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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ETRAVIRINE

ALEEM AHAMED[1], KRISHNAMURTHY. G[1*], BHOJYA NAIK H. S[2] & RAMESHA S[2]

[1*] Department of Chemistry, Sahyadri, Science College, Jnana Sahyadri, Shimoga-577201, Kuvempu University, Karnataka, India, [2]Department of Studies and Research in Industrial Chemistry, School of Chemical, Sciences, Kuvempu University, Shankaraghatta 577451, Karnataka, India. Email: gkmnaik_sahyadri@yahoo.co.in

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ABSTRACT

A novel rapid, sensitive and reproducible mass compatible, ultra performance liquid chromatographic method was developed for quantitative determination of etravirine in active pharmaceutical ingredients and its dosage forms. Etravirine is a medicine used for the treatment of Human Immunodeficiency Virus (HIV) infection. It belongs to a group of anti-HIV medicines called non-nucleoside reverse transcriptase inhibitors (NNRTIs). The method is applicable to the quantification of related compounds and assay of etravirine drug. Chromatographic separation of drug from the possible impurities and the degradation products was achieved on an YMC's UltraHT *Pro* C18 50 x 3.0 mm, 2.0µm column; the gradient elution achieved with in 7.0 min. 0.1 % formic acid in water and Acetonitrile was used as mobile phase. The flow rate was 0.8 mL/min, column temperature 40°C and the detection was done at 310 & 250 nm. The above developed UPLC method was further subjected to hydrolytic, oxidative, photolytic and thermal stress conditions. The performance of the method was validated according to the present ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness.

Keywords*:* Etravirine; Forced degradation; Validation.

INTRODUCTION

Ultra performance liquid chromatography is a new category of analytical separation science that retains the practicality and principles of HPLC, while increasing the overall interlaced attributes of speed, sensitivity and resolution, which utilizes sub-2-µm particles for the stationary phase. Smaller particles provide not only increased efficiency, but also the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Hence this technique has gained considerable attention in recent days for pharmaceutical analysis. In this preset work the sub-2-µm particle technology has been applied for the method development and method validation of related compounds and assay determination of etravirine API and its dosage forms.

Etravirine is a diarylpyrimidine (DAPY), a type of organic molecule with some conformational isomerism that can bind the enzyme reverse transcriptase in multiple conformations, allowing for a more robust interaction between etravirine and the enzyme, even in the presence of mutations [1], etravirine can be used by patients infected with HIV that is resistant to other NNRTIs [2].

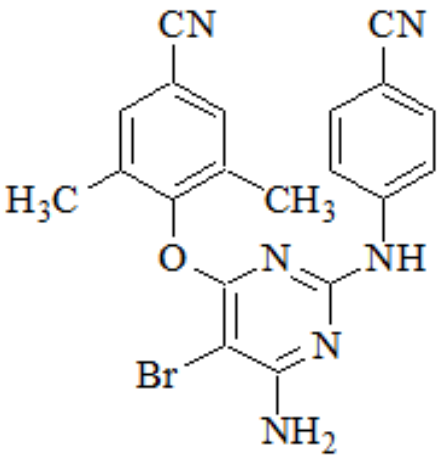
When HIV infects a CD4 cell in a person's body, it copies its own genetic code into the cell's DNA. In this way, the cell is then "programmed" to create new copies of HIV. HIV's genetic material is in the form of RNA. In order for it to infect CD4 cells, it must first convert its RNA into DNA. HIV's reverse transcriptase enzyme is needed to perform this process.

NNRTIs, also known as "non-nucleosides" or "non-nukes" for short, attach themselves to reverse transcriptase and prevent the enzyme from converting RNA to DNA [3, 4]. In turn; HIV's genetic material cannot be incorporated into the healthy genetic material of the cell, and prevents the cell from producing new virus [5].

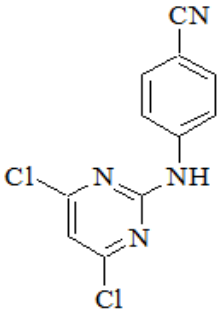
The objective of the work is to develop an economic, time-efficient, RP-UPLC method and demonstrate its stability-indicating capabilities by forced degradation followed with method validation. In this study, the kinetics of degradation of etravirine in solution and in solid state has been studied since it is a part of developmental strategy under the ICH requirements and is carried out under more severe conditions. These studies provides valuable information on stability of drug, the degradation pathways and storage of drug, and also helps in the validation of analytical methods to be used in stability studies [6-8]. Currently there are limited number of LC methods were reported for the determination of etravirine in pharmaceutical preparations. Mass spectrometry and LC have been reported for the determination of assays in human and rat plasma and its application in pharmacokinetic studies [9, 10] and some more methods for simultaneous determination of etravirine, and other antiretroviral drugs [11-13]. However there are no methods reported in the literature for the quantification etravirine and its related compounds. Hence a reproducible stability – indicating RP UPLC method was developed for the quantitative determination of etravirine and its seven impurities. This paper mainly deals with the forced degradation of etravirine drug product under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat, humidity and light. This paper also deals with the validation of the developed method for the accurate quantification of impurities and assay of etravirine in bulk drug samples.

