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ANALYTICAL METHOD VERIFICATION –A TITRIMETRIC CASE STUDY

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ABSTRACT

The ISO 17025 testing laboratories have to verify the selected methods before introducing them for routine analysis.This application note provides an illustration for the verification of titrimetric methods.The Grubbs , Cohrans and Horwitz formula are used in this study.

Keywords: Verification, Precision, Competence, Cohran Test, Horwitz Formaula, Standard Deviation

1. INTRODUCTION

Laboratories complying to ISO 17025 are required under clause 7.2.1.5 – to verify that it can properly perform methods before introducing them by ensuring that it can achieve the required performance. The ISO 17025 standard defines verification in Clause 3.8 as 'verification : provision of objective evidence that a given item fulfils specified requirements'. Three examples for this definition are also given in the clause. However, Example 2: Confirmation that performance properties or legal requirements of a measuring system are achieved is being taken for this study.

When a method is tobe verified, laboratory is required to demonstrate that it can achieve certain specific performance characteristics, but not all, established during the validation specified in clause 7.2.2.3. The minimum objective evidence for verification include specificity, repeatability intermediate precision LoD and LoQ obtained from actual laboratory data with the matrices to which the method being applied.

2. METHODS & MATERIALS

AOAC guidelines lists some performance characteristics for method verification.All are not equally applicable for all kinds of test methods.In titration methods, the specificity of reaction , repeatability and within laboratory precision can be considered.

Specificity : Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix.It includes the methodology such as identification , assay and purity tests.

The specificity depends on the basic principles of reaction and instrumentation.

If the lab samples are identical to those in the standard method and if any difference in the instrumentation do not impact specificity, no verification is needed.For example , in argentometric titration of chloride determination , the basic principle is to produce a precipitate of AgCl and hence no verification is required.

If the lab samples differ from those in the standard method, verification same as those required for validation is to be performed.

If the differences between instruments could affect specificity, the verification activity needed only deal with the unique aspects of instrument.For example, the different resolution or detection systems in ICP-OES may result in different interferences.

Repeatability: Represents precision under same operating conditions over a short interval of time .This means in repeatability, the independent test results are obtained with same method on identical test items in the same

laboratory by the same operator using the same equipment within short intervals of time.

Intermediate Precision: Precision represented as within laboratory variations such as different days,different analysts,different equipment.In this study time different intermediate precision is discussed.

Acceptability of Data: The acceptability criteria for repeatability and intermediate precision are determined by applying Grubbs , Cohran and Horwitz formula.

Grubbs Method

This method involves determining the outlier using Grubbs equation.

Grubbs test is a simple technique to quantify the outlier in the set of data points.It is based on a normal distribution and a test statistic is calculated from the most extreme data point using the formula

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$G_{exp} = (X_{max} - X_{mean}) / s$ (for highest data point) $G_{exp} = (X_{mean} - X_{min}) / s$ (for lowest data point)

A value can be regarded as an outlier if the statistic G value (G_{exp}) is greater than critical value $G_{(\alpha,n)}$ (Table-1).Then the mean and standard deviation are recalculated excluding the outlier.

Table-1 Critical values for Grubbs Test

n	$G_{(\alpha,n)}$ for α = 1 %	$G_{(\alpha,n)}$ for α = 5%	n	$G_{(\alpha,n)}$ for α = 1 %	$G_{(\alpha,n)}$ for α = 5 %
3	1.15	1.15	15	2.71	2.41
4	1.49	1.46	16	2.75	2.44
5	1.75	1.67	17	2.79	2.47
6	1.94	1.82	18	2.82	2.50
7	2.1	1.94	19	2.85	2.53
8	2.22	2.03	20	2.88	2.56
9	2.32	2.11	21	2.91	2.58
10	2.41	2.18	22	2.94	2.60
11	2.48	2.23	23	2.96	2.62
12	2.55	2.29	24	2.99	2.64
13	2.61	2.33	25	3.01	2.66
14	2.66	2.37	**α** - probability of incorrectly rejecting the suspected outlier **n-** number of samples in the data set.		

Cohrans Test

For a given set of **p** standard deviations ,all calculated from the same number of replicate(**n**) results , Cohran's test statistic C is

$$C = s^2_{max} / \sum s^2$$

where s-standard deviation

It is assumed that data points in all groups are normally distributed,sample size in each group are same and Cohran's test is used for maximum variance only.The critical Cohrans value are given in Table-2.

(i) If a test statistic is less than or equal to its 5% critical value,the item tested is accepted as correct.

(ii) If the test statistic is greater than its 5% critical value and less than or equal to its 1% critical value ,the item tested is called a straggler and is indicated by a single asterisk.

(iii) If the test statistic is greater than its 1% critical value,the item is called a statistical outlier and is indicated by a double asterisk.

The time different standard deviation is calculated by

$$S_{[T]} = \sqrt{\frac{1}{t(n-1)}} \sum_{j=1}^{t} \sum_{k=1}^{n} (y_{jk} - \overline{y})^2$$

Where t-number of days and n-replicate per day.

