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On-line preconcentration and determination of ketamine and norketamine by micellar electrokinetic chromatography Complementary method to gas chromatography/mass spectrometry

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Abstract

We have investigated a rapid, simple, and highly efficient on-line preconcentration method using in micellar electrokinetic chromatography (MEKC) for the analysis of abused drugs. Ketamine is an anesthetic that has been abused as a hallucinogen. We applied the sample sweeping technique first to ketamine and its major metabolite, norketamine, and separated the analytes with MEKC. Several of the sweeping MEKC parameters to effect successful separations, such as the concentration of sodium dodecyl sulfate (SDS), the injection time, and the applied voltage were optimized. The improvements in the number of theoretical plates under the different separation conditions are presented clearly in a three-dimensional representation. The limits of detection were 2.8, 3.4, and 3.3 ng/mL for ketamine, norketamine, and ketamine-D_4, respectively. The enrichment factor for each compound was within the range of 540–800. Experimental results are in agreement with those of analysis conducted by gas chromatography/mass spectroscopy (GC/MS). Therefore, we believe that sweeping, combined with MEKC, represents a suitable complementary method to GC/MS for use in clinical and forensic analyses of ketamine and norketamine.

Keywords: Ketamine; Norketamine; Sweeping; Micellar electrokinetic chromatography; On-line preconcentration

1. Introduction

Ketamine is familiar to emergency physicians as a dissociative anesthetic that has been abused as a hallucinogen for almost 30 years. Ketamine produces effects similar to phencyclidine (PCP) in conjunction with the visual effects of lysergic acid diethylamide (LSD) [1]. Ketamine is available as either a powder or a liquid; in its powdered form, it can be inhaled nasally, smoked, or mixed into drinks; in its liquid form, it can be injected or applied to, for example, cigarettes. Ketamine is metabolized to at least two compounds of pharmacological interest. First, ketamine undergoes *N*-demethylation mediated to form norketamine in the liver. Then, norketamine's cyclohexanone ring undergoes oxidative metabolism to form dehydronorketamine. Current techniques for analyzing ketamine include the use of high-performance liquid chromatography (HPLC) [2–5] and gas chromatography in conjunction with mass spectroscopy (GC/MS) [6]. These approaches almost always employ liquid–liquid extraction (LLE), solid-phase extraction (SPE), or solid-phase microextraction (SPME) techniques to obtain the target substances.

Capillary electrophoresis (CE) is a separation method – based on a physical process quite different from that of chromatography – that has been the focus of much attention for developing new analytical methodologies [7–9]. CE is a powerful technique that is simple, provides rapid results, has high efficiency, resolution, and sensitivity, and involves low sample consumption; additionally, many CE instruments are available commercially. CE is a rapidly growing separation technique that is being applied in bioscience, pharmaceuticals, environmental, food science, and forensic research [10]. Micellar electrokinetic chromatography (MEKC), which is one of the basic modes of CE, has become a popular technique for improving CE separation efficiency for both neutral and

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charges analytes [11]. Unfortunately, the benefits provided by the high number of theoretical plates obtained with CE can be overshadowed by the low sensitivity of UV detection systems. Because of the small dimensions of a CE capillary – the typical inside diameter (I.D.) and length are 25–100 μm I.D. and 40–80 cm, respectively – only very small sample volumes may be loaded onto the column. Additionally, for most common optical detection techniques, CE suffers from a drastically reduced pathlength relative to, for example, LC. Overcoming the poor sensitivity of CE with on-line sample preconcentration has been the focus of many investigations [12–14]. For example, Quirino and Terabe [15–18] found that neutral compounds could be analyzed effectively when utilizing the technique of MEKC combined with stacking. In 1998, they reported a sweeping method that can effect infiltration of analytes into the pseudostationary phase of the sample zone by applying an electric potential [19]. This technique is a new one for the on-line sample concentration of neutral or charged analytes in MEKC [20,21]. The sample solution does not need to be prepared in a low-conductivity matrix, but the conductivity equal to or higher than the running micellar solution is favored.

