List of publication

1. Sojitra C, Tehare A, Dholakia C, Sudhakar P, Agarwal S, Singh KK. Development and validation of residual solvent determination by headspace gas chromatography in Imatinib Mesylate API. SN Applied Sciences. 2019 Mar 1;1(3):233.

2. Joshi VM, Sojitra C, Sasane S, Shukla M, chauhan R, chaubey V, Jain S, Shah K, Mande HM, Soman SS, Pamidimukkala PS. Practical and Efficient Synthesis of 2-Thio-imidazole derivative-ZY12201: A Potent TGR5 Agonist. Organic Process Research & Development. 2020 Jul 30.

3. Sojitra C, Agarwal S, Dholakia C, Sudhakar P, Singh KK. Quantification of Hydrazine Hydrate in Imatinib Mesylate at Genotoxic level by Chromatographic Method. Indian drugs. December 2019. 56 (12)

4. Sojitra C, Tehare A, Dholakia C, Sudhakar P, Agarwal S, Singh KK. Development of Chromatographic Method for Determination of Impurities in Solid Dispersion of Dasatinib. Brazilian Journal of Analytical Chemistry, 2018, 5(21), pp 19-29

5. Sojitra C, Dholakia C, Sudhakar P, Agarwal S, Singh KK. Identification of degradation impurity of novel TGR5 receptor agonist by force degradation study. Canadian journal of chemistry (Under communication)

Poster presentation

1. Chandrakant Sojitra, Chintan Dholakia, Padmaja Sudhakar, Sameer Agarwal, and Kumar K. Singh.” Quantification of hydrazine hydrate in imatinib mesylate at genotoxic level by chromatographic method” in ramanbhai patel 9th international symposium on “Advances in new drug discovery & development “ at Zydus Corporate park Feb 6-8, 2020.

Research Article

Development and validation of residual solvent determination by headspace gas chromatography in Imatinib Mesylate API

Chandrakant Sojitra[1,2,3] · Ajay Tehare[1] · Chintan Dholakia[1] · Padmaja Sudhakar[2] · Sameer Agarwal[3] · Kumar K. Singh[1]

Abstract
An accurate, precise, robust and sensitive method was developed for residual solvents determination by fast static headspace gas chromatography (HSGC) with flame ionization detector in Imatinib Mesylate API. Residual solvents in drug substances are quantified using gas chromatography with headspace. As per regulatory guidelines, residual solvents must be controlled for release any batches of active pharmaceutical ingredients (API). This paper includes the development and validation of HSGC method for the determination of residual solvents specifically methanol, acetone, dichloromethane, n-hexane, ethyl acetate and pyridine in Imatinib Mesylate API. Imatinib Mesylate is a specific inhibitor of BCR-ABL tyrosine kinase. DB-624 capillary column, 30 m long × 0.53 mm internal diameter, the 3 µm film thickness was used for analysis. To minimize degradation, injector temperature was set at 170 °C. The initial oven temperature was kept at 35 °C for 2 min and used ramp 1 at a rate of 4 °C min^{-1} to temperature of 80 °C hold for 0 min and used ramp 2 at a rate of 40 °C min^{-1} to a final temperature of 230 °C for 12 min hold time. Nitrogen was selected as a carrier gas. 1-Methyl-2-Pyrrolidinone (NMP) was used as a sample solvent. The method can be readily used to determine defined residual solvents present in a various range of APIs, intermediates, excipients and drug products.

Keywords Residual solvents · Imatinib Mesylate · Headspace gas chromatography · Flame ionization detector · Method validation

1 Introduction

Residual solvents or volatile organic solvents are used or formed during the manufacturing of pharmaceutical drug substances, intermediates, excipients or pharmaceutical drug product [1]. The solvents are toxic, have no therapeutic importance and affect the quality and stability of drug substances and drug products so they are not desirable in the final product [2–4]. Although it is difficult to remove completely with the common techniques used in practical manufacturing process such as increased process temperature or/and decreased pressure, they need to be minimized. However, depending on the nature of the API, residual solvents and drying condition of the process, some amount of residual solvents traces can be retained in the final drug substances or drug product. Thus, acceptable levels of many residual solvents are included in regulatory guideline; particularly in guideline Q3C issued by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s42452-019-0233-x) contains supplementary material, which is available to authorized users.

