IV INTERNATIONAL SCIENTIFIC-PRACTICAL CONFERENCE
17 - 18 May 2018, Moscow

Сеченовский Университет

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Development of paper-based microfluidic devices for presumptive drug detection

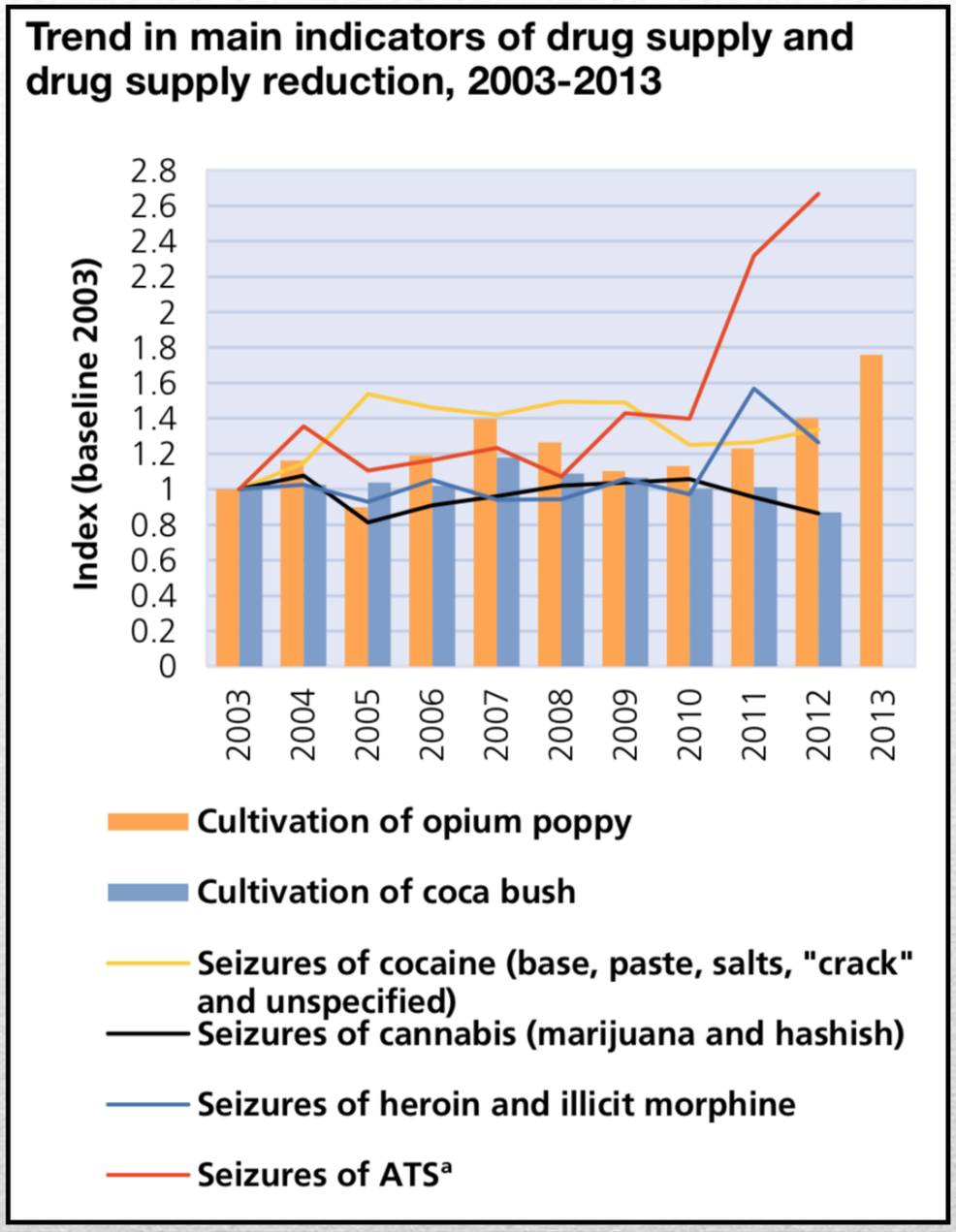
Giacomo Musile

Unit of Forensic Medicine
Dept of Diagnostics and Public Health
University of Verona

Seized drugs

http://www.threesheetsnw.com/blog/2009/08/ecstasy-tablets-worth-720000-seized-from-boat-near-neah-bay/

https://www.todayonline.com/world/asia/police-bust-kl-drug-syndicate-masterminded-student-nigeria



[a] *Including amphetamine, "ecstasy"-type substances, methamphetamine, non-specified ATS, other stimulants and prescription stimulants. For the categories of other stimulants and prescription stimulants, seizures reported by weight or volume only are included.*

UNODC World Drug Report 2014

Analysis of drugs in forensic laboratories

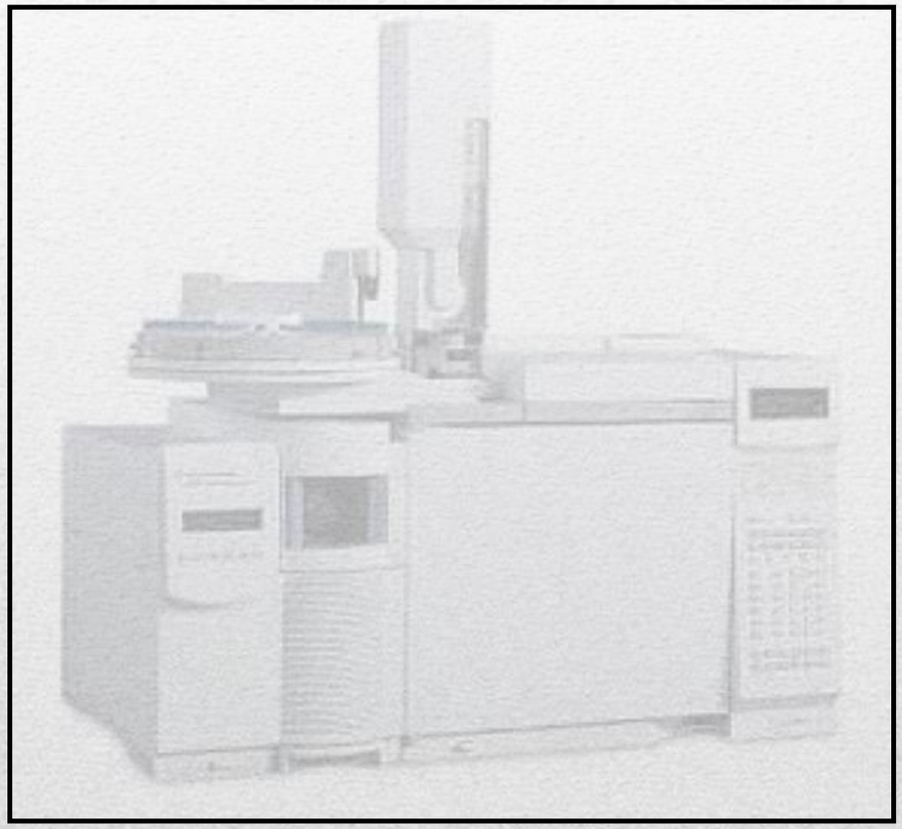
Presumptive tests

Identifying step

Analysis of drugs in forensic laboratories

Presumptive tests on-site

Identifying step



Drawbacks of common presumptive methods

Expensive to maintain
Requires skilled handler

Dangerous if
used improperly
Fairly expensive

Bulky
Fairly expensive
(40,000 $)

Aim of the experimental work

- Development of an inexpensive on-site method able to detect drugs of abuse

FIU
FLORIDA INTERNATIONAL UNIVERSITY

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Microfluidics Paper-based Analytical Devices (µPADs)

Angewandte International Edition Chemie

GDCh A Journal of the Gesellschaft Deutscher Chemiker

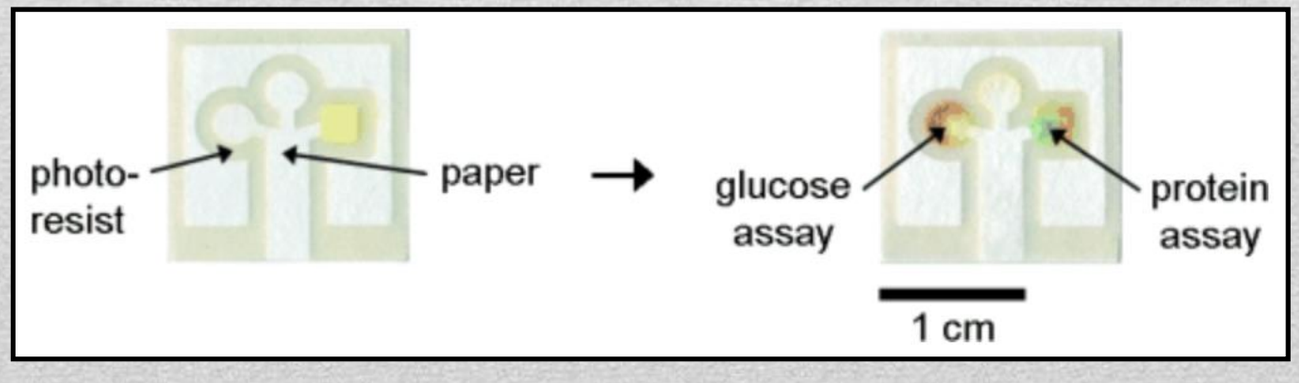
Communication | Full Access

Patterned Paper as a Platform for Inexpensive, Low-Volume, Portable Bioassays*

Andres W. Martinez, Scott T. Phillips Dr., Manish J. Butte Dr., George M. Whitesides Prof.

