Original Article

International Journal of Forensic Science
Volume 4 Number 1, January–June 2021

Green Extraction Method for Oxazepam Drug from Complex Matrices

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How to cite this article:
Shivpoojan Kori/Green Extraction Method for Oxazepam Drug from Complex Matrices/International Journal of Forensic Science. 2021;4(1):15–19.

Abstract

A number of benzodiazepine drugs viz. Diazepam, Flunitrazepam, Lorazepam, and Oxazepam, etc. have been used in the commission of such drugs facilitated sexual assault crimes and date rape case. Literature assessment reveals that only a few analytical methods are reported for the estimation of OXZ. Oxazepam drug was effectively extracted using micellar solution of Brij-58 (3mM) / Ethanol (1:1) solution. Limit of detection (LOD) and Limit of quantification (LOQ) are 0.6 ng/mL and 2.26 ng/mL of UV-Vis Spectrophotometric technique respectively. The linearity range (5-25ng/mL) for Oxazepam was obtained best with Coefficient of determination (R2=0.9998) and its wavelength of detection was 285 nm. A novel, safe and sensitive spectrophotometric method for estimation of Oxazepam drug in complex matrices viz. human blood, milk and urine have been developed and validated according to ICH guideline. This method can be scaled up in forensic science and toxicological laboratories.

Key words: Extraction; Oxazepam; surfactant; Complex matrices; Spectrophotometric technique.

Introduction

The illegal exploit of drugs has grown to be one of the most severe social problems. Clearly, there is an urgent need to expand analytical techniques that would identify such drugs in order to avoid the increasing incident of drug abuse. In the earlier period few decades, research articles have been coming up on drugs related to date-rape drugs, drug-facilitated sexual assault and drug-facilitated crimes (1–3). A number of benzodiazepine and non-benzodiazepine drugs viz. Diazepam, Flunitrazepam, Lorazepam, Ketamine, Oxazepam, Eszopiclone and Zolpiclone etc. have been used in the commission of such terrible and anti-social crimes. These drugs fall under the class of depressant and have an unfavorable psychological and physiological effect ultimately resulting in drowsiness, amnesia, confusion, and impaired memory. Oxazepam (OXZ) is a medicinal compound in the major current pharmacopoeia and extensively used as anxiolytic, being regarded as the prototype for the 3-OH-1, 4-BZD derivate. Oxazepam (Fig. 1) is also the pharmacologically active metabolite of many 1, 4-BZD derivatives, and metabolized to the inactive glucuronide. Literature assessment reveals that only a few analytical methods are reported for the estimation of OXZ.[1]

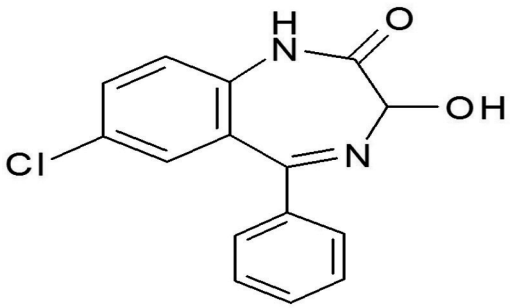

Fig. 1: Molecular structure of Oxazepam.

There have been reports pertaining to the

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extraction and there after detection of drugs and their metabolites viz. zolpidem, trizolam, cocaine, opiates and alkaloids of cannabis from urine and blood.[4] In context to forensic domain, the 'surfactant novel chemistry' can be applied, where in extraction of various polar and non polar components can be facilitated using surfactant aggregates. The utilization of surfactants in low consumption natural organic solvent based sample preparation methods, (for example, surfactant as an emulsifier, surfactant rich phase as an extraction medium, ion pair based extraction, hemimicelle/ admicelle extraction, solid phase microextraction with micellar desorption)[5] is a promising approach to limit the issues emerging from preliminary steps, which are the sensitive stages in analytical estimations. In this study efforts were made to develop a simple and economic UV-Visible Spectrophotometric method for the estimation of OXZ in complex matrices.

