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Research Article

MICROEXTRACTION TECHNIQUES IN ANALYSIS OF DRUGS

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ABSTRACT
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. In many cases only a small fraction of the initial analyte is extracted for analysis. The extraction efficiency is determined by the partitioning of analyte between the sample matrix and the extraction phase. The higher the affinity the analyte has for the extraction phase relative to the sample matrix, the greater the amount of analyte extracted. Microextraction techniques represent an important contribution to the improvement of sample preparation performance, which especially addresses the issues of miniaturization, automation, onsite analysis, and time efficiency. Sample preparation is essential for isolating desired components from complex matrices and greatly influences their reliable and accurate analysis. Solid-phase microextraction (SPME) is a new and effective sample preparation technique. Fibers and capillary tubes coated with an appropriate stationary phase are usually used for SPME, but alternative microextraction techniques are also used.

Keywords: Analyte, affinity, microextraction, SPME, stationary phase.

INTRODUCTION

Microextraction is defined as an extraction technique where the volume of the extracting phase is very small in relation to the volume of the sample, and extraction of analytes is not exhaustive. In most cases only a small fraction of the initial analyte is extracted for analysis. The extraction efficiency is determined by the partitioning of analyte between the sample matrix and the extraction phase. The higher the affinity the analyte has for the extraction phase relative to the sample matrix, the greater the amount of analyte extracted[1]. Thus while the goal of microextraction is to extract based on equilibrium partitioning i.e. the goal is to extract asnear as possible to 100% of the analytes from a sample[2].

Partitioning is controlled by the physicochemical properties of the analyte, the sample matrix and the extraction phase. Where sample matrix and extraction phase composition are constant, the degree of partitioning and hence the percentage of analyte extracted will be constant also. Because partitioning is not dependent on analyte concentration, quantification of sample concentration may be determined from absolute amount extracted. Microextraction of drugs has to date, found its greatest application with the technique of solid-phase microextraction (SPME) and in particular fibre SPME. SPME is a relatively new sample preparation method, which has the potential to significantly simplify sample preparation, and integrate it with sample analysis[1].

Microextraction techniques represent an important contribution to the improvement of sample preparation performance, which especially addresses the issues of miniaturization, automation, onsite analysis, and time efficiency. Actually, different types of microextraction techniques were reported in literature a long time ago[3,4], but the field gained in significance with the invention of solid-phase microextraction (SPME) in 1990[5],which later became commercially available. In the commercial version of this technique, a small diameter fiber coated with a small volume of stationary phase is placed either in an aqueous or a gaseous sample. The analytes partition into the stationary phase and are subsequently thermally desorbed in the injector of a gas chromatograph (GC). During recent years, SPME has gained substantially in popularity, and from this point, several alternative approaches have been

introduced, such as in-tube SPME designed primarily for high-performance liquid chromatography (HPLC)[6]. In parallel to the development of SPME, attention has also been directed to the utility of small volumes of liquids for analytical extractions, namely liquid-phase microextraction (LPME). This field was basically initiated in 1996 when the use ofsmall droplets of organic solvents suspended from the tip of amicro-syringe was described for the first time[7,8], and thisapproach was subsequently refined by implementing the use ofporous hollow fibers for protection of the extracting liquids[9].

Types of microextraction techniques[10]

1. Solid phase extraction (SPE)
2. Solid phase microextraction (SPME)
3. Liquid phase microextraction (LPME)
4. Molecularly imprinted polymers (MIPs)
5. Turbulent flow chromatography (TFC)

1. SOLID PHASE EXTRACTION

Solid phase extraction (SPE) is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type, of analyte from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantify the amount of analyte(s) in the sample[11].

Principles of solid-phase extraction[12]

It consists of mainly four steps.

1. Conditioning

Before extraction of analytes can begin, the sorbent bed must be prepared so that it will make intimateand effective surface contact with the liquid sample solution. Most commonly, conditioning is accomplished by passing a small volume of methanol or acetonitrile through the SPE extraction tube. Some of this organic solvent is adsorbed on the surface of the sorbent particles, making the surface more hydrophilic and thus more compatible with a primarily aqueous sample solution. Without such treatment the surface of many common sorbents is hydrophobic and is poorly wetted by the hydrophilic sample solution. The polar liquid flows in small channels through the solid phase without making the necessary close surface contact. The conditioning step also serves to elute any adsorbed organic impurities from the SPE bed.

2. Adsorption

The liquid sample is passed through the packed SPE device with the aid of a gentle vacuum (applied to the end of the column), applied pressure or a pump. The flow rate should be reasonably constant. The flow rate and sorbent bed dimensions will depend on the particle size of the solid extractant. Very small particles (e.g., 10 mm) are more efficient than columns packed with larger particles (e.g.,50–100 mm). Therefore, a smaller bed of the smaller particles may be used.

3. Washing

The most common type of SPE is where organic analytes are extracted from an aqueous sample. The purpose of the washing step is to remove salts and other non-extracted material as completely as possible without eluting any of the desired analytes. Water alone is often the appropriate wash solution, but some solutes may be partially retained by the SPE column and only slowly washed off by water alone. In such cases water containing 10–20% of anorganic solvent might be a better wash liquid. Ofcourse, the wash solution must not contain a percentage of organic solvent high enough to elute the sample analytes.

4. Elution

In the elution step the adsorbed analytes are removed from the solid extractant and are returned to a liquid phase that is suitable for analytical measurement. Most commonly, the eluting phase is an organic liquid, although it is often possible to thermally desorb analytes with the aid of a gas stream. It is usually better to select an eluting solvent that is miscible with water, otherwise the effluent may contain two liquid phases. It is common practice to remove as much of the water as possible from the column just before the elution step. This can be accomplished by applying gentle vacuum for a few minutes or by passing compressed air or nitrogen through the column. Occasionally centrifugation is used to remove liquid from the column.

Solid phase extraction theory[13]

How compounds are retained by the sorbent

➢ **Reversed phase**

Reversed phase separations involve a polar (usually aqueous) or moderately polar sample matrix (mobile phase) and a nonpolar stationary phase. The analyte of interest is typically mid- to nonpolar. Several SPE materials, such as the alkyl- or aryl-bonded silicas (**LC-18, ENVI-18, LC-8, ENVI-8,LC-4,** and **LC-Ph**) are in the reversed phase category.

(Polar - liquid phase, non-polar modified solid phase)

Hydrophobic interactions

- Nonpolar-nonpolar interactions

- Van-der Waals or dispersion forces.

