S-metolachlor
Collaborative Trial Report

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

CIPAC/5420/R

Report to CIPAC by Shandong Binnong Technology Co., Ltd
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Note: Only 10 of them returned the results.

2. Sample information

Sample	Quantity	Batch	Declared Content of AI
S-metolachlor TC-1	100 mL	202411059	976 g/kg
S-metolachlor TC-2	100 mL	202411130	980 g/kg
S-metolachlor EC-1	100 mL	202411090	872 g/kg
S-metolachlor EC-2	100 mL	202412007	877 g/kg
S-metolachlor EC-3	100 mL	202412086	874 g/kg

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

3. Method description

3.1 Outline of method

S-metolachlor content is determined by normal phase HPLC on a chiral column using UV detector at 230 nm and external standardization. The S-isomers percentage is also 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

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. Weigh duplicate (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 CA1 and CB1).

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 12.1 min
	S2 isomer: about 13.3 min
	R1 isomer: about 14.5 min
	R2 isomer: about 16.0 min

3.4 Procedure

(i) Weigh duplicate (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 S1 and S2).

(ii) Determination. Inject in duplicate 10 µl portions of the calibration solutions and of the sample solutions in the following sequence:

C_{A1}, S_1, S_1, C_{B1}, S_2, S_2, C_{A1}, ……

Measure the relevant peak areas.

(iii) Calculation.
The S-metolachlor content is calculated as follows:

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

$$S\ isomers\ content\ (g/kg) = \frac{A_S \times f}{w}$$

$$R\ isomers\ content\ (g/kg) = \frac{A_R \times f}{w}$$

$$\text{S} - \text{metolachlor content}\ (g/kg) = S\ isomers\ content + R\ isomers\ content$$

$$S\ isomers\ percentage\ (\%) = \frac{S\ isomers\ content}{S - metolachlor\ content} * 100$$

Where:
f_i = single response factor of S isomers or R isomers
f = average response factor of S isomers or R isomers
A_C = area of S isomers or R isomers peak in S-metolachlor in calibration solution
A_S = area of S isomers peak in S-metolachlor sample solution
s = mass of S-metolachlor calibration solution (mg)
w = mass of S-metolachlor sample taken (mg)
P = purity of S isomers or R isomers in S-metolachlor standard (g/kg)

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

4. Deviations and remarks

Lab	Lab 1	Lab 2	Lab 3	Lab 5	Lab 6	Lab 7	Lab 8	Lab 11	Lab 12	Lab 13
HPLC Model	Shimadzu Prominence, LC-20AD	Waters Corporation ,	Thermo Scientific Vanquish	Agilent 1260 Infinity II	Agilent 1260 Infinity	Agilent 1260 Infinity II	Shimadzu	Agilent1260 Infinity Ⅱ	Agilent 1260	Agilent 1200
Column Information	CHIRAL PAK AY-H, 250mm× 4.6mm×5μm	CHIRAL PAK AY-H, 250mm×4.6 mm×5μm	CHIRALPAK AY-H, 250mm×4.6 mm×5μm	Daicel Chiralpak AY-H 250mmx4.6 mm x 5um.	Chiralpak AY-H 250x4.6 mm 5um (new)	Daicel CHIRALPAK AY-H (250 x 4.6 x 5)	Daicel CHIRALPAK IG (250mmx4.6m mx5um)	Daicel CHIRALPAK AY-H (250mm×4.6m m×5μm)	CHIRALPAK AY-H (250mm×4.6m m× 5μm)	AY-H (250mm× 4.6mm×5 μm)
Column Temperature (ºC)	30	30	30	30	30	30	30	30	30	30
Detector Wavelength (nm)	230	230	230	230	230	230	230	230	230	230
Flow rate, ml/min	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Injection Volume (μL)	10	10	10	10	10	10	10	10	10	10

