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# The clinical applications of drugs and their metabolites analysis in biological fluids and commonly used analytical techniques for bioanalysis: review

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**Background:** This study provides an overview of the clinical applications of drugs and their metabolites analysis in biological fluids and identifies commonly used analytical techniques for bioanalysis.

**Methods:** Original open-access articles published between 31 October 2005 and 31 October 2020 in Google Scholar, MEDLINE, PubMed, and Embase were reviewed, and pertinent findings of the individual studies were pooled and presented using tables. This review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).

**Results:** Fifteen studies met the eligibility criteria and were included in the review. These studies show that qualitative identification and quantitative determination of drugs and their metabolites in biological fluids are important for therapeutic drug monitoring (TDM), pharmacokinetic and pharmacodynamic studies, drug abuse control, and forensic and toxicological analyses. Spectroscopic, electrochemical, and hyphenated and nonhyphenated chromatographic techniques are used to analyse drugs and their metabolites in biological fluids. However, hyphenated techniques are the preferred analytical methods because of their sensitivity, selectivity, accuracy, reproducibility, efficiency, and rapid analysis.

**Conclusion/Recommendations:** Bioanalysis is important for pharmacokinetic and pharmacodynamic studies, therapeutic drug monitoring, forensic and toxicological analyses, and drug abuse control using different bioanalytical techniques. However, hyphenated techniques are the most commonly used bioanalytical techniques. Bioanalysis of drugs and their metabolites needs to be improved to provide good medical and pharmaceutical care to patients, to confirm forensic and toxicological cases, and to control drug abuse.

## KEYWORDS

drug, medicine, bioanalysis, biological fluids, analytical techniques

## 1 Introduction

Bioanalysis is a subdiscipline of pharmaceutical analysis that focuses on the analysis of drug substances and their metabolites in biological fluids using bioanalytical techniques (Hansen et al., 2011). Drug analysis in biological fluids has been important for many years and has become the cornerstone of developing and formulating new chemical entities. However, most recently, patient care has included the identification and quantification of drugs and their metabolites in various biological systems (blood, plasma, serum, saliva, urine, skin, hair, and organ tissue (Wood, 1999; Thompson et al., 2002; Hansen et al., 2011).

The field of bioanalysis has matured significantly since early studies on drug metabolism using many simple and advanced techniques, and current bioanalysis is well-equipped to address modern challenges (Thompson et al., 2002). However, the analysis of drugs and their metabolites in biological fluids is complex for several reasons: drugs and their metabolites are found in complex biological matrices. The concentrations to be measured are often in the microgram to nanogram or picogram levels. These matrices normally contain large amounts of endogenous compounds, which can interfere with the chemical and physical analytical methods used to detect and determine materials of pharmacological interest. Consequently, unless an ultra-specific method of analysis is available for the substance of interest, physical separation of that substance from other interfering substances is usually necessary before quantitative determination can be achieved. To this end, most drug analyses involve different steps, such as sample collection, sample pre-treatment, separation from the matrix, detection, and analysis (Food and Drug Administration, 2001; Wells, 2003; Hansen et al., 2011).

Biological fluids serve as invaluable sources of information for various diagnostic, research, and therapeutic purposes in medicine and biology. Collecting these fluids with precision and care is crucial to ensure the integrity of the samples and the reliability of the subsequent analysis (Food and Drug Administration, 2001; Wells, 2003). Samples of biological fluids were collected from extracellular fluids, namely, blood (whole blood, serum, or plasma), cerebrospinal fluid (CSF), saliva, amniotic fluid, ocular fluid, pleural fluid (from the sac surrounding the lungs), urine, pericardial fluid (from the sac surrounding the heart), peritoneal fluid, also called ascetic fluid (from the abdomen), and synovial fluid (a fluid that is found in joint cavities). However, blood, plasma, urine, and serum are the fluids of choice (Wells, 2003; Food and Drug Administration, 2001; Thompson et al., 2002; Ritscher et al., 2019). A good bioanalysis starts with appropriate sample collection procedures and sample preparation, which are often the keys to successful analytical results. It has a direct impact on accuracy, precision, and quantification limits and is often the rate-determining step for many analytical methods. The sample preparation stage of the analysis is often the most critical and difficult part of the process, both in terms of the time involved and the difficulty of extracting the desired analyte from the matrix. In addition, each matrix has unique challenges (Hansen et al., 2011; Ritscher et al., 2019).

