A Generic Extraction Method for Basic Drugs of Abuse from Oral Fluid Samples

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Abstract

The ultimate aim of this work is to evaluate the complexity and simplicity of the methods required in a commercial laboratory to extract a range of basic drugs from a buffered saliva sample. Saliva collection is a robust high integrity and reliable method for on site sampling, where sample contamination or adulteration is a difficult problem. Each class of drug is extracted sequentially using an optimised method and the results are quantified using a Varian HPLC system. The method described will allow the extraction of a range of drugs from oral fluid with a single SPE cartridge.

Removal of Oral Collection Device Additives

The integrity of saliva samples in a clinical campaign is of utmost importance. All of the major saliva sampling devices contain a preservative solution with stabiliser and buffer to prevent testing prior to testing. One of the major components in the preservative solution is Tween 20, which is a non-ionic surfactant that can adsorb onto the analytes and potentially affect the results of analysis in oral fluid samples.

In this study a series of well known and fluid SPE devices were tested against Plexa DAS for Tween 20 removal properties. The most optimised method was then used to assess the removal of the most commonly testing devices in the industry. The Varian® device is the only device that Plexa DAS appears to remove Tween contamination in the flow through format, whereas other methods are not efficient at the high levels of contamination.

Introduction

Plexa DAS is a highly engineered hydrophilic polymer which contains cation exchange sites and other oligomeric characteristics which allow for excellent phase transfer of analytes out of saliva matrices. Specialised engineered pores exclude endogenous material such as saliva proteins and extraneous substances allowing solution of analytes in oral fluid samples.

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The work being undertaken is investigating the possibility of using a single generic method for the extraction of a range of basic drugs (Opiates, Benzodiazepine, Amphetamine and Cocaine). A generic method would have the advantage of reducing the number of solutions and solvents required and reduce the pressure on a limited sample volume. The extraction methodology is based on the use of Varian’s Plexa DAS SPE cartridge.

The best results are obtained when the solutions are left to flow through the Plexa DAS SPE cartridge under gravity. A small amount of preservative solution can be applied to the flow if the solvent required.

The method is set up as follows:

1. Apply sample
2. Washing
3. Elution

The solutions are concentrated to reduce the volume of the extract before analysis. The extracts are then concentrated until the final concentration is 100 µl of volume.

Applying Sample

A 3.8 ml aliquot of raw saliva is drawn into a micro pipette and placed into a 1.5 ml Eppendorf vial. 600 µl of buffered Saliva (1 ml, Methanol, phosphate buffer, Mix and then centrifuge)

Column Conditioning

1 ml, Methanol

Load Sample

100 µl of buffered saliva (pH 5.5 for 1 ml, Methanol, phosphate buffer, Mix and then centrifuge)

Elution

100 µl, 65% Methanol/25% Milli-Q water/10% Ammonium Hydroxide 1 ml, 65% Methanol/25% Milli-Q water/10% Ammonium Hydroxide

The method which is currently being developed takes account of the various cut off levels of the different drugs classes and the actual volume of saliva present in the buffered solution (3.8 ml of buffered saliva contains 15% of saliva). The extraction method and Analytical Parameters

The extraction efficiency was determined by running a set of six calibration standards (n=6), against a set of four concentration standards (n=12), the table below outlines the percentage recovery and reproducibility. The extraction efficiency was determined by running a set of six calibration standards (n=6), against a set of four concentration standards (n=12), the table below outlines the percentage recovery and reproducibility. The extraction efficiency was determined by running a set of six calibration standards (n=6), against a set of four concentration standards (n=12), the table below outlines the percentage recovery and reproducibility.