

Development of a LC-MS/MS method for simultaneously determination of 30 pesticides in *Chenpi*

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1. Overview

A method was proposed in this paper for determination of 30 pesticides in *Chenpi* with solid phase extraction.

2. Introduction

In August 2019, the Chinese Pharmacopoeia Commission issued public a draft of Pharmacopoeia 2020. It lists 30 forbidden pesticides in Traditional Chinese Medicines(TCM). 30 pesticides and residues are not supposed to be detected (no higher than the requirement of quantitative limit) by LC-MS/MS. Referring to the fifth method of pesticide residue determination method in public draft 2341 of **Chinese Pharmacopoeia** (2015), the method for determination of banned multi pesticide residues in TCM(plants), an analytical method for 30 banned pesticides and residues in *Chenpi* based on LCMS-8050 triple quadrupole liquid chromatography-mass spectrometry was established.

3. Methods and Materials

Standards of 30 pesticides were diluted with acetonitrile to an appropriate concentration and then analyzed by LC-MS/MS. As an LC-MS/MS system, UHPLC was coupled to triple quadrupole mass spectrometer (Nexera X2 with LCMS-8050, Shimadzu Corporation, Kyoto, Japan).

Separation was achieved using a Shim-pack Velox C18 column (100mmL., 2.1mmL.D., 2.7um particles) and column oven temperature was maintained at 40 C. Samples were eluted at flow rate 300uL/min with a binary gradient system; the mobile phase consisted of A-0.1% formic acid+5mM ammonium acetate aqueous solution, B-0.1% formic acid acetonitrile:A(95:5,V/V). LC-MS/MS with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.



Figure 1 LCMS-8050 triple quadrupole mass spectrometer

High Speed Mass Spectrometer

Ultra Fast Polarity Switching

- 5msec

Ultra Fast MRM

- Max. 555 transition /sec

4. Result

4-1. Method development for pesticides

Full scan measurement by flow injection analysis (FIA) was conducted to determine the optimum ionization polarity of target compounds followed by MRM transition optimization by FIA.

UHPLC conditions (Nexera X2 system)

Column: Shim-pack Velox C18 100mm × 2.1mmL.D., 2.7 um

Mobile phase A:0.1% formic acid+5mM ammonium acetate aqueous solution

B: B-0.1% formic acid acetonitrile:A(95:5,V/V)

Flow rate: 0.3 mL/min

Time program: B conc.30%(0 -1 min) -100%(12-14 min) - 30%(14.1-17min)

Injection vol.: 2 uL

Column temperature: 40 °C

MS conditions (LCMS-8050)

Ionization: ESI, Positive MRM mode

Table 1. MRM transitions of Pesticides

No.	Compound	Retention time	Precursor	Product	Collision energy/eV	No.	Compound	Retention time	Precursor	Product	Collision energy/eV
1	Methamidophos	0.74	142.05	94	-14	16	phorate-sulfone	5.111	293.2	170.95	-12
			142.05	124.95	-16				293.2	115	-26
			304.2	217	-22				293.2	247	-8
2	Fenamiphos	5.753	304.2	201.95	-36	17	coumsphos	7.787	363.1	226.8	-26
			304.2	234	-17				363.1	306.8	-21
			336.2	266	-20				256.1	139.9	-24
3	Fenamiphos sulfone	3.192	336.2	188	-27	18	phosfolan	1.664	256.1	167.9	-17
			320	233	-25				300	174.05	-13
			320	171.2	-33				213.15	89.15	-17
4	Fenamiphos sulfoxide	2.02	320	292.05	-15	19	phosphamidon	2.207	300	127	-27
			320	233	-25				213.15	116	-12
			247.1	109	-18				208.1	116.2	-8
5	fonofos	7.775	247.1	136.9	-11	20	aldicarb	2.234	208.1	88.9	-16
			323	114.95	-30				240.2	86	-21
			323.1	170.95	-15				240.2	223	-8
6	sulfotep	7.953	323.1	97.05	-39	21	aldicarb-sulfone	0.94	223.1	86	-15
			222.1	123	-22				223.1	76	-8
			238.2	163	-13				207.1	89	-15
7	carbofuran	3.387	222.1	165	-13	22	aldicarb-sulfoxide	0.78	207.1	132	-8
			238.2	181.1	-11				224.1	127	-15
			220.2	167	-9				224.1	193	-9
8	carbofuran -3-hydroxy	1.246	220.2	107	-30	23	monocrotopos	0.837	224.1	127	-15
			220.2	163	-13				259.1	89.15	-16
			220.2	107	-30				259.1	60.95	-33
9	ethametsulfuron-methyl	3.852	411.1	196.1	-16	24	demeton	4.597	243.1	130.9	-21
			411.1	168	-28				243.1	96.9	-32
			382	167	-17				321	170.95	-10
10	metsulfuron-methyl	3.154	382	199	-21	25	ethoprophos	5.954	305.1	187	-12
			358	141.2	-18				305	96.95	-40
			271.3	158.9	-14				312.1	235.9	-15
11	chlorsulfuron	3.523	271.3	96.9	-36	26	terbufos-sulfone	6.138	312.1	270.1	-7
			271.3	130.9	-23				291.10*	231	-16
			314	120	-26				291.1	121	-31
12	cadusafos	7.507	314	162	-16	27	terbufos-sulfoxide	4.992	197.1	46.1	-23
			314	120	-26				197.1	117.05	-29
			261.05	75	-12				197.1	152.1	-20
13	isazafos	7.015	261.05	47.1	-37	28	isocarbofos	5.146	332.15	231.1	-15
			261.15	75	-12				332.15	273	-8
			277.1	97	-32						
14	phorate	7.971	277.1	198.95	-10	29	chlordimeform	0.979			
			277.1	170.9	-13						
			277.1	142.9	-20						
15	phorate-sulfoxide	3.757	277.1	170.9	-13	30	isofenphos-methyl	7.717			
			277.1	142.9	-20						

