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Trace analysis of Azo bis (isobutyronitrile) in pharmaceuticals by capillary gas chromatography with flame ionization detection

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ABSTRACT

A capillary gas chromatographic method using flame ionization detection was developed and validated for the trace analysis (ppm level) of Azo bis (isobutyronitrile) in pharmaceutical drug substance. The method utilizes a megabore capillary column with 5% Phenyl-95%methylpolysiloxane stationary phase. A dissolve-and-injection approach was adopted for sample introduction in a split mode (1:1). Methanol is used as sample solvent. A limit of detection of about 0.5ppm and limit of quantitation of about 1.8ppm were achieved for the Azo bis (isobutyronitrile) in drug substance samples. The method optimization and validation are also discussed in this paper.

KEYWORDS

Pharmaceutical analysis;
Azo bis (isobutyronitrile);
Validation.

INTRODUCTION

Recently, the potential health hazards of trace amounts of Azo bis (isobutyronitrile) in pharmaceuticals have attracted the attention of regulatory authorities. It is known to be potent carcinogenic as well as mutagenic compound. Its presence in the pharmaceutical products may be the result of leftover material, as it is widely used as catalyst in most of the free radical reactions.[1,2] As the limit for the compound have not been reported[3,4] the concentration of these compounds are expected to be controlled as per the TTC. Till date no quantitative method was reported. Therefore, it is of great importance to develop analytical methods that are sensitive enough and meet all the regulatory requirements.

The pure Azo bis (isobutyronitrile) is a solid at ambient temperature with a melting point around 102 °C. Therefore, it is feasible quantify these compounds by gas chromatography. The analysis of the Azo bis (isobutyronitrile) 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 Azo bis (isobutyronitrile) in pharmaceuticals using capillary GC with flame ionization detection (FID). The limit of detection and limit of quantitation were determined to be about 0.5ppm and 1.8ppm 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

Azo bis (isobutyronitrile) was purchased from Aldrich Chemical (Milwaukee, WI, USA). Chromatographic grade methanol was purchased from Lichrosolv Merck specialities private limited (Worli, Mumbai, India). This study also involves three proprietary Dr. Reddy's Laboratories Pharmaceutical Research & Development compounds.

Preparation of solutions

The stock solutions of Azo bis (isobutyronitrile) were prepared by dissolving 100mg of compound in sample solvent. The solution was further diluted by transferring 0.2ml of the solution to 10ml. The standard solution was prepared by pipetting 25µL of the diluted solution 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 J&W Scientific DB-5 column with a dimension of 30 m × 0.32 mm and a film thickness of 1 µm. Helium was used as carrier gas at a constant flow of 1.7ml/min. The GC oven temperature program utilized an initial temperature of 60 °C and an initial holding time of 4 min, then increased at 5 °C/min to 120 °C with a hold time of 10 min, then increased at 35°C/min to 250 °C. The final temperature was held for 15 min.

A flame ionization detection (FID) system was used. The H_2, air, makeup flows were kept at 30, 300 and 40 mL/min, respectively. The detector temperature was set at 240 °C.

The samples were injected with the Agilent 6890 series auto sampler. The inlet temperature was kept at 220 °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 4-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 may allow a relatively large injection volume without significant deterioration in column efficiency.

The effect of injection volume on the quantitation of the Azo bis (isobutyronitrile) was investigated by injecting between 1 µL and 5 µL of the standard solution containing 10ppm of Azo bis (isobutyronitrile). The results show that the peak widths of Azo bis (isobutyronitrile) 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 4 µL in our experiments. An injection volume of 4 µL was chosen for this method.

The effect of inlet temperature on the drift of the baseline was investigated. The inlet temperature was varied from 100 to 240 °C. An aliquot of 3 µL of the sample was injected in the split mode. The results show that at an inlet temperature more than 240°C there is a drift in the baseline which will effect the quantification of Azo bis (isobutyronitrile). An inlet temperature of 220 °C was chosen, which allowed smooth baseline and also a better peak shape of the compound.

This method utilizes a dissolve-and-inject approach for the analysis. Several factors were con-

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sidered 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 Azo bis (isobutyronitrile) at 0.5ppm level, the purity of sample solvent is critical. It has been observed in our laboratory that the chromatographic grade solvents are generally suitable. The tested sample concentration of drug substances was in the range of 40–100 mg/mL. The use of methanol was successfully used for one of the in-house compounds for the residue analysis (Figure 1). The Azo bis (isobutyronitrile) showed reasonable stability in the methanol solution. This is important because many pharmaceuticals have better solubility in alcohols.

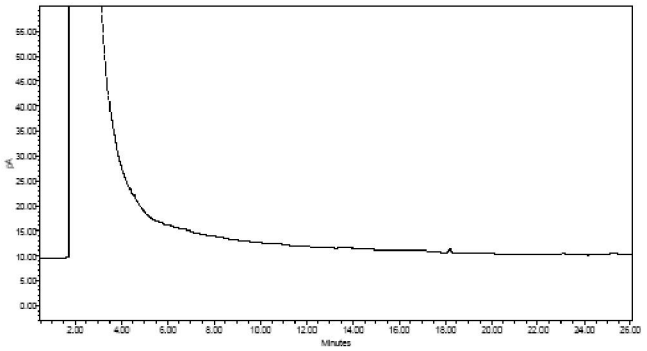

Figure 1 : Chromatogram of sample solvent

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, accuracy at limit of quantification and linearity was done.

The detection limit (LOD) of the method for the Azo bis (isobutyronitrile) was estimated from a chromatogram of a solution containing about 0.5ppm. From the chromatogram, a signal-to-noise ratio of 2.8 was obtained for Azo bis (isobutyronitrile. 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 1.8ppm for Azo bis (isobutyronitrile) (Figure 2) based on the precision and accuracy data discussed below.

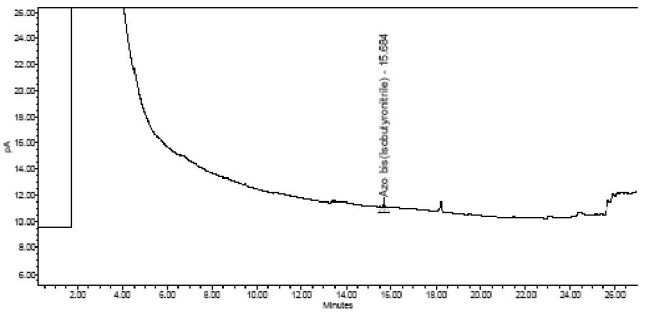

Figure 2 : Chromatogram of a standard solution containing 1.8ppm of Azo bis(Isobutyronitrile)

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 10ppm respectively of Azo bis (isobutyronitrile). For six injections of the solution, the R.S.D. of the peak area of Azo bis (isobutyronitrile) was 5.0%. Accuracy of the method was determined by analyzing drug substance samples spiked with limit of quantification amount of the Azo bis (isobutyronitrile) and the recovery was found to be 99%. Linearity of the method was established from limit of quantification to 30ppm at a six point curve and the correlation was found to be 0.999. Because this method uses the dissolve-and-inject approach, for every sample injection, about 150 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 cleaned after every sequence of 15–20 injections.

CONCLUSION

A simple and sensitive GC method has been developed and validated for the trace analysis of Azo bis (isobutyronitrile) in pharmaceuticals. The mini validation was performed. 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 0.5ppm of Azo bis (isobutyronitrile) in pharmaceutical products.

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