Recently Developed Techniques for Extraction of Drugs and Medicines during Chromatographic Determination in Toxicological Analysis

Akira NAMERA [a, *], Akihiro NAKAMOTO [b], Takeshi SAITO [c], Manami NISHIDA [d]

[a] Department of Forensic Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Kaumi 1-2-3, Minami-ku, Hiroshima 734-8551, Japan

[b] Scientific Investigation Laboratory, Hiroshima Prefectural Police Headquarters, Kohnan 2-26-3, Naka-ku, Hiroshima 730-0825, Japan

[c] Department of Emergency and Critical Care Medicine, Tokai University School of Medicine, Shimokasuya 143, Isehara, Kanagawa 259-1143, Japan

[d] Hiroshima University Technical Center, Kaumi 1-2-3, Minami-ku, Hiroshima 734-8551, Japan

Author to whom correspondence should be addressed: E-mail; namera@hiroshima-u.ac.jp

1. Introduction

Some problems have been encountered in the chromatographic determination of drugs and pharmaceuticals in biological materials, such as low concentration of analytes, a complicated matrix, and the limited sample volume available for analysis. Overcoming these problems is one of the most challenging tasks in toxicological analysis. Endogenous interferences must be removed, and the analytes must be concentrated prior to chromatographic determination. These problems cannot be overcome even by improving the performance of analytical equipment. In clinical, toxicological, and environmental analyses, the process involving purification, concentration, and derivatization of analytes is called "sample preparation [1]." Liquid-liquid extraction (LLE) has been widely used for sample preparation because of its usefulness and low cost of operation. In the LLE technique, analytes are systematically extracted and purified by using the chemical characteristics of the analytes. However, a large amount of extraction solvent is required, and the extraction process is complicated. Moreover, the organic solvents used in this technique are toxic to both humans and the environment. The procedure is also time-consuming, and the number of samples that can be simultaneously treated is limited. The formation of the emulsion during extraction is also a critical problem. To overcome these problems, solid-phase extraction (SPE) was developed. In the SPE technique, silica gel or polymer resins is embedded in a cartridge as a solid adsorbed material. The analytes are adsorbed onto the surface of the sorbent when the sample solution flows through the cartridge. Endogenous interferences can be removed by passing an aqueous solution through the cartridge. Sorbent materials can be selected according to the behavior of the analytes. The LLE and SPE methods involve laborious, intensive, and expensive preparatory procedures. Moreover, in both these methods, the eluated solution finally has to be removed by evaporation.

To avoid these complicated and time-consuming steps, new techniques have been recently developed to reduce the size of the previously used devices or inject all the adsorbed analytes into

the analytical equipment. In this chapter, some unique and useful techniques and devices for the chromatographic determination of drugs and pharmaceuticals in biological materials are introduced.

2. Technique

2.1. Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) is a unique and solvent-free sample preparation technique, which was first developed by Pawliszyn and coworkers at the University of Waterloo in 1990 [2]. This technique uses a syringe-like device, which consists of a fiber assembly and a holder (Figure 1). The extraction coating (ca. 0.6 µl) is deposited at the tip of a thin stainless wire in the fiber assembly and is transferred to a needle in the fiber assembly by pulling the plunger out. The coating is exposed to the headspace of the sample vial or immersed in the sample solution in order to extract the analytes from the sample solution. After extraction is complete, the coating is withdrawn into the needle by pulling the holder back, and the extract is then injected into a GC or an LC. The analytes are thermally desorbed in the injection port of the GC, or dissolved in the solvent and injected into the LC (Figure 2). One of the advantages of SPME is that the adsorbed analytes can be completely injected into the analytical equipments. Analytes can be extracted by SPME by using only an extraction vial, therefore, contamination of the analytes is prevented during the sample extraction procedure. The coating is repeatedly used 50–100 times.

The basic principle of SPME is described in book [3]. In brief, the analytes are transferred from the sample solution to the coating according to distribution constants. The amount of extracted analyte is increased by increasing the exposure or immersed time until an equilibrium is achieved between the coating and the sample solution. The amount of analytes adsorbed or absorbed depends on their concentration in the sample solution; and uantification of analytes can be

carried out by using the SPME technique. In headspace SPME, analytes are extracted from the headspace of the sample solution; this technique has high selectivity, because only target analytes and not endogenous interfering substances such as proteins and lipids are extracted. SPME is suitable for the analysis of volatile compounds; it is particularly suitable for the simultaneous determination of compounds with low and high boiling points.

The SPME technique has been used to extract a wide range of analytes from different matrices (urine, plasma, and blood). Many volatiles, illegal drugs, and pharmaceutical drugs have been extracted from biological samples such as blood, plasma, and urine by using the SPME technique, and some reviews and books on this technique have been published [4-7]. Thypical chromatogram extracted amphetamines in human hair by using SPME is shown in Figure 3.

2.2. Stir bar sorptive extraction (SBSE)

The stir bar sorptive extraction (SBSE) technique, which is based on the same principle as SPME, was developed by Sandra and coworkers [8]. During SBSE, the extraction coating is deposited on the surface of the magnetic stirring bar; this deposition position is different from that in SPME, in which the extraction coating is deposited at the tip of the stainless wire (Figure 4). The amount of sorbent used in SBSE is ca. 125 µl, which is greater than that used in SPME. However, the coatings are limited to commercially available polydimethylsiloxane (PDMS). During extraction of analytes from an aqueous sample, the SBSE bar is immersed in the sample and stirred for the optimal period. After extraction, the bar is removed from the sample solution and wiped with a soft tissue. The analytes are thermally desorbed in the injection port of the GC. During LC analysis, the analytes are desorbed by dipping the bar in the solvent. The desorption process is relatively slow due to the large amount of sorbent used. The biggest drawback of the SBSE technique is that specialized equipment (a thermal desorption unit) is necessary for the thermal

desorption of the analytes.

The SBSE technique has been used to extract a wide range of analytes in different matrices (urine, plasma, and blood). For example, several drugs such as local anesthetics, methadone, and cocaine and cocaine metabolites have been extracted from biological samples such as blood, plasma, and urine by using the SBSE technique [9].

