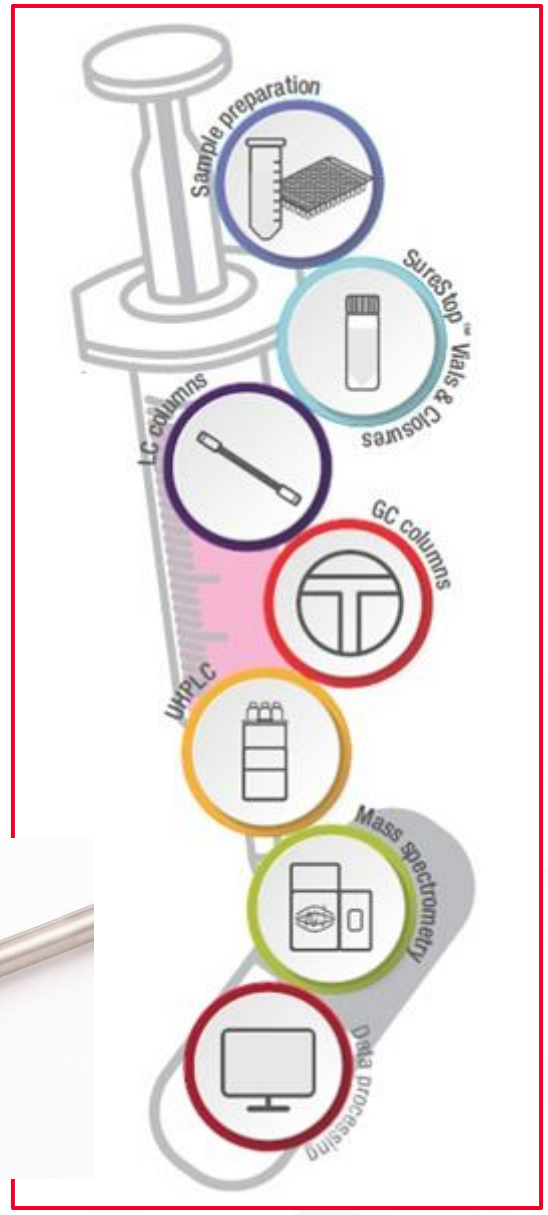
Spotrebný materiál pre testovanie metabolitov liečiv

Sabína Lociová


Titan3
PTFE-L 0.45µm
NYLON 0.2µm


HyperSep SLE


Sample preparation
SureStop™ Vials & Closures
LC columns
GC columns
UHPLC
Mass spectrometry
Data processing


HyperSep Retain-CX


SOLAµ
Thermo


Titan3
NYLON 0.2µm
PTFE-L 0.45µm


HyperSep SLE


HyperSep RetainCX


SOLAµ
Thermo

Kde všade?

Forenzná analýza

Forenzná toxikológia
- krv
- sérum
- moč
- sliny

Doping
- krv
- sérum
- moč
- sliny

Post-mortem
- krv
- sérum
- moč
- sliny

Výskum

- krv
- moč
- sérum

Potraviny

- ryby
- mäso
- Mlieko
- med

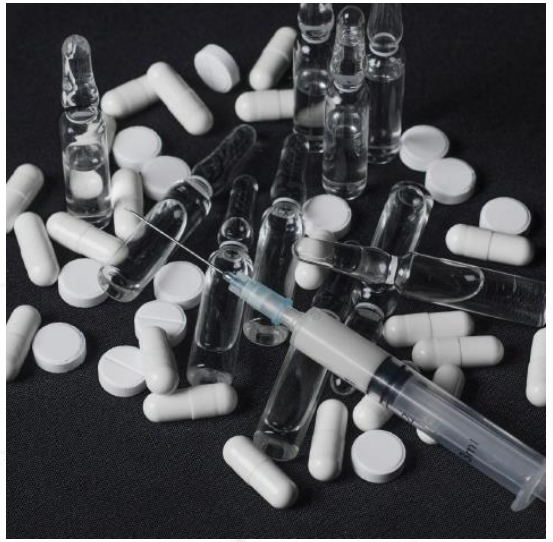
Environment

- voda

Úprava vzorky

Výzva

- Komplexné vzorky a matrice
- Objem vzoriek
- Predpisy a legislatíva
- Etický pohľad
- Náklady

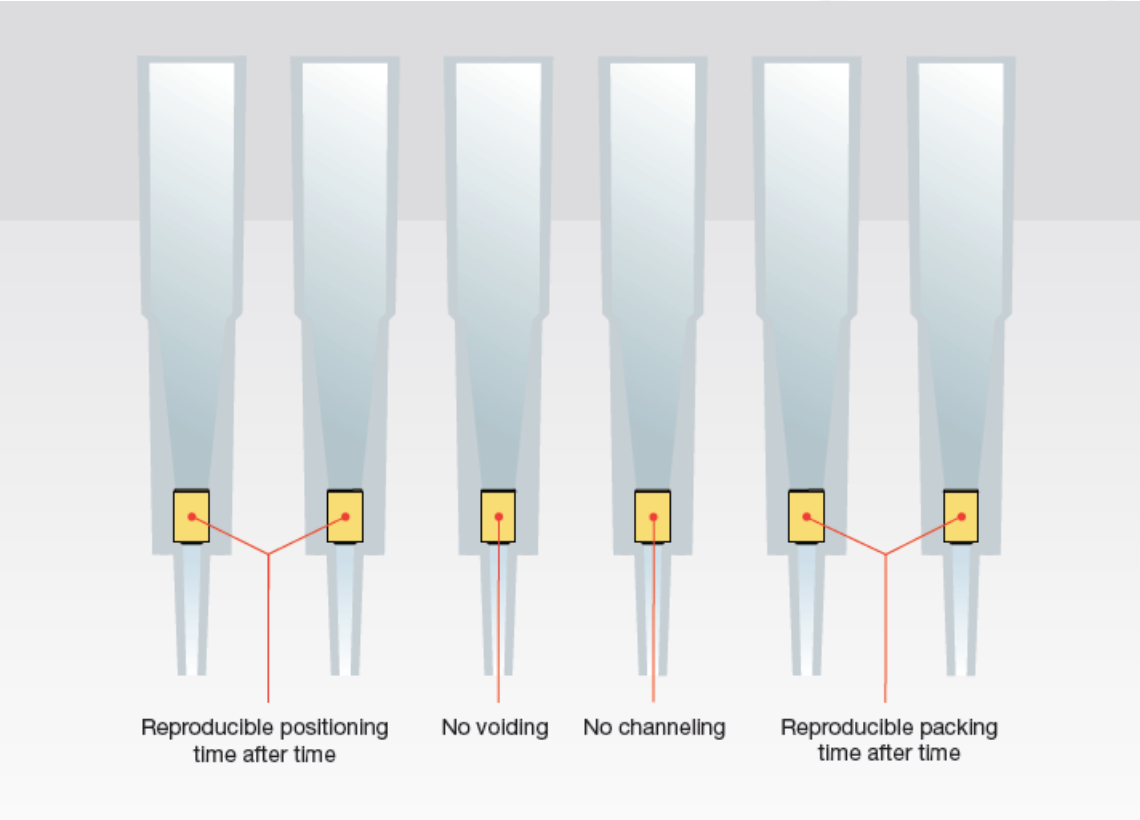


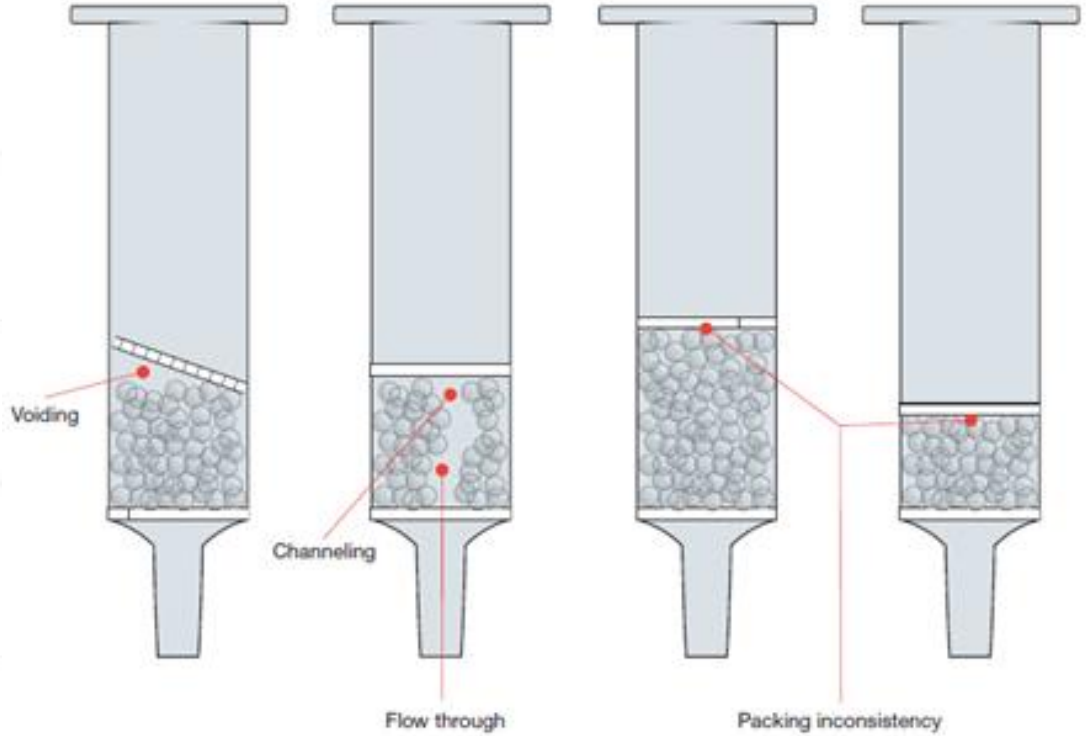
Požiadavky

- Reprodukovateľnosť
- Robustnosť
- Citlivosť
- Selektivita
- Rýchlosť
- Koncentračný rozsah

