SYSTEMATIC REVIEW

Morphine Analysis in Biological Samples: A Systematic Review

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Abstract

Background: The analysis of morphine in biological samples is pivotal in clinical and forensic toxicology and indicates drug exposure, metabolism, and toxicological profile.
Methods: This systematic review explores the recent analytical techniques that have used the detection and quantification of morphine in forensic toxicological investigations. Articles were collected from PubMed, Scopus and Google Scholar electronic databases from 2011 until 30th September 2024. They were searched using a systematic search of English keywords including: "Morphine" OR "Analysis" OR "Analytical techniques" OR "Analytical innovations" OR "Methods" AND "Biological samples" OR "Biological matrices". The selection criteria were based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta- Analyses).
Results: From 1200 articles detected in the early systematic search, 30 articles met the inclusion criteria and included in this study. The results showed that the advanced hyphenated analytical methods couple with mass spectrometry (MS) such as Gas Chromatography- Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) and related tandem GC-MS and LC-MS with recent sample preparation methods such as Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) and Dispersive Liquid-Liquid MicroExtraction (DLLME) are the most common analytical methods for detection of morphine in biological samples.
Conclusion: Due to increase of morphine abuse as a worldwide concern, use of advanced analytical techniques with high sensitivity and precision in forensic toxicology setting should be recommended.

Keywords: Morphine, Analysis, Biological samples, Forensic toxicology

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INTRODUCTION

Morphine, a potent opioid derived from the opium plant (*Papaver somniferum* L.), and which is one of the main ingredients of opium. Morphine widely used as a potent analgesic in severe pain management especially in patients undergoing a surgical operation, and for palliative cancer-related pain [1,2]. Also, its use and abuse has a significant impact on human health and society [1]. It is an alkaloid with chemical formula $C_{17}H_{19}NO_3$ and acts as an agonist on μ-opioid receptors, providing analgesic, euphoria, drowsiness, constipation, respiratory depression, nausea, vomiting, and in severe cases, overdose leading to coma or death effects [1,2]. Morphine metabolizes primarily in the liver through glucuronidation and sulfation and producing active and inactive metabolites like morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) [1,2].

Morphine abuse and misuse have become a global concern issue with a substantial rise in opioid-related deaths and addiction cases over the past few decades. Globally, opioid abuse including morphine, is a significant public health concern. According to the world drug report (2022), from 345 million subjects experience substance abuse, at least 61 million cases estimates have opioids abuse [3]. In Europe, the mortality rate due to drug overdoses is estimated at 22.6 deaths per million population aged 15-64 [4]. Also, opioids, are involved in most of the drug-induced deaths reported in Europe in 2017 [4]. In Iran, opioid abuse, mostly opium, is prevalent, leading to various health, social, and economic challenges. The lifetime rates of opiate abuse were between 1.2 and 8.6% in different parts of the country [5]. Drug trafficking represents a major challenge for Iran. The geographical location of the country, particularly its porous 1,923 km-long Eastern border with Afghanistan - the world's largest illicit opium producer - and Pakistan, has turned it into a major transit country for illicit drugs. Iran accounted for 74% of the world's opium seizures and 25% of the world's heroin and morphine seizures in 2012 (6).

Morphine abuse is linked to addiction, co-morbidities, and death. It is a precursor of opioids analgesics, among which the most representative drug are heroin, codeine. Morphine analysis in biological samples has been applied in forensic

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cases as an indicator of opium, heroin abuse and overdose and in pharmacokinetic or pharmacodynamic investigations. Therefore, advancements in analytical techniques for detecting of morphine in biological samples play a crucial role in forensic and clinical toxicology [7,8]. Analytical innovations sheds light on the evolving landscape of morphine detection and its forensic implications, emphasizing the importance of continuous research and collaboration across disciplines to address the challenges posed by morphine abuse effectively. The present article will provide an overview on recent developments of analytical methods for the detection of morphine in biological samples in the fields of forensic and clinical toxicology.

METHODS

Search Strategy

We performed a systematic review to determine the recent instrumental analytical methods which have been developed for morphine analysis in biological samples. Articles were searched from PubMed, Scopus and Google Scholar electronic databases from 2011 until 2024 (30th September). They were searched using a systematic search of English keywords including: “Morphine” OR “Analysis” OR “Analytical techniques” OR “Analytical innovations” OR "Methods" AND “Biological samples” OR “Biological matrices". The selection criteria were based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta- Analyses).

