CHEMISTRY 5 (INSTRUMENTAL)

AGRICULTURAL BIOTECHNOLOGY, LEVEL 2

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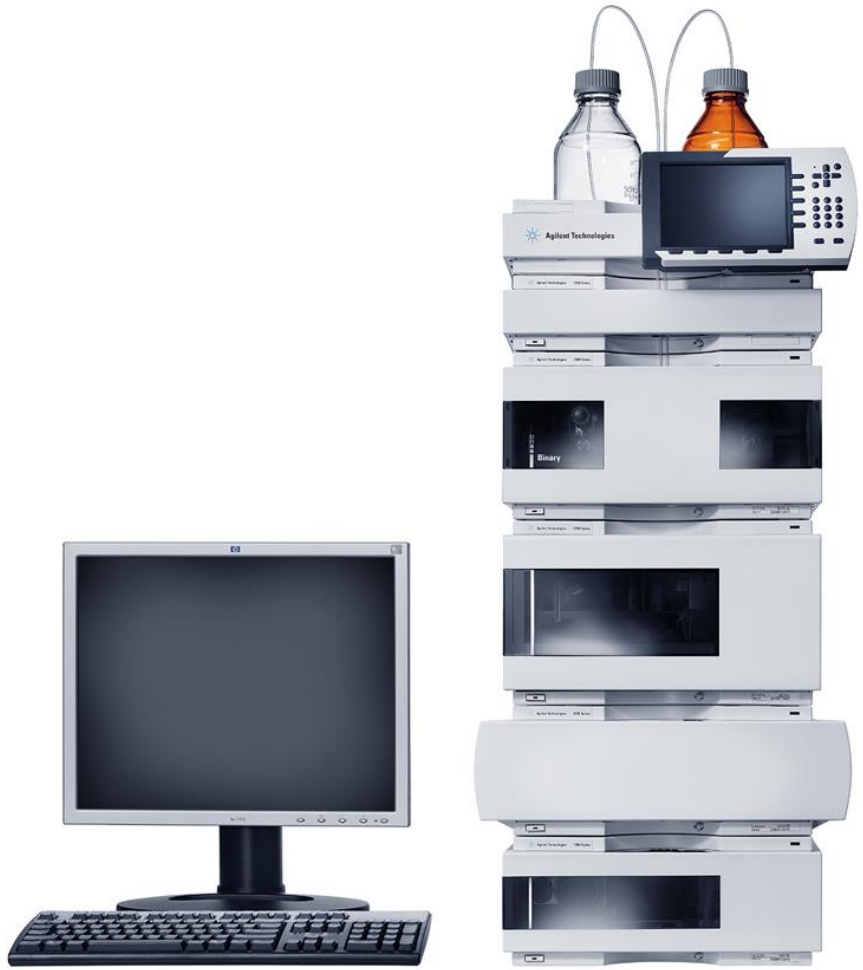
CONTENTS

WHAT IS HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)?

- HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector.

- Compounds are separated by injecting a sample mixture onto the column. The different component in the mixture pass through the column at differentiates due to differences in their partition behavior between the mobile phase and the stationary phase. The mobile phase must be degassed to eliminate the formation of air bubbles. .



LIQUID CHROMATOGRAPHY

- Chromatography in which the mobile phase is a **liquid**.
  - The liquid used as the mobile phase is called the "**eluent**".
- The stationary phase is usually a solid or a liquid.
- In general, it is possible to analyze any substance that can be stably dissolved in the mobile phase.

FOUR TYPES OF LIQUID CHROMATOGRAPHY

1. Partition chromatography
2. Adsorption, or liquid-solid
3. chromatography
4. Ion exchange chromatography
5. Size exclusion, or gel, chromatography

Modes of High-Performance Liquid Chromatography (Very Important)

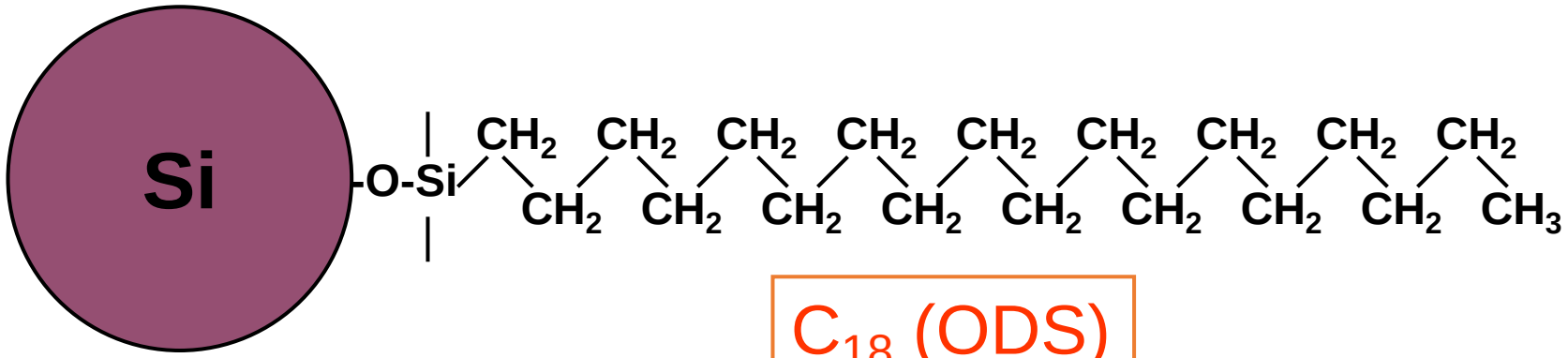
Types of Compounds	Mode	Stationary Phase	Mobile Phase
Neutrals Weak Acids Weak Bases	Reversed Phase	C18, C8, C4 cyano, amino	Water/Organic Modifiers
Ionics, Bases, Acids	Ion Pair	C-18, C-8	Water/Organic Ion-Pair Reagent
Compounds not soluble in water	Normal Phase	Silica, Amino, Cyano, Diol	Organics
Ionics Inorganic Ions	Ion Exchange	Anion or Cation Exchange Resin	Aqueous/Buffer Counter Ion
High Molecular Weight Compounds Polymers	Size Exclusion	Polystyrene Silica	Gel Filtration- Aqueous Gel Permeation- Organic

In general, packing material produced by chemically bonding hydrophobic (low-polarity) functional groups to a silica gel substrate is used as the stationary phase.

The most widespread of such packing materials is a type called "ODS", which is formed by bonding octadecyl groups ($-C_{18}H_{37}$) to the surface of silica gel. The structure of this material is illustrated above.

In addition to ODS, packing materials produced by bonding octyl groups, which have a short aliphatic chain, phenyl groups, and cyanopropyl groups are commercially available, and are used in cases where a different separation selectivity from that of ODS is required. Also, the support material is not limited to silica gel. For example, materials formed by bonding octadecyl groups to the surface of a resin are also available.

- C_{18} (ODS) type
- C_8 (octyl) type
- C_4 (butyl) type
- Phenyl type
- TMS type
- Cyano type



C_{18} (ODS)

Chromato-graphy / -graph / -gram / -grapher

Chromato**graphy**:	Analytical technique
Chromato**graph**:	Instrument
Chromato**gram**:	Obtained “picture”
Chromato**grapher**:	Person

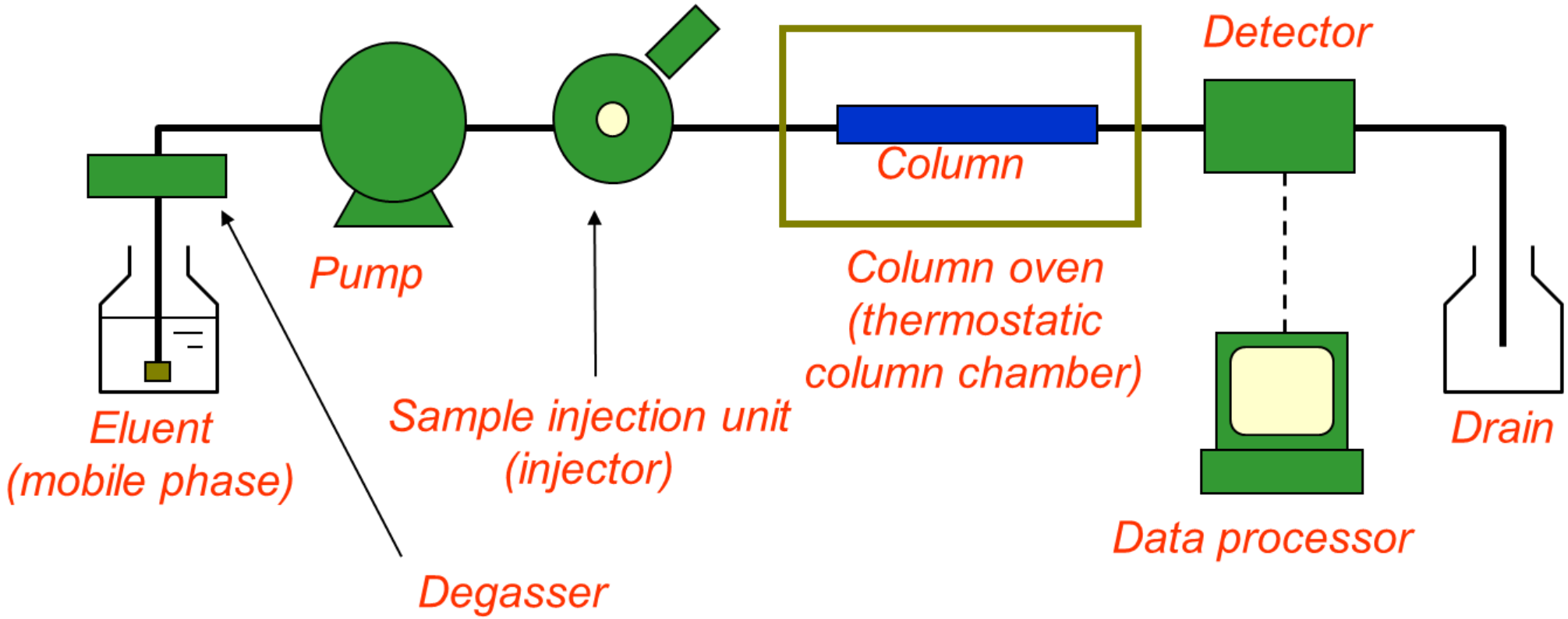
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)?

