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Y. Hida · M. Matsumoto · K. Kudo · T. Imamura
N. Ikeda

Identification of beverages and beverage stains by GC/MS using aroma components as indicators

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Abstract A method to identify brown coloured beverages or beverage stains for criminological purposes was devised using aroma components as indicators. The examined beverages comprised three brands each of beer, coffee, cola and black tea, two brands of oolong tea, and four brands of green tea. Aroma components in each sample were efficiently concentrated with a porous polymer beads column (Porapak Q) and were analyzed by gas chromatography/mass spectrometry (GC/MS) using n-octyl alcohol as the internal standard. Specific aroma components were found in each beverage and beverage stain, for example cis-terpin hydrate in cola, and thus identification of beverages or beverage stains became feasible through the detection of these specific components. The present method was applied efficiently to two practical criminological cases.

Key words Beverage · Aroma components · Porous polymer beads · GC/MS

Introduction

Brown coloured beverages such as beer, coffee, cola and various kinds of tea are popular drinks, and are often found at crime scenes. As the analysis of blood stains or hairs left at crime scenes, identification of these beverages or beverage stains is useful to reconstruct the crime or to ascertain the victim's behavior before death. Several studies have been reported on the aroma components contained in beverages [1, 2]. However there have been no reports on the discrimination between beverages or between beverage stains from the aspect of forensic science.

Y. Hida · K. Kudo · T. Imamura · N. Ikeda (✉)
Department of Forensic Medicine, Faculty of Medicine,
Kyushu University 60, Fukuoka 812–82, Japan
FAX: +81 (92) 642 6126

Y. Hida · M. Matsumoto
Forensic Science Laboratory,
Fukuoka Prefectural Police Headquarters, Fukuoka 812–77, Japan

We previously developed a simple GC/MS method for discrimination between distilled liquors using aroma components as the indicators [3]. We applied this technique to the identification of aroma components within brown coloured beverages or beverage stains, and we were then able to successfully discriminate between beverages.

Materials and methods

Reagents

Porous polymer beads, Porapak Q (50–80 mesh), were purchased from Waters (Milford, MA, USA). Diethyl ether, n-octyl alcohol and other reagents were of analytical grade. The following marketed beverages were examined in this study as beverage and beverage stain samples.

Beverage and beverage stain samples

Three brands each of beer, coffee, cola and black tea, two brands of oolong tea, and four brands of green tea were examined as beverage samples. One of the brands selected for each beverage was used as beverage stain samples.

Extraction of aroma components

The same extraction procedure as described in our previous paper on the analysis of aroma components in distilled liquors was used [3]. A 10-ml volume of each beverage was diluted with 10 ml of deionized water, and applied to a 2 × 30 cm column packed with 40 ml of porous polymer beads. The column was washed with 100 ml of deionized water and the adsorbed aroma components were eluted with 40 ml of diethyl ether. The eluate was concentrated to about 10 μl under a stream of nitrogen. Of this solution 1 μl was mixed with 1 μl of internal standard (IS) solution (1 μg of n-octyl alcohol in diethyl ether) and the mixture was submitted to GC/MS.

The extraction procedure for aroma components within beverage stains was as follows: a beverage stain on a cotton cloth equivalent to a 5-ml volume of beverage was sonicated with 100 ml of deionized water, and the solution was applied to a porous polymer beads column, as described above. The column was washed with 100 ml of deionized water and the adsorbed aroma components were eluted with 40 ml of diethyl ether. The eluate was concentrated to about 10 μl under a stream of nitrogen and 1 μl of this solution was mixed with 1 μl of IS solution and submitted to GC/MS.

