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Letters to *Analytical Chemistry*

Multiplexed Detection of Metabolites of Narcotic Drugs from a Single Latent Fingermark

Pompi Hazarika,[†] Sue M. Jickells,[‡,||] Kim Wolff,[§] and David A. Russell*[,†]

School of Chemistry, University of East Anglia, Norwich, Norfolk, United Kingdom NR4 7TJ, Department of Forensic Science and Drug Monitoring, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, United Kingdom, SE1 9NH, Addiction Department, Institute of Psychiatry, King's College London, 4 Windsor Walk, Denmark Hill, London, United Kingdom SE5 8AF

An immunoassay based technique is used for the detection of psychoactive substances in the sweat deposited within fingermarks of a narcotic drug user. Magnetic particles functionalized with antimorphine and antibenzoylecgonine antibodies were used for the detection of a metabolite of heroin (morphine) and a metabolite of cocaine (benzoylecgonine), respectively. The drug metabolites were detected individually as well as simultaneously from a single fingermark. The images of the fingermarks obtained using brightfield and fluorescence microscopy were of high evidential quality with resolution to enable identification of an individual in addition to providing information on drug usage.

Fingermarks are still the key piece of evidence in criminal investigations since they are an internationally recognized and an established means of human identification[1,2] Fingermarks are unique to each person, and they remain unchanged during an individual's lifetime. Their uniqueness is defined by careful study of their ridge characteristics such as bifurcation, enclosure, island, and ridge ending.[2] When a finger comes into contact with a surface, a fingermark is left behind. Some of these marks are visible, but some are invisible to the naked eye. These latter marks are termed latent fingermarks. Such fingermarks require a chemical or physical method in order to visualize the invisible marks.[3] The most commonly used method for fingermark visualization is powdering,[4] although other conventional methods including vacuum metal deposition,[5] amino acid sensitive reagents such as ninhydrin,[6] and cyanoacrylate (superglue) fuming[2] are also widely used. Recently, there has been increasing interest in fingerprinting research in order to find various ways of sensitive detection of fingermarks.[6−9] Several research groups are working on developing methods for fingermark imaging[10−12] as well as detecting quantities of drugs, explosive residues, and other exogenous substances in fingermarks.[13−17] Our work has focused on the development of methods for the detection of narcotic drugs and drug metabolites that have been excreted in the sweat deposited with latent fingermarks of drug users.[18−20] We have shown previously that gold nanoparticles[18] and magnetic particles[19,20] functionalized with antibodies against the drugs and metabolites can be used to detect the metabolite of nicotine (cotinine), the main psychoactive component of cannabis (Δ^9-tetrahydrocannabinol), the synthetic opioid-methadone and its major metabolite 2-ethylidene-1,5-dimethyl-3,3,-diphenylpyrrolidine (EDDP), and the metabolite of cocaine (benzoylecgonine). The images obtained of the fingermarks were of high resolution so that the identity of the individual could be established as well as gaining lifestyle information of that person.

* To whom correspondence should be addressed. E-mail: d.russell@uea.ac.uk.
[†] University of East Anglia.
[‡] Department of Forensic Science and Drug Monitoring, King's College London.
[§] Addiction Department, Institute of Psychiatry, King's College London.
[||] Current address: Centre for Staff and Educational Development, University of East Anglia, Norwich, Norfolk, United Kingdom NR4 7TJ.

