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Regarding the Manufacturing of the Medicine Omalizumab, How Extensively is the Overall Efficacy of Omalizumab Regarding Asthma and Allergies Maintained?

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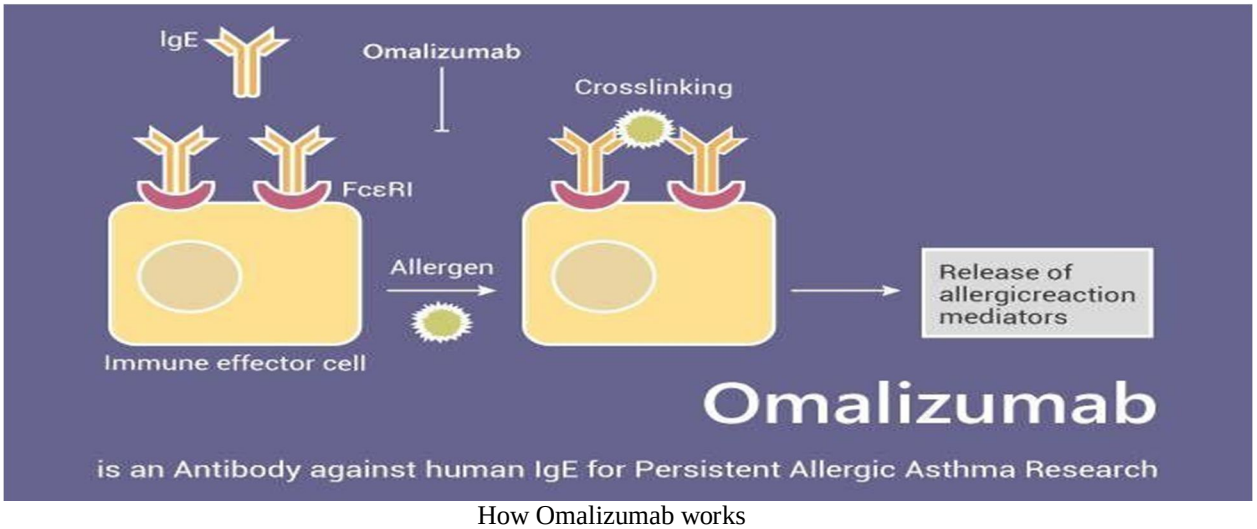
Abstract: ***A medication that can be used to treat inflammation caused by allergies and asthma, omalizumab, is really a special medication. Omalizumab's efficacy has been previously tested in both real-life and controlled settings, in previous studies. In those studies Omalizumab is found to be both safe and efficient, for asthma and allergies, even for prolonged usage. But my question is, how this safety and efficiency is maintained in omalizumab throughout its manufacturing process. Here, I will be looking at and explaining the process of how omalizumab is maintained, through my insight from an internship at Kashiv Biosciences, a biosimilar pharmaceuticals company.***

I. ACKNOWLEDGEMENTS

Thank you for the guidance of Aisha Abdulkarimu from the University of Maryland in the development of this research paper. Thank you also for the resources and guidance from KashivBiosciences, LLC.

II. INTRODUCTION TO OMALIZUMAB

When an anaphylactic allergy or asthma occurs, antibodies (a type of protein, built up byamino acids) form. These antibodies are called immunoglobulin or IgE. Whenever an antigen (toxic/alien substance)enters the body IgE forms and chemically attaches to the foreign substance. This causes swelling and inflammation, causing the allergic reaction. Omalizumab is amedication that binds to the IgE, and stops it from binding to the antigen, which in turn helps stop the allergic reaction. So that is how omalizumab works, but what about its manufacturing processes? Some drugs can often be inefficient, and not manufactured with proper standards, resulting in a final product thatboth might not work, and could be potentially hazardous to the recipient. With the experience I got with my internship, I can hypothesize that omalizumab is very extensively tested throughout the entire manufacturing process to maintain its safety and efficacy.



How Omalizumab works

During my internship at kashiv Biosciences, I was able to see their entire manufacturing process from start to finish. It all begins with the initial research of the medication. The research and development team then builds a set of assessments for that substance, to ensure quality. Quality control then tests the medication with those assessment methods, which include, Hydrophobic Interaction Chromatography (HIC), Size Exclusion Chromatography (SEC), and Enzyme Linked Immuno-Sorbent Assay (ELISA). As the manufacturing of the drug begins, quality control still keeps testing it, to make sure the drug substance is working all the way. Throughout this processboth good documenting and manufacturing processes are kept up, with accurate and visible records of all activity, and constant maintenance of equipment, to maintain overall safety.

