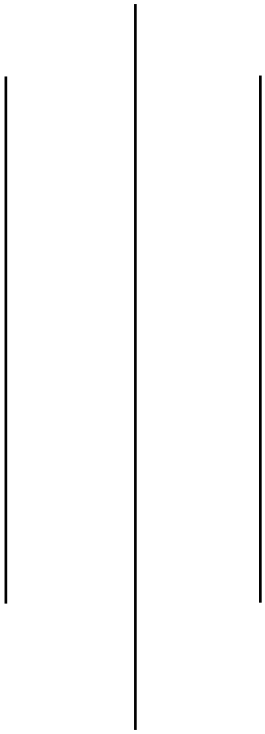
AMVP/076-77-02

Guideline on Analytical Method Validation on Non-pharmacopoeial Products for Regulatory Approval



Government of Nepal

Department of Drug Administration

National Medicines Laboratory

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Contents

Abbreviations

abs.	Absorbance
AMV	Analytical Method Validation
API	Active Pharmaceutical Ingredient
COA	Certificate of Analysis
Conc.	Concentration
DAC	Drug Advisory Committee
DAD	Diode Array Detector
DDA	Department of Drug Administration
FDC	Fixed Dose Combination
FPP	Finished Pharmaceutical Products
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
LT	Less than
MA	Method of Analysis
Med.	Medium (dissolution)
mg	milligram
MT	More Than
NLT	Not Less Than
NML	National Medicines Laboratory
NMT	Not More Than
NPP	Non pharmacopoeial product
SIM	Stability Indicating Method
SOP	Standard Operating Procedure
Std.	Standard
UV	Ultra Violet
VIS	Visible
WHO	World Health Organization

Preface

This guideline has been prepared to aid on method selection, execution of validation performances, analytical method approval, verification of approved method and documents that are required for submission during registration/market authorization of non-pharmacopoeial products. The document will support both regulatory body and manufacturer by standardizing the analytical method validation process.

Different international guidelines such as USFDA, ICH, WHO, USP BP, IP and publications are taken as a reference for the preparation of this guideline.

This guideline is a revised and updated form of "Protocol on analytical method validation for non-pharmacopoeial products for regulatory approval" and has been prepared by the support of WHO. It deals with the categorization of non pharmacopoeial medicinal products which is completely revised and taken as a basis for selection of method for analytical method-modification, validation performances using HPLC, UV-VIS spectroscopy, titration and microbiological methods. In addition to this, it deals with the verification procedure for validated approved method and development of alternative method.

The overall goal of this guideline is to support in achieving the highest practicable method of analysis, updated, simplified guidelines for the process of evaluation of quality control documents of non pharmacopoeial products to ensure safety, quality and efficacy of non pharmacopoeial products for the protection of public health of the country as envisaged by the Drug Act 2035 and Drug Category Rules, 2043. The guideline will also help DDA and NML in regulation and evaluation of non-pharmacopoeial products.

This guideline will be updated periodically as per the requirement

1.0 Background:

Drug Category Rules 2043, Rule 6 has provision to determine category and related test and analytical method for drugs which are not mentioned in recognized pharmacopoeia and encyclopedia by Department of Drug Administration (DDA) upon consultation with Drug Advisory Committee (DAC). To execute the provision of above mentioned rule, Analytical Method Validation Committee (AMVC) was formed as per the decision of DDA in 2072/11/21. Thereafter, "Protocol on Analytical Method Validation of Non-pharmacopoeial Product (NPP) for Regulatory Approval" was developed and approved by DAC on 2073/10/13. Based on this protocol, Analytical Method Validation Committee has been conducting method validation activities so far.

The categorization of drug (drug standard) and related analytical method is determined as per the recommendation by Analytical Method Validation Committee followed by final approval by DAC. This protocol is limited to Assay and Dissolution by HPLC method only. Therefore, the protocol has been revised and updated with additional scope of activities and additional provisions. The revised guideline encompasses conventional as well as modern analytical techniques including microbiological analysis with additional provisions like verification procedures, alternative method etc.

2.0 Objective:

2.1 To provide the documented evidence that whether the analytical method submitted by pharmaceutical industry is suitable for the analytical operation.

2.2 To revise existing document and include new documents required during the submission of NPP.

2.3 To select the appropriate method available to AMV Committee of different non-pharmacopoeial methods and develop the product (quality control) specification and standard analytical method for non-pharmacopoeial drug product.

2.4 To recommend DDA, the appropriate method for approval from DAC.

3.0 Scope:

This guideline applies to

- all registered non-pharmacopoeial products that are imported and locally produced
- all pharmaceutical manufacturers those apply for marketing authorization of non-pharmacopoeial product
- procedural requirement for preparation, adaptation and approval of document on NPP
- analytical method such as assay, dissolution by conventional and modern analytical technique (HPLC, UV-VIS and titration, etc), microbiological analysis (sterility test, endotoxin test and microbial limit test, etc).

4.0 Category of the non-pharmacopoeial product:

- Products requiring document evaluation and testing – Category 1, 2, 3, 4, 5.3 and the products mentioned in Clause 7

- Products requiring document evaluation only- Category 5.1, 5.2
- Products requiring submission of document only – Category 6

4.1 Category 1

The monograph of the active pharmaceutical ingredient (API) and dosage form are not available in recognized Pharmacopoeia.

4.2 Category 2

The monograph of API is available in recognized pharmacopoeia but dosage form is not available.

4.3 Category 3

Monograph of API and different dosage form/ salt form available in pharmacopoeia.

4.4 Category 4

Monograph of API and dosage form available in pharmacopoeia but not available in fixed dose combination.

4.5 Category 5

Immunosuppressant/Immuno-modulator drug, Cytotoxic drug, transdermal patches; products not absorbed systemically and external dosage forms except ophthalmic, otic and nasal use

4.6 Category 6

Biological products (Vaccines, Monoclonal antibodies, Polyclonal antibodies, rDNA products and bio-similar products) etc.

(For descriptive information refer ANNEX I)

5.0 Method development & selection:

5.1 For assay and dissolution, analytical method can be referred from reliable literature. For Dissolution test condition, updated USFDA or equivalent database should be referred, if available.

5.2 Category 1 – The method should be analyzed on WHO recommended innovator / comparator product or SRA approved product. The method should be stability indicating using HPLC or modern analytical technique.

5.3 Category 2 – The method can be referred from monograph of API in Pharmacopoeia as far as possible or from reliable literature. If conventional method such as titration and UV method is mentioned, it can be changed to HPLC method or modern advanced technique but HPLC method or modern analytical technique cannot be changed to conventional method.

5.4 Category 3 – The method should be selected from similar dosage form as far as possible. If not available, the method of other dosage form can also be referred. If UV method is mentioned, it can be changed to HPLC method or modern advanced technique but HPLC method or modern analytical technique cannot be changed to UV method.

If the monograph of API and dosage form (e.g. tablet, liquid etc.) is available but the salt form of API in dosage form is different from the available monograph, (e.g. diclofenac sodium to diclofenac potassium, etc.) analytical method should be based on the salt form available in the pharmacopoeial monograph and analytical method validation is not required but if the method is not applicable then analytical method validation should be done. If the base form is available in pharmacopoeia but salt form is not available analytical method should be based on the base form available in the pharmacopoeial monograph and analytical method validation is not required but if the method is not applicable then analytical method validation should be done.

5.5 Category 4 – The method should be selected from individual monograph as far as possible. If not applicable, alternative methods can also be referred. The dissolution test parameter in case of tablet/capsule dosage form should be as mentioned in the individual monograph (should be narrowed but not wider e.g. if dissolution time is 45 minutes, it can be varied to 30 minutes with justifications, same is the case for RPM). If UV method is mentioned, it can be changed to HPLC method or modern advanced technique but HPLC method or modern analytical technique cannot be changed to UV method.

5.6 Category 5 & 6 - As per relevant requirement.

6.0 Required document for evaluation by AMV Committee

All of the documents including analytical method validation protocol and report, references literature, at least 3 month stability study during submission of sample for testing, product license, raw data, chromatogram spectra/printout, traceability report of standard, certificate of analysis, method of analysis, calibration date of equipment, batch number of culture media, lot number of reference culture, daily observation record, etc. calculations along with general required document as mentioned in ANNEX II: 2.7, 2.8, 2.9, 2.10 and ANNEX X (Recommended acceptance criteria for microbiological quality of non-sterile dosage form)

6.1 Additional document

6.1.1 For Category 1, comparative study of assay and dissolution with innovator/ comparator product or SRA approved product.

6.1.2 For Category 4, comparative study of dissolution test profile with method mentioned in the individual monograph.

6.1.3 For modified release dosage form, comparative study of dissolution profile with innovator/ comparator product or SRA approved product. Similarity/ dissimilarity factor should be submitted.

7.0 Water soluble Multivitamins, Enzymes, and Mineral containing multi-ingredient product:

This guideline is applicable to water soluble multivitamins, enzymes, mineral containing multi-ingredient product having upper limit of assay not more than 130% of stated amount.

8.0 Exemption

The pharmaceutical products which are identified by DDA for not requiring to do method validation shall be exempted for the purpose of this guideline. Example- Stringent Regulatory Authorities (SRA) approved products.

9.0 Selection of Performance Characteristics

The selection of performance characteristics for specific analytical method shall be selected as per ANNEX,

For Assay & Dissolution by HPLC - ANNEX III, 3.1

For Assay & Dissolution by UV-VIS Spectroscopy - ANNEX III, 3.2

For Assay & Dissolution by titrimetric analysis - ANNEX III, 3.3

For microbiological analysis, Sterility Test, Microbial Limit Test and Endotoxin Test: ANNEX III, 3.4

Microbiological Quality of Non-sterile dosage form - ANNEX X

10.0 Products having multiple strengths

If the product is of multiple strengths, testing is carried out for lowest and highest strength.

11.0 Format for document submission:

The document should be submitted to DDA in a prescribed format as per ANNEX XI.

12.0 Preliminary Screening

Preliminary screening shall be done by DDA (industry section for national industry and import section for foreign industry) as per ANNEX IV before submission of document to AMV Committee.

13.0 Document Evaluation

Document evaluation shall be done by AMV Committee as per SOP NPV/076-77/SOP-02 for study of documents of non pharmacopoeial products for regulatory approval (ANNEX V, Procedure 5.2).

14.0 Numbering System

Numbering system and publication of approved method shall be done as per SOP NPV/076-77/SOP-02 for study of documents of non pharmacopoeial products for regulatory approval (ANNEX V, Procedure 5.4, 5.5)

15.0 Verification of the method:

15.1 Published method shall be implemented by pharmaceutical industries following the method verification process as in clause 15.2.

15.2 Verification of method is demonstrated by meeting system suitability, specificity, accuracy and system precision (repeatability) established for the specified method but not limited to these parameters.

15.3 For microbiological analysis, at least accuracy and specificity shall be done for verification.

15.4 Verification document shall be submitted to AMV Committee for marketed product prior renewal of product registration certificate/ import registration certificate.

15.5 Verification document shall be submitted to AMV Committee after publication of approved method for marketing authorization (product registration certificate/ import registration certificate).

16.0 Alternative method:

16.1 Alternative method can be developed in case of non-compliance with the Clause 15.0.

16.2 In case of non-compliance, the industry shall submit the documented evidence to AMV Committee regarding the reason for non-compliance.

16.3 Alternative method shall be developed, validated and documented, demonstrating statistically equivalence to the approved method and should be submitted to AMV Committee for evaluation through change control SOP (NPV/076-77/SOP-03)

16.4 In the event of dispute between the alternative method and initial method, the initial method is alone authoritative.

17.0 Revision of method

The approved method shall be revised and re-approved following the SOP of change control as per ANNEX VI.

18.0 Revalidation

Revalidation may be necessary in the following circumstances:

- Changes in the synthesis of the drug substance;
- Changes in the composition of the finished product;

- Changes in the analytical procedure.

