



Sveučilište u Zagrebu

PRIRODOSLOVNO-MATEMATIČKI FAKULTET
Kemijski odsjek
Poslijediplomski sveučilišni studij - Analitička kemija



Pregled analitičkih tehnika u analizi farmaceutika u bioti

R. Álvarez-Ruiz i Y. Picó, Trends Environ. Anal. Chem. 25 (2020) e00082

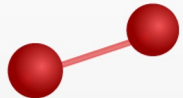
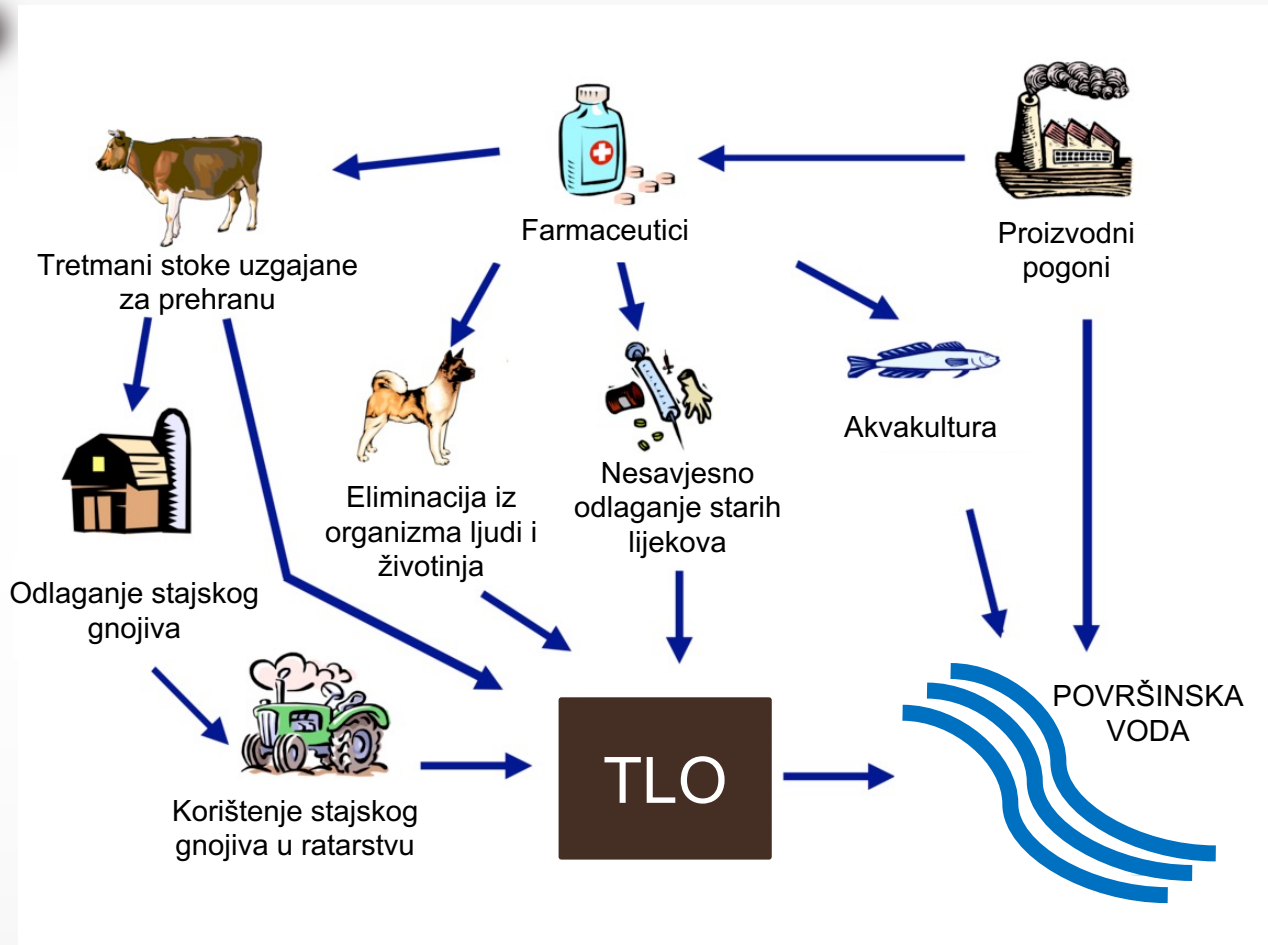
Klaudija Ivanković, mag. appl. chem.