Criteria for Selecting Articles

After studying all abstracts of the articles, unrelated articles were excluded, possible related articles were identified, and their full texts were extracted. The criteria for the inclusion of articles in the study were studies related to the identification and analysis of morphine in human biological samples and articles restricted to the English language. The animal, *in vitro* studies and meta-analysis articles were excluded from the study.

Finally, the selected characteristics of analytical methods among selected articles were extracted and showed in the data extraction table.

RESULTS

Data Extraction

In the first stage of the search, 1200 articles were reviewed and then 640 articles remained after removing the duplicate ones. Then, the titles of articles were screened, 530 articles were entered to second stage. One hundred and ten (110) articles were deleted because lacking of sufficient data about validation of method of analysis. Finally, we included 30 articles that met the inclusion criteria (Figure 1). Also, table 1 shows the characteristics of analytical methods among selected 30 articles which included in this review.

Importance of morphine analysis in biological samples in clinical and forensic toxicology

Analysis of morphine is important in clinical practice to ensure its therapeutic efficacy, minimize adverse effects, and rational treatment regimens to individuals. Monitoring of the morphine levels can help healthcare professionals for effective pain management while reducing the side effects associated with opioid administration [8,9]. Morphine can be detected in biological samples such as blood (serum and plasma), urine and hair by various analytical techniques [10]. The ccommon analytical methods include immunoassays and chromatography [such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS)]. These analytical methods provide qualitative and quantitative information about morphine levels in the body [10,11]. They are essential in clinical toxicology, forensic investigations, and pain management practices to ensure proper use and prevent adverse effects associated with opioid use and confirmation of cause of death in overdose and poisoning [8-11]. Different biological matrices have unique advantages for morphine detection. Blood samples provide real-time information about recent drug use, while urine samples are commonly used for routine drug screening due to ease of collection and longer detection windows. Hair samples has a historical record of drug exposure over an extended period [12]. Analysis of drugs like morphine and its metabolites in bio-samples can help establish a timeline of drug exposure, determine the cause of death in fatalities involving opioid overdose, identify drug abuse patterns, and provide evidence in criminal proceedings [13].

Types of Biological samples in morphine analysis

Blood sample

Blood sample is a fundamental biological fluid for the analysis of morphine, offering valuable data in drug exposure and pharmacokinetics. In forensic cases, the detection of morphine in blood plays a crucial role in various investigations, such as drug-related crimes and poisonings, postmortem analysis, and monitoring of opioid use disorders. Blood samples provide a direct measure of the concentration of morphine present in the systemic circulation, reflecting recent drug exposure [7, 14].

Detection of morphine in blood is essential for determining drug-related fatalities, impaired driving cases, and drug abuse monitoring. Proper collection and handling of blood samples are crucial to avoid contamination and ensure accurate results. Among in this review, 6 studies were conducted on blood samples. For example, Manca et al. (2023) describe an ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS-MS) method for the simultaneous quantification of morphine, oxycodone, oxymorphone, hydrocodone, hydromorphone, methadone and fentanyl in several tissues including blood plasma. The method has been applied on postmortem samples. This method is validated and fitted the recommendations of international guidelines for postmortem pharmacological/toxicological studies [15].

Orfanidis et al. (2021) developed an UHPLC-tandem mass spectrometry (MS-MS) method for the detection and quantification of 84 drugs and pharmaceuticals in postmortem blood. The target analytes including pharmaceuticals (like antipsychotics, antidepressants), drugs of abuse including opiates, cocaine, cannabinoids, amphetamines, benzodiazepines and new psychoactive substances. Sample pretreatment was applying a modified

QuEChERS single step. UHPLC-MS-MS analysis took place on a C18 column with a gradient elution over 17 min. The method was found to be selective and sensitive, offering limits of detection (LOD) ranging from 0.01 to 9.07 ng/mL. The method performed satisfactorily to the analysis of postmortem blood from chronic drug abusers [16].

Urine sample

Urine samples are commonly utilized in the analysis of morphine due to their non-invasive nature, ease of collection, and longer detection window compared to blood samples. The detection of morphine in urine plays a pivotal role in forensic investigations, drug monitoring programs, and clinical settings, providing valuable information about drug intake and metabolism [11].

