Pharmaceutical and Biomedical Sciences Journal
Volume 4(1), 2022, 1-12

The Development of Derivative Method analysis 1,4 Benzodiazepines in Biological Matrix using High-Performance Liquid chromatography *(HPLC)* and Liquid Chromatography-Mass Spectrometry

Annisa Larasati Putri, Supandi*, Umar Mansur
Department of Pharmacy, Faculty of Health Sciences, UIN Syarif Hidayatullah Jakarta.

*Corresponding author: supandi@uinjkt.ac.id

Received: February 03, 2022; Accepted: July 07, 2022

Abstract: 1.4 benzodiazepine derivatives are the benzodiazepine class that is used most frequently in clinical as a sedation agent, anxiolytic, and antiepileptic. The high tense of benzodiazepine consumption can cause addiction and misappropriation. That matter causes benzodiazepine and its metabolites often find in toxicology clinical and forensic. This systematic review aimed to identify an efficient sample preparation procedure and an accurate analysis method to determine benzodiazepine existence in various biological matrices. According to the PRISMA flow diagram's chart, twenty-four reviewed articles systematically pointed out that plasma and urine utilization gave good accuracy results and recovery than other biological matrices. Stir bar sorptive extraction (SBSE) method development in the biological matrix using *vinylpyrrolidone ethylene glycol dimethacrylate polymer* gave efficient results with the amount of recovery *of* 96%, and HPLC instrument utilization was still selective and sensitive with LOD until 12ng/mL and LOQ 36 ng/mL.

Abstrak: Derivat 1,4 benzodiazepin merupakan golongan benzodiazepin yang paling sering digunakan dalam klinis sebagai agen sedasi, anxiolitik, dan antiepilepsi. Tingginya konsumsi benzodiazepin dapat menyebabkan kecanduan dan penyalahgunaan. Hal tersebut menyebabkan benzodiazepin dan metabolitnya sering dijumpai dalam kasus toksikologi klinis dan forensik. *Systematic review* ini bertujuan untuk mengidentifikasi prosedur preparasi sampel yang efisien dan metode analisis yang akurat untuk menentukan adanya benzodiazepin dalam berbagai matriks biologi. Berdasarkan alur diagram PRISMA, 24 artikel yang di *review* secara sistematis menunjukan bahwa penggunaan plasma dan urin memberikan hasil akurasi dan *recovery* yang baik dibanding matriks biologi lainnya. Pengembangan metode ekstraksi SBSE dari matriks biologi menggunakan polimer *vinylpyrrolidone ethylene glycol dimethacrylate* memberikan hasil yang efisien dengan *recovery* sebesar 96% dan penggunaan instrumen HPLC masih selektif dan sensitif dengan LOD hingga 12 ng/mL dan LOQ 36 ng/mL

Keywords: alprazolam, analysis method, biological matrix, clonazepam, diazepam, lorazepam, and oxazepam.

DOI: 10.15408/pbsj.v4i1.24629

1. INTRODUCTION

The worldwide prevalence of Anxiety disorders patients in 2012 reaches 3.6% population, with the level of female patients at 4.6% higher than that of males that around 2.6% (Wordl Health Organization, 2017). In Indonesia, based on Basic Health Research's report, the population prevalence of men

tal disorder health in depression and anxiety cases for fifteen years old and over are 9.8% of its total population (Riskesdas, 2018). Anxiety patient is supplied with benzodiazepine as second-line therapy (DiPiro *et al.*, 2020). Benzodiazepine is a drug that works by binding $GABA_A$ receptors at subunits α, β, and γ (Batlle *et al.*, 2019). Generally, the biggest class of benzodiazepine and is frequently used in clinical are 1.4 benzodiazepin derivatives such as alprazolam, diazepam, clonazepam, lorazepam, and oxazepam (Szatkowska *et al.*, 2014). Around 77.2% of patients are prescribed benzodiazepine class medicine as an additional therapy for other mental illnesses and *for* off-label use without prior anxiety diagnosis (Guina & Merrill, 2018). Those drugs' high consumption can cause addiction and misappropriation in criminal act. Benzodiazepine and its metabolites are often found in clinical cases and forensic toxicology (Uddin *et al.*, 2014).

In terms of detecting the body content, there is a need for an accurate analysis method and efficient sample extraction procedure to determine benzodiazepine existence in various biological matrices. The biological matrix used in the analysis is 1.4 benzodiazepine involving plasma, serum, breast milk, urine, and hair (Persona *et al.*, 2015). Quantitative and qualitative method analysis development in biological matrix allows for medicine quality guarantee, helps to optimize chronic dose, monitor 1.4 benzodiazepine derivates concentration in biological liquid during the therapy, identify pharmacokinetics change, verify submission, and detect misappropriation presence (Olędzka *et al.*, 2015).