pragolab

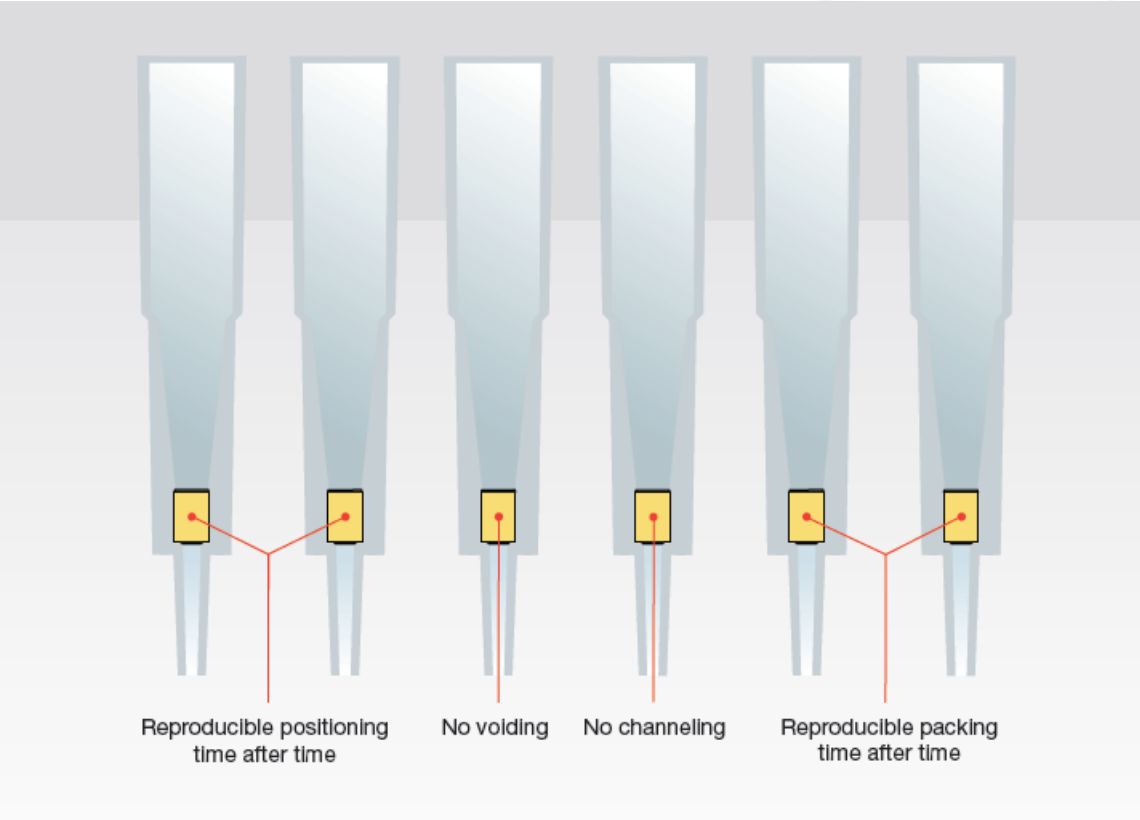
SOLAμ vs. klasické SPE

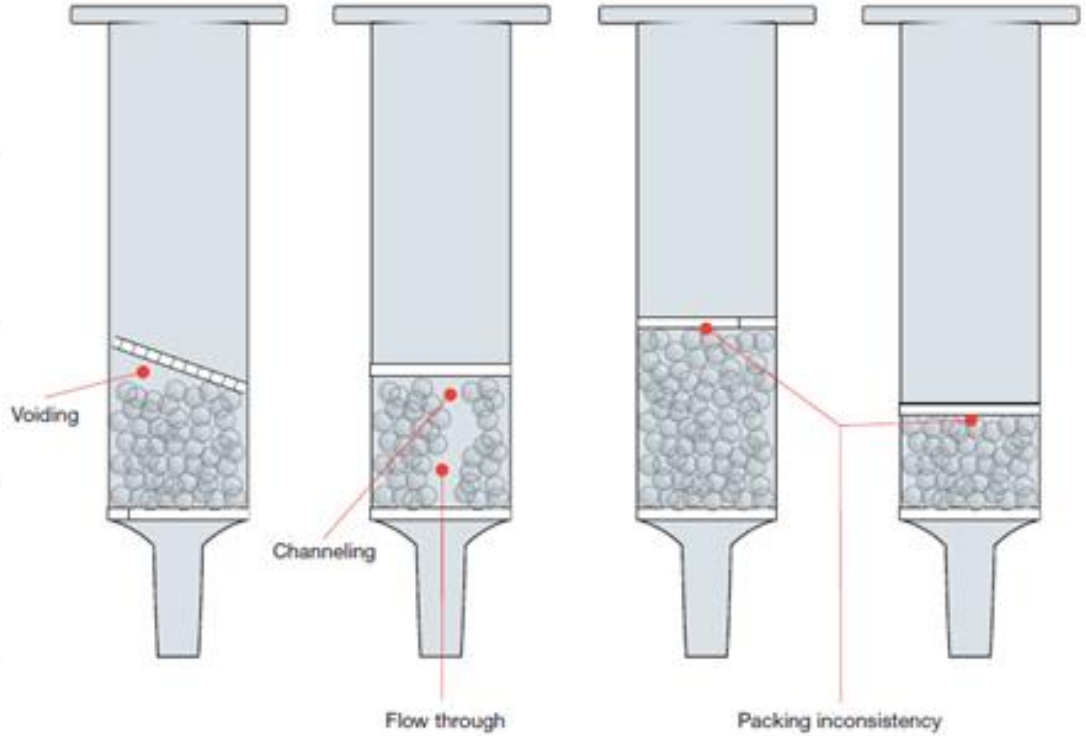




SOLAμ SPE design – limiting issues associated with conventional SPE formats

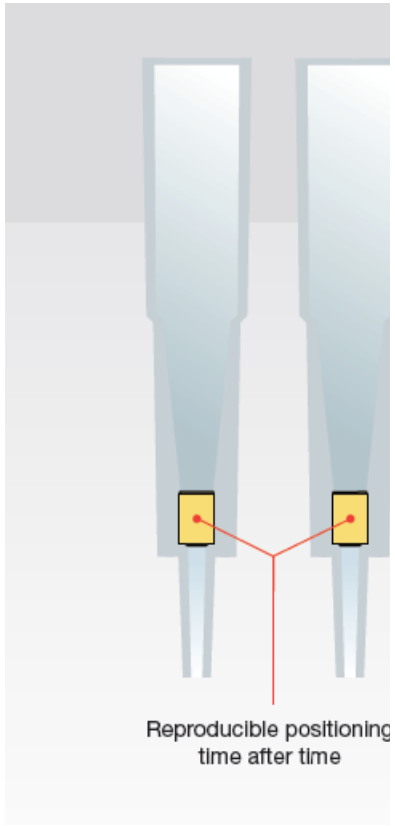
SOLAμ vs. klasické SPE

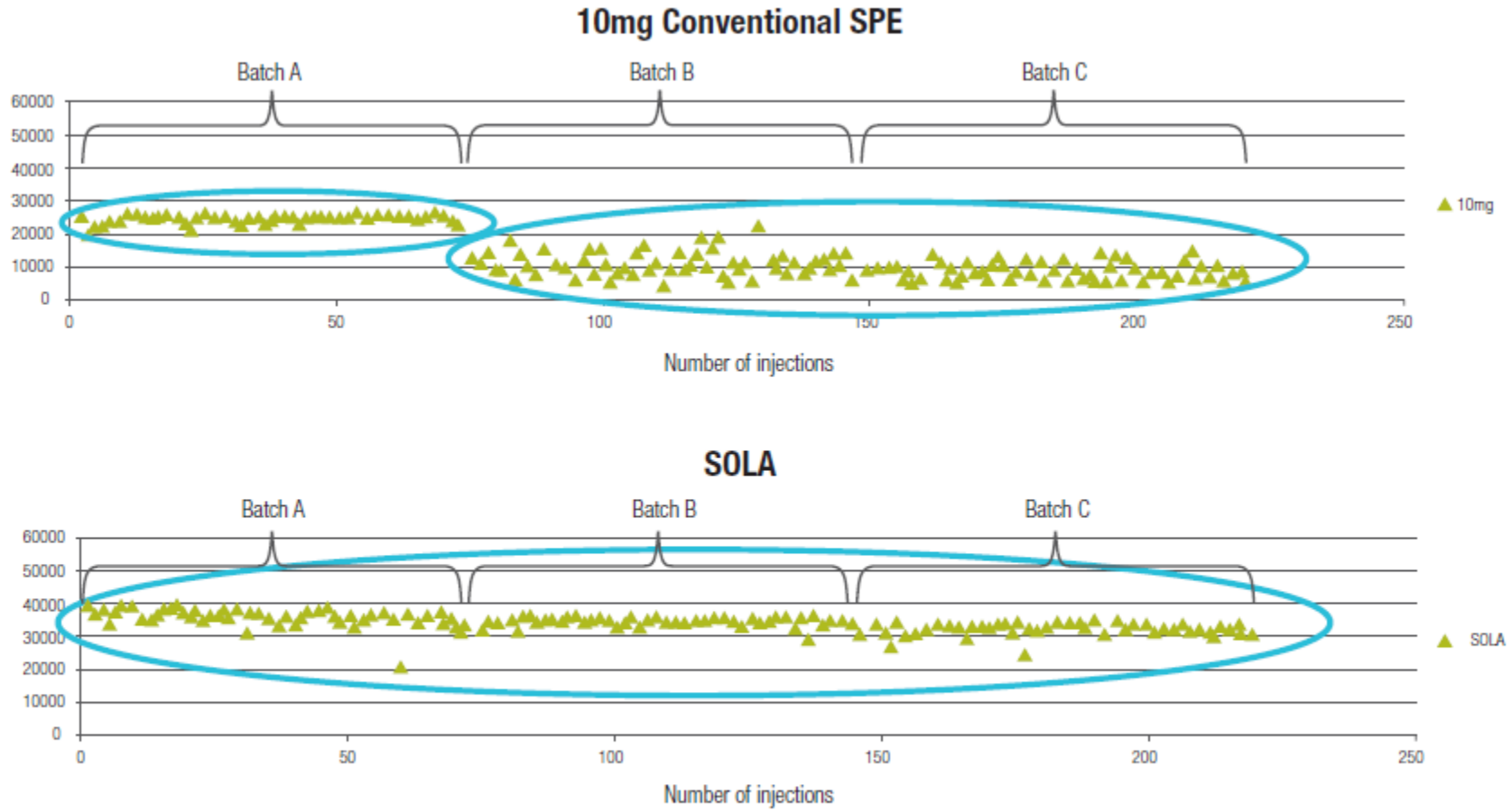


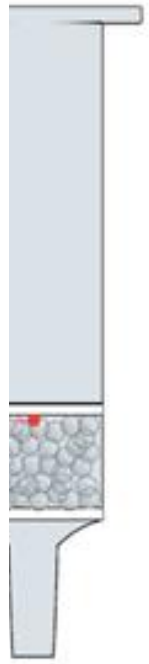


SOLAμ SPE design – limiting issues associated with conventional SPE formats

SOLAμ vs. klasické SPE

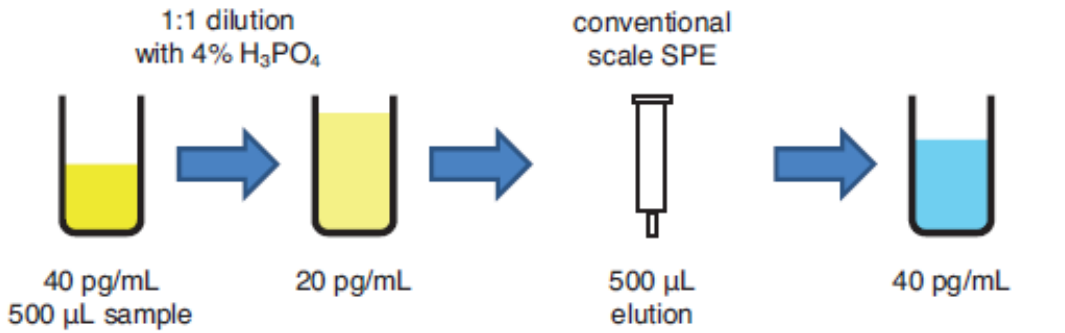




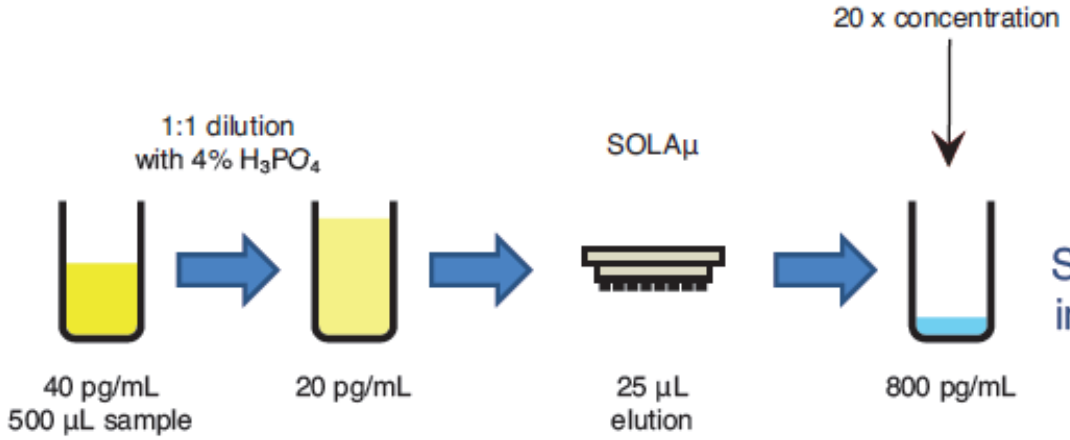


Batch to batch reproducibility of SOLA compared to a conventional SPE product

Mixed-Mode, Weak Anion-Exchange, Solid-Phase Extraction Method for the Extraction of Niflumic Acid from Human Plasma



The problem;
improvement in assay sensitivity required



SOLAµ solution;
SOLAµ for up to 20 times increase in concentration of sample without changes to workflow

Sample preparation protocol

Sample pre-treatment

500 µL of human plasma diluted 1:1 with 4% phosphoric acid

Sample preparation

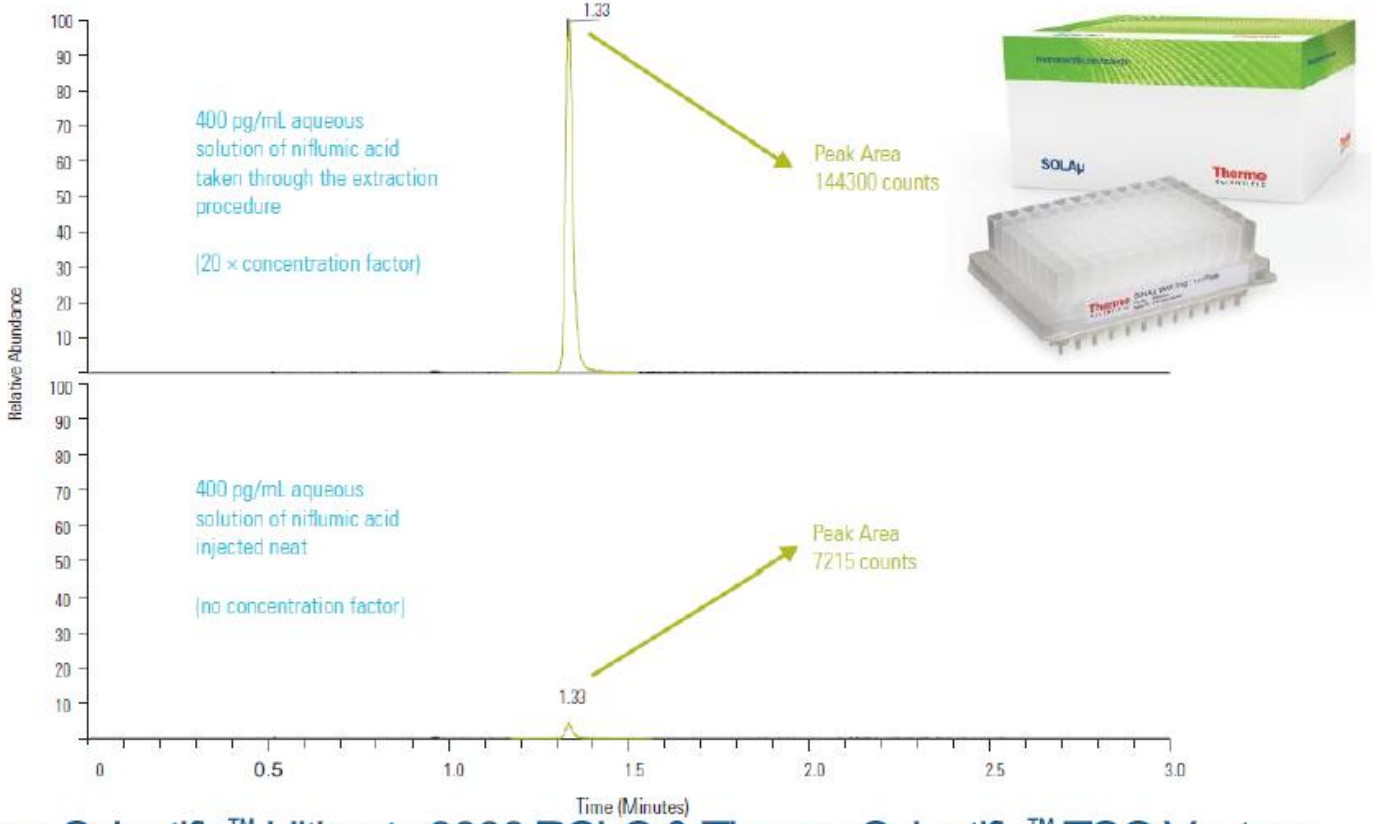
Compound(s):	niflumic acid, niflumic acid d5 (IS)
