Anal Bioanal Chem (2013) 405:3945–3952
DOI 10.1007/s00216-012-6569-3

ORIGINAL PAPER

Time-course measurements of caffeine and its metabolites extracted from fingertips after coffee intake: a preliminary study for the detection of drugs from fingerprints

Kenji Kuwayama · Kenji Tsujikawa · Hajime Miyaguchi · Tatsuyuki Kanamori · Yuko T. Iwata · Hiroyuki Inoue

Received: 9 July 2012 / Revised: 11 November 2012 / Accepted: 12 November 2012 / Published online: 28 November 2012

Abstract The aim of this study was to determine whether an ingested drug and its metabolites could be detected in the subject's fingerprints. Caffeine (CF) was chosen as the model drug. Three healthy subjects were asked to consume a cup of coffee (ca. 100 mL) containing 80 micro micro mg CF as the total dose, which is the normal amount in one cup of coffee. After washing hands with water to remove external contaminants, each subject pressed the index fingertip to a collecting matrix just before consuming the test cup of coffee, and then again pressed the index fingertip to the collecting matrix after 1, 3, 5, and 7 h. The time curve of the amounts of CF and its metabolites—theobromine (TB), paraxanthine (PX), and theophylline (TP)—in fingerprints and blood was determined using liquid chromatography/tandem mass spectrometry (LC/MS). A filter paper wetted with water (50 μL) was an efficient collecting matrix for extracting the analytes from the fingertip. With optimized sample preparation and LC/MS conditions, the total operating time, from taking the fingerprints to obtaining the analytical result, was approximately 10 min. The lower limits of quantification for CF, TB, PX, and TP were 0.5, 5, 0.5, and 5 ng/fingerprint, respectively. The amount of CF or PX determined in fingerprints obtained over 7 h after coffee intake was significantly greater than the amount determined in fingerprints taken before drinking coffee. Fingerprints were a more efficient source for drug testing than other biological samples, such as blood and sweat, because the procedures for sampling and extracting the drugs were simpler and took less time. The method could be used to prove drug intake in criminal investigations.

Keywords Fingerprint · Drug · Sweat · Caffeine · LC/MS

Published in the special paper collection *Forensic Toxicology* with guest editors Kazuhito Watanabe and Satoshi Chinaka.

K. Kuwayama (✉) · K. Tsujikawa · H. Miyaguchi · T. Kanamori · Y. T. Iwata · H. Inoue
National Research Institute of Police Science, 6-3-1, Kashiwanoha,
Kashiwa, Chiba 277-0882, Japan
e-mail: kuwayama@nrips.go.jp

Introduction

Fingerprint ridge patterns have been known to be useful for individual identification for over 100 years [1–3], and they are often used in criminal investigations [4, 5]. A fingerprint consists of not only the external contamination on the fingertip but also of secreted sweat which develops within the body [5]. Since only portions of an ingested drug and its metabolites are excreted via sweat [6, 7], a fingerprint can contain trace amounts of the drug and its metabolites. In a criminal investigation, drug detection through fingerprints would provide valuable information about the relationship between a suspect and particular drugs. The success of such a technique would depend on the state of the fingerprints at the scene of a crime and the amount of drug in the fingerprints. Drug detection from fingerprints has been investigated using mass spectrometry [8], infrared spectroscopy [9], and immunochemical approach [10]. However, the analytical methods have some problems from the viewpoints of sampling of fingerprints, sample pretreatment, detection sensitivity, and selectivity of compounds for practical applications in criminal investigation. The purpose of this study was to determine the feasibility of detecting an administered drug and its metabolites from fingertips after washing fingers to remove the external contaminants completely.

In a criminal investigation, taking a fingerprint is easier than obtaining other biological samples such as blood and urine. Although drug usage can be easily proved by analyzing

blood or urine, a physician is required to draw blood for the sample or an observer needs to be present to prevent falsification of a urine sample. Trial procedures specify where and how blood and urine samples may be taken. Furthermore, biological samples must be obtained promptly because drugs and their metabolites are continuously metabolized and excreted. In contrast, a fingerprint can be recovered on sites even by a nonspecialist. Moreover, the procedure is simple, rapid, and noninvasive. Although sampling of oral fluids for drug testing is also simple, proper storage of samples to prevent drug degradation and complicated extraction of drugs from biological matrices are required because oral fluids have many biological components containing digestive enzymes [11, 12].

