Original Research Article

Etravirine lipid nanoparticles: Synthesis and validation using specific RP-HPLC technique

Abstract:

Introduction: A common, exact & economical reverse phase-high performance liquid chromatographic approach for coinciding measurement of anti-retroviral medicine, Etravirine in the formulation of nanoparticle has been devised. This approach is a revolutionary analytical methodology for detecting substance at the same time.

Methods: Validation & Optimization chromatographic conditions were completed as per the guidelines of ICH. The disconnection was done on a column measuring 4.6 × 250mm using methanol & pH 2.7 of a buffer as the mobile phase in the ratio 85:15 at a flow rate of 0.8ml/min. The wavelength was 310nm & the column temperature was kept at 26°C.

Results: The plots were created with linearities of 10, 20, 30, 40, and 50 MCG to determine the peak value of etravirine, paracetamol, and the internal standard of paracetamol at 20MCG.

Conclusion: The approach was, precise, accurate & rapid with excellent inter- & intra-day accuracy. The approach was also useful for characterization & coinciding measurement of medicines in formulation of nanoparticles.

Keywords- Antiretroviral drugs, Etravirine, HPLC, Nano formulation, Nanoparticles.

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1. Introduction

Nanoparticles are solid particles or particulate dispersions with diameters ranging from 10 to 1000nm. A nanoparticle matrix is used to dissolving, encapsulating, entrapping, encapsulating, or attaching a medicine. Depending on the manufacturing procedure, nanospheres, nanoparticles or nano capsules may be created. Nano capsules are matrix structures in which the medication is equally distributed, while nanospheres are cavity systems in which the medicine is confined inside a cavity surrounded by a specific polymer membrane due to their capacity to circulate for an extended length of time and pick a specific organ, biodegradable nanoparticles, especially those bound along with hydrophilic polymers such as polyethylene glycol, have been explored as possible drug delivery innovations in recent years [1-4]. Particle size, characteristics, and releasing of pharmacologically active substances are the primary aims of producing nanoparticles like a delivery technique. This is done in order to produce site-specific drug activity at a therapeutically appropriate rate and dosage rule. Toxic or side effects can be decreased or eliminated by using liposomes as possible carriers. However, liposomes have intrinsic disadvantages like minimum encapsulation competence, fast leakage of water-soluble medicines into the blood, and inadequate strength. Polymeric nanoparticles whereas, have

several distinct benefits over liposomes. They may, for example, aid to enhance the drugs stability or proteins & also have beneficial controlled capabilities. The following are some of the benefits of employing nanoparticles as a medication delivery system: [5-6].

- **i.** Nanoparticle size and surface properties may be readily changed after parenteral delivery to provide both passive and active medication targeting.
- **ii.** They regulate & maintain the release of drugs throughout transit & at the surface of localization, modifying organ release & subsequent clear of the medication in order to maximize pharmacological therapeutic effectiveness and reduce adverse effects.
- **iii.** The matrix elements may easily alter controlled release & particle disintegration properties. The loading of drug is quite high, & pharmaceuticals may be integrated into arrangement without causing any chemical reactions; an important element in caring the drug action.
- **iv.** Targeting ligands may be coherent to the particles surface to provide site-specific targeting, or magnetic guiding can be used.
- **v.** The method may be employed for a variety of delivery routes as like oral, nasal, parenteral, intra-ocular, and so on.

Although the introduction of combination antiretroviral treatment (cART) in 1996 has played a critical part in lowering mortality & morbidity associated with HIV infection, there are still significant obstacles in the field of antiretroviral therapy. Although cART may lower the HIV payload to minute levels, it is impossible to eliminate the virus from infected people because to factors like as drug resistance, long-term drug treatment, toxicity, & strong protein binding, which leads to poor drug pharmacokinetics. Most crucially, the difficulty of most anti-retroviral drugs to enter tissues & other biological aspects of the body plays a critical part in HIV tenacity in body even when the patient is on combination antiretroviral treatment, resulting in the establishment of viral reservoirs [7-10].

