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Identification of Coffee Fragrances Using Needle Trap Device-Gas Chromatograph/Mass Spectrometry (NTD-GC/MS)

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A fast and simple sampling and sample preparation device, (NTD) has been developed and applied to sample and analyze volatile components from ground coffee beans. Coffee fragrances and other volatile organic compounds (VOCs) were sampled by the NTD and then analyzed by gas chromatograph-mass spectrometry (GC/MS). Divinylbenzene (DVB) particles (80/100 mesh size) were the sorbent bed of the NTD. More than 150 volatile components were first identified based on the database of the mass library and then finally 30 fragrances including caffeine were further confirmed by comparing experimental retention indices (*i.e.* Kovat index) with literature retention indices. Total sampling time was 10 minutes and no extra solvent extraction and/or reconstitution step need. Straight *n*-alkanes (C6-C20) were used as retention index probes for the calculation of experimental retention indices. In addition, this report suggests that an empty needle can be an alternative platform for analyzing polymers by pyrolysis-GC/MS.

Key Words : Sorbent tube, Microextraction, Needle trap device, Coffee fragrance, Kovat index

Introduction

The analysis of volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) is very important due to the fact that those compounds are ubiquitous and often harmful to human health.[1] Among them, natural and synthetic fragrances are now widely used as major additives in many industries such as cosmetics, perfume, air freshener, foods, and household products because some people are attracted to fragrant smell. However, others are very sensitive to especially "synthetic" fragrant volatiles even with very low threshold concentration (ppb~ppm). Since many diseases including asthma, breathing problem, coughing, fatigue, and eye irritation are strongly associated with those fragrances, health facilities in North America ban the use of any fragrance to staff, patients and even visitors in the buildings.[2,3] Therefore, analyzing VOCs is crucial both for developing human care products and for clearing the health effect of fragrances and other VOCs to human.

Liquid-liquid extraction (LLE)[4] is one of the most conventional techniques to extract and analyze fragrances and other VOCs from different matrixes. Then there has been a strong effort for researchers in analytical chemistry field to develop an easier and cost effective format for analysis of VOCs. Solid-phase microextraction (SPME),[5] inside needle capillary adsorption trap (INCAT),[6] membrane extraction with a sorbent interface (MESI),[7] and stir bar sorptive extraction (SBSE)[8] are now widely used in VOC analysis. Generally, the analysis of most fragrances and other volatile organic compounds (VOCs) has been achieved mainly by sampling the headspace with those miniaturized samplers except SBSE technique.

Since the first introduction to sample preparation field in the late 1990s, the use of the SPME fiber is getting popular as a tool to analyze VOCs, SVOCs and fragrances.[9] The SPME technique provides an easier and a faster way to transfer VOCs enriched on its fiber coating into a hot injector of a gas chromatograph equipped with mass detector (GC/MS) which is one of the most suitable instruments for identifying and/or quantifying VOCs until now. Sampling VOCs and its enrichment step is also simple; expose a SPME fiber to a gaseous or aqueous sample and waits until fragrances or other VOCs reaches equilibrium in concentration on the fiber's coating. Furthermore, sampling selectivity can be adjustable by considering the partition coefficient of target fragrances and other VOCs to a proper coating material. This simplicity is pro for the most of the sampling situations but is sometimes con in the case of that one needs to represent the most of fragrances, VOCs and other SVOCs which might be present in a sample.

If the representation of analytes is an issue, the use of sorbent packed tubing can be an alternative choice because sorbent tubes can trap all gaseous analytes passing through it once there is no breakthrough. But the use of sorbent tubing takes extra steps of sample preparation like a reconstitution step followed by solvent extraction before the introduction of analytes to the analytical instrument like a GC. A stand-alone heater needs to use to get thermal desorption and in many cases, the cryogenic refocusing should be used to attain sharp chromatogram.[10] Needle trap device (NTD)[11-16] introduced recently to overcome these disadvantages over the conventional sorbent tubing. The NTD is a miniaturized format of sorbent tubing but it takes the most advantages of SPME techniques described above. Especially, sampling fragrances by the NTD without breakthrough gives an idea of the relative distribution of fragrances and other VOCs

present in a sample.

