Emamectin Benzoate

Collaborative Study

Small scale collaborative study for the

Determination of Emamectin Benzoate in

TC by HPLC

Report to CIPAC by CHIPAC

Method Developed by

Hebei Xingbai Agricultural Technology Co., Ltd.

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1. Participants

Small Scale Collaborative Study for the determination of Emamectin Benzoate TC by HPLC was organized by CHIPAC, and participated by 3 labs. All the 3 laboratories provided their results, which are presented in the following sections.

Index	Contact	Lab
Lab 1	Hongxia Li	Nutrichem Laboratory Co., Ltd
Lab 2	Jianzhong Yu	Institute of Quality Safety and Nutrition of Agricultural Products, Zhejiang Academy of Agricultural Sciences
Lab 3	Wendy Wang	Jiangsu Agrochem Laboratory Co., Ltd.

2. Active Ingredient: General Information

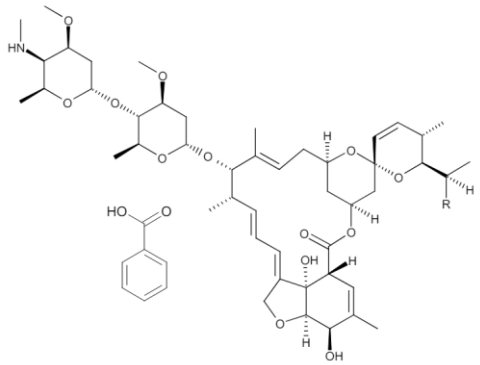
ISO common name: Emamectin benzoate

CAS No.: Emamectin benzoate: 155569-91-8

Emamectin B_{1a} benzoate: 138511-97-4

Emamectin B_{1b} benzoate: 138511-98-5

Structure:



R = CH_2CH_3 for emamectin B1a benzoate

R = CH_3 for emamectin B1b benzoate

Molecular mass: Emamectin B_{1a} benzoate:1008.3

Emamectin B_{1b} benzoate: 994.2

Empirical formula: Emamectin B_{1a} benzoate: $C_{56}H_{81}NO_{15}$

Emamectin B_{1b} benzoate: $C_{55}H_{79}NO_{15}$

3. Samples

Three test samples and Emamectin benzoate analytical standard were sent to the participants:

(1) Emamectin benzoate tech. sample (TC-1)
(2) Emamectin benzoate tech. sample (TC-2)
(3) Emamectin benzoate tech. sample (TC-3)
(4) Emamectin benzoate, reference standard

All participants sent back their results in time.

4. Method

4.1 Scope

The determination of Emamectin benzoate active ingredient content was assayed in technical material (TC).

4.2 Outline of method

Emamectin benzoate is determined by high performance liquid chromatography on a reversed phase column (C_{18}) with UV detection at 245 nm and external standardization.

4.3 Procedure for the collaborative trial

The samples were analyzed on two different days, each day involving duplicate injections of duplicate weights. Both test and reference solutions were freshly prepared on each day.

5. Analytical conditions

Lab	Instrument	Column	Flow Rate (ml/min)	Column Temp. (℃)	Wavelength (nm)	Injection Volume (μl)	Mobile phase (v/v)
1	Agilent 1200	ZORBAX RR Extend-C_{18} (250×4.6 mm, 3.5 μm)	1.0	30	245	5	Methanol : acetonitrile : ammonia water=25 : 55 : 20
2	Shimadzu Nexera UHPLC LC-30A	Agilent ZORBAX Eclipse XDB-C_{18} (250×4.6 mm, 5 μm)	1.2	30	245	5	Methanol : acetonitrile : ammonia water=25 : 55 : 20
3	Agilent 1260	Agilent Extend C_{18} (250×4.6 mm, 5 μm)	1.2	25	210	5	Methanol : acetonitrile : ammonia water=25 : 55 : 20

6. Deviations and Remarks

For lab 1, the particle size of column was 3.5 μm which was different from the method requirement. And the flow rate was revised as 1.0 ml/min accordingly which was different from 1.2 ml/min required. This deviation was justified as a minor change.

7. Evaluation and Discussion

7.1 Evaluation of the Quality of Data and Chromatograms

The data obtained from each laboratory was visually reviewed and no significant deviation regarding the chromatography which might affect the analysis results was founded.

Therefore, all data sets were included within the statistical assessment. The report below contains statistical evaluations with the full set of 3 participating laboratories.

