农药分析数据的质量与实验室质量控制

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产品质量分析的重要要素

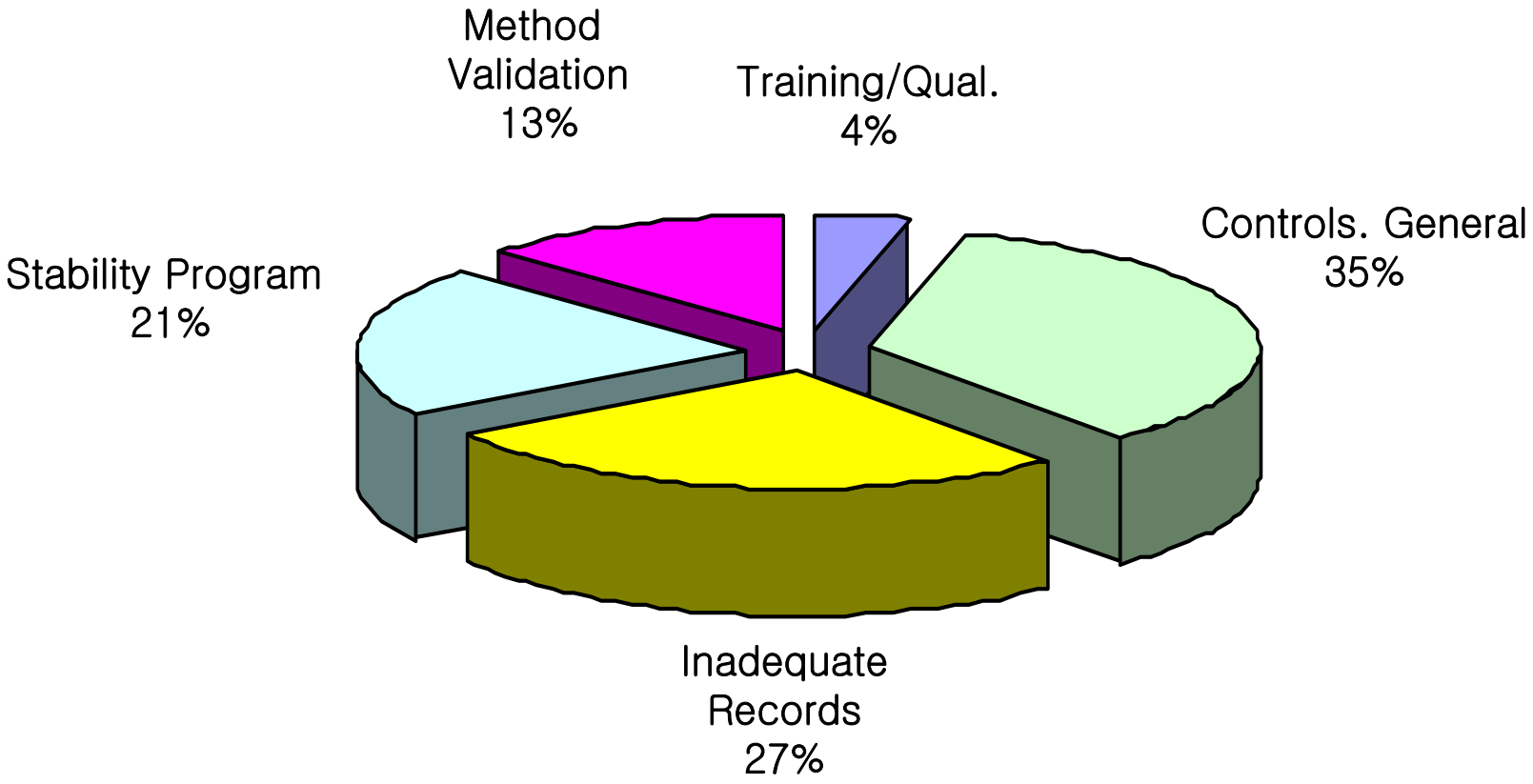
- 可靠的分析方法： CIPAC、AOAC、 国标、行业标准、经过验证的企业标准、权威文献报道的方法

- 可靠的分析实验室质量控制手段： 内部质量控制IQC； 外部质量控制

- 公认的原则： 采用标准分析方法；开发并验证用于质量控制的分析方法、使用实验室熟练掌握的分析方法

FDA Systems Based Inspection: Laboratory System

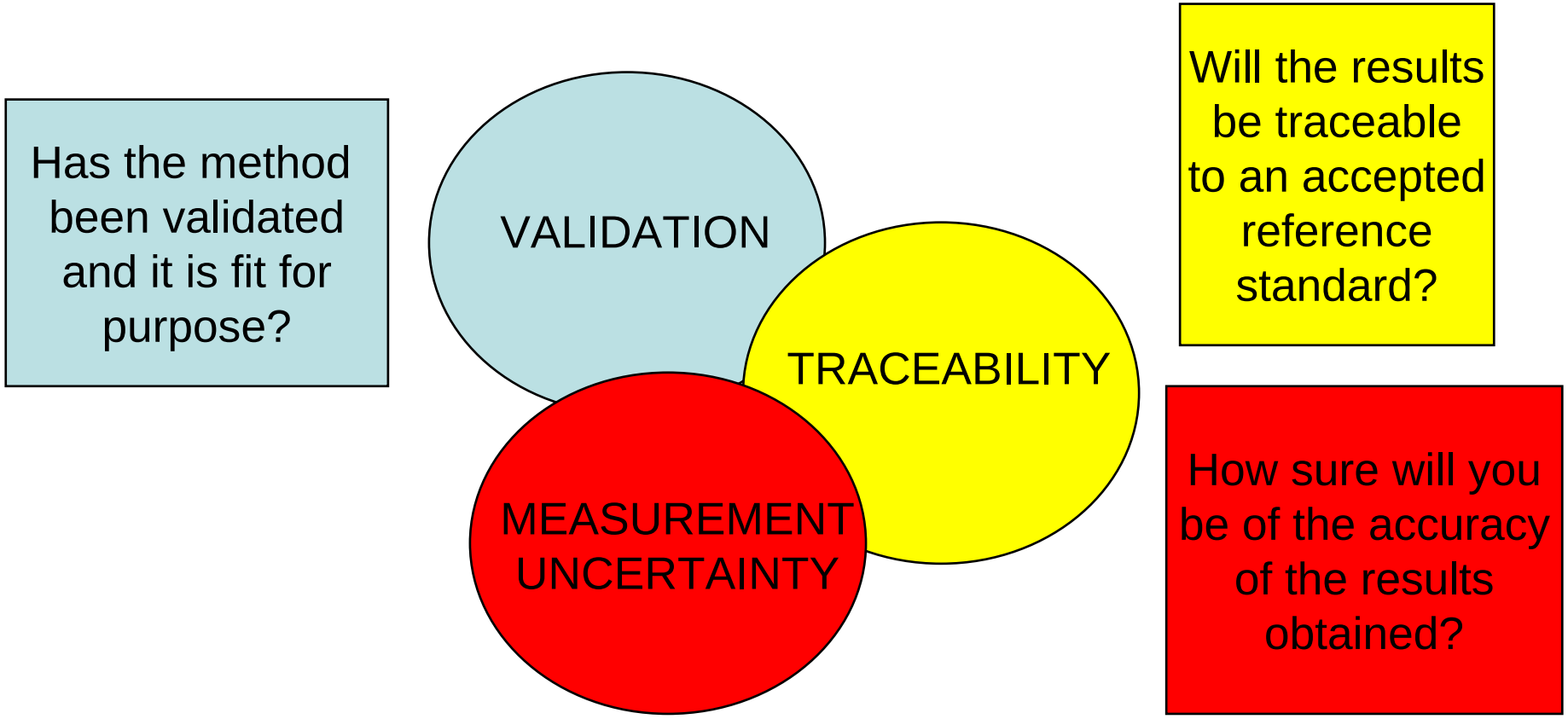
Feb – July 2002: 212 Inspections (US)



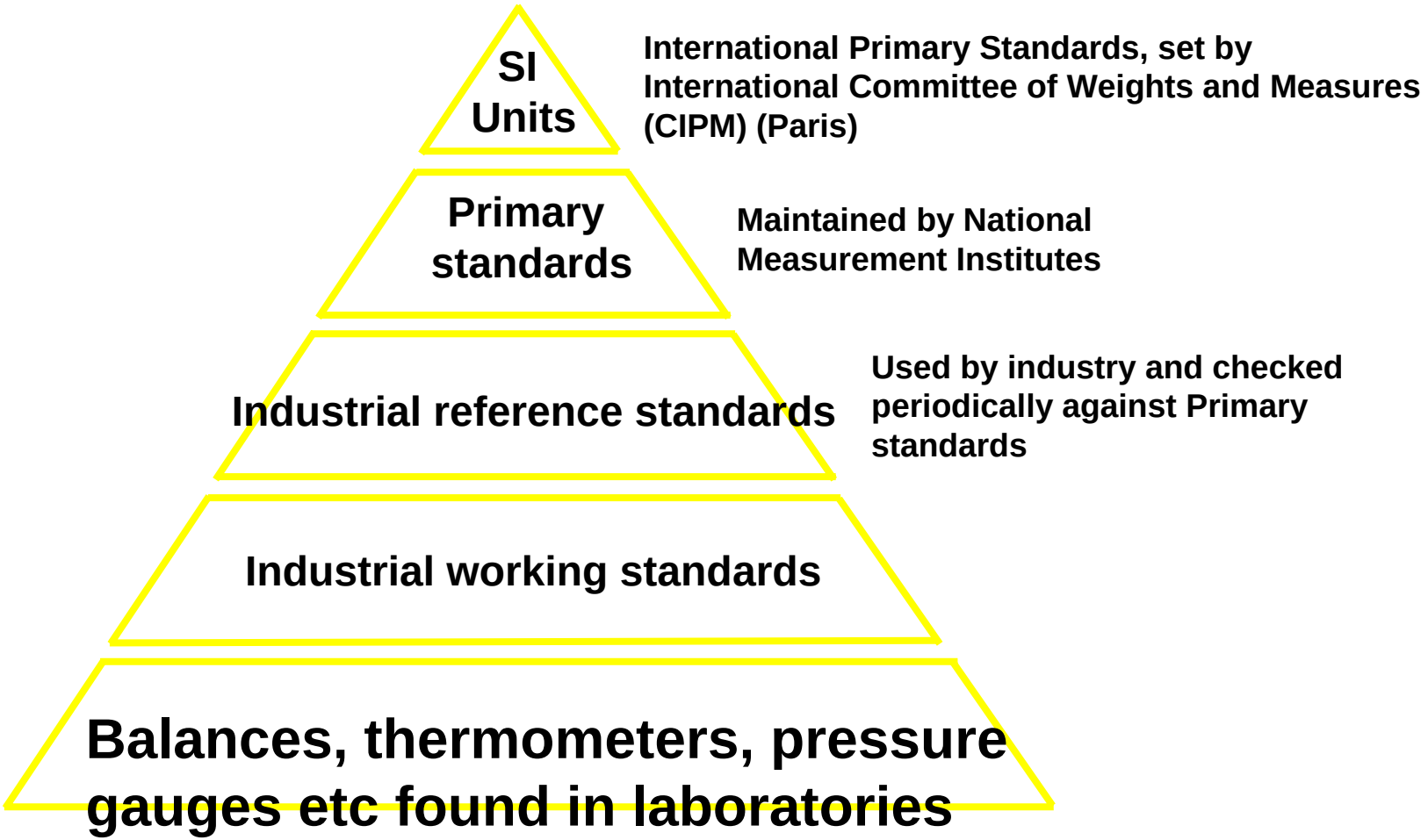
* Reference: Albinus D' Sa, FDA, CDER Office of Compliance, from AAPS, Nov. 2002 presentation.

