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A REVIEW ON ANALYTICAL METHODS FOR ESTIMATION OF RALTEGRAVIR

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ABSTRACT

Raltegravir is the first antiretroviral drug in the class of integrase inhibitors approved for the treatment of Human immunodeficiency virus type 1 (HIV-1) in combination with other antiretroviral agents in the treatment experienced adults with evidence of ongoing viral replication and resistance to multiple antiretroviral drugs. Raltegravir inhibits HIV integrase to prevent the viral genome being incorporated into the human genome. They are generally administered as tablets. For determination of Raltegravir in bulk and pharmaceutical dosage form, several analytical methods including UV, HPLC, UPLC, LC-MS and HPTLC techniques are reported in literature. For qualitative and quantitative estimation of Raltegravir analytical methods can be used and also for the related degradants in bulk formulations and biological fluid. It is used alone or with other HIV medications to help control HIV infections. The present paper illustrates the review on analytical methods which involves the estimation of the antiviral drugs. This detailed review includes examination of analytical methods published during 2008 to 2020 using various techniques. The review also illustrates the scope and limitations of many published analytical methods for analysis of Raltegravir. Such detailed review will be of great help to the researcher who is working on Raltegravir drug.

INTRODUCTION

Raltegravir is an antiretroviral drug produced by Merck & Co., used to treat HIV infection. It received approval by the U.S. Food and Drug Administration (FDA) on 12 October 2007, the first of a new class of HIV drugs, the integrase inhibitors. Raltegravir inhibits HIV integrase to prevent the viral genome being incorporated into the human genome. Raltegravir is primarily metabolized by glucuronidation Integrase is essential for viral replication as it mediates the integration of the viral DNA genome into the host DNA resulting in the establishment of the permanent provirus. Persistent efforts have resulted in the discovery of Raltegravir (Isentress, MK-0518), the first integrase inhibitor approved by US Food and Drug Administration for the treatment in HIV-1 infected patients. Numerous clinical studies with raltegravir have found it to be safe and effective in treatment naïve as well as treatment experienced patients. Adverse events associated with raltegravir based therapy are milder compared to previously available regimens. Raltegravir is metabolized primarily via glucuronidation mediated by uridine diphosphate glucuronosyl transferase and has a favorable pharmacokinetics independent of age, gender, race, food, and drug-drug interactions. Within a short period of time of its introduction, raltegravir has

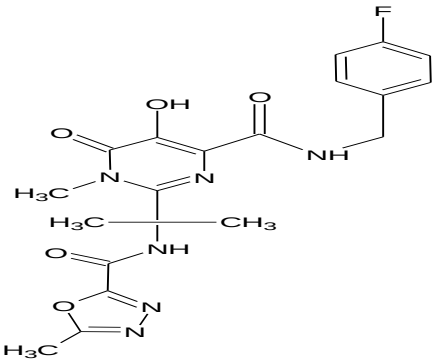
been included as one of DHHS recommended preferred regimen for the treatment of HIV-1 infection in treatment naïve patients.

CHEMICAL CHARACTERISTICS

Raltegravir in pharmaceutical industry is found as potassium salt, and most used as Raltegravir potassium. Raltegravir potassium is a White to off-white powder and its soluble in water, while it is slightly soluble in methanol, very slightly soluble in ethanol and acetonitrile, insoluble in isopropanol. The melting range for Raltegravir potassium is between 155-157° C, while the melting range for Raltegravir is 216 °C.

CHEMICAL TAXONOMY

This compound belongs to the class of organic compounds known as pyrimidine carboxylic acids and derivatives. These are compounds containing a pyrimidine ring which bears a carboxylic acid group.



N-[2-[4-[(4-fluorophenyl)methylcarbamoyl]-5-hydroxy-1-methyl-6-oxopyrimidin-2-yl]propan-2-yl]-5-methyl-1,3,4-oxadiazole-2-carboxamide

Molecular Formula: $C_{20}H_{21}FN_6O_5$

Molecular weight: 444.4 g/mol

PHARMACOKINETIC STUDIES

Absorption: Absorbed from the gastrointestinal tract.