Here, the NTD was used to sample and identify fragrant components present in a ground coffee bean so as to demonstrate the capability of NTD as a faster and convenient sampler for analyzing VOCs. Coffee was chosen because it contains a wide range of volatile analytes and many different types of functional groups of organic compounds such as aldehydes, carboxylic acids, phenols and pyrazines which affects the final aroma quality of a coffee.[17]

Experimental

Reagents and Materials. The straight chain *n*-alkane mixture of C8-C20 (40 mg/L) in hexane and toluene was purchased from Sigma Aldrich (Seoul, Korea). Reagent grade hexane and heptanes were also purchased from Sigma Aldrich and used without further purification. Divinylbenzene (DVB) sorbent (HaySept Q, 80/100 mesh) was purchased from Restek (Seoul, Korea). Ground coffee bean (Ethiopia SIDAMO 100, produced and packed by Lotte Chilsung, Korea) was purchased from a local grocery store. High purity gases of helium, hydrogen, air were purchased from a local gas product supplier (Dongsung, Gyeongbuk, Korea) and were used to operate a GC system. Amber vials (20 mL) and caps equipped with polytetrafluoroethylene (PTFE)/silicone septa were purchased from Supelco (Seoul, Korea). 22 gauge stainless steel needles (O.D. 0.71 mm, I.D. 0.39 mm) were purchased locally. Epoxy (harden within 5 minutes, Quick Set, 3 M Korea, South Korea) was used to fix and hold sorbent particles in place.

Needle Trap Device (NTD) Preparation. A packing set-up for the NTD preparation and its structure were depicted in Figure 1. A blunt type stainless steel needle (22 gauze, Clover Science, Daegu, South Korea) was used to prepare a needle trap device (NTD). DVB sorbent particles (SB) was packed inside the needle (see the Figure 1(a)) with an aid of aspirator (model A-3S, Eyela). Vacuum gauge (VG) monitored the pressure drop during sorbent packing process. The vacuum pressure starts around −0.4 bar at the beginning of packing sorbent particles and ended up around −0.9 bar at the end of packing process (with 1 cm long sorbent bed). Stainless steel spiral plug (SP) and a 5 min Epoxy (presented by dots at the end of the sorbent bed in Figure 1(b)) was used to fix sorbent particles in place. A side hole (SH, 0.3 mm id) was drilled 3 cm apart from the end tip of the needle, which is a passage of eluent gas (*i.e.* Helium) during an injection step so as to help transfer trapped analytes efficiently into a GC separation column.[11] To condition a NTD, it was placed in a hot GC injector (270 °C) for ~30 minutes. More detailed explanation for both the preparation and the sample transfer technique can be found elsewhere.[13]

Retention Index Probes and Kovat Index. The retention probes (straight n-alkane mixture, Hexane and heptane) in gaseous state were sampled and injected into a hot GC injector (kept at 250 °C) using a NTD. Alkane peaks were identified based on MS library and spiking of a pure *n*-alkane. Retentions times of *n*-alkanes were used to calculate

