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Review Article

Modification of the QuEChERS Method for Drug Analysis in Biological Sample: A Review

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Abstract
Drug analysis in biological samples remains a significant challenge due to the complex matrix composition that can interfere with extraction and detection processes. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, initially developed for pesticide residue analysis, has been increasingly adapted for forensic toxicology, particularly in drug extraction from biological matrices. This review explores various modifications of the QuEChERS method applied to blood, urine, and liver samples, focusing on the effectiveness of different solvents, buffer salts, and sorbents in enhancing extraction efficiency. Furthermore, it highlights current challenges such as matrix effects and the critical importance of method validation to ensure reliability and reproducibility. By analyzing recent developments and trends in QuEChERS applications, this review offers valuable insights into optimizing extraction protocols for forensic purposes. The findings aim to support the advancement of more efficient, robust, and accessible analytical techniques in forensic toxicology, ultimately contributing to more accurate and reliable drug detection in complex biological samples.

Keywords: QuEChERS, drugs, biological samples, forensics

1. INTRODUCTION

Drug abuse remains a growing global problem, with approximately 300 million people using drugs annually, as reported by the United Nations Office on Drugs and Crime (UNODC) [1]. Reliable analytical methods are essential for the detection of drugs in biological matrices such as blood, urine, and other biological tissues. Conventional extraction techniques, such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) have been widely used, but have limitations such as solvent consumption, emulsion formation, and high operational costs [2]-[4]. To overcome these challenges, the QuEChERS method was introduced as an alternative extraction technique that offers simplicity, cost-effectiveness, and environmental friendliness.

Although the QuEChERS method was initially applied to the analysis of pesticides [5]-[14], subsequent studies have shown its potential in forensic toxicology, especially in the extraction of drugs from biological matrices [15][16]. The main advantage of this method lies in the principle of dispersed LLE that enables high-efficiency separation of target compounds in a relatively short time. Compared to pesticide analysis, the extraction of pharmaceuticals in biological matrices is often more complex due to stronger interactions between the analyte and matrix components, such as proteins and phospholipids.

Modifications to the QuEChERS method in forensic toxicology often include the use of specific sorbents such as C18, primary secondary amine (PSA), and zirconium-based sorbents to remove interferences from more complex matrix components, such as phospholipids and proteins [16][17]. In addition, the use of different buffer salts, such as sodium chloride, sodium acetate or sodium citrate, can improve analyte stability. These modifications increase the selectivity and sensitivity of the extraction without the need for lengthy purification steps, which are often required in conventional methods such as LLE or SPE [16]. In addition to being quick, easy, cheap, effective, rugged, and safe, the QuEChERS method in drug analysis offers advantages in terms of compatibility with various detection techniques, including chromatography and spectrophotometry. This enables multi-residue analysis in a single extraction, which is particularly important in forensic studies where various drug compounds must be detected at once. Therefore, with proper modification and