Volume of Distribution: Approximately 83% bound to human plasma protein and is minimally distributed into red blood cells (blood-to-plasma partitioning ratio of 0.6).

Metabolism: In feces, only raltegravir was present, most of which is likely derived from hydrolysis of raltegravir-glucuronide secreted in bile as observed in preclinical species. Two components, namely raltegravir and raltegravir-glucuronide, were detected in urine and accounted for approximately 9 and 23% of the dose, respectively. The major circulating entity was raltegravir and represented approximately 70% of the total radioactivity, the remaining radioactivity in plasma was accounted for by raltegravir-glucuronide.

Route of Elimination: Through feces and urine.

Clearance: The major mechanism of clearance of raltegravir in humans is glucuronidation mediated by UGT1A1, the renal clearance of unchanged drug is a minor pathway of elimination of raltegravir (9% of total dose).

Analytical methods for Raltegravir for Estimation in Bulk drug & pharmaceutical formulation

UV Visible Spectrophotometry

Rapid and easy analytical methods are needed due to increasing number of multicomponentformulations, biotherapeutic products and samples of complex matrix in que. Number of Ultraviolet (UV) spectrophotometric methods used for these purpose. Different types of UV spectrometric methods developed on the basis of principle of additivity, absorbance difference, processing absorption spectra. The aim of this review is to present information on simultaneous equation method, difference spectrophotometry, derivative spectrophotometry, absorbance ratio spectra, derivative ratio spectra, successive ratio - derivative spectra, Q-absorbance ratio method, absorptivity factor method, dual wavelength method, absorption factor method, multivariate chemometric methods, and isosbestic point method

UV spectrophotometer is a highly simple instrument which makes it easier to couple with other analytical instrument such as RP-HPLC. Apart from this one UV spectrophotometric method had been conveniently adopted to develop a new better analytical method. Such method transfer and instrument compatibility are facilitated with UV spectroscopy UV spectrophotometric method based on simultaneous equation method and area under curve has been reported. Apart from

these uv spectrophotometric by chemometric approach has also been reported.

Bhavar G B et al., developed and validated a new simple spectrophotometric method for estimation of raltegravir potassium in bulk and pharmaceutical formulations. UV spectra of standard and tablet solutions of the drug in water found to be same. The UV spectrum of RALP in water has maximum absorption 331.6 nm. Thus obeyed Beer–Lambert's law in the concentration range of 1–100 μg/mL with coefficient of correlation (R2) was found to be 0.9999. The developed method found to be precise as %RSD values for intraday and interday precision were found to be less than 2%. The method was also found to be accurate indicated by % recoveries ranging from 99.1 to 100.1%. The recovery was obtained with values close to the 100 % of theoretical at three different concentrations. The results of analysis validated as per ICH guidelines.

Siddartha et al., developed and validated spectrophotometric method for estimation of Raltegravir in pure and pharmaceutical dosage form. In this method Raltegravir shows maximum absorbance found to be at 334nm using 0.1N NaOH as a solvent. Attempt has been made to develop rapid, economic, precise, accurate and sensitive analytical method for Raltegravir in pure pharmaceutical dosage form. Beer's law obeyed in concentration ranging from 10 to 60μg/ml. of Raltegravir with correlation coefficient 0.999 shows that absorbance was linear with concentration. The proposed method validated as per ICH Q2 (R1) guidelines for precision (Repeatability and Intraday and interday precision), linearity, accuracy and recovery. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.311μg/ml and 0.941μg/ml respectively by simple UV spectroscopy. The proposed method has been validated and effectively adopted for routine quality control of Raltegravir in bulk and formulated dosage form.

Sateesh Babu Dhulipalli et al.,developed and Validation of Chromatographic Method for Related Substances of Raltaglavir in Raltaglavir Tablets by Using Quality by Design (Qbd) Approach. The method was optimized by using an Inertsil (C18 x 2.5 μ) reverse phase column by following DoE approach. By employing DoE, a multivariate approach was carried out for Flow rate, Column Temperature, Organic solvent ratio in mobile phase and Buffer PH. A two level full factorial design is employed and statistical analysis of the experimental data is used to determine significant influential chromatographic parameters. The experimental data for optimization of USP resolution is critical ATP. Organic phase is identified as critical parameter interacting with the Flow and pH to achieve the desired resolution of NLT 1.8. The method was validated according to ICH guidelines for Accuracy, Precision, Linearity, Range, Specificity, Ruggedness and Robustness.

