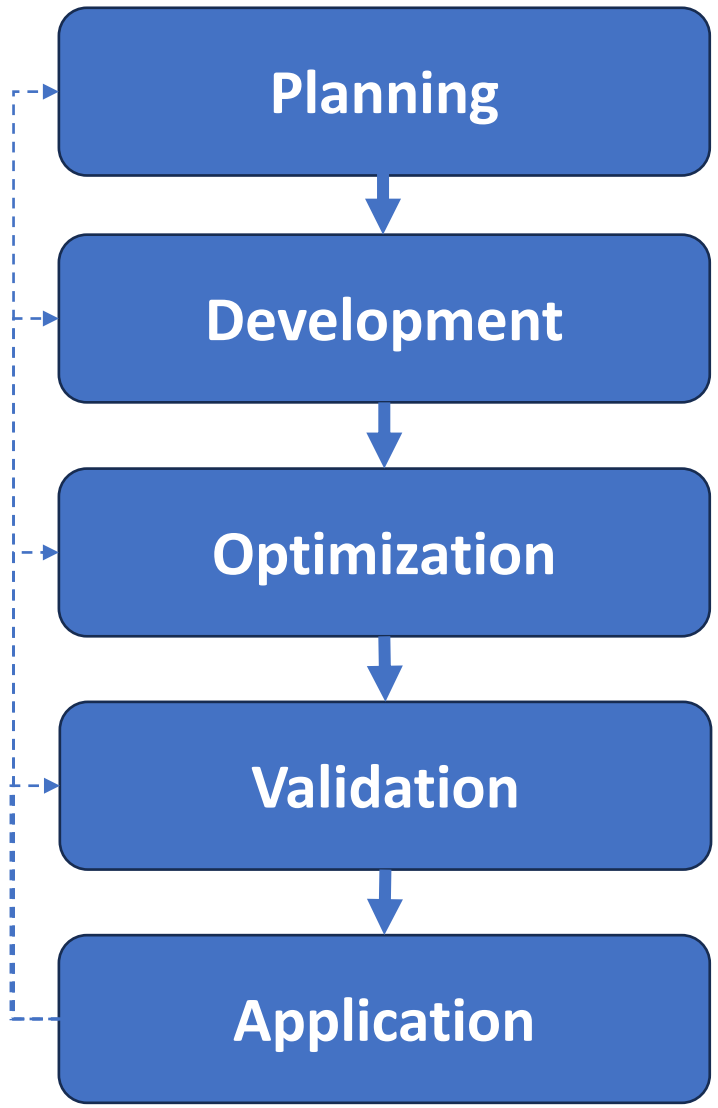
Validation of HS-SPME-GC-MS/MS methods for water T&O

Tri Kaloudis
Laboratory of Organic Micropollutants, Water Quality Dept., EYDAP SA, Greece
Institute of Nanoscience & Nanotechnology, NCSR Demokritos, Greece

WaterTOP Training School "Microextraction in T&O analysis: fundamentals and applications
TUC, Chania, Greece, 20-22/09/2023

LABORATORY OF ORGANIC MICROPOLLUTANTS
EYDAP
DEMOKRITOS NATIONAL CENTER FOR SCIENTIFIC RESEARCH
WaterTOP COST ACTION CA18225
cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
Funded by the European Union
LEAD LABORATORY OF ENVIRONMENTAL ANALYSIS NCSR DEMOKRITOS

Analytical methods: from planning to application



Requirements,
Resources

Setup, preliminary results

Design of experiments,
optimized conditions

Performance evaluation

Quality control

Planning: requirements of water T&O analytical methods

Consumer requirements (EU DWD, National Water Authorities):

- Method can detect/quantify T&O below the Odor Threshold Concentration (OTC).
- Reasonable accuracy and precision (e.g., <25% RSD and bias).
- Reasonable measurement uncertainty (e.g., <50% Uexp).

Laboratory requirements:

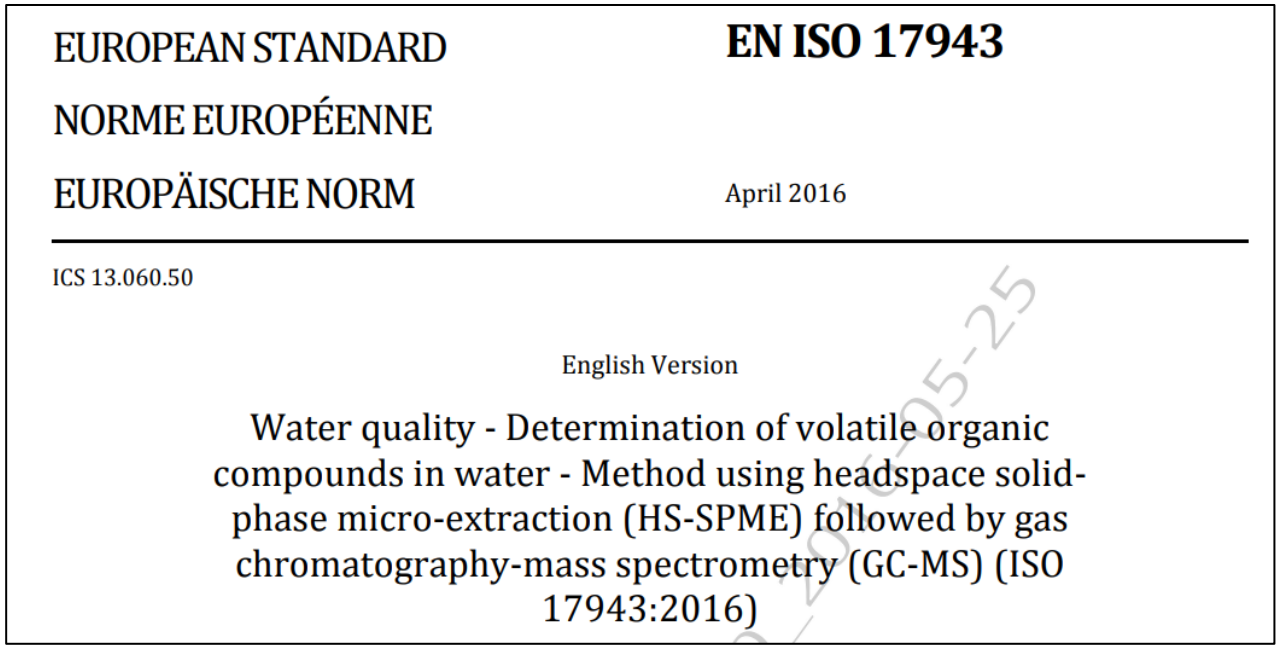
- Simple procedure.
- Low level of analytical skills-expertise.
- Low equipment and operational costs.
- Fast.
- High throughput.

Method development example: SPME-GC-MS for geosmin & MIB

- Sample volume 10ml (20ml SPME vials)
- NaCl (salting out)
- HS-SPME (50/30 µm DVB/CAR/PDMS, Stableflex)
- Automated SPME (CombiPAL)
- GC-MS and GC-MS/MS alternatives
- Non-polar and semi-polar columns.
- Run-time 35min including SPME (time-saver).

Extraction temperature	**60 ºC**
Equilibration time	10 min
Extraction time	10 min
Agitation	300 rpm
Fiber position	10mm, "fiber depth from bottom"

Example SPME conditions



EUROPEAN STANDARD **EN ISO 17943**

NORME EUROPÉENNE

EUROPÄISCHE NORM April 2016

ICS 13.060.50

English Version

Water quality - Determination of volatile organic compounds in water - Method using headspace solid-phase micro-extraction (HS-SPME) followed by gas chromatography-mass spectrometry (GC-MS) (ISO 17943:2016)

Based on EN ISO 17093, modified (GC-MS/MS)

LABORATORY OF ORGANIC MICROPOLLUTANTS

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Method development example: SPME-GC-MS for geosmin & MIB

Parameter	Setting
Injection	Spitless (2 min), 1µl
Desorption temp.	250 °C (injector temp)
Fiber cleaning	In injector, split, 2-10min
Column flow	Constant flow, 1 mL/min He
Col. oven equil. time	5 min
Temp. gradient	50 °C (hold 1 min) → 250 °C- 15 °C/min (hold 6 min) Total 19.33min

Example GC conditions

Compound	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	CE (collision energy-ev)
Geosmin	9.81	95	67*	10.0
		95	55	10.0
		95	93	10.0
MIB	7.64	112	97*	10.0
		112	69	10.0
		112	83	10.0
	*Quantitation ion			