Etravirine (ETR), a member of the diarylpyrimidine chemical family, is a nonnucleoside reverse transcriptase inhibitor of the second generation (NNRTI) [10]. It prevents HIV replication by directly attaching to and destroying the reverse transcriptase enzyme's catalytic regions. Because of the drug's intrinsic molecular flexibility, it may withstand the binding site modifications generated by viral mutations, resulting in a greater genetic blockade to resistance [11]. Etravirine is active towards NNRTI-resistant & the HIV wild type strains & has been shown in the DUET-1 research to have an improved capacity to suppress the treatment-experienced individuals of the virus with resistance to more NNRTIs [12]. Different detection platforms and analytical approaches, & LSPR (localized surface Plasmon resonance) nanotechnology have been investigated [13].

Ultraviolet-Visible spectrophotometry, HPLC, Liquid chromatography attached with mass spectrometric recognition, biomolecular fluorescence complementation analysis, and confocal microscopy were used in the luminescence switch on detection assay [14] and were utilized for chemical identification in biological and non-biological fluids [15]. Several High-Performance Liquid Chromatography techniques have been used to estimate ETR in dosage forms and human plasma [16-17].

The most medically developed non-viral gene delivery technology is lipid nanoparticles (LNPs). Lipid nanoparticles carry nucleic acids in a safe and efficient manner, removing a significant impediment to the development and application of genetic treatments. Lipid nanoparticles have a

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high efficiency of nucleic acid encapsulation, improved penetration, low cytotoxicity & immunogenicity. These above features make lipid nanoparticles outstanding applicants for nucleic acid approach [18].

2. Methods and Materials

i. Standard drugs

Arene Life Sciences Limited produced the etravirine.

ii. Reagent & chemicals

Filtered water and methanol was obtained from Finer Chemical Ltd. and Rankem Chemicals provided acetonitrile.

iii. Instruments

Ultraviolet (UV) lamp (Elico SL-196), HPLC columns (Hypersil™ ODS C18, (5µ, 150*4.6 mm), HPLC (Analytical Technologies), sonicator (Analytical tech. ltd.), software (Analchrome, Clarity) as well as detector (UV lamp, Analytical Technologies).

2.1 Preparation of mobile phase

500 mL of 100% acetonitrile HPLC class was autoclaved for ten minutes in an ultra-sonic water bath & vacuum purified through a 0.45 mm nylon filter.

i. Diluent

Diluents including mobile phase are employed.

ii. Standard preparation

Measure 25 mg of etravirine correctly and transport to a volumetric flask (25 ml). Sonicate approximately 10 ml of solvent to solubilize. Reduce the temperature of the liquid to room temperature then dilute with solvent to level. 1 ml solution was poured in a volumetric flask with 10 ml capacity and diluted the solution with diluent, repeat the same with same sol. (4 mL) in a volumetric flask (10 mL) & to level this with the diluent, dilute it again.

iii. Sample preparation

Calculate 25 mg of etravirine correctly, then transport to a volumetric flask containing 25ml. Dissolving, combine about 10 ml of solvent and sonicate. Reduce the temperature of the solution to room temperature, 1 ml solution was poured in a volumetric flask with 10 ml capacity and diluted the solution with diluent, repeat the same with same sol. (4 mL) in a volumetric flask (10 mL) & to level this with the diluent, dilute it again.

Optimized chromatographic conditions

Table 1 – **Chromatographic condition**

Run time	5 min
Flow rate	1 ml/min
Injection volume	10 µl
Column temperature	35°C
Wavelength	271nm
Column	Hypersilods C18 (150*4.6 mm), 5 µ

2.2 Method validation

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The subsequent factors were considered while validating the analytical technique for etravirine in mass form.

2.3 System suitability

The chromatograms of the standard formulations (6 duplicate doses) & peak area sensitivities for the analyte peak were determined, as well as the system suitability parameters.

2.4 Accuracy

To determine the accuracy, three distinct quantities of the analyte were created independently, namely 50%, 100%, and 150%, and chromatograms were collected for each.

2.5 Precision

Six injections of the standard solution were made, as well as the area of each injection was determined using HPLC. The region of six duplicate injections was determined to have a percent relative standard deviation (RSD) within the prescribed ranges.

