

# Completely Automated LC/MS/MS Analyses of Drugs and Metabolites Using Disposable Pipette Extraction (DPX)

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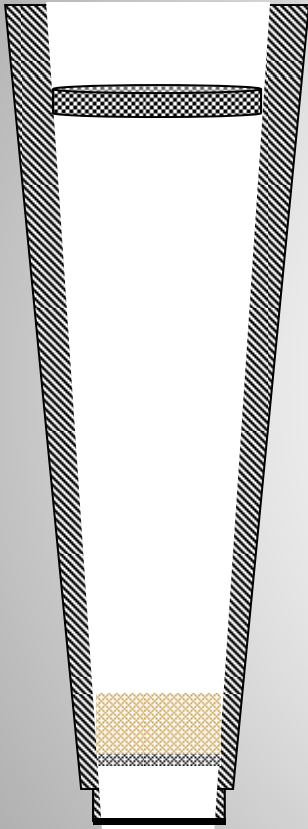
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# Outline

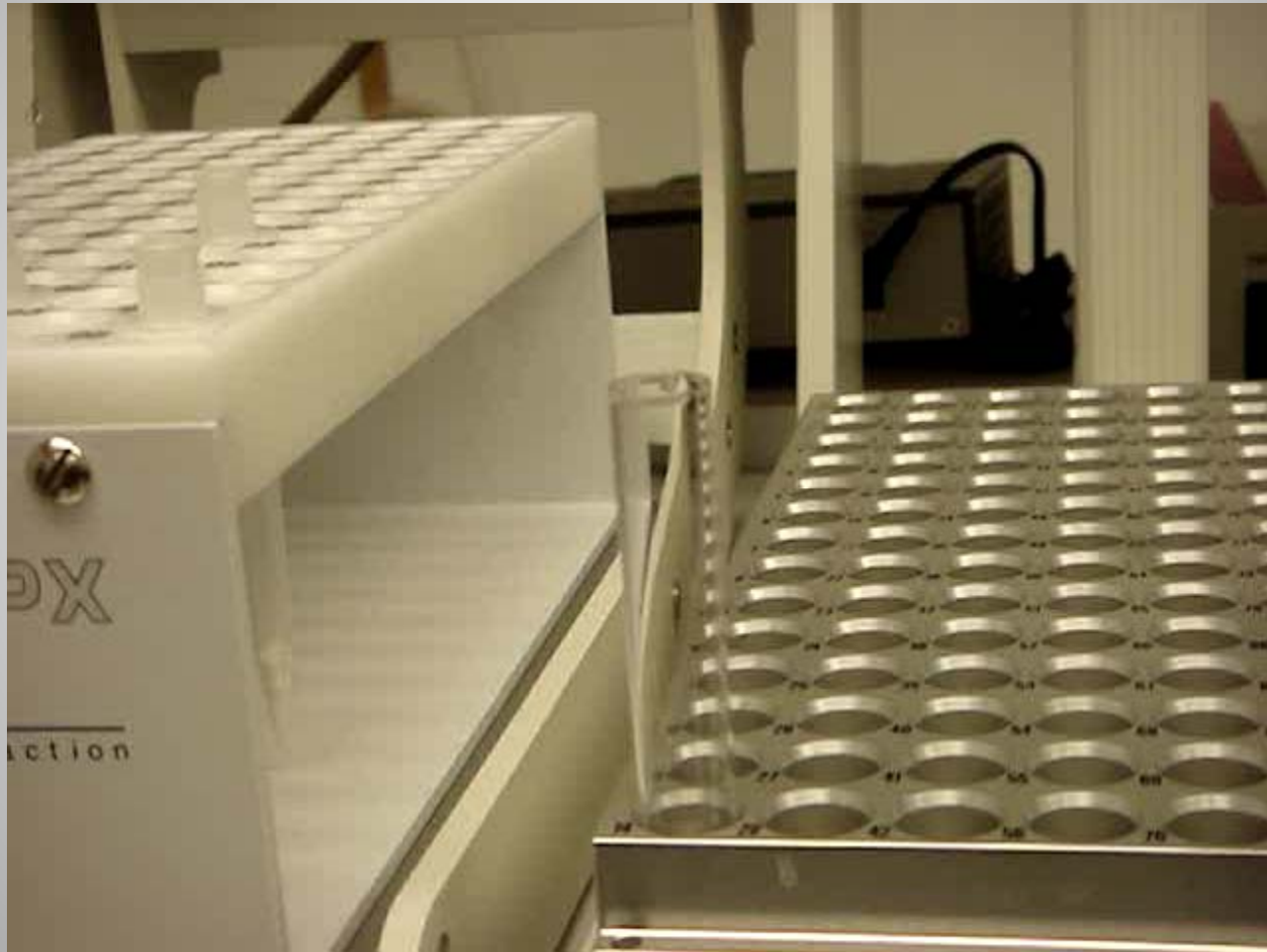
- Disposable Pipette Extraction (DPX)
  - Extraction process
  - Automation
- DPX products
  - Reverse phase (RP)
  - Cation exchange (CX)
  - Weak anion exchange (WAX)
  - Cleanup Tips/Sample Collection Tips (SC-Tips)
- Rapid and Comprehensive Screening Results
- Conclusions