GC/MS conditions

The apparatus used was a Shimadzu QP-1100EX gas chromatograph-mass spectrometer equipped with a multiple-ion detector. A fused-silica capillary column (25 m × 0.53 mm I.D., 1.0 μm film thickness), coated with Shimadzu CBP-20 bonded polyethylene glycol stationary phase, was used. The carrier gas was helium at a flow rate of 30 ml/min. The operating temperatures were as follows: column 50° C for 2 min then 5° C/min up to 200° C, injection port 250° C, separator 250° C and ion source 250° C. The ionization energy was 70 eV.

Identification of aroma components within beverages or beverage stains

Aroma components detected in the beverages or beverage stains were identified by comparing their mass spectra with those within the data base (NIST library) installed into the GC/MS system, and the specific aroma components selected as indicators for each beverage were further identified by comparing the retention times with those of the authentic compounds.

Results and discussion

Extraction of aroma components from beverages and beverage stains

We first applied the liquid-liquid extraction procedure, used for the analysis of flavor components from food and plants [4] to our samples. However, a large quantity of caffeine was found to interfere with the detection of the small amount of aroma components contained within cola, coffee and tea. Guo et al. [5] extracted aroma components from green tea and black tea by a method of simultaneous distillation and extraction (SDE method) using the Linkens-Nickerson apparatus. Vitzthum et al. [6] detected pyridines and pyrazines from black tea by supercritical CO_2 extraction under pressure and GC/MS. These methods are not in practical use, because the control of the pressure and temperature is strictly necessary for the SDE method, and because the apparatus for the supercritical CO_2 extraction procedure is too expensive.

Solid-phase extraction is useful for selective extraction of target compounds from various matrices, such as biological samples or food, and has become more popular for the analysis of abused drugs [7, 8]. Shimoda et al. [9] successfully used a porous polymer beads column to concentrate aroma components in foods. We therefore applied our simple extraction procedure previously used for the analysis of aroma components within distilled liquors [3], and discovered that a large quantity of caffeine could be successfully removed, allowing the small amount of aroma components to be efficiently concentrated.

Identification of aroma components within beverage or beverage stain samples

A representative chromatogram of a coffee sample is shown in Fig. 1. A large amount of caffeine was removed and many aroma components were efficiently concentrated by the porous polymer beads column.

The characteristic aroma components detected in beverages and beverage stains are listed in Table 1. In beer samples, 21 aroma components were identified, in coffee samples 18 and in cola samples 6. Three characteristic aroma components were detected in all the tea samples, and 2, 9 and 3 characteristic aroma components were detected in the black tea, oolong tea and green tea samples, respectively. Some of the characteristic aroma components detected in each beverage were also detected in the respective beverage stain.

