DETERMINATION OF SEVERAL PESTICIDE RESIDUES AND DISINFECTION BY-PRODUCTS IN DRINKING WATER BY CHROMATOGRAPHY

HUYNH THAI KIM NGAN, PHẠM HUU ANH
Centre for Applications of Nuclear Technique in Industry; 01, DT 723, Dalat, Lamdong
Email: nganhtk@canti.vn; kimngan270979@gmail.com

Abstract: Pesticides are extensively used to control pests and destroy weeds, so they are ubiquitous contaminants accumulating in our environment. Such residues can be determined by using various chemical methods among which the most common are based on gas chromatography or liquid chromatography. This study describes a validation method for the analysis of pesticide residues and disinfection by-product in drinking water by extraction and clean-up by solid phase extraction and analysis by chromatography. Method validation was carried out on drinking water matrices following SANTE/11813/2017 of the European Commission. 21 compounds obtained acceptable recoveries (71 –120%) with good repeatability (RSD < 20%) and the method minimum detection qualification LOQ of compounds ranged from 0.02 –0.2 μg/L and is lower than the maximum residue levels (MRL) of National technical regulation on drinking water quality (QCVN:01-2009/BYT)).

Keywords: *SANTE, RSD, MRL, LOQ, QCVN*

1. INTRODUCTION [1,2,4]

Water is a critical resource on which life depends. Addressing issues of drinking water quality and understanding and managing safe water supplies require a thorough understanding and evaluation of all types of elements and factors that affect its composition. Loading of contaminants to drinking water sources occurs through many paths, natural and anthropogenic. Thus, the impact of water quality on human health is highly complex due to the many factors that influence its chemical nature.

Humans are potentially exposed to thousands of natural and synthetic chemicals every day. These substances reach the body via food or water, or by adsorption from the environment through the skin and lungs. Pesticides are extensively used to control pests and destroy weeds, so they are ubiquitous contaminants accumulating in our environment and inevitably reaching humans as a result. Some pesticides are highly toxic and nonbiodegradable, and persist in the environment over very long periods. As consequence, their use and their presence in foods and water are subject to stringent regulations. Pesticide residues have been monitored for regulatory compliance purposes at levels potentially exceeding their maximum allowed concentrations in surface, ground, and drinking water. Such residues can be determined by using various chemical methods among which the most common are based on gas chromatography or liquid chromatography. However, identifying individual compounds in complex matrices with detection limits meeting the requirements of legal regulations on pesticides in water usually entails labor-intensive extraction and cleanup prior to their instrumental analysis.

Gas chromatography (GC) is the chromatographic technique of choice for the determination of pesticide residues in environmental samples due to its very high selectivity and resolution, good accuracy and precision, a wide dynamic concentration range, and a high sensitivity. The electron capture detector (ECD) was one of the first selective detectors to be extensively used with organochlorine and pyrethroid pesticides). The ECD is extremely sensitive to polychlorinated (and other polyhalogenated) pesticides, for which it provides detection limits (DLs) at the nanogram-per-liter level. The number of pesticides analyzed by GC–MS has been greatly expanded and it is now possible to simultaneously determine more

than 100 compounds even with a single quadrupole mass analyzer operating in the single-ion monitoring (SIM) mode by virtue of the intrinsically high resolving power and peak intensities of capillary GC.

Until the last decade, applications of Liquid chromatography (LC) to pesticide analysis usually focused on individual compounds or compound groups that were not amenable to GC analysis such as low volatile, thermally unstable, or (very) polar pesticides. The UV detector has been the most widely used in the LC determination of pesticides despite its low sensitivity, which requires preconcentrating the analytes. The introduction of the diode-array detector (DAD) has enabled the acquisition of spectra from fast-eluting peaks.

Solid-phase extraction (SPE) has gained popularity for sample preparation of pesticides from water. It offers the advantages of short analysis time, cleaner extracts, enhanced trace enrichment, higher chemical selectivity, lack of emulsions, reduced consumption of organic solvents, and the possibility for automation. Several papers describe the use of C18 bonded silica cartridges, and Carbopack cartridges for the extraction of acidic herbicides from water samples. The techniques of automated column switching and on-line SPE coupled to liquid chromatography have been reported for the determination of acidic herbicides both in drinking water and in surface water. Other researchs have focused on the separation and quantification techniques for acidic pesticides.

