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VALIDATION OF NEW MULTIRESIDUE METHOD FOR PYRETHROID INSECTICIDES IN TIAN HUA FEN (*Radix trichos* Anthis)

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ABSTRACT

The analysis of several pyrethroid insecticides in the Chinese herbal medicine (CHM) Tian Hua Fen (*Radix trichos* Anthis) by multiresidue (ML) gas chromatography (GC) method is described. Insecticides were extracted from Tian Hua Fen (5g) by petroleum ether and cleaned up by natural aluminum oxide; purified extracts were analyzed by GC using electron capture detection (ECD). The extraction method has shown good recovery on various spike standard levels (1, 0.1 and 0.01 mg kg^{-1}). Pyrethroid insecticides are quantified using backed column gas chromatography. Good linearity ranges ($r>0.99$) were observed for all compounds. The average recoveries (over 82%), standard deviation, coefficient of variation (<16) and variances of recoveries were calculated for each analyte for each fortification level independently. Individual detection limits were in the range 0.0007–0.0028 mg kg^{-1}. Limits of quantification varied from 0.0022 to 0.0092 mg kg^{-1}. Method performance was characterized and validated according to (ISO 17025 and/or GLP). Selectivity, accuracy, precision, repeatability, reproducibility, limit of detection and limit of quantification were within acceptable ranges.

INTRODUCTION

Tian Hua Fen (*Radix trichos* Anthis) means "Heavenly Flower Powder" it is one of the Chinese Herbal Medicines (CHMs) that have been widely used to: 1. Clears heat and generates fluids (for irritability, dry lips, dry mouth, dry tongue, thirst, desire to drink cold beverages and cough with thick sputum or blood streaked sputum, for wasting and thirsting disorders) Dan Bensky (2004). 2. Dispels pus and resolves toxicity (for breast abscesses or carbuncles and sores with redness, swelling, pain and pus) Him Che. Yeung (1983). 3. Clears lung heat, transforms phlegm, moistens lung (for cough with thick, sticky sputum that is difficult to expectorate and signs of dryness) John K. Chen and Tina T. Chen (2003). Tian Hua Fen could be prepared in different formulas with dosage depends on the formulation type, Dan Bensky (1990), Him Che. Yeung (1983), Qiao Yi (2000). Tian Hua Fen is a traditional Chinese medicine which is gaining in popularity, like other CHMs. In China, there are more than 1,000 traditional manufacturers turning out 4,000 different products-about half the drugs China consumes, Lancet (2000). Herbal remedies with known mild pharmaceutical effects and minimum side effects have won respect and look promising, Max Wichtl (2004). To meet the demand of Tian Hua Fen in abroad, it is exported world wide, but the safety of the CHMs is generally unknown. The cultivation of CHMs usually requires the use of pesticides to reduce pest damage. Improper use of pesticides not only pollutes the cultivation soil, and ground water, but also leads to

accumulation of pesticides in the plants; pesticides may therefore be present in the CHMs. Recently some Chinese herbal medicine has been subjected to frequent quality control checks for pesticide residues used for the control of pests in Chinese herbal medicine Jalal *et al.* (2001). Selecting a proper method is a cornerstone in pesticide residue analysis, Chromatographic methods are the most suitable for pesticide residue analysis, D. Barcelo (1993), Th. Cairns and J. Sherma (1992), Z.A. Grosser *et al.* (1993), J. Tekel, and J. Kovacicova (1993). When the history of pesticide application in unknown, multiresidue (MR) method is the ideal choice, because it requires little sample treatment and minimizes reagent consumption, same time it could determine many analytes in a single step. The definition of the term multiresidue is being revised by IUPAC J. Vessman *et al.* (2001). Multiresidue method could be applied either by using High performance liquid chromatography (HPLC) or by using GC techniques. Since GC is the basic technique in modern multiresidue methods for analysis of pesticides, W. Specht *et al.* (1995), Y. Nakamura *et al.* (1994), Sanino *et al.* (1995), D.M. Hostege *et al.* (1991), PAM (1994), D.M. Holstege *et al.* (1994), S.J. Lehotay, and K.I. Eller (1995), a GC multiresidue method was adapted to determine a several pyrethroid insecticides in Tian Hua Fen. To date, many multiresidue analytic methods have been reported; some of them require sophisticated and expensive instruments for extraction, cleanup or even for detection, H. Obana *et al.* (2001), J. W. Pensabene *et al.* (2000), M. Okihashi *et al.* (1998), E. Ueno *et al.* (2004). Such methods will not be applicable in various laboratories around the world, especially in the developing countries, where most of the laboratories is not well equipped and only contains the minimum required facilities for pesticide residue analysis. No single report was found in the literature to deal with the multiresidue of pyrethroid insecticide in Tian Hua Fen. The objective of this study was to develop and validate a simple, reliable and efficient new multiresidue method that doesn't require expensive and sophisticated instruments for sample preparation and detection. The method based on good laboratory practice (ISO 17025 and/or GLP.) criteria, for the determination of several pyrethroid insecticides commonly applied to Tian Hua Fen.

