Analytical Method Development And Validation For The Quantification Of Evocalcet In Tablet Formulation

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ABSTRACT

Evocalcet is a new oral calcimimetics drug that works in a manner similar to Cinacalcet hydrochloride in order to decrease the release of parathyroid hormone by acting on the calcium receptors on parathyroid gland cells. On March 23, 2018, the Japanese Ministry of Health, Labor, and Welfare (MHLW) authorized the manufacture and sale of Evocalcet (trade name ORKEDIA) for the treatment of secondary hyperparathyroidism in dialysis patients.

It was necessary to create an analytical method to determine the content of Evocalcet in its bulk formulation because it is a novel and non-pharmacopoeial medication. A standard procedure by HPLC technique for the determination of Evocalcet in its bulk formulation was developed and validated as per ICH guidelines, Q2 (R1). A simple HPLC method was developed. A mixture of phosphate buffer pH 3.0 ± 0.05 and acetonitrile in the ration 50:50 v/v was used as mobile phase. The sample was separated using Octadecyl silane (C18, 25 cm x 4.6 mm, 5 µm) bonded to porous silica HPLC column maintained 30 degree column oven temperature. The sample volume injected was 20 µl and measured at UV detector 255 nm. Retention time was about 3.5 minutes at 1.0 ml per minute flow rate. The developed method is specific, linear (R^2 > 0.9997), precise and accurate and robust. Limit of detection and quantitation were found to be 0.1814 µg/ml and 0.5496 µg/ml respectively.

Keywords: Evocalcet, calcimimetics, Hyperparathyroidism, Analytical method, Dosage forms, ICH guidelines, HPLC.

INTRODUCTION

Background:

In the formulation of any new drug molecule, analysis is an important component. There are two types of analysis, one qualitative analysis and other quantitative analysis. Identification of components or analyte of mixture or sample is qualitative analysis whereas, in quantitative analysis, there is determination of amount of components or analyte of mixture or sample. There is no need for validation for compendial dosage forms because the procedure will already be established. However, a suitable and validated method must be provided for the analysis of the drug(s) in the bulk and tablet dosage forms for non-compendial products. To determine the amount of drug content in a dosage form when an appropriate method is not available, a straightforward, sensitive, accurate, precise, and repeatable approach must be developed.

When it comes to the research, development, and production of new pharmaceutical products, the creation of appropriate analytical methods and their validation are crucial. Good manufacturing practices guidelines states different elements need to be fulfilled by the pharmaceutical industries. Among them validation is one of the key elements to fulfill the requirement of current good manufacturing practices (cGMP) and good laboratory practices (GLP). GMP is the quality aspects for both production and quality control. It ensures that the processes necessary for production and laboratory testing are clearly defined, validated, reviewed, and documented. The staff, location, and supplies are appropriate for the production. GMP has legal components, covering responsibilities for, manufacturing, for testing, for distribution and addressing to product defects and market complaints. Use of suitable and validated method for testing and ensuring the quality and the content of the developed dosage products in the pharmaceuticals industry is the integral part of the compliance. It is essential to create novel analytical techniques, particularly for those products that are not included in pharmacopoeia. During the process of drug discovery, release to market, and development, which results in a marketing approval, the creation of sound analytical method(s) is of the utmost importance. It is now important to validate the new analytical technique after development.

Analytical analysis of bulk drug materials, intermediates, drug products, drug formulations, contaminants and degradation products, the pharmaceuticals and their metabolites, is crucial in the field of pharmaceutical research. Analytical assay techniques have been part of compendial monographs from the inception of formal pharmaceutical analysis, with the goal of defining the quality of bulk drug products by establishing limits for the quantity of their active ingredient. There are different techniques of analytical methods. Titrimetry, spectrometry, chromatography, and capillary electrophoresis are some of the test methods used in contemporary monographs.

The process of method development demonstrates that an analytical method is appropriate for application. Information on numerous phases and parameters, such as accuracy, precision, linearity, limit of detection, limit of quantification, specificity, range, and robustness, are provided through the validation of analytical methods. Validation should be carried out in accordance with regulatory standards like the ICH standards. To measure the drug

ingredients, various instrumental approaches are available. Among them, the HPLC method is chosen because it is quite exact.

