Using sweeping micellar electrokinetic chromatography to analyze Δ^9-tetrahydrocannabinol and its major metabolites

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ABSTRACT

We have applied sweeping micellar electrokinetic chromatography (sweeping-MEKC) to the simultaneous determination of Δ^9-tetrahydrocannabinol (THC) and its major metabolites, 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH). We monitored the effects of several of the sweeping-MEKC parameters, including the proportion of organic modifier, the concentration of sodium dodecyl sulfate (SDS), the pH, and the sample injection volume, to optimize the separation process. The optimal buffer for the analysis of the three analytes was 25 mM citric acid/disodium hydrogenphosphate (pH 2.6) containing 40% methanol and 75 mM SDS. Under the optimized separation parameters, the enrichment factors for THC, THC-COOH, and THC-OH when using sweeping-MEKC (relative to MEKC) were 77, 139, and 200, respectively. The limits of detection (LODs) for the three compounds in standard solutions ranged from 3.87 to 15.2 ng/mL. We combined the sweeping-MEKC method with solid-phase extraction to successfully detect THC, THC-COOH, and THC-OH in human urine with acceptable repeatability. The LODs of these analytes in urine samples ranged from 17.2 to 23.3 ng/mL. Therefore, this sweeping-MEKC method is useful for determining, with high sensitivity, the amounts of THC and its metabolites in the urine of suspected THC users.

1. Introduction

Δ^9-Tetrahydrocannabinol (THC) is the major psychoactive component of marijuana. It is present in various parts of the plant, most abundantly in the dried flowers and trichome of the female [1]. When administered orally or by smoking, THC can cause euphoria, hallucinations, difficulties in concentration, and impairment of memory [2]. THC is metabolized to 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), which is further oxidized to 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) by cytochrome 450 isoenzymes in the liver. Because this metabolism occurs quickly, THC belongs to the class of short-half-life drugs. Unmodified THC is barely detectable in urine, while THC-OH accounts for 2% of a dose; the most abundant urinary metabolite is THC-COOH [1], which is present in urine as its glucuronide (THC-COOH-glucuronide). Because THC-COOH-glucuronide standards are not available, its presence in body fluids cannot be determined readily [2]. THC-COOH-glucuronide can, however, be hydrolyzed (through basic or enzymatic hydrolysis) to THC-COOH prior to routine analysis [3]. In this manner, the presence of THC-COOH can be used to verify illicit THC use.

There is a need to detect and determine the levels of THC and its metabolites in biological samples. The abuse of marijuana is widespread and increasing; in Taiwan, owning and using marijuana, other than for medical purposes, is illicit. Several methods have been developed for the determination of THC and its metabolites, mostly through gas chromatography (GC) combined with mass spectrometry (MS) in the electron impact (EI) or chemical ionization (CI) modes [4–7]. Notably, gas chromatography/tandem mass spectrometry (GC/MS/MS), which provides full scan information from a single, selected parent ion, is a preferable technique for obtaining forensic evidence [8]. These methods are usually used for the routine screening of THC and its metabolites after positive testing through immunoassays. Although GC/MS is a powerful technique for the detection and quantification of abused drugs, it remains necessary to derivatize the analytes after liquid–liquid or solid-phase extraction (SPE). Because such derivatization is time-consuming and complicated, it may deteriorate the analytical results.

The combination of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) with liquid chromatography/mass spectrometry (LC/MS) or liquid

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chromatography/tandem mass spectrometry (LC/MS/MS) [3,9–14] allows the detection of THC and its metabolites without the need for a derivatization step. Most studies employing LC/MS or LC/MS/MS have focused on the analysis of THC-COOH because it is the major metabolite found in the blood, urine, and hair [10–14]. A few papers [3,15] have described the use of tandem mass spectrometry to confirm the presence of THC-COOH-glucuronide. In addition, the simultaneous analyses of THC, THC-OH, and THC-COOH in plasma samples after liquid–liquid extraction or SPE have also been reported [9,16]. Although LC/MS/MS methods have several advantageous features, the analytical instruments required are not generally present in common laboratories. Because capillary electrophoresis (CE) provides good separation efficiency and rapid analyses with minimal sample requirements, it has also drawn great attention from analytical chemists in forensic science [17]. Thus, we suspected that CE would be very suitable for the analysis of THC and its metabolites without the need for derivatization. To the best of our knowledge, only one report describes the use of non-aqueous CE with electrochemical detection for the determination of cannabinoids in hair [18].

