APARATURA
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Automation and robotization of samples preparation methods for chemical analysis

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ABSTRACT:

This article reviews the methods of sample preparation for chemical analysis in which the preparation process is automated and coupled to the measuring device. The paper exemplifies methods of both liquid and solid sample preparation, including elements of an automated and robotic process.

Automatyzacja i robotyzacja metod przygotowania próbek do analiz chemicznych

Słowa kluczowe: przygotowanie próbek, automatyzacja, robotyzacja

STRESZCZENIE:

Przedmiotem artykułu jest przegląd metod przygotowania próbek do analizy chemicznej, w których proces przygotowania próbki jest zautomatyzowany i sprzężony z przyrządem pomiarowym. Zaprezentowano przykłady metod przygotowania próbek ciekłych i stałych z elementami automatyzacji i robotyzacji tego procesu.

1. INTRODUCTION

Before every analysis analysts have to ask themselves a few important questions: What kind of sample is being analysed? What kind of analytes, if any, does a sample contain and what is their quantity? Which method can be applied to determine the analyte? As soon as those questions are answered, new questions arise: How to prepare a sample for analysis? How to remove interferents? A series of such questions and answers enables an analyst to choose an appropriate method or methods of analysis of the tested material or exclude those which cannot be applied because of various restrictions. The procedure of determining an analyte/analytes in a sample consists of a few stages. Figure 1 shows a scheme of an analytical procedure.

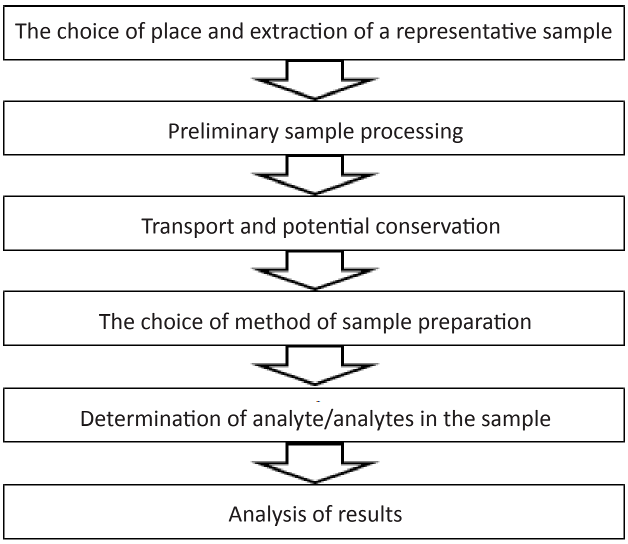

Figure 1 A scheme of a procedure of determining analytes [1]

The complexity of a sample matrix which contains an analyte demands adopting a specific strategy from an analyst. Appropriate sample extraction and its preparation for analysis are two very important stages leading to achieving an analytical success. Every time any quantitative or qualitative change in the sample composition during the preparation stage should be carefully monitored. The stage of sample preparation constitutes approximately 60% of the whole analysis time. The work performed during this stage is very important because it determines whether the results will be correct or not [2].

Errors made during the stages of an analytical procedure may be connected with the extraction of a non-representative sample and its inappropriate preparation. A high quality of sample preparation and shortening its preparation time can be achieved as a result of an automated process, combined with injecting an analytical sample directly into an analyser. Currently, automation is very popular in chemical analyses of materials with complex matrices, which contain a very low quantity of or an impermanent analyte. Automated sample preparation should guarantee that the preparation will not be time-consuming, will be methodologically simple and will result in the extraction of analytes from the analysed material. Automated sample preparation is a solution complying with the principles of green analytical chemistry [3, 4]. Additionally, the possibility of performing the subsequent stages of the analysis by automatons or robots limits significantly the participation of an operator, who frequently makes errors, for instance, when injecting the sample into a chromatographic column.

2. ON-LINE AND OFF-LINE MODES OF SAMPLE PREPARATION FOR ANALYSIS

There are two modes of sample preparation: on-line and off-line. An on-line sample preparation mode involves using devices for sample preparation connected directly to the analyser. This helps to avoid losses of an analyte contained in the sample and makes it possible to achieve results in real time. It is important in case of analysis performed for technological processes control. An undesired result of the analysis shows that the technological process is unreliable, and the person controlling the process may react in an appropriate way. One disadvantage to an on-line mode is the fact that the whole prepared sample is injected into the analyser and neither the analysis can be repeated nor the analytes can be determined with another method. Another drawback is a high cost of the equipment and its maintenance. This mode is usually automated.

In an off-line mode, the device used for sample preparation is not directly connected to the analyser, and the sample is most frequently injected to the analyser by an analyst. It is the main reason of the low accuracy and repeatability of results when determining analytes in this mode [5]. Table 1 compared the times of sample preparation in on-line and off-line modes in a solid phase extraction. The analysis was performed using HPLC-DAD-MS. In both cases, the analysis lasted 25 minutes.

