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Review

Microplates in liquid chromatography – New solution in clinical research? – A review

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ABSTRACT

Microplates are routinely used in Radio- or Immuno-assays. Recently, microplates have found use not only in analytical but also in the pre-analytical phase in bioanalyses (sample storage, sample preparation). New connection of this technology to liquid chromatography could be economical, fast and simple solution for many routine laboratories handling large sequences of biological samples. This review summarises the application of microplates in bioanalytical laboratories. Different types of sorbents, materials and shapes of microplates are discussed, and the main advantages and disadvantages of microplates used in clinical research are presented.

Contents

1. Introduction

The requirements for biomedical analyses are continuously increasing. The groups of patients for statistical surveys have to be larger, which means that clinical research laboratories have to

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process a larger amount of samples. Timesaving and effective methods are used more often. The consumption of only small amounts of the sample and small volumes of the chemicals used is necessary. Laboratory practices have been increasingly focused on the miniaturisation of the sample preparation process because it provides a solution for the requirements such as high performance, rapid analysis with a low running cost, and minimal environmental pollution [1].

During the last decade, microplates (MCPs) have become of increasing interest. There are many manufacturers that design microplates with various shapes and different materials. Recently, MCPs are entering the liquid chromatography (LC) market. These plates are used in LC auto-samplers rather than the vials; they are intended for single-use and are starting to be one of the most important components in the sample preparation procedure and sample storage.

2. MCPs in liquid chromatography

In the last decade, MCPs began to enter the fields of liquid chromatography and the pre-analytical phase (all the operations which should be done before analysis – sample takeover, transport, storage and sample preparation procedure) connected with these techniques.

One of the first connections of LC with MCPs was presented in patent no: 10/563266 [2].

Charlton and Galarza [3] used MCPs for stationary phase screening and development of a method for the separation of a protein mixture. Specially adapted MCPs were constructed that have an outlet in the base beneath each well. These outlets allow the passage of fluid through the well and the adsorbent. Furthermore, the use of these high throughput experimental techniques increases the number of adsorbents or process conditions that can be evaluated, potentially resulting in substantial time and cost savings in process development activities. The results from this study indicate that the separation of proteins in column chromatography is well comparable with the separation of proteins developed under the same conditions in the 96-well chromatography MCPs study. Therefore, the validity of using a MCPs-based approach for the initial process development activities was demonstrated. The primary advantages of LC include its ability to separate, identify and quantify substances with different structures over a wide concentration range during a single analysis with high sensitivity and automation possibilities.

In the beginning of the 21st century, manufacturers of chromatographic equipment began to develop systems equipped with samplers for MCPs; it was the key step in the development of a new generation of instruments for chromatographic techniques. Similarly, this development was an important step in the development of pre-analytical procedures that were usually connected to the analysis of biological samples.

Using MCPs rather than vials is very advantageous, especially for bioanalyses:

- MCPs are intended for a single use.
- The samples are usually stored in a dark, cool place in the auto-sampler and are protected against evaporation by special lids.

In the last years, there has been considerable progress in coupling chromatographic techniques with mass spectrometry detection (MS) [4]. The use of MS directly in medicinal research is an important tool for contributing to diagnoses.

In the last decade, the modern drug discovery processes involved high-throughput screening processes on considerable numbers of samples. In this case, HPLC–MS was used to confirm the presence and purity of potential target compounds. HPLC–MS has already been demonstrated to be an effective tool for characterising pharmaceutical compounds, and this technique is currently being optimised for speed to process the large number of samples that are created during the drug discovery process. The MCPs (96-, 384-, or 1536-well) are the ubiquitous standard for sample manipulation during drug discovery [5]. The important advantage is the ability to inject samples by an auto-sampler directly from the MCPs which eliminates the need for any reformatting steps. The inherent high-speed capabilities of an MS system have been exploited by operating the system in a parallel fashion, which allows a single MS to simultaneously collect data from multiple HPLC systems.

Combining these two analytical techniques with modern sample preparation procedures is important, especially in the analysis of biological samples.

MCPs are the solution of choice because they enable large sequences of samples to be handled with minimal mistakes while using small volumes of solvents and samples (see Fig. 1).

