WiSys TECHNOLOGY FOUNDATION™

WiSys Ref: T130020

Colorimetric Presumptive Illicit Drug Detection

Overview:

For decades, color tests have provided criminalists an inexpensive, efficient way to determine if suspected controlled substances found in the field require further investigation. With over 200 controlled substances in Wisconsin alone, law enforcement rely heavily on the use of rapid color tests in the field to determine probable cause for arrest and subsequent substance identification by crime laboratory specialists and confirmatory testing.

Existing color tests are currently used to detect the presence of single active ingredients within controlled substances such as cocaine, heroin, and marijuana. However, there are currently no available tests which are effective and reliable in detecting multiple drugs as well as newer desginer synthetic drugs including synthetic cathinones. Hence there is a clear and unmet need for development of a colorimetric field test with utility for detecting the presence of existing Scheduled compounds including cocaine along with emergent drug species such as synthetic cathinones and cannabinoids.

Technology:

Researchers at the University of Wisconsin – Platteville have developed a rapid, inexpensive color test for the presumptive identification of multiple classes of controlled substances including synthetic cathinones, cannabinoids, opiates and stimulants including cocaine.

This novel, aqueous reagent test can be delivered in an industry-accepted plastic test pouch containing an ampoule of chemical reagents necessary for preliminary testing of controlled substances. Test reagents can be tailored to detect specific target compounds of interest and as new derivatives are developed.

Research and Development Status:

Prototype tests have been developed and tested internally for use in detection of synthetic cathinones (WuCo1/WuCo2) and cocaine (WuCo3) with low type 1 and type 2 error observed (Appendix 1).

WiSys' two-stage synthetic cathinone test (WuCo1/WuCo2) has been subsequently tested internally by both the Wisconsin State Crime Laboratory (WSCL) in Milwaukee, WI and the Northeastern Illinois Regional Crime Laboratory (NIRCL) in Chicago, IL demonstrating effectiveness in the detection of 17 different synthetic cathinones, phenethylamines and tryptamines (Table 1). See Appendix 2 for a complete data set. Vidoes of trials conducted at NIRCL are avaiable upon request.

In addition to WuCo1/WuCo2 and WuCo3, further test kits are under development for detection of synthetic cannabinoids.

Table 1. Third party validation by State Crime Laboratories

Test Substance	WSCL (positive/negative)	NIRCL (positive/negative)	Drug Class
Cathine	Negative*	NT	Cathinone
Cathinone	Negative*	NT	Cathinone
Dimethylone (bk-MDDMA)	Positive	NT	Synthetic cathinone
Ethylone	Positive	NT	Synthetic cathinone
3,4-methylenedioxypyrovalerone (MDPV) – active ingredient in bath salts ***	Positive	Positive	Synthetic cathinone

Methcathinone	Positive		Substituted cathinone
Methylone ***	Positive	Positive	Synthetic cathinone
Pentylone	NT**	Positive	Synthetic cathinone
2,5-dimethoxy-4-chloroamphetamine (DOC)	Positive	NT	Phenethylamine/ amphetamine
2,5-dimethoxy-4-ethylphenethylamine (2C-E)	Positive	NT	Phenethylamine
4-ethylmethcathinone	NT	Positive	Synthetic cathinone
4-fluoromethcathinone	Positive	NT	Synthetic cathinone
4-methylethcathinone	Positive	Positive	Synthetic cathinone
4-methylmethcathinone (4MMC aka mephedrone)***	NT	Positive	Synthetic cathinone
5-methoxy-diisopropyltryptamine (5-MeO-DiPT aka Foxy Methoxy)	Positive	NT	Tryptamine
5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO-MiPT, analogue of Foxy Methoxy)	Positive	NT	Tryptamine
25B-NBOMe	NT	Positive	Phenethylamine
25C-NBOMe	NT	Positive	Phenethylamine
25I-NBOMe	NT	Positive	Phenethylamine

* WuCo2 reagent does not produce a yellow result on the cathinone or cathine parent compound

** NT = Not Tested

*** three most commonly available synthetic cathinones up to 2011

Applications:

Rapid detection of illicit drugs for field officers seeking probable cause related to potential controlled substances including synthetic cathinones, cocaine, phenethylamines and tryptamines.