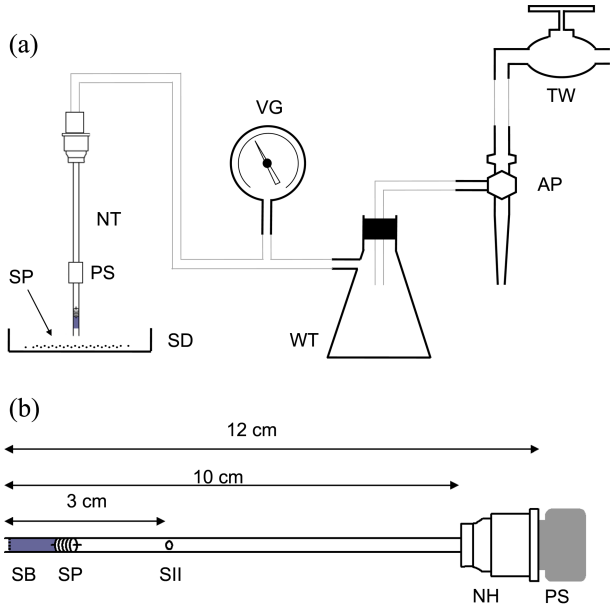

Figure 1. NTD packing set-up using aspirator (a) and the schematic structure of the NTD (b); AP: aspirator, TW: tap water, VG: vacuum gauge, WT: water trap, SD: sorbent dish, SP: sorbent particles, NT: NTD, SB: sorbent bed packed inside a needle, SP: stainless steel spiral plug, SH: side hole, NH: needle head, PS: PTFE sealer. The line in gray color indicates PTFE tubing connection.

Kovat index of coffee aromas with the following equations.[18] Kovat inex is the most widely used as retention index probes when a GC oven temperature increases linearly. This is also called as linear temperature programmed retention index (LTPRI).

$$\text{LTPRI, } I = 100(n) + 100\left[\frac{\{t_r(\text{unknown}) - t_r(n)\}}{\{t_r(n+1) - t_r(n)\}}\right]$$

Where, $t_r(n)$ is the retention time of a normal alkane having n carbons and $t_r(n+1)$ is the retention time of an alkane having $(n+1)$ carbons. A LTPRI value of an analyte will be one of the rational numbers between $100n$ and $100(n + 1)$.

Sampling Coffee Aromas and Instrumental Condition. To sample coffee fragrances and other VOC components, first, ground coffee bean (~1 g) was placed in an amber vial so those volatile components from coffee filled the vial. Syringe pump (Kloehn, Nevada, USA, purchased from Revodix, Gyeonggi, Korea) aspirated those volatiles from the vial's headspace through a NTD so those fragrances and other VOCs were enriched on sorbent bed inside the NTD. The sampling flow rate was fixed at 1 mL/min which did not cause breakthrough. Reportedly, no breakthrough was observed for benzene up to ~2 mL/min of sampling flow rate.[11] Sample volume was 10.0 mL and otherwise, it will be noticed. GC/MS (Agilent 6890) equipped with mass spectrometer (5975C MSD, Agilent) was used to separate and identify coffee fragrant compounds enriched on sorbent bed of the NTD. Separation column was the nonpolar stationary phase (HP-5MS, 30 m × 0.25 mm I.D., 0.25 μm). Mass scan range was

Table 1. Retention times of straight-chain *n*-alkanes (C6-C20)

n-Alkanes	Retention Times, min.
Hexane (C6)	2.40
Heptane (C7)	4.37
Octane (C8)	6.08
Nonane (C9)	9.19
Decane (C10)	12.45
Undecane (C11)	15.60
Dodecane (C12)	18.56
Tridecane (C13)	21.33
Tetradecane (C14)	23.93
Pentadecane (C15)	26.39
Hexadecane (C16)	28.73
Heptadecane (C17)	30.93
Octadecane (C18)	33.11
Nonadecane (C19)	35.06
Eicosane (C20)	37.08

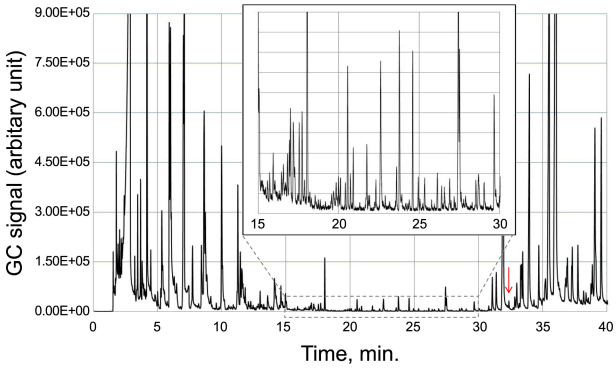

Figure 2. Total ion current (TIC) chromatogram of aromas and other VOCs released from Coffee; Inset is the zoom-in window for 15-30 min interval to show that many peaks are well-resolved. Y-scale is enlarged 12 times than the original chromatogram. The peak pointed by an arrow is identified as caffeine.