The extraction procedure choice of 1,4 benzodiazepines suitable in the biological matrix is a critical step in the analysis process because it directly affects the resulting quality (Fernández *et al*., 2016). Several extraction method approaches that are used to extract analyte in the biological matrix have been reported in various literature, such as using liquid-liquid extraction (LLE), Solid Phase Extraction (SPE), Stir bar sorptive extraction (SBSE), dispersive microextraction solid phase (DMSPE), Dispersive liquid-liquid microextraction (DLLME), and so on (Asgharinezhad *et al*., 2014; Olędzka *et al*., 2015; Unceta *et al*., 2010). Nowadays, the most effective analysis method used in 1.4 benzodiazepines analysis in the biological matrix is HPLC and LCMS with detector modifications such as UV-Vis, Diode Array Detector (DAD), and MS. HPLC has still become an accurate analysis method with affordable instrument cost. However, LCMS gives specificity and sensitivity in analyte analysis better because it only requires a smaller amount of injected samples than HPLC. This literature study aimed to study 1,4 benzodiazepines analysis method, a study that contains about the method that can be used to extract analytes from the biological matrix and detect accurate compound content in biological matrix qualitatively and quantitatively using High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometer (LCMS).

2. MATERIAL AND METHODS

2.1 Search strategy

Systematic reviews or meta-analyses in this research tended to be qualitative that used the meta-synthesis approach method. Publication journal search used acadedatabasesbase in Garuda, Science Direct, Pubmed, MDPI, Wiley, and Springerlink during January-February 2021. Keywords used in the journal search were analysis method, biological matrix, alprazolam, diazepam, clonazepam, lorazepam, and oxazepam. The applied journal search was the full-text articles that could be accessed and published from 2015 to 2020.

2.2 Inclusion criteria and exclusion criteria

Inclusion criteria in this systematic review were (1) experimental analysis chemical journal, (2) using English and Indonesian language, (3) Q1-Q3 category and Sinta 1-Sinta 3, (4) single substance analysis biological matrix as well as simultaneous in the biological matrix by using HPLC method or LCMS, and (5) Biological matrices used included blood plasma, blood serum, urine, breast milk, and hair. Journal that was a review article and 1,4 benzodiazepine derivatives compounds only as an internal standard and exclusion criteria.

3. RESULTS AND DISCUSSION

3.1 Plasma

Blood plasma is the most common biological matrix used for the bioanalysis method. Blood plasma utilization as the central matrix in bioanalysis gives benefits drug can be well-detected in its intact conditions or metabolic shape. The extraction method

uses microextraction dispersive liquid-liquid with surfactant help (SA-DDLME) is expanded to extract 1.4 benzodiazepine derivatives in plasma *(Molaei et al*., 2015). This method was based on the droplet dispersion of extraction solvent in an aqueous solution. The things that affected this method's extraction efficiency included type and volume extraction solvent, surfactant type and concentration, pH samples, and added salt concentration. The used solvent type was 1-octanol. 1-octanol high polarity showed better ability and dispersion in the liquid phase.

The optimal volume for extraction was gained in 70 µL 1-octanol for 500 µL plasma. Besides solvent choice, the surfactant type choice that would be used also could affect extraction result quality. Cetyl Trimethyl Ammonium Bromide (CATB) surfactant gave the highest efficiency with 2mmol/L concentration because of droplet formation occurrence that caused mash enhancement into the organic phase. SA-DLLME Extraction method combined with HPLC-UV gave relevant and validated results indicating 84-96% recovery, high PF and ER%, wide dynamic linear range, low LOD, and appropriate RSD. Although it gave a relevant result, there were some lacks in DLLME method development related to the applied solvent. Dispersive solvent application, usually its analyte partition coefficient into extraction solvent experienced degradation and frequently chlorinated and had the poisonous potential for human and enthe environment.

The development of extraction method 1.4 benzodiazepine derivatives in plasma using stir bar sorptive extraction by covering vinylpyrrolidone-ethylene glycol dimethacrylate polymer was reported in Torabizadeh *et al*., (2016) research. This method had an absorbed extraction principle, in which solute was extracted into a polymer layer in a magnetic stirrer. Extraction is controlled by solute partition coefficient among polymer layer and sample matrix and phase ratio among polymer layer and sample volume (David & Sandra, 2007). Vinylpyrrolidone-ethylene glycol dimethacrylate polymer choice as a stir bar coating because it gives better polar-characteristic in the layer, allowing the extracted compound to lower $K_{o/w}$ (Torabizadeh *et al*., 2016).

There was polarity enhancement of the stir bar layer to extract simultaneous diazepam and its active metabolite in the plasma sample. The stir bar that would be used was prepared in combination with ultrasound-assisted desorption of fluids. Generally, this method utilized agitation mode for the desorption step by stirring in a certain amount of solvent for a while. The extraction process optimum condition was obtained by using 250 µl methanol at a temperature of 55^{o}C for ten minutes. The extraction process happened fastly because ultrasonic agitation implementation contributed to the fast desorption step. Moreover, using sonication at a high temperature had the potential to damage the polymer layer in the stir bar. In the optimal condition, analytical performance from the stir bar sorptive extraction method combined with HPLC-UV showed 96% recovery, excellent linearity for diazepam (36-1200 ng/mL), with a coefficient correlation of 0.9986, a detection limit of 12 ng/mL, and quantification limit 36 ng/mL (Torabizadeh *et al*., 2016). The results are shown in the Table 1.

Table 1. Bioanalytical Parameters of Derivatives 1,4 Benzodiazepine in Plasma.