This study describes a validation method for the analysis of residues pesticides and disinfection by-product in drinking water by extraction and clean-up using solid phase extraction and analysis by chromatography. Method validation was carried out on drinking water matrices following SANTE/11813/2017 of the European Commission. The analytical method was validated from blank drinking water prior to actual analysis. To validate the analytical method, recovery percentage (R%) was established by fortification of the pure standard. Recovery experiments were performed by comparing true amount which represent 100% recovery with those of extracted samples, where analysts should have been added at least three different concentrations. Method limit of quantification (LOQ) was determined by repeating 12 times of drinking water samples at a spike concentration lower than MRL concentration, performed at 2 different days, 6 samples per day. SANTE/11813/2017 defines LOQ as the smallest standard concentration that the method can determine with repeatability (RSD_r), reproducibility (RSD_R) less than 20%, recovery efficiency (R) obtained from 70 - 120%, and the method means when the LOQ is equal to or less than the allowed MRL.

2. DETERMINATION OF SEVERAL PESTICIDE RESIDUES IN DRINKING WATER BY GC/ECD

Pesticides and their breakdown products directly or indirectly reaching soil may dissipate by various means, including mobilization to the atmosphere through volatilization or dust, transport in water though leaching and run-off, and degradation through abiotic or biotic processes. Similarly, pesticides and their breakdown products directly or indirectly reaching water (surface water such as streams, ponds, dams and wetlands, or ground water), may be dissipated from the water column by processes including adsorption to sediment, degradation in the water column or sediment, and volatilization. Before dissipating, they may be transported elsewhere, e.g. downstream or back to the land through irrigation or flooding. Ground water may ultimately return to the surface through pumping or in springs. Residues of more persistent substances may remain indefinitely in soil and sediment, adsorbed to organic matter or clay particles. Provided adsorption is not too strong, prior to degradation, pesticide residues may be taken up by plants or animals and may therefore enter the food web, even if not directly applied to the produce. [1,3]

EXPERIMENTAL SECTION

Materials and Reagents. Standards for pesticide, used as quantitative and fortificated, were purchased from Supelco. All other chemicals were HPLC and ACS reagent grade and purchased from Fisher Scientific. All standard and reagent solutions were prepared in glass containers with either ground-glass stoppers or Teflon- lined screw caps purchased from Fisher Scientific.

GC/ ECD Conditions. The studied analyted were separated and determined in Agilent 7890A gas chromatography**.** A GC capillary column of DB-5 (30 m × 0.32 mm id., 0.25mm thickness, 5% phenyl/ 95% dimethylpolysiloxane; Agilent Technologies, Inc.) was used for method development and sample analysis. We ran the temperature program initially held at 80°C for 1 min, then ramped at a rate of 10°C/ min to 280°C, and held at the final temperature for 10 min. Nitrogen was used as carrier gas at a rate of 1.2 mL/ min. The temperature of the electron capture detector was set at 300°C, and the inlet temperatures of the GC were at 270°C, respectively. A 1μL aliqout of the sample extract or standard was injected in splitless mode by an autosampler.

Table 1. Method limit of quantification (LOQ) of pesticide residues

No	Compound	Group	LOQ, µg/L	%R (n=12)	RSDr (n=6)	RSD_R (n=12)	MRL, µg/L
1	Hexachlorobenzen	OC	0.02	87.00	8.37	12.76	1
2	Lindane	OC	0.02	103.00	4.00	6.49	2
3	Alachlor	OC	0.02	88.00	5.86	5.77	20
4	Heptachlor	OC	0.02	82.00	1.25	13.94	0.03
5	Aldrine	OC	0.02	105.00	4.81	8.00	0.03
6	Heptachlor epoxide	OC	0.02	79.00	4.50	3.40	0.03
7	Chlordane	OC	0.02	86.00	5.66	10.30	0.2
8	Dieldrin	OC	0.02	90.00	3.84	3.97	0.03
9	DDT	OC	0.02	90.00	9.16	8.79	2
10	Metolachlor	OC	0.02	95.00	4.09	14.86	10
11	Methoxychlor	OC	0.02	111.00	7.26	11.96	20
12	Permethrine	Pyre	0.20	81.00	5.14	6.39	20
13	Clorotoluron	PhU	1.00	107	9.17	8.27	30
14	Isoproturon	PhU	1.00	83	2.69	5.42	9
15	2,4 - D	PhA	1.00	110	8.39	4.67	30
16	2,4 DB	PhA	1.00	102	4.95	5.53	90
17	2,4,5 - T	PhA	1.00	86	8.9	6.43	9

