采用MP程序验证CIPAC－AOAC方法

What is MP？

- Multi－Procedure： 实验室装备1－2根色谱柱分析尽可能多的农药， 建立的分析方法与CIPAC－AOAC方法等效。

- 背景： CIPAC和AOAC方法均经过专门的方法验证（method validation）， 分析实验室可以简便采用（Adoption）， 采用时建议进行简化的方法验证。
- 背景2： CIPAC、AOAC方法对每个农药的分析方法的色谱柱不同， 一些为填充柱。 分析实验室为充分适应该分析方法， 必须再实验室频繁更换色谱柱， 或者做很多方法验证工作， 效率很低。
- 解决途径： MP

MP基本原理与过程

1 SST　System Suitability Test

2 专一性测试：

3D－光谱、

无干扰物质证明：空白制剂浓缩物、两根不同色谱柱对同一物质分析比较

3 测试重复性 （不是进样重复性！）、样品均匀性考察（同一样本的三个不同部位）

4 线性考察： 相关系数(r>0.997)、相对残差的标准偏差Srr<0.02

5 批次分析

6 与权威数据比较 （FAO/IAEA 共有10个以上实验室参加验证）

7 日常应用、方法稳定性

Research Route 研究路线

1.Selection of suitable pesticide formulations. 选择农药品种

2.Review of literature, AOAC and CIPAC method, and inquiring of methods in the local country and other organization. CIPAC等方法查询

3.Preparation of samples, pesticide standards and reagents. 实验准备

4.QA/QC procedure exercise and training. 实验室QA QC

5.Identification of active ingredient. 定性分析

6.Method validation. MP方法的验证

7.Regular use of the methods developed for the analysis of samples taken from the market. MP方法的日常应用

8.Discussion and conclusion of the analysis．讨论

Advantages of the Application of MP Methods

- Larger sample output of laboratories with limited instrumentation
- Lower cost of analysis

Test compounds for System Suitability Test for columns CP-Sil 8Cb and DB-1701

CP-Sil 8Cb	**DB-1701**
Undecane	2-Chlorophenol
2,4 Dimethylphenol	Undecane
2,6 Dimethylaniline	2,4 Dimethylaniline
Tetradecane	1-Undecanole
1-Undecanole	Tetradecane
1-Methylnaphthalene	Acenaphthylene
Hexadecane	Pentadecane
Composition of test mixture:	**250 µ g/ml of each in hexane**

System Suitability Test for CP-Sil 8Cb

Compound	t_R (sec)	t_R' (sec)	k(sec)	N_{eff}/m	W_h (sec)	T	Rs	As
2-chlororp	184,0	82,4	0,8	710,01	1,88	1,02	_	1,11
Undecane	230,8	129,2	1,3	1274,78	2,20	0,98	13,5	1,08
2,4 dim.aniline	302,4	200,9	2,0	1572,28	3,08	1,00	16,0	1,04
1-Undecanol	632,9	531,3	5,2	1787,98	7,64	1,01	36,4	1,43
Tetradecane	716,3	614,7	6,1	2457,32	7,54	1,05	6,5	1,03
Acenaphthylene	950,6	849,0	8,4	2685,85	9,96	1,04	15,8	1,02
Pentadecane	1127,4	1025,8	10,1	2613,69	12,20	1,01	9,4	1,01

System Suitability Test for DB-1701

Compound	t_R (sec)	t_R' (sec)	k(sec)	N_{eff}/m	W_h (sec)	T	Rs	As
Undecane	79,2	42,4	1,2	520,9	1,1	1,01	_	1,06
2,4 dim.phenol	181,9	145,1	3,9	1067,3	2,7	1,02	31,7	1,04
2,6 dim.aniline	205,8	169,0	4,6	1099,1	3,1	1,02	4,9	1,05
Tetradecane	257,6	220,8	6,0	981,6	4,3	1,01	8,3	1,02
1-undecanole	296,8	260,0	7,1	1195,5	4,6	1,01	5,2	0,99
1-met.naphthalene	378,2	341,4	9,3	1146,9	6,1	1,00	9,0	1,01
Hexadecane	688,6	651,8	17,7	1060,3	12,2	1,00	20,0	1,00

PARAMETERS FOR METHOD VALIDATION OF THE ACTIVE SUBSTANCE

Method validation data should address the following issues according to EU legislation and CIPAC guidelines:

- ✓ Linearity of response for the analyte in the method.

