Again,
The Importance of **Validation**
To Deliver **Reliable** Data

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WHAT IS THE ROLE OF AN ANALYTICAL CHEMIST?

Chemicals make up everything we use or consume

- What is is (qualitative analysis)
- How much it is (quantitative analysis)
- Structure

To deliver reliable data

Quality of manufactured products depends on proper chemical proportions and measurement of the constituents

Quantity determination has big impact on ECONOMICS

QUALITY CONTROL

WHAT DO ANALYTICAL CHEMISTS DO?

Analytical chemists work to improve the reliability of existing techniques

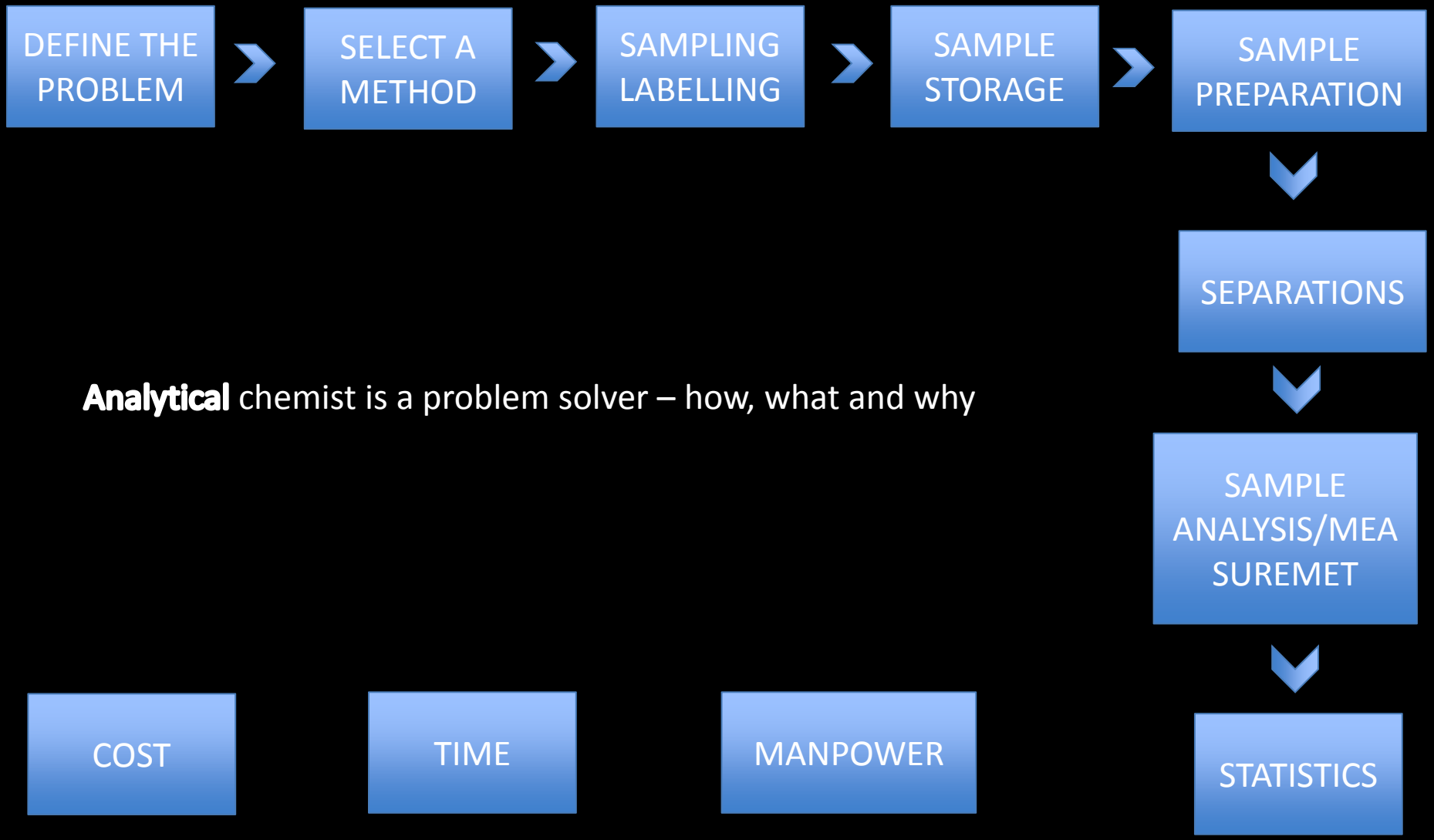
Analytical measurements are always improving

New research to discover new principles and utilization of discoveries

- Food
- Forensic
- Medecine
- Industry
- Environment

- Metabolomics
- Proteomics
- Lipidomics

THE ANALYTICAL WORKFLOW



Analytical chemist is a problem solver – how, what and why

SAMPLING – a major difficulty

An analysis is usually performed on a very small sample – mg, couple of g
How stable is the analyte/s?

