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Gas Chromatographic Determination of Prochloraz in Ten Herbal Medicines

Jialun Wu[1]*, Jing Zhao[1], Tianyu Wang[1], Aoran Chen[1] and Jian Xue[2]

[1]*Ministry of Agriculture Key Laboratory of Molecular Biology of Crop, Pathogens and Insects, Institute of Pesticide and Environmental Toxicology, Zhejiang University, Hangzhou 310029, China*
[2]*Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193 China*

Abstract

A method for determining prochloraz in ten herbal medicines is described. Pesticide standards were fortified into herbal medicines at 3 levels (0.005, 0.01 and 0.05mg/kg). Prochloraz reacts with pyridine hydrochloride and generates a hydrolysate, the 2,4,6-trichlorophenol which responded intensively in GC-ECD. The results showed average recoveries were between 76.6% and 105.1%. The method evidenced good accuracy and precision for monitoring prochloraz in ten herbal medicine samples. The limit of quantification (LOQ) was 0.005mg/kg. It is linear from 0.005 mg/L to 2.000mg/L. The regression equation is y=3816.1x-7.7835, R=0.9997.

Keywords: Prochloraz; Fungicide; Gas chromatography; 2,4,6-Trichlorophenol; Herbal medicine

Introduction

Herbal Medicines have been prevailing for thousands of years in China, which are the treasure to all human beings. With the increasing demand for herbal medicines, most of them are cultivated in fields. Plant disease, pest insects and weeds, which results in yield loss and lower quality, are the primary limitation to the production. Currently, it is a main method to use pesticides in medicinal herb fields for controlling the damage caused by Plant disease, pest insects and weeds. Prochloraz (N-propyl-N-[2-(2,46-trichlorophenoxy)-ethyl] imidazole-1-carboxamide) (Figure 1) is a non-systemic imidazole fungicide, an ergosterol biosynthesis inhibitor with contact and translaminar, protectant and eradicant activity. It is used in agriculture and horticulture against various plant diseases, especially Ascomycetes and Fungi Imperfecti. It is used to control foliar diseases of cereals (*Pseudocercosporella*, *Pyrenophora*, *Rhynchosporium* and *Septoria spp.*), field crops (such as *Alternaria*, *Botrytis*, *Pyrenopeziza* and *Sclerotinia* in oilseed rape, *Ascochyta* and *Botrytis* in legumes, *Pyricularia* in rice), fruit (blossom blight) and vegetables (anthracnose) [1]. In china, it is also used in medicinal herb fields.

Several analytical methods of prochloraz residues have been reported. Most of them were on vegetable, fruit, and cereals [2-15], some is on soil and water[16,17]. It is not found on herbal medicines. Ginseng Rodix ET Rhizoma , Rhizoma Atractylodis Macrocephalae, Isatidis Radix, Curcumae Rhizoma, Dioscoreae Rhizoma, Crataegi Fructus, Acanthopanacis Senticosi Radix, Lonicerae Japonicae Flos, Lorydalis Rhizoma and Radix Astragali are conventional Chinese herbal medicines that are artificially planted. Prochloraz is a popularly used fungicide in these medicinal herb fields. The object of this work was to develop a method for determining prochloraz in herbal medicines. It is significant to find, control prochloraz residue and improve the quality of herbal medicines.

Experimental

Apparatus

a. Gas chromatograph.—Agilent 7890A equipped with an ECD detector (Agilent Technology Company, USA).

b. Vacuum pump.— SHB-III-A(Hangzhou Dawei Scientific Ltd., PRC).

c. Rotary evaporator.—LABOROTA 4010(Heidolph Instruments, Germany).

d. Ultrasonic Machine.—KQ5200DE (KunShan Ultrasonic Instruments Co., Ltd, PRC).

e. Grinder.—DD-01(Wenling Linda Machine Ltd., PRC).