Hendericky et al. described a method for screening the enzymatic racemase activity using a fast chiral LC separation and ion spray-MS as the detection technique. After incubation of the substrate, the 96-well MCPs were centrifuged to remove the cell material. The enantiomers were separated on a Crownpak CR+ column within 1 min. A Gilson 215 auto-sampler with 889 multiple injection probe was used to inject the samples into the LC system. The total analysis time for a 96-well microplate was 56 min and inter-day reproducibility was within 15%. [6]. Important advantage of this method is that over 12,000 samples were analysed using this method without a loss in the performance of the system.

In reference [7], the authors evaluated LC/MS analyses of samples that required manual transfers of the wells of interest from a 1536-well plate into a 384-well plate. Currently, the commercially available 1536-well plate auto-samplers are not compatible with LC/MS systems. The authors modified their CTC PAL auto-sampler to support injection from up to twenty-four 1536-well plates. This modification allows the authors to cherry-pick any sample from up to 36,864 wells on the auto-sampler.

Dear et al. [8] used ultra-performance liquid chromatography (UHPLC) coupled to MS, and in this work, the ability of a MCPs imager (instrument for MCPs reading based on various conditions) to act as a suitable radio detection system for UHPLC methods is assessed. The system demonstrates robustness and sensitivity comparable to a MCPs scintillation counter, which is typically used for off-line radio detection of metabolites. The MCPs imager was also used to undertake the successful metabolite profiling of actual samples, which included two investigational drug candidates, using

Comparison of solvent consumption using different types of MCPs

Type of MCPs	MCPs Quantity	Assay Volume	Total Solvent Consumption
96-Well	1 075	200µl	20.7L
384-Well	269	50µl	5.1L
1536-Well	81	7µl	871mL
3456-Well	32	1µl	110mL

N = 100 000 samples

Fig. 1. Comparison of reagent usage for 100,000 samples.

both 96- and 384-well yttrium silicate MCPs. Coefficient of Variation (CV) of intra-plate precision was generally less than 3%. CV of precision for all UPLC chromatographed analytes was less than 7%.

3. MCPs materials

MCPs are composed of various materials based on their intended use. Advantages and disadvantages of each material will be discussed.

The most common material is *polystyrene* (*PS*), which is the most used material in optical detection. The MCPs can be coloured white by the addition of titanium dioxide for optical absorbance or luminescence detection, or they can be coloured black by the addition of carbon for fluorescent detection. Untreated PS surface is hydrophobic in nature and binds biomolecules through passive interactions. PS is suitable primarily for the immobilisation of large molecules, such as antibodies, that have large hydrophobic regions that can interact with the surface. Disadvantage of MCPs made of this material instead of vials in LC is possible release of some compounds which can interfere with MS detection.

Polycarbonate is inexpensive and easy to mould, and it has been used in single-use MCPs for the polymerase chain reaction (PCR) for DNA amplification. Possible important advantage is resistance of this material against high centrifugation speed. Polycarbonate MCPs should be one of the solutions for centrifugation of the samples with high speed in pre-analytical procedure before LC analysis.

Polypropylene (*PP*) is an ideal material for plates exposed to wide changes of temperature (storage at −86 °C and evaporation at +50 °C). This material has excellent properties for long-term storage of chemical compounds and biological samples.

PTFE (*polytetrafluorethylene*) is used for inert non-stick MCPs. PTFE is very non-reactive, partly because of the strength of carbon–fluorine bonds. MCPs made of PTFE can be sterilised using thermal or chemical methods and can be used in a wide range of temperatures (max. 300 °C).

Cyclo-olefins (*COC*) are currently being used to produce MCPs that transmit ultraviolet light for use in newly developed assays. Important advantages of MCPs manufactured from 100% COC polymer are superior optical clarity, thermal stability, biocompatibility, low auto-fluorescence, flatness and chemical resistance.

The most common manufacturing process for MCPs is injection moulding, which is used for PS, PP and COC. Vacuum forming can be used with softer plastics, such as polycarbonate.

Vinyl (*PVC*) MCPs are economical plates with main disadvantage which is incompatibility with automation procedures. This type of MCPs is usually used for complement fixation, agglutination, and blood typing.

Glass coated MCPs have the same properties as polypropylene MCPs and are coated with a 200 nm thick layer of silicone dioxide, which eliminates the need to use glass limited volume inserts with plastic MCPs. Very important advantage of this material is suitable usage for pharmaceutical applications where PS or polypropylene MCPs are not applicable. This material is chemically and thermally resistant and will tolerate temperatures from −80 °C to 80 °C.

