

Accreditation of a speciation method : example of methylmercury analysis in sediment

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Context

- Increasing interest for speciation analysis in the field of environmental monitoring (for example for mercury speciation)
- « Progressive » introduction of speciation in reglementary text or directives (organotin compounds for example among priority substances in WFD)
- Routine laboratories could then be more and more implied and accreditation could be required



Two possibilities for accreditation on speciation methods :
standardisation (CEN-ISO) or intralaboratory method validation

Context

- ISO 17025 standards offers the possibility to laboratories to be accredited for their ability to adapt, develop, conceive methods
 - Method Validation is a key parameter



Flexible accreditation – Type II or III

- brgm laboratories have recently been accredited by COFRAC for this activity
- Recent needs at brgm for mercury speciation



opportunity to apply validation procedure to methylmercury analysis in sediment

Requirements for flexible accreditation (type II or III)

> **A laboratory should have written two procedures**

- Description of the global management of the activity
 - Responsibilities
 - Competences
 - Traceability
 - ...
- Validation procedure
 - example in BRGM :
 1. Development of the method
 2. Drafting of a provisional standard operating procedure
 3. Validation of the method following SOP after choice of validation criteria
 4. Decision : comparison of results with objectives

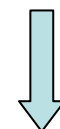
Analytical method

- Methylmercury analysis in sediments
- Method adapted from Liang & al (1994) and from Cossa & al (IFREMER)
 - Acid digestion
 - Dichloromethane extraction and back extraction in water
 - Ethylation and trapping on TENAX
 - Thermal desorption and GC AFS (Tekran 2500)
- Installation – development of the method in the laboratory
- Provisional SOP
- Validation criteria : linearity, LOQ, precision, trueness, specificity, (uncertainty)
- Reference material : estuarine sediment CRM 580

Validation : linearity

- Linearity test according to standard NFT90210 (ethylation to detection)
- 5 methylmercury levels : 0, 100, 200, 300, 400 pg injected
- 3 series (consecutive days)

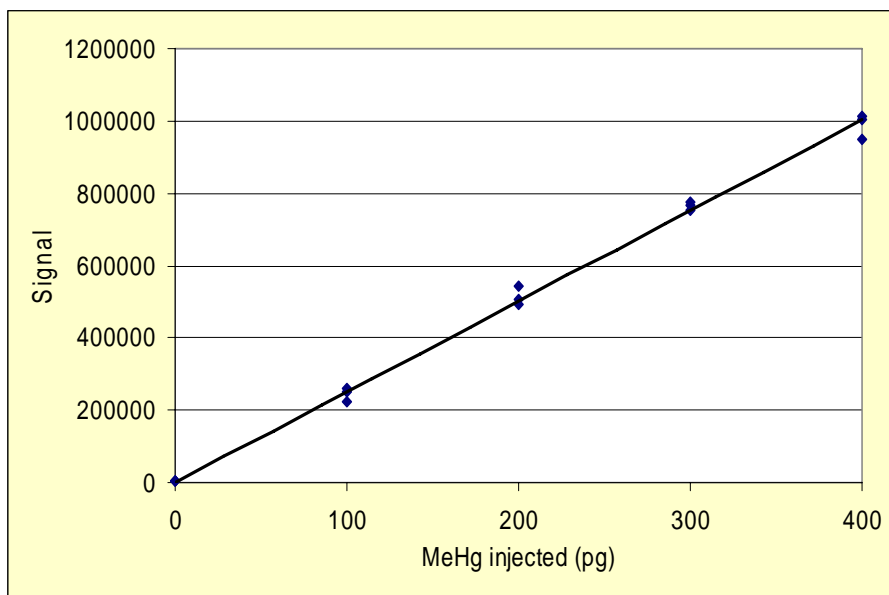
MeHg (pg)	Day 1	Day 2	Day 3
0	4944	6362	2731
100	224547	252160	261855
200	506233	493678	542311
300	776415	751712	765399
400	948929	1002022	1013178



Linearity validated up to 400pg MeHg injected by :

- Visual inspection
- Comparison of variances (test 90210)

Equivalent to 120 ng/g
(200mg of sample and 1ml extract ethylated)



Validation : limit of quantification

- 10 complete analyses of a standard solution (including acid digestion, ethylation, GC AFS)
 - Equivalent to 5 pg injected or 1.5 ng/g
- Repeatability conditions

- Results
 - No statistical difference between average and theoretical value (5pg) – Test NFT90210
 - Standard deviation less than 20%

