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Characteristics, Complexation and Analytical Methods of Darunavir

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

What is it? Darunavir is a protease inhibitor used in the treatment of HIV infection. It is an important drug of therapy cocktail for patients infected with the virus. On the market there are darunavir ethanolate tablets of 75, 150, 300, 400, 600 and 800mg, because this is the most stable form. It is commercialized by Janssen-Cilag with the name Prezista™.
Why we started? This drug has low water solubility and poor bioavailability, therefore requires administration in doses relatively high to the success of the therapeutic effect. The complexation of drugs by using cyclodextrin is welcome in this respect to improve the solubility and hence increase the dissolution rate of poorly soluble drugs. A monograph about this compound has not been described, thus it is an extremely important quality control of darunavir to demonstrate its effectiveness and safety.
What we did? Some existing analytical techniques have been discussed in this manuscript, focusing on bioanalytical and pharmaceutical quality control applications.
What we found? This review showed the published analytical methods reported for the determination of darunavir and discuss about its characteristics and complexation with cyclodextrin.

Keywords: Antiretroviral; analytical methods; complexation; cyclodextrin; darunavir; quality control.

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1. INTRODUCTION

Protease inhibitors were a major therapeutic advance in the mid-1990s for the treatment of HIV infection, which resulted in increased life expectancy for patients who had failed therapies in earlier. Darunavir, the newest protease inhibitors, is a non-peptide synthetic analogue of amprenavir [1], which was approved by the U.S.FDA in June 2006 and in February 2007 the European Commission approved its marketing [2]. It is effective in patients with experiments in antiretroviral treatments, such as those with HIV-1 strains that are resistant to more than one protease inhibitors [3].

Darunavir (Fig. 1) has the molecular formula $C_{27}H_{37}N_3O_7S$. Group highlighted in Fig. 1 shows part of the structure which binds to the active center of the protease, responsible for rapid association and slow dissociation, which increases the effectiveness and duration of action against the viral protease. The affinity of this union is 100 times higher than that of amprenavir, another protease inhibitor [4].

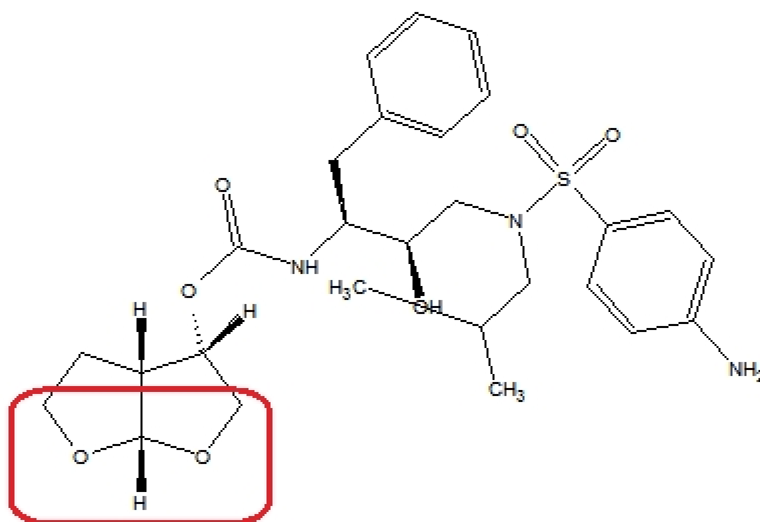

Fig. 1. Chemical structure of darunavir with emphasis in the group responsible for its action

Protease inhibitor drugs are one of the pillars of the cocktail therapy [5] and darunavir is the new generation of synthetic non-peptidic protease inhibitor [6].

Most newly-discovered chemical entities, despite the high therapeutic activity, have low water solubility and poor bioavailability, leading to poor absorption in the gastro intestinal tract [7]. The important characteristics of a molecule that needs to be considered for effects positive anti-HIV are, among others, solubility and stability in biological fluids. When these properties are unfavorable for the development of specific medicines, processing and alternative formulation can be employed to achieve maximum therapeutic gain [8].

Some studies associating darunavir to some systems or other antiretroviral agents are being evaluated, aiming to increase its bioavailability. The complexation of darunavir to β-cyclodextrin showed increased solubility against the free drug. In the production of pellet

formulations with microcrystalline cellulose and kappa-carrageenan, the bioavailability of darunavir was substantially improved in kappa-carrageenan pellets, when compared with microcrystalline cellulose pellets [9]. The bioavailability of darunavir is 37%, but when combined with ritonavir increases to 82% [4].