MATERIALS AND REAGENTS

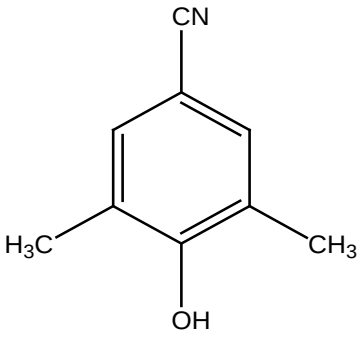
Active pharmaceutical ingredient and its related impurities (Fig.1) were procured from Manus Aktteva, India. Commercially available Intelence in 100 mg tablets was used for the dosage form analysis. Acetonitrile purchased from Merck, Darmstadt, Germany. Formic acid, purchased from spectrochem. HPLC grade water was prepared from Millipore and Milli-Q purification system.



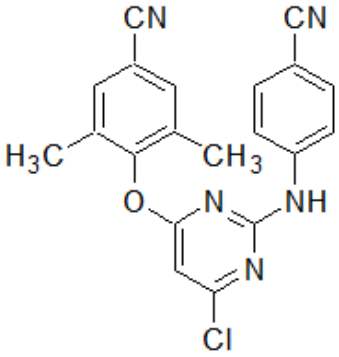
ETRAVIRINE



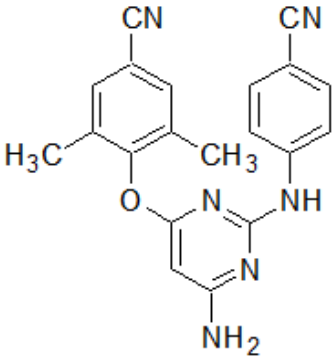
IMP A - 4[(4, 6-Dichloro-pyrimidin-2-ylamino)-benzonitrile



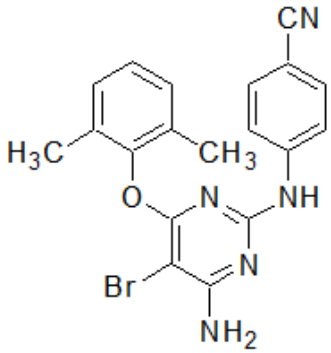
IMP B – 4-Hydroxy-3,5-dimethyl-benzonitrile



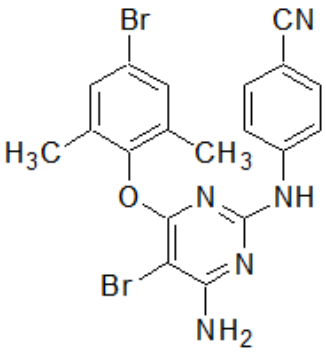
IMP C - 4-[[6-Chloro-2-[(4-cyanophenyl)amino]-4-pyrimidinyl]oxy]-3, 5-dimethyl benzonitrile.



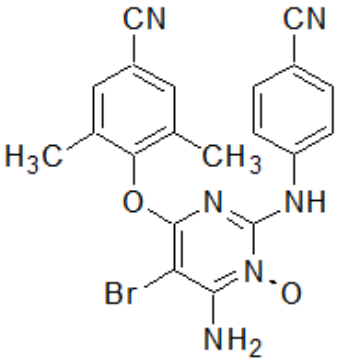
IMP D - 4-[[6-Amino-2-[(4-cyanophenyl)amino]-4-pyrimidinyl]oxy] -3, 5-dimethyl benzonitrile.



IMP E - 4-(4-Amino-5-bromo-6-(2,6-dimethylphenoxy)pyrimidin-2-ylamino) benzonitrile.



IMP F - 4-(4-Amino-5-bromo-6-(4-bromo-2,6-dimethylphenoxy)pyrimidin-2-ylamino) benzonitrile.



IMP G - 4-[[6-Amino-5-bromo-2-[(4-cyanophenyl)amino]-1-oxido-4- pyrimidinyl]oxy]-3,5-dimethylbenzonitrile.

Fig. 1: Structures of Etravirine and its impurities

Equipments

The Ultra performance liquid chromatograph (Waters) used was equipped with Photo diode array detector with gradient elution capacity and an auto sampler with data handling system (Empower software) on lenovo computer.

Chromatographic conditions

The chromatographic separation was achieved using a gradient method on an YMC's UltraHT *Pro* C18 50 x 3.0 mm, 2-µm column; the gradient Liquid chromatographic method employs solution **A** and solution **B** as mobile phase. The solution **A** contains 0.1 % formic acid in water and solution **B** is HPLC grade acetonitrile. The flow rate was 0.8 mL/min. The UPLC gradient program was set as Time / % Solution B: 0/35, 0.29/35, 4.18/60, 4.88/80, 5.50/80, 5.51/35 & 7.0/35. The column temperature was maintained at 40 °C, sample compartment temperature was maintained at 5 °C and the detection wavelength was 310 nm for identified and unidentified impurities & 250 nm for Impurity B. The injection volume of 1 µL.

