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Sample Preparation for GC-MS

Dipl.- Ing. Petra Gerhards
Regional Marketing Manager Columns and Consumables, EMEA
Thermo Fisher Scientific, Dreieich/Germany

Sample Preparation

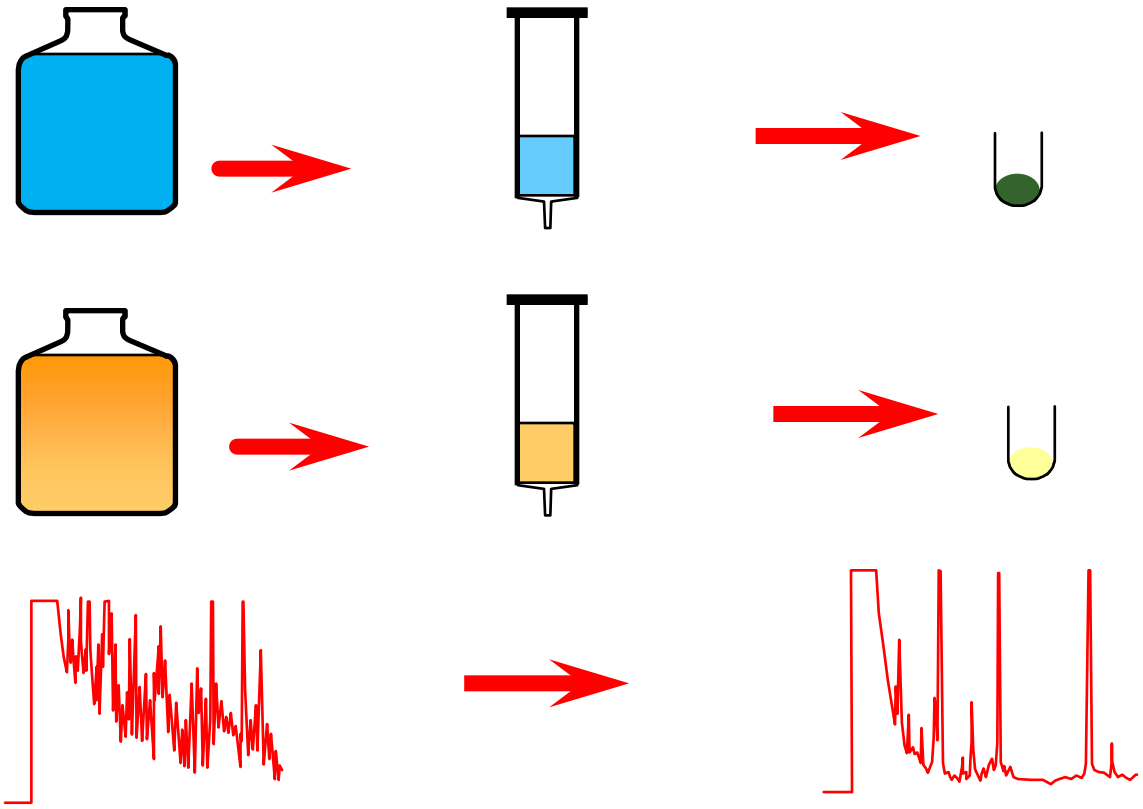
- Objectives of sample preparation
- Types of sample preparation
  - Fundamentals for different sorbents
  - Polymeric Sorbents
  - Silica based Sorbents
- Fundamentals of solid phase extraction (SPE)
- Solid phase extraction products
  - Protocols
  - Real examples

Objectives of Sample Preparation

Concentrate the analyte

Clean up the sample matrix

Analytical compatibility

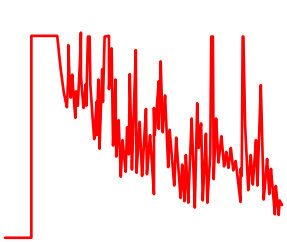


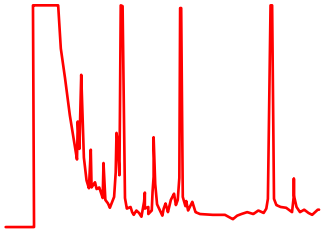
Not for use in diagnostic procedures

Liquid/Liquid Extraction (LLE)

- Does not concentrate the analyte
- Provides a cleaner extract than precipitation
- Analytical compatibility is questionable
  - Note every drug is extracted with the given/chosen solvent for LLE
  - The solvent is always for a large variety of drugs, special drugs like THC require special sample preparation