Glass MCPs are designed for high-sensitivity detection, including fluorescent and luminescent detection and scintillation counting, where extremely low backgrounds with no cross-talk are required; this material is chemically stable and biologically inert. These plates are excellent for evaporation and long-term storage [9,10,11]. Glass MCPs are suitable for LC with MS detection.

Composite MCPs, such as filter plates, SPE plates, and even some advanced PCR plate designs, use multiple components that are moulded separately and subsequently assembled into a finished product. ELISA plates may now be assembled from twelve separate strips of eight wells, which makes only partially using a plate easier. This design saves cost for the scientist. A microplate typically has 6-, 12-, 24-, 48-, 96-, 384- or even 1536- sample wells that are arranged in a 2:3 rectangular matrix. Some MCPs have even been manufactured with 3456- or even 9600-wells, and an "array tape" product has been developed that provides a continuous strip of MCPs embossed on a flexible plastic tape [12,13].

4. Well shapes

During the introduction of the MCPs to the market, manufacturers began developing different well shapes (see Fig. 2). Each well shape was designed for a specific application, as follows:

Flat bottom – works well for reagent injection while providing the optimal optical characteristics – reading plate readers, cell culture applications.

C-bottom – similar to flat bottom well but with a slight rounded curve before the bottom.

U-bottom – improves washing in ELISAs, enhances sensitivity in fluorescent applications and facilitates the observation of agglutination reactions.

Round-bottom enhances washing in ELISAs while retaining high-quality optical characteristics for improved mixing and washing.

Flat bottom with radius edge – enhances washing in ELISAs, works well for reagent injection and provides optimal optical characteristics.

V-bottom – easier to remove all content [14,15].

5. 384-Well plates

The 96-well plate has been firmly established as the universal format for high-throughput screening in drug discovery. However, there is a growing interest in reducing assay volumes, increasing sample throughput, and increasing sample density, which all result in lower costs. Therefore, the increased interest is in the next logical format, the 384-well MCP.

There are some practical hurdles for preventing simple migration in the 384-well format, including a lack of assay sensitivity in the smaller volumes, surface tension problems that result in mixing issues, and format incompatibilities between library compounds that are stored in 96-well plates and their adaptation to 384-well plates. When downsizing the 96-well plate assays to 384-well plate assays, there are problems encountered where adjustments can usually be made. For example, PS plates are hydrophobic, and the flow of water over the surface at a high flow rate can trap air bubbles on the surface of the well. It is difficult to see the bubbles, and larger trapped bubbles can result in the well being overfilled, which could cause cross-well contamination. Trapped air in a well will distort optical-based measurements and can be problem in sample injection in LC auto samplers. Sometimes, the mixing of liquids requires more time in the smaller volume wells [16].

6. MCPs lids, sealings, films, tapes and well caps

The liquid in the MCPs should be protected against evaporation and contamination, during both long-term storage and in the sampler before analysis.

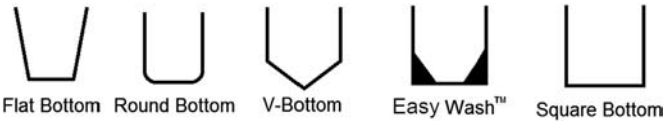

Fig. 2. Different well shapes.

There are various shapes and sizes of lids, films and tapes, which are composed of different materials, which are commercially available. The use of these materials and shapes depends on the application of the MCP.

The commonly used materials for the caps and films include silicon, PS, PP or its combination, aluminium in combination with stainless steel pins, and the films are usually made of polyvinyladene chloride coated PP etc.

Films and tapes are usually used for storage, PCR, microscopy, culturing and protection. Possible advantage is LC needle locking during sample injection.

Sealing mats are engineered to maintain a strong seal at all times on MCPs, and they are compatible with low temperatures such that the seals remain strong, even under extreme conditions.

Well cap mats protect the contents of the well during short- or long-term storage of chemicals or compounds and they are chemically resistant; pierceable thermoplastic elastomer well caps are excellent for the storage of compounds to −80 °C [17,18]. These well caps are excellent for MCPs stored in auto samplers before LC analysis.

Some well caps contain tiny holes in each well, which provides important advantages, such as a better injecting of samples. Furthermore, a microplate covered with a well cap mat is mechanically stable and the samples are protected in case of shaking. The disadvantage of well cap mats compared to tapes and films is the reduction of the sample volume (each well cap mat should be inserted into each well).

