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Reliable screening of pesticide residues in maternal and umbilical cord sera by gas chromatography-quadrupole time of flight mass spectrometry

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The widespread use of pesticides induces heavy adverse effects on human health, especially for the pregnant women and the newborns. In this study, a screening method has been developed for the determination of multi-pesticides in maternal and umbilical cord sera. All pesticides in sera were collected using solid phase extraction (SPE), and analyzed by gas chromatography-quadrupole time of flight mass spectrometry (GC-QTOF MS). To set up the quality criteria, a database of 50 pesticides was created and the accurate masses of 3 up to 5 representative ions with their intensity ratios were included for each pesticide. In addition, a novel "identification points" (IPs) system relying on the accurate MS^1 and MS^2 spectra was used to interpret the data for each suspected pesticide. The methodology was then applied to a pair of maternal and umbilical cord sera. A total of six pesticide residues were screened out successfully. In conclusion, GC-QTOF MS combined with an accurate mass database seemed to be one of the most efficient tools for systematic pesticide analysis.

pesticide residues, screening, maternal and umbilical cord sera, gas chromatography-quadrupole time of flight mass spectrometry

1 Introduction

With the widespread use of pesticides, pesticide residues have posed high risks both to the environment and human beings [1–4]. Facing such a serious crisis, pesticide exposure to human health has been studied in various populations over the past several decades [5–9]. Thereinto, pesticide residues in pregnancy have been paid more attention [10, 11], since many pesticides not only bring harm to the pregnant woman [12–14], but also can reach the fetus through the transplacental transport and cause the behavioral disorders and learning disabilities in its future growth [15–18].

For the pesticide monitoring, both gas chromatography (GC) [19–21] and liquid chromatography (LC) [22–24] with mass spectrometry (MS) have been extensively used. However, focusing on the pesticide residues in human, electron capture detector (ECD), rather than MS, has been taken as the main detector because of its particular sensitivity to halogens, like polychlorinated biphenyls (PCBs) and dichlorodiphenyl trichloroethane (DDT) etc. [25, 26]. Triple quadrupole mass spectrometry (TQ MS) and ion trap mass spectrometry (IT MS) also have been applied to the analysis of pesticides in umbilical cord blood serum [10, 27]. Nevertheless, the low resolution and the low scan rate limited their further application to the simultaneous screening of multi-residues in trace. With the development of MS technique, QTOF MS attracts more expectation in the analysis of complicated matrix [28, 29]. As an advanced technique,

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the main advantage of QTOF MS realizes the full spectrum acquisition with higher resolution and sensitivity. Abundant and accurate ions from MS^1 and MS^2 spectra bring an active power in the structural elucidation of compounds.

In this work, we developed a screening method for multi-pesticide residues using GC-QTOF MS. The MS^1 spectra were utilized for the quick identification of the suspected pesticides, and the MS^2 spectra were for the structural confirmation. In addition, a database of 50 pesticides and a novel IPs system relying on the accurate mass spectra were created as a guideline to the reliability of the screening process. Finally, the methodology was applied successfully for the pesticide residues in the sera.

2 Experimental

2.1 Materials

Fifty references of pesticides were purchased from J&K Scientific Ltd (Beijing, China). Standard solutions of pesticides were prepared in *n*-hexane (HPLC grade) supplied by Dikma Technologies Inc. (Lake Forest, USA). Dichloromethane, methanol, and water were all in HPLC grade and purchased from Fisher Scientific Company LLC (Fair Lawn, USA). Bond Elut C18 cartridges (200 mg, 3 mL) and the empty cartridges (3 mL) were purchased from Agilent Technologies Inc. (Santa Clara, USA). Anhydrous sodium sulfate (AR) and ammonium sulfate (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Serum collection

Serum samples were provided from the Yijishan Hospital of Wannan Medical College. The maternal and umbilical cord blood were collected at delivery. The supernatant sera were aspirated out and stored at −20 °C until processed for the pesticide analysis.

2.3 Instrumentation

All measurements were performed with Agilent 7200 accurate-mass GC-QTOF MS instrument (Santa Clara, USA), using a fused silica DB-35MS capillary column of 30 m × 0.25 mm i.d. The injector was operated at 250 °C in splitless mode and helium (purity > 99.999%) was used as the carrier gas at 1.5 mL/min. The GC oven temperature was programmed from an initial temperature of 80 °C held for 1 min, ramped at 25 °C/min to 170 °C, and then at 6 °C/min to final 300 °C held for 10 min, resulting in a total run time of 46.3 min. Injection volume was 1 μL. The other optimized parameters included a transfer line temperature of 300 °C and an ion source of 250 °C. TOF for MS was operated at 5.0 spectra/s acquiring the mass range *m/z* 50–600 and about 13500 (FWHM). The MS/MS conditions were fixed for each compound with a quadrupole for isolation of precursor ion at a medium MS resolution and a linear hexapole collision cell with nitrogen at 1.5 mL/min as the collision gas. Perfluorotributylamine (PFTBA) was utilized for daily MS calibration. MassHunter Acquisition B.06 and MassHunter Qualitative/Quantitative Analysis B.05 were applied to the control of equipment and the treatment of data files.

2.4 Method validation

Individual stock solutions were prepared by dissolving 10 mg of each pesticide into a 10 mL *n*-hexane and stored at −20 °C until used. The working standards were prepared by serial dilution of stock solutions with *n*-hexane and ranged from 1 to 100 ng/mL. After analysis of these working standard solutions, calibration curves were then established by plotting the peak areas versus the concentrations of these standards. The limit of detection (LOD) was established as the concentration of each analyte that generated a response with a signal-to-noise ratio (S/N) > 3. The precision was evaluated by calculating the coefficient of variation (CV) for two replicate standard solutions prepared at three concentration levels (1, 10 and 100 ng/mL).