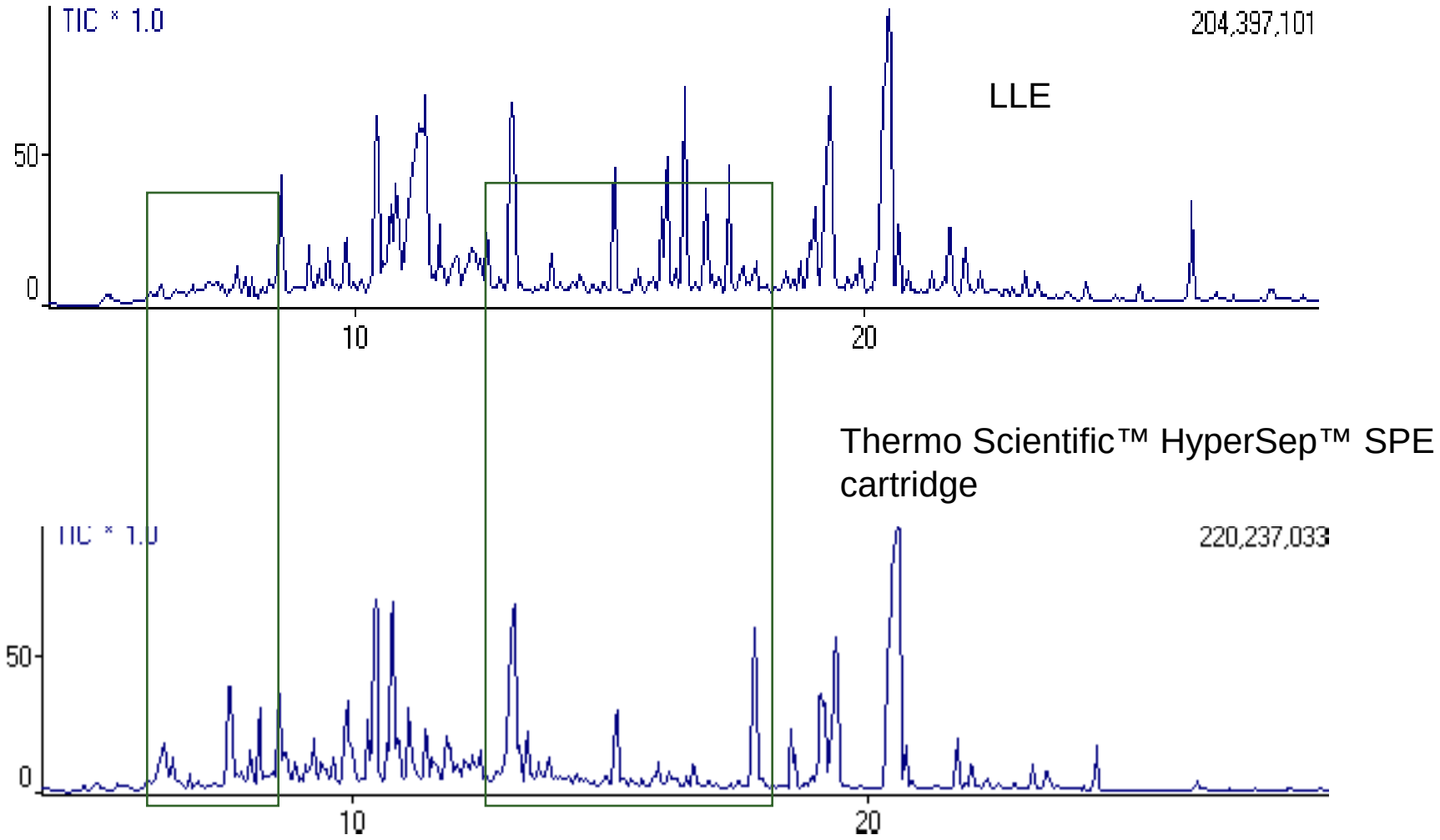
Analytical compatibility





Liquid/Liquid Extraction (LLE) with Chlorobutane - Comparison

LLE extracts a lot of hormones and steroids, SPE is MUCH cleaner!

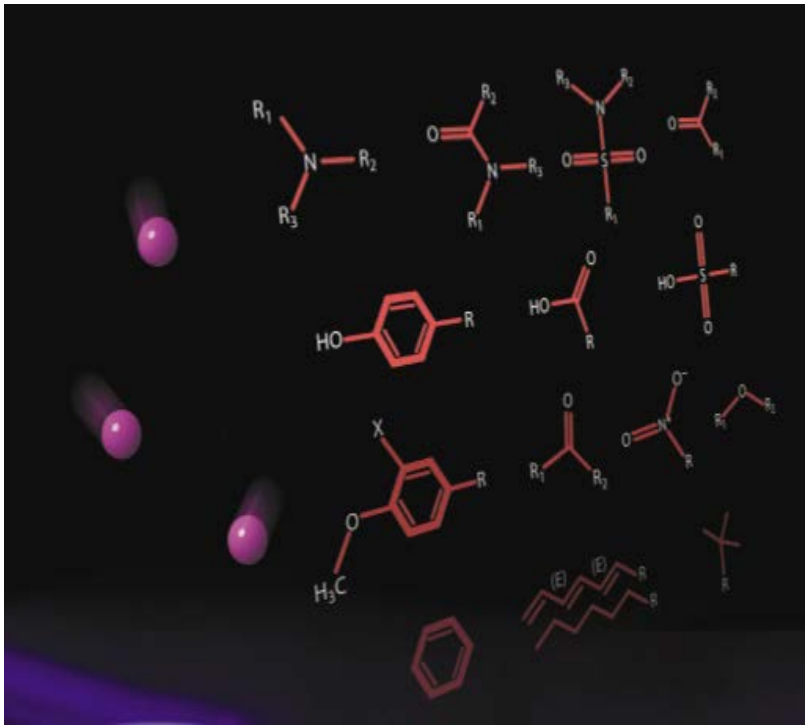


Solide Phase Extraction (SPE)

- Capable of concentrating the analytes
  - Up to 2ml of plasma or 3 ml of urine can be used, compared to a 1:1 extraction with LLE
  - Concentration factor after SPE process is 1:60
- Yields very clean extracts
- Extracts are analytically compatible

SPE Fundamentals

- Analytes, sample Matrix and SPE sorbent
- Chemistry of extraction protocols
- Troubleshooting and technical comments



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SPE Fundamentals

Extraction modes

- Nonpolar
- Polar
- Ion exchange
- Covalent*
- Affinity*

Format considerations

- Sample size
- Processing speed
- Device capacity

* Applies to liquid chromatography

How Does Chemistry in SPE Work?