Several of analytical techniques which were discussed in this article focused on application in quality control aiming at efficient and dynamic analysis, because the darunavir does not present monograph described in Brazilian Pharmacopoeia [10], in Portuguese Pharmacopoeia [11], USP 33 [12] and BP [13].

The significant increase in the number of drugs available, as well as the advancement and transformation of technologies used in the production of these have increased the interest of different sections of the society linked to health (regulatory agencies, governments, pharmacists) in the search for measures to ensure the integrity of the product available to the patient, taking the concepts of quality control outside the frame simply industrial but regulator.

The development of effective analytical methods for quality control of marketed drugs is extremely important and aims to provide reliable information about the nature and composition of the materials under analysis [14].

This review will examine the published analytical methods reported for determination of darunavir and discuss about its characteristics, complexation to β-cyclodextrin and methods of analysis of darunavir.

2. DARUNAVIR

Darunavir has become interesting for some reasons.

Cytotoxicdoses are much higher than therapeutic doses, allowing a wide margin of safety to darunavir. Its spectrum is very broad and it is effective against all subtypes of HIV-1 and HIV-2. It is also more active against the most resistant HIV compared to other protease inhibitors [3,4,15], and its robustness against the known mechanisms of HIV resistance is also superior to other available protease inhibitors.

Induction and selection of mutations which confer resistance to this drug seems to be slower and more difficult, which would allow it to maintain its antiviral effect unaltered for long periods [16].

It was observed that darunavir is well tolerated, with adverse effects lower than those protease inhibitors. The monotherapy presents efficacy no less than the triple therapy with darunavir and two reverse transcriptase nucleoside analogues [4].

There are three known forms of darunavir: Ethanolate, hydrate and amorphous [6]. The ethanolate and hydrate forms exist in the form of crystals, whereas the non-solvated form is amorphous. When in the formed crystalline forms exist solvent molecules, water, salt, excipient or impurity has a pseudo polymorphic behavior and it can occur during handling, processing and storing. The tendency of a molecule to form solvates is related to the molecular structure, standards of hydrogen bonding and crystal packing. The solvent serves to stabilize the structure and desolvation process results in the formation of an amorphous form.

Darunavir commercialized is called Prezista® [4] and it is in the form ethanolate (molecular weight of 593.73g mol^{-1}) because it is the most stable form, but the environmental conditions can trigger its conversion into other forms [6]. On the market are found tablets darunavir ethanolate of 75, 150, 300, 400, 600 and 800 mg with the name of Prezista™ and it is not suitable for pediatric use [5].

Despite all the advantages of darunavir (cited above), it has low solubility in water, which leads to poor availability and therefore the need for high doses to the success of the therapeutic effect. Over time, patients who use this medication daily can suffer from intoxications. Drugs with high dose and light colateris effects, such as darunavir, with prolonged use can cause side-effects to patients.

3. COMPLEX DARUNAVIR: β-CYCLODEXTRIN

These types of drugs require frequent administration in relatively high doses being the major cause of non-adherence to treatment and an obstacle to the fulfillment of pharmacotherapy [5]. Moreover, there are important issues of drug adverse reactions and drug interaction associated with antiretroviral therapy. After chronic treatment even the moderate toxicity can lead to serious complications. Treatment failure not only affects the quality of life of patients, but also contributes significantly to the economic burden of the health system [8].

Only 12 antiretroviral drugs have been approved for use in children (as opposed to the 25 approved for adults). In this context, note the need for the development of antiretroviral drug encapsulation and delivery strategies; work on the technological aspects in order to reduce the dosing frequency and improve compliance of existing pharmacotherapy [5]. Therefore, there is great interest in developing efficient methods, reliable, cost effective and scalable to increase the oral bioavailability of poorly soluble drugs in water [7].These methods are becoming complementary to the development of new drugs [5].

Cyclodextrins are cyclic oligosaccharides, composed of glucose units united by α-1,4 linkages, originating from the degradation of starch by the enzyme glycosyltransferases, synthesized by some micro-organisms. Those which are obtained with higher income are commonly known as cyclodextrins natural and contain six, seven or eight glucose units, being denominated α-cyclodextrin, β-cyclodextrin and γ-cyclodextrins, respectively. The β-cyclodextrin (Fig. 2) is the most widely used in commercial formulations currently available.

