SPR-003-V1.2 The Determination of Benomyl in Fruits and Vegetables (HPLC Method)

1 SCOPE AND APPLICATION

This method is applicable to the analysis of benomyl in fruits and vegetables with a reporting limit of 0.5 ppm (µg/g) in the sample.

2 REFERENCES

Determination of Benomyl in Pea Plants, Apples and Saskatoon Berries by HPLC/UV Analysis. Version P-RE-029-97(1)-BEH, Health Canada, Pest Management Regulatory Agency, Laboratory Services Subdivision, Ottawa, Ontario.

3 HEALTH AND SAFETY

WARNING

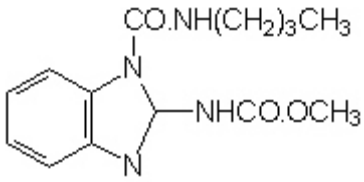
The toxicity or carcinogenicity of each reagent and standard used in this method has not been precisely defined. Each chemical must be treated as a potential health hazard; therefore, exposure to these chemicals must be reduced to a minimum through the use of protective clothing, fume hoods, proper disposal of waste, etc. Appropriate safety procedures are documented in the current CFIA Laboratory Safety Manual. Pertinent information in the Material Safety Data Sheet (MSDS) on each chemical in regards to its potential hazard and control measures required must be consulted and evaluated prior to its use.

4 PRINCIPAL AND THEORY

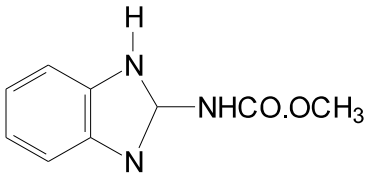
4.1 A representative sample is blended with ethyl acetate. The extract is filtered and evaporated to a small volume. HCl is added and the acidified mixture is heated at 80˚C for one hour to hydrolyze benomyl to carbendazim. After one wash with hexane and another one with ethyl acetate, the acidic aqueous phase is made basic by the addition of sodium carbonate solution. The resulting carbendazim is extracted with ethyl acetate. The ethyl acetate extract is evaporated. The residue is dissolved in methanol and passed

through a Florisil Sep Pak cartridge. Analysis is performed by high pressure liquid chromatography with a photo diode array detector.

4.2 The Structures of Benomyl and Carbendazim



Benomyl



Carbendazim

5 STANDARDS AND REAGENTS

5.1 Standards

Standards are prepared from the purest analytical material available from either the manufacturer or commercial suppliers, typically 99% or higher. The Nanogen codes are used for identification purposes.

Nanogen Code	Identity, Purity	Source
BEH	Benomyl, 99.30%	Pestanal
CAZ	Carbendazim, 99.80%	Pestanal
FUB	Fuberidazole, 99.0%	Pestanal

All solutions prepared from analytical standards are kept in amber vials and stored at 4˚C when not in use. The weight of each standard solution is recorded in order to keep track of solvent loss due to evaporation.

5.1.1 Stock Standard Solutions, BEH and CAZ, (100 µg/mL)
Accurately weigh approximately 10 mg of BEH and CAZ into individual 100 mL amber volumetric flask. Dissolve in ~80 mL of methanol and sonicate for 10 minutes. Dilute to volume with methanol and mix well.
Note: It has been noticed that no more than ~20 mg of BEH and CAZ can be dissolved completely in 100 mL of methanol.

5.1.2 Spiking Standard Solution, BEH, (10 µg/mL)
Using the exact concentration of the stock standard solution, corrected for purity, make an appropriate dilution so that the spiking solution is exactly 10 µg/mL in methanol. Mix well. A 500 µL aliquot of this spiking standard solution in a 10 g of sample represents a 0.5 µg/g BEH spike. The corresponding concentration of the final extract in the LC vial is 2 µg/mL BEH which is equivalent to 1.32 µg/mL CAZ.

5.1.3 Working Standard Solutions, CAZ, (0.66 - 4.0 µg/mL)
Using the exact concentration of the stock standard solution, corrected for purity, make appropriate dilutions so that the working solutions are exactly 0.66, 1.32, 2.0, and 4.0 µg/mL in mobile phase. Add the internal standard working solution (FUB) such that the internal standard concentration is 1 ug/mL. Mix well.