Example MS/MS conditions

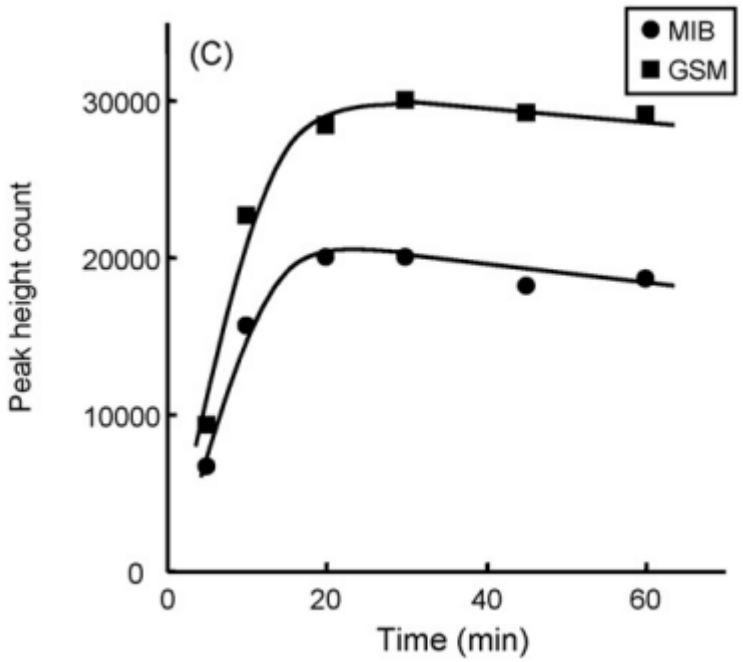
SPME optimization

SPME parameters may need to be optimized, e.g., equilibration/extraction temperature/time, NaCl, agitation etc.

1. Define the **“Response”** to be optimized, e.g., peak area, peak height, recovery, reproducibility etc…
2. Define the **“Factors”** (parameters) to be studied.
3. Evaluate the **effects** of factors on the response.
4. Identify **significant** factors.
5. Develop a **model** that predicts the response from significant factors.
6. Determine the values of significant factors for **optimal** response.
7. **Verify** the optimum conditions.

Optimization strategies

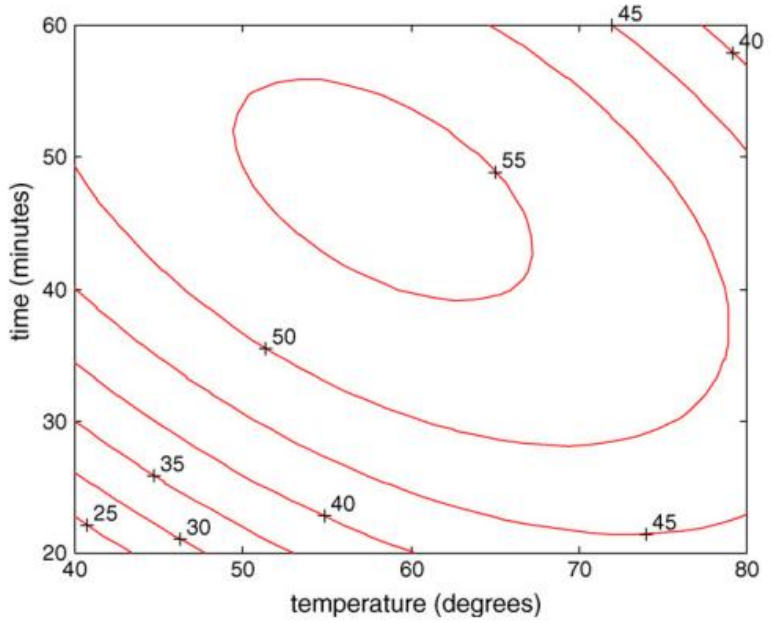
One factor at a time (OFAT)



Saito et al., J. Chromat. A., 2008

Other factors are set at set at pre-defined values.
Cannot detect interaction effects.
Large number of experiments.

Design of Experiments (DoE)



Leardi et al., Analytica Chimica Acta 2009

DoE: "A branch of applied statistics that deals with planning, conducting, analyzing, and interpreting controlled tests to evaluate the factors that control the value of a parameter or group of parameters." *ASQ*

Models the whole experimental domain.
Can detect interaction effects.
Small number of experiments.

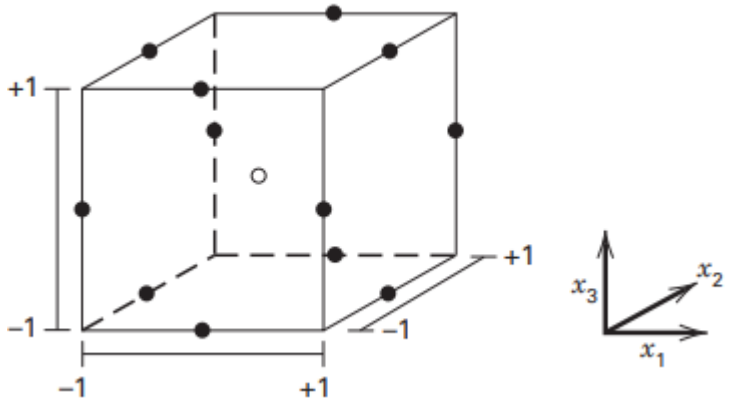
Response surface designs: BB and CCD

A Three-Variable Box–Behnken Design

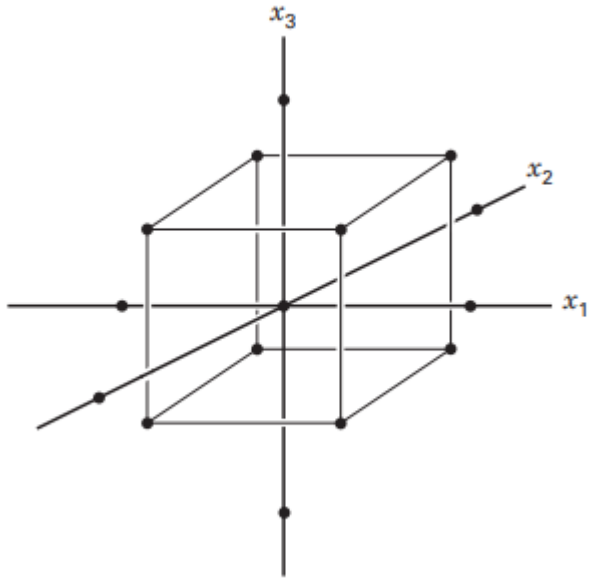
Run	x_1	x_2	x_3
1	−1	−1	0
2	−1	1	0
3	1	−1	0
4	1	1	0
5	−1	0	−1
6	−1	0	1
7	1	0	−1
8	1	0	1
9	0	−1	−1
10	0	−1	1
11	0	1	−1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

Example of a 3 factor BB design

Montgomery, Design and analysis of experiments 9th Ed. 2017



BB3



CCD3

Available Response Surface Designs

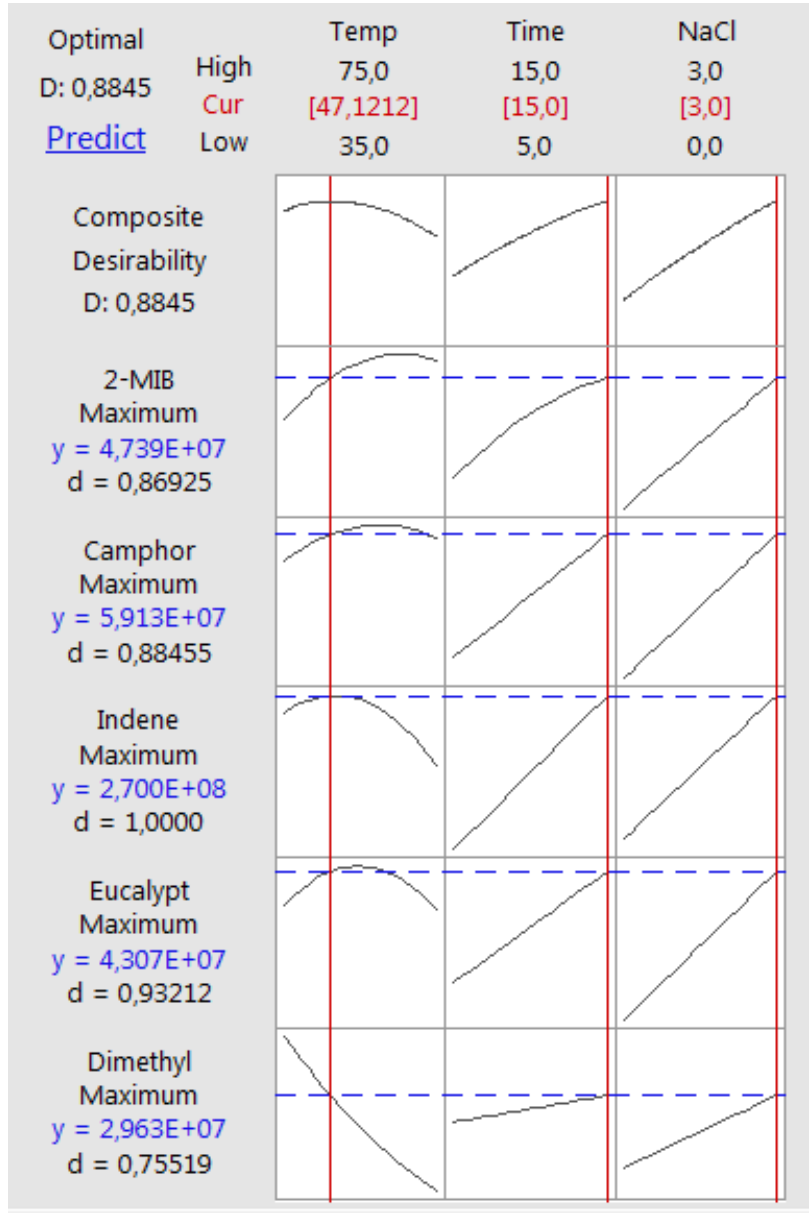
Design		Continuous Factors								
		2	3	4	5	6	7	8	9	10
Central composite full	unblocked	13	20	31	52	90	152			
	blocked	14	20	30	54	90	160			
Central composite half	unblocked				32	53	88	154		
	blocked				33	54	90	160		
Central composite quarter	unblocked							90	156	
	blocked							90	160	
Central composite eighth	unblocked									158
	blocked									160
Box-Behnken	unblocked		15	27	46	54	62		130	170
	blocked			27	46	54	62		130	170

