Multi-residues Analysis for 88 Pesticides and Metabolites from Soil by Gas Chromatography-time - of - Flight Mass Spectrometry

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An easy and fast Gas Chromatography-time-of-Flight Mass Spectrometry (GC-TOF-MS) method for 88 pesticides and metabolites identification and quantitation from soil was developed. Most widely used pesticides from group of pesticides, like herbicide, insecticide, fungicide were analyzed. The soil samples were extracted with acetonitrile acidified with 1% acetic acid; then extract was buffered with anhydrous magnesium sulfate and sodium acetate and then was cleaned-up by solid-phase extraction (SPE) with anhydrous magnesium sulfate and primary-secondary amine (PSA) . The final extract was directly injected in GC. Analysis of extracts was carried out by GC-TOF-MS within 29.2min run time. The GC method was validated for soil in term of linearity, accuracy, limit of detection (LOD) and limit of quantification (LOQ). Good linearity with correlation coefficients (r^2) higher than 0.98 was obtained. For 60 analytes recoveries were in range of 70-120% and LOQ values in range of 0.01- 0.05mg/kg. Instrument LOD values generally varied from 0.5 to 0.8ng . mL^{-1}. The developed method was successfully tested on five soil samples from different cultivated lands, with and without known history in terms of pesticide use.

Keywords: Soil; Pesticide residues; Multi-residues analysis; Gas chromatography–Time-of-flight Mass Spectrometry, Solid-phase extraction

The monitoring of pollution levels for pesticide residues in agricultural products (vegetable), soil, water and other components of agricultural systems is a major concern for the environment. Pesticides and their degradation products are typically found in soil either from direct application or indirect from crop spraying. Adsorption is the main mode of interaction between pesticides in soils, although hydrogen bonding, ionic bonding and van der Waals forces play roles as well, albeit smaller and more pesticide specific roles [1-3]. The degree to which pesticides bind to soil is also determined by ageing of the chemical, or the time of contact between the chemical and soil and subsequent stronger association over time [2, 4, 5]. Additionally, the amount of organic (humus) versus inorganic (minerals, rock fragments, etc.) present content can affect how pesticides interact with soil [3]. All the above interactions between soil and pesticides will ultimately determine how much the pesticide residue is bio-available, or how much the bound residue can be taken up by plants and/or wildlife and ultimately the by human population [2]. The quantity of pesticide residues accumulated in the plants is correlated with the soil retention capacity. More heavily contaminated plants grown on soils are those with high capacity to retain pesticides. This explains the stronger contamination of plants grown on soils with clayey texture than those grown on sandy soils [1-7]. Among the most significant migration effects and processes by which pesticides are evaporated on the soil surface and with other chemical components (detergents, heavy metals, etc.), with which the soil structural compounds interact, are released into groundwater [8-12]. The raising of pesticides to the soil surface occurs due to convection and evaporation conditional. The evaporation of pesticides is more advanced in moist soils than dry ones and the pesticides moving in the groundwaters occurs along with the stream of water that infiltrates. After a few years of use, the pesticides can be found in the wells to a depth greater than 50m [1, 6, 12].

The growing cultivation of organic vegetables and fruits in recent years primarily involves the knowledge of the soil history in terms of pollutants content, especially of pesticide residues content. On the other hand, the knowing of the pollutants content in order to create an effective soil remediation system is imperative [6].

Preparation procedures of soil samples for pesticides analysis are a challenge due to the complex nature of the matrix, and are usually associated with many steps in sample preparation and take long times. Different organic extractive solvents have been proposed in recent years (i.e. methanol, ethanol, acetone and acetonitrile) and many extraction procedures, such as Soxhlet, liquid-liquid extraction, solid phase extraction, supercritical fluid extraction, automated microwave extraction, have been tested [6, 7, 13-17].

A crude extract that have large amounts of matrix co-extractives is obtained after extraction of soil sample. The matrix masks target compounds are leading to the instrument contamination if no clean-up is applied. As a consequence, a clean-up step is required, such as SPE or GPC [7,13-15], but unfortunately, very often this clean-up step reduces recoveries or removes target compounds together with matrix components.

Gas chromatography – Mass Spectrometry (GC-MS) has become the primary approach to analyze all classes of GC – amenable pesticides from different matrix, even from soil samples [2, 6, 7, 13-16].