The analytical system itself must be properly built, maintained, calibrated, and verified before beginning the work of methods validation. The test method, supporting documentation, and acceptance criteria must all be specified for each of the validation characteristics in this document. Specific values must be based on references from the ICH, US FDA, USP, and relevant literature.

Analytical Method Validation:

The process of confirming the analytical testing strategy utilized for a particular test which is reasonable for its expected use is referred to the method validation. The method used to conduct the analysis is referred to as the analytical technique. The procedures required to carry out each analytical test should be thoroughly explained. This may involve—but is not limited to—the preparation of the sample, the reference standard, and the reagents; usage of the equipment; creation of the calibration curve; application of the calculation formulae; etc. [1]

In the pharmaceutical industry, analytical method development gives important information on the potency of a drug, the drug's bioavailability, the drug's stability and also its effects.

Analytical methods should be validated unless the method employed is included in the relevant pharmacopoeia or other recognized standard reference. [2]

Validation is a concept developed in the United States in 1978. The concept of validation has been broaden over the years to achieve many activities like from analytical methods used to control quality of drug substances and drug products up to computerized systems for clinical trials, process control or labelling. Validation is best seen as a necessary and prime part of cGMP. [3]

Analytical method validation is defined as the process of establishing documented evidence which provides a high degree of assurance that a specific process such as analytical test method, will consistently produce a product supported by assay results meeting its predetermined specification and quality attributes (i.e. accuracy, precision etc.) [4] The process of doing multiple evaluations is known as method validation, and it is used to determine whether an analysis methodology demonstrates the expected explanation in an appropriate manner and is set up to provide accurate, legal measurements.

HPLC is one of the most widely used analytical procedures out of all the different analytical techniques. It has a number of benefits over traditional chromatographic methods. HPLC makes precise and quick identification and determination of a variety of natural and synthetic substances possible because of its ease of use and effectiveness. It has a wide range of applications in terms of quantitative and qualitative estimation in many various disciplines, including pharmaceutical, environmental, forensic, food and flavor, and therapeutic. HPLC's only drawbacks are its high cost and length of time.

Before beginning validation investigations, the methodology and goal of the analytical techniques should be properly stated and understood. This understanding is obtained from scientifically-based method development and optimization studies. Validation data must be generated under a protocol approved by the sponsor following current good manufacturing practices with the description of methodology of each validation characteristic and predetermined and justified acceptance criteria, using qualified instrumentation. Protocols for

both drug substance and product analytes or mixture of analytes in respective matrices should be developed and executed.

A suitable standard technique should be established for producing reliable analytical results from the competent laboratory. It can only happen if the analytical procedure is validated. Validation of analytical methods is a crucial prerequisite for carrying out the chemical evaluation. Method validation is a process of carrying out several tests to ensure that an analytical test system is appropriate for its intended uses and is able to produce documentation supporting its appropriateness. The validation test should include products excipients that could affect test results in order to accurately evaluate method parameters. Validation of analytical methods is therefore product-specific.

The common method for the development and validation of the analytical method is completed by the following process.

- Planning the appropriate method that must be developed.
- The information related to the work should be collected.

- Qualitative and quantitative analytical methods that can be performed in the lab should be developed.
- The procedure for testing the sample should be created.

A fundamental requirement to play out with the chemical assessment is validation that deals with the analytical procedure. Method validation is a process that involves carrying out several evaluations to see if a method of analysis demonstrates the proper expected explanation and is equipped to provide legitimate, legal measurements. According to the rules and recommendations, the approach should offer useful information that guarantees the product's quality. Such results are determined using many tests on the material. A thoroughly tested procedure should meet each requirement. Testing of the excipients and a focus on standard testing conditions should be part of the validation of the analytical method. These circumstances demonstrate that the analytical method's validation is product-specific.