In this paper, we describe a simple and highly sensitive method for the detection of ketamine and its major metabolite, norketamine, in urine using the techniques of on-line preconcentration and sample sweeping, and combined with MEKC. We have optimized several electrophoresis parameters to effect successful separations, such as the concentration of sodium dodecyl sulfate (SDS), the injection time, the applied voltage, and the temperature. We provide a three-dimensional representation to present a clear visualization of the improvements in the number of theoretical plates with respect to the different separation conditions. We determined the optimal separation conditions for this method and decreased the amount of sample consumed and the separation time. Finally, we also compare the results of this analytical approach with those obtained when using MEKC, sweeping MEKC, and GC/MS.

2. Experimental

2.1. Chemicals

Ketamine hydrochloride (K·HCl, 1 mg/mL methanol), norketamine hydrochloride (NK·HCl, 1 mg/mL methanol), and the internal standard, [$^2H_4$]ketamine hydrochloride (ketamine-d_4, K-D_4·HCl, 1 μg/mL methanol), were obtained from Radian International. Fig. 1 displays their structures. SDS was purchased from Sigma (St. Louis, MO, USA). Disodium hydrogen phosphate (Na_2HPO_4) and sodium hydroxide (NaOH) were purchased from Fluka (Buchs, Switzerland). Citric acid was obtained from Merck (Darmstadt, Germany). Methanol, dichloromethane, *n*-hexane, isopropanol, acetic acid, ammonium hydroxide, acetone, and phosphoric acid were obtained in analytical grade

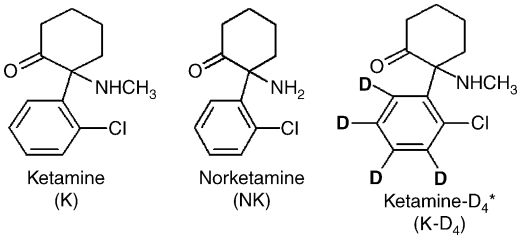

Fig. 1. The structures of ketamine, norketamine and ketamine-D_4.

(Aldrich). Water was purified by using a Milli-Q water system (Millipore, Bedford, MA, USA) and filtered through a 0.22 μm filter. All of the urine samples were donated by the Command of the Army Force of Military Police, Forensic Science Center, Taiwan.

2.2. Apparatus

A Beckman P/ACE 5500 capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) was used to effect the separations. A diode-array detector was employed for detection. Separations were performed in a 47 cm (40 cm to detector) × 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 System Gold software controlled the P/ACE instrument and allowed data analysis. The separation capillary was preconditioned prior to use with 1 M NaOH for 30 min, 0.1 M NaOH for 30 min, and then deionized water for 30 min. The sample was injected hydrodynamically and then a negative voltage was applied with the micellar background electrolyte (BGE) at both ends of the capillary to effect separation. Between runs, the capillary was flushed sequentially with 0.1 M NaOH, water, and BGE for 10 min each. The optimal buffer (pH 2.6) consisted of 25 mM citric acid/disodium hydrogenphosphate.

2.3. Sweeping and separation procedures

The column we used was a bare fused-silica capillary that we conditioned initially using a low-pH micellar electrolyte. The electroosmotic flow was suppressed by the low pH (2.6). Samples were pressure-injected at 0.5 psi. The detection wavelength was set at 200 nm. The neutral sample moved slowly because the velocity of the electroosmotic flow was very slow. The inlet and outlet of the capillary were placed in vials containing the BGE, and a negative voltage (15–30 kV) was applied. After the anionic micelles entered the sample zone, sweeping and separation were achieved through MEKC [21]. Stock sample solutions were prepared in methanol at a concentration of 100–1000 ppm. Different sample concentrations were obtained by diluting concentrated samples while keeping the sample matrix as 25 mM citric acid/disodium hydrogen phosphate and a low percentage of organic solvent (around 5–10%, v/v).

