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APPLICATION NOTE

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Determination of cocaine on banknotes using innovative sample preparation coupled with multiple calibration techniques

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Abstract

A method using innovative sample preparation was developed for determination of cocaine on banknotes. Aqueous extraction of cocaine from banknotes was performed using a sonication-enhanced technique. Quantitation of cocaine was achieved using high performance liquid chromatography (HPLC) with UV detection at 230 nm, whereas identification was accomplished utilizing gas chromatography with mass spectrometry (GC-MS). Multiple calibration techniques, including the external calibration method (ECM), internal standard method (ISM), and standard addition method (SAM) were incorporated into the experimental design to simultaneously determine cocaine contents and assess matrix effects. Statistical paired *t* tests confirmed that matrix effects were not significant with the sample preparation employed. No damage to the features of the banknotes was observed from the extraction procedure. Extraction efficiency, spike recovery, and detection limit were also determined. The unique experimental design allowed for ECM, ISM, and SAM to concurrently determine the contents of cocaine on banknotes collected around Metro-Detroit. The concentration range of cocaine was from 1.58 to 14.7 μg per note, with an average of 6.96 μg per note. The method is simple and suitable for drug analysis and forensic science applications.

1 | INTRODUCTION

The World Drug Report 2020 released by the United Nations revealed that drug abuse around the world has been on the rise.[1] The widespread contamination of paper currency is believed to be due to the innumerous extent of cocaine distribution, transaction, and consumption.[2–4] Reports have shown that banknotes were contaminated with cocaine residue.[2–7] Cocaine is often snorted up the nose through rolled-up bills. Sorting machines in financial institutions may also cause cross-contamination, with an average detected value of 2.67 ng per note.[2,8] The presence of cocaine on paper currency provides an interesting and comprehensive framework of drug testing, research in forensic science, and of educational instruction in analytical chemistry.[2–5,9,10] The chemical structure of cocaine is shown in Figure 1.

Analysis of cocaine on paper currency commonly consists of an extraction followed by instrumental analysis using GC-MS and HPLC instruments.[6,7,11–14] Cocaine has low solubility in water (0.17 g/100 ml), so most methods employed organic solvents to extract cocaine from banknotes.[7,12–15] Components with low polarity (e.g., dyes and fibers) in the organic extracts could interfere with the determination of cocaine. The use of organic solvents also causes damage to the

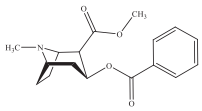


FIGURE 1 Chemical structure of cocaine

holographic marks and loosens the metallic foil used to check against counterfeit money.[3] Although cocaine is known to be relatively non-polar, its hydrochloride salt, which is a common form of street-market cocaine, is polar and quite water soluble.[15] Due to this solubility, it is possible to avoid the destruction of banknotes by using water to extract cocaine.[6,9] However, it is not clear if aqueous extraction coupled with sonication would damage the unique features of banknotes that are used to check counterfeit money.

Another challenge involved in cocaine analysis is the development of a proper procedure to minimize matrix effects of the samples. Matrix effects can be detrimental to an analysis with an instrument (e.g., HPLC) as components of the sample, other than the analyte, can alter the signal from the instrument. This influence from other species can hinder the method's sensitivity and accuracy. The matrix effects are typically handled using SAM, which requires a linear response to the analyte. The SAM is especially appropriate when the sample composition is unknown or complex, which may attenuate or enhance responses of analytes.[16] Quantitation of cocaine was often accomplished using ECM and ISM.[6,7,9,14] Both methods are not ideal to account for the matrix effects, though the ISM is useful for the variation of signal responses of analytes or of the instrument among runs. Little is known about the matrix effects associated with aqueous extraction coupled with HPLC measurements. It remains undetermined if the quantities of cocaine analyzed by ECM, ISM, and SAM are statistically different.

