Food and Agriculture Organization
of the United Nations

FAO SPECIFICATIONS

FOR PLANT PROTECTION PRODUCTS

BENOMYL (AGP:CP/324)

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Rome, 1995

CONTENTS

DISCLAIMER

FAO specifications are developed with the basic objective of ensuring, as far as possible, that pesticides complying with them are satisfactory for the purpose for which they are intended. However, the Group on Pesticide Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent wishes to emphasize that, owing to the complexity of the problem involved, questions such as the suitability of pesticides for the control of a particular pest must be decided at national or provincial level. These specifications should not be assumed to be an endorsement of the use of a particular compound for a given purpose by either the Group of Experts or FAO.

Accordingly, neither the Food and Agriculture Organization of the United Nations (FAO) nor the members of the Group on Pesticide Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent warrant that pesticides complying with these specifications are suitable for control of any given pest or for use in any particular area.

Furthermore, the preparation and use of pesticides complying with these specifications are not exempt from any safety regulation or other legal or regulatory provision applicable thereto. Neither FAO nor any member of the FAO Group of Experts shall be liable for any injury, loss, damage or prejudice of any kind that may be suffered as a result of the preparation or use of pesticide complying with these specifications.

Additionally, the Group of Experts wishes to warn users of specifications that improper field mixing and/or application of pesticides can result in either a lowering or complete loss of their efficacy. This holds true even in cases where such pesticides comply with the specifications indicated.

Accordingly, the Group of Experts and/or FAO can accept no responsibility for the consequences of improper field mixing and/or application.

INTRODUCTION

From time to time, FAO publishes booklets of specifications for technical materials and related formulations of plant protection products. Revisions of, and additions to, already published specifications will be issued when necessary, but revisions may be printed in the *FAO Plant Protection Bulletin* during the interval between editions.

The specifications contained herein have been carefully reviewed and agreed by the Group on Pesticide Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent after consultations with official government scientists, the pesticides industry through GIFAP (Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques or, in English, International Group of National Associations of Manufacturers of Agrochemical Products) and, where appropriate, with individual manufacturers.[1]

FAO has published a *Manual on the development and use of FAO Specifications for Plant Protection Products*, FAO Plant Production and Protection Paper No. 173, Rome 2002 (Revised First Edition available only on the FAO home page of the Internet at: http://www.fao.org/pest-and-pesticide-management/en/).

This manual contains detailed definitions and other essential background information on basic procedures and technical principles adopted by the group on Pesticide Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent, such as:

1. Categories of Specifications (Section 3.1 of the Manual)

FAO Tentative Specifications (Code 'S/T', formerly 'ts') are those which have been recommended by FAO as preliminary specifications and which are based on minimum requirements. The methods of analysis cited are normally supplied by the manufacturer or may already have been published or be the subject of collaborative work.

FAO Provisional Specifications (Code 'S/P', formerly 'S') are those for which more evidence of the necessary parameters is available and where some collaborative study of the methods of analysis has been carried out.

FAO (full) Specifications (Code 'S/F', formerly 'S').
Specifications that have all necessary requirements together with CIPAC (full) methods, or other collaboratively studied (proven) methods.[2, 3]

Wherever possible, standards for apparatus and common names for pesticides are those approved by the International Organization for Standardization (ISO).

2. Expression of active ingredient content (Section 4.2.5 of the Manual)

- for solids, liquid technical materials, volatile liquids (of maximum boiling point 50 ° C) and viscous liquids (with minimum kinematic viscosity of 1 x 10^3 m^2/s at 20 ° C) the FAO Specification shall be based on expression of the content as g/kg;

- for all other liquids the active ingredient content of the product shall be declared in terms of g/kg *or* g/l at 20 ° C. If the customer requires both g/kg *and* g/l at 20 ° C, then in case of dispute the analytical results shall be calculated as g/kg.

