Journal of Pharmaceutical Research and Innovation (JPRI)
Volume 4, Issue 2, July 2024
e-ISSN: 2583-0023

Development and Validation of HPLC Method for Estimation of Ibandronic Acid in Tablet Dosage Form

Sammi Akter[1], ArghyaProsun Sarkar[2], Bidduth Kumar Sarkar[3], Nilay Saha[4], Anita Rani Chowdhury[5], Sozoni Khatun[6], Tonima Enam[7], Musab Shahariar[8], Lamia Hasan Joarder[9], Sukalyan Kumar Kundu[10*]

[1,4,10] Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh
[2,6,7,8,9] Department of Pharmacy, Islamic University, Kushtia, Bangladesh
[3] Department of Pharmacy, Comilla University, Cumilla, Bangladesh
[5] Department of Pharmacy, Jagannath University, Dhaka, Bangladesh
*Corresponding Author Email: skkbd415@juniv.edu

Abstract

Ibandronic acid is a highly efficacious bisphosphonate that effectively impedes the process of osteoclast-mediated bone resorption in postmenopausal women with osteoporosis. Our investigation aimed to establish a cost-effective high-performance liquid chromatography (HPLC) method for the accurate and dependable quantification of Ibandronic Acid. It is imperative to note that this particular compound is not included in the official pharmacopoeial compendium. Employing a C18 column (Hypersil BDS recommended), the compound was isolated utilizing a mobile phase containing pentanesulfonic acid sodium salt, EDTA, TEA, and orthophosphoric acid. The flow rate was set at 1 mL/min, with detection wavelength at 200 nm. Employing a calibration curve, the amount of Ibandronic acid present in tablet form was determined. The methodology expounded in this research was validated in compliance with the International Council for Harmonisation (ICH) guidelines for various parameters, encompassing linearity, accuracy, precision, robustness, and specificity. The linearity of concentrations, specifically at 0.19, 0.32, 0.51, 0.64, 0.76, and 0.96 mg/mL, exhibited a strong correlation coefficient (r) of 0.999. Ibandronic Acid displayed a retention time of 4.58 ± 0.45 min. The specificity assessment indicated the absence of impurities. The Limits of Detection (LOD) and quantification (LOQ) values were determined to be 0.021026880 and 0.06371782, respectively. This methodology can be effectively utilized for routine quality control evaluation in the analysis of Ibandronic Acid in tablet preparations.

Keywords

Bisphosphonate, HPLC, Ibandronic Acid, Mobile Phase, Osteoclast, Validation

INTRODUCTION

Ibandronate, also known as ibandronic acid, belongs to the third generation of bisphosphonates. It is a powerful compound that contains nitrogen and is specifically developed to improve the therapeutic effectiveness of bisphosphonate treatment.[1,2] Bisphosphonates (BPs) are widely regarded as the most efficacious pharmacological interventions for addressing skeletal disorders characterized by substantial bone resorption. These ailments include Paget's disease, bone metastases, myeloma, osteoporosis, as well as several genetic disorders impacting pediatric populations.[3] Metastatic bone disease is a frequently encountered presentation in persons with advanced malignant illnesses, including breast cancer, prostate cancer, multiple myeloma, and lung carcinoma. It affects a significant percentage of patients, ranging from 30% to 90%. Hence, ibandronate could potentially serve as an advantageous and well-tolerated adjunctive pharmacological intervention for the alleviation of severe bone pain.[4] The incidence of fractures resulting from osteoporosis is increasing in diverse geographical areas worldwide. The incidence of hip fractures globally was recorded at 1.7 million in 1990, with a projected increase to 21 million by the year 2050. The phenomenon of escalating bone loss is sometimes referred to as "the silent epidemic" and "the silent thief" in academic discourse.[5] Hence, Ibandronic acid plays a crucial role in the therapeutic management of Paget's disease, postmenopausal osteoporosis, and corticosteroid-induced osteoporosis, which are characterized by bone abnormalities, possible metastasis, malignant hypocalcemia, and various other medical disorders.[6] The formulation commonly employed is that of Ibandronate sodium salt. BM 21.0955, which is also referred to as ibandronate, exhibits similarities to zoledronic acid, minodronic acid, and risedronic acid.[3][7] The initial documentation of ibandronate as a therapeutic intervention for canine osteoporosis was recorded in the scientific literature in 1993.[7] The therapeutic index has a wide range due to the relatively low toxicity of excesses and the potential for a half-life as long as 157 hr. It is crucial to initiate a thorough discourse with patients concerning the probable manifestation of upper gastrointestinal side events, hypocalcemia, musculoskeletal discomfort, osteonecrosis of the jaw, atypical femur fractures, and severe renal dysfunction.It has been discovered that bisphosphonates containing nitrogen, such as ibandronate, can induce death in hematological cancer cells by inhibiting crucial elements of

