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Development and Validation of RP- HPLC Method for the Determination of Raltegravir In Human Plasma

P. Srirama Murthy[1], V.V. Panakala Rao[1] and G. Rama Babu[2]*

1. Department of Chemistry, SKVT College, Rajahmundry-533103, E.G.Dt, A.P, **INDIA**
2. S.M.B.T.A & S.N. Degree College, Veeravasaram-534245, W.G.Dt, A.P, **INDIA**

Email: rbgodasi@rocketmail.com, srirammpasupuleti@gmail.com, vvpankalarao@gmail.com

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ABSTRACT

A rapid, specific and accurate high performance liquid chromatographic method for the determination of Raltegravir in human plasma using metronidazole as internal standard has been developed and validated. The separation was performed on a Phenomenex C_{18} (5µm; 250 X 4.6mm) column. The composition of the mobile phase is 60:40 (v/v) of 10 mM phosphate buffer (pH 3.5± 0.05) and acetonitrile. The peaks were detected by UV-Visible detection at a wavelength of 268 nm. The extraction process involved a simple liquid-liquid extraction technique using methyl-t-butyl ether. The method showed good linearity in the range of 40.0-4003.9 ng mL^{-1} with a sensitivity (limit of detection) of 40.0 ng mL^{-1} using 200 µL of K_2EDTA plasma. The mean recovery of Raltegravir from all the quality control samples is 78.71% with a coefficient of variation of 1.5% and recovery of internal standard was 73.2%. The intra-day accuracy at three levels of quality control samples ranged from 96.8 - 102.2% with a precision of 3.5 to 5.8%. The inter-day accuracy ranged from 94.3-103.5% with a precision of 2.7-7.7%. The peaks were well separated from the plasma interferences. The method is successfully validated as per FDA guidelines in human plasma containing K_2EDTA as an anti-coagulant.

Keywords: Raltegravir, antiretroviral, HIV-1 integrase inhibitor, HPLC.

INTRODUCTION

Raltegravir potassium is chemically, "N- [(4-Fluorophenyl) methyl]-1,6-dihydro5-hydroxy-1-methyl-2-[1-methyl-1-[[(5-methyl-1,3,4-oxadiazol-2-yl) carbonyl] amino] ethyl] -6-oxo-4- pyrimidinecarboxamide monopotassium salt" [1]. Raltegravir is a human immunodeficiency virus integrase strand transfer inhibitor, is indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus (HIV-1) infection [2].

Literature survey revealed that a few analytical methods have been reported for the determination of raltegravir in pure drug and in pharmaceutical dosage forms using HPLC [3-7] and LC-MS [8-11] either in single or in combined forms. Rambabu Kuchi *et al* [12] and Satyanarayana *et al* [13] developed HPLC methods for the analysis of Raltegravir in tablet dosage forms. One of the method reported by Jean Francois Jourdil *et al* [14] described an LC–MS/MS method in which 3 antifungals and one active

metabolite (posaconazole, voriconazole, itraconazole, and hydroxy-itraconazole), 5 antibiotics (daptomycin, ciprofloxacin, oxacillin, levofloxacin, and rifampicin), an antineoplastic agent (imatinib), and an antiretroviral (raltegravir) in human plasma were extracted using protein precipitation. Lauriane Goldwirt *et al* [15] developed and validated a precise and accurate high-performance liquid chromatography (HPLC) method with photodiode array detection for raltegravir.In this paper, we described a simple liquid-liquid extraction technique for the determination of Raltegravir using Metronidazole as internal standard. The sensitivity of the method is 40.0ng mL^{-1}. The method is developed in human plasma containing K_2EDTA as the anti-coagulant. The developed method was validated according to the FDA guidelines [16].

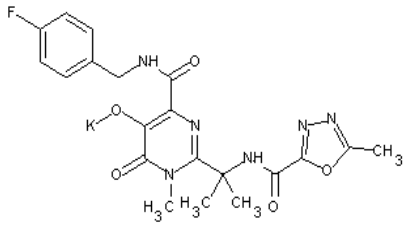

Fig.1. Structure of Raltegravir

MATERIALS AND METHODS

The list chemicals and reagents along with the instrumentation used for the experiment are given in table-1. The optimized chromatographic conditions are given in table-2.