3. Tolerance on content (Section 4.2.7 of the Manual)

A declared content of active ingredient must be included in all specifications, and one of the problems immediately arising is the level of tolerance acceptable about the nominal figure. The tolerance is influenced by (a) the reproducibility of the method of analysis, (b) the sampling error and (c) the manufacturing variance.

Allowable variations in analytical results (i.e. tolerances in content of active ingredient) with respect to specific pesticide consignments are intended to cover reasonable variations in the contents of active ingredients. For examples of such tolerances, see the table in Section 4.2.7 of the Manual.

4. Containers/packaging

FAO guidelines are in preparation.

Containers shall comply with pertinent national and international transport and safety regulations.

<u>Technical materials, dustable powders and granules</u>

Containers shall be suitable, clean, dry and as specified, and shall not adversely affect, or be affected by, the contents, but shall adequately protect them against external conditions.

<u>Wettable powders</u>

The product shall be packed in suitable, clean, dry containers as specified in the order. The container shall provide all necessary protection against compaction, atmospheric moisture, loss by vaporization and/or contamination to ensure that the product suffers no deterioration under normal transit and storage conditions.

The product shall be protected by an adequate moisture barrier. This may be a suitable bag of polyethylene or alternative means of giving equal or better protection.

<u>Solutions and emulsifiable concentrates</u>

Containers shall be lined, where necessary, with a suitable material, or the interior surfaces shall be treated to prevent corrosion and/or deterioration of the contents.

Additional information should be given in all specifications where particular pesticides present problems in packaging.

5. Biological information

Phytotoxicity

No test can be specified to cover the possible phytotoxicity of a formulation to all crops. When a crop is not mentioned in the instructions for use, purchasers should check with the supplier that the material is suitable, always provided that such a use is not restricted or legally forbidden.

Wetting of crops

The dilute spray should satisfactorily wet the leaves of the specified crops when used in accordance with the instructions. Test method MT 53.2, CIPAC F, p.162, may be useful.

[1] *Should national pesticide specifications developed from these approved FAO specifications deviate from them, the National Authority responsible for making such changes is requested to inform the FAO Plant Protection Service of the nature of, and the reasons for, the modifications.*

[2] *Methods of analysis and miscellaneous techniques referred to in these specifications have been developed and adopted by CIPAC (Collaborative International Pesticides Analytical Council Ltd.). See CIPAC Handbooks 1 (1970), 1A (1980), 1B (1983), 1C (1985), D (1988), E(1993), CIPAC Proceedings 1980 and 1981, obtainable from Black Bear Press Limited, King's Hedges Road, Cambridge CB4 2PQ, England. The page numbers of specific methods are given in parentheses in the specifications. Copies of methods not yet published can be obtained from the FAO Plant Protection Service.*

[3] *Information on standard waters for laboratory evaluation of pesticidal formulations will be found in CIPAC Monograph 1,* Standard Waters and an FAO Survey on Naturally Occurring Waters *(1972), Black Bear Press Limited, King's Hedges Road, Cambridge CB4, England.*

SUBMISSION OF DRAFT SPECIFICATIONS TO FAO

Any organization, commercial firm or interested individual is encouraged to submit relevant specifications, or proposals for revision of existing specifications, for pesticide products for consideration and possible adoption by FAO. Correspondence should be addressed to the Pesticides Control Officer, Plant Production and Protection Division, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy.

General guidelines on preparing draft specifications are given in Plant Production and Protection Paper 173, *Manual on the Development and Use of FAO Specifications for Plant Protection Products, Revised First Edition,* FAO, Rome, 2002 (available only on the FAO home page of the Internet at: http://www.fao.org/pest-and-pesticide-management/en/).

Specifications which are considered suitable for further processing are assigned priorities and circulated to appropriate organizations and specialists to comment. Comments, together with other relevant information, are then reviewed in detail by the Group on Specifications of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent. The drafts are converted into FAO Provisional Specifications, or full FAO Specifications.

