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Quantification of organophosphate insecticides in drinking water in urban areas using lyophilization and high-performance liquid chromatography–electrospray ionization-mass spectrometry techniques

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ABSTRACT

A sensitive method for the quantification of eight organophosphate pesticides in water samples at the ng L^{-1} concentration level has been developed. These organophosphates include pesticides, insecticides and herbicides used in agricultural applications. A lyophilization with simple solvent extraction followed by selective analysis using a liquid chromatography–mass spectrometry method was used. This method was accurate (≥98.9%), as it possessed limits of detection and quantification in the 4.9–51 and 16.5–171 ng L^{-1} ranges, respectively. Furthermore, the coefficients of variations (≥0.999) were less than 8.2% at the low ng L^{-1} end of the linear range of the method. In addition, the percentage recovery of all pesticides at the 0.1 μg L^{-1} levels ranged from 96% to 103%. This method was then used for the quantification of organophosphates in drinking and bore water samples collected from different parts of urban areas. We subsequently found detectable levels of monocrotofos, imedacloprid, triazofos, atrazine, propanil, quinolfos and metribuzin in more than 23% of the water samples analyzed.

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1. Introduction

Organophosphate (OP) pesticides are widely used as insecticides in both agricultural and residential settings worldwide because they exhibit a common mechanism of action, i.e., inhibition of cholinesterase, an essential nervous system enzyme [1]. These pesticides tend to be commonly metabolized to a dialkylphosphate moiety consisting of a phosphate and two ethyl or methyl esters relatively quickly and excreted primarily in the urine. Nearly all these metabolites have similar solubilities and toxic properties [1]. They have achieved great popularity because of their effectiveness as insecticides and their lack of persistence in the environment. In India, the current use of OP pesticides, including herbicides and insecticides, is at a maximum [2].

Exposure to OP pesticides typically occurs through multiple routes (i.e., food, air, soil or water); however, the dominant routes of exposure among individuals vary to a large extent, making it difficult to assess exposure to OP pesticides. For instance, dietary intake represents the major source of pesticide exposure to children, and this exposure may increase pesticide-related health risks in children in comparison to adults [3]. In many epidemiologic studies, markers of exposure in biological samples have been measured to estimate the absorbed dose in humans [4–8].

OP pesticides, including herbicides, can also leach into ground water. According to the United States Geological Survey (USGS), at least 143 different pesticides and twenty-one transformation products have been found in ground water, including pesticides from every major chemical class. Over the past two decades, pesticides have been found in the ground water of more than 43 states. Contamination of ground water is of concern because ground water supplies 50% of the U.S. population with drinking water [9]. During one survey in India, 58% of drinking water samples drawn from various hand pumps and wells around Bhopal were found to be contaminated with organochlorine pesticides at levels above the environmental protection agency (EPA) standards [9]. Once ground water is polluted with toxic chemicals, it may take many years for the contamination to dissipate or be cleaned up. Cleanup may also be very costly and complex, if not impossible [9].

Several methods exist in the literature for the detection of OP pesticides. For example, the OP pesticide residue levels in the Ganga River in Narora (U.P.), India have been reported [10]. To carry out this analysis, the authors used high performance liquid chromatography (HPLC) analysis of water samples from a liquid extraction procedure and found the presence of several OP pesticides, such as dimethoate and methyl parathion. Additionally, a gas liquid chromatograph equipped with an electron capture

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detector (ECD) and a nitrogen phosphorous detector (NPD) and employing a capillary column has also been used for published analyses of OP pesticides in rainwater in India [11]. A gas chromatographic (GC) analysis showed the presence of certain OP pesticides (dimethoate, malathion, methyl-parathion and chlorpyrifos) in wastewater that was used for agriculture purposes in Ghaziabad City, India [12]. A liquid chromatography/atmospheric pressure chemical ionization mass spectrometry method was used for the determination of several OP pesticides (mevinphos, dichlorvos, azinphos-methyl, azinphos-ethyl, parathion-methyl, parathion-ethyl, malathion, fenitrothion, fenthion, chlorfenvinphos and diazinon) in groundwater [13].

Diazinon, chlorpyrifos, propargite, paraquat, dimethoate and methomyl have been reported in polluted groundwater because of their suspected involvement in Parkinson's Disease (PD) and also because at least 10% of the US population has been exposed to these pesticides at some point [14]. This study was the first that indicated that the use of agricultural pesticides and consuming well water contaminated with pesticides plays a role in the etiology of PD [14].

One specific micro-extraction method, termed ultrasound-assisted emulsification-micro-extraction (USAEME), has been successfully used for the extraction of OP pesticides in water and orange juice samples [15]. The QuEChERS (quick, easy, cheap, effective, rugged and safe) sample preparation method was applied to quantify about 180 analytes in various cereal grain matrices (corn, oat, rice and wheat) and provided good analytical results (recoveries in the range of 70–120% and RSDs < 20%) [16]. Additionally, the degradation of pesticides in water at low pressure using UV photolysis and advanced oxidation processes have been developed for the removal of pesticides from drinking water [17].

The limit of detection (LOD) reported [13,15,18–23] for the determination of pesticides in drinking water samples at certain confidence intervals was not as low as our developed method reported here. Additionally, all of these reported methods were not simple, sensitive, cost-effective or time saving. Therefore, there is a great need to develop a simple, sensitive and cost-effective method for the analysis of commonly used OP and herbicides in the water of developing countries. Hence, in this paper, we describe a very sensitive, selective, accurate, simple and cost-effective method for the determination of eight commonly used OP pesticides and herbicides in Indian drinking water samples using a method that employs lyophilization followed by liquid chromatography–mass spectrometry.

2. Experimental

2.1. Materials

All certified standards of pesticides, insecticides and herbicides were purchased from Sigma–Aldrich Chemical Co. (USA). Water, methanol, acetonitrile (LC–MS grade) and formic acid (analytical grade) were also obtained from Sigma–Aldrich (USA). All reagents were made fresh in LC–MS grade water or solvent before use.

2.2. Preparation of standard

2.2.1. Stock solution-1

Individual stock solutions at 10 mg L^{-1} of the pesticides of interest (monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin) were prepared in 10 mL volumetric flasks with acetonitrile. The stock solutions were then divided into aliquots, sealed in ampoules and stored at −40 °C.

