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Rapid and sensitive detection of drugs of abuse in sweat by multiplexed capillary based immuno-biosensors†

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Rapid and sensitive detection of drugs of abuse plays an important role in monitoring of drug use and treatment compliance. Sweat based drug analysis shows great advantages due to its non-invasive nature. However, most of the related methods developed to date are qualitative, slow, or costly, which significantly hinders their application in field use. Here we report rapid, sensitive, quantitative detection of drugs of abuse in sweat based on capillary arrays combined with competitive enzyme-linked immunosorbent assay. Using four common drugs of abuse, methadone, methamphetamine, amphetamine, and tetrahydrocannabinol, spiked in artificial sweat as a model system, we demonstrate rapid, quantitative, and multiplexed detection of the four drugs in ~16 minutes with a low sweat volume (~4 μL per analyte) and a large dynamic range (methadone: 0.0016 ng mL^{-1}–1 ng mL^{-1}; METH: 0.016 ng mL^{-1}–25 ng mL^{-1}; amphetamine: 0.005 ng mL^{-1}–10 ng mL^{-1}; THC: 0.02 ng mL^{-1}–1000 ng mL^{-1}). In addition, we show that the detection range can be tuned for different applications by adjusting the competitors' concentrations. Our work paves a way to develop an autonomous, portable, and cost-effective device for hospital testing, workplace drug-use screening, roadside testing, and patient monitoring in drug rehabilitation centers.

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1. Introduction

The use and abuse of potentially-addictive substances has become a national and global crisis.[1,2] Approximately 25 million people in the United States (US) alone used drugs of abuse in the past month, as reported by the latest National Survey on Drug Use and Health.[3] The social (>70 000 deaths every year) and economic costs ($200 billion annually in healthcare and treatment) of drug use and abuse are immense.[4] The likely legalization of cannabis across the US will only worsen this crisis. Thus, rapid, sensitive, and on-site detection of drugs of abuse is essential for hospital testing, workplace drug-use screening, roadside testing, and patient monitoring in drug rehabilitation centers. To be maximally useful, results of drug detection would be quantitative and provide information, not just about the presence or absence of the substance, but also about the level of the substance that is present in the system.

Biological sources for drug analysis include blood, urine, saliva, hair, sweat, and exhaled breath.[5] Normally, blood analysis provides an accurate approach to tracking drug dosage for hours, because the parent compounds of the drugs can be found in blood. However, it is invasive and causes pain and intense stress in patients. Urine can also be tested for the parent compounds as well as metabolites of various drugs. One of the main benefits of the urine test is that it is non-invasive and specimen can be collected within minutes. Hair testing is the best long-term drug monitoring method, since hair grows slowly and drugs can be detected months or even years after drug is ingested. However, the processing time for hair testing is much longer than blood or urine testing. Alternatively, sweat analysis shows great advantages in drug detection as a non-invasive diagnosis method. It can record drug abuse history over a long period (up to 14 days),[6] depending on the specific substance being examined. The distinctive

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† Electronic supplementary information (ESI) available: Fig. S1 Picture of the cartridge. Fig. S2 Capillary arrangement for drug detection. Table S1 Recovery rate of multiplexed drug detection with one drug absent. Table S2 Recovery rate of multiplexed drug detection with mixed drug-HRP conjugates. See DOI: 10.1039/c9an02498k

characteristics of secretion, accessibility, and abundance in biomolecules make sweat an ideal candidate for drug monitoring.

The most widely used method in sweat testing is to use a sweat patch,[7–11] which accumulates drugs or metabolites *via* a semipermeable membrane and is subsequently sent to a lab for analysis. However, the whole sweat collection and assay process takes 7–10 days. Recent research on imaging of fingerprint sweat using magnetic particles was shown to provide a fast and on-site approach to detecting drugs and user identification simultaneously.[12,13] However, only qualitative or binary information (*i.e.*, yes/no) can be obtained. Wearable sensors provide another way to detect drugs in sweat.[5,14,15] Those sensors usually consist of electrical components for signal transduction and data transmission. They are portable, fast, and convenient. The main challenges for those sensors are limited sensitivities and different detection mechanisms may be needed for different drugs in order to achieve detection specificity. In contrast, conventional analytical techniques, such as gas chromatography and liquid chromatography coupled with mass spectrometry, can provide superior sensitivity and selectivity for drug detection in sweat, but they require expensive instruments and tedious sample pretreatment.[16] Immunoassay based on 96-well plates combined with colorimetry, fluorescence, and chemiluminescence has also been employed as one of the primary tools for quantitative detection of drugs and the associated metabolites.[17,18] Long assay time and high cost make such assays more suitable for laboratory use (for example, methadone detection kit from Neogen Corporation ($230 dollars per 96-well plate) takes longer than 1.5 hours for analyte quantification[19]).

