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A competitive enzyme immunoassay for the quantitative detection of cocaine from banknotes and latent fingermarks

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ABSTRACT

A sensitive and versatile competitive enzyme immunoassay (cEIA) has been developed for the quantitative detection of cocaine in complex forensic samples. Polyclonal anti-cocaine antibody was purified from serum and deposited onto microtiter plates. The concentration of the cocaine antibody adsorbed onto the plates, and the dilution of the cocaine-HRP hapten were both studied to achieve an optimised immunoassay. The method was successfully used to quantify cocaine in extracts taken from both paper currency and latent fingermarks. The limit of detection (LOD) of 0.162 ng mL^{-1} achieved with the assay compares favourably to that of conventional chromatography–mass spectroscopy techniques, with an appropriate sensitivity for the quantification of cocaine at the low concentrations present in some forensic samples. The cEIA was directly compared to LC–MS for the analysis of ten UK banknote samples. The results obtained from both techniques were statistically similar, suggesting that the immunoassay was unaffected by cross-reactivity with potentially interfering compounds. The cEIA was used also for the detection of cocaine in extracts from latent fingermarks. The results obtained were compared to the cocaine concentrations detected in oral fluid sampled from the same individual. Using the cEIA, we have shown, for the first time, that endogeneously excreted cocaine can be detected and quantified from a single latent fingermark. Additionally, it has been shown that the presence of cocaine, at similar concentrations, in more than one latent fingermark from the same individual can be linked with those concentrations found in oral fluid. These results show that detection of drugs in latent fingermarks could directly indicate whether an individual has consumed the drug. The specificity and feasibility of measuring low concentrations of cocaine in complex forensic samples demonstrate the effectiveness and robustness of the assay. The immunoassay presents a simple and cost-effective alternative to the current mass spectrometry based techniques for the quantitation of cocaine at forensically significant concentrations.

1. Introduction

The supply and consumption of illicit drugs such as cocaine is a growing societal problem on a global scale. In order to combat the effect of usage and trade, many countries implement laws that allow the seizure of circumstantial evidence associated with cocaine trafficking [1].

It has been documented that a significant proportion of paper currency in general circulation is contaminated with cocaine [2–8]. Adherence of cocaine to the banknote can be due to surface contamination and entrapment of drug crystals between fibres of the note [9], while indirect transfer of cocaine is possible through contact with other notes or bank sorting machines [10]. Cocaine trading, using cash, and administration *via* a rolled up banknote can lead to direct exposure of banknotes to the crystalline drug powder [11]. It is thought that a concentration of cocaine on a note that is beyond that of the general circulation can be associated with drug use or trade and thus provide incriminating evidence [12,13]. Quantitative methods which enable the differentiation between 'drug money' and general currency, that are suitable for daily analysis in a forensic laboratory are crucial in providing such evidence. It is therefore of interest that a greater range of suitable methods for this type of analysis be developed.

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Interest has also grown in recent years in gaining information from latent fingermarks beyond suspect identification. Additional information in a forensic context, such as the use by or recent exposure of an individual to illicit drugs, could provide enhanced evidence between a fingermark and a suspect. The successful detection of cocaine and other drug compounds in latent fingermarks is known using a number of different analytical approaches, and is particularly well-described for prints artificially doped with the compound of interest [14–17]. However, recent advances by our research group using an immuno-labelling approach based on the use of fluorescently labelled, antibody-functionalised magnetic particles have shown the detection of drug metabolites endogenously produced in the latent fingermarks of drug users [18–20]. In addition, Rowell et al. were able to show isolated patches of cocaine contamination in a fingerprint obtained from an individual being treated at a drug addiction centre using surface assisted laser desorption/ionisation time-of-flight mass spectrometry (SALDI-TOF-MS). The non-homogenous pattern of cocaine contamination in the print was thought to be due to the subject's fingertip coming into contact with the drug prior to the time of sampling [16]. Analytical methods for the detection of these types of cocaine residues in latent fingermarks could be used in a forensic context, as their presence would provide further evidence of a suspect's involvement in drug use or trade.

At present, gas chromatography, liquid chromatography or thermal desorption coupled with mass spectrometry (MS) are most often employed for the detection of cocaine extracted from banknotes [4,5,8,11,21]. Alternative methods include ion-mobility spectrometry [7,22], electrochemiluminescence [23], and Raman microspectroscopy [9]. The immunodetection of cocaine on banknotes is limited to the application of strip-based immunoassays originally developed for qualitative detection of the drug in urine [24,25]. The detection of cocaine extracted from doped fingerprints is similarly focused on MS-based approaches, such as desorption electrospray ionisation (DESI)-MS [15] and SALDI-TOF-MS [16] or Raman spectroscopy [14,17].

