

Laboratories Division, Georgia Department of Agriculture	<b>Title: SCREENING OF FRUITS, VEGETABLES, AND FEEDS FOR CARBAMATES - STRATEGIC DIAGNOSTICS INC.'S, InQuest® OP/CARBAMATE SCREEN</b>	Page <u>1</u> of 5
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I. **PURPOSE** - The purpose of this document is to detail the standard operating procedure for the screening of cholinesterase inhibiting carbamate insecticides in fruits, vegetables, and feeds using the SDI Food Prep Kit and InQuest OP/Carbamate Screening Kit.

**NB** - This is a screening technique designed to determine presence or absence of cholinesterase inhibiting pesticides, therefore, positive results may indicate presence of a broad range of cholinesterase inhibiting compounds necessitating confirmation of compound identity and concentration by P-703 - HPLC with Post column derivitization.

II. **SCOPE** - This standard operating procedure should be employed on all samples of fruits, vegetables, and feeds analyzed by the pesticide enforcement laboratories for carbamate insecticides.

III. **RESPONSIBILITY** - It is the responsibility of the Laboratory Associate to assure that all personnel analyzing fruits, vegetables, and feeds for carbamate insecticides follow this standard operating procedure without exception.

IV. **REFERENCES** -

Bull. Environ. Contam. Toxicol. (1990)45:120-124

A New Sample Preparation System for Immunoassay Detection of Pesticides on Fruit. Poster presented at the California Pesticide Residue Workshop, February, 1994. Jourdan, Scutellaro, Hayes, and Herzog. Ohmicron.

V. **RELATED DOCUMENTS** -

Intra-Laboratory Method Validation for the Screening of Fruits, Vegetable and Feeds for Carbamate Residues using the SDI Food Prep and InQuest OP/Carbamate Screening Kit., Ga. Dept. of Agri. - Chemical Laboratories Div. Farrow and Nuga. 1996.

VI. **DEFINITIONS** -

**Extract Cleanup Reagent** - A Food Prep Kit reagent (pre-packaged in tubes) containing a powdered adsorptive polymer mixture which adsorbs interfering phenolic compounds such as flavenoids.

**Salt Reagent** - A Food Prep Kit reagent (in a bulk bottle) used to effect separation of the aqueous and organic phases (water: acetone) so that an aliquot of the organic phase can be obtained.

**Measuring Scoop** - The scoop used to add the Salt Reagent to the Extract Cleanup Reagent tube.

**Buffer/Chromogen Reagent** - A OP/Carbamate Kit reagent consisting of buffer salts and color indicator reagent (pre-packaged in tubes).

**NB** - After this is mixed with the sample extract from the Food Prep Kit, this reagent will compete with any carbamate present for reaction with the Cholinesterase Reagent.

**Cholinesterase Reagent** - An OP/Carbamate reagent containing lyophilized acetyl cholinesterase and preservatives (pre-packaged in blue top tubes).

**NB** - The Cholinesterase Reagent will complex with the Chromogen to form a dark blue to indigo color in the absence of a cholinesterase inhibitor which might other wise out- compete the Chromogen for the Cholinesterase resulting in reduced intensity of color.

VII. **LABORATORY ANALYTICAL PROCEDURE**

**THEORY** - Fruit, vegetable, and feed samples are extracted with acetone to yield an acetone: water: sample extract which is filtered, aliquot, and cleaned of phenolic compounds using the SDI Food Prep Kit Extract Cleanup Reagent. The aqueous and

organic acetone mixture is partitioned by the addition of the SDI Food Prep Kit Salt Reagent. An aliquot of the organic acetone supernatant is transferred to a borosilicate tube and evaporated just to dryness under nitrogen. The dried extract is dissolved in the SDI OP/Carbamate Screen Wash Solution A, diluted with water, and transferred to the SDI OP/Carbamate Screen Buffer/Chromogen Reagent and the subsequent mixture is transferred to the SDI OP/Carbamate Screen Cholinesterase Reagent. A stop solution is added. The mixture is then transferred to a borosilicate tube, diluted, and an absorbance is taken on a UV/Visible Spectrophotometer. The sample and the historical absorbances of blank matrices are compared and the sample is determined to be positive if the absorbance of the sample is significantly less than that of matrix blanks. The sample is considered to be negative if the absorbance reading is greater than that of the historical absorbances indicating no presence of a cholinesterase inhibitor at a detectable level.