In this study, caffeine (CF) was chosen as the model for fingerprint detection of an ingested drug and its metabolites. Because CF is abundant in beverages, such as coffee and tea, it serves as a convenient model from the aspects of health and ethics. Since a cup of coffee normally contains 40 to 150 micro mg of CF [13, 14], it should not be difficult to detect CF in a subject's body fluids after the subject has had a cup of the beverage. CF's metabolites are important as markers of its intake; paraxanthine (PX), which can be detected in blood and urine, is the main metabolite of CF [15, 16]. CF, a trimethylxanthine, is metabolizedwo by t reactions (Fig. 1). One is the demethylation of the three *N*-methyl moieties and the other is oxidation at the 8-C position of the xanthine structure to the corresponding uric acid structure. Because the reactions occur successively, many different metabolites can be detected in blood and urine.

The simultaneous analyses of CF and its metabolites are usually performed using gas chromatography (GC) [17], GC/mass spectrometry (MS) [17, 18], liquid chromatography (LC) [19–22], or LC/MS [23]. GC is superior to LC for separating CF and its metabolites. Before a GC analysis, any free N in the xanthine structure must be *N*-alkylated; xanthines with free N positions are not completely vaporized in the GC injector. Although *N*-propyl derivatization of dimethylxantines enhances the detection sensitivity of GC and GC/MS, the reactions are time consuming and labor intensive [18]. In contrast, CF and its metabolites, particularly PX and TB, were not well separated by LC, and various mobile phases were used to separate the analytes in previous studies [19–23]. Because the analytes in fingerprints are present in only trace amounts, a highly sensitive analysis is required to detect drugs in them. The use of LC/MS permits sensitive analysis of most water-soluble compounds, and rapid simultaneous analysis of many compounds because it affords not only chromatographic separation but also mass separation. Therefore, in this study, CF and its metabolites in fingerprints were analyzed using LC/MS.

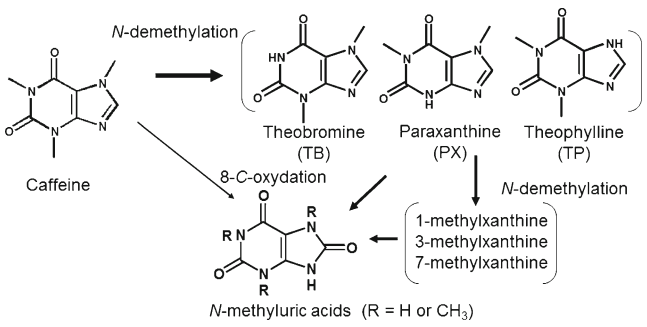

Fig. 1 CF metabolic pathways

This was a preliminary study for the detection of drugs from fingerprints of persons suspected of drug intake in criminal investigations. The relationship between the time elapsed after consuming coffee and the amounts of CF and its metabolites on a subject's fingertip was determined.

Materials and methods

Materials

CF, its deuterated analog CF-D9, theobromine (TB), PX, theophylline (TP), and LC/MS grade methanol (MeOH) were purchased from Wako Pure Chemical Co. (Osaka, Japan). Instant coffee powder was supplied by Nestle Japan Ltd. (Kobe, Japan), filter papers with a diameter of 21 micro mm were obtained from Kiriyama Glass Co. Ltd. (Tokyo, Japan), and lancets for the blood pricks were obtained from Sanwa Kagaku Kenkyusho Co. Ltd. (Nagoya, Japan).

Coffee intake and CF secretion

The experiments to measure CF and its metabolites in fingerprints and blood after coffee intake were carried out under the approval of an ethics committee at the National Research Institute of Police Science (Kashiwa, Japan). Healthy subjects were asked to eat and drink nothing containing CF for 12 h before beginning the experiment. Their fingerprints and blood were taken just before they drank a measured amount of coffee. They washed their hands ordinarily with tap water and hand soap for a minimum of 10 s and then soaked both index fingers in distilled water for 10 s, to completely remove any external contamination on the surface of their fingers. The washing procedure was performed before every fingerprint sampling. After wiping their fingers with a clean towel and keeping their hands open in the air at room temperature for 30 s to facilitate sweat secretion, they pressed their both index fingertips for 30 s to filter paper previously wetted with 50 μL of distilled

water in plastic dishes (diameter, 40 μmm). The procedure was repeated to examine the variations in the amounts of analytes extracted from the fingertips. Blood (ca. 15 μL) was drawn by pricking any one of fingers except for index fingers with a lancet for self-collection. A cup of coffee was prepared by dissolving instant coffee powder containing 80 micro mg CF in hot water (ca. 100 mL). The three subjects (two men in their 30s and one woman in her 20s) consumed the coffee within 10 min to take 80 micro mg CF as the total dose. Their fingerprints and blood samples were collected 1, 3, 5, and 7 h after they consumed the coffee.