Mobile phase Heptane+ Ethanol (v/v)	94+6	94+6	94+6	94+6	94+6	94+6	94+6	94+6	94+6 Add by pipette ethanol (60 ml) to heptane (940 ml)	94+6
Retention Time, min	S1 isomer: 13.1 min S2 isomer: 13.7 min R1 isomer: 15.4 min R2 isomer: 16.0min	S1 isomer: 11.8 min S2 isomer: 13.0 min R1 isomer: 14.2 min R2 isomer: 15.8 min	S1 isomer: 12.3 min S2 isomer: 13.2 min R1 isomer: 14.8 min R2 isomer: 15.6 min	S1 isomer: 12.8 min S2 isomer: 13.7 min R1 isomer: 15.1 min R2 isomer: 15.8 min	S1 isomer: 12.2 min S2 isomer: 12.6 min R1 isomer: 14.3 min R2 isomer: 14.7 min	S1 isomer: 13.3 min S2 isomer: 14.2 min R1 isomer: 16.0 min R2 isomer: 16.8 min	S1 isomer: 15.3 min S2 isomer: 15.7 min R1 isomer: 16.4 min R2 isomer: 17.5 min	S1 isomer: 12.1 min S2 isomer: 13.3 min R1 isomer: 14.5 min R2 isomer: 16.0 min	S1 isomer: 12.6 min S2 isomer: 13.9 min R1 isomer: 15.3 min R2 isomer: 17.1 min	S1 isomer: 14.8 min S2 isomer: 16.1 min R1 isomer: 18.2 min R2 isomer: 19.9 min

Remark:

Lab 1：1. Retention times observed are different from stated in method.

Less separation between the isomers, but they are still well resolved. Retention times observed were: S1 isomer: 13.086, S2 isomer: 13.681, R1 isomer: 15.397, R2 isomer: 16.041.

Lab5: The batch number on the COA is identical to the batch numbers of the samples that have arrived, but the batch numbers are not correct in the Excel table that was already pre-entered

Lab8: peaks are not good seperated, it is difficult to weigh 25 mg (to the nearest 0.1 mg), it take a long time to equilibration the system.

5. Statistical evaluation

The values highlighted in yellow indicate Cochran/Grubbs outliers, while those in red denote stragglers.

Table 1-1 Results of S-metolachlor content in TC-1

	Day1		Day2		Average	Si
Lab1	976.31	1006.79	1039.52	978.56	1000.30	25.63406
Lab2	973.25	987.49	972.43	979.49	978.16	6.038865
Lab3	987.74	982.00	981.97	980.01	982.93	2.892672
Lab5	958.06	962.20	957.34	964.14	960.44	15.30175
Lab6	986.38	982.99	993.23	993.20	988.95	4.430467
Lab7	970.46	984.80	1012.99	988.80	989.27	15.30175
Lab8	1035.26	946.71	1004.99	996.30	995.82	31.82589
Lab11	973.28	973.68	974.20	975.23	974.10	0.733394
Lab12	972.34	981.57	971.10	976.39	975.35	4.088808
Lab13	979.81	977.30	973.13	977.77	977.00	2.426815

Table 1-2 Results of S-isomers percentage in TC-1

	Day1		Day2		Average	Si
Lab1	887.04	888.43	885.81	885.97	886.81	1.045476
Lab2	887.09	887.08	887.08	887.01	887.06	0.034617
Lab3	890.83	890.97	888.93	889.06	889.95	0.955262
Lab5	889.80	889.66	889.91	889.99	889.84	0.123657
Lab6	885.98	885.27	887.16	886.93	886.33	0.759041
Lab7	887.80	887.82	887.82	887.87	887.83	0.027786
Lab8	888.15	888.16	887.13	883.56	886.75	1.887113
Lab11	888.08	887.76	887.42	886.04	887.32	0.776322
Lab12	888.53	888.62	888.24	888.49	888.47	0.14141
Lab13	887.71	887.72	887.17	887.11	887.43	0.289258

