Journal of Applied Pharmaceutical Science Vol. 10(04), pp 075-099, April, 2020
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2020.104012
ISSN 2231-3354

Forced degradation study of efonidipine HCl ethanolate, characterization of degradation products by LC-Q-TOF-MS and NMR

Charu P. Pandya, Sadhana J. Rajput*

Department of Pharmaceutical Quality Assurance, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Center of Relevance and Excellence in New Drug Delivery System, Government of India, Vadodara, Gujarat, India.

ARTICLE INFO

Received on: 23/09/2019
Accepted on: 24/12/2019
Available online: 04/04/2020

Key words:
Forced degradation, efonidipine, preparative HPLC, LC-Q-TOF-MS, NMR and IR.

ABSTRACT

Efonidipine HCl Ethanolate is an antihypertensive drug with 1,4 dihydropyridine and phosphinane derivative. Forced degradation study was performed in Efonidipine as per the guidelines by International Conference on Harmonization (ICH) Q1A (R2). Extensive degradation and slight degradation were observed in alkaline and photolytic conditions, respectively, whereas acidic, oxidative, and thermal conditions did not show any degradation. Degradation products were separated on Thermo Hypersil BDS C18 column (250 × 4.6 mm, 5 µ), mobile phase in gradient mode using ammonium acetate buffer and acetonitrile with detection at a wavelength of 254 nm. Six degradation products in alkaline condition and four degradation products in photolytic condition were identified by HPLC and characterized by mass spectrometry using LC-Q-TOF-MS, and degradation pathway was proposed. This is the typical case of degradation, where co-solvent methanol reacts with Efonidipine to form pseudo degradation products such as DP1, DP4, DP5, and DP6. Three degradation products DP1, DP3, and DP4 in alkaline condition were isolated by preparative HPLC and were characterized by LC-Q-TOF-MS, $^{1}H/^{13}C$ NMR, and IR techniques. By characterization with these techniques, DP1 is characterized as 3-2-(N-benzylanilino)ethyl 3-oxo-2,2-dimethylpropyl hydrogen 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) pyridin-3-yl-3-phosphonate, DP3 is characterized as 2-(N-benzyl-N-phenylamino) ethanol, and DP4 is characterized as 3-methoxy-2,2-dimethylpropyl hydrogen 1,4-dihydro-2,6-dimethyl-5-methyloxycarbonyl-4-(3-nitro)phenylpyridin-3-yl-3-phosphonate. The developed method was validated as per guidelines by ICH with respect to linearity, accuracy, precision, limit of detection, and robustness.

INTRODUCTION

Efonidipine HCL Ethanolate (EFO) is a new calcium channel blocker with dihydropyridine and phosphinane derivative. It blocks both T-type and L-type calcium channels (Hikaru and Koki, 2002; Masuda and Tanaka, 1994; Nakano *et al*., 2010). It has a slow onset and longer duration of action. In a patient with essential hypertension, it causes an increase in renal blood flow, a decrease in renal vascular resistance, and an increase in glomerular filtration rate. It chemically consists of 2-(N-benzylanilino) ethyl 5-(5, 5-dimethyl-2-oxo-1, 3, 2^5}-dioxaphosphinan-2-yl)-2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3-carboxylate, ethanol, hydrochloride with molecular formula $C_{36}H_{45}ClN_3O_8P$, and molecular weight 714.19 g/mole (Pubchem, 2019). Efonidipine has pKa (basic) of 2.33 and log P is 5.35 (Drugbank, 2019). It was approved in 1995 as a brand name Landel®. It is approved for marketing in India by Drug controller general India to Zuventus Pharma as Efnocar®. The HPLC method development of EFO has been reported (Kumar *et al*., 2017). The LC-MS/MS method has been reported for the development of EFO in human plasma for pharmacokinetic applications and its stereospecific determination (Liu *et al*., 2015; 2016). Literature has been reported on spectroscopic studies on the interaction of efonidipine with bovine serum albumin (Wang *et al*., 2008), and the development considerations

