



CAPILLARY GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF BENZYL ALCOHOL IN INJECTABLE FORMULATIONS

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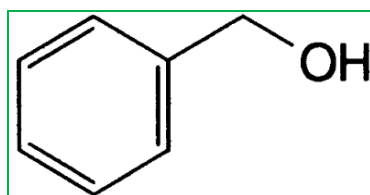
ABSTRACT

A Simple, precise and accurate capillary gas chromatographic method has been developed for determine benzyl alcohol in injectable formulations (pafelo) ampoules. The method enables to inject the substance without extraction. Separation and quantification are achieved on a fused silica capillary column (30 m x 0.53 mm id), coated with dimethylpolysiloxane with FID. The ability of the system to resolve Benzyl alcohol peak from interfering compound is good using temperature program. The method was validated with respect to specificity, linearity, accuracy, precision, limit of detection and limit of quantitation. The validation acceptance criteria were met in all cases. Linearity and recovery data were obtained in spiked placebo studies. The method displays excellent linearity over concentration range 25 – 175 µg/ml. The quantification limit is 13.3 µg/ml and the detection limit equal to 2.67 µg/ml.

Keywords: Benzyl alcohol, injectable formulation, Pafelo®, Capillary gas liquid Chromatography.

INTRODUCTION

Benzyl alcohol or Phenyl methanol¹



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Benzyl alcohol is commonly used as an antibacterial agent (bacteriostatic preservative) in parenteral pharmaceutical formulations (medications) at low concentration, it used in concentration 0.9 – 2.0 %². It is oxidized rapidly in healthy individuals to benzoic acid, conjugated with glycine in the liver and excreted as hippuric acid.

Benzyl alcohol preservation has been reported to cause toxic effect in High concentration³. High concentration can result in toxic effects including respiratory failure, vasodilation, hypotension, convulsions and paralysis. Newborns, especially if critically ill, may not metabolize benzyl alcohol as readily as adults. Report in the early 1980s of sixteen neonatal deaths associated with the use of saline flush solutions containing benzyl alcohol preservative led to recommendations to avoid its use in neonates.³

Therefore it is very important to monitor benzyl alcohol in its dosage form. The assay of benzyl alcohol in pharmaceutical preparation was not reported either in USP⁴ nor in BP¹ or others pharmacopeias.

Different methods have been reported in the Literature for Identification and Quantitation of benzyl alcohol using GC-MS in serum⁵ or in nasal spray and parenteral using the techniques of high performance liquid chromatography reversed phases.^{6,7}

Therefore we report a new GC method for determination of benzyl alcohol in injectable dosage form pafello® which included Pancuronium bromide as active ingredient and sufficient quantities of sterile excipients for 2 ml included. Benzyl alcohol included as preserve agent.

MATERIALS AND METHODS

Materials

All chemical were of analytical reagent grade.

- Benzyl alcohol special grade, Merck, batch K41694587
- Ethanol LC, Merck
- Pafelo® dosage form Batch No. A 80081 Mfg 09/2009 was obtain from the commercial market.
- Placebo was obtained from Pafelo® manufacturing company (Avenzor).
- Water LC, Merck

Instrumentation

GC analyses were performed on GAS CHROMATOGRAPH GC-2014 SHIMADZU coupled with FID.

The capillary column (30m x 0.53 mm Optima-1-5.00 µm). The Column coated with nonpolar stationary phase consist of 100 % dimethylpolysiloxane, confirm with USP G1 and G2, the film thickness is equal or less than 3 µm film.



- split less manual injection system
- Injector temperature 250 C°
- Temperature program mode with initial oven temperature 110 C°
- After maintaining that temperature for 3 min the oven temperature was increased at rate of 14 C°/min to reach the final temperature of 250°C. This temperature was maintained constant for 2 minutes.
- Run Tim: 15 minutes
- Carrier gas: Helium
- Flow rate: 5 ml
- Burner gas: H₂/air
- FID: 300 C°
- Pump pressure: 200 atm

Preparation of stock and standard solution

A Stock solution of benzyl alcohol (10 mg/ml) was prepared in water, a standard series from this stock solution was prepared in concentration of 25, 50, 75, 100, 125, 150 and 175 µg/ml, all solution were used on the day of preparation.

