Method for the Determination of Captan Metabolites in Milk and Bovine Tissue Samples by Gas Chromatography with Mass-Selective Detection

Dan Kukla and Larry A. Wiebe*

Western Research Center, Environmental Chemistry Section, Zeneca Agricultural Products, 1200 South 47th Street, Richmond, California 94804-0023

Geoffrey S. Davy and Naresh C. Atreya

Zeneca Limited, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY, United Kingdom

Susan Clark

Morse Laboratories, Inc., 1525 Fulton Avenue, Sacramento, California 95825

A method for the simultaneous determination of residues of five possible metabolites of captan, 1,2,3,6-tetrahydrophthalimide (THPI), *cis*-3-hydroxy-1,2,3,6-tetrahydrophthalimide (C3), *cis*-5-hydroxy-1,2,3,6-tetrahydrophthalimide (C5), *trans*-3-hydroxy-1,2,3,6-tetrahrahydrophthalimide (T3), and *trans*-5-hydroxy-1,2,3,6-tetrahydrophthalimide (T5), has been developed. Tissue and milk samples are macerated with acetone and centrifuged. An aliquot of the extract is diluted with ethyl acetate, and the solution is dried over anhydrous sodium sulfate. The residuum obtained after evaporation of the solvent under a stream of dry nitrogen is partitioned between hexane and acetonitrile to remove lipids. After a silica column cleanup, the metabolites are derivatized by heating with *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 10% trimethylchlorosilane. The trimethylsilyl derivatives are quantitated by gas chromatography with mass-selective detection. The mean recoveries and coefficients of variation (CV) from the analysis of 145 fortified milk, fat, kidney, liver, and muscle samples were as follows: THPI, 91.1% (CV 15); T3, 93.4% (CV 16); T5, 92.6% (CV 17); C3, 95.1% (CV 14); and C5, 88.5% (CV 19), respectively. The fortification levels ranged from 0.01 to 1.0 μg/g.

Keywords: *Captan; metabolites; analysis; milk/tissues*

INTRODUCTION

Captan is a fungicide that was first introduced in 1951 in the United States. It is used to control certain fungal diseases of fruits, nuts, and ornamental crops, and as a seed treatment to control certain seed rots and damping-off diseases. Captan is registered for use on a number of crops. Therefore, animal feed may contain finite residues of captan and could result in residues appearing in meat and milk. Meat and milk methods for the determination of captan and captan metabolites have been published (Erney and Gilvydis, 1992; Onley, 1977). However, neither of these methods was designed to determine the hydroxylated captan metabolites discussed below. The method described here was developed to determine the levels of captan metabolites in tissues and milk from animals that may have consumed treated crops. This method is not intended for the analysis of captan.

Metabolism studies in animals using ring-labeled captan (unpublished) have identified the major captan metabolites to be 1,2,3,6-tetrahydrophthalimide (THPI) and 3- and 5-hydroxy-THPI. Both 3- and 5-hydroxy-THPI metabolites can exist as the *cis* or *trans* isomers. Early metabolism studies, which used direct determination of the compounds by gas–liquid chromatography (GLC), were unable to differentiate clearly between *cis* and *trans* isomers. Therefore, a method giving greater resolution was needed. Investigation of various possible derivatizing reagents showed that *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) produced the best results.

The chemical structures of captan [*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide], 1,2,3,6-tetrahydrophthalimide (THPI), *trans*-3-hydroxy-1,2,3,6-tetrahydrophthalimide (T3), *trans*-5-hydroxy-1,2,3,6-tetrahydrophthalimide (T5), *cis*-3-hydroxy-1,2,3,6-tetrahydrophthalimide (C3), and *cis*-5-hydroxy-1,2,3,6-tetrahydrophthalimide (C5) are shown in Figure 1.

MATERIALS AND METHODS

Reagents. Acetone, acetonitrile, ethyl acetate, hexane, and toluene were of a purity that was suitable for trace-level analyses. *N,O*-Bis(trimethylsilyl)trifluoroacetamide containing 10% trimethylchlorosilane (Regisil RC-3, also known as BSTFA + 10% TMCS) was obtained from Regis Chemical Co. Anhydrous reagent-grade sodium sulfate was supplied by Alameda Chemical. Bond Elut LRC disposable solid-phase extraction columns (10 mL) containing 500 mg of unbonded silica were obtained from Varian Associates. Dimethylchlorosilane (DMCS) was supplied by Pierce Chemical Co.

Nomenclature. (IUPAC and CAS names and CAS Registry numbers have been provided by the author.)

THPI: The IUPAC name is 1,2,3,6-tetrahydrophthalimide. The CAS name is 3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione. The CAS Registry number is 85-40-5.

T3. The IUPAC name is *trans*-3-hydroxy-1,2,3,6-tetrahydrophthalimide. The CAS name is 3a,4,7,7a-tetrahydro-4-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (3a.alpha.,4.alpha.,7a.alpha).