BB and CCD designs (Minitab)

Multiple response optimization based on desirability functions

Std Order	Run Order	PtType	Extr. Temp. (°C)	Extr. Time (min)	NaCl g/10ml
8	1	2	75	10	3
10	2	2	55	15	0
11	3	2	55	5	3
15	4	0	55	10	1,5
1	5	2	35	5	1,5
4	6	2	75	15	1,5
2	7	2	75	5	1,5
3	8	2	35	15	1,5
14	9	0	55	10	1,5
5	10	2	35	10	0
12	11	2	55	15	3
9	12	2	55	5	0
13	13	0	55	10	1,5
7	14	2	35	10	3
6	15	2	75	10	0

BB3 design for optimization of peak area of 21 T&O by HS-SPME-GC/MS



Avagianos et al., Proceedings CEST 2017

Validation

Validation: provision of objective evidence that a given item fulfils **specified requirements**, where the specified requirements are adequate for an **intended use** (VIM 3).

Specified requirements: Customers, end-users, guidelines, regulations.

Example of specified requirements for the determination of geosmin

Performance parameter	Expressed as	Requirement
Precision	$\%RSD_r$ $\%RSD_R$	<25 <25
Accuracy	% mean recovery	75-125
LOQ	Minimum validated level (ng/L)	<3 ng/L
LOD	LOD, S/N>3 (RMS)	<3 ng/L
Uncertainty	Uexp, k=2	<50%
Validation levels: LOQ, 10ng/L		

Validation plan (protocol)

Day:	1	2	3
ID	**Thanos**	**Alina**	**Tri**
1A	1 ng/L	1 ng/L	1 ng/L
2A	1 ng/L	1 ng/L	1 ng/L
3A	1 ng/L	1 ng/L	1 ng/L
4A	1 ng/L	1 ng/L	1 ng/L
5A	1 ng/L	1 ng/L	1 ng/L
6A	1 ng/L	1 ng/L	1 ng/L
1B	10 ng/L	10 ng/L	10 ng/L
2B	10 ng/L	10 ng/L	10 ng/L
3B	10 ng/L	10 ng/L	10 ng/L
4B	10 ng/L	10 ng/L	10 ng/L
5B	10 ng/L	10 ng/L	10 ng/L
6B	10 ng/L	10 ng/L	10 ng/L

Determination of geosmin (HS-SPME-GC-MS/MS)

- Samples: Bottled water, e.g., Zagori LOT number xxxxx
- Spike with geosmin CRM 10 µg/L, LOT number xxxx
- Validation levels: 1ng/L (A) and 10ng/L (B)
- Use the Lab's Standard Operating Procedure

Estimates per spike level:

- %RSDr : Within-day precision (repeatability, pooled estimate)
- $\%RSD_R$: Overall precision (all days)
- % mean Recovery: all days
- LOQ/LOD, needed (<1 ng/L) : Repeat at LOQ/LOD level.

All raw and processed data should be kept in records and be traceable.

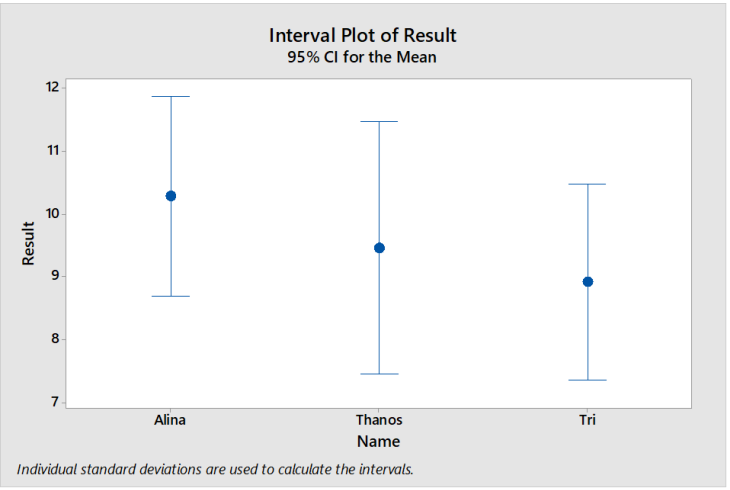
Interpretation of results

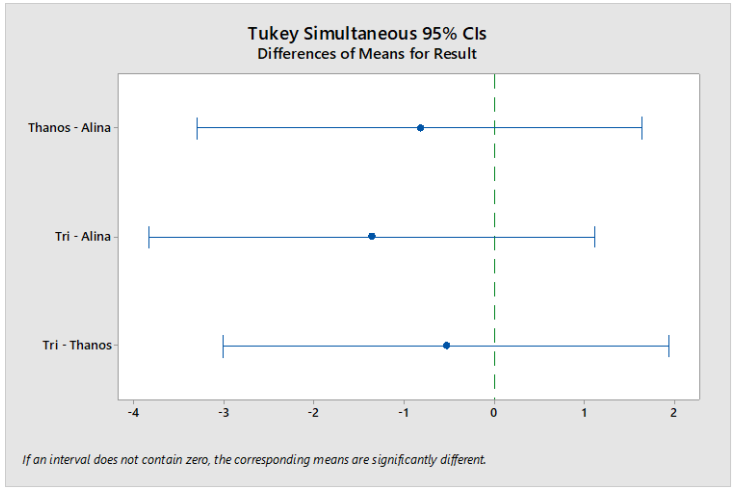
Spike level = 10ng/L

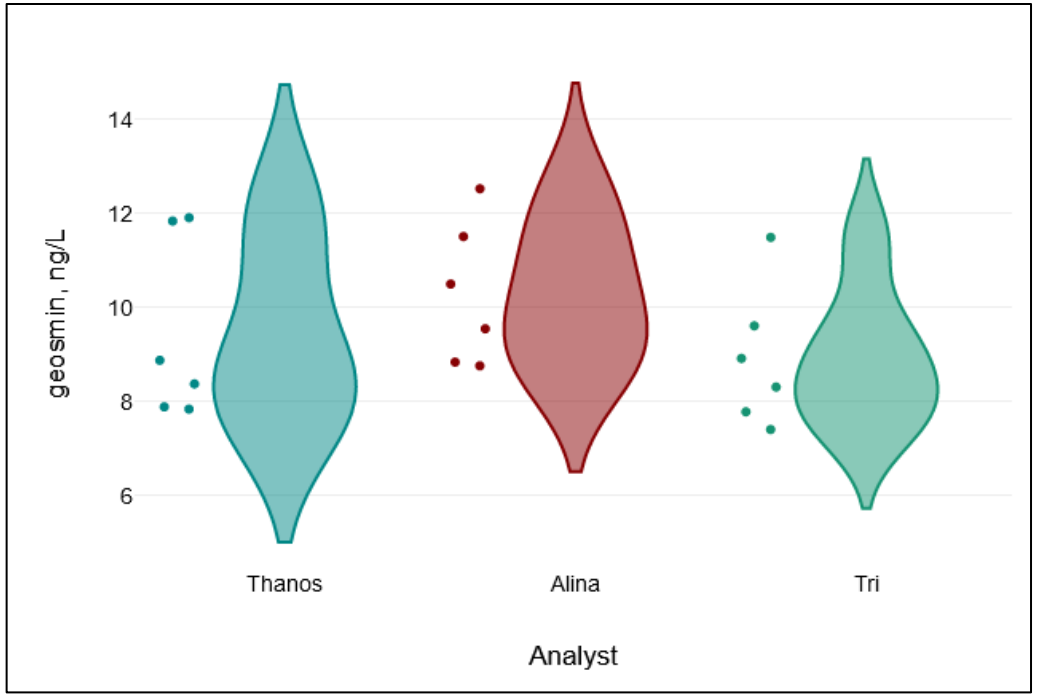
Variable	Name	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Result	Alina	6	0	10.282	0.619	1.517	8.764	8.823	10.025	11.764	12.525
	Thanos	6	0	9.458	0.780	1.909	7.844	7.880	8.631	11.857	11.910
	Tri	6	0	8.923	0.606	1.485	7.411	7.693	8.616	10.083	11.491

Simulated data

Question: Is Tri an outlier?