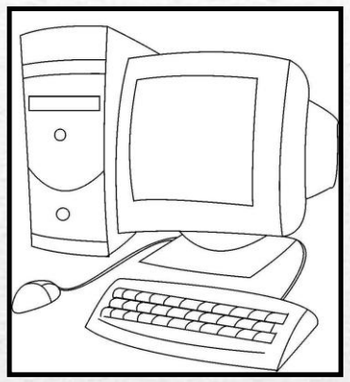
First published: 09 January 2007 | https://doi.org/10.1002/anie.200603817 | Cited by: 1151

* This research was supported by the National Institutes of Health (NIH) (GM06536 ... More

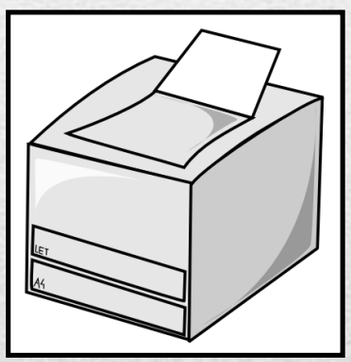


How it’s made

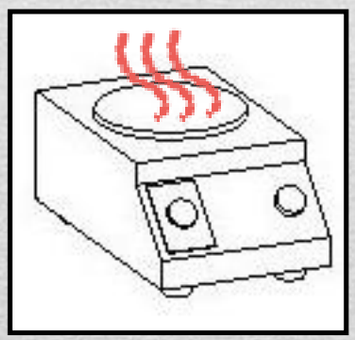
Design

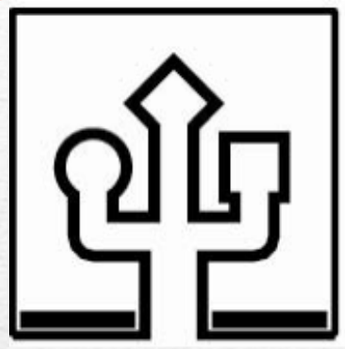


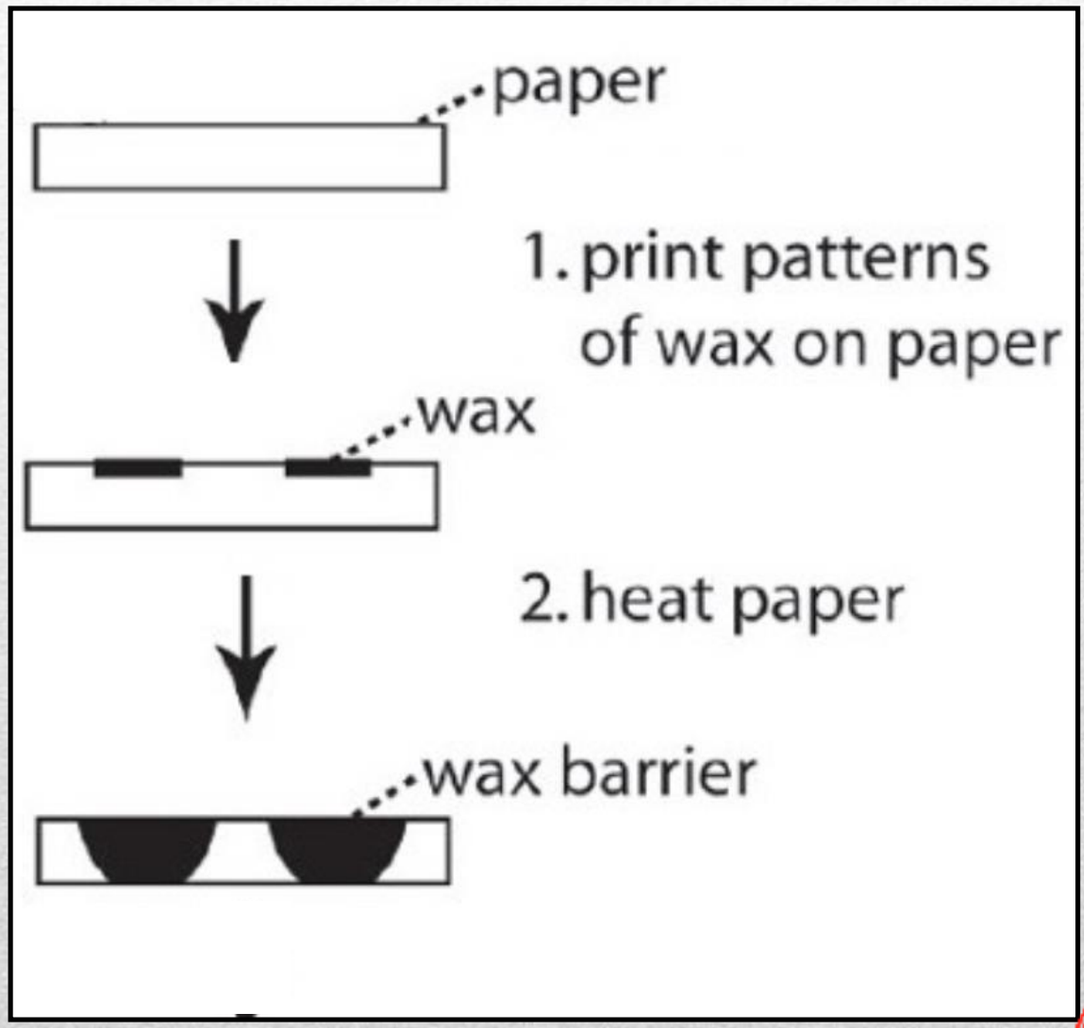
Print



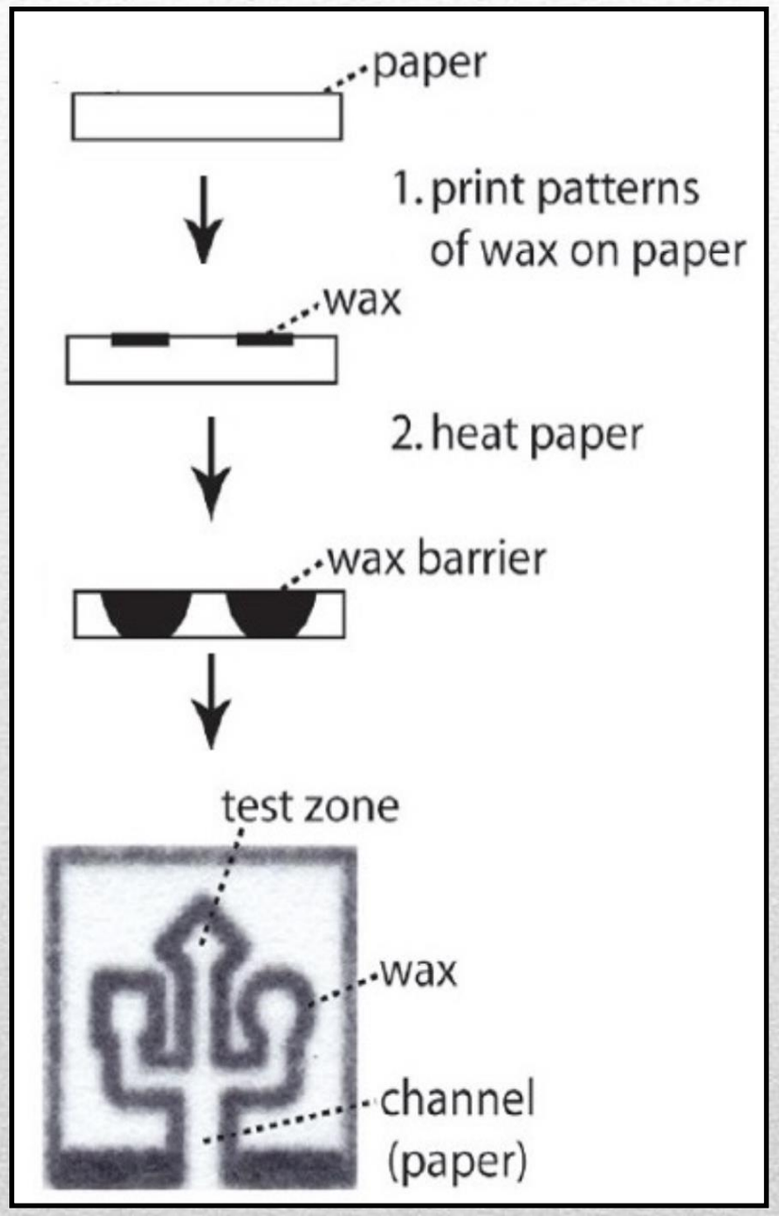
Heat the ink to create hydrophobic barriers





