Application of MonoSpin Series

GL Sciences

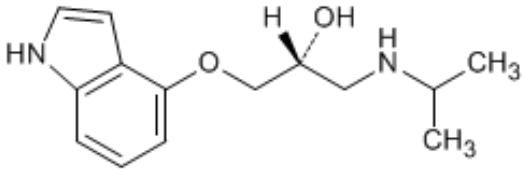
Application list of MonoSpin™ series

▪ **Basic drugs in plasma:**	**MonoSpin™ C18**
▪ **Amphetamines in urine:**	**MonoSpin™ C18**
▪ **Desaltration of peptides:**	**MonoSpin™ C18**
▪ **Fractionation of peptides:**	**MonoSpin™ SCX**
▪ **Pesticides in urine:**	**MonoSpin™ CBA**
▪ **PA-sugar chain**	**MonoSpin™ NH2**
▪ **Catecholamines in urine:**	**MonoSpin™ PBA**
▪ **Glyphosate in drink**	**MonoSpin™ TiO**

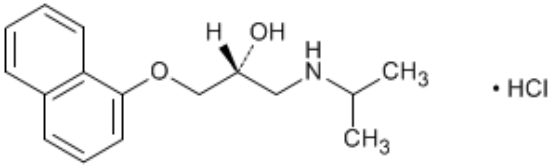
GL Sciences

Extraction of basic drugs from plasma (MonoSpin™ C18)

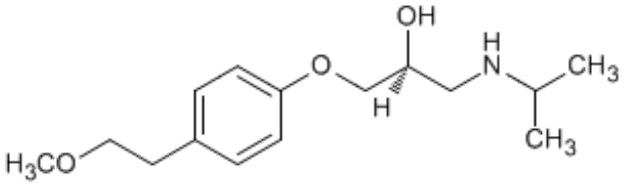
Basic drugs



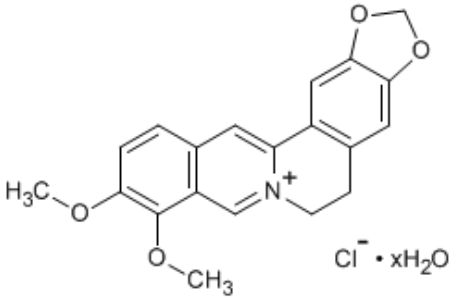
Pindolol
Log P : 1.75
pKa : 9.25



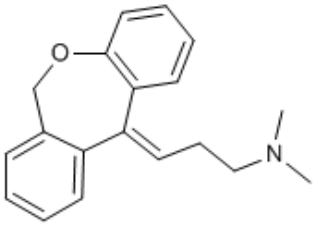
Propranolol
Log P : 0.74
pKa : 9.5



Metoprolol
Log P : 1.69
pKa : 9.7



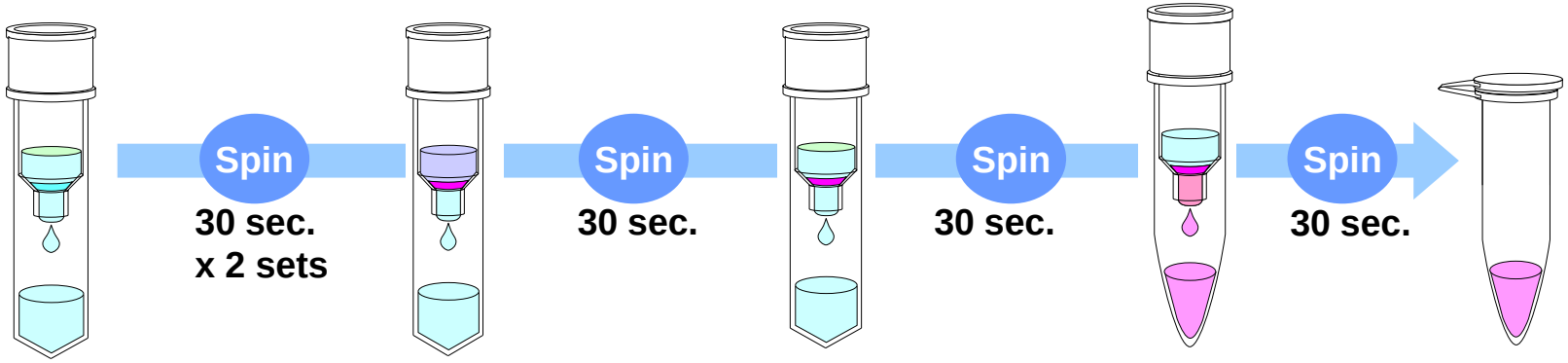
Berberine
Log P : 2.08
pKa : 8.7



Doxepin
Log P : 4.29
pKa 8.0

GL Sciences

Protocol of MonoSpin™ C18 for extraction of basic drugs.



1. Conditioning

300 µL of methanol

→

300 µL of 1 % NH_4OH in water

2. Adsorption

300 µL of Sample solution

Sample solution	
matrix (Plasma)	**990 µL**
+ STD solution	**10 µL**
	1,000 µL

3. Rinsing

300 µL of 1 % NH_4OH in water

4. Elute

300 µL of 5 % formic acid in methanol

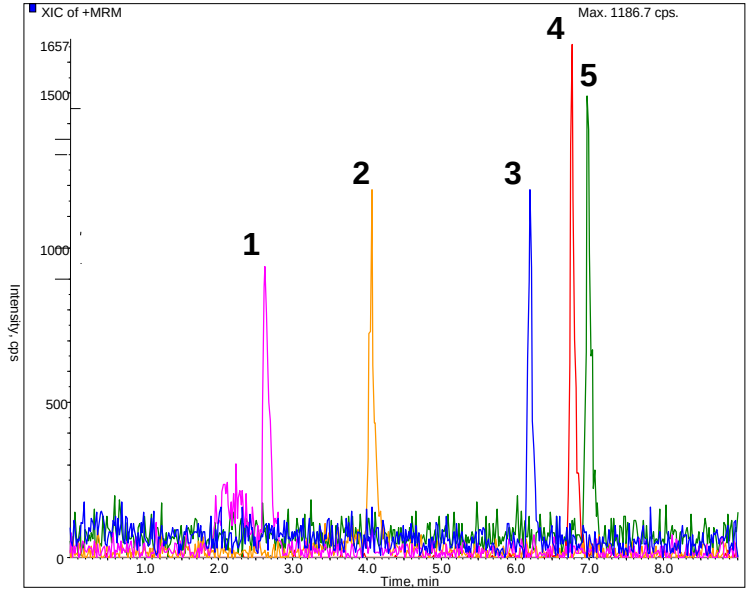
Extract

Spin : 5,000 rpm for 30 seconds

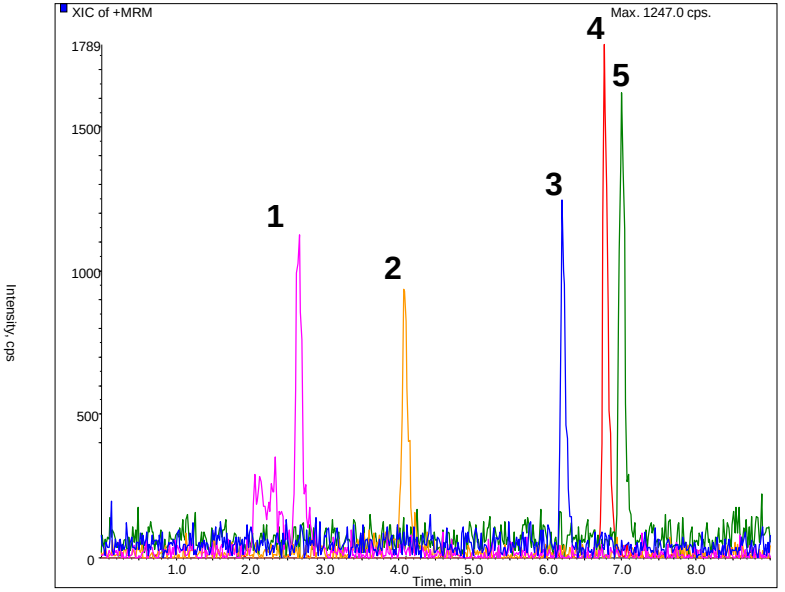
GL Sciences

Extraction of basic drugs from plasma (MonoSpin™ C18).

