

# **DETERMINATION OF CARBADOX (AS QUINOXALINE-2-CARBOXYLIC [QCA] RESIDUES IN SWINE LIVER AND MUSCLE TISSUES AFTER DRUG WITHDRAWAL**

The procedure described is the FDA-CVM regulatory method and is on file at the FDA in Rockville, MD.

## **Principle of the Method**

A tissue sample is hydrolysed in an alkaline medium, cooled and the hydrolysate acidified. The hydrolysate is extracted three times with ethyl acetate. The combined organic layers are extracted with a citric acid buffer, and the buffer is passed through an ion-exchange column. QCA is eluted from the column with 14% methanol which is then partitioned with chloroform. The chloroform is evaporated, and the residue is derivatized with n-propanol/sulfuric acid reagent to form the propyl ester. The derivative is extracted into toluene and measured using gas chromatography with electron capture detection.

## **Validation of the Method**

The most recent validation study was reported on April 28, 1998 by Dr. Joe Boison at the

Centre for Veterinary Drug Residues  
Health of Animals  
Canadian Food Inspection Agency  
116 Veterinary Rd.  
Saskatoon, SK  
Canada S7N 2R3

The Centre for Veterinary Drug Residues (CVDR), laboratory facility of the Canadian Food Inspection Agency (CFIA), conducted the chemical analysis component of a Depletion Study of Quinoxaline-2-Carboxylic Acid (QCA) Residue from Liver and Muscle Tissue of Growing Swine at Intervals Following Oral (In-Feed) Medication with Carbadox, sponsored by Pfizer Animal Health, Lee's Summit, MO.

The objective of which was to determine the concentrations of QCA in liver and muscle tissues of growing swine fed carbadox (Mecadox®, Pfizer Animal Health) at 50 grams/ton (55 ppm), and to determine the depletion profile of QCA following withdrawal of carbadox from the feed.

The calibration range in method CBX-SPO8 was from 5-30 ppb with an estimated detection limit of 2 ppb for QCA in muscle and liver. However, because the tolerance level for QCA in the target tissue, liver, is 30 ppb, the

calibration range in this study was extended from 30-70 ppb in order to satisfy one of the basic criterion for the validation of the analytical method, that the calibration curve be suitable for the determination of the analyte of interest at 0.5, 1, and 2X the concentration of interest (15, 30, and 60 ppb)

Gas chromatograms showing the retention parameters and typical detector responses for QCA standard solutions of concentrations of 5, 10, 25, 50, and 70 ppb used for constructing the calibration curve and generation regression parameters for the determination of QCA in swine liver and muscle tissue are shown in Figure 1.

Mean Regression line with its 95% Confidence limits for the 3 sets of calibration curves presented as shown in Figure 2.

The detection limit or limit of detection (LOD) of an analytical method is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value.

Figures 3A – 3D show typical chromatograms of a 1, 2, 3, and 5 ppb QCA standard after Derivatization with propanol/sulfuric acid to form the propyl ester. The lowest concentration of the CBX-PFIZ calibration method is 5 ppb. It can be seen from Figure 3 that it is possible to detect 2 ppb QCA sample very easily with this method. Any analyte detected in a sample extract that has a concentration lying between 2 ppb and 5 ppb would be classified as “Not Quantifiable” and designated NQ. Any response detected as the retention parameter for QCA that is below the 2 ppb limit of detection is considered to be “Non Detectable” and designated ND.

The Limit of Quantitation (LOQ) of the analytical procedure is the lowest amount of the analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

To determine the LOQ, a series of standard calibration curves were plotted and mean regression parameters from these calibration curves were used to calculate the LOQ as specified in the method at 5 ppb.

The Recovery (absolute) of QCS from swine muscle and liver tissue is defined as the ratio of the amount of QCA found experimentally (i.e., after extraction and cleanup) by interpolation from an external standard calibration curve (i.e., using standards that have not been subjected to the extraction-cleanup procedure) to the theoretical amount added to the sample that was analyzed. For example, if a control muscle or liver tissue was fortified with QCA at 30 ppb and the detector response obtained from this sample extract was found after interpolation from the calibration curve to contain 25 ppb, then the Absolute Recovery is calculated at  $25/30$  (i.e., 0.83). This translates into a Recovery Factor ® of 0.83 or 83% Recovery.

An external calibration standard set was run together with each set of fortified sample and/or samples containing physiologically-induced QCA, a Recovery standard were always included in each run in order that a Recovery Factor could be calculated. Table 1 shows that mean absolute recoveries ranging from 103% to 70% for swine liver and from 73% to 68% for swine muscle tissue are achievable over the range of concentrations studied. It permits QCA to be analyzed with mean recoveries of  $70 \pm 3\%$  from swine muscle tissue and  $86 \pm 16\%$  from swine liver tissue.