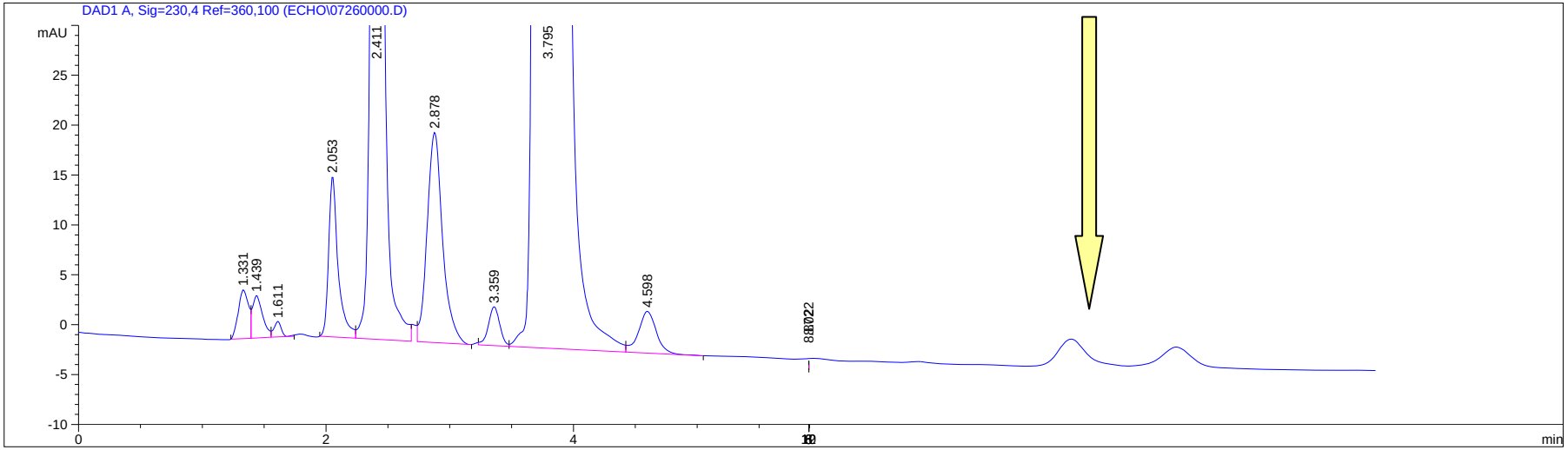
  An estimation of the precision of the procedure.

