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Note

Determination of amitrole by ion-pair high-performance liquid chromatography

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Residues of the herbicide amitrole (3-amino-1,2,4-triazole) in fruit and vegetables can be estimated by various colorimetric methods employing N-(1-naphthyl)-ethylenediamine[1], 8-amino-1-naphthol-3,6-disulphonic acid (H-acid)[2] or 1,8-dioxynaphthalene-3,6-disulphonic acid (chromotropic acid)[2] for coupling with the diazotized amine. Agrawal and Margoliash[3] studied the parameters of the coupling reaction of amitrole and H-acid, and pointed out that the presence of excesses of reagents, especially nitrous acid, caused variable colour intensities and instability of the coloured products.

Pribyl *et al.*[4] concluded that direct high-performance liquid chromatography (HPLC) of amitrole was not possible due to its lack of absorption in the ultraviolet or visible range, and they proposed analysis by thin-layer chromatography after reaction with 5-dimethylaminonaphthalene-1-sulphonyl chloride (dansylation).

The method described in this paper is based on a combination of the Storherr and Burke[1] procedure for extraction and clean-up, and the optimum H-acid coupling described by Agrawal and Margoliash[3], followed by chromatographic clean-up on polyamide and ion-pair HPLC. The polyamide clean-up was a modification of a method for the determination of synthetic acid food colouring matters[5].

EXPERIMENTAL

Extraction and clean-up

Potatoes and fodder beet samples were first extracted with ethanol, then adsorbed on resin. This was followed by acetonitrile and acid digestion clean-up according to the Storherr and Burke[1] procedure, except that the amounts of reagents and sample were halved. The final volume was adjusted to 10 ml with water. The final concentration was 3.5 *M* sulphuric acid.

Colour development and polyamide clean-up

Aliquots (2 ml) corresponding to 20 g of sample were transferred to small

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tubes. Each sample was diazotized with 0.1 *M* sodium nitrite (0.5 ml) with constant magnetic stirring at room temperature (*ca.* 25°C). After exactly 2 min, 0.1 *M* sulphamic acid (0.5 ml) was added followed by 1 m*M* H-acid in 50% ethanol (0.5 ml, monosodium salt of H-acid; Fluka, Buchs, Switzerland). The pH was adjusted to 4–5 with ammonia (3 *M*). A column was prepared by suspension of 1 g of polycaprolactam (Polyamide CC 6, grain size <0.16 mm; Macherey, Nagel & Co, Düren, G.F.R.) in water (25 ml) for 15 min. The suspension was poured onto a small cotton plug in a chromatographic tube (15 mm I.D.) and rinsed with water followed by 0.15 *M* ammonia in 95% methanol (5 × 10 ml). The column was further rinsed with water (5 × 10 ml) and the sample was transferred onto the column by rinsing with water (6 × 10 ml). The coloured reaction products were eluted with 0.15 *M* ammonia in 95% methanol (10 ml) and the eluate was concentrated to 1–0.5 ml. The final volume was adjusted to 5 ml with methanol–water (9:11). Standards were prepared from 3.5 *M* sulphuric acid solutions of amitrole (≤99%, Pestanal; Riedel-de Haën AG, Seelze-Hannover, G.F.R.) by the same colour development and polyamide clean-up procedures.

HPLC

The HPLC conditions were: instrument, Waters Model M-6000 A (Waters Assoc., Milford, MA, U.S.A.); column, 10-μm μBondapak C_{18}, microparticulate silica (Waters Assoc.), 3.9 mm × 30 cm; mobile phase, methanol–water (9:11 or 13:27), and 0.005 *M* counter-ion (PIC reagent A, tetrabutylammonium phosphate; Waters Assoc.), flow-rate, 1.2 or 1.5 ml min^{-1}; detector, 546 nm (Model 440, Waters Assoc.); sample size, 10 or 20 μl; amplifier range 0.05 or other appropriate setting. The peak height responses were recorded. The response of the standard was linear with concentration within the range of interest.

RESULTS AND DISCUSSION

The amount of H-acid reagent was in excess of amitrole on a molar basis up to 40 μg of amitrole. HPLC of the products of colour formation showed two peaks with retention times 4.0 min (A) and 4.7 min (B) (Fig. 1). For both peaks linearity was found for the colour formation in range from 0 to 25 μg of amitrole. Amounts of amitrole above 25 μg resulted in two additional peaks at 7.2 min (C) and 9.2 min (D). With increasing amounts of amitrole the heights of peaks A and B decreased with a simultaneous increase in that of D, and the colour turned from red to purple. This change of reaction mechanism is illustrated in Fig. 2 by a log–log plot of the HPLC peak heights *versus* the amount of amitrole.

