Extraction of Illicit Drugs and Pesticides from Liver Tissue Using ISOLUTE® SLE+ Prior to GC/MS Analysis

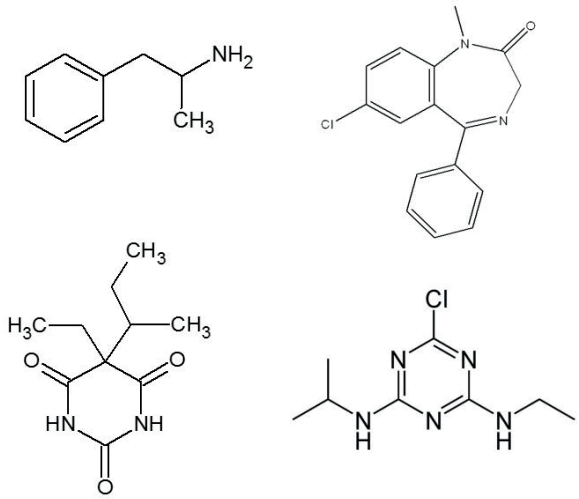

Figure 1. Structures of amphetamine, diazepam, butabarbital and atrazine respectively.

Introduction

This application note describes the extraction of a broad range of analytes from liver tissue matrix prior to GC/MS analysis using ISOLUTE® SLE+ supported liquid extraction columns. A protocol has been developed that allows the simultaneous extraction of various drugs of abuse classes: amphetamines, barbiturates, benzodiazepines, cocaine, ketamine, THC. In addition to these drug panels, simultaneous extraction of carbamate, organochlorine, organophosphate, pyrethroid, and triazine pesticide classes is demonstrated.

ISOLUTE SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

This application note describes an effective and efficient ISOLUTE SLE+ protocol optimized for the 1 mL capacity column format. The simple sample preparation procedure delivers clean extracts, good recoveries and RSD values and LLOQ of 50 ppb, suitable for screening applications.

Analytes

Amphetamine, Bendiocarb, Methamphetamine, Propanil, MDMA, Chlorothalonil, Atrazine, Butabarbital, Secobarbital, Ketamine, Malathion, Phenobarbital, Cocaine, Methadone, THC, Bifenthrin, Diazepam, Nitrazepam, Midazolam, Clonazepam, Estazolam, Alprazolam and Triazolam.

Sample Preparation Procedure

Format:

ISOLUTE SLE+ 1 mL Sample Volume Column, part number 820-0140-C

Sample Pre-treatment:

Weigh 200 mg of liver and place in 7 mL BeadRuptor tubes containing 2.8 mm ceramic beads. Add methanol:water (50:50, v/v, 1.8 mL). Add internal standard mix (500 ppb, 10 µL in methanol). Load tubes into the Bead Ruptor 24 and homogenize using the following program: 1 cycle for 30 seconds at 5.3 m/s.

Transfer the homogenized liver into 2 mL Eppendorf tubes and place in a micro centrifuge for 10 minutes at 13,300 rpm.

Sample Loading:

Load 500 µL of the pre-treated liver supernatant onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Extraction:

Apply dichloromethane (DCM) (2.5 mL) and allow to flow under gravity for 5 minutes. Collect in an appropriate glass tube containing 100 µL HCl in methanol (0.2 M). This acts to stabilize free-base analytes in the solvent prior to evaporation.

Apply a second aliquot of DCM (2.5 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent into the collection tube.

Evaporation and Derivatization:

Evaporate the extract in a stream of air or nitrogen (ambient, 20 to 40 L/min).

Reconstitute the extracts with ethyl acetate (200 µL) and vortex for 20 seconds before transferring to high recovery GC vials. Evaporate the extract in a stream of air or nitrogen using a SPE Dry (ambient room temperature, 20 to 40 L/min).

Reconstitute extracts with ethyl acetate (25 µL) and MSTFA (25 µL). Vortex mix, then heat on a block for 30 minutes at 80 °C to complete derivatization.