HPLC/ DAD Conditions. The HPLC apparatus was equipped with a ZORBAX-C18 150mm x 4.6mm column, 5mm particle size (Agilent Technologies). The gradients applied were, sequence: 30%B, 30min linear to 76%B, 35min linear to 100%B, and 35–45 min isocratic 100% B (A-H_2O, B – Methanol).

Organochloride (OC) and pyrethroid (Pyre) pesticide residues sample preparation. Pesticide residues are extracted from a water sample by passing 0.5L of sample water through a cartridge containing a solid matrix with a chemically bonded C-18 organic phase (strata-C18, 500mg/6mL, Phenomenex). The organic compound are eluted from the cartridge with 4ml of acetonitrile. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high resolution fused silica capillary column of a gas chromatography/ electron capture detector (GC/ECD) system. [7]

Phenoxy acid (PhA) and phenyl urea (PhU) sample preparation. A measured sample volume of approximately 500 mL is adjusted to pH 12 with 1N sodium hydroxide, shaken, and allowed to set for 1hr to hydrolyze chlorinated esters. The sample is acidified with H_3PO_4, filtered, and chlorinated acids are extracted from a 20 mL aliquot. The 20 mL aliquot is pumped (using a vacuum extraction manifold, Phenomenex) through a strata-C18 cartridge, the

organic compounds are eluted from the cartridge with small quantities of methanol. The analytes are separated and measured by photodiode array detection (HPLC-DAD). [8].

Recovery tests.The samples (500 ml) were spiked with pesticide in at levels of once, once and a half and twice time the LOQ of pesticide in drinking water (0.02, 0.03, and 0.04 µg/L for OC; 0.2, 0.3 and 0.4 µg/L for Pyre; 1, 1.5, and 2 µg/L for PhA and PhU), six replications of each point were done for the recovery tests of pesticide in drinking water.

RESULTS AND DISCUSSION

Method limit of quantification (LOQ). The results in Table 1 show that the LOQ of pesticide is less than the MRL of National technical regulation on drinking water quality (QCVN:01-2009/BYT)

The precision of method was determined as repeatability and reproducibility. The precision was expressed in terms of RSD. The reproducibility was performed on three consecutive days. Pesticide was added to drinking water samples at levels of once, once and a half, twice of LOQ (C1, C2, C3) and each sample was analyzed, based on the described procedure to revaluate the precision of the analytical method. The results are shown in Table 2. The recovery was measured by comparing peak areas of the spiked samples with those of the related the standard calibration curve. The mean recoveries of 18 replicates ranged of 71% to 120% with relative standard deviations (RSD_r) of 1.19% to 9.17%. The producibility (expressed as RSD_R) ranged of 1.22% to 8.97%.

Table 2. Precision of method pesticide residues

No	Items	C1, µg/L		C2, µg/L		C3, µg/L		RSD_R
		%R1	RSD_{r1}	%R2	RSD_{r2}	%R3	RSD_{r3}	
		(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=18)
1	Hexachloro benzen	87	8.37	96	8.21	89	7.97	8.18
2	Lindane	103	4	113	3.92	83	3.81	3.91
3	Alachlor	88	5.86	116	5.59	116	5.2	5.56
4	Heptachlor	82	1.25	109	1.23	94	1.19	1.22
5	Aldrine	105	4.81	105	4.72	94	4.58	4.7
6	Heptachlor epoxide	79	4.5	91	4.41	120	4.29	4.4
7	Chlordane	86	5.66	102	5.55	114	5.39	5.53
8	Dieldrin	90	3.84	95	3.76	95	3.66	3.75
9	DDT	90	9.16	84	8.98	96	8.72	8.96
10	Metolachlor	95	4.09	98	4.01	74	3.9	4
11	Methoxychlor	111	7.26	105	7.12	108	6.91	7.1
12	Permethrine	81	5.14	93	6.84	87	6.42	6.18
13	Clorotoluron	107	9.17	103	8.99	96	8.73	8.97
14	Isoproturon	83	2.69	89	2.63	120	2.56	2.63
15	2,4 - D	110	8.39	77	8.22	76	7.99	8.2
16	2,4 DB	102	4.95	105	4.85	93	4.71	4.84
17	2,4,5 - T	86	8.9	71	8.72	94	8.47	8.7

