Estimation of Alcohol Content in Homeopathic Formulations by Gas Chromatography

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Abstract: The alcohol content in homeopathic formulations was estimated by gas chromatography. The storage period of these formulations is sometimes very long in the market[1]; during this period there may be loss of alcohol from packed formulations. Consequently these preparations were selected to compare via gas chromatographic analysis the actual alcohol content to the alcohol percentage indicated on the label of the products. The method of analysis was developed and validated as per ICH Q3C guidelines[2,3]. The percentage of alcohol detected was found to be consistent with the labels' claims. The correlation coefficient for linearity was 0.99, which was within acceptance criteria (i.e., this should not be less than 0.95). Accuracy was determined at three levels by adding 50%, 100% and 150% standard solution to equal percent solutions of test preparation. The results found percentage mean recoveries of 100.26, 99.81 and 99.73 respectively, which were within the acceptance criteria; i.e., 80% to 120% as per ICH Q3C guidelines[3]. Precision study was done for system, method and intermediate precision. The result obtained for overall RSD was 0.584, which was within the acceptance criteria (i.e., should not be more than 15.0 %). Specificity was tested by injecting blank, test and standard preparations, and no peak for the blank sample was observed.

Keywords: homeopathic formulations, alcoholic concentrations of; gas chromatography assessment of homeopathic preparation alcohol content.

1. Introduction

Homeopathic medicine is becoming popular, primarily for treating chronic diseases in India, China, the United States, and in many other European countries. Liquid homeopathic remedies frequently contain alcohol[1]. The FDA allows higher levels of alcohol in these remedies than it allows in conventional drugs. However, no adverse effects from alcohol levels have been reported to the FDA[3]. Homeopathic remedies in high dilution, taken under the supervision of trained professionals, are generally considered safe and unlikely to cause severe adverse reactions. Tinctures are made from a variety of botanical and zoological substances. They are diluted in varying percentages of alcohol and then sit for thirty days (according to HPUS standard). Tinctures have crude material in them and therefore may not be safe for oral consumption. Preparations are stored for long periods of time with no expiration date. A consequent concern is whether the alcohol content of these preparations declines during this long storage period. Thus there is a need to estimate the percentage of alcohol remaining in these formulations after prolonged storage. For the present study the formulations used were manufactured thirteen years ago (1996 to 1999).

In the present study, a simple and highly sensitive gas chromatographic method with packed column was developed to determine the percentage of alcohol. The conditions for sample preparation and analysis were optimized, and the method was validated in terms of linearity, specificity, accuracy, precision and recovery [4,5,6].

2. Experimental

2.1.Chemicals and Test formulations.

2.1.1 Chemicals

Pure Ethanol (99.9%). Other chemicals used were nitrogen gas, hydrogen gas. Deionized water used for all experiments was of Milli-Q quality (Millipore Corp, USA).

2.1.2 Test formulations.

Table 1
Manufacturing details of Homeopathic test formulations.

Sr. No.	Homeopathic test formulation	Manufacturing Date
1	*Agnus Castus*	May 1999
2	*Ambra Grisea*	July 1997
3	*Argentum Metallicu*	June 1996
4	*Alumen*	July 1997
5	*Berberis Aquifolium.*	July 1997

2.2. Instrumentation and chromatographic conditions

A Perkin Elmer Clarus 500 Chromatographic system for chromatographic analysis with manual injector was utilized. Chromatographic separations were performed on packed column (1/8 inch diameter and 2 meters long RUN Chromatograph) maintained at 85°C. The oven temperature was maintained at 85°C. The carrier gas used was nitrogen gas. Hydrogen gas and air was used for ignition of flame. The flow rate was 25 mL/min, and the sample injection volume was 0.05µL. Injector (Perkin Elmer) temperature was 150°C. The detector system (Perkin Elmer) used was a Flame Ionisation Detector (FID). The FID detector temperature was 200°C.

2.3. Sample preparation

2.3.1 Preparation of standard stock solution.

Stock was prepared by diluting 12.5 ml of ethanol (99.9%) in a 50 ml. volumetric flask with Millipore water. This was considered as the 200% stock solution. From the stock solution 20%, 50%, 80%, 100%, 120% and 150% solutions were prepared.

For estimation of percent alcohol 100% solution from stock solution was used.

2.3.2 Method development

Table 1
Optimized method for standard ethanol (99.9%)

Method		Observations	Inference
Oven temperature	:85	The Peak was observed with little tailing	Sharp peak observed
Time	:10		
Injector temperature	:150		
FID temperature	:200		
Carrier gas (N_2) flow	:25ml/min		

The peak area was calculated from this chromatogram and was considered as 100 % for further calculations. The equal injection volume of test preparations were injected by making the same dilutions as that of standard ethanol.