(1) Faulds, H. *Nature* **1880**, *22*, 605.
(2) Saferstein, R. *Criminalistics: An Introduction to Forensic Science*, 9th ed.; Prentice Hall, Inc.: Upper Saddle Rive, NJ, 2006.
(3) Oden, S.; Von Hofsten, B. *Nature* **1954**, *173*, 449–450.
(4) Sodhi, G. S.; Kaur, J. *Forensic Sci. Int.* **2001**, *120*, 172–176.
(5) Theys, P.; Turgis, Y.; Lepareux, A.; Chevet, G.; Ceccaldi, P. F. *Int. Criminal Police Rev.* **1968**, *217*, 106–108.
(6) Jelly, R.; Patton, E. L.; Lennard, C.; Lewis, S. W.; Lim, K. F. *Anal. Chim. Acta* **2009**, *652*, 128–142.
(7) Ifa, D. R.; Jackson, A. U.; Paglia, G.; Cooks, R. G. *Anal. Bioanal. Chem.* **2009**, *394*, 1995–2008.
(8) Zhang, M.; Girault, H. H. *Analyst* **2009**, *134*, 25–30.
(9) Jickells, S. M. *Meas. Control* **2008**, *41*, 243–247.
(10) Tang, H. W.; Lu, W.; Che, C. M.; Ng, K. M. *Anal. Chem.* **2010**, *82*, 1589–1593.
(11) Liu, J.; Shi, Z.; Yu, Y.; Yang, R.; Zuo, S. *J. Colloid Interface Sci.* **2010**, *342*, 278–282.
(12) Becue, A.; Moret, S.; Champod, C.; Margot, P. *Forensic Sci. Int.* **2009**, *191*, 36–41.
(13) Ifa, D. R.; Manicke, N. E.; Dill, A. L.; Cooks, R. G. *Science* **2008**, *321*, 805.
(14) Bhargava, R.; Schwartz Perlman, R.; Fernandez, D. C.; Levin, I. W.; Bartick, E. G. *Anal. Bioanal. Chem.* **2009**, *394*, 2069–2075.
(15) Mou, Y.; Rabalais, J. W. *J. Forensic Sci.* **2009**, *54*, 846–850.
(16) Ng, P. H.; Walker, S.; Tahtouh, M.; Reedy, B. *Anal. Bioanal. Chem.* **2009**, *394*, 2039–2048.
(17) Chen, T.; Schultz, Z. D.; Levin, I. W. *Analyst* **2009**, *134*, 1902–1904.
(18) Leggett, R.; Lee-Smith, E. E.; Jickells, S. M.; Russell, D. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 4100–4103.
(19) Hazarika, P.; Jickells, S. M.; Wolff, K.; Russell, D. A. *Angew. Chem., Int. Ed.* **2008**, *47*, 10167–10170.
(20) Hazarika, P.; Jickells, S. M.; Russell, D. A. *Analyst* **2009**, *134*, 93–96.

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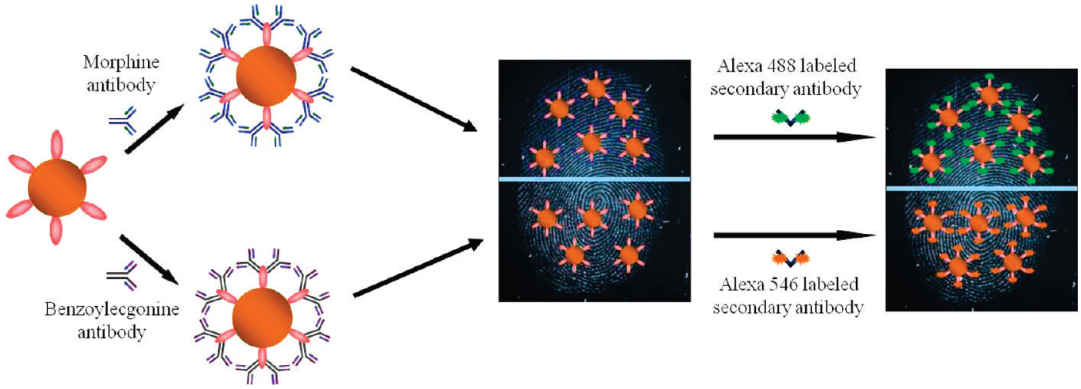

Figure 1. Schematic representation of the detection of two drug metabolites from a single fingermark using antibody−magnetic particle conjugates. Antimorphine antibody functionalized magnetic particles and antibenzoylecgonine antibody functionalized magnetic particles were prepared by combining antimorphine antibody and antibenzoylecgonine antibody to the recombinant fusion Protein A/G coated magnetic particles, respectively. Then, the conjugates were incubated over two separate sections of the fingermark that had been collected on a glass microscope slide. Excess particles were removed using a magnet. Subsequently, two secondary antibody fragments tagged with Alexa Fluor 488 and Alexa Fluor 546 dye were incubated over the two separate portions of the fingermark. After removal of excess antibodies, the fingermark was imaged using a fluorescence stereomicroscope.