The aims of this study were to develop innovative sample preparation for simultaneous determination of cocaine on banknotes using the ECM, ISM, and SAM and to assess matrix effects and evaluate if the contents of cocaine determined by the ECM, ISM, and SAM were statistically different. An aqueous, sonication-enhanced technique was employed to extract cocaine from paper currency. The features of the banknotes tested were examined by a financial institution to evaluate a possible destructive effect of the procedure to the banknotes. Quantitation of cocaine was achieved using HPLC, whereas identification was accomplished using GC-MS. The statistical paired *t* test was further employed to determine if the results obtained from the ECM, ISM and SAM were significantly different. In addition, extraction efficiency, spike recovery, and detection limit were determined. The unique experimental design allowed for ECM, ISM, and SAM techniques to concurrently determine the contents of cocaine on banknotes collected around Metro-Detroit.

2 | MATERIAL AND METHODS

2.1 | Chemicals and reagents

Cocaine standard (1.000 mg/ml in acetonitrile, Cerilliant, Round Rock, USA) was used to prepare working standard solutions. Deionized water obtained from the Milli-Q Gradient unit (Millipore, Burlington, USA) was used to prepare all aqueous solutions. Hexane (+95%, spectrophotometric grade) was obtained from Aldrich (St Louis, USA). Methanol (HPLC grade, 99.9%), acetonitrile (99.95%, Chromplete grade), ammonium acetate (99.0%, ACS certified grade), and sodium hydroxide (ACS certified grade) were obtained from Thermo Fisher Scientific (Waltham, USA). Anthracene (≥ + 99.0%, Sigma-Aldrich, St Louis, USA) was used as an internal standard (I.S.). Nitrogen gas (99.998%, prepurified grade) and helium gas (99.999%, ultra-high purity grade) were obtained from PuritPlus Specialty Gas Company (Indianapolis, USA).

2.2 | Sample collection, extraction, and preparation

Two uncirculated $1 banknotes were obtained from a financial institution and assumed to not be exposed to cocaine. The uncirculated banknotes were used to determine the spike recovery of cocaine. The other $1 and $5 banknotes were collected randomly from the Metro-Detroit area. Each note was folded and placed in a 50 ml polyethylene centrifuge tube, and 20.00 ml of Milli-Q water was added to completely submerge the banknote. The centrifuge tubes were capped and placed in a beaker filled with distilled water. The beaker, containing the tubes, was placed in an ultrasonic water bath and sonicated at room temperature for 10 min. After sonication, 10.00 ml of the aqueous sample was quantitatively transferred from the centrifuge tube to a 60 ml separation funnel; 50.0 μl of 0.050 M sodium hydroxide solution was then added to the aqueous sample in the separation funnel. The solution was mixed for 20 s. The sodium hydroxide solution was added to the aqueous extract to decrease the polarity of cocaine hydrochloride salt ($C_{17}H_{21}O_4NH^+Cl^-$) by neutralization. The reaction product ($C_{17}H_{21}O_4N$) is less polar than its hydrochloride salt and thus is readily extracted by hexane. Cocaine in the aqueous sample was extracted with 4.00 ml of hexane by vortexing the liquid in the separation funnel for 1 min. After the first extraction, the aqueous phase was collected into a beaker and the hexane phase was collected into a glass vial. The same extraction procedure was used to perform two additional extractions for the same aqueous sample. All hexane extracts were collected into the same glass vial, and this sample was evaporated to dryness at ambient temperature under nitrogen. The dried sample was reconstituted with 1.00 ml acetonitrile. The reconstitution solution of each sample was treated according to Table 1. The solutions listed in Table 1 were prepared using micropipettes.

2.3 | Instrumental analysis

2.3.1 | HPLC analysis

The quantitation of cocaine was carried out using an HPLC system (LC-10AD binary pump, DGU-14A degasser, SIL-HT autosampler) with a photodiode array detector (SPD-M10A) (Shimadzu, Kyoto, Japan). The mobile phase was prepared by combining acetonitrile (CH_3CN) and an

TABLE 1 Sample preparation for quantitative analysis of cocaine

Sample ID	Volume of reconstitution solution (ml)	Volume of cocaine working standard[a] (ml)	Volume of I.S. solution[a] (ml)	Volume of acetonitrile (ml)	Total volume (ml)
S_0	0.300	0.000	0.100	0.100	0.500
S_1	0.300	0.100	0.100	0.000	0.500