3.DETERMINATION OF DISINFECTION BY-PRODUCTS (PHENOLs AND HALOACETIC ACID) IN DRINKING WATER USING GC/ECD

Disinfection of drinking water has been in practice since the early twentieth century. Chlorine has been most widely used in many water-treatment plants to inactivate microorganisms and maintain a residual concentration through the water distribution system. Despite obvious advantages in terms of controlling microbes in drinking water, chlorination also has disadvantages because of the formation of disinfection by-products (DBPs) through contact between natural organic matter (NOM) and disinfectants. Some of these products such as phenols and haloacetic acids (HAAs) have been known to cause cancer and other toxic effects to human beings [1].

EXPERIMENTAL SECTION

Materials and Reagents. Standards for DBPs, used as quantitative and fortificated, were purchased from Supelco. The derivatizing reagents, pentafluorobenzil bromide, were from Aldrich),K_2CO_3, anhydrous ACS grade, was purchased from Fisher Scientific. All other chemicals were HPLC and ACS reagent grade and purchased from Fisher Scientific. All standard and reagent solutions were prepared in glass containers with either groundglass stoppers or Teflon lined screw caps purchased from Fisher Scientific.

GC/ ECD Conditions. The studied analyted were separated and determined in Agilent 7890A gas chromatography**.** A GC capillary column of DB-5 (30 m × 0.32 mm id., 0.25mm thickness, 5% phenyl/ 95% dimethylpolysiloxane; Agilent Technologies, Inc.) was used for method development and sample analysis. We ran the temperature program initially held at 35°C for 1 min, then ramped at a rate of 10°C/ min to 280°C, and held at the final temperature for 10 min. Nitrogen was used as carrier gas at a rate of 1.2 mL/ min. The temperature of the electron capture detector was set at 300°C, and the inlet temperatures of the GC were at 270°C, respectively. A 1μL aliqout of the sample extract or standard was injected in splitless mode by an autosampler.

Sample preparation. A 500 mL volume of sample is adjusted to pH 1.5 and cleaned and concentrated by strataX-AW cartridge (500 mg/6 mL, Phenomenex). The HAAs and phenols that have been eluted into the organic phase (methanol) are then converted to their methyl esters by the addition of pentafluorobenzil bromide (PFBBr) followed by slight heating. The acidic extract is neutralized by a back extraction with a saturated solution of sodium bicarbonate and the target analytes are identified and measured by capillary column gas chromatography using an electron capture detector (GC/ ECD). [3,5,6,9,10]

Recovery tests.The samples (500 ml) were spiked with phenols and HAAs in at levels of once, once and a half and twice time the LOQ of pesticide in drinking water (0.02, 0.03, and 0.04 µg/L), six replications of each point were done for the recovery tests of pesticide in drinking water.

RESULTS AND DISCUSSION

Method limit of quantification (LOQ). The results in Table 3 show that the LOQ of phenols and HAAs is less than the MRL of National technical regulation on drinking water quality (QCVN:01-2009/BYT)

Table 3. Method limit of quantification (LOQ)

No	Items	Group	LOQ, µg/L	%R (n=12)	RSDr (n=6)	RSD_R (n=12)	MRL, µg/L
1	2,4,6 Triclorophenol	Phenol	0.02	87.00	8.37	12.76	1
2	Pentachlorophenol	Phenol	0.02	103.00	4.00	6.49	2
3	Acid dicloroacetic	HAA	0.02	88.00	5.86	5.77	20
4	Acid tricloroacetic	HAA	0.02	82.00	1.25	13.94	0.03

Table 4. Precision of method

No	Items	C1, µg/L		C2, µg/L		C3, µg/L		RSD_R
		%R1	RSD_{r1}	%R2	RSD_{r2}	%R3	RSD_{r3}	
		(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=18)
1	2,4,6 Triclorophenol	73	5.72	74	5.6	102	5.4	5.59
2	Pentachlorophenol	74	4.47	76	4.38	76	4.3	4.37
3	Acid dicloroacetic	80	9.56	84	9.37	78	9.1	9.35
4	Acid tricloroacetic	87	13.63	88	13.36	114	13.0	13.33

The precision of method. The reproducibility was performed on three consecutive days.