2.2.2. Stock solution-2 (1 μg mL^{-1})

Stock solution of 1 μg mL^{-1} standard mixtures of monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin was prepared by diluting 1 mL of each standard from stock one into 10-mL acetonitrile in a 10-mL volumetric flask. The stock solutions of these standards were stored at −20 °C.

2.2.3. Working solutions

From, eleven working standard sets for monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin (0.1, 0.2, 1, 2, 5, 10, 30, 50, 100, 150 and 250 ng mL^{-1}) were prepared to encompass the entire linear range of the method by using the serial dilution technique [24,25]. The standard sets were divided into aliquots, sealed in ampoules and stored at −40 °C until use. These standards were then used for the validation of method (determination of limit of detection (LOD), limit of quantification (LOQ), recovery experiment and linearity experiment).

2.2.4. Laboratory reagent blanks

Before extraction of the water samples, the purchased LC–MS grade water was tested by LC–MS/MS using a similar extraction method that was used for the recovery experiment, and the water was found to be free from pesticide residues.

2.2.5. Spiking concentration of standards

Pure water was spiked with standards of each compound (monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin) at different levels (0.1, 0.2, 1, 2, 5, 10 and 30 ng mL^{-1}).

2.2.6. Working standard solution (SD)

Seven solutions of different spiking concentrations of monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin (ranging from 0.1 to 150 ng mL^{-1}) were prepared in acetonitrile using the serial dilution technique for the construction of the linear curve. These spiking standard curves were used throughout the study to maintain accuracy and precision in sample analysis.

2.3. Data processing and analysis

The peaks of the standards, the spiking samples and the field samples were integrated using Analyst software (version 1.4.2). The noise of the peaks was subtracted and the 5-point smoothing of peaks was carried out using this software for accurate integration and analyte measurement. Microsoft EXCEL® and SPSS software were used for the interpretation of the data and for reporting the results in this paper. The retention time (RT), signal to noise (S/N) ratio and area of standards were calculated, and their concentrations were automatically downloaded by the Analyst software.

2.4. Percentage recovery

The LC–MS grade water (free from pesticides) was spiked with standards of monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin at different spiking levels. The percentage recovery and relative standard deviation (RSD) from these experiments are shown in Table 2. Excellent extraction efficiency was obtained for all of these pesticides.

2.5. Sampling

Thirteen water samples were collected from an urban city (Hyderabad, India): six samples were collected from the Municipal

Corporation (MC) water supply and seven samples were collected from bore water at different locations throughout the city.

2.6. Sample preparation

Pesticide-free LC–MS grade water and reagent blanks were prepared identically. All samples, reagents, and standards were brought to room temperature, and then 5-mL of water was pipetted into 20-mL test tubes. The water was then spiked with a mixture of different pesticides at different concentrations (0.1, 0.2, 1, 2, 5, 10 and 30 ng mL^{-1} of monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin). The water was subsequently mixed and allowed to equilibrate for approximately 60 min. The tubes were then placed in a methanol bath and held at −100 °C for at least 10 min. Once the samples were frozen, they were placed in a lyophilizer at −109 °C. The vacuum status was checked and the samples were left for 6 h to ensure complete dryness. The samples were then removed from the lyophilizer for extraction.

2.6.1. Extraction

Three milliliters of acetonitrile was added to each tube, mixed for 2 min on a vertex shaker and aliquoted into 15-mL centrifuge tubes. In the second step, samples were extracted with 3 mL acetonitrile for 2 min and then transferred to the first extract. When the extraction was complete, all of the tubes were centrifuged for 8 min at 3000 rpm. Next, the supernatant solution was transferred into a new set of 15-mL tubes for drying and placed in a TurboVap at 20 °C under 5 psi nitrogen and completely dried. The dry residues were reconstituted in 1-mL acetonitrile for analysis.

2.7. Instrumentation

The eight pesticides were analyzed using a 4000-QTRAP triple-quadrupole hybrid mass spectrometer (Applied Biosystems MDS Sciex, USA) equipped with a liquid chromatography (LC, Shimadzu, LC 20 AD, binary pump) and operated in the positive turbo ion spray (ESI) mode. The LC chromatography was fitted with a reverse phase column.

2.7.1. Mass spectrometer configuration

The analysis of the analytes was carried out in the multiple-reaction monitoring (MRM) positive ESI mode with high resolution. The interface heater was held at the temperature of 550 °C and the ion-spray (IS) voltage used was 5500 eV. A full auto tune of the mass spectrometer was performed before the analysis of every set of samples. A full scan of the mass spectra of all pesticides were recorded in order to select the most abundant mass to charge ratio (m/z) ion (Q1) using continuous infusion of each pesticide in the positive ionization mode of ESI. The product mass spectra were obtained with continuous infusion of each analyte, so Q1, corresponding to the protonated precursor ion, remained constant. The most abundant product ion for each compound was then selected for MRM analysis.

At least two of the most intense product ions were isolated: one ion was used for quantification, whereas the other was used for confirmation, as per the three principle criteria given for mass spectrometry studies of OP pesticides [26–28]. The optimization of the source dependent parameters, such as nebulizing gas (GS1), heating gas (GS2) and curtain gas, were carried out in the flow injection analysis (FIA) mode. The GS1, GS2 and curtain gas pressures were then maintained at 35, 40 and 25 psi, respectively, during the entire study. Furthermore, the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision exit potential (CXP) were used as per the required sensitivity of the method (Table 1).

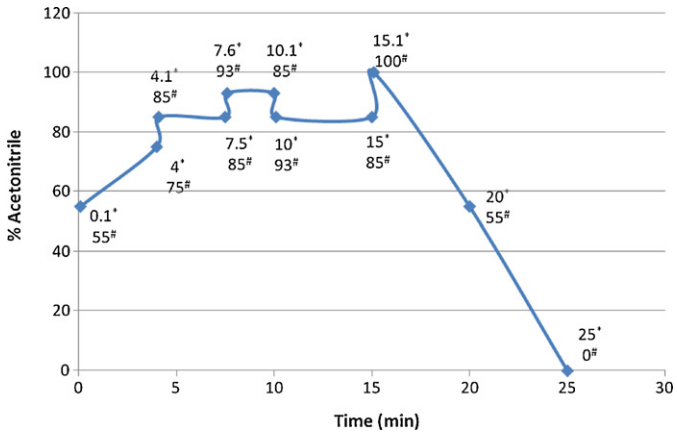

Fig. 1. Different gradient profiles of % acetonitrile (ACN) in 0.1% formic acid in water at different time intervals.

