APPENDIX 10: GUIDELINES FOR THE SUBMISSION OF PROTOCOL OF ANALYSIS AND ANALYTICAL METHOD VALIDATION DOCUMENTS

10.1 Guidelines for the Submission of Protocol of Analysis

I. General Requirements

1. The Protocol of analysis must be in a standard format that contains information as stated below:-
    a. Product name
    b. Name and address of manufacturer
    c. Name, signature and designation of authorized person
    d. Effective date
    e. Review date

2. Protocol of analysis must consist of all test methods and specifications that are carried out by the manufacturer. Standard pharmacopoeias, for example, BP/USP can be used as references. The tests and specifications in the pharmacopeias are the minimum requirements.

3. Photocopies of methods/ methods directly copied from pharmacopoeias are not acceptable. Manufacturers can use methods from those standard references but must have their own written and detailed procedure.

4. Manufacturers must confirm that all test methods in their protocol of analysis perform as expected. Copies of chromatograms (HPLC/GC/TLC), UV spectrum etc must be submitted together with the protocol of analysis.

5. Protocol of analysis must be properly ordered with proper numbering for all tests and specifications.

6. All references stated in the protocol of analysis must be submitted and clearly labeled.

7. Protocol of analysis submitted must be in either Bahasa Malaysia or English. Protocol of analysis in other languages will be rejected.

8. An authorized copy of latest certificate of analysis for the product concern must be submitted with the protocol of analysis.

II. Specific Requirements

1. Identification test
    a. List of equipment and apparatus required.
    b. List of chemical/ reagents
    c. Preparation of sample and standard solutions.
    d. Details of method and procedures.
    e. Specification and acceptance criteria

2. Physical test (friability, uniformity of weight, pH, viscosity, etc).
    a. List of equipment required together with test parameters
    b. Sample preparation (if any)
    c. Specification and acceptance criteria

3. Disintegration test
    a. Equipment required
    b. Test parameters
    c. Test medium
    d. Specification

4. Dissolution test
    a. Equipment and apparatus required
    b. List of chemical / reagents required
    c. Test parameters i.e. type and volume of dissolution medium, rotation rate, temperature of solution and time
    d. Preparation of dissolution medium, preparation of sample and standard solution (if any), etc
    e. Type and method of analysis (HPLC, UV, etc) and test procedures. For example, if HPLC method is used, test method has to include the preparation of mobile phase, brand and type of column used, run time, detector used (UV, RI, etc), injection volume, system suitability test and other parameters
    f. Typical chromatograms / UV spectrum for sample & standard solution, system suitability etc
    g. Complete formula for calculation. For example, 'slow release' products calculation must include quantity of active substance in the medium volume which have been taken out for analysis
    h. Test specification

5. Impurities / degradation / purity test
    a. List of equipment and apparatus required
    b. List of chemical and reagents required
    c. Preparation of sample and standard solutions
    d. Detailed method and procedures
    e. Complete formula for calculation
    f. Typical chromatogram of system suitability test, sample & standard solutions if applicable
    g. Specification / acceptance criteria

6. Assay and uniformity of content
    a. List of equipment and apparatus required
    b. List of chemical and reagents required
    c. Preparation of sample and standard solution
    d. Detailed method and procedures
    e. Complete formula for calculation
    f. Typical chromatogram/spectrum of system suitability test, sample & standard solutions if applicable
    g. Specification / acceptance criteria

7. Pyrogen / abnormal toxicity test
    a. List of equipment, apparatus, glassware and reagents required
    b. Preparation of sample solution and injection dose
    c. Test method & procedure
    d. Test interpretation
    e. Test specification

8. Bacterial Endotoxins Test (LAL)
    a. List of apparatus, glassware and reagents required
    b. Preparation of standard solution, LAL reagent/substrate and sample

c. Determination of MVD (Maximum Valid Dilution) and endotoxin limit
d. Detailed test procedure
e. Calculation and interpretation of test result
f. Test specifications

9. Microbial Limit Test

9.1 Determination of microbial contamination test
i. List of apparatus and culture required
ii. Preparation of test medium and growth promotion test
iii. Sample preparation including method for neutralizing of preservatives for samples that contain preservatives
iv. Complete test procedure by 'surface spread' for bacteria and 'pour plate' for fungi
v. Colony counting
vi. Specification and acceptance criteria

9.2 Test for specified microorganisms and total viable aerobic count
i. List of apparatus and culture required
ii. Preparation of test medium and growth promotion test
iii. Sample preparation including method for neutralizing of preservatives for samples that contain preservatives
iv. Complete test procedure for each of specific microorganism involved
v. Observation on colonies presence
vi. Specifications and acceptance criteria