5. Normal phase

Normal phase SPE procedures typically involve a polar analyte, a mid- to nonpolar matrix (e.g. acetone, chlorinated solvents, and hexane), and a polar stationary phase. Polar-functionalized bonded silicas (e.g. **LC-CN, LC-NH2,** and **LC-Diol**), and polar adsorption media (**LC-Si, LC-Florisil, ENVI-Florisil,** and **LC-Alumina**) typically are used under normal phase conditions. Retention of an analyte under normal phase conditions is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface. These include hydrogen bonding, pi-pi interactions, dipole-dipole interactions, and dipole-induced dipole interactions, among others. A compound adsorbed by these mechanisms is eluted by passing a solvent that disrupts the binding mechanism-usually a solvent that is more polar than the sample's original matrix.
(Nonpolar- liquid phase, polar modified solid phase)
Hydrophilic interactions

- Polar-polar interactions
- Hydrogen bonding
- Pi-pi interactions
- Dipole-dipole interactions
- Dipole-induced dipole interactions.

6. Ion exchange

Electrostatic attraction of charged group on compoundto a charged group on the sorbent's surface.

7. Adsorption

(Interactions of compounds withunmodified materials), Hydrophobic and hydrophilic interactions may applyDepends on which solid phase is used.

Mechanism of solid phase extraction process[13]

The most common retention mechanisms in SPE are based on van der Waals forces ("non-polar interactions"), hydrogen bonding, dipole-dipole forces ("polar" interactions) and cation-anion interactions ("ionic" interactions). Reversed phase involves a polar or moderately polar sample matrix (mobile phase) and a nonpolar stationary phase. The analyte of interest is typically mid- to nonpolar. Retention of organic analytes from polar solutions (e.g. water) onto these SPE materials is due primarily to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the sorbent surface. These nonpolar – nonpolar attractive forces are commonly called van der Waals forces or dispersion forces. The nonpolar solvent, which can disrupt the forces between the sorbent and compound, is used to elute an adsorbed compound from a reversed phase SPE tube or disk. Normal phase involve a polar analyte, a mid- to non-polar matrix (e.g. acetone, chlorinated solvents and hexane) and a polar stationary phase. Retention of an analyte under normal phase conditions is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface. These include hydrogen bonding, л- л interactions, among others. A compound adsorbed by these mechanisms is eluted by passing a solvent that disrupts the binding mechanism, usually a solvent that is more polar than the sample's matrix.

Solid phase extraction process[13,14]

The SPE process can provide samples that are in solution, free of interfering matrix components and concentrated enough for detection. Solid phase extraction is achieved through the interaction of three components: the sorbent, the analyte and the solvent. The analyte must be attracted more strongly to the sorbent than to the matrix. The best solid phase extraction mechanism and procedures are defined by the characteristics of the analyte in the sample. The steps of the solid phase extraction process are shown in Fig. 1.

Step 1: Select the proper SPE tube or Disk

- **Selecting SPE tube size**

Criterion for the selection of SPE tube size is mentioned in table no.1

- **Selecting SPE disk size**

Criterion for the selection of SPE tube size is mentioned in table no.1

- **Selecting an SPE tube:Bed weight**

Reversed phase, normal phase, and adsorption-type procedures
The mass of the compounds to be extracted should not be more than 5% of the mass of the packing in the tube. In other words, if you are using a 100mg/1mL SPE tube, do not load more than 5mg of analytes.

Step 2: Condition the SPE tube or Disk

To condition the SPE tube packing, rinse it with up to one tube-full of solventbefore extracting the sample. For disks, use a volume of 5-10 ml.

Step 3: Add the sample

Accurately transfer the sample to the tube or reservoir, using a volumetric pipette or

micropipette. The sample must be in a form that is compatible with SPE.

Step 4: Wash the packing
If compounds of interest are retained on the packing, wash off unwanted, un-retained materials using the same solution in which the sample was dissolved, or another solution that will not remove the desired compounds. Usually no more than a tube volume of wash solution is needed, or 5-10 ml for SPE disks.

Step 5: Elute the compounds of interest
Rinse the packing with a small volume (typically 200µl to 2 ml depending on the tube size, or 5-10 ml depending on the disk size) of a solution that removes compounds of interest, but leaves behind any impurities not removed in the wash step. Collect the eluate and further prepare as appropriate.

Applications of SPE[12]

- **Biological fluids**

The compatibility of SPE coupled with HPLC has been reflected in growing popularity of SPE–HPLC on-line. A number of papers in the area of SPE–HPLC document that it is a well established technique.
Eg. Determination of methylated arsenic species in human urine.

- **Waters**

An interesting example of a simple combination of sample trapping with a non-separation analytical technique was presented by Ackerman et al. [189] when they determined polycyclic aromatic hydro-carbons (PAHs) isolated from water using SPE combined with a direct in-situ measurement by fluorescence /phosphorescence. Various pollutants were determined in aqueous samples after SPE or SPME.

- **Food, beverages and agricultural**

The use of SPE in the determination of various chemicals in food has increased rapidly in the last decade and SPE methods have replaced many of the traditional methods of sample pretreatment.As an example, the bulk of lipid content of fats and oil in foods has complicated the analysis of pesticide residues as well as other chemical contaminants. The recent examples of food analysis for volatile flavor compounds show ongoing use of SPE–GC methods but especially an increase in the use of SPME–GC. Examples of the first type of analysis include trace-level determination of polar flavor compounds in butter by SPE with polymeric sorbent.

- **Air and gas**

The analysis of air and gaseous samples can be subdivided into analysis of volatile compounds (VOCs) and of particulate matter (PM). The method classical SPE followed by GC analysis. The examples include the analysis of volatile hydro-carbons, volatile organic sulfur compounds.

2. SOLID PHASE MICROEXTRACTION (SPME)

Microextraction techniques have been regarded as themost attractive for the pretreatment of complex samplematrices prior to chromatographic and capillaryelectrophoretic processes because they enable rapid analysis at low operating costs and with no environmental pollution.The recent trend in sample preparation processes focuses onhow to miniaturize the process and which medium to use forthe extraction and pre-concentration of sample components.In this section, I review in detail fiber SPME, in-tube SPME(or capillary microextraction), solid-phase dynamic extraction(SPDE), microextraction in a packed syringe (MEPS) andstir-bar-sorptive extraction (SBSE), all of which have beenwidely used for forensic, clinical and pharmaceuticalanalysis. Fiber SPME is the most widely used technique. IntubeSPME was developed primarily to extend SPME tohigh-throughput applications and automated instrumentation.SBSE was developed to increase the sensitivity of SPME.
Various new affinity SMPE sorbents, includingimmunosorbents and MIPs, have been used for the specificpreparation of samples and are also described in this section. Other new microextraction techniques, such as liquid-liquidmicroextraction (liquid-phase[15-17] or single-drop[18] microextraction), solvent bar microextraction[19] and liquidmembrane microextraction,[20-22] are not reviewed here,since they have been recently reviewed[23,24].

Principle of solid phase microextraction
SPME is based on a modified syringe which contains stainless steel microtubing within its syringe needle. This microtubing has an about 1-cm. fused-silica fiber tip which is coated with an organic polymer. The coated silica fiber can be moved between two positions, inside and outside the needle, with a plunger as in the case of a normal syringe the diameter of the syringe needle housing the microtubing and coated silica fiber is not much increased in comparison with a normal GC syringe. Thus, by means of this simple equipment several steps of sample preparation are combined in

one device. Extraction and enrichment of the analyteis completed by the coating in the position outside the syringe needle. Penetration of the septum of a GC injection port is possible if the fiber was withdrawn into the syringe needle. Desorption of the analyte and transfer to the capillary is performed after again moving the fiber to the position outside the syringe[25,26].