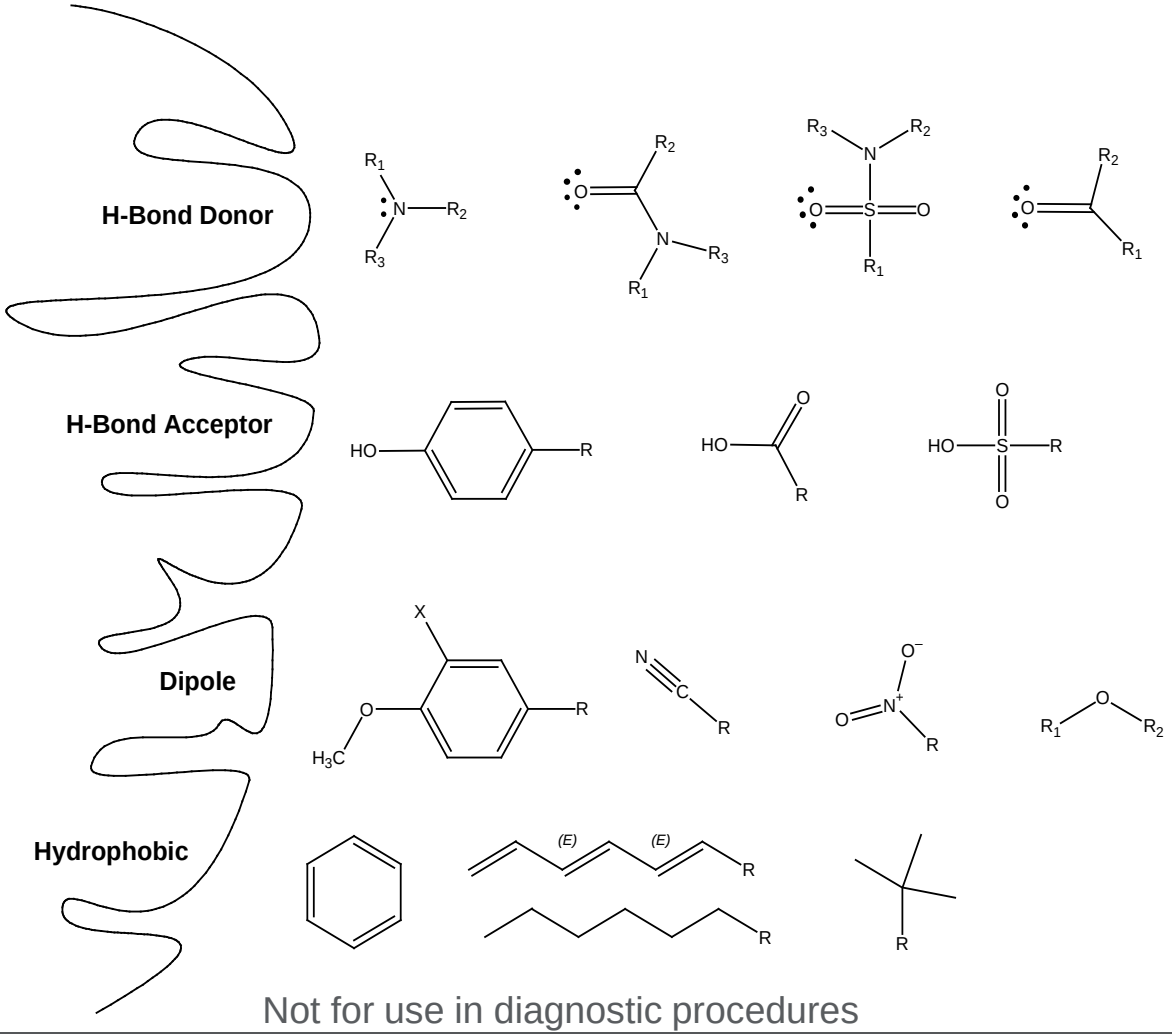
$$\text{Log SP} = c + mV_x + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H$$

$a\Sigma\alpha_2^H$ H-Bond Donor

$b\Sigma\beta_2^H$ H-Bond Acceptor

$s\pi_2^H$ Dipole

rR_2 Hydrophobic



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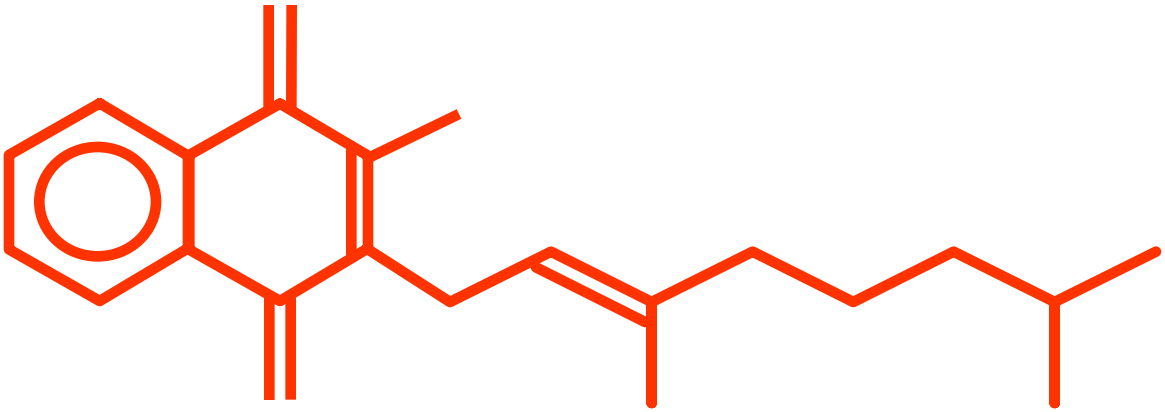
Analyte Fundamentals

