C1_006_PF: SIMPLE AND RAPID MEASUREMENT OF ETHANOL IN PERFUME BY PORTABLE RAMAN SCATTERING SPECTROMETER: POTENTIAL PERFUME ETHANOL POISON FOR INFANTS

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Abstract: Perfume commonly contains ethanol as solvent of the fragrant components as well as for promoting evaporation of the fragrance. Ethanol content can vary over a wide range from 50 to 99% (v/v). Toxicity of ethanol in infant is of concern both from direct ingestion or adsorption through the infant's skin. Lethal dose of ethanol in infant by ingestion is 3g/kg infant weight. Therefore, information of the ethanol content in perfume is necessary in order for parents to be aware of the potential toxic risk of the product to their child. A rapid and convenient method for the determination of ethanol content in perfume product will be useful as an indicator of the ethanol toxicity. This work aims to apply Raman scattering technique as a rapid and direct method for measurement of the ethanol content in commercial perfume products. Gas chromatography with FID was used as the comparison method to validate the method. Linear calibration curves from the Raman scattering and GC-FID methods were $y = (7.7\pm0.1)x + (114.1\pm5.8)$ and $y = (1781.1\pm24.7)x + (569.8\pm878.6)$, respectively, with good coefficient of determination ($R^2 > 0.99$). There was no statistically significant difference between the two methods ($p > 0.05$). The measured ethanol content in four local perfume products were in the range 66.0 to 77.0% (v/v). The Raman scattering method provides high sample throughput of 120 samples/h, employing a convenient portable Raman scattering spectrometer.

Introduction: Ethanol is a component contained in various products, such as alcoholic beverages (1), fuels (2), and household products (3), including personal care products (4). In this work, we focus on the ethanol content in perfume products. Ethanol is a major component in perfume ranging from 50 to 99% (v/v), depending on the perfume manufacturer and percentage of fragrance (3). However the content of ethanol is not commonly included in the composition of the perfume products, in addition there has not been labelled amount of ethanol on its description.

Ethanol content in household product is important since its toxicity especially for infants by exposure or ingestion. There are reports of ethanol toxicity by skin adsorption leading to death of infants (5-7). It is due to composing of lower keratin of infant skin than that from adult, thus it was lower tolerate of ethanol adsorption (6, 8). Also, ingestion of overdose ethanol by infants was mentioned to cause death due to hypoglycemia condition (3, 9, 10). Figure 1 presents the schematic of the condition of hypoglycemia caused by gluconeogenesis, Figure 1(a) is the normal gluconeogenesis with no disturbance of metabolism by ethanol. Pyruvate is normally converted to glucose and lactate, but when ethanol is present, it can block the gluconeogenesis since ethanol metabolism requires nicotinamide adenine dinucleotide (NAD) for conversion to acetate (see Figure 1(b)). This leads to reduction of the major pathway of gluconeogenesis leading to hypoglycemia, especially in the case of infants (3, 9, 11). Details are given in Figure 1. Lethal dose of ethanol in an infant by ingestion is 3 g ethanol per kg of infant weight (9, 10).