Advantages of solid-phase microextraction[27-29]

The main advantage of SPME is simplicity,rapidity, solvent elimination.

1. High sensitivity
2. Small sample volume
3. Low cost.

Design of SPME device

SPME is a modified syringe-like instrument (Figure 2). The fused silica fiber, having a small size and cylindrical shape, is connected to stainless-steel tubing that is used to provide additional mechanical strength to the fiber assembly for repeated sampling. This stainless-steel tubing is connected toa specially designed syringe-like instrument. The fused silica fiber is coated with a relatively thin film of several polymeric stationary phases. The fiber assembly is reusable and replaceable. Supelco (www.sigmaaldrich.com) provides seven different types of fibers. The small size and cylindrical geometry of fiber has some advantages such as easy placement of the sorbent fibercoating into a sample or headspace above the sample to extract the analytes; also it can be easily placed in desorption chamber of GC or interphase of the HPLC without any modification of GC or HPLC. Plunger movement and timing must be controlled carefully to perform adsorption and desorption correctly. It is very important for field sampling to prevent loss of analyte during transport. To do this, the needle opening of SPME devise must be sealed by using a septum and/or by cooling the needle[30]. The SPME device is shown in figure no. 2.

Working with SPME device

During the SPME operation, the fiber is first drawn into the syringe needle, then lowered into the vial (which is sealed with a septum type cap) by pressing the plunger. The fused silica fiber of suitable coating is used which is dependent upon the nature of the analyte. The fiber should be cleaned before analyzing any sample so as to remove contaminants that give a high background in the chromatogram. Cleaning can be performed in the desorption chamber of HPLC by running solvent. Now the cleaned fiber coating is exposed to a sample matrix for a predetermined, fixed period, which results in the adsorption of the analyte on the fiber coating (Figure 2). This extraction can be performed in twoways[30]: 1) headspace SPME or HS-SPME where fiber is exposed in the vapor phase above a gaseous, liquid, or solid sample: 2) direct immersion DI-SPME, where the fiber is directly immersedin liquid samples[31].

➢ MATERIALS AND METHODS

Before the SPME device was used it was necessary to condition the fibres, this was achieved by exposing the fibres to the GC injector port at 250 ◦C for 1h.Several fibre blanks were run to ensure the fibres were fully conditioned and that no interferences from the fibres were present in GC chromatograms. Once this had been completed successfully a variety of standard solutions containing individual PAHs were made. Eventually, it is hoped the device will be used to extract up to 16 PAH compounds simultaneously, however, for the purpose of optimising extractionand desorption conditions it was decided that working with individual compounds would make extractions both easier and quicker. When using SPME it is important that the device is placed into the hottest part of the GC injector while desorption is taking place, therefore, a series of desorption's of a standard solution containing 9.98 ng μL−1 naphthalene were carried out withdesorption taking place at different depths in the injector, all other factors were kept constant. It was found that a depth of 3 cm in the injector port was optimal so this depth was used in all further experiments.

Investigations into the various parameters governing extraction efficiency were then carried out using standard solutions of the PAHs naphthalene, chrysene, fluorene and BaP and samples of de-ionised water spiked with these compounds. These compounds were chosen because they cover a range of PAH physiochemical properties, naphthalene being one of the smallest and most volatile with two aromatic rings, and BaP one of the largest and least volatile of the PAHs of interest with five fused aromatic rings. Generally, at ambient temperature, headspace SPME can only be used to extract compounds with Henry's law constants above90 atm.cm3mol−1, this includes three-ring PAHs or more volatile analytes (Zhang and Pawliszyn, 1993). Therefore, it was decided to concentrate on the parameters that affect the extraction of the compounds of interest by immersion extraction at this stage[32].

Applications of SPME in various fields

- **Environmental applications:**

In the early developmental period the majority of applications were in environmental chemistry. Mostly organic compounds[33-40] have been studied, and pesticides, herbicides and other biologically active compounds in aqueous samples[41-43].

- **Applications in food chemistry**

Food analysis is important for the evaluation of nutritional value, for quality control of fresh and processed products and the monitoring of food additives and other toxic contaminants. In general, flavor is sensitive to compositional alterations in the case of food (fruit, wine, etc.)[44,45].

- **Applications to biological fluids**

Sample preparation is one of the most critical steps in the analysis of biological fluids and compounds in biological matrices[46].

3. LIQUID PHASE MICROEXTRACTION (LPME)

Sample preparation can include cleanup procedures for very complex (dirty) samples. This step must also bring the analytes to a suitable concentration level[47]. LPME is a solvent-minimized samplepretreatment procedure of LLE, in which only several lLit of solvent are required to concentrate analytes from various samples rather than hundreds of ml needed intraditional LLE. It is compatible with capillary gas chromatography (GC), capillary electrophoresis (CE) and HPLC. In LPME, extraction normally takes place into a small amount of a water-immiscible solvent (acceptor phase) from an aqueous sample containing analytes (donor phase)[48].

Advantages

- It is a rapid, simple, solvent free and sensitive method for the extraction of analytes;
- It is a simple, effective adsorption/desorption technique;
- It is compatible with analyte separation and detection by high-performance liquid chromatography withultraviolet detection (HPLC - UV);
- It provides linear results for a wide range of concentrations of analytes;
- It has a small size, which is convenient for designing portable devices for field sampling; and,
- It gives highly consistent, quantifiable results from very low concentrations of analytes.

Disadvantages

- Relatively low recommended operating temperature (generally in the range 240–280^{0}C);
- Their instability and swelling in organic solvents (greatly restricting their use with HPLC);
- Fiber breakage;
- Stripping of coatings; and,
- The bending of needles and their expense[49].

Types of liquid phase microextraction

It can be divided into three main categories
(1) Single-drop microextraction (SDME)
(2) Dispersive liquid–liquid microextraction (DLLME)
(3) Hollow-fiber microextraction (HF-LPME).

1. Single-drop microextraction (SDME)

SDME, using typically 1–3 lL of an organic solvent at the tip of a micro syringe, has evolved from LPME. Afterextraction, the micro drop is retracted back into the syringe and transferred for further analysis
In practice, two main approaches can be used to perform SDME:
a) Direct immersion (DI)-SDME
b) Headspace (HS)-SDME

a) Direct immersion (DI)-SDME

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

b) Headspace(HD)-SDME

In HS-SDME, a micro drop of appropriate solvent is placed in the headspace of the sample solution or in aflowing air sample stream to extract volatile analytes.Headspace (HD) – SDME is shown in figure no. 3.

Advantage

A wider variety of solvents to choose from.

Disadvantage

Extraction and injection have to be performed separately, using different apparatus[50,51].

