***Original Research Article***

# A Novel Ultra Performance Liquid Chromatographic Method for the Estimation of Lercanidipine in Bulk and Tablet Dosage Form

**Short title:** Novel Lercanidipine estimation method using UPLC


**Abstract**

In this present study an accurate reverse phase ultra-performance liquid chromatography (RP-UPLC) method has been developed, validated and applied to stability indicating studies to determine Lercanidipine HCL in bulk and marketed dosage form. Optimized chromatographic conditions were achieved by using Waters Acquity BEH C18 (2.1 x 50mm, 1.7m) UPLC column. Empower 2 is a software, dihydrogen Orthophosphate Buffer : Methanol (40 : 60) as eluent at flow rate 0.3 ml/min. PDA detection was performed at 254nm. The developed method was validated and stability study was conducted as per ICH guidelines. The retention time was found at 0.503 min. The method shows linearity over a range of 1 µg/ml to 60 µg /ml with the obtained correlation coefficient is 0.999. The LOD and LOQ values were found 0.025 and 0.05 µg /ml. The acidic and peroxide stressed study shows more degradation of 6.23% and 3.03%. The present developed method was found stability indicating, reliable, validated method was applied for the routine analysis of lercanidipine in bulk drug and the pharmaceutical formulations.



**Key Words:** Lercanidipine, UPLC, method development, ICH guidelines, stability study


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## 1. INTRODUCTION

Lercanidipine, a dihydropyridine calcium-channel blocker, is used alone or with an angiotensin-converting enzyme inhibitor, to treat hypertension, chronic stable angina pectoris, and Prinzmetal's variant angina[1]. Chemically lercanidipine is (RS)-2[(3,3-Diphenylpropyl)(methyl)amino]-1,1-dimethylethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate[2], the chemical structure was shown in Figure 1. Lercanidipine helps the heart perform more efficiently by lowering blood pressure. It does not significantly affect on calcium channels in the atrioventricular node, and hence does not decrease heart rate, in contrast to the non-dihydropyridine calcium channel blockers verapamil and diltiazem[3,4]. The intestine absorbs lercanidipine slowly but completely. It has a total bioavailability of 10%, or up to 40% if administered after a fatty meal5, due to its extensive first-pass impact. The maximum blood plasma levels are obtained after 1.5 to 3 hours. The chemical is rapidly absorbed by the tissues and binds to lipid membranes, forming a depot. The circulating fraction is nearly fully attached to plasma proteins (>98%).[6,7]. It is entirely digested in the liver and has a half-life of 8 to 10 hours in the body. The antihypertensive impact lasts at least 24 hours due to the depot effect. The urine excretes 50% of the substance.[8,9]. To uncover the faults in the stated approaches, an exhaustive literature study on lercanidipine estimate methods was undertaken as a significant aspect of the current research work. The lercanidipine was found to be effective whether used alone or in combination[10,11] with other antihypertensive drugs. There was relatively little published work on lercanidipine estimation using HPLC, and each approach had its own set of drawbacks, including inadequate chromatograms and ambiguity in various areas. One method[12], for example, had a linearity of 20-80 g/mL, which is considered very high and calls into doubt the sensitivity. Another method[13] revealed an excessively lengthy retention time (9 minutes). The pH of the mobile phase was kept at 3 in another method[14]. This acidic pH can shorten the analytical column's shelf life. In another technique,[15] a large amount of acetonitrile (90 percent) was used, which was unreasonable due to its expensive cost. The linearity of 6-40 g/mL, as found in a recent HPLC method[16], can be considered a narrow limit. A review of the literature indicated that no ultra-high-performance liquid chromatographic method has been published to date. Ultra-performance liquid chromatography's advantages over high-performance liquid chromatography in terms of turnaround time, process dependability, method sensitivity, and drug specificity stimulate the use of LC techniques for a wide range of drug active chemical groups.[17]. Almost every approach presented has its own drawbacks; therefore, it is necessary to overcome all potential disadvantages and produce a reliable, cost-effective, and simple method for estimating lercanidipine. Keeping the aforementioned facts in mind, the current study's goal was to design a simple, reliable, quantitative approach for lercanidipine testing, as well as to validate the method[18] according to ICH criteria.

