Small scale collaborative trial on the validation of the method for the determination of Ethephon in TC, TK and SL

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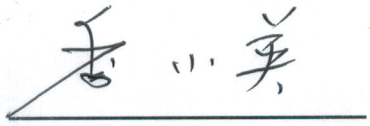

Study Director: Xiaoying Ji

Shaoxing Eastlake High-Tech Co., Ltd

Address: No.359, Jiangzhong Road,Doumen Street,

Yuecheng District, Shaoxing, Zhejiang P.R.China

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Table of Content

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1. Ethephon method description

1.1 Outline of method

The content of ethephon is determined by ion chromatography using sodium carbonate and sodium hydrogen carbonate as eluent.

1.2 Apparatus and reagents

High performance ion chromatograph equipped with a electrolytic conductivity detector and an injection system capable of injecting 25 µl.

Electronic integrator or data system

Ion Exchange column: Dionex IonPac AS23, 250 x 4.0 mm (i.d.), or equivalent

Guard column: Dionex IonPac AG23, 50 x 4.0 mm (i.d.), or equivalent.

Analytical balance, accurate to ± 0.1 mg

Sodium carbonate: AR or GR

Sodium hydrogen carbonate: AR or GR

Ultrapure water

1.3 IC condition

Eluent:	7.2 mMNa_2CO_3+9.0 mM $NaHCO_3$
Flow rate:	1.0 mL/min
Current of inhibitor:	70 mA
Temperature of detector cell:	35C°
Temperature of column:	30C°
Mode of injection:	PushFull
Volume of Injection:	25µL
Frequency of data sampling:	5.0 Hz
Run time:	13 min
Retention time:	9.5 min

1.4 Procedure

(i) Preparation of Calibration sample. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (w mg) to contain about 120 mg of Ethephon into a brown volumetric flask (100 ml). Add ultrapure water to the mark and mix thoroughly. Transfer 5.00 mL of the above solution into a 50 mL volumetric flask, add ultrapure water to the mark and mix thoroughly. (Calibration solutions CA and CB).

(ii) Preparation of Ethephon sample. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (w mg) to contain about 120 mg of Ethephon into a brown volumetric flask (100 ml) (for TC, melt the sample at 95C° and mix well). Add ultrapure water to the mark and mix thoroughly. Transfer 5.00 mL of the above solution into a 50 mL volumetric flask, add ultrapure water to the mark and mix thoroughly. (Sample solutions S1 and S2).

(iii) Determination of ethephon

(a) Equilibration of the system. Pump sufficient mobile phase through the column to equilibrate the system. Inject 25 µl portion of calibration solution CA until the response obtained from two consecutive injections deviate by less than 1.5%. Then inject 25 µl portion of calibration solution CB. The response factor for this solution should not deviate by more than 1.5% from that for calibration solution CA, otherwise prepare new calibration solutions.

(b) Determination. Inject in duplicate 25 ul portions of each sample solution bracketing them by injections of the calibration solutions as follows: calibration solution CA, sample solution S1, sample solution S1, calibration solution CB, sample solution S2, sample solution S2, calibration solution CA, and so on. Measure the relevant peak areas.

(iv) Calculation

Determine the peak area of Ethephon and calculate the mean value of response factors from the calibration solutions bracketing the injections of the sample solutions and use this value for calculating the Ethephon content of the bracketed sample solutions. The Ethephon content is the mean value of two sample solutions.