- ✓ Interference – free separation (specificity)
- ✓ Repeatability

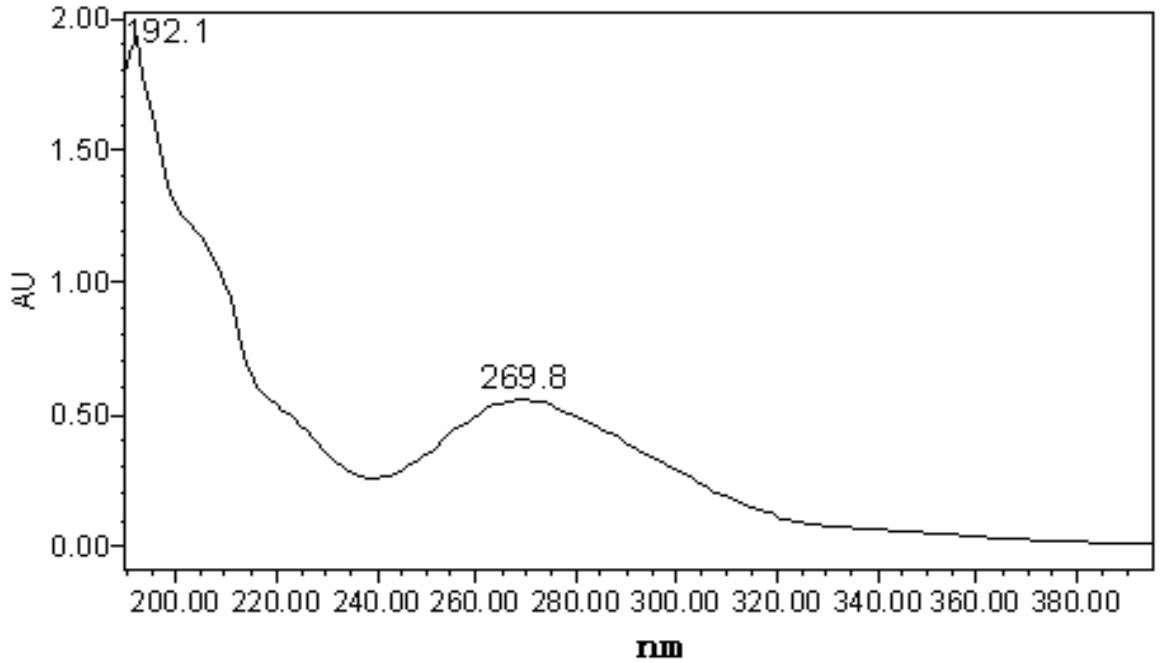
Specificity 考察

- Blank solution 空白制剂： 提取、浓缩后进样无干扰
- 不同色谱柱对同一样本分析结果的考察： 色谱流出物无干扰的证明
- 光谱、分离手段等

Blank Solution

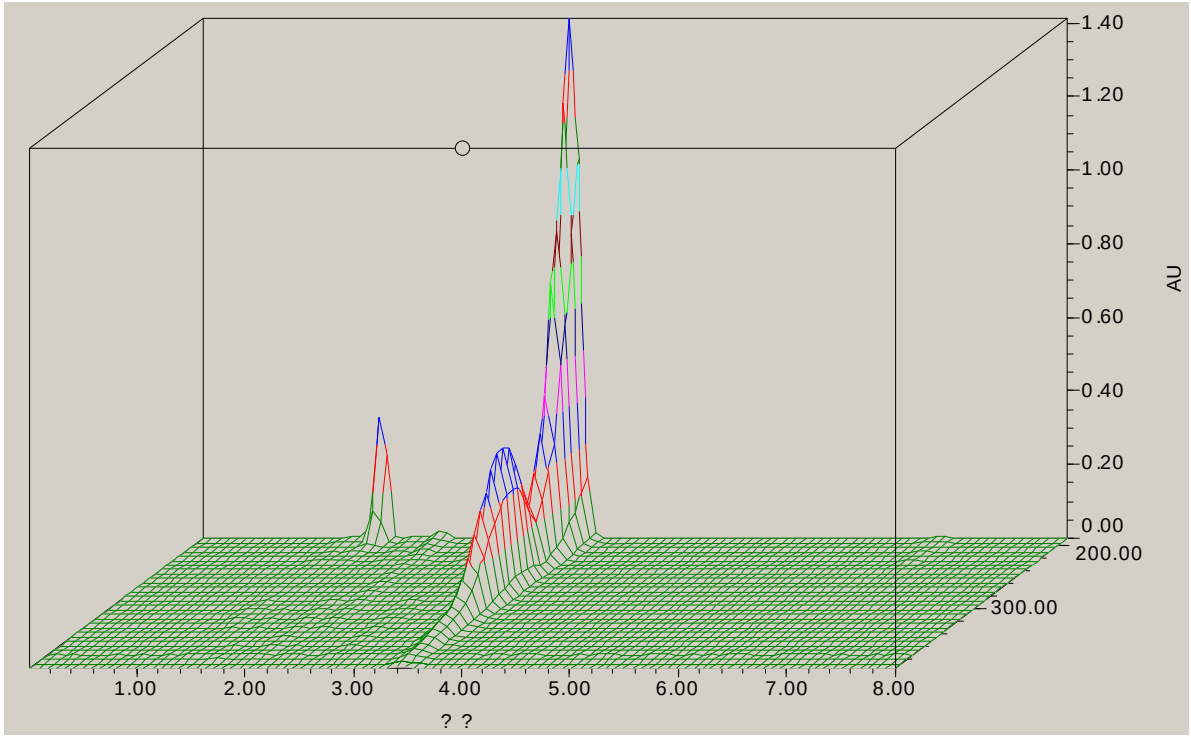


Interference Peak should be lower than 0.3%



Fenitrothion

UV of fenitrothion determinded by 3D mode



内标物的选择： 相对固定2－3种内标， 用于分析所有农药

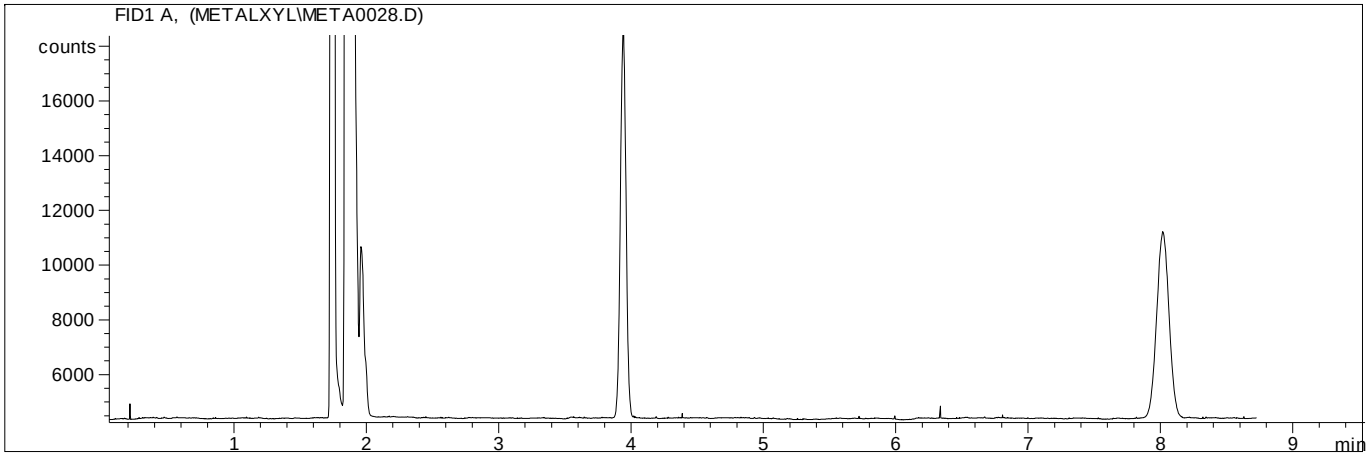


Figure 1 CP-8 column, 224 degree column temp (Diethyl phthalate + META)

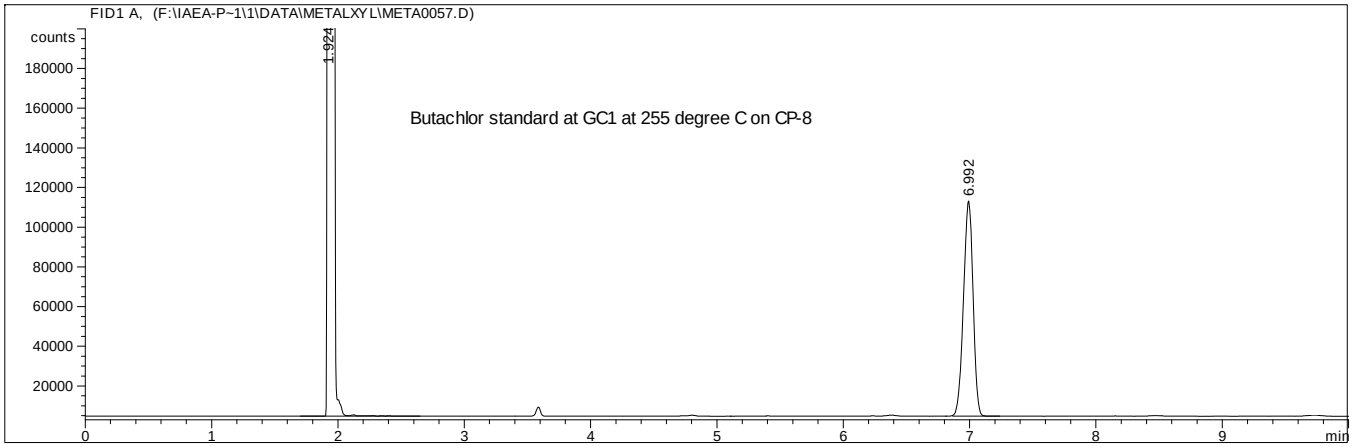


Figure 3 Butachlor standard at GC1 (dibutyl phthalate as internal st.)

Internal standars

- Diethyl- phthalate (DEF)
- Benzyl benzoate (BB)
- Dibuthyl phthalate (DBF)
- Diphenyl phthalate (DFF)
- Squalane ?
- missing IS at pyrethroid range

Repeatability of injections for alachlor:

	Alachlor	dipentyl phthalate			