2. Dispersive liquid-liquid microextraction (DLLME)

This technique that uses1Lit volumes of extraction solvent along with a few ml of dispersive solvents. Inthis method, a cloudy solution is get formed when anappropriate mixture of extraction and dispersive solventsis injected into an aqueous sample containing the analytesof interest. Hydrophobic solutes are rich in the extraction solvent, which is

dispersed into the bulk aqueous solution. After centrifugation, analytes in the settled phase can be determined by using conventional analytical techniques.
In DLLME, the dispersive solvent plays a key role that helps extraction solvent form fine droplets in aqueous samples, representing about 97–99% of the total volume of the extraction mixture. Compared to other methods, abundant surface contact between fine droplets and the analyte in DLLME speeds up the mass-transfer processes of analytes from aquatic phase to organic phase, which not only greatly enhances extraction efficiency but also overcomes the problem of the time taken.

Advantages

1. Simplicity of operation
2. Rapidity
3. Low cost
4. High recovery
5. High enrichment factor
6. Very short extraction time (a few seconds)[52].

3. Hollow-fiber microextraction (HF-LPME)

They used the basic principle of supported liquid membrane (SLM), for the first time, in simple, inexpensive, disposable extraction units for the liquid-liquid-liquid microextraction (LLLME) utilizing polypropylene HFs as the membrane.
The sample vial is filled with the aqueous sample. A short piece of a porous HF may be either a rod with a closed bottom or a u-shape where both ends are connected to guiding tubes. Prior to extraction, the HF is first dipped in the organic solvent for a few times to immobilize solvent in the pores, and excess solvent is removed. The solvent is immiscible with water to ensure that it remains within the pores during the extraction with no leakage to the aqueous sample. The organic solvent forms a thin layer within the wall of the HF. The extraction solvent must be compatible with the HF so that the pores in the wall of the HF can be filled completely.
The acceptor solution then fills the lumen of the HF. This acceptor solution can be an organic solvent (the same as that used for the organic solvent in HF pores) resulting in a two-phase extraction system, or the acceptor solution may be an acidic or alkaline aqueous solution, resulting in a three-phase extraction system. In the two-phase LPME system, the target analytes are extracted from the aqueous sample and into the organic solvent (acceptor solution) present both in the porous wall and inside the lumen of the HF[53].

Applications

All LPME techniques can be utilized effectively for extraction of target analytes from various sample solutions[54].

4. MOLECULARLY IMPRINTED POLYMERS (MIPS)

Molecularly imprinted polymers (MIPs) are polymers prepared in presence of a template [55,56] that serves as a mould for the formation of template complementary binding sites. Thus, MIPs can beprogrammed to recognize a large variety of target structures with antibody-like affinities and selectivities[57].MIP is based on the formation of a complex between an analyte (template) and a functional monomer. In the presence of a large excess of a cross-linking agent, a three-dimensional polymer network[58] is formed. After polymerization process, the template is removed from the polymer leaving specific recognition sites complementary in shape, size and chemical functionality to the template molecule. Usually, intermolecular interactions like hydrogen bonds, dipole–dipole and ionic interactions between the template molecule and functional groups present in the polymer matrix drive the molecular recognition phenomena. Thus, the resultant polymer recognizes and binds selectively only the template molecules[59].

Molecular imprinting process

Template design and monomer selection are two of the mostcritical features of the molecular imprinting process. For analytical applications, the **"MIP Rule of 6"** should be followedwhen possible:

- Never use the analyte as a template unless there is absolutely no alternative
- Make rational choices about which regions of an analyte are likely to command the best types of interaction in a low dielectric medium (organic solvent) and then incorporate these elements in an analog of the analyte molecule
- Select monomers that are likely to form strong interactions in the chosen solvent (e.g., Bronsted acids or bases/H-donors or acceptors/nonpolar groups, etc.)—this will increase capacity and influence homogeneity of the binding cavities.
- Choose templates and monomers that will be soluble in the porogenic solvent to be used in the polymerization-this

may seem obvious but it sometimes requires carryingout solubility tests
- Ensure as far as possible that the template–monomer mixture is stable and does not undergo side reactions under the polymerization conditions.
- Consider the nature of the matrix from which the analyte will eventually be extracted when selecting the cross-linking monomer - a range of di- or tri-unsaturated cross linking monomers with varying chemistries are available to create the porous organic network material[60-64]. Process of preparation of MIPs is shown in figure no. 4.

Methodologies applied for the preparation of MIPs
The main methodologies applied for the preparation of MIPs are based on covalent, non-covalent,semi-covalent, and metal-mediated interactions[66].

a) Covalent imprinting
Covalent imprinting, or the pre-organized approach[67], involves the formation of reversible and easily cleavable covalent bonds between the template molecule and one, or more, polymerizable monomers prior to the polymer synthesis[68].

Advantages
1. The monomer/template complexes are stable and stoichiometric.
2. wide variety of polymerization conditions can be used.

Disadvantages
1. slow release and binding of templates[69].

b) Non-covalent imprinting
In non-covalent imprinting the prearrangement between the template and the functionalmonomer(s) occurs by non-covalent interactions such as hydrogen bonding, ionic interactions, π – π interactions, hydrophobic interactions or Van der Waals forces[70].

Advantages
- Easy preparation of the template/monomer complex.
- Easy removal of the templates from the polymers.
- Fast binding of templates to MIPs.

Disadvantages
- The template/monomer complex is not stable and in order to minimize the non-specific binding sites the conditions ofpolymerization must be carefully chosen[69].

c) Semi-covalent imprinting
This approach includes all the procedures in which the template is covalently bound to a polymerizable group for polymer synthesis but template rebinding takes place by noncovalent interactions (Fig. 10)[71-73].

d) Metal-mediated interactions
Metal ions can play different roles in imprinting; they can be used as templates or as components of the template functionalmonomer interaction. Metal ion imprinting can be achieved by cross-linking preformed polymers bearing complexing ligands, or polymerizing specific metal complexes with polymerizable ligands[74,75].

- **Applications of MIPS**
  1. Their use as tailor-made separation materials,
  2. Their use in organic synthesis and enzyme technology as catalytically active polymers or enzyme mimics.
  3. As sensors in biosensor-like configurations[76].
  4. Scheme outlining the main applications envisaged for MIPs are shown in figure no.5[77].

5.Turbulent flow chromatography (TFC)
Turbulent Flow Chromatography (TFC)is a technique that combines high-throughput and high reproducibility by means of separating analytes from various matrices with reduced sample handling. The sample can be injected directly onto a narrow diameter column (0.5 or 1.0 mm) packed with large particles (30–60 m) at a high flow rate (higher than 1 ml min−1) helping creating a very high linear velocity inside the turbulent flow column. Under turbulent flow conditions the improved mass transfer across the bulk mobile phase allows for all molecules to improve their radial distribution, however, under these conditions a laminar zone around the stationary phase particles still exists, where diffusional forces still dominate the mass transfer process[78]. Molecules with low molecular weight diffuse faster than molecules with a high molecular weight, forcing large molecules to quickly flow to waste while retaining the small analytes. The retained compounds are then back-flushed and focused on theanalytical column for chromatographic separation. It is extremely important to effectively avoid interferences from the matrix on the analysis of a contaminant. The optimization of the different on-line extraction steps is crucial, as parameters like mobile phase composition,

flow rates and extraction time windows will affect recovery and extraction efficiency in general[79].