Reagents and materials

- Solvents.— All organic solvents, e.g., acetone, dichloromethane, petroleum ether, hydrochloric acid and concentrated sulfuric acid, analytical grade (made in China).
- Sodium sulfate anhydride.— Analytical grade and heated 8 h at 450°C and stored in a tightly capped bottle until used.
- Celite 545.— (Fluke).
- Pesticide standards solution.—Prochloraz which was provided by Dr. Ehrenstorfer. 500mg/L dissolve in methanol. 2,4,6-trichloro-phenol were provided by DIMEE TECHNOLOGY in Hexane. Pyridine hydrochloride was provided by Hangzhou Faojing Fine Chemical Industry Ltd., Zhejiang, PRC.
- Herbal medicines. —buy from market or supplied by Medicinal herb GAP plantation.

Extraction

Dried samples were pulverized and passed through a 20-mesh screen. Weigh 4g sample in a 250 ml screw-stop sample bottle, add 5 ml

*Corresponding author: Jialun Wu, Ministry of Agriculture Key Laboratory of Molecular Biology of Crop., Pathogens and Insects, Institute of Pesticide and Environmental Toxicology, Zhejiang University, Hangzhou 310029, China, Tel: 0086 0571 86971221; Fax number: 0086 0571 86971221; E-mail: jlwu@zju.edu.cn

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1 mol/L hydrochloric acid and 60 ml acetone, ultrasonic extract for 30 minutes. Vacuum filtrate with Buchner funnel (add a filter paper and suitable celite545). Elute the sample bottle with 40ml acetone. Collect filtrate into a 250 ml flask and concentrate to 3~5ml in a rotary vacuum evaporate at 45°C. The remaining solution was transferred to a 250 ml separating funnel, add 40 ml 10% sodium chloride water solution. Extract with 2×50ml dichloromethane. Collect organic phase which passed through 10g sodium sulfate anhydrous into a 250ml Florence flask and concentrate to 5~10 ml in a rotary vacuum evaporate at 48°C. Transfer concentrated solution into a 50ml round-bottom flask, rinse the Florence flask with 20ml dichloromethane, combine extracts carefully and concentrate to almost 1 ml in a rotary vacuum evaporate at 48°C then evaporate to completely dry with soft nitrogen stream.

Hydrolysis and cleanup

Add 2g pyridine hydrochloride into the flask, connecting with a condenser pipe. Heated up to 220~240°C in a thermostatic oil bath for 1 hour. Wash the condenser pipe with 10 ml distilled water after cooling. Put the stopper on the flask and shake to dissolve completely. Transfer the solution into a 250 ml separating funnel and elute the flask several times with 30 ml 0.01 mol/L sodium hydroxide solution, collect all solution to the separating funnel. Decontaminate with 2 x 20mL petroleum-ether/toluene (4:1, v/v), and the organic phase was discarded. Adding 3 ml 1 mol/L hydrochloric acid, extract with 2 x 30mL petroleum ether, collect organic phase which passed through 10 g sodium sulfate anhydrous into another 250 ml separating funnel. Add 10 ml concentrated sulfuric acid into 250 ml separating funnel, shake for 1 minute. Discard concentrated sulfuric acid layer. Repeat 2 times until concentrated sulfuric acid layer is clear. Wash organic phase with 2×40 mL 10% sodium chloride water solution discard water solution. Collect organic phase into the 250 ml flask and concentrate to almost 1 ml in a rotary vacuum evaporate at 48°C then evaporate to completely dry with soft nitrogen stream. Add 2 ml n-hexane and