The Accuracy of an analytical method measures the closeness of agreement between the acceptable value (it may be the conventional true value or an accepted reference value) and the one that was experimentally found.

Results of the accuracy experiments summarized in Table 1 indicate that QCA added to swine muscle and liver tissue can be determined with acceptable levels of accuracies  $\pm 20\%$  of true concentration) over the entire range of the calibration curve.

A stability study conducted to assess the stability of QCA propyl ester in the injector vials during a typical analysis indicated that this analyte is very stable in the presence or absence of tissue matrix.

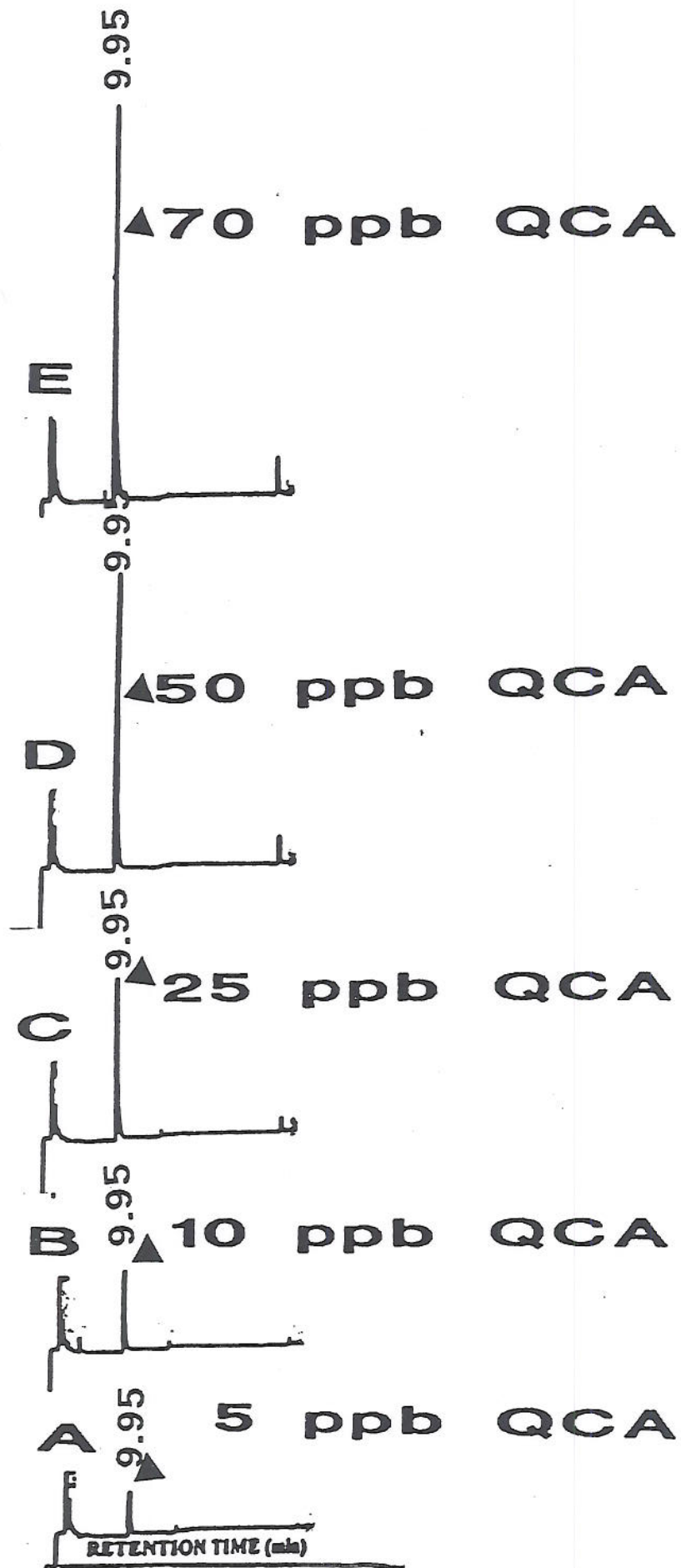


Figure 1.