2.6 Robustness

Robustness evaluation, purposeful changes in temperature & flow rate were conducted to determine the technique's effect.

2.7 Range & linearity

The analytical method's linearity is determined by injecting solutions in to chromatograph in range between 10 to 60 gm (25 to 150 percent) of the test concentration, comprising a minimum of 6 various concentration.

2.8 Ruggedness

Validate the analytical method's robustness by assaying six separate sample formulations from the same batch using a separate HPLC system by a different investigator.

3. Result and discussion

3.1 Standard preparation

Measure 25 mg of etravirine carefully and transfer to a 25 ml volumetric flask. Sonicate approximately 10 ml of the solvent mixture to dissolve. Bring the solution to room temperature & dilute with a solvent combination to volume. Transfer 1 ml of the preceding solution to a 10 ml volumetric flask, then dilute to volume with diluent, repeat with 4 ml of the preceding solution in a 10 ml volumetric flask & dilute to volume with diluent.

3.2 Validation

- **Accuracy**

  At 150, 100, and 50 percent concentrations, etravirine rates of recovery are estimated to be 99.17, 99.8, and 98.6 percent, respectively. The drug's recovery rate ranges from 99 to 102 percent.

Table 2 - Etravirine accuracy results

Level of concentration	Added amount (mg)	Detected amount (mg)	% Recovery	Average % recovery
50%	12.5	12.3 12.3	98.4 98.4	98.6

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		12.4	99.2	
100%	25	24.95 24.97 24.98	99.02 99.1 99.13	99.08
150%	37.5	37.3 37.4 37.2	99.4 99.22 98.90	99.17

Table 3 - % Etravirine recovery		
Added concentration (mg)	**Concentration present (mg)**	**Average % recovery**
27	26.80	94.78

- **Precision**

  The precession had a percent RSD value of 2.0%, indicating that the suggested approach is precise and reliable.

Table 4 - Precision results of etravirine	
Sample. No (Dose)	**Etravirine peak area**
1	538.54
2	531.14
3	520.78
4	528.32
5	530.28
6	533.23
Mean	532.470
SD	2.9382080992
%RSD	0.58

- **Linearity**

  The response was found to be linear. For etravirine, the correlation coefficient was determined to be 0.999. The data is shown in Table 5. Graph 1 shows the etravirine linearity curve.

Table 5 - Etravirine linearity result		
% Level	**Concentration (µg/ml)**	**Peak area**
27	11	163.008
50	20	339.13
75	30	499.27
100	40	670.498
125	50	844.534
150	60	1029.54
Y intercept		16.95
Correlation co-efficient (R^2)		0.999
Linearity range	10-60	
Slope		17.1489

