

Determination of benzyl bromide as a genotoxic impurity in donepezil hydrochloride using extraction technique by gas chromatography

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ABSTRACT

A gas chromatographic method has been developed for the determination of Benzyl bromide in Donepezil hydrochloride drug substance. The method was optimised based on the basis of solubility of Benzyl bromide. The method was validated as per ICH guideline in terms of LOD, LOQ, Method precision, accuracy and specificity. The LOD and LOQ values were found to be 6.6 ppm (6.6 µg/mL) and 19.9 ppm (19.9 µg/mL) respectively.

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KEYWORDS

Chromium (III) determination;
Isatin;
Surfactant;
Igneous rock;
Beer's law.

INTRODUCTION

Donepezil hydrochloride is a new anti-Alzheimer drug. It is the potent acetylcholine esterase inhibitor. Chemically 2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4piperidiny]methyl]-1H-inden-1-one hydrochloride. It has an empirical formula of C₂₄H₂₉NO₃HCl and molecular weight of 415.96. Donepezil hydrochloride was the first piperidine type reversible based inhibitor of the enzyme acetylcholinesterase. It has been approved for the symptomatic treatment of mild to moderate Alzheimer's disease (22).

Various methods in the literatures involve determination of Donepezil in human plasma by HPLC^[1-4], UV Spectrophotometry^[5,6], Donepezil in pharmaceutical dosage by HPLC^[7-19]. However no method is available for content of benzyl bromide in Donepezil by Gas chromatography. In the present work we have developed a new, simple precise, accurate method for determination of benzyl bromide in Donepezil hydrochloride by gas chromatography in bulk drug. In synthesis

of Donepezil hydrochloride involves benzyl bromide as a raw material which is an genotoxic alert. Hence evaluation of Benzyl bromide is necessary. Benzyl bromide is a pungent colourless liquid causes eye and skin irritation on exposure. It is classified as hazardous by definition of hazardous communication standard (29 CFR 1910.1200). Under the authority of the federal advisory committee act (FACA) the national advisory committee for acute exposure guideline levels for hazardous substance (NAC/AGEL committee) has been established to identify, review and interpret relevant toxicological and other scientific data(23).

EXPERIMENTAL

Chemicals

Benzyl bromide was purchased from Aldrich chemicals; LC grade cyclohexane was purchased from rankem. Samples of donepezil hydrochloride are received from local market. Structure of donepezil hydrochloride and benzyl bromide are shown in Figure 1

and Figure 2.

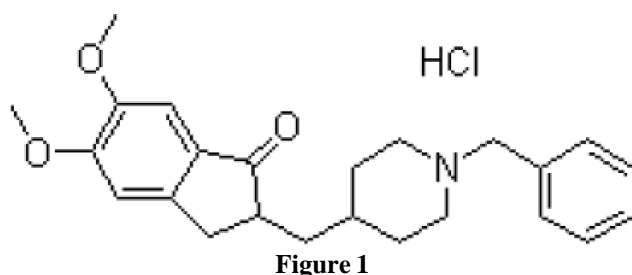


Figure 1

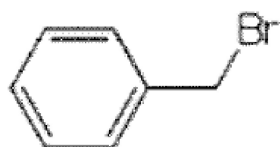


Figure 2

Equipment

Analysis was carried on a GC system equipped with liquid auto sampler (Perkin Elmer 500 gas chromatograph with auto sampler) with Flame ionisation detector and Helium as a carrier gas.

Chromatographic conditions

Analysis was done on DB-5 (5% phenyl 95% methylpolysiloxane) column of 30 m length, 0.53 mm inner diameter and 5 μ m film thickness. Injector temperature and detector temperature were kept as 220°C and 260°C respectively. The detector attenuation is kept at -6(1) for maximum response. After optimisation the split was decided as 2:1 for 5 μ L injection. Flow of carrier gas (Helium) is kept at 5.0 psi at constant pressure mode. The GC oven temperature is kept isocratic as 150°C for 30 min.