Principle of TFC

Turbo Flow methods are based on the direct injection of biological samples without previous extraction or treatment a column packed with large particles. These large particles have an additional level of selectivity via the stationary phase chemistry added to them. After the sample is injected onto a TurboFlow column the high flow rate (cf. 1.5 – 5.0ml/min) generates turbulent flow conditions inside the column. Since 100% aqueous mobile buffers are used, the small analyte molecules are retained via diffusion into the particle pores, while the proteinaceous material is washed to waste. Once the compounds of interest are extracted from the biological matrix, they are eluted from the Turbo Flow column onto the analytical column with a volume of solvent, which has been stored in a holding loop. The holding loop should have a volume at least ten times that of the Turbo Flow column and is typically filled with organic mobile phase (for reversed stationary phase) or pH buffered solutions (for ion exchangephases). As the analytes are released from the Turbo Flow column they are transferred with the pumping solvent (at a considerably lower flow rate than that used during loading) through the tee rotor-seal in the second valve and mixed with the pumping solvent from the analytical system[80]. Schematic representation of on-line turbulent-flow column-switching is shown in figure no. 6.

Online turbulent-flow column-switching

A schematic diagram of the on-line TFC-LC/MS instrument set-up based on column-switching and fast HPLC is shown in Fig. (12). A pump was used to deliver a high flow through a hydrophilic–lipophilic balanced (HLB) reversed-phase column to load and wash the sample, and subsequently to flush and equilibrate the extraction column. Solvent A was used as the solvent for this pump. An Agilent 1100 HPLC system (equipped with a binary pump, an on-line degasser, an auto-plate-sampler, and a thermostatically controlled column compartment) was used to deliver a gradient flow to elute the analytes from the extraction column and to perform the separation on a fast HPLC column. Two Rheodyne six-port switching valves were used for the column-switching purposes. The L and E in the center of the each six-port valve designate “load” or “elute” positions for the flow path[81].

Applications of TFC

- In food and environmental analysis.
- In the handling of biological samples containing a large amount of proteins, such as blood plasma[82,83–88].
- Isolation of veterinary drugs and growth promoters from food[89].
- A solution that has gained wide use, particularly in the clinical field, to increase throughput on such systems[90].

CONCLUSION

Solid phase extraction is a widely used sample-preparation technique for isolation, concentration, clean-up and medium exchange. SPME is a technique for extraction of organic compounds from gaseous, aqueous, and solid matrices. All LPME techniques can be utilized effectively for extraction of target analytes from various sample solutions. Imprinted polymers are now well established as materials for molecular recognition, chromatographic separation, and analytical sample enrichment but their use as active biomedical devices is still in the early stages of development. The on-line TFC-LC/MS method was suitable for TCMs pharmacokinetic study at a low dose level.

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Table 1: Selecting SPE tube size

If Your Sample is	Use Tube Size
< 1mL	1ml
1mL to 250mL and the extraction speed is not critical	3ml
1mL to 250mL and a fast extraction procedure is required	6ml
10mL to 250mL and higher sample capacity is needed	12, 20, or 60ml
< 1 liter and extraction speed is not critical	12, 20, or 60ml