Table-2 Critical upper limit for(C_{UL}) Cohran's test (Where p-number of days/operator and n-number of replicates in each day/group)

p	N=2		N=3		N=4		N=5		N=6	
	1%	5%	1%	5%	1%	5%	1%	5%	1%	5%
2	-	-	0.995	0.975	0.979	0.939	0.959	0.906	0.937	0.877
3	0.993	0.967	0.942	0.871	0.883	0.798	0.834	0.746	0.793	0.707
4	0.968	0.906	0.864	0.768	0.781	0.684	0.721	0.629	0.676	0.590
5	0.928	0.841	0.788	0.684	0.696	0.598	0.633	0.544	0.588	0.506
6	0.883	0.781	0.722	0.616	0.626	0.532	0.564	0.480	0.520	0.446

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7	0.838	0.727	0.664	0.561	0.568	0.480	0.508	0.431	0.466	0.397
8	0.794	0.680	0.615	0.516	0.521	0.438	0.463	0.391	0.423	0.360
9	0.754	0.638	0.573	0.478	0.481	0.403	0.425	0.358	0.387	0.329
10	0.718	0.602	0.536	0.445	0.447	0.373	0.393	0.331	0.357	0.303
11	0.684	0.570	0.504	0.417	0.418	0.348	0.366	0.308	0.332	0.281
12	0.653	0.541	0.475	0.392	0.392	0.326	0.343	0.288	0.310	0.262
13	0.624	0.515	0.450	0.371	0.369	0.307	0.322	0.271	0.291	0.243
14	0.599	0.492	0.427	0.352	0.349	0.291	0.304	0.255	0.274	0.232
15	0.575	0.471	0.407	0.335	0.332	0.276	0.288	0.242	0.259	0.220
16	0.553	0.452	0.388	0.319	0.316	0.262	0.274	0.230	0.246	0.208
17	0.532	0.434	0.372	0.305	0.301	0.250	0.261	0.219	0.234	0.198
18	0.514	0.418	0.356	0.293	0.288	0.240	0.249	0.209	0.223	0.189
19	0.496	0.403	0.343	0.281	0.276	0.230	0.238	0.200	0.214	0.181
20	0.480	0.389	0.330	0.270	0.265	0.220	0.229	0.192	0.205	0.174
21	0.465	0.377	0.318	0.261	0.255	0.212	0.220	0.185	0.197	0.167
22	0.450	0.365	0.307	0.252	0.246	0.204	0.212	0.178	0.189	0.160
23	0.437	0.354	0.297	0.243	0.238	0.197	0.204	0.172	0.182	0.155
24	0.425	0.343	0.287	0.235	0.230	0.191	0.197	0.166	0.176	0.149
25	0.413	0.334	0.278	0.228	0.222	0.185	0.190	0.160	0.170	0.144

Horwitz formula

Horwitz equation is an empirical relationship between the concentration of the analyte and the precision of the method. The relative standard deviation(RSD) varies with concentration , C , the dimensionless mass fraction.The approximate value for predicted relative standard deviation is calculated by the formula

Predicted RSD, **PRSD = C $^{-0.15}$**

The maximum acceptable limit of RSD shall be twice the PRSD from Horwitz equation.

3. DISCUSSION

The method verification is done as follows.

1. **Suitability of Test Conditions**: Reagents, glasswares and equipments AR Grade potassium chromate, silver nitrate and CRM Grade Sodium chloride, calibrated glasswares and calibrated electronic balance with accuracy ensured by intermediate checks are used.
2. **Specificity**: Since the basic reaction is the formation of the precipitate of AgCl , no verification is required.
3. **Repeatability**: A Iodised salt sample was homogenized and analysed seven times in a day.The data evaluation is given in Table-3 and Table-4.

Table-3 Repeatability Data Evaluation

Sl No	Weight of Sample (g)	Make up Volume (ml)	Volume Pipette(ml)	Volume Burette(ml)	Sodium chloride Content
1	1.1137	100	10	18.8	98.4986
2	1.1353	100	10	19.5	100.2223
3	1.0440	100	10	17.6	98.3677
4	1.0783	100	10	18.2	98.4855
5	1.1124	100	10	18.8	98.6403
6	1.1175	100	10	19.0	99.2079

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7	1.2359	100	10	21.0	99.1462
mean	1.119586	100	10	18.98571	98.93836
Standard Deviation , S = 0.655403					
Grubbs Test G_{max} = 1.959014 G_{min} = 0.870697 $G_{critical}$ = 1.94 As $G_{max} > G_{critical}$ Data 2 is an outlier					

4. Recalculated data

Table-4 Repeatability Data Evaluation (Recalculated**)**

Sl No	Weight of Sample	Volume Make up	Volume Pipette	Volume Burette	Sodium chloride
1	1.1137	100	10	18.8	98.4986
2	1.0440	100	10	17.6	98.3677
3	1.0783	100	10	18.2	98.4855
4	1.1124	100	10	18.8	98.6403
5	1.1175	100	10	19.0	99.2079
6	1.2359	100	10	21.0	99.1462
mean	1.116967	100	10	18.9	98.72437
Standard Deviation S = 0.361678					
Grubbs Test G_{max} = 1.336917 G_{min} = 0.986145 $G_{critical}$ = 1.82 No outlier.Data set acceptable.					