2.7.2. Mass-spectrometer instrument-control by the analyst program

The Analyst 4.1.2 software controlled the MRM operational mode in the 4000 Q-Trap mass spectrometer used. The operational procedure of the instrument was also controlled by the same Analyst software. This program set the instrument to acquisition by ESI in the positive mode for the analysis of the various analytes. The operational details for the precursor ions and product ions are shown in Table 1. Different DP, CE, EP and CXP parameters were applied for the selection of confirmation and quantification ions of each analyte depending on their physical and chemical properties. Maximum sensitivity for quantification ions, however, was achieved at different energy sets given in Table 1. Precursor ions were therefore isolated using the maximum sensitivity, specificity and linear dynamic range. The product ions of the precursor ions were then used for confirmation, adding to the selectivity of the analysis (Table 1).

2.7.3. Liquid chromatography conditions (LC)

An LC equipped with a triple-quadrupole ion trap hybrid mass detector operating in the MRM mode was used for this study. Chromatographic separation was achieved for the eight pesticides by a liquid chromatograph equipped with a Phenomenex C_{18} reversed phase column with an ID of 5-μm and dimensions of 50 m × 4.68 mm. 10-μl samples were injected using a Shimadzu auto-sampler fitted with a Hamilton 100-μl syringe. Different gradient compositions of 0.1% formic acid in water and acetonitrile at a flow rate of 0.5 mL min^{-1} were used, but the best sensitivity and separation for all the compounds of interest were achieved using the different gradient compositions shown in Fig. 1. The column oven temperature was held constant at 20 °C.

2.8. Quantification

2.8.1. Correlation coefficient (r)

Calibration curves were prepared with seven different concentrations (0.1, 1, 5, 10, 25, 50 and 150 ng mL^{-1}) for each OP pesticide, insecticide or herbicide (monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin) in each analytical run. The concentration of each compound was then plotted against the area of the pesticide to determine the correlation coefficient and percentage accuracy of this method at low concentrations [24,25]. The calibration standard concentration curves were used during the entire study to insure the linear range of analysis for

Table 1
The isolated precursor and product ions of different pesticides in multiple reaction monitoring (MRM) using different energy profiles.

Pesticides	Precursor ion (Q-1)	Product ion (Q-2)	[a]DP	[b]CE	[c]EP	[d]CXP	Duel time	[e]RT (min)
Atrazine	216.20	174.20	78.90	23.50	3.65	7.92	60	2.80
Propanil	218.10	162.20	47.20	23.50	2.94	8.15	60	3.53
Imedacloprid	256.10	209.00	54.90	19.29	3.51	2.93	60	1.66
Triazofos	314.10	162.20	40.00	24.80	5.05	7.54	70	4.79
Ethion	385	171	62.10	13.80	4.60	4.40	100	7.36
Quinolfos	299.17	146.90	58.00	31.00	3.50	7.30	50	5.54
Monocrotofos	224.10	127.10	52.30	17.50	5.30	10.70	30	1.28
Metribuzin	215.3	187.1	31.00	25.60	7.40	4.50	30	2.25

[a] DP, declustering potential.
[b] CE, collision energy.
[c] EP, entrance potential.
[d] CXP, collision exit potential.
[e] RT, retention time.

every run. The lowest standard concentration used for the construction of linearity was at the lowest spiked level for each pesticide. The unknown sample concentrations in drinking water were determined using linear regression analysis of the calibration plot slopes and intercepts. The obtained correlation coefficient for all pesticides was calculated to be $\leq$0.999.

2.9. Method validation

2.9.1. Sample analysis protocol for quality assurance

Thirteen water samples were studied in one typical batch for confirmation and quantification. Seven standard samples and two blank reagents were also used for this analysis. In one batch, the first, ninth and twenty-third samples were blank, whereas the second through eighth samples were at different concentrations for the standard curve. The thirteen study samples were then put in positions ten through twenty-two.

2.9.2. Limit of detection (LOD)

For this study, the point at which the measured value was considered reliable was when it was larger than the uncertainty associated with it, also called the LOD. In this method, the analytical LOD was calculated as per the earlier reported method [24,25].

2.9.3. Limit of quantification (LOQ)

For this study, the lower level where measurements became quantitatively meaningful was called the LOQ and was calculated as per the earlier reported method [24,25].

2.9.4. Percentage recovery

The recoveries of the method were determined by spiking water samples free of pesticides with different known concentrations of reference standards. The recovery of each pesticide was calculated at each of the known concentration levels by comparing the measured concentrations with the spiked concentrations, as per the reported method [25]. A ratio of 1.00 indicated 100% recovery.

2.9.5. Accuracy

In order to determine accuracy, seven water samples were fortified with different concentrations of OP pesticides, mixed and allowed to stand for 1 h so that all the pesticides were thoroughly dissolved in the water before extraction. A pesticide-free water sample served as the negative control in these experiments. The accuracy was calculated by spiking water samples with known concentrations of the reference standards, analyzing the samples, and then comparing the calculated and expected concentrations. Linear regression analysis was carried out on linearity curves of the calculated concentrations versus the expected concentrations. After performing analysis, an obtained slope of 1.0 indicated 100% accuracy [24,25].

2.9.6. Precision

The precision of this method was determined by calculating the percentage RSD of spiked samples at seven concentrations (0.1–30 ng mL^{-1}). At least six repeat measurements were then used in this calculation.

2.9.7. Selectivity

The degree of selectivity of the analytical method was determined by using LC–MS/MS in MRM transition mode. The chromatographic sensitivity increased when the LC was interfaced with a MS/MS detector and was selected for this study as the most selective analytical technique [1,25]. This technique is capable of separating the OP compounds on the basis of the selected ion. In addition, the background noise was easily removed with high selectivity using this technique, which increased the signal intensity.

