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SHORT COMMUNICATION

Determination of genotoxic alkyl methane sulfonates and alkyl paratoluene sulfonates in lamivudine using hyphenated techniques

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Abstract Two highly sensitive methods for the determination of genotoxic alkyl methane sulfonates (AMSs) and alkyl paratoluene sulfonates (APTSs) in lamivudine using hyphenated techniques have been presented. AMSs were determined by GC–MS method using GSBP-INOWAX (30 m × 0.25 mm × 0.25 μm) column. Temperature program was set by maintaining at 100 °C initially for 3 min, then rised to 220 °C at the rate of 15 °C/min and maintained at 220 °C for 16 min. N,N-dimethyl formamide was used as diluent. APTSs were determined by LC-MS using Zorbax, Rx C8, 250 mm × 4.6 mm, 5 μm column as stationary phase. 0.01 M ammonium acetate is used as buffer. The mixture of buffer and methanol in 75:25 (v/v) ratio was used as mobile phase A and mixture of buffer and methanol in 5:95 (v/v) ratio was used as mobile phase B. The gradient program (T/%B) was set as 0/28, 16/50, 17/100, 23/100, 27/28 and 40/28. Both the methods were validated as per International Conference on Harmonization guidelines. Limit of quantitation was found 1.5 μg/mL for AMSs and was in the range of 1.0–1.5 μg/mL for APTSs.

1. Introduction

Synthesis of drug substances often involves the use of reactive reagents and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity and are to be controlled based on the maximum daily dose [1]. These limits generally fall at low μg/mL levels and hence conventional HPLC, GC methods (or final drug substance methods) are not suitable for their determination. Hyphenated techniques like GC–MS and LC–MS combine physical separation capabilities of chromatography (GC or HPLC) with the mass analysis capabilities of mass

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spectrometry and have high sensitivity and specificity over conventional HPLC and GC methods. Their applications are oriented towards the potential identification and quantitation of trace level of impurities in drug substances [2].

Lamivudine (LMD) is chemically known as (2R-Cis)-4-Amino-1-[2-(hydroxy methyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone. It is used to treat HIV (Type 1) and hepatitis B [3]. In the manufacturing process of LMD, methane sulfonic acid (MSA) and paratoluene sulfonic acid (PTSA) are used as reagents and three alcohols (viz. methanol, ethanol and isopropanol) are used as solvents and hence genotoxic methyl methane sulfonate (MMS), ethyl methane sulfonate (EMS) and isopropyl methane sulfonate (IPMS), methyl paratoluene sulfonate (MPTS), ethyl paratoluene sulfonate (EPTS) and isopropyl paratoluene sulfonate (IPPTS) may exist as impurities in lamivudine drug substance. Based on maximum daily dose of LMD (300 mg/day), these are to be controlled at a limit of 5 μg/mL.

In literature, some analytical methods using hyphenated techniques for the determination of AMSs [4–7], APTSs [5,6,8] and LMD [9–13] were reported. However, no method was reported for the determination of AMSs and APTSs in LMD. Hence, the present work is aimed towards the development of rapid, specific and robust methods for the determination of AMSs and APTSs in LMD at trace level concentration.

2. Experimental

2.1. Chemicals and reagents

MMS, EMS and IPMS were purchased from Acros organics, Geel, Belgium. MPTS and EPTS were purchased from Aarti Drugs Ltd., Mumbai, India. N,N-dimethyl formamide, ammonium acetate, acetonitrile and methanol were procured from Merck, India. IPPTS and pure samples of LMD were obtained from synthetic division of Hetero Drugs Ltd. (R&D), Hyderabad, India.

2.2. Preparation of stock solutions

N,N-dimethyl formamide was used as diluent in GC–MS method. MMS, EMS and IPMS stock solutions were prepared by dissolving 10 mg each individually in 10 mL of diluent. The mixture solution, 1000 μg/mL with respect to 200 mg/mL of LMD, was prepared by diluting the appropriate volumes of above stock solutions with diluent.