Table-1: Chemicals & Reagents &Instrumentation

Chemicals & Reagents	Grade (Manufacturer)
Raltegravir and Metronidazole	M/s Aurobindo Pharma Limited
Ortho phosphoric acid and Potassium dihydrogen phosphate	Analytical Reagent Grade (Merck ltd)
Milli-Q water	In house
HPLC Configuration	Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Autosampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. SCL-10Avp System Controller was used to control the components of the system. Data analysis carried out using LC Solutions Version 1.23 software. Mobile Phase: a mixture of 60 parts of 10 mM mixed phosphate buffer (pH 3.5± 0.05) and 40 parts of acetonitrile.

Preparation of Standard Solutions: A stock solution of Raltegravir is prepared in a mixture of acetonitrile and water (50:50) such that the final concentration is approximately 1000.97µg mL^{-1}. Stock solution of Metronidazole (approx 1 mg mL^{-1}) is prepared in a mixture of acetonitrile and water (50:50). The solutions were stored below 10°C. Aqueous stock dilution of Raltegravir is prepared in diluent solution (mixture of 50: 50 % v/v of acetonitrile: HPLC Grade water).

Sample Preparation: Aqueous stock dilutions were prepared initially. 0.5 mL of each aqueous stock dilution is transferred into a 10 mL volumetric flask. The final volume is made up with screened drug-free K_2EDTA human plasma and mixed gently for 15 min to achieve the desired concentration of calibration curve standards. These calibration curve standards labeled from CC-01 to CC-08. Similarly, quality control samples were prepared in plasma such that the final concentrations were 40.2, 120.3, 2148.1 and 2902.8ng mL^{-1} respectively. These samples are labeled as Lower limit of quantification (LLOQ), Low quality control (LQC), median quality control (MQC) and high quality control (HQC) respectively. Each of these spiked calibration standards and quality control samples were distributed in disposable polypropylene

micro centrifuge tubes (2.0 mL, eppendorf) in volume of 0.3 mL and stored at -70°C until analysis.

Extraction procedure: The extraction of the plasma samples involved a simple liquid-liquid extraction technique. For processing, the stored spiked samples were withdrawn from the freezer and allowed to thaw at room temperature. An aliquot of 200 µL is then transferred to pre-labeled polypropylene centrifuge tubes (2.0 mL.) 50 µL of internal standard dilution (50 µg/mL) is then added and vortexed for ten seconds. 2.5 mL of methyl tertiary butyl ether is then added. The tubes were further vortexed for 10 min at 2200 rpm on a vibramax unit and then centrifuged at 4000 rpm for 5 min at 10^0C. 2mL of supernatant is then transferred into prelabelled polypropylene tubes and allowed to evaporate to dryness under nitrogen gas at constant temperature of 40 ^{o}C. The dried residue is reconstituted in 150 µL of mobile phase, vortexed thoroughly and transferred into shell vials containing vial inserts for analysis. 50µL of the samples is then injected into the system for analysis. The auto sampler temperature is maintained at 4^{o}C throughout the analysis. The column temperature oven is maintained at ambient temperature.

RESULTS AND DISCUSSION

Method development and optimization of the chromatographic conditions: Since LLE technique is known to give good sample clean-up and little or no noise (due to matrix components [17]), these authors adopted LLE technique. Interferences in LLE technique are due to the presence of phospholipids in the human plasma matrix [18]. For developing the method for the assay of Raltegravir, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all the other conditions constant. In order to get sharp peaks and base line separation of the components, the author has carried out a number of experiments by varying the commonly used solvents, their compositions and flow rate.Forideal separation of the drug under isocratic conditions, mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases on C_{18} stationary phase. A binary mixture of 10 mM phosphate buffer (pH 3.5± 0.05) and acetonitrile in the ratio 60:40 (v/v) was proved to be the most ideal of all the combinations with better defined and resolution chromatographic peaks, free from tailing. A mobile phase flow rate of 1.4 mL/min. was found to be suitable in the study range of 0.5 -1.5 mL min^{-1}.The detection of the drug was monitored at 268 nm.A representative chromatogram for the separation of Raltegravir is shown in fig. 2. In the optimized conditions (table 2), the retention times for Raltegravir and Metronidazole were found to be3.33 and 6.43 min respectively.