19.0 Repeal and Savings

19.1 The old method will be repealed after its revision and if the product becomes pharmacopoeial.

19.2 Protocol for the Guidance and Recommendation of documents for non-pharmacopoeial product for National Regulatory Approval, 2073 is hereby repealed.

19.3 All the actions taken under "Protocol for the Guidance and Recommendation of documents for non- pharmacopoeial product for Regulatory Approval, 2073" shall deemed to have been performed or taken under this protocol.

ANNEXES

ANNEX I

Descriptive information of category

Categories	Characteristic	Sub categories	Method development and selection
Category 1	The monograph of the active pharmaceutical ingredient (API) and dosage form are not available in recognized Pharmacopoeia.	N/A	The method should be analyzed on WHO recommended innovator / comparator product or SRA approved product. The method should be stability indicating using HPLC or modern analytical technique such as GC, AAS, etc. The document evaluation and testing should be done.
Category 2	The monograph of API is available in recognized pharmacopoeia but dosage form is not available.	N/A	The method can be referred from monograph of API in Pharmacopoeia as far as possible or from reliable literature*. The document evaluation and testing should be done.
Category 3	Monograph of API and different dosage form/ salt form available in pharmacopoeia.	**3.1**. Monograph of API and one of the dosage form available in Pharmacopoeia. e.g. tablet may be available (solid dosage form) but capsule, dispersible, chewable, inserts, buccal, sublingual, mouth dissolving not available etc. or vice-versa	The method should be selected from similar dosage form as far as possible. If not available, the method of other dosage form can also be referred. The document evaluation and testing should be done.

		Liquid dosage form like solution may be available but suspension, drops, syrup powder for oral suspension not available or vice versa	
		3.2. One or more Dosage form with similar base (diclofenac) available but different salt, complex**,** isomer, not available. diclofenac sodium tablet and diclofenac potassium tablet etc.) - Base form is available in pharmacopoeia but salt form is not available in pharmacopoeia or vice versa.	As described for sub category 3.1 If the monograph of API and dosage form (e.g. tablet, liquid etc.) is available but the salt form of API in dosage form is different from the available monograph, (e.g. diclofenac sodium to diclofenac potassium, etc.) analytical method should be based on the salt form available in the pharmacopoeial monograph and analytical method validation is not required but if the method is not applicable then analytical method validation should be done. If the base form is available in pharmacopoeia but salt form is not available analytical method should be based on the base form available in the pharmacopoeial monograph and analytical method validation is not required but if the method is not applicable then analytical method validation should be done.
Category 4	Monograph of API and dosage form available in pharmacopoeia but not available in fixed dose combination.	N/A	The method should be selected from individual monograph as far as possible. If not applicable, alternative methods can also be referred. The dissolution test parameter in case of tablet/capsule dosage form should be as

			mentioned in the individual monograph (should be narrowed but not wider e.g. if dissolution time is 45 minutes, it can be varied to 30 minutes with justifications, same is the case for RPM). The document evaluation and testing should be done.
Category 5	Immunosuppressant/ Immuno-modulator drug, Cytotoxic drug, transdermal patches; products not absorbed systemically and external dosage forms except ophthalmic, otic and nasal use	5.1Immunosuppressant/Immuno-modulator drug, Cytotoxic drug, transdermal patches	The document evaluation should be done
		5.2 Products not absorbed systemically and external dosage	The document evaluation should be done.
		5.3 External dosage forms such as ophthalmic, otic and nasal use	Document evaluation and testing should be done.
Category 6	Biological products (Vaccines, Monoclonal antibodies, Polyclonal antibodies, rDNA products and bio-similar products), etc.	N/A	The document should be submitted but evaluation and testing is not done.
If conventional method like titration and UV method is mentioned, it can be changed to HPLC method or modern advanced technique but HPLC method or modern analytical technique cannot be changed to UV method for all categories except Category 1. For assay and dissolution, reliable literature can be referred. For dissolution test condition, update USFDA or equivalent database should be referred if available.			

ANNEX II

2.1 Checklist for Assay

Checklist for document study of analytical method validation

(Assay Checklist)

Brand name: Registration number:

Composition: Registration date: Date:

Product License: Stability Study (atleast 3 months):

Manufactured by: Submitted by:

Method validation of :

- Assay - Dissolution -Related substances: -Any other impurities

S.No.	Documents	Yes	No	Remarks
a.	Summary Validation Report/Protocol no.			
b.	Analytical Method Reference (IP/BP/USP/JP Any other literature)			
c.	Instruments used and calibration date			
d.	Reagents used and Grades			
e.	Reference standard (Traceability)			
	Primary			
	Secondary			
f.	Resolution standard (Traceability)			
g.	Internal standard			
h.	Analytical Method			
1	Reagent preparation			
2	Diluent			
3	Mobile Phase Preparation			
4	Standard Preparation			
5	Sample Preparation			
6	Microbiological Quality			

Analytical Method validation parameters

S.No	Parameters	Requirements	Documents / Raw data: Chromatogram with detail chromatographic condition /Spectrum/Print out	Documents / Raw data: Calculation with formula	Remarks
a.	**Specificity**				
1	Blank values: Diluents	Resolution: NLT 1.5 /blank interference NMT 1%			
2	Sample solution without active	Resolution: NLT 1.5 /placebo interference NMT 2%			
b.	**Linearity & Range**	r2 $\geq$ 0.99			
c.	**Repeatability**	RSD $\leq$ 2.0 %			
d.	**Intermediate Precision**	RSD $\leq$ 3.0 %			
e.	**Accuracy**	98 % to 102 % (HPLC) / 95 % to 105 % (UV)& titration			
f.	**Robustness(optional for titration)**				
1	**Deliberate variation**	changes should be within the limits that produce acceptable chromatography & UV spectrum			
2	**Solution Stability (HPLC)**	98% to 102% in comparison to the freshly prepared solutions			
g.	**System Suitability test (HPLC)**				
1	Theoretical plates	NLT 2000			
2	Tailing factor	NMT 2.0			
3	RSD of five/six replicate injections	NMT 2.0 %			
4	Resolution between two peaks	NLT 2.0			
5	RSD (for UV)	NMT 3.0%			

Name of the authorized person:

Signature and Date:

2.2 Checklist for Dissolution

Checklist for document study of analytical method validation
(Dissolution Checklist)

S.No.	Documents	Yes	No	Remarks
a.	Summary Validation Report/Protocol no.			
b.	Analytical Method Reference (IP/BP/USP/JP Any other literature)			
c.	Instruments used and calibration date			
d.	Reagents used and Grades			
e.	Reference standard (Traceability)			
	Primary			
	Secondary			
f.	Resolution standard (Traceability)			
g.	Internal standard			
h.	Analytical Method			
1	Reagent preparation			
2	Diluent			
3	Mobile Phase preparation			
4	standard preparation			
5	sample preparation			

Analytical Method validation parameters

S.No	Parameters	Requirements	Documents		
			Raw data		
			Chromatogram with detail chromatographic condition /Spectrum/Print out	Calculation with formula	Remarks
a.	**Specificity**				
1	Blank values: Diluents	Resolution: NLT 1.5 /blank interference NMT 1%			
2	Sample solution without active	Resolution: NLT 1.5 / placebo interference NMT 2%			
b.	**Linearity & Range**	r2 ≥ 0.98			
c.	**Repeatability**	RSD ≤ 2.0 %			
d.	**Intermediate Precision**	difference in the mean value for dissolution results between any two conditions using same strength should not exceed an absolute 10% at time points with <85% dissolved and does not exceed 5% for time points > 85%			
e.	**Accuracy**	recovery 95 % to 105 % of amount added			
f.	**Robustness**				
1	**Deliberate variation**	changes should be within the limits that produce acceptable chromatography & UV spectrum			
2	**Solution Stability**	98% to 102% in comparison to the freshly prepared solutions			
g.	**System Suitability test**				
1	Theoretical plates	NLT 2000			
2	Tailing factor	NMT 2.0			
3	RSD of five/six replicate injections	NMT 2.0			
4	Resolution between two peaks	NLT 2.0			

Name of the authorized person:

Signature and Date:

2.3 Checklist for Sterility Test

Checklist for document study of analytical method validation (Sterility Test Checklist)				
S. No.	**Documents**	**Yes**	**No**	**Remarks**
1	Reference culture used			
2	Analytical Method			
2.1	Sample preparation			
2.1.1	Number of containers sampled			
2.1.2	Quantity taken from each container			
2.2	Batch no. of media and its preparation			
2.3	Details of <100cfu inoculum			
2.3.1	Name of organism			
2.3.2	Preparation and verification of <100cfu inoculum or COA (Lot No. of <100cfu)			
2.3.3	Passage used			
3	Chemical and biological indicator used			
4	Method used			
4.1	Membrane filtration			
4.2	Direct Inoculation			
5	Batch size			
6	Type of filter used			
7	Washing cycle by diluting fluid			
Analytical Method validation parameters				
S. No.	**Parameters**	**Requirement**	**Raw data & Calculation**	**Remarks**
1	**Environmental monitoring: Exposure plate/Test Tube**	No growth		
2	**Specificity (Growth promotion test)**			
2.1	Positive control : Aerobic bacteria and anaerobic bacteria(*Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium sporogenes or Bacteroides vulgatus, Bacillus subtilis, Clostridium sporogenes)*	Growth is visually comparable to that obtained on the same medium previously tested and approved.		
2.2	Batch no. of media:			
2.3	Fungi *(Aspergillus brasiliensis, Candida albicans, Bacillus subtilis)*	Growth is visually comparable to that obtained on the same medium previously tested and approved.		
3	**Accuracy (Product Positive Control)**			
3.1	Growth of organism in the presence of sample	Growth is visually comparable to the positive control tube.		
3.2	For Penicillin and Cephalosporin using β-Lactamase			
4	**Negative Control**	No growth		
		Name of the authorized person: **Signature and Date:**		

2.4 Checklist for Microbial Limit Test

Checklist for document study of analytical method validation (Microbial Limit Test Checklist)				
S. No.	**Documents**	**Yes**	**No**	**Remarks**
1	Reference culture used			
2	Analytical Method			
2.1	Batch no. of media and its preparation			
2.2	Details of <100cfu inoculum			
2.2.1	Name of organism			
2.2.2	Preparation and verification of <100cfu inoculum or COA (Lot No. of <100cfu)			
2.2.3	Passage used			
3	Method used			
3.1	Membrane filtration			
3.2	Plate count method			
3.3	Most probable number method			
4	Chemical and biological indicator used			
Analytical Method validation parameters				
S. No.	**Parameters**	**Requirement**	**Raw data & Calculation**	**Remarks**
1	**For Total aerobic microbial count**			
1.1	**Specificity (Growth promotion test)**			
1.1.1	Positive control : Bacteria(*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis)*	Growth obtained must not differ from the calculated cfu of the standardized inoculum by a factor > 2		
1.1.2	Batch no. of media:			
1.1.3	Fungi *(Aspergillus brasiliensis, Candida albicans)*	Growth obtained must not differ from the calculated cfu of the standardized inoculum by a factor > 2		
1.2	**Accuracy (Product Positive Control)**			
1.2.1	Growth of organism in the presence of sample	Growth obtained must not differ from standard inoculum (positive control) and sample plate by a factor > 2		
1.3	**Negative Control**	No growth		
1.4	**Intermediate Precision**	15-35%		
2	**For Test for specified microorganisms**			
2.1	**Specificity (Growth promotion test)**			
2.1.1	Positive control : Bacteria(*Staphylococcus aureus, Pseudomons aeruginosa, Escherichia coli , Salmonella enterica spp., Shigella boydii, Clostridium sporogenes)*	For luxuriant organism, recovery ≥ 50% and inhibitory organism, recovery = 0%		
2.1.2	Batch no. of media:			
2.1.3	Fungi *(Candida albicans)*	For luxuriant organism, recovery ≥ 50% and inhibitory organism, recovery = 0%		
2.2	**Accuracy (Product Positive Control)**			
2.2.1	Growth of organism in the presence of sample	Specified microorganism must be detected with the colony morphology & indication reaction as described.		
2.3	**Negative Control**	No growth		
		Name of the authorized person: **Signature and Date:**		

2.5 Checklist for Endotoxin analysis

Checklist for document study of analytical method validation
(Endotoxin Test Checklist)

S. No.	Documents	Yes	No	Remarks
1	Method			
2	Lot No. of Control Standard Endotoxin			
3	Control Standard Endotoxin used			
4	Lot No. of Lysate			
5	Sensitivity of Lysate			
6	Test for interfering factors with raw data			
7	Endotoxin Limit with raw data calculation			

Name of the authorized person:
Signature and Date:

2.6 Format: List of inadequate documents

Sample Name:

Manufacturer:

Submitted by:

List of Inadequate documents:

1.