	Std As,i	**Istd Ais,i**	**Ratio, Yi**	**Rt (As)**	**Rt (i.s.)**
	991444	627791	1,5793	9,488	16,130
	958259	612297	1,5650	9,492	16,143
	962088	609235	1,5792	9,492	16,142
	980667	618783	1,5848	9,488	16,138
	971149	613721	1,5824	9,490	16,142
Mean	972721	616365	1,5781	9,490	16,139
SD	13586	7259	0,0077	0,002	0,005
%CV	**1,4**	**1,2**	**0,5**	**0,02**	**0,03**

Linearity of response for Chlorpyrifos methyl

Slope a:	0,3054
Intercept b:	0,0087
r:	0,9998
sYrel:	0,0058
Calibration Equation:	**y=0,3054x+0,0087**
Column:	CP-Sil 8Cb

线性考察举例

浓度比	Peak 1	Peak 内标	Peak Ratio
0.51296	231240	745507	0.310178
0.51296	237668	761721	0.312015
0.83108	405285	773240	0.524139
0.83108	410041	782121	0.524268
0.99926	503018	809940	0.621056
0.99926	505447	803818	0.628808

回归统计	
Multiple R	0.999462
R Square	0.998924
Adjusted R Square	0.998655
标准误差	0.005256
观测值	6