Table-2. Optimized Chromatographic Conditions

Parameter	Value
Column	Phenomenex C_{18}(4.6 X 250 mm, 5µ)
Mobile phase	10 mM potassium di hydrogen phosphate pH 3.5 buffer and acetonitrile (60:40 v/v)
Flow rate	1.4 mL/min
Run time	9.0 min
Column oven temperature	35 ± 2^0C
Auto sampler temperature	10^0C
Volume of injection	50 µL
Detection wave length	268 nm
Retention time of Raltegravir	3.33 min.
Retention time of Metronidazole	6.43 min.

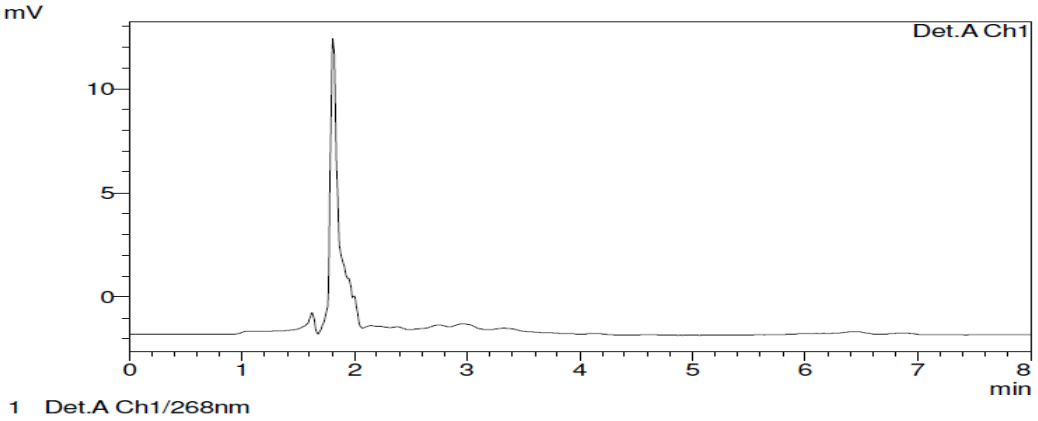

Fig. 2.1 Chromatogram of the extracted blank plasma sample

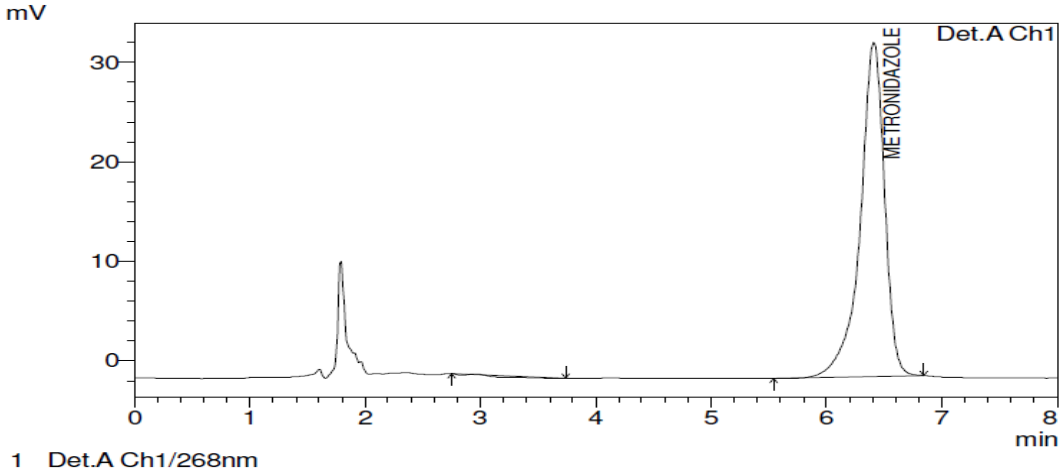

Fig. 2.2 Chromatogram of Metronidazole (IS) spiked blank plasma sample

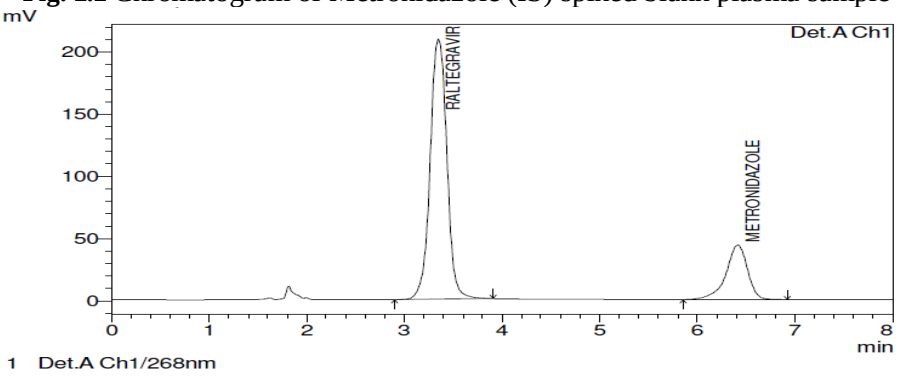

Fig. 2.3 Chromatogram of RALTEGRAVIR (drug) and Metronidazole (IS) spiked blank plasma sample

Validation of the Method: The quantitative HPLC-UV method was validated to determine selectivity, calibration range, accuracy and precision, limit of detection (LOD), limit of quantitation, % recovery, short term, long term, freeze–thaw, and auto sampler stability. The method was validated as per FDA guidelines.

Carry over test: Cary over test was determined by sequentially injecting the extracted blank plasma sample, the extracted ULOQ standard and again, the extracted blank plasma sample and then calculating the percentage of the residual analyte and the internal standard carried over to the latter blank plasma sample.

Screening of plasma lots and specificity: The selectivity of the present method was evaluated by checking the blank K_2EDTA plasma (without spiking with Raltegravir) obtained from different blood donors. Six different lots of blank plasma were screened and all of them were shown no interference in the blank plasma at the retention time of analyte and the internal standard. The same human EDTA plasma lots, free of interfering substances, were used for the preparation of the calibration curve standards and QC samples in the validation study. This result shows that all matrices under study meet the selectivity criteria (100%).

Linearity: The linearity of the method was determined by using bulk spiked plasma samples using the method of least squares. The standard curves were linear in the range of 40.0 - 4003.9 ng mL^{-1} as shown in Fig 3. The regression coefficient (r^2) is in the range of 0.9879 - 0.9999.

