reproducibility and linearity over a wide range of xanthine levels. The Bond Elut extraction procedure, when used in conjunction with the modified HPLC procedure presented here, provides for the complete analysis of 10 samples in a total of 60–70 min, from extraction to chromatographic results. This fact, when considered with the simplicity of the method and the selectivity with which xanthines are separated from interfering compounds, makes the method advantageous for any laboratory involved in the routine analysis of caffeine.

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Determination of Triforine in Fruit Crops as *N,N'*-Bis(pentafluorobenzoyl)piperazine

A method was developed for the determination of triforine and its piperazine metabolites in fruit. The procedure involves hydrolysis to piperazine, isolation of the piperazine by ion-exchange chromatography, and determination of the pentafluorobenzoyl derivative by gas–liquid chromatography with electron capture detection. The minimum limit of detection was 0.05 ppm, and recoveries from five crops fortified at the 0.10-ppm level averaged 91%.

Triforine [*N,N'*-piperazine-1,4-diylbis[1-(2,2,2-trichloroethyl)formamide] is a systemic fungicide registered for use on several crops. Piperazine and *N*-[2,2,2-trichloro-1-(piperazin-1-yl)ethyl]formamide (TF/2) have been identified as metabolites in barley plants (Rouchaud et al., 1978). Piperazine has also been found to be present in aqueous solutions of triforine after irradiation with UV light (Buchenauer, 1975). One method for the analysis of triforine residues involves extraction with acetone, followed by acid hydrolysis and gas–liquid chromatographic determination of the liberated chloral hydrate (Bourke et al., 1977). While this procedure is satisfactory for the parent compound, TF/2 would be underestimated and piperazine would not be determined. Triforine and its metabolites may be analyzed by three separate determinations performed after chloroform extraction, partitioning with 0.1 N HCl, and hydrolysis of the chloroform extract, aqueous HCl phase, and solid residue (Rouchaud, 1977). The present method was developed to provide a relatively simple procedure capable of determining the total residue of triforine and its metabolites in a single analysis.

MATERIALS AND METHODS

Materials. Cation-exchange resin, Dowex 50 w X8, 100–200 mesh (Sigma Chemical Co., St. Louis, MO), was purified by washing with alkali and acid as described previously (Newsome, 1974). Ion-exchange columns were prepared containing a 3-mL settled volume of resin (Newsome, 1974) and were washed sequentially with 1 N NaOH (15 mL), water until neutral, 1 N HCl (15 mL), and water until neutral.

Silicic acid for adsorption chromatography (Woelm, activity I, 100–200 μm) was purchased from ICN Pharmaceuticals Inc., Cleveland, OH, and was deactivated by the addition of 5% water.

Pentafluorobenzoyl chloride reagent was prepared by dissolving pentafluorobenzoyl chloride (0.1 mL, PCR Research Chemicals, Inc., Gainesville, FL) in 10.0 mL of dichloromethane.

The *N,N'*-bis(pentafluorobenzoyl)piperazine standard was prepared by stirring piperazine (Aldrich Chemical Co., Inc., Milwaukee, WI, 0.4 g, 4.7 mmol) and pentafluorobenzoyl chloride (2.8 g 12.6 mmol) in a solution of acetonitrile (10 mL) and 2 M K_2CO_3 (10 mL) for 1 h. The solid that precipitated during the reaction was removed by filtration, washed with water and then hot acetone, and air-dried to yield 1.2 g (53%) of white powder. The product, mp 293–296 °C, gave a strong molecular ion at m/z 475 on electron impact mass spectrometry and a single peak on gas–liquid chromatography. The reference standard was dissolved in toluene and diluted serially in toluene to give a solution containing 50 pg/μL for gas–liquid chromatography.

Triforine, labeled as 99.6% pure, was obtained from Celamerck, Gmbh Co., Ingelheim/Rhein, West Germany. Solutions used for fortification were prepared in dimethyl sulfoxide and were added to samples in volumes of 0.1 mL.

Analytical Procedure. Samples of homogenized crop (10.0 g) were weighed in 125-mL flasks and refluxed with 1 N HCl (25 mL) for 2 h. The cooled hydrolysate was filtered through Whatman No. 1 paper on a Büchner funnel, and the solids were rinsed with 1 N HCl (15 mL).

The filtrate was transferred to a 1.5 × 20 cm chromatographic tube containing 3 mL of cation-exchange resin and permitted to percolate through it at a flow of approximately 20 mL/h. The column was washed with 1 N NaCl (10 mL) and then piperazine eluted with saturated (35 °C) $NaHCO_3$ (5 mL).

