Title of the Assignment:

The Selection and Use of Reference Materials in Pharmaceutical and Clinical Laboratories.

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Table of Content

1. Introduction:

Reference materials have significant role in scientific experiment because of its wider range of use in different purposes in research especially pharmaceutical and medical laboratories. There are different criteria are established for selecting and using of reference materials according to intend to be use. As the scientific development increasing day by day there is another issue also considers for the development and that is the reliability which is the conjunction of two words accuracy and precision where accuracy is greatly related with analytical value of the reference material. We know that closeness between the results from measurement and true value is termed as accuracy and true value comes from the analysis of reference materials. For the complexity of use of the reference materials there are some guidelines was established by the International Standard Organization (ISO), World Health Organization (WHO), European Commission (EC) and US Food and Drug Administration (FDA) for the selection of reference material and for future use. In pharmaceutical laboratory Reference materials having great role in the proficiency testing, reliability of the measurement but in clinical laboratory

2. Early development of Reference Material:

Reference materials have an extensive range of use in analytical chemistry since analytical chemistry was developed in 19^{th} century. During that time all chemicals were not much more purified as like 21^{st} century so suitable purified materials as primary standard materials had used for analysis. According to Stoeppler, M. and Bowen, H.J. (2001) , Gay-lussac (1824) has been first recommended the primary standard for that purpose and it was As (III) oxide additionally Sorensen (1887) was acknowledged some criteria for selecting primary chemical standard and which was then modified by Wagner (1903). In 1880 a group of analytical chemist in London took an initial step to introduce biological RM for determination of amount of fat in milk. For the fulfillment of increasing demand for RM the US National Bureau of Standards (NBS) now National Institute of Standard and Technology (NIST) was established in 1901(Stoeppler, M. and Bowen, H.J. 2001) and In 1906 NBS took a program to supply reference materials for comparing the analytical results between laboratories and different countries in industrial

uses (Zschunk, 2001). According to Bell (1951) the US Pharmacopoeia introduce a method for testing purity and quality in volume VI issued in 1880 but there was no description about use of reference materials for testing purity up to 1950 for pharmaceuticals. The only biological references were few sera until 1950 but the pioneer was Humphry Bowen for developing biological matrices RMs and prepared 100 kg kale *(Brassica oleracea)* in 1960 (Stoeppler, M. and Bowen, H.J. (2001) which was influenced for further development of RMs and enhance the planning, distribution and analysis of materials. Now a day each and every section of chemistry and applied chemistry as well as branches of chemistry is depends on RMs for measuring quality and are used for method validation, calibration, estimation of measurement uncertainty, and training, internal quality control (QC) and external quality assurance (QA) purposes.

3. Definitions of different types of Reference Materials:

3.1. Reference Material (RM):

According to ISO guide (30), Reference materials are the substances which are satisfactorily identical and constant with respect to some specified properties and have been recognized to be in favor of purpose in an analytical practice (Emons et al., 2006). Usually reference materials can be found as a pure or mixed gas, liquid or solid and widely used in pharmaceutical, clinical, food industries, cosmetics and research laboratories for measurement and calibration process.

3.2. Certified Reference Material (CRM):

According to ISO Guide (33), Reference materials accompanied by an official document and the values of the property are eligible through a suitable procedure which develops the traceability to a true detection level and the certified values are go with an uncertainty statement at a declared level of confidence (Deak,A. 1999).

3.3. Standard Reference Material (SRM):

According to May, W et al (2000) SRM is the CRM which is issued by the NIST and having specified certificates of analysis that report the results of their characterization and provide information regarding the appropriate uses of the material.

3.4. Pharmaceutical Reference Materials (PRM):

According to Pragst, F and Kulpmann, W.R. (2000), Pharmaceutical reference materials are produced as per guidelines to monographs of international or national pharmacopoeia like European Pharmacopoeia (EP) or the United States Pharmacopoeia (USP). These are high grade and certified standard because of their purpose of use.