The method of sample preparation generally depends on the available analytical technique and the physical characteristics of the analytes under investigation. The purpose of sample preparation is to remove many endogenous interferences from the analyte and to ensure that the injection matrix is compatible with the chromatographic system (Van der Heijden et al., 2009; Hansen et al., 2011).

The choice of analytical methods and separation techniques for the analysis of drugs in biological fluids may depend on the type of sample matrix to be analysed, the physicochemical properties of the drug(s) and its metabolites, the chemical structures of the drug, the range of concentrations to be measured, the stability of the drug(s), the objective of the analysis (clinical, forensic), and the degree of experience of the analyst (Mark, 2003; Fríguls et al., 2010). Therefore, this study provides an overview of the clinical applications of drugs and their metabolites analysis in biological fluids and identifies the most effective analytical techniques for bioanalysis.

## 2 Methodology

### 2.1 Search strategy

Different databases (PubMed, Medline, Embase, and Google Scholar) were used. Articles that were published between 31 October 2005 and 31 October 2020 were identified using keywords. The entire search was conducted using stepwise procedures from 15 October 2020, to 10 November 2020, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 1) (Moher et al., 2009).

Keywords: (clinical application OR clinical importance OR clinical purpose) AND (analysis OR test) AND (drugs OR pharmaceutical products OR medicines OR medical products) AND (biological fluids OR biological matrices) AND (analytical techniques OR analytical methods).

### 2.2 Eligibility criteria

Original open-access articles in PDF format published within the past 15 years and studies clearly described their sample size, sampling drugs, the purpose of the study, biological fluids taken for bioanalysis, analytical techniques used for bioanalysis, and major findings were included in the review, whereas book reviews, abstracts, reviewed articles, and unrelated articles were excluded.

### 2.3 Data extraction

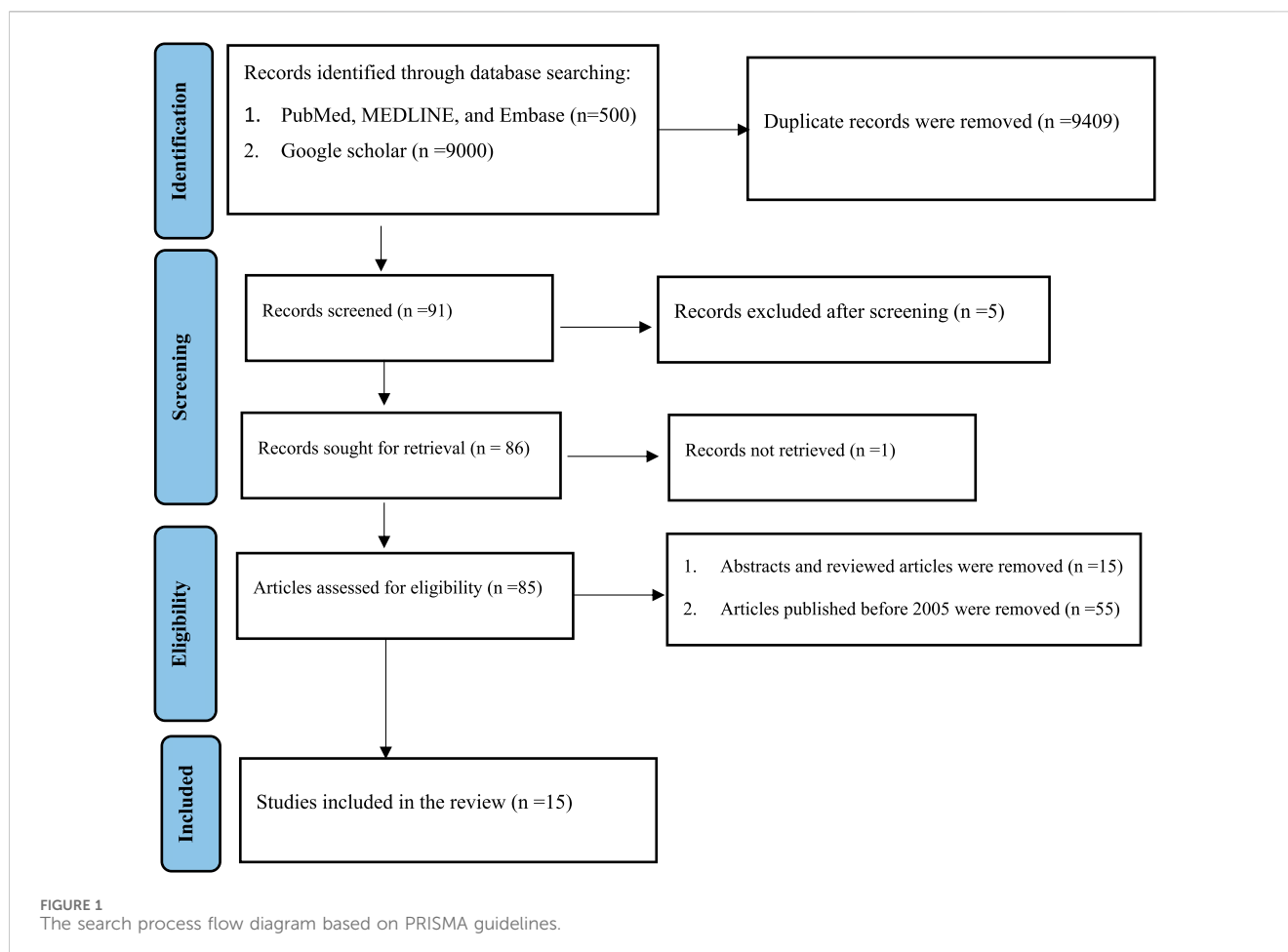
Important and relevant findings were extracted from the original articles using inclusion and exclusion criteria.