*Corresponding Author
Sadhana J. Rajput, Department of Pharmaceutical Quality Assurance, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Center of Relevance and Excellence in New Drug Delivery System, Government of India, Vadodara, Gujarat, India.
E-mail: sjrajput @ gmail.com

Original Article

Stress Degradation Studies of Riociguat, Development of Validated Stability Indicating Method, Identification, Isolation and Characterization of Degradation Products by LC-HR-MS/MS and NMR Studies

Charu P Pandya, Sadhana J Rajput*

Department of Pharmaceutical Quality Assurance, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Center of Relevance and Excellence in New Drug Delivery System, Government of India, Vadodara, Gujarat, INDIA.

ABSTRACT

Aim: The present study reports the degradation behavior of new antihypertensive drug Riociguat under various stress conditions as per International Conference on Harmonization guidelines ICH, Q2(R1). **Materials and Methods:** Riociguat was subjected to stress degradation under hydrolytic (acidic, alkaline and neutral), oxidative, photolytic and thermal stress conditions to investigate the inherent stability. A rapid, accurate, precise and robust HPLC method was developed on Waters Symmetry C_{18} Column (150mm X 4.6 mm, 5μ) using isocratic elution of 10 mm ammonium acetate buffer pH 5.7 and acetonitrile in the ratio of 70:30 with the flow rate at 1.0 mL/min.The detection was performed at 254nm. **Results:** The drug was found to be degraded in alkaline and oxidative condition whereas it was stable under acidic, neutral hydrolytic, thermal and photolytic conditions. Two degradation products (DP1, DP2) under alkaline condition and one under oxidative condition (DP3) were characterized by LC-HR-MS/MS with accurate mass measurements. Degradation products (DP1, DP2 and DP3) were isolated by preparative HPLC and were characterized by ^{1}H NMR, ^{13}C NMR, APT and IR Techniques. **Conclusion:** Using spectral data analysis, alkaline degradation product DP1 wascharacterized as 2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)-N5-methylpyrimidine-4,5,6-triamine and DP2 was characterized as 2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)-6-amino-7-methyl-7H-purin-8(9H)-one while oxidative degradation product DP3was characterized as methyl 2-(1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl)-4,6-diaminopyrimidin-5-ylmethylcarbamate-N-oxide.The developed chromatographic method was validated in terms of specificity, linearity, accuracy, precision as per ICH guidelines. The robustness of the method was studied with 2-level fractional factorial design 2^4-1.

Key words: Riociguat, Stress degradation, RP-HPLC, LC-HR-MS/MS, Preparative HPLC, NMR.

INTRODUCTION

Riociguat (RIO) (Figure 1a) is the first drug which belongs to the class of soluble guanylate cyclase stimulator.[1,2] Nitric oxide when it binds to soluble guanylate cyclase, results in the synthesis of Cyclic Guanosine Monophosphate (cGMP) which regulates the mechanism of blood pressure. Pulmonary hypertension is characterized by impaired synthesis of nitric oxide and insufficient stimulation of nitric-oxide-s guanylate cyclase- guanosine monophosphate (cGMP) pathway. RIO has dual mode of action.[3] It stimulates sGC to nitric oxide and stabilizes NO-sGC binding thereby it causes relaxes of vascular smooth muscle. It has antiproliferative and antifibrinolytic effects. RIO

Submission Date: 05-04-2019;
Revision Date: 12-06-2019;
Accepted Date: 10-09-2019

DOI: 10.5530/ijper.53.4s.159

Correspondence:
Prof. Sadhana J Rajput,
Department of Pharmaceutical Quality Assurance, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Center of Relevance and Excellence in New Drug Delivery System, Government of India, Vadodara-390002, Gujarat, INDIA.
Phone: +91 9662060501
E-mail: sjrajput@rediffmail.com

www.ijper.org

RJC

RASĀYAN *J. Chem.*
Vol. 11 | No. 3 |1042 - 1049 | July - September | 2018
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SEPARATION AND CHARACTERIZATION OF MAJOR OXIDATIVE IMPURITY IN FIMASARTAN DRUG SUBSTANCE