Preparation of standards

A working standard of etravirine and its related compounds were prepared by appropriate weighing and respective dilution of impurity and reference standards in a mixture of mobile phase **A** & mobile phase **B** in the ratio of 10: 90 (% v/v) to yield a final concentration of Etravirine = 0.5 µg/mL, IMP A = 0.5 µg/mL, IMP B = 0.5 µg/mL, IMP C = 0.5 µg/mL, IMP D = 0.5 µg/mL, IMP E = 0.5 µg/mL, IMP F = 0.5 µg/mL & IMP G = 0.5 µg/mL.

Preparation of Samples

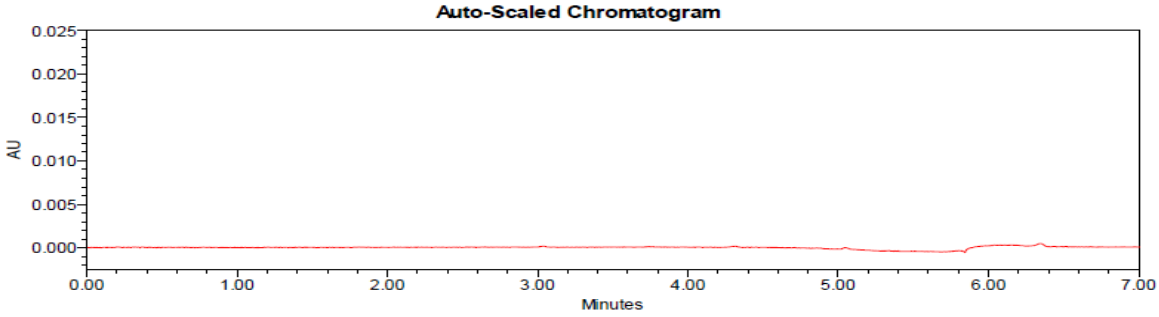
Five (n=5) etravirine 100 mg Intelence tablets were weighed, and the pellets were transferred into a clean, dry mortar. Pellets equivalent to 50 mg of the drug were dissolved in 100 mL of a mixture of mobile phase **A** & mobile phase **B** in the ratio of 10: 90 (% v/v). The resulting solution that was filtered through a 0.22 µm membrane filtered and used or a sample concentration equivalent to 0.5 mg/mL active pharmaceutical ingredient was prepared.

Degradation Studies

Specificity is the ability of method to measure the analyte response in the presence of its potential impurities and degradation products [13]. The specificity of the developed RP-UPLC method of etravirine was carried out in presence of its seven potential impurities, namely IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G.

Forced degradation studies were performed on for etravirine bulk drug (Fig. 2). Intentional degradation was attempted with stress conditions of UV light (254 nm), heat & humidity (60 °C at 70 % RH), acid (0.1 N HCl), base (0.1 N NaOH) and oxidation (3 % H_2O_2) to determine the ability of the proposed method to separate etravirine from its impurities and degradation products generated during forced decomposition studies . For heat and light studies, study period was 7 days where as for acid, base and oxidation it was 24 hrs. Peak purity test was carried out on the stressed samples by using PDA. Related compounds studies were carried out for stress samples against qualified reference standard. Related compounds were also calculated for bulk sample by spiking with its impurities at its specification level (0.1 %).

BLANK AT 310 nm



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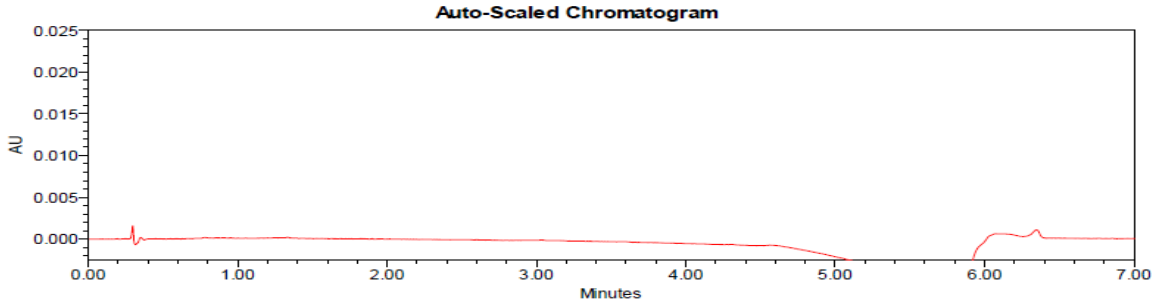
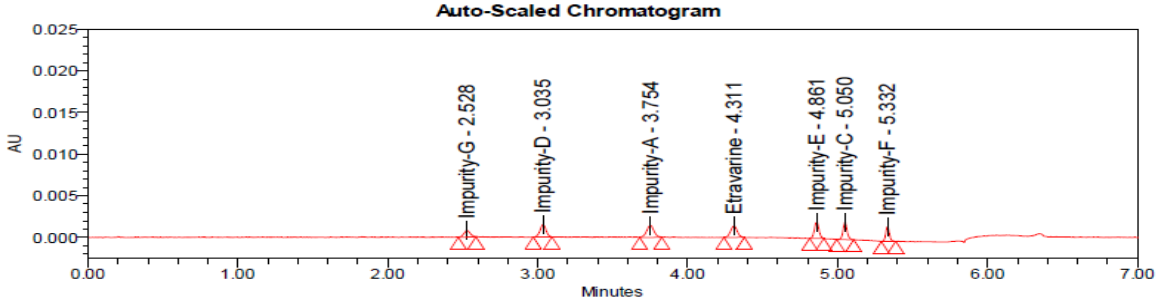


