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Stability, Degradation Kinetics and in vitro Bioequivalence Studies of Valsartan Tablets.

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Abstract
The aim of this study was to evaluate the stability of valsartan in-house tablets in two different packs CFB and Aclar for the estimation of shelf life and degradation constant. The formulation was subjected to stability studies as per ICH Q1A(R). The degradation process of the in-house formulation in the stated packs appeared to follow first order reaction. The shelf life of the formulation was found out to be approx. 6 and 4 years in CFB and Aclar pack, respectively. There was no significant change from the initial values in the drug content during the stability studies in both the packs, hence, the formulation was found to be stable during the course of the study. The in-house tablet was compared with an innovator product (under same conditions) for their bioequivalence by evaluating % drug dissolved in multimedia. Similarity factor for both the products was found to be 61, 76 and 81 in 0.1N HCl, acetate buffer pH 4.5, 0.067 M phosphate buffer pH 6.8, respectively thereby proving the bioequivalence of the in-house product with that of the marketed product.

Key Words
Valsartan, ICH, shelf life, bioequivalence, stability testing, similarity factor.

Introduction
Valsartan is 3-methyl-2-[pentanoyl-[[4-[2-(2*H*-tetrazoyl-5-yl)phenyl]phenyl]methyl]amino] butanoic acid (Figure 1) with empirical formula $C_{24}H_{29}N_5O_3$ and molecular weight is 435.519 g/mol. It is a potent, orally active nonpeptide tetrazole derivative and selectively inhibits Angiotensin II Receptor type 1[1]. The drug is widely used in treatment of diseases like hypertension, heart failure myocardial infarction, diabetic nephropathy, its beneficial effects being related to the inhibition of angiotensin II by blockade of AT_1 receptor[2].

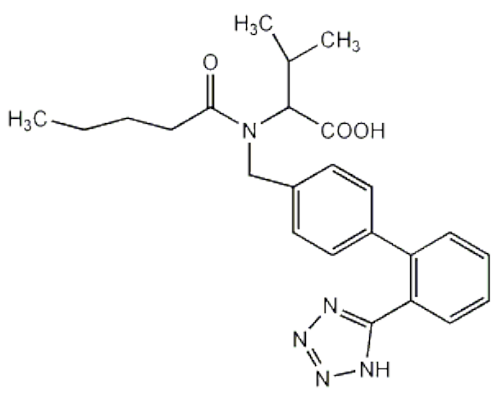

Figure 1: Chemical structure of Valsartan

Valsartan is rapidly absorbed orally and forms one inactive metabolite in the body namely valeryl 4-hydroxy valsartan that accounts for about 9% of the dose and is inactive in hypertension[3]. It contains acid (pKa=4.73) and carboxylic (pKa=3.9) groups making the compound soluble in the neutral pH range. Hence, it exists as solution at physiological pH values as the undissociated acid, the mono-anion and the di-anion. On increasing the pH from 4 to 6 the solubility of valsartan increases by a factor of about 1000, but it favors the anionic form and decreases lipophilicity, hence the rate of absorption of valsartan is influenced by intestinal pH along the gastro-intestinal tract. *In vitro* dissolution is complete and rapid at pH 5.0 and above[4]. According to International Conference on Harmonization, guidelines, the degradation of active compounds (drugs) cannot exceed 5% of the initial value during shelf life[5]. Since valsartan contains carboxylic groups, this suggests that hydrolysis and oxidation likely be the probable causes of degradation. Stability testing is a primary tool used to assess expiration dating and storage conditions for pharmaceutical products. International Conference on Harmonization recommended some stability guidelines that were developed as a cooperative effort between regulatory agencies and industry officials from Europe, Japan and United States[6]. Proper design, implementation, monitoring and evaluation of the studies are linked to the establishment and assurance of safety, quality and

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efficacy of the drug product from early phase development through the lifecycle of the drug product[7]. According to FDA guidelines drug absorption from a solid dosage form after oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two steps, in vitro dissolution may be relevant to the prediction of in vivo performance (IVIVC). For curves to be similar f2 values should be between 50 to 100 and f1 should be between 1 to 15. But EMEA guidelines[8] state that for products to be similar f2 values should be between 50 to 100.