The current good manufacturing practices suggest that quality should be built into the product, and testing alone cannot be relied on to ensure product quality. Pharmaceutical products need to maintain high quality in order to provide safe and effective usage. From the analytical point of view, analytical methods used to test these products should have quality attributes built into them. Validation ensures these quality attributes are built into the method. Validation of analytical methods is an essential but time consuming activity for most analytical laboratories. But it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end. The analytical methods need to be validated or revalidated before initial use of the method in routine analysis, when transferred from one laboratory to another, whenever the conditions or method parameters for which the method has been validated change and change is outside the original scope of the method. One of the most acceptable testing method is by chromatographic technique. Chromatography is defined as a procedure by which solutes are separated by dynamic differential migration process in a system consisting of two or more mobile phases, one of which moves continuously in a given direction and in which the individual substances exhibit different mobilities by reason of differences in absorption, partition, solubility, vapor pressure, molecular size or ionic charge density. When mobile phase used is liquid the type of chromatography is called liquid

chromatography. High performance liquid chromatography (HPLC) is a modern form of liquid chromatography that uses small particle columns through which the mobile phase is pumped at high pressure. The separation of components depends on the extent of interaction between the solute component and the stationary phase. The component that has lowest affinity for the stationary phase will elute first. HPLC is becoming a preferred method of analysis among various analytical methods for pharmaceuticals. HPLC methods provide rapid analysis, greater sensitivity, high resolution, and easy sample recovery, precise and reproducible results.

Validation of analytical procedure is the legal requirement and is mandatory for non compendial products to perform. ICH guidelines [Q2 (R1)] have set the guidelines for the validation of the analytical method.

The validation of the analytical methods for the product must be performed for the following test:

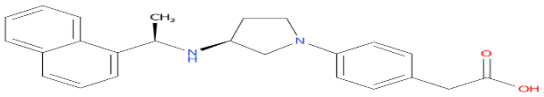
i. Identification
ii. Test for impurities
iii. Assay
iv. Dissolution

1.1. Product for which the method to be validated:

1.2.

Evocalcet Tablets

Molecular structure:



Molecular formula: $C_{24}H_{26}N_2O_2$

Molecular weight: 374.5 g/mol.

Chemical name: {4-[(3S)-3-{[(1R)-1-(Naphthalen-1-yl) ethyl] amino} pyrrolidin-1-yl] phenyl} acetic acid

Evocalcet is a newly approved calcimimetic drug. It is similar to Cinacalcet. Evocalcet has been developed to improve defects of Cinacalcet for management of SHPT. [5] A daily dose ranging from 1 to 8 mg of Evocalcet is administered orally by combining 1 and 2 mg tablets, and the dose can be increased up to 12 mg/day. [6] Manufacturing and marketing authorization for Evocalcet (trade name ORKEDIA) was granted by from Japan's Ministry of Health, Labor and Welfare (MHLW) on March 23, 2018 for treatment of secondary hyperparathyroidism in patients on dialysis. [7]

Evocalcet is a new oral calcimimetics drug that suppresses parathyroid hormone secretion by acting on the calcium receptors on parathyroid gland cells in a similar way to that of Cinacalcet hydrochloride. Evocalcet is another new calcimimetic agent for oral use developed to address the issues reported with cinacalcet use. [8]

Evocalcet being a new molecule, compendial method for the analysis of this drug is not available. So development and validation of a suitable method is essential for the routine estimation of Evocalcet in bulk and tablet dosage forms. It is crucial to have a precise, focused, trustworthy, and affordable approach for detecting Evocalcet in pharmaceutical dosage forms and bulk drugs. The development of a High Performance Liquid Chromatographic (HPLC) method had been made in an effort to measure the presence of Evocalcet in pharmaceutical formulations.

Physical properties of Evocalcet: The material, Evocalcet received from the vendor (Kaifeng Pharmaceutical (Group) Company Limited) is off-white powder. It is soluble in DMF, Slightly soluble in acetonitrile and ethanol, very slightly soluble in water.

MATERIALS AND INSTRUMENTS

Materials:

Evocalcet tablets (1 mg) under the R&D phase in QbD Pharmaceuticals Pvt. Ltd. was taken as a sample. EVOCALCETERUI 1 mg was taken as a reference product. Reagents and chemicals used were of HPLC grade and made of Qualigens/Merck. Evocalcet working standard was obtained from the supplier of API (Kaifeng Pharmaceutical Company Limited).