In this work, we show the use of antibody−magnetic particle conjugates for the sole detection of metabolites of heroin and cocaine, i.e., morphine and benzoylecgonine, respectively, in the fingermarks of drug users. Additionally, we have taken the concept of drug detection and imaging of fingermarks a step forward as we show, for the first time, that the two drug metabolites morphine and benzoylecgonine can be detected simultaneously from a single fingermark. This latter development may be of particular interest to the addiction field since drug addicts frequently use different combinations of substances at the same time, for example, the combination of heroin and cocaine, colloquially known as "speedballing".

EXPERIMENTAL SECTION

Magnetic particles (ca. 1 μm) coated with recombinant Protein A/G were purchased from Bioclone Inc., USA. The magnetic particles (200 μL; 20 mg/mL) were cleaned by washing with 1 mL of 100 mM phosphate buffer (57.7 mM Na_2HPO_4, 42.3 mM NaH_2PO_4, pH 7.0). The antimorphine antibody functionalized magnetic particle conjugates were prepared by mixing 102.6 μL (1.95 mg/mL) of antimorphine antibody (Fitzgerald Inc., USA) with 200 μL of the magnetic particles. The mixture of the antimorphine antibody and the magnetic particles was incubated overnight at 4 °C with constant stirring. A magnetic separator (Bioclone Inc.) was used to separate the antibody bound magnetic particle conjugates from the unbound antibodies. The unbound antibodies were then removed using a micropipet. The antibody−magnetic particle conjugates were washed five times with 1 mL of 100 mM phosphate buffer. Finally, the conjugates were resuspended in 800 μL of phosphate buffer. The antibenzoylecgonine antibody functionalized magnetic particle conjugates were prepared using the same procedure as described above but by mixing 39.2 μL (5.1 mg/mL) of antibenzoylecgonine antibody (Fitzgerald Inc., USA) with 200 μL of magnetic particles.

For the collection of fingermarks, glass microscope slides were cleaned using methanol (HPLC grade). Then, volunteers were asked to apply fingermarks, one mark to each glass slide. The presence of a latent fingermark on a glass slide was later confirmed by obtaining an image of the fingermark using brightfield microscopy. The collected fingermarks were stored in the dark and used within 2 weeks of collection.

For the immunoassays, a delimiting pen (Dako Pen, Dako, Denmark) was used to draw an outline around a fingermark on the glass slide to retain the antibody−magnetic particle conjugate solution over the fingermark. For the detection of a single analyte, 100 μL of either antimorphine magnetic particle conjugates or antibenzoylecgonine magnetic particle conjugates was applied to the fingermark. The slide was then incubated for 30 min at 37 °C in a wet chamber (consisting of moist tissues surrounding the glass slide; all contained within a covered Petri dish). After the incubation period, the excess magnetic particle conjugates were removed with a magnetic wand (Tetra Scene of Crime Inc., USA). Subsequently, 100 μL of a 50 μg/mL solution of antimouse secondary antibody fragment ($F(ab')_2$ fragment of goat antimouse IgG), tagged with either Alexa Fluor 488 or Alexa Fluor 546 dye (Invitrogen) was applied to the fingermark and again incubated for 30 min at 37 °C in the wet chamber. Excess secondary antibodies were removed by washing the mark twice with 200 μL of 100 mM phosphate buffer. The fingermark was left to dry for about 5 min before being imaged with a fluorescence stereomicroscope.

For the simultaneous detection of two analytes from a single fingermark, the delimiting pen was again used to draw an outline around a fingermark. However, additionally, the pen was used to partition the fingermark into two separate sections. The schematic representation of the detection method is shown in Figure 1.

For the detection, 100 μL of the antimorphine magnetic particle conjugates was applied to one section of the fingermark, while 100 μL of the antibenzoylecgonine magnetic particle conjugates was applied to the second section of the same fingermark. The glass slide was then incubated at 37 °C for 30 min in the wet chamber, after which the excess particles were removed using the magnetic wand. Subsequently, a secondary antibody fragment labeled with Alexa Fluor 488 dye (100 μL; 50 μg/mL) was applied to the section of the fingermark containing the antimorphine antibody particle conjugates while a secondary antibody fragment labeled with Alexa Fluor 546 dye (100 μL; 50 μg/mL) was applied