Determination of Valsartan in biological samples and pharmaceutical preparations has been reported in the literature using various analytical techniques but there is no study related to stability of valsartan in tablets and determination of degradation constant at room temperature & determination of half life and shelf life of valsartan tablets in two packing types.

The aim of this study was to carry out accelerated and long-term stability studies as per ICH guidelines in two packs, CFB and Aclar. According to these guidelines analytical test procedures for stability samples should be fully validated and the assays should be stability indicating[9]. Hence the present studies were carried out on our in-house tablet dosage form (Valsartan tablet 320 mg) at 30˚C± 2˚C and 65% ± 5% RH for twelve months and 40˚C ± 2˚C and 75% ±5% RH for six months as India falls under climatic zone III. The parameters evaluated were % loss on drying, % drug content, and % drug dissolved. As valsartan falls in BCS Class III (highly soluble and low permeable), the in-house tablet dosage form was compared with an innovator product (both under similar conditions at room temperature) and similarity & difference factor were calculated to prove the bioequivalence between the two. In the current work, a validated HPLC-UV method is utilized to carry out the kinetic investigation by finding the order of reaction with respect to gradually changing time and temperature conditions.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents used were of analytical grade and were of the highest purity unless indicated otherwise. Valsartan working standard was obtained as gift sample from Ranbaxy Research Laboratories, Gurgaon and was certified to contain 99.7% purity. HPLC-grade methanol and acetonitrile were obtained from Fisher Scientific. Water was purified using a Milli-Q system (Millipore, Tokyo, Japan). Other reagents and solvents were HPLC grade or the highest grade commercially available and used without further purification.

Equipments

The liquid chromatographic system employed for the assay was Agilent HPLC (1100 series) fitted with binary pumping system and UV detector. HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Data collection and integration was accomplished using EmPower software. UV spectrophotometer used was Perkin Elmer (1105 series). Other instruments used include sonicator (Bransonic Cleaning Company), the pH of solution was adjusted using digital pH meter (Mettler Toledo). Dissolution apparatus used was from Distek 2100A series. Buffers and other solutions were filtered through 0.45µ filtration membrane nylon filters (Millipore). Storage for stability was done in Environmental Stability Chamber (TH80 S), Thermo Lab. Instrument, Mumbai.

Chromatographic Conditions for Assay

Separations were carried out on a Thermo-hypersil ODS column, (150mm X 4.6mm, 5µ, Agilent, USA) with the mobile phase consisting of a filtered mixture of water, acetonitrile and glacial acetic acid in the ratio of 500:500:1. Flow rate was kept at 1.0 mL/min with an analysis time of 10 min and column oven temperature 25˚C. The sample temperature was kept at 25 ± 2˚C in an auto sampler with UV detector at 273nm[10].

Tablet Formulation

The in-house tablet corresponds to the Valsartan coated tablet 320 mg were manufactured in our pharmaceutics laboratory. Main excipients of the tablet formulation were croscarmellose sodium, magnesium stearate, silicified microcrystalline cellulose, colloidal silicon dioxide, polyvinyl alcohol, titanium dioxide, macrogol, talc and iron oxide yellow. The innovator tablet corresponds to ‘DIOVAN’ 320 mg manufactured by Novartis, batch number B5010.

Packaging Types

Two packaging types were used in our pharmaceutics laboratory:

CFB (Cold Form Blister): the main components were oriented polyamide nylon film; adhesives, polyvinyl chloride (PVC) film, and aluminium soft temper foil. Aclar Pack: the main components were acetone, butyl alcohol, carbon tetrachloride, 1,2-dichloroethane, ethyl acetate, ethyl alcohol, ethyl ether, ethylene oxide, formic acid, gasoline, acids, methanol, toluene, osmium tetra oxide and plastisolve.

Assay of Valsartan 320 mg Tablet

Standard solution of Valsartan (100 µg/mL) was prepared in diluent consisting of water: acetonitrile in the ratio of 50:50. The five replicates injections (20 µL) of standard solution were injected and the % relative standard deviation for five replicates came out to be less than 2%. Sample solution was prepared by weighing 20 tablets and their average weight was calculated. Tablets were crushed into a fine powder and quantity of tablet powder equivalent to about 100mg of valsartan was weighed and transferred into a 100 mL volumetric flask. About 50 mL diluent was added and sonicated for 30 minutes with occasional stirring, made volume up to the mark with diluents and mixed. Diluted 5 mL of this solution to 50 mL with diluent and filtered through 0.45 µ nylon filter.