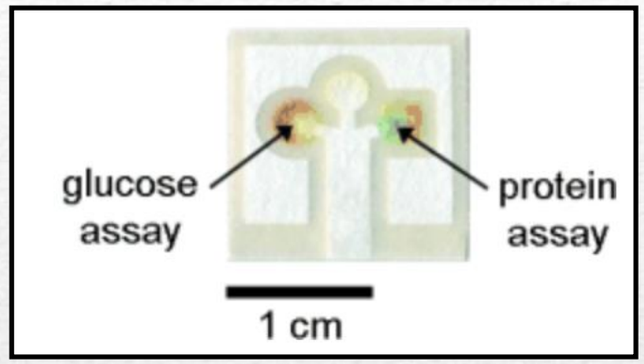


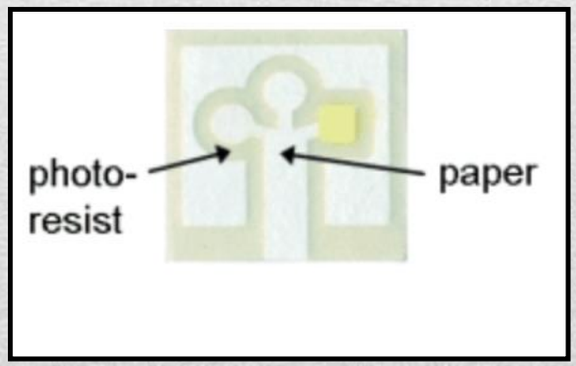
How it's made



Capillary Action

< 5 min





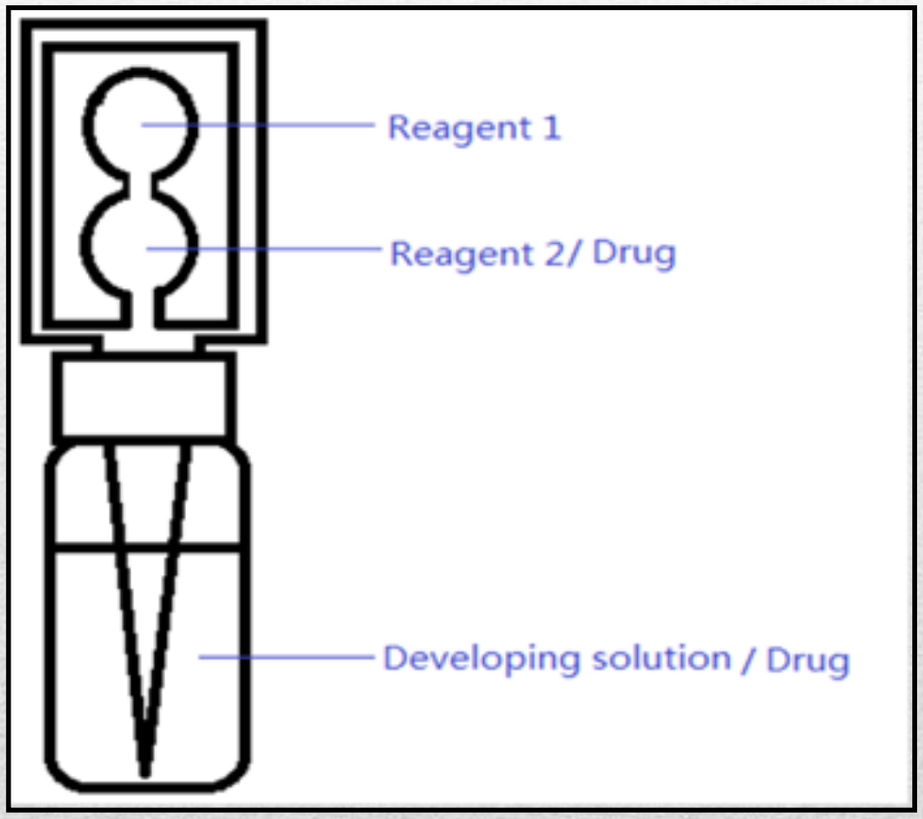
Why this approach?

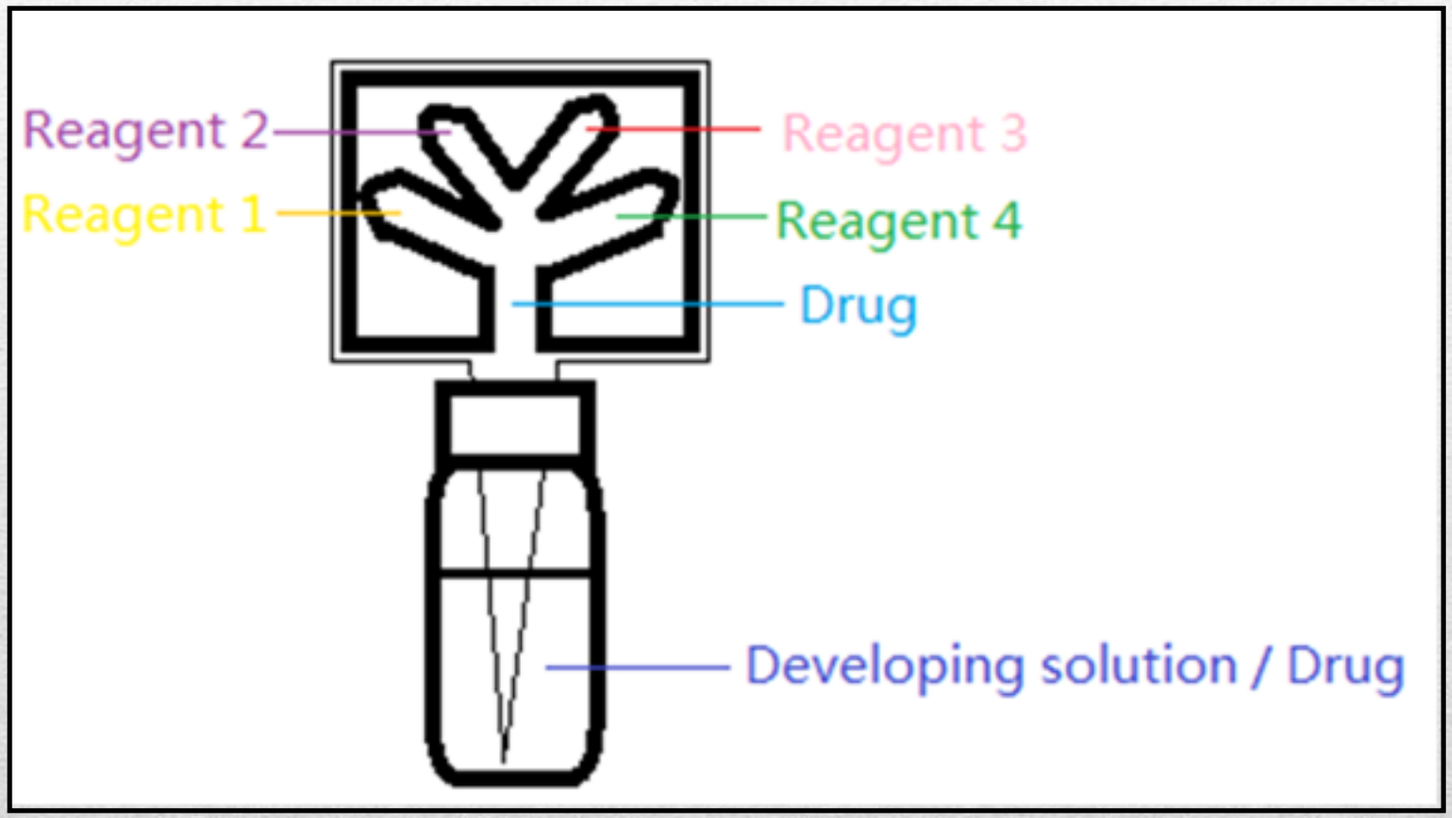
SIMPLE!

