COZART®

The validation and use of an accredited routine method for the simultaneous analysis of opiates in oral fluid by GC-MS.

Peter Akrill*, Claire Reid, Lisa Wilson, Amy Reed, Gail Cooper and Chris Hand
Cozart Bioscience Ltd., 92 Milton Park, Abingdon, Oxfordshire, UK.

INTRODUCTION

The analysis of drugs of abuse in oral fluid poses a challenge due to limited sample volume available for analysis and the requirement to detect drugs at lower concentrations. Confirmation analysis techniques such as GC-MS and LC-MS are crucial to prove unequivocally the presence of particular drugs in oral fluid. This is particularly the case for analysis of opiates, since a positive opiate screen could be as a result of the use of declared prescription or over-the-counter medication, such as codeine, or through the illicit use of heroin.

Increasingly important in workplace drug testing in particular is the need to assure the testing organisation and the client being tested of the quality of the testing and analysis program. Within the UK this is best demonstrated by compliance with and accreditation to the ISO17025:2005 standard.

The need to detect drugs at the low levels that could be present in oral fluid samples, from the low volume of sample available for analysis require particularly optimised systems for these analyses.

AIMS

The aim of the work described here was to develop a sensitive and robust method suitable for the routine analysis of codeine (COD), morphine (MOR), 6-acetylmorphine (6AM) and dihydrocodeine (DHC) in oral fluid for use in a laboratory accredited to ISO17025:2005. Following full validation it was planned to use this method to analyse samples collected from a criminal justice setting.

ANALYTICAL METHOD

The collected oral fluid was diluted within the Cozart® Oral Fluid Collector 1:2 in buffer. 500 µL of this buffered oral fluid was taken for analysis. 1 mL of pH6 0.1M phosphate buffer and 20 µL of 1 µg/ml solution of the deuterated analogues of each analyte were added to this. Figure 1 summarises the solid phase extraction method followed using 50mg Bond Elut Certify columns.

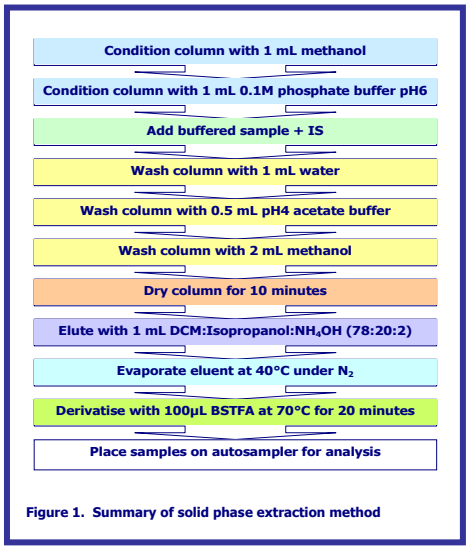

Figure 1. Summary of solid phase extraction method

Calibration standards were prepared by spiking blank collected oral fluid with the appropriate volume of a methanolic standard solution to give calibrators at the concentrations of 0, 15, 30, 60, 90, 120 and 180 ng/mL in neat oral fluid (3 times lower in the buffered oral fluid). Each extracted batch of 20 samples included two analytical quality control samples and one blank sample. A vial containing ethyl acetate was placed on the autosampler along with the calibrators, controls and samples for analysis and was injected between each sample to demonstrate the absence of carryover.

Analytical Column: Restek RTX-5Sil MS (30m x 0.25mm x 0.25µM)

Gas Chromatograph Parameters

Injector Temperature: **290°C**

Oven Programme
Initial Temperature: **150°C (hold for 0.5 min.)**
Ramp 1: **10°C/min until 300°C (hold for 4 min.)**
Total Run Time: **19.5 min.**

Mass Spectrometric Detector Parameters

Ionisation Mode: **Electron Impact (70eV)**
Dwell Time: **50msec**
Source Temperature: **230°C**
Quad Temperature: **150°C**