Table 2-1 Results of S-metolachlor content in TC-2

	Day1		Day2		Average	Si
Lab1	992.35	951.95	971.96	974.82	972.77	14.33411
Lab2	970.41	978.77	977.68	975.54	975.60	3.216404
Lab3	977.43	971.32	982.52	992.50	980.94	7.762262
Lab5	950.38	959.96	952.78	944.10	951.80	5.674234
Lab6	973.56	976.80	991.93	990.44	983.18	8.101101
Lab7	982.25	971.47	984.18	969.88	976.95	6.330444
Lab8	965.04	959.48	955.40	974.70	963.65	7.236967
Lab11	977.49	976.68	974.39	974.32	975.72	1.395632
Lab12	970.90	980.77	970.69	983.96	976.58	5.894733
Lab13	978.45	982.84	975.38	981.32	979.50	2.852082

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

	Day1		Day2		Average	Si
Lab1	881.43	880.45	882.34	882.49	881.68	0.817418
Lab2	883.33	883.24	883.28	883.26	883.28	0.032263
Lab3	888.11	888.26	886.01	885.80	887.04	1.145341
Lab5	886.28	886.39	886.96	886.65	886.57	0.260746
Lab6	884.32	881.73	883.50	883.45	883.25	0.943016
Lab7	884.27	884.30	884.38	884.35	884.33	0.043404
Lab8	884.37	884.70	887.12	884.01	885.05	1.218584
Lab11	884.37	884.16	884.04	884.43	884.25	0.157457
Lab12	885.22	884.87	885.01	885.00	885.02	0.128167
Lab13	884.39	884.38	884.21	884.42	884.35	0.082556

Table 3-1 Results of S-metolachlor content in EC-1

	Day1		Day2		Average	Si
Lab1	874.36	872.88	867.44	864.69	869.84	3.936135
Lab2	880.31	869.85	866.03	877.84	873.51	5.795895
Lab3	882.68	870.40	882.31	850.81	871.55	12.95327
Lab5	846.06	856.49	848.31	851.75	850.65	3.933565
Lab6	860.90	858.38	878.66	876.80	868.68	9.112861
Lab7	863.26	880.90	862.03	860.70	866.72	8.234148
Lab8	868.22	920.01	888.98	887.69	891.22	18.54181
Lab11	867.36	867.55	865.11	862.91	865.73	1.893701
Lab12	856.04	862.77	867.80	854.25	860.21	5.408715
Lab13	872.74	876.67	876.02	870.23	873.92	2.598223

Table 3-2 Results of S-isomers percentage in EC-1

	Day1		Day2		Average	Si
Lab1	885.49	885.07	885.05	885.19	885.20	0.173802
Lab2	884.60	884.54	885.10	884.74	884.75	0.216771
Lab3	890.20	890.34	887.74	887.73	889.00	1.269403
Lab5	888.19	888.27	888.48	888.57	888.38	0.153599
Lab6	883.51	883.05	884.99	884.72	884.07	0.807629
Lab7	886.24	886.14	886.20	886.21	886.20	0.034836
Lab8	885.20	885.13	884.48	884.51	884.83	0.336261
Lab11	886.05	886.09	885.98	887.64	886.44	0.695467
Lab12	886.29	886.42	886.37	886.48	886.39	0.070324
Lab13	886.06	886.14	886.17	886.07	886.11	0.045098

Table 4-1 Results of S-metolachlor content in EC-2

	Day1		Day2		Average	Si
Lab1	882.65	880.83	882.73	887.45	883.41	2.451524
Lab2	885.12	890.79	899.70	884.85	890.11	6.019936
Lab3	884.02	879.48	871.29	877.04	877.96	4.591834
Lab5	860.99	858.45	871.59	876.49	866.88	7.420499
Lab6	866.77	867.17	895.43	887.44	879.20	12.55764
Lab7	886.19	895.05	889.37	875.95	886.64	6.939803
Lab8	907.93	920.04	950.33	926.46	**926.19**	15.44222
Lab11	869.92	871.96	870.03	869.96	870.47	0.863337
Lab12	875.33	882.21	876.31	872.38	876.56	3.570748
Lab13	883.53	878.69	877.09	878.03	879.34	2.487284