50-300 amu. The temperature of column was initially set at 40 ºC for 3.0 min and then ramped up to 250 ºC with a rate of 5 ºC/min. The total GC run time was 60 min. The carrier gas flow rate was kept constant at 1.0 mL/min.

Results and Discussion

Retention Index Probe. Retention times of retention index probes (straight chain n-alkane mixture (C8-C20) and hexane (C6) and heptanes (C7)) were summarized on the Table 1. These retention times were used to calculate experimental retention indices (RI_{Exp}) of coffee fragrances and other VOCs. The retention probes were also injected by SPME fiber (DVB coated fiber, Aldrich, Seoul Korea) because it was of interest to compare retention times of analytes injected by both samplers. For C6-C20 retentions index probes, both samplers shows the same times of retention, which implies that the desorption efficiency of a NTD is comparable with SPME fibers. Based on relative abundance, C9-C17 among C8-C20 alkanes are more clearly observed even though the same concentrations were injected (data not shown).

Coffee Aroma Identification. Total ion current (TIC) chromatogram for coffee aroma is shown in Figure 2. With 60 minute time span, ~150 peaks are identified based on MS library hit result but eventually 30 peaks are identified. Tentatively identified analytes were listed on Table 2. Those aromas were identified first by the database of the MS library and then further confirmed with the retention index. Experimental retention indices (RI_{Exp}, *i.e.* Kovat index) were determined using the LTPRI formula (shown in experimental section) and then used to compare with literature retention index (RI_{Lit}) to confirm them. Spiking pure analyte is the last step to firmly identify a VOC but this step was not performed because this was not our scope here. The TIC chromatogram clearly shows that the abundance for more volatile components is higher than semi-volatile components. After amplifying y axis by ~12 times, the inset in the Figure 2 clearly shows that many peaks are well-resolved, which proves that the DVB packed NTD is an efficient sampling device to analyze and indentify fragrances and other VOCs from a complex matrix with GC/MS. The caffeine (indicated by an arrow) was eluted at 32.36 min and its experimentally obtained Kovat index is 1765 while the literature Kovat index value is 1780. The difference between experimental and literature values is quite normal in this kind of qualitative analysis.

Limitation and Promising Capability. It was difficult to identify late-eluted analytes (*i.e.* semi-volatiles) because retention index probes longer than C20 were not available at that time. Also, highly abundant peaks after 35 minutes could make it worse to identify peaks in low abundance. It was found that the most peaks after ~35 minutes were originated from epoxy (used as sorbent fixer) and oil stein. This oil was used as a lubricant when the NTD's side hole was machine-drilled. Those highly abundant unwanted peaks should be removed to identify (low concentration) semi-volatile components accurately. Removal of those peaks could be done by cleaning an empty needle before packing it with sorbent particles. Cleaning process would be simple: a needle can be sonicated in soap solution for ~20 minutes and then was baked in a hot GC injector (450 ºC) for ~30 minutes. The black trace in Figure 3 is the chromatogram from an empty needle after those cleaning steps. The peaks in this black trace are thought to be from oil stein inside a needle. The chromatogram of epoxy was also shown in gray trace in Figure 3. The epoxy resin and hardener was mixed well and then filled inside the empty needle used above. Epoxy fragment peaks could be the peaks which are not overlapped with peaks in black trace. Even though peaks from epoxy debris and oil lubricant were hassles for the identification purpose in this study, this unwanted situation suggests an interesting experiment; an empty needle could be a good platform to analyze synthetic materials like polymer. In other words, an empty needle itself can be applied as an alternative injection technique of polymer into a GC/MS for pyrolysis (Py-GC/MS)[1] because this technique

Table 2. Tentatively identified aromas and other VOCs released from ground coffee beans