Analite	Analysis Methode	Linearity	Accuracy (%)		Precision (%)		LOD	LOQ	*Recovery* (%)	Matrix Effect (%)	Referensi
			Intraday	Interday	Intraday	Interday					
Lorazepam	SA-DLLME, HPLC-UV	0.994	-	-	-	-	2.1 µg/L	10.0 µg/L	90.5	-	Molaei *et al.*, (2015)
Diazepam	Protein presipitation, LCMS-ESI	0.992	11.5	10.2	2.9	2.7	1.0- 100 ng/mL	1.0 ng/ml	102 ±1.2	112 ± 1.3	Gong *et al*., (2015)
Clonazepam and Diazepam	Protein presipitation, LCMS-ESI	0.9993 and 0.9972	10.7 and -11.5	11.8, and -10.3	2.3	3.2 and 3.0	-	0.313-0.630 ng/ml	-	4.7, and 3.9	Domingues *et al.*, (2015)
Alprazolam, oxazepam, and diazepam	Sistem SPE-DLLME, HPLC-UV	0.995, 0.992, and 0997	-	-	6.1, 7.3, and 4.6	-	0.4-0.7 µg/L	1.5-2.5 µg/L	86,.84, and 90	-	Mashayekhi & Khalilian, (2016)
Diazepam	SBSE using *vinylpyrrolidone ethylene glycol dimethacrylate* polimer, HPLC-UV	0.9986	-	-	2	3	12 ng/mL	36 ng/mL	96	-	Torabizadeh *et al* (2016)
Oxazepam and lorazepam	HF-LPME, LCMS-ESI	0.9991 and 0.9988	-	-	4.2 and 4.5	4.6 and 4.8	0.5- 0.7 µg/L	1.2-1.5 µg/L	-	-	Nazaripour *et al* (2016)
Alprazolam, clonazepam, and oxazepam.	Protein presipitation, LCMS-ESI	1.0	91.4, 85.3, and 92.9	89.9, 110, and 83.8	10.4, 10.4, and 13.1	10.3, 12.9, and 9.2	0.9-1.9 ng/mL	7.8 ng/mL	79.1-119	109	Arora *et al.*, (2016)
Alprazolam and clonazepam	Automatic SPE LCMS-ESI	0.9982 and 0.9994	-	-	1.12-4.54	-	0.573-0.688 µg/L	2.29-2.75 µg/L	75.9-122	76-93	Zhong *et al.*, (2016)
Lorazepam and Clonazepam	DLLME-EME, HPLC-UV	0.98	-	-	5.6 and 5.8	6.4 and 6.9	7.0-15.0 ng/mL	25.0 and 450 ng/mL	38 and 49	-	Hemmati *et al.*, (2017)
Diazepam	LLE, LCMS-ESI	0.99	101.4	101.4	5.4	7.2	-	0.5 ng/mL	81.9	93	Kim *et al* (2017)
Alprazolam, Clonazepam and Lorazepam	LLE, LCMS-ESI	0.993	-19.2 – 16.6	-14.3 – 16.2	2.2- 12.1	1.8- 14.4	-	0.5– 1.0 ng/mL	79.2-86 ± 5.1	-5.4, -22.0, and -18.2	Furugen *et al.*, (2019)(Furugen et al., 2019)

3.2 Serum

Serum utilization as a biological matrix is less liked because serum utilization as a biological matrix gives less analyte sample volume than plasma utilization (Sherwood, 2016). In 2016, Samanidou *et al*. developed a fabric phase sorptive extraction (FPSE) method to analyze benzodiazepine in serum using HPLC. FPSE is an extraction technic with a flexible fiber substrate and a high-efficiency sorbent layer lowered from sol-gel, which is mobilization in substrate surface (Kabir & Furton, 2019). FPSE media layered with PEG sol-gel triggered an interaction between hydrogen bonding with analyte if the media contains hydrogen bonding donor and acceptor. The higher the total amount of hydrogen bonding donor and acceptor in an analyte, the higher possibility of its analyte sorbent interaction, resulting in a higher extraction efficiency (Samanidou *et al*., 2016).

The stuck porosity in sol-gel sorbent and cellulose fiber substrate characteristic permeability resulted in fast drug extraction from blood serum and reached extraction balance in a short time. This FPSE extraction method gave a recovery of 40.12-62.8% analyte from the serum matrix. That result pointed out that the method is less efficient in extracting 1.4 benzodiazepines in blood serum than the protein precipitation method, which gives a recovery of 98.05-101.01% (Ali *et al*., 2020) (Table 2). However, this method is concluded can be used for routine analysis of therapeutic drugs or forensic purposes.

Extraction of 1.4 benzodiazepine derivates in serum with protein precipitation reported by S. N. Ali *et al*., (2020) gave a more efficient result with the recovery amount 98.05-101.01% with a detection limit of 19.76 ng/mL and LOQ of 0.0598 µg/mL. Extraction was conducted by using 9 mL centrifuged acetonitrile in ten minutes at 10000 rpm speed. That method could extract the target analyte from serum well without significant disturbance from serum endogen component. That protein precipitation extraction method can be concluded that the developed method is sensitive and specific with a high-reliability index.