Table 1. SIM Parameters

Analyte	SIM Ions
Dihydrocodeine	**373**, 282, 315
Dihydrocodeine-d_6	**379**, 288, 318
Codeine	**371**, 234, 343
Codeine–d_3	**374**, 237, 346
Morphine	**429**, 324, 287
Morphine-d_3	**432**, 327, 290
6-Acetylmorphine	**399**, 340, 287
6-Acetylmorphine-d_3	**402**, 343, 290

Figure 2. Instrument Parameters for the Agilent 6890N Gas Chromatograph and 5973 Mass Spectrometer

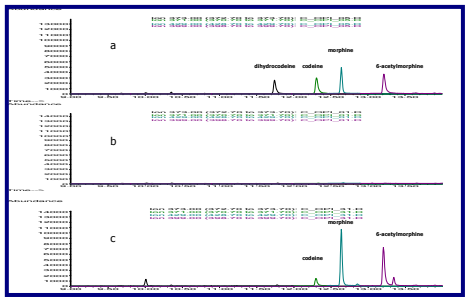

Figure 3. SIM Chromatograms of extracted oral fluid samples a) oral fluid spiked at 120 ng/mL; b) blank oral fluid; c) oral fluid sample collected from a clinical setting with COD 29 ng/mL, MOR 185 ng/mL 6AM 147 ng/mL

VALIDATION METHOD

The validation of the method was carried out to determine the following:

LINEARITY

The linearity was determined by the analysis of 4 standard curves over the working range of the method (LOQ – 180 ng/mL).

LIMIT OF DETECTION

The limit of detection (LOD) was determined as the concentration level at which the peak height was three times the background.

LIMIT OF QUANTITATION

The limit of quantitation (LOQ) was determined as the concentration level at which the concentration could be quantified and the peak could be positively identified.

ROBUSTNESS

The robustness of the method was assessed by determining the variation in retention time of the analytes over the course of an analytical run.

PRECISION

The precision of the method was determined by the analysis of an analytical control sample spiked with each analyte at 60ng/mL. The precision was assessed by the analysis of four separate analytical runs, each containing 10 replicates of the spiked oral fluid control.

VALIDATION RESULTS

Table 2. Summary of Method Validation Data

			COD	DHC	MOR	6AM
Linearity	Range (ng/ml)		5 - 180	5 - 180	5- 180	5-180
	Corr. Coeff	Mean	0.9965	0.9965	0.9970	0.9978
		Std. Dev.	0.0031	0.0038	0.0034	0.0005
Limit of Detection (ng/mL)			2	5	1	5
Limit of Quantitation (ng/mL)			5	5	5	5
Robustness	Retention Time CV (%)		0	0	0.10	0.03
Precision	Inter-assay CV (%)		8.5	9.1	7.9	6.6
	Intra-assay CV (%)		4.7	4.8	4.2	4.5

FIELD STUDY

The suitability of this method to analyse 'real' samples was evaluated by analysing 437 samples collected from a criminal justice setting. The samples were collected using the Cozart® Oral Fluid Collector and sent to the laboratory for analysis.

RESULTS

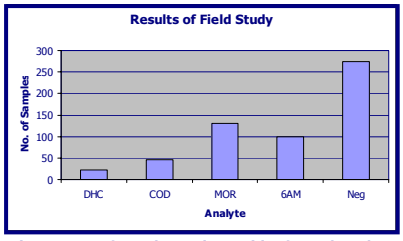

Figure 4. No. of samples testing positive for each analyte

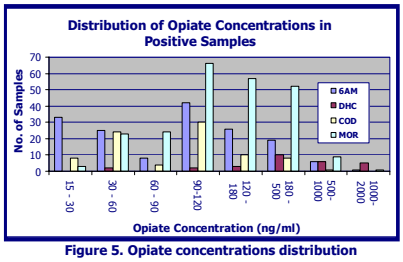

Figure 5. Opiate concentrations distribution

CONCLUSION

The method is sufficiently sensitive and robust to be used for the routine analysis of oral fluid samples for opiates. The validation of the method has shown that it can be used routinely in a laboratory accredited to ISO17025:2005.