Charu P. Pandya[1] and Sadhana J. Rajput[1,*]

[1]Department of Pharmaceutical Quality Assurance, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Center of Relevance and Excellence in New Drug Delivery System, Government of India, Vadodara (Gujarat) 390002,
*E-mail: sjrajput@gmail.com

ABSTRACT

In the stress degradation studies of Fimasartan, one major unknown oxidative degradation impurity was identified by LC-MS. This impurity was separated by preparative HPLC. By spectral data analysis (^{1}H NMR, ^{13}C NMR, DEPT, MS/MS and IR), this impurity is characterized as 2-(1-((2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl)-2-butyl-1,6 –dihydro-4-methyl-6-oxo-pyrimidin-5-yl)-N,N-dimethylacetamide. The details of stress studies, identification, isolation, characterization, formation and mechanism of this impurity are discussed and presented here.

Keywords: Fimasartan, Degradation, Identification, Isolation, Characterization

INTRODUCTION

Fimasartan (FIMA) (Fig.-1) is an antihypertensive agent. It is ninth non-peptide angiotensin II receptor antagonist (ARB)[1]. It is also used for the treatment of heart failure. Fimasartan acts by blocking angiotensin II receptor type I (AT1 receptor). Fimasartan is pyrimidin-4(3H)-one derivative of losartan which is obtained by replacement of imidazole ring in losartan. Fimasartan has higher potency and longer duration than losartan. Fimasartan was approved in South Korea in September 9, 2010. It is marketed as Kanarb by Boryung Pharmaceuticals in Korea. It is available as a tablet for oral use which contains 60 mg or 120 mg of Fimasartan potassium trihydrate[2]. It is approved in India by CDSCO in 2016. HPLC method has been developed for evaluation of stability and simultaneous determination of fimasartan and amlodipine in tablet dosage form[3]. UPLC tandem mass chromatographic method has been reported for determination of fimasartan in human plasma[4]. Literature has been reported on LC-MS method development for the estimation of fimasartan in human plasma[5-7].Literature has been reported on pharmacokinetics and metabolite profiling of fimasartan[8].

Recently we have developed a stability indicating method development of Fimasartan[9]. Major degradation was observed in oxidative condition. The objective of this study was to identify the major degradation product in oxidative condition after its isolation and characterization by mass, NMR and IR.

EXPERIMENTAL

Chemical Reagents and Solutions

Fimasartan (FIMA) standard drug was obtained from Angene Chemical Ltd (China). HPLC grade Acetonitrile was purchased from Rankem Pvt. Ltd., Mumbai. Chemicals used in the analysis were potassium dihydrogen ortho phosphate(AR grade), ortho phosphoric acid, formic acid purchased from Loba Chemie Pvt. Ltd., Mumbai. Hydrogen peroxide (H_2O_2) 30% v/v was purchased from S.D. Fine Chemical Ltd, Mumbai.

Preparation of Mobile Phase

10 mm phosphate buffer (pH 3) was prepared by dissolving 1.37 g of potassium dihydrogen phosphate in sufficient double distilled water to produce 1000ml and then the pH of the buffer was adjusted to 3.0 with

http://dx.doi.org/10.31788/RJC.2018.1132083

INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

IDENTIFICATION, SEPARATION AND CHARACTERIZATION OF POTENTIAL DEGRADATION PRODUCTS IN ACOTIAMIDE DRUG SUBSTANCE

Charu P. Pandya, Sadhana J. Rajput *
Department of Pharmaceutical Quality Assurance, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Center of Relevance and Excellence in New Drug Delivery System, Government of India, Vadodara, Gujarat, India
*Corresponding Author Email: sjrajput@gmail.com

Article Received on: 07/01/19 Approved for publication: 28/02/19

DOI: 10.7897/2230-8407.1003106

ABSTRACT

In the stress degradation studies of Acotiamide, two degradation products in alkaline conditions were identified by LC-MS. These impurities were isolated using preparative high performance liquid chromatography. By spectral data analysis (^{1}H NMR, ^{13}C NMR, MS, and IR), degradation products are characterized as 2-hydroxy-4,5-dimethoxy benzoic acid, and 2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxylic acid . The details of stress studies, identification, isolation, characterization, formation and mechanism of degradation products are discussed and presented here.