Dissolution of Valsartan 320 mg tablet

Standard solution of valsartan was prepared by weighing and dissolving 65 mg of valsartan standard in 100 mL 0.067 M phosphate buffer pH 6.8. Then 5 mL of this stock solution was diluted to 200 mL with the respective media and filtered through 0.45 µ nylon filter giving the actual concentration of about 16 µg/mL. Sample solution was prepared by adding one tablet to 1000 mL dissolution vessel containing the dissolution media. 10 mL of the sample was withdrawn at each time point, 5 mL of which was diluted to 100 mL with the dissolution media and filtered through 0.45 µ nylon filter and analyzed by UV spectrophotometer at 248 nm.

Dissolution Conditions

The dissolution of valsartan for both the innovator and the in-house formulation was carried out in three Medias: phosphate buffer pH 6.8, acetate buffer pH 4.5, 0.1N HCl. The dissolution conditions involved USP XXIII paddle (Apparatus 2) apparatus with 1000 mL of dissolution media at rotational speed of 50 rpm and temperature 37±0.5˚C. 10 mL of the sample was withdrawn at the end of 10, 15, 30 and 45 minutes. The collected samples were analyzed through UV-visible spectrophotometer at 248 nm.

Calculations

The stability samples were analyzed for every month for first three months and every three month for six months at 30˚C±65% RH and at 40˚C±75% RH (accelerated stability studies) in two packaging's: CFB and Aclar, to derive the degradation constant, and to predict the shelf life in the respective packaging types at the room temperature using Arrhenius equation. Each injection for the concentration was injected in duplicate and the mean concentration of valsartan was calculated at each condition. At the selected temperatures (303K and 313K) for six months, the degradation appeared to follow 1st order kinetics as is evident from the straight line obtained from the plot between log C and time.

Equation for the first order reaction is given by:

$$\log_{10} Ct = [-k/2.303]\, t + \log_{10} C_o$$

Where, k is the degradation rate constant, C is the concentration at time t, C_o is the concentration at time t= 0

After determining the order of the reaction the degradation rate constant was measured from the slope of the lines (slope= -k/2.303) at each elevated temperature. The effect of temperature on the rate of reaction was obtained by drawing Arrhenius plot given by logarithmic equation as:

$$\text{Log } k = \frac{-E_a}{2.303R} + \log A$$

In exponential form as:

$$k = A\, e^{-Ea/RT}$$

Where, k is degradation rate constant, A is the frequency factor (kcal mole^{-1}), E_a is the energy of activation (kcal mole^{-1}), R is the gas constant (1.987 cal K^{-1} mol^{-1}) and T is the absolute temperature (K).

From the plot, value of k at 25˚C was determined and was used to calculate shelf life using the equation

$$t_{0.9} = \frac{0.1052}{k_{25}}$$

Where, $t_{0.9}$ is the time required for 10% degradation of the drug and is referred as shelf life.

The same plot was used to calculate the half life at 25˚C using the equation:

$$t_{1/2} = \frac{0.693}{k_{25}}$$

Where, $t_{1/2}$ is the time required for 50% degradation of the drug and is referred to as half life.

To have an estimate about the bioequivalence between our in-house formulation and the innovator product the similarity factor (f2) and difference factor (f1) were calculated as follows:

$$f2 = 50 \cdot \log \{[1 + (1/n)\Sigma_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \cdot 100\}$$

$$f1 = \{[\Sigma_{t=1}^{n} | R_t - T_t |]/[\Sigma_{t=1}^{n} R_t]\} \cdot 100$$

Where, n= the number of time points, R_t is the mean percent dissolved of a reference product, T_t is the mean percent drug dissolved of a test product.