STD in extraction buffer



Extraction by MonoSpin™ C18 from plasma



HPLC condition

System : LC800, API-3000
Column : Inertsil ODS-4 (5 µm 100 mm x 2.1 mm I.D.)
Eluent : A) 0.1%HCOOH in H_2O、B)0.1%HCOOH in CH_3CN
A/B=80/20-12 min-40/60, v/v
Flow Rate : 200 µL/min
Col. Temp : 40 ℃
Detection : MS/MS (API-3000:ESI, Pos, MRM)
Injection Vol. : 1µL
Sample : 1.Pindolol, 2.Metoprolol, 3.Propranolol, 4.Berberine
5.Doxepin

1. Pindolol (m/z 249.0>116.0)
2. Metoprolol (m/z 260.2>116.2)
3. Propranolol (m/z 268.2>116.0)
4. Berberine (m/z 280.2>107.1)
5. Doxepin (m/z 336.2>292.3)

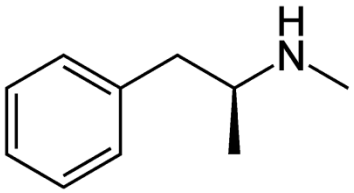
GL Sciences

Application list of MonoSpin™ series

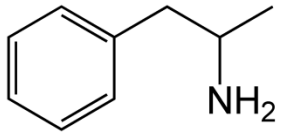
▪Basic drugs in plasma:	MonoSpin™ C18
▪Amphetamines in urine:	MonoSpin™ C18
▪Desaltration of peptides:	MonoSpin™ C18
▪Fractionation of peptides:	MonoSpin™ SCX
▪Pesticides in urine:	MonoSpin™ CBA
▪PA-sugar chain	MonoSpin™ NH2
▪Catecholamines in urine:	MonoSpin™ PBA
▪Glyphosate in drink	MonoSpin™ TiO

GL Sciences

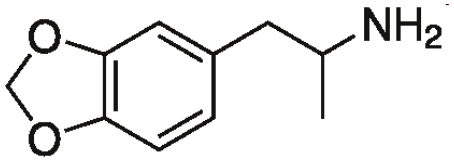
Extraction of amphetamines from urine using a MonoSpin™ C18.



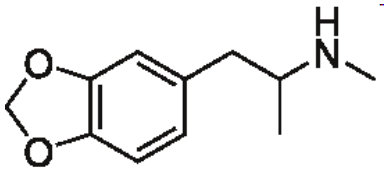
Methamphetamine(MA)



Amphetamine(AP)



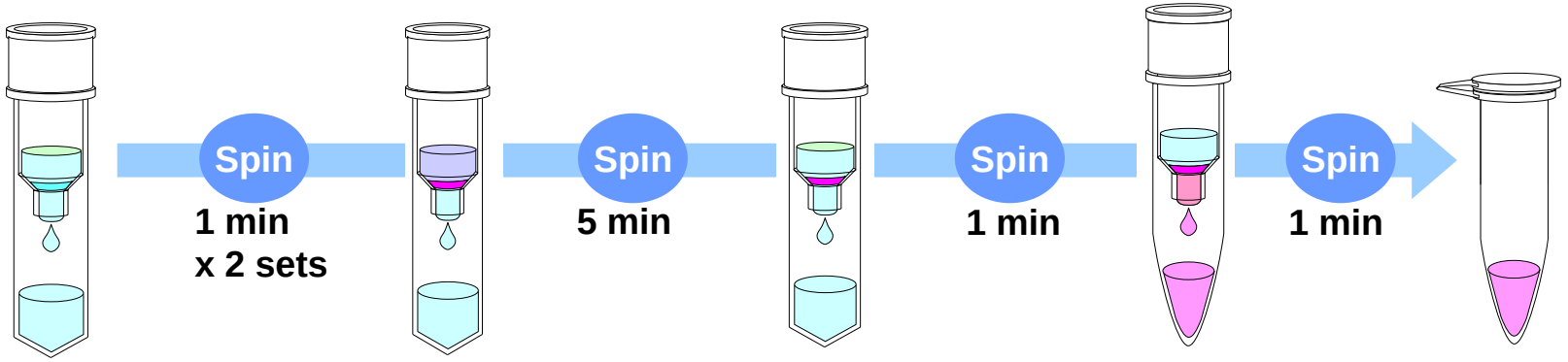
3,4-Methylenedioxyamphetamine(MDA)



3,4-Methylenedioxymethamphetamine(MDMA）

GL Sciences

Protocol of MonoSpin™ C18 for extraction of amphetamines form urine.



1. Conditioning

500 µL of acetonitrile

→

500 µL of water

2. Adsorption

910 µL of Sample solution

3. Rinsing

300 µL of water

4. Elute

100 µL of 5 % formic acid in Acetonitrile

Extract

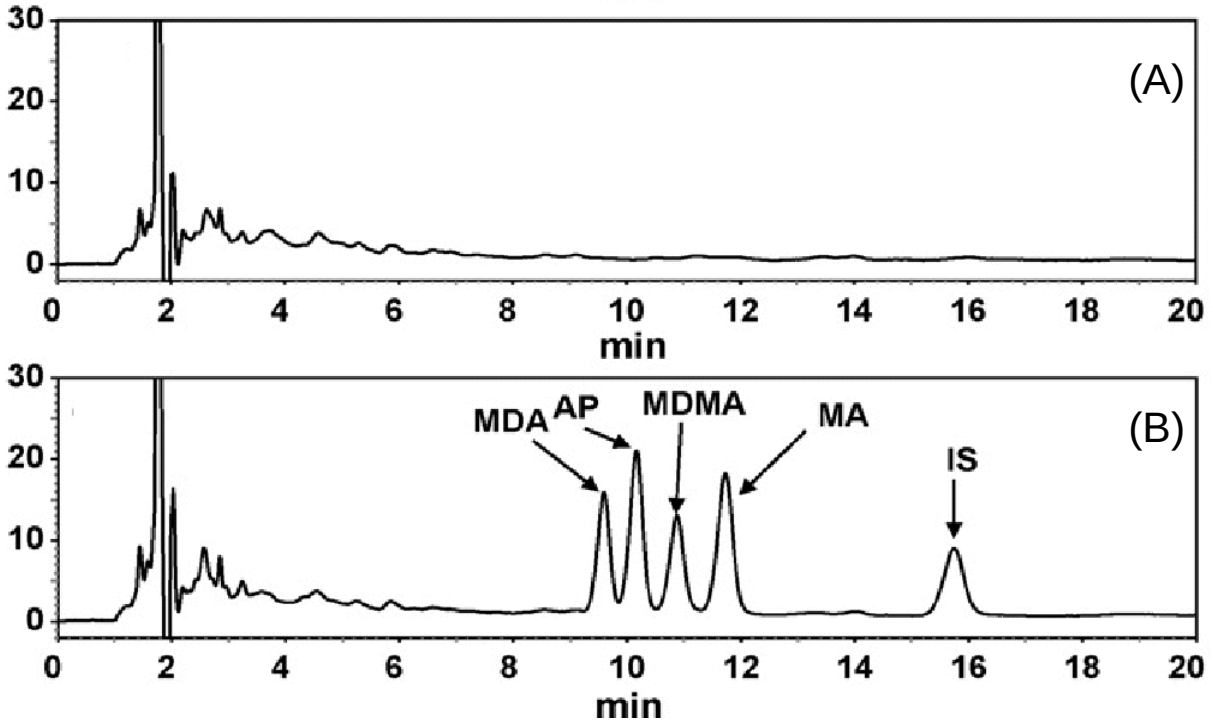
Sample solution

matrix (urine)	500 µL
Alkaline buffer(pH12)	400µL
+ STD solution	10 µL
	910 µL

Spin **: 5,000 rpm**

GL Sciences

Extraction of amphetamines from urine using a MonoSpin™ C18.