Annapurna et al., developed and validated a New three Analytical Techniques such as Zero order, first order derivative spectroscopy and differences spectroscopy for the Assay of Raltegravir in tablets (Anti-HIV Drug). Spectrophotometric techniques such as zero order (D_0), first-order derivative ($D_!$) and difference spectroscopic methods have been developed in water and phosphate buffers (pH 3,pH 4,and pH 5) for the determination of Raltegravir. Raltegravir has obeyed Beer-Lambert's law 1–150 μg/mL in zero order spectra(D_0) and 10–150 μg/mL in both first-order derivative ($D_!$) and difference spectroscopic methods have been developed for the determination of Raltegravir in pharmaceuticals dosage forms. Raltegravir has shown a wide range of linearity in all the methods , are simple, precise, accurate, economical and useful for the quality control testing of Raltegravir in pharmaceutical formulations.and all the methods were validated as per the ICH guidelines. These simple methods can be successfully applied for the assay of Raltegravir in tablets.

COLORIOMETRIC METHOD

Panigrahy Uttam Prasad et al., developed and validated the colorimetric method for the estimation of Raltegravir in pharmaceutical formulation and human

biological fluids using 3-methyl-2-benzothiazolinone hydrazone (MBTH) reagent. Based on oxidative coupling reaction of MBTH reagent with Raltegravir by using ferric chloride solution & HCl to produce bluish green chromogen at 572nm. Beer Lambert's law was obeyed over the concentration range between 80-220 µg/mL.The linear regression equation were found to be 0.004x+ 0.007 having r2=0.999. The method shows good precision & ruggedness with accuracy in between 99.85% -100.43% with % RSD less than 2%. The optical characteristics and various statistical reports were reported successfully and there was no interference of any excipients and reagents.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid. High performance liquid chromatography (HPLC) is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product.[1] The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase. Depending upon the partition behaviour of different components, elution at different time takes place. [2] The sample compound with the greater affinity to the stationary layer will travel slower and for a shorter distance in comparison to compounds with less affinity which travel faster and for a longer distance. [3]

The High Performance Liquid Chromatography is more versatile than gas chromatography since

(a) it is not limited to volatile and thermally stable samples, and

(b) the choice of mobile and stationary phases is wider.

Krishnaveni Nagappann, Sonam Patel et al., developed and validated linear Reverse-phase High-performance Liquid Chromatographic (RP-HPLC) method for the estimation of raltegravir potassium in the bulk and pharmaceutical dosage form. The chromatographic system employs a reverse phase shim-pack C18 column, (150 x 4.6 mm; 5 µ) using the mobile phase acetonitrile: (0.05 M) ammonium acetate buffer, (pH-4 adjusted with glacial acetic acid) in the proportion of 50:50 v/v, delivered at a flow rate of 0.8 ml/min with the detection wavelength of 271 nm. The developed method resulted in the retention of raltegravir at 4.31 min. Raltegravir potassium exhibited linear relationship (r2> 0.9999) over the analytical range 10-50 µg/ml. The precision was exemplified by a relative standard deviation of 1.60 %. The percentage recovery was found to be in the range of 100-102 %, during accuracy studies. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) was found to be 0.104 µg/ml and 0.315 µg/ml, respectively. An accurate, precise and linear RP-HPLC method was developed and validated for the quantitative estimation of raltegravir potassium in (20 mg, 50 mg) tablet as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

