Determination of Selected Organophosphorus Insecticides and Their Oxides in Tea and Soil by HPTLC

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Abstract. A determination method of selected organophosphorus insecticides and their oxides (Parathion-methyl and Dimethyl-paraoxon; Parathion and Paraoxon; Chlorpyrifos and Chlorpyrifos-oxon.) in tea and soil was developed with high performance thin-layer chromatography (HPTLC). The acetonitrile was applied to extract tea sample and the tandem column of solid-phase extraction was to clean samples up. The soil sample was extracted with ethyl acetate and not to be clean-up. The processed samples were directly applied as bands to glass-backed silica gel 60F254 HPTLC plates. The plates were developed by AMD with 7-step for tea samples and 2-step for soil samples. Evaluation of the developed HPTLC plates was performed densitometrically. Three fortification levels of the samples were conducted in the test. In this method, the detection limits of parathion-methyl, dimethyl-paraoxon, parathion, paraoxon and chlorpyrifos-oxon were from 3.0×10⁻⁹ g to 1.0×10⁻⁸ g in different development systems. The chlorpyrifos was from 7.0×10⁻⁹ g to 2.0×10⁻⁸ g. Recoveries of the pesticides residues from tea were 68.98-116.73%, and the soil were 69.44 –120.00 % except the chlorpyrifos. The relative standard deviations were 4.02–17.50 % for tea, and 3.11–14.20 % for soil except the chlorpyrifos. The \( R_f \) value and the validation data were given. The development by AMD in this method was discussed.

Keywords: HPTLC; AMD; Organophosphorus insecticides; Determination.

1. Introduction

Tea is a popular beverage rich in many components that are beneficial to human health. It also can contain the residues of pesticide used as pest control methods to protect tea tree from the insect or the phytopathogen. The pesticide residues in tea are not only from the plants but also from the soil where the plants grow up in [1-2]. An important task of the administration is to determine the pesticide residues in tea and in soil. Many studies reported on the organophosphorus residues detection in tea garden or other matrix by GC [2-13] or HPLC [14-19] focused on the parental pesticides in recent years. The pesticides, however, may degrade through oxidative mechanisms in environment. Not all the organophosphorus is more toxic than their oxides, such as parathion and paraoxon[20]. Methods only detecting the parental organophosphorus compounds usually missed to detect their oxides. Moreover, tea is a plant with complex matrix that usually causes interference in the determination process. It is a tough work to process tea sample contained organophosphorus residues to be satisfactory for detection by GC or HPLC. They are generally requested expensive instruments, work-hard preprocesses, and long-time determination. It makes the determinations not be used commonly. The routine task usually requests the methods fast, low cost, and easy operation. High performance thin-layer chromatography (HPTLC) as efficient tool for pollutants evaluation in environment has the advantages above. Studies on analysis of the parental organophosphorus compounds residues in the
foods, plants or the tissue of human by HPTLC have been reported [21-25]. It is seldom reported the residues of organophosphorus and their oxides in the tea garden determined by HPTLC. AMD system designed for automated multiple development in HPTLC offers improved precision and reliability. The methods by HPTLC with AMD are not affected by environmental factors greatly during the development procedure. This work tried to explore a method to determine the selected organophosphorus insecticides and their oxides in tea and soil by HPTLC with AMD.

2. Material and Methods

2.1. Regents and Apparatus
Chemicals used were of analytical grade. Standards of Parathion-methyl (99.0%), Dimethyl-paraoxon (98.0%), Parathion (98.0%), Paraoxon (99.0%), Chlorpyrifos (99.0%), Chlorpyrifos-oxon (98.0%), were purchase from Dr. Ehrenstorfer Gmbh – Bgm (Germany). High performance thin-layer chromatography (HPTLC) system contains silica gel 60 F254 plates (20×10 cm, 0.2 mm layer thickness, MN, Germany), TLC Scanner 3 linked to winCATS software (CAMAG) for densitometer, the Linomat 4 sample band applicator (CAMAG, Muttenz, Switzerland) for application, 100 μL syringe (Hamilton, Bonaduz, Switzerland), glass twin-trough chamber (20 ×10 ×4 cm; CAMAG), and AMD instrument (AMD 2, CAMAG). Supelclean ENVI-Carb (6 mL) and ENVI-FLORISIL (3mL) (Supelco, USA) were constructed Solid-phase extraction (SPE) columns.