Table 1 The comparison of preparation time of a water sample containing pharmaceuticals for analysis in on-line and off-line modes [6]

SPE in an off-line mode	SPE in an on-line mode
Sample volume 100 ml	Sample volume 100 ml
Preliminary sample preparation *(2 minutes)*	Preliminary sample preparation *(2 minutes)*
Column preparation *(18 minutes)*	Column preparation *(8 minutes)*
SPE EXTRACTION *(70 minutes)*	SPE EXTRACTION *(35 minutes)*
Drying of SPE column deposit *(5 minutes)*	Valve switch
Elution of analytes *(20 minutes)*	
Solvent evaporation *(200 minutes)*	
Evaporation of the remnants *(30 minutes)*	
TOTAL TIME *345 minutes*	**TOTAL TIME *45 minutes***

3. AUTOMATED MINIATURE DEVICES FOR SAMPLE PREPARATION

An automated stage of sample preparation increases the analytical capacity of a laboratory and has a positive impact on the accuracy and precision during the determination of analytes. At the same time, it prevents humans from being exposed to chemical and biological hazards. Apart from fully automated stages of the sample preparation and its injection into the analyser, another important aspect are miniaturised analysers used during the analytical process. The application of miniature (mobile) analysers makes it possible to perform a chemical microanalysis of samples. Such devices are able to determine the analyte in the extraction spot which eliminates the loss of the analyte or the pollution of the sample during its transportation. The most frequently used method of preparation of complex matrix samples is solid-phase or liquid-phase micro extraction. The classic liquid-phase micro extraction methods are constantly improved and modified so that they meet the standards of green analytical chemistry. Commonly applied liquid-liquid extraction, which uses solvents in microlitre or millilitre volumes, has numerous variations [7]. It includes Single Drop Microextraction (SDME), which is a highly selective method with low values of analytes determination, very simple and with a high speed of extraction, requiring a low--volume sample. The method uses chromatographic micro syringes, which produce a drop hanging at the end of the needle [1]. The possibility of an automated process is very important, since it allows for achieving highly accurate measurements and repeatability of determination.
Single-drop micro extraction (SDME) can be performed by xyz Cartesian robots, which can work in three dimensions. A xyz Cartesian robot has a side (y axis), horizontal (x axis) and vertical (z axis) positioning mechanisms. Figure 2 depicts a scheme of a Cartesian robot for a single-drop micro extraction.

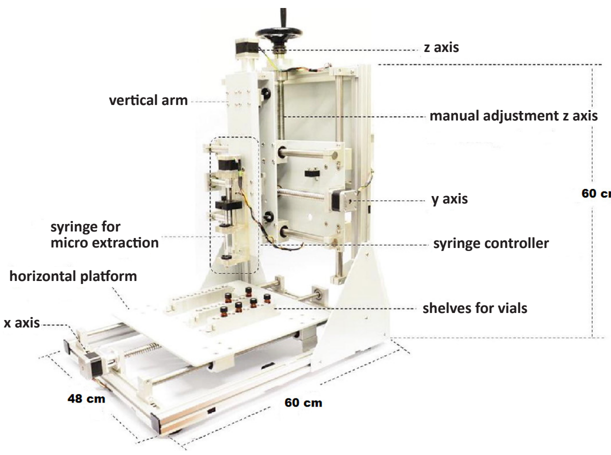

Figure 2 A xyz Cartesian robot [8]

This is a robot for performing solid-phase and liquid-phase micro extraction, initial concentration of analytes and combining an on-line sample preparation stage with liquid or gas chromatography. The whole procedure of sample preparation and its injection into an analyser is automated. Such a robot was used, for example, during the preparation of sewage samples for the analysis of polycyclic aromatic carbohydrates. Apart from SDME of a solvent, the robot makes Hollow Fiber Liquid Phase Microextraction (HF-LPME) and Microextraction by Packed Sorbent (MEPS) possible [8].
Medina et al. [9] conducted SDME and determined triazine derivatives in coconut water with an automated robot coupled to a liquid chromatograph. Automated micro extraction process was conducted by a xyz Cartesian robot Arduino controlled by a suitable programme. The robot is equipped with a micro syringe controller, which facilitates filling, injecting and rinsing the syringe.

Forming a drop with a certain volume is an automated procedure programmed by an analyst. After micro extraction, the micro syringe is connected through a six-port valve with a chromatographic column. After injecting the sample, the valve returns to its initial position, the micro extraction system is cleaned and another extraction is prepared. Figure 3 depicts a scheme of an automated SDME-LC procedure.