2.5 Sample preparation

Frozen serum samples were thawed at room temperature and then vortexed to ensure homogeneity prior to the analysis. A 2 mL aliquot of serum was transferred to a test tube and added with 2 mL saturated ammonium sulfate solution. The mixture was vortexed for 10 s and then placed in the refrigerator at 4 °C for 10 min. After protein precipitation, the mixture was centrifuged at 12000 r/min for 10 min and 3 mL of the supernatant liquid was aspirated for SPE.

A C18 SPE cartridge was pre-rinsed and activated with 4 mL dichloromethane, 4 mL methanol and 4 mL water. After conditioning, the cartridge was not allowed to dry and the supernatant was loaded into the SPE cartridge. The cartridge was then washed with 4 mL water, dried with vacuum suction, and connected to a cartridge that was filled with 2 g anhydrous sodium sulfate and pre-rinsed by 4 mL dichloromethane beforehand. The tandem cartridges were eluted with 4 mL dichloromethane. The entire volume of effluent was collected and dried to completion under a gentle stream of nitrogen at 25 °C. The residue was reconstituted in 50 μL *n*-hexane, vortexed for 30 s, and transferred to a 200 μL glass vial for GC-MS analysis.

3 Results and discussion

3.1 Development of pesticide screening

Workflow of screening

A GC-QTOF method for the multi-residues in vegetable has

been reported in our previous study [21]. A single accurate ion from the MS^1 spectra with its retention time was utilized to quickly find out the suspected pesticide. In this work, the screen method was performed based on the multi-representative ions from a pesticide. The identification criteria were referred to the European Commission (EC) guideline [30]. The presence of representative ions at the expected retention time was measured at their accurate masses and the attainment of their Q/q_i intensity ratios was within the specified tolerances ($Q/q < 2$, deviation ± 10%; 2–5, ± 15%; 5–10, ± 20%; ⩾10, ± 50%). Q/q_i is the ratio between the most abundant ion (Q) and every one of the other measured ions (q_i).

In the traditional application [31], Q/q_i ratios were used as the only approach to confirm a positive finding after the agreement in the retention time and accurate ion masses of a suspected pesticide between the sample and the reference. However, facing the real sample, the extraneous mass spectral peaks arising from the matrix effect can pose a serious problem for the pesticide finding. Thus, the further verification is necessary which required a higher selectivity to discriminate between the analyte peak and other chromatographic peaks. For this requirement, the high-resolution MS^2 full mass spectra acquired by GC-QTOF MS were taken into account. The suspected pesticide underwent the collision-induced dissociation (CID). Thereafter, at least two representative product ions were calculated elemental compositions and to be compared the experimental masses with the theoretical values. The most abundant ion from the MS^1 full scan spectra was selected preferentially as the precursor ion in order to achieve the best sensitivity. Other measured ions that could generate the most abundant product ion would also be considered.

Creation of database

A database that includes the representative ions of each pesticide was required for the pesticide identification firstly. As an example in this study, a total of 50 pesticide references, such as organophosphate insecticides, organochlorine insecticides, organic fluorine insecticides, pyrethroid insecticides, and azole fungicides, were collected. The spectrum of each pesticide was obtained and then at least 3 ions were selected for each pesticide taking into account the sensitivity and selectivity. Table 1 showed the exact masses of the representative ions for each pesticide. For some pesticides, it was feasible to use up to 5 ions giving extra reliability to the identification process.

3.2 Application to the maternal and umbilical cord sera

The pesticides in serum were collected using SPE, which has been universally adopted for the modern residue analysis [25–27, 32]. Before SPE, equivoluminal saturated $(NH_4)_2SO_4$ solution was used to remove the abundant proteins. At the end of SPE, anhydrous sodium sulfate as an inert drying agent was used to remove traces of water from the effluent. The conditions of extraction were optimized to meet the demand of this study.

MS^1 identification

The procedure of MS^1 identification was based on two approaches: (1) retention time; (2) mass window of extracted ion chromatogram (EIC) and Q/q_i ratio evaluation. The precision of retention time was evaluated by the GC-MS measurements of all test pesticides, showing that the coefficient of variation for each pesticide's retention time was between ± 0.00 and ± 0.04 min. Considering the effect induced by the different matrices, the retention time window was optimized to 0.25 min to avoid a false negative finding.

As another important factor, the influence of the mass extraction window was studied, selecting the values between 1 and 1000 mDa. And this case was illustrated in the finding of piperonyl butoxide at 1 ng/mL, where different mass extraction windows were used 1, 10, 100 and 1000 mDa (Figure 1). Comparing four EICs at *m/z* 176.0832, "Q" ion of piperonyl butoxide, the mass window of 10 mDa obviously presented a better *S/N* improved greatly by the decrease of the background noise. Another case of fenhexamid at 50 ng/mL also gave the same result for its "q" ion at *m/z* 176.9743 (Figure 1). The excessive narrow mass window like 1 mDa, especially for the less abundant ions, could cause the sharp drop of ion intensity and the split of peak shape. On the other hand, a wide mass window also led to a failure because of the interferences from the co-eluting or interfering compounds. Like piperonyl butoxide and fenhexamid, the difference of their retention times (19.35 min for piperonyl butoxide and 19.38 min for fenhexamid) and representative ion masses (*m/z* 176.0832 from piperonyl butoxide and *m/z* 176.9743 from fenhexamid) were 0.03 min and 0.8911 Da. When the mass window was set to 500 mDa or above, they might not be distinguished and the inappropriate Q/q_i confirmatory ratios would be achieved.