T5. The IUPAC name is *trans*-5-hydroxy-1,2,3,6-tetrahydrophthalimide. The CAS name is 3a,4,7,7a-tetrahydro-5-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (3a.alpha.,5.alpha.,7a.alpha).

C3. The IUPAC name is *cis*-3-hydroxy-1,2,3,6-tetrahydrophthalimide. The CAS name is 3a,4,7,7a-tetrahydro-4-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (3a.alpha.,4.beta.,7a.alpha).

0021-8561/95/1443-1224$09.00/0

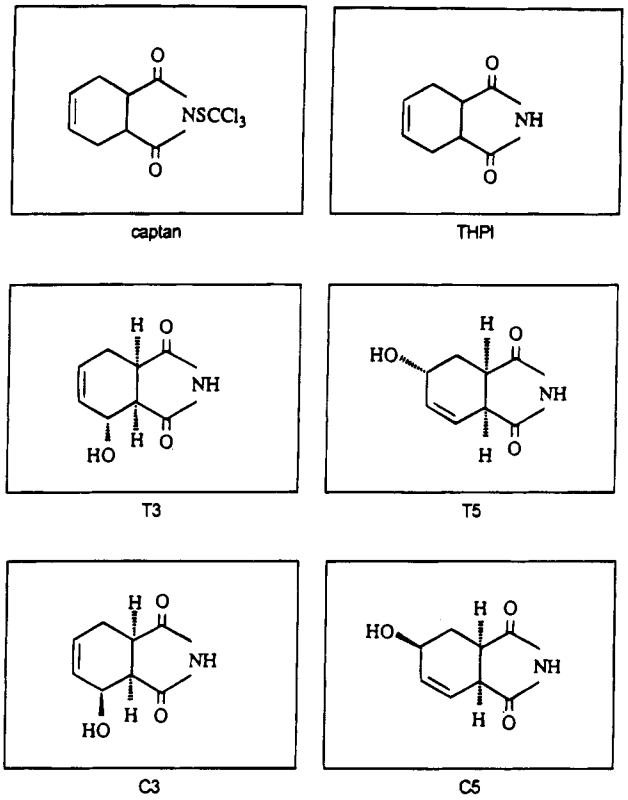

Figure 1. Structures of captan and five metabolites.

C5. The IUPAC name is *cis*-5-hydroxy-1,2,3,6-tetrahydrophthalimide. The CAS name is 3a,4,7,7a-tetrahydro-5-hydroxy-1*H*-isoindole-1,3(2*H*)-dione (3a.alpha.,5.beta.,7a.alpha).

Safety Precautions. Personnel untrained in the routine safe handling of chemicals and good laboratory practices must not attempt to use this procedure. Information on any specific chemical regarding physical properties, hazards, toxicity, and first-aid procedures can be found on the Material Safety Data Sheet (MSDS) accompanying the chemical or available from the supplier. In general, always wear safety glasses with side shields, work in a well ventilated area, avoid inhaling vapors, and avoid contact of the chemicals with skin and clothing. Flammable solvents should always be kept away from potential sources of ignition.

Reference Materials. The reference standards were synthesized, characterized, and purified by Zeneca Ag Products; they are available from Zeneca Ag Products, Richmond, CA. The purities of THPI, T3, T5, C3, and C5 were 99.7, 99.0, 97.0, 98.0, and 99.5%, respectively. Their Zeneca reference codes are ASW-1369, MSW-1198, MSW-1200, MSW-1197, and MSW-1199, respectively. Ion abundances generated from the mass spectra for the derivatized analytes are shown in Tables 3–7.

Separate stock solutions of the five analytes were prepared at concentrations of 1000 μg/mL in acetonitrile. These solutions were used to prepare two intermediate calibration solutions containing all five analytes combined, at concentrations of 1.0 and 0.1 μg/mL in acetonitrile. These two intermediate calibration solutions were used to prepare four concentration levels of working calibration standards, specifically, 0.1, 0.05, 0.025, and 0.005 μg/mL. These standards were prepared in autosampler vials when a set of samples was ready for derivatization and were derivatized alongside the samples.

Analytical Procedure. *Sample Preparation.* Frozen milk samples were allowed to thaw completely; the temperature of the milk was not allowed to exceed 8 °C. Prior to subsampling, the samples were well mixed by shaking vigorously. Frozen tissue samples were ground with dry ice in a Hobart chopper; dry ice was added to maintain tissues in a frozen condition. The samples were allowed to thaw for a minimum period before being broken up, subsampled, and extracted.

Sample Extraction—Milk. A 25-g aliquot was weighed into a 200-mL centrifuge bottle. Appropriate samples were fortified at this stage. For example, to fortify milk at 0.01 μg/g, 250 μL of an acetonitrile solution that contains each of the five analytes at 1 μg/mL was added. Each sample was blended with 75 mL of acetone for 5 min using a high-speed laboratory blender (PowrPulse homogenizer; Craven Labs). The macerate was centrifuged at 1000 rpm for 6 min. The supernate was decanted into a funnel containing a plug of glass wool, and the filtrate was collected in a 100-mL glass-stoppered cylinder. A reagent-blank sample was started at this stage by taking 75 mL of acetone. The reagent-blank sample was taken through all stages of the method.