In this review, 13 studies were conducted on urine samples. For example, Ebrahimi Rahmani et al. (2018) described a magnetic molecularly imprinted polymer (MIP) for selective extraction of morphine from urine and plasma samples. Fe_3O_4 nanoparticles were coated with SiO_2 -NH_2. The MIP was coated on the Fe_3O_4 /SiO_2 -NH_2 surface by the copolymerization of methacrylic acid and ethylene glycol dimethacrylate in the presence of morphine as the template molecule. The recoveries from plasma and urine samples were in the range of 84.9-105.5 and 94.9-102.8%, respectively. They concluded that by using the magnetic MIP, morphine can selectively, reliably, and in low concentration be determined in biological samples with high-performance liquid chromatography and UV detection (HPLC-UV). The LOD and LOQ of the method were 0.03 µg/mL and 0.08 µg/mL, respectively [14].

Tahmasebi et al. (2022) developed a new method of morphine detection in urine samples using the β-glucuronidase-dendrimer poly amidoamine (PAMAM) enzyme hybrid system. The PAMAM dendrimer was synthesized based on silica and the β-glucuronidase enzyme was replaced inside its dendrimer cavities and the compound was released into a urine sample containing morphine. The LOD, LOQ and recovery were reported as 6.5ng/mL, 9.59ng/mL and 98%, respectively [17].

Cao et al. (2019) established a rapid and sensitive screening method using a competitive fluorescence immunoassay for the qualitative and quantification of morphine in urine sample. In this method, hapten was prepared as a covalent conjugating a morphine derivative