Matrix:	human plasma
	SOLAµ WAX 96 well plate (60209-005)
Condition:	200 µL methanol
Equilibrate:	200 µL 4% phosphoric acid
Load:	apply sample at 0.5 mL/min
Wash:	200 µL 25 mM ammonium acetate (pH4)
	200 µL 70% methanol (pH4)
Elute:	2 × 12.5 µL 50/50 methanol/acetonitrile with 2% ammonia

Direct injection of eluent

HPLC system:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system
Column:	Thermo Scientific™ Accucore™ RP-MS HPLC column 50 mm × 2.1 mm 2.6 µm (17626-052130)
Guard column:	Thermo Scientific™ Accucore™ RP-MS Defender™ guard cartridge (17626-012105) Thermo Scientific™ Uniguard™ drop-in guard holder (852-00)
Mass spec system:	Thermo Scientific™ TSQ Vantage™ Triple Stage Quadruple mass spec

pragolab

20 x pre-concentration of Niflumic Acid in Human Plasma



Thermo Scientific™ Ultimate 3000 RSLC & Thermo Scientific™ TSQ Vantage MS using Accucore RPMS Column, 2.6 µm × 2.1 mm × 50 mm

	Precision Data for Niflumic Acid Peak Area Ratio (%RSD) n = 18	Recovery of Niflumic Acid (%)	Matrix Effects (%)
QC Low (0.4ng/mL)	1.31	89.9	8.63
QC High (30ng/mL)	1.06	94.0	3.21

