Development and Validation of a Liquid Chromatographic Method for Monitoring of Process-Related Synthetic Organic Impurities of Profenofos in Technical Products

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Abstract

A simple and rapid reversed-phase high-performance liquid chromatographic method for the monitoring of process-related synthetic organic impurities of profenofos (PFS) is developed. Impurities are separated and determined on a reversed-phase Hypersil C_{18} column using gradient elution of 50mM ammonium formate buffer–acetonitrile as a mobile phase and detection at 230 nm at ambient temperature. The method is validated with respect to accuracy, precision, linearity, and limits of detection and quantitation. The method is found to be suitable not only for monitoring the reactions involved in the process development of PFS, but also quality assurance, as it can detect impurities at the level of 1.5×10^{-8} g.

Introduction

O-(4-bromo-2-chlorophenyl) *O*-ethyl-*S*-propyl phosphorothioate (CAS No. 41198-08-7), commonly known as profenofos (PFS), is a broad-spectrum organophosphorous pesticide and acts as a nonsystemic insecticide and acaricide with contact and stomach action. It is generally used to control insects and mites on cotton, maize, sugar beet, soybean, potato, tobacco, and other crops (1,2). It is synthesized from 2-chloro phenol as a starting material in a laboratory (3). The authors' laboratory has studied extensively the process development of PFS, during which a host of intermediates were produced. It is likely that the small quantities of these unreacted intermediates left over during a variety of chemical reactions may finally decrease the yield and quality of the finished products. Thus, there is a need to develop suitable analytical methods to separate, identify, and determine the process-related organic impurities of PFS during process development and quality control.

A thorough literature search has revealed that numerous methods have been reported for multiresidue analysis of PFS in fruits, vegetables (4,5), medicinal plants (6), eggs (7), food stuffs (8), and water (9,10). A gas–liquid chromatographic method using a flame-ionization detector is generally used for the determination of PFS in technical products (11). To the best of our knowledge, no analytical methods are available for monitoring the process-related organic impurities of PFS to monitor the synthetic procedures in a laboratory. In the present study, reported are the development and validation of a simple and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method for separation and determination of process-related organic impurities of PFS during the process development and quality control.

Experimental

Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Glass-distilled and deionized water (Nanopure, Barnsted, Dubuque, IA), HPLC-grade acetonitrile (Ranbaxy, SAS Nagar, India), and ammonium formate (S.D. Fine Chem, Mumbai, India) were used. PFS reference standard and impurities [viz., 2-chloro phenol (I), 4-bromo-2-chlorophenol (II), *O*-(4-bromo-2-chlorophenyl) *O*,*O*-diethyl phosphorothioate (III), tertiary alkyl ammonium salt of *O*-(4-bromo-2-chloro-phenyl) *O*-ethyl phosphorothioate (IV), 2-chloro-4,6-dibromophenol (IIA), *O*-(2-chloro-4,6-dibromophenyl)-*O*,*O*-diethyl phosphorothioate (IIIA), tertiary ammonium salt of *O*-(2-chloro-4,6-dibromophenyl) *O*,*O*-diethyl phosphorothioate (IVA), *O*-(2-chloro-4,6-bromophenyl) *O*-ethyl-*S*-propyl phosphorothioate (VA), and profenofos isomer (PFS iso)], synthesized in our laboratory (all are having > 98% purity), were used.

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Apparatus

The HPLC system was composed of two LC-10 ATvp pumps, an SPD-10Avp photodiode array detector, an SIL-10AD vp auto injector, DGU-12A degasser, and SCL-10A Vp system controller (all from Shimadzu, Kyota, Japan). A reversed-phase Hypersil C_{18} column (ThermoQuest, Runcorn, U.K.) (25-cm × 4.6-mm i.d, 5-µm particle size) was used for separation. The chromatographic and integrated data were recorded using an HP-Vectra (Hewlett-Packard, Waldron, Germany) computer system.

Chromatographic conditions

The mobile phase was 0.05M ammonium formate buffer–acetonitrile (50:50, v/v), initially. Later, a linear gradient by increasing the concentration of acetonitrile to 75% within 4 min and 80% in 15 min was used. It was maintained for 20 min until it came to the initial condition at 25 min. Before delivering it into the system, the mobile phase was filtered through 0.45-µm poly(tetraflouroethylene) filter and degassed using vacuum. The analysis was carried out under gradient conditions using a flow rate of 1.0 mL/min at room temperature (28ºC). Chromatograms were recorded at 230 nm using an SPD-10A vp photodiode array detector.

Analytical procedures

Solutions of PFS standard and samples were prepared by dissolving 5.0 mg of each in 10 mL of mobile phase. These stock solutions were further diluted to desired concentrations for studying precision, accuracy, and linearity. Amounts of 20 µL of each standard and sample solutions were injected and chromatographed under identical conditions. The percentage of each impurity was calculated from the peak areas of the respective compounds.