Note: Two identical portions (0.300 ml each) were taken from the reconstitution solution of each sample, where one portion (S_1) was with addition of the cocaine standard solution and the other (S_0) without the cocaine standard addition. The internal standard was added to both portions (S_0 and S_1).
[a]The cocaine concentration of the working standard was 50.0 μg/ml. The concentration of anthracene as an internal standard (I.S.) was 0.0122 mg/ml.

ammonium acetate solution (CH_3COONH_4, 0.009403 M, pH 6.3) with a ratio (v/v) of 75:25 CH_3CN:CH_3COONH_4.[17] Prior to its use, the mobile phase was filtered through a pore size of 0.45-μm nylon membrane (Millipore, Burlington, USA) and degassed for 10 min. Isocratic elution was used at a flow rate of 1.00 ml/min. A C8 column (particle size 5 μm, 150 mm length × 4.6 mm i.d., Prevail Alltech, Nicholasville, USA) was used with an injection volume of 20 μl. The detection of cocaine and the internal standard was performed at 230 nm.[18] All samples were run in triplicate. The reproducibility of the instrumental signals as peak areas of cocaine was evaluated by calculating the standard deviation of the peak areas of the triplicate runs. The average percent standard deviation was 0.69%. The reproducibility of the instrumental signals as ratios of peak areas of cocaine versus peak areas of the internal standard was also evaluated by calculating the standard deviation of the ratios of the triplicate runs. The average percentage standard deviation was 0.63%. The variation of instrumental signals was similar for either the peak areas of cocaine or the ratios of peak areas of cocaine against peak areas of internal standard among the triplicate runs. The low percent standard deviation values indicate that the peak areas of both cocaine and the internal standard agreed well among the triplicate runs.

2.3.2 | GC-MS analysis

The identification of cocaine in reconstituted solutions of each sample was performed using a GC-MS instrument (Agilent 7870 GC and Agilent 5977 MS, Santa Clara, USA). The method used a HP-5 ms column (30 m × 0.25 mm × 0.25 μm, Agilent, Santa Clara, USA) with a temperature program (85°C for 3 min, ramp at 22°C/min to 260°C) modified from a previous study.[9] The temperature at the sample inlet was kept at 280°C. Helium was used as a carrier gas at 1.0 ml/min. The sample injection volume varied from fractions of 1 μl with a split-mode up to two microliters with a splitless mode, depending on the concentrations of cocaine in the samples. The electron energy of the ion source for the mass spectrometer was 70.0 eV. Mass spectra were scanned from 50 to 650 m/z at a rate of 1.5 scans/s.

2.4 | Quantitation of cocaine

Three cocaine working standard solutions (i.e., 2.50, 50.0, and 100.0 μg/ml) were prepared by diluting appropriate volumes of the cocaine standard (1.000 mg/ml) with acetonitrile. These working standards were used to prepare a series of calibration standard solutions in a concentration range from 0.125 to 80.0 μg/ml. Anthracene (I.S., 0.0122 mg/ml) was added to each calibration standard solution with a final concentration of 2.44 μg/ml. The calibration standard solutions were analyzed by the HPLC instrument. The peak area values of cocaine were corrected by subtracting the average peak area of a blank, which contained the internal standard and acetonitrile.

2.4.1 | External calibration method (ECM)

The calibration curve was constructed using the corrected peak areas of cocaine against the cocaine concentrations of the calibration standard solutions. The following equation was obtained through a linear regression analysis with a R^2 value of 0.9996.

$$Y = 49876\,X \tag{1}$$

where Y is the corrected peak area and X is the concentration (μg/ml) of cocaine. The content of cocaine per banknote (μg/note) was calculated using the equation below:

$$\mu g\,\text{cocaine/note} = \frac{Y}{49876}(0.500\,mL)\left(\frac{1}{1.00\,mL\,reconstitution\,solution}\right)x\left(\frac{20.00\,mL}{10.00\,mL}\right) \tag{2}$$

2.4.2 | Internal standard method (ISM)

The calibration curve was obtained by plotting ratios of the corrected peak areas of cocaine to the peak areas of anthracene against the cocaine concentrations of the calibration standard solutions. The following equation was obtained through a linear regression analysis with a R^2 of 0.9966.