The main objective of this study was to further the rapid, sensitive, and quantitative detection of drugs of abuse in sweat. In our former study, we developed an automated microfluidic system.[20] Hereby, competitive binding as well as multiplexed detection was applied and combined to the system for the first time. Four common drugs of abuse, methadone, methamphetamine (METH), amphetamine, and tetrahydrocannabinol (THC) were spiked in artificial sweat as a model system. Rapid and quantitative detection with ~4 μL sweat in ~16 minutes was demonstrated. The results illustrated dynamic range of 0.0016 ng mL^{-1}–1 ng mL^{-1} for methadone, 0.016 ng mL^{-1}–25 ng mL^{-1} for METH, 0.005 ng mL^{-1}–10 ng mL^{-1} for amphetamine, and 0.02 ng mL^{-1}–1000 ng mL^{-1} for THC. Cross-activity among four drugs was also determined. Our study provides promising strategy for rapid and on-site sweat detection and multi-drug analysis.

2. Materials and methods

2.1. Reagents

Methadone antibody (20-1576), methamphetamine antibody (10-2731), amphetamine antibody (10-1509), tetrahydrocannabinol antibody (10-T43E), methadone-horseradish peroxidase (methadone-HRP, 80-1208), methamphetamine-HRP (65-IM52), amphetamine-HRP (65-IA52), and tetrahydrocannabinol-HRP (65-IT60) were purchased from Fitzgerald. Methadone solution in methanol (M007), methamphetamine solution in methanol (M009), amphetamine solution in methanol (A007), and tetrahydrocannabinol solution in methanol (T4764) were bought from Sigma-Aldrich. ELISA plate-coating buffer (1× PBS, DY006), reagent diluent concentrate (10% BSA in 10× PBS), and Quantikine ELISA wash buffer (25×, WA126) were purchased from R&D Systems. The chemiluminescent substrate (SuperSignal ELISA Femto Substrate, 37074) was purchased from Thermo Fisher. Artificial eccrine perspiration (pH = 4.5, 1700-0020) was bought from Pickering Laboratories.

2.2. Capillary based immune-biosensor

The automated microfluidic system applied in this study consists of a disposable sample/reagent reservoir plate (for sample/reagent storage), a disposable cartridge having a capillary array (for ELISA reaction), and an automated machine equipped with a liquid pump, a robotic arm, and an imaging module as well as the software that controls the system, reads the chemiluminescence signal, and analyzes data.[20] The disposable cartridge was made of polystyrene through the injection molding method (see Fig. S1†). Each cartridge contained 12 capillaries for multiplexed detection of up to 12 analytes (see Fig. 1(A)). Each capillary had an inner diameter of 0.8 mm and a volume of 8 μL. During the experiment, all reagents and sweat samples were pre-loaded into the sample/reagent reservoir plate and were sequentially withdrawn into the capillaries where ELISA reaction occurred. Automated cartridge movement, and reagent introduction and ejection were achieved by the robotic arm. Signal was recorded through an imaging module after the end of ELISA reaction. In this study, competitive enzyme-linked immunosorbent assay (ELISA) where two kinds of molecules competed to bind on the capillary was applied to the microfluidic system for the first time. Besides, quantifying multiple drugs simultaneously is important in real detection as two or more drugs may be present in sweat of a drug user. Here, we demonstrated multiplexed drug detection of four illicit substances.

2.3. Drug detection protocols

We used competitive ELISA to detect drugs (or drug-related metabolites) in sweat. As illustrated in Fig. 1, the inner surface of each capillary was first coated with the antibody for a certain analyte. Then, mixed solution containing the free drug (*i.e.*, analyte) and the drug-HRP conjugate (*i.e.*, competitor) competed for the limited amount of antibody. With the increased analyte concentration, the amount of drug-HRP conjugates bound to the antibody decreases, resulting in a decreased chemiluminescence signal. In the current study, four widely abused drugs, methadone, METH, amphetamine, and THC were used as the model system.

Preparation. The capillary inner surface was coated with the corresponding antibody by incubating the antibody diluted