Chromatograms of amphetamines extracted from normal and drug containing urine samples by using the MonoSpin C18: (A) normal urine, and (B) drug-containing urine (5g/mL)

＜LC Condition＞
Column: ODS (150 X 4.6 mm I.D.)
Elution: Acetonitrile/0.1% phosphate 20mM sodium octanesulfate=25/75
Flow rate=1ml/min
injection: 10 mL
Detector: UV 210 nm
Sample: Methamphetamine(MA)
Amphetamine(AP)
3,4-Methylenedioxymethamphetamine(MDMA)
3,4-methylenedioxyamphetamine(MDA)

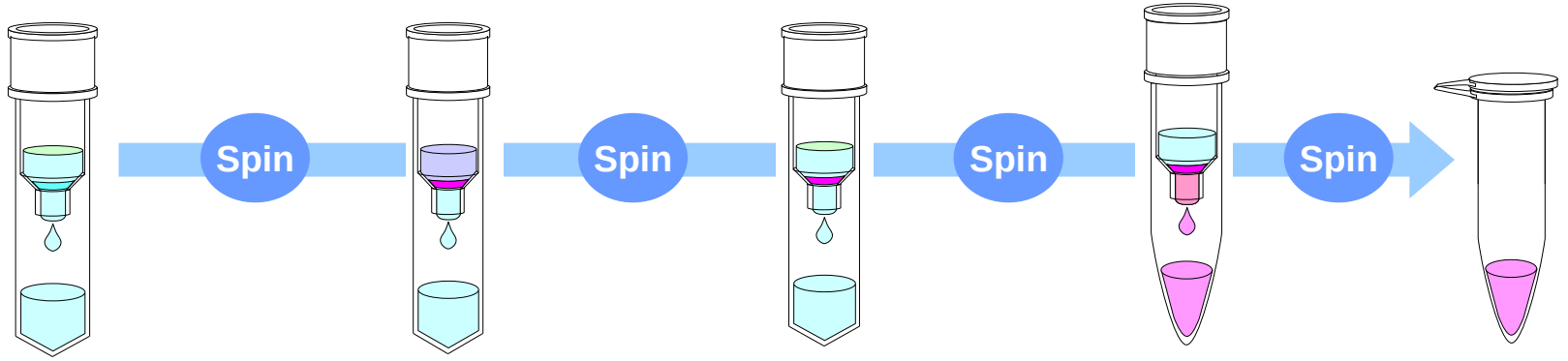
GL Sciences

Application list of MonoSpin™ series

▪Basic drugs in plasma:	MonoSpin™ C18
▪Amphetamines in urine:	MonoSpin™ C18
▪Desaltration of peptides:	MonoSpin™ C18
▪Fractionation of peptides:	MonoSpin™ SCX
▪Pesticides in urine:	MonoSpin™ CBA
▪PA-sugar chain	MonoSpin™ NH2
▪Catecholamines in urine:	MonoSpin™ PBA
▪Glyphosate in drink	MonoSpin™ TiO

GL Sciences

Protocol of MonoSpin™ C18 for desaltration of peptides sample.



1. Conditioning

300 µL of actonitrile

→

300 µL of 1% HCOOH in water

2. Adsorption

500 µL of Sample solution

3. Rinsing

200 µL of 1 % HCOOH in water

4. Elute

200 µL of 60% Acetonitrile (1% HCOOH)

Extract

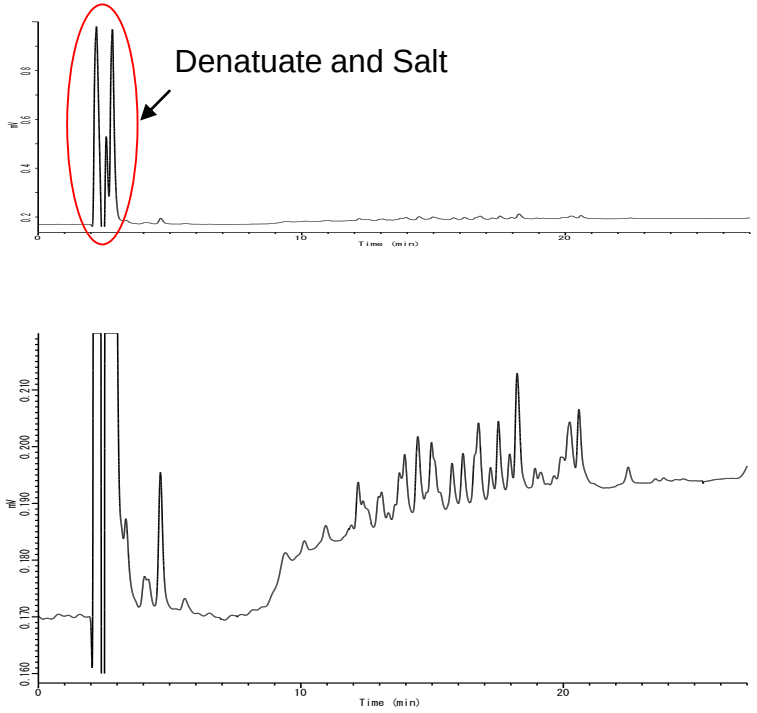
Sample solution
1mg/mL Trypsin digested BSA
(Add 1% HCOOH)

Spin **: 5,000 rpm for 1 min**

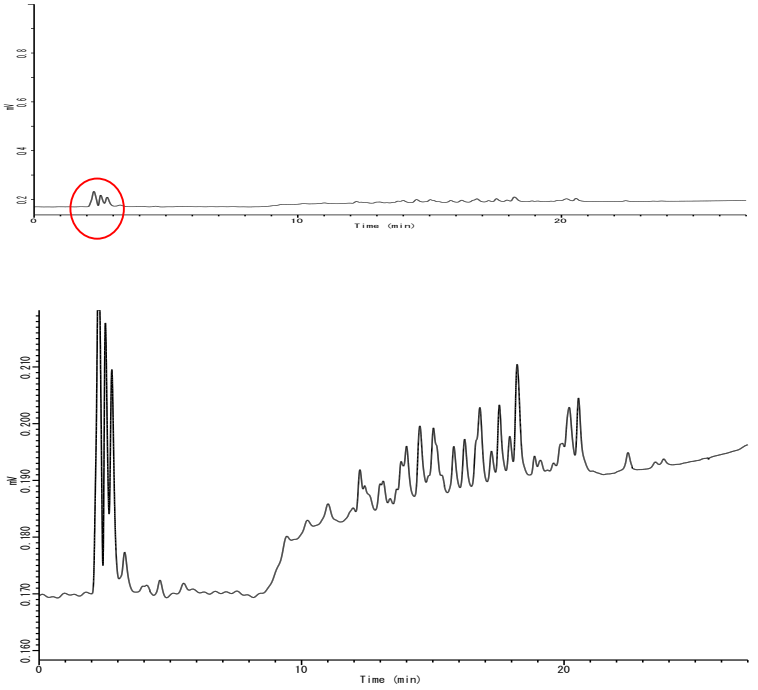
GL Sciences

Desaltration of peptides using a MonoSpin™ C18.

Before



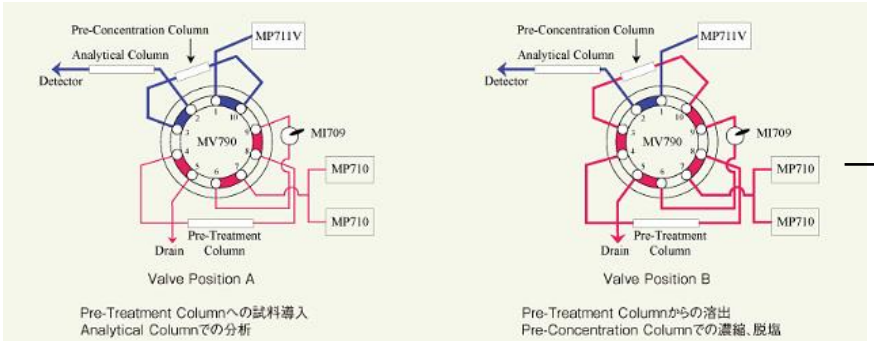
After

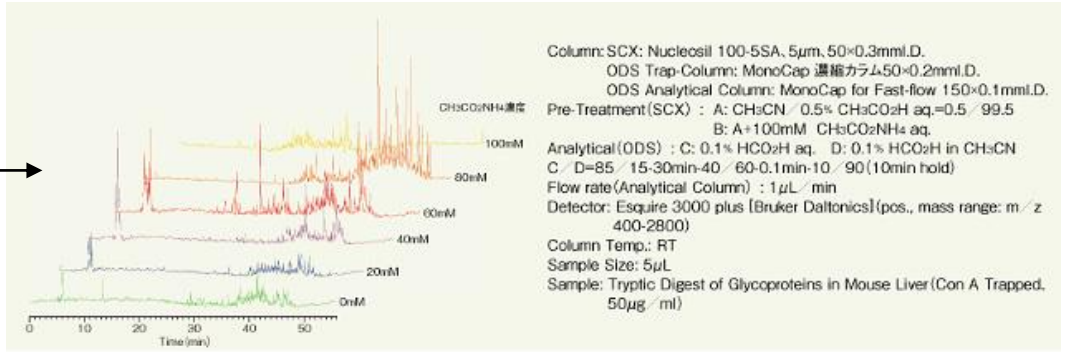


HPLC Condition
Sample: digested BSA 2 uL
Column: Inertsil ODS-3 (3um 2.1 x 150 mm)
Eluent: A: Water(0.1%TFA)
B: ACN(0.1% TFA)
Grad A:B=90:10 - 5min 90:10 - 20min - 50:50
Detection UV 210 nm
Flow rate 0.2 mL/min
Temp 40℃

GL Sciences

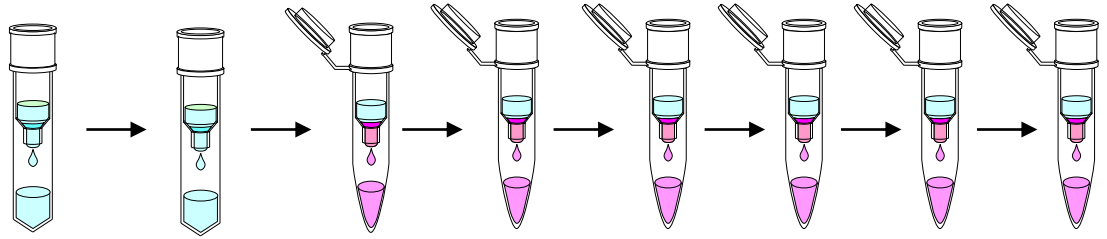
Fractionation of peptide using a MonoSpin™ SCX.





