A rapid validated UV-HPLC method for the simultaneous determination of the antiretroviral compounds darunavir and raltegravir in their dosage form

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ABSTRACT

**Introduction.** A rapid, simple and sensitive high-performance liquid chromatography (HPLC) method with ultraviolet detection has been developed for quantification of darunavir and raltegravir in their pharmaceutical dosage form.

**Material and methods.** The assay enables the measurement of both drugs with a linear calibration curve \( R^2 = 0.999 \) 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 35°C. 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.

**Results.** 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%.

**Conclusions.** 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.

**Keywords:** Darunavir, HAART, HPLC, raltegravir, validation study

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Determinación simultánea de los compuestos antirretrovirales darunavir y raltegravir en su forma farmacéutica mediante un método rápido y validado de UV-HPLC

RESUMEN

**Introducción.** Un método rápido, sencillo y sensible de cromatografía líquida de alto rendimiento (HPLC) con detección ultravioleta ha sido desarrollado para la cuantificación simultánea de darunavir y raltegravir en su forma farmacéutica.

**Material y métodos.** La determinación se llevó a cabo empleando una columna Tracer Excel 120 ODSB (15x0.4.6 cm) C18 a 35°C. La longitud de onda empleada fue de 254 nm. La fase móvil fue una mezcla de una disolución tampón dihidrogeno fosfato de sodio 0,037 M, acetonitrilo y metanol (40:50:10, v/v/v) con un flujo de 2,0 mL/min. El fármaco nevirapina (50 mg/L) fue usado como patrón interno.

**Resultados.** La exactitud, repetibilidad intra-día \( n = 5 \) y precision inter-día \( n = 3 \) fueron considerados satisfactorios, siendo la exactitud de -4.33 a 3.88% y las precisiones intra-día e inter-día, 0.25% y 4.42% respectivamente en caso de darunavir. Raltegravir intra-día e inter-día precisiones más bajas de 1.01 y 2.36%, respectivamente y valores de exactitud entre -4.02 y 1.06%.

**Conclusión.** La determinación de darunavir y raltegravir en su forma farmacéutica se realizó con una desviación máxima del 4%. El método es rápido, fácilmente implantable y ofrece buenos resultados.

**Palabras clave:** Darunavir, HAART, HPLC, raltegravir, validación
INTRODUCTION

The most used antiretroviral therapy against HIV virus is based in the use and combination of three drugs from at least two different families. This combination is called “Highly Active Antiretroviral Therapy” (HAART). The effectiveness of HAART therapy is increasing and reducing its toxicity thanks to the development of new drugs. Usually, two drugs are coming from the families of inhibitor protease (IP) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). Darunavir is an example of inhibitor protease and rilpivirine and etravirine are NNRTIs. Another important family of drugs used in HAART therapy is the integrase inhibitor with examples as dolutegravir.

A quality control of the dosage form of the antiretroviral compounds can be done in the health care services to assure the quality of the medication. To assess the quality and chemical stability of the administered drugs to the Hospital patients could be an improvement in the treatment by controlling possible lost and degradations of the drugs. Also, the quality control can be mandatory to assure the correct concentrations of the prepared magistral formula in the health care services for paediatric or HIV+ patients with dysphagia who need special oral preparations.

Quantification of the drugs is done usually by Liquid Chromatography tandem mass spectroscopy (HPLC-MS/MS), Ultra Performance Liquid Chromatography (UPLC) or High Performance Liquid Chromatography (HPLC) when blood samples are analyzed. HPLC-MS/MS or UPLC are really effective techniques, but the cost of these analyses is high and most research and clinical laboratories have not these equipments. In case of dosage form analysis also spectrophotometric methods can be developed but HPLC is the main used analytical technique to quantify drugs as the antiretroviral compounds because it is needed less expensive equipment and is more usual to have it in the clinic laboratories where the determination of the drugs in plasma can be done.

The simultaneous determination can simplify the HPLC analysis in routine having one method to carry with the quality control of the maximum number of drugs in the minimum time as possible. To the best of our knowledge, there are not works where dosage forms of darunavir and raltegravir are analyzed simultaneously. Darunavir has been analyzed by Sanyanarayana et al. with a retention time of 5.86 minutes, Patel et al. with 5.02 minutes and Kumar et al. with a retention time of 3.99 minutes. For raltegravir HPLC estimation methods, 4.3 minutes was the obtained retention time by Sudha et al. The development of a method to quantify simultaneously both antiretroviral compounds can be an improvement in the quality control of their dosage form.

