"Gas Chromatography Mass Spectrometry Determination of Alcohol in Herbal Medicines Available to the Herbalists"

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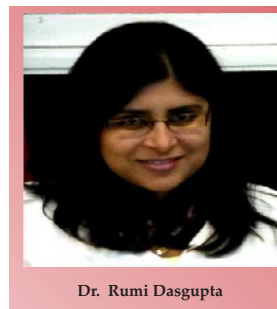University Affiliation: University of East London.

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Abstract

Background: Herbal medicine is the most widely accepted system of medicine used for curing and prevention of various diseases. People all over the globe use herbal medicines. Due to its acceptability by people, as these medicines are made from herbs, the safety, efficacy and clinical trials of herbal medicines are generally not carried out. The herbal tinctures which are prescribed by the qualified herbalists specify the amount of alcohol present in the tincture. Alcohol is used as a solvent for making tinctures. This study aims to analyse the percentage of alcohol present in the herbal tinctures as specified in the labels.

Method: To carry out the analysis gas chromatography with flame ionisation detector has been used with a ZB – Wax Plus wax column. The method is simple and sensitive.

Results: The results suggest that herbal tinctures of Melissa officinalis which claims to have an alcoholic content of 25%, 30%, 40%, 45%, 66% and 70% contains higher amount of ethanol in it than it is prescribed in the labels. It suggests Melissa officinalis containing 66% of alcohol contains 88% of ethanol and 0.09 ppm of methanol in it. The ethanol content is lower than it is prescribed in the labels of the herbal tinctures of Chamomile. A very high amount of methanol, i.e. 1.9 ppm of methanol is present in herbal tincture of Chamomile containing 45% of alcohol.

Conclusion: Due to the presence of different active components in the herbal tinctures, it may be possible that the peaks obtained can be of some of those active components present in the herbal tincture. It is also possible that these active components are overlapping with the peaks of methanol and ethanol. It is difficult to segregate the peaks of active components which are overlapping with that of methanol and ethanol peaks. To confirm it, further analysis is required, which can be carried out by using Gas Chromatography Mass Spectrometer Method or HPLC technique.

Keywords: Herbal medicines, Alcohol, Gas Chromatography, Flame Ionisation Detector.

Thesis Question: Is the ethanol content of the Melissa officinalis and Matricaria reticutita medicines prescribed by the herbalists like the content prescribed in the label?

Thesis Answer: The amount of ethanol varies with tincture preparation and active compounds present. The peaks also suggest the presence of methanol content in the tinctures. The amount higher than 1 ppm suggests that there is a significant amount of methanol present.

THESIS SUMMARY

Introduction

Herbal medicine is the most common and widely accepted system of medicine. It is due to its natural nature. Herbal practioners prescribe these medicines to their patients. Because of its nature, herbal medicines are sold in the market without conducting tests for safety, efficacy and clinical trials, unlike the pharmaceutical drugs[1]. Thus, many plants which are poisonous in nature affect the patient adversely by triggering adverse reactions in the body. Alcohol is used as a solvent for the preparation of herbal medicines. It has been observed that the content and amount of the content is generally not mentioned in the labels of the herbal medicines. The aim of this project is to test the amount of alcohol present in the herbal medicines which is prescribed by the herbalists, mentioned in the labels and to identify the contaminants present in the herbal

medicines, if any, by using gas chromatography flame ionisation detector technique. Herbal medicines which will be used for the analysis are generally prescribed by the herbal practioners and taken by the people. Gas chromatography with flame ionization detector is a definitive technique, hence it will be used to separate present in the herbal medicines along with the percentage of alcohol present in the herbal medicines.

There are several systems of herbal medicine which governs the practice of herbal medicine. They are: Chinese Herbal Medicine or Chinese Herbology, Siddha and Ayurvedic systems from South Asian Countries, Herbal medicine system based on Roman and Greek sources, Unani – Tibb medicine and Shamanic herbalism. There are diverse types of herbal medicines which are used across the globe for the treatment of various diseases. The common types of medicinal uses are herbal teas, herbal tinctures, fluid & solid extracts, poultices and essential oils[2].

Traditionally, herbal medicines are prepared in standardized ways, which varies as per the plants utilized and the condition they are being treated. The standardized methods include infusions, decoctions, tinctures and macerations. These tinctures are prescribed by the herbal practioners to their patients.