Standard stock preparation

Benzyl bromide solution was prepared by diluting 55 mg of Benzyl bromide to 50 mL with Cyclohexane. Further 5 mL of this solution was diluted up to 50 mL with Cyclohexane. Further 3 mL of above solution was diluted up to 50 mL with Cyclohexane (equivalent to 66 ppm with respect to sample concentration 100 mg per ml as per maximum daily dosage 23 mg i.e TTC = 1.5/daily dosage grams).

Standard preparation

Transfer 8 mL of purified water in a stoppered test tube and added 2 mL of standard stock solution stopper the test tube vortex for 3 min in cyclomixer. Sepa-

rate the upper organic layer, inject the organic layer.

Sample preparation

Weighed about 200 mg of Donepezil hydrochloride drug substance and add 8 mL of purified water in a stoppered test tube dissolve the sample by gentle shaking and add 2 mL of Cyclohexane, stopper the test tube vortex for 3 min in cyclomixer. Separate the upper organic layer, inject the organic layer

RESULTS AND DISCUSSION

Method development and optimization

Benzyl bromide is a liquid at ambient temperature with a boiling point of 198°C. Solvents used for development were Methylene chloride, Toluene and Cyclohexane. Cyclohexane is finalised as the diluents as Benzyl bromide shows good response compared to Methylene chloride and Toluene. Benzyl bromide is soluble in Cyclohexane and insoluble in water whereas Donepezil hydrochloride is soluble in water and insoluble in Cyclohexane. Hence Extraction method is employed so that Donepezil hydrochloride gets soluble in water

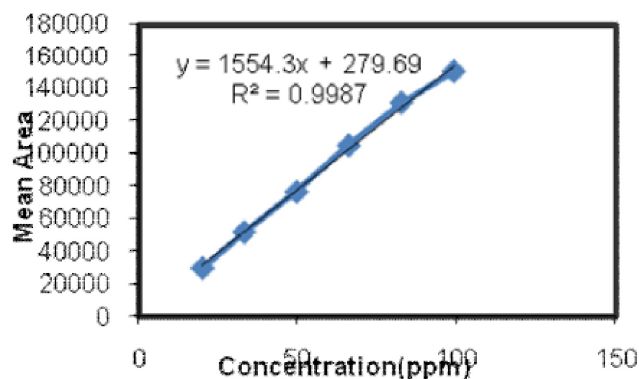


Figure 3 : Linearity of benzyl bromide

TABLE 1 : Results for linearity of benzyl bromide

%Level	Conc	Area
LOQ	19.9	29867
50	33	51789
75	49.5	76554
100	66	105188
125	82.5	131445
150	99	150699
Correlation co-efficient		0.998701
intercept		279.6859
slope		1554.341