MATERIALS AND METHODS

Chemicals and reagents

Insecticide analytical standards (Fenpropathrin, Cypermethrin, Fenvalirate and Deltamethrin) were purchased from Sumitomo (Chemical Ltd, China) and were certified at least 98% pure. Petroleum ether (at 30-60 °C) and ethyl acetate were for pesticide residues (Barcelona, Spain). Anhydrous sodium sulfate and natural aluminum oxide were of analytical grade (Lan qi reagents and chemical factory, China). The standard solutions are individually prepared in petroleum ether at (ca. 100 - 120 mg kg^{-1}) concentration. The standard cumulative solutions were prepared by mixing suitable volumes of individual standard solutions and diluting with petroleum ether for the recovery study.

Apparatus and chromatography

GC–ECD system: A Shimadzu GC-9A gas chromatograph was used. The chromatograph was fitted with ^{63}Ni electron-capture detector and on-column injector connected to a C-R3A (Shimadzu) reporting integrator. A glass column packed with 5% OV-101 on 60-80 mesh Chromosorb W. AW-DMCS (1.5 m × 3.0 mm I.D.) was used. The injector and detector were operated at 280 and 290°C, respectively. Nitrogen was used as a carrier gas with flow rate at 70 ml / min, the injection volume was (0.6 µl) and the oven temperature was held constant at 260 °C.

The collection of the samples

Tian Hua Fen was purchased at a local market in Hangzhou, China and we confirmed that the concentrations of pesticide residues were below detectable levels with the proposed method.

Extraction

A high-speed electric mixer (Polytron-Aggregate, Kinematica, Germany) was used to homogenize Tian Hua Fen (5 g) at 7000 rpm for 3 min with 80 ml of petroleum ether. The mixture was filtered through a glass funnel containing 5 g anhydrous sodium sulfate; then washed with two (10 ml) portions of petroleum ether. All the fractions were collected in a concentration flask and concentrated to about 5 ml using rotary vacuum evaporation at 40 °C.

Clean-up

A chromatographic column (25 cm × 1.5 cm id) was prepared by adding 2 g anhydrous sodium sulfate, 5 g natural aluminum oxide and 2 g anhydrous sodium sulfate consequently and then pre-washed by 20 ml petroleum ether / ethyl acetate (95 + 5). The extract was poured into the column and petroleum ether (80 ml) was used for eluting the insecticides into a concentration flask. Rotary vacuum evaporator at 40 °C was used to get 10 ml final amount for detection.

Recovery assays

Untreated Tian Hua Fen samples, once crushed and homogenized, were spiked with insecticides. Recovery assays were performed at 0.01–1 mg kg^{-1}. The samples were allowed to equilibrate for 60 min prior to extraction, and then processed according to the above procedure. Five replicates were analyzed at each fortification level.

RESULTS AND DISCUSSION

In this experiment, Standard Operating Procedures (SOPs) was prepared according to ISO 17025 section # 4.2.3, 5.4 to insure the quality of the results.

Calibration Function

Pyrethroid insecticide standard solutions were prepared at three levels (0.01, 0.1and 1 mg kg^{-1}) in petroleum ether and assayed on three separate occasions. Standard curves were constructed for each insecticide by plotting peak height versus concentration; line was fitted by using linear regression. table 1 demonstrates that all curves are linear over the selected range (ISO 17025 section # 5.5.6).

Table 1. Mean values of Slope, Intercept and Regression Coefficient (r^2) for Pyrethroid Insecticides

Insecticide	Slope (peak height units per mg kg^{-1})	Intercept	r^2
Fenpropathrin	41.0	0.5	0.995
Cypermethrin	36.9	0.0	0.998
Fenvalirate	39.5	-0.6	0.998
Deltamethrin	27.3	-0.1	0.997

Matrix Function

In order to determine the matrix effect on linearity response, Tian Hua Fen control extracts were fortified with concentrations ranged from 0.01 to 1 mg kg^{-1} prior to GC analysis. Matrix standard curves for the tested insecticide were constructed as above. Results in table 2 demonstrate that, curves are linear over the tested concentration range.