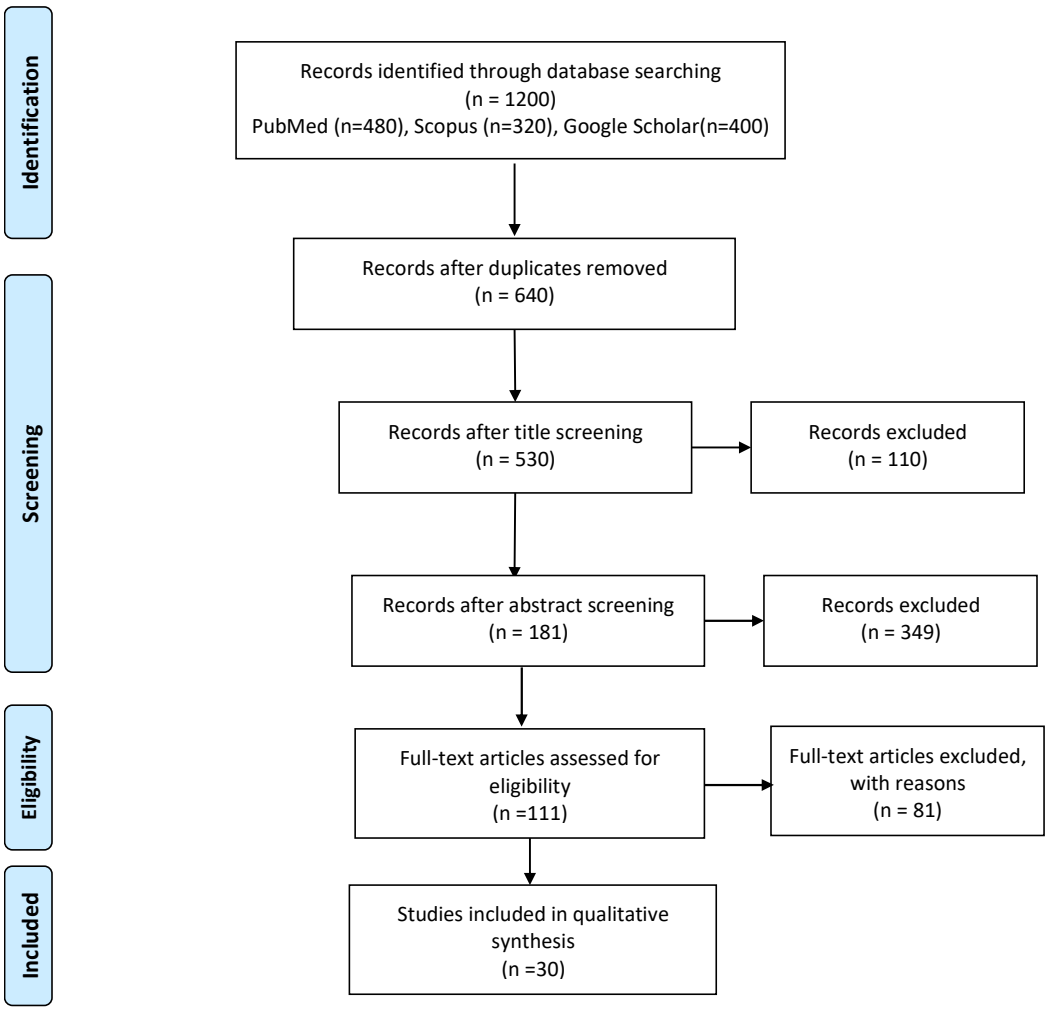

Table 1. Distribution of the studied cases according to the type of ingested antihypertensive (n=105)

Table 1. Summary of selected studies included in the systematic review on morphine analysis in biological samples

First Author (Publication Year)	Biological sample(s)	Sample preparation method	Instrumental method	Recovery (%)	LOD	LOQ	Reference No.
Ebrahimi Rhamani et al. 2018)	Urine	MMIP	UHPLC-DAD	94.9	0.03 µg /mL	0.08 µg/ mL	14
Manca et al. (2023)	Blood	QuEChERS	UHPLC–MS-MS	101	0.009 ng/mg	0.027 ng/mg	15
Suárez-García et al. (2023)	Hair	SPE	GC-MC	88.1	0.15ng/mg	0.2 ng/mg	24
Malaca et al. (2019)	Saliva	QuEChERS	UHPLC–MS-MS	93.1	1.4 ng/ml	4.7 ng/ml	45
Scendoni et al. (2022)	Nail	SPE	UHPLC	95	0.02 ng/mg	0.05 ng/mg	1
Orfanidis et al. (2023)	Blood	QuEChERS	UHPLC -MS-MS	79	1.23 ng/ml	3.69 ng/ml	16
Soltaninejad et al. (2023)	Hair	DLLME	GC-MC	99	0.12 ng/mg	0.39 ng/mg	22
Zhuo et al. (2020)	Hair	LLE	LC-MS-MS	72	0.02 ng/mg	0.05 ng/mg	46
Ke et al. (2020)	Urine	DLLME	Immunoassay	97	6.5 ng/ml	9.59 ng/ml	33
Cao et al. (2019)	Urine	-	Immunoassay	100	1 ng/mL	3 ng/mL	8
Hansen et al. (2021)	Blood/Muscle	SPE	UHPLC-MS-MS	96	0.002 mg/kg	0.005 mg/kg	47
Akhunov et al. (2021)	Blood	SPE	LC-MS-MS	100	0.025µg /mL	0.050µg/ mL	48
Kang et al. (2022)	Urine	SPE	LC-MS-MS	90	50 ng/ml	100 ng/ml	18
Gürler et al. (2022)	Hair	SPE	LC-MS-MS	99	0.11 ng/mg	0.50 ng/mg	23
Akcan et al. (2020)	Saliva	SPE	LC-MS-MS	95	19.91 ng/mg	59.73 ng/mg	27
Barroso et al. (2011)	Blood	SPE	GC-MC	101	2.5 ng/ml	12.5 ng/ml	25
Zhang et al. (2021)	Urine	LLE	Electrochemical sensor	108	0.03ng/mg	0.1ng/mg	49
Tahmasebi et al. (2021)	Urine	LLE	FTIR	98	0.027ng/mg	0.073ng/mg	17
Isbell et al. (2015)	Urine	LLE	CE-MS	99	1.95µg /mL	3.5µg/ mL	50
Truver et al. (2018)	Saliva	SPE	LC-MS-MS	95	5 ng/ml	10ng/ml	28
Lu et al. (2020)	Urine	SPE	UHPLC-MS-MS	80.4	0.02 µg/ L	0.2 µg/ L	43
Wang et al. (2020)	Urine	SPE	LC-MS-MS	93	1.2 ng/ml	2.5 ng/ml	51
Boonchaleaw et al. (2021)	Blood	QuEchERS	LC-MS-MS	80	0.05 ng/ml	0.8 ng/ml	52
Buratti et al. (2023)	Nail	SPE	LC-MS	90	0.01 ng/ml	0.1 ng/ml	53
Alahyari et al. (2018)	Urine	DLLME	HPLC	95.5	25 µg /mL	100 µg /mL	37
Simao et al (2022)	Urine	SPE	GC-MS-MS	90	1 ng/mL	3 ng/mL	39
Baciu et al. (2016)	Urine	SPE	Capillary electrophoresis	76	20 ng/ml	50 ng/ml	54
Kovatsi et al. (2011)	Vitreous humor	SPE	GC-NPD	76	0.89 µg /mL	2.68µg /mL	55
Júnior etal. (2022)	Blood	QuEchERS	UHPLC–MS-MS	120	10 ng/mL	16 ng/mL	56
Franzin et al. (2024)	Bile	SPE	LC-MS-MS	100	0.2 µg/mL	1.1 µg/mL	42