Keywords: Acotiamide, Degradation, Potential degradation product, Identification, Separation and Characterization.

INTRODUCTION

Acotiamide (ACOT) is chemically N-[2-[di(propan-2-yl)amino]ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxamide;trihydrate;hydrochloride.

ACOT is used in functional dyspepsia[1]. It controls upper gastrointestinal tract to inhibit symptoms resulting from hypomotility and delay in gastric emptying. It produces action in stomach through inhibition of muscarinic receptors M1 and M2 and cause increase in release of acetylcholine and inhibits activity of acetylcholine esterase [2].ACOT is used in the combination of esomeprazole to improve the symptoms of functional dyspepsia [3,4].

The presence of impurities can have a significant impact on the product quality, safety and efficacy, hence the percentage level of impurities need to control in the drug substance as well as a drug product [5, 6]. The literature review reveals that there were few analytical methods available for determination of acotiamide by HPLC[7], LC-MS-MS[8], UPLC-Q-TOF-MS [9, 10], identification of degradation products of acotiamide by UPLC/ESI-quadrupole TOF-tandem MS[11].

Recently we have developed stability indicating method of acotiamide[12]. Major degradation products were formed in alkaline conditions. To the best of our knowledge there are no reports on isolation and characterization of degradation products of acotiamide. The present research work describes identification of degradation products in the alkaline condition after its isolation and characterization by LC-MS, NMR and IR.

MATERIALS AND METHODS

Chemicals, Reagents and Solutions

Acotiamide hydrochloride trihydrate (ACOT) bulk drug was provided by Hetero Drugs Pvt. Ltd. HPLC grade acetonitrile was procured from Rankem Ltd, Mumbai. HPLC grade triethylamine and formic acid were procured from Loba Chemie Pvt. Ltd., Mumbai. Analytical grade hydrochloric acid (HCl), sodium hydroxide (NaOH) were procured from S D Fine Chem. Ltd. Mumbai, India.

Mobile Phase was prepared by 1ml of Triethylamine in 1000 ml of double distilled water and adjusting the pH with 2 ml of formic acid.

Instrumentation and Chromatographic Conditions

HPLC-PDA

Waters Alliance 2695 separation module equipped with Waters 2996 Photo diode Array detector (PDA) was used. Data acquisition and integration was processed with Emchem 2 software. Thermo Hypersil BDS C-8 column (250 X 4.6 mm i.d., 5µ particle size) was used. The mobile phase was composed of 0.1 % triethylamine with 0.2 % formic acid: acetonitrile with the gradient program: time / % of MP-B was 20/20, 21/20, 25/40, 35/20. Detection was done at 282 nm with flow rate of 1ml/min. The column oven was maintained at 40ºC.

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 9, 2018

Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD RP-HPLC METHOD OF ACOTIAMIDE

CHARU P. PANDYA, SADHANA J. RAJPUT*

Quality Assurance Laboratory, Centre of Relevance and Excellence in Novel Drug Delivery System, Faculty of Pharmacy, Shri G. H. Patel Pharmacy Building, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara, 390002, Gujarat, India
Email: sjrajput@gmail.com

Received: 22 Jan 2018 Revised and Accepted: 17 Jul 2018

ABSTRACT

Objective: The objective of present work was to develop and validate simple, precise, accurate and specific stability indicating method for determination of acotiamide in presence of its degradation products.