The major concern when using CE in forensic analyses of real samples is the lack of sensitivity of UV detection toward illicit drugs, the concentrations of which are usually low because of drug metabolism. Several methods have been developed, however, to increase the sensitivity and enhance the detection limits of CE. Quirino and Terabe reported a number of on-line preconcentration techniques to improve the sensitivities of CE and micellar electrokinetic chromatography (MEKC) [19–21]. Because these techniques are simple to use, provide high sensitivity, and do not require additional equipment, they have found increasing uses. In addition, sweeping-MEKC provides satisfactory results when used as an on-line preconcentration technique for hydrophobic drugs [22,23]; e.g., the analysis of lysergic acid diethylamide (LSD) in mouse blood [22] and ketamine and norketamine in human urine [23]. These studies revealed the practicality of the method in terms of the linearity of the quantitative data, the repeatability of the measurements, and the applicability to real samples.

In this paper, we describe a sweeping-MEKC method for determining THC, THC-OH, and THC-COOH. The proportion of organic modifier, the concentration of sodium dodecyl sulfate (SDS), the pH, and the sample injection volume were all optimized to increase the sensitivity. We observed sensitivity enhancements when using sweeping-MEKC, rather than MEKC, to analyze these compounds. Combining the optimized sweeping-MEKC conditions with SPE for sample clean-up allowed us to analyze THC and its metabolites in human urine samples.

2. Experimental

2.1. Chemicals

All reagents and chemicals were of analytical grade. Δ^9-Tetrahydrocannabinol (THC, 1.0 mg/mL in methanol), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH, 100 μg/mL in methanol), and 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH, 1.0 mg/mL in methanol) were obtained from Cerilliant Corporation (Round Rock, TX, USA); their chemical structures are provided in Fig. 1. SDS was purchased from Sigma (St. Louis, MO, USA). Disodium hydrogenphosphate (Na$_2$HPO$_4$) and sodium hydroxide (NaOH) were purchased from Fluka (Buchs, Switzerland). Citric acid was obtained from Merck (Darmstadt, Germany). Methanol was purchased from Echo Chemical (Miaoli, Taiwan). Ethyl acetate was purchased from Grand Chemical (Bangkok, Thailand). Phosphoric acid and glacial acetic acid were purchased from J.T. Baker (Phillips-
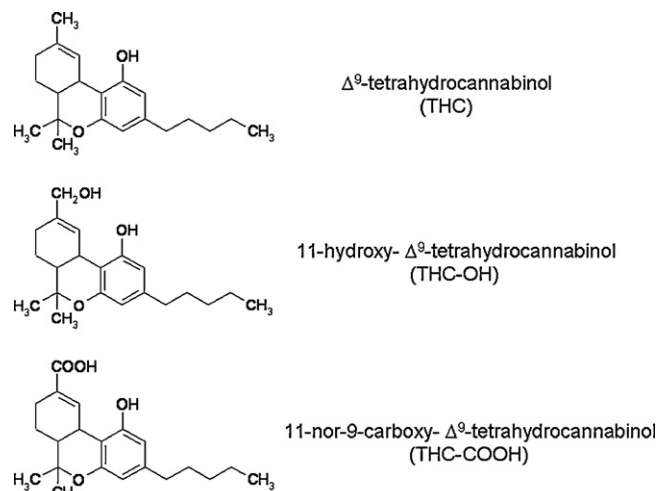

Fig. 1. Molecular structures of THC, THC-OH, and THC-COOH.

burg, NJ). Hexane was obtained from BDH Chemicals (Poole, Dorset, England). Water was purified through a Milli-Q water system (Millipore, Milford, MA, USA). The blank urine samples were collected from a young female volunteer; urine samples obtained from suspected THC users were donated by the Command of Army Force Military Police, Forensic Science Center, Taiwan.