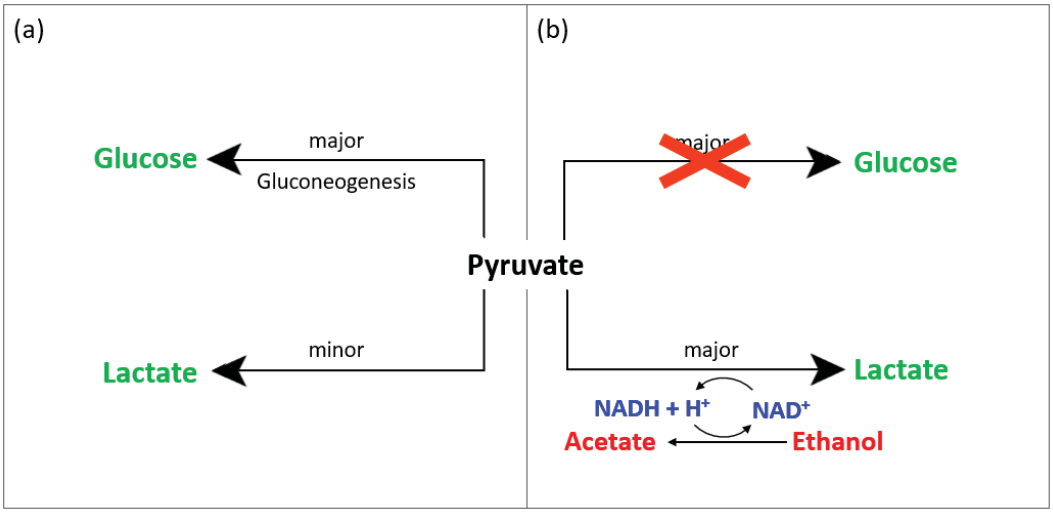

Figure 1. Schematic of ethanol metabolism and its effect to gluconeogenesis. (a) Normal gluconeogenesis and (b) inhibition in response to presence of ethanol. This picture was modified from references (3, 11).

Method of ethanol measurement: Determination of ethanol content in perfume products has rarely been reported. Only the fragrance components have been analyzed by gas chromatography (GC) (12-14), high liquid performance chromatography (HPLC) (15, 16), infrared spectroscopy (IR) (13, 17, 18) and capillary electrophoresis (CE) (19). Gas chromatography is a conventional method for the determination of ethanol due to its high sensitivity and accurate analysis. The drawbacks of this technique are destruction of sample, time consuming and technical skill for operation of the instrument. Raman scattering technique is an alternative method that has many advantages. Raman scattering is vibrational spectroscopy and can identify molecular structure from vibration modes that changes the polarization of bonding. It is convenient and user-friendly with rapid measurements and non-destructive of samples. The aim of this work is to employ a portable Raman instrument as a rapid method for the determination of ethanol content in perfume products as marker for possible toxic level to infant.

Table 1. Different methods for ethanol determination with their advantages and drawbacks

Sample Type	Method	Analysis time	Advantage	Drawback	Ref.
Alcoholic beverage	GC-FID	12 min	High sensitivity Accurate analysis	Time of analysis, sample preparation, high skill operation	(1)
	HPLC-FID	10 min	High sensitivity Accurate analysis	Time of analysis, sample preparation, high skill operation	(20)
	UV-NIR	**NA*	Direct analysis with less sample steps of pretreatment	Methanol interference	(21)
Perfume	Raman	**NA*	Non-destructive sample Short analysis time Reliable result	High cost of instrument	(22)
	Raman	*0.5 min*	*Non- destructive sample* *Short analysis time* *Reliable result*	*High cost of instrument*	*This work*

**NA*: Not Available

Methodology:
Chemicals: Standard ethanol (99.9% purity) was obtained from QRëC (Rawang, Malaysia). Ultrapure water (18.2 MΩ-cm) was from Siemens ultrapure water system (Evoqua Water Technologies, PA, USA).
Raman instrument: A portable PeakSeeker™ model (Agiltron Inc., Woburn, MA, USA) was employed. The excitation diode laser was at 785 nm with maximum output of 300 mW. Detector was a charge coupled device (CCD) with a reject filter of the Rayleigh scattering (785 nm). The instrument was equipped with liquid sample holder and RSIQTM software.
GC–FID instrument: The GC–FID instrument was an Agilent 6890 model (Agilent Technologies, Palo Alto, CA, USA). The GC capillary column was HP–INNOWax polyethylene glycol column (30 m length × 0.25 mm I.D. × 0.25 μm film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The GC oven temperature program was as follow: initial temperature, 40 °C; increased to 250°C at 100°C/min; total run time of 12 min. The detector temperatures was 250°C. The sample injection volume was 1.0 μL with split ratio of 50:1.
Perfume sample preparation: Four samples of commercial perfume purchased in the Bangkok area were employed in this research. The perfume liquids were analyzed directly as shown in Figure 2. Samples were directly transferred to the GC vial and capped (Figure 2(a)) for GC–FID analysis or to the quartz cuvette (Figure 2(b)) for Raman scattering analysis.