- PRINCIPLE,
- INSTRUMENTATION,
- APPLICATIONS

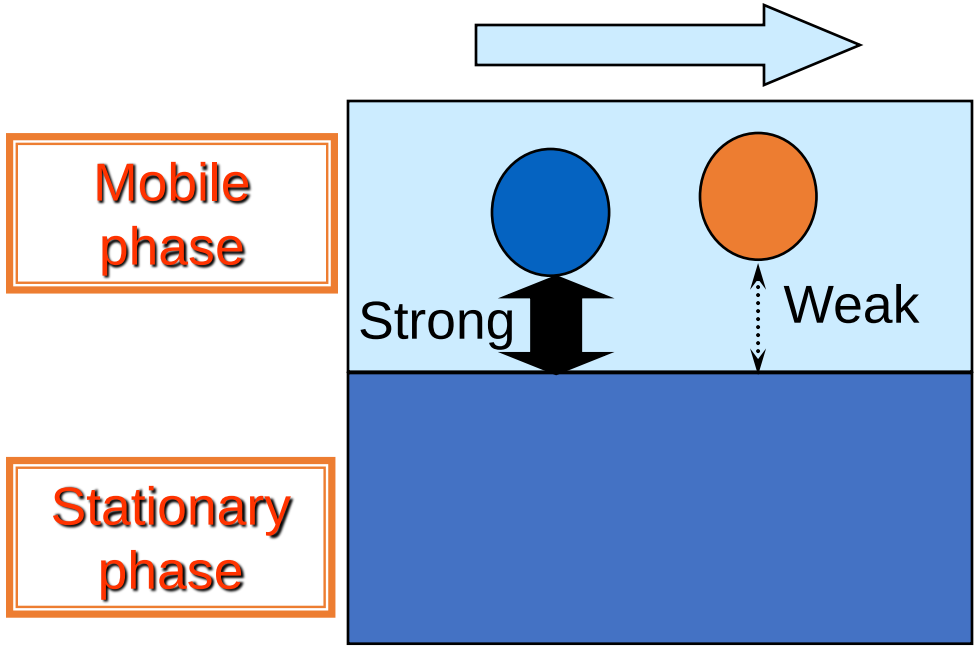
PRINCIPLE

- The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material define their time “on-column”. Hence, different constituents of a sample are eluted at different times. Thereby, the separation of the sample ingredients is achieved.
- A detection unit (e.g. UV detector) recognizes the analytes after leaving the column. The signals are converted and recorded by a data management system (computer software) and then shown in a chromatogram. After passing the detector unit, the mobile phase can be subjected to additional detector units, a fraction collection unit or to the waste.

Flow Channel Diagram for High Performance Liquid Chromatograph



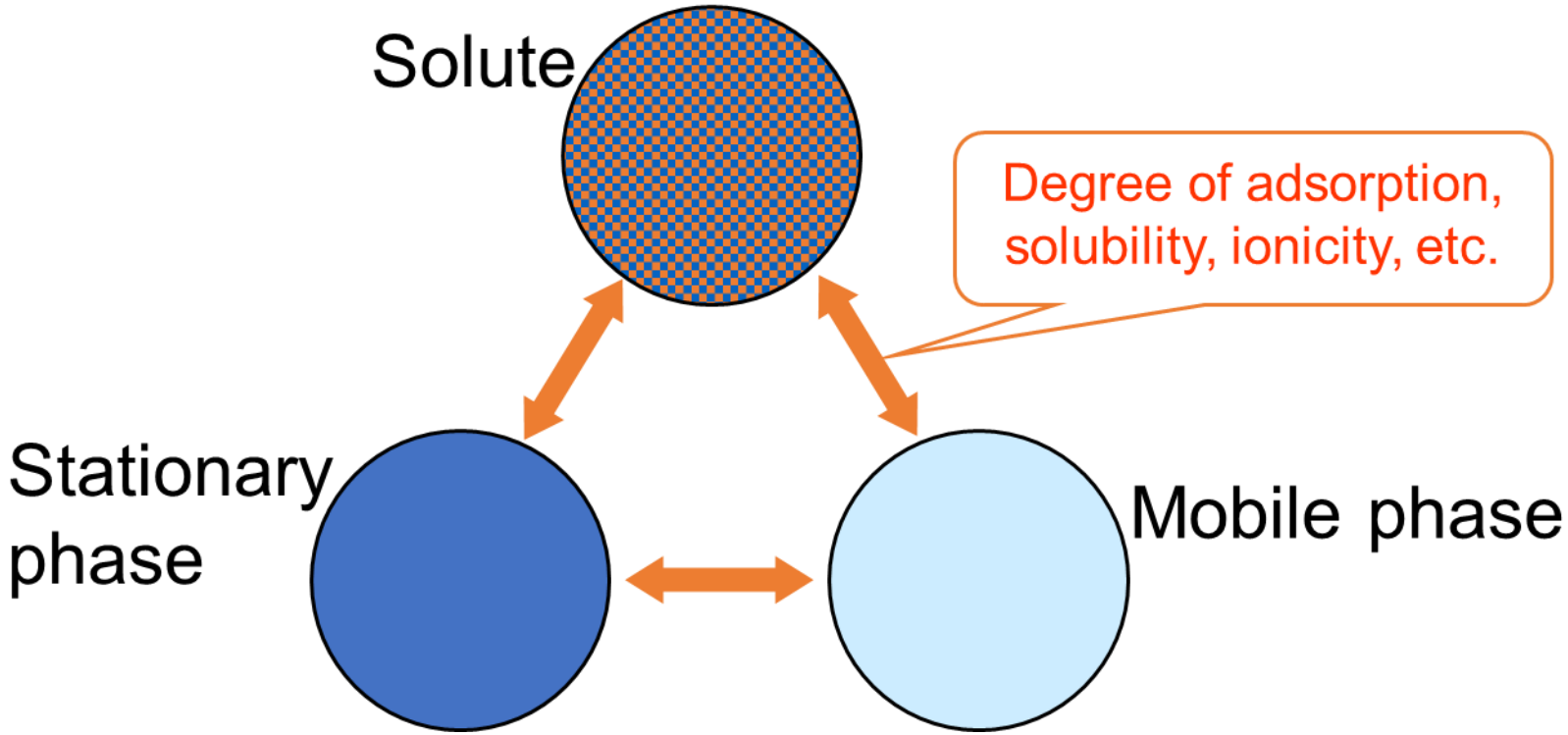
Mobile Phase / Stationary Phase



- A site in which a moving phase (mobile phase) and a non-moving phase (stationary phase) make contact via an interface that is set up.
- The affinity with the mobile phase and stationary phase varies with the solute. → Separation occurs due to differences in the speed of motion.

Interaction Between Solutes, Stationary Phase, and Mobile Phase

- Differences in the interactions between the solutes and stationary and mobile phases enable separation.



INSTRUMENTATION

The essential components of HPLC instrumentation include:

1. Solvent
2. Solvent Delivery System (Pump)
3. Injector
4. Sample
5. Column
6. Detectors (Diode Array)
7. Waste Collector
8. Recorder (Data Collection)

HPLC Analysis Parameters

Mobile Phases

Flow Rate
Composition

Injection Volume

Column
Oven Temperature

Wavelength
Time Constant

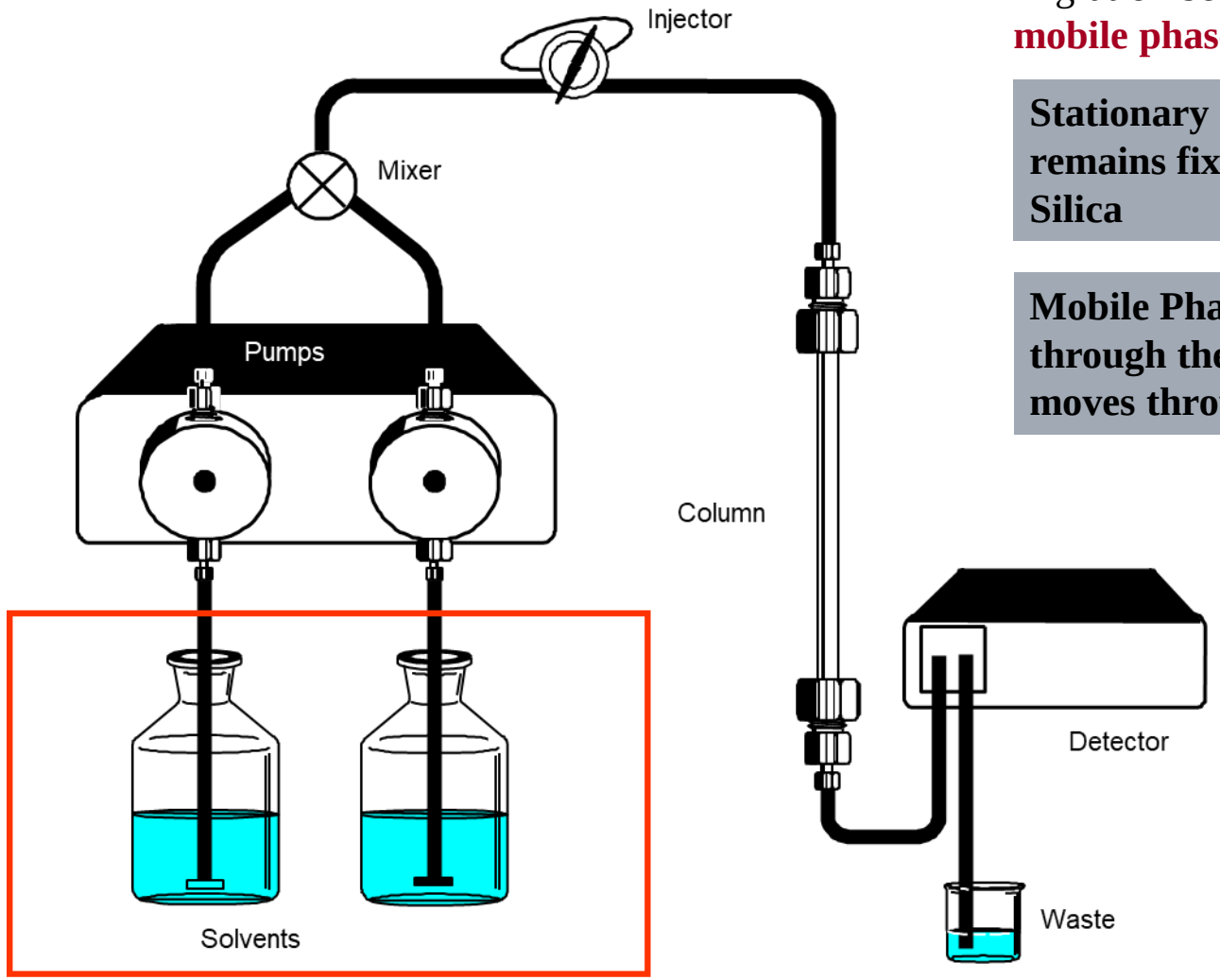
How the HPLC works

Separations

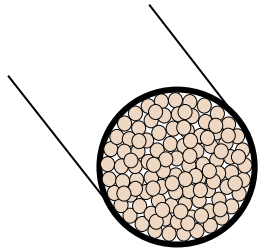
Separation in based upon differential migration between the **stationary** and **mobile phases**.

Stationary Phase - the phase which remains fixed in the column, e.g. C18, Silica

Mobile Phase - carries the sample through the stationary phase as it moves through the column.