According to the retention time and Q/q_i ratios, a total of eight suspected pesticides in a pair of maternal and umbilical cord sera were shown in Table 2. One "+" represented one Q/q in the specified ratio and retention time tolerances. Obviously, the more "+" signs presented, the more reliability it was for the pesticide identification. As an example in Figure 2, four ion chromatograms of hexachlorobenzene in maternal serum were extracted at *m/z* 283.8096 (Q), 285.8067 (q_1), 281.8126 (q_2), and 141.9372 (q_3). Subsequently, their experimental Q/q_i ratios were calculated as 1.16, 1.80 and 5.08 of the reference ratios respectively. Among them, Q/q_2 1.80 compared to the reference ratio 2.03 was out of the specified tolerance (deviation of ± 10%). Therefore, only two "+" were got in final. Additionally, in order to avoid the missing, a compound that exceeded the tolerance of Q/q_i ratios but the "Q" ion and all "q" ions

Table 1 The representative ion masses selected for the identification of 50 pesticides

Compound	Retention time (min)	Formula	Representative ion (Da)				
			Q	q_1 (Q/q_1)	q_2 (Q/q_2)	q_3 (Q/q_3)	q_4 (Q/q_4)
Biphenyl	6.16	$C_{12}H_{10}$	154.0777	153.0699 (2.06)	152.0621 (2.65)	76.0308 (7.63)	
Etridiazole	6.74	$C_5H_5Cl_3N_2OS$	182.9181	210.9494 (1.13)	212.9465 (1.64)	184.9152 (1.44)	
Pentachlorobenzene	7.41	C_6HCl_5	249.8486	251.8456 (1.63)	247.8515 (1.67)	214.8797 (4.74)	
Chlorpropham	8.86	$C_{10}H_{12}ClNO_2$	127.0183	171.0082 (3.09)	213.0551 (5.59)	129.0154 (3.22)	
Propoxur	8.90	$C_{11}H_{15}NO_3$	110.0362	111.0441 (15.15)	152.0832 (16.95)	81.0335 (19.23)	
Cadusafos	9.08	$C_{10}H_{23}O_2PS_2$	158.9698	96.9508 (1.17)	130.9385 (1.97)		
Hexachlorobenzene	9.73	C_6Cl_6	283.8096	285.8067 (1.27)	281.8126 (2.03)	141.9372 (5.00)	
Ethoxyquin	9.97	$C_{14}H_{19}NO$	202.1226	174.0913 (1.36)	145.0886 (6.49)		
Pronamide	10.39	$C_{12}H_{11}Cl_2NO$	172.9555	174.9526 (1.62)	144.9606 (3.03)	239.9977 (13.16)	
Diazinon	10.48	$C_{12}H_{21}N_2O_3PS$	137.0709	179.1179 (1.06)	199.0631 (2.79)		
Dinitramine	10.77	$C_{11}H_{13}F_3N_4O_4$	305.0856	261.0594 (1.91)	244.0566 (2.65)		
Simazine	10.80	$C_7H_{12}ClN_5$	173.0463	186.0541 (2.55)	201.0776 (2.91)		
Carbofuran	11.23	$C_{12}H_{15}NO_3$	164.0832	149.0597 (1.44)	131.0491 (3.48)		
Pentachlorobenzonitrile	11.34	C_7Cl_5N	274.8438	276.8409 (1.65)	272.8468 (1.67)	241.8720 (11.24)	
Vinclozolin	12.16	$C_{12}H_9Cl_2NO_3$	212.0028	178.0499 (1.00)	197.9872 (1.40)	186.9586 (1.52)	
Pirimicarb	12.17	$C_{11}H_{18}N_4O_2$	166.0975	72.0444 (2.68)	238.1425 (10.87)		
Chlorpyrifos-methyl	12.61	$C_7H_7Cl_3NO_3PS$	285.9256	287.9226 (1.47)	124.9821 (2.39)	78.9943 (3.04)	
delta-BHC	12.73	$C_6H_6Cl_6$	180.9373	182.9344 (1.05)	218.9110 (2.48)	216.9140 (3.22)	108.9606 (3.04)
Propanil	12.80	$C_9H_9Cl_2NO$	160.9794	162.9764 (1.60)	217.0056 (15.38)	164.9735 (10.31)	
Chlorothalonil	12.87	$C_8Cl_4N_2$	265.8781	263.8810 (1.25)	267.8751 (2.11)		
Tolclofos-methyl	13.00	$C_9H_{11}Cl_2O_3PS$	264.9850	266.9820 (2.93)	124.9821 (5.38)	249.9615 (6.10)	
Ametryn	13.02	$C_9H_{17}N_5S$	227.1199	212.0964 (1.31)	170.0495 (1.90)		
Parathion-methyl	13.12	$C_8H_{10}NO_5PS$	109.0049	124.9821 (1.5)	78.9943 (1.77)		
Diethofencarb	13.58	$C_{14}H_{21}NO_4$	124.0393	151.0264 (1.29)	225.0996 (1.38)	207.0890 (3.94)	
Dacthal	13.61	$C_{10}H_6Cl_4O_4$	300.8801	298.8831 (1.32)	302.8772 (2.13)		
Chlorpyrifos	13.66	$C_9H_{11}Cl_3NO_3PS$	96.9508	196.9196 (1.36)	198.9167 (1.36)	257.8943 (1.91)	
Dichlofluanid	13.88	$C_9H_{11}Cl_2FN_2O_2S_2$	123.0137	223.9498 (5.92)	167.0637 (2.83)		
Pirimiphos-ethyl	13.94	$C_{13}H_{24}N_3O_3PS$	168.0590	180.1131 (1.31)	318.1036 (1.75)	304.0879 (2.50)	333.1271 (2.89)
Fenthion	14.31	$C_{10}H_{15}O_3PS_2$	278.0195	124.9821 (3.39)	109.0049 (3.52)		
o,*p*-Dicofol	14.49	$C_{14}H_9Cl_5O$	138.9945	110.9996 (2.29)	140.9916 (3.06)		
Isocarbophos	14.78	$C_{11}H_{16}NO_4PS$	135.9977	120.0206 (1.22)	121.0284 (1.51)		
Procymidone	15.44	$C_{13}H_{11}Cl_2NO_2$	96.0570	283.0161 (1.75)	285.0132 (2.78)	67.0542 (1.12)	
trans-Chlordane	15.51	$C_{10}H_6Cl_8$	372.8254	374.8225 (1.04)	370.8284 (2.33)	376.8195 (1.97)	
cis-Chlordane	15.81	$C_{10}H_6Cl_8$	372.8254	374.8225 (1.06)	376.8195 (1.95)	370.8284 (2.28)	
Hexaconazole	16.43	$C_{14}H_{17}Cl_2N_3O$	82.0400	213.9947 (1.27)	83.0478 (1.31)	215.9917 (2.02)	
p,*p*'-DDE	16.58	$C_{14}H_8Cl_4$	245.9998	247.9968 (1.62)	176.0621 (3.10)	315.9375 (2.72)	
Oxyfluorfen	16.63	$C_{15}H_{11}ClF_3NO_4$	252.0393	300.0034 (2.69)	317.0061 (4.17)	361.0323 (6.99)	
Buprofezin	16.72	$C_{16}H_{23}N_3OS$	105.0573	106.0651 (1.40)	175.0774 (1.56)	172.1029 (2.99)	
Bupirimate	17.13	$C_{13}H_{24}N_4O_3S$	208.1444	273.1016 (1.31)	193.1573 (1.37)	166.0975 (1.99)	
Isoprothiolane	17.77	$C_{12}H_{18}O_4S_2$	117.9905	188.9675 (2.13)	161.9804 (2.20)		
Endrin	17.81	$C_{12}H_8Cl_6O$	262.8564	264.8535 (1.59)	260.8594 (1.54)	81.0335 (1.14)	
o,*p*'-DDT	18.05	$C_{14}H_9Cl_5$	235.0076	237.0046 (1.59)	165.0699 (1.89)	212.0388 (8.55)	
Endosulfan II	18.69	$C_9H_6Cl_6O_3S$	159.9842	238.8378 (1.95)	234.8438 (1.95)	236.8408 (1.31)	169.9685 (1.39)
Tris(2-butoxyethyl)PhosPhate	18.83	$C_{18}H_{39}O_7P$	124.9998	85.0648 (1.12)	57.0699 (1.57)		
p,*p*'-DDT	19.25	$C_{14}H_9Cl_5$	235.0076	237.0046 (1.61)	165.0699 (1.84)	212.0388 (6.99)	
Piperonyl Butoxide	19.35	$C_{19}H_{30}O_5$	176.0832	177.0910 (4.17)	149.0597 (5.13)		
Fenhexamid	19.38	$C_{14}H_{17}Cl_2NO_2$	97.1012	178.9713 (4.17)	176.9743 (2.59)		
Triazophos	19.87	$C_{12}H_{16}N_3O_3PS$	162.0662	161.0584 (1.05)	96.9508 (1.93)	172.0870 (1.48)	
Triphenylphosphate	20.55	$C_{18}H_{15}O_4P$	325.0624	77.0386 (2.76)	94.0414 (4.10)	326.0702 (1.19)	
Fenpropathrin	20.76	$C_{22}H_{23}NO_3$	181.0648	97.1012 (1.43)	265.0734 (3.63)	209.0835 (1.85)	