3. Discussion

This method was developed for testing the quality of drinking water and fixing the maximum residue limit (MRL) for pesticides in water. As of the publication of this report, India still did not have its own MRL for OP pesticides in drinking water because there was a lack of sensitive and cost-effective methods for the analysis of OP pesticides in water. Therefore, India follows the European drinking water standard of 0.1 $\mu g\,L^{-1}$ for individual pesticides in multi-residue analysis and 0.5 $\mu g\,L^{-1}$ for total pesticides [10]. The purpose of this study was to assess the presence of OP compounds in drinking water, to fix an MRL level and correlate it to OP pesticide exposure in the general population for health risk assessment. This study is necessary as the general population is exposed to OP pesticides through food and water on a daily basis and no study has yet been reported on the health risk of OP exposure in India. Therefore, we developed a sensitive, simple and cost-effective method for OP compound analysis in water samples for the determination of OP exposure in the general population of India.

The extraction process for this study was very simple, accurate and easy. In the case of water samples, several methods have been used to extract OP compounds from water, but most liquid–liquid extractions do not optimally extract these compounds. In our method, acetonitrile was used directly to extract the analyte from lyophilized samples at neutral pH, which prevented loss of compounds and gave better recovery as compared to other methods [29]. The solvent had an important role in liquid–liquid and solid–liquid extraction, and its effect depended upon medium, pH and the polarity of the samples.

It is very difficult to analyze pesticides in water samples due to the polarity of the OP compounds. Polar/non-polar interaction mechanisms prevented us from using polar solvents such as methanol and acetonitrile for the extraction of these compounds directly from water. Therefore, lyophilization was used to com-

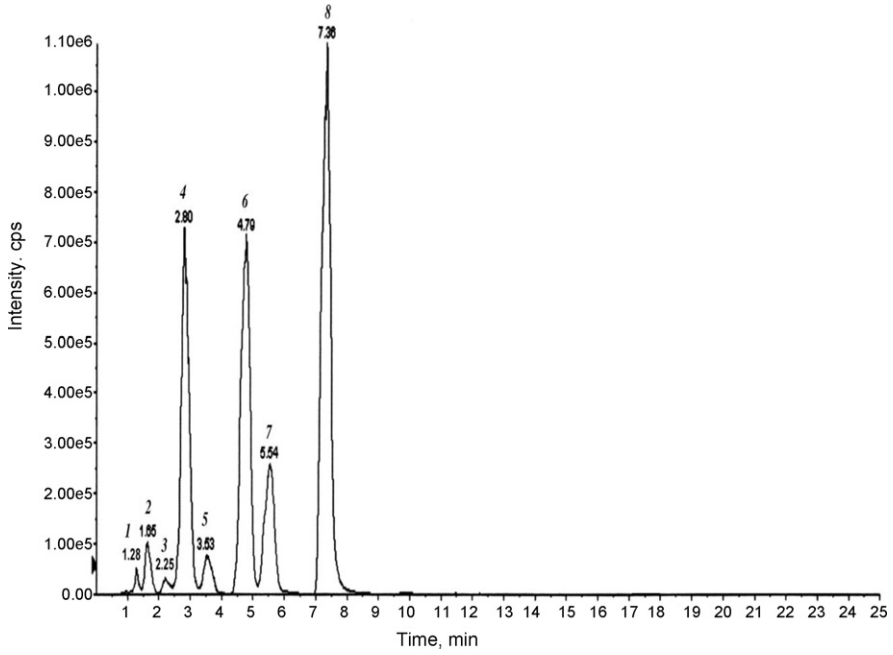

Fig. 2. Total ion chromatogram (TIC) of spiked water samples at 10 μg L^{-1} levels each: (1) monocrotofos (RT; 1.65), (2) imedacloprid (RT; 1.65), (3) metribuzin (RT; 2.25), (4) atrazine (RT; 2.80), (5) propanil (RT; 3.53), (6) triazofos (4.29), (7) quinolfos (RT; 5.54) and (8) ethion (RT; 7.36).

pletely dry the samples and acetonitrile solvent was then used for the extraction of OP compounds. This method worked well, as it achieved good recoveries at the lowest concentration level (0.1 μg L^{-1}). We also observed lower LOD and LOQ, as well as higher percentage recovery and good precision in comparison with other reported methods [15,18–23]. For example, the solid phase extraction (SPA)–gas chromatography–mass spectrometry method has been reported [15] for the isolation of different kinds of pesticides, but the LOD and percentage recovery were higher than in our described method. Similarly, several methods have been reported for the isolation and quantification of OP compounds [15,18–20,22,23], which were not as simple or as cost-effective as the method described here.

In several reported methods [8,9,14,17], GC and GC–MS/MS techniques were used for the analysis of OP pesticides, insecticides and herbicides in water samples. However, we used a LC–MS/MS hybrid technique for the analysis of these pesticides. The 4000-Q-Trap LC–MS/MS technique was preferable for quantification and confirmation of OP compounds due to the highly polar nature of these compounds. For analysis of OP compounds on GC, several complex methods have been used for the isolation of analytes from water samples; therefore, there is a chance for the loss of compound during these complex extraction procedures. Lyophilization was a found to be a very good technique for removing water from the samples [30–32] for extraction. Therefore, the lyophilization technique was used for removal of water from samples in our method. A good stability of OP compounds has been reported during the lyophilization process and there was no loss of compound [30–33], which strengthened our approach.

The sensitivity and reproducibility was the most important tool for analyzing the analytes on an MS detector because the ions could be extracted to remove interfering impurities and noise from the samples using a 5-point smoothing factor. Different compositions of the mobile phase were used on the basis of the physical and chemical properties of the OP compounds of interest.

The isolated ions of the OP standards are shown in Table 1. First, the full scan spectra were recorded using manual tuning. In the full scan spectra, the characteristic stable ions were then isolated for MRM transition for confirmation and quantification of eight pesticides in water samples. The detail isolated ion, different energy parameters and retention times (RTs) for MRM transitions are also shown in Table 1.