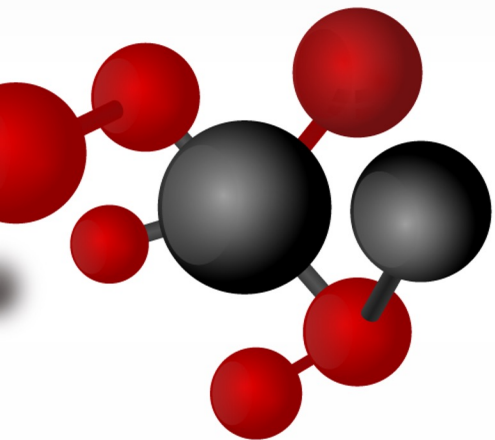
Institut Ruđer Bošković

Zavod za istraživanje mora i okoliša,

Laboratorij za analitiku i biogeokemiju organskih spojeva

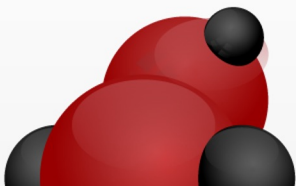




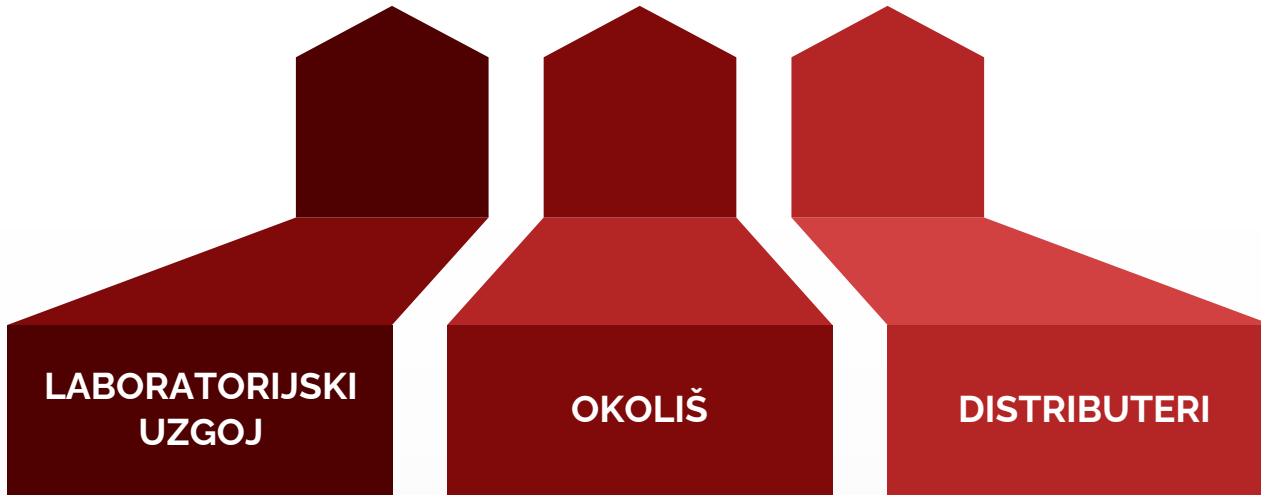


01

**PRIKUPLJANJE I PRIPREMA
UZORKA**



PRIKUPLJANJE UZORAKA



- bioakumulacija, toksičnost, opći utjecaj zbog izloženosti

- stvarna izloženost biote u određenom području

- ljudska izloženost putem prehrane



PRIPREMA UZORAKA



HOMOGENIZACIJA

- analiza cijelog tijela ili više jedinki
- ČVRSTI UZORCI:
usitnjavanje

LIOFILIZACIJA

