QUANTIFICATION OF IMPURITY-G CONTENT IN LEVETIRACETAM INJECTION (100mg/mL) BY USING RP-HPLC TECHNIQUE

ABSTRACT

Highly sensitive method for the quantification of impurity-G content in Levetiracetam samples by using RP-HPLC method has been presented in the present paper. Quantification of Impurity-G content in Levetiracetam sample by HPLC with UV Detector. Impurity-G was determined by RP-HPLC method using Waters X-Terra MS-C18 (250x4.6mm, 5µm) column as stationary phase. Column temperature maintained 45°C and sample cooler temperature 5°C, Injection volume 50µL, Flow rare was 1.0 ml/min, Impurity-G was detected using UV detector at the wavelength of 200nm and run time was 30 minutes. The mobile phase used pH 3.0 buffer and acetonitrile in the ratio of (850:150 v/v) isocratic mode. The method validation has been carried as per International Conference on Harmonization guidelines (ICH). Limit of quantitation (LOQ) was found 0.4122 µg/mL for impurity-G.

Key words: impurity-G, Levetiracetam, RP-HPLC method, Validation and limit of quantitation.

1.0 INTRODUCTION

Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and finalized formulations is one of the most challenging tasks of pharmaceutical analytical chemists under industrial environment [1]. The presence of unwanted or in certain cases unknown chemicals, even in small amounts, may influence not only the therapeutic efficacy but also the safety of the pharmaceutical products [2]. For these reasons, all major international pharmacopoeias have established maximum allowed limits for related compounds for both bulk and formulated APIs. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product [3-4].

Levetiracetam is an antiepileptic drug used in treatment of epilepsy, partial onset, myoclonic or tonic-clonic seizures. It is S-enantiomer of Levetiracetam. Its chemical name is (S)-2-(2-oxopyrrolidin-1-yl) butanamide. It acts by binding to SV2A (synaptic vesicle glycoprotein 2A)

and inhibits presynaptic calcium channels reducing neurotransmitter release and acting as a neuromodulator [5-7]. Its empirical formula is $C_8H_{14}N_2O_2$, and molecular weight 170.212 g/mol. Levetiracetam is a white to off-white free-flowing crystalline powder. Levetiracetam is very soluble in water and freely soluble in chloroform, methanol and ethanol; polymorphism has not been observed in induction studies. Its structural formula is shown in **Figure 1**.

Impurity-G controlled in drug substance nevertheless this impurity also monitor in Levetiracetam drug product, due to drug product formulations using different types of excipients. These excipients may be reacts with Levetiracetam to form impurity-G. Hence impurity-G specifications limit not more than 0.10%, as per maximum daily dose of Levetiracetam and ICH in New Drug Products Q3b (R2).

Reverse phase HPLC method used during drug substance analysis, no extraction procedure needed for extraction of the impurities and main analyte while per USP and EP monograph (drug substance), but these API methods were not appropriate for formulations. For formulations, extraction process is appropriate for the extraction of the impurities as well as main analyte as drug product containing excipients and those excipients may bind with drug substance.

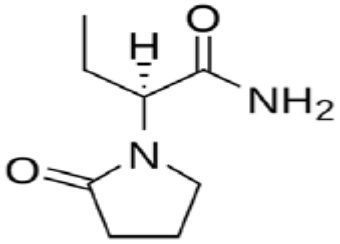

Figure 1. Chemical structure of Levetiracetam

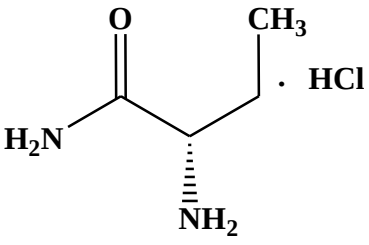

Figure 2. Chemical structure of impurity-G

In literature, until now no RP-HPLC no analytical method was reported for the determination of impurity-G in Levetiracetam injection formulation. Hence the author was aimed towards the

development of rapid, specific and robust method for the determination of impurity-G in Levetiracetam injection at trace level concentration.

