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Review

# Achievements in robotic automation of solvent extraction and related approaches for bioanalysis of pharmaceuticals

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ABSTRACT

Currently, the growing demand on quick, easy and ecological sample pretreatment methods is unquestionable. Such challenge involves also approaches focusing on the analysis of pharmaceuticals and other endogenous compounds in biological matrices, termed as *Bioanalysis*.

Solvent extraction such as liquid-liquid extraction (LLE), derived liquid phase microextraction (LPME) and related approaches such as solid liquid extraction (SLE), proved to be applicable in bioanalysis, as numerous papers have been published in this field. However, their manual performances may suffer from a long-term and laborious preparation, due to the inherent complexity of the biological samples. A high sample-throughput (enabling measurement of tens or hundreds of samples on a daily basis) can be achieved when automation of sample pretreatment is performed, resulting in decreased imprecision and low waste production of hazardous solvents and risky biological materials. Here, robotic systems have a key role, especially when multiple processing (e.g., 96-well plate format) and coupling to modern analytical instrumentation (e.g. LC-MS) are combined.

A thorough overview on the up-to-date automations of LLE, LPME, SLE and solid LLE via robotics, is therefore presented. Pharmaceuticals and related compounds determined in classical liquid biological samples (i.e. plasma/serum, whole blood, urine, saliva etc.) and modern dried matrix spots (DMS) were considered as analytes of interest. The methodologies were critically compared to manual setups and among themselves.

## 1. Introduction

Analysis of small compounds (e.g., drugs, metabolites etc.) or large biomolecules (e.g., macromolecules, peptides, proteins, DNA etc.) in biological samples are the main scopes of *Bioanalysis*, either ex-vivo or in-vivo [1–3]. With the term bioanalytical methods, a series of analytical and clinical methodologies have been described, covering various fields of health and life science, such as pharmacokinetics, bioequivalence studies, clinical chemistry, molecular biology, toxicology and anti-doping control.

In bioanalysis, composition of the biological sample represents the major problem as it is often of perplexing character [4]. Here, interfering components can affect chromatography negatively [5], as well as signal response, especially when highly selective analysis such as tandem mass spectrometry (MS/MS) is used (i.e., ion suppression/enhancement) [5–10]. This phenomenon is more critical when analytes must be quantified at low concentration levels (e.g., ng $mL^{-1}$) [11]. Further issues originate from the instability of the biological sample and their potential hazardous properties [2, 12, 13]. In addition, regarding method features, impureness and toxicity of reagents/solvents, numerously repeated and thus boring pipetting or retained memory effect (e.g., cross well contamination in 96-well plate format) may contribute toward problematic analysis as well [12, 14–16]. To this intent, high emphasis should be given to the effective, convenient, rapid and last but not least safe sample preparation, which is still very challenging for many researchers [17].

So far liquid-liquid extraction (LLE) has been frequently used in bioanalysis [2, 17]. To its benefit, it has relatively simple assembly compared to SPE, providing pure extracts. Clean extraction products exhibit reduced back-pressure in the chromatographic column and lower background noise for the signal response, thus better sensitivity is achieved. Apart from this, spacious tuning of chemical conditions suitable for determination of a variety of analytes is feasible [7, 18–25]. On the other hand, large amounts of (biological) sample and/or toxic organic solvents toward increased waste production, repeated mixing and centrifugation toward prolonged preparation time [2], or difficulties to couple with modern analytical instruments (e.g., LC-MS), can be addressed as LLE limitations. The loss of analyte concentration is also

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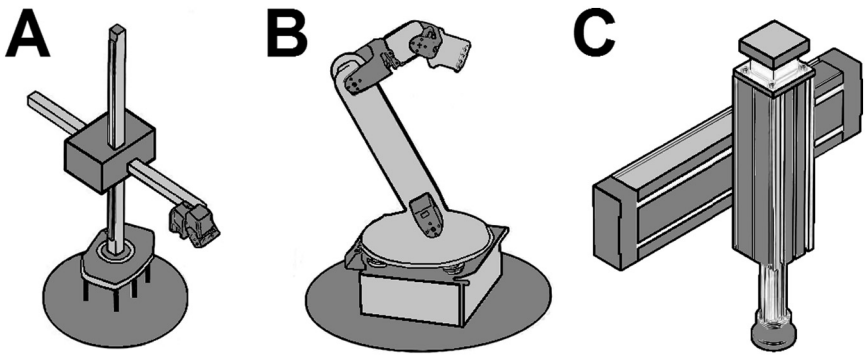


**Fig. 1.** Robotic modules based on (A) cylindrical, (B) anthropomorphic and (C) Cartesian configurations, frequently used for sample preparation in bioanalysis of pharmaceuticals and related compounds.

feasible when trace analysis is carried-out [17].