- Polarity
- Metabolism
- Concentration
- Stability



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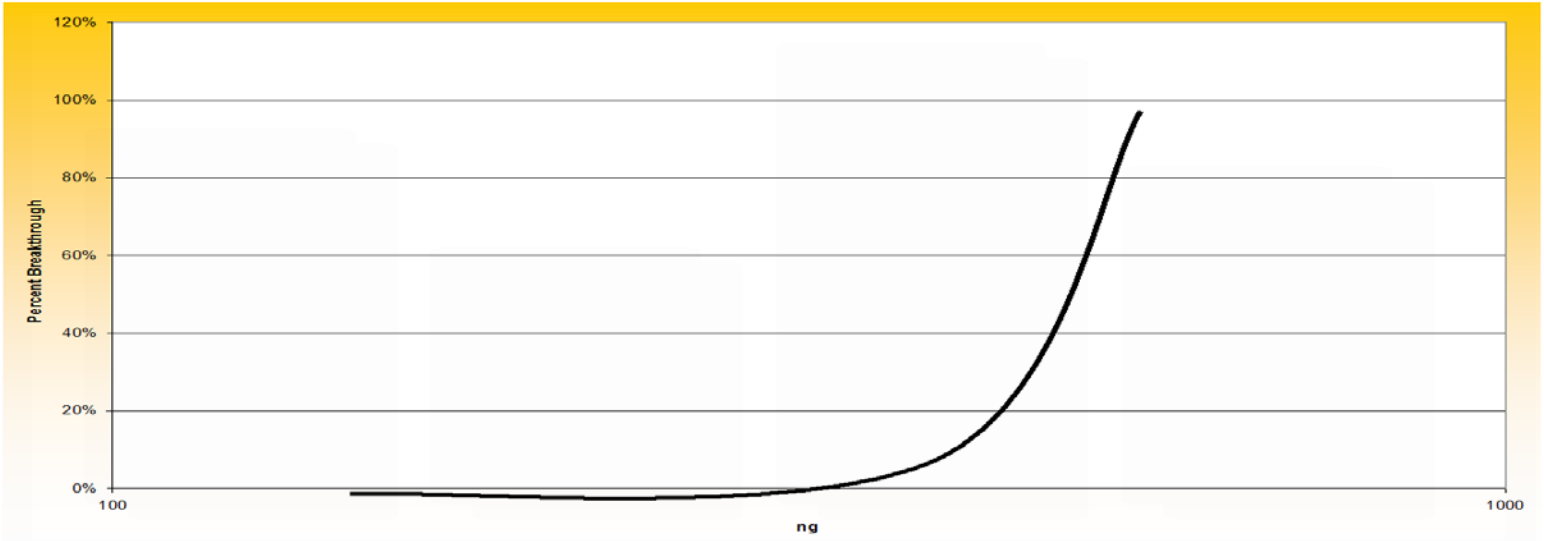
Concentration

- What is the maximum concentration expected?
- What is the capacity of sorbent?
- Are there any matrix interferences?



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Sorbent Capacity

- Capacity is dependent on sorbent type, bed volume and chemistry
- General rule is 4 – 10% of the bed volume on non-polar chemistries
- Certificates of analysis generally indicate sorbent capacity

Thermo Scientific™ HyperSep™ and SOLA™ SPE Sorbents

- Silica based → HyperSep cartridges
- Polymeric based → SOLA cartridges

$$\text{Log SP} = c + mV_x + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H$$

Not for use in diagnostic procedures

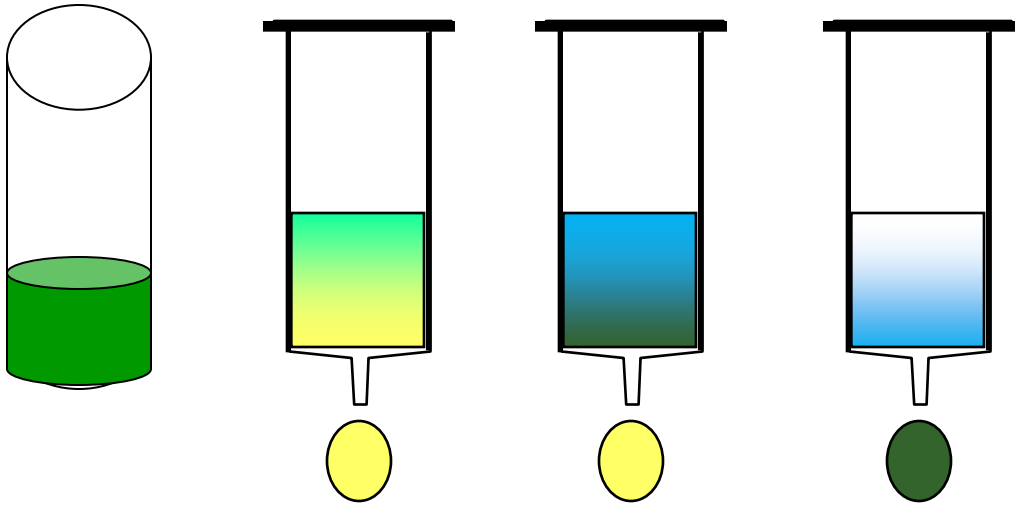
Silica Based Sorbents

- Non-polar (Hydrophobic) phases
  - C18(ec), C18, C8, C4 etc.
- Polar and ion-exchangers
  - CN, Phenyl, SAX, NH2, SCX, SI
- Mixed phases
  - C8 / SCX

Not for use in diagnostic procedures

Troubleshooting

- Sample pre-treatment → Right pH?
- Conditioning → Is the flow slow enough to activate the sorbent?
- Sample application → pH correct, flow correct?
- Washing → Break through?
- Elution → Solvents correct, enough elution steps?