Overseeing all this is a quality assurance department, which makes sure that all the other departments are doing all their steps correctly to make sure the final product is of right quality. They also make sure that all the documentation and practices are up to date with the US Food and Drug Administration (FDA). There are also a group of engineers who are generally hired to maintain the facilities, and make sure that they don't get contaminated. Overall, the most important part of the process in checking the quality of the drug are the methods - HIC, SEC, andELISA - all of which I will be discussing later on.

Omalizumab has been used as a medication for allergies and asthma for several decades at thispoint, and has also been widely tested to work through previous studies. It has been tested for children, adults, severe allergies, allergy desensitization, and more.

III. OMALIZUMAB LITERATURE REVIEW

As previously mentioned, Omalizumab is used as a medication for asthma. In the observational study, *Real-life Efficacy of omalizumab After 9 Years of Follow-up*, Omalizumab had been tested as a treatment for patients with inadequately controlled asthma. Over the course of 9 years, results were measured on an index that primarily focused on activity limitation, symptoms, environmental stimuli, and emotional function. The final results from this particular study shows no concerns of safety in the continued use of omalizumab. There were no changes in liver function parameters, blood count, or creatinine, and no patient showed local or systemic side effects. Furthermore no asthma related emergencies were documented for the test subjects, over the last 5 years of the study. From this it can be said that omalizumab was safe and efficientenough to prevent symptoms of uncontrolled asthma, during the study. While it has been observed to be effective in this study, it has not been tested or proved against severe cases of asthma. Moreover it is very likely that this drug may affect individuals differently depending onboth the severity and type of allergy. In *Treatment of Childhood Asthma With Anti-Immunoglobulin E Antibody (Omalizumab),* omalizumab was tested as a treatment for children with asthma. A total of 334 males and females, ages 6 to 12, who had moderate-severe asthma and required inhaled corticosteroids, an anti-inflammatory drug, were selected for this study. They were given equivalent doses of beclomethasone dipropionate (BDP), an allergy medication equivalent to inhaled corticosteroids,and then treated with omalizumab. Only half of the subjects were treated with omalizumab.

According to the results of this experiment more participants in the omalizumab groupo were able to decrease their BDP dosage and it was withdrawn completely from 55% of them, compared to only 39% with the regular group. Overall there were no issues with the omalizumab treatment and there were no serious treatment-related side-effects. So it can be said from this study that omalizumab proved safe and effective for children with asthma, while also reducingthe requirements for the inhaled corticosteroids.

In *Effectiveness of omalizumab in patients with severe allergic asthma with and without chronic rhinosinusitis with nasal polyps: a PROXIMA study post hoc analysis*, omalizumab wastested on a 2-part study conducted in Italy in adult patients with severe allergic asthma. Patientseligible for add-on omalizumab were treated for a period of 12 months. The variables that were tested were asthma control, lung function, and the exacerbation rate. These outcomes were compared between a group with comorbid CRSwNP (a type of sinonasal inflammation) and onewithout it. Of the 123 subjects included in the analysis 17 were in the CRSwNP group. Overall, while the baseline clinical data was similar in both CRSwNP and regular groups, the CRSwNP had more numerically favorable results (35.7% vs. 23.0%) for improvements in the 3 variable conditions. While the sample size of people might have been limited in this observational study,it shows that in a realistic setting, omalizumab as an add-on for severe allergic asthma, was highly effective in improving lung function, asthma control, and in reducing exacerbations, including for patients with CRSwNP.

Omalizumab is also frequently used to treat chronic spontaneous urticaria (csu), chronic hives. In *Profile of omalizumab in the treatment of chronic spontaneous urticaria*, omalizumab's relation to csu (chronic spontaneous urticaria) is detailed. The omalizumab attaches to the IgE and disables them, which helps stop/reduce with the hives, similar to how it does for asthma.

This study provides solid reasoning suggesting that omalizumab is efficient in treating csu as an add-on therapy. The study also details how omalizumab could pose a potential risk to pregnantwomen and nursing infants, who are both in a fragile state. It is only advisable to use the medication when the situation is severe and the potential benefits far outweigh the risks.

Furthermore, while omalizumab has been tested on adults with csu adults, not much informationis yet available on the efficacy of omalizumab for children with csu.