Sample preparation

To extract the analytes in the fingerprints, 50 μL of an aqueous solution of 200 ng/mL CF-D9 as the internal standard and 1.4 mL of the mixture of MeOH and water (1:4, *v*/*v*) were added in the plastic dish with the filter paper. The dish was shaken at 10 Hz for 3 min, after which the solution was transferred to a 2-mL tube and centrifuged at 15,000×*g* for 1 min. The supernatant (25 μL) was analyzed using LC/MS. To extract the analytes from blood, a 1.5-mL tube with the blood (5 μL), an aqueous solution of 200 ng/mL CF-D9 (45 μL), and MeOH (100 μL) was shaken at 30 Hz for 5 min and centrifuged at 15,000×*g* for 3 min in duplicate. The supernatant (50 μL) was transferred to another 1.5-mL tube, and water (100 μL) was added in duplicate. The tube was shaken at 30 Hz for 5 min and centrifuged at 15,000×*g* for 3 min. The supernatant (7.5 μL) was analyzed using LC/MS.

LC/MS conditions

Shiseido Nanospace SI-2 (Tokyo, Japan) and Thermo Fisher Scientific TSQ Quantum Ultra (San Jose, CA) instruments were used for LC/MS. Chromatographic separation was achieved using an octadecylsilyl column (Imtakt Unison UK-C18; 150×3.0 μmm i.d., 3 micro m, Kyoto, Japan) at 40 °C. The mobile phase consisted of an aqueous solution of ammonium acetate (5 mM) containing formic acid (0.5 %), and MeOH (65:35, *v*/*v*). The flow rate was maintained at 0.35 mL/min. The switch valve was changed from the waste line to the MS line at 1 min. Electrospray ionization was performed in positive ionization mode. The $[M+H]^+$ of the targeted compounds (CF, TB, PX, and TP) were selected as the precursor ion. Each of the compounds in the extract of fingerprint was identified with the retention time and the product ion scan spectrum of the authentic standards. Selected reaction monitoring (SRM) was used to quantify each of the compounds after the identification. The precursor and product ions monitored for the compounds were *m*/*z* 195>138 (CF), 181>67 (TB), 181>124 (PX and TP), and 204>144 (CF-D9). The MS parameters for the analysis were as follows: spray voltage, 4000 V; sheath gas (N_2) pressure, 6 Pa; auxiliary gas (N_2) pressure, 0.5 Pa; capillary temperature, 230 °C; vaporizing temperature, 450 °C; tube lens offset, 100 V; collision gas (Ar) pressure, 0.2 Pa; and collision energy, 30 eV. Data acquisition and instrument control were performed using Xcalibur software (Thermo Fisher Scientific Corp.).

Quantification of analytes

CF, TB, PX, and TP were dissolved in distilled water to make aqueous solutions of 100 μg/mL. The four solutions were combined and diluted to make eight aqueous solutions of the standard mixture (0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 μg/mL). Aliquots of 5 μL of the standard mixture's aqueous solutions were spiked onto filter paper without fingerprints, and the analytes were extracted as described in "Sample preparation." The concentration of analytes in calibration solution was converted to the amount in fingerprint (nanograms per fingerprint). The calibration curves, constructed according to an internal standard method using deuterated CF, covered the range between 0.025 and 50 ng/fingerprint. The range of the calibration curves for each analyte covered the highest concentration of the analyte measured in the fingerprints. The accuracy of the quantifications was evaluated by the percent deviation (%DEV) from the nominal concentration using the formula: %DEV=100×(measured concentration−added concentration)/added concentration, where the measured concentration is the concentration calculated using a calibration curve, and the added concentration is the concentration in the samples spiked with standard solutions. The precision of the measurements was expressed by the coefficient of variation of the values from four measurements. The lower limit of quantification (LOQ) was defined as the lowest spiked amount to meet signal-to-noise ratio of >10 and the accuracy obtained from a calibration curve, i.e., <15 %, with the coefficient of variation in the peak areas of <15 %. The lower limit of detection was defined as the lowest spiked amount to meet signal-to-noise ratio of >3.

Matrix effects

To examine the matrix effects that originated from the fingerprint components, 5-μL aliquots of the aqueous solutions of 1 and 10 μg/mL standard mixture were spiked onto drug-free fingerprints on the wet filter paper. The drug-free fingerprints were prepared by pressing fingertips onto filter paper wetted with 50 μL of water after a drug-free subject washed his fingers as described in "Coffee intake and CF secretion." We confirmed in advance that the amount of the each analyte was less than the LOQ. As controls, 5-μL aliquots of the same aqueous solutions (1 and 10 μg/mL standard mixture) were spiked onto fingerprint-free filter

papers. The matrix effects were evaluated by comparing the peak areas of the analytes extracted from the filter papers with and without fingerprints.