Step gradient elution in 2D LC

MonoSpin® SCX

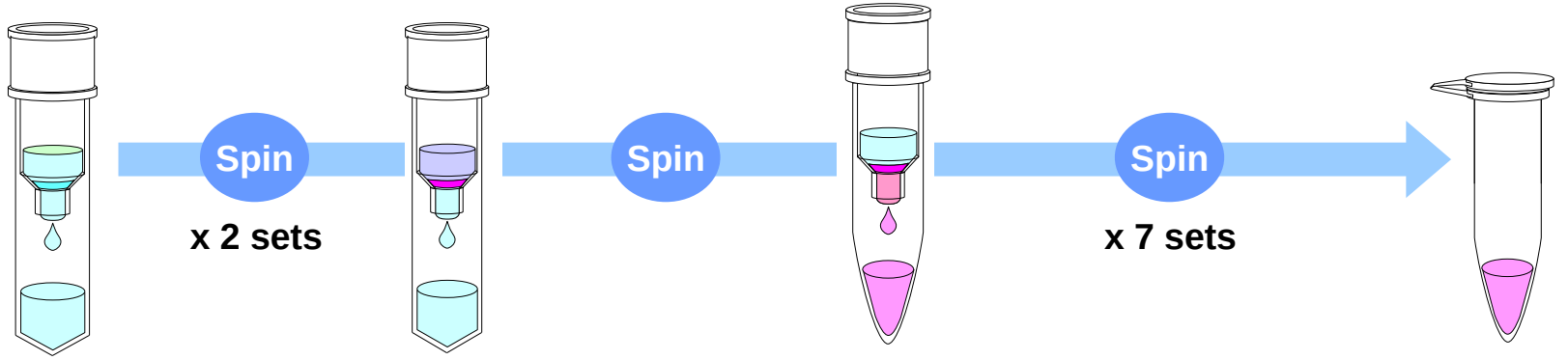


Step gradient elution

Step gradient elution in MonoSpin SCX

GL Sciences

Protocol in fractionation of peptide using a MonoSpin™ SCX.



1. Conditioning

300 µL of
10 mM SPB

2. Adsorption

500 µL of
Sample solution

Sample solution
1mg/mL Trypsin digested BSA
The sample was treatment by MonoSpin C18, then dry the sample and redissolve in the sodium phosphate buffer(pH3.0)

3. Elute

1st 200 µL of SPB containing 31mM NaCl
2nd 200 µL of SPB containing 31mM NaCl
3rd 200 µL of SPB containing 62mM NaCl
4th 200 µL of SPB containing 125mM NaCl
5th 200 µL of SPB containing 250mM NaCl
6th 200 µL of SPB containing 500mM NaCl
7th 200 µL of SPB containing 1M NaCl

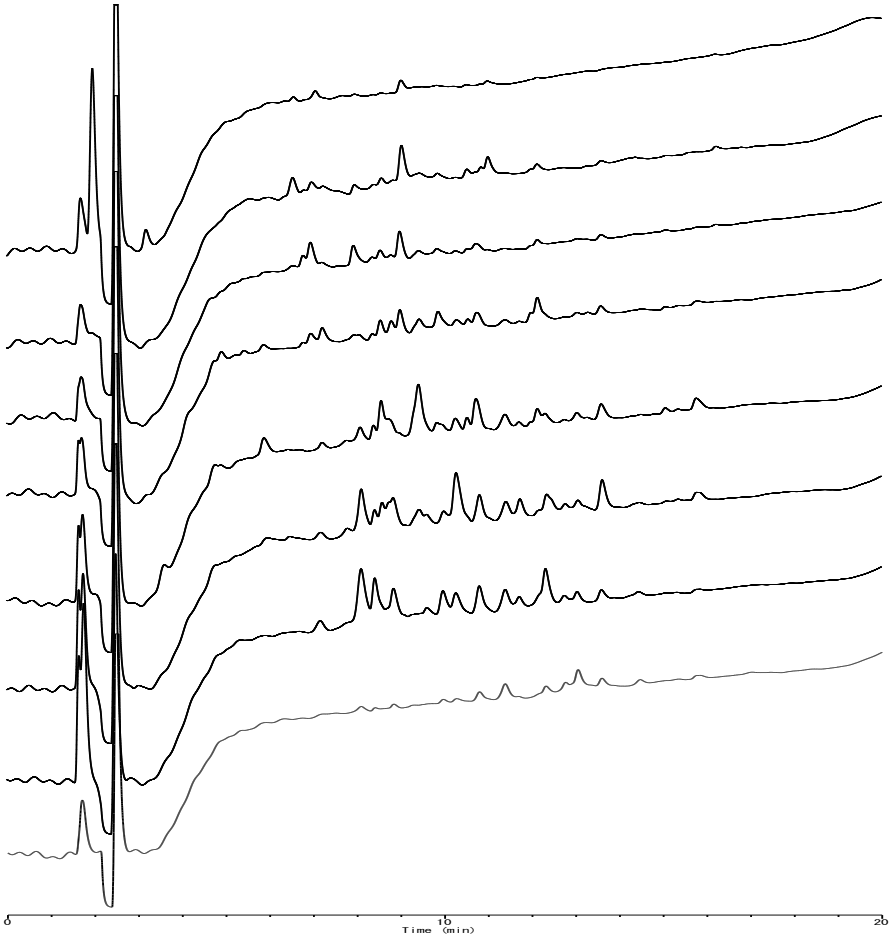
Extract

Spin : 5,000 rpm for 1 min

SPB:Sodium Phosphate Buffer(pH3)

GL Sciences

Fractionation of peptide using a MonoSpin™ SCX.



Elution

10mM buffer (Through)

31mM NaCl

62mM NaCl

125mM NaCl

250mM NaCl

500mM NaCl

1M NaCl

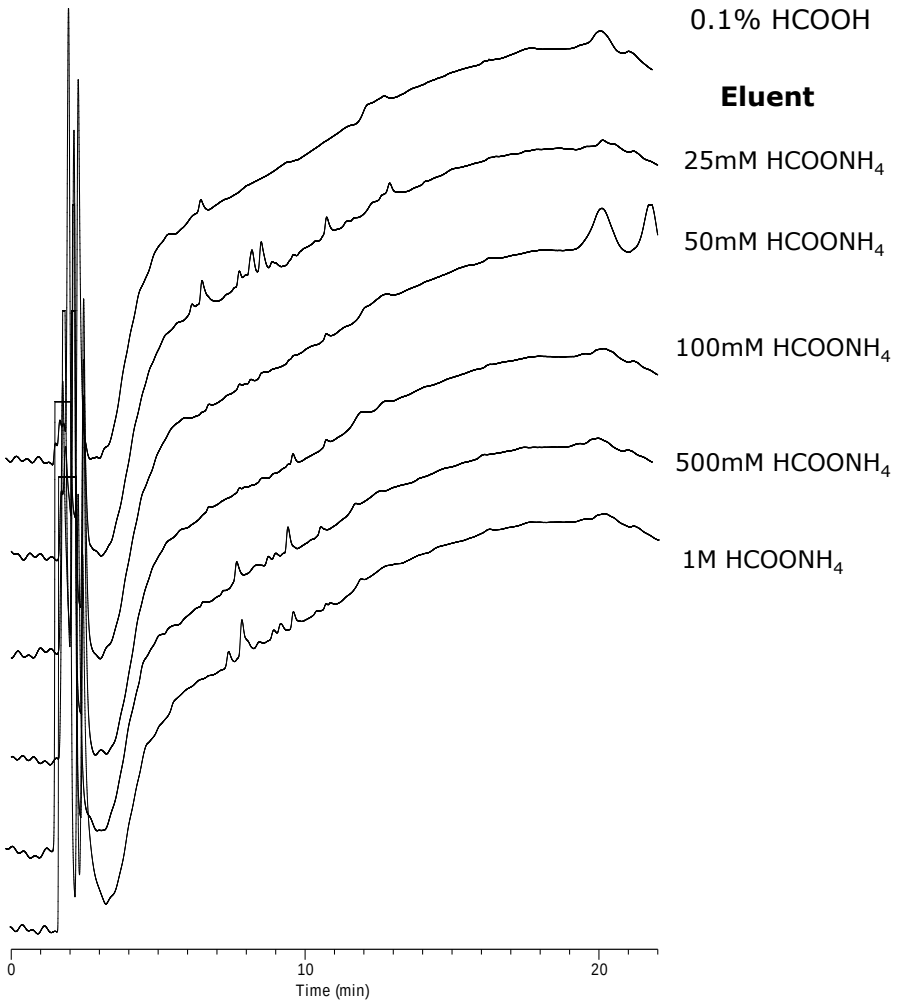
50% ACN

HPLC Condition
Sample: digested BSA 2 uL
Column: Inertsil ODS-3 (3um 2.1 x 150 mm)
Eluent: A: Water(0.1% HCOOH)
B: ACN(0.1% HCOOH)
Grad A:B=90:10-20min-50:50
Detection UV 210 nm
Flow rate 0.2 mL/min
Temp 40℃