方差分析

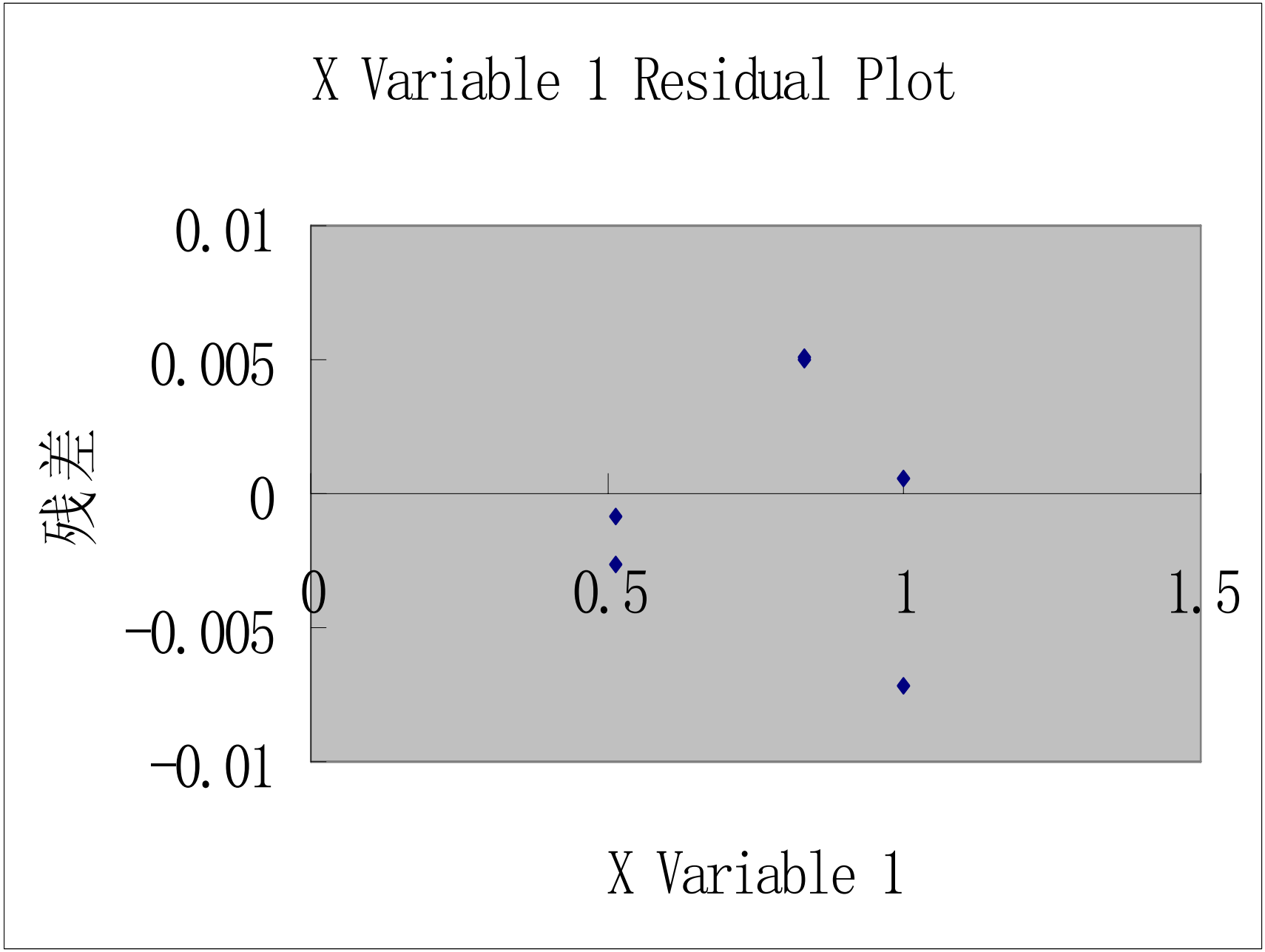
	df	SS	MS	F	Significance F
回归分析	1	0.102624	0.102624	3714.488	4.34E-07
残差	4	0.000111	2.76E-05		
总计	5	0.102734			

RESIDUAL OUTPUT

观测值	预测 Y	残差	标准残差	相对残差
1	0.312841	-0.00266	-0.56641	-0.00851
2	0.312841	-0.00083	-0.17581	-0.00264
3	0.519158	0.00498	1.059324	0.009593
4	0.519158	0.00511	1.086826	0.009842
5	0.628232	-0.00718	-1.52641	-0.01142
6	0.628232	0.000576	0.122471	0.000916

Srr 0.008931

Srr<0.01 or 0.02 Acceptable!


