Research article

Carbitol as adulterant in menthol; analytical method for quantitative analysis of adulteration

Indrajit Sen[1], Deepak Shrivastava[2], Manjeet Aggarwal[3] and Rakesh Kumar Khandal[4,*]

[1] International Rice Research Institute, South Asia Regional Center, Varanasi, India
[2] Plastics Technology—School of Chemical Technology, H.B Technical University, Kanpur, India
[3] National Institute of Food Technology Entrepreneurship and Management, Kundli, Haryana, India
[4] India Glycol Ltd, Noida, Utter Pradesh, India

* Correspondence: Email: drrakeshkhandal@gmail.com; Tel: +919599433590.

Abstract: India is the largest exporter of menthol, a highly valued natural volatile compound obtained from peppermint. However, natural plant menthol, which often found to be adulterated. The standard methods used for quantitative analysis of menthol are not good enough to identify certain adulterants. One of the commonly used adulterants in menthol is found to be carbitol which is nothing but diethylene glycol monoethyl ether or 2-(2-Ethoxyethoxy) ethanol. This adulterant cannot be detected properly with the gas chromatography method as prescribed in the IS (Indian Standards) 3134:1992 method for this purpose. The present paper deals with the development of a method using gas chromatography with mass spectrometry (GC-MS) which is easy and simple to determine adulteration of carbitol in menthol. It has been demonstrated that by using this method developed here can easily detect the presence of carbitol in menthol at a level as low as 1 μg/mL with linearity correlation coefficient >0.999 for the concentration range of 5.0 to 100 μg/mL. This method is found to be robust and easy to adapt.

Keywords: menthol; carbitol; GC-MS

1. Introduction

Menthol is a major agriculture derived product meant for food, drugs, pharmaceutical, personal care, nutritional and functional foods etc., produced in northern part of India. The suppliers exports menthol in bulk to countries all over the world. It is a product in high demand and hence, supplies

fall short of the demand. As a result, the traders adulterate it with substances that are cheap and those which cannot be detected by analyst [1–4].

The quality of menthol is checked and duly certified at the time of dispatch as per the method (IS: 3134:1992) prescribed by regulators in India. As per this method, the following parameters are meant to ascertain the contamination as also the purity of the menthol: a) gas chromatography (GC) with flame ionization detector and packed column to ascertain purity. b) several identification tests include identification tests, freedom from thymol and other phenols, solubility and nonvolatile matter by GC to ensure no contamination [5].

The suppliers always look for the adulterants that go undetected during the above mentioned test as prescribed in the IS 3134:1992 method. Taking advantage of the fact that a GC method suffers from the drawback of being only an indication technique, the adulterant having similar physico-chemical nature, many go undetected. Various types of compounds that exhibit similar physical (polarity) characteristics therefore would be candidates for adultering menthol. Almost all types of adulterants get deducted by these methods. However, there is no method mentioned in the above specification by which we can detect the presence of carbitol at very low level. Carbitol (a structure is shown in Figure 1) is one of the most adulterated compounds in menthol. Consequently, food and drug regulatory authorities are recommending to estimate the carbitol in menthol [6,7]. The present study pertains to the development of an easy, simple and confirmatory method to estimate carbitol in menthol quantitatively. This method was validated according the international guideline described in International Council for Harmonisation (ICH) [8].
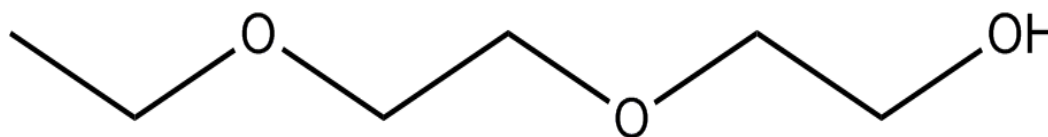

Figure 1. Chemical Structure of Carbitol.

2. Materials and methods

2.1. Reagents and chemicals

Acetone (LCMS grade) was purchased from SD fine chemicals, Mumbai, India. Carbitol of purity >99% was obtained from Sigma Aldrich, India.

Menthol with purity >99% was procured from Sigma Aldrich, India and it was used for calibration. For analysis, commercial menthol samples were obtained from ten different market in Delhi/NCR region.

2.2. Standard preparation stock solution for carbitol

Approximately 10 mg of carbitol standard was taken in a calibrated 100 mL volumetric flask and the volume was made up with HPLC grade acetone. The stock solution of 100 μg/mL was used for further studies.