In the study, *Efficacy of omalizumab in reducing latex allergy*, a case of a young boyaffected by severe persistent allergic asthma and rhinitis is shown. The boy presented mild-moderate symptoms after latex exposure, and started omalizumab therapy to treat his severe asthma. The study evaluates the latex allergy again after 6 years of treatment. After those 6 years, a reduction in latex sensitization was confirmed by a decrease in latex positivity on IgE tests, andit was hypothesized that it was due to the omalizumab binding the Fc3 sub-unit of free IgE molecules, reducing the binding of free specific IgE to the high-affinity IgE receptor (FceRI), leading to a reduction in the density of specific IgE. To conclude, the findings in the study show that omalizumab has an important role in reducing the symptoms and potential for desentization for a latex allergy. This study also indicates a possibility of omalizumab use for different allergies and therapy.

In another study, *Long-term "real-life" safety of omalizumab in patients with severe uncontrolled asthma: A nine-year study*, omalizumab was tested on its safety for consumption bypatients. In the study, asthmatic patients treated with omalizumab in a real-life setting (up to 9 years) were evaluated retrospectively, and adverse events/reasons for discontinuation were recorded. 39.1% of patients who discontinued treatment dropped out within the first year, for non-treatment related issues. Only 6 patients left because of treatment related adverse events. Butoverall, a long-term treatment with omalizumab appeared to be well tolerated in the real-life setting for more than half the patients. The omalizumab treatment for consecutive years did not increase the risk of side effects, and most predominantly anaphylaxis. This highlights that omalizumab can be a safe long term treatment, while not increasing chances of side effects, or anaphylaxis, with the majority of all patients leaving treatment because of issues not related withthe treatment.

IV. OMALIZUMAB TESTING METHODS

I was able to see the full pharmaceutical drug manufacturing process at my internship at Kashiv Biosciences. After the proper research is completed for the drug, samples are tested through multiple tests to determine if the drug is what it is, and if it is up to standard for usage. Inthat process quality control tests the medication with Hydrophobic Interaction Chromatography (HIC), Size Exclusion Chromatography (SEC), and Enzyme Linked Immuno-Sorbent Assay (ELISA) tests.