2.2. Apparatus

A Beckman P/ACE MDQ CE system (Beckman, Fullerton, CA, USA) was used to effect the separations. A diode-array detector was employed for detection. Separations were performed in a 60 cm (50 cm effective length) × 50 μm I.D. fused-silica capillary tube (Polymicro Technologies, Phoenix, AZ, USA). The capillary tube was assembled in the cartridge format. A personal computer using 32 Karat software controlled the P/ACE instrument and allowed data analysis. Prior to use, the separation capillary was preconditioned sequentially with methanol (10 min), 1 M HCl (10 min), deionized water (5 min), 1 M NaOH (10 min), and then deionized water again (5 min). In the sweeping-MEKC mode, the capillary was flushed with water (3 min) and separation buffer (5 min) between runs.

2.3. Preparing standards, calibration curves, and urine samples

Stock standard solutions of THC (1.0 mg/mL), THC-COOH (100 μg/mL), and THC-OH (1.0 mg/mL) were obtained in methanol and refrigerated at 4 °C. Prior to analysis in the sweeping-MEKC mode, the stock solution was diluted to 10 μg/mL using 25 mM citric acid/disodium hydrogenphosphate buffer (pH 2.6, 1.06 mS/cm) as the standard dilution solution. In a pH 2.6 citric acid/disodium hydrogenphosphate buffer, the molar ratio of citric acid to disodium hydrogenphosphate is ca. 4:1. The working standard was then diluted with the same buffer to the desired concentration. In the MEKC mode, the sample was diluted with methanol to 80 μg/mL.

Calibration curves were obtained after preparing solutions of the standards individually at 0.1, 1.0, 3.0, 4.0, and 5.0 μg/mL in the citric acid/disodium hydrogenphosphate buffer. Each sample was analyzed five times. The human urine samples were collected and stored at −20 °C; when required for an assay, they were thawed and warmed to room temperature. Note that the samples were not filtered or centrifuged after being warmed to room temperature.

2.4. Method procedures

The column used was a bare fused-silica capillary that was conditioned initially using a low-pH electrolyte. The electroosmotic flow was suppressed by the low pH (pH 2.6). In the MEKC procedure, samples were pressure-injected at 3.45 kPa for 3 s. The detection wavelength was set at 210 nm. The separation was effected using the micellar buffer under a negative applied potential (−20 kV). In the sweeping-MEKC procedure, the capillary was filled initially with micellar buffer and then samples were pressure-injected at 3.45 kPa for 180–420 s. Finally, a negative voltage (−20 kV) was applied to begin the sweeping process. The anionic SDS micelles moved toward the outlet; all of the hydrophobic analytes were swept by the SDS, resulting in the separations occurring in the MEKC mode [21].

2.5. Solid-phase extraction

For urine sample analysis, drug-free samples (3 mL) were spiked with solutions of THC, THC-OH, and THC-COOH (100 μg/mL, 20 μL). The samples were hydrolyzed using 10 M potassium hydroxide (KOH, 300 μL) at 60 °C for 15 min. Glacial acetic acid (400 μL) was added to the samples, which were then acidified to pH 4.3 with 50 mM phosphoric acid (3 mL) prior to extraction.

Bond Elut Certify column-type cartridges for SPE were obtained from Varian (Harbor City, CA, USA). The SPE procedure was performed following the instructions provided with the cartridge, but with some slight modifications. The cartridges ($10 \, cm^3$/300 mg) were first conditioned with methanol (2 mL) and 50 mM phosphoric acid (2 mL). The prepared sample was then loaded through the cartridge. The cartridge was washed with 50 mM phosphoric acid (9 mL) and then with a solution consisting of 70% 50 mM phosphoric acid and 30% methanol (5 mL). After drying under full vacuum, the cartridge was rinsed with hexane (1 mL). The elution solution comprised 80% hexane and 20% ethyl acetate (3 mL); the eluate was dried under a stream of nitrogen gas while heating at 40 °C. For assaying, the residue was dissolved in a citric acid/disodium hydrogenphosphate buffer (pH 2.6, 25 mM, 200 μL).