5.1.4 Standards in Matrix, CAZ, (1.32 µg/mL)

Spiking with 1.0 mL of the 1.32 µg/mL CAZ working standard solution to 1.0 mL of the matrix blank at the end of step 8.3.16. Mix well. Evaporate to a 1.0 mL volume to make a standard of 1.32 µg/mL CAZ in Matrix.

5.1.5 Internal Standard Stock solution, FUB, (330 µg/mL)
Accurately weigh 3.3 mg of FUB into a 10 mL volumetric flask. Dilute to volume with methanol.

5.1.6 Internal Standard Working Solution, FUB, (10 µg/mL)
Using the exact concentration of stock standard solution, corrected for purity, make appropriate dilutions so that the working solution is 10 µg/mL FUB in methanol. Mix well.

5.2 Reagents

5.2.1 Ethyl acetate ($CH_3COOC_2H_5$), distilled in glass grade
5.2.2 Hexane (C_6H_{14}), distilled in glass grade
5.2.3 Methanol (CH_3OH), distilled in glass grade
5.2.4 Purified water, from Milli-Q system or equivalent
5.2.5 Hydrochloride acid (HCl), concentrated, ACS grade
5.2.6 Sodium carbonate (Na_2CO_3), ACS grade
5.2.7 Sodium sulphate (Na_2SO_4), anhydrous, granular, ACS grade

5.3 Solutions and Materials

5.3.1 0.1N HCl - Add 8.33 mL of concentrated HCl to a 1 liter volumetric flask containing 500 mL of purified water, dilute to the mark with more purified water and mix well.

5.3.2 1M. Na_2CO_3. - Weigh 106 g of Na_2CO_3 into a 1 litre volumetric flask. Dissolve in approximately 900 mL of purified water, dilute to volume with more purified water and mix well.

6 APPARATUS

6.1 Labware

6.1.1 Glass Mason jar, 500 mL
6.1.2 Buchner funnel, 80 mm i.d.
6.1.3 500 mL vacuum flask
6.1.4 Flat boiling flask, stopper size 24/40, 500 mL
6.1.5 Volumetric flask, 1000 mL
6.1.6 Centrifuge tube, 50 mL.
6.1.7 Separatory funnel, 250 mL
6.1.8 Funnel L/S, 90 mm.
6.1.9 Graduated centrifuge tube, 15 mL
6.1.10 Disposable Pasteur pipettes
6.1.11 Glass beakers, 250 mL.
6.1.12 Volumetric pipettes of various sizes

6.1.13 Graduated pipettes of various sizes
6.1.14 Digital syringe, 500 µL
6.1.15 Filter paper #1, 7 cm diameter, Whatman
6.1.16 Autosampler vial and cap, to fit autosampler
6.1.17 Indicator Strips pH 0-14 from BDH. or equivalent
6.1.18 Florisil Sep Pak Plus, Waters, P.N. WAT020525 or equivalent
6.1.19 Supelco Visiprep system or equivalent

6.2 Auxiliary Equipment

6.2.1 Analytical balance, 4 decimal places, Sartorius Model 1702 mp8 or equivalent
6.2.2 Electronic balance, 3 decimal places, Mettler Model PM400 or equivalent
6.2.3 Rotary evaporator, equipped with water bath set at 40˚C and cryogenic cooling system, Büchi R or equivalent
6.2.4 Vacuum System, Büchi Model V-503 or equivalent
6.2.5 Circulating cooling bath, filled with 1:1 ethylene glycol: water, Neslab Model RTE 111 or equivalent
6.2.6 Analytical evaporator, with bath set at 50˚C for solvent evaporation and 80˚C for hydrolysis, Organomation Model 112 N-EVAP or equivalent
6.2.7 Milli-Q water system, Millipore Ltd. or equivalent
6.2.8 Vortex test tube mixer
6.2.9 Homogenizer, Dupont Model 17105 Omni Mixer Homogenizer or equivalent
6.2.10 Ultrasonic bath
6.2.11 Food processor, Robot Coupe Model R2 or equivalent

6.3 Analytical Instrumentation

6.3.1 HPLC - Waters Millennium system with M6000a series pump, 712 series WISP, TCM and 996 photodiode array detector or equivalent
6.3.2 HPLC Column - Supelco Supelcosil LC-ABZ+plus 25 cm x 4.6 mm analytical column or equivalent
6.3.3 Detection wavelength: Results are calculated using the maximum absorption wavelength for benomyl (285 nm).