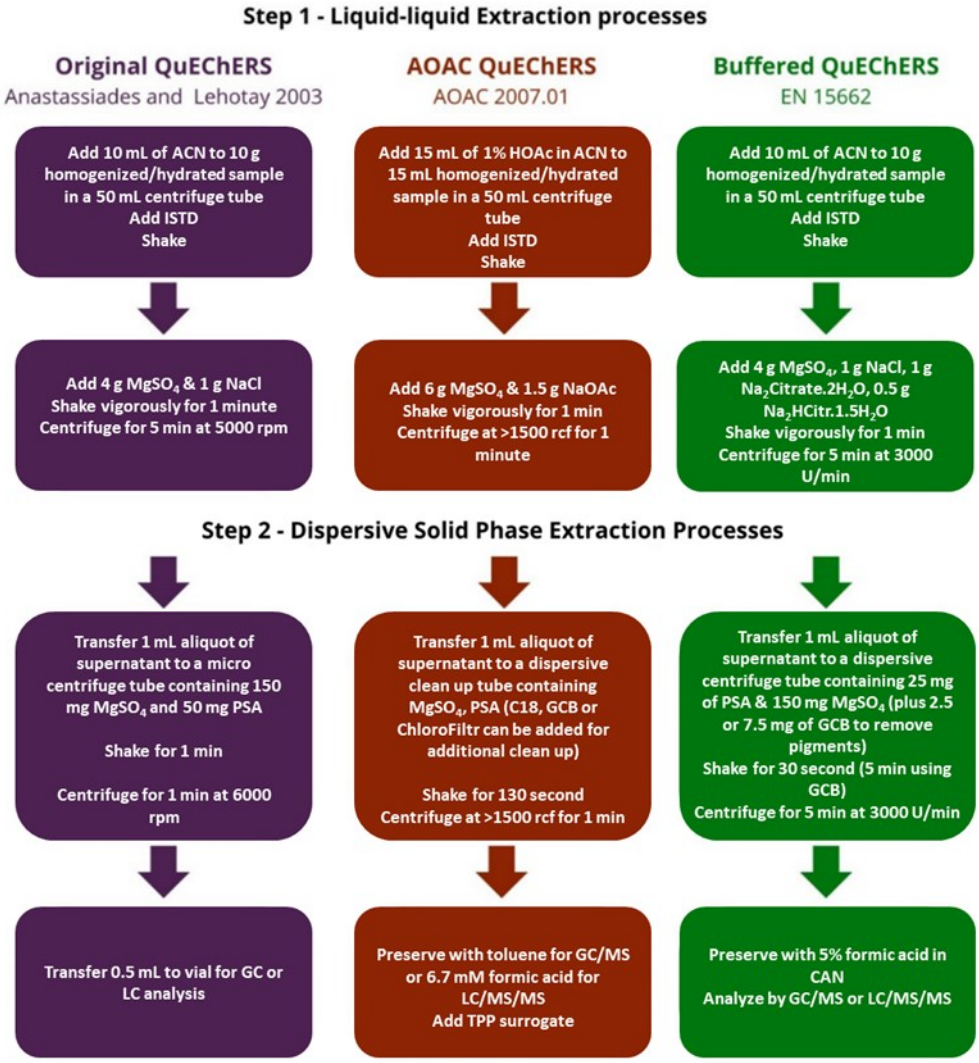

Figure 1. Schematic for original QuEChERS, AOAC 2007.01, and EN 15662 sample preparation. Adapted from the QuEChERS Information Booklet UCT Pesticide Residue Analysis [25].

optimization, QuEChERS becomes a highly reliable extraction method for drug analysis in forensic toxicology [18].

Several studies have made various modifications to the QuEChERS method, particularly in the aspects of solvent selection, use of buffer salts, and sorbent variation, with the aim of improving the efficiency of drug extraction in biological samples [19][20]. These modifications were made to overcome the challenges posed by the complexity of biological matrices. Therefore, this review aims to synthesize the key findings from various studies that have developed and modified QuEChERS extraction methods in forensic drug analysis. Furthermore, this review compares the effectiveness of various approaches in improving analyte recovery and selectivity, evaluate practical limitations that may arise, as well as propose mitigation strategies to overcome the drawbacks of these methods. Thus, the results of this review are expected to provide more comprehensive insights for researchers and practitioners in selecting and developing extraction methods that are more reliable, efficient, and suited to the needs of forensic toxicology analysis.

2. DEVELOPMENT OF THE QUECHERS METHOD

The QuEChERS method is an extraction technique designed to provide a fast, easy, cheap, effective, robust, and safe solution in the analysis of chemical residues in various matrices [16][20][21]. This method was first introduced by Anastassiades

et al. [5] to analyze pesticide residues, with the main principle of acetonitrile (ACN) and magnesium sulfate ($MgSO_4$) based extraction. Acetonitrile serves as the main solvent capable of extracting various organic compounds, while $MgSO_4$ is used to remove interfering components such as sugars, lipids, organic acids, steroids, proteins, pigments, and excess water in one extraction stage. As its development, the QuEChERS method underwent various modifications to improve the extraction efficiency, especially in the recovery of pH-dependent analytes. One important development is the addition of buffer salts in the AOAC 2007.01 method [22], which aims to maintain the stability of analytes during extraction. In addition, the European Standard method EN 15662 adopts a variety of buffer salts such as citrate, which not only helps maintain pH but also extends the application of QuEChERS to different types of compounds and more complex matrices [23].