分析中的三个重要因素

When expressing results of analytical measurements you will need to bear in mind the 3 inter-linking parameters of:



可溯源性通过一系列的比较来实现



何时进行方法验证

新方法开发	F[1,2,3]
现有方法的适应（新的基质等）	F[1,2,]F or P[3]
标准方法的改良	P or F[1]
质量控制显示分析方法有偏离	P or F[1]
不同实验室之间方法交换	P[1] or E[2]
仪器、操作人员改变	P[1]
新的试剂与配件	P[1]
已验证方法长时间未采用	P[2]
实验室管理或相关的改变	P[2]
新的协作研究方法	P[2]
经过验证但未经协作研究验证	P + E[2]
文献报道、有方法的特征参数	P + E[2]
文献报道、无方法的特征参数	F[1,2,3]

F: full validation; E: extensive validation; P: partial validation;

农药分析方法验证的内容（1）

准确性 Accuracy: 与真值的偏离程度

线性范围 Linearity: 分析的可靠范围（定量分析的基础）

精确性 Precision: 结果之间的接近程度

农药分析方法验证的内容（2）

灵敏度 Sensitivity: 不同浓度样本的响应大小

特异性 Specificity: 分析物定性的考察

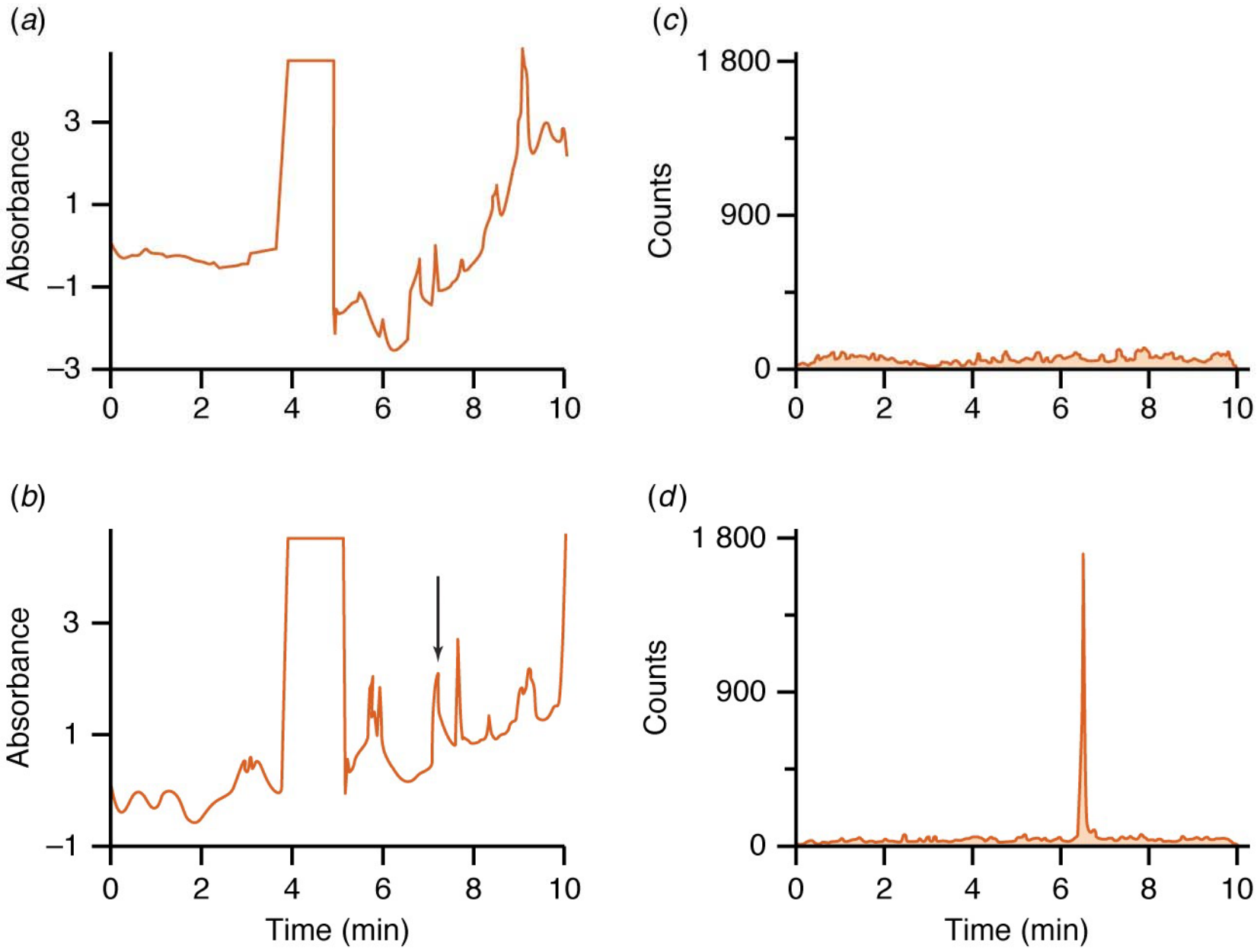
添加回收率Recovery: 测定样本中分析物全部的能力

重现性 Reproducibility

稳定性 Stability 分析方法各步骤中分析物稳定性

抗干扰能力

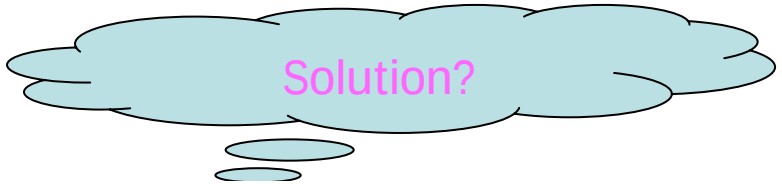
分析范围


(a)
Absorbance
3
1
−1
−3
0
2
4
6
8
10
(c)
Counts
1 800
900
0
0
2
4
6
8
10
(b)
Absorbance
3
1
−1
0
2
4
6
8
10
Time (min)
(d)
Counts
1 800
900
0
0
2
4
6
8
10
Time (min)

	Quantification and analysis of a.i. in technical material	Quantification and analysis of significant impurities (>0.08 % and substances of toxicological concern below this level) in technical material	Qualitative analysis of low level impurities (<0.08 %) in technical material	Quantification and analysis of a.i. in a matrix (formulation)		Quantification and analysis of a.i. in drinking water (0.1 $\mu g\ l^{-1}$)
				High Concentration (≥1 %w/w)	Low Concentration (≤1 %w/w)	
Accuracy	✓	✓	x	✓	✓	✓
Repeatability	✓	✓	x	✓	✓	✓
Reproducibility	Where the method is to be used in other laboratories reproducibility should be addressed					
Specificity	✓	✓	✓	✓	✓	✓
LOD	x	x	✓	x	✓	✓
LOQ	x	x	x	x	✓	✓
Linearity	✓	✓	x	✓	x	✓
Range	x	✓	x	✓	✓	✓
Robustness	Robustness should be addressed as part of the method development					

Deviation from the AOAC-CIPAC procedure

- Regulatory laboratories are forced to deviate from the CIPAC procedure if they have to analyze a number of different pesticide products. as they cannot change columns and eluents after each product or on daily basis due to cost and time restrictions.
- deviation: at the determination step? sample preparation? extraction can be carried out according to the CIPAC or AOAC procedure ; use of large amount of very expensive analytical standards?



Application of CIPAC method:

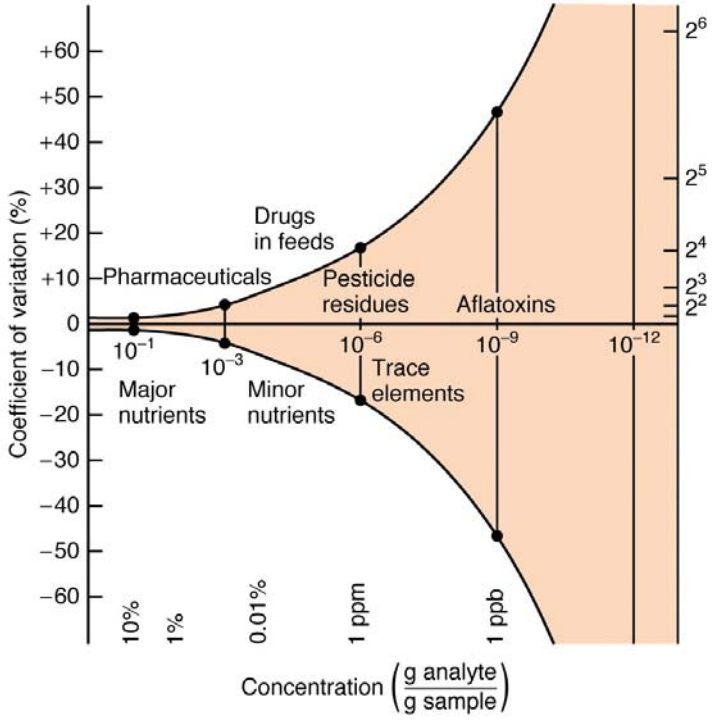
- Blank formulation or no interference proof are necessary when apply with new formulations.

- no interface proof by different columns etc.

Method Validation for the active substance

- 特异性：干扰物< 3% area
- 线性： ＋ — 20%：r>0.99： 3×2 or 1*5level
- *Accuracy: 回收率, 需要测定干扰物质影响和方法精密度。*
- *重复性： 至少5个重复， 符合改良的*Horwitz 方程。

- % Analyte Proposed acceptable F
- (Horwitz value x 0.67) %
- RSDR = 2(1-0.5 logC)
- 100 1.34 ; 50 1.49
- 20 1.71; 10 1.90
- 5 2.10; 2 2.41
- 1 2.68; 0.25 3.30



Method Validation for relevant impurities

- 特异性： 证明在有效成分、或者其它杂质存在时可以检出某一杂质
- 线性范围
- 准确性： 回收率
- 精密度
- LOQ

不同含量下 回收率要求

Guideline confidence intervals for % mean recovery from preparations, based on consultation with Industry, are as follows.

% active (nominal)	mean % recovery	% impurities(nominal)	mean % recovery
>10	98-102	>1	90-110
1-10	97-103	0.1-1	80-120
<1	95-105	<0.1	75-125
0.01-0.1	90-110		
<0.01	80-120		

SST:

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

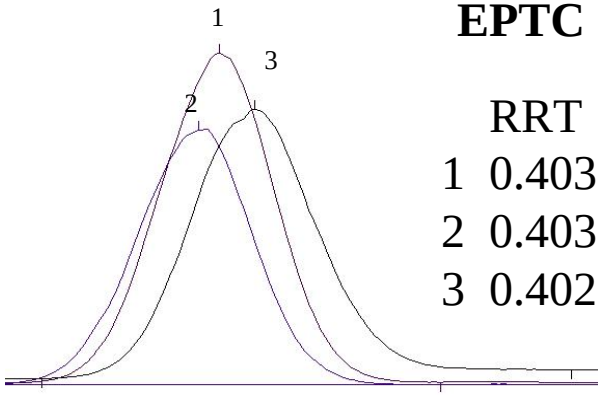
These suggested limits may be used as a reference to set up the initial system suitability criteria in the early method development process.

GC：系统测试

- 进样重复性的测试
  - 系统稳定后，进行系统测试SST
  - 取标样和内标的混合物，重复进样5次
- 计算 SD和CV
  - 要求：（对于不分流进样）
- （标样/内标）比值　　CV≤1%（较难的化合物最差≤2%）
- 单个峰　　CV≤5%（最差≤10%）
- 保留时间　CV≤0.5%

Table 1 System performance check					5-Jun-06		
Raw data file:		IAEATEB2		Evaluation based on:		peak area	
		Solution ID	Tebuconazole	Di-cyclohexyl Phthalate			
Analyst	Run No	Injected	Std As,i	Istd Ais,i	Ratio, Yi	Rt (AS)	Rt (As)
RVO	005B0401	Tebu 1	144.56032	175.80035	0.82230	5.159	7.602
	005B0405	Tebu 5	141.04211	169.97841	0.82976	5.162	7.605
		Mean	**143.3894**	**173.9048**	**0.8246**	**5.160**	**7.603**
		SD	**1.3800**	**2.3037**	**0.0030**	**0.001**	**0.002**
		CV	**0.0096**	**0.0132**	**0.0037**	**0.000**	**0.000**

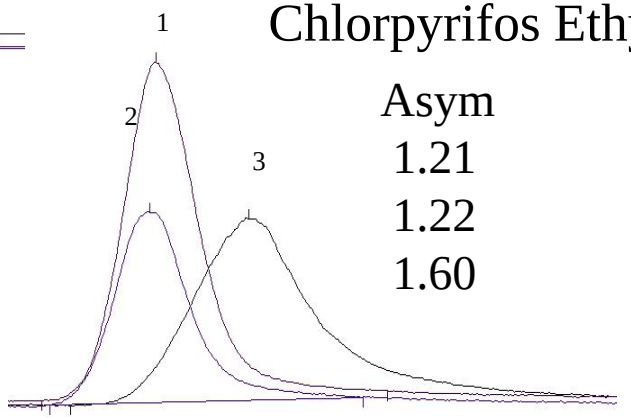
Change of peak shape and RRT



EPTC

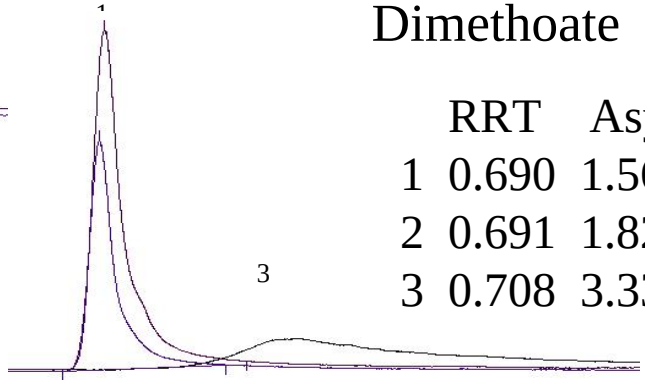
	RRT	Asym
1	0.403	0.92
2	0.403	1.01
3	0.402	0.95