7 SAMPLE PREPARATION

The Calgary Laboratory is not responsible for initial sampling procedures. Representative samples supplied by Client Divisions are collected according to established guidelines.

7.1 Prepare the edible portions of the sample as per guidelines set out by Health Canada, e.g., remove core and stem of apples from a sample.
7.2 Blend the entire sample (1 kg) in food processor for two minutes.
7.3 Transfer each homogenate to a glass bottle (large Mason jar) and freeze until required for analysis.
7.4 Thaw frozen homogenates and mix well before taking any subsample.

8 PROCEDURE

8.1 Extraction

8.1.1 Weigh ~10 g sample into a 500 mL Mason jar. Record weight to nearest 0.01 g.
8.1.2 Spike a blank sample with 500 µL of BEH spiking standard (10 µg/mL) to yield a 0.5 ppm (µg/g) BEH spike that is equivalent to a 0.33 µg/g CAZ spike. Allow the spiked sample to sit for 15 minutes before extraction.

8.1.3 Add 150 mL of ethyl acetate and blend for 10 minutes in the Omni mixer, at medium speed.
8.1.4 Filter the extract under vacuum through a Buchner funnel fitted with a 7 cm #1 filter paper into a 500 mL vacuum flask
8.1.5 Transfer filtrate into a 500 mL flat bottomed boiling flask.
8.1.6 Rinse the Mason jar with 3 x 20 mL of ethyl acetate and add the rinsing through the Buchner funnel/vacuum flask. Combine all rinsing in the same flat bottomed boiling flask.
8.1.7 Evaporate on a Rotovap to ~1 mL.
8.1.8 Quantitatively transfer sample extract to a 50 mL centrifuge tube using 10 mL ethyl acetate.

8.2 Hydrolysis

8.2.1 Rinse flask with 25 mL of 0.1 N HCl and add to the sample extract in the centrifuge tube. Shake vigorously for 1 minute.
8.2.2 Place centrifuge tubes in 80˚C water bath and heat for 1 hour.
8.2.3 Remove centrifuge tubes from the water bath and allow to cool to room temperature.

8.3 Clean-up

8.3.1 Quantitatively transfer contents of each centrifuge tube to a 250 mL separatory funnel using hexane.
8.3.2 Extract with 1 x 20 mL of hexane. Separate and discard the hexane (upper phase).
8.3.3 Extract with 1 x 50 mL of ethyl acetate. Separate and discard the ethyl acetate (upper phase).
8.3.4 Add 25 mL of 1M Na_2CO_3 to the acidic aqueous phase and mix. Check the pH with a pH strip. It should be $\geq$ 9.
8.3.5 Extract the now basic solution with 4 x 40 mL of ethyl acetate, collecting and pooling the ethyl acetate extracts for each sample in a 500 mL boiling flask passing through Na_2SO_4 (~20 g Na_2SO_4 on top of a plug of glass wool inside a glass funnel). Discard the aqueous phase.
8.3.6 Evaporate the ethyl acetate extracts on a Rotovap to ~1 mL.
8.3.7 Add 20 mL of methanol and evaporate on a Rotovap to ~1 mL.
8.3.8 Transfer quantitatively to a 15 mL graduated centrifuge tube using methanol and evaporate on a N-Evap to ~0.5 mL.
8.3.9 Prepare the Florisil Sep Pak Plus clean-up columns: Tightly fix the Sep Paks to the top of the Visiprep and place a 10 mL reservoir on top of each Sep Pak. Rinse each Sep Pak with 2 x 10 mL of methanol.
8.3.10 Place a 15 mL graduated centrifuge tube under each Sep Pak column. Quantitatively transfer the sample onto the Sep Pak using methanol.
8.3.11 Elute with 10 mL of methanol, using a flow rate of about 3 drops per second.
8.3.12 Evaporate down to ~0.5 mL..