Three molecular properties, namely polarity, volatility and thermal lability, have an influence on the recovery and detection of pesticides. Polarity varies from lipophilic - non-ionic pesticides and synthetic pyrethroids (e.g. deltametrin) to hydrophilic - water soluble organophosphorous pesticides. Many pesticides are thermally labile (e.g. dicofol, captan, folpet) being degraded in the GC system due to the temperature of the injector and of the column [16, 17].

The aim of this study is the applicability evaluation of an easy extraction method and a fast GC TOF-MS method for

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routine analysis of pesticide residues from soil. The developed method presented in this paper use a GC-TOF MS technique, which offers many advantages in term of selectivity and sensitivity for low quantities of pesticides in dirty extracts such as vegetables, soils and sediments. The LECO Pegasus GC Time-of-Flight Mass Spectrometer (TOF-MS) offers unique advantages for reducing analysis time, taking full advantage of Fast GC techniques [6, 16-18]. Complicated mixtures of co-eluting compounds from samples are rapidly analyzed using Automated Peak Find and Deconvolution algorithms. Deconvolution not only assigns the proper spectra to each co-eluting component, but also assigns the correct signal intensity to each component, allowing the quick and reliable quantification of the co-eluting species [18].

Experimental part

Reagents and materials

Pesticide analytical standards were purchased from Sigma-Aldrich (Munich, Germany). The stock solution (1000μg/mL) for each pesticide was prepared in pure toluene and stored at -18°C. An intermediary standards mixture (5μg/mL) was prepared from stock solutions by dilution with isooctane:toluene 9:1, v/v. Working standard solutions were prepared by dilution with *iso*-octane:toluene 9:1, v/v. Solution of hexachlorbenzene (HCB) (0.2μg/mL) prepared in *iso*-octane:toluene 9:1, v/v was used as internal standard (IS).

All solvents (acetonitrile, toluene, *iso*-octane, acetic acid – all analytical grade), anhydrous magnesium sulfate, sodium chloride, sodium acetate (reagent grade) were purchased from Sigma-Aldrich (Munich, Germany). Primary-secondary amine (PSA) (reagent grade) and PSA dispersive SPE Clean-up tubes (dSPE) were purchased from Supelco, USA.

Soil sampling and storage

Five samples of soil (ca 200g each) were collected from cultivated areas where were used pesticides treatments. Soil sample were collected using a steel soil sampler (soil core 0.06m - diameter) at 0-10cm depth [19].

Each soil sample was packed in a plastic bag and freeze at -18°C. Each sample was homogenized and divided in two portions before analysis. One portion was used to detect and quantify the target compounds under the experimental conditions described above, and the other portion was divided in 10g samples that were used to check the performance of the method.

Sample preparation

10g of soil sample were accurately weighted into a 250mL polypropylene centrifuge tube and mixed with 10mL water and 20mL acetonitrile acidified with 1% acetic acid. After the addition of HCB (0.2μg/mL), the residues were extracted using an Ultraturax homogenizer (15.000rpm for 1min). Then, 4g $MgSO_4$, 1g NaCl and 1g CH_3COONa were added and the resulting mixture was shaken for 1min and then centrifuged for 5min at 4000rpm. An aliquot of the acetonitrile extract (6mL) was transferred to a 20mL polypropylene centrifuge tube and 150mg PSA and 900mg $MgSO_4$ were added. The mixture was shacking by hand for few minutes, centrifuged and then 1 mL from the resulting extract (ca 4mL) was analyzed by GC TOF-MS [13].

GC-TOF-MS analysis

An LECO Pegasus Time-of-Flight Mass Spectrometer (TOF-MS) (USA) equipped with an Agilent 6890 series gas chromatograph with two ovens, Agilent 7683 series Autosampler and a split/splitless capillary injector port, was used. Chromatographic separation was achieved on two capillary columns, first RXi-MS 30m x 0.25mm x 0.25μm (Restek, USA) and second BPX50 1.6m x 0.1m x 0.1μm (SGE Analytical Science, Australia). The injector temperature was 250°C and splitless injection was performed using helium as carrier gas with a flow rate of 1.2mL/min. The ovens temperatures were programmed as follow: oven 1 - 80°C (2 min), 20°C/min to 180°C (0 min), 5°C/min to 220° C (0min), 25°C/min to 300°C (11min); oven 2 - 110°C (2 min), 20°C/min to 210°C (0 min), 5°C/min to 250°C (0 min), 25°C/min to 330°C (10min) without modulation. The injection volume was 1μL. The mass spectrometer was operated in electron ionization mode (EI) and full scan mode monitoring between m/z 40 and m/z 450, with ionization energy of 70eV and acquisition rate of 15 spectra/second. The transfer line temperature was kept at 280°C.

