

Analysis of clonazepam in oral fluid by SPE-C18 and HPLC-UV-DAD

Abstract

One of the most common illness in Chilean population are related with stress and anxiety, in that context Benzodiazepines are one of the most used medications in order to treat this symptoms. In this group one of the most consumed is Clonazepam, however the use of this Benzodiazepine also has secondary effects such as drowsiness, dependence and addiction. In this scenario is very important develop analytical technics in order to analyze the content Clonazepam in dosage forms and in biological fluids, such as urine, blood and oral fluid. Saliva or strictly referred to as oral fluid has recently gained a special interest due to its many advantages such as simple collection, due to is a non-invasive method, and the results of the analysis reflects recent consumption of this compound. Due to all this facts, and in order to satisfy the legal requirements, a novel, rapid and reliable methodology for the determination of Clonazepam in oral fluid is implemented using HPLC-UV-DAD liquid chromatography, and SPE extraction. This method appears to be suitable for Clonazepam analysis in oral fluid with accurate and consistent results.

Keywords: benzodiazepines, clonazepam, SPE, HPLC

Volume 5 Issue 5 - 2017

Sonia A Rojas,¹ Boris E Duffau,¹ Marcela Escobar²

¹Institute of Public Health of Chile, Chile

²Faculty of Pharmacy, University of Valparaiso, Chile

Correspondence: Sonia A Rojas, Laboratory of Illicit Substances, Institute of Public Health of Chile, Faculty of Pharmacy, University of Valparaiso, Instituto Salud Pública de Chile, Marathon 1000, Santiago, Chile, Email srojas@ispch.cl

Received: June 25, 2017 | **Published:** July 27, 2017

Abbreviations: SPE, solid phase extraction; HPLC, high performance liquid chromatography; UV-DAD, ultraviolet/ diode array detection; CITUC, toxicological information center catholic university; ISPCH, institute of public health of chile

Introduction

The analysis of drugs of abuse is a necessity in different areas and with emphasis on the regulatory nature to be able to have affordable tools that allow fulfilling these ends. That is why within the biological matrices that are used in the forensic field are commonly known blood and urine, likewise the use of saliva matrix or strictly speaking oral fluid presents important advantages such as its collection is not invasive, adulteration is more difficult, the risk of contracting an infection is lower than the blood sample, can be taken by untrained staff and better reflects recent drug use.^{1,2} Solid phase extraction has multiple advantages compared to other extraction techniques, such as the process is automated so it is possible to minimize the errors of the analyte process, deliver extracts of cleaner samples and the amount of solvent used less.³ A special concern of our society has the licit and illicit drugs, since although the acquisition of these substances is regulated by law, indiscriminate, irresponsible and in some cases under-diagnosed, results in a pattern of abuse being observed. Such is the case of abuse of Clonazepam, whose therapeutic purpose and pharmacological properties are mainly to be anxiolytics, hypnotics and anticonvulsants however, abuse of this drug has become a public health problem, endorsed by studies that indicate that on average there are around 300 cases of annual poisonings by Clonazepam reported to CITUC,⁴ which is consistent with the one of the most seized drug in Chile according to data provided by the ISPCH's illicit drug laboratory.⁵

Case presentation

Standards and reagents: Certified standard Clonazepam USP, purity 99.9%. The samples are reconstituted in methanol by the chromatographic quality of the MERCK brand. For the mobile phase K_2HPO_4 , KH_2PO_4 and triethylamine all in analytical grade purchased by Merck were used.

Preparation of the sample and extraction of clonazepam

After collection of a pool of saliva samples, 0.5mL of oral fluid is then taken, and then 0.5mL of known solution of Clonazepam and 0.5mL of distilled water is added. After vigorous stirring for 5 minutes, the analyte is extracted into the sample in four steps which used the vacuum extraction system of SPE, where each cartridge C-18 is conditioned with 2mL of methanol and 2mL of distilled water. Subsequently 1mL of sample and 1mL of distilled water is added. Elution of the analyte of interest remaining in the cartridge is collected using 1mL of 50:50 methanol/distilled water. The sample is then left in a nitrogen concentrator at a temperature of 40°C with a light flow for 20 minutes. Finally, the analyte of interest is reconstituted with 250µL of methanol and then added in a chromatography vial. This procedure is presented in Figure 1.

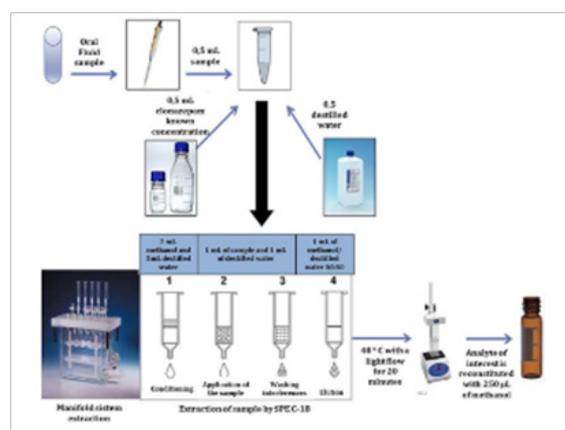


Figure 1 Scheme of sample preparation of saliva using solid phase extraction (SPE).

Chromatographic conditions

20 µL of extracted sample was injected into an HPLC-UV-DAD Agilent Technologies 1100 series®, using a column RP-18 endcapped

(5 μ m), 150mm-4.6 (Purospher® STAR), mobile phase contained 40% v/v Acetonitrile and 60% v/v Buffer K₂HPO₄/KH₂PO₄ adjusted to pH 8.5 with Triethylamine. Flow of this mobile phase was established at 1mL/min. The wavelength selected was 245nm the complete run time was about 11 minutes.

Discussion

A fast extraction method is presented, that allowed to us obtain clean extracts in a short time and with a minimum amount of solvent, and the proposed extraction method also can process several samples simultaneously by using the Manifold® vacuum filtration system. Those potential interferences are retained in the C-18 SPE cartridge and then Clonazepam can be eluted easily. In that context we concluded that the use of HPLC-UV-DAD and extraction by SPE-C18 for samples of oral fluid becomes a powerful tool for the determination of Clonazepam, becoming in an very powerful option in order to a rapid examination and diagnostic in the field of occupational safety, treatment and rehabilitation of drug use, as well as in the monitoring of drivers in accordance with the regulations of road safety in several countries.

Acknowledgements

The authors thank Institute of Public Health of Chile for providing all materials for this study.

Conflicts of interest

The author declare no conflict of interest.

References

1. Fernández AA, Font AM, Fité MS, et al. Drogas de abuso en saliva de conductores: Aspectos médico-legales. *Rev Esp Med Leg.* 2008;34(1):3–10.
2. Persona K, Madej K, Knihnicki P, et al. Analytical methodologies for the determination of benzodiazepines in biological samples. *J Pharm Biomed Anal.* 2015; 113:239–264.
3. Uddin MN, Samanidou VF, Papadoyannis IN. Validation of SPE-HPLC determination of 1,4-benzodiazepines and metabolites in blood plasma, urine, and saliva. *J Sep Sci.* 2008;31(21):3704–3717.
4. Paris E. Epidemiology of intoxication cases that originated calls to the Toxicology Information Centre (CITUC) of the Catholic University of Chile. Metropolitan Region. *Cuad Med Soc.* 2005;45:256–268.
5. Rojas PS, Duffau B Fuentes. Perfil De Las Principals Benzodiazepinas Incautadas En Chile. Congreso de la sociedad de toxicología de Chile. 2014.