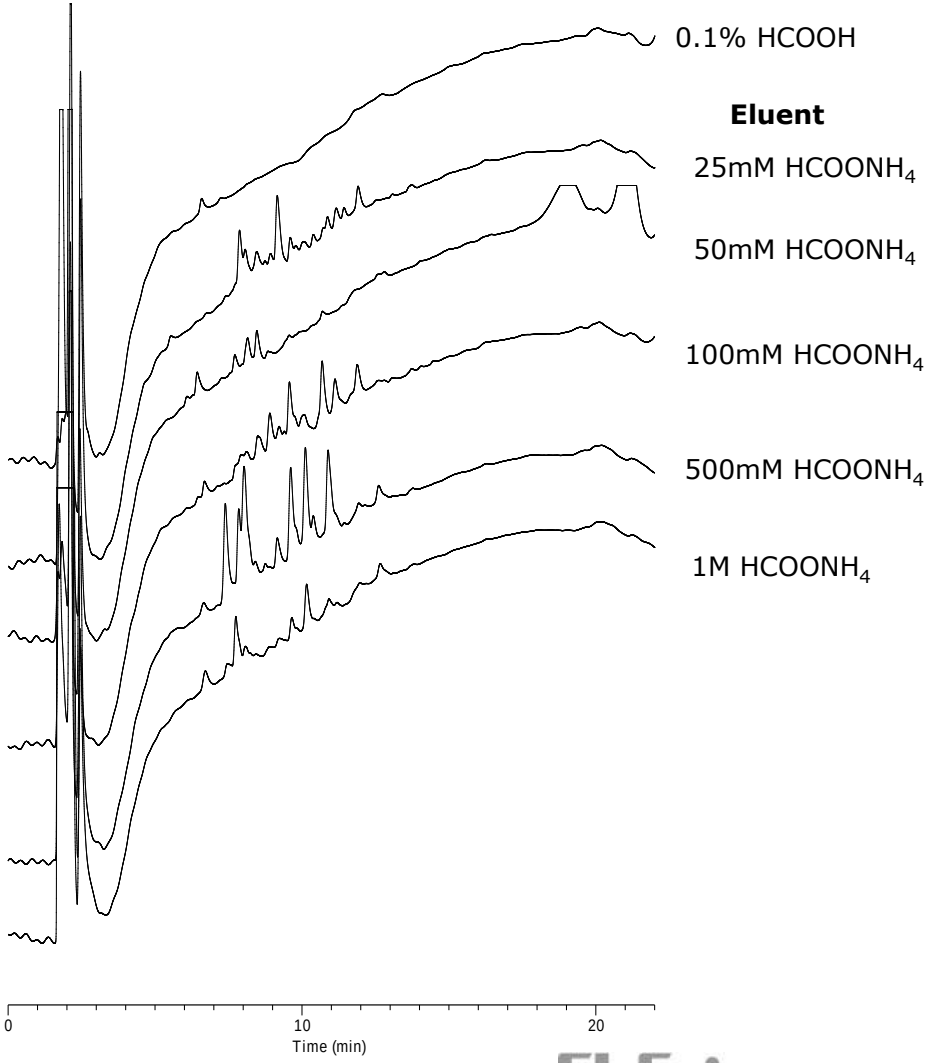
GL Sciences

Effect of organic sorvent additation for peptides recovery

0% CH_3CN



Eluent + 10% CH_3CN



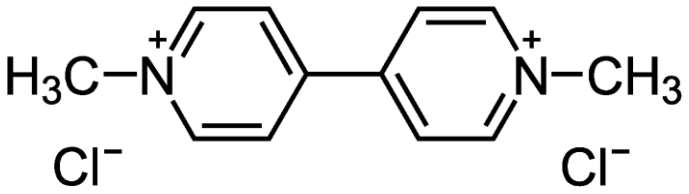
GL Sciences

Application list of MonoSpin™ series

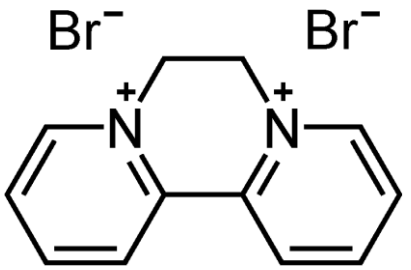
▪Basic drugs in plasma:	MonoSpin™ C18
▪Amphetamines in urine:	MonoSpin™ C18
▪Desaltration of peptides:	MonoSpin™ C18
▪Fractionation of peptides:	MonoSpin™ SCX
▪Pesticides in urine:	MonoSpin™ CBA
▪PA-sugar chain	MonoSpin™ NH2
▪Catecholamines in urine:	MonoSpin™ PBA
▪Glyphosate in drink	MonoSpin™ TiO

GL Sciences

Extraction of pesticides from urine using a MonoSpin™ CBA.

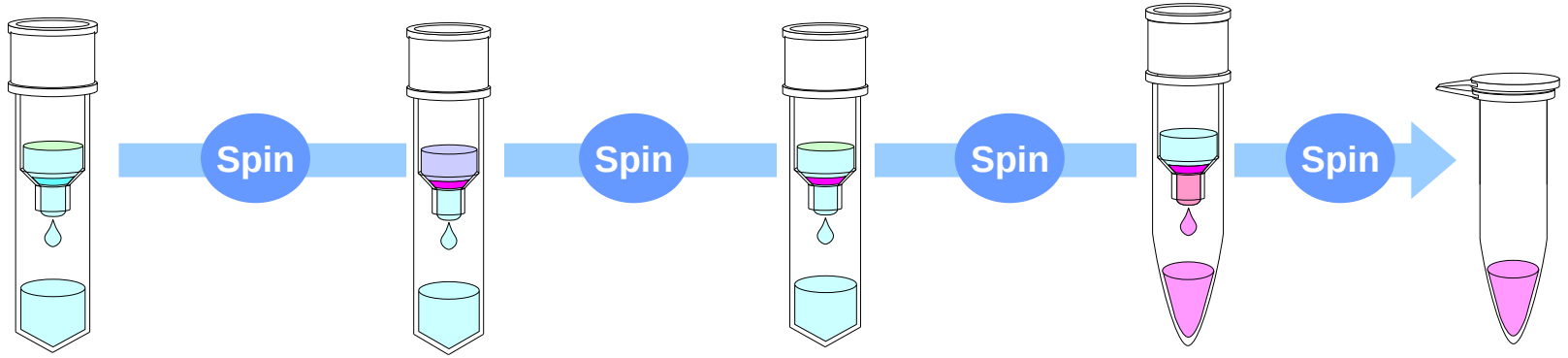


Paraquat



Diquat

Protocol of MonoSpin™ CBA for extraction of pesticides from urine.