3. Results and discussion

3.1. Optimizing conditions for separation in sweeping-MEKC

In the initial sweeping-MEKC experiments, we found that the three analytes migrated together and appeared as a single peak when SDS was present in the citric acid/disodium hydrogenphosphate buffer (pH 2.6) in the absence of any organic solvent. The presence of an organic additive has the effect of varying the retention factors of the analytes and potentially improving the resolution. After adding organic solvents to the running buffer, we observed that the resolution of the analytes improved progressively (Fig. 2). When 30% (v/v) methanol was added to the buffer, the three analytes remained incompletely separated; 40% (v/v) methanol provided the optimal resolution—further addition promoted the separation, but the baseline deteriorated, the enhanced efficiency decreased, and long analysis times were required. Therefore, we added 40% (v/v) methanol to the separation buffer in our subsequent analyses.

We examined the effect of varying the SDS concentration in the running buffer in terms of signal enhancement. Fig. 3 reveals that increasing the concentration of SDS from 50 to 100 mM increased the signal intensities for THC, THC-COOH, and THC-OH. Nevertheless, when 100 mM SDS was used, the separation of the analytes was poor. We found that 75 mM SDS in the running buffer provided the optimal balance between resolution and signal enhancement.
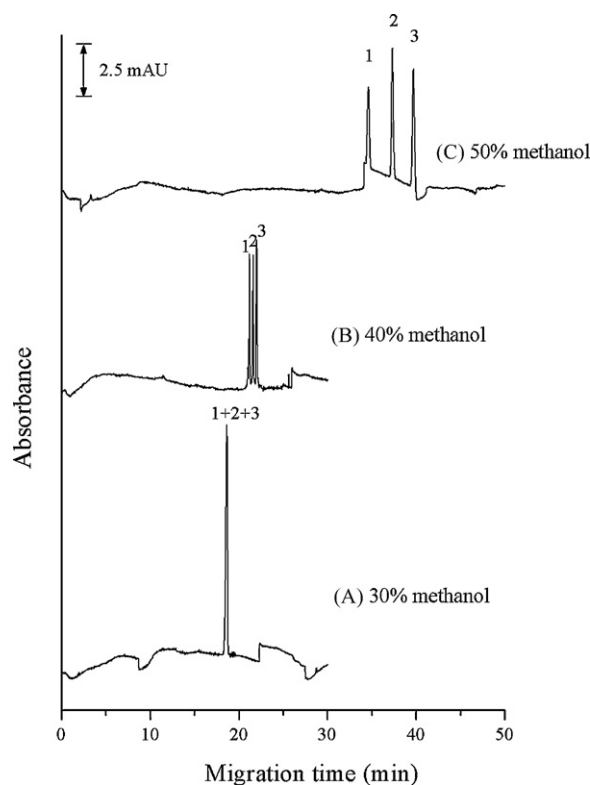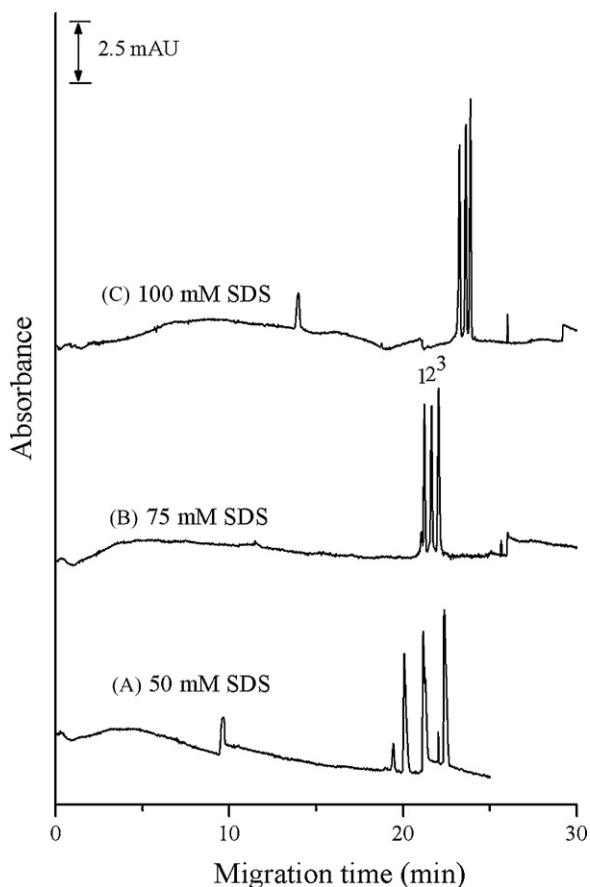