Recovery of analytes spiked to fingertips

The four standard's aqueous solutions (100 μg/mL, each) were combined with MeOH to prepare the standard mixture's MeOH solutions of 1 and 10 μg/mL. To examine the recovery of analytes from fingertips, 5-μL aliquots of the two standard mixture's MeOH solutions of 1 and 10 μg/mL were placed on clean fingertips known to contain analytes in amounts lesser than the LOQs. After allowing the solvent to evaporate under air-drying, the fingertips were pressed onto filter papers wetted with water (50 μL) for 30 s. As a control, 5-μL aliquots of the two standard mixture's MeOH solutions (1 and 10 μg/mL) were spiked onto fingerprint-free filter papers. The recovery was calculated from the percentages in the peak areas of analytes spiked onto the filter paper.

Analyte extraction from fingertips in various sampling conditions

To examine the effect of the collecting matrices on the amount of analytes extracted from fingertips, fingerprints were taken on paraffin sheets, polyethylene terephthalate (PET) sheets, and dry filter paper 3 h after coffee intake. Furthermore, we compared the extractions from filter paper wetted with 50 μL of distilled water and unwetted filter paper. Finally, direct extraction of the analytes from fingertips was measured: a drop of distilled water (100 μL) was gently placed on the index fingertip by using a micropipette, and the droplet was recovered from the fingertip after 30 s. The peak areas corresponding to the analytes extracted using various sampling methods were compared.

To examine the effect of contact time with the wet filter paper on the extraction of CF and PX from the fingertip, both index fingers were pressed on wetted filter paper for a definite period of time (1, 5, 10, 20, 30, or 60 s) 3 h after coffee intake. The fingers were pressed to filter paper wetted with 50 μL of water for 1 s and then washed in distilled water for 10 s. After waiting for 30 s to facilitate sweat secretion, they were pressed to other filter paper for 5 s. In this way, fingerprints with the contact times of 10, 20, 30, and 60 s were obtained sequentially.

Statistical analysis

At each sampling point, fingerprints from each subject were taken from both index fingers in duplicate as described in "Coffee intake and CF secretion," and the four measurements were used in the statistical analysis. The significant differences between the amounts of analytes extracted from the same subject's fingertips before and after coffee intake were calculated by Student's t test ($p<0.05$, $n=4$).

Results and discussion

LC/MS analysis of CF and its metabolites

TB, PX, and TP are dimethylxanthines, with each pair of two *N*-methyl moieties positioned differently, as shown in Fig. 1. The product ion spectra of PX and TP using $[M+H]^+$ (m/z 181) as the precursor ion showed the same

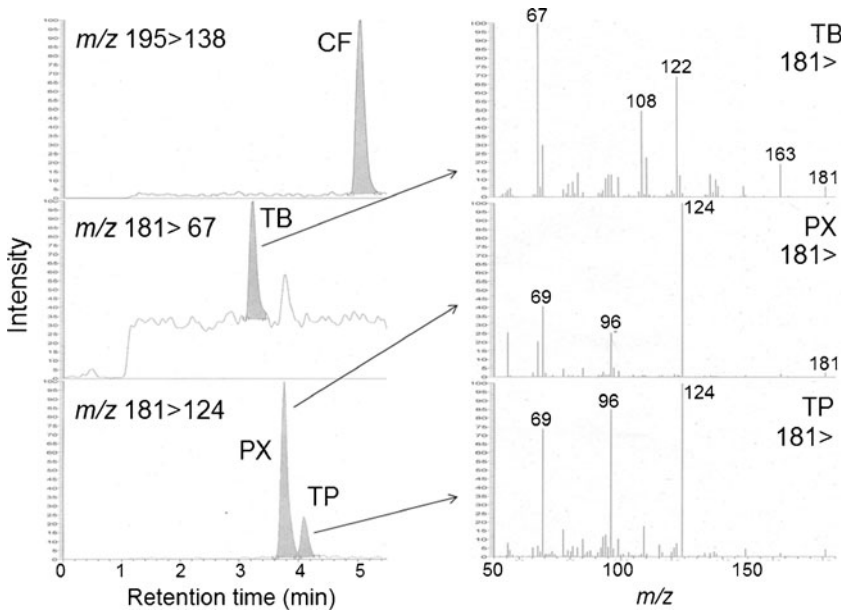

Fig. 2 SRM chromatograms and product ion spectra for CF and its metabolites. The data were obtained from a calibration solution (0.5 μg/mL, each). $[M+H]^+$ of each compound was selected as the precursor ion. The precursor and product ions monitored for each compound were m/z 195>138 (CF), 181>67 (TB), and 181>124 (PX and TP)

characteristic fragment ions (*m/z* 69, 96, and 124) (Fig. 2). In order to identify and quantify PX and TP, a baseline separation was required. We tested different columns and compositions of the mobile phase and found that the strong elution effect of acetonitrile (the mobile phase) impeded the separation of the two isomers. A subtle adjustment of the composition of mobile phases containing MeOH yielded the best separation of the two isomers; the composition is described under "LC/MS conditions." The SRM chromatograms of the analytes are shown in Fig. 2. The LC conditions enabled rapid simultaneous analysis of CF, TB, PX, and TP (5.5 min/sample) at a low-flow pressure (less than 20 MPa), which was available in conventional LC instruments.