2.2. Materials
The tea and the soil were collected from the tea garden in Anhui agricultural university, Hefei, China. The tea samples were stored at 4℃ in air-tight containers and abraded to 40 mesh when prepared the sample. The soil was ground to 40mesh after dry in the air.

2.3. Method

2.3.1. Standard Solutions. Dissolving 5.0 mg standard chemicals in acetonitrile in a 5 mL volumetric flask prepared the standard solutions. Then, stepwise dilution with the same solvent yielded solutions containing 1 ×10−5 and 1× 10−6 g·mL−1. The mixed standard solution was combined by proper volumes of each solution when required.

2.3.2. AMD Development. The plates were developed for 7 steps with the mobile phase systems using AMD for tea samples solutions and 2 steps for soil samples solutions at 25±4˚C and 60±20 % relative humidity for a migration of 4.5-5.5 cm. (Table 1)

| Component | AMD system for tea sample | AMD system for soil sample |
|-----------|---------------------------|---------------------------|
| Steps     | Migration (mm) | A | B | C | D | Migration (mm) | B | D |
| 1         | 25 | 85 | 15 | 15 | 25 | 85 | 15 |
| 2         | 32 | 10 | 10 | 10 | 50 | 10 | 90 |
| 3         | 39 | 10 | 10 | 10 | 80 | 10 | 90 |
| 4         | 45 | 10 | 10 | 90 | 90 |     |    |
| 5         | 48 | 10 | 90 |    |    |    |    |
| 6         | 51 | 10 | 90 |    |    |    |    |
| 7         | 60 | 10 |    |    |    |    |    |

A = methanol, B = t-butyl methyl ether, C = dichloromethane, D = n-hexane; all proportions are percentages.

2.3.3. Preparation of Sample Solutions. 2.5 g prepared tea were weighted and were fortified to furnish tea containing 0.2, 1, and 2.0 mg·kg⁻¹ mixed standards. Then, ultrasonic vibrated the samples for 1 min and balanced them for 4 h. After that, added 30 mL acetonitrile into the samples and trembles for 1 h. All samples were filtered before concentrated to 1 mL. The SPE tandem column (an ENVI-CARB column followed by a ENVI-FLORISIL column) were pre-treated by rinsing with 5 mL hexan–actone (1 + 1, v/v). Before 4 mL hexan–actone (1 + 1, v/v) was injected into the column, the extract was
applied to through the column. Collected the eluate and evaporated it to 1.0 mL under a stream of nitrogen gas at room temperature.

The soil samples was prepared by adding the mixed standard solution into 5.0 g of the dried soil to achieve sample containing 0.1, 0.5, and 1.0 mg·kg⁻¹ mixed standards. The balanced procession was same as the tea samples. The samples were shaken in an ultrasonic apparatus for 10min after adding 30, 20, 20 mL ethyl acetate. Then, they were filtered and the filtrate was combined and concentrated to 1 mL.

2.3.4. Validation Procedures. Each standard solution were spotted on an HPTLC plate. The plate was developed, dried in air and scanned at 320nm for Chlorpyrifos-oxon and 290nm for the other five substances. The validation of the analytical procedure was followed according to the International Conference on Harmonization (ICH) guidelines (CPMP/ICH/381/95; CPMP/ICH/281/95). The method was validated for precision, repeatability, and accuracy. Accuracy of the methods were tested by performing recovery studies. For the determination of limit of detection (LOD) and limit of quantitation (LOQ), different dilutions of the standard solutions were applied along with acetonitrile as the blank and calculated on the basis of signal-to-noise ratio.

3. Results and Discussion

3.1. Development by AMD

The chromatogram map of the standards developed by AMD system was seen in the figure1. The selected organophosphorus pesticides were developed by AMD with 7 steps for tea samples (figure2) and the 2 steps for soil samples (figure3). The values of the Rf and the maximum absorb waves of the organophosphoruses in the systemes were given in table 2. The target substances were not separated only by one-step both in the two development systems although many mobile phase systems with different base solvents were used in this study. It is considered that the organophosphoruses were greatly difference from their oxides in the polarity.

![Figure 1. The chromatogram of standards for 2-step in AMD system. (80ng/spot)](image)
Figure 2. The chromatogram of tea samples in AMD development system (1 mg·kg\(^{-1}\) spiked level).
b. blank

Figure 3. The chromatogram of soil samples in AMD development system (1 mg·kg spiked level).