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

	Day1		Day2		Average	Si
Lab1	884.03	884.18	883.80	883.78	883.95	0.165917
Lab2	884.19	883.97	884.12	884.22	884.13	0.09471
Lab3	889.93	890.01	887.21	887.13	888.57	1.402215
Lab5	887.64	887.65	887.75	887.80	887.71	0.066117
Lab6	882.22	882.48	884.20	884.49	883.35	1.007698
Lab7	885.68	885.69	885.57	885.55	885.62	0.061632
Lab8	885.27	884.93	884.35	884.56	884.77	0.351089
Lab11	885.34	885.39	885.27	884.82	885.20	0.226099
Lab12	886.07	885.97	886.03	886.01	886.02	0.034951
Lab13	885.45	885.53	885.50	885.55	885.51	0.037464

Table 5-1 Results of S-metolachlor content in EC-3

	Day1		Day2		Average	Si
Lab1	883.77	883.88	883.59	899.18	887.61	6.685729
Lab2	883.72	873.24	867.47	877.88	875.58	5.971938
Lab3	869.67	873.33	868.64	867.84	869.87	2.099687
Lab5	869.39	855.67	871.86	873.82	867.68	7.112227
Lab6	850.37	852.80	878.95	880.94	865.76	14.22107
Lab7	879.77	893.10	872.81	882.42	882.02	7.293949
Lab8	837.69	913.20	779.04	811.89	835.45	49.46513
Lab11	873.27	873.27	876.57	875.53	874.66	1.438326
Lab12	867.74	868.05	870.04	870.39	869.06	1.171102
Lab13	878.13	873.96	874.99	871.45	874.63	2.393161

Table 5-2 Results of S-isomers percentage in EC-3

	Day1		Day2		Average	Si
Lab1	884.14	883.94	883.84	883.88	883.95	0.113222
Lab2	884.06	884.05	884.21	884.24	884.14	0.083453
Lab3	890.06	890.15	887.08	887.08	888.59	1.512764
Lab5	887.88	887.84	887.85	887.86	887.86	0.016066
Lab6	882.83	882.45	884.45	883.47	883.30	0.75616
Lab7	885.63	885.54	885.58	885.64	885.60	0.043734
Lab8	885.06	885.28	884.93	884.58	884.96	0.254262
Lab11	885.28	885.32	885.02	884.98	885.15	0.149716
Lab12	885.86	886.04	886.00	885.84	885.94	0.088259
Lab13	885.40	885.43	885.49	885.39	885.43	0.03901