Sample Extraction—Tissues (Except Fat). A 10-g aliquot was weighed into a 200-mL centrifuge bottle. Appropriate samples were fortified at this stage. Each sample was blended with 50 mL of acetone for 5 min using a high-speed laboratory blender. The homogenizer was stopped when necessary to remove entrapped tissue with a clean instrument. The macerate was centrifuged at 1000 rpm for 6 min. The supernate was decanted into a 100-mL graduated cylinder. The matrix plug at the bottom of the centrifuge bottle was broken up after 10 mL of acetone was added. The bottle was centrifuged at 1000 rpm for 6 min. The supernate was decanted into the funnel containing a plug of glass wool, and the filtrate was added to the graduated cylinder. The volume was adjusted to 60 mL using acetone. The cylinder was stoppered and inverted several times to mix the contents.

Sample Extraction—Fat. The procedure for tissues was followed except that the volume of acetone was increased to 60 mL owing to the liability for emulsions to be formed. After homogenization, the samples were centrifuged at 2000 rpm for 15 min. The supernate was then decanted from the fat into a 100-mL cylinder; no volume adjustment was made. The cylinder was stoppered, and the contents were mixed.

The solubility of the fat in acetone was determined. A 10-mL aliquot of the acetone extract was placed in a 15-mL graduated centrifuge tube and was subjected to solvent evaporation until a constant volume was obtained using an N-EVAP solvent reduction system (Organomation) with the water bath maintained at 30–35 °C. The observed volume of fat was used to correct the reported result.

Solvent Partition. After extraction, the solvent partition was performed. An aliquot of the acetone supernate (2.0 g = 8 mL for milk and 1.0 g = 6 mL for tissue) was placed in a 125-mL separatory funnel, and 25 mL of ethyl acetate was added. The solution was shaken for 1 min and filtered through 25 g of anhydrous sodium sulfate into a 50-mL glass, screw-top, centrifuge tube. The separatory funnel was washed with two 5-mL amounts of ethyl acetate, and each wash was passed separately through the sodium sulfate into the centrifuge tube. The centrifuge tube was placed into the N-EVAP solvent reduction system filled with water at 35 °C. With the nitrogen flow rate set so as to cause a 2-mm depression on the surface of the solvent, the solvent volume was reduced to 0.1–0.2 mL. The sample was finally taken to dryness manually with a stream of pure, dry nitrogen delivered onto the semiliquid residue through the tip of a disposable Pasteur pipet.

The residuum in the tube was dissolved in 5 mL of hexane with the help of a vortex mixer. After 5 mL of acetonitrile was added, the tube was closed using a cap with a fluorocarbon liner and shaken for 1 min. The phases were allowed to separate, and the hexane layer was removed with a Pasteur pipet and discarded. After 5 mL of hexane was added to the tube, the procedure was repeated. The volume of the acetonitrile was reduced to 0.1–0.2 mL by use of the N-EVAP maintained at 35 °C, and then the tube was taken to dryness with a stream of pure, dry nitrogen as described above. The residuum was dissolved in 2 mL of toluene/ethyl acetate (95:5 v/v) with vortexing.

Adsorption Column Cleanup. The required number of silica Bond Elut LRC solid-phase extraction columns was placed into a Vac Elut SPS 24 vacuum manifold (Analytichem International Inc.) fitted with stopcocks (Baxter Scientific Products). With the manifold set to the "waste" position, the columns were conditioned with approximately 10 mL of toluene, which was drawn down slowly at a rate of 2 to 3 drops per second to the level of the column frit. The manifold stopcocks were used to

Table 1. Retention Times and Ions Monitored for Derivatized Captan Metabolites

derivatized analyte	m/z	retention time (min)	ion ratio[a]
THPI	208[b] and 223	8.11	0.81
T3	296[b] and 311	8.77	0.36
T5	296 and 311[b]	9.02	0.43
C3	296[b] and 311	9.20	0.048
C5	296[b] and 311	9.38	0.31

[a] Ion ratios were determined from the analysis of a 0.1 μg/mL reference standard. The value was determined by dividing the lesser ion abundance by the greater ion abundance. [b] Denotes the greater abundant ion.

control the flow and to stop the flow when the solvent reached the top of the frit.

The toluene/ethyl acetate (95:5 v/v) solution containing the analytes was transferred to the extraction column by means of a Pasteur pipet, and the solvent was drawn down to the level of the frit as already described. After 5 mL of toluene/ethyl acetate (90:10 v/v) was added to the 50-mL centrifuge tube, the tube was swirled, and the solvent was transferred to the extraction column and drawn down to the level of the frit.