2.3. Preparation of calibration standard and matrix-matched calibration solutions

From the carbitol standard solution having concentration of 100 μg/mL, appropriate aliquots were taken and further diluted with acetone so as to give a series of calibration standard solutions having carbitol concentration range of 5.0, 10.0, 20.0, 50.0 and 100.0 μg/mL respectively and matrix-matched calibration standards were prepared by fortifying with high purity menthol standard. This resulted in standard solution at 6 levels of concentration ranges: 5.0, 10.0, 20.0, 50.0 and 100.0 μg/g. A standard of carbitol of 0.1 μg/mL concentrations was prepared for estimation of limit of detection (LOD) of the instrument. We spiked 1 μg/mL concentration level of carbitol standard into the 1 g of menthol sample for determination of LOD for the method. Sample preparation was followed as illustrated in below and final concentration was made 0.1 μg/g. All solutions were stored at 2 °C to 8 °C until analysis [9].

2.4. Sample preparation

Approximately 1 g of homogenized ten menthol samples from different sources were weighed accurately into the 10 mL calibrated volumetric flask and made the volume with LCMS grade acetone.

2.5. Sample preparation for recovery

For recovery studies, 1 g of menthol samples were spiked with 5.0, 10.0 and 20.0 μg/g of carbitol reference standard and accuracy of the method was estimated by calculating percent recovery [10].

2.6. GC-MS analysis

Gas chromatography analysis was carried out using Agilent Technologies 7890 B GC system, equipped with an Agilent Technologies 7683 series auto sampler, Mass selective detector Model 5977 B network, and a glass capillary VF-Wax MS, ID: 0.25 mm, length: 30 m, film thickness: 0.25 μm; (Agilent CP9205, USA); helium carrier gas (99.9999% purity) with the flow rate of 1.0 mL/min; injection temperature 280 °C; transfer line temperature 300 °C; ion source temperature 230 °C; MS Quadrupole temperature 150 °C; ion mode: electron ionization (Scan mode/Selective ion monitoring mode); solvent delayed 3 mins; oven temperature program 50 °C for 2 mins, @ 5 °C/min to 180 °C for 2 mins to 220 °C @ 10 °C/min hold for 6 mins. Ions selected for selective ion monitoring (SIM) mode were 45, 31 and 59 m/z (mass to charge ratio) and full scan mode mass were selected from m/z of 40 to 550. The splitless injection was done at a volume of 1 μL by auto sampler [11].

3. Results and discussion

3.1. Gas chromatographic separation

A simple, selective, precise and an accurate method was developed for the determination of carbitol adulteration in the menthol sample using EI GC-MS (Electron Ionization Gas

chromatography-Mass spectrometer). Using the chromatographic conditions as above mentioned, we found a well resolved peak of carbitol at the retention time (R.T) 17.02 minutes as shown in Figure 2.
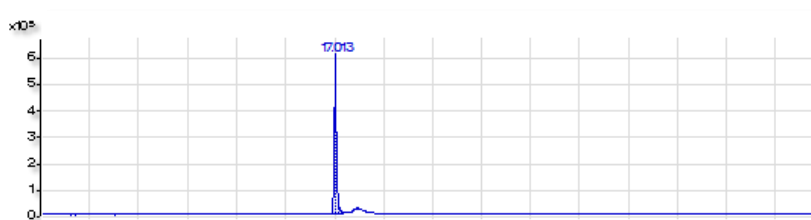

Figure 2. Total ion chromatogram of carbitol in menthol.