AGP:CP/324

Supersedes AGP: CP/204 (1984)

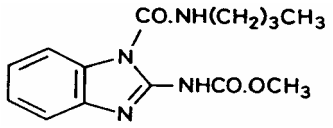
BENOMYL

methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate

INFORMATION

COMMON NAME: benomyl (ISO)

STRUCTURAL FORMULA:



EMPIRICAL FORMULA: $C_{14}H_{18}N_4O_3$

RMM: 290.3

CAS REGISTRY NUMBER: 17804-35-2

CIPAC CODE NUMBER: 206

CHEMICAL NAMES:

methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate (IUPAC)

methyl [1-[(butylamino)carbonyl]-1*H*-benzimidazol-2-yl]carbamate (CA)

BENOMYL TECHNICAL

FAO Specification 206/TC/S/F (1992)

1. DESCRIPTION

The material shall consist of benomyl together with related manufacturing impurities and shall be a fine white powder free from visible extraneous matter and added modifying agents.

2. ACTIVE INGREDIENT

2.1 Identity tests (206/TC/(M)/2, CIPAC D, p.14)*

An identity test is required if the identity of the active ingredient is in doubt.

2.2 Benomyl (205/TC/(M)/3, CIPAC D, p.15)

The benomyl content shall be declared (not lest than 950 g/kg) and, when determined, the content obtained shall not differ from what declared by more than ± 20 g/kg.

3. IMPURITIES

3.1 Water (MT 30.1, CIPAC F, p.91)

Maximum: 3 g/kg.

3.2 2,3-diaminophenazine**

Maximum: 0.5 mg/kg of the benomyl content found under 2.2.

3.3 2-amino-3-hydroxyphenazine**

Maximum: 0.5 mg/kg of the benomyl content found under 2.2.

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* An additional identity test is available from the Plant Protection Officer, FAO Plant Production and Protection Division.

** Methods available from the Plant Protection Officer, FAO Plant Production and Protection Division. Click here to download Analysis of Impurities

BENOMYL WETTABLE POWDERS

FAO Specification 206/WP/S/F (1992)

[Benomyl wettable powder containers should not be stored at temperatures above 50 ° C or in direct sunlight]

1. DESCRIPTION

The material shall consist of a homogeneous mixture of technical benomyl, complying with the requirements of FAO specification 206/TC/S/F (1992), together with filler(s) and any other necessary formulants. It shall be in the form of a fine powder, free from visible extraneous matter and hard lumps.

2. ACTIVE INGREDIENT

2.1 Identity tests (206/WP/(M)/2, CIPAC D, p.16*

An identity test is required if the identity of the active ingredient is in doubt.

2.2 Benomyl (206/WP/(M)/3, CIPAC D, p.16)

The benomyl content shall be declared (g/kg) and, when determined, the content obtained shall not differ from that declared by more than the following amounts.

Declared content	Permitted tolerance
Up to 500 g/kg	± 5% of the declared content
Above 500 g/kg	± 25 g/kg

3. IMPURITIES

3.1 2,3-diaminophenazine**

Maximum: 0.5 mg/kg of the benomyl content found under 2.2.

3.2 2-amino-3-hydroxyphenazine**

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* An additional identity test is available from the Plant Protection Officer, FAO Plant Production and Protection Division.

** Methods available from the Plant Protection Officer, FAO Plant Production and Protection Division.

Click here to download Analysis of Impurities

Maximum: 0.5 mg/kg of the benomyl content found under 2.2.

4. PHYSICAL PROPERTIES

4.1 pH range (MT 75.2, CIPAC F, p.206)

pH range: 5.0 to 8.0.

4.2 Wet sieve test (MT 59.3, CIPAC F, p.179)

Maximum: 0.5% retained on a 75 µm test sieve.

4.3 Suspensibility (MT 15.1, CIPAC F, p.45. Notes 1 and 2)

A minimum of 50% of the benomyl content found under 2.2 shall be in suspension after 30 min in CIPAC Standard Water C at 30 ° C (Notes 3 and 4)

Alternatively, if the buyer requires other CIPAC Standard Waters to be used, then this shall be specified when ordering.