Key Benefits:

- One pouch, field-deployable presumptive drug test capable of easily and quickly identifying the presence of a variety of controlled substances including synthetic narcotics, stimulants, opiates, cannabinoids and future derivatives;
  - Tests are designed to detect all Scheduled compounds containing an amine group (a signature functional group in all synthetic cathinones), indoline functional groups, as well as azabicyclo and morphinan groups (groups commonly present in scheduled drugs);
  - Test reagents have been shown to possess high specificity and accuracy when testing for both well documented and emergent drug species and are stable for at least 15 months at room temperature;
- WuCo1/WuCo2 is capable of detecting over 20 different synthetic cathinones. Two-stage color test technology reduces likelihood of false positives.
- WuCo3 has shown promise in specifically detecting cocaine with higher accuracy than traditional modified Scott's test.

Intellectual Property:

WiSys Technology Foundation currently holds rights to a U.S. (14/329714) pending patent as well as a pending International PCT application (PCT/US2014/046398).

Inventors:

Chuck Cornett, Ph.D., UW-Platteville. Dr. Cornett has 25+ years' experience in analytical and radionuclear chemistry with research interests and expertise in forensics and criminalistics. Dr. Cornett's research group is focused on the study of current topics of interest to regional, state, and federal crime laboratories in the areas of controlled substance identification, ignitable liquid analysis, and the recovery of latent prints

Joseph Wu, Ph.D., UW-Platteveille. Dr. Wu has more than twenty-five years' experience in chemical engineering and analytical chemistry with research interests in electrochemical sensor technology for industrial, environmental, and

forensic applications. Dr. Wu's research group is also focused on the use of nanomaterials for enhancement of sensor performance.

Appendix 1

Preliminary data generated using T130020: two-stage synthetic cathinone test (WuCo1/WuCo2) as well as one-stage cocaine test (WuCo3).

Table 1. WuCo1/WuCo2 Testing

Two Stage Color Chart	**Synthetic Cathinone Two Stage test**	
Name	**WuCo1**	**WuCo2**
cathinone hydrochloride	+	-
2-methoxymethcathinone	+	+
methedrone	+	+
butylone hydrochloride	+	+
3-methylbuphedrone hydrochloride	+	+
3,4 dimthylethcathinone hydrochloride	+	+
3,4 methylenedioxy-α-pyrrolidnopropiophenone hydrochloride	+	+
bupropion hydrochloride	+	+
diethylcathinone hydrochloride	+	+
isopentedrone hydrochloride	+	+
chlorpheniramine maleate	+	+
diphenhydramine hydrochloride (Benadryl)	+	+
cocaine hydrochloride	+	-
ketamine hydrochloride	+	-
codeine	+	+
PCP (phencyclidine)	+	+
quinine sulfate	+	+
pseudoephedrine hydrochloride	+	+
(+-) - metoprolol	-	+
1-methylindole -3- carboxylic acid	-	-
MDPA (3,4-methylenedioxy-N-propyl-amphetamine)	-	+
lidocaine	-	+
1H - benzotriazole	-	-
1S, 2R (+) ephedrine hydrochloride	-	+
3,4 - dimethoxymethamphetamine hydrochloride	-	+
(±)-amphetamine	-	-
6-acetylmorphine	-	+
6-acetylcodeine	-	+
androstenedione	-	-
butalbital	-	-
gamma-butyrolactone	-	-
butabarbital	-	-

