

A-1 METHODOLOGY FOR ACTIVE INGREDIENT BENALAXYL - M (D- ISOMER) CONTENT

A-1.1 DETERMINATION OF TOTAL ISOMERS (D AND L) OF BENALAXYL USING GC METHOD

A-1.1.1 Method of analysis:

Reagents:

- Acetone – AR grade
- Internal standard – Tri phenyl methane, pure grade
- Benalaxyl (Sum of D and L isomer) Reference standard of known purity

Preparation of internal standard solution:

About 750 ± 50 mg of triphenyl methane was weighed into a 100 ml volumetric flask and made up to the mark with acetone. This is used as stock solution.

Preparation of reference standard solution:

About 50 mg of Benalaxyl (Sum of D and L isomer) reference standard of known purity was weighed into a 50 ml volumetric flask, added 5.0 ml of internal standard solution into the volumetric flask. Sonicated for 15 minutes made up the mark using acetone, and coded as (CS1). The solution is used for GC analysis.

Preparation of sample solution:

A 1250 ± 50 mg of sample was weighed into a 50 ml volumetric flask in two replicates (S1 and S2) and added 5.0 ml of internal standard solution into each volumetric flask and made up the mark using acetone, further sonicated for 15 minutes then filtered using 0.45 µ filter paper. The filtrate is used for GC analysis coded as S1 and S2 in duplicate analysis.

Sample analysis:

Injected in the sequence CS1, S1R1, S1R2, CS1, S2R1 and S2R2 analyzed for Benalaxyl (D and L) total isomer content.

Estimation:

The sample as well as reference standard solution was injected in gas chromatograph and Flame Ionization Detector. The peak integration and peak area calculation was carried out using GC – Solution software.

A-1.1.2 Chromatographic Parameters for Benalaxyl (Sum of D and L isomer) Content:

Instrument - Shimadzu GC-2010 PF Gas Chromatograph with Flame ionisation detector, and GC solution software

Column - DB-1 Mega bore (30mm length x 0.53 I.D., 1.0 µm film thickness).

Detector - Flame Ionisation Detector (FID)

Temperature conditions

Oven Programme

Temp 1	-	100°C hold 2 min
Temp 2	-	30°C /min to 280°C hold 4 min
Injector	-	290°C
Detector	-	300°C

Gas flow rate

Name of the gas	Flow rate ml/min
Nitrogen	15
Makeup	30
Hydrogen flow	40
Air Flow	300

Injection volume	-	1.0 µl
Split ratio	-	1:5

Retention time (approximate)

Tri Phenyl Methane (*Internal standard*) - 7.5 minutes
Benalaxyl (Sum of D and L isomer) (*Standard*) - 8.5 minutes

A-1.1.3 Formula for Calculation:

$$\text{Benalaxyl (Sum of D and L isomer) content (\% w/w)} = \frac{H_w \times M \times P \times I_r}{H_s \times w \times I_q}$$

Where

H_s = Peak area of Benalaxyl (Sum of D and L isomer) in the reference standard Solution (µv-sec)

H_w = Peak area of Benalaxyl (Sum of D and L isomer) in the sample solution (µv-sec)

M = Mass of Benalaxyl (Sum of D and L isomer) in the reference standard solution (mg)

w	=	Mass of sample taken (mg)
P	=	Purity of Benalaxyl (Sum of D and L isomer) reference standard (%)
I _r	=	Peak area of internal standard in the calibration solution (μV-sec)
I _q	=	Peak area of internal standard in the sample solution (μV-sec)

A-1.2 SEPARATION AND DETERMINATION OF BENALAXYL (D AND L) ISOMER CONTENT USING HPLC METHOD:

A-1.2.1. Method of Analysis:

Reagents:

- n-Hexane
- Isopropyl alcohol
- Benalaxyl (Sum of D and L isomer) Reference standard of known purity
- Benalaxyl -M (D- isomer) Reference standard of known purity

Preparation of reference standard solution:

A 20 ± 2 mg of Benalaxyl (Sum of D and L isomer) reference standard of known purity was weighed into a 20 ml volumetric flask, dissolved and brought up to the mark using n-hexane : Isopropyl alcohol (70:30) solution and coded as C2.

