of the University of Córdoba and collected by both farmers and regional administration workers.

2.4. Sample extraction

An amount of 2 g of olive oil containing 500 ppb of internal standard (Bromophos-methyl) were dissolved in 2 ml of *n*-hexane and supplied with 20 ml of acetonitrile (two extractions with 10 ml each), the mixture being vigorously shaken in a Selecta Vibromatic-384 mechanical shaker for 20 min, centrifuged on a Selecta-1000 apparatus and refrigerated in order to favour layer separation, a small amount of anhydrous sodium sulphate (ca. 0.1 g) being added to the dry extract (the acetonitrile layer) at the end.

2.5. Clean-up

The previous extract was placed in a heart-shaped flask and the solvent evaporated to dryness at a low pressure at 45 °C in a Heidolph-Laborata 4000 rotary evaporator from Heizbad WB. Then, the dried extract was dissolved in 25 ml of 94:6 (v/v) petroleum benzine–diethyl ether mixture. The resulting solution was passed through a Florisil column that was pre-activated with 25 ml of the previous solvent mixture. Then, two 25 ml portions of 94:6 and 85:15 (v/v) solvent mixtures, and 55 ml of 50:50 (v/v) mixture, were used to elute the herbicides. The final solution was collected in a 100 ml heart-shaped flask and evaporated to dryness in the rotary evaporator, the residue being reconstituted in 1 ml of acetone for GC–ECD or 0.5 ml of cyclohexane for GC–MS/MS.

2.6. GC–ECD conditions

A Fisons Instruments Series II 83600-00 gas chromatograph equipped with an electron capture detector (Model ECD-800, 50 V, 1 μs, Nickel-63 370 MBq, also from Fisons Instruments) and a Cold On-Column injector was used. A non-polar 2 m, 0.32 mm i.d. column and a Teknokroma TRB-5MS 30 m, 0.32 mm i.d. column of 0.25 μm film thickness were also used. The optimized chromatographic

conditions were as follows: carrier and make-up gas, nitrogen; sample size, 1 μl; column head pressure, 10 psi; oven temperature, 60 °C (hold 2 min), followed by a 10 °C min^{-1} ramp to 170 °C (hold 2 min), a 3 °C min^{-1} ramp to 200 °C (hold 1 min) and a 10 °C min^{-1} ramp to 250 °C (hold 5 min); detector temperature, 330 °C.

2.7. GC–MS/MS conditions

A CP-3800 gas chromatograph from Varian (Sunnyvale, CA) equipped with a 1079 PTV injector containing a plug of Carbofrit (Restek, Bellefonte, PA) and coupled to a Saturn-220 Ion-Trap MS/MS detector, also from Varian, was used in conjunction with a 2 m, 0.32 mm i.d. non-polar column and a Varian CPSil8CB 30 m, 0.25 mm i.d. column of 0.25 μm film thickness. The chromatographic conditions were as follows: injector temperature, 70 °C (hold 0.5 min), followed by a 100 °C min^{-1} ramp to 300 °C (hold 15 min); carrier gas, helium more than 99.999% at 1.0 ml min^{-1}; sample size, 7 μl; column head pressure, constant flow of 1 ml min^{-1}; oven temperature, 70 °C (hold 3.5 min), followed by a 25 °C min^{-1} ramp to 180 °C (hold 10 min) and a 4 °C min^{-1} ramp to 300 °C (hold 10 min). The GC–MS transfer line temperature was 280 °C; the MS source pressure and temperature were 10^{-6} mbar and 200 °C, respectively; the electron impact ionization energy was 70 eV; and the scan rate 1.5 scans s^{-1}. The ion-trap mass spectrometer was operated in both the electron ionization (EI) and chemical ionization (CI) modes. The filament emission current was 90 μA for EI and 30 μA for CI. The reagent used for chemical ionization was methanol. Finally, the MS–MS process was conducted in the collision-induced dissociation (CID) mode, using non-resonant excitation.