4-bromo-2,5-dimethoxyphenethylamine	-	-
Two Stage Color Chart	**WuCo1**	**WuCo2**
4-chloro-2,5-dimethoxyphenethylamine hydrochloride	-	-
(±)-N-ethylamphetamine	-	+
hydrocodone	-	-
hydromorphone hydrochloride	-	+
heroin	-	+
LSD (lysergic acid diethylamide)	-	-
morphine	-	+
methamphetamine	-	-
MDA (3,4-methylenedioxyamphetaine)	-	-
methaqualone	-	+
methylephedrine	-	-
norcodine	-	+
oxycodone hydrochloride	-	+
phentermine	-	+
XLR-II	-	-
UR-144	-	-
AM 1248	-	-
(-)-delta9-THC	-	-
testosterone	-	-

Note: the results above are all for 50 micrograms of drug standards.
"-" negative or no color change
"+" positive or color change

Table 2. WuCo3 Testing

One Stage Cocaine Test	Cocaine One Stage test
Name	**WuCo3**
cathinone hydrochloride	yellow
2-methoxymethcathinone	yellow
methedrone	yellow
butylone hydrochloride	yellow
3-methylbuphedrone hydrochloride	yellow
3,4 dimthylethcathinone hydrochloride	yellow
3,4 methylenedioxy-α-pyrrolidnopropiophenone hydrochloride	Yellow
bupropion hydrochloride	Yellow
diethylcathinone hydrochloride	Yellow
isopentedrone hydrochloride	yellow
chlorpheniramine maleate	green
diphenhydramine hydrochloride (Benadryl)	green
cocaine hydrochloride	blue
ketamine hydrochloride	yellow/green
codeine	colorless
PCP (phencyclidine)	yellow
quinine sulfate	green
pseudoephedrine hydrochloride	yellow
(+-) - metoprolol	yellow
1-methylindole -3- carboxylic acid	colorless
MDPA (3,4-methylenedioxy-N-propyl-amphetamine)	yellow/green
lidocaine	colorless
1H - benzotriazole	colorless
1S, 2R (+) ephedrine hydrochloride	colorless
3,4 - dimethoxymethamphetamine hydrochloride	yellow
(±)-amphetamine	colorless
6-acetylmorphine	colorless
6-acetylcodeine	yellow
androstenedione	colorless
butalbital	colorless
gamma-butyrolactone	colorless
butabarbital	colorless
One Stage Cocaine Test	**Cocaine One Stage test**
Name	**WuCo3**
cannabidiol	colorless
4-chloro-2,5-dimethoxyphenethylamine hydrochloride	colorless
(±)-N-ethylamphetamine	colorless

hydrocodone	yellow
hydromorphone hydrochloride	colorless
heroin	yellow
LSD (lysergic acid diethylamide)	yellow/green
morphine	colorless
methamphetamine	colorless
MDA (3,4-methylenedioxyamphetaine)	colorless
methaqualone	colorless
methylephedrine	colorless
norcodine	colorless
oxycodone hydrochloride	colorless
phentermine	colorless
XLR-II	colorless
UR-144	colorless
AM 1248	colorless
(-)-delta9-THC	colorless
testosterone	colorless

Note: the results above are all for 50 micrograms of drug standards.
"-" negative or no color change
"+" positive or color change

Appendix 2 – Third Party Validation using WuCo1/WuCo2

Methodology. For stage 1 testing, 0.05-1mg of test substance is added into ampoule 1 (from a single pouch) followed by addition of one drop of WuCo1 test reagent. A blue color change is indicative of a positive result and presence of a synthetic cathinone (Figure 1). Positive results from stage 1 testing are confirmed with stage 2 testing. For stage 2 testing, 0.25mL of WuCo2 reagent is added into a fresh ampoule followed by addition of 0.25mL of chloroform. Sample is mixed, resulting in the formation of two layers. The bottom layer should be colorless. 0.05-1mg of test substance is then added to ampoule containing two layers and mixed. Following an incubation period of 10 seconds, results are observed. A yellow color change in the bottom layer is indicative of a positive result and presence of a synthetic cathinone (Figure 1).