✉ Chandrakant Sojitra, chandrakant.sojitra@yahoo.com; ✉ Sameer Agarwal, sameeragarwal@zyduscadila.com | [1]API Division, Cadila Healthcare Limited, Sarkhej-Bavla N.H. No. 8 A, Changodar, Ahmadabad 382210, India. [2]Department of Chemistry, Faculty of Science, M.S.University of Baroda, Baroda 390002, India. [3]Zydus Research Centre, Cadila Healthcare Ltd., Sarkhej-Bavla N.H. No. 8 A, Moraiya, Ahmadabad 382210, India.

SN Applied Sciences (2019) 1:233 | https://doi.org/10.1007/s42452-019-0233-x

Received: 29 August 2018 / Accepted: 6 February 2019 / Published online: 14 February 2019

SN Applied Sciences
A SPRINGER NATURE journal

ORGANIC PROCESS RESEARCH & DEVELOPMENT
OPR&D

pubs.acs.org/OPRD Article

Practical and Efficient Synthesis of 2-Thio-imidazole Derivative—ZY12201: A Potent TGR5 Agonist

Vivek M. Joshi, Chandrakant Sojitra, Santosh Sasane, Mrigendra Shukla, Rakesh Chauhan, Vipin Chaubey, Sarika Jain, Kalpesh Shah, Hemant Mande, Shubhangi Soman, Padmaja Sudhakar Pamidimukkala, Shailesh R. Shah, Bipin Pandey, Kumar K. Singh, and Sameer Agarwal*

Cite This: https://dx.doi.org/10.1021/acs.oprd.0c00234 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

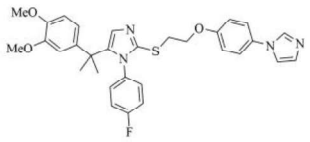
ABSTRACT: Early scalable process development for the synthesis of **ZY12201**, a novel TGR5 receptor agonist, as a potential clinical candidate is described. A practical, efficient, and scalable synthetic route provided **ZY12201** in seven steps and 32% overall yield. The key step involves an inexpensive acetic acid-mediated cyclization of thiourea **6** for the construction of 2-thio-imidazole derivative **7**. The developed process demonstrated cost-effective, high-yielding, kilogram-scalable, and environmentally friendly synthesis of ZY12201. This high-yielding route enabled us to rapidly synthesize large quantities of **ZY12201** in 99% purity to support *in vivo* and toxicity studies.

KEYWORDS: *TGR5, TGR5 agonist, type 2 diabetes, 2-thio imidazole, 1,2-dibromo ethane*

INTRODUCTION

Diabetes is increasing at an alarming rate with approximately more than 463 million people affected worldwide, and the number of patients is expected to reach 700 million by the year 2045.[1] Diabetes caused at least USD 760 billion in health expenditure in 2019, which is 10% of total spending on adult treatment. With the rising incidence of obesity, a major risk factor for the onset of type 2 diabetes, this metabolic disorder represents a major health concern and is commonly termed as "Diabesity".[2] Although a range of antidiabetic agents are available, still there is a high unmet medical need.[3−5]

Takeda G protein-coupled receptor 5 (TGR5), also known as GPR 131, or GPBAR1, is a bile acid G protein-coupled receptor primarily expressed in monocytes, macrophages, lung, spleen, and intestinal tract and is activated by bile acids.[6,7] It has been suggested that bile acids induce glucagon-like peptide-1 secretion from primary intestinal enteroendocrine cells by increasing the intracellular cAMP levels *via* the TGR5 receptor.[8,9] The activation of TGR5 receptors in brown adipose tissue has been proposed to increase energy expenditure through the induction of type 2 iodothyronine deiodinase (D2).[10] Recent studies suggest that the activation of TGR5 in macrophages may play a key role in the pathogenesis of atherosclerosis.[11] TGR5 is being investigated as an attractive therapeutic target for the treatment of obesity and its highly associated type 2 diabetes. Recently, we have reported the identification of several novel TGR5 agonists,[12,13] including the discovery of 2-((2-(4-(1*H*-imidazol-1-yl)-phenoxy)ethyl)thio)-5-(2-(3,4-dimethoxyphenyl)propan-2-yl)-1-(4-fluorophenyl)-1*H*-imidazole (**ZY12201**) as a potent, selective, orally bioavailable, and efficacious TGR5 agonist (Figure 1).[14] The 2-thio-imidazole derivative **ZY12201** was found to be a highly potent TGR5 agonist (hTGR5 EC_{50} = 57 pM, mTGR5 = 62 pM) with a favorable pharmacokinetic profile. The compound was found to have excellent glucose-lowering effects *in vivo* during an oral glucose tolerance test in DIO C57BL/6 mice with ED_{50} of 7.9 mg/kg and ED_{90} of 29.2 mg/kg. Thus, an efficient synthetic route was needed to cater large quantities of **ZY12201** to support further preclinical studies including *in vivo* and toxicity studies.



