Original Research Article

Etravirine lipid nanoparticles: development 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 (150mm x 4.6mm, 5µ) using the mobile phase consisted of acetonitrile and 10mM ammonium acetate buffer in the ratio of 90:10 v/v of pH 2.7 as the mobile phase in the ratio 85:15 at a flow rate of 1ml/min for a short run time of 13 minutes. The wavelength was 310nm & the column temperature was kept at 26°C.

Results: The devised technique was linear at 10, 20, 30, 40, as well as 50 g/ml, with a regression coefficient of 0.999 for each of these concentrations. For etravirine, the LOD readings were 0.514 g/ml, whereas the LOQ readings were 1.713 g/ml. Etravirine was shown to have a recovery rate of 98.8%.

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, RP-HPLC, Nano formulation, Nanoparticles.

1. Introduction

Nanotechnology is an incredibly growing field in the era of modern science. It is a booming field of this 21st century. The role of Nano technology is becoming very crucial in nearly every aspect of life ranging from cosmetics to advanced bio technological approaches. Nanotechnology is the field of science that mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule. In simple terms, Nanotechnology is the design, characterization, production and application of structures, devices, and systems by controlled manipulations of size and shape at the nanometer scale [1]. Nanoparticles are solid particles or particulate dispersions with diameters ranging from 10 to 1000nm. Use of nanoparticles of certain size, shape and morphology is also economical, and they have great potential and stability [2]. A nanoparticle matrix is used in dissolving, encapsulating, entrapping, encapsulating, or attaching a medicine. Nanomaterials have shown unique and considerably changed physical, chemical, and biological properties compared to their bulk counterparts [3, 4].

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 [5-8]. Particle size, characteristics, and releasing of pharmacologically active substances are the primary aims of producing nanoparticles like a delivery technique. This is done to produce site-specific drug activity at a therapeutically appropriate rate and dosage rule. The following are some of the benefits of employing nanoparticles as a medication delivery system: [9, 10].

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 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.

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 [11].

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 high efficiency of nucleic acid encapsulation, improved penetration, low cytotoxicity & immunogenicity. These above features make lipid nanoparticles outstanding applicants for nucleic acid approach. Lipid nanoparticles are used in treatment of HIV treatment [12].

Whereas combined treatment with antiretroviral therapy (HAART) is successful in decreasing viral loads to undetected levels in the blood of HIV-infected patients and extending life, considerable quantities of drug-resistant HIV persist in the lymphoid cells [13]. A originally developed LNP (lipid nanoparticle) preparedness usually contains HIV protease inhibitors which rapidly accumulate in HIV-2 infected macaques' lymph nodes, first near the site of injection and then throughout all nodes, delivering substantially higher concentration of drug in lymph nodes than oral or subcutaneously soluble drugs [14]. In monkey HIV models, LNP-mediated improved medication delivery considerably lowered viral load and delayed the pace of CD4+ T-cell decline [15].

Etravirine (ETR), a member of the diarylpyrimidine chemical family, is a nonnucleoside reverse transcriptase inhibitor of the second generation (NNRTI) [16]. 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 [17]. 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 [18]. Different detection platforms and analytical approaches, & LSPR (localized surface Plasmon resonance) nanotechnology have been investigated [19].

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 [20] and were utilized for chemical identification in biological and non-biological fluids [21]. Several High-Performance Liquid Chromatography techniques have been used to estimate ETR in dosage forms and human plasma [22].

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, 150mm x 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

The mobile phase used was acetonitrile and 10 mM ammonium acetate buffer (pH 4.5) in the ratio of 90:10 v/v. The ammonium acetate buffer was prepared by dissolving 770 mg of ammonium acetate in 1000 ml of HPLC grade water and pH 2.7 was adjusted to using glacial acetic acid. Before use the mobile phase was filtered through millipore membrane filter paper and degassed for 15 minutes by sonication.

i. Diluent

Diluents including mobile phase are employed.

ii. Standard solution

A standard stock solution of etravirine (1 mg/ml) was prepared by dissolving 100 mg of etravirine in 100 ml diluent solution. Working standard solutions was prepared from stock solution by proper dilution with the same solvent system.

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	13 min
Flow rate	1 ml/min
Injection volume	10 µl
Column temperature	26°C
Wavelength	310nm
LOD	0.514 µg/ml
LOQ	1.713 µg/ml
Column	Hypersilods C18 (150mm x 4.6 mm), 5 µ

2.2 Method validation

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 replicate 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 µg (25 to 150 %) 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 (µg)	Detected amount (µg)	% Recovery	Average % recovery
50%	12.5	12.3 12.3 12.4	98.4 98.4 99.2	98.6
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 (µg)	Concentration present (µg)	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