Not only has the extraction method evolved, but also the types of matrices used have diversified. Initially applied to pesticide residues in food, the method was later adapted for various other applications, including in forensic toxicology. Plossl et al. (2006) [18] were among the researchers who developed the QuEChERS method for the analysis of pharmaceuticals in blood samples, demonstrating that the principles originally applied in food analysis can be modified for the extraction of drug compounds in biological matrices.

These three methods have similar basic steps, but the modification lies in the use of salts and sorbents to improve the extraction efficiency (Fig. 1). In general, the QuEChERS extraction process consists of two steps (Fig. 2). First, liquid-liquid extraction using acetonitrile and salt to create a partition between the organic and aqueous phases. Second, cleaning by dispersive-SPE (d-SPE) using $MgSO_4$ to remove water [5][22][23] and sorbents such as PSA to bind interfering compounds such as fatty acids and pigments [12][24]. With the combination of these steps, the QuEChERS method becomes a flexible, efficient, and customizable extraction method for different types of analytes in diverse matrices.

3. QuEChERS METHOD FOR DRUG ANALYSIS

QuEChERS has been approved as a sensitive, effective, renewable, and relatively easy-to-use method for the qualitative and quantitative analysis of drugs and toxins. Because it does not require special equipment to clean the sample and takes less time. This method has great potential in analyzing clinical and legal samples [18]. There have been many reports on the development and modification of the QuEChERS method for drug analysis (Table 1). This study evaluates various parameters that affect the performance of the QuEChERS method, namely solvent selection, buffer salt addition, and sorbents. The selection of optimal conditions involves a balance between simplicity, ease of application, speed, selectivity, and validation parameters. Advances in analytical instrumentation, particularly gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-

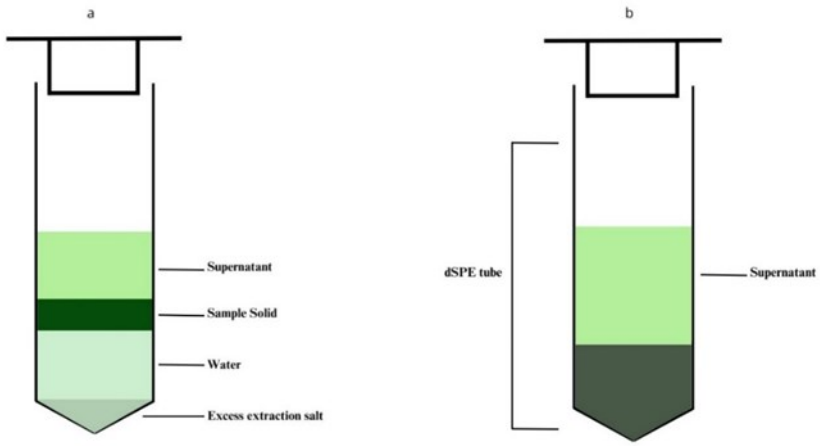

Figure 2. Schematic diagram of (a) liquid-liquid extraction and (b) solid phase extraction.

MS/MS), have expanded the analytical scope of QuEChERS while maintaining selectivity levels. By optimizing the purification process, matrix interference and instrument contamination can be minimized, resulting in optimal analyte purification [26].