4.4 Persistent foam (MT 47.2, CIPAC F, p.152. Note 5)

Maximum: 25 ml after 1 min.

4.5 Wetting of the product without swirling (MT 53.3.1, CIPAC F, p.165)

The product shall be completely wetted in 1 min without swirling.

5. STORAGE STABILITY

5.1 Stability at 45 ° C (MT 46.1.1, CIPAC F, p.149)

After storage at 45 ± 2 ° C for 21 days, the determined average active ingredient content must not be lower than 97% relative to the determined average content found before storage (Note 6) and the product shall continue to comply with 4.1, 4.2 and 4.3.

NOTES

1. This test will normally be carried out only after the heat stability test 5.1.

2. Chemical assay is the only fully reliable method of measuring the mass of active ingredient still in suspension. However, simpler methods such as gravimetric and solvent extraction determination may be used on a routine basis provided that these methods have been shown to give equal results to those of the chemical assay method. In case of dispute the chemical method shall be the 'Referee method'.

3. Unless another temperature is specified.

4. *The product should be tested at the highest and lowest rates of use recommended by the supplier, provided this is consistent with the conditions given in the method MT 15.1, CIPAC F, p.45.*

5. *The mass of sample to be used in the test should correspond to the highest rate of use recommended by the supplier.*

6. *Samples of the product taken before and after the storage stability test should be analysed together after the test to reduce the analytical error.*

BENOMYL WATER DISPERSIBLE GRANULES

FAO Specification 206/WG/S/F (1992)

[Benomyl water dispersible granules containers should not be stored at temperatures above 50 ° C or in direct sunlight]

1. DESCRIPTION

The material shall consist of a homogeneous mixture of technical benomyl, complying with the requirements of FAO specification 206/TC/S/F (1992), together with fillers and any other necessary formulants. It shall be in the form (Note 1) of granules that will be applied after disintegration and dispersion in water. The product shall be dry, free flowing and free from visible extraneous matter and hard lumps.

2. ACTIVE INGREDIENT

2.1 Identity tests (206/WP/(M)/2, CIPAC D, p.16)*

An identity test is required if the identity of the active ingredient is in doubt.

2.2 benomyl (206/WP/(M)/3, CIPAC D, p.16)

The benomyl content shall be declared (g/kg) and, when determined, the content obtained shall not differ from that declared by more that the following amounts.

Declared content	Permitted tolerance
Up to 500 g/kg	± 5% of the declared content
Above 500 g/kg	± 25 g/kg

3. IMPURITIES

3.1 2,3-diaminophenazine**

Maximum: 0.5 mg/kg of the benomyl content found under 2.2.

3.2 2-amino-3-hydroxyphenazine**

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* Additional identity tests and methods available from the Plant Protection Officer, FAO Plant Production and Protection Division.

** Methods available from the Plant Protection Officer, FAO Plant Production and Protection Division.

Click here to download Analysis of Impurities 

Maximum: 0.5 mg/kg of the benomyl content found under 2.2.

4. PHYSICAL PROPERTIES

4.1 pH range (MT 75.2, CIPAC F, p.206)

pH range: 5.0 to 8.0.

4.2 Wetting of the product without swirling (53.3.1, CIPAC F, p.967)

The product shall be completely wetted in 10 sec without swirling.

4.3 Wet sieve test (MT 167, CIPAC F, p.416)

Maximum: 0.5% retained on a 75 µm test sieve.

4.4 Suspensibility (MT 168, CIPAC F, p.417. Notes 2 and 3)

A minimum of 50% of the benomyl content found under 2.2, shall be in suspension after 30 min in CIPAC Standard Water C at 25 ° C (Note 4).

Alternatively, if the buyer requires other CIPAC Standard Waters to be used, then this shall be specified when ordering.

4.5 Persistent foam (MT 47.2, CIPAC F, p.152. Note 5)

Maximum: 25 ml after 1 min.