3.3 Urine

Urine is one of the biological matrices that becomes a choice for screening and identifying a drug that has not known its concentration and metabolite found higher in the urine than in plasma or serum (Namera *et al*., 2007). For pulling target analyte in urine, there was a need for a proper extraction method to obtain high purification. The SPE-DLLME extraction method was extended by Mashayekhi & Khalilian, (2016) to see reliability and implementation in analyzing benzodiazepine in urine. SPE-DLLME is a combination technic that benefits SPE and DLLME excellence methods. These include their simplicity, low solvent utilization, cost, low extraction time, and high recovery in a complex matrix. In their research, Mashayekhi & Khalilian, (2016) compared SPE, DLLME, and SPE-DLLME methods. The SPE-DLLME method had more excellence than other methods, such as urine samples extracted using SPE-DLLME. It only needed dilution became 1:1 using deionization water to decrease the matrix effect, so it did not need dilution too often. Sorbent C18 was selected to bind the analyte in the SPE process and used 1.5 mL methanol to elute the analyte. In the DLLME process, dichloromethane solvent was chosen as an optimum extraction solvent because it gave the highest recovery compared with other used solvents. Extraction method development combined with SPE-DLLME to analyze benzodiazepines (Oxazepam, Alprazolam, and Diazepam) in a low amount proven by LOD of 0.07-0.3 µg/L, LOQ of 0.7– 1.0 µg/L, and recovery of 91-95.5%.

Table 2. Bioanalytical Parameters of Derivatives 1,4 Benzodiazepine in Serum

Analyte	Analysis Method	Linearity	Accuracy		Precision		LOD	LOQ	*Recovery* (%)	Matrix effect (%)	References
			Intraday	Interday	Intraday	Interday					
Alprazolam, diazepam, and lorazepam.	FSPE, HPLC-DAD	0.99	92.5	107.0	11.9	13.1	0.01 ng/µL	0.03 ng/µL	40.12, 41.8, and 62.8	-	Samanidou *et al* (2016)
Alprazolam	Protein precipitation, HPLC-UV	0.9984	-	-	0.818-1.383	-	19.76 ng/ml	0.0598 µg/ml	98.05-101.01	-	S. N. Ali *et al* (2020)

Table 3. Bioanalytical Parameters of Derivatives 1,4 Benzodiazepine in Urine

Analyte	Analysis Method	Linearity	Accuracy		Precision		LOD	LOQ	*Recovery* (%)	Matrix effect (%)	References
			Intraday	Interday	Intraday	Interday					
Lorazepam	SA-DLLME, HPLC-UV.	0.995	-	-	11.3	-	1.5 µg/mL	10.0 µg/mL	87.2	-	Molaei *et al* .(2015)
Alprazolam, clonazepam, and diazepam.	SPE, LCMS-ESI	0.99	-	-	15	-	0.01-0.5 ng/mL	-	107, 83, and 91	92, 80, and 100	Liang *et al.* (2015)
Alprazolam, oxazepam, and diazepam.	Sistem SPE-DLLME, HPLC-UV	0.998, 0.996, and 0.999	-	-	3.2-6.2	-	0.07-0.3 µg/L	0.7– 1.0 µg/L	92.5, 91, and 95.5	-	Mashayekhi & Khalilian, (2016)
Oxazepam and lorazepam.	HF-LLME, LCMS-ESI	0.9994 and 0.9991	-	-	4.1 and 4.3	4.3 and 4.6	0.3- 0.5 µg/L	0.6-0.9 µg/L	88.0-102.0	-	Nazaripour *et al.* (2016)
Lorazepam and Clonazepam	DLLME-EME, HPLC-UV.	0.98-0.99	-	-	5.0 and 5.4	5.8 and 6.5	5.0- 12.0 ng/mL	18.0 and 40.0 ng/mL	39.0 and 52.0	-	Hemmati *et al.* (2017)
Diazepam, Oxazepam, and Alprazolam.	Protein precipitation, LCMS-APCI	0.98	95.65, 98.80, and 100.37	-	4.95-5.32	5.39, 3.28, and 7.78	20-500 ng/mL	20 ng/mL	20	-	Dunlop *et al.* (2017)
Alprazolam, Clonazepam, Diazepam, Lorazepam, and Oxazepam.	Protein precipitation, LCMS-ESI	-	-	-	7.0, 9.0, 6.0, and 8.0	-	5.0-10.0 ng/mL	-	125, 72, 100.0, 128.0, and 174.0	-	Kahl *et al.* (2019)
Diazepam	SPE, HPLC-UV	0.9934	-	-	-	5.7	0.15 µg/L	0.54 µg/L	-	83.6– 92.9	Ghani *et al.* (2020)
Oxazepam, Lorazepam, Clonazepam, Alprazolam, and Diazepam.	SPE, LCMS-ESI	0.99	-	-	-	-	2.5-1.0 µg/mL	5-20 µg/L	-	25	Sofalvi *et al.* (2020)
Diazepam and Oxazepam	SLE, LCMS-ESI	0.99	-	-	-	0.1 – 0.3	1.0-4.0 ng/mL	-	33 and 35	-61 and -27	Wong *et al* .(2020)

Follow-fiber liquid-phase microextraction method automatically developed to extract lorazepam and oxazepam in urine gives a recovery of 88.0-102.0%, a detection limit of 0.2-0.3 μg/mL (Nazaripour *et al*., 2016). The primary principle in this HF-LPME method was based on analyte separation in the sample (donor) that diffused in the hollow fiber with the prior capillary style that has already been optimized with immiscible organic solvent (acceptor). Then, it is reextracted to the organic acceptor phase with a concentration gradient between two organic solvents (Gjelstad & Pedersen-Bjergaard, 2013). Three-phase HF-LPME based on two immiscible organic solvents gave better extraction efficiency than two phases in optimal conditions. Three-phase HF-LPME is suitable for acids or bases to be analyzed by HPLC.