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

$$\text{Ethephon content} = \frac{f \times H_w}{w} \quad \text{g/kg}$$

where:

f_i = individual response factor
f = mean response factor of bracketing calibration injections
H_s = peak area of Ethephon in the calibration solution
H_w = peak area of Ethephon in the sample solution
s = mass of Ethephon reference standard in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of Ethephon reference standard (g/kg)

2. Participants and sample distribution

Participants

Index	NAME	ORGANIZATION	Address
Lab1	Xiangdong Shao	BioGuide Technologies Co., Ltd.	Building 8, IFST-CAAS, 2 Yuanmingyuan West Road, Haidian District, Beijing, China
Lab2	Wenhan Yang	Pesticides Test Laboratory of Shen Yang Research Institute of Chemical Industry Co., Ltd.	No.8, Shen Liao Dong Road, Tie Xi district, Shenyang, China
Lab3	Xiaoying Ji	Shaoxing Eastlake High-Tech Co., Ltd	No.359, Jiangzhong Road, Doumen Street, Yuecheng District, Shaoxing, Zhejiang, China

Sample information

Sample	Quantity	Batch	Declared Content of AI
ethephon TC1	50 g	E202011011	Min. 93%
ethephon TC2	50 g	E202010030	Min. 93%
ethephon TK1	50 mL	F202011011	75%
ethephon TK2	50 mL	F202010030	75%
ethephon TK1	50 mL	2020110111	40%

ethephon TK2	50 mL	2020100130	40%

3. Deviations and remarks

Lab 1 increased the flow rate to 1.2 mL/m to produce similar retention time as specified in the method.

4. Statistical evaluation

Table 1. Results of the analysis of AI content in the TC1

Lab	Day1 (%)		Day2 (%)		Average Yi	Yi^2	Standard Deviation Si	Si^2
	1	2	1	2				
BioGuide	93.32	93.38	93.25	93.20	93.29	8702.8693	0.0791	0.0063
SYRIC	94.22	94.02	93.88	93.94	94.01	8838.5729	0.1499	0.0225
EASTLAKE	94.02	94.54	93.86	94.31	94.18	8870.7598	0.3016	0.0910

Table 2. Results of the analysis of AI content in the TC2

Lab	Day1 (%)		Day2 (%)		Average Yi	Yi^2	Standard Deviation Si	Si^2
	1	2	1	2				
BioGuide	93.07	93.21	93.11	93.31	93.18	8681.8246	0.1091	0.0119
SYRIC	94.04	93.83	94.16	94.17	94.05	8845.2142	0.1587	0.0252
EASTLAKE	93.63	93.61	93.88	93.50	93.65	8770.7896	0.1609	0.0259

Table 3. Results of the analysis of AI content in the TK1

Lab	Day1 (%)		Day2 (%)		Average Yi	Yi^2	Standard Deviation Si	Si^2
	1	2	1	2				
BioGuide	76.47	77.55	76.17	76.65	76.71	5884.6903	0.5928	0.3514
SYRIC	76.24	75.82	75.51	76.01	75.90	5760.0933	0.3062	0.0937
EASTLAKE	75.99	76.20	76.01	76.03	76.06	5784.4266	0.0944	0.0089

Table 4. Results of the analysis of AI content in the TK2

Lab	Day1 (%)		Day2 (%)		Average Yi	Yi^2	Standard Deviation Si	Si^2
	1	2	1	2				
BioGuide	76.16	76.48	76.01	76.20	76.21	5808.3785	0.1940	0.0376
SYRIC	76.20	75.63	75.23	76.00	75.76	5739.9596	0.4268	0.1822
EASTLAKE	75.59	75.60	75.56	75.88	75.66	5723.7355	0.1503	0.0226

Table 5. Results of the analysis of AI content in the SL1

Lab	Day1 (%)		Day2 (%)		Average Yi	Yi^2	Standard Deviation Si	Si^2
	1	2	1	2				
BioGuide	41.18	41.28	41.28	41.14	41.22	1699.1808	0.0698	0.0049
SYRIC	41.74	41.65	41.22	40.84	41.36	1710.9019	0.4154	0.1725
EASTLAKE	40.80	40.43	41.01	41.10	40.84	1667.6734	0.3013	0.0908