2.3. Liquid-phase microextraction (LPME)

Liquid-phase microextraction (LPME) is a miniaturized version of the traditional LLE technique based on passive diffusion. Single-drop solvent extraction was first introduced in 1996 as a type of LPME [10] (Figure 5). In this technique, a droplet of an organic solvent is suspended at the tip of the needle of a microsyringe. The droplet is exposed to the headspace of the sample vial, or immersed in the sample solution. After extraction, the droplet is withdrawn into the needle of the syringe, and the extract is then directly injected into a GC or an LC. This method has many advantages. Different kinds of solvents can be used, and a low volume of extraction solvent is sufficient. In addition, the operational cost is low, and preconditioning is not necessary. However, this method has some critical drawbacks; (1) if the rotational speed is increased in an attempt to reduce the extraction time, the droplet may be detached from the tip of the needle of the microsyringe; (2) an emulsion may form in the biological samples; (3) this method is not suitable for the extraction of highly volatile compounds because it would require the use of high-boiling-point solvents.

In order to overcome these drawbacks, Rasmussen and coworkers developed another LPME technique involving the use of a hollow fiber [11]. In this technique, the acceptor phase is placed inside the hollow fiber. The hollow fiber is then placed in the sample solution for 15–60 min. After extraction, the acceptor solution is collected by a microsyringe and injected into a GC

or an LC. Two-phase and three-phase LPMEs in which hollow fibers are employed have been used to extract analytes from the sample solution (Figure 5). In the two-phase LPME technique, organic solvents insoluble in water are usually used as the acceptor solution. The solution may be directly injected into the GC, or may be evaporated and dissolved in an aqueous solution before being injected into the LC or CE. Therefore, the two-phase LPME technique is suitable for the extraction of nonpolar compounds from an aqueous sample. Since the mass transfer of analytes depends on partition coefficients, hydrophobic analytes can possibly be extracted by the two-phase LPME technique. However, this technique is not suitable for the extraction of polar compounds. In the three-phase LPME technique, a porous hollow fiber is impregnated with an organic solvent, and an aqueous acceptor solution is introduced into the hollow fiber. The analytes are transferred through the thin layer of the organic solvent in the fiber and then transferred to the acceptor solution. This extraction rate (recovery) is controlled by the partition coefficients of the analytes.

The LPME technique has been used to extract a wide range of analytes in different matrices (urine, plasma, and blood). For example, several drugs such as methamphetamine, diazepam, and naproxen have been extracted from biological samples such as blood, plasma, and urine by using the LPME technique [12, 13].

2.4. Pipette-tip extraction

Pipette-tip extraction is a miniaturized version of the conventional solid-phase extraction technique. Sorbent is packed in the top of the 0.2 to 1.0ml - volume type micropipette. Analytes are extracted by repeated aspiration and dispensing of the sample solution. The extraction procedure is simple and easy because no specialized equipment is required; the mechanism of the transfer of the analytes in this procedure is the same as that in the conventional SPE technique. Usually, the sorbent is washed and pre-activated with methanol and water, respectively. The

sample solution is passed through the pipette by aspiration and dispensing using the micropipetter. If the recovery is low, the aspiration and dispensing processes are repeated. After extraction, the adsorbed analytes in the sorbent are eluted with the solvent. The evaporation step is omitted in order to concentrate the analytes. The eluate can be directly injected into a GC or an LC so that even a small volume of the solvent is desorbed analytes from the sorbent.

Many types of pipette tips are commercially available, such as ZipTip (Millipore), OMIX (Varian), MonoTip (GL Sciences), and NuTip (Glygen) (Figure 6). The amount of sorbent that can be packed into ZipTip and NuTip pipette tips during the extraction of analytes from a sample solution is small; therefore, a limited amount of analytes is adsorbed and the rest overflows from the tip. The amount of sorbent that can be packed into OMIX and MonoTip pipette tips is greater than that into ZipTip or NuTip pipette tips. As a result, a larger sample volume can be used to concentrate the analytes. Most tips have been developed for the extraction of peptides and proteins [14]. These tips have the potential for the extraction of drugs or pharmaceutics in biological samples [15].

In ZipTip pipette tips, the polymer that is coated over silica beads is fixed to the top of the pipette tip. The amount of polymer used is approximately 0.6 μl, and the maximum amount of analytes absorbed is 400–5000 ng. The sorbent types used are C4, C18, hydrophilic resins, and chelate resins obtained by immobilized metal affinity chromatography.

In NuTip pipette tips, the sorbent is coated on the inner surface of the pipette. The amount of sorbent used is 30–75 μg, and the maximum amount of analytes absorbed is 1–4 μg. Many sorbent types such as C4, C18, hydrophilic resins, and NH_2 are available.

In Mono Tip, the monolithic silica is fixed the top of the 200ul pipette tip. The sorbent is 2.8mm diameter and 1mm thickness (mesopore size of 15nm and through-pore size of 15-25um) The limit of the absorbed amount of analytes is up to ug order. The absorbed analytes is eluted with less

than 50ul. The sorbent types are available; only silica and C18. Some applications have been reported to extract of proteins and drugs [16-19].

In OMIX pipette tips, the monolithic sorbent is fixed to the top of the 100-µl pipette tip. The amount of sorbent used is approximately 0.38–2.4 mg, and the maximum amount of analytes absorbed is up to 80 µg. The absorbed analytes are eluted with less than 50 µl of the solvent. Many sorbent types such as C4, C18, hydrophilic resins, and ion-exchange resins are available [20, 21].

2.5. Microextraction in packed syringe (MEPS)

Microextraction in packed syringe (MEPS) is a new technique for sample preparation and sample handling [22]. In the MEPS technique, approximately 1 mg (0.5~2 mg) of a solid packing material is packed inside a syringe (100–250 µL) as a plug or between the barrel and the needle as a cartridge (Figure 7). This assembly can be used for sample preparation without any modifications and/or can be connected to an autosampler for online analysis of the GC or LC. The sample is prepared by drawing and dispensing the contents of the syringe When the sample passes through the sorbent, the analytes are adsorbed by the sorbent. The concentration of the analytes in the sorbent is increased by repeating the drawing and dispensing steps. While repeating these steps, the sorbent is washed in order to remove endogenous interferences such as proteins and lipids. Next, the analytes are eluted with organic solvents or the mobile phase of the LC and directly introduced into the LC or GC (Figure 8). In the MEPS technique, the sorbents used for the sample preparation are the normal-phase sorbent (silica), reversed-phase sorbents (C2, C8, and C18), the mixed-mode sorbent (C8 + SCX), and the ion-exchange sorbent (SCX). The sample processing, extraction, and injection steps of the MEPS technique, which are carried out using the same syringe, can be fully automated by using the autosampler. The MEPS technique significantly reduces the

volumes of the solvent and the sample required for the extraction. Sample preparation by the MEPS technique is very promising for various reasons: (1) the sample can be easily prepared, (2) the sample preparation is a fully automated online procedure, (3) the sample preparation requires less time, and (4) the cost of sample analysis using this technique is minimal compared to that using the conventional SPE technique.

