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## REVIEW ARTICLE

### Micro extraction Techniques in Analysis of Drugs

Dr. R. Vani<sup>1\*</sup>, Rafia Tabassum<sup>2</sup>, Saba Khan<sup>3</sup>, Dr M.sunitha<sup>4</sup>

<sup>1</sup>Head of Department, Pharmaceutical Analysis and QA, Shadan Women's College of Pharmacy, Hyderabad

<sup>2</sup>Post-Graduate Student, Shadan Women's College of Pharmacy, Hyderabad

<sup>3</sup>Post-Graduate Student, Shadan Women's College of Pharmacy, Hyderabad

<sup>4</sup>Principal, Shadan Women's College of Pharmacy, Hyderabad

#### ABSTRACT

Analytical methods comprise of a few stages including; examining, test preparation, analysis, figurings and measurable assessment of the outcomes. Among these means, test arrangement is the most time consuming. Consequence of studies demonstrated that more than 60% of examination time is spent for test planning. Test arrangement takes follows two principle points: Sample tidy up and fixation. The present pattern of micro extraction procedures is gone for a solid and precise examination of contaminants from complex examples. Micro extraction is an extraction system where the volume of the extricating stage is little in connection to the volume of the specimen, and extraction of analytes is not thorough. Among them, SPE is the most prominent for medication examination and has turned into a fundamental device in labs everywhere throughout the world. It has additionally generally supplanted more seasoned methods. The advancement of SPE has been quick and went with numerous upgrades. One of these upgrades is MIPs. Due to their particular and specific properties, their utilization will most likely be more extensive later on, particularly in scientific, clinical, pharmaceutical and biochemical investigations. The on-line TFCLC/MS technique was appropriate for TCMs pharmacokinetic learn at a low measurement level. Later on, miniaturized scale channel SPME will be connected in mechanization as the covering could be put on the internal surface of the smaller scale channel and just a little measure of test will be required. This review will attempt to provide an overview as well as a theoretical and practical understanding of the use of microextraction technologies for drug analysis.

**Keywords:** Analytes, affinity, microextraction, SPME, stationary phase, MIPs, TCMs.

#### ARTICLE INFO

##### AUTHOR DETAILS

**Dr R. Vani**

Head of Department,  
Pharmaceutical Analysis and QA,  
Shadan Women's College of Pharmacy, Hyderabad

**MS-ID: AJMPS3564**



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**1. Introduction**

**Principle of Extraction:**

The extraction effectiveness is dictated by the partitioning of analyte between the specimen lattice and the extraction stage.

$$\text{Partition coefficient } k = \frac{\text{CONCENTRATION OF SOLUTE IN PHASE 1}}{\text{CONCENTRATION OF SOLUTE IN PHASE 2}}$$

The higher the fondness the analyte has for the extraction stage with respect to the example lattice, the more noteworthy the measure of analyte extracted.

Thus while the objective of miniaturized scale extraction is to extricate in view of balance dividing [i.e. the objective is to remove as close as would be prudent to 100% of the analytes from a sample]. Partitioning is controlled by the physicochemical properties of the analytes, the specimen framework and the extraction stage. Where test grid and extraction stage piece are consistent, the level of parceling and consequently the rate of analyte separated will be steady. Since apportioning is not subject to analyte fixation, evaluation of test focus might be resolved from outright sum extricated.

**2. Classification Micro extracton**

Classification Of Micro extraction Techniques(Mets)

- Solid Phase Extraction
- Solid Phase Microextraction (SPME)
- Liquid Phase Microextraction (LPME)
- Molecularly Imprinted Polymers (MIPS)
- Turbulent Flow Chromatography (TFC)

**Solid phase extraction:**

Solid phase extraction (SPE) is an extraction method that uses a strong stage and fluid stage to seclude one or onetype of analyte from an answer. It is generally used to tidy up a specimen before utilizing a chromatographic or other logical strategy to quantify the measure of analyte(s) in the example

