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Short communication

Sensitive derivatization methods for the determination of genotoxic impurities in drug substances using hyphenated techniques

Nanduri V.V.S.S. Raman*, Adapa V.S.S. Prasad*, Kura Ratnakar Reddy

Hetero Drugs Ltd. (R&D), Plot No. B. 80 & 81, APIE, Balanagar, Hyderabad 500018, India

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ABSTRACT

Six sensitive derivatization methods for the determination of genotoxic impurities in selected drug substances were developed using hyphenated techniques. Some of the raw materials, reagents and reaction intermediates of the selected drug substances were identified as genotoxic impurities through DEREK software for windows. The genotoxic impurities which are amenable for derivatization were selected as substrates. Derivatizing agents were selected based on the functional groups of the genotoxic impurities. The chemistry involved in the derivatization was explained with suitable mechanisms. An appropriate hyphenated technique viz. LC–MS and GC–MS was opted based on the sensitivity and aromaticity of the derivatized genotoxic impurities. All the methods were validated as per International Conference on Harmonization guidelines. Correlation coefficient values were found about 0.99. The obtained % R.S.D values from replicate injections in the range of 2.3–4.8 and % recoveries of the added impurities in the range of 83.7–101.7 ensured the precision and accuracy, respectively.

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1. Introduction

The emphasis on impurities has been emerged in the current pharmaceutical analysis due to their toxicological effects [1,2]. Genotoxic impurities have the potential to cause cancer in humans [3]. The European Medicines Agency guideline on the limits of genotoxic impurities [4] proposes a threshold of toxicological concern value (1.5 μg/day) to define a common exposure level that do not pose a risk of significant carcinogenicity. Hence, it is necessary to determine the genotoxic impurities in drug substances. However, the direct determination of some of the genotoxic impurities leads to erroneous results as they react rapidly, decompose, hydrolyse and/or oxidize partially. Thus, derivatization [5] can be opted to convert them into particular chemical forms that are more compatible with the chromatographic environment. Even though, mass spectrometers provide specificity and sensitivity, direct determination using mass spectrometers alone cannot survive the purposes due to the wide range of volatilities, ionizations and stabilities of genotoxic impurities. Hence, derivatization along with hyphenated techniques can be preferred.

Literature survey [6–11] reveals that derivatization was focussed less on the determination of genotoxic impurities in drug substances. Hence, five drug substances [12] were selected and some of their raw materials, reagents and reaction intermediates were identified as genotoxins through DEREK software for windows [13]. Accordingly, six derivatization methods viz. (1) (S)-tert-butyl-3-oxo-1-phenylpropylcarbamate (TBC) in maraviroc by LC–MS (method-1), (2) methyl hydrazine carboxylate (MHC) in aprepitant by LC–MS (method-2), (3) methyl-2-(2-chloro-1-iminomethyl) hydrazine carboxylate (MIC) in aprepitant by LC–MS (method-3), (4) ethylchloroformate (ECF) in mosapride citrate dihydrate by LC–MS (method-4), (5) 2,3,4,5-tetrafluorobenzoyl chloride (TFC) in levofloxacin hemihydrate by GC–MS (method-5) and (6) formaldehyde in tetrabenazine by GC–MS (method-6) using hyphenated techniques were developed and validated.

2. Experimental

2.1. Materials

All the drug substances, (S)-tert-butyl-3-oxo-1-phenylpropylcarbamate, methyl hydrazine carboxylate, methyl-2-(2-chloro-1-iminomethyl) hydrazine carboxylate, 2,3,4,5-tetrafluorobenzoyl chloride and N-methylmethanamine were obtained from synthetic division, Hetero Drugs Ltd (R&D),

* Corresponding authors.
E-mail addresses: raman@heterodrugs.com, nanduri.raman@gmail.com (N.V.V.S.S. Raman), prasad.a@heterodrugs.com, avssprasad@rediffmail.com (A.V.S.S. Prasad).