Instruments:

A Shimadzu (Japan) HPLC system (i-Series, LC-2050 C 3D) with PDA deterctor, and Agilent Technology (1260 Infinity II) HPLC system (German made) auto-sampler with Ultraviolet detector were used. The drug analysis data were acquired and processed using LC solution (Shimadzu, Japan), and Open Lab (Agilent) software.

METHODOLOGY

Method: **High Performance Liquid Chromatography**

Chromatographic conditions:

The sample was scanned from 400 nm to 200 nm and found the maximum absorption at 255 nm. Hence the detection wavelength was selected as 255 nm. The chromatograms were obtained using LC solution and Open lab system. The chromatographic condition were optimized. The peaks were detected using Octadecylsilane, 250 mm x 4.6 mm, 5 micron column with a mobile phase of buffer and acetonitrile. The samples were injected with a 20 microliter at a flow rate of 1.0 ml/min, and retention time observed at about 3.5 minutes duration, and at 30^0C temperature.

Preparation of mobile phase:

Mobile phase constitute 50 volumes of buffer solution prepared by dissolving 7.0 g of potassium dihydrogen orthophosphate in 1000 ml of water and adjusted pH of the solution to 3.0 ± 0.05 with dilute orthophosphoric acid or sodium hydroxide solution and 50 volumes of acetonitrile. The mobile phase was filtered through 0.45 µm membrane filter and degassed in a sonicator for about 3 minutes.

Diluent: 30 % Acetonitrile

Preparation of standard solution: (10 ppm) (100 % of target concentration)

About 20 mg of Evocalcet standard was weighed and transferred to 100 ml volumetric flask. About 70 % of diluent was added and dissolved with the aid of sonication for 15 minutes.

The solution was allowed to cool and volume was made up to the mark. 1 ml of this solution was pipetted out and diluted to 20 with the same diluent, mixed and filtered through 0.2 µm Nylon membrane filter.

Preparation of sample solution:

20 tablets were accurately weighed and the average weight was calculated. The tablets were grinded to a fine powder with the help of mortar and pestle. Then, the amount of powder containing 1 mg of Evocalcet was weighed and transferred to 100 ml volumetric flask. About 70% of the diluent was added and dissolved with the aid of sonication for about 15 minutes with the aid of sonication. The sample was allowed to cool and diluted to the volume with the same diluents to make the final concentration of working sample equivalent to 100 % of the target concentration.

Development and validation of HPLC method:

The study was conducted to obtain a convenient method for the determination of Evocalcet in tablet dosage formulations by High Performance Liquid Chromatographic Technique. The method was validated for the different parameters as per ICH Q2 (R1) guidelines.

RESULTS AND DISCUSSION

High performance liquid chromatographic method was developed for the determination of Evocalcet in the bulk formulation and the method was validated as per ICH guidelines. The results obtained are discussed hereunder.

Specificity:

Specificity test was carried out by preparing the formulation without active product ingredient and checking the positive or negative interference due to placebo on results of analytical method.

Specificity was determined by injecting 20 µl separately, the diluents as blank solution, placebo solution, standard solution and test sample solution.