7.2 Determination of emamectin benzoate

The statistical evaluation of the data was accomplished following the “Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods”, according to DIN ISO 5725. The testing for outliers/stragglers of the laboratory mean values were not performed.

In Tables 1-7 and Figures 1-9, respectively, the full set of all laboratories (3 participants) are reported. A comparison of the RSD_R of this collaborative study with the unmodified Horwitz equation showed that the reproducibility relative standard deviation (RSD_R) is lower than the Horwitz value for all the products (see in Table 5-7). The Horwitz Ratio (HorRat) for each sample was found within the desired range (0.3-1.0). Due to the universal applicability of the method, this collaborative trial is acceptable.

8. Conclusions

From the results shown above, the method can be considered applicable for the determination of emamectin benzoate contents in TC. CHIPAC propose that a full scale collaborative trial might be conducted.

Table 1 Results of analysis of Emamectin benzoate content in the TC1

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi^2	Standard Deviation S_i	Si^2
	1	2	1	2				
Lab 1	969.4	965.5	970.2	968.9	968.5	937984.7	2.081	4.330
Lab 2	956.3	954.2	955.1	952.6	954.5	911121.4	1.540	2.371
Lab 3	974.6	976.0	971.9	972.2	973.7	948040.4	1.983	3.934

Table 2 Results of analysis of Emamectin benzoate content in the TC2

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi^2	Standard Deviation S_i	Si^2
	1	2	1	2				
Lab 1	967.4	972.3	970.1	971.8	970.4	941664.6	2.226	4.95
Lab 2	958.9	956.8	959.2	954.0	957.2	916317.2	2.390	5.714
Lab 3	974.2	977.3	976.0	970.4	974.5	949616.0	3.029	9.18

Table 3 Results of analysis of Emamectin benzoate content in the TC3

	Day1(g/kg)		Day2(g/kg)		Average Yi	Yi^2	Standard Deviation S_i	Si^2
	1	2	1	2				
Lab 1	972.0	968.1	974.5	970.9	971.4	943554.1	2.662	7.09
Lab 2	952.6	958.2	956.8	953.2	955.2	912367.4	2.743	7.521
Lab 3	971.7	972.0	976.4	973.8	973.5	947631.1	2.156	4.650

Table 4 Ratio of B_{1a} to B_{1b} of Emamectin benzoate in the TC1, TC2 and TC3

	TC1				TC2				TC3			
	Day1		Day2		Day1		Day2		Day1		Day2	
	1	2	1	2	1	2	1	2	1	2		
Lab 1	74.3	73.2	75.8	76.2	76.9	80.2	76.8	77.4	77.4	73.3	73.0	76.0
Lab 2	65.9	65.6	65.3	65.9	65.6	65.4	66.1	65.4	65.9	65.9	65.5	65.4
Lab 3	70.8	70.9	71.1	71.4	70.8	70.7	70.8	70.5	69.5	70.0	69.9	69.9

Table 5 Statistics of the results of TC1 (Emamectin benzoate)

S_1=Sum Yi	2896.7
S_2=Sum Yi^2	2797146.6
S_3=Sum Si^2	10.64
No. Lab P	3
No. Determination n	4
Average Y=S_1/P	965.6

$Sr^2=S_3/P$	3.545	Sr	1.883
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n$	97.21	SL	9.859
$S_R^2=S_r^2+S_L^2$	100.75	SR	10.037
Repeatability $r=2.8*S_r$	5.272		
Reproducibility $R=2.8*S_R$	28.10		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.1950		
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	1.0395		
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	2.011		
HorRat	0.5		

Table 6 Statistics of the results of TC2 (Emamectin benzoate)

S_1=Sum Yi	2902.1		
S_2=Sum Yi^2	2807597.8		
S_3=Sum Si^2	19.84		
No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	967.4		
$Sr^2=S_3/P$	6.61	Sr	2.572
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n$	79.47	SL	8.915
$S_R^2=S_r^2+S_L^2$	86.09	SR	9.278
Repeatability $r=2.8*S_r$	7.201		
Reproducibility $R=2.8*S_R$	25.98		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.2659		
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	0.9591		
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	2.010		
HorRat	0.5		

Table 7 Statistics of the results of TC3 (Emamectin benzoate)

S_1=Sum Yi	2900.0
S_2=Sum Yi^2	2803552.6
S_3=Sum Si^2	19.26
No. Lab P	3
No. Determination n	4

