S-metolachlor
Collaborative Trial Report

Small scale collaborative trial for the determination of S-isomers Percentage in S-metolachlor TC and EC

Report to CIPAC by Shandong Binnong Technology Co., Ltd
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1. Participants and sample distribution

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Lab2	Wendy Wang	Jiangsu Agrochem Laboratory Co., Ltd.	No.98, Minjiang Road, Hi-Tech Development Zone Changzhou, Jiangsu, China
Lab3	Shen Peng	JiangSu EverTest Co., Ltd.	No. 31-1, Hengjing Road, Economic and Technological Development Zone, Nanjing, Jiangsu, China

2. Sample information

Sample	Quantity	Batch	Declared Content of AI
S-metolachlor TC-1	100 g	202401105	975 g/kg
S-metolachlor TC-2	100 g	202402078	975 g/kg
S-metolachlor EC-1	100 g	202401090	975 g/kg
S-metolachlor EC-2	100 mL	202401176	860 g/kg
S-metolachlor EC-3	100 mL	202402113	860 g/kg

S-metolachlor analytical standard, 98.7 % purity, with S-isomers purity 87.2%.

3. Method description

3.1 Outline of method

S-isomers and R-isomers percentage in S-metolachlor is determined by normal phase HPLC on a chiral column using UV detector at 230 nm. The S-isomers percentage in S-metolachlor is calculated.

3.2 Apparatus and reagents

HPLC system with UV

Column, Daicel CHIRALPAK AY-H (250mm × 4.6mm × 5 µm), or equivalent.

Electronic integrator or data system

Ultrasonic bath

Metolachlor standard of known purity

S-metolachlor standard of known purity

Heptane, HPLC grade

Ethanol HPLC grade

Mobile phase Heptane - Ethanol, 94+6 (v/v). Add by pipette ethanol (60 ml) to heptane (940 ml); degas before use.

Calibration solution (racemate solution). Weigh (to the nearest 0.1 mg) about 25 mg of

the racemate metolachlor standard into a volumetric flask (25 ml). Add heptane (about 15 ml) into the flask, place the flask in an ultrasonic bath for 2 min. Allow to cool to ambient temperature. Dilute to volume with heptane. Mix thoroughly. (Solutions Cm).

Calibration solution (S-metolachlor standard solution). Weigh (to the nearest 0.1 mg) about 25 mg of the S-metolachlor standard into a volumetric flask (25 ml). Add heptane (about 15 ml) into the flask, place the flask in an ultrasonic bath for 2 min. Allow to cool to ambient temperature. Dilute to volume with heptane. Mix thoroughly. (Solutions Cs).

3.3 HPLC condition

Column temperature	30°C
Detector wavelength	230 nm
Injection volume	10 µl
Flow rate	0.6 ml/min
Run time	25 min
Retention time	S1 isomer: about 14.8 min
	S2 isomer: about 16.2 min
	R1 isomer: about 17.8 min
	R2 isomer: about 19.8 min

3.4 Procedure

(i) Preparation of sample solution. Weigh (to the nearest 0.1 mg) into a volumetric flask (25 ml) sufficient sample to contain approximately 25 mg of S-metolachlor. Add heptane (about 15 ml) into the flask, place the flask in an ultrasonic bath for 2 min. Allow to cool to ambient temperature. Dilute to volume with heptane. Mix thoroughly. (Solutions S).

(ii) Determination. Inject in duplicate 10 µl portions of the calibration solutions (Cm and Cs) and of the sample solutions (S_1, S_2, ……) in the following sequence:

C_m, C_s, S_1, S_2, S_3, S_4, C_m, C_s, S_5, S_6……

Measure the relevant peak areas.

(iii) Calculation.

$$P_S = \frac{H_S \times 100}{H_S + H_R}$$

$$P_R = (100 - P_S)$$

where:

H_S = peak area of total S-isomers

H_R = peak area of total R-isomers
P_S = Total S-isomers percentage (%)
P_R = Total R-isomers percentage (%)