1- peak shape after the system maintenance;
2 – after injection of 20 sample extracts;
3 – after injection of 40 sample extracts



Chlorpyrifos Ethyl

	Asym
1	1.21
2	1.22
3	1.60



Dimethoate

	RRT	Asym
1	0.690	1.56
2	0.691	1.82
3	0.708	3.33

System suitability tests (SSTs)

标准曲线

- 斜率 Slope (可置信范围？)
- 截距 Intercept (可置信范围？
- 相关系数 Correlation Coefficient (R^2>0.997)
- 方差 Variance
- 相对残差的标准偏差 Standard deviation of relative residual (SS< 0.01 or 0.02)

残差在结果评价中的应用

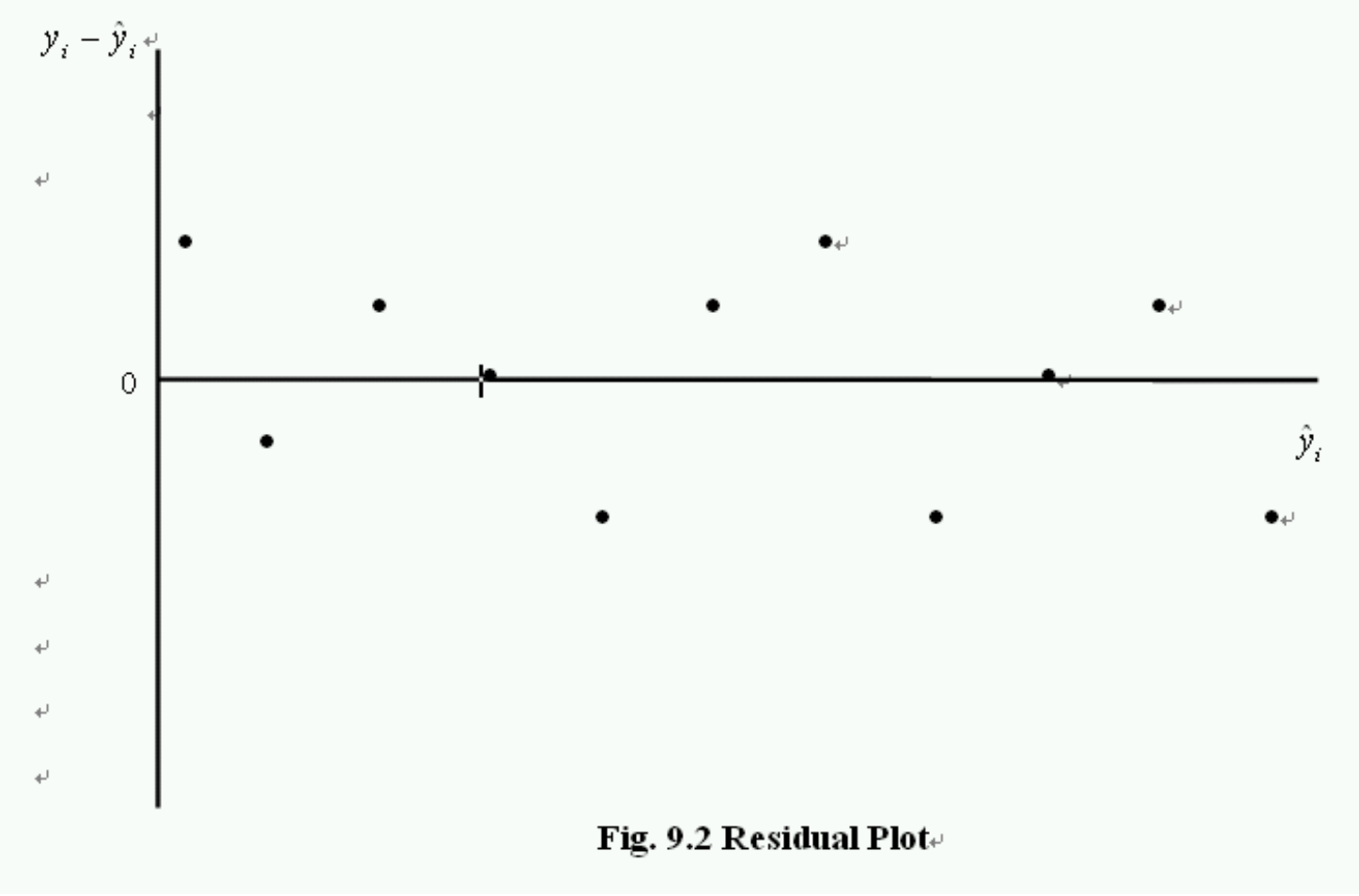


Fig. 9.2 Residual Plot

Run No	Standard	f_a
	solution ID	C_{ai}
004B0501	Tebuconazole 1	0.29123426
004B0502	Tebuconazole 1	0.29123426
005B0801	Tebuconazole 2	0.24258232
005B0802	Tebuconazole 2	0.24258232
006B1001	Tebuconazole 3	0.19303416
006B1002	Tebuconazole 3	0.19303416
	Slope a:	**3.404088617**
	Intercept b:	**0.007455547**
	r:	**0.9999**
	sYrel.:	**0.0028**

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

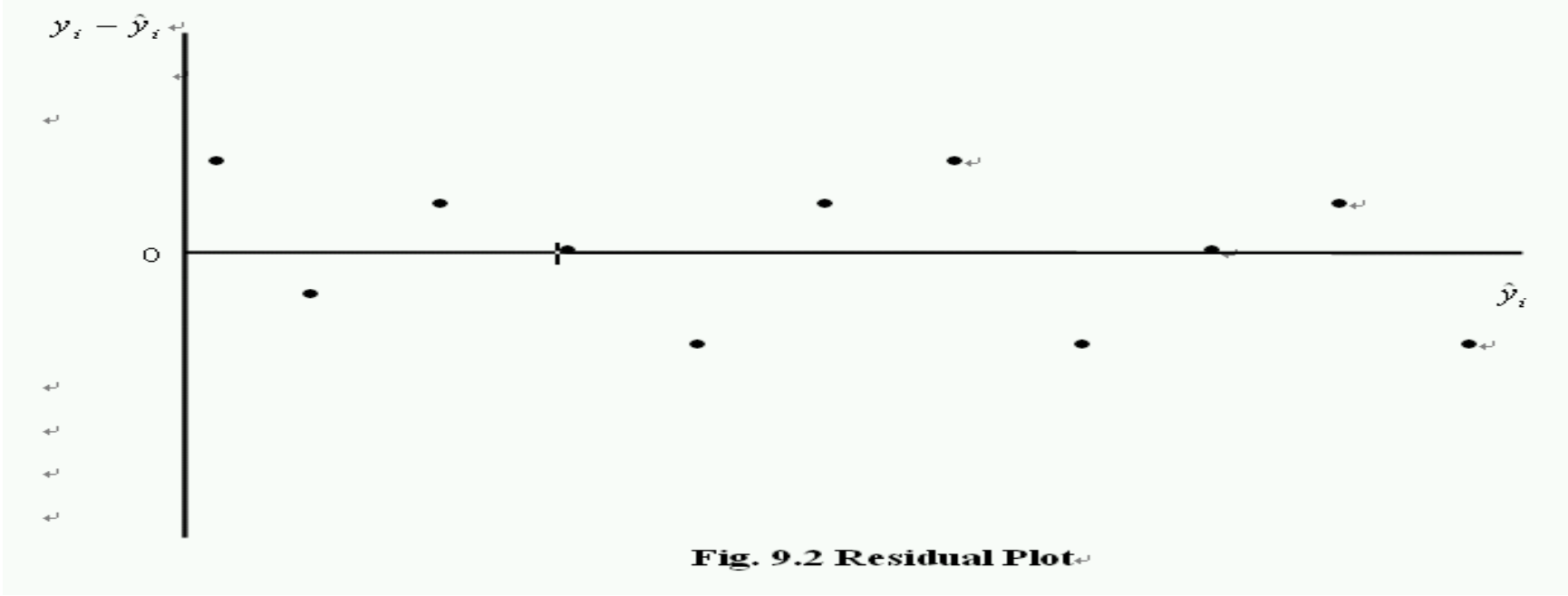


Fig. 9.2 Residual Plot

An ideal residual plot should be random!
Standard deviation of relative residual, namely Srr, should be <0.02

Example of Srr calculation

Conc. ratio	Peak 1	Peak Area of IS	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

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			

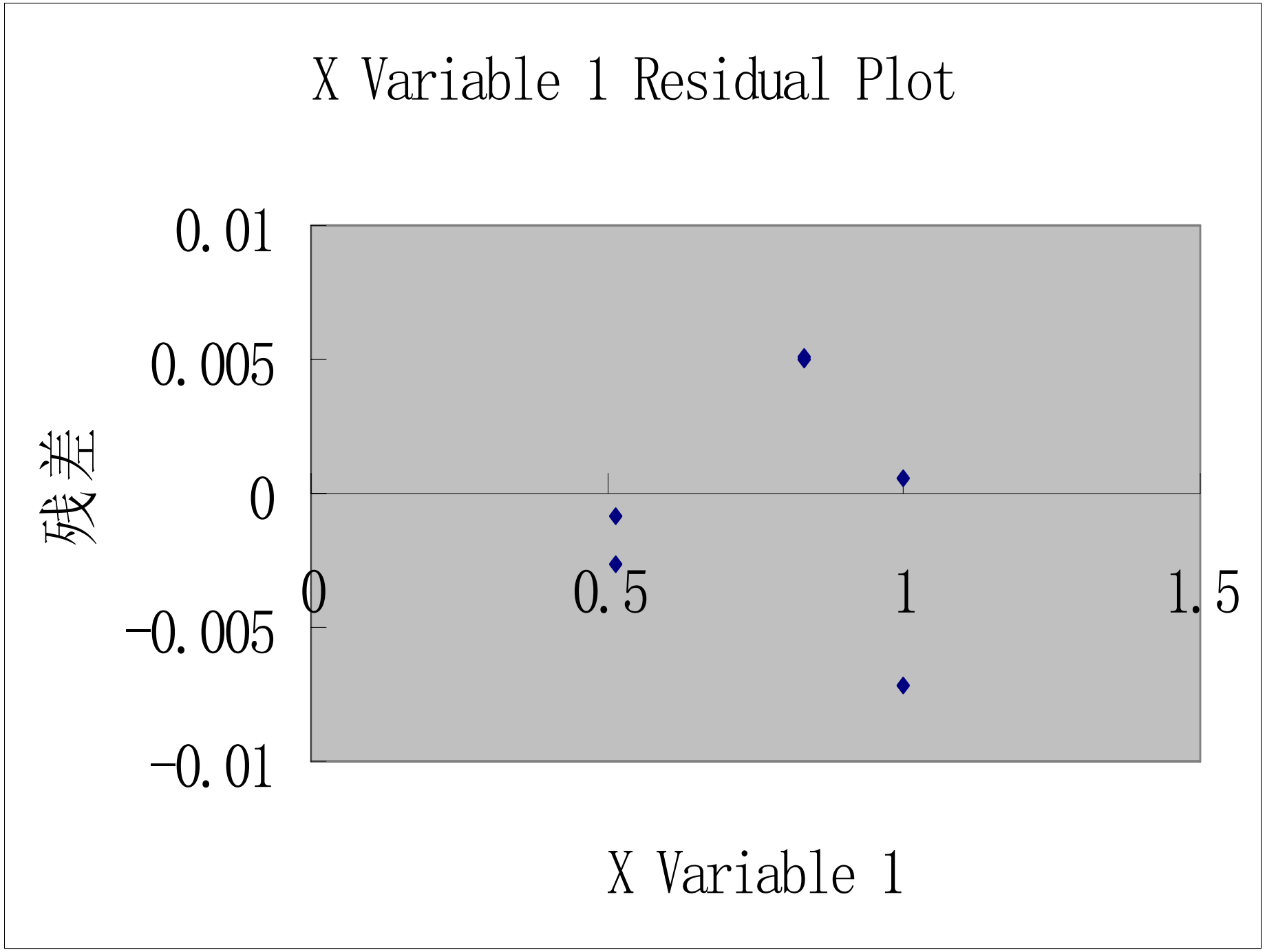
	Coefficients	SD	t Stat	P-value	Lower 95%	Upper 95%	下限 95.0%	上限 95.0%
Intercept	-0.01984	0.008584	-2.31119	0.081925	-0.04367	0.003994	-0.04367	0.003994
Slope	0.648552	0.010641	60.9466	4.34E-07	0.619007	0.678097	0.619007	0.678097

RESIDUAL OUTPUT

Observe	Pred. Y	Residual	ST residual	Relative Residual
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