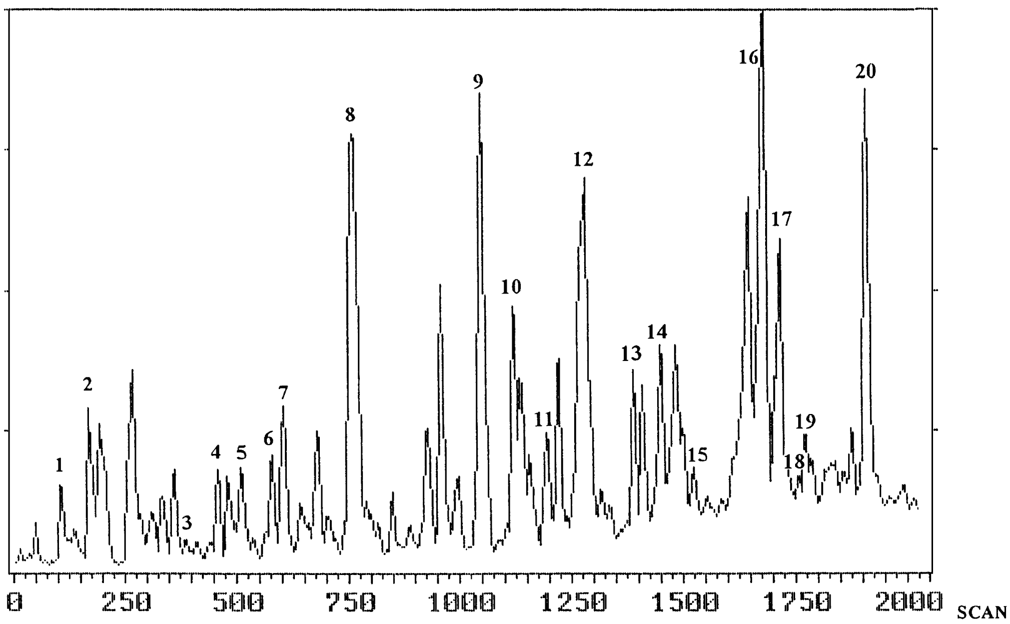

Fig. 1 Total ion chromatogram of the extract from a coffee sample. 1 = Pyridine, 2 = Diethylbenzene, 3 = 1,3,5-Triethylbenzene, 4 = Furfural, 5 = 4-Acetylpyrazole, 6 = Internal standard (I.S.), 7 = 5-Methyl-2-furfural, 8 = Furfuryl alcohol, 9 = 2,6-Di-tert-butyl-p-cresol (BHT), 10 = Maltol, 11 = 1H-Pyrrole-2-carboxaldehyde, 12 = 1-Methyl-2-pyrrolecarboxaldehyde, 13 = 1-(2-Hydroxy-5-methylphenyl)-ethanone, 14 = 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 15 = 5-Hydroxy-2-methylpyridine, 16 = Triethyl citrate, 17 = 5-(Hydroxymethyl)-2-furaldehyde, 18 = Vanillin, 19 = Caffeic acid, 20 = Catechol

Table 1 Characteristic aroma components detected in beverages and beverage stains

Aroma components	RRT	Beer		Coffee		Cola		Black tea		Oolong tea		Green tea	
		1	2	1	2	1	2	1	2	1	2	1	2
Ethyl isovalerate	0.17	●											
Pyridine	0.29			●									
3-Methyl-1-butanol	0.33	●											
Ethyl hexanoate	0.35	●	●										
Diethylbenzene	0.44	●		●				●	●				
1,3,5-Triethylbenzene	0.74			●									
Ethyl octanoate	0.75	●											
Furfural	0.86			●	●								
Benzaldehyde	0.94	●	●										
4-Acetylpyrazole	0.94			●									
Internal standard (I.S.)	1.00												
5-Methyl-2-furfural	1.10			●									
Isobutyric acid	1.10	●											
5,5-Dimethyl-2(5H)-furanone	1.17	●											
Diethylene glycol monoethyl ether	1.19									●	●	●	●
Phenylacetaldehyde	1.22	●	●										
cis-β-Terpineol	1.23					●							
Furfuryl alcohol	1.34	●	●	●	●					●	●		
Isovaleric acid	1.34	●											
4-Ethylbenzaldehyde	1.35							●	●	●	●	●	●
α-Terpineol	1.37					●							
β-Phenylethyl acetate	1.59	●											
4-Ethylacetophenone	1.61							●	●	●	●	●	●
3,4-Dimethylacetophenone	1.68							●	●	●	●	●	●
Hexanoic acid	1.71	●	●							●			
2-Phenylethanol	1.82	●	●										
Maltol	1.94	●		●	●					●			
4-Isopropylbenzyl alcohol	1.97							●	●				
Benzylidenemalonaldehyde	2.02					●							
1H-Pyrrole-2-carboxaldehyde	2.05			●	●					●			
Octanoic acid	2.11	●	●							●	●		
cis-Terpin hydrate	2.19					●	●						
1-Methyl-2-pyrrolecarboxaldehyde	2.22			●	●								
1-(2-Hydroxy-5-methylphenyl)-ethanone	2.38	●		●									
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	2.46	●	●	●						●		●	
Decanoic acid	2.49	●	●										
5-Hydroxy-2-methylpyridine	2.60			●	●								
Levulinic acid	2.60					●							
trans-p-Coumaric acid	2.71									●		●	
Triethyl citrate	2.86			●	●								
5-(Hydroxymethyl)-2-furaldehyde	2.91	●		●	●	●				●			
Vanillin	2.97			●	●								
Caffeic acid	3.00	●		●									
Catechol	3.24			●	●								