…. to design
…. to make
…. to learn
…. to use
…. to interpret
…. to pay for

Materials & Methods

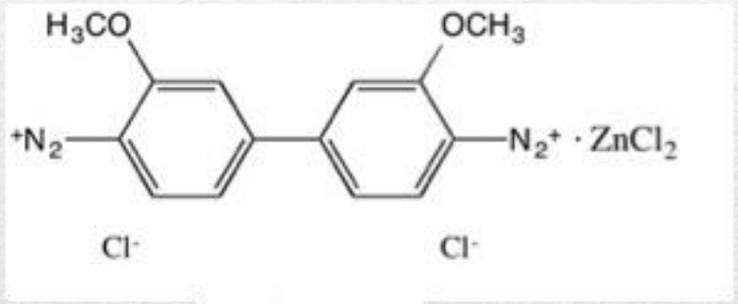
TESTING DESIGN

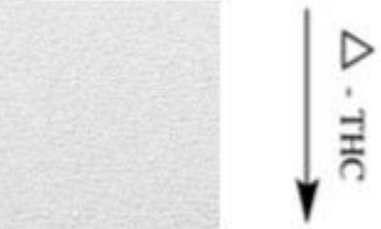


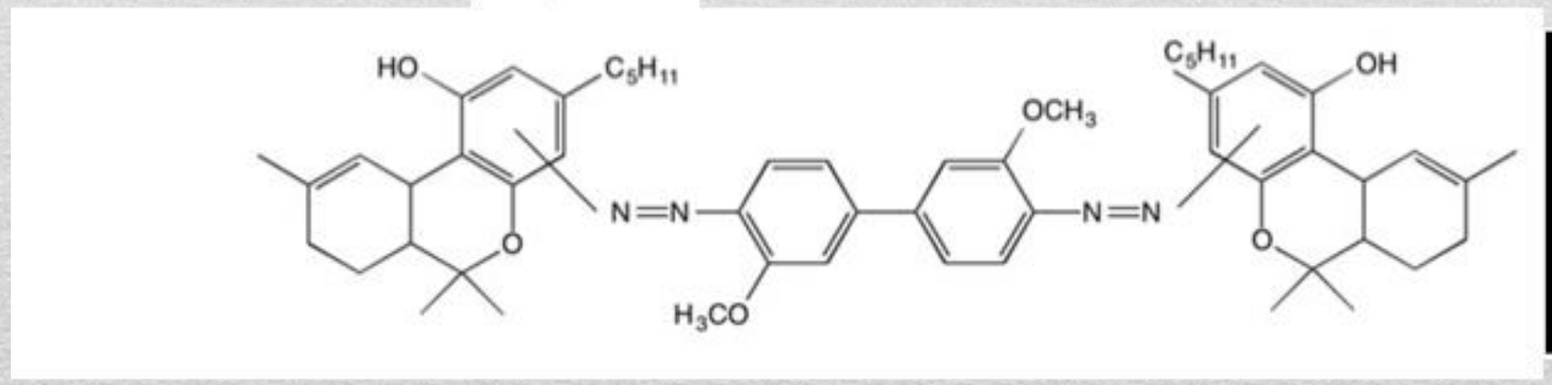


Method development

Fast Blue B/ Sodium Sulfate Reagent







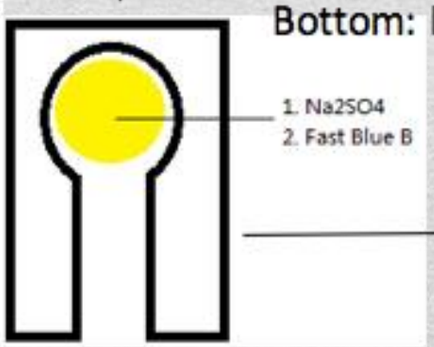
Reagent

Fast Blue B, 10mg/mL

Na2SO4, 100mg/ mL

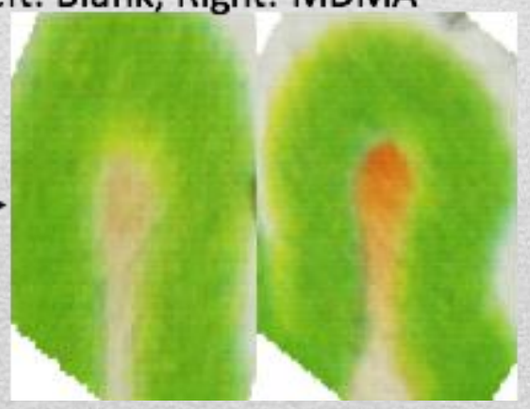
Target

Methamphetamine, MDMA



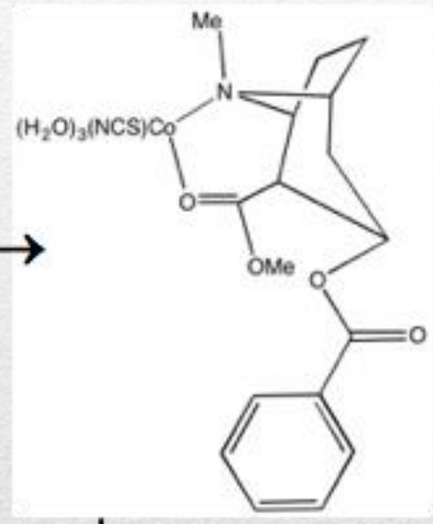
Top: Left: Blank; Right: Methamphetamir

Bottom: Left: Blank; Right: MDMA



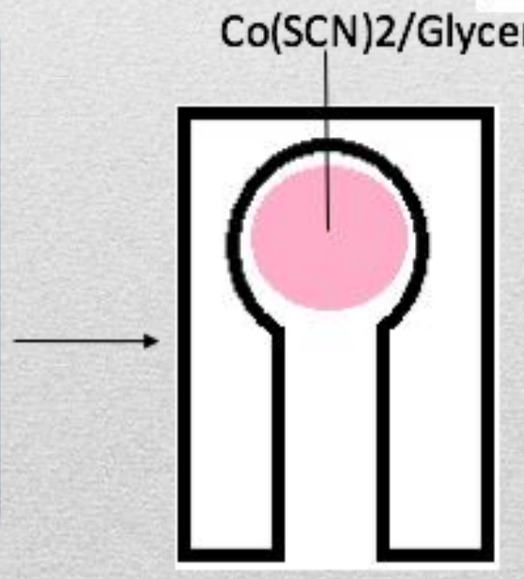
Method development

Modified Scott's Reagent

$$[Co(SCN)(H_2O)_5]^+ + 3SCN^- + 2R_3NH^+ \leftrightarrow$$ 

$$+ 5H_2O$$

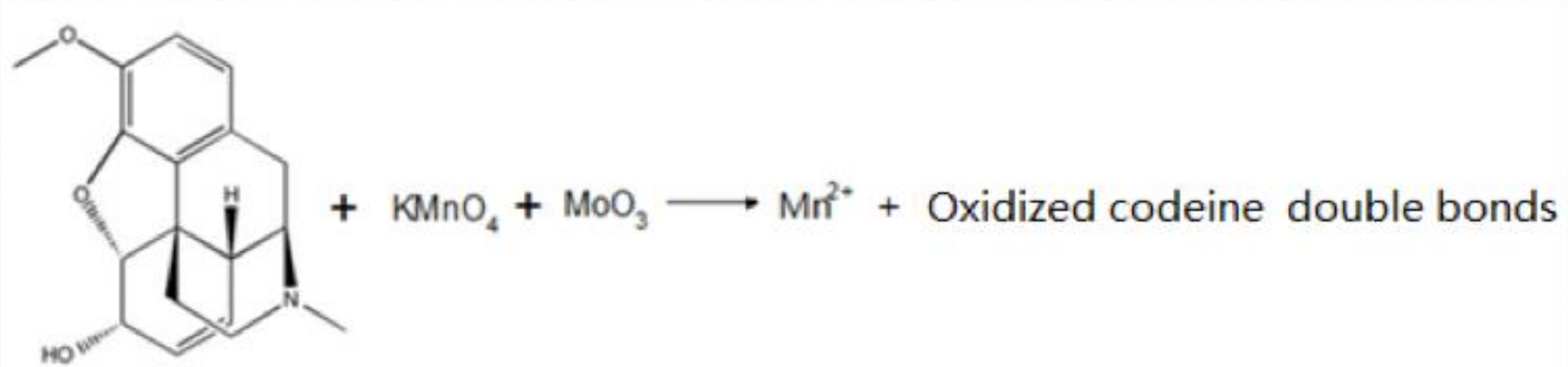
Reagent
Co(SCN)2,
100mg/mL,300mL
/ Glycerol 200 mL
Target
Cocaine



Left: Blank; Right: Cocaine

Method development

Potassium permanganate/ Molybdic acid Reagent



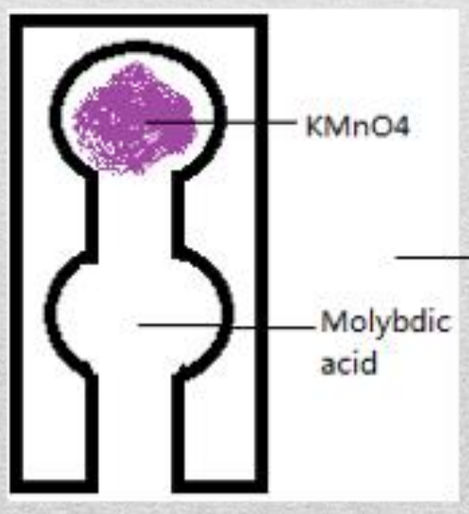
Reagent

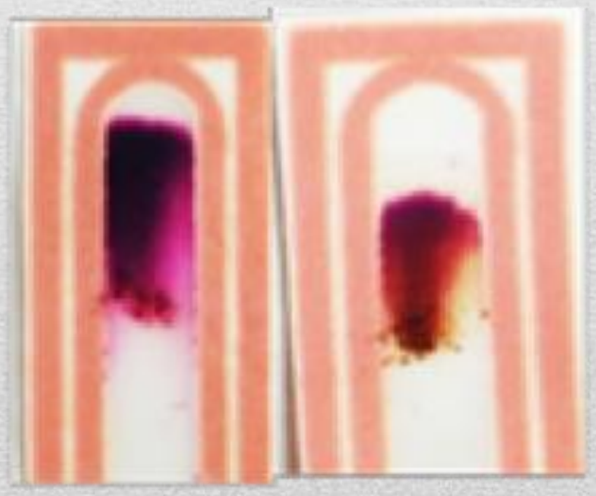
KMnO4 (Paste)