Preparation of sample solution:

A 500 ± 10 mg of sample 1 and sample 2 was weighed into a 20 ml volumetric flask, dissolved and brought up to the mark using n-hexane: Isopropyl alcohol (97:3) solution and analysis coded as S1 and S2 in single analysis

Sample analysis:

Injected in the sequence C2, S1 and S2 and analyzed for Benalaxyl Isomer ratio content.

Estimation:

The sample as well as reference standard solution was injected in HPLC-UV detector. The peak integration and peak area calculation was carried out using LC – solution software.

A-1.2.2 HPLC Parameters Benalaxyl (D and L) Isomer Separation parameter:

Instrument	:	Shimadzu – Prominence HPLC series equipped with LC-20AT pump module, CTO-20A oven module SPD-20A UV-VIS detector and interfaced with LC solution software
Column	:	Chiracel OD (250 mm x 4.6 mm i.d x 10μm particle)
Mobile Phase	:	n-Hexane: Isopropanol (97:3)

Flow Rate : 0.5 mL/min

Column Temperature : Ambient

Detector Wave Length : 264 nm

Injection Volume : 20µL

Retention Time (Approximately)

D - Isomer (Methyl-N-phenylacetyl-N-2, 6-xylyl-D-alaninate) : 10.6 min. (Active)

L - Isomer (Methyl-N-phenylacetyl-N-2, 6-xylyl-L-alaninate) : 11.6 min.

A-1.2.3 Formula for calculation of Benalaxyl-D-Isomer and L-Isomer content:

Benalaxyl-D-Isomer : L-Isomer ratio = % A : %B

$$\% A = \frac{\text{Area A}}{\text{Total Area}} \times 100$$

$$\% B = \frac{\text{Area B}}{\text{Total Area}} \times 100$$

Where

Area A = Peak area of Benalaxyl D-Isomer in the sample solution (µv-sec)

Area B = Peak area of Benalaxyl L- Isomer in the sample solution (µv-sec)

Total Area = Area A + Area B

A-1.2.4 Formula for Calculation of Benalaxyl-M (D isomer) Content:

The content of active ingredient Benalaxyl-M (D isomer) in the test material was calculated on the basis of assay and enantiomeric ratio

$$\text{Benalaxyl-M (D-Isomer) content (\%w/w)} = \frac{\{\text{Benalaxyl Total (D and L) Isomer Content \%w/w} \times \text{Benalaxyl D-Isomer (\% A)}\}}{100}$$

Blank Titration:

About 25 ml each of lead acetate solution was taken into first two absorbers. Similarly, 25 ml each of methanolic potassium hydroxide solution was added to the last two gas absorbers. The whole assembly was connected and cooling water circulation was initiated to the condenser. About 50 ml of glacial acetic acid was added through the inlet tube. The rate of gas bubbling was adjusted to 5-10 bubbles per second. Then immediately 25 ml of Sulphuric acid was added to the flask through the inlet tube. It was heated and the refluxing was continued for 2 hours from the commencement of heating. At the end of the digestion the absorbers was quickly disconnected from the assembly. The contents from the absorber3 and 4 (potash traps) was carefully transferred into 500 ml Erlenmeyer flask. The traps was rinsed with distilled water and transferred into the same flask. This solution was neutralized by using one drop of phenolphthalein indicator and dilutes acetic acid. This neutral solution was titrated with standard iodine solution by starch indicator.

Calculation:

$$\text{Mancozeb a.i. content (\%w/w)} = \frac{(V_1 - V_2) \times N \times 13.551}{M}$$

Where

V_1 = volume, in ml, of standard iodine solution required for titration for the sample

V_2 = volume, in ml, of standard iodine solution required for blank determination

N = normality of the standard iodine solution and

M = mass, in g, of the sample taken for the test

Specification with Method of Analysis of Benalaxyl-M Technical

PROPOSED INDIAN STANDARD SPECIFICATION FOR BENALAXYL-M TECHNICAL

1. SCOPE

This standard prescribes the requirements for methods of sampling and test for Benalaxyl-M Technical.

2. REFERENCE

The following Indian Standards are necessary adjuncts to this standard

IS. No	Title
1070 : 1992	Reagent grade water (Third revision)
6940 : 1982	Methods of test for pesticides and their formulations (first revision)
IS : 8190 - (Part-I) -1988	Requirements for packaging of pesticides : Part 1 solid pesticides (second revision)
10946 : 1996	Method of sampling for technical grade pesticides (first revision)

3. REQUIREMENTS

3.1 Description

The material shall be in the form of cream colour crystalline powder. It shall be free from extraneous matter.