Tiwari R N et al., developed and validated RP-HPLC method for the simultaneous estimation of the Lamivudine (LAM) and Raltegravir (RAL) in laboratory prepared binary mixture. Separation was achieved on phenomenex C18 column (150 X 4.6 mm id, 5µ particle size) and mobile phase was composed of 75% methanol: 15% Acetonitrile: 10 % (0.05mM) phosphate buffer (at pH 3.0), with flow rate 1.2 ml/min at 254nm. Developed method was optimized by using Box Behnken Design (BBD) in response surface methodology (RSM). The independent variables such as the concentration of methanol, pH in mobile phase and flow rate were selected for the optimization and Retention time (Rt) were

used as responses for both drugs. Derringer's desirability function was used to concurrently optimize the selected responses. The LOD and LOQ were found to be 1.04 and 3.18 µg/ mL for LAM and 0.36 and 1.08µg/mL of RAL. The percentage recoveries were found to be less than 2% for LAM and RAL. Retention time of LAM and RAL was 3.13±0.07 and 7.27±0.01 minutes respectively. The developed and optimized method was fully validated. The validated method further can be potentially used for estimation of these drugs in combined dosage form.

Uluri Krishna Dutta Tejaswi, R. Govinda Rajan et al., reverse phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of the combined tablet formulation of lamivudine (LAM) and raltegravir (RAL) in dosage forms and its API. Chromatographic separation was achieved on inertsil ODS C18 5 µm (4.6 X 150 mm) using a mobile phase (MP) consisting of a mixture of mixed orthophosphoric acid (OPA): acetonitrile (ACN) in the ratio 50:50 v/v which was determined at 242 nm respectively.The assay of LAM and RAL was performed with tablets, and the % assay was found to be 100.12 and 99.89 which shows that the method is useful for routine analysis. The linearity of LAM and RAL was found to be linear with a correlation coefficient of 0.998 and 0.999 shows that method is capable of producing good sensitivity. The retention time of LAM and RAL was 1.99 min and 4.34 min respectively; linearity range was found to lie from 15 µg/ml to 75 µg/ml for LAM, 30 µg/ml to 150 µg/ml for RAL with a correlation coefficient of 0.999 respectively. Forced degradation studies were conducted in acidic, basic, thermal, photolytic and peroxide where all the degradation peaks were monitored. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of LAM and RAL in bulk and tablet dosage form. The validated economical method was applied for forced degradation study of LAM and RAL tablet

HPLC WITH FLUORESCENCE DETECTION

Talameh J A, et al.,developed HLPC method with fluorescence detection for the accurate determination of the first licensed HIV integrase inhibitor raltegravir in human plasma. A 500 L plasma sample was spiked with delavirdine as internal standard and subjected to liquid–liquid extraction based on a previously described assay i.e. using hexane/methylene chloride (1:1, v/v%) at pH 4.0. HPLC was performed using a Symmetry Shield RP18 column (150mm×4.6 mm), a gradient elution of acetonitrile −0.01% (v/v) triethylamine in water adjusted to pH 3.0 at a flow rate of 1 mL/min and a fluorimetric detector set at 299 and 396nm as excitation and emission wavelengths, respectively. The retention time was 5.0 min for internal standard and 6.4 min for raltegravir. Calibration curves were linear in the range 5–1000 ng/mL and the accuracy of quality control samples in the range 10–750 ng/mL varied from 98.3 to 99.1% and 98.3 to 101.0% of the nominal concentrations for intra-day and day-to-day analysis, respectively with a precision of 6.3% or less. Among the other antiretroviral drugs which can be given in association to HIV infected patients, none was found to interfere with internal standard or raltegravir. The described assay was developed for the purpose of therapeutic drug of this HIV integrase inhibitor.

HPLC WITH PHOTODIODE ARRAY DETECTION

Poirier J M et al., developed high-performance liquid chromatography (HPLC) method with photodiode array developed and validated for raltegravir, a human immunodeficiency virus integrase strand transfer inhibitor (HIV-1 INSTI). Plasma (300L) was extracted with dichloromethane/hexane 50:50 (v/v) after addition of the internal standard, 6,7-dimethyl-2,3-di(2-pyridyl) quinoxaline. The compounds were separated using a dC18 column and detected with ultraviolet detection at 320 nm. The limit of quantification was 10 ng/mL for raltegravir. The method was linear and validated over a concentration range of 0–10,000 ng/mL. The

intra-day precision ranged from 3.1 to 12.3%, while the intra-day accuracy ranged from −15.0 to −0.5%, the inter-day precision and accuracy were less than 7%. The mean recovery was 76.8%. Application to clinical samples taken from patients treated with raltegravir indicated that the method is suitable for measuring plasma concentrations of raltegravir in pharmacokinetic studies of clinical trials.