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Hyderabad, India and characterized by using UV, IR, NMR and Mass spectral analysis. 2,4-dinitrophenyl hydrazine (2,4-DNP) and aniline were obtained from Sigma-Aldrich (Saint Louis, MO 63103, USA). Ethylchloroformate, formaldehyde, benzaldehyde, pyridine, methanol, acetonitrile, ammonium formate, ammonia and hydrochloric acid were procured from Merck Specialities Pvt. Ltd (Mumbai, India).

2.2. Instrumentation

LC–MS analysis (methods 1–4) was carried out on LCMS-2010 EV single quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan) having LCMS solution software. Interface, curve dissolvation line and detector voltages were fixed as 4.5 kV, 5.0 V and 1.75 kV, respectively. Nebulization gas flow was 1.5 l/min interface, curve dissolvation line and heat block temperatures were kept as 250, 250 and 200 °C, respectively. Method-1 was run in ESI −ve mode whereas and methods 2–4 in ESI +ve mode.

GC–MS analysis (methods 5 and 6) was carried out on a GC system coupled with quadrupole mass spectrometer equipped with AOC-5000 autoinjector (GCMS-QP2010 model, Shimadzu Corporation, Kyoto, Japan). Helium was used as carrier gas. Mass spectrometric conditions include ion source temperature as 250 °C, interface temperature as 230 °C, detector gain as 1.9 KV, threshold as zero. Selective Ion Monitoring mode was selected for validation studies.

2.3. Chromatographic conditions

The chromatographic conditions for six developed methods are briefly given below.

Method-1 conditions include, column: Develosil C8 UG-5, 150 mm × 4.6 mm, 3.5 μm (Nomura Chemical Co., Ltd, Seto, Aichi, Japan), mobile phase: 30:70 (v/v) mixture of water and acetonitrile, flow rate: 0.8 mL/min, injection volume: 10 μL, column temperature: 27 °C, auto sampler temperature: 5 °C, run time: 20 min, m/z = 428.15 [M−1].

Method-2 conditions include, column: Inertsil ODS-3, 100 × 4.6, 5 μm (GL Sciences Inc., Tokyo, Japan), mobile phase: 50:50 (v/v) mixture of buffer (0.01 M ammonium formate) and acetonitrile, flow rate: 0.4 mL/min, injection volume: 20 μL, column temperature: 35 °C, auto sampler temperature: 10 °C, run time: 30 min, m/z = 179.05 [M+1].

Method-3 conditions include, column: Zorbax SB-C18, 150 mm × 4.6 mm, 5 μm (Agilent Technologies, Santa Clara, CA, USA), mobile phase: Gradient containing A (0.01 M ammonium formate, pH adjusted to 7.0 with ammonia) and B (methanol) with a program of Time (min)/% B as 0.01/60, 6/60, 7/90, 20/90, 21/60 and 30/60, flow rate: 0.4 mL/min, injection volume: 20 μL, column temperature: 35 °C, auto sampler temperatures: 10 °C, run time: 30 min, m/z = 175.05 [M+1].

Method-4 conditions include, column: Develosil ODS UG-5, 150 mm × 4.6 mm, 5 μm, mobile phase: 40:60 (v/v) mixture of buffer (0.01 M ammonium formate, pH adjusted to 7.0 with ammonia) and acetonitrile, flow rate: 0.8 mL/min, injection volume: 10 μL, column temperature: 27 °C, auto sampler temperature: 20 °C, run time: 25 min, m/z = 166.0 [M+1].

Method-5 conditions include, column: Stabilwax, 30 m × 0.32 mm × 1.0 μm (Restek Corporation, Bellefonte, PA, USA), flow rate: 1.4 mL/min, split: 1:5, injection volume: 1.0 μL, temperature program: initially 100 °C for 2 min, rise to 220 °C at the rate of 10 °C/min and hold for 16 min, injector temperature: 220 °C, run time: 30 min, m/z = 177 {M−OCH_3].