Analytical validation

The method for analysis of CF and its metabolites extracted from fingertips was validated (Table 1). The accuracy (−4.3 to 3.9 %) and precision (<9.6 %) were within the acceptable range according to the guidelines of the US Food and Drug Administration [24]. The matrix effects of the fingerprints were examined by comparing the peak areas of the analytes spiked onto the filter papers, both with and without the drug-free fingerprints. Neither ion suppression nor enhancement was observed at 5 and 50 ng/fingerprint. In fingerprints, interference from the components would be minor, even less than that encountered in biological samples, such as blood and sweat, and neither a complicated extraction nor even deproteinization is required before the analysis. Therefore, fingerprint testing has advantages for forensic drug determinations over testing of conventional biological samples. In this study, samples extracted with the mixture of MeOH and water (1:4, *v/v*) were injected into an LC/MS instrument without prior concentration of the extracted samples. In the optimized sample preparation and under "LC/MS conditions," the total operating time from taking the fingerprints to obtaining the analytical result was approximately 10 min. In a criminal investigation, rapid testing for drugs in biological samples is important to determine the relationship between a suspect and drugs. If more sensitive detection of drugs is necessary, the sample extract can be condensed under a nitrogen flow, although the preparation time will increase. In this study, just 25 μL from the 1.5-mL sample extract was injected into the LC/MS instrument, without concentrating the samples. If the sample extract were to undergo maximum concentration (60 times), and if most of that sample was injected, the LOQs of the analytes would improve. Needless to say, the use of highly sensitive analytical instruments is also important for detecting trace amounts of analytes. The most sensitive commercially available MS instrument allows drug detection at the sub-femtogram level [25, 26]. A combination of highly concentrated samples and a highly sensitive MS instrument would enable detection of trace amounts of drugs.

Approximately 70 % of the amounts (5 and 50 ng) of analytes applied to fingertips were recovered. The rest of the analytes was assumed to have remained on the fingertips. The recovery from the spiked analytes was sufficient to detect the analytes extracted from fingertips after the subjects had ingested coffee. When the peak areas of analytes were compared between the standard solution and the extracts from filter paper where the standard solution was spiked, the differences were negligible values. Therefore, the adsorption of analytes to filter paper did not affect the recovery of analytes. When 50 ng of analytes were applied to fingertips and the fingers were washed as described in "Coffee intake and CF secretion," the amount of the each analyte was less than the LOQ. Therefore, it was confirmed that the external contamination was removed completely by the washing procedure.

Table 1 Analytical validation of CF and its metabolites

Compound	Spiked (ng/FP)	Accuracy (%)	Precision (%)	LOQ (ng/FP)	LOD (ng/FP)	Matrix effect (%)[a]	Recovery (%)[b]
CF	5	−4.3	8.4	0.5	0.05	101.1±5.6	81.0±6.0
	50	0.3	2.3			96.6±3.8	74.7±4.3
TB	5	3.9	4.4	5	0.5	116.0±19.7	80.2±12.2
	50	−3.3	3.6			98.1±8.1	78.7±3.5
PX	5	−1.7	5.9	0.5	0.05	100.3±13.0	79.8±9.6
	50	1.3	1.4			96.9±4.2	76.2±4.9
TP	5	3.3	9.6	5	0.5	78.4±10.6	67.1±5.9
	50	−1.3	1.8			95.1±6.3	72.8±4.2

FP fingerprint, *LOQ* limit of quantification, *LOD* limit of detection

[a] The peak areas from analytes spiked to filter papers with and without fingerprints were compared. The values represent the average±standard deviation of four measurements

[b] The peak areas from analytes spiked to fingertips and filter papers were compared. The values represent the average±standard deviation of four measurements

Table 2 Effects of the composition of the collecting matrices on the extraction of CF and its metabolites from fingertips

Collecting matrix	Peak area (%)[a]			
	CF	TB	PX	TP
Paraffin sheet	8.3±0.0	10.7±1.9	11.2±0.8	ND
PET sheet	0.4±0.2	ND	0.4±0.4	ND
Dry filter paper	7.2±0.7	9.9±3.3	8.5±0.4	ND
Filter paper wetted with 50 μL water	100±8.1	100±9.9	100±8.6	Trace
Direct droplet extraction[b]	45.1±15.5	36.9±10.6	39.6±11.9	Trace