The specificity of mass spectrometry is capable of eliminating interfering components and matrices in the water sample extracts, which in turn provides lower detection limits for the method. In our study, analysis in simple scan mode resulted in recurring interferences for many of the analytes. These specificity requirements precluded the use of single quadrupole and ion trap mass spectrometry. To increase sensitivity for the quantification at the picogram level of this method, we shifted to MRM transition analysis. The obtained isolated ions for different pesticides in the MRM mode are shown in Table 1. The isolation of ions was carried out in a similar fashion as per the previously reported method [26]. To increase the sensitivity of the MS/MS analysis, different DP, EP, FP and CE parameters were used, but the highest sensitivity was achieved by the energy profile shown in Table 1.

The detailed precursor and product ions for each analyte for quantification are shown in Table 1. The confirmation ions were isolated at *m*/*z* 167, 199, 175, 131, 127, 163, 286 and 132 for monochrotofos, ethion, imedacloprid mertibuzin, propanil, quinolfos, triazofos and atrazine, respectively, by using different energy parameters.

Certain OP compounds proved to be amenable to separation by LC. Different solvent gradient methods were used, but the best separation of all pesticides was achieved using the LC–MS/MS method described earlier in this paper. The solvent gradient was fixed to accommodate the physical and chemical properties of the pesticides (Fig. 1). The total ion chromatograms of monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin (10 μg L^{-1} spiked level) are shown in Fig. 2.

The pesticide-free water samples were spiked with different concentrations of standard (i.e., monocrotofos, imedacloprid, triazofos, ethion, atrazine, propanil, quinolfos and metribuzin). The inter-day percentage recovery (Table 2), standard deviation (SD) and RSD were determined as per the reported methodology [24,25]. In addition, excellent extraction efficiencies were obtained for all these compounds. The obtained percent recoveries for all these pesticides were found to be in the range of 96–103% of the stan-

Table 2
Inter-day percentage recovery (mean) and % RSD of pesticides at different spiked concentrations.

Spiked concentration (ng mL^{-1})		0.10	0.20	1.00	2.0	5.0	10.0	30.00
Atrazine	SD	4.15	4.65	2.24	7.59	5.35	3.4	0.812
	% RSD	4.09	4.76	2.23	7.33	5.54	3.42	0.815
	% Recovery	101	97	100	103	96	100	99
	N	7	7	7	7	7	7	7
Propanil	SD	4.70	2.29	4.19	1.74	6.31	2.17	1.74
	% RSD	4.55	2.27	4.26	1.76	6.52	2.12	1.74
	% Recovery	103	100	98	99	96	100	100
	N	8	8	8	8	8	7	7
Imedacloprid	SD	3.21	6.80	1.44	4.65	8.21	1.36	1.04
	% RSD	4.87	2.94	3.03	2.67	2.19	2.58	0.544
	% Recovery	99	98	101	99	97	101	99
	N	7	7	7	7	7	7	6
Triazofos	SD	4.98	2.27	3.01	2.62	2.16	2.55	0.547
	%RSD	4.82	2.94	3.03	2.67	2.19	2.58	0.544
	% Recovery	103	101	99	98	98	98	100
	N	7	7	7	7	7	7	6
Ethion	SD	6.44	2.89	0.516	3.20	3.09	6.54	1.93
	% RSD	6.69	2.81	0.507	3.20	3.14	6.36	1.94
	% Recovery	96	103	101	100	98	102	99
	N	6	6	6	6	6	6	6
Monocrotofos	SD	6.24	3.79	6.27	1.76	6.38	2.73	0.867
	% RSD	6.21	3.69	6.43	1.75	6.57	2.76	0.864
	% Recovery	100	102	97	100	97	98	100
	N	7	7	7	7	7	7	7
Quinolfos	SD	5.98	1.54	4.36	1.74	6.38	4.01	0.722
	% RSD	5.76	1.56	4.35	1.75	6.51	4.08	0.717
	% Recovery	103	99	100	99	98	98	100
	N	7	7	7	7	7	7	7
Metribuzin	SD	2.30	2.56	3.39	4.10	7.29	6.22	3.19
	% RSD	2.26	2.60	3.31	4.25	7.28	6.06	3.21
	% Recovery	101	98	102	96	100	102	99
	N	7	7	7	7	7	7	7

RSD, relative standard deviation; SD, standard deviation and *N*, number of replicates.

dard value [25]. At the lowest spiked level of 0.1 μg L^{-1} of all the pesticides in water, the absolute recoveries were 96%, indicating the selectivity and precision of this method. The obtained RSD was below 8% for all compounds, which further reinforced the importance, sensitivity, precision and selectivity of this method. The obtained *S*/*N* ratios were 25, 7, 46, 23, 34, 20, 11 and 12 for monocrotofos, imedacloprid, atrazine, triazofos, ethion, quinolfos, propanil and metribuzin, respectively, at the 0.1 μg L^{-1} spiked level.

Seven replicate measurements of seven point calibration curves were prepared using seven different spike concentrations (0.1, 1, 5, 10, 25, 50 and 150 μg L^{-1}) plotted against area and evaluated by linear square regression. The observed *r* values for all pesticides are shown in Table 3. The corresponding precision was below 2%, and the correlation coefficient was greater than 0.999 for all these compounds [24,25]. Furthermore, a100% accuracy was achieved, which was indicative of the high degree of accuracy of the employed method [25].

Fig. 3 shows the correlation diagram plotted between spiked triazofos concentrations and the measured triazofos concentrations (μg L^{-1}). The observed correlation coefficient was 0.1 with a slope of 0.9999, indicating excellent agreement between the spiked and measured values of triazofos (Table 3). Similar observations were achieved for all the pesticides of interest. These data suggested that the manual and laboratory errors were not significant.

The accuracy of the method was also assessed by calculating the coefficient of determination (r^2) by spiking different concentrations of pesticides in pesticide-free water samples and plotting against the area of the concentration (Table 3). The observed accuracy for all compounds was ≥99.99%.