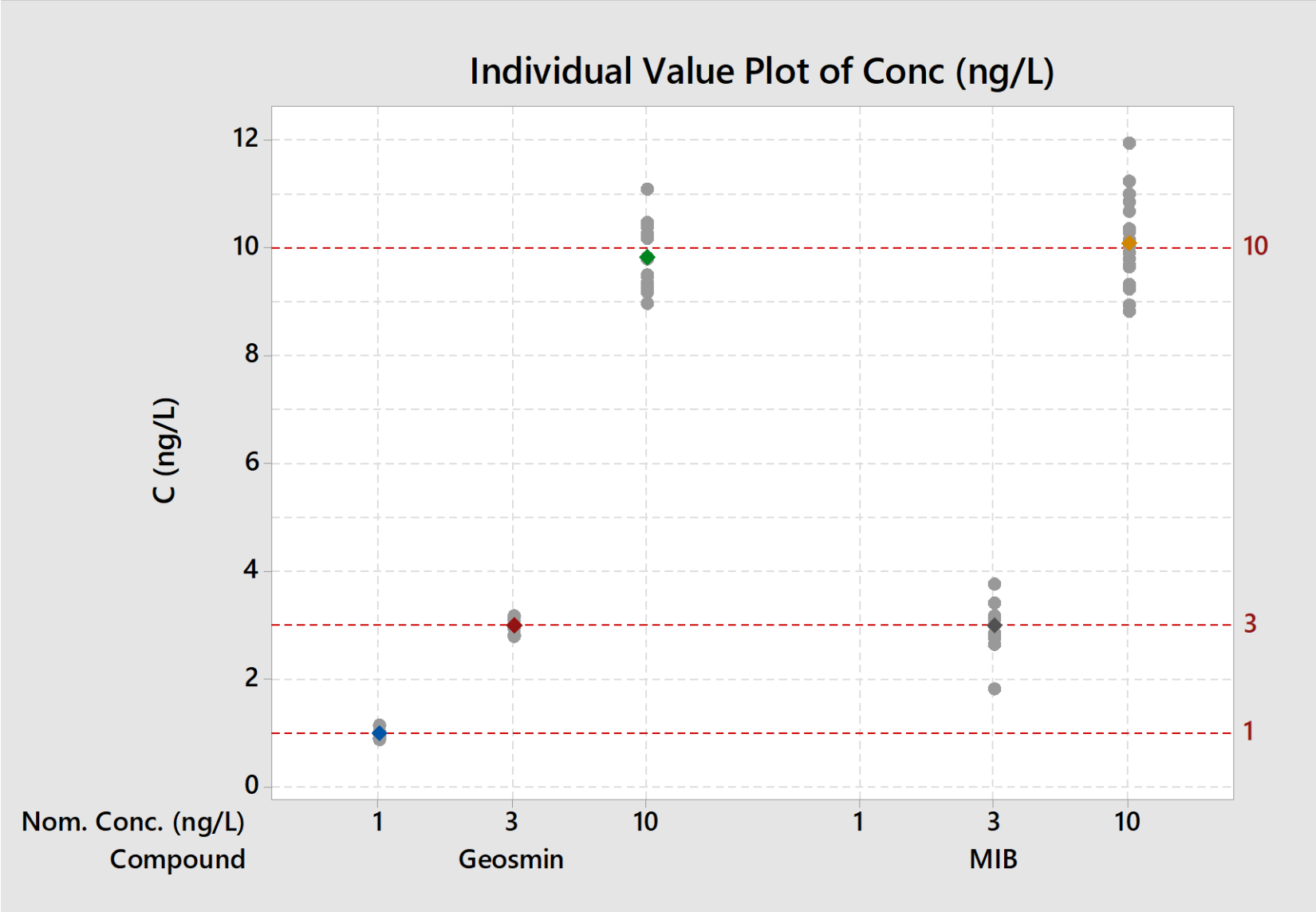




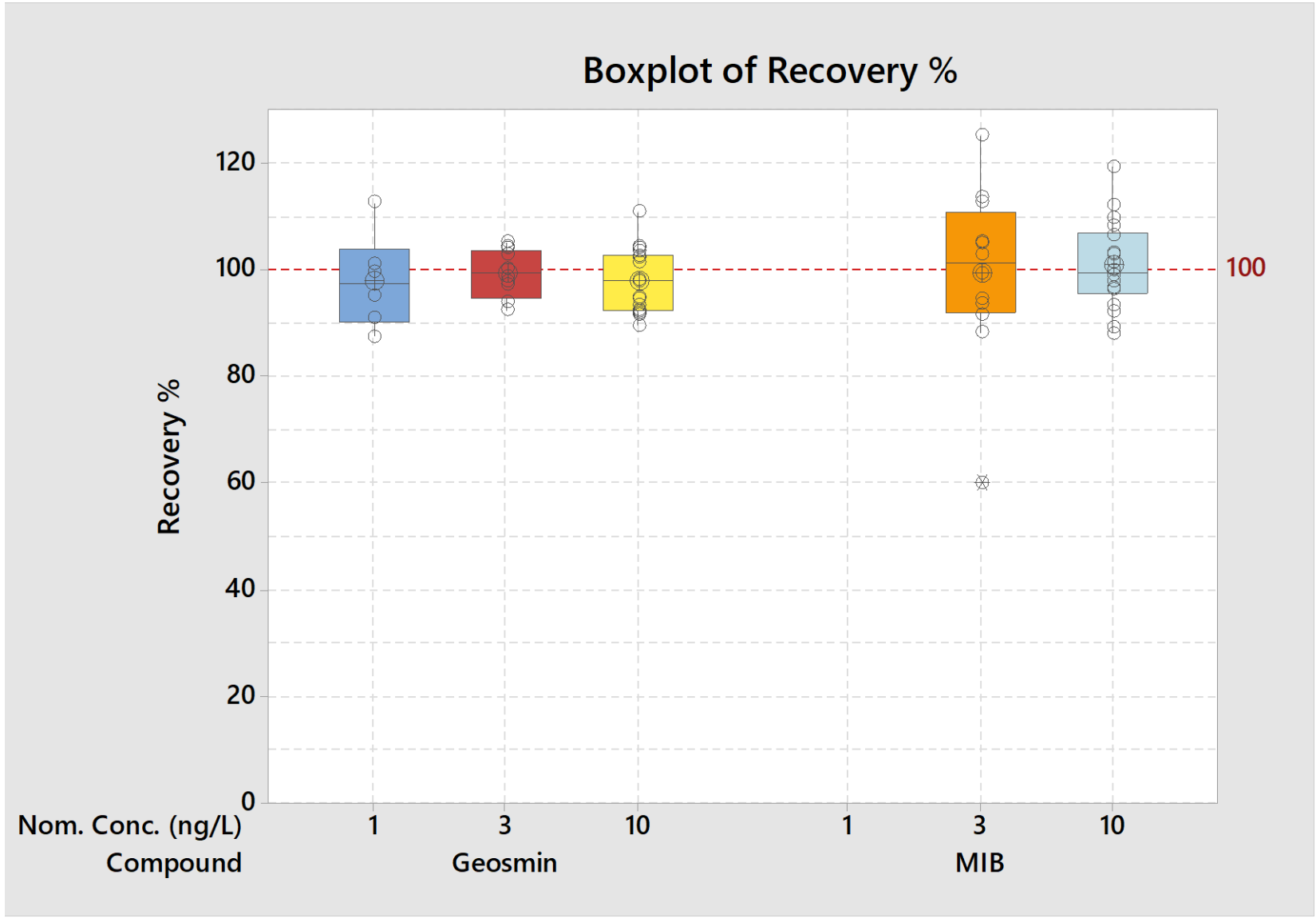


Visualize data. Check for statistical significance.