Table 6. Results of the analysis of AI content in the SL2

Lab	Day1 (%)		Day2 (%)		Average Yi	Yi^2	Standard Deviation Si	Si^2
	1	2	1	2				
BioGuide	41.09	40.98	40.78	40.90	40.94	1675.9005	0.1351	0.0182
SYRIC	42.21	42.16	41.16	41.62	41.79	1745.9945	0.4960	0.2460
EASTLAKE	40.45	40.84	40.79	40.58	40.67	1653.8194	0.1811	0.0328

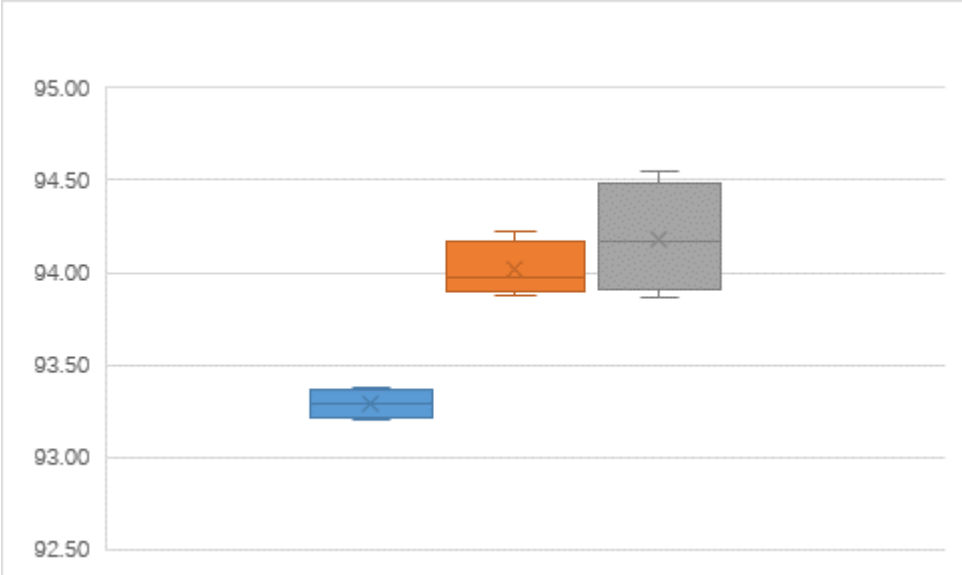

Figure 1. Graphical presentation of TC1 data

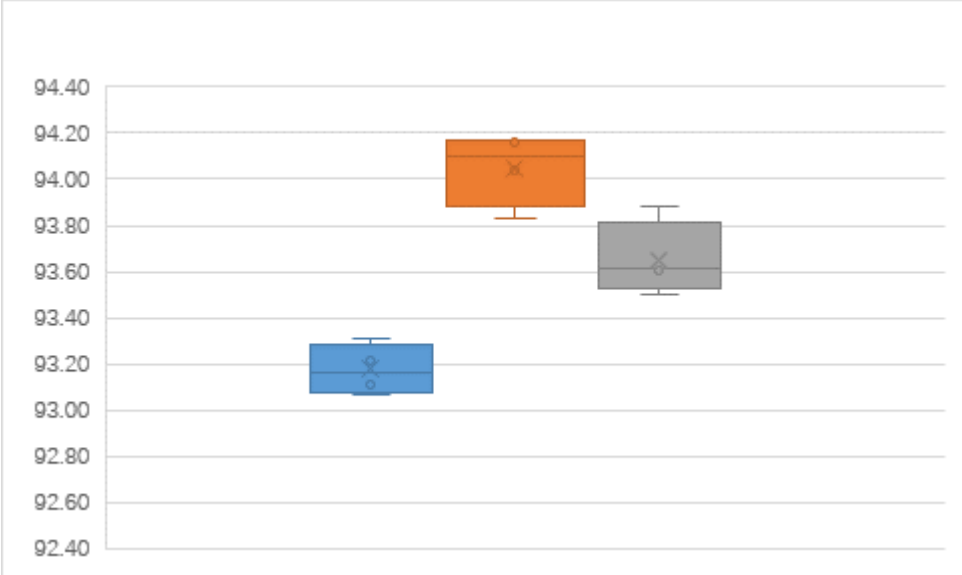

Figure 2. Graphical presentation of TC2 data

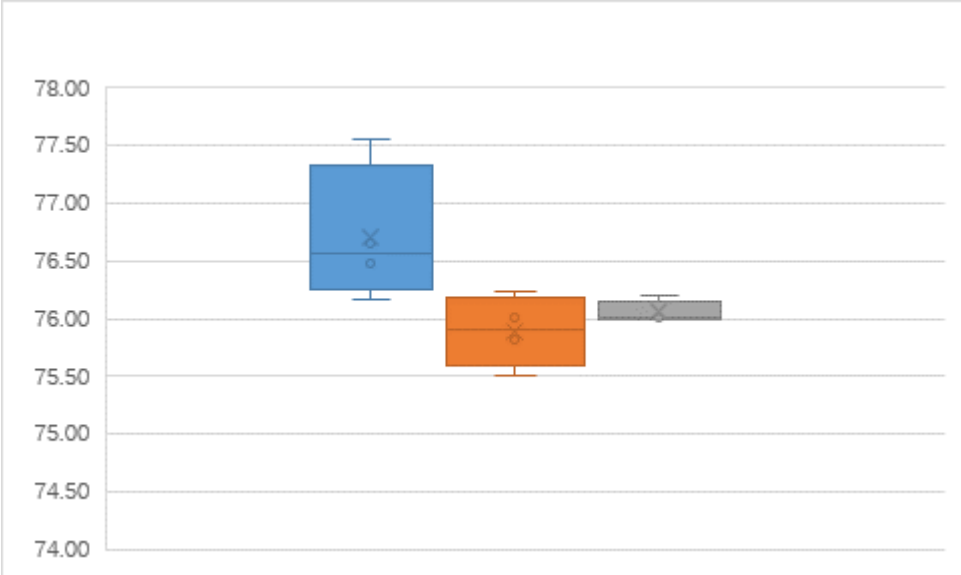

Figure 3. Graphical presentation of TK1 data

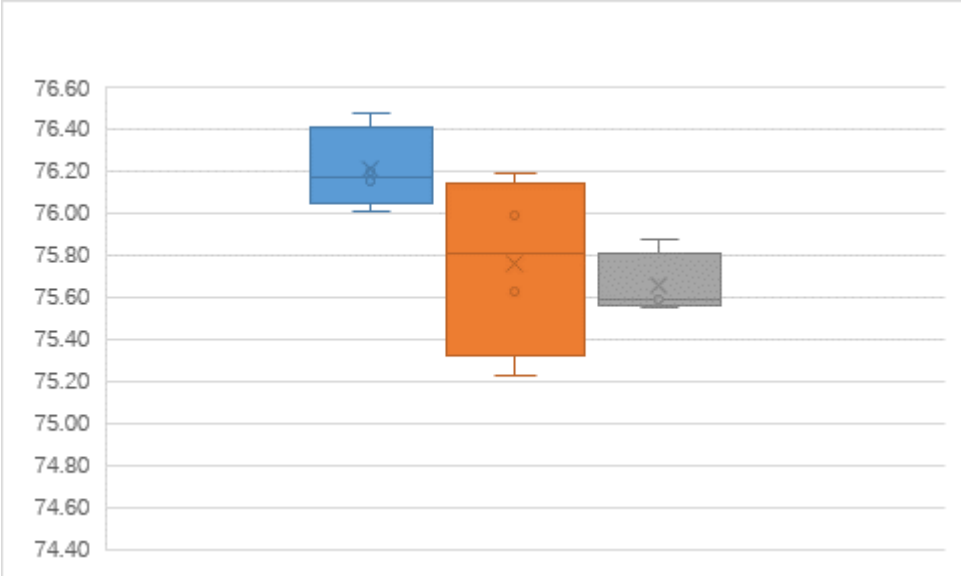

Figure 4. Graphical presentation of TK2 data