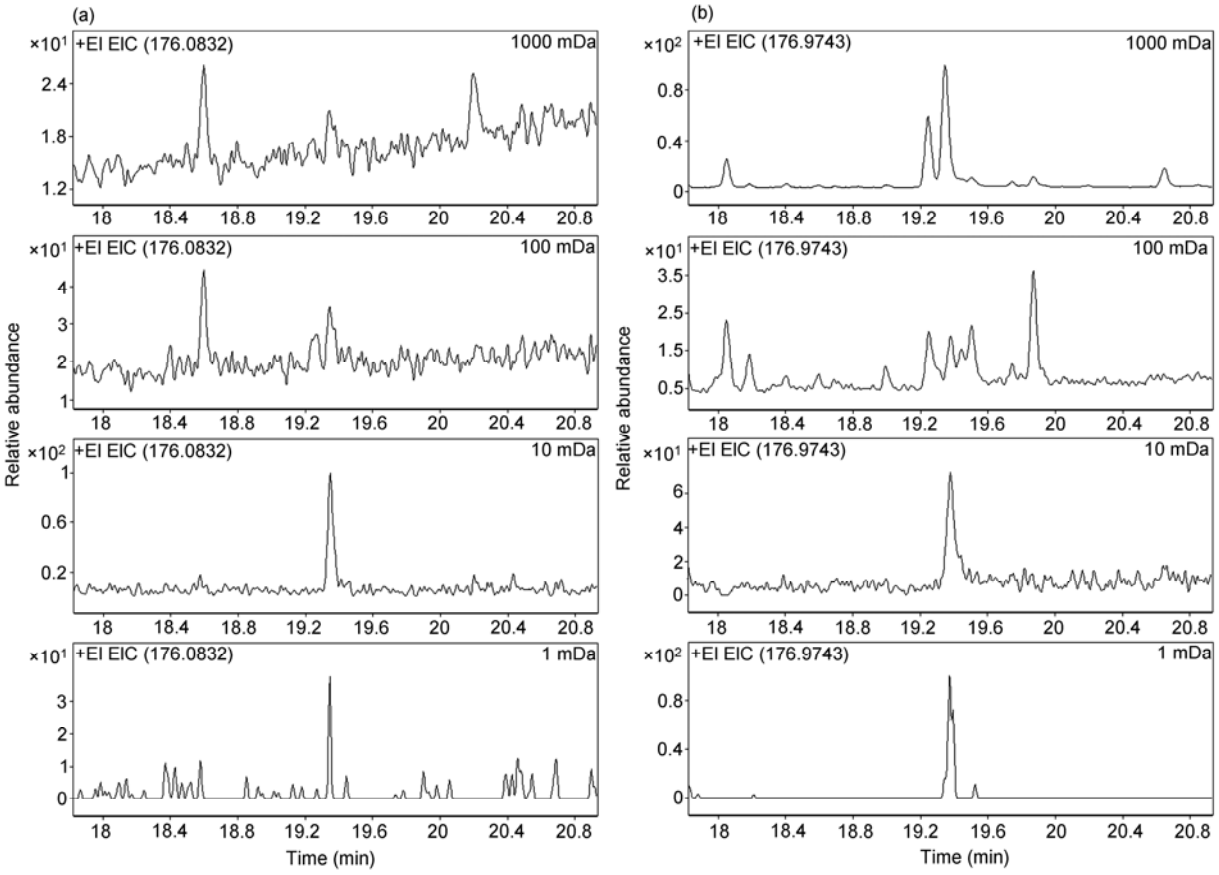

Figure 1 The multi-EICs of piperonyl butoxide (a) and fenhexamid (b) with mass windows of 1, 10, 100 and 1000 mDa.

