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Simultaneous Analysis of Cocaalkaloids and Sugars in Illicit Cocaine Using Capillary Electrophoresis

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ABSTRACT: Most illicit cocaine is adulterated with other substances such as sugars and polyhydric alcohols or local anesthetics. Various sugars have been detected in seized cocaine. Analysis of sugars, polyhydric alcohols, and cocaalkaloids yields helpful information that aids in identification of the sample seized as well as the possible route of sales. We analyzed illicit cocaine directly using capillary electrophoresis. As a result, we were able to separate and detect sugars and polyhydric alcohols and cocaalkaloids using a combination of Micelle electrokinetic chromatography (MEKC) and indirect UV detection.

KEYWORDS: forensic science, cocaine, sugar, polyhydric alcohol, adulterant, capillary electrophoresis

Cocaine abuse is increasing in Japan. Most illicit cocaine is augmented with sugars and polyhydric alcohols or local anesthetics by dealers to enhance profit (1,2). Sugars and polyhydric alcohols have especially been detected in illicit cocaine. If sugars, polyhydric alcohols, and cocaalkaloids are analyzed, useful information about sample identity and possible sales routes can be obtained. Sugars and polyhydric alcohols are analyzed using gas chromatography (3,4) and liquid chromatography. However, a derivative and an RI detector are necessary, because sugars and polyhydric alcohols have high polarity and weak UV absorption (5–8). The analysis of sugars in heroin samples has been reported using an RI detector (9,10). These previous methods required sample clean up.

In recent years, capillary electrophoresis (CE) has developed rapidly, allowing for the analysis of inorganic ions, organic acids, proteins, and drugs (8). Sugars, not their derivatives, have been analyzed directly (11–15). The method employed is indirect detection. They can be detected in spite of being a weak UV absorption substance, however they are detected as a negative peak. The pk_a of sugars and polyhydric alcohols are at pH 11~14 (15). Therefore, when the pH of the buffer was raised to around 12, separation occurred.

On the other hand, various drugs have been separated using MEKC. Weinberger and Lurie analyzed impurities in heroin and cocaine using dodesyl sodium sulfate as the surface active reagent (16). Trenerry analyzed cocaine using cetyltrimethylammonium bromide (CTAB) (17). But these methods could not detect sugars and polyhydric alcohols.

In this study, we used MEKC and indirect UV detection to analyze several sugars, polyhydric alcohols and cocaalkaloids without preparatory treatment. This method can analyze the sample directly without forming derivatives, does not require any sample clean up, and does not detect any anomer peak of reducing sugars. It will prove useful for indicating illicit sale routes.

Experimental

Materials

D-glucose was obtained from Junsei Chemistry (Tokyo, Japan). Lactose, mannitol, sucrose, myo-inositol, procaine·HCl, and D-ribose (IS) were obtained from Wako Junyaku (Osaka, Japan). Cocaine·HCl was obtained from Sankyo Pharmaceutical (Tokyo, Japan). Ecgonine and benzoylecgonine were synthesized from cocaine·HCl. Potassium hydrogenphthalate, potassium sorbate, and sodium benzoic acid for VR (Visualization reagent) were obtained from Kanto Chemical (Tokyo, Japan). CTAB was obtained from Tokyo Kasei (Tokyo, Japan). Caffeine was obtained from Kanto Chemical (Tokyo, Japan). Ephedrine·HCl and methylephedrine·HCl were obtained from Dainihon Pharmaceutical (Tokyo, Japan). Quinine·HCl was obtained from Iwaki Pharmaceutical (Tokyo, Japan). Cinnamoylcocaine was used as the impurity of seized cocaine. Doubly deionized water was prepared from a Milli-Q system (Millipore, Bedford USA). Capillaries used were from GL Science (Japan). New capillaries were conditioned for 5 min with buffer. Buffer was filtered with a 0.2 μm filter before using.

Apparatus

The separating procedures were carried out on a Hewlett-Packard HP^{3D} capillary electrophoresis instrument (Warldbronn, Germany). The conditions were as follows: Column—fused silica, effect length 85 cm, internal diameter 75 μm; Injection—pressure 50 mbar 3.7 sec; Voltage—negative 27 kV; Detector—signal 310 nm. reference 200 nm; and Vial—2 mL glass vial.

Sample Preparation

A 7.5 mg quantity of IS was dissolved in 100 mL of water. Each of the sugars and polyhydric alcohols (1–1.5 mg) was added to 10 mg illicit cocaine which did not contain sugars and polyhydric alcohols. They were dissolved in 980 μL water and 20 μL IS solution was added. These constituted the measurement samples.