The mixture of water and acetonitrile in the ratio of 65:35 v/v was used as diluent in LC-MS method. MPTS, EPTS and IPPTS stock solutions were prepared by dissolving 10 mg each individually in 10 mL of diluent. The mixture solution, 1000 μg/mL with respect to 50 mg/mL of LMD, was prepared by diluting the appropriate volumes of above stock solutions with diluent as above. A blend solution was also prepared by spiking 1000 μg/mL of APTSs to 50 mg/mL of LMD and is used for method development.

2.3. GC–MS conditions

GC–MS analysis was carried out on GCMS-QP2010 system (Shimadzu Corporation, Japan) having GCMS solution software. The instrument was run in EI mode. GSBP-INOWAX column (30 m × 0.2 mm i.d. × 0.25 μm film, Agilent Technologies, USA) was used as stationary phase. 1.5 μL volume with 1:5 split inlet was selected for injection. The GC oven temperature program was set by maintaining at 100 °C initially for 3 min, then rised to 220 °C at the rate of 15 °C/min maintained at 220 °C for 16 min. The injection temperature, GC–MS interface temperature and ion source temperature were 200, 240 and 240 °C, respectively. Helium was used as the carrier gas with a flow rate of 1.46 mL/min. The ionizing energy was 70 eV. The mass detector gain is 1.5 kV.

2.4. LC–MS conditions

LC–MS analysis was carried out on Shimadzu LCMS-2010 EV system (Shimadzu Corporation, Japan) having LCMS solution software in electro spray ionization (positive) mode. Zorbax, Rx C8 column (250 mm × 4.6 mm, 5 μm, Agilent Technologies, USA) was used as stationary phase. 0.01 M ammonium acetate is used as buffer. The mixture of buffer and methanol in 75:25 (v/v) ratio was used as mobile phase A and that of buffer and methanol in 5:95 (v/v) ratio was used as mobile phase B. The gradient program (T/%B) was set as 0/28, 16/50, 17/100, 23/100, 27/28 and 40/28. The flow rate of the mobile phase was kept at 1.0 mL/min. The injection volume was set as 50 μL. Column oven temperature and auto sampler temperature were set as 50 °C and 20 °C, respectively. Interface, curve dissolvation line (CDL) and detector voltages are 4.5 kV, 5.0 V and 1.75 kV, respectively. Interface, CDL and heat block temperatures were 250, 250 and 200 °C, respectively. Nabulizing gas flow was 1.5 L/min.

3. Results and discussion

3.1. GC–MS method development

LMD is soluble in N,N-dimethyl formamide (DMF) and hence it was used as diluent. AMSs mixture solution was initially run through using DB-1 column (100%-Dimethylpolysiloxane). The resolution between MMS and IPMS is not adequate in this column. Then, this column was replaced by DB-5 column and the same result was found. Finally, GSBP-INOWAX column was used and good resolutions were observed. An optimum injection volume of 1.5 μL was chosen. The split ratio was fixed as 1:5 depending on the detector response. An initial column temperature of 100 °C was found to be optimum. The elution order was observed from the total ion chromatogram (Fig. 1) in SCAN mode using AMSs mixture (1.5 μg/mL each) and the individuals were also confirmed using the National Institute of Standard Technology mass spectral library. Validation was done in Selective Ion Monitoring (SIM) monitoring for m/z ions at 110 for MMS, 124 for EMS and 138 for IPMS.

3.2. LC–MS method development

A blend solution containing APTSs and LMD was run in literature method [8]. LMD eluted too early and hence the flow rate of the mobile phase was reduced from 1.5 mL/min to 1.0 mL/min. In this condition LMD eluted at an optimum retention time, but the retention times of APTSs were drastically increased. Hence, the gradient program (T/%B) was fine tuned to 0/28,