The MEPS technique has been used to extract a wide range of analytes in different matrices (urine, plasma, and blood). For example, several drugs such as local anesthetics and their metabolites, methadone, and cocaine and cocaine metabolites have been extracted from biological samples such as blood, plasma, and urine by using the MEPS technique [23, 24].

2.6. Spin column extraction

A spin column is a new device used for sample extraction. The spin column is packed with a monolithic silica disk. Monolithic silica is being used as a new type of separation material for HPLC. Conventional materials used as separation materials for SPE are similar to those used for HPLC. However, monolithic silica has potentials for use in drug extraction from biological materials and the subsequent purification of these drugs. In our previous study, we packed monolithic silica into a capillary glass tube (internal diameter (ID): 0.2 mm) and manufactured the extraction device by connecting a microsyringe to the capillary column [25, 26]. However, sample filtration was required to avoid blockage within the tube, and thus, only one sample could be obtained from each batch processing cycle. In order to overcome these problems, a monolithic silica disk (ID: 4.3 mm, thickness: 1.5 mm) was packed into a spin column (Figure 9), wherein the structure of monolithic silica combined the support body and the surface area for each unit volume is wide by comparing with a particle-type silica. Handling procedures such as sample loading, washing, and elution of the target drugs are carried out by column centrifugation. In addition,

many samples can be processed simultaneously. This method has many advantages: its operational procedure is simple, it requires a low elution volume, and it does not require solvent evaporation. When the manifold was used for extraction of analytes, the scientists may watch the manifold and the column not to dry them. In this spin column, the scientists only wait for the centrifugation time and the next step will be prepared during the time.

The spin column extraction technique has been used to extract a wide range of analytes in different matrices (urine, plasma, and blood). For example, several drugs such as amphetamines (Figure 10), naphazoline, amitraz and its metabolites, and pharmaceuticals have been extracted from biological samples such as blood, plasma, and urine by using the spin column extraction technique [27-29].

3. Conclusion

Even though there have been rapid advances in devices used for the analysis of samples, it is necessary for the sample to be prepared accurately. Before carrying out toxicological analysis, the target analytes in biological materials must be purified and their concentrations must be known. Since LLE and SPE are still widely used for sample preparation, potential applications of many drugs and pharmaceuticals have not been discovered. It is also expected to increase the application using the method of the introduction in this chapter, and to be used for the routine analysis. We intend to develop a novel and practicable extraction method in the future.

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Figure regends

Figure 1 Diagram of SPME Fiber Assembly and Holder

Figure 2 Typical extraction procedure of SPME from the headspace

Figure 3 Typical chromatogram of amphetamines extracted from hair by headspace-SPME
AMP-PF:amphetamine propylformate, MA-d_5-PF:pentadeuterated methamphetamine propylformate, MA:methamphetamine propylformate,

Figure 4 Diagram of the stir bar of SBSE

Figure 5 Schema of single droplet LPME, two-phase PLMS, and three-phase LPME

Figure 6 Diagram of pipette tip extraction device;