Chromatography

Figure 2 shows MRM chromatograms of 30 pesticides, including organophosphorus, carbamates and its metabolites. It took 17 minutes per one LC-MS/MS analysis, including column rinsing, and excellent separation and high sensitive detection were obtained.

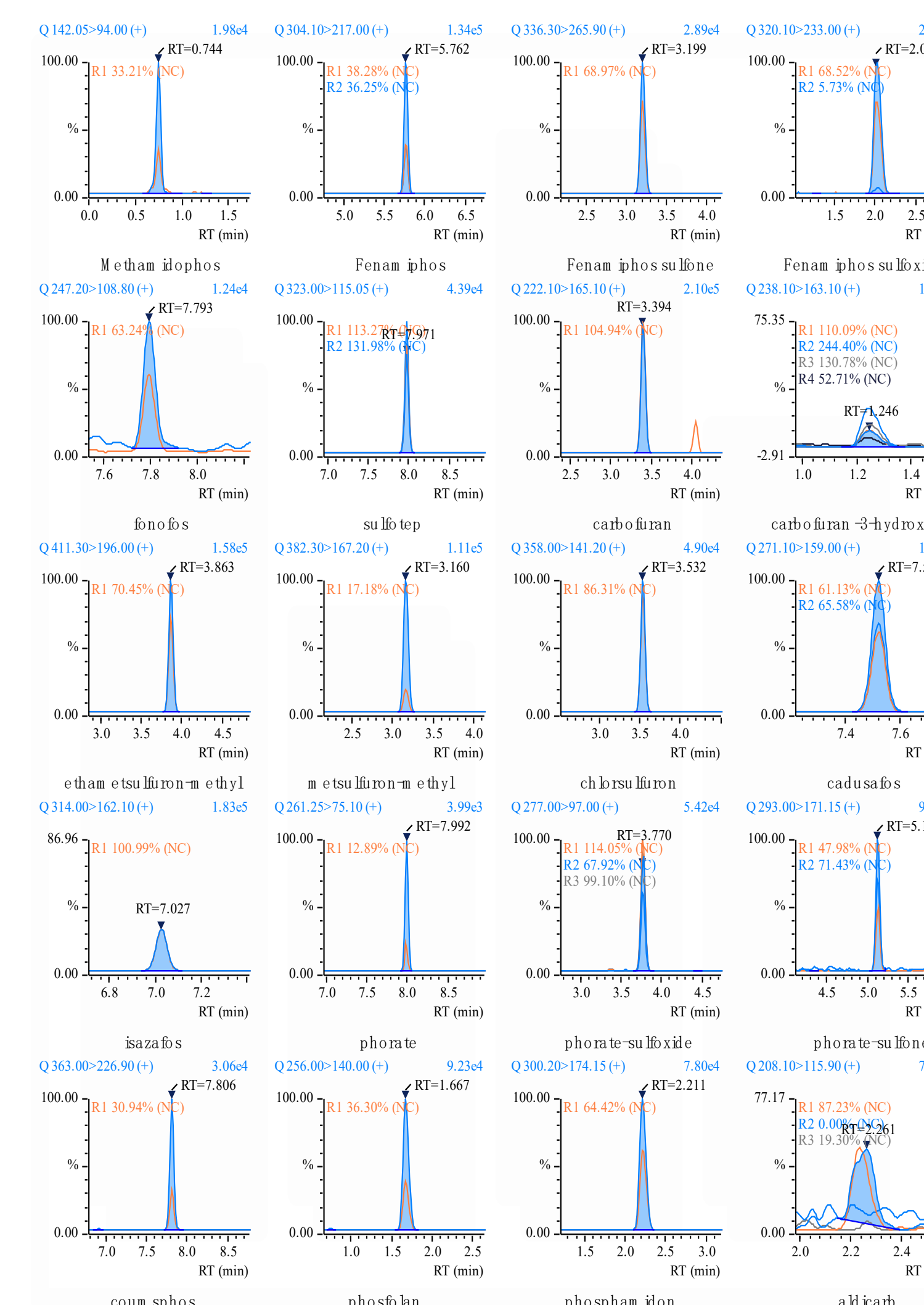


Figure 2 Mass Chromatgrams of typical 30 pesticides in *Chenpi* matrix (Level 1 concentration)