The following test preparations were injected: *Agnus castus, Ambra grisea, Argentum metallicum, Alumen, Berberis aquifolium.*

2.4 Method Validation

Method validation assays were carried out according to the United States Food and Drug Administration (US FDA) bio-analytical method validation guidance [2,3,4,5,6].

2.4.1 Specificity

12.5ml of accurately measured ethanol (99.9%) was diluted with water in a 50ml volumetric flask to make a 200% solution. From this a 100% solution was prepared. 0.05µL quantity of this solution was injected. For the blank sample, Millipore Water was used and an equal quantity was injected.

2.4.2 Precision

2.4.2.1 System precision

Six replicates of the standard 100% solution were injected as per the described method. From that, mean, standard deviation (SD), percent relative standard deviation (percent RSD) were calculated.

2.4.2.2 Method Precision

Six replicate injections of test solutions were injected as per the method. The amount of residual solvents, mean, SD, %RSD were calculated and recorded.

2.4.2.3 Intermediate Precision

The same procedure was repeated, followed by method precision on a different day, by a different analyst, using the same lot of sample as that in method precision, and the mean, SD, percent RSD was calculated and recorded.

2.4.3 Accuracy

From the 200% stock solution, 50%, 100%, 150% solutions were prepared and a three level study was performed. The amount found and percent recovery at each level was calculated. Mean percent recovery and percent RSD was calculated.

2.4.4 Linearity

From the 200% stock solution, 50%, 80%, 100%, 120%, 150% solutions were prepared. The runs of all samples were taken and area under the peaks was considered for the linearity study.

3. Result and Discussion

3.1 Result for 100% standard et hanol solution.

The area under the peak was found to be 4909550.25; this area was considered as 100% and accordingly the other test areas were calculated. See Figure 1: Chromatogram for Standard ethanol.