The six organophosphoruses were also separated successfully by an ascending development with 2 steps in the two-trough chamber (Table 2). This development method can be used to determine the targets in the soil. The LOD and the LOQ in the ascending development were lower than that in the AMD system with 7-steps but higher than that in AMD development system with 2-steps (Table 2). This development system, however, were affected by the environmental factors, such as the humidity and the temperature, greatly. The repeatability of \( R_f \) values was not satisfied. The repeatability in AMD system was better than in ascending system.

The background noise of the stationary fluctuated after the 7-step development in the AMD system. It influenced the detection limits in the AMD system. However, the target substances were not separated from the impurities in the tea using 2-steps development whether by the AMD or by the ascending development (figure 2). There are two base solvents in the AMD mobile phase system for 7-step, e.g. the dichloromethane and the t-butyl methyl ether (Table 1). The dichloromethane was suit to separate the parathion from the impurity numbered 7 in the figure 2. The t-butyl methyl ether was to separate the parathion-methyl from the impurity 7. It is found in this system that more development steps cannot help the separation of the targets insecticides and chemicals in tea as the impurities while the migration is good for the separation. But long distance migrated for a long time and the mobile phase solvents were exhausted if the migration was over the 7.0 cm in our test. The separation of the soil sample has good results by AMD system with 2 steps and one base solvent of t-butyl methyl ether (figure 3).

Table 2. The values of the Rf and the validation parameters for the quantification of the pesticides.

| Substances                   | Maximum Wavelength (nm) | AMD system | Ascending system a |
|------------------------------|-------------------------|------------|--------------------|
| Dimethyl-paraoxon paraaxon   | 286                     | 0.1 10 30  | 0.1 3 10 0.1 5 15 |
| Chlorpyrifos- oxon           | 320                     | 0.4 15 45  | 0.4 5 15 0.3 10 30 |
| Parathion- methyl            | 290                     | 0.7 10 30  | 0.5 3 10 0.5 5 15 |
| Parathion                    | 292                     | 0.8 10 30  | 0.6 3 10 0.6 5 15 |
| Chlorpyrifos                 | 287                     | 0.9 20 60  | 0.8 7 20 0.8 15 45 |

| For tea samples (7-steps) Rf | LOD (ng) | LOQ (ng) | Rf | LOD (ng) | LOQ (ng) |
|------------------------------|----------|----------|----|----------|----------|
| For soil samples (2-steps) Rf | LOD (ng) | LOQ (ng) |    |          |          |
|------------------------------|----------|----------|----|----------|----------|
|------------------------------|----------|----------|----|----------|----------|

| a) Mobile phase 1: t-butyl methyl ether / n-hexane=85/15 (v/v), migration= 2.5cm for first step. Mobile phase 2: t-butyl methyl ether / n-hexane=10/90 (v/v), migration= 6cm for second step. |
| b) LOD= limit of detection |
| c) LOQ= limit of quantitation |
| d) Rf: specific retardation factors |
3.2. Validation of the Sample Analysis

The values of $R_f$, and method validation data were shown in table 2 and the calibration data investigated from AMD with 2-step were given in table 3. The coefficient of variation (CV%) of Instrument precision was from 0.97% to 1.63%, it was from 1.03% to 1.71% for standard repeatability and from 1.29% to 1.97% for sample repeatability. Validation parameters were investigated by the analysis of tea sample fortified with 1 mg·kg$^{-1}$ of the organophosphates. The recoveries were 70.80-111.96% with the coefficient of variation of 3.48-15.89% for the intraday precision, and were 80.86-110.36% with the coefficient of variation of 5.21-13.67% for interday precision. The data indicated a good precision and repeatability of the method.

The data of accuracy validated by recoveries given in the Table 4 indicated that the average recovery of the tea samples is 68.98-116.73% with a high CV of 17.50%, and a low CV of 4.02% in the tea samples. It is 69.44–120.00 % with a high CV of 14.20%, and a low CV of 3.11 % in the soil samples except the chlorpyrifos. The validation data were satisfactory generally for the pesticides residues analysis in the tea and the soil.