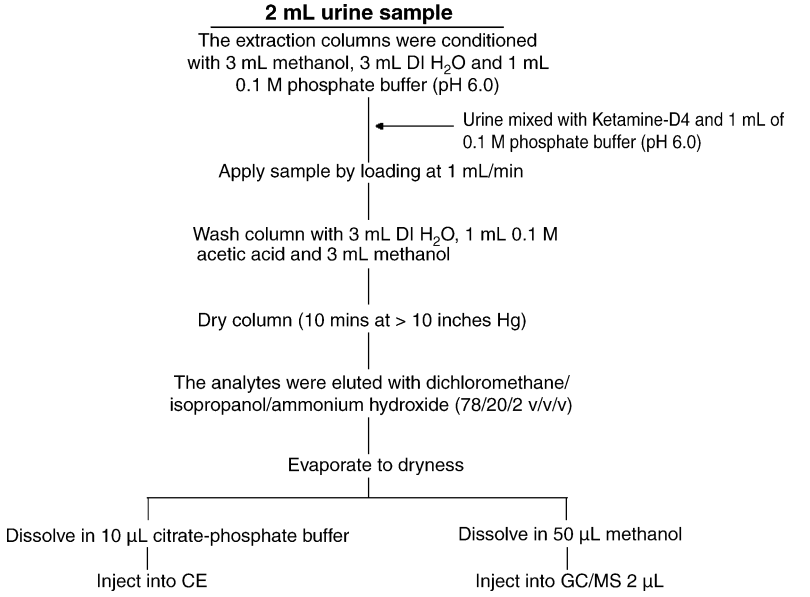

Fig. 2. The procedures used for sample preparation for urine by solid-phase extraction of urine.

2.4. GC/MS apparatus and method

A Hewlett-Packard (HP; Palo Alto, CA, USA) system was used for gas chromatography/mass spectrometry (GC/MS). It consisted of an HP 6890 series GC, an HP 5973 quadrupole mass-selective detector, and an HP 7683 auto-injector; data were collected using an HP Chem Station computer system. Helium was the carrier gas and was used at a flow-rate of 1 mL/min. The injector temperature was 250 °C. A Zebron ZB-5 MS fused-silica capillary column (30 m × 0.25 mm I.D.; 0.25 μm film thickness of 5% phenylmethylsilicone) provided the analytical separation. The retention times for ketamine, norketamine, and ketamine-d_4 (I.S.) were 9.87, 9.60, and 9.84 min, respectively. The oven temperature was programmed as follows: beginning at 120 °C (held for 1 min), the temperature was ramped to 200 °C at 15 °C/min and then held for 2 min. Next, it was ramped to 250 °C at 18 °C/min and then finally held at that temperature for 5.0 min. The total analysis time was 16.12 min. The MS system was operated in electron ionization and selected ion monitoring (SIM) modes. The spectrometer was operated under the following conditions: SIM mode; ionization energy, 70 eV; the ion temperature was maintained at 280 °C; 40–300 u at 1.84 scans/s.

2.5. Solid-phase extraction procedure

The cartridges (column type, LRC) were obtained from Varian (CA, USA). The cartridges were conditioned with methanol (3 mL), water (3 mL) and 0.1 M phosphate buffer (pH 6.0; 1 mL). The urine sample (2 mL) was mixed with ketamine-d_4 (100 μL) and 0.1 M phosphate buffer (pH 6.0; 1 mL). The column was washed with deionized water (3 mL), 0.1 M acetic acid (1 mL), and methanol (3 mL), and then it were dried under vacuum for 10 min. The analytes were eluted with dichloromethane/isopropanol/ammonium hydroxide (78:20:2, v/v/v). The clean organic phase was then evaporated to dryness. The residue was dissolved in methanol (50 μL) and a sample (2 μL) was injected into the GC/MS system. Fig. 2 provides detailed procedures.