The LOD and LOQ were determined as per the previously reported method [24,25]. The LODs (μg L^{-1}) were found for monocrotofos (0.0051), imedacloprid (0.0049), triazofos (0.0287), quinolfos (0.0161), ethion (0.051), atrazine (0.02), propanil (0.0133) and metribuzin (0.0063) respectively (Table 4). The LOQs were as follows: monocrotofos: 0.0178, imedacloprid: 0.0165, triazofos: 0.0957, ethion: 0.171, atrazine: 0.0668, propanil: 0.0446,

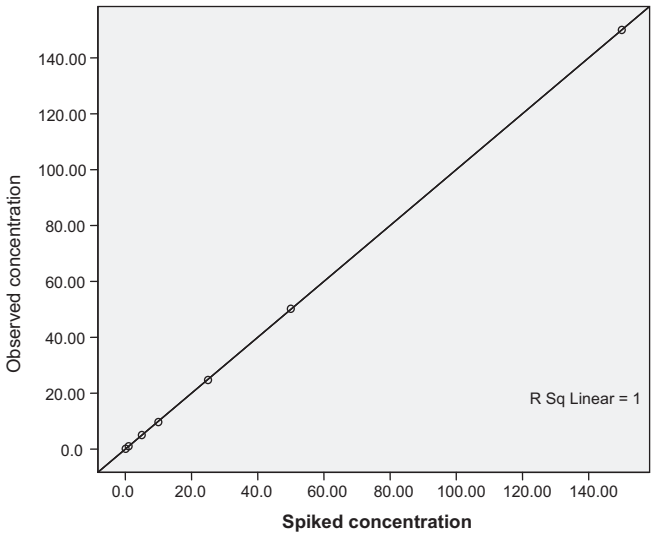

Fig. 3. Linear regression diagram plotted between spiked triazofos concentrations and the measured triazofos concentrations (μg L^{-1}).

Table 3
Accuracy determination using the correlation coefficient of spiked samples at different concentrations with uncertainties parameter (slope, intercept and standard error in slope).

Compounds	Concentrations (ng mL^{-1})	[a]r	Slope	Intercept	[b]r^2 (mean)	N	%RSD	% Accuracy	[c]SES
Atrazine	0.1, 1, 5, 10, 25, 50 and 150	1	0.9999	−0.01297	0.999	7	0.320	100	0.002
Propanil	0.1, 1, 5, 10, 25, 50 and 150	0.9999	1.000	−0.00236	0.999	7	0.0264	99.99	0.001
Imedachlorid	0.1, 1, 5, 10, 25, 50 and 150	1	0.9993	−0.19779	0.999	7	0.0248	100	0.030
Triazofos	0.1, 1, 5, 10, 25, 50 and 150	0.9999	0.9999	−0.00798	0.999	7	0.0263	99.99	0.002
Ethion	0.1, 1, 5, 10, 25, 50 and 150	1	0.9993	−0.07987	0.999	7	0.0143	100	0.002
Monocrotofos	0.1, 1, 5, 10, 25, 50 and 150	0.9996	0.989	−0.16634	0.999	7	0.0224	99.96	0.017
Quinolfos	0.1, 1, 5, 10, 25, 50 and 150	0.9997	0.989	−0.38747	0.999	7	0.0203	99.97	0.009
Metribuzin	0.1, 1, 5, 10, 25, 50 and 150	0.9994	1.005	−0.1935	0.999	7	0.056	99.99	0.004

RSD, relative standard deviation; N, number of replicates.
[a] r, correlation coefficient.
[b] r^2, determinations of coefficient.
[c] SES, standard error in slope.

quinolfos: 0.0161 and metribuzin: 0.0212 ng mL^{-1}. High accuracy with a high degree of precision at low levels of detection was also found (Table 4). The observed LOD of our method was lower than other reported methods [8,9,13,15,18–20,22,23], except for ethion. In addition, LOQs were reported in our described method that had better analytical significance in comparison to other reported methods for quantification.

The comparison of the LODs is shown in Table 5. The detection limits for the different OP pesticides ranged from 0.5 to 100, 100 to 5000, 10 to 32, 1 to 10 and 10 to 32 ng L^{-1}, and the RSD for the analytical method was 1–20% (Table 5). Our obtained RSDs were better than those reported in the literature for methods with higher LODs. Our achieved LODs ranged from 4.9 to 28.2 ng L^{-1}, except for ethion (51 ng L^{-1}), which was higher than almost all reported methods shown in Table 5. The one reported LOD ranged from 1 to 10 ng L^{-1} [20], which was much lower than our LODs because they used very complex and costly analytical methods (GC–MS/MS + isotopic dilution + solid-phase extraction, Table 5). It is very difficult to adopt this costly and complex method for use by developing countries in the analysis of pesticides in water samples due to a lack of funds and analytical expertise.

Our method was more sensitive, simple, cost-effective and more selective than previously published methods for measuring various pesticides in water samples. For example, typical LODs in the literature were higher than our observed LODs. Additionally, the imprecision associated with our measurements was typically lower than in other studies. Furthermore, our study showed that LC–MS/MS in the ESI positive mode with MRM ion preparation is the best technique for the confirmation, as well as quantification, of OP pesticides at the μg L^{-1} level.

This method was used for the analysis of OP pesticide and herbicides in drinking water from bore and MC supply water from different parts of the city of Hyderabad in India to estimate the exposure of the general population and for assessment of associated health risks, especially in different age groups of children.

Table 4
LOD, LOQ, % accuracy, and coefficient of determination for eight pesticides.

Name of pesticides	[a]r	[b]LOQ	[c]LOD	Co-variation	% Accuracy
Atrazine	0.999	0.066	0.020	0.127	99.9
Propanil	0.988	0.044	0.013	0.058	98.9
Imedacloprid	0.990	0.016	0.004	0.051	99.0
Triazofos	0.999	0.095	0.028	0.036	99.9
Ethion	0.999	0.171	0.051	0.016	99.9
Monocrotofos	0.994	0.017	0.005	0.414	99.4
Quinolfos	0.999	0.053	0.016	0.365	99.9
Metribuzin	0.999	0.021	0.006	0.207	99.9

[a] r, correlation coefficient.
[b] LOD, limit of detection.
[c] LOQ, limit of quantification.

