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Quantitative determination of a trace level of carcinogenic agent methyl iodide in naratriptan hydrochloride by GC using ECD detection

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ABSTRACT

A simple and a quantitative GC method for the determination of trace level of methyl iodide; a suspected carcinogen in naratriptan hydrochloride drug substance(API) has been developed and validated. Methyl iodide being toxic, it is very important to control the level of the same in the final API. The GC column used was AT-WAX, 30m×0.53mm, 1.0µm film thickness and the instrument employed for the method development and validation was Agilent6890N GC equipped with electron capture detector(ECD). In the developed method the limit of detection(LOD) of methyl iodide was about 0.25ppm and the limit of quantification was about 0.8ppm(LOQ). The method was validated with respect to precision, linearity and accuracy. The developed method can be very well employed and can be used in the quality control laboratories to monitor and control the trace levels of methyl iodide in naratriptan hydrochloride API.

KEYWORDS

Gas chromatography (GC);
Electron capture detector (ECD);
Validation and quantification;
Active pharmaceutical ingredient (API).

INTRODUCTION

Iodomethane, commonly called methyl iodide and commonly abbreviated "MeI", is a dense volatile liquid. It is colorless, although it is light sensitive and may develop a purplish tinge caused by iodine, thus it is usually stabilized by storage over copper metal. Methyl iodide is widely used in organic synthesis to deliver a methyl group into a structure, a process called methylation, usually via SN^2 substitution. It is naturally emitted by rice plantations in small amounts. Iodomethane is an excellent reagent for methylation, the iodide leaving group in MeI may cause side reactions, as it is a powerful nucleophile. Finally, being highly reactive, MeI is more dangerous for laboratory workers than related chlorides and bromides.

As MeI is known to be toxic and is a cancer suspect agent[1-4] the prolonged and repeated contact with this chemical may be harmful and could be fatal if swallowed, inhaled or absorbed through skin. This product can cause irritation to skin, eyes and respiratory tract and affects central nervous system. MeI is used during the synthetic manufacture of naratriptan hydrochloride (anti migraine drug) in the penultimate stage involves methylation step. It is very important to monitor and control the MeI, suspected carcinogen in the final API. Few GC methods were reported in the literature as early as in the year 1993 by Kanno et al. and in year 1997 by Ekdahl et al. for the determination of MeI using FID and ECD detection techniques[5-6]. The present research work describes the development of a sensitive method for the trace level determination of MeI in pharmaceutical drug substance. Attempts were made to develop a sensitive GC method for the quantitative

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determination of carcinogenic agent MeI in naratriptan hydrochloride API using ECD detection.

EXPERIMENTAL

Chemicals and reagents

Samples of MeI and naratriptan hydrochloride (Figure 1) were received from process research department of Custom Pharmaceutical Services of Dr.Reddy's Laboratories Ltd., Hyderabad, India. HPLC grade water was purchased from Merck, Mumbai, India.

Instrumentation

The GC system, employed in the chiral method development and validation was GC6890N series (Agilent technologies, Foster city, CA, USA) equipped with an electron capture detector(ECD). The output signal was monitored using chemstation software (Agilent) on Pentium computer (Digital equipment co., Houston, USA).

Sample preparation

The stock solution of MeI was prepared separately by dissolving the appropriate amounts of the substance in water used as a diluent. The target analyte concentration of naratriptan hydrochloride was fixed as 50mg mL^{-1}.

Chromatographic conditions

The chromatographic column used was AT-WAX, 30m×0.53mm, 1.0µm film thickness (Alltech, Deerfield, IL, USA). The carrier gas used was nitrogen at a flow of 4mL min^{-1}. The flow rate of the mobile phase was 1.0mL min^{-1}. The injection volume was 0.2µL and spilt mode was used during sample injections.

RESULTS AND DISCUSSION

Method development

The objective of the present work is to develop a sensitive and quantitative method for the determination of trace level of MeI present in naratriptan hydrochloride API. Various stationary phases were employed during method development namely DB-624, DB-5 and AT-Wax. During various trails the satisfactory response for methyl iodide peak was obtained on AT-Wax column not on the other stationary phases. Very good response for MeI peak and the stable base line were obtained when the water is used as diluent during the standard and test preparations.

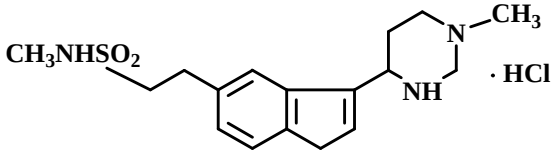


Figure 1 : Chemical structure of naratriptan hydrochloride

Optimized chromatographic conditions

The optimized gas chromatographic conditions are as follows:

- Column : AT-WAX, 30m×0.53 mm, 1.0µm Film thickness
- Temperature program : 40°C(12 min Hold) to 220°C@ 30°C/min (5 min hold)
- Detector : ECD
- Injection temperature : 180°C
- Detection temperature : 280°C
- Carrier gas : Nitrogen
- Carrier flow : 4.0mL min^{-1}
- Mode of injection : Split
- Split ratio : 3:1
- Injection volume : 0.2µl
- Diluent : Water
- Run time : 23min