Herbal alcoholic tinctures are usually used herbal medicines. Generally, tinctures are easily taken up by the body than water – soluble teas. Due to the presence of alcohol, tinctures can be preserved as compared to herbal teas or water soluble herbal medicines. In general, alcohol is a better solvent than water as the plant constituents dissolve in alcohol more readily than water. Tinctures are the most commonly used methods for treatment. It is a fluid extract prepared from the herbs[1]. A tincture is a water and alcohol extract, which is used when plants have active chemicals which are not very water soluble. The alcoholic percentage helps to determine the shelf – life of a tincture. Alcoholic percentage or content is directly proportional to the shelf – life of tincture. Alcoholic percentage differs from plant to plant as some active compounds have higher affinity for alcohol while some have higher affinity for water. Mostly vodka, rum or 90 – 180 proof grain alcohols is used for the preparation of tinctures[3].

A standard 4:1 tincture indicates 4 parts of liquid and 1 part of plant part used. One year shelf – life tincture can be prepared by using 40% alcohol, i.e. 80 proof vodka or rum without water. Approximately a cup of tincture can be prepared by mixing two ounces of herbs, four ounces of distilled water and four ounces of 180 proof alcohols into a container. Once the mixing is done, the container is sealed and stored at room temperature for about four weeks. After two weeks, the mixture is filtered to remove the plant parts and then pouring the tincture in a cleaned container and sealing it. This technique uses a higher plant to liquid ratio; the tinctures are usually lower than infusions and decoctions.

Herbs depend on its bioavailability factor as they are introduced in the body through topical or oral routes, unlike the pharmaceutical drugs which can be directly introduced in the blood stream[3][4].

Bioavailability plays an important role in delivering the dose of active compounds as it defines the amount of active substances absorbed into the bloodstream after oral doses.

Aims & Objectives

The aim of my project, as a toxicologist, is to analyse the percentage of alcohol present in the herbal medicines available to the herbalists as prescribed in the labels. These herbal medicines are generally prescribed by the qualified herbal practioners to their patients. The analysis will be done by using gas chromatography with flame ionization detector instrument. This technique has been chosen because it is a definitive technique and can easily identify the components present in the herbal medicines[4].

This experiment helped in the analysis of the amount of percentage which is present in the herbal tincture as claimed by the manufacturers and to check if there are any impurities like methanol, iso – butanol, propan – 1 – ol, etc. present. Since tests for safety, efficacy and clinical trials are not carried out for herbal medicines, this experiment is helping us to assess the authenticity of the content which is claimed by the manufacturers.

This was carried out looking for a suitable solvent for the analysis and an internal standard which has a different retention time than methanol and ethanol. An internal standard is required for the comparison of the data and to validate the results. The calibration of methanol and ethanol are then carried out. Methanol calibration was done because methanol is the commonly present impurity and ethanol calibration is done because ethanol is used as a solvent for the herbal extracts or medicines.

Once the calibration was obtained different samples were taken and diluted as per the requirement and were run on GC. The data obtained gave us the peak area and the retention time helped to sort out the methanol, ethanol and the acetonitrile (the internal standard) from the chromatogram obtained after the analysis. Further calculation of the percentage of alcohol present was carried out by comparing the peak area and the calibration curve of methanol and ethanol.

Materials and Methods

Materials which will be used for analysis are the herbal medicines which are generally prescribed by the herbalists. The herbal medicines which are going to be used for this project are Lemon Balm (Melissa officinalis) and Chamomile (Matricaria reticutita). For the analysis, herbal medicines which are generally prescribed by the qualified herbalists have been used[5][6].

Gas chromatography flame ionisation detector is a technique which is used to separate the compounds present in the herbal medicine and identifies the compounds which has been separated at a molecular level[6]. Gas chromatography works on the separation principle where components present in the herbal medicine will get separated into individual substances when excited. The excited gases are carried through a column with an inert gas. As the separated

substances emerge from the column opening, they flow into mass spectrometer. Gas chromatography has many advantages like it can detect volatile compounds that are present in the sample. It requires less time to carry out the analysis and the results are obtained within 1 – 100 minutes. This technique is good for the separation of different components present in the sample. The precision of gas chromatography is very high. The equipment is simple to handle[6][7][8].