There were some parameters that affected the extraction efficiency of three-phase HF-LPME based on two immiscible organic solvents such as acceptor solvent selection, salt addition, hollow fiber long, stirring speed, and extraction time. The acceptor solvent that was used in extraction was acetonitrile because it provided high recovery. Acetonitrile as an acceptor solvent had low solubility in n-dodecane and effectively in fiber lumen during the extraction phase without membrane leakage and solvent loss because of evaporation. In HF-LLLME, n-dodecane is usually used as SLM (supported liquid membrane) based on two immiscible organic solvents. For getting a high preconcentration compound, it is usually added with trioctylphosphine oxide (TOPO) compound that is efficient to transfer polar organic analytes with low pKa and $K_{O/W}$ because of hydrogen bonding formation between TOPO and analyte (Nazaripour *et al.*, 2016).

The effect of NaCl concentration escalation was because it could decrease analyte solubility in an aqueous solution as a result, it increased their partition into the organic phase. Sodium chloride optimum concentration that gave high efficiency was 7.5% b/v. Hollow fiber length was 7 cm which was selected because it gave a high relative top wide. In HF-LLLME, sample centrifugation had an essential role in upgrading extraction efficiency. Extraction could be accelerated by stirring the sample because of Nernst diffusion layer thickness reduction and continuous exposure from extraction surface to sample. An efficient extraction process is shown in centrifugation speed with a total of 100 rpm in 30 minutes, proven by good recovery (88.0-102.0%) (Nazaripour *et al*., 2016). The results are shown in the Table 3.

Furugen *et al*., (2019) developed a method to detect and benzodiazepine quantification in breast milk using the LLE extraction method and LC-MS-MS instrument. The breast milk sample needed sample preparation by adding 10 μl IS and 100 μl buffer borat (pH 9, 0.1M) before extracting it in the LLE method. LLE method using ethyl acetate 1500 μl that was vortexed for 10 minutes and centrifuged at 3900 rpm for 15 minutes in temperature 4 ^{o}C gave recovery of 57.5-83.8%. LLOQ and breast milk around 0.25 until 0.5 ng/mL with accuracy and precision fulfill the requirement and can be accepted. This method succeeds in detecting alprazolam content in breast milk in breastfeeding mothers who are routine to get alprazolam therapy.

The extraction method developed by Furugen *et al*., (2019) has excellence in that the sample needed is smaller, that is 100 μl and gives good sensitivity. The prior reported method requires a bigger sample (500 μl) and uses the SPE extraction method with the same extraction result quality as this method (Marchei *et al*., 2011). The excellence provided, like a small amount of sample and fast extraction time, makes this method have the potential to be applied to evaluate the protein binding of 1,4 benzodiazepine derivatives in breast milk.

3.5 Hair

Hair is one of the biological matrices that is used in forensic analysis. Hair utilization in the analysis is beneficial compared to plasma and urine utilization in which its broad detection index to analyze drugs and their use as a retrospective evaluation of drug use history (Gambelunghe *et al*., 2017; Pragst *et al*., 2019). Complexity in hair matrix needed further cleaning process so that there was no interference with the sample extract during the analysis. Generally, the sample extraction method in the efficient hair matrix uses the SPE method (Esmaeili-Shahri & Es'haghi, 2015; Licata *et al.,* 2016). Shin *et al*., (2019) promoted the analysis method of seventy-five potentially abused drugs, including benzodiazepine, simultaneously in hair with LCMS. The dispersive-SPE method that was used gave a recovery of 96.3-102.2% with a detection limit of 2.01-10.0 pg/mg. Hair samples originated from 11 drug abuse suspects, including alprazolam (39.4 pg/mg), diazepam (2.0 pg/mg), and clonazepam (131.2 pg/mg), and LOQ 2.0 pg/mg successfully identified by using the method. The results are shown in the Table 4.

A method that was expanded by Shin *et al.*, (2019) had excellence because it only required a smaller sample (10 mg) to be extracted. However, it gave good sensitivity, selectivity, reproductivity, and stability that can be accepted than Licata *et al*., (2016) research that required a 50 mg sample. Methanol was selected as a solvent because it gave high extraction efficiency and hair sample ultrasonication for one hour at 50 °C temperature that was not affected 75 analytes stability. This method can be applied for fast and accurate screening to detect drug abuse in forensic and clinical toxicology with a small required sample.

4. CONCLUSION

According to the conducted review regarding 1.4 benzodiazepine derivatives method development in the biological matrix using High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometer (LCMS), in conclusion, the biological matrix that is primarily used in bioanalysis 1.4 benzodiazepine derivatives were blood plasma and urine. Utilization of Blood plasma and urine provides better accuracy and recovery than other biological matrixes, 80-102%. The efficient extraction method in term of extraction time, solvent needs, and a result that was given for extracting 1.4 benzodiazepines in the biological matrix was SBSE using *vinylpyrrolidone ethylene glycol dimethacrylate* polymer that was indicated by recovery acquisition reached 96%. Each analysis instrument has its benefits to identify accurate and sensitive target compounds. 1.4 benzodiazepine derivatives analysis in the biological matrix using HPLC instrument still gives efficient result, selective, and sensitive with LOD 12 ng/mL and LOQ 36 ng/mL.