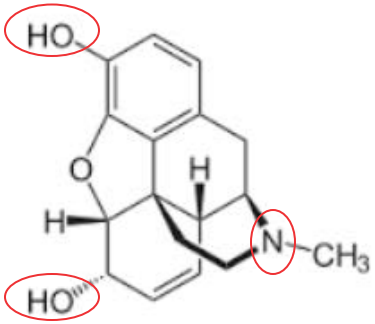
HyperSep Based On Cation Exchanger Mechanism

If the substance contains NH or NH_2 or OH groups

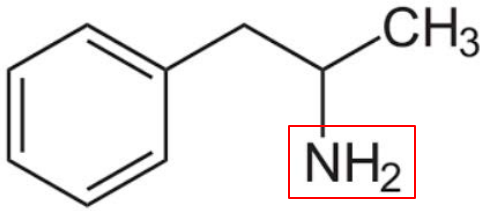
- Recommend HyperSep Verify, SCX or WCX will work for these functional groups
- If the substance is a little bit more polar (containing OH groups), recommend SCX
- If the substance is a little bit more non polar, recommend WCX

For ion exchange mechanisms the target is to charge the functional groups.

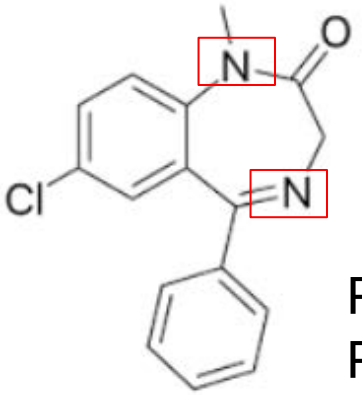
Basic Drugs, Where Charges Can Be Applied



Morphine → SCX or Verify



Amphetamine → WCX or Verify



Diazepam → WCX or Verify

For a broad extraction of Drugs of Abuse → Verify
For a specific extraction of groups → SCX or WCX

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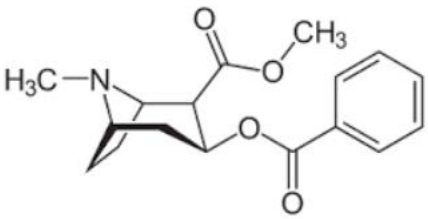
Zwitterions

If a compound contains NH or NH_2 plus CO, OH or COOH groups recommend HyperSep WCX if the NH or NH_2 group is not steric hindered.

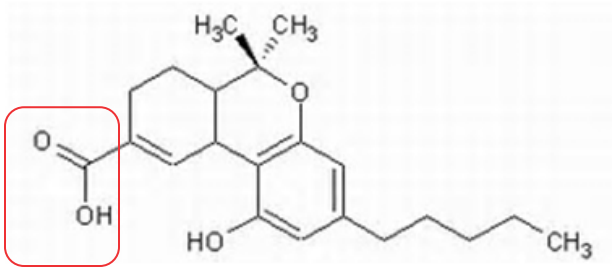


Steric hindered N in Ecgonine Alakloide from Cocain and Benzoylecgonine, main metabolite

If it is steric hindered, use HyperSep C18 or HyperSep C18 acid wash, C8 can also be an option.

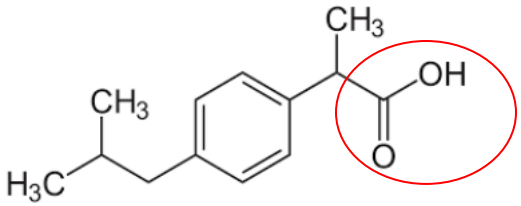
A method development set would be a good choice, containing HyperSep C18, C8 and Verify.

Acidic Drugs



THC carboxylic acid
More hydrophobic interaction

Mostly hydrophobic → C18
For GC sample has to be evaporated and derivetized and reconstitution is done on Hexan: Ethylacetate 75:25

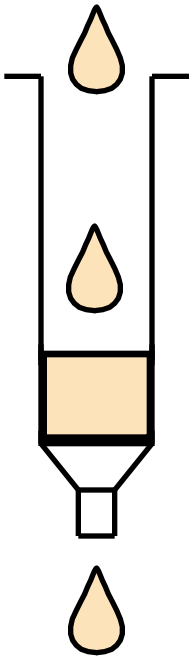


Ibuprofen, acidic drug → C18 or Verify AX

Cation Exchanger SOLA 10mg/1ml For Plasma Generic Protocol

Low volumes only!

Condition



400 µl Methanol
let it react for 2 min.

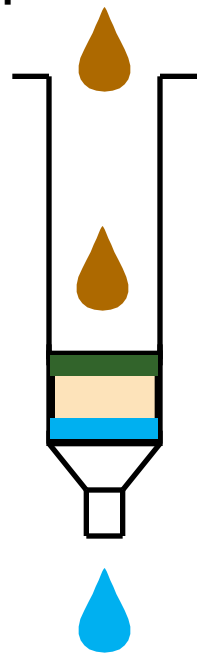
Equili. with

400 µl 0.1 M acetic acid

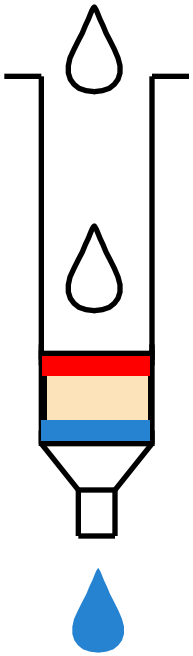
200 µl plasma dilt.