Figure 1: Chromatogram of diluent as blank solution

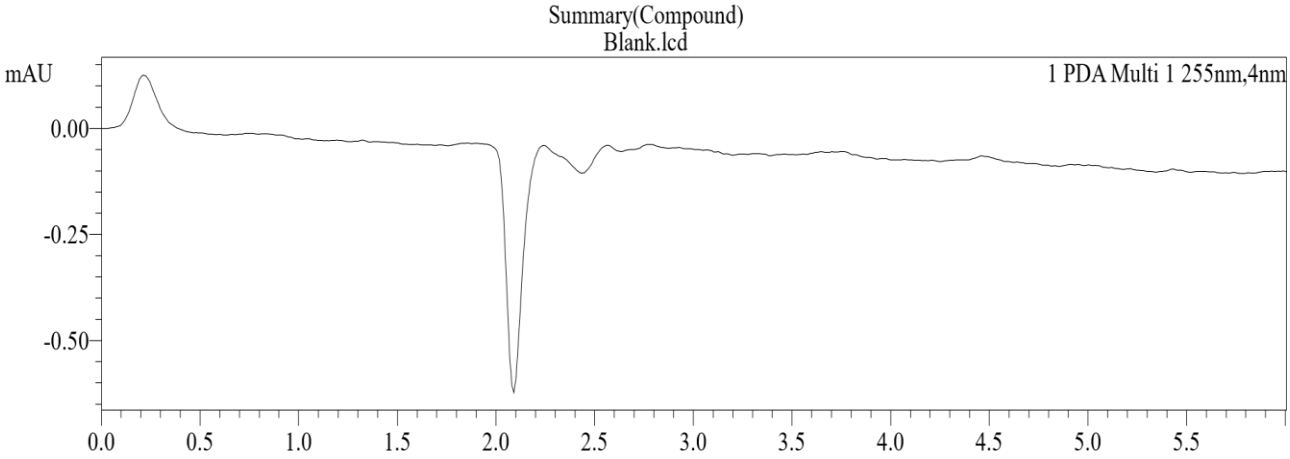


Figure 2: Chromatogram of placebo solution

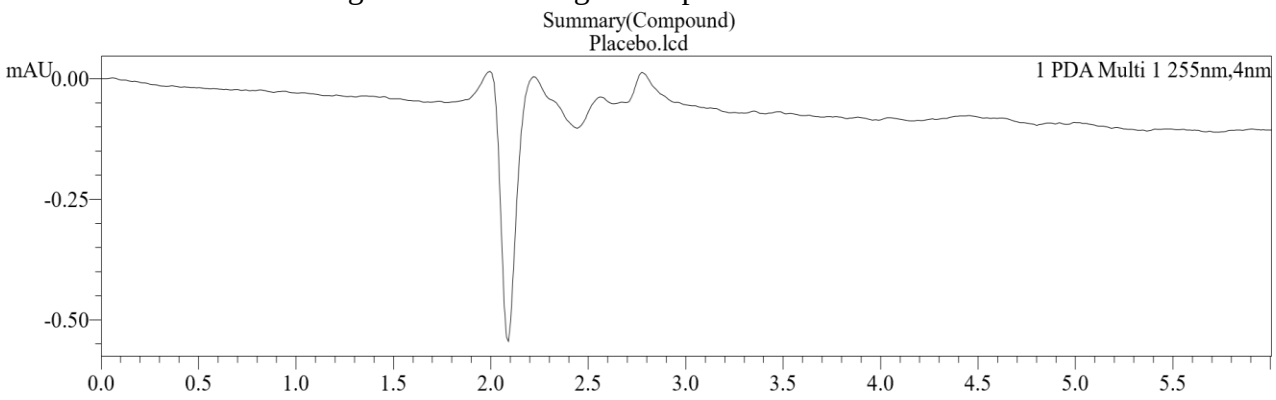


Figure 3: Chromatogram of Sample (Evocalcet Tablet)