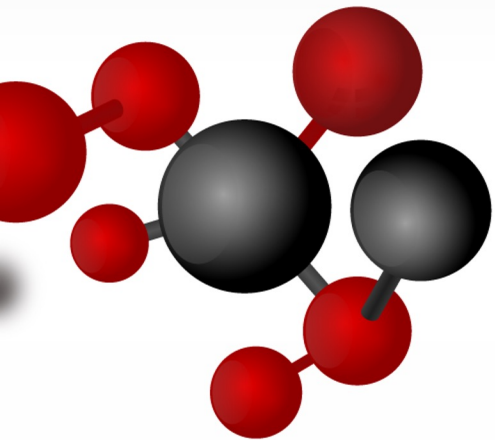
- eliminacija vode

CENTRIFUGIRANJE

- dobivanje plazme iz krvi
- taloženje proteina

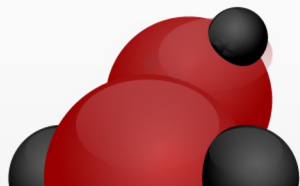
DODATAK β - GLUKURONIDAZE

- hidroliza glukuronida



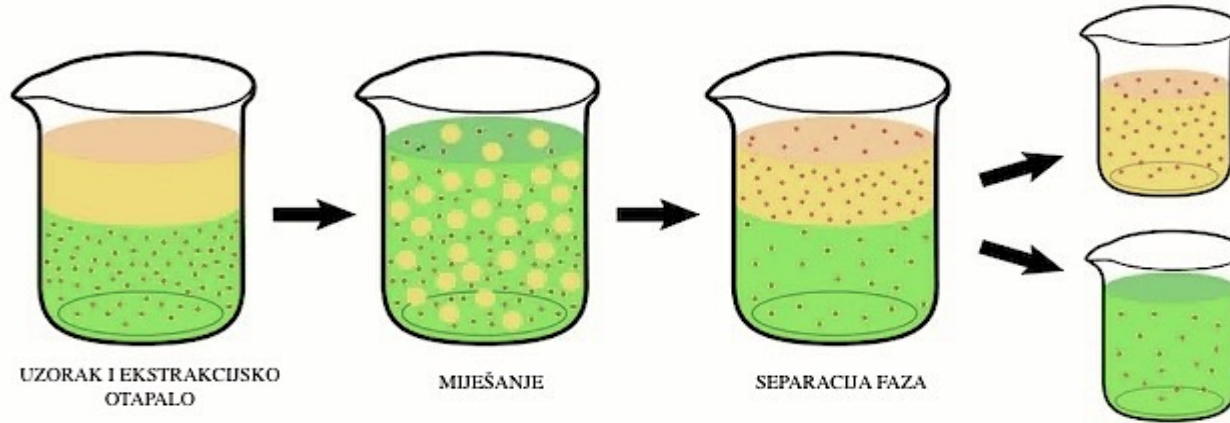
02

EKSTRAKCIJA FARMACEUTIKA





EKSTRAKCIJA TEKUĆE-TEKUĆE (LLE)



ANALIZIRANA BIOTA



Plazma
ribe



Jetra ribe



Mozak
ribe



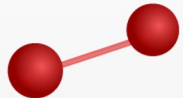
Beskralježnjaci



Meko tkivo
školjke



- Aceton
- Acetonitril
- Metanol
- Smjesa otapala
- Dodatak kiseline ili baze





EKSTRAKCIJA NA ČVRSTOJ FAZI (SPE)