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## 2. MATERIALS AND METHOD

Pharmaceutical grade working standard Lercanidipine HCL (99.94% purity) were obtained from Abbot India Pvt, Ltd, Goa, India. The methanol was provided by Loba Chemicals in Mumbai, India, and was of HPLC quality. SD-fine chemicals in Mumbai provided analytical grade dipotassium hydrogen phosphate, orthophosphoric acid, and hydrochloric acid. HPLC quality distilled water was delivered by BVK technology services in Hyderabad, India.

### 2.1 Instrumentation operations

The study of lercanidipine was carried out using a Waters Acquity BEH C18 (2.1 x 50mm, 1.7m) UPLC column. Empower 2 is a software package that includes an auto sampler and a

PDA detector was used. 0.1 mg sensitivity Afcoset ER-200A analytical balance was utilized. The pH meter (Adwa – AD 1020), and a Labline India's Ultra sonicator model 1.5L(H). The isocratic elution technique with the flow rate of 0.3ml/min was maintained. The PDA detector was used to detect the signal at 254 nm.

Comment [u5]: References?

**2.2. Preparation of 0.05M Phosphate buffer Solution:**

6.8 grammes of potassium dihydrogen orthophosphate are placed into a 1 litre beaker, dissolved, and diluted with HPLC Grade water up to 1000ml.

**2.3. Preparation of Mobile Phase:**

Phosphate buffer 400 mL, methanol 600 mL are well combined and degassed in an ultrasonic water bath for 15 minutes. Under vacuum filtration, the resulting solution was filtered through a 0.45m filter. The mobile phase employed in this experiment is a 40:60 (v/v) mixture of 10mM potassium dihydrogen orthophosphate and methanol.

**2.4. Sample & Standard Preparation for the Analysis**

The Lercanidipine hydrochloride standard was transferred to a 10ml volumetric flask, dissolved in methanol, and made up to volume with mobile phase. To get 10g/mL of lercanidipine, 1ml of the above-mentioned solution was transferred into a 100ml volumetric flask and filled to the mark with the mobile phase.

**2.5. Assay of Lercanidipine HCL in marketed Dosage Form**

Twenty tablets were ground and thoroughly triturated. A quantity of powder equivalent to 100 mg of pharmaceuticals was transferred to a 100 ml volumetric flask, and 10 ml of methanol was added to dissolve the content, followed by 60 mL mobile phase and 15 minutes of sonication. The volume was then increased to 100 ml with the same solvent. Then, using HPLC grade methanol, 10 mL of the aforementioned solution was diluted to 100 mL. To remove the gas, the solution was filtered through a membrane filter (0.45 m) and sonicated. The stock solution (1.0 mL) was transferred to five 10 mL volumetric flasks and the volume was increased to 10 mL using the same solvent system. The prepared solution was injected into the UPLC system in five duplicates, and the results were recorded. The peak regions of a duplicate injection of the standard solution into the UPLC system were also recorded.

**2.6.Study of validation parameters**

***2.6.1. System Suitability***

Many analytical processes include system suitability testing as part of the process. The tests are founded on the idea that the equipment, electronics, analytical activities, and samples to be studied are all part of a larger system that may be evaluated. The parameters for the system suitability test were established as follows.

***2.6.2. Accuracy***

Recovery studies were carried out to evaluate the correctness of the created approach by adding a fixed amount of standard medicine of lercanidipine HCL, in order to ascertain the accuracy of the suggested method. To generate the 80 percent, 100 percent, and 120 percent levels of the sample, different amounts of the lercanidipine tablet solution of concentration were spiked. Percentage recovery values were determined using this information.

Comment [u6]: References?

### *2.6.3.Precision*

*Repeatability*

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of Five replicates of a fixed amount of drug. Lercanidipine HCL (API). The percent relative standard deviation was calculated for Lercanidipine HCL.

*Intermediate precision*

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Lercanidipine HCL revealed that the proposed method is precise.