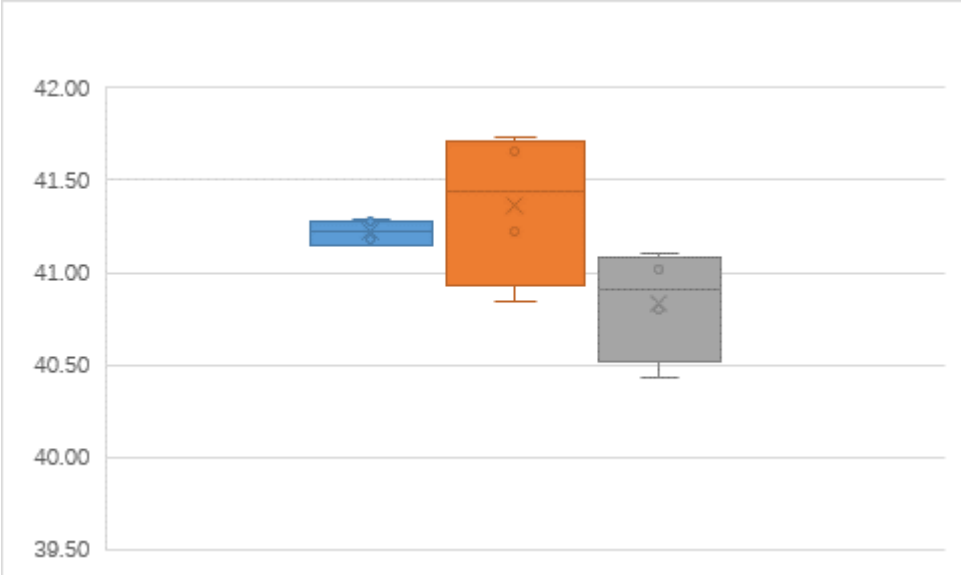

Figure 5. Graphical presentation of SL1 data

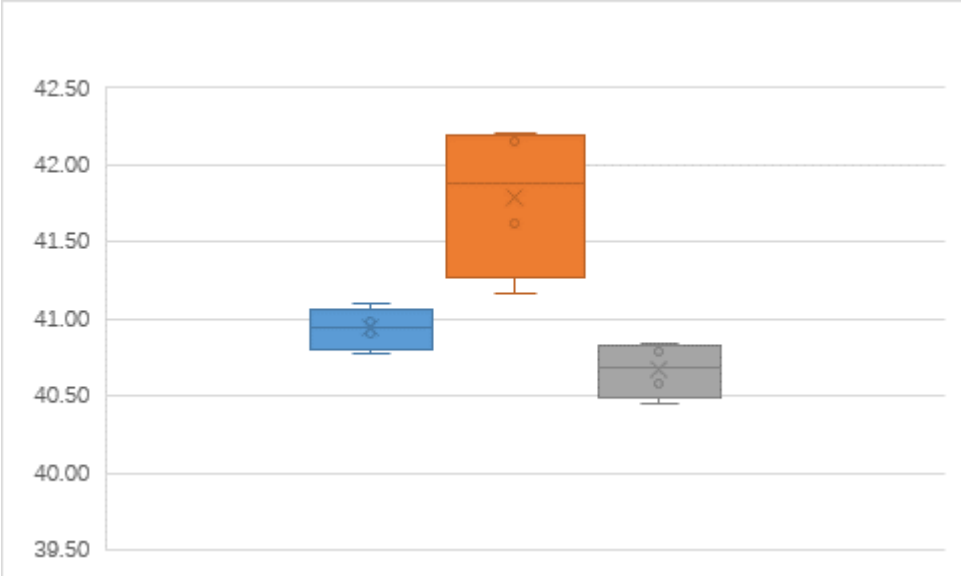

Figure 6. Graphical presentation of SL2 data

Table 7. Statistics of the results of TC1

S_1=sum Yi	281.49
S_2=sum Yi^2	26412.20202
S_3=sum Si^2	0.1197
No. Lab P	3
No. Determination n	4
Average Y=S_1/P	93.83