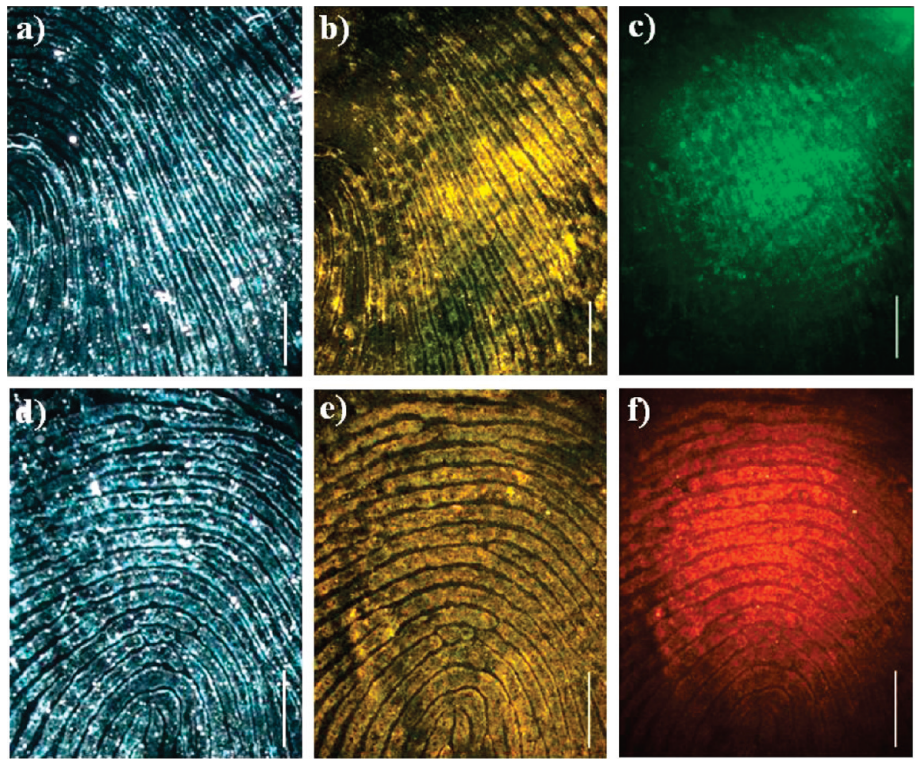

Figure 2. Detection of morphine and benzoylecgonine in two separate fingermarks. (a and b) Brightfield images of a fingermark section for the detection of morphine obtained before and after incubation of antimorphine antibody functionalized magnetic particles, respectively. (c) Shows the fluorescence image of the same fingermark section obtained for morphine detection after subsequent application of Alexa Fluor 488 dye labeled secondary antibody fragments. (d and e) Brightfield images of a section of a different fingermark for the detection of benzoylecgonine obtained before and after incubation of antibenzoylecgonine antibody functionalized magnetic particles. (f) Shows the fluorescence image of the fingermark section obtained for benzoylecgonine detection after subsequent application of Alexa Fluor 546 dye labeled secondary antibody fragments. Scale bars are 2 mm.

to the other section of the fingermark containing the antibenzoylecgonine antibody particle conjugates. The secondary antibodies were incubated over the fingermark at 37 °C for 30 min in the wet chamber. After the incubation period, excess secondary antibodies were removed and each fingermark section was washed separately twice with 200 μL of 100 mM phosphate buffer. After leaving the fingermark to dry for about 5 min, the stereomicroscope was used to image the fingermark.

Brightfield and fluorescence microscopy images were obtained using a SteREO Lumar.V12 stereomicroscope (Carl Zeiss Europe). A brightfield image of the fingerprint was obtained using a halogen lamp (Carl Zeiss, Cold-light source KL 2500 LCD 115 V), and the fluorescence image was obtained using a 120 W Mercury Vapor Short Arc lamp (Exfo, X-Cite 120). The fluorescence images were obtained using a 470 nm excitation filter (40 nm bandpass) and 525 nm emission filter (50 nm bandpass) for the Alexa fluor 488 dye tagged secondary antibody fragments, while a 550 nm excitation filter (25 nm bandpass) and 605 nm emission filter (70 nm bandpass) were used for the Alexa fluor 546 dye tagged secondary antibody fragments. All images were captured with an AxioCam HRc CCD camera and processed using Axio Vision software (Carl Zeiss). The imaging of the two sections of the fingermark was performed simultaneously. Brightfield microscopy was used to image the whole fingermark, and subsequently, magnified images of the two different sections of the fingermark were obtained. For fluorescence microscopy, multichannel acquisition was used to generate a fluorescence image of the whole fingermark with separate color for the two sections of the fingermark. The images of the two sections of the fingermark at higher magnification were obtained by imaging the two sections separately.

