Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(3):390-400

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Determination Of Clips Generated by Acid Hydrolysis of Trastuzumab for Injection by Sodium Dodecyl Sulfate Capillary Gel Electrophoresis

Dhruv Kumar*, Chimaji Aher and Anita Desai

Formulation Analytical Development Laboratory (FD ADL), Integrated Project Development (IPD), Cipla Ltd., Mumbai - 400083, India

---

ABSTRACT

Sodium dodecyl sulfate capillary gel electrophoresis (reduced and non- reduced) is often used as a purity release assay for monitoring clips in monoclonal antibodies (mAbs). Our criteria for the development of acid hydrolysis of Trastuzumab for injection (Cipla 302) included quantification of clips by both reduced and non- reduced capillary gel electrophoresis thereby comparing its similarity with that of the innovator drug product (Herceptin®). Some of the sample preparation parameters including concentration of HCl, incubation temperature and comparison of Cipla 302 with Herceptin® were investigated. It was observed that a slightly acidic condition and increase in incubation temperature greatly increased the degradation of reduced and non-reduced mAbs. Using this method, it was found that Cipla 302 was almost in the pure form when subjected to harsh conditions like incubation at 50° C and in 0.01 N HCl. The optimal sample preparation conditions to carry out the acid hydrolysis were found to be as follows: (1) concentration of HCl: 0.01N (2) incubation temperature: 70° C. This method was found to be linear in terms of increasing concentration and increasing temperature.

Keywords: Capillary gel electrophoresis; Acid hydrolysis; Temperature incubation; Comparison of mAbs

---

INTRODUCTION

Trastuzumab (rhuMAb HER2 or trade name: Herceptin®) is a CDR (complimentary domain receptor) grafted (humanized) recombinant IgG1 (immunoglobulin1)-subclass antibody that binds to the extracellular region of the Human Epidermal Growth Factor Receptor 2 tyrosine kinase (HER 2, also known as *neu* or C-*erb B*-2). HER 2 over expression correlates with a poor prognosis in a number of cancers. Herceptin as HER2 had been approved for the treatment of early-stage breast cancer and metastatic breast cancer. With its focus on affordable medicine, Cipla is currently developing a biosimilar trastuzumab for injection. We have characterized Cipla 302 by a variety of complementary characterization methods, which shows Cipla 302 to be similar to that of the Herceptin® as detailed in Table 1.

Cipla 302 is a sterile lyophilized product packaged in a sterile borosilicate glass vial. After reconstitution with bacteriostatic sterile water for injection, it results in a colorless liquid solution at pH 6 at a trastuzumab concentration of 21 mg/ ml. Each Cipla 302 drug product vial contains 150 mg of trastuzumab formulated with polysorbate 20, a histidine- histidine HCl buffer system and alpha, alpha- trehalose dihydrate. The drug product composition is detailed in Table 2.

Table 1: Results of Physicochemical characterization of Cipla 302 against Herceptin®

Attribute	Methods	Herceptin®	Cipla 302
Primary Structure	Peptide Mapping by LC- MS	-	Identical
	Peptide Mapping by HPLC- UV	-	Comparable
Intact Mass	LC- MS	148220.22 (G0F/G1F)	148221.91 (G0F/G1F)
Reduced mass	LC- MS	HC= 50765.43 (G0F)	HC= 50765.98 (G0F)
		LC= 23495.89	LC= 23495.70
Glycosylation	HPLC- FLR	-	Similar
Size Heterogenicity	Non Reduced CE SDS	IgG= 97.81%	IgG= 96.18%
	Non Reduced SDS PAGE	-	Similar
Purity	Reduced CE SDS	LC= 28.86%	LC= 28.65%
		HC= 69.67%	HC= 70.18%
	Reduced SDS PAGE	99.50%	99.50%
	HPLC- SEC	99.69%	97.76%
Charge Heterogenicity	Charge Variants Analysis	-	Similar
	CE-IEF (Isoelectric point)	8.67	8.67
Protein Content	by UV E1%	20.11mg/ml	21.01 mg/ml
pH	-	6.79	6.2
UV Spectrum		Complies (280nm)	Complies(279)
N- Terminal amino acid sequence		Heavy Chain: EVQLVESGGGLVQPG	Heavy Chain: EVQLVESGGGLVQPG
		Light Chain: DIQMTQSPSSLSASV	Light Chain: DIQMTQSPSSLSASV

Table 2: Composition of Cipla 302 Drug products

Component	Quantity (mg/ml)
Trastuzumab	22 mg
L-Histidine	0.32 mg
L-Histidine hydrochloride	0.54 mg
Alpha alpha- trehalose dehydrate	20 mg
Polysorbate 20	0.09 mg
pH	6

With current study we aim to determine the suitability of sodium dodecyl sulfate capillary gel electrophoresis [1-3] method for the determination of clips in Trastuzumab for injection (Cipla 302) and its comparison to innovator product. Forced hydrolysis studies using the optimized acid hydrolysis method and elevated temperature was performed on different batches of Cipla 302 in order to support comparability between different batches.

MATERIALS AND METHODS

Materials

Herceptin® (B3435B01) was purchased commercially and formulated Cipla 302 (BMF1/302/10003) was used for the characterization. SDS-MW gel buffer (M307123), acidic wash solution (0.1N HCl, M012473) and basic wash solution (0.1N NaOH, M101475) were obtained from Beckman Coulter. MES, Sodium dodecyl sulphate, β-mercapto ethanol, Iodoacetamide, N, N methylene bis acrylamide, N, N, N', N' Tetramethylethylenediamine, Ammonium per sulphate and Comassie brilliant blue- 250 were purchased from Sigma, Spruce street, England. Sodium hydroxide, Formaldehyde, Silver nitrate and Sodium thio sulphate were purchased from S D fine- chem limited, Mumbai- 30. Methanol, Glacial acetic acid and Hydrochloric acid were purchased from Fisher Scientific, Mumbai. Also, Ethanol (Hayman limited, Essex, England), Sodium carbonate and Acryl amide (Merck, Mumbai), and Tris (Sisco Research Laboratory, Mumbai) were also used in this study.