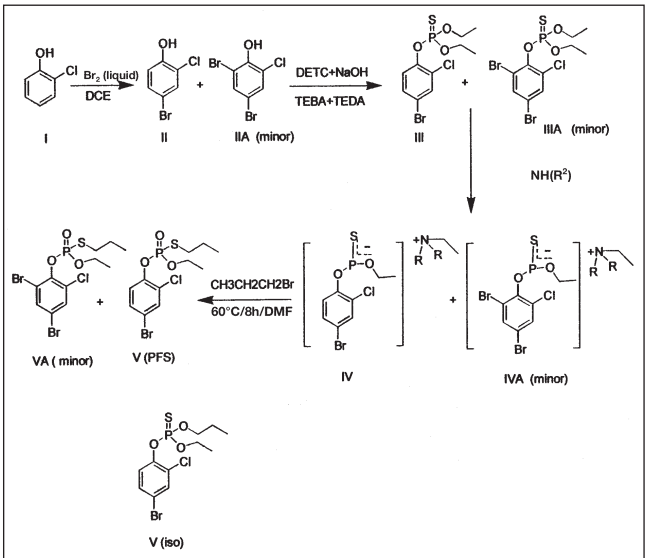

Figure 1. Synthetic reactions followed for preparation of PFS in a laboratory.

Table I. Gradient Elution Program Optimized for Separation of PFS and Its Process-Related Impurities

Time (min)	%50mM Ammonium formate	%Acetonitrile
0.01	50	50
4.0	25	75
15.0	20	80
20.0	20	80
25.0	50	50
30.0	50	50

Results and Discussion

Figure 1 shows the chemical reactions generally followed in the synthesis of PFS in a laboratory. It can be seen from Figure 1 that 2-chloro phenol (I) as a starting material is reacted with liquid bromine in dichloroethane (DCE) to form 4-bromo-2-chlorophenol (II). It is further reacted with *O*,*O*-diethyl thiophosphoryl chloride in aqueous sodium hydroxide and DCE in presence of trimethyl benzyl ammonium chloride and triethylene diamine as phase-transfer catalysts to form *O*-(4-bromo-2-chloro-

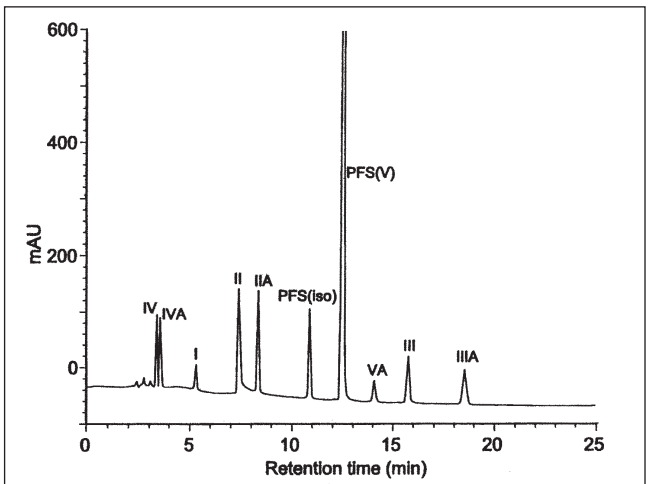

Figure 2. Typical chromatogram of a synthetic mixture containing PFS (500 µg/mL) (V) and its related impurities: I, II, IIA, III, IIIA, IV, IVA, VA, and PFS(iso) of 2.5 µg/mL each.

Table II. Retention Data of PFS and Its Process-Related Intermediates

Compound	t_R	k'	RRT	Tailing factor	Rs	Theoretical plates (N)	λmax (nm)
I	5.31	3.25	0.42	1.04	10.98	68,534	216,223
II	7.39	4.91	0.60	1.07	13.52	165,541	224,232
IIA	8.39	5.71	0.67	1.10	6.48	167,335	221,231
III	15.80	11.65	1.26	1.11	6.55	217,000	225,231
IIIA	18.61	13.89	1.48	1.02	9.45	212,218	218,223
IV	3.38	1.71	0.27	1.20	1.75	24,102	222,215
IVA	3.52	1.82	0.28	1.15	0.75	20,457	216,222
PFS	12.52	9.01	1.00	1.09	8.26	218,373	222,228
PFS(iso)	10.88	7.80	0.860	1.09	2.32	227,983	226,222
VA	14.10	10.28	1.12	1.02	6.85	203,442	222,230

* Abbreviations: t_R = retention time, RRT = relative retention time, and Rs = resolution.