Fig. 2. Effects of (A) 30, (B) 40, and (C) 50% methanol on the sweeping-MEKC performance. Conditions: separation buffer, 25 mM citric acid/disodium hydrogenphosphate buffer (pH 2.6) containing 75 mM SDS; sample concentration, 5 μg/mL; injection, 3.45 kPa for 120 s; separation voltage, −20 kV; detection at 210 nm; effective capillary length, 50 cm. Peak identification: (1) THC; (2) THC-COOH; (3) THC-OH.

In the acidic buffer at pH 2.6, the electroosmotic flow (EOF) was suppressed; although we might expect no migration of neutral analytes under such conditions, interaction with the negatively charged SDS micelles allowed the neutral analytes to proceed to the outlet under a reversed-polarity voltage. On the basis of the formula $l_{sweep} = l_{inj} [1/(1 + k)]$, where k is the retention factor and l_{sweep} and l_{inj} are the sweeping and injection lengths, respectively, higher SDS concentrations improved the sweeping efficiency because of the higher phase ratio, resulting in a larger retention factor that shortened the swept zone. Nevertheless, higher surfactant concentrations resulted in poorer resolution. Therefore, in the following experiments, we added 75 mM SDS to the running buffer.

We examined the influence of the pH of the buffer while maintaining the concentration of SDS and the proportion of organic modifier constant. The results were satisfactory at pH 2.6, but the baseline became unstable and the analytes did not achieve baseline separation at either pH 3.6 or 4.6. Thus, to obtain accurate and quantifiable results, we performed the following experiments using the running buffer at pH 2.6.

While injecting samples at a pressure of 3.45 kPa, we examined the influence of the injection time in the range from 180 to 420 s. The peak heights of the analytes increased upon increasing the injection time to 300 s, but did not increase further after that point. The sweeping process involves the surfactants decreasing the volume of the analytes in a large volume of sample down to a narrow zone: so-called sweeping enhancement. A larger value of l_{inj} provides a higher value of l_{sweep}, but the resolution worsens if the surfactant's concentration is maintained at a constant level. The maximum value of l_{inj} is also limited by the capillary length; if too much sample is introduced into the capillary, there will be insufficient capillary length remaining to perform the separation
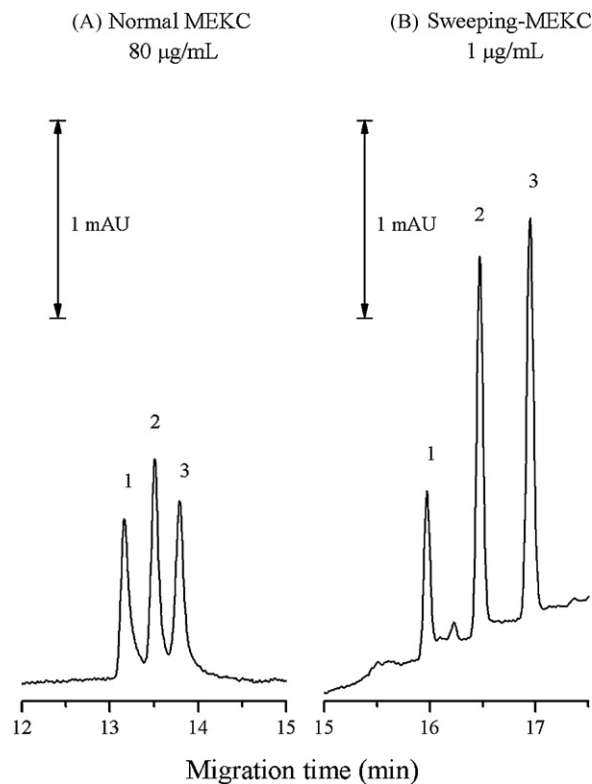
Fig. 3. Effects of (A) 50, (B) 75, and (C) 100 mM SDS on the sweeping-MEKC performance. Conditions: separation buffer, 25 mM citric acid/disodium hydrogen-phosphate buffer (pH 2.6) containing 40% methanol. Other conditions were the same as those used to obtain Fig. 2.