Cyclodextrins are currently classified as excipient and have been used to develop pharmaceuticals, particularly due to their complex properties, which provide increased solubility and consequent increased dissolution rate of poorly soluble drugs, stability, reduced irritation and a reduction or elimination of odors or flavors in liquid formulations [17].

Changes in physicochemical properties of the guest molecules can be identified by analytical methods that can detect the formation of complexes. To characterize the formation of inclusion complex in solid state methods can use thermal methods, infrared spectroscopy, scanning electron microscopy and analysis of the dissolution properties, already in the liquid state nuclear magnetic resonance spectroscopy, UV visible spectroscopy, and phase solubility diagram [17].

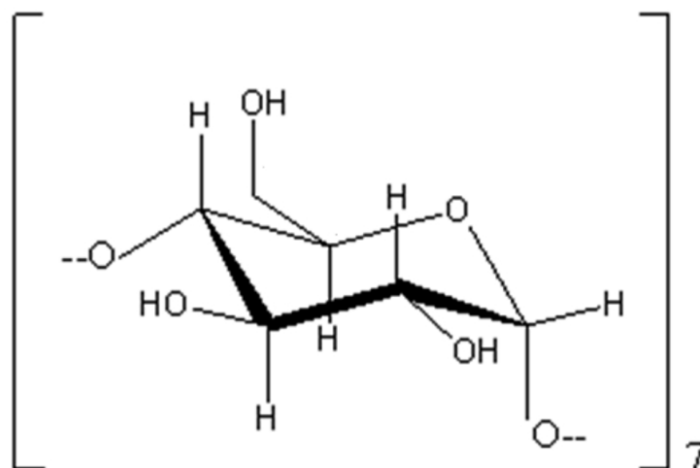

Fig. 2. Chemical structure of β-cyclodextrin

The incorporation of cyclodextrins in pharmaceutical systems is a consolidated reality. Advances in industrial technology allow the manufacture of natural cyclodextrins with high purity. The β-cyclodextrin is quoted at about U.S. $ 5/Kg. It is probable that the number of formulations containing this adjuvant will be broadened considerably in the coming years [18].

Thus, complexing properties, possibility to characterize the complex formed both in solid and in liquid, being the most widely used in commercial formulations, easy manufacture of β-cyclodextrin, scalable and low price were decisive characteristics in choosing the complexation of darunavir to β-cyclodextrin.

Today and increasingly, the choice of analytical methods, reagents, materials and systems used in laboratories is essential in the application of new technology on the market, outside the walls of laboratories. The preference for less polluting, cheaper, scalable systems and accessible reagents are essential in the spread and exploitation of science. The scientific community should always evaluate the socio-economic impact of analytical decisions.

The combination of theoretical and experimental techniques confirmed the formation of an inclusion complex between darunavir and β-cyclodextrin by our research group. Complexation was obtained in purified water in proportion of 1:1. In theoretical studies, complex darunavir: β-cyclodextrin formed showed increased solubility in about 600 times against the free drug.

4. METHODS OF ANALYSIS

The development of effective and reliable analytical methods for quality control of marketed drugs is extremely important and aims to provide reliable information about the nature and composition of the materials in question [14]. Validation is an important part of the quality assurance program and aims to demonstrate that the analytical method is suitable for the intended proposal and that it is safe to be executed [19], being the procedures included in the standards of Good Manufacturing Practices (GMP) required by U.S. FDA, and applied in

pharmaceutical industries and should also occur according to good laboratory practice (GLP) [20].

It is known that the polymorphism of drugs, the ability of a substance to exist as two or more crystalline forms, having different arrangements and/or conformations of molecules in the crystalline structure [6], affect the therapeutic activity by presenting differences in pharmacological properties. Thus, knowledge of the polymorphic form of darunavir used in laboratory tests is important for detecting possible analytical differences.

Darunavir has not been described monograph in the researched pharmacopoeias [10-13]. Dissolution studies, for example, are extremely important because they provide rate data available dissolved drug to be utilized by the organism.

We performed a literature search on methods for analysis of darunavir and were found to detect the drug in tablets by thin layer chromatography (TLC), UV spectrophotometry, infrared spectroscopy (IR) and high performance liquid chromatography (HPLC) [21,22], in plasma by HPLC [3,15], ultra efficiency liquid chromatography coupled to mass spectrometry (UHPLC-MS) [23] and HPLC coupled with mass spectrometry (HPLC-MS) [4]. It was found the HPLC-MS method for simultaneous detection of darunavir and other antiretroviral drugs in plasma [2, 24-28] and in blood, saliva and tissue [29].