The results showed the presence of pesticide residues in bore water, as well as in the MC supply water. A total of seven pesticides were detected in these water samples, but ethion was not among those detected in the samples. Among the different groups of pesticides, OP pesticides were present in the range of 0.002–0.768 μg L^{-1} with maximum concentrations for triazofos (0.768 μg L^{-1}) and metribuzin (2.51 μg L^{-1}) in their respective groups (Table 6). Almost 100% of the samples showed the presence of triazofos and monocrotofos, while only 64% of the water was contaminated with metribuzin. Quinolfos, atrazine, propanil and imedacloprid were detected in 46%, 38%, 23% and 23%, respectively, in bore water samples. The presence of other OP pesticides (quinolfos, atrazine, propanil and imedacloprid) were in the range of 0.0926–0.381 μg L^{-1} in the MC water, while in bore water the range of these pesticides was 0.127–0.496, well above the MRL (0.1 μg L^{-1}) for individual pesticides in multi-residue analysis. The quinolfos, atrazine, propanil and imedacloprid detected in 16%, 33%, 16% and 33% in MC water samples. The level of most of the analyzed pesticides in MC water did not exceed the MRL of 0.1 μg L^{-1} fixed for multi-residue analysis of individual pesticides, except triazofos. Student's t-test analysis of pesticides showed there was no significant difference between pesticides concentration in the MC water supply and the bore water supply. In addition, there was no significant correlation of pesticides concentration between the MC water and the bore water.

Most of the reported studies have been concerned with the presence of OP pesticides in river, rain and waste water in India, while no studies have reported on the measurement of commonly used OP pesticide concentrations in drinking water. One example reported the concentrations of the OP pesticides dimethoate and methyl parathion at 0.20 and 0.41 μg L^{-1}, respectively, in the Ganga River water in Narora (U.P.), India [10]. Similarly, OP pesticides reported in Indian rainwater were in the range of 0.050–4.000 μg L^{-1} and showed maximum contamination with cypermethrin (1.000 μg L^{-1}) and monocrotofos (4.000 μg L^{-1}). Almost 80% of the samples showed residues above the MRL of 0.5 μg L^{-1} fixed for multi-residues, and on the basis of single pesticides, 16–50% of the samples contained residues above the MRL value of 0.1 μg L^{-1} [11]. The OP pesticides dimethoate and methyl parathion were also detected at concentration levels of 0.41–0.56 μg L^{-1} and 0.16–0.50 μg L^{-1}, respectively, in Ganga River water near Kanpur, India [34].

The presence of these pesticides in water might be due to surface water runoff, which can carry pesticides from areas such as agricultural fields and residential properties into lakes, rivers and reservoirs. These areas can then carry pesticides through the soil into underground water supplies. For example, improperly disposed pesticides can move through the soil and reach drinking water supplies, while improperly applied termiticides can seep into private well water [35]. Without proper safeguards, pesticides have

Table 5
Comparison of different reported methods and our described method.

Analytes	Matrices	Sample preparation technique	References	[a]LOD ranged	[b]LOQ ranged	Recovery ranged	[c]RSD ranged
[d]OP + herbicides	River water	Solid-phase extraction + GC coupled with time-of-flight MS	[19]	0.5–100 ng L^{-1}	2–185 ng L^{-1}	[e]NR	≥20%
OP	Water + juice	Ultrasound-assisted emulsification-micro-extraction	[15]	5.3–10.0 ng L^{-1}	NR	80.0–110.0%,	1.6–13%.
OP	Ground water	Automated on-line liquid – solid extraction + LC–MS (APCI)	[18]	5 and 37 ng L^{-1}	NR	NR	NR
Pesticides	Waste water	GC–MS/MS + isotopic dilution + solid-phase extraction	[20]	1–10 ng L^{-1}	NR	71% and 118%	1–15%
OP	Water	Micro liquid extraction + HPLC	[22]	100–5000 ng L^{-1}	NR	99.9–117.6%	4.7% at 200 μg L^{-1}
OP	Water	SPE + GC	[23]	10–32 ng L^{-1}	NR	77.5–99.1%.	NR
OP + herbicides	Drinking water	Lyophilization + LC–MS/MS (Q-Trap)	Our method	4.9–51 ng L^{-1}	16–171 ng L^{-1}	96–103%	0.544–8.2

[a] LOD, limit of detection.
[b] LOQ, limit of quantification.
[c] RSD, relative standard deviation.
[d] OP, organophosphorus.
[e] NR, not reported.

Table 6
Pesticide concentrations in urban water samples.

Pesticides	Nature of water samples	Mean concentrations (ng mL^{-1})	Ranges (ng mL^{-1})	SE[a]	*N*[b]
Trizofos	MC[c]	0.30167	0.107–0.486	0.0550	6
Trizofos	Bore	0.33986	0.142–0.768	0.0822	7
Monochrotofos	MC	0.034167	0.002–0.088	0.0102	6
Monochrotofos	Bore	0.083857	0.008–0.14	0.0237	6
Metribuzin	MC	0.07048	0.0408–0.131	0.0223	4
Metribuzin	Bore	0.67243	0.172–2.51	0.3210	7

[a] SE, standard error.
[b] *N*, number of samples.
[c] MC, municipal corporation.

the potential to seriously threaten many groundwater supplies in the United States (U.S.). Approximately 50% of the U.S. population obtains its drinking water from groundwater sources, and as much as 95% of the population in agricultural areas uses groundwater as its source of drinking water. In the USA, less than 2% of wells sampled in multi-state studies were found with pesticide concentrations above the established maximum contaminant level (MCL) [36]. Due to repeated detection of various pesticides in U.S. wells, the U.S. Environmental Protection Agency (EPA) recently proposed a State Management Program (SMP), which would control or ban pesticides with the greatest potential to contaminate groundwater [37].

4. Conclusions

We have developed a highly sensitive and selective method for quantifying eight pesticides in water samples at low levels of determination. Our method employs a lyophilization followed by simple solvent extraction and analysis using a selective LC–MS/MS. This method was found to be selective and have good precision and sensitivity. The lower limit of method validation and determination were in the parts per trillion (ppt) ranges with percentage RSD values of <8%. The vital point of our method was its simple, cost-effective and time saving nature. We effectively saved solvent and time without compromising sensitivity, selectivity or precision of this method; thus, this method may be used for the analysis of pesticides in drinking water samples for cumulative exposure to OP pesticides, especially in developing countries where the unemployment and economic crisis is severe. Furthermore, we found detectable concentrations of commonly used agricultural and residential pesticides in urban water samples. These data confirmed the usefulness of our method in detecting environmental exposure to a variety of pesticides. These data were informative, but not conclusive, because sample size was low. We plan to further estimate the exposure of OP pesticides in different age groups of children through drinking water and food by applying this method.

