Journal of Environmental Protection and Ecology 7, No 1, 196–203 (2006)

METHOD VALIDATION – REQUIREMENT OF ISO/CEI 17025 FOR ACCREDITED AIR POLLUTION LABORATORY FROM INCD ECOIND ROMANIA

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Abstract. To assure the comparability of the results of the measurements done for the transboundary pollution quantification, the results must to be obtain using standardised/reference methods or own methods which were validated. Accredited until 2002 by RENAR – The Romanian Accreditation Association, in concordance with ISO EN 45001 and ISO Guide 25, Air Pollution Laboratory from National Research and Development Institute for Industrial Ecology – ECOIND will recently obtain the ISO/CEI 17025 accreditation for 37 laboratory tests in the field of emission and air quality measurements. When a laboratory has developed its own methods these method must to be validated that means to asses: the detection limit, the quantification limit, the working range and the linearity, the sensitivity, the recovery and the accuracy (trueness and precision) of the methods. The precision (repeatability and reproducibility) can be estimated/calculated on the results obtained in a collaborative inter-laboratory experiment. The results obtained for the validation of couples chemical methods developed by the laboratory: hydrochloric acid (inorganic chloride compounds), ammonia, chromium(VI), phenol are presented.

Keywords: accreditation, validation, emission, imission (air quality), detection limit, quantification limit, working range, linearity, sensitivity, recovery and accuracy, trueness, precision, repeatability and reproducibility.

AIMS AND BACKGROUND

Accredited until 2002, by RENAR – The Romanian Accreditation Association, in concordance with ISO EN 45001 and ISO Guide 25, Air Pollution Laboratory from National Research and Development Institute for Industrial Ecology – ECOIND will recently obtain the ISO / CEI 17025 accreditation for 37 laboratory tests in the field of emission and air quality measurements.

To assure the comparability of the results of the measurements done for the transboundary pollution quantification, the results must to be obtain using standardised/reference methods or own methods which were validated.

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Validation means: 'confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled. '

Method validation means: 'The process of establishing the performance characteristics and limitations of a method and the identification of the influences which may change these characteristics and to what extent. The process of verifying that a method is fit for purpose, i.e. for use for solving a particular analytical problem'.

Verification means: 'confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. '

EXPERIMENTAL

The steps for to establishing the performance parameters of the method are:

a. **Confirmation of identity and selectivity/specificity**: to establish that the measured property attributed to the analyte is due to the analyte and to assess the reliability of measurements in the presence of interferences. The selectivity of a method is usually investigated by studying its ability to measure the analyte of interest in test portions to which specific interferences have been deliberately introduced.

For hydrochloric acid was studied the interferences of couple substances and were established the limit values up where from the method (i.e. Table 1) for ammonia determination or what to do to eliminate the interferences (i.e. to eliminate the interference of cyanides in inorganic chlorides / hydrochloric acid determination is necessary to add hydrogen peroxide).

Table 1. Interferences for ammonia

Interference substance	Ratio ammonia : interference substance
Sulphur dioxide – SO_2	1 : 200
Nitrogen dioxide – NO_2	1 : 100
α-Naphthylamine	1 : 50
Aniline	1 : 50
Al^{3+}	1 : 50
Cyanide –CN^-	1 : 10
Urea	1 : 10
Ni^{2+}	1: 5
Fe^{3+}	1 : 1
Hydrogen sulphide/sulphides – H_2S / S^{2-}	1 : 0.5
Hydroxilamine – NH_2-OH	1 : 0.25
Hydrochloric acid	no interferences
Ca^{2+}	

Ratio ammonia:interference substance up to which there are no interferences.

b. **Limit of detection / Method Detection Limit (MDL)** (Tables 2 and 3): to establish which is the lowest concentration of the analyte or property value that can be confidently detected by the method. Analysing 10 independent samples blanks and 10 independent samples blanks fortified at lowest acceptable concentration and taking the photometric readings was established the MDL. Usually as analyte concentration corresponding to: mean sample blank value + 3s OR mean sample blank value + 4.65s OR 2.821 s; OR 0 + 3s.