Methods

Chemicals and Instruments

The surfactants (Brij-58, Brij-35 and SDS) and other chemical reagents (ethanol, acetonitrile, HCl, NaOH, buffers, methanol, ethanol, acetone, acetonitrile; chloroform and isopropanol) used in the study are described previously. ANXOZAP 10 (10 mg Oxazepam IP) were procured from Piramal healthcare Ltd. Baddi, Himachal Pradesh (India). Purity of OXZ has been checked by FT-IR.

Instruments: Double beam Uv-visible spectrophotometer: The Uv-vis spectra were recorded with Shimadzu UV-VIS 2550 spectrophotometer having range from 1100-200 nm. Two stoppered rectangular quartz cells (sample and reference) with 1 cm path length were placed in cuvette holder maintained at room temperature.

FT-IR, (the Agilent Cary 630 FT-IR Spectrometer) with Agilent Microlab FT-IR software was used, Sartorius BSA-224S-CW electronic balance (S/N: 27390945).

Standard stock solution

Standard stock solution of OXZ, 1000 µg/mL was prepared in ethanol (99.9%) and stored in a refrigerator at 4°C. one tablets of OXZ were weighed and finely powdered. An accurately weighed portion of the powder (89 mg), equivalent to 10 mg of OXZ, was dissolved in 10 mL of ethanol and ultrasonicated for 5 minutes respectively and filtered. The working solutions were prepared by diluting the stock solution prior to use.

Sample preparation and Extraction

Commercial Milk samples used in the study were procured from a supermarket (India), and drug-free human Blood, human Urine was obtained from a healthy volunteer and kept at –20°C until further analysis. The extraction procedure of OXZ used here was a modification of the technique reported.[6,7] A known concentration (spiked concentrations i.e. 5.0 ng/mL, 10 ng/mL, 25 ng/ mL, 50 ng/mL, 100 ng/mL) of the OXZ drug was added in various complex matrices, namely Blood, Milk and Urine. The spiked samples (1.0 mL) were allowed to stand overnight, so that an even and homogeneous distribution of the drug can take place. The samples were then extracted by adding 1.0 mL of conventional solvents (ethanol, methanol, acetone, hexane, isopropanol) and surfactant-based formulations (aqueous Brij-35 (3mM), aqueous Brij-58 (3mM), aqueous Brij-35 aqueous, Brij-58 (10mM), Brij-35 (3mM)/Ethanol (1:1), (10mM), Brij-58 (3mM)/Ethanol (1:1) solution. It was followed by vortexing for 5.0 min. In order to disturb the drug protein binding and denaturing the proteins, 50 µL of HCl (35%) and TCA were added simultaneously.

The solutions were kept in a water bath shaker maintained at a temperature of 37±0.5°C for 5.0 min. Subsequently, samples were ultrasonicated and centrifuged at 3000 rpm for 5.0 min. A volume of 1.0 mL of the supernatant was filtered and transferred to a volumetric flask. The filtrates were suitably diluted and then diluents were analysed spectrophotometrically against a representative blank.

Selection of solvents and absorption maxima

For selection of preferential solvent, OXZ was dissolved in different solvents viz. methanol, ethanol, acetone, acetonitrile, chloroform and isopropanol. Absorbance spectrums were studied carefully during UV-Vis spectrophotometry. Among all the spectra obtained with varied solvents, the spectrum with ethanol (EtOH) was found to be better as OXZ was stable and showed very clear absorbance at λmax. =285 nm (Figure 2)

Analytical validation parameters

Linearity was investigated using five sets of OXZ-ethanolic solutions (5, 10, 15, 20, and 25 ng/mL). Investigations were carried out on three times in a

day and the obtained data was analysed by linear regression (Table 1, Figure 3).