Data visualization – individual values



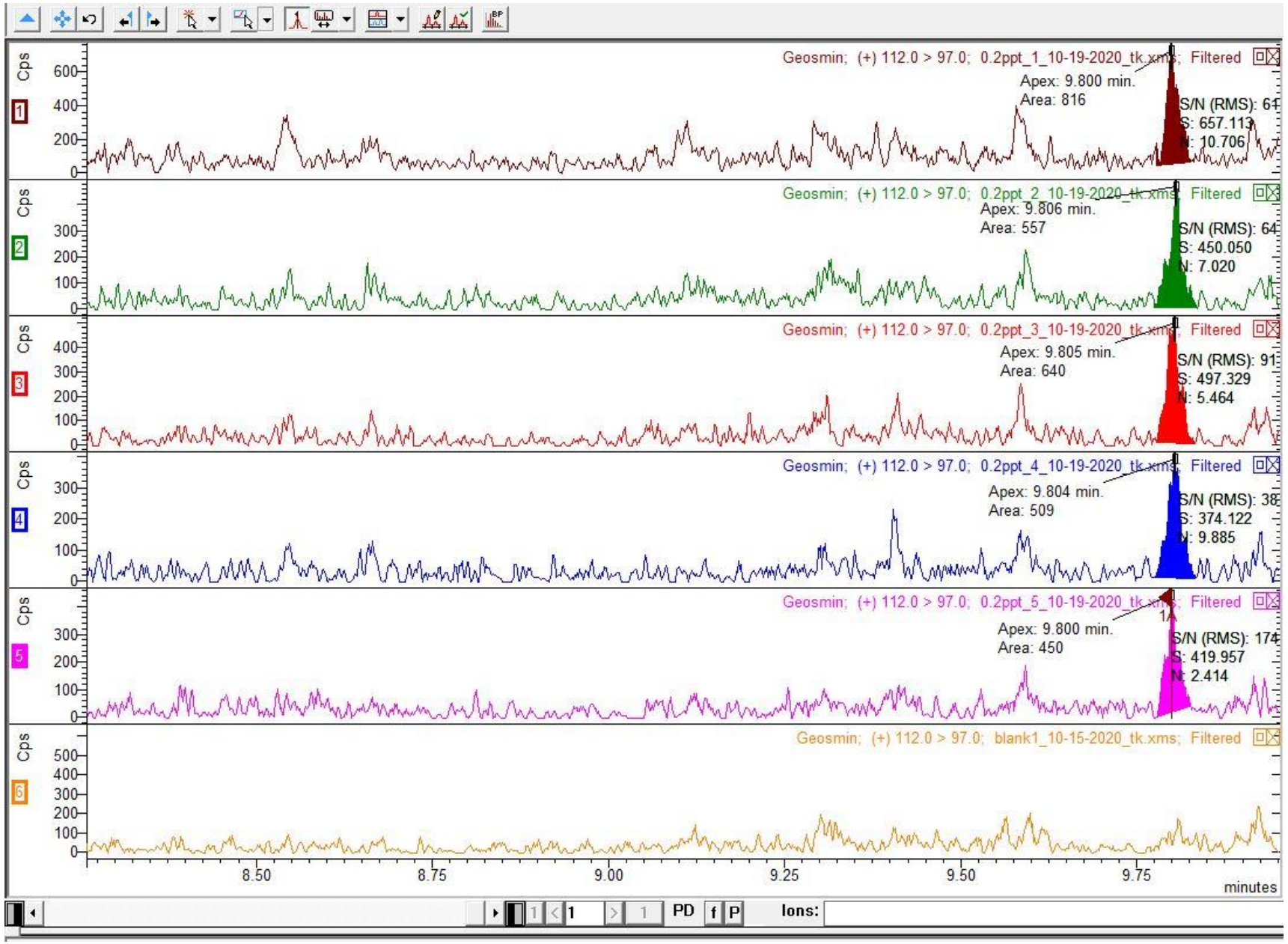
Data visualization – % recovery boxplots



Summary of validation results

Compound	Conc. Level (ng/L)	N	Mean (ng/L)	Mean %Rec.	$\%RSD_R$	%RSDr
Geosmin	0.5	6*	0.554	110.9	-	5.75
Geosmin	1.0	6*	0.978	97.8	-	9.09
Geosmin	3.0	12	2.979	99.3	4.72	3.65
Geosmin	10	18	9.794	97.9	6.01	4.23
MIB	3	12	2.979	99.3	16.45	17.15
MIB	10	18	10.079	100.8	8.12	8.27
			Specifications	75-125	<25	<25
				✓	✓	✓

Limit of Detection



- Replicates of samples spiked with 0.2 ng/L geosmin.
- MS/MS method (m/z 112->97).
- Visible peak.
- S/N>3 (but S/N not a good metric in MS/MS).

LOD depends on instrument condition.

LOQ is always more important than LOD.

Blank sample

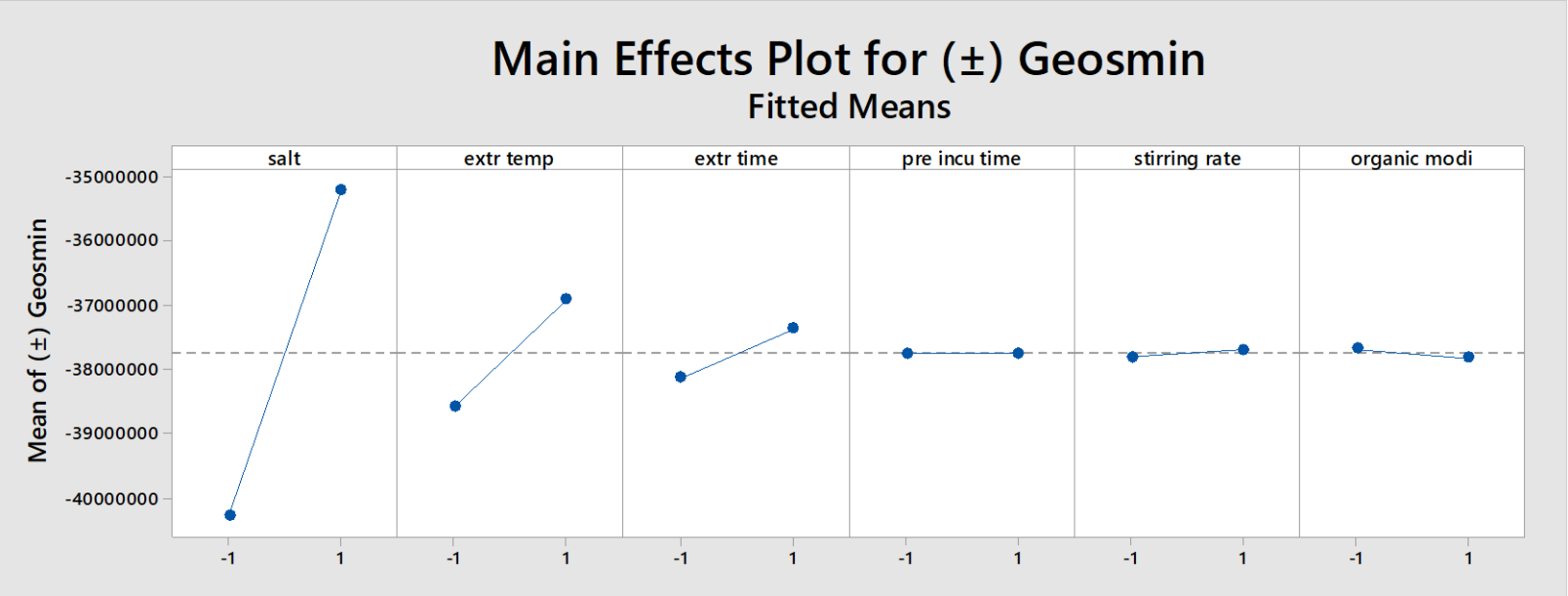
Robustness

The capacity of an analytical procedure to produce unbiased results when small changes in the experimental conditions occur.

Especially important for manual SPME, where e.g., temperatures and times may vary from the target settings.

DoE to evaluate effects of factors which are not precisely controlled.

- Significant effects: Take measures to control these factors.
- Non-significant effects: Methods is robust in the range studied.



Example: Effects of various factors on peak area of geosmin, Pluckett-Burman screening DoE.

Routine application

Example of analytical sequence.

Run	Sample
1	Blank 1
2	Blank 2
3	Calibration 1
4	Calibration 2
5	QC 1
6	Sample 1
7	Sample 2
8	Sample 3
……	…….
15	Sample 10
16	Calibration 3
17	QC 2
13	Sample 11
….	….