# Disposable Pipette Extraction (DPX)

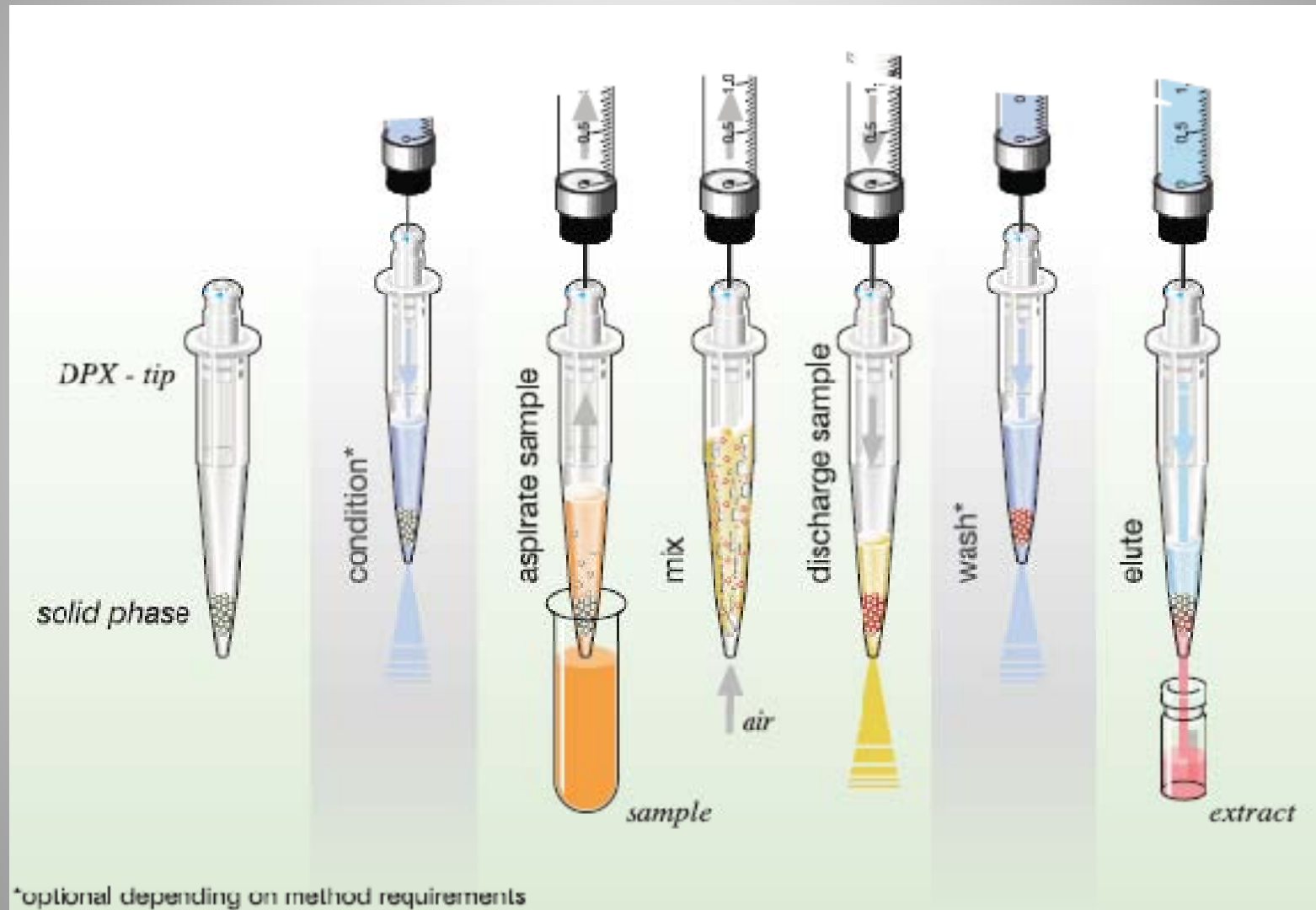


- Extractions are rapid—app. 3 minutes
- Flow is bi-directional
- Loosely contained sorbent—mixing
- No conditioning steps required
- Extraction efficiencies based on equilibration time, not flow rate
- Use less sorbent--less solvent required
- Minimal solvent waste generated
- Readily automated