3.4 Breast Milk

Table 4. Bioanalytical Parameters of Derivatives 1,4 Benzodiazepine in Hair

Analite	Analysis Method	Linearity	Accuracy		Precision		LOD	LOQ	Recovery (%)	Matrix effect (%)	Referensi
			Intraday	Interday	Intraday	Interday					
Diazepam, Oxazepam, Clonazepam, and Alprazolam.	SPE with CTAB, HPLC-UV/DAD	0.9938-0.9990	-	-	7.07, 4.27, 5.88, and 6.88	-	0.0097-0.032 mg/L	-	84.90-90.50	-	Esmaeili-Shahri & Es'haghi, (2015)
Alprazolam, Clonazepam, Diazepam, and Lorazepam.	dSPE, LCMS-ESI	0.990	9.6, 8.0, 3.8, and 13.0	-	9.4; 8.1, 9.6, and 9.9	11.4, 9.2, 10.3, and 13.7	5.0-10.0 pg/mg	10.0-20.0 pg/mg	93.0, 90.4, 84.4, and 40.6	-23.3, -30.2, -15.2, and -35.0	Licata *et al* (2016)
Alprazolam, Diazepam, Lorazepam, and Oxazepam.	Digesti with buffer acid VMA- T M3, LCMS-ESI	0.99	12.5, 8.2, 1.7, and 10.7	13.3, 7.0, 2.5, and 8.6	14.8, 13.3, 5.7, and 85	4.8, 7.2, 3.7, and 10.1	0.03-0.02 ng/mg	0.06-0.1 ng/mg	76, 82.8, 72.4, and 71.4	86.2, 97.9, 95.6, and 85.3	Pichini *et al* (2016)
Diazepam and Oxazepam	Protein presipitation, UHPLC-ESI-MS.	-	84-107	-	13.0–23.1	-	0.5- 250 pg/mg	0.5-2.5 pg/mg	-	-	(Wang *et al.*, 2017)
Alprazolam, Clonazepam, Diazepam, Lorazepam and Oxazepam	Q-sep dispersive solid-phase extraction, LCMS-ESI	0.9951	100.4, 102.7, 95.7, 98.2, and 104.8	100.9, 102.9, 98.9, 100.2. and 102.4	6.4, 9.9, 6.0, 6.6, and 5.7	10.9, 12.1, 6.8, 11.5, and 9.8	2.0-10.0 pg/mg	2.0 pg/mg	100.0, 96.3, 97.8, 101.4. and 102.2	8.8, 14.6, 10.7, 7.7, and 7.8	Shin *et al* (2019)

5. REFERENCES

Ali, S. N. *et al.* (2020) 'Liquid chromatographic method for simultaneous determination of alprazolam with NSAIDs in bulk drug, pharmaceutical formulation and human serum', *Pakistan Journal of Pharmaceutical Sciences*, 33(1), pp. 121–127. doi: 10.36721/PJPS.2020.33.1.REG.121-127.1.

Arora, B. *et al.* (2016) 'Development and validation of an ESI-LC-MS/MS method for simultaneous identification and quantification of 24 analytes of forensic relevance in vitreous humour, whole blood and plasma', *Drug Testing and Analysis*, 8(1), pp. 87–98. doi: 10.1002/dta.1797.

Asgharinezhad, A. A. *et al.* (2014) 'Dispersive micro-solid-phase extraction of benzodiazepines from biological fluids based on polyaniline/magnetic nanoparticles composite', *Analytica Chimica Acta*, 844, pp. 80–89. doi: 10.1016/j.aca.2014.06.007.

Batlle, E. *et al.* (2019) '1,4-Benzodiazepines and New Derivatives: Description, Analysis, and Organic Synthesis', *Medicinal Chemistry*. doi: 10.5772/intechopen.79879.

David, F. and Sandra, P. (2007) 'Stir bar sorptive extraction for trace analysis', *Journal of Chromatography A*, 1152(1–2), pp. 54–69. doi: 10.1016/j.chroma.2007.01.032.

Domingues, D. S., Souza, I. D. de and Queiroz, M. E. C. (2015) 'Analysis of drugs in plasma samples from schizophrenic patients by column-switching liquid chromatography-tandem mass spectrometry with organic-inorganic hybrid cyanopropyl monolithic column', *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 993–994, pp. 26–35. doi: 10.1016/j.jchromb.2015.04.040.

Dunlop, S. *et al.* (2017) 'An atmospheric pressure chemical ionisation liquid chromatographic–tandem mass spectrometry method for the analysis of benzodiazepines in urine', *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1064(August), pp. 22–27. doi: 10.1016/j.jchromb.2017.08.023.