3.5. Working RM:

According to Pan Xiu Rong and Zhao Min (1999), working RMs are those which are usually used in routine analysis of instrument, calibration of analytical method and for assessment of the analytical techniques. Working RM is also known as the secondary reference materials or quality control materials in pharmaceutical and clinical labs.

4. Traceability of RMs:

According to International Vocabulary of basic and general terms in Metrology (VIM) Popescu, Ileana Liliana et al (2002) stated that "Traceability is the property of the result of a measurement or the value of a standard whereby it can be related to stated reference, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties"

If reference materials are used for calibration they establish traceability of chemical measurements. Every measurement, calibration, reference materials, quality issues, accreditation, methods should be traceable regarding with international standards. According to Bievre, P.De (2000) traceability of reference material is also referred to as "Trackability" due to its importance. He also stated that one of the traceability functions is situated within the traceability series, as the reference material is utilized as an additional 'internal standard'. Then, the value approve by the reference material is vital

for set up the traceability of the calculated value of an unknown sample. In the next function, the reference material may be used as an 'amount standard'. According to Gills, T.E (1999) there are two components regarding traceability, these are traceability measurements with SI unit and traceability linkage to other national measurement institutes and international measurements.

5. Selection of RMs in Pharmaceutical and clinical Laboratories:

According to Wise,S.A. and Emons,H. (2007) The 10th International symposium on Biological and Environmental Reference Materials (BERM 10) was held for discussion about proper use of reference materials for biological purposes and the selection criteria also discussed on that symposium. As the different organization prepared lots of pharmaceutical and clinical reference materials then the selection of well characterized reference materials is difficult. During the selection of reference material there are some points should be considered according to pharmaceutical and clinical use like, availability of RM, suitability of RM in favor of uncertainty, certifying methods, date of certification, expiration date, sampling size, storage condition and special instruction if necessary. Gancberg,D. (2008) stated that there are some general guidelines were established by ISO Guide 32 (17) and 33 (17) and Eurachem Guide (19) for selecting and use of reference materials.

5.1. Selection criteria:

As the selection of RMs is very difficult for analytical purpose though during the selection of RMs the following basic information are considered for the purpose of use.

Matrix of RM: For selection of RM, the matrix of RM must have the similar with the determinant because if a wrong matrix is use that may seem to be wrong result.

Form of RM: Reference materials are generally different physical properties like solid, gas and liquid. Use of these forms depends upon the measurement carried out and the principle of measuring method.

Certified value and uncertainty of RM: The Certified value and uncertainty of RM should consider in the economical factor, as seeking higher accuracy is time consuming and matter of cost so according to Rong, Pan Xiu and Min, Zhaon (`1999), metrologists use one third ($1/3^{rd}$) principle when choosing metrological standard that means the uncertainty of metrological standard must not exceeds 1/3 of uncertainty of measurements and may be taken as a reference of choosing RMs.

Homogeneity and Stability of RM: Homogeneity of RM is essential because as the practical sampling size is less than the RM sampling size, it may create the heterogeneous error. Stability of RM is important because of its validity time must meet with the long term quality control.

5.2. Requirement for Using RM:

As the purpose of using RM in pharmaceutical and clinical laboratories the procedure of use of the RMs is preliminarily prepared and the procedure may contain the information regarding with analytical method, operation procedure and the measuring conditions. In that instances the measured results are provided based on the measuring data and the uncertainty of certified value of RM used. These requirements are very important for the successful analysis in pharmaceutical and clinical labs.

6. Purpose of Reference Materials in Pharmaceutical and Clinical Laboratories:

Reference materials usually use for the following purposes in the Pharmaceutical a clinical laboratories, these includes:

6.1. Validation of Methods:

In pharmaceutical laboratory, usually a method is validated by the use of CRMs and the values are known as certified values. Validation of the pharmaceutical analysis method is the proof of sufficient compliance between the results for the determinant contents of a CRM achieved by use of the analytical method under proper investigation and the certified values. This certified value should be accurate and precise that is it must be

reliable. Now further analyses which are carried out with the sample are compared with the certified values.

