COLUMN AND THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF 5-AMINO-4-CHLORO-2-PHENYL-3(2H)-PYRIDAZINONE

K. ĎULÁK, J. KOVÁČ AND P. RAPOŠ

Research Institute of Agrochemical Technology, Bratislava-Predmestie (Czechoslovakia)

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5-Amino-4-chloro-2-phenyl-3(2H)-pyridazinone, common name pyrazon (PCA), is the active ingredient of a new herbicide known under the commercial names Pyramin or Burex. Technical PCA is contaminated by 4-amino-5-chloro-2-phenyl-3-(2H)-pyridazinone (iso-PCA) formed simultaneously by the reaction of 4,5-dichloro-2-phenyl-3(2H)-pyridazinone (PCC) with ammonia.

Until now only two authors are known to have dealt with analytical methods for PCA. DRESCHER[1], who described a method for PCA residue determination in plant and soil, and MEINECKE et al.[2], who dealt with quantitative PCA analysis in urine of experimental animals. The infrared spectra were described by BOURDAIS[3].

It is necessary to differentiate PCA from its isomer (iso-PCA) in a technical mixture, because in the application doses the iso-PCA present has no herbicidal effect and therefore it is considered as ballast[4].

The similarity of the chemical reactions of the constituents of the technical product, *i.e.* PCA, iso-PCA and unreacted PCC, their physical properties, low solubility in the solvents, reduces the choice of suitable methods for their selective determination. The most frequently occurring impurities in the technical mixture are reducible on a Hg-dropping electrode, and under the conditions shown in Table I they give well developed polarographic waves, but due to the coincidence of the two isomers it is not possible to use the method without preliminary separation.

TABLE I

HALF-WAVE POTENTIALS OF PCC, iso-PCA AND PCA IN 70% METHANOL

Compound	pH	$E_{1/2}$ (Volts)
PCC	7.0	—1.64
Iso-PCA	11.8	—1.86
PCA	11.8	—1.93

The same is true for absorption curves in the U.V. region. For these reasons a chromatographic method, with subsequent spectrophotometric evaluation, was chosen for the determination of the PCA content in the technical product. Aluminium oxide was used as adsorbent for this type of compound as recommended by SNYDER[5].

EXPERIMENTAL

The solvents were all analytical reagent grade.

Aluminium oxide for chromatography Brockmann's II (neutral) "Reanal" with 5 % water was used.

Standards were prepared by the Division of Organic Chemistry of our institute. PCA, m.p. 204–206° (Kofler); iso-PCA, m.p. 142–143.5° (Kofler); PCC, m.p. 162–163° (Kofler).

Thin-layer chromatography

The carrier glass plate (10 × 24 cm) was layered (1.4–1.7 mm thick) with aluminium oxide without a binder in the usual way. The solvent system used was chloroform–methanol (99:1). Each plate was developed by ascending chromatography in a suitable tank under conditions of equilibrium. Technical samples (180–200 mg) were chromatographed simultaneously with 150 mg of the synthetic mixture. In 100 ml calibrated flasks, the weighed amounts were dissolved in hot chloroform and after cooling immediately diluted with chloroform to the mark. 0.5 ml aliquots of these two solutions corresponding to 0.75–1 mg were applied quantitatively with a micropipette on the starting points. These points were on a line about 1.5 cm from the lower edge of the plate and the distance between the two points was 5 cm.

Development lasted 25–30 min; after this time the solvent front was found to be 2–3 cm from the top of the plate. The plate was taken out of the tank and air dried. The dried plate was observed under U.V. light. The separated compounds appeared as light-green fluorescent spots. The positions of the separated compounds (Table II) were marked off with a fine knife and the adsorbent from the marked places was transferred quantitatively into 20 ml glass extraction tubes. The isolated compounds were extracted with 5 ml of methanol into 50 ml calibrated flasks and diluted to the mark with water.

TABLE II

R_F VALUES FOR PCC, iso-PCA AND PCA

Compound	R_F
PCC	0.85
Iso-PCA	0.62.
PCA	0.19

Spectrophotometric measurements of the solutions obtained by the thin-layer chromatography and column chromatography were identical.

Column chromatography

The chromatographic tube was 80 cm long × 0.9 cm I.D., fitted at the lower end with a finely ground stopcock. The upper part formed a 250 ml reservoir. One quarter of the chromatographic tube was filled with benzene, a pad of cotton-wool was inserted above the stopcock and 6 g aluminium oxide poured into the tube.

The technical sample was finely crushed, and 50 mg was mixed with small

amounts of adsorbent and then quantitatively transferred to the top of the prepared column. This thoroughly dispersed sample formed a 0.5 cm layer with the adsorbent.

The column was washed with 125 ml benzene; 125 ml 5 % chloroform in benzene; 125 ml 10 % chloroform in benzene and so on (with every 125 ml solution added, we increased the amount of chloroform by 5 %).

The ratio of 6 g of adsorbent to the basic amount of solvent (125 ml) was found experimentally and depended on the activity of the adsorbent, on the height of the column and on the particle size. Flow rate of the solvent was 1.3–1.6 ml per min. Changes in the flow rate influenced the shape of the elution curves. Too fast a flow rate resulted in elongated elution curves, whereas a slow one prolonged the whole procedure.

For perfect discernment of PCC from iso-PCA whose elution peak (Fig. 1) is very close, 10 ml fractions of the eluate had to be withdrawn. When determining the PCA in the technical sample 25 ml fractions were withdrawn, because the elution wave of PCA was sufficiently distant from the waves of the accompanying compounds (especially iso-PCA).
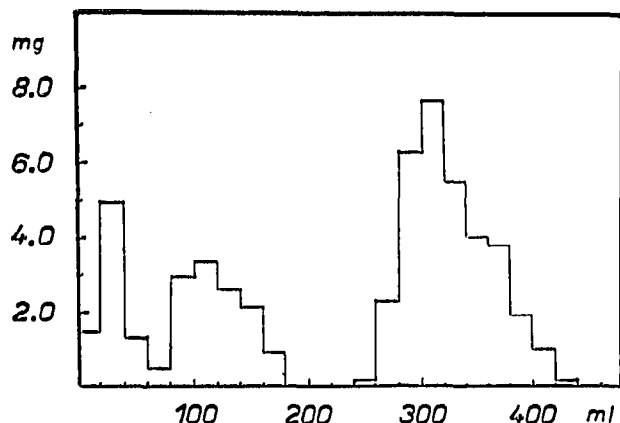

Fig. 1. Chromatogram of a synthetic mixture. Single components are given in the following order: PCC, iso-PCA and PCA. The amount of the components (in mg) corresponds to 20 ml fractions.

All fractions were evaporated to dryness. Like fractions were dissolved in methanol and all put into one 2 l calibrated flask. The methanolic solution was diluted with water so that the ratio of methanol to water was 2:8. For determination of PCC a methanolic solution was used.

As the solubilities of PCC, iso-PCA and PCA in water at 20° were substantially higher (Table III), the sample cannot be separated from the solution.

TABLE III

SOLUBILITY OF PCC, iso-PCA AND PCA IN WATER AT 20°

PCC	0.031 g/l
Iso-PCA	0.161 g/l
PCA	0.392 g/l

A complete analysis of PCA by this method requires approximately 8–10 h.

For the confirmation of this procedure infrared spectroscopy was used to study the single isolated fractions. It was found, that the infrared spectrum of the standard PCA in Nujol has its absorption bands at 656, 699, 707 (double), and 888 cm^{-1}, whereas the infrared spectra of fractions corresponding to PCA had similar absorption bands at 660, 701, and 902 cm^{-1}. The spectra of solutions were identical. There were two crystalline modifications of the same material, which we proved by X-ray diffraction. Modification "A" changes into "B" by heating to 150–170°. This phenomenon can be observed under a microscope. The process is reversible and by recrystallization we obtain again modification "A".

Spectrophotometric determination of isolated PCC, iso-PCA and PCA

The isolated compounds can be determined by various methods. Ultraviolet spectrophotometric determination was used because all the mentioned compounds have their absorption spectrum in this region (Fig. 2) and because the measurement is simple and precise enough. Molar absorptivities of the compounds are given in Table IV. The Lambert–Beer law is valid for methanol and water–methanol solutions at the wavelength of the absorption maximum.
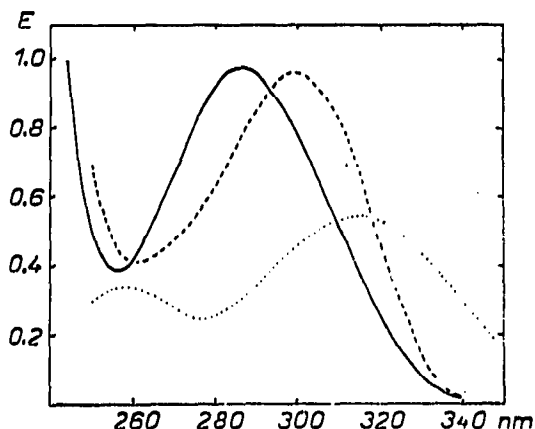

Fig. 2. Ultraviolet spectra of methanolic solutions of PCC (· · ·), iso-PCA (- - -), and PCA (———), at a concentration of 10^{-4} M.

TABLE IV

MOLAR ABSORPTIVITIES OF PCC, ISO-PCA AND PCA

	Wavelength (nm)	ε (l·gmole^{-1}·cm^{-1})
PCC	315	$5.4 \cdot 10^3$
Iso-PCA	300	$9.6 \cdot 10^3$
PCA	284	$9.7 \cdot 10^3$

TABLE V

SOME ANALYSES OF KNOWN MIXTURES OF PREDETERMINED COMPOSITION USED FOR CHECKING THE COLUMN METHOD

	Taken		Found		Taken		Found		Taken		Found	
	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%
PCC	7.10	14.36	6.94	14.04	6.91	12.97	6.46	12.13	2.80	5.39	2.13	4.14
Iso-PCA	12.06	24.40	12.25	24.78	12.01	22.55	12.28	23.05	7.59	14.76	8.03	15.61
PCA	30.27	61.24	29.65	59.98	34.35	64.48	34.45	64.67	41.04	79.85	40.73	79.19
Σ	49.43	100.0	48.84	98.80	53.27	100.0	53.19	99.85	51.43	100.0	50.89	98.94

RESULTS AND DISCUSSION

Both chromatographic procedures, which were tried on model mixtures (for typical results, see Table V) differ in time and amount of work. Both procedures can be used for the simultaneous determination of PCC, iso-PCA and PCA contents, and this was found to be advantageous for the study of the reaction kinetics of PCA.

Thin-layer chromatography is quicker but requires greater skill. Column chromatography takes more time but is simple and therefore more suitable for routine analyses. Both procedures are equivalent as far as their accuracy and precision is concerned.

SUMMARY

Two methods for the chromatographic separation of technical 5-amino-4-chloro-2-phenyl-3(2H)-pyridazinone (PCA) were developed. These methods also permit the quantitative determination of its isomer (4-amino-5-chloro-2-phenyl-3-(2H)-pyridazinone, iso-PCA) and 4,5-dichloro-2-phenyl-3(2H)-pyridazinone (PCC). The mobile phase for the thin-layer chromatography was chloroform with 1 % methanol, while for the column chromatography benzene with increasing chloroform content was used. In both methods aluminium oxide was used as adsorbent. The isolated compounds were dissolved in methanol and diluted with water. The absorbances were measured at the wavelength of absorption maximum.

It was found that PCA has two crystalline modifications and that the compounds described are polarographically active.

REFERENCES

1 N. DRESCHER, *Vorträge anlässlich der Wissenschaftlichen Aussprache über chemische Unkrautbekämpfung in Zuckerrüben mit Pyramin, BASF, Ludwigshafen am Rhein, 8.–9. Januar, 1964.*
2 K. H. MEINECKE, H. FROHBERG AND E. SCHULZE, *Vorträge anlässlich der Wissenschaftlichen Aussprache über chemische Unkrautbekämpfung in Zuckerrüben mit Pyramin, BASF, Ludwigshafen am Rhein, 8.–9. Januar, 1964.*
3 J. BOURDAIS, *Bull. Soc. Chim.,* 9 (1964) 2124.
4 P. RAPOŠ, J. SYNAK AND P. WINTERNITZ, *Chem. Zvesti,* 19 (1965) 403.
5 L. R. SNYDER, *J. Chromatog.,* 17 (1965) 73.