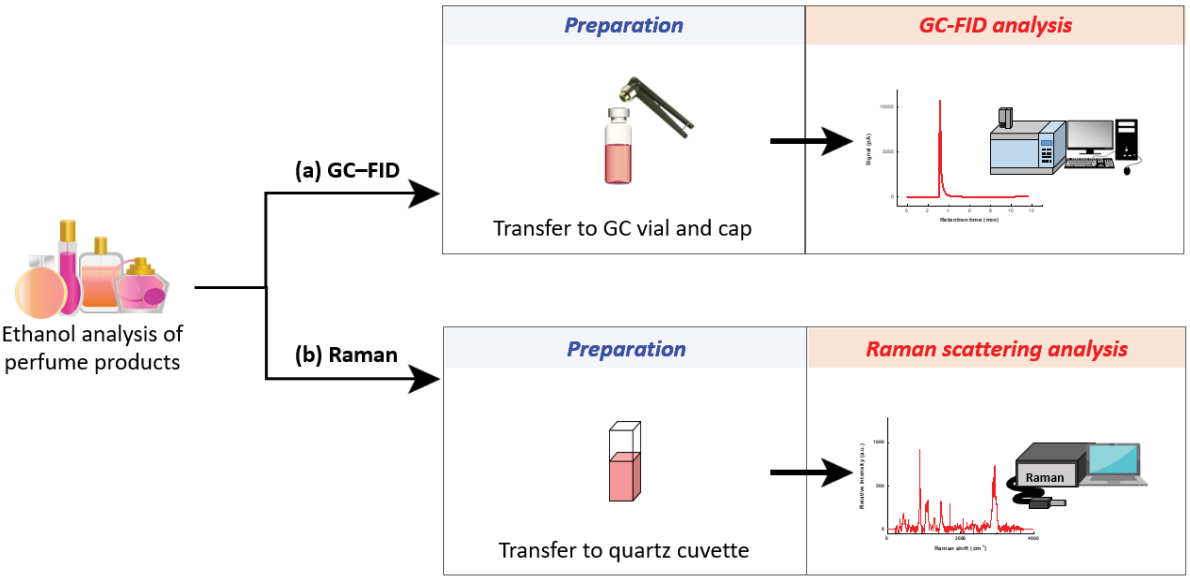

Figure 2. Schematic of ethanol measurement in perfume by (a) GC–FID and (b) Raman scattering.

Results and discussion: Measurements of ethanol by Raman scattering and GC–FID are presented in this section, including analytical characteristics, results of the measurements of the four perfume products, method comparison in terms of analysis time, and evaluation of lethal potential of perfume ethanol content to an infant.
Analytical characteristics: The GC–FID and portable Raman spectrometer were used for construction of linear calibration curves of standard ethanol solutions. An example of ethanol response obtained from the two methods are given in see Figure 3A, for an ethanol sample of 70.0% (v/v). Figure 3A(a) shows a chromatogram of ethanol with retention time of 3.16 min. Figure 3A(b) is a Raman signal of ethanol where 882 cm^{-1} is the C–C stretching of ethanol. The calibration curve of standard ethanol solutions was y = (1781.1±24.7)x + (569.8±878.6) for the GC–FID method with x from 0.0 – 70.0% (v/v) was obtained, where x axis is for the ethanol content, % (v/v) and y is for the peak area. The calibration line for Raman scattering was y = (7.7±0.1)x + (114.1±5.8) with x from 1.0 – 99.9% (v/v), where x axis is for the ethanol content, % (v/v)

and y is for the relative intensity. The two methods gave good coefficient of determinations (R^2 > 0.99).