Acid hydrolysis

251.1 µl of purified water was mixed with 6 µl of 0.5N HCl in 2.0 ml centrifuge tube. Then, 42.9 µl of Cipla 302 (21 mg/ ml) was added to the mixture to get sample working concentration of 3 mg/ ml and mixed thoroughly on a vortex mixer followed by centrifugation for 30 seconds. Finally, the sample tube was kept for hydrolysis by incubating it at 70°C for 30 minutes in a water bath. Later, the above sample solution was buffer- exchanged (desalted) in MES- SDS sample buffer (25 mM MES, 1% SDS, pH 6.5) using Amicon ULTRA- 0.5 ml 3 kDa centrifugal filters. For achieving buffer- exchange, 100 µl of acid hydrolyzed sample was mixed with 100 µl of MES- SDS sample buffer in 3 kDa tubes and centrifuged at 12000 rpm at 10° C for 15 minutes. After centrifugation,

again 100 µl of MES- SDS sample buffer was added and centrifuged at 12000 rpm at 10° C for 15 minutes. At least 4 such centrifugation cycles were conducted to ensure complete buffer exchange. Then the sample solution volume was adjusted upto the 100 µl mark in same Amicon tube with MES- SDS sample buffer and mixed thoroughly.

Reduced sample preparation

25 µl of desalted sample solution was mixed with 55 µl of MES- SDS sample buffer in a 0.5 ml centrifuge tube. Following this, 2 µl of β- mercapto ethanol was added and the solution was mixed thoroughly on a vortex mixer followed by centrifugation for 30 seconds. The sample tube was kept for incubation at 65°C for 5 minutes in a water bath. After incubation, it was cooled for 3 minutes at room temperature, vortexed and then centrifuged for 30 seconds to remove any air bubble prior to loading in CE vials for injection.

Non-reduced sample preparation

25 µl of desalted sample solution was mixed with 55 µl of MES- SDS sample buffer in a 0.5 ml centrifuge tube. Following this, 10 µl of 250 mM iodoacetamide was added and the solution was mixed thoroughly on a vortex mixer followed by centrifugation for 30 seconds. The sample tube was kept for incubation at 65°C for 5 minutes in a water bath. After incubation, it was cooled for 3 minutes at room temperature, vortexed and then centrifuged for 30 seconds to remove any air bubbles prior to being filled in the CE vials for injection.

Instrumentation

CE- SDS experiments were conducted in the Beckman Coulter P/ACE™ MDQ capillary electrophoresis instrument equipped with a photodiode array (PDA) detector and the 32 Karat software (version 8.0). The Amersham Biosciences mini VE polyacrylamide gel electrophoresis equipment was used for gel electrophoresis. The Bio- Rad molecular imager gel doc system was used for scanning the gel.

Method

The analysis method was described in the Beckman Coulter IgG Purity/ Heterogeneity kit (Cat. No. A10663) which had methods to resolve reduced and non-reduced IgG by size and to quantify the heterogeneity and impurities which may exist in the IgG formulation. A bare fused silica capillary of 50 mm internal diameter with an effective length of 21 cm and total length of 31.2 cm was used for analysis. Prior to sample injection and separation, the capillary was rinsed with basic solution for 3 mins at 70 psi, acidic solution for 1 min at 70 psi, water for 1 min at 70 psi, and SDS gel buffer for 10 min at 70 psi. After the rinses, the capillary and electrode ends were dipped twice in separate water vials prior to sample injection at 5 kV. Another water dip was performed after injection followed by separation at 15 kV. During separation, pressure was applied to both ends of the capillary at 20 psi. Detection was performed at 214 nm using a photo diode array (PDA) detector. The method for reduced and non-reduced capillary gel electrophoresis is mentioned in the Table 3.

Table 3: Method for reduced and non-reduced capillary gel electrophoresis

Step	Event	Value	Duration	Comments
1	Rinse- Pressure	70 psi	3 mins.	0.1 N NaOH rinse to clean capillary surface.
2	Rinse- Pressure	70 psi	1 min.	0.1 N HCl rinse to neutralize silanol groups.
3	Rinse- Pressure	70 psi	1 min.	Water rinse to remove the acid residues.
4	Rinse- Pressure	70 psi	10 mins.	SDS gel rinse to fill capillary with SDS gel.
5	Wait			Water dip to clean capillary tip.
6	Wait			Water dip to clean capillary tip.
7	Inject- Voltage	5 kV	20 secs.	Sample injection.
8	Wait			Water dip to avoid sample carry over.
9	Separate- Voltage	15 kV	30 mins.	SDS gel for separation.

Study design

In the first step, we optimized the experimental conditions to induce forced hydrolysis of Cipla 302 with different concentrations (0.1 N to 3 N) of HCl solutions. Rapid precipitation of the monoclonal antibody (mAb) was observed upon incubation in 3 N HCl solution. Precipitation persisted post incubation in diluted HCl concentrations down to 0.4 N and manifested during sample preparation for CE-SDS. No precipitation was observed below 0.1 N HCl. Forced degradation was conducted in 0.01 N HCl solutions to obtain a time-course of the degradation process. The optimization process is outlined in Figure 1 below.

