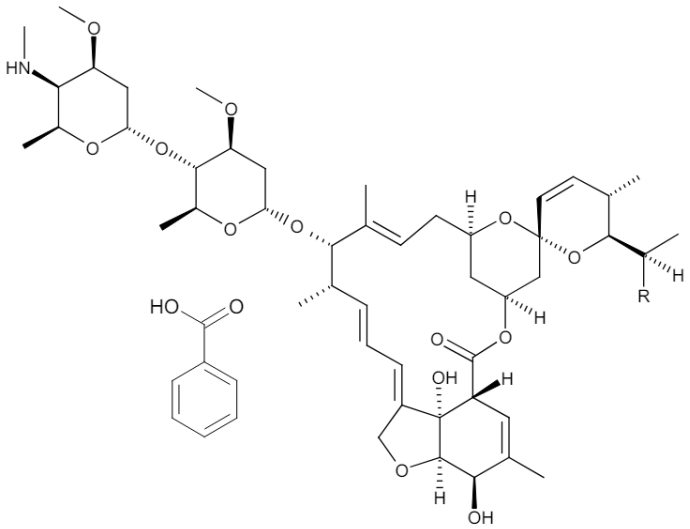
# EMAMECTIN BENZOATE

XXX



R = $CH_2CH_3$ for emamectin B1a benzoate

R = $CH_3$ for emamectin B1b benzoate

| | |
|---|---|
| *ISO Common Name* | Emamectin benzoate |
| *Chemical Name* | (4’’R)-4’’-deoxy-4’’-(methylamino) avermectin B1 benzoate |
| *CAS Number* | Emamectin benzoate: 155569-91-8<br>Emamectin $B_{1a}$ benzoate: 138511-97-4<br>Emamectin $B_{1b}$ benzoate: 138511-98-5 |
| *Empirical formula* | Emamectin $B_{1a}$ benzoate: $C_{56}H_{81}NO_{15}$<br>Emamectin $B_{1b}$ benzoate: $C_{55}H_{79}NO_{15}$ |
| *Molecular mass* | Emamectin $B_{1a}$ benzoate:1008.3<br>Emamectin $B_{1b}$ benzoate: 994.2 |
| *m.p.* | 141-146 ºC |
| *v.p* | 0.004 mPa (21 ºC) |
| *Solubility* | In water 0.024 g/L at 25 ºC; soluble in acetone and methanol |
| *Stability* | Stable to hydrolysis at pH 5,6,7 and 8 (25 ºC). Photodegrades rapidly. |

| | |
|---|---|
| *Description* | White to off-white powder |
| *Formulation* | - |

# EMAMECTIN BENZOATE TECHNICAL

XXX/TC/(M)/-

**1 Sampling.** Take at least 100 g.

## 2 Identity tests

**2.1 HPLC.** Use the HPLC method below. The relative retention time of the emamectin benzoate in the sample solution should not deviate by more than 1.5% from that of the calibration solution.

**2.2 Infrared.** Prepare potassium bromide discs for the technical sample and emamectin benzoate reference substance. Scan the discs from 4000 to 400 $cm^{-1}$. The spectrum from the sample should not differ significantly from that of the reference substance.

## 3 Emamectin Benzoate

**OUTLINE OF METHOD**

Emamectin benzoate is determined by reversed phase high performance liquid chromatography using UV detection at 245 nm and external standardization.

**REAGENTS**

*Emamectin benzoate:* reference standard of known purity

*Acetonitrile:* HPLC grade

*Methanol:* HPLC grade

*Ammonium hydroxide:* HPLC grade, 25%~30%

*Ammonia solution:* Ammonium hydroxide : water= 1 : 300 (v/v)

*Water:* HPLC grade

**Calibration solutions.** Weigh in duplicate (to the nearest 0.1 mg) into a volumetric flask (100 ml) about 50 mg of emamectin benzoate standard (*s* mg). Dilute to volume with methanol. Mix thoroughly. Filter a portion of each sample solution with a 0.45 µm filter prior to analysis (calibration solutions $C_A$ and $C_B$).

## APPARATUS

*High performance liquid chromatograph* equipped with a UV detector capable for operation at 245 nm, a constant-temperature column compartment and an injection system capable of injecting 5 µl.

*Column stainless steel* 250 x 4.6 mm (i.d.), packed with $C_{18}$ 5 µm or equivalent with the same selectivity.

*Filtering apparatus* disposable plastic syringes (or equivalent) fitted with 0.45 µm filters.