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Results and Discussion

Selection of Visualization Reagent (VR)

The VR was selected as follows: potassium hydrogenphthalate, sodium benzoic acid, and potassium sorbate were each examined (13). Electrophoretic mobility of potassium hydrogenphthalate was moderate, while sodium benzoic acid and potassium sorbate were both slow (8,18). As a result, the three types did not differ much in degree of separation. However sodium benzoic acid and potassium sorbate showed interference near the IS peak, thus potassium hydrogenphthalate was selected as the VR.

The pK_a of each of the sugars and polyhydric alcohols is in the 11–14 range (15). Thus the pH was changed in this range, and the separations were examined. As a result, separation of sugars improved when pH increased (Fig. 1). All sugars separated at pH of 12.5, but the S/N ratio was lower at pH values higher than 12.5. Thus pH 12.5 was used.

Selection of Organic Solvent

We added organic solvent to the buffer, and examined its influence on the peak shape of cocaalkaloids. As shown in Fig. 2, peak shape improved as the concentration of acetonitrile increased, with optimum shape at 15%. Therefore, the concentration of acetonitrile was set at 15%. It was thought that cocaalkaloids were taken into the micelle when acetonitrile increased. As a result, their mobilities were slowly increased because of having a positive charge on the micelle. Therefore peak shape improved.

Selection of CTAB Concentration (MEKC)

There have been reports of sugars analyzed using CE (11–15). But separation of cocaalkaloids was not successful. Therefore, MEKC is necessary, since cocaalkaloids do not separate by a change in pH alone. The critical micelle concentration of CTAB is 0.92 mM (25°C) (8). The cocaalkaloids were therefore examined at a lower micelle concentration (0.5 mM) and at higher micelle concentrations. The results (see Fig. 3), show that cocaalkaloids did not separate at 0.5 mM. They did separate at CTAB concentrations higher than 2 mM. Separation improved as the CTAB became higher. The peak of benzoylecgonine separated from the other peaks at 10 mM. Separation of cocaalkaloids improved when the concentration of CTAB was higher than 10 mM, but sensitivity of sugars decreased. Bromide ion has absorption at low UV wavelength (200 nm). Sugars also have absorption at low UV wavelength. Therefore sensitivity of sugars decreased as bromide ion increased. Thus the limit of CTAB concentration was set at 10 mM.

Selection of Na_2HPO_4 as Buffer Component

Potassium hydrogenphthalate, the VR, does not possess any buffering effect at pH 12.5. Therefore, we checked the influence of Na_2HPO_4 which does have a buffering effect at pH 12.5. As a result, separation of sugars improved as the concentration of Na_2HPO_4 increased, and all sugars separated at 8 mM (Fig. 4). It was thought that stacking was occurring to increase phosphoric acid, resulting in improved separation. However the sensitivity decreased because of the effects of indirect detection. Thus the con-

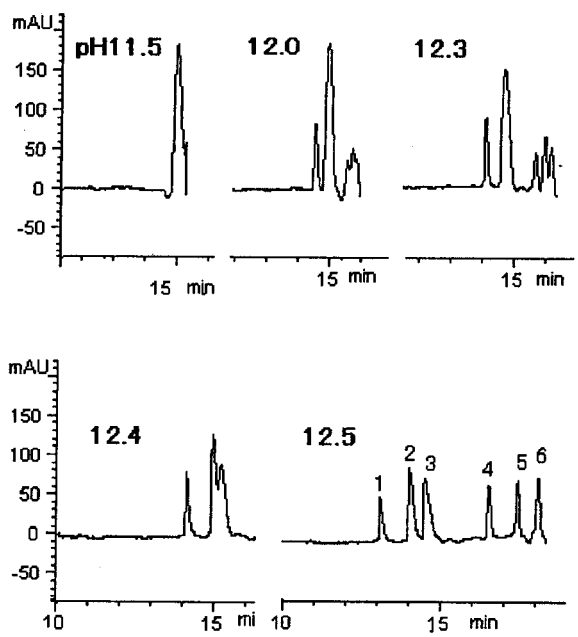

FIG. 1—*Effect of pH, Conditions; Buffer: 15% (v/v) acetonitrile in 8 mM Na_2HPO_4/5 mM phtalate/10 mM cetyltrimethylam monium bromide; Capillary: fused silica effect length = 85 cm, id = 75 μm, Voltage: negative 27 kV; Temperature: 15°C; Detector wavelength: signal 310 nm, reference 200 nm, 1:Ribose, 2:Glucose, 3:Lactose, 4:Sucrose, 5:Mannitol, 6:Inositol.*