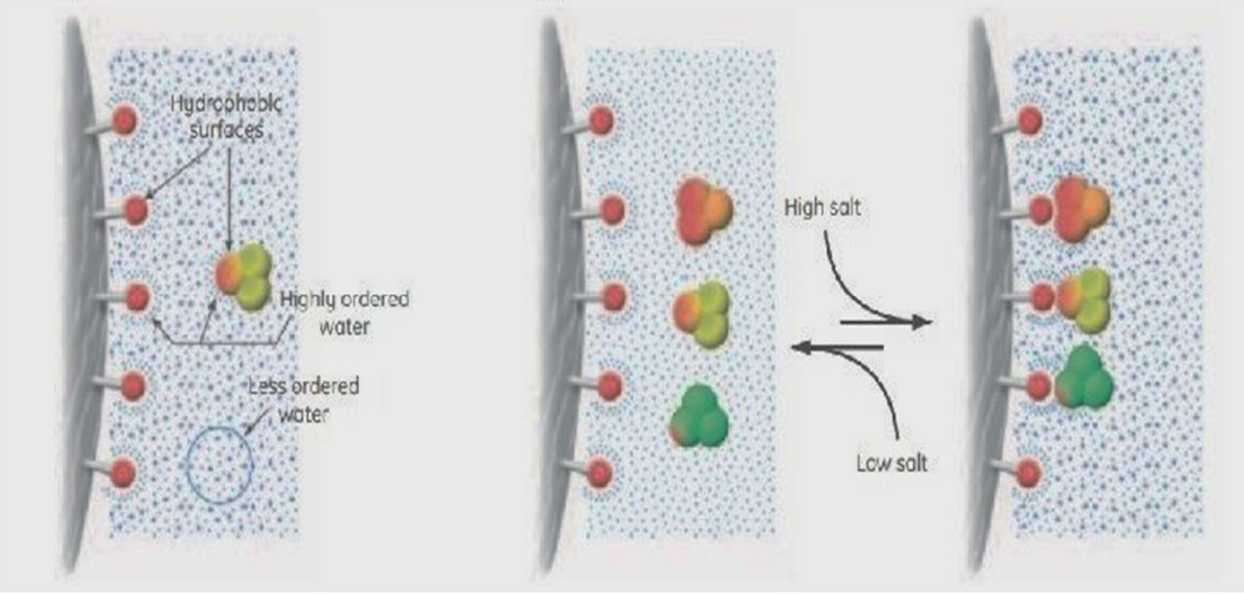


Diagram of HIC

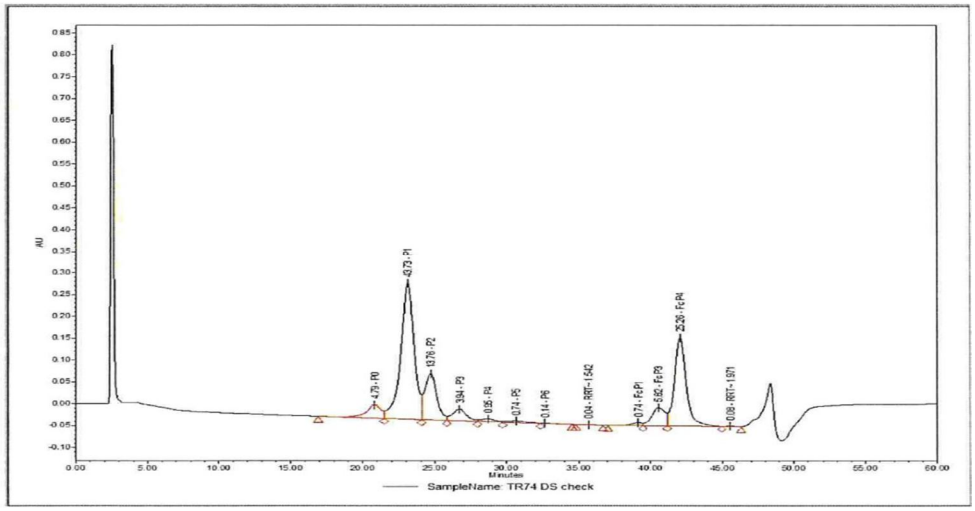
V. HYDROPHOBIC INTERACTION CHROMATOGRAPHY

The HIC (Hydrophobic Interaction Chromatography) method separates and purifies protein molecules based on how hydrophobic they are. In HIC, the sample protein molecules areintroduced into the column containing a high-salt buffer, and the molecules can be eluted out in the order of increasing hydrophobicity using a decreasing salt gradient. HIC uses a HPLC (High Performance Liquid Chromatography) machine with a pump, an autosampler, a UV detector, anda suitable data acquisition system.

HPLC Machine

HIC COLUMN

HIC also uses an HIC Column, e.g., TOSOH Bioscience TSKgel Phenyl-5PW, 7.5 X 75 mm, 10 µm column. For the mobile phase it uses a 2M Ammonium Sulfate with 20mM Tris in water (pH7.5) and 20mM Tris in water (pH 7.5). The run time is 60 minutes with the flow rate being 1 ml/min, and detection by UV at 215 nm to determine the purity of the samples. The samples are prepared at 1 mg/mL using a digestion buffer and digested by papain enzyme at 37 °C for 6 hrs. The prepared sample is injected into the column by HPLC injector and separated from the column by the mobile phase running through the system by the HPLC pump. The separated molecules pass through the detector and the UV signal is captured in the form of a chromatogram by data acquisition software. In the chromatogram the percent peak area of each peak represents the amount of that molecule present in the sample. This result is checked to see if the drug is within its designated parameters for the selected molecule.



Example of representative chromatogram in full scale (for HIC)

