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Note

Simple thin-layer chromatography method for detection of pentachlorophenol in sawdust and woodshavings

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Sawdust and woodshavings are extensively used in agriculture as a cheap source of litter for broiler chickens, turkeys, ducks, pigs and cattle. This material usually comes from the external layer of a tree that has been treated with pentachlorophenol (PCP) and its sodium salt which have excellent fungicidal properties and are used extensively in wood preserving solutions.

PCP is extremely toxic to young pigs[1], and its poisoning cases in cats have been reported in Perth[2], and in the Central Veterinary Laboratory[3]. Suspected PCP poisoning cases in chickens, pheasants and guinea pigs which have been reared on PCP treated sawdust and woodshavings beddings have also been found in the latter laboratory[4].

PCP in sawdust or woodshavings is usually determined by a gas–liquid chromatographic (GLC) method following an ion-exchange chromatography[5], which is not easily accessible and unsuitable for large batch analysis. A simple thin-layer chromatography (TLC) method is reported here for detection of PCP. PCP is extracted from sawdust into an acetic acid–methanol (1:9; v/v) mixture, the compound is then converted into chloranil by brief warming with concentrated nitric acid. The resulting chloranils are then spotted on thin-layer plates to separate from oxidised products of lower chlorophenols. The chloranils are detected by spraying a citric acid solution of tetramethyl-*p*-diaminodiphenylmethane (tetrabase). A blue oxidation product (quinoidal ion) of tetrabase is formed under heat, which colour intensity is proportional to the *O*- and *p*-chloranils formed.

EXPERIMENTAL

Materials

PCP was recrystallised from a technical product with ethanol–water mixture in the presence of decolorising carbon. A stock solution of 1 mg/ml was prepared in chloroform. Working standards of 10, 25, 50 and 100 μg/ml were prepared from the stock. Tetrabase reagent was prepared by dissolving 2.5 g of tetramethyl-*p*-diaminodi-

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phenylmethane and 10 g of citric acid in 10 ml of distilled water and dilute to 500 ml with distilled water. Silica gel G plates (20 × 20 cm²) were used. All solvents and chemicals used were AR grade.

Method

An amount of 1 g of specimens was weighed into a 50-ml beaker and cut to fine pieces with scissors. For feedstuffs specimens, they were ground into powder form first before extraction. PCP was extracted from the specimens with 10 ml acetic acid–methanol mixture at 55°C for 10 min. The extracts were then filtered into 10-ml graduated centrifuge tubes with Whatman No. 1 filter paper. The specimens were washed with another 5 ml of the acetic acid–methanol mixture, filtered and added to the previous fractions. Both specimens and standards (1 ml working standards) were blown down to dryness under a gentle stream of nitrogen in a 37°C water bath. A 0.1-ml volume of concentrated nitric acid was then added to each tube, stoppered and boiled for 3 min in a water bath. The tubes were then cooled and the volume was made up to 0.5 ml with methanol. Samples of 10 μl were spotted on the thin-layer plate. The plates were run in dichloromethane for 30 min and dried. Then they were sprayed with tetrabase reagent, dried at ambient temperature and developed at 110°C for 10 min.

RESULTS AND DISCUSSION

Solvent systems

A few solvent systems had been tried to find a better separation between PCP and the lower chlorophenols. The systems tried were: acetone–hexane (20:80 and 30:70); acetone–toluene (20:80 and 30:70); dichloromethane; chloroform and a chloroform–acetone–diethylamine (5:4:1) mixture. The toluene system gave hazy spots and no colour was obtained with the diethylamine system. The results obtained for other systems in terms of R_F values of oxidised chlorophenols are shown in Table I.

TABLE I

R_F VALUES OF CHLOROPHENOLS IN DIFFERENT SOLVENT SYSTEMS AFTER NITRIC ACID OXIDATION

Standards of 100 μg/ml were used. Figures in brackets indicate very weak colour spots. Solvent systems: I = acetone–hexane (20:80) fraction, II = acetone–hexane (30:70) fraction, III = chloroform and IV = dichloromethane. 2,3,4-Trichlorophenol, *o*-chlorophenol, *p*-chlorophenol, *p*-nitrophenol and dinitrobenzene do not give any colour reaction even at 500 μg/ml level.