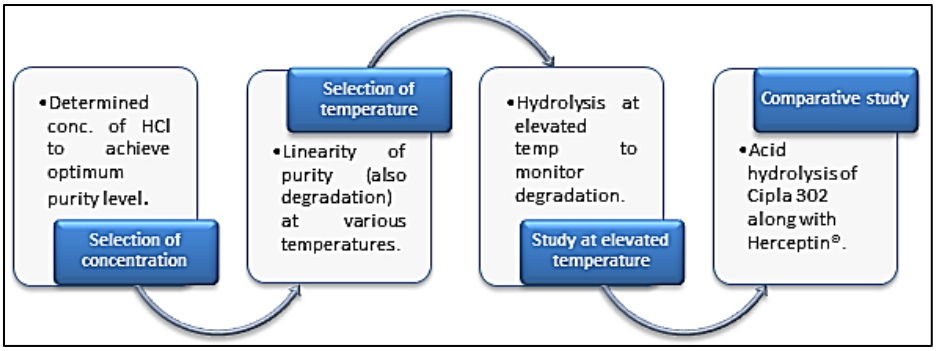

Figure 1: Study design of acid hydrolysis of Trastuzumab for injection

Further, we confirmed the purity (or degradation) of our molecule at various temperatures which ranges from 5°C to 80°C for 30 mins to check the linearity of purity (or degradation). This data showed how stable our molecule was in the acidic conditions at different temperatures. Also, our molecule was subjected to plain hydrolysis (without addition of HCl) at 80°C and was analyzed at various intervals to get information about its degradation pattern.
Cipla 302 and the Herceptin® drug product were subjected to acid hydrolysis with 0.01N HCl at 70°C and samples were analyzed by CE- SDS and SDS- PAGE methods at different time intervals (15 mins, 30 mins, 60 mins and 120 mins) to assess the forced hydrolysis rates of the two mAbs. The same sample preparation procedure was used for CE- SDS and SDS- PAGE to enable correlation of the data obtained by both CE-SDS and SDS-PAGE [4,5].

RESULTS AND DISCUSSION

In this study, Cipla 302 and Herceptin® were subjected to reduce and non-reduced CE- SDS method to detect forced hydrolysis product. In the red uced sample, preparation, β- mercapto ethanol was used to break the disulphide bond between the light chain and heavy chain which led to the separation between them (Figure 2a). In the non-reduced sample preparation, IgG was alkylated which stabilizes the disulphide bond. Due to alkylation, the free thiol groups of cysteine are blocked and it prevents the molecule from forming intermolecular disulfide bond resulting in an intact IgG (Figure 2b). Thus, it decreases the production of light chain and heavy chain fragments.
From the as such reduced data of Cipla 302, it was found that the area % of light chain, heavy chain and non-glycosylated heavy chain was 28.79%, 69.62% and 0.28% respectively, thus showing optimum purity (%LC + %HC) of the molecule. On the other hand, from the as such non reduced data, area % of intact IgG was 95.37% and non-glycosylated IgG was 0.22%. In this data, all the impurities such as LC, HC, HL, HH and 2H1L were all well resolved from the intact IgG.

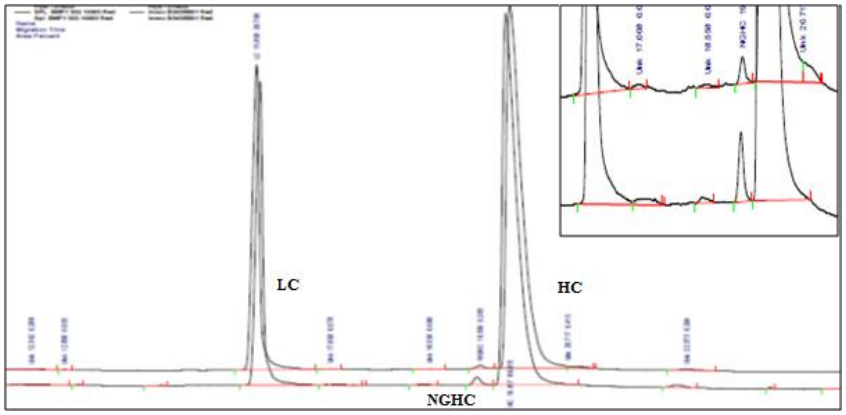

Figure 2a: Comparison of as such reduced Herceptin® and Cipla 302. The inset highlights a zoomed- in region of the electropherogram

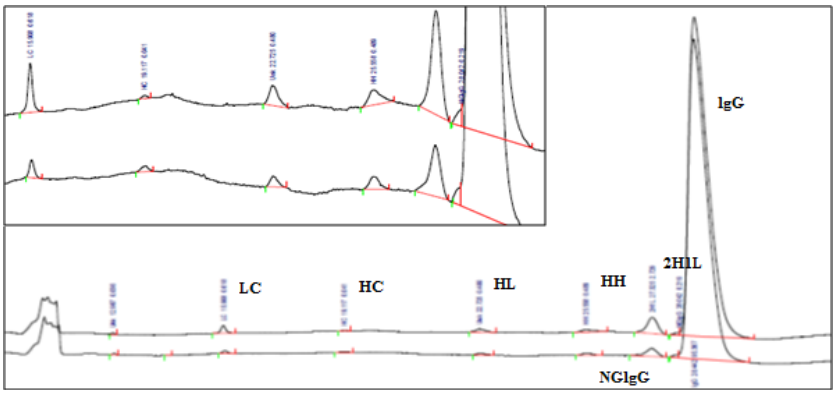

Figure 2b: Comparison of as such non-reduced Herceptin® and Cipla 302. The inset highlights a zoomed- in region of the electropherogram

Further, generation of clips was studied through acid hydrolysis by mixing the sample with different concentrations of HCl ranging 3N to 0.01N at 70°C for 30 minutes. Precipitation was observed from 3N to 0.4N HCl. After this, study was carried out from 0.3N to 0.01N HCl. In 0.3N HCl treated sample, the non-reduced profile was slightly degraded whereas the reduced profile showed area % of 30% for LC and 41.75% for HC comprising purity of 71.75% (LC + HC). Further, study was carried out with 0.2N, 0.1N, 0.05N and 0.01N HCl in which a linear increase in the purity was observed under both reduced and non-reduced conditions (Figure 3b). For 0.01N HCl treated sample, area% of intact IgG was 85.82% whereas that of LC and HC was 28.4% and 61.03% comprising overall purity of 89.43% (LC + HC). Thus, final concentration of 0.01N HCl was decided based on the above study whose results are displayed in the (Figure 3b) Table 4. Also a linear graph of decreasing purity with increasing concentration can be seen in the Figure 4.