**Mechanism of Solid Phase Extraction Process:**

The most widely recognized maintenance components in SPE depend on vander Waals powers ("nonpolar interactions"), hydrogen holding, dipole-dipole strengths ("polar" cooperations) and cation anion interactions ("ionic" connections). Switched stage includes a polar or reasonably polar specimen network (portable stage) and a nonpolar stationary stage. The analyte of intrigue is ordinarily mid-to nonpolar. Retention of natural analytes from polar arrangements (e.g. water) onto these SPE materials is expected essentially to the alluring strengths between the carbon hydrogen bonds in the analyte and the useful gatherings on the sorbent surface. These nonpolar – nonpolar appealing powers are generally called vander Waals strengths or scattering powers. The nonpolar dissolvable, which can disturb the forces between the sorbent and compound, is utilized to elute an adsorbed compound from a switched stage SPE tube or circle. Typical stage include a polar analyte, a mid-to nonpolar

lattice (e.g. CH<sub>3</sub>)<sub>2</sub>CO, chlorinated solvents and hexane) and a polar stationary stage. Maintenance of an analyte under ordinary stage conditions is essentially because of connections between polar utilitarian gatherings of the analyte and polar gatherings on the sorbent surface. These incorporate hydrogen bonding, π-π associations, among others. A compound adsorbed by these instruments is eluted by passing a dissolvable that upsets the coupling component, for the most part a dissolvable that is more polar than the specimen's framework.

**Solid Phase Extraction Process**

The SPE procedure can give tests that are in arrangement, free of meddling framework parts and thought enough for identification. Solid phase extraction is accomplished through the collaboration of three parts: the sorbent, the analyte and the dissolvable. The analyte must be pulled in more emphatically to the sorbent than to the framework. The best strong stage extraction instrument and systems are: characterized by the qualities of the analyte in the specimen.

The SPE process can be performed in a two ways:

On-line-In disconnected SPE eluate from the cartridge is brought into the chromatograph by method for an infusion valve.

Off-line-In on-line SPE the extraction cartridge is embedded as a component of chromatographic equipment, as circles or high weight stream of the portable stage.

**Steps of Solid Phase Extraction Process:**

**Step 1:** Select the proper SPE tube or Disk, Selecting SPE tube size

**Table-1:** Criterion for the selection of SPE tube size

If your sample size is <1ml	Use tube size 1ml
1mL to 250mL and the extraction speed is not critical	3ml
10mL to 250mL and higher sample capacity is needed	12, 20 or 60 ml
1mL to 250mL and a fast extraction procedure is required	6 ml

**Table 2:** Criterion for the selection of SPE disk size

If Your Sample is 100mL to 1 liter	Use Disk Size 47mm
>1 liter and higher sample capacity is needed	90 mm

**Selecting an SPE tube:** Bed weight Reversed stage, ordinary stage, and adsorption-sort methods. The mass of the mixes to be separated ought not be more than 5% of the mass of the pressing in the tube. As it were, whether you are utilizing a 100mg/1mL SPE tube, don't stack more than 5mg of analytes.

**Step 2:**

Condition the SPE tube or Disk -To condition the SPE tube pressing, flush it with up to one tube-brimming with solvent before removing the specimen. For circles, utilize a volume of 5-10 ml.

**Step 3:**

Add the specimen -Precisely exchange the specimen to the tube or supply, utilizing a volumetric pipette, micropipette. The example must be in a shape that is perfect with SPE.

**Step 4:**

Wash the pressing -In the event that mixes of intrigue are held on the pressing, wash off undesirable, un-held materials utilizing an indistinguishable arrangement from a part of which the specimen was broken up, or another arrangement that won't expel the sought mixes. Normally close to a tube volume of wash arrangement is required, or 5-10 ml for SPE circles.

**Step 5:** Elute the mixes of intrigue- Flush the pressing with a little volume (normally 200µl to 2 ml contingent upon the tube size, or 5-10 ml relying upon the plate size) of an answer that evacuates mixes of intrigue, however abandons any pollutions not expelled in the clothes washer step. Gather the elute and further get ready as suitable.

**Applications of SPE**

**Biological liquids** - Various papers in the zone of SPE–HPLC report that it is a settled system. Eg. Assurance of methylated arsenic species in human pee.