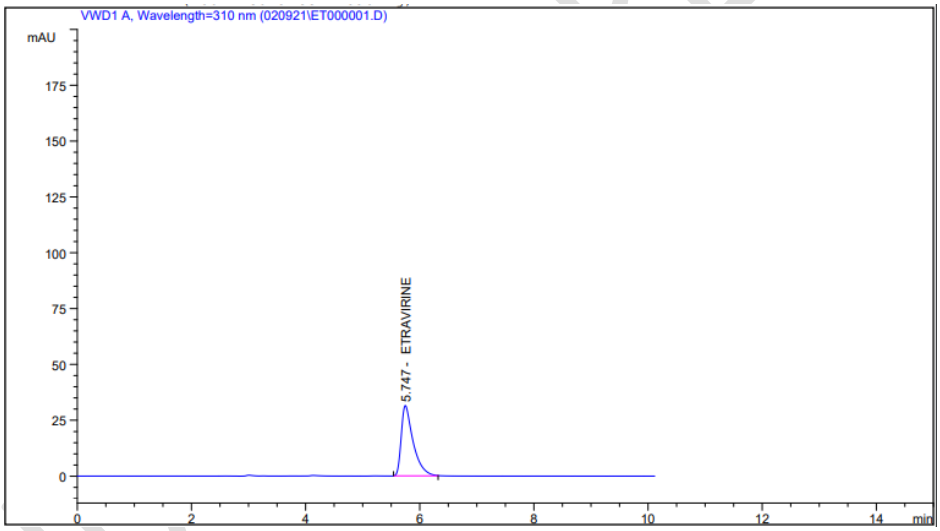
- **Linearity**

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

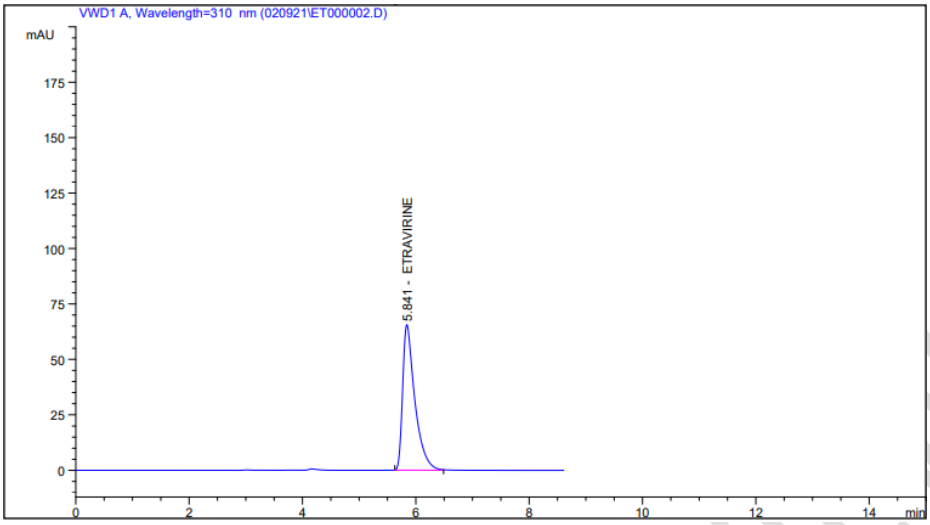
% Level	Concentration (µg/ml)	Peak area
27	11	163.008
50	20	339.13
75	30	499.27
100	40	670.498
125	50	844.534
150	60	1029.54
Y intercept		16.95
Correlation co-efficient (R^2)		0.999
Linearity range	10-60	
Slope		17.1489

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



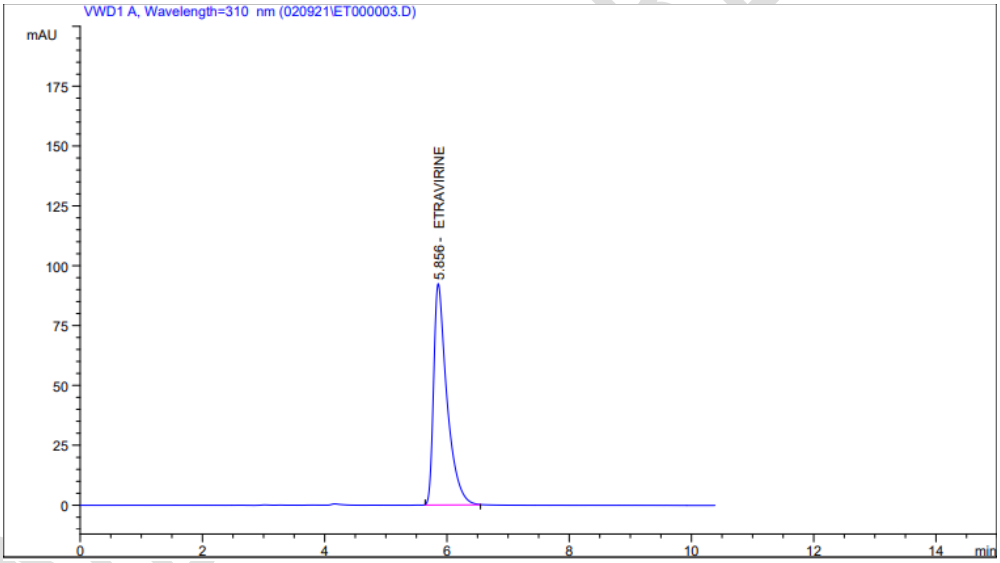
Graph 1 - linearity graph of etravirine at 10 µg

The Graph 1 describes the linearity of 10 µg where the retention time of peak value of etravirine is 5.747.