Fig. 4. Comparison between the normal MEKC and sweeping-MEKC methods. (A) Normal MEKC conditions: separation buffer, 75 mM SDS in 25 mM citric acid/disodium hydrogenphosphate buffer (pH 2.6) containing 40% methanol; sample concentration, 80 μg/mL; injection, 3.45 kPa for 3 s. (B) Sweeping-MEKC conditions: separation buffer, 75 mM SDS in 25 mM citric acid/disodium hydro-genphosphate buffer (pH 2.6) containing 40% methanol; sample concentration, 1.0 μg/mL; injection, 3.45 kPa for 300 s.

after sweeping, resulting in poor resolution or overlapping peaks. The additional sample injection volume also causes signal broadening, rather than an increase in intensity. Thus, the sample injection time must be optimized in terms of providing adequate separation efficiency and enhanced sensitivity. For our experiments, a sample injection time of 300 s was optimal performed at 3.45 kPa.

3.2. Sweeping enhancement

The electropherograms in Fig. 4 reveal the enhanced detection of THC and its metabolites when using sweeping-MEKC rather than normal MEKC. For the sweeping-MEKC method, the optimal separating conditions involved a buffer of 25 mM citric acid/disodium hydrogenphosphate (pH 2.6) containing 40% methanol in 75 mM SDS, with sample injection at 3.45 kPa for 300 s, providing a 15-cm sample length for THC and its metabolites. The analytes in Fig. 4 migrated earlier than those in Fig. 3 because of the extended injection time used in Fig. 4. The peak heights of the three analytes in Fig. 4 also varied, with the peak height of THC being smaller than the peak heights of the other two analytes; this situation differs from that observed in Fig. 3. Because the sweeping efficiencies were different in these figures, the sensitivities for the detection of the analytes differed accordingly. For the normal MEKC method, the running buffer was the same as the separation buffer for sweeping-MEKC, but the sample was introduced into a narrow zone, i.e., at 3.45 kPa for 3 s to obtain a 1.5-mm sample length. The enhancement factors for sweeping-MEKC over MEKC (defined as the peak height multiplied by the sample dilution factor) for the detection of

THC, THC-COOH, and THC-OH were 77, 139, and 200, respectively. These high sensitivity enhancements suggest that the sweeping-MEKC method is a powerful tool for improving the detectability of these analytes.

3.3. Calibration curve and detection limits

Table 1 lists the calibration curve equations, limits of detection (LODs), and relative standard deviations (RSDs) of the migration times of THC and its metabolites under our optimal sweeping-MEKC conditions. The linear range in the calibration curve was 0.1–5 μg/mL for all of the analytes, with each coefficient of determination (r^2) being greater than 0.9977. The LODs (S/N = 3) for the three analytes ranged from 3.87 to 15.2 ng/mL. The RSDs of the migration times were all less than 0.39%. These results clearly indicate that the sweeping-MEKC method for analyzing THC and its metabolites provides adequate linearity, enhanced sensitivity, and acceptable repeatability.