Figure 1 Graphical presentation of S-metolachlor content in TC-1

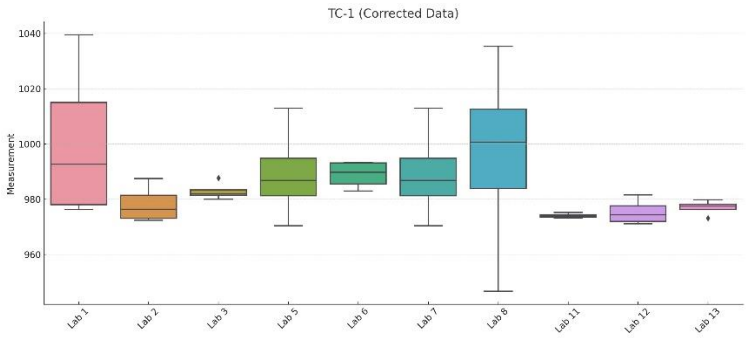


Figure 2 Graphical presentation of S-metolachlor content in TC-2

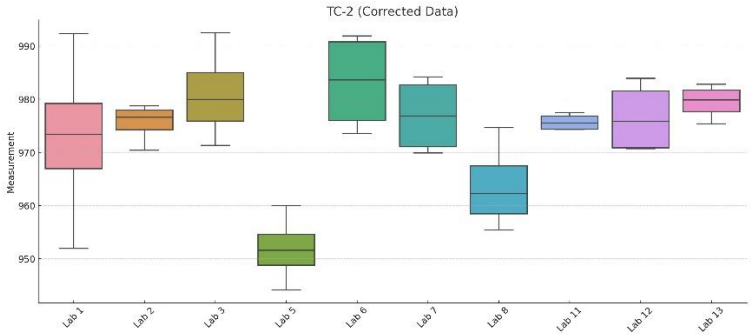


Figure 3 Graphical presentation of S-metolachlor content in EC-1

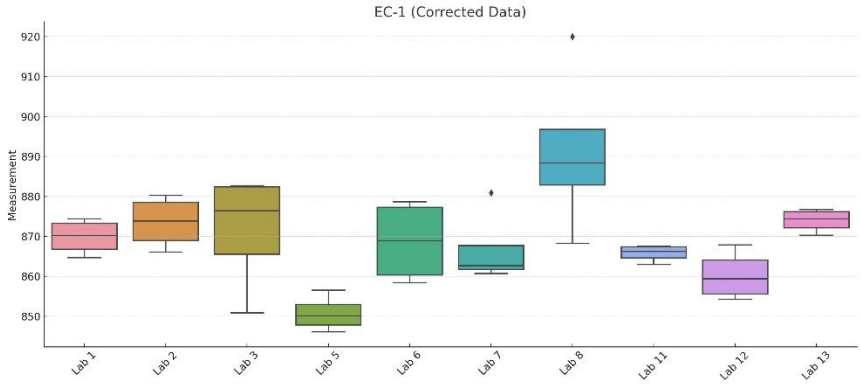


Figure 4 Graphical presentation of S-metolachlor content in EC-2

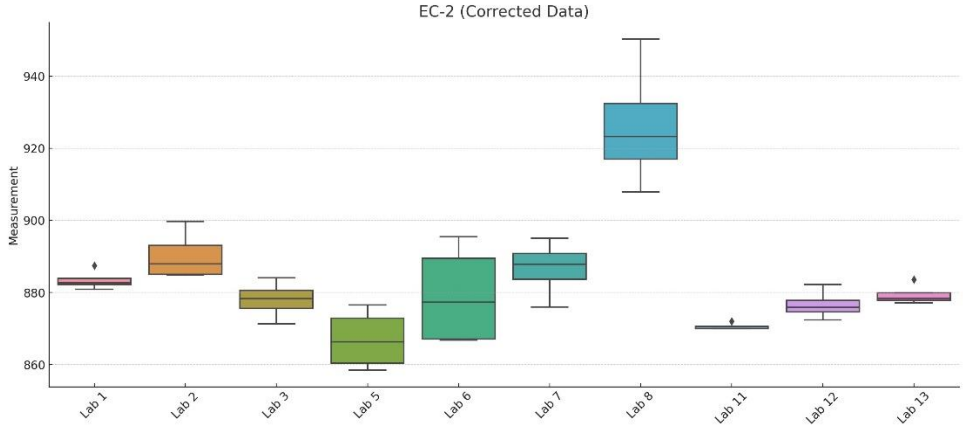
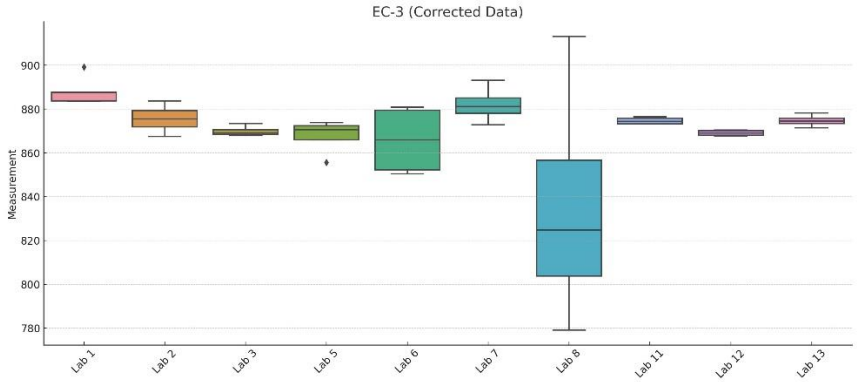