**Limitations:** The OP/Carbamate Screen will detect organophosphate and carbamate pesticides to different degrees. The OP/Carbamate Screen provides screening results. Positive results must be confirmed using P-703.

#### **SAFETY EQUIPMENT REQUIRED-**

- Lab Coat
- Safety Glasses
- Latex Gloves
- Exhaust Hood

#### **REAGENTS REQUIRED-**

- RaPID Prep<sup>®</sup> Food Prep Kit reagent: Extract Cleanup Reagent and Salt Reagent
- InQuest<sup>®</sup> OP/Carbamate Screen reagents: Wash A, Buffer/Chromogen Reagent, and Cholinesterase Reagent
- Supplies can be ordered: Strategic Diagnostics Inc., 128 Sandy Drive, Newark, Delaware 19713 USA. 302-456-6789, 302-456-6782 fax.
- Acetone, Ultra Resi-Analyzed
- Distilled Water
- 200 microgram/ml carbaryl spiking standard
- 0.5 % sulfuric acid stop solution

#### **GLASSWARE REQUIRED -**

- 50 ml KIMAX Brand Graduated Griffin Beakers
- 12 X 75 mm Borosilicate Culture Tubes
- 20 X 150 mm Borosilicate Culture Tube
- 50 ml KIMAX Single Metric Scale Graduated Cylinder

#### **CONSUMABLES REQUIRED -**

- Whatman # I Qualitative 11.0 cm., diam., Filter Paper
- Disposable Transfer Pipets, 7 ml capacity

#### **EQUIPMENT REQUIRED -**

- Milton Roy UV/Vis Spectrophotometer, Model.' Spectronic 1201 equipped with an Ambient
- Flowcell Accessory. (Ambient Flowcell Specifications: Cell Path length = 10 mm, Cell Material = Quartz, and Cell Volume = 500 microliters)
- Vortex Genie 2
- Mettler PM4000 Top Loading Balance

#### **METHODOLOGY -**

1. To a rated 1 liter stainless steel blender cup add 100 grams (fruits and vegetables) or 50 grams (feeds) and 200 ml acetone. Blend the sample for 3 minutes. Let the sample settle for 3 minutes. (For feeds, hydrate the sample with 50 ml of distilled water by adding the water directly to the blender cup before blending.)

2. Using a 7 ml disposable transfer pipette, transfer ~ 20 ml of the extract to Whatman # 1, 11.0 cm qualitative filter paper folded half and then half again fitted into a 50 ml griffin beaker. Allow ~ 15 - 20 ml to be filtered into the beaker. Discard filter paper and immediately cover the beaker to stop evaporation of the acetone.

NB - Steps 1 and 2 of this method are the same as P-702 (modified Luke), therefore, the extract can be obtained from P-702 for filtering.

3. Transfer a 4 ml aliquot of the filtered sample extract to a Food Prep Kit Extract Cleanup

Reagent tube and vortex for 20 seconds. (Vortex setting is "touch" and at the midpoint between 4 and 5 on the setting dial.)

4. Add to the Extract Reagent Cleanup tube one scoop of the Food Prep Kit Salt Reagent and again vortex for 20 seconds. Allow to sit for 5 minutes to allow the aqueous and organic acetone phases to partition. (Vortex setting is "touch" and at the midpoint between 4 and 5 on the setting dial.)

5. With an Eppendorf Reference pipette (100 - 1000 microliter volume range) transfer 100 microliters of the supernatant acetone extract from the partitioned Extract Reagent Cleanup tube to a 12 X 75 mm borosilicate glass culture tube.

6. Evaporate the 100 microliter acetone extract just to dryness under nitrogen.  
NB - Be sure that all the acetone has evaporated to assure that the acetone solvent tolerance (0.5%) of the OP/Carbamate Screen Cholinesterase Reagent is not exceeded.

