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Research Article

Investigating the Factors Having the Important Role in the Standardization of Herbal Distillates' Methanol Content

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Abstract

Blurred vision leading to nervous blindness is the only symptom of chronic methanol intoxication that occurs following prolonged drinking some kinds of herbal distillates and has been motivated serious anxieties. The methanol content of these products is not usually measured which may be due to the lack of applicable standards in this regard. In this study, the methanol concentrations of some kinds of herbal distillates were quantified by two different methods to define the most effective factors in the determination of methanol permitted dose of these products. In this study, the methanol contents of 60 different samples were examined by a newly designed kit and Gas Chromatography methods, and finally, based on gained results some recommendations were presented about how standardization of herbal distillates' methanol content. All samples have different concentrations of methanol that in some cases, their methanol content seems enough for the creation of chronic methanol intoxication. Also, access to an appropriate and efficient national standard for controlling of methanol content of herbal distillates seems to be necessary.

Keywords: Chromotropic acid; Herbal distillates; Methanol; Methanol concentration; Methanol intoxication

Introduction

Methanol is a colorless alcohol with a characteristic smell that has many extensive industrial and household uses [1]. Land plants' growth and their biologic activities have the most contribution to methanol production in nature [2-7]. This process is mostly formed by degradation of the pectic substances of the middle lamella of plant cells. Pectin degradation is one of the essential stages of plants' cell walls development during their growth [7-9]. The plants' cell walls are distinguished into two types based on their own molecular weight that each of them has different potential for methanol production [8,10]. In the presence of Pectin Methylesterase Enzyme (PME), high methoxyl pectin is converted into low one by releasing methanol, that in fruits and vegetables is accompanied with the softness of their cell walls (ripening) [7,11,12]. This alcohol can also produce from enzymatic cleavage of lignin, demethylation of DNA and protein repair pathways [8,10]. Likewise, all physical and biological stresses can increase its production in plant [2,6,7,13-16]. Therefore, it has many physiologic roles in plant biology and so, its presence in plants and their products is fully logical and expected.

According to accepted standards, the presence of 120-460 mgL^{-1} (with mean 140mgL^{-1}) methanol in kinds of fresh and canned juices is naturally permitted [17-21]. So, its existence in herbal distillates shows their reality [22]. Herbal distillates are usually colorless liquids and obtained from the condensed steam of different parts of medicinal plants. They usually consist of water as the main factor, different essences, drugs, and organic compounds, and are frequently being used for different purposes in food regimen of some countries. There are many reports about the presence of high levels of methanol in these products. Prolonged drinking of such drinks can lead to chronic type of methanol intoxication. So, measuring and decreasing of methanol in them is recently considered [18-22]. The only reported symptom of this type of intoxication is apparently the blurred vision leading to nervous blindness [22].

Unfortunately, despite the extended usage of herbal distillates in Iran, there is not any applicable standard about methanol allowable concentration in them. This may be due to the lack of necessary standards and access to an inexpensive and simple diagnostic method in this regard [18-20,22]. Direct detection or determination of methanol needs advanced and very expensive apparatus (usually gas chromatography or GC), high technical knowledge or experience. Sometimes, the difficult and troublesome of techniques of sample preparation make their usage impossible in common labs [7,23-28]. Therefore, access to a cheap and sensitive method along with the needed standards for the quality control of the methanol content of the herbal distillates seems to be necessary.

Methanol's oxidization is lead to formaldehyde formation that can be determined using different chemical methods such as Chromotropic Acid (CA) one [29]. This method has been recommended as a reference method to measure methanol in alcoholic drinks by Association of Official Analytical Chemists (AOAC) [30]. This method has three steps. Methanol firstly oxidizes into formaldehyde and then, formic acid by potassium permanganate in acidic medium. In the next step, the violet color of the excess Manganese (Mn) is faded by sodium hydrogen sulfite through transforming violet $Mn7^{+}$ to colorless $Mn2^{+}$ to see the possible positive result. At the final step, the formic acid is reduced to formaldehyde

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to react with CA in hot concentrated sulfuric acid vicinity that is accompanied by the appearance of the violet complex. The intensity of appeared color depends on the samples methanol level that can be determined at 575nm.

Recently, an Iranian company has produced two qualitative and quantitative diagnostic kits with easy application based on this method to determine and measure the methanol content of herbal distillates that the efficacy of them has been proven in previous studies [18-20,22]. Therefore, the formulation of a suitable standard regarding the permitted dose of methanol in these products can be effective for their control at this time. So, the aim of the current study was the quantification of methanol in some kinds of herbal distillates by two chemical and GC methods to introduce the factors having an important role in the standardization of their methanol content.