ANALIZIRANA BIOTA



Plazma i
žuč ribe



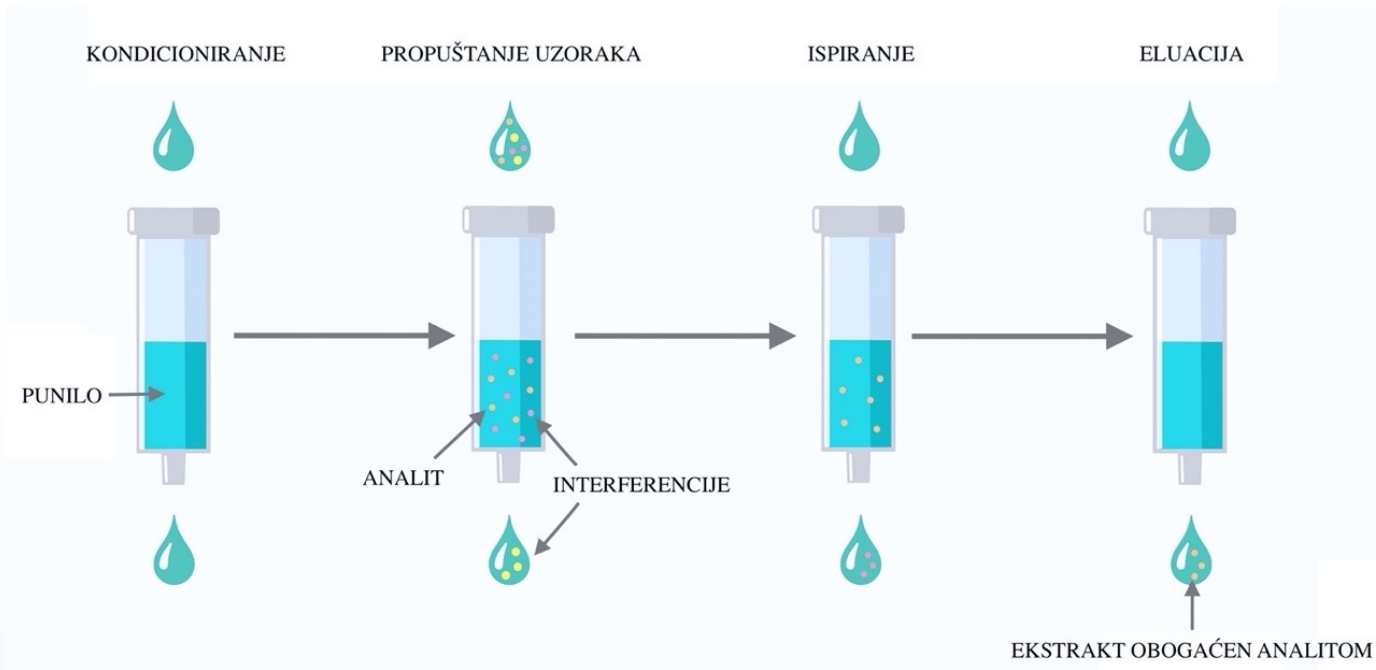
Jetra ribe



Mozak
ribe



Beskralježnjaci





“Quick Easy Cheap Effective Rugged Safe”