Sample preparation protocol

Sample pre-treatment

500 µL of human plasma diluted 1:1 with 4% phosphoric acid

Sample preparation

Compound(s):	niflumic acid, niflumic acid d5 (IS)
Matrix:	human plasma
	SOLAμ WAX 96 well plate (60209-005)
Condition:	200 µL methanol
Equilibrate:	200 µL 4% phosphoric acid
Load:	apply sample at 0.5 mL/min
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Mass spec system:	Thermo Scientific™ TSQ Vantage™ Triple Stage Quadruple mass spec

Increased speed and sample throughput of opioid analysis from human urine using micro-elution solid phase extraction

SOLA 10 mg Vol (µL)	SOLA 10 mg Time (min)		SOLAµ 2 mg Vol (µL)	SOLAµ 2 mg Time (min)
500	5	Condition with methanol	200	5
500	5	Equilibrate with water	200	5
1000	5	Load pre-treated sample	1000	10
500	5	Wash with 0.1% formic acid (aq)	200	5
500	5	Wash with 0.1% formic acid (methanol)	200	5
Place a collection plate under the SPE device to capture the extract				
2 x 200	5	Elute with MeOH/ACN/TEA (45/45/10)	2 x 25	5
Post-extraction processing requirements				
-	-	Dilute with water	50	1
n/a	30	Evaporate under nitrogen	-	-
100	5	Reconstitute with mobile phase	-	-

Figure 3. Method details for SOLA SCX and SOLAµ SCX showing each step, volume of solvent required, and length of time in minutes for each step.

LC conditions

Column	Thermo Scientific™ Hypersil GOLD™ aQ, 3 µm, 100 x 4.6 mm
Flow rate	1 mL/min
Column temperature	30 °C
Injection details	20 µL
Injection wash solvent 1	Water
Injection wash solvent 2	45:45:10 (v/v/v) IPA / acetonitrile/acetone

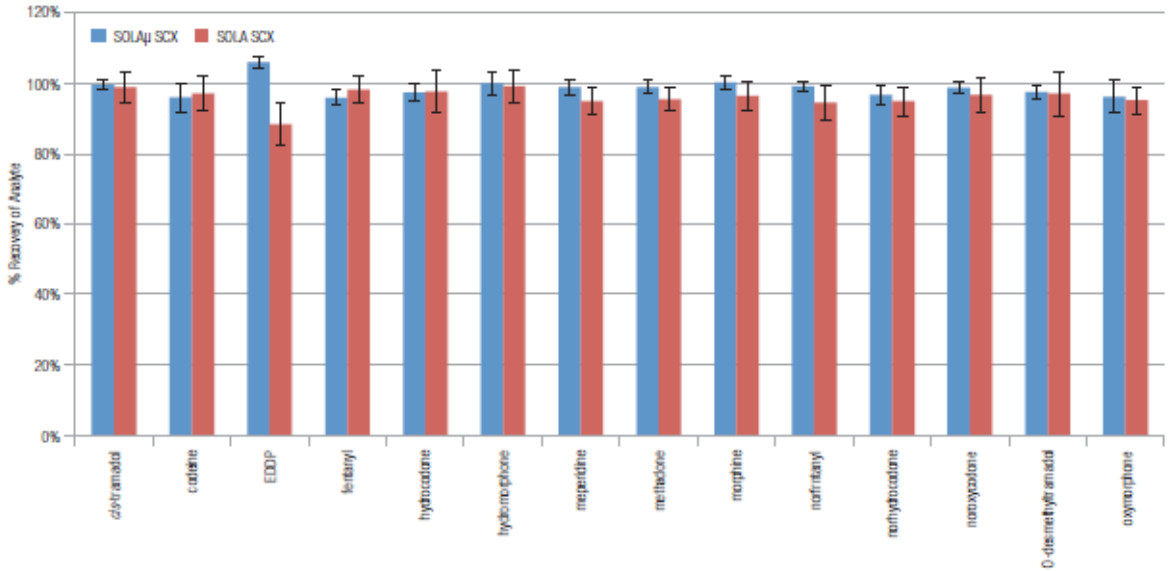
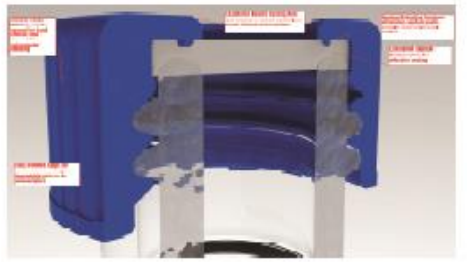


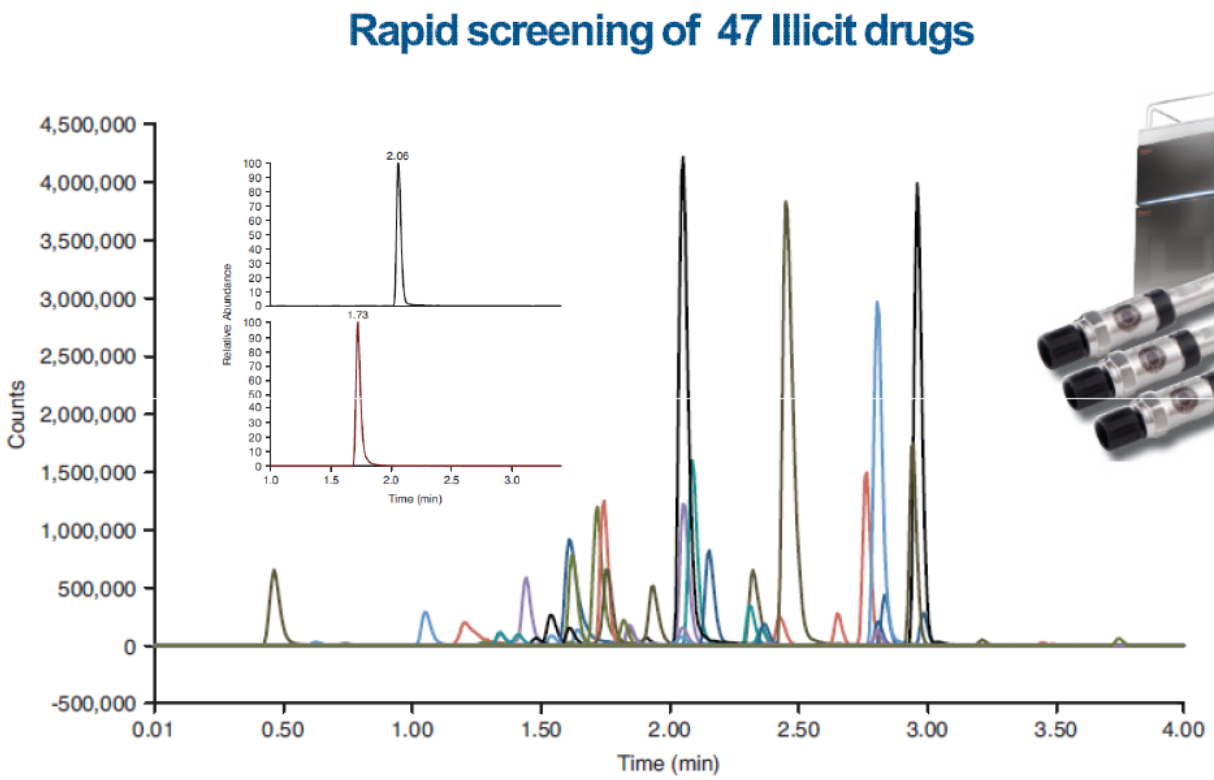
Figure 6. Comparison of SPE recovery between SOLA SCX and SOLAµ SCX.

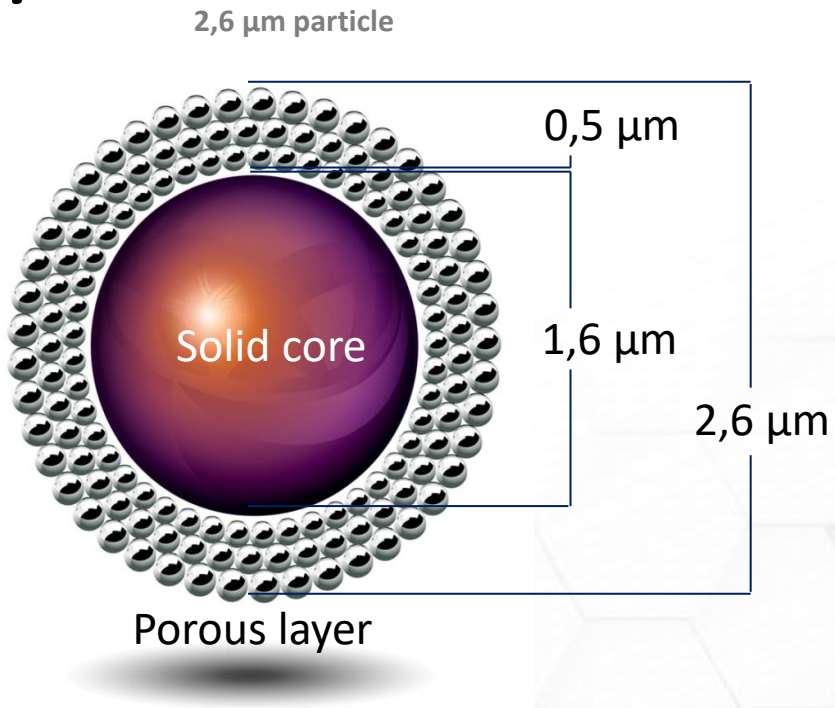
„Sample handling“

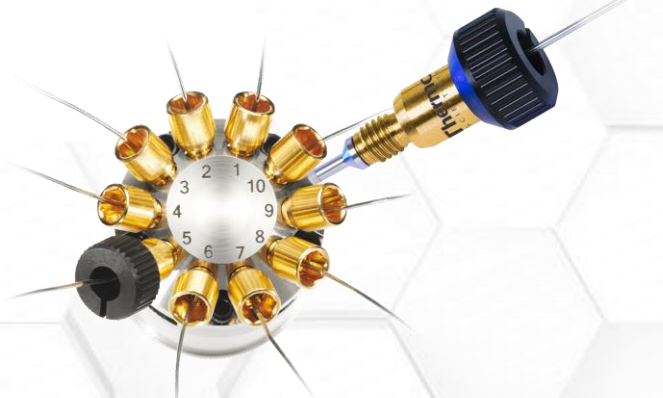
- Thermo Scientific™ Virtuoso™ Vial Identification System
- SureStop™ vials



