Analytical Method Validation

Method

Ion-exchange and size exclusion chromatography methods was validated for linearity, accuracy, precision, limit of detection and limit of quantification, in accordance with the International Consensus on Harmonization guidelines (ICH Q2R1).

Linearity, Limit of Detection and Limit of Quantification:

Linearity of this method was evaluated by spiking five concentrations of trastuzumab (0.6, 0.8, 1, 1.2 and 1.4 mg/mL) in NaCl and injecting into the chromatographic instrument. A calibration curve was plotted for peak area *versus* drug concentration and a coefficient of determination (R^2) was determined by linear least-square regression analysis. The limit of detection (LOD) was calculated as 3.3 (σ/S), where σ is the standard deviation of the y-intercept and S is the slope of the calibration curve. Similarly, the limit of quantification (LOQ) was calculated as 10 (σ/S).

Precision and Accuracy:

The precision of the method was assessed as the intra-day precision (repeatability) and the inter-day precision (intermediate precision), and given as relative standard deviations (RSDs). To this end, intra-day precision was determined using 9 samples with high concentration levels (3 samples of 1.4 mg/mL), medium concentration levels (3 samples of 1 mg/mL), and low concentration levels (3 samples of 0.6 mg/mL). These samples were prepared and injected into the chromatography system on the same day. The inter-day precision was estimated from the analysis of standard solutions at three concentration levels (0.6, 1, and 1.4 mg/mL) over 3 successive days. Three samples of each concentration were prepared and analyzed daily.

Accuracy was carried out with calculation of the average recovery value (%) by analyzing three standard solutions at three concentration levels representing the high (1.4 mg/mL), the medium (1 mg/mL), and the low (0.6 mg/mL) level of the linear range of calibration curve.

Results

As shown in Tables 1 and 2, the method can provide quantification of cetuximab in the linearity range with good precision and accuracy. The obtained results are in accordance with ICH guideline.

Size Exclusion Chromatography

Table 1 : Linearity, LOD and LOQ for Size Exclusion chromatography.

Figure of merit	Value
Pratical linear range (mg/mL)	0.6 - 1.4
Slope (a)	106.29
Intercept (b)	-0,72
Determination coefficient (R^2)	0.9997
Limit of Detection (LOD)	0.04 mg/mL
Limit of Quantification (LOQ)	0.13 mg/mL

Table 2 : Accuracy and Precision of the Size-exclusion method.

Concentration tested (mg/mL)	Accuracy - Recovery %	Precision (%)	
		Intra-day	Inter-day
0.6	102.10 (1.37)	0.30	1.39
1	100.36 (0.38)	0.11	0.38
1.4	99.95 (0.18)	0.11	0.18

Ion-Exchange Chromatography

Table 3 : Linearity, LOD and LOQ for plotting peaks area against trastuzumab concentration in mg/mL by Ion-exchange chromatography.

Figure of merit	Value
Pratical linear range (mg/mL)	0.6 - 1.4
Slope (a)	44.83
Intercept (b)	-0.46
Determination coefficient (R^2)	0.9988
Limit of Detection (LOD)	0.03 mg/mL
Limit of Quantification (LOQ)	0.10 mg/mL

Table 4 : Accuracy and Precision of the Ion-exchange method.

Peaks	Concentration tested (mg/mL)	Mean peak area (%)	Accuracy - Recovery %	Precision (%)	
				Intra-day	Inter-day
	0.6	4.95 (0.27)	102.29 (2.08)	1.02	2.23
Acidic peaks	1	5.57 (0.36)	99.58 (1.42)	0.83	1.43
	1.4	5.67 (0.10)	98.89 (2.49)	0.98	2.52
	0.6	93.25 (0.10)	96.83 (1.53)	0.87	1.54
Main peak	1	92.45 (0.15)	99.10 (1.54)	0.60	1.55
	1.4	91.98 (0.08)	100.68 (0.83)	0.74	0.82
	0.6	1.81 (0.12)	101.32 (1.48)	1.38	1.61
Basic peaks	1	2.08 (0.16)	98.24 (1.25)	1.20	1.27
	1.4	2.35 (0.05)	99.38 (2.26)	1.42	2.27