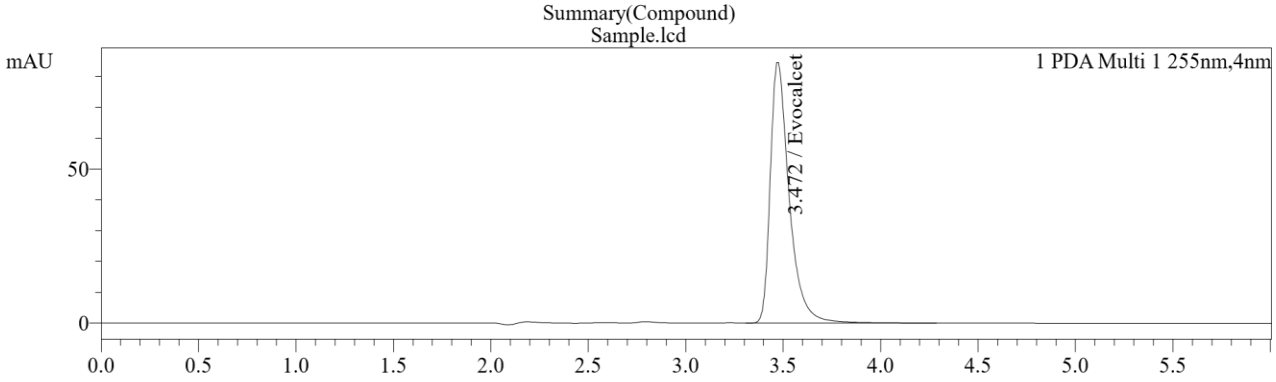


Figure 4: Chromatogram of Evocalcet standard

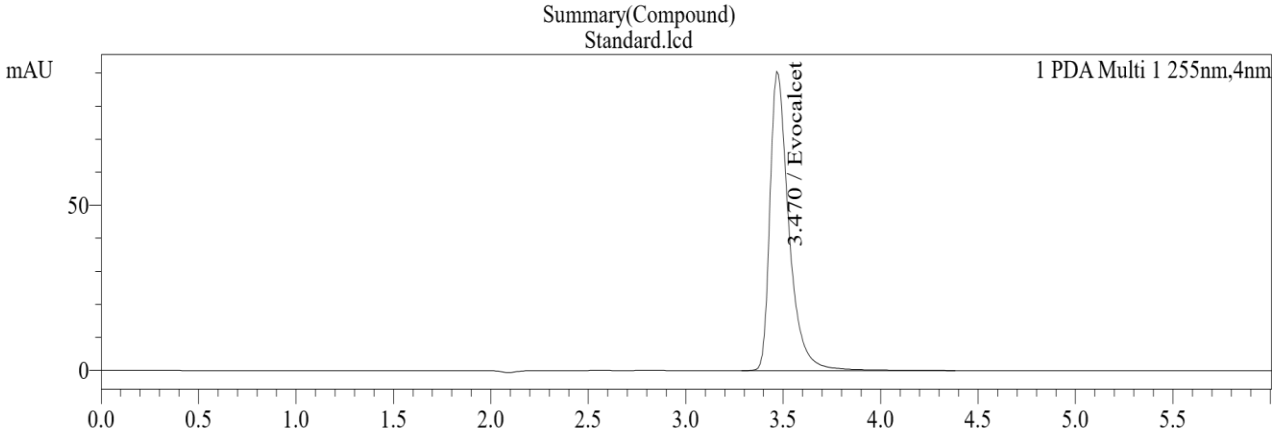


Table 2: Specificity (data)

Title	Sample Name	Sample ID	Ret. Time	Area	Number of theoretical Plate (USP)	Tailing Factor
Blank	Evocalcet	Specificity	0.000	0	--	--
Placebo	Evocalcet	Specificity	0.000	0	--	--
Sample	Evocalcet	Specificity	3.472	575469	5430	1.536
Standard	Evocalcet	Specificity	3.470	613146	5410	1.534
Average			3.471	594307	5420	1.535
%RSD			0.035	4.483	0.269	0.071
Maximum			3.472	613146	5430	1.536
Minimum			3.470	575469	5410	1.534
Standard Deviation			0.001	26641	15	0.001

There is no interference of blank and placebo. The result is summarized in Table 3.

Table 3: Specificity (summary)

Sample	**Peak area**	**Interference (%)**
Blank	-	0.00 %
Placebo	-	0.00 %
Evocalcet standard	575469	NA
Evocalcet sample	613146	NA

7.1. Linearity:

20 µl of prepared 5 linear concentration samples were injected and calculated the slope, intercept and regression coefficient by linear regression and relative standard deviation.

Table No. 4: Linearity data

Sample	**Conc. (µg/ml)**	**Peak Area**	**Peak Area correction**	**Response factor (RF)**	**% response factor compared to target level response factor.**
1.	6	371392.00	362333.66	60388.94	100.95
2.	8	496208.00	484105.37	60513.17	101.16
3.	10	613165.00	598209.76	59820.98	100.00
4.	12	740737.00	722670.24	60222.52	100.67
5.	14	850417.00	829675.12	59262.51	99.07
% y-intercept compared to target concentration response.					**0.58 %**

Calculation:

Correction factor= $\frac{Nominal\ weight}{Actual\ weight}$

Peak area correction= *Peak area x Correction factor*

Response factor=$\frac{Peak\ area}{Concentration}$

% response factor compared to target level response factor= $\frac{RF}{RF\ of\ target\ conc.(10\ ppm)}$