Chlorophenols	*Solvent systems*			
	I	*II*	*III*	*IV*
PCP	0.34	0.45	0.64	0.6
2,3,4,5-Tetrachlorophenol	(0.34)	(0.45)	(0.66, 0.7)	(0.61, 0.68)
2,3,4,6-Tetrachlorophenol	0.31	0.4	0.61, (0.17, 0.71)	0.54, (0.12, 0.61, 0.65, 0.68)
2,3,5,6-Tetrachlorophenol	long blue strip	0.45, (0.32, 0.39)	0.64, (0.71)	0.61, (0.68)
2,3,5-Trichlorophenol	(0.23)	(0.39)	(0.53)	(0.46, 0.53)
Tetrachlorohydroquinone	0.34	0.45	0.64	0.6

Specificity and sensitivity

The method was sensitive to detect down to 2 μg/g of PCP in the specimens when 20 μl oxidised extract was applied onto the plate. Sensitivity down to 80 ng was achieved by reducing the amount of methanol added to the tubes. Standards of concentrations 2–150 μg could be used to read against the specimens. Tests had been carried out on the lower chlorophenols, *p*-dinitrophenol and *o*-dinitrobenzene. The results show that the test is highly selective for PCP, and most of the lower chlorophenols do not give the blue colour even when 500 μg/ml standards were used (see Table I). Tetrachlorophenols gave blue spots with similar R_F values and could interfere with the detection of PCP.

A typical thin-layer chromatogram of chloranils obtained by this method is shown in Fig. 1.

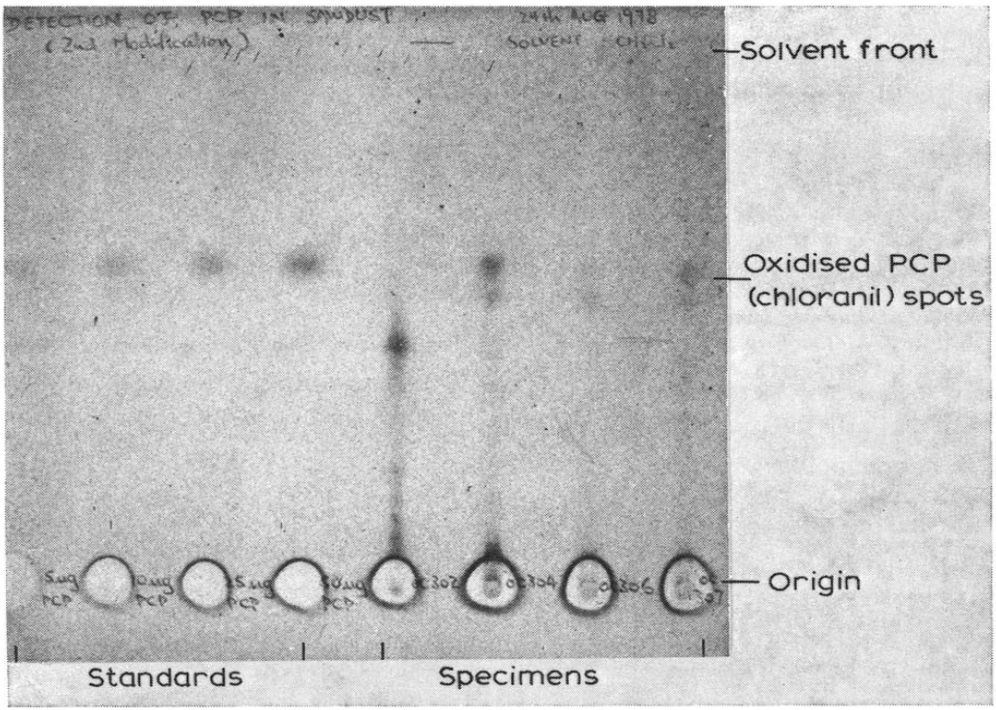

Fig. 1. A typical thin-layer chromatogram of oxidised PCP (chloranil) obtained by this method. Solvent dichloromethane.

PCP levels in different specimens had been obtained by both this method and the ion exchange–GLC method[5]. The results obtained were very similar ($t = 0.028$, $n = 14$). This method is much simpler and easier to carry out than the GLC method, and the whole procedure only takes about 90 min to give results. Though this method is less effective in separating tetrachlorophenols from PCP and the colour produced is only selectively good for PCP but not specific for it, this could be also an advantage for this method, as (1) tetrachlorophenols are also toxic to animals[6], the detection of

both compounds in the same specimen may be necessary; expecially when tetrachlorophenols are usually found in the technical PCP products, (2) tetrachlorophenols and other lower chlorophenols are metabolites of PCP in animals and bacteria in soil[7], the measurement of those break-down products at the same time may reflect the starting PCP level.

CONCLUSION

PCP can be separated and measured quantitatively by TLC. This TLC method is recommended for detection of PCP in sawdust, woodshavings and feedstuffs at field stations or veterinary investigation centres where no expensive instrumentation is installed, and also for batch analysis due to its low cost, rapidity and simplicity.

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