$S_r^2=S_3/P$	0.0399	**Standard Deviation of Repeatability S_r**	0.1997
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.2161	S_L	0.4648
$S_R^2=S_r^2+S_L^2$	0.2559	**Standard Deviation Reproducibility S_R**	0.5059
Repeatibility r=2.8*Sr	0.5593		
Reproducibility R=2.8*S_R	1.4166		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.2129		
Relative Standard Deviation of Reproducibility $SD_R=S_R*100/Y$	0.5392		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/100)]	2.0193		

Table 8. Statistics of the results of TC2

S_1=sum Yi	280.88		
S_2=sum Yi^2	26297.82845		
S_3=sum Si^2	0.0630		
No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	93.63		
$S_r^2=S_3/P$	0.0210	**Standard Deviation of Repeatability S_r**	0.1449
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.1857	S_L	0.4309
$S_R^2=S_r^2+S_L^2$	0.2067	**Standard Deviation Reproducibility S_R**	0.4546
Repeatibility r=2.8*Sr	0.4056		
Reproducibility R=2.8*S_R	1.2729		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.1547		
Relative Standard Deviation of Reproducibility $SD_R=S_R*100/Y$	0.4856		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/100)]	2.0199		

Table 9. Statistics of the results of TK1

S_1=sum Yi	228.66
S_2=sum Yi^2	17429.21017
S_3=sum Si^2	0.4541

No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	76.22		
$S_r^2=S_3/P$	0.1514	**Standard Deviation of Repeatability S_r**	0.3890
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.1493	**S_L**	0.3864
$S_R^2=S_r^2+S_L^2$	0.3007	**Standard Deviation Reproducibility S_R**	0.5483
Repeatibility r=2.8*Sr	1.0893		
Reproducibility $R=2.8*S_R$	1.5354		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.5104		
Relative Standard Deviation of Reproducibility $SD_R=S_R*100/Y$	0.7194		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/100)]	2.0834		

Table 10. Statistics of the results of TK2

S_1=sum Yi	227.63		
S_2=sum Yi^2	17272.07366		
S_3=sum Si^2	0.2424		
No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	75.88		
$S_r^2=S_3/P$	0.0808	**Standard Deviation of Repeatability S_r**	0.2843
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.0673	**S_L**	0.2594
$S_R^2=S_r^2+S_L^2$	0.1481	**Standard Deviation Reproducibility S_R**	0.3848
Repeatibility r=2.8*Sr	0.7959		
Reproducibility $R=2.8*S_R$	1.0774		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.3746		
Relative Standard Deviation of Reproducibility $SD_R=S_R*100/Y$	0.5071		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/100)]	2.0849		

Table 11. Statistics of the results of SL1

S_1=sum Yi	123.42
S_2=sum Yi^2	5077.75606
S_3=sum Si^2	0.2682

No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	41.14		
$S_r^2=S_3/P$	0.0894	**Standard Deviation of Repeatability S_r**	0.2990
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.0517	**S_L**	0.2273
$S_R^2=S_r^2+S_L^2$	0.1411	**Standard Deviation Reproducibility S_R**	0.3756
Repeatibility r=2.8*Sr	0.8371		
Reproducibility $R=2.8*S_R$	1.0516		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.7267		
Relative Standard Deviation of Reproducibility $SD_R=S_R*100/Y$	0.9129		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/100)]	2.2861		