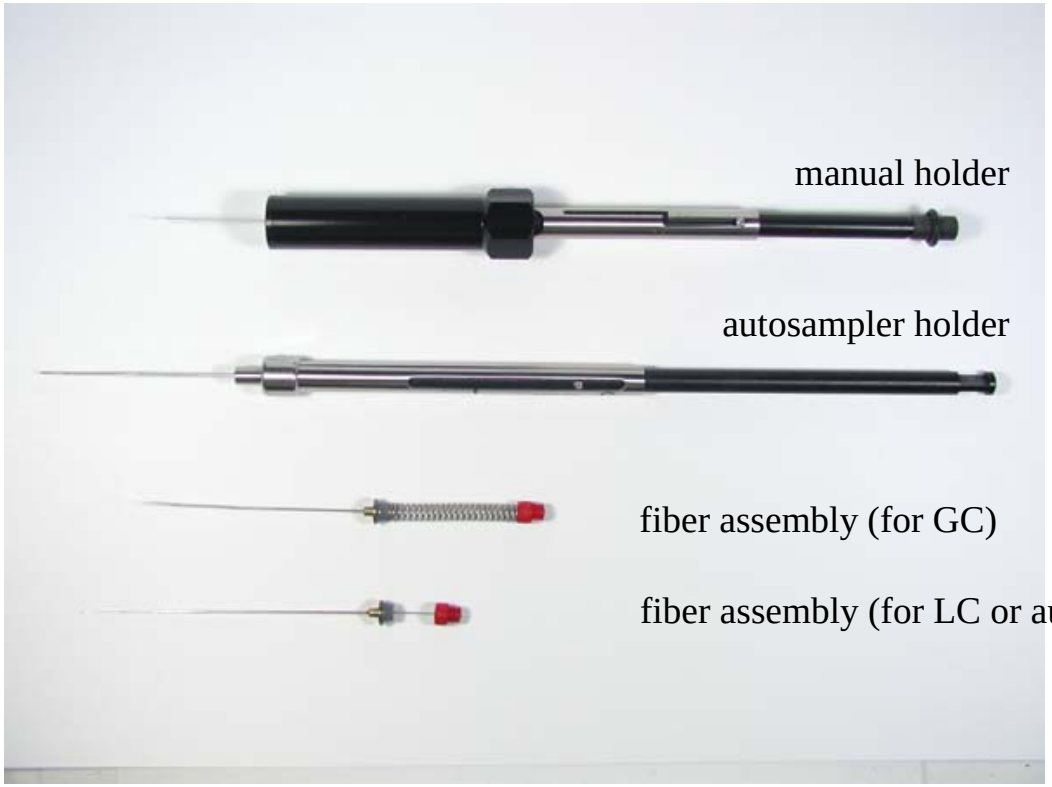
Figure 7 Diagram of MEPS

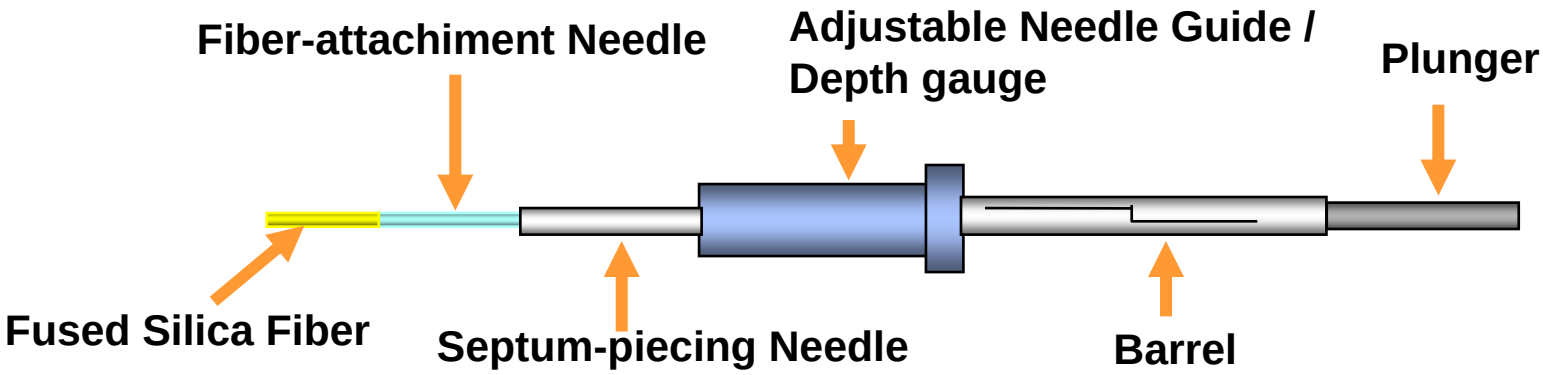
Figure 8 Typical extraction procedure of MEPS from the sample solution

Figure 9 Diagram of spin column packed in monolithic silica disk

Figure 10 Typical chromatogram of amphetamines extracted from urine by a spin column
MDA:3,4-methylenedioxyamphetamine, MAP:amphetamine,
MDMA:3,4-methylenedioxymethamphetamine, MA:methamphetamine

Figure 1


manual holder
autosampler holder
fiber assembly (for GC)
fiber assembly (for LC or autosampler)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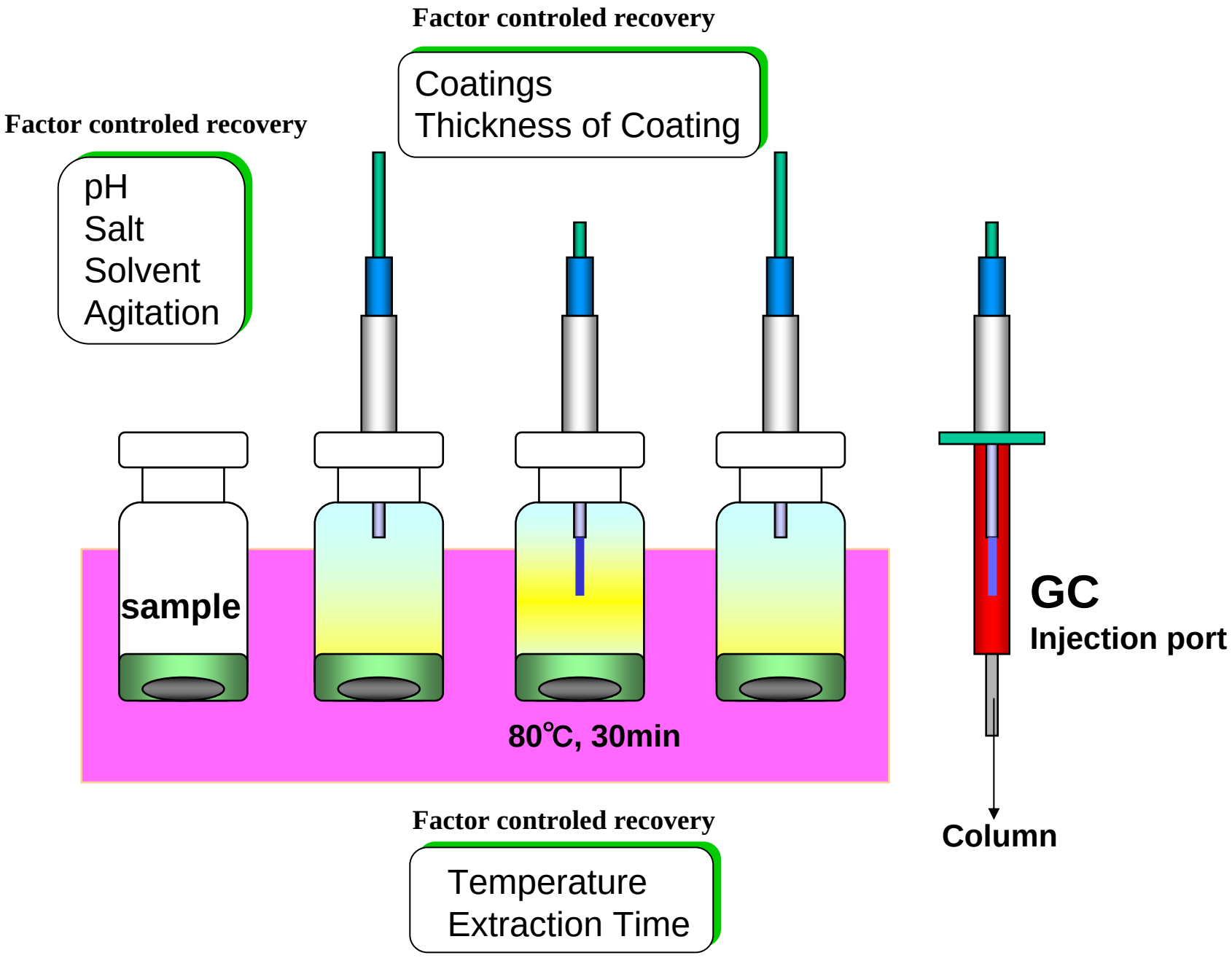
Adjustable Needle Guide / Depth gauge
Plunger
Fused Silica Fiber
Septum-piecing Needle
Barrel

Figure 2


Factor controled recovery
Coatings
Thickness of Coating
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80℃, 30min
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Factor controled recovery
Temperature
Extraction Time