Journal of Pharmaceutical Research and Innovation (JPRI)
Volume 4, Issue 2, July 2024
e-ISSN: 2583-0023

the mevalonate pathway, including farnesyl diphosphate synthase, farnesyl diphosphate, and geranylgeranyl diphosphate.[8, 9] The incorporation of these constituents is crucial in enabling the post-translational prenylation mechanism of GTP-binding proteins, such as Rap1.[9] The absence of prenylation negatively affects the functional capacity of these proteins, resulting in the onset of apoptosis specifically in the case of Rap1.[9]The induction of caspase-3 activation, a crucial modulator of the apoptotic pathway, was observed during treatment with ibandronate.[10] The chemical structure of Ibandronic acid is shown in Figure 1.

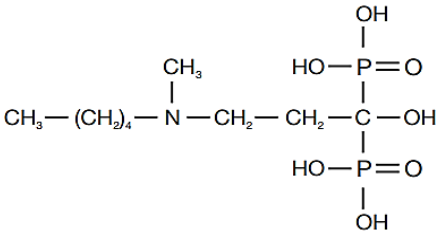

Figure 1. Chemical structure of Ibandronate

Due to its highly effective separations and frequently high detection sensitivity, HPLC is the separation technique most commonly used in pharmaceutical and biomedical analysis today. The development and validation of HPLC techniques are indispensable for innovating, developing, and manufacturing pharmaceutical drugs, as well as for conducting numerous other human and animal studies.[11]The primary aim of our research was to create and authenticate a straightforward, expeditious, reliable, and meticulous high-performance liquid chromatography technique for quantifying ibandronic acid in tablet formulations.

MATERIALS AND METHODS

Chemicals and Solvents

Ibandronic acid was acquired from Sonali Scientific Store, acetonitrile (HPLC grade) was obtained from Sigma-Aldrich, Germany, di-potassium hydrogen phosphate (HPLC grade) was obtained from Scharlab S.L., Spain, and orthophosphoric acid (AR grade) was obtained from S.D. Fine Chemicals Ltd., Mumbai, India. Nipa Pharma Ltd., Bangladesh, supplied water of HPLC quality.

Preparation of Buffer Solution and Sample Solution

1.75 grams of sodium pentanesulfonic acid and 100 milligrams of EDTA were dissolved in 900 milliliters of water, followed by the addition of 6 milliliters of triethylamine (TEA). The resulting solution was then diluted to a final volume of 1 liter and the pH was adjusted using orthophosphoric acid, serving as the mobile phase. The diluent utilized in this study was water.

A total of twenty-five tablets underwent a process involving weighing and subsequent grinding, leading to the formation of a powdered substance. Approximately 32 milligrams of Ibandronic acid were placed into a 50-milliliter volumetric flask. Subsequently, around 30 milliliters of diluent were added to the flask, followed by sonication for a duration of 20 minutes. Post-sonication, the mixture was cooled to room temperature, further diluted to achieve the desired volume, and thoroughly homogenized. The resulting solution was then purified using suitable filter paper, becoming the sample solution with an Ibandronic acid concentration of 0.64 milligrams per milliliter.An aliquot of the solution was filtered through a 0.22-micrometer membrane filter into a vial designed for high-performance liquid chromatography (HPLC).

