

Residues Analysis and Dissipation Kinetics of Three Herbicides; Mesotrione, Ametryn and MCPA-Na in Maize and Soil Using LC-MS/MS

Pang K and Jiye Hu*

Department of Chemistry and Biological Engineering, University of Science Technology Beijing, Beijing, 100083, PR China

*Corresponding author: Jiye Hu, Department of Chemistry and Biological Engineering, University of Science Technology Beijing, Beijing, 100083, PR China, Tel: +86 82376002; Fax: +86 1082376002; E-mail: jyhu@ustb.edu.cn

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Abstract

A liquid chromatography tandem mass spectrometry method with modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation was developed for the simultaneous determination of Mesotrione, Ametryn, and MCPA-Na. The mean recoveries were ranged from 72% to 102% ($R^2 < 14.4\%$). A study of dissipation kinetics conducted under open field conditions at two sites during 2016. The half-lives 0.7-14.7 d for mesotrione, 1.2-16.1 d for ametryn and 1.1-3.2 d for MCPA-Na. The terminal residues of three pesticides in maize grain were lower than the maximum residue limits (MRLs) set by China (0.01 mg/kg for mesotrione, 0.05 mg/kg for MCPA-Na) and US (0.05 mg/kg for ametryn) at harvest time as well as at pre-harvest (after apply 96 d and 71 d).

Additional the chronic dietary risk was evaluated using risk quotients method based on Chinese dietary pattern. That were significantly lower than RQ (risk quotient)=1. On the basis of supervised field trial data and dietary exposure risk assessment results, the MRLs values of ametryn were recommended as 0.05 mg/kg for maize grain and maize plant in China, respectively.

These results not only gave insights about the analytes but also contributed to environmental protection and food safety.

Keywords: Chromatography; Spectrometry; Kinetics

Introduction

The herbicide mesotrione (Figure 1) is a triketone herbicide that was first reported by Syngenta in 2001. It was widely used to pre-emergence and post-emergence control of annual broadleaved weeds in field maize [1]. It have low octanol-water partition coefficients ($\log K_{ow} < 0.11$) showing that is relatively hydrophilic and highly solubility (15 g/L at pH 7.20°C). In subchronic and chronic oral studies, ocular lesions, liver and kidney effects, and/or body weight decrements were the major adverse effects seen in rat, mouse, and dog. These results show that the determination of mesotrione in food and environment ecosystem is very important, so that several researchers attention to determination of the mesotrione and its metabolites in non-edible and/or edible matrices [2,3].

Ametryn (Figure 1) is a selective herbicide belonging to the triazine family and widely used for killing annual grasses or weeds [4]. Triazine derivatives (atrazine, terbuthylazine, ametryne, and terbuthryne) are considered one of the most important classes of chemical pollutants due to their toxicity and high resistance [3], genotoxic activity and few hematological alterations [5]. Although ametryn could quickly degrade by newly bacteria; JW-1 [6] but 0.05 mg/kg and 19.9-32.1 ng/kg of ametryn residues were determined at maize, sugarcane crops and shellfish [7], respectively.

MCPA-Na (Figure 1) belonging to phenoxy-hydroxy-acid family herbicide that was widely used to cornmaizewheat fields and so on. Although phenoxyherbicides are relatively slightly toxic LD50 value for MCPA is 700 mg/kg of body weight of rat males [8], their ubiquitous

distribution and prolonged exposure may impose a substantial health risk on subjects living or working in rural areas. So it is important to determination of MCPA-Na in ecosystem. It was reported that the method of determination for MCPA [9], but it was not found that the method of directly determination of MCPA-Na in maize grain, maize plants and soil.

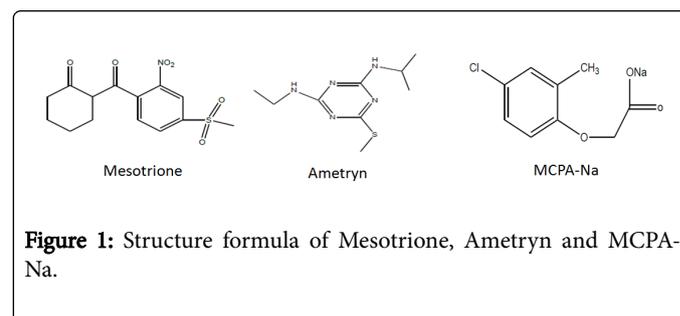


Figure 1: Structure formula of Mesotrione, Ametryn and MCPA-Na.