Figure 3

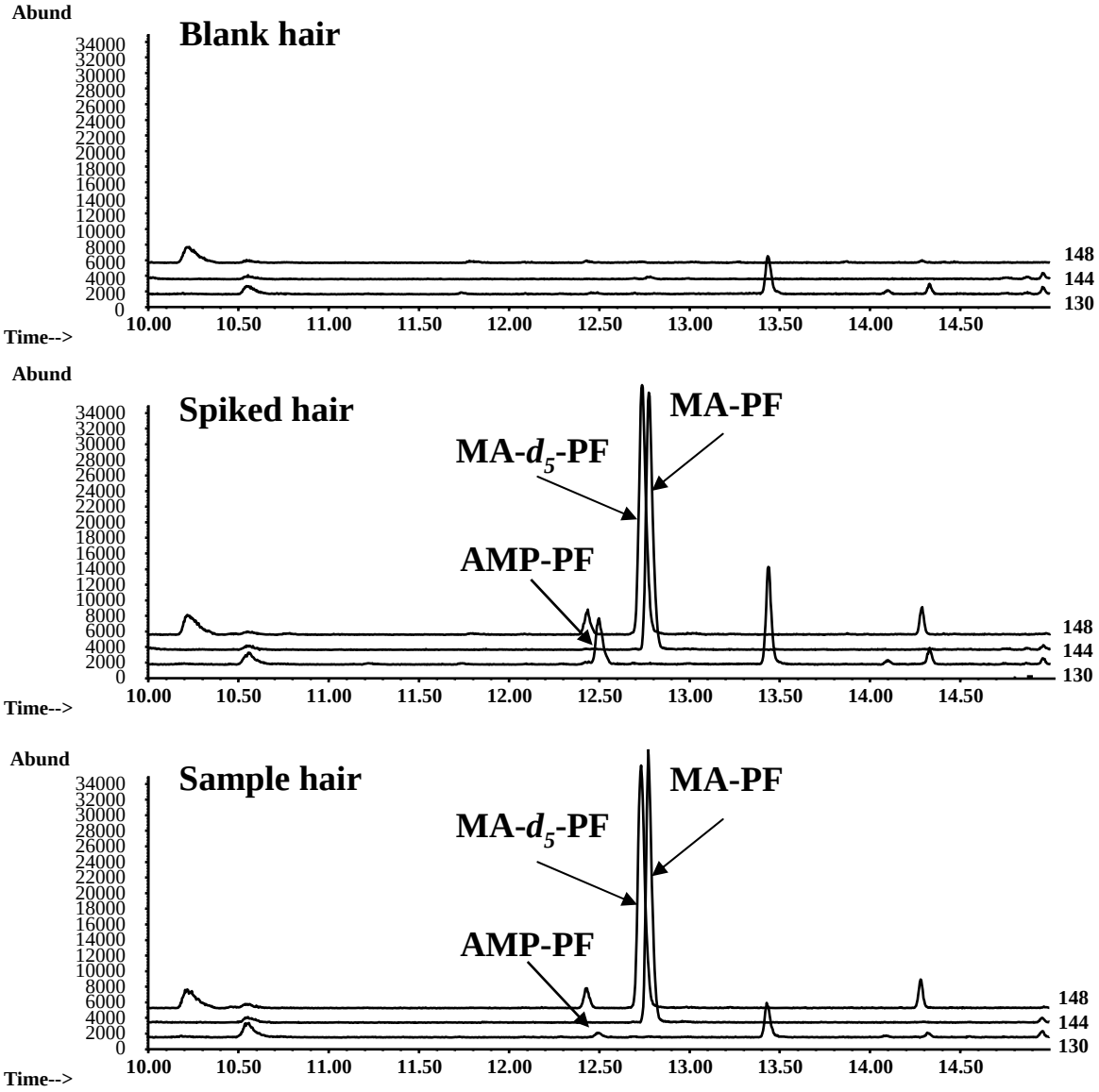
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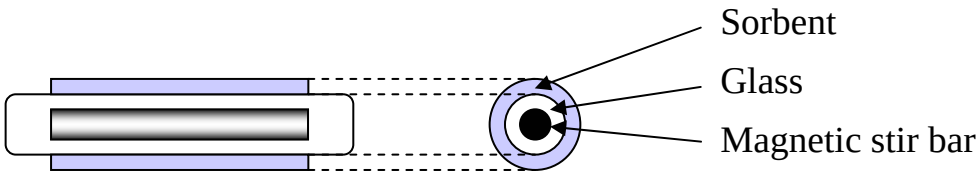
Figure 4



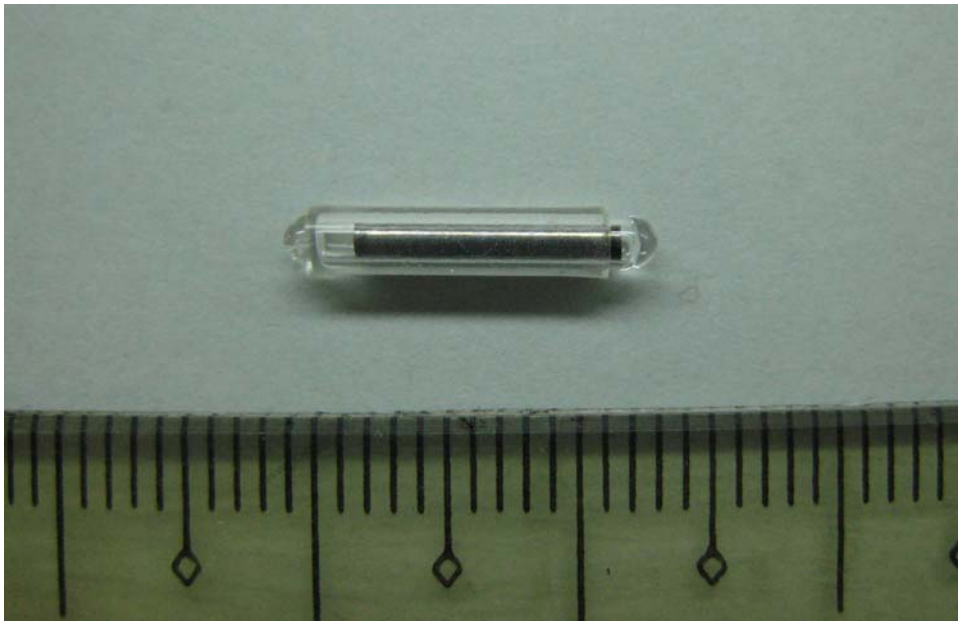
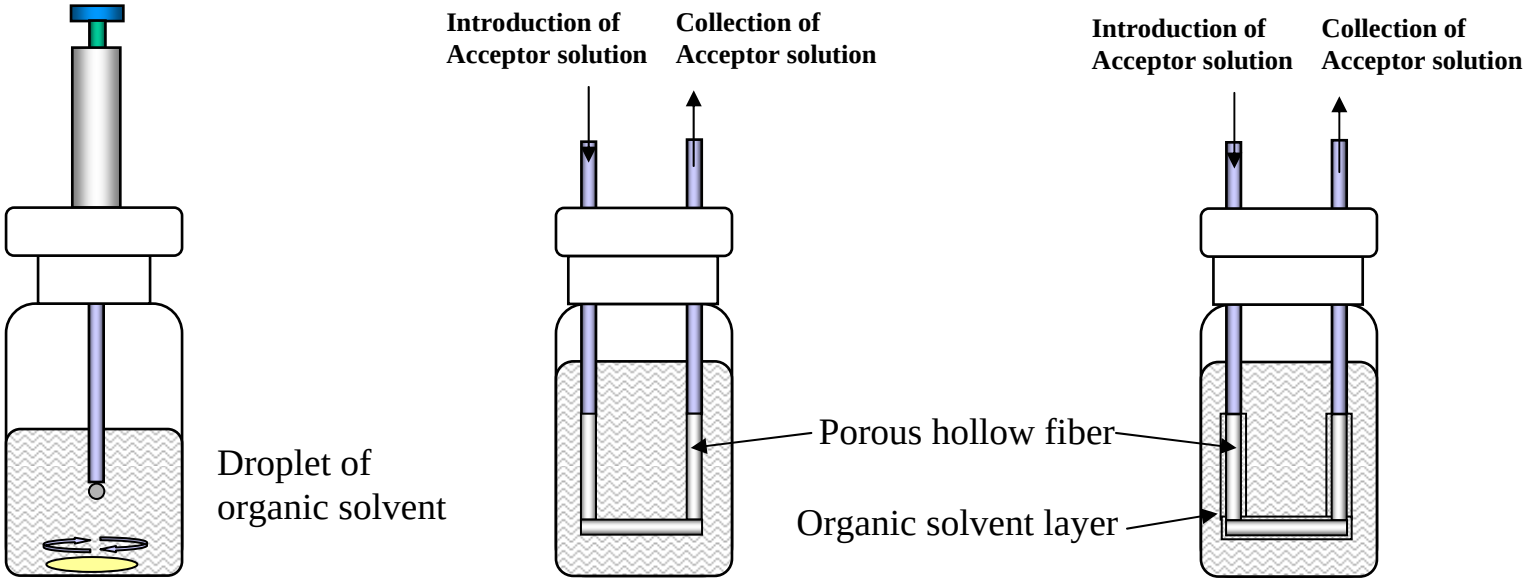