Chromatographic Analysis and Chromatogram with working standard

The medicine underwent analysis via a High-Performance Liquid Chromatography (HPLC) machine produced by Shimadzu, a renowned manufacturer based in Japan. The High-Performance Liquid Chromatography (HPLC) system was comprised of a quaternary low-pressure gradient pump, a Photodiode array (PDA) detector equipped with a temperature-controlled autosampler, and a column oven. A chromatographic examination was conducted utilizing a Hypersil BDS C-18 column featuring a 250 4.6 mm inner diameter and a particle size of 5m. The isocratic elution method was chosen, utilizing a flow rate of 1.0 mL/min. The detection wavelength employed was set at 200 nanometers, the injection volume was 10 L, and the run duration lasted for 10 min. The mobile phase was prepared with great care and underwent a thorough process of ultrasonic degassing for 10 minbefore its utilization. The column underwent equilibration by allowing the mobile phase to flow through the system for a minimum period of 40 min. The column and High-Performance Liquid Chromatography (HPLC) equipment were maintained at a temperature of 35°C.

The formulation of the system suitability solution and the functioning standard was carried out in accordance with the previously indicated methodology. The aforementioned procedure was employed to acquire accurate measurements of Ibandronic acid, yielding values of 9.6 mg, 16.0 mg, 25.6 mg, 32.0 mg, 38.4 mg, and 48 mg. Subsequently, the aforementioned volumes were placed into individual volumetric flasks with a capacity of 50 mL each, resulting in concentrations of 30%, 50%, 80%, 100%, 120%, and 150% respectively. The injection of each solution (20 µL) was carried out using an auto-injector into the column, with a flow rate of 1.0 mL/min of mobile phase. The resulting chromatogram was then recorded and may be observed in Figure 2. The chromatogram displayed in Figure 2demonstrates a high level of quality, indicating its suitability for both qualitative and quantitative analysis of the Ibandronic Acid tablet dosage form. The retention period of the chromatogram was determined by analyzing multiple duplicates, yielding a value of 4.58±0.45 min. The construction of the calibration graph involved graphing the concentrations of the drug against the corresponding areas (µV) of the chromatogram, as depicted in Figure 3. The

Journal of Pharmaceutical Research and Innovation (JPRI)
Volume 4, Issue 2, July 2024
e-ISSN: 2583-0023

resulting graph exhibited a linear relationship. The regression equation for the curve was determined to be y = 547,075.57915x - 2494.75261, with a correlation coefficient (R) of 0.99979. The method was employed for the quantification of Ibandronic acid in tablet formulations.

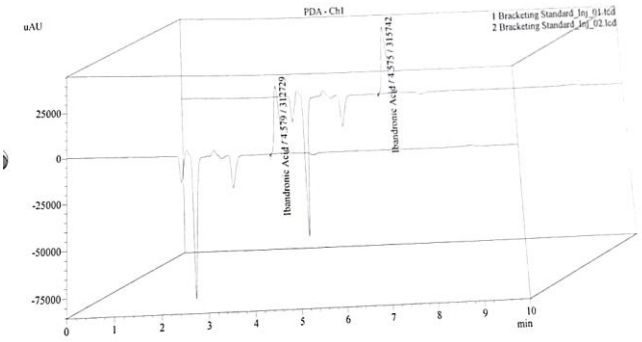

Figure 2. This study presents a representative chromatogram depicting the elution profile of Ibandronic Acid, with a retention time of 4.58±0.45 minutes, obtained under optimized experimental conditions (Calibration Plot).

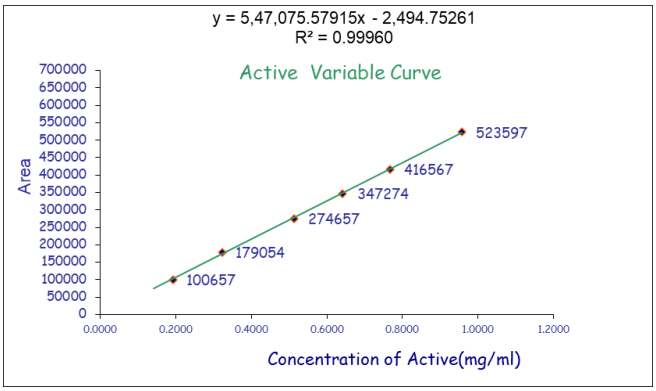

Figure 1. Calibration curve for Ibandronic acid only (Working standard).

Validation of the Proposed Method

The present study conducted a comprehensive investigation of various parameters including system appropriateness, linearity, specificity, accuracy, precision, range, and robustness. The objective was to validate the proposed High-Performance Liquid Chromatography (HPLC) method following the guidelines provided by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

System suitability: To ensure the validity of the technique, it was crucial to evaluate the suitability of the system. The achievement was attained through the computation of the Relative Standard Deviation (RSD) of the peak area derived from six replicates of the standard. The results and discussion section presents the findings and relevant analyses.