Table 2. Method detection limit values for Cr^{6+}

	Absorbance	Concentration (µg)
Spiked level	0	0
Sample 1	–0.0008	–0.1159
Sample 2	–0.0005	–0.0982
Sample 3	0.0002	–0.0569
Sample 4	0.0006	–0.0645
Sample 5	0.0045	0.1628
Sample 6	0.0048	0.1804
Sample 7	0.0024	0.0902
Sample 8	0.0022	0.09
Sample 9	0.001	0.0452
Sample 10	0.0012	0.0455
Mean	0.00156	0.02786
Standard deviation – s	0.001923	0.106515
3s	0.005769	0.319546
5s	0.009615	0.532576
6s	0.011538	0.639091
10s	0.019231	1.065152

			µg	µg/ml
MDL	+ 3s	0.007329	0.347406	0.013896
LoQ	+ 5s	0.011175	0.560436	0.022417
	+ 6s	0.013098	0.666951	0.026678
	+10s	0.020791	1.093012	0.04372

For MDL were made verifications for 5 criteria recommended by EPA and Wisconsin Department of Natural Resources. Laboratory Certification Program:

1) MDL × 10 > spiked level: OK/NO?;
2) MDL < spiked level: OK/NO?;
3) MDL < MDL from legislation: OK / NO?;

4) Ratio signal/noise: < 10;

5) Mean recovery %: > 90%; acceptable/not acceptable.

c. **Limit of quantitation / Method Quantitation Limit (LoQ)** (Tables 2 and 3): to establish which is the lowest concentration of the analyte that can be determined with an acceptable level of repeatability, precision and trueness. Analysing 10 independent samples blanks and 10 independent samples blanks fortified at various analyte concentration close to the MLD and taking the photometric readings was established the LoQ, usually as analyte concentration corresponding to mean sample blank value + 5s OR mean sample blank value + 6s OR mean sample blank value + 10s.

Table 3. MDL for Cr^{6+}

	Absorbance	Concentration (μg)	
Spiked level	–	0	
Sample 1	0.0125	0.6682	
Sample 2	0.0123	0.6564	
Sample 3	0.0112	0.5899	
Sample 4	0.0112	0.5899	
Sample 5	0.0104	0.5798	
Sample 6	0.0114	0.5906	
Sample 7	0.0119	0.6125	
Sample 8	0.0120	0.6490	
Sample 9	0.0138	0.7465	
Sample 10	0.0136	0.7420	
Mean	0.01203	0.64248	
Standard deviation – s	0.001072	0.062067	
		μg	μg/ml
MDL (2.821 s)	0.007329	0.347406	0.013896
LoQ (10 × s)	0.01072	0.62067	0.024827
Checks if spiked level it's too great	spiked level < 10 MDL	0.5 < 1.7 OK	
Checks if spiked level it's too small	spiked level > MDL	0.5 > 0.175091 OK	
Ratio S/N	11.22	10.35	
Final MDL established	0.50 μg Cr^{6+} in the 25-ml volumetric flask that means 0.010 absorbance units and represents 0.02 μg Cr^{6+}/ml solution		
Final LoQ established	1.10 μg Cr^{6+} in the 25-ml volumetric flask that means 0.022 absorbance units and represents 0.044 μg Cr^{6+}/ml solution		
Sensibility	0.02 μg Cr^{6+}/ml solution that means 0.010 absorbance units		

d. **Working and linear ranges**: to determine the range of analyte concentrations over which the method may be applied. The lower one can be MLD or LoQ and the upper one depends on the instrument response system. Usually the working range is the range in which the calibration curve is linear and is established making the calibration curve in minimum 5 points of different concentrations.

e. **Accuracy – trueness** (Table 4): the closeness of a result to a true value / an accepted reference value and is expressed in terms of bias.

Trueness can be established by two techniques: checking against Certified Reference Materials (CRM) or from another characterised and standardised method.

We applied checking against Certified Reference Materials (CRM) or standard reference solutions (SRS) with known concentration used in QC (control charts).

Table 4. Recovery/trueness for ammonia

No det.	CRM 14 / 2001, 2.932 μg NH_3/ml confidence range 2.383-3.480 μg NH_3/ml solution	CRM 15 / 2001, 8.070 μg NH_3/ml confidence range 7.748-8.392 μg NH_3/ml solution	CRM 19 / 2002, 8.28 μg NH_3/ml confidence range 7.66-8.89 μg NH_3/ml solution
1	2.899	7.562	8.130
2	2.934	7.597	8.153
Media	2.916	7.58	8.14
R%	99.47	93.92	98.33

f. **Accuracy – precision: repeatability and reproducibility** (Tables 5, 6, 7 and 8): how close results are to one another, expressed by measures such as standard deviation or relative standard deviation which describe the spread of the results.

Repeatability and reproducibility are usually dependent on concentration level.

We organised collaborative trials for each method and two concentration levels and the statistical interpretation of the results was made according ISO 5725:1994 Part 1 and Part 2.