### 2.4 Data analysis

After extraction, the pertinent findings of the individual studies were pooled and presented in a table.

## 3 Results

Fifteen studies related to the analysis of drugs in biological fluids and analytical techniques used for bioanalysis were searched; these studies focused on the importance of drug analysis in biological



fluids, including therapeutic drug monitoring, pharmacokinetic and pharmacodynamic drug studies, drug abuse control, and forensic and toxicological analyses. In addition, studies focusing on the analytical techniques used in bioanalysis were included (Table 1).

## 4 Discussion

Bioanalysis represents a multifaceted field that is critical for unraveling the complexities of human health and disease. It is conducted in hospital laboratories, forensic toxicology laboratories, and doping control laboratories. Bioanalytical data obtained in these settings are highly important for therapeutic drug monitoring (TDM), pharmacokinetic and pharmacodynamic drug studies, drug abuse control, and forensic and toxicological analyses (Cingolani et al., 2004; Larson and Richards, 2009; Li et al., 2010; Kumar et al., 2013; Al Za'abi et al., 2013; Parsons et al., 2014; Ciltas et al., 2015; Lu M et al., 2016; Ebers et al., 2017; Ningrum et al., 2018; Ritscher et al., 2019; Ritscher et al., 2020).

Bioanalysis is crucial for TDM in many hospital laboratories. Recently, TDM has become an essential tool for guiding rational clinical drug use and aiding the management of patients receiving drug therapy to alleviate or prevent disease. TDM combines pharmacokinetic and pharmacodynamic knowledge to optimize personalized drug therapy, which can improve treatment

outcomes, reduce drug toxicity, and avoid the risk of developing drug resistance (Campbell and Bode, 1994; Al Za'abi et al., 2013; Ciltas et al., 2015).

Personalised medicine is a medical approach that optimises treatment efficacy and reduces adverse effects by designing the best treatment plan specifically for each patient (Kumar et al., 2013; Lu et al., 2016). The pharmacological response to a drug given in a selected dosage regimen depends on various factors, such as patient compliance, age, sex, body type, metabolic status, lifestyle, environmental factors, underlying diseases, drug interactions, bioavailability, pharmacokinetics, and the protein-binding ability of the drug(s) (Larson and Richards, 2009; Al Za'abi et al., 2013; Ebers et al., 2017; Ritscher et al., 2019).

Currently, TDM approaches depend on the pharmacokinetic/pharmacodynamic variability of patients, which causes variations in the dose-response relationship among different patients (Larson and Richards, 2009; Ritscher et al., 2020). It is important to know how quickly drugs are metabolised; that is, whether a patient's daily doses of a drug are positively or negatively correlated with its metabolites, and the concentration of its metabolites also increases. Generally, pharmacokinetics (ADME) analysis determines how these four criteria influence performance and pharmacological activity, such as drug distribution in plasma and tissue (Humphrey, 1996; Kumar et al., 2013; Parsons et al., 2014; Ciltas et al., 2015; Lu et al., 2016).