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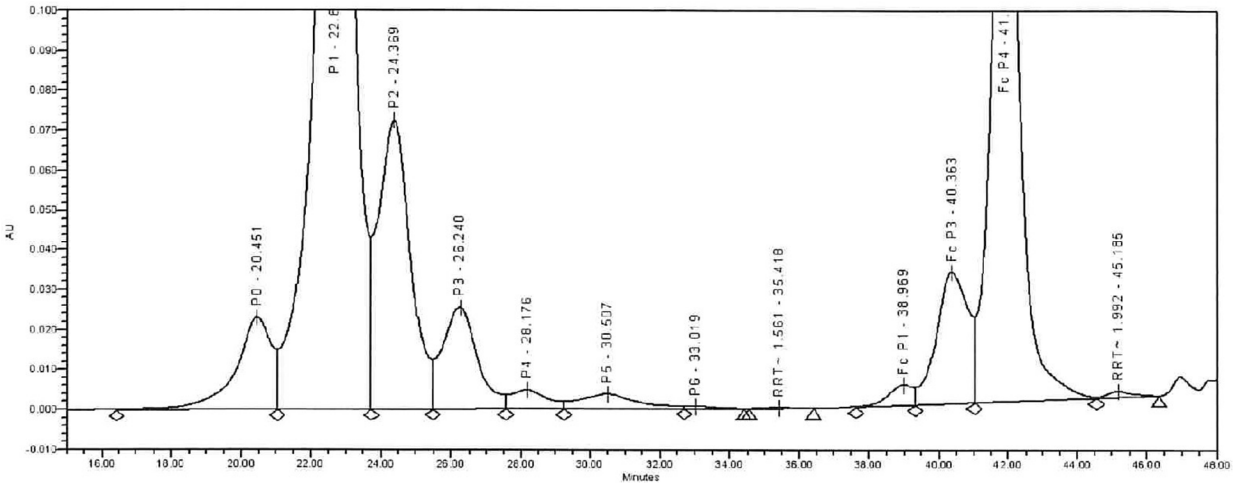
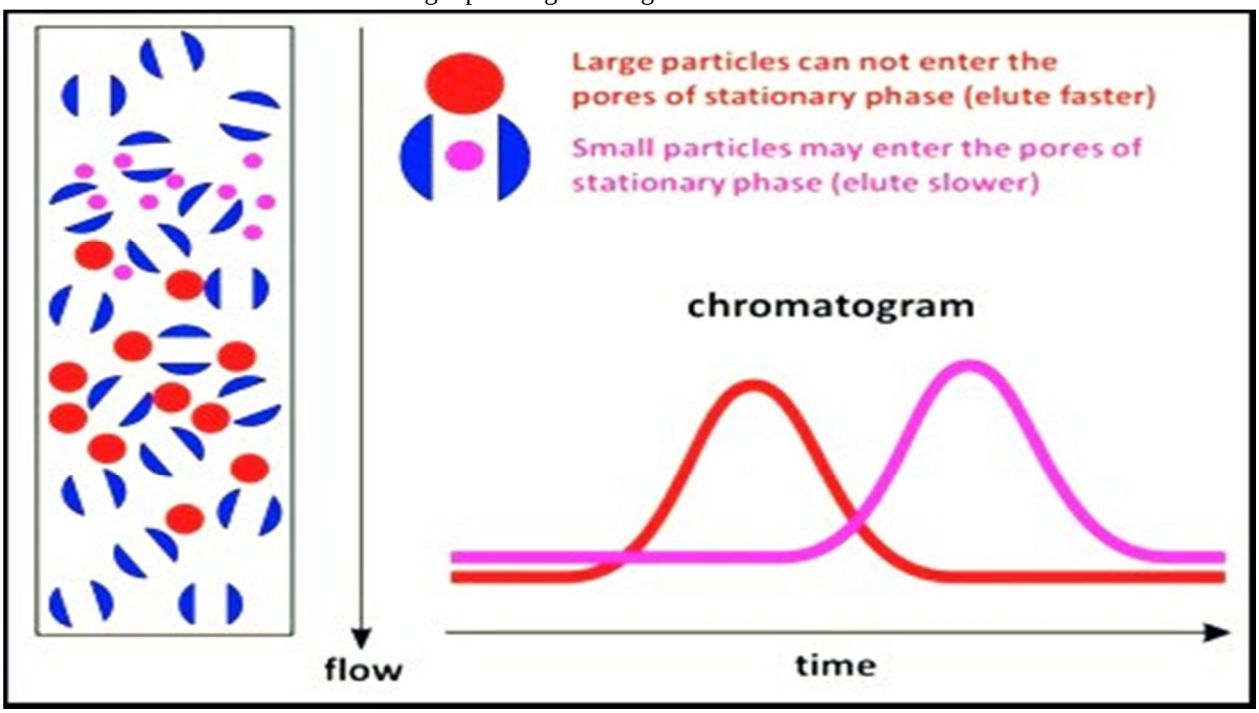


Example of representative chromatogram in zoom scale (for HIC)