Calibration curves

Triplicate 1 µl injections were made for each concentration and the peak area versus concentration was plotted to obtain the calibration graph.

Pharmaceutical Preparation

Each commercial injection dosage form of Pafelo® (Ampoule) has a volume of 2 ml and contains 2 mg Pancuronium bromide and usually 0.01g/ml of benzyl alcohol. The content of 20 ampoules was poured into backer and then 1 ml of the solution was transferred in to 100 ml volumetric flask, dilute to 100 ml with water.

Recovery studies

Appropriate known quantities of Benzyl alcohol (12.5, 10 and 7.5 mg) each one was spiked to 100 ml placebo solution. Triplicate injections of these spiked sample extracts and the standard extracts were made in the GC. Comparisons of the integrate area of peaks resulting from the injected reference standard with those resolution from the spiked sample were used to calculate the percentage recovery of benzyl alcohol in each case.

RESULTS AND DISCUSSION

A typical gas chromatogram obtained from analysis of benzyl alcohol. The benzyl alcohol was resolved in with retention time of 11.48 min. In Fig.1 are typical chromatograms showing separation of benzyl alcohol from dosage form (Pafelo®).

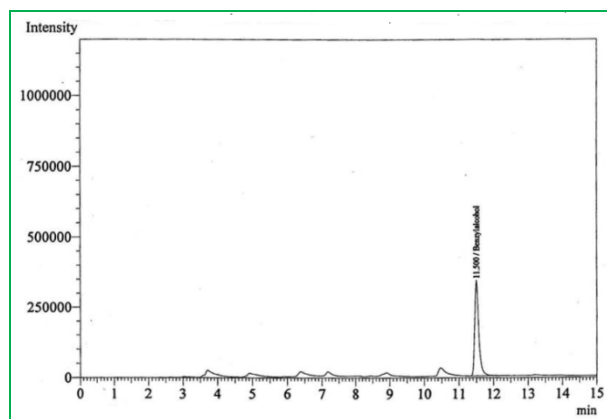


Figure 1: Chromatogram of pafelo® sample

While the Chromatogram of placebo which prepared in the manufacturing company doesn't show any significant peak at the benzyl alcohol retention time (Fig.2)

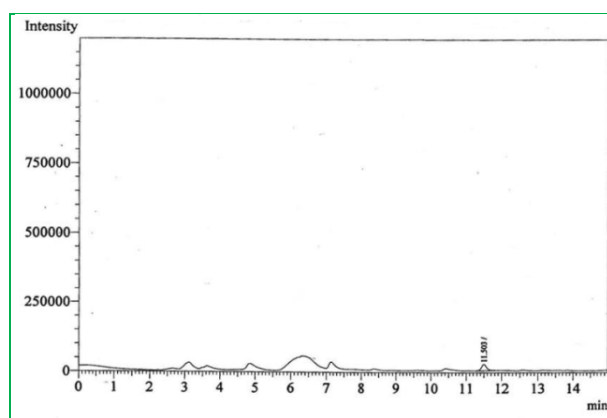


Figure 2: Chromatogram of pafelo placebo.

While the chromatogram in Fig.3 show the peak of Standard solution of benzylalcohol.

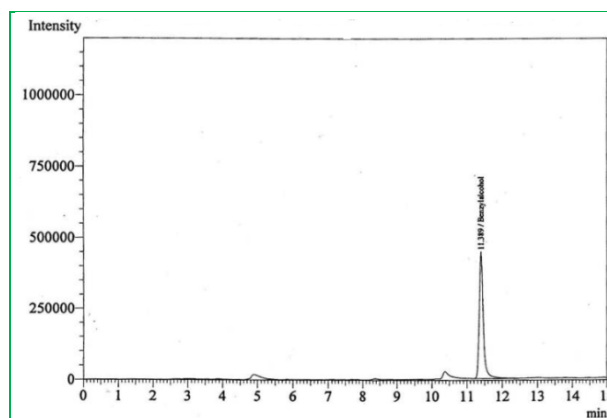


Figure 3: Chromatogram of Benzyl alcohol standard solution (75 µg/ml)

The GC conditions described gave complete elution with run time of 15 min. The described method was validated throw establishing repeatability, recovery and specificity of the assay, and determined the detector linearity and limit of detection of benzyl alcohol.