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GC Conditions

Instrument
Agilent 7890A with QuickSwap

Column
Agilent J&W DB-5, 30 m x 0.25 mm ID x 0.25 µm

Carrier
Helium 1.2 mL/min (constant flow)

Inlet
300 °C, Split (5:1), Septum purge flow: 3 mL/min

Injection
1 µL

Wash Solvents
Methanol and ethyl acetate

Oven
Initial temperature 55 °C

Ramp 25 °C/min to 325 °C, hold for 3.2 minutes

Post Run
Backflush for 1.6 minutes (2 void volumes)

Transfer Line
300 °C

MS Conditions

Instrument
Agilent 5975C

Source
230 °C

Quadrupole
150 °C

MSD mode
SIM

SIM Parameters

Table 1. Ions acquired in the Selected Ion Monitoring (SIM) mode (5 minute solvent delay)

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	Amphetamine-D_5	96	197	
1	Amphetamine	91	192	
2	Bendiocarb	223	238	
3	Methamphetamine	130	206	91
4	Propanil	200	130	
4	MDMA	130	250	
4	Butabarbital	341	300	
4	Atrazine	200	215	
5	Chlorothalonil	300	341	
5	Secobarbital	297	367	109
6	Ketamine	180	182	
6	Malathion	125	173	158
6	Phenobarbital	146	117	100
7	Methadone	72	82	85
7	Cocaine	182	82	94
8	THC-D_3	389	374	
8	THC	386	371	
9	Bifenthrin	181	166	
10	Diazepam-D_5	289	261	287
10	Diazepam	256	283	284
10	Nitrazepam	352	353	
11	Midazolam	310	312	
11	Clonazepam	352	387	
12	Estazolam	259	293	77
12	Alprazolam	279	308	77
13	Triazolam	313	238	315

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Results

Blank liver supernatant was spiked at 1000 ppb for recovery testing; typical recovery data is shown in Figure 4. The protocol described offered reproducible recovery with most RSD values <10%.

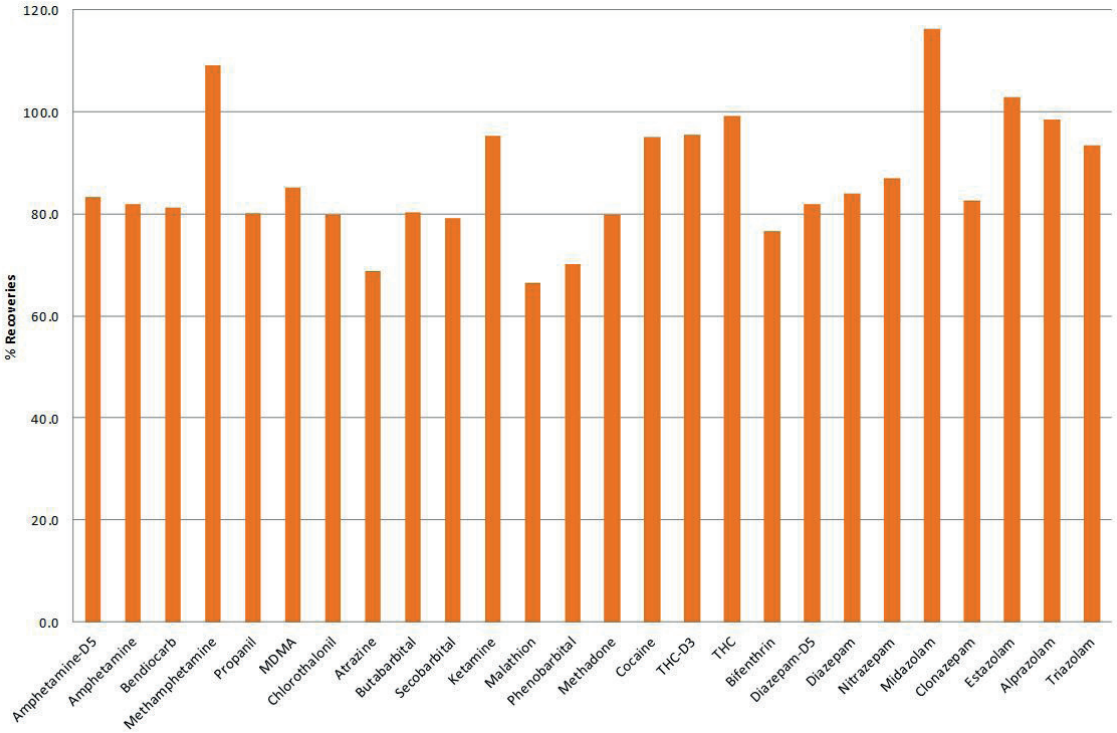

Figure 2. Chart demonstrating typical analyte recoveries.