6.2. Calibration of Instruments:

Calibration with certified reference materials is special for the methods that are normally analyzed by solid samples. There are two types of calibration method is normally recognized

Direct Method: In the direct calibration method a calibration curves are use directly resulting from the usage of CRMs of some sorts of materials. If the analytical procedures are routinely used an uneconomically high consumption of the expensive CRM is need for that purpose.

Indirect Method: In the indirect analytical calibration method there is an internal reference materials or quality control materials in pharmaceutical industries which are produced in the internal laboratory techniques and then are calibrated with the original CRMs and thus there is a metrological relationship is developed. Further calibration of the sample is then carried out with the internal reference materials of quality control materials.

6.3. Measurement of Uncertainty:

Uncertainty can be defined according to International vocabulary of basic and general terms in metrology (VIM), is the parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand. Estimation of bias is one of the most important factors for the method validation. The uncertainty of the certified reference values should be compatible with the reliability requirement. In the selection of CRM the level of uncertainty should be considered in the purpose of use and availability, physical and chemical suitability is also taking into account.

6.4. Accuracy testing of an analytical procedure:

There are several ways for testing the accuracy of an analytical method. CRMs may be use directly or indirectly that is use of an intermediate reference standards or QC reference materials.

Introduction of a Validated Method: In this method an already validated method is used for the accuracy testing. That means the analytical results are compared with the certified values and the closeness between the certified values and the test result indicates the accuracy. Here one thing should be considered during the test is that the laboratory conditions. Laboratory condition should be specified. Any deviation of the laboratory test results from the certified values of the CRM cannot be qualified to the analytical method in practice. In meaningful differences observed in the certified values of the content and the data obtained from the test results then the test method is checked for determining the fault and recover the problem and this is until the satisfactory results is obtained.

Continuous Testing of Accuracy: In this process, continuous testing is done constantly with different interval. A CRM or a test sample is analyzed by calibrating with the CRMs regularly at certain period of time in the same procedure as analytical samples. In case of any significant deviation it's easily find out the problem in the sample testing.

6.5. Proficiency Testing:

According to accreditation of the pharmaceutical analysis, the proficiency proof of analytical laboratories being carried out in different measurements by means of appropriate tests are necessary because of the regulatory bonded with the law. For this instances CRMs are used in different ways,

Proficiency Testing by Interlaboratory Comparisons: Accrediting association arranges an interlaboratory test and dispenses pre characterize material; the participating laboratories should able to meet the prearranged limit of analyte contents in the materials. Due to high cost of CRMs working RM or quality control materials are frequently used for the proficiency testing. This proficiency testing is carried out constantly between the laboratories due to the maintenance of high quality product production. In pharmaceutical industry there is a quality control chart is used for determining the quality of the pharmaceutical active ingredients.

6.6. Quality Assurance and Quality Control:

Pharmaceutical ingredients that are controlled in quality are allowed for the long tine used in the pharmaceutical purposes. This is done by the proficiency testing and a quality control chart is used for comparing the values obtained from the test result and the certified values. If there is any deviation from the quality control chart then the pharmaceutical ingredient should not use in further process. Caroll, T.A. et al (2003) stated that, a method was established by the Westgard for the proficiency testing and the rules are known as Westgard's rules. According to Westgard's rule Clinical reference are out of control i.e. should not use in the subsequent conditions;

Rule a: (1-3s). If 1 controls inspection is more than 3s ahead any side of the mean.

Rule b: (2-2s). If 2 successive control inspection are more than 2s from the mean and both in the same side of the mean.

Rule c: (4-1s). If 4 successive control inspection are more than 1s in same side of the mean.

Rule d: (R-4s). The dissimilarity of the largest and the smallest observations is over 4s.

Rule e: (10X). If 10 successive control observation is laid same side of the mean.

Rule f: (1-2s). If 1 control value go beyond 2s on any side of the mean, its "warning sign" but nor out of control.