Validation

The calibration graph for peak area of benzyl alcohol versus the concentration (table 1) was constructed using the above conditions, the graph showed a linear response for the range 25-175 µg/ml benzyl alcohol (figure 4). The correlation coefficient (r) of the resulting calibration curve was 0.9999.

The equation of this curve was $y = 47597 X$ and applied to calculate the unknown benzyl alcohol concentration in the pafelo dosage form while x is the concentration of benzyl alcohol and y is the peak area.

Table 1: Peak area and concentration of the calibration curve

Concentrations of Benzyl alcohol µg/ml	Peak area average (n=3)
0	63706
25	1188610
50	2376130
75	3555332
100	4753550
125	5952959
150	7125544
175	8350080

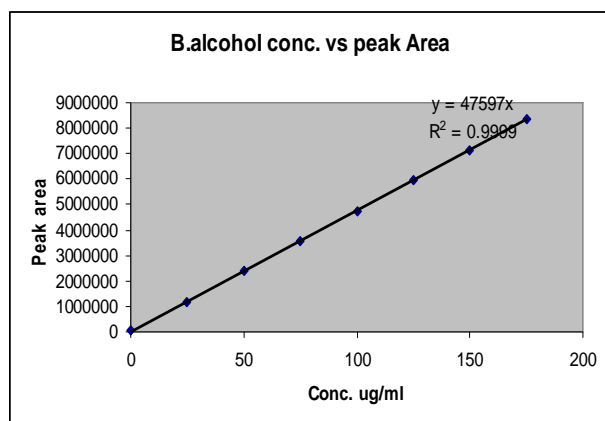


Figure 4: Benzyl alcohol concentration versus peak area

Recovery

The analytical recovery was performed by analyzing reference sample that were spiked with placebo, the total recovery of benzyl alcohol from the spiked injection formulation was $99.6 \pm 1.3 \%$ (Table 2)

Table 2: Application of the proposed method to the determination of Benzyl alcohol in spiked pafelo[®] placebo.

Spiked concentration	Peak area average	Concentration found	% recovery (n=3)
75 µg/ml	3499878	73.8	98.4
100 µg/ml	4731005	99.5	99.5
125 µg/ml	6001311	126.2	100.9
			$99.6 \pm 1.3 \%$

Selectivity

Throw the monitoring of standard solution of benzyl alcohol in the presence of all pharmaceutical active ingredient and excipients in Pafelo[®] enabled to checked the selectivity of the method. The ability of the analytical system to resolve benzyl alcohol from the potential interfering components was good. So it was no interference due to the formulation components.

Sensitivity

The sensitivity of the method was determined through the applied of equation $\Delta Q / \Delta R$ which is used for determination of Limit of detection (LOD) and limit of Quantization (LOQ), while the LOD and the LOQ were 2.67 and 13.3 µg /ml respectively.

Pharmaceutical application

The proposed method was applied to determine the Benzyl alcohol in the Pafelo[®] dosage form, the analytical result summarized in tab 3.

Table 3: Performance data for the proposed method

Parameter	Proposed GC method
Concentration range	25-175 µg/ml
Regression equation	Peak area = 47597 x concentration
Correlation Coefficient	0.999
Application	Parenteral dosage form (Pafelo [®])
% Recovery	99.6 ± 1.3

CONCLUSION

Anew GLC method was proposed for selective quantitative of Benzyl alcohol in Parenteral dosage form, the method is suitable to applied in quality control analysis of benzyl alcohol containing injection formulations.

The method validation demonstrated good precision, specificity and accuracy with acceptable recovery and chromatographic resolution.

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Prof. Dr. J. Al-Zehouri graduated from Damascus University, College of Pharmacy at 1984 and got the Dr.rer.nat in pharmacy from MLU in Germany at 1991. He is having 21 years of teaching experience at Damascus University, College of pharmacy and several universities like Philadelphia, KSU, IUST, and SIUST) since 2005 he became a prof. of Pharmaceutical analytical chemistry, he got the head dept. of Analytical and Food chemistry at 2009, his most researches was in pharmaceutical analysis (Drug Control).