High Performance Liquid Chromatograph



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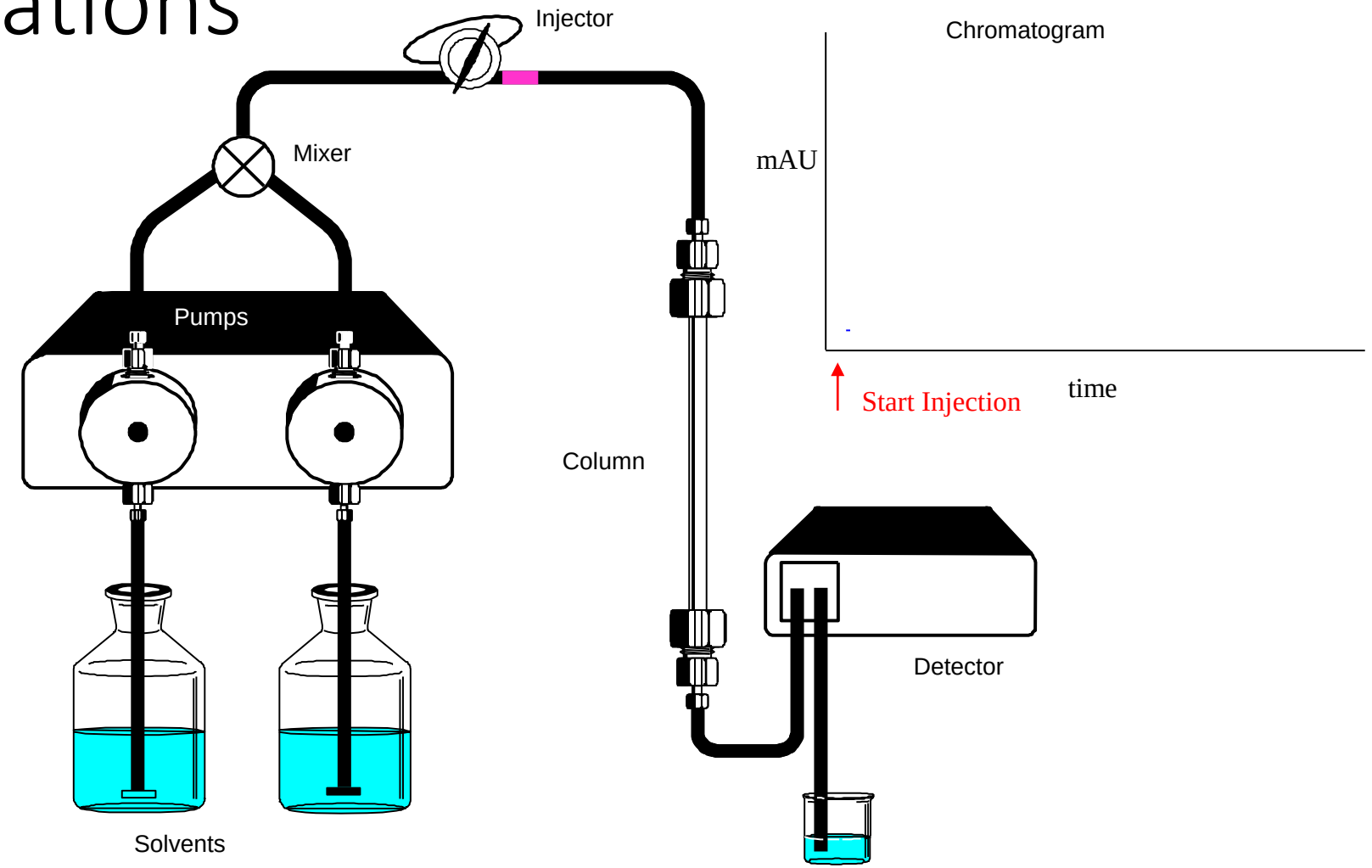

High Performance Liquid Chromatograph

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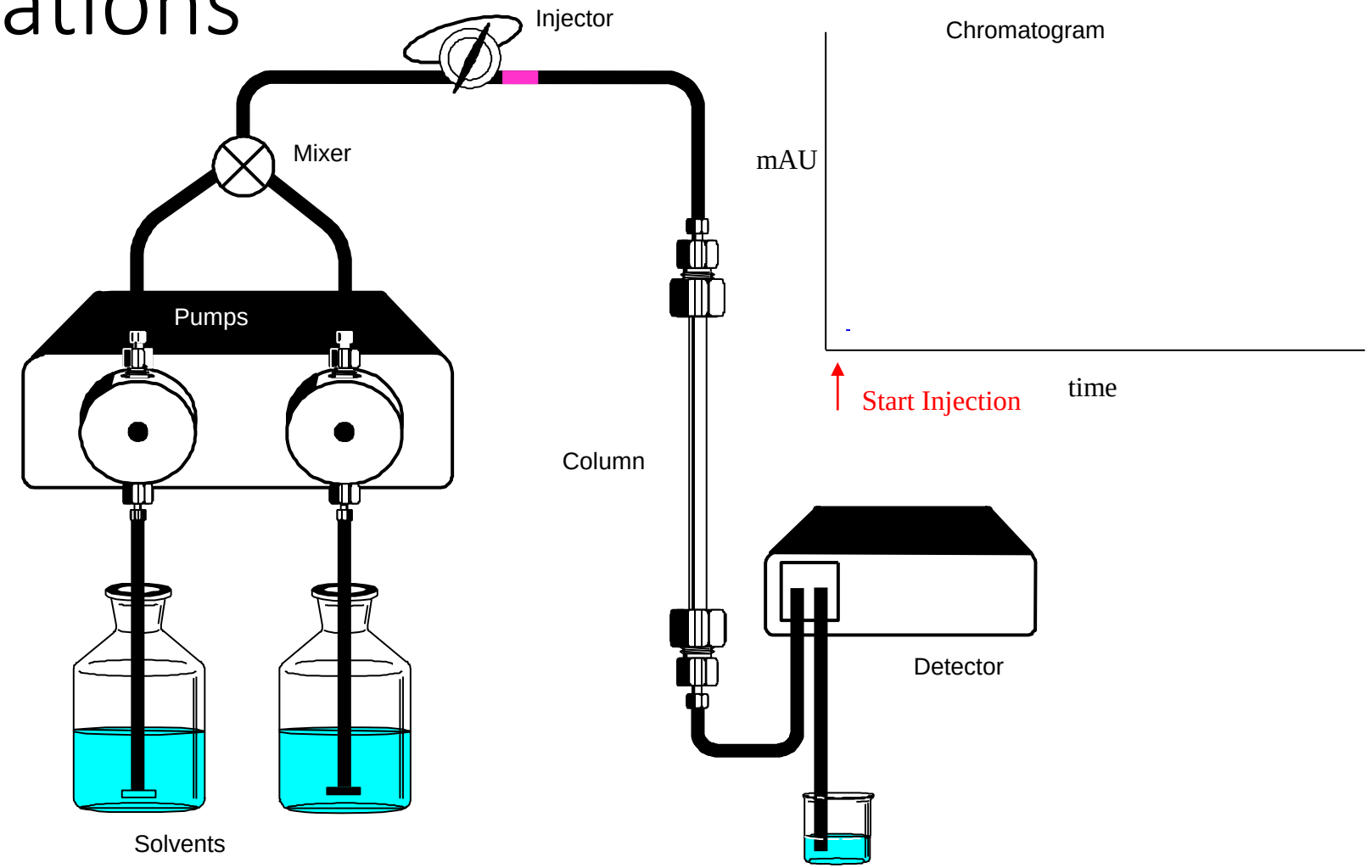

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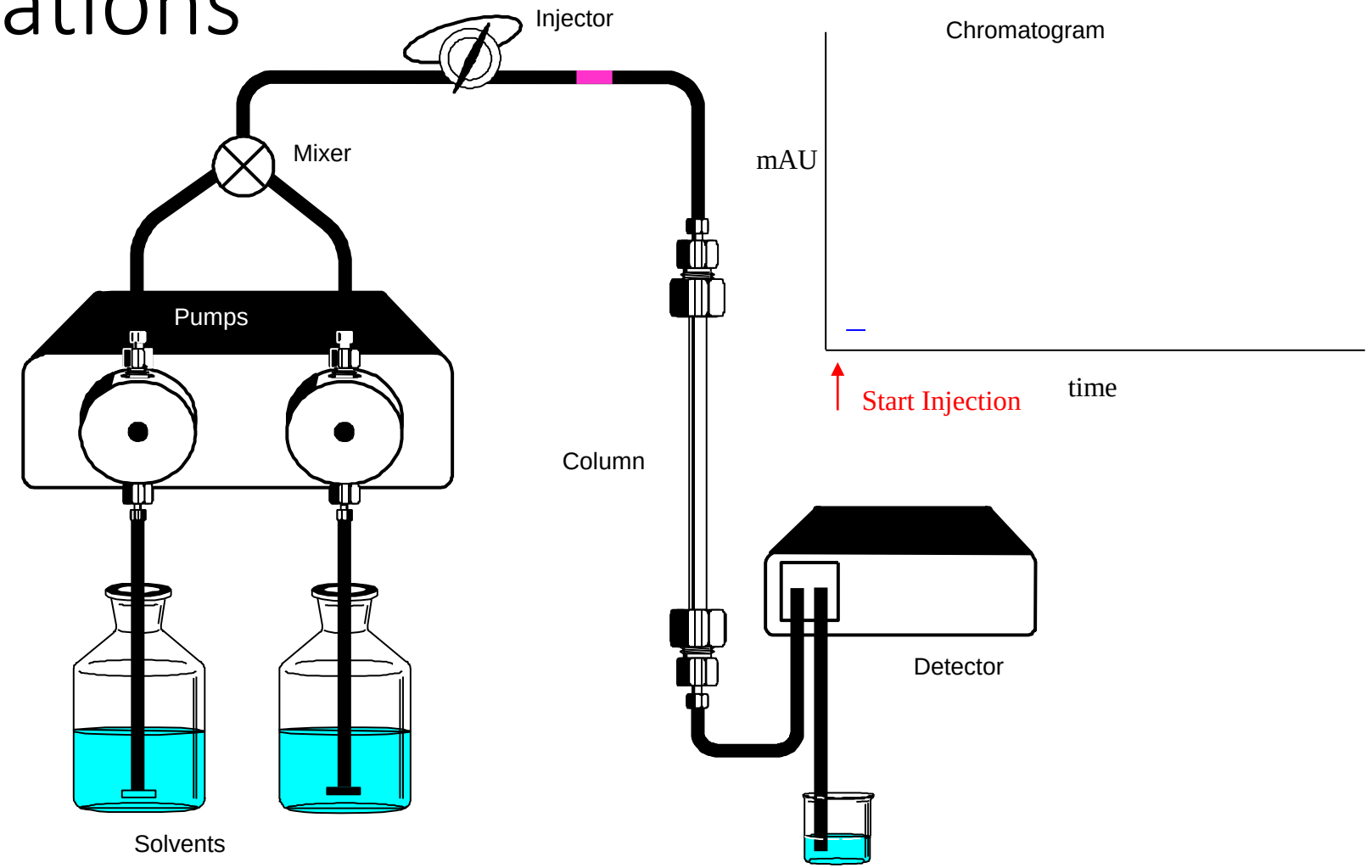