3. Results and discussion

3.1. Optimization of the extraction and clean-up steps

3.1.1. Extraction

A mixture of acetonitrile and *n*-hexane was found to be the most effective choice of extraction solvent. Adding a small amount of *n*-hexane and refrigerating the samples favored retention of triglycerides in the *n*-hexane layer. The minimum volume of acetonitrile providing acceptable recoveries was 20 ml (two extractions with 10 ml each) and the optimum extraction time for all herbicides 20 min. Fig. 1 clearly illustrates that recoveries obtained using two extractions with 10 ml acetonitrile each are higher than those achieved for a single extraction with 20 ml.

3.1.2. Clean-up

Because the acetonitrile layer inevitably contained a small amount of triglycerides after liquid–liquid partitioning, it had to be cleaned up with Florisil. Prior to passage through the column, however, the acetonitrile layer must be freed of any water by using anhydrous sodium sulphate

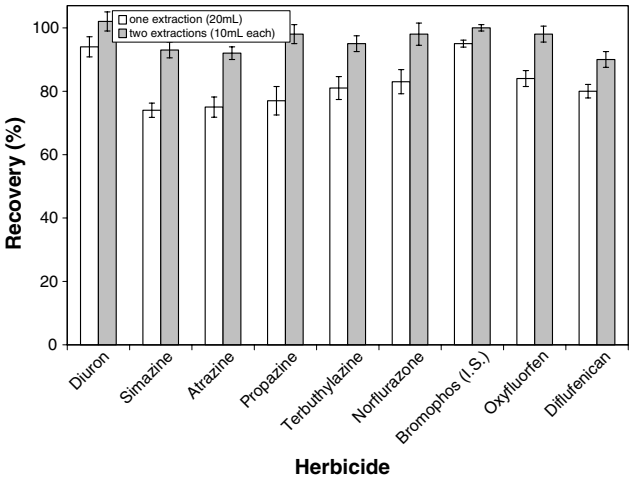

Fig. 1. Results obtained for recovery tests using a single extraction with 20 ml of acetonitrile or two extractions with 10 ml acetonitrile each.

in order to avoid deactivation of the Florisil. The optimum amount of Florisil was determined from the GC–ECD chromatographs of Fig. 2, which were obtained with 2–5 g of polymer. As can be seen, too much Florisil (5 g) resulted in incomplete elution of the herbicides, whereas too little (2 g) led to co-extraction of other compounds that hindered determination of some analytes. Also, an amount of 4 g of Florisil provided poor recoveries (in the region of 50%), so we chose to use 3 g in subsequent tests.

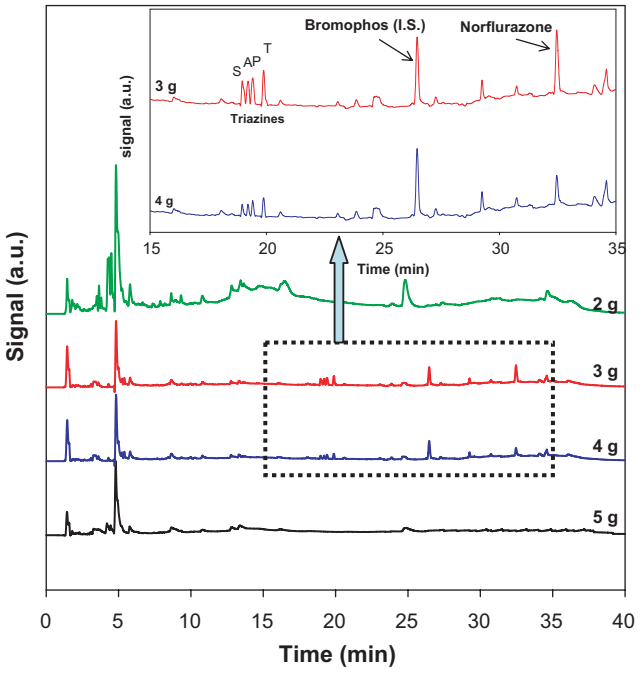

Fig. 2. Influence of the amount of Florisil used on the clean-up efficiency and recovery of herbicides. Elution order for triazines is simazine (S), atrazine (A), propazine (P) and terbuthylazine (T).

3.2. Recovery of herbicides

Recoveries were calculated from quintuplicate analyses of samples that were spiked at five times the limit of quantitation (LOQ) and at the MRL for each analyte, and found to be 92–98% for triazines, 98% for oxyfluorfen and norflurazone, 90% for diflufenican and 102% for diuron at both concentration levels. The corresponding relative standard deviations (RSDs, $n = 5$) were all less than 7%.