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

4. Deviations and remarks

Lab	Lab 1	Lab 2	Lab 3
HPLC Model	Hitachi Primaide	Agilent 1260	Shimadzu LC20AD
Column Information	CHIRALPAK AY-H, 250mm×4.6mm×5μm	CHIRALPAK AY-H, 250mm×4.6mm×5μm	CHIRALPAK AY-H, 250mm×4.6mm×5μm
Column Temperature (ºC)	30	30	30
Detector Wavelength (nm)	230	230	230
Flow rate, ml/min	0.5	0.6	0.6
Injection Volume (μL)	10	10	10
Mobile phase Heptane+Ethanol (v/v)	94+6	94+6	94+6
Retention Time, min	S1 isomer: 14.8 min S2 isomer: 16.1 min R1 isomer: 17.8 min R2 isomer: 19.9 min	S1 isomer: 12.9 min S2 isomer: 13.8 min R1 isomer: 15.4 min R2 isomer: 16.1 min	S1 isomer: 16.6 min S2 isomer: 17.9 min R1 isomer: 20.3 min R2 isomer: 22.0 min

Note: Lab 1 used a different flow rate of 0.5 mL/min to obtain the consistent retention time.

5. Statistical evaluation

Table 1 Results of the analysis of S isomers percentage in the TC-1

	DAY-1		DAY-2		Average Yi (%)	Yi^2	Standard Deviation, Si	Si^2
LAB-1	88.58	88.62	88.58	88.58	88.59	7848.494887	0.021149267	0.000447292
LAB-2	88.84	88.84	88.71	88.72	88.78	7881.498623	0.073117861	0.005346222
LAB-3	87.91	87.76	87.75	87.91	87.83	7714.200542	0.087228068	0.007608736

Table 2 Results of the analysis of S isomers percentage in the TC-2

	DAY-1		DAY-2		Average Yi (%)	Yi^2	Standard Deviation, Si	Si^2
LAB-1	88.78	88.78	88.73	88.74	88.76	7877.987079	0.023093025	0.000533288
LAB-2	88.97	88.96	88.89	88.90	88.93	7908.586441	0.040253294	0.001620328
LAB-3	88.08	88.13	88.06	88.09	88.09	7759.727752	0.029408922	0.000864885

Table 3 Results of the analysis of S isomers percentage in the EC-1

	DAY-1		DAY-2		Average Yi (%)	Yi^2	Standard Deviation, Si	Si^2
LAB-1	88.65	88.57	88.61	88.60	88.61	7850.895425	0.032204103	0.001037104
LAB-2	88.82	88.82	88.72	88.72	88.77	7879.583166	0.058221007	0.003389686
LAB-3	87.88	87.96	87.95	87.82	87.90	7726.598301	0.065263967	0.004259385

Table 4 Results of the analysis of S isomers percentage in the EC-2

	DAY-1		DAY-2		Average Yi (%)	Yi^2	Standard Deviation, Si	Si^2
LAB-1	88.65	88.63	88.66	88.66	88.65	7859.054142	0.013682292	0.000187205
LAB-2	88.82	88.81	88.71	88.71	88.76	7878.828323	0.057966746	0.003360144
LAB-3	87.91	87.96	87.98	87.85	87.92	7730.51719	0.059477436	0.003537565

Table 5 Results of the analysis of S isomers percentage in the EC-3

	DAY-1		DAY-2		Average Yi (%)	Yi^2	Standard Deviation, Si	Si^2
LAB-1	88.64	88.61	88.61	88.61	88.62	7852.738909	0.014187873	0.000201296
LAB-2	88.81	88.81	88.72	88.72	88.76	7879.144008	0.048658819	0.002367681
LAB-3	87.95	87.84	87.89	87.89	87.89	7725.274179	0.044266927	0.001959561