RESULTS AND DISCUSSION

For the detection of the drug metabolites morphine and benzoylecgonine, fingermarks were provided on glass microscope slides by volunteers from a community based drug treatment service. Initial experiments were performed to confirm the presence of either the metabolites of cocaine or heroin, i.e., benzoylecgonine and morphine, respectively, in the fingermarks of a volunteer.

In the first instance, antimorphine antibodies conjugated to recombinant fusion Protein A/G coated magnetic particles were used to confirm the presence of morphine in a fingermark from the volunteer. The results obtained for detection of morphine are shown in Figure 2a–c. After application of the antimorphine magnetic particle conjugates to the fingermark, the color of the mark changed from gray (Figure 2a) to yellow/brown (Figure 2b). The color change is a result of the binding of the antibody conjugated magnetic particles to the morphine antigen present within the sweat of the fingermark and, hence, confirms the presence of morphine in the fingermark. The detection of morphine was further confirmed by the fluorescence image of the fingermark which was obtained by the binding of the fluorescently tagged secondary antibody fragments to the primary antibody magnetic particles bound to the fingermark and imaged

using fluorescence microscopy (Figure 2c). A further experiment was performed using antibenzoylecgonine conjugated magnetic particles for the detection of benzoylecgonine in a fingermark from the same volunteer. The results are shown in Figure 2d–f. In this instance, following application of the antibenzoylecgonine magnetic particle conjugates to the fingermark, the color of the print changed from gray (Figure 2d) to yellow/brown (Figure 2e). A positive fluorescence image of the fingermark was also obtained using fluorescence microscopy (Figure 2f). The images shown in Figure 2e,f shows the presence of the benzoylecgonine antigen in the sweat within the fingermark. These experiments suggest that the individual who provided the fingermarks was a user of both the narcotic drugs heroin and cocaine. Importantly, the detection of metabolites of the drugs, rather than the drugs themselves, suggests that the individual was a user of the narcotics rather than the subject of accidental contact with the drugs from an external source. It should be noted that the presence of morphine in the fingermark could potentially result from an individual taking a codeine based medication or through the direct administration of morphine itself. The presence of benzylecgonine, however, directly confirms that the fingermark came from a user of cocaine. The fingermark images obtained were of high resolution and clearly showed the secondary level details of the fingerprint ridge pattern such as bifurcation and ridge ending that are used by fingerprint experts to confirm the identity of an individual. Closer inspection of the fingermarks, shown in Figure 2e,f, also revealed third level details such as sweat pores, visible as dark spots along the ridge length, which are also important for identification purposes.

Control experiments were performed to show the specificity of the antimorphine and the antibenzoylecgonine antibodies used for the detection of metabolites of the drugs heroin and cocaine, respectively. The negative control experiments were carried out using fingermarks from volunteers who were not taking any drugs. The fingermark images obtained after application of the antibody–magnetic particle conjugates and the secondary antibody fragments show neither any apparent color change by brightfield microscopy nor a positive image of the fingermarks when visualized using fluorescence microscopy (Figure S-1 and S-2 in the Supporting Information). Thus, the results suggest that the antimorphine antibody functionalized magnetic particles and the antibenzoylecgonine antibody functionalized magnetic particles bind specifically to morphine and benzoylecgonine antigen, respectively, present in the fingermarks of the drug users.

Following the successful detection of morphine and benzoylecgonine in the fingermarks of the volunteer, a further experiment was performed to establish whether multiple drug metabolites could be detected simultaneously from a single fingermark. The method used for the simultaneous detection of both morphine and benzoylecgonine from a single fingermark is shown schematically in Figure 1. In the first instance, two different antibody–magnetic particles were prepared by conjugating antimorphine and antibenzoylecgonine antibodies to recombinant fusion Protein A/G coated magnetic particles. The conjugates were then applied to two separate sections of a fingermark simultaneously. After incubation and removal of excess particles using a magnetic wand, secondary antibody fragments labeled with