Results and Discussion

The design of the formal stability studies for valsartan 320 mg tablet was based on the knowledge of the behavior and properties of the valsartan, and from stability studies on the active substance and experience gained from pre-formulation studies and investigational pharmaceutical products. At the selected temperatures of 303K (30˚C) and 313K (40˚C) in both the packs, CFB and Aclar, the degradation followed 1st order kinetics as is evident from the straight line obtained from the plot between log percent drug remaining and time as in Figure 2 and 3. A decrease in concentration (percent drug remaining) was observed with time which was more in case of Aclar pack than in CFB. From the straight line equation obtained from the plot, the degradation constants at two temperatures were calculated, the slope of the straight line being equal to –k/2.303 (Table 1). A plot of Log k (observed) value at the elevated temperature versus the reciprocal of temperature (1/T x 1000K), Arrhenius plot was obtained as shown in Figure 4 and 5. An extrapolation of the Arrhenius plot gives the information about the degradation process at the room temperature (25±2˚C). The first order E_a, $t_{1/2}$, $t_{90\%}$ (shelf life) as well as value of A at 25˚C were calculated and mentioned as in Table 2. Drug content in the products at the accelerated and long term stability conditions were determined by stability indicating HPLC assay method that was validated as per ICH guidelines. There was no significant change from the initial value in accelerated and long term stability conditions in both the packs as shown in Table 3 and 4. The dissolution studies on the initial and the stability subjected batches in both the packs did not show much difference. It indicated that the release pattern was largely unaffected by the long term and accelerated stability conditions in the two packs. Even though there was change in the loss on drying the release pattern was more or less unaltered. Hence, the formulation was found to be stable. The degradation rate constant for CFB and Aclar pack was found to be 0.00004529/day and 0.00006166/day, respectively and the shelf life was found to be approximately 6 years in CFB and 4 years in Aclar packaging clearly indicating that the formulation was more stable in CFB as compared to Aclar pack. For the comparison of bioequivalence between the in-house formulation and the innovator product, dissolution of 12 units each of both the products at the same room temperature condition was done in three medias: 0.1N HCl, acetate buffer pH 4.5, and 0.067 M phosphate buffer pH 6.8 at the time intervals of 10 min, 15 min, 30 min and 45 min. (Table 5) They were analyzed by using UV spectrophotometer at wavelength 248 nm. Their mean dissolution values were calculated and compared by calculating similarity and difference factor in the three medias and f2 values were found to be greater than 50 as in Table 6.

Conclusion

Stability testing is interwoven through the entire lifecycle of a drug product. A detailed knowledge of the stability requirements and the impact on the other areas like container closure, process changes etc. are needed to properly design and evaluate stability studies in order to ensure that the product is stable throughout its stated shelf life in the given container and closure. This also ensures minimal delays and minimizes costs in developing a new drug product. The developed Valsartan tablet was stable during its stability studies with a shelf life of about 6 years in CFB pack and about 4 years in Aclar pack. Also the formulation was bioequivalent in comparison with an innovator product. This ensures that in the same subject an essentially similar plasma concentration time course will result in essentially similar concentration at the site of action and thus in an essentially similar effect given by the in-house preparation as with the marketed formulation.

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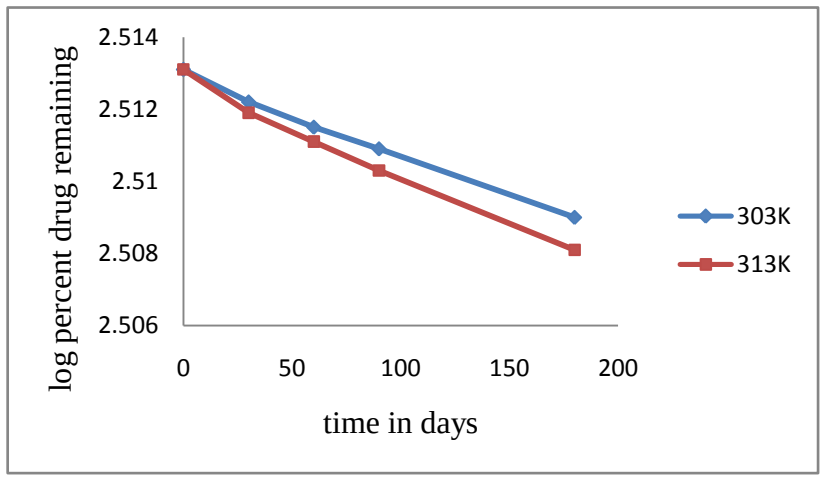

Figure 2: First order kinetics for the degradation of valsartan tablet in CFB pack at two conditions.