**Waters** - Different poisons were resolved in fluid examples after SPE or SPME.

**Food, refreshments and rural** -The utilization of SPE in the assurance of different chemicals in sustenance has expanded quickly in the most recent decade and SPE strategies have supplanted a significant number of the customary techniques for test pretreatment. For instance, the majority of lipid substance of fats and oil in sustenances has confounded the examination of pesticide deposits and also other compound contaminants. The late cases of sustenance investigation for unstable flavor mixes indicate continuous utilization of SPE– GC strategies however particularly an expansion in the utilization of SPME–GC.

**Air and gas** -The investigation of air and vaporous examples can be subdivided into examination of unpredictable mixes (VOCs) and of particulate matter (PM).

**Solid Phase Microextraction (SPME)**

Microextraction strategies have been regarded as the most appealing for the pretreatment of complex test networks prior to chromatographic and capillary electrophoresis processes because they empower quick investigation at low operating costs and with no environmental pollution. The recent incline in sample preparation processes focuses on how to scale down the procedure and which medium to use for the extraction and pre-centralization of sample components.

**Principle of Solid Phase Micro extraction:**

SPME depends on an altered syringe which contains stainless steel microtubing inside its syringe needle. This microtubing has an about 1-cm. melded silica fiber tip which is covered with a natural polymer. The covered silica fiber can be moved between two positions, inside

and outside the needle, with a plunger as in the case of a typical syringe the measurement of the syringe needle lodging the microtubing and coated silica fiber is very little expanded in comparison with an ordinary GC syringe. Thus, by method for this straightforward hardware several steps of test readiness are consolidated in one gadget.

Extraction and improvement of the analyte is finished by the covering in the position outside the syringe needle. Penetration of the septum of a GC injection port is conceivable if the fiber was pulled back into the syringe needle. Desorption of the analyte and exchange to the slim is performed after again moving the fiber to the position outside the syringe.

**Advantages of Solid-Phase Micro extraction:**

- Simplicity, rapidity, dissolvable end.
- High affectability
- Small test volume
- Low cost.

**Design of SPME Device**-SPME is an adjusted syringe-like instrument. The combined silica fiber, having a small estimate and round and hollow shape, is connected to stainless-steel tubing that is utilized to provide additional mechanical quality to the fiber assembly for rehashed examining. This stainless-steel tubing is associated to a specially composed syringe-like instrument. The fused silica fiber is covered with a generally thin film of a few polymeric stationary stages. The fiber gathering is reusable and replaceable. The small estimate and round and hollow geometry of fiber has some advantages, for example, simple position of the sorbent fiber coating into a specimen or headspace above the sample to extricate the analytes; likewise it can be easily placed in desorption load of GC or interphase of the HPLC with no alteration of GC or HPLC. Plunger development and timing must be controlled painstakingly to perform adsorption and desorption accurately. It is essential for field examining to forestall loss of analyte during transport. To do this, the needle opening of SPME device must be fixed by utilizing a septum or potentially by cooling the needle.

**Working With SPME Device :**

Amid the SPME operation, the fiber is first drawn into the syringe needle, then lowered into the vial (which is fixed with a septum sort top) by squeezing the plunger. The fused silica fiber of appropriate covering is utilized which is needy upon the way of the analyte. The fiber ought to be cleaned before breaking down any specimen in order to evacuate contaminants that desorption chamber of HPLC by running dissolvable. Presently the cleaned fiber covering is presented to a sample matrix for a foreordained, settled period, which brings about the adsorption of the analyte on the fiber covering. This extraction can be performed in two ways ;

- Headspace SPME or HS-SPME where fiber is uncovered in the vapor stage over a vaporous, fluid, or strong example.
- Direct Immersion DI-SPME, where the fiber is specifically immersed in fluid tests.

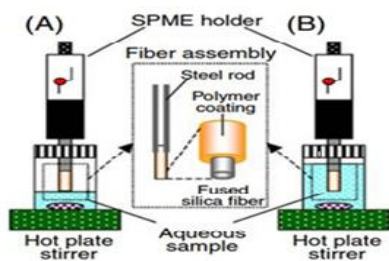


Fig 1:SPME (A) HS-SPME. (B)DI-SPME

### Applications of SPME In Various Fields:

**Environmental applications**-In the early formative time frame the majority of applications were in ecological science. For the most part natural mixes have been considered, and pesticides, herbicides and other organically dynamic mixes in aqueous tests.