Linearity: The linearity of the analytical method was evaluated through the implementation of three regression studies. The first analysis focused on Ibandronic acid (API) in its pure form, without the presence of any excipients. The second analysis involved Ibandronic acid together with a constant concentration of excipients. Lastly, the third analysis encompassed Ibandronic acid with changing quantities of excipients. Two subsequent tests were done to examine the potential interaction between the standard drug and the excipients.

Limit of Quantification (LOQ): It is a parameter used in analytical chemistry to determine the lowest concentration of an analyte that can be reliably quantified with a certain method or instrument.

The determination of the Limit of Quantification (LOQ) was conducted by employing the standard deviation of the response and the slope technique. The method of linearity was performed within the specified concentration range for the reference sample solution. A graphical representation illustrating the correlation between concentration in milligrams per milliliter (X-axis) and peak response (Y-axis) was generated. The determination of the Limit of Quantification (LOQ) entailed the application of diverse statistical measures, such as the correlation coefficient, the regression line's slope, and the standard deviation of the regression line.

The Limit of Detection (LOD): The determination of the Limit of Detection (LOD) was performed by employing two methods: the standard deviation of response and the slope technique. The method of linearity was carried out within a specified concentration range in the reference sample solution. A graph was built to visually represent the relationship between concentration in milligrams per millilitre (mg/mL) on the X-axis and peak response on the Y-axis. The determination of the limit of detection (LOD) encompassed the application of several statistical metrics, such as the correlation coefficient, the regression line slope, and the regression line standard deviation.

Range: The concept of "range" refers to the difference between the highest and lowest values in a given set of data. The range of values is typically found using linear research. The determination of the analytical technique's range has been conducted by taking into account the upper and lower amounts of the analyte that are found within the sample. This conclusion guarantees that the technique demonstrates a suitable degree of precision, accuracy, and linearity.

Specificity: The assessment of the procedure's specificity was performed by precisely quantifying the analyte in the presence of additional constituents, such as excipients, that are expected to be found in a pharmaceutical formulation. The concentration of the analyte in the test sample was determined by the utilization of a regression equation, as depicted in Figure 3.

Placebo effect: The placebo effect is a phenomenon in which individuals experience a perceived improvement in their symptoms or condition after receiving an inactive substance or treatment, such as a sugar pill or sham procedure. The phenomenon of the placebo effect was investigated through the implementation of High-Performance Liquid Chromatography (HPLC) to analyze and

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compare the blank, placebo, and active solution.

Accuracy: The concept of accuracy is of paramount importance in academic research and analysis. The first stage of evaluating the drug in the developed product entailed ascertaining the precision of the method. To facilitate the execution of the experiment, a matrix including a placebo devoid of any active substance was constructed. The excipients utilized in the formulation of the Ibandronic Acid tablet, except the active pharmaceutical ingredient (API), were employed to replicate the Ibandronic Acid sample. The simulated sample was generated at three distinct concentrations, namely 80%, 100%, and 120% of the anticipated concentration. Subsequently, each concentration was subjected to analysis in triplicate through the use of High-Performance Liquid Chromatography (HPLC).

Precision: The assessment of a method's precision for validation was determined based on its repeatability, intermediate precision, and reproducibility. The assessment of repeatability precision was conducted through six iterations, each performed at a consistent amount of test sample concentration within a uniform solution. The determination of intermediate precision was conducted by many analysts, with different equipment, over multiple days within the confines of the same laboratory, as indicated by the HPLC measurements. The reproducibility of the HPLC method was confirmed by conducting additional tests employing analysts who had not previously participated in the repeatability and intermediate precision experiments. In this verification process, six determinations were performed consecutively in a separate laboratory.