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References

[1] R. Bravo, L. Caltabiano, G. Weerasekera, D.W. Ralph, C. Fernandez, L. Needham, A. Bradman, D.B. Barr, J. Exp. Anal. Environ. Epidemiol. 14 (2004) 249.
[2] P.C. Abhilash, N. Singh, J. Hazard. Mater. 165 (2009) 1.
[3] CFSAN FDA Office of Plant and Dairy Foods: FDA Pesticide Residue Monitoring Program 1994–2002, http://vm.cfsan.fda.gov/~dms/pesrpts.html.
[4] K.A. Cook, Pesticides in the US Food Supply, 1995, www.ewg.org/reports/fruit/Contents.html.

[5] Centers for Disease Control, National Report on Human Exposure to Environmental Chemicals, 2003, March, www.cdc.gov/exposurereport/2nd/.
[6] Centers for Disease Control, National Report on Human Exposure to Environmental Chemicals, 2002, Available: www.cdc.gov/nceh/dls/. Accessed, January 2002.
[7] W. Denise, D.B. Barr, P. Mendola, Environ. Health Perspect. 111 (2003) 1939–1946.
[8] A.M. Branum, G.W. Collman, A. Correa, S.A. Keim, W. Kessel, C.A. Kimmel, et al., Environ. Health Perspect. 111 (2003) 642.
[9] United States Geological Survey, report pesticides in the nation's streams and ground water, 1992–2001, http://pubs.usgs.gov/fs/2006/3028/pdf/fs2006-3028.pdf.
[10] Z. Rehana, A. Malik, M. Ahmad, Mutat. Res./Genetic Toxicol. 343 (1995) 137.
[11] B. Kumari, V.K. Madan, T.S. Kathpal, Environ. Monit. Assess 133 (2007) 467.
[12] M.I. Ansari, A. Malik, Environ. Toxicol. 24 (2009) 103.
[13] S. Lacorte, D. Barceló, J. Chromatogr. A 754 (1996) 125.
[14] N.M. Gatto, M. Cockburn, J. Bronstein, A.D. Manthripragada, B. Ritz, Environ. Health Perspect. 117 (2009) 1912.
[15] C. Jia, X. Zhu, L. Chen, M. He, P. Yu, E. Zhao, J. Sep. Sci. 33 (2009) 244.
[16] K. Mastovska, K.J. Dorweiler, S.J. Lehotay, J.S. Wegscheid, K.A. Szpylka, J. Agric. Food Chem. (2009), 22 December On line PMID: 20028018.
[17] S. Sanches, M.T. Barreto Crespo, V.J. Pereira, Water Res. 44 (2010) 1809.
[18] S. Lacorte, D. Barceló, Anal. Chem. 68 (1996) 2464.
[19] V. Matamoros, E. Jover, J.M. Bayona, Anal. Chem. 82 (2010) 699.
[20] S. Öllers, P. Heinz, S.F. Philipp, R.M. Stephan, J. Chromatogr. A. 911 (2001) 225.
[21] D. Ortelli, P. Edder, C. Corvi, Anal. Chem. Acta 520 (1–2) (2004) 33.
[22] L. He, X. Luo, H. Xie, C. Wang, X. Jiang, K. Lu, Anal. Chim. Acta 655 (2009) 52.
[23] X. Zhu, J. Yang, Q. Su, J. Cai, Y. Gao, J. Chromatogr. A 1092 (2005) 161.
[24] Laboratory Procedure Manual, CDC, Method No.: 11-OD, Organic Analytical Toxicology Branch, Division of Laboratory Sciences, CDC, National Center for Environmental Health, Atlanta, USA.
[25] D.B. Barr, J.R. Barr, V.L. Maggio, R.D. Whitehead Jr., M.A. Sadowski, R.M. Whyatt, L.L. Needham, J. Chromatogr. B 778 (2002) 99.
[26] K.D. Miller, P. Milne, AOAC Int. 91 (2008) 181.
[27] S.N. Sinha, R. Pal, A. Dewan, M.M. Mansuri, H.N. Saiyed, Int. J. Mass Spectrom. 253 (2006) 48.
[28] S.N. Sinha, P.K. Kulkarni, N.M. Desai, S.H. Shah, G.M. Patel, M.M. Mansuri, D.J. Parikh, H.N. Saiyed, J. Chromatogr. A 1065 (2005) 315.
[29] V.K. Dua, S.N. Sinha, V.P. Sharma, J. Chromatogr. B 708 (1998) 316.
[30] W.J. Driskell, D.B. Barr, M.D. Beeson, I.R. Harmon, The American Society for Mass Spectrometry Conference, Dallas, TX, June, 1999, Poster No. 167.
[31] A.N. Oglobline, H. Elimelakh, B. Tattam, R. Geyer, G.E. O' Donnell, G. Holder, Analyst 126 (2001) 1037.
[32] R. Whyatt, D.B. Barr, Environ. Health Perspect. 109 (2001) 417.
[33] I. Ferrer, D. Barceló, J. Chromatogr. A 737 (1996) 93.
[34] Z. Rehana, A. Malik, M. Ahmad, Mutat. Res./Genetic Toxicol. 367 (1996) 187.
[35] Aspelin, A.L., Pesticides industry sales and usage, 1992 and 1993 market estimates: U.S. EPA, Office of Pesticides Programs, Biological and Economic Analysis Div., Economic Analysis Branch Report 733-K-94-001, p. 33, 1994.
[36] Pesticides in Groundwater. Volume 2 of the series Pesticides in the Hydrologic Environment. Barbash, J.E. and E.A. Resek. Ann Arbor Press, Inc., Chelsea, MI. pp. 425, 1996.
[37] Federal Register. Wednesday, June 26, 1996. Part VII. Environmental Protection Agency. 40 CFR Parts 152 and 156.