With the vacuum manifold in the "collect" position, 5 mL of ethyl acetate was added to the column to elute the analytes, and the eluate was collected in a 13 × 100 mm, screw-cap, culture tube equipped with a fluorocarbon-lined cap.

The volume of the ethyl acetate was reduced to 0.1−0.2 mL using the N-EVAP equipment with the bath at 30−35 °C. The evaporation of the samples was completed manually one at a time with a stream of pure, dry nitrogen delivered through the tip of a Pasteur pipet. Each tube was capped immediately to minimize contact of the residuum with water vapor in the ambient air. After 1 mL of dry acetonitrile was added, each tube was immediately recapped and vortex mixed for 30 s to dissolve the residuum. The matrix to solvent ratio is 2 g/mL for the milk and 1 g/mL for the tissues.

Derivatization. A Pasteur pipet was used to transfer the acetonitrile sample extract to an autosampler vial (Hewlett-Packard). This was done one sample at a time, and each vial was capped and crimp-sealed immediately after the transfer. The working calibration standards (see above) were prepared; precautions were taken to minimize exposure to the air.

When the derivatization was to be performed, the cap from a sample or working calibration standard vial was removed, 70 μL of BSTFA + 10% TMCS was added, and the vial was recapped immediately with a new cap. One vial was completed at a time. The vials were inverted to mix the contents and placed in a heating block for 30 min at 115 ± 2 °C. The samples and standards were submitted for GLC analysis within 24 h of derivatization.

Instrumentation. A Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with an HP 5970 mass-selective detector operating in the selective ion mode was used. Injections were made using an HP Model 7873A autosampler capable of "rapid" (≤0.1 s) injections. The capillary inlet was fitted with a 78 × 4 mm i.d. silanized borosilicate "straight" glass insert (HP 19251-60540) lightly packed in the middle with a 5−6-mm silanized glass wool plug. The insert was silanized by treating it with 5% dimethylchlorosilane in hexane for 10 min and allowing it to air-dry in a fume hood. The instrument was fitted with a 10 m × 0.18 mm i.d. fused-silica capillary column bonded with a 0.3-μm film thickness of Durabond (DB) 17 (J&W Scientific, Inc.). The carrier gas was helium at a column head pressure of 6 lb/in.^{2}. The inlet temperature was 230 °C, and the mass-selective detector (MSD) interface temperature was 238 °C. The column was held at 60 °C for 1 min after injection, programmed to 265 °C at 20 °C/min, and held for 5.5 min. The volume injected was 1 μL, and the inlet-purge time was 0.78 min. The retention times and ions monitored for quantitation are given in Table 1.

Calibration and Analysis. Before the start of the analysis, several injections of the 0.05 μg/mL standard were made to determine the actual retention times of the analytes and to ensure that the system was operating correctly. At the start of an analysis, the 0.05 μg/mL standard was injected followed by three injections of any sample so as to equilibrate the column. The four working calibration standards were injected next in the sequence 0.1, 0.05, 0.025, and 0.005 μg/mL. The samples were then injected starting with the reagent blank; the 0.05 μg/mL standard was injected after every third sample. The concentration of the standard used throughout the run was selected to be similar to the level expected in the samples, if known, or at 0.025 μg/mL if no residues were expected.

If the response of a sample was found to be above the response range of the calibration standards, the sample was diluted to be within the range and reassayed. Dilutions were made using acetonitrile that had been heated at 115 ± 2 °C for 30 min with derivatizing agent as if it was a sample.

Example chromatograms of milk, muscle, and liver sample analysis are shown in Figures 3, 5, and 7, respectively.

Calculations. A calibration graph was prepared for each analyte using the peak heights recorded for the working calibration standards; zero was included as a data point. However, none of the standards significantly deviate from linearity, and calculation of a sample residue can be made using a standard response factor. The graph was used to determine the migrograms of analyte in each sample. The results for fat samples were corrected for the observed solubility of fat in the extract as follows, where V is the volume of fat in 10 mL of acetone extract:

$$\text{corrcd residue} = \text{calcd residue}\,[(10 + V)/10]$$

Alternative GC Column. We have found a 12 m × 0.2 mm i.d., 0.33-μm film thickness, Ultra 1 from Hewlett-Packard Co., Santa Clara, CA, to be a satisfactory alternative column. The conditions were the same as those given for the Durabond 17 column except that, after the column was held for 1 min at 60 °C after injection, the oven temperature was raised at 20 °C/min to 190 °C and held for 5.5 min. The temperature was then raised to 270 °C at 15 °C/min and held for 1 min. The retention times were as follows: THPI, 6.82 min; T3, 8.31 min; T5, 8.52 min; C3, 8.57 min; and C5, 8.93 min.

RESULTS AND DISCUSSION

The present method, in which the metabolites are determined after derivatization with BSTFA, represent a considerable advance over the earlier procedure in which the metabolites were determined directly by gas−liquid chromatography. The present method has been used to analyze in excess of 600 samples arising from a bovine animal-feeding study and a national market-basket survey of residues of the breakdown products of captan in whole milk.

