SHIMADZU

WP 533

Determination of Pesticides in Dog Collars by On-line Supercritical Fluid Extraction/Chromatography Mass Spectrometry

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1. Overview

- Evaluation of SFE as a sample preparation technique for dog collars
- Pesticides were extracted from the collar surface and focused onto an SFC column for online analysis.

2. Introduction

There has been growing concern about flea collar pesticide effects on animal and human health. Tetrachlorvinphos (TCVP) is an organophosphate insecticide that works by affecting the central nervous system. TCVP is an EPA listed possible carcinogen still used in dog collars. Other collars in the US use a combination of pesticides, such as flumethrin and imidacloprid. On-line supercritical fluid extraction – supercritical chromatography-mass spectrometry (SFE-SFC-MS) is a new analytical technique for the extraction, separation, and detection of compounds in a single analysis that limits the need for extensive manual sample preparation. The extracted compounds are trapped directly onto an analytical column for chromatographic analysis. This technique was applied to the analysis of the above pesticides in store bought dog collars.

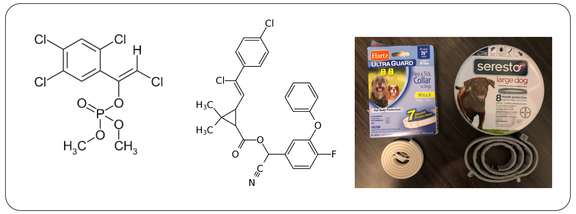


Figure 1 Structures of TCVP and Flumethrin

3. Sample Prep/System Configuration

Polymeric matrix dog collars are embedded with pesticides that are continuously released in low concentrations. CO2 is unable to penetrate this matrix, but is able to extract pesticides from the surface of this matrix. A small piece of the dog collar was simply cut and placed into an extraction vessel for analysis. Repeated extractions were made over time to examine the extraction profile of the pesticides from the dog the collar. The use of a SFE system configuration with two back pressure regulators allows the amount of extracted material to be adjusted between the column or waste through the adjustment of both pressures..

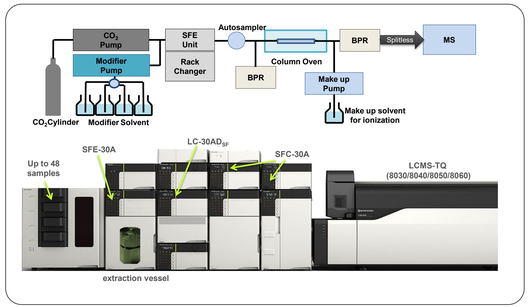


Figure 2 SFE-SFC-MS system configuration (Nexera UC)

4. Method

4-1. Tetrachlorvinphos Extraction Profile

SFE conditions

SFC A: 24.9 MPa
SFC B: 25.0 MPa
Flow: 5.0 mL/min 100% CO2
Static extraction: 3 minutes
Dynamic Extraction: 3 minutes
Ambient temperature

SFC conditions

Column: Naphthyl, 4.6 x 250 mm, 35℃
Mobile phase A: CO2
B: MeOH
Flow rate: 3 mL/min
Time program: 2-20% B over 15 minutes
PDA: 254 nm
Sample size: 302 mg

5. Results

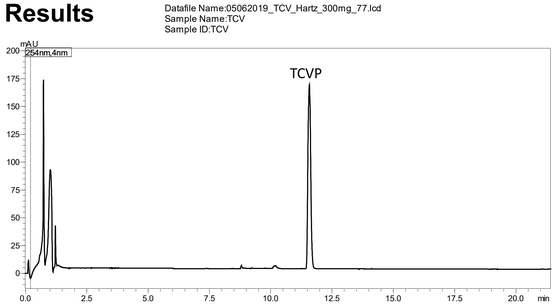
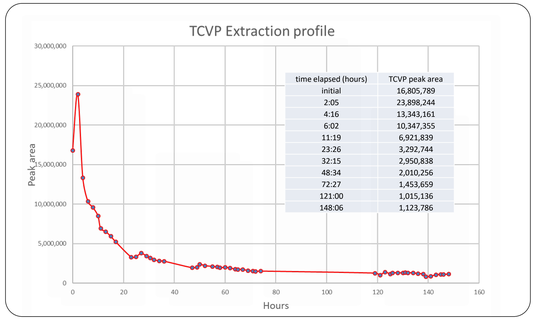
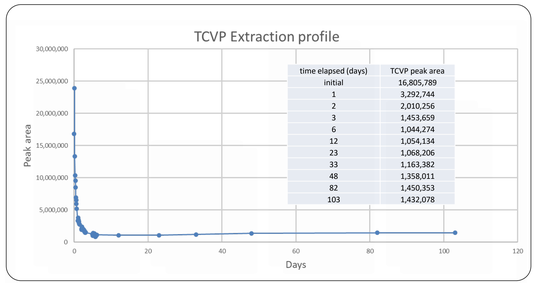


Figure 3 SFE-SFC chromatogram of TCVP from dog collar



time elapsed (hours)	TCVP peak area
initial	16,805,789
2:05	23,898,244
4:16	13,343,161
6:02	10,347,355
11:19	6,921,839
23:26	3,292,744
32:15	2,950,838
48:34	2,010,256
72:27	1,453,659
121:00	1,015,136
148:06	1,123,786

Figure 4 TCVP extraction profile over 6 days



time elapsed (days)	TCVP peak area
initial	16,805,789
1	3,292,744
2	2,010,256
3	1,453,659
6	1,044,274
12	1,054,134
23	1,068,206
33	1,163,382
48	1,358,011
82	1,450,353
103	1,432,078

Figure 5 TCVP extraction profile over 103 days

6. Method

6-1. Flumethrin Isomer extraction

Flumethrin consists of a complex mixture of stereoisomers and contains three chiral carbons. The tested collar ingredients lists a maximum ratio of 66% for the transZ-1 isomer and a minimum ratio of 34% for the transZ-2 ratio.

SFE conditions

SFC A: 24.9 MPa SFC B: 25.0 MPa
Flow: 5.0 mL/min 100% CO2
Static extraction: 3 minutes
Dynamic Extraction: 3 minutes
Ambient temperature

SFC-MS conditions

Column: Chiralpak IA-3, 4.6 x 250 mm, 35℃
Mobile phase 90/10 CO2/MeOH
Flow rate: 3 mL/min
Makeup flow: 0.1 mL/min 0.1% w/v Ammonium Formate in MeOH
MS: 526.9>266.95 ESI positive
Sample size: 6.2 mg

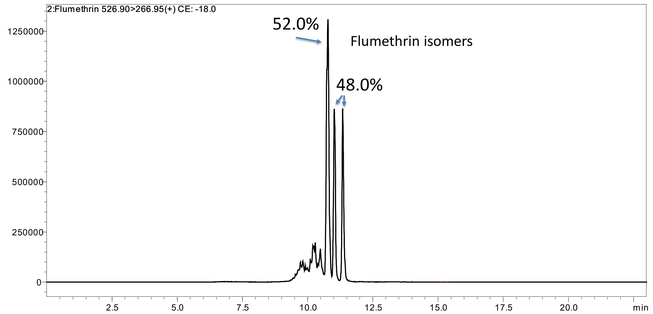


Figure 6. SFE-SFC-MS chromatogram of dog collar containing flumethrin

6. Conclusions

SFE-SFC was proven to be a viable method to determine pesticides in polymeric dog collars.

This sample preparation technique could also be applied to collars containing a mixture of isomers and obtained isomer ratio values that were consistent with label claim.

Consistent amounts of TCVP were released from the dog collar after 48 hours until 103 days.