$$Y = 0.2201\,X \tag{3}$$

where Y is a ratio of the corrected peak area of cocaine to the peak area of anthracene (I.S.) and X is the concentration (μg/ml) of cocaine. The content of cocaine per banknote (μg/note) was calculated using the equation below:

$$\mu g\,\text{cocaine/note} = \frac{Y}{0.2201}(0.500\,\text{ml}) \times \left(\frac{1}{1.00\,\text{ml reconstitution solution}}\right) \times \left(\frac{20.00\,\text{ml}}{10.00\,\text{ml}}\right) \quad (4)$$

2.4.3 | Standard addition method (SAM)

The concentration of cocaine in the reconstitution solution, C_x, can be calculated using the following equation[16]:

$$C_x = \frac{C_s V_s A_0}{(A_1 - A_0)V_x} \quad (5)$$

where C_s is the concentration of the working standard (50.0 μg/ml), V_s is the volume (0.100 ml) of the working standard added, V_x is the volume (0.300 ml) of the reconstitution solution, and A_0 and A_1 are the signals (e.g., peak area) of S_0 and S_1 in Table 1, respectively. The quantity of cocaine (Q) on each banknote can be calculated using the following equation:

$$Q\left(\frac{\mu g}{\text{note}}\right) = C_x(1.00\,\text{ml reconstitution solution}) \times \left(\frac{20.00\,\text{ml}}{10.00\,\text{ml}}\right) \quad (6)$$

2.5 | Spike recovery and extraction efficiency

2.5.1 | Spike recovery

The spike recovery was examined using two uncirculated $1 banknotes with the procedure described previously. One had 1.00 μg of cocaine added, and the other had 5.00 μg added. The two samples were subjected to the sample preparation procedure followed by HPLC analysis. The percent recovery of the method can be calculated using the equation below:

$$\%\,\text{Spike Recovery} = \frac{\text{grams of cocaine determined}}{\text{grams of cocaine initially added}} \times 100\% \quad (7)$$

2.5.2 | Extraction efficiency

The extraction efficiency of cocaine was investigated using a $1 bill that was under general circulation at the time of sample collection. The bill was first treated according to the sample preparation procedure. The note was then removed from the centrifuge tube, rinsed with Milli-Q water, air-dried on a paper towel, and subjected to the sample preparation procedure a second time. Both solutions obtained were analyzed by the HPLC method. The percent extraction efficiency was estimated by comparing the quantity (Q_1, μg/note) of cocaine from the first extraction with that (Q_2, μg/note) from the second extraction:

$$\%\,\text{Extraction Efficiency} = \left(\frac{Q_1 - Q_2}{Q_1}\right) \times 100\% \quad (8)$$

2.6 | Detection limit

To determine the detection limit (DL) of this method, a blank containing 2.44 μg/ml anthracene (I.S.) and acetonitrile (solvent) was measured nine times by HPLC. The signals of the blank were used to calculate the standard deviation. The DL is given by the following equation[16]:

$$DL = \frac{3s}{m} \quad (9)$$

Where m is the slope of a calibration curve and s is the standard deviation of signals of a blank for Equation 9.

3 | RESULTS AND DISCUSSION

3.1 | Quantitation of cocaine

The quantitation of cocaine was performed using the ECM, ISM, and SAM. Based on the designed procedure, all three of these calibration methods can be applied to one obtained dataset. Moreover, these methods can be used individually or in tandem, depending on the analyst's preference. Strong linear relationships were observed between the HPLC response signals over a wide concentration range of cocaine (0.125 to 80.0 μg/ml). As shown in Table 2, cocaine was detected on all the banknotes collected, in amounts ranging from 1.58 to 14.7 μg per note, with an average of 6.96 μg per note. Cocaine contamination on United States paper currency has been reported in previous studies. Jenkins reported the presence of cocaine on 92% of US $1 notes randomly collected from five United States cities in a range of 0.01 to 922.72 μg per note, with a median value of 1.37 μg per note.[14] Zuo et al. showed that cocaine was detected in 67% of the

TABLE 2 Quantities of cocaine on the banknote samples determined using SAM, ECM, and ISM