7. MCPs in the pre-analytical phase

Time of analysis is only a small part of the time that must elapse from the surgery up to the moment when the physician obtains the result of the analysis. Laboratory tests include the preparation of the patients, sending the collected material to the lab, and storage before analysis in the laboratory, which comprise the pre-analytic period. Sample preparation is a fundamental procedure for the analysis of a biological material. The implementation of sample preparation depends on the overall success of the analytical determination – both in qualitative and quantitative terms. Selecting appropriate sample preparation methods influences not only the precision of the substance (analyte) determination but also the possibility of its determination. In the analysis of biological samples, it is practically impossible to avoid the use of sample preparation methods. In many cases, methods must be combined [19,20,21,22,23]. The entire process can be modelled as a set of individual operations, which are selected based on certain requirements, such as the sample complexity, sample volume, amount of impurities, etc. The use of some methods appears to be trivial. It is necessary to realise practical requirements, such as when handling large sequences of samples that have to be processed in a relatively short time. In this regard, it is necessary to consider the extra demands on the automation and accuracy in terms of Good Laboratory Practice. The modern method's group of processing biological material usually includes solid phase extraction (SPE), particularly its miniaturisation (MCPs – SPE) [24,25,26]. The use of new media in combination with the MCPs, which offers the possibility of processing 96 or more samples in one extraction step, is particularly suitable for large sequences. The very low consumption of samples and solvents is also an important advantage [27,28,29,30].

The use of MCPs in the pre-analytical period is very easy. Purchasing expensive equipment is not necessary, and the samples can be processed similar to when using test tubes. The holder for the MCPs is a common piece of equipment for centrifuges and vacuum concentrators.

MCPs can be used in the full range of the pre-analytical period for both sample storage and sample preparation.

7.1. Long-term sample storage

MCPs are optimal for the storage of samples, especially coupled with low volume and high throughput techniques (HPLC, UHPLC, immune-assays, etc.) [31].

As mentioned previously, PP is an ideal material for plates exposed to wide changes of temperature (storage at −86 °C and evaporation at +50 °C). This material has excellent properties for long-term storage. Each microplate should be covered with a special seal lid, which protects against contamination and evaporation of these stored samples.

COC polymer MCPs are suitable for cold storage, incubation and thermo-cycling. Black coloured MCPs can be used for light sensitive analytes.

7.2. Sample storage prior to analysis

The storage of samples during the pre-analytical stage is important, especially in the determination of unstable and photosensitive analytes. The extract is usually an organic solvent, which should be selected with consideration of the MCPs material. Chemical stability and resistance of the material of the microplate organics solvent is important, specifically in MS detection.

Samples are usually stored in HPLC auto samplers before analysis. It is important to maintain the samples in the appropriate storage conditions because a considerable amount of time always elapses between the analysis of the first and the last sample (depends on the number of wells in one plate and the time of analysis).

The samples should be stored in a dark, cool place that is protected against evaporation. Some modern auto samplers allow these conditions [32,33].

In cases where chemical resistance and thermal stability are important, glass MCPs are ideal, for example in UHPLC and MS applications.

7.3. Sample centrifugation

Centrifugation is also an important part of the pre-analytical procedure. This process is always used in the sample preparation procedure after sample admission and its storage before the extraction procedure. Furthermore, centrifugation is a very important step in various (SPE, LLE, etc.) sample preparation techniques.

The centrifugation of MCPs is generally very easy. Modern centrifuges allow the rotors to be exchanged with holders for the MCPs, or there are centrifuges equipped with MCPs holders that can be removed and changed without removing the rotors (Eppendorf, Heraeus, etc.).

The centrifugation speed is limited by the material, shape and thickness of the MCPs. For example, polypropylene MCPs can be centrifuged with a maximum speed $6000 \times g$ and the working temperature ranges from −86 °C to +100 °C. Polystyrene MCPs are very fragile and not suitable for high centrifugation speed (over $3000 \times g$). One solution to this problem will be the possible usage of polycarbonate MCPs which will be resistant to high centrifugation speed as was observed in polycarbonate test tubes case suitable for ultracentrifugation.

7.4. Sample purification and crystallisation

Protein crystallisation and purification are used in biology and physical geochemistry.

Protein crystallisation and purification MCPs are manufactured for high-throughput protein crystal growth and screening. Each

well of a microplate typically holds somewhere between tens of nanoliters to several millilitres of liquid.