Figure 5



Single droplet LPME

Two-phase LPME

Three-phase LPME

Figure 6

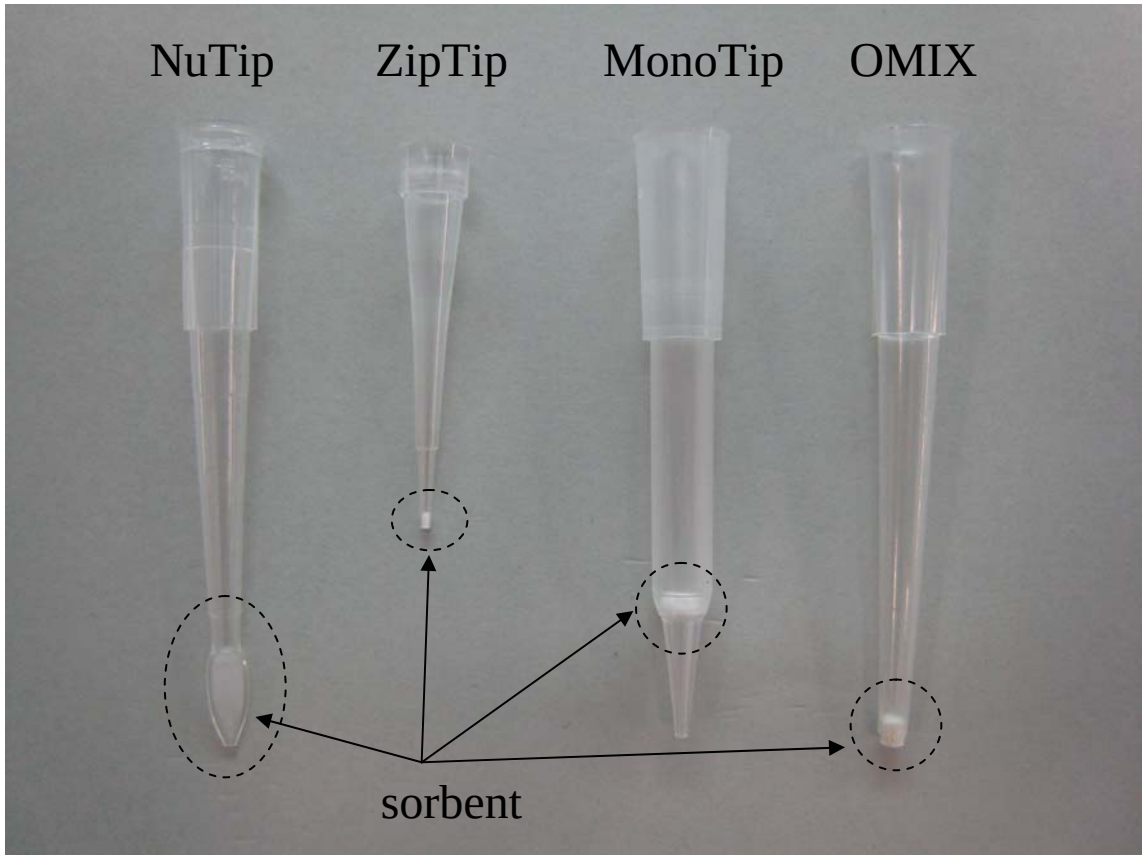


Figure 7

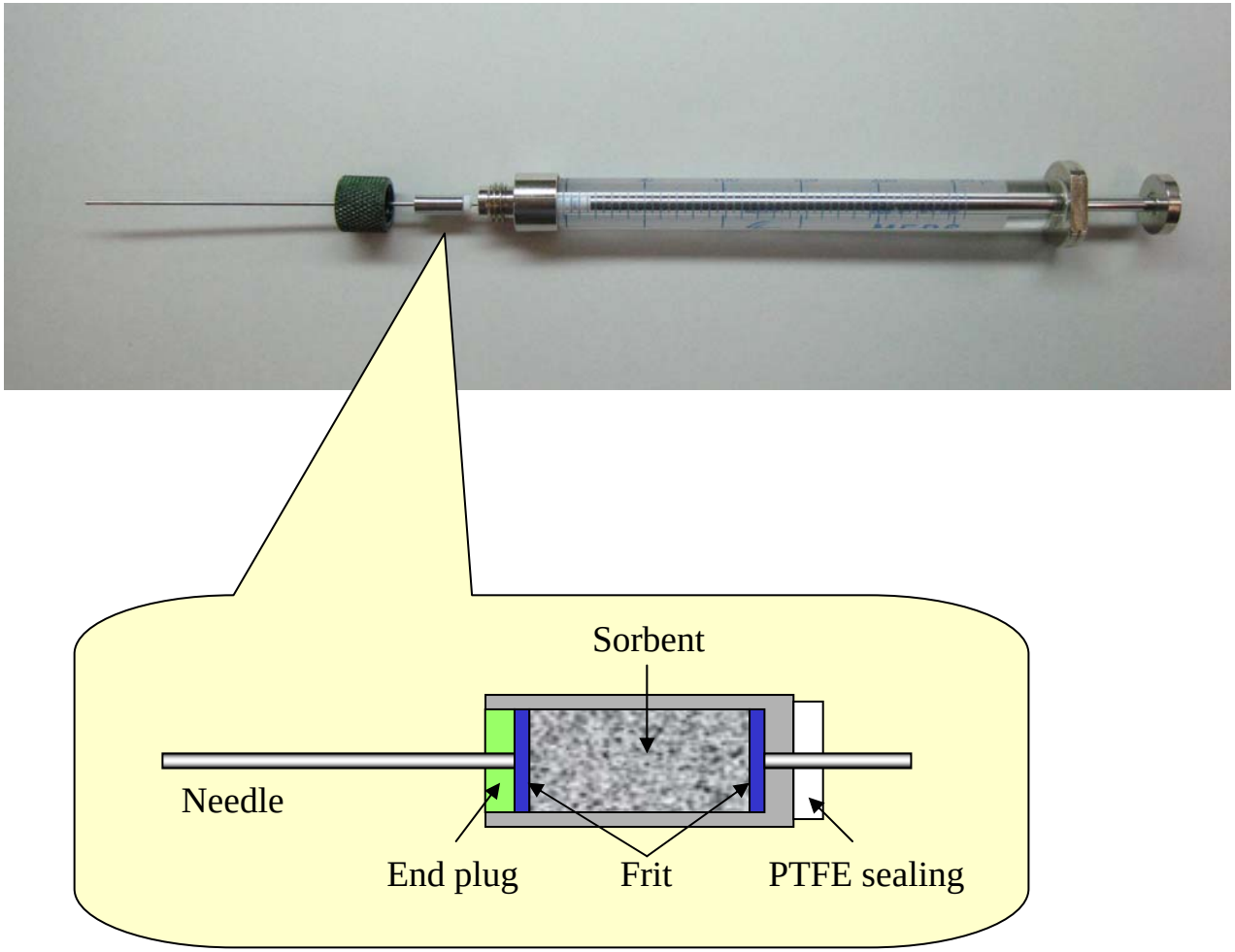