HPLC WITH UV

Talameha J A et al., developed Quantifying the HIV-1 integrase inhibitor raltegravir in female genital tract secretions using high-performance liquid chromatography with ultraviolet detection. Understanding the pharmacokinetics of drugs in peripheral body compartments, such as the genital tract, is particularly important in the infectious diseases arena. However, extracting drugs from small volumes of viscous, proteinacious substances like cervicovaginal fluid is particularly challenging. The goal of this study was to develop a method to quantify raltegravir, an HIV-1 integrase inhibitor, in the female genital tract. The method included sample preparation with perchloric acid followed by solid-phase extraction,separation with reverse-phase high-performance liquid chromatography, and detection with an ultraviolet wavelength of 218 nm. The method was linear from 0.05 to 10.0 mg/L, with minimal endogenous interference. The method was accurate (1.2–11.0% deviation) and precise (1.1–12.6% CV) for both within and between-day analyses. The ability to detect raltegravir in the female genital tract is essential for future investigations of raltegravir as an agent for prevention of HIV acquisition, and this method will be used for clinical studies further evaluating pharmacokinetic–pharmacodynamic relationships in this body compartment

High Performance Thin Layer Chromatography

HPTLC is a fast separation technique and flexible and to analyze a wide variety of samples. This technique is very advantageous it is simple to handle and requires a short time to analyze. It is suitable for qualitative and quantitative analysis. Chromatographic with the advancement of the technique, high performance thin layer chromatography (HPTLC) emerged as an important instrument in technique method

HPLC AND HPTLC

Sudha T et al., a high performance liquid chromatography and high performance thin layer liquid chromatography method were developed and validated for the quantitative determination of Raltegravir potassium in pharmaceutical dosage form. The different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ) were determined according to the international conference of harmonization ICH 2QB guidelines. In RP-HPLC method the drugs were resolved using a mobile phase at phosphate buffer pH-3.0: methanol (45:55%v/v) with pH adjusted to 3.0 using phosphoric acid on a C18 symmetry (150mmX4.6mm,5µ) column in isocratic mode. The retention time of Raltegravir was 4.3 min. In HPTLC method the chromatograms were developed by using a mobile phase of Toluene: ethyl acetate: methanol: glacial acetic acid (4: 5: 0.6: 0.4%v/v) on precoated plate of silica gel 60F254 and quantified by densiometric absorbance mode at 218nm. The Rf value of Raltegravir was 0.12. Recovery value at 98.36% to100.18%, %RSD of 0.9213 and correlation coefficient (linear dynamic range) at r2=0.9998 shows that the developed methods were accurate and precise. These methods can be employed for the routine analysis of tablets containing Raltegravir potassium

Ultra Performance Liquid Chromatography

Ultra performance liquid chromatography (UPLC) is a new category of separation technique it is a principles of liquid chromatography. Combination of UPLC with a tandem mass spectrometer (MS/MS) appears to be a suitable approach that gives sensitivity and selectivity for the rapid determination of an analysis at low concentration in complex matrices.