3.2 Result for determination of percent alcohol in homeopathic formulations.

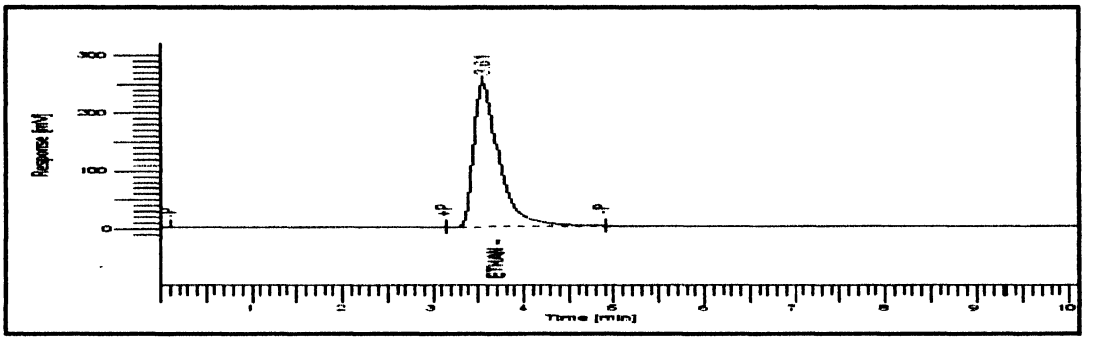

Fig 1. Chromatogram for standard ethanol

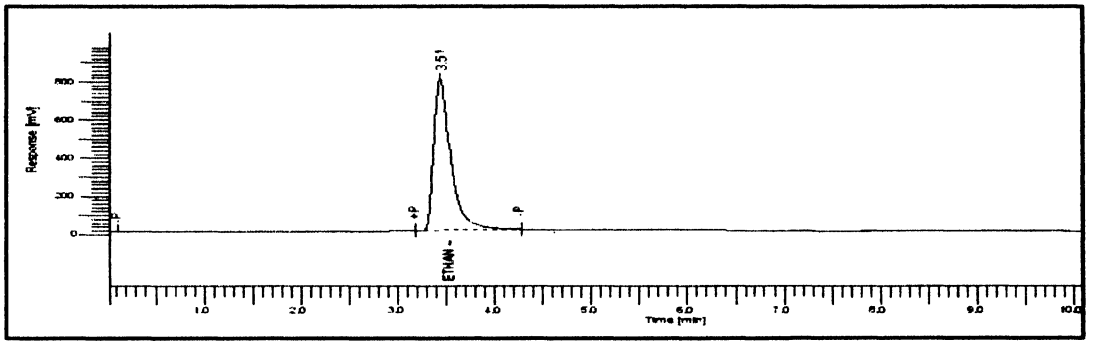

Fig 2. Chromatogram for *Agnus castus*

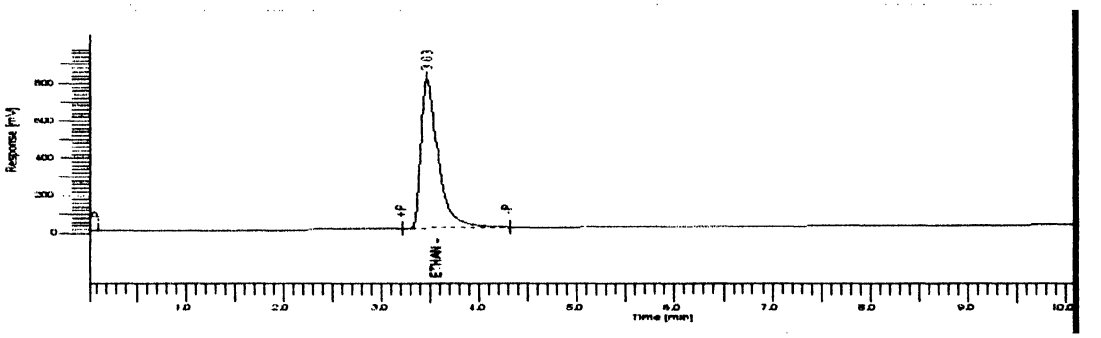

Fig 3. Chromatogram for *Ambra grisea*

Table 2
Percent alcohol determination data for test formulations

Test (Homeopathic Formulation)	Area(μV)	Percent area found
Agnus Castus	4467446.43	90.99502
Ambra Grisea,	4406892.25	89.76163
Argentum Metallicu,	4389894.26	89.41541
Alumen	4390632.78	89.43045
Berberis Aqui olium.	4325786.52	88.10963