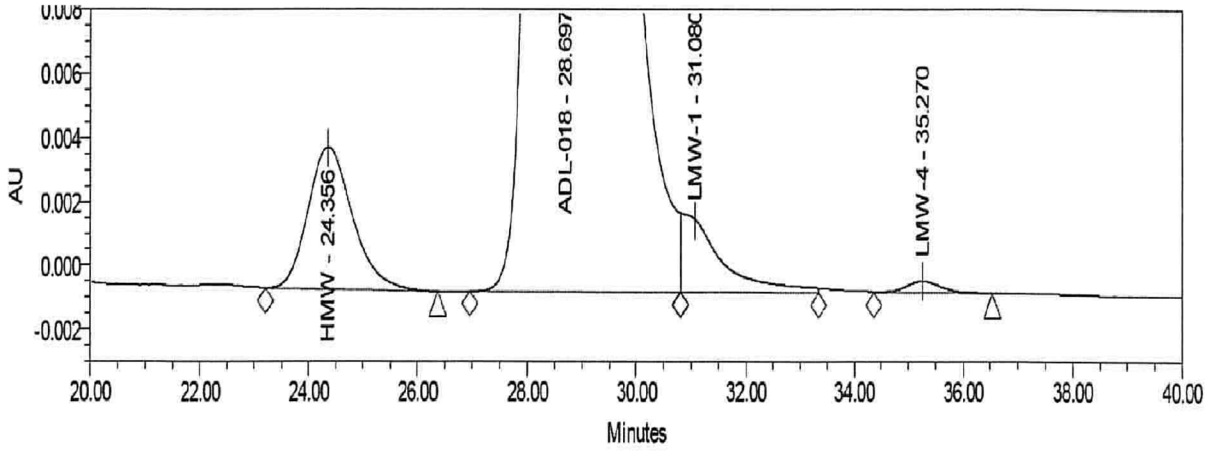
VI. SIZE EXCLUSION CHROMATOGRAPHY

During the SEC (Size Exclusion Chromatography) method of testing, molecules are separated based on size as they pass through a gel column composed of beads with a defined pore size. In simple terms, the larger molecules will take a shorter path through the matrix due to exclusion or limited access of pore space compared to smaller molecules. Smaller molecules can move through the pores and around the beads and thus have a longer pathlength through the column.



A cartoon illustrating the theory behind size exclusion chromatography

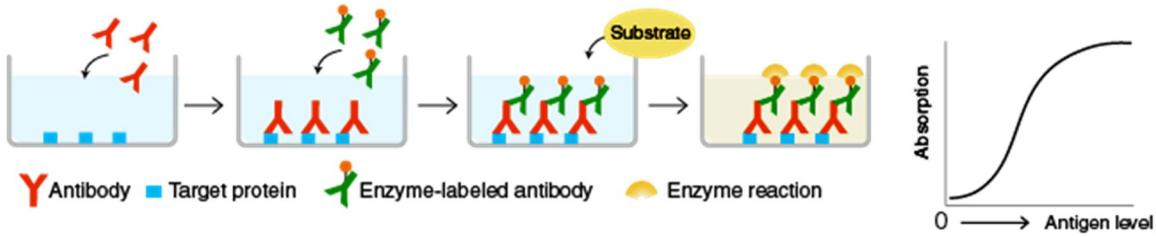
The SEC method uses a HPLC machine equipped with a pump, an autosampler, and an UV detector and suitable data acquisition system. It also uses a TOSOH Bioscience TSKgel G3000SW XL , 7.8 mm ID X 30 cm Length, 5 µm column, an isocratic elution using a mixture of pH 6.5 100mM phosphate, 100 mm Sulfate buffer as mobile phase with run time of 45 minutes and flow rate of 0.3 ml/min, and detection by UV at 215 nm. Samples are then preparedat 1 mg/mL using the mobile phase as diluent. The results for SEC on a chromatogram consists of high molecular weight peaks, monomer peaks, and low molecular weight peaks. These results are monitored to make sure the drug is within its designated parameters.



Example chromatogram for SEC

VII. ENZYME LINKED IMMUNO-SORBENT ASSAY

The principle of ELISA (Enzyme Linked Immuno-Sorbent Assay) is antigen-antibodyinteraction. In ELISA, the specific antibodies associate or bind to their target antigen. The substrate can bind to the enzyme, only when the interaction takes place.