4.6 Dustiness (MT 171, CIPAC F, p.425, Gravimetric method)

Maximum: 12 mg collected dust (Note 6).

4.7 Flowability (MT 172, CIPAC F, p.430)

100% of the product shall pass through a 5 mm test sieve after 20 drops of the sieve.

5. STORAGE STABILITY

5.1 Stability at 45 ° C (MT 46.1.1, CIPAC F, p.149)

After storage at 45 ± 2 ° C for 21 days, the determined average active ingredient content must not be lower than 97% relative to the determined average content found before storage (Note 7) and the product shall continue to comply with 4.1, 4.3, 4.4, 4.6 and if required 4.2, 4.5 and 4.7.

NOTES

1. To describe a specific product, it is recommended to add information about the form (e.g. irregular shape, nearly spherical, cylindrical, ...) and to state the nominal size range.

2. *The product should be tested at the highest and lowest rates of use recommended by the supplier, provided this is consistent with the conditions given in method MT 168.*

3. *Chemical assay is the only fully reliable method of measuring the mass of active ingredient still in suspension. However, simpler methods such as gravimetric and solvent extraction determination may be used on a routine basis, provided that these methods have been shown to give equal results to those of the chemical assay method, In case of dispute the chemical method shall be the 'Referee method'.*

4. *Unless another temperature is specified.*

5. *The mass of sample to be used in the test should correspond to the highest rate of use recommended by the supplier.*

6. *The optical method MT 171 usually shows good correlation with the gravimetric method and can, therefore, be used as an alternative where the equipment is available. Where the correlation is in doubt, it must be checked with the product to be tested. In case of dispute the gravimetric method shall be used.*

7. *Samples of the product taken before and after the storage stability test should be analysed together after the test to reduce the analytical error.*

2-BENZIMIDAZOLE CARBAMIC ACID, METHYL ESTER (IN-E965 (MBC))

DETERMINATION OF PHENAZINES (DAP AND AHP) BY REVERSED PHASE LIQUID CHROMATOGRAPHY (RPLC) WITH FLUORESCENCE DETECTION

1. Principle

A portion of MBC is dissolved in an aqueous hydrochloric acid solution and precipitated with saturated sodium chloride. The filtrate is analyzed for 2,3-diaminophenazine (DAP) and 2-amino-3-hydroxyphenazine (AHP) by reversed phase liquid chromatography. The eluted compounds pass through a post-column reagent delivery module and mix with a basic buffer-solution. The compounds are then detected with a fluorescence detector. The detector response for the sample is compared and quantitated against the response of a standard solution of phenazines.

2. Applicability

This method is applicable to the determination of DAP and AHP in MBC technical in the range of 0.1 to 5 ppm

3. Limitation

None

4. Sensitivity, Precision and Accuracy

A. SENSITIVITY

The detection limit for DAP and AHP is 50 ppb (0.05 ppm) based on signal to noise ratio.

B. PRECISION

1. Single Operator
The average analysis (X), standard deviation (s) and 95% confidence limits (95% CL) established for the single operator precision of the method were as follows:

	X	s	95% CL	%RSD
DAP, ppm	2.30	0.155	± 0.40	6.8
AHP, ppm	0.68	0.142	±0.36	21

The above data were calculated from six replicate analysis of one sample performed by one technician over a period of one day.

2. Multiple Operator
Not currently available

C. ACCURACY

A spiking and recovery study was carried out by spiking an MBC technical sample at two different levels. The average recovery was 90% for DAP and 115% for AHP.

Component	Baseload ppm	ppm added	Actual ppm	Recovery %
DAP	2.10	0.685	2.76	96.4
AHP	0.45	0.52	1.12	128.8
DAP	2.10	1.37	3.25	83.9
AHP	0.45	1.04	1.43	94.2

NOTE: Linearity
The linearity of DAP and AHP was determined by spiking four different levels into MBC. The results of this study show DAP is linear up to approx. 5 ppm and AHP to approx. 4 ppm.