Pesticide was added to drinking water samples at levels of once, once and a half, twice of LOQ (C1, C2, C3) and each sample was analyzed, based on the described procedure to revaluate the precision of the analytical method. The results are shown in Table 4. The recovery was measured by comparing peak areas of the spiked samples with those of the related the standard calibration curve. The mean recoveries of 18 replicates ranged of 73% to 114% with relative standard deviations (RSD_r) of 4.3% to 13.6%. The producibility (expressed as RSD_R) ranged of 4.37% to 13.33%.

4. REAL SAMPLE ANALYSIS.

The method was later applied to determine the pesticide residue and by-product disinfection levels in ten samples collected from the raw source water in Dalat city, Vietnam. The extraction of each products was performed in triplicate (n= 3). None of analyte was detected in real sample from the results of GC-μECD and HPLC/DAD analyses.

5. CONCLUSIONS

Based on US EPA method, the method validation was carried out on drinking water matrices following SANTE/11945/2015 of the European Commission. In addition, this method is simple inexpensive, efficient, and has the important advantage that it requires only small volume of solvent. 21 compounds had acceptable recoveries (71 –120%) with good repeatability (RSD < 20%) and the method minimum detection qualification LOQ of compounds ranged from 0.02 –0.2 μg/L and was lower than the maximum residue levels (MRL) of National technical regulation on drinking water quality (QCVN:01-2009/BYT). This method is applicable to the determination of the following analytes in finished drinking water, drinking water during intermediate stages of treatment, and raw source water.

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XÁC ĐỊNH DƯ LƯỢNG HÓA CHẤT BẢO VỆ THỰC VẬT VÀ SẢN PHẨM PHỤ TRONG NƯỚC SINH HOẠT BẰNG KỸ THUẬT SẮC KÝ.

HUỲNH THÁI KIM NGÂN, PHẠM HỮU ANH

Trung tâm Ứng dụng kỹ thuật hạt nhân trong công nghiệp, Viện Năng lượng nguyên tử Việt Nam,
Số 01-Đường ĐT723, Phường 12, Đà Lạt, Lâm Đồng.
Email: nganhtk@canti.vn; kimngan270979@gmail.com

Tóm tắt: Nước là một nguồn tài nguyên quan trọng cho cuộc sống. Giải quyết các vấn đề về chất lượng nước uống và và quản lý nguồn cung cấp nước an toàn đòi hỏi sự hiểu biết và đánh giá kỹ lưỡng về tất cả các yếu tố ảnh hưởng đến thành phần của nó. Hóa chất bảo vệ thực vật (HCBVTV) được sử dụng rộng rãi để kiểm soát sâu bệnh và tiêu diệt cỏ dại, vì vậy chúng là những chất gây ô nhiễm phổ biến trong môi trường của chúng ta. Dư lượng HCBVTV có thể được xác định bằng cách sử dụng các phương pháp hóa học khác nhau trong đó phổ biến nhất là sắc ký khí hoặc sắc ký lỏng. Bài báo đưa ra phương pháp xác định các hóa chất bảo vệ thực vật và sản phẩm phụ trong nước sinh hoạt bằng phương pháp chiết pha rắn kết hợp phương pháp sắc ký. Thẩm định phương pháp được thực hiện trên nền mẫu nước sinh hoạt theo hướng dẫn của SANTE/11813/2017. Tất cả các chất xác định trong phương pháp có LOQ 0.02 –0.2µg/L nhỏ hơn giá trị MRL quy định của QCVN 01/2009-BYT, hiệu suất thu hồi trong khoảng 71-120% với độ lặp lại RSD <20%.

Từ khóa: *SANTE, RSD, MRL, LOQ, QCVN*