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Rapid Determination of Residual Ethanol in Perfumery Products Using Headspace Gas Chromatography-Mass Spectrometry

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Abstract: The aim of this study is to determine the alcohol residue in perfumery products claimed to be alcohol-free using the automated headspace gas chromatography-mass spectrometry (HS-GC-MS) method. This method successfully determined the residual ethanol in 26 perfumery products to be in the range of 7.31- 611.64 µg/mL. The linear concentration range is 0.2-1000 ppm and the correlation coefficient is relatively good (R^2=0.998). The results show that the method has an excellent measurement precision (RSD < 3.8%) and accuracy recovery of 97.3 ± 3.7%. The analysis requires no sample pre-treatment and the method is very simple, reliable and rapid. This method provides an invaluable analytical tool for the quality control of perfumery products and can be used in routine analysis.

Key words: Ethanol · Headspace GC-MS · Perfume · Validation

INTRODUCTION

Ethanol is widely used as a solvent in all kinds of household, personal care and pharmaceutical products with direct exposure to the human skin. These products include cosmetics, skin cares, perfumes, mouthwashes, hair spray products and pharmaceutical preparations [1, 2]. Perfumes contain many ingredients such as water and fragrances, with ethanol comprising the highest percentage of 50-80%. Ethanol containing fragrances are the most common vehicles used in preparing fine perfumes. It has the advantage of fast drying rate and provides a strong lift of the fragrance after its application to the skin [3]. Besides being used as a solvent, ethanol is also used as an anti-bacterial agent [1]. The antimicrobial effect of ethanol is based on protein denaturation [4] and thus has excellent bactericidal and fungicidal activity [5]. During the topical application of ethanol, the most prone organ for the adverse effect seems to be the skin. Hence, the disadvantages of ethanol are it will dry out the skin, one of the causes of cutaneous intolerance or allergic contact dermatitis which cause skin irritation and inflammation [6, 7].

Ethanol is often purposely made poisonous by the addition of methanol, where it is known as SDA (Specially Denatured Alcohol). Many consumers are concerned about the usage of these alcohols in products such as in perfumes. Ethanol may cause the acne to inflame as it dries out the skin's top layers, which inhibit the skin's natural ability to shed skin properly and keep the pores open, leading to more clogged pores. In general, it has been proven to cause allergic contact dermatitis. However, scientific studies indicate that topical use is safe *per se* [1, 8, 9].

There is a worldwide incentive to reduce the use of volatile organic chemical (VOC) such as ethanol in perfume products and fragrances and instead develop water based or non-VOC fragrances and perfumes [3]. The replacement of ethanol based product is considered as a step forward in this direction. The advantage of the new formulation is elimination of the problems caused by VOC restrictions imposed by many countries, objections by environmental groups, consumer concern on infant safety and objections to alcohol in some countries based on religious ground [3]. In most Islamic countries, the consumers are concerned on the usage of ethanol in their

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perfume products. They prefer to buy products with label stating 'alcohol free' or 'no alcohol'. In fact, in Muslim countries such as Middle East, Malaysia and Indonesia, Muslim consumers demand for these products. Hence, it is important to develop fast and reliable methods to detect ethanol content residue in these products and at the same time, consumer would like to know the ethanol content in the perfume products available in the market which carry this claim. At the moment, there is no minimum level of ethanol residue set for these 'alcohol free' products.

For alcohol determination, accurate and reasonably rapid ethanol detection is the most important analytical procedure. The determination is required for quality control and investigations of products produced. Nowadays, gas chromatography (GC) is a common and preferred method used to detect alcohol in products [10, 11, 12]. GC is widely used in determining alcohol in alcoholic beverages [11] and blood samples [13, 14, 15] because it has excellent selectivity with capillary columns of various chemical film coating and good sensitivity with flame ionization detection (FID) or mass spectrometry (MS) [10].

Headspace gas chromatography (HS-GC) is an effective technique for determining volatile compounds in samples [10]. The principle of this method is based on sampling the equilibrated vapor phase (headspace) above the liquid or solid sample in a closed vial and quantifying the volatile compounds in the headspace by GC [16]. This method has several advantages whereby the impact and the interference of non-volatile compound in the sample during the determination of the volatile compound of interest can be reduced significantly [17], extending column life and preventing injector contamination [18].