1. Conditioning

200 µL of PPB

2. Adsorption

600 µL of Sample solution

3. Rinsing

200 µL of PPB

4. Elute

200 µL of
1% HCl
- 39% Acetonitrile
- 60% H_2O

Extract

Sample solution

	matrix (Urine)	200 µL
	containing pesticide(0.05~10 µg/mL)	
+	PPB	400 µL
		600 µL

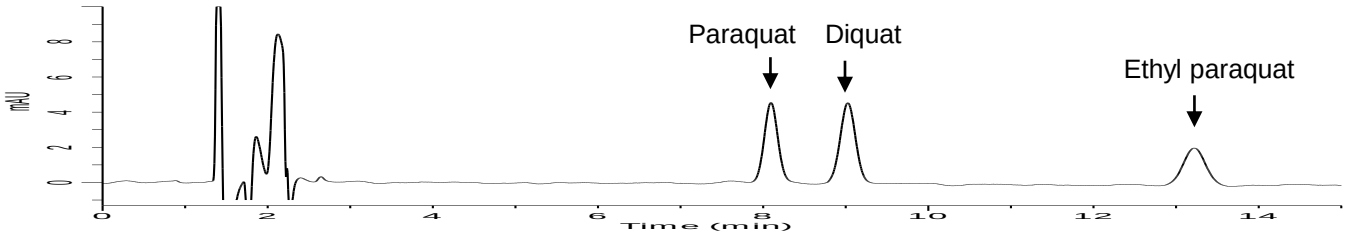
Spin **: 10,000 rpm for 30 sec**

PPB:10mM Potasium Phosphate Buffer(pH7.0)

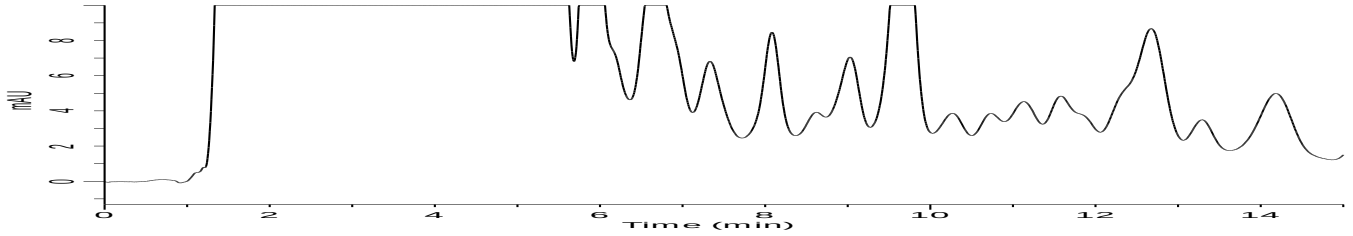
GL Sciences

Extraction of pesticides from urine using a MonoSpin™ CBA.

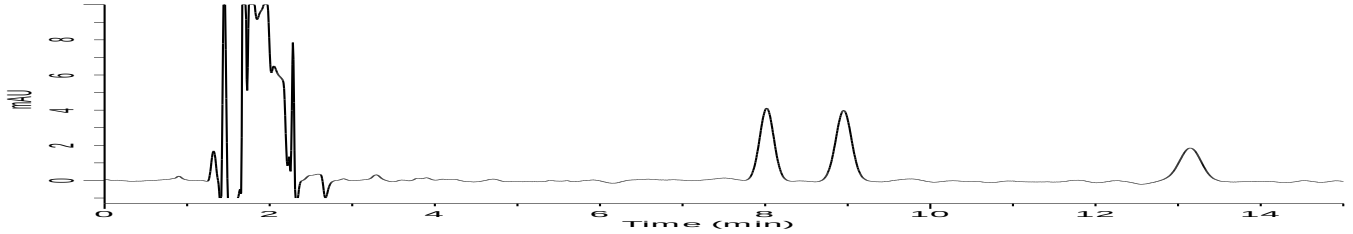
STD (each 1µg/mL)



Urine + Pesticides (each 1µg/mL)



Extraction by MonoSpin CBA



HPLC conditions

Column : Inertsil ODS3 (5 µm, 4.6 mm I.D. × 150 mm; GL Sciences), Eluent : 0.2 M phosphoric acid, 0.1 M diethyl amine, 7.5 mM IPCC-08 (IPCC-08, Sodium 1-Octanesulfonate) / Acetonitrile = 89 / 11

Flow rate : 1 mL/min, Column Temp. : 40 ℃, Detection : PDA 290 nm, Injection : 50 µL

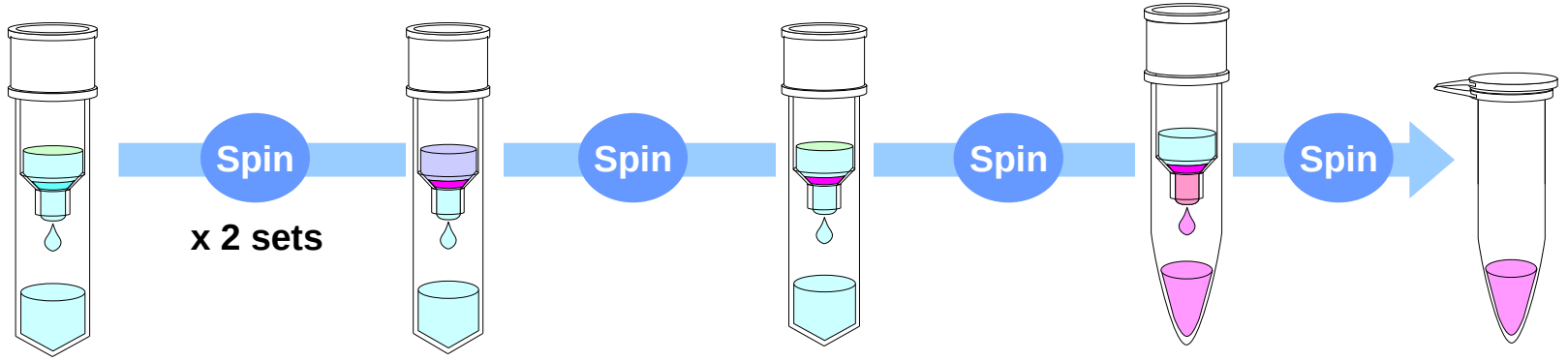
GL Sciences

Application list of MonoSpin™ series

▪Basic drugs in plasma:	MonoSpin™ C18
▪Amphetamines in urine:	MonoSpin™ C18
▪Desaltration of peptides:	MonoSpin™ C18
▪Fractionation of peptides:	MonoSpin™ SCX
▪Pesticides in urine:	MonoSpin™ CBA
▪PA-sugar chain	MonoSpin™ NH2
▪Catecholamines in urine:	MonoSpin™ PBA
▪Glyphosate in drink	MonoSpin™ TiO

GL Sciences

Protocol of MonoSpin™ NH2 for extraction of PA-sugar chain.



1. Conditioning

200 µL of
Water (0.1% TFA)
→
200 µL of
90% Acetonitrile
(0.1% TFA)

2. Adsorption

500 µL of
Sample solution

3. Rinsing

200 µL of
90% Acetonitrile
(0.1% TFA)

4. Elute

200 µL of
1% conc. ammonia
in water

Extract

Sample solution

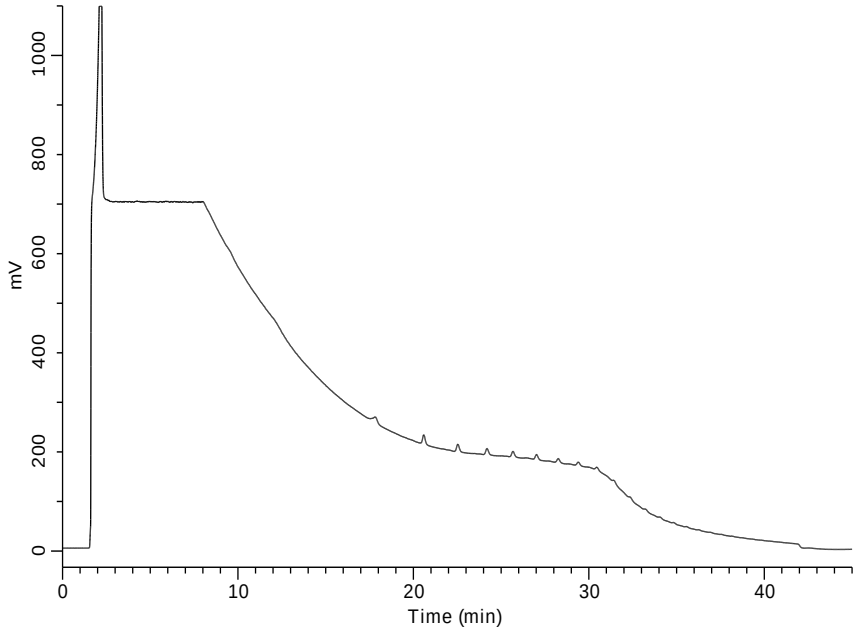
Sample(sugar chain)	**50µL**
0.1% TFA in Acetonitrile	**450µL**

Spin **: 5,000 rpm for 30 second**

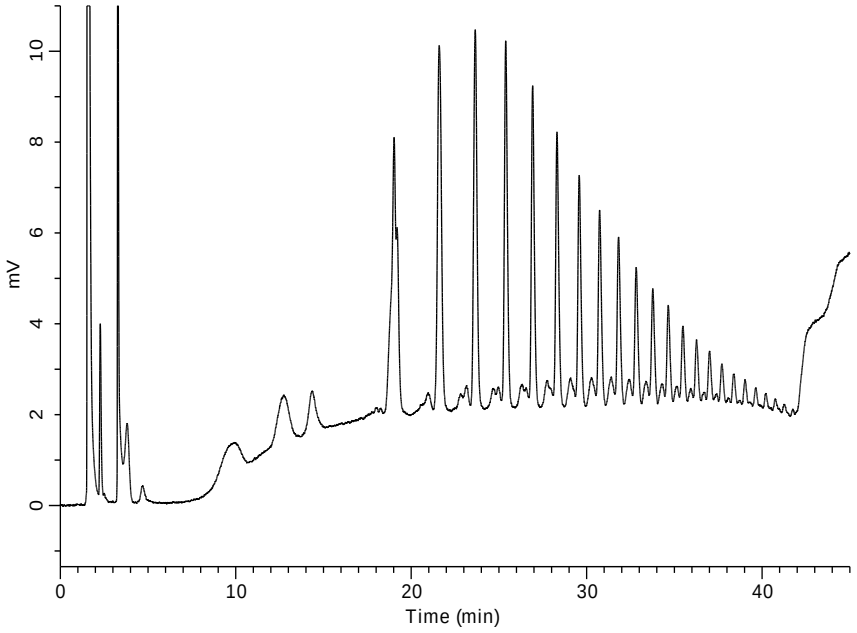
GL Sciences

Extraction of PA-sugar chain using a MonoSpin™ NH2.

