HPLC

High Performance Liquid Chromatography

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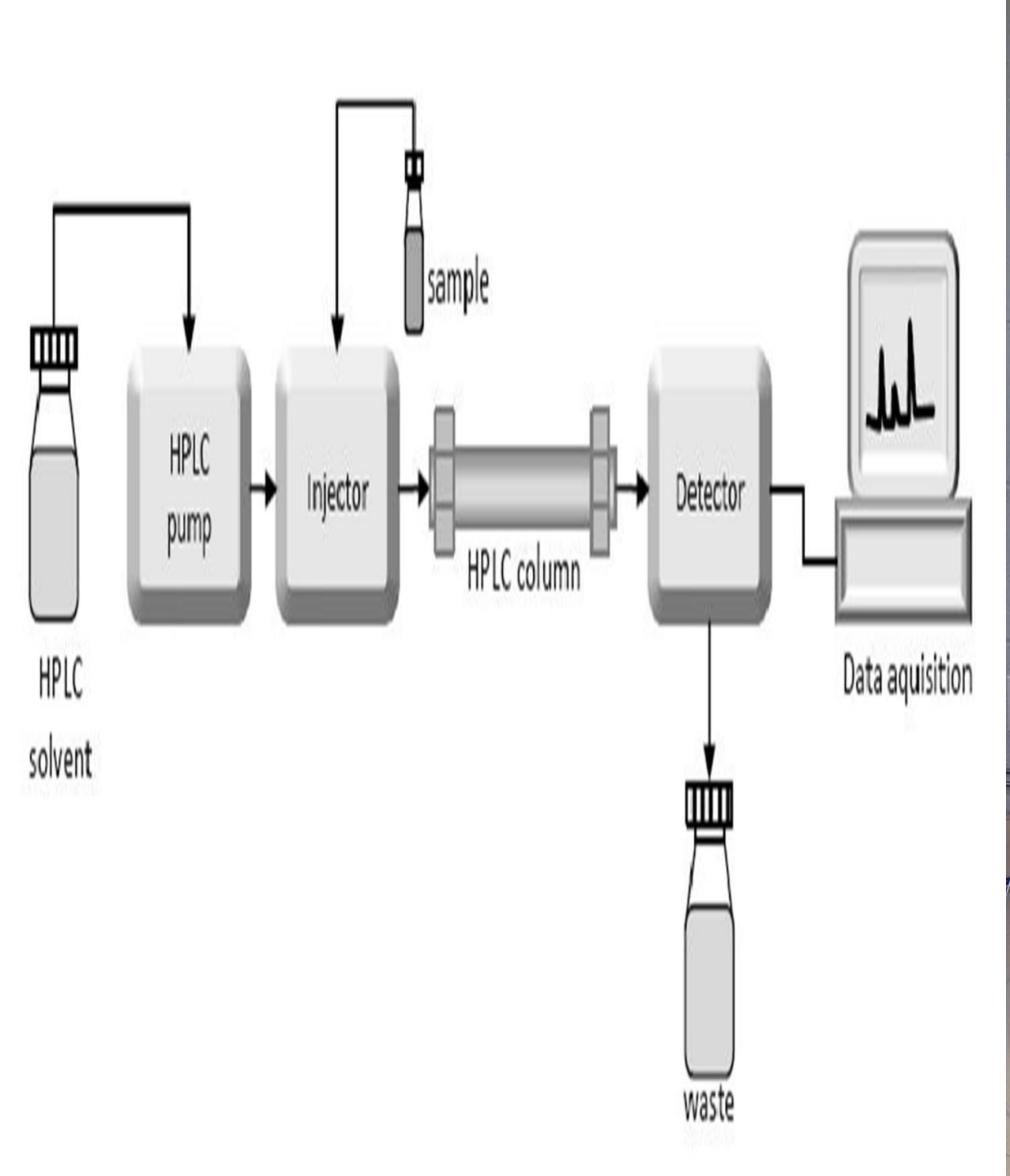
HPLC is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds which are not easily volatalised, thermally unstable and have high molecular weights.

HPLC

1) Solvent Reservoir
2) Pump
3) Sample Injection System
4) Column
5) Detector
6) Data Collection Device

Components


sample
HPLC pump
Injector
HPLC column
Detector
Data aquisition
HPLC solvent
waste


Agilent Technologies
Quaternary

- ✓ These are inert containers for mobile phase storage and transport.
- ✓ Generally transparent glass bottles are used so that so as to facilitate visual inspection of mobile phase level inside the container.
- ✓ Glass filters are provided inside for removal of particulate impurities in the mobile phase if any.
- ✓ The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.