3.2. Mass spectrometry

For the purpose of evaluating the fragment ions and the intensity of the signals, the reference standard solution of carbitol was injected using Electron Ionization (EI) mode at 70 eV (electron volts) of the mass spectrometer detector through chromatographic technique. The confirmation of standard carbitol was carried out with help of National Institute of Standards and Technology (NIST) 17 library [11,12]. The representative mass spectrum of carbitol using GC-MS is given in Figure 3. For SIM mode quantification, we have selected quantifier ion m/z is 45 and the qualifier ion m/z are 31, 59, which is exactly same with mass spectra of carbitol as given in NIST 17 library. The results of carbitol contents in menthol samples are shown in Table 1. Carbitol was present in all menthol samples from all the sources. The concentration of carbitol in the samples were obtained in the range of 10.28 to 41.11 μg/g.
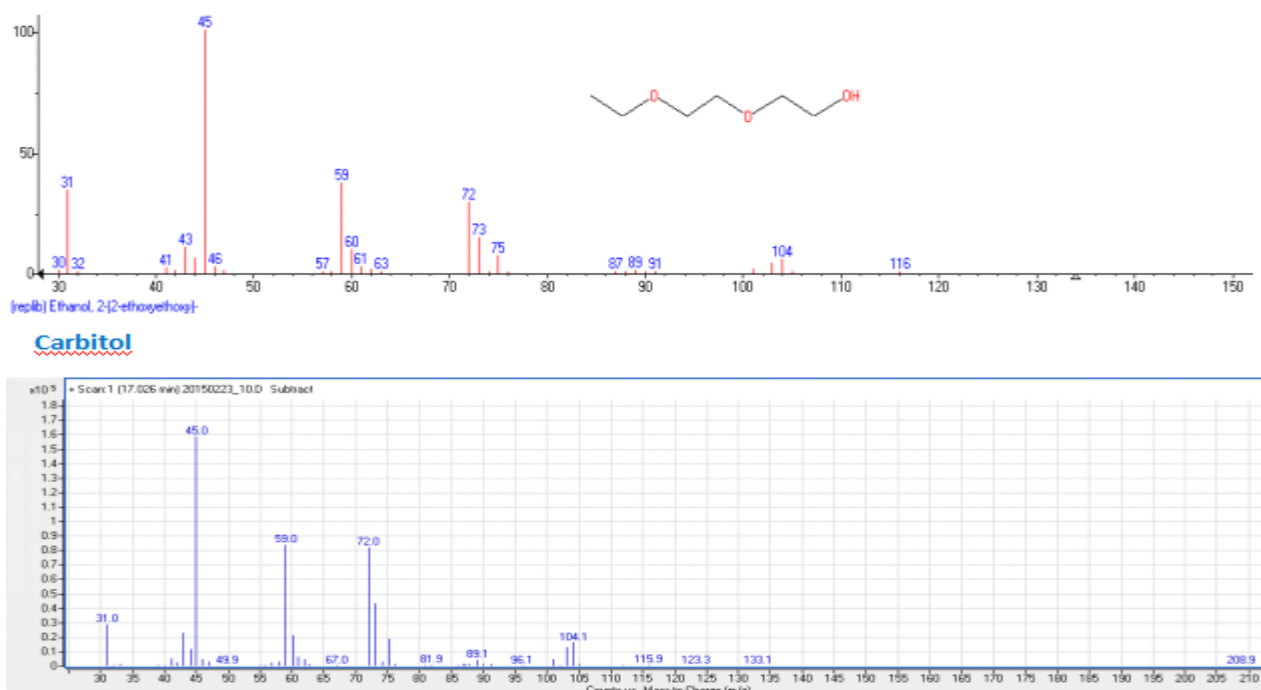

Figure 3. Mass spectra of carbitol in menthol sample and matching form NIST library.

Table 1. Determination of carbitol in menthol samples from different market in Delhi/NCR.

Sr. No.	Sample Source	Compound	Concentration (µg/g) (Avg. of three replicates)
1	Source-01	Carbitol	25.06
2	Source-02	Carbitol	30.71
3	Source-03	Carbitol	15.94
4	Source-04	Carbitol	10.28
5	Source-05	Carbitol	18.49
6	Source-06	Carbitol	40.86
7	Source-07	Carbitol	39.80
8	Source-08	Carbitol	41.11
9	Source-09	Carbitol	13.63
10	Source-10	Carbitol	34.97

3.3. Selectivity/specificity

The selectivity or specificity of an analytical method for carbitol in menthol is shown in Figure 2. As per IS: 3134:1992 method, analysis of menthol is recommended to be done by using gas chromatography techniques. The physical properties of carbitol are quite similar to that of menthol and because of this the adsorption behavior of the both menthol and carbitol on the column surfaces is always similar. Consequently, the separation of these molecules appears to be difficult using IS: 3134:1992 method [5]. The chromatogram (Figure 2) indicates that the developed method was successful in separation of carbitol in complex menthol matrix [13]. The peak of the chromatograms is also confirmed by the m/z value of standard carbitol with NIST library (Figure 3).

3.4. Limit of detection (LOD) and limit of quantification (LOQ)

LOD was determined by considering signal to noise (S/N) ratio of 3:1 for the strongest mass transition with respect to the background noise obtained from the blank sample whereas LOQ was determined similarly by considering signal to noise ratio (S/N) ratio of 10:1 (Table 2). Based on the mean noise level for the ten injections of the matrix blank of menthol sample, the LOD of the instrument was calculated as 0.1 µg/g as well as lowest detection limit for the method was calculated 1.0 µg/g and confirmed using standard solutions of carbitol with concentration of 0.1 µg/mL. The lowest concentration levels that could be quantified with reproducible values for menthol was determined as 0.5 µg/g for the instrument and LOQ for the method was calculated 5 µg/g [9]. The results were further confirmed by injecting standard solution of having concentration 0.5 µg/mL.