No significant interferences were detected in the analyses of blanks containing all reagents and extracts of untreated plant material. The detection limit defined as twice the baseline noise was 0.005–0.01 mg kg^{-1} in potatoes or fodder beets. The limit depended on the condition of the HPLC column and on the nature of the sample. Peaks A and B were both suitable for estimation of residues, and the sensitivities were *ca.* 14 mm of recorder scale per ng amitrole injected at a detector range setting of 0.05 and recorder scale 1 mV. However, the formation of the derivatives of amitrole with H-acid depends on the quality of the preceding clean-up. Sometimes the original Storherr and Burke[1] procedure fails to yield sufficient recovery due to losses in the

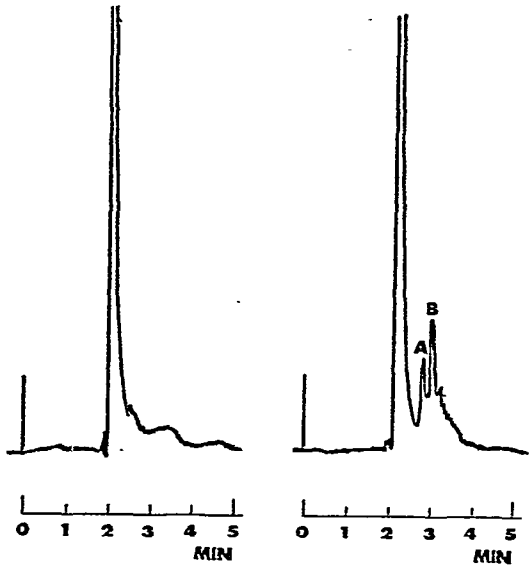

Fig. 1. HPLC chromatogram of potato samples: untreated control (left) and fortified sample (0.05 mg kg^{-1}). Peaks: A and B = H-acid–amitrole. Conditions: mobile phase, methanol–water (9:11), flow-rate 1.5 ml min^{-1}; sample size, 10 μl; amplifier range setting, 0.05.

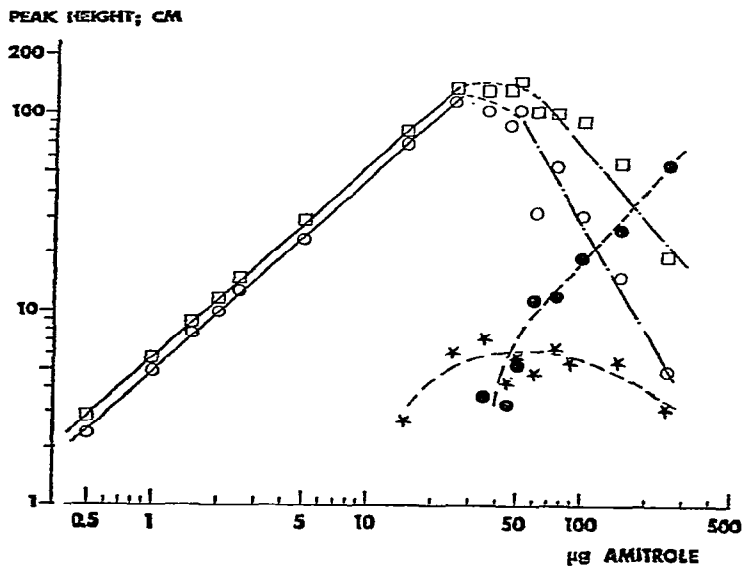

Fig. 2. Dependence of HPLC peak heights of coloured H-acid–amitrole derivatives A (○), B (□), C (★) and D (●) on amount of amitrole added in coupling reaction, shown in the log–log system. Conditions: mobile phase, methanol–water (13:27), flow-rate 1.2 ml min^{-1}; sample size, 20 μl; amplifier range setting reference, 0.05.

clean-up steps or inhibition of the colour formation. This type of problem will, of course, be reflected in the present HPLC procedure.

The repeatability of the colour development and polyamide clean-up of 2 μg amitrole standards was checked by performing eight replications. The coefficient of variation of peak A was 5.8% and of peak B 3.8%. It was found that hydrochloric acid could be used instead of sulphuric acid in the colour development as described by Agrawal and Margoliash[3] without significant changes in the colour intensity. The polyamide clean-up gave coloured derivatives which were stable indefinitely if stored at 5°C and protected from light.

Upon modification this method may also be applicable to the extension of other spectrophotometric procedures for the determination of pesticide residues into HPLC procedures, allowing better selectivity and possibly multiresidue detection.

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