Table 2. Values of Slope, Intercept and Regression Coefficient (r^2) for Pyrethroid Insecticides in Matrix

Insecticide	Slope (peak height units per mg kg^{-1})	Intercept (peak height)	r^2
Fenpropathrin	46.1	0.4	0.995
Cypermethrin	45.0	-0.5	0.998
Fenvalirate	42.7	-0.5	0.997
Deltamethrin	30.3	-0.1	0.996

Due to the presence of matrix, rise in peak height over the concentration range was clearly observed. Two tailed paired t-test was used to compare the standard curve in matrix against that in solvent, results indicated that *t* calculated is greater than *t* critical at the 95% confidence level (table 3). To compensate matrix effect, samples were analyzed with post-extraction fortified control Tian Hua Fen and the results calculated from this control (ISO 17025 section # 5.9).

Table 3. Results Comparison of Matrix Standard Curves to Solvent Standard Curves Using Paired *T* Test (2 Tailed)

Insecticide	t_{calc}	t_{crit}
Fenpropathrin	7.762	4.303
Cypermethrin	5.452	4.303
Fenvalirate	11.442	4.303
Deltamethrin	8.943	4.303

Precision

Recovery data from the fortified controls was used to measure repeatability and reproducibility variation of the method at 0.01, 0.1 and 1 mg $kg^{-1}$and expressed as % CV. Each analytical batch comprised of 10 negative control samples, and the test was repeated on three occasions at each concentration. All the recoveries obtained were within the acceptable range (70-120) see table 4 (ISO 17025 section # 5.4).

Accuracy

Accuracy was described as the overall recovery (table 4), it is the mean recovery obtained for each compound, at each fortification level, for all observations (ISO 17025 section # 5.4).

Table 4. Reproducibility of Pyrethroid Insecticides Recovery Values and the Accuracy of the Method at 0.01, 0.1 and 1 mg kg^{-1}

Insecti-cide	Fortifica-tion level (mg kg^{-})	Batch 1		Batch 2		Batch 3		Overall		
		mean	%CV	mean	%CV	mean	%CV	mean	%CV	n
Fenpro-pathrin	1	97	11	93	7	100	9	97	9	9
	0.1	98	10	99	7	99	11	99	8	9
	0.01	103	9	103	4	105	13	104	8	9
Cyper-methrin	1	90	8	90	4	90	6	90	6	9
	0.1	89	4	97	5	88	5	91	6	9
	0.01	94	7	95	4	90	4	93	5	9
Fenva-lirate	1	83	7	88	5	91	12	87	8	9
	0.1	89	8	92	15	86	5	89	10	9
	0.01	84	8	92	14	96	6	91	10	9
Delta-methrin	1	90	9	86	9	90	8	89	8	9
	0.1	101	8	92	15	108	6	100	11	9
	0.01	84	7	89	6	83	8	85	7	9

Sensitivity

Slope, intercept and correlation coefficient (r^2) was used to express the sensitivity of the method, for each compound, over the range 0.01 to 1 mg kg^{-1} (table 2). All the results obtained were within the acceptable limits according to ISO/IEC 17025.

Selectivity

Peak retention time was used to determine the selectivity of the method, no interference from co-extractives was found to interfere with the tested pesticide (figure 1). Good resolution was obtained by the method; table 5 gives the retention times of each pyrethroid insecticides when analyzed by GC.