ND not detected, *trace* under LOQ

[a] The percentages to the peak areas in wet filter papers were calculated. The values represent the average±standard deviation of four measurements

[b] A drop of water (100 μL) was placed on an index finger, and the droplet was recovered after 30 s

Influence of the sampling method of fingerprints on analyte extraction from fingertips

The influence of the collecting matrix used for fingerprinting on the extraction of CF and its metabolites was examined. The quantity of the analytes extracted 3 h after coffee intake differed according to the sampling methods (Table 2). The use of a paraffin sheet and dry filter paper helped extract more analyte than did PET sheet, which is attributed to the collecting matrix's affinity for the analytes. Filter paper wetted with water extracted more than ten times as much analyte as dry paper. We consider both the water solubility of the analytes and the increased area of contact afforded by the penetration of water into the grooves of the fingertips to be the contributing factors. Although a solution composed of both organic and ionic solvents could extract the analytes even more efficiently, skin contact with any liquid other than water is contraindicated for health reasons. In addition, the use of water without any organic and ionic solvents allowed us to inject the extracted samples directly into the LC/MS instrument, without inserting a step to remove the extra solvent. To extract the analytes directly with water, a drop of water was placed on the index finger and the droplet recovered after 30 s. Although the direct sampling method also extracted analytes efficiently, it was unsuitable because it was difficult to hold the droplet on the fingertip for a sufficiently long time.

The longer the contact time, the greater the amount of analytes extracted, although the rate of that increase declined with time (Fig. 3). When contact times were less than 10 s, the amount of analyte extracted varied greatly; long contact times enabled reproducible extraction of analytes. The amounts of analytes extracted from fingertips in contact for 30 s measured approximately 80 % of those for 60 s; the contact time of 30 s was adopted to minimize the burden imposed on the subjects.

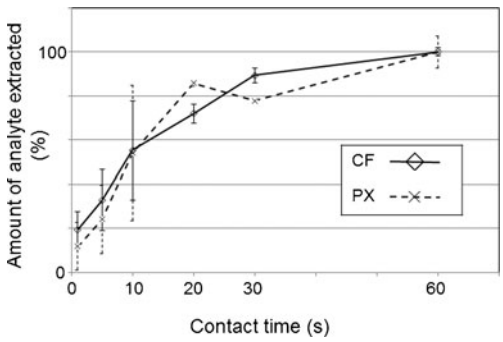

Fig. 3 Relationship of the length of the fingerprint contact period to the wet filter and the extraction of CF and PX. Three hours after coffee intake, the fingertips were pressed to a filter paper wetted with 50 μL of water for 1, 5, 10, 20, 30, and 60 s. The percentages of the amounts of analytes extracted in each contact time to that in contact time of 60 s were calculated. The values represent the average±standard deviation of four measurements

Time lapse between ingestion and appearance of CF

Although fingertip-extracted CF and its main metabolite, PX, increased gradually after coffee intake (Fig. 4), TB

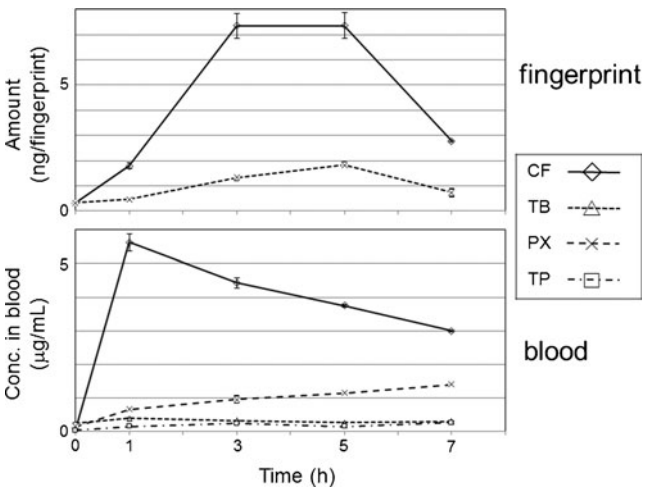

Fig. 4 Typical time courses for CF and its metabolites to appear in fingerprint and blood after coffee intake. Fingerprint and blood were taken just before coffee intake and 1, 3, 5, and 7 h afterward. The values represent the average±standard deviation of four measurements