Table 12. Statistics of the results of SL2

S_1=sum Yi	123.39		
S_2=sum Yi^2	5075.71446		
S_3=sum Si^2	0.2971		
No. Lab P	3		
No. Determination n	4		
Average $Y=S_1/P$	41.13		
$S_r^2=S_3/P$	0.0990	**Standard Deviation of Repeatability S_r**	0.3147
$S_L^2=[(P*S_2-S_1^2)/P(P-1)]- S_r^2/n$	0.3154	**S_L**	0.5616
$S_R^2=S_r^2+S_L^2$	0.4144	**Standard Deviation Reproducibility S_R**	0.6438
Repeatibility r=2.8*Sr	0.8811		
Reproducibility $R=2.8*S_R$	1.8025		
Relative Standard Deviation of Repeatability $RSD_r=S_r*100/Y$	0.7651		
Relative Standard Deviation of Reproducibility $SD_R=S_R*100/Y$	1.5652		
Horwitz RSD_R (Hor)=2^[1-0.5*log(Y/100)]	2.2862		

5. Conclusion

From the results shown above, the method can be considered applicable for the determine of ethephon contents in TC, TK and SL. We propose that a full scale collaborative trial might be conducted.

6. Figures

Fig.1 HPIC chromatogram of blank

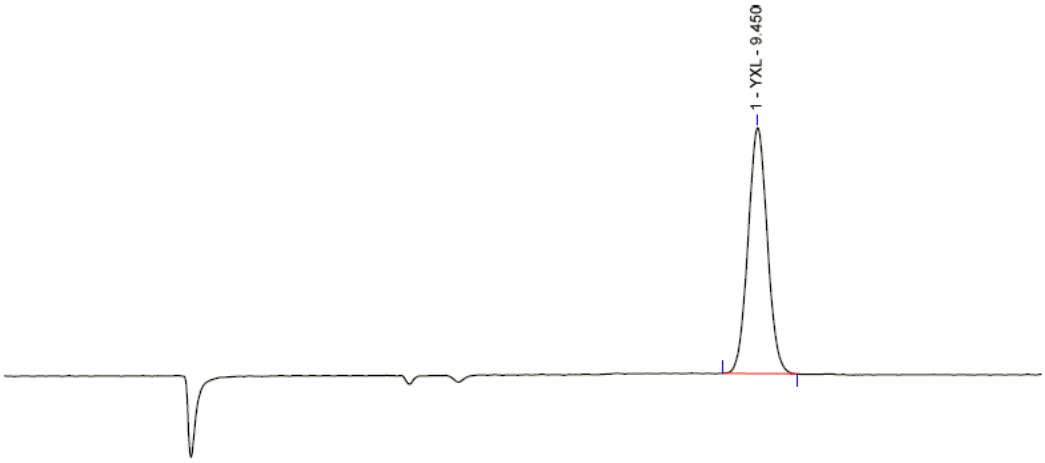

Fig.2 HPIC chromatogram of Ethephon standard

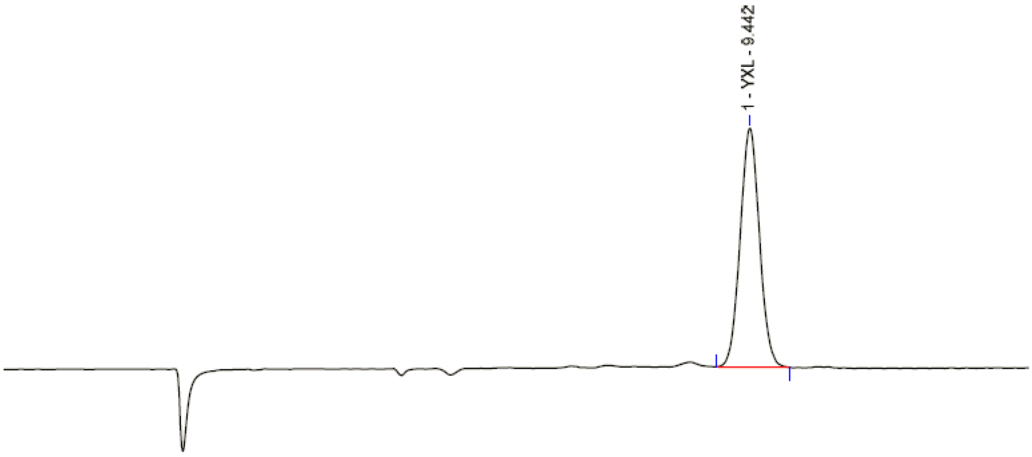

Fig.3 HPIC chromatogram of Ethephon TC

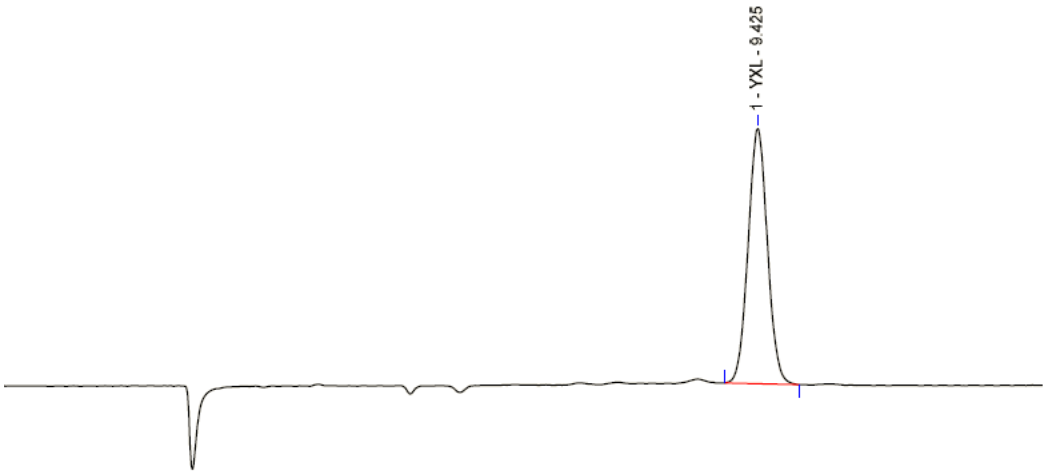

Fig.4 HPIC chromatogram of Ethephon TK

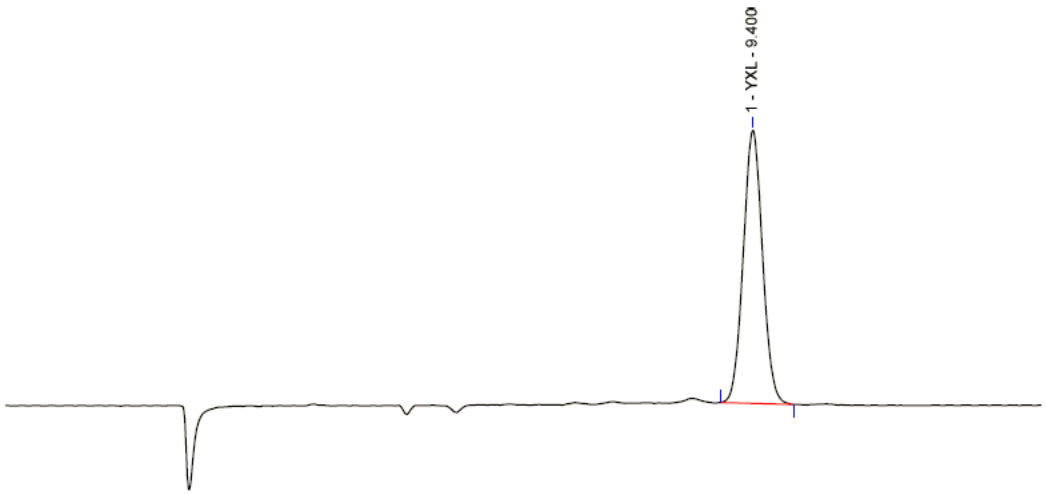

Fig.5 HPIC chromatogram of Ethephon SL