Validation of the method

The method was validated for the following parameters: linearity, matrix effect, limit of quantification, specificity, repeatability, reproducibility and uncertainty.

Linearity

Seven level calibration standard solutions for each pesticide (0.01; 0.02; 0.05; 0.1; 0.2; 0.5; 0.8μg/mL) were prepared by dilution of intermediary standards mixture (5μg/mL) with *iso*-octane:toluene 9:1, v/v. At each solution HCB (0.2μg/mL) was added.

Linearity range was between 0.01-0.8mg/kg. The evaluation of calibration curves linearity was done both in solvent (*iso*-octane:toluene, 9:1 v/v) and spiked matrix at concentration 0.01μg/mL, 0.02μg/mL, 0.05μg/mL, 0.1μg/mL, 0.2μg/mL, 0.5μg/mL and 0.8μg/mL. Each solution was injected four times (n = 4) and relative standard deviation (RSD), average peak areas, calibration curve equation and correlation coefficients (r^2) were calculated. The linearity was tested both in solvent and in soil matrix being different for many pesticides.

Matrix effect

A fortified sample at 0.1mg/kg was analyzed and the analyte concentrations were calculated. A matrix effect was evaluated as follows:

EM (%) = (analyte concentration in matrix - analyte concentration in solvent)/analyte concentration in matrix * 100

Limit of detection (LOD)

The LODs for each pesticide were determined by six replicate injections at lowest and detectable levels (0.005μg/mL, 0.008μg/mL, 0.01μg/mL, 0.02μg/mL) and were calculated from the STDEV of the average peak areas by formula:

$$LOD\ (ng/mL) = (y_0 + 3STDEV)/s$$

where y_0 is matrix calibration curve interception, STDEV is standard deviation of the peak areas with lowest concentration and s is the slope of calibration curve areas/concentration.

Limit of quantification (LOQ)

The LOQ is the lowest validated spike level meeting the method performance acceptability criteria (mean recoveries in the range 70-120%, with an RSD ≤ 20%). LOQ was determined for each pesticide being calculated from STDEV of the average peak areas by formula:

$$LOQ\ (ng/mL) = (y_0 + 10STDEV)/s$$

where y_0 is matrix calibration curve interception, STDEV is standard deviation of the peak areas with lowest validated concentration and s is the slope of calibration curve areas/ concentration.

Specificity

GC-TOF-MS technique was considered as a specific analysis method if it can provide at least 3 ionic fragments with m/z > 100 for identification and quantification. Therefore, for each analyte one quantification ion and two qualifying ions which have to respect the ratio m/z in the range of 30% were determined.

Precision (repeatability)

Repeatability is expressed as standard deviation (RSD %) for 4 recovery measurements corresponding to four levels of fortification (0.01μg/mL, 0.02 μg/mL, 0.05μg/mL, 0.1μg/mL). RSD was calculated for each level of fortification. RSD must be smaller than 20%. The mean recovery at each fortification level must be in the range 70-120%.

Reproducibility

Fortified samples at the level of 0.1μg/mL were analyzed in different days and in different operating conditions. Recovery and standard deviation were calculated for each analyte. Acceptability conditions were the same as for repeatability, i.e. RSD < 20% and mean recovery between 70-120%.

Uncertainty

Uncertainty was calculated taking into account both systematic errors and the random (repeatability, reproducibility) and was determined using a confidence interval of 95%. Calculated uncertainty for GC-TOF MS developed method was ± 50%.

Results and discussions

A wide range of pesticides like herbicide, insecticide and fungicide having different polarities were selected. The selected pesticides are mostly used and have tendency to bio-accumulate via soil in vegetables.

The proposed multi-residues method was optimized for routine analysis, meeting essential requirements such as rapidity and small volumes of solvent consumption. The sample preparation time is short compared with other techniques which involves extraction and clean-up steps. Clean-up step was simplified (like in QuEChERS technique [13]) and in tandem with sample extraction step employed a remarkably preparation samples technique suitable for GC TOF-MS analysis despite the complexity of the matrix [16, 17, 20].

Figure 1 presents the Total Ion Chromatogram (TIC) of few analytes between 950s and 1350s and two metabolites tetrahydrophtalimide and phtalimide, obtained by analyzing of blank soil sample spiked at 0.1 μg/mL with a mixture consisting of 88 pesticides.