2.0 Experimental

Chemicals and reagents

(S)-2-Aminobutyramide Hydrochloride [impurity-G], orthophosphoric acid, potassium hydroxide, 1-decane sulfonic acid sodium salt purchased from Merck, Mumbai, India. Acetonitrile and Milli-Q water HPLC grade procured from Merck, India.

Preparation of Buffer:

Weighed accurately 1.2245 g of 1-decane sulfonic acid sodium salt into 1000 mL of water, containing 1.3 mL of phosphoric acid, adjusted the pH 3.02 with aqueous potassium hydroxide solution and mix well.

Preparation of Mobile Phase:

Prepared a mixture of 150 mL of Acetonitrile and 850 mL of buffer in the ratio of 15:85 (%v/v). Filtered through 0.45 membrane filtered and sonicated to degas.

Preparation of diluent:

Mobile phase was used as diluent.

Preparation of Standard solution:

Weighed accurately 2.061 mg of Levetiracetam impurity-G standard in 20 mL of volumetric flask, added 10 mL of diluent sonicated to dissolve, diluted to volume with diluent and mixed well. Transferred 1.0 mL of this solution into 50 mL volumetric flask, diluted to volume with diluent and mixed well.

Preparation of Sensitivity solution:

Transferred 2.0 mL of standard solution into 10 mL volumetric flask, made up to the mark with diluent and mixed well.

Preparation of Placebo solution

Transferred 1.0 mL of placebo into a 50 mL volumetric flask, with the help of suitable hypodermic needle and syringe, made up to the mark with diluent and mixed well.

Preparation of Sample solution

Transferred 1.0 mL of sample into a 50 mL volumetric flask, with the help of suitable hypodermic needle and syringe, made up to the mark with diluent and mixed well.

Preparation of spiked sample solution

Transferred 1.0 mL of sample solution into a 50 mL volumetric flask, added 2.0 ml of impurity-G stock solution made up to the mark with diluent and mixed well.

Chromatographic conditions

RP-LC analysis was carried out on Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower^2 software, wavelength 200 nm. Waters X-Terra MS-C18 (250x4.6mm, 5µm) column was used as stationary phase. The mobile phase used pH 3.0 buffer and acetonitrile in the ratio of (850:150 v/v) isocratic mode. The flow rate of the mobile phase was kept at 1.0 mL/min. The injection volume was set as 50µL. Column oven temperature and auto sampler temperature were set as 45°C and 5°C, respectively.

3.0 Method development

A spiked sample solution containing impurity-G and Levetiracetam was run with column temperature 25°C. Placebo peaks interference was observed at impurity-G peak. Further trial was taken with increase the column temperature from 25°C to 35°C , in this trial the resolution between Levetiracetam placebo peaks and impurity-G peak very less. Next trial was taken column temperature changed from 35°C to 45°C. In this condition Levetiracetam placebo peaks are well separated from impurity-G peak. Hence, the elution order was observed from the chromatogram **(Figure 7)** Levetiracetam solution spiked with impurity-G (2.0 µg/mL).