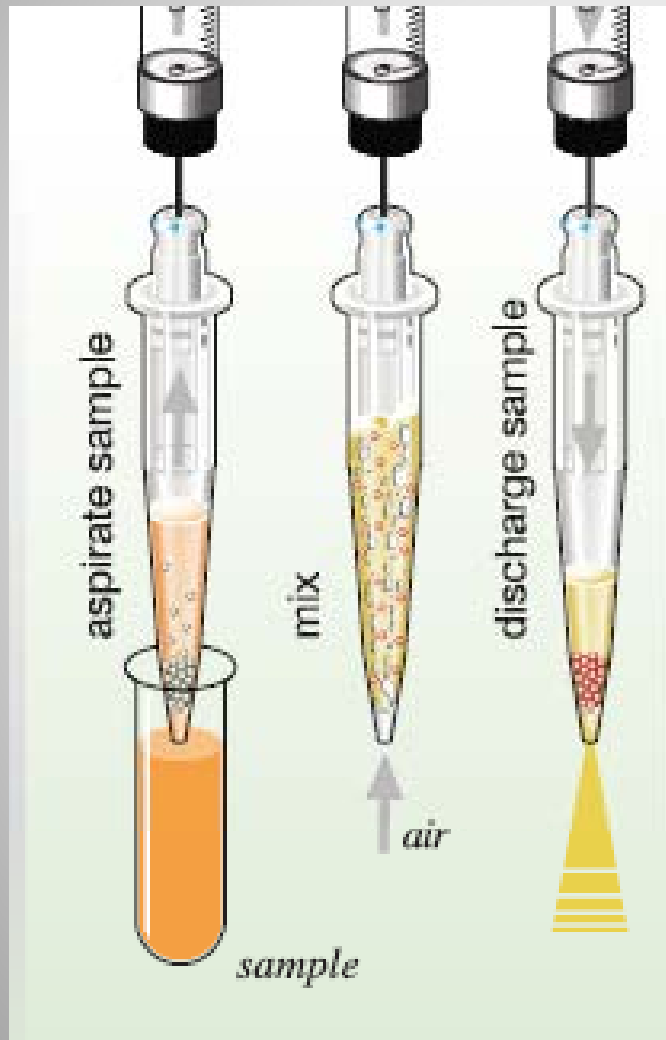
# DPX High Performance SPE (GEL)



# Automated DPX Extraction Process



# Automated DPX Cleanup Process



## Cleanup Tips:

Aspirate sample solution as shown.

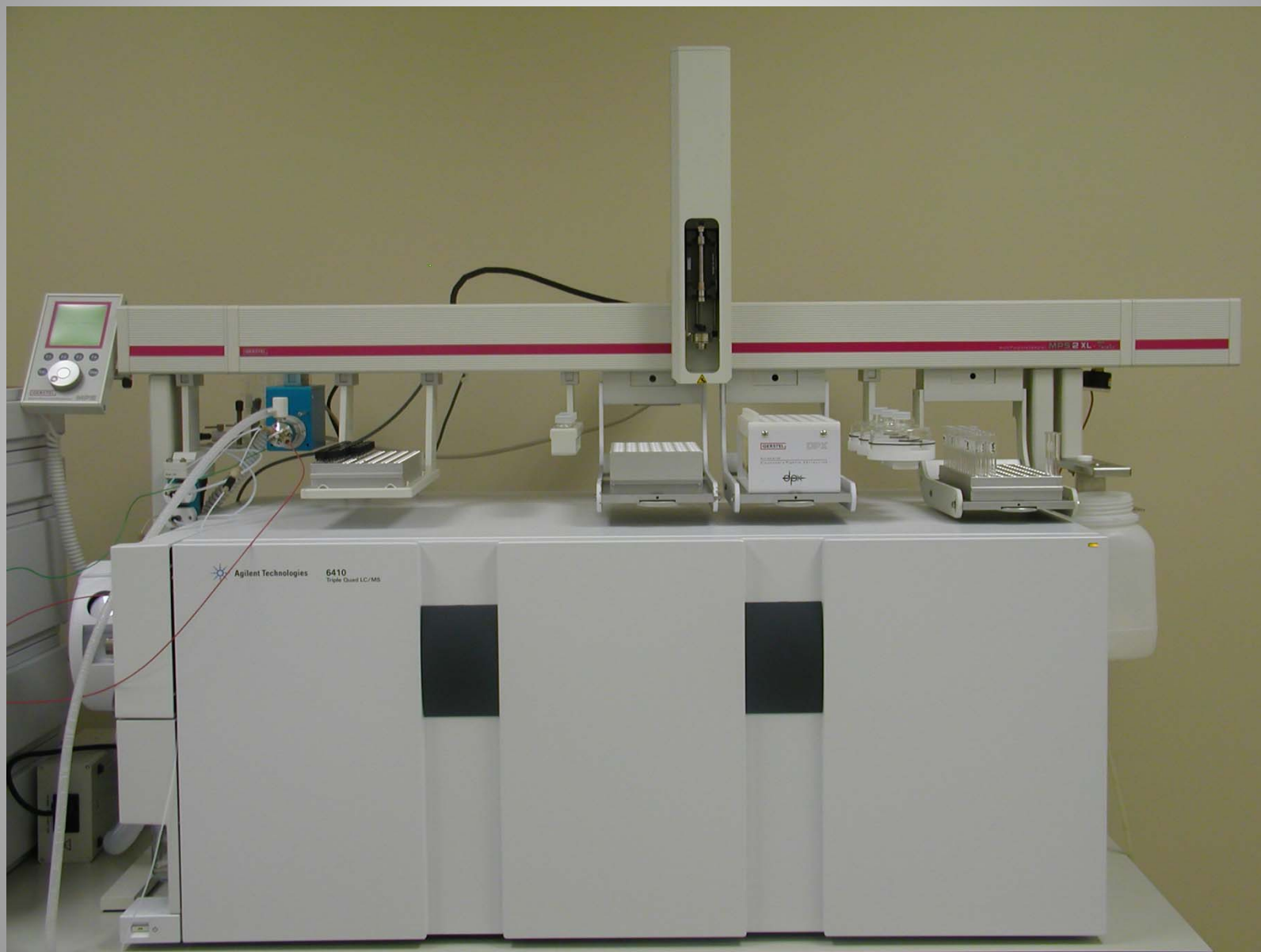
- Extract sample matrix (cleanup)