### *2.6.4. Linearity*

Standard lercanidipine standard solution was prepared as previously stated, and an aliquot of these solutions was diluted with mobile phase in seven different strengths to provide a solution with lercanidipine concentrations ranging from 1 to 60 µg/ml. After preparing a calibration curve employing concentration versus peak area, the resulting values were subjected to regression analysis. The connection between concentration and peak area in the examined range should be linear, with a correlation coefficient of at least 0.999.

**Comment [u7]:** References?

### *2.6.5.Limit of detection*

To reach a concentration of final LOD sample solution, suitable amount of sample solution of the lercanidipine from the primary stock solution was accurately diluted to 100 ml with mobile phase. The aforementioned solution was further diluted sequentially to achieved a final concentration of 0.025 µg/ml. The lercanidipine solution was introduced into the UPLC system after filtering. For the computation of the limit of detection, the signal to noise (S/N Ratio) value must be 3. The solution for LOD study were made as fixed, injected three replicates and the injection region was estimated for each injection. This study was performed in six replicates of injections; the percent RSD was calculated.

### *2.6.6.Limit of quantitation*

To reach a concentration of final LOQ sample solution, suitable amount of lercanidipine aliquot from the primary stock solution was accurately diluted to 100 ml with mobile phase. And the prepared solution was further diluted sequentially to achieved a final concentration of 0.05 µg/ml. For the computation of the limit of detection, the signal to noise (S/N Ratio) value must be 10. The solution for LOD study were made as fixed, injected three replicates and the injection region was estimated for each injection. This study was performed in six replicates of injections; the percent RSD was calculated.

### *2.6.7.Method Robustness*

This method was studied to test the capacity of the developed method to remain stable on the deliberate changes of the various optimised parameters. Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), wavelength of detection (± 2nm) & organic phase content in mobile phase (± 2%) were studied to determine the robustness of the method.

### 2.7.Stability studies

The API (Lercanidipine HCL) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation, photolytic degradation and oxidative degradation. For all the degradation conditions the final concentration was prepared to 10 µg/ml with mobile phase and was injected into the HPLC system.

*2.7.1.Acid degradation*

The API was exposed to acidic conditions by using *30 ml of 0.1 N HCl and was refluxed in a water bath at $60^0$C for 4 hours.*

*2.7.2.Basic degradation*

The API was exposed to acidic conditions by using *30 ml of 0.1 N NaOH and was refluxed in a water bath at $60^0$C for 4 hours.* The resulting solution was injected into the chromatographic system, and analysed.

*2.7.3.Thermal degradation*

The drug was mixed with water and refluxed in a *water bath at $60^0$ c for 6 hours uninterruptedly*. The resulting solution was injected into the chromatographic system, and analysed.

*2.7.4.Photolytic degradation*

10 mg of pure lercanidipine solution was taken in a clean & dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. The resulting solution was injected into the chromatographic system, and analysed.

*2.7.5.Oxidative degradation*

The prepared lercanidipine solution was exposed to oxidative degradation conditions by using *3% $H_2O_2$ & then kept as such in dark for 24 hours.* The resulting solution was injected into the chromatographic system, and analysed.

## 3. RESULTS AND DISCUSSION

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Different chromatographic settings were used to establish an accurate, linear, specific stability indicating UPLC technique for the measurement of Lercanidipine HCL. The current study preferred isocratic elution. Several variables, including mobile phase composition, column type, mobile phase pH, and diluents, were changed throughout the early studies. In order to acquire a suitable mobile phase composition for method optimization, various solvent and buffer proportions were tested. Finally, lercanidipine was eluted with excellent peak shape using a mobile phase of phosphate buffer: methanol (60:40) with a flow rate of 0.4 mL/min. Lercanidipine was discovered by PDA detection at 254 nm. The retention time was 0.503 minutes, indicating that the substance under study eluted quickly. In compliance with ICH recommendations, the established technique was validated. The optimised chromatogram is presented in Figure 2.