TABLE 1 Studies on drugs and their metabolites in biological fluids and bioanalytical techniques.

Country	Sample size (n)	Sampling drugs	Purpose of the study	Types of samples and analytical techniques	Major findings	References
Iran	9 Death cases	Morphine	Addiction	Plasma and HPLC-F	<ul style="list-style-type: none"> <li>Morphine was detected at 4.75 min of Rt</li> <li>Morphine conc. (ng/mL) was 13.6–233</li> </ul>	Khansari et al. (2006)
Uganda	40 Patients	Methadone	Abuse (n = 3) TDM (n = 37)	Urine and GC/MS	<ul style="list-style-type: none"> <li>m/z ratio D3-EDDP, 280, 265; EDDP, 277, 262, 276</li> <li>Conc. 40 ng/mL and 2000 ng/mL</li> <li>85.8% of patients were compliant</li> <li>14.2% of patients were non-compliant</li> </ul>	Larson and Richards (2009)
China	48 Patients	Digoxin	Serum TDM	Serum and HPLC-MS/MS	<ul style="list-style-type: none"> <li>m/z ratio 798.6/651.5 and 802.6/654.5 for digoxin and digoxin-d3, respectively</li> <li>14.6% (7/48) conc.0.5–0.9 ng/mL</li> <li>77.1% (37/48) higher than the target range</li> <li>8.3% (4/48) lower than the target range</li> <li>27.1% (13/48) had digoxin concentrations <math>\geq 2</math> ng/mL</li> </ul>	Li et al. (2010)
Scotland	32 Patients	Opioids	Addiction	Urine and HPLC-MS/MS	<ul style="list-style-type: none"> <li>17 (53.1%) tested positive for morphine, codeine, and their metabolites</li> <li>7 (21.9%) and 6 (18.8%) were +ve for 6-mono acetyl- morphine and norcodeine, respectively</li> <li>From 34.4%, cases               <ol style="list-style-type: none"> <li>Morphine conc. range:14 ng/mL-799 ng/mL</li> <li>Codeine conc. range:2 ng/mL-20,000 ng/mL</li> </ol> </li> </ul>	Asmari and Anderson (2007)
Japan	26 Death cases	New-generation antidepressants	Addiction	Plasma and GC-MS	<ul style="list-style-type: none"> <li>88.5% (23) were positive for some of the drugs studied</li> <li>The drug retention time: norfluoxetine (11.89 min), fluoxetine (12.75 min), venlafaxine (13.46 min), mirtazapine (15.79 min), sertraline (19.06 min), paroxetine (21.18 min), olanzapine (22.44 min)</li> <li>The conc. was: 1.2 <math>\mu\text{g/mL}</math> for fluoxetine, norfluoxetine, and venlafaxine</li> <li>0.2 <math>\mu\text{g/mL}</math> for mirtazapine, sertraline, and paroxetine</li> <li>0.1 <math>\mu\text{g/mL}</math> for olanzapine</li> <li>The cause of death is either overdose, Suicide (hanging), or complication of medication</li> </ul>	Pietracci et al. (2013)
Oman	151 Patients	Valproic acid phenytoin carbamazepine, and phenobarbital	TDM	Plasma and Fluorescence	<ul style="list-style-type: none"> <li>50% were below, 37% were within, and 13% were above the therapeutic range</li> <li>70% no clear reasons for plasma concentrations to lie outside the therapeutic range</li> <li>24.2%, seizure due to poor compliance</li> <li>42.7%, no change in the drug therapy/dosing</li> <li>62.7% increase in the seizures frequency</li> </ul>	Al Za'abi et al., 2013

(Continued on following page)

TABLE 1 (Continued) Studies on drugs and their metabolites in biological fluids and bioanalytical techniques.