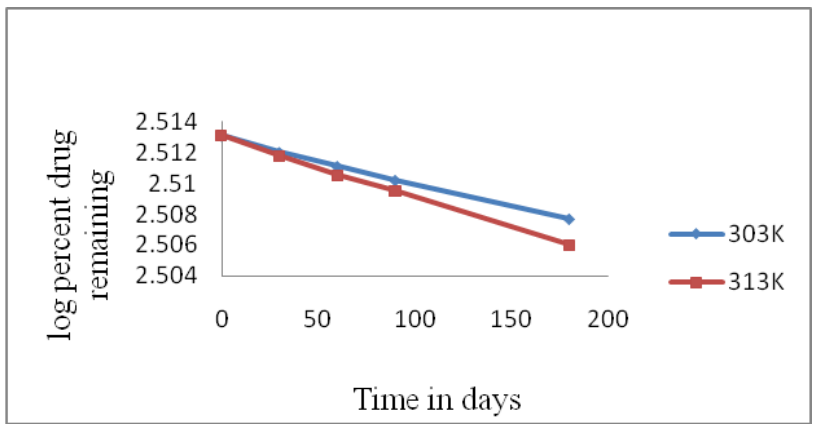

Figure 3: First order kinetics for the degradation of Valsartan tablet in Aclar pack at two conditions:

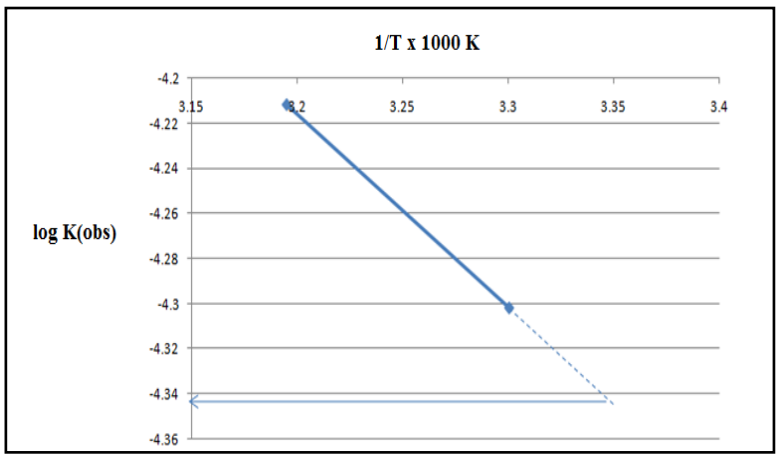

Figure 4: Arrhenius plot for the degradation of Valsartan tablet in CFB pack and its extrapolation to predict the degradation at room temperature (25±2˚C).

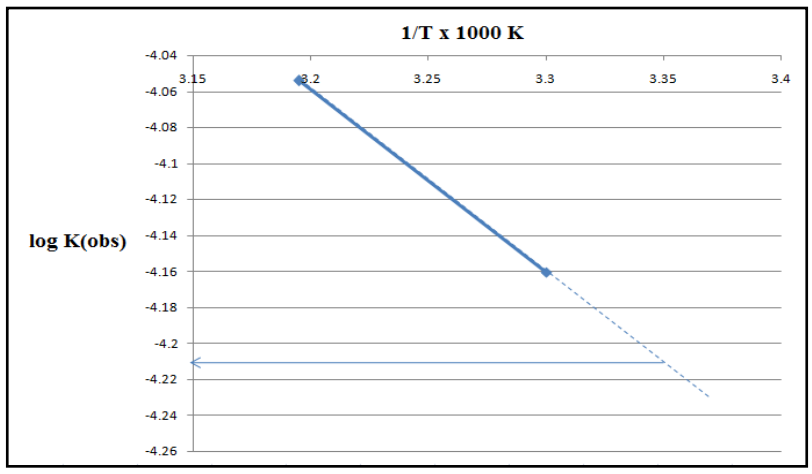

Figure 5: Arrhenius plot for the degradation of Valsartan tablet in the Aclar pack and its extrapolation to predict the degradation at room temperature (25±2˚C).

