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# Gas Chromatography-Mass spectrometric Determination of Benzyl alcohol in Injectable Suspensions

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## ABSTRACT

*A simple and reliable Gas chromatography-Mass spectrometer method was developed for the determination of Benzyl alcohol in injectable suspension. Methanol was used for extraction solvent and co-extractive was removed by filtrated through anhydrous sodium sulfate. The method was validated by determining parameters such as, specificity, linearity, limit of detection, limit of quantitation, precision, recovery and robustness. The method was found to be specific against matrix interferences. Linearity was evaluated over the concentration ranges of 0.1 µg/ml to 10 µg/ml and correlation coefficient was more than 0.999. Both the inter day and intra day precision of the system and method were determined. Recovery data obtained by fortifying three matrices at 0.5 µg/g, 1.0 µg/g and 2.0 µg/g with ranged between 98 to 105 % and the relative standard deviation (RSD) was obtained below 5%. Limit of detection and quantitation of Benzyl alcohol were 0.05 and 0.1 µg/g.*

**Keywords:** Benzyl alcohol, Preservative, injectable suspension, Gas chromatography-Mass spectrometer.

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## INTRODUCTION

Benzyl alcohol is frequently used as an antimicrobial preservative or co-solvent in a varity of pharmaceutical injection formulations. The main toxic oxidation product, benzaldehyde, arises from the oxidation of benzyl alcohol upon long-term storage or heat sterilization of parenteral dosage forms containing benzyl alcohol, if oxygen is not excluded totally by nitrogen flashing. The presence of this potential impurity needs to be monitored owing to its reactivity and toxicity [1]. Exposure to excessive amounts of benzyl alcohol has been associated with toxicity (hypotension, metabolic acidosis), particularly in neonates, and an increased incidence of

kernicterus, particularly in small preterm infants. Several methods have been reported for the quantitative determination of benzyl alcohol in parenterals, which include spectrophotometry [2], gas chromatography [3,4,5,10], HPLC [6,7]. The United States Pharmacopoeia (8) limits the presence of benzaldehyde in benzyl alcohol to a level of 0.2%, when determined by the HPLC method. The British Pharmacopoeia (9) states that benzyl alcohol intended for use in the manufacture of parenteral dosage forms should be in the limit and describes a GC method for its determination in the raw material. This present work was to develop and validate a selective and confirmatory method for determination of Benzyl alcohol in injectable suspension, based on Gas Chromatography-Mass Spectrometry technique.

## MATERIALS AND METHODS

### *2.1 Reagents and chemicals*

Methanol (HPLC grade) was purchased from SD fine chemicals, Mumbai, India. Reagent grade anhydrous sodium sulfate was obtained from SD fine chemicals, Mumbai, India. Benzyl alcohol with purity >99% were obtained from Fluka chemicals, USA. Stock solutions of Benzyl alcohol at 100µg/ml was prepared by dissolving standard Benzyl alcohol (>99%) in methanol and storing the solution at -20°C. Working standard solutions were prepared by diluting the stock solutions to 10µg/ml in methanol. Calibration standards were prepared by adding appropriate volumes of standard working solution with methanol at 7 levels in the ranged between 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/ml. 1 µl of the each standard was injected onto the GC-MS by auto sampler.

Injectable suspensions (ARISTOSPAN®-20, KENALOG®-40, KENALOG®-20) were collected from the local market of Delhi (India).

### *2.2 GC/MS analysis*

Gas chromatography analysis was carried out using Agilent Technologies 6890 N network GC system, equipped with a Agilent Technologies 7683 series auto sampler, Mass selective detector Model 5973 network, and a glass capillary DB-5, ID: 0.32 mm, length: 30 m, film thickness: 0.25 µm; packed with non-polar polymer [(5%-phenyl)-methylpolysiloxane] (J & W Scientific 122-5532, USA).; a helium carrier gas flow. 1.0 ml/min; injection temperature 280°C; transfer line temperature 300°C, ion source temperature 230°C, MS Quard temperature 150°C; ion mode electron ionization mode (scan mode/SIM mode); oven temperature program 50°C for 5min, @ 10°C/min to 180°C, post run: 280°C held for 2 min (total run time: 18 min); Splitless injection at a volume of 1 µl by auto sampler.

