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# Application of Statistical Tools on Analytical Method Validation & its Method Transfer of Biological Product Pegylated Interferon Alfa 2a by Cytopathic Effect Assay

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***Abstract :*** *Quality is always a prerequisite when looking at any pharmaceutical product. Therefore, drugs must be manufactured to the highest quality standards and its quality control results must be inter- laboratory reproducible. The purpose of this effort is to provide an introduction and overview of the analytical method validation of one of the biotech product of our organization at the commercial name of UNIPEG 180 mcg containing pegylated interferon alfa 2a injection as per instructions and guidelines & requirements of the U.S. Food and Drug Administration (FDA), World Health Organization (WHO) & United States Pharmacopoeia.*
*In our study analytical method validation of pegylated interferon alfa 2a has been performed inside the scientific research laboratory (SRD) and its method transfer activity has also been successfully done to the QC lab of our organization. The method is precise with CV% <35% and also the confidence interval was calculated using student t-test & the results were found within the 80-125% from the first analyst results which are within range from 302 MIU/mg to 473 MIU/mg.*

***Keywords -*** *Method Validation, Method Transfer, Cytopathic Effect Assay & Virus Titer Determination*

## I. Introduction

In-situ testing of interferon alfa 2a is a method of choice unlike in vivo and in vitro methods and hence it is also a globally acceptable method for the testing of interferon alfa 2a. There are other methods like ELISA for the testing of interferon alfa 2a, but the disadvantage with this method is that it not only estimates the potency of live proteins but also the dead proteins as well. As a result we may give false high results in terms of estimated potency. Whereas in CPE assay we accurately estimate only live proteins that are required to be determined.

In Pakistan, currently testing of interferon alfa 2a (pegylated) is being outsourced because of the reason that product technology is quite expensive.

In the analytical method of interferon alfa 2a (pegylated) vesicular stomatitis virus is being used which is a pathogenic organism because as per regulations of biosafety in microbiological and biomedical laboratories (BMBL), it falls under biosafety level (BSL) 2. So there are regulatory complexities involved in import of the virus, as the VSV is highly vulnerable to zoonotic diseases. And the most acute one disease is FMDV i.e. foot and mouth disease.

Cell lines used in the analytical method validation of interferon alfa 2a (pegylated) are being procured from American Type Culture Collection (ATCC) center, which is a time taking & difficult to procure activity due to regulatory inquiries.

Unlike the conventional microbiological product, handling with biological products is comparatively a difficult job. It requires especial training, expertise and level of knowledge to deal with in order to avoid high variability in analytical results.

## II. Assay Validation

[1] , [2] , [3] & [4]
Performed assay validation as follows;

### 1.1 Accuracy/Biasness (Validation of Assay Lay Out):

In order to reduce the bias due to non-random pipetting order or plate edge effects should be eliminated by randomizing the plates lay out & by avoiding the use of edge well respectively. So for this purpose perform the bioassay as follows;

- Perform bioassay by randomizing the plates lay out using MDBK cell line
- Perform bioassay by randomizing the plates lay out using VERO cell line
- Perform bioassay with unsealed micro plate
- Perform bioassay with sealed micro plate
- Perform bioassay with path check option activated

- Perform bioassay with path check option de-activated

**1.2 Specificity (Choice of Cell Line):**

Perform the CPE assay as per the procedure mentioned above with MDBK and VERO cell lines to ensure the most sensitive response to the interferon preparations to be assayed

**1.3 Specificity (Choice of Response):**

To ensure the most sensitive response perform the staining with Crystal Violet and Neutral Red staining solutions as per procedure shown above.

**1.4 Precision:**

**1.4.1 Repeatability: (Transfer of analytical procedure)**

- Run bioassay in triplicate with same lot of sample by two different but experienced analysts using two different equipments i.e. SpectraMax plus 384 microplate readers and ELISA reader respectively.
- The assay results should not differ by more than 80-125% of the first analyst's results.

**1.4.2 Reproducibility: (Transfer of analytical procedure)**

- Run bioassay in triplicate with same lot of sample by alternate analyst of two different labs i.e. Scientific Research & Development Lab and Quality Control lab using two different equipments i.e. SpectraMax plus 384 microplate readers and ELISA reader respectively.
- Results should not differ by more than 80-125% from the first analyst's results.