X Variable 1 Residual Plot
0.01
0.005
0
-0.005
-0.01
残差
0
0.5
1
1.5
X Variable 1

Batch Analysis for alachlor in CP-Sil 8Cb

ai content	ai contnet in	mean of duplicate	Reference Values
	g/l	injections	(Batches)
423,9	473,06	**473,36**	**481,00**
424,8	474,03		
442,0	493,74	**494,06**	**482,00**
442,6	494,38		
423,3	472,45	**471,84**	**482,00**
422,2	471,02		
430,5	480,88	**482,67**	**483,00**
433,7	484,47		
432,8	483,38	**482,02**	**481,00**
430,3	480,65		

T－Test 检验？

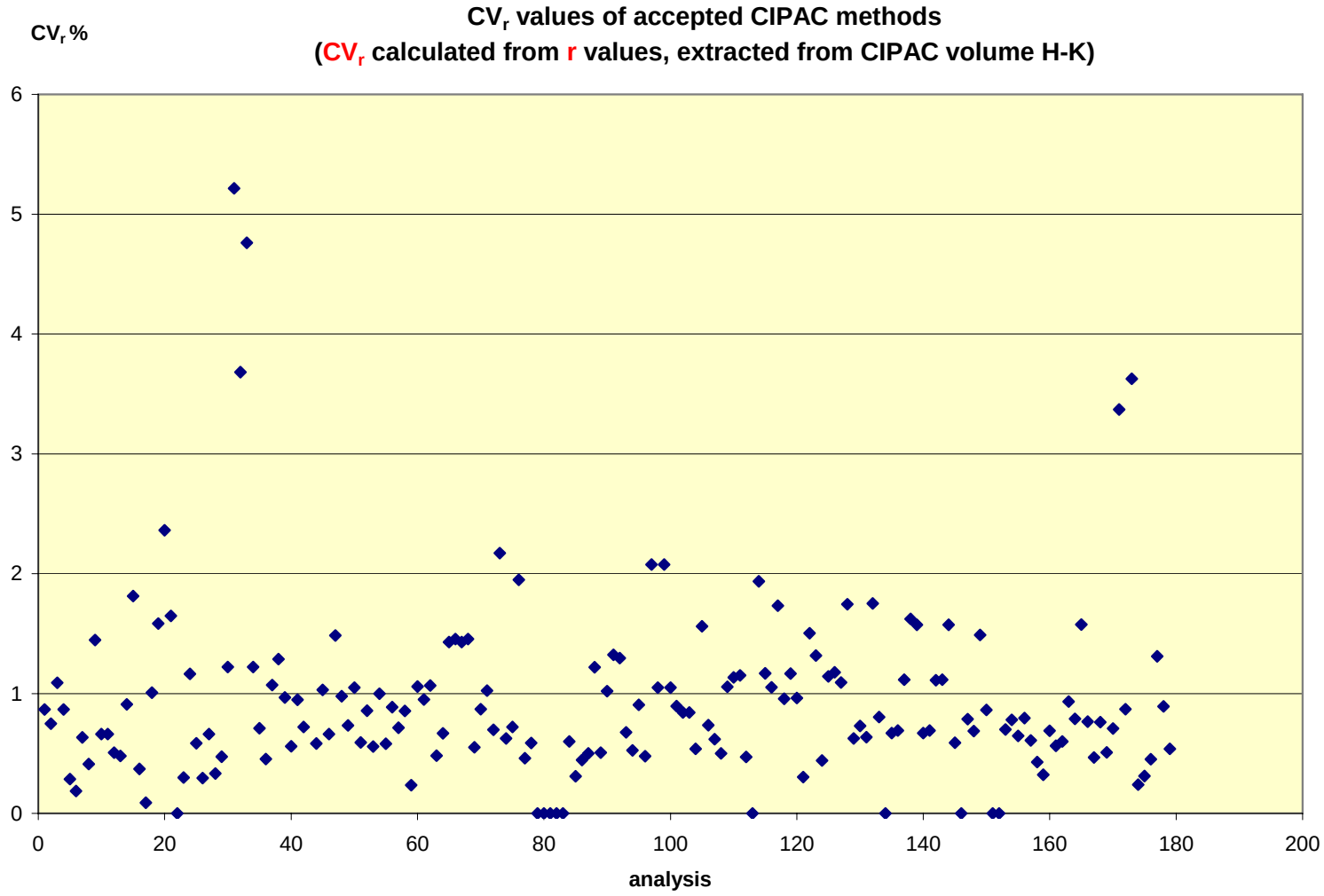
Comparison of regression lines for chlorpyrifos methyl

Time	Regression Equation
1st year 2004	**y=0,3054x+0,0087**
2nd year 2005	**y=0,3497x-0,015**
Routine Analysis-Day 1	**y=0,3223x-0,0093**
Routine Analysis-Day 2	**y=0,3233x-0,0121**
Routine Analysis-Day 3	**y=0,3237x-0,0118**