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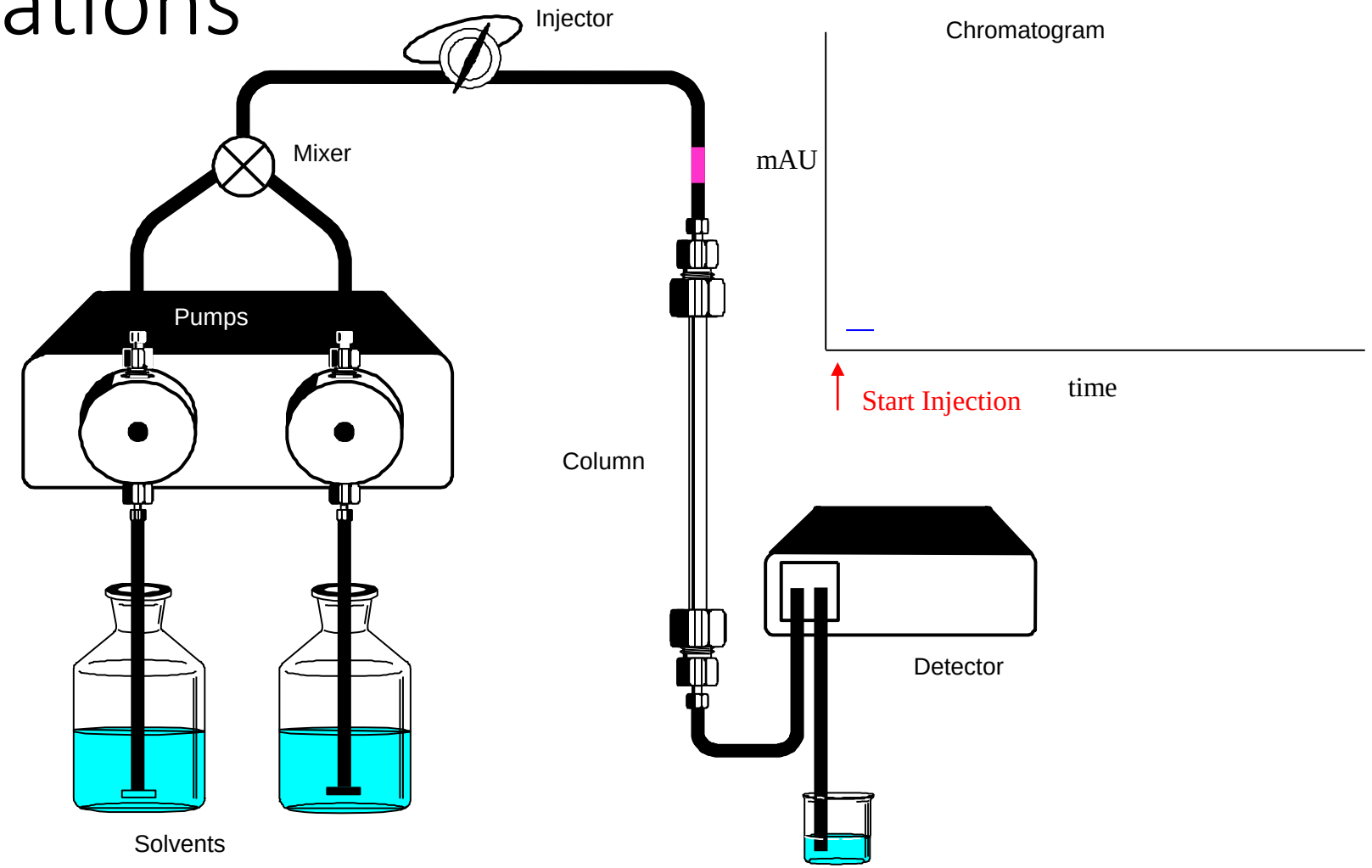

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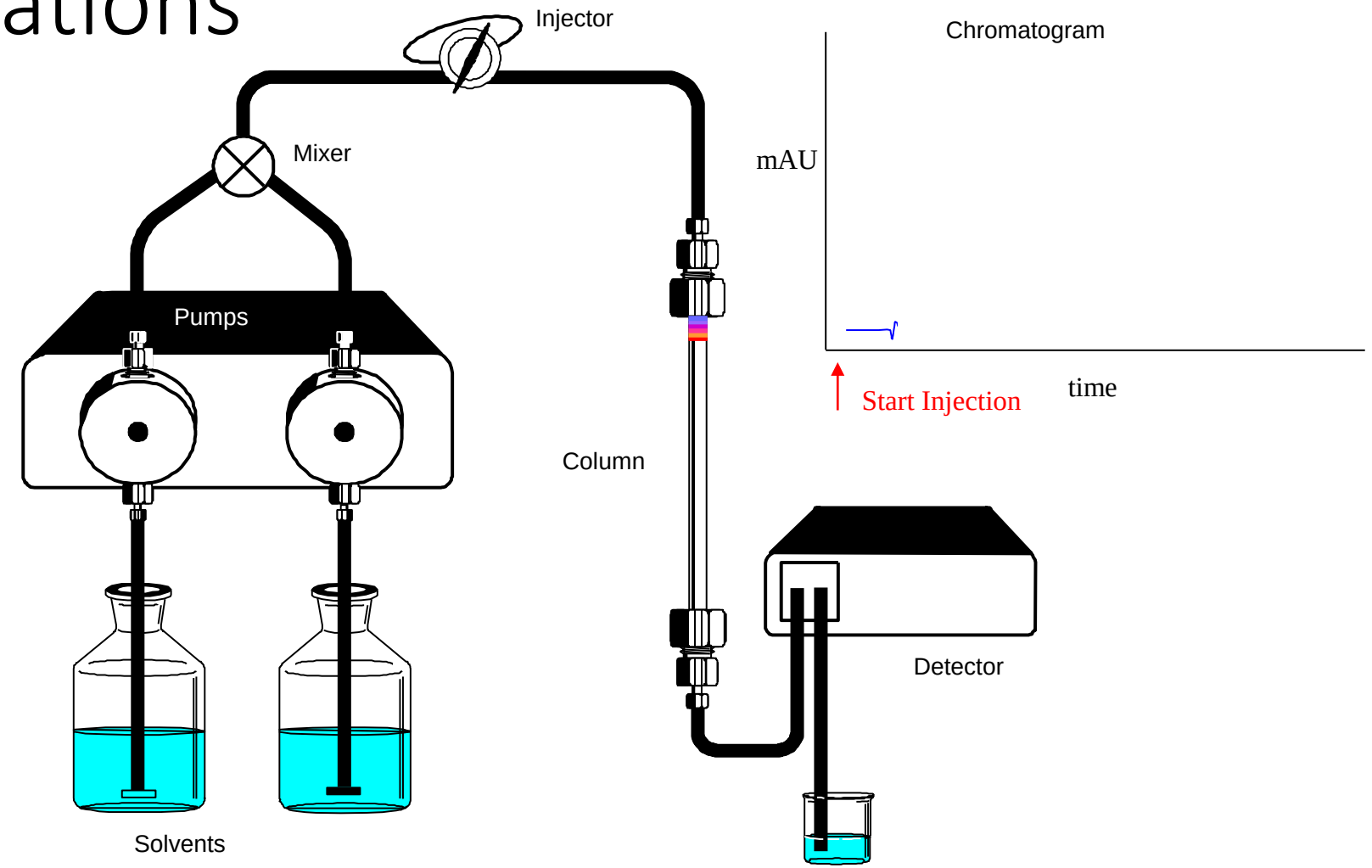

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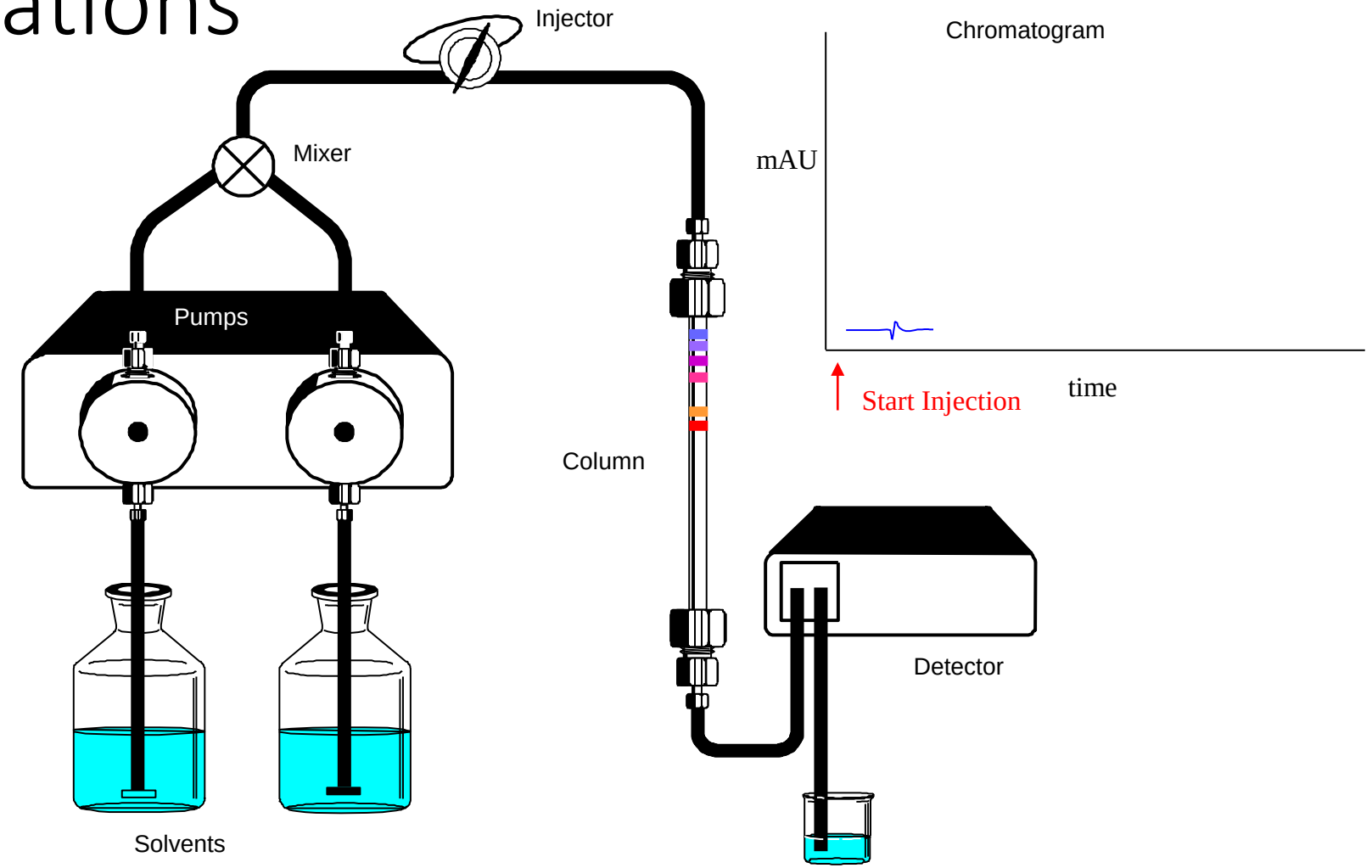