UPLC

Rami Reddy B V et al., An Isocratic, reverse-phase Ultra performance liquid

chromatographic (RP-UPLC) method was developed for the determination of assay of Raltegravir Potassium, an active pharmaceutical ingredient used to treatment of HIV. The chromatographic separation was achieved on UPLC with BEH Shield 100x2.1mm, 1.7µm column. The UPLC method employs for assay, a mixture of Sodium perchlorate (0.2g in 1000mL of water, pH 2.5±0.05 with perchloric acid) and acetonitrile in the ratio of 65:35(v/v) used as Mobile Phase. The flow rate was kept 0.3mL/minute, injection volume 0.3µL, column temperature kept at 30°C and the detection wavelength was monitored at 240nm. In the developed UPLC method the raltegravir peak is homogeneous and it is well separated from all other known and unknown impurities. The drug was subjected to stress conditions such as hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in Acid, base and Oxidative stress conditions. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.2%. The developed RP-UPLC method was validated as per ICH guidelines. Sarif Niroush Konari , Jane T. Jacob demonstrated the technique to be simple and quick approach of stability indicating UPLC technique established for the simultaneous determination of newly invented anti-HIV drug combination of raltegravir and lamivudine in bulk and pharmaceutical combo within a 4 min of chromatographic run. UPLC separation of the two drugs attained with a BEH Shield RP18 (2.1 mm,100 mm, 1.7 mm) as stationary phase, analytical column using buffer potassium dihydrogen orthophosphate pH 3 adjusted with orthophosphoric acid:Methanol (30:70, %v/v) as mobile phase in isocratic mode at a flow rate of 0.230 ml/min. The column maintained at an ambient temperature. Detection of two drugs examined at 254 nm using a PDA detector WHO study reveals approximately 2.1 million people recently infected with HIV, including 240,000 children below 15 years of age in the year 2013. This method of analysis could be applied for the safety, efficacy and quality of the drug in a cost effective manner The established methods validated as per ICH guideline and stability study revealed that the technique was useful in monitoring drug stability. It could be applied for routine analysis in Hospital research institutions in therapeutic drug monitoring for clinical trials, Bioanalytical Laboratory, dissolution studies of the formulations, in quality control division of pharmaceutical company and in accredited testing laboratories

Table No.1 Summary of spectroscopic methods used in analysis of Raltegravir and its combination(s)

Sl.No	Name of drug	max	Method	Concentration/ Range R2	LOD/LOQ & Recovery
1	Raltegravir	334nm	UV-Visible spectroscopy	10 to 60µg/ml	0.311µg/ml 0.941µg/ml
2	Raltegravir potassium	331.6 nm	UV- Visible spectroscopy	1–100 µg/mL	99.1 to 100.1%.
3	Raltegravir potassium	323-333 nm	UV-Visible spectroscopy	3-55 µg/ml	0.91 µg/ml 1.18 µg/ml 99.36 to 102.31%.

Table No.2

Summary of HPLC and LC/MS methods used in analysis of Raltegravir and its Combination

Sl. No	Drug	Column	Mobile Phase	Flow Rate	Retention Time	Detector	Concentration Range	R2/LOD/ LOQ Recovery
1	Raltegravir potassium	Shim-pack C18 column	acetonitrile: (0.05 M) ammonium acetate buffer, (pH-4 adjusted with glacial acetic acid)	0.8 ml/min	4.31 min	271 nm	10-50 µg/ml	(r2> 0.9999) 0.104 µg/ml and 0.315 µg/ml
2	Lamivudine and Raltegravir	Phenomenex C18 column	Methanol: Acetonitrile: (0.05Mm)Phosphate buffer (75:15 :10)	1.2ml/ min	2 min and 6.34 min	254nm	10-50 µg/ml	1.04 and 3.18 µg/ mL for 0.36 and 1.08µg/mL.
3	Raltegravir and Lamividine	Inertsil ODS C18	mixed orthophosphoric acid (OPA): acetonitrile (ACN) in the ratio 50:50 v/v		1.99 min and 4.34 min	242 nm	15 µg/ml to 75 µg/ml for LAM, 30 µg/ml to 150 µg/ml for RAL	0.25 mg/L
4	Raltegravir	Symmetry Shield RP18 column	Acetonitrile −0.01% (v/v) triethylamine in water adjusted to pH 3.0	1ml/ min	5.0 min	299 and 396nm	10-50 µg/ml	0.104 µg/ml and 0.315 µg/ml
5	Darunavir and raltegravir	Tracer Excel 120 ODSB (15x0.4.6 cm) column	mixture of 0.037 M sodium dihydrogen phosphate buffer, acetonitrile and methanol (40:50:10, v/v/v)	2ml/ min	1.7 minutes 2.2 minutes.	254 nm	5–100 mg/L.	0.25 mg/L
6	Raltegravir and Maraviroc	Symmetry C18 Column (150mm x 4.6mm)	Acetonitrile : Phosphate buffer (60:40v/v)	1.0ml/ min	2.42 min 3.71min	239nm	10-50 µg/ml 5-25 µg/ml	97-102%