This method will be used to determine the alcoholic content of the herbal medicines as it gives accurate results. It is a definitive tool; hence this method is chosen to carry out this experiment. These experiments will help to analyse how much percentage of alcohol the herbal medicine contains and if there is any impurity present. Since tests for safety, efficacy and clinical trials are not conducted for herbal medicines, this experiment will help to assess the authenticity of the information provided in the labels of the herbal medicines and safety of the herbal medicines[9][10][11].

Gas Chromatography Flame Ionisation Detector which has been used for the analysis is from the Agilent Technologies 7890A GC System with a ZB – Wax Plus wax column. The length of the column which has been used was 30 m, the internal diameter was 0.25 mm and the thickness of the film was 0.25 μm. The temperature range was 20 C (minimum) to 250 C (maximum).

To choose a solvent for the dilutions, absolute methanol and ethanol was run by diluting the chemicals in water. Before running the samples, water was run as a blank to eliminate the presence of unwanted or leftover organic compounds. The standards for methanol and ethanol was made by using absolute methanol and ethanol and diluting it to 1:10 ratio for methanol and 1:1000 ratio for ethanol by using distilled water. To make 1:10 dilution for methanol and 1:1000 dilutions for ethanol, following calculations were used:

$$C_1V_1 = C_2V_2$$

Where,

C1= Concentration of the stock
V1= Volume of the stock
C2= Concentration of the solution
V2= Volume of the solution

Different concentrations were made by using the above diluted solutions. To it the 1:10 diluted acetonitrile was added as acetonitrile was used as an internal standard. The standards were then run and readings were recorded.

The samples of medicines with different alcoholic content were then taken and a dilution of 1:10 ratio and 1:1000 ratio dilutions were made for methanol and ethanol analysis respectively. Then the samples were centrifuged to get a clear solution as particulate matters were present. After centrifuging the internal standard, i.e. acetonitrile was added and then the samples were run. The observations were then recorded.

A range of concentration ranging from 0 – 5 ppm were made for 1:10 dilution of methanol calibration curve and 0 – 100% were made for 1:1000 dilution of ethanol calibration curve. The analysis will be carried out by comparing the concentrations in the calibration curve to determine the alcoholic content of the herbal medicines[10][11]. Acetonitrile was used as an internal standard as the retention time of acetonitrile is different from that of methanol & ethanol and it does not overlap with the retention time of methanol and ethanol[4][12][13][14]. A 1:10 dilution of acetonitrile was used for the analysis of the samples. 500 μl of acetonitrile was taken in a 5-ml volumetric flask and then distilled water was added till the mark to make 5 ml of 1:10 dilution of acetonitrile solution[4][5][15][16]. The results in Table 1 give us the calibration for methanol and results in Table 2 is for the calibration of ethanol. The calibration was carried out by making a dilution of 1:10 for methanol and 1:1000 for ethanol. A dilution of 1:10 was carried out for methanol because the concentration of methanol in the solution is present in trace amounts. To detect those trace amounts of methanol a 1:10 dilution was carried out for methanol. Ethanol, on the other hand, gives a very sharp peak and hence a dilution of 1:1000 was carried out for ethanol.

Results

The retention time of acetonitrile was 7.16 mins, ethanol was 5.82 mins and methanol was 4.89 mins. Once the chromatograms were obtained integration method was used for the quantitation or the values of the peaks. The parameters were then set.

Once the parameters were set the APPLY option was clicked and then OK was clicked. This gave the retention time of the peaks and the peak areas which helped in the determination of the content of the alcohol present in the herbal tincture samples.

The results were obtained from the calibration curve. The results of the analysis are as follows:

1. Ethanol Content of Melissa officinalis: (Table 1)

CONCENTRATION AS PRESCRIBED	ETHANOL CONTENT (%)
25% Melissa	52
30% Melissa	48
40% Melissa	72
45% Melissa	82
66% Melissa	88
70% Melissa	79

2. Methanol Content of Melissa officinalis: (Table 2)

CONCENTRATION AS PRESCRIBED	METHANOL CONTENT (ppm)
25% Melissa	0.1
30% Melissa	0.3
40% Melissa	0.1
45% Melissa	1.7
66% Melissa	0.09

70% Melissa   0.2

3. Ethanol Content of Chamomile: (Table 3)
CONCENTRATION AS PRESCRIBED   ETHANOL CONTENT (%)
45% Chamomile   34
60% Chamomile   2
66% Chamomile   18

4. Methanol Content of Chamomile: (Table 4)
CONCENTRATION AS PRESCRIBED   METHANOL CONTENT (ppm)
45% Chamomile   1.9
60% Chamomile   0.6
66% Chamomile   0.1.