Before treatment



After treatment



Pyridilyamino derivative of 1-20 mer glycose oligomer

Conditions
Column: NH2 column 5um (4.6 x 250 mm)
Eluent: (A) MilliQ/CH3CN =5/95 0.1 % FA
(B) MilliQ/CH3CN=95/5 0.1 % FA
Gradient: A/B=90/10-10min-90/10-40min-60/40
Flow rate: 1ml/min
Detection: FL Em 320nm, Ex 400nm
Injection: 5ul

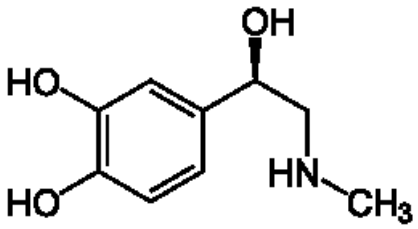
GL Sciences

Application list of MonoSpin™ series

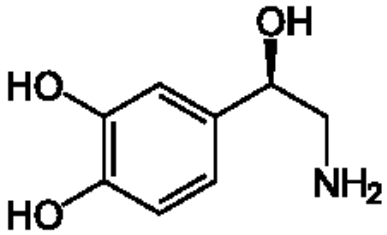
▪Basic drugs in plasma:	MonoSpin™ C18
▪Amphetamines in urine:	MonoSpin™ C18
▪Desaltration of peptides:	MonoSpin™ C18
▪Fractionation of peptides:	MonoSpin™ SCX
▪Pesticides in urine:	MonoSpin™ CBA
▪PA-sugar chain	MonoSpin™ NH2
▪Catecholamines in urine:	MonoSpin™ PBA
▪Glyphosate in drink	MonoSpin™ TiO

GL Sciences

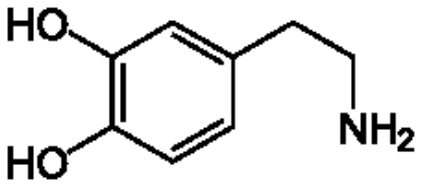
Extraction of catecholamines using a MonoSpin™ PBA.



Epinephrine



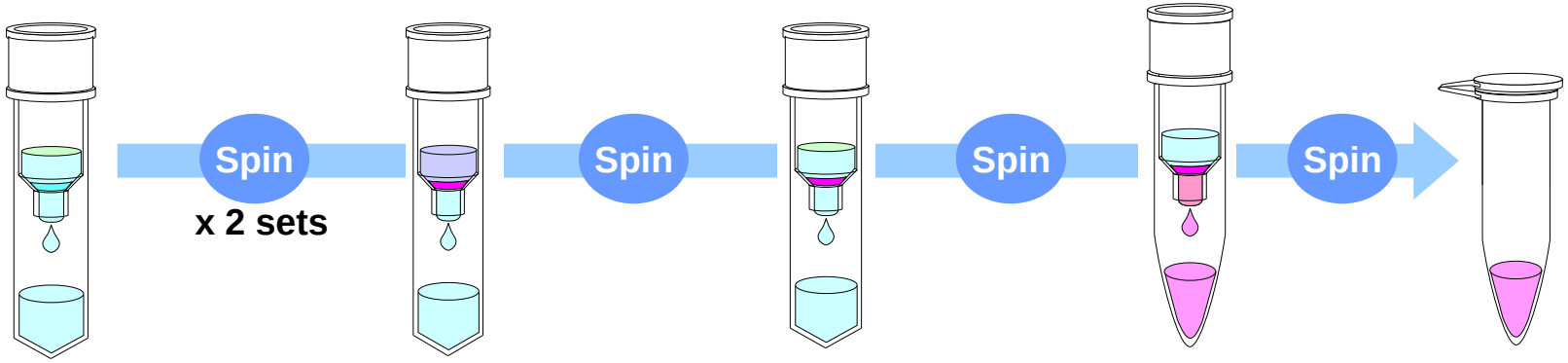
Noradrenalin



Dopamine

GL Sciences

Protocol of MonoSpin™ PBA for extraction of catecholamines from urine.



1. Conditioning

200 µL of
1% Acetic acid
→
200 µL of
Sodium phosphate buffer (pH8.0)

2. Adsorption

250 µL of
Sample solution

3. Rinsing

200 µL of
Sodium phosphate buffer (pH8.0)

4. Elute

300 µL of
1% Acetic acid

Extract

Sample solution

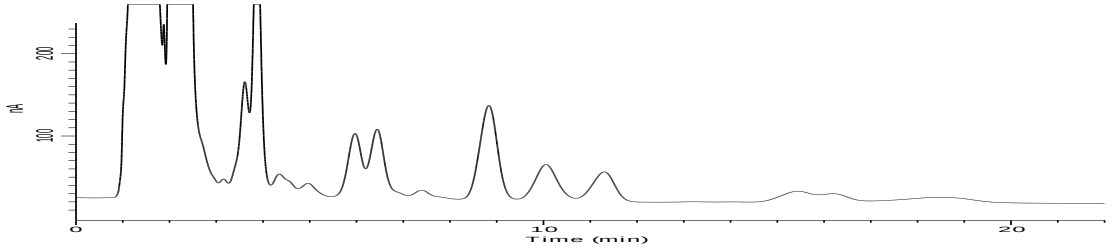
Urine	200 µL
+ Sodium phosphate buffer(pH8.0)	50 µL
	1,000 µL

Spin : 10,000 rpm for 1min

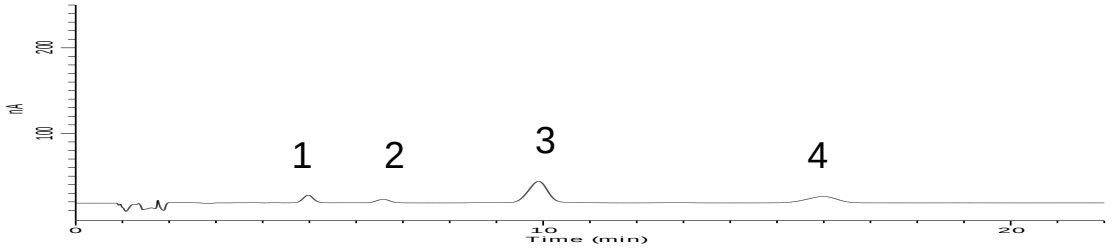
GL Sciences

Extraction of catecholamines using a MonoSpin™ PBA.