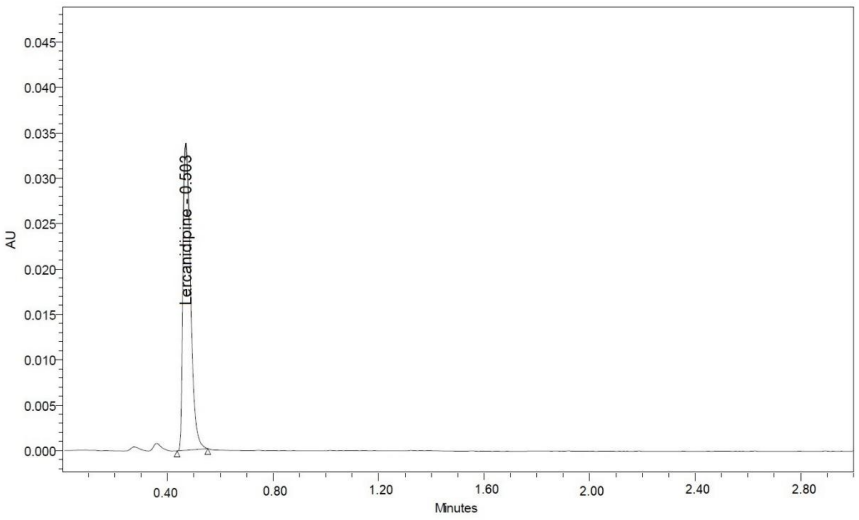



**Figure 2.** Optimised UPLC chromatogram of Lercanidipine.

Validation trials were conducted using the currently established optimal approach for Lercanidipine. The current method was effectively used to conduct a quantitative study of the marketed tablet dosage form of Lercanidipine, with a percentage assay of 98.10 percent in the marketed capsule dosage form. The assay result was confirmed to be within acceptable limits. Figure 3 shows the chromatogram of the commercially available tablet dosage form, and Table 1 shows the results.

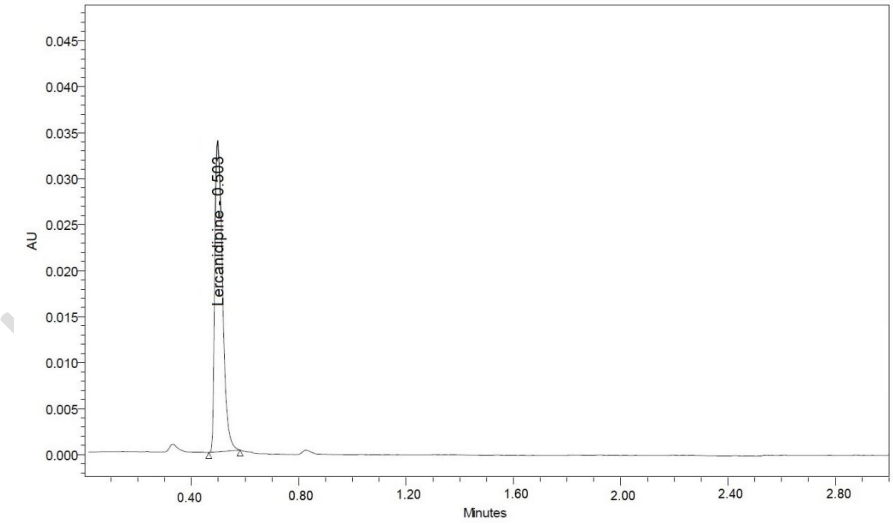



**Figure 3.** UPLC chromatogram of marketed Lercanidipine tablet dosage form.

**Table** 1. Assay of marketed Lercanidipine marketed formulations

| Formulation | Labelled claimed | Amount obtained* | Percentage purity* |
|---|---|---|---|
| Actavis tablets (20 mg Lercanidipine), | 20 mg | 19.62 mg | 98.10% |

*average of three replicates

In the repeatability and intermediate precision studies, the percent RSD was 0.91 and 0.87, respectively. Repeatability and intermediate precision data were found to be adequate and within acceptable limitations in terms of accuracy (percent RSD). The findings of the precision study revealed that the suggested methodology was found to be precise. The results of the precision investigation were also reported in Table 2. The accuracy of the proposed method was proven in the study (mean percent recovery was found to be 98.42, and the percent RSD was determined to be less than 2%). The percent recovery was determined to be within the acceptable range, indicating that the devised approach is accurate. Lercanidipine has detection and quantitation limits of 0.05 g/ml and 0.25 g/ml, respectively. The sensitivity of the suggested approach was demonstrated by the obtained limit of detection and quantitation values. By looking at a number of variables, a system suitability study was undertaken to ensure the analytical measuring equipment's efficient performance (retention time, peak area, theoretical plate, tailing factor). The relative standard deviations for peak area, theoretical plates, tailing factors, and retention length were all 0.27 percent, 0.33 percent, 0.93 percent, and 0.09 percent, respectively, showing that the system is suitable for carrying out the current approach for lercanidipine estimate.