EKSTRAKCIJSKE SOLI

- Magnezijev sulfat
- Natrijev klorid
- Acetatni pufer
- Citratni pufer

ANALIZIRANA BIOTA



Ribe



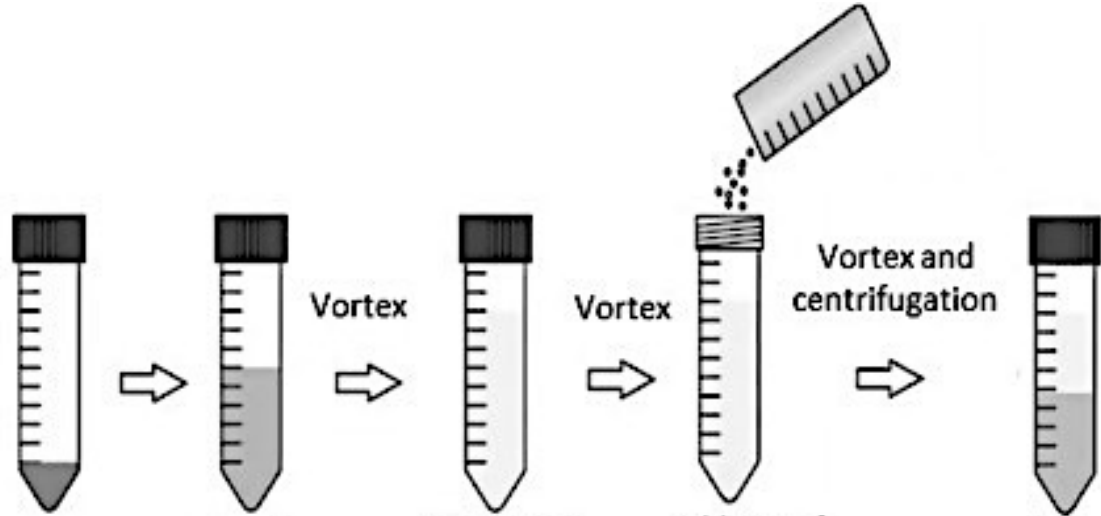
Biljke



Beskralježnjaci

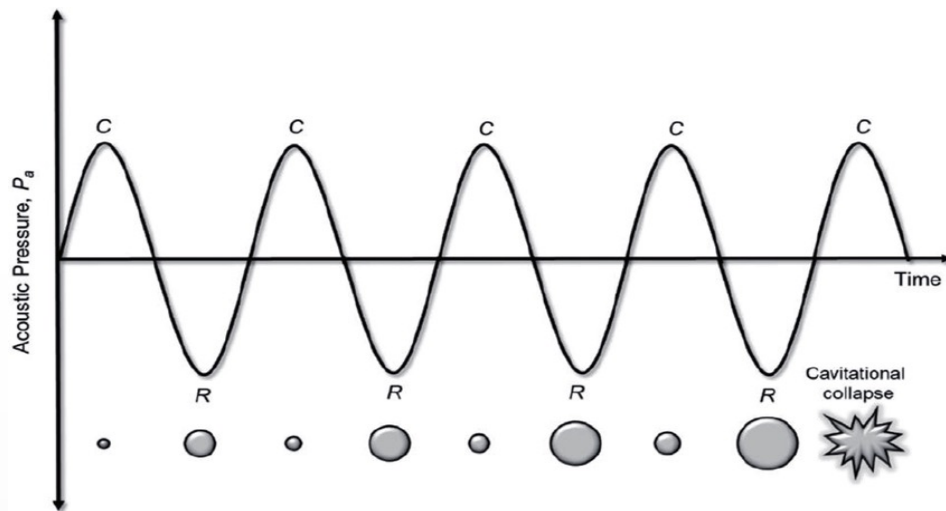
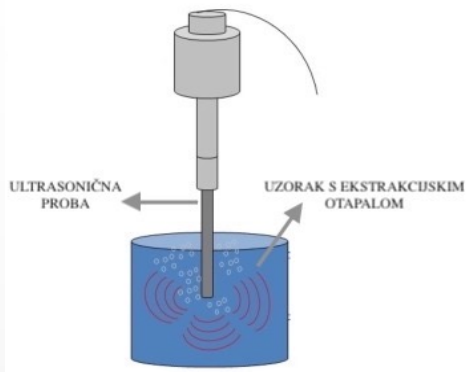
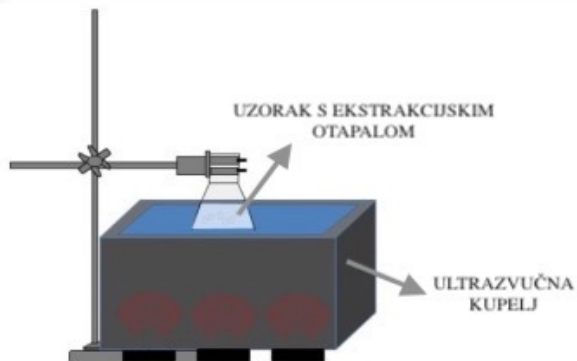


Meko tkivo
školjke





EKSTRAKCIJA POTPOMOŽNUTA ULTRAZVUKOM (USE)



ANALIZIRANA BIOTA



Razna
tkiva ribe



Biljke



Beskralježnjaci



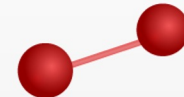
Meko tkivo
školjke

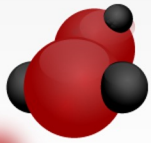


Ptice

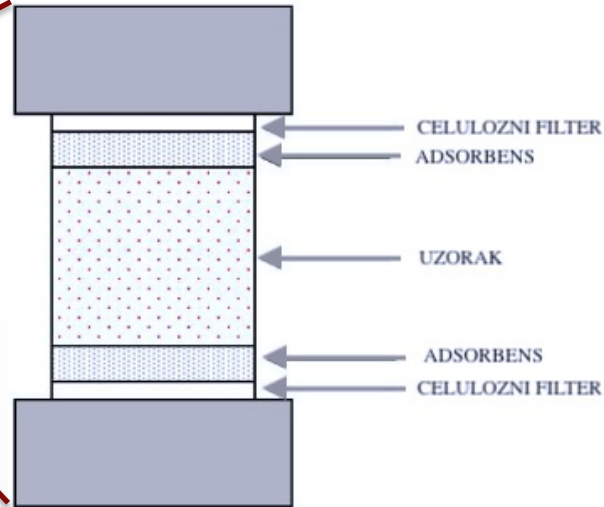


Alge





EKSTRAKCIJA POTPOMOŽNUTA VISOKIM TLAKOM (PLE)