Country	Sample size (n)	Sampling drugs	Purpose of the study	Types of samples and analytical techniques	Major findings	References
India	178 Patients	Warfarin and 7-OH warfarin	PK Analysis	Plasma and HPLC	<ul style="list-style-type: none"> <li>Plasma warfarin (<math>\mu\text{g/mL}</math>) ranged from 2.2-10.3</li> <li>Plasma 7-OH warfarin (<math>\mu\text{g/mL}</math>) ranged 0.18-2.1</li> </ul>	Kumar et al. (2013)
USA	26 Patients	Rifapentine	PK Analysis	Plasma and HPLC-MS/MS.	<ul style="list-style-type: none"> <li>The RPT was eluted at 1.40 min</li> <li>The concentration of RPT, was 70,367 ng/mL</li> </ul>	Parsons et al. (2014)
					<ul style="list-style-type: none"> <li>The quality control levels were defined as b/n 150 ng/mL and 70,000 ng/mL</li> </ul>	
Turkey	10 Patients	Diclofenac	TDM	Serum and Voltammetry	<ul style="list-style-type: none"> <li>The conc. diclofenac was 11.12 <math>\mu\text{g/mL}</math></li> <li>No oxidation compounds</li> <li>The conc. of diclofenac was of 2–20 <math>\mu\text{g/mL}</math></li> </ul>	Ciltas et al. (2015)
Taiwanese	151 Patients	Olanzapine	TDM/Clinical efficacy	Plasma and HPLC- Coulometry	<ul style="list-style-type: none"> <li>The mean <math>C_{OLZ}</math> levels were <math>37.0 \pm 25.6</math></li> <li>70% (68/97) OLZ levels <math>&gt;22.77</math> ng/mL</li> </ul>	Lu et al., 2016
Tanzania	41 Patients	Levofloxacin	TDM	Plasma and HPLC-UV	<ul style="list-style-type: none"> <li>Levofloxacin identified, Rt 6.19 min</li> <li>The median <math>C_{max}</math> was 5.86 (3.33–9.08 <math>\mu\text{g/mL}</math>)</li> <li>Only 13 of 41 (31.3%) patients had <math>C_{max} &gt;</math> the lower limit (<math>C_{max} \geq 7.55</math> <math>\mu\text{g/mL}</math>)</li> <li>Outcomes cure in 16 (41%) and</li> <li>Treatment completion 10 (25.6%)</li> </ul>	Ebers et al. (2017)
Indonesia	82 Patients	Metformin	TDM	Plasma and HPLC-UV	<ul style="list-style-type: none"> <li>Metformin conc., 0.587–2.168 <math>\mu\text{g/mL}</math></li> <li>32.1% of them had metformin <math>C_{ss}</math> min</li> <li>84.1% had <math>C_{ss}</math> max. of metformin within the recommended therapeutic range</li> </ul>	Ningrum et al. (2018)
Italy	3 Patients	Cocaine (COC)	Forensic analysis	Plasma and HPLC-MS/MS	<ul style="list-style-type: none"> <li>Cocaine conc., 19–108 ng/mL</li> </ul>	Cingolani et al. (2004)
Germany	56 Patients	Antihypertensive drugs	Adherence	Urine and HPLC-MS/MS	<ul style="list-style-type: none"> <li>59% were completely adherent (all drugs detectable in urine)</li> <li>29% were partially adherent (1 drug undetectable in urine)</li> <li>13% were non adherent (<math>&gt;1</math> drug undetectable in urine)</li> </ul>	Ritscher et al. (2020)
Germany	20 Patients	Antihypertensive drugs	TDM/Adherence	Plasma and HPLC-MS/MS	<ul style="list-style-type: none"> <li>9 were treated with bisoprolol, conc. of 3.3–53.8 ng/mL (8.14–44.6 ng/mL)</li> <li>7 with metoprolol, 8–110.8 ng/mL (3.74–267 ng/mL)</li> <li>1 with nebivolol, 0.36 and 1.08 ng/mL</li> <li>10 with HCT, 15.5–606.3 ng/mL (7.44–298 ng/mL)</li> <li>8 with torasemide, 17.6–1829.2 ng/mL and</li> <li>2 with spironolactone, 14.0–91.2 ng/mL</li> <li>24% were out of the therapeutic range</li> <li>100% of all measured conc. were above the lower dose-related concentration</li> </ul>	Ritscher et al. (2019)

HCT, hydrochlorothiazide; TDM, therapeutic drug monitoring; Rt, retention time; PK, pharmacokinetics; F, fluorescence.

It is well established that only unbound free drugs can bind to receptors to produce the desired effect. Serum drug concentrations and pharmacological responses are significantly correlated with dose-serum drug concentrations for some drugs. Therefore, monitoring serum concentrations of these drugs is beneficial for patient management, particularly when they have very narrow therapeutic ranges (Larson and Richards, 2009; Ritscher et al., 2019).

