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Screening for cocaine on Euro banknotes by a highly sensitive enzyme immunoassay

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ABSTRACT

This study focused on quantitative detection of cocaine on Euro banknotes in Germany. A sensitive direct competitive immunoassay was developed and optimized with a limit of detection (LOD) of 5.6 ng/L. Exhaustive cocaine extraction by solvent was tested using different methanol concentrations and buffered solutions. Cross-reactivity studies were performed to determine the degree of interference of cocaine metabolites with the immunoassay. Sixty-five Euro banknotes obtained from different districts in Berlin were evaluated. A 100% contamination frequency with cocaine was detected. A comparison between the amount of cocaine extracted by cotton swabbing of one square centimeter of the banknote showed a good correlation for lower contamination levels. This assay showed high sensitivity of detecting pg of cocaine per 1 cm^2 of one banknote by swabbing 1 cm^2: 0, 14, and 21 pg/cm^2. Moreover, three notes of different denominations revealed high cocaine concentration; 1.1 mg/note, and twice 55 μg/note.

1. Introduction

Cocaine consumption continues to grow globally. An estimated 21 million people were reported to be consumers of cocaine in 2012 [1]. Usage in Western countries remains the highest. In North and South America cocaine consumption levels increased by 1.8% and 1.2%, respectively, while the percentage dropped by 0.3% in Central and Western Europe [1].

Banknotes can be contaminated by numerous drugs: cocaine, methamphetamine, heroin, morphine, codeine, and others [2,3]. Cocaine contaminates nearly all types of banknotes including US dollars, UK pounds, and Euros [4], where the contamination frequencies reached 100% with UK and US banknotes. An average of 155 [4], 19.9 [5]. and 28.75 [4] μg/note was detected on Spanish, British and American banknotes, respectively.

One source of contamination can be through direct contact, a massive amount of cash being involved in drug trafficking with the same people handling the cocaine powder and the money. Another source of direct contamination is rolling up the banknote for sniffing the powder through the tube formed. Yet, there are also indirect pathways. Indirect contamination occurs by the contact of “clean” banknotes with the cocaine-contaminated ones in automated teller machines (ATM) and cash counting machines and has been considered a major source of contamination [6–8]. In the attempt to detect cocaine on banknotes, several analytical techniques have been employed. Chromatographic methods rank as the most common ones since coupling with mass spectroscopy provide the high sensitivity required [4]. For example, GC-MS reached a LOD for cocaine of 1 ng/mL [2], and the sensitivity increased with MS/MS coupling and positive-ion chemical ionization mass spectrometry producing LODs of 0.15 ng/note [9] and 1 ng/note [10], respectively. Liquid chromatography (LC) coupled with MS/MS indicated higher sensitivity than GC-MS/MS with a detection limit of pg per Euro banknotes as reported by Bones et al. [3].

Other analytical techniques like spectrofluorometry [11], electrochemiluminescence sensor [12], aptamer-based electrochemiluminescence biosensor [13], capillary electrophoresis (CE) with electrochemiluminescence (ECL) detection [14], CE with UV detection [15], and thermal desorption tandem mass spectrometry were also used for cocaine determination on banknotes [16].

Extraction of cocaine from bank notes has been carried out by different methods such as direct thermal desorption, vacuum systems, and solvent extraction [4]. Solvent extraction can be an effective method with reduced damage of the banknotes. The used solvents in

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extraction were chloroform [10], sodium acetate buffer [11], acetic acid [13,14], acetonitrile [2,15,17], and methanol [3,5,9,18,19]. Methanolic extraction has been described by Esteve-Turrillas et al., [9] showing a recovery rate of 98 ± 3% of the cocaine spiked. The note was rolled in a tube and solvent extracted, by agitation either using a vortex [9], mixing roller [11] or ultra-sonication bath [15,20]. De-ionized water was tested for extraction, too and good recoveries of 95%, and 93%, respectively, were obtained [20,21], a second extraction step not yielding any more recovered cocaine.

The direct competitive enzyme immunoassay has the advantage due to its high selectivity of enabling for determinations of analytes in matrix-loaden samples without the requirement of sample preparation. Highly sensitive, specific and accurate immunoassays have been described for analyzing complex samples such as urine or oral fluids [22–24]. A direct competitive enzyme immunoassay was developed by van der Heide et al. for detection of cocaine on banknotes and fingerprints, where the LOD was 162 ng/L [5]. The competitive immunoassay was validated by analyzing the same samples by LC-MS which yielded similar results [5].

In this study, our aim was to develop an extremely sensitive competitive enzyme immunoassay (ELISA) to be able to quantify cocaine on Euro banknotes circulating in Germany. Extraction should be non-destructive and optimized in terms of time and equipment requirements. Excellent sensitivity was supposed to result from a careful optimization of the assay and by optimizing the synthesis of the enzyme conjugate. Sixty-five Euro bills were collected from different areas in Berlin and analyzed and the results classified with regard to circulation time and denomination.