3. Results and discussion

3.1. Optimizing the conditions for separation by sweeping MEKC

SDS is the most commonly additive used for MEKC during its separation. Fig. 3 displays typical MEKC chromatograms of ketamine (K), norketamine (NK), and ketamine-d_4 (K-D_4) that were separated in the presence of different concentrations of SDS. In Fig. 3, in addition to SDS, the buffer also consisted of 25 mM citric acid/disodium hydrogenphosphate (pH 2.6). As indicated in chromatogram of Fig. 3a, when 25 mM SDS was used, the separation of the analytes within 5 min was poor. When 50 mM SDS was used, however, the separation (Fig. 3b) began to improve as a result of increased interactions between the analytes and SDS micelles. The separation of the analyte was optimized (Fig. 3c) at an SDS concentration of 75 mM. In the acidic buffer solution (pH 2.6), the electrophoretic mobility of the neutral analytes toward the outlet (anode) is provided by the negative charged SDS micelles. The migration sequence of analytes to the outlet is based on their interaction with SDS. Thus, NK with the highest interaction with SDS migrated first. Under these conditions, we observed migration times in the following order: NK (peak 2) < K (peak 1) < K-D_4 (peak 3). When the concentration of the SDS was 100 mM (Fig. 3d), peaks K and K-D_4 became broad and overlapped.

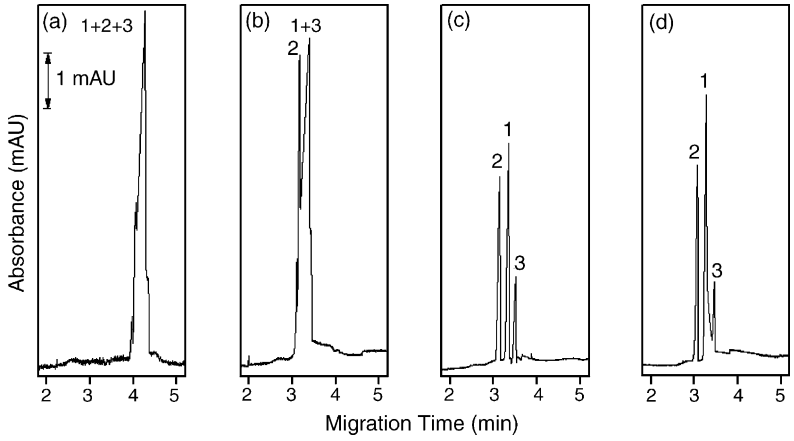

Fig. 3. Effects that different SDS concentrations have on MEKC separations: (a) 25 mM; (b) 50 mM; (c) 75 mM; and (d) 100 mM. Conditions: capillary, 47 cm long (40 cm to detector), 50 μm I.D.; 25 mM citrate/phosphate buffer (pH 2.6); applied voltage, −25 kV; detection wavelength, 200 nm; temperature, 25 °C; injection time, 4 s (0.5 psi); sample concentrations: 50, 30, and 20 ppm for K (peak 1), NK (peak 2), and K-D_4 (peak 3), respectively.

Fig. 4 illustrates the effects of different injection times on the analyte's resolution during sweeping MEKC separation. We performed hydrodynamic injection at a pressure at 0.5 psi, injected the sample solution into the capillary for 90, 120, 150, 180, or 210 s, and then applied a −25 kV potential to effect sweeping MEKC separation. The concentration enhancement of the analytes increased as the injection time increased. Injecting the sample for 150 s provided an excellent separation efficiency (Fig. 4c), but longer injection times led to incomplete peak separation; peaks 1 (K) and 2 (NK) gradually overlapped as the injection time increased, which would not allow qualitative analyses in a forensic environment.

The influence that the applied voltage had on the sweeping MEKC separation was examined in the range of potential from −15 to −30 kV (data not shown). Clearly, an applied voltage of −25 kV provided the optimal separation. Joule heating occurs upon increasing the applied voltages and results in the occurrence of diffusion phenomena, which leads to poor separation at −30 kV. Finally, we examined the effect that temperature had on the separation condition by varying the capillary temperature from 18 to 30 °C (data not shown). We found that the resolution reduced at 30 °C, so we chose 25 °C as an optimum separation temperature.