STANDARD AT 310 nm



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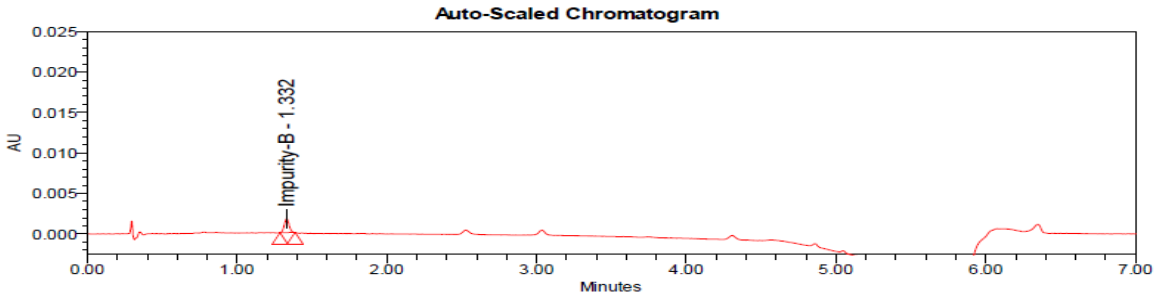
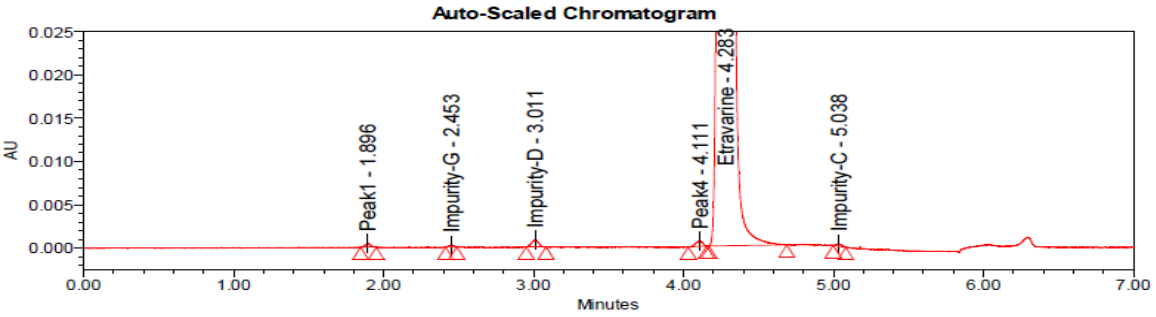
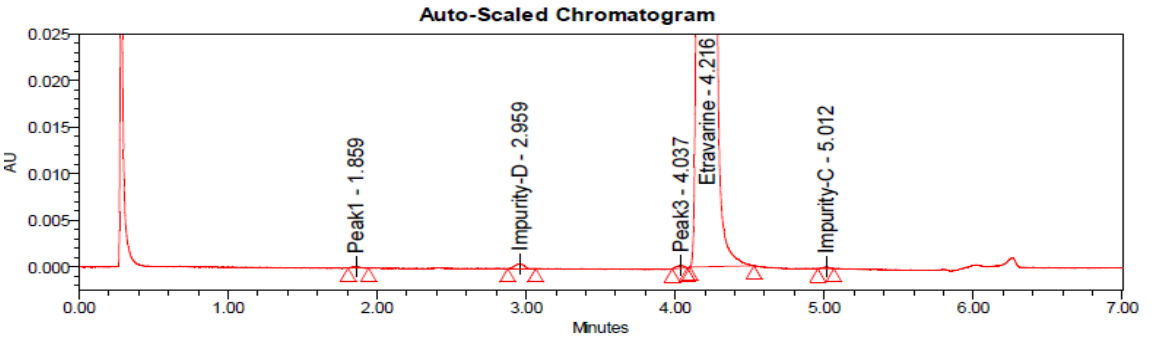