2.

3.

4.

5.

6.

Recommendation:

1.

2.

3.

3.

4.

5.

Name of Authorized Person:

Date:

2.7 **Parameters to be checked for the dosage form for the non pharmacopoeial products.**

Product Specification

S.No.	**Parameters to be checked**	Dosage form
1.	Description, Identification, Uniformity of weight, Disintegration test, Friability, Dissolution, Uniformity of content (if required), Assay, Microbiological quality, Water content (if required), Related substances (if required), Leak test, Any other additional tests if required, storage condition, pack size.	Tablet
2.	Description, Identification, Uniformity of weight, Disintegration test, Dissolution, Uniformity of content (if required), Assay, Microbiological quality, Water content (if required), Related substances (if required), Leak test, Any other additional tests if required, storage condition, pack size.	Capsule
3.	Description, Identification, Uniformity of volume, Uniformity of weight, Assay, Microbiological quality, Water content (if required), pH, Related substances (if required), Leak test, Any other additional tests if required, storage condition, pack size.	Liquid, Powder for oral suspension
4.	Description, Identification, Filled weight variation, Assay, pH, Related substances (if required), Leak test, Any other additional tests if required, storage condition, pack size.	Cream, Gel & Ointment
5.	Description, Identification, Uniformity of weight, Assay, Microbiological quality, Water content (if required), pH, Related substances (if required), Any other additional tests if required, Seal test (only for sachets), storage condition, pack size.	Oral Powder
6.	Description, Identification, Uniformity of weight, Microbiological quality, Water content (if required), pH, related substances (if required), Any other additional tests if required, leak test, storage condition, pack size.	Suppository
7.	Description, Identification, Uniformity of volume, Assay, Uniformity of content (if required), pH, related substances (if required), Bacterial endotoxin, sterility test, particulate matter, Any other additional tests if required, leak test, storage condition, pack size.	Sterile preparation
8.	Description, Identification, Uniformity of volume, Assay, Uniformity of content (if required), pH, related substances (if required), particulate matter, Any other additional tests if required, leak test, storage condition, pack size.	Non-sterile preparation
9.	Description, Identification, Filled weight variation, Assay, pH, sterility test, isotonicity test, Related substances (if required), Leak test, Any other additional tests if required, storage condition, pack size.	Sterile eye ointment

2.8 Checklist of Product Specification if similar molecule is available in Pharmacopoeia.

S.No.	Parameters	**Monograph available in pharmacopoeia**		**Tolerance Limit**	
		Yes (If Yes, Name of product and Name of Pharmacopoeia)	No	Pharmacopoeial product	Non pharmacopoeial product
1.	API standard				
2.	Description				
3.	Average weight				
4.	Uniformity of weight				
5.	Disintegration test				
6.	Limit of water content if necessary				
7.	Limit of Assay				
8.	Method of analysis of Dissolution if necessary				
9.	Limit of Dissolution if necessary				
10.	Method of analysis of Content Uniformity if necessary				
11.	Limit of Content Uniformity if necessary				
12.	Limit of Related Substance if necessary				
13.	Method of analysis of Related Substance if necessary				
14.	Any other tests if required				

2.9 Analytical Method Validation checklist.

S. No.	Parameters	Yes	No	Remarks
1.	Analytical Method Reference (IP/BP/USP/JP/Any other literature)			
2.	Reagents used and Grade			
3.	Reference standard traceability			
4.	Analytical Method ✓ Reagent Preparation ✓ Diluents ✓ Mobile phase preparation ✓ Standard preparation ✓ Sample preparation			
5.	Chromatogram, Spectrum & Calculation with formula should be submitted where needed.			
6.	Analytical method validation			

2.10 Analytical Method Validation checklist. (To be filled by authorized person of industry)

S.No.	Parameters	Limit	Requirements	Yes	No	Remarks
1.	**Specificity**	Resolution: NLT 1.5	Should be investigated by injecting the blank (solvent)/ placebo (matrix solution), standard solution, sample solution to demonstrate the absence of interference with the elution of analytes.			
2.	**Linearity** **Assay** **Dissolution**	 $r^2 \geq 0.99$ $r^2 \geq 0.98$	Standard solutions should be prepared at minimum of 5/6 concentrations within the range of typically 80%, 100 %, 120 %, of target concentration.			
3.	**Range**		Assay of drug substances (80 % to 120 % of the test concentration) Content Uniformity (minimum 70% to 130 % of the test concentration) Dissolution testing (+/-20 % over the specified range)			
4.	**Repeatability**	RSD $\leq$ 2.0 %	For instrument precision determinations of five replicate of reference standard should be made. For the method at least nine determinations covering specified range of 3 concentration and 3 replicates should be made.			
5.	**Intermediate Precision** **Assay** **Dissolution**	RSD $\leq$ 3.0 % The diff. in the mean value for dissolution results between any two conditions using the same strength should not exceed an absolute 10 % at time points with < 85 % dissolved nor exceed 5 % for time points >85 %.	Test procedure Intermediate precision (within-laboratory variation) should be demonstrated by at least two analysts, using at least two HPLC/UV-vis spectrophotometer on different days and evaluating the relative percent purity data across the two systems of triplicate sample of one concentration. Dissolution test should be performed by two analysts using two different dissolution test apparatus on different days.			

2.10 Analytical Method Validation checklist. (To be filled by authorized person of industry) contd…..

S.No.	Parameters	Limit	Requirements	Yes	No	Remarks
6	**Accuracy** **Assay** **Dissolution**	 98 % to 102 % (HPLC) 95 % to 105 % (UV) 95 % to 105 %	 Spiked samples should be prepared at three concentrations over the range of 80 %, 100 % and 120 % of the target concentration. Three individually prepared triplicates at each concentration will be analyzed.			
7 7.1 7.2	**Robustness** **Deliberate variation** **Stability of the standard and sample solution**	 Changes should be within the limits that produce acceptable chromatography & UV spectrum 98.0 % to 102.0 % in comparison to the freshly prepared solutions	 The investigation of robustness can be done by change of flow rate of the mobile phase, change of temperature of column, change of composition of the mobile phase, change in the pH of the mobile phase and use of different column. Solutions of drug product should be analysed in comparison to the fresh prepared solutions stored at room temperature in auto sampler and stored at 2 - 8 °C, in refrigerator at least 24 hours.			
8	**System Suitability test**	Theoretical plates (NLT 2000) Tailing factor (NMT 2.0) rsd (NMT 2.0 %)	System suitability tests should be performed on HPLC systems to determine the accuracy and precision of the system by injecting five/ six injections of a solution containing analyte (standard solution) at 100% of test concentration. Determine relative standard deviation (rsd) of the replicate injections, theoretical plate and tailing factor.			

Note: Every page should be signed with date by the authorized person with company stamp.

Authorized Person:
Signature:
Name:
Designation:
Stamp:
Date:

ANNEX III

3.1 Performance Characteristic for Assay & Dissolution by HPLC

1. Analytical Performance Characteristics

Procedure: Before undertaking the task of methods validation, it is necessary that the analytical system itself should be adequately designed, maintained, calibrated, and validated. All personnel who will perform the validation testing must be properly trained. For each of the validation characteristics in this document should defines the test procedure, documentation, and acceptance criteria. Specific values are taken from the ICH, U.S. FDA, USP and pertinent literature as references.

1.1. Specificity

1.1.1. Test procedure:

The specificity of the assay and dissolution method should be investigated by injecting the blank (solvent/dissolution medium), placebo (matrix solution), standard solution, sample solution to demonstrate the absence of interference with the elution of analytes.

1.1.2. Documentation:

Print chromatograms/Spectrum

1.1.3. Acceptance criteria:

The excipient compounds must not interfere with the analysis of the targeted analyte. Placebo interference in dissolution should not exceed 2% and blank interference in dissolution should not exceed 1%.

1.2. Linearity

1.2.1. Test procedure:

Linearity will be determined by preparing reference standard (API) of at least five different concentrations within the range of 80 % to 120 % of the target concentration for assay and by preparing standard solution or spiked solution or by method of standard addition ranging in concentration from less than the lowest expected concentration to more than the highest concentration during release for dissolution. The method of standard preparation and the number of injections should be same as used in the final procedure. Linearity curve will be plotted for peak area response or absorbance against concentration. The linear relationship will be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares.

1.2.2. Documentation:

Print the chromatogram and record the results on a datasheet. Calculate the mean, standard deviation, and Relative Standard Deviation (RSD) for each concentration. Plot concentration (x-axis) versus mean response (y-axis) for each concentration. Calculate the regression equation and coefficient of determination (r2). Record these calculations on the datasheet.

1.2.3. Acceptance criteria:

The correlation coefficient for minimum of five concentration levels should be ≥0.99 for the range of 80% to 120% of the target concentration for assay and should be ≥0.98 for dissolution. The y-intercept must ≤ 2% of the target concentration response. A plot of response factor versus concentration must show all values within 2.5% of the target level response factor, for concentrations between 80% and 120% of the target concentration.

1.3. Range

Range is an expression of the lowest and highest level of analyte that have been demonstrable to be determinable with acceptable precision, accuracy and linearity. For the assay of a drug substance or a finished product: normally from 80% to 120% of the test concentration; for content uniformity, covering a minimum of 70% to 130% of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified; and for dissolution testing: +/-20 % over the specified range, eg: for control release product covering a region from 30% after 1 hr & up to 90% after 24 hr, validated range would be 10% to 110% of label claim.

1.3.1. Test procedure:

The data obtained during the linearity and accuracy studies will be used to assess the range of the method.

The precision data used for this assessment is the precision of the three replicate samples analyzed at each level in the accuracy studies.

1.3.2. Documentation:

Record the range on the datasheet.

1.3.3. Acceptance criteria:

The acceptable range will be defined as the concentration interval over which linearity and accuracy are obtained per the above criteria, and in addition, that yields a precision of ≤ 3% RSD.

1.4. Accuracy

1.4.1. Test procedure:

Spiked samples will be prepared by addition of analyte of known purity (reference substance) at three concentrations over the range of 80 %, 100 % and 120 % of the target concentration. Three individually prepared replicates at each concentration will be analyzed. When it is (Spiked samples) difficult to prepare, use a low concentration of a known standard.

1.4.2. Documentation:

Print the chromatogram. For each sample, report the theoretical value, assay value, and percent recovery. Calculate the mean, standard deviation, RSD, and percent recovery for all samples. Record results on the datasheet.

1.4.3. Acceptance criteria:

100 ± 2% is typical for an assay of an active ingredient in a drug product over the range of 80 to 120% of the target concentration. The measured recovery in case of dissolution is typically 95 % to 105 %.

1.5. Precision

1.5.1 Repeatability

1.5.1.1 Test procedure:

Repeatability of system and method should be performed. For instrument precision determinations of five replicate of reference standard should be made. For the method nine determinations covering specified range of 3 concentration and 3 replicates should be made or six determinations at 100 % of the test concentration. For dissolution purpose, nine determinations covering specified range of 3 concentration and 3 replicates should be made or minimum of six determinations at 100 % of the test concentration. The demonstration of the repeatability for the dissolution step is conducted by performing the dissolution step on separate units of a well characterized dosage form or equivalent composite.

1.5.1.2 Documentation:

Record the retention time, peak area on the datasheet. Calculate the mean, standard deviation, and RSD.