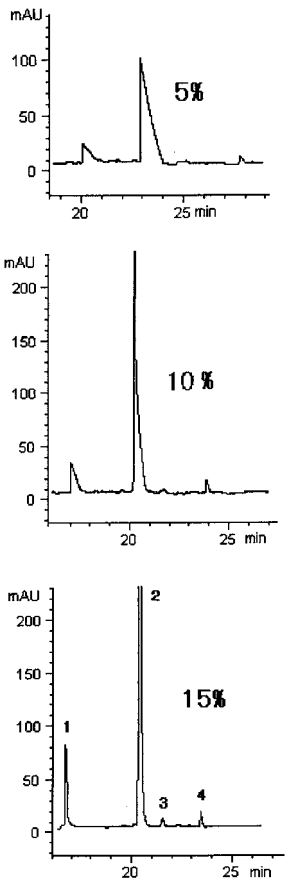

FIG. 2—*Effect of acetonitrile, Conditions; Buffer: 8 mM Na_2HPO_4/5 mM phtalate/10 mM cetyltrimethylammonium bromide, Capillary: fused silica, effect length = 85 cm id = 75 μm. Voltage: negative 27 kV, Temperature: 15°C, Detector wavelength: signal 310 nm, reference 200 nm, 1:Benzoylecgonine, 2:Cocaine, 3:Cis-cinnamoylcocaine, 4:Trans-cinnamoylcocaine.*

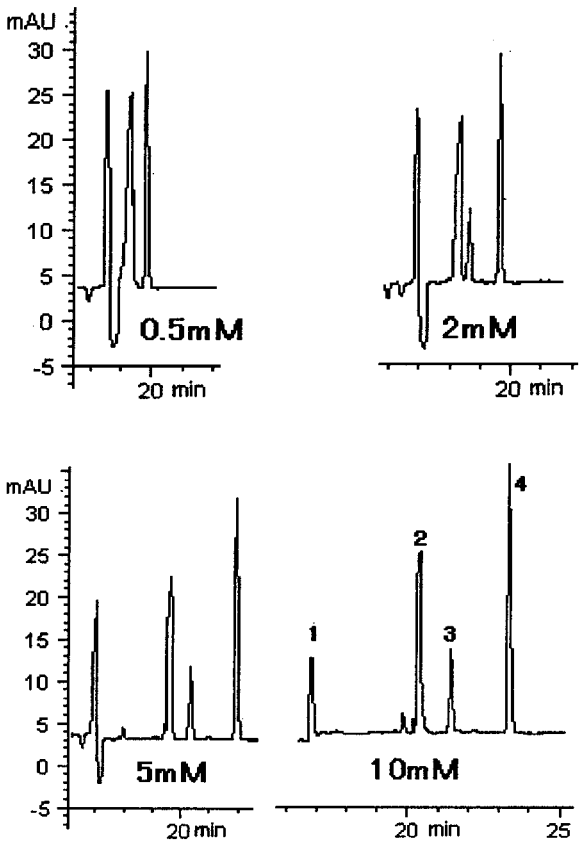

FIG. 3—*Effect of CTAB, Conditions; Buffer: 15% (v/v) acetonitrile in 8 mM Na_2HPO_4/5 mM phtalate, Capillary: fused silica, effect length = 85 cm, id = 75 μm, Voltage: negative 27 kV, Temperature: 15°C, Detector wavelength: signal 310 nm, reference 280 nm. 1:Benzoylecgonine, 2:Cocaine, 3:Cis-cinnamoylcocaine, 4:Trans-cinnamoylcocaine.*

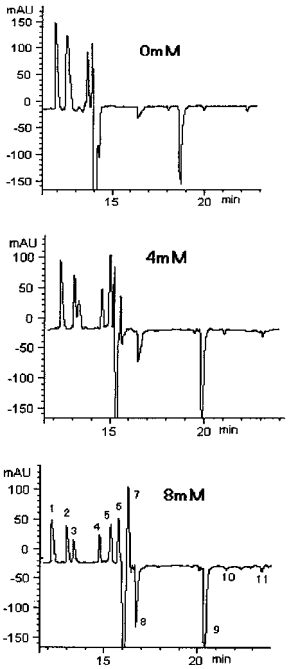

FIG. 4—*Effect of Na_2HPO_4 concentration, Conditions; Buffer: 15% (v/v) acetonitrile in 5 mM phtalate/10 mM cetyltrimethylammonium bromide, Capillary: fused silica. effect length = 85 cm, id = 75 μm, Voltage: negative 27 kV, Temperature: 15°C, Detector wavelength: signal 310 nm. reference 200 nm 1: Ribose, 2:Glucose, 3:Lactose, 4:Sucrose, 5:Mannitol, 6:Inositol, 7:Electroosmotic flow, 8:Benzoylecgonine, 9:Cocaine, 10:Cis-cinnamoylcocaine, 11:Trans-cinnamoylcocaine.*

centration of Na_2HPO_4 used, 8 mM, was the minimum concentration at which the sugars separated.