Mettler weighing balances with a least count of 0.0001g and 0.001mg for weighing of samples and standards respectively. Calibrated ‘A’ grade glassware of Borosil procured from local market.

### *2.3 Sample preparation:*

Approximately 2±0.5 g of homogenized test sample (injectable suspension) was weighed accurately and transferred into the separatory funnel. Extract the sample with 60 ml of methanol and passed through filter containing 20g of anhydrous $Na_2SO_4$ into 250 ml round-bottom flask.

Extraction was repeated twice with 50 ml of methanol each time. Evaporate methanol solution in rotary evaporator at 40°C, made the dilution to 2 ml.

## RESULTS AND DISCUSSION

### *3.1 Gas chromatographic separation*

A comparatively simple, sensitive and an accurate method was developed for the determination of Benzyl alcohol residues in injectable suspension using EI GC-MS (Electron Ionization Gas chromatography-Mass spectrometer). Using the chromatographic conditions as mentioned above, a well resolved peak for Benzyl alcohol was obtained at 10.6 minutes of the injection in the electron ionization mode, which shown in Figure 1. For avoiding matrices which has come in extraction solution with Benzyl alcohol, we delayed solvent up to 5 min.

### *3.2 Extraction Procedure*

For the extraction of Benzyl alcohol from the injectable suspension, a simplified extraction procedure was developed as compared to the other extraction procedure which has published earlier. Since Benzyl alcohol is soluble in methanol, it was taken as extraction solvent for extracting the residues of Benzyl alcohol from injectable suspension. Any moisture which might have been co-extracted along with the Benzyl alcohol residues were removed by passing solution through filter containing anhydrous sodium sulfate.

### *3.3 Mass Spectrometry*

For the purpose of evaluating the fragment ions and the intensity of the signals, the reference standard solution of Benzyl alcohol was injected using EI mode at 70 eV of the mass spectrometer detector through chromatography technique. The standard Benzyl alcohol was confirmed with NIST library of GC-MS. The mass spectrum of Benzyl alcohol using GC-MS is given in Figure 2. Form the mass spectrometric data, it can be seen the Quantifier ion m/z is 79 and the Qualifier ion m/z is 108 and 109, which is exactly same with the mass spectra of Benzyl alcohol in NIST library.

### *3.4 Method Performance Characteristics*

*3.4.1 Selectivity/specificity*: The selectivity or specificity of analytical method for Benzyl alcohol in injectable suspension is represented in Figure 1. The chromatogram indicates that the developed method was successful in separation of Benzyl alcohol in injectable suspension. The peak of the chromatograms is also confirmed by the m/z value of standard Benzyl alcohol with NIST library of GC-MS. The peak area of Benzyl alcohol obtained the RSD value was within 2% at ppm level. The retention time had good reproducibility, within RSD 0.05%. Table 1 shows the results for selectivity and specificity of Benzyl alcohol in injectable suspension.

*3.4.2 Linearity and Range:* The calibration curve for Benzyl alcohol was prepared by plotting peak area against the concentration of standard Benzyl alcohol at seven levels in blank solvent. It was linear in the range of 0.1µg/ml, 0.2 µg/ml, 0.5 µg/ml, 1.0 µg/ml, 2.0 µg/ml 5.0 µg/ml and 10.0 µg/ml gave linear response over the studied range of concentration, and the least squares linear regression analysis of the data provided excellent correlation coefficient (r) of more than 0.999. The linear equations, correlation co-efficient and RSD values are presented in Table 2.