3.4. Separating and determining THC and its metabolites in urine samples

To determine low concentrations of THC, THC-COOH, and THC-OH in a urine sample, we employed SPE prior to performing sweeping-MEKC to eliminate interference from other components in the urine sample and to enhance the signals of the analytes. The complexity of biological samples such as urine and blood usually makes it difficult to analyze them without pretreatment. The matrix effect in real samples can decrease the detection sensitivity or cause

Table 1
Ranges of linearity, calibration curve formulas, coefficients of determination (r^2), LODs, limits of quantification (LOQs), RSDs, and sensitivity enhancements for THC, THC-OH, and THC-COOH when using the sweeping-MEKC method.

|	THC	THC-COOH	THC-OH
Sweeping-MEKC			
Range of linearity (ng/mL)	100–5000	100–5000	100–5000
Calibration curve	$y = 37,400x - 6700$	$y = 48,900x + 1600$	$y = 56,100x + 4000$
Coefficient of determination	0.9977	0.9979	0.9989
LOD ($S/N = 3$; ng/mL)	15.2	5.64	3.87
LOQ ($S/N = 10$; ng/mL)	50.6	18.8	12.9
RSD (%; $n = 5$)			
(a) Migration time (min)	0.26	0.34	0.39
(b) Peak area	8.1	4.0	4.6
Sensitivity enhancement	77	139	200

the signals of the analytes to overlap with those of unknown compounds. Thus, we performed SPE to reduce the matrix effect from the urine sample. It was necessary for us to hydrolyze THC-COOH-glucuronide to THC-COOH prior to performing SPE to determine its concentration in the urine sample. Fig. 5 presents the sweeping-MEKC electropherograms obtained for the urine samples after basic hydrolysis and SPE treatment. Although we observed no signals for the analytes in the blank urine sample, signals for THC and its metabolites are clearly visible in the electropherograms of the urine sample spiked with these analytes. We confirmed the identities of these peaks by comparing the migration times with those of the standard samples. The electropherograms also reveal that basic hydrolysis and SPE treatment allowed us to analyze the presence of these compounds in urine samples without other unknown analytes interfering in the sweeping-MEKC process. Under the optimized conditions, the recoveries for THC, THC-COOH, and THC-OH were 53.7, 63.6, and 83.9%, respectively.