Chromatografické kolóny





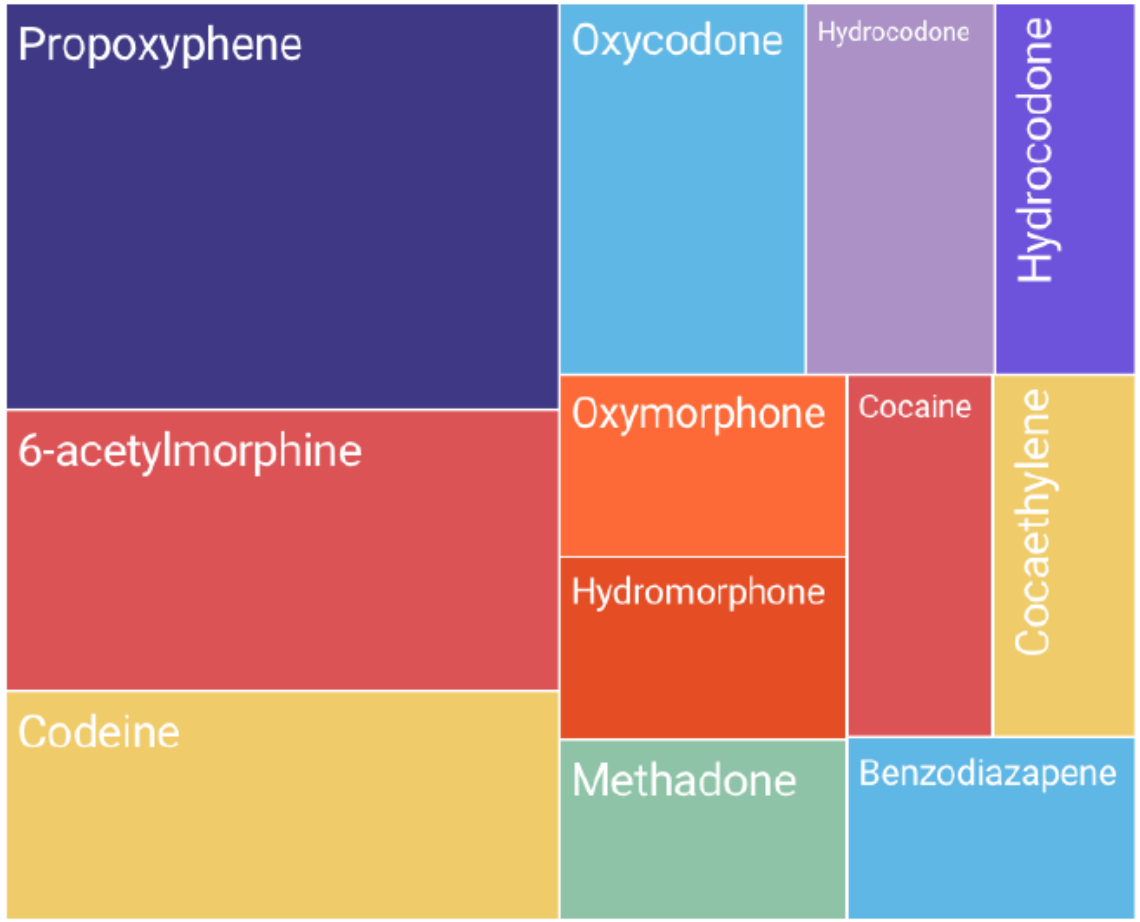


Accucore Vanquish, Vanquish and Viper

- rýchla separácia
- lepšia separácia
- vyššia priepustnosť
- nulový mŕtvy objem

Forenzná analýza

- LC-MS/MS Method*



pragolab

thermoscientific

Forensic toxicology quantitation of 30 benzodiazepines in whole blood using a high-resolution, accurate-mass (HRAM) mass spectrometer

Aplikácia:

- široká škála benzodiazepínov a ich metabolitov v 1 analýze
- krátka metóda
- minimálna úprava vzorky

Chromatografické podmienky:

Injection Volume:	100 µL
Column Temperature	40°C
Analytical Column	Accucore phenyl-hexyl, 2.6 µm, 100 x 2.1 mm
Run Time	10 minutes
Mobile Phases	A) 2 mM ammonium formate with 0.1% of formic acid in water B) 2 mM ammonium formate with 0.1% of formic acid in methanol/acetonitrile 50/50 V/V

pragolab

Compound Name	Internal Standard	Type	Weighting	R^2	LOQ (ng/mL)	Recovery	Matrix Effects
1-Hydroxymidazolam	Bromazepam D4	QUAD	1/X	0.9919	2	73%	94%
3-Hydroxybromazepam	Bromazepam D4	QUAD	1/X	0.9943	2	77%	102%
7-Aminoclonazepam	Bromazepam D4	QUAD	1/X	0.9938	5	56%	86%
7-Aminoflunitrazepam	Bromazepam D4	QUAD	1/X	0.9963	5	55%	77%
Alpha-hydroxyalprazolam	Bromazepam D4	QUAD	1/X	0.9951	2	77%	97%
Alprazolam	Bromazepam D4	QUAD	1/X	0.9922	2	63%	79%
Bromazepam	Bromazepam D4	QUAD	1/X	0.9947	2	65%	107%
Chlordiazepoxide	Bromazepam D4	QUAD	1/X	0.9931	5	56%	75%
Clobazam	Bromazepam D4	QUAD	1/X	0.9942	2	59%	99%
Clonazepam	Bromazepam D4	QUAD	1/X	0.9938	2	81%	86%
Clotiazepam	Bromazepam D4	QUAD	1/X	0.9947	2	111%	114%
Desalkylflurazepam	Bromazepam D4	QUAD	1/X	0.9938	2	63%	96%
Diazepam	Bromazepam D4	QUAD	1/X	0.994	2	107%	106%
Estazolam	Bromazepam D4	QUAD	1/X	0.9939	2	51%	83%
Flunitrazepam	Bromazepam D4	QUAD	1/X	0.9924	2	57%	83%
Flurazepam	Bromazepam D4	QUAD	1/X	0.9938	2	92%	74%
Loprazolam	Bromazepam D4	QUAD	1/X	0.9922	2	69%	76%
Lorazepam	Bromazepam D4	QUAD	1/X	0.9956	2	90%	98%
Lormetazepam	Bromazepam D4	QUAD	1/X	0.9945	2	63%	104%
Medazepam	Bromazepam D4	QUAD	1/X	0.9943	2	55%	78%
Midazolam	Bromazepam D4	QUAD	1/X	0.9944	2	71%	75%
Nitrazepam	Bromazepam D4	QUAD	1/X	0.9931	2	61%	88%
Norclobazam	Bromazepam D4	QUAD	1/X	0.9955	2	96%	91%
Nordiazepam	Bromazepam D4	QUAD	1/X	0.9941	2	54%	94%
Oxazepam	Bromazepam D4	QUAD	1/X	0.9961	2	61%	90%
Prazepam	Bromazepam D4	QUAD	1/X	0.9959	5	46%	81%
Temazepam	Bromazepam D4	QUAD	1/X	0.9947	2	66%	100%
Tetrazepam	Bromazepam D4	QUAD	1/X	0.9937	2	65%	75%
Zolpidem	Zolpidem D6	QUAD	1/X	0.9949	2	58%	88%
Zopiclone	Zolpidem D6	QUAD	1/X	0.9925	2	68%	78%

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Quantitation of THC and THC Metabolites in Blood Using SOLAμ SPE Plates and the TSQ Quantiva Triple Quadrupole Mass Spectrometer for Forensic Analysis

- Analýza THC a 4 hlavných emtabolitov – stanovenie užitia THC
- Jednoduchá, ekonomicky nenáročná a jednoducho automatizovateľná metóda prípravy vzorky
- Robustná metóda s limitovaným matricovým efektom
- LOQ: 0,2 ng/ml pre THC, THC-OH a THC-COOH,
  0,5ng /mL pre THC-glucuronide
  2ng/ml pre THC-COOH-glucuronide.

Chromatografické podmienky:

Injection Volume:	50 µL
Column Temperature	room temperature
Analytical Column	Accucore RP-MS 2.6 µm, 100 x 2.1 mm
Run Time	5 minutes
Tray Temperature	15 °C
Mobile Phases	A) Water with 0.1% Formic Acid
	B) Acetonitrile with 0.1% Formic Acid

Extraction recovery of sample preparation method and matrix effects obtained for blood samples spiked to concentrations of low, medium, and high QC samples:

	Recovery (%)			Absolute Matrix Effect (% Recovery)			Relative Matrix Effect (% Recovery)		
Analyte	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
THC	58.3	52.5	49.9	81.7	53.3	46.1	111	111	107
THC-OH	29.0	30.0	27.1	61.8	64.1	56.5	88.1	106	100
THCCOOH	81.7	67.6	63.6	63.6	50.2	45.5	98.6	83.5	88.6
THC-glucuronide	69.9	55.7	53.8	58.6	49.7	52.1	89.5	82.6	90.4
THCCOOH-glucuronide	25.6	26.2	28.7	131	140	110	120	107	105

Výskum

Fast, Reproducible LC-MS/MS Analysis of Dextromethorphan and Dextrorphan

Kimberly Phipps, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Application Note 20685

Sample Preparation		Part Number
Compound(s):	Dextromethorphan, dextromethorphan-d_3, and dextrorphan	
Matrix:	Plasma	
Plate type: Conditioning stage:	Thermo Scientific SOLA CX Apply 500 µL of methanol, then 500 µL 0.1% formic acid in water to the SPE plate	60309-002
Application stage:	Apply all supernatant to the SPE plate at a flow rate of 0.5 mL/min	
Washing stage:	Apply 500 µL of methanol / water (40:60 v/v) to the SPE plate	
Elution stage:	Apply 4 × 250 µL 5% ammonia in methanol to the SPE plate and dry well	
Additional stage:	Dry down under nitrogen and reconstitute in 200 µL acetonitrile / water (50:50 v/v). Mix well.	