Methods: An isocratic RP-HPLC method has been developed using C-8 Thermo Hypersil BDS Column (250 x 4.6 mm i.d., 5µparticle size) with the mobile phase composition of acetonitrile: 0.1 % triethylamine in 0.2% formic acid (30: 70) at column oven temperature of 40 °C. The flow rate was 1.0 ml min^{-1} and effluent was detected at 282 nm. The method was validated in terms of linearity, accuracy, precision, LOD (Limit of Detection), LOQ (Limit of Quantification) and robustness as per ICH guidelines.

Results: The method was found to be linear in the range of 10-60µg/ml. Limit of detection and limit of quantification was found to 0.36µg/ml and 1.10 µg/ml.% Recovery was found to be in the range of 99.45%-99.75%and precision less than 2%. The developed method was successfully applied for estimation of Acotiamide in marketed tablet formulation and percentage assay was found to be 100.45%. Acotiamide was subjected to stress degradation under acid, base, neutral hydrolysis, oxidation, dry heat, photolysis conditions. Significant degradation was observed in acid and base degradation.

Conclusion: The developed RP-HPLC method was simple, rapid, accurate, precise and stability indicating for the estimation of Acotiamide in bulk and tablet dosage form.

Keywords: Acotiamide, Reverse Phase High Performance Liquid Chromatography(RP HPLC), Stability Indicating Assay Methods (SIAM's), Stress Degradation, ICH Q1A(R2), Q2R1

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i9.24925

INTRODUCTION

Acotiamide (ACOT) is N-[2-[di(propane-2-yl)amino]ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl)amino]-1,3-thiazole-4-carboxamide; trihydrate; hydrochloride. Acotiamide is a new prokinetic agent [1]. It is approved in Japan in 2013 [2]. It causes an increase in the release of acetylcholine thereby it exerts gastroprokinetic activity through acting as an antagonist on the M1 and M2 muscarinic receptors in the enteric nervous system. It inhibits anticholinesterase activity. It may also act directly on the gut and indirectly on the central nervous system by way of brain-gut axis. Studies have shown that acotiamide could enhance gastric emptying and gastric accommodation. Acotiamide could be a promising agent in the treatments with functional dyspepsia [3, 4].

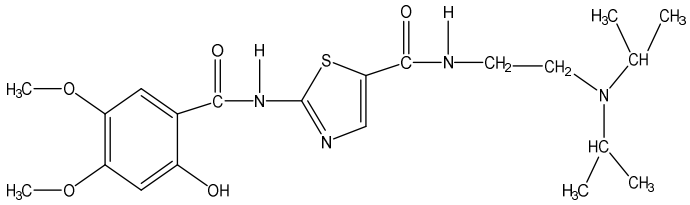

Fig. 1: Structure of acotiamide

The presence of impurities can have a significant impact on the product quality, safety and efficacy, hence the percentage level of impurities need to control in the drug substance as well as a drug product. Information on the stability of molecule aids in the selection of proper storage conditions which is the pre-requisite of documentation of drug profile. Force degradation study is an important parameter in pharmaceutical research and development to predict stability of drug. Stress testing helps in identification of degradation products and degradation behaviour of drug [5, 6]. The literature reveals there were few analytical methods available for determination of acotiamide by HPLC [7], LC-MS-MS [8], UPLC-Q-TOF-MS [9, 10], stability indicating the method by UPLC-Q-TOF-MS (gradient mode) [11], this reported method based on UPLC method. In the present study, a simple, rapid, precise, isocratic, accurate, cost-effective stability indicating liquid chromatographic method was developed by HPLC for the determination of Acotiamide in tablet dosage forms and validated as per ICH guidelines [12, 13].