ANALIZIRANA BIOTA



Razna
tkiva ribe



Biljke



Beskrležnjaci



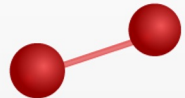
Meko tkivo
školjke



Ptice



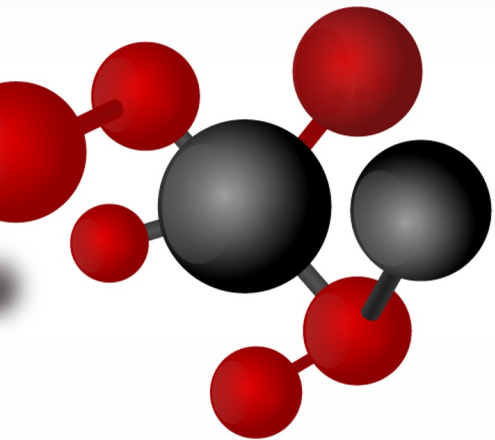
Planktoni





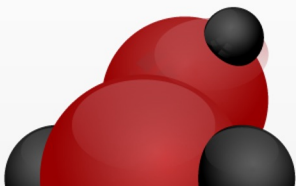
TEHNIKA	JEDNOSTAVNO	BRZO	JEFTINO	"ZELENO"	PONOVLJIVOST	ANALITIČKI POVRATI
LLE	✓	✗	✓	✗	✗	
SPE	✓	✗	✗	✓	✓	
QuEChERS	✓	✗	✓	✓	✗	?
USE	✓	✗	✓	✗	✗	
PLE	✓	✓	✓	✓	✓	

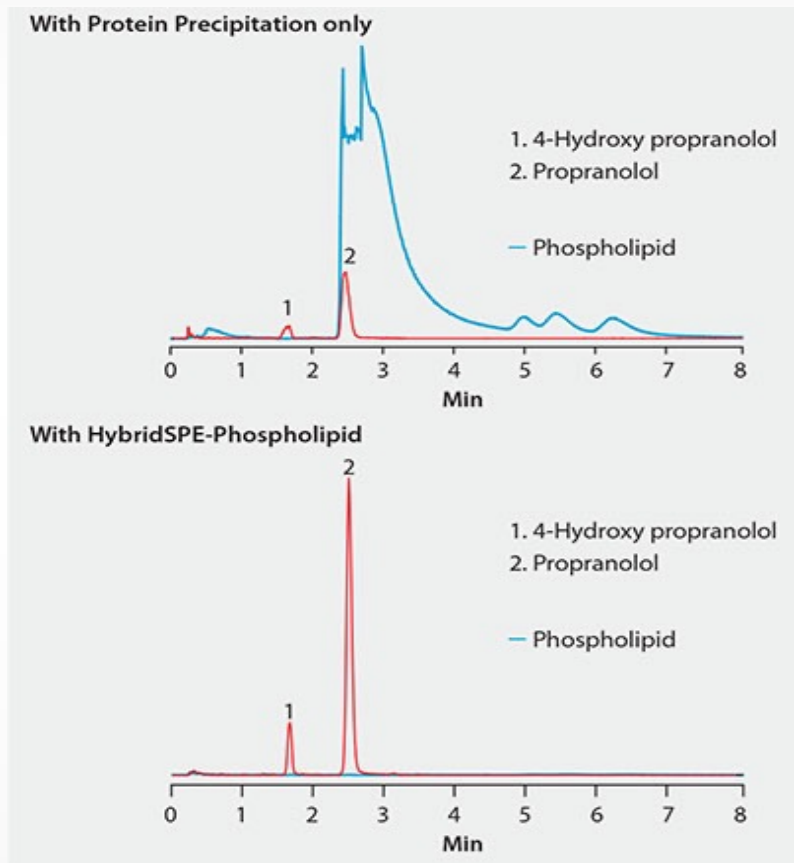




03

**PROČIŠĆAVANJE I
UKONCENTRIRAVANJE**







1. Kolonska kromatografija

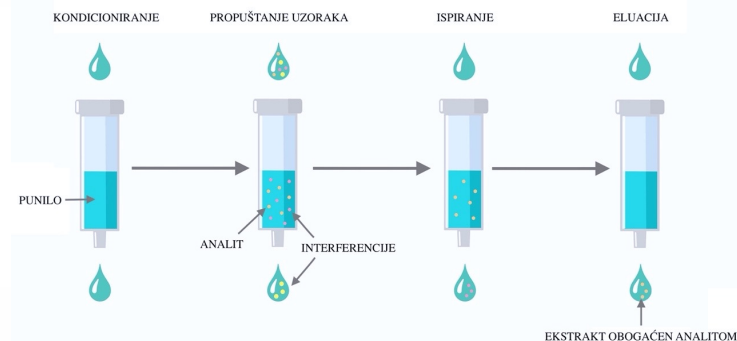
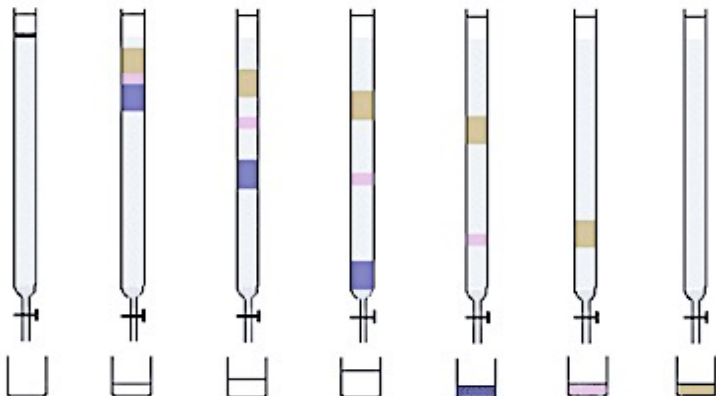