Table 5. Repeatability and reproducibility for HCl/Cl⁻

Concentration (μg/ml)	p	Mean	s_r		s_R		Conclusions
			(μg/ml)	(%)	(μg/ml)	(%)	
2.49	7	2.4912	0.0849	3.41	0.1736	6.97	$s_{r\ relative}$ = 2.0% and $s_{R\ relative}$ = 4.0% reported to the concentration existing in the solution photometred
6.248	7	6.2445	0.1168	1.87	0.3888	6.23	
9.06	9	9.1593	0.1554	1.70	0.2411	2.63	
16.937	7	16.9371	0.2328	1.37	0.4896	2.89	
18.34	7	18.2894	0.3502	1.91	0.4795	2.62	
504.84	7	505.2731	16.1697	3.20	18.4663	3.65	
504.84	9	503.7263	10.1065	2.01	12.9516	2.57	
Mean	–	–	–	2.21	–	3.94	

Note: p – number of laboratories.

Table 6. Repeatability and reproducibility for ammonia

Concentration (μg/ml)	p	Mean	s_r		s_R		Conclusions
			(μg/ml)	(%)	(μg/ml)	(%)	
2.932	7	2.9579	0.1229	4.15	0.3288	11.12	$s_{r\ relative}$ = 3.0% and $s_{R\ relative}$ = 8.0% reported to the concentration existing in the solution photometred
8.070	7	7.6675	0.1732	2.26	0.7915	10.32	
8.28	7	8.2877	0.1641	1.98	0.3409	4.11	
Mean	–	–	–	2.80	–	8.52	

Note: p – number of laboratories.

Table 7. Repeatability and reproducibility for phenol

Concentration (μg/ml)	p	Mean	s_r		s_R		Conclusions
			(μg/ml)	(%)	(μg/ml)	(%)	
3.69	9	3.7503	0.1577	4.20	0.2371	6.32	$s_{r\ relative}$ = 3.0% and $s_{R\ relative}$ = 6.0% reported to the concentration existing in the solution photometred
9.40	9	9.1887	0.2577	2.80	0.5165	5.62	
13.50	9	13.4309	0.3363	2.50	1.2046	8.97	
16	9	16.4883	0.4184	2.54	1.3530	8.21	
110	9	108.9647	2.4898	2.28	4.0834	3.75	
Mean	–	–	–	2.86	–	6.57	

Note: p – number of laboratories.

Table 8. Repeatability and reproducibility for Cr^{6+}

Concentration (μg/ml)	p	Mean	s_r		s_R		Conclusions
			(μg/ml)	(%)	(μg/ml)	(%)	
2.65	7	2.6139	0.2036	7.79	0.2214	8.47	$s_{r\ relative}$ = 8.0% and $s_{R\ relative}$ = 10.0% reported to the concentration existing in the solution photometred for Cr^{6+} concentration < 10 μg/ml
21.25	7	21.1749	0.5397	2.55	0.8055	3.80	$s_{r\ relative}$ = 3.0% and $s_{R\ relative}$ = 5.0% reported to the concentration existing in the solution photometred for Cr^{6+} concentration > 10 μg/ml

Note: p – number of laboratories.

CONCLUSIONS

The results summarised in Tables 2 to 8 show that:

– the MDL and LoQ are sufficient low for the concentration we analyse usually and the 5 criteria recommended by EPA and Wisconsin Department of Natural Resources (Laboratory Certification Program) were OK;

– the Working and Linear Ranges: was established by the calibration curve in minimum 5 points of different concentrations;

– the trueness: the values obtained are in the confidence interval of CRM or SRS;

– repeatability and reproducibility: it were obtained values comparable with the value obtained by other method.

It were elaborated for each method the Method Documentation Protocol which contains:

– First page: the title in Romanian, English and French, the date of validation by the general manager of INCD ECOIND and other aspects included in any first page of a method standard;

– Second page: FOREWORD where is made a short presentation of the method and its limitations, the signature of the general manager of INCD ECOIND and a table for the next review;

– Third page and so on: the description of the method:
  – scope and domain of application;
  – normative references;
  – principle;
  – reagents and materials;
  – apparatus and equipment;
  – sampling;
  – interferences;
  – analytical procedure description;
  – calibration curve;
  – calculation and expression of the results;
  – performance characteristics of the method: MDL, LoQ, sensibility, repeatability and reproducibility.

The Method Documentation Protocol refers to:

– Hydrochloric acid / inorganic chloride by spetrophotometric method with mercury thiocyanate;

– Phenol by ultraviolet spetrophotometric method;

– Ammonia by spetrophotometric method with Nessler reagent;

– Chromium^{6+} by spetrophotometric method with diphenylcarbazide.

Received 5 July 2004
Revised 18 October 2004