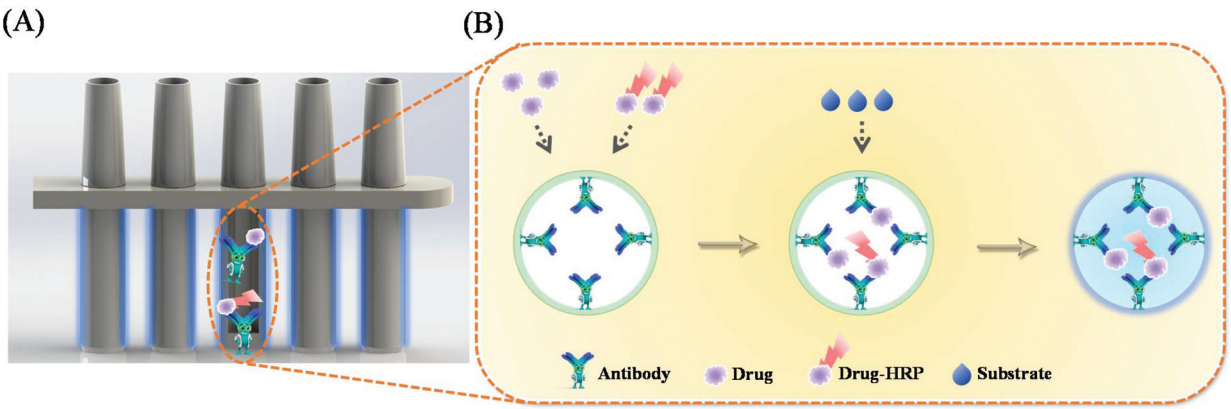

Fig. 1 Schematic of a disposable cartridge used in drug detection. It consisted of 12 capillaries, each of which had an inner diameter of 0.8 mm and a volume of 8 μL. The cartridge was mounted on a robotic arm for automated movement. Liquid was withdrawn into or ejected out of the capillaries by an external liquid pump. (B) Illustration of competitive ELISA for drug detection. Antibody was first coated on the inner surface of a capillary. The free drug (*i.e.*, target analyte) and corresponding drug-HRP conjugate (*i.e.*, competitor) were mixed and withdrawn into the capillary, where they competed for limited antibodies on the surface. For details of the capillary allocation for drug detection in the current study, refer to Fig. S2.† For details of automated device operation, refer to ref. 20.

with ELISA plate-coating buffer inside the capillary for 1 hour, followed by washing with 1× wash buffer for 20 s. The concentration of the antibody used to coat the sensor surface should be optimized. Insufficient antibody concentration leads to a low antibody surface density, which, in turn, causes an uneven antibody distribution and a decrease in detection signal (see, for example, Fig. 2(A), (B) and (D)). On the other hand, in the presence of excessive antibody on the surface, drug and drug-HRP bind to it without competition, which deteriorates the detection sensitivity and limits the dynamic range (*i.e.*, it makes the signal to saturate easily – see Fig. 2(C) for example). Therefore, the optimal concentration for antibody for each target analyte should be the lowest possible antibody concentration (in order to save the antibody) that can generate the largest difference in chemiluminescence signal between the lowest and the highest drug concentration. As shown in Fig. 2, the optimal antibody concentration was determined to be 20 μg mL^{-1} for methadone, 15 μg mL^{-1} for METH, 10 μg mL^{-1} for amphetamine, and 20 μg mL^{-1} for THC.

After antibody incubation, the capillaries were blocked with 1× reagent diluent (1% BSA in 1× PBS) for 40 minutes at room temperature. Finally, washing was conducted again to remove unbound molecules. Solutions containing the analytes (*i.e.*, free drug molecules) were diluted with artificial sweat in order to prepare free drug standards at serial concentrations. All reagents such as drug-HRP conjugates, wash buffer, and substrate were pre-loaded into the wells on the sample/reagent reservoir plate.

Drug detection. Actual drug detection was performed as follows. (1) An equal volume of free drug standards was manually mixed with drug-HRP solutions of pre-determined concentrations, which competed with the free drug molecules in binding to the antibodies on the capillary surface, in wells of the sample/reagent reservoir plate. (2) 8 μL of mixture solution was withdrawn into the capillary and incubated inside the capillary for 15 minutes. (3) The solution was ejected from the capillary to the waste well on the sample/reagent reservoir plate, followed by rinse with 1× wash buffer twice (20 seconds for each wash). (4) The chemiluminescent substrate was withdrawn into the capillaries and the chemiluminescent images were recorded by a CMOS camera after 3 seconds of incubation. The entire detection time (*i.e.*, from loading samples to recording results) was approximately 16 minutes. The sensor arrangement in a cartridge to detect each individual drug is illustrated in Fig. S2 (A).†

Signal analysis. ImageJ software was used to analyze the images. Blue chemiluminescence was extracted and its intensity counts along the capillary longitudinal direction was recorded and averaged among 150 pixels (about 3.7 mm in length along the capillary center line),[20] which was related to free drug concentrations *via* the inhibition curve (IC), *i.e.*, the inhibition ratio B/B_0(%) as a function of the analyte (free drug) concentration, where B is the signal of a drug at a certain concentration and B_0 is the signal of the drug at zero concentration.

2.4. Characterization of cross-reactivity

In order to develop a sensor array that can quantify the multiple analytes simultaneously, it is important to examine their cross-reactivity. Using the methadone as an example, methadone was the target analyte and channels of the capillary sensor were coated with methadone antibody first. After blocking, serial concentrations of the challenging analytes, METH, amphetamine, or THC, were individually mixed with methadone-HRP and withdrawn into the capillaries. The challenging analyte molecules (*e.g.*, METH) competed with methadone-