MATERIALS AND METHODS

Chemicals, reagents and solutions

Acotiamide hydrochloride trihydrate (ACOT) bulk drug was provided by Hetero Drugs Pvt. Ltd. Acogut tablets 100 mg were purchased from local pharmacy. HPLC grade methanol and acetonitrile were purchased from Rankem Ltd, Mumbai. HPLC grade triethylamine and formic acid were purchased from Loba Chemie

Chromatographia
https://doi.org/10.1007/s10337-018-3617-y

ORIGINAL

Stress Degradation Studies of Anagliptin, Development of Validated Stability Indicating Method, Degradation Kinetics Study, Identification and Isolation of Degradation Products

Charu P. Pandya[1] · **Sadhana J. Rajput**[1]

Received: 14 July 2018 / Revised: 7 September 2018 / Accepted: 14 September 2018

Abstract

A gradient-specific stability indicating HPLC method was developed and validated for the determination of the antidiabetic agent anagliptin in laboratory mixtures. Reversed-phase chromatography was performed using a Shimadzu LC-20 AD pump (binary), Shimadzu PDA M-20A diode array detector, and Waters Symmetry C-18 column (150×4.6 mm, 3.5 µm) maintained at a column oven temperature of 40 °C with UV detection at 247 nm. A gradient program was run at flow rate of 1 mL min^{-1}. Mobile phase A consisted of a mixture of acetate buffer(10 mm) pH 5/methanol/acetonitrile in the ratio of 90:5:5. Mobile phase B consisted of a mixture of acetate buffer (10 mm) pH 5/methanol/acetonitrile in the ratio of 50:25:25. The method was validated according International Conference of Harmonization (ICH) guidelines. Linearity was observed in the concentration range of 10–120 µg/mL with regression coefficient r^2(0.999). The LOD was found to be 7.8 µg/mL and LOQ was found to be 22.68 µg/mL. Anagliptin was subjected to stresses such as acidic, alkali, oxidation, photolysis, and thermal conditions. The proposed method was validated as per ICH guidelines and was found to be accurate, precise, and specific. The drug showed significant degradation in alkaline and oxidative conditions. Alkaline and oxidative degradation followed first-order kinetics. Degradation rate constant and half-lives were determined. Degradation products in alkaline and oxidative conditions were identified by LC–MS. One major degradation product was isolated from each condition by preparative HPLC. These degradation products were characterized by ^{1}H NMR, ^{13}C NMR, DEPT, D_2O exchange, MS/MS, HRMS, and IR techniques. From the spectral data the alkaline degradation product was characterized as 1-{2-[1-(2-methylpyrazolo[1,5-*a*] pyrimidine-6-carboxamido)-methyl-propan-2-yl-amino]acetyl}pyrrolidine-2-carboxamide. The oxidative degradation product was characterized as *N*-[2-({2-[(2*S*)-2-cyanopyrrolidin-1-yl]-2-oxoethyl}amino)-2-methylpropyl]-2-methylpyrazolo-[1,5-*a*] pyrimidine-*N*-oxido-6-carboxamide.

Keywords RP-HPLC · Anagliptin · Gradient stability indicating assay · Identification · Isolation · ICH Q1A(R2) · Q2R1

Abbreviations

ICH	International Conference of Harmonization
HPLC	High-performance liquid chromatography
NMR	Nuclear magnetic resonance
HRMS	High-resolution mass spectroscopy
IR	Infrared
PDA	Photo diode array
UV	Ultraviolet
LC–MS	Liquid chromatography–mass spectroscopy
RT	Retention time
% DR	% Drug remaining after degradation
SD	Standard deviation
% RSD	% Relative standard deviation

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10337-018-3617-y) contains supplementary material, which is available to authorized users.

✉ Sadhana J. Rajput
sjrajput@gmail.com

[1] Department of Pharmaceutical Quality Assurance, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Center of Relevance and Excellence in New Drug Delivery System, Government of India, Vadodara, Gujarat 390002, India

Introduction

Diabetes mellitus is a metabolic disorder in which there is elevation of blood glucose levels. Anagliptin (ANA) is a dipeptidyl peptidase IV inhibitor that was approved for use in the treatment of type II diabetes in Japan in 2012.