## Sample Collection Tips (SC-Tips):

Add sample to "top" of tip, then aspirate solvent.

- precipitate proteins
- extract sample matrix (cleanup)
- filter extract
- improve sample integrity  
(bar code labels)

# Automated DPX for LC/MS/MS



# Sample Prep for Automated DPX

## ➤ Blood specimens:

- 1) Spike sample with internal standard
- 2) Precipitate proteins with acetonitrile (skip for SC-Tips)
- 3) Centrifuge and transfer to clean labeled tube
- 4) Place on sample tray rack of MPS

## ➤ Urine specimens:

- 1) Spike samples with internal standard
- 2) Perform hydrolysis if needed
- 3) Transfer set volume to clean labeled tube
- 4) Place on sample tray rack of MPS

# Automated DPX Method

## ➤ DPX-RP and DPX-WAX

- Wet the DPX sorbent using 250µL of 30% acetonitrile (10% provides better recoveries of drugs using reversed phase mechanisms) in water.
- Aspirate the entire sample solution and mix with 1.3mL of air.
- Equilibrate for 20 seconds before dispensing the entire contents to waste.
- Wash the DPX sorbent by dispensing 500µL of 10% acetonitrile in water into the top of the tip.
- Elute benzos by dispensing 500µL of 100% acetonitrile (or 50% methanol in acetonitrile for benzos using DPX-WAX) through the top of the DPX tip into 2mL autosampler vials.

## ➤ DPX-CX

- Add 100 µL of 0.1 M HCl (or 1 M HCl for benzos for ion exchange due to low pKa values)
- Wet the DPX sorbent using 250µL of 30% acetonitrile in water.
- Aspirate the entire sample solution and mix with 1.3mL of air.
- Equilibrate for 20 seconds before dispensing the entire contents to waste.
- Wash the DPX sorbent by dispensing 500µL of 10% acetonitrile in water through the top of the tip.
- Wash by dispensing 500µL of 100% acetonitrile through the top of the tip.
- Elute benzos by dispensing 500µL of 4% ammonium hydroxide in acetonitrile/methanol (50/50)

# DPX Methods

## DPX Extraction Methods

- Reversed phase (RP)
  - styrene divinyl benzene
  - pi-pi interactions
- Cation exchange (CX)
  - sulfonated divinyl benzene
  - pi-pi interactions
  - Reverse phase
- Weak anion exchange (WAX)
  - polyamino divinyl benzene
  - pi-pi interactions
  - hydrogen bonding

## Rapid Cleanup Method

- Sample Collection Tips (SC-Tips)
  - Protein precipitation in the tip
  - Removes salt and water
- Cleanup Tips (aspirate sample solution)
  - Removes salt and water
  - Optional: removes organic acids and phospholipids that cause ion suppression!

# Sample Collection Tips (SC-Tips)

Protein precipitation and “clean up” in one step!  
60 second extraction for blood and hydrolyzed urine



# LC Parameters

HPLC: Agilent 1200 HPLC

HPLC Column: Zorbax Eclipse XDB-C18 RRHT column (2.1 x 50mm, 1.8  $\mu$ m)

LC/MS: Agilent 6410 Triple Quadrupole Mass Spectrometer with electrospray source

Autosampler: GERSTEL MPS 2XL with Active Washstation

Injection: 6 port (0.40mm) Cheminert C2V injection valve; 20  $\mu$ L stainless steel sample loop;  
2.5  $\mu$ L injection volume

## LC Parameters

Mobile Phase A = 5mM ammonium formate with 0.05% formic acid. Mobile Phase B = Methanol with 0.05% formic acid.