**Applications in food chemistry** -Nourishment investigation is essential for the assessment of nutritional esteem, for quality control of crisp and processed items and the checking of food additives and other harmful contaminants. In general, flavor is delicate to compositional adjustments on account of nourishment (natural product, wine, etc.).

**Applications to biological fluids**-Test arrangement is a standout amongst the most critical steps in the investigation of organic liquids and compounds in natural networks.

### Liquid Phase Micro extraction(LPME)

Test readiness can incorporate cleanup techniques for exceptionally intricate (filthy) specimens. This progression should likewise convey the analytes to a suitable focus level. LPME is a solvent-minimized sample pretreatment procedure of LLE, in which just a few  $\mu\text{L}$  of solvent are required to think analytes from different specimens instead of hundreds of  $\text{mL}$  required in traditional LLE. It is compatible with slender gas chromatography (GC), capillary electrophoresis (CE) and HPLC. In LPME, extraction typically happens into a small measure of a water-immiscible solvent (acceptor stage) from a fluid sample containing analytes (giver stage).

### Advantages

- It is a quick, straightforward, dissolvable free and sensitive strategy for the extraction of analytes;
- It is a straightforward, effective adsorption/desorption method;
- It is good with analyte separation and recognition by high performance liquid chromatography with bright identification (HPLC - UV);
- It gives straight outcomes to a wide range of convergences of analytes;
- It has a little size, which is convenient for outlining convenient gadgets for field examining; and,
- It gives exceptionally reliable, quantify able results from low fixations of analytes.

### Disadvantages

- Relatively low recommended operating temperature (by and large in the extend 240–2800C);
- Their insecurity and swelling in organic solvents (incredibly limiting their use with HPLC);
- Fiber breakage;

- Stripping of coatings; and,
- The twisting of needles and their expense.

### Types of Liquid Phase Micro extraction

It can be isolated into three primary classes

- (1) Single-drop micro extraction (SDME)
- (2) Dispersive liquid-liquid micro extraction (DLLME)
- (3) Hollow-fiber micro extraction (HF-LPME).

### Single-Drop Miniaturized Scale Extraction (SDME):

SDME, utilizing regularly 1–8  $\mu\text{L}$  of an organic solvent at the tip of a small scale syringe, has evolved from LPME. After extraction, the microdrop is withdrawn again into the syringe and transferred for further examination.

**By and by, two primary methodologies can be used to perform SDME:**

- a) Direct drenching (DI)- SDME
- b) Headspace (HS)- SDME

**In DI-SDME**, a drop of a water-immiscible solvent is suspended specifically from the tip of a micro syringe needle inundated in the aqueous sample.

### (b) Headspace (HD)-SDME

In HS-SDME, a small scale drop of appropriate solvent is put in the headspace of the sample arrangement or in an flowing air sample stream to concentrate unstable analytes.

**Advantage:** A more extensive assortment of solvents to look over. Extraction and infusion must be performed independently, utilizing diverse contraption.

### Dispersive Fluid Micro extraction (DLLME)

This system that uses  $\mu\text{L}$  volumes of extraction dissolvable alongside a couple  $\text{mL}$  of dispersive solvents. In this strategy, a cloudy solution is get shaped when an appropriate mixture of extraction and dispersive solvents is injected into a fluid specimen containing the analyte of intrigue. Hydrophobic solutes are rich in the extraction dissolvable, which is dispersed into the mass watery arrangement. After centrifugation, analytes in the settled phase can be dictated by utilizing conventional analytical techniques. In DLLME, the dispersive dissolvable plays a key role that helps extraction dissolvable frame fine droplets in fluid examples, representing about 97–99% of the aggregate volume of the extraction blend. Contrasted with other methods, bounteous surface contact between fine beads and the analyte in DLLME accelerates the mass exchange procedures of analytes from oceanic stage to natural stage, which not just enormously improves extraction productivity additionally, defeats the issue of the time taken.