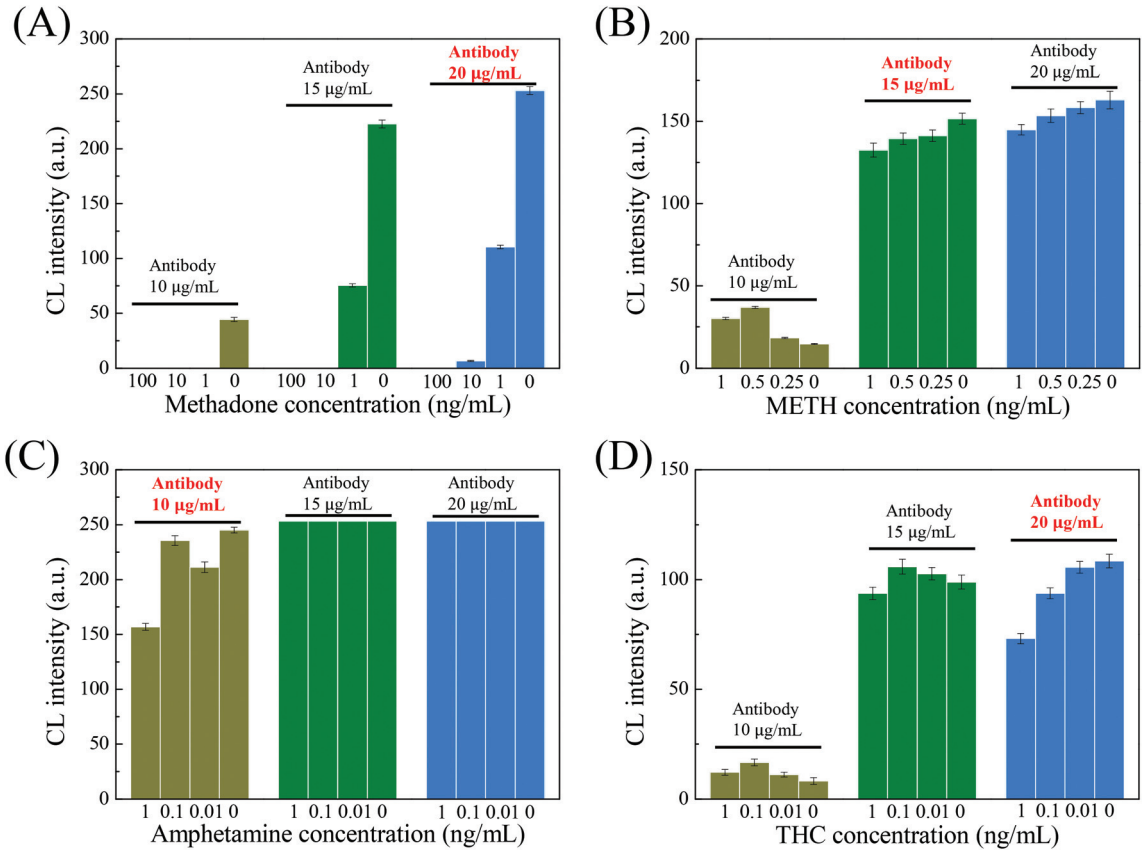

Fig. 2 Optimization of the antibody concentration used to coat the capillary inner surface. (A) Methadone. Drug concentration from 0 to 100 ng mL^{-1} competed with 500× diluted methadone-HRP. Optimal antibody concentration was 20 μg mL^{-1}. (B) METH. Drug concentration from 0 to 1 ng mL^{-1} competed with 2000× diluted METH-HRP. Optimal antibody concentration was 15 μg mL^{-1}. (C) Amphetamine. Drug concentration from 0 to 1 ng mL^{-1} competed with 2000× diluted amphetamine-HRP. Optimal antibody concentration was 10 μg mL^{-1}. Note that the slight decrease in the 0.01 ng mL^{-1} signal is due to the reason that it is below our detection limit with 2000× diluted amphetamine-HRP. (D) THC. Drug concentration from 0 to 1 ng mL^{-1} competed with 2000× diluted THC-HRP. Optimal antibody concentration was 20 μg mL^{-1}. Error bars are obtained with three inter-cartridge measurements. CL: chemiluminescence.

HRP for the methadone antibody coated on the capillary surface. Cross-reactivity is calculated as follows:[21]

$$\text{Cross-reactivity } (\%) = [\text{IC}(\text{methadone})_{50}]/[\text{IC}(\text{challenging analyte})_{50}] \times 100\%, \quad (1)$$

where $[IC_{50}]$ is the half-maximum inhibition concentration.

2.5. Multiplexed detection of drugs

In order to demonstrate multiplexed drug detection, four drugs at three different levels (high, intermediate, and low concentrations) were spiked into sweat. Four capillaries on a cartridge coated with four different antibodies were used to detect the four drugs. Four drug-HRP conjugates were prepared individually at 2000×, 4000×, 15 000×, and 4000× dilution for methadone-HRP, METH, amphetamine-HRP, and THC-HRP, respectively, for the detection of the corresponding drugs. The sensor arrangement in a cartridge for multiplexed drug detection is illustrated in Fig. S2(B).†

Furthermore, to improve convenience in practical utility, individual drug-HRP was replaced with a mixture of drug-HRPs with the final concentration equivalent to 2000×, 4000×, 15 000×, and 4000× dilution for methadone-HRP, METH-HRP, amphetamine-HRP, and THC-HRP, respectively. The remaining procedures were the same as the experiments in the multiplexed drug detection described previously.