Table 1: Degradation rate constant (K_{obs}), half life ($t_{1/2}$), shelf life ($t_{90\%}$) and regression coefficient (R^2) for valsartan tablet in CFB and Aclar packs at 30˚C± 65% RH and 40˚C±75% RH.

Pack	Temp.(K)	1/T x 1000(K^{-1})	Slope	k_{obs}(day^{-1})	log k	$t_{1/2}$(days)	$t_{90\%}$(days)	R^2 for 1st order plot
CFB	303	3.3003	-0.00002167	0.000049898	-4.301	13888.33	2122.33(5.8yrs)	0.995
	313	3.1948	-0.00002667	0.000061413	-4.2117	11284.26	1724.39(4.7yrs)	0.995
Aclar	303	3.3003	-0.00003000	0.000069090	-4.1606	10030.40	1532.78(4.19yrs)	0.997
	313	3.1948	-0.00003833	0.000088282	-4.0541	7849.84	1199.56(3.29yrs)	0.998

Table 2: Degradation kinetics of Valsartan Tablet at room temperature in the two packs.

Degradation Kinetics at 25±2˚C	**CFB**	**Aclar**
Log K_{25} (Room Temperature)	-4.342	-4.210
Degradation Rate Constant K_{25} (day^{-1})	0.00004529	0.00006166
Activation Energy, E_a (kcal $mole^{-1}$)	28.466	28.038
Half life, $t_{50\%}$(years)	41.92	30.79
Shelf life, $t_{90\%}$(years)	6.4	4.7
Arrhenius frequency factor, logA(kcal $mole^{-1}$)	1.87	1.92

Table 3: Evaluation of in-house formulation at stability study in CFB pack.

Condition	Sampling period	Parameters evaluated			
		%w/w LOD	%Drug Content remaining	log percent drug remaining	% dissolution (45min)
30˚C±2˚C,	Initial	2.01	101.8	2.0077	97
65 ± 5 % RH	1Month	2.25	101.6	2.0069	97
	2 Month	2.42	101.5	2.0065	97
	3Month	2.37	101.3	2.0056	96
	6Month	2.51	100.9	2.0039	96
	9Month	2.49	100.2	2.0009	95
	12Month	2.68	99.7	1.9987	94
40˚C ± 2˚C,	Initial	2.01	101.8	2.0077	97
75 ± 5 % RH	1Month	2.31	101.5	2.0065	97
	2Month	2.43	101.3	2.0056	96
	3Month	2.54	101.2	2.0052	96
	6Month	2.52	100.7	2.003	95

Table 4: Evaluation of in-house formulation at stability in Aclar pack.

Condition	Sampling period	Parameters evaluated			
		%w/w LOD	%Drug Content remaining	log percent of drug remaining	% dissolution (45min)
30˚C±2˚C,	Initial	2.01	101.8	2.0077	97
65 ± 5 % RH	1Month	2.27	101.6	2.0069	97
	2 Month	2.45	101.3	2.0056	96
	3 Month	2.49	101.2	2.0052	96
	6 Month	2.52	100.6	2.0026	95
	9 Month	2.53	100	2	94
	12 Month	2.69	99.5	1.9978	94
40˚C ± 2˚C,	Initial	2.01	101.8	2.0077	97
75 5 ± 5% RH	1Month	2.33	101.5	2.0065	96
	2 Month	2.45	101.2	2.0052	96
	3 Month	2.57	101	2.0043	96
	6 Month	2.56	100.1	2.0004	95

Table 5: Percentage drug release of the two products in different medias.

% Drug Dissolved				
Innovator	**10 min**	**15 min**	**30 min**	**45 min**
0.1N HCl	12	20	32	40
4.5 acetate buffer	61	72	82	86
6.8 phosphate buffer	93	94	95	95
In-house Product	**10 min**	**15 min**	**30 min**	**45 min**
0.1N HCl	18	26	39	44
4.5 acetate buffer	64	75	84	89
6.8 phosphate buffer	90	93	95	97

Table 6: f2 and f1 values for the in-house and innovator product.

Media	f2	f1
0.1 N HCl	61	22
pH 4.5 acetate buffer	76	4
0.067 M, pH 6.8 phosphate buffer	81	2

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