**MEAN REGRESSION CURVE FOR CALIBRATION CURVE DATA  
GENERATED ON OCT 20 & 21 1997**

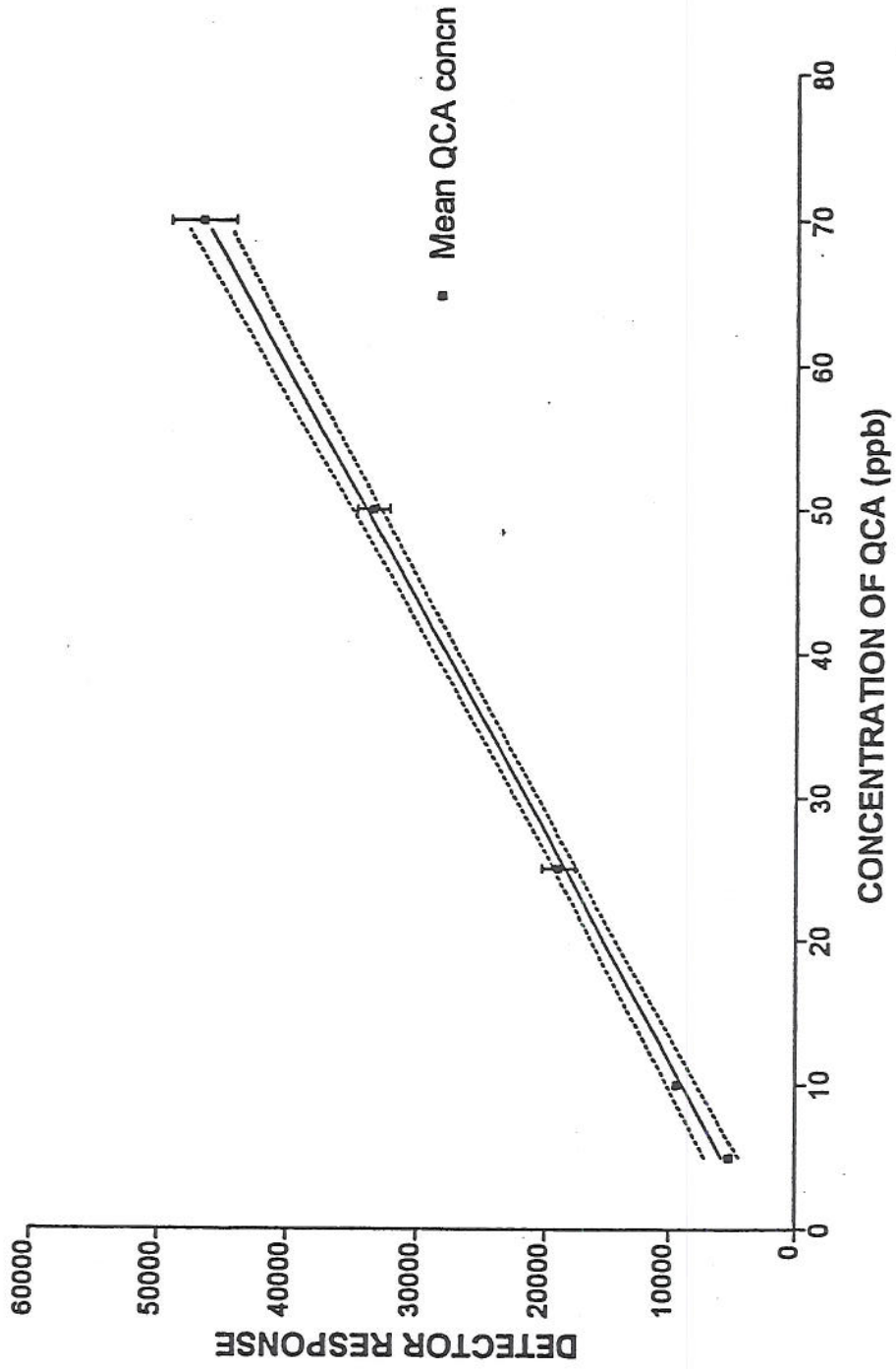


Figure 2

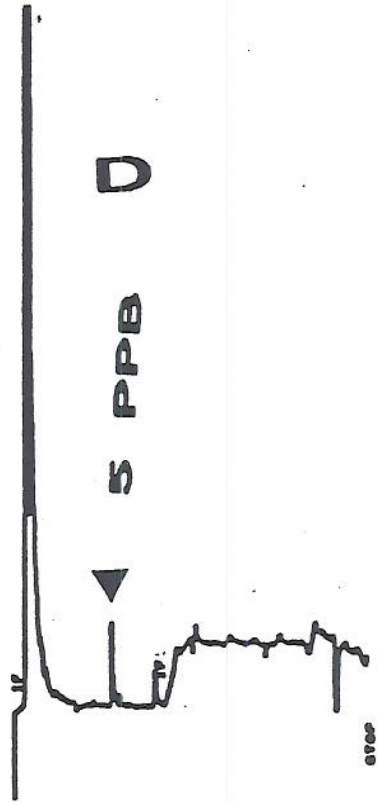
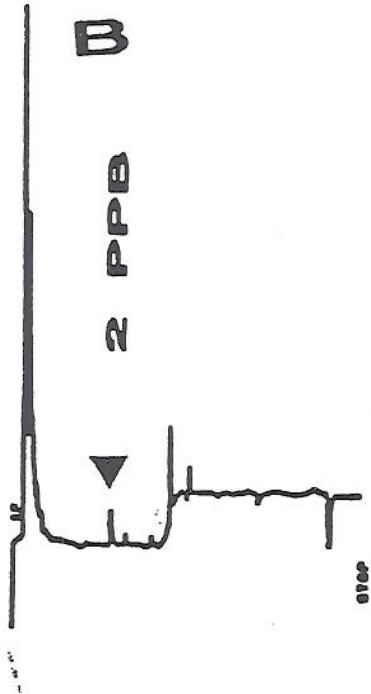
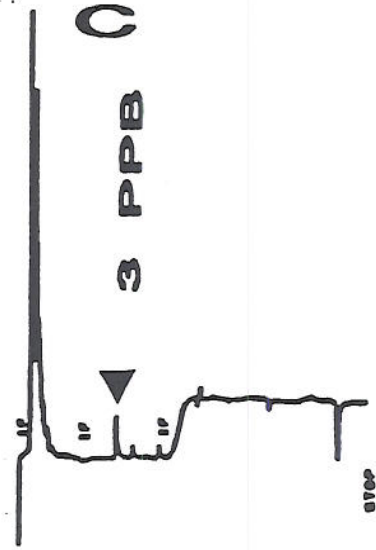
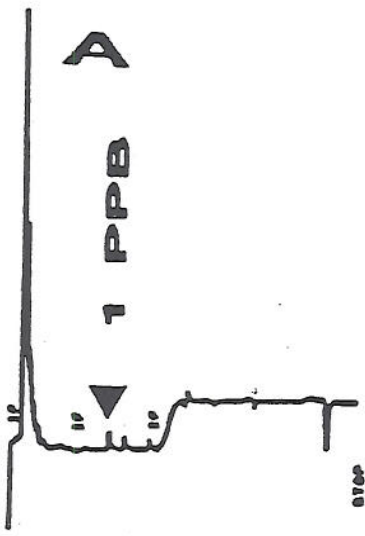


Figure 3.

Table 1.

Recovery of QCA from control swine liver tissues fortified at 15, 30, and 60 ppb with QCA

Date of Anal.	Recovery of QCA from swine liver tissue fortified at 15 ppb (Analysed Jan 13, 1998)					Recovery of QCA from swine liver tissue fortified at 30 ppb (Analysed Jan 14, 1998)					Recovery of QCA from swine liver tissue fortified at 60 ppb (Analysed Jan 15, 1998)					
	# of analysis (n)	Absolute Recovery (%)		Recovery Factor	Accuracy (%)	# of Analysis (n)	Absolute Recovery (%)		Recovery Factor	Accuracy (%)	# of Analysis (n)	Absolute Recovery (%)		Recovery Factor	Accuracy (%)	
		[QCA] found (ppb)	%QCA Recov'd				[QCA] found (ppb)	%QCA Recov'd				[QCA] found (ppb)	%QCA Recov'd			[QCA] found (ppb)
Total # of Samples	6					6					6					
MEAN ± SD		15.4 ± 0.7	103 ± 4	0.92	16.8 ± 0.7	+6 to +19	25.8 ± 1.5	86 ± 5	0.88	29.3 ± 1.7	-12 to +5	41.8 ± 1.9	69.7 ± 3.2	0.76	55.0 ± 2.5	-14 to -3
%C.V.		5	4		4		6	6		6		5	5		5	

[QCA] is the concentration of QCA interpolated directly from the calibration curve.  
 [QCA]<sub>1</sub> is the concentration of QCA found in the sample after applying the recovery factor.  
 % Accuracy is defined as  $([QCA]_{100} - \text{Fortified Concentration Level}) / \text{Fortified Concentration Level} \times 100$   
 % Recovery is defined as  $[QCA] / \text{Fortified Concentration Level} \times 100$

Recovery of QCA from control swine muscle tissues fortified at 15, 30, and 60 ppb with QCA