2. GPC

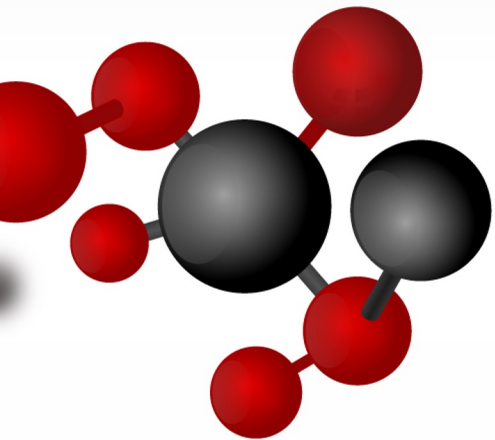


3. (d)SPE

- Polimerni gel, silika gel, aluminijev oksid
- Potrebno ukoncentriravanje

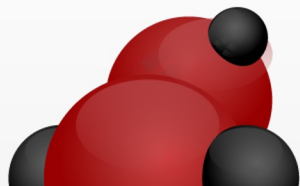


- hidrofilne i lipofilne interakcije (Oasis HLB)
- kationski izmjenjivači (Oasis MCX)
- anionski izmjenjivači (SAX)
- π - π , dipol-dipol i hidrofobne interakcije (Strata X)
- primarne i sekundarneaminske skupine (PSA)



04

INSTRUMENTNA ANALIZA

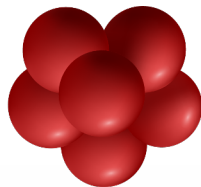




KROMATOGRAFSKA SEPARACIJA I MASENOSPEKTROMETRIJSKA DETEKCIJA

91%

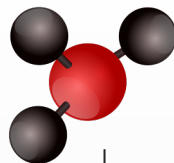
LC-MS



- Kromatografija obrnute faze
- HPLC ili UPLC
- Elektroraspršenje
- Trostruki kvadrupol (QqQ)

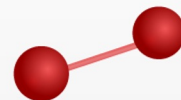
- Analizator s vremenom preleta spregnut s kvadrupolom (QTof) i Orbitrap

- Kemijska ionizacija ili ionizacija ubrzanim elektronima
- Kvadrupol (Q)
- **Derivatizacija**



9%

GC-MS



IDENTIFIKACIJA

LC ili GC sustav
Separacija analita na osnovi afiniteta prema mobilnoj/stacionarnoj fazi

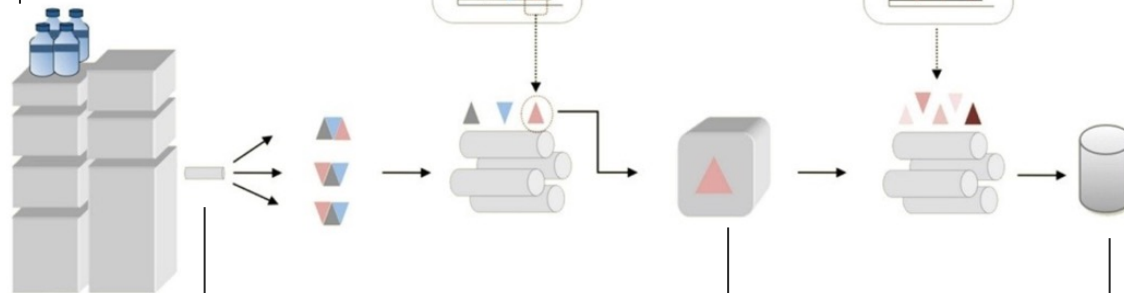
Analizator
Separacija roditeljskog spoja od ostalih prisutnih spojeva

Fragmentacija
praćenje odabranih fragmenata roditeljskog spoja koji su specifični i selektivni