1.5.1.3 Acceptance criteria:

RSD should be less than 2% for the assay and dissolution of finished products.

1.5.2 Intermediate Precision

1.5.2.1 Test procedure

Intermediate precision (within-laboratory variation) will be demonstrated by two analysts, using two HPLC/ UV-Vis spectrophotometer on different days and evaluating the relative percent purity data across the two HPLC systems.

For dissolution testing purpose, if possible intermediate precision can be evaluated using a well characterized lot of drug product with tight content uniformity. If this type of lot is not available, premeasured placebo and active ingredients may be used to identify intermediate precision. The dissolution procedure on the same sample may be run by at least two different analysts from the same laboratory, with each analyst preparing the standard solutions and the medium and following the defined quantification procedure.

1.5.2.2 Documentation:

Print the chromatogram. Record the relative % purity (% area) of each concentration on the datasheet.

Calculate the mean, standard deviation, and RSD for the operators and instruments.

1.5.2.3 Acceptance criteria:

The assay results obtained by two operators using two instruments on different days should have a statistical RSD ≤ 3%.

For dissolution, a typical acceptance criteria is the difference in mean value for dissolution results between any two conditions, using the same strength, does not exceed an absolute 10 % at time points with < 85 % dissolved and does not exceed 5 % for time points > 85 %.

1.6. Limit of Detection: (Not necessary for assay)

1.6.1. Test procedure:

The lowest concentration of the standard solution will be determined by sequentially diluting the sample. Five replicates should be made from this sample solution.

1.6.2. Documentation:

Print the chromatogram. Record the lowest detectable concentration and RSD on the datasheet.

1.6.3. Acceptance criteria:

The ICH references recommend a signal-to-noise ratio of 3:1.

1.7. Limit of Quantitation (Not necessary for assay)

1.7.1. Test procedure:

Limit of quantitation can be determined based on the standard deviation of the response and the slope with the instrumental response obtained from the linearity. Establish the lowest concentration at which an analyte in the sample matrix can be determined with the accuracy and precision required for the method in question. This value may be the lowest concentration in the standard curve. Make six replicates from this solution.

1.7.2. Documentation:

Print the chromatogram and record the lowest quantified concentration and RSD on the datasheet. Provide data that demonstrates the accuracy and precision required in the acceptance criteria.

1.7.3 Acceptance criteria:

The limit of quantitation for chromatographic methods has been described as the concentration that gives a signal to noise ratio (a peak with height at least ten times as high as the baseline noise level) an RSD of approximately 10% for a minimum of six replicate determinations.

1.8. System Suitability

1.8.1. Test procedure:

System suitability tests should be performed on HPLC systems to determine the accuracy and precision of the system by injecting five injections of a solution containing analyte at 100% of test concentration. The following parameters will be determined:

- Theoretical Plate count
- Tailing factors,
- Resolution if required , and
- Reproducibility (percent RSD of retention time, peak area, and height for five injections).

1.8.2. Documentation:

Print the chromatogram and record the data on the datasheet

1.8.3. Acceptance criteria:

Retention factor (k): the peak of interest should be well resolved from other peaks and the void volume; generally k should be ≥2.0.

Resolution (Rs): Rs should be ≥2 between the peak of interest and the closest eluted peak, which is potentially interfering (impurity, excipient, and degradation product).

Reproducibility: RSD for peak area, height, and retention time will be 1% for five injections.

Tailing factor (T): T should be ≤2.

Theoretical plates (N): ≥2000.

NOTE: Number of TP(N) depends upon molecules in compound and Mobile Phase viscosity in controversial cases (justify with scientific reason and data)

1.9. Robustness:

1.9.1 Deliberate variation

1.9.1.1 Test procedure:

HPLC analysis parameter may include variation in mobile phase composition (eg: buffer or surfactant concentration, pH, deaeration), flow rate, wavelength, column temperature & multiple columns. UV analysis parameter may include change in wavelength. Dissolution parameter may include variation in medium composition, volume, agitation rate, sampling time & temperature. These parameters may be evaluated one factor at a time or simultaneously as part of a factorial experiment.

1.9.1.2. Documentation

Print the chromatogram. Record all the variations.

1.9.1.3 Acceptance criteria

Changes should be within the limits that produce acceptable chromatography & UV spectrum.

1.9.2. Stability of Standard and sample solutions

1.9.2.1 Test procedure:

Stability of the sample solution will be performed by analysing test solutions stored in auto sampler (at least 24 h) and stored at 2 - 8 °C in refrigerator (at least 24 hour) with the freshly prepared standard solutions.

In case of dissolution, the stability of the standard is analysed over the specified period of time (at least the time of the entire dissolution procedure) using a freshly prepared standard solution at each time interval for comparison.

1.9.2.2. Documentation

Print the chromatogram. Stability should be documented by a table with mean values.

1.9.2.3. Acceptance criteria

The acceptable range for standard and sample solution stability is typically between 98% and 102% compared with the initial analysis of standard and sample solution.

Acceptance criteria for the study of analytical method validation document

S.No.	Parameters	Requirement
a.	**Specificity**	
1	Blank values: Diluents	Resolution: NLT 1.5 /blank interference NMT 1%
2	Sample solution without active	Resolution: NLT 1.5 /placebo interference NMT 2%
b.	**Linearity & Range**	r2 $\geq$ 0.99
c.	**Repeatability**	RSD $\leq$ 2.0 %
d.	**Intermediate Precision**	RSD $\leq$ 3.0 %
e.	**Accuracy**	98 % to 102 % (HPLC)
f.	**Robustness**	
1	**Deliberate variation (**mobile phase composition, flow rate, wave length, column temperature, etc) (Agitation rate, volume, sampling time and temperature)	changes should be within the limits that produce acceptable chromatography
2	**Solution Stability**	98% to 102% in comparison to the freshly prepared solutions
g.	**System Suitability test**	
1	Theoretical plates	NLT 2000
2	Tailing factor	NMT 2.0
3	RSD of five/six replicate injections	NMT 2.0
4	Resolution between two peaks	NLT 2.0

3.2 Performance Characteristic for Assay & Dissolution by UV-VIS Spectroscopy

1. Analytical Performance Characteristics:

This document is mainly focused on validation of quantitative determination of main component of drug product (but can also be used for quantitative determination of drug substance and impurity). Specific values are taken from the USP and pertinent literature as references.

1.1. Specificity

1.1.1. Test procedure:

The specificity of the assay method should be investigated by placebo (matrix solution) standard solution, sample solution run separately and standard solution containing a mixture of the component being analyze should also be run i.e. taking a scan of spectrum of wavelength bracketing the λ_{max} of the main component to demonstrate the absence of interference to the analytes. The λ_{max} should be noted for each of analyte peaks and check for its resolution from the nearest peak.

1.1.2. Documentation:

Print spectrum.

1.1.3. Acceptance criteria:

The excipients should not interfere with the analysis of the targeted analyte.

1.2. Linearity

1.2.1. Test procedure:

Linearity should be determined by preparing standard solution of at not less than five different concentrations within the range of 80 % to 120 % of the target concentration. The method of standard preparation and the number of determination should be same as used in the final procedure. Linearity curve should be plotted for absorbance response against concentration. The linear relationship will be evaluated by appropriate statistical methods e.g. least squares regression,

1.2.2. Documentation:

Record the results on a datasheet. Calculate the mean, standard deviation, and Relative Standard Deviation (RSD) for each concentration. Plot concentration (x-axis) versus mean response (y-axis) for each concentration. Calculate the regression equation and coefficient of determination (r^2). Record these calculations on the datasheet.

1.2.3. Acceptance criteria:

The correlation coefficient for minimum of five concentration levels should be ≥0.99 for the range of 80 to 120% of the target concentration for assay and should be ≥0.98 for dissolution. The y-intercept must ≤ 2% of the target concentration response.

1.3. Range

For the assay of a finished product, normally from 80 to 120 percent of the test concentration should be used; for content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified; and for dissolution testing: ± 20 % over the specified range is used.

1.3.1. **Test procedure:**

The data obtained during the linearity and accuracy studies will be used to assess the range of the method.
The precision data used for this assessment is the precision of the three replicate samples analyzed at each level in the accuracy studies.

1.3.2. **Documentation:** Record the range on the datasheet.

1.3.3. **Acceptance criteria:**

The acceptable range will be defined as the concentration interval over which linearity and accuracy are obtained per the above criteria, and in addition, that yields a precision of ≤ 3% RSD.

1.4. Accuracy

1.4.1. Test procedure:
Spiked samples will be prepared at three concentrations over the range of 80 %, 100 % and 120 % of the target concentration. Three individually prepared replicates at each concentration will be analyzed. When it is (Spiked samples) difficult to prepare, use a low concentration of a known standard.

1.4.2. Documentation:

For each sample, report the theoretical value, assay value, and percent recovery. Calculate the mean, standard deviation, RSD, and percent recovery for all samples. Record results on the datasheet.

1.4.3. Acceptance criteria:
The acceptable range should be 95 % to 105 % for assay and dissolution over the range of 80 to 120% of the target concentration.

1.5. Precision

1.5.1 Repeatability

1.5.1.1 Test procedure:

Repeatability of analytical method should be performed by measuring the concentration of six independently prepared sample solution at 100% of assay test concentrations. It can also be determined by measuring concentration of three replicate of separate sample solution at

different concentrations (i.e. nine determinations covering specified range of 3 concentration and 3 replicates should be made or six determinations at 100 % of the test concentration). For dissolution purpose, nine determinations covering specified range of 3 concentration and 3 replicates should be made or six determinations at 100 % of the test concentration or 2 or 3 determinations on each of 3 days should be performed.

1.5.1.2 Documentation

Record the spectrum and maximum absorbance at the target wavelength on the datasheet. Calculate the mean, standard deviation, and RSD.

1.5.1.3 Acceptance criteria:

RSD should be ≤ 2% for the assay and dissolution of finished products.

1.5.2 Intermediate Precision

1.5.2.1 Test procedure

Intermediate precision (within-laboratory variation) will be demonstrated by two analysts, using two UV-Visible systems on two different days and evaluating the relative percent purity data across the two Spectrophotometer systems.

The dissolution procedure on the same sample may be run by at least two different analysts from the same laboratory, with each analyst preparing the standard solutions and the medium and following the defined quantification procedure.

1.5.2.2 Documentation:

Record the relative % purity (% area) of each concentration on the datasheet.

Calculate the mean, standard deviation, and RSD for the operators and instruments.

1.5.2.3 Acceptance criteria:

The assay results obtained by two operators using two instruments on different days should have RSD ≤ 3%.

1.6. Limit of Detection: (Not necessary for assay)

1.7. Limit of Quantitation (Not necessary for assay)

1.8. Robustness:

It is the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters. Robustness provides some indication of the reliability of an analytical method during normal usage. It can be determined by measuring the stability of analyte under specified storage condition and small variation in wavelength.

1.9. System suitability:

A system suitability test of the spectrophotometric system can be performed before each validation experiment by measuring absorbance of six replicate reading of standard preparation, evaluate % RSD of standard reading.

Acceptance criteria for system suitability, % RSD of standard reading should be not more than 3.0%, it should be full filled during all validation parameter.