% y-intercept compared to target concentration response= $\frac{y-intercept}{PA\ of\ target\ conc.(10\ ppm)}$

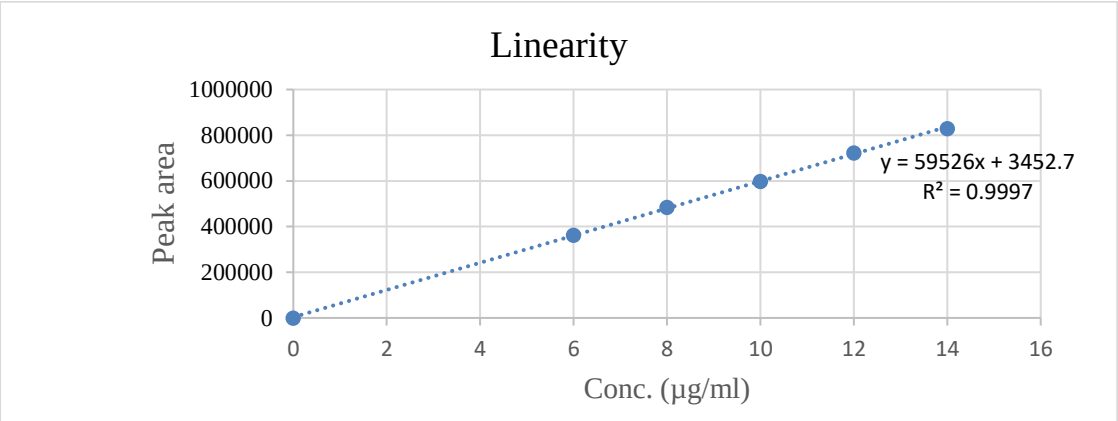


Figure 5: Graphical representation of Linearity

The linearity equations obtained was y= 59526x + 3452.7. The Correlation coefficient (R2) was found to be 0.9997. The Y-intercept compared to target concentration response was found ≤ 2 %.

7.2. Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or as an accepted reference value and the value measured. (ICH Q2 (R1)) This is sometimes termed trueness.

Table No. 5: Accuracy using drug products (% Recovery)

Average peak area of standard solution: 594062				
% Level	Amount of API added (mg equivalent of Evocalcet)	Average PA of sample	Accuracy	Recovery %
80 %	0.8	487779	81.2	101.5
	0.8	476996	81.3	101.6
	0.8	470984	80.8	101.0
100 %	1	572398	98.1	98.1
	1	572811	98.9	98.9
	1	573413	98.6	98.6
120 %	1.2	699228	120.4	100.3
	1.2	697043	119.9	99.9
	1.2	697889	120.8	100.7
			Average:	100.1

Table No. 6: Accuracy using drug substances (% Recovery)

Average peak area of standard solution: 615090				
% Level	Amount of API added (mg)	Average PA of sample	Accuracy	Recovery %
80 %	16	473987	78.7	98.3
	16	495103	80.2	100.2
	16	479778	79.1	98.9
100 %	20	602575	99.5	99.5
	20	627643	100.2	100.2
	20	606802	99.7	99.7
120 %	24	717403	118.6	98.8
	24	732839	120.6	100.5
	24	712238	118.2	98.5
			Average:	99.4

The recovery for the test is within 98.0 % to 102.0 % from both methods, using drug substances as well as drug product.

7.3. Precision:

7.4.1 Repeatability:

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Repeatability of system and method was performed. For instrument precision determinations of five replicates of reference standard were made. For the method nine determinations covering specified range of 3 concentration and 3 replicates were made.

Table 7: System precision

Injections	Peak Area observed (HPLC I)	Peak Area observed (HPLC II)
1	593421	587.05
2	593948	586.98
3	597049	587.98
4	593205	588.19
5	592689	587.16
Average	594062	587.47
Stdev	1729.6	0.6
RSD %	0.3 %	0.1

7.4.2 Intermediate Precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc. It was performed using two different HPLC make in two different days.