Observations

The standard calibration curve for ethanol suggests that there may be some error while preparing the concentrations. The calibration curve of methanol shows a straight line with $R^2 = 0.990$. It suggests that the preparation of concentrations of methanol calibration curve was fine with not much error. The standard calibration curve is made by diluting the sample with water.

The results in Table 1 suggest that herbal tinctures of Melissa officinalis which claims to have an alcoholic content of 25%, 30%, 40%, 45%, 66% and 70% contains higher amount of ethanol in it than it is prescribed in the labels. This suggests either there is a more number of active components of plants are present for which more ethanol has been used to make it soluble or maybe there was some fault in the Gas Chromatography instrumentation. The above table suggests Melissa officinalis containing 66% of alcohol contains 88% of ethanol and 0.09 ppm of methanol in it.

The percentage of alcohol present in the sample of Melissa officinalis against response or ethanol peak area with ethanol content is 48% - 88% suggests that ethanol content is more in the herbal tincture of Melissa officinalis with 66% of alcohol (88%). The herbal tincture with 30% of alcohol has lesser ethanol content (48%) as compared to the others, though the overall results show increased ethanol content in the tinctures with different alcoholic content. Figure 4 shows the chromatogram of ethanol content in the sample of Melissa officinalis with 66% of alcohol content as prescribed in its label.

The comparison of the methanol content in different samples of Melissa officinalis with methanol content range lies between 0.07 – 0.3 ppm suggests that the tincture with 70% alcohol has more amount of methanol (0.3 ppm) in it and the tincture with 25% of alcohol has the lowest methanol content (0.07 ppm).

The percentage of alcohol present in the sample of Chamomile in a range of ethanol content in Chamomile lies between 18 – 34%. It suggests that the tincture with 45% of alcohol has more ethanol content (34%) and the tincture with 66% of alcohol has fewer amounts (i.e. 18%) of ethanol in it.

The percentage of alcohol present in the sample of Chamomile against the methanol peak area with methanol content ranges between 0.1 – 1.9 ppm suggests that the tincture with 45% of alcohol has 1.9 ppm of methanol and the tincture of Chamomile with 66% of alcohol contains 0.1 ppm of methanol.

It also suggest that the ethanol content is lower than it is prescribed in the labels of the herbal tinctures of Chamomile. This suggests that maybe the medicine has less active compounds present in it and has a placebo effect on the patients or due to the breakdown of Gas Chromatography Instrumentation the results obtained were not accurate. A very high amount of methanol, i.e. 1.9 ppm of methanol is present in herbal tincture of Chamomile containing 45% of alcohol. Generally, 1 ppm is the maximum level till which methanol can be present. The amount higher than 1 ppm suggests that there is a significant amount of methanol is present. Methanol is a common adulterant present in the herbal tinctures, as tinctures contain alcohol as a solvent.

Conclusion

The accuracy of the results is not very high. It is due to the breakdown of the instrument and maybe error was there while making the dilutions. The calibration curve was carried out without the addition of the internal standard. The second sets of reading could not be taken due to the breakdown of the instrumentation. A further analysis is required to confirm the results and identify the different peaks that were found during the analysis as Gas Chromatography Flame Ionisation Technique detects any organic components present in the sample but a Mass Spectrometer or HPLC system is required for the identification of all the organic compounds detected by the Gas Chromatography.

Further work needs to be done for the confirmation of the results. HPLC and/or a Mass Spectrometer can be used to identify the different components of the herbal tinctures. Since the Gas Chromatography Flame Ionisation Detector cannot reveal the molecular formula of the components, hence one can be never be sure if the peak is for methanol and ethanol exclusively. Due to the presence of different active components in the herbal tinctures, it may be possible that the peaks obtained can be of some of those active components present in the herbal tincture. It is also possible that these active components are overlapping with the peaks of methanol and ethanol. It is difficult to segregate the peaks of active components which are overlapping with that of methanol and ethanol peaks. To confirm it, further analysis is required, which can be carried out by using Gas Chromatography Mass Spectrometer Method or HPLC technique.

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