1:1 with dist. water,

add 20 µl acetic acid, pH 4



Interference-elution

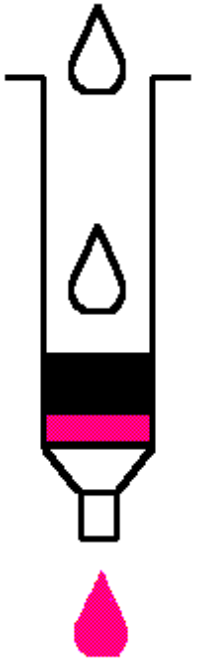


400 µl 0.1 M acetic acid

200 µl methanol

Dry with strong vacuum 2 min.

Elution



2 x 200 µl

NH_3/methanol

1 : 9

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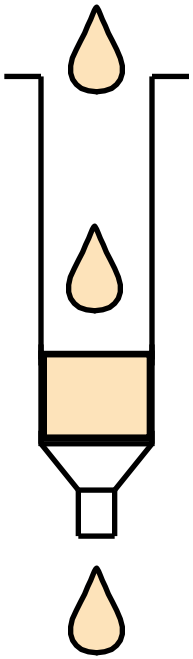
SOLA HRP 10mg/1ml For Plasma THX Example

Condition



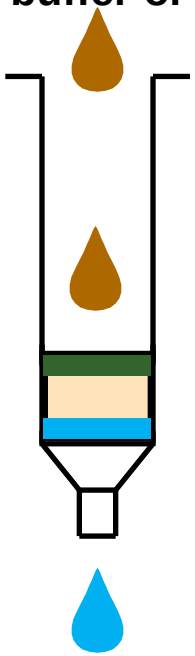
400 µl Methanol
let it react 2 min.

Followed by 400 µl neutral buffer

Ammonia acetate, in some cases water will be enough

200 µl plasma dilt.

1:1 with buffer or dist. Water ,

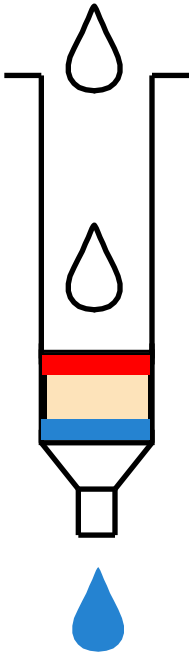


400 µl buffer + 3 % isopropanol

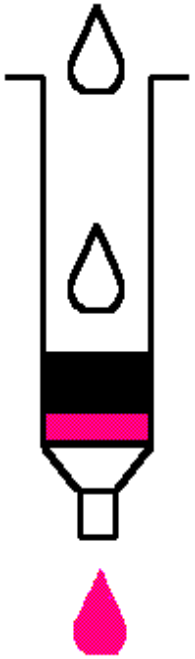
400 µl buffer

Dry with strong vacuum 2 min.