Esmaeili-Shahri, E. and Es'haghi, Z. (2015) 'Superparamagnetic Fe3O4@SiO2 core-shell composite nanoparticles for the mixed hemimicelle solid-phase extraction of benzodiazepines from hair and wastewater samples before high-performance liquid chromatography analysis', *Journal of Separation Science*, 38(23), pp. 4095–4104. doi: 10.1002/jssc.201500743.

Fernández, P. *et al.* (2016) 'Optimization of ultrasound assisted dispersive liquid-liquid microextraction of six antidepressants in human plasma using experimental design', *Journal of Pharmaceutical and Biomedical Analysis*, 124, pp. 189–197. doi: 10.1016/j.jpba.2016.02.041.

Furugen, A. *et al.* (2019) 'Quantification of eight benzodiazepines in human breastmilk and plasma by liquid-liquid extraction and liquid-chromatography tandem mass spectrometry: Application to evaluation of alprazolam transfer into breastmilk', *Journal of Pharmaceutical and Biomedical Analysis*, 168, pp. 83–93. doi: 10.1016/j.jpba.2019.02.011.

Gambelunghe, C. *et al.* (2017) 'Norcocaine and cocaethylene distribution patterns in hair samples from light, moderate, and heavy cocaine users', *Drug Testing and Analysis*, 9(2), pp. 161–167. doi: 10.1002/dta.1903.

Ghani, M., Zayeri, Z. and Maleki, B. (2020) 'Glutathione-stabilized Fe3O4 nanoparticles as the sorbent for magnetic solid-phase extraction of diazepam and sertraline from urine samples through quantitation via high-performance liquid chromatography', *Journal of Separation Science*, pp. 1–20. doi: 10.1002/jssc.202000938.

Gjelstad, A. and Pedersen-Bjergaard, S. (2013) 'Perspective: Hollow fibre liquid-phase microextraction - principles, performance, applicability, and future directions', *Scientia Chromatographica*, 5(3), pp. 181–189. doi: 10.4322/sc.2014.003.

Gong, W. *et al.* (2015) 'Simultaneous quantification of diazepam and dexamethasone in plasma by high-performance liquid chromatography with tandem mass spectrometry and its application to a pharmacokinetic comparison between normoxic and hypoxic rats', *Molecules*, 20(4), pp. 6901–6912. doi: 10.3390/molecules20046901.

Guina, J. and Merrill, B. (2018) 'Benzodiazepines I: Upping the Care on Downers: The Evidence of Risks, Benefits and Alternatives', *Journal of Clinical Medicine*, 7(2), p. 17. doi: 10.3390/jcm7020017.

Hemmati, M., Rajabi, M. and Asghari, A. (2017) *A twin purification/enrichment procedure based on two versatile solid/liquid extracting agents for efficient uptake of ultra-trace levels of lorazepam and clonazepam from complex bio-matrices*, *Journal of Chromatography A*. Elsevier B.V. doi: 10.1016/j.chroma.2017.09.045.

Kabir, A. and Furton, K. G. (2019) *Fabric phase sorptive extraction: A new genration, green sample preparation approach*, *Solid-Phase Extraction*. Elsevier Inc. doi: 10.1016/B978-0-12-816906-3.00013-3.

Kahl, K. W., Seither, J. Z. and Reidy, L. J. (2019) 'LC-MS-MS vs ELISA: Validation of a Comprehensive Urine Toxicology Screen by LC-MS-MS and a Comparison of 100 Forensic Specimens', *Journal of analytical toxicology*, 43(9), pp. 734–745. doi: 10.1093/jat/bkz066.

Kim, D. H. *et al.* (2017) 'Development of a simple and sensitive HPLC-MS/MS method for determination of diazepam in human plasma and its application to a bioequivalence study', *Translational and Clinical Pharmacology*, 25(4), pp. 173–178. doi: 10.12793/tcp.2017.25.4.173.

Liang, C. *et al.* (2015) 'Identification and quantification of 34 drugs and toxic compounds in blood, urine, and gastric content using liquid chromatography with tandem mass spectrometry', *Journal of Separation Science*, 38(10), pp. 1680–1690. doi: 10.1002/jssc.201401300.

Licata, M. *et al.* (2016) 'Hair testing in clinical setting: Simultaneous determination of 50 psychoactive drugs and metabolites in headache patients by LC tandem MS', *Journal of Pharmaceutical and Biomedical Analysis*, 126, pp. 14–25. doi: 10.1016/j.jpba.2016.04.015.

Marchei, E. *et al.* (2011) 'Simultaneous analysis of frequently used licit and illicit psychoactive drugs in breast milk by liquid chromatography tandem mass spectrometry', *Journal of Pharmaceutical and Biomedical Analysis*, 55(2), pp. 309–316. doi: 10.1016/j.jpba.2011.01.028.

Mashayekhi, H. A. and Khalilian, F. (2016) 'Development of Solid-Phase Extraction Coupled with Dispersive Liquid-Liquid Microextraction Method for the Simultaneous Determination of Three Benzodiazepines in Human Urine and Plasma', *Journal of Chromatographic Science*, 54(6), pp. 1068–1073. doi: 10.1093/chromsci/bmw031.

Molaei, K. *et al.* (2015) 'Surfactant-assisted dispersive liquid-liquid microextraction of nitrazepam and lorazepam from plasma and urine samples followed by high-performance liquid chromatography with UV analysis', *Journal of Separation Science*, 38(22), pp. 3905–3913. doi: 10.1002/jssc.201500586.