## III. Method Transfer [2]

It is also called as transfer of analytical procedure (TAP). Analytical method transfer is typically managed under an internal transfer protocol that details the parameters to be evaluated in addition to the predetermined acceptance criteria that will be applied to the results. Transfer studies usually involve two or more laboratories or sites (originating lab and receiving labs) executing the preapproved transfer protocol. A sufficient number of representative test articles (e.g., same lot(s) of drug substance or drug product) are used by the originating and receiving laboratories. The comparative studies are performed to evaluate accuracy and precision, especially with regard to assessment of inter laboratory variability. In cases where the transferred analytical procedure is also a stability indicating method, forced degradation samples or samples containing pertinent product-related impurities should be analyzed at both sites

## IV. Figures And Tables

### 1.1 Summary Sheet of Accuracy & Specificity Results of Bioassay

| Sr. # | Validation Criteria | Test Description | Results (MIU/mg) | R-Square Value |
|---|---|---|---|---|
| 1 | Accuracy & Specificity | Single run of bioassay using MDBK cell lines with crystal violet staining procedure (Templat-1) | 364 | 0.976 |
| 2 | Accuracy & Specificity | Single run of bioassay using VERO cell lines with crystal violet staining procedure (Templat-2) | 371 | 0.977 |
| 3 | Accuracy & Specificity | Single run of bioassay using MDBK cell lines with neutral red staining procedure (Templat-3) | 325 | 0.994 |
| 4 | Accuracy & Specificity | Single run of bioassay using VERO cell lines with neutral red staining procedure (Templat-4) | 321 | 0.995 |
| 5 | Accuracy | Single run of bioassay with unsealed micro plate | 389 | 0.971 |
| 6 | Accuracy | Single run of bioassay with sealed micro plate | 381 | 0.977 |
| 7 | Accuracy | Single run of bioassay with path check option activated | 379 | 0.978 |
| 8 | Accuracy | Single run of bioassay with path check option de-activated | 381 | 0.977 |

### 1.2 Summary Sheet of Repeatability & Reproducibility Results of Bioassay

| Sr. # | Validation Criteria | Test Description | Results (MIU/mg) | R-Square Value |
|---|---|---|---|---|
| 1 | Repeatability | Run# 1 of bioassay (Analyst-1 from SRD lab using SpectraMax plus 384 microplate reader) | 378 | 0.977 |
| **Acceptance Criteria (80%-125%):** | | **302 MIU/mg to 473 MIU/mg** | | |
| 2 | Repeatability | Run# 2 of bioassay (Analyst-1 from SRD lab using SpectraMax plus 384 microplate reader) | 353 | 0.938 |
| 3 | Repeatability | Run# 3 of bioassay (Analyst-1 from SRD lab using SpectraMax plus 384 microplate reader) | 383 | 0.977 |
| 4 | Repeatability | Run# 1 of bioassay (Analyst-2 from SRD lab using ELISA reader microplate reader) | 394 | 0.917 |
| 5 | Repeatability | Run# 1 of bioassay (Analyst-2 from SRD lab using ELISA reader | 359 | 0.945 |

| | | | | |
|---|---|---|---|---|
| | | microplate reader) | | |
| 6 | Repeatability | Run# 1 of bioassay (Analyst-2 from SRD lab using ELISA reader microplate reader) | 409 | 0.964 |
| 7 | Reproducibility | Run# 1 of bioassay (Analyst-2 from QC lab using ELISA reader microplate reader) | 322 | 0.996 |
| 8 | Reproducibility | Run# 2 of bioassay (Analyst-2 from QC lab using ELISA reader microplate reader) | 399 | 0.993 |
| 9 | Reproducibility | Run# 3 of bioassay (Analyst-2 from QC lab using ELISA reader microplate reader) | 427 | 0.995 |