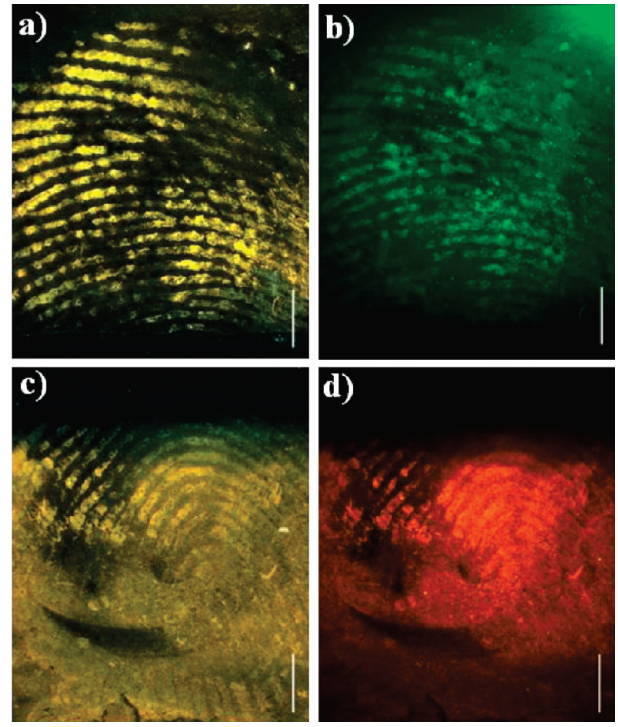

Figure 3. Detection of morphine and benzoylecgonine in two separate sections of a single fingermark. (a) Brightfield and (b) fluorescence images of the upper portion of a fingermark used for the detection of morphine; images obtained after incubation of antimorphine antibody functionalized magnetic particles and Alexa Fluor 488 dye labeled secondary antibody fragments to one-half of the fingermark. (c) Brightfield and (d) fluorescence images of the lower portion of the same fingermark used for the detection of benzoylecgonine; images obtained after incubation of antibenzoylecgonine antibody functionalized magnetic particles and Alexa Fluor 546 dye labeled secondary antibody fragments to the second half of the same fingermark. Scale bars are 2 mm.

Alexa Fluor 488 and 546 dyes were applied to the two sections of the fingermark, respectively.

The results of the simultaneous detection of multiple analytes from a single fingermark are shown in Figure 3. Panels a and b of Figure 3 are brightfield and fluorescence images, respectively, of the upper portion of the fingermark used for the detection of morphine. Panels c and d of Figure 3 are brightfield and fluorescence images, respectively, of the lower portion of the fingermark used for the detection of benzoylecgonine. The typical color change of the fingermark to yellow/brown due to binding of the antibody magnetic particles was observed for both sections of the fingermark (Figure 3a,c). This binding suggests detection of the morphine and benzoylecgonine in the fingermark. This result was further confirmed by the fluorescence images obtained for the upper and lower portions of the fingermark (Figure 3b,d respectively). The brightfield and fluorescence images confirmed the successful detection of the drug metabolites morphine and benzoylecgonine simultaneously from a single fingermark. The fingermark images obtained also showed secondary as well as tertiary level details that could be used by fingerprint experts to confirm identification of the individual.

The application of a mixture of the antimorphine conjugated magnetic particles and antibenzoylecgonine conjugated magnetic particles to a fingermark of the drug user for the simultaneous

detection of the two drug metabolites was also considered. However, a "positive result" shown by a change of color of the fingermark to yellow/brown in the brightfield image would only indicate that the individual had taken at least one of the drugs; it would not enable the discrimination between the two drugs.

CONCLUSIONS

In this work, we have shown that a metabolite of heroin, i.e., morphine, can be detected in the sweat deposited within a latent fingermark. Additionally, it has been shown, for the first time, that metabolites from two different drug classes, i.e., morphine and benzoylecgonine, can be detected simultaneously in a single fingermark using white light and/or a fluorescence light source. The fingermark images obtained provided information on drug usage of an individual. The high resolution images would also enable the identification of the individual to be established. The particle treated fingermarks can be stored for future use without compromising their quality. This simple and fast method paves the way for the simultaneous detection of multiple substances of forensic importance from a single fingermark within a short space of time. It is possible that this versatile method will find applications in forensic science, policing, and homeland security.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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