Optimization and Validation of an ELISA kit for the Quantification of Four Interferon-Beta (IFN-ß) Marketed Compounds in Human Serum

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Introduction

Interferons (IFNs) are a group of cytokines released by host cells in response to pathogens such as viruses, bacteria, or tumors. There are three classes of Interferons: Type I, Type II, and Type III. IFN-β belongs to Type I IFNs and includes 2 subtypes: IFN-β-a1 and IFN-β-b1. IFN-β is used to treat Multiple Sclerosis[1].

Four trademark compounds (Rebif®, Avonex®, Betaseron®, and Extavia®) were independently validated using a commercial ELISA kit[2] over a range of 5-200 pg/mL. Assay parameters were evaluated and optimized to improve the performance for the individual compounds

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Materials and Methods

Specific reagents used in the assay and procedure are summarized in Table 1. The assay quantitates Human (IFN-β) in serum by sandwich enzyme linked immunosorbent assay (ELISA) and has been developed to measure low/basal levels of INF- β. The Interferon binds to plates coated with capture antibody and the detection is accomplished using a biotinylated secondary antibody followed by streptavidin conjugated to horseradish peroxidase (HRP). Tetramethy-benzidine (TMB) is the substrate. Prior to preparing the standards and QCs of the fortified solutions in human serum, it was necessary to screen the matrix in order to identify the matrices with below lower limit of quantitation (BQ) levels of endogenous analyte .

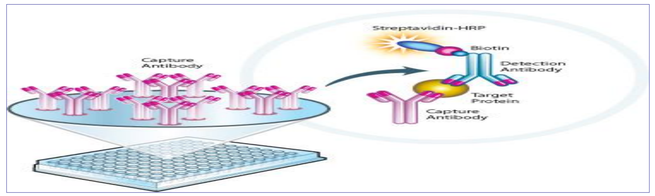

Figure 1. ELISA assay design

Reagent	96-well plate		
	standards	Quality controls	Validation samples
Diluted Antibody (µL)	50	50	50
Sample buffer (µL)	50	50	50
Sample (µL)	50	50	50
Cover plate and place on a plate shaker set at 450 rpm for 2 hours			
Wash plate 3x with wash buffer			
Diluted HRP (µL)	100		
Cover plate and place on a plate shaker set at 450 rpm for 30 minutes			
Wash plate 4x with wash buffer			
TMB Substrate (µL)	100		
Cover plate and incubate at room temperature for 30 minutes protected from			
1N H_2SO_4 stop solution	100		
Read the plate using absorance reader at 450 nm			

Table 1. Assay procedure

Results

The validation parameters of accuracy, precision, robustness, freeze-thaw stability, long-term stability, and bench-top stability were evaluated for each compound. The intra-assay and inter-assay (pooled) precision (%CV) and accuracy (%RE) for each validation sample concentration was ≤ 20% (≤ 25% for LLOQ and ULOQ) for the four compounds. The inter-assay total error (|%CV| + |%RE|) was < 30% (< 40% for LLOQ).