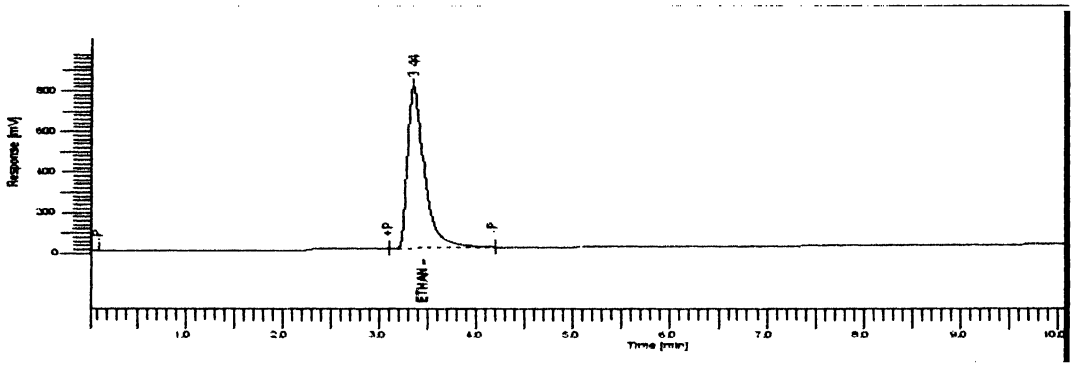

Fig 4. Chromatogram for *Argentum metallicum*

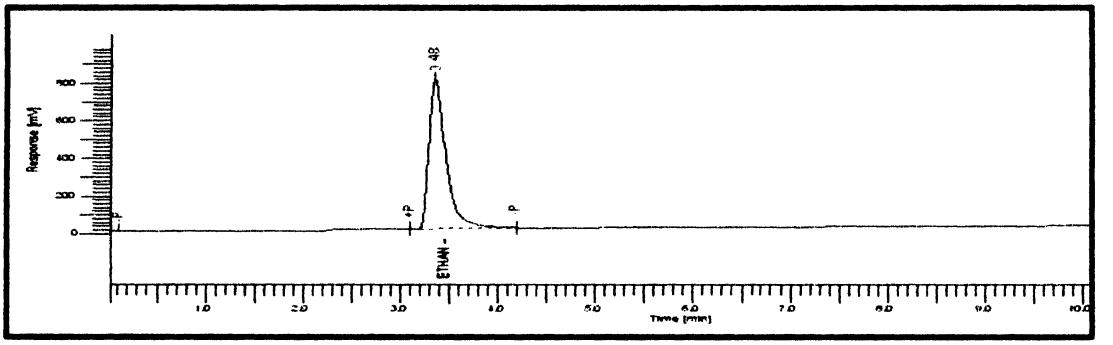

Fig 5. Chromatogram for *Alumen*

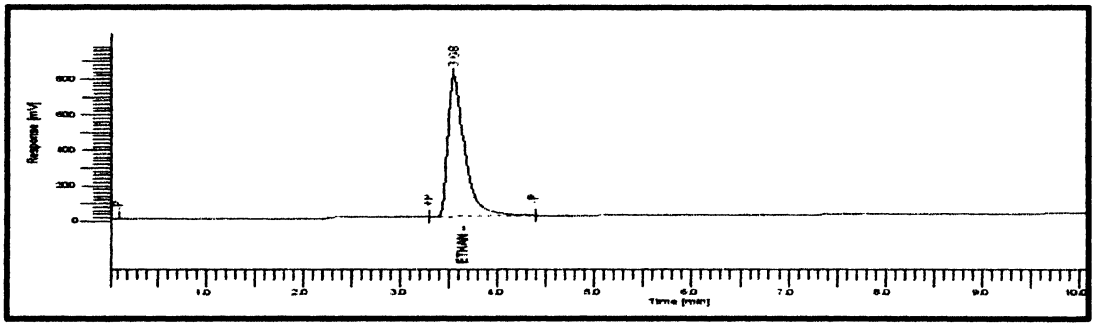

Fig 6. Chromatogram for *Berberis aquifolium*

3.2.1 Chromatograms for test formulations.
See Figures 2-6.

Acceptance criteria: Overall relative standard deviation should not be more than 15.0 %.

3.3 Results for method validation

3.3.1 Specificity

There was no interference of blank with the main peak and no interfering peak from blank at the retention time of analyte peaks.

3.3.2 Precision

See Table 3: Data for Precision study.

3.3.3 Accuracy

See Table 4: Accuracy data.

Acceptance Criteria: Percent recovery at each level and percent mean recovery should be "between" 80-120%.

Table 3
Data for Precision Study

Sample No.	Peak Area (μV)			
	System precision	Method precision	Intermediate Precision	
			Analyst I	**Analyst II**
1	4963829.37	4347596.42	4963829.37	4905486.21
2	4987347.49	4386921.24	4987347.49	4952163.16
3	4913567.37	4396458.89	4913567.37	4923215.56
4	4997453.19	4402589.42	4997453.19	4932568.45
5	4994573.94	4369348.23	4994573.94	4895632.21
6	4938649.26	4356594.56	4938649.26	4889563.54
Mean	**4965903.437**	4376584.793	4965903.437	4916438.188
SD	**33951.1057**	22227.75527	33951.1057	23905.95321
RSD	**0.683684372**	0.507879004	0.683684372	0.486245373
			Overall mean	**4941170.813**
			Overall SD	**28928.52946**
			Overall RSD	**0.584964873**

Acceptance criteria: Overall relative standard deviation should not be more than 15.0 %.

Table 4
Accuracy Data

Level	Amount added	Amount Recovered	Recovery (%)	Mean Recovery (%)
I	50	49.78	99.56	100.26
	50	50.64	101.28	
	50	49.97	99.94	
II	100	98.49	98.49	99.81
	100	101.62	101.62	
	100	99.31	99.31	
III	150	150.14	100.09	99.73
	150	149.63	99.75	
	150	149.02	99.35	
	Overall mean		**99.93**	
	Overall SD		**0.979**	
	Overall RSD		**0.98**	

Acceptance Criteria: %Recovery at each level and % mean recovery should be "between" 80-120%

Table 5
Linearity data for 100% ehtanol solution

Concentration (%)	Area (μV)
0	0
20	959357.59
50	2556763.44
80	4001957.14
100	4909550.25
120	6290833.39
150	7834458.58
200	9710290.61

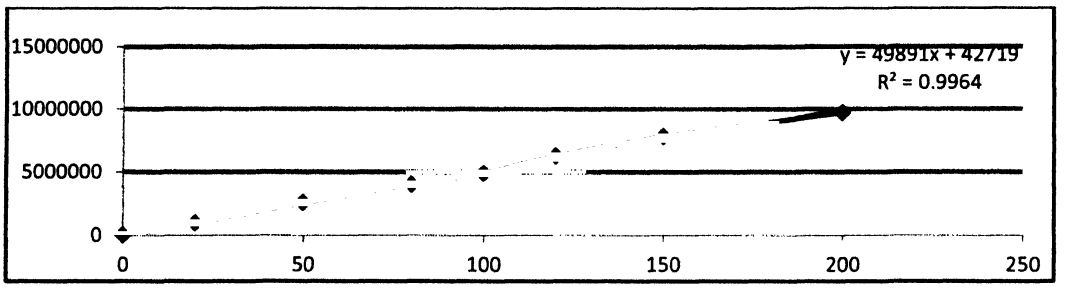

Fig 7. Graph for linearity data of 100% ethanol solution.
The correlation of coefficient was within acceptance criteria; i.e., 0.9964.
Acceptance criteria: The correlation of coefficient should not be less than 0.95.

3.3.4 Linearity
See Table 5: Linearity data for 100% ethanol solution.

4. Conclusion

It was concluded that the percentage of alcohol found in all five homeopathic formulations was within the label claim limit. No large variation in the percentage of alcohol content was found after long storage. All the validation parameters are under the acceptance range and therefore the method is suitable for the routine analysis of alcohol content in the homeopathic formulations.

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