This research aims to develop a sensitive, reliable, easy to use and rapid procedure for the determination of alcohol residue in perfumery products claimed to be alcohol-free using the HS-GC-MS method and to evaluate the ethanol content in these products. To our knowledge, no study on the detection of ethanol residue in alcohol-free perfume products has been carried out. The precision and accuracy for ethanol quantification by the proposed method were also evaluated.

Experimental

Materials and Reagents: Perfumes were purchased from supermarkets and departmental stores located in Kuala Lumpur and Putra Jaya, Malaysia. Fragrances and perfume compounds were obtained from local suppliers. All chemicals used were reagent grade. The internal standard used was acetonitrile (ACN) and external standard was ethanol. Both were obtained from Merck (Darmstadt, Germany).

Instrumentation and Chromatographic Condition: All identification was accomplished using Agilent 7890A GC, interfaced to Agilent 5975C Mass Spectrometer, equipped with headspace sampler (Agilent G1888). The GC capillary column was DB Wax (0.25mm x 0.25µm x 30m). The carrier gas and make-up gas were compressed helium. Processing and interpretation of mass spectra were carried out with Enhanced MSD Chemstation with the Wiley mass spectral library as well via the NIST AMDIS software using the NIST mass spectral search program for the NIST/EPA/NIH mass spectral library.

HS-GC Conditions: The initial gas flow was 1 ml/min constant. During the thermal desorption process, the inlet temperature was set at 250°C with a sample split ratio of 5:1 to reduce the sample volume introduced into the GC column. The column oven temperature was initially set at 50°C and ramped to a temperature of 220°C at 50 C/min and held at 220°C for 5 min (total analysis time 10 min). The equilibrium temperature and time were 80 °C and 10 min. The temperature of the sample loop and the transfer line were 90°C and 100°C, respectively. The injection time was 1 min and the loop fill and equilibration were 0.5 min and 0.2 min, respectively. The vial pressure was 15 psi. During the heating process, high shaking of the HS vial was applied. The Agilent 5975C MSD was operated in the Electron Impact Mode (EI) and scanned over the mass range of 35 to 450 Dalton.

Validation Method

Linearity: An aqueous stock solution of 500 mg/mL of ethanol was prepared. Linearity was verified by analyzing 11 standard ethanol solutions from 0.2 to 1000 µg/mL, which were prepared by stepwise dilution of stock solution. An internal standard solution of acetonitrile (ACN) at concentration of 1000 µg/mL was added into the standard and samples to improve the injection precision. Ethanol in the sample is measured by the area ratios of ethanol to ACN. The volume of sample, standard and recovery used in this study is 0.5 mL.

Limit of Detection/ Limit of Quantification (Lod/loq) Based on Standard Deviation (Sd) of the Blank: LOQ is quantified by measuring the variability of the background response directly [21]. Five replicate numbers of blank

samples (without ethanol) were measured and SD of these measurements was calculated. The area under the ethanol was integrated. The LOD and LOQ formula determined based on the SD of the blank response are presented below:

LOQ = (10 x SD of blank response)/ slope of the curve
LOD = (3.3 x SD of blank response)/ slope of the curve

Precision: Precision of the method was evaluated by the degree of consistency between concentrations of ethanol in perfumery products (n=5).

Accuracy: The accuracy of the method was carried out by determining the ethanol recovery. The recoveries were determined by adding ethanol concentrations of 25, 50, 100, 500, 750 and 1000 µg/mL into perfume sample followed by ACN with a final concentration of 1000 ig/mL. All measurements were carried out in triplicates.

Statistical Analysis: The concentration averages and standard deviations were calculated for ethanol in each sample. Statistics were performed with Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Equilibration Time for Headspace Condition: To determine the equilibration time to the ethanol mass transfer from a perfume sample, the equilibration temperature used was 80°C. We used 0.5mL of sample in this study. Larger sample volume is helpful in increasing the sensitivity in the headspace measurement [10]. However it was difficult to get full evaporation of the volatile components even though the temperature of the headspace used was high. Some of the volatile components were still trapped in the condensate phase in the headspace vials. As shown in Figure 1, the amount of ethanol released from the perfume sample reached almost plateau within 10 min. Hence, we used 10 min equilibration time as the value for our headspace condition.