Currently QuEChERS [10] for sample preparation and coupled with gas chromatography-tandem quadrupole (GC-MS/MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) is widely using to determination for multi-residues of pesticide and/or its metabolism [11].

This study mainly aimed at (1): to develop the method for simultaneous determination of mesotrione, ametryn, and MCPA-Na in soil, maize grain, and maize plant, using the QuEChERS sample preparation method with rapid resolution liquid chromatography electro-spray ionization tandem mass spectrometry (RRLC-ESI-MS/MS), (2): Investigating dissipation behaviours and residue distributions

of the three pesticides under the open field conditions using the established method, and (3): Assessing dietary risk probability of these pesticides in maize grain based on field trial data and relevant toxicological parameters, (4): to recommend the suitable MRL value of ametryn in maize grain and maize plant in China, and also providing scientific information for proper application of these herbicides in maize field ecosystem.

Materials and Methods

Reagents and chemicals

Three standards were from commercial sources as available: mesotrione (97.8%) from Fluka of Sigma-Aldrich Co.Ltd., Ametryn (97.9%) from Shanghai Pesticide Research Institute Co.Ltd. (Shanghai, China), and MCPA-Na (99.2%) from Beijing QinChenYiXin Co. Ltd. (Beijing, China). Acetonitrile and formic acid of LC grade were purchased from Fisher Scientific (Far Lawn, New Jersey). Sodium chloride (NaCl), anhydrous magnesium sulfate (MgSO₄), and acetic acid of analytical grade were purchased from Beijing Chemical Reagents Company (Beijing, China). C18 (50 μ m, 60 \AA), MWCNT (SSA>200 m², NO:XFM13) were purchased from Agela Technologies (Tianjing, China).

Field trials

The field trials including the dissipation and residue experiments were carried out at two sites (site A of Beijing: 116.46E, 39.92N and site B of Shandong province: 120.42E, 36.58N) in China in the year 2016 according to NY/T 788-2004 issued by the Ministry of Agriculture, P. R. China. The properties of site A were as follows: organic matters of 2.7%, pH 6.73 and cation exchange capacity of 29.7 cmol/kg and one of site B were as follows: organic matters of 3.9%, pH 7.32 and cation exchange capacity of 16.7 cmol/kg. During the entire experimental period, the average temperature of site A and site B were 28°C and 25°C, the average rainfall were amounted 240-400 mm and 200-380 mm, respectively.

To investigate the dissipation and residues of mesotrione, ametryn and MCPA-Na in maize plant and soil, the WDGs of 60% mesotrione-ametryn-MCPA-Na (10% mesotrione, 40% ametryn and 10% MCPA-Na) was sprayed on the surface of maize and the bare soil at a dose of 1080 g a.i./ha (grams active ingredient/hectare, 1.5 times the recommended dosage). Each experiment plot was 30 m² and a buffer zone was used to separate between the plots of different treatments. Maize plant and soil samples were collected randomly from three replicate plots on 2 h, 1, 3, 5, 7, 10, 14, 21, and 30 d after application.

To investigate the terminal residues of mesotrione, ametryn and MCPA-Na, a low-dose level of 1080 g a.i./ha (recommended high dosage) and a high-dose level of 1620 g a.i./ha (1.5 times the recommended high dosage) were applied to the final residual experimental plots by using a portable sprayer for one time. Maize plant, maize grain, and soil samples were collected after application at 71 d and 96 d (pre-harvest 25 d and 0 d). All the collected samples were stored in dark at -20°C for further analysis.

Sample preparation by QuEChERS

The soil (5 g), maize grains (5 g), and maize plants (2 g) samples were weighed into a 50 mL PTFE centrifuge tube, separately. Then, 5% (v:v) acetic acid and acetonitrile 10 mL and 5 mL water was added and

shaking vigorously for 1 min, and this was extracted for 15 min at 30°C air bath with shaken and then anhydrous NaCl (1 g) and anhydrous MgSO₄ (2 g) were added and vortexed vigorously for 1 min. After centrifugation at 4000 rotation per minute for 3 min, 1 mL of the supernatant was transferred into 5.0 mL centrifuge tube that was containing 100 mg MgSO₄ and 60 mg C18 for soil, 150 mg MgSO₄, 60 mg C18 and 5 mg MWCNT for maize plants and maize grains. The extract was filtered with 0.22 μ m nylon membrane filters for RRLC-ESI-MS/MS analysis.