Acceptance criteria for the study of analytical method validation document

S. No.	Parameters		Requirement
a.	**Specificity**		
	1	Blank values: Diluents, Sample solution without active(placebo) API	No interference in the elution zone (λmax**)** of the active ingredient from the blank/diluent, or the placebo impurities/degradants.
	2	Mixed Sample solution (if degradant standards are available, specificity can be demonstrated by addition of these compounds to the analyte API.	Resolution: NLT 1.5(assure that there is no interferences)
b.	**Linearity & Range**		r2 ≥ 0.99
c.	**Repeatability**		RSD ≤ 2 %
d.	**Intermediate Precision**		RSD ≤ 3.0 %
e.	**Accuracy**		95 % to 105 %
f.	**Robustness**		
	1 2	**Stability of standard and sample solution** **Small variation in wavelength**	98 % to 102 % Changes should be within the limits that produce acceptable UV spectrum.
g.	**System suitability**		RSD ≤ 3.0 %

3.3 Performance Characteristic for titrimetric analysis

1. Analytical Performance Characteristics

Procedure:

The titrimetric method of analysis is a nonspecific method the validation of titrimetric method applies to analytical method validation used auto-titrator, Potentiometer using different kind of electrodes, pH meter, the qualification of instruments, electrode in such case will be the prime job. And application is able to determine the component of interest precisely and accurately.
For a qualified system most important will be titer of the titrand as well as performance of the electrode (if used)

For instrumental analyses, the recommendations for establishing the validity of the calibration curve will be a part of method validation:

The titrant to be used in this validation has to be standardized first against a primary standard. Primary standards are commercially available substances with the following characteristics:

- Clearly defined composition and high degree of purity.
- Accurately weighable (not hygroscopic, insensitive to oxygen and/or CO2).
- Stable in solutions and easily soluble in adequate solvents.
- Rapid and stoichiometric reaction with the titrant.

1.1. Specificity

1.1.1 Test procedure:

The specificity of the assay method should be investigated by performing titration of the blank (solvent)/ placebo (matrix solution) standard solution, sample solution to demonstrate the absence of interference to the analyte.

1.1.2. Documentation:

Print the data it titration is carried out from pH meter/potentiometer.

1.1.3. Acceptance criteria:

The excipient (matrix) compounds should not interfere with the analysis of the targeted analyte.

1.2. Linearity

1.2.1. Test procedure:

Linearity can also be investigated for the method as a whole and thus becomes an investigation of trueness as a function of the concentration of the analyte.

Linearity should be determined by preparing samples of at least five different concentrations within the range of 80 % to 120 % of the target concentration. The method of standard preparation and the number of determination should be same as used in the final procedure for Test method. The volume of titrant consumption obtained (consumption of volume should be 30 to 90% of the burette volume to avoid refilling of the burette) is plotted against the respective sample size which determines the analyte concentration per single analysis. A linear regression is performed on these data. The regression line is described by the formula y

= a + bx, where a represents the intercept on the y-axis and b is the slope of the regression line.

Note: If volume consumption is less than 10ml, micro burette should be used.

1.2.2. Documentation:

Record the results on a datasheet. Calculate the mean, standard deviation, and Relative Standard Deviation (RSD) for each concentration. Plot concentration (x-axis) versus mean response (y-axis) for each concentration. Calculate the regression equation and coefficient of determination (r^2). Record these calculations on the datasheet.

1.2.3. Acceptance criteria:

The correlation coefficient for minimum of five/six concentration levels should be ≥0.995 for the range of 80 to 120% of the target concentration. The y-intercept must ≤ 2% of the target concentration response. A plot of response factor versus concentration must show all values within 2.5% of the target level response factor, for concentrations between 80 and 120% of the target concentration.

1.3. Range

For the assay of a drug substance or a finished product: normally from 80 to 120 percent of the test concentration; for content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified; and for dissolution testing: +/-20 % over the specified range

1.3.1. Test procedure:

The data obtained during the linearity and accuracy studies will be used to assess the range of the method.

The precision data used for this assessment is the precision of the three replicate samples analyzed at each level in the accuracy studies.

1.3.2. Documentation:

Record the range on the datasheet.

1.3.3. Acceptance criteria:

The acceptable range will be defined as the concentration interval over which linearity and accuracy are obtained per the above criteria, and in addition, that yields a precision of ≤ 3% RSD.

1.4. Accuracy

1.4.1. Test procedure:

Spiked samples will be prepared at three concentrations over the range of 80 %, 100 % and 120 % of the target concentration. Three individually prepared replicates at each concentration will be analyzed. When it is (Spiked samples) difficult to prepare, use a low

concentration of a known standard. Consumption of titrant of is 30 to 90% of the burette volume. A refilling of the burette should be avoided.

1.4.2. Documentation:

For each sample, report the theoretical value, assay value, and percent recovery. Calculate the mean, standard deviation, RSD, and percent recovery for all samples. Record results on the datasheet.

1.4.3. Acceptance criteria:

100 ± 2% is typical for an assay of an active ingredient, in a drug product over the range of 80 to 120% of the target concentration. The measured recovery in case of dissolution is typically 95 % to 105 %.

1.5. Precision

1.5.1. Repeatability

1.5.1.1. Test procedure:

For the method repeatability, nine determinations covering specified range of 3 concentration and 3 replicates should be made or six determinations at 100 % of the test concentration. /auto-titrator Consumption of titrant should be equivalent to 90% of the burette volume. A refilling of the burette should be avoided.

1.5.1.2. Documentation

Record the data if titration involves instruments like pH meter/potentiometer print out the data and data sheet. Calculate the mean, standard deviation, and RSD.

1.5.1.3. Acceptance criteria:

RSD should be NMT 1% for drug substances and drug products, less than NMT 2% for the assay

1.5.2. Intermediate Precision

1.5.2.1 Test procedure

Intermediate precision (within-laboratory variation) will be demonstrated by two analysts on different day and evaluating the relative percent purity data across the systems on different instrument.

1.5.2.2 Documentation:

Record the relative % purity of each concentration on the datasheet. Calculate the mean, standard deviation, and RSD for the operators and instruments.

1.5.2.3 Acceptance criteria:

The assay results obtained by two operators using two instruments on different days should have a statistical RSD ≤ 3%.

1.6. Limit of Detection: (Not necessary for assay)

1.7. Limit of Quantitation (it is not necessary for assay)

1.8. Robustness: Optional.

Robustness measures the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters. Robustness provides some indication of the reliability of an analytical method during normal usage. Parameters, which will be investigated by small variation in solution temperature, pH etc. may be evaluated.

Acceptance criteria for the study of analytical method validation document

S.No.		**Parameters**	**Requirement**
1.		**Specificity**	
	i.	Blank values: Diluents/solvent	
	ii.	Sample solution without active	
2		**Linearity & Range**	$r^2 \geq 0.99$
3.		**Repeatability**	RSD $\leq$ 2.0 %
4.		**Intermediate Precision**	RSD $\leq$ 3.0 %
5.		**Accuracy**	95.0 % to 105 %
6.		**Robustness (Optional)**	

3.4 Performance Characteristic for microbiological analysis

1. Performance Characteristics (Sterility test & Microbial Limit Test)

1.1 Specificity

Specificity is the capability of the method to resolve or measure a range of microorganisms. Freedom from interference from excipients or active pharmaceutical ingredients, degradation products or impurities must be noted as part of a recovery (accuracy) study. Where selecting an appropriate culture medium is part of the study, the properties of the medium against selective, non-selective and mixed cultures must also to be considered.

A low number of specified <100 CFU is appropriate. All challenge microorganisms should be recovered. Where atypical colony morphology is observed, supporting identification should be considered.

In the case of total aerobic microbial count, *Staphylococcus aureus*(ATCC 6538), *Pseudomonas aeruginosa*(ATCC 9027), *Bacillus subtilis*(ATCC 6633), *Candida albicans*(ATCC 10231) and *Aspergillus brasiliensis*(ATCC 16404) is to be used according to the media.

In the case of test for specified micro-organisms, *Staphylococcus aureus*(ATCC 6538), *Pseudomonas aeruginosa*(ATCC 9027), *Escherichia coli* (ATCC 8739), *Salmonella enterica spp. enterica serotype typhimurium* (ATCC 14028) or *Salmonella enterica spp. enterica serotype abony* (NCTC 6017), *Shigella boydii* (ATCC 8700 or NCTC 12985), *Candida albicans*(ATCC 10231) and *Clostridium sporogenes* (ATCC 11437 or ATCC 19404) is to be used according to the media.

In the case of sterility test, *Staphylococcus aureus*(ATCC 6538), *Pseudomons aeruginosa*(ATCC 9027), *Clostridium sporogenes (*ATCC 19404), *Bacteroides vulgatus* (ATCC 8482), *Bacillus subtilis*(ATCC 6633 or NCIMB 8054), *Aspergillus brasiliensis*(ATCC 16404), *Candida albicans*(ATCC 10231 or ATCC 2091 or NCYC 854) is to be used according to the media.

Acceptance Criteria: Growth obtained on solid medium must not differ from the calculated cfu of the standardized inoculum by a factor > 2. If the growth is luxuriant, the recovery rate should be ≥ 50% and if the growth is inhibitory, the recovery rate is 0%. The recovery rate is considered as 100% for bacteria growth on Soyabean Caesin Digest Agar and fungus growth on Sabouraud Dextrose Agar.

Liquid media under test should be considered suitable if the growth of the organism is comparable to that obtained on the same medium, previously tested and approved. For the test for specified microorganism, surface spread method is to be used and the growth obtained should be comparable to that on the same medium previously approved.

In order to prevent any phenotypic changes in the strains used, the organisms used in the test should not be more than 5 passages made from the original culture.

Note: Specificity can also be demonstrated by COA of particular batch of culture media and micro-organisms.

1.2 Accuracy

Accuracy is the closeness of agreement between the measured value and the "true" or expected measure or reaction across the range of the test. This can be assessed by determining the recovery of known quantities of a microorganism that has been added to a sample.

This is done by addition of the organisms of less than 100 cfu to the final diluent of the sample. The organism is mentioned in the Specificity.

Acceptance Criteria: Growth obtained on solid medium must not differ from the calculated cfu of the standardized inoculum by a factor > 2. If the growth is luxuriant, the recovery rate should be ≥ 50% and if the growth is inhibitory, the recovery rate is 0%. The recovery rate is considered as 100% for bacteria growth on Soyabean Caesin Digest Agar and fungus growth on Sabouraud Dextrose Agar.

Liquid media under test should be considered suitable if the growth of the organism is comparable to that obtained on the same medium, previously tested and approved. The growth of the organism in the medium in the presence of the sample (product positive control) should be visually comparable to the positive control tube. In the case of Penicillin and Cephalosporin, β-lactamase should be used for sterility test.

For the test for specified microorganism, surface spread method is to be used and the growth obtained should be comparable to that on the same medium previously approved. At the time of mixing, add each test organism in the prescribed growth medium. The specified micro organism must be detected with the colony morphology and indication reaction as described.

If the test specimen is known to contain any of the below mentioned antimicrobial substances then use the corresponding inactivating agent to neutralize the antimicrobial activity.

Table 1.2 : Antimicrobial substances with corresponding inactivating agents

Antimicrobial substances	**Inactivator**	**Concentration**
Phenolics, Parahydroxy benzoate (Parabens)	Polysorbate 80	30 g per litre
Iodine, Quarternary ammonium compound (QACs)	Lecithin Sodium lauryl sulphate	3 g per litre 4 g per litre
Alcohol, Aldehydes, Sorbates	Dilution	-
Mercurial halogens	Sodium thiosulphate	5 g per litre

1.3 Intermediate Precision

The degree of precision of test results obtained by the analysis of the samples under a variety of typical test conditions such as different analysts on different days, apparatus & reagent lots.

This parameter is not required for sterility test and test for specified micro-organisms.

Acceptance criteria: Typical level of precision shall be 15% to 35% RSD.

1.4 Robustness

Robustness is the reliability of a method or test to withstand small (but deliberate) variations in method parameters. Example- reagent volume, incubation time or ambient temperature providing an indication of its reliability during normal usage.

2. Endotoxin Test (Gel Clot method)

2.1 Calculation of Endotoxin Limit

The endotoxin limit for a given test preparation is calculated from the expression K/M, where M is the maximum dose administered to an adult (taken as 70 kg for this purpose) per kg per hour and K is the threshold pyrogenic dose of endotoxin per kg of body mass. The value of K is 5.0 EU/kg for parenteral preparations except those administered intrathecally, and is 0.2 EU/kg for preparations intended to be administered intrathecally.