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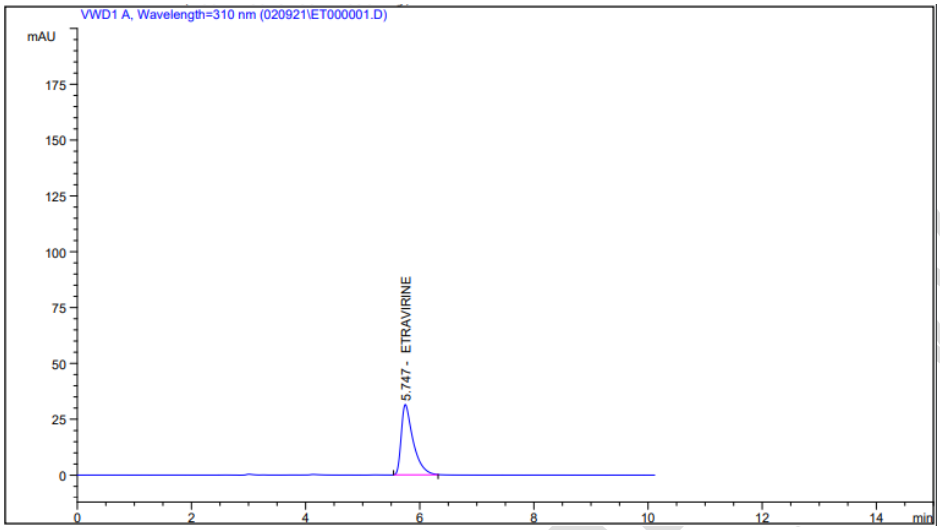
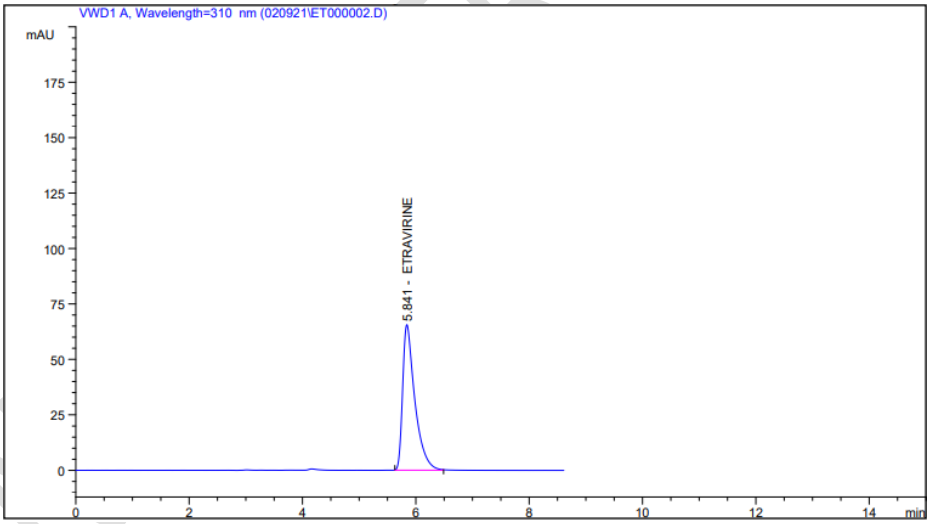
Linearity graphs for Etravirine at 10, 20, 30, 40, and 50 MCG, respectively.



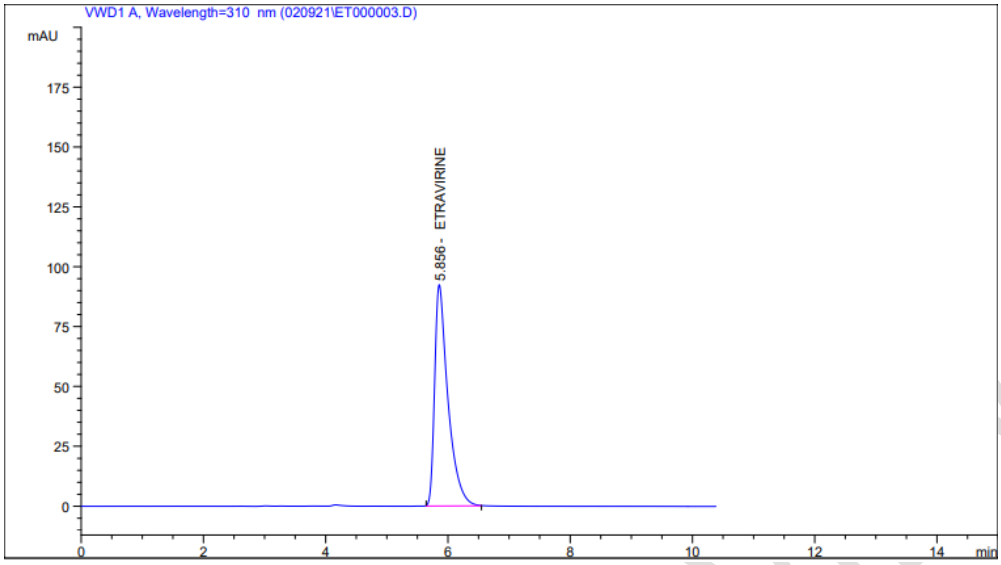
Graph 1 - linearity graph of etravirine at 10 MCG

The Graph 1 describes the linearity of 10 MCG where the peak value of etravirine is 5.747.



