

# Extraction of Benzodiazepines from Various Matrices using Supported Liquid Extraction (SLE) and LC-MS/MS Analysis

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## Introduction

Supported Liquid Extraction (SLE) is a 96-well high throughput technