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Note

High-performance liquid chromatographic separation of cyromazine and its metabolite melamine

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Cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) (I, Fig. 1) is an insect growth regulator with specific activity against dipterous larvae. It is used to control fly larvae on animals and is also recommended against leafminers of the *Liriomyza* genus. When used on ornamental plants and vegetables, cyromazine exhibits a systemic action, with a strong translaminar effect when applied to the leaves; applied to the soil it is taken up by roots and translocated acropetally[1].

In both plants and animals, cyromazine undergoes degradation, the cyclopropyl group being eliminated to form melamine (1,3,5-triazine-2,4,6-triamine) (II, Fig. 1). This metabolite is of great importance owing to its potential carcinogenicity[2] even though at high dosages; therefore, in pesticide tolerances for cyromazine, the residue limits are established as the sum of the active ingredient (a.i.) and its metabolite.

Gas chromatographic[3,4] and liquid chromatographic[5–7] methods have been developed for the determination, in different matrices, of melamine, which also originates from the degradation of *s*-triazine herbicides and is also employed in the manufacture of thermosetting plastics for houseware.

For the determination of cyromazine and melamine residues, gas chromatographic methods have recently been reported[8,9], and also a high-performance liquid chromatographic (HPLC) method[10].

In this paper, the HPLC separation of cyromazine from melamine by means of columns with different mechanisms is reported.

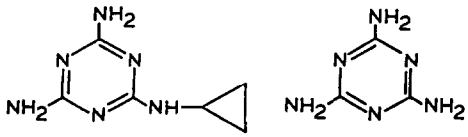

Fig. 1. Cyromazine (I) and its major metabolite melamine (II).

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EXPERIMENTAL

Apparatus

A Varian (Palo Alto, CA, U.S.A.) Model 5020 liquid chromatograph was used, fitted with a UV-100 variable-wavelength UV–VIS detector and a Rehodyne injection valve (50-μl loop), connected to an HP 3390 A reporting integrator (Hewlett-Packard, Avondale, PA, U.S.A.).

Chromatography

Hibar RP-8 and RP-18 (250 mm × 4.0 mm I.D.; 10 μm) (Merck, Darmstadt, F.R.G.) and Whatman (Clifton, NJ, U.S.A.) Partisil 10 SAX (250 mm × 4.6 mm I.D.; 10 μm) columns were used. The mobile phase was acetonitrile–$0.5 \cdot 10^{-3}$ *M* sulphuric acid (in different ratios) for the RP columns and phosphate buffer (at different pH values) for the SAX column. The flow-rate was 1 ml/min for the RP columns and 0.8 ml/min for the SAX column; the detector was always operated at 214 nm.

Chemicals and materials

Acetonitrile was of HPLC grade (Carlo Erba, Milan, Italy) and potassium dihydrogen phosphate, phosphoric acid, sodium hydroxide and sulphuric acid were of analytical-reagent grade (Carlo Erba). Water was distilled twice and filtered through a Milli-Q apparatus (Millipore, Molsheim, France) before use. The pH of the buffer solution, (10^{-2} M KH_2PO_4) was adjusted to the required value with H_3PO_4 or NaOH. The analytical standard of cyromazine (99.3%) was obtained from Ciba Geigy (Milan, Italy); melamine (99.5%) was purchased from Carlo Erba.

RESULTS AND DISCUSSION

In the FDA method[10], the separation of cyromazine from melamine was achieved with a normal-phase (amino-bonded) column and acetonitrile–water (95:5, v/v) as the mobile phase; on the reversed-phase columns this mobile phase did not allow, in any ratio, the separation of the two compounds. By replacing the water in the mobile phase with a $0.5 \cdot 10^{-3}$ M sulphuric acid, the separation of cyromazine from melamine was achieved (Table I). On reducing the percentage of sulphuric acid in the mobile phase, the retention times of I and II increased (more on the RP-8 than on the RP-18 column). Analogous results were achieved with methanol–sulphuric acid as eluent, but the peak sharpness was poorer than with acetonitrile. The best resolution was achieved with the RP-8 column by using acetonitrile–sulphuric acid (50:50, v/v) as the mobile phase (Fig. 2). Under these conditions, the detection limits for cyromazine and melamine were 0.02 and 0.01 ppm, respectively; the calibration graphs were constructed by plotting peak height *vs.* concentration; good linearity was achieved in the range 0–2 ppm with correlation coefficients of 0.9997 and 0.9998 for I and II, respectively.

With the SAX column, at a mobile phase pH of 3, cyromazine and melamine were hardly retained and their separation was impossible. On increasing the pH, the retention times of I and II also increased (Table I); at pH 4.5 a good separation was achieved (Fig. 2). A further increase in pH did not result in a better separation, and the peak sharpness, especially with regard to cyromazine, became poorer. The detection

TABLE I

RETENTION TIMES OF CYROMAZINE AND ITS METABOLITE MELAMINE WITH DIFFERENT COLUMNS AND ELUENTS

Column	*Mobile phase*	*Flow-rate (ml/min)*	*Retention time (min)*	
			Melamine (II)	*Cyromazine (I)*
	Acetonitrile–sulphuric acid:			
RP-8	40:60	1.0	4.34	5.63
	50:50	1.0	5.39	6.89
	60:40	1.0	6.31	8.00
RP-18	50:50	1.0	4.26	5.44
	60:40	1.0	6.33	8.03
	70:30	1.0	8.08	10.19
	Buffer:			
SAX	pH 3.0	0.8	3.30	3.67
	pH 4.5	0.8	4.64	6.64
	pH 5.5	0.8	7.26	10.02

limits at pH 4.5 were similar to those obtained with RP-8 and reported above.

The possibility of achieving the separation of cyromazine from melamine by means of columns with different mechanisms may be useful in residue determinations, either as a confirmatory assay or to overcome the problem of interfering compounds in different samples.

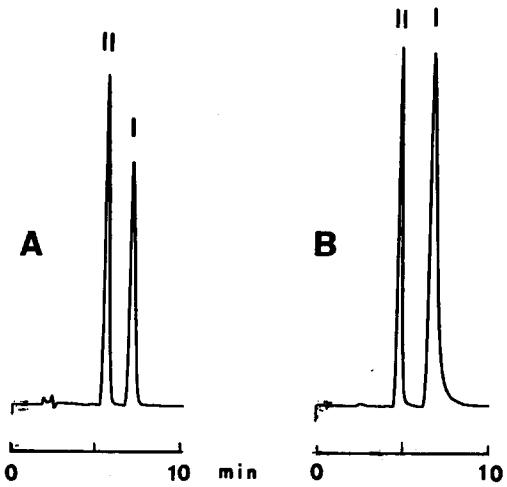

Fig. 2. Chromatography of cyromazine (I) and melamine (II). (A) RP-8 column; mobile phase, acetonitrile–0.5 · 10^{-3} M sulphuric acid (50:50, v/v); flow-rate, 1 ml/min; detection, UV at 214 nm. (B) SAX column; mobile phase, phosphate buffer (pH = 4.5); flow-rate, 0.8 ml/min; detection, UV at 214 nm.

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