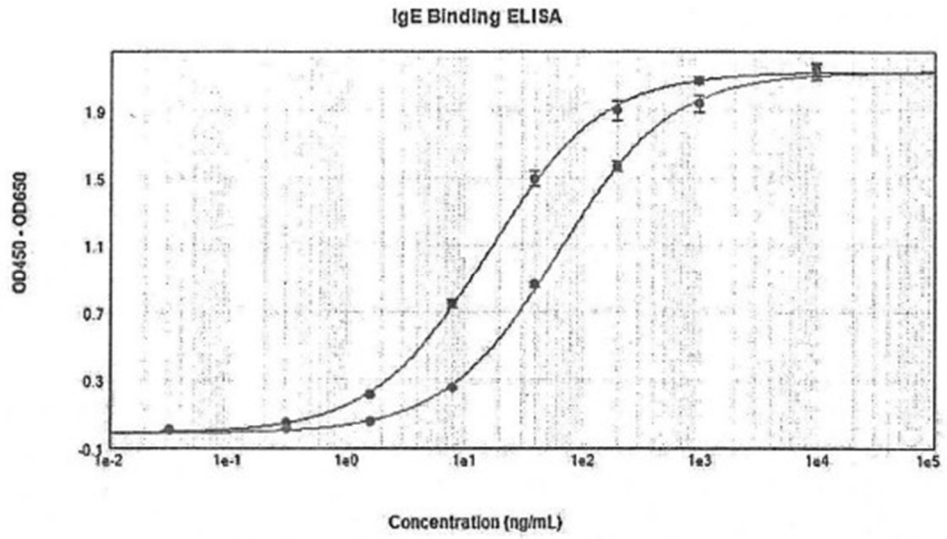
ELISA diagram

In ELISA, first the Human IgE is coated onto a polystyrene 96 well microplate, followed by a blocking step, ADL-018 is allowed to bind to the surface bound IgE. This bound complex is detected using HRP conjugated recombinant ProteinA/G. Tetramethyl benzidine (TMB) is usedas a substrate for HRP and the color intensity produced is directly proportional to the amount if ADL-018 bound to the captured IgE. Color intensity is measured by absorbance at 450nm and650nm using Molecular devices, spectramax plate reader.

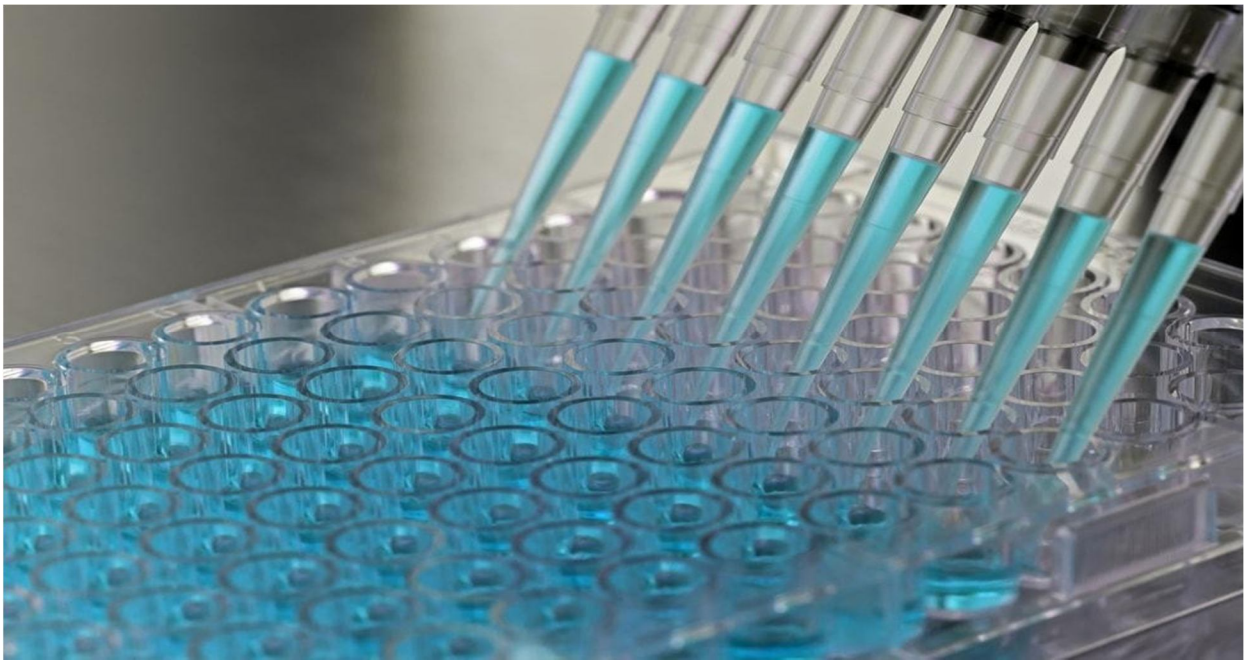
Spectramax plate reader

Then, the recombinant human IgE (100µL) is pipetted to each well of the 96 well assay plate, sealed and incubated overnight at 2-8°C. Next, the serially diluted reference standard and test samples (100 µL) are transferred and incubated for 120±10minutes. Then, the plate is washed three times with a 300 µL washing buffer using a plate washer, followed by the addition of 100uL peroxidase conjugated recombinant protein A/G and incubates for 60±5 minutes.The plateis washed three times with 300 µL washing buffer using a plate washer, followed by addition of 100 µL TMB substrate and incubate for 20-25 min. Finally, the stop solution (100 µL) is added and the absorbance measured at 450nm and 650nm. The 650nm background is subtracted from the 450nm absorbance values to derive the background corrected optical density (OD) values.