Analysis of 3 portions of one homogenized batch by HPLC

sample	A	average	portion	area	concentration /ppm	content (g/kg)
1(1)	9460.6	9462.15	1	9462.15	546.456	370.22
1(2)	9463.7		2	9998.45	588.502	375.89
2(1)	10048.3	9998.45	3	8773.2	492.442	368.87
2(2)	9948.6				**Cmax-Cmin**	**7.025**
3(1)	8798.6	8773.2			**3.31*r/2.8**	**35.464**
3(2)	8747.8				**r: =2.8* Sr**	

CVr values of accepted CIPAC methods (data are compiled from CIPAC Handbooks H-K



Addition test of 36% EC of Dimethoate

remark	Weight(g)	A	average of A	Con.(ppm)
standard	0.05136	8680		513.6
addition inj1		10506.7	10483.3	620.302
addition inj2		10459.9		
blank inj1	0.809	9636.5	9637.8	570.273
blank inj2	0.809	9639.1		
standard	0.05136	8815.1		513.6

Q=0. 992

标准曲线与样本分析

- 建议： 标准曲线与样本分析同时进行， 进样顺序应该随机安排。
- Or: Inject a median concentration of standard to regularly check the peak areas are under statistic control.

对MP方法的讨论

- MP程序建立的方法可以分析绝大多数农药产品，固定了色谱柱、内标， 提高实验室效率和容量。
- 农药制剂空白非常重要， 有条件时可以作为考察无干扰证明的有力依据。 建立提取浓缩后进样分析。
- 对线性方程的考察不应局限于相关系数。 残差分布、Srr应该考察
- 可以用于开发新的方法。 不同色谱柱证明无干扰、或者与权威数据比较

SST of HPLC

5.1 Default Values from Regulatory Guidelines

There are numerous guidelines which detail the expected limits for typical chromatographic methods. In the current FDA guidelines on "Validation of Chromatographic Methods" , the following acceptance limits are proposed as initial criteria:

Parameter	**Limit**
Capacity factor	k' > 2
Injection precision	RSD < 1% for n ≥ 5
Resolution	Rs >2
Tailing factor	T ≤ 2
Theoretical plate	N > 2000

These suggested limits may be used as a reference to set up the initial system suitability criteria in the early method development process.

2.3 Linearity check of response

Chlorpyrifos methyl as an example

Slope a:	0,3054
Intercept b:	0,0087 =0? statistically
r:	0,9998 >0.997?
Srr:	0,0058 <0.01 or 0.02?
Calibration Equation:	**y=0,3054x+0,0087**
Column:	CP-Sil 8