Sample name	Fortified concen.(mg/kg)	Recovery(%)			Average recovery(%)	RSD(n=3)/%
Ginseng Rodix ET Rhizoma	0.005	102.5	97.1	103.8	101.1	3.5
	0.010	106.3	96.2	98.7	100.4	5.2
	0.050	103.8	100.2	108.2	104.1	3.9
Rhizoma Atractylodis Macrocephalae	0.005	91.2	93.4	95.1	93.2	2.1
	0.010	96.2	92.2	93.0	93.8	2.3
	0.050	102.4	98.8	97.9	99.7	2.4
Isatidis Radix	0.005	86.7	85.7	89.1	87.2	2.0
	0.010	107.1	93.4	94.1	98.2	7.9
	0.050	108.4	106.7	100.3	105.1	4.1
Curcumae Rhizoma	0.005	79.4	85.4	83.6	82.8	3.7
	0.010	93.9	94.2	91.1	93.1	1.8
	0.050	96.7	95.1	97.8	96.5	1.4
Dioscoreae Rhizoma	0.005	76.8	74.8	78.2	76.6	2.2
	0.010	79.8	79.2	80.7	79.9	0.9
	0.050	86.1	83.7	90.8	86.9	4.2
Crataegi Fructus	0.005	88.8	86.0	84.3	86.4	2.6
	0.010	91.9	89.2	93.4	91.5	2.3
	0.050	90.8	96.4	95.8	94.3	3.3
Acanthopanacis Senticosi Radix	0.005	81.2	83.1	85.5	83.3	2.6
	0.010	97.6	96.6	92.2	95.5	3.0
	0.050	106.4	105.2	100.7	104.1	2.9
Lonicerae Japonicae Flos	0.005	86.2	88.0	84.7	86.3	1.9
	0.010	92.5	99.5	93.1	95.0	4.1
	0.050	88.4	96.4	95.5	93.4	4.7
Lorydalis Rhizoma	0.005	83.6	85.1	85.7	84.8	1.3
	0.010	102.8	98.4	98.1	99.8	2.6
	0.050	95.2	93.3	95.6	94.7	1.3
Radix Astragali	0.005	79.8	93.3	86.0	86.4	7.8
	0.010	91.8	88.5	90.4	90.2	1.8
	0.050	85.7	96.2	95.3	92.4	6.3

Table 1: Recoveries of prochloraz in ten medicinal herb samples.

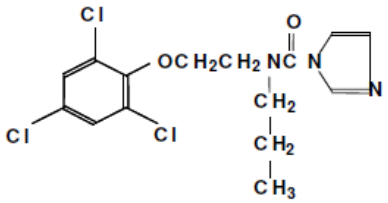

Figure 1: Structural formula of prochloraz.

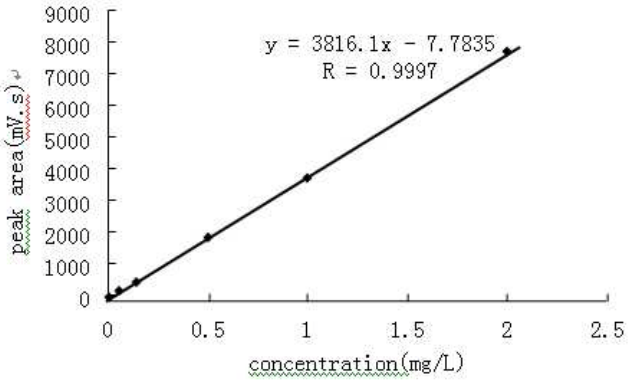

Figure 2: Calibration curve for prochloraz (concentration from 0-2.0 mg/L).

mixed for determination.

Condition of gas chromatograph

The analysis of 2,4,6-trichlorophenol was carried out by GC using an Agilent 7890A with an ECD, a capillary chromatographic column SE-54, 30m×0.32mm×0.25μm film thickness. The temperature program applied as follows: 100°C for 1 min, 100-160°C at 15°C /min, 160°C for 3 min, 160-180°C at 2°C /min, 180°C for 5 min, 180-250°C at 30°C /min and 250°C for 5 min. The injector port temperature was 250°C, detector temperature, 300°C; Nitrogen carrier gas flow rate, 1 ml/min. up gas, 30ml/min and septum purge flow to appropriate levels, 3ml/min. The injection port type: splitless, inject amount was 1 μl.

Standard sample

Respectively add 0.005, 0.010, 0.050, 0.500, 1.000, 2.000mg/L standard sample into a round-bottle flask and evaporate to completely dry with nitrogen. Next processing step according to 2.4 and 2.5 described.