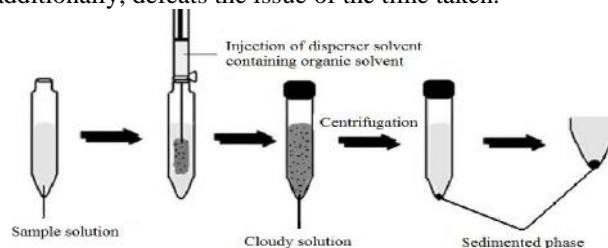


Fig 2:Different steps of DLLME

### Advantages

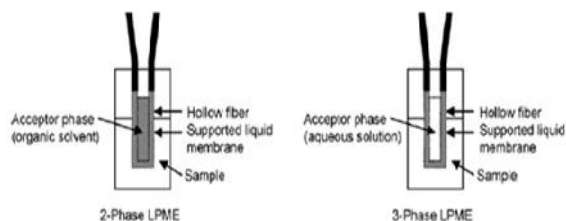
- Simplicity of operation
- Rapidity
- Low cost
- High recuperation

- High advancement calculate
- Very short extraction time (a few moments).

**Hollow Fiber Micro extraction (HF-LPME):**

They utilized the fundamental standard of supported liquid layer (SLM), surprisingly, insimple, reasonable, dispensable extractionunits for the fluid microextraction (LLLME) using polypropylene HF's as themembrane.

The example vial is loaded with the aqueoussample. A short bit of a permeable HF may be either a pole with a shut base or a u-shapewhere both finishes are associated with guidingtubes.



**Fig 3:**2-phase and 3-phase LPME

Before extraction, the HF is first dipped in the natural dissolvable for a couple times to immobilize dissolvable in the pores, and excess solvent is expelled. The dissolvable is immiscible with water to guarantee that it stays inside the pores amid the extraction with no spillage to the fluid example. The natural solvent forms a thin layer inside the mass of the HF. The extraction dissolvable must be compatible with the HF so that the pores in the mass of the HF can be filled completely. The acceptor arrangement then fills the lumen of the HF. This acceptor arrangement can be an organic dissolvable (the same as that utilized for the organic dissolvable as a part of HF pores) bringing about a two-phase extraction framework, or the acceptor solution might be an acidic or soluble aqueous solution, bringing about a three-stage extraction system. In the two-stage LPME framework, the target analytes are separated from the aqueous sample and into the natural dissolvable (acceptor solution) introduce both in the permeable divider and inside the lumen of the HF.

**Applications**

All LPME strategies can be used successfully for extraction of target analytes from different example arrangements.

**Table 3:** Chosen Applications For Medication Examination Utilizing LPME And Other Related Micro extraction Procedures

Analyte	Matrix	Extraction mode	Analytical method
Anti-fouling agent	Water	DI-SDME	GC-ECD
Hypericin, Hyperforin	Plasma , Serum	DI-SDME	HPLC-UV-FLD
Local anaesthetic	Urine , Plasma, Serum	DI-SDME	HPLC-UV
Fentanyl	Urine , Plasma, Serum	DI-SDME	HPLC-UV

Cocaine and its metabolites	Saliva	HF-LPME	GC-PDHID
Basic drugs	Plasma , Urine	HF-LPME	GC-NPD
Anti-malarial agents (Chloroquine)	Urine, Tablet	DI-SDME	HPLC-UV

**Molecularly Imprinted polymers (MIPs)**

Molecularly engraved polymers (MIPs) are polymers arranged in nearness of a template that serves as a shape for the development of template reciprocal restricting locales. Thus, MIPs can be programmed to perceive a large variety of target structures with counter acting agent like affinities and selectivities. MIP is based on the arrangement of a complex between an analyte (template) and a practical monomer. In the presence of a vast abundance of a cross-linking agent, a three-dimensional polymer network is framed. After polymerization handle, the template is expelled from the polymer leaving particular acknowledgment destinations reciprocal in shape, size and substance usefulness to the template atom. More often than not, intermolecular interactions like hydrogen bonds, dipole-dipole and ionic connections between the template particle and useful groups present in the polymer framework drive the molecular acknowledgment marvels. Along these lines, the resultant polymer perceives and binds selectively just the layout particles.