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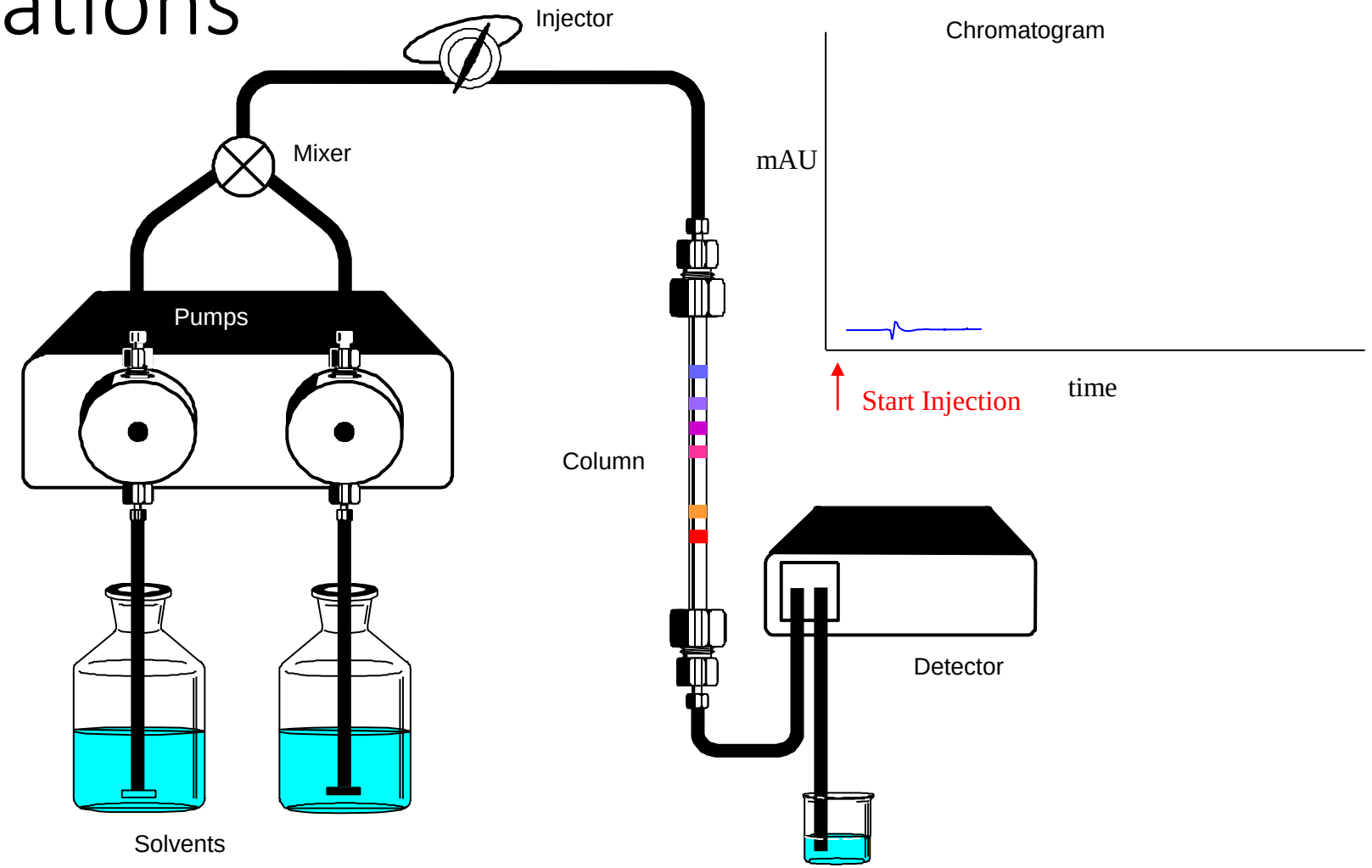

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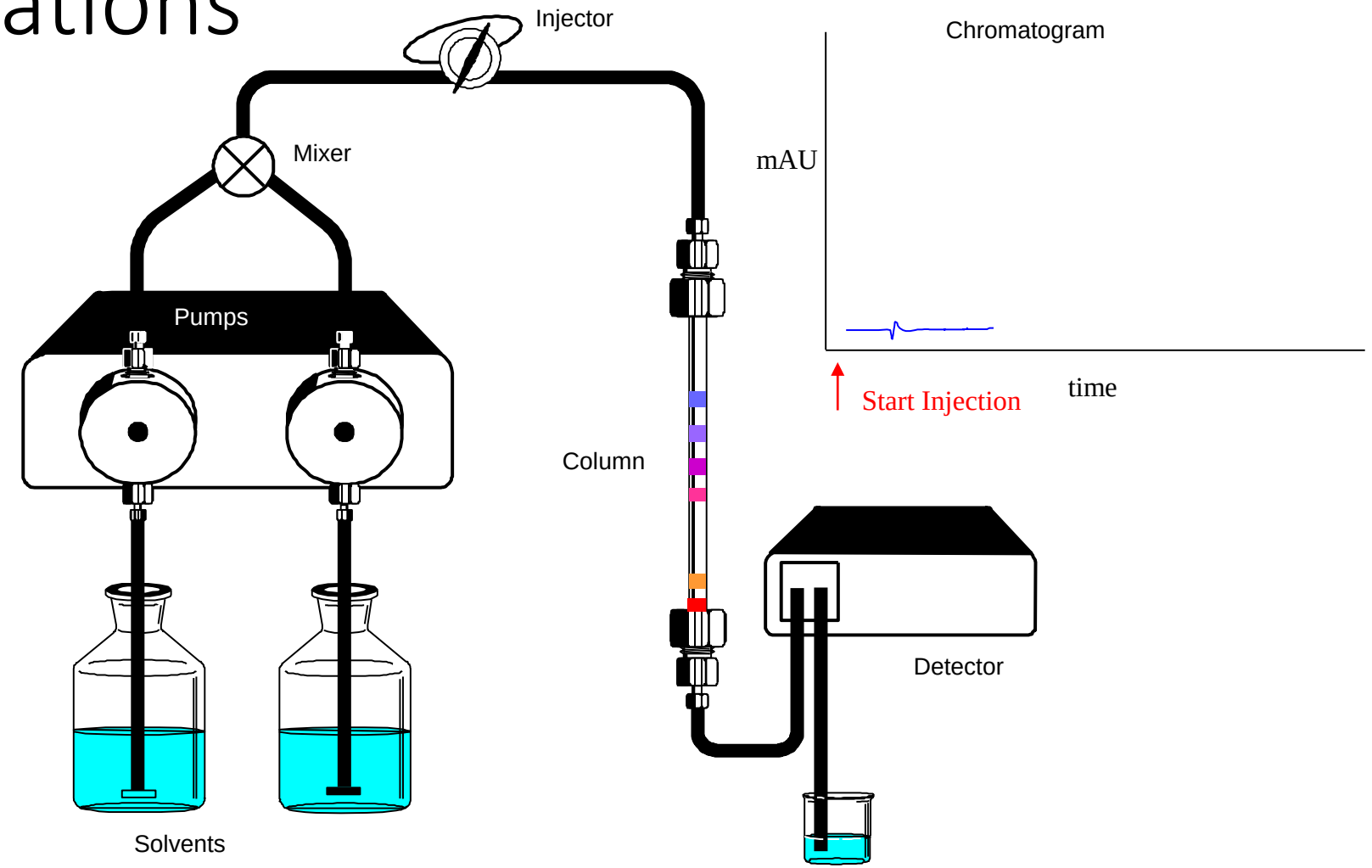

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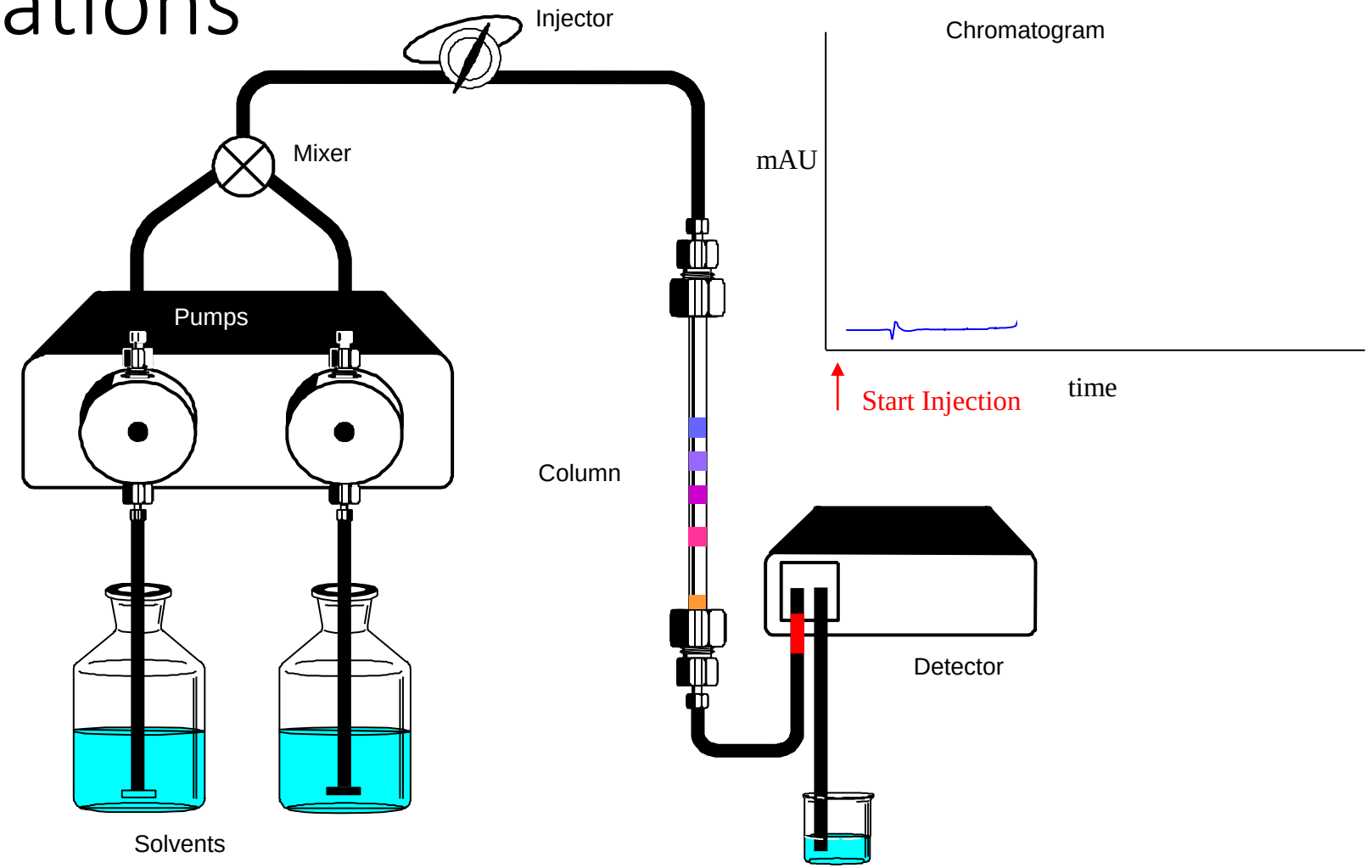