Results and Discussion

The method used demonstrated acceptable performance for the prochloraz residue analysis in ten herbal medicines. Under the chromatographic conditions described, good linear and correlation coefficient was achieved for the compound studied. Replicates (n=3) of six standard pesticide solutions of different concentrations were found to be linear in the range from 0.005 to 2.0 mg/L for the chromatographic techniques (Figure 2). The regression equation for the calibration curve was y=3816.1x-7.7835. The correlation coefficients obtained for the prochloraz was 0.9997. The limit of quantification (LOQ) for prochloraz under study was 0.005 mg/kg. Validation of the method for this fungicide was performed by fortified recovery study with prochloraz at 0.005, 0.01 and 0.05 mg/kg levels. For analyses by this method, average recovery ranges of prochloraz from ten herbal medicines were 76.6-105.1%. Relative standard deviations (RSD) were 0.9-7.9%. The detail results are shown in Table1. Some typical chromatograms of 2,4,6-trichloro-phenol standard, fortified 0.5 mg/kg prochloraz with no herbal medicines and solvent check unfortified prochloraz are shown in Figure 3-5. Typical chromatograms of herbal medicine samples untreated and fortified 0.5 mg/kg of prochloraz are shown in Figure 6, 7.

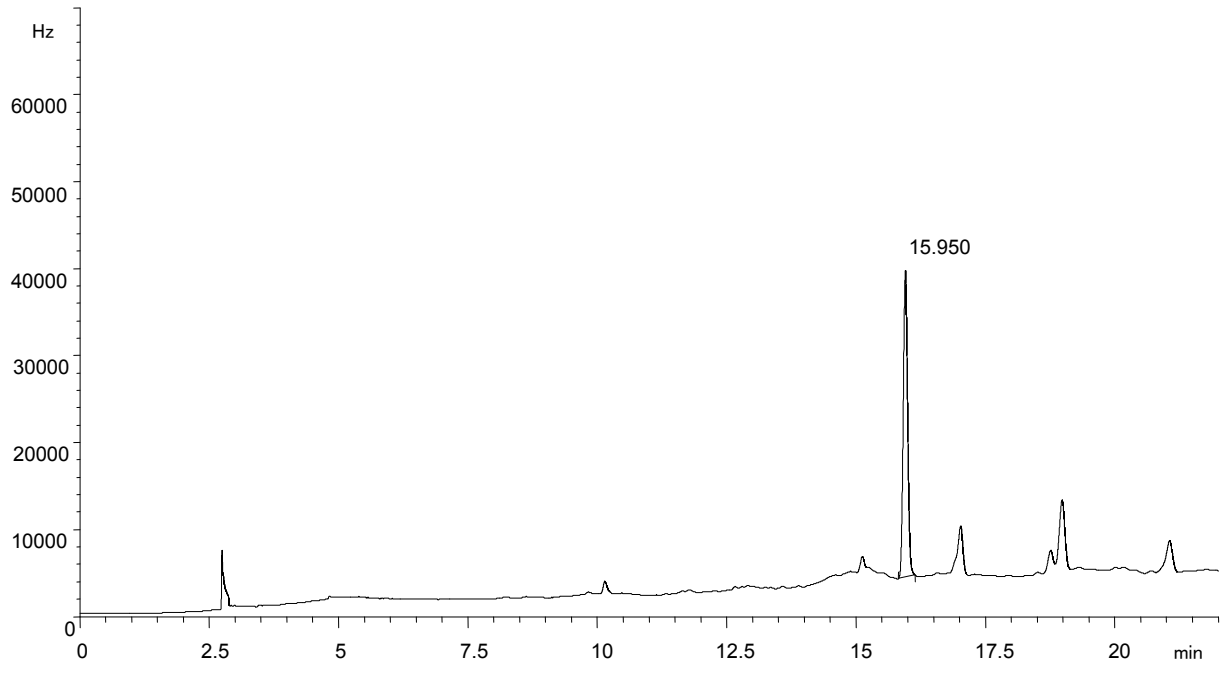

Figure 3: Chromatogram of 0.5mg/kg 2,4,6-trichloro-phenol.