Repetition	Result (pg)
1	4,88
2	5,13
3	4,80
4	5,03
5	5,29
6	5,43
7	5,03
8	5,08
9	5,09
10	5,71
Average	5,15
SD(%)	5,24



Estimated limit of quantification : 1.5 ng/g (200mg – 1ml ethylated)
Possibility to lower this value by increasing sample weight or volume of extract ethylated

Validation : precision

- Repeatability estimated with CRM 580 at 4 different levels (4 repetitions)

	MeHg (ng/g)				Average	SD (%)
	Repetition					
CRM 580	1	2	3	4		
50 mg	78,9	58,0	68,3	61,0	66,5	14,0
100 mg	74,8	71,5	64,1	82,1	73,1	10,2
200 mg	89,3	65,0	84,2	81,4	80,0	13,1
300 mg	91,5	76,9	70,2	79,1	79,4	11,2

- Intralaboratory reproductibility calculated with data from 4 days and two different operators (one sample CRM580 200 mg)

	MeHg (ng/g)				Average	Calc. Intralab. Reprod.(%)
	Repetition					
	1	2	3	4		
Day 1	89,3	65,0	84,2	81,4	80,0	10.3%
Day 2	91,5	76,9	70,2	79,1	79,4	
Day 3	57,7	70,8	76,0	77,3	70,4	
Day 4	67,1	73,7	62,3	60,7	65,9	



The variability seems mainly due to repeatability
Repeatability should be improved



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Validation : trueness

- Evaluation with data from reproducibility study
- CRM 580
 - Certification : 75.5 ± 3.7 ng/g (k=2)
 - Reproducibility study : 73.9 ± 5 ng/g (k=2 / n=16)
- Test of trueness

$$\left| \frac{\bar{x} - V_r}{\sqrt{u_x^2 + u_{CRM}^2}} \right| \leq 2$$



No observed bias for the method with CRM 580

Validation : specificity

- 6 sediment samples from different origin spiked with known amounts of methylmercury standard solution
- Spiking just before acid digestion : only evaluation of possible matrix effect on the analysis

Sample	Origin	MeHg (ng/g)			Spike recovery (%)
		Result Without spike	Spike	Result after spiking	
1	estuarine	2,3	19,7	19,6	88
1	estuarine	1,7	5,0	5,4	74
2	sea	0,0	4,6	4,2	92
3	river	6,0	19,7	26,0	101
3	river	4,4	9,8	13,5	93
4	sea	0,0	19,9	19,1	96
5	sea	0,0	9,8	7,7	78
5	sea	0,0	4,8	3,4	70
5	sea	0,0	19,3	16,1	84
6	sea	0,0	4,8	4,5	94

Recovery results and slope-test for recovery curve correct

Good specificity of the method



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Uncertainty

- Evaluation by combining :
 - Uncertainty of laboratory result estimated by reproductibility
10%
 - Uncertainty on CRM580 material (certified reference material used for evaluation of trueness)
 $= 0.5 * 3.7 / 75.5 = 2.5 \% (k=1)$
- Quadratic sum

Evaluation of uncertainty : 25%

coverage factor $k=2$

level : around 75 ng/g

matrix : estuarine sediment

mean of evaluation : intralaboratory



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Validation : summary

Characteristic	Results - Comments	Decision
Calibration curve	Linear up to 400 pg (120 ng/g sediment)	OK
Limit of quantification	Lower than 1,5 ng/g	OK comparison with literature data
Specificity	No matrix effect identified (6 different samples)	OK
Repeatability	Around 10%	Higher than some literature results - Accepted with taking into account in uncertainty
Trueness	No bias (CRM 580)	OK
Reproductibility	10-15%	See repeatability
Uncertainty	25% (k=2)	

COFRAC audit

- Positive results with some remarks and some complementary work asked by auditors
 - Other reference material could have been used to reinforce demonstration
 - Important comment from auditors on sample preparation : brgm SOP indicates freeze drying with reference to many publication : not enough .



New analysis needed to test potential effect of freeze drying on results

Conclusion

- Diffusion of speciation analysis in « routine » or « commercial » laboratories is perhaps not for tomorrow...but it will certainly come in the next years
- It seems important that research laboratories (who sometimes have long experience) could have a look on what will be done in the framework of accreditation on speciation analysis.
- Harmonization of validation procedure is needed
- Experience on freeze-drying effect ?