Date of Anal.	Recovery of QCA from swine muscle tissue fortified at 15 ppb					Recovery of QCA from swine muscle tissue fortified at 30 ppb					Recovery of QCA from swine muscle tissue fortified at 60 ppb					
	# of analysis (n)	Absolute Recovery (%)		Recovery Factor	Accuracy (%)	# of Analysis (n)	Absolute Recovery (%)		Recovery Factor	Accuracy (%)	# of Analysis (n)	Absolute Recovery (%)		Recovery Factor	Accuracy (%)	
		[QCA] found (ppb)	%QCA Recov'd				[QCA] found (ppb)	%QCA Recov'd				[QCA] found (ppb)	%QCA Recov'd			[QCA] found (ppb)
Total Sample	12					12					12					
MEAN ± SD		10.9 ± 1.4	73 ± 9	0.71 ± 0.09	15.5 ± 3.1	-15 to +36	21.6 ± 3.3	72 ± 11	0.71 ± 0.09	30.4 ± 2.8	-10 to +17	40.6 ± 4.5	60 ± 7	0.71 ± 0.09	57.7 ± 7.1	-21 to +11
%C.V.		13	12	13	20		15	15	13	9		11	10	13	12	

[QCA] is the concentration of QCA interpolated directly from the calibration curve.  
 [QCA]<sub>1</sub> is the concentration of QCA found in the sample after applying the recovery factor.  
 % Accuracy is defined as  $([QCA]_{100} - \text{Fortified Concentration Level}) / \text{Fortified Concentration Level} \times 100$   
 % Recovery is defined as  $[QCA] / \text{Fortified Concentration Level} \times 100$

**CBX-PFIZ**

No.	3
Authorized by	<i>[Signature]</i>
Date	1997/09/30

**A STUDY SPECIFIC PROCEDURE (SSP) FOR  
THE DETERMINATION OF CARBADOX (AS  
QUINOXALINE-2-CARBOXYLIC ACID [QCA])  
RESIDUES IN SWINE LIVER AND MUSCLE TISSUES  
AFTER DRUG WITHDRAWAL**

Canadian Food Inspection Agency, Centre for Veterinary Drug Residues  
Health of Animals Laboratory, 116 Veterinary Road, Saskatoon, SK S7N 2R3

**Reference:** Lynch, M.J. & Bartolucci, S.R. J. Assoc. Off. Anal. Chem., 65, 66-70,  
1982 (Reference: CVDR METHOD'S MANUAL CBX-SP08).

**1. Scope:**

This method is applicable to the determination of carbadox (CBX) and its metabolites as quinoxaline-2-carboxylic acid (QCA) in swine liver and muscle tissues. It is being used to quantify the concentrations of QCA residues in liver and muscle tissues obtained from growing swine fed carbadox (Mecadox® 10, Pfizer Animal Health) at 50 grams/ton (55 ppm), and to determine the depletion profile of QCA following withdrawal of carbadox from the feed [This version is specifically designed for Study #2522A-60-97-077].

**2. Principle:**

A tissue sample is hydrolysed in an alkaline medium, cooled and the hydrolysate acidified. The hydrolysate is extracted three times with ethyl acetate. The combined organic layers are extracted with a citric acid buffer, and the buffer is passed through an ion-exchange column. QCA is eluted from the column with 14% methanol which is then partitioned with chloroform. The chloroform is evaporated, and the residue is derivatized with n-propanol/sulfuric acid reagent to form the propyl ester. The derivative is extracted into toluene and measured using gas chromatography with electron capture detection.

1997/09

Version CBX-PFIZ

page 1 of 10

Prepared by: J. Boison

Date:

*[Signature]* SEP 30 '97

Reviewed by: V. Martz

Date:

*[Signature]* 1997/09/30

Approved by: J. MacNeil

Date:

*[Signature]* 1997/09/30

Effective Date:

SEP 30 1997

**PFIZER STUDY #2522A-60-97-077  
CERTIFIED ORIGINAL COPY**



### 3. Apparatus:

- 3.1 Notes: See Appendix "A" for Specific Identification Numbers for each capital equipment included in the Study.
- 3.1.1 All volumetric glassware used throughout this method is class A.
- 3.2 Centrifuge tube: 50 mL polypropylene, disposable (T4158-50R, Falcon Brand, Baxter/Canlab, Mississauga, ON).
- 3.3 Centrifuge tubes: 10 mL glass, disposable with teflon-lined screw cap.
- 3.5 Pasteur pipettes: 15 cm glass, disposable,
- 3.6 Volumetric flasks, 10 and 100 mL.
- 3.7 Round bottom flasks: 250 mL with 24/40 joint.
- 3.8 Separatory funnels: 125 mL with teflon stopcock.
- 3.9 Separatory funnel rack: (#S9179-11, Baxter/Canlab).
- 3.10 SPE reservoir: 8 mL with frit (#C612102, Chromatographic Specialties, Brockville, ON).
- 3.11 SPE reservoir: 75 mL with adaptors (#9434 and #9430, Baxter/Canlab).
- 3.12 GC column: SPB 50, 30 X 0.32, 0.5  $\mu$ m film thickness (Supelco).
- 3.13 Micropipettors: adjustable, 10-100  $\mu$ L and 100-1000  $\mu$ L.
- 3.14 Liquid dispensers: adjustable, 1-10 mL and 5-50 mL.
- 3.15 Repeater pipette: 1-50 mL (Eppendorf, Fisher Scientific, Edmonton, AB).
- 3.16 Heating module: Reacti-Therm Heating Module #P18870 with aluminum block drilled to accept 50 mL centrifuge tubes (Chromatographic Specialties).
- 3.17 Sand bath: variable temperature (Reacti-Therm Heating Module #18870, Chromatographic Specialties).

