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Trace level determination of t-butyl alcohol and t-butyl chloride by GC in dolasetron mesylate

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We herein report the trace level determination of t-butyl alcohol and t-butyl chloride by GC of dolasetron mesylate using a DB-502 column. This method was developed based on an oven programming approach using a nitrogen gas as the mobile phase. The detection limit for the t-butyl alcohol and t-butyl chloride using our developed method was 1.5 ppm. The quantification limit for the t-butyl alcohol and t-butyl chloride using our developed method was 4.5 ppm respectively. Our method is also compatible with the GC-MS (mass spectrometry) technique using helium gas mobile phase instead of nitrogen gas. The successful separation of t-butyl alcohol and t-butyl chloride were confirmed by determination of their corresponding specific molecular masses. We expect that our method will be applicable for the trace level determination of t-butyl alcohol and t-butyl chloride during the control of manufacturing processes, and for use in rapid analysis for quality control in the pharmaceutical industry. Finally, this method was validated according to the International Council on Harmonization Validation Guidelines Q2 (R1).

Keywords: GC, DB-502, t-butyl alcohol, t-butyl chloride, GC-MS.

Introduction

Dolasetron mesylate is an indole derivative with antiemetic activity[1]. As a selective serotonin receptor antagonist, dolasetron mesylate competitively blocks the action of serotonin at 5HT3 receptors, resulting in suppression of chemotherapy and radiotherapy-induced nausea and vomiting. One dose is usually administered once or twice daily and lasts 4 to 9 h. This drug is removed from the body by the liver and kidneys. t-Butyl alcohol was used in the synthesis of dolasetron mesylate and t-butyl chloride which was a by-product of t-butyl alcohol reacts in acidic condition like hydrochloric acid. The structures of t-butyl alcohol and t-butyl chloride were given in Fig. 1.

t-Butyl alcohol [2] (TBA) is found in ginger; it is colourless liquid or white solid, with a camphor-like odor. It is very soluble in water and miscible with ethanol and diethyl ether. As per the Toxtree (Estimation of Toxic hazard – A decision tree approach, version 2.6.6, t-butyl alcohol was not genotoxic; however, the structural alerts shows some positive results

t-butyl alcohol t-butyl chloride

Fig. 1. Chemical structures of t-butyl alcohol and t-butyl chloride.

and the limit of t-butyl alcohol was considered as 15 ppm based on threshold toxicological of concern (TTC).

t-Butyl chloride[3] (TBC) is the organochloride with the formula $(CH_3)_3CCl$. It is a colorless, flammable liquid. It is sparingly soluble in water, with a tendency to undergo hydrolysis to the corresponding TBA. As per the Toxtree (Estimation of Toxic hazard – A decision tree approach, version 2.6.6, t-butyl chloride was not genotoxic; however, the structural alerts shows some positive results and the limit of t-butyl chloride was considered as 15 ppm based on TTC.

Till date, the detection and quantification of t-butyl alco-

hol and t-butyl chloride was using GC-MS (mass spectrometry) estimations. However, previous reports[1–16] in this area describe the analysis of the t-butyl alcohol, and do not address the detection or quantification of t-butyl chloride.

In contrast, the simultaneous determination of t-butyl alcohol and t-butyl chloride in pharmaceutical drug substances has not yet been reported. We therefore selected this technique for the purpose of this study, and employed a DB-502 capillary column for the determination of these compounds.

Materials and methods:

Materials:

All samples were received from China. t-Butyl alcohol and t-butyl chloride, dimethyl sulfoxide, n-butyl acetate, methanol, ethanol, acetone, isopropyl alcohol, acetonitrile, dichloromethane, n-hexane, ethyl acetate, tetrahydrofuran, toluene, n-heptane, 2-butanone, cyclohexane, methyl tertiary butyl ether, methyl isobutyl ketone and diisopropyl ether solvents were purchased from Fisher Scientific (Mumbai, India). The DB-502 GC capillary column was obtained from LCGC (Hyderabad, India). USP grade water was employed throughout, and was prepared using a Metrohm Elga water purifier (Metrohm, Switzerland). The nitrogen/helium gas cylinder was procured from Indo gas agencies (Tamilnadu, India). Development and validation studies were carried out using 7890A gas chromatograph equipped with 7693auto sampler (Agilent technologies, Singapore). Finally, a 5973C GC-MS (Agilent technologies, Singapore) was utilized for molecular mass identification of each t-butyl alcohol and t-butyl chloride peaks.

Preparation of solutions:

Preparation of standard stock solution:

The standard stock solution was prepared by weighing the 37.5 mg of t-butyl alcohol and t-butyl chloride in methanol (50 mL). Further, 5 mL of this solution was diluted to 100 mL with methanol.

Preparation of standard solution:

The standard solution was then prepared by diluting a portion of the standard stock solution (0.5 mL) to 100 mL with diluent. The concentration of the standard solution was 15 ppm of t-butyl alcohol and t-butyl chloride with respect to the analyte concentration.

Preparation of sample solution:

The sample solution was prepared by weighing 500 mg of sample in 2 mL of methanol. For preparation of the spiked sample solutions, the 500 mg of sample was weighed and added the 2 mL of standard solution.