The results of (1) fortification experiments that were made to validate the method prior to initiation of the animal-feeding study, (2) the recoveries from bovine tissues and milk obtained during the animal-feeding study, and (3) a concurrent analyte, storage-stability study are summarized in Table 2.

The extractability of the analytes using the procedure described in this method was demonstrated in a goat metabolism study using ^{14}C-labeled captan.

The structures of the derivatized analytes are (1) THPI, a mono substituted *N*-trimethylsilyl derivative, and (2) T3, T5, C3, C5, disubstituted *N*- and *O*-trimethylsilyl derivatives.

The success of the method is critically dependent upon the derivatization step. The method is designed to minimize the levels of water or coextractives in the sample that could interfere with the derivatization. The N-EVAP solvent removal procedure consumes a significant portion of the method time, but it is included because it has been shown to be reliable during the analysis of a large number of samples. The common laboratory rotary evaporator has caused problems and

Table 2. Summary of Recoveries from Fortified[a] Bovine Tissues and Milk

analyte	tissue	no. of samples	range	mean	CV[b]
THPI	fat	22	72–109	89.6	11.9
	kidney	21	75–135	91.5	13.5
	liver	21	64–95	78.5	12.0
	muscle	23	75–115	91.8	13.6
	milk	58	49–126	95.7	14.0
T3	fat	22	72–123	96.3	14.4
	kidney	21	80–150	97.4	18.7
	liver	21	50–95	75.6	16.2
	muscle	23	65–120	97.6	12.0
	milk	58	52–120	95.6	12.0
T5	fat	22	73–112	95.3	11.7
	kidney	21	79–116	93.0	11.1
	liver	21	50–95	69.9	16.8
	muscle	23	56–120	96.5	15.3
	milk	58	52–121	98.1	12.6
C3	fat	22	73–121	88.1	14.3
	kidney	21	83–113	96.3	10.4
	liver	21	60–115	83.9	17.3
	muscle	23	29–120	93.7	19.8
	milk	58	70–118	98.1	9.9
C5	fat	22	65–138	91.6	23.8
	kidney	21	68–115	86.8	14.5
	liver	21	48–115	69.3	24.9
	muscle	23	56–120	93.3	15.1
	milk	58	46–114	93.1	13.2

[a] Fortification range 0.01–1.0 ppm. [b] Coeffecient of variation.

Table 3. Relative Ion Abundances Generated from a Full-Scan Spectra of 50 ng of the THPI Trimethylsilyl Derivative Produced on the HP 5970A Mass-Selective Detector

m/z	rel abundance	*m/z*	rel abundance
73	28	80	99
75	46	107	31
77	48	207	45
78	21	208	56
79	100	223	36

Table 4. Relative Ion Abundances Generated from a Full-Scan Spectra of 50 ng of the T3 Trimethylsilyl Derivative Produced on the HP 5970A Mass-Selective Detector

m/z	rel abundance	*m/z*	rel abundance
73	96	243	23
75	59	295	20
77	23	296	100
79	32	297	25
142	45	311	31
242	20		

Table 5. Relative Ion Abundances Generated from a Full-Scan Spectra of 50 ng of the T5 Trimethylsilyl Derivative Produced on the HP 5970A Mass-Selective Detector

m/z	rel abundance	*m/z*	rel abundance
72	11	105	13
73	10	106	22
74	13	151	10
75	66	167	16
77	22	168	90
78	16	169	16
79	15	296	12
100	13		

should not be used because (a) the large surface area of the glass walls may be detrimental to subsequent complete removal of the analytes, (b) the venting of the system may introduce sufficient moisture from the ambient air to interfere with the derivatization, and (c) the analytes may be lost by volatilization. Besides

Table 6. Relative Ion Abundances Generated from a Full-Scan Spectra of 50 ng of the C3 Trimethylsilyl Derivative Produced on the HP 5970A Mass-Selective Detector

m/z	rel abundance	*m/z*	rel abundance
51	5	127	20
52	4	133	4
59	12	142	50
61	6	143	7
66	5	147	14
70	6	151	5
72	12	154	15
73	99	168	12
74	11	242	13
75	47	243	4
76	4	283	7
77	21	296	100
78	24	297	26
79	49	298	11
80	4	311	4
100	10		