Finally, we used the sweeping-MEKC method combined with basic hydrolysis and SPE to analyze real urine samples obtained from suspected THC users. We could clearly distinguish the peak for THC-COOH in the urine sample within 20 min [Fig. 6(A)]. Fig. 6(B) displays the electropherogram of this urine sample spiked with a THC-COOH standard (540 ng/mL); the signal for THC-COOH did indeed increase relative to that in Fig. 6(A). We determined that the concentration of THC-COOH in this urine sample from the suspected THC user was 170 ng/mL. The presence of THC-COOH was confirmed using GC/MS analysis.
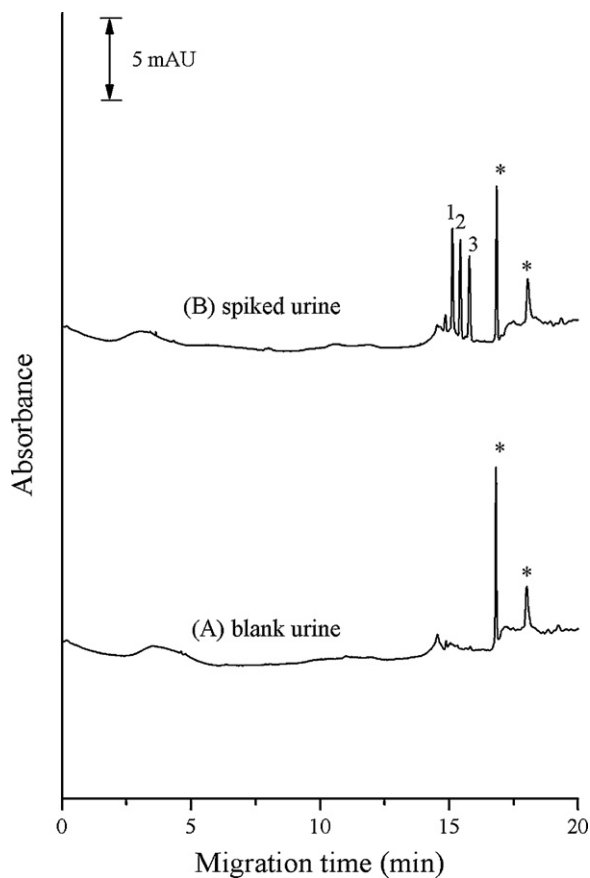
Fig. 5. Sweeping-MEKC electropherograms of SPE-extracted samples of (A) blank urine and (B) blank urine spiked with analytes (0.67 μg/mL). The analyses were performed according to the optimized conditions of the sweeping-MEKC method; *unknown. Other capillary conditions were the same as those used to obtain Fig. 2.
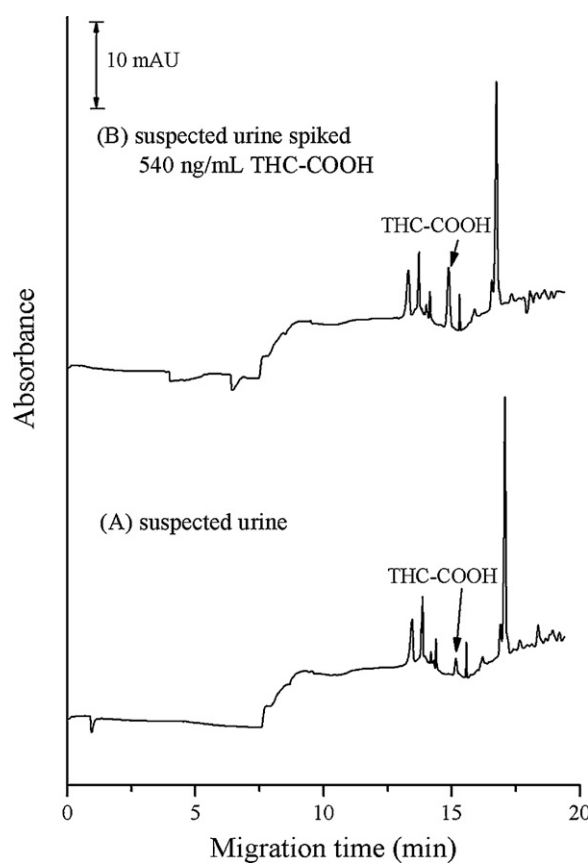
Fig. 6. Sweeping-MEKC electropherograms of SPE-extracted samples of (A) the urine of a suspected marijuana user and (B) the same urine sample spiked with a THC-COOH standard (540 ng/mL). Other capillary conditions were the same as those used to obtain Fig. 2.

4. Conclusions

Applying sweeping-MEKC is an acceptable means of performing the on-line concentration and separation of THC, THC-COOH, and THC-OH within 20 min. Under the optimized separation parameters, the enrichment factors for THC, THC-COOH, and THC-OH under sweeping-MEKC (relative to MEKC) were 77, 139, and 200, respectively; the corresponding LODs ranged from 3.87 to 15.2 ng/mL and the repeatability was acceptable. We also analyzed a urine sample from a suspected THC user; the sweeping-MEKC method after SPE revealed that the level of THC-COOH was 170 ng/mL. The total analysis time including sample preparation was ca. 80 min. Unlike the traditional GC/MS technique, this new method does not require a complicated derivatization process to obtain low detection limits. Combining the optimized sweeping-MEKC conditions with SPE for sample clean-up allowed the analysis of THC and its metabolites in human urine samples. Our results suggest that this sweeping-MEKC method will be a useful tool for other forensic or drug metabolism analyses.

Acknowledgments

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