MATERIAL AND METHODS

Chemicals and reagents. Darunavir was kindly provided by Cilag (Schaffhausen, Switzerland). Raltegravir was supplied by Merck (Rahway, USA) and nevirapine was provided by Boehringer Ingelheim (Ingelheim, Germany). The dosage form of raltegravir is called Isentress® and it is from the laboratory Merck Sharp and Dohme (Kenilworth, USA) containing 400 mg of the drug per each tablet. Prezista® is the dosage form of darunavir and it can be presented with different concentrations, in example 400, 600 and 800 mg per tablet and it is manufactured by Janssen-Cilag International N.V. (Beese, Belgium).

HPLC-grade acetonitrile and water were obtained from Teknokroma (Barcelona, Spain) and HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate was provided from Panreac (Barcelona, Spain). Dimethyl sulfoxide (DMSO) was acquired from Acofarma (Terrassa, Spain).

Chromatographic conditions. The employed equipment was a HPLC system (Shimadzu, Kyoto, Japan) consisted of a pump (model LC-20AD), degasser (model DGU-20A), autosampler (model SIL-20AC), thermostated column compartment (model CTO-10AS), and an UV-visible detector (model SPD-M20A). Data were acquired and processed with LCSolution® software from Shimadzu Corporation (Kyoto, Japan). Separation of the compounds was achieved by using a Tracer Excel 120 ODSB column (5 μm; 150 x 4.6 mm) with a guard column packed Ultraguard SAXE18 (10 x 3.2 mm). Chromatographic conditions were carried out by using a mobile phase [consisting of a mixture of potassium dihydrogen phosphate buffer solution (pH 4.3; 0.037 M), acetonitrile and methanol] in 40:50:10, v/v/v proportion, pumped at a constant flow rate of 2.0 mL/min. The column was maintained at 35°C, the injection volume was 10 μL and the eluents were monitored at a wavelength of 254 nm.

Preparation of stock solutions and standards solutions. Stocks solutions containing 5,000 mg/L of darunavir, 2,500 mg/L of raltegravir and 5,000 mg/L of nevirapine were prepared in methanol and were stored for less than one month at 4 °C in the dark. Each day, fresh raltegravir and darunavir working solutions were prepared by diluting the stock solutions with methanol to obtain concentrations of 500 and 50 mg/L. Likewise, nevirapine working solutions were further diluted with methanol to a final concentration of 500 mg/L.

In order to validate the analytical method, calibrators and quality control (QC) samples were prepared. Calibrators were mobile phase samples containing known concentrations of darunavir and raltegravir. These calibrators were used for construction of a calibration curve consisting of a blank sample (matrix sample processed without IS), a zero sample (matrix sample processed with IS), and 8 nonzero samples covering the expected range, including the lower limit of quantification (LLOQ). Darunavir and raltegravir calibrators were prepared by diluting working solutions with mobile phase each day to obtain concentrations of 5, 10, 20, 40, 50, 60, 80 and 100 mg/L. QC samples were three of known concentrations of each drug
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Figure 1  Chromatogram with IS, raltegravir and darunavir (80 mg/L of each drug) on top; Isentress with IS chromatogram in the middle and Prezista with IS chromatogram at the bottom.
in mobile phase. These controls spanned the calibration curve, encompassing concentrations at 10, 40 and 80 mg/L.

**Validation of the analytical method.** The method validation procedure was carried out following the guidelines for Bioanalytical Method Validation published by the FDA\(^1\) and the European Medicines Agency (EMA)\(^2\).

a) **Specificity and selectivity.** The specificity of the method was evaluated with regard to interference, by presence of any other excipient, with the detection of the analyte and/or the IS. Six different mobile phases were prepared to evaluate the interference potential. Zero sample was analysed to check for the absence of interference with the analyte. The interference peak should be less than 5% of the peak area for both analyte and IS.

b) **Linearity.** Complete calibration curves of each drug (8 concentrations; 5, 10, 20, 40, 50, 60, 80 and 100 mg/L) were analysed on three separate days. Calibrations curves were generated by plotting the peak area ratio of darunavir and raltegravir to that of the IS. The linear regression equation was generated by the application of weighted (1/concentration) least square regression analyses. This equation was used to calculate the concentrations of the QCs. Slope, intercept, and coefficient of determination (\(R^2\)) were calculated for each calibration curve. Also the value of the relative standard deviation of the response factor was calculated. Calibration range linearity from 5 to 100 mg/L was concluded if \(r\) was greater than 0.99 for all calibration curves.