Subsequently, the blank value is subtracted from the background corrected OD values. A 4-parametric logistic curve of reference standard and test samples is created, and the relative potency is derived using the equation- % Relative potency = Estimated relative potency (Global Fit) x 100. Here one could see if the parameters are met for the drug, similar to how the chromatograms are checked to see if the parameters are met in the HIC and SEC assays.



Example (IgE Binding ELISA)



ELISA sampling in process

VIII. TO CONCLUDE

In conclusion, omalizumab has been proven to treat patients with asthma and allergies, inthe previous studies, and is extensively tested using all the above methods and more to ensure thequality of the drug. This ensures that further expansions of the drug will be safe and possible in the future. Through this it can be stated that omalizumab goes through many procedures that are meant to guarantee its efficacy, and thus is very extensively maintained, and hence fairly safe medication to use.

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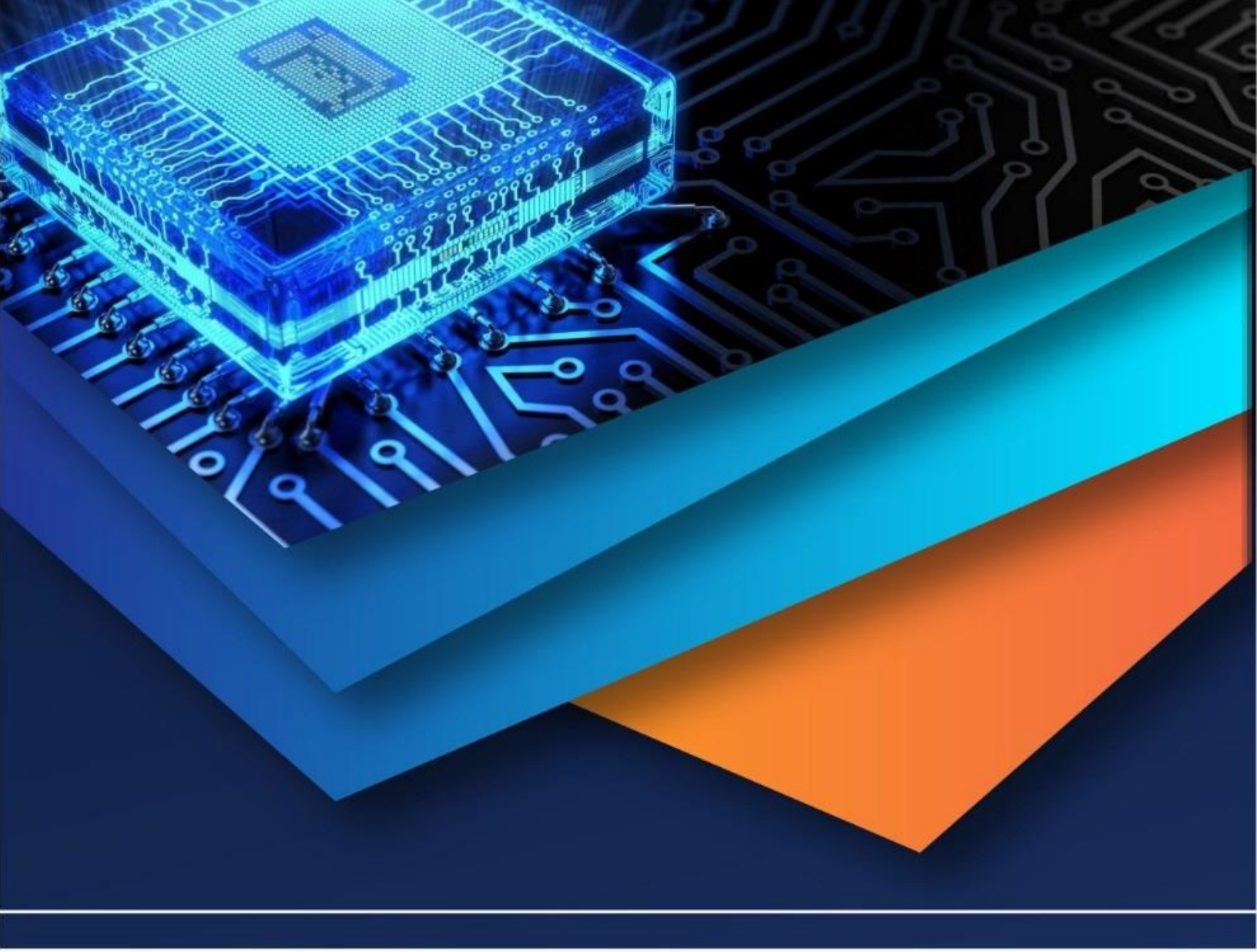

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