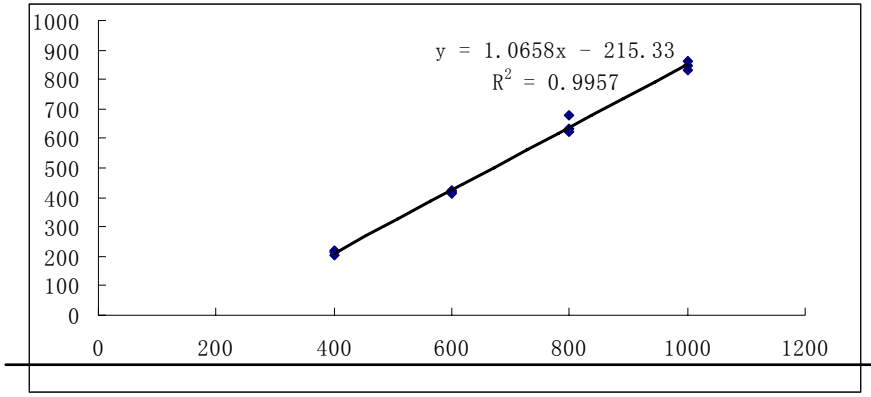
Srr: 0.008931

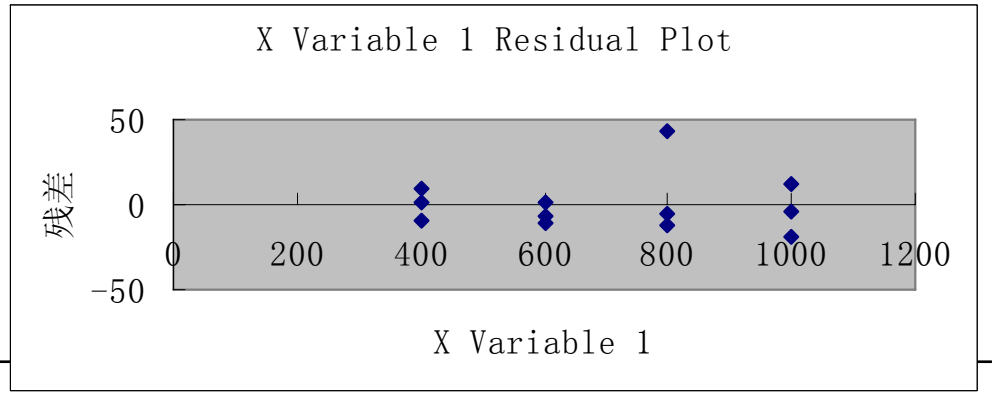
Srr<0.01 or 0.02 Acceptable!


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

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

Solution?

- ✓Bracketing method for calibration ?

Q: 双柱验证－定量分析的例子

- 假设采取内标法测定某杂质，**(CVra = 1.5%).** 对同一个提取液的含量分别进行3次测定。

- **CPSIL8CB 0.535 mg/ml ：**
- **CPSIL5CB 0.562 mg/ml.**

- Q：两次的结果是否有差异？ －> 是否色谱柱中有干扰物？

$$t = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{s_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

- The calculated t value is 4.018,
- $t2\alpha=0.05$, $\nu=4$ = 2.776

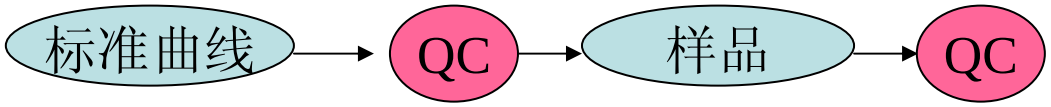
- The difference is significant (5.9238%). The results indicate the possibility of impurity.

Summary of most commonly used QC activities

- Analysis of reagent blank
- Analysis of blank samples
- Duplicate analysis
- System suitability tests （SST）
- Spike or Recovery samples
- Efficient use of control charts
- Blind samples
- Participation in collaborative studies, proficiency tests

如何使用质控样品

- （1）批质控样品



- （2）由监督人员使用的盲样

- （3）将质控样品检测结果标在质控图中

接受和拒绝检测结果的规则

（1）如果3个浓度的质控样（平行样的均值）中*均在2S*范围，接受此批结果。

（2）如果3个浓度的质控样（平行样的均值）中*有一个在2S*之外，下列规则中任何一条成立则判断为失控而拒绝结果：

其中一个浓度的样品（平行样的均值）超出3S界限

三个浓度中有两个（平行样的均值） 超出2S界限，并均值的同侧

三个浓度中有两个（平行样的均值） 超出2S界限，并分布在均值的两侧

某一个浓度的质控样品，累计连续7次以上结果在均值的同侧

SOP of QA QC：
File：4391.pdf

Effective Date: 1/15/2009
Revision Date: 1/15/2009
Revision Author: A. Niculescu
GC-001-2.10

Quality Assurance/Quality Control in the GC Pesticides Laboratory

1. SCOPE AND APPLICATION

1.1. This SOP details the Quality Assurance (QA) and Quality Control (QC) procedures for the GC-Pesticides Work Group.

1.2. Quality Assurance consists of all of the practices undertaken in a laboratory to insure the data generated are as accurate and precise as possible. It includes not only quality control measures, but can be as specific as the cleaning of glassware and preparation of standards. This SOP will concentrate on Quality Control measurements that are used to measure and track the Quality Assurance in the GC Pesticides lab. It will touch briefly on some general guidelines for QA. Refer to the individual preparation or analysis SOPs for more specific details on QA.

1.3. Most of these QA/QC practices described are common throughout the Chemistry Section.

实验室质量控制

实验室内质控

- 自我控制
- 发现随机误差和新出现的系统误差
- 评价分析质量的稳定性
- 是分析的基础、必需、常规

实验室间质控

- 外部质控
- 发现系统误差和实验室间数据的可比性
- 评价实验室的测试系统和分析能力
- 有效的校核是参加与标准实验室的比对

外部质量控制手段

- 初步实验室间研究： 由两个或多个实验室参加， 评价一种方法， 确定其是否具备条件作为协作研究的对象。

- 实验室间检测能力测试 Performance Test：

  分析经仔细制备的均匀样本， 以证实和考核实验室或分析人员的试验水平。

CNAS—GL02

能力验证结果的统计处理和能力评价指南

Guidance on Statistic Treatment of Proficiency Testing Results and Performance Evaluation

International Advisory Committee

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The Second International Proficiency Testing Conference

PTconf

The Second Announcement

Sibiu, România

(15)16th – 18th September, 2009

Structural changes from G13 and Guide 43 parts 1 and 2

- Technical Requirements come before Management Requirements
- One part with 3 Informative Annexes rather than two parts
  - A: Types of proficiency testing
  - B: Statistical methods for proficiency testing
  - C: Selection and use of proficiency testing

ISO 17043

Introduction – Types of ILC's - PT

a. evaluation of the performance of laboratories and monitoring laboratories' continuing performance;
b. identification of problems in laboratories and initiation of actions for improvement;
c. establishment of the effectiveness and comparability of test or measurement methods;
d. provision of additional confidence to laboratory customers;
e. identification of interlaboratory differences;
f. education of participating laboratories based on the outcomes of such comparisons;
g. validation of uncertainty claims;

Introduction – Types of ILC's ***not*** PT

h. evaluation of the performance characteristics of a method;

i. assignment of values to reference materials and assessment of their suitability for use in specific test or measurement procedures; and

j. support for statements of the equivalence of measurements of National Metrology Institutes through "key comparisons" and supplementary comparisons conducted on behalf of the International Bureau of Weights and Measurement (BIPM) and associated regional metrology organizations.

国际能力验证要求

- 国际指南**ISO/IEC**指南**43**：**1997**。正在由**ISO/CASCO-WG28**进行修订，修订后将变更为国际标准，代号为**ISO/IEC17043**，预计在**2010**年发布；
- **ISO13528**的发布。该标准经过多年的准备和讨论，于**2005**年发布实施。为能力验证提供统计上的支持。**ISO/IEC 17011**：**2004**《合格评定-认可机构通用要求》（**GB/T27011-*2005***）
- **ISO/IEC** 指南**43**《利用实验室间比对的能力验证》（**GB/T15483**，**IDT**）
- **ISO13528**：**2005**《利用实验室间比对的能力验证中的统计方法》
- **ISO/IEC 17025**：**2005**《检测和校准实验室能力的通用要求》（**GB/T27025-2008**）
- **ISO15189**：**2003**《医学实验室-质量和能力的专用要求》

ILAC和APLAC要求及其动态

- **APLAC-MR001** 《建立和保持**APLAC** 多边互认协议的程序要求》中规定，签署互认协议认可机构能力验证工作必须符合**ILAC-P9**的要求，同时，也要求认可机构符合相应的能力验证要求；
- **ILAC- P9**《参加国家和国际能力验证活动的政策》也进行了修订，新文件中给出了能力验证的频次和子领域划分的相关指导；
- **ILAC-G13** 《能力验证计划提供者的能力要求指南》，正在修订；
- **ILAC-G22** 《应用能力验证作为对检测能力认可的手段》，从实验室如何参加能力验证的角度提出了相关要求；
- **APLAC-PT001**等系列文件，既有改进也有新制定的。

农药产品质量检测与判定

- 原药含量分析
- 制剂含量分析
- 杂质分析

tolerance limits for pesticide products

- According the FAO Manual[i], the active ingredient content of technical materials should be expressed as:
- "The [ISO common name] content shall be declared (not less than ...g/kg) and, when determined, the *mean* measured *content shall not be lower than* the declared content."

- The active ingredient content of technical concentrates and formulated pesticides should be expressed as:
- “The [ISO common name] content shall be declared (g/kg or g/l at 20 ± 2° C,) and, when determined, the *mean measured content shall not differ from* that declared by more than the following *tolerances*:”

Table 1. Tolerance limits for active ingredients of pesticide products

Declared content in g/kg or g/l at 20±2°C	Tolerance
up to 25	± 15% of the declared content for homogeneous formulations (EC, SC, SL, etc.), **or** ± 25% for heterogeneous formulations (GR, WG, etc.)
above 25 up to 100	± 10% of the declared content
above 100 up to 250	± 6% of the declared content
above 250 up to 500	± 5% of the declared content
above 500	± 25 g/kg or g/l
Note In each range the upper limit is included	

Where the formulation contains more than one active ingredient, specifications must be provided for all active ingredients present.

- As the tolerances for pesticide product generally correspond to the 95% confidence level,
- thus standard deviation of the variability of active ingredient content, ST, can be derived from the tolerance intervals (T) specified for the mean measured content of the product: ST = T/1.96,
- (for practical purposes ST = T/2 may be used).

$$S_{\overline{m}} = \sqrt{S_T^2 - S_{\overline{C}}^2}$$

$$S_{\overline{C}} = \frac{S_{Ra}}{\sqrt{n}}$$

Table 2. Tolerance intervals and performance characteristics of CIPAC methods for some pesticide formulations

Active ingredient	Formu-lation	± Tolerance %	± Tolerance g/kg	Content, g/kg	r	R	S_R g/kg	CV_r%	$S_R/\sqrt{2}$	$S_{\bar{m}}$ g/kg
Glyphosate	SG		25	731	9 to 11	11 to 14	3.92-5.00	0.54	2.70-3.53	12.26
			25	874	8	14	5.00	0.33	3.53	12.59
Flusilazole	WG	6	*11.82*[a]	197	7	16	5.71	1.27	4.04	4.48
	EC	5	*12.85*	257	12	16	5.71	1.67	4.04	5.16
			0	396	16	25	8.93	1.44	6.31	7.89
Methomyl	WP	6	*14.64*	244	9	14	5.00	1.32	3.54	6.58
	UL	5	*14.9*	298		8	2.86		2.02	7.33
Metsulfuron-methyl	WG	6	*12.72*	212	6 to 9	6 to 9	2.14-3.21	1.52	1.52-2.28	6.08
		5	*30.35*	607	15 to 19	19 to 20	7.14	1.12	5.05	14.64
Profenofos	EC	5	*22.4*	448	8	15	5.36	0.64	3.79	10.78
		5	*17.6*	352	9	23	8.21	0.91	5.81	6.85
Propineb	WP		25	700	10	29	10.36	0.51	7.32	10.44
Quinclorac	WP	5	25	500	23	26	9.29	1.64	6.57	10.94
	WG		25	762	26	42	15.00	1.22	10.61	7.08
	SC	6	*13.14*	219	12	18	6.43	1.96	4.55	4.93
Triazophos	EC	5	*21.65*	433	12	12	4.29	0.99	3.03	10.62
			25	570	12	24	8.57	0.75	6.06	11.22

within laboratory Sra ≤ 1.5 CIPAC Sr
: (Sra ~ Sr).