Robustness: The concept of robustness refers to the ability of a system or process to withstand and adapt to various disturbances or uncertainties without significant loss. The robustness of the method was evaluated through the execution of a stability investigation on a sample solution containing Ibandronic Acid, which was maintained at ambient temperature (20-25ºC) for different periods (0hr, 24hr, and 48hr). Varying periods (0, 24, 48 hr) at ambient temperature (20°C-25ºC) to assess the stability of Ibandronic Acid. The relative standard deviation was calculated to assess the stability of the sample solution for a duration of 48 hr. The current investigation revealed a disparity in the assay for the sample solution, with values of 0.97 and 1.24 in relation to the ICH limit (not more than 2).[12]This finding suggests that the working sample solution remained stable for a minimum duration of 48 hr.

RESULTS

In this study, a High-Performance Liquid Chromatography (HPLC) approach was described for the estimation of Ibandronic Acid in tablet dosage form. It should be noted that the method for Ibandronic Acid is not included in the pharmacopoeia. Therefore, an in-house method was designed and validated for the accurate measurement of Ibandronic Acid. The user has provided a range of numbers, specifically 13-15. An optimum condition (Table 1) for the HPLC response with Ibandronic Acid was achieved through a series of trial and error experiments. The measured value of RT was determined to be 4.58±0.45 min, as shown in Fig 2. The HPLC approach was subsequently verified to assess the tablet dosage form of Ibandronic Acid.The assessment of linearity in the analytical procedure was conducted through three distinct investigations. Initially, a regression analysis was performed on Ibandronic Acid utilizing a range of concentrations. Additionally, a regression analysis was conducted on Ibandronic Acid, focusing on concentrations ranging from 20% to 120%. Throughout the investigation, the concentration of excipients remained constant. Finally, a regression analysis was conducted on Ibandronic Acid using varying doses of excipients. The linear regression equation, y = 547,075,57915x - 2494.75261 (R^2 = 0.999), was derived from the plot of the results (Fig 3), which represents the relationship between the concentration of Ibandronic Acid and the corresponding values of y. The observed response exhibited a linear dependence on the concentration of Ibandronic Acid. The regression line's linearity is further supported by the correlation coefficient (R^2 = 0.999). It is crucial to note that the HPLC method provided for the determination of Ibandronic Acid demonstrated linearity within the range of 0.192-0.958 mg/mL, as depicted in Fig 3. The determined values for the limit of detection (LOD) and Limit of Quantification (LOQ) were 0.021026880 and 0.06371782, respectively.

Table 1. Optimized chromatographic conditions Ibandronic Acid

Test	Condition
Mobile Phase	Acetonitrile: Buffer (40:60V/V), Isocratic
Diluent	Mobile phase
Column	C18 (Dimension : 25 cm × 4.6 mm), 5 µm
Wavelength	200 nm
Column oven	35
Flow rate	1.0 mL/min
Detector	PDA
Injection volume	10 µL
Run time	10

To assess the method's specificity, the monitoring process involved the examination of a standard solution of Ibandronic Acid, as well as its tablet form, a blank sample, and placebo materials (consisting of excipients). The analysis of standard and tablets in High-Performance Liquid Chromatography (HPLC) revealed a prominent peak with a retention time of 4.58±0.45 min. This peak was observed when the standard and tablets were analyzed individually. However, no peak was observed at this retention time for the blank and placebo samples.

The assessment of accuracy involved conducting nine determinations at three distinct concentration levels that spanned the intended range of analysis, which was 0.322-

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0.958mg/mL. Three repetitions of each concentration were used (Table 2). Based on these findings, it was determined that the recovery values for estimating fell within the recommended range of 98% to 102% as specified by the ICH percentage recovery guideline.[12] Hence, it was demonstrated that the proposed methodology exhibited a high degree of accuracy in the analysis of the pharmaceutical compound known as Ibandronic Acid.

Table 2. Percent recovery of Ibandronic Acid from simulated tablet contents

TEST DATA SHEET-01					
Ibandronic Acid:					
Spiked level	**Sample No.**	**Amount added (mg)**	**Amount found (mg)**	**Recovery (%)**	**Average recovery (%)**
60%	1	16.0	15.76	98.5	99.0
	2	15.9	15.96	100.4	
	3	16.1	15.79	98.1	
90%	1	31.9	31.71	99.4	99.4
	2	32.0	32.03	100.1	
	3	32.0	31.57	98.7	
120%	1	47.9	48.51	101.3	101.1
	2	48.1	48.46	100.7	
	3	48.0	48.60	101.2	