Materials and Methods

In this study, the methanol contents of 60 different samples (ten kinds of herbal distillates from six diverse companies) were examined by both chemical (kit) and GC methods, and finally, the obtained results were used to investigate the principles must be considered for their standardization.

Apparatus

A Gas Chromatography (GC) device (YL 6100 GC model, South Korea) was applied to determine of methanol. The GC system equipped with a Flame Ionization Detector (FID) and TR-25, capillary column (30m×0.53mm×1.5µm). A 10µL Hamilton syringe (made by Australia) was used to inject samples. Also, a spectrophotometer (6405 UV/VIS Jenway, England) is used for performance of tests by chemical kit.

Chemicals

The needed methanol and ethanol for preparation of standard solutions for GC method was prepared with analytical grade from Merck (Darmstadt, Germany) and used without further purification. A newly designed (by the author) kit that has been produced by an Iranian company (Arya Mabna Tashkhis Co., Tehran, Iran) was used in the chemical method of methanol measuring. This kit contains five reactants (shown by A, B, C, D and E), five methanol standards with 0, 12.5, 25, 50 and 100 mgL^{-1} concentrations and an instruction brochure available in the pack. Also, the 60 different industrial herbal distillates (*Mentha spicata L.*, *Mentha pulegium L.*, *Urtica dioica L.*, *Trigonella foenum-graecum L.*, *Cichorium intybus L.*, *Alhagi maurorum L.*, *Anethum graveolens L.*, *Rosa damascena L.*, *Citrus aurantium L.*, and *Salix aegyptiaca L.*) were prepared from different local commercial stores (Rasht, Gilan province, Iran). Their producing and expiring dates were nearly close together (maximum one-month) whereas, the difference was nearly up to five months in products of all different companies. These samples were known selected based on some specific characteristics (such as origin tissue, amount and circumstances of using, etc.) to better interpret the gained results and the needed basics are discussed more easily. Also, deionized double Distillated Water (DW) was used for preparatin of all standards solutions and dilution of the samples.

Preparation of samples and Standard solutions

Five standard solutions containing 0, 12.5, 25, 50 and 100 mgL^{-1} of methanol with 100mgL^{-1} ethanol (as the internal standard) was prepared by a serial method to use in GC method. For determination of samples' methanol by GC, some ethanol solution was added into 10mL of each sample to obtain 100mgL^{-1} concentration of it. Whereas, for chemical method, one volume of each sample (triplicate) was diluted with four volume of DW (1:4 ratio) to obtain 1:5 dilution ratio to examine as double.

The kit procedure

According the kit brochure, 0.2mL of each standard and all diluted samples were poured into separated previously labeled test tubes with 50µL of reactants A and B (sulfuric acid and potassium permanganate solutions, respectively) and well shaken. After five minutes, reactant C (sodium hydrogen sulfite) was added and the mixture was shaken hardly until became colorless. 50µL of reactant D (CA solution) and one mL of reactant E (concentrated sulfuric acid) were then added to the tubes and shaken. After spontaneously cooling down at room temperature, their absorbance was read at 575nm in contrast of DW blank. Finally, the methanol content of samples was computed in comparison to the standard curve by multiplication of the result into the dilution factor (five).

GC procedure

Helium carrier gas (at a linear 6ml min^{-1} rate) was used for methanol separation. All standards and samples were directly injected (2µL with 1:20 split ratio) to GC apparatus as triplicate at 80°C column oven temperature (isothermal condition). The injector port and detector temperatures were 200 and 300°C, respectively. Then, the obtained results were corrected based on internal standard peak and finally, the average of three replicate results were used as last results for next calculations.

Results

Methanol content of all samples was determined using both GC and chemical methods. The results are shown in Table 1. According to the kit manufacturer's claim (in the brochure), the kit limit of quantification (LOQ) is 7mgL^{-1} in herbal distillates. As it is seen in Table 1, all samples had methanol more than the mentioned LOQ.

Discussion

Due to the important roles of methanol in land plant physiology, its existence in all herbal distillates is expected and shows their reality [2,6,7,13-16]. The occurrence of blured vision leading to nervous blindness as a consequence of prolonged drinking some kinds of herbal distillates and existence of varying concentrations of methanol in them has been provoked serious anxieties [21,22,35]. Due to methanol hazardous effects on the human body, its determination in these products should be enforced as a major step of quality control in their production process. This measurement had not been possible due to the lack of required standards and the inaccessibility to easier and less complicated techniques [18-20,22]. Full conformity of our results (Table 1) with the previous ones [19,22] indicates the used kit efficacy (accuracy and precision) in this regard and as a result, developing the necessary standards about methanol content of herbal distillates seems to be a necessity.

All of our examined samples had different amounts of methanol and from this point of view, the obtained results in this study is

Table 1: The comparison gained results of kit and GC methods. The results were shown based on mg L^{-1}.