Table 2: Selecting SPE disk size

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

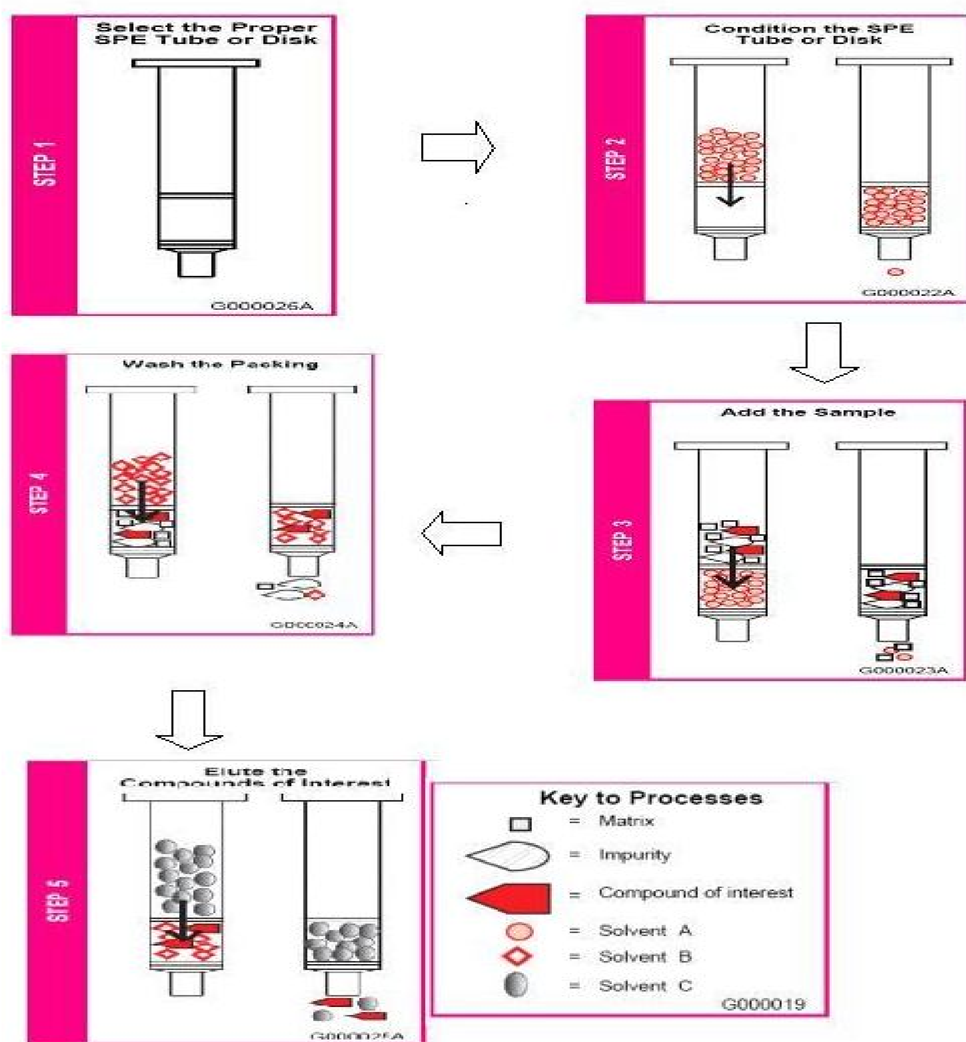

Fig. 1: Steps of solid phase extraction process

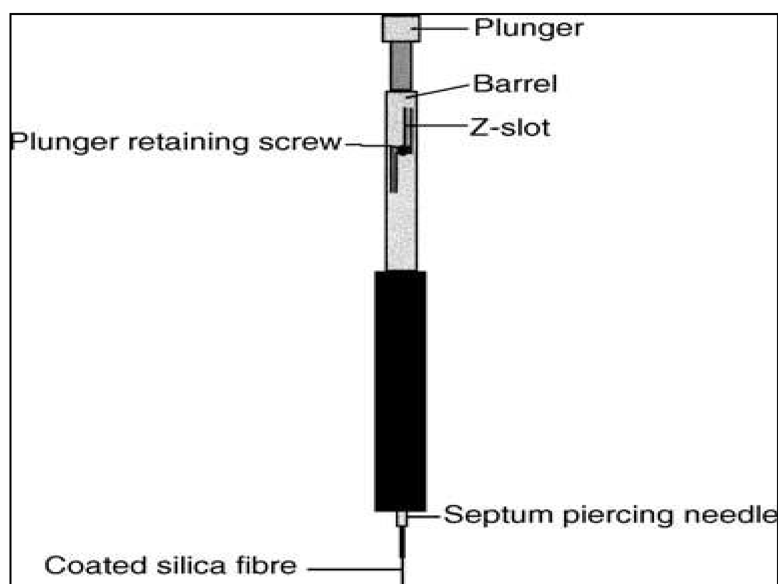

Fig. 2: SPME device[32]

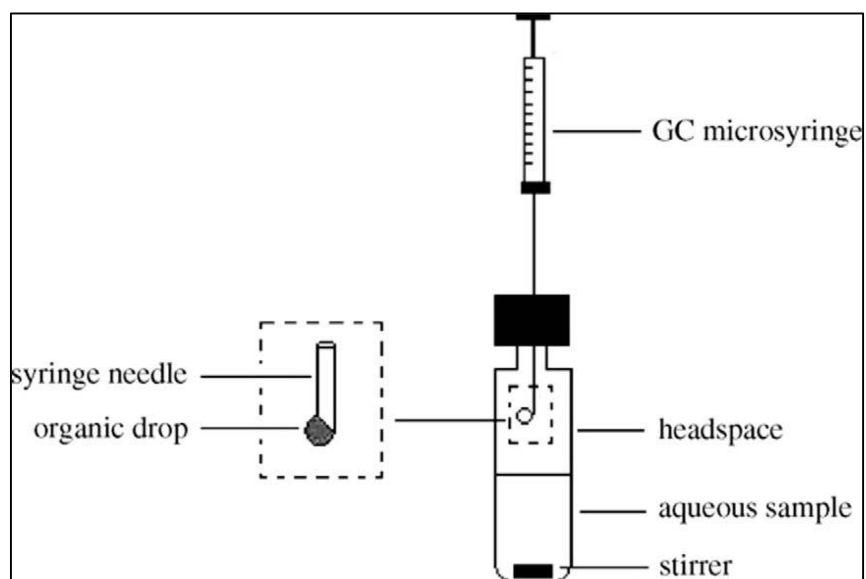

Fig. 3: Headspace single drop microextraction

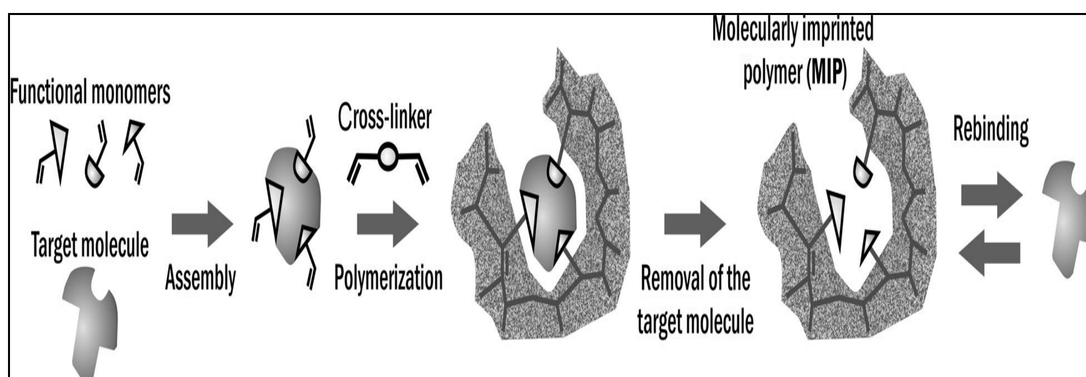

Fig. 4: Schematic representation of the molecular imprinting process[65]

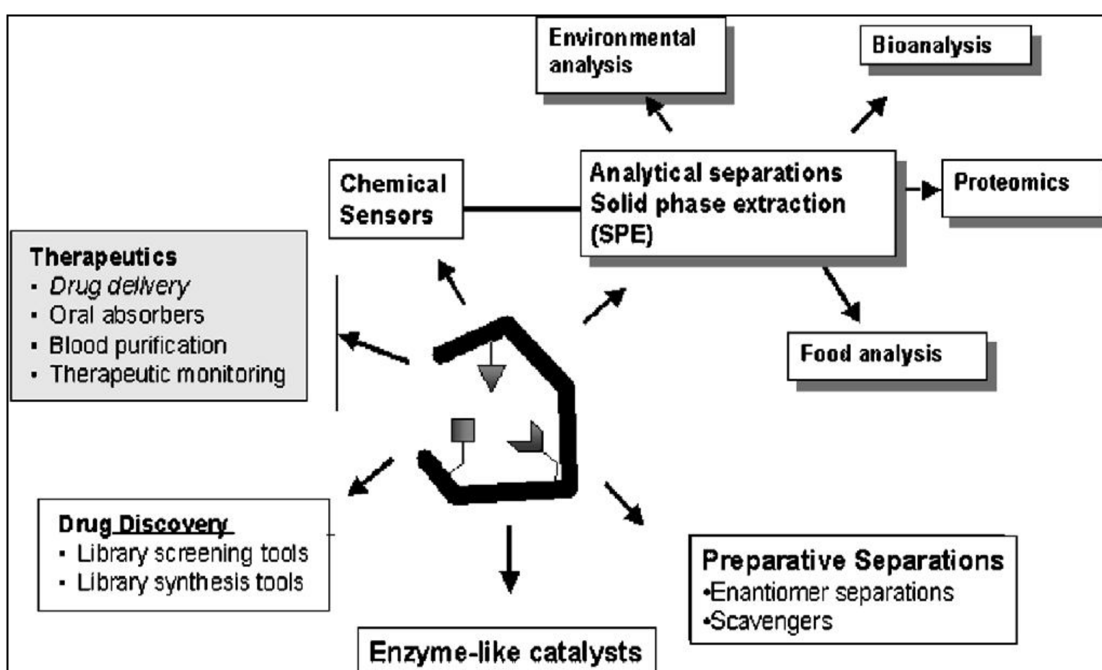

Fig. 5:Scheme outlining the main applications envisaged for MIPs[77]