Note: For residue analysis, accept calibration if r ≥0.995 and Srr < 0.1

contribution of residual plot in regression

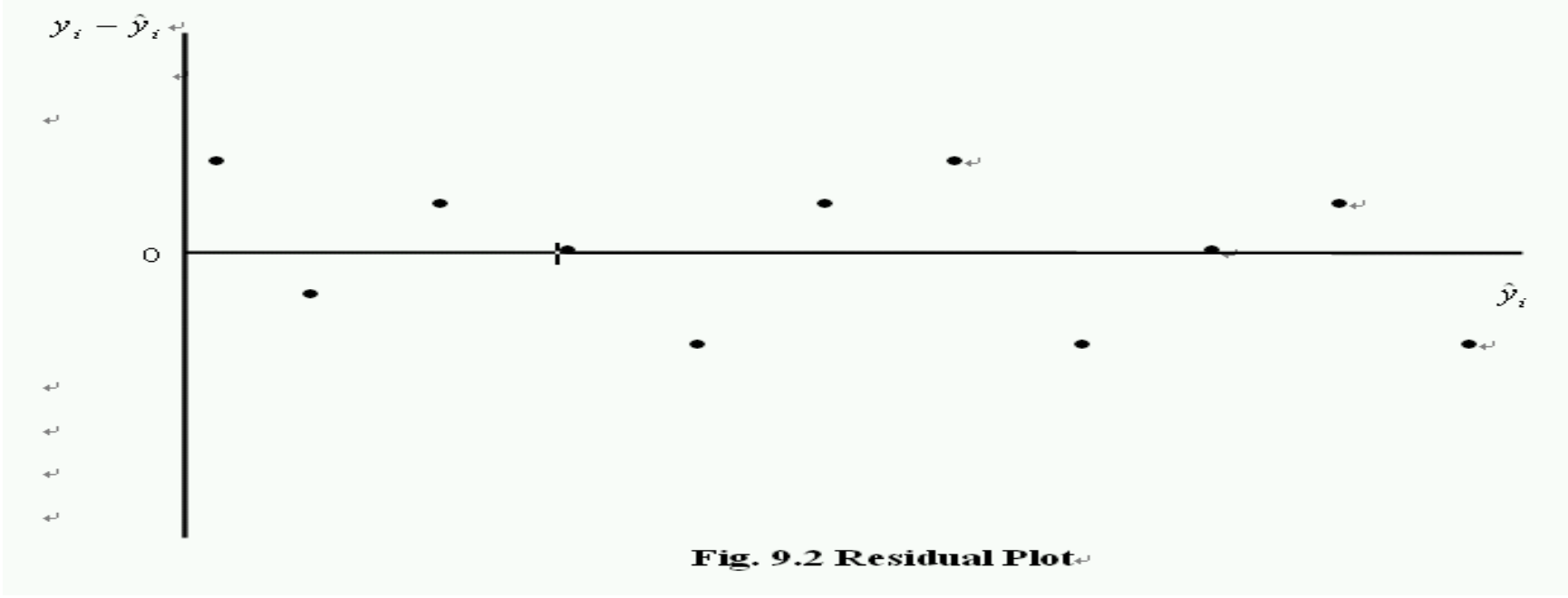


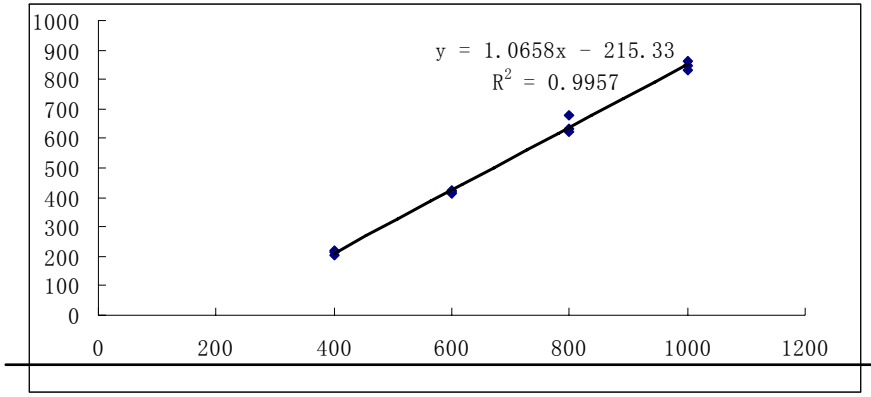
Fig. 9.2 Residual Plot

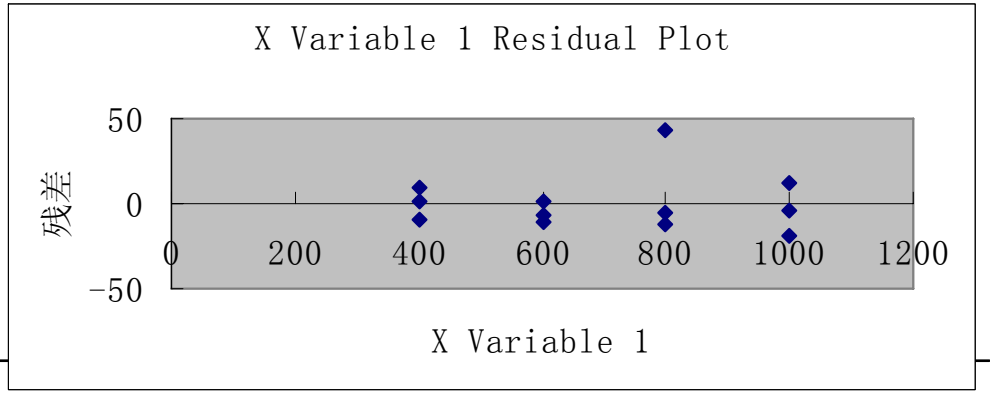
An ideal residual plot should be random!

Standard deviation of relative residual, namely Srr, should be <0.02

Another example of bad calibration

r>0.997?; b=0?; and residual pot? ; Srr<0.02?





	Coefficients	标准误差	t Stat	P-value	Lower 95%	Upper 95%	下限 95.0%	上限 95.0%
Intercept	-215.333	16.27713	-13.2292	1.16E-07	-251.601	-179.066	-251.601	-179.066
X Variable 1	1.065833	0.02215	48.11808	3.63E-13	1.016479	1.115187	1.016479	1.115187

Check co-elutes on columns by two different polar columns (two methods)

Method A			Method B or reference method			
Replicate 1	Replicate 2	Average	Replicate 1	Replicate 2	Average	Difference
0.532	0.545	0.539	0.518	0.524	0.521	0.018
0.52	0.53	0.525	0.538	0.523	0.531	-0.006
0.535	0.531	0.533	0.527	0.519	0.523	0.01
0.517	0.526	0.522	0.513	0.531	0.522	-5E-04
0.529	0.523	0.526	0.521	0.528	0.525	0.002
					Average	0.005
					SDdif	0.009
					tcalc=	1.127
					tcrit=	2.776