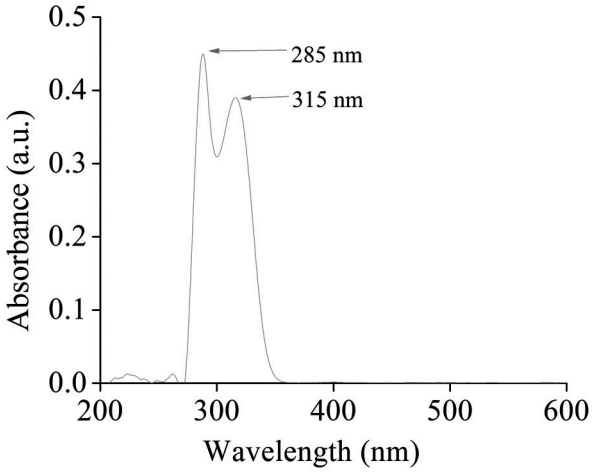

Fig. 2: Spectrum of ethanolic-OXZ solution.

Table 1: (a)Analytical characteristics of OXZ drug by proposed double beam UV-Visible spectrophotometer.

Parameters	UV-visible spectrophotometer Oxazepam
λmax, nm	285
Beers law limit, ng/mL	5-25
Molar absorptivity, L/mol cm	0.45728 x 104
Linearity range, ng/mL	5-25
Regression equation (Y=mx + c)	Y= 0.0159x + 0.0387
Coefficient of determination (R^2)	0.9998
Sandell's sensitivity (ng/cm^2/0.001 a.u.)	0.06269
Limit of quantification, LOQ (ng/mL)	2.26
Limit of detection, LOD (ng/mL)	0.6

Data; Mean ± SD (n=3)

(b) Reproducibility, accuracy and precision data evaluated.

OXZ Actual conc. (ng/mL)	Intra-day measured concentration (ng/mL)±SD; R.S.D % (n=3)	Recovery (%)	Inter-day measured concentration (ng/mL) ±SD; R.S.D % (n=3)	Recovery (%)
5	4.8 ± 0.05; 1.04	96.0	4.9 ± 0.08; 1.6	98.0
10	9.9 ± 0.1; 1.01	99.0	9.8 ± 0.1; 1.08	98.0
15	14.7 ± 0.44; 2.99	98.0	14.8 ± 0.3; 2.02	98.6
20	19.0 ± 0.9; 4.7	95.0	19.5± 0.8;3.2	97.5
Average recovery (%)		97.0 ±1.8		98.0±0.4

Data; Mean ± SD (n=3)

The value of molar absorptivity is 0.45728 x 104 L/mol cm respectively. The value of Sandell's sensitivity is 0.06269 ng/cm2/0.001/au. LOD and LOQ values are 0.6 ng/mL and 2.26 ng/mL respectively (8). Method accuracy was assessed by the standard addition method at 3 levels i.e. stock solution was used to prepare four dilutions viz. 5.0, 10, 15, 20 ng/mL.

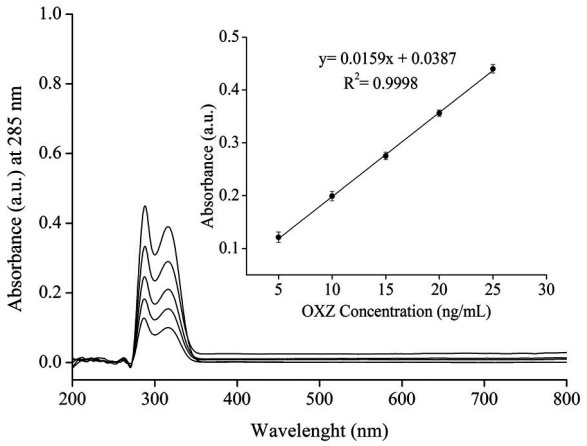

Fig. 3: UV-Visible spectrum of standard OXZ solutions (5-25ng/mL) inset calibration curve.

Four different concentrations i.e. 5.0-20 ng/mL were studied on the same day and the values

Table 2: Concentration of OXZ recovered from spiked matrices i.e. human Blood, Urine and Milk using surfactant system.