Molybdic acid (Paste)

Target

Codeine





Left: Blank; Right: Codeine

Method development

Cobalt (II) thiocyanate / NaOH Reagent

$$[Co(SCN)(H_2O)_5]^+ + 3SCN^- + 2R_3NH^+$$

$$\leftrightarrow \text{Complex of CoSCN/Ketamine} + 5H_2O$$

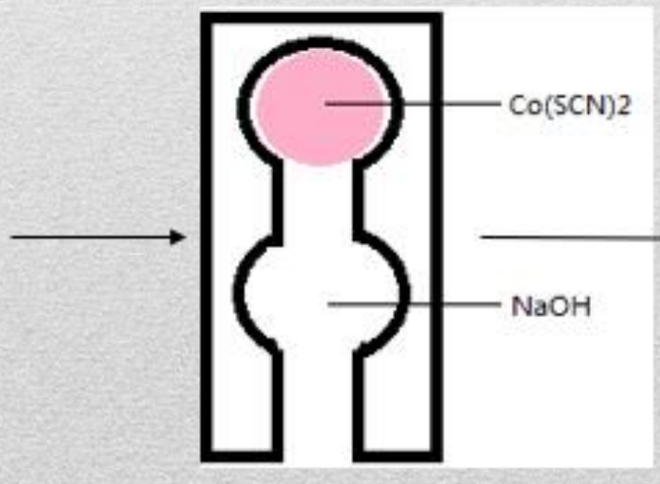
Reagent

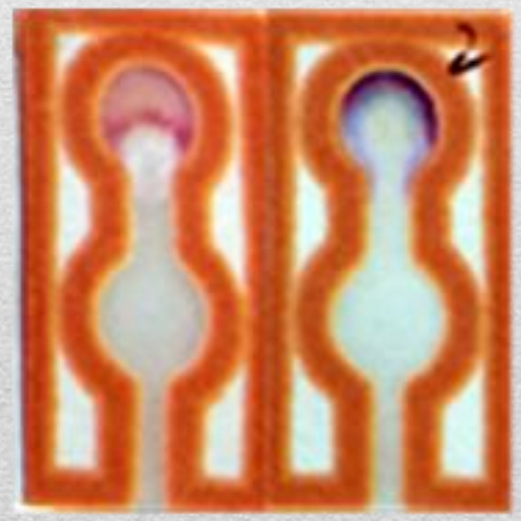
Co(SCN)2, 100 mg/mL

NaOH, 2%

Target

Ketamine

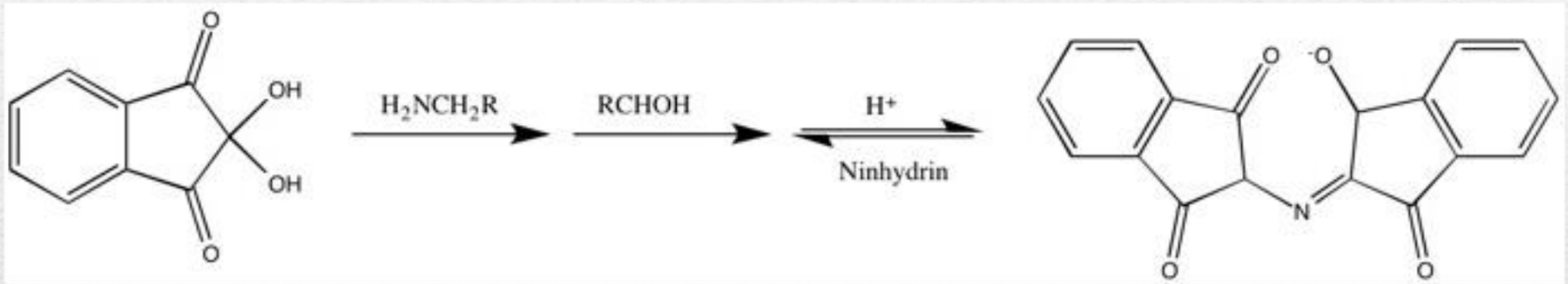




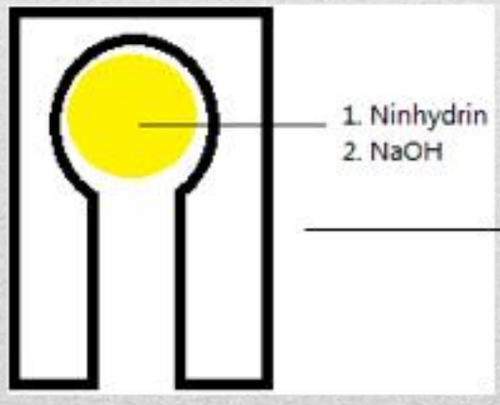
Left: Blank; Right: Ketamine

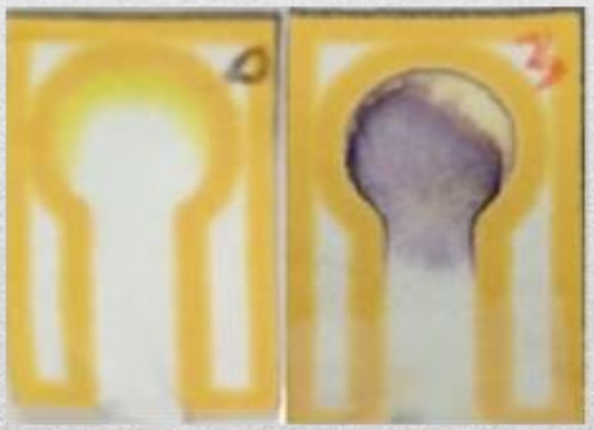
Method development

Ninhydrin/ NaOH Reagent



Reagent
Ninhydrin, 50mg/mL
NaOH, 1M
Target
Amphetamine





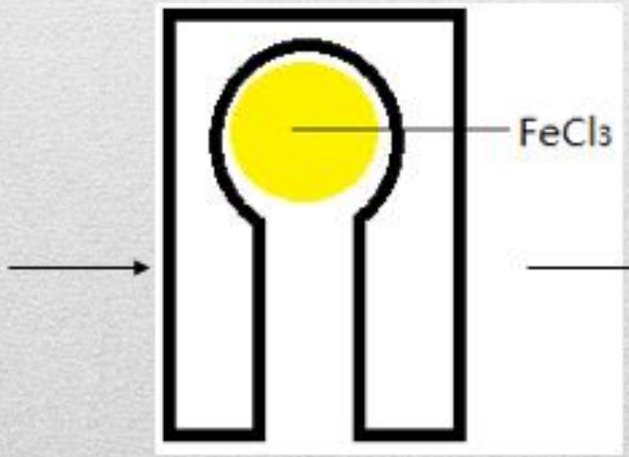
Left: Blank; Right: Amphetamine

Method development

Iron (II) Chloride Reagent

$$2\ Fe_2Cl_6 + 2\ H_2O \rightarrow 4\ FeCl_2 + 4HCl + O_2$$

Reagent
FeCl3, 100 mg/mL
Target
Morphine



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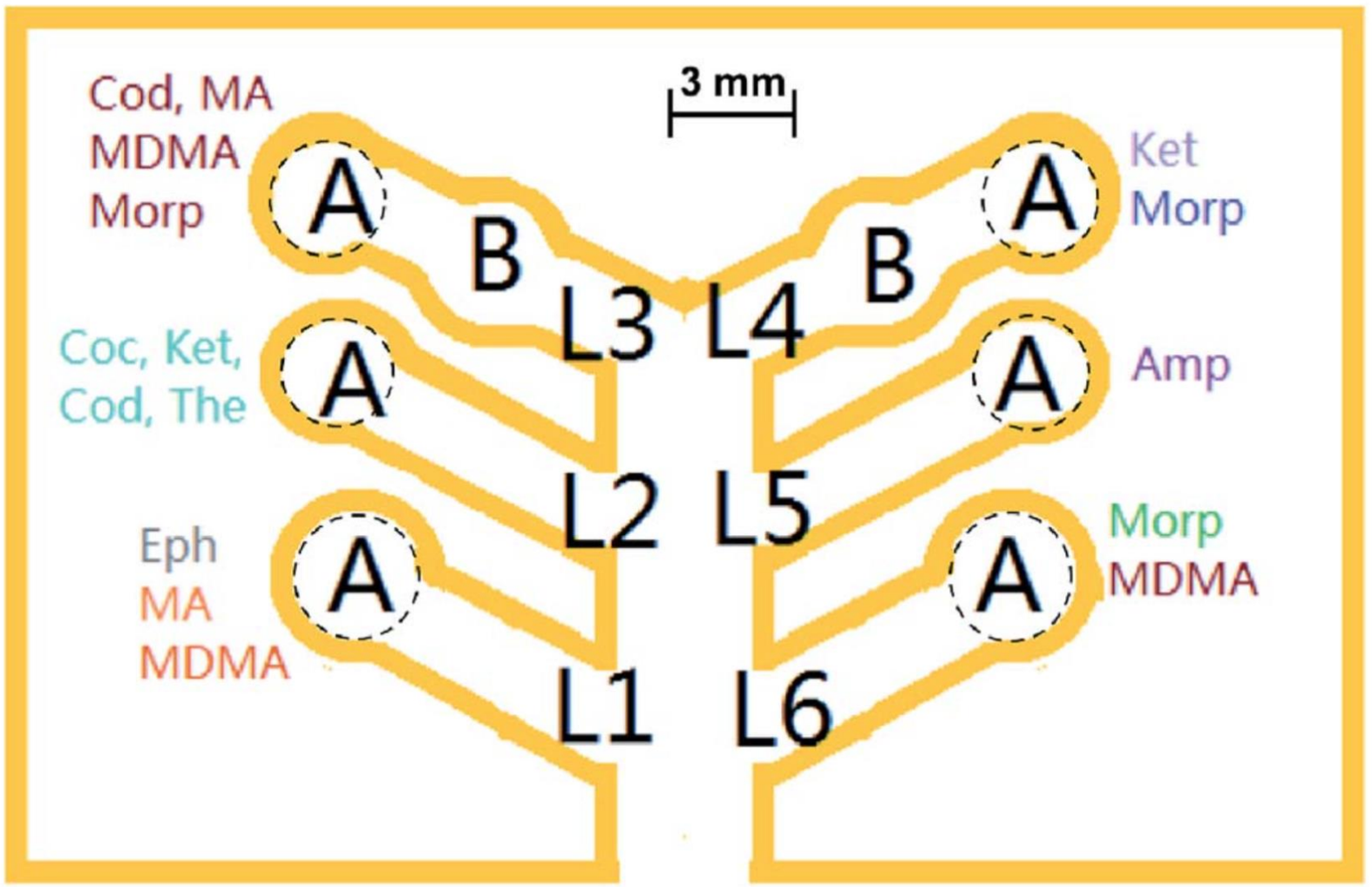
Materials & Methods

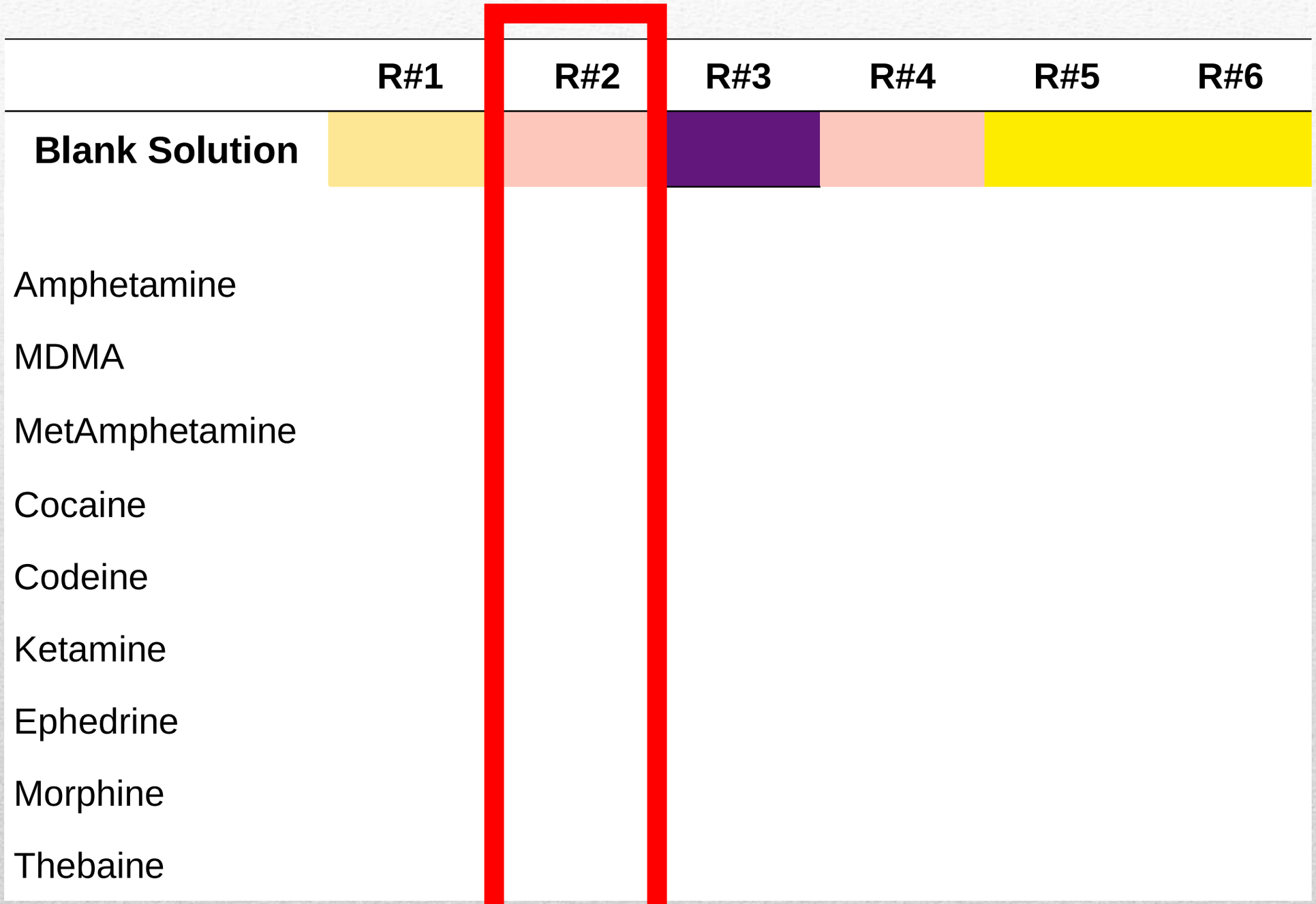


A position: 0.5 µL drug solution
B, C, D positions: 0.5 µL reagent solution

Reagents:

1. Fast Blue B
2. $Co(SCN)_2$ (Acid)
3. $KMnO_4$ MoO_4