Solvent Reservoir

Pumps provide constant flow of mobile phase to the column under constant pressure.

Variations in flow rates of the mobile phase effect elution time of sample components and result in errors.

Pumps

- ✓ Injectors are used to provide constant volume injection of sample into the mobile phase stream. .
- ✓ May be auto-sampler or manual

Sample Injection System

- A column is a stainless steel tube packed with stationary phase.
- between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 μm.
- Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.

Columns

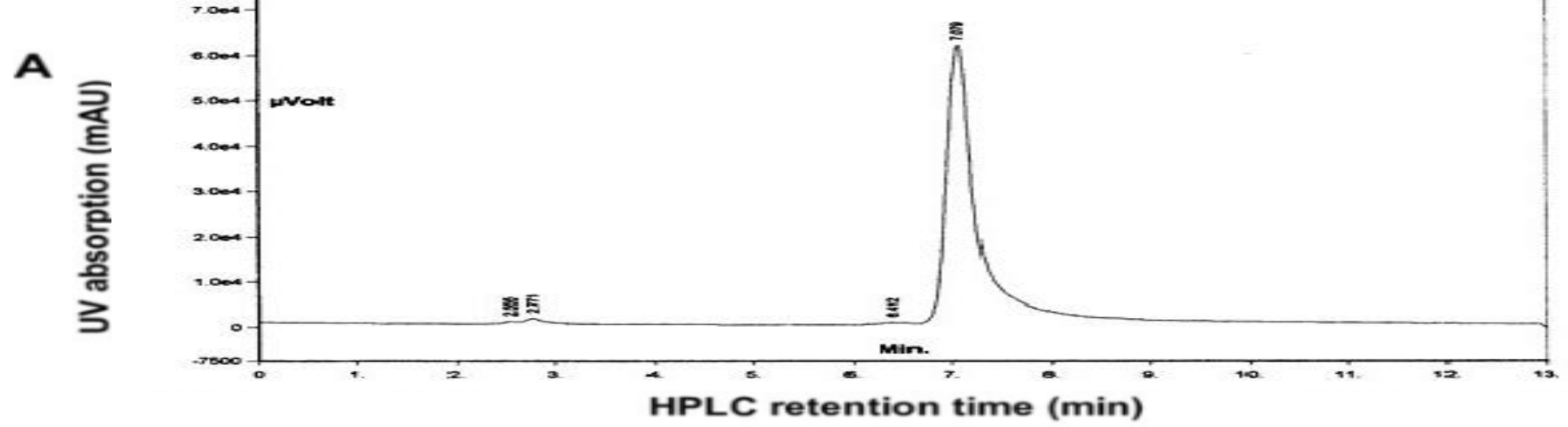
- ✓ located at the end of the column
- ✓ A detector gives specific response for the components separated by the column
- ✓ Commonly used detectors are UV-spectroscopy, fluorescence and mass-spectrometric