10. Sterility test
a. List of apparatus required
b. List of biological and chemical substance required:-
i. Culture medium
ii. List of rinsing solution, buffer solution and diluent
iii. Neutralizing agent (if any)
iv. List of specific type cultures required
c. Method used (e.g. membrane filtration method, direct inoculation, etc)
d. Method of preparation of the following solutions/materials:-
i. Culture medium (e.g. Fluid Thioglycollate Medium and Soyabean Casein Digest Medium)
ii. Rinsing solution, buffer solution and diluents
iii. Neutralizing agent (if any)
iv. Microorganism culture
e. Growth promotion test for medium used in sterility testing (specific aerobes, anaerobes and fungi)
f. Preparation of sample solution (including neutralizing procedure of antimicrobial agent for antibiotic samples and samples which contain preservatives)
g. Complete test procedure for sterility test
h. Specifications and acceptance criteria
i. Validation procedure & validation data (if applicable)

11. Microbiology assay
a. List of apparatus required
b. List of biological and chemical substances required
c. Procedure for the preparation of following solutions/substances:-
i, Culture mediums

ii. Rinsing solutions
iii. Buffer solutions
iv. Diluents
v. Microorganism culture used in assay

d. Test method (e.g. agar diffusion, turbidimetric, randomized block, dose, etc)
e. Test procedure
   i. Preparations of solutions containing antimicrobial agents which may be present in the sample to be tested (if applicable)
   ii. Preparation of standard solutions (including any steps to counteract the antimicrobial properties of any preservatives, etc present in the sample)
   iii. Preparation of test solutions (including any steps to neutralize the antimicrobial properties of any preservatives, etc present in the sample)
   iv. Dilution schemes for test and standard solutions
   v. Application of test & standard solutions (volume, latin squares, etc)
   vi. Incubation temperature & time
   vii. Procurement of test data
f. Complete calculation for the test including ANOVA tablet and other data showing validity of test results
g. Specifications and acceptance criteria

10.2 Guideline for submission of analytical method validation documents.

1. Introduction

The requirements for the submission of the analytical method validation data and documents by the industry to the Drug Analysis Division, National Pharmaceutical Regulatory Agency (NPRA) are presented in this guide

All the analytical validation done by the industry should be in accordance to ASEAN and ICH Technical Requirements Guidance Documents specifically:-

Q2A: Text on validation of analytical procedures, 1994
Q2B: Validation of analytical procedure: methodology, 1996

2. Requirements

The industry is required to submit the following documents for evaluation by NPRA:-

a. Analytical method protocol for the testing of the raw materials (only the active pharmaceutical ingredients (API) and preservatives if any). This should include the specifications and certificate of analysis. All analytical test procedures where possible should be in accordance with the official monograph of that ingredient in the latest edition of the official pharmacopoeia such as British Pharmacopoeia, United States Pharmacopoeia and WHO.

b. Analytical method validation protocol for the finished product. The protocol of analysis should be in accordance with NPRA's guidelines for the submission of protocol of analysis.

c. Protocol for the analytical method validation procedure carried out on the finished product. This procedure should include all details about the validation process including preparation of all solutions used – standards, samples, placebo etc,

detection methods, test conditions, equipment used, statistical analysis & evaluation, calculations etc.

Types of analytical procedures to be validated includes:-

i. Identification tests
ii. Quantitative tests for impurities' content
iii. Limit tests for control of impurities
iv. Quantitative tests of the active ingredient in the sample
v. Pyrogen/ Bacterial endotoxin test
vi. Sterility test

A brief description of the type of tests considered in this document is provided below:-

Identification tests are intended to ensure the identity of an active ingredient in the sample. This is normally achieved by comparison of a property of the sample e.g. spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard.

Testing for impurities can be either a quantitative test or a limit test for the impurity in the sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.
Assay procedures are intended to measure the content of active pharmaceutical ingredient present in a given sample. The analytical data submitted must be able to support the claim that the analytical method employed has been validated.

Pyrogen Test and Limulus Amebocyte Lysate Test -
Relevant validation data for pyrogen test and Limulus Amebocyte Lysate Test include product independent data such as equipment validation, validation of temperature system, lysate sensitivity and product dependent validation data such as inhibition/ enhancement studies and validation for routine LAL tests according to the type of LAL test method employed eg. Gel Clot method, quantitative end point method or quantitative kinetic method.

Sterility testing applied to products that are required to be sterile. A satisfactory result indicates that no contaminating microorganism has been found in the sample examined in the condition of the test. For sterility testing it is imperative that the testing procedure adopted by the manufacturers include all aspects of validation of the testing method including the precautions against microbial contamination.

d. Complete set of data obtained from the validation process. These include all raw data such as weights used, chromatograms, tabulated sets of value as well as graphs, statistical analysis & evaluation, calculations & formulae etc. Summary of data will not be accepted. Acceptance criteria for each characteristic/ parameter should also be submitted. For products tested using analytical methods described in official pharmacopeias, users are not required to validate accuracy and reliability of these methods, but must submit data verifying their suitability under actual conditions of use.

1. Certificate of analysis of three (3) recent batches of the finished product.
2. Certificate of analysis for one batch of API used in the product.
3. Summary on the validation process together with conclusion reached.