The repeatability precision was assessed using six separate measurements of a constant test concentration (0.642mg/mL) of a uniform solution (Table 3) containing Ibandronic Acid. The RSD values were computed based on the determinations as mentioned earlier, and afterward, the resultant RSD value was assessed to determine if it fell within the prescribed limit of not more than 2% as specified in the ICH guideline [16]. In the current study, the Relative Standard Deviation (RSD) was determined to be 0.689% (Table 3), which falls within the acceptable limit (not more than 2%) specified by the International Council for Harmonisation (ICH) guideline.[12] Therefore, the reproducibility of the current analytical method for Ibandronic Acid was determined. Furthermore, it was discovered that the intermediate accuracy, repeatability, and system applicability requirements aligned with the International Council for Harmonisation (ICH) standard.[12] The provided sample solution was subjected tovarying periods (0, 24, 48 hr) at ambient temperature (20°C-25ºC) to assess the stability of Ibandronic Acid. The relative standard deviation was calculated to assess the stability of the sample solution for the duration of 48 hr. The current investigation revealed a disparity in the assay for the sample solution, with values of 0.97 and 1.24 in relation to the ICH limit (not more than 2).[12] This finding suggests that the working sample solution remained stable for a minimum duration of 48 hr.

Table 3. Relative standard deviation of six determinations of Ibandronic acid contents in simulated tablet amount.

Sample	**Concentration (mg/mL)**	**Peak Area (µV)**	**% of Ibandronic Acid**	**RSD (%)**	**ICH limit of RSD (%)**	**Remarks**
01	0.64	345597	98.81	0.689	NMT 2.0	Repeatability of Ibandronic acid measurements are compiled
02	0.64	350868	100.63			
03	0.64	347175	99.26			
04	0.64	349716	99.98			
05	0.64	352296	100.41			
06	0.64	348932	99.76			

DISCUSSION

Ibandronic Acid is classified as a third-generation amino-bisphosphonate, possessing properties that inhibit bone resorption and hypercalcemia. The product is now being developed and distributed on a global scale. However, Ibandronic Acid has not yet been included in any recognized pharmacopoeia, thus lacking an established procedure for quality control of Ibandronic Acid. Efforts are being made by researchers to identify an appropriate technique for the quantification of Ibandronic Acid. This paper introduces a novel, straightforward, and economical approach. The findings of this study demonstrate that the current approach successfully detects Ibandronic Acid and effectively separates it from its excipients in a quantitative manner, as shown in Table 4. The percentage of Ibandronic Acid recovered in the absence and presence of excipients was determined to be within the acceptable range specified by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline[12] (refer to Table 4). This indicates that the developed method is capable of selectively quantifying Ibandronic Acid.

e-ISSN: 2583-0023

Table 4. Results of specificity

TEST DATA SHEET-01				
Observation (Peak Purity)				
Sample Name	Resolution	Peak purity Index	Single Point Threshold	Remarks
API Solution	0.00	0.999992	0.999310	Impurity Not Detected
Sample solution	0.00	0.999988	0.999240	Impurity Not Detected
	Observation (No interfering peak)			
Blank solution	No interfering peak			
Placebo solution	No interfering peak			

CONCLUSION

An HPLC method was devised and subsequently validated to analyzeIbandronic Acid in a prepared tablet. The method that was devised underwent validation for multiple characteristics by the principles set forth by the International Council for Harmonisation (ICH). These factors include selectivity, precision, accuracy, linearity, limit of detection, and limit of quantification. The results met the criterion for acceptance. Hence, it can be asserted that the proposed methodology is straightforward, precise, cost-effective, secure, and suitable for the regular assessment of Ibandronic acid in tablet formulations. The HPLC approach proposed in this study has the potential to offer robust scientific data for future pharmacological investigations conducted both *in vitro* and *in vivo.*

Acknowledgments

The authors wish to thank the Department of Pharmacy, Jahangirnagar University, Bangladesh.

Conflict of interest

The authors have no conflicts of interest to declare.

Abbreviations

BDS- Base Deactivated Silica; EDTA-Ethylenediamine tetraacetic acid; TEA- Triethylamine; GTP- Guanosine triphosphate; Rap1- Ras-proximate-1; API- Active Pharmaceutical Ingredient

Ethics approval and consent to participate

Not applicable

Funding

This is a self-funded research work.

PATIENT CONSENT

None.

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