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Experimental Article

Development and validation of a stability indicating HPLC method for the simultaneous analysis of lopinavir and ritonavir in fixed-dose combination tablets

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الملخص

الأهداف: تحديد الاستقرار الدوائي بطريقة بسيطة، ودقيقة، وسريعة عن طريق تحليل الاستشراب السائل عالي الدقة في نفس الوقت لدوائي "لوبي نافير" و"ريتو نافير" مجتمعين في جرعة دواء واحدة.

طرق البحث: تم تطوير التحديد الكمي لاستقرار دوائي "لوبي نافير" و"ريتو نافير" المضادة للفيروسات مجتمعين في جرعة دواء واحدة، بطريقة تحليل الاستشراب السائل عالي الدقة.

النتائج: تم التحقق من صحة الاستقرار الدوائي بهذه الطريقة وثبتت دقتها النوعية، وحساسيتها، وسرعتها، وصمودها وقوتها. لقد تم تعريض دوائي "لوبي نافير" و"ريتو نافير" لمختلف ظروف الإجهاد المتسارعة، من أجل الوصول لنتائج استقرار دوائي دقيقة.

الاستنتاجات: نستنتج من هذه الدراسة أن هذه الطريقة يمكن تطبيقها لمراقبة الجودة الروتينية لدوائي "لوبي نافير" و"ريتو نافير" مجتمعين في جرعة دواء واحدة، وكذلك الدواء السائب. كما يمكن استنتاج أن هذه الطريقة المقترحة ذات حساسية ودقة عاليتين، ويمكن تطبيقها بنجاح لتقييم موثوق به لمعرفة مقدار المواد الدوائية الفعالة الموجودة بالمنتجات التجارية لدوائي "لوبي نافير" و"ريتو نافير".

الكلمات المفتاحية: تدرك؛ تحليل الاستشراب السائل عالي الدقة؛ لوبي نافير؛ التحقق من الطريقة؛ ريتونافير

Abstract

Objectives: A simple, accurate, precise and rapid stability indicating HPLC method for simultaneous determination of Lopinavir and Ritonavir in combined dosage forms.

Methods: A validated stability indicating reversed phase high-performance liquid chromatographic method was developed for the quantitative determination of two antiviral drugs viz. lopinavir (LPV) and ritonavir (RTV) on Agilent TC C18 (2) 250 × 4.6 mm, 5 µ column using mobile phase composition of acetonitrile: 0.05 M phosphoric acid (55: 45, v/v) at a flow rate of 1.2 ml/min.

Results: Quantification was achieved with ultraviolet detection at 240 nm. The retention time obtained for ritonavir was at 4.35 min and for lopinavir was at 6.68 min. The result obtained with the detector response was found to be linear in the concentration range of 8–48 µg/ml for lopinavir and 2–12 µg/ml for ritonavir. This method has been validated and shown to be specific, sensitive, precise, linear, accurate, rugged, robust and fast. LPV and RTV were subjected to different accelerated stress conditions. The degradation products, were well resolved from the pure drug with significantly different retention time values.

Conclusion: It is concluded that this method can be applied for routine quality control of LPV and RTV in tablet dosage forms as well as in bulk drug. Hence the proposed method is highly sensitive, precise and accurate and it successfully applied for the reliable quantification of active pharmaceutical ingredient (API) content in the commercial formulations of lopinavir and ritonavir.

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Keywords: Degradation; High-performance liquid chromatography; Lopinavir; Method validation; Ritonavir

Introduction

Lopinavir, (2*S*) -*N*-[(2*S*, 4*S*, 5*S*) -5-[2- (2, 6-dimethylphenoxy) acetamido]-4-hydroxy-1,6-diphenylhexan -2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl) butanamide.[1] Ritonavir,1,3-thiazol-5-ylmethyl *N*-[(2*S*,3*S*,5*S*)-3-hydroxy-5-[(2*S*)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl] methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl] carbamate.[2] Lopinavir (LPV) is a protease inhibitor that has been co-formulated with a low dose of ritonavir (RTV) to improve its pharmacokinetic properties, resulting in substantially increased plasma exposure that maintains high drug levels throughout a 12-h dosing interval.[3–5] The chemical structures of drugs are shown in (Figure 1).