Name of herbal distillate	Method	A	B	C	D	E	F	Mean	Difference
Mentha Spicata L.	GC	247	285	313	417	272	231	294	5
	kit	259	271	329	401	260	216	289	
Mentha pulegium L.	GC	177	199	185	249	250	228	215	1
	kit	178	198	191	245	243	232	214	
Urtica dioica L.	GC	216	478	313	268	296	204	296	2
	kit	218	461	324	255	283	222	294	
Trigonella foenum-graecum L.	GC	248	255	251	249	229	236	245	2
	kit	236	265	244	254	233	225	243	
Cichorium intybus L.	GC	213	188	163	223	214	265	209	13
	kit	222	202	179	231	219	281	222	
Alhagi maurorum L.	GC	371	283	261	485	292	91	297	5
	kit	355	278	255	491	287	86	292	
Anethum graveolens L.	GC	55	355	220	696	395	126	308	2
	kit	57	356	232	679	399	139	310	
Rosa damascena L.	GC	70	115	59	99	86	65	82	4
	kit	79	119	62	95	91	68	86	
Citrus aurantium L.	GC	211	178	191	204	221	200	201	3
	kit	213	186	193	212	218	203	204	
Salix aegyptiaca L.	GC	201	213	189	194	195	222	202	1
	kit	193	218	201	187	192	229	203	

fully similar with previous reports [18-22]. As it is visible in table 1, the methanol content of a particular herbal distillate produced by different companies may be different together that it can be due to the lack of the needed standards to control methanol in these products [22]. Perhaps, rose water (*Rosa damascena L.* distillate) is the only herbal distillate that has an applicable national standard (including methanol content) from far years back in Iran that is probably due to its religious usages. Some efforts have been recently use this standard for other kinds of herbal distillate that cannot has practically benefit. In the following, it will be discussed that various factors (including origin tissue for preparation of distillate, genetic and race differences, climate and weather conditions, age of the used plant, applied distillation method, etc.) affect the amount of methanol in a herbal distillate and in this regard, some recommendations will be presented for defining the admissible dose of methanol in these products.

As it is visible in Table 1, the mean of methanol content of rose water samples in our study was compatible with the national standard of Iran (less than 100mgL^{-1}). Rose water is prepared from an especial genus of Rose family flowers' petals (un-photosynthetic section of flower) and so, the presence of very low amounts of methanol in it is quite predictable. In the industrial production process of rose water, huge amounts of the flower with their sepal and Peduncle (the green and photosynthetic sections of flower) are poured into large distillation tanks. The most part of a rose flower consists of large petals, and green tissues (sepal and Peduncle) are formed a very small part of it and this can justify the presence of small concentrations of methanol in this product. On the other hand, for promotion of productivity and increase of final product's quality, the pickup of the flowers is usually done after 48 hours of dehydration (stress) that is accompanied by the increase of methanol production in the plant [2,6,7,13-16]. But, the methanol level of the B company sample (Table 1) was more than the allowable dose and is not expectable and more investigation is required for final judgment.

Existence of more methanol contents in two other examined flowers' distillates (orange blossom/*Citrus aurantium L.* and pussy willow/*Salix aegyptiaca L.*) (Table 1) was expected and is probably due to their flower structures. In contrast rose flower, in the orange blossom and pussy willow flowers, the number and cross-section of petals are very limited and small. Also, their sepals and Peduncles and even the ovaries have been enlarged and intensified, which leads to the presence of relatively more amounts of green and photosynthetic tissues containing methanol in the mass of flowers used in the distillation process. Therefore, existence more methanol in their distillates than the rose water is awaited [22].

The *Mentha spicata L.* (mint), *Mentha pulegium L.* (pennyroyal), *Urtica dioica L.* (Nettle), *Trigonella foenum-graecum L.* (Fenugreek), *Cichorium intybus L.* (chicory), *Alhagi maurorum L.* (camel thorn) and *Anethum graveolens L.* (dill) distillates are prepared from their aerial green parts (frequently green leaves and stems) and occasionally root or mix of them (like chicory). The aerial organs have higher amounts of methanol and this increases the amount of methanol in the final product (Table 1). However, other factors also affect their methanol contents.

For example, different amounts of methanol are found in distillates produced from different parts of a unique chicory plant. Because, in addition to the difference in the pectin structure of various plant species, the pectin content of each tissue of plant is diverse too. Therefore, each plant can have an exclusive pectin structure with different compositions of it in its diverse parts and tissues and in this regard, the difference in the production of methanol by different tissues of a plant is justified [18,19,22,31]. For example, the production rate of methanol in the root is far less than the green leaves and stems [32-34]. So, because of the intense photosynthetic activity, the methanol concentration of distillates produced exclusively from green sections of the plant (like mint) is usually very high [17-22,35], While the low production of methanol in flowers depends on their cell walls' pectin degradation.