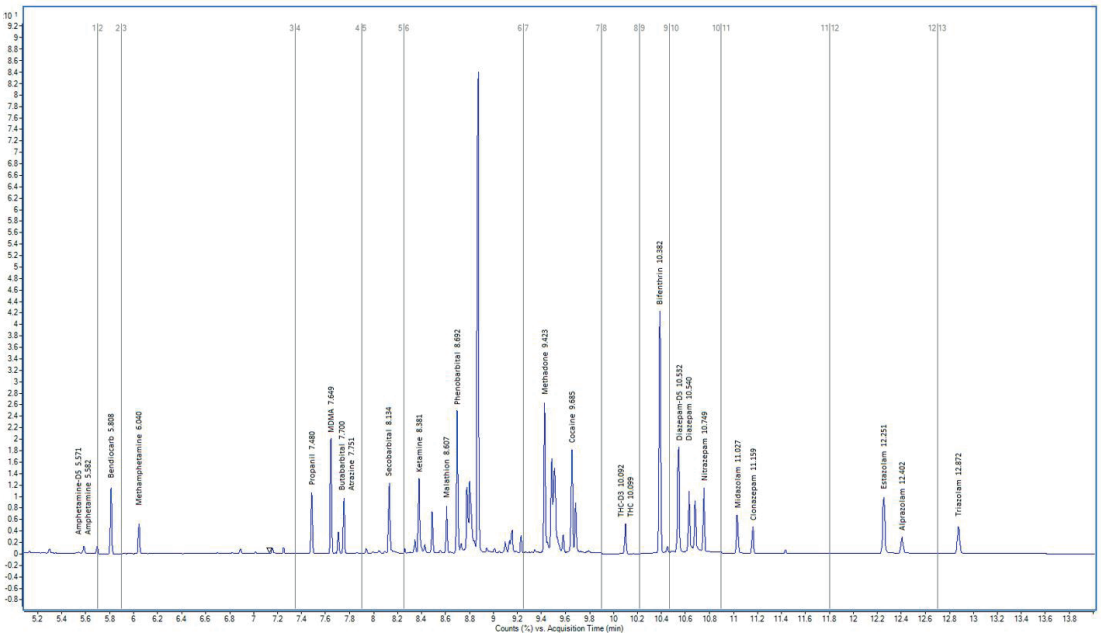

Figure 3. Total Ion Chromatogram of extracted analytes spiked into liver at 250ppb

Calibration Curves

Liver supernatant was spiked prior to extraction, at concentrations of 50, 100, 250, 500, 1000 and 2500 ppb for each analyte to create calibration curves. The internal standards were spiked at 500 ppb for each level. The curves are shown in Figure 4.