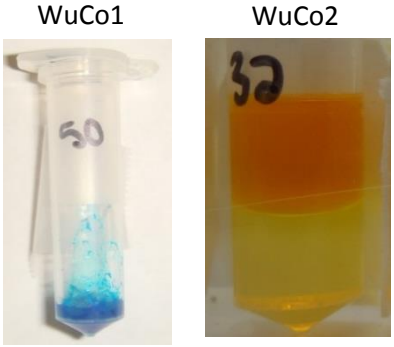


Figure 1. Expected color change indicative of positive result and presence of synthetic cathinone using WuCo1 and WuCo2

Testing at Wisconsin State Crime Laboratory (WSCL). Initial third party validation was carried out at WSCL in 2014. A total of 13 test substances were assessed using the WuCo2 test reagent.

Figure 2 below shows positive results obtained from testing of 6 synthetic and substituted cathinones alongside a negative control and parent compounds (cathine and cathinone). While positive results were seen with all synthetic/substituted cathinones, WuCo2 reagent did not produce a positive result on either parent compound.

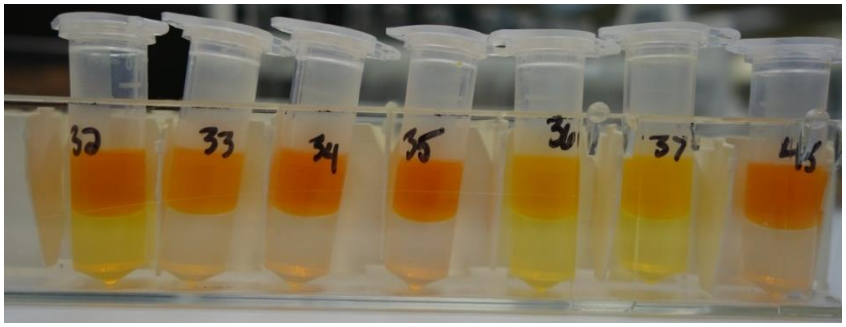


Figure 2. WuCo2 generates positive results for synthetic/substituted cathinones (#32, 34, 36 and 37).

From left, #32 (methcathinone), #33 (cathinone), #34 (4-fluoromethcathinone), #35 (cathine), #36 (methylone), #37 (4-methylethcathinone) and #45 (negative control).

In addition to those synthetic cathinones shown above, a further 7 test substances were evaluated using WuCo2 and compared to a negative control. As shown in Figure 3, WuCo2 reagent yielded positive results for all 7 synthetic cathinones and tryptamines tested.

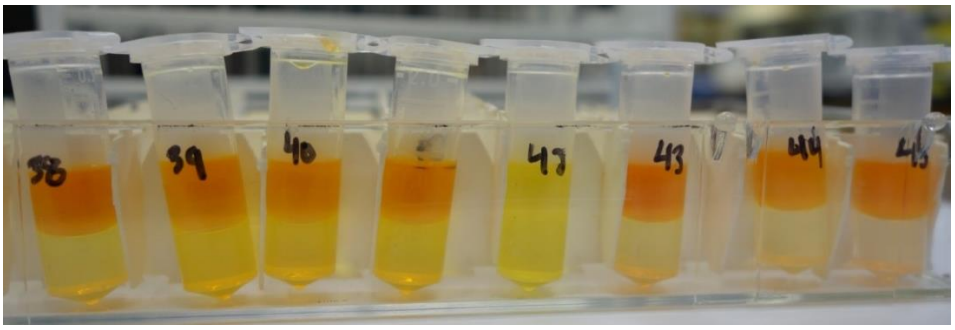


Figure 3. WuCo2 generates positive results for synthetic cathinones and tryptamines (#38 – 44).

From left, #38 (MDPV), #39 (5-methoxy-diisopropyltryptamine), #40 (5-methoxy-N-methyl-N-isopropyltryptamine), #41 (2,5-dimethoxy-5-chloroamphetamine), #42 (2,5-dimethoxy-4-ethylphenethylamine), #43 (ethylone), #44 (dimethylone) and #45 (negative control).