Namera, A., Yashiki, M. and Kojima, T. (2007) 'Analysis of drugs in biological fluids using SPME', pp. 510–526. doi: 10.1039/9781847550149-00510.

Nazaripour, A. *et al.* (2016) 'Automated hollow-fiber liquid-phase microextraction followed by liquid chromatography with mass spectrometry for the determination of benzodiazepine drugs in biological samples', *Journal of Separation Science*, 39(13), pp. 2595–2603. doi: 10.1002/jssc.201600015.

Olędzka, I. *et al.* (2015) 'Simultaneous separation of eight benzodiazepines in human urine using field-amplified sample stacking micellar electrokinetic chromatography', *Journal of Analytical Toxicology*, 39(6), pp. 436–443. doi: 10.1093/jat/bkv042.

Persona, K. *et al.* (2015) 'Analytical methodologies for the determination of benzodiazepines in biological samples', *Journal of Pharmaceutical and Biomedical Analysis*, 113, pp. 239–264. doi: 10.1016/j.jpba.2015.02.017.

Pichini, S. *et al.* (2016) 'Ultra-high-pressure liquid chromatography tandem mass spectrometry determination of antidepressant and anxiolytic drugs in neonatal meconium and maternal hair', *Journal of Pharmaceutical and Biomedical Analysis*, 118, pp. 9–16. doi: 10.1016/j.jpba.2015.10.016.

Pragst, F. *et al.* (2019) 'Hair analysis of more than 140 families with drug consuming parents. Comparison between hair results from adults and their children', *Forensic Science International*, 297, pp. 161–170. doi: 10.1016/j.forsciint.2019.01.039.

Riskesdas, K. (2018) 'Hasil Utama Riset Kesehata Dasar (RISKESDAS)', *Journal of Physics A: Mathematical and Theoretical*, 44(8), pp. 1–200. doi: 10.1088/1751-8113/44/8/085201.

Samanidou, V. *et al.* (2016) 'Simplifying sample preparation using fabric phase sorptive extraction technique for the determination of benzodiazepines in blood serum by high-performance liquid chromatography', *Biomedical Chromatography*, 30(6), pp. 829–836. doi: 10.1002/bmc.3615.

Sandmire, H. F., Austin, S. D. and Bechtel, R. C. (2017) *Depression and Other Common Mental Disorder, WHO*.

Sherwood, L. (2016) 'Human physiology from cells to systems Ninth Edition', *Appetite*.

Shin, Y. *et al.* (2019) 'Simultaneous determination of 75 abuse drugs including amphetamines, benzodiazepines, cocaine, opioids, piperazines, zolpidem and metabolites in human hair samples using liquid chromatography–tandem mass spectrometry', *Biomedical Chromatography*, 33(9). doi: 10.1002/bmc.4600.

Sofalvi, S. *et al.* (2020) 'Development and validation of an LC–MS-MS method for the detection of 40 benzodiazepines and three Z-drugs in blood and urine by solid-phase extraction', *Journal of Analytical Toxicology*, 44(7), pp. 708–717. doi: 10.1093/jat/bkaa072.

Szatkowska, P. *et al.* (2014) 'Analytical methods for determination of benzodiazepines. A short review', *Central European Journal of Chemistry*, 12(10), pp.

994–1007. doi: 10.2478/s11532-014-0551-1.

Torabizadeh, M. *et al.* (2016) 'Preparation of a novel sorptive stir bar based on vinylpyrrolidone-ethylene glycol dimethacrylate monolithic polymer for the simultaneous extraction of diazepam and nordazepam from human plasma', *Journal of Separation Science*, 39(7), pp. 1316–1325. doi: 10.1002/jssc.201501273.

Uddin, M. Nasir, Victoria F, Samanidou and Ioannis N Papadoyannis (2014) 'An Overview on Total Analytical Methods for the Detection of 1,4-Benzodiazepines', *Pharmaceutica Analytica Acta*, 05(06), pp. 1–13. doi: 10.4172/2153-2435.1000303.

Unceta, N. *et al.* (2010) 'Development of a stir bar sorptive extraction based HPLC-FLD method for the quantification of serotonin reuptake inhibitors in plasma, urine and brain tissue samples', *Journal of Pharmaceutical and Biomedical Analysis*, 51(1), pp. 178–185. doi: 10.1016/j.jpba.2009.07.015.

Wang, X. *et al.* (2017) 'Deposition of diazepam and its metabolites in hair following a single dose of diazepam', *International Journal of Legal Medicine*, 131(1), pp. 131–141. doi: 10.1007/s00414-016-1429-x.

Wong, J. K. Y. *et al.* (2020) 'A high-throughput and broad-spectrum screening method for analysing over 120 drugs in horse urine using liquid chromatography–high-resolution mass spectrometry', *Drug Testing and Analysis*, 12(7), pp. 900–917. doi: 10.1002/dta.2799.

Zhong, Q. *et al.* (2016) 'Automatic on-line solid-phase extraction with ultra-high performance liquid chromatography and tandem mass spectrometry for the determination of ten antipsychotics in human plasma', *Journal of Separation Science*, 39(11), pp. 2129–2137. doi: 10.1002/jssc.201600129.