Figure 8

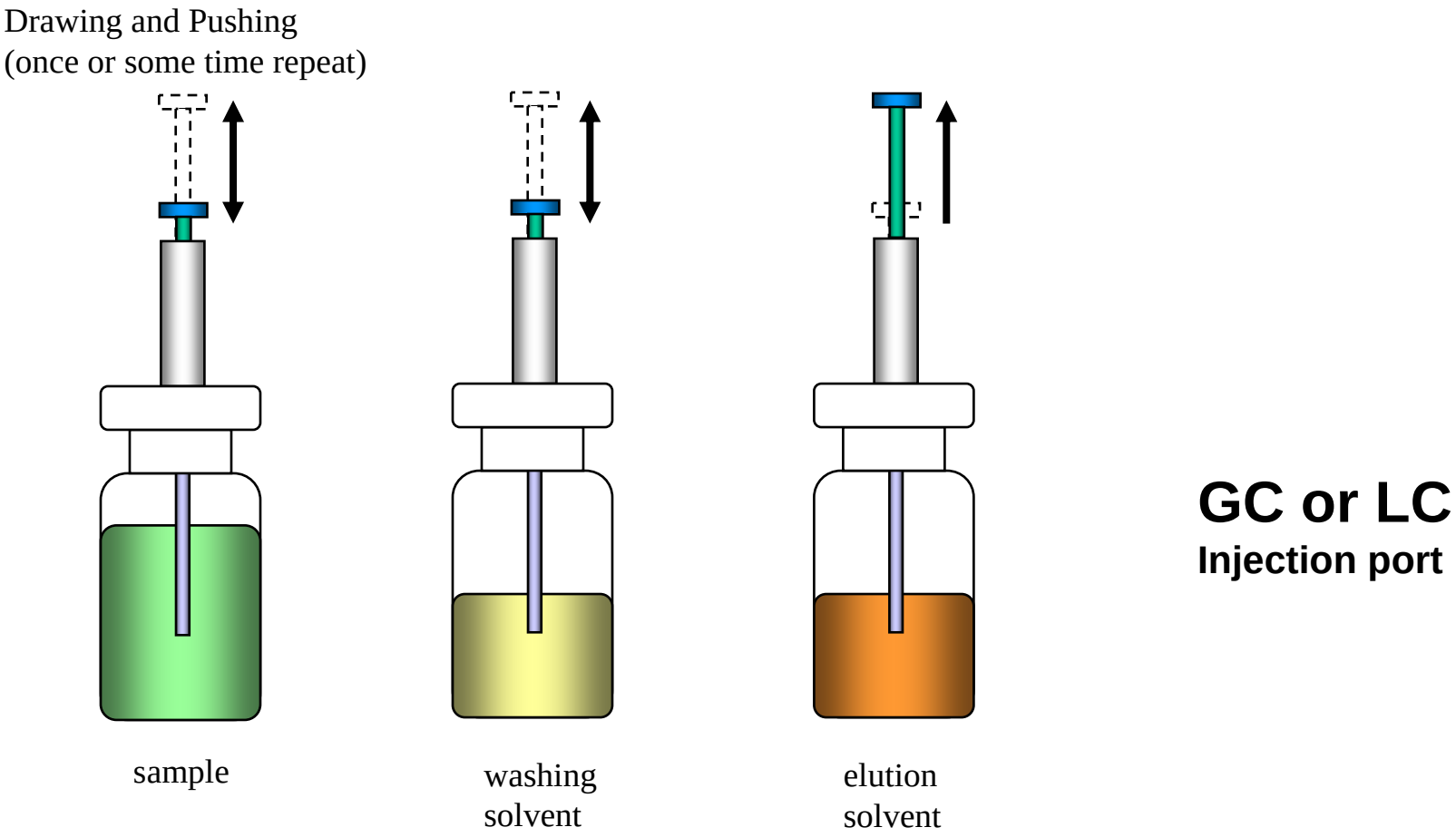

Drawing and Pushing
(once or some time repeat)
sample
washing
solvent
elution
solvent
GC or LC
Injection port