2. Materials and methods

2.1. Reagents and materials

The cocaine-binding antibody was an anti-benzoylecgonine (cocaine) monoclonal antibody [clone IP3G2] (mouse IgG1), from GeneTex (Irvine, CA, USA) purchased from Acris Antibodies GmbH (now OriGene EU, Herford, Germany; cat. no. MAB4029, 3.75 mg/mL). The secondary antibody, a polyclonal anti-mouse IgG from sheep (cat. no. R1256P, 2.2 mg/mL), was also obtained from Acris. Cocaine hydrochloride, Ph Eur, BP, USP, cryst, extra pure, Cat. No. 1.02562.0005 was obtained from Merck KGaA, Darmstadt, Germany, norcocaine hydrochloride (Cat. no. COC-266-HC) from Lipomed (Weil am Rhein, Germany), benzoylecgonine tetrahydrate (D745) was obtained from the National Measurement Institute of Australia, North Ryde, NSW, 1670, Australia. Horseradish peroxidase (HRP, EIA grade) was obtained from Roche. N,N-Dicyclohexylcarbodiimide (DCC, puriss), tetrabutylammonium borohydride (purum, ≥97%), hydrogen peroxide solution ≥30% (TraceSELECT®), N-hydroxysuccinimide (NHS, 98%) N,N-dimethylformamide (DMF, ≥99.5%), sodium phosphate dibasic dehydrate (purum p.a.), sodium phosphate monobasic dihydrate (BioUltra, ≥99%), sodium chloride (BioUltra, ≥99.5%), potassium phosphate monobasic (BioUltra, ≥99.5%), potassium phosphate dibasic (BioUltra, 99%), potassium sorbate (purum p.a.), for the preparation of buffers were from Sigma-Aldrich. Tween™ 20 (pure) and 3,3′,5,5′-tetramethylbenzidine (TMB, research grade) were obtained from Serva. Guardian™ Peroxidase Conjugate Stabilizer/Diluent was from ThermoFisher Scientific. Sulfuric acid 95–97% (both Baker-analyzed grade), isopropanol (Baker HPLC analyzed) and methanol (HPLC gradient grade) were purchased from Mallinckrodt Baker (now Avantor Performance Material, Griesheim, Germany). Ethyl acetate and $NaHCO_3$ were supplied by Merck KGaA. PD-10 desalting columns were obtained from GE Healthcare and high-binding microtiter plates (MTPs) with 96 flat-bottomed wells were purchased from Greiner Bio-One (Frickenhausen, Germany). Ultrapure reagent water for buffers and solutions was obtained from a Milli-Q® Reference water purification system (Merck Millipore, Darmstadt, Germany).

2.2. Preparation of standard solutions

Standard solutions were prepared gravimetrically by dissolving 4.50 mM of cocaine hydrochloride, benzoylecgonine, ecgonine methyl ester, norcocaine in methanol and kept as a stock solution. Compounds were diluted in the range of $100–10^{-3}$ µg/L with Milli-Q water as required.

2.3. Sample collection

Euro banknotes with denominations of 5, 10, 20, and 50 € were or withdrawn from automated teller machines (ATM), or were received in supermarkets. Each banknote was kept isolated in a plastic bag before extraction. After the extraction of cocaine from the Euro banknotes, they were dried and recirculated into the market.

2.4. Cocaine extraction method

Each note was rolled and placed in a 15 mL polypropylene bottle which already contained 10 mL of different water: methanol mixtures. The bottle was placed in an ultrasonic bath for 10 min, followed by rinsing with 5 mL methanol, and then the methanolic solution was added to the first extract. To determine the remaining amount of cocaine on the banknotes, another extraction was carried out with the same method. Afterward, the extracted solution was evaporated to dryness at 60 °C under reduced pressure, reconstituted in 1 mL methanol and centrifuged for 15 min at 14,000 rpm. Several methanolic extract/Milli-Q water dilutions with ratios: 1:10, 1:50, 1:100, 1:500, 1:1000 and 1:10000, were prepared.

Van der Heide et al. [5], using a competitive enzyme immunoassay for cocaine detection on banknotes found various contaminants including sweat, dust, and grease. As co-extractants, they could interfere with the immunoassay. Therefore, the solvent extract was evaporated to dryness and then reconstituted in 1 mL Milli-Q water, which does not dissolve all co-extractants. Furthermore, the effect methanol sometimes shows on the antibody [33] is eliminated. Due to the high sensitivity, the extract could be diluted prior to the assay, decreasing possible matrix effects as described by van der Heide et al. [5,25].

2.5. Preparation of the protein conjugate

The protein conjugate was prepared by the NHS/DCC activated ester (carbodiimide) method [26]. 5.00 mg (13.8 µmol) of benzoylecgonine (BEC), 7.96 mg (69.2 µmol) of N-hydroxysuccinimde (NHS) and 14.28 mg (69.2 µmol) of N,N′-dicyclohexylcarbodiimide (DCC) were weighed. Each compound was dissolved in 72.4 µL of anhydrous N,N-dimethylformamide (DMF) under argon. After total dissolution, the NHS and DCC solutions were added to the BEC solution. The mixture was then shaken at 750 rpm, overnight at 20 °C, followed by centrifugation for 15 min at 14,000 rpm. 15.7 µL of benzoylecgonine activated ester was added drop wise to 200 µL of HRP (0.02 µmol in 0.1 M sodium bicarbonate buffer solution). Afterward, the mixture was shaken for another four hours at 20 °C. Then, it was centrifuged at 14,000 rpm for 15 min, followed by gel permeation chromatography purification on a PD-10 desalting column. Two eluent drops were collected in each well of a microtiter plate. Absorbance was measured at 403 nm and the contents of the wells with the highest absorbance were collected and mixed with Guardian™ solution (HRP stabilizer) and stored at 4 °C.

2.6. Immunoassay procedure

The direct competitive cocaine immunoassay was performed in transparent high-binding 96-well microtiter plates. Each well was coated with 200 µL of 1 mg/L anti-mouse IgG in PBS. The microtiter plate was kept overnight on a plate shaker at 750 rpm, followed by