7. With an Eppendorf Reference pipette (100 - 1000 microliter), add 1 ml of the OP/Carbamate Screen Wash A to the evaporated extract and vortex for 10 seconds to reconstitute the extract in the wash solution. (Vortex setting is "touch" and at the midpoint between 4 and 5 on the setting dial.)

8. With an Eppendorf Reference pipette (100 - 1000 microliter), add 1 ml of distilled water to the reconstituted extract and again vortex for 10 seconds. (Vortex setting is "touch" and at the midpoint between 4 and 5 on the setting dial.)

9. Pour the 2 ml reconstituted extract into the OP/Carbamate Screen Buffer/Chromogen Reagent tube tapping slightly to assure all droplets are deposited into the tube. Replace the tubes' lid, invert once and vortex for 15 seconds. (Vortex setting is "touch" and at the midpoint between 4 and 5 on the setting dial.) Allow to stand for 2 minutes.

10. Pour the contents of the mixed OP/Carbamate Screen Buffer/Chromogen Reagent tube into the OP/Carbamate Screen Cholinesterase Reagent tube tapping slightly to assure all droplets are deposited into the tube. Vortex for 20 seconds. (Vortex setting is "touch" and at the midpoint between 4 and 5 on the setting dial.) Allow to sit exactly 20 minutes.

Nil - This is a good time to turn on the UV/Vis Spectrophotometer and set the wavelength to 350 nm to allow for 20 to 30 minutes of time for the instrument to warm up during the wait period.

11. Immediately after the 20 minute period, add 230 microliters of the 0.5 % sulfuric acid stop solution, vortex for 15 seconds and transfer the contents of the Cholinesterase Reagent tube to a 20 X 150 mm borosilicate glass culture tube rinsing the Cholinesterase Reagent tube once with 5 ml of distilled water from a pre-measured 15 mL of distilled water in a 25 ml graduated cylinder or dispensette. Pour the remaining distilled water from the graduated cylinder or dispensette into the borosilicate glass culture tube. Vortex for 15 seconds. (Vortex setting is "touch" and 8 on the setting dial.)

12. Read the absorbance of the contents of the 20 X 150 mm borosilicate glass culture tube after 30 minutes of elapsed time from the point at which the Buffer/chromogen/reconst. was poured into the Cholinesterase Reagent and vortexed on the Spectronic 1201 per the instructions for analysis with the Spectronic 1201.

#### QUALITY CONTROL:

Samples will be analyzed in batches of not more than 8 samples with one OP/Carbamate Screen Blank (no Food Prep Kit) and one OP/Carbamate Spike (4 ppm Carbaryl) in water accompanying each batch.

A) OP/Carbamate Screen Blank - The screen blank is a matrix similar to those being tested which has previously been determined to be free of cholinesterase inhibiting compounds. These are processed with the samples in the same way as the samples and used for benchmarking for the determination of positive and negative test results.

B) OP/Carbamate Matrix Spiked (4 ppm Carbaryl) - In step 1 of the method add 2 ml of a 200 microgram / ml carbaryl spike standard directly into the blender cup to the same matrix used as the OP/Carbamate Screen Blank. Then add the 200 ml of Acetone and proceed with the method as this were a sample. This will yield an extract with the Cholinesterase inhibiting ability at the Limit of Detection of the screening procedure.

The absorbance measurements will be recorded on control sheets for both A) and B) above so that absorbance readings outside of established control limits will alert the analyst of problems with a batch. All analytical data will be recorded on the SDI Carbamate Screen **Control Sheet**.

Quality control of the Mettler PM4000 Top Loading Balance is contained in P-702.

#### INTERPRETATION OF RESULTS:

If the absorbance measurement of the sample is equal to or less than that of the matrix spike, then the sample is considered to be positive and P-703 must be used to contain results. The result for this will be reported using the reporting guidelines of P-703.

If the absorbance measurement of the sample is greater than that of the matrix spike, then the sample is considered to be negative and the carbamate result will read: Negative for carbamates by P-704.

***NOTE: The time required to determine the results of the described procedure above should not exceed 3 hours from start to finish.***