Recently, liquid phase microextraction (LPME), a novel solvent extraction technique, was developed [26] based on downscaling of extractant consumption from mL or hundreds of μL to > 100 μL or even to few μL or nL per assay [27]. LPME has proved to be applicable in bioanalysis, as reported in several recent reviews [1, 3, 17, 28–30]. Even though LPME faced some of the aforementioned LLE drawbacks, tedious handling of chemicals at μL volumes or the need for operator's presence may accompany manual LPME as well.

Classical liquid biological samples (i.e., blood, serum/plasma, urine, saliva, cerebrospinal fluid, tears etc.) could be processed in various applications via modified and amended LLE or LPME protocols, as new devices or designs have been introduced. The introduction of *Dried Matrix Spots* (DMS) as a novel type of biological sample collection and storage was a milestone in modern bioanalysis [31]. The related solvent extraction methodologies termed as solid liquid extraction (SLE) or solid LLE were used for DMS analysis [32]. However, apart from benefits, such as high stability, easy storage and transport, DMS face prolonged overall analysis, due to their preparation from liquid samples while manual DMS pretreatment requires additional actions (e.g., punching).

To overcome the mentioned difficulties, automation of solvent extraction and related approaches was a logical consequence.

## 2. Toward automated solvent extraction bioanalysis

Today's clinical laboratories frequently deal with numerous samples collected over different periods of time (days, weeks, months etc.), obtained from clinical trials [33], pharmacokinetic and toxicological studies, anti-doping analysis etc. [34–37]. Therefore, rapid assays are essential, especially if long-lasting investigation (e.g., bioequivalence studies) has to be carried out [38]. A step forward to facilitate and speed up the analysis, is the use of hyphenated instruments with inherent selectivity: liquid chromatography-mass spectrometry (LC-MS) or gas chromatography–mass spectrometry (GC–MS) [39]. However, manual sample preparation may lengthen the overall analysis time, since the errors from operation workload need to be excluded [38, 40]. In other words, sample preparation may be the main rate-limiting-step, especially when a rapid analysis and detection combination (i.e., LC-MS) are applied [41]. Moreover, potentially hazardous biological samples (whole blood, plasma, urine etc.) or toxic (volatile) solvents may endanger the health of the laboratory staff [39].

Multiple sample processing can dramatically shorten batch time analysis consisted of hundreds of samples while keeping similar or even better reproducibility compared to manual performance [41–43]. Thus, automation is more favourable and convenient for routine (clinical) analysis [44] or method development [45].

Automation of solvent extraction techniques (LLE, LPME) and related sample preparation approaches (SLE, SLLE) via robotics has brought novel and smart insights toward state-of-the-art analytical arrangements. Several authors overviewed robotics and laboratory automation for drug analysis [46–49]. However, the majority of the described applications involves classical liquid biological samples.

In this review, the recent developments in the field of robotic solvent extraction bioanalysis, including related approaches, are highlighted, covering the literature till the beginning of 2018. Various types of robotic systems are presented and thoroughly discussed based on their geometry configuration (i.e., cylindrical, humanoid and Cartesian robots) for analysis of classical liquid biological samples (blood samples, urine, saliva etc.) and modern DMS [31, 50, 51]. Pharmaceuticals quantified in biological matrices were chosen as target compounds but the information provided applies to all fields of bioanalysis (i.e. clinical, toxicological, doping control etc.) as well.

## 3. Robotic systems for solvent extraction bioanalysis

The laboratory robot (also liquid handler, workstation) emulates manual steps with aim to perform unattended preparation actions as: transport of objects (glassware, plates, racks etc.), aspiration-&-injection of liquids, mixing, extraction, reconstitution of dried product, transfer to the HPLC system [43, 49] etc. Cylindrical (robotic arm rotates around a focal point in 365°), anthropomorphic (robotic arm simulates movement of human arm via multiple joints), and Cartesian (robotic arm moves in x,y,z-axis) robots are frequently used for the pharmaceutical analysis as depicted in Fig. 1 [46–48].

In the following sections, all approaches will be classified according to such geometric arrangements. Applications and performances for robotic automation of solvent extraction and related approaches are compared in Tables 1–3.

### 3.1. Classical biological liquid samples

#### 3.1.1. Cylindrical robots

Zymate™ system (developed by Zymark™ corporation) [52] can be considered as the one of first robotic setup used in drug analysis. The early models were capable of common laboratory operations like lifting or positioning objects and pouring solvents. Higher speed, better tactile sensing, simultaneous hand-&-wrist motion or lower system collision were achieved by Zymate II™ [53], which was later upgraded to PyTechnology II™ ensuring easier programming and being able to schedule fast serialisation analysis [54].

Generally, such system is equipped with a programmable mechanical arm rotating 360° on its base and a controller or CPU (Fig. 2). The arm is able to carry either an exchangeable gripper (with pair of fingers) or a pipettor (with fixed stainless-steel cannula or disposable pipette tips). The gripper and pipettor serve for transporting objects and to aspirate-transfer-&-inject solvents among stations within the working bench-area, respectively [43, 49, 55–57]. In several works [34, 57–61],