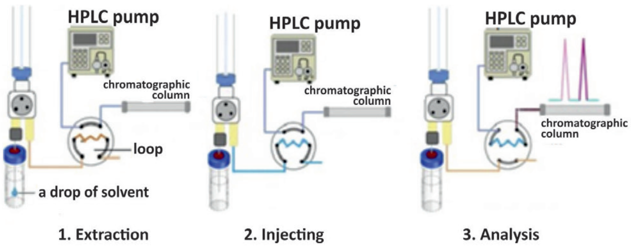

Figure 3 A scheme of an automated SDME-LC procedure [9]

Another frequently applied technique of preparation of liquid and solid samples for analysis is Solid Phase Microextraction (SPME), which is increasingly being automatised. SPME is used for quick and accurate analyses in clinical laboratories in Clinical Laboratory Automation Modules (CLAM). An automated device for sample preparation is coupled to LC/MS apparatus and controlled by a computer programme. The system was used to analyse medicines with a detectability limit amounting to 2 ng/ml [10]. The CLAM-LC-MS/MS module was applied to analyse steroids and cardiac antiarrhythmic medications in human plasma, among other things. The system is highly efficient, accurate and guarantees repeatability as well as increased safety for lab employees [11]. The system enables laboratories to check the concentration of medication in blood during treatment, to track biological and pharmacokinetic variability of medications in real time and it was used to analyse steroids in human plasma, among others [12].

The application of the CLAM module has accelerated the sample preparation tenfold as compared to the traditional procedure and has simplified time-consuming and complex procedures performed in an off-line mode.

Markes International offers an automated system of sample extraction of volatile and medium volatile organic compounds and their initial concentration from solid, liquid and gas samples before GC-MS analysis with the CENTRI apparatus. Apart from SPME the apparatus uses adsorption of analytes in a HiSorb™ sorbent from liquids and headspace with their subsequent thermal desorption. CENTRI makes it possible to remove interferents, including water, from the sample matrix and to achieve detectability at ppt level. CENTRI can be a convenient solution for environmental and medical laboratories which use the GC-MS method, in which the phase of sample preparation for analysis is time-consuming [13].

An automated device for sample extraction and injecting has been used to analyse volatile organic compounds in sewage. The device is programmed to extract sewage samples from a flow cell to vials and then injects the samples to an analytic device. Such a solution helps to control potential pollutants in real time. Placing a lot of vials in the dispenser enables us to conduct continuous unsupervised analyses. A gas chromatograph with a FID and/or TOF MS detector has been used in the analysis. FID is used for continuous pollution monitoring and TOF MS is used to conduct an analysis confirming and identifying the pollutants [14].

4. AUTOMATED SOLID PHASE EXTRACTION

Solid Phase Extraction (SPE) is a popular method of sample preparation for analysis. It is being increasingly used in an on-line mode combined with High Performance Liquid Chromatography (HPLC). In this type of configuration, an extracting column is a fore column in which analytes are extracted. He et al. [15] suggest an automated SPE-HPLC on-line method with a RAM (*Restricted Access Media*) extracting column for routine determination of amisulpride in human plasma. A RAM column prevents micro-particles (including proteins) from entering these parts of sorbent where the analyte is retained. Integrated software was used to control the pumps in the SPE and HPLC systems, a 6-way valve and an automated dispenser. The software takes into consideration the flow of solvents, the proportions of the components of the mobile phase for each working cycle of the device and a working sequence of an automated dispenser. Figure 4 shows a scheme of an automated SPE-HPLC system.

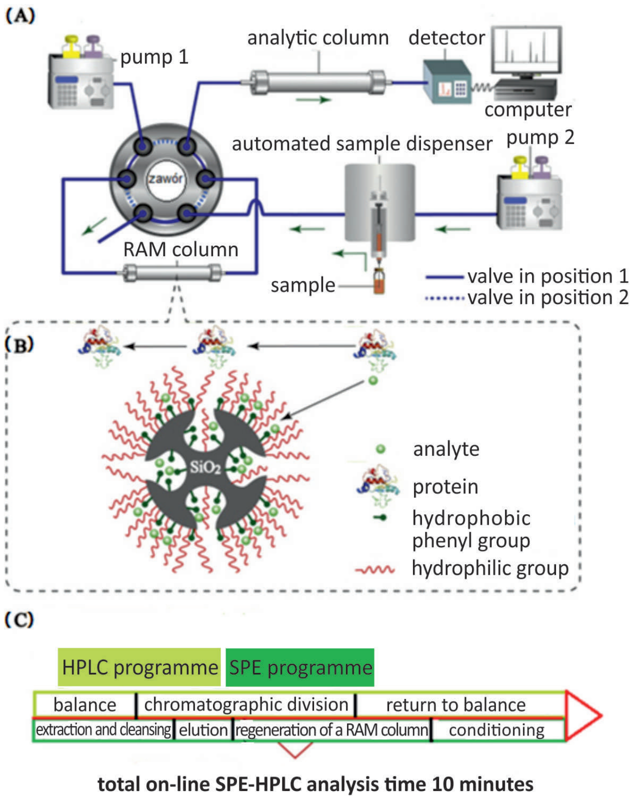

Figure 4 A scheme of an automated SPE-HPLC system (A), the principle of extraction and cleansing of a RAM column (B), the idea of the whole SPE-HPLC procedure (C) [15]