<b>Time(min)</b>	<b>Flow(mL/min)</b>	<b>Pressure(bar)</b>	<b>%Solv Ratio B</b>
0	0.5	600	5
0.5	0.5	600	5
1.5	0.5	600	30
3.5	0.5	600	70
4.5	0.5	600	95
6.5	0.5	600	95
7.5	0.5	600	5

# Project: Develop a Comprehensive Screen for Over 40 Drugs and Metabolites in Urine

Opiates

Opioids

Benzodiazepines

Barbiturates

Analgesics

Stimulants

Hallucinogens

Muscle relaxants/Anti-Convulsants

Analysis—use dynamic MRMs with Agilent LC/MS/MS

Challenge—sample preparation that is fast, efficient and comprehensive

# Comprehensive screening using LC/MS/MS

## Sample Collection Tips (SC-Tips)

- Opiates—6MAM, codeine, hydrocodone, hydromorphone, morphine, oxycodone, oxymorphone
- Opioids—fentanyl, methadone, buprenorphine, EDDP, norbuprenorphine, norfentanyl
- Benzos—hydroxyalprazolam, lorazepam, nordiazepam, oxazepam, temazepam, diazepam, alprazolam, flunitrazepam, 7-aminoflunitrazepam, nitrazepam
- Barbs—amobarbital, butabarbital, butalbital, pentobarbital, phenobarbital, secobarbital
- Analgesics—propoxyphene, norpropoxyphene, tramadol, o-desmethyltramadol

# Comprehensive screening using LC/MS/MS

## Sample Collection Tips (SC-Tips)

- Anticonvulsants/muscle relaxants—gabapentin, carisoprodol, meprobamate
- Hallucinogens—COOH-THC, PCP
- Stimulants—amphetamine, benzoylecgonine, cocaine, MDA, MDMA, methamphetamine, methylphenidate

## Overview of DPX Extraction Methods

### DPX-RP:

- Ideal for acidic and neutral drugs in urine
  - recoveries greater than app. 80% for benzos
  - presence of acetonitrile in “sample solution” limit recoveries

### DPX-CX:

- Ideal for basic drugs in urine and blood
- Good recoveries for many acidic and neutral drugs
- 2 elutions necessary for acidic/neutral and basic drugs
- low recoveries for a couple of compounds

### DPX-WAX:

- Good for almost all drugs
- Ion exchange causes need for second elution step for acidic drugs (COOH-THC)

# LC/MS/MS—Cleanup Tips in urine

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor Voltage	Collision Energy	Retention Time	%Recovery
6-MAM	328.2	165.0	110	40	2.476	89
Oxycodone	316.2	241.0	110	30	2.392	91
Oxymorphone	302.2	227.1	110	33	1.620	73
Codeine	300.2	152.0	110	75	2.297	95
Hydrocodone	300.2	199.0	110	28	2.446	113
d3-morphine	289.2	152.1	110	75	1.414	100
Morphine	286.2	152.1	110	75	1.426	72
Hydromorphone	286.2	185.0	110	33	1.881	74
Buprenorphine	468.4	414.0	220	35	4.131	59
Norbuprenorphine	414.3	187.0	180	40	3.631	68
d5-Fentanyl	342.3	188.1	140	20	3.680	100
Fentanyl	337.2	188.2	140	25	3.691	59
Methadone	310.2	265.1	130	12	4.256	75
EDDP	278.2	234.1	160	30	3.807	81
Norfentanyl	233.1	150.1	100	20	3.046	79
a-OH-alprazolam	325	297	120	30	4.369	128
Lorazepam	321	275	140	20	4.447	150
Clonazepam	316	270	120	25	4.228	121
Flunitrazepam	314	268	160	30	4.283	123
Alprazolam	309	281	160	25	4.483	117
Temazepam	301	255	120	35	4.565	96
Oxazepam	287	269	120	15	4.444	142
Diazepam	285	257	160	25	4.811	78
Nitrazepam	282	236	160	25	4.243	101
Nordiazepam	271	165	160	30	4.691	79
d5-nordiazepam	276	213	160	30	4.674	100