The recoveries are acceptable for many analytes, but are poor for fenitrothion (39%), phorate (59%), chlorothalonil (68%), endosulfan (alpha isomer) (65%), chlorpyrifos-ethyl (60%). These values indicate a possible extraction problem that could be solved either by increasing the extraction time or, maybe, by optimization of the quantity of PSA sorbent and $MgSO_4$ used in clean-up step.

A summary of average recovery (n = 5) of blank soil sample spiked at 0.1 μg/mL is shown in figure 2.

A well-known problem in multi-residues analysis was the GC determination of thermally -labile pesticides, such as captan, folpet, tolylfluanid, chlorothalonil or dichlofluanid. These pesticides may be degraded at high pH values and high temperature, during sample preparation, in GC inlet or on the active sites of the GC system [21]. As seen, the folpet and captan was not quantified as intact parent compound just as metabolites tetrahydrophtalmida (captan´s metabolite) and phtalimmida (folpet´s metabolites). One explanation could be that these

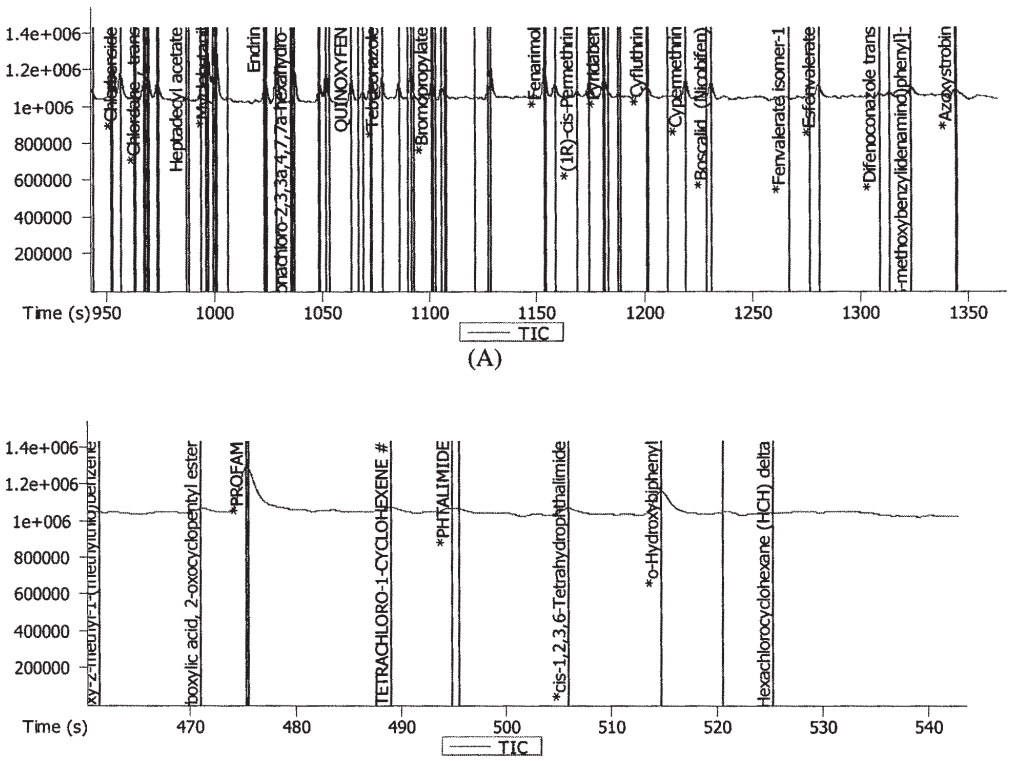


Fig. 1. TIC chromatograms: (A) for analytes with RTs between 950s and 1350s, and (B) for two metabolites tetrahydrophtalimide and phtalimide, identified and quantified at RT 505.9s, respective 494.8s.