Interference-elution



Elution



2 x 200 µl

Buffer * + ACN

Evaporate, derivetize and reconstitute in

Hexan 75:Ethylacetate 25

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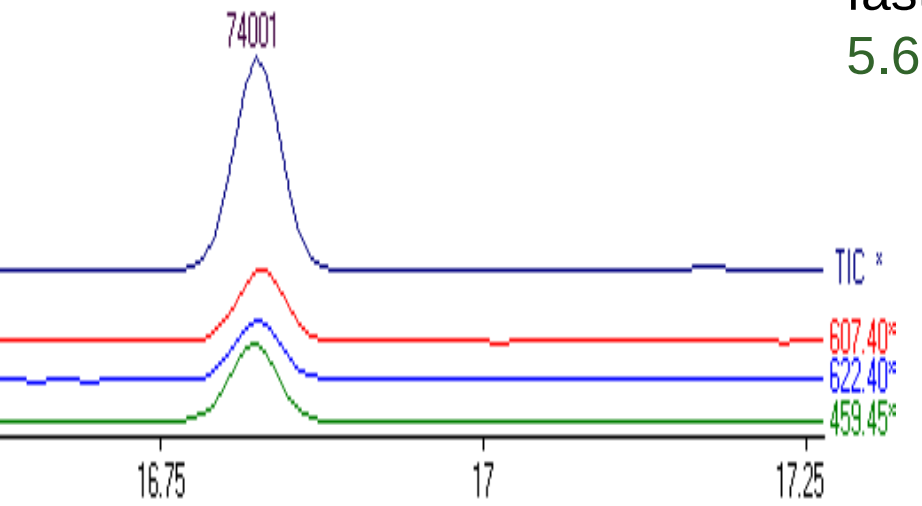
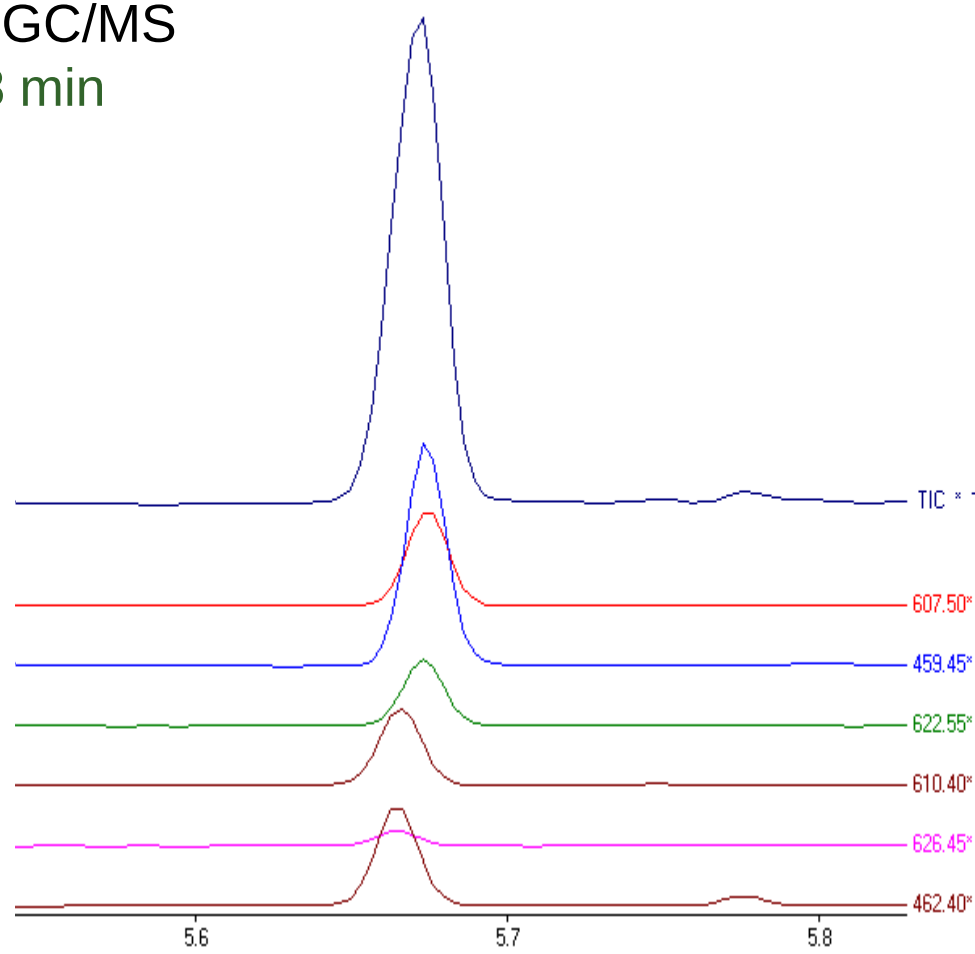
THC Sample After Derivatization



Urine sample
normal GC/MS
16.78 min

Deuterated standard
fast GC/MS
5.68 min



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HyperSep Verify 200 mg For Urine Generic Protocol