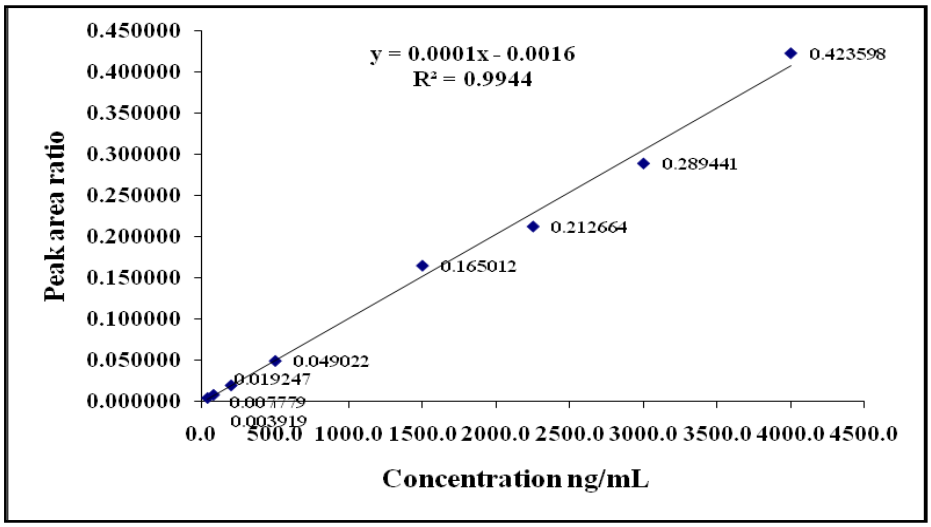

Fig 3: Calibration curve for RALTEGRAVIR (RALT CC1)

Precision and Accuracy: Accuracy and precision were evaluated from replicate analyses (n = 6) of Quality-control samples containing Raltegravir at different concentrations. All the results were found within the acceptable limit of precision (2.7-7.7%), and accuracy (94.3 -106.0%).

Recovery of the drug: From the knowledge of the areas of the extracted QC samples with equivalent aqueous samples, the percent recoveries were calculated. Mean Recovery for Raltegravir was found to be in the range of 77.7% - 81.0% (Mean Recovery: 78.71%).

Recovery of the internal standard: From the areas of the extracted IS samples with equivalent aqueous IS samples, the percent recovery of the IS (Metronidazole) was calculated. The mean recovery obtained for Metronidazole was found to be 73.2 %)

Stability of the drugs in stock solution

Short-term stability: Stock solutions of about 1.0 mg mL^{-1} of Raltegravir and Metronidazole (IS) were freshly prepared in methanol and a portion of the freshly prepared stock solutions (Stability samples), kept at a room temperature of ~25^0C for 6.5 h. The remaining portions of the above stock solutions were left in refrigerator below 10^0C, which can use as comparison samples. The Short-term stability of the stock solution of Raltegravir and Metronidazole in methanol was measured by comparing the mean of the responses of six replicates of the stability samples with that of the six replicates of the comparison samples. After keeping for 6.5 h at a room temperature of ~ 25^0C, the percent stabilities were found to be be 99.7 and 96.2 % for Raltegravir and Metronidazole respectively.

Long-term stability: Stock solutions (1mg mL^{-1}) of Raltegravir and Metronidazole were prepared freshly in methanol and stored below 10^0C in a refrigerator for 9 days. After recommended period of storage both drug and internal standard solution allowed to reach room temperature and samples with appropriate dilution were prepared. The long-term stock solution stability of Raltegravir and Metronidazole in methanol was determined by comparing the mean responses of six replicates of the stability samples with that of six replicates of comparison samples. After keeping for 9 days, the percent stabilities obtained were 95.7 % and 97.8 % respectively for Raltegravir and Metronidazole.

Stability of Drugs in Biological Matrix

Freeze-thaw stability: The Freeze-thaw stability of Raltegravir in human plasma was determined by measuring the assay precision and accuracy of the LQC and HQC samples over three freeze thaw cycles along with six replicates of the freshly spiked (FS) quality control samples (Comparison samples) at low and high concentration levels (known from a freshly prepared calibration curve). After completion of each cycle the samples were analyzed and the results were compared with that of zero cycle. The percent stability at low and high quality control concentration levels were calculated by comparing the mean of the concentrations of stability samples with the comparison samples. The Freeze-thaw stability values for the calibration curve standards of Raltegravir in plasma after 3 FT cycles were 96.8 % and 95.2 % at low and high concentrations respectively. All the stability results were found within the acceptable limit.

Bench top stability: The bench top stability of Raltegravir in human plasma was determined at 25^0C for 9.0 h by comparing the ratio of means of the concentrations for LQC and HQC. Six replicates of the freshly prepared quality control samples (Comparison samples) at low and high QC concentration levels (known from a freshly prepared calibration curve) were analyzed. The percent stability at low and high QC concentration levels was calculated by comparing the mean of the concentrations of the stability samples with that of the comparison samples. The Bench top stability values of Raltegravir in plasma after 9.00 hrs were 98.7 % and 101.4 % at low and high concentrations respectively.