Average $Y=S_1/P$	966.7		
$Sr^2=S_3/P$	6.420	Sr	2.534
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]-S_r^2/n$	98.52	SL	9.926
$S_R^2=S_r^2+S_L^2$	104.94	SR	10.244
Repeatability $r=2.8*S_r$	7.094		
Reproducibility $R=2.8*S_R$	28.68		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.2621		
Relative Standard Deviation of Reproducibility $RSD_R=S_R*100/Y$	1.0597		
Horwitz $RSD_R(Hor)=2^{[1-0.5*log(Y/1000)]}$	2.010		
HorRat	0.5		

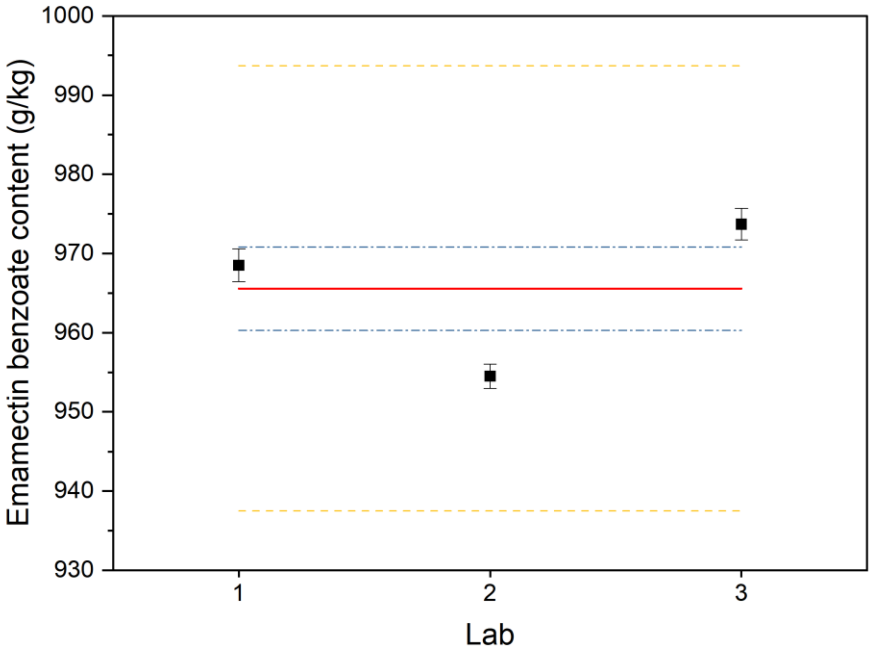


Figure 1. Graphical presentation of TC1 data (Emamectin benzoate)

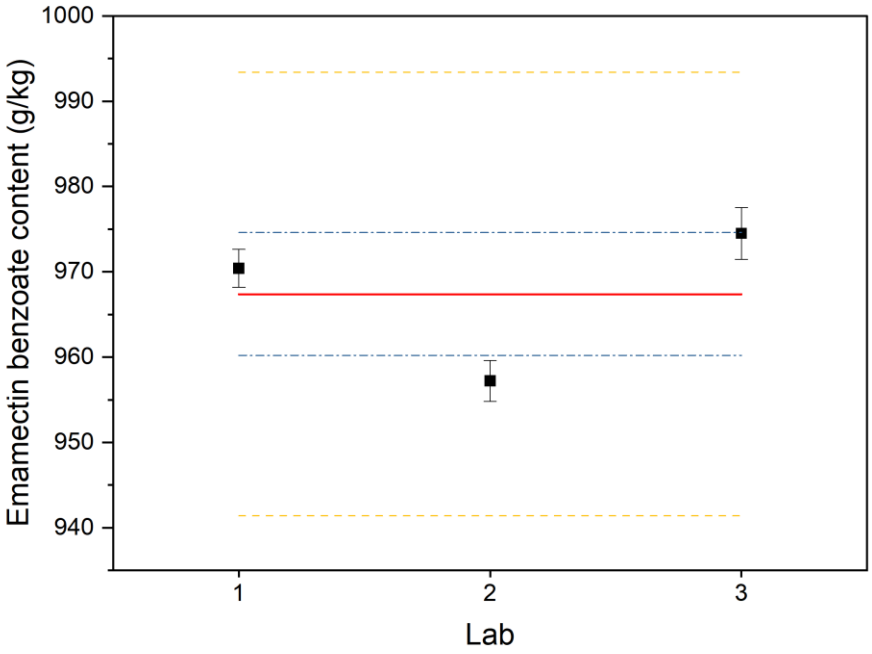


Figure 2. Graphical presentation of TC2 data (Emamectin benzoate)