In the works [34,35], protein crystallisation was investigated because it purifies specific proteins from otherwise impure mixtures using 96-well MCPs to achieve a highly pure protein as an active enzyme.

7.5. Sample evaporation and chemical resistance

Sample evaporation and in mobile phase reconstitution are key steps before injecting the sample into the HPLC system. This procedure can be used for sample pre-concentration, and this technique can be used for the reconstitution of analytes in the mobile phase. During this process, the target analytes in the MCPs (usually dissolved in organic solvent) are heated, shaken or centrifuged. An inert chemical microplate material is required, especially in MS detection.

PP is a smooth material with a crystalline structure, which provides it with high resistance to breakage and chemical/thermal influences. Therefore, MCPs manufactured from PP are ideal for use where temperatures from −196 °C to +121 °C are experienced.

PS has an amorphous structure, is relatively fragile and has a lower chemical and thermal resistance than PP between −20 °C and +60 °C [36].

The 100% cycle-olefin polymer MCPs exhibit thermal stability from −80 °C to 120 °C and broad chemical resistance.

7.6. Filtration before LC analysis

Filtration is a part of the sample preparation procedure and clean-up procedure, especially if we are working with biologic materials [37]. There are various commercially available MCPs with filters composed of different materials and pore sizes, such as PP, cellulose acetate, cellulose nitrate, hydrophilic polyvinylidenfluoride – PVDF, hydrophobic PVDF, and glass fibres. The most common pore sizes are 0.45 μm and 0.20 μm (for example, Pall Corporation, Ann Arbor, USA).

Filtration MCPs are available in various well shapes and volumes, such as 100 μl for 384-well, 150 μl, 350 μl, 800 μl and 2 ml for 96-well and 10 ml for 24-well etc.

Various types of MCPs vacuum manifolds for filtration and solid phase extraction (SPE) of samples were developed (one is in Fig. 3). Manifold usage is very simple and vacuum helps pass the samples through filters or SPE sorbents. Using MCPs filters and SPE phases is improving throughput and reducing costs for a number of biological sample preparation and clean-up procedures.

7.7. Protein precipitation

The majority of the target analytes in biological fluids (especially in serum) are transported by binding proteins. The elimination of these proteins and releasing the target analytes is one of the important principles. Deproteinisation is possible by 3 basic principles, which include *enzymatically* by specific protein (protein kinases), *physically* by extreme temperatures, by ultrasound, and *chemically* with organic solvents, acids, etc. [38].

Special MCPs for protein precipitation are commercially available. FF (fast Flow) Protein Precipitation MCPs are based on removing proteins from plasma via precipitation with acetonitrile and vacuum filtration. The plate has a special membrane configuration that retains the acetonitrile/sample mixture during the mixing or inoculation steps (GE Healthcare UK, Canadian Life-science, Canada, Whatman, USA) [39].

7.8. SPE phases

Solid phase extraction is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type of, analyte from a solution. SPE is usually used to clean up a sample before using a chromatographic or other analytical method. Solid phase extraction procedures are used not only to extract traces of organic compounds from environmental samples but also to remove the interfering components of the complex matrices to obtain a cleaner extract that contains the analytes of interest [40,41].

MCPs sorbents are prepared using various chemistries, for example: Octadecyl, Octyl, Phenyl, Cyclohexyl, Butyl, Ethyl, Methyl, Cyanopropyl, Silica Gel Florisil Alumina (Neutral) Alumina (Acidic) Alumina (Basic) Diol Carboxylic Acid Benzenesulphonic Acid Aminopropyl Pri/Sec Amine Diethylamino Quaternary Amins (see Table 1) etc.

These sorbents are presented in various amounts and volumes of plate wells, usually 2–200 mg and 1–2 ml (see Table 2).

The primary advantage of using SPE MCPs is handling large sequences of the samples in one extraction procedure; in SPE extraction, using MCPs is advantageous, especially during sorbent activation and the elimination of interferences combined with the use of a multichannel pipette. The primary disadvantage is the inequality of sorbent in each well, which can cause elution or activation of each well at different speeds. This fact is primarily a problem in sorbents that must not dry out, which makes using a multichannel pipette pointless.