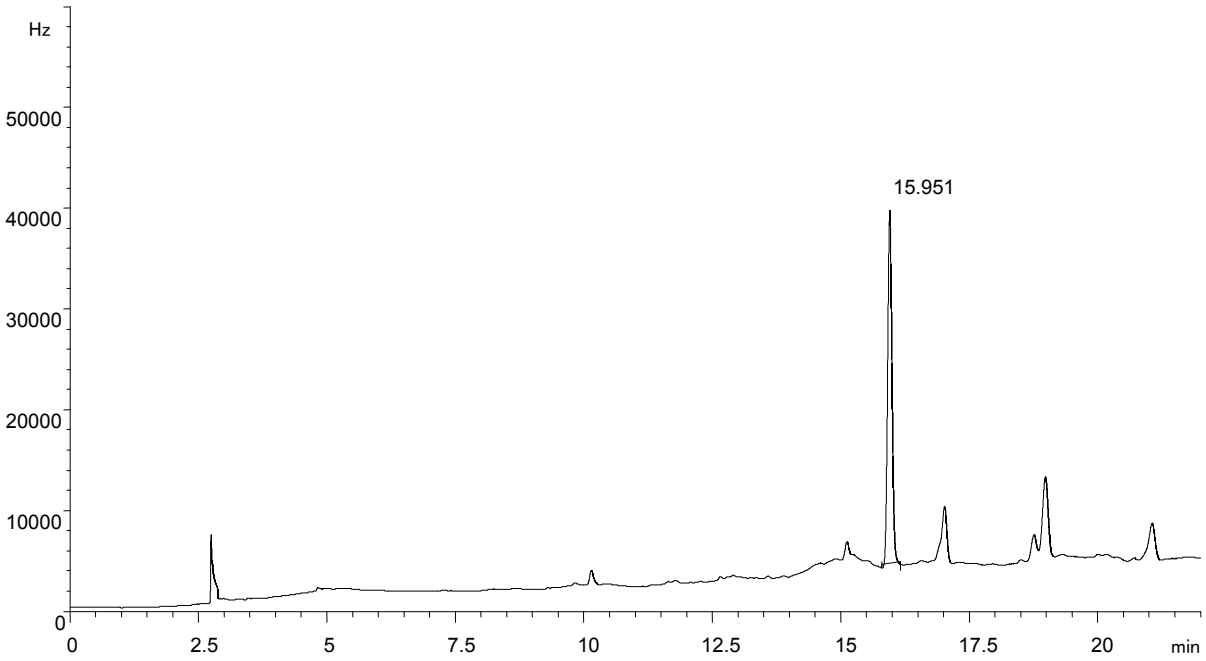

Figure 4: Chromatogram of fortified 0.5mg/kg of prochloraz standard (no sample).