3. Results

3.1. Detection of individual drugs

The sensing performance of the device in drug detection was systematically investigated. Each individual drug with various concentrations in sweat was analyzed and the entire analysis took ~16 minutes to complete. The capillary arrangement is illustrated in Fig. S2(A).† Three cartridges were used for each drug to generate the error bars. The corresponding inhibition curve is plotted in Fig. 3. Averaged relative standard deviation is 0.6% for methadone, 1.4% for METH, 0.4% for amphetamine, and 1.0% for THC, showing good reproducibility

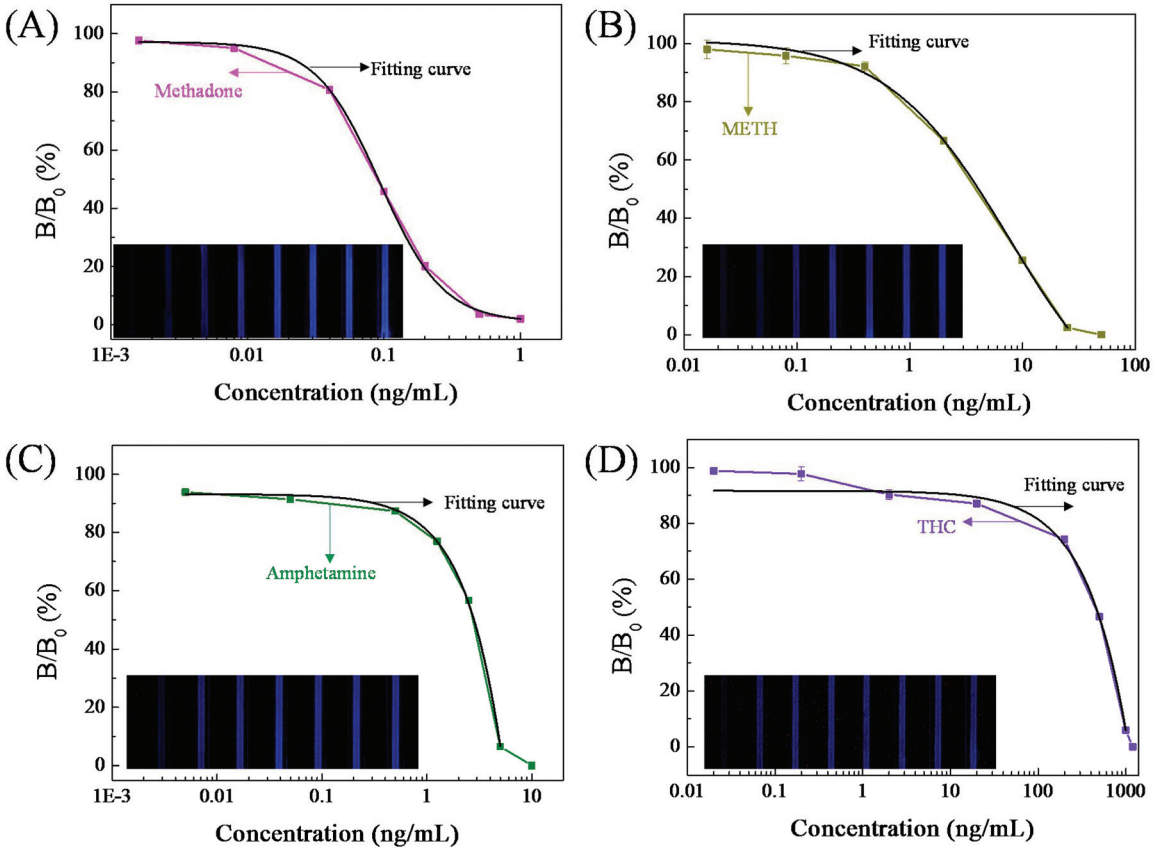

Fig. 3 Inhibition curves for four drugs of abuse. (A) Methadone. Dynamic range: 0.0016 ng mL^{-1}–1 ng mL^{-1}. (B) METH. Dynamic range: 0.016 ng mL^{-1}–25 ng mL^{-1}. (C) Amphetamine. Dynamic range: 0.005 ng mL^{-1}–10 ng mL^{-1}. (D) THC. Dynamic range: 0.02 ng mL^{-1}–1000 ng mL^{-1}. Solid black lines are the logistic curve fit. Error bars are obtained with three inter-cartridge measurements. Insets show the exemplary images for drugs of various concentrations, from which the chemiluminescence signal are extracted. The concentration of the competitor was 2000×, 4000×, 15 000×, and 4000× dilution from the original concentration provided by the vendor (Fitzgerald) for methadone-HRP, METH-HRP, amphetamine-HRP, and THC-HRP, respectively.