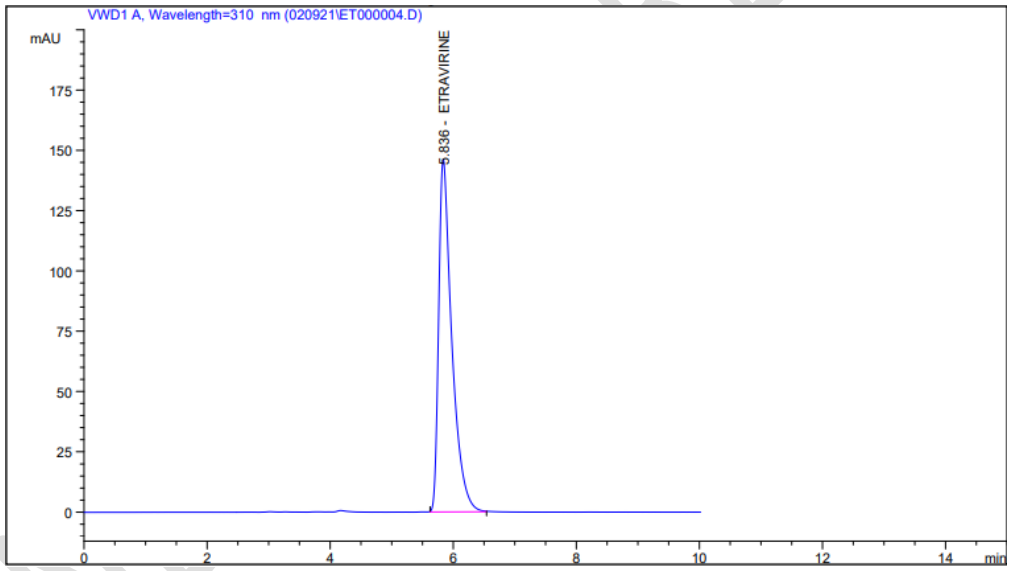
Graph 2 – linearity graph of etravirine at 20 MCG

The Graph 2 indicate the linearity of 20 MCG, where the peak value of etravirine is shown as 5.841.



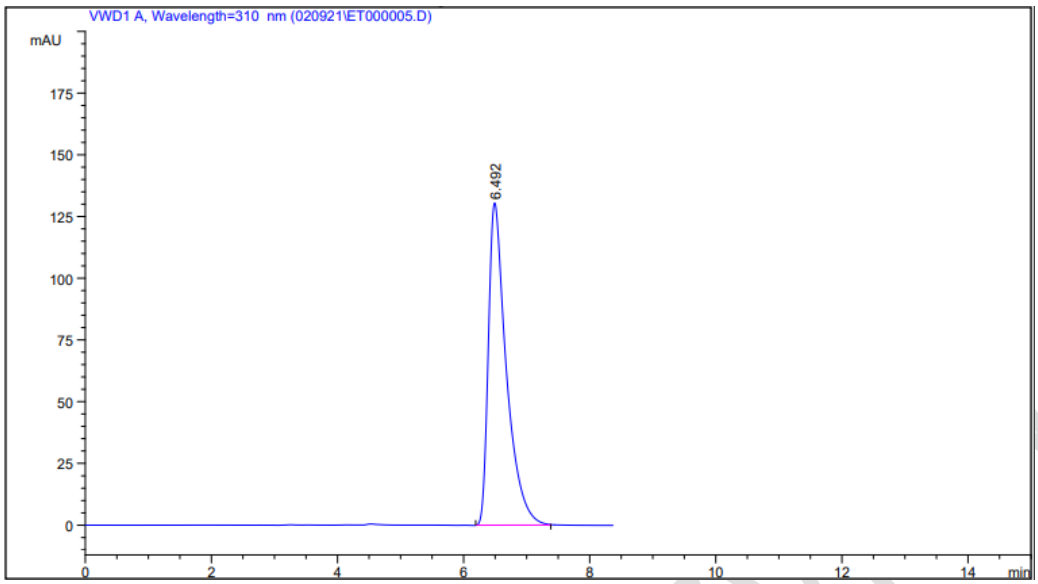
Graph 3 - linearity graph of etravirine at 30 MCG

The Graph 3 describes the linearity of 30 MCG, where the peak value of etravirine is 5.856.