96-Well Plate-to-Plate Gravity Fluorous Solid-Phase Extraction was presented by Zhang and Lu [44] – large particle size (125–210 μm) fluorous silica gel bonded with a $-SiCH_2CH_2C_8F_{17}$ stationary phase has been employed for gravity-driven fluorous solid-phase extraction (F-SPE) on two types of 96-well plates. The products collected in the 96-well receiving plates are directly concentrated on a GeneVac vacuum centrifuge. This simple and highly efficient plate-to-plate F-SPE technique has been demonstrated in the purification of four 96-compounds; approximately 80% of products in each library have greater than 85% purity after F-SPE without performing a chromatographic separation.

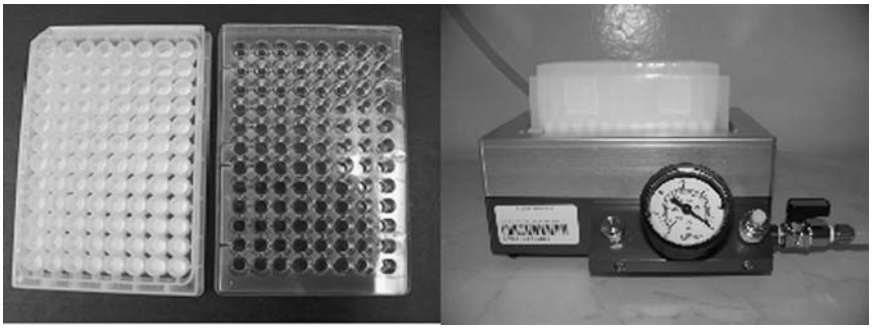

Fig. 3. MCP for filtration (left) and vacuum manifold with SPE MCPs (right) (Pall Corporation, Ann Arbor, USA).

Table 1
Common SPE MCPs sorbents (Phenomenex, Sopachem, Waters, Sigma Aldrich).

Sorbent	Analytes
ABW, cation-anion mixed mode	Extraction or fractionation of complex mixtures
C18-E octadecyl bonded endcapped silica	Hydrophobic (i.e.) those that contain carbon chains
C18-T octadecyl bonded endcapped silica	Large molecules
C18-U octadecyl bonded endcapped silica	Hydrophobics that exhibit slight polar attributes
C8 octyl bonded endcapped silica	Extremely hydrophobic compounds
CN cyanopropyl bonded endcapped silica	Polar compounds such as those that contain hydroxyl groups
Eco-screen normal phase sorbent with sodium sulphate to remove any excess water	Hydrocarbons from environmental samples
LC – diol diol bonded silica	Normal phase extraction of polar compounds
Melamine	Melamine and cyanuric acid
NH_2 aminopropyl bonded silica	Acidic compounds (i.e.) strong acids
PAH	Polycyclic aromatic hydrocarbons (PAHs)
Phenyl	Hydrophobic and aromatics
SAX quaternary amine binder silica with Cl− counterion	Weakly acidic compounds
Screen-A	Acidic drugs from biological matrices
Screen-C	Basic drugs from biological matrices
Screen-C GF	Basic drugs viscous biological samples matrices
SCX sulphonic acid bonded silica with Na+ counterion	Weakly basic compounds
SDB-L	Hydrophobic and aromatics
SI-1 Silica	Polar analytes such as those that contain amino groups
WCX (mixed-mode weak cation exchange)	Basic compounds, particularly strong bases
HCX, HCX-3 and HCX-5 (non-polar and strong cation exchange retention)	Basic drugs from biological fluids
HCX-Q combines non-polar and weak cation exchange	Quaternary amine or strongly basic drugs from biological fluids
HAX combines non-polar and strong anion exchange	Acidic drugs from biological fluids
Oasis HLB: hydrophilic–lipophilic-balanced reversed-phase	Sorbent for acids, bases and neutrals
Oasis MCX: mixed-mode cation exchange	Sorbent for bases
Oasis MAX: mixed-mode anion exchange	Sorbent for acids
Oasis WCX: mixed-mode weak cation exchange	Sorbent for strong bases and quaternary amines
Oasis WAX: mixed-mode weak anion exchange	Sorbent for strong acids
LC-Alumina-A alumina-based packing acidic pH ~5	Anion exchange and adsorption extraction of polar compounds, such as vitamins
LC-Alumina-B alumina-based packing basic pH ~8.5	Polar compounds, and cation exchange
LC-Alumina-N alumina-based packing neutral pH ~6.5	Polar compounds with pH adjustment, cation or anion exchange of vitamins, antibiotics, essential oils, enzymes, glycosides, and hormones
ENVI-Carb graphitised carbon-based	Polar and nonpolar compounds
ENVI-Carb C graphitised carbon-based packing nonporous, surface area 10 m^2/g, 80/100 mesh	Polar and nonpolar compounds
ENVI-chrom resin-based packing – 80–160 μm spherical particles	Polar aromatic compounds such as phenols from aqueous samples also nonpolar to midpolar aromatic compounds
LC-Florisil Florisil®-based packing – Magnesium silicate 100/120 mesh particles	Polar compounds, such as alcohols, aldehydes, amines, drugs, dyes, herbicides, pesticides, PCBs, ketones, nitro compounds, organic acids, phenols, and steroids
ENVI-Florisils Florisil®-based packing – Magnesium silicate	Polar compounds, such as alcohols, aldehydes, amines, drugs, dyes, herbicides, pesticides, PCBs, ketones, nitro compounds, organic acids, phenols, and steroids