*Electronic integrator or data system*

## PROCEDURE

**(a) Liquid chromatographic conditions (typical):**

| | |
|---|---|
| *Column* | stainless steel, 250 x 4.6 mm (i.d.), packed with $C_{18}$, 5 µm, or equivalent |
| *Mobile phase* | methanol : acetonitrile : ammonia solution, 25:55:20(v/v/v) |
| *Column temperature* | 30°C ± 2°C |
| *Flow rate* | 1.2 ml/min |
| *Detector wavelength* | 245 nm |
| *Injection volume* | 5 µl |
| *Retention time* | Emamectin $B_{1b}$: approximately 16.0 min, Emamectin $B_{1a}$: approximately 22.0 min. |
| *Run time* | 35 min |

**(b) System equilibration**. Inject 5 µl portion of calibration solution $C_A$ until the response factors (*fi*) obtained for two consecutive injections differ by less than 1.5%. Then inject 5µl portions of calibration solution $C_B$. The response factor (*fi*), for two consecutive injections should not deviate by more than 1.5% from that of solution $C_A$, otherwise prepare new calibration solutions.

**(c) Sample preparation.** Prepare solutions in duplicate for each sample. Weigh

(to the nearest 0.1 mg) sufficient sample (w mg) to contain about 50 mg of emamectin benzoate into a volumetric flask (100 ml). Dilute to the volume with methanol. Mix thoroughly. Filter a portion of each sample solution with a 0.45 μm filter prior to analysis (sample solutions $S_1$ and $S_2$).

**(d) Determination.** Inject in duplicate 5 μl portions of each sample solution bracketing them by injections of the calibration solutions as follows:

$C_A$, $S_1$, $S_1$, $C_B$, $S_2$, $S_2$, $C_A$, ...

**(e) Calculation**. Calculate the mean value of each pair of calibration response factors *f*, bracketing the two injections of a sample, and use this value for calculating the emamectin benzoate contents of the bracketed sample injections.

$$f_i = \frac{s \times P}{Hs}$$

$$\text{Content of Emamectin benzoate} = \frac{H_W \times f}{W} \text{ (g/kg)}$$

where:
$f_i$ = individual response factor
$f$ = mean response factor
$H_s$ = peak area of emamectin (peak $B_{1b}$+$B_{1a}$) in the calibration solution
$H_w$ = peak area of emamectin (peak $B_{1b}$+$B_{1a}$) in the sample solution
$s$ = mass of emamectin benzoate reference standard in the calibration solution (mg)
$w$ = mass of sample taken (mg)
$P$ = purity of the emamectin benzoate reference standard (g/kg)

Calculate the ratio of $B_{1a}$ to $B_{1b}$ of emamectin benzoate in the sample:

$$\alpha(B_{1a}/B_{1b}) = \frac{H_{W_{B1a}}}{H_{W_{B1b}}}$$

where:
$\alpha(B_{1a}/B_{1b})$ = ratio of $B_{1a}$ to $B_{1b}$ of emamectin benzoate in the sample
$H_{W_{B1a}}$ = peak area of emamectin (peak $B_{1a}$) in the sample solution