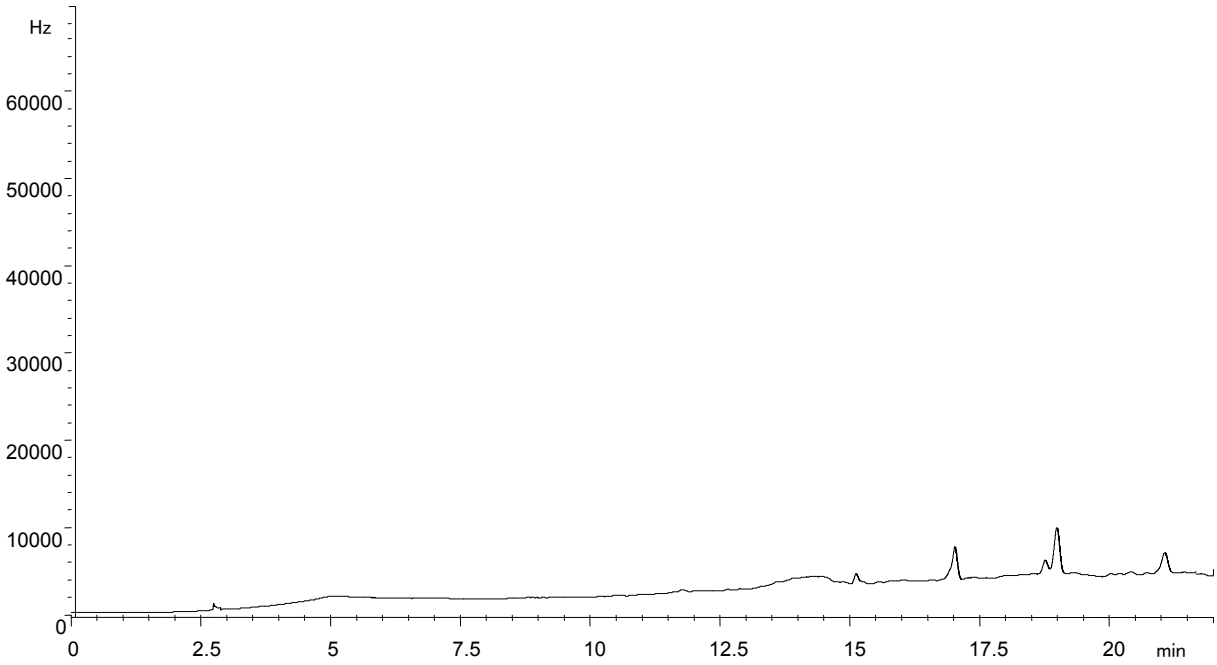

Figure 5: Chromatogram of solvent check of unfortified prochloraz.

In hydrolysis, the rate that prochloraz hydrolyzes to 2,4,6-trichlorophenol would influence reliability and veracity of the method. On the basis of stechiometry, the prochloraz is hydrolysed with pyridine hydrochloride to break down all components to 2,4,6-trichlorophenol. This hydrolysate is then cleaned-up by extraction into the aqueous layer with alkali and re-extraction into petroleum ether after acidification. Total 2,4,6-trichlorophenol residues are determined by gas chromatography with electron capture detector, and the results are expressed as prochloraz equivalents by correcting the measured 2,4,6-trichlorophenol concentration for the molecular weight factor of 1.9. The tested result of prochloraz standard showed the conversion rate was above 98% under the test condition.

FAO and WHO reported MRLs of prochloraz in cereal grains and rape seed were 0.05 mg/kg (France), in mushroom were 0.05

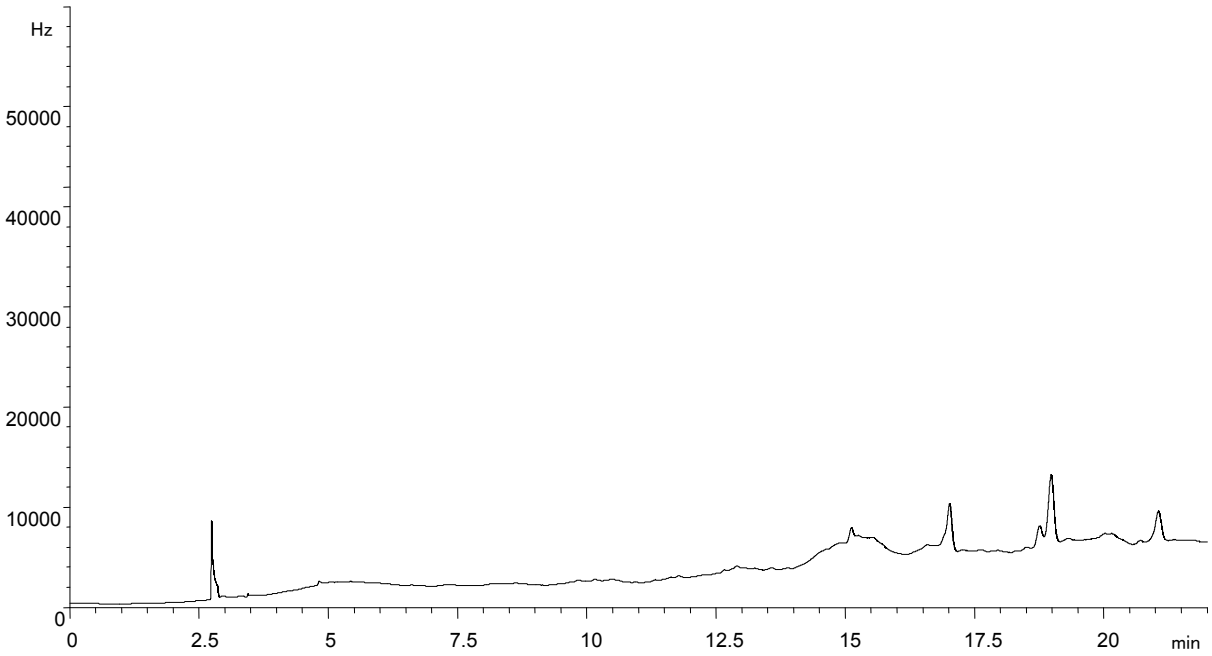

Figure 6: Chromatogram of Rhizoma Curcumae unfortified prochloraz.