Immunoassays such as competitive enzyme immunoassays (cEIAs), where free analyte from a sample competes with a labelled analyte for available antibody binding sites on a micro-titer plate, are also commonly used for the detection of illicit compounds in biological samples such as blood and urine [26]. Assays of this nature offer excellent sensitivity and specificity in a simple to use and cost effective manner making them highly suited for forensic analysis, without requiring specialised chemicals or advanced instrumentation.

The aim of the present study was to develop a sensitive cEIA specifically designed for the quantitation of cocaine in forensic samples such as latent fingermarks and paper currency. Ten Bank of Scotland (UK) banknotes obtained from general circulation were tested for cocaine using the developed cEIA method as a preliminary indication of efficacy. The assay was validated by comparison with analysis of the same extracted samples by LC–MS. Further, the cEIA was used for the detection of cocaine in ten latent fingermarks. The fingermarks were obtained from five test subjects being treated for the habitual use of drugs, including cocaine, at a Methadone Treatment Clinic. The concentrations determined in the extracted fingermark samples were compared with the cocaine concentrations quantified by GC–MS in oral fluid samples collected from the same individuals.

2. Experimental

2.1. Materials

All reagents were of analytical grade, purchased from Sigma-Aldrich (UK) and used as received unless otherwise stated. Polyclonal anti-cocaine antibody was obtained in 1 mL units of rabbit serum from Europa Bioproducts (UK) and purified as reported below. 'Slide-A-Lyzer' mini dialysis units (10,000 MWCO), 'NAb Protein A plus' spin columns, Coomassie brilliant blue R250, bromophenol blue sodium salt, ammonium persulfate, 96-well Nunc C8 Maxisorp microtiter plates and 3,3′,5,5′ tetramethylbenzidine (TMB) substrate solution, 50 mL centrifuge tubes and 1.5 mL Eppendorf microcentrifuge tubes were all purchased from Thermo Scientific (UK). Novex Sharp unstained protein standard was purchased from Invitrogen (UK), and N,N,N′-tetramethylethylenediamine (TEMED) was purchased from Bio Rad (UK). Cocaine standard (Cerilliant; 1 mg mL^{-1} in acetonitrile) and cocaine-horseradish peroxidase conjugates (cocaine-HRP) were purchased from LGC Standards (UK) and Randox (UK), respectively. Ten Scottish banknotes of £10 and £20 denominations from general circulation were kindly provided by the Scottish Crime and Drug Enforcement Agency (SCDEA). Ten fingermark samples on glass microscope slides were obtained from five volunteers attending a Methadone Treatment Clinic.

UV–vis absorption spectra were recorded on a Hitachi U-3000 spectrophotometer. All centrifugation steps were performed using a Beckman Coulter Allegra X-22R centrifuge. Microtiter plate absorbance intensity readings were recorded using a Perkin Elmer Wallac Envision 2103 multilabel microplate reader. The confirmation analysis of the banknote extracts was performed using a Thermo LTQ Orbitrap Discovery high resolution accurate mass LC–MS running in full scan positive ion electrospray mode. The orbitrap was operating at a mass resolution of 30,000 FWHM at m/z 400.

2.2. Anti-cocaine antibody purification

Polyclonal anti-cocaine antibody was isolated from the supplied rabbit serum samples by immunoprecipitation followed by Protein A chromatography. Ammonium sulfate precipitation was performed using standard methodology [27], prior to dialysis in mini-dialysis units for 2 h against 2 L of 10 mM phosphate buffer (pH 7.4; PB). Immunochromatography was performed using Protein A spin columns with a sample capacity of $\leq$500 μL in accordance with the manufacturer's instructions. Three columns were used to accommodate the 900 μL of solution to be purified. Briefly, the columns were equilibrated before use by washing twice with 400 μL of 10 mM phosphate-buffered saline (150 mM NaCl, pH 7.2; PBS). Wash solutions were removed from the columns by centrifugation for 1 min at 4000 × g. The solution containing the antibody was added at 300 μL per column, and the columns incubated at room temperature on a rotary mixer for 10 min. The columns were then washed three times with 400 μL of 10 mM PBS (pH 7.2). The antibody was eluted from the columns using three 400 μL additions of 0.1 M glycine (pH 2.0). Each of the resulting three fractions was neutralised with 40 μL of 1.0 M tris–HCL buffer (pH 8.5). The presence of antibody in the first and second fractions was determined by UV–vis spectrophotometry at A_{280} and confirmed by SDS-PAGE [28]. The two fractions were combined and dialysed in mini-dialysis units for 2 h against 2 L of 10 mM PB (pH 7.4), and the solutions stored at −20 °C.

2.3. Banknote extraction

Extracts from the banknotes were obtained using a modified version of the method described by Esteve-Turrillas et al. [3]. Each banknote was rolled and placed in a 20 mL glass test tube with 15 mL of methanol. The tube was sealed and vortex-mixed for 5 min. The banknote was then removed from the tube and rinsed with a further 5 mL of methanol. The two extract volumes (15 and 5 mL) were combined to make up the 20 mL final volume. The