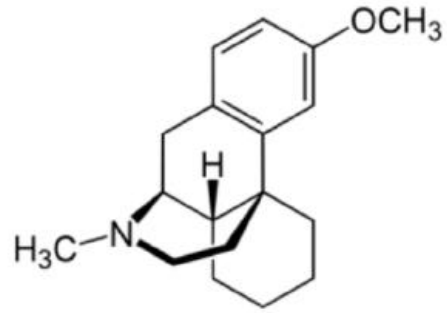


Figure 1. Dextromethorphan

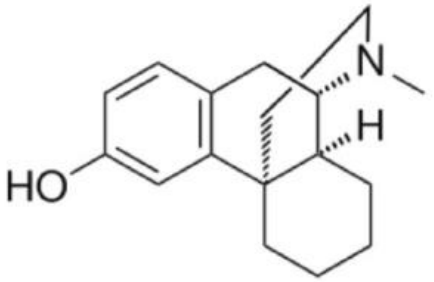


Figure 2. Dextrorphan

Separation Conditions			Part Number
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC HPLC System		
Column:	Accucore C18 2.6 µm, 50 × 2.1 mm		17126-052130
Mobile phase A:	Water + 0.1% formic acid		
Mobile phase B:	Acetonitrile + 0.1% formic acid		
Gradient:	Time (min)	%B	
	0	5	
	1	95	
	1.01	5	
	2	5	
Flow rate:	1.4 mL/min		
Column temperature:	40 °C		
Pressure:	360 Bar		
Injection details:	2 µL		

MS Conditions	
Instrumentation:	Thermo Scientific™ TSQ Vantage™ MS
Ionization conditions:	HESI
Polarity:	Positive
Spray voltage (V):	5000
Vaporizer temperature (°C):	450
Sheath gas pressure (Arb):	60
Aux gas pressure (Arb):	40
Capillary temp (°C):	300
Collision pressure (m Torr):	1.5
Q1 (FWHM):	0.7
Q3 (FWHM):	0.7
Compound transition details are provided in Table 1.	

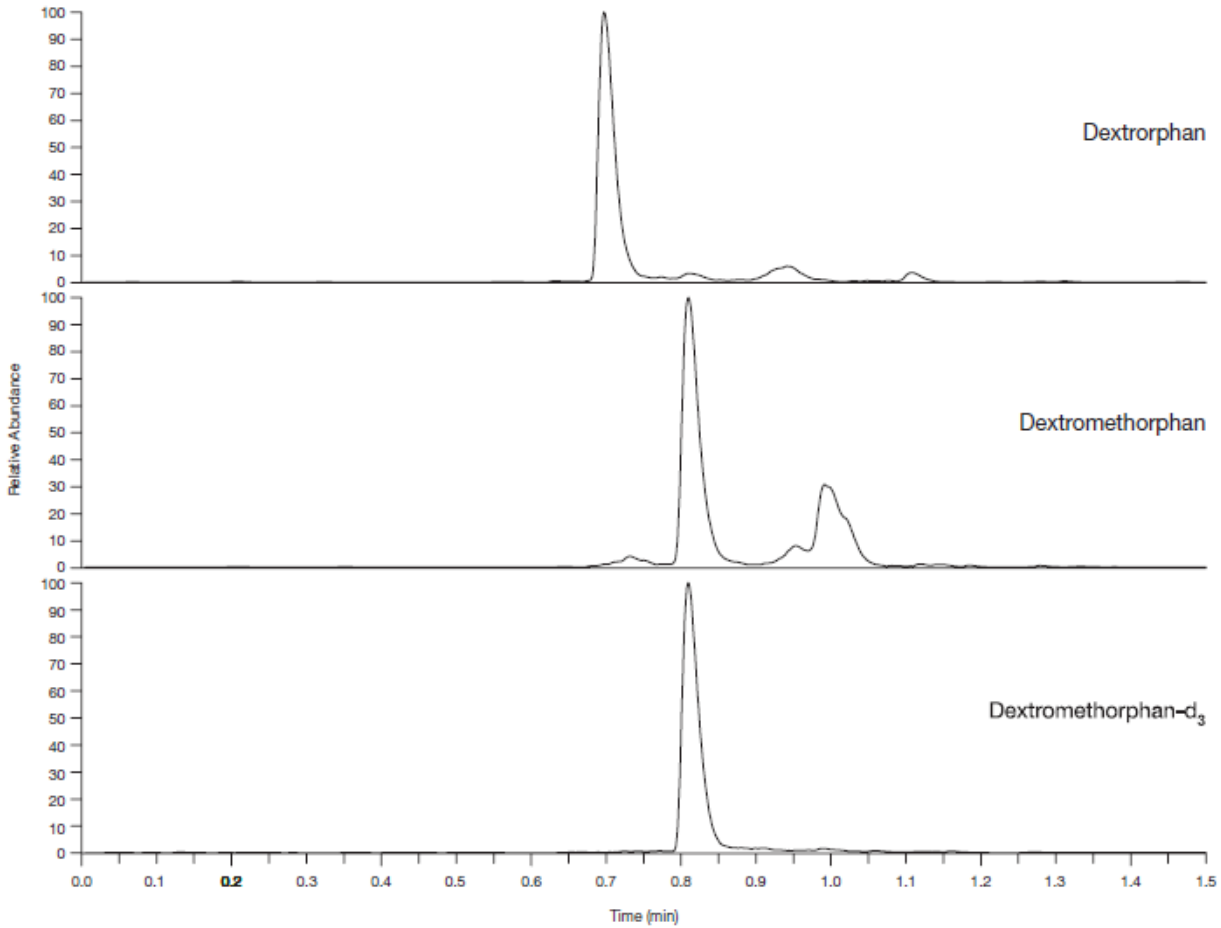
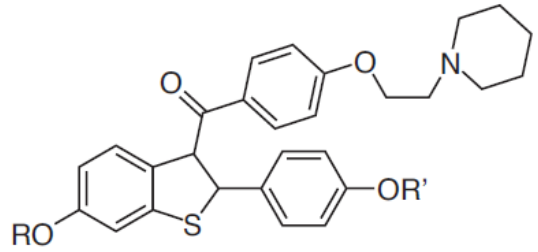


Figure 3: Representative chromatogram of dextromethorphan and dextrorphan SRM, extracted from plasma at 2.5 ng/mL

LC-MS/MS Method for the Determination of Raloxifene and its Glucuronide Metabolites from Human Plasma Using SPE Micro Elution

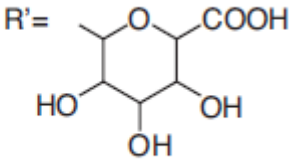
Krishna Rao Dara, Dr. Tushar N. Mehta, Centre of Excellence for Asia Pacific Laboratory
Thermo Fisher Scientific, Ahmedabad, India

Application Note 21002

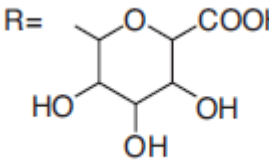


a) RAL, R =R'= H

b) R4G, R =H;= R'=



C) R6G, R=



; R' =H

SOLAμ SCX 2 mg/1 mL 96-well plate

Sample Pretreatment

A standard spiking stock solution of RAL, R4G, and R6G was prepared in methanol at a concentration of 0.1 mg/mL separately. An internal standard stock solution (d4-raloxifene) was prepared in methanol at a concentration of 0.1 mg/mL.

Blank human plasma (295 µL) was added to 300 µL of 2.0% formic acid. For standards and quality control (QC) samples, 6 µL of standard spiking solution and 20 µL of internal standard solution were added to 295 µL of human plasma. For blanks, 26 µL of water was added.

Extraction Procedure

Condition:	200 µL methanol
Equilibrate:	200 µL water
Application:	Load pre-treated sample
Wash 1:	200 µL water with 2.0% formic acid
Wash 2:	200 µL methanol
Elution:	2 × 75 µL methanol with 5.0% ammonia
Dilution:	Add 50 µL of water with 6.0% formic acid to each sample

Separation Conditions

Recommended instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000RS Rapid Separation System
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Acetonitrile + 0.1% formic acid
Mode:	Gradient (refer to Table 1)
Flow rate:	0.5 mL/min
Column temperature:	30 °C
Injection details:	10 µL

Time (min)	% B
0.0	20
6.0	80
6.2	20
7.5	20

Table 1: Mobile phase gradient

MS Conditions

Instrumentation: TSQ Vantage MS

The MS conditions and compound transition details are given in Tables 2 and 3.