# LC/MS/MS—Cleanup Tips in Urine

Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor Voltage	Collision Energy	Retention Time	%Recovery
d5-propoxyphene	345.3	271.2	120	5	4.210	100
Propoxyphene	340.3	58.0	120	5	4.220	78
Norpropoxyphene	326.2	252.1	90	5	4.251	70
Tramadol	264.2	58.0	120	5	3.164	70
o-desmethyltramadol	250.1	189.1	100	10	3.283	65
d7-Carisoprodol	268.2	183.1	60	3	4.49	100
Carisoprodol	261.2	176.1	60	3	4.50	72
Meprobamate	219.1	158.0	60	0	3.709	73
Gabapentin	172.1	154.0	100	10	2.504	61
COOH-THC	345	193.0	110	30	5.512	47
d5-PCP	249.3	164.3	40	15	3.609	100
PCP	244.3	91.2	110	25	3.62	71
Amphetamine	136.2	91.1	80	15	2.583	50
Cocaine	304.2	182.1	140	20	3.151	145
Benzoylcegonine	290.3	168.3	120	15	3.041	89
Methylphenidate	234.2	84.1	120	20	3.206	103
MDEA	208	135.0	100	15	2.806	120
MDMA	194	163.0	100	10	2.643	85
MDA	180	163.3	100	15	2.58	79
Methamphetamine	150.2	91.1	100	15	2.642	65
d5-Amphetamine	141.1	93.0	80	15	2.573	100

# LC/MS/MS—Cleanup Tips in Blood with WAX

<b>Opiates</b>	<b>% Recov.</b>	<b>%CV</b>
d3-morphine	64.58	NA
Morphine	77.44	4.62
Oxymorphone	71.22	4.82
Hydromorphone	66.68	8.51
Codeine	73.45	8.08
Oxycodone	73.44	4.31
Hydrocodone	73.44	3.45
6-MAM	76.57	5.86

<b>Hallucinogens</b>	<b>% Recov.</b>	<b>%CV</b>
d5-PCP	76.84	NA
PCP	77.95	1.37
COOH-THC	24.39	NA

<b>Opioids</b>	<b>% Recov.</b>	<b>%CV</b>
d5-Fentanyl	74.54	NA
Fentanyl	77.54	2.08
Norfentanyl	74.12	5.03
Buprenorphine	69.28	1.05
Norbuprenorphine	61.77	5.48
Methadone	79.13	2.10
EDDP	88.57	0.95

<b>Analyte</b>	<b>% Recov.</b>	<b>%CV</b>
d5-pentobarbital	64.82	NA
Phenobarbital	74.71	4.67
Butobarbital	61.85	17.60
Butalbital	73.45	14.23
Amobarbital/ Pentobarbital	71.43	1.71
Secobarbital	86.66	9.03

# LC/MS/MS—Cleanup Tips in Blood

<b>Benzos</b>	<b>% Recov.</b>	<b>%CV</b>
d5-nordiazepam	86.24	NA
Nordiazepam	87.10	5.79
Diazepam	80.36	12.24
Clonazepam	84.67	7.05
Nitrazepam	89.89	4.47
Flunitrazepam	86.46	4.67
a-OH-alprazolam	68.96	3.26
Oxazepam	88.81	3.06
Lorazepam	90.08	3.38
Alprazolam	71.65	5.27
Temazepam	85.34	5.22

<b>Anticonvulsants</b>	<b>% Recov.</b>	<b>%CV</b>
d7-Carisoprodol	82.05	NA
Carisoprodol	85.15	5.54
Meprobamate	79.74	4.04
Gabapentin	18.39	17.60

<b>Analgesics</b>	<b>% Recov.</b>	<b>%CV</b>
d5-propoxyphene	73.38	NA
Propoxyphene	80.79	3.56
Norpropoxyphene	318.90	12.51
Tramadol	62.42	6.56
o-desmethyltramadol	76.36	2.28

<b>Stimulants</b>	<b>% Recov.</b>	<b>%CV</b>
d5-Amphetamine	72.12	NA
Amphetamine	69.23	5.38
Methamphetamine	81.30	1.60
MDMA	82.68	4.36
MDEA	78.72	5.31
Benzoylecgonine	73.03	3.10
Cocaine	70.01	8.16
Methylphenidate	80.71	3.94
MDA	69.96	10.08