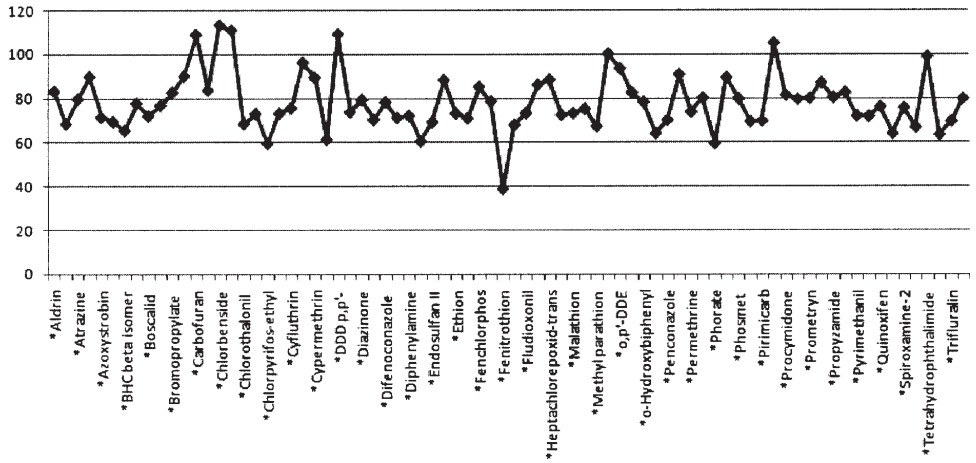


Fig. 2. Percentage of recovery rates at 0.1μg/mL for a part of studied pesticide

compounds are rapidly hydrolyzed at high *p*H values and high temperature, especially in samples that contains water. Another possible explanation could be that the temperature conditions from GC method are too high and the run time is too long for them [22].

Tolylfluanid and dichlofluanid were un-quantified and the causes could be due to preservation of the samples, the complexity of the matrix or that in the clean-up step, PSA sorbent associated with matrix effect, interfered in the quantification of these pesticide. The same explanation could be accepted even for chlorothalonil which has lower recovery rate (68%) than in other matrices similarly extracted and analyzed. The other pesticides that were not quantified were: dimetomorph, endrin, esfenvalerat, heptaclor isomer1, metalaxyl, phosphamidon and chlordane cis, most of them being nonpolar and organochlorinated pesticide. In this case it could be the effect of the sample homogenization time, which should be increased at 2 min [6, 15]. As expected, the addition of acetic acid-sodium acetate to the extraction solvent resulted in no change for recoveries of the organochlorines and pyrethroids [8]. As it can be seen, most chlorinated pesticides have lower recoveries than the other pesticides, one reason could be due to the purification step. However, the values of obtained recoveries are satisfactory considering the complexity of the matrix. On the other hand, the purification step is more than necessary on such matrix, and the simplicity of the entire extraction process that can be done by a person without a technical qualification in this field (especially in a monitoring program), makes this drawback (low recoveries) to be accepted in gas chromatographic routine analysis of pesticides in soil [6, 13, 15, 16].

Method validation

The method was validated using one single chromatographic run used for the analyze of 88 pesticides by GC-TOF-MS in full scan mode. Ecological samples soil with known history in terms of pesticide usage having the pesticides residues concentration lower than 0.010mg/kg as blank soil samples were used.

Calibration curves, linearity, LOD and LOQ

The calibration curves correlation coefficients were higher than 0.98 for all compounds. Sample sets for soil sample - 5 spiked blank samples at 0.010mg/kg (corresponding to low LOQ level), 5 spiked blank samples at 0.020mg/kg (intermediate level if LOQ of 0.010mg/kg cannot be obtained), 5 spiked blank samples at 0.050mg/kg, 5 spiked blank samples at 0.100mg/kg (superior level) and 2 un-spiked samples for each level (0.010, 0.020, 0.050, 0.100mg/kg) were analyzed. In order to prove that HCB was suitable as Internal Standard, the LOD for HCB was determined by ten times injection of 0.005μg/mL HCB solution. Retention time and S/N ratio were determined and the established LOD was 0.004μg/mL. This Internal Standard is appropriate for pesticide residues analysis from soil considering that MRL in vegetables for HCB was 0.010g/kg and in soil matrix the HCB concentration is much higher. Moreover, the instrument can identified lower concentration than HCB´s MRL in vegetables.

The LODs for each pesticide were determined by six replicate injections at four lowest and detectable levels (0.005μg/mL, 0.008μg/mL, 0.010μg/mL, 0.020μg/mL) and was calculated using STDEV and calibration curves.

The RSD values (%, n = 6) of spiked blank samples at 0.050μg/mL, correlation coefficients and equation of calibration curves are presented in table 1.

Matrix effect

In GC and GC-MS the sample matrix usually affects the chromatographic response of the pesticides compared with that obtained in the matrix-free solvent. This effect may be difficult or impossible to eliminate because it appears from several physical and chemical processes. The matrix-matched calibration may reduce or compensate this effect [20].

Matrix effect may increase or decrease detector responses, compared with those produced by simple solvent solutions of the analyte. Also, the slope of matrix-matched calibration curves against those of the calibration curves in solvent may be compared. In figure 3 the matrix effect is illustrated by comparing the matrix-matched peaks against those of the solvent peaks, all three at 0.1μg/mL level. It can be seen from figure 3 that the tolylfluanid peak is missing and the DDT peak is too high in soil (matrix) calibration comparatively with the calibration in solvent.