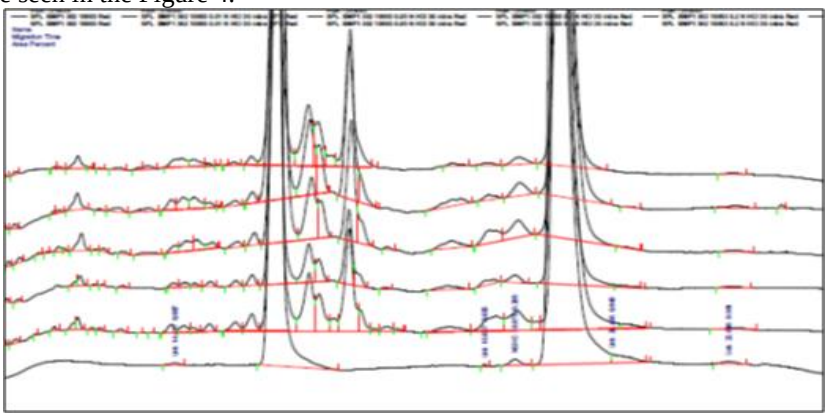

Figure 3a: Incubation of reduced Cipla 302 at different concentrations of acid

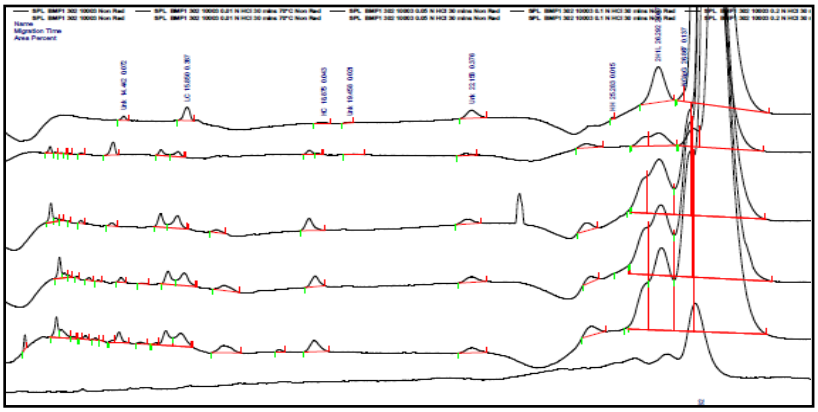

Figure 3b: Incubation of non-reduced Cipla 302 at different concentrations of acid

With the decreasing concentration of HCl, the area % of light chain (eluted around 15 mins) was slightly affected whereas that of heavy chain (eluted around 19 mins) degraded the most in the reduced condition. The clips were generated immediately after the LC at around 16 mins (RRT 1.03 with respect to LC) and a major amount of clip at around 16.5 mins (RRT 1.07 with respect to HC) and they increased with the increasing concentration of HCl. Under the non-reduced conditions, major degradation was observed in the NGIgG region, 2H1L, HC and LC fragments with decreasing area % of intact IgG. Area% of non-glycosylated profile was also increased but got merged with the unknown impurity adjacent to it.

Table 4: Linear degradation of reduced and non-reduced Cipla 302 with increasing concentration of HCl; Finally, acid hydrolysis was carried out at 0.01 N HCl

	LC	HC	Purity (LC+HC)	IgG
0.01 N HCl, 70°C, 30 Mins.	28.40%	61.03%	89.43%	85.82%
0.05 N HCl, 70°C, 30 Mins.	29.78%	57.36%	87.14%	83.73%
0.1 N HCl, 70°C, 30 Mins.	30.65%	55.42%	86.07%	80.62%
0.2 N HCl, 70°C, 30 Mins.	27.87%	50.88%	78.75%	74.92%
0.3 N HCl, 70°C, 30 Mins.	30.00%	41.75%	71.75%	Slight degradation
0.4 N HCl, 70°C, 30 Mins.	Precipitation after incubation.			
0.5 N HCl, 70°C, 30 Mins.	Precipitation after incubation.			
1 N HCl	Precipitation.			
3 N HCl	Precipitation.			

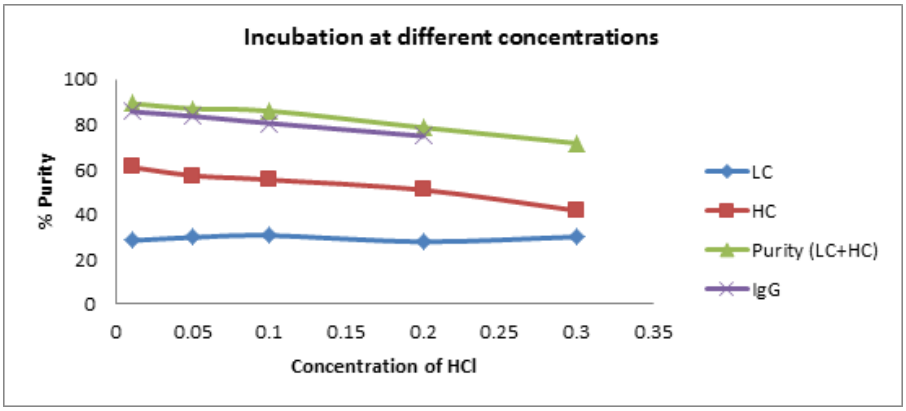

Figure 4: Linear degradation of light chain (LC), heavy chain (HC), purity (LC + HC) and intact IgG of Cipla 302 at various concentrations of HCl

Further, after deciding the final concentration, acid hydrolysis was carried out at different temperatures ranging from 5°C to 80°C for 30min. In this study, the overall area % of reduced and non-reduced sample was not affected at 5°C, room temperature, 37°C and 50°C with 0.01N HCl for 30 mins. This data shows that Cipla 302 is very much in the pure form in such acidic conditions upto 50°C for 30 mins. At 50°C, the purity mAb was just 1.43% and 2.17% less under the reduced and non- reduced condition (Figure 5a and 5b) than the as such sample. Further as the temperature increases, the purity of LC, HC, and IgG decreased upto 25%, 45% and 74% at 80°C after 30 min.