Apparatus and conditions

All experiment were performed with Agilent 1260 infinity RRLC system (Agilent Technologies, CA, USA) with a Agilent Poroshell 120 EC-C18 (50 mm \times 3.0 mm i.d., 2.7 μ m). The RRLC system was coupled on-line to Agilent 6420 triple quadrupole mass spectrometer (Agilent Technologies) equipped with an electro-spray ionization source. Three compounds were separated with a mobile phase consisting of 0.2% formic acid and acetonitrile. The injection volume for all samples was 5 μ L and the column temperature was 30°C. The MS operating conditions were optimized as positive and negative electro-spray voltage 4000 V, nebulizer pressure 35 psi, drying gas (N₂) flow rate 10.0 L/min, and dry gas temperature 325°C. MS experiments were performed in multi-reaction monitoring (MRM) mode using optimized Q1/Q3 (m/z) values for the particular compound (Table 1).

Dietary exposure risk assessment

For the safe application of mesotrione, ametryn and MCPA-Na, dietary exposure risk assessment was studied on the basis of risk quotients (RQ) method. The chronic dietary exposure risk assessment was estimated by calculating RQc as follows functions (1), (2) (You et al. 2017).

$$\text{NEDI} = \sum(\text{Fi} \times \text{SMTRi}) \quad (1)$$

$$\text{RQc} = \text{NEDI} / (\text{ADI} \times \text{bw}) \quad (2)$$

Where i is the i-th food index of all foods used for chronic dietary exposure risk assessment, NEDI (mg/kg bw) is the international estimated daily intake, Fi (kg) is the reference intake of i-th food, bw (kg) is the average body weight, SMTRi is supervised trials median residue for Fi and ADI (mg/kg bw) is the acceptable daily intake (Table S1).

Results and Discussion

Optimization of separate conditions

For mesotrione, ametryn and MCPA-Na, negative and positive ion modes were compared and negative ion mode for mesotrione and MCPA-Na, positive ion mode for ametryn were more sensitive. Different proportion of mobile phase (75:25, 50:50, 25:75 of acetonitrile and 20 mmol ammonium acetate water (v:v)) were compared and the proportion of mobile phase 25:75 (v:v) of 20 mmol ammonium acetate water (v:v), since under the this condition not only have a good effective separation and peak style but also more earlier retention time, the retention time were 0.663 min for mesotrione, 0.839 min for ametryn and 1.307 min for MCPA-Na, respectively (Figure 2).

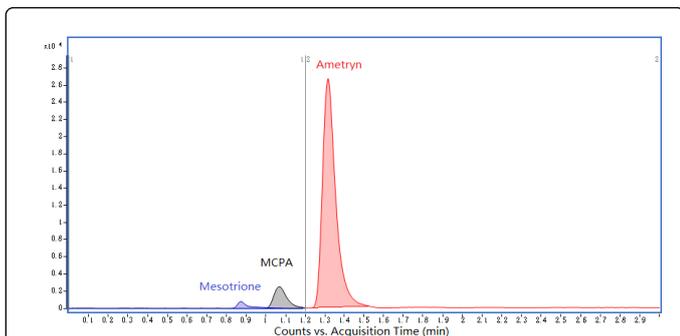


Figure 2: Liquid Chromatograph of the mesotrione, ametryn and MCPA-Na.

Recovery test

The fortified levels were chosen as 0.1, 1.0 and 2.0 mg/kg for spiked soil matrix, and 0.01, 0.1 and 1.0 mg/kg for spiked maize grain, respectively. All of the tests were carried out with five replicates. Table 1 showed the detailed recoveries data for all three herbicides in three matrices. The recoveries for three herbicides were in the range of 72%-105% (80%-102% for soil, 72%-104% for maize grain, and 78%-105% for maize plant). RSDs were below 14.4% for all cases (4.7%-14.4% for spiked soil, 5.0%-7.0% for spiked maize grain, and 7.6%-11.9% for spiked maize plant). The results of the recovery test show that this method conformed to analysis for pesticide with desirable recovery and accuracy in soil, maize grain and maize plant samples.