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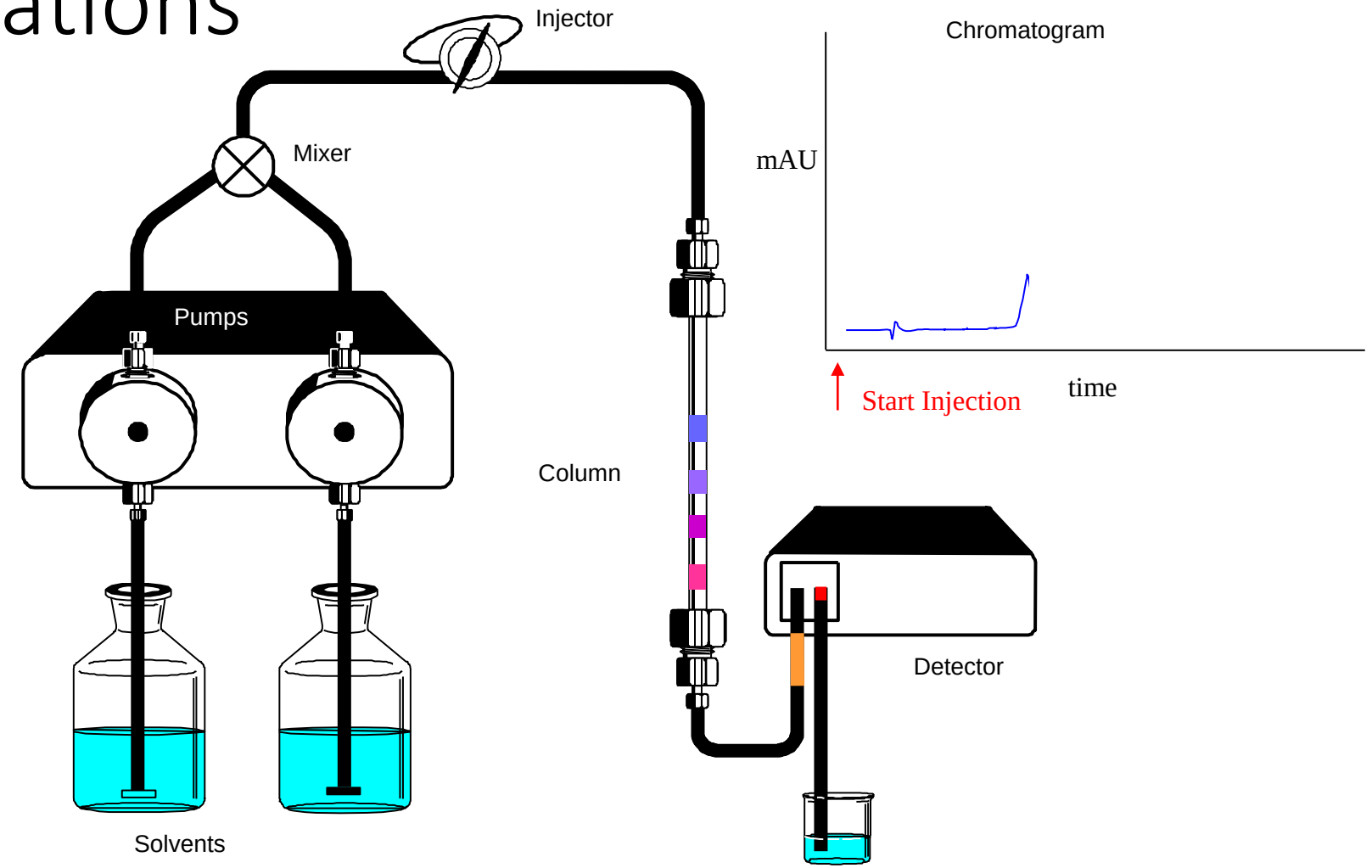

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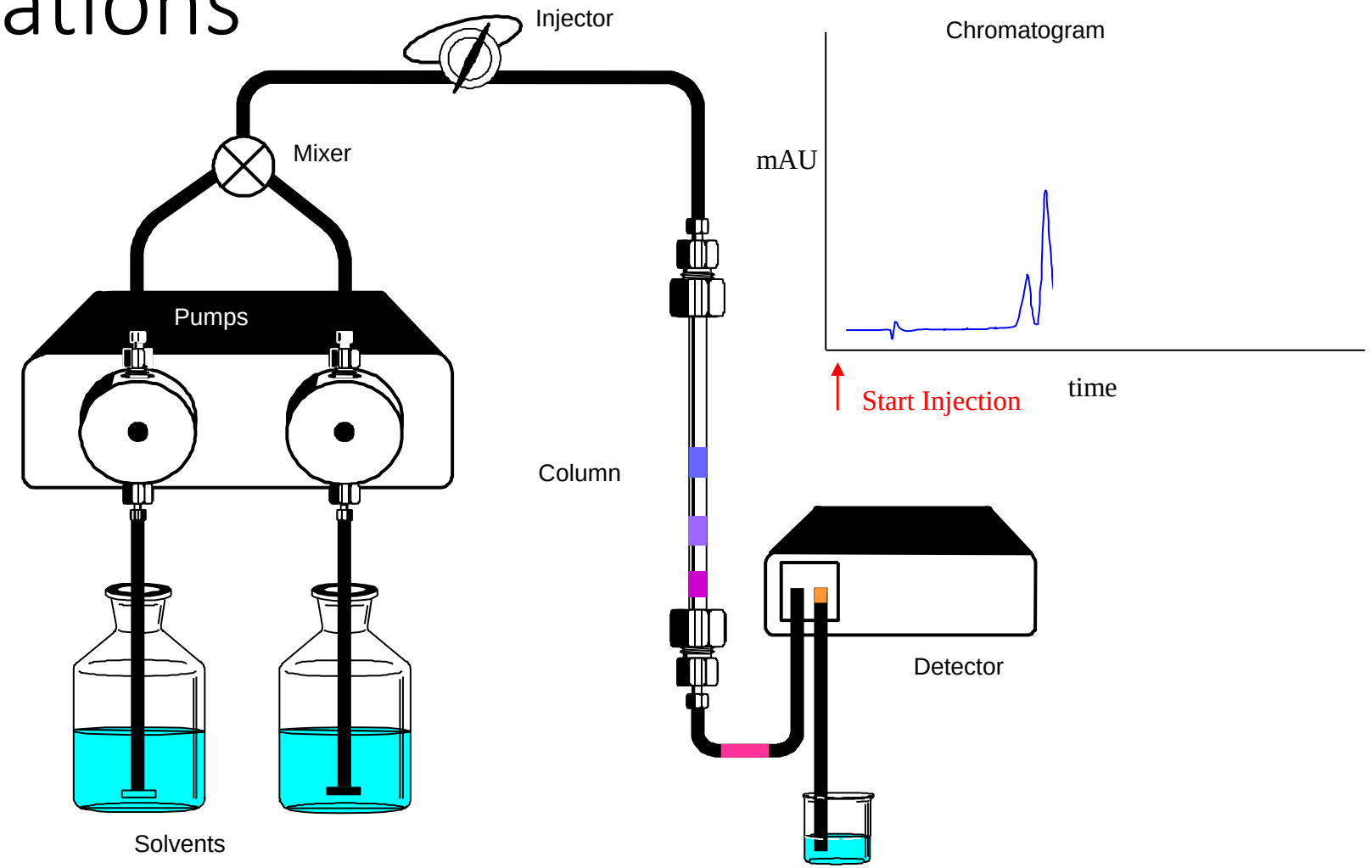

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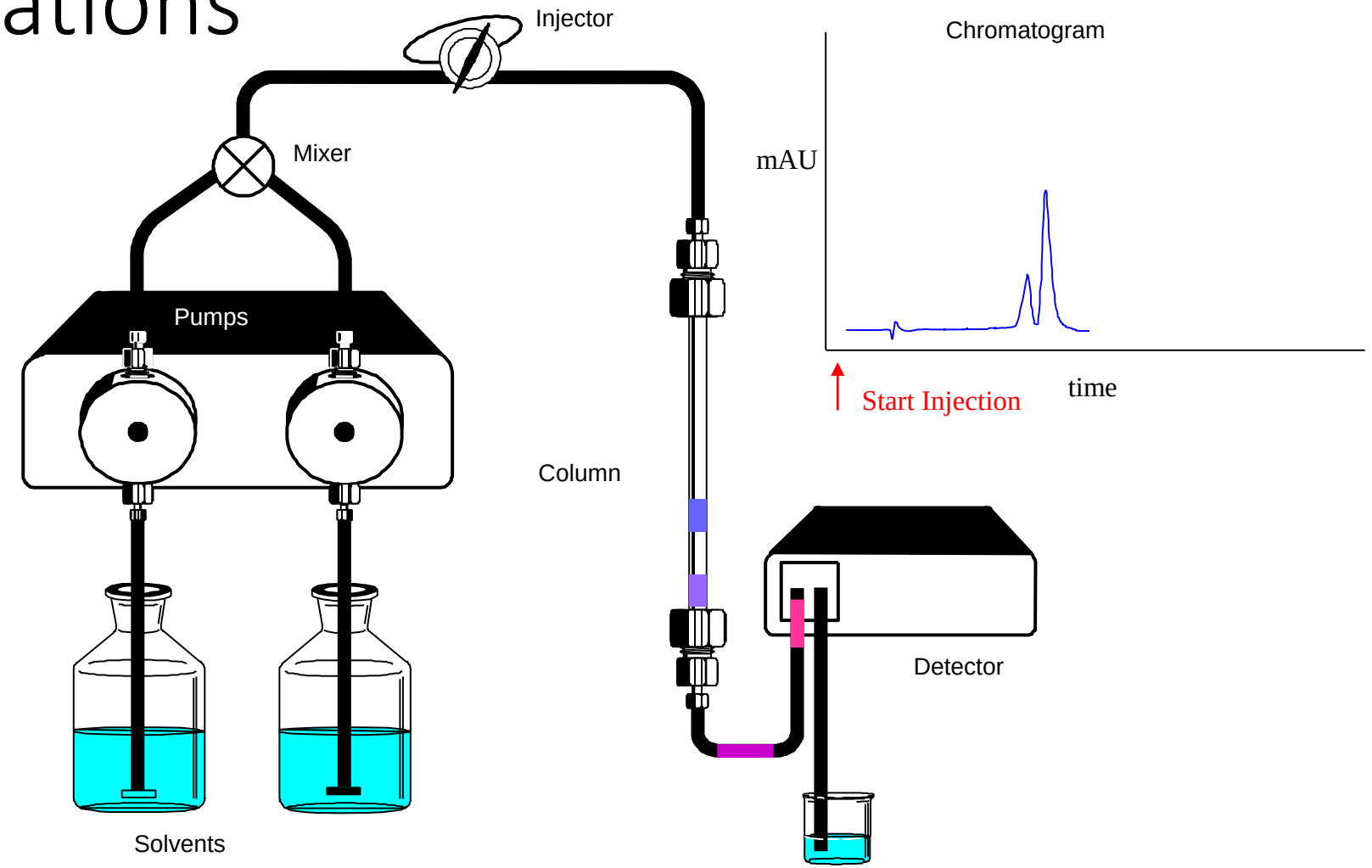