At 70°C, the purity of the Reduced (LC + HC) and non-reduced was 83.79% and 85.82% which was much above 80% which we had decided as the limit for acid hydrolysis considering forced degradation limit of HPLC. Thus 70°C was decided as the standard temperature for this study. The data of all the temperature related study is shown in the Table 5. Also a linear graph of decreasing purity with increasing temperature can be seen in the Figure 6. The profile of reduced and non-reduced sample was same as that of the trial taken for deciding the concentration. Same clips were increased which were mentioned earlier at around 16 mins and 16.5 mins. Thus, it concluded that concentration and temperature has effect on the same region of IgG, generally clips at the same area after LC as that observed in the concentration study.

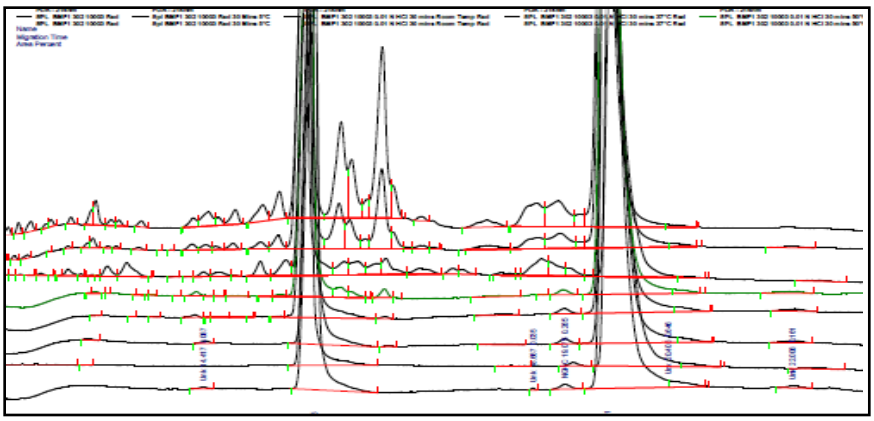

Figure 5a: Incubation of reduced Cipla 302 at different temperatures

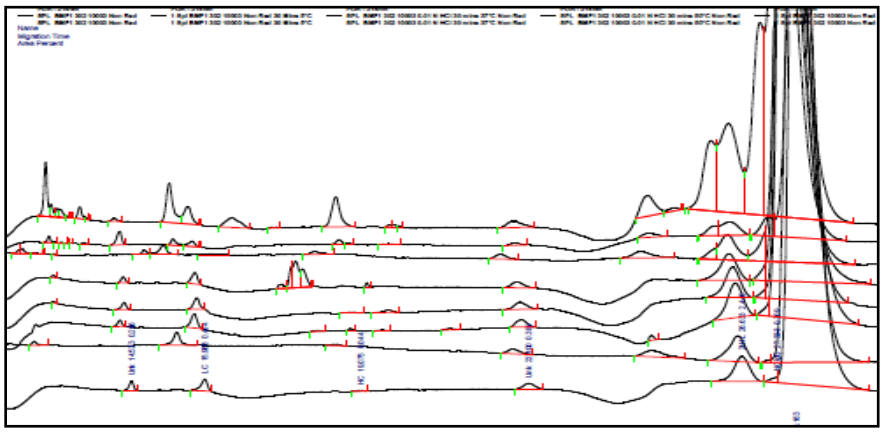

Figure 5b: Incubation of non-reduced Cipla 302 at different temperatures

Table 5: Linear degradation of reduced and non-reduced Cipla 302 with increasing temperature; finally, acid hydrolysis was carried out at 70° C with purity around 84%

	LC	HC	Purity (LC+HC)	IgG
0.01 N HCl, 5°C, 30 Mins.	28.56%	69.92%	98.48%	94.55%
0.01 N HCl, (RT) 25°C, 30 Mins.	29.28%	69.52%	98.80%	96.37%
0.01 N HCl, 37°C, 30 Mins.	29.26%	69.40%	98.66%	96.31%
0.01 N HCl, 50°C, 30 Mins.	29.01%	68.23%	97.23%	94.14%
0.01 N HCl, 60°C, 30 Mins.	27.82%	64.48%	92.30%	92.06%
0.01 N HCl, 70°C, 30 Mins.	27.64%	56.15%	83.79%	85.82%
0.01 N HCl, 80°C, 30 Mins.	24.46%	44.74%	69.20%	74.25%

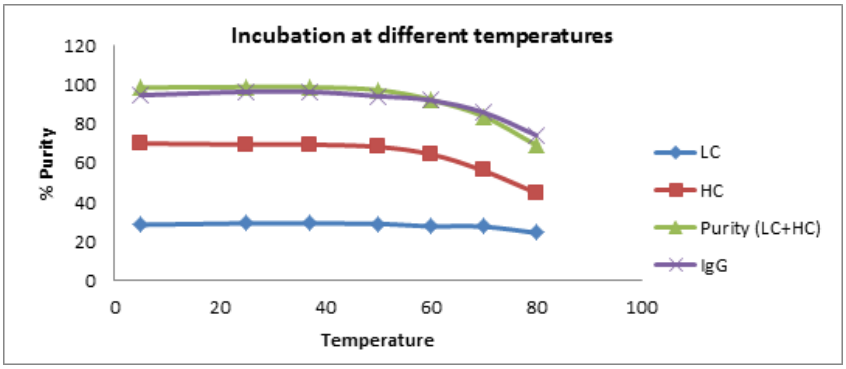

Figure 6: Linear degradation of light chain (LC), heavy chain (HC), purity (LC + HC) and intact IgG of Cipla 302 at various temperatures