Table 7. Relative Ion Abundances Generated from a Full-Scan Spectra of 50 ng of the C5 Trimethylsilyl Derivative Produced on the HP 5970A Mass-Selective Detector

m/z	rel abundance	*m/z*	rel abundance
59	5	117	6
73	100	147	5
74	11	151	4
75	29	152	14
77	10	168	13
78	19	296	15
79	13	297	4
100	5	311	4
103	5		

taking care to avoid contact of the samples with moisture, it is important to ensure that the contents of the acetonitrile storage bottle are handled in such a way as to minimize contact of the solvent with ambient air. The quality of the derivatization agent is important, not only for the success of the derivatization but also for freedom from background peaks. Supplies should be obtained directly from the manufacturer who has established a two-year expiration date for unopened material and will not ship outdated material. It is recommended that for each set of samples a new 1-mL ampule of derivatization agent be opened. After the ampule has been opened, the contents should be transferred to an autosampler vial, which should be immediately capped. The 70-μL aliquots are removed from the vial when needed by piercing the vial cap with the syringe needle. The derivatized analytes are nominally stable for 24 h. Unforeseen delays (e.g., instrument malfunction) have led to instances of milk samples being analyzed up to 48 h and longer after derivatization. While the instrument was being repaired, these samples were refrigerated in autosampler vials with unpunctured caps. Successful analysis of the samples followed. On several occasions, in one laboratory that was using an alternative solvent evaporation procedure, where water contamination may have been a problem, the response of the fortified controls gave recoveries that were lower than expected. The remedy, as concluded by the success of the reanalysis of the fortified control samples, was to add 30 μL of additional derivatization reagent, reheat the sample vials, and reanalyze the samples.

At the gas–liquid chromatography stage, it is important that the HP Model 7673A autosampler is used. This high-speed injector is capable of making injections taking about 0.1 s. It was found that quantitative analysis was unsuccessful when the injections were made

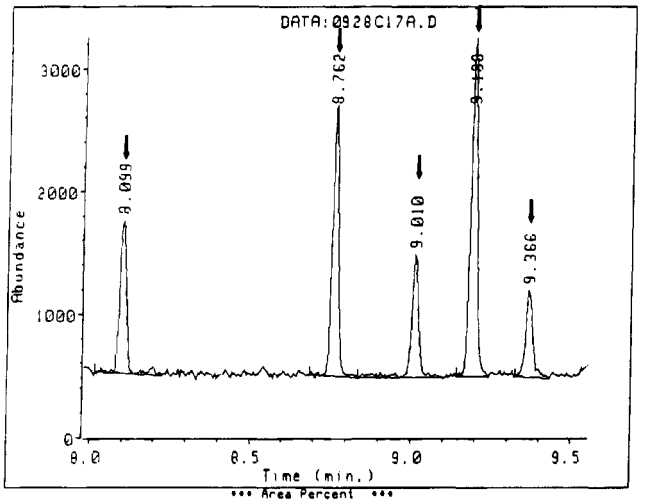

Figure 2. Selected ion chromatogram showing a calibration solution containing 0.02 μg/mL each of THPI (8.1 min), T3 (8.8min), T5 (9.0 min), C3 (9.2 min), and C5 (9.4 min).

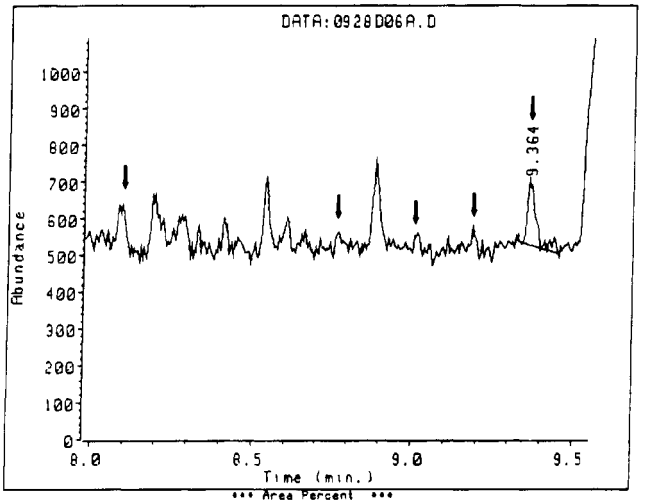

Figure 3. Selected ion chromatogram showing a control milk (2 g/mL).

either manually or made by a slower autoinjector. The syringe is adversely affected by the excess derivatization agent in samples and standards. A metal plunger that is corroded by the hydrochloric acid generated from the derivatization agent will tend to become immobilized in the glass barrel. This is not normally a problem within a set of samples of injections made on a regular basis as would be done during an autosequence. The following syringe-wash cycle was included in the autosampler program: wash bottle 1, two washes with acetonitrile followed by wash bottle 2, two washes with acetonitrile. However, after a set of samples has been run, if the syringe is allowed to remain motionless for several hours, the metal-to-glass surface interface of the syringe may be damaged beyond recovery. A new syringe must be installed before running a set of samples if efforts to manually wash the used syringe are not successful. The borosilicate glass inlet should be silanized as described. It is recommended that a new column be used for this analysis. It is acceptable for a used column to be employed only if an injection of derivatized standards produces a chromatogram similar in appearance and response to that given in Figure 2. The 60 °C initial oven temperature should not be significantly altered. The technique employed for the sample introduction is commonly identified as the Grob splitless solvent effect, for which the initial oven temperature is 10–15 °C below the boiling point of the injected solvent. This technique is critical to the success of this method.