Peak #	RT (min)	Identified Chemicals	CAS #	RI_{Exp}	RI_{Lit}	Reference
1	2.79	Acetic acid	000064-19-8	620	603	A
2	3.23	2,3-Pentanedione	000600-14-6	642	674	B
3	3.45	3-Hydroxy-2-butanone	000513-86-0	653	680	B
4	3.80	1,4-Diazine	000290-37-9	671	704	B
5	4.18	Pyridine	000110-86-1	691	695	B
6	4.48	1-Hydroxy-2-butanone	005077-67-8	707	739	B
7	5.25	2,3-Butanediol	000513-85-9	752	789	C
8	5.40	2-Methyl-3-oxo-Tetrahydro furan	003188-00-9	761	776	B
9	5.91	Methylpyrazine	000109-08-0	791	794	B
10	6.07	2-Furancarboxaldehyde	000098-01-1	800	800	B
11	7.02	Acetoxyacetone	000592-20-1	830	839	B
12	7.09	2-Furanmethanol	000098-00-0	833	827	B
13	7.73	Butyrolactone	000096-48-0	853	865	B
14	8.42	2-Acetylfuran	001192-62-7	875	876	B
15	8.61	2,5-Dimethylpyrazine	000123-32-0	881	887	B
16	8.63	2,6-Dimethylpyrazine	000108-50-9	882	882	B
17	8.72	2-Ethylpyrazine	013925-00-3	885	890	C
18	10.07	2-Furancarboxaldehyde	000620-02-0	927	926	B
19	11.20	Phenol	000108-95-2	961	967	B
20	11.26	2-Acetoxymethylfuran	000623-17-6	963	967	B
21	11.45	2-Ethyl-6-methylpyrazine	013925-03-6	969	978	B
22	11.56	2-Ethyl-5-methylpyrazine	013360-64-0	973	978	B
23	11.58	2,3,5-trimethyl pyrazine	014667-55-1	973	974	B
24	12.99	dl-Limonene	000138-86-3	1017	1015	B
25	14.10	2-Ethyl-3,5-dimethylpyrazine	013925-07-0	1052	1059	B
26	14.60	Larixinic acid	000118-71-8	1068	1070	C
27	18.02	Decanal	000112-31-2	1182	1182	B
28	27.43	Dodecanoic acid	000143-07-7	1544	1547	B
29	32.36	Caffeine	000058-08-2	1765	1780	B
30	33.43	Muskolactone	000106-02-5	1816	1841	A

Reference: A: http://www.pherobase.com/database/kovats/kovats-range-600-700.php#. B: http://webbook.nist.gov. C: http://www.flavornet.org/info/513-85-9.html

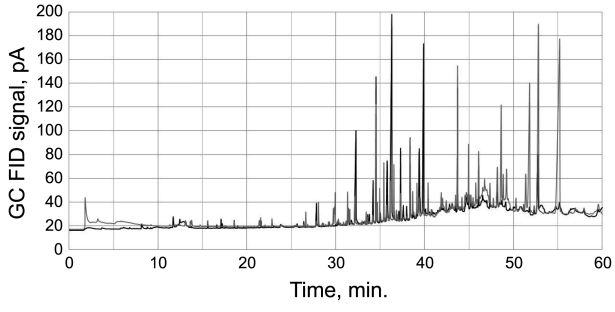

Figure 3. Example chromatogram of an empty needle (black trace) and the needle after filled the Epoxy (gray trace). For this study, GC/FID equipped with a DB-5 column (30 m × 0.25 mm I.D., 0.25 mm) was used. All GC conditions including the temperature programming are the same as the GC/MS. See text for details.

does not need an extra stand alone heater (for thermal desorption and/or decomposition) and a cost high cryogenic refocusing apparatus. Well-resolved peaks in example chromatograms in Figure 3 prove the capability of NTD as sample introduction platform for pyrolysis-GC/MS technique.

Concluding Remarks

A fast, simple, and convenient sampling and sample preparation device, NTD has been developed and applied to sample and analyze volatile components from ground coffee beans. The sampling time was only 10 minutes. No liquid extraction needs. More than 150 peaks were observed and 30 components were clearly identified based on MS library and retention index (Kovat index). Despite of some drawbacks (difficultness of identification of lately eluted component), this report demonstrates the promising potential of the needle trap device (NTD) as an alternative miniaturized sampler to investigate volatile components from many different matrixes. In future, it would be an interesting study to analyze fragments from bio-tissue and/or synthetic materials after thermal breakdown with an empty needle.

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