Uncertainty

The uncertainty ranges must take into consideration all sources of errors. The measurement uncertainty of each pesticide are made as combination of: uncertainty of preparing the stock standard solution, uncertainty of sample preparation, uncertainty produced by GC system, uncertainty produced by recovery/repeatability percent, uncertainty produced by reproducibility percent and uncertainty provided by calibration curve. Finally, this combined uncertainty was multiplied with k, for a confidence interval of 95%.

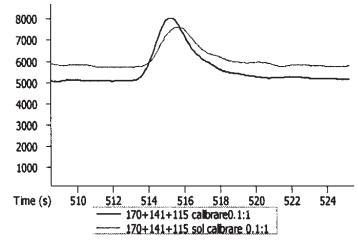
Combined uncertainty values in percentages ranging from 11.54 to *trans* permethrin at 46.23 for bromophos ethyl, but overall, all uncertainty values of validated pesticides are below 50%, which is accepted in multi-residues analysis.

Name	Absolute R.T. (s)	Equation	Correlation Coefficients	RSD%
*Profam	475.28	y= +938.86x - 2886.46	0.99761	8.3
*Phtalimide	494.86	y= +337.597x - 19393.6	0.99592	6.4
*cis-1,2,3,6-Tetrahydrophthalimide	505.91	y= +400.961x - 5592.64	0.99675	8.2
*o-Hydroxybiphenyl	514.77	y= +647.074x - 1937.88	0.99747	2.3
*Trifluralin	573.91	y= +174.408x + 1353.62	0.99840	5.7
*Diphenylamine	577.37	y= +1047.97x - 3370.27	0.99743	8.7
*Chlorpropham	589.03	y= +568.254x - 2004.88	0.99802	9.2
*Phorate	610.67	y= +1621.23x + 15315.4	0.99629	-
*alpha-Hexachlorocyclohexane (HCH)	628.79	y= +443.318x + 1421.92	0.99703	7.0
*Hexachlorobenzene (HCB)	631.39	SI	SI	SI
*Carbofuran	646.90	y= +417.38x + 55.7332	0.9967	6.8
*Atrazine	654.76	y= +159.111x + 610.755	0.99566	7.8
*Diazinone	667.68	y= +316.323x + 24.087	0.99763	3.4
*Propyzamide	674.94	y= +537.893x - 1296.01	0.99811	9.3
*BHC beta isomer	678.21	y= +538.39x - 6458.49	0.99549	15.6
*Pyrimethanil	692.46	y= +754.588x - 736.78	0.99554	8.4
*Phosmet	705.18	y= +34.0091x - 445.162	0.99506	17.8
*Chlorothalonil	710.77	y= +202.756x - 3281.8	0.99545	17.5
*Pirimicarb	714.10	y= +1322.97x - 3683.7	0.99853	2.7
*Hexachlorocyclohexane (HCH) delta	735.28	y= +261.229x - 471.475	0.99638	6.8
*Spiroxamine-1	748.07	y= +828.189x - 2886.13	0.99894	8.7
*Chlorpyrifos-methyl	755.39	y= +242.378x - 2179.32	0.99852	6.48
*Vinclozoline	759.86	y= +231.827x - 1062.81	0.99801	7.8
*Metribuzin	766.72	y= +217.99x - 895.179	0.99481	17.3
*Methyl parathion	772.38	y= +348.911x - 1560.97	0.99421	19.5
*Metalaxyl	775.64	y= +826.552x - 6389.13	0.99796	5.3
*Heptachlor	781.04	y= +199.125x - 1811.8	0.99306	-
*Heptachlor:2	781.64	y= +58.9544x + 168.183	0.99498	16.8
*Fenchlorphos	782.43	y= +260.087x - 116.209	0.99552	18.7
*Phosphamidon (Z)	782.90	y= +42.4571x + 509.479	0.99339	-
*Prometryn	783.30	y= +299.512x - 867.039	0.99735	8.8
*Spiroxamine-2	791.63	y= +804.703x - 2188.75	0.99848	7.2
*Pirimiphos methyl	793.96	y= +231.402x - 52.4472	0.99680	6.8
*Fenitrothion	811.41	y= +266.43x - 2332.38	0.99571	-
*Malathion	816.60	y= +282.927x - 137.978	0.99754	3.7
*Dichlofluanid	822.53	y= +405.667x - 4740.37	0.99324	-
*Chlorpyrifos-ethyl	830.19	y= +485.85x + 590.901	0.99681	18.5
*Aldrin	843.04	y= +1024.75x - 1407.35	0.99861	2.8
*Fenthion	845.77	y= +309.089x - 583.086	0.99760	7.1
*Parathion-ethyl	850.70	y= +432.104x - 3011.81	0.99723	16.5
*Triadimefon	853.03	y= +1000.22x + 5878.46	0.99629	16.3
*Pendimethaline	892.99	y= +158.044x + 1177.56	0.99681	10.4
*Cyprodinil	903.18	y= +428.902x - 369.107	0.99655	17.4
*Penconazole	910.24	y= +500.623x - 3382.19	0.99756	11.0
*Tolylfluanide	913.37	y= +482.162x - 13737.5	0.99525	19.7
*Heptachlorepoxid-trans	924.03	y= +147.477x + 474.979	0.99664	13.2
*Quinalphos	925.82	y= +316.027x - 911.688	0.99762	4.9
*Procymidone	931.82	y= +941.863x - 3893.32	0.99776	2.2
*Bromophos-ethyl	941.34	y= +1657.53x - 5158.74	0.99665	7.1
*Chlordane	952.60	y= +33.2415x + 308.455	0.99513	-
*o,p'-DDE	952.99	y= +597.381x - 1174.48	0.99749	10.2
*Chlorbenside	956.79	y= +1810.31x - 8094.64	0.99646	7.1
*Chlordane , trans	967.45	y= +36.2731x + 697.14	0.99612	9.5
*Endosulfan I	969.45	y= +57.6132x + 1798.24	0.99315	19.0
*Endosulfan II	969.65	y= +253.119x - 269.329	0.99716	18.8
*Mepanipyrim	974.04	y= +484.009x - 1427.34	0.99744	6.2
*Fludioxonil	994.02	y= +215.733x - 765.536	0.99707	7.1
*Buprofezin	996.29	y= +585.542x - 1274.87	0.99799	5.5
*Kresoxim-methyl	997.42	y= +906.67x - 2496.78	0.99782	2.3
*Myclobutanil	999.68	y= +296.032x - 1467.8	0.99684	16.0