$H_{W_{B1b}}$ = peak area of emamectin (peak $B_{1b}$) in the sample solution

**Repeatability r** = XXX-XXX g/kg at an active ingredient content of XXX – XXX g/kg

**Reproducibility R** = XXX-XXX g/kg at an active ingredient content of XXX – XXX g/kg

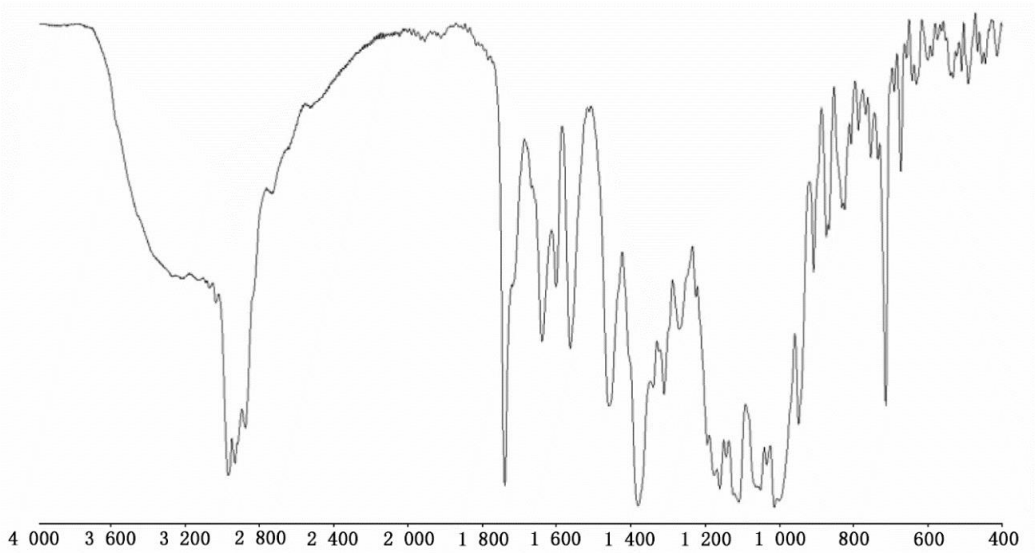



**Fig. 1** FTIR spectrum of emamectin benzoate standard

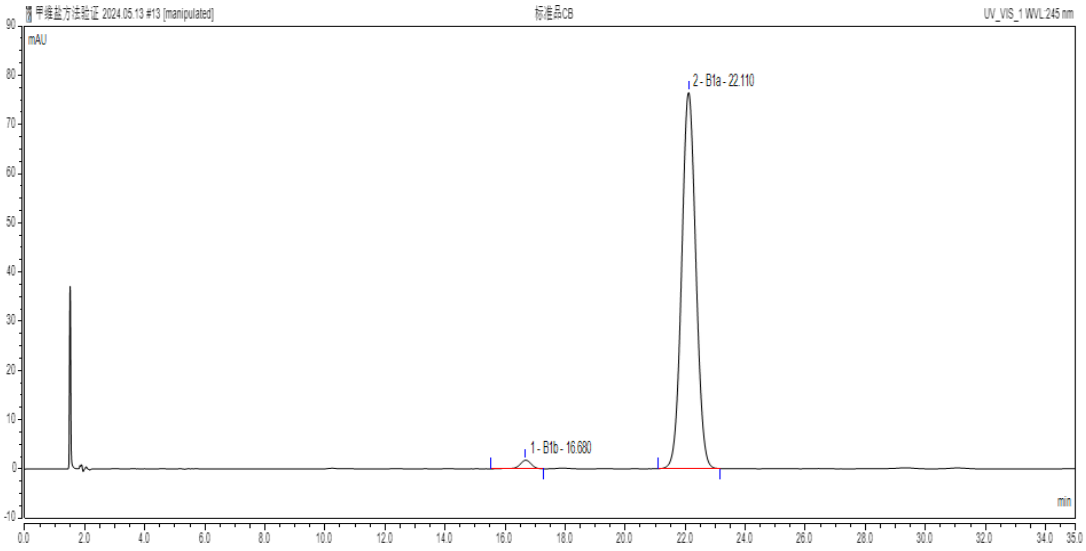



**Fig. 2** HPLC Chromatogram of emamectin benzoate standard

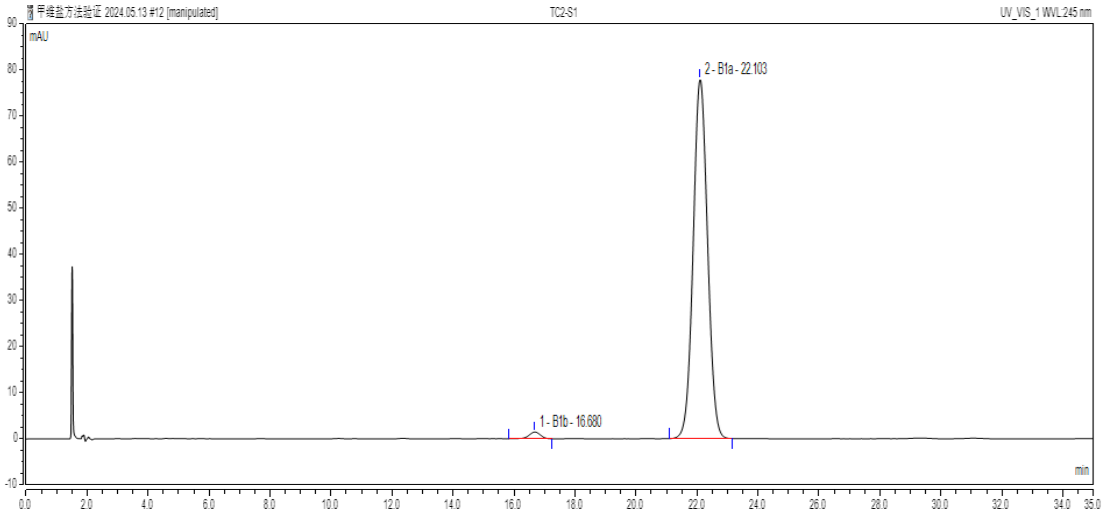



**Fig. 3** HPLC Chromatogram of emamectin benzoate TC