c) **Limit of detection and lower limit of quantification.** The assay sensitivity was evaluated by determining the limit of detection (LOD) and the LLOQ. LOD was defined as the lowest concentration required to give a signal equal to the blank plus 3 times standard deviation of the blank (S/N = 3), whereas theoretical LLOQ was the lowest drug concentration required to give a signal equal to the blank plus 10 times standard deviation of the blank (S/N = 10), and acceptable accuracy and precision data. In our case, LLOQ was adapted to covert the desired range concentration.

d) **Precision and accuracy.** Accuracy and precision were assessed by determining darunavir and raltegravir concentration at LLOQ and in QC samples at 10, 40 and 80 mg/L, measuring five replicates per concentration on three different days. Precision, expressed as the relative standard deviation (RSD), was calculated as the standard deviation for intra-day and inter-day runs divided by the average for those runs. Accuracy was determined by the mean relative error (MRE) from the theoretical concentrations, calculated as the absolute value of 100 minus the average estimated concentration divided by theoretical concentration. For each concentration, both RSD and MRE should be lower than 15% except for LLOQ, where they should not deviate by more than 20%.

e) **Solutions stability.** Stock and working solutions of the IS nevirapine were also found to be stable stored at 2-8°C for 25 days and for short term stability at room temperature\(^1\). Stock and working solution of darunavir in methanol were also found to be stable for short-term stability at room temperature and long-term stability at 5°C\(^1\). Stock solution of raltegravir was found stable at 4°C for four months\(^1\) and the short-term stability was also assured for 8 hours\(^1\). Stability of the calibrators in the mobile phase was measured by HPLC for darunavir, raltegravir and the internal standard nevirapine at 2, 4, 6 and 24 hours.

**Applicability of analytical method.** This assay was used to quantify the amount of darunavir and raltegravir in their pharmaceutical dosage form Prezista\(^8\) and Isentress\(^8\), respectively. A tablet of Prezista\(^8\) contains 400 mg of darunavir. A tablet of Isentress\(^8\) includes 600 mg of raltegravir. Tablets were made powder in a mortar and then an appropriate amount of the powder to add 100 mg of each drug was weighted and put it into a volumetric flask of 100 mL with 80 mL of DMSO. Then, samples were sonicated for 30 minutes and DMSO was added to the volumetric flask until 100 mL, reaching a 1000 mg/L theoretical concentration (M) of darunavir or raltegravir. Samples were initially diluted to the half with mobile phase (M\(_1\)) and then, 50 \(\mu\)L of this diluted sample (M\(_1\)) and 50 \(\mu\)L of the IS solution of 500 mg/L were added to 400 \(\mu\)L of the mobile phase to get a theoretical concentration of 50 mg/L in darunavir or raltegravir and in the IS.

**RESULTS**

**Validation method.**

a) **Specificity and selectivity.** Different columns, mobile phases and fluxes were tested finding the best retention time with good resolution employing the selected conditions. Chromatograms obtained with blank sample not shown any interference. Also, the three compounds are clearly separated in mobile phases and fluxes were tested finding the best retention time with good resolution employing the selected conditions. Chromatograms obtained with blank sample not shown any interference. Also, the three compounds are clearly separated

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Calibration curves parameters for darunavir and raltegravir.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image" alt="Calibration curves parameters for darunavir and raltegravir." /></td>
</tr>
<tr>
<td>Day</td>
<td>Darunavir</td>
</tr>
<tr>
<td></td>
<td>(a \times 10^{-3})</td>
</tr>
<tr>
<td>1</td>
<td>9.7</td>
</tr>
<tr>
<td>2</td>
<td>-1.0</td>
</tr>
<tr>
<td>3</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Linearity Equation, \(y = a + b\cdot C\); \(a\) = y-intercept of the line; \(b\) = slope of the line; \(C\) = drug concentration (mg/L); \(r\) = coefficient of determination.
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Limit of detection and lower limit of quantification.
Lower limit of detection was detected as 0.25 mg/L for darunavir and for raltegravir. The calibration curve was prepared using the relationship between the areas of the calibration and the internal standards peaks in front of the concentration of each drug. The study of the lower limit of quantification is presented in table 2. All the obtained values accomplish the parameter to be acceptable having accuracy lower than acceptable value <20% described in the Guidelines being the maximum value a 10.82%.