*3.4.3 Limit of detection (LOD) and limit of quantitation (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 6:1. Based upon the mean noise level for the ten injections of the matrix blank of injectable suspension sample, lowest detection limit of the instrument was calculated as 0.05µg/g and confirmed using standard solutions with concentration of 0.05µg/g and the lowest concentration levels that could be quantified with reproducible values obtained on injecting 6 replicates of the same concentration as 0.1µg/g and further confirmed by injecting 6 replicates of matrix matched standard solution of Benzyl alcohol having concentration of 0.1µg/g.

*3.4.4 Precision:* Precision studies were carried out for both intra-day and inter-day repeatability and reproducibility (Table 3). Spiked known concentration of standard Benzyl alcohol into the sample of injectable suspension and made at different concentration levels i.e. 0.2 µg/g and 0.5 µg/g respectively were injected five replicates on the same day and the same number of times on three subsequent days by three different analysts. The low %RSD 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.

*3.4.5 Recovery study:* The recovery of Benzyl alcohol in spiked samples was calculated to study the effect of matrix on the determination of Benzyl alcohol. The recovery studies were carried out at three different concentrations. For this three different portions of pre-analyzed different injectable suspension samples were spiked with 0.5 µg/g, 1.0 µg/g and 2.0 µg/g respectively in six replicates on three different days and then extracted and determined by the same method as mentioned earlier. The recoveries of Benzyl alcohol from the injectable suspension 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% to 105% , are shows in Table 4.

*3.4.6 Robustness:* Robustness of the method was determined by analyzing the same set of spiked samples (i.e. samples spiked at concentration levels of 0.1 µg/g, 0.2 µg/g and, 0.5 µg/g) under different parameters; such as same column chemistry from different manufacturers, different analysts, and different injection volumes. The method was found to be robust even with small changes in analytical conditions: change in flow rate (± 0.05 ml/min), a change in injector temperature (± 2°C), use of same column from different manufacturers (HP5, DB5, CP-sil 8 CB). Under all of these conditions, the analytical values of the spiked samples were not affected and it was in accordance with the actual values.

**Table 1: Fragment ions produced using EI mode, specificity and selectivity of Benzyl alcohol**

| Compounds | Category | Mode | R.T (min) | Parent ion, m/z | Product ions, m/z |
|---|---|---|---|---|---|
| Benzyl alcohol | Preservative | EI | 10.6 | 79 | 108, 109 |

**Table 2 Comparison of matrix matched calibration with standard calibration, and repeatability data for the Benzyl alcohol**.

| Standard | Solvent calibration | | | Repeatability (% RSD) | LOD (µg/g) | LOQ (µg/g) |
|---|---|---|---|---|---|---|
| | Slope | y-imtercept | $R^2$ | | | |
| Benzyl alcohol | 186000 | -9140 | 1 | 1.53 | 0.05 | 0.1 |

**Table 3. Intra-day and Inter-day precision data for the proposed method for Benzyl alcohol residues in three samples of injectable suspension**

| Concentration of Benzyl alcohol (µg/g) | Sample No | Day 1 | | Day 2 | | Day 3 | | Intra-assay | |
|---|---|---|---|---|---|---|---|---|---|
| | | Benzyl alcohol conc. obtained (µg/g) n=5 | % RSD | Benzyl alcohol conc. obtained (µg/g) n=5 | % RSD | Benzyl alcohol conc. obtained (µg/g) n=5 | % RSD | Benzyl alcohol conc. obtained (µg/g) n=5 | % RSD |
| 0.2 | 1 | 0.204 | 3.41 | 0.198 | 2.84 | 0.197 | 2.97 | 0.199 | 3.85 |
| | 2 | 0.201 | 3.24 | 0.204 | 2.79 | 0.199 | 2.82 | 0.201 | 2.54 |
| | 3 | 0.198 | 3.54 | 0.201 | 3.21 | 0.203 | 3.85 | 0.199 | 3.28 |
| 0.5 | 1 | 0.499 | 1.85 | 0.503 | 1.62 | 0.501 | 2.25 | 0.499 | 1.79 |
| | 2 | 0.500 | 1.54 | 0.500 | 1.87 | 0.498 | 2.15 | 0.500 | 2.14 |
| | 3 | 0.499 | 2.13 | 0.500 | 1.99 | 0.499 | 1.47 | 0.502 | 1.63 |