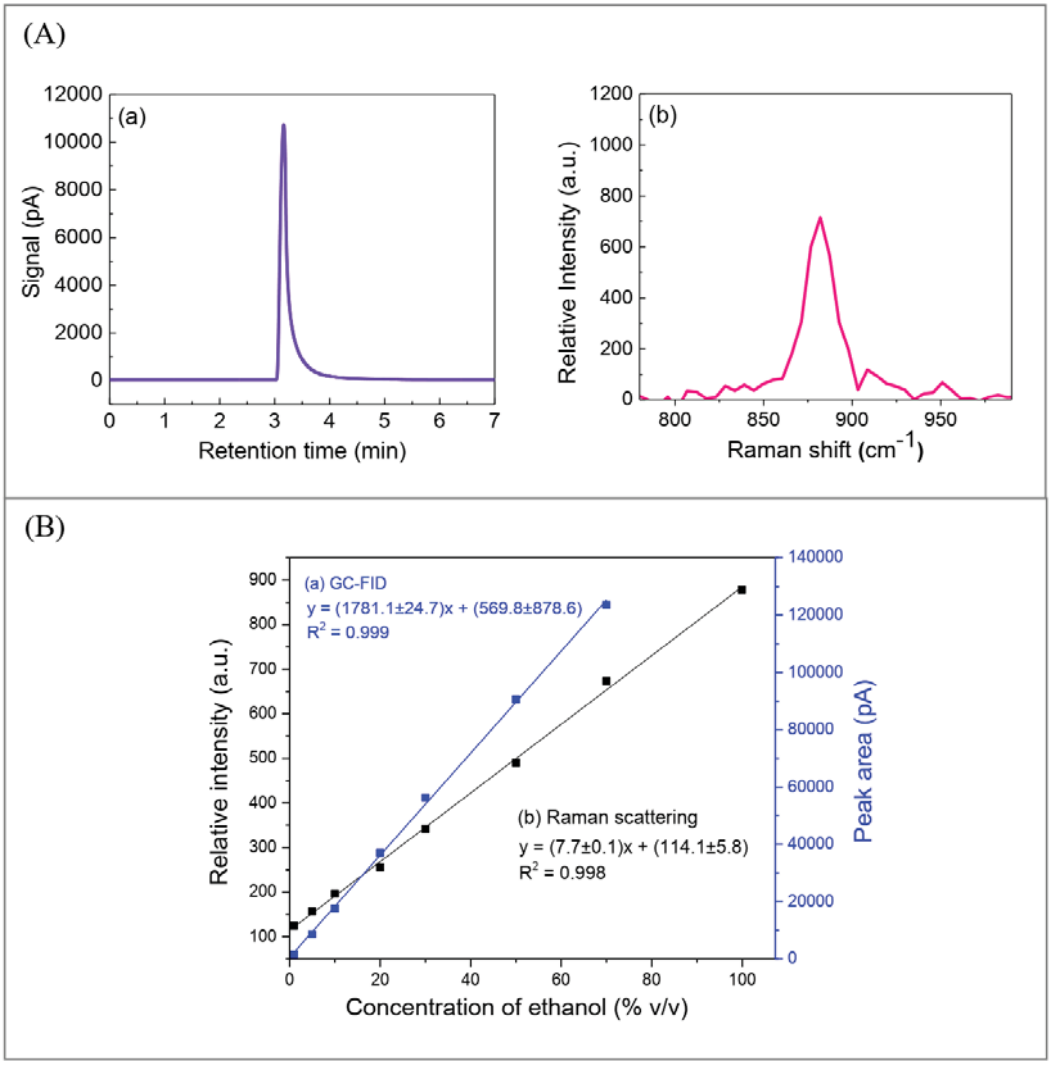

Figure 3. (A) An example of **(a)** chromatogram peak and **(b)** Raman scattering peak of ethanol at concentration of 70.0% (v/v). **(B)** Calibration curves of standard ethanol solutions for the two methods: **(a)** GC method, 1.0 – 70.0% (v/v) and **(b)** Raman scattering method, 1.0 – 99.9% (v/v).

Application of the method for determination of ethanol content in perfume products: Four commercial perfume products were analyzed. Direct measurement of the ethanol content was carried by the two methods. Ethanol content in four perfume samples were 70.6%, 70.0%, 64.3% and 71.5% (v/v) and 75.1%, 77.2%, 66.0 and 77.0% (v/v) by GC–FID and Raman scattering methods, respectively, as shown in Figure 4. The difference (GC–FID – Raman) of the measured ethanol content were -6.2%, -9.8%, -2.6% and -7.5% for samples; P1, P2, P3, P4, respectively. It can be noted that lower content for the GC–FID method was observed for all samples. However, the two methods showed no statistically different ethanol content for all four samples using paired *t*-test at 95% confidence level (p > 0.05).