Graph 4 - linearity graph of etravirine at 30 MCG

The Graph 4 shows the linearity of 40 MCG, where the peak value of etravirine is shown as 5.836.



Graph 5 - linearity graph of etravirine at 40 MCG

The Graph 5 shows the linearity of 50 MCG, where the peak value of etravirine is shown as 6.492.

- **Robustness:**
  Minor purposeful modifications in various experimental variables like temperature (10°C) and flow rate (0.2 ml) had no significant effect on etravirine peak area and retention duration, demonstrating that the suggested approach is robust, as shown in Tables 6 and 7.

Table. 6 - Etravirine's robustness findings (changes in flow rates)

No.	Flow rate	Peak area of etravirine	Avg. standard deviation	percentage RSD
1	0.8 mL/min	282.208 285.301	283.75 2.1870	0.77
2	1 mL/min	353.80 362.597	358.198 6.220	1.73
3	1.2 mL/min	324.453 331.65	328.051 5.089	1.55

Table. 7 - Etravirine's robustness findings (changes in the temperature)

S. No.	Temp.	Peak area of Etravirine	Avg. Standard Deviation	percentage RSD
1	25° C	529.788 524.673	527.230 3.616	0.685
2	35° C	362.597 353.80	358.198 6.220	1.73
3	45° C	470.584 476.412	473.498 4.1210	0.87

- **Ruggedness**
  The approach is rugged at various time periods, and it had no significant effect on the peak area, recoveries, or retention duration of any of the aforementioned medications, showing that the suggested method, as shown in Table 8, is rugged.

Table. 8 - Etravirine ruggedness results

Ruggedness	Etravirine peak area
Day 1	414.763

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Day 2	410.383
Day 3	402.921
Day 4	407.399
Day 5	408.500
Day 6	409.639
Average	400.9350
SD	0.691
% RSD	2.81595

- **LOD "(limit of detection)"**
  It is obtained by analysing models with specified analyte concentrations as well as determining the lowest level at which the analyte can be consistently identified. It is determined by using the following formula:

  "LOD=3.3×SD/b",

  where, Standard deviation of peaks of medicine is denoted by SD, and the gradient of a calibration curve is denoted by b. Etravirine has a 0.514 µgmL-1 of limit of detection.

- **LOQ "(limit of quantification)"**
  Usually, it is established via sample analysing with the well-known concentration levels as well as determining the lowest level where the analyte could be measured with sufficient precision as well as accuracy. The following equation is used to determine it:

  "LOQ=10×SD/b"

  where, Standard deviation of peaks of medicine is denoted by SD, and the gradient of a calibration curve is denoted by b. Etravirine has a 1.713 µgmL-1 of limit of detection.

Table. 9 - LOD and LOQ results of etravirine

Parameters	Arthemether
LOQ	1.713µg/ml
LOD	0.514µg/ml

- **Validation summary**

Table 10 - Etravirine validation summary

No.	Parameter	Criteria of acceptance	Obtained results
1	Precision	%RSD NMT2	98.85%
2	Accuracy	Recovery 98-102%	0.55%
3	Linearity		10-60 µg/ml
4	Theoretical plates	NLT 200	8100
5	Ruggedness	% RSD NMT 2	0.691
6	LOQ		1.713
7	LOD		0.514 µg/ml
8	Specificity	No interference	No impurity

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Conclusion

The RP-HPLC technique was used to develop and validate the etravirine method. Hypersil C18 (4.0 mm,151 mm, 6 m) was employed for the estimate. Acetonitrile was employed as the mobile phase at a flow rate of 1 ml/minute with a retention duration of 1.8 min, at a maximum wavelength of 271 nm. Etravirine has a linearity range of 10 to 60 gml-1. The average recovery rate was 98.8%, that falls in the range of 98-99%. The correlation co-efficient was 0.999 and the % RSD was 0.55%, which is in the acceptable range. These findings demonstrate that the approach is exact, responsive, economical, as well as tough. HPLC is a quicker process. The suggested approach may be used to accurately determine the dose form of both drug products and tablets. The approach was determined to be appropriate for normal laboratory testing with a high level of precision as well as accuracy.

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.

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