Table No.3 Summary of HPTLC methods used in the analysis of Raltegravir and its combination(s)

Sl.No	Name of drug	Mobile phase	Rf value	Concentration	LOD/LOQ & Recovery
1	Raltegravir Pottasium	Toluene:ethyl acetate:methanol: glacialacetic acid (4:5:0.6:0.4%v/v)	0.12	0.104 µg/ml and 0.315 µg/ml	98.36% to100.18%,

Table No.4 Summary of UPLC methods used in analysis of Raltegravir and its combination(s)

S.no	Drug	Column	Mobile Phase	Flow Rate	Retention Time	Detector	Concentration Range	R2/ LOD/ LOQ
1	Raltegravir Potasium	BEH Shield 100x2.1mm, 1.7µm column	Sodium perchlorate (0.2g in 1000mL of water, pH 2.5±0.05 with perchloric acid) and acetonitrile in the ratio of 65:35(v/v)	0.3mL/ min	6min	240nm	55mg/ml	1.99-4.34 mg/ml
2	Raltegravir and Lamivudine	BEH Shield RP18 (2.1 mm _ 100 mm, 1.7 mm),	buffer potassium dihydrogen orthophosphate pH 3 adjusted with orthophosphoric acid:methanol (30:70, %v/v)	0.230 ml/min.	0.50min 1.00min	254 nm	Standard solution of 45, 90 mg/ml of LMV and RAL	0.396 - 0.248 mg/ml 1.350 - 0.81 mg/ml) 50, 100 and 150%

SPECTROSCOPIC METHOD

UV HPLC

Servicio de Farmacia and team developed and validated a high-performance liquid chromatography (HPLC) method with ultraviolet detection for quantification of darunavir and raltegravir in their pharmaceutical dosage form. The assay enables the measurement of both drugs with a linear calibration curve (R2 = 0.999) over the concentration range 5–100 mg/L. The determination was performed on an analytical Tracer Excel 120 ODSB (15x0.4.6 cm) column at 35ºC. The selected wavelength was 254 nm. The mobile phase was a mixture of 0.037 M sodium dihydrogen phosphate buffer, acetonitrile and methanol (40:50:10, v/v/v) at a flow rate of 2.0 mL/min Nevirapine (50 mg/L) was used as internal standard. Accuracy, intra-day repeatability (n = 5), and inter-day precision (n = 3) were found to be satisfactory, being the accuracy from -4.33 to 3.88% and precisions were intra-day and inter-day, 0.25% and 4.42% respectively in case of darunavir. Raltegravir intra-day and inter-day precisions lower of 1.01 and 2.36%, respectively and accuracy values bet from -4.02 to 1.06%. Determination of the darunavir and raltegravir in their dosage form was done with a maximum deviation of 4%. This analytical method is rapid, easily implantable and offers good results.