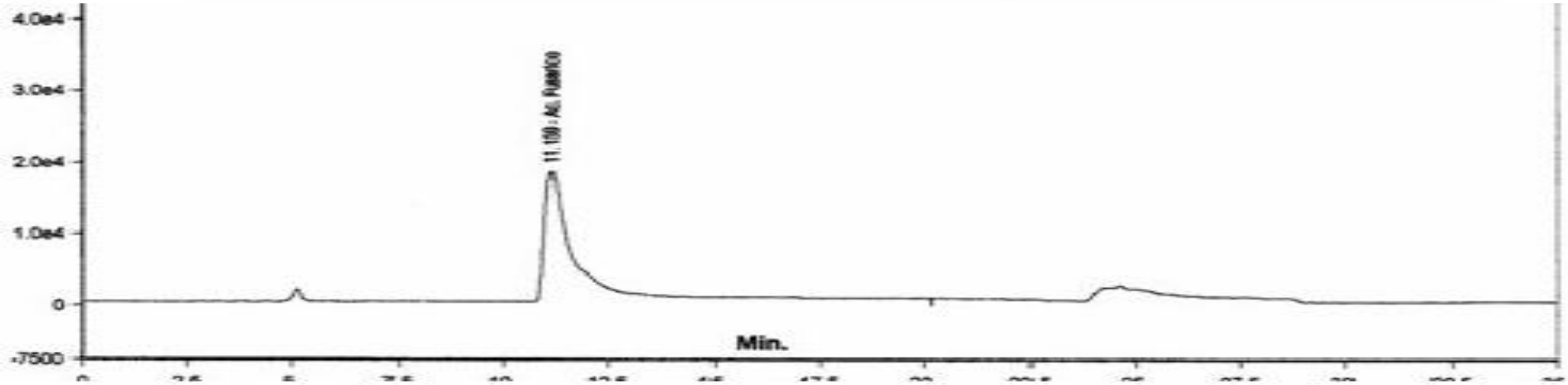
Detector

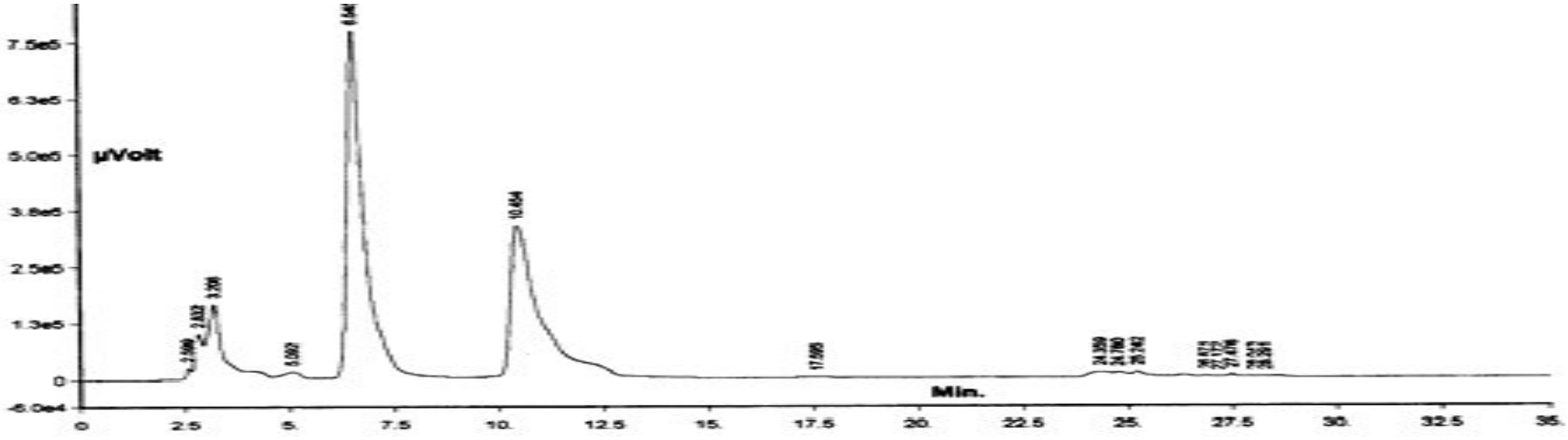
The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