Table 2
Selected SPE MCPs producers and their products.

Producer	Product name	Sorbent[a]	Sorbent mass range (mg)
Agilent Technologies	Bond ELUT 96-Square Well,	13	20–100
Sigma Aldrich, Supelco	Discovery® SPE 96-Well Plates	14	25–100
Applied Separation	Spe-ed 96 (96-well) Cartridges	21	25–200
3M Corporation	Empore™ 96-Well Plates	3	10, 14
Waters Corporation	μElution Plates	6	2
Waters Corporation	Oasis® microplate	5	5–60
United Chemical Technologies, Inc.	96-Well SPE Plates	50	50–300
Thermo Scientific	SPE 96-well plate HyperSep(tm)-96	12	10–500
Avantor Performance Materials	Speedisk 96-Well Plates	6	20
Phenomenex	Strata-X	21	10–100
Provair Sciences	Microlute™	>56	10–100
Macherey-Nagel Inc.	Chromabond® Multi-SPE	40	25–100
Orochem Technologies	Orpheus	26	25–200
Argonaur (IST Ltd.)	Isolute-96-well plate	30	10–100

[a] Number of available different sorbents published in internet pages of producers.

8. Discussion

The discovery of MCPs and their use in the bioanalytical sciences can be taken as the first step towards the miniaturisation of the methods in various medical science branches (immunology, cytology, biochemistry and genetics). The exponential development of MCPs has driven manufacturers to produce new shapes and sizes of the plates composed of different materials. MCPs have found use in the other science and non-science fields. One of the key advantages of MCPs is their very simple usage.

This review provides a general overview of the materials, shapes and various possibilities of usage of MCPs in biological

research combined with liquid chromatography with critical comment of their advantages and disadvantages.

Fast technical developments in HPLC are resulting in improvements of sample preparation methods. High throughput analysis is becoming more important; therefore, the use of ultra-fast separation coupled with rapid and simple pre-analytical procedures is being established in common clinical and pharmaceutical laboratories. The most powerful combination is the use of MCPs. In this review, it was shown that MCPs combined with LC analytical methods can be effectively used to process large sequences of samples in a minimal amount of time and with minimal sample volumes.

The inherent advantages of MCPs and LC coupling include the single methodology, automation, high-throughput, economy, low sample and solvents consumption and minimal sample manipulation.

MCPs can be used in the pre-analytical phase very easily and can completely replace test tubes. Common techniques such as centrifugation, evaporation, and filtration can be used only with changing of holders for test tubes to MCPs. From our own experience, the transfer of the column SPE method to MCPs SPE procedure is easy, and saves time and money [32].

Because of the various types of cups and covers and the temperature resistance, MCPs are suitable for long-term sample storage or storage before analysis. The MCPs technology is a powerful technique, whose usage remains a challenge in modern bioanalytical methods in clinical research and will undoubtedly yield improvements that will lead to a better monitoring of diseases, can help its diagnoses and patient care.

9. Conclusions

Microplate technology is a new trend in miniaturisation, especially in the pre-analytical procedure connected to HPLC/UHPLC techniques. This technology is a green method, and a time and money saving solution for handling large sequences of samples. This technology could be a progressive tool in common routine and research laboratories because of its simplicity (no special expensive instruments are needed); furthermore, it is easy to use and allows methods to be easily transferred. In the future, it is possible that microplates could replace vials and test tubes that are currently used in bioanalysis.

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