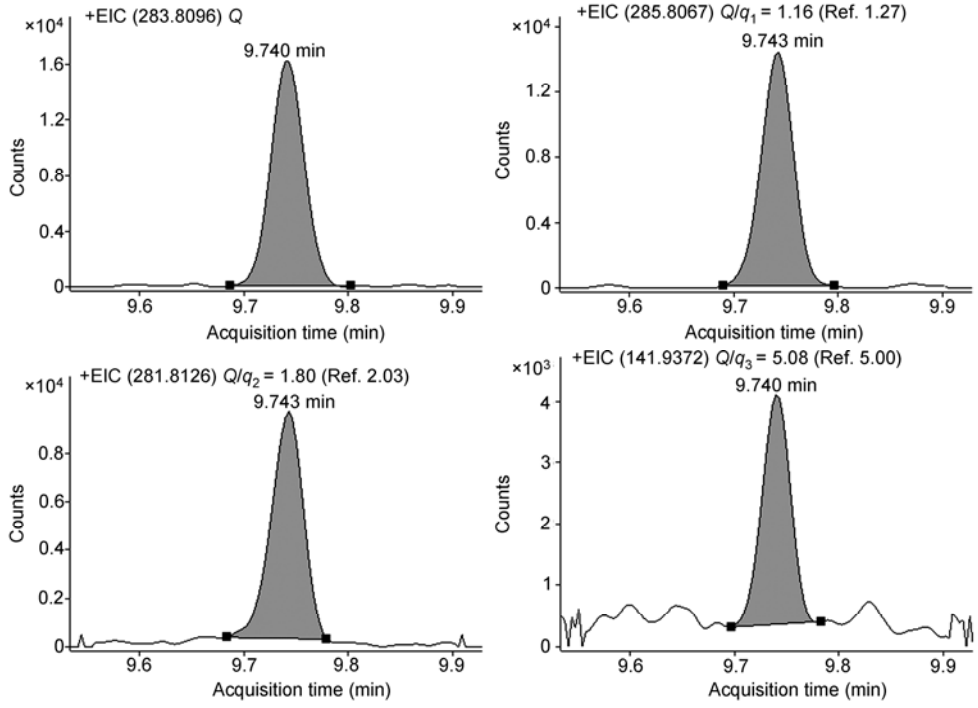

Figure 2 EICs of hexachlorobenzene in the MS^1 identification of the maternal serum.

positively found was also included, like chlorothalonil in Table 2.

MS^2 confirmation

False positive or false negative finding occurs frequently in the pesticide screening, due to the matrix effect. In this study, QTOF MS in MS^2 scan mode provided an efficient approach for the structural confirmation of the suspected pesticide. In contrast to the MS^1 scan mode, MS^2 scan mode has the advantages of the MS/MS transitions to elucidate the suspected or unknown structure, and present the less-disturbed product ions. It improves mass accuracy, ion selectivity, and confirmation capability.

Here, eight suspected pesticides from MS^1 identification were conducted MS^2 analysis. Two MS/MS transitions with their optimal collision energies for each pesticide were picked according to our previous study [21]. As an illustration of the MS^2 confirmation for hexachlorobenzene in Figure 3, the proposal product ions *m/z* 283.8 > 213.8719 and *m/z* 281.8 > 211.8749 were well found in the MS^2 spectra with the absolute mass errors 1.9 and 1.1 mDa, respectively. Likewise, another five of eight suspected pesticides were positively confirmed. But the absence of product ions *m/z* 285.9 > 93.0100 and *m/z* 124.9 > 47.0491, 265.8 > 230.9092 and *m/z* 263.8 > 167.9402 indicated the suspected compounds were not chlorpyrifos-methyl and chlorothalonil as supposed (Table 2).

By our observation, in some cases, MS^2 scan mode based on the ion separation of low-resolution quadrupole failed to achieve a "pure" MS^2 spectrum. For example, many high abundant product ions were found at low masses in the MS^2 spectrum of *m/z* 281.8 (Figure 3). These unexpected ions were generated by a precursor ion *m/z* 281.0534 from a co-eluted compound. The mass difference of 0.7577 Da failed to remove *m/z* 281.0534 from *m/z* 281.8111 in the quadrupole. And the both of them were submitted to perform CID, resulting in a combo MS^2 spectrum. Fortunately, the identification of the proposal ion *m/z* 281.8 > 211.8749 was not disturbed in this case. Otherwise, another MS/MS transition should be taken place for the structural confirmation.

3.3 Validation of the screening results

Identification points (IPs)

As it reported, the EC Guideline introduces an "identification points" system (IPs) to ensure effective and reliable confirmation of residues in the identification and quantification of organic residues [33]. The main advantage of using IPs is that the verification of identity could be done in a well-described and internationally accepted way.