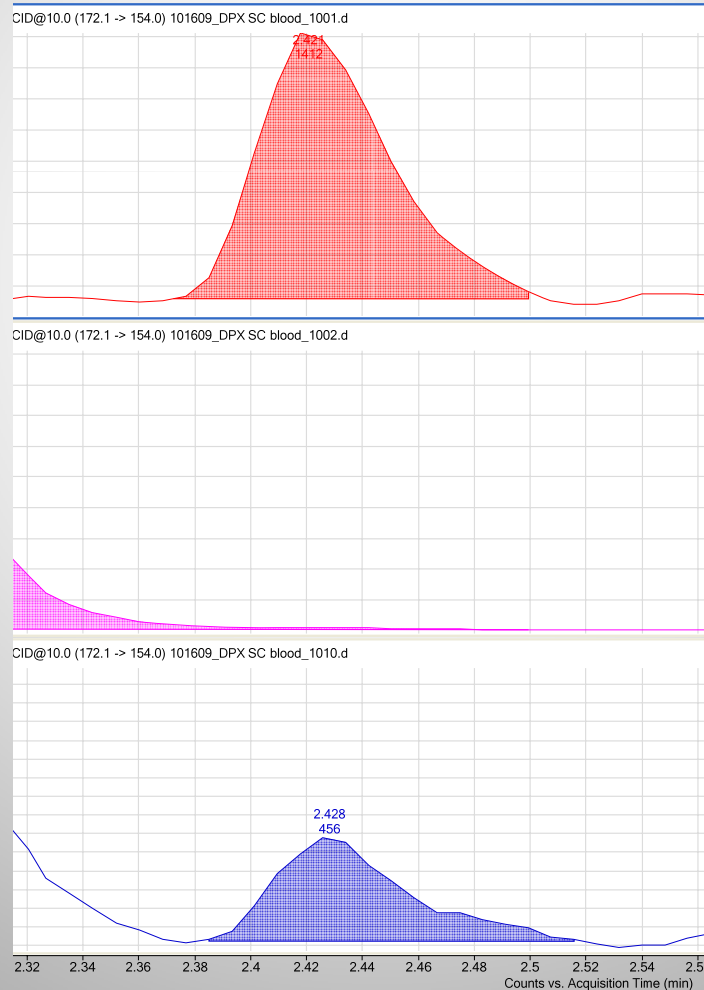
# LC/MS/MS—Cleanup Tips in Blood

## Comparison of Gabapentin at 20 ng/mL in whole blood

Top: neat standard

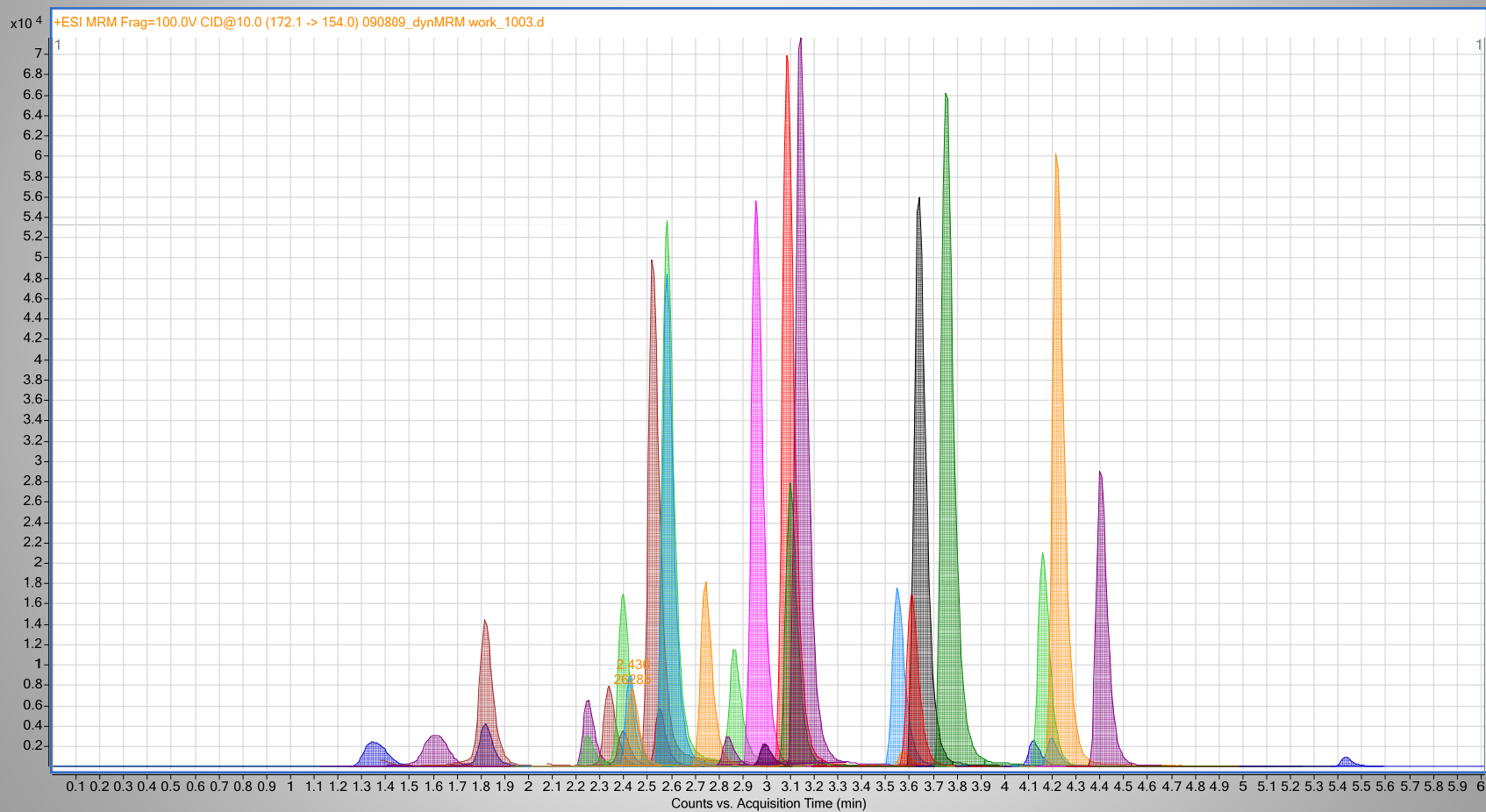
Middle: ACN protein precipitated

Bottom: ACN dried w MgSO<sub>4</sub> then WAX