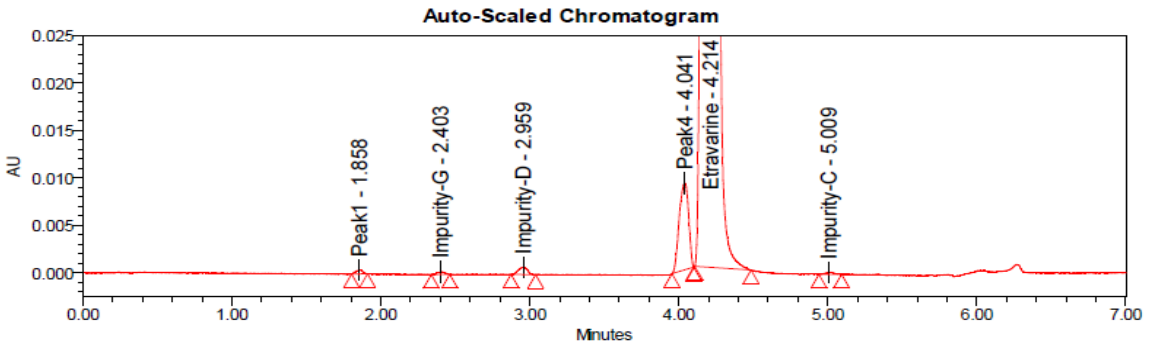
UNSTRESSED SAMPLE AT 310 nm



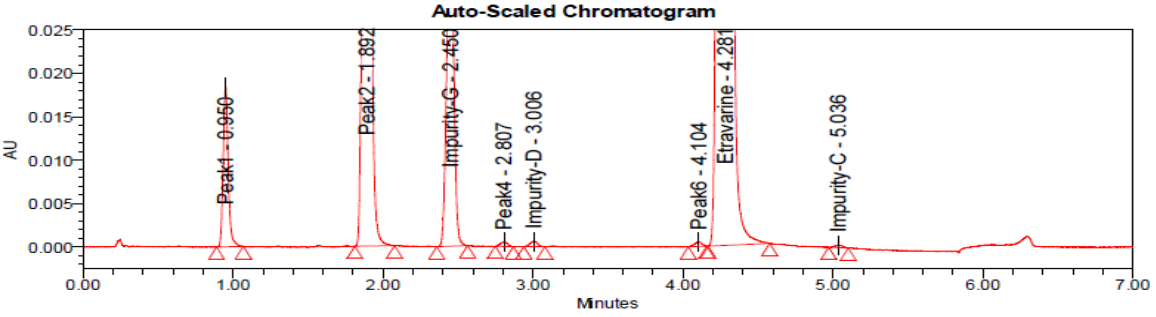
PEROXIDE DEGRADATION



ACID HYDROLYSIS



BASE HYDROLYSIS



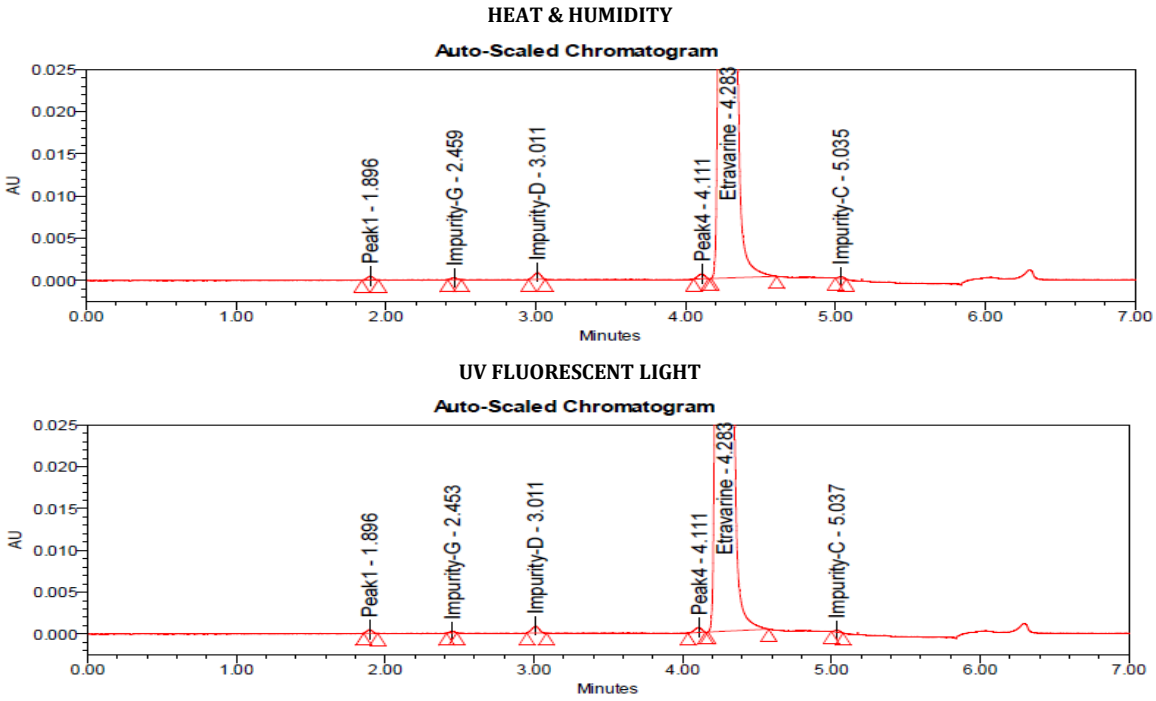


Fig. 2: Chromatograms of forced degradation studies

Method Validation

System & Method Precision

The system precision is indicated by the repeatability of multiple injections and indicates the performance of the UPLC instrument under the prescribed chromatographic conditions. The variance of the values obtained is represented as the percent relative standard deviation (% RSD). A working standard solution of etravirine and its related compounds was consecutively injected six times under the same analytical conditions. The % RSD of peak areas, difference of retention times, tailing factor (T) column efficiency (N) and resolution (R) are calculated. The intermediate precision of the method was also evaluated using one unspiked sample and 6 independent sample preparations spiked with a 100 % of the target concentrations as defined by the method. The samples were injected using a different instrument and column.