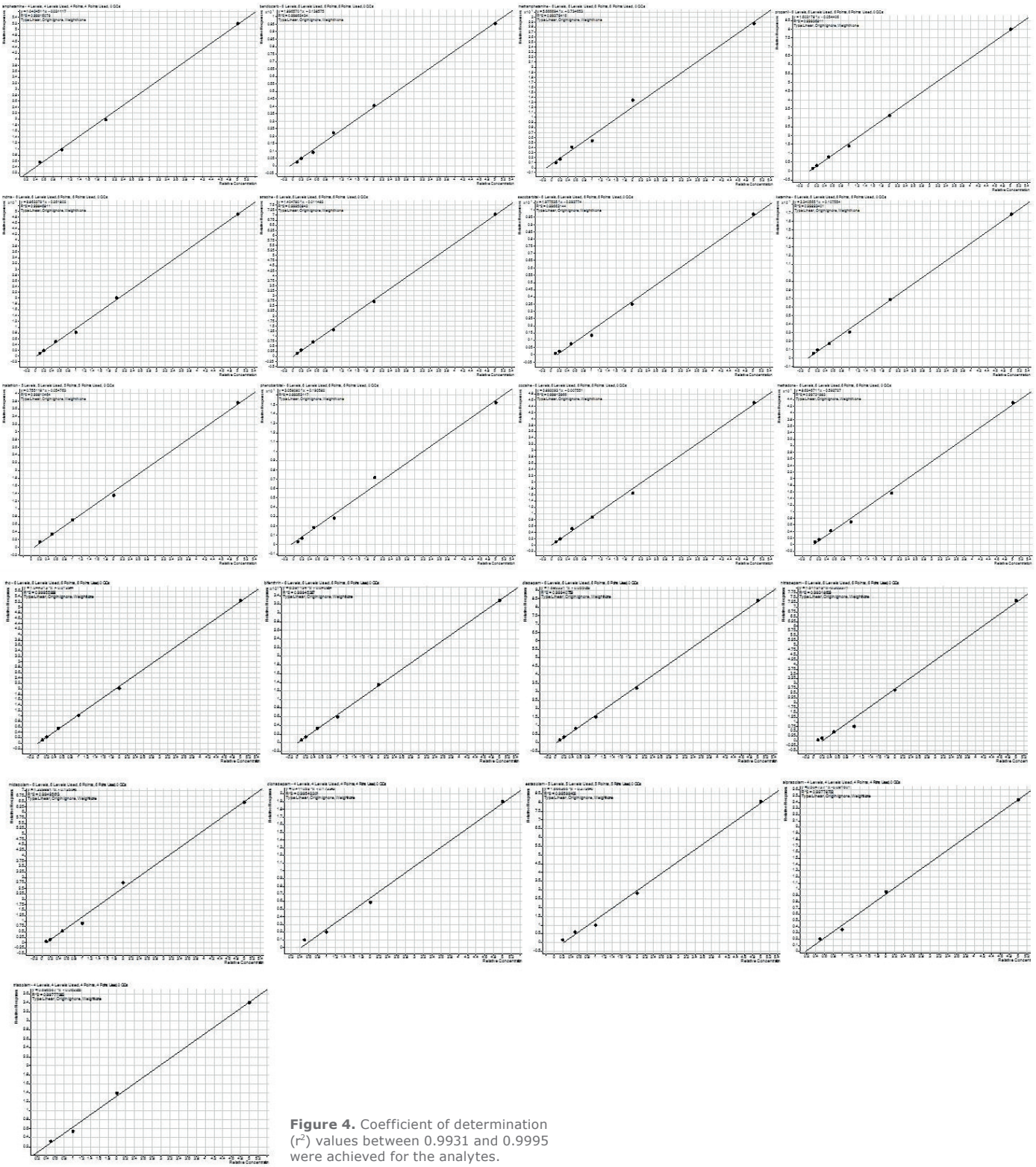

Figure 4. Coefficient of determination (r^2) values between 0.9931 and 0.9995 were achieved for the analytes.

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Table 2. Lower Limits of Quantitation (LLOQ) using the ISOLUTE® SLE+ procedure described in this application note

Drug Analyte	LLOQ (ppb)
Amphetmamine	50
Bendiocarb	50
Methamphetamine	50
Propanil	50
MDMA	50
Chlorothalonil	50
Atrazine	50
Butabarbital	50
Secobarbital	50
Ketamine	50
Malathion	100
Phenobarbital	50
Cocaine	50
Methadone	50
THC	50
Bifenthrin	50
Diazepam	50
Nitrazepam	50
Midazolam	50
Clonazepam	250
Estazolam	100
Alprazolam	250
Triazolam	250

Additional information

All solvents were HPLC grade.

0.2M HCl in methanol: Add 200 µL concentrated HCl solution (37%) to 11.8 mL methanol. Mix thoroughly.

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE SLE+ 1 mL Sample Volume Column	30
PPM-48	Biotage PRESSURE+ 48 Positive Pressure Manifold	1
SD-9600-DHS	SPE Dry Sample Evaporator	1

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