5. Special Apparatus

(Equivalent apparatus may be substituted)

High performance liquid chromatograph, such as the following modular system:

- Eluent pump (Beckman Model 110A)
- Buffer (post column) pump (Applied Biosystems Model 400A)
- Fluorescence Detector (McPherson Model FL-749) equipped with a high sensitivity accessory (HSA)
- Column, 150 x 4.6 mm Inertsil C8 (Metachem)
- Hewlett-Packard 1050 Autosampler

- Hewlett-Packard 3350 Lab Automation System (LAS) or equivalent computing system

- Solvent filters, Cat. No. XX10 047 00, Millipore Corporation
- Filter Discs, for organic solvents, Cat. No. FHUP-047 00, Millipore Corp.
- Filter flasks and funnel, Cat. No. KT93825-47 and KT93840-4035, VWR Scientific
- Sample filters, 0.45 um, PTFE, Cat. No. 44525-PC, Scientific Resource Inc.
- Burrel wrist action shaker, Cat. No. 57040-027, VWR Scientific
- Repipet, 10 ml, Cat. No. 13-687-49N, VWR Scientific

6. Reagents

(Reagent grade except as noted)

- Triethylamine, (TEA), 99%
- Acetonitrile, HPLC grade
- Phosphoric Acid, HPLC grade, 85%
- Hydrochloric Acid, concentrated, ACS grade, 12 N
- Hydrochloric acid, 1.0 N
  Add gradually 83 ml of concentrated HCl to 917 ml of water. Label and date. The solution is stable for one year.
- Ascorbic Acid
- Sodium Chloride
- Water, ASTM Type ll
- 2,3-diaminophenazine (DAP), reference standard, IN-M959, Dupont Agric. Products
- 2-amino-3-hydroxyphenazine (AHP), reference standard, IN-F2703
- Sodium acetate trihydrate, “Baker Analyzed”

ELUENT (TEA/CH_3CN)

- Add gradually 1700 ml of water to a 2000 ml beaker.
- While stirring the solution, add (syringe) 0.20 ml (200 ul) of triethylamine to the beaker. Keep the tip of the syringe below the surface of the solution during the addition process.
- Calibrate a pH meter using pH 4 and pH 7.
- Adjust the pH to 3.75 using H3PO4.
- While stirring the solution, add gradually 300 ml of CH3CN.
- Filter (0.45 um) the solution quickly so the CH3CN and the TEA do not evaporate.
- Label the eluent, date, and store in a tightly capped bottle. The solution is stable for at least three months.

The pH and/or CH3CN concentration may be adjusted to optimize peak separation.

ACETATE BUFFER

- Dissolve 27.2 g ± 0.05 of sodium acetate trihydrate in 1000 ml of water.
- Adjust the pH to 5.0 with concentrated HCl.
- Filter (0.45 um) the solution, label and indicate the date. The solution is stable for at least three months.

SAMPLE SOLVENT

- Weigh (to the nearest 0.01 g) 1.0 g of ascorbic acid into a 200 ml volumetric flask.
- Dilute to volume with 1.0 N HCl.
- Transfer to a repipet bottle and label. Prepare this solution daily. The solution is stable for 24 hours.

NACL, SATURATED

- Add approximately 500 g of NaCl (or enough to saturate the solution) to a 1000 ml bottle.
- Make to volume with water.
- Stir for at least two hours.

DAP STOCK SOLUTION (APPROX. 20 UG/ML)

- Weigh (to the nearest 0.0001) 0.01 g of DAP reference standard into a 500 ml volumetric flask.
- Make to volume with CH3CN.
- Ultrasonicate the solution for at least 5 minutes.
- Label the flask, with the date and concentration (ug/ml) corrected for purity.
- Store the solution in an amber bottle and in a dark place. The solution is stable for at least four months.