- Initial blanks to clean the fiber, no impurities.
- Bracketing calibration samples every 10 samples at max.
- QC samples every 10 samples at max.
- End of sequence with calibration, QC and blank.

- Blank, QC and calibration samples are in bottled water matrix.

Certified Reference Materials

Certificate of Analysis

(+/-)-GEOSMIN,1X1ML,100UG/ML,MEOH

Certified Reference Material

Description

Product ID	CRM47522
Lot	**LRAB9720**
Expiration Date	August 2021
Manufacturing Date	August 2018
Storage Conditions	Room Temperature
Solvent/Matrix	METHANOL

Certified Values

Analyte	*Units*	*Certified Value* [1,4]	*Raw Material Purity,%*	*Analytical Value* [6]	*Elution order*	*Raw Material Lot*	*CAS*
Geosmin	µg/ml	100.0 ± 1.8	98.25	94.5	2	BCBS9505V/ DSL5567	16423-19-1

Additional Information:

Analytical Method Parameters:

Column: SLB-5MS, 30 m x 0.25 mm x 0.25 µm df , Flow: 1.0 ml/min
Inlet: 240 oC, Split Ratio: 15:1
70 oC (2 min) to 260 oC (3 min) at 30 oC/min
Detector: MSD-SIM mode, Tx line: 260 oC, Q: 150 oC
Injection Volume: 1.0 µl

SIGMA-ALDRICH®
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800-325-5832
TechService@millipore sigma.com www.sigma-aldrich.com

Description

Lot	**LRAB9720**
Expiration Date	August 2021
Manufacturing Date	August 2018
Storage Conditions	Room Temperature
Solvent/Matrix	METHANOL

1 **Metrological traceability:** Traceable to the SI and higher order standards from NIST through an unbroken chain of comparisons. The balance used to weigh raw materials is accurate to +/-0.0001 g and calibrated regularly using mass standards traceable to NIST. All dilutions were performed gravimetrically. Additionally, individual analytes are traceable to NIST SRMs where available and specified above.
4 Ucrm - Uncertainty values in this document are expressed as Expanded Uncertainty (Ucrm) corresponding to the 95% confidence interval. Ucrm is derived from the combined standard uncertainty multiplied by the coverage factor k, which is obtained from a t-distribution and degrees of freedom. K=2 unless specified. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long term stability, and short term stability (transport). The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies. The mathematical representation of the Ucrm calculation is as follows:

$$u_{CRM} = \sqrt{u^2_{char} + u^2_{hom\ eqeneity} + u^2_{stability}}$$

k: Coverage factor derived from a t-distribution table, based on the degrees of freedom of the data set. Assume 2.0 for a **Confidence interval ≈ 95%**

6 Analytical Value- For QC verification of the certified value only- not to be used in calculations. Represents the analytical data obtained by comparison to a standard as analyzed by the method described in the CoA or another acceptable method. The result may differ from the certified value and UCRM based on method uncertainty as well as the uncertainty associated with the standard used for comparison.

Traceability: The standard was manufactured under an ISO/IEC 17025:2017 certified quality system. The balance used to weigh raw materials is accurate to +/- 0.0001g and calibrated regularly using mass standards traceable to NIST. All dilutions were preformed gravimetrically. Additionally, individual analytes are traceable to NIST SRMs where available and specified above.

Homogeneity: Homogeneity was assessed in accordance with ISO 17034:2016. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared using a one-way analysis of variance approach as described by TNI EL-V3-2009 Appendix A.2. See Instructions for minimum sub-sample size.

Expiration is at end of month given on certificate and label.

MSDS [illegible]

THIS PRODUCT WAS DESIGNED, PRODUCED AND VERIFIED FOR ACCURACY AND STABILITY IN ACCORDANCE WITH **ISO/IEC 17025:2017 (ANAB Cert AT-1467)** and **ISO 17034:2016 (ANAB Cert AR-1470)**.

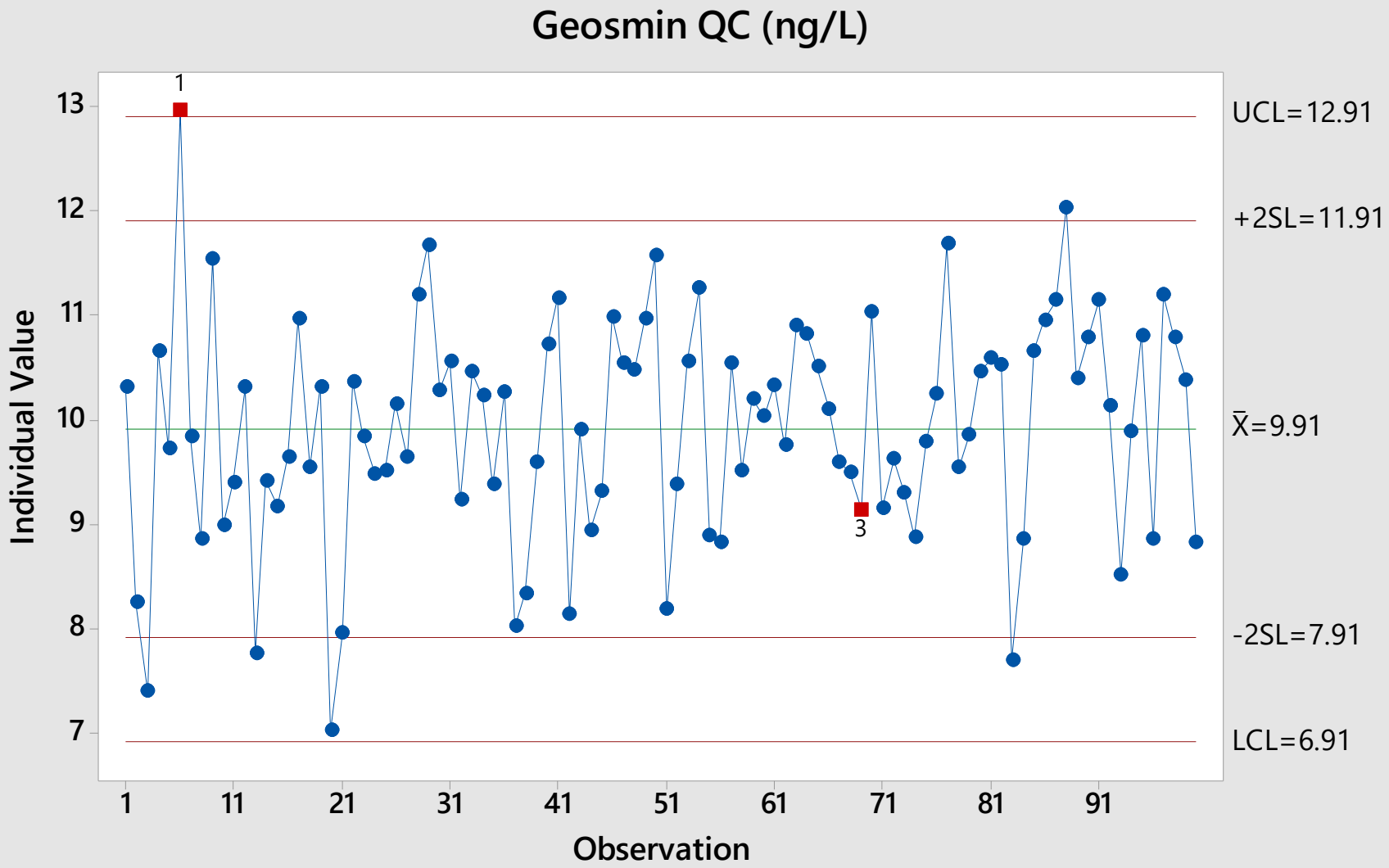
Andy Ommen - QC Manager

Mark Pooler - QA Supervisor

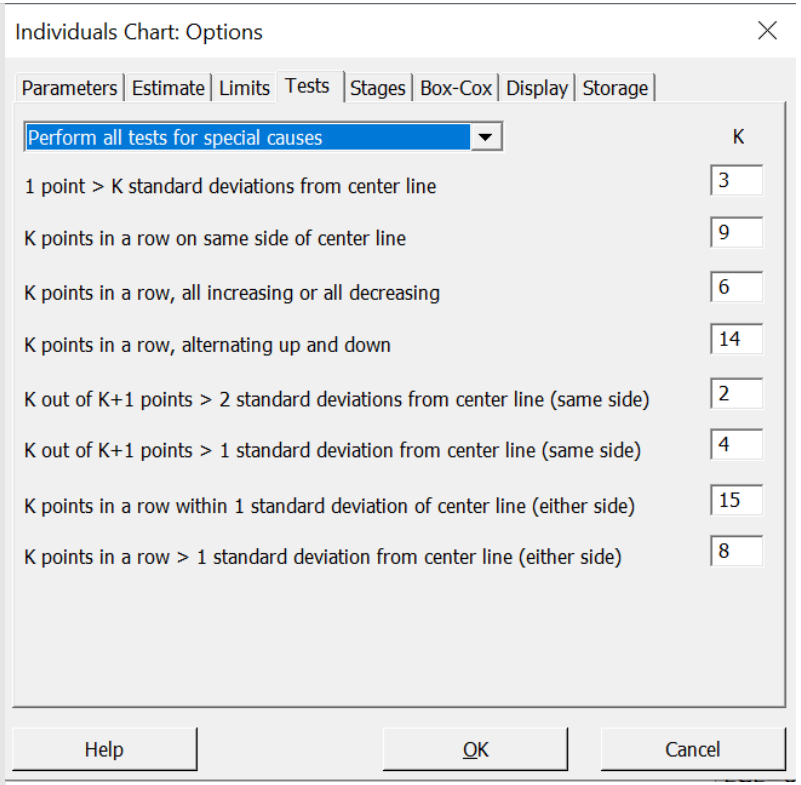
Certification Date March 13, 2020
Version 0-3132020