Testing at Northeastern Illinois Regional Crime Laboratory (NIRCL). Further third party validation was carried out at the NIRCL in August 2015. A total of 10 test substances were evaluated using the WuCo1/WuCo2 two-stage test. Tests were conducted in the presence of Gina Romano, Forensic Scientist in Drug Chemistry/Toxicology at NIRCL.

As shown in Figure 4, WuCo1 and WuCo2 were successfully used to detect a series of solid powder standards of synthetic cathinones.

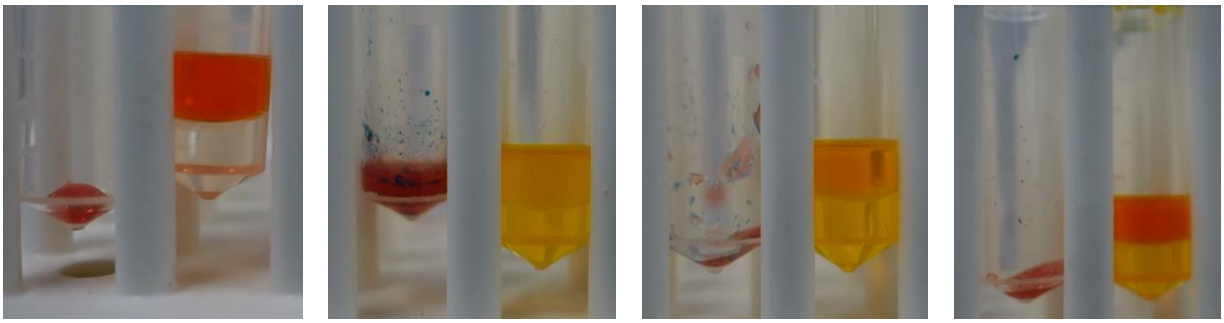
Compound	WuCo1 Color (Bottom Layer)	WuCo2 Layer (Bottom Layer)	Outcome
Methylone	Blue	Yellow	Positive
4-methylethcathinone	Blue	Yellow	Positive
Pentylone	Blue	Yellow	Positive
MPDV	Blue	Yellow	Positive
4-ethylmethcathinone	Blue	Yellow	Positive
4-MMC	Blue	Yellow	Positive
Ethylone (street case)	Blue	Yellow	Positive
Control	Dark orange	Clear	Negative

Control | 4-methylethcathinone | Control | Ethylone street case

Figure 4. WuCo1/WuCo2 two stage test generates positive results for synthetic cathinones

In addition to synthetic cathinones, a series of phenethylamines (e.g. alphabet drugs) were evaluated using WuCo1/WuCo2. As seen in Figure 5, WuCo1 and WuCo2 were successfully used to detect all test substances evaluated.

Compound	WuCo1 Color (Bottom Layer)	WuCo2 Layer (Bottom Layer)	Outcome
25B-NBOMe	Blue	Yellow	Positive
25C-NBOMe	Blue	Yellow	Positive
25I-NBOMe	Blue	Yellow	Positive
Control	Dark orange	Clear	Negative



Control 25-NBOMe 25C-NBOMe 25I-NBOMe

Figure 5. WuCo1/WuCo2 two stage test generates positive results for phenethylamines

Stability of WuCo1/WuCo2, two-stage colorimetric field test. The shelf life of test reagents was evaluated during the third party testing conducted at the NIRCL in August 2015. The ability WuCo1/WuCo2 test reagents stored at room temperature for a period of 15 months was tested in comparison to fresh reagent. Results are shown in Figure 6. Both fresh reagent as well as reagent stored for 15 months resulted in positive results using 4-methylethcathinone as a test substance demonstrating shelf life stability of WuCo1/WuCo2 for at least 15 months at room temperature (Figure 6).

Compound	WuCo1 Color (Bottom Layer)	WuCo2 Layer (Bottom Layer)	Outcome
4-methylethcathinone (using fresh reagent)	Blue	Yellow	Positive
4-methylethcathinone (15mth old reagent)	Blue	Yellow	Positive

Control 4-methylethcathinone (using fresh reagent) 4-methylethcathinone (using 15mth old reagent)