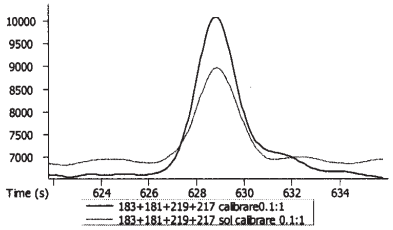
Table 1
RSD (%, n=6) SPIKED AT 0.05 ppm , CORRELATION COEFFICIENTS AND EQUATION OF CALIBRATION CURVES

*Dieldrin	1000.82	y= +577.153x - 2553.76	0.99791	12.2
*DDD p,p'-	1001.21	y= +295.062x - 558.447	0.99851	7.3
*Endrin	1023.79	y= +43.6781x + 337.55	0.99242	-
*Ethion	1028.92	y= +1389.8x - 3696.1	0.99720	11.3
*o,p'-DDT	1036.18	y= +158.132x + 530.627	0.99350	18.5
*Benalaxyl	1051.83	y= +628.496x - 900.011	0.99458	16.2
*Quinoxyfen	1063.62	y= +390.928x - 308.673	0.99549	8.6
*Fenhexamide	1068.95	y= +372.786x - 3571.78	0.99706	7.8
*Propargite	1073.01	y= +292.385x - 4346.21	0.99751	9.0
*Tebuconazole	1077.80	y= +422.179x - 1169.47	0.99772	9.6
*Carbosulfan	1085.66	y= +167.039x - 4521.05	0.99353	18.5
*Bifenthrin	1091.26	y= +877.496x - 1103.49	0.99779	3.3
*Bromopropylate	1101.05	y= +303.023x + 794.844	0.99733	9.5
*Phosalone	1127.62	y= +321.206x - 4208.21	0.99536	20.0
*Cyhalothrin trans	1128.75	y= +441.979x - 1403.98	0.99814	10.6
*Fenarimol	1153.66	y= +563.117x - 2844.84	0.99789	9.7
*Azinphos-ethyl	1158.59	y= +219.01x - 2684.81	0.99537	11.1
*cis-Permethrin	1168.78	y= +164.01x + 1859.49	0.99551	12.0
*Permethrine	1174.44	y= +365.569x - 925.725	0.99828	9.6
*Pyridaben	1181.37	y= +743.727x - 2132.82	0.99814	10.5
*Cyfluthrin	1201.61	y= +256.073x + 947.582	0.99599	14.6
*Dimethomorph (E)	1210.87	y= +271.96x + 633.572	0.99595	-
*Cypermethrin	1219.26	y= +289.051x - 812.02	0.9967	10.9
*Boscalid (Nicobifen)	1231.05	y= +768.646x - 785.149	0.99557	14.7
*Fenvalerate isomer	1267.08	y= +12.1434x + 970.429	0.99728	-
*Esfenvalerate	1280.67	y= +456.129x - 2270.79	0.99825	-
*Difenoconazole trans	1309.04	y= +51.7863x + 986.654	0.99639	10.6
*Difenoconazole	1313.44	y= +74.9777x + 301.068	0.99461	14.7
*Azoxystrobin	1344.20	y= +87.3343x + 24.8072	0.99502	12.2