Ethanol Separation in the Headspace Analysis on a Perfumery Product: Figure 2 shows a GC chromatogram from HS analysis on a perfumery product, in which ethanol was found in the vapor phase. Within less than 4 min, the ethanol and ACN were clearly detected with a sharp peak chromatogram and good separation from the other volatile components under the specified GC

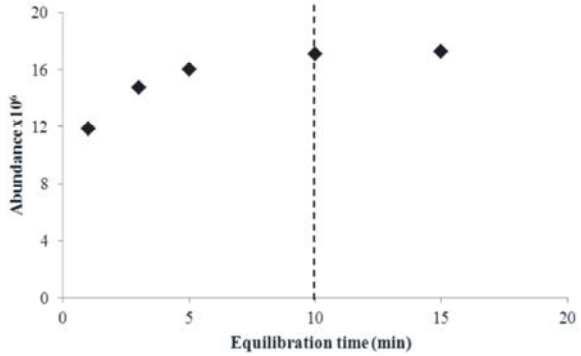

Fig. 1: The amount of ethanol released from the perfume sample within 10 min. Retention time (min)

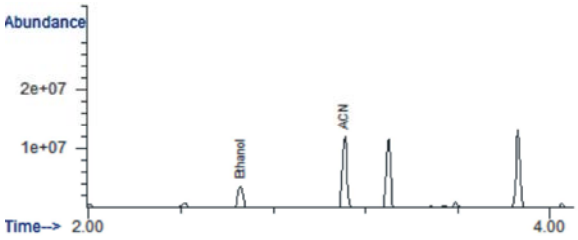

Fig. 2: GC chromatogram from HS analysis on a perfumery product.

conditions. The retention time for ethanol and ACN revealed by this method was at 2.659 and 3.112 min, respectively. For the column cleaning, another 3 min was allowed to evaporate all the volatiles components in the column. Thus, the HS-GC method was able to eliminate the interference from the non-volatile and volatile components found in the perfumery product samples.

The usage of the mass spectrometer detection is an added value as it combines the relative mobility of a substance in a carrier gas with the relative atomic mass and compares those values to a series of standards. It compares the polarity and size of molecules to standards to determine the ethanol species present [18]. GC-MS provides unequivocal qualitative analysis for ethanol from its three major mass fragments namely *m/z* 31 (base peak), 45 and 46 molecular ion [19]. Another study identified ethanol's ion at *m/z* 31, 45 and 29, while ACN (internal standard) was represented by ions *m/z* 39, 40 and 41 [15]. Quantification of ethanol in perfumery products was carried out using ion 45, because this ion demonstrated sharp peak, less tailing and fronting phenomena. Similar quantification at the same ion was conducted by [18]. In this study, the ethanol ions were represented by ions *m/z* 31, 45 and 46, while ACN by ions 39, 40 and 41.

Validation of the Method

Linearity: Internal standard calibration was employed in the determination of ethanol to compensate sample injection variations and minimize matrix effect. In the present work, equal amount of the internal standard (ACN) was added to all calibration standards and unknown samples. From the HS-GC-MS analysis, the standard calibration curve from 0.2-1000 µg/mL was obtained and a good linear regression coefficient R^2= 0.998 was achieved.

Limit of Detection/ Limit of Quantification (Lod/loq): The LOQ and LOD for this method were obtained by analyzing 8 blank samples and the SD of blank samples area was calculated. The LOD and LOQ concentrations were at 0.11 µg/mL and 0.32 µg/mL, respectively.

Precision: The precision is the degree of repeatability consistency between measurements. The results within-run precision (repeatability) show relative standard deviations (RSD) in 5 measurements of the same sample to be less than 3.8%, indicating that the technique was reproducible which also meet the requirement of 10% in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Similar values were obtained by other researchers for alcohol residue quantification of 4.5 - 6.9% in fish [20], 5.9 - 7.7% in gelatin capsules [10] and <7.9% in cooked meal containing alcoholic drinks [18].

Accuracy: To validate the accuracy of the method, a set of sample solutions was prepared by spiking 0.5 mL of perfume with different concentrations (25, 50, 100, 500, 1000 µg/mL) of pure ethanol. The original perfume sample without added ethanol contains 229.00 µg/mL of ethanol was used as a reference. Comparison on the concentration of the added amount of ethanol in perfume samples with those determined by this method demonstrated a consistently high and accurate recovery of 97.3 ± 3.7% (Table 1). The range of the recoveries was between 94 - 104%. From these measurements, it indicates that this method is useful for the determination of ethanol in perfumery product samples.