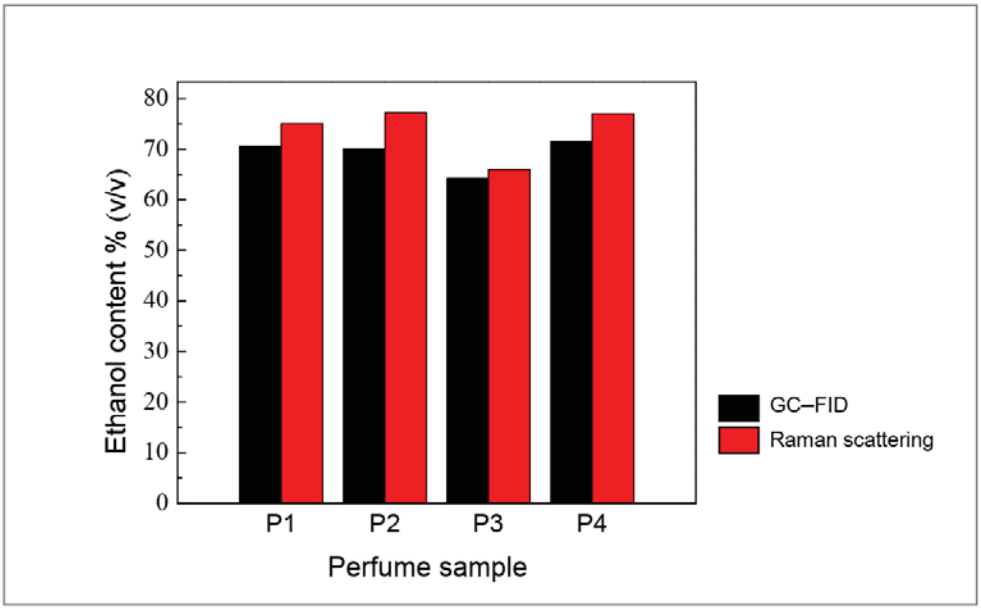

Figure 4. Bar graphs of measured ethanol contents in % (v/v) in four perfume products (P1, P2, P3 and P4) using GC–FID and Raman scattering.

Method comparison in terms of analysis time: As mentioned in the Introduction, information on the ethanol content in perfume product is important. The analysis time for the GC–FID and Raman scattering methods was compared. Since both methods employ the perfume sample directly, instrumental analysis time is the main factor. Raman scattering required only 0.5 min analysis time, whereas GC–FID run took 12 min. Sample throughput by Raman and GC–FID methods were 120 and 5 samples/h, respectively. Raman scattering is much simpler and more rapid than the GC method.

Evaluation of lethal potential of perfume ethanol content to an infant: As reported by Jepsen and Ryan (9) and Shulman (10), lethal dose of ethanol for an infant by ingestion was 3g/kg weight of infant. Thus, if the mean content of perfume samples (P1–P4) from the Raman scattering data is 73.8% (v/v), the volume of sample that can cause death to an infant is 9.0 mL, for an infant of 3.2 kg weight.

Note: New born infant weight of 3.2 kg was from the data of WHO Child Growth Standards (23).

Conclusion: The measurement of ethanol content in perfume products by GC–FID and Raman scattering methods were carried out. The analytical characteristics and method for ethanol measurement of perfume products were described. The Raman scattering method is more rapid and convenient than the GC–FID method. Analysis time of the Raman scattering method is only 0.5 min, compared to 12 min for GC–FID. Throughput analysis is 120 samples/h for Raman scattering method, with only 5 samples/h for the GC–FID method. The measured ethanol content in four perfume products were in the range 66.0 – 77.0% (v/v), using Raman scattering analysis. Thus calculated potential lethal volume of perfume to a 3.2 kg infant by ingestion is about 9.0 mL, based on lethal dose of 3g/kg infant weight.

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Acknowledgements: This research project is supported by Forensic Science Unit, Medical and Graduated Education Division, Department of Chemistry, and Central Instrument Facility, Faculty of Science, Mahidol University. Portable Raman instrument was kindly provided by Dr. Noppadon Nuntawong.