3.5. Linearity and range

The calibration curve for carbitol was prepared by plotting peak area against the concentration of carbitol in menthol and in blank solvent. The calibration standards were run in six replicates [10–12]. The calibration curve was prepared using the pure standard was to be found linear in the range of 5.0–100.0 µg/mL with correlation coefficient (r) of >0.9990. The

calibration curve for the matrix-matched standards was also found to be linear with correlation coefficient (r) of ≥0.9965 (Table 2).

Table 2. Selectivity, specificity, linearity and LOD & LOQ of carbitol and general GC-MS information.

Compound	R.T (mins)	Quantifier ion, m/z	Qualifier ions, m/z	LOD (μg/g)	LOQ (μg/g)	Solvent Calibration (R^2)	Matrix Calibration (R^2)
Carbitol	17.02	45	31, 59	1.0	5.0	0.9990	0.9965

3.6. Precision

Precision studies were carried out for both intra-day and inter-day repeatability and reproducibility by measuring the concentrations in seven replicates are presented in Table 3 [9,10,13]. Menthol samples were spiked at different concentration levels 5.0, 10.0 and 20 μg/g respectively and then solutions were injected on the same day and the same number of times on two subsequent days by different analysts. The low %RSD (Relative Standard Deviation) value obtained for intra-day and inter-day variation within the acceptable norms showed that the proposed method is precise and can be adopted for analysis.

3.7. Recovery study

The recoveries of carbitol in spiked samples were calculated to study the effect of menthol matrix on the determination of carbitol [14]. The recovery studies were carried out at spiked level 5.0,10.0 and 20.0 μg/g concentration of carbitol in menthol sample respectively and then prepared the sample and determined by the same method as mentioned earlier. The solutions were injected in seven replicates on two different days. The recoveries of carbitol from the menthol samples were evaluated on the basis of the comparison of the theoretical concentration level of the spiked solutions with the observed concentration gave acceptable and good percent recoveries found in the range of 98.5–103.6% are shows in Table 3.

Table 3. Intra-day and inter-day precision and recovery data for the proposed method for carbitol residues in two samples of menthol.

Spiking level μg/g	Sample	Day 1			Day 2		
		Amount calculated μg/g	% Recovery	% RSD	Amount calculated μg/g	% Recovery	% RSD
5.0	1	4.95	99.00	1.85	5.02	100.40	1.98
	2	5.13	102.7	1.56	5.17	103.4	1.58
10.0	1	10.24	102.4	1.60	10.36	103.6	1.71
	2	9.85	98.5	1.07	10.02	100.2	1.08
20.0	1	20.29	101.4	1.12	20.18	100.9	1.29
	2	20.13	100.6	1.48	20.22	101.1	1.14

3.8. Robustness

Robustness of the method was determined by analyzing the same set of spiked samples (i.e. samples spiked at concentration levels of 5.0, 10.0 and, 20.0 µg/g) under different parameters; such as same column chemistry from different manufacturers, different analysts, and different injection volumes [11,12,14]. The method was found to be robust even with small changes in analytical conditions: Change in flow rate (±0.1 mL/min), a change in injector temperature (±2 °C), use of same column from different manufacturers (DB wax. and VF wax). Under all of these conditions, the analytical values of the spiked samples were not affected and it was in accordance with the actual values.

4. Conclusions

The studies presented here, following conclusion may be drawn: (i) Electron Ionization Gas chromatography-Mass spectrometer (EI-GCMS) method developed for carbitol is found to be a rapid method with excellent chromatography separation, lowest limit of detection and lowest limit of quantification reported so far. (ii) The method yields high precision, accurate and sensitive quantification by using simple sample preparation method, is advantageous. (iii) Carbitol shows the quantifier ion m/z 45 with qualifier ion m/z 31 and 59. (iv) Despite using simple sample preparation method, no interferences were observed from the matrix.

We believe that the current developed method is easy to adapt and can be used for ascertaining contamination or adulteration of carbitol in menthol, which is almost difficult to identify using current IS 3134:1992 method. The method is suitable for all the types of grades menthol available in the market.

Conflict of interest

The authors declare no conflict of interest.

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