The HPLC-MS allows simultaneous and rapid determination of multiple analytes in a single run. Moreover, their high sensitivity facilitates the use of small sample volumes and determination of analytes in other matrices where the concentration is low [2]. However, according to Takahashi and collaborators (2007) and Goldwirt and collaborators (2007) the equipment of HPLC-MS is very expensive and unavailable in laboratories, so the need to develop alternative methods such as HPLC. But, the mobile phase used by Takahashi and collaborators (2007) this technique includes 50mM phosphate buffer, which does not contribute to a greater dynamic pharmaceutical industry because it requires longer preparation time, your life is low having the necessity of preparations more frequent and promotes a decrease in the time of use of the column because of the interactions. Now, Goldwirt (2007) and collaborators used water and acetonitrile in the mobile phase. However, the detection time of darunavir was 17.5min, a long time aiming the faster flow of the laboratory and industry.

In the technique of HPLC-MS some authors [2,4,24,26,29] also resorted to the use of buffer in the mobile phase, which provides the questions mentioned above about appreciate the most dynamic logistics of the pharmaceutical industry.

The Table 1 shows some methods for determination of darunavir in pharmaceuticals and biological fluids.

Table 1. Methods for determination of darunavir in pharmaceuticals and biological fluids

Method	Conditions	Detection System	Matrices	Reference
TLC	The migration chromatography was performed on silica gel plates 60F 254(20x 20cm), thickness of 0.25mm and a mobile phase containing methanol and purified water (70:30, v/v), adjusted to pH3.0 with glacial acetic acid.	Ultraviolet in 365 nm	Raw material and tablets	[21]
UV	Solutions in ethyl alcohol.	Ultraviolet in 268 nm	Raw material and tablets	[21]
IR	Pellets using potassium bromide and quantification through the carbonyl band.	Infrared between 1757-1671 cm^{-1}	Raw material and tablets	[21,22]
HPLC	Phenomenex Luna CN C18 column (5µm, 4.6x250 mm). The mobile phase consisted of purified water+ 0.1% acetic acid: acetonitrile+ 0.1% acetic acid (60:40, v/v) and flow rate of 1.0mL min^{-1}.	Ultraviolet in 268 nm	Raw material and tablets	[21]
HPLC	Radial-Pak Nova-Pak C18 column (4 µm, 8x100 mm, Waters) protected by Guard-Pak Inserts Nova-Pak C18 pre column. Separations were performed at 30ºC. The mobile phase consisted of 39% 50mM phosphate buffer (pH 5.9), 22% methanol and 39% acetonitrile. Flow rate of 1.8 ml min^{-1}.	Ultraviolet in 205 nm	Plasma	[3]
HPLC	20ºC on a C8 plus Satisfaction column (5µm, 3x250 mm, Cluzeau) protected by a guard column (3x15 mm) of the same phase and an A-103xfilter (Cluzeau). The mobile phase consisted of a mixture of acetonitrile:water (40:60, v/v) with a flow rate set at 0.6mL min^{-1}.	Ultraviolet in 266 nm	Plasma	[15]
HPLC	Symmetry Shield RP18 (4.6x150mm) column, 0.1% orthophosphoric acid and acetonitrile 50:50 (v/v) as mobile phase. The flow rate used was 1.5mL min^{-1} and controlled temperature of 25ºC.	Ultraviolet in 265 nm	Tablets	[30]
HPLC	RP Inertsil ODS-3V C18 (4,6x250 mm) column and 0.02 M dipotassium hydrogen ortho phosphate + 0.02 M potassium dihydrogen ortho phosphate in water and acetonitrile 40:60(v/v) as the mobile phase. The flow rate was 1.0 ml min^{-1}.	Ultraviolet in 265 nm	Tablets	[31]
HPLC	X-Bridge C18 (4.6x150 mm) column and buffer solution 0.01 M ammonium formate (pH.3.0) and acetonitrile 55:45 (v/v) in isocratic mode, as mobile phase. The flow rate was 1.0 ml $min^{-1}$and the column temperature of 30°C.	Ultraviolet in 265 nm.	Tablets	[32]
UELC-MS	Waters Acquity UELC BEH C18 (1.7 µm, 2.1x50 mm) column was maintained at 40°C with an alarm band of ± 6°C in the column oven. For gradient elution, the mobile phase solvent consisted of (A) 0.5% formic acid in water and (B) 0.5% formic acid in acetonitrile: methanol (70:30, v/v) and the flow rate was maintained at 0.3 mL min^{-1}. Up to 0.4 min, the ratio of A and B was kept at 50:50 (v/v) and from 0.4 min to 1.2 min the ratio was changed to 30:70 (v/v), before returning to the starting conditions [A:B, 50:50 (v/v)] up to 1.6 min.	Ionization and detection was carried out on a Quattro Premier XE Mass spectrometer (Waters) equipped with ion spray interface and operating in positive ion mode. Quantitation was performed using multiple reaction monitoring (MRM) mode to monitor precursor → product ion transitions for darunavir m/z 548.1 → 392.0	Plasma	[23]
HPLC-MS	X-Bridge™ C18 column (2.1×100mm, 3.5 um) maintained at 65°C. The mobile phase consisting of acetonitrile (A) and 3 mM ammonium acetate/formic acid 0.1	Triple quadruple Quattro micro (Micromass). The electrospray ionization occurred in positive mode. The	Plasma	[4]