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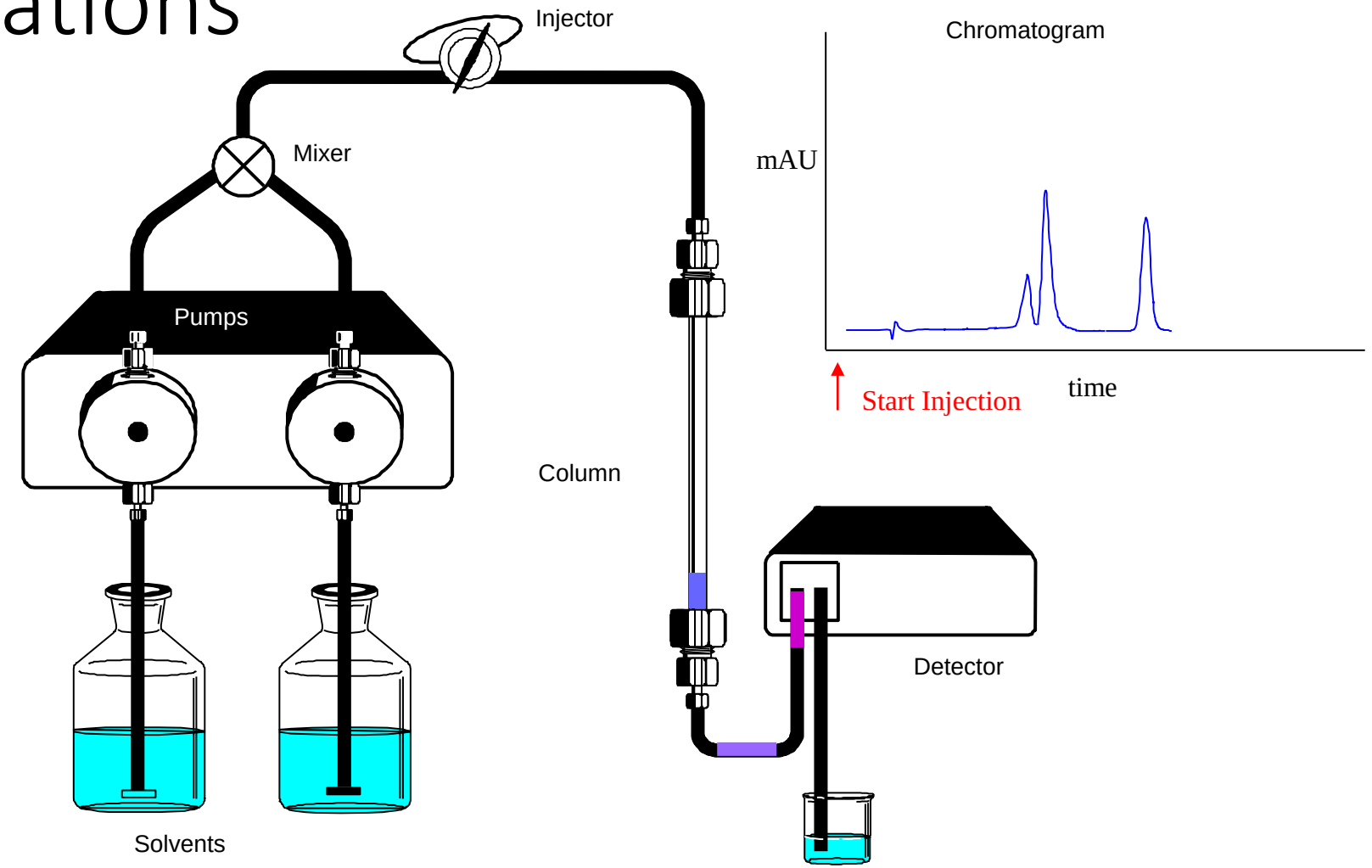

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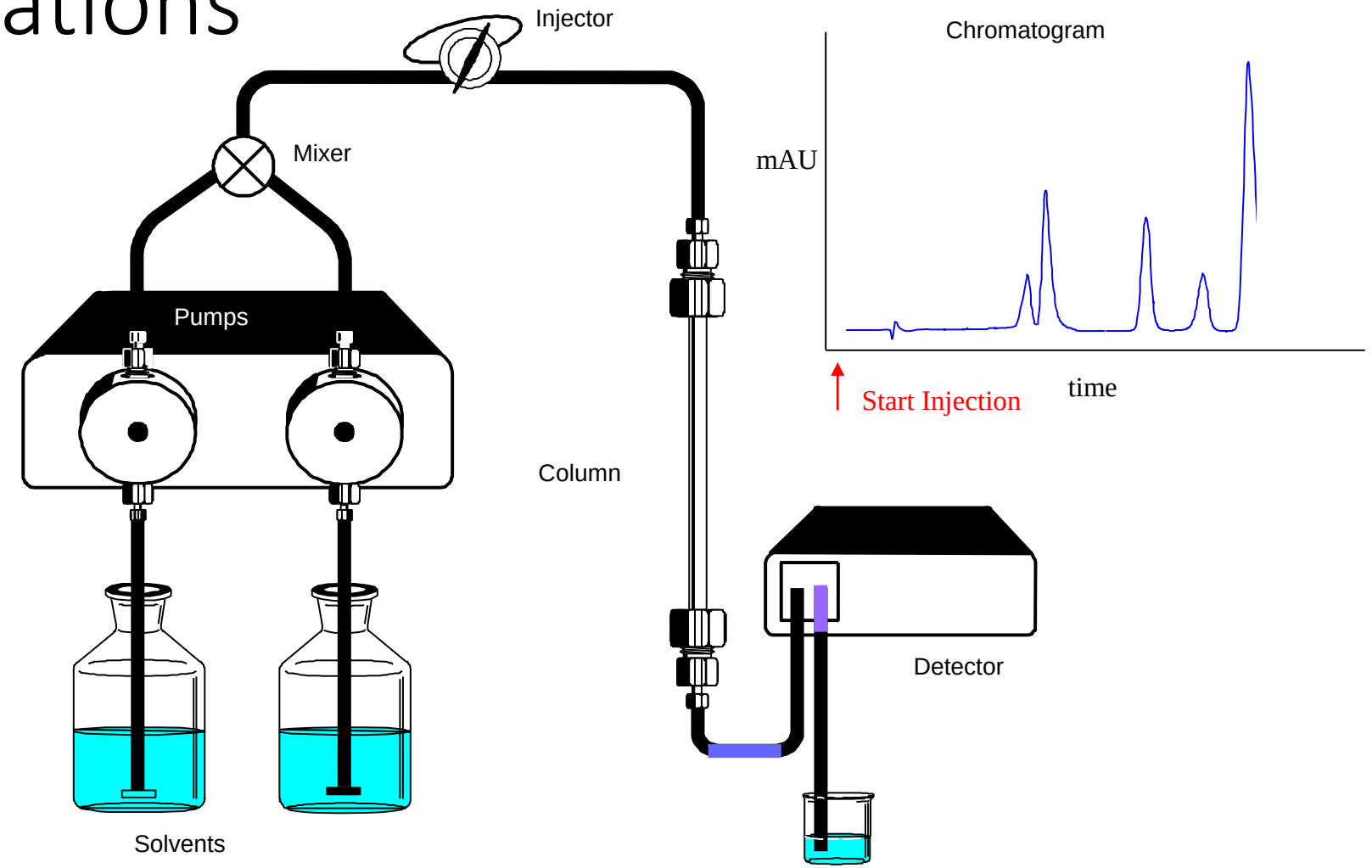

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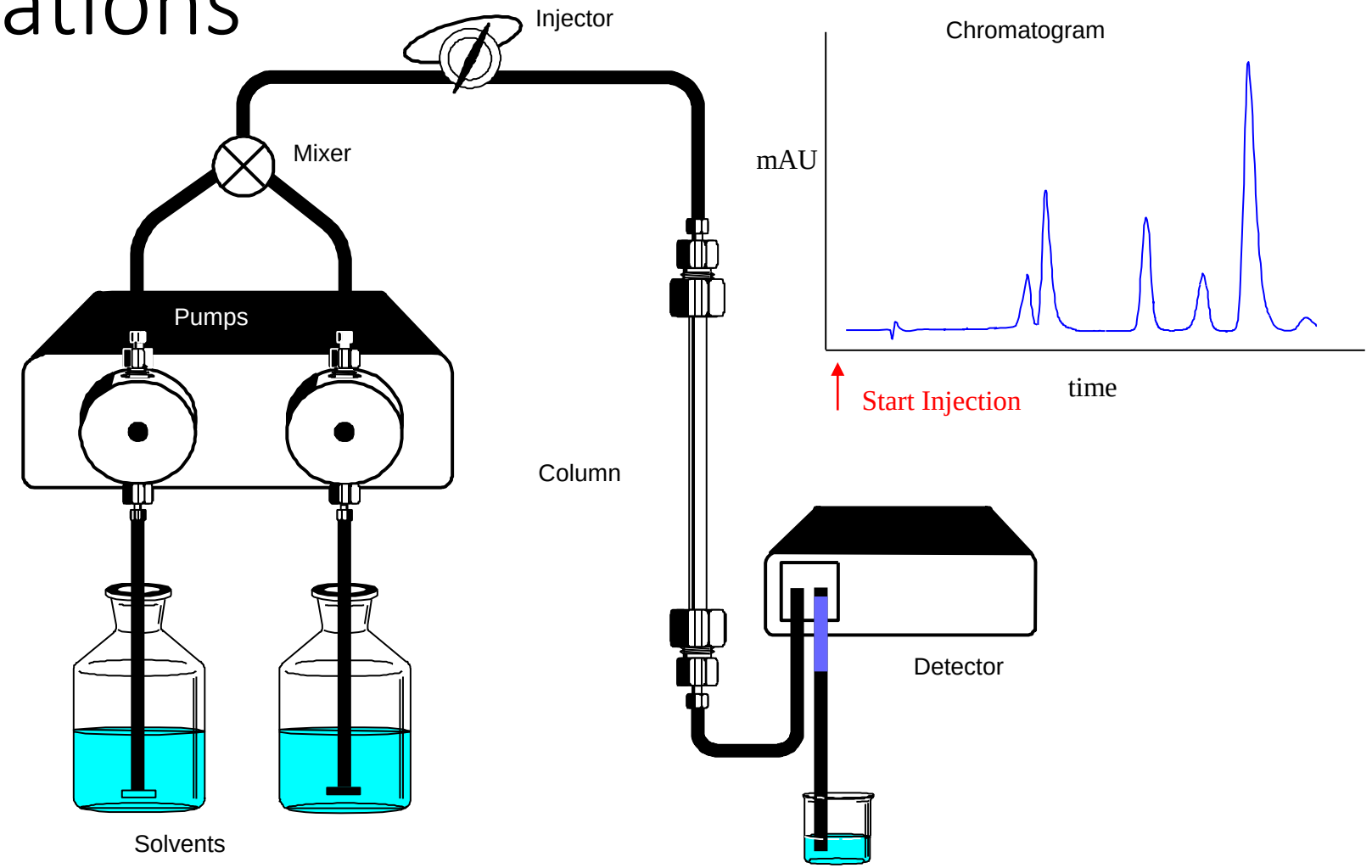