Table No. 8: Method and intermediate precision

Level %	Day I: HPLC I			Day II: HPLC II		
	Average Peak Area of sample	Recovery	Recovery %	Average Peak Area of sample	Recovery	Recovery %
80 %	487401	81.1	101.4	469.46	79.74	99.6
	476325	81.0	101.3	448.50	78.9	98.6
	468185	80.4	100.5	466.00	79.91	99.9
100 %	572898	98.86	98.9	588.61	101.36	101.4
	573646	99.08	99.1	595.46	101.36	101.4
	574398	98.83	98.8	577.48	99.44	99.4
120 %	700456	120.62	100.5	693.73	118.82	99.0
	696045	119.76	99.8	687.69	119.03	99.2
	696911	120.68	100.6	690.76	118.89	99.1
	Average		100.1	Average		99.7
	Stdev		0.99	Stdev		1.01
	RSD %		0.99 %	RSD %		1.02 %

The relative standard deviation (RSD %) was found to be less than 2.0 %

7.4. Limit of detection and Limit of quantitation:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Detection limit was determined by using standard deviation of the response and the slope as:

DL= $\frac{3.3\sigma}{S}$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Quantitation limit was determined using standard deviation of the response and the slope as:

QL= $\frac{10\,\sigma}{S}$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

Detection limit and quantitation limit was found 0.1814 µg/ml and 0.5496 µg/ml respectively.

7.5. Robustness:

This was evaluated by small, deliberate change in chromatographic conditions like, change in column oven temperature, change in mobile phase concentration, change in flow rate, and change in wavelength.

It was observed that there were no deliberate changes in the chromatogram and the recovery which demonstrate that the method developed is robust. The deliberately changed parameters and the results obtained are summarized in the Table No. 9.

Table No. 9: Results of robustness study

Parameters	Level	Retention time	Number of theoretical plates	Peak area	Recovery %
Temperature (^{0} C)	25	3.422	4655	575342	99.2
	35	3.495	5111	572624	98.4
Mobile phase concentration (Buffer: ACN) (v/v)	55:45	4.305	5278	568951	98.7
	45:55	3.016	4297	576429	98.6
Flow rate (ml/min)	1.5	2.315	3226	385309	99.0
Wavelength (nm)	253	3.449	4793	552560	99.0
	257	3.449	4799	585156	99.0
Change in column	150 mm	2.192	2653	607304	99.5

7.6. System suitability:

System suitability tests was performed on HPLC systems to determine the accuracy and precision of the system by injecting five injections of a solution containing analyte at 100% of test concentration. System suitability test was performed as per USP guidelines on the chromatograms. The studied parameters to verify the optimum conditions and the obtained results are summarized in Table No.10.

Table No. 10: System suitability

Parameters	Limits	Observed results
Tailing factor	Not more than 2.0	1.502
Theoretical plates	Not less than 2000	4883
Relative standard deviation	Not more than 2.0 %	0.291

7.7. Solution stability:

Stability of the sample solution was performed by analysing test solutions stored in auto sampler and stored at 2 - 8 °C in refrigerator (at least 24 hours) with the freshly prepared standard solutions.

Table No. 11: Solution stability

Solution storage conditions	Peak area	Recovery %
At auto-sampler	604867	100.6
At 2-8^0 C	604184	100.5
At room temperature	607430	101.0

The recovery % of the assay sample stored at different storing conditions are within 98-102 %.

7.8. Filter compatibility:

The objective of this study was to provide guidance on filter selection during method development and validation with a special emphasis on analyte binding to syringe filters. Different syringe filters like 0.22 µm nylon filter, 0.45 µm nylon filter, 0.22 µm PTFE filter and 0.45 µm PTFE filters were evaluated.

Table No. 12: Filter compatibility

Membrane Filter	Level	Peak area	Recovery %
Nylon	0.22 µm	535645	101.0
	0.45 µm	574090	98.9
PTFE	0.22 µm	570455	101.9
	0.45 µm	574224	100.4

CONCLUSION

Evocalcet is a new calcimimetic drug. Testing method for the determination of its content in the bulk formulation is not available. It is non compendial product. Hence, the analytical method by High performance liquid chromatographic technique was developed and validated as per ICH guidelines. The method is precise, accurate, simple and less time consuming.

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