4. $Co(SCN)_2$ (Basic)
5. Ninhydrin
6. $FeCl_3$

Method development



Method development: Common Powders & Adulterants

Cross Reactivity

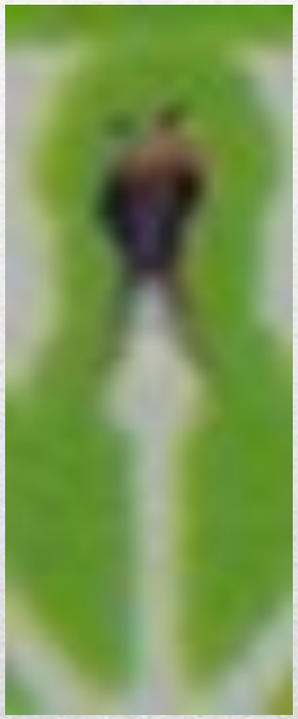
	R#1*	R#2	R#3	R#4	R#5	R#6
Blank Solution						
Positive Solution						
1. King Artur Flour Gluten Free Multipurpose Flour	NCC	**NCC**	NCC	NCC	NCC	NCC
2. Arm & Hummer Pure Baking Soda		**NCC**	NCC	NCC	NCC	NCC
4. Publix Pure Granulated Sugar Extrafine	NCC	**NCC**	NCC	NCC	NCC	NCC
5. Publix Confectioners Sugar	NCC	**NCC**	NCC	NCC	NCC	NCC
6. Rumford Aluminium Free Baking Powder	NCC	**NCC**	NCC	NCC	NCC	NCC
7. Shower Bath Salt Absorbent Body Powder	NCC	**NCC**	NCC	NCC	NCC	NCC
8. Winn Dixie Iodized Salt	NCC	**NCC**	NCC	NCC	NCC	NCC
9. Antacid Tablet	NCC	**NCC**	NCC	NCC	NCC	NCC

	R#1*	R#2	R#3	R#4	R#5	R#6
Blank Solution						
Positive Solution						
Adulterants						
Quinine	NCC		NCC	NCC	NCC	NCC
Lidocaine	NCC	NCC		NCC	NCC	NCC
Procaine					NCC	NCC
Caffeine	NCC	NCC	NCC		NCC	NCC
Diluents						
Dimethylsulfone	NCC	NCC	NCC	NCC	NCC	NCC
Lactose	NCC	NCC	NCC	NCC	NCC	NCC
Sucrose	NCC	NCC	NCC	NCC	NCC	NCC
Mannitol	NCC	NCC	NCC	NCC	NCC	NCC
Inositol	NCC	NCC	NCC	NCC	NCC	NCC
Starch	NCC	NCC	NCC	NCC	NCC	NCC

