**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

**I.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.

**II.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

**Gexp = (Xmax – Xmean) / s**  (for highest data point) **Gexp = (Xmean – Xmin) / s** (for lowest data point)

A value can be regarded as an outlier if the statistic G value (Gexp) is greater than critical value G(α,n)

(Table-1).Then the mean and standard deviation are recalculated excluding the outlier.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **n** | **G(α,n) for α = 1 %** | **G(α,n) for α = 5%** | **n** | **G(α,n) for α = 1 %** | **G(α,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. | | |

**Table-1 Critical values for Grubbs Test**

**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 = s2max /∑s2**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.

1. If a test statistic is less than or equal to its 5% critical value,the item tested is accepted as correct.
2. 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.
3. 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] =**

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

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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 |
| 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 |

**Table-2 Critical upper limit for(CUL) Cohran’s test** (Where **p**-number of days/operator and **n**-number of replicates in

each day/group)

**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****.*

**III.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.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 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 |
| 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 Gmax = 1.959014 Gmin = 0.870697 Gcritical = 1.94  As Gmax > Gcritical Data 2 is an outlier | | | | | |

**Table-3 Repeatability Data Evaluation**

**Recalculated data**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 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 Gmax = 1.336917 Gmin = 0.986145 Gcritical = 1.82  No outlier.Data set acceptable. | | | | | |

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

**4.Within laboratory Precision**

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

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 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 |
|  | 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 | ∑(X-M)2 = 3.179376 | |
| SD-Time S[T] = 0.514731 | | | RSD[T] = 0.5249 | | |  |

**Table-5 Intermediate Precision Data Evaluation**

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.

|  |  |  |  |
| --- | --- | --- | --- |
| 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 |

|  |
| --- |
| **Sl. No** |

**Table-6 Verification Documentation**

**IV.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.

**V.CONFLICT OF INTEREST**

The author has no conflict of interest.

**VI.REFERENCE**

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