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Note

Analysis of some chlorinated pyridine isomers in mixtures by reversed-phase high-performance liquid chromatography

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2,3,5,6-Tetrachloropyridine (TCP) and pentachloropyridine (PCP) isomers are important intermediates in the manufacture of pharmaceuticals, pesticides, herbicides, dyes and plastics. These compounds are obtained by vapour-phase chlorination of pyridine at relatively high temperatures. Some lower chlorinated pyridine isomers are also known to form during the process. Therefore, for monitoring the progress of the reaction, a complete analysis is necessary. Methods to characterize these compounds are available[1–6], but a lack of information[7] is seen in the literature for their separation and analysis. Recently, we have carried out the separation and identification of chloropyridine isomers in mixtures by gas chromatography–mass spectrometry (GC–MS)[8], in which only the quantitative determination of PCP is described. Quantitative determination of TCP could not be accomplished due to its incomplete separation. A simple, rapid and selective separaration and quantitative determination of TCP and PCP using reversed-phase high-performance liquid chromatography has now been developed and the results are reported here.

EXPERIMENTAL

A Waters Assoc. high-performance liquid chromatograph equipped with a Model 6000A solvent delivery system, a U6K injector, a UV detector (254 nm) and a Shimadzu chromatopak EIA integrator was used. A μBondapak C_{18} column (30 cm × 3.9 mm I.D.; 10 μm particle size) was employed.

Reagents and materials

2,3,5,6-Tetrachloropyridine and pentachloropyridine were obtained from Fluka (Switzerland) and were used as such, without any further purification. Spectroscopic grade chloroform (Sarabhai M chemicals, India), ethanol ("pro analysis", Merck, Darmstadt, F.R.G.), phenacetin (BDH, U.K.) and double-distilled water in all-glass apparatus were used.

A water–ethanol–chloroform (13:11:1) solvent system was freshly prepared before use.

Procedure

An aliquot of the mixture containing TCP and PCP was injected with a 10-

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μl Hamilton syringe and chromatographed at a flow-rate of 1.4 ml/min (1800 p.s.i.). The analysis was carried out at room temperature (27°C) under isocratic conditions.

RESULTS AND DISCUSSION

Many solvent systems have been tried to achieve good separation of chloropyridines. The best resolution of these compounds has been obtained using a water–ethanol–chloroform eluent. Poor peak shapes and longer retention times have been observed when common solvent modifiers, such as methanol, acetonitrile, etc., are used. To overcome this difficulty, ethanol[9] and chloroform[10] are chosen.

Fig. 1 shows a typical chromatogram of the separation of TCP and PCP in mixtures. The corresponding peaks are identified by injecting pure samples. Quantitative studies have been carried out by the standard addition method[11]. This method is generally employed for the determination of one or two components in a mixture.

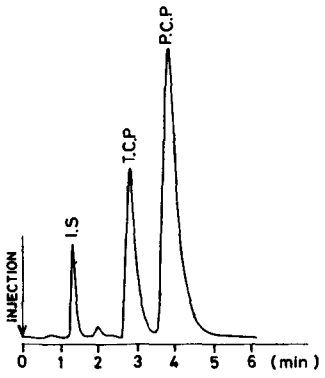

Fig. 1. Typical chromatogram showing the separation of PCP, TCP and the internal standard.

In the present method, the sample containing fixed amounts of TCP, PCP and internal standard (I.S.) is chromatographed and the peak heights are measured. The peak-height ratio of TCP and PCP, with respect to the I.S., is determined using eqns. 1 and 2

$$R_{TCP} = \frac{\text{Peak height of TCP}}{\text{Peak height of I.S.}} \quad (1)$$

$$R_{PCP} = \frac{\text{Peak height of PCP}}{\text{Peak height of I.S.}} \quad (2)$$

Known amounts of TCP and PCP are then added and the sample is rechromatographed. The addition of known amounts of TCP and PCP to the sample increases their respective peak heights, whereas the peak height of the I.S. remains constant.

The increase in peak height corresponds to the amount added and their proportions are calculated using eqns. 3 and 4

$$R_{\text{TCP (added)}} = \left[\frac{\text{Peak height of TCP after adding known amount}}{\text{Peak height of I.S.}}\right] - R_{\text{TCP}} \quad (3)$$

$$R_{\text{PCP (added)}} = \left[\frac{\text{Peak height of PCP after adding known amount}}{\text{Peak height of I.S.}}\right] - R_{\text{PCP}} \quad (4)$$

Finally, the concentrations of TCP and PCP present in the sample are calculated using eqns. 5 and 6

$$\text{Quantity of TCP} = \frac{\text{Quantity of TCP added}}{R_{\text{TCP (added)}}} \times R_{\text{TCP}} \quad (5)$$

$$\text{Quantity of PCP} = \frac{\text{Quantity of PCP added}}{R_{\text{PCP (added)}}} \times R_{\text{PCP}} \quad (6)$$

By employing the above-mentioned procedure, the validity of the method is tested against various synthetic mixtures of TCP and PCP. Phenacetin is used as the internal standard and peak heights are employed for the purpose of calculation. The results are given in Table I. It can be seen that the percentage relative errors are less than 1.14 and 1.05 for TCP and PCP, respectively.

The method is successfully applied for the determination of TCP and PCP in chlorinated pyridine residue and the results are shown in Table II. It can be seen from the results in Table II that the standard deviation is better than 0.88 and 1.47 for TCP and PCP, respectively.

TABLE I

ANALYSIS OF STANDARD MIXTURES

Sample mixture No.	*Amount taken (mg)*		*Amount found (mg)**		*Error (%)*	
	TCP	*PCP*	*TCP*	*PCP*	*TCP*	*PCP*
I	19.30	19.01	19.08	19.21	1.14	1.05
II	40.02	30.11	39.75	29.88	0.67	0.76
III	70.09	10.04	69.80	9.97	0.40	0.60
IV	52.41	25.72	51.90	25.58	0.97	0.54
V	36.25	48.46	35.87	48.04	1.05	0.87
VI	24.25	57.80	23.93	57.20	0.03	1.18
VII	40.54	60.85	47.89	60.67	1.11	0.30
VIII	63.71	37.15	62.97	36.89	1.16	0.71

* Average of three injections.

TABLE II

ANALYSIS OF TCP AND PCP IN CHLORINATED RESIDUE

Sample mixture No.	*Amount of TCP found (mg)**	*S.D.*	*Amount of PCP found (mg)**	*S.D.*
I	58.27		15.85	
	58.66		16.09	
	58.89	0.50	16.12	1.10
	57.98		15.64	
	58.25		15.73	
II	59.43		17.01	
	57.01		17.17	
	57.99	0.88	16.20	0.76
	58.18		17.24	
	58.65		16.89	
III	92.32		18.69	
	91.86		19.23	
	90.91	0.47	19.05	1.47
	91.53		18.98	

* Average of three injections.

This method appears to be simple, rapid, selective and requires only about 15 min for complete analysis.

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