3.2 The material shall comply with the requirements specified in Table 1.

TABLE - 1
Requirements for Benalaxyl-M Technical
(clauses 3.2 and 7.1)

S.No (1)	Characteristic (2)	Requirement (3)	Method of test, Ref. to	
			Annex of this standard (4)	Clause of IS. 6940(5)
i)	Benalaxyl-M content, percent by mass, Min	95	A	
ii)	Moisture content, percent by mass, Max	0.5	-	4.1
iii)	Acidity as H ₂ SO ₄ , percent by mass, max.	0.5	-	11.2

4. PACKING

The material shall be packed according to the requirements given in IS 8190 (Part-1).

5. MARKING

5.1 The containers shall bear legibly and indelibly the following information and other additional information, as is required under the *Insecticides Act, 1968* and Rules framed thereunder :

- a. Name of the material;
- b. Name and address of the manufacturer;
- c. Batch number
- d. Date of manufacture;
- e. Date of expiry
- f. Net mass of contents;
- g. Nominal test item content, percent (m/m); and
- h. A cautionary notice worded as in the *Insecticides Act, 1968* and Rules framed thereunder; and
- i. Any other information required under the *Standards of Weights and Measures (Packaging Commodities) Rules, 1977*.

5.2 BIS certification marking

The product may also be marked with standard mark.

5.2.1 The use of the standard mark is governed by the provisions of Bureau of *Indian Standards Act, 1986* and the rules and regulations made thereunder. The details of the conditions under which the license for the use of standard mark may be granted to manufacturers or procedures may be obtained from the Bureau of Indian Standards.

6. SAMPLING

Representative sample shall be drawn as prescribed in IS 10946.

7. TESTS

7.1 Tests shall be carried out in accordance with the method referred in col 4 and 5 of table 1.

7.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (see IS 1070) shall be employed in test.

Note : 'Pure Chemical' shall mean chemicals that do not contain impurities which affects results of analysis.

ANNEX – A
 [Table 1, SI No. (i)]
DETERMINATION OF BENALAXYL-M CONTENT

- 01 **Chromatographic conditions :-**
 Instrument: Gas Chromatograph equipped with FID Detector and PTV injector system.
 Column : ZB – 5,30 meter, ID 0.32 mm ,Film Thickness 1.0µ m
 Oven Temp : 45°C-5 min-10°C/ min -280°C -15 min HOLD
 Injector Temp. : 40°C -14°C / Sec- 280°-1min Hold – 40°C
 Injector : PTV
 Detector Temp.: 320 °C
 Carrier Helium Flow : 1.5 ml / min
 Hydrogen Flow : 35 ml / min
 Zero Air Flow : 350 ml / min
 Makeup gas Nitrogen: 30 ml / min
 Split flow : 45 ml / min
 Run Time : 40 Min.
 Injection Size : 0.5µl
- 02 **Retention time :**
 Internal Standard (DEP) : 22.40 min
 Benalaxyl-M : 30.20 min.
- 03 **Internal Standard** : Diethyl Phthalate
- 04 **Preparation of standard solution:** Accurately weigh about 50 mg of internal standard (DEP) and 50 mg of Benalaxyl-M standard in 25 ml volumetric flask. Dissolve and makeup the volume with Dichloromethane (MDC).
- 05 **Preparation of Sample solution:** Accurately weigh about 50 mg of internal standard (DEP) and 50 mg of Benalaxyl-M sample in 25 ml volumetric flask. Dissolve and makeup the volume with Dichloromethane (MDC).
- 06 **Calculations:**
- $$RF = \frac{W1}{W2} \times \frac{A2}{A1}$$

$$\text{Assay (\% m/m)} = \frac{W4}{W3} \times \frac{A3}{A4} \times RF \times \text{Purity of Benalaxyl-M Standard.}$$

Where,

W1= Weight of Benalaxyl-M in standard solution

W2= Weight of Internal Standard (DEP) in standard solution

W3= Weight of Benalaxyl-M in sample solution

W4= Weight of Internal Standard (DEP) in Sample solution

A1= Area of Benalaxyl-M peak in standard solution

A2 =Area of Internal Standard peak in standard solution

A3 = Area of Benalaxyl-M peak in sample solution

A4 = Area of Internal Standard in sample solution

07 **Note: Retention time may vary column to column and instruments to instruments.**