Figure 5 Graphical presentation of S-metolachlor content in EC-3



Summary of the statistical results：

We identified one outlier for TC1, two stragglers for TC2, one straggler for EC1, and one outlier for EC2 and EC3. The RSD_R values for the five samples, based on data from all ten participants, were 1.60, 1.15, 1.48, 2.01 and 2.32, respectively. After excluding all the outliers, the final statistical results were calculated as follows:

Table 5-1 Summary of the results of S-metolachlor Content

	TC-1	TC-2	EC-1	EC-2	EC-3
x	983.93	973.67	869.21	878.96	874.10
L	9	10	10	9	9
S_r	11.61	7.15	8.76	6.19	6.67
S_R	13.28	11.20	12.88	9.07	9.14
r	32.51	20.02	24.52	17.31	18.67
R	37.19	31.36	36.06	25.39	25.60
RSD_r	1.18	0.73	1.01	0.70	0.76
RSD_R	1.35	1.15	1.48	1.03	1.05
$RSD_{R(Hor)}$	2.01	2.01	2.05	2.05	2.05
HorRat	0.68	0.58	0.74	0.51	0.52

(values given in units of g/kg)

Table 5-2 Summary of the results S-isomers percentage

	TC-1	TC-2	EC-1	EC-2	EC-3
x	887.78	884.48	886.14	885.48	885.49
L	10	10	10	10	10
S_r	0.83	0.67	0.54	0.57	0.55
S_R	1.45	1.68	1.64	1.71	1.73
r	2.32	1.87	1.53	1.58	1.53
R	4.07	4.71	4.59	4.79	4.83
RSD_r	0.09	0.08	0.06	0.06	0.06
RSD_R	0.16	0.19	0.19	0.19	0.20
$RSD_{R(Hor)}$	2.04	2.04	2.04	2.04	2.04
HorRat	0.08	0.10	0.10	0.10	0.10

(values given in units of g/kg)

6. Conclusion

All HorRat values obtained for the determination of **S-metolachlor content** ranged from **0.51 to 0.74** after outliers were excluded, meeting the acceptable criterion of **0.3 to 1.0**. No outliers were found in the determination of **S-isomers percentage**, and data from all 10 participants were included in the statistical analysis. The HorRat values for S-isomers percentage ranged from **0.08 to 0.10**, which is well below the threshold of **0.3**. This narrow variation is considered acceptable, as the test involves percentage determination rather than content determination.

Based on the results above, the method is deemed suitable for the determination of both **S-metolachlor content** and **S-isomers percentage** in S-metolachlor **TC** and **EC**

formulations. We propose that this method be adopted as a **provisional CIPAC method**.