For radiopharmaceutical products not administered intrathecally, the endotoxin limit is calculated as 175/V, where V is the maximum recommended dose in ml. For intrathecally administered radiopharmaceuticals, the endotoxin limit is obtained by the formula 14/V. For formulations (anticancer products) administered on as per square meter of body surface, the formula is K/M, where K=2.5 EU per kg and M is the (maximum dose/m^2/hour x 1.80 m^2)/70 kg.

2.2 Sensitivity of the lysate.

Confirm the labelled sensitivity of each new batch of lysate prior to use in the test using at least one vial of each batch of lysate. Prepare a series of dilutions of CSE to give concentrations of 2λ, λ, 0.5λ and 0.25λ, where λ is the labelled sensitivity of the lysate in EU per ml. Perform the test in duplicate and include a negative control consisting of water BET. At least the final dilution in each series must give a negative result.

Dilution	**Result**
2 λ	+
λ	+ / -
0.5 λ	+ / -
0.25 λ	-

+ = positive (gel clot present), - = negative (gel clot absent)

Calculate the average of the logarithms of the lowest concentration of endotoxin in each series of dilutions for which a positive result is found. The geometric mean end-point concentration is the measured sensitivity of the lysate in EU/ml, which is calculated using the following expression:

Geometric mean end-point concentration = antilog ($\sum e/f$)

where, $\sum e$ = sum of the log end-point concentrations of the series of dilutions used;
f = number of replicate test-tubes.
This average gives the estimated lysate sensitivity which must lie between 0.5λ and 2λ

2.3 Test for interfering factors.

The possibility of interference with the bacterial endotoxins test by certain factors should be borne in mind. For validation of the test results it must be demonstrated that the test preparation does not inhibit or enhance the reaction or otherwise interfere with the test. The validation must be repeated if the lysate vendor or the method of manufacture or the formulation of the sample is changed. Dilution of the test preparation with water BET is the easiest method for overcoming inhibition.

The allowable dilution level or Maximum Valid Dilution (MVD) is dependent on the concentration of the product, the endotoxin limit for the product and the lysate sensitivity. It is calculated by the following expression:

$$MVD = \frac{\text{Endotoxin limit X Concentration of the test solution*}}{\lambda}$$

where, λ is the labelled sensitivity of the lysate (EU/ml).

* Concentration of the test solution is expressed as mg/ml in case the endotoxin limit is specified by weight (EU/mg), or as Units/ml in case the endotoxin limit is specified by Unit (EU/Unit), or as 1.0 ml/ml in case the endotoxin limit is specified by volume (EU/ml).

2.4 Preparation of test solutions.

Prepare replicates of solutions A to D as indicated in the table.

Table 2.4: Preparation of test solutions

Solution	**Final concentration of added CSE in the solution**	**Number of replicates**
A	-	4
B	2λ	4
	λ	4
	0.5λ	4
	0.25λ	4
C	2λ	2
	λ	2
	0.5 λ	2
	0.25λ	2
D	-	2

Solution A = Solution of the product at a dilution at or below MVD (test solution).
Solution B = Test solution spiked with indicated CSE concentrations (Positive Product Control; PPC).
Solution C = Standard solution with indicated CSE concentrations in water BET.
Solution D = Water BET (Negative Control; NC).

Carry out the procedure in receptacles such as tubes, vials or wells of micro-titre plates.

2.5 Acceptance Criteria:

The test for interfering factors is valid if

(a) solutions of series A and D give negative results;

(b) the results obtained with solutions of series C confirm the labelled sensitivity of the lysate;

(c) the geometric mean of the end-point concentration of solutions of series B is not more than 2 λ or not less than 0.5 λ.

If the result obtained is outside the specified limit, the test preparation under examination is acting as an inhibitor or activator. The interfering factors may be eliminated by further dilution (not greater than MVD), filtration, neutralisation, inactivation or by removal of the interfering substances. The use of a more sensitive lysate permits the use of greater dilution of the preparation under examination.

ANNEX IV

Preliminary Screening of the document for AMV

Name of the Product: **Composition:**
Manufactured by: **Submitted by:**
Product License:
Category:
Registration no:

Assay: ☐ **Dissolution:** ☐
HPLC ☐ **Chemical** ☐ **Microbiology** ☐

S.No.	Parameter to be Performed	Assay	Dissolution	Remarks
1.	Specificity			
2.	Linearity and range			
3.	Precision			
3.1	Repeatability			
3.2	Intermediate precision			
4.	Accuracy			
5.	Solution stability			
6.	Robustness			
7.	System suitability			
9.	Checklist (Annex 2.8, 2.9, 2.10)			
10.	Microbiological quality document (Annex X)			
11.	Format (as per Annex XI)			

Modified release product/Category 1: Innovator/ Comparator product data

File accepted ☐ **File rejected** ☐ **Reason: _____**

Forwarded to Validation committee: _____ Date: _____

Received:

Signature: _____
Name: _____
Designation: _____
Date: _____

1. System suitability and Robustness Test are optional for UV-Visible spectrophotometric and titration method of analysis.
2. Solution stability test is optional for Titration method of analysis.

ANNEX V

SOP for study of documents of non pharmacopoeial products for regulatory approval

	Department of Drug Administration **National Medicines Laboratory** **Analytical Method Validation Committee**	**NPV/076-77/SOP-02**
SOP for study of documents of non pharmacopoeial products for regulatory approval		

1. **Purpose:**

   To provide the documented evidence that whether the analytical method submitted by the pharmaceutical industry is suitable for the analytical operation.

2. **Objective:**

   To evaluate the available validated analytical method and give recommendation to DDA for the approval of the Product (Quality Control) specification and standard analytical method of non pharmacopoeial product.

3. **Scope:**

   This will provide procedure for the study of documents related to analytical method validation of non pharmacopoeial product

4. **Responsibility:**

   The entire committee member will be responsible for the guidance and recommendation regarding the parameters for the product specification and analytical profile of the non pharmacopoeial product.

5. Procedure:

5.1 Procedure for the incoming documents in the committee :

i. First the pharmaceutical company registers the document of non pharmacopoeial product along with the analytical method validation test report to Department of Drug Administration. Domestic pharmaceutical company registers the document to Industry section and foreign pharmaceutical company registers the document to import section through importers.

ii. The authorized person from Industry section and Import section will fill preliminary screening form (ANNEX IV) before registration.

iii. The documents should be submitted to AMV Committee after getting the product license from DDA.

iv. From Industrial section and Import section, the authorized person prepares note for suggestion *(Tippani & Aadesh in Nepali)* and submits the document file to Director General, DDA.

v. The document will be sent to Analytical method validation committee for adequacy check as per the guideline.

vi. The document will be registered in Entry Register Book which contains all the information regarding the entry date and remarks of the documents. The numbering of Entry Book Register will be ERB-AMV/fiscal year-Number. The format of the document entry book will be as follows:

S.No.	Date	Product Name	API Name	Category of product	Company Name	Document Submitted by	Checked By	Date	Remarks	Status

5.2 Procedure for the checking of the documents

i. The received product application along with analytical method validation will be distributed to all the member of the committee.

ii. The committee member will check all the parameters of the documents and checklist filled by the company using the internal check list (ANNEX II, 2.1, 2.2, 2.3, 2.4, 2.5).

iii. All the documents required and acceptance criteria are available in the internal check list.

iv. If there is some deficiency and mistakes in the documents, the committee will decide about the deficiencies and errors of the document and fill the form as per ANNEX II, 2.6. The committee will correspondence the manufacturers/importers about their deficiencies in written form as per ANNEX IX.

v. In case of product requiring document evaluation only, document will be studied as per guideline. The method will be evaluated and published in phase wise manner through post marketing surveillance.

vi. The committee will recommend for the analysis of sample after obtaining the complete documents from the manufacturers/importers.

vii. Letter will be issued to NML for Testing as per ANNEX VIII.

5.3 Procedure for selection of method by AMV Committee

i. There should be at least three method (if available) with reference to reliable literature.

ii. The method should be selected from the one which is simple and easy to perform. The method should be stability indicating using HPLC or modern analytical technique.

iii. It should be safe to handle (less hazard to person and environment).

iv. The instruments /equipment and reagent should be readily available.

v. The method should be robust i.e. no deliberate change on changing environment, Specific, precise and should produce accurate result.

vi. The testing of the method should be under taken on at least three different product manufactured of same dosage, from three different manufacturer as far as possible from selected method.

vii. The method by modern advanced technique is preferable, if the equipment is readily available.

5.4 Procedure for the analysis of the finished product and approval of the report

i. The domestic pharmaceutical company/importers will be informed to deposit the required amount of payment for the analysis as per letter (ANNEX VIII).

ii. The required number of sample (the product) and required documents will be submitted to NML for analysis.

iii. The testing of the method should be under taken on at least three different product manufactured of same dosage, from three different manufacturer as far as possible using recommended method from the committee and report of analysis will be issued to AMV committee.

iii. AMV committee will discuss on the report and evaluate the result. The committee will prepare Product Specification (Quality Control) and Analytical profile.

iv. Committee will send a document (*Tippani* file) to DDA along with Product (Quality Control) Specification and Analytical profile for the final approval.

v. The analytical method will be approved by the DDA and the method is forwarded to DAC for final approval.

vi. After approval from DDA, the analytical report is forwarded to corresponding manufacturer/ importer through AMV Committee.

vii. The method is published prior to approval from DAC with the Disclaimer Statement "Subject to Approval from DAC". The disclaimer will be removed after approval of the method by DAC.

5.5 Procedure for the numbering of the document

i. The name of the approved method from the DDA will be given as **Analytical Profile No. Letters of generic name of sample/Fiscal Year/AP Number.**

ii. Numbering of the Analytical Method Validation Guideline will be as **AMVP/FiscalYear-Revision Number.** For e.g. AMVP/076/077-01

iii. Numbering of SOP will be as **NPV/Year/SOP-Number.** For e.g. NPV/073/SOP-01.

iv. If the analytical profile is revised through Change Control SOP(NPV/076-77/SOP-03), the fiscal year, the numbering of method shall be same as previous with revision number. For e.g. If the numbering of Analytical Profile of Chlorzoxazone and Paracetamol tablet is Chl Para 073/074/AP025 and if the document is revised in the fiscal year 074-75 then the numbering will be as Chl Para 074/075/AP025-01.

ANNEX VI

SOP for Change Control

	Department of Drug Administration **National Medicines Laboratory** **Analytical Method Validation Committee**	**NPV/076-77/SOP-03**
SOP for Change Control Procedure		

1. **Objective:**

   To describe the Procedure and Instructions to identify changes analytical procedures approved by Department of Drug Administration (DDA) or Drug Advisory Committee(DAC) and evaluation and implementation of change control.

2. **Scope:**

   This SOP applies during the changes in analytical procedures approved by Department of Drug Administration (DDA) or Drug Advisory Committee(DAC).

3. **Responsibility:**

S.N.	Responsibility	Activity
1	AMV Committee member	To review and study the document provided by initiator. To recommend for changes in analytical procedures approved by DDA or DAC.
2	Co-ordinator of AMV Committee	To recommend for changes to Director General of DDA
3.	DDA Director General	To recommend for changes to the DAC Committee for the approval of changes in the analytical method approved by DAC Committee.
4.	DAC Committee	To approve the changes in the analytical method proposed by AMV committee.
5.	Member Secretary	To record the details of change control

4. Procedure:

4.1 The initiator (NML, DDA, AMV Committee, and Industry) shall identify the requirement of changes.

4.2 Proposal for Change:

4.2.1 The initiator should initiate the change as per change request form NPV/076-77/F-01

4.2.2 Reason for change should be specific and clearly highlighted. The cost/quality benefits should be mentioned.

4.3 Evaluation of Change by AMV Committee

4.3.1 Member secretary shall enter the details of change in Change Control Register (NPV/076-77/F-02) by assigning Change Control Code as Serial No./Fiscal Year. (For eg. 001/2071/72).

4.3.2 The change request form shall be discussed among AMV Committee members and evaluated by AMV Co-ordinator for its completeness, feasibility and the action to be carried out before implementation of changes.

4.3.3 If the change is applicable, AMV Co-ordinator shall forward the method for its approval to the Director General along with the required documents.