Ionizacija
Separirani analiti se prevode u plinsku fazu i ioniziraju

Roditeljski spoj

Detektor

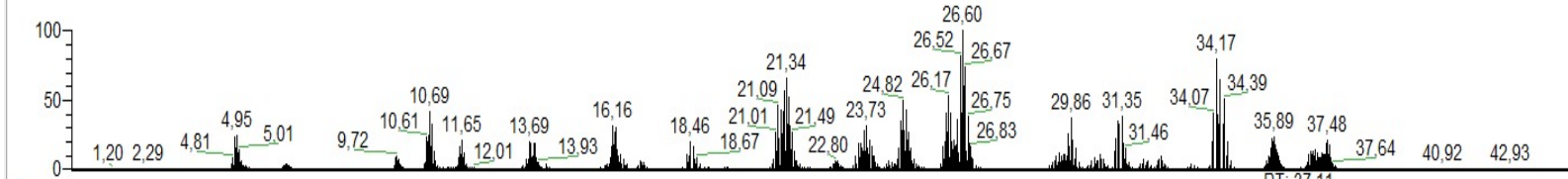


KVANTIFIKACIJA

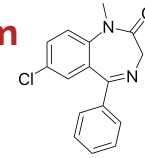


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NL: 3,09E7
TIC MS SED_metodaE_01

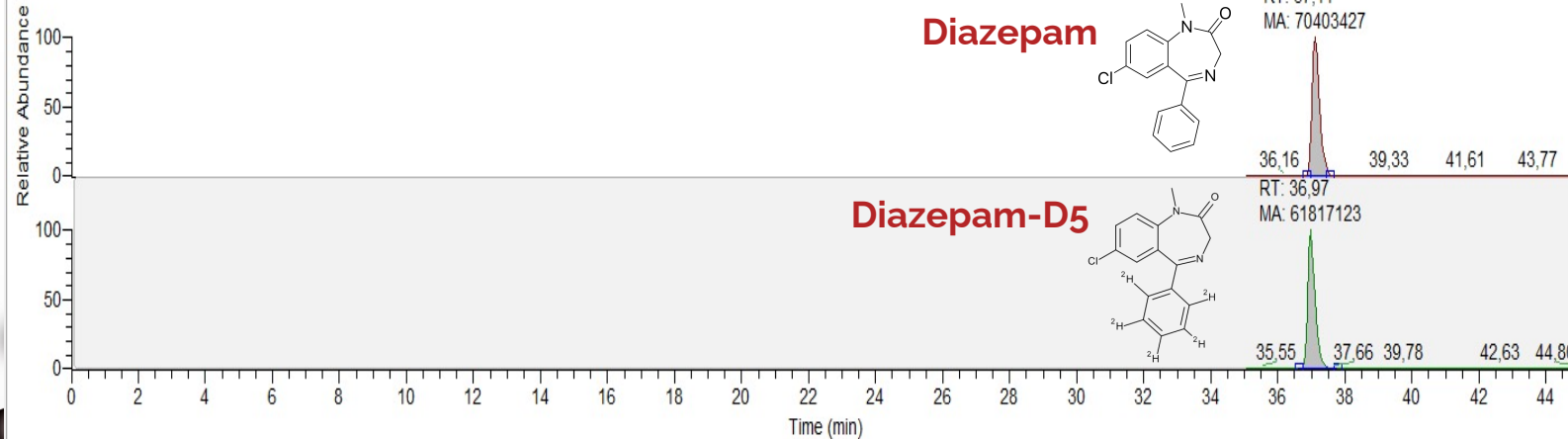


Diazepam

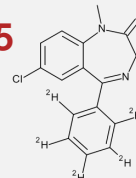


RT: 37,11
MA: 70403427

NL: 4,53E6
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SRM ms2
285,100@cid28,00
[192,508-193,508] MS
SED_metodaE_01

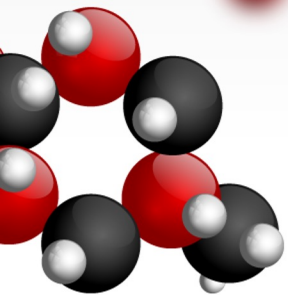


Diazepam-D5



RT: 36,97
MA: 61817123

NL: 4,04E6
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SRM ms2
290,100@cid32,00
[197,536-198,536] MS
SED_metodaE_01



**ZAHVALJUJEM
NA PAŽNJI!**