**Table 4. Percent recovery of Benzyl alcohol from three different injectable suspension samples analyzed on different days (n=3)**

| Spiking level µg/g | Sample No | Day1 | | Day 2 | | Day 3 | |
|---|---|---|---|---|---|---|---|
| | | Amount calculated µg/g | % Recovery | Amount calculated µg/g | % Recovery | Amount calculated µg/g | %Recovery |
| 0.5 | 1 | 0.498 | 99.6 | 0.497 | 99.4 | 0.490 | 98.0 |
| | 2 | 0.492 | 98.4 | 0.501 | 100.2 | 0.492 | 98.4 |
| | 3 | 0.485 | 97.0 | 0.489 | 97.8 | 0.499 | 99.8 |
| 1.0 | 1 | 0.964 | 96.4 | 0.998 | 99.8 | 1.003 | 100.3 |
| | 2 | 0.983 | 98.3 | 0.992 | 99.2 | 1.009 | 100.9 |
| | 3 | 0.971 | 97.1 | 0.998 | 99.8 | 1.000 | 100.0 |
| 2.0 | 1 | 1.954 | 97.7 | 2.098 | 104.9 | 2.042 | 102.1 |
| | 2 | 1.742 | 87.1 | 1.976 | 98.8 | 2.009 | 100.4 |
| | 3 | 1.885 | 94.2 | 1.894 | 94.7 | 2.021 | 101.1 |