**Molecular Engraving Process**

Format plan and monomer choice are two of the most critical elements of the Atomic engraving process. For analytical applications, the "MIP Rule of 6" ought to be followed when conceivable:

Never utilize the analyte as a template unless there is totally no alternative.

Make levelheaded decisions about which regions of an analyte are likely to command the best sorts of interaction in a low dielectric medium (organic solvent) and after that fuse these elements in a simple of the analyte molecule

Select monomers that are likely to form solid connections in the chosen solvent (e.g., Bronsted acids or bases/H-benefactors or acceptors/nonpolar groups, and so on.)— this will increase capacity and impact homogeneity of the restricting pits.

Choose formats and monomers that will be dissolvable in the porogenic solvent to be utilized as a part of the polymerization— this may appear glaringly evident yet it sometimes requires doing dissolvability tests

Ensure quite far that the template-monomer blend is stable and does not experience side reactions under the polymerization conditions.

Consider the way of the grid from which the analyte will in the end be extracted while selecting the cross linking monomer - a scope of di- or triune saturated cross connecting



monomers with changing sciences are available to make the permeable natural system material.

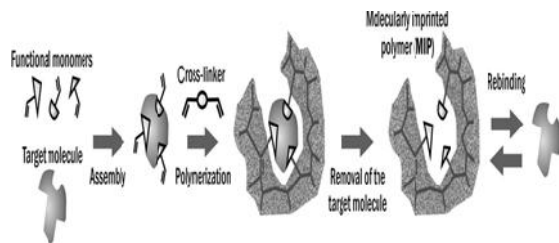


Fig 4: Procedure of readiness of MIPs

1. Their utilization as tailor-made separation materials,
2. Their utilization in natural combination and enzyme innovation as catalytically active polymers or compound copies.
3. As sensors in biosensor-like configuration
4. Plot sketching out the principle applications conceived for MIPs is appeared in figure.

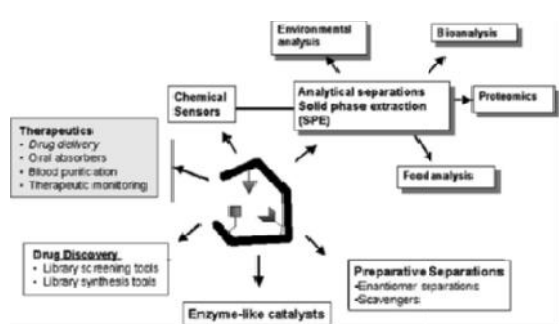


Fig 5: Scheme outlining the main application envisaged for MIPs

### Turbulent Flow Chromatography (TFC)

Turbulent Flow Chromatography (TFC) is a technique that joins high-throughput and high reproducibility by method for separating analytes from different frameworks with reduced sample taking care of.

#### Principle of TFC –

Turbo Flow techniques depend on the direct injection of organic examples without previous extraction or treatment a section pressed with large particles. These vast particles have an additional level of selectivity by means of the stationary phase science added to them. After the sample is infused onto a Turbo Flow column the high stream rate (cf. 1.5 – 5.0 ml/min) generates turbulent stream conditions inside the column. Since 100% watery versatile buffers are utilized, the little analyte atoms are retained by means of dissemination into the molecule pores, while the proteinaceous material is washed to waste. Once the mixes of intrigue are extracted from the organic network, they are eluted from the Turbo Flow segment onto the analytical segment with a volume of solvent, which has been put away in a holding circle. The holding circle ought to have a volume no less than ten times that of the Turbo Flow section and is typically loaded with natural portable stage (for reversed stationary stage) or pH buffered solutions (for particle exchange phases). As the analytes are discharged from the Turbo Flow column they are exchanged with the

pumping solvent (at an impressively bring down stream rate than that utilized amid stacking) through the tee rotor seal in the second valve and blended with the pumping dissolvable from the investigative system.