**Table** 2. Summary of validation parameters

| Parameters | Lercanidipine |
|---|---|
| Linearity range (µg/ml) | 1-60 |
| Co-relation co-efficient | 0.999 |
| LOD µg/ml | 0.025 |
| LOQ µg/ml | 0.05 |
| Repeatability (% RSD) | 0.44 |
| Intermediate precision (% RSD) | 0.86 |
| Accuracy (Mean % recovery) | 98.42 |

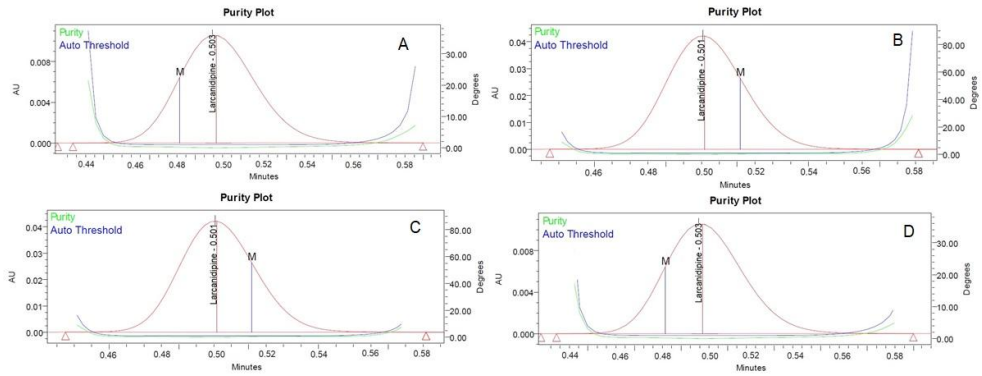
A system appropriateness analysis was performed to assure the analytical measuring equipment's efficient operation by looking at a variety of criteria (retention time, peak area, theoretical plate, tailing factor). Peak area, theoretical plates, tailing factors, and retention length all had relative standard deviations of 0.27 percent, 0.33 percent, 0.93 percent, and 0.09 percent, respectively, indicating that the system is suitable for carrying out the current approach for lercanidipine estimation. The robustness of the lercanidipine solution was tested by changing three parameters from the chromatographic conditions: mobile phase composition (± 2%), flow rate (± 0.1ml/min), and detection wavelength (±2 nm), and the percent RSD of the tailing factor, which was used as a tool parameter, was found less than 2, which confirms the robustness of the developed method as listed in the table 3.

**Table 3** Results of the Robustness study of Lercanidipine

| Change in parameter | % RSD of tailing factor |
|---|---|
| Flow (0.45 ml/min) | 0.09 |
| Flow (0.35 ml/min) | 0.56 |
| More Organic(+2%) | 0.12 |
| Less Organic(-2%) | 0.19 |
| Wavelength of Detection (256 nm) | 0.44 |
| Wavelength of detection (252 nm) | 0.42 |



The lercanidipine stability study was conducted by force degradation tests which was carried out in a variety of stressed circumstances, including acid, alkali, oxidation, thermal, and photolytic environments. the exception of photolytic stressed conditions, remaining other stressed conditions degradation was observed. Degradation was observed 6.23 percent in acidic strained conditions, 2.43 percent in alkaline stressed conditions, and 3.03 percent in peroxide stressed conditions and for heat degradation was found to be 12.11 percent. The details of the peak purity chromatograms and purity angle and purity threshold values were included in the Table 4 and Figure 4. The stressed degradation sturdy results revealed that the degradation percentages were within the limits and as per the ICH stressed degradation study guidelines. The details of the results of stability study were illustrated in the Table 4 and UPLC chromatograms were shown in Figure 5.