4.4 Implementation by AMV Co-ordinator:

4.4.1 On completing all the procedures, AMV Co-ordinator shall formally change the status in Change Control Record and formally close the Change Control Procedure.

5 Abbreviation:

DAC: Drug Advisory Committee

DDA: Department of Drug Administration

NML: National Medicines Laboratory

6 Reference: Pharmaceutical Guidelines

7 Records:

7.1 Change Request Form

7.2 Change Control Record

	National Medicines Laboratory **Analytical Method Validation Committee**	**NPV/076-77/F-01**

Change Request Form

1	Change Initiated by	
2	Existing Condition*	
3	Proposed Change*	
4	Justification/Impact of Change*	
5	Supporting Data (If Required)	Enclosed/Not Enclosed
6	Signature: Designation: Organisation: Date submitted to AMV Committee:	
7	Comment from AMV Committee Regulatory Notification/approval Type of analysis to be carried out (if any extra analysis is required, give details): Change Control No: Signature of AMVC Co-ordinator : Date :	Validation Status Affected/Not Affected
8	Date Forwarded to DDA Director General for approval (for AMV method)	

*Whenever applicable, append supporting documentation.

S.N.	Date	Change control No.	Originating Organisation	Product/ Document	Details of Change	Date of Implementation	Status	Remarks
1.								
2.								
3.								
4.								
5.								
6.								
7.								
8.								
9.								
10.								
11.								
12.								
13.								
14.								
15.								
16.								
17.								
18.								

ANNEX VII

Guideline on Degradation Reactions for specificity determination

1.0 Acid hydrolysis

Expose the Sample in aqueous acid or acidified solvent/ Heat/reflux or UV radiation. The exposure on stressed condition and solution strength (strength of base) for base hydrolysis and exposure time may be determined by the pharmaceutical laboratory as per the physiochemical characteristic of the molecule/dosage form.

2.0 Base hydrolysis:

Expose the Sample in aqueous base / basic solvent /Heat/reflux or UV radiation. The exposure on stressed condition and solution strength (strength of base) for base hydrolysis and exposure time may be determined by the pharmaceutical laboratory as per the physiochemical characteristic of the molecule/dosage form.

3.0 Oxidation:

Treat with H_2O_2/ UV irradiation, solution strength and exposure time should be determined by pharmaceutical laboratory.

4.0 Light decomposition (photolysis):

Expose to high-intensity UV light in suitable increment which can be determined by pharmaceutical laboratory.

5.0 Thermal decomposition (pyrolysis):

Expose heat to suitable temperature with appropriate increments, and optimum time should be determined by pharmaceutical laboratory with scientific justification.

Acceptance Criteria:

If possible, degradants spiked placebos can be used in addition to peak purity to demonstrate that the degradants are resolved from the analyte. Evaluation whether the chromatograms/ spectra of the sufficiently degraded spiked placebo overlaid with the degraded placebo under each degradation condition and that of un- degraded API, drug product.

It is possible to identify no co-elution with degradation peaks and other impurities using HPLC method coupled with DAD. For FDC (e.g. with more than one API), individual active solutions should be made for each component.

ANNEX VIII

Format of letter issued to NML for Testing

औषधि व्यवस्था बिभाग

राष्ट्रिय औषधि प्रयोगशाला

औषधि परिक्षण बिधि पुस्टिकरण समिति

मिति :

श्री राष्ट्रिय औषधि प्रयोगशाला,

बिजुलीबजार ,काठमाडौँ

बिषय :नमुना परिक्षण बारे

उपरोक्त बिषयमा Analytical Method Validation Committee मा प्राप्त …….....

.....……को tnemucod study सम्पन्न भैसकेकोले सो कम्पनी बाट ..... ……मा प्राप्त नमुना) B.N.:……..……..; MD/ED: …… (को परिक्षण गरिदिन हुन अनुरोध गर्दछु |

संयोजकको नाम

पद

(संयोजक)

औषधि परिक्षण बिधि पुस्टिकरण समिति

ANNEX IX

Format of letter issued to manufacturers/importers

Department of Drug Administration

National Medicines Laboratory

Analytical Method Validation Committee

Date:

To,

……..

C/O……

Sub: Analytical Method Validation

Dear Sir,

With reference to above mentioned subject, it is requested to provide sample*/ documents of

…….....

as mentioned below.

1.

2.

3.

4.

5.

Name of Co-ordinator of AMV Committee

Post

***Note:**

- For analysis in NML, sample should be submitted along with Product specification, Certificate of Analysis, Method of Analysis, working standard, Certificate of Analysis of working standard & atleast 3 month stability study.
- For microbiological testing, additional copies of above mentioned documents should be submitted.
- Sample Analysis fee should be submitted along with the documents and a copy of this letter to NML.

ANNEX X

Recommended acceptance criteria for microbiological quality of non-sterile dosage form

Route of Administration	TAC (cfu/g or cfu/ml)	TFC (cfu/g or cfu/ml)	Specified microorganisms
Non aqueous oral	10^3	10^2	Absence of *E. coli* (1g or 1ml)
Aqueous oral	10^2	10	Absence of *E. coli* (1g or 1ml)
Rectal	10^3	10^2	--
Oral, mucosal, gingival, Nasal, Auriculur	10^2	10	Absence of *Staphylococcus aureus* (1g or 1ml) Absence of *Pseudomonas aeruginosa* (1gor 1ml)
Vaginal	10^2	10	Absence of *Pseudomonas aeruginosa* (1g or 1ml) Absence of *Staphylococcus aureus* (1g or 1ml) Absence of *Candidia albicans* (1g or 1ml)
Transdermal patch(limits of one patch including adhesive layer and backing	10^2	10	Absence of *Staphylococcus aureus* (1g or 1ml) Absence of *Pseudomonas aeruginosa* (1g or 1ml)
Inhalation use (special requirements apply to liquid preparation for nebulization)	10^2	10	Absence of *Staphylococcus aureus* (1g or 1ml) Absence of *Pseudomonas aeruginosa* (1g or 1ml) Absence of Bile-tolerant Gram negative bacteria (1g or 1ml)

ANNEX XI

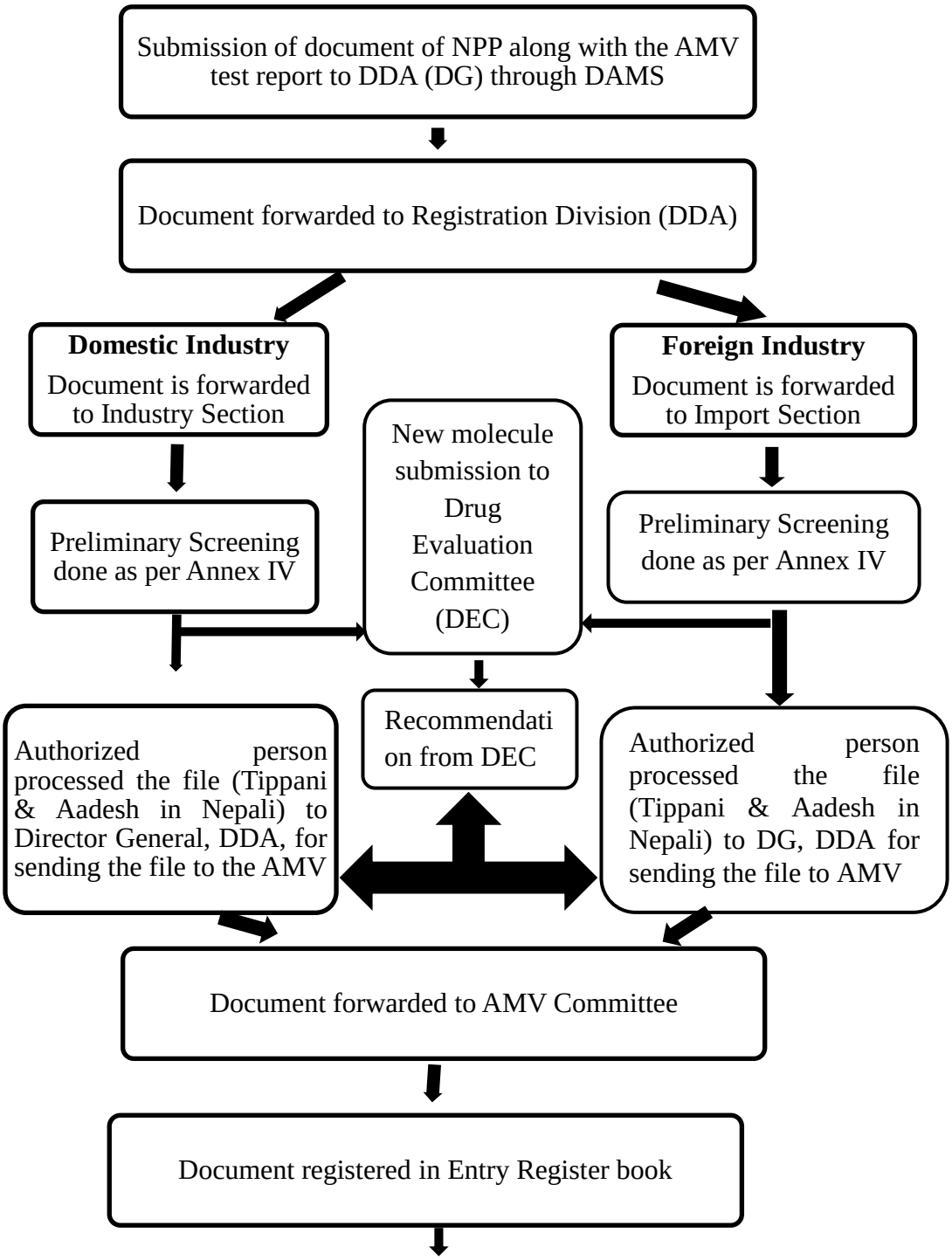
Format of the document to be submitted for Analytical Method Validation

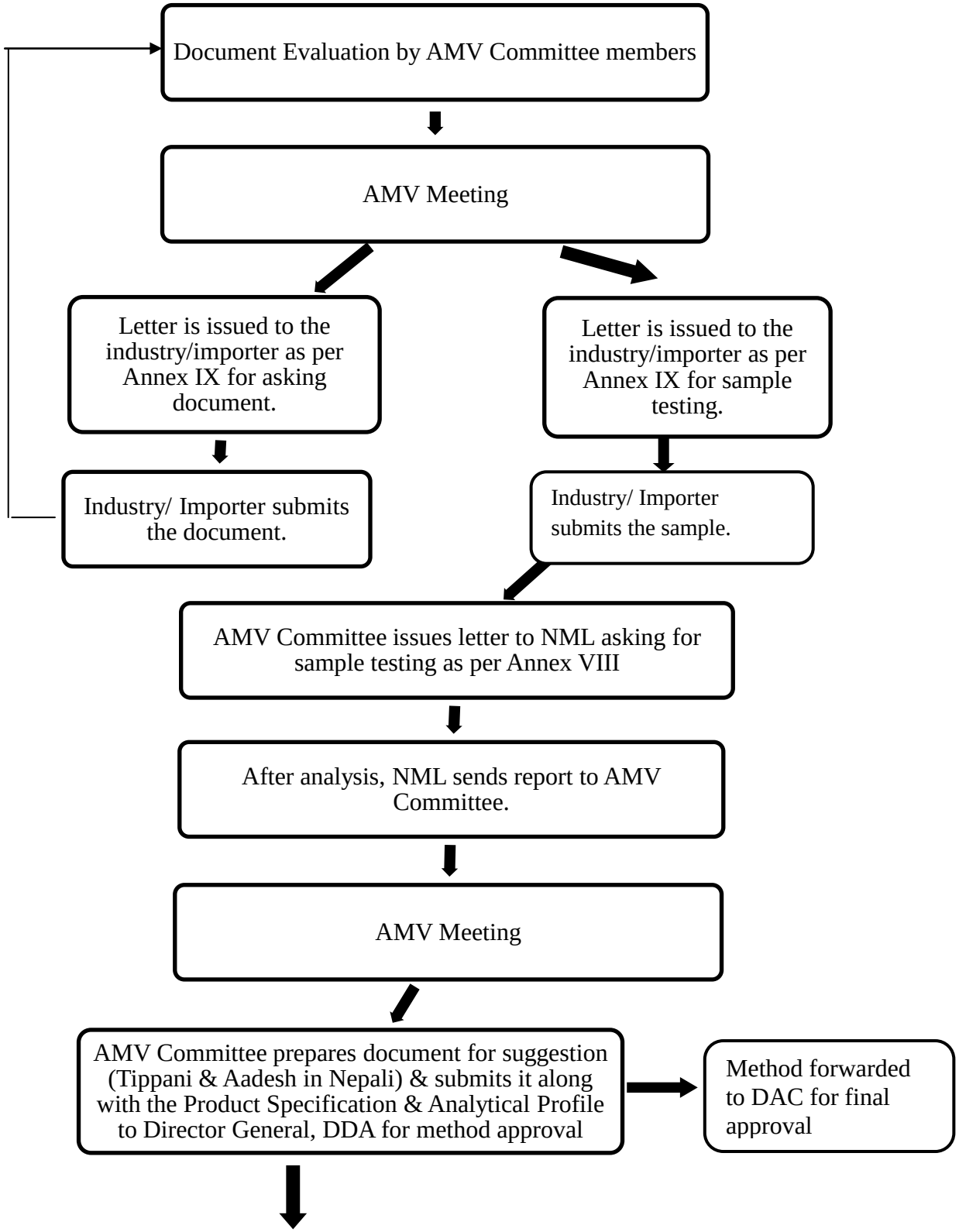
The documents should be submitted in the hard file. The document should be properly separated with separator containing tab. The prescribed format/order of the document to be submitted for Analytical Method Validation is as follows:

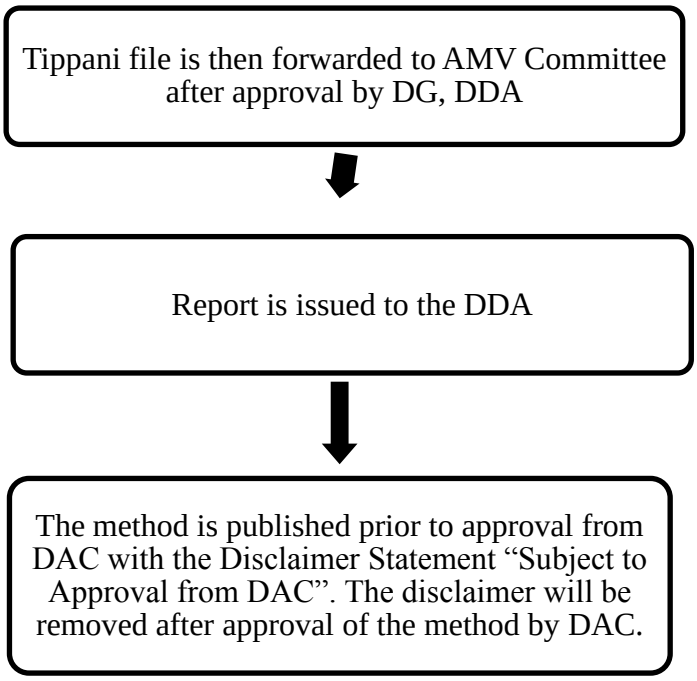
1. "Application of the method validation of the drug (Schedule 2)" duly filled and authorized.
2. Product License (Schedule 5) relating to Sub-rules (2) and (3) of Rule 4 of Drugs Registration Rules, 2038 (1981).
3. Annex II, 2.7, 2.8, 2.9, 2.10 of Guideline on Analytical Method Validation on Non-pharmacopoeial Products for Regulatory Approval duly filled and authorized.
4. Table of content
5. Product Specification, method of analysis, analytical method reference (if applicable).
6. Reagent used, instruments calibration record, reference material record.
7. Analytical Method Validation Protocol.
8. Analytical Method Validation Report including all the analytical method validation parameters with calculation, chromatogram, raw data, etc.
9. Any other documents as required.

ANNEX XII

Flow chart of AMV process




Document Evaluation by AMV Committee members
AMV Meeting
Letter is issued to the industry/importer as per Annex IX for asking document.
Letter is issued to the industry/importer as per Annex IX for sample testing.
Industry/ Importer submits the document.
Industry/ Importer submits the sample.
AMV Committee issues letter to NML asking for sample testing as per Annex VIII
After analysis, NML sends report to AMV Committee.
AMV Meeting
AMV Committee prepares document for suggestion (Tippani & Aadesh in Nepali) & submits it along with the Product Specification & Analytical Profile to Director General, DDA for method approval
Method forwarded to DAC for final approval


Tippani file is then forwarded to AMV Committee after approval by DG, DDA
Report is issued to the DDA
The method is published prior to approval from DAC with the Disclaimer Statement “Subject to Approval from DAC”. The disclaimer will be removed after approval of the method by DAC.

GLOSSARY OF TERMS

GLOSSARY OF TERMS

Acceptance criteria: Numerical limits, ranges, or other suitable measures used to determine the acceptability of the results of analytical procedures.

Accuracy: Expresses the closeness of agreement between the value found and the value that is accepted as either a conventional true value or an accepted reference value. It may often be expressed as the recovery by the assay of known, added amounts of analyte.

Active pharmaceutical ingredient (API):

Also known as drug substance, it is component that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure of any function of the body of man or other animals.

Analytical performance characteristics:

A term used by the USP, analytical performance characteristics refer to those characteristics of an analytical method that define its performance as an analytical technique. These performance characteristics include accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range.

Approved Method The document forwarded by AMV committee with suggestion, undergoes discussion between DDA and DAC and will be finalize by DAC as a formal document.

Blank: A sample or standard of a particular matrix or composition without analyte.

Calibration curve: A plot of standard solution concentration, on the x-axis, versus instrument response, on the y-axis.

Comparator: The finished pharmaceutical product with which a product to be compared. The comparison may be by means of bioequivalence studies or clinical studies of safety and/or effectiveness.

Forced degradation condition (stressed condition):

A molecule (API)/excipient/finished product is allowed to change chemically over time/soon by the action/reaction of light, temperature, pH, water.

Drug product: The combination of API and excipients processed into a dosage form and marketed to the public. Common examples include tablets, capsules, and oral solutions. Also referred to as finished product or dosage form. Drug substance.

Filter compatibility: A comparison of filtered to unfiltered solutions in a methods validation to determine whether the filter being using retains any active compounds or contributes unknown compounds to the analysis.

Fixed Dose Combination (FDC):

A combination of two or more actives in a fixed ratio of doses. This term is used generically to mean a particular combination of actives irrespective of the formulation or brand. It may be administered as single entity products given concurrently or as a finished pharmaceutical product.

Forced degradation: Is a process that involve degradation of the sample drug product or API at condition more severe than accelerated conditions.

Formulation: The recipe describing the quantity and identity of API and excipients making up a drug product.

Innovator Drug: a drug for which a New Drug Application (NDA) has been submitted to a regulatory authority and marketing authorisation granted.

Linearity: Evaluates the analytical procedure's ability (within a given range) to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. Linearity is usually expressed as the confidence limit around the slope of the regression line.

Matrix (sample matrix): The components and physical form with which the analyte of interest is intimately associated. In the case of drug product, the matrix is the combination of excipients in which the active ingredient is diluted and formed within.

Non-pharmacopoeial product: If the categorization and test or analytical method of any drug has not been mentioned in the pharmacopoeia or the encyclopedia pursuant to rule 4 and 5 of Drug Categorization Regulation, 2043 such drug is known as non-pharmacopoeial product.

Percent relative standard deviation (% RSD):

A measure of the relative precision of an analytical method for a given set of measurements. % RSD is calculated by dividing the standard deviation for a series of measurements by the mean of the same sets of measurements and multiplying by 100. % RSD (σ n1/ mean) * 100. Large % RSDs for a series of measurements indicate significant scatter and lack of precision in the technique.

Placebo: A formulation containing all ingredients of a drug product except the active ingredient for which the method is being developed.

Protocol: An approved documented procedure when executed, will demonstrate the ability of the subject method to perform as intended.

Raw data: Raw data are the original records of measurement or observation. Raw data may include, but are not limited to, printed instrument output, electronic signal output, computer output, hand-recorded numbers, digital images, hand-drawn diagrams, and so on. Raw data are proof of the original measurement or observation and by definition cannot be regenerated once collected.

Reagent blanks: Reagents used during the analytical process (including solvents used for extraction or dissolution) are analysed in isolation in order to see whether they contribute to the measurement signal. The measurement signal arising from the analyte can then be corrected accordingly.

Reference standard: A highly purified compound that is well characterized. It is used as a reference material to confirm the presence and/or amount of the analyte in samples. Related compounds. Categorized as process impurities, degradants, or contaminants found in finished drug products.

Reliable literature: International, regional or national standards or other recognized specifications that contain sufficient and concise information on analytical method.

Sample blanks: These are essentially matrices with no analyte. They are difficult to obtain but such materials are necessary to give a realistic estimate of interference that would be encountered in the analysis of test samples.

Specific: Measure only the desired component without interference from other species that might be present; separation is not necessarily required.

Specification: The quality control standards (e.g., tests, analytical procedures, and acceptance criteria) provided in an approved application to confirm the quality of drug substances, drug products.

Specificity: The ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as impurities, degradation products, and excipients. There must be inarguable data for a method to be specific.

Spiked material: These are material or solutions, which have been fortified with the analyte(s) of interest.

Spiked placebo: Preparation of a sample to which known quantities of analyte are added to placebo material

Spiking: The addition of know amounts of a known compound to a standard, sample, or placebo, typically for the purpose of confirming the performance of an analytical procedure or the calibration of an instrument.

SRA WHO recognizes the scientific evaluation of finished pharmaceutical products (FPPs) that has been carried out by **stringent regulatory authorities** (SRAs), which apply similarly stringent standards for quality, safety and efficacy to those recommended by WHO.

Stability: is determined by comparing the response and impurity profile from aged standards or samples to that of a freshly prepared standard and to its own response from earlier time points. These are short-term studies and are not intended to be part of the stability indication assessment or product stability program**.**

Stability indicating methodology:

A validated quantitative analytical procedure or set of procedures that can detect the changes with time in the pertinent properties (e.g., active ingredient, preservative level, or appearance of degradation products) of the drug substance and drug product Stability indicating assay. An assay that accurately measures the component of interest [the active ingredient(s) or degradation products] without interference from other degradation products, process impurities, excipients, or other potential interfering substances.

Standard and sample solution stability.

Established under normal benchtop conditions, normal storage conditions, and sometimes in the instrument (e.g., an HPLC auto sampler) to determine if special storage conditions are necessary, for instance, refrigeration or protection from light.

Stressed studies: See Forced degradation studies.

System suitability: Evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factors, resolution, and precision of standard peak areas, and comparison to a confirmation standard, capacity factors, retention times, theoretical plates, and calibration curve linearity.

Tailing factor: A measure of peak asymmetry. Peaks with a tailing factor of 2 are usually considered to be unacceptable due to difficulties in determine peak start and stop points which complicates integration. Tailing peaks are an indication that the chromatographic conditions for a separation have not been properly optimized.

Test method: An approved, detailed procedure describing how to test a sample for a specified attribute (e.g., assay), including the amount required, instrumentation, reagents, sample preparation steps, data generation steps and calculations use for evaluation.

Theoretical plates: A dimensionless quantity used to express the efficiency or performance of a column under specific conditions. A decrease in theoretical plates can be an indication of HPLC column deterioration.

Titrand: is the species of interest during a titration. When a known concentration and volume of titrant is reacted with the analyte, it's possible to determine the analyte concentration.

Titrant: is a solution of known concentration that is added (titrated) to another solution to determine the concentration of a second chemical species. The titrant may also be called the titrator, the reagent, or the standard solution.