3.2. Three-dimensional representation of the effects

The number of theoretical plates changed as a function of the conditions of the many different experiments, i.e., the injection time, SDS concentration, applied voltage, and temperature; Fig. 5 provides a clear visualization of these data for K and NK in three-dimensional representation. Fig. 5a indicates the plate numbers for K and NK, respectively, in the range from 1.0×10^5 to 3.6×10^5. We have fitted continuous analytical functions to the experimental values to guide the eye; they indicate that the optimized plate numbers for K and NK of 3.48×10^5 and 2.81×10^5, respectively, occur for injection times in the neighborhood of 150 s at an SDS

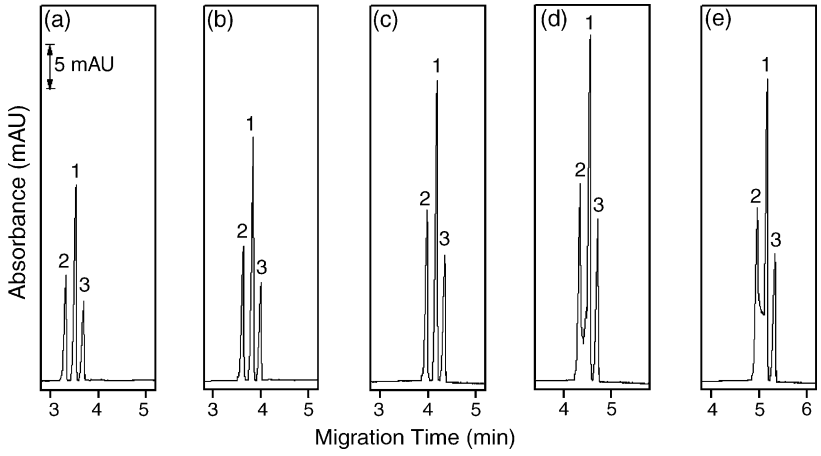

Fig. 4. Effects that different injection times have on sweeping MEKC separations. (a) 90 s, (b) 120 s, (c) 150 s, (d) 180 s, and (e) 210 s. Conditions: SDS concentration, 75 mM; sample concentrations: 500, 300, and 200 ppb for K (peak 1), NK (peak 2), and K-D_4 (peak 3), respectively. Other conditions are the same as those in Fig. 3.