An aliquot of the $NaHCO_3$ eluate (1.0 mL) was placed in a 15-mL centrifuge tube containing 2 M Na_2CO_3 (9 mL)

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Table I. Recoveries of Triforine Added to Various Commodities

triforine added, ppm	triforine found, ppm[a]				
	blueberry	cranberry	strawberry	plum	peach
0	0.028	0.025	0.024	0.023	0.030
0.050	0.077 (97)[b]	0.070 (90)			
0.10	0.13 (97)	0.12 (95)	0.11 (89)	0.12 (94)	0.11 (82)
0.50	0.47 (89)	0.50 (95)			
1.0			0.93 (91)	0.89 (87)	
5.0			4.2 (84)	4.2 (84)	4.6 (91)
10					9.0 (90)

[a] Values are the means of duplicate determinations. [b] Values in parentheses are the percentage recoveries, corrected for background.

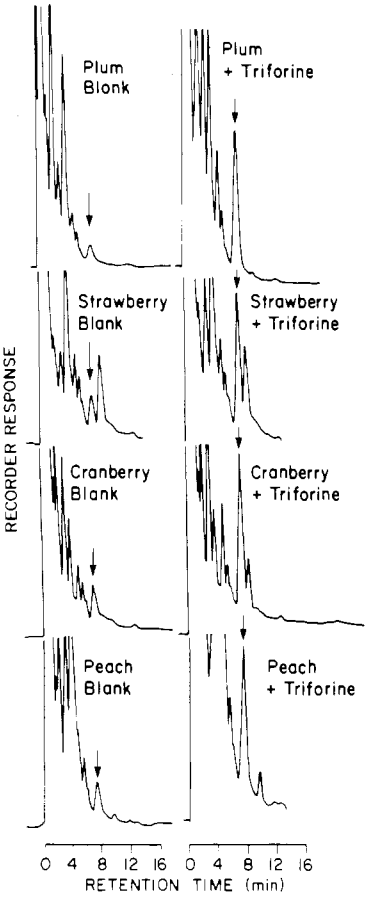

Figure 1. Gas–liquid chromatograms of various commodities analyzed with and without fortification with 0.10 ppm of triforine. The arrow indicates the retention time of the *N,N'*-bis(pentafluorobenzoyl)piperazine standard.

and shaken on an orbital shaker for 30 min with 1% pentafluorobenzoyl chloride reagent (1.0 mL). After reaction, the mixture was extracted with hexane (2 × 4 mL), and the combined extracts were evaporated to dryness with a stream of nitrogen.

The residue from the derivatization was taken up in dichloromethane (1.0 mL) and applied to a 2-g column of 5% deactivated silicic acid in dichloromethane. Elution was commenced with 1% 2-propanol in dichloromethane and the first 20 mL discarded. The next 10-mL fraction was collected, and an aliquot (1.0 mL) was evaporated to dryness with nitrogen and then taken up in toluene (1.0 mL) for analysis by gas–liquid chromatography.

Gas–liquid chromatography was carried out on a Hewlett-Packard 5700 A chromatograph fitted with a ^{63}Ni electron capture detector and 6 fit × 4 mm i.d. glass column packed with 3% SE-30 on Chromosorb W, HP, 80–100 mesh. Operating parameters were as follows: injection port temperature, 250 °C; column temperature, 225 °C; detector 300 °C; argon–methane (95:5) carrier gas flow rate, 30 mL/min. Under these conditions, the injection of 0.1 ng of *N,N'*-bis(pentafluorobenzoyl)piperazine resulted in a peak with a retention time of 7.25 min, producing a response of 80% full scale deflection at an attenuator setting of 16. Samples were quantitated by comparison of their peak height with that of a standard.

RESULTS AND DISCUSSION

The hydrolysis of triforine to piperazine in 1 N HCl was quantitative within 1 h in the absence of sample. In the presence of a sample matrix, a further 1-h digestion was required to give reproducible results. Although recovery studies on the TF/2 metabolite were not conducted, it is assumed that it would yield a quantitative amount of piperazine since it is already a partial hydrolysis product of the parent compound. Pentafluorobenzoylation of the resulting piperazine was employed to provide a derivative which could be detected easily by electron capture after gas–liquid chromatography. Under the biphasic reaction conditions used, derivatization was complete within 30 min. Excess reagent and interferences were removed by subsequent chromatography on silicic acid.

Typical chromatograms of various commodities analyzed for triforine are presented in Figure 1. An apparent residue was present in all samples analyzed. As shown by the data in Table I, the background value remained relatively constant from one crop to another, with a mean of 0.026 ppm. The minimum detectable limit, defined as twice the background, was 0.052 ppm. The fortification levels for each crop were chosen to encompass the tolerances established in the United States. These tolerances are 0.1 ppm on blueberry and cranberry, 2 ppm on strawberry, 3 ppm on plum, and 8 ppm on peach. The data in Table I indicate that satisfactory recoveries were obtained over the range of concentrations studied.

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