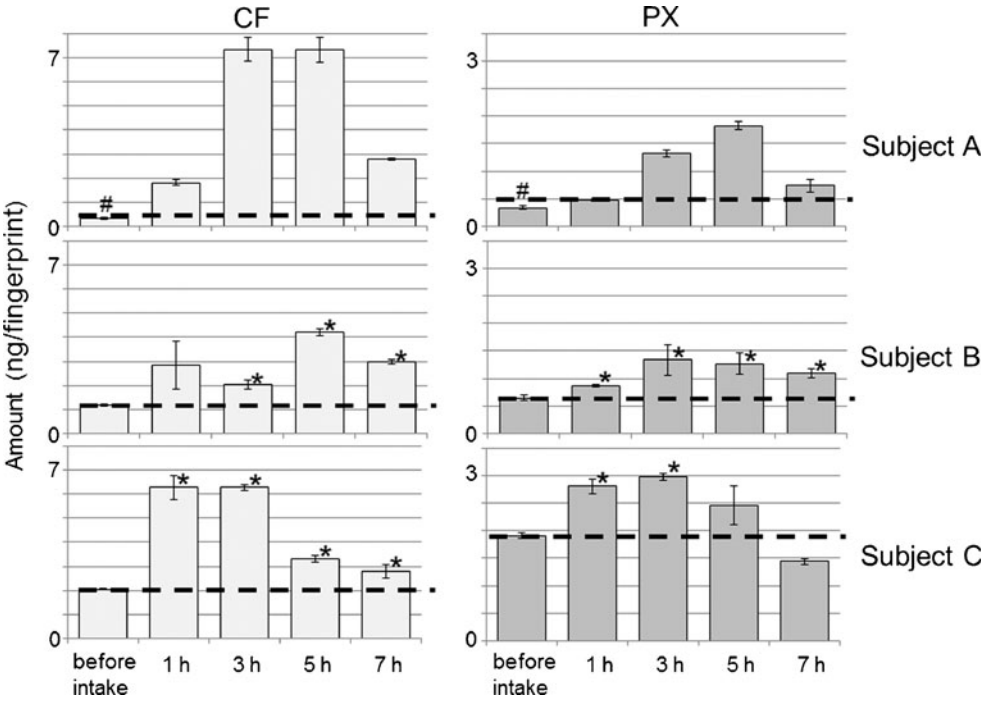

Fig. 5 Amounts of CF and PX found in fingerprints before and after coffee intake. The values represent the average±standard deviation of four measurements. *Number sign* represents a value under the LOQ. *Dashed lines* represent the values obtained from fingerprints before coffee intake (subjects B and C) or the LOQ (subject A). *$p<0.05$, a significant difference compared with amount before coffee intake

and TP were detected at trace levels below the LOQs. CF in the blood increased rapidly after coffee intake and decreased gradually, and the increase in its metabolites were slower than the increase in CF itself. For a rapidly metabolizing drug, a fingerprint could be a source superior to blood for detecting it after an extended interval from the time of ingestion. As the components of a fingerprint are contained in sweat, that secretion might also be an efficient biological sample for drug testing. However, collecting an adequate volume (ca. 15 μL) of sweat from a hand encased in a plastic glove took more than 30 min. Drug testing from sweat is hardly applicable to criminal investigations, since latent sweat stains at the scene of a crime are rare. In contrast, fingerprints can be sought and found on various materials at a crime scene, and they are a useful tool to associate a particular person to the crime. Fingerprints are an efficient source for drug testing, because sampling and extraction from fingertips are simpler and quicker than from other biological samples, such as blood and sweat.

CF and PX in fingerprints as markers to determine CF intake

Because the instant coffee used in this study contained no TB, PX, or TP, the appearance of PX in fingerprints after coffee intake may be attributed to the metabolism of CF. Detection of CF and its metabolites was important for demonstrating CF absorption in the tissues. In all three test subjects, the amounts of either CF or PX found in fingerprints taken as late as 7 h after coffee intake were significantly greater than those found before coffee intake (Fig. 5). Comparing the amounts of CF and PX in fingerprints from the same subjects before and after coffee intake allowed us to determine whether CF equivalent to at least a cup of coffee had been ingested. CF and PX were, however, detected from fingerprints just before coffee intake, although the subjects had been asked not to take CF for more than 12 h before the experiment. Because the external contaminants were excluded by washing fingers completely, the analytes detected before coffee intake were considered to derive from CF intake over 12 h before the experiment. The amounts of CF and PX in fingerprints before coffee intake varied so greatly among individuals that it was impossible to determine cutoff values for CF and PX that could indicate whether coffee had been drunk or not; thus, the amounts of CF and PX may depend on each individual's normal intake of CF. From the aspects of health and ethics, CF contained in a cup of coffee served as a convenient model for this study on drug detection.