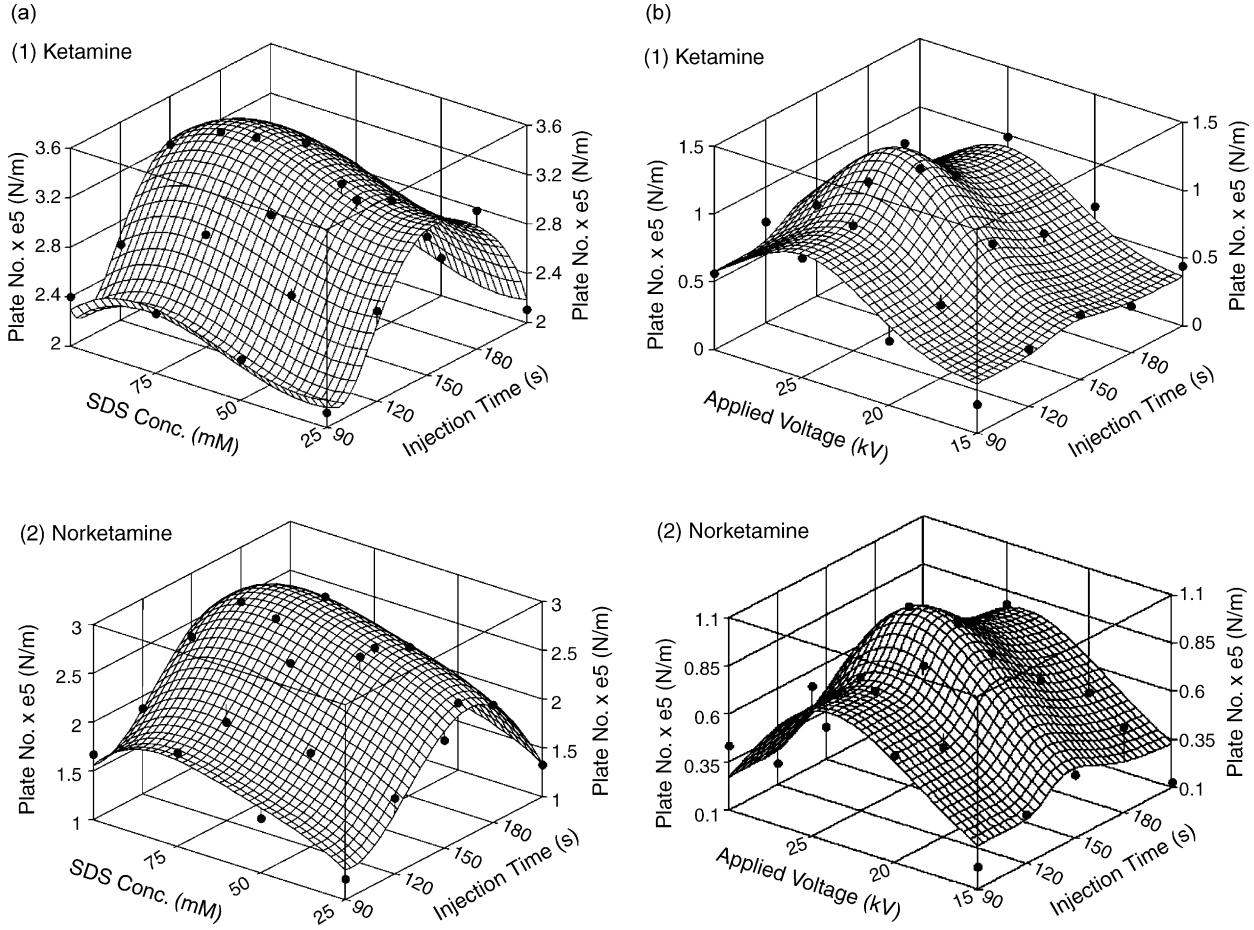

Fig. 5. Three-dimensional representation of the effects that (a) SDS concentration and injection time, (b) applied voltage and injection time have on the number of theoretical plates for (1) ketamine and (2) norketamine.

concentration of 75 mM. Fig. 5b illustrates the plate numbers for K and NK, respectively, as a function of injection time and applied voltage. By comparing the sub-figures in Fig. 5, we find that the SDS concentration is the most important condition, more so than the applied voltage or temperature (data not shown), for affecting the plate number of the separation. In comparison, the temperature effect is minimal. We believe that such a three-dimensional representation is useful for determining a range of the optimized conditions for CE separation.

3.3. Comparing MEKC and sweeping MEKC

Fig. 6 depicts the analysis of K and NK by MEKC and sweeping MEKC methods. The concentrations of the analytes K, NK, and K-D_4 were 50, 30, and 20 ppm, respectively in Fig. 6a. However, the sample concentration was diluted 100-fold used in Fig. 6b. Under these conditions, K, NK, and K-D_4 had ca. ∼760-, ∼540-, and ∼800-fold enhancements in their detection sensitivities, respectively, relative to those obtained in Fig. 6a. Table 1 presents values for the range of linearity, coefficient of determination (r^2), limit of detection (LOD), RSD, and the number of theoretical plates for K, NK, and K-D_4 using the MEKC and sweeping MEKC methods; in addition, we compare these values with those obtained when using the GC/MS method. The results indicate that the sweeping MEKC method provides better results than do the other methods for the separation of these analytes.