To prevent adverse reactions and drug toxicity, which can occur when the drug concentration in the blood exceeds the maximum therapeutic concentration, and when the dosage is customised to achieve maximum efficacy, it may also be more beneficial to adjust the dose based on the serum drug concentration rather than routine patient assessment for these medications, which have very narrow therapeutic ranges. Therefore, therapeutic drug monitoring is a cost-effective healthcare approach. However, more rapid, precise, and advanced analytical techniques are needed to successfully apply TDM, particularly at low sample concentrations (Cingolani et al., 2004; Larson and Richards, 2009; Li et al., 2010; Kumar et al., 2013; Al Za'abi et al., 2013; Lu et al., 2016; Ebers et al., 2017; Ningrum et al., 2018).

TDM is tested for concentration after a certain period of drug administration at corresponding time intervals, and drug administration is controlled according to the drug's therapeutic effect at different treatment stages in combination with the drug treatment window (Kumar et al., 2013; Parsons et al., 2014; Lu et al., 2016; Ebers et al., 2017).

A patient's venous blood sample (whole blood, plasma, serum, etc.) is collected and analysed to measure the concentrations of drugs and their metabolites in blood samples. First, the identification of the drug in the blood sample confirms whether the patient has taken the drug or not. This information is important because not all patients comply with the prescribed medication. Second, the exact concentration of the drugs and their metabolites measured in the blood samples confirmed that the amount of drug was appropriate. This refers to the individualisation of dosage by maintaining blood drug concentration within a target range to optimize efficacy and reduce the risk of adverse side effects by monitoring drug concentrations in the blood (Kumar et al., 2013; Parsons et al., 2014; Lu et al., 2016; Ritscher et al., 2020).

Bioanalysis is also very important for forensic and toxicological analyses in forensic and toxicology laboratories (Khansari et al., 2006; Asmari and Anderson, 2007; Pietracci et al., 2013). Drug analysis in forensic and toxicology laboratories is performed by taking biological fluids as samples following the death of any person to aid in legal investigations to determine the actual cause of death using different bioanalytical techniques. Several studies have used blood, urine, brain tissue, etc., to determine the presence of compounds, such as opioids, antidepressants, antipsychotic drugs, and cocaine, for forensic and toxicological analysis and have also been used to investigate the abuse of opioids, antidepressants, antipsychotic drugs, cocaine, and other toxic substances (Flanagan et al., 1995; Hoja et al., 1997; Cingolani et al., 2004; Khansari et al., 2006; Asmari and Anderson, 2007; De Castro et al., 2009; Pietracci et al., 2013).

The analyte is typically unknown in forensic toxicology. Therefore, samples were first screened for the presence of drugs or drugs of abuse. In the case of a positive sample, the presence of the drug or the drug of abuse is confirmed using a second bioanalytical

method. Due to the serious legal consequences of forensic cases, particular emphasis is placed on the quality and reliability of bioanalytical results. The work always involves the application of at least two different analytical methods (screening and confirmation) based on different physical or chemical principles (Campbell and Bode, 1994; Cingolani et al., 2004; Li et al., 2010; Kumar et al., 2013; Al Za'abi et al., 2013; Ciltas et al., 2015; Ebers et al., 2017; Ningrum et al., 2018; Ritscher et al., 2019).

Bioanalysis is highly challenging because most target pharmaceutical substances are present in blood, urine, and saliva samples at very low concentrations. Typically, the concentration is low at the nanogram level; however, in some cases, target pharmaceuticals must be detected at the picogram level. This requires high operator skill and highly sensitive instrumentation (Cingolani et al., 2004; Li et al., 2010; Ningrum et al., 2018). In addition, target pharmaceuticals coexist with a broad range of endogenous compounds that are naturally present in biological samples, which can be thousands of different components, and many of them can be present at high concentrations. Therefore, before the final measurement using the sensitive instrument, target drugs must typically be isolated from the biological matrix for bioanalysis to be successful. Therefore, experience and skills in sample preparation and the selection of bioanalytical techniques are extremely important in bioanalysis (Kumar et al., 2013; Al Za'abi et al., 2013; Ciltas et al., 2015; Ebers et al., 2017; Ritscher et al., 2019).