LOD: Limit of detection
LOQ: Limit of quantification
MMIP: Magnetic molecularly imprinted polymer
UHPLC-DAD: Ultra-high performance liquid chromatography (UHPLC) coupled with diode array detection
GC-MC: Gas chromatography–mass spectrometry
FTIR: Fourier-transform infrared spectroscopy
QuEChERS: Quick, Easy, Cheap, Effective, Rugged and Safe
SPE: Solid Phase Extraction
UHPLC-MS-MS: Ultra-high performance liquid chromatography tandem mass spectrometry
DLLME: Dispersive liquid-liquid microextraction
LLE: Liquid-liquid extraction
GC-NPD: Gas chromatography–nitrogen phosphorous detector
LC-MS: Liquid chromatography–mass spectrometry
CE-MS: Capillary electrophoresis–mass spectrometry

with Bovine serum albumin (BSA). In the immunoassay, monoclonal antibodies labeled with a signal indicating dye, fluorescein isothiocyanate (FITC). The fluorescein intensity decreases in the presence of morphine molecules due to the competitively binding to antibodies against hapten. The linearity range of 0.2 μg/mL-2.5 μg/mL, along with a LOD 1 ng/mL. The developed method produced comparable results to the standard GC-MS-MS method [8].

Kang et al. (2022) developed an on-site method that enabled determination and quantitation of morphine in urine sample in 3 min. The method consisted of a simple extraction process, using a fast dansyl-derivatization and direct analysis by a miniature tandem mass spectrometer. MS/MS of derivatized morphine produced a highly abundant product at *m/z* 285 in positive ion mode. LOD and LOQ of the method were 50 ng/mL and 100 ng/mL, respectively [18].

Hair sample

Hair analysis has been mainly used to verification of drug use history in cases of drug abuse/addiction, drug-facilitated crime, doping control and clinical, environmental, occupational and postmortem toxicology [19]. Hair sample are increasingly recognized as valuable specimen for the detection of morphine and other drugs over an extended period. The analysis of morphine in hair offers unique advantages in forensic investigations. Hair samples can provide a historical record of drug exposure over several months, offering insights into chronic drug use patterns [20]. Collection of hair samples is non-invasive and can be easily performed, making it a preferred matrix for drug testing in various settings. External factors such as environmental exposure to drugs can lead to false-positive results and require careful consideration during analysis [20].

Drugs get deposited in hair sample through blood circulation by various mechanisms. The deposited drug is much stable and can be detected after a longer period of time as compared with other biological samples, such as saliva, blood, and urine. Also, segmental analysis can detect multiple or single drug administration [21].

Soltaninejad et al. (2024) established a simple, sensitive and specific GC-MS method for the identification and quantitation of selected opioids (tramadol, methadone, morphine, and codeine) in hair samples. After external decontamination of hair samples, with hydrochloric acid (0.1 M) and incubated on a magnetic stirrer at 56°C for 16 hours. The liquid-liquid extraction(LLE) with Chloroform-isopropanol (ratio: 80:20 V/V) was utilized. The sample was derivatized with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and analyzed by GC-MS. The LOD ranged from 0.12 to 0.21 ng/mg. Extraction efficiency varied from 91.8 to 102.4% [22].