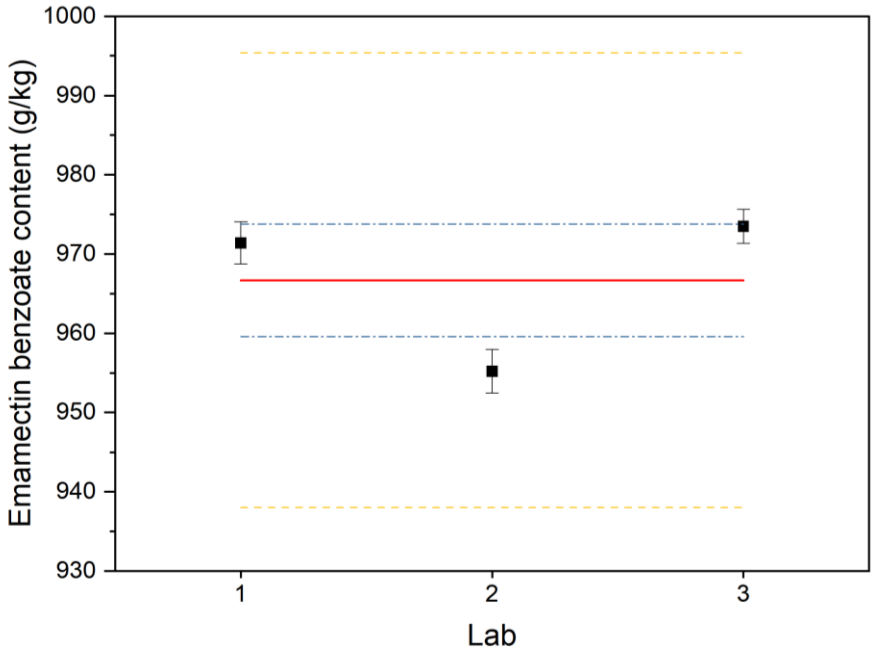


Figure 3. Graphical presentation of TC3 data (Emamectin benzoate)

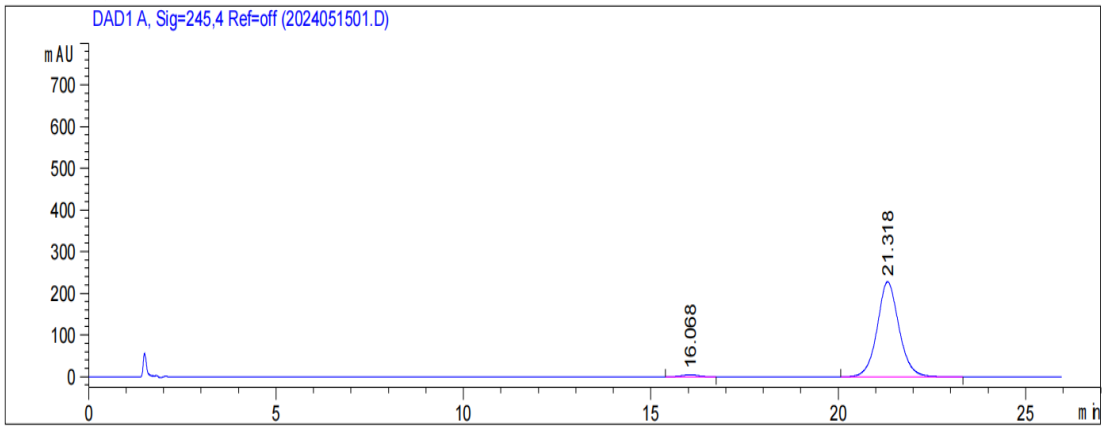


Figure 4. HPLC chromatogram of Emamectin benzoate standard (Lab 1)