Figure 1 Graphical presentation of TC-1 data

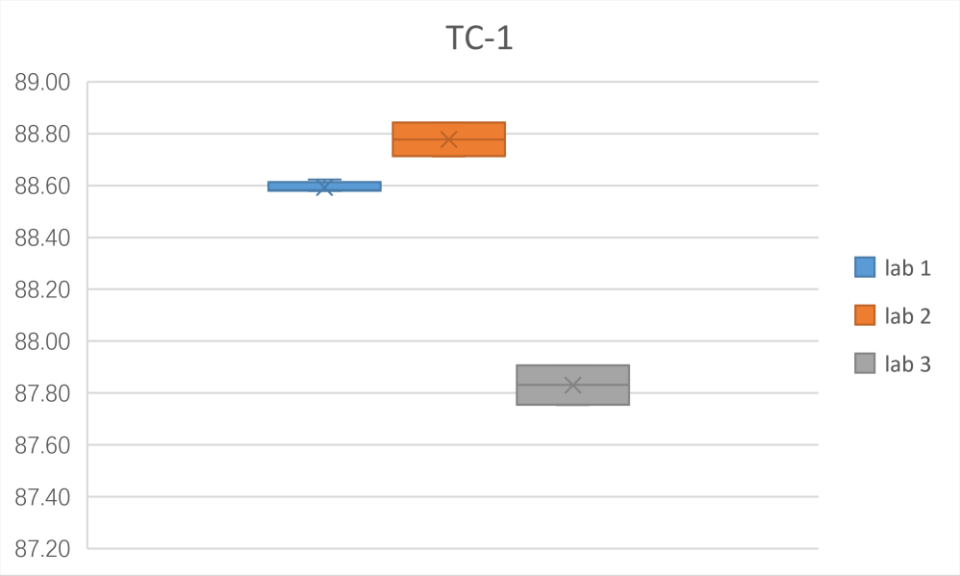

Figure 2 Graphical presentation of TC-2 data

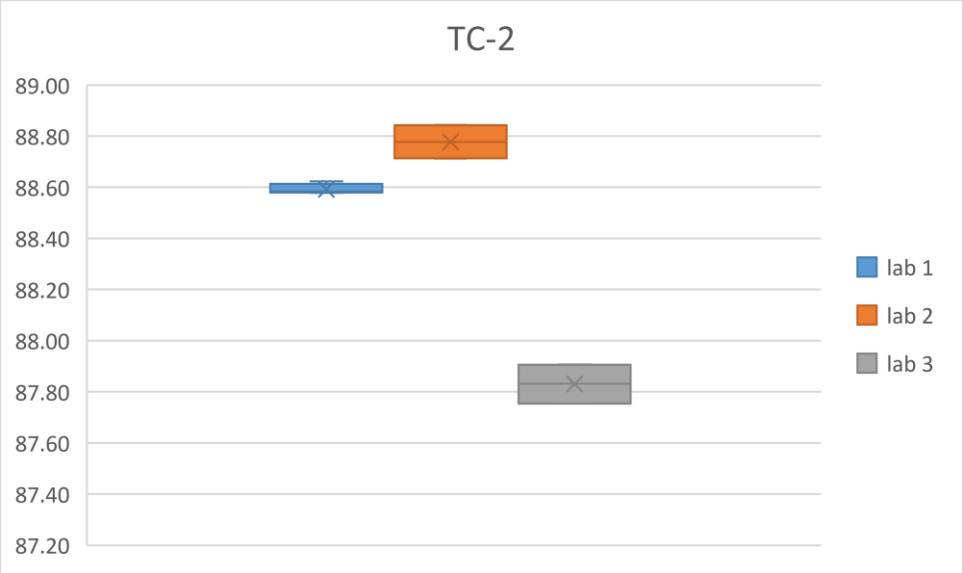

Figure 3 Graphical presentation of EC-1 data

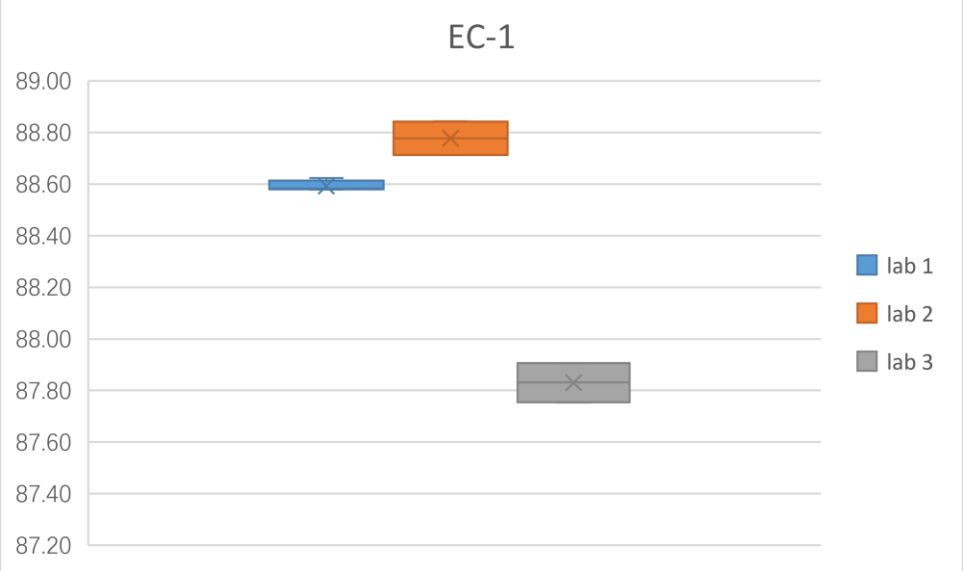

Figure 4 Graphical presentation of EC-2 data

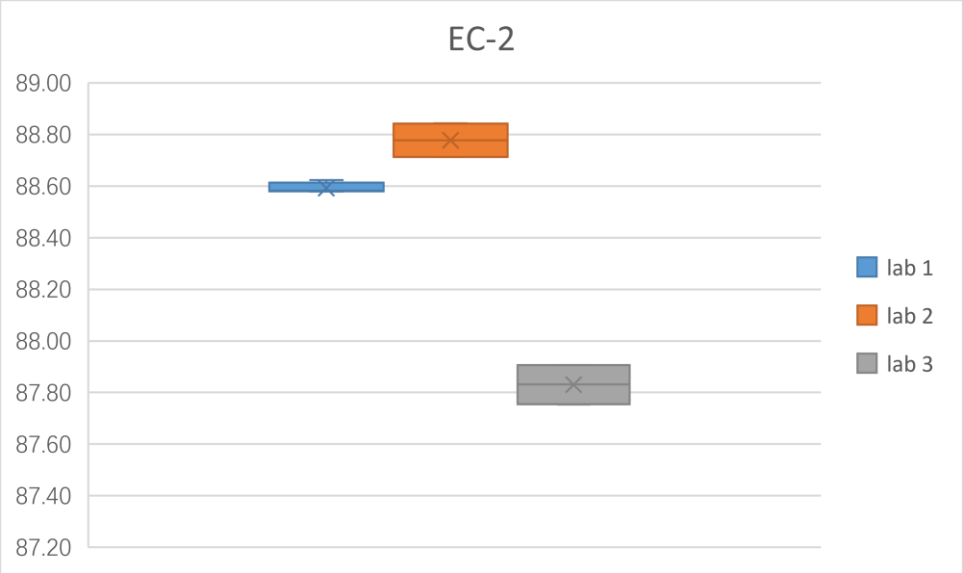

Figure 5 Graphical presentation of EC-3 data