Solid
Liquid
gas

Homogeneous
heterogeneous

Solid
Liquid
gas

Representative sample

MINIMIZE
Contamination
Loss
Decomposition
Matrix change

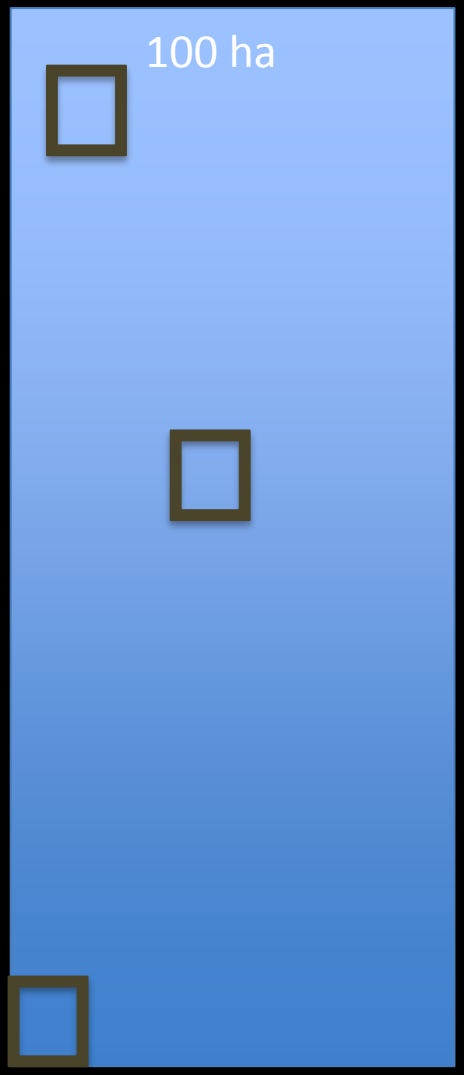
Adding
conservants
Protectants
Inert atmosphere

gross sample

Laboratory sample

analytical sample

SAMPLING EXAMPLE



Material:
Square A: 23 plants, 68 tubers, 8-45 g
Square B: 18 plants, 76 tubers, 13-60 g
Square C: 30 plants, 51 tubers, 22-58 g