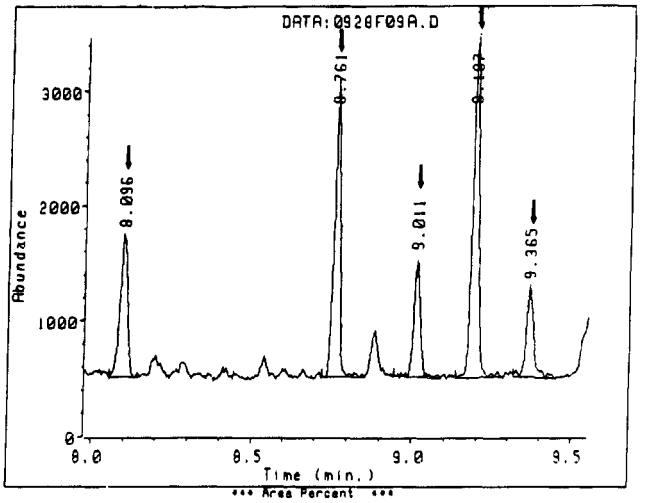

Figure 4. Selected ion chromatogram showing a control milk (2 g/mL) fortified at 0.02 μg/g each of THPI, T3, T5, C3, and C5.

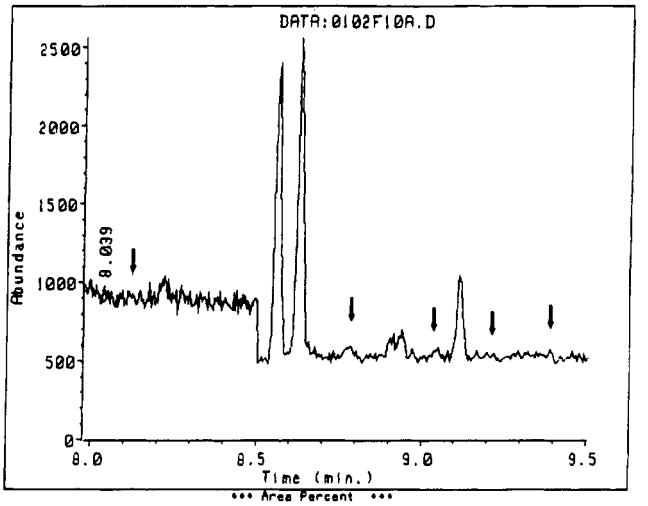

Figure 5. Selected ion chromatogram showing a control muscle (1 g/mL).

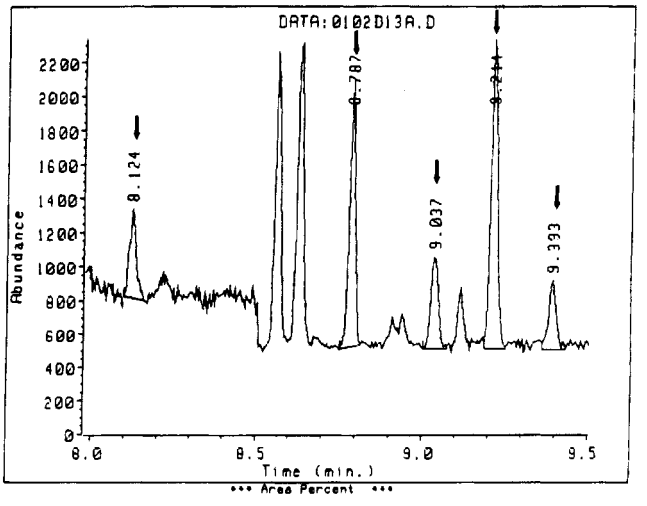

Figure 6. Selected ion chromatogram showing a control muscle (1 g/mL) fortified at 0.01 μg/g each of THPI, T3, T5, C3, and C5.

Analysis of samples in two laboratories has shown that no significant responses from impurities interfered

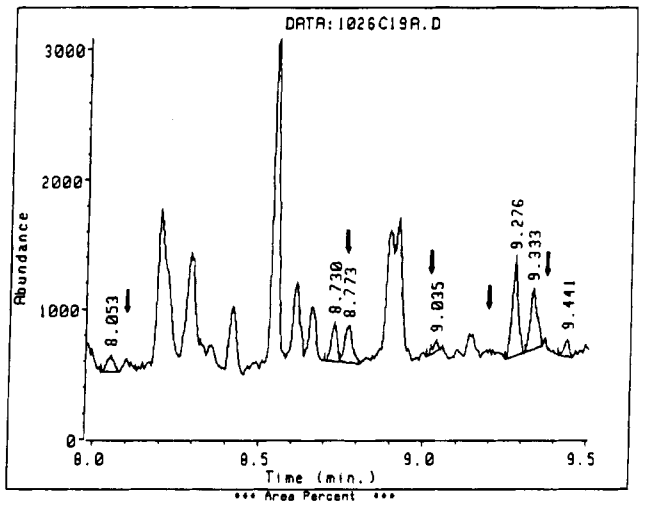

Figure 7. Selected ion chromatogram showing a control liver (1 g/mL).