Matrix	Addition Concentration (mg/kg)	Mesotrione		Ametryn		MCPA-Na		LOQs (mg/kg)
		Recoveries (%)	RSDs (%)	Recoveries (%)	RSDs (%)	Recoveries (%)	RSDs (%)	
Soil	0.1	88	4.7	94	2.3	86	2.1	0.1
	1.0	95	14.4	102	3.4	90	4.9	
	2.0	80	10.3	84	11.8	101	8.2	
Maize grain	0.01	72	6.4	84	4.7	77	3.0	0.01
	0.1	94	7.0	100	4.1	104	1.6	
	1.0	103	5.0	98	2.0	100	2.9	
Maize plant	0.1	86	10.4	87	4.4	78	3.1	0.1
	0.5	11	11.6	105	4.1	103	3.9	
	1.0	95	11.9	91	5.7	93	5.8	
	2.0	86	7.6	88	4.7	94	1.6	

Table 1: Recoveries, RSDs and LOQs of three herbicides (mesotrione, ametryn and MCPA-Na) in soil, maize grain and maize plant.

Quantitation and matrix effects

Quantification in this study was performed with matrix-matched standards (Figure S1-S3). The correlation coefficient (R²) of matrix-matched calibration was higher than 0.98 in the range of 0.02-2 mg/kg for all matrix, indicating that a linearity was obtained (Table S2). The LODs were from 0.57 to 2.7 µg/L for mesotrione, from 0.37 to 3.3 µg/L for ametryn and from 0.37 to 0.95 µg/L for MCPA-Na, respectively. The LOQs in soil maize grain and maize plant were 0.1, 0.01 and 0.1 mg/kg for three herbicides, respectively. The results indicated that the established method was sensitive and accurate enough for determination of the three analytes. All of the RRLC-ESI-MS/MS MRM chromatogram of mesotrione, ametryn, and MCPA-Na in soil matrix, maize grain matrix and maize plant matrix were shown at Figures S4-S6.

Dissipation of three herbicides in different ecosystems under field conditions

The initial residues of mesotrione were 0.13 mg/kg and 1.88 mg/kg in soil of site A and site B, and 3.80 mg/kg and 5.26 mg/kg in maize plant of two sites, respectively. The corresponding the half-lives of

mesotrione were 14.7 d in soil of site B and 0.7 d and 1.3 d in maize plant of two sites (Table S3). After apply herbicides, the residues of mesotrione were below the LOQs at about 5 d and 7 d in maize plant, and 1 d and 30 d in soil of site A and site B, respectively.

The initial residues of ametryn were 0.54 mg/kg and 9.66 mg/kg in soil of site A and site B, and 39.1 mg/kg and 33.0 mg/kg in maize plant of two sites, respectively. And the corresponding the half-lives of ametryn were 12.1 d and 16.1 d in soil of site A and site B, and 2.6 d and 1.2 d in maize plant of two sites (Table 2). The amounts 90% of initial concentration of ametryn in maize plant and soil of site A was dissipated at 10 d and 21 d, and site B was 5 d and 30 d, respectively. The residues of ametryn were below the LOQs at about 21 d and 14 d in maize plant of site A and site B, and 21 d in soil of site A, respectively.

The initial residues of MCPA-Na were 0.13 mg/kg and 2.05 mg/kg in soil of site A and site B, and 20.68 mg/kg and 5.21 mg/kg in maize plant of two sites, respectively. And the half-lives of MCPA-Na were 3.2 d in soil of site B, and 2.5 d and 1.1 d in maize plant of two sites, respectively. This result was close to 7 d in soil, and 0.48 - 6.74 d in wheat plant [12].