In this work, novel criteria for IPs were also attempted to validate the MS^1 identification and the MS^2 confirmation on the basis of GC-QTOF MS. In the MS^1 screening, one Q/q ratio at the proposal retention time can get 1.5 IPs under the supposed conditions. In the MS^2 confirming, the relationship between IPs and mass accuracy of the product ion was described as follows: if the absolute error between the experimental accurate mass and the calculated mass of the product ion was no more than 2 mDa, the product ion could obtain 1.5 IPs; if the absolute mass error was between 2 and 10 mDa (include 10 mDa), it gained 1.0 IPs; if the absolute mass error was between 10 and 20 mDa (include 20 mDa), it gained 0.5 IPs; if the absolute mass error was more than 20 mDa, it gained 0 IPs. In a positive screening, at least 4 IPs should be gained for a pesticide. According to the new criterion, IPs about the pesticide screened out from the maternal and cord sera were listed in Table 3. Taking hexachlorobenzene in the maternal serum as an example, it got 3 IPs resulting from two Q/q_i ratios in the MS^1 identification.

Table 2 The results of pesticide screening in the maternal and umbilical cord sera

Compound	MS^1 identification		MS^2 confirmation					
	M [a]	I [b]	Precursor ion (Da)	Proudct ion (Da)	$Exp_{(M)}$ (Da)	Error (mDa)	$Exp_{(I)}$ (Da)	Error (mDa)
Biphenyl	++	+	154.1	153.0699	153.0702	0.3	153.0690	–0.9
			153.1	152.0621	152.0625	0.4	152.0628	0.7
Pentachlorobenzene	++	++	249.9	214.8797	214.8778	–1.9	214.8788	–0.9
			248.0	212.8827	212.8826	–0.1	212.8795	–3.2
Hexachlorobenzene	++	+++	283.8	213.8719	213.8738	1.9	213.8730	1.1
			281.8	211.8749	211.8760	1.1	211.8722	–2.7
Chlorpyrifos-methyl	+	+	285.9	93.0100	/	/	/	/
			124.9	47.0491	/	/	/	/
Chlorothalonil	/	/	265.8	230.9092	/	/	/	/
			263.8	167.9402	/	/	/	/
trans-Chlordane	+++	+++	372.8	265.9032	265.8959	–7.3	265.9020	–1.2
			271.9	236.8408	236.8489	8.1	236.8410	0.2
p,*p*'-DDE	+++	++	246.1	176.0621	176.0651	3.0	176.0609	–1.2
			215.8	245.9998	245.9938	–6.0	246.0008	1.0
Triphenylphosphate	+	++	325.0	169.0648	169.0641	–0.7	169.0648	0
			214.9	168.0570	168.0563	–0.7	168.0572	0.2

a) Maternal serum; b) infant serum.

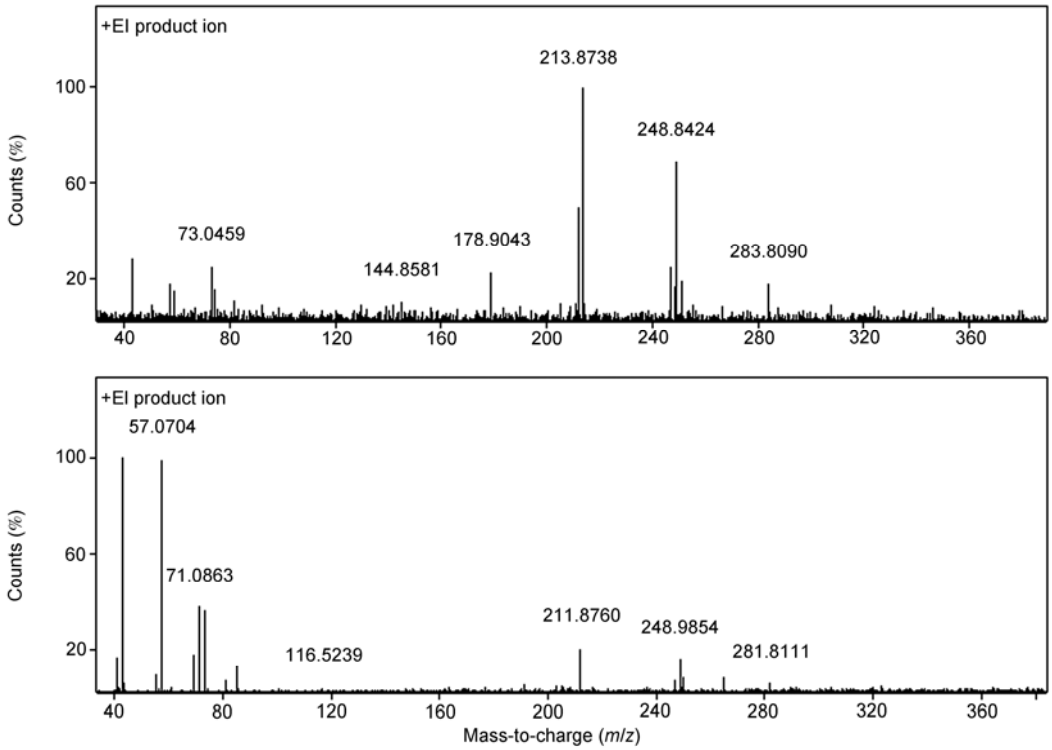

Figure 3 MS/MS spectra of hexachlorobenzene in the MS^2 confirmation from the maternal serum.

Table 3 Validations and concentrations of pesticides in a pair of the maternal and umbilical cord sera

Compound	IPs_M	IPs_I	Linear range (ng/mL)	R^2	LOD (ng/mL)	CV (%)			Concentration (ng/mL)	
						1 ng/mL	10 ng/mL	100 ng/mL	M	I
Biphenyl	6.0	4.5	1–100	0.9982	0.1	2.08	3.36	0.59	1.68	0.89
Pentachlorobenzene	6.0	5.5	1–100	0.9925	0.1	1.39	1.50	1.68	0.10	0.09
Hexachlorobenzene	6.0	7.0	1–100	0.9948	0.1	3.94	2.04	3.14	0.22	0.20
trans-Chlordane	6.5	7.5	1–100	0.9949	0.1	9.61	6.10	4.05	0.13	0.16
p,*p*′-DDE	6.5	6.0	1–100	0.9935	0.1	7.59	4.13	5.36	0.11	0.13
Triphenylphosphate	4.5	6.0	1–100	0.9916	0.1	1.26	1.41	7.63	0.40	0.48

Another 3 IPs were obtained from the two MS/MS transitions (1.5 IPs for 283.8 > 213.8738, 1.5 IPs for 281.8 > 211.8760). Therefore, total 6 IPs can be earned after the analysis of GC-QTOF MS. While 1 IPs for chlorpyrifos-methyl and 0 IPs for chlorothalonil, both less than 4 IPs meant the negative findings in this screening case.