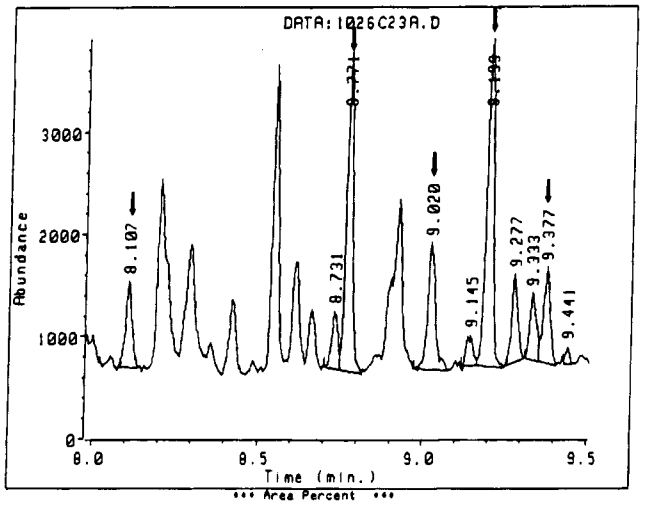

Figure 8. Selected ion chromatogram showing a control liver (1 g/mL) fortified at 0.01 μg/g each of THPI, T3, T5, C3, and C5.

with quantitation of the analyte peaks. However, on occasion, small responses from impurities would interfere with the quantitation of THPI and C5. The background responses observed were generally equivalent to less than the limit of quantitation of the analytes. The C5 interference was attributed to coextractives from the sample matrix. The THPI interference was attributed to coextractives from the sample matrix and to THPI present in the sample from laboratory contamination of samples with captan. Technical captan had been handled in the same laboratory that was used to prepare control samples found to contain the THPI interference. Captan readily breaks down to THPI in some matrices. Captan, formulated captan, captan-treated samples, or concentrated solutions of captan should not be present in the same laboratory used to prepare or analyze samples for THPI. Captafol should also not be present in the laboratory. In a third laboratory that has run this method, all samples, standards, or blanks containing the derivatization agent gave chromatograms that had large background peaks; one peak significantly interfered with the C5 analyte. This interference can arise from either (1) off-specification derivatization agent or (2) cold-trapped contaminants present in the GC inlet system that react in situ with the derivatization agent and subsequently volatilize. As a preventative measure, prior to the start of the method, 70 μL of the derivatization agent from a newly opened vial may be added to 1 mL of acetonitrile and the sample analyzed. If large background peaks are observed, the GC inlet system may be contaminated and should be cleaned. Also, before samples are analyzed using this method, a chromatogram of derivatized standards similar in appearance to the ones shown in Figure 2 should be generated. One commonly overlooked source of contamination, which can come from the HP 5890 instrument itself, is due to a dirty lower inlet seal. This seal is a metal disk with a small hole in the center that is located in the bottom of the injection port. It is accessed by removing the retaining nut inside the oven. Replacement with a new seal, or cleaning of the used seal, may eliminate much of the background.

If a residue is found, verification of a suspected analyte can be made by an ion ratio technique with two ions monitored for each analyte (see Table 1). The relative abundance of each ion is automatically stored by the Hewlett-Packard workstation. By following the workstation operating instructions, the relative abundances can be obtained for the suspected analyte. Then the relative abundances can be obtained for the same analyte in the working calibration solution that is the closest in concentration to the extract containing the suspected sample residue. Compare the ratio of the two ions monitored for both the sample extract and the standard. If the ratios are nearly the same (within ±10% of each other), the analyte identity is confirmed. If the ratios are significantly different (greater than ±40% of each other), the analyte is not confirmed. If the ratio differences are intermediate (between ±10 and ±40%), another confirmation technique should be conducted either by use of an alternative column, by reextraction and reanalysis of the sample, or by combination of the options.

The time required for the analysis of five samples is approximately three 8-h days. If the samples have not been reduced in volume to less than 1 mL during the N-EVAP solvent evaporation steps that occur (1) just before the solvent partition, (2) just after the solvent partition, or (3) after the adsorption column cleanup, they can be successfully analyzed if they have been capped and stored at 4 ± 3 °C for no more than two days.

CONCLUSION

This method describes the analysis of five possible captan metabolites in meat and milk. No other method that can determine T3, T5, C3, and C5 has been published.

LITERATURE CITED

Erney, D. R.; Gilvydis, D. M. Determination of Tetrahydrophthalimide in Milk by Gas Chromatography and Nitrogen Selective Detection. *J. High Resolut. Chromatogr.* **1992**, *15* (11), 773–5.

Onley, J. H. Gas-Liquid Chromatographic Determination of Captan and Two Metabolites in Milk and Meat. *J. Assoc. Off. Anal. Chem.* **1977**, *60* (3), 679–81.

Received for review December 28, 1994. Accepted February 6, 1995.[⊗]

JF940009I

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1995.