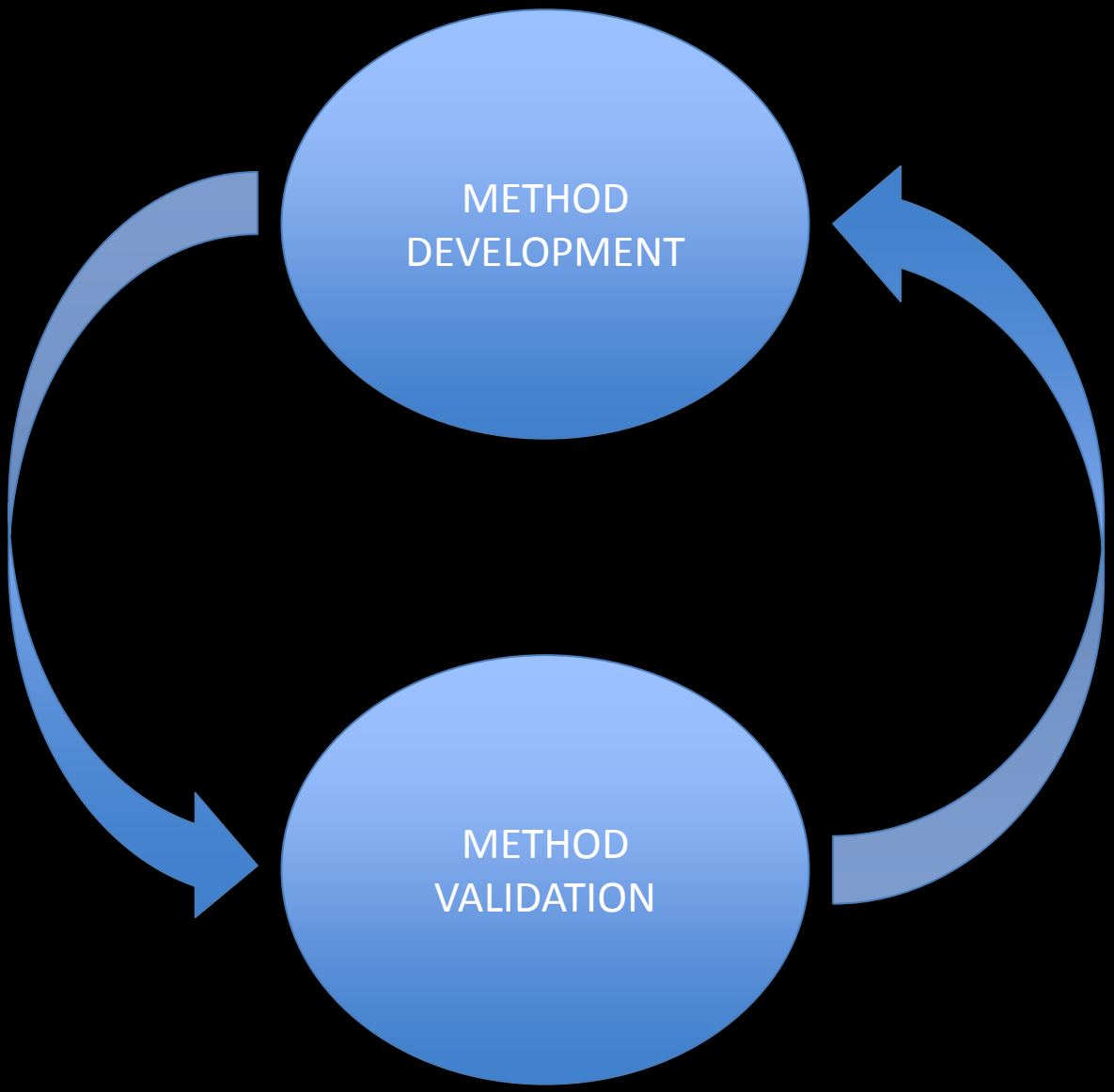
Analytical sample size: 50 mg

Results:
Square A: 578 - 3,654 mg/100g
Square B: 86 – 5,876 mg/100g
Square C: 1,956 – 4,347 mg/100 g

Median: 2,854 mg/100g
Standard deviation: 1,145 mg/100g

Random picks:
985,2
4111,9
2974,3
2198,4
3854,9

METHOD DEVELOPMENT & VALIDATION



Develop a method which meets desired parameters

ISO 17025

PROVE the method works and delivers the required results

ANALYTICAL RESULTS

All results must be **RELIABLE** because wide-impact decision might be made upon them with great economic/legal/scientific consequences

Quality assurance in the analytical lab

Every measurement has some imprecision associated with it

Good laboratory practice (GLP)

Every method should have as narrow as possible the random and systematic errors

MAIN VALIDATION PARAMETERS

TRUENESS / ACCURACY
REPEATABILITY
REPRODUCIBILITY
INTERMEDIATE PRECISION
LINEARITY / CALIBRATION
SELECTIVITY
RECOVERY
LIMIT OF DETECTION
LIMIT OF QUANTITATION
ROBUSTNESS
RUGGEDNESS
RANGE
SENSITIVITY
STABILITY
LIMIT OF DETECTION
SPECIFICITY
UNCERTAINTY

EXAMPLE: VALIDATION PROTOCOL

Analyze one analyte in one matrix on different days using at least two operators

		matrix A									
		matrix blank		matrix		matrix + 25 % spike		matrix + 75 % spike		matrix + 125 % spike	
day 1	operator A										
day 2	operator A										
day 3	operator A										
day 4	operator A										
day 5	operator A										
day 6	operator B										
day 7	operator B										
day 8	operator B										
day 9	operator B										
day 10	operator B										

Best: use isotopically labelled standards and also certified reference materials or at least in-house reference material

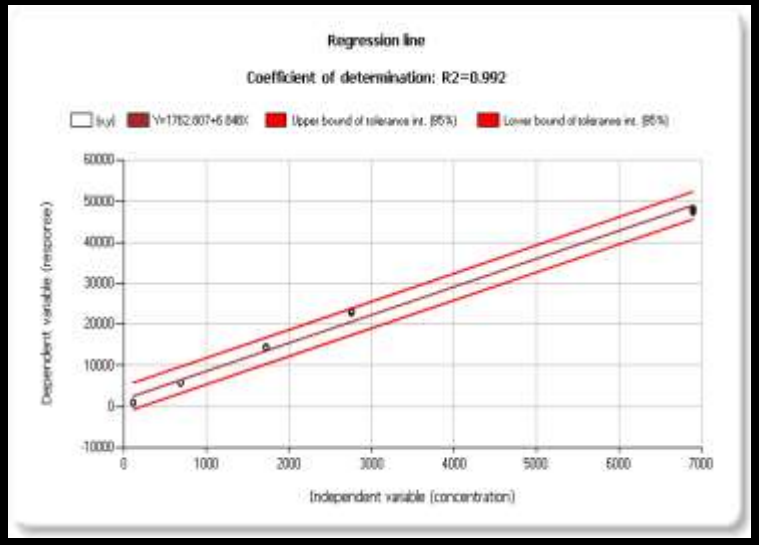
EXAMPLE – Validation report (part 1)