A literature survey reveals analytical methods like UV spectrophotometric,[6–9] HPTLC,[10–12] HPLC,[13–15] LC-MS for simultaneous determination of lopinavir and ritonavir in pharmaceutical dosage forms and biological fluids[16–19] are reported. However, no references are reported so far for the stability indicating simultaneous determination of said drugs by HPLC method. It is needed to determine the intrinsic stability of a drug substance in formulation and to establish degradation pathways of drug substances and drug products. It is also necessary to understand the chemical properties of drug molecules. So it was planned to develop and validate simple, rapid and precise stability indicating HPLC method for simultaneous estimation of said drugs in combined dosage form. Few reports were published concerning the degradation behavior of RTV and its forced degradation products, of which, recent report used Stability-indicating HPLC and HPTLC method and LC-MS-MS. The current study is comparable to the reported work.[20,21] In addition, the active pharmaceutical ingredient eluted earlier at 4.35 min and 6.68 min in the proposed study as compared to 4.82 and 9.0 min in the reported method. The parent drug stability test guidelines (Q1A) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability indicating.[22–24]

Experimental

Materials and Methods

LPV and RTV were procured as a gift samples by Emcure Pharmaceuticals Ltd., Pune India. All the reagents used were of HPLC grade were purchased from MERCK, India. The commercially available tablets containing a combination of RTV-50 mg and LPV -200 mg were procured from pharmacy. All the solutions for analysis were prepared and analyzed freshly.

Instruments

Agilent technologies 1260 LC system with gradient pump connected to DAD UV detector and Agilent TC C18 (2) 250 × 4.6 mm, 5 µ column, LC-GC AGN204PO balance was used for all weighing.

Method development

Chromatographic conditions

Chromatographic separation was achieved on Agilent TC C18 (2) 250 × 4.6 mm, 5 µ column using mobile phase composition of acetonitrile: 0.05 M phosphoric acid (55: 45, v/v) at a flow rate of 1.2 ml/min with 240 nm UV detection. A typical absorption spectrum of LPV and RTV as shown in Figure 2. The retention time obtained for RTV was at 4.35 min and for LPV was at 6.68 min as shown in Figure 3. Diluent was prepared by mixing 550 ml of acetonitrile, 450 ml of 0.05 M phosphoric acid, filtered through 0.45 µm and degassed before use.

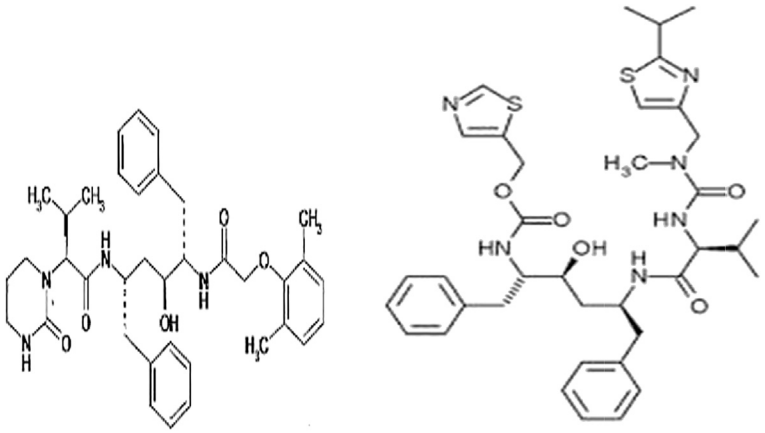

Figure 1: Chemical structure of Lopinavir and Ritonavir.