In-injector stability or wet extract stability: In-injector stability of RALTEGRAVIR in plasma was estimated by storing the processed QC samples (Stability samples) for 48.00 h at 10^0C in the auto sampler and then analyzing them along with six replicates of freshly prepared quality control samples (Comparison samples) at low and high concentration levels (known from a freshly prepared calibration curve). The percent In-injector stability was calculated from the mean of the concentrations of stability samples stored at 10^0C for 48.00 h in the auto sampler and the mean of the comparison samples. The In-injector stability values of Raltegravir in human plasma after 48.00 h were 97.1% and 107.2% at low and high concentrations respectively.

Dry Extract stability: The stability of the extracted and dried samples of RALTEGRAVIR from plasma (without reconstitution), was measured using stored replicates (six) of the dried stability samples at < 10^0C for 32 h, by analyzing after appropriate reconstitution along with six replicates of freshly prepared quality control samples (Comparison samples) at low and high concentration levels (known from a freshly computed calibration curve). The percent stability is the mean concentrations of QC samples stored below 10^0C for 32 h and that of the comparison samples. The dry extract stability values of RALTEGRAVIR at a temperature below 10^0C for 32 h were 93.2% and 95.1% at low and high concentrations respectively.

Long-term stability in plasma matrix: For finding out the long-term stability of RALTEGRAVIR in human plasma the stability samples were stored at a temperature of -70ºC for 9.00 days. Then they were analyzed along with six replicates of the freshly prepared quality control samples (Comparison samples) at low and high concentration levels. The low and high concentration levels were read from the calibration curve. The quality control samples and the calibration curve standards were prepared by spiking the freshly prepared drug dilutions in screened blank plasma. The percent stabilities at low and high QC concentration levels were calculated by using the mean of the concentrations of the stability samples and the mean of the

comparison samples. The long-term stability values obtained for RALTEGRAVIR in human EDTA plasma at a temperature of -70°C for 9.00 days were 105.7% and 99.7% at low and high concentrations respectively.

Dilution Integrity: To determine the influence of dilution on the integrity of samples, six aliquots of 8007.8 ng mL^{-1} of Raltegravir were prepared. The samples were subjected to two fold and four fold dilution (n = 6) with drug free human plasma to bring them within the calibration range. The samples were processed, analyzed and the concentrations obtained were compared with theoretical values. The precision for dilution integrity of Raltegravir at 25 and 50 percent dilution were found to be 4.0% and 3.1% respectively. The accuracy for dilution integrity of Raltegravir at 25 and 50 percent dilution were found to be 99.2% and 101.0% respectively. The summary of the results of validation parameters were presented in table 3.