3.3. GC–ECD calibration

All herbicides were analyzed as such by GC–ECD. By exception, diuron had to be previously transformed into its metabolite (1,4-dichloro-2-isocyanato benzene) in the PTV injector of the GC–MS/MS equipment. Because the on-column injector of our GC-ECD instrument was not amenable to heating, diuron gave a non-Gaussian peak by effect of its decomposing through the column, so it could not be quantified with the proposed method.

Table 2 lists the limit of quantitation (LOQ, at a signal-to-noise ratio of 10), linear range and correlation coefficient (R) as obtained from calibration curves, as well as the standard deviations for repeatability (RSD_r, $n = 5$) and reproducibility (RSD_R, $n = 6$), for the studied analytes. A comparison with the MRLs of these compounds in olives and olive oil (see Tables 1 and 2) reveals that they are very similar; therefore, a more sensitive and especially selective method is required in order to meet current regulations as the electron capture detector does not afford unequivocal confirmation of identity and is often subject to matrix interferences (Gonçalves et al., 2006).

3.4. GC–MS/MS tests

Table 3 shows the GC–MS/MS conditions, and the quantitation and confirmation ions, used for each analyte. Table 4 compares the linear ranges, LOQs (S/N = 10) and correlation coefficients (r^2) as obtained from calibration curves, as well as the relative standard deviations for repeatability (RSD_r, $n = 5$) and reproducibility (RSD_R, $n = 6$), for the analytes. As can be seen, the results meet the regulations currently in force. Also, the ability to record the full mass spectrum allows each target herbicide to be unequivocally identified.

3.5. Applications

The applicability of the proposed method was checked by spiking various herbicide-free olive oils at the EU's MRL for each analyte. The standard deviations thus

Table 2
Linearity range, limit of quantitation (LOQ), recovery (Rec), relative standard deviation for repeatability (RSD_r), and relative standard deviation for reproducibility (RSD_R) of the analytes as determined by GC–ECD

Compound	Linear range (μg kg^{-1})	R	RSD_r (%)	RSD_R (%)	Rec (%)	LOQ (μg kg^{-1})	MRL[a] (μg kg^{-1})
Diuron	Non-Gaussian peak	–	–	–	–	–	200
Simazine	50–2000	0.9994	4	7	93	50	100[b]
Atrazine	50–2000	0.9997	5	9	92	50	100
Propazine	50–2000	0.9985	4	8	98	50	Not regulated
Terbuthylazine	40–2000	0.9987	3	5	95	40	50
Norflurazone	40–2000	0.9979	3	6	98	40	50

[a] MRL, maximum residue limits for the herbicides in olives.
[b] The use of simazine in olive groves was banned in Spain in 2003.

Table 3
MS/MS conditions for the herbicides analyzed

Compound	RTW[a] (min)	Precursor ion (m/z)	Ion[b]	CID[c]	CID RF (m/z)	Product ion-quantifiers (m/z)	Product ion-qualifiers (m/z)
Diuron[d]	8.11	187	EI	75	75	124	124, 187, 159, 97, 88
Simazine	13.03	202	CI	71	80	104	104, 174, 146, 202, 132
Atrazine	13.16	216	CI	80	90	174	174, 146, 104, 132, 216
Propazine	13.26	230	CI	85	110	188	188, 146, 230, 174
Terbuthylazine	13.73	230, 214	CI EI	57, 92	80, 100	174 150 + 178	174 150, 178, 214,169
Norflurazone	30.47	303	EI	100	100	234	145, 234, 302, 260
Bromophos IS[e]	21.48	331	EI	75	90	286	284, 286, 316
Oxyfluorfen	27.00	300	EI	100	95	132	132, 223
Diflufenican	31.76	394	EI	82	150	374	266, 374

[a] RTW, retention time window.
[b] Ion, ionization mode; CI, chemical ionization with methanol; EI, electron ionization.
[c] CID, collision-induced dissociation. Amplitude, V.
[d] Detected as a intermediate: 1,4-dichloro-2-isocyanato benzene.
[e] IS, Internal Standard.