phenyl)*O*,*O*-diethyl phosphorothioate(III). Later, it is treated with diethylamine in water to get a tertiary alkyl ammonium salt of *O*-(4-bromo-2-chloro-phenyl) *O*-ethyl phosphorothioate(IV) and realkylated with *n*-propyl bromide to give *O*-(4-bromo-2-chlorophenyl) *O*-ethyl-*S*-propyl phosphorothioate(V), which is popularly known as PFS. During this process, there is a possibility for the formation of small quantities of 2-chloro-4, 6-dibromophenol (IIA), along with 4-bromo-2-chlorophenol (II), which in turn may be converted into *O*-(2-chloro-4,6-dibromophenyl)-*O*,*O*-diethyl phosphorothioate (IIIA), tertiary alkyl ammonium salt of *O*-(2-chloro-4,6-dibromophenyl) *O*,*O*-diethyl phosphorothioate (IVA), and *O*-(2-chloro-4,6-bromo phenyl) *O*-ethyl-*S*-propyl phosphorothioate (VA). Thus, there is a great necessity to develop analytical methods to monitor the synthetic reactions of PFS for process development and quality control in industry.

Optimization of chromatographic conditions

In preliminary experiments, all the process impurities and PFS were subjected to separation by RP-HPLC using a Hypersil C_{18} column with methanol–water–acetic acid (80:19.5:0.5, v/v/v) as the eluent. Most of the impurities were not separated under these conditions. Later, 0.05M ammonium formate buffer and acetonitrile were selected as eluent. When the concentration of acetonitrile was varied in the range of 30–50% (v/v), it was found that tertiary alkyl ammonium salts of profenofos (IV) and bromoprofenofos (IVA) were merged with each other, whereas other compounds (III and IIIA) eluted at larger retention times. Thus, the elution step, starting with 50% of both 0.05M ammonium formate buffer and acetonitrile followed by a linear gradient with increasing concentration of acetonitrile, was selected. The gradient program is shown in Table I. Figure 2 shows a typical chromatogram of a synthetic mixture containing 500 µg/mL of PFS and 2.5 µg/mL each of all the process impurities. It can be seen from Figure 2 that all process impurities were well separated from PFS. The peaks were identified by injecting and comparing with the retention times of the individual compounds. The online UV spectra were recorded for all the compounds using photodiode array detector and found that the absorption maxima (λ_{max}) are in the range of 225–235 nm. A wavelength of 230 nm was selected to monitor these compounds. The retention time, capacity factor (k'), relative retention time (RRT), tailing factor, resolution, theoretical plates, and wave length of maximum absorption (λ_{max}) of PFS and its process impurities are recorded in Table II.

Table III. Precision Data for Spiking 0.5% of All Process Intermediates to PFS

	Retention time		Peak response	
Compound	Average	%RSD	Average	%RSD
I	5.28	0.21	70,780	1.41
II	7.37	0.11	151,250	2.16
IIA	8.31	0.31	132,364	2.26
III	15.74	0.08	218,520	1.79
IIIA	18.51	0.12	192,045	1.27
IV	3.36	0.96	179,548	1.73
IVA	3.52	0.24	211,845	1.93
V	12.46	0.07	10,870,353	0.85
PFS(iso)	10.85	0.09	258,493	0.90
VA	14.04	0.09	94,857	1.33

Precision

The precision of the method was checked by spiking 0.5% (w/w) of all process intermediates to PFS and injecting five times the solution. Chromatographic precision, expressed as relative standard deviation (RSD), was calculated for retention time, and peak area of all compounds were found to be not more than 0.31 and 2.26, respectively. The precision data are given in Table III.

Accuracy

Recovery studies were conducted by analyzing PFS (500 µg/mL), to which all process impurities (0.5% nominally) were spiked at six levels in the range of 25% to 150%. The recovery of impurities was expressed for each concentration as the mean