3.4. Separating and determining of ketamine and norketamine in suspect urine samples

Finally, we have used the sweeping MEKC method combined with SPE, was compared it with the GC/MS method, to analyze real urine samples obtained from suspected K users. First, we attempted to analyze the urine sample without extraction or sweeping, but we could not obtain a signal for K or NK (Fig. 7a). Next, we applied the same conditions as those used to obtain Fig. 7a, but with an injection time of 150 s; the resulting separation remained poor, but peaks for the target of analytes gradually appeared (Fig. 7b). Then, when we utilized SPE in conjunction with sweeping, we were able to clearly distinguish peaks for K, NK, and K-D_4 from the urine sample within 5 min (Fig. 7c). The concentrations of K and NK are 61.2 and 55.4 ppb, respectively. We also compared these results with those obtained by GC/MS for

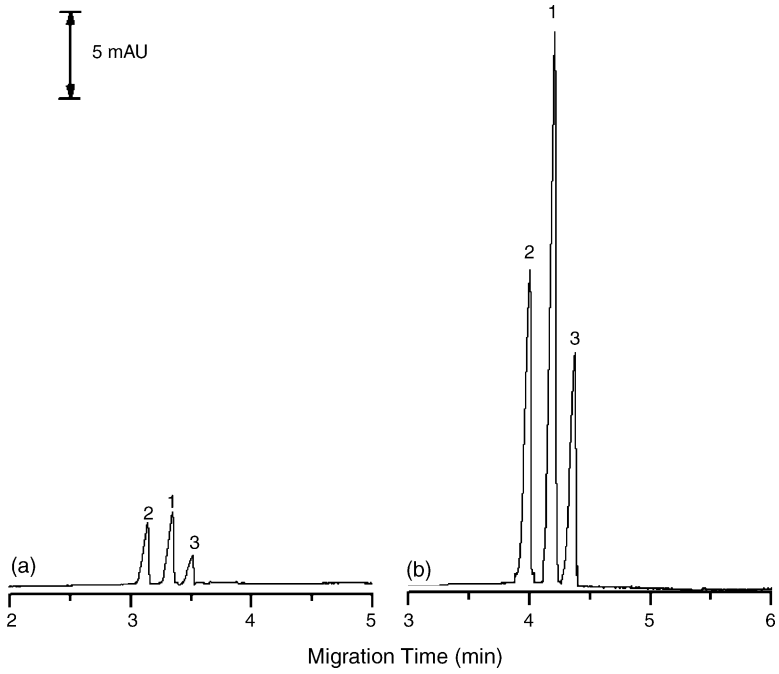

Fig. 6. Analysis of ketamine and norketamine by (a) MEKC and (b) sweeping MEKC methods. Sample concentrations: (a) 50, 30, and 20 ppm for K (peak 1), NK (peak 2), and K-D_4 (peak 3), respectively and (b) 500, 300, and 200 ppb for K (peak 1), NK (peak 2), and K-D_4 (peak 3), respectively. Other conditions are the same as those in Figs. 3 and 4.

Table 1
Values of the range of linearity, coefficient of determination (r^2), limit of detection (LOD), RSD, and the number of theoretical plates for ketamine, norketamine, and ketamine-d_4 during separation by MEKC, sweeping/MEKC and GC/MS, respectively

	Ketamine	Norketamine	Ketamine-d_4
MEKC			
Range of linearity (μg/mL)	5–500	5–500	5–500
Coefficient of determination	$r^2 = 0.9921$	$r^2 = 0.9963$	$r^2 = 0.9938$
LOD (S/N = 3) (μg/mL)	1.1	1.2	1.9
RSD (%; $n = 5$)			
(a) Migration time	3.12	4.74	3.87
(b) Peak area	4.22	3.85	4.66
Number of theoretical plates (N/m)	2.58×10^5	2.45×10^5	2.41×10^5
Sweeping MEKC			
Range of linearity (ng/mL)	5–500	5–500	5–500
Coefficient of determination	$r^2 = 0.9957$	$r^2 = 0.9984$	$r^2 = 0.9961$
LOD (S/N = 3) (ng/mL)	2.8	3.4	3.3
RSD (%; $n = 5$)			
(a) Migration time	2.11	2.03	1.89
(b) Peak area	1.76	1.92	2.04
Number of theoretical plates (N/m)	3.48×10^5	2.81×10^5	3.18×10^5
GC/MS			
Range of linearity (ng/mL)	10–1000	10–1000	10–1000
Coefficient of determination	$r^2 = 0.9992$	$r^2 = 0.9991$	$r^2 = 0.9993$
LOD (S/N = 3) (ng/mL)	5.4	7.1	4.5
RSD (%; $n = 5$)			
(a) Retention time	1.01	1.03	1.0
(b) Peak area	2.11	1.99	2.01