Surfactant System	Spiked Conc. (ng/mL)	Conc. in Urine (ng/mL)	R (%)	Conc. in Milk (ng/mL)	R (%)	Conc. in Blood (ng/mL)	R (%)
Brij-58 (3mM) / Ethanol (1:1)	5.0	5.1 ± 0.08	102	4.8 ± 0.01	96.0	4.3 ± 0.02	86.0
	10	9.74 ± 0.1	97.4	8.11± 0.2	81.1	10.01 ± 0.4	100.1
	25	22.3± 0.8	89.2	21.4± 0.9	85.6	22.1± 2	88.4
	50	50.1± 1.0	102.2	48.4± 1	96.8	47.2± 2	94.4
	100	94± 4.0	94.0	96.0 ± 3	96.0	95.3± 2	95.3
Average, R%			97±5.5		91.1±7.3		93±5.6

of relative standard deviation (% RSD) were calculated to determine intra-day precision. The accuracy was assessed as % Recoveries at each concentration level. (R2=0.999).

Results and Discussion

Determination of OXZ from complex matrices

It can be seen from Table 2 that Brij-58 possesses enhanced entrapment efficiency in comparison to other solvent systems. It was deciphered that among all the extraction media used, Brij-58 (3mM) /Ethanol (1:1) showed the highest entrapment efficiency viz. 89-102%, 81.1-96% and 86-100.1% from Urine, Milk and Blood respectively (Table 2, Figure 4).

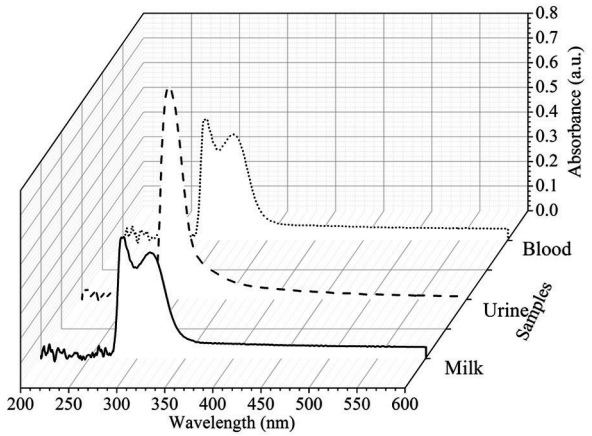

Fig. 4: UV-Visible spectrum of extracted OXZ from Spiked (10 ng/mL) samples using surfactant extractant.

Table 3: Statistical Analysis Data.

Parameters	Samples		
	Urine	Blood	Milk
No. of value	5	5	5
Mean±SD	96.96±5.5	91.10±7.2	92.60±5.6
SE	2.4	3.2	2.6
One Sample t Test			
Actual mean	96.96	91.10	92.60
Discrepancy	-96.96	-91.10	-92.60
95% CI of discrepancy	90.10 to 103.8	82.09 to 100.1	85.60 to 99.60
P value (2 tailed)	< 0.0001	< 0.0001	< 0.0001
Significant (alpha=0.05)	Yes	Yes	Yes

This enhanced extraction efficiency may be explained on the basis of type and nature of the extraction media used, polarity, CMC and Hydrophilic to Lipophilic Balance (HLB). Discussion of enhanced extraction efficiency was explained and described in my previous published research articles (9–11).

Statastics Analysis

One-way ANOVA and one sample t-test have been performed using SPSS. The f-ratio and P-value values were 9.612 and 0.000725 of one-way ANOVA test. One sample t-test values were enlisted in Table 3. The result is significant at P<0.05.

Conclusion

The objective of present work is to propose an efficient and effective extraction method for Oxazepam and can be scale up the protocol in forensic chemical and toxicological laboratories. Therefore, the proposed method could be used for the determination of OXZ in complex matrices. It was also deciphered that OXZ showed an enhanced solubility in Brij-58 (3mM)/ EtOH surfactant based formulations. Brij-58 (3mM) with ethanol as co-solvent showed best efficient recovery values in human urine, milk and human blood etc. This proposed protocol can also be scaled up in forensics, pharmaceutical and industrial fields.

Recommendations

We recommend further analysis to utilize the multicomponent array of surfactant assemblies in aqueous or non-aqueous media for an efficient extraction of Benzodiazepam drugs from complex matrices and to study the interaction mechanism between drugs and surfactant solutions.

Abbreviations

OXZ: Oxazepam; EtOH: ethanol; CMC: Critical micelle concentration; HLB: Hydrophilic to Lipophilic Balance

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