Gürler et al. (2022) developed a sensitive method for the identification and quantification of morphine, codeine, 6-monoacetylmorphine (6-MAM), heroin, tetrahydrocannabinol, amphetamine, 3,4-methylenedioxyamphetamine, buprenorphine, methamphetamine, 3,4-methylenedioxy-N-methylamphetamine, and cocaine in human hair by LC-MS/MS. The sample preparation step includes washing, standard addition, liquid-phase extraction with methanol, and solid-phase extraction steps. The LOD values of each substance ranged from 0.11-0.87 ng/mg, and the linearity was quite good (r^2>0.99). The concentration ranges for quantification were 0.50 and 8.0 ng/mg for all substances. The intraday and interday accuracy and precision values of this method were acceptable (<12.81%) and the recovery was found to be between 93.72%-104.78% at different concentrations [23].

In another study, Suárez-García et al. (2023) was analyzed hair samples from chronic opioid users in beginning and cessation of a controlled drug program over a 6-month period. Morphine, codeine and 6-MAM were analyzed by GC/MS (LOQ = 0.2 ng/mg). They showed the traces amount of morphine, codeine and 6-MAM still remained in the newly growing hair segments after cessation of opioid use. After 2 months, still 27 % of the users tested positive, and at 4 months, 20 % were positive but only for 6-MAM. However, after six months of withdrawal, the results were negative for all opioids [24].

Saliva sample

Saliva specimen has emerged as a non-invasive sample for the detection of morphine. It is offering real-time monitoring capabilities and ease of collection [25]. Drug concentrations in saliva can vary based on factors such as saliva flow rate and individual metabolism, requiring careful interpretation of results [25]. Contamination of saliva with food, beverages, or smoking can affect the accuracy of sample analysis and must be mitigated during collection and analysis [25]. Standardizing collection protocols and analytical methods is essential to ensure consistent and reliable results in drug analysis in saliva [26].

Akçan et al. (2020) established a fast, accurate and cost-effective method by Surface-Enhanced Raman Spectroscopy (SERS) for analysis of Heroin and its metabolites (morphine, M3G and 6-MAM) in saliva sample. The results showed that heroin and its metabolites can be detected and quantified in saliva samples using a SERS-based system [27].

Truver et al. (2018) developed and validated a comprehensive analytical method for the detection and quantification of morphine, 6-MAM, buprenorphine, synthetic opioids (U-47700, U-49900, U-50488, AH-7921, MT-45, W-18 and W-15) in saliva by solid-phase extraction (SPE) followed by LC-MS-MS. The LOD and LOQ were 5 ng/mL and 10 ng/mL, respectively. Linearity was observed between 10 and 500 ng/mL ($R^2 \geq 0.9959$) [28].

Vitreous humor sample

Vitreous humor (VH) is a gelatinous fluid that is largely composed of water contained in the posterior chamber of the eye. It has been used in various forensic applications, primarily for the evaluation of postmortem interval and for postmortem toxicological analysis [29]. Since most of the drugs present in the blood are detected in VH after crossing the blood-retinal barrier. From this view, VH is an alternative sample in forensic toxicology analysis [29, 30]. The specific drugs/substances that are detected in vitreous humor include amphetamines, cocaine, Delta-9-tetrahydrocannabinol (THC), alcohols, benzodiazepines, barbiturates, antidepressants, and opioids. VH analysis offers special advantages in comparison with other biological samples

including easy to collect, less prone to postmortem redistribution, relatively simple matrix. Also, it has with little or no pretreatment and shows sample stability in postmortem toxicology. Disadvantages of VH include a limited sample volume and the limited interpretative value of analytical results [29].

The use of VH as an alternative sample to blood was investigated for the detection of heroin, morphine and 6-MAM in post-mortem samples [31].

Analytical techniques for morphine analysis

Immunoassays rely on the interaction between antibodies and antigens, providing a rapid and cost-effective method for morphine analysis. Immunoassays [both homogeneous immunoassays include enzyme immunoassay (EIA), fluorescent polarization immunoassay (FPIA) and kinetic interaction of microparticles in solution (KIMS) and heterogenous immunoassays include radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA)] were applied for drug detection and they can be used to screen rapidly a large number of samples for the potential presence of a drug group [33].

In forensic toxicology, immunoassay methods have been instrumental in identifying morphine presence in various biological samples, such as blood, urine, sweat, hair, tissue homogenates, blood stains and saliva and it can detect morphine at very low concentrations, aiding in accurate quantification [32].