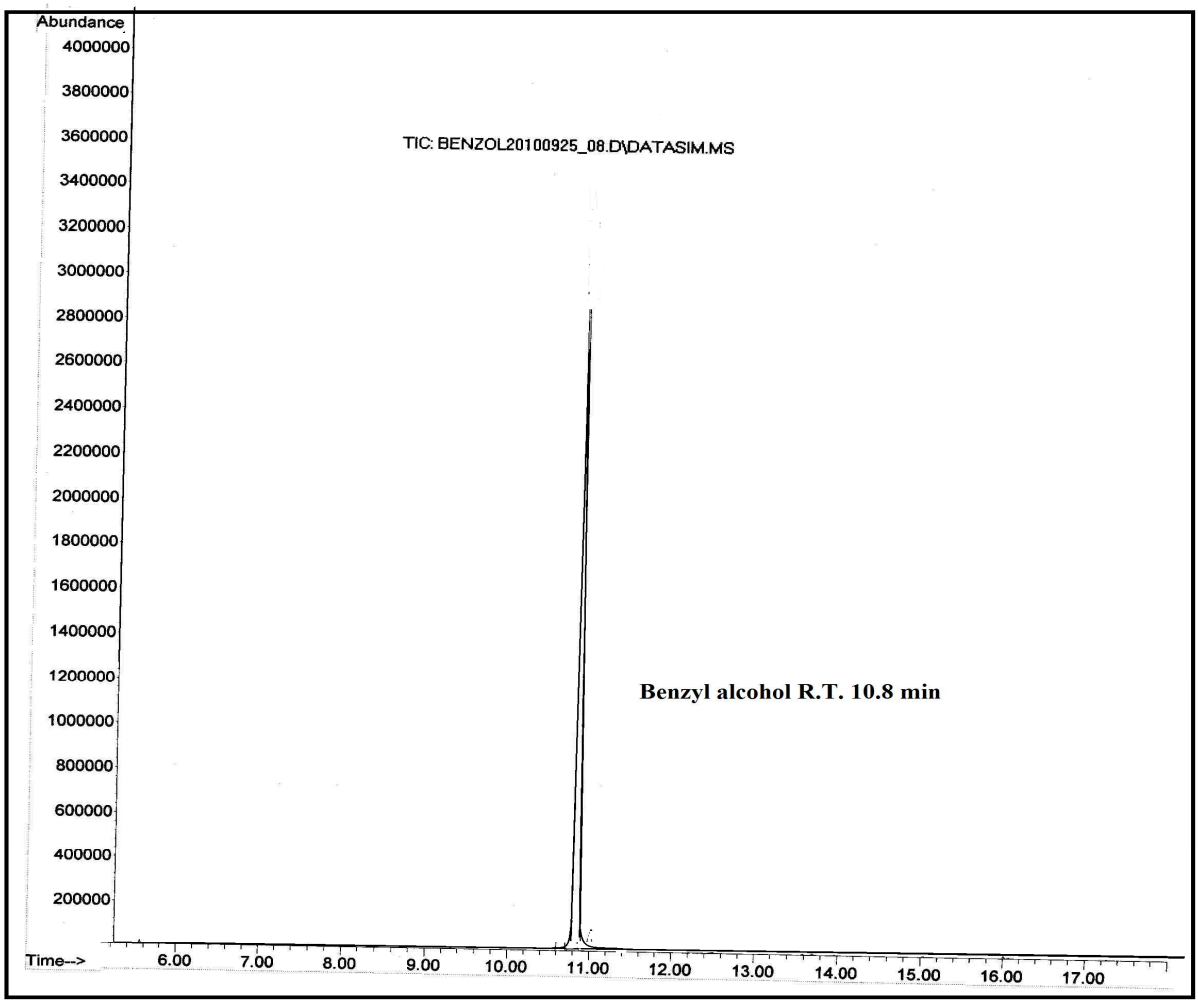
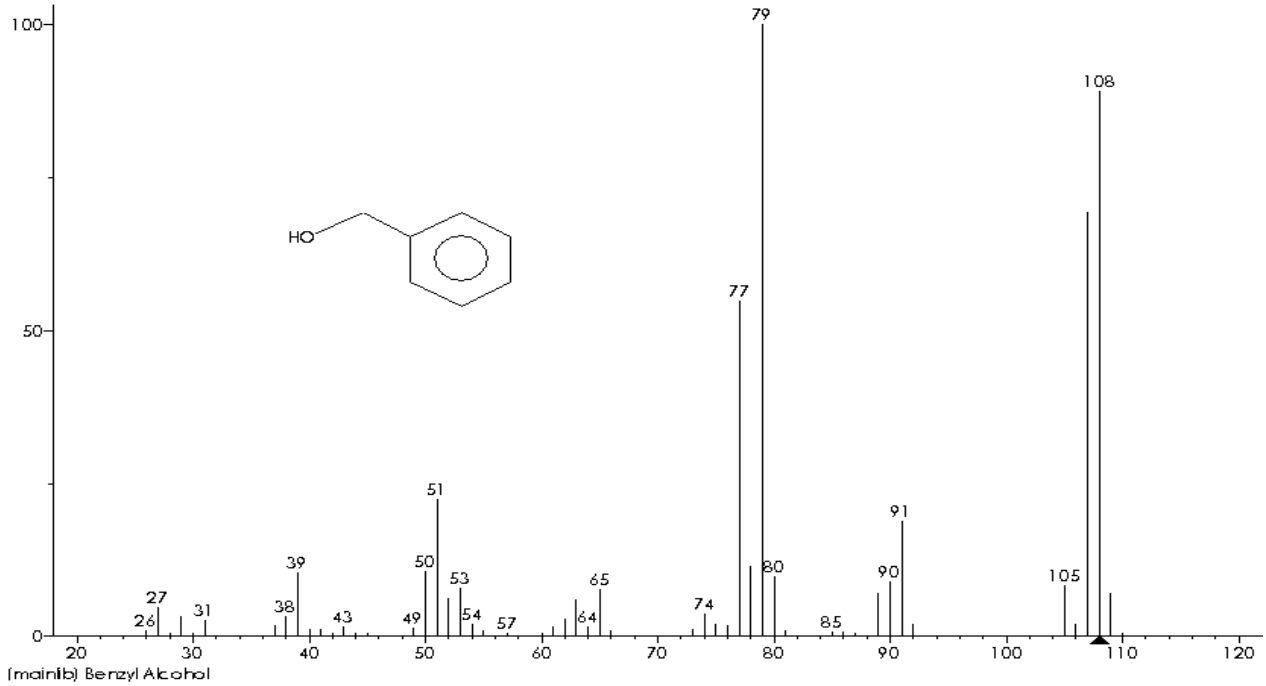