Some of the biological samples used in testing the QuEChERS method are blood, urine, and liver. Dybowski et al. [27] and Jacqueline et al. [28] performed QuEChERS extraction on blood samples to test the effectiveness of the method in extracting analytes such as Δ9-tetrahydrocannabinol and antidepressant compounds. Both used the same type of solvent, ACN, because it is able to precipitate proteins moderately and is soluble in water, so both are good for extraction procedures [29]-[31]. However, Dybowski et al. [27] used the original model by adding $MgSO_4$ and NaCl for buffer salts [5]. While Jacqueline [28] chose NaOAc and $MgSO_4$ (AOAC 2007.1 model) as buffer salts [17]. The addition of buffer salts aims to improve the separation process [28]. The selection of buffer salts is very important to be adjusted to the analysis conditions. If the analyte is stable at various pH, then $MgSO_4$ and NaCl are more suitable than $MgSO_4$ and NaOAc [16][32]. After the extraction process, the samples were analyzed using chromatography to see the effectiveness of the extraction. The validation results showed that the QuEChERS method was effective for drug extraction in the blood matrix. The QuEChERS method is said to be fast, easy, cheap, effective, robust, and safe as evidenced by the method validation results. Validation parameters include recovery which indicates the extent to which a method could extract and remeasure the amount of analyte in the sample. The recovery validation requirement is in the range of 90–120% [33]. Then for LOD and LOQ values indicated by RSD, which should be < 20% [28][33]. It can be said that the QuEChERS method has great potential to be used as a method of extracting drugs in blood samples. This is because the analytical results from both studies have met the method validation requirements. However, it is important to consider the selection of solvent volume, buffer salt type, and sorbent according to the analysis conditions used.

Extraction of drugs in urine matrix has been done [39][40][43]. Methadone was extracted from urine using ethyl acetate (EtOAc) solvent as it is considered simpler and safer than acetonitrile. Keep in mind that the selected solvent has a wide range of multivariate experiments for different types of analytes [43]. Meanwhile, the extraction of Z drug and matamfetamine from urine used ACN solvent. These three studies chose $MgSO_4$ and NaCl buffer salts to increase the extraction efficiency to isolate the target analytes from interfering substances. The method validation results showed the percent recovery of each study for methadone, Z drug, and methamphetamine were 67%, 93–99%, and 93–100% respectively and RSD < 20%. Although methadone has a recovery of 67% from a value that is generally 90–120%, according to Anzillotti et al., a recovery of 60–70% into non-moderate and polar compounds is acceptable for the QuEChERS method [44]. Therefore, the QuEChERS method is effective for the extraction of drugs such as methadone, Z drug, and methamphetamine in urine matrix.

In forensic toxicology, when blood and urine are not available, liver can be used as an alternative biological specimen. Because this organ is easily retrieved at autopsy and homogenized for drug extraction. In addition, the concentration of basic drugs in the liver is known to be 10 to 100-fold higher compared to blood samples [45]. Usui has tested liver samples to extract analytes of diazepam class drugs [42]. ACN was chosen because this solvent is the most commonly used basic solvent for the extraction of various analytes and minimizes the interference of the co-extracted matrix [46]. The buffer salt added was $MgSO_4$ and CH_3COONa. The buffer salt in the form of sodium acetate acts as a buffer, to maintain pH stability during extraction [16]. The extraction process combined with chromatography provides optimal results, as shown by the validation parameters that meet the acceptance requirements.

Sorbent selection is critical in the modified QuEChERS extraction method for different types of drugs in biological samples such as blood, urine, saliva, gastric fluid, and liver (see more in Table 2). Sorbent selection is based on the chemical nature of the drug and the complexity of the biological matrix to improve extraction efficiency by removing interfering compounds [28]. PSA is used to remove fatty acids and sugars [47][48]. Reported results

Table 1. QuEChERS methods for drug extraction in biological samples.