Calibration

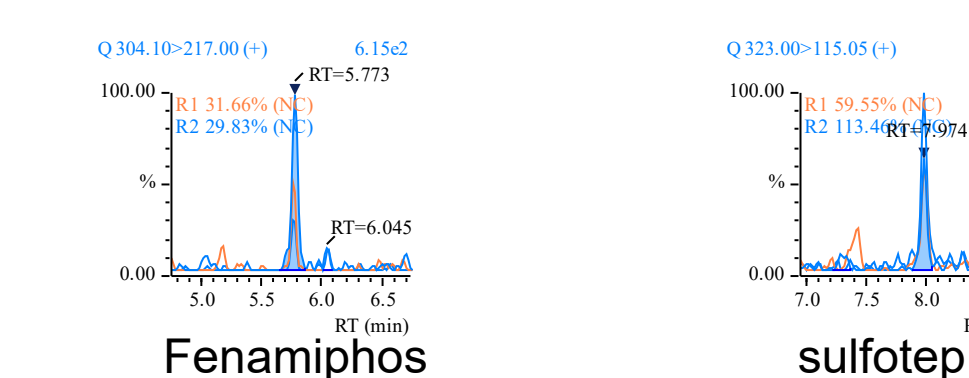
A series of 30 pesticides spiked at 5,10,25,50,75,100 ng/mL (calculated by methamidophos) were prepared by adding 30 mix standard stock solution to blank *Chenpi* matrix. The dilution series of these compounds were analyzed. All compounds were detected at ppb level with excellent linearity (Table 2, Figure 2).

Table 2 Linearity 30 compound

No.	Compound	Coefficient (r)	Range(µg/L)	Accuracy%
1	Methamidophos	0.9997	5-100	97.6-102.6
2	Fenamiphos	0.9999	2-40	97.9-102.3
3	Fenamiphos sulfone	0.9996	2-40	95.7-105.2
4	Fenamiphos sulfoxide	0.9995	2-40	94.7-104.2
5	fonofos	0.9999	2-40	97.7-102.9
6	sulfotep	0.9999	2-40	97.6-102.4
7	carbofuran	0.9998	5-100	97.7-102.3
8	carbofuran -3-hydroxy	0.9996	5-100	93.4-105.1
9	ethametsulfuron-methyl	0.9996	5-100	96.7-104.0
10	metsulfuron-methyl	0.9985	5-100	93.7-111.0
11	chlorsulfuron	0.9998	5-100	97.4-104.6
12	cadusafos	0.9999	2-40	98.8-102.0
13	isazafos	0.9999	1-20	98.3-102.6
14	phorate	0.9997	2-40	93.2-105.6
15	phorate-sulfoxide	0.9997	2-40	96.9-104.2
16	phorate-sulfone	0.9994	2-40	96.6-105.3
17	coumsphos	0.9998	5-100	97.0-102.3
18	phosfolan	0.9999	3-60	97.9-102.3
19	phosphamidon	0.9999	5-100	98.0-102.2
20	aldicarb	0.9969	5-100	86.2-109.8
21	aldicarb-sulfone	0.9988	5-100	91.4-106.0
22	aldicarb-sulfoxide	0.9995	5-100	96.4-104.3
23	monocrotopos	0.9997	3-60	95.8-102.4
24	demeton	0.9997	2-40	88.9-110.9
25	ethoprophos	0.9998	2-40	96.6-102.0
26	terbufos-sulfone	0.9991	2-40	94.1-105.0
27	terbufos-sulfoxide	0.9999	2-40	98.3-102.2
28	isocarbofos	0.9981	10-200	87.3-109.6
29	chlordimeform	0.9999	2-40	96.0-103.8
30	isofenphos-methyl	0.9995	4-80	94.3-106.7

4-2. Quantitative Analysis of real world sample

Chenpi sample were obtained from local market in Beijing. *Chenpi* was homogenated and stored in a ventilated, dry environment at 4 °C temperature. Sample was extract with acetonitrile and purified with *InertSep HLB SPE column*. Finally, it was filtered through a 0.2um filter and analyzed by LC-MS/MS. The result shows that Fenamiphos and sulfotep were detected in *Chenpi* sample, and the concentration was far more lower than the maximum residue limit(25 µg/L).



5. Conclusions

After extraction, 30 kinds of pesticides and metabolites in *Chenpi* can be screened and determined quickly and accurately in 30 minutes by using *inertsep HLB* solid phase extraction column. The linearity and detection limit of the method were investigated. The results showed that the linearity of 30 pesticides in the detection range was good, the correlation coefficient was greater than 0.995, the detection limit of the instrument was 0.001-2.37 µg / L, the quantitative limit was 0.001-4.95 µg / L, and the precision of the instrument was within 5.40%, which met the requirements of the 2341 draft of **Chinese Pharmacopoeia** (2015).

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