ZY12201

Figure 1. TGR5 receptor agonist.

Our original medicinal chemistry synthesis of **ZY12201** (Scheme 1),[14,15] began with commercially available 2-(3,4-dimethoxyphenyl) acetonitrile. Key intermediate **2** was obtained from 2-(3,4-dimethoxyphenyl) acetonitrile *via* a four-step sequence in 56% overall yield. Other synthetic routes of **2** involved harsh conditions, multistep synthesis with cumbersome workup, difficult product isolation, and unsat-

Received: May 18, 2020
Published: July 30, 2020

QUANTIFICATION OF HYDRAZINE HYDRATE IN IMATINIB MESYLATE AT GENOTOXIC LEVEL BY CHROMATOGRAPHIC METHOD

Sojitra C.[a,b,c], Agarwal S.[b,*], Dholakia C.[a], Sudhakar P.[c], and Singh K. K.[a]

(Received 29 June 2019) (Accepted 15 November 2019)

ABSTRACT

Hydrazine hydrate has genotoxic effect in nature and so it should be controlled down as Potential Genotoxic Impurity (PGI). Being polar molecule, hydrazine hydrate ($N_2H_4H_2O$) has no chromophores present in structure which can follow Lambert beer law, thus it is difficult to analyze. The present work described an accurate and highly sensitive reversed-phase liquid chromatography-UV derivatization method for determination of hydrazine in imatinib mesylate drug substance. The method of quantification was developed by attaching chromophores to hydrazine with derivatization, which helped to increase sensitivity. The derivatization of hydrazine hydrate was performed using 1% methanolic solution of benzaldehyde which acts as derivatizing agent. The derivatized product 1,2-dibenzylidenehydrazine gives maximum absorbance at 300 nm and at this wavelength no interference of solvents and other impurities are noted. Limit of detection for developed method was 0.002 µg/g. The developed method was validated to determine hydrazine content and can be used in quality control for commercial batch release of imatinib mesylate drug substances with a genotoxic specification limit level 1.87 µg/g by HPLC.

Keywords: Hydrazine, Imatinib Mesylate, Potential Genotoxic Impurity (PGI), Chromatographic Method, Method Validation

INTRODUCTION

Hydrazine hydrate is a colorless flammable liquid which has pungent ammonia-like odour and is very dangerous to handle in solution[1,2]. Hydrazine hydrate is highly reactive and posses carcinogenic activity in nature, inspite of these limitations it is used in manufacturing of numerous intermediates and pharmaceutically active ingredients in bulk productions[3,4]. Structural characteristics present in hydrazine hydrate are mainly responsible for the genotoxicity. Moreover, metabolites produced from metabolism of hydrazine hydrate have synergistic effect on genotoxic potential of hydrazine hydrate[5]. At highly reactive methyl diazonium ions and methyl free radicals are formed when hydrazine hydrate is intercalate with DNA which further cause cellular damage[6]. In addition hydrazine hydrates reacts with endogenous formaldehyde and tends to produce formaldehyde hydrazone which is also genotoxic in nature[7]. Alkylating compounds such as diazomethane produced as metabolites of hydrazine hydrate and also account for the known genotoxicity[8]. Hydrazine hydrate has been employed as reducing agent in various reactions, for example Knorr synthesis,[9,10] Gabriel synthesis[11] and Wolff-Kishner reaction[12] and should be controlled at Therapeutic Threshold Concentration (TTC) limit[13,14]. Estimation of hydrazine hydrate is very difficult as it does not possess chromophores which can be detected on UV spectroscopy or on HPLC nor ionizable group for LC-MS nor carbon atoms for flame ionization detection (GC). Due to these reasons, derivatization has a strategic advantage in development of highly selective and sensitive method for determination of hydrazine hydrate. For determination and quantification of hydrazine hydrate, various methods are reported such as GC-MS,[15,16] LC-MS/MS[4,17,18]. High performance Liquid chromatography[19-21] and ion chromatography.[22] Most of the methods use derivatization for estimation of hydrazine hydrate e.g, Zhang et.al[23]. developed a method having 0.25 ppm detection limit using 2-hydroxy-1-naphthalaldehyde as a derivatizing agent, while Tamas et.al[24]. have developed a method using benzaldehyde as a derivatizing agent in allopurinol with solid phase extraction for sample preparation, but no method is available for hydrazine to quantify at a TTC level by HPLC.