8.3.13 Add 250 µL of internal standard (10 µg/mL FUB) so that its final concentration will be 1 µg/mL.
8.3.14 Bring volume to 2.5 mL with mobile phase. The final represented sample concentration is 4 g/mL.
8.3.15 Transfer into a HPLC autosampler vial and analyzed by HPLC-UV.
8.3.16 Use 1 mL of the blank extract as the Matrix Blank and another 1 mL for the preparation of Matrix Standard.
8.3.17 A typical run will consist of the 4 standards, a blank, a spike, a matrix standard and the samples. The first run each month should include a duplicate spike for precision testing.

8.4 Determination

Note: HPLC column may degrade and lead to inadequate chromatography.

8.4.1 Mobile Phase: 30% acetonitrile in water
8.4.2 Flow rate: 1.0 mL/min
8.4.3 Injection volume: 20 µL
8.4.4 Retention time of Carbendazim : ~6 minutes.
8.4.5 Retention time of Fuberidazole is ~12 minutes.
8.4.6 Run a set of solvent standards at 0.66, 1.32, 2.0 and 4.0 µg/mL CAZ before and after the samples.

9 CALCULATIONS

Calculations are performed by entering data into the Excel spreadsheet named BEHnnnn.XLS, where nnnn is the current sample year. A summary of the calculations made is given as follows:

9.1 The area count of the Carbendazim peak is corrected by dividing by the area count of the FUB peak and multiplying by the average area count of the FUB peaks for the entire run.

9.2 If the blank is positive, the area count of the blank is to be subtracted from the area counts of the matrix standards as well as from the spikes.

9.3 The four standards from section 5.1.3 are run at the beginning and the end of the analysis. A linear regression analysis is performed on the area counts against concentrations of the standards. Both sets of standards are used for the calculation.

9.4 The slope (m) and the “y intercept” (b) are being calculated.

9.5 The concentration of each extract is calculated from the formula:
Concentration = (net area counts-b)/m.

9.6 The sample concentration is then calculated using the dilution factors and sample weight.

10 CRITICAL CONTROL POINTS

	Section	Control Point	Acceptable Control
10.1	5.1.1 5.1.2	BEH is not readily soluble.	Great care must be taken to transfer quantitatively.
10.2	8.4	Degradation of HPLC column may occur with the consequence that other peaks merge with that of the analyte. Monitor HPLC column performance and replace when necessary.	The performance of HPLC column must be monitored/replaced.

11 PERFORMANCE STANDARDS

11.1 Analytical range is 0.5 µg/g to 1.6 µg/g. Sample with results higher than 1.6 µg/g needs to be retested at a lower weight or the sample is split at step 8.1.8 and an appropriate aliquot is taken.

11.2 Linearity
Correlation coefficient of a standard curve should be 0.99 or better. Otherwise, samples must be re-analyzed.

11.3 Spike Recovery
Recoveries should be in the 58% to 85% range. (Recoveries higher than 85% are more likely due to result from a spiking error than a very high recovery.) If the % recovery of the spiked sample is beyond the range, analysis should be repeated.

11.4 Limit of Detection (LOD) is estimated to be 0.17 µg/g and Limit of Quantitation (LOQ) is estimated to be 0.51 µg/g.

11.5 Reporting Limit is 0.5 µg/g, a level corresponding to the spike run with the samples.

11.6 Results are to be reported to the limit of up to three significant figures and up to the first decimal (e.g., 123 µg/g, 12.3 µg/g, 1.2 µg/g, or 0.5 µg/g).

12 METHOD REVISION HISTORY

Version	Date	Description	Author
SPR-003-V1.1	12 Dec 2000	Detection limit lowered	Doug Baker

Version	Date	Description	Author
SPR-003-V1.2	37804	Change to the use of the photo-diode array detector. Expanded method to fruits and vegetables.	Doug Baker