Linearity

The linearity is determined by the ability of the method to obtain test results, which are directly proportional to the concentration of the compounds of interest in the sample. Stock solutions were serially diluted to produce solutions containing concentration levels from QL to 160% with respect to impurity specification limits of 0.1 %. The calibration curve was drawn by plotting the peak areas of etravirine; IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G versus its corresponding concentrations. The % RSD value of the slope and Y intercept of the calibration curve was calculated.

Quantification limit (QL) and Detection Limit (DL)

The lower end of the linear range was considered to be the QL for the method. The QL concentrations were determined by injecting diluted standard solution to a level such that % RSD was not more than 10 %, precision study was also carried at the QL level by injecting six individual preparations of etravirine, IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G and calculating the % RSD of the area. The DL was theoretically calculated from QL using the following expression. DL = QL/3

Accuracy

Etravirine sample solution was spiked with impurity standard solutions containing IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G at three concentration levels corresponding to QL 100 % and 160 % of analyte concentration. The % recovery is the amount of the compound of interest analyzed as a percentage of the theoretical amount present in the medium were calculated from the slope and the Y intercept of the calibration curve.

Robustness

Deliberate variations in critical method parameters were done to assess the robustness of the related compounds method to evaluate method reliability. The flow rate of the mobile phase was 0.8 mL/min, to study the effect of flow rate on the resolution; it was changed by 0.1 unit from 0.7 to 0.9 mL/min. The effect of column temperature on resolution was studied at 35 and 45 °C instead of 40 °C.

Solution Stability

The stability of the analyte was established for standard and sample solutions under conditions as prescribed in the method. The purpose of this procedure was to determine the time during which the standard and sample solutions remain stable. In this validation, three solutions were studied: Stock standard solution, Working standard solution and Sample solution.

RESULTS AND DISCUSSIONS

Method development and optimization

Etravirine drug substance has a reported pKa of 3.5 and logP value of 5.2 [14]. The main aim of the chromatographic method is to achieve the separation of precursors, intermediates and the main component etravirine. From the UV profiling it was found that the suitable wavelength for the etravirine drug and its related impurities is 205 and 310 nm except for IMP B (205 & 250 nm). Hence it was concluded to work at dual wavelengths, anticipating the possible base line interferences at lower wavelength 310 nm was selected as the detection wavelength for the quantification of etravirine, its identified and unidentified impurities and 250 nm for IMP B. A change in mobile phase pH from 5.0 to 5.2 will cause the retention of etravirine to increase from 4.5 minutes to 5.6, an almost 11 to 12 % increase. However, at a mobile phase pH of 2.0, there is a negligible change in retention with an increase of 0.2 pH units. When developing a reversed phase method for basic compounds, like etravirine, you can expect a more robust method when using acidic mobile phases. Based on the experimental data & the opted wavelength it was found formic acid is suitable

The chromatographic separation was achieved on an YMC's UltraHT *Pro* C18 50 x 3.0 mm, 2-µm Column. The gradient liquid chromatographic method employs solution A and Solution B as mobile phase. Mobile phase **A** contains 0.1 % formic acid in water and mobile phase B is HPLC grade acetonitrile. The flow rate was 0.8 mL/min. The UPLC gradient program was set as Time / % Solution B: 0/35, 0.29/35, 4.18/60, 4.88/80, 5.50/80, 5.51/35 & 7.0/35. The column temperature was maintained at 40 °C, sample compartment temperature is maintained at 5 °C and the detection wavelength was 310 nm for identified and unidentified impurities & 250nm for Impurity B. The injection volume 1 µL. The peak shape of etravirine was found to be symmetric and well separated by its potential process impurities and degradants. In the optimized conditions, Etravirine, IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G. were well separated with a resolution greater than 3.5 and the typical retention times for Etravirine, IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G. were about 4.31, 3.75, 1.34, 5.05, 3.04 4.86, 5.33 and 2.53 respectively. The system suitability results were tabulated and the developed method for Etravirine and its impurities IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G. was found to be specific. (Table 1)

Results of Forced Degradation

Forced degradation samples were analyzed with a sample concentration of 0.5 mg/mL etravirine with above mentioned chromatographic conditions using a PDA detector to monitor the homogeneity and purity of the etravirine peak. Degradation was not observed under stress condition like, heat & humidity (60 °C & 70 % RH for 7 days) oxidative (3 % H_2O_2 at RT for 24 hours) and light exposure in solid state and liquid state. Very mild degradation of drug material was observed during acid hydrolysis (0.1 N HCl 24 hours at 80 °C) however the drug is more susceptible to base hydrolysis (0.1 N NaOH 24 hours at 60 °C) leading to the formation of IMP G (Fig. 2). The assay studies were carried out for the stress samples against an etravirine qualified reference standard. The mass balance (%assay + % sum of all related compounds + % sum of all degradants) were calculated for all of the stressed samples and were found to be more than 95 %. Peak purity test results obtained from PDA confirm that the etravirine peak was homogeneous and pure in all analyzed stress samples, which confirms the stability indicating power of the developed method