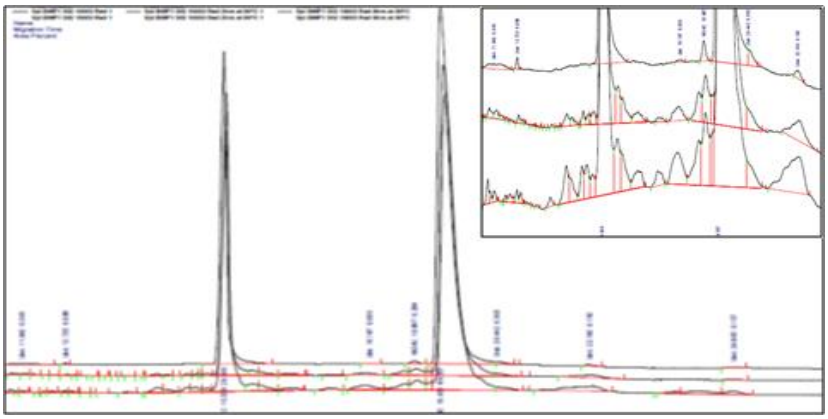

Figure 7: Incubation of reduced Cipla 302 at elevated temperature. The inset highlights a zoomed- in region of the electropherogram

Table 6: Linear degradation of reduced Cipla 302 at different time intervals of elevated temperature. For intact IgG, a completely degraded pattern was observed

	LC	HC	Purity (LC+HC)
As such	29.31%	69.34%	98.65%
80°C, 2 Hrs.	27.90%	65.02%	92.92%
80°C, 6 Hrs.	27.06%	58.12%	85.18%
80°C, 24 Hrs.	27.49%	44.76%	72.25%

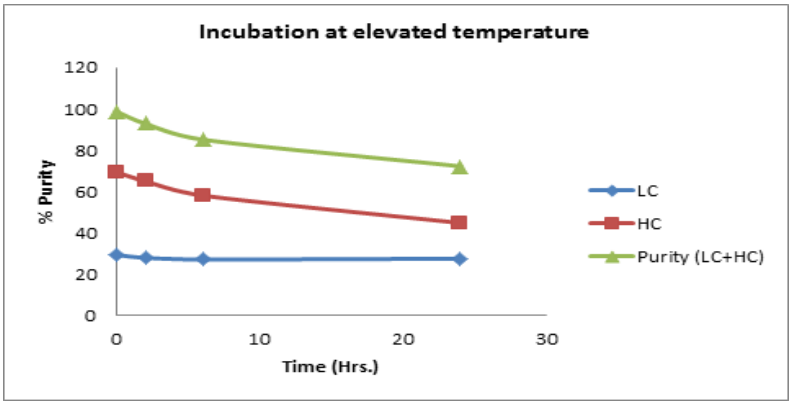

Figure 8: Linear degradation of light chain (LC), heavy chain (HC) and purity (LC + HC) of Cipla 302 at different time intervals of elevated temperatures

Also, when Cipla 302 was subjected to plain hydrolysis at 80°C upto 6 hours to check the degradation pattern of the molecule it was observed that in the reduced condition again the area % of LC was slightly affected as compared to HC. The area % of HC degraded from 69.34% (as such) to 58.12% after 6 hours of incubation at 80°C. On the other hand, non-reduced sample slightly degraded after 2 hours and completely there after thus after 6 hours it showed purity of 27.06% and 58.12% of LC and HC respectively comprising total purity (LC + HC) of 85.18%. Details of intervals are shown in the Table 6 and the linear degradation with increasing interval of time is shown in the Figure 9. In this study degradation was observed all over the profile with major ones around 14 mins before LC, around 16 mins after LC and around 18 mins adjacent to NGHC which can be seen from the Figure 7. Also denatured IgG was observed after HC in the increasing concentration with the increase in the interval of time (Figure 8).

After deciding the concentration and temperature, Cipla 302 along with Herceptin® was subjected to acid hydrolysis and was analyzed after time intervals of 15 mins, 30 mins, 60 mins, and 120 mins. When these as such samples were loaded in the reduced condition, it was observed that the NGHC of Herceptin® was around 0.44% more than Cipla 302 whereas in the non-reduced condition area % of 2H1L impurity of Cipla 302 was almost 1.2% more than Herceptin®. Still the overall purity of Cipla 302 and Herceptin® under reduced and non-reduced conditions remained almost similar.

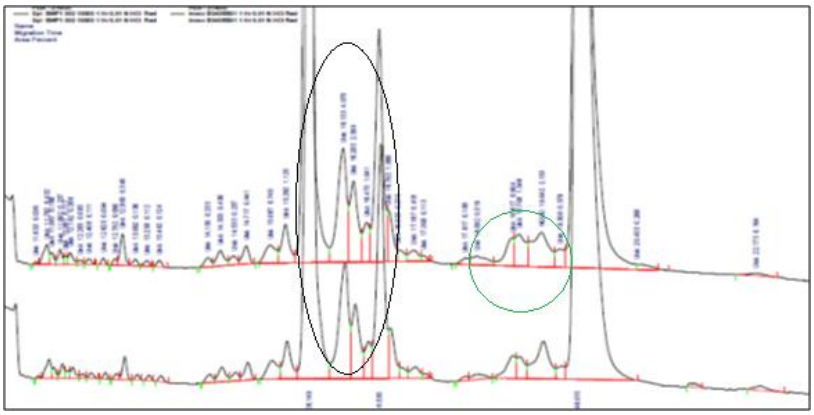

Figure 9a: Electropherogram of reduced Herceptin® and Cipla 302 after acid hydrolysis at 70° C for 1 hour. Clips seen between light chain and heavy chain in the electropherogram resembled with that of the SDS PAGE as shown below