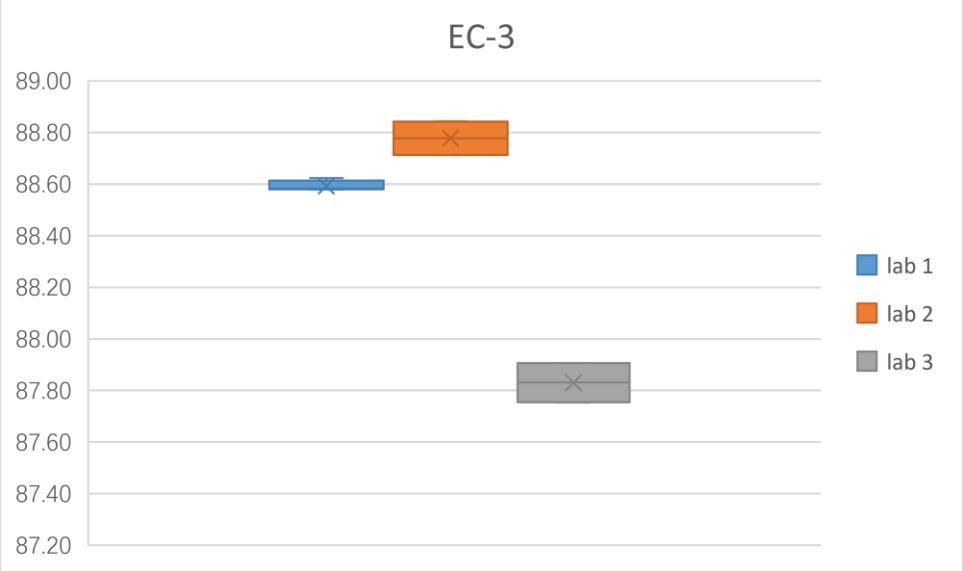

Table 6 Statistics of the results of TC-1

S_1=sum Yi	265.200058		
S_2=sum Yi^2	23444.19405		
S_3=sum Si^2	0.013402249		
No. Lab P	3		
No. Determination n	4		
Average Y=S_1/P	88.40001932		
S_r^2=S_3/P	0.004467416	**Standard Deviation of Repeatability, S_r**	0.066838734
S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n	0.250784622	**S_L**	0.500784008