Parameter	Setting
Ion Source Type	HESI-2
Polarity	Positive
Spray voltage (V)	4000
Vaporizer temperature (°C)	400
Sheath gas pressure (Arb)	45
Ion Sweep gas pressure (Arb)	0
Auxiliary gas pressure (Arb)	12
Capillary temperature (°C)	375
Declustering voltage (V)	0
Collision pressure (mTorr)	1.5
Scan width (*m/z*)	0.2
Scan time (s)	0.1
Q1 (FWHM)	1.2
Q3 (FWHM)	1.2

Table 2: TSQ Vantage MS conditions

Compound	RAL	R4G	R6G	d4-RAL (IS)
Parent (*m/z*)	474.2	650.2	650.2	478.2
Products (*m/z*)	112.1	112.0	112.0	116.1
Collision energy	28	40	40	28
S-lens	203	145	145	111

Table 3: Compound transition details

Hypersil GOLD PFP 3 µm, 100 × 3 mm

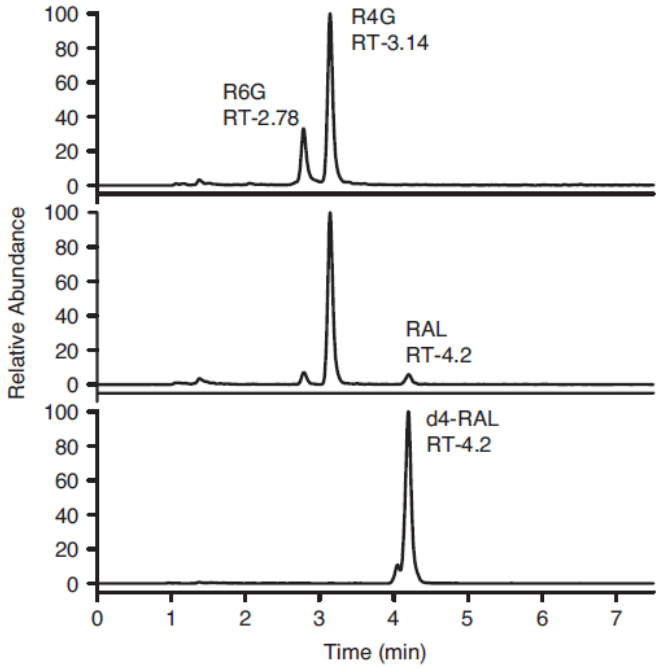
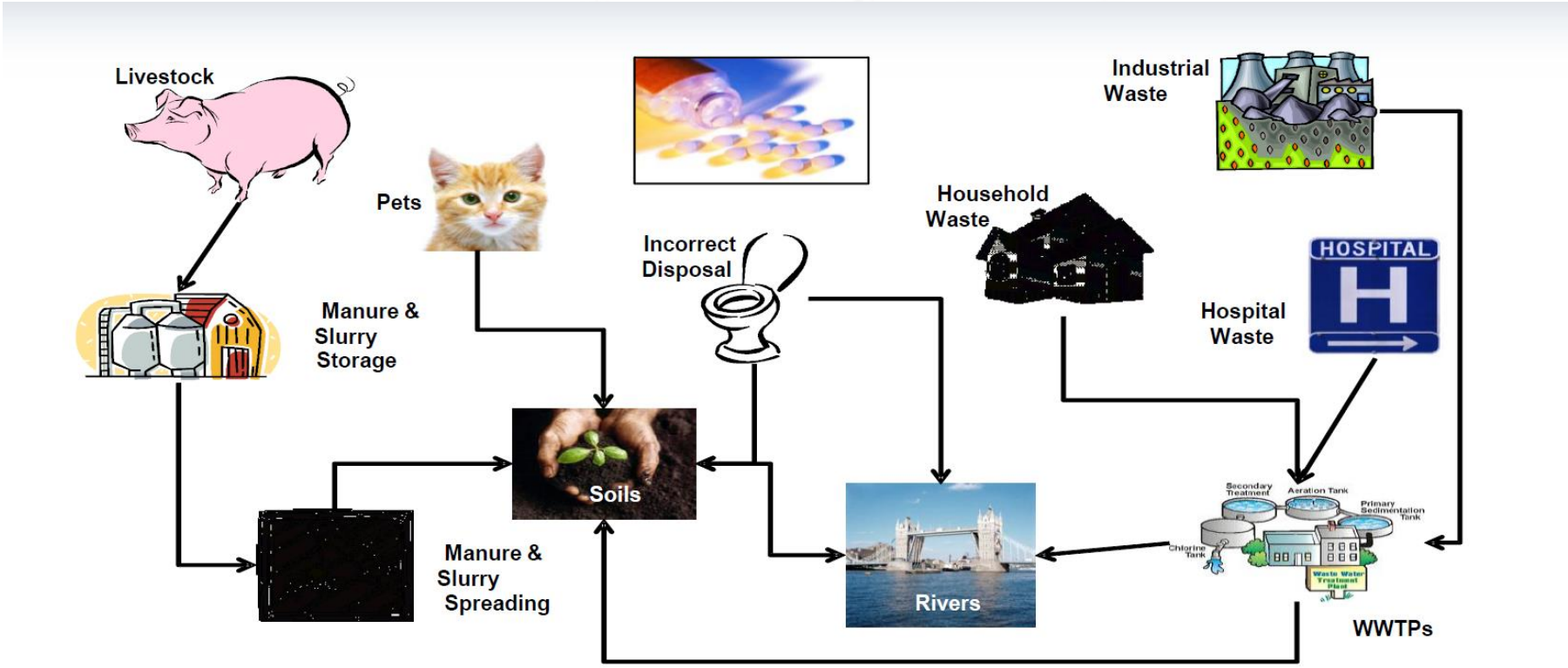


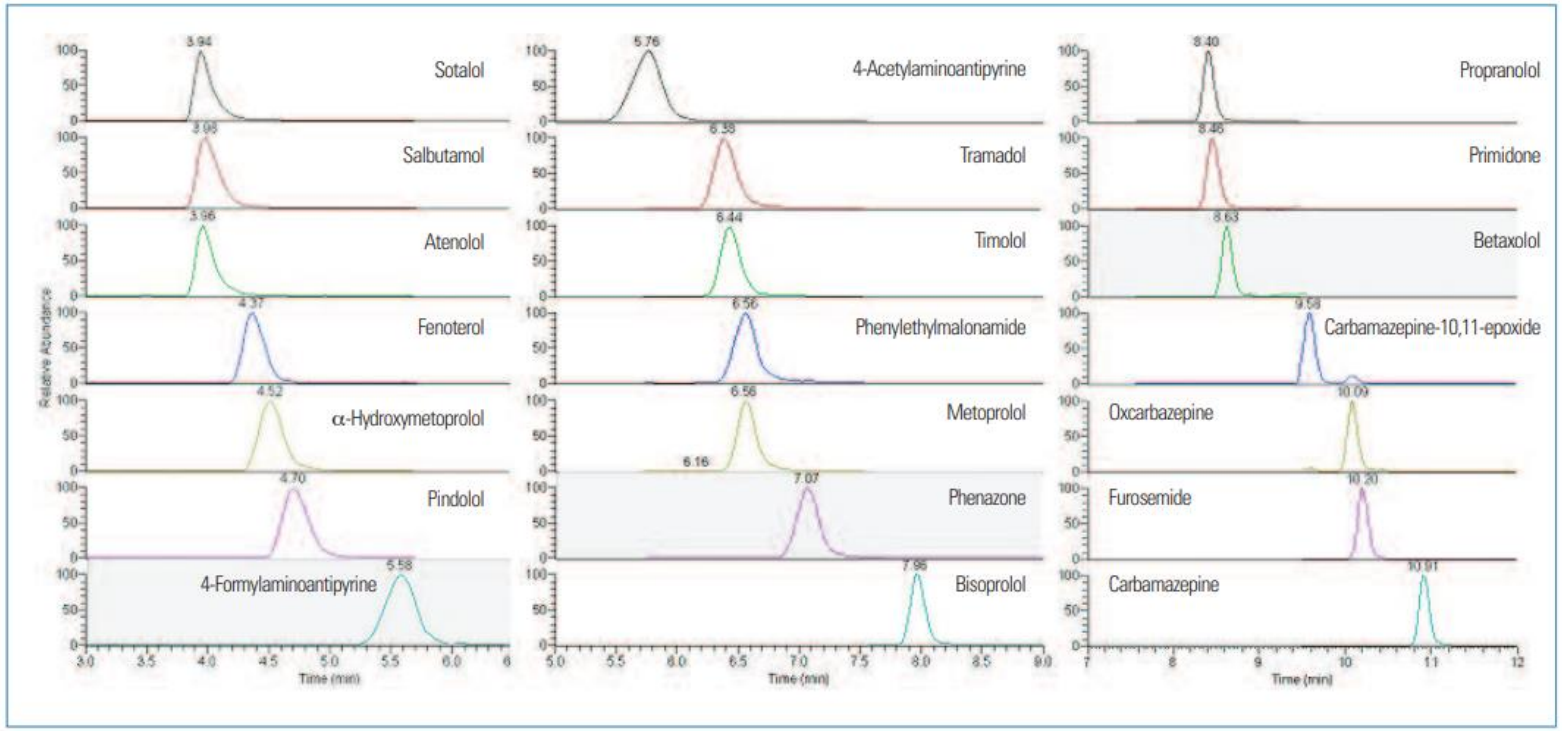
Figure 5: Representative SRM chromatograms of R4G and R6G (top) and RAL (middle), extracted from human plasma at the respective LLOQ levels along with d4-RAL (bottom) (ISTD)

Farmaceutiká vo vode

Pharmaceuticals in water is a current ‘hot topic’ in water analysis.



Analysis of Basic and Acidic Pharmaceutical Products in Drinking Water Using Online Sample Preparation and LC-MS/MS



SRM chromatograms of a selection of basic and neutral analytes at a concentration of 50 ng/L monitored in positive ESI.

