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Note

Determination of thiabendazole residues in marmalades by high-performance liquid chromatography

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Thiabendazole [2-(4-thiazolyl)benzimidazole] (TBZ) is a post-harvest fungicide for the protection of citrus fruits and bananas¹. Most European (Austria, Belgium, France, Great Britain, Italy, The Netherlands, Switzerland) and North American countries (Canada, U.S.A.) authorize the utilization of this stable product at concentrations of 6–10 mg/kg for citrus fruits and 3 mg/kg for bananas. No norms are specified for marmalades prepared with these fruits. We wished to monitor the levels of TBZ residues in commercial products made from cooked fruits.

Many methods have been proposed for the qualitative and quantitative determination of TBZ on or in treated fruits^{2–10}, but not for determination in marmalades. As for the analysis of other preservatives^{11,12}, high-performance liquid chromatography (HPLC) was chosen for this problem.

EXPERIMENTAL

Reagents

Ethyl acetate, methanol, ammonium nitrate, 1.0 *N* sodium hydroxide solution and 0.1 *N* hydrochloric acid were obtained from Merck. Ammonia solution (25%) was obtained from UCB.

Sodium acetate–sodium chloride solution was prepared by dissolving 33 g of sodium acetate (Merck) and 200 g of sodium chloride (Merck) in sufficient distilled water to make 1000 ml.

A 1000 ppm stock standard solution of thiabendazole (Merck, Sharp & Dohme) in ethyl acetate was prepared and working standard solutions were prepared by dilution of the stock solution with methanol.

Apparatus

HPLC was carried out with a Pye Unicam Type PCXPD instrument with a dual-piston reciprocating pump, Rheodyne injection valve (Model 7120; 20 μ l) and a variable-wavelength detector with a (Macherey, Nagel & Co.). Philips PM 8251/02 recorder. The column packing was Polygosil 60/7.5/18.

Procedures

Extraction. The two methods chosen for extraction were based on the Merck

method² for the spectrofluorimetric determination of TBZ residues in orange marmalades and Tafuri *et al.*'s method¹⁰ for the HPLC determination of TBZ in fruits.

For curds (artificial marmalades) and lemon marmalades, a 20-g amount of homogenized sample, 10 ml of sodium acetate–sodium chloride solution and 10 ml of ethyl acetate are placed in a 50-ml centrifuge tube, which is agitated mechanically for 10 min and centrifuged. The ethyl acetate fraction is transferred into a 50-ml calibrated flask (repeat twice) and diluted to 50 ml with ethyl acetate. A 20-ml aliquot is evaporated to dryness using a rotary evaporator, 2 ml of methanol are added to the residue and 20 μ l of the resulting solution are injected on to the chromatographic column.

For orange marmalades, to the ethyl acetate extract obtained as above are added 10 ml of 1.0 *N* sodium hydroxide solution. After vigorous agitation for 5 min, the alkaline phase is discarded and TBZ is extracted by agitation with 10 ml of 0.1 *N* hydrochloric acid. To the separated acid extract are added 1 ml of 1.0 *N* sodium hydroxide solution, 5 ml of sodium acetate–sodium chloride solution and 25 ml of ethyl acetate. The mixture is agitated mechanically for 10 min and centrifuged. The ethyl acetate fraction is transferred into a calibrated flask and diluted to 50 ml with ethyl acetate. The procedure is then continued as for curds and lemon marmalades.

Chromatography. The mobile phases were an aqueous solution containing 0.8% (m/v) of ammonium nitrate and 0.7% (v/v) of ammonia (buffered solution)–methanol (1:1) for curds, and buffered solution–methanol (3:2) for marmalades.

The flow-rate in the column was 1.0 ml/min, the column temperature was 22–26°C, the detection wavelength was 305 nm and peak-height measurement was used for quantitation.

RESULTS

The selectivity and sensitivity with the proposed buffered mobile phase are superior to those with the non-buffered mobile phase of Tafuri *et al.*¹⁰ (Table I).

TABLE I
CHROMATOGRAPHIC CHARACTERISTICS IN TWO DIFFERENT SYSTEMS

Solutions containing 40 ppm of TBZ.

Procedure	Retention time (min)	Peak height (cm)	Peak width at half-height (cm)
Non-buffered system ¹⁰	7.3	3.85	1.29
Buffered system (this work)	8.3	16.70	0.34

Using the proposed conditions, the column performance remained constant throughout 2 months of daily use. The linearity of response is excellent between 1 and 60 ppm. The detection limit is 0.1 ppm.

Table II gives recoveries for a concentration of 2.5 ppm. Ten commercial products (two curds, three lemon marmalades and five orange marmalades) were examined by this method. These samples were free from TBZ (less than 0.1 ppm).

TABLE II

RECOVERIES OBTAINED WITH THE PROPOSED PROCEDURE

Extraction procedures as described under *Extraction*. 2.5 ppm thiabendazole.

Sample	Mobile phase	Recovery (%)
Pure solution	Buffered solution-methanol (1:1)	96.27 (86.30-97.50)
Curds	Buffered solution-methanol (1:1)	87.20 (85.00-88.79)
Lemon marmalades	Buffered solution-methanol (3:2)	85.90 (83.70-89.00)
Orange marmalades	Buffered solution-methanol (3:2)	84.66 (82.31-86.67)

The time required for a determination is about 30 min. With a limit of detection of 0.1 ppm and a recovery of more than 85%, the proposed procedure should be a good method for routine use.

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