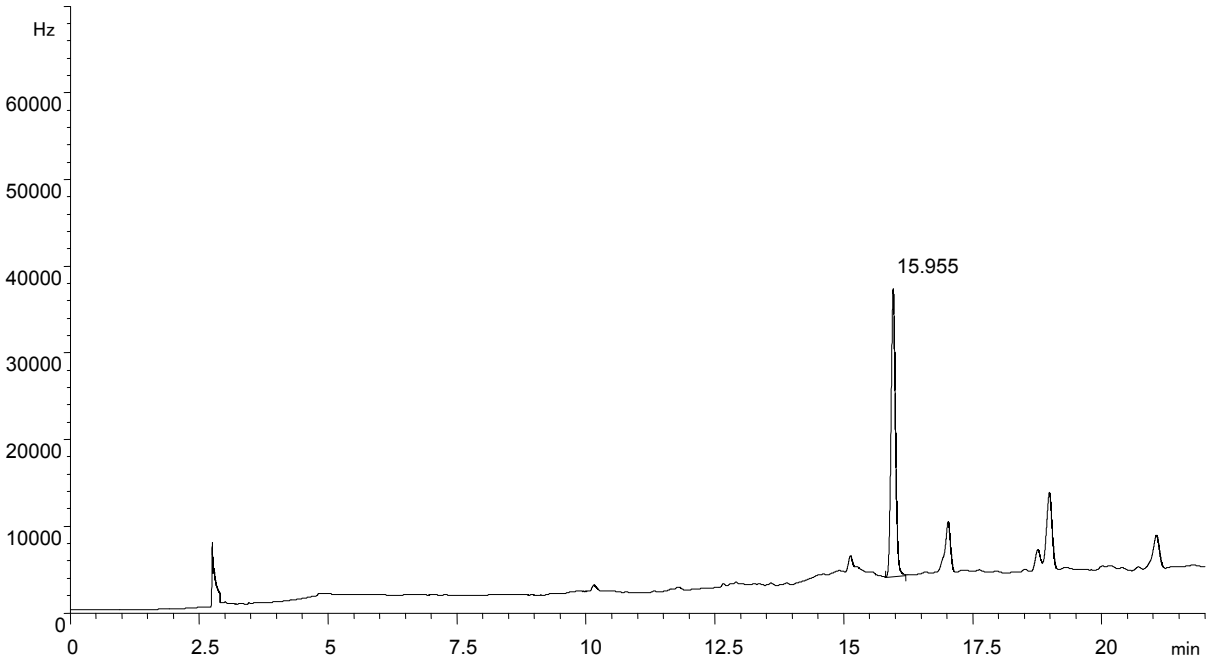

Figure 7: Chromatogram of Rhizoma Curcumae fortified with 0.5mg/kg of prochloraz.

mg/kg (The Netherlands), in other products were 0.01 mg/kg (The Netherlands)[18]. By now, no any MRL of prochloraz was established in herbal medicines. In view of many countries usually set 0.01 mg/kg as a criteria for no MRL products, such as "Positive list system of Japan", the method is available for herbal medicines. Paoli et al (1997) reported a GC-ECD method for determining 2, 4, 6-trichlorophenol as prochloraz in vegetable, fruit and wheat, and the LOQ were 0.01-0.5 mg/kg. Blasco et al (2001) developed a LC-MS and LC-UV method for determining prochloraz in fruits and vegetables. The LOQ was 0.1 mg/kg (LC-MS) and 0.5 mg/kg (LC-UV). Polese et al (2006) described results of prochloraz, which determined as 2,3,4-trichlorophenol, and its metabolite residue in ginger. The LOQ of GC-ECD method was 0.1 and 0.2 mg/kg, respectively. The method of this paper is more sensitive compared with published papers in terms of the LOQ. The method described here has been successfully used to determine prochloraz in

ten Chinese herbal medicines. The GC-ECD method should be readily applicable to other Chinese herbal medicines.

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