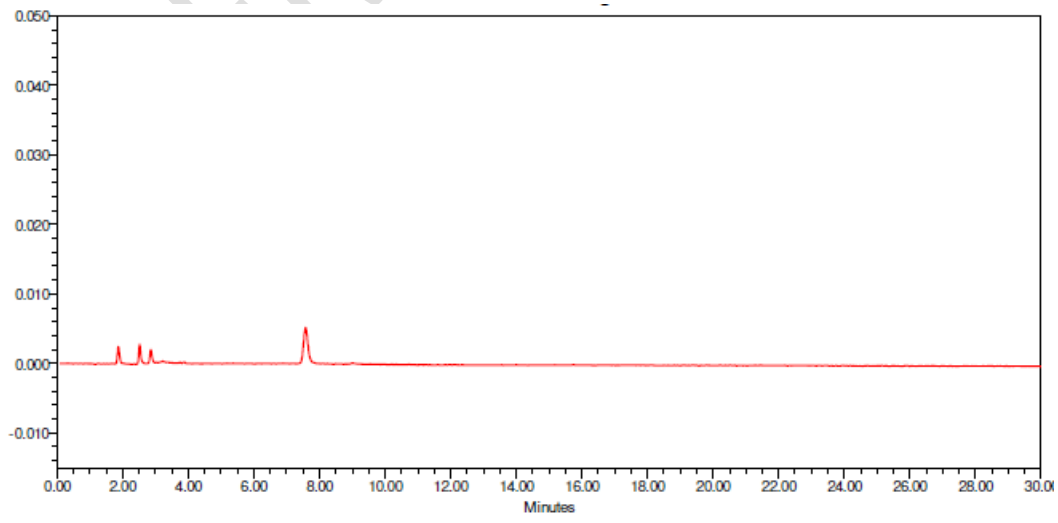

Figure 3. typical chromatogram of blank

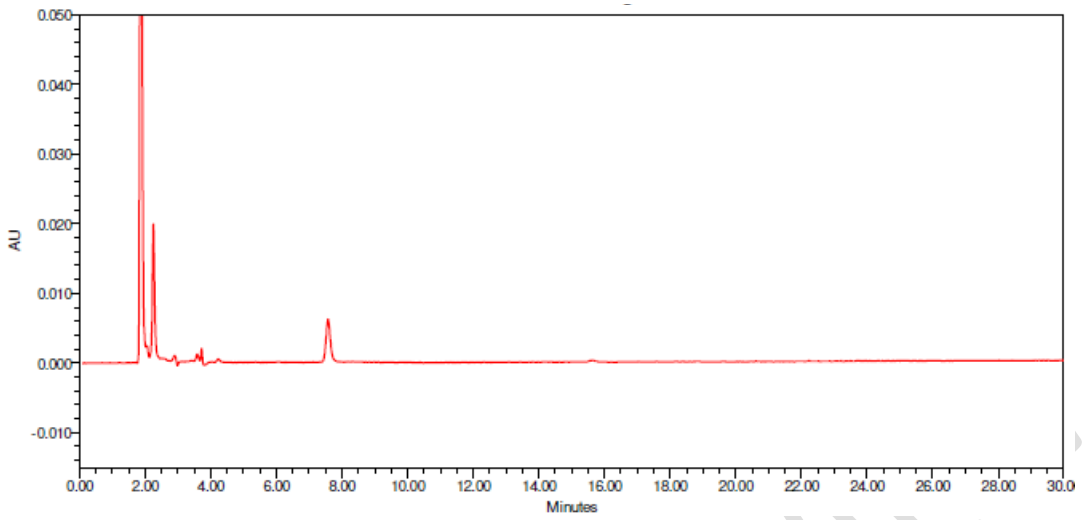

Figure 4. typical chromatogram of placebo

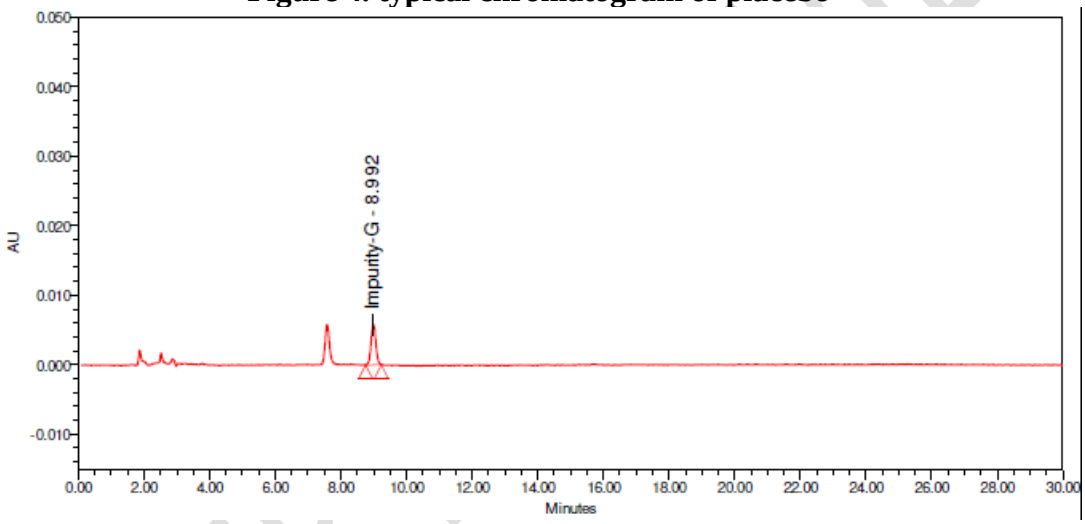

Figure 5. typical chromatogram of standard

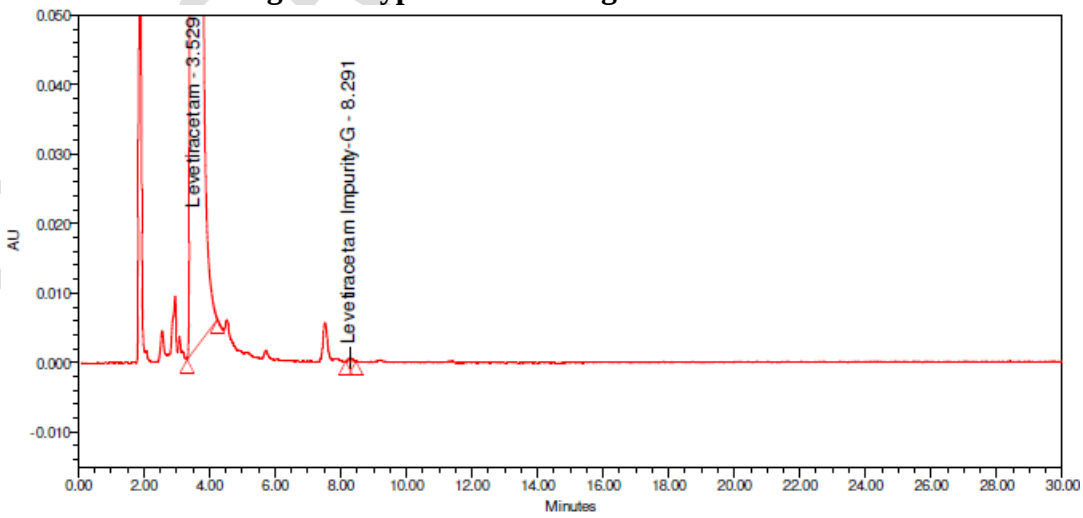

Figure 6. typical chromatogram of As such sample

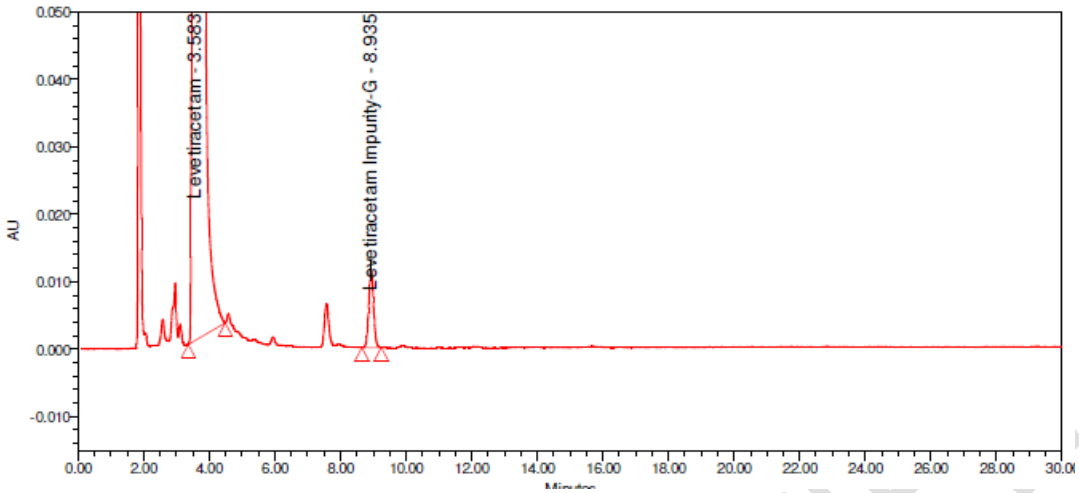

Figure 7. Impurity-G spiked chromatogram of Levetiracetam

4.0. Method validation

4.1 Specificity

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **(Figure 3)** showed no peak at the retention time of impurity-G peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of impurity-G in Levetiracetam injection formulation. Similarly chromatogram of placebo solution **(Figure 4)** showed no peaks at the retention time of impurity-G peak. This indicates that the placebo used in sample preparation do not interfere in estimation of impurity-G in Levetiracetam injection formulation.

4.2 Precision

4.2.1 System Precision

Perform the analysis of reference solution (Diluted standard) six times and determine the percentage relative standard deviation of peak area of replicate injections of impurity-G.