Example Certificate. We do not endorse any commercial products.

Quality control



At least one estimated historical parameter is used in the calculations.



Customization of Westgard rules in Minitab

Which QC chart rules?
How rules should be used?

QC samples must be independent from calibration samples.

Simulated data

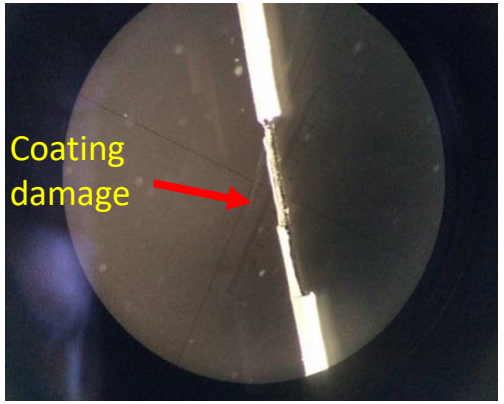
Mistake-proofing (poka-yoke)

Suction piston graduated pipettes, glass (sub-sampling)

Single-use clear glass SPME vials, Teflon liner.

Merlin Microseal "duckbill" valve.

Amber glass vials, 100ml, ground glass stoper (100ml), for sampling.

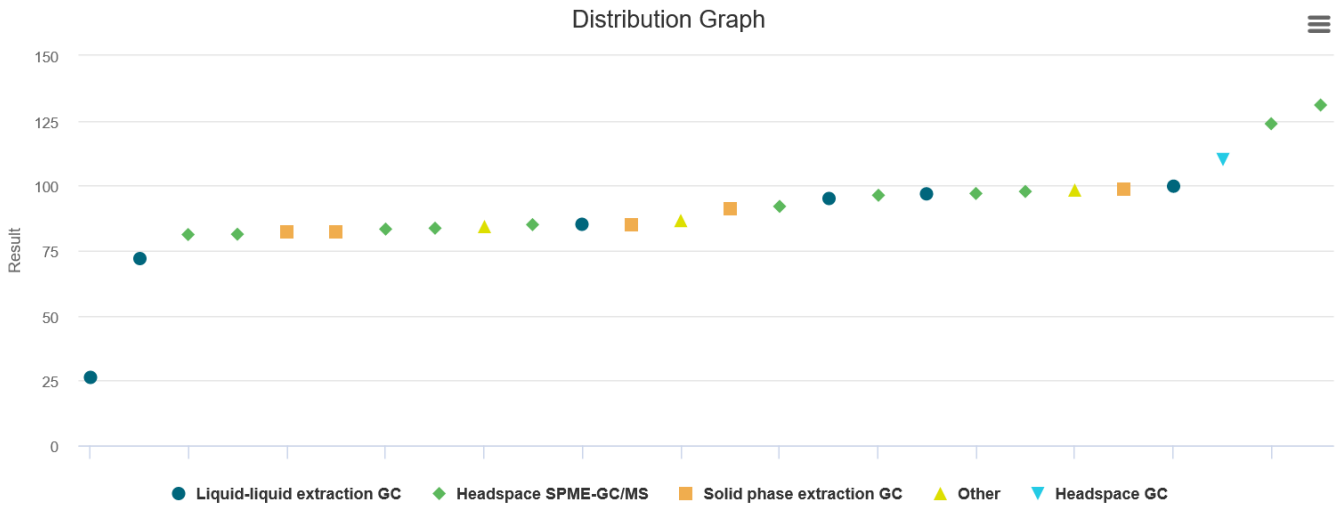


SPME fiber inspection

- No storage of solvents or standard solutions in GC-MS room.
- Pre-baking of NaCl.
- Separate room for preparation of calibration and QC samples.
- Preventive maintenance.

Proficiency Testing Schemes

Analyte	Analyst	Result	Your reference	Ux Units	Performance score	Score type	Number of results	Uncertainty of Assigned value	SDPA	Expanded SDPA	Assigned value	Median	Mean	Robust SD	SD
Geosmin	Dr	97.76	Headspace SPME-GC/MS	ng/L	0.30	Z Score	26	0.366	14.04	N/A	93.60	88.77	90.20	11.115	18.525
Methyl isoborneol	Dr	97.56	Headspace SPME-GC/MS	ng/L	0.28	Z Score	24	0.366	14.04	N/A	93.60	96.82	95.09	10.381	22.032



When available, use PT schemes which are accredited by ISO ISO/IEC 17043.

Search for PT schemes in https://www.eptis.org/

Evaluate your results. Usually,

$$Z - score = \left[\frac{x_i - x_a}{\sigma_{\mathrm{PT}}}\right] < 2$$

PT schemes for untargeted detection of T&O in water are also available.

Uncertainty of measurement

Measurement uncertainty: non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used. [JCGM 200:2008 (VIM) 2.26]

JCGM 104:2009

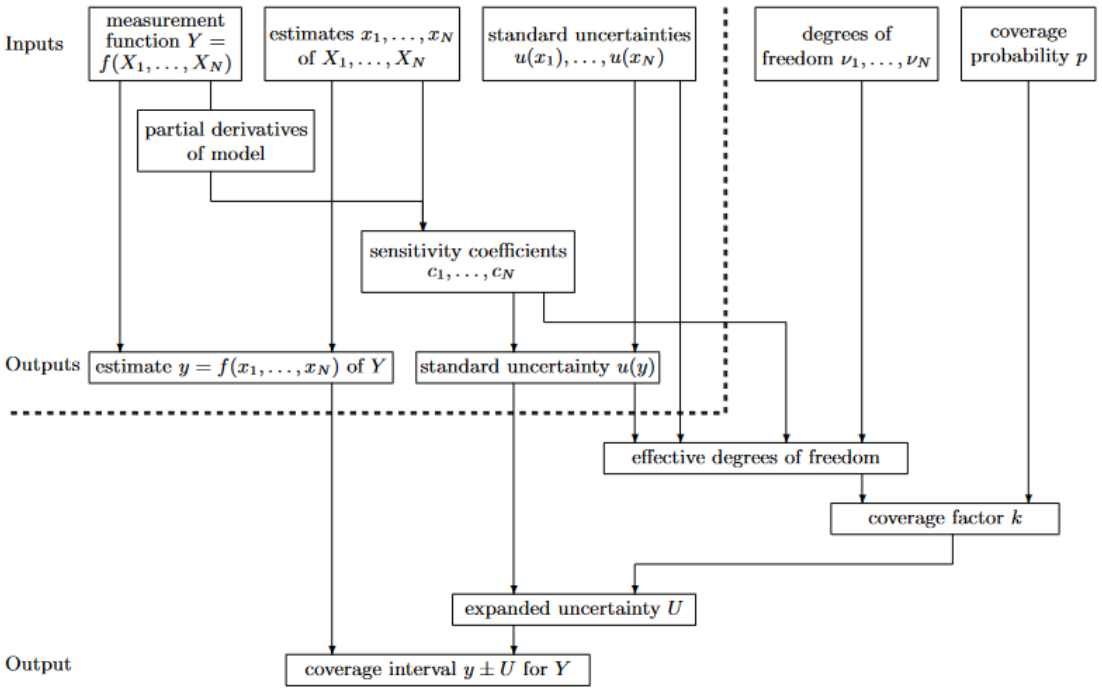


Figure 7 — Measurement uncertainty evaluation using the GUM uncertainty framework, where the top-left part of the figure (bounded by broken lines) relates to obtaining an estimate y of the output quantity Y and the associated standard uncertainty $u(y)$, and the remainder relates to the determination of a coverage interval for Y

https://www.bipm.org/en/committees/jc/jcgm/publications

Uncertainty of measurement – The Nordtest approach

Standard uncertainty (u_c) is estimated from two contributions:

1. **Within-laboratory reproducibility,** e.g., from QC samples:

$$u(Rw) = s_{Rw}$$

2. **Method and laboratory bias:**

$$u(c_{recovery}) = \sqrt{u_{conc}^2 + u_{vol}^2},$$

where u_{conc} is the uncertainty of the CRM concentration (in the certificate) and u_{vol} is the uncertainty of the volume of spiked standard (from pipette calibration certificate.

$$RMS_{bias} = \sqrt{\frac{\sum_{i=1}^{N}(100\% - recovery_i)^2}{N}} \text{ and } u_{(bias)} = \sqrt{RMS_{bias}^2 + u(c_{recovery})^2}$$

$$u_c = \sqrt{u(Rw)^2 + u_{(bias)}^2}$$

Expanded uncertainty, 95% confidence (k=2): $\boldsymbol{Uexp = 2 \cdot u_c}$

Results are expressed as $(\boldsymbol{measured\ value \pm 2 \cdot U_{exp}})$

NORDTEST NT TR 537 edition 4 2017:11

Estimating uncertainty with MUkit

MEASUREMENT UNCERTAINTY ESTIMATION

Step	Action	Geosmin	9/17/2023
1	Specify Measurand	Measurand: Concentration Concentration range: 1 - 10 ng/l Sample Type (Matrix): Potable water Analysis Principle (Analyzer etc.): HS-SPME-GC-MS/MS Sample preparation: HS-SPME	
2	Quantify within-laboratory reproducibility, $u(R_w)$ Control sample that covers all the steps in the analytical process	**Control samples:** Sample Type (Matrix): Potable bottled water Zagori Period of measurements: 9/23/2020 - 10/6/2020 Number of control samples: 15 Average concentration: 9.111 ng/l Standard deviation, s_{Rw} : 0.49 % $u(R_w) = s_{Rw}$ = 0.49 %	
3	Quantify method and laboratory bias, $u(bias)$	**Method and laboratory bias from recovery tests:** Standard solution concentration: 100 ug/L Standard uncertainty of standard solution concentration, $u(conc)$: 1.00 % Standard uncertainty of standard solution volume, $u(vol)$: 0.76 % Recovery test count, N : 15	
4	Convert components to standard uncertainty	$u(R_w)$ = 0.49 % $u(bias)$ = 15.16 %	
5	Calculate combined standard uncertainty, u_c	$u_c = \sqrt{u(Rw)^2 + u(bias)^2}$ = 15.17 %	
6	Calculate expanded uncertainty, U	$U = 2 \cdot u_c$ = 31 %	

i	1	2	3	4	5	6	7	8
Recovery of added analyte, $Recovery_i$	118.60 %	113.30 %	115.10 %	110.60 %	112.40 %	109.60 %	107.90 %	122.60 %
Approximated concentration								
Standard solution added								
Date	9/23/2020	9/23/2020	9/23/2020	9/23/2020	9/23/2020	9/23/2020	9/23/2020	10/5/2020

i	9	10	11	12	13	14	15
Recovery of added analyte, $Recovery_i$	125.80 %	121.20 %	110.50 %	117.90 %	105.60 %	105.40 %	111.60 %
Approximated concentration							
Standard solution added							
Date	10/5/2020	10/5/2020	10/5/2020	10/5/2020	10/5/2020	10/5/2020	10/5/2020

$u(c_{recovery}) = \sqrt{u(conc)^2 + u(vol)^2}$ = 1.26 %

$RMS_{bias} = \sqrt{\frac{\sum_{i=1}^{N}(100\% - Recovery_i)^2}{N}}$ = 15.11 %

$u(bias) = \sqrt{RMS_{bias}{}^2 + u(c_{recovery})^2}$ = 15.16 %

9/17/2023

Summary of the method's measurement uncertainties

Method information

Method name	Geosmin
Measurand	Concentration
Sample Type (Matrix)	Potable water
Analysis Principle (Analyzer etc.)	HS-SPME-GC-MS/MS
Sample preparation	HS-SPME

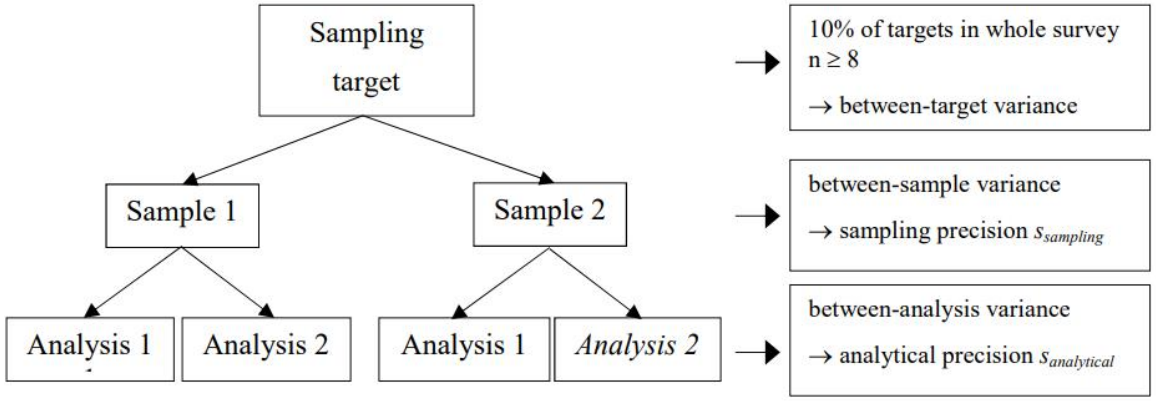
Calculated Uncertainties at Different Measurand Levels

Concentration range (ng/l)	Within-lab Reproducibility Data	u (Rw)	Bias Data	u (bias)	Combined standard uncertainty	Expanded uncertainty
1-10	Control sample covering the whole analytical process	0.49 %	Recovery Test	15.16 %	15.17 %	31 %

Näykki et al., J. Chem. Metrology, 2014
https://www.syke.fi/envical/en

Uncertainty from sampling - empirical (top-down) approach

Figure 2: A balanced design



Balanced experimental design for empirical estimation of uncertainty (i.e. two-stage nested design), using the 'duplicate method'. Removal of *Analysis 2* on Sample 2 would result in the more cost-effective unbalanced design [Fig D2(b)], discussed in Note 2 above.

Table 3: Uncertainty contributions in the empirical approach

Process	Effect class*	
	***Random** (precision)*	***Systematic** (bias)*
Analysis	Analytical variability (combined contribution of random effects)	Analytical bias (combined effect of bias sources)
Sampling	Sampling variability (dominated by heterogeneity and operator variations)	**Sampling bias** (combined effect of selection bias, operator bias etc.)

*The differentiation of random from systematic effects can depend on the context. A systematic effect in measurements by one organisation (e.g. analytical bias) can also be considered a random effect when viewed in the context of the consensus value from an inter-organisational proficiency test.

$$u = s_{meas} = \sqrt{s_{sampling}^2 + s_{analytical}^2}$$

In a survey across various sampling targets:

$$x = X_{true} + \varepsilon_{target} + \varepsilon_{sampling} + \varepsilon_{analytical}$$

$$s_{total}^2 = s_{between-target}^2 + s_{sampling}^2 + s_{analytical}^2$$

Table 4: Estimation of uncertainty contributions in the empirical approach

Process	Effect class	
	Random	***Systematic** (bias)*
Analysis	*duplicate analyses gives precision under repeatability conditions*	e.g. *validation data or CRM*
Sampling	*Duplicate samples*	*Reference sampling target, inter-organisational sampling trial*

Four classes of effects that contribute to the uncertainty of measurements, and methods for their estimation.

Source: Eurachem, UfS 2019 https://www.eurachem.org/

Validation of HS-SPME-GC-MS/MS methods for water T&O

Tri Kaloudis
Laboratory of Organic Micropollutants, Water Quality Dept., EYDAP SA, Greece
Institute of Nanoscience & Nanotechnology, NCSR Demokritos, Greece

WaterTOP Training School "Microextraction in T&O analysis: fundamentals and applications
TUC, Chania, Greece, 20-22/09/2023

LABORATORY OF ORGANIC MICROPOLLUTANTS
EYDAP
DEMOKRITOS NATIONAL CENTER FOR SCIENTIFIC RESEARCH
WaterTOP COST ACTION CA18225
cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
Funded by the European Union
LEAD LABORATORY OF ENVIRONMENTAL ANALYSIS NCSR DEMOKRITOS