$S_R^2=S_r^2+S_L^2$	0.255252039	**Standard Deviation Reproducibility, S_R**	0.505224741
Repeatability, r=2.8*Sr	0.187148455		
Reproducibility, R=2.8*S_R	1.414629274		
Relative Standard Deviation of Repeatability, $RSD_r=S_r*100/Y$	0.075609411		
Relative Standard Deviation of Reproducibility, $RSD_R=S_R*100/Y$	0.571521075		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/1000)]	2.881407727		
HorRat	0.198347867		

Table 7 Statistics of the results of TC-2

S_1=sum Yi	265.7775759		
S_2=sum Yi^2	23546.30127		
S_3=sum Si^2	0.0030185		
No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	88.5925253		
$S_r^2=S_3/P$	0.001006167	**Standard Deviation of Repeatability, S_r**	0.066838734
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.197076387	**S_L**	0.443932863
$S_R^2=S_r^2+S_L^2$	0.198082553	**Standard Deviation Reproducibility, S_R**	0.445064662
Repeatability, r=2.8*Sr	0.088816367		
Reproducibility, R=2.8*S_R	1.246181053		
Relative Standard Deviation of Repeatability, $RSD_r=S_r*100/Y$	0.035804523		
Relative Standard Deviation of Reproducibility, $RSD_R=S_R*100/Y$	0.502372701		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/1000)]	2.880464464		
HorRat	0.174406839		

Table 8 Statistics of the results of EC-1

S_1=sum Yi	265.2733661
S_2=sum Yi^2	23457.07689

S_3=sum Si^2	0.008686175		
No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	88.42445536		
$S_r^2=S_3/P$	0.002895392	**Standard Deviation of Repeatability, S_r**	0.053808845
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.211264211	**S_L**	0.459634867
$S_R^2=S_r^2+S_L^2$	0.214159603	**Standard Deviation Reproducibility, S_R**	0.462773814
Repeatability, r=2.8*Sr	0.150664765		
Reproducibility, $R=2.8*S_R$	1.295766678		
Relative Standard Deviation of Repeatability, $RSD_r=S_r*100/Y$	0.060852899		
Relative Standard Deviation of Reproducibility, $RSD_R=S_R*100/Y$	0.523355006		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/1000)]	2.881287861		
HorRat	0.181639264		

Table 9 Statistics of the results of EC-2

S_1=sum Yi	265.3374305		
S_2=sum Yi^2	23468.39965		
S_3=sum Si^2	0.007084914		
No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	88.44581017		
$S_r^2=S_3/P$	0.002361638	**Standard Deviation of Repeatability, S_r**	0.048596688
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.207231132	**S_L**	0.455226462
$S_R^2=S_r^2+S_L^2$	0.20959277	**Standard Deviation Reproducibility, S_R**	0.45781303
Repeatability, r=2.8*Sr	0.136070726		
Reproducibility, $R=2.8*S_R$	1.281876483		
Relative Standard Deviation of Repeatability, $RSD_r=S_r*100/Y$	0.054945156		
Relative Standard Deviation of Reproducibility, $RSD_R=S_R*100/Y$	0.517619805		

Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/1000)]	2.881183141
HorRat	0.179655294

Table 10 Statistics of the results of EC-3

S_1=sum Yi	265.2737624		
S_2=sum Yi^2	23457.1571		
S_3=sum Si^2	0.004528537		
No. Lab P	3		
No. Determination n	4		
Average Y=S_1/P	88.42458745		
S_r^2=S_3/P	0.001509512	**Standard Deviation of Repeatability, S_r**	0.038852444
S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n	0.216671992	**S_L**	0.465480388
S_R^2=S_r^2+S_L^2	0.218181504	**Standard Deviation Reproducibility, S_R**	0.46709903
Repeatability, r=2.8*Sr	0.108786844		
Reproducibility, R=2.8*S_R	1.307877285		
Relative Standard Deviation of Repeatability, RSD_r=S_r*100/Y	0.043938508		
Relative Standard Deviation of Reproducibility, RSD_R=S_R*100/Y	0.528245643		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/1000)]	2.881287213		
HorRat	0.183336684		

A Grubbs test were run on all determinations, and no outlier was found.