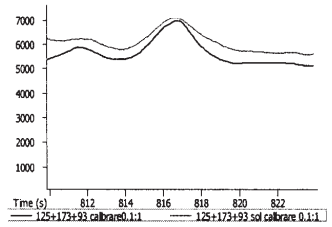
Table 1 (cont.)
RSD (%, n=6) SPIKED AT 0.05 ppm , CORRELATION COEFFICIENTS AND EQUATION OF CALIBRATION CURVES



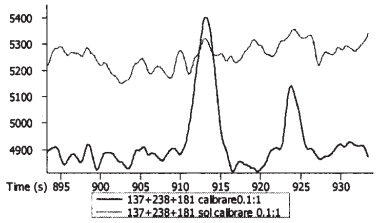
o-Hydroxybiphenyl



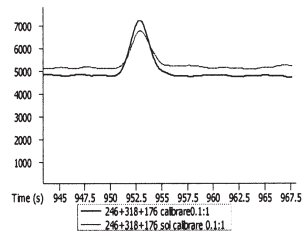
alpha HCH



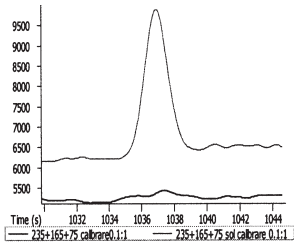
Malathion



Tolylfluanide



o, p' DDE



o,p' DDT

Fig. 3. Comparative peaks of 6 pesticides (o-Hydroxybiphenyl, alpha HCH, malathion, tolylfluanide, o, p' DDE and o, p' DDT) for matrix effect evaluation

Real samples

The validated method was used for the analysis of five soil samples known as a challenging analytical task for determination of pesticide residues level due to the heavy matrix impact.

Three samples were collected from three farms specializing in the fruit and vegetables growing, a sample is taken from a field which for three years has been uncultivated and other sample is taken from a field located near a decommissioned pesticide deposit.

Table 2
POSITIVE RESULTS FOUND IN SOIL SAMPLES

Soil Sample	Pesticide Residues found	Concentration (Mg/kg)
Contaminated field	o,p' DDT	1.035
	p,p' DDD	0.030
	pendimethaline	0.024
Farm1	buprofezin	0.453
	ethion	0.022
Uncultivated field	buprofezin	0.120
	p,p' DDD	0.026
	carbosulfan	0.035
Farm2	o,p' DDE	0.034
	azoxistrobin	0.026
	bromopropylate	0.011
Farm3	cyhalothrintrans	0.034
	beta HCH	0.018
	bifentrin	0.096

Table 2 gives a representative overview of pesticides found in soil samples and their concentration. All pesticides were detected by using at least three transitions and were confirmed by checking the calibrated ion ratios.

Higher values were obtained for buprofezin and bifenthrin pesticides that are used as insecticides in farming and fruit growing. As was expected in samples taken from the land located near the contaminated area, have been obtained high values of DDT. Subsequently samples were analyzed carrots grown on this land and have been obtained exceeding the maximum limits set by EU regulations.

Conclusions

The GC method was fully validated for 60 of the 88 pesticides proposed for validation, from all chemical classes. The values of evaluated validation parameters were accepted for sixteen pesticides, except the recovery rates, that were slightly outside of the 70-120% range, but in the routine analysis a recovery range between 60% and 140% it was accepted. However, these values are an indicator of a possible extraction problem that could be improved, in a first step, by increasing the extraction time at 2 minutes. Two pesticides have recovery rates below of accepted range and ten pesticides were un-quantified. This situation could be due to the interference of matrix co-extractive compounds. In order to prove the suitability of developed method in routine analysis, five real soil samples were analyzed and positive results were founded.

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