Determination of Measurement Temperature

We examined several measurement temperatures for separation of the compounds. Separation degree, along with a stable baseline, improved as the temperature was lowered. However cocaine and reducing sugars were decomposed at high temperature (7,20,21). For example, cocaine is hydrolyzed to benzoylecgonine and ecgonine. Reducing sugars are isomers to other sugars. The temperature control of the apparatus has a lower limit of 15°C. Therefore, the temperature was set at 15°C.

Determination of Capillary Length

Separation degree improved as capillary length increased. Measurement time was excessive when the length of the capillary was greater than 85 cm (effect length). Thus 85 cm was used.

Running Conditions

We examined each component used in the separation of sugars, polyhydric alcohols, and cocaalkaloids separately, employing capillary electrophoresis. The VR, pH, buffer component, and concentration were first tested. Concentration of surface active reagent, the type and concentration of organic solvent, measurement temperature, length of capillary, and conditioning method were also tested. The following running conditions were selected: Buffer—15% (v/v) acetonitrile in 8 mM Na_2HPO_4/5 mM phthalate/10 mM CTAB/pH = 12.5; Capillary—fused silica, effect length 85 cm, internal diameter 75 μm; Voltage—negative 27 kV; temperature—15°C; and detector wavelength—signal 310 nm, reference 200 nm. Electrophoretic mobilities of cocaine adulterants such as ephedrine and local anesthestics are shown in Table 1. Ecgonine, sugars, and polyhydric alcohols have the negative charge of hydroxyl groups at the high alkaline levels. Therefore they have high mobilities in buffer. Cocaine and adulterants are positively charged in the micelle and therefore have lower mobilities. So all substances separate.

Examination of Conditioning

Reproduction is influenced by the conditioning method of the CE. Optimum run conditions were found to be: 0.1 M HCl (6 min) 0.1 M NaOH (3 min) Buffer (5 min) stabilization time 1 (min). The RSDs of migration time and area ratio are shown in Table 2.

Linearity

We made a standard solution and calibration curve for sugars. Correlation coefficient, limit of detection, and linearity range for sugars and cocaine · HCl are shown in Table 3.

Recovery

Sugars and polyhydric alcohols were added to pure cocaine, and the recovery measured. The concentrations were determined from

TABLE 1—*Electrophoretic mobilities of cocaine adulterants.*

Compound	Mobility ($\times 10^{-4}$ cm^2 v^{-1} s^{-1})	Compound	Mobility ($\times 10^{-4}$ cm^2 v^{-1} s^{-1})
Ecgonine	4.51	Benzoylecgonine	2.63
Ribose (IS)	3.64	Methylephedrine	2.55
Glucose	3.44	Amphetamine	2.52
Lactose	3.33	Methylamphetamine	2.41
Sucrose	2.98	Procaine	2.28
Mannitol	2.84	Benzocaine	2.24
Inositol	2.78	Lidocaine	2.18
EOF*	2.71	Cocaine	2.11
Caffeine	2.67	Cis-cinnamoylcocaine	2.01
PPA†	2.65	Qunine	1.94
Ephedrine	2.63	Trans-cinnamoylcocaine	1.84

* Electroosmotic flow.
† Phenylpropanolamine.

TABLE 2—*Repeatability of the method (*n = *6).*

	RSD%	
Compound	Mt*	Area†
Glucose	0.6	2.2
Sucrose	0.6	2.0
Inositol	0.7	2.3
Lactose	0.6	3.5
Mannitol	0.7	2.0
Cocaine	1.1	1.5

* Migration time.
† Area ratio (sample/IS).

TABLE 3—*Limit of detection and linearity for sugars and cocaine.*

Compound	LOD*% (w/w)	Linearity Range†	r^2
Glucose	0.5	0.5–10	0.9993
Sucrose	0.5	0.5–10	0.9991
Lactose	0.5	0.5–10	0.9981
Inositol	1	0.1–10	0.9967
Mannitol	2	0.5–10	0.9956
Cocaine	0.03	0.1–18‡	0.9998

* Limit of detection in synthetic mixtures of cocaine · HCl and surgars.
† mg/mL.
‡ As cocaine · HCl.