**Figure 1: Total Ion Chromatogram of Benzyl alcohol spiked in Injectable sample**




**Benzyl alcohol**

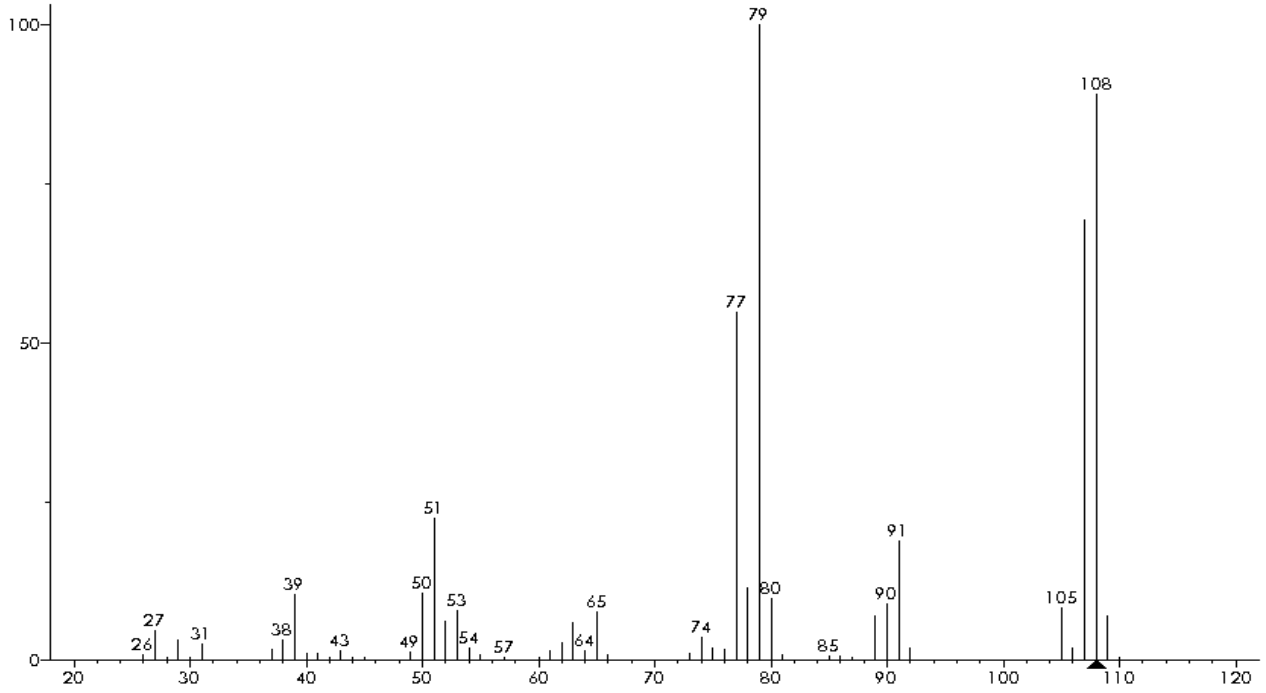



**Figure 2: Mass Spectra of Benzyl alcohol in the spiked sample and matching form NIST Library.**

## CONCLUSION

Benzyl alcohol is used as a preservative in injectable suspension but it should be at ppm level because at high concentration it caused toxic to human health. A validated gas chromatography-Mass spectrometer method has been developed for the determination of Benzyl alcohol in injectable suspension at ppm level. The work described in this paper has shown that the analytical method developed is precise, accurate, sensitive and robust for the determination of Benzyl alcohol. The method is specific to the analysis of Benzyl alcohol in injectable suspension without any matrix interference. This method is also appropriately useful for the determination of Benzyl alcohol in the preservative free injectable suspension.

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