Compound	Matrix	Site	Dissipation kinetics	R ²	Half-lives
					(d)
Mesotrione	Soil	A	-	-	-
		B	C = 0.7285e -0.047t	0.8435	14.7
	Maize plant	A	C = 3.0148e -0.531t	0.9737	1.3
		B	C = 2.5746e -0.974t	0.9453	0.7
Ametryn	Soil	A	-	-	-
		B	C = 1.039e -0.217t	0.9425	3.2
	Maize plant	A	C = 5.4622e -0.274t	0.9037	2.5
		B	C = 3.306e -0.619t	0.9904	1.1
MCPA-Na	Soil	A	C = 0.4226e -0.057t	0.8636	12.2
		B	C = 7.3623e -0.043t	0.9096	16.1
	Maize plant	A	C = 35.531e -0.269t	0.986	2.6
		B	C = 30.389e -0.568t	0.9991	1.2

Table 2: Dissipation kinetics and half-lives of the mesotrione, ametryn and MCPA-Na in different ecosystems under field conditions.

Terminal residues of three herbicides in different ecosystems

The MRLs of mesotrione and ametryn were 0.01 mg/kg, and 0.05 mg/kg in maize set by China (2014 year), respectively. And for MCPA-Na, the MRLs was 0.05 mg/kg in maize set by US. In this works, the terminal residues of three herbicides were below LOQs at pre-harvest 25 d and at harvest (after apply 71 d and 96 d) maize grain, maize plant and soil samples [13-15]. The result shown that it was safe for maize consumption not only at the harvest with an interval of 96 d after apply but also 71 d after apply according to the recommended dosages. All of the RRLC-ESI-MS/MS MRM chromatograms of mesotrione, ametryn, and MCPA-Na in soil samples, maize grain samples and maize plant samples at the pre-harvest 25 d and at the harvest were shown at Figures S7-S12.

Chronic dietary exposure risk assessment and recommend MRL for ametryn

Chronic dietary exposure risk assessments of specific pesticides need to be conducted for all registered crops that are subject to risk assessment (IPCS, 2009). The registered crops of mesotrione, ametryn and MCPA-Na in China and their corresponding MRLs recommended by different countries or international organizations were listed in Table S3. The MRLs of three pesticides was used as STMR for calculation. NEDI values to perform dietary exposure risk assessments.

The RQc value was calculated by the equations (1,2), and the results are showed at Table S4. Where the ADI value for mesotrione, ametryn

and MCPA-Na were 0.01, 0.01 and 0.1 mg/(kg bw). The RQc values were 0.026 for mesotrione, 0.104 for ametryn and 0.003 for MCPA-Na that were significantly lower than RQ=1. The result show that the risk of mesotrione, ametryn, MCPA-Na for maize at the recommend dosage was negligible to humans within scope of ADI.

There are various registration data for ametryn in several vegetables and countries, for example, MRLs values in maize was 0.05 mg/kg by registered US and Japan. And our field trial data was also shown that all of the final residues were lower than LOQ (0.01 mg/kg) at two sites (Beijing and Shandong) [16,17]. Hence, the MRLs of ametryn were recommended as 0.05 mg/kg for maize grain and maize plant to Chinese consumers.

Conclusion

In this work, a simple and rapid analytical method based on QuEChERS methodology with LC-MS/MS for simultaneous determination of mesotrione, ametryn and MCPA-Na in soil, maize grain and maize plant was developed and validated. By changing the amount each of Florisil, PSA and C18, in the QuEChERS method, three herbicides (mesotrione, ametryn and MCPA-Na) could be quantified in different matrices, including soil, maize grain, and maize plant. The method met the requirement of analysis for all selected analytes with satisfactory recoveries in the range of 72%-104% as well as RSDs lower than 14.4%. Dissipation study and terminal assay were also conducted by this method at two sites in the year of 2016 with WDGs. In different matrix, the half-lives of mesotrione, ametryn and MCPA-Na were ranged from 0.6 d to 14.7 d, from 1.2 d to 16.1 d and from 1.1 d to 3.2 d, respectively. Moreover, the result of the dietary exposure risk assessment for mesotrione, ametryn and MCPA-Na in China was indicated that the RQc value for three pesticides were all below 1 (RQc were 0.03 for mesotrione, 0.056 for ametryn and 0.414 for MCPA-Na). This result implying that the dietary exposure risk of mesotrione, ametryn and MCPA-Na used in maize grain were acceptable to Chinese consumers. Meanwhile, MRLs values of ametryn were proposed as 0.05 mg/kg for maize grain and maize plant based on supervised field trial data and dietary exposure risk assessment results.

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