TABLE 4—*Recoveries from synthetic mixture.*

Compound	Recoveries % (w/w)
Glucose	92.8
Sucrose	108.1
Lactose	95.5
Inositol	103.8
Mannitol	103.8
Cocaine	97.0

the calibration curve. As the results show in Table 4, all recoveries were about 92.8% or greater. Many sugars and polyhydric alcohols are added to illicit cocaine, and cocaine purity on the street is very high (22). Actually, the quantities of sugars and polyhydric alcohols were determined in seized cocaine using this method. They were 2.6–9.7% (w/w). The recovery of cocaine using this method was the same as the HPLC method (23).

References

1. Marnell T. Drug identification bible 2nd ed.: AERA-CHEM 1993;316.
2. Izonsei Yakubutu Kenkyuusya. Cocaine: Izonsei Yakubutu Kenkyuusya 1992;8.
3. Barni Comparini I, Centini F, Pariali A. Simultaneous detection of narcotics, adulterants, and dilutents in street samples by means of gas chromatography with capillary columns. J Chromatgr 1983;279:609–13.
4. Barni Comparini I, Centini F, Pariali A. Simultaneous detection of narcotics, adulterants, and dilutents in street samples. J High Resol Chromatogr & Chromatogr Commun 1984;7:140–1.
5. Touruinokoukandobunseki LC Application News: Yokokawa Anaytical Systems 1992:No. 3.
6. Sweeley CC, Bentley R, Makita M, Wells WW. Gas-liquid chromatography trimethylsilyl derivatives of sugars and related substance. J Amer Chem Soc 1963;85:2497–507.
7. Hiroshi U, Shigeo S, Mituya T, Minoru N. Eiseikagaku Kousyuueiseigaku: Asakura syoten 1988;9.
8. Susumu H, Shigeru T. Capillary denkieido kisotojissai: Koudansya 1995.
9. Wheals BB, White PC. In situ modification of silica with amines and its use in separating sugars by high-performance liquid chromatography. J Chromatogr 1979;176:421–5.
10. White PC, Jane I, Scott A, Connett BE. Use of high-performance liquid chromatogrphy to quantitate the opiate and sugar content of illicit heroine preparation. J Chromatogr 1983;265:293–300.
11. Kuhn H, Paulus A, Gassmann E, Widmer HM. Influence of borate complex on the electrophoretic behavior. Anal Chem 1991;63:1541–7.
12. Garner TW, Yeung ES. Indirect fluorescence detection of sugars separated by capillary zone electrophoresis with visible laser excitation. J Chromatogr 1990;515:639–44.
13. Vorndran AE, Oefner PJ, Scherz H, Bonn GK. Indirect UV detection of carbohydrates in capillary zone on electrophoresis. Chromatographia 1992;33:163–8.
14. Lee YH, Lin TI. Determination of carbohydrate by high-performance capillary electrophoresis with indirect with absorbance detection. J Chromatogr B 1996;681:87–97.
15. Klockw A, Paulus A, Figueiredo V, Amadò R, Widmer HM. Determination of carbohydrate in fruit juices by capillary electrophoresis and high-performance liquid chromatography. J Chromatogr 1994;680:187–200.
16. Weinberger R, Lurie IS. Micellar electrophoresis capillary chromatography of illicit drug substance. Anal Chem 1991;63:823–7.
17. Trenery VC, Robertson J, Wells RJ. The determination of cocaine and related substance by micellar electrokinetic capillary chromatography. Electrophoresis 1994;15:103–8.

18. Heiger DN. Capillary denkieidounyuumon: Yokokawa Analytical Systems 1994;32.
19. Heiger D, Weinberger R. Application note: Yokokawa Analytical Systems 1995:5963–1138E.
20. Fletcher SM, Hancock VS. Potential errors in benzoylecgonine and cocaine analysis. J Chromatogr 1981;206:193–5.
21. Fujio E, Kazutoshi N. Tansuikabutu. Asakura syoten 1969;51–2.
22. Barrio G, Saavedra P, De La Fuente L, Royuela L. Purity of cocaine seized in Spain, 1985–1995: Variations by weight, province and year of seizure. Forensic Sci Int 1997;85:15–28.
23. Noggle T Jr, Clark CR. Liquid chromatography identification of cis- and trans-Cinnamoy[1] cocaine in illicit cocaine. Anal Chem 1982;65: 756–61.

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