### 1.3 Repeatability using SpectraMax Plus 384 and ELISA reader Microplate Reader in SR & D lab

| **Analyst-1** | **Analyst-2** | **Analyst-1** | | **Analyst-2** | |
|---|---|---|---|---|---|
| 378 | 394 | | | | |
| 353 | 359 | Mean | 371.33 | Mean | 387.33 |
| 383 | 409 | Standard Error | 9.28 | Standard Error | 14.81 |
| | | Median | 378.00 | Median | 394.00 |
| | | Standard Deviation | 16.07 | Standard Deviation | 25.66 |
| | | Sample Variance | 258.33 | Sample Variance | 658.33 |
| | | Skewness | -1.55 | Skewness | -1.09 |
| | | Range | 30.00 | Range | 50.00 |
| | | Minimum | 353.00 | Minimum | 359.00 |
| | | Maximum | 383.00 | Maximum | 409.00 |
| | | Sum | 1114.00 | Sum | 1162.00 |
| | | Count | 3.00 | Count | 3.00 |
| | | Confidence Level (95.0%) | 39.93 | Confidence Level (95.0%) | 63.74 |
| | | %RSD | 4.33 | %RSD | 6.62 |

### 1.4 Reproducibility using SpectraMax Plus 384 Microplate Reader b/w SR & D lab & QC lab

| **Analyst-1 from SR & D Lab** | **Analyst-2 from QC Lab** | **Analyst-1 from SR & D Lab** | | **Analyst-2 from QC Lab** | |
|---|---|---|---|---|---|
| 378 | 322 | | | | |
| 353 | 399 | Mean | 371.33 | Mean | 382.67 |
| 383 | 427 | Standard Error | 9.28 | Standard Error | 31.39 |
| | | Median | 378.00 | Median | 399.00 |
| | | Standard Deviation | 16.07 | Standard Deviation | 54.37 |
| | | Sample Variance | 258.33 | Sample Variance | 2956.33 |
| | | Skewness | -1.55 | Skewness | -1.23 |
| | | Range | 30.00 | Range | 105.00 |
| | | Minimum | 353.00 | Minimum | 322.00 |
| | | Maximum | 383.00 | Maximum | 427.00 |
| | | Sum | 1114.00 | Sum | 1148.00 |
| | | Count | 3.00 | Count | 3.00 |
| | | Confidence Level (95.0%) | 39.93 | Confidence Level (95.0%) | 135.07 |
| | | %RSD | 4.33 | %RSD | 14.21 |

## V. Conclusion

Perform all bioassays as shown above in assay validation using parallel line curve analysis with PLA 4-P Fit criteria. Additional acceptance criteria for these tests should be as under;

Biological assays are frequently analyzed with the help of the parallel-line method.

With the help of the parallel-line model the statistical validity of the following hypotheses is tested:

1. The dose-response relationship is linear for the standard and sample preparation.
2. The dose-response curve has a significant slope.
3. The dose-response curves of the standard and sample preparation are parallel.

The statistical analysis of an assay will produce a potency ratio that expresses the potency of the unknown sample in terms of the standard potency.

The parallel-line procedure has several advantages compared to traditional single-point assay. Due to the check of the hypotheses mentioned above

1. a linear dose-response correlation is not only assumed but also proven
2. a dose-independent relative potency is obtained.

The disadvantages of the parallel-line method are the more extensive assay setup (standard and sample preparations have to be measured at different concentrations) and the complex statistical analysis of the results.

Further acceptance criteria of bioassay are mentioned below;

- Coefficient of determination i.e. $R^2$ value should not be less than 0.900.
- CV should not be more than 35% in both sample and standard replicates.

### 1.1 Conclusion of Repeatability:

Confidence interval was calculated using student t test. Also the results are within the 80-125% from the first analyst results which is in range from 302 MIU/mg to 473 MIU/mg and hence it is concluded that statistically derived results are complying with the predetermined acceptance criteria, and therefore accepted for repeatability criteria.

### 1.2 Conclusion of Reproducibility:

Confidence interval was calculated using student t test. Also the results are within the 80-125% from the first analyst results which is in range from 302 MIU/mg to 473 MIU/mg and hence it is concluded that statistically derived results are complying with the predetermined acceptance criteria, and therefore accepted for reproducibility criteria.

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## References

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