Method-6 conditions include, column: Stabilwax, 30 m × 0.32 mm × 1.0 μm, flow rate: 1.44 mL/min, split: 1:5, temperature program: 38 °C initially for 8 min, rise to 220 °C at the rate of 15 °C/min and hold for 9.9 min, injector tempearture: 220 °C, runtime: 30 min, Head space conditions: incubation temperature: 60 °C, syringe temperature: 140 °C, incubation time: 20 min, injection load: 1.0 mL, HS program run time: 30 min, m/z = 59 [M−C_2H_5O].

2.4. Sample preparations

The standard and sample preparations are given below.

In method-1, diluent was prepared by mixing 0.10% 2,4-DNP in acetonitrile and 0.01% (v/v) hydrochloric acid in ethanol in 1:1 (v/v) ratio. The standard solution (1.25 ppm) was prepared by dissolving 5.0 mg of TBC in 100 mL of diluent and further diluting 5 μL to 100 mL with diluent. Test sample solution was prepared by dissolving 20 mg of test sample in 10 mL of diluent.

In method-2, standard solution (12 ppm) was prepared by taking 25 mg of MHC into 50 mL volumetric flask containing 1.0 mL of benzaldehyde, dissolving and diluting to volume with diluent (methanol). 12 μL of this solution was further diluted to 100 mL with diluent. Test sample solution was prepared by dissolving 50 mg of drug substance in 10 mL volumetric flask containing 0.20 mL of benzaldehyde. The mixture was dissolved and diluted to the mark with diluent.

In method-3, standard solution (12 ppm) was prepared by taking 25 mg of MIC into 50 mL volumetric flask containing 0.5 mL of N-methylmethanamine, dissolving and diluting to volume with diluent (methanol). 12 μL of this solution was further diluted to 100 mL with diluent. Test sample solution was prepared by dissolving 50 mg of test sample in 10 mL of volumetric flask containing 0.10 mL of N-methylmethanamine. The mixture was dissolved and diluted to the mark with diluent.

In method-4, standard solution (100 ppm) was prepared by taking 5.0 mg of ECF into 100 mL volumetric flask containing 0.50 mL of aniline and 0.50 mL of pyridine, dissolving and diluting to volume with diluent (methanol). 0.80 mL of this solution was diluted to 100 mL with diluent. Test sample solution was prepared by dissolving 40 mg of test sample in 10 mL of volumetric flask containing 0.05 mL of aniline and 0.05 mL of pyridine amd diluting upto the mark with methanol.

In method-5, standard solution (2 ppm) was prepared by taking 5.0 mg of TFC into 100 mL volumetric flask, dissolved and diluted to volume with diluent. 0.20 mL of this solution was diluted to 100 mL with diluent (methanol). Test sample solution was prepared by dissolving 250 mg of test sample in 5 mL of diluent. This solution was mixed for 15 min.

In method-6, diluent was prepared by transferring 10 g of PTSA in 1000 mL volumetric flask, dissolving and diluting to the mark with ethanol. Blank solution was prepared by transferring 0.40 mL of 0.1 N HCl solution and 2.0 mL of diluent into a 20 mL head space vial, septum was placed and cappped immediately. Standard solution (30 ppm) was prepared by taking 50 mg of formaldehyde into 50 mL volumetric flask, dissolving and diluting to volume with diluent. 30 μL of this solution was diluted to 100 mL with diluent. 2.0 mL of this solution and 0.40 mL of 0.1 N HCl were transfreed into into a 20 mL head space vial, placed the septum and capped immediately and heated at 60 °C for 20 min. Test sample solution was prepared by transferring 20 mg of test sample into a 20 mL head space vial containing 0.40 mL of 0.1 N HCl solution and 2.0 mL of diluent. Septum was placed and capped immediately and heated at 60 °C for 20 min.

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