Table 1: Forced degradation results

Stress Condition	IMP A	IMP B	IMP C	IMP D	IMP E	IMP F	IMP G	Total Impurities	% Assay	Mass Balance
Acid Hydrolysis	ND	ND	0.03	0.09	ND	ND	0.04	1.3	98.1	99.4
Base Hydrolysis	ND	ND	0.03	0.06	ND	ND	5.58	19.5	78.6	98.1
Oxidative Degradation	ND	ND	0.02	0.06	ND	ND	ND	0.1	99.5	99.6
Thermal degradation	ND	ND	0.01	0.07	ND	ND	0.02	0.2	99.0	99.2
Photolytic degradation	ND	ND	0.02	0.07	ND	ND	0.01	0.2	98.7	99.9

Results of Method Validation

Precision

The injection (system) precision was evaluated by performing six replicate injections for the standard etravirine and its related compounds at 100 % working standard concentration. The % relative standard deviation of 6 injections was calculated, the % RSD for etravirine, IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G. are found to be 1.30, 1.30, 1.37, 1.81, 1.56, 0.39, 0.87 and 1.99% respectively. The RSDs of the % recovery values meet the requirement of not more than 10 % for all impurities. (Table 2)

Linearity

For all seven impurities and etravirine, a linear calibration curve was obtained ranging from QL to 0.16 %. The analytical data and linearity results for etravirine, IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G are tabulated in (Table 2).

The coefficient of determination (r^2) is 0.9975, 0.9990, 0.9978, 0.9999, 0.9997, 0.9997, 0.9980 and 0.9987 respectively, which meets the specification for the r^2 value of Not less than 0.99, confirming the linearity of the method.

Table 2: Summary of Method Validation

Validation Parameter	ETRA	IMP A	IMP B	IMP C	IMP D	IMP E	IMP F	IMP G
System Precision								
% RSD of peak area	1.30	1.30	1.37	1.81	1.56	0.39	0.87	1.99
% Difference of Retention time (last two std)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
% Difference of Retention time (last std and check std)	0.000	0.001	0.000	0.001	0.000	0.001	0.001	0.000
Resolution	6.23	8.4	-	3.58	6.37	7.64	6.02	-
Tailing Factor	0.94	0.93	1.06	1.14	1.04	1.02	1.10	1.12
Column efficiency	44198	27616	8215	189902	29895	129093	245422	12970
Linearity								
Slope	7987850	9258796	6902752	7024056	8279906	8362019	5739805	4984011
Intercept	390	-212	80	95	27	-112	34	-41
r^2	0.9975	0.9990	0.9978	0.9999	0.9997	0.9997	0.9980	0.9987
RRF	1.000	1.159	-	0.879	1.037	1.047	0.719	0.624
Quantitation limit(µg/mL)	0.0214	0.0216	0.0218	0.0318	0.0214	0.0208	0.0309	0.04128
Detection limit(µg/mL)	0.0071	0.0072	0.0073	0.0106	0.0071	0.0069	0.0103	0.01376
Accuracy Mean % Recovery at								
QL	NA	91.3 %	97.8 %	96.4 %	84.2 %	91.2 %	94.7 %	112.6 %
100 %		91.1 %	95.5 %	100.2 %	91.3 %	93.7 %	103.8 %	116.9 %
160 % of target		91.9 %	97.7 %	96.3 %	94.3 %	94.4 %	101.5 %	112.2 %
Intermediate Method Precision								
% RSD	1.25	1.48	1.60	1.82	1.55	1.28	1.22	2.38
Stability of Solutions								
Stock Standard Solution (5±3°C)	4 weeks	4 weeks	4 weeks	4 weeks	4 weeks	4 weeks	4 weeks	4 weeks
Working Standard Solution (5±3°C)	7 days	7 days	7 days	7 days	7 days	7 days	7 days	7 days
Working Standard Solution (Room temp)	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours
Sample Solution (5±3°C)	72 hours	72 hours	72 hours	72 hours	72 hours	72 hours	72 hours	72 hours
Sample Solution (Room temp)	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours	24 hours

Quantification limit (QL) and Detection limit (DL)

The quantification limit (QL) and detection limit (DL) of etravirine and its related impurities are tabulated (Table 2).

Accuracy

The related compounds of etravirine can also be determined accurately over a concentration range varying from QL to 160 % of their respective target analyte concentrations when in etravirine sample solution. The percentage recovery for the related compounds IMP A, IMP B, IMP C, IMP D, IMP E, IMP F & IMP G. was ranged from 83.24 to 118 (Table 2).

Robustness

In all the deliberate varied conditions (flow rate and column compartment temperature) the resolution between etravirine and its impurities was greater than 2.0, illustrating the robustness of method.