Linearity.
Linearity is demonstrated in each of the calibration curves with $R^2$ value it was greater than 0.999 the three validation days for darunavir and for raltegravir (table 1). Also, the value of the standard deviation of the response factor was calculated for each compound being 0.034 in case of darunavir validation and 0.052 for raltegravir.

Table 2
Intra-day and inter-day precisions and accuracy at LLOQ level.

<table>
<thead>
<tr>
<th>Theoretical concentration (mg/L)</th>
<th>Intra-day precision and accuracy</th>
<th>Inter-day precision and accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean observed concentration (SD) (mg/L)*</td>
<td>Precision (RSD, %)</td>
<td>Accuracy (MRE, %)</td>
</tr>
<tr>
<td>Darunavir 5</td>
<td>4.46 (0.01)</td>
<td>0.15</td>
</tr>
<tr>
<td>Raltegravir 5</td>
<td>4.98 (0.18)</td>
<td>3.51</td>
</tr>
</tbody>
</table>

*Results expressed as mean (SD) from 5 replicates.

Table 3
Intra-day and inter-day precisions and accuracy for darunavir.

<table>
<thead>
<tr>
<th>Theoretical concentration (mg/L)</th>
<th>Intra-day precision and accuracy</th>
<th>Inter-day precision and accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean observed concentration (SD) (mg/L)*</td>
<td>Precision (RSD, %)</td>
<td>Accuracy (MRE, %)</td>
</tr>
<tr>
<td>10</td>
<td>9.74 (0.02)</td>
<td>0.25</td>
</tr>
<tr>
<td>40</td>
<td>41.73 (0.08)</td>
<td>0.20</td>
</tr>
<tr>
<td>80</td>
<td>79.26 (0.06)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Results expressed as mean (SD) from 5 replicates.

Table 4
Intra-day and inter-day precisions and accuracy for raltegravir.

<table>
<thead>
<tr>
<th>Theoretical concentration (mg/L)</th>
<th>Intra-day precision and accuracy</th>
<th>Inter-day precision and accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean observed concentration (SD) (mg/L)*</td>
<td>Precision (RSD, %)</td>
<td>Accuracy (MRE, %)</td>
</tr>
<tr>
<td>10</td>
<td>10.42 (0.10)</td>
<td>1.01</td>
</tr>
<tr>
<td>40</td>
<td>39.98 (0.34)</td>
<td>0.85</td>
</tr>
<tr>
<td>80</td>
<td>79.15 (0.36)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*Results expressed as mean (SD) from 5 replicates.
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Table 5  Quantification data of darunavir content in Prezista® tablets.

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/L)</td>
<td>48.05</td>
<td>51.30</td>
<td>51.17</td>
</tr>
<tr>
<td>SD (mg/L)</td>
<td>0.28</td>
<td>0.54</td>
<td>1.07</td>
</tr>
<tr>
<td>Prezista ± SD (mg)</td>
<td>384 ± 2</td>
<td>410 ± 4</td>
<td>409 ± 9</td>
</tr>
<tr>
<td>Deviation from theoretical value (%)</td>
<td>-4.00</td>
<td>2.50</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Table 6  Data obtained from the quantification of raltegravir content in Isentress® tablets.

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/L)</td>
<td>49.23</td>
<td>48.98</td>
<td>49.93</td>
</tr>
<tr>
<td>SD (mg/L)</td>
<td>0.05</td>
<td>0.20</td>
<td>0.54</td>
</tr>
<tr>
<td>Isentress ± SD (mg)</td>
<td>591 ± 1</td>
<td>588 ± 2</td>
<td>599 ± 6</td>
</tr>
<tr>
<td>Deviation from theoretical value (%)</td>
<td>-1.55</td>
<td>-2.04</td>
<td>-0.3</td>
</tr>
</tbody>
</table>

d) Precision and accuracy. The rest of the studied calibration standards are also inside of the acceptable range (with a precisions and accuracy <15%). The major value of intra-day precision in case of darunavir was 0.25% and inter-day precisions were between 0.75 and 4.42% and accuracy was between -4.33 and 3.88% (table 3). Raltegravir parameters are also inside of the acceptable values having an intra-day precisions lower of 1.01%, and the inter-day values of this parameters also below 2.36%. Finally, accuracy values for raltegravir QC are between -4.02 and 1.06% (table 4).

e) Solution stability. The stability of the calibrators and quality controls was measured and it was confirmed that all the employed compounds are 24 hours stable in the mobile phase with concentration values of 108% and 95% of the initial values for darunavir and raltegravir, respectively. Also, the stability of nevirapine was studied, showing a 98% of the initial value of the drug after 24 hours. Thus, all the calibrators have shown acceptable values of short term stability in the mobile phase (auto sampler stability).