### Table 3. Calibration data and the working range for the quantification of six pesticides by AMD with 2-step.

| Substances          | Regression equation $^a$ | $r$  | RSD $^b$ [%] | Working range [ng] |
|---------------------|--------------------------|------|--------------|---------------------|
| Dimethyl-paraoxon   | $Y=3.19+0.680X-0.000X^2$ | 0.99928 | 1.88 | 10-130 |
| paraoxon            | $Y=0.7021+0.924X-0.001X^2$ | 0.99834 | 2.70 | 10-130 |
| Chlorpyrifos- oxon  | $Y=6.631+0.41X-0.000X^2$ | 0.99746 | 3.00 | 15-130 |
| Parathion- methyl   | $Y=6.628+0.782X-0.001X^2$ | 0.99872 | 2.13 | 10-130 |
| Parathion           | $Y=-1.301+0.817X-0.000X^2$ | 0.99804 | 2.95 | 10-130 |
| Chlorpyrifos        | $Y=0.302+0.153X-0.000X^2$ | 0.98294 | 8.51 | 20-130 |

$^a$ $X$ = Amount of pesticide (ng), and $Y$ = peak area.
$^b$ RSD = Relative standard deviation.

### Table 4. The recoveries of the six organophosphates from the three levels fortifications.

| Substances          | Tea samples | Soil samples |
|---------------------|-------------|--------------|
|                     | Level [mg kg$^{-1}$] | Recoveries [%] | CV[%] | Level [mg kg$^{-1}$] | Recoveries [%] | CV[%] |
| Dimethyl-paraoxon   | 0.2         | 116.73      | 9.83 | 0.1         | 99.28        | 8.57  |
|                     | 1.0         | 85.819      | 12.26 | 0.5         | 69.44        | 6.21  |
|                     | 2.0         | 80.93       | 10.39 | 1.0         | 91.06        | 5.57  |
| paraoxon            | 0.2         | 111.90      | 11.79 | 0.1         | 89.63        | 10.21 |
|                     | 1.0         | 90.22       | 6.77  | 0.5         | 95.05        | 5.02  |
|                     | 2.0         | 81.79       | 9.12  | 1.0         | 71.56        | 3.11  |
| Chlorpyrifos- oxon  | 0.2         | 79.54       | 8.90  | 0.1         | 120.00       | 13.20 |
|                     | 1.0         | 80.60       | 10.28 | 0.5         | 119.13       | 9.81  |
|                     | 2.0         | 76.00       | 4.02  | 1.0         | 100.09       | 8.90  |
| Parathion- methyl   | 0.2         | 99.73       | 6.52  | 0.1         | 108.62       | 10.83 |
|                     | 1.0         | 110.35      | 9.83  | 0.5         | 86.46        | 5.47  |
|                     | 2.0         | 83.07       | 11.5  | 1.0         | 73.51        | 4.95  |
| Parathion           | 0.2         | 70.80       | 12.26 | 0.1         | 115.95       | 14.20 |
|                     | 1.0         | 88.49       | 5.21  | 0.5         | 101.15       | 6.98  |
|                     | 2.0         | 84.38       | 9.12  | 1.0         | 73.16        | 7.40  |
| Chlorpyrifos        | 0.2         | 68.98       | 17.50 | 0.1         | 45.00        | 18.39 |
|                     | 1.0         | 82.02       | 6.28  | 0.5         | 54.41        | 9.89  |
|                     | 2.0         | 92.10       | 6.79  | 1.0         | 54.01        | 9.16  |
4. Conclusions
1) The HPTLC analysis of the organophosphorus and their oxides residues in the tea and in the soil was studied in order to develop a fast, low cost, less quantity of the solvents and easy to operation method. The detection limits of parathion-methyl, dimethyl-paraoxon, parathion, paraoxon and chlorpyrifos-oxon were from $3 \times 10^{-9}$ g to $1.0 \times 10^{-8}$ g in different development systems. The chlorpyrifos was from $7 \times 10^{-9}$ g to $2.0 \times 10^{-8}$ g. Recoveries of the pesticides from tea were 68.98–116.73%, and the soil were 69.44 –120.00 % except the chlorpyrifos. The relative standard deviations were 4.02–17.50 % for tea, and 3.11–14.20 % for soil except the chlorpyrifos. All the validation data satisfied the routine work for the determination of selective pesticides.