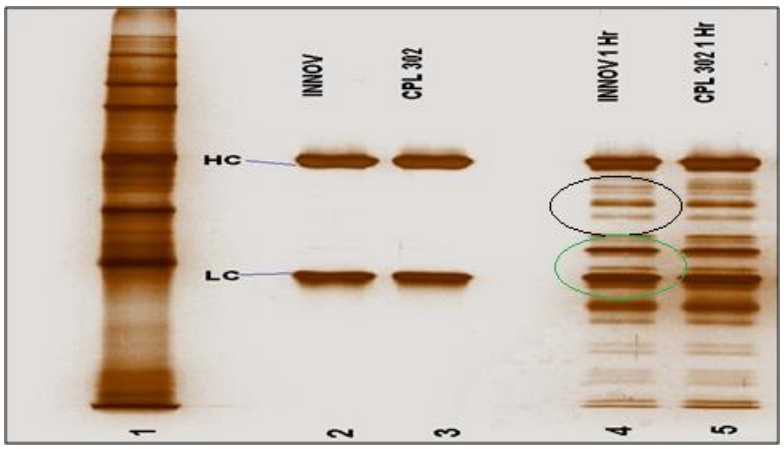

Figure 9 b: SDS- PAGE scan of reduced Herceptin® and Cipla 302 after acid hydrolysis at 70° C for 1 hour. Clips seen between light chain and heavy chain in the gel resembled with that of the electropherogram as shown above

After 120 mins of incubation, area % of LC was reduced by 3.95% in Herceptin® and by 3.44% in Cipla 302 and that of HC, was affected the most by degradation of 35.33% in Herceptin® % and by 36.06% in Cipla 302. Detailed results of degradation after intervals are shown in the Table 7. In the profile (Figure 9a) the region around 16 mins and 16.5 mins was the most degraded where the clips were supposed to be formed, major being around 16.5 mins. The NGHC also increased with interval of incubation but got merged with the co- eluting impurity. The sample which was used for reduced CE- SDS was also loaded on SDS- PAGE. After silver staining the gel, very sharp band was observed above LC which is circled in black in figure 10b. In the electropherogram (Figure 9a) the black circled region which was the most degraded, resembled with the band on SDS-PAGE (Figure 9 b) which is circled in black. Also, around NGHC the co-eluting impurity in the electropherogram also reflected in the silver staining of SDS-PAGE circled in green colour .Thus, both the data from reduced SDS-PAGE and reduced CE-SDS resembled with each other which showed that the clips were generated at the same site of the molecule.

Even under non reducing conditions, same sample was loaded on CE-SDS and SDS-PAGE. In this, the area% of intact IgG decreased by 27.4% in Herceptin® and by 28.17% in Cipla 302 after 120 mins. Detailed results of degradation after intervals are shown in Table 7. As mentioned earlier, 2H1L impurity was more intense in Cipla 302 as compared to Herceptin®, it was also seen that the 2H1L band in the non-reduced gel of Cipla 302 (Figure 10b- lane 5) was more intense than that of Herceptin® (Figure 10b- lane 4). This intensity is incircled in blue in both electropherogram (Figure 10a) and SDS- PAGE (Figure 10b) scan. The impurities like LC, HC, HL, HH and 2H1L were well resolved from intact IgG even in SDS-PAGE like that of CE-SDS which also showed that the clips were generated at the same site of the molecule. Like reduced condition, even in non-reduced condition, data obtained from CE-SDS and SDS-PAGE resembled with each other of both Herceptin® and Cipla 302.

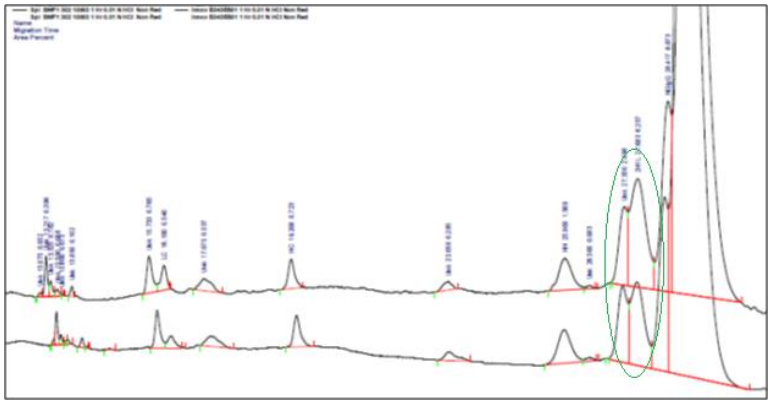

Figure 10a: Electropherogram of non-reduced Herceptin® and Cipla 302 after acid hydrolysis at 70° C for 1 hour. Fragments which resolved from non-reduced CE- SDS like LC, HC, HL, HH and 2H1L resembled with that of the SDS PAGE

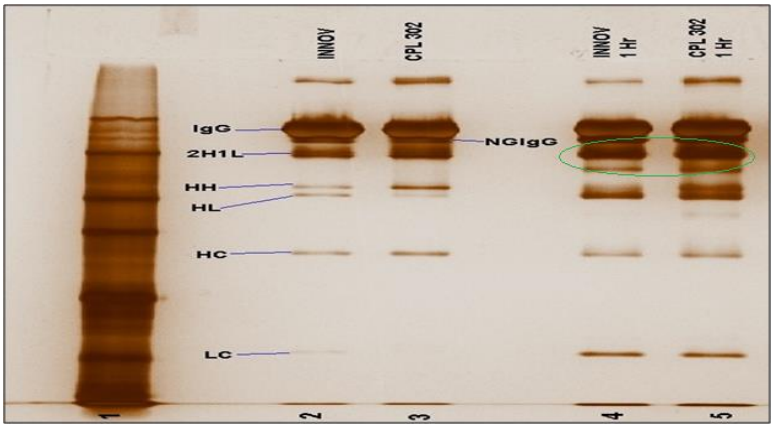

Figure 10b: SDS- PAGE scan of non-reduced Herceptin® and Cipla 302 after acid hydrolysis at 70° C for 1 hour. Fragments which resolved from non-reduced CE- SDS like LC, HC, HL, HH and 2H1L as shown above, resembled with SDS PAGE