Table: 1 System Precision data for impurity-G

Injection No	impurity-G
1	59174
2	60852
3	59784
4	58995
5	59321
6	58745
Mean area	59478
SD	757.5161
%RSD	1.27

The %RSD of peak area for Impurity-G was found to be 1.27% which is below 5.0% indicates that the system gives precise result.

4.2.2 Method Precision

Precision of the impurity was determined by injecting six sample solutions spiked with impurities (impurity-G) at specification level. The samples were prepared as per the method and the result for precision study is tabulated in **Table: 2.**

Table: 2. Results of method precision

Inj. No	impurity-G recovery (%)
1	98.6
2	99.6
3	98.2
4	100.1
5	98.6
6	99.7
Mean (%)	99.13
SD	0.76
% RSD	0.77

The method precession was performed with impurity-G spiked at specification level. The % recovery for each of the spiked sample preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The data were shown in **Table 2.**

4.3 Limit of detection (LOD) & Limit of Quantitation (LOQ)

A solution containing 0.1360 µg/ml of Impurity-G standard was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were

detected in all the three injections.

A solution containing 0.4122 µg/mL of Impurity-G standard was injected six times. The RSD of areas, deviations of each six replicates from the linear regression curve and average deviation for each standard were calculated. The results are presented in the following tables:

Table: 3 LOQ for Carbon Disulfide

S.No	Impurity-G
1	11233
2	10797
3	11554
4	11249
5	11403
6	11896
Average	**11355**
Std.Dev	**366.625**
%RSD	**3.2**

The limit of quantitation and limit of detection values obtained for Impurity-G was within the acceptance criteria.

4.4 Linearity

The linearity is determined by injecting the solutions in duplicate containing impurity-G ranging from LOQ to 200% of the specified limit. Perform the regression analysis and determine the correlation coefficient and residual sum of squares. Determine the response factor for each impurity with respect to Levetiracetam. Report the linearity range as the range for determining the impurities. Results obtained are in the table & figure show the line of best fit for peak area versus concentration.

Table: 4 Linearity of detector response impurity-G

Level (%)	**Concentration (ppm)**	**Mean Area**
LOQ	0.412	10578
75	1.551	88339
100	2.061	117785
125	2.576	147536
150	3.091	176689
200	4.122	237745
R^2 Value		1.000
Slope		60661.45
Intercept		-9874.11