- 3.18 Vortex mixer: variable speed.
- 3.19 Mechanical shaker: two speed, flat bed (Eberbach #6000 - Baxter/Canlab)
- 3.20 Nitrogen evaporator: N-Evap (Organomation Associates, Inc., Berlin, MA).
- 3.21 SPE vacuum manifold: Visiprep 12 port (#5-7030 Supelco Canada, Oakville, ON).
- 3.22 Electronic Balance: 0.001 g sensitivity.
- 3.23 Beckman CS-6KR Refrigerated Centrifuge: floor model with 50 mL tube carriers, capable of 1000 x g.
- 3.24 Rotary evaporator: Büchi Rotovapor RE 121 equipped with a Büchi 461 thermostated water bath (Brinkmann Instruments Ltd., Rexdale, ON).
- 3.25 GC system: 5890 gas chromatograph equipped with electron capture detector, 7673A autosampler and 3396A integrator (Hewlett-Packard).

#### 4. Reagents:

##### 4.1 Notes:

- 4.1.1 Should lot numbers for reagents etc., change during the course of the study, such changes will be made on an added Appendix to this SSP.
- 4.1.2 All water used throughout method was purified by reverse osmosis followed by deionization, absorption and filtration.

4.2 Cation exchange resin, AG MP-50 100-200 mesh (#143-0841, Bio-Rad Laboratories, Mississauga, ON, Lot # 54224A). Preparation: using a large buchner funnel fitted with two #4 Whatman filter papers, wash 200 g of resin with 2 x 500 mL methanol, 2 x 500 mL water, and 500 mL 1 M HCl. Prepared resin can be stored, up to one month, in an amber jar under 1 M HCl.

4.3 Cation Exchange column preparation: Place a frit in an 8 mL SPE column and attach the column to a vacuum block. Fill the column to within 1 cm of the top with the AG MP-50 resin slurry. Allow the resin to settle, using vacuum assist. Discontinue vacuum when 1 M HCl level is just above the resin bed. Do not allow the resin to become dry or column must be re-packed. Attach a 75 mL reservoir to the column with an adaptor.

- 4.4 Sodium hydroxide, pellets, reagent grade (Fisher Scientific).
- 4.4.1 Sodium sulfate, anhydrous, reagent grade (#B10264-34, BDH Inc., Lot #115213-37554).
- 4.4.2 Citric acid monohydrate, reagent grade (Fisher Scientific, Lot # 974690).
- 4.5 Solvents:
- 4.5.1 Chloroform, distilled in glass (Caledon, ON, Lot # 20226)  
**CAUTION: Chloroform is a suspect Carcinogen - To be used in fumehood with protective gloves. See MSDS for more information**
- 4.5.2 Ethyl acetate, distilled in glass (Caledon, ON, Lot # 22339)
- 4.5.3 Methanol, distilled in glass (Caledon, ON, Lot # 702045)
- 4.5.4 Toluene, distilled in glass (Fisher Scientific).
- 4.5.5 1-Propanol, distilled in glass (Caledon, ON, Lot #110047). Dry with sodium sulfate.
- 4.5.6 Sulfuric acid, reagent grade (Fisher Scientific).
- 4.5.7 Hydrochloric acid, reagent grade (J. T. Baker).
- 4.5.8 Silyl-8 column conditioner (Pierce brand, # P38014, Chromatographic Specialties Lot # 97070262).
- 4.6 1 M citric acid: Dissolve 21.0 g citric acid in 75 mL water and dilute to 100 mL.
- 4.7 0.5 M citric acid buffer: to 100 mL 1 M citric acid add approximately 55 mL 5 M sodium hydroxide to give pH  $6.00 \pm 0.10$  then dilute to 200 mL.
- 4.8 1 M HCl: Add 83 mL HCl to 500 mL water and dilute to 1 L.
- 4.9 14% methanol: Dilute 140 mL methanol to 1 L with water.
- 4.10 1-Propanol/sulfuric acid (97:3): Combine 150  $\mu$ L sulfuric acid with 5 mL 1-propanol. Prepare fresh as dictated by expiration dates on prepared samples.
- 4.17 3 M sodium hydroxide: dissolve 120 g sodium hydroxide in water and dilute to 1 L.