	M (B) and followed by a gradient elution program. It began with 60% mobile phase A and 40% mobile phase B for 1.5 min. Then for 0.5 min the flow was increased to 100% mobile phase A and maintained for 1.5 minutes. For finally, 0.1 min fell to 60% mobile phase A and 40% mobile phase B maintained at this rate for 1.9 min. Flow 0.4 mL min^{-1}.	transitions *m/z* of ions detected were 548.3> 392.4 for darunavir. The data processing was performed using the Mass LynxTM V 4.0 software.		
HPLC-MS	Phenomenex Luna Phenyl–Hexyl (5 µm, 2×50 mm) column. Methanol:water (97:3, v/v), 10mM ammonium acetate and 0.1% acetic acid as mobile phase. Flow rate 0.5 mL min^{-1}.	Tandem mass spectrometry detector in multiple reaction monitoring (MRM).	Plasma	[26]
HPLC-MS	Waters Atlantis^{TM}-d C18 column (3 µm, 2.1×50 mm). Separations are performed using a gradient program with 2 mM ammonium acetate and acetonitrile both containing 0.1% formic acid.	Electrospray ionization - triple quadrupole mass spectrometry using the selected reaction monitoring detection in the positive mode.	Plasma	[24]
HPLC-MS	Agilent Zorbax® XDB C-8 (1.8 µm, 3x50 mm) column. Mobile phase A consisted of 0.01% formic acid in water and B consisted of 0.01% formic acid in acetonitrile. A linear gradient elution was performed from 35 to 65% mobile phase B over 6 min, with 1 min at 100% mobile phase B for column washing, followed by 3 min of re-equilibration time using 36% mobile phase B. Over the first 6 min, the gradient mobile phase flow rate increased from 0.65 to 0.75 mL min^{-1}.	Agilent quadruple 1100 mass spectrometer, fitted with an electrospray ionization (ESI) source and operated in the positive ionization mode. The darunavir was detected by their positive ion *m*/*z* 548.2.	Plasma	[25]
HPLC-MS	Atlantis T3 C-18 column (3µm, 2.1x150 mm) (Waters), protected by a Security Guard with C-18 (3x4 mm) pre-column (Phenomenex,) at 35°C. Chromatographic run was performed with a gradient: the mobile phase A was composed by water (+0.05% formic acid) and mobile phase B by acetonitrile (+0.05% formic acid).	Electrospray ionization (ESI), positive polarity ionization.	Plasma	[27]
HPLC-MS	Gradient [ACN:water (0.05% formic acid) 5:95 and 80:20, v/v] was used as mobile phase on a reverse-phase C18 column at a flow rate of 400 µl min^{-1}.	Triple-quadruple mass spectrometer, positive ionization mode, selective reaction monitoring (SRM).	Plasma	[28]
HPLC-MS	Phenomenex Gemini C18 column (2x150 mm) with a Phenomenex Security guard Gemini C18 pre column (2x4.0 mm). Gradient [700mL of methanol with 440mL of 10 mM acetic acid and 860mL of 10 mM ammonium acetate solution in water:methanol 85:15 and 15:85, v/v] was used as mobile phase.	Ions were created at atmospheric pressure and transferred to an API 3000 triple quadruple mass spectrometer (Sciex). Multiple reaction monitoring (MRM) in positive mode was used for drug quantification.	Blood, saliva and tissue	[29]
HPLC-MS	Reversed-phase C18 column (5 µm, 2x150 mm) with a gradient using an acetate buffer (pH 5) and methanol, at a flow rate of 0.25 mL min^{-1}.	Triple quadruple mass spectrometer was operated in the positive ion mode and the multiple reaction monitoring (MRM) was used.	Plasma	[2]