[a] Cadila Healthcare Limited, API Division, Sarkhej-Bavla N.H. No. 8 A, Changodar, Ahmedabad - 382210, Gujarat, India;
[b] Zydus Research Centre, Cadila Healthcare Ltd. Sarkhej-Bavla N.H. No. 8 A, Moraiya, Ahmedabad - 382210, Gujarat, India;
[c] Department of Chemistry, Faculty of Science, M.S.University of Baroda, Baroda - 390002, Gujarat, India
*For Coresspondence E-mail: sameeragarwal@zyduscadila.com

Article

Br. J. Anal. Chem., **2018**, *5* (21), pp 19-29
DOI: 10.30744/brjac.2179-3425.2018.5.21.19-29

Development of Chromatographic Method for Determination of Impurities in Solid Dispersion of Dasatinib

Chandrakant Sojitra[1,2,3]*, Ajay Tehare[1], Chintan Dholakia[1], Padmaja Sudhakar[2], Sameer Agarwal[3]* and Kumar K. Singh[1]

[1]Cadila Healthcare Limited, API Division, Sarkhej-Bavla N.H. Nº 8 A, Changodar, Ahmedabad – 382 210, India
[2]Department of Chemistry, Faculty of Science, M. S. University of Baroda, Baroda – 390 002, India
[3]Zydus Research Centre, Cadila Healthcare Ltd. Sarkhej-Bavla N.H. Nº 8 A, Moraiya, Ahmedabad – 382 210, India

An accurate, fast, precise and economic gradient reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for quantitative determination of process and degradation related impurities in the solid dispersion of dasatinib drug substance. The optimum separation was achieved by Sunniest C18, 250 x 4.6 mm, 5 µm column at 35 °C. The mobile phase A was 20 mM ammonium acetate buffer (pH 5.0) and mobile phase B was composed of methanol:buffer:acetonitrile (90:5:5) (%, v/v/v); the chromatographic analysis was performed with gradient condition detecting the related substances at wavelength 310 nm at flow rate of 1.2 mL/min. The resolution for dasatinib and six related components was found to be greater than 2.0 for any pair of impurities. The stability indicating nature of the method was demonstrated by performing force degradation studies. Significant degradation was observed when the solid dispersion of dasatinib was subjected to oxidation, thermal and photo degradation, while the drug substance was stable in acid and alkali degradation. Relative standard deviation obtained for the system precision and method precision studies was less than 5%. The accuracy of the method was demonstrated by performing recovery studies through spiking studies. The developed method was validated for linearity, specificity, accuracy, precision, limit of detection, limit of quantitation and robustness studies; it can be used in quality control for commercialization of solid dispersion of dasatinib drug substances and performing stability studies.

Keywords: Dasatinib, chromatographic method, stress testing, method validation.

INTRODUCTION

Dasatinib is an inhibitor of multiple tyrosine kinases, inhibiting the growth of chronic myeloid leukemia and acute lymphoblastic leukemia cell lines overexpressing BCR-ABL [1,2]. Dasatinib is an approved drug, sold under the brand name Sprycel, and is a chemotherapy medication used for the treatment of chronic myelogenous leukemia and acute lymphoblastic leukemia [3].

Stability testing is an integral part of the new drug development process because it provides quality of drug substances in different storage condition having variable temperature and humidity. As per the International Conference on Harmonization (ICH) Guideline Q1A(R2) [4], the shelf life of any drug substances is determined by stability studies. The quantification of impurities and dasatinib API is required to be determined using stability indicating chromatographic method, as suggested by the previously mentioned ICH guideline and United State Pharmacopoeia (USP) [5]. However, a few methods have been used for quantification of major tyrosine kinase inhibitors i.e. imatinib, dasatinib and nilotinib in human plasma [6-17].

*chandrakant.sojitra@yahoo.com; sameeragarwal@zyduscadila.com
https://orcid.org/0000-0002-7039-3028