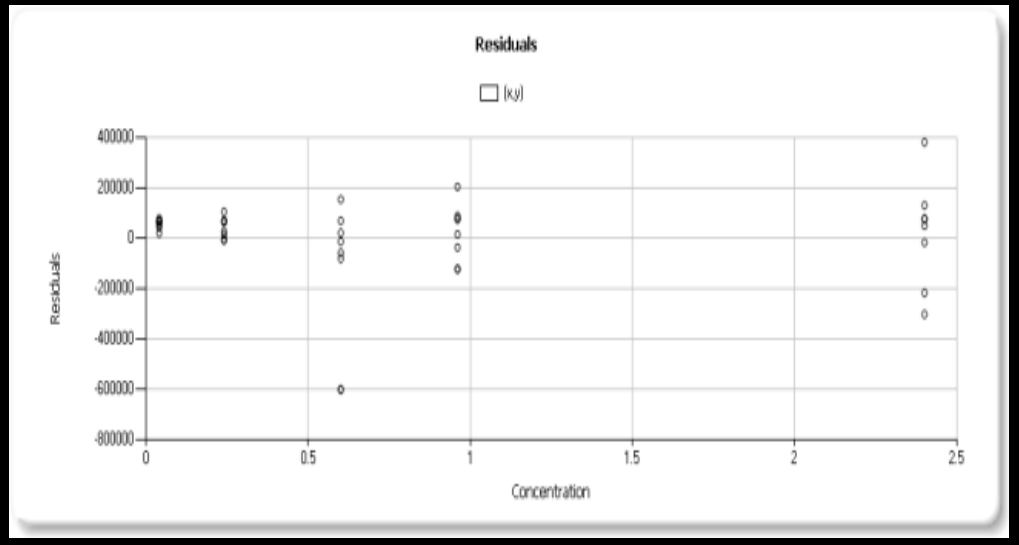
Working range

Matrix	Analyte [unit]	Working range*	
		Min	Max
A	Vitamin A (all-*trans* + 13-*cis*) [RE/100g]	1060	2130
	Vitamin E (α-tocopherol) [TE/100g]	11	20
B	Vitamin A (all-*trans* + 13-*cis*) [RE/100g]	820	1720
	Vitamin E (α-tocopherol) [TE/100g]	1.6	3.0
C	Vitamin A (all-*trans* + 13-*cis*) [RE/100g]	890	1980
	Vitamin E (α-tocopherol) [TE/100g]	8.7	17.5
D	Vitamin A (all-*trans* + 13-*cis*) [RE/100g]	1200	2100
	Vitamin E (α-tocopherol) [TE/100g]	2.9	5.9

Calibration / Linearity

Analyte	Unit	Concentration range		Slope		Intercept		Coefficient of determination R^2	Standard deviation of residuals
		Min	Max	Central value	Slope=0 ? (Y/N)	Central value	Intercept=0 ? (Y/N)		
Vitamin A	RE/100g	114	6896	6.848	N	1762	N	0.992	1531.7
Vitamin E	TE/100g	0.53	32	87650	N	103975	N	0.991	97874





EXAMPLE – Validation report (part 2)

Matrix A

Analyte	Sample	Unit	Number of days * number of replicates	Median	Repeatability				Intermediate reproducibility			
					SD(r)	CV(r) [%]	r	r% [%]	SD(iR)	CV(iR) [%]	iR	iR% [%]
Vitamin A	Unspiked	RE/100g	8/2	1063.68	23.37	2.2	64.78	6.1	31.12	2.9	86.25	8.1
	Spiked		8/2	2127.71	16.78	0.8	46.51	2.2	59.22	2.8	164.16	7.7
Vitamin E	Unspiked	TE/100g	8/2	11.53	1.04	9.0	2.88	25.0	1.44	12.5	3.98	34.5
	Spiked		8/2	20.27	0.47	2.3	1.31	6.5	1.48	7.3	4.09	20.2