TLC = thin layer chromatography, UV = UV spectrophotometry, IR = infrared spectroscopy, HPLC = high performance liquid chromatography, UELC-MS = ultra efficiency liquid chromatography coupled to mass spectrometry, HPLC-MS = High performance liquid chromatography coupled to mass spectrometry

5. CONCLUSION

Darunavir is a promising drug and is becoming increasingly interesting for its pharmacological properties, however it presents problems of solubility and high dosage forms are required to achieve the desired affect. The complexation with β-cyclodextrin is a simple, scalable and inexpensive alternative to increase the solubility of darunavir.

The Quality Control developed in the pharmaceutical industry to identify the active substance content, to study the physical and chemical characteristics of the drug are essential to ensure final product quality.

Darunavir does not have standardized methods of analysis in official compendiums. The HPLC-MS allows simultaneous and rapid determination of multiple analytes, but the HPLC-MS equipment is very expensive and unavailable in laboratories, so there is a need to develop alternative methods such as HPLC, IR or TLC.

This fact justifies new research in this area for the development and validation of analytical methods and socio economic impact of the analytical decisions should be considered, because the cost of the analysis involves the cost of medication to the patient.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. McCoy C, Darunavir A. Nonpeptidic Antiretroviral Protease Inhibitor. Clin. Ther. 2007;29(8):1559-76.
2. Heine R, Alderden-Los CG, Rosing H, Hillebrand MJX, Gorp EC, Huitema ADR, et al. Fast and simultaneous determination of darunavir and eleven other antiretroviral drugs for therapeutic drug monitoring: Method development and validation for the determination of all currently approved HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun. Mass Spectrom. 2007;21:2505-14.

3. Takahashi M, Kudaka Y, Okumura N, Hirano A, Banno K, Kaneda T. The Validation of Plasma Darunavir Concentrations Determined by the HPLC Method for Protease Inhibitors. Biol. Pharm. Bull. 2007;30(10):1947-49.
4. García SP, Tunica DG, Serra MB. Development and validation of a method for determination of darunavir in plasma by LC-MS/MS. Rev. Clin Lab. 2011;4(3):127-33. Spanish.
5. Sosnik A, Chiappetta DA, Carcaboso AM. Drug delivery systems in HIV pharmacotherapy: What has been done and the challenges standing ahead. J. Controlled Release. 2009;138:2-15.
6. Gyseghem EV, Stokbroekx S, Armas HN, Dickens J, Vanstockem M, Baert L, et al. Solid state characterization of the anti-HIV drug TMC114: Interconversion of amorphous TMC114, TMC114 ethanolate and hydrate. Eur. J. Pharm. Sci. 2009;38:489-97.
7. Sinha S, Ali M, Baboota S, Ahuja A, Kumar A, Ali J. Solid Dispersion as an Approach for Bioavailability Enhancement of Poorly Water-Soluble Drug Ritonavir AAPS Pharm Sci Tech. 2010;11(2):518-27.
8. Sharma P, Garg S. Pure drug and polymer based nanotechnologies for the improved solubility, stability, bioavailability and targeting of anti-HIV drugs. Adv. Drug Delivery Rev. 2010;62:491-502.
9. Thommes M, Baert L, Van't Klooster GM, SchuellerL, Rosier J, Kleinebudde P. Improved bioavailability of darunavir by use of K-carrageenan versus microcrystalline cellulose as pelletisation aid. Eur J Pharm Biopharm. 2009;72(3):614-20.
10. Farmacopeia Brasileira. 5th ed. Atheneu: São Paulo; 2010.
11. Farmacopeia Portuguesa. 8th ed. Infarmed: Lisboa; 2005.
12. USP. The United States Pharmacopeia. 33st ed. United States Convention: Rockville; 2010.
13. BP, British Pharmacopoeia. Her Majesty’s Stationary Office: London; 2010.
14. La Roca MF, Sobrinho JLS, Nunes LCC, Neto PJR. Development and validation of analytical method: Important step in the production of medicines. Rev. Bras. Farm. 2007;88(4):177-80.
15. Goldwirt L,Chhuna S, Rey E, Launay O, Viard J, Pons G, et al. Quantification of darunavir (TMC114) in human plasma by high-performance liquid chromatography with ultra-violet detection. J. Chromatogr. B. 2007;857:327-31.
16. Liaño JP,Tenorio CH. Chemical characteristics, mechanism of action and antiviral activity of darunavir. Enferm. Infecc. Microbiol. Clín. 2008;26:3-9.
17. Lyra MA, Alves LDS, Fontes DAF, Soares-Sobrinho JL, Rolin-Neto PJ. Analytical tools applied to the characterization of drug-cyclodextrin complexes inclusion. Rev. Ciênc Farm. Basic Apl. 2010;31(2):117-24.
18. Cunha-Filho MSS, Sá-Barreto LCL. Use of cyclodextrin in forming inclusion complexes interessefarmacêutico. Rev. Ciênc Farm. Basic Appl. 2007;28:1-9.
19. International Conference On Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva; 2005.
20. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J. Chromatogr. A. 2003;987:57-66.
21. Kogawa AC, Salgado HRN. Development of analytical methods for the qualitative analysis of darunavir tablet. Rev. Ciênc Farm. Basic Apl. 2013;34(2):207-13.