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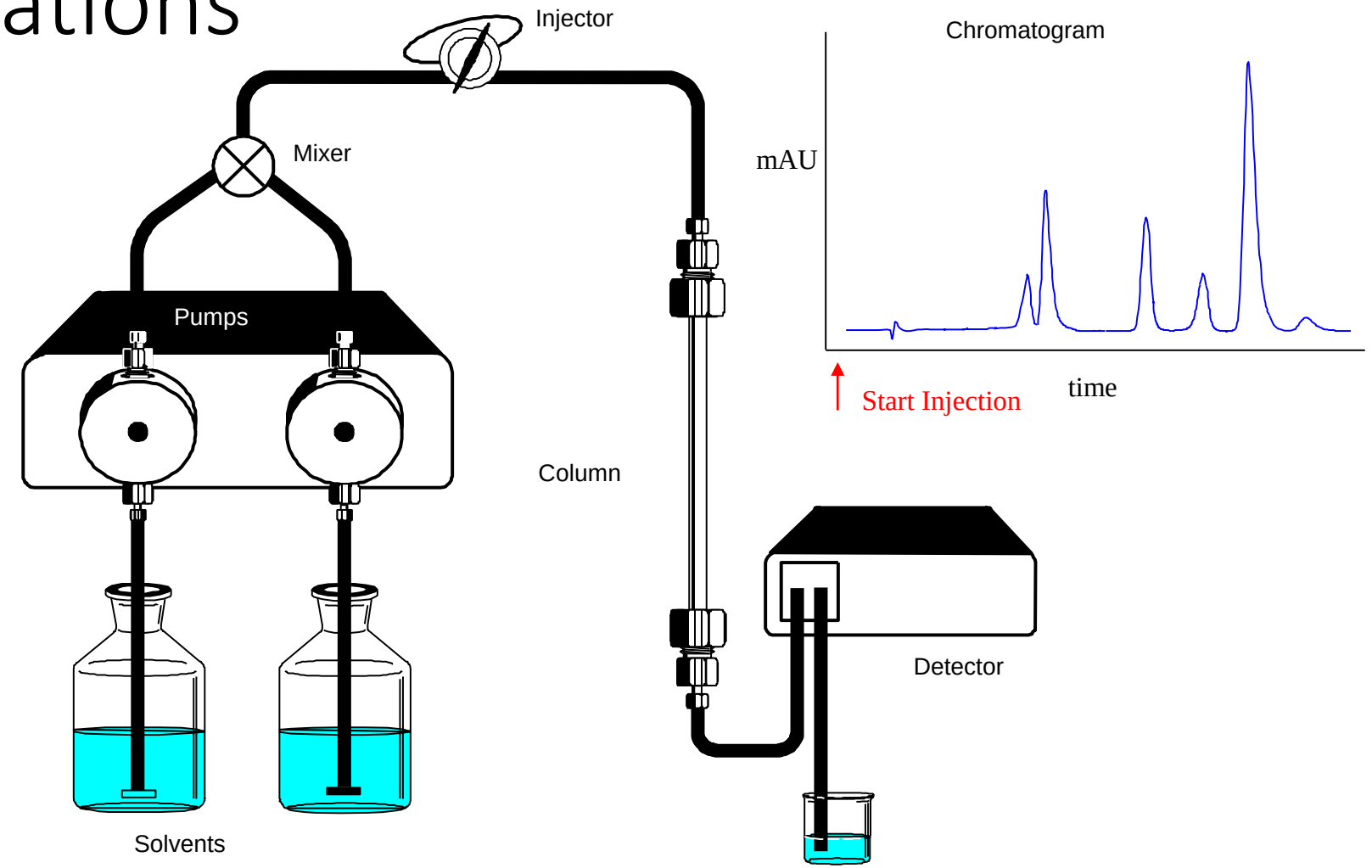
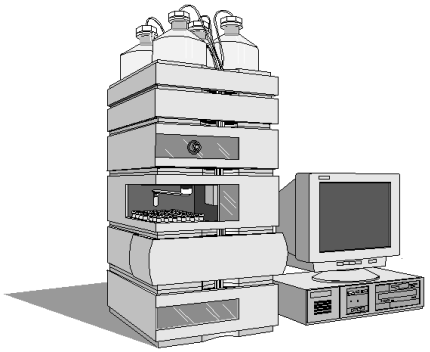
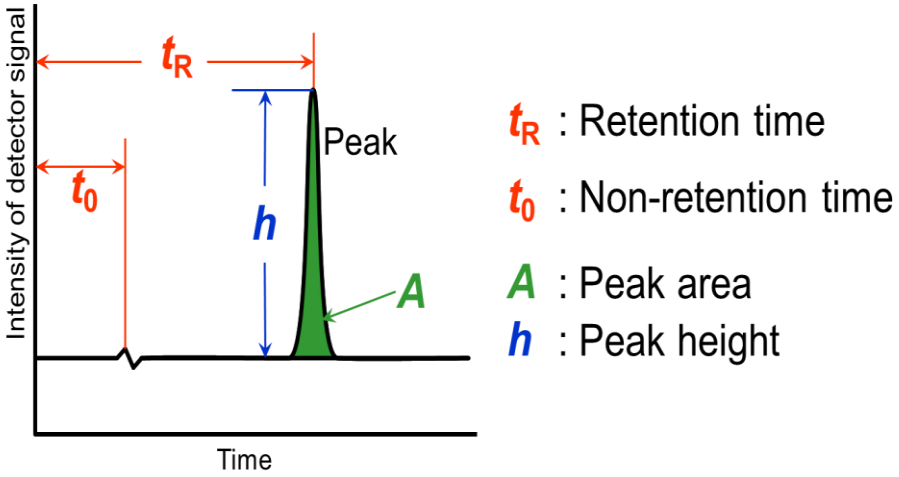


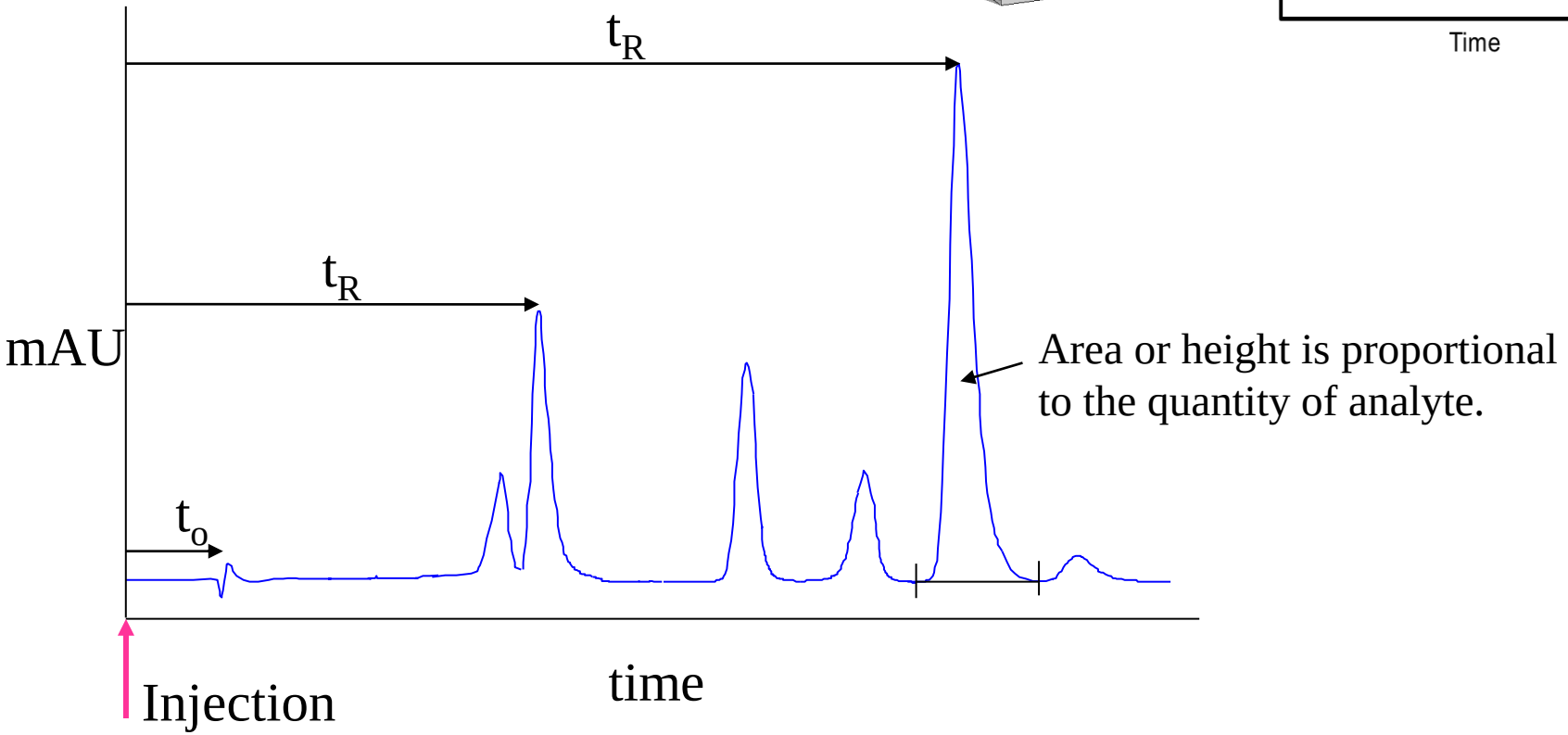
The Chromatogram

t_o - elution time of unretained peak

t_R- retention time - determines sample identity







APPLICATIONS

Some common applications for HPLC technique:

- This technique is used for chemistry and biochemistry research analyzing complex mixtures, purifying chemical compounds, developing processes for synthesizing chemical compounds, isolating natural products, or predicting physical properties. It is also used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate product stability and monitor degradation.
- In addition, it is used for analyzing air and water pollutants, for monitoring materials that may jeopardize occupational safety or health, and for monitoring pesticide levels in the environment. Federal and state regulatory agencies use HPLC to survey food and drug products, for identifying confiscated narcotics or to check for adherence to label claims.

HPLC Applications

Chemical

polystyrenes
dyes
phthalates

Bioscience

proteins
peptides
nucleotides

Pharmaceuticals

tetracyclines
corticosteroids
antidepressants
barbiturates

Consumer Products

lipids
antioxidants
sugars

Environmental

polyaromatic hydrocarbons
Inorganic ions
herbicides

Clinical

amino acids
vitamins
homocysteine

Advantages of High-Performance Liquid Chromatography

High separation capacity, enabling the batch analysis of multiple components

Superior quantitative capability and reproducibility

Moderate analytical conditions

- Unlike GC, the sample does not need to be vaporized.

Generally high sensitivity

Low sample consumption

Easy preparative separation and purification of samples

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