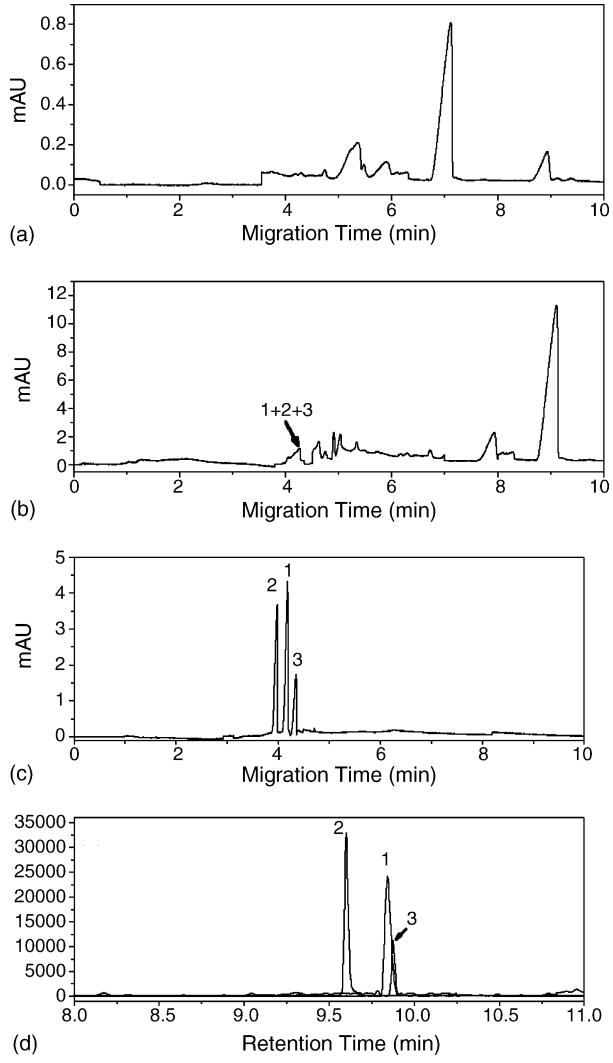

Fig. 7. Electropherograms and GC/MS traces for the analysis of a urine sample of a suspected ketamine user via (a) MEKC; (b) sweeping MEKC; (c) solid-phase extraction and sweeping MEKC; and (d) selective ion current profile measured using GC/MS methods. Conditions are the same as those in Figs. 3 and 4.

the same sample (Fig. 7d). Although the separation remained similarly as that in Fig. 7c, the analysis time was almost twice that required for using the sweeping MEKC technique.

4. Conclusions

In this study, we have demonstrated successfully the use of on-line sample preconcentration for determining the presence of K and NK by sweeping MEKC, which is an easy, rapid, and efficient technique. We have presented our results in a three-dimensional plot to provide a clear depiction of the conditions that effect the optimal separation. Under the optimized separation parameters, the analysis times for K, NK, and K-D_4 were less than 5 min, which is much faster than similar results obtained by GC/MS. The optimized parameters for the sweeping MEKC method were: running buffer, 25 mM citrate/phosphate (pH 2.6); applied voltage, −25 kV; temperature, 25 °C; SDS concentration, 75 mM. The limits of detection were 2.8, 3.4, and 3.3 ng/mL for K, NK, and K-D_4, respectively, and the enrichment factor for each compound fell within the range of 540–800. Accordingly, sweeping in conjunction with MEKC represents a good method that is complementary to GC/MS for use in clinical and forensic analyses.

Acknowledgements

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