Results: Mixtures

R. Ninhydrin

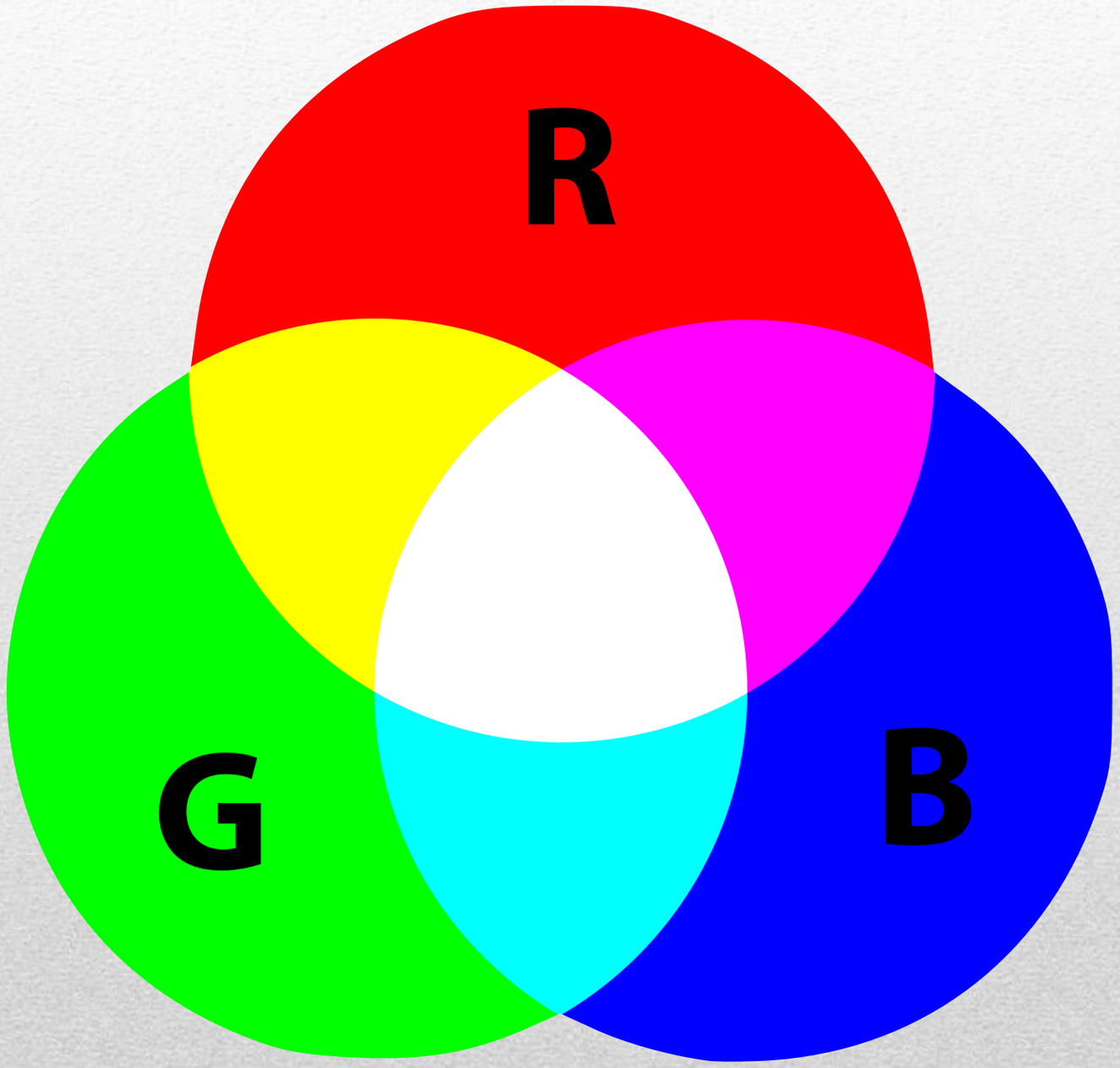
Amphetamine 100 g/L
Lactose 100 g/L
Sucrose 100 g/L
Mannitol 100 g/L
Caffeine 100 g/ L
Dimethylsulfone 100 g /L



R. Ninhydrin

Amphetamine 100 g/L

Quantitative approach?



Quantitative approach

Regression Equations & Minimum Detectable Quantity (MDQ)

$$A_{\mathrm{X}} = -\log \frac{(I_{\mathrm{X}})_{\mathrm{c}}}{(I_{\mathrm{X,w}})_{\mathrm{c}}} = -\log R_{\mathrm{X}}$$

$$\mathrm{MDQ} = \frac{3 \times \mathrm{SD}_{\mathrm{intercept}}}{\mathrm{slope}}$$

Regression analysis & MDQs

Drugs	Relationship	Linear Range, µg/µL	Regression equation	R^2	Instr. MDQ, µg	Visually MDQ, µg
Morphine	A_R and C	12.5 - 100	**y** = (0.0053 ± 0.0004) **x** + (0.0074 ± 0.0057)	0.9807	3.2	4.7
MDMA	A_B and C	25 - 100	**y** = (0.0082 ± 0.0009) **x** - (0.0219 ± 0.0239)	0.9630	8.7	11
Amphetamine	A_G and C	12.5 - 75	**y** = (0.0069 ± 0.0002) **x** + (0.0014 ± 0.0029)	0.9980	1.2	4.6
MA	A_B and C	12.5 - 50	**y** = (0.0110 ± 0.0013) **x** + (0.0280 ± 0.0205)	0.9576	5.6	6.2
Ketamine	A_{RGB} and C	25 - 75	**y** = (0.0016 ± 0.0001) **x** - (0.0053 ± 0.0022)	0.9766	4.1	11
Ephedrine	A_B and C	6.25 - 25	**y** = (0.0144 ± 0.0015) **x** - (0.0060 ± 0.0116)	0.9680	2.4	3.1

µPADs method Vs NIJ method

Drugs	PROPOSED METHOD MDQ, µg (Visual)	NIJ, µg
Cocaine*	5	60
Morphine	3	200
Ephedrine		—
Amphetamine	4	10*
MDMA	15	—
MetAmphetamine	30	5*
Ketamine	10	1250*
Codeine*	5	50

Conclusions

- An inexpensive, rapid and sensitive method has been developed
  - The method is able to detect in few minutes, few micrograms of drugs using micrograms of reagents
  - Multichannel system permits to merge information from different reactions increasing the specificity of the method
- Overall we expect this procedure to greatly benefit forensic testing, customs and other applications where quick portable testing of unknown powders is necessary.

Analytical Methods

PAPER

View Article Online
View Journal

The development of paper microfluidic devices for presumptive drug detection

Giacomo Musile,[ab] Ling Wang,[a] Jashaun Bottoms,[a] Franco Tagliaro[b] and Bruce McCord*[a]

Cite this: DOI: 10.1039/c5ay01432h

Received 3rd June 2015
Accepted 5th August 2015

DOI: 10.1039/c5ay01432h

www.rsc.org/methods

A paper microfluidic device has been developed for the presumptive testing of seized drugs in forensic casework. The procedure involves creating hydrophilic channels on chromatographic paper using wax printing and thermal lamination. The channels are connected to a single stem that draws an unknown analyte solution up into 6 different lanes. A different colorimetric reaction occurs within each lane, permitting the multiplexed detection of a variety of different compounds, including cocaine, opiates, ketamine, and various phenethyl amines. Furthermore, the linear orientation of the lanes permits series of reactants to be placed in each channel, enhancing stability and permitting sequential interaction with the analyte as the solvent front passes through each individual reagent. The resultant device was characterized for sensitivity and tested with a variety of common interferences and drug diluents. It should prove a useful device for screening seized drugs.

спасибо!

Grazie!

Microfluidics

It is the science and technology of systems that process or manipulate small amounts of fluids (10^{-9} to 10^{-18} litres), using channels with dimensions of tens to hundreds of micrometers.

Nature. 2006 Jul 27;442(7101):368-73. The origins and the future of microfluidics. Whitesides GM.

http://folk.ntnu.no/fossumj/eit/

Microfluidics Paper-based Analytical Devices (µPADs)

Angewandte International Edition Chemie

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Patterned Paper as a Platform for Inexpensive, Low-Volume, Portable Bioassays*

Andres W. Martinez, Scott T. Phillips Dr., Manish J. Butte Dr., George M. Whitesides Prof.

First published: 09 January 2007 | https://doi.org/10.1002/anie.200603817 | Cited by: 1151

* This research was supported by the National Institutes of Health (NIH) (GM06536 ... More

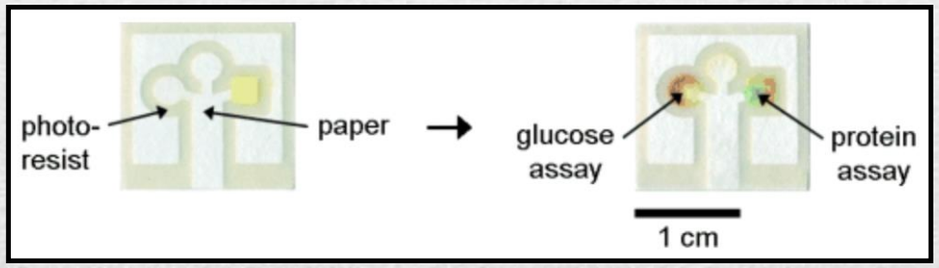
Understanding Wax Printing: A Simple Micropatterning Process for Paper-Based Microfluidics

Emanuel Carrilho*†‡, Andres W. Martinez† and George M. Whitesides*†
Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, and Instituto de Química de São Carlos, Universidade de São Paulo, 13566-590 São Carlos-SP, Brazil

Anal. Chem., **2009**, *81* (16), pp 7091–7095
DOI: 10.1021/ac901071p
Publication Date (Web): July 15, 2009

* To whom correspondence should be addressed. E-mail: gwhitesides@gmwgroup.harvard.edu (G.M.W.); emanuel@iqsc.usp.br (E.C.)., † Harvard University., ‡ Universidade de São Paulo.