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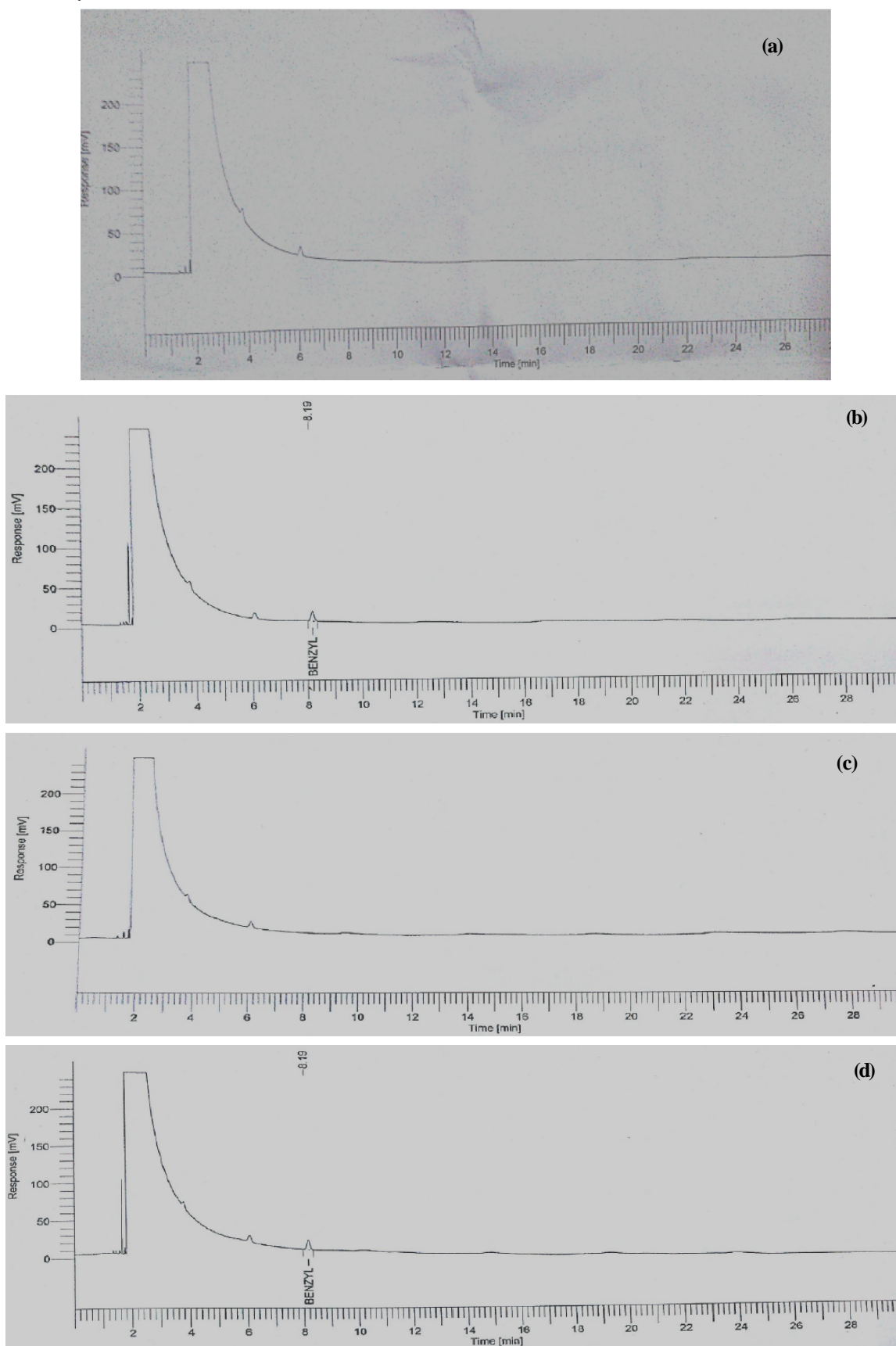


Figure 4 : Typical chromatograms of (a) Blank (b) Standard (c) Sample (d) spiked sample

and content of Benzyl bromide gets extracted in Cyclohexane. The experiment was initially carried out on DB-1 column but was replaced by DB-5 column for sharper peak. The effect of injection volume and split was observed and optimised up to 5 μ L with a split of 2:1 for sample concentration of 100mg/mL.

Method validation

The method validation work was conducted according to the ICH guidelines(20-21). The Validated method parameters include specificity, accuracy, sensitivity, precision, linearity, robustness, ruggedness and solution stability. LOD, LOQ values were obtained by preparing a series of known concentration solutions of increasing concentration and plotted a graph of concentration against area of analyte. LOD and LOQ values were found to be 6.6 ppm and 19.9 respectively for the sample concentration of 100 mg/mL. Linearity of the method was determined by preparing and analyzing a series of 7 standard solutions to cover the concentration range of LOQ to 132ppm for benzyl bromide. The linearity correlation coefficient was found to be 0.9987

The method is precise which is indicated by the low % relative standard deviation of six replicate standards, which was 2.130. The accuracy of method was determined by spiking the samples at 50 %, 100 % and 150 % level. Method also validated for solution stability at room temperature. Standard solution of benzyl bromide at concentration of 66ppm was injected at regular intervals up to 3days at room temperature. The recovery was in the range of 95-105% which confirms solution is stable.

CONCLUSIONS

A simple and sensitive GC method has been developed and validated for the trace analysis of benzyl bromide in pharmaceuticals. The validation has been conducted according to ICH guidelines. Compared with the previously reported methodologies, this method utilizes a FID detector, which is readily available in most of the testing laboratories in pharmaceutical industry and relatively simple to use. This method is sensitive enough to detect 6.6ppm and quantify 19.9ppm level of benzyl bromide in pharmaceutical drug substances.

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