$$S_L = \sqrt{S_{Sp}^2 + S_A^2}$$

$$S_{R\bar{a}} = \sqrt{\frac{S_{ra}^2}{2} + \frac{S_r^2}{2}}$$

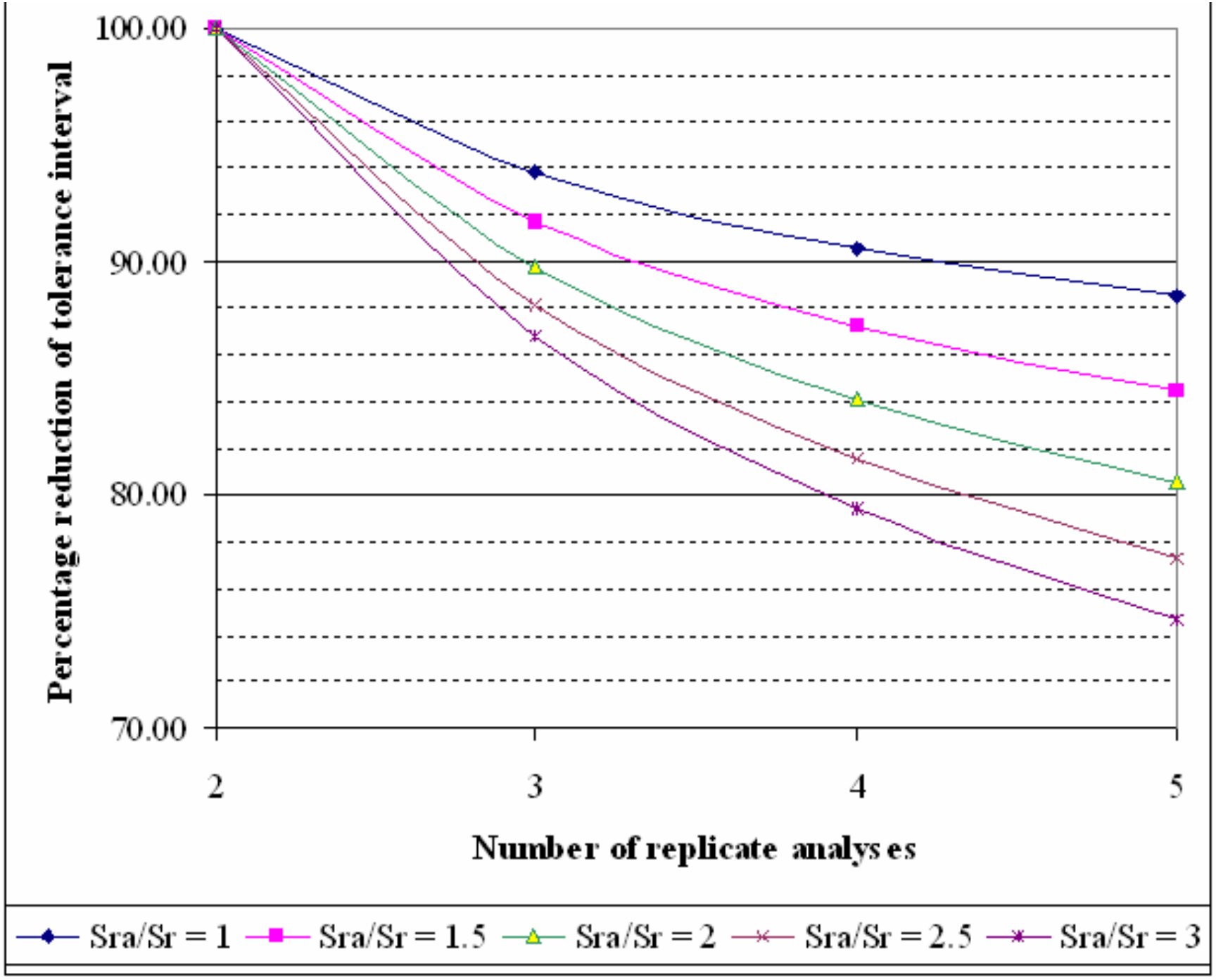
$$S_T^{'} = \sqrt{S_{R\bar{a}}^2 + S_{\bar{m}}^2}$$

$$T = 2ST'$$

possibility of reducing ST

Table 3. $S_{R\bar{a}}$ values calculated for various S_{ra} and n assuming $S_r = 1$

	S_{Ra} values				
n	$S_{ra} = 1$	$S_{ra} = 1.5$	$S_{ra} = 2$	$S_{ra} = 2.5$	$S_{ra} = 3$
2	1.00	1.27	1.58	1.90	2.24
3	0.91	1.12	1.35	1.61	1.87
4	0.87	1.03	1.22	1.44	1.66
5	0.84	0.97	1.14	1.32	1.52


100.00
90.00
80.00
70.00
Percentage reduction of tolerance interval
2
3
4
5
Number of replicate analyses
Sra/Sr = 1
Sra/Sr = 1.5
Sra/Sr = 2
Sra/Sr = 2.5
Sra/Sr = 3

- **Testing the minimum active ingredient content of technical active ingredient**

- The FAO Specification declares the minimum a.i. content, e.g. 950 g/kg. The mean a.i. content should not be significantly lower than the declared content.

$$\mu \leq \bar{x} + \frac{ts}{\sqrt{n}}$$

Testing the minimum active ingredient content of technical active ingredient

$$q_{\bar{x},\mu} = \frac{\left|\bar{x} - \mu\right|}{X_n - X_1}$$

X < U 时

Table A9a. Significance limits ($q_{\bar{x},\mu}$) for the differences between the mean of a sample and a hypothetical mean

Significance limits[2] for the difference between the mean of a sample and a hypothetical mean μ

Test quotient: $\frac{|\bar{x} - \mu|}{x_N - x_1}$

x_N is the highest, x_1 the lowest value of a sample of size N

2α	0,10	0,05	0,02	0,01	0,002	0,001
N						
2	3,157	6,353	15,910	31,828	159,16	318,31
3	0,885	1,304	2,111	3,008	6,77	9,58
4	0,529	0,717	1,023	1,316	2,29	2,85
5	0,388	0,507	0,685	0,843	1,32	1,58
6	0,312	0,399	0,523	0,628	0,92	1,07
7	0,263	0,333	0,429	0,507	0,71	0,82
8	0,230	0,288	0,366	0,429	0,59	0,67
9	0,205	0,255	0,322	0,374	0,50	0,57
10	0,186	0,230	0,288	0,333	0,44	0,50
11	0,170	0,210	0,262	0,302	0,40	0,44
12	0,158	0,194	0,241	0,277	0,36	0,40
13	0,147	0,181	0,224	0,256	0,33	0,37
14	0,138	0,170	0,209	0,239	0,31	0,34
15	0,131	0,160	0,197	0,224	0,29	0,32
16	0,124	0,151	0,186	0,212	0,27	0,30
17	0,118	0,144	0,177	0,201	0,26	0,28
18	0,113	0,137	0,168	0,191	0,24	0,26
19	0,108	0,131	0,161	0,182	0,23	0,25
20	0,104	0,126	0,154	0,175	0,22	0,24

Impurity tests

- if the measured impurity is significantly exceeds the specified limit or not.

$$m \leq \bar{x} - \frac{ts}{\sqrt{n}}$$

实验室数据的质量

测量值的组成

- **Observed value**:

  xi = m + B + e

- where
- ***M:*** *平均值*
- ***B*** is bias ;
- ***e*** is 随机误差;
- μ is 真值; often not known

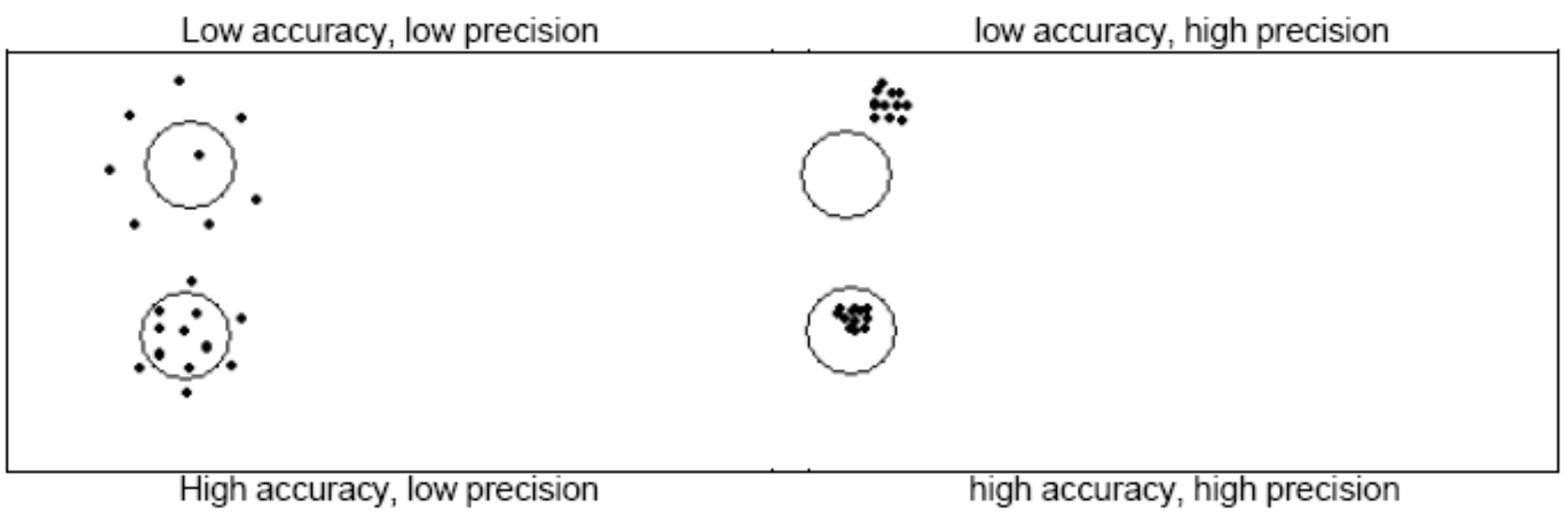
单个实验室内部的误差来源

1- 每个分析测试员之间的差异,

2- 仪器之间的差异,

3- 实验试剂与消耗品之间的差异,

4- 一定时间内以上1、2、3的差异,



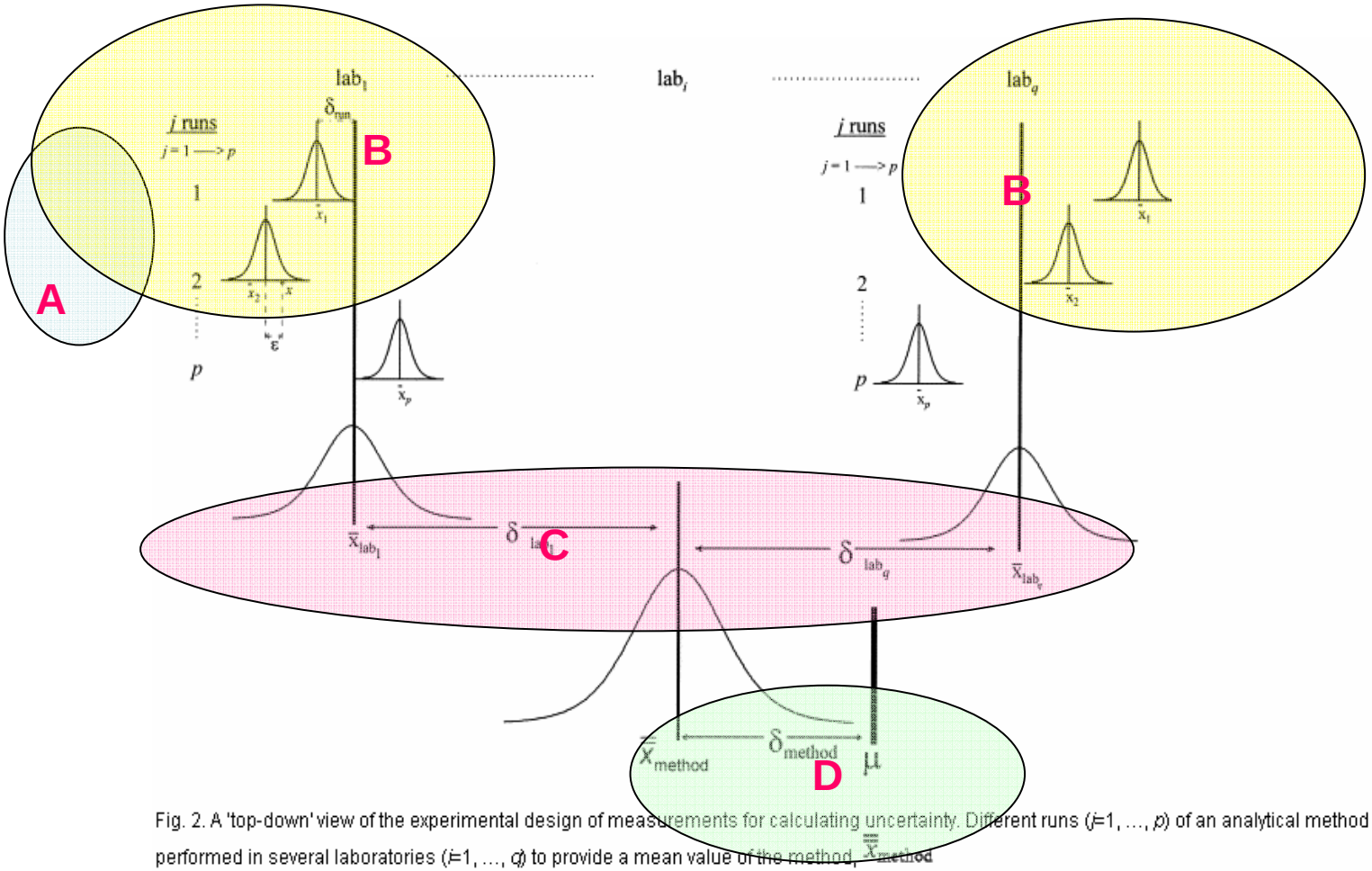


Fig. 2. A 'top-down' view of the experimental design of measurements for calculating uncertainty. Different runs (i=1, …, p) of an analytical method are performed in several laboratories (i=1, …, q) to provide a mean value of the method, $\bar{\bar{x}}_{method}$

. The random error of individual results, ε, the run bias, δ_{run}, the laboratory bias, δ_{lab}, and the method bias, δ_{method}, are shown.