Quantitative results

The calibration curves showed excellent linearity (R^2 > 0.9900) for six pesticides within the range of 1–100 ng/mL (Table 3). Analysis of the highest level of working standard was followed by injection of pure methanol, and no significant carryover effect was observed. LOD at S/N > 3 was 0.01 ng/mL for each pesticide. The precision expressed as the CV was all less than 10%. Assuming all pesticides were collected in the process of SPE extraction without loss, the mean concentrations of six pesticide residues in the maternal and umbilical cord sera were calculated and the results were listed in Table 3. Besides four organochlorine pesticides (pentachlorobenzene, hexachlorobenzene, trans-chlordane and *p*,*p*′-DDE), biphenyl and triphenylphosphate were also found in the sera. The structural stability and lipid solubility (Log$P$ > 3) cause these residues resistant to environmental breakdown and accumulate in maternal bodies [34, 35]. Although the placental barrier has long been considered as a protective barrier to the fetus against noxious agents, the residues in maternal blood, due to their good lipid solubility, can cross the placental barrier. Therefore, it was found less difference whether residue types or residue levels between maternal and umbilical cord sera.

4 Conclusions

In this study, a novel screening method for multi-pesticides in serum was developed using GC-QTOF MS and a pair of maternal and umbilical cord sera as the real examples were analyzed. For the identification, a database of 50 pesticides

was created and 3 up to 5 representative ions from the MS^1 full mass spectra were included for each pesticide. For the structural confirmation, the high-resolution MS^2 full mass spectra were acquired by GC-QTOF MS. The representative product ions of the suspected pesticide were calculated and compared with the reference. Additionally, an IPs system relied on the accurate MS^1 and MS^2 spectra was introduced to interpret the data for each suspected pesticide.

Summarizing, GC-QTOF MS demonstrated a strong power in the analysis of pesticide residues. The availability of high-resolution full scan mass spectra throughout each GC-QTOF MS chromatogram, narrow mass extraction window and accurate mass measurements provided qualitative information to ascertain whether pesticide residues were present in samples.