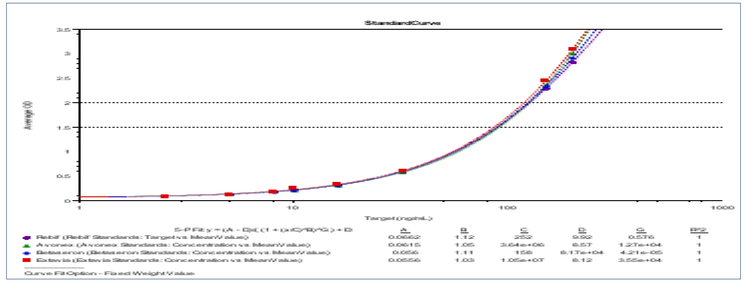

Figure 2. Representative Calibration Curves for the four IFN-β

Compound	Statistic	Nominal Concentration (pg/mL)				
		LLOQ 5.000	QCL 15.00	QCM 60.00	QCH 150.0	ULOQ 200.0
Rebif®	n	36	36	36	36	36
	Mean Bias (%RE)	-15.5	-3.6	-4.9	-4.8	-8.7
	Interbatch (%CV)	20.5	9.4	6.9	4.0	5.8
	|Mean| + Interbatch	36.064	12.989	11.822	8.772	14.504
Avonex®	n	36	36	36	36	36
	Mean Bias (%RE)	3.5	1.8	2.6	6.7	7.1
	Interbatch (%CV)	14.5	7.6	8.8	9.3	7.8
	|Mean| + Interbatch	18.019	9.318	11.396	16.039	14.869
Betaseron®	n	36	34	35	36	36
	Mean Bias (%RE)	-1.8	0.3	0.5	3.7	3.9
	Interbatch (%CV)	7.1	5.4	4.9	3.7	7.8
	|Mean| + Interbatch	8.879	5.681	5.425	7.376	11.716
Extavia®	n	36	36	36	36	35
	Mean Bias (%RE)	-10.0	-8.2	-10.1	-9.5	-8.9
	Interbatch (%CV)	11.1	5.3	7.5	6.4	3.5
	|Mean| + Interbatch	21.168	13.528	17.567	15.887	12.365

Table 2. Total Error for Precision and Accuracy

Sample	Unspiked Matrix	Spiked matrix at the QCL concentration (15.0 pg/mL)			
		Rebif	Avonex	Betaseron	Extavia
		%RE	%RE	%RE	%RE
1	<LLOQ	-2.00	-4.00	-11.3	-18.0
2	<LLOQ	8.00	9.33	5.33	2.67
3	<LLOQ	12.0	-6.67	13.3	14.0
4	<LLOQ	-17.3	-5.33	17.3	10.7
5	<LLOQ	-31.3*	16.7	NC	-2.00
6	<LLOQ	12.7	-12.0	-10.0	-13.3
7	<LLOQ	-50.7*	3.33	6.67	14.0
8	<LLOQ	5.33	10.0	-37.9*	-32.0*
9	<LLOQ	9.33	-25.3*	-5.33	-4.00
10	<LLOQ	11.3	-38.9*	13.3	18.7
LLOQ = 5.00 pg/mL NC = not calculated * = Mean value outside acceptance criteria: RE ± 20% of nominal					

Table 3. Selectivity evaluation of the four IFN-β

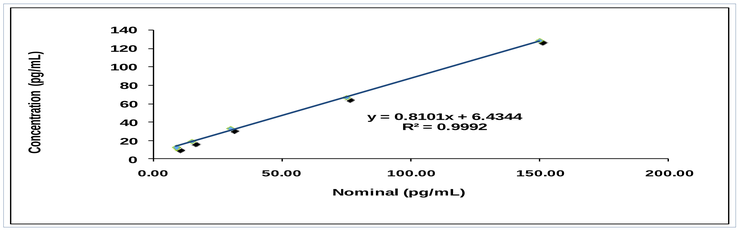

Figure 3. Dilution linearity of Betaseron

(pg/mL)		
Nominal	Concentration	%RE
150	128	-14.7
75.0	66.4	-11.5
30.0	32.9	9.67
15.0	18.7	24.7
9.00	12.2	35.6
Dilution of sample prepared from high stock was shown to be linear down to 30.0 pg/mL which represents 83,333 fold dilution.		

Table 4. Dilution linearity of Betaseron

Validation Experiment	Outcome			
	Rebif®	Avonex®	Betaseron®	Extavia®
Short Term Stability (Bench)	4 Hours	5 Hours	4 Hours	4 Hours
Short Term Stability (4°C)	24 Hours	48 Hours	24 Hours	4 Hours
Freeze Thaw Stability (-80°C)	4 Cycles	4 Cycles	2 Cycles	3 Cycles
Selectivity	No interference observed	No interference observed	No interference observed	No interference observed
Hemolysis	No hemolysis effect observed	No hemolysis effect observed	No hemolysis effect observed	No hemolysis effect observed
Dilution (fold)	10	10	83,333	100
Long-Term Freezer Stability (-80°C)	153 Days	70 Days	61 Days	59 Days

Table 5. Validation summary of the four IFN-β

Conclusions

Four sensitive assays for the detection of Rebif®, Avonex®, Betaseron® and Extavia® were developed, optimized, and validated over a range of 5-200 pg/mL. The methods were reliable and robust, and considered suitable for the analyses of pharmacokinetic studies in human serum.

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Literature cited

1. Weinstock-Guttman B, Ramanathan M, Zivadinov R. Interferon-beta treatment for relapsing multiple sclerosis. Expert Opin Biol Ther. 2008 Sep:8(9):1435-47.

2. PBL interferon Source. Verikine-HS™ Human INF-β Serum Elisa Kit (Product#41415-1).

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