AHP STOCK SOLUTION (APPROX. 100 UG/ML)

- Weigh (to the nearest 0.0001 g) 0.01 g of AHP reference standard into a 100 ml volumetric flask
- Add (50 ml graduate) 30 ml of CH3CN and 30 ml of water to the flask.
- Add (pipet) 2 ml of concentrated HCl
- Dilute to volume with water
- Ultrasonicate to dissolve
- Label the flask. Indicate the date and record the concentration (corrected for purity). The solution is stable for two months if stored in the dark.

2-Benzimidazole carbamic acid, methyl ester (IN-E965) (MBC)

7. Special Safety considerations

PRODUCT HAZARDS

MBC may irritate the eyes, nose, throat and skin. It may cause skin irritation and sensitization. Use ventilation that is adequate to keep airborne concentrations below exposure limits .In case of contact with skin, immediately wash with soap and water. In case of eye contact, immediately flush with water for at least 15 minutes.

PROCEDURE HAZARDS

Handle syringes with care to avoid skin puncture
Acetonitrile is flammable and toxic. Avoid breathing vapors or skin contact
HCl and H3PO4 are corrosive. Avoid eye or skin contact. Do not breathe vapors or mist.
DAP and AHP are mutagenic compounds and should be handled with gloves in a well ventilated area.
Triethylamine is flammable and corrosive. Avoid contact to eyes, skin or clothing.
Wash thoroughly after handling any of the above compounds.

8. Procedure

OPERATING CONDITIONS

Pumps

Eluent pump: Beckman Model 110A
1.0 ml/min (TEA/CH_3CN)

Buffer pump: ABI Model 400A
0.5 ml/min (acetate buffer)

Expected retention times:		
	DAP	approx. 4.2 minutes
	AHP	approx. 9.3 minutes

Detector and power supply

Excitation wavelength	404 nm (may vary 1-2 nm, optimize for best sensitivity)
Emission filter	550 nm
Gain	0.6
Suppression	0 (ccw)
Range	0.03-0.01
Time	5

OPERATING CONDITIONS

<u>Excitation Wavelength Optimization:</u>

- Remove the emission filter (550 nm) from the high sensitivity accessory.
- Set the PMT gain between 600-800 volts.
- Rotate the excitation wavelength knob slowly, from 390 nm to 410 nm.
- Peak for maximum energy.
- The theoretical wavelength should be 404 nm.
- After optimizing the excitation wavelength, install the emission filter into the high sensitivity accessory.

The system must warm up for at least 15 minutes. It is best to start the system and allow it to equilibrate before beginning sample preparation.

SYSTEM WARMUP

- Turn on the power supply and ignite the lamp.
- Turn on the photometer.
- Set the lamp power to 8 and allow it to warm up for at least 15 minutes.
- Adjust the GAIN so the meter reads 0.0-0.1.
- Change the RANGE from 0.03 to 0.01.
- Adjust the Suppression if necessary for the meter to read 0.1-0.2.
- Leave the detector ON at all times.

CALIBRATION

Prepare the working standard daily:

Add (pipet) 5.0 ml of DAP Stock standard and 1.0 ml of AHP Stock Standard to a 100 ml volumetric flask and dilute to volume with water. Mix and label the flask.

Sampling

Samples should be taken in clean, dry containers.

Sample Analysis

BLANK

Prepare a “blank”by weighing (to the nearest 0.0001) 1 g ± 0.005 of a retained MBC sample (known to be low in both DAP and AHP) into a 2oz amber bottle.

Add (repipet) 10 ml of sample solvent and swirl until the MBC completely dissolves. (See comment 3)

Add gradually 30 ml of saturated NaCl solution.

Shake for 2 ± 0.5 minutes on a Burrell wrist action shaker

Immediately (within 5 minutes) filter (0.45 um) the solution into an HPLC vial for analysis.

SPIKE

Prepare a “spike”by weighing (to the nearest 0.0001) 1 g ± 0.005 of the same MBC used above.

Add (pipet) 2 ml of the working standard into the bottle.

Add (repipet) 10 ml of sample solvent and swirl until the MBC completely dissolves.

Add gradually 28 ml of saturated NaCl solution.