Banknote denomination	Quantity of cocaine (μg/note)			
	SAM	ECM	ISM	Mean
$1	2.23	2.16	2.20	2.20
$1	11.7	11.3	11.0	11.3
$1	3.48	3.32	3.19	3.33
$1	1.43	1.78	1.61	1.58
$5	14.7	14.5	15.1	14.7
$5	8.80	8.84	8.22	8.62

circulated banknotes with all denominations collected in amounts ranging from ~2 ng to 49.4 μg per note.[6] With similar sample preparations, our results agree well with the data as reported by these previous studies. Evidently, US banknotes are widely contaminated, and the levels of cocaine on individual banknotes vary greatly from nanograms to micrograms per note. Several mechanisms have been proposed to explain paper currency tainted by cocaine, including contamination during drug deals and the use of rolled up bills for snorting. The drug is also thought to be transferred indirectly from one contaminated bill to others during counting in financial institutions.[2,8]

3.2 | Evaluation of matrix and destructive effects

For a given banknote, the contents of cocaine computed by the ECM, ISM, and SAM were similar, as shown in Table 2. Evidently, the initial aqueous and subsequent hexane extractions were effective to greatly reduce the complexity of the sample matrix. As a result, interferences from other substances were potentially minimized.

To check if the features of the banknotes tested were damaged during the aqueous extraction coupled with sonication, the banknotes were brought to a financial institution. A series of tests were conducted according to the protocol set by the institution. The tests included examining the banknotes' appearance and texture, touching their surfaces, and viewing their unique features under white light and UV light. The banknotes were confirmed to not be damaged by the experimental procedure, as no damage was found on the tested currency.

Paired *t* tests gave $t_{calculated}$ values of 0.709, 0.582, and 0.956 with respect to SAM and ECM, ISM and ECM, and ISM and SAM, respectively.[16] All $t_{calculated}$ values were less than the value of t_{table} (2.571) for 95% confidence and five degrees of freedom, which showed that the quantities of cocaine determined by the three calibration methods were not significantly different.[16] The results indicated that the matrix effects were insignificant with the sample preparation used.

3.3 | Confirmation of cocaine by GC-MS

GC-MS was used to confirm the identification of cocaine in the samples by comparing the mass spectrum of each sample with that of a cocaine standard (10.0 μg/ml) under the same GC-MS conditions. Figure 2 shows a representative mass spectrum of the sample and the spectrum of the cocaine standard solution. The sample spectrum and