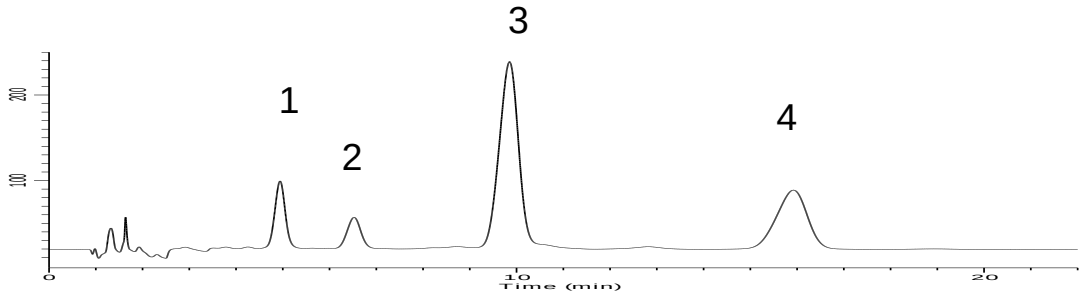
Before Treatment



After treatment
Sample volume 500μL,
Elution volume 500μL



After treatment
Sample volume 500μL,
Elution volume 50μL



＜LC Condition＞
Column: Inertsil ODS-3 (150 X 2.1 mm I.D.)
Elution: 50 mM phosphate buffer(pH5.6) 50mg/L EDTA 600 mg/L IPCC-008-10% Methanol
Flow rate=300 ml/min, injection: 5 μL, Detector: ECD703 plus Diamond electrode +800mV Ag/AgCl
Temp. 35℃, Sample 1:Noradrenaline, 2:Adrenalin, 3:DHBA, 4:Dopamine

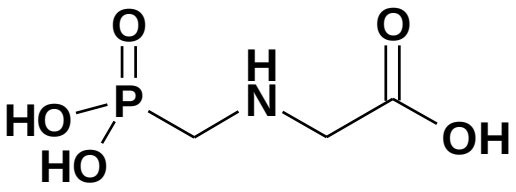
GL Sciences

Application list of MonoSpin™ series

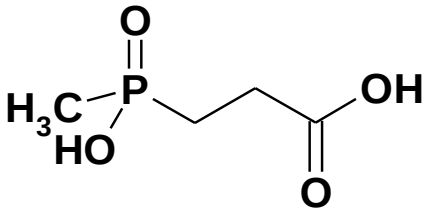
▪Basic drugs in plasma:	MonoSpin™ C18
▪Amphetamines in urine:	MonoSpin™ C18
▪Desaltration of peptides:	MonoSpin™ C18
▪Fractionation of peptides:	MonoSpin™ SCX
▪Pesticides in urine:	MonoSpin™ CBA
▪PA-sugar chain	MonoSpin™ NH2
▪Catecholamines in urine:	MonoSpin™ PBA
▪Glyphosate in drink	MonoSpin™ TiO

GL Sciences

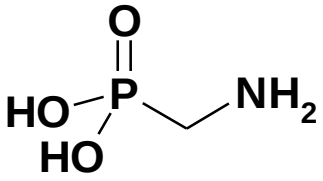
Extraction of Glyphosates from drinks using a MonoSpin™ TiO.



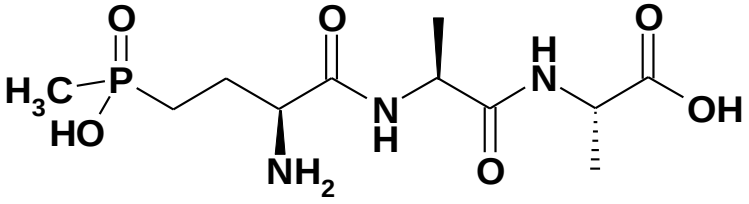
Glyphosate



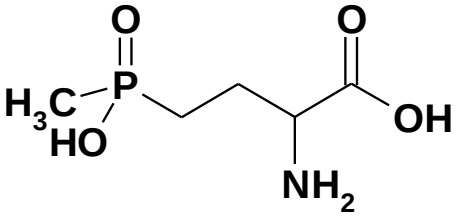
MPPA



AMPA



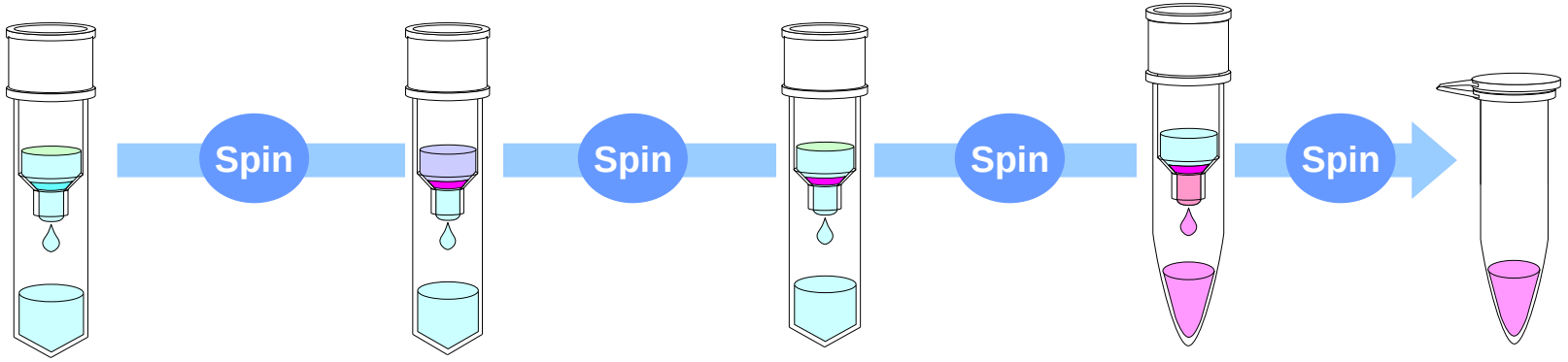
Bialaphos



Glufosinate

GL Sciences

Protocol of MonoSpin™ TiO for extraction of glyphosates from drink.



1. Conditioning

200 µL of
50% Acetonitrile
(0.1% TFA)

2. Adsorption

250 µL of
Sample solution

3. Rinsing

100 µL of
50% Acetonitrile
(0.1% TFA)

4. Elute

50 µL of
5% conc. Ammonia
in water

Extract

Sample solution

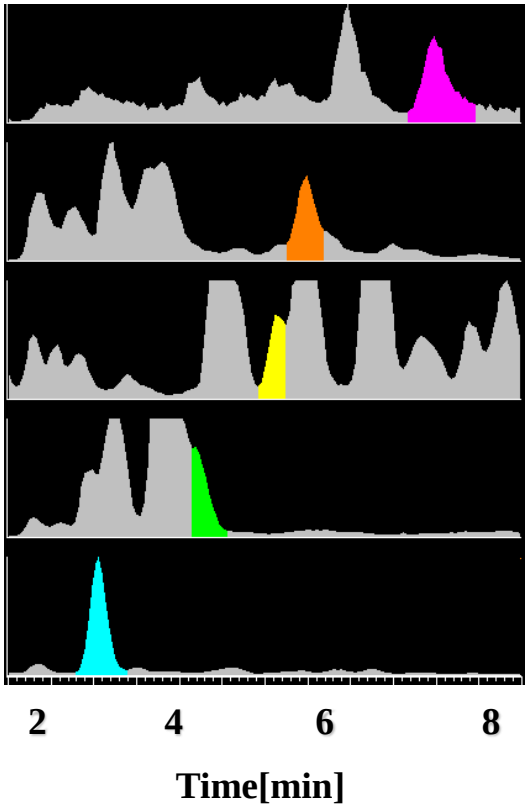
	Sample	**50 µL**
+	**0.1% TFA in water**	**200 µL**
		250 µL

Spin **: 5,000 rpm for 1min**

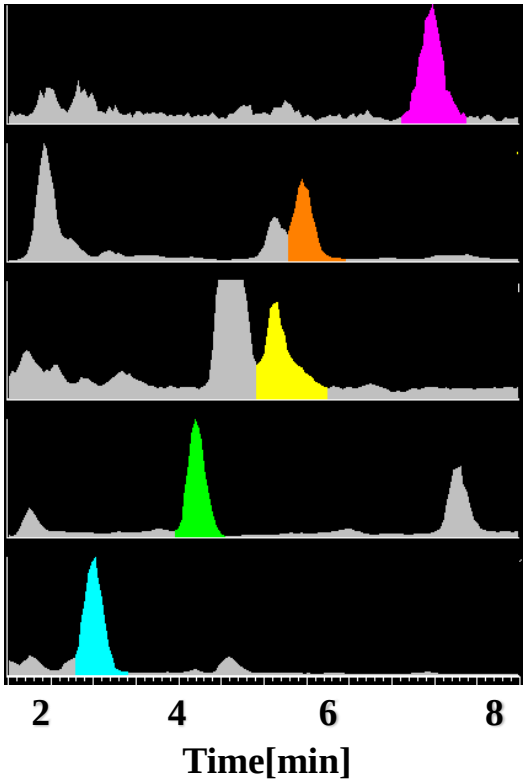
GL Sciences

Extraction of Glyphosates from drinks using a MonoSpin™ TiO.

Before pretreatment



Extraction by MonoSpin Tio



Bialaphos

Glyphosate

MPPA

Glufosinate

AMPA

＜LC Condition＞
Column: ODS Column (150 x 2.1 mm I.D.)
Elution: MeOH/20 mM HCO_2NH_4 (pH3.0) = 15/85
Flow rate=200 µl/min
Injection: 5 µL
Detector: SIM
Sample: Bialaphos, Glyphosate, MPPA, Glyfosinate, AMPA

GL Sciences

GL Sciences

info@glsciences.eu