Published online: 24 September 2018

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 6, 2018

Original Article

STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF CLEVIDIPINE BUTYRATE IN SYNTHETIC MIXTURE

CHARU P. PANDYA, SADHANA J. RAJPUT*

Quality Assurance Laboratory, Center of Relevance and Excellence in Novel Drug Delivery System, Faculty of Pharmacy, Shri G. H. Patel Pharmacy Building, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara 390002, Gujarat, India
Email: sjrajput@gmail.com

Received: 21 Mar 2018 Revised and Accepted: 15 May 2018

ABSTRACT

Objective: To develop and validate stability indicating HPTLC method for determination of clevidipine butyrate in synthetic mixture.

Methods: The present study deals with development and validation of stability indicating HPTLC method for estimation of clevidipine butryate. Chromatographic separation was performed on aluminum plate pre coated with Silica Gel 60 F254 using toluene: ethyl acetate (8:2) as mobile phase. TLC scanner was set at wavelength of 370 nm.

Results: Retention factor R_f of clevidipine was found to be 0.49. The method was validated as per ICH guidelines. Calibration curve was in the range of 1000-6000ng/band. The correlation coefficient was found to be 0.999. The precision expressed by RSD was less than 2%. The accuracy of method was confirmed by recovery studies using standard addition method and recovery was found to be 99.03-99.57%. The drug was subjected to ICH prescribed hydrolytic, oxidative, photolytic and thermal stress conditions. Clevidipine and its degradation products were well resolved under experimental conditions. The method was validated according to ICH guidelines. The drug showed significant degradation in alkaline and acidic condition and slight degradation in oxidative condition. The drug was stable in thermal condition.

Conclusion: A new, Simple, Accurate, Precise, Sensitive and economic stability indicating HPTLC method has been developed and validated for the determination of clevidipine and can be employed for stability indicating analysis.

Keywords: Clevidipine butryate, Stability indicating HPTLC method, ICH Guidelines

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i6.26110

INTRODUCTION

Clevidipine butyrate (CLEVI) is dihydropyridine L-type calcium channel blocker that is selective for vascular smooth muscle [1]. It is indicated for reduction of blood pressure when oral therapy is not possible [2]. It is available as lipid emulsion for intravenous infusion and is approved in US. It is marketed as Cleviprex by The Medicines Company [3]. It is effective in the treatment of both preoperative and post operative hypertension in adult cardiac surgery patients with rapid onset and short duration of action. Chemical name of CLEVI is methyl 5-{[(butanoyloxy) methoxy] carbonyl}-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate.
Literature survey reveals determination of CLEVI and its metabolite by HPLC method [4]. LC-MS/MS methods are reported for determination of CLEVI [5-7]. Various methods are reported for determination of related substances of clevidipine butyrate by HPLC method [9-11].

Force degradation studies have an important role in the development of pharmaceuticals. ICH guidelines requires that stability of samples should be analysed by stability indicating assay method which is to be developed by stress testing in conditions like hydrolytic, oxidative, thermal and photolytic and validated. To the best of our knowledge stability indicating method of clevidipine by HPTLC method has not been reported. HPTLC method has several advantages over HPLC methods. It is economic, samples can be analysed with shorter run time, low mobile phase consumption per sample. It facilitates automatic sample application and scanning to the plate, can handle large no. of samples at a time and is sensitive.

The objective of the present study was to develop simple, economical, specific, precise, accurate and reproducible HPTLC method development for the determination of clevidipine butyrate in bulk and synthetic mixture. The developed analytical method was validated for linearity, accuracy, precision, sensitivity and robustness as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Clevidipine butyrate (CLEVI) was purchased from Angene Chemical Ltd, China. Toluene, methanol, ethyl acetate of HPLC grade was purchased from SD Fine Chemical Ltd., Mumbai Pvt. Ltd.