Figure 9

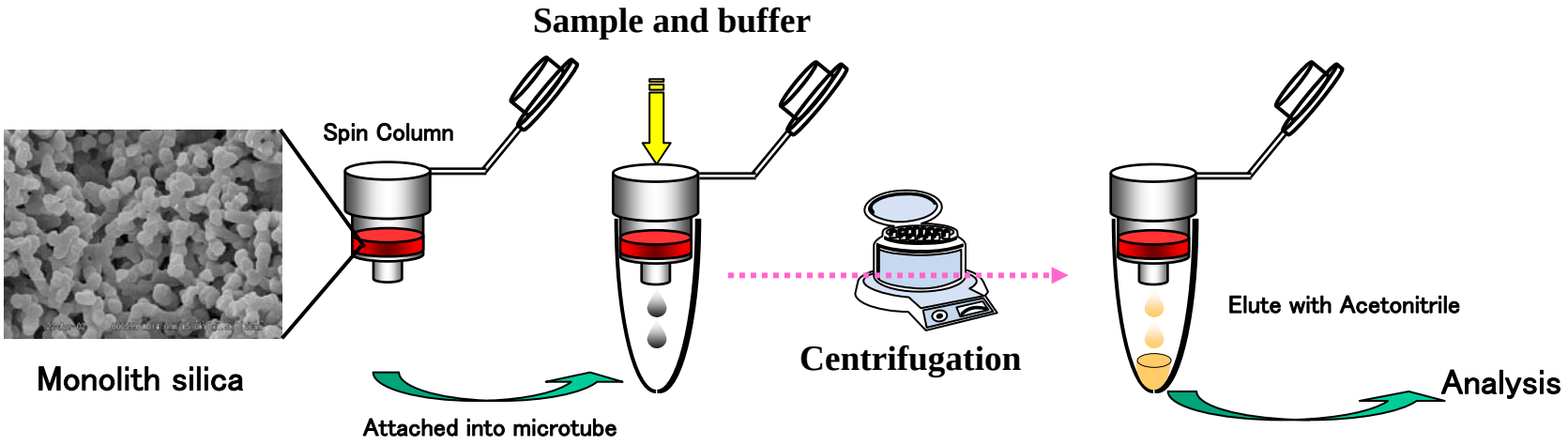
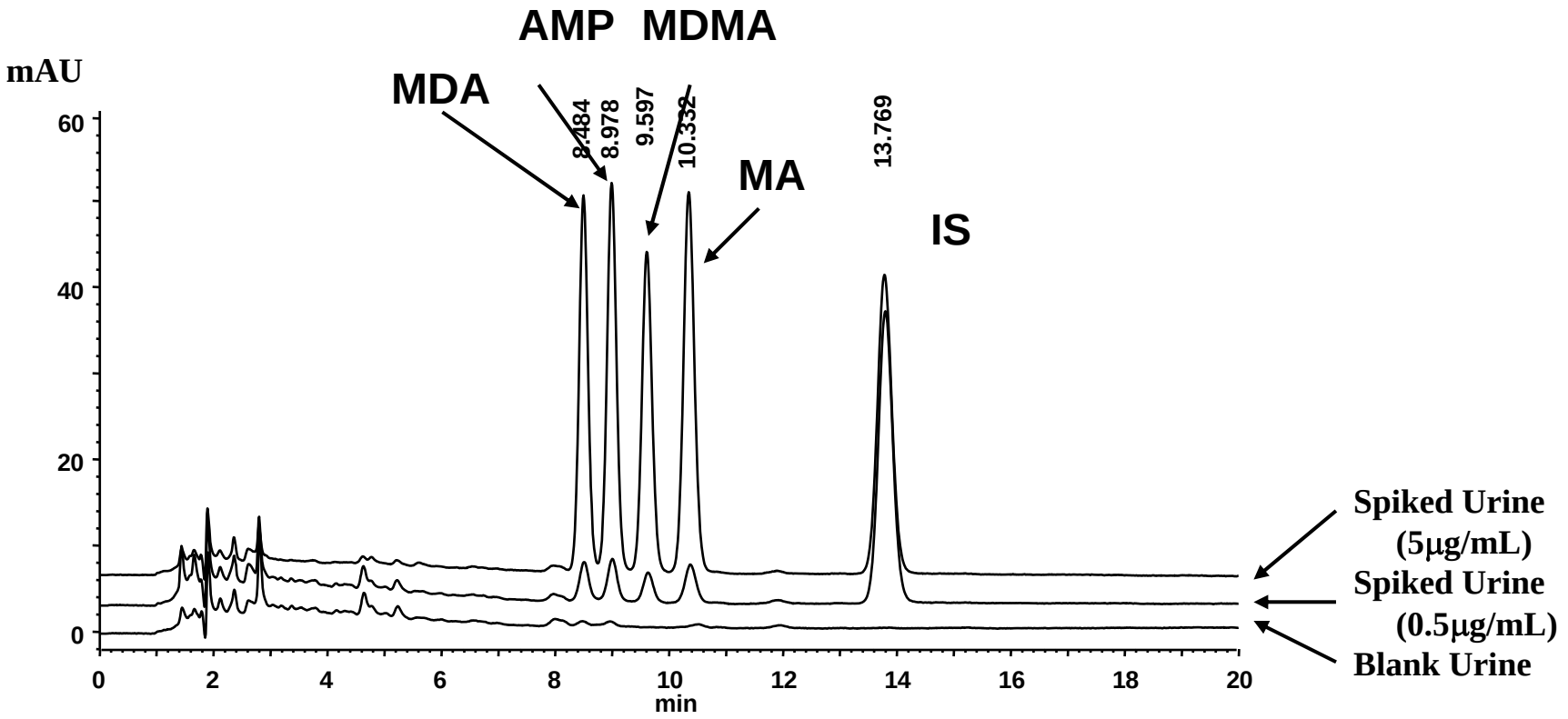

Sample and buffer
Spin Column
Monolith silica
Attached into microtube
Centrifugation
Elute with Acetonitrile
Analysis

Figure 10


AMP
MDMA
MDA
MA
IS
mAU
8.484
8.978
9.597
10.332
13.769
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
min
Spiked Urine (5μg/mL)
Spiked Urine (0.5μg/mL)
Blank Urine