Sample	Analytes	Extraction solvent	Buffer salt	Result	Ref
Blood and Urine	Benzodiazepines	ACN	$MgSO_4$ + NaCl	Recovery: 51–346%	[2]
Blood	13 opioids, cocaine and cocaethylene	$C_4H_8O_2$	$MgSO_4$ + NaCl	Recovery: 52.4–95.0% LOD : 6.4–81.7 ng/mL LOQ : 31.2–125.0 ng/mL RSD: 15%	[3]
Blood	40 drugs	MeCN	$MgSO_4$ + NaCl	Recovery: 89.9–103.7% LOD : 5.6–17.2 ng/mL LOQ : 11.3–39.0 ng/mL RSD: < 20%	[18]
Blood	>90 drugs and toxins	ACN	$MgSO_4$	Recovery: 39–127% LOD: 0.04–1.53 ng/mL LOQ: 5.00 ng/mL RSD: 0.5–12.7%	[34]
Blood	13 drugs for forensic purposes	ACN	$MgSO_4$ + NaCl	Recovery: 99.5–99.9% LOD : 0.01–0.10 ng/mL RSD: 0.03–0.20%	[35]
Blood	35 drugs and their metabolites	ACN	$MgSO_4$, NaCl, $C_6H_9Na_3O_9$, $C_6H_5Na_3O_7$	Recovery: 96% LOD: 3 ng/mL LLOQ: 5 ng/mL RSD: 9.0–19.3%	[36]
Blood	Δ9- tetrahydrocannabinol and its metabolites	ACN	$MgSO_4$ + NaCl	Recovery: 94.0–98.9% LOD : 0.011 ng/mL LOQ : 0.033 ng/mL RSD : 3.76–4.21%	[27]
Blood	63 drugs and pesticides	ACN	$MgSO_4$ + NaOAc	Recovery: 61–118% LOD: < 2 ng/mL LOQ:< 20 ng/mL RSD: < 25%	[37]
Blood	28 psychotropic drugs	ACN	$MgSO_4$ + CH_3COONa	Recovery: 85.9% RSD: < 20%	[38]
Blood	20 antidepressants	ACN	NaAc + $MgSO_4$	Recovery: 86.7–96.7% LOD dan LOQ: 10 ng/mL RSD: < 20%	[28]
Urine	Methadone and methadone metabolites (EDDP)	$C_4H_8O_2$	$MgSO_4$ + NaCl	Recovery: 67% LOD: 29.1 ng/mL LOQ: 97 ng/mL	[29]

Table 1. *Cont.*

Sample	Analytes	Extraction solvent	Buffer salt	Result	Ref
Urine	Z-drugs	Methanol	$MgSO_4$	Recovery: 93–99% LOD : < 1 ng/mL LOQ : < 2 ng/mL RSD: 1.0–3.5%	[39]
Urine	Methamphetamine	ACN	$MgSO_4$ + NaCl	Recovery: 93–100% LOD : 0.36 ng/mL LOQ : 1.09 ng/mL RSD: < 15%	[40]
Urine, saliva and gastric juices	TMDL and TNP antidepressants	Methanol	Na_2HCitr-$1.5H_2O$ dan $Na_3Citrate$-$_2H_2O$	Recovery: 88–114% LOD: 10.41–12.64 ng/mL LOQ: 29.53–38.31 ng/mL RSD: 1.13–1.23%	[41]
Liver	Lormetazepam, Medazepam, Mid-azolam, Nitraze-pam, Oxazepam, dan Prazepam	ACN	$MgSO_4$ + CH_3COONa	Recovery: 92–112% LOD: 0.29–1.16 ng/mL LOQ: 5.00 ng/mL RSD: 4.1–9.9%	[42]

TMDL: tramadol; TNP: tianeptine; ACN: acetonitrile; EtOAc: ethyl acetate; $MgSO_4$: magnesium sulfate; NaCl: sodium chloride; CH_3COONa or NaOAc: sodium acetate; $C_6H_9Na_3O_9$: Trisodium citrate dihydrate; $C_6H_5Na_3O_7$: sodium citrate; Na_2HCitr-$1.5H_2O$: dinatrium hydrogen citrate sesquihydrate; Na_3Citr-$2H_2O$: trisodium citrate.