22. Kogawa AC, Salgado HRN. Development and validation of infrared spectroscopy method for the determination of darunavir in tablets. Physical Chemsitry. 2013b;3(1):1-6.
23. Gupta A, Singhal P, Shrivastav PS, Sanyal M. Application of a validated ultra performance liquid chromatography–tandem mass spectrometry method for the quantification of darunavir in human plasma for a bioequivalence study in Indian subjects. J. Chromatogr. B. 2011;879:2443-53.
24. Fayet A, Béguin A, Zanolari B, Cruchon S, Guignard N, Telenti A, et al. A LC–tandem MS assay for the simultaneous measurement of new antiretroviral agents: Raltegravir, maraviroc, darunavir, and etravirine. J. Chromatogr. B. 2009;877:1057-69.
25. Rezk NL, White NR, Jennings, SH, Kashuba ADM. A novel LC–ESI-MS method for the simultaneous determination of etravirine, darunavir and ritonavir in human blood plasma. Talanta. 2009;79:1372-78.
26. Martin J, Deslandes G, Dailly E, Renaud C, Relequet V, Raffi, et al. A liquid chromatography–tandem mass spectrometry assay for quantification of nevirapine, indinavir, atazanavir, amprenavir, saquinavir, ritonavir, lopinavir, efavirenz, tipranavir, darunavir and maraviroc in the plasma of patients infected with HIV. J. Chromatogr. B. 2009;877:3072-82.
27. D'avolio A, Simiele M, Siccardi M, Baietto L, Sciandra M, Bonora S, et al. HPLC–MS method for the quantification of nine anti-HIV drugs from dry plasma spot on glass filter and their long term stability in different conditions. J. Pharm. Biomed. Anal. 2010;52:774-80.
28. Else L, Watson V, Tjia J, Hughes A, Siccardi M, Khoo S, et al. Validation of a rapid and sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. J. Chromatogr. B. 2010;878:1455-65.
29. Heine R, Rosing H, Gorp ECM, Mulder JW, Steeg WA, Beijnen JH, et al. Quantification of protease inhibitors and non-nucleoside reverse transcriptase inhibitors in dried blood spots by liquid chromatography–triple quadrupole mass spectrometry. J. Chromatogr. B. 2008;867:205-12.
30. Ganduri RB, Lanka RA, Pamidi S, Peddareddigari JR, Rao JVLNS. New RP-HPLC method for the determination of darunavir in tablet dosage form. Asian J Pharm Res. 2011;1:10-4.
31. Satyanarayana L, Naidu SV, Rao MN, Kumar A, Suresh K. The Estimation of Darunavir in Tablet dosage form by RP-HPLC. Asian J Res Pharm Sci. 2011;1:74-6.
32. Reddy BVR, Jyothi G, Reddy BS, Raman NVVSS, Reddy KSC, Rambabu C. Stability-Indicating HPLC Method for the Determination of Darunavir Ethanolate. J Chromatogr Sci. 2013;51:471-76.

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