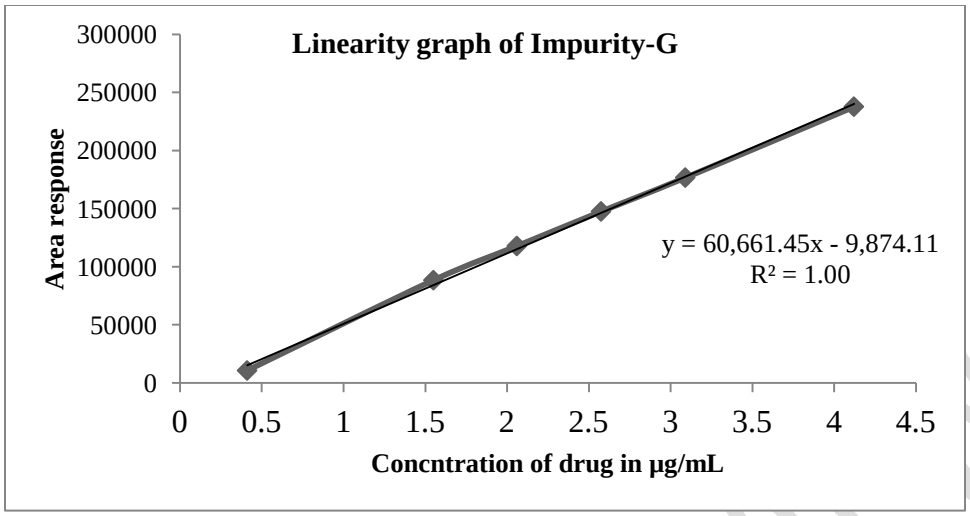

Figure 8. Linearity of detector response for impurity-G

The linearity results for Impurity-Gin the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

4.5 Accuracy

Recovery of Impurity-G in Levetiracetam was performed. The sample was taken and varying amounts of Impurity-G representing LOQ to 150 % of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in **Table 5.**

Table: 5 Accuracy study of Impurity-G

S.No.	Component Name	% Recovery level		
		LOQ	100%	150%
1	Impurity-G	100	95.6	96.1
2		100	96.6	96.8
3		95.2	96.6	96.4
Avg. Recovery		**98.4**	**96.2667**	**96.4333**

4.6 Solution stability of analytical solutions:

Standard and sample solutions were kept for about 48 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of "old" standard solutions with freshly prepared standard solutions.

Table 6. Results for solution stability of standard

Time Interval (hrs)	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24	1.05	1.4

Table 7. Results for solution stability of sample at room temperature

Time Interval (hrs)	% recovery	% recovery difference
Initial	100.5	NA
24	101.9	1.4

Table 8. Results for solution stability of sample in Refrigerator

Time Interval (hrs)	%Assay	% of Assay difference
Initial	100.5	NA
24	101.0	0.5

From the above results, it is concluded that standard and sample solutions are stable up to 24 hours in both the conditions (bench top and refrigerator).

5.0 Results & Discussion

A simple, economic, accurate and precise HPLC method was successfully developed. In this method it was carried out by using Waters X-Terra MS-C18 (250x4.6mm, 5µm)**.** Injection volume of 50 µL is injected and eluted with the mobile phase used pH 3.0 buffer and acetonitrile in the ratio of (850:150 v/v) isocratic mode, which is pumped at a flow rate of 1.0 ml/min. Column temperature 45°C and sample temperature 5°C. Detection was carried out at 200 nm. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of selectivity, accuracy, linearity and precision.

For selectivity the chromatograms were recorded for standard and sample solutions of Levetiracetam and impurity-G. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of impurity-G in Levetiracetam. There is no interference of diluent and placebo at impurity-G peak. The limit of detection (LOD) and limit of quantitation (LOQ) for Impurity-G standard 0.1360 µg/mL and 0.4122 µg/mL respectively.

The linearity results for impurity-G in the specified concentration range LOQ to 200% are found satisfactory, with a correlation coefficient greater than 0.99. Calibration curve was plotted and correlation co-efficient for impurity-G found to be 1.000 respectively.

The accuracy studies were shown as % recovery for impurity-G at specification level. The limit of % recovered shown is in the range of 80% and 120% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

For Precision studies six (6) replicate injections were performed. %RSD was determined from the peak areas of impurity-G. The acceptance limit should be not more than 10 and the results were found to be 0.77% within the acceptance limits.

6.0 Conclusion

The proposed RP-LC method that can quantify impurity Impurity-G in Levetiracetam at trace level concentration have been developed and validated as per ICH guidelines. The effectiveness of the method was ensure by the specificity, precision, Linearity and accuracy. Hence, the method well suit for their intended purposes and can be successfully applied for the release testing of Levetiracetam into the market.

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.

7.0 References

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