among cartridges. Due to the competitive nature of the analyte and its competitor (*i.e.*, drug-HRP), the higher the analyte concentration, the lower the chemiluminescence signal. In general, all inhibition curves follow a logistic relationship with $R^2 > 0.99$ (x is in the units of ng mL^{-1}):

$$y = 0.87 + 96.23/(1 + (x/0.09)^{1.87}) \text{ for methadone;}$$

$$y = -35.27 + 136.48/(1 + (x/7.63)^{0.81}) \text{ for METH;}$$

$$y = -1.31 + 94.46/(1 + (x/1473.04)^{1.2}) \text{ for amphetamine, and}$$

$$y = -2.14 + 93.79/(1 + (x/5.95)^{0.92}) \text{ for THC.}$$

These curves will be used as a calibration curve in the subsequent tests. With the above inhibition curves, the detection limit ($IC_{(100\text{-}3\delta)}$, δ is the standard deviation of artificial sweat without analyte and is 0.05%, 1.3%, 2.4%, and 2.8% for methadone, METH, amphetamine, and THC, respectively) is estimated to be 1.6 pg mL^{-1} for methadone, 142 pg mL^{-1} for METH, 35 pg mL^{-1} for amphetamine, and 20 pg mL^{-1} for THC, indicating the high sensitivity of the device (while being rapid), which are similar to or even better than the commercial products and previous work, which are usually on the order of 100–1000 pg mL^{-1}.[19]

3.2. Tuning of the dynamic range

Since many drugs of abuse have been legalized in many states in the US (and in Canada), the presence of drugs and their corresponding metabolites in sweat of a drug user does not necessarily establish the case of illegal drug use – it all depends on the appropriate cut-off. For example, the typical concentration of a drug in sweat of someone who uses a drug can be as large as >10 ng mL^{-1}.[7–10] Therefore, it is important that the dynamic range of a sensor be adjustable to cover both sides of the cut-off values, should those cut-off values be established. Fig. 4 shows that the dynamic range of the drugs (especially methadone, METH, and amphetamine) can be tuned by changing the concentration of the corresponding competitor (*i.e.*, drug-HRP). With the increased concentration of drug-HRP, the entire inhibition curve shifts to a higher drug concentration range. This is because a higher concentration of the drug is needed to compete with the higher concentration

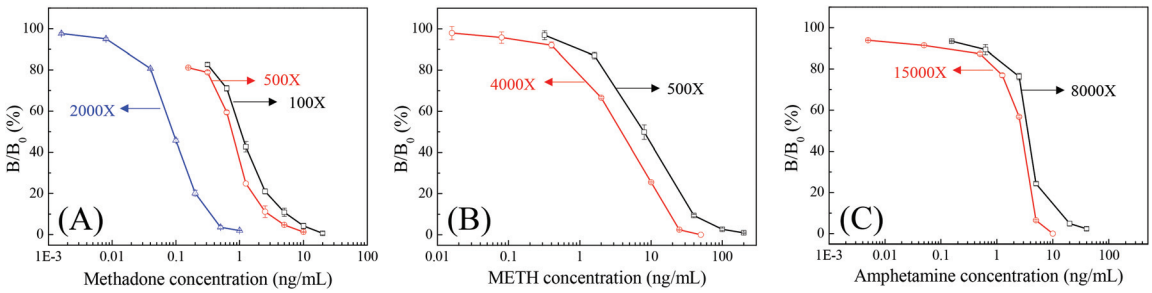

Fig. 4 Dynamic range of methadone, METH, and amphetamine can be shifted to a higher concentration by using a higher concentration of the corresponding competitor, *i.e.*, drug-HRP. The dilution factors for the drug-HRPs are shown in the figure. Error bars are obtained from three inter-cartridge measurements.

of drug-HRP in order to generate the same chemiluminescence signal. As shown in Fig. 4, methadone concentration up to 1 ng mL^{-1}, 10 ng mL^{-1}, and 20 ng mL^{-1} can be quantitatively analyzed with 2000×, 500×, and 100× dilution of methadone-HRP, respectively. Similarly, the upper limit of METH is shifted from 25 ng mL^{-1} to 200 ng mL^{-1} with 8× higher METH-HRP concentration, and the upper limit of amphetamine is shifted from 10 ng mL^{-1} to 40 ng mL^{-1} by changing the dilution factor of amphetamine-HRP from 15 000× to 8000×. Note that the detection range of THC is sufficiently wide (see Fig. 3(D)) and therefore does not need any further adjustment.

3.3. Cross-reactivity of capillary based immunoassay sensor

In order to perform multiplexed detection of drugs in sweat, we need to first examine the cross-reactivity of our capillary based sensors. The capillary coated with the antibody intended for detecting one particular drug (*i.e.*, target analyte) is challenged with various concentrations of the other three drugs (*i.e.*, challenging analytes). In our study, we used the concentration of 10 000 ng mL^{-1}, 5000 ng mL^{-1}, 400 ng mL^{-1}, 80 ng mL^{-1}, and 1.6 ng mL^{-1} for the challenging analytes in sweat. The cross-reactivity is calculated according to eqn (1) and the corresponding results are presented in Table 1. Overall, the sensors exhibit excellent specificity with the cross-reactivity far less than 1%. One exception is the 3.1% cross-reactivity of METH sensor to amphetamine, which is due to the structural similarities between METH and amphetamine.