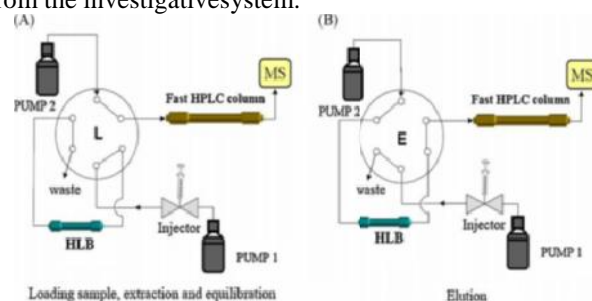


Fig 6: Schematic representation of on-line turbulent-flow column-switching

### Online Turbulent-Flow Column-Switching:

A schematic chart of the on-line TFCLC/MS instrument set-up in view of column switching and quick HPLC is appeared in Fig. A pump was utilized to convey a high flow through a hydrophilic-lipophilic balanced (HLB) turned around stage section to load and wash the specimen, and in this manner to flush and equilibrate the extraction segment. Solvent A was utilized as the dissolvable for this pump. An Agilent 1100 HPLC framework (furnished with a binary pump, an on-line degasser, an auto plate-sampler, and a thermostatically controlled section compartment) was utilized to deliver an angle stream to elute the analytes from the extraction segment and to perform these separation on a quick HPLC segment. Two Rheodyne six port exchanging valves were used for the section exchanging purposes. The L and E in the focal point of the every six-port valve designate "stack" or "elute" positions for the flow way.

#### Applications of TFC

- In sustenance and ecological investigation.
- In the treatment of natural samples containing a lot of proteins, such as blood plasma.
- Isolation of veterinary medications and growth promoters from sustenance.
- A arrangement that has increased wide use, particularly in the clinical field, to increase throughput on such system.

### 3. Future Trends

Micro extraction techniques – To enhance the execution of micro extraction techniques, computerization should be brought into test arrangement, and will be a noteworthy research subject later on. Computerization may bring about better reproducibility contrasted with manual strategies. At present, a few computerized strategies have been created in plant tests, including direct drenching and headspace SPME, and SPME combined with chromatographic analysis, and so on. Later on, micro channel SPME will be connected in mechanization as the covering could be set on the inward surface of the micro channel and just a little measure of test will be required. In any case, full robotization of micro extraction procedures gives off an impression of being extremely troublesome for plant tests. This is because of the way that specific specimen

pretreatments are vital preceding micro extraction of plant tests at present, and that robotization of these example pretreatment forms (mixing, homogenization, filtration, and centrifugation) is extremely troublesome. Keeping in mind the end goal to enhance mechanization, future work will concentrate on direct micro extraction examination of plant tests, for example, saffron without pretreatment steps.

#### 4. Conclusion

It is outstanding that specimen planning is a standout amongst the most basic strides in the assurance of follow contaminations in various ecological frameworks. As of late, example arrangement strategies have been altogether moved forward. As the utilization of pharmaceuticals is expanding, more specimen planning methods are being created. The scaling down of examining strategy and the need to decrease testing time and dissolvable volume has prompted the improvement of different microextraction strategies. The presentation of ionic fluid based and sol-gel arranged ionic fluid extraction stage, which can be utilized for all the micro extraction strategies will give a superior enhancement calculate, higher recuperation, low location confine and higher extraction throughput because of their remarkable properties, for example, unimportant vapor weight, great warm dependability, and high consistency. Among them, SPE is the most well-known for medication examination and has turned into a fundamental device in labs everywhere throughout the world. It has additionally to a great extent supplanted more seasoned methods. The advancement of SPE has been quick and went with numerous enhancements. One of these changes is MIPs. Due to their particular and specific properties, their utilization will most likely be more extensive later on, particularly in measurable, clinical, pharmaceutical and biochemical investigations. All LPME methods can be used viably for extraction of target analytes from different example arrangements. Engraved polymers are currently entrenched as materials for sub-atomic acknowledgment, chromatographic partition, and systematic example advancement yet their utilization as dynamic biomedical gadgets is still in the early phases of improvement. The on-line TFCLC/MS strategy was reasonable for TCMs pharmacokinetic learn at a low measurements level.

#### 5. Acknowledgement

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