CERTIFIED ORIGINAL COPY

4.18 5 M sodium hydroxide: dissolve 200 g sodium hydroxide in water and dilute to 1 L.

## 5. Standard Solutions:

### 5.1 Notes:

5.1.1 Quinoxaline-2-carboxylic acid (QCA) reference standard was obtained as a gift from Pfizer Inc., Eastern Point Road, Groton, CT. [Lot #014585-033-01] or from Aldrich [Lot #11015PQ]

5.2 Stock standard A (20 µg/mL):  
Dissolve 2 mg QCA in methanol and dilute to 100 mL with methanol. Prepare monthly. Store at 2- 8°C.

5.3 Intermediate standard B (4 µg/mL):  
Dilute 2 mL of the 20 µg/mL stock standard to 10 mL with methanol. Prepare biweekly. Store at 2 - 8°C.

5.4 Working standard C (2 µg/mL):  
Dilute 1 mL of the 20 µg/mL stock standard A to 10 mL with methanol. Prepare biweekly. Store at 2 - 8°C.

5.4 GC standards (5, 10, 25, 50, and 70\* ppb tissue equivalent):  
Steps 6.12 and 6.13.

Note: This calibration concentration range will be used for the sole purpose of providing validation data for the method in order to satisfy the 0.5, 1.0, and 2.0 x the concentration of interest guidelines required in CVM's method validation protocol. The concentration range for calibration during the actual sample analyses may, however, be modified to reflect the actual range of concentrations of carbadox residues likely to be found in the incurred samples.

5.5 Fortified tissue (30 ppb tissue equivalent) for recovery correction:  
Step 6.2.

## 6. Extraction, Clean-up and concentration of QCA:

### 6.1 Notes:

6.1.1 The analyst should follow the safety procedures as outlined in the Procedure for Handling, Storage and Disposal of Chemicals and

CERTIFIED ORIGINAL COPY  
100-000000-077

Hazardous Waste, and the CVDR Procedure, Safe Working Practices.

- 6.2 Alkaline hydrolysis of carbadox (CBX) to quinoxaline-2-carboxylic acid (QCA)  
Weigh  $5 \pm 0.005$  g diced/homogenized tissue into a 50 mL centrifuge tube.  
Fortify one blank tissue using 75  $\mu$ L stock standard C (30 ppb tissue equivalent) for tissue recovery correction.
- 6.3 Add 10 mL 3 M sodium hydroxide, cap and vortex 3 seconds, loosen cap and place in heating block at  $105^\circ \pm 5^\circ\text{C}$  for 20 minutes. Remove from heat source carefully, tighten cap and swirl to wash down walls and cap. Replace on heat source and continue hydrolysis for 15 minutes. Cool the tissue hydrolysate in ice for 10 minutes. [NOTE: This is a convenient stopping point. Let the sample sit overnight and continue the next day].
- 6.4 [Next day]. Add 4 mL of concentrated HCl to the hydrolysate followed by 15 mL ethyl acetate, cap, shake at high speed for 3 minutes and centrifuge for 5 minutes at  $800 \times g$  at  $-5 \pm 5^\circ\text{C}$ .
- 6.5 Extraction of QCA into ethyl acetate  
Transfer the organic layer to a 125 mL separatory funnel. Repeat the ethyl acetate extraction twice with no additional HCL and combine the organic layer in the separatory funnel.
- 6.6 Back extraction of QCA from ethyl acetate into pH 6.0 citrate buffer  
Add 5 mL freshly prepared citrate buffer, stopper, shake 30 seconds and let stand for 10 minutes. Drain the aqueous layer into a 50 mL polypropylene tube. Repeat the citrate extraction and combine the aqueous layers.
- 6.7 Acidify the aqueous layer using 2 mL concentrated HCl and mix.
- 6.8 Clean-up and concentration of QCA by ion exclusion chromatography  
Pour the acidified aqueous layer into the 75 mL reservoir and let it run through the anion exchange column to waste. Rinse the 50 mL polypropylene tube with 5 mL 1 M HCl and add the rinse to the column, letting it run to waste. Rinse the anion exchange column with 25 mL 1 M HCl to waste.  
[NOTE: DO NOT LET THE LIQUID LEVEL DROP BELOW THE TOP OF THE RESIN BED].
- 6.9 Place a 50 mL polypropylene tube in the vacuum block to collect eluate. Elute the QCA off the cation exchange column using 50 mL 14% methanol. Slight vacuum may be used to adjust flow to 2 - 4 mL/minute. The column may run dry at this point.