Table-3. Summary of the validation parameters

PARAMETERS	RESULTS
Screening of plasma lots and specificity	No significant interfering peaks were observed at the retention time of analyte (RALTEGRAVIR) and Internal standard (IS; Metronidazole).
Calibration Curve	**Calibration range:** 40.0-4003.9 ng/mL; **Accuracy:** 95.9-106.2 %**NominalPrecision:** 3.4-11.5 %CV **Correlation coefficient (r):** 0.9879-0.9999
Recovery	**RALTEGRAVIR:** Mean of % Recovery-78.71, % CV for % Recovery – 1.5 **Metronidazole (IS):** % Recovery- 73.20
Precision (%CV)	**Within-batch** (LLOQ QC): 4.9 to 8.8 LQC, MQC & HQC: 2.7 to 7.7 **Between-batch** (LLOQ QC): 6.5 LQC, MQC & HQC: 4.9 to 6.8
Accuracy (% Nominal Conc.)	**Within-batch** (LLOQ QC): 98.23 - 105.3 L, M & HQC: 94.3 - 106.0 **Between-batch** (LLOQ QC): 102.1 L, M & HQC: 97.2 to 103.1
Short term stock solution stability (6.5 hrs)	**RALTEGRAVIR Percent stability:** 99.7% **Metronidazole (IS) Percent stability:** 96.2%
Long term stock solution stability (9.00 Days)	**RALTEGRAVIR Percent stability:** 95.7% **Metronidazole (IS) Percent stability:** 97.8%
Freeze – Thaw Stability (3 Cycles)	**% Nominal:** 101.3 – 107.1 **% CV:** 1.6 – 4.8 **Percent stability:** At LQC Level: 96.8 At HQC Level: 95.2
Bench top stability (9.00 hrs)	**% Nominal:** 100.7 – 104.1 **% CV:** 2.7 – 3.9 **Percent stability:** At LQC Level: 98.7 At HQC Level: 101.4
In injector stability (48.00 hrs)	**% Nominal:** 97.9 – 107.0 **% CV:** 1.8 – 7.6 **Percent stability:** At LQC Level: 97.1 At HQC Level: 107.2%
Dry extract stability (32.00 hrs)	**% Nominal:** 94.9 – 108.7 **% CV:** 3.8 – 6.1 **Percent stability:** At LQC Level: 93.2 At HQC Level: 95.1
Long term stability in plasma (9 days at-70°C)	**% Nominal:** 97.8 – 108.7 **% CV:** 4.1 – 8.7 **Percent stability:** At LQC Level: 105.7 At HQC Level: 99.7
Dilution Integrity	**At 50% dilution:** Precision: 3.1 %CV Accuracy: 101.0 %Nominal **At 25% dilution:** Precision: 4.0 %CV Accuracy: 99.2 %Nominal

CONCLUSIONS

A HPLC method was developed and validated for the determination of Raltegravir in human plasma. The extraction process was a simple LLE procedure using methyl-t-butyl ether. This assay requires only a small volume of plasma (200 µL). There is no carryover effect. Matrix effects are not observed. In conclusion, method validation following FDA guideline indicated that the developed method had high sensitivity with an LLOQ of approximately 40.0 ng mL^{-1}, acceptable recovery, stability, specificity and excellent efficiency with a total running time of 9.0 min per sample, which is significantly economical when compared to the cost of LC-MS/MS analysis.

REFERENCES

[1] A. Savarino, A historical sketch of the discovery and development of HIV 1 integrase inhibitor, *Expert Opin. Investig. Drugs,* **2006**, 15(Suppl 12), 1507-1522.

[2] www.rxlist.com

[3] NL Rezk, NW Angela, DM Kashuba, An accurate and precise high performance liquid chromatography method for the rapid quantification of the novel HIV integrase inhibitor raltegravir in human blood plasma after solid phase extraction, *Anal Chim Acta,* **2008,** 628, 204-213.

[4] JM Poirier, P. Robidou, P Jaillon, Quantification of the HIV-integrase inhibitor raltegravir in human plasma by high performance liquid chromatography with fluorescence reduction, *J Chromatogr B,* **2008,** 867, 277-281.

[5] S Notari, C. Tommasi C, E. Nicastri, R. Bellagamba, M. Tempestilli, LP Percillo, Simultaneous determination of maraviroc and raltegravir in human plasma by HPLC-UV method, *Inter Union Biochem Mol Biol*, **2009**, 61, 470-475.

[6] JA Talameh, NLRezk, ADM Kashuba. Quantifying the HIV-1 integrase inhibitor raltegravir in female genital tract secretions using high performance liquid chromatography with ultraviolet detection, *J Chromatogr B,* **2010**, 878, 92-96.

[7] V.Furlan, AM Taburet, MD Cruz, Barrail- A.Tran, L. Goldwirt, Quantification of raltegravir in human plasma by high performance liquid chromatography with photodiode array detection, *J Chromatogr B,* **2010,** 878: 456-460.

[8] EP Acosta, MC Long, C. Bennetto-Hood A., sensitive HPLC-MS-MS method for the determination of raltegravir in human plasma, *J Chromatogr B,* **2008,** 867: 165-171.

