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

Adrian King[1], Paul A Boguszewski[2], James Stratta[2]

1) Synergy Health Laboratory Service, Brecon Road, Abergavenny NP7 7RX. Tel: +44 (0) 1873 856688 2) Varian UK Ltd, 10 Mead Road, Yarnton, Oxford OX5 1QU. Tel: +44 (0) 1865 291500

Abstract

The ultimate aim of this work is to minimise the variety and complexity 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 manipulation or adulteration is difficult. Normally each class of drug is extracted separately using an optimised method for each particular class of drugs, this has two major disadvantages:

1. A large selection of solutions and solvents are required.
2. Limited sample volume combined with low drug concentrations.

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, Methadone 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.

Introducing Plexa DAS: Hydrophilic Sorbent for Oral Fluid SPE

Plexa DAS is a highly engineered hydrophilic polymer which contains cation exchange sites and other oligomeric chemistries which allow for excellent phase transfer of analytes out of saliva matrices. Specially engineered pores exclude endogenous material such as salivary proteins and excellent elution profiles allow isolation of analytes in small elution volumes.

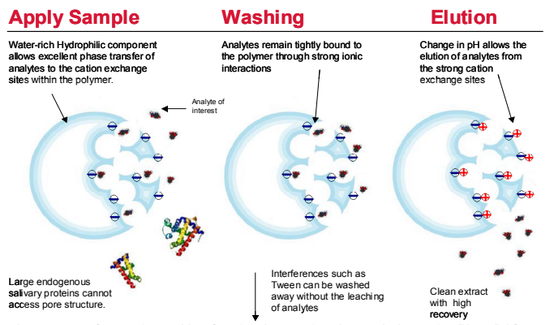


Figure 1: The three stages of extraction and key functional properties observed when using Plexa DAS.

Removal of Oral Collection Device Additives

The integrity of saliva sample is a vital component of drug analysis. All of the major saliva sampling devices contain a preservative solution which stabilises and buffers testing samples prior to testing. One of the major components in the storage liquor; Tween 20, is known to cause issues with ion suppression and column build-up.

In the study below, a series of well known oral fluid SPE devices were tested against Plexa DAS for Tween removal properties. The top chromatogram control was device fluid from one of the most common testing devices in the industry, the Orasure® device. It is very clear that Plexa DAS shows no Tween contamination in the final elution extract, whereas other materials still retain and elute high levels of the preservative.

Mobile Phase : A: 0.1% Formic Acid B: Methanol
Isocratic: t = 0-3 min 20% A : 80% B
Column: Pursuit XRs $^{2.8}$ C18 30 x 2.0 mm

All samples dried and reconstituted in 100 µL of 1:1 0.1% Aq formic acid: MeOH

MS conditions:

Compound	Q1	Q3	CE
Tween 20	227.1	95.0	15.0V

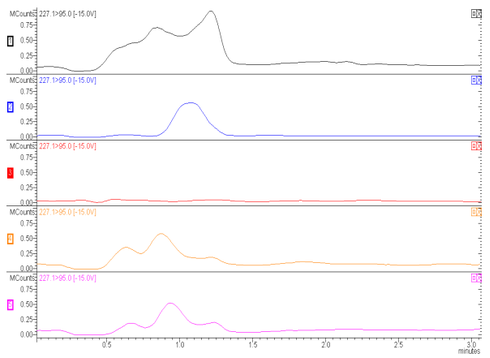


Figure 2: LCMS responses for Tween 20 in elution fractions of several extraction devices including Plexa DAS.

Extraction Method and Analysis Parameters

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 (600µl of buffered saliva contains 150µl of saliva).

	Cut-Off limit (ng/ml)	Calibration range (ng/ml)
Opiates (ex. 6-MAM)	40	10-100
6-MAM	4	1-10
Benzodiazepine	5	0.5-20
Methadone	20	5-50
Amphetamines	30	5-100
Cocaine	8	1-20

Table 1. Cut off limits and calibration ranges used for the different drug classes

The method is set up as follows:

	Plexa DAS (1mL 30mg)
Sample Pre-treatment	600µl of buffered Saliva 500µl of 0.1M, pH6 phosphate buffer. Mix and then centrifuge
Column Conditioning	1ml, Methanol 1ml, Water
Load sample	
SPE Cartridge wash	1ml, 0.1M Hydrochloric acid 1ml, 60:40 Methanol/0.1M hydrochloric acid Dry 10 minutes
Elution	150µl, 50:50 Acetonitrile/Methanol 2x 150µl, 50:50:2 Acetonitrile/ Methanol/ Ammonium Hydroxide

The samples were evaporated to dryness and reconstituted in 90:10, 5mM Ammonium Acetate (aq)/ Methanol solution. The best results are obtained when the solutions are left to flow through the Plexa DAS SPE cartridge under gravity. A small amount of positive pressure can be applied to aid the flow if and when required.