Shake for 2 ± 0.5 minutes on a Burrell wrist action shaker.

Immediately (within 5 minutes) filter (0.45 um) the solution into an HPLC vial for analysis.

SAMPLE

Prepare the sample by weighing (to the nearest 0.0001) 1 g ± 0.005 of the MBC sample into a 2 oz bottle.

Add (repipet) 10 ml of sample solvent and swirl until the MBC completely dissolves. (see Comment 3)

Add gradually 30 ml of saturated NaCl solution.

SAMPLE ANALYSIS

Shake for 2 ± 0.5 minutes on a Burrell wrist action shaker.

Immediately (within 5 minutes) filter (0.45 um) the solution into an HPLC vial for analysis.

Load samples and standards into the auto injector and start the analysis.

INSTRUMENT SHUTDOWN

Turn off the recorder and cap the pen.

Switch the eluent to a FLUSH solution of 75% CH3CN and 25% water.

Switch the post column to a FLUSH solution of 95% water and 25% CH3CN.

Flush both pumps for at least 30 minutes, then turn the pumps off..

Leave the detector ON.

CALCULATIONS

$$\frac{A \times B \times C \times D \times 10^6}{}$$

DAP, ppm = (E –F) x G x H x I

where,

A = area of DAP in the sample (from the chromatogram)
B = grams of DAP in the stock std. (approx. 0.01 g)
C = ml of stock solution in working standard (5 ml)
D = ml of working standard in the spike (2 ml)
E = area of DAP in the spike (from the chromatogram)
F = area of DAP in the blank (from the chromatogram)
G = volume of DAP stock solution (500 ml)
H = volume of DAP working standard (100 ml)
I = sample weight (g)

Simplified DAP calculation:

$$\text{DAP,(ppm)} = \frac{A \times B \times 200}{(E-F) \times I}$$

$$\text{AHP,ppm} = \frac{J \times K \times L \times D \times 10^6}{(M- N) \times O \times H \times I}$$

where

J = area of AHP in the sample (fro the chromatogram)
K = grams of AHP in the stock std. (approx. 0.01 g)
L = ml of stock solution in working standard (1 ml)
D = ml of working standard in the spike (2 ml)
M = area of AHP in the spike (from the chromatogram)
N = area of AHP in the blank (from the chromatogram)
O = volume of AHP stock solution (100 ml)
H = volume of AHP working standard (100 m^{l0}
I = sample weight (g)

Simplified AHP calculation:

$$\text{AHP,ppm} = \frac{J \times K \times 200}{(M-N) \times I}$$

Report results to the nearest whole ppm.

9. Quality Control

Analyze a retained control sample with every set of samples and monitor the results using appropriate statistical control techniques (e.g. CUSUM, Shewart, ANOVA, etc.). If results fall outside established limits, perform the appropriate

corrective action before proceeding with sample analysis. Possible corrective actions may include, but are not limited to the following:

Check instrument settings.

Check expiration dates of standards, and reprepare if necessary.

Reprepare eluent

Reanalyze the control sample in duplicate. If results are still outside the established limits, notify supervisor.

10. Comments

1. If a large number of samples are analyzed daily, leave the detector ON at all times as the detector operates better if left ON all the time.

2. The MBC sample used for the blank and the spike should have been determined to contain less than 0.1 ppm AHP and 0.5 ppm DAP per the condition of this method.

3. Benomyl will not completely dissolve in the sample solvent.

4. For Benomyl AHP and DAP analyses, use Benomyl in the blank and spiked standard calibration solution.

5. The retention times for AHP and DAP in MBC are different from that of Benomyl.

6. In the case of MBC, all the MBC is dissolved in the sample solution which has a matrix effect on the retention times of AHP and DAP.

7. For Benomyl analysis, recovery was 98% for DAP and 113% for AHP.
Expected retention times (Benomyl): DAP = approx. 3.8 minutes
AHP = approx. 8.5 minutes

11. Reference

Dupont MSDS #DUOO5715 for MBC.