Table IV. Recovery Data from Analyzing PFS with Its Process Impurities

	Nominal 0.5% of impurity spiked to PFS range (%)					
	25	50	75	100	125	150
Amount added (µg/mL)	0.625	1.25	1.875	2.5	3.125	3.750
%Recovery (± %RSD)*						
I	98.65 ± 1.30	97.62 ± 2.08	97.62 ± 0.29	103.36 ± 1.15	101.02 ± 0.62	101.73 ± 0.98
II	99.48 ± 3.92	100.39 ± 0.73	102.54 ± 2.35	98.80 ± 1.22	94.72 ± 2.75	104.59 ± 3.35
IIA	98.25 ± 2.30	96.32 ± 1.20	101.20 ± 0.52	97.24 ± 1.50	96.85 ± 1.63	99.78 ± 1.20
III	99.53 ± 1.54	103.1 ± 5.85	93.30 ± 2.26	91.99 ± 2.26	88.40 ± 2.82	86.30 ± 0.84
IIIA	89.53 ± 1.23	93.1 ± 2.85	93.30 ± 2.26	91.99 ± 2.65	98.40 ± 1.82	96.30 ± 1.84
IV	88.85 ± 0.54	90.1 ± 3.55	92.85 ± 1.26	93.99 ± 0.89	101.23 ± 3.82	103.30 ± 0.95
IVA	90.43 ± 2.62	89.98 ± 1.92	94.30 ± 1.50	96.99 ± 1.26	98.85 ± 0.87	101.30 ± 1.35
PFS(iso)	99.30 ± 1.64	101.1 ± 0.85	98.30 ± 0.45	97.99 ± 0.87	96.40 ± 2.52	102.50 ± 1.13
VA	89.43 ± 2.42	93.1 ± 2.21	93.30 ± 1.16	97.99 ± 2.56	102.40 ± 2.62	99.30 ± 2.44

* $n = 3$.

Table V. Linearity Data for Six Solutions of Impurities

Compound	Range (µg/mL)	Regression equation	r^2	LOD (ng/mL)
I	1.25–7.50	$y = 27{,}919x + 3673$	0.999	109
II	1.25–7.50	$y = 59{,}011x + 236$	0.999	13
IIA	1.25–7.50	$y = 51{,}372x + 1651$	0.999	27
III	1.25–7.50	$y = 80{,}664x + 3369$	0.999	59
IIIA	1.25–7.50	$y = 73{,}456x + 6631$	0.999	80
IV	1.25–7.50	$y = 71{,}263x + 7745$	0.999	50
IVA	1.25–7.50	$y = 83{,}004x + 6850$	0.998	54
PFS(iso)	1.25–7.50	$y = 103{,}556x + 2996$	0.999	30
VA	1.25–7.50	$y = 34{,}873x + 3191$	0.999	126

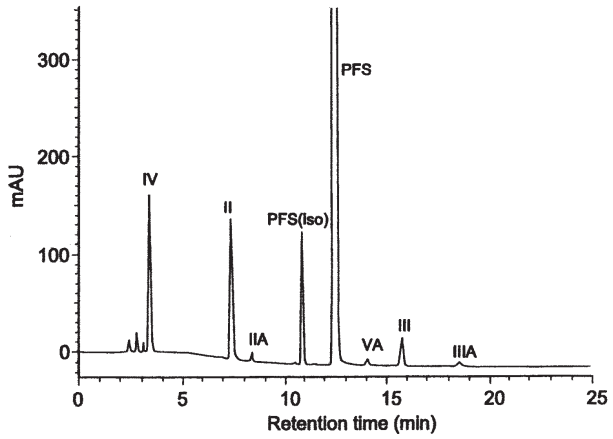

Figure 3. Typical chromatogram of a technical sample of PFS. For identification of peaks, see Figure 1.

percentage ratio between the measured amount and the actual value. The recoveries were between 88% and 104%, with RSDs less than 4%. The data are shown in Table IV.

Linearity

Six solutions of different concentrations of impurities ranging from 1.25–7.5 µg/mL were prepared for calibration and each one injected in triplicate ($n = 3$). The data were subjected to statistical analysis using a linear-regression least-squares method, and the peak areas of the individual impurities were found to be linear with respect to the concentration. The regression equations ($y = mx + c$) and the correlation coefficients (r^2) of all impurities are given in Table V.

Limits of detection and quantitation

The limits of detection (LOD) and quantitation (LOQ) were determined by measuring the magnitude of analytical background response [mean 0.044 mAU, RSD = 6.8% ($n = 4$)] by injecting blank samples. By substituting the mean value in the formula [signal to noise ratio (s/n) = 2 × height of peak/100 × base line noise] the s/n was calculated for each compound by injecting a series of solutions until the s/n 3.3 for LOD and 10 for LOQ were obtained. The LOD values thus determined are shown in Table V. The technical samples of PFS were analyzed five times, and the amounts of II, IIA, III, IIIA, IV, VA, and PFS(iso) were found to be 0.06%, 0.04%, 0.56%, 0.05%, 1.60%, 0.15%, and 0.68%, respectively. The typical chromatogram of a technical sample of PFS is shown in Figure 3. The technical sample of PFS was stored in the mobile phase for 24 h and analyzed by HPLC. No significant changes were observed in the chromatogram of PFS.

Conclusion

These results indicate that the RP-HPLC method developed and validated is useful to separate and determine the low levels of all process-related synthetic organic impurities of PFSs in process development and quality control. The method is accurate, precise, and offers good sensitivity to detect as low as 1.5×10^{-8} g of process impurities in technical products of PFS.

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