LIQUID CHROMATOGRAPHY- MASS SPECTROSCOPY

Wang Ling-Zhi et al., Raltegravir is a highly efficacious inhibitor of HIV integrase. Large pharmacokinetic variability has been reported in clinical trials and this could be due to glucuronidation of raltegravir, the only reported metabolism pathway. In order to precisely evaluate and monitor the raltegravir and raltegravir glucuronide simultaneously, a novel, sensitive and robust liquid chromatography tandem mass spectrometric method was developed and validated for simultaneous determination of raltegravir and raltegravir glucuronide in human plasma. A simple protein precipitation with acetonitrile was utilized for plasma sample preparation prior to analysis. Baseline chromatographic separation was achieved on a ZORBAX Eclipse XDB-C8 using gradient elution mode. The run time was 9min at a constant flow rate of 0.4ml/min. The mass spectrometer was operated under a positive electrospray ionization condition. Excellent linearity ($r2 \geq 0.9997$) was achieved for raltegravir and raltegravir glucuronide in the range of 2–2000 nmol/l. The average recovery of raltegravir and raltegravir glucuronide was 105.8% and 102.2%, respectively. The precision (coefficient of variation) was 1.6–6.6% for raltegravir and 2.1–6.9 for raltegravir glucuronide, respectively. The accuracy was 98.6–106.1% for raltegravir and 96.3–100.3% for raltegravir glucuronide. The plasma samples were tested to be stable after nine freeze–thaw cycles and exposure to room temperature for 24 h. This well-validated assay was applied for the quantification of raltegravir and raltegravir glucuronide in plasma samples within 24 h after a single oral dose of 400 mg raltegravir in six healthy subjects. Gupta A et al., developed and validated a selective and rapid high-performance liquid chromatography–tandem mass spectrometry method for the quantification of raltegravir using raltegravir-d3 as an internal standard (IS).The analyte and IS were extracted with methylene chloride and n-hexane solvent mixture from 100 mL human plasma. The chromatographic separation was achieved on a Chromolith RP-18e endcapped C18 (100 mm,4.6 mm)column in a runtime of 2.0min. Quantitation was performed in the negative ionization mode using the transitions of m/z 443.1-316.1 for raltegravir and m/z 446.1-319.0 for IS. The linearity of the method was established in the concentration range of 2.0–6000 ng/mL. The mean extraction recovery for raltegravir and IS was 92.6%and91.8%,respectively,and the IS-normalized matrix factors for raltegravir ranged from0.992 to 0.999. . The LLE procedure afforded highly selective separation of the analytes from endogenous components enabling quantification of 0.01–40 ng on-column per sample injection employing100 mL plasma samples The proposed validated LC–MS/MS assay provided are liable and rugged approach for the quantitation of RAL in human plasma in the negative ionization mode. The method was extensively validated for matrix effect and stability under different storage conditions. It was successfully applied in a clinical study and the reproducibility of the assay was demonstrated by incurred sample reanalysis. Takahashi M et al., A Conventional LC-MS Method Developed for the Determination of Plasma Raltegravir Concentrations Raltegravir belongs to a new class of antiretrovirals acting for a human immunodeficiency virus (HIV)-1 integrase inhibition. Clinical trials demonstrated potent antiviral activity in both therapy naive & experienced patients. Raltegravir has become an important component of combination treatment regimens used to treat patients with multidrug-resistant HIV-1. The quantification of raltegravir in human plasma is important to support clinical studies and determine pharmacokinetic parameters of raltegravir in HIV-1 infected patients. The LC-MS/MS superfine system was developed to determine plasma concentration of raltegravir The system needs to be delicately set and the equipment is very expensive. Developed a conventional LC-MS method to overcome these difficulties. Subsequently the method validated by estimating the precision and accuracy for inter and intraday analysis in

the concentration range of 0.010-7.680m g/ml. The calibration curve was linear in this range. Average accuracy ranged from 97.2 to 103.4%. Relative standard deviations of both inter and intraday assays were less than 10.4%. Recovery of raltegravir was more than 80.6%. These resultsindicate our newly developed method achieves the same level of reproducibility and accuracy as the LC-MS/MS method.

Tiwari Ravi N et al., aim of the present investigation was to carry out stress degradation studies on raltegravir according with International Conference on Harmonization (ICH) Q1A(R2) guideline. The drug was subjected to hydrolytic (acid, alkaline, and neutral), oxidative, thermal, and photolytic stress. Raltegavir showed a labile behavior in acidic, basic, and neutral stress while it was stable in oxidative, photolytic, and thermal stress conditions. In total, five degradation products (DP) were formed, which were separated on a C-18 column employing a gradient HPLC method. To elucidate the structure of degradation products, initially a complete mass fragmentation pathway of the drug was established with the help of multi-stage mass spectrometry (MSn) and mass spectrometry=time of flight (MS=TOF) accurate mass studies. Then, stressed samples were subjected to LC–MS=TOF studies, which provided their fragmentation pattern and accurate masses. The mass spectral data were employed to characterize the DPs and assign structures to them. The total information was also used to establish the degradation pathway of the drug.

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