[9] LA Decosterd, A Fayet, A. Beguin, B. Zanolari, S Cruchon, N. A.Guignard, LC Tandem MS assay for the simultaneous measurement of new antiretroviral agents: raltegravir, maraviroc, darunavir and etravirine, *J Chromatogr B,* **2009,** 877:1057- 1069.

[10] RT Heine, MJ Hillebrand, H Rosing, ECV Gorp, JW Mulder, JH Beijnen, AD Huitema, Quantification of the HIV-intergrase inhibitor raltegravir and detection of its main metabolite in human plasma, dried blood spots and peripheral blood mononuclear cell lysate by means of high performance liquid chromatography tandem mass spectrometry, *J Pharm Biomed Anal,* **2009,** 49:451-458.

[11] LZ Wang, LS Lee, WL Thuya, GH Soon, LR Kong, PL Nye, EJ Lee, C Flexner, BC Goh, Simultaneous determination of raltegravir and raltegravir glucuronide in human plasma by liquid chromatographytandem mass spectrometric method, *J Mass Spectrom,* **2011,** 46:202-208.

[12] K. Rambabu, K. Balamurali Krishna, Sambasiva Rao, New RP - HPLC Method Development and validation for Analysis of Antiviral drug Raltegravir, *International Journal of Research in Pharmaceutical and Biomedical sciences,* **2011,** 2 (1), 132-135.

[13] L. Satyanarayana, S.V. Naidu, M. Narasimha Rao, C. Ayyanna, Alok Kumar, The Estimation of Raltigravir in Tablet dosage form by RP-HPLC, *Asian J. Pharm. Ana,* **2011**; 1(3), 56-58.

[14] Jean Francois Jourdil, Julia Tonini, Francoise StankeLabsque, Simultaneous quantitation of azole antifungals, antibiotics, imatinib and raltegravir in human plasma by two-dimensional high-performance liquid chromatography–tandem mass spectrometry, *Journal of Chromatography B,* **2013,** Volumes 919-920, 1 March, 1-9.

[15] LaurianeGolwirt, AurelieBarrail-Tran, Maria Da Cruz, Anne-Marie Taburet, Valerie Furlan, Quantification of raltegravir (MK0518) in human plasma by high-performance liquid chromatography with photodiode array detection, *Journal of Chromatography B*, **2010,** 878(3-4), 456-460.

[16] Guidance for Industry, Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research, **2001**.

[17] Ann Van Eeckhaut, KatrienLanckmans, Sophie Sarre, Ilse Smolders, Yvette Michotte, Validation of bioanalytical LC–MS/MS assays: Evaluation of matrix effects, *J. Chromatogr. B,* **2009,** 877, 2198–2207.

[18] A. Omnia, Ismaiel, RTianyi, G. Zhang, Jenkins and H. Thomas Karnes, Investigation of endogenous blood plasma phospholipids, cholesterol andglycerides that contribute to matrix effects in bioanalysis by liquidchromatography/mass spectrometry, *J. Chromatogr. B,* **2010,** 878, 3303–3316.

AUTHORS' ADDRESSES

1. **Dr. P. Sriramamurthy**
S.G. Lecturer, Department of Chemistry,
S.K.V.T.College, Rajahmundry-533103, E. G. Dt, A.P, India
E-mail: srirammpasupuleti@gmail.com, Ph: 9848064332

2. **V. V. Panakala Rao**
S.G. Lecturer, Department of Chemistry,
S.K.V.T.College, Rajahmundry-533103, E. G. Dt, A.P, India
E-mail: vvpankalarao@gmail.com, Ph: 0883-2410723, 9705433111

3. **Dr. G. Rama Babu**
Reader, Department of Chemistry,
S.M.B.T.A.V & S.N.Degree College, Veeravasaram-534245, W. G. Dt, A.P, India
E-mail: rbgodasi@rocketmail.com, 08816-285656, 9441170125