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Note

High-performance liquid chromatographic determination of pyridazinones in waste waters. II

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In a previous paper, the properties and determination of 5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone (PCA) and 4,5-dichloro-2-phenyl-3(2*H*)-pyridazinone (PCC) by gas chromatography (GC)[1] were described. To obtain sufficient information we have to combine the techniques of gas–liquid chromatography and high-performance liquid chromatography (HPLC). The liquid chromatographic studies of similar compounds were described by Harzer and Barchet[2] using a reversed-phase system and by Crathorne and Watts[3].

The aim of this work was to develop an HPLC method for the determination of pyridazinones in waste waters with the characteristics of applicability to very small amounts, a speed compatible with routine measurement requirements and reliability. We have compared our results statistically with GC results obtained previously[1].

EXPERIMENTAL

Reagents

MicroPak CN (10 μm) was purchased from E. Merck (Darmstadt, G.F.R.). Standards of PCA and PCC were obtained from the Research Institute of Agrochemical Technology (Bratislava, Czechoslovakia).

All solvents were of analytical-reagent grade and were distilled prior to use.

Instrumentation

A Packard liquid chromatograph equipped with a valve injector, a UV detector and a W + W recorder was used. Detection was carried out at 254 nm.

Procedure

The method commonly used for determining pyridazinones in waste waters is solvent extraction[1]. The concentrated chloroform extracts were injected into the (15 cm × 2 mm I.D.) column with a sample valve. Different mobile phases were used for PCA and PCC because of their different polarities, *viz.*, 2% methanol in chloroform–

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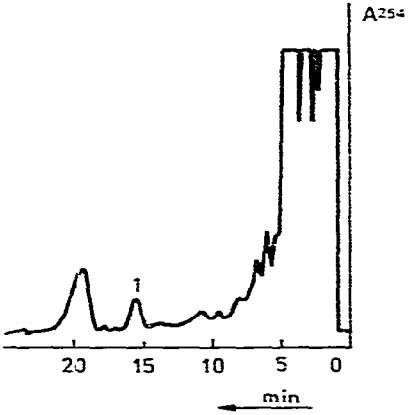
cyclohexane (1:1) and 10% chloroform in cyclohexane, respectively. Recovery experiments were performed in the same way using 1 mg/l standard solutions of PCA and PCC[1]. The results were evaluated by linear least-squares fitting[2] of the curves of amount of PCA or PCC injected against peak area. The concentrations of the standard solutions were 0.5–10.0 mg of PCA or PCC in 10 ml of chloroform and the calibration graphs were linear.

RESULTS AND DISCUSSION

The MicroPak CN column was found to be optimal, the shapes of the peaks of PCA and PCC being symmetrical. The selectivity of the separation systems used is demonstrated in Figs. 1 and 2, which show typical HPLC traces for the quantitative analysis of waste water extracts.



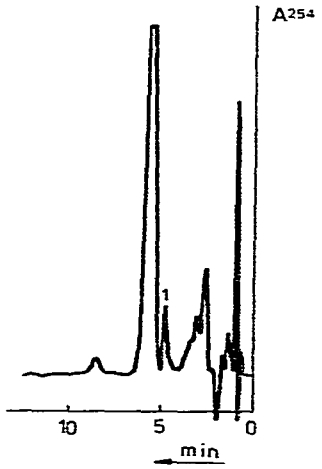


Fig. 1. HPLC results for determination of PCA (peak 1) in a concentrated chloroform extract of waste water. Flow-rate: 0.74 ml/min.

Fig. 2. HPLC results for determination of PCC (peak 1) in a concentrated chloroform extract of waste water. Flow-rate: 0.76 ml/min.

PCA gives a peak with a capacity ratio of 11.7 in 2% methanol in chloroform–cyclohexane (1:1) as the mobile phase and PCC a capacity ratio of 5.0 in 10% chloroform in cyclohexane.

The calibration graphs in Fig. 3 are straight lines with slopes of 0.143 for PCA and 0.255 for PCC, within a 1% deviation range, and pass through the origin. Each point was measured three times and average values have been plotted.

The amounts of PCC and PCA in waste water samples are given in Table I. The samples were the same as in the previous paper[1]. The limits of detection of injected standard were found to be 40 ng of PCC and 50 ng of PCA in 10 μl of solution.

An important problem arises comparing mean values ($\bar{x}_1$, $\bar{x}_2$) obtained by GC and HPLC for PCA and PCC in the same sample. There are two possibilities: (1) the HPLC and GC results are identical, which can be formulated statistically as a null

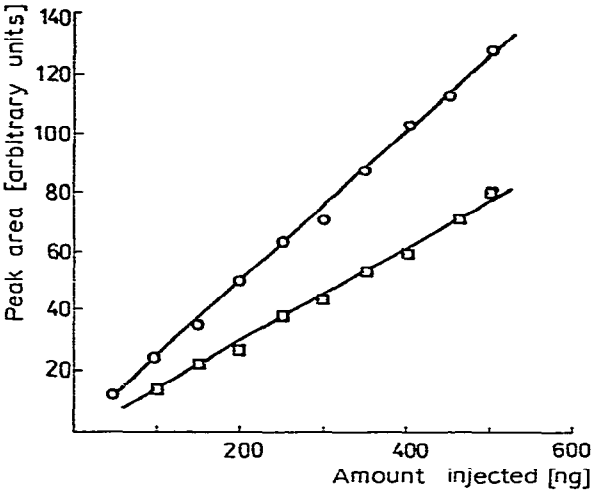


Fig. 3. Calibration graphs for the determination of PCA (□) and PCC (○).

hypothesis (H_0), or (2) the HPLC and GC results are different, which is formulated as a second hypothesis (H_1). If the null hypothesis is valid, a variable t, which is defined as

$$t = \frac{|\bar{x}_1 - \bar{x}_2| \sqrt{n-1}}{\sqrt{s_1^2 + s_2^2}} \quad (1)$$

must be less than the value $t_{\nu;\alpha}$ given in statistical tables[5], where ν is the number of degrees of freedom, calculated from the number of measurements ($n = 10$) as $\nu = 2(n - 1)$, and α is the probability of validity of hypothesis H_1 or H_0. In our case we chose a 95% level of probability ($\alpha = 0.05$)[5].

From the experimental data for PCA sample I:

$\bar{x}_1 = 1.69$ $\quad s_1 = 0.18$ (GC result[1])
$\bar{x}_2 = 1.43$ $\quad s_2 = 0.21$ (HPLC result)

the variable t was calculated according to eqn. 1 as $t_{calc} = 0.59$. In the statistical tables[5] the value $t_{8;0.05} = 2.306$ was found. It is obvious that calculated variable t is less than tabulated value and we can assume with 95% probability that the null hypothesis can be accepted, which means that the results of the GC and HPLC

TABLE I

RESULTS OF DETERMINATION OF PCA AND PCC IN WASTE WATERS

Sample	*PCA (mg/l)**	*PCC (mg/l)**
I	1.43 ± 0.21	4.02 ± 0.32
II	0.82 ± 0.16	4.21 ± 0.35

* Mean ± standard deviation (n = 5).

methods are not significantly different. It is therefore predictable that in all instances random errors will predominate. The null hypothesis can be also accepted in all PCC determinations that were tested in the same manner.

The results indicate that the use of HPLC for the determination of PCA and PCC in waste waters is potentially useful. The method gives reproducible results and allows the detection of trace amounts of PCA and PCC.

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