Recently, Ke et al. (2020) developed a new, sensitive and fast method for morphine detection in urine samples based on the surface plasmon resonance imaging (SPRi) technique according to an indirect competitive immunoassay. The qualitative and quantitative analysis could be completed in 20 minutes. The mixture of morphine at different concentrations and morphine antibody at a certain concentration as the mobile phase was reacted with morphine BSA fixed on a chip surface in a competitive way. The LOD and LOQ were as 9.59 ng/mL and 6.5 ng/mL, respectively [33].

Chromatographic-based analytical methods are the most frequent techniques for morphine detection in different samples in forensic investigations, clinical toxicology, and pharmaceutical analysis. Techniques like HPLC and GC coupled with advanced detectors have significantly enhanced the sensitivity of morphine detection [34-36]. These methods can detect trace amounts of morphine in complex biological matrices, increasing the likelihood of identifying the substance even at low concentrations [34, 35].

HPLC is particularly popular for morphine analysis due to its high sensitivity and ability to separate compounds efficiently. By using a suitable detector, such as UV-VIS or mass spectrometry, morphine can be detected and quantified accurately [37].

In a study, the detection of morphine in fingernails from forensic autopsies using immunohistochemistry (IHC), with confirmation by UHPLC coupled with high-resolution mass spectrometry (UHPLC-HRMS) has been developed. In IHC method, a primary antibody specific to morphine and a secondary antibody conjugated to horseradish peroxidase was used. Then, UHPLC-HRMS and GC-MS analysis showed that a morphine concentration range between 0.35- 1.23 ng/mg in the fingernail and 360-472 ng/mL in the blood samples in different groups. Also, most of those matrices were positive for codeine, methadone, 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 6-MAM [1].

Alahyari et al. (2018) developed a dispersive liquid-liquid microextraction (DLLME) combined with HPLC with photo diode array detector (HPLC-PDA), as a new and sensitive method for determination of morphine, codeine and methadone in postmortem urine samples. Opioid analyzed by HPLC-PDA using a Eurospher® C_{18} column. recovery of morphine, codeine, and methadone were in the range of 175–215.8 and 87.5–107.9%, respectively. LOD for the analytes was in the range of 10–25 µg /L [37].

GC-MS is another chromatography technique that can be used for simultaneous analysis of opioids including morphine, especially when coupled with mass spectrometer detector (MSD). The volatile nature of morphine derivatives makes them suitable for analysis using GC-MS [38].

Simao et al (2022) develop a fast, selective and accurate method for the simultaneous determination of morphine, codeine, 6- acetylcodeine, 6-MAM, tramadol and fentanyl in urine samples using GC coupled to tandem mass spectrometry (GC-MS-MS). The analysis includes the use of microextraction by packed mixed-mode sorbent with minimal use of solvents for sample preparation. The method was validated in urine samples, with the ability to detect and quantify all analytes with good linearity (1 – 1000 ng/mL for all analytes, except for fentanyl (10–1000 ng/mL)). Low LOQs obtained (1 ng/ mL for all analytes; and 10 ng/mL for fentanyl) [39].

In recent years, LC-MS or tandem mass spectrometry (LC-MS-MS) has become increasingly important in the field of clinical and forensic toxicology, systematic toxicological analysis, substance abuse screening testing, and control of doping. LC–MS-MS has used for analyzing of non-volatile, hydrophilic and thermolabile compounds that were not sufficiently covered by the GC–MS [40, 41]. Also, LC-MS-MS using triple quadrupole or ion trap instruments have been established for comprehensive screening approach based on HRMS analysis using benchtop time-of-flight (TOF) MS instruments for multi-target screening and/or quantification of drugs, poisons, and or their metabolites in various biosamples [41]. LC-MS-MS has been used of detecting morphine at pico or femtogram concentrations in biosamples. In this review, 17 papers were discussed the LC-MS-MS technique for morphine analysis in the field of forensic toxicology and drug screening testing.

Franzin et al. (2024) presented a LC–MS-MS method to quantify 108 drugs and metabolites in postmortem bile sample including opioids, amphetamines, benzodiazepines, cocaine derivatives, barbiturates, z-drugs, and psychedelics. The proposed method showed an appropriate selectivity, specificity, accuracy, and precision (precision < 15%; accuracy < 100 ± 15%). The LOD and LOQ for morphine were 0.2 µg/L and 1.1 µg/L, respectively [42].