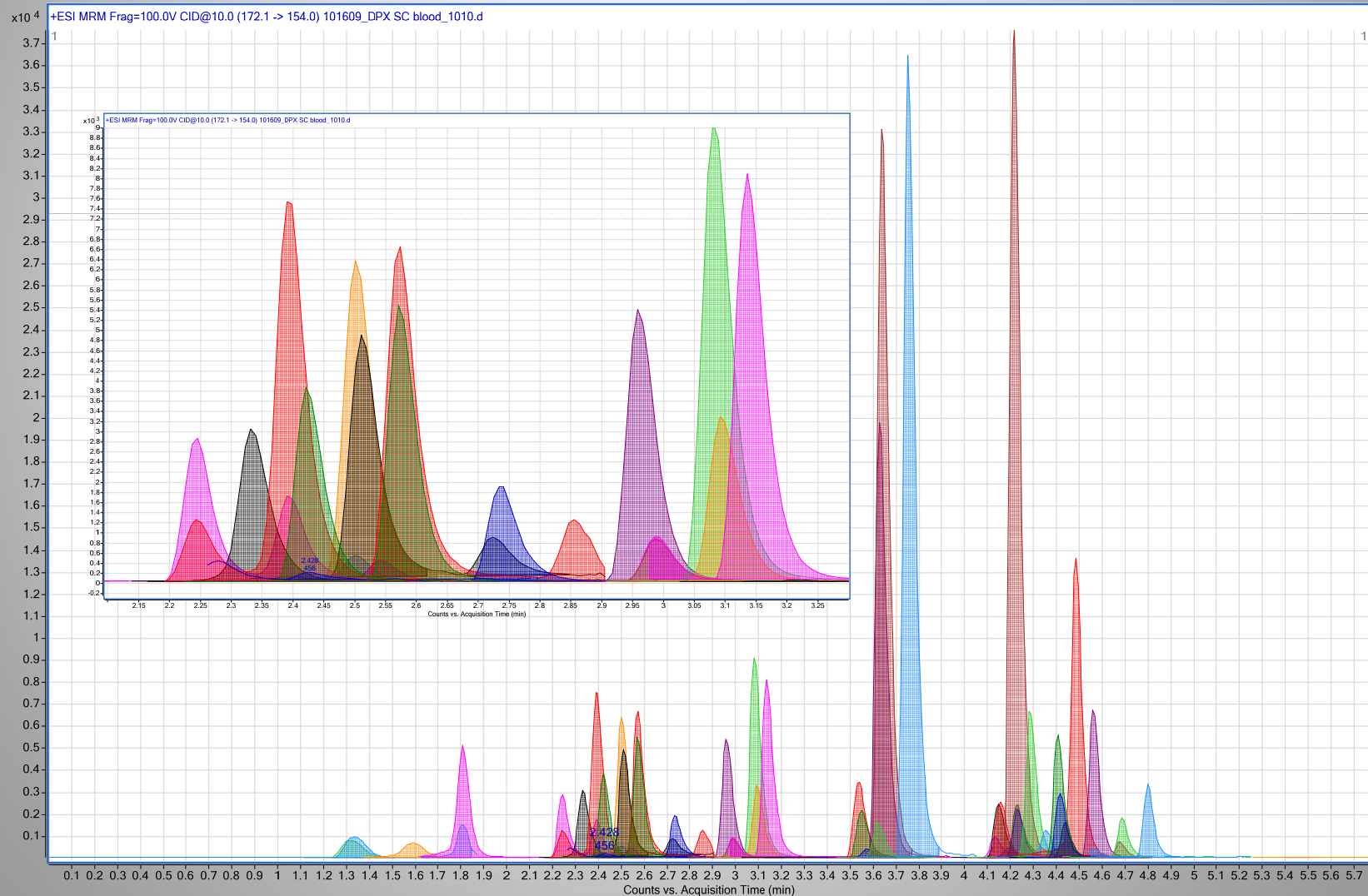
# LC/MS/MS—Cleanup Tips of Urine

## QQQ ESI dynMRM Traces



# LC/MS/MS—Cleanup Tips of 20 ng/mL Whole Blood

## QQQ ESI dynMRM Traces



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