revealed that the use of acetonitrile as a solvent and the combination of PSA and $MgSO_4$ as sorbents are optimal conditions in the extraction method [28][34][35][41][42]. These two parameters are proven to provide the best results in increasing the efficiency of separating analytes from biological matrices, reducing interference from impurity compounds, and increasing the acquisition of the desired analytes [28]. While C18 (non-polar phase) is effective in adsorbing hydrophobic compounds such as Δ9-tetrahydrocannabinol (THC). The combination of $MgSO_4$ and C18 is often applied to remove water as well as lipids [48]. Thus, the selection of an appropriate sorbent largely depends on the polarity, chemical structure of the drug, and matrix characteristics, which play an important role in improving the selectivity and sensitivity of the extraction method in forensic analysis. which has an effect on the recovery rate [34].

The most common extraction method applied in various forensic laboratories is LLE. Although this technique has long been used, LLE has some significant drawbacks that can affect efficiency and practicality in forensic analysis. One of the main challenges in this method is the high requirement of sample volume and the amount of organic solvent needed for the extraction process, making the procedure time-consuming and labor-intensive. In addition, LLE methods often suffer from limitations in terms of extraction efficiency, especially when dealing with complex matrices that can hinder optimal analyte separation [43]. Not only that, the potential contamination of laboratory equipment and the impact on the environment due to the use of large amounts of organic solvents are also issues that need to be considered [16]. Therefore, although still widely used, LLE is starting to be replaced by more modern and environmentally friendly extraction methods, such as QuEChERS which offers higher efficiency and sensitivity with lower solvent consumption [5].

Such as the study conducted by Asl et al. [43], which compared the performance of the modified QuEChERS extraction method with conventional extraction methods such as LLE. Based on the results obtained, the methadone recovery rate using QuEChERS was obtained at 67%. This value is significantly higher compared to 49% in the LLE

Table 2. Sorbents used for modified QuEChERS extraction.

Sample	Analytes	Sorbents	Ref
Blood	40 drugs	PSA dan NH_2	[18]
Blood	90 drugs and toxins	PSA dan C18EC	[34]
Blood	13 opioids, cocaine and cocaethylene	PSA	[35]
Blood	Δ9- tetrahydrocannabinol and its metabolites	C18	[27]
Blood	63 drugs and pesticides	$MgSO_4$ dan C18	[37]
Blood	20 antidepressants	PSA dan $MgSO_4$	[28]
Urine, saliva and gastric juices	TMDL and TNP antidepressants	PSA dan $MgSO_4$	[41]
Liver	Lormetazepam, Medazepam, Midazolam, Nitrazepam, Oxazepam, dan Prazepam	PSA dan C18EC	[42]

PSA: primary secondary amine

method. However, although QuEChERS offers various advantages, this method still has some practical limitations that need to be considered in forensic toxicology, especially related to matrix effects and interference from biological components [16]. Samples such as blood and urine contain proteins, phospholipids, and inorganic salts, which can interact with the analytes, thus decreasing the extraction recovery efficiency [18]. Some drugs with high polarity or complex chemical properties have low recovery in the QuEChERS method [43]. Although QuEChERS can overcome some of these interferences through the use of sorbents such as PSA and C18, protein and phospholipid residues can still inhibit detection in LC-MS/MS, causing ion suppression that reduces the sensitivity of the analysis [34]. Studies by Usui et al. showed that without proper sorbent selection (e.g. PSA or C18), the recovery of drugs in blood can vary greatly (39–127%) due to uncontrolled matrix effects [34]. Extraction optimization strategies such as proper solvent selection, suitable buffer salts, more selective sorbents, and the use of LC-MS/MS techniques with isotope internal standards, can help improve analyte stability during extraction [28].