Cite this: *Anal. Chem.* 81, 16, 7091-7095
RIS Citation GO



"…. Certainly a revolutionary concept with many extensions possible…."

Angew. Chem. Int. Ed. 10.1002/anie.**2018**02336

REVOLUTIONARY CONCEPT ?

Microfluidics Paper-based Analytical Devices (µPADs)

Martinez et al (2007) **Angew. Chem. Int. Ed.** 46, 1318 –1320

Carrilho, E et al (2009) **Anal Chem,** Vol. 81, No. 16,

pH and Nitrite ion

Lopez-Ruiz N et al (2014) **Anal Chem** 86:9554–9562

Human Blood typing

Khan MS et al (2010) **Anal Chem** 82:4158–4164
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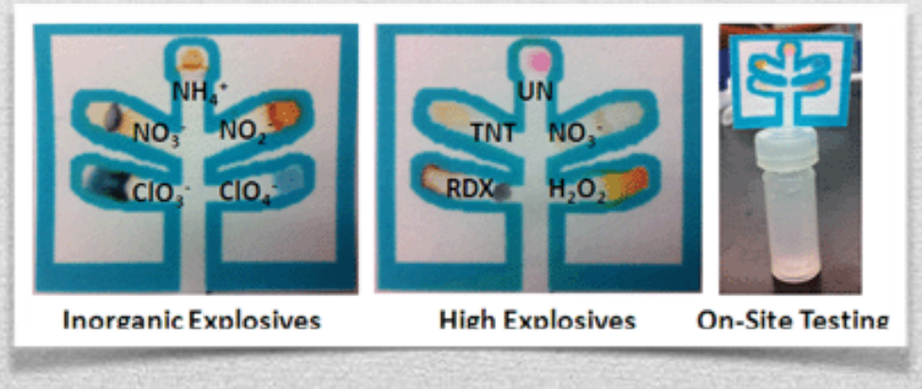
Jarujamrus P et al (2012) **Analyst** 137:2205–2210
Su J et al (2012) **Cellulose** 19:1749–1758

Elisa Test

Cheng C-M et al (2010) **Angew Chem** Int Ed 49:4771–4774

Peters KL et al (2015) ***Anal. Methods*** 10.1039/C4AY01677G



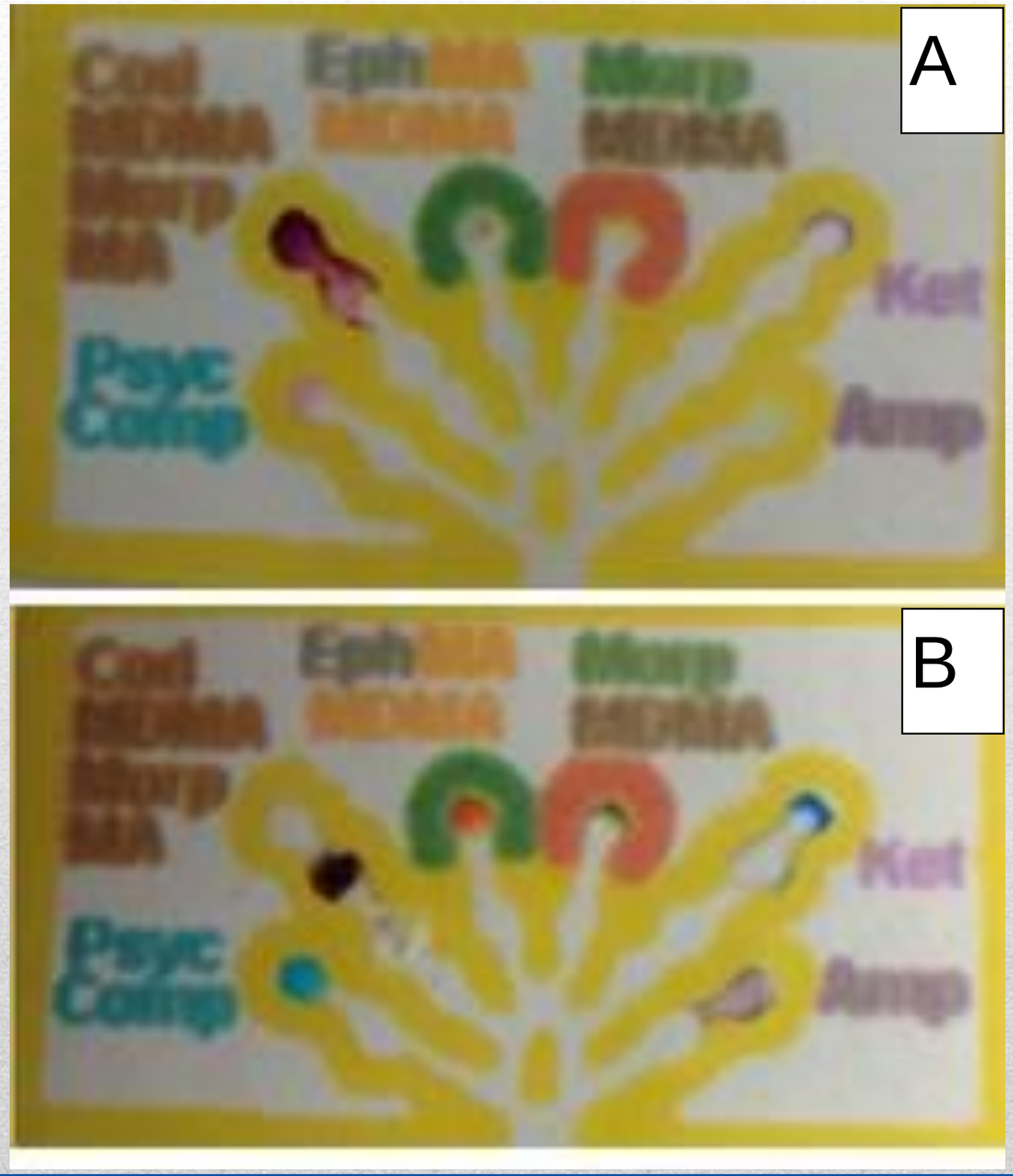
October 2012 to October 2014: over 1000 articles

Cate et al. (2015) **Anal Chem** 87:19–41

Stability

Reagent	Drugs	1 month
Fast Blue B Reagent	MDMA	**ok**
Fast Blue B Reagent	MA	**ok**
Ninhydrin Reagent	Amphetamine	**ok**
Iron (III) Chloride	Morphine	**ok**
Cobalt Thiocyanate (Acid)	Cocaine	**ok**
Cobalt Thiocyanate (Basic)	Ketamine	**ok**
Molybdic Acid	Codeine	**ok**

Results



Sample composition:
H_2O / $(CH_3)_2CO$

Sample composition:
lane 1. Cocaine
lane 2. Codeine
lane 3. MDMA
lane 4. Morphine
lane 5. Ketamine
lane 6. Amphetamine

Results: Mixtures

R. Ninhydrin

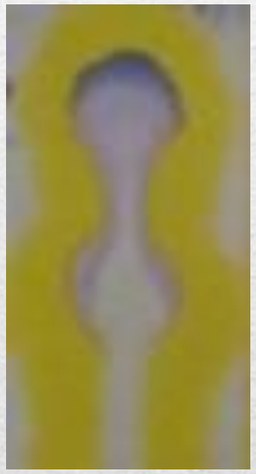
Amphetamine 100 g/L
Lactose 100 g/L
Sucrose 100 g/L
Mannitol 100 g/L
Caffeine 100 g/ L
Dimethylsulfone 100 g /L

R. Ninhydrin

Amphetamine 100 g/L

R. $Co(SCN)_2$ (Basic)

Amphetamine 100 g/L



R. $Co(SCN)_2$ (Basic)

Ketamine 100 g/L

R. $Co(SCN)_2$ (Basic)

Amphetamine 100 g/L
Ketamine 100 g/L
MetAmphetamine 100 g/L
MDMA 100 g/L

µPADs method Vs NIJ method

Drugs	LOD, mg/mL (Visual)	MDQ, µg (RegEq)	NIJ, µg
Cocaine*	5	1,4	60
Morphine	3	3,2	200
Ephedrine		2,9	—
Amphetamine	4	3,5	10*
MDMA	15	0,8	—
MetAmphetamine	30	5,8	5*
Ketamine	10	6,0	1250*
Codeine*	5	2,0	50