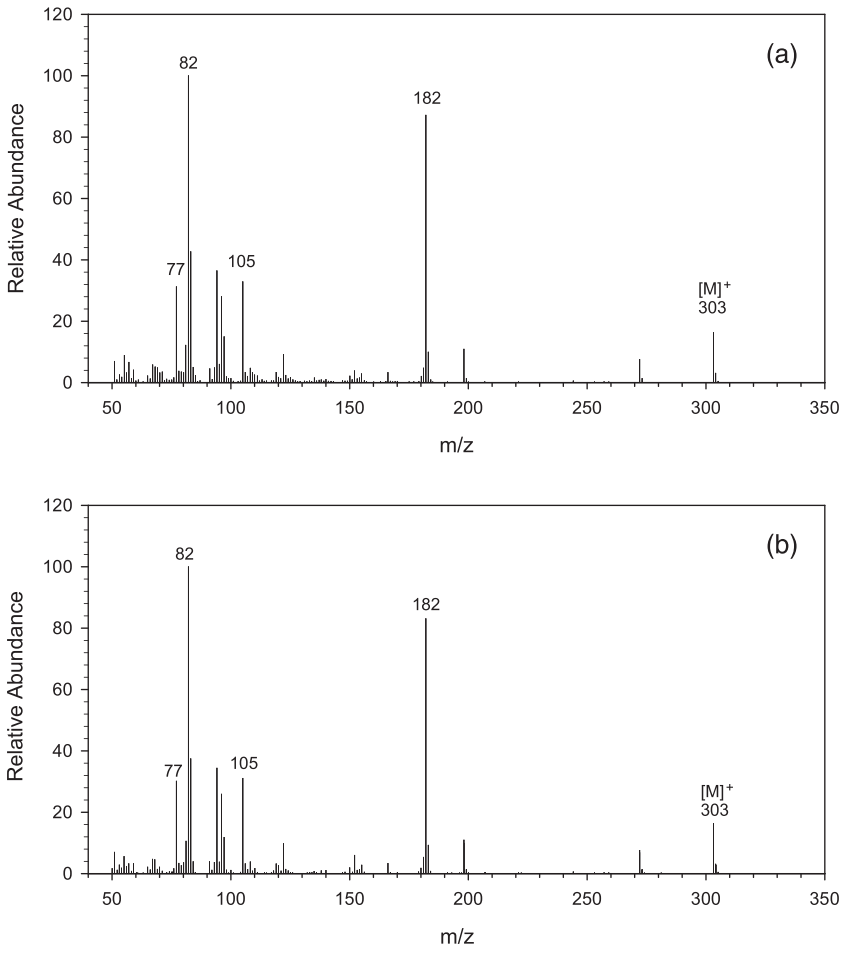


FIGURE 2 Comparison of the GC-MS mass spectra: (a) mass spectrum of the reconstitution solution for a $1 bill sample; (b) mass spectrum of the cocaine standard solution (10.0 mg/L). The mass spectrum of the sample on the top was matched to that of the cocaine standard solution on the bottom by comparison of their major fragments and the molecular ion (m/z 303) of cocaine. The mass spectrum of the sample was also matched to that of the GC-MS library to confirm the identity of cocaine.

that of the standard were matched by major fragment ions (m/z 77, 82, 105, and 182) and the molecular ion of cocaine (m/z 303). These ions are unique for the cocaine mass spectrum, and their corresponding chemical structures are listed in Table 3.[19] The mass spectra of the samples were also matched to that of the GC-MS library to confirm the identity of cocaine.

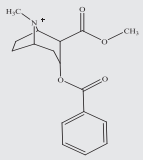
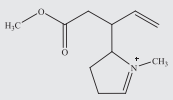
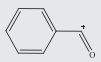
3.4 | Spike recovery and extraction efficiency

The mean values of the spike recovery determined by the ECM, ISM, and SAM were 57.5%, 46.3%, and 50.6%, respectively. The percent efficiency of extraction values obtained with the three calibration methods were 82.5 for ECM, 83.0 for ISM, and 83.4 for SAM.

3.5 | Detection limits (LD)

The average values of LD for ECM and ISM were 14.1 and 16.3 ng/ml, respectively. The mean value of LD when considering both the ECM and ISM was 15.2 ng/ml.

TABLE 3 Mass to charge ratios (m/z) of major fragments and the molecular ion of cocaine and their corresponding chemical structures[19]

Mass to charge ratio (m/z)	Chemical structures of major fragments and the molecular ion of cocaine
303 (molecular ion of cocaine)	
182	
105	
82	
77	

4 | CONCLUSIONS

A sonication-enhanced, non-destructive technique was employed to extract cocaine from paper currency. Quantitation and identification of cocaine on banknotes were conducted using HPLC and GC-MS, respectively. The experimental design incorporated the ECM, SAM, and ISM to simultaneously determine the contents of cocaine on banknotes and assess the sample matrix effects. Strong linear relationships were observed among the HPLC response signals over a wide concentration range of cocaine (0.125 to 80.0 mg/ml), with a mean detection limit of 15.2 ng/ml. The cocaine contents on the banknotes collected around Metro-Detroit were found in a range of 1.58 to 14.7 μg per note, with an average of 6.96 μg per note. Statistical paired *t* tests confirmed that the matrix effects were not remarkable because the cocaine contents determined by the three calibration methods were not significantly different. The matrix effects were greatly minimized with the sample preparation procedure developed in this study. The features of the banknotes were not damaged with aqueous extraction coupled with sonication. With the unique experimental design, ECM, ISM, and SAM can be applied individually or tandem to an obtained dataset for quantitation of cocaine. The method is simple and suitable for drug analysis and forensic science applications.

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CONFLICT OF INTERESTS

The authors have no conflicts of interests to declare.

DATA AVAILABILITY STATEMENT

Author elects to not share data.

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