3.4. Multiplexed drug detection

In the studies that we have conducted so far, each individual drug was added to sweat and analyzed by our sensor. In reality, multiple drugs (and the associated metabolites) may be present in sweat of a drug user. Therefore, it is important to quantify multiple drugs in sweat simultaneously. To demonstrate the multiplexed drug detection capability of our sensors, a drug mixture containing all four drugs was prepared to compete with individual drug-HRP conjugate for the detection of the corresponding drugs (see the arrangement of the capillary array in Fig. S2(B)†). By using the chemiluminescence signal and the calibration curves in Fig. 3, the concentration of a target drug in sweat can be quantified. The quantification and the recovery rate for each drug are shown in Fig. 5 and Table 2. Overall, the recovery rate (defined as the ratio between the observed and spiked concentration) is between 75% and 120% with an average of 106% among all concentration levels, which proves the quantitative analysis capability and the reliability of our sensors. Furthermore, we performed the drug detection by using the mixed drug solution with one drug absent in order to confirm the cross-reactivity. The corresponding results are shown in Table S1.† Again, no cross-reactivity was observed (*i.e.*, the sensors reported zero concentrations for the missing drugs).

In the experiment above, the drug-HRP conjugate was prepared individually for each target drug to be detected. In practice, it is more convenient and cost-effective to prepare all drug-HRP conjugates in one solution so that only one storage chamber (or vial) is needed instead of four in an eventual device. To test the feasibility, we prepared the mixture of drug-HRP conjugates containing all four competitors to compete with drug mixture that contained all four drugs. Again, by using the chemiluminescence signal and the calibration

Table 1 Cross-reactivity of four drug sensors

Target analyte	Challenging analyte	Cross-reactivity (100%)
Methadone	METH	<0.001%
	Amphetamine	<0.001%
	THC	<0.001%
METH	Methadone	<0.04%
	Amphetamine	3.1%
	THC	<0.04%
Amphetamine	Methadone	<0.02%
	METH	0.07%
	THC	<0.02%
THC	Methadone	<0.4%
	METH	<0.4%
	Amphetamine	<0.4%

The final concentration of competing drug conjugates was 2000×, 4000×, 15 000×, and 4000× for methadone-HRP, METH-HRP, amphetamine-HRP, and THC-HRP, respectively, in corresponding sensors.

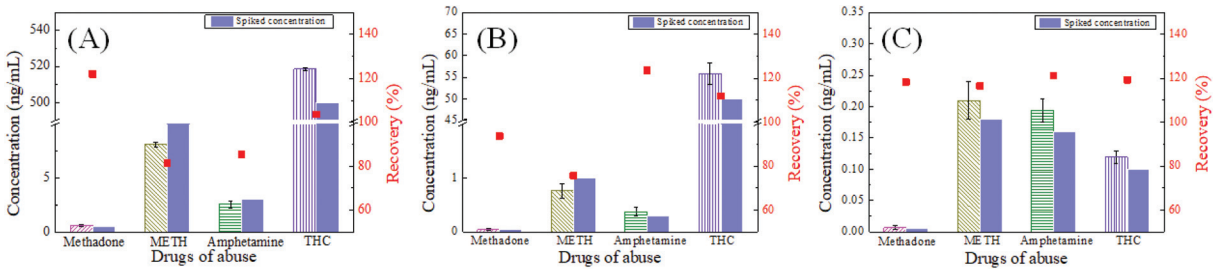

Fig. 5 Multiplexed detection of four drugs, methadone, METH, amphetamine, and THC. The four drugs were spiked in sweat at high (A), intermediate (B), and low (C) level of concentrations. The drug concentration is calculated by using the calibration curves in Fig. 3 and the chemiluminescence signals detected by the capillary sensors. Error bars are obtained with four measurements (see Fig. S2(B)† for the capillary arrangement). The recovery rates are also presented. See Table 2 for the details of the spiked concentrations, calculated concentrations, and the recovery rates.

Table 2 Recovery rate of multiplexed drug detection

	Drug	Spiked (ng mL^{-1})	Calculated (ng mL^{-1})	Recovery rate (%)	Averaged recovery rate (%)
High level	Methadone	0.5	0.61 ± 0.05	122.0	98
	METH	10	8.12 ± 0.22	81.2	
	Amphetamine	3	2.56 ± 0.31	85.2	
	THC	500	518.40 ± 1.01	103.7	
Intermediate level	Methadone	0.05	0.047 ± 0.03	93.6	101.2
	METH	1	0.76 ± 0.13	75.6	
	Amphetamine	0.3	0.37 ± 0.08	123.6	
	THC	50	55.9 ± 2.48	111.8	
Low level	Methadone	0.006	0.007 ± 0.002	118.3	118.9
	METH	0.18	0.21 ± 0.03	116.6	
	Amphetamine	0.16	0.19 ± 0.02	121.3	
	THC	0.1	0.12 ± 0.01	119.3	

curves in Fig. 3, the drug concentration in sweat was quantified. The quantification and the recovery rate for each drug are shown in Table S2.† We notice that some individual recovery rate can be as low as 52% (for METH) and as high as 180% for THC, suggesting that the mixed drug-HRP conjugate method still needs improvement for quantitative analysis of drugs and currently it can only be used in semi-quantitative measurement of drugs.