A = Acidic peak purity , B=Alkaline peak purity, C=Peroxide peak purity, D= Thermal peak purity

**Figure 4**. Peak purity UPLC chromatograms of the Lercanidipine at different stressed conditions.

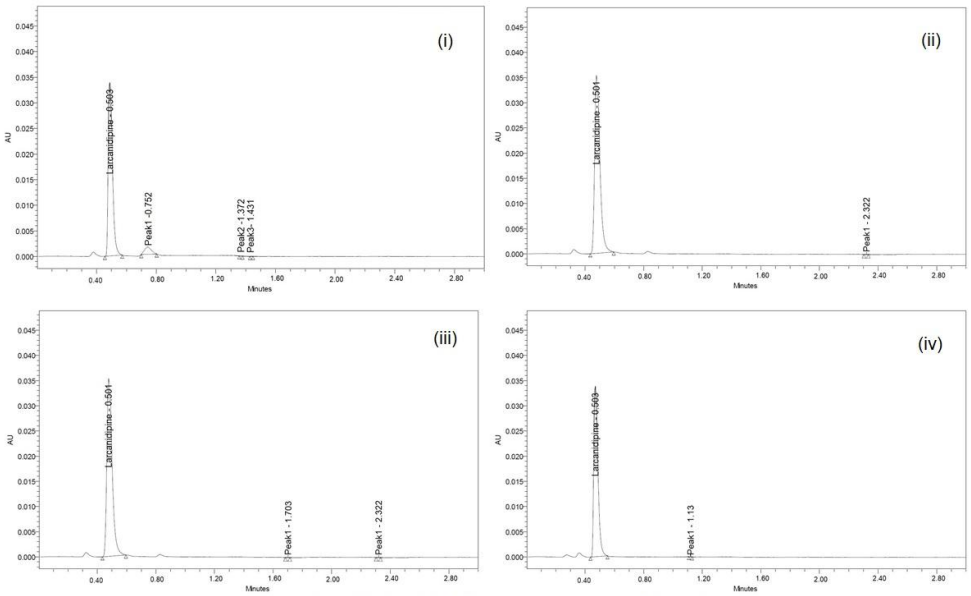



**Figure 5**. Stressed degradation UPLC chromatograms of lercanidipine.

**Table 4.** Degradation results for lercanidipine solution

| Sample Name | Lercanidipine | | | | |
|---|---|---|---|---|---|
| | Mean Area* | % Degraded | Purity Angle | Purity Threshold | Peak purity |
| Acid | 401563 | 6.23 | 0.216 | 1.429 | Passes |
| Base | 522923 | 2.43 | 0.543 | 1.241 | Passes |
| Peroxide | 511742 | 3.03 | 0.336 | 1.203 | Passes |
| Thermal | 553534 | 2.11 | 0.422 | 1.315 | Passes |
| Photo | 581303 | 0.23 | 0.081 | 1.015 | Passes |

## 4. CONCLUSION

In comparison to other developed HPLC methods, the current developed approach for lercanidipine employing UPLC is considered to be creative. Because the whole analysis time was 0.503 minutes, the current method can be considered rapid depending on the experiment. In all types of force degradation studies, the lercanidipine approach showed reduced degradation. Because of the distinct separation of isavuconazole from other degraded peaks, the current approach is referred to as "stability indicating." The current method is the first "fast" UPLC method for reporting stability indicators. All validation parameters yielded findings that were acceptable according to the ICH Q2B criteria. Finally, it can be stated that the newly proposed method must be used for quality control and routine analytical tests of Lercanidipine in the marketed tablet dosage form.

**Abbreviations**

**API**: Active Pharmaceutical ingredient. **HPLC:** High performance liquid chromatography**,** **UPLC:** Ultra performance liquid chromatography; **UV** : Ultra Violet, **LC**: Liquid chromatography, **ICH:** International conference on harmonization; **PDA:** Photo diode array; **LOD:** Limit of detection; **LOQ:** Limit of quantitation; **SD:** Standard deviation; **RSD:** Relative standard deviation.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors



**References**

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