Analytical technique is a set of techniques that allow us to qualitatively and quantitatively determine the analyte in a sample. Bioanalytical techniques are analytical techniques used to determine drugs or substances in biological matrices, such as urine, serum, and plasma. Drugs in biological fluids are identified and quantified using various analytical techniques, including spectroscopic, electrochemical, and hyphenated and nonhyphenated chromatography techniques (Li et al., 2010; Kumar et al., 2013; Ritscher et al., 2020).

Spectroscopic techniques that use the relationship between the amount of light absorbed or emitted and the concentration of the reference drug can be used to determine the concentration of the drug in biological fluids. Because of its high selectivity, low detection limits, and ability to detect drug concentrations ranging from ng/mL to pg/mL, the fluorescence technique is a spectroscopic method that has been widely used in biological fluid analysis. However, the use of fluorescence methods has become limited because only certain classes of compounds exhibit fluorescence. Furthermore, derivative spectrophotometry offers an alternative approach for enhancing the sensitivity and specificity of mixture analyses with spectral overlap (Sotomayor et al., 2008; Al Za'abi et al., 2013).

High-performance liquid chromatography voltammetry and coulometry, are considered alternatives to spectroscopy and chromatography for determining drug concentrations in biological fluids (Gergov et al., 2009). These techniques are based on the continuous variation of the potential applied across the electrode-solution interface, and the resulting current is recorded. The current is a quantitative measurement related to the analyte concentration. The applied potential acts as the driving force for electrochemical reactions such as analyte reduction or oxidation. With the use of simple and affordable instrumentation, electrochemical methods offer high sensitivity and low detection limits. However, the application of these approaches to the analysis

of drug concentrations in biological fluids is limited because of a lack of analysis of related substances (Gergov et al., 2009; Santos et al., 2009).

In recent years, chromatographic methods have become highly versatile for detecting and determining most drug concentrations in biological fluids. Hyphenated or nonhyphenated chromatographic techniques are preferred over other analytical techniques in terms of their sensitivity, selectivity, accuracy, reproducibility, small sample volumes, rapid analysis, and high separation efficiency (Gergov et al., 2009; Pietracci et al., 2013; Al Za'abi et al., 2013).

Gas chromatography (GC), but not high-performance liquid chromatography (HPLC), is limited to volatile and thermally stable compounds or molecules that undergo derivatization reactions to produce thermally stable products. HPLC is one of the most commonly used nonhyphenated methods; it is not dependent on the volatility of compounds and is very versatile for detecting and determining most drug concentration levels in biological fluids (Khansari et al., 2006; Asmari and Anderson, 2007; Larson and Richards, 2009; Santos et al., 2009; Pietracci et al., 2013; Kumar et al., 2013; Ciltas et al., 2015; Lu M et al., 2016; Ebers et al., 2017; Ningrum et al., 2018).

Extremely low concentrations of some drugs in biological fluids necessitate the combination of various chromatographic and spectroscopic techniques; such combinations are called hyphenated techniques. The hyphenation system provides the best drug identification and quantification through increasingly complex separations to optimize the sample response and discriminate against interferences using different detectors, accurate and rapid analysis, the best sample throughput, and the best degree of automation. Thus, it provides powerful methods for analyzing complex samples and provides more information than individual techniques. It is a multidisciplinary analysis method to avoid incorrect results, it saves time because two or more analyses can be run at the same time, sample preparation time is reduced, and it provides accurate analytical information without cross-contamination (Kallner, 1999; Valcárcel et al., 1999; Kantharaj et al., 2003; Pulido et al., 2003).

In hyphenation systems, chromatographic methods are largely used for separation, whereas interfaced methods are mostly used for quantification and detection. Spectrophotometric detectors, electrochemical detectors, and mass spectrometry are by far the most frequently employed in HPLC systems. HPLC coupled with UV/vis detectors, diode array detectors (DADs), and fluorescence detectors (Khansari et al., 2006; Ebers et al., 2017; Ningrum et al., 2018).

UV/vis detectors are most often used in HPLC-based quantitative analysis because they are easy to use, affordable, and capable of evaluating a wide range of compounds. However, its sensitivity and selectivity are low for some stronger substances. Although DADs are more complex and costly than UV/vis detectors, they allow the analyte's UV absorption spectrum to be registered, thus increasing the identification power of HPLC. Additionally, DADs allow the evaluation of chromatographic peak purity, which is useful for determining the separation efficiency of HPLC systems (Khansari et al., 2006; Gergov et al., 2009; Ebers et al., 2017; Ningrum et al., 2018).