5. Within laboratory Precision

The same homogenized sample was analysed for next six days in triplicate.The data evaluation is given in Table-5.

Table-5 Intermediate Precision Data Evaluation

Day	Weight of Sample	Volume Burette	Result(X)	(X-M)	$(X-M)^2$	Cohrans Value
Day-1	1.7126	28.8	98.1244	-0.07613	0.005796	**0.6511** With P=6,N=3 and α =0.05 Cohran Critical Value = **0.72** No outlier .All data set acceptable.
	1.0923	18.3	97.7574	-0.44313	0.196367	
	1.1644	19.7	98.7198	0.519267	0.269638	
Mean (M)	1.3231	22.26667	98.20053	Sum Difference2 = 0.471801		
Day-2	1.0151	17.3	98.4538	1.014133	1.028466418	
	1.0492	17.7	98.4462	0.006533	4.26844E-05	
	1.2819	21.4	97.4190	-1.02067	1.041760444	
Mean(M)	1.1154	18.8	98.43967	Sum Difference2 = 2.070269547		
Day-3	1.2615	21.1	97.6066	-0.22603	0.051091068	
	1.1665	19.6	98.0518	0.219167	0.048034028	
	1.0736	18.0	97.8395	0.006867	4.71511E-05	
Mean(M)	1.1672	19.5667	97.83263	0.099172247		
Day-4	1.2064	20.3	98.1255	0.2989	0.089341	
	1.0918	18.3	97.7429	-0.0837	0.007006	
	1.0637	17.8	97.6114	-0.2152	0.046311	
Mean(M)	1.120633	18.8	97.8266	0.142658		
Day-5	1.2411	20.9	98.2012	-0.03603	0.001298	

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	1.1735	19.9	98.8888	0.651567	0.424539	
	1.2186	20.4	97.6217	-0.61553	0.378881	
Mean(M)	1.211067	20.4	98.23723	0.804719		
Day-6	1.0934	18.4	98.1332	0.19215	0.036921622	
	1.2106	20.3	97.78508	-0.15597	0.024326641	
	1.0483	17.6	97.90487	-0.03618	0.001308992	
Mean(M)	1.117433	18.76667	97.94105	0.062557256		
Total Mean	1.146347	19.26667	98.05544	$\sum(X-M)^2$ = 3.179376		
SD-Time $S_{[T]}$ = 0.514731			$RSD_{[T]}$ = 0.5249			

Predicted RSD (Horwitz equation), PRSD = 1.003

The maximum acceptability limit (2PRSD), MAL = 2.006 (approx 2). Since $RSD_{[T]}$ < MAL , all data are also acceptable as per Horwitz calculation.

Thus, in this test method, the acceptability criteria can be fixed at a RSD of 2.0 which can be used for evaluating the competence of another operator.

The verification study can be summarized in Table-6.

Table-6 Verification Documentation

Attributes	Reference Method IS 253	Within Lab	Remarks/Comment
Equipments	1.Volumetric Flask , 100 ml 2.Burette , 50 ml 3.Pipette , 10 ml 4.Electronic balance	1.Volumetric Flask , 100 ml (Calibrated) 2.Burette , 50 ml (Calibrated) 3. Pipette , 10 ml (Calibrated) 4.Electronic balance , readability 0.0001 g (calibrated)	Verified
Reagents	1.Potassium chromate 2.Silver nitrate 3.Silver nitrate solution (approx. 0.1 N) 4.Sodium chloride	1.Potassium chromate , AR Grade 2.Silver nitrate , AR Grade 3.Silver nitrate solution , 0.1 N standardized using Sodium chloride solution 4.Sodium chloride , CRM	Verified
Specificity (Basic Principle)	Precipitation of silver chloride	Since basic reaction is the same , no verification required	N/A
Repeatability	--	Data acceptable Grubbs Method	Verified
Intermediate Precision	--	Data acceptable Cohran Test	Verified
Acceptability Limit	--	RSD = ± 2 (apporx.) Horwitz Method	Verified

4. CONCLUSION

The ISO 17025 standard requires the laboratories to verify methods but does not provide any guidelines to fulfils it. This paper suggest a method to verify titration method.

The analytical data are successfully evaluated for method verification under clause 7.2.1.5 of ISO 17025 standard. This is done in simple and easily understandable way.

The laboratories can follow the evaluation method used in this study not only for method verification, but also for measurement uncertainty and ensuring validity of their test results.

5. CONFLICT OF INTEREST

The author has no conflict of interest.

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