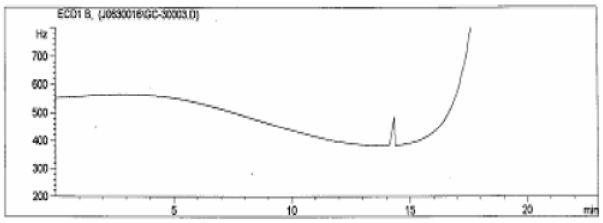
Method validation

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the same homogenous sample under prescribed conditions[7]. The system and method precision for MeI was checked at its specification level(i.e. not more than 1.0ppm of MeI in naratriptan hydrochloride API). The percentage RSD of method repeatability and injection repeatability(n=6) for MeI was found to be 7.6 and 8.3 respectively, confirms good precision of the method.

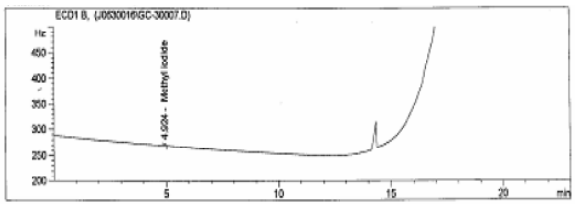
Linearity

The linearity of an analytical procedure is its ability (within a given range)to obtain test results, which are directly proportional to the concentration of the analyte in the sample[8]. The linearity of the method for MeI



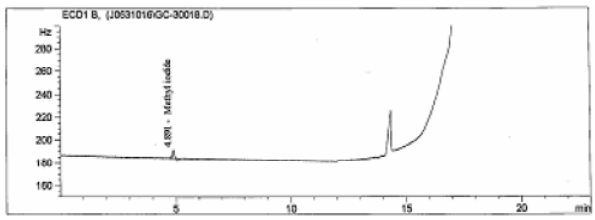
Time(min)

(i) Blank chromatogram



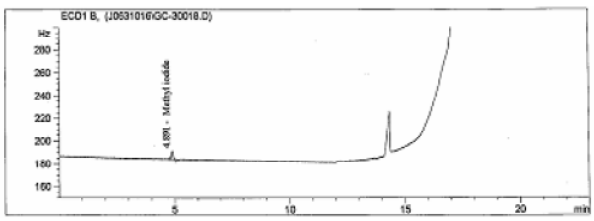
Time(min)

(ii) Typical GC chromatogram representing the LOD solution



Time(min)

(iii) Typical GC chromatogram representing the LOQ solution



Time(min)

(iv) Typical spiked GC chromatogram representing the LOQ solution of MeI

Figure 2 : Typical GC chromatograms

was checked at five different concentration levels i.e. from LOQ(0.8ppm) to 200%(i.e. 2.0ppm of MeI), which is with respect to target analyte concentration. The coefficient of regression of the calibration curve obtained was more than 0.900, thus confirming the linearity of the developed method.

Accuracy/Recovery

Standard addition and recovery experiments were conducted to determine the accuracy of the present method, for the quantification of MeI present in bulk samples of naratriptan hydrochloride. The study was carried out spiking the 0.8, 1.0 and 1.5ppm of MeI in naratriptan hydrochloride at target analyte concentration (i.e. 50mg mL^{-1}). The percentage recoveries of MeI were ranged from 65% to 71%(general acceptance criteria at trace levels: 50 to 150% recovery) in the bulk samples of naratriptan hydrochloride thus proving the accuracy of the developed method.

Limit of detection and limit of quantification

The limit of detection(LOD) represents the concentration of analyte that would yield a signal to noise ratio of 3[9]. Limit of detection for MeI was found to be 0.25ppm for 0.2 L injection volume. The limit of quantification represents the concentration of analyte that would yield a signal to ratio of 10[9]. Limit of quantification (LOQ) for MeI was found to be 0.8ppm for 0.2µL injection volume.

Ruggedness

The ruggedness of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under variety of normal test conditions such as different labs, analysts, instruments, and reagents of different lots. The standard addition and recovery experiments of MeI carried out in naratriptan hydrochloride bulk samples at the same concentration levels tested in laboratory A were again carried out at laboratory B using different instrument by different analyst. The data obtained from the laboratory B was well within the acceptance variation of the results obtained in laboratory A, thus proving the method ruggedness.

CONCLUSION

A simple, sensitive and accurate GC method was described for the quantitative determination of trace level of MeI present in the bulk samples of naratriptan hydrochloride using AT-Wax column. The method was sufficiently validated and showing satisfactory data for all the method validation parameters tested. The developed method can be well employed in the pharmaceutical quality control laboratories to monitor and control quantitatively the trace levels of MeI present in the bulk samples of naratriptan hydrochloride. The above developed method can also be applied to other API's for the trace level determination of MeI.

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