- 6.10 Transfer the methanolic eluate to a 125 mL separatory funnel. Acidify using 1 mL conc. HCl. Add 35 mL chloroform, shake 30 seconds, and let stand until clear (approximately 10 minutes). The chloroform layer should be clear (if an emulsion is formed add 1 mL HCl, shake 20 seconds and let stand for another 10 minutes). Drain the chloroform layer into 250 mL round bottom flask. Repeat the chloroform extraction and combine the chloroform extracts.
- 6.11 Roto-evaporate the chloroform extract to dryness at  $60 \pm 5^\circ\text{C}$ . Rinse the round bottom flask into 10 mL glass centrifuge tube using 3 x 1 mL methanol
- 6.12 Preparation of external standards and samples for chemical derivatization  
Pipet 12.5, 25, 62.5  $\mu\text{L}$  of Working standard C, 62.5 and 87.5  $\mu\text{L}$  aliquots of Intermediate standard B into separate 10 mL glass centrifuge tubes. These samples correspond to 5, 10, 25, 50, and 70 ppb tissue equivalents of CBX as QCA respectively.  
[Note: The range of calibration concentrations indicated above is primarily for providing validation data for the method and may be modified during the course of the actual analyses of experimental samples to reflect the actual range of concentrations of carbadox residues that are found].
- 6.13 Evaporate these samples together with the tissue samples from Step 6.11 above to dryness under nitrogen at  $60 \pm 5^\circ\text{C}$ .
- 6.14 Derivatization of QCA extracts and standards to form the propyl ester (QPE)  
Add 200  $\mu\text{L}$  freshly prepared 1- propanol/sulfuric acid (97:3) to the tissue residue and chemical standards, cap, and vortex 20 seconds. Warm in sand bath for 5 -10 seconds and roll to wet the interior wall. Let stand in sand bath at  $105 \pm 5^\circ\text{C}$  for 45 minutes. Remove from heat let stand for 1 minute.
- 6.15 Add 1 mL water and 2 mL toluene, cap, shake for 1 minute and centrifuge for 2 minutes at 500 x g. If the toluene layer is cloudy, repeat the vortex and centrifugation steps. Transfer the clear toluene layer into 2 mL HP autosampler vial and seal using a teflon-lined septum.

## 7. GC Determination:

### 7.1 Notes:

- 7.1.1 The GC system should be conditioned by first injecting a positive carbadox sample from a previous run to improve overall response. Also, if each standard is injected between two samples, the correlation coefficient of the standard curve is improved. GC parameters are set to allow 45 minutes

between each injection to enable system to equilibrate.

7.2 GC parameters:

Oven temperature program:	195°C for 15 minutes, ramp to 250°C at 40°C/minute and hold for 20 minutes. Equilibration time, 7 minutes.
Injection port temperature:	280°C.
Detector temperature:	360°C.
Carrier gas:	Ultra high purity helium, 1.8 mL/min.
Detector make-up gas:	Argon/methane (95:5, 60 mL/min.
Configuration:	Grob splitless with He purge after 1.8 min.
Injection volume:	2 µL.

7.3 Load standard and sample vials into autosampler. Initiate analysis sequence.

7.4 Suitability criteria for acceptability of chromatograms: Retention times of carbadox should be in the range of  $9.9 \pm 0.8$  minutes. Minor tailing of the peaks is acceptable, but excessive tailing accompanied by a decrease in peak height is an indication of a contaminated injection liner, which should be replaced.

7.5 After each run inject 2 µL of Silyl-8 column conditioner followed by 2 µL of acetone.

8. Calculation:

8.1 Using linear regression, determine the slope, y-intercept and regression coefficient for the standard curve of concentration in ppm (X) versus peak height in cm (Y). Correlation coefficient should be  $> 0.995$ .

8.2 Calculate the concentration of carbadox (as QCA) in the fortified and samples as follows:

$$\text{CBX (ppm)} = \frac{(\text{Sample peak height}) - (\text{y-intercept})}{\text{Slope of calibration curve}} \times \frac{5}{\text{Tissue wt.}}$$

8.3 Correct sample results for the recovery where R is the % recovery for the fortified sample.

$$\text{CBX}_{\text{cor}} (\text{ppm}) = \frac{\text{CBX}(\text{ppm}) \times 100}{R}$$