Table 4
Linearity range, limit of quantitation (LOQ), recovery (Rec), relative standard deviation for repeatability (RSD_r), and relative standard deviation for reproducibility (RSD_R) of the analytes as determined by GC–MS/MS

Compound	Linear range (μg kg^{-1})	R	RSD_r (%)	RSD_R (%)	Rec (%)	LOQ (μg kg^{-1})
Diuron	3–500	0.9985	6	8	102	3
Simazine	2–500	0.9983	5	8	93	2
Atrazine	1–500	0.9987	4	11	92	1
Propazine	1–500	0.9985	6	8	98	1
Terbuthylazine	0.5–500	0.9976	5	10	95	0.5 CI
	2–500					2 EI
Norflurazone	1–500	0.9958	7	9	98	1
Bromophos IS[a]	–	–	–	–	100	1
Oxyfluorfen	2–500	0.9967	5	11	98	2
Diflufenican	0.5–500	0.9964	5	10	90	0.5

[a] IS, Internal standard.

obtained ($n = 5$) were all less than 10%. No similar work on the determination of herbicides in olive oil had been reported at the time the present project was started. During the four years it has been conducted, the proposed GC–MS/MS method has been applied to more than three thousand samples of virgin or organic olive oil. The results can be summarized as follows: the analysis of the 2001 season revealed the presence of simazine at levels overly exceeding its MRL in a large number of samples (15% of all studied); moreover, concentrations of this herbicide over the range 235–350 μg kg^{-1} were frequently encountered in the contaminated samples, which were set aside for refining. These results prompted a broad campaign intended to disseminate useful information and provide effective training in this respect for farmers. As a consequence, the number of contaminated samples encountered in the 2002 season, and their herbicide levels, were markedly reduced with respect to the previous one. The use of simazine was banned in 2003, which resulted in a further reduction in the extent of contamination by this herbicide; in fact, this compound was rarely detected, and only at levels well below its MRL. A number of oil samples were found to contain terbuthylazine the most common and highly concentrated among the studied herbicides, followed by diuron. This latter herbicide is very commonly applied to olive crops and was found, as its metabolite, in high proportions that exceeded its MRL in less than 3% of all samples. On the other hand, norflurazone, which is scarcely used by Andalusian olive growers, was rarely detected, and oxyfluorfen was present in few samples and at very low levels in any case. By contrast, diflufenican, which is commonly used in the region, was encountered in many samples, but generally at very low levels below its MRL in most samples. In any case, organic olive oil exhibited lower levels of all herbicides much lower than the respective MRLs or even their complete absence.

Application of the proposed GC–MS/MS method has been extended to 80 pesticides, with LOQs of a few parts-per-million and excellent repeatability and reproducibility in all cases. Other herbicides used by olive growers were studied in previous work (Aramendía et al., 2006). Amitrole and glyphosphate are the targets of various HPLC–MS/MS methods currently under development. Because oil is a non-polar matrix, however, these pesticides pose less serious contamination problems.

4. Conclusions

The proposed extraction-clean-up method provides acceptable recoveries for the herbicides most commonly used on olive trees in Spain. The fact that the final extract contains very little oil residue thanks to the efficient clean-up procedure used allows it to retain its separation efficiency after many injections. Moreover, the low solvent volume and short analysis time required per sample make it especially economical.

GC–ECD does not afford reliable identification and quantitation of the herbicides in such a complex matrix as olive oil; furthermore, the LOQs it provides are inadequate or too close to the MRLs for the herbicides. On the other hand, the GC–MS/MS technique provides excellent selectivity and LOQs, which allows the simultaneous confirmation of identity and quantitation of the herbicides at low levels in olive oil.

Diuron was detected as its metabolite (1,4-dichloro-2-isocyanato benzene, which is also the metabolite for linuron). The type of injector used dictates whether this herbicide can be accurately quantified.

Bad agricultural practices were found to be origin of the herbicide contamination present in the olive oils. Disseminating good practices and transferring the required technology to agricultural laboratories, which were two objectives of this work, allowed the proportion of samples containing herbicides above their MRLs to be reduced below 3% and their levels in such samples to be decreased very markedly with respect to the year our Project was started (2001).

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