3 Ml urine +30 µl

ß-Glucuronidase

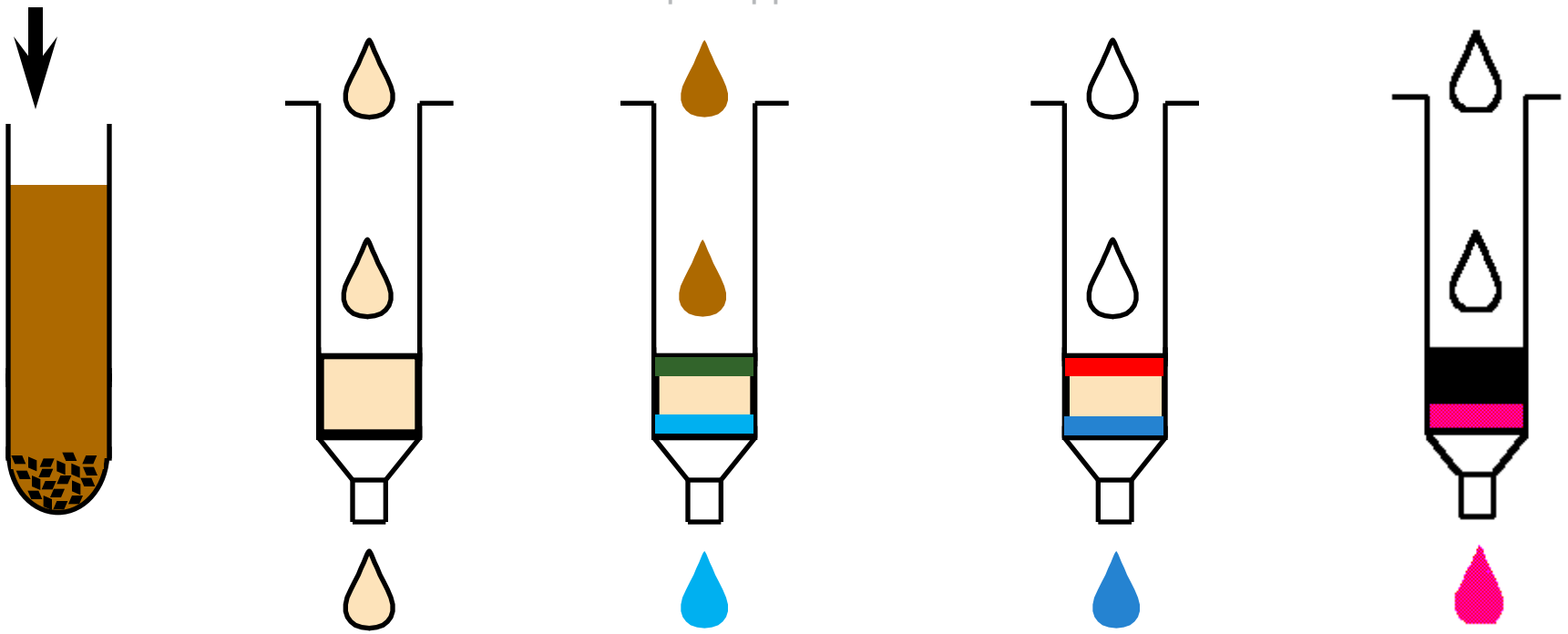
Incubate at 56 ° C for 60 min

Analyte elution with

2 x 2 ml

NH_3/methanol

Sample application



Conditioning with 3ml methanol

pre-equilibration with

diluted phosphoric acid

Interference elution with 3 ml

Diluted phosphoric acid, 0.5 ml 0.1 N acidic acid and

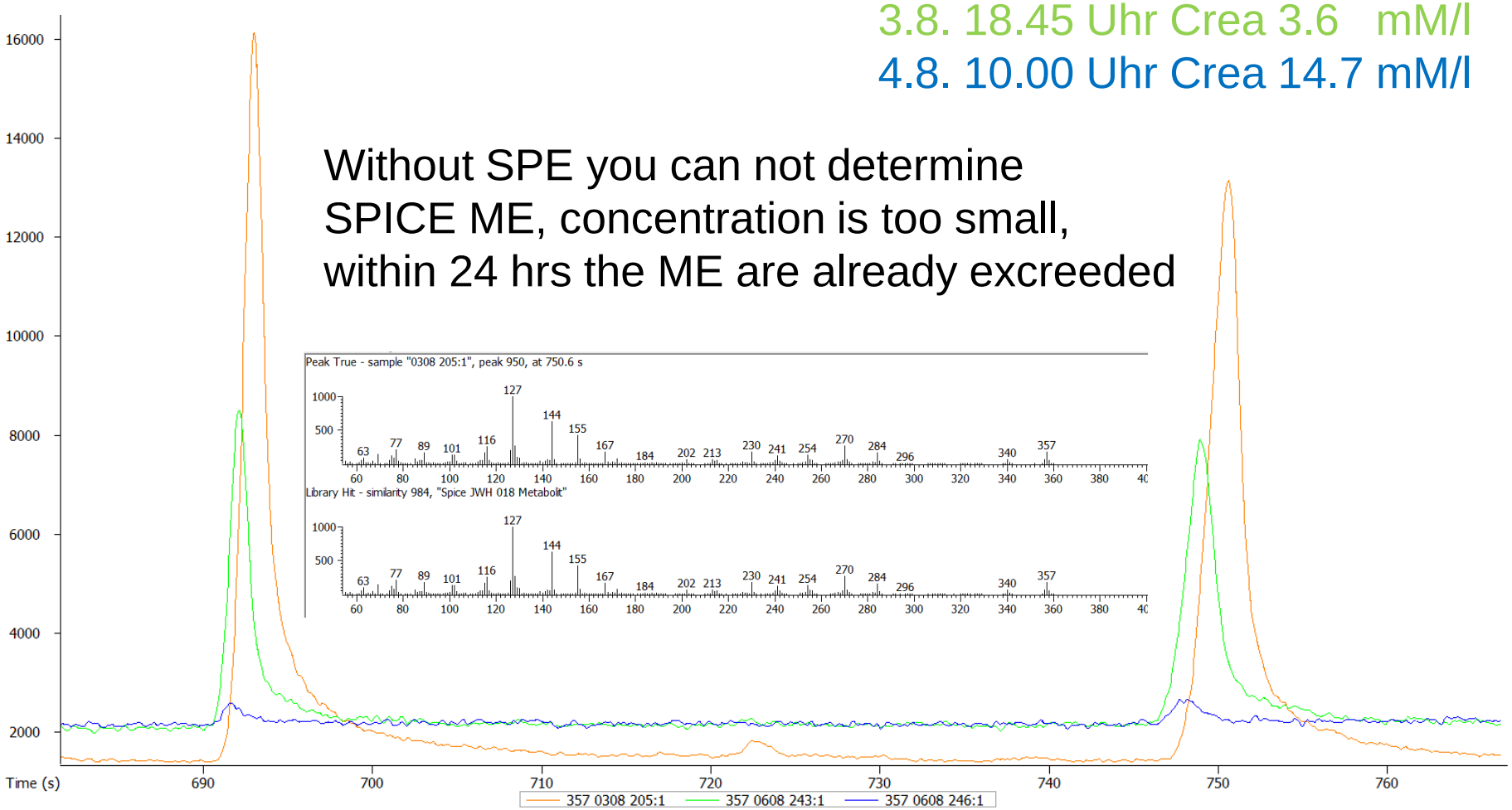
1ml methanol

Not for use in diagnostic procedures

SPICE Urine WITH ME OF Synthetic Cannabinoides

3.8. 11.20 Uhr Crea 45.2 mM/l
3.8. 18.45 Uhr Crea 3.6 mM/l
4.8. 10.00 Uhr Crea 14.7 mM/l

Without SPE you can not determine SPICE ME, concentration is too small, within 24 hrs the ME are already excreeded



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Any Questions?

Do you want to receive detailed SPE protocols?
We have protocols ready for drugs of abus analysis.
Others could be assembled upon request.
Please send an e-mail to
petra.gerhards@thermofisher.com and specify
if required for GC or LC.

Do you have additional questions
or do you want to talk to an expert from Thermo Fisher Scientific?
Please send an E-Mail to analyze.eu@thermofisher.com
and we will get back to you.