Trueness / Recovery

Analyte	Sample	Unit	Number of days	Ref. value	Uncertainty of ref. value	Median of results	Rec [%]	SD(Rec)	Rec=100 % ? (Y/N)	SD(Rec) corrected
Vitamin A	Spiked at 100 % intrinsic level	RE/100g	8	1027.82	negligible	1051.96	102.3	0.010	Y	0.010
Vitamin E	Spiked at 100 % intrinsic level	TE/100g	8	9.597	negligible	8.67	90.3	0.021	N	0.053

Uncertainty

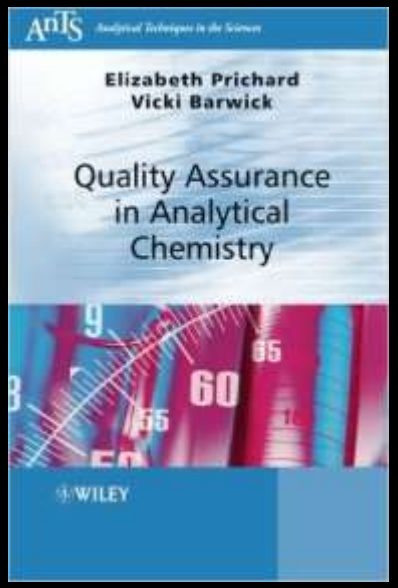
Analyte	Sample	Unit	Median	CV(iR) [%]	RSD(Rec) corrected [%]	Standard uncertainty		Expanded uncertainty	
						u	Relative u [%]	U	Relative U [%]
Vitamin A	Spiked at 100 % intrinsic level	RE/100g	1051.96	3.0	1.02	32.93	3.1	65.86	6.3
Vitamin E	Spiked at 100 % intrinsic level	TE/100g	8.67	8.3	5.88	0.88	10.2	1.76	20.4

EXAMPLE – Validation report (part 3)

Fitness-for-purpose

Performance characteristic	Analyte	Target value	Measured value	Target achieved
LOD-LOQ	Vitamin A	≤10 μg RE/100g ≤ 0.02 mg TE/100g	5.15 μg RE/100g 0.02 mg TE/100g	Y
	Vitamin E	CVr [%] <10% for dry food CVr [%] <15% for dry food	≤9% ≤14.2%	Y Y
Repeatability	Vitamin A	CVr [%] <5%	≤2.2%	Y
	Vitamin E	CVr [%] <10% for dry food CVr [%] <15% for dry food	≤9% ≤14.2%	Y Y
Intermediate Reproducibility	Vitamin A	iR[%] <15%	≤10.5%	Y
	Vitamin E	iR [%] <35% for dry food iR [%] <60% for dry food	≤34.5% ≤60.7%*	Y Y
Trueness/Recovery	Vitamin A	rec [%] between 80 to 110%	99.5%≤rec≤104%	Y
	Vitamin E	rec [%] between 80 to 110%	84%≤rec≤90.4%	Y

BOOK RESOURCES 1



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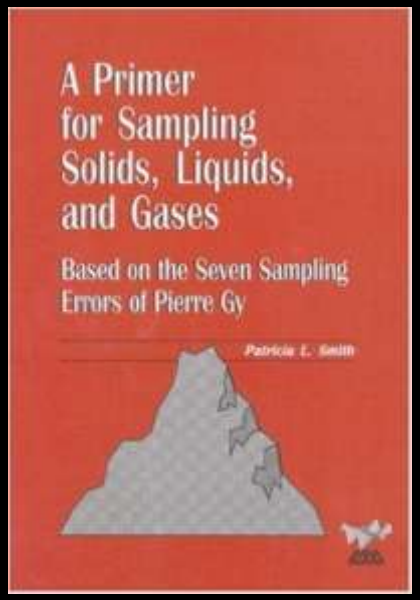
BOOK RESOURCES 2



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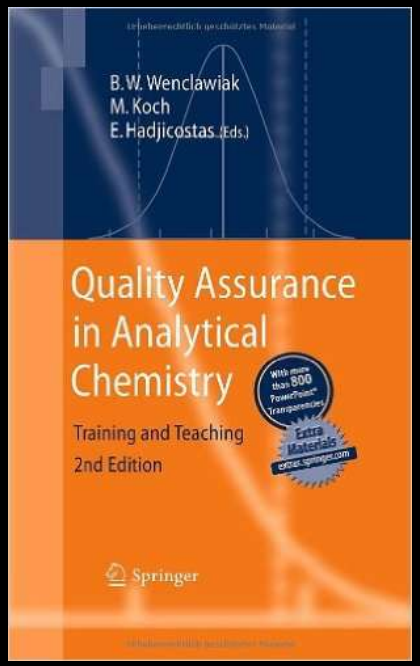

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