Although drug detection from fingerprints has already been reported by Rowell et al. [8], those fingerprints were taken after the subjects had touched their foreheads and thus, the fingertips were coated with sebum. Those prints contained not only fingertip secretions but also external contamination on the fingertip surface. In this study, we removed external contaminants by washing the fingertips thoroughly. We then quantified the amounts of analytes in fingerprints obtained at definite periods of time elapsed from ingestion. To the best of our knowledge, this report is the first to relate the time course after ingestion of a drug to the amount recovered.

Conclusions

Herein, we reported an efficient method for extracting CF and its metabolites from the fingertips after the subjects had ingested coffee. Sample preparation and LC/MS conditions were optimized to take into account the burden on the subjects and the speed of the method. When wet filter paper was used as a collecting matrix to extract the analytes from fingertips and analyte extracts dissolved with the mixture of MeOH and water (1:4, *v*/*v*) were injected directly into an LC/MS instrument, without prior concentration of the samples, the total operating time—from taking the fingerprints to obtaining the analytical result—was approximately 10 min. The LOQs of CF, TB, PX, and TP were 0.5, 5, 0.5, and 5 ng/fingerprint, respectively. In all three subjects, the amounts of either CF or PX in the fingerprints taken in the 7-h period after coffee intake were significantly greater than those in the fingerprints taken before coffee intake. Compared with other biological samples, such as blood and sweat, fingerprints are a good source for extracting drugs for testing because fingerprints can be obtained and prepared easily. The amounts of ingested drugs and their metabolites in fingerprints could be useful makers for determining whether an individual has taken drugs.

Acknowledgment This work was supported in part by a Grant-in-Aid for Young Scientists (B) (24790649) from Japan Society for the Promotion of Science.

References

1. Faulds H (1880) Nature 22:605
2. Faulds H (1894) Nature 50:548
3. Herschel WJ (1894) Nature 51:77–78
4. Galton F (1892) Finger prints. Macmillan, London
5. Lee HC, Gaensslen R (2001) Advances in fingerprint technology. CRC Press, Boca Raton
6. Bush DM (2008) Forensic Sci Int 174:111–119
7. Barnes AJ, Smith ML, Kacinko SL, Schwilke EW, Cone EJ, Moolchan ET, Huestis MA (2008) Clin Chem 54:172–180
8. Rowell F, Hudson K, Seviour J (2009) Analyst 134:701–707
9. Ng PH, Walker S, Tahtouh M (2009) Anal Bioanal Chem 394:2039–2048
10. Hazarika P, Jickells SM, Russell DA (2009) Analyst 134:93–96
11. Lee D, Milman G, Schwope DM, Barnes AJ, Gorelick DA, Huestis MA (2012) Clin Chem 58:1101–1109
12. Coulter C, Garnier M, Moore C (2012) J Anal Toxicol 36:413–417
13. Stavric B, Klassen R, Watkinson B, Karpinski K, Stapley R, Fried P (1988) Food Chem Toxicol 26:111–118
14. US Food and Drug Administration (2007) Medicines in my home: caffeine and your body. US Food and Drug Administration, Silver Spring
15. Tang-Liu DD, Williams RL, Riegelman S (1983) J Pharmacol Exp Ther 224:180–185
16. Lelo A, Miners JO, Robson R, Birkett DJ (1986) Clin Pharmacol Ther 39:54–59
17. Kumazawa T, Sato K, Seno H, Ishii A, Suzuki O (1994) Forensic Sci Int 68:53–67
18. Regal KA, Howald WN, Peter RM, Gartner CA, Kunze KL, Nelson SD (1998) J Chromatogr B: Biomed Sci Appl 708:75–85
19. Muir KT, Kunitani M, Riegelman S (1982) J Chromatogr 231:73–82
20. Naline E, Flouvat B, Advenier C, Pays M (1987) J Chromatogr 419:177–189
21. Tanaka E (1992) J Chromatogr 575:311–314
22. Kizu J, Watanabe S, Yasuno N, Arakawa Y, Uzu S, Kanda S, Komoda F, Iwata T, Hayakawa H, Hayakawa T, Imai K (1999) Biomed Chromatogr 13:15–23
23. Kanazawa H, Atsumi R, Matsushima Y, Kizu J (2000) J Chromatogr A 870:87–96
24. US Food and Drug Administration (2001) Guidance for industry bioanalytical method validation. US Food and Drug Administration, Rockville
25. Momoh P, Fandino A, Aisawa E, Schlabach T, Miller K, Stafford G (2010) Technical overview: ifunnel technology for enhanced sensitivity in tandem LC/MS. Agilent Technologies, Inc, Santa Clara
26. Romanelli A, Olson L, Biesenthal T, Ghobarah H (2012) Technical note: the AB SCIEX Triple Quad™ 6500 system for bioanalysis—a new level of sensitivity. AB SCIEX, Framingham