7. Figures

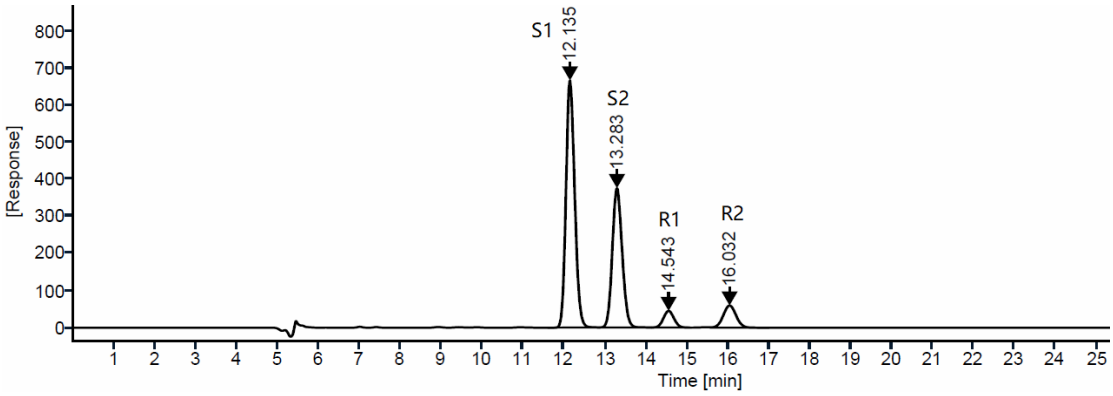


Fig 2 HPLC chromatogram of S-metolachlor standard

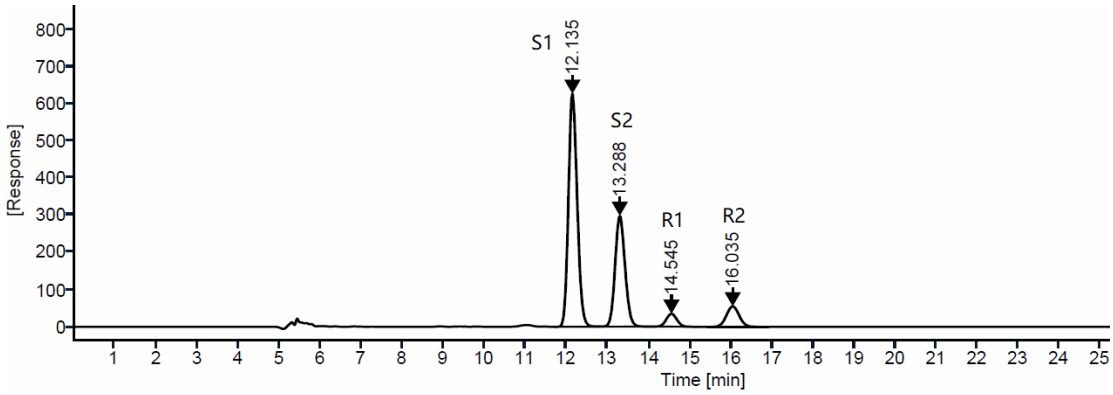


Fig 3 HPLC chromatogram of S-metolachlor TC

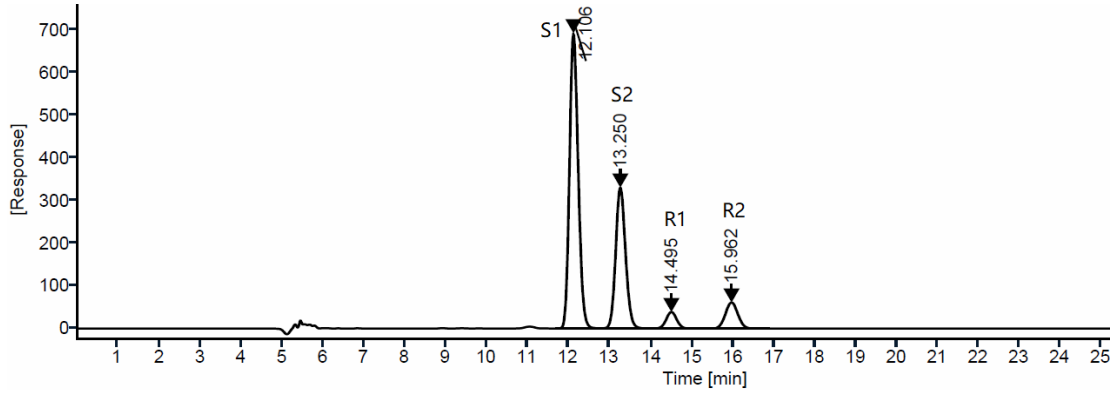


Fig 4 HPLC chromatogram of S-metolachlor EC