Method:

The chromatographic conditions of GC; the column used was DB-502 (60 m×0.32 mm×1.8 μm). The oven temperature program was with the initial temperature about 40°C through the hold time of 5 min. The rate was 5°C/min and the temperature was about to 70°C by means of hold time of 5 min. The rate was 15°C/min and the temperature was about to 170°C by means of hold time of 2 min. The rate was 40°C/min and the temperature was about to 220°C by means of hold time of 4.08 min. The total run time was 30 min. Injection mode was split with split ratio of 10:1. The column flow rate was 3.5 mL/min. The injection port temperature was 200°C and the detector temperature was 250°C. The injection volume is 2 μL. The mobile phase was nitrogen for GC and the mobile phase for GC-MS was helium. All other parameters were maintained same as the chromatographic conditions of GC.

For the specificity study, each solvent was weighed approximately 10–20 mg in vial and added the methanol. Each solvent vial was closed tightly. The chromatograms of the standard solutions are shown in Fig. 2.

Results and discussion

Method development:

In recent years, several trials have been performed involving the investigation of different capillary stationary phases for the detection of t-butyl alcohol. However, we selected the DB-502 capillary column as the stationary phase for our study due to its unique properties. In DB-624, DB-1, DB-5, DB-FFAP, DB-wax columns, there was no separation between t-butyl alcohol and t-butyl chloride peaks. Hence, we selected the typical column viz. DB-502 which was ideal polarity for light hydrocarbons and aromatics. To improve the resolution, the column length was to increase to 60 m. The column length has been selected for this study was 60 m length with the internal diameter of 0.32 mm along with 1.8 micron particle size.

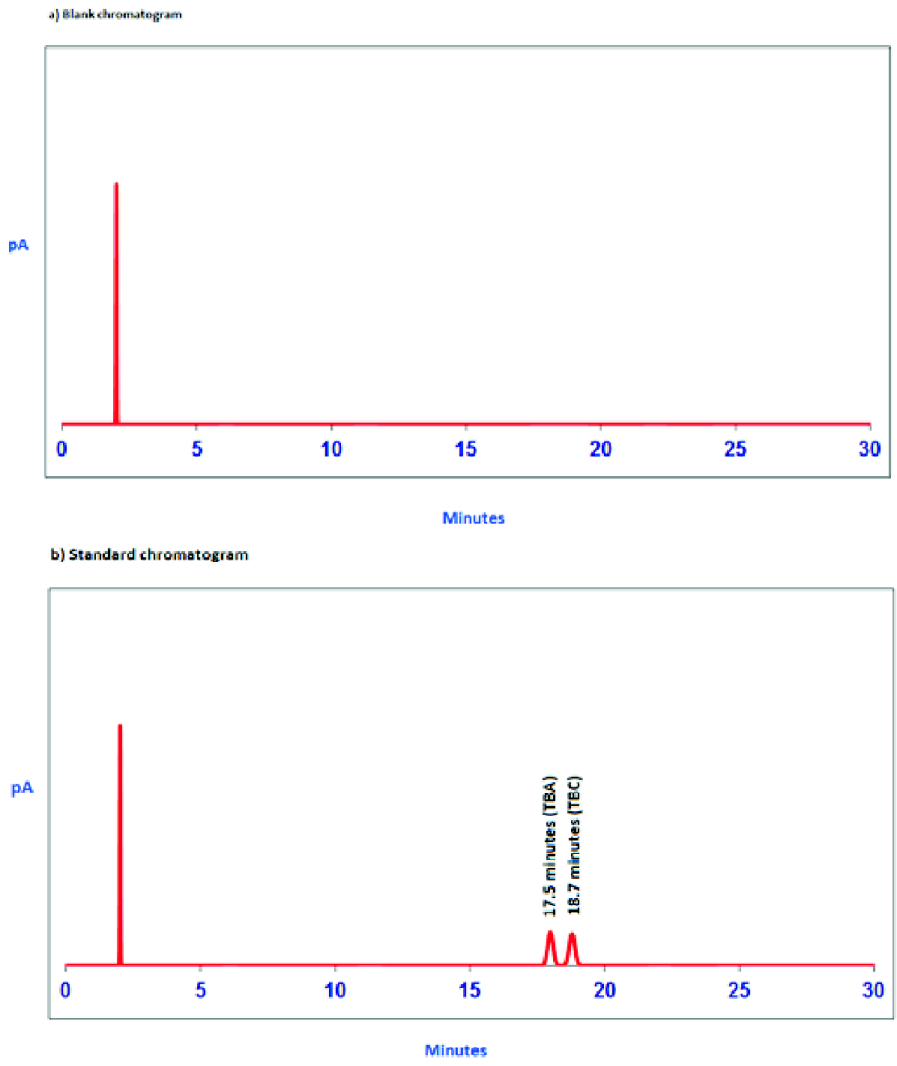

Fig. 2. The chromatograms of the blank and standard solution.