Likewise, the genetic, racial differences and many other possible variables can be affected in methanol content of plants and consequently, their products. For example, Fenugreek belongs to the Fabaceae family that coexists with nitrogen stabilization bacteria and naturally has a high metabolic rate and hence produces more methanol than other plants. Or, temperature and climatic conditions can influence the plant growth and methanol storage [8,22,32,36], which is well recognizable in comparison of gained results of camel

thorn and Fenugreek distillates (Table 1). Although both of them are members of Fabaceae family, camel thorn mostly grows in the desert and even saline lands and prolonged dehydration (stress) is naturally associated with increased methanol concentration than another family member (Fenugreek) which is specific for mild regions [22]. So, the existence of difference in methanol content of a specific herbal distillate prepared from a unique plant can be due to their particular climatic regions [12,22].

The age of plant is also the other affecting factor in the methanol content of herbal distillate, because young and growing tissues have more methanol than the others (like dill) [2,6,7,13-16]. For these reasons, the confirmation of A and F companies dill and camel thorn (respectively) distillate's methanol content (Table 1) requires further investigation.

Likewise, the methanol content of plants with thicker wooden beds (like mint and pennyroyal) is more than herbaceous plants. In this case, the more activity of intra-cellular PME enzyme and consequently, more autolysis of plant tissues' cell walls are the source of more production and storage of methanol. The technical factors of the production process also can increase methanol content of the product.

In this regard, the methanol concentration of herbal distillates can be considered an important factor in assessing the originality of a distillate. So, the methanol content of a real herbal distillate should never be zero. However, the maximum decreasing of methanol in these products is desired. Despite the much heterogeneity and differences in methanol production and storage capacity in different plants, how we can use a constant amount of methanol as the allowable dose in all herbal distillates?

Perhaps paying attention to the pattern of herbal distillates' consumption is more important than their methanol content in defining allowable methanol concentration because drinking of only some kinds of these products practically causes blurred vision leading to nervous blindness. So, for better interpretation and a more accurate conclusion in this study, herbal distillates have been divided into three groups according to their type of use.

The first group of them includes kinds of distillates prepared from flowers. Some of them have naturally low amounts of methanol (rose water) and some other has more concentrations of it (orange blossom and pussy willow). Except for spraying some of them (especially rose water) to the air or wash the surfaces in some religious ceremonies, all of them are used in occasional as only aromatic or flavoring products in very low volume directly or mixed in water, juices, and syrups. Also, they may be used in making some types of foods and candies. In all these cases, heat and dilution hardly decrease their methanol content and intoxication possibility. Therefore, regardless of their methanol content, none of them are practically hazardous or able to cause intoxication.

The second group of distillates is prepared from green leaves and stems. So, the existence of huge concentrations of methanol in them is expected (mint and pennyroyal distillates). They are occasionally drunk for their therapeutic properties in low volume as directly or diluted with water or syrups. Therefore, although their prolonged and direct drinking can theoretically lead to the chronic type of methanol intoxication, their usual consumption pattern protects the consumers from it.

The third group of distillates (like dill, Nettle, Fenugreek, chicory and camel thorn) is usually prepared from green leaves, stems and the aerial organs of the plant. These organs have high concentrations of methanol. On the other hand, dill and Nettle are always green and growing. Or, camel thorn is special for desert and tropical regions. Hence they have physiologically high reserves of methanol in their tissues. But their consumption pattern is the most important factor in the creation of chronic methanol intoxication because large volumes of them should be daily consumed in a long period of time (from weeks to several months) to gain therapeutic effects. So, they can potentially cause the chronic type of methanol intoxication and are harmful.

Since a national standard always covers the minimum quality of a product, the application of a unique concentration of methanol as a permitted dose for all kinds of herbal distillates not only has not any benefit but also can fade the producers' facility. Because reducing methanol in these products requires a lot of money and time. Apparently, the first and second groups of mentioned distillates can have a higher permitted dose of methanol than the third group's distillates and it has not any conflict with public health. However, factory standards can play an important role in promoting the quality of each company's product. But, the third group of distillates is potentially dangerous, so their allowable concentration of methanol (national standard) should be at the least possible. However, achieving such a level of quality is very difficult and high-cost.

Conclusion

The obtained results have shown, all examined herbal distillates have different concentrations of methanol that in some cases, can cause to create chronic methanol intoxication. Also, it seems, the creation and development of an appropriate and efficient national standard for controlling of methanol content in them require the interdisciplinary co-operation of different specialists. Because this matter should be considered from different aspects to ensure the health of consumers of such products.

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