Luminescence and fluorescence detectors are the most common in HPLC, and they are used either for direct analyte detection or for

detecting analyte derivative products. Fluorescence detectors are widely used in bioanalysis because of their better sensitivity than that of absorption-based detection. The use of fluorescence detectors in HPLC systems is an intriguing strategy to exploit their increased sensitivity. The importance of very sensitive detectors in HPLC systems is clearly demonstrated by the extremely small quantity of samples using this technique. Fluorescence detectors are therefore suitable for this purpose (Khansari et al., 2006).

Another type of detector that is widely used in HPLC for bioanalysis is the electrochemical detector. The separation capabilities of HPLC are combined with the attractive features of electrochemical techniques, including sensitivity, precision, and inexpensive instrumentation, for electrochemical detection. Electrochemical detection techniques can be helpful in HPLC because their sensitivity is higher than that of fluorimetric methods. Additionally, electrochemical detectors are more versatile because they do not require chemical derivation steps because electroactive substances are more common than fluorescent substances (Ciltas et al., 2015; Lu et al., 2016).

However, electrochemical detection fundamentally differs from spectrophotometric detection because it alters the sample by oxidation or reduction of the analyte. This characteristic causes the electrode surface to be passivated by redox reaction intermediates or products, which is a limitation of electrochemical detectors in HPLC. When determining organic species, like drugs, whose redox processes frequently involve numerous intermediates that can firmly adsorb onto the electrode surface, this restriction of electrochemical detection is particularly noticeable (Khansari et al., 2006; Gergov et al., 2009; Ciltas et al., 2015; Lu et al., 2016).

High-performance liquid chromatography-mass spectrometry-mass spectrometry (HPLC-MS/MS) is the most effective and efficient technique (Katagi et al., 2001; Rivier, 2003). HPLC systems through a chromatographic column allow separating compounds in relation to polarity and affinity to the column, while a mass spectrometer generates multiple ions from the sample and separates these ions according to their specific mass-to-charge ratio, recording the relative abundance of each ion type (Hubert et al., 2002; Matuszewski et al., 2003; Asmari and Anderson, 2007; Siddiqui et al., 2009; Parsons et al., 2014).

HPLC-MS/MS is now the gold standard for TDM and offers many advantages in its application in TDM: sensitivity, precision, accuracy, speed of analysis, simple preparation, and determination of multiple analytes in one short run (Cingolani et al., 2004; Li et al., 2010).

Gas chromatography coupled with mass spectrometry (GC-MS) (Bressolle et al., 1996; Mark, 2003; Larson and Richards, 2009; Pietracci et al., 2013), high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) and ultraviolet (HPLC-UV) (Ebers et al., 2017; Ningrum et al., 2018), high performance liquid chromatography with atomic emission spectrometry (HPLC-AES) (Khansari et al., 2006), and high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD) were also used for the identification and quantification of drugs and their metabolites in biological fluids (Katagi et al., 2001; Link et al., 2007; Lu et al., 2016).

## 5 Potential future indications

Bioanalysis of drugs and their metabolites need to be improved to provide good medical and pharmaceutical care to patients, to confirm forensic and toxicological cases, and to control drug abuse. In addition, newly developed or advanced bioanalytical techniques are required to obtain more reliable, accurate, and precise results by overcoming the challenges associated with bioanalysis.

## 6 Conclusion

Identification and quantification of drugs and their metabolites in various biological fluids (whole blood, plasma, serum, saliva, urine, etc.) using different bioanalytical techniques are used for therapeutic drug monitoring (TDM), pharmacokinetic and pharmacodynamic drug studies, drug abuse control, and forensic and toxicological analyses. Spectroscopic, electrochemical, and hyphenated or nonhyphenated chromatography techniques are used for bioanalysis. However, hyphenated techniques such as GC-MS, GC-MS/MS, HPLC-MS, HPLC-ECD, HPLC-MS/MS, and HPLC-AES are the most commonly used bioanalytical techniques for bioanalysis.

## Author contributions

BM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision,

Validation, Writing—original draft, Writing—review and editing. MY: Supervision, Validation, Writing—original draft, Writing—review and editing. KA: Conceptualization, Data curation, Formal Analysis, Methodology, Supervision, Validation, Writing—original draft, Writing—review and editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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