Analytical method validation:

In terms of method validation, the system suitability and precision, limit of detection (LOD), limit of quantification (LOQ), linearity and range, recovery, specificity, robustness, and solution stability of chloride were determined as per the International Council on Harmonization (ICH) validation guidelines Q2 (R1)[17].

System suitability and precision:

To determine the precision of this analytical[17], the standard solution was injected into the chromatograph six times and the percent relative standard deviation (%RSD) was calculated. The obtained %RSD of <1% for TBA and <3% for TBC indicates that this system was precise (%RSD limit = ≤15%)[17].

Method precision:

To determine the precision of this method, six spiked sample solutions were initially prepared. Using the above described method, the %RSD for the TBA and TBC content were <0.5% for the method precision (limit = <5%)[17], and was within 1.0% for the intermediate precision when performed by different analysts, on different columns and instruments on different days.

Limit of detection and limit of quantification:

The limit of detection, LOD, and limit of quantification, LOQ were determined based on the signal to noise (S/N) ratio method as outlined in the ICH guideline Q2 (R1). Upon

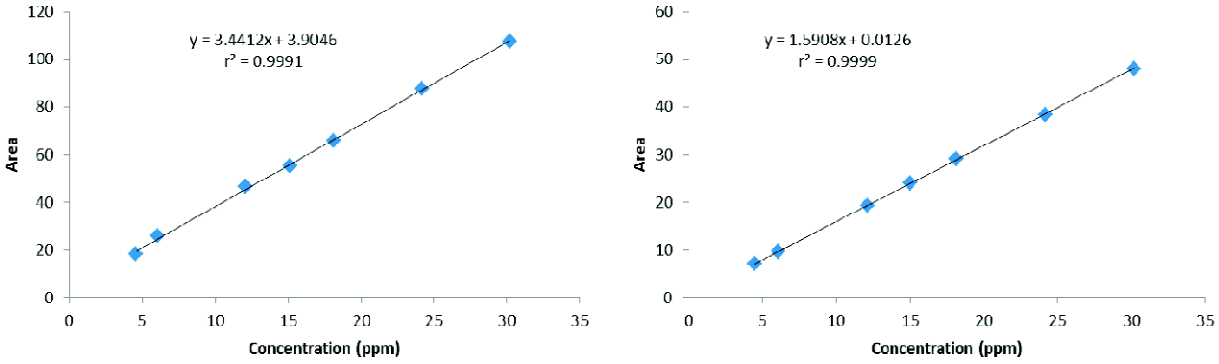

Fig. 3. Linearity plot of t-butyl alcohol and t-butyl chloride.

injecting the solution sequence of predetermined known concentrations (TBA and TBC), the S/N ratio for the LOD was determined to be 3:1, while that of the LOD was determined to be 10:1. Thus, the LOQ for t-butyl alcohol and t-butyl chloride were 1.5 ppm, respectively; the LOQ for TBA and TBC were 4.5 ppm, respectively. These results indicate that the method was sufficiently sensitive for determination of the TBA and TBC content in dolasetron mesylate drug substance.

Linearity and range:

The linearity[17] tests were performed from the LOQ to 200% of this limit for an analyte concentration. The results of this test and the corresponding correlation coefficient were close to 1, indicating that the developed method was indeed linear over the defined concentration range of TBA and TBC.

Accuracy:

The accuracy of an analytical procedure for detecting the TBA and TBC in LOQ, 50%, 100% and 150% levels for dolasetron mesylate drug substance were 89–101%, respectively. These results indicate that our developed method was accurate for the present analytical system, as the mean accuracy value was within the standard 80–120% limit.

Specificity:

Specificity of our method was determined by examination of the interference. There was no interference observed either from the blank or from all solvents. Furthermore, the results of the spiking of solvents with the sample indicate that the TBA and TBC were not co-eluted with the other solvents. These results confirm the specificity/homogeneity of our developed method.

Robustness:

We then examined the effect of chromatographic conditions on the resolution between the t-butyl alcohol and t-butyl chloride. As the original nitrogen gas flow rate was 3.5 mL/min, we varied the flow rate from 3.4 to 3.6 mL/min to investigate its effect on the resolution. In addition, the column oven temperature was set at 40, 35, or 45°C to examine the effect of temperature. Interestingly, the resolution was >1.0 under all conditions studied, thus demonstrating the robustness of our method.

Solution stability:

Finally, the solution stability was determined by examination of a freshly prepared standard solution in a sealed vial at 25°C over 24 h. The %Difference between the peak area of t-butyl alcohol and t-butyl chloride at 0 h and 24 h less than 15.0 for standard solution[17]. The results obtained indicate that the standard solution was stable under these conditions.

Conclusions

We herein reported the versatile gas chromatograph (GC) method for the simultaneous quantitative determination and separation of the t-butyl alcohol and t-butyl chloride in dolasetron mesylate drug substance. More specifically, a DB-502 column was employed in our precise and accurate method, yielding acceptable and repeatable recoveries in addition to low limits of detection and quantification. This authenticated method is expected to be applicable in the regular analysis of t-butyl alcohol and t-butyl chloride in quality control laboratories of pharmaceutical drug substances.

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