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1 Banerjee K, Utture S, Dasgupta S, Kandaswamy C, Pradhan S, Kulkarni S, Adsule P. Multiresidue determination of 375 organic contaminants including pesticides, polychlorinated biphenyls and polyaromatic hydrocarbons in fruits and vegetables by gas chromatography-triple quadrupole mass spectrometry with introduction of semi-quantification approach. *J Chromatogr A*, 2012, 1270: 283–295
2 Herrera Rivera Z, Oosterink E, Rietveld L, Schoutsen F, Stolker L. Influence of natural organic matter on the screening of pharmaceuticals in water by using liquid chromatography with full scan mass spectrometry. *Anal Chim Acta*, 2011, 700: 114–125
3 Garcia-Ac A, Segura PA, Viglino L, Fürtös A, Gagnon C, Prévost M, Sauvé S. On-line solid-phase extraction of large-volume injections coupled to liquid chromatography-tandem mass spectrometry for the quantitation and confirmation of 14 selected trace organic contaminants in drinking and surface water. *J Chromatogr A*, 2009, 1216: 8518–8527
4 Hernandez F, Portoles T, Pitarch E, López FJ. Target and nontarget screening of organic micropollutants in water by solid-phase microextraction combined with gas chromatography/high-resolution time-of-flight mass spectrometry. *Anal Chem*, 2007, 79: 9494–9504
5 Nanita SC, Padivitage NLT. Ammonium chloride salting out extraction/cleanup for trace-level quantitative analysis in food and biological matrices by flow injection tandem mass spectrometry. *Anal Chim Acta*, 2013, 768: 1–11
6 Bolaños PP, Moreno JL, Shtereva DD, Frenich AG, Vidal JL. Development and validation of a multiresidue method for the analysis of 151 pesticide residues in strawberry by gas chromatography coupled to a triple quadrupole mass analyzer. *Rapid Commun Mass Spectrom*, 2007, 21: 2282–2294
7 Cervera MI, Portoles T, Pitarch E, Beltrán J, Hernández F. Application of gas chromatography time-of-flight mass spectrometry for target and non-target analysis of pesticide residues in fruits and vegetables. *J Chromatogr A*, 2012, 1244: 168–177
8 Ferrer I, Garcia-Reyes JF, Mezcua M, Thurman EM, Fernánndez-Alba AR. Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography-time-of-flight mass spectrometry. *J Chromatogr A*, 2005, 1082: 81–90
9 Goni F, Lopez R, Etxeandia A, Millán E, Amiano P. High throughput method for the determination of organochlorine pesticides and polychlorinated biphenyls in human serum. *J Chromatogr B*, 2007, 852: 15–21
10 Mariscal-Arcas M, Lopez-Martinez C, Granada A, Olea N, Lorenzo-Tovar ML, Olea-Serrano F. Organochlorine pesticides in umbilical cord blood serum of women from Southern Spain and adherence to the Mediterranean diet. *Food Chem Toxicol*, 2010, 48: 1311–1315
11 Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, Hore P, Robson MG. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. *Sci Total Environ*, 2010, 408: 790–795
12 Barraza-Vazquez A, Borja-Aburto Victor H, Bassol-Mayagoitia S, Monrroy A, Recio-Vega R. Dichlorodiphenyldichloroethylene concentrations in umbilical cord of newborns and determinant maternal factors. *J Appl Toxicol*, 2008, 28: 27–34
13 Harley KG, Huen K, Schall RA, Holland NT, Bradman A, Barr DB, Eskenazi B. Association of organophosphate pesticide exposure and paraoxonase with birth outcome in Mexican-American women. *PLoS One*, 2011, 6: e23923
14 Wang YR, Zhang M, Wang Q, Yang DY, Li CL, Liu J, Li JG, Li H, Yang XY. Exposure of mother-child and postpartum woman-infant pairs to DDT and its metabolites in Tianjin, China. *Sci Total Environ*, 2008, 396: 34–41
15 Zhao G, Xu Y, Li W, Han G, Ling B. Prenatal exposures to persistent organic pollutants as measured in cord blood and meconium from three localities of Zhejiang, China. *Sci Total Environ*, 2007, 377: 179–191
16 Pathak R, Suke SG. Ahmed RS, Tripathi AK, Guleria K, Sharma CS, Makhijani SD, Mishra M, Banerjee BD. Endosulfan and other organochlorine pesticide residues in maternal and cord blood in North Indian population. *Bull Environ Contam Toxicol*, 2008, 81: 216–219
17 Lafiura KM, Bielawski DM, Posecion Jr NC, Ostrea Jr EM, Matherly LH, Taub JW, Ge Y. Association between prenatal pesticide exposures and the generation of leukemia-associated T(8;21). *Pediatr Blood Cancer*, 2007, 49: 624–628
18 Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fitó N, Carrizo D, Romieu I, Antó JM, Grimalt JO. Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. *Clin Exp Allergy*, 2006, 36: 1236–1241
19 Sharma E, Mustafa Md, Pathak R, Guleria K, Ahmed RS, Vaid NB, Banerjee BD. A case control study of gene environmental interaction in fetal growth restriction with special reference to organochlorine pesticides. *Eur J Obstet Gynecol Reprod Biol*, 2012, 161: 163–169
20 Dewan P, Jain V , Gupta P, Banerjee BD. Organochlorine pesticide residues in maternal blood, cord blood, placenta, and breastmilk and their relation to birth size. *Chemosphere*, 2013, 90: 1704–1710
21 Zhang F, Yu CT, Wang WW, Fan RJ, Zhang ZX, Guo YL. Rapid simultaneous screening and identification of multiple pesticide residues in vegetables. *Anal Chim Acta*, 2012, 757: 39–47
22 Diaz R, Ibáñez M, Sancho JV, Hernández F. Qualitative validation of a liquid chromatography-quadrupole-time of flight mass spectrometry screening method for organic pollutants in waters. *J Chromatogr A*, 2013, 1276: 47–57
23 Gomez-Ramos MM, Ferrer C, Malato O, Agüera A, Fernández-Alba AR. Liquid chromatography-high-resolution mass spectrometry for pesticide residue analysis in fruit and vegetables: Screening and quantitative studies. *J Chromatogr A*, 2013, 1287: 24–37
24 Masiá A, Ibáñez M, Blasco C, Sancho JV, Picó Y, Hernández F. Combined use of liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry in systematic screening of pesticides and other contaminants in water samples. *Anal Chim Acta*, 2013, 761: 117–127
25 Asawasinsopon R, Prapamontol T, Prakobvitayakit O, Vaneesorn Y, Mangklabruks A, Hock B. The association between organochlorine and thyroid hormone levels in cord serum: A study from northern Thailand. *Environ Int*, 2006, 32: 554–559
26 Covaci A, Jorens P, Jacquemyn Y, Schepens P. Distribution of PCBs and organochlorine pesticides in umbilical cord and maternal serum.

Sci Total Environ, 2002, 298: 45–53
27 Liao HT, Hsieh CJ, Chiang SY, Lin MH, Chen PC, Wu KY. Simultaneous analysis of chlorpyrifos and cypermethrin in cord blood plasma by online solid-phase extraction coupled with liquid chromatography-heated electrospray ionization tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, 2011, 879: 1961–1966
28 Kang WY, Zhang F, Su Y, Guo YL. Application of gas chromatography-quadrupole-time-of-flight-mass spectrometry for post-target analysis of volatile compounds in *Fructus Amomi*. *Eur J Mass Spectrom*, 2013, 19: 103–110
29 Hong SS, Kang WY, Su Y, Guo YL. Analysis of trace-level volatile compounds in fresh turf crop (*Lolium perenne L.*) by gas chromatography quadrupole time-of-flight mass spectrometry. *Chinese J Chem*, 2013, 31: 1329–1335
30 Hernandez F, Portolés T, Pitarch E, López FJ. Target and nontarget screening of organic micropollutants in water by solid-phase microextraction combined with gas chromatography/high-resolution time-of-flight mass spectrometry. *Anal Chem*, 2007, 79: 9494–9504
31 Nunez O, Gallart-Ayala H, Ferrer I, Moyano E, Galceran MT. Strategies for the multi-residue analysis of 100 pesticides by liquid chromatography-triple quadrupole mass spectrometry. *J Chromatogr A*, 2012, 1249: 164–180
32 Covaci A, Schepens P. Solid phase disk extraction method for the determination of persistent organochlorine pollutants in human body fluids. *Anal Lett*, 2001, 34: 1449–1460
33 Hernández F, Ibáñez M, Sancho JV, Pozo OJ. Comparison of different mass spectrometric techniques combined with liquid chromatography for confirmation of pesticides in environmental water based on the use of identification points. *Anal Chem*, 2004, 76: 4349–4357
34 Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*, 2001, 46: 3–26
35 Waliszewski SM, Aguirre AA, Infanzon RM, Siliceo J. Persistent organochlorine pesticide levels in maternal blood serum, colostrum, and mature milk. *Bull Environ Contam Toxicol*, 2002, 68: 324–331