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# LAB-IN-SYRINGE AUTOMATED DISPERSIVE LIQUID-LIQUID EXTRACTION OF ANTIBIOTICS
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# A XC9+ Syringe pump is used upright, the piston distance is 30 mm, syringe size is 2.5 mL
# Ports on the syringe pump head valve are used for:
# 1 HPLC // 2 Standard // 3 NADES // 4 Buffer
# 5 SAMPLE // 6 WATER // 7 Isopropanol for cleaning // 8 AIR // 9 WASTE
# A Trinket M0 chip is used to control a stirring motor via pin 0
# It is activated by sending 'R' plus the duty cycle percentage (0-99)
# COM13: Syringe pump, COM23: Trinket, COM10: Autosampler
# H HPLC, V valve R speed
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```
VolSample = 1700
VolNADES = 200
VolAir = 50
VolBuffer = 200
```

```
Speed = 20 #99 #70 earlier #60
Time_Extraction = 180 #30
Time_Separation = 240 #20
depth = 115
rack = 2
SamplePos = 1
Routine 'AIM')
```

```
# ===== DLLME
# 1 HPLC // 2 Standard // 3 NADES // 4 Buffer
# 5 SAMPLE // 6 WATER // 7 Isopropanol for cleaning // 8 AIR // 9 WASTE
```

```
If( Option_DLLME == 1)
# First position on autosampler with standard
StartPos = 2
# Number of samples to be measured
No_positions = 15
```

```
# Cleaning of needle in water, position 1
depth = 115
rack = 2
SamplePos = 1
Routine 'AIM')
```

```
SamplePos = StartPos
# Loop for number of solutions to be measured
Loop(No_positions)
```

```

# Cleaning the autosampler tube with new solution
rack = 2 ##### Changed
Routine 'AIM')
Routine 'CleanAutosampler')

##### ADDED
#Loop(5) # Optimization parameter change VolNADES (100, 150, 200, 250, 300)

# Loop of method repetition for the same sample
Loop(3)

# Cleaning of syringe with isopropanol
Routine 'CleanSyringe' once with 500 µL from head valve port 7
Routine 'CleanSyringePlusTransferLine' once with 200 µL from head valve port 7

# Cleaning the syringe with water
VolClean = 1000
ValvePos = 6
Repeat = 1
Routine 'CleanSyringe')
VolClean = 500
Repeat = 1
Routine 'CleanSyringePlusTransferLine')

# Cleaning the syringe with the new sample
Routine 'AIM')
VolClean = 500
ValvePos = 5
Routine 'CleanSyringe')

#### EXTRACTION =====
### 1 HPLC // 2 Air // 3 NADES // 4 Buffer
### 5 SAMPLE // 6 WATER // 7 Isopropanol for cleaning // 8 AIR // 9 WASTE

Aspirate VolSample µL at 20000 µL/min from head valve port 5      Aspiration of sample
Wait 1 s

# Stirring ON
Send_serial(port = 'COM23', message = 'R15\r')
Aspirate VolBuffer µL at 10000 µL/min from head valve port 4      Aspiration of buffer
Wait 0.5 s
Aspirate 25 from head valve port 8                                Aspiration of air
Aspirate VolNADES at 5000 µL/min from head valve port 3          Aspiration of NADES
Wait 3 s

```

Aspirate VolAir μL at 20000 $\mu\text{L}/\text{min}$ from head valve port 8
Send_serial(port = 'COM23', message = 'R' + str(Speed) + '\r')
Wait Time_Extraction s

Aspiration of air

Extraction time

Send_serial(port = 'COM23', message = 'R15\r')
Wait 2 s

Phase separation

Send_serial(port = 'COM23', message = 'R0\r')

Wait Time_Separation)

If(OptionUseHPLC == 1)

Store some extract in transfer line

Send_serial(port = 'COM23', message = 'V1\r') # LOAD

Wait 1 s

Dispense 240 μL at 2000 $\mu\text{L}/\text{min}$ to head valve port 1

Wait 2 s

Trigger HPLC and switch injection valve

Send_serial(port = 'COM23', message = 'V1\r')

LOAD

Wait 3 s

Send_serial(port = 'COM23', message = 'H1\r')

Wait 2 s

Send_serial(port = 'COM23', message = 'V0\r')

INJECT

Wait 9 s

Send_serial(port = 'COM23', message = 'H0\r')

If_end()

Empty syringe at 20000 $\mu\text{L}/\text{min}$ to head valve port 9
syringe content

Discharge remains of

Wait 6.5 min

Waiting time for HPLC -

LIS synchronization

Loop_end()

Changing the optimization parameter

#VolNADES += 50

#Loop_end()

SamplePos = SamplePos + 1

Loop_end()

If_end()

===== Routines

If(Option_Store_System_Clean == 1)

VolClean = 700

ValvePos = 6

Repeat = 1

Routine 'CleanSyringe')

ValvePos = 7

```
Repeat = 2  
Routine 'CleanSyringe')  
If_end()
```

Routine define 'CleanTube')

Aspirate VolClean μL at 15000 $\mu\text{L}/\text{min}$ from Cavo_XR.valve(ValvePos)

Wait 0.5 s

Empty syringe at 25000 $\mu\text{L}/\text{min}$ to head valve port 9

Waste

Routine_end()

Routine define 'CleanSyringePlusTransferLine')

Loop(Repeat)

Cavo_XR.valve(ValvePos) # Choose water or cleaning solvent

Send_serial(port = 'COM23', message = 'R99\r') # Stirrer on

Aspirate VolClean μL at 20000 $\mu\text{L}/\text{min}$

Wait 0.5 s

Send_serial(port = 'COM23', message = 'R0\r') # Stirrer off

Empty syringe at 1000 $\mu\text{L}/\text{min}$ to head valve port 1

Waste

Loop_end()

Routine_end()

Routine define 'CleanSyringe')

Loop(Repeat)

Cavo_XR.valve(ValvePos) # Choose water or cleaning solvent

Send_serial(port = 'COM23', message = 'R99\r') # Stirrer on

Aspirate VolClean μL at 20000 $\mu\text{L}/\text{min}$

Wait 0.5 s

Send_serial(port = 'COM23', message = 'R0\r') # Stirrer off

Empty syringe at 30000 $\mu\text{L}/\text{min}$ head valve port 9

Waste

Loop_end()

Routine_end()

Routine define 'AIM')

Send_serial(message='move_z(0)\r', port='COM10')

Wait 2 s

Send_serial(message='move_sample('+str(rack-1)+','+str(SamplePos s+'))\r', port='COM10')

Wait 3 s

Send_serial(message='move_z('+str(depth)+')\r', port='COM10')

Wait 1 s

Send_serial(message='move_z('+str(depth)+')\r', port='COM10')

Wait 1 s

Routine_end()

Routine define 'CleanAutosampler')

Aspirate three times new solution and air segments

Loop(3)

Send_serial(message='move_z('+str(depth)+')\r', port='COM10')

Wait 3 s

Aspirate 100 μL at 25000 $\mu\text{L}/\text{min}$ head valve port 5

```
Send_serial(message='move_z(0)\r', port='COM10')
Wait 2 s
Aspirate 100 µL at 25000 µL/min head valve port 5
Loop_end()
Send_serial(message='move_z('+str(depth)+'')\r', port='COM10')
# Discharge to waste
Aspirate 500 µL
Empty syringe head valve port 9
Routine_end()
```