Column: C18, 2.1x50mm
Mobile phase: A – 5mM Ammonium Acetate with 0.001% Formic acid
B –Methanol with 0.001% Formic acid

Injection Volume: 60µl
Instrument: Agilent 6410MS/MS

The Gradient Profile for the LC.

Time (minutes)	B (%)	Flow (ml/min)
0	10	0.4
1	10	0.4
5	100	0.4
7.5	100	0.4
7.6	10	0.4
12.5	10	0.4

Typical Total Ion Chromatogram

The MS has been segmented to allow increased sensitivity for all the analytes. For each analyte the MS is monitoring for both quantification and qualification ions, also for each compound its deuterated analogue is included to act as the internal standard. A typical chromatogram is shown below and the retention times are listed in table 2.

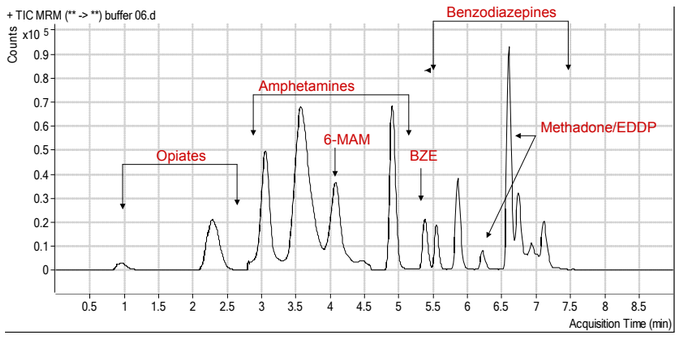


Figure 3: Representative chromatogram of the drugs extracted by the Plexa DAS SPE cartridge.

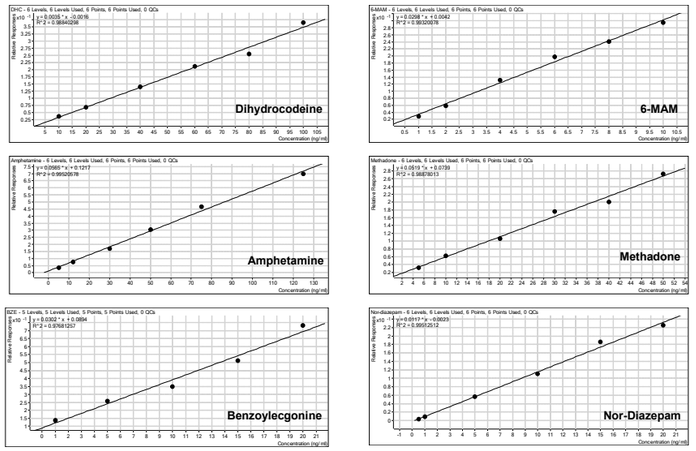
The extraction efficiency was determined by running a set of the top calibration standard (n=6), against a set of non-extracted top calibration standards (n=6), the table below outlines the percentage recovery and reproducibility.

	Retention time (min)	% Recovery	%RSD
Morphine	0.8	82	15
Codeine	2.4	96	2.0
Dihydrocodeine	2.3	92	4.3
6-MAM	4.3	74	14
Amphetamine	3.1	75	5.5
Methamphetamine	3.6	93	2.8
MDA	3.8	78	8.1
MDMA	4.2	88	5.3
MDEA	4.9	97	3.3
Benzoylecgonine	5.4	95	2.8
Methadone	6.6	98	1.5
EDDP	6.2	31	7.0
Flunitrazepam	6.7	88	5.2
Aminoflunitrazepam	5.5	77	2.9
Aminonitrazepam	5.2	67	9.9
Diazepam	7.2	90	5.9
Nor-Diazepam	7.0	91	7.7
Temazepam	6.9	80	3.3

Table 2. Retention times and extraction efficiencies for the drugs that were investigated

Calibration

As well as determining the extraction efficiency we investigated the useable dynamic range of the calibration curves. As the calibrations curves are based on the saliva content no dilution factors are necessary when quantifying the samples. The calibration curves were constructed with at least two of the calibrators present being lower then the cut-off concentration, the following calibration curves are representative of the typical curve from each drug class investigated.



Summary

This initial work indicates that it is possible to use a single generic method for a range of basic drugs. The recoveries are all acceptable ranging from 67 to 98% with the exception of EDDP which has a recovery of only 30%. However the poor recovery is off set by the good sensitivity of EDDP and it can still be quantified down to the 5ng/ml level. The reproducibility of the extraction is also acceptable with %RSD ranging from 1.5 to 15% for all the drugs.

There was an attempt to incorporate cannabis into the extraction, however this approach has ceased at present. The main reason for this is the poor performance of the Opiates and in particular Morphine, which had irreproducible recoveries (%RSD ca. 40%) and significant loss of signal due to ion suppression. Also the cut-off levels for cannabis (1ng/ml) means that it requires a more optimised extraction method to recovery sufficient material for the analysis.