A: Sr; B: run bias; C: lab bias; D: method bias

测量不确定度的来源

在实际分析工作中，不确定度典型的来源包括：

1）对样品的定义不完整或不完善；

2）分析的方法不理想；

3）取样的代表性不够；

4）对分析过程中环境影响的认识或控制不完善；

5）对仪器的读数存在偏差；

6）分析仪器计量性能（灵敏度、分辨力、稳定性等）上的局限性；

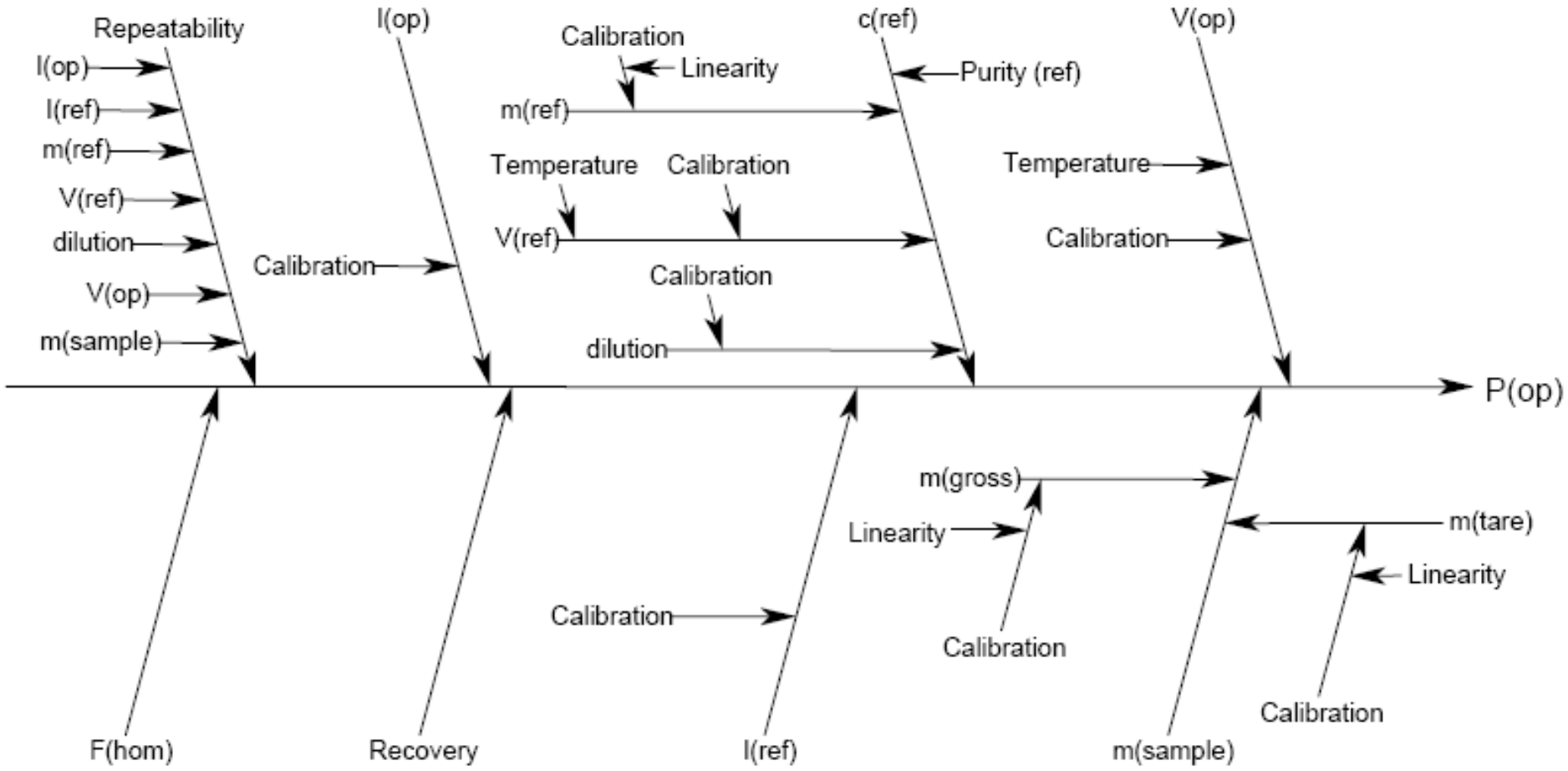
7）标准物质的标准值不准确；

8）引进的数据或其他参量的不确定度；

9）与分析方法和分析程序有关的近似性和假定性；

10）在表面上看来完全相同的条件下，分析时重复观测值的变化等。

Figure A4.2: Uncertainty sources in pesticide analysis



结果精密度的表述

- X = Average(Xi) +(-) t * Sigma * / Squareroot(n)
- n＝测定次数； Sigma：方法的重复性； t：一定容错概率下包含因子； Xi：平均值

不确定度计算： 例

PART 2 – PROPOSED EXTENSION OF CAC-GL 59-2006:

PRACTICAL AND SIMPLIFIED MU ESTIMATION BASED ON TOP-DOWN APPROACHES

Underlying principles, formulas and statistics for PT based estimation of MU

Within-laboratory reproducibility standard deviation is combined with estimates of the method and laboratory bias using PT data:

$$U' = k * u' = \sqrt{u'(R_W)^2 + u'(bias)^2}$$

where:

$$u'(bias) = \sqrt{RMS'_{bias}{}^2 + u'(C_{ref})^2}$$

and:

$$RMS'_{bias} = \sqrt{\frac{\sum (bias'_i)^2}{m}}$$

and:

$$u'(C_{ref}) = \frac{\sum_i \frac{S'_{Ri}}{\sqrt{n_i}}}{m}$$

不确定表述实例

Table A4.4: Uncertainties in pesticide analysis

Description	Value x	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)$	Remark
Repeatability(1)	1.0	0.27	0.27	Duplicate tests of different types of samples
Bias (*Rec*) (2)	0.9	0.043	0.048	Spiked samples
Other sources (3) (Homogeneity)	1.0	0.2	0.2	Estimations founded on model assumptions
P_{op}	--	--	0.34	Relative standard uncertainty

$$\frac{u_c(P_{op})}{P_{op}} = \sqrt{0.27^2 + 0.048^2 + 0.2^2} = 0.34$$

$$\Rightarrow u_c(P_{op}) = 0.34 \times P_{op}$$

实验室内部控制手段

- 方法验证中采用已知 repeatability σ_r and reproducibility σ_R .
- 及时监测 accuracy, σ_r and σ_R parameters
- 统计学原理和手段.
- 有力工具之一:

统计控制图 The control charts

质量控制图 **Control charts**

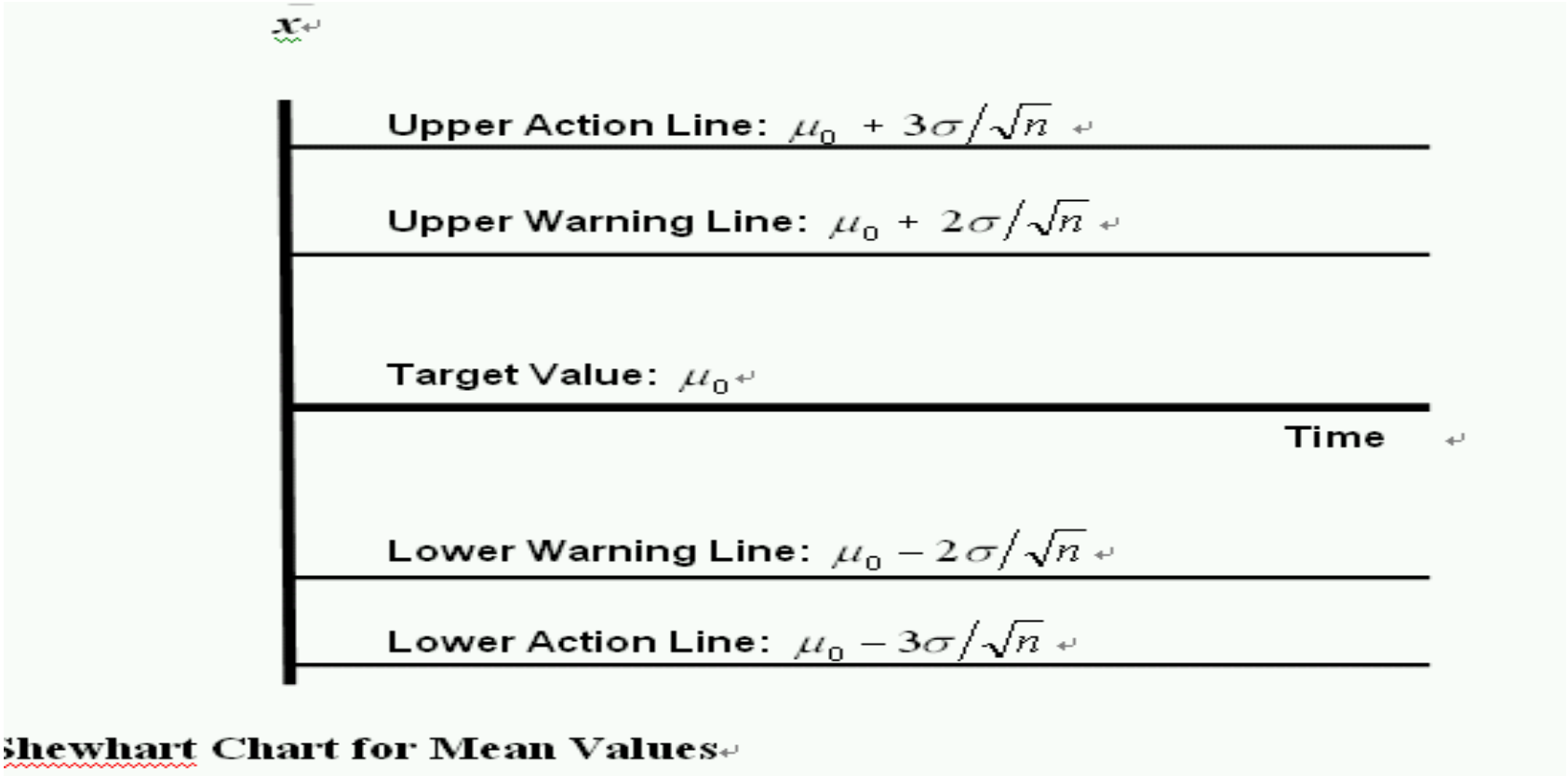
Before charting

application of outliers test; Dixon’s Q and Grubbs for screning of the data

After

application of the classical statistics.

统计控制图示意



Shewhart Chart for Mean Values

For 95% confidence limits: $m = \bar{x} \pm 2\sigma/\sqrt{n}$

For 99.7% confidence limits: $m = \bar{x} \pm 3\sigma/\sqrt{n}$

单一样本的测试 - I

- 一定时间内结果稳定性? ————》 统计控制图

- 如果试验结果不能提供充足数据建立统计控制图：
  - 平行分析次数
  - 添加回收、线性关系

单一样本的测试 - II

- 建立 CD值：critical difference.

- CR＝f * σ * sqrt(2).

  – f (CR factor) depends on the probability level to be associated with the critical difference and on the shape of the distribution.

单一样本的测试 - III

- the repeatability limit $r=2.8\ \sigma_r$
- the reproducibility limit $R=2.8\ \sigma_R$.

For R and r, the probability level is 95% and we assume an approximately normal distribution.

  - Under these conditions, *f* is 1,96 and *f* sqrt2 is 2,77 (we use a rounded value of 2,8).