The success of the QuEChERS method in extracting pesticides [5], encouraged scientists to develop this method for the extraction of pharmaceuticals [18]. In general, the basic principles of these methods are the same. The selection of solvents, buffer salts, and sorbents is tailored to the sample matrix. In pesticide analysis, the main matrix analyzed is food and agricultural products, such as fruits and vegetables, so the main focus of this extraction is the removal of pigments and fats [5][11]. Whereas in drug analysis, biological matrices such as blood, urine, and liver have more complex protein and phospholipid contents, which can interact with the target compounds and interfere with extraction [18]. Therefore, additional modifications such as the selection of appropriate solvents, the use of buffer salts, and special sorbents are needed to overcome biological matrix interference. These modifications should be customized based on the characteristics of the sample and analyte to achieve optimal results in the absence of significant interfering compounds [16][17]. In addition, the QuEChERS method may result in higher detection limits for certain drugs,

requiring the use of chromatography in the form of LC-MS/MS to increase sensitivity [36].

4. PROSPECTS AND FUTURE DIRECTIONS

Modifications to the QuEChERS method provide benefits in the analysis of drugs in biological samples, particularly in the forensic field. To ensure the validity of evidence used in judicial proceedings, the method offers a number of improvements over traditional extraction methods. In the long term, modifications to the QuEChERS method also have the potential to support automation in forensic laboratories, which will speed up the analysis process and reduce the possibility of human error. The future of this method looks promising for reducing cost, time and effort in forensic case handling. This review provides perspectives and future directions regarding the potential of the QuEChERS extraction method for drug analysis in biological samples.

The QuEChERS method has become the first choice in pesticide residue analysis as it is known for its simple and rapid extraction process [5][20]. In the forensic field, the adaptation of the QuEChERS method enables expressive processing of biological samples, which is particularly important in emergency situations such as overdose cases [3]. Future development focuses on further simplification to improve efficiency, especially in handling large sample volumes with minimal operational costs [35].

Modifications to the QuEChERS method have opened up opportunities to analyze a broader spectrum of drugs, including psychoactive substances, narcotics, and prescription drugs [2][18][28][34][42]. Further development efforts can be focused on simplifying the method into ready-to-use kits or procedures, so as to improve the work efficiency of forensic laboratories in analyzing various types of biological samples [42].

The flexibility of the QuEChERS method allows integration with advanced detection techniques such as LC-MS/MS or GC-MS, thus meeting various needs of forensic analysis [26][43]. This combination is particularly useful in detecting compounds at trace concentration levels, which is often a challenge in forensic cases with limited samples or the presence of drug metabolic processes [39].

The consistency and reliability of the analytical results are critical in a forensic context, where analytical results have legal consequences. Therefore, it is necessary to develop specific validation protocols for modifications to the QuEChERS method, so as to produce data that is accurate, consistent, and acceptable as valid evidence in judicial proceedings [16].

It is necessary to automate or miniaturize the QuEChERS method to obtain accuracy in forensic analysis [49]. Therefore, this method can be applied more efficiently, both in the laboratory environment and in the field, and can provide faster and more stable results, especially for cases that require immediate handling [16][40].

5. CONCLUSIONS

Based on the results of the literature review, it can be concluded that the modification of the QuEChERS method provides a more efficient approach in the analysis of drugs in biological samples. Various innovations have been made to improve the extraction, purification, and sensitivity of this method, such as the use of new sorbents, variations in extraction solvents and buffer salts, and more sensitive detection techniques. Despite significant progress, the method still has some limitations, including limited selectivity to certain compounds, potential matrix interferences that can affect the accuracy of results, as well as the need for further validation in different types of biological samples. Future research should focus on further simplification to improve method efficiency, optimization of extraction parameters to improve reproducibility, and integration of more advanced analytical techniques to improve sensitivity and selectivity.

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The research conceptualization, data and resource collector, data analysis and writing the original draft preparation by S. N. R. The supervision, data curation, validation, and helping in data analysis was supported by S. K. R. E. P.. S. K. R. E. P. helped in review and editing the final draft. A. F. helped in validation, review and editing the draft. M. H. Z. A. helped in data curation, supervision, and review the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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