6.7. Production of Secondary Reference Materials:

As the original CRMs are highly expensive so it is very difficult to perform the laboratory testing. For this instances a secondary reference materials also known as the quality control materials or working RM or in-house RM is produced by the own laboratory and some characteristics should be maintained during the production of secondary reference materials or quality control materials.

7. Suitability of the Reference Materials for Assessment:

According to Walker, R. (1999), a code of behavior for assessing the suitability of RMs in pharmaceutical and clinical laboratories is defined and these are discussed below. Appropriateness and the fitness of purpose are greatly considered during the time of assessment. Factors to be considered include the following:

1. The suitability of a reference material depends on the details of the analytical specification. The features those are important:
    - Determinant
    - Calculation vary
    - Matrix equivalent and prospective interferences
    - Sample volume
    - Homogeneity and constancy
    - Calculation of Uncertainty
    - Measurement of assigning values
2. The authenticity of the ‘certification’ and uncertainty data
3. Information details of the manufacturer and the substance.
4. Certified with the confirmation of report.
5. Established way of the production of the RMs with superiority standards such as ISO Guides or ILAC.

All or some of the requirements may be specified by the pharmaceutical specification though it normally specified by the pharmaceutical analyst to use professional judgment in the pharmaceutical laboratories.

8. Some Examples of the Reference Materials used in the Pharmaceutical and Clinical laboratories:

According AOAC International (2010) Examples of the reference materials that are frequently used in the pharmaceutical and clinical laboratories are noted below:

Examples of RMs represent measures:

a) Certified pure water that are normally used for the calibration of Viscometer;
b) Intrinsic concentration of the substances in the Cholesterol that is absent in the Human serum preferably used only as a determination of precision control material;
c) A portion of Dioxin contained Fish tissue used as a calibrator.

Examples of RMs represent measures insignificant property:

a) color chart indicating one or more specified colors;
b) A precise nucleotide chain contained by the DNA compound;
c) Urine having 19-androstenedione

Example of the CRM

> Human serum which is assigned with quantity value for the concentration of cholesterol and associated measurement uncertainty stated in an accompanying certificate, used as a calibrator or measurement trueness control material.

Some commercially available human reference materials are Human serum, Human urine, Blank human serum, Drug free urine, Defiibrinated plasma (Pragst, F. and Kulpmann, W.R., 2000) used for the negative control test in clinical laboratories.

9. Conclusion:

Reference materials and the certified reference materials are applied in different important purpose in the pharmaceutical and clinical laboratories but the selection criteria are somewhat difficult. Selection of the RMs is fully depends upon the intended to be use and the analyst who carried out the analysis. Use of RMs is varied between the two laboratories as well as among analyst. In pharmaceutical and clinical laboratories, a CRM is accepted because of the better matching with the samples as uncertainty in the certified property may be preferred. Uncertainty is determined then the difference between the composition of CRM with the samples. Traceability is also considered during the selection process and the proficiency testing among laboratories is also successfully established by the proper selection of the CRMs. RMs having great variety role in the method validation, instrumental calibration, traceability and uncertainty of the RMs, proficiency testing and the quality control and quality assurance department for the qualitative and quantitative analysis. Different types of pharmaceutical and clinical reference materials are found commercially and these are accreditated by the accreditation organization internationally. The updated knowledge and skill of the pharmaceutical and clinical analyst are required for selecting the proper RMs as it intended to be use and for calculating the uncertainty of the measuring results.

10. Abbreviations:

RM= Reference Material

CRM= Certified Reference Material

SRM= Standard Reference Material

PRM= Pharmaceutical Reference Materials

ISO= International Standard Organization

WHO=World Health Organization

EC = European Commission

FDA= Food and Drug Administration

NBS = National Bureau of Standards

NIST= National Institute of Standard and Technology

USP= United States Pharmacopoeia

EP= European Pharmacopoeia

VIM= International Vocabulary of basic and general terms in Metrology

BERM = Biological and Environmental Reference Materials

QC= Quality Control

QA= Quality Assurance

ILAC= International Laboratory Accreditation Cooperation

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