Basic / Neutral Pharmaceuticals	Atenolol
	Betaxolol
	Bisoprolol
	Carbamazepine
	Fenoterol
	Furosemide
	Metoprolol
	Oxcarbazepine
	Phenazone
	Pindolol
	Primidone
	Propranolol
	Salbutamol
	Sotalol
	Timolol
	Tramadol
Metabolites of Basic / Neutral Pharmaceuticals	4-Acetylaminoantipyrine
	4-Formylaminoantipyrine
	α-Hydroxymetoprolol
	Carbamazepine-10,11-epoxide
	Phenylethylmalonamide
Acidic Pharmaceuticals	Bezafibrate
	Diclofenac
	Fenoprofen
	Gemfibrozil
	Indomethacin
	Ketoprofen
	Naproxen

thermoscientific

APPLICATION BRIEF 65118

The VetDrugs Explorer Collection: screening and quantitation of multi-class veterinary drug residues in animal matrices with a comprehensive workflow solution

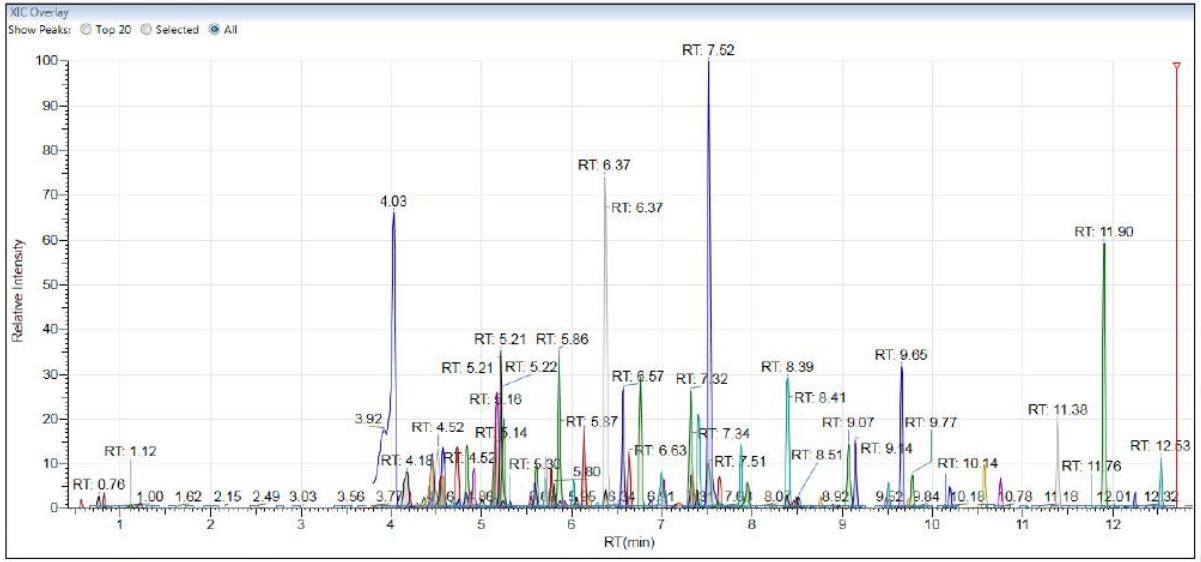


Figure 4: Total extracted ion chromatogram of salmon extract at 1× STC.

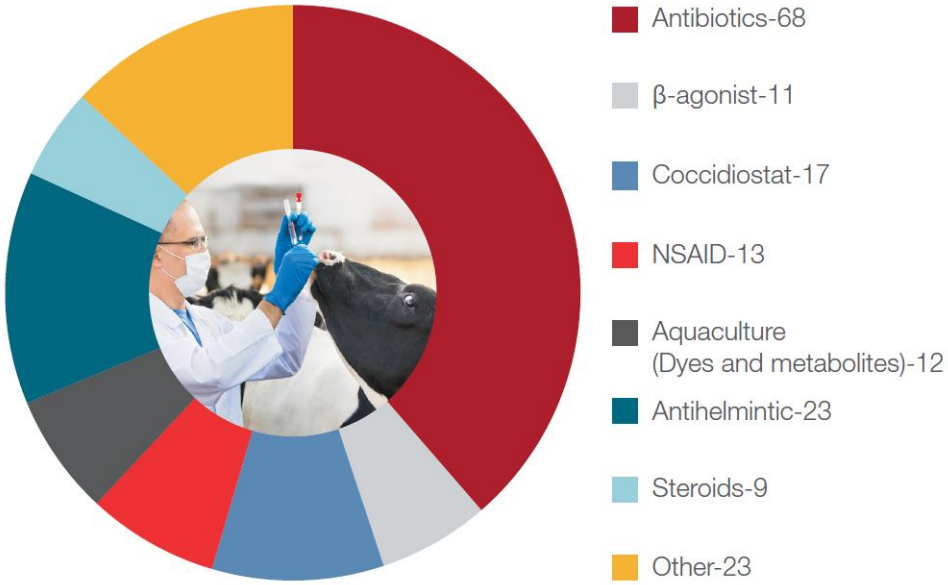


Figure 3: Compound classes with number of analytes evaluated during the development of the VetDrugs Explorer Collection.

QuEChERS

5 g homogenized sample (bovine or fish fillet) in 50 mL extraction tube

↓

Add ammonium oxalate/EDTA solution (0.5 mL)

↓

Add internal standards and acetonitrile to final volume of 15 mL

↓

Add 5 g anhydrous sodium sulphate, vortex

↓

Wait 30 min, centrifuge @ 4500 rpm for 10 min

↓

Decant supernatant, add 500 mg CEC18 dSPE material, shake 15 min

↓

Centrifuge for 5 min at 4500 rpm

↓

Remove 3 mL, add 1 mL H_2O, mix and filter with 0.45 µm PTFE filter

↓

Transfer to autosampler vial and inject 2 µL

Figure 1: Extraction procedure for bovine muscle and salmon (fillet). Further details for milk are included in the VetDrugs Explorer Collection.

Table 2. LC pump gradient.

Time (minutes)	Flow rate (mL/min)	%B
0.0	0.30	2
2.0	0.30	2
3.0	0.30	20
11.0	0.30	100
13.0	0.40	100
14.4	0.40	100
14.5	0.35	2
16.0	0.30	2
17.0	0.30	2

Chromatografické podmienky:

Injection Volume:	2 µL
Column Temperature	40 °C
Analytical Column	Accucore VDX, 100 × 2.1 mm × 2.6 µm
Run Time	17 minutes
Tray Temperature	15 °C
Mobile Phases	A) Water with 0.05% Formic Acid
	B) 50% Acetonitrile 50% Methanol
	5% Water with 0.05% Formic Acid

MS API:

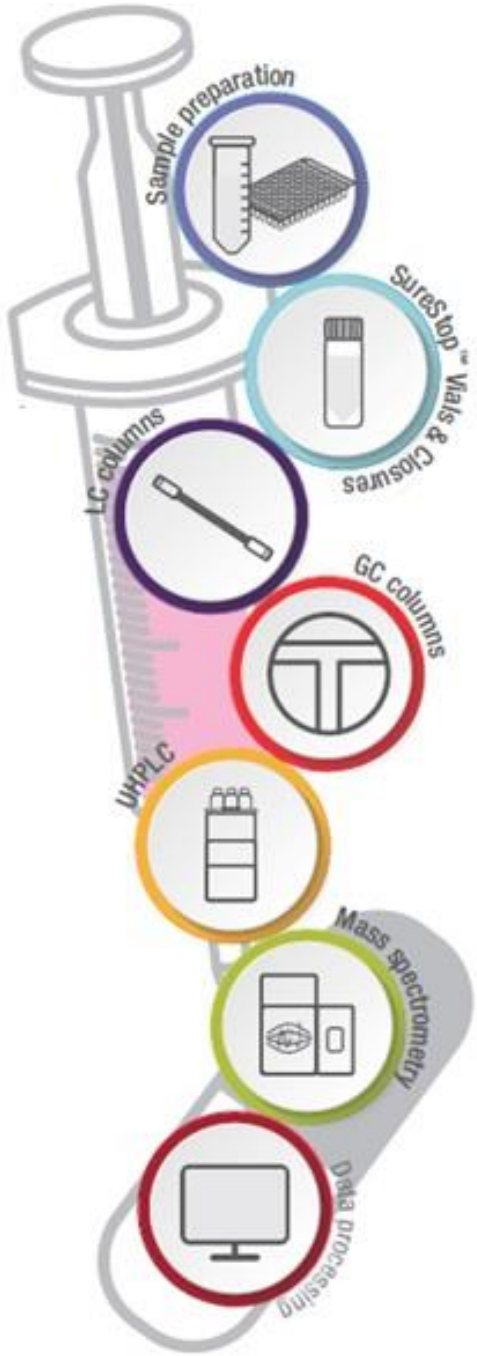
Negative Voltage	2500 V
Positive Voltage	3500 V
Sheath Gas	50 Arb Units
Auxiliary Gas	13 Arb Units
Sweep Gas	1 Arb Unit
Ion Transfer	
Tube Temperature	310 °C
Vaporizer Temperature	350 °C

Forenzná toxikológia

- SOLA™/SOLAμ™ SPE Plates and Cartridges
- HyperSep™ SPE Cartridges and Plates
- HyperSep™ Retain Cartridges
- Accucore™ Biphenyl LC Columns
- Accucore RP-MS LC Columns
- TraceGOLD™ GC Columns and Guard Columns

Výskum – návykové látky

- SOLA/SOLAμ SPE Plates andCartridges
- HyperSep Verify CX Cartridges
- Accucore Biphenyl LC Columns
- Hypersil GOLD™ LC Columns
- TraceGOLD GC Columns



Výskum – liečivá

- SOLA/SOLAμ SPE Plates and Cartridges
- WebSeal Well Plates and Mats
- Accucore™ C18 LC Columns
- Acclaim 120 C18 Columns

Kontrola potravín

- QuEChERS
- Target2™ PTFE Syringe Filters
- Accucore VDX LC Columns

Kontrola vody

- HyperSep Cartridges
- Hypersil GOLD™ aQ LC Columns
- Accucore™ C18 LC Columns

pragolab

Ďakujem za pozornosť!