Solution Stability

The Stock standard solution, Working standard solution and Sample solution were prepared as per the method, after dispensing an amount for the testing of initial time , the solutions were stored in volumetric flasks and kept in refrigerator (5 ±3 °C) prior to the testing at each time interval of 1st week, 2nd week, 3rd week & 4th week for Stock standard solution and 24 hours, 48 hours, 72 hours and 7th day for Working standard solution and Sample solution, the flasks were taken out of the refrigerator, allowed to equilibrate to room temperature before use.

The % recovery of each analyte meets the requirement of 90 to 110 % after 4th week for Stock standard solution; however working standard is stable up to 7th day. No extra peaks detected, no peaks disappeared and no peak areas are increased or decreased by more than the respective QL level after 72 hours in case of sample solution. Therefore sample solution was found to be stable for 72 hours. However working standard and sample stored at room temperature showed a stability of 24 hours.

CONCLUSION

A stability indicating UPLC related compounds method was developed for the quantification of etravirine and its potential impurities in active pharmaceutical ingredients and its dosage forms. The developed method is specific, precise, accurate, linear and robust for etravirine and its impurities. Degradation products formed during forced decomposition studies were very well separated from analyte peak, which demonstrates that the developed method was specific and stability indicating. The run time of 7 minutes indicates excellence of sub 2µ particle size in terms of speed and selectivity. This method can be used to carry out the analysis of etravirine drug product in regular quality check and stability samples.

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REFERENCES

1. Das K, Clark AD, Lewi PJ, Heeres J, De Jonge MR, Koymans LM, Vinkers HM, Daeyaert F, Ludovici DW, Kukla MJ, De Corte B, Kavash RW, Ho CY, Ye H, Lichtenstein MA, Andries K, Pauwels R, De Béthune MP, Boyer PL, Clark P, Hughes SH, Janssen PA, and Arnold E: Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. J. Med. Chem 2004; 47: 2550–2560.
2. Van Herrewege Y, Vanham G, Michiels J, Fransen K, Kestens L, Andries K, Janssen P and Lewi P: A series of diaryltriazines and diarylpyrimidines are highly potent nonnucleoside reverse transcriptase inhibitors with possible applications as microbicides, Antimicrob. Agents Chemother 2004; 48: 3684–3689
3. Mordant C, Schmitt B, Pasquier E, Demestre C, Queguiner L, Masungi C, Peeters A, Smeulders L, Bettens E, Hertogs K, Heeres J, Lewi P and Guillemont J: Synthesis of novel diarylpyrimidine analogues of TMC278 and their antiviral activity against HIV-1 wild-type and mutant strains. Eur. J. Med. Chem 2007; 42: 567–579
4. Goebel F, Yakovlev A, Pozniak AL, Vinogradova E, Boogaerts G, Hoetelmans R, de Béthune MP, Peeters M and Woodfall B: Short-term antiviral activity of TMC278--a novel NNRTI--in treatment-naive HIV-1-infected subjects. AIDS 2006; 20: 1721–1726
5. Fang C, Bauman JD, Das K, Remorino A, Arnold E and Hochstrasser RM: Two-dimensional infrared spectra reveal relaxation of the nonnucleoside inhibitor TMC278 complexed with HIV-1 reverse transcriptase. Proc Natl Acad Sci USA 2007; 105: 1472-1477
6. ICH, text on Validation of Analytical Procedures, Q2A
7. ICH Validation of Analytical Procedures, Methodology Q2B
8. References for Validation: USP 30 – NF 25
9. Abobo CV, Wu L, John J, Joseph MK, Bates TR and Liang D: LC-MS/MS determination of etravirine in rat plasma and its application in pharmacokinetic studies. J. Chromatogr B Analyt Technol Biomed Life Sci.2010; 878(30):3181-3186
10. Heine RT, Rosing H, van Gorp EC, Mulder JW, Beijnen JH and Huitema AD. J. Phar. Bio. Anal 2009; 49: 393
11. Rezk NL, White NR, Jennings SH and Kashuba AD: A novel LC-ESI-MS method for the simultaneous determination of etravirine, darunavir and ritonavir in human blood plasma. Talanta 2009; 79: 1372
12. Quaranta S, Woloch C, Paccou A, Giocanti M, Solas C and Lacarelle B: Validation of an electrospray ionization LC-MS/MS method for quantitative analysis of raltegravir, etravirine, and 9 other antiretroviral agents in human plasma samples. Ther Drug Monit 2009; 31: 695-702
13. D'Avolio A, Baietto L, Siccardi M, Sciandra M, Simiele M, Oddone V, Bonora S and Di Perri G: An HPLC-PDA method for the simultaneous quantification of the HIV integrase inhibitor raltegravir, the new nonnucleoside reverse transcriptase inhibitor etravirine, and 11 other antiretroviral agents in the plasma of HIV-infected patients. Ther Drug Monit 2008; 30: 662-669
14. Frenkel YV, Clark Jr AD, Das K, Wang YH, Lewi PJ, Janssen PAJ and Arnold E: Concentration and pH dependent aggregation of hydrophobic drug molecules and relevance to oral bioavailability. J. Med. Chem 2005; 48: 1974.