Trade Science Inc.

September 2009 Volume 8 Issue 3

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 8(3) 2009 [346-349]

Trace analysis of mesityl oxide and diacetone alcohol in pharmaceuticals by capillary gas chromatography with flame ionization detection

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Received: 26th May, 2009 ; Accepted: 5th June, 2009

ABSTRACT

A capillary gas chromatographic method using flame ionization detection was developed and validated for the trace analysis (ppm level) of mesityl oxide and diacetone alcohol in pharmaceutical drug substance. The method utilizes a megabore capillary column with bonded and crosslinked polyethylene glycol stationary phase. A dissolve-and-injection approach was adopted for sample introduction in a split mode (1:1). Water is used as sample solvent. A limit of detection of about 1.5 and 2 ppm and limit of quantitation of about 6 and 10 ppm were achieved for the Mesityl oxide and Diacetone alcohol in drug substance samples. The method optimization and validation are also discussed in this paper.

KEYWORDS

Pharmaceutical analysis;
Mesityl oxide and Diacetone alcohol.

INTRODUCTION

Recently, the potential health hazards of trace amounts of mesityl oxide and diacetone alcohol in pharmaceuticals have attracted the attention of regulatory authorities. These are known to be potent carcinogenic compounds[1-3]. Their presence in the pharmaceutical products may be the result of leftover starting materials, or formed as by-products between acid, base (often used as a counter ion) and ketone (often used as solvents in manufacturing process). Although official guidelines[4-5] have not been established, the concentration of these compounds are expected to be controlled at a level less than or equal to 10ppm .Therefore, it is of great importance to develop analytical methods that are sensitive enough and meet all the regulatory requirements.

The pure mesityl oxide and diacetone alcohol are liquids at ambient temperature with a boiling point around 130 °C and 170°C respectively. Therefore, it is feasible to separate and quantify these compounds by gas chromatography. The analysis of the mesityl oxide and diacetone alcohol using HPLC is not straightforward because of the specific chemical and physical properties of these compounds.

This short communication describes a simple and sensitive method for the determination of mesityl oxide and diacetone alcohol in pharmaceuticals using capillary GC with flame ionization detection (FID). The limit of detection and limit of quantitation were determined

to be about 2ppm and 10ppm per gram of API, respectively. The method utilizes a dissolve-and-inject approach for sample preparation and introduction. The samples were injected in the split mode and quantitation was achieved using a single point external standard calibration.

EXPERIMENTAL

Instrumentation

An Agilent 6890 GC (Agilent, Palo Alto, CA, USA) equipped with an auto sampler was used in the experiment. Data acquisition and processing were conducted using the Waters Empower software.

Chemicals

Mesityl oxide and Diacetone alcohol were purchased from Aldrich Chemical (Milwaukee, WI, USA). HPLC grade equivalent water was obtained from an in-house Millipore Milli-Q-Gradient ultrapure water system (Millipore, Billerica, MA, USA). This study also involves two proprietary Dr. Reddy's Laboratories Pharmaceutical Research & Development compounds.

Preparation of Solutions

The stock solutions of mesityl oxide and diacetone alcohol were prepared by dissolving 5.8µL and 5.3µL each of the compounds in sample solvent. The diluted stock solution (standard solution) was prepared by pipetting 10µL each of the stock solutions into a 10-mL volumetric flask and diluting to volume with sample solvent. The sample solution was prepared by accurately weighing about 50 mg of the drug substance into a 2-mL GC vial and adding 1.0 mL of sample solvent.

Operating Conditions

The GC separation was conducted on an Alltech AT-WAX column with a dimension of 30 m × 0.53 mm and a film thickness of 1 µm. Helium was used as carrier gas at a constant flow of 2.6psi. The GC oven temperature program utilized an initial temperature of 50 °C and an initial holding time of 6 min, then increased at 8 °C/min to 115 °C with a hold time of 6 min, then increased at 35°C/min to 220 °C. The final temperature was held for 10 min.

A flame ionization detection (FID) system was used. The H2, air, makeup flows were kept at 30, 350 and 30 mL/min, respectively. The detector temperature was set at 260 °C.

The samples were injected with the Agilent 6890 series auto sampler. The inlet temperature was kept at 110 °C. A straight glass injection liner with glass wool was obtained from Restek, (Restek, Bellefont, PA, USA). The samples were injected in a split mode (1:1) with a 1-µL injection volume unless otherwise specified.

RESULTS AND DISCUSSION

Method Development and Optimization

The main challenge was to achieve the desired detection and quantitation limit using the most commonly available instrument, i.e. a gas chromatograph with a FID system. To obtain the desired sensitivity, one approach is to increase sample amount injected into the GC system. The adoption of a megabore capillary GC column (0.53 mm I.D.) with a high capacity bonded stationary phase seems to be the obvious choice. Suitable initial column temperature in combination with a moderate inlet temperature (120 °C) may allow a relatively large injection volume without significant deterioration in column efficiency.

The effect of injection volume on the quantitation of the mesityl oxide and diacetone alcohol was investigated by injecting between 0.5 µL and 3 µL of the standard solution containing 10ppm each of mesityl oxide and diacetone alcohol. The results show that the peak widths of mesityl oxide and diacetone alcohol are independent of injection volume within the tested range. Further studies were not done to determine the maximum injection volume that the chromatographic system could handle because interfering peaks from the sample solvent started being detected when the injection volume was greater than 2 µL in our experiments. An injection volume of 1 µL was chosen for this method as it will not over load the column.

The effect of inlet temperature on the drift of the baseline was investigated. The inlet temperature was varied from 100 to 200 °C. An aliquot of 1 µL of the sample was injected in the split mode. The results show that at an inlet temperature more than 130°C there is a

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drift in the baseline which will effect the quantification of diacetone alcohol. An inlet temperature of 110 °C was chosen, which allowed smooth baseline.