6. Conclusion

No outlier was found in the data set.

All RSD_R values were lower than the criteria calculated using the Horwitz equation. All HorRat values were smaller than 0.3.

From the results shown above, the method can be considered applicable for the determination of S isomers percentage in S-metolachlor TC and EC. We propose that a full scale collaborative trial might be conducted.

7. Figures

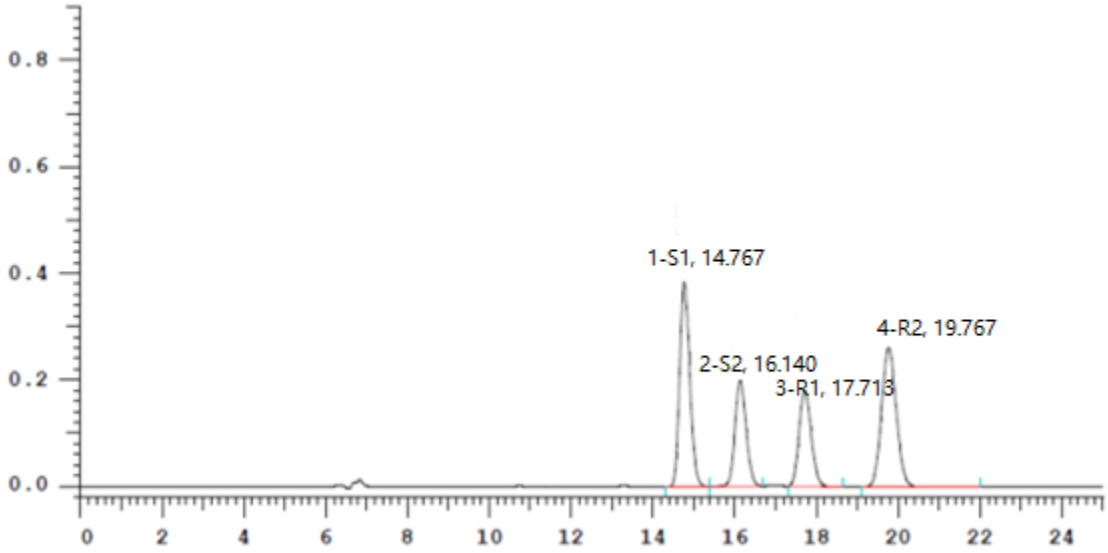

Fig 1 HPLC chromatogram of metolachlor racemate standard

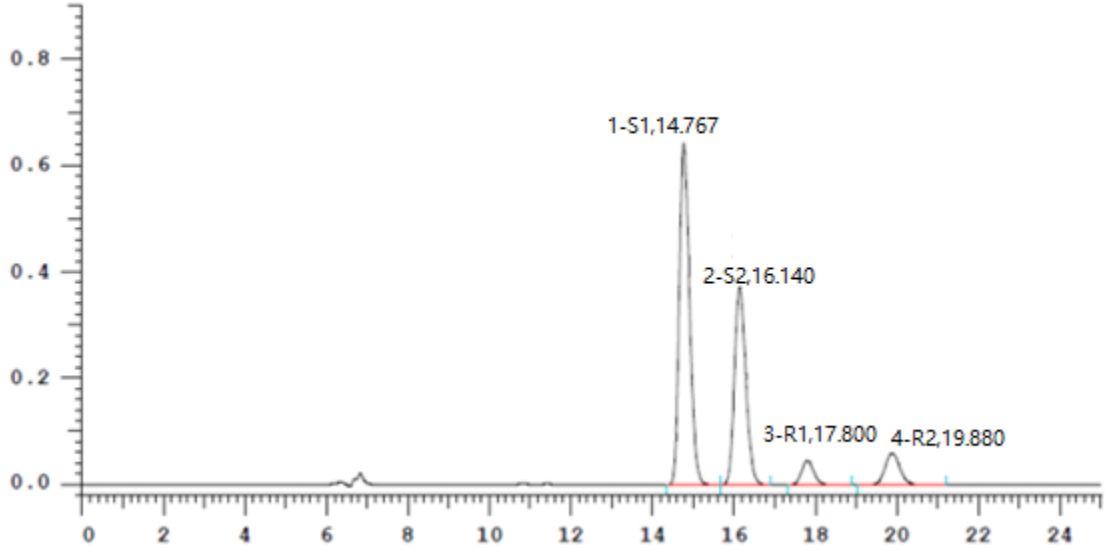

Fig 2 HPLC chromatogram of S-metolachlor standard

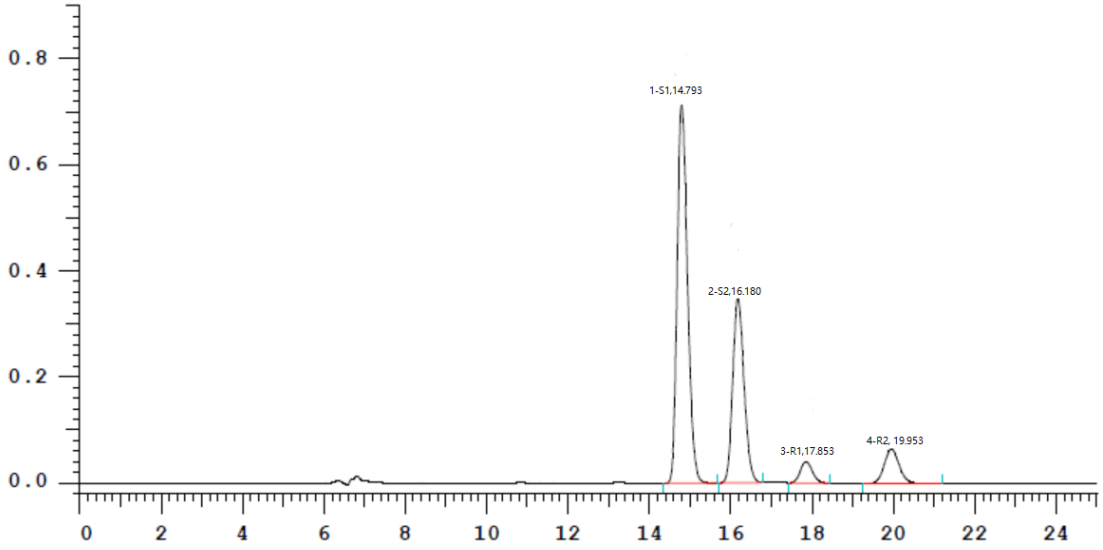

Fig 3 HPLC chromatogram of S-metolachlor TC

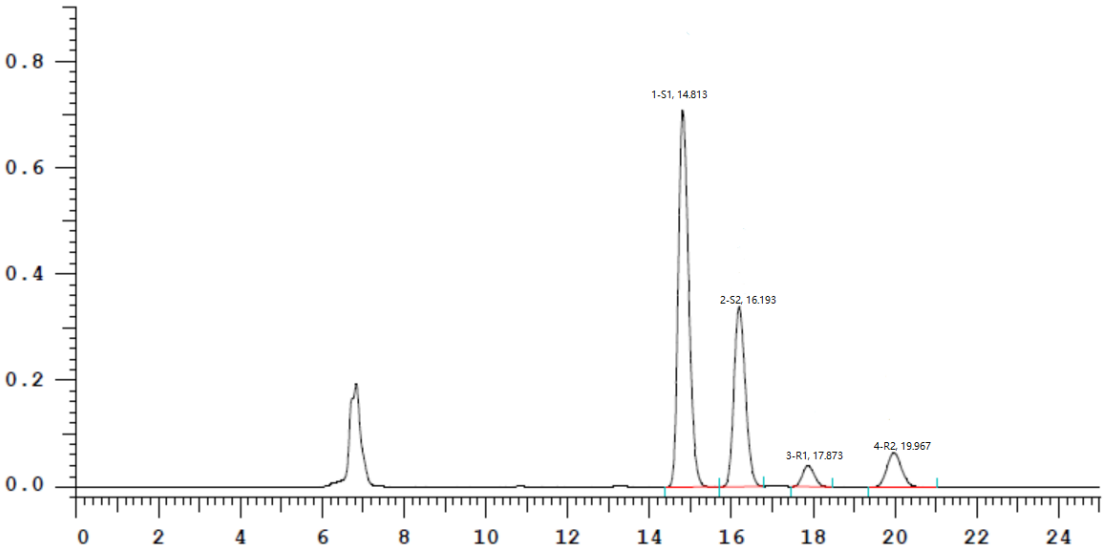

Fig 4 HPLC chromatogram of S-metolachlor EC