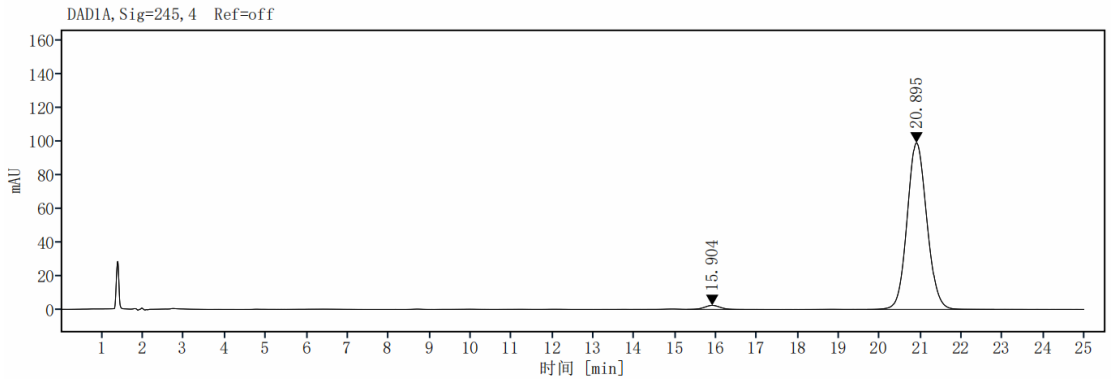


Figure 5. HPLC chromatogram of Emamectin benzoate standard (Lab 2)

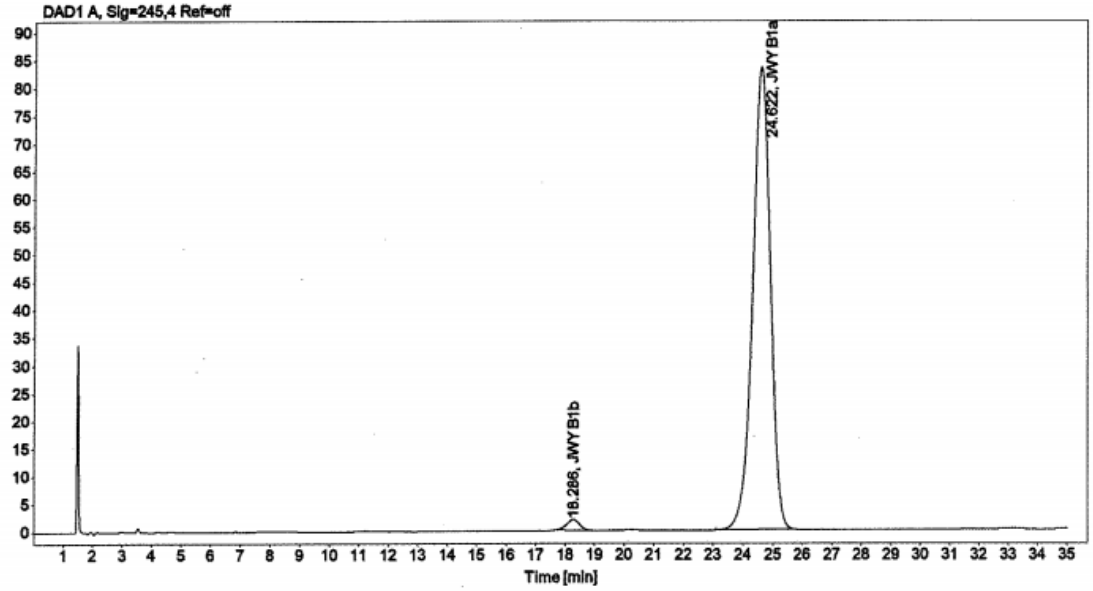


Figure 6. HPLC chromatogram of Emamectin benzoate standard (Lab 3)

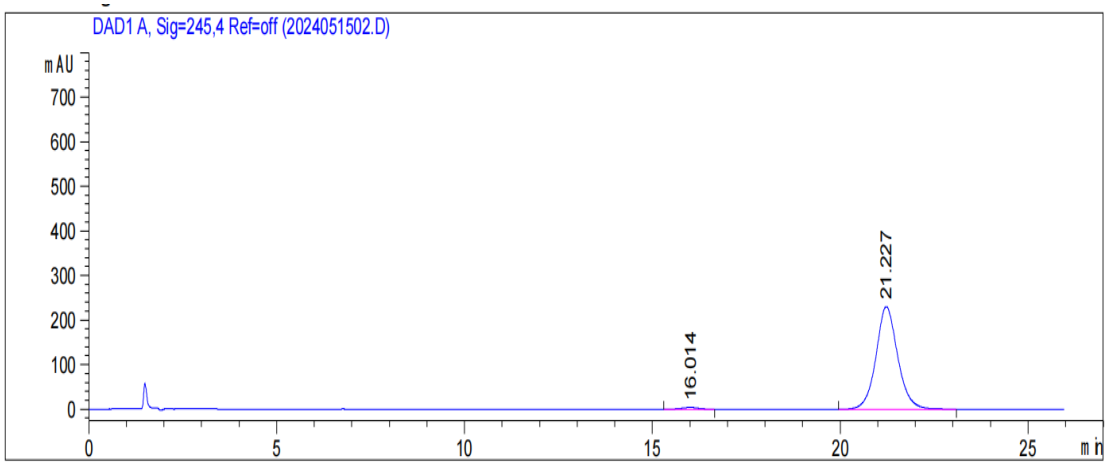


Figure 7. HPLC chromatogram of Emamectin benzoate TC (Lab 1)