附 录 B
（资料性附录）
9种磺酰脲类除草剂精密度数据

表B 9种磺酰脲类除草剂精密度数据

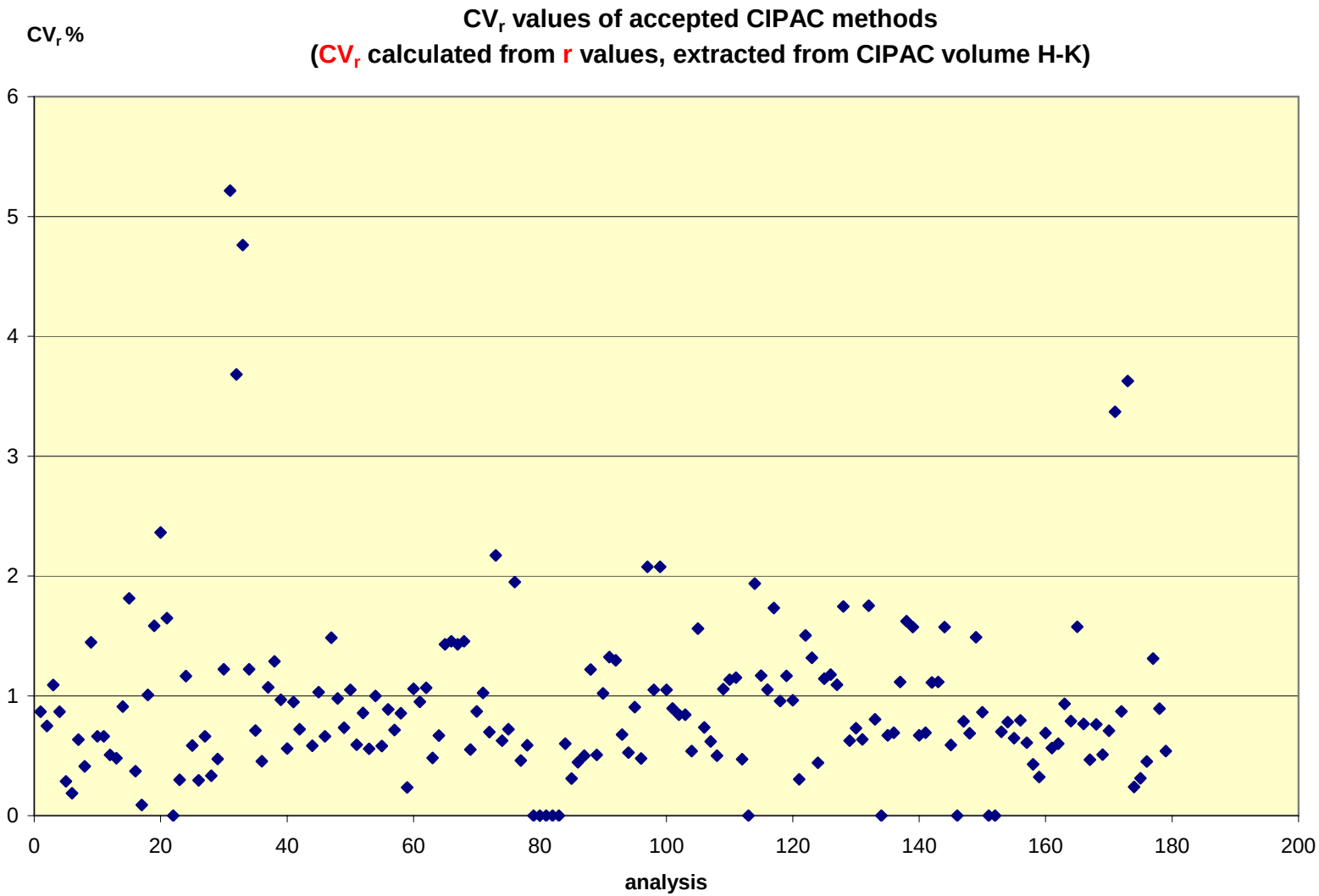
序号	农药名称	添加水平 mg/kg	重复性限 r	再现性限 R	添加水平 mg/kg	重复性限 r	再现性限 R
1	烟嘧磺隆	0.01	0.001 1	0.001 9	1	0.06	0.11
2	噻吩磺隆	0.01	0.000 9	0.001 2	1	0.10	0.15
3	甲磺隆	0.01	0.000 8	0.001 8	1	0.08	0.14
4	甲嘧磺隆	0.01	0.000 5	0.001 4	1	0.12	0.17
5	氯磺隆	0.01	0.001 1	0.001 1	1	0.12	0.14
6	胺苯磺隆	0.01	0.001 0	0.001 6	1	0.09	0.13

where:

f_i = response factor
f = average response factor
H_s = area of sulfotep peak in the calibration solution
H_w = area of sulfotep peak in the sample solution
I_r = area of internal standard peak in the calibration solution
I_q = area of internal standard peak in the sample solution
q = mass of internal standard in the sample solution
r = mass of internal standard in the calibration solution
s = mass of sulfotep in the calibration solution (g)
w_a = mass of the intact smoke tin (g)
w_b = mass of the empty smoke tin (g)
P = purity of sulfotep reference substance (g/kg)

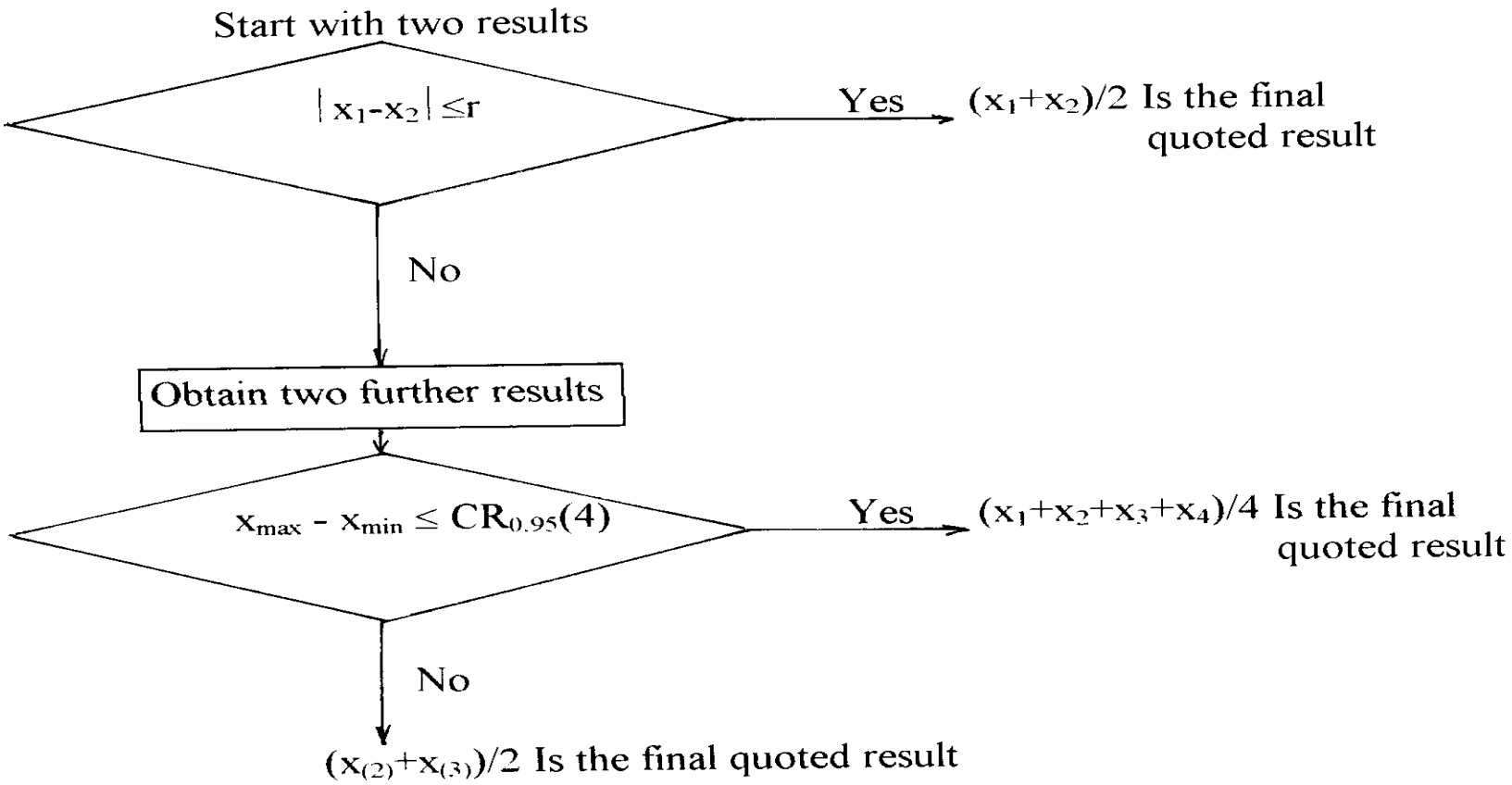
Repeatability r = 11 g/kg at 172 g/kg active ingredient content (small tins)
= 3 g/kg at 178 g/kg active ingredient content (large tins)

Reproducibility R = 28 g/kg at 172 g/kg active ingredient content (small tins)
= 10 g/kg at 178 g/kg active ingredient content (large tins)


CVr values of accepted CIPAC methods
(CVr calculated from r values, extracted from CIPAC volume H-K)
CVr %
6
5
4
3
2
1
0
0
20
40
60
80
100
120
140
160
180
200
analysis

测试结果的评价与表达

- σ_r and σ_R of the measurement method are known.



Where

$x_{(2)}$ is the second smallest result

$x_{(3)}$ is the third smallest result

例： 分析结果的有效性与表示

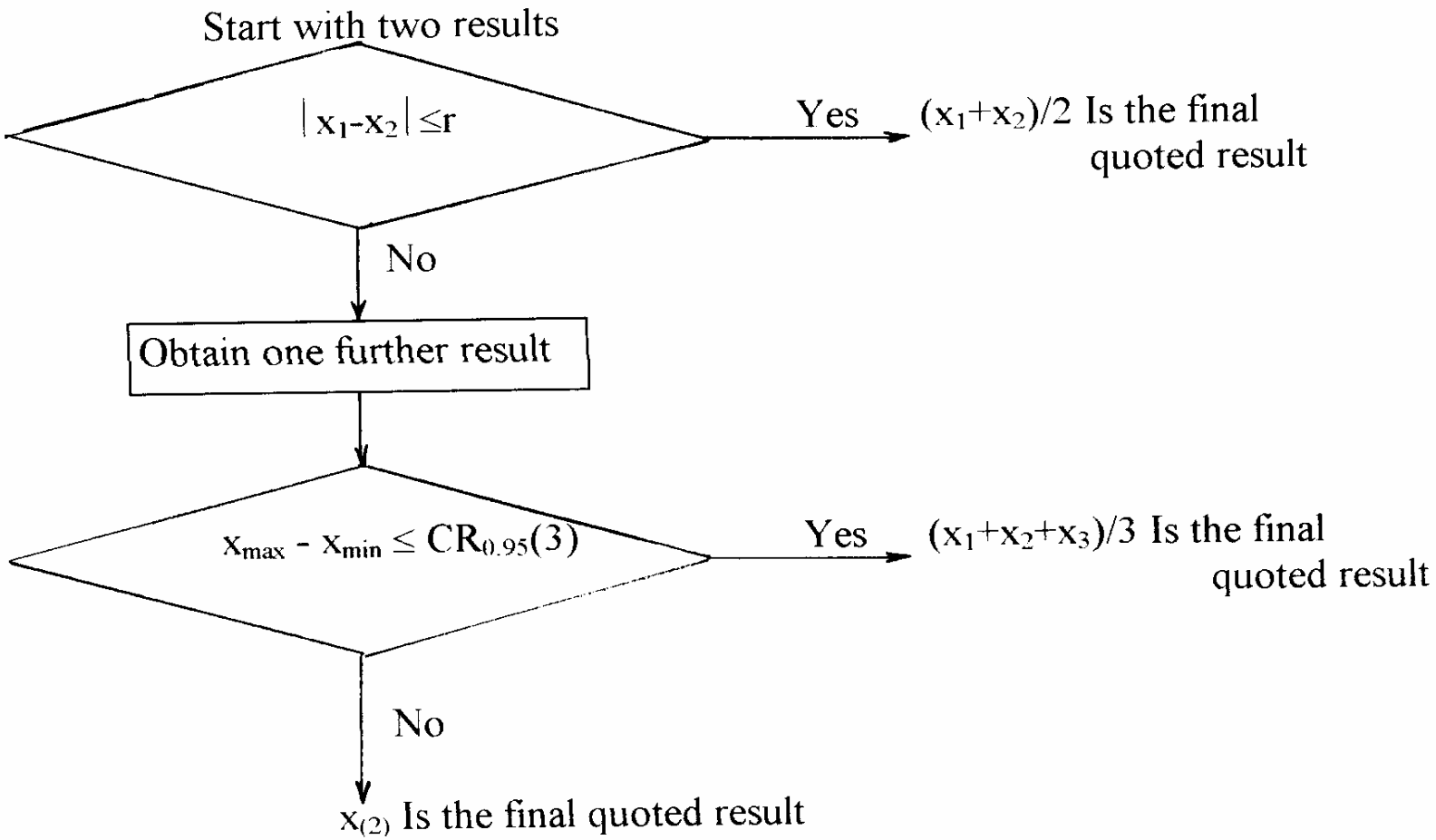
- 乐果乳油分析 (CIPAC E p.69).