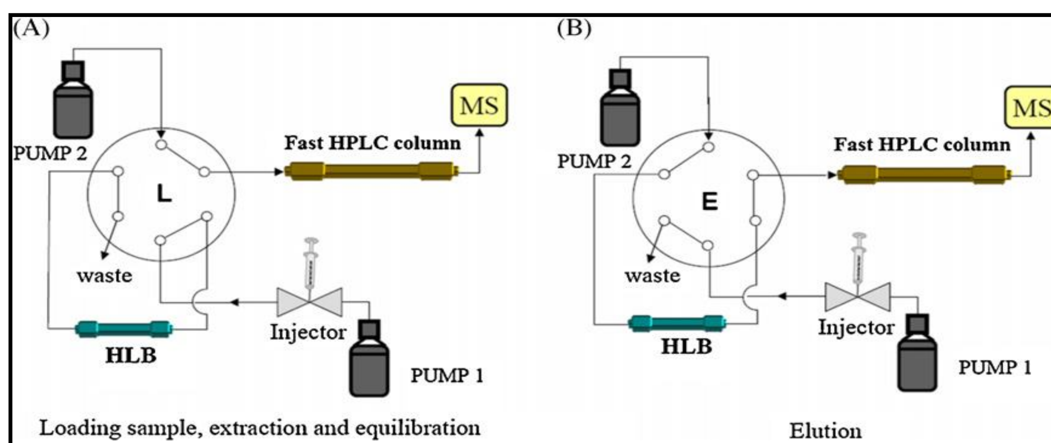

Fig. 6: Schematic representation of on-line turbulent-flow column-switching

REFERENCES

1. ArthurCL,Pawliszyn J. Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal. Chem. 1990;62:2145.
2. Louch D,Motlagh S,Pawliszyn J. Anal. Chem. 1992;64:1187.
3. Murray DAJ. Rapid microextraction procedure for analyses of trace amounts of organic compounds in water by gas chromatography and comparisons with macroextraction methods. J. Chrom.1979;177:135–140.
4. Thielen DR,Olsen G,Davis A,Bajor E,Stefanovski J,Chodkowski J. An evaluation of microextraction/capillary column gas chromatography for monitoring industrial outfalls. J. Chromatogr.Sci.1987;25:12–16.
5. Arthur CL,Pawliszyn J. Solid phase microextraction with thermal desorption using fused silica optical fibers. Anal. Chem. 1990;62:2145–48.
6. Eisert R,Pawliszyn J.Automated in-tube solid phase microextraction coupled to high performance liquid chromatography. Anal.Chem. 1997;69:3140–47 .
7. Liu H,Dasgupta PK. Analytical chemistry in a drop. Solvent extraction in a micro drop.Anal. Chem. 1996;68;1817–21 .
8. Jeannot MA,Cantwell FF. Solvent microectraction into a single drop. Anal Chem. 1990;68;2236–40.
9. Pedersen-Bjergaard S ,Rasmussen KE. Liquid-liquid microextraction for sample preparation of biological fluids prior to capillary electrophoresis. Anal.Chem.1999;71;2650–56
10. Istvan B, Kiado B,Kezirat L.Modern analytical techniques in the pharmaceutical & bioanalysis.2011;3126-31.
11. Zwir-Ferenc A, Biziuk M. Solid Phase Extraction Technique – Trends,Opportunities and Applications.Polish J. of Environ. Stud.2006;15:677
12. Urbe I, Ruana J. J. Chromatogr. 1998;778:337.
13. SupelcoBulletinGuide to Solid Phase Extraction,Sigma-Aldrich Co 910;1998.
14. Sabikh, Jeannot R., Rondeaub. Multi-residue methods using solid phaseextraction techniques for monitoring priority pesticides, including triazines and degradation products, in ground water. J. Chromatogr.2000;885:217.
15. Rasmussen KE,Bjergaard SP,Krogh M, Ugland HG, Gronhaug T.J. Chromatogr.2000;873:3.
16. Anderson S,Halvorsen TG,Bjergaard SP,Rasmussen KE.J.Chromatogr. 2002;963:303.
17. Ho TS,Halvorsen TG,Bjergaard SP,Rasmussen KE. J.Chromatogr.2003;998: 61
18. Psillakis E,Kalogerakis N. Trends Anal. Chem. 2002;21:53.
19. Jiang X, Lee HK. Anal. Chem.2004;76:5591.
20. Jonsson OB, Nordlof U, Nilsson UL. Anal. Chem.2003;75;3174.
21. Basheer C, Suresh V, Renu R, Lee HK. J. Chromatogr.2004;1033:213.
22. Yang R, Xie W.Forensic Sci. Int.2004;139:177.
23. Kataoka H, Lord HL.In Sampling and Sample Preparation for Field and Laboratory; Pawliszyn, J. Ed Elsevier 2002: 779.
24. Wells DA, Loyd TL. In Sampling and Sample Preparation for Field and Laboratory; Pawliszyn, J. Ed.; Elsevier 2002:837.
25. Belardi RG, Pawliszyn J. Water Pollut. Res. J. Can. 1989;24:179.
26. Arthur CL, Pawliszyn J. Anal. Chem. 1990;62:2145.
27. Wu J, Lord HL,Pawliszyn J. Talanta.2001;54:655.
28. Wu J, Lord HL, Pawliszyn J, Kataoka H. J. Microcol2000;12:255.
29. Walles M, Mullett WM, Levsen K, Borlak J, Wunsch G,Pawliszyn J. J Pharm. Biomed. Anal., 2002;30;307.
30. Kataoka H,Lord HL,Pawliszyn J.Application of solid-phase microextraction in food analysis.J. of Chromatography2000;880:35–62.
31. Zhang Z,Pawliszyn J. Quantitative extraction using an internallycooled solid phase microextraction device.Anal. Chem. 1995;67:34–43.
32. Amanda JK, Readman JW, Zhou JL.The Application of Solid phase microextraction (SPME) to the analysis of Polycyclic aromatic Hydrocarbons (PAHs) Environmental Geochemistry and Health 2003;25:69–75
33. Fattore E, Benfenati E, Fanelli R. J. Chromatogr.1996;737:85.