This method utilizes a dissolve-and-inject approach for the analysis. Several factors were considered in selection of a sample solvent, including the purity, its ability to dissolve the analyte, and its chemical compatibility with the compounds of interest. To detect the mesityl oxide and diacetone alcohol at 2ppm level, the purity of sample solvent is critical. It has been observed in our laboratory that the HPLC grade solvents are generally suitable. The tested sample concentration of drug substances was in the range of 40–100 mg/mL. The use of water was successfully used for one of the in-house compounds for the residue analysis (Fig 1). The mesityl oxide and diacetone alcohol showed reasonable stability in the aqueous solution. This is important because many pharmaceuticals are in salt forms, which sometimes show limited solubility in pure organic solvents.

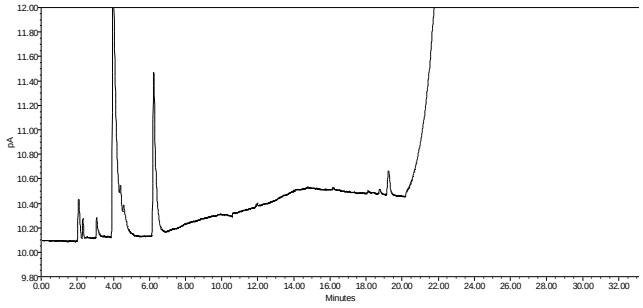

Figure 1 : Chromatogram of sample solvent

Method Validation

A Critical parameters of validation was done for the developed work. The validated method parameters include establishment of limit of detection, limit of quantification, precession at limit of quantification and accuracy at limit of quantification was done.

The detection limit (LOD) of the method for the mesityl oxide and diacetone alcohol was estimated from a chromatogram of a solution containing about 1.5ppm and 2.0ppm respectively. From the chromatogram, a signal-to-noise ratio of 2.8 and 3.0, was obtained for mesityl oxide and diacetone alcohol respectively. In the pharmaceutical industry, the quantitation limit (LOQ) was defined as the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The LOQ was determined to be 6ppm and 10ppm respectively for mesityl oxide and diacetone alcohol (Fig 2) based on the precision and accuracy data discussed below.

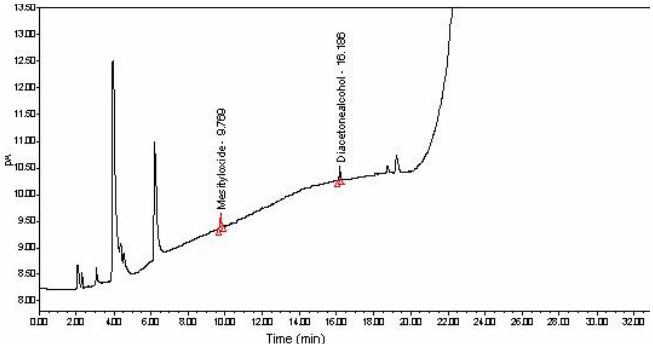

Figure 2 : Chromatogram of a standard solution containing 6ppm and 10ppm of mesityl oxide and diacetone alcohol respectively.

The experimental results also show that this method has excellent precision without using an internal standard. Multiple injections were made for the standard solutions containing 6ppm and 10ppm respectively of mesityl oxide and diacetone alcohol. For six injections of the solution, the R.S.D. of the peak area of mesityl oxide was 7.8% and for diacetone alcohol was 8.2%

Accuracy of the method was determined by analyzing drug substance samples spiked with limit of quantification amount of the mesityl oxide and diacetone alcohol. The recovery was 122% for mesityl oxide and 91% for diacetone alcohol Because this method uses the dissolve-and-inject approach, for every sample injection, about 500 µg of the drug substance is introduced in the injection port. The accumulation of drug substance may have negative effect on the recovery. Therefore the injection liner should be replaced after every sequence of 15–20 injections.

CONCLUSION

A simple and sensitive GC method has been developed and validated for the trace analysis of mesityl oxide and diacetone alcohol in pharmaceuticals. The mini validation was performed. Compared with the previously reported methodologies, this method utilizes a FID detector, which is readily available in most of the quality control testing laboratories in the pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect 1.5ppm of mesityl oxide, 2.0ppm

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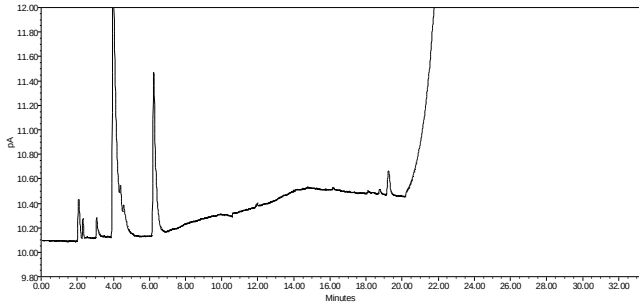

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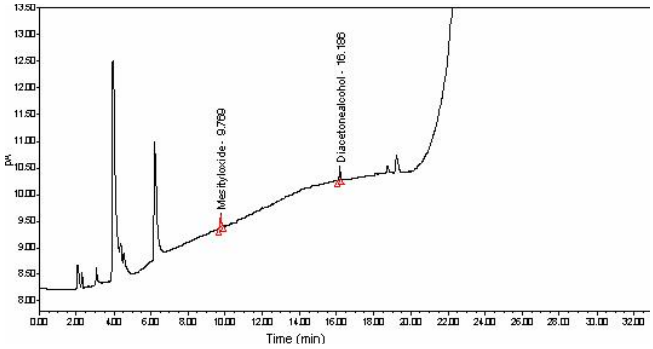

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