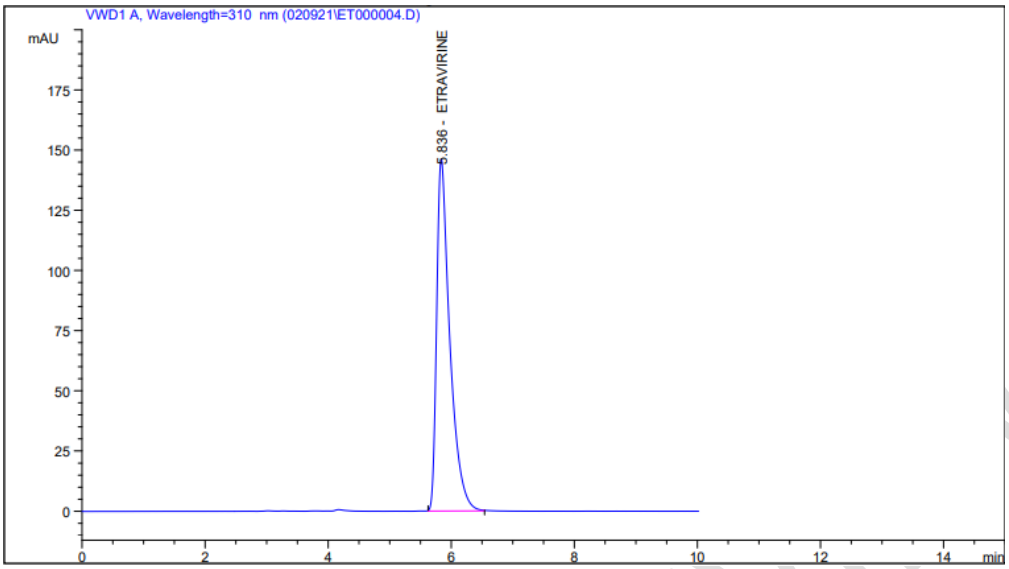
Graph 2 – linearity graph of etravirine at 20 µg

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



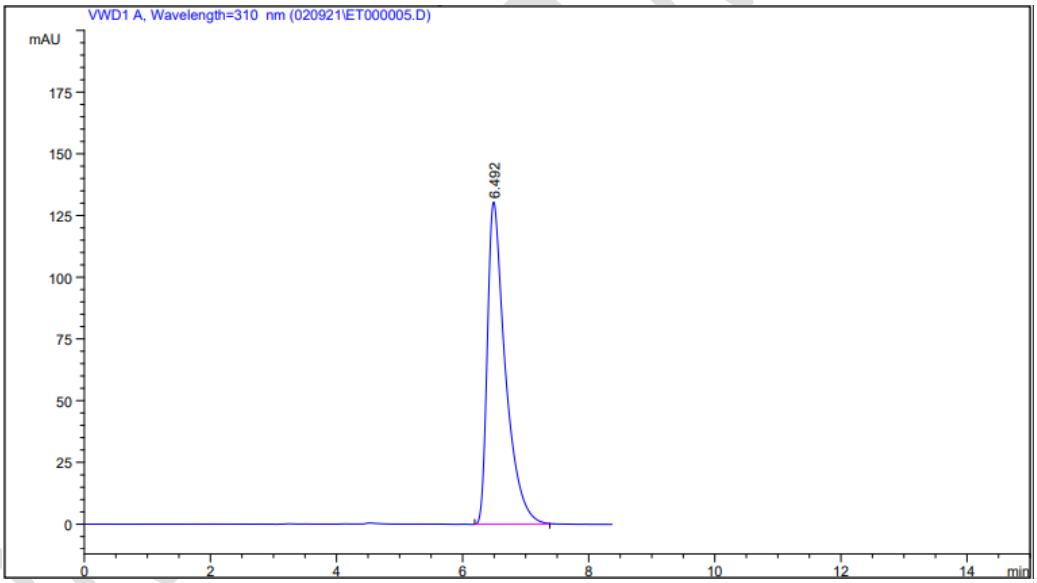
Graph 3 - linearity graph of etravirine at 30 µg

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



Graph 4 - linearity graph of etravirine at 30 µg

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



Graph 5 - linearity graph of etravirine at 40 µg

The Graph 5 shows the linearity of 50 µg, where the retention time of 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	524.673 - 529.788	527.230 ± 3.616	0.685
2	35° C	353.80 - 362.597	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
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",

- **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"

Table. 9 - LOD and LOQ results of etravirine

Parameters	Etravirine
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

Conclusion

The RP-HPLC technique was used to develop and validate the etravirine method. Hypersil C18 (150 mm x 4.6 mm, 5 µ) was employed for the estimate. Etravirine has a linearity range of 10 to 50 µg/ml. 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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