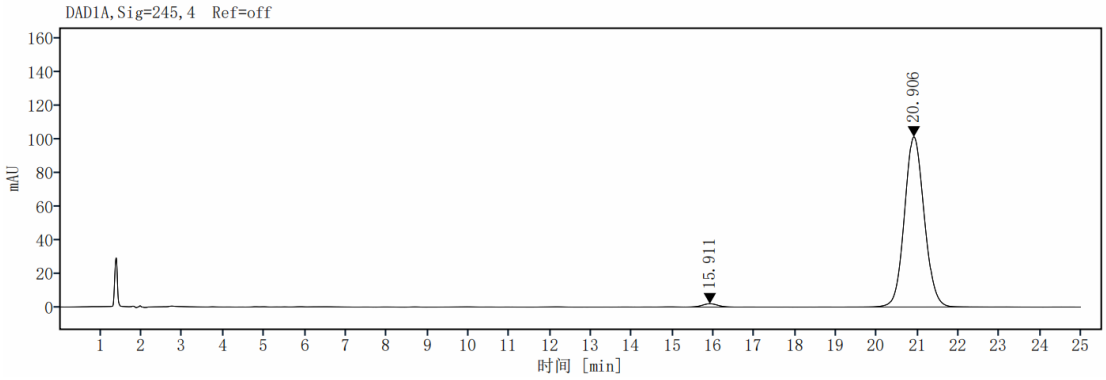


Figure 8. HPLC chromatogram of Emamectin benzoate TC (Lab 2)

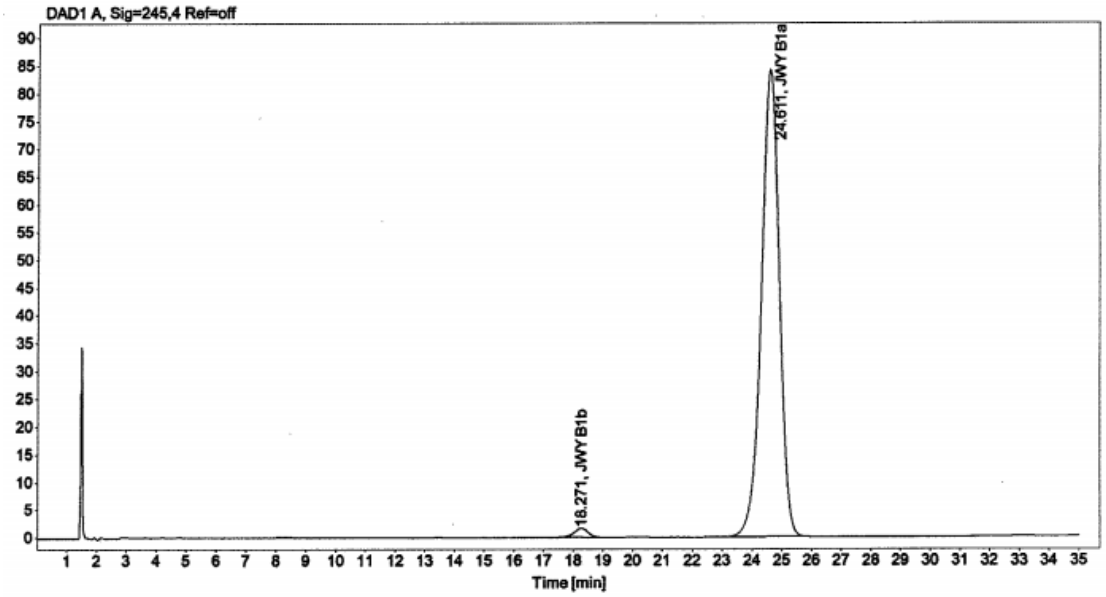


Figure 9. HPLC chromatogram of Emamectin benzoate TC (Lab 3)