1: beverages; 2: beverage stains; ●: detected; I.S.: n-Octyl alcohol

Selection of specific aroma components as indicators

From among the characteristic aroma components shown in Table 1, aroma components which can be used as indicators for discrimination between beverages were selected and these are listed in Table 2. As indicators for beer, 13 specific aroma components were selected, for coffee 10 and for cola 5. For black tea, oolong tea and green tea, 5, 7 and 5 specific aroma components were selected respectively. Among these indicators, some components underlined in Table 2 were also useful as indicators for discrimination between beverage stains. Discrimination between beverages or between beverage stains can be accomplished quickly when using these indicators.

Case reports

Case 1

A robber struck a hotel cloakroom attendant on the head with a beer bottle which was full of beer, and both the robber and the ho-

Table 2 Useful aroma components selected as indicators for discrimination between beverages

Beverages		Characteristics aroma components			
Beer		ethyl isovalerate	3-methyl-1-butanol	ethyl hexanoate	ethyl octanoate
		benzaldehyde	isobutyric acid		5,5-dimethyl-2(5H)-furanone
		phenylacetaldehyde	isovaleric acid		β-phenylethyl actetate
		hexanoic acid	2-phenylethanol		decanoic acid
Coffee		pyridine	1.3,5-triethylbenzene	furfural	4-acetylpyrazole
		5-methyl-2-furfural			1-methyl-2-pyrrolecarboxaldehyde
		5-hydroxy-2-methylpyridine	triethyl citrate	vanillin	catechol
Cola		cis-β-terpineol	α-terpineol		benzylidenemalonaldehyde
		cis-terpin hydrate			levulinic acid
Tea	Black tea	diethylbenzene	4-ethylbenzaldehyde		4-ethylacetophenone
		3,4-dimethylacetophenone			4-isopropylbenzyl alcohol
	Oolong tea	diethylene glycol monoethyl ether		furfuryl alcohol	4-ethylbenzaldehyde
		4-ethylacetophenone	3,4-dimethylacetophenone		trans-p-coumaric acid
		octanoic acid			
	Green tea	diethylene glycol monoethyl ether			4-ethylbenzaldehyde
		4-ethylacetophenone	3,4-dimethylacetophenone		trans-p-coumaric acid

tel man were splashed with the beer. The robber was arrested shortly after. One week later, identification of a beverage stain on the robber's cardigan was required in order to help prove his culpability, and the present method was applied to this sample. Although many unknown peaks supposedly derived from his cardigan appeared on the chromatogram, benzaldehyde, phenylacetaldehyde, hexanoic acid, and 2-phenylethanol selected as indicators of beer, and characteristic aroma components of beer such as furfuryl alcohol and octanoic acid were detected in the extract from his cardigan. Therefore, grounds for his involvement in this crime, in which a beer bottle was used, were provided.

Case 2

A 50-year-old man was found dead in a car where some cans of drink including black tea, oolong tea and green tea were found opened beside him. Identification of 7 ml of brown coloured liquid in his stomach was required in order to ascertain his behavior just prior to death and the present method was applied to the sample. 4-Ethylbenzaldehyde, 3,4-dimethylacetophenone, 4-isopropylbenzyl alcohol and 4-ethylacetophenone selected as indicators of black tea, and other characteristic aroma components of black tea were detected in the extract from his stomach contents. It was ascertained that the victim had drunk black tea prior to his death.

In conclusion the present method using aroma components as indicators seems to be valuable for the discrimination between beverages or between beverage stains for criminological purposes.

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