4. Discussion

The abuse of illicit and addictive drugs has become a worldwide crisis, leading to huge social and economic costs. Rapid and sensitive drug detection shows the potential to guide clinical decision-making on diagnostics and monitoring, as well as patient screening. Compared to other biological sources, like blood and hair, sweat illustrates distinctive characteristics of being easily accessible and non-invasive as the ideal drug detection candidate. Immunoassay based on 96-well plates has been applied as one of primary tools for drug detection *in vitro*. It is simple and does not require expensive instruments. Besides, many labels such as fluorescence,[22] colorimetry,[23] quantum dots,[24] and chemiluminescence,[25] are developed and optimized to increase the detection sensitivity. The quantitative detection of cocaine through competitive enzyme immunoassay was reported in ref. 26. A limit of detection of 162 pg mL^{-1} was achieved, which was comparable to that of conventional gas chromatography/liquid chromatography-mass spectrometry techniques. However, the whole assay took more than 4 h and 100 μL costly reagent was applied in each procedure. A study based on quantum dots-labeled antibody fluorescence immunoassays for the detection of morphine shortened the detection time to 1 h, but the detection limit of 270 pg mL^{-1} still needed improvement.[27] Some commercial products in Neogen Corporation (United States) furtherly improves drug detection sensitivity in 1.5 h.[19]

Here, we demonstrated a rapid tool for detection of drugs of abuse in low-volume sweat based on the capillary biosensor. Four common illicit drugs, methadone, METH, amphetamine, and THC in artificial sweat, were detected in around 16 minutes and 4 μL sweat to achieve the detection limit of 1.6 pg mL^{-1} for methadone, 142 pg mL^{-1} for METH, 35 pg mL^{-1} for amphetamine, and 20 pg mL^{-1} for THC. The performance of previous related work regarding to sample volume, analysis time as well as detection limit was shown and compared in Table 3. Because of low surface-to-volume ratio of wells compared to the capillary, 96-well plate immunoassays are difficult to be optimized greatly.

Table 3 Performance of previous related drug detection work

Drug	Platform	Analytical time	Sample volume	Detection limit
Methadone	Colorimetry	~1.5 h	10 μL	50 pg mL^{-1} (ref. 19)
METH	Colorimetry	~1.5 h	10 μL	1 ng mL^{-1} (ref. 19)
THC	Colorimetry	~1.5 h	10 μL	100 pg mL^{-1} (ref. 19)
Cocaine	Colorimetry	>4 h	50 μL	162 pg mL^{-1} (ref. 26)
Morphine	Quantum dots	>1 h	100 μL	270 pg mL^{-1} (ref. 27)
Amphetamine	Colorimetry	>1.5 h	25 μL	10 ng mL^{-1} (ref. 28)
Methadone	Colorimetry	>1.5 h	—	25 ng g^{-1} (ref. 29)
Amphetamine	Colorimetry	>1.5 h	—	20 ng g^{-1} (ref. 29)
METH	Colorimetry	>1.5 h	—	20 ng g^{-1} (ref. 29)
METH	Colorimetry	2 h	10 μL	14.9 pg mL^{-1} (ref. 30)
Cocaine	Colorimetry	>50 min	25 μL	60 pg mL^{-1} (ref. 31)

In the near future, we will explore the following directions. First, we will test more drugs and increase the multiplicity of drug detection. Second, the detection protocol will be improved to shorten the entire assay to 5–10 minutes. Third, a smaller cartridge with smaller capillaries will be developed to further reduce the sample/reagent consumption. Finally, an integrated sweat collection module will be developed to automatically collect and transfer the sweat to the cartridge for detection.

5. Conclusion

We have developed sensitive and quantitative technique for rapid and on-site detection of multiple drugs of abuse using a microfluidic capillary based sensor array and a competitive ELISA protocol. Our results show that the device is able to complete the assay of four drugs in sweat in ~16 minutes with only 4 μL of sweat for each drug. The detection range is between a few pg mL^{-1} to tens-hundreds of ng mL^{-1} and can be tuned for different applications.

Conflicts of interest

The authors declare the following competing financial interest (s): M. K. K. O. and X. F. are co-founders of and have an equity interest in Optofluidic Bioassay, LLC. G. K., M. A. I., and X. F. are co-founders of and have an equity interest in Arborsense Inc.

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