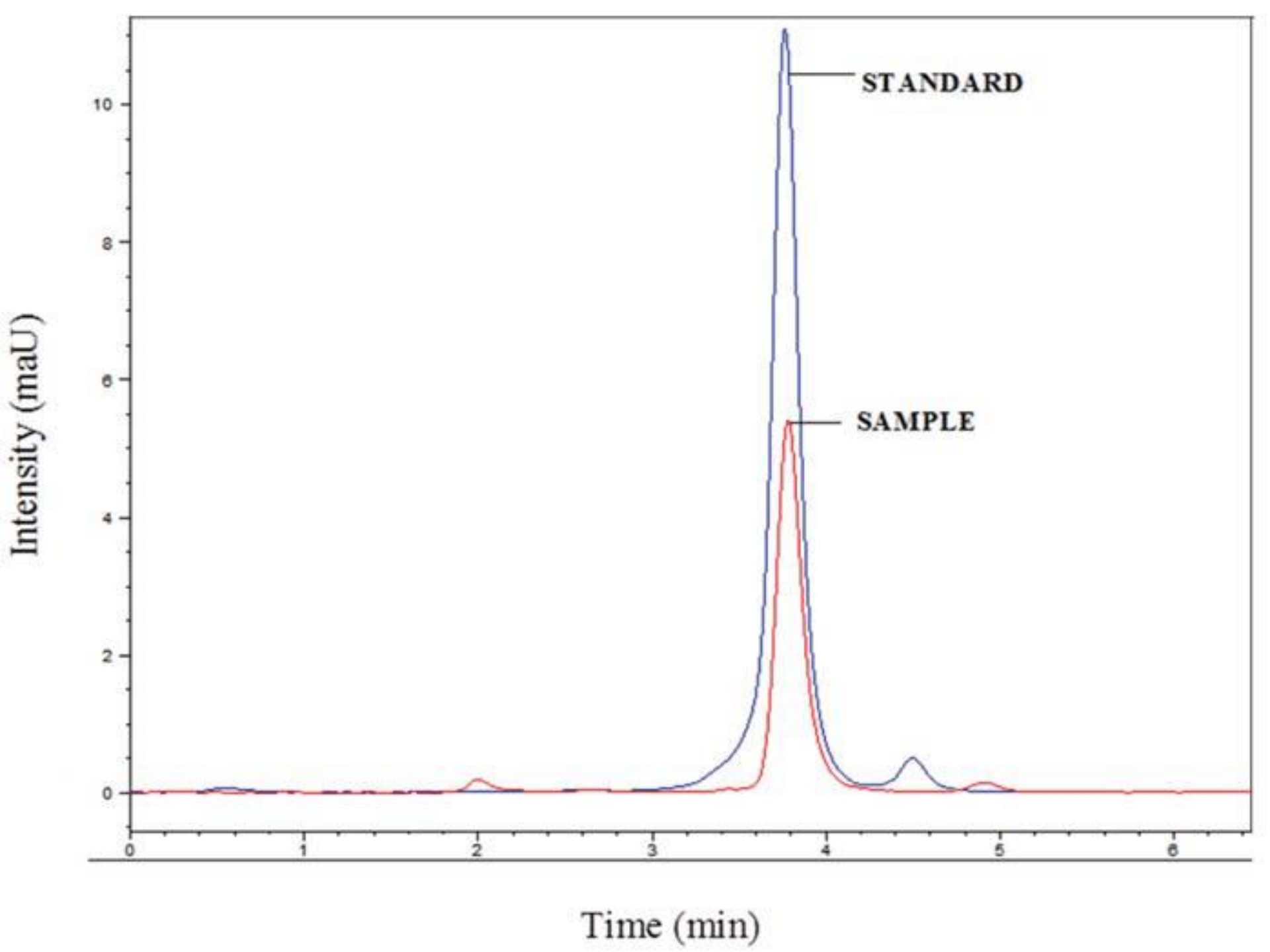
Data Collection Devices

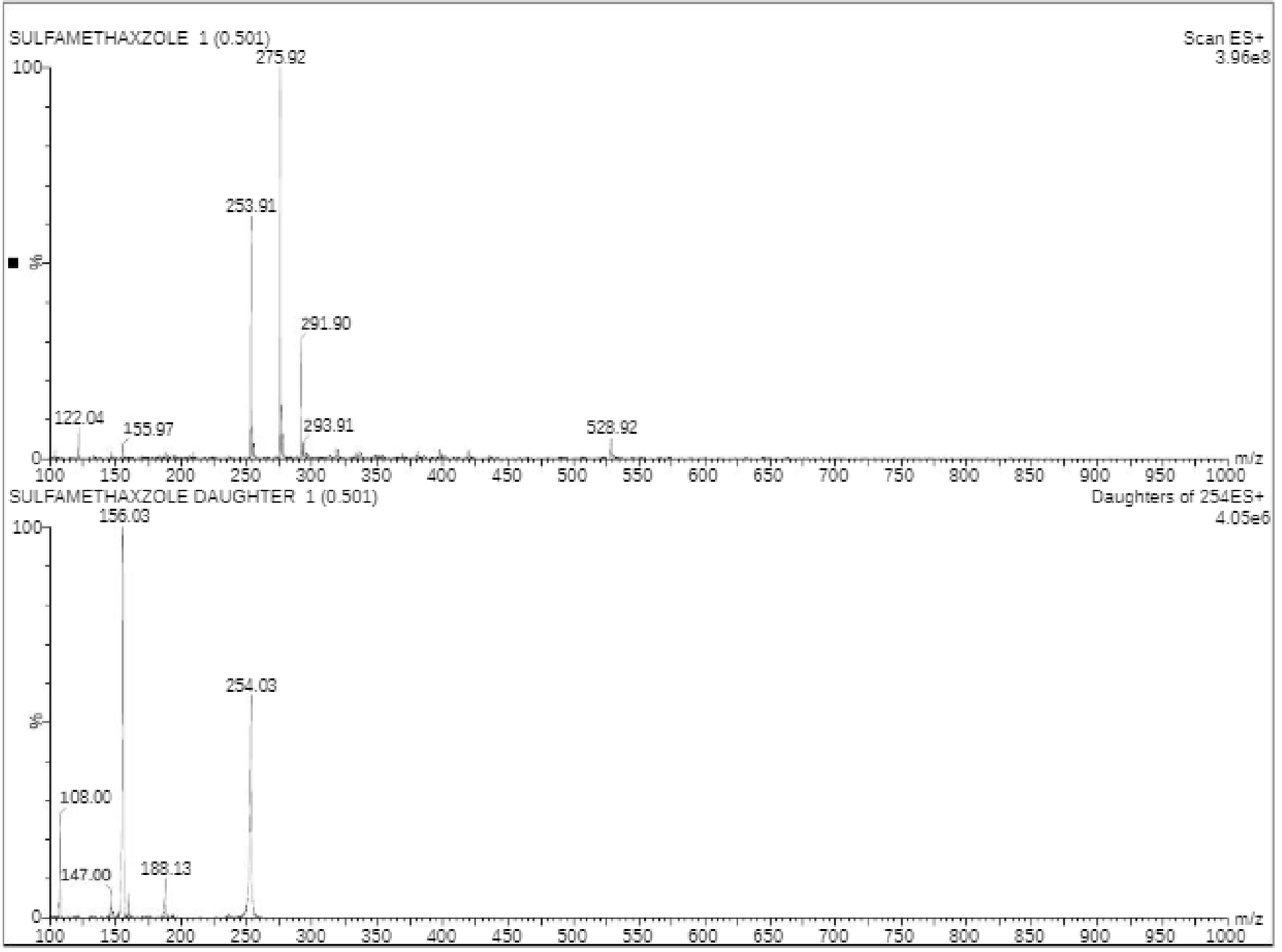
A


UV absorption (mAU)
µVolt
Min.
HPLC retention time (min)


Min.


µVolt
Min.


STANDARD
SAMPLE
Intensity (maU)
Time (min)
0
2
4
6
8
10
0
1
2
3
4
5
6


SULFAMETHAXZOLE 1 (0.501)
Scan ES+
3.96e8
100
%
0
275.92
253.91
291.90
122.04
155.97
293.91
528.92
m/z
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000
SULFAMETHAXZOLE DAUGHTER 1 (0.501)
Daughters of 254ES+
4.05e6
100
%
0
156.03
254.03
108.00
147.00
188.13
m/z
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000

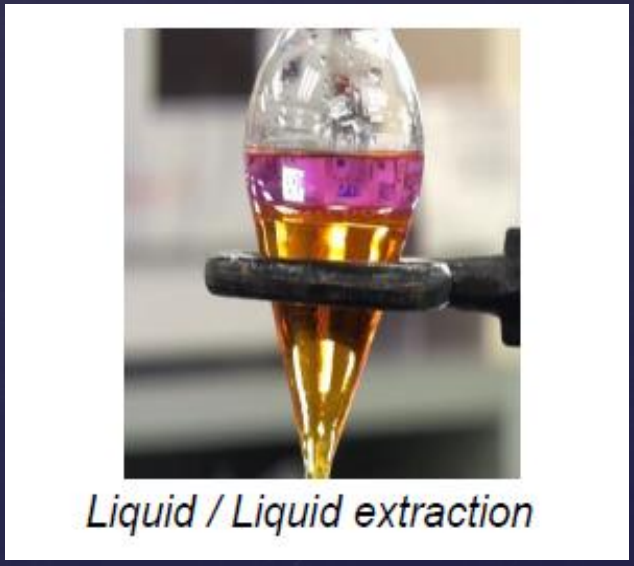
There are many techniques used, each with their own benefits. Examples of widely used techniques are;

- Dilution
- Centrifugation
- Filtration
- Precipitation
- Liquid extraction
- Liquid/Liquid Extraction
- Solid Phase Extraction (SPE)

Sample Preparation

Solid samples such as food or soil samples require extraction before analysis. Typically this is performed by homogenising the sample followed by solvent extraction. This is simple but time consuming and can result in a very complex extract.

- Liquid / Liquid extraction is a technique for extraction of samples based partitioning of compounds between a polar and non-polar solvent.
- Excellent for clean up of biological samples and removes more matrix components



Liquid / Liquid extraction

Liquid / Liquid Extraction

- Solid Phase Extraction (SPE) is a targeted extraction technique for isolation of a compound(s) from complex matrices e.g. biological samples.

- Separation is achieved by the affinity of the compound(s) of interest for the active components of the stationary phase.

Silica beads or polymeric material is bonded with specific functional groups to create the stationary phase.

Solid Phase Extraction (SPE)

Poor extraction methods can cause serious problems with chromatography, detection and robustness of the instruments.

SPE can provide very clean extracts which prevents such issues.