Lu et al. (2020) developed a rapid and green sample pretreatment method for extracting of psychoactive drugs using a Graphene oxide–Fe_3O_4 (GO–Fe_3O_4) nanocomposite

as magnetic sorbent to extract the eight psychoactive drugs from urine samples. The analytes are morphine, 6-MAM, amphetamine, methamphetamine, codeine, cocaine, dolantin and benzoylecgonine (BZE), which were determined by UHPLC-MS-MS. This method has high selectivity for the target analytes. The LOD and LOQ for morphine were 0.02–0.2 µg/ L and 0.05–0.5 µg/ L, respectively. The linear ranges were calculated 0.1–1000 µg/L for 6-MAM and codeine, and 0.5–1000 µg/L for morphine and BZE. The recoveries ranged in 80.4–105.5% [43].

Sample Preparation Methods

Several methods are commonly used for morphine analysis in biosamples including liquid-liquid extraction (LLE), solid-phase extraction (SPE), and liquid-phase microextraction (LPME). Novel techniques such as solid-phase microextraction (SPME), dispersive liquid-liquid microextraction (DLLME), and QuEChERS(Quick, Easy, Cheap, Effective, Rugged, and Safe) have gained popularity for their ability to efficiently extract morphine from complex biological matrices. Automation and high-throughput methods have also been developed to fast sample preparation, allowing for the analysis of a large number of samples in a shorter time course [44]. In this review, SPE (16 papers), QuEChERS (5 papers), LLE (4 papers) and DLLME (4 papers) were the common methods for morphine sample preparation.

DISCUSSION

In this systematic review, the effectiveness of various analytical methods such as LC-MS-MS, UHPLC-MS-MS, GC-MC, HPLC and immunoassays for detection and quantification of morphine in biological samples in forensic and clinical toxicology fields has been evaluated. Immunoassays compared to other analytical techniques, are often more cost-effective and require minimal sample preparation. However, these methods have slightly lower accuracy compared to chromatography and mass spectrometry-based method. The advancement of analytical techniques, such as chromatography hyphenated techniques such as LC-MS-MS and GC-MS-MS have significantly enhanced the sensitivity, selectivity, and accuracy of morphine analysis in various biological samples [41-44]. Also, in this review, recent sample preparation methods were used for the sample pretreatment for morphine analysis in biosamples. Sample preparation techniques for morphine analysis in biological matrices has advantages and limitations, and the choice of the technique often depends on factors such as sample volume, analyte concentration and desired sensitivity [44]. A variety of biological samples were examined in these studies. For example, urine samples offer a longer detection window for morphine compared to blood, allowing for the monitoring of drug use over an extended period [11].

Collaborative efforts between forensic scientists, toxicologists, and legal professionals are essential to ensure the integrity and reliability of morphine analysis in forensic investigations. By using of high tech analytical tools and interdisciplinary collaborations, forensic laboratories can improved the drug and poison analysis and enhance research capabilities with the highest standards in forensic and clinical toxicology fields.

CONCLUSION

In recent years, sophisticated and high tech instrumental analytical methods are used as an indispensable part of bioanalysis of morphine in the forensic and clinical toxicology. Nodaway, sensitive, specific and green chemistry methods such as LC-MS-MS, GC-MS-MS, UHPLC-MS-MS with DLLME, magnetic solid-phase extraction and QuChERS techniques as sample pretreatment methods have been performed for morphine analysis in clinical and postmortem biological samples. By the way, improvement in all the three most important parts of bioanalysis including the sample preparation, the separation techniques and the detection for morphine analysis has been utilized. With the recent improvements in analytical technology for LC and GC- hyphenated techniques providing a high separation efficiency and sensitivity are available in a shorter time of analysis. The new achievements in instrumental analytical technologies are used to determine morphine at low or very low concentrations in very complex biosamples.

LIMITATION

There are some limitations in our study. This review was based on English language articles and other articles with non-English languages were not included in this review. Secondly, news and conference abstracts exclusion may leads to some notable missing data.

Conflict of interest

The authors state that there is no conflict of interests.

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