5. SUPERCRITICAL FLUID EXTRACTION

Supercritical Fluid Extraction (SFE) is a very good method of analyte extraction from solids and complies with the principles of green chemistry. It is characterised by a short procedure time, relatively low energy consumption and the application of safe solvents [16]. Automation is frequently applied in this method. The analysed material is placed in an extraction cell and then conditions for extraction are programmed. The obtained extract can be collected in a vial for an off-line analysis or can be directly injected into a chromatographic column.

SFE has been applied, for instance, to prepare a sample for analysis combined with ultra-fast liquid chromatography (SFE-UHPLC). Piperine was determined in black pepper with carbon monoxide as an extracting medium [17]. The device used for this purpose guarantees self-operating work with a possibility of modification in order to prepare a lot of samples.

Supercritical Fluid Extraction is an alternative to classic extraction of analytes with a solvent. Furthermore, it makes it possible to extract analytes which can be sensitive to high temperatures or remnants of a solvent [18].

6. EXAMPLES OF ROBOTS USED FOR SAMPLE PREPARATION

A CSDA10F YASKWA dual-arm robot has two arms which can simulate human dexterity and is adapted to work with various laboratory devices. It is programmed to service various accessories and can use one of its arms, for example, to hold a sample and the other one to perform a specific analytic activity. Dual-arm robots perform the same activities as an analyst in a lab; however, their precision and accuracy is higher. The robot prepares solutions, can dilute the samples, and operates two electronic pipettes with different volumes, for instance, 5–200 µL and 50–1000 µL [19]. The robot can use a manual pipette or a glass vial independently, or place a sample in a homogeniser. After preparing a sample, the robot transfers it to an analyser. Every analysis is programmed and can be found in the catalogue of analyses. Thanks to this fact, in order to determine an analyte in a specific sample it is only necessary to apply a previously programmed procedure. Figure 5 depicts a CSDA10F robot.

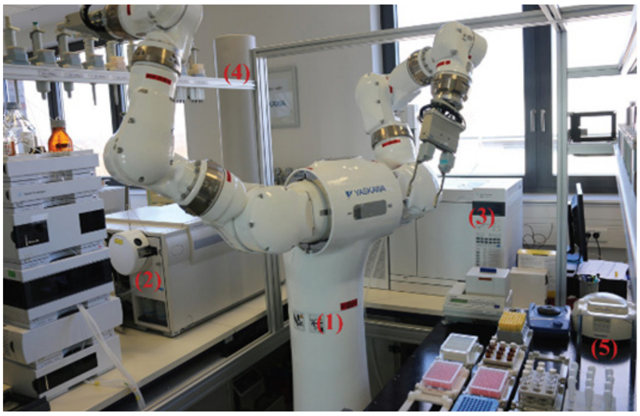

Figure 5 A dual-arm CSDA10F robot (1) lifting a pipette, (2) LC/MS, (3) GC/MS, (4) a shelf with pipettes, (5) a sample preparation stand before transferring them to an analyser [19]

A graphic user interface shows individual activities the robot has to perform after being programmed by an analyst. Thanks to the user-friendly software, even an operator not very knowledgeable in IT may successfully programme an analytical procedure. CSDA10F has been used for determining proline, among other things [19].

A dual-arm SDA10F robot with a FS100 (YASKWA) controlling unit – see Figure 6, has been used, for example, for determining cholesterol levels in a biliary endoprosthesis with GC-MS [20]. The robot performs all stages of sample preparation in compliance with a manual procedure, including diluting, filtering and transferring the sample, among other things, as well as all tasks of an automated dispenser connected with dosing the sample to an analyser [20].

Figure 6 A dual-arm SDA10F robot pipetting with a manual multi-channel pipette [20]

7. CONCLUSIONS

Analytical laboratories are increasingly applying automated sample preparation for analysis. They use robots which transfer samples to an analyser in an on-line mode, and, therefore, increase the analytic capacity of the laboratory. Automation and robots shorten the analytic procedures and improve the quality of the results of the analyses. The latter can be achieved as a result of limiting the number of errors which can be made by an analyst. Programmed and automated analytic procedures are significantly more accurate than the procedures performed manually.

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