Applicability of analytical method. This validated method has been used to determine the darunavir and raltegravir amount in their marketed pharmaceutical forms. The obtained chromatograms for both compounds are presented in figure 1. Data obtained from these analyses are presented in tables 5 and 6 for darunavir and raltegravir, respectively. In case of darunavir, good results of precision are shown (from -4.00 to 2.50) and they are even improved in raltegravir analysis (from -2.04 to -0.13).

DISCUSSION

Data obtained from the analysis of the tablets are inside of the acceptable ranges with deviations from the theoretical value lower than a 4%. Thus, the applicability of this method is demonstrated and it can be used in the quality control by UV-HPLC of the darunavir and raltegravir dosage forms. Raltegravir has just one dosage form and it was studied. Darunavir have more than one but they can be successfully studied with this method solving the appropriate amount of the tablet.

The validation procedure has been reached following the FDA and EMA Guidelines. Linearity, precision and accuracy of this newest method are inside of the appropriate ranges to assure the quality of the determination of darunavir and raltegravir. The short-term stability of the calibrators and QC controls in the auto sampler has been also demonstrated. Also, good linearity values have been shown and acceptable parameters of precision and accuracy have been found.

The method has an important improvement in front the rest of the published methods, because its retention time obtained for darunavir is clearly minor than the previous published works10-12 and also, in case of raltegravir the retention time applying this method is minor than the previous one13. These facts mean that our new UV-HPLC method shows better cost-effectiveness ratio in comparison with the previous published darunavir or raltegravir determination methods.

A validated method to determine the concentration of darunavir and raltegravir has been developed with good values of precision and accuracy. This method can be implanted in routine analysis of quality control by UV-HPLC of darunavir and raltegravir concentrations in their dosage form with low retention times and run time minor than four minutes.

ACKNOWLEDGEMENTS

Authors want to thanks Cilag, Merck and Boehringer Ingelheim for the kindly provide of the raw material.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING

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REFERENCES

1. Ruela Correa JC, D’Arcy DM, dos Reis Serra CH, Nunes Salgado HR.
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Initially nevirapine, raltegravir and darunavir were studied one by one.

**Figure 1** Conditions. Column: Tracer Excel. Flow: 1 mL min⁻¹. Mobile phase (%): TPI: 43; MeCN: 35; MeOH: 22.
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Figure 2

Figure 3
Conditions. Column: Tracer Excel. Flow: 1 mL min⁻¹. Gradien Time 0-6 min Mobile phase (%): TPI: 60; MeCN: 20; MeOH: 20. Time 6-8 min Mobile phase (%): TPI: 57; MeCN: 14; MeOH: 29.
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Then, mixtures with the three compounds were also studied.

Figure 4  Conditions. Column: Tracer Excel. Flow: 1 mL min⁻¹. Mobile phase (%): TPI: 40; MeCN: 42; MeOH: 18.

Figure 5  Conditions. Column: Tracer Excel. Flow: 2 mL min⁻¹. Mobile phase (%): TPI: 40; MeCN: 40; MeOH: 20.
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Figure 6  Conditions. Column: Tracer Excel. Flow: 2 mL min⁻¹. Mobile phase (%): TPI: 50; MeCN: 50.

Figure 7  Conditions. Column: Mediterranean SEA18. Flow: 2 mL min⁻¹. Mobile phase (%): TPI: 50; MeCN: 40; MeOH: 10.

*Shoulders are due by the column.
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Then, the best resolution peaks were obtained by using the following conditions:

Figure 8
Conditions. Column: Mediterranean SEA18. Flow: 2 mL min⁻¹. Mobile phase (%): TPI: 40; MeCN: 50; MeOH: 10.

Figure 9
Conditions. Column: Tracer Excel. Flow: 2 mL min⁻¹. Mobile phase (%): TPI: 40; MeCN: 50; MeOH: 10.