- r = 7 g/kg at 463 g/kg a.i. content.
- R = 20 g/kg at 463 g/kg a.i. content (18 results)

- We obtain 2 test results: x_1= 451 and x_2= 457 g/kg
- $|x_1-x_2|$= 6 < r, so the final quoted result is

(451 +457)/2 = 454 g/kg.

当测定费时、花费较大时结果的评价



Where

$x_{(2)}$ is the second smallest result

例： 分析结果的有效性与表示（续）

- The next time we obtain: x_1= 453 and x_2=468 g/kg
- $|x_1-x_2|$= 15 > r. We shall obtain 2 further results.
- x_3= 460 and x_4= 450 g/kg,
  - the $CR_{0.95(4)} = f(4)\ \sigma_r$.
  - $r = 2.8\sigma_r$, σ_r = r/2.8 = 7/2.8 = 2.5 g/kg
- *f*(4) = 3.6, therefore $CR_{0.95(4)} = 3.6*2.5 = 9$

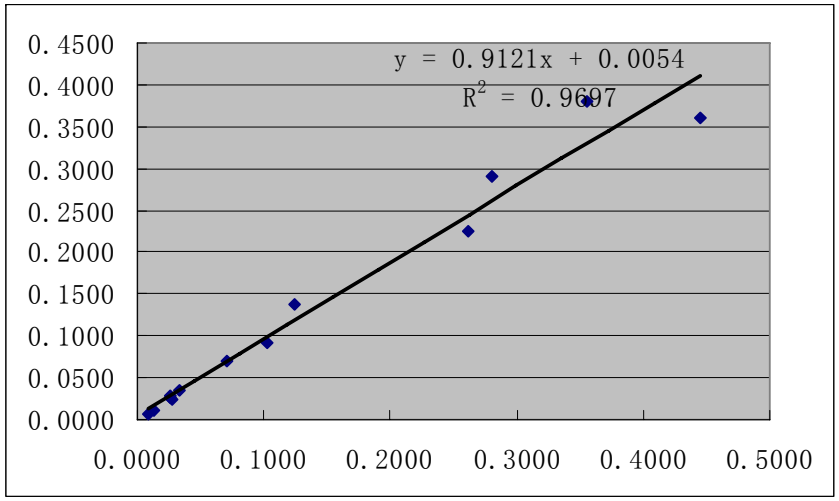
- We calculate $x_{max} - x_{min}$ = 468 -450 = 18 > 9
- In this case the median （中值） of the 4 results is reported as the final quoted result. 450, 453, 460, 468,
- so x = (453 + 460)/2 = 456 g/kg .

- 如果$x_{max} - x_{min} < CR$, 则结果应表示为四次测定的平均值。

方法 A与B的比较研究

	Method A			Method B or reference method			
	Replicate 1	Replicate 2	Average	Replicate 1	Replicate 2	Average	Difference
87	0.532	0.545	0.5385	0.518	0.524	0.521	0.0175
88	0.52	0.53	0.525	0.538	0.523	0.5305	-0.0055
89	0.535	0.531	0.533	0.527	0.519	0.523	0.01
90	0.517	0.526	0.5215	0.513	0.531	0.522	-0.0005
91	0.529	0.523	0.526	0.521	0.528	0.5245	0.0015

s_{Ara}	0.007447	Average	0.0046
		SD_{dif}	0.009127
		t_{calc}=	1.126991
		t_{crit}=	2.776



	Lab 1	Lab 2	R_1-R_2
1	0.0266	0.0259	0.0007
2	0.0256	0.0279	-0.0023
3	0.0710	0.0709	0.0001
4	0.0334	0.0352	-0.0018
5	0.0087	0.0062	0.0025
6	0.0123	0.0118	0.0005
7	0.0269	0.0249	0.0020
8	0.2810	0.2910	-0.0100
9	0.3550	0.3800	-0.0250
10	0.2610	0.2240	0.0370
11	0.1030	0.0920	0.0110
12	0.1240	0.1370	-0.0130
13	0.4450	0.3610	0.0840

分析同一样本的重复性考察

- 对同一样本的三个部位分析结果应符合:

  Cmax-Cmin< 3.31*r/2.8

每两个重复分析间至少满足：

Amax—Amin≤3.64*CV*Xmean

分析同一样本的三个部位的考察

RATIO of AS/IS	Content	Average	SD	CV
1.12616	39.35703	39.28324	0.10436	0.26565
1.12193	39.20945			
1.10382	38.39576	38.31078	0.12017	0.31366
1.09893	38.22581			
1.11266	38.75563	38.59627	0.22537	0.58391
1.10351	38.43691			
		Cmax-Cmin:	0.97245	
		3.31*r/2.8:	1.773214	
		r=1.5		

实验室内部两组结果的比较研究

- group 1 ⟶ n_1 results giving a mean of y_1
- group 2 ⟶ n_2 results giving a mean of y_2
- the SD of (y_1- y_2) is:

$$\sigma = \sqrt{\sigma_r^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

- and the critical difference for |y_1- y_2| at 95% probability level is:

$$CD = 2.8\sigma_r \sqrt{\frac{1}{2n_1} + \frac{1}{2n_2}}$$

两个实验室间对同一样本分析结果的比较:

- lab. 1 ⟶ n_1 results giving a mean of y_1
- lab. 2 ⟶ n_2 results giving a mean of y_2
- under repeatability conditions, the SD (y_1-y_2) is: $\sigma = \sqrt{\sigma_L^2 + \frac{1}{n_1}\sigma_r^2 + \sigma_L^2 + \frac{1}{n_2}\sigma_r^2}$

- and the critical difference for $|y_1-y_2|$ is:

$$CD = \sqrt{(2.8\sigma_R)^2 + (2.8\sigma_r)^2\left(1 - \frac{1}{2n_1} - \frac{1}{2n_2}\right)}$$

	Repeatability standard deviation S_r	Reproducibility standard deviation S_R	Between-laboratories variability S_L
Split levels (Youden pairs)	$\sqrt{\frac{\sum(D_i - \bar{D})^2}{2(p-1)}}$	$\sqrt{\frac{1}{p-1}\sum_i^p(\bar{x}_i - \bar{\bar{x}})^2 + \frac{S_r^2}{2}}$	$\sqrt{\frac{1}{p-1}\sum_i^p(\bar{x}_i - \bar{\bar{x}})^2 - \frac{S_r^2}{2}}$
Uniform-level	$\sqrt{\frac{\sum D_i^2}{2p}}$	$\sqrt{\frac{\sum(x_i - \bar{x})^2}{N-1}}$	Similar for both

D: Difference between the 2 values obtained in one laboratory
p: number of laboratories or differences.
N: number of data of all laboratories.
These formulae are valid for n = 2 replicates. If more are performed, please refer to the I

实例: A, B, C 三人在不同的天数对粉剂的五个部分进行分析， 结果如下：

A d1	B d1	C d1	A d2	B d2	C d2	A d3	C d3
0.43	0.51	0.5	0.51	0.49	0.47	0.49	0.52
0.52	0.5	0.48	0.5	0.48	0.49	0.55	0.52
0.5	0.53	0.48	0.48	0.51	0.51	0.54	0.5
0.49	0.5	0.52	0.49	0.47	0.54	0.44	0.49
0.51	0.52	0.46	0.56	0.49	0.5	0.51	0.5

- **1) A, B, C** 三个人实验的重复性**?**
- Sa,ν=12 0.037193 %； CV = 0.074189
- Sb,ν=8 0.013964 % CV = 0.027928
- Sc,ν=12 0.021370 % CV = 0.042854

- **2)** 如何评价**A B C** 三人的测定**?**

- **3)** 建立实验室内部重复性、再现性**?**
  Sr =？ SR＝？

$$S_r = \sqrt{\frac{\sum d^2}{2n}}$$

- **5) Are the Sr and SR values significantly different?**

	A d1	B d1	C d1	A d2	B d2	C d2	A d3	C d3
Ave	0.49	0.512	0.488	0.508	0.488	0.502	0.506	0.506
SD	0.035355	0.013038	0.022804	0.031145	0.014832	0.025884	0.043932	0.013416
Var	0.00125	0.00017	0.00052	0.00097	0.00022	0.00067	0.00193	0.00018

Q5: 检查异常数据: 统计分析前

The suspect population is the A d3 measurements:

Dixon test 0.55 0.54 0.51 0.49 0.44

$$r_{10} = (x_n - x_{n-1})/(x_n - x_1) \quad \text{or} \quad (x_2 - x_1)/(x_n - x_1)$$

$r_{10} = 0.454545$

$r_{10.\,0.05}$ 0.642 $r_{10} < r_{10,\,0.05}$ critical

The 0.44 % value is not an outlier

Q5: Outliner test:Grubb's test:

Grubb's test:

$$G'_{lowest} = (\bar{x} - x_1)/s \text{ or } C'_{highest} = (x_n - \bar{x})/s \qquad (3.5)$$

- If the test statistics G is $\leq G_{crit,0.05}$ (5% critical value) the item tested is accepted as correct.
- If $G_{crit,0.05} < G \leq G_{crit,0.01}$ the item is called straggler
- If $G > G_{crit,0.01}$ the item is a statistical outlier.

calculate *s* with all data points, see A11 for critical values

$G'_{lowest} = 1.502$,

$G'_{0.05} = 1.672$

The 0.44 % value is not an outlier

Table A.13

Q5: 三个人的分析水平是否相当？

Apply Cochran test to verify that the 8 sets of measurements may come from the same population:

$$g = \frac{S_{\max}^2}{\sum_{i=1}^{p} S_i^2} \qquad (3.8)$$

g = 0.00193/0.00591 = 0.326565

read critical value from table A13.1 at p=8 (number of data sets, and n=5 (number of replicate measurements): $g_{0.05} = 0.391$

0.391> 0.32

The measurements may come from the same population, that is there is no significant difference between the performance of analysts.

Note: for such comparison, that is comparing the results of a series of replicate measurements, the F-test cannot be applied!

Q5: 实验室内重复性

$$s_p = \sqrt{\frac{(s_1^2 x df_1) + (s_2^2 x df_2) + \ldots + (s_n^2 x df_n)}{df_1 + df_2 + \ldots + df_n}}$$

- The average repeatability standard deviations and corresponding coefficient of variations of the individual analysts (calculated from their measurements made on different days) are:
- SA, ν=12 0.037193 % CV = 0.074189
- SB, ν=8 0.013964 % CV = 0.027928
- SC, ν=12 0.021370 % CV = 0.042854

What is the within laboratory repeatability (Sr) of the method?

It is the average of the variations obtained by all analysts:

Pooled standard deviation

$$S_p = \sqrt{\frac{(s_1^2 x\, df_1) + (s_2^2 x\, df_2) + \ldots + (s_n^2 x\, df_n)}{df_1 + df_2 + \ldots + df_n}}$$

$$df_p = df_1 + df_2 + \ldots + df_n$$

The $df = \nu$ of each set of measurement in this case is 5-1=4. The $\nu_p = 8*4=32$!

$S_p = S_r = 0.02718$

What is the within laboratory reproducibility (SR) of the method?

$$s = \left\{ \frac{\sum_i \left(x_i - \bar{x}\right)^2}{n-1} \right\}^{\frac{1}{2}}$$

- The within laboratory reproducibility of the method is the SD of all measurements calculated with eq. 2.5: SR= 0.02632

- Note: Sr ≤ SR!

Q5: 评价室内重复性和室间再现性

Apply F-test to decide (if it is not obvious):

F= 1.0672

Apply two sided test at P = 0.95, read F_{crit} at

$F_{32/39} \sim F_{30/40} = 1.74$ $F_{40/40} = 1.69$

P = 0.9, read F_{crit} at

$F_{32/39} \sim F_{30/40} = 1.54$ $F_{40/40} = 1.51$

The difference is not significant!

样本是否均匀？

OR are the mean values obtained significantly different?

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
A d1	5	2.45	0.49	0.00125
B d1	5	2.56	0.512	0.00017
C d1	5	2.44	0.488	0.00052
A d2	5	2.54	0.508	0.00097
B d2	5	2.44	0.488	0.00022
C d2	5	2.51	0.502	0.00067
A d3	5	2.53	0.506	0.00193
C d3	5	2.53	0.506	0.00018

ANOVA

Source of Variation	*SS*[1]	*df*	*MS*[2]	*F*[3]	*P-value*[4]	*F crit*
Between Groups	0.00336	7	0.00048	0.649746	0.711786	2.312738
Within Groups	0.02364	32	0.000739			
Total	0.027	39				

问题与交流讨论

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Science net 博客：http://www.sciencenet.cn/blog/canpingp2222.htm