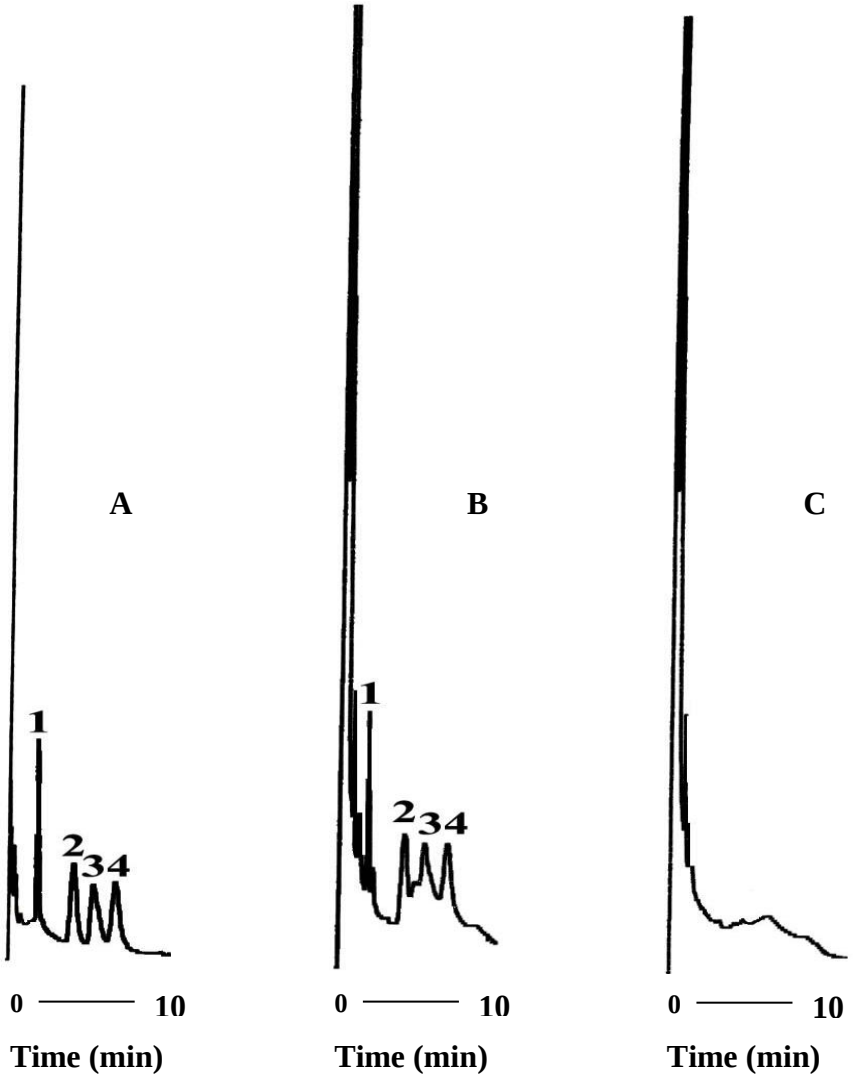

Figure 1. Representative gas chromatograms of (A) Insecticide standards (B), Tian Hua Fen sample fortified with pyrithroid insecticide 0.01 mg kg^{-1}. Peak identities are Fenpropathrin (1), Cypermethrin (2), Fenvalerate (3), and Deltamethrin (4). (C) Control Tian Hua Fen.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection is calculated using the formula:

Noise peak height (mean x 3 SD) / Peak height of standard x Standard concentration. Table 5 demonstrates LOD and LOQ for all pyrethroid insecticides at 0.01 mg kg^{-1} (ISO 17025 section # 5.4, 5.5.6)**.**

Table 5. Limit of Detection and Limit of Quantitation of Pyrethroid Insecticide on Gas Chromatograph by Electron Capture Detector.

Insecticide	Retention time (min)	LOD mg kg^{-1}	LOQ mg kg^{-1}
Fenpropathrin	3.07	0.0007	0.0022
Cypermethrin	5.7	0.0020	0.0067
Fenvalirate	7.73	0.0028	0.0092
Deltamethrin	9.72	0.0022	0.0075

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ISO 17025 section # 4.2.3, 5.4 Quality system/quality manual Test methods and method validation.

ISO 17025 section # 5.4 Test method and method validation.

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تقييم كفاءة وشرعية طريقة جديدة لتحليل المتبقيات العديدة للمبيدات الحشرية البيروثرويدية في عشبة TIAN HUA FEN (*Radix trichos* Anthis)

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يشرح البحث طريقة تحليل المبيدات الحشرية البيروثرويدية علي احدي الاعشاب الطبية الصينية Tian Hua Fen (Radix trichos Anthis) باستخدام طريقة جديدة لتحليل المتبقيات العديدة بواسطة تقنية الكروماتوجرافي. استخلصت المبيدات من العشب (٥جم) بواسطة اثيرالبترول وتمت عملية التنقية باستخدام اكسيد الالومينيوم المتعادل, كما واستخدم جهاز الكروماتوجرافي الغازي المجهز بكشاف اللاقط الالكتروني (ECD) والمزود بعمود زجاجي ذات التعبئة بمادة (5% OV-101) لتقدير المتبقيات في المستخلص النقي. تم الحصول علي نسب استرجاع جيده للعينات المقواة بعدة مستويات من المبيدات القياسية ($1, 0.1$ and 0.01 mg kg^{-1}) وذلك باستخدام طريقة الاستخلاص والتنقية المذكورة اعلاه. أظهرت النتائج المتحصل عليها مدي خطية جيد ($r>0.99$), ومتوسط استرجاع (over 82%) ونسبة انحراف معياري (<16) جيدين لكل المركبات المستخدمة في الاختبار, كما وتم الحصول علي حد ادني للتقدير LOD بين 0.0007 و 0.0028 ملجم/كجم وحد ادني للقياس LOQ بين 0.0022 و 0.0092 ملجم/كجم. تمت عملية تقييم مميزات الطريقة والمصادقة علي كفائتها وادائها بحسب (ISO 17025 and/or GLP) حيث اضهرت النتائج ان كل الخطوات المتعلقة بتقييم كفائة واداء الطريقة مثل: الانتقائية و الدقة والضبط والتكرارية وغيرها, كانت في المدي المقبول لهذا النوع من التحاليل.