Evaluation of Ethanol in Perfumery Products: The HS-GC-MS method used in this work was applied to perfumery products purchased in the market and obtained from the local suppliers. With the methodology described, in this study, 26 samples claimed to be alcohol-free perfumery products can be analyzed without sample pretreatment (Table 2). The products were categorized according to the label on the package namely fragrance, perfume compound, perfume and atar perfume.

All the products contain less than 1000 µg/mL ethanol and the range of alcohol detected in the products was between 7.31 – 611.64 µg/mL. The fragrance and perfume compounds tested are used as ingredients in perfumes and personal care products and contained ethanol in the range of 45.95 - 238.55 µg/mL. In the perfume category, generally the ethanol content was lower than 50 µg/mL except sample P3 (611.6 µg/mL) and P4 (125.9 µg/mL). In addition, 8 perfume samples (P5-P12) which carry the halal logo and claimed to be alcohol-free were found to have less than 40 µg/mL ethanol content. Very interesting to note that atar perfume category which claimed to be alcohol-free and used by many Muslims for religious purpose contain various concentrations of ethanol below 100 µg/mL, except sample A4 (142.15 µg/mL) and A6 (140.70 µg/mL). It is believed, even though the ethanol is not used as an ingredient in the perfumery products, the residual alcohol present is possibly due to

Table 1: Mean recovery and relative standard deviation of the HS-GC-MS method

	Ethanol concentration (µg/mL)			
Sample no.	Original content	Added amount	Detectedamount	Recovery(%)
1	229 ± 0.9	25	253 ± 6	96.0
2	229 ± 0.9	50	281 ± 8	104.0
3	229 ± 0.9	100	323 ± 3	94.0
4	229 ± 0.9	500	693 ± 12	94.4
5	229 ± 0.9	750	953 ± 84	96.5
6	229 ± 0.9	1000	1215 ± 40	98.6
Mean Recovery (%)				97.3
RSD (%)				3.7

Table 2: Ethanol content present in the perfumery products

Sampel No.	Ethanol content detected (µg/mL)	Sampel No.	Ethanol content detected (µg/mL)
Fragrance		Perfume	
F1	201.91 ± 1.85	P1	17.11 ± 0.15
F2	84.75 ± 1.48	P2	43.37 ± 0.08
		P3	611.64 ± 4.72
Perfume		P4	125.91 ± 1.24
compound		P5*	7.31 ± 0.66
PC1	238.55 ± 0.69	P6*	28.03 ± 0.63
PC2	97.40 ± 3.70	P7*	32.53 ± 1.45
PC3	174.15 ± 2.75	P8*	13.93 ± 0.02
PC4	45.95 ± 1.92	P9*	20.86 ± 0.40
PC5	71.06 ± 2.83	P10*	16.16 ± 0.45
PC6	71.06 ± 2.83	P11*	8.11 ± 0.01
		P12*	11.10 ± 1.18
Atar			
perfume			
A1	31.19 ± 0.99		
A2	37.67 ± 0.27		
A3	96.05 ± 2.16		
A4	142.15 ± 1.58		
A5	28.45 ± 0.71		
A6	140.70 ± 3.47		

* Carry halal logo on the package.

the carried over from raw material of the fragrance or the perfume compounds. Ethanol containing fragrances are the most common vehicles and carriers used in preparing fine perfumes [3]. Even though the ethanol used in the extraction of essential oil and fragrance has been removed, the ethanol residue still remained and can be detected using the HS-GC-MS technique.

CONCLUSION

It is concluded that HS-GC-MS is a simple, fast and sensitive method for the routine analysis of residual ethanol in perfumery products. 26 samples of perfumery products were analysed and the ethanol concentration detected was ranged from 7.31-611.64 µg/mL. The conditions selected for the HS-GC-MS revealed that this technique has high linearity and reproducibility. The results demonstrated that the method is reliable. In addition, the external standard quantification can be used in the HS-GC-MS when the sample matrix does not interfere with the analyte. The findings from this study can be used as reference by consumer and the regulators to set the maximum allowable limit in these products.

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