Instrumentation and chromatographic conditions

Linomat 5 sample applicator (Camag, Switzeland), twin trough chamber (20 X 10 cm; Camag, Switzerland), TLC scanner IV(Camag, Switzerland), winCATS version 1.4.6 software (Camag, Switzerland), Hamilton microlitre syringe (Linomat syringe 659.0014, Hamilton Bonaduz Schweiz, Camag, Switzerland), UV chamber (Camag, Switzerland), precoated silica gel 60F254 aluminium plates (20X 20 cm,100 mm thickness; E. Merck, Darmstadt, Germany) were used in the study.

Optimized chromatographic conditions

Standard solutions and sample solutions were applied to the HPTLC plates from the bottom and 10 mm from the side edges in the form of bands with the band length of 6 mm on the pre-coated silica gel aluminium plate 60 F 254 (20x 20 cm), 100 µm thickness, using Camag Linomat V sample applicator. The mobile phase was toluene: ethyl acetate (8:2). Mobile phase components were mixed prior to use and the development chamber was left to saturate with mobile phase vapor for 20 min before each run. Ascending method development was carried out to a migration distance of 80 mm. TLC plates were dried in a current of air with air dryer. Scanning was performed at wavelength of 370 nm reflectance absorbance mode, slit dimension 6 X 0.3 mm, micro), scanning speed 20 mm/sec, data resolution 100 µm/step, optical filter second order, filter factor (Savitsky Golay 7). The source of radiation was deuterium lamp emitting a continuous UV spectrum of 200 to 400 nm. Data was integrated with WINCATS software.

INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF FIMASARTAN IN PRESENCE OF DEGRADATION PRODUCTS

Charu P. Pandya, Dr. Sadhana J.Rajput*

Quality Assurance Laboratory,Center of Relevance and Excellence in Novel Drug Delivery Systems, Faculty of Pharmacy, Shri. G.H. Patel Building, The Maharaja Sayajirao University of Baroda, Fatehgunj, Vadodara-390002, Gujarat, India.

ARTICLE INFO

Article history
Received 30/11/2017
Available online
06/12/2017

Keywords
Fimasartan,
RP-HPLC,
Stability Indicating Assay Method (SIAM's),
ICH Q1A(R2),
Q2R1.

ABSTRACT

A simple, isocratic, specific and sensitive stability-indicating high-performance liquid chromatographic method was developed and validated for the determination of fimasartan in synthetic mixture. Fimasartan is used to treat hypertension. Reverse phase chromatography was performed on Shimadzu LC-20AD pump (binary) and Shimadzu PDA-M20A Diode Array Detector using Hypersil BDS C18 column (250 x 4.6 mm, 5µm) mobile phase containing Phosphate buffer pH3 :Acetonitrile (50:50, v/v) with a flow rate of 1ml/min. Detection was done at wavelength 262nm. Linearity was observed in the concentration range of 5-30µg/mL (R^2=0.999) with regression equation y=78487x+66095. The LOD was found to be 1.54µg/ml and LOQ was found to be 4.67µg/ml. Fimasartan was subjected to stress conditions such as acidic, alkaline, oxidation, photolysis and thermal degradations. The proposed method was validated as per ICH guideline and was found to be accurate, precise and specific. The degradation products peaks were well resolved from the standard drug peak and hence this method can be used for quality control of fimasartan. The drug showed significant degradation in alkaline and oxidative condition. Degradation products in alkaline and oxidative conditions were identified by LC-MS. Oxidative degradation followed first order kinetic. Degradation rate constants and half -lives were determined.

Corresponding author
Dr. Sadhana J.Rajput
Quality Assurance laboratory,
Center of Relevance and Excellence in Novel Drug Delivery Systems,
Faculty of Pharmacy, Shri. G.H. Patel Building
The Maharaja Sayajirao University of Baroda,
Fatehgunj, Vadodara-390002, Gujarat, India

*Please cite this article in press as **Charu P. Pandya** et al. Validated Stability Indicating RP-HPLC Method for the Determination of Fimasartan in Presence of Degradation Products. Indo American Journal of Pharmaceutical Research.2017:7(11).*