2) To avoid the interference of factors in the development procedure and obtain a good repeatability in the test, two AMD systems with 7-step for tea samples and 2-step for soil samples were conducted. The results indicated both methods could be applied to quantitatively analyze the residues of three organophosphorous insecticides and its oxides in tea and soil.

3) Dichloromethane and t-butyl methyl ether were two base solvents used in the AMD system for tea sample to separate the targets from the impurity to realize less development steps in AMD system achieved good repeatability. t-Butyl methyl ether is also advantageous for the separation of targets homologue in the test.

References
[1] S. M. Waliszewski, O. Carvajal, S. Go´mez-Arroyo, O. Amador-Munoz, R. Villalobos-Pietrini, P. M. Hayward-Jones, R. Valencia-Quintana, Bull Environ. Contam. Toxicol., 81 (2008) 343
[2] A. Bishnu, K. Chakrabarti, A. Chakraborty, T. Saha, Environ. Monit. Assess. 149 (2009) 457
[3] A. Ozbey, U. Uygun, Food chemistry, 104(2007) 237.
[4] S. Moinfar, M. H. Hosseini, J. Hazardous materials, 169(2009) 907.
[5] H. R. Yoon, E. J. Lee, M.K. Park, J.H. Park, Chromatographia, 47(1998) 587.
[6] Z.Y. Lou, Z.M. Chen, F.G. Lou, F.B. Tang, G.M. Liu, Chinese J. Chromatogr., 26 (2008) 568. (In Chinese.)
[7] J.B. Dong, J.H. Wang, X.Y. Lu, Ch.Y. Xu, J.J. Yang, J. Beijing Univers. Chem. Tech., 34 (2008) 472. (In Chinese)
[8] C. López-Blanco, S. Gómez-Álvarez, M. Rey-Garrote, B. Cancho-Grande, J. Simal-Gándara, Anal. Bioanal. Chem., 383 (2005) 557.
[9] P.X. XU, D.X. Yuan, SH.M. Zhong, Q. M. Lin, Environ. Monit. Assess, 87(2003)155.
[10] Y. Picó, C. Kozmutza, Anal. Bioanal. Chem., 389 (2007)1805.
[11] I. Martinez Salvador, A. Garrido Frenich, F. J. Egea Gonzalez, J. L. Martinez Vidal, Chromatographia, 64 (2006)667.
[12] M. J. Gonzalez-Rodriguez, F. J. Arrebola Liebanas, A. Garrido Frenich, J. L. Martinez Vidal, F. J. Sanchez Lopez, Anal. Bioanal. Chem., 382 (2005) 164
[13] N. Manikandan, S. Seenivasan, M. N. K. Ganapathy, N. N. Muraleedharan, R. Selvasundaram, Food Chemistry, 113(2009)522.
[14] H. John, F. Worek, H. Thiermann, Anal. Bioanal. Chem., 391 (2008)97.
[15] Y. Lu, X. Sun, S.Y. Ji, J.F. Wang, Y.F. Zhao, P.X. Xu, Environ. Monit. Assess. 130 (2007)281.
[16] B.J. Bhadbhade, P.K. Dhakephalkar, S.S. Sarnaik, P.P. Kanekar, Biotechnology Letters 24(2002)647.
[17] P. Salm, P. J. Taylor, D. Roberts, J. de Silva, J. Chromatogr. B, 877(2009) 568.
[18] M.P. García de Llasera, M. L. Reyes-Reyes, Food Chemistry, 114(2009)1510.
[19] P. Calza, C. Massolino, E. Pelizzetti, Journal of Photochemistry and Photobiology A: Chemistry, 199(2008)42
[20] R.L. Mundy, M.C. Bowman, J.H. Farmer, T.J. Haley, Arch. Toxicol. 41(1978)111
[21] Y.D. Yue, R.Zhang, W. Fang, F. Tang, J. AOAC International, 91(2008) 1210.
[22] K. Futagami, Ch. Narazaki, Y. Kataoka, H. Shuto, R. Oishi J. Chromatogr. B. 704 (1997) 369.
[23] A. K. ski, Z. H. Kudzin, W. Ciesielski, J. Drabowicz, J. Chromatogr. A 831(1999)321
[24] M. Curini, A. Laganà, B. M. Petronio, M. V. Russo, Talanta, 27(1980)45.
[25] S. R. Sharma, R. P. Singh, S. R. Ahmed, Ecotoxic and Environ. Safety, 11(1986) 229