Table 7: Comparative data of acid hydrolyzed Herceptin® and Cipla 302 showing similarity between both of them. % IgG of Cipla 302 was 1.2% less than the Herceptin® whereas the % NGHC of Herceptin was 0.44% more than Cipla 302 which can be seen from the figure 2a and 2b

Innovator: B3435B01				
	LC	**HC**	**Purity (LC+HC)**	**IgG**
As such	29.06%	68.89%	97.95%	97.25%
0.01 N HCl, 70°C, 15 Mins.	28.12%	63.95%	92.07%	93.66%
0.01 N HCl, 70°C, 30 Mins.	25.89%	58.00%	83.89%	89.16%
0.01 N HCl, 70°C, 60 Mins.	24.06%	48.72%	72.78%	81.06%
0.01 N HCl, 70°C, 120 Mins.	25.11%	33.56%	58.67%	69.85%
Cipla 302: BMF1/ 302/ 10003				
	LC	**HC**	**Purity (LC+HC)**	**IgG**
As such	28.79%	69.62%	98.41%	95.37%
0.01 N HCl, 70°C, 15 Mins.	28.02%	63.16%	91.18%	91.66%
0.01 N HCl, 70°C, 30 Mins.	25.38%	58.15%	83.53%	86.62%
0.01 N HCl, 70°C, 60 Mins.	26.15%	46.02%	72.17%	79.03%
0.01 N HCl, 70°C, 120 Mins.	25.35%	33.56%	58.91%	67.20%

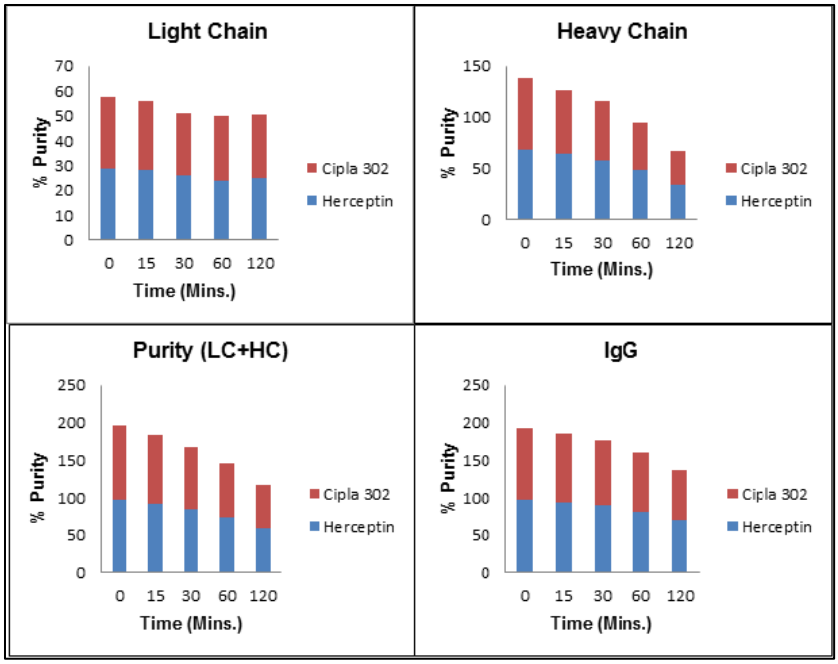

Figure 11: a: Light chain; b: Heavy chain; c: Purity (LC + HC); d: Intact IgG; Comparison of acid hydrolyzed Herceptin® and Cipla 302 showing similarity of light chain (LC- fig. 11 a), heavy chain (HC- fig. 11 b), purity (LC + HC- fig. 11. c) and intact IgG- fig. 11 d between both of them

Thus, after subjecting Cipla 302 to acid hydrolysis along with Herceptin ®, it was observed that Cipla 302 was similar to Herceptin® in terms of area% of light chain, heavy chain, purity (LC + HC) and intact IgG (Figure 11). The degradation pattern and the generation of clips on acid hydrolysis were seen on the same site in both Cipla 302 and Herceptin®.

CONCLUSION

In this study, we are providing a thoroughly controlled acid hydrolysis method for the quantification of clips to the biotechnology industry to assess the purity and heterogeneity of IgG under reduced and non- reduced conditions.
Under non-reduced conditions, the assay resolved the 1) IgG, 2) non- glycosylated IgG, 3) 2 heavy chains and 1 light chain, 4) 2 heavy chains, 5) 1 light chain, 1 heavy chain, 6) 1 heavy chain, 7) 1 light chain and some unknown impurities between them. Under reduced conditions, the assay resolved the 1) heavy chain, 2) non- glycosylated heavy chain 3) light chain and the lower-molecular-weight impurities that eluted between the light chain and heavy chain, as well as higher-molecular-weight impurities that are larger than the heavy chain.
In the above acid hydrolysis method, some of the sample preparation parameters including concentration of HCl, incubation temperature, incubation duration and comparison of Cipla 302 with Herceptin® were investigated. It was observed that a slightly acidic condition and increase in incubation temperature greatly increased the degradation of reduced and non -reduced mAbs.
Also, the data obtained from reduced and non-reduced CE- SDS was comparable to that of the reduced and non-reduced SDS- PAGE respectively (in terms of purity and generated clips), in both Cipla 302 and Herceptin® which was considered as an important standard for the quantitation of clips.

REFERENCES

[1] J Zhang; S Burman; S Gunturi JP Foley. *J Pharm Biomed Anal*, **2010**, 53(5):1236-1243.
[2] LY Liu; C Ratnayake; J Chapman; N Dontha; S Choo; MP Reddy. Beckman Coulter Application Information A-1973A.
[3] DJ Kroon; S Goltra; B Sharma. *J Capillary Electrop*, **1995**, 2(1):34-39.
[4] N Stackhouse; AK Miller; HS Gadgil. *J Pharm Sci*, **2011**, 100(12):5115-5125
[5] J Vlasak, R Ionescu. Fragmentation of monoclonal antibodies. InMAbs, Taylor & Francis, **2011**, 3(3), 253-263.