34. Stack MA, Fitzgerald GO, Connell S, James JK. Chemosphere2000; 41:1821.
35. Abalos M, Prieto X, Bayona JM. J. Chromatogr. 2002;963:249.
36. Lambropoulou DA, Sakkas VA, Albanis TA. Anal. Chem. Acta2002;468:171.
37. Diaz A, Ventura F, Galceran MT. Anal. Chem.2002;74:3869.
38. Diaz A, Ventura F, Galceran MT.J. Chromatogr. 2002;963:159.
39. Tombesi NB, Freije H.J. Chromatogr. 2002;963:179.
40. Tamiri T. Presented at the 51st Conference on MassSpectrometry and Allied Topics, Montreal, June 2003.
41. Sarrion MN, Santos FJ, Galceran MT. J. Chromatogr.1999;839:159.
42. Navalon A, Prieto A, Araujo L, Vilchez JL. J. Chromatogr. 2002;946:239.
43. Moeder M, Schrader S, Winkler M, Popp P. J. Chromatogr. 2000;873:95.B
44. Vas G, Lorincz G.Acta Aliment.1999;28:95.
45. Vas G, Koteleky K, Farkas M, Dobo A, Vekey K. Am. J. Enol.Vitic.1998; 49; 100.
46. Imaizumi M, Saito Y, Hayashida M, Takeichi T, Wada H, Jinno K. J. Pharm. Biomed. Anal.2003;30:1801.ull
47. Arthur CL, Pawliszyn J. Anal. Chem. 1990;63:2145
48. Liu H, Dasgupta PK. Anal. Chem. 1996;68:1817.e
49. KumarGA, Malik AK, Tewary DK, Singh B. Anal. Chim. Acta 2008;610: 1.
50. Jeannot MA,Cantwell FF. Anal. Chem. 1997;69:235.
51. Liu H,Dasgupta PK. Anal. Chem. 1996;68:1817.ti
52. Jahromi EZ,Bidari A, Assadi Y, HosseiniMRM, Jamali MRM. Anal. Chim. Acta 2007;585;305.
53. King S,Meyer JS,Andrews ARJ. J. Chromatogr. 2002;982:201n5[[
54. Yazdi AS,Amiri A. Liquid-phase microextraction. Trends in Analytical Chemistry. 2010;29:1.
55. Sellergren B. Molecularly imprinted polymers, manmade mimics of antibodies and their applications in AnalyticalChemistry, in: Techniques and Instrumentation in AnalyticalChemistry. Elsevier 2001:23.
56. Sellergren B. Imprinted polymers with memory for smallmolecules, proteins, or crystals, Angew. Chem., Int. Ed. 2000;39:1031–1037.
57. Sellergren B. Enantiomer separations using designedimprinted chiral phases, in: G. Subramanian (Ed.), ChiralSeparation Techniques,Wiley-VCH,Weinheim,2001;2:153–184.
58. Ramstrom O, Mosbach K. Synthesis and catalysis by molecularly imprinted materials.Curr. Opin. Chem. Biol. 1999;3:759–764.
59. Annamma, KM,Mathew B. Design of 2,4-dichlorophenoxyacetic acid imprinted polymer withhigh specificity and selectivity. Mater. Sci. Appl. 2011;2:131–140.
60. Sellergren B.Anal. Chem.1994;66:1578–82.
61. Bereckzi A,Tolokan A,Horvai G,Horvath V, Lanza F, Hall AJ, Sellergren, BJ. Chromatogr.2001;930:31–8.
62. Martin P, Wilson ID,Morgan DE, Jones GR, Jones K.Anal. Commun. 1997;34:45–7.
63. Venn RF, Goody RJ. Chromatographia 1999;50:407–14.
64. Walshe M,Howarth J,Kelly MT, O'Kennedy R, Smyth MR. J. Pharm. Biomed. Anal.1997;16:319–25.
65. Opik A, Menaker A, Jekaterina R, Vitali S.Molecularly imprinted polymers: a new approach to the preparationof functional materials.2009;58:3–11
66. Alexander C,Andersson HS,Andersson LI,Ansell RJ,Kirsch N,Nicholls IA, O'Mahony J, Whitcombe MJ. J. Mol.Recognit.2006;19:106.
67. Anderson LI.J. Chromatogr.2000;745:3.
68. Wulff G, Sarhan A.Angew. Chem.1972;84:364.
69. Dr.Istvan,Kiado B, Budapest, Kezirat L.Modern analytical techniques in the Pharmaceutical and bioanalysis.2011;31:34
70. Norrlow O, Glad M, Mosbach KJ. Chromatogr.1984;29:29.
71. Whitcombe MJ, Rodriguez ME, Villar P,Vulfson EN. J. Am.Chem. Soc., 1995;117:7105.
72. Whitcombe MJ,Rodriguez ME,Vulfson, EN. In Separationfor Biotechnology3, Pyle, D. L. (Ed.); Royal Society of

ChemistryCambridge. 1994:565-571.
73. Whitcombe MJ,Vulfson EN.In Molecularly Imprinted Polymers:Man-Made Mimics of Antibodies and their Applications inAnalytical Chemistry, Techniques and Instrumentation in AnalyticalChemistry, Sellergren, B. (Ed.); Elsevier: 2001;23:203-212.
74. Murray GM, Jenkins A, Bzhelyansky A, Uy OM.JohnHopkins Appl.Tech. Diggest1997;18:464.
75. Al-Kindy S, Badi R, Diaz García ME.Anal. Lett., 2002;35:1763.
76. Wulff G, Minavik MJ. Liq.Chromatogr. 1990;13:2987-3000
77. Borje SA, Chris JA. Molecularly imprinted polymers: A bridge toadvanced drug delivery. 2005:1733–1741.
78. Michopoulos F, Edge AM,Theodoridis G,Wilson ID. J. Sep. Sci.2010;33:1472.
79. Herman JL, Poster no. 72, 27th Montreux Symposium on LC/MS, Montreux, Switzerland, November 2010.
80. Quinn HM, Takarewski JJ. Int. Patent Number WO97/16724, 1997.
81. Kasprzyk-Hordem B, Dinsdale RM, Guwy AJ. J. Chromatogr. A 2007;1161: 132.
82. Michopoulos F, Edge AD, Theodoridis G,Wilson ID. J. Sep. Sci. 2010;33:1472.
83. Xin GZ,Zhou JL,Qi LW, Li CY,Liu P,Li HJ,Wen XD, Li P. J. Chromatogr.B: Analyt. Technol. Biomed. Life Sci. 2010;878:435.
84. Kasper DC, Herman J,De J,Mechtler VTP, Metz TF,Shushan B, RapidCommun.Mass Spectrum. 2010;24:986.
85. Breaud AR,Harlan R,Di Bussolo JM,McMillin GA,Clarke W, Clin. Chim.Acta 2010;411:825.
86. Harlan R,Clarke W, Di Bussolo JM, Kozak J,Straseski J, Li Meany D, ClinChim. Acta 2010;411:1728.
87. Bunch DR,Heideloff C,Ritchie JC,Wang S, J. Chromatogr. B:Analyt. Technol.Biomed. Life Sci.2010;878:3255.
88. Mueller DM, Duretz B, Espourteille FA,Rentsch KM. Anal. Bioanal. Chem.2011;400:89.
89. Kinsella B,O'Mahony J,Malone E,Moloney M,Cantwel H,Furey A, DanaherM. J. Chromatogr. A 2009;1216:7977.
90. Berube M. Application note: 458, Thermo Scientific(www.thermo.com/appnotes).