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Notes

Separation and Determination of Process-related Impurities of Industrial Chlorpyriphos by Reversed-Phase High-Performance Liquid Chromatography

Ramisetti Nageswara RAO,† Mariappanadar VAIRAMANI, Sara KHALID, and Syed Naseeruddin ALVI

HPLC Group, Division of Analytical Chemistry, Indian Institute of Chemical Technology, Hyderabad-500 007, India

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Introduction

O,*O*-Diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, known as chlorpyriphos (CPP) [CAS 2921-88-2], is one of the non-systemic organophosphorous pesticides that has a broad range of insecticidal activity: contact, stomach action, and respiratory action. It is used not only for the control of flies, household and various crop pests in soil and on foliage, but also against ectoparasites on cattle, sheep and poultry.[1,2] It is produced in large quantities by the condensation of sodium salt of 2-hydroxy-3,5,6-trichloropyridine (HTCP) with diethyl thionyl phosphoryl chloride (DETC).[3] Our laboratory has studied two different processes to obtain HTPC: i) the vapor phase chlorination of pyridine and ii) the condensation of trichloroacetyl chloride (TCAC) with acrylonitrile (ACN). The development of these two processes has required analytical methods for monitoring the related impurities of CPP, not only to follow the progress of chemical reactions but also to determine its quality.

Chemical methods by which CPP content is determined by phosphorous or nitrogen estimation suffer from interference from DETC and other related impurities.[4] Many methods based on gas chromatography (GC), thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) have been reported for determination of organophosphorus pesticides in biological as well as environmental matrices.[5-17] Husain *et al*. have developed gas and normal-phase liquid chromatographic methods for determination of chloropyridines in different process streams of CPP.[18] However, these methods do not address the problems of separation and quantitative determination of the hydrolysis product *viz*., HTCP, which is likely to be present in industrial products of CPP. Further, the normal-phase HPLC method developed by Husain *et al*. may not be compatible with samples of aqueous nature and would require prior treatment. The objective of this work was to develop and validate an alternative analytical method that allows the simultaneous detection and determination of related impurities of CPP by reversed-phase HPLC. Synthetic impurities include monochloropyridine (MCP), dichloropyridine (DCP), tetrachloropyridine (TCP), pentachloropyridine (PCP), HTCP, and DETC. In this paper, we describe a reversed-phase HPLC method using a Shimpac C_{18} column with acetonitrile–0.1% aqueous acetic acid (for improving peak characteristics and reducing the peak tailing) (60:40 v/v) as mobile phase at ambient temperature for separation and determination of process related impurities of CPP not only in the industrial products but also with different formulations.

† To whom correspondence should be addressed.
E-mail: rnrao@iict.ap.nic.in

Experimental

Materials

Samples of CPP, HTCP, MCP, DCP, TCP, PCP, and DETC were a kind gift from Vantech (Hyderabad, India). HPLC grade acetonitrile and acetic acid were obtained from Ranbaxy (Mumbai, India). The mobile phase and solutions were prepared in deionized double distilled water. Stock solutions of the compounds were prepared in the mobile phase and stored at room temperature. Fresh working solutions were prepared daily. All solutions were filtered (0.45 μm) and degassed by passing helium.

Apparatus

An HPLC system composed of two LC-10 AT Vp pumps, an SPM-10Vp diode array detector, an SIL-10 ADVp auto sampler, a DGU-12 A degasser, and SCL-10Avp system controller (all from Shimadzu, Kyoto, Japan) was used. A reversed phase C_{18} (Shimadzu) column (250 mm × 4.6 mm i.d., particle size 5 μm) was used for separation. The chromatographic and integrated data were recorded using the HP-Vectra (Hewlett-Packard, Waldbron, Germany) computer system.

Chromatographic conditions

The mobile phase was acetonitrile–0.1% aqueous acetic acid (60:40 v/v). Samples were dissolved in the mobile phase and the analysis was carried out under isocratic conditions at a flow-rate of 1 ml/min. Chromatograms were recorded at an absorption wavelength of 288 nm using diode-array detection.

Analytical procedure

Samples (10 mg) were dissolved in the mobile phase (10 ml); a 20-μl volume of each sample was injected and chromatographed under the above conditions. Synthetic mixtures and the industrial samples were analyzed under

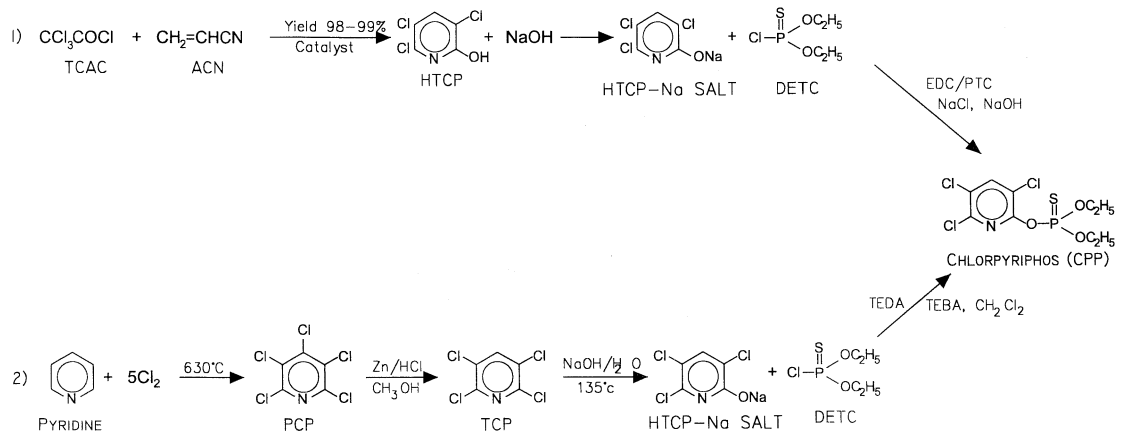

Fig. 1 Chemical reactions involved in preparation of CPP by two different processes.

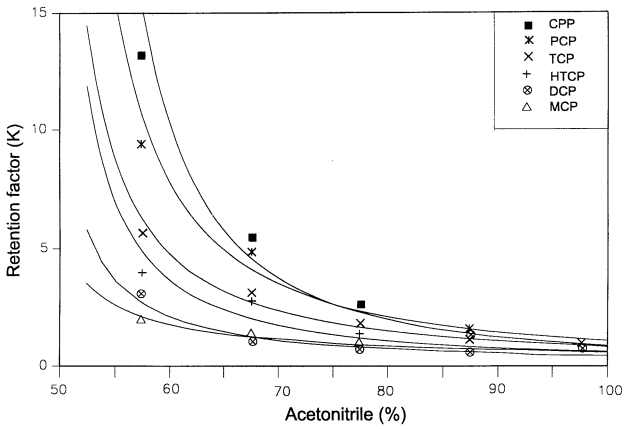

Fig. 2 Effect of concentration of acetonitrile on retention of CPP and its impurities.

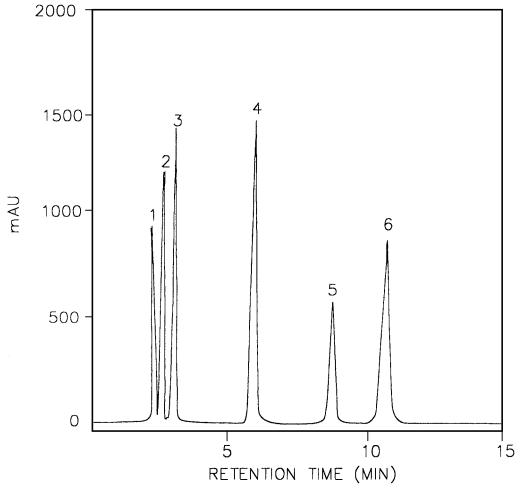

Fig. 3 HPLC chromatogram of a synthetic mixture containing 1, MCP (10 μg); 2, DCP (10 μg); 3, HTCP (10 μg); 4, TCP (10 μg); 5, PCP (12 μg); and 6, CPP (10 μg). For conditions see text.

identical conditions. The amounts of CPP and related impurities were calculated from the areas of the corresponding peaks in the chromatogram.

Results and Discussion

The chemical reactions involved in the two different processes followed for production of CPP are shown in Fig. 1. The figure shows that the step involving the condensation of HTCP and DETC is common to both the processes. During the course of this reaction, if the conditions are not controlled properly, DETC may undergo dimerisation at higher temperatures, reducing the yield and quality of CPP. The figure also shows that HTCP is obtained either by hot chlorination of pyridine or by reacting TCAC with ACN. It is possible that the unreacted chlorinated pyridines may be left over in small quantities in the finished products of CPP, affecting not only the quality but also the environment significantly. Thus, it is important to determine their levels in the finished products of CPP for assurance of its quality. To find the optimum separation conditions for a synthetic mixture containing CPP and its related impurities *viz*; MCP, DCP, TCP, PCP and HTCP, the influence of separation parameters such as concentration of ACN in the eluent on their retention was studied. ACN was used as an organic modifier of the mobile phase. The dependence of the retention factor (k) of the compounds under investigation on the content of ACN is shown in Fig. 2. When the concentration of ACN was at 60%, all the compounds and CPP were eluted and separated from one another. Figure 3 shows the HPLC chromatogram of a synthetic mixture containing CPP and its related impurities. The figure shows that all the chloropyridines are well separated from CPP. The peaks were identified by comparing the retention times with those of authentic standards injected separately.

Accuracy

The accuracy of the method was evaluated by analyzing independently prepared solutions of CPP spiked with different levels of impurities against the standard solutions of the same concentrations as external standards. The recovery data expressed as an average percent of triplicate injections are recorded in Table 1. Table 1 shows that the method is accurate for determination of process-related impurities of CPP quantitatively.

Precision

The precision of the method was investigated with respect to repeatability and intermediate precision. For intra-day

Table 1 Recovery data for standard mixtures

Compound	Abbreviation	Taken,%	Found,%[a]	Error,%
Chlorpyriphos	CPP	95.47	95.58	0.12
Monochloropyridine	MCP	1.20	1.16	3.33
Dichloropyridine	DCP	0.83	0.78	6.02
Tetrachloropyridine	TCP	1.05	1.09	3.80
Pentachloropyridine	PCP	0.92	0.97	5.43
Hydroxytrichloropyridine	HTCP	0.52	0.49	5.76

a. Average of three determinations.

Table 2 Intra-day and day-to-day precision of the related impurities of industrial chlorpyriphos

Compound	Concentration/ μg ml^{-1}	Intra-day RSD, % (n = 15)	Day-to-day RSD, % (n = 15)
MCP	5	6	5
DCP	4	5	5
TCP	2	3	3
PCP	3	4	5
HTCP	3	3	4

Table 3 Response data

Compound	Concentration/ μg ml^{-1}	Relative response factor	RSD, %[a]
CPP	950 – 990	2.33	1.45
MCP	1 – 10	2.93	1.75
DCP	1 – 10	1.00	1.97
TCP	5 – 15	7.83	1.08
PCP	3 – 15	2.34	1.63
HTCP	1 – 10	1.04	2.56

a. Average of three determinations.

Table 4 Typical composition of industrial chlorpyriphos

Compound	Content/g kg^{-1}[a]	RSD, %[a]
CPP	978.8 – 990.6	1.45
MCP	0.1 – 0.2	1.75
DCP	0.1 – 0.3	1.97
TCP	0.1 – 0.3	1.85
PCP	0.2	1.63
HTCP	0.5 – 2.5	2.56

a. Average of three determinations of five batches.

precision, three concentrations of each compound were analyzed in three independent series on the same day. Within each series, every sample was injected fifteen times. For an estimate of the day-to-day precision (inter-day precision), similar samples were analyzed on 3 consecutive days. Each sample was injected fifteen times. Table 2 summarizes the relative standard deviations (RSD) of the peak areas of the compounds under study. Generally acceptable repeatability of the areas within one day and day-to-day was observed. At the lowest concentration, the RSD of the area was between 3.05 and 4.62%, while at higher concentration, RSDs of about 1.48% or less were found. Data of the absolute and relative retention times obtained in a series of fifteen consecutive injections also showed acceptable repeatability when analyzed not only on the same day but also on three consecutive days.

Specificity

The specificity of the method was evaluated to ensure separation of all the process related impurities that are likely to be present in the technical products of CPP. The specificity of the method was demonstrated by assaying a solution of CPP of 1.0 mg/ml spiked with small amounts of MCP, DCP, TCP, PCP and HTCP. The method demonstrated not only the resolution between CPP and the potential impurities but also the resolution among the related impurities.

System suitability

System suitability of the method was evaluated by analyzing the symmetry of the CPP peak, theoretical plates of the column and the resolution between the peaks of CPP and other impurities. The concentration of CPP was selected at about 1.0 mg/ml to assure symmetry below 3.5 and to assume sufficient sensitivity for detecting low concentration impurities. The following parameters were summarized from runs with different lots of CPP: asymmetry <3.5, theoretical plates >6000, and resolution >2.

Linearity, range, limit of quantification and detection

Solutions of CPP at 0.1%, 1%, 10%, 50%, 70%, 80%, 100% and 150% of the specified concentration were assayed in duplicate using a reference material as external standard. The method exhibited acceptable linearity from 1% to 150%. The least-squares linear regression equation obtained was $y = 1.008x - 0.1464$, where x is the expected response and y is the observed response. The coefficient of correlation is 0.997 and the standard deviation of the line is 1.68. The method is considered to be linear. The range was determined to be 10% to 150%. In order to determine the linearity of MCP, DCP, TCP, PCP and HTCP in low concentrations the solution of CPP (impurities were spiked to a 0.5 mg/ml) at 0.05%, 0.1%, 0.2%, 0.5%, 1.0% (w/w). The mixtures were assayed in duplicate and the relative areas of impurities to that of CPP were plotted against the expected concentration. The least squares linear regression equation for HTCP is $y = 1.03x - 0.075$, where x is the expected percent concentration based on sample preparation and y is the observed percent concentration based on HPLC analysis. The coefficient of correlation was 0.983. The detector response to different compounds was determined as relative response factors and is given in Table 3. At 1.0 mg/ml, the limit of detection for impurities was around 5×10^{-9} g.

The quality of CPP in different batches of industrial products obtained from industry was thoroughly checked. On comparison with the retention times of authentic standards, the peaks were identified and their contents were determined quantitatively. The levels of impurities and the purity of CPP were calculated. The content of CPP was found to be in the range of 978 – 990 g/kg, while the total amount of impurities determined to be 10 – 22 g/kg. The results are summarized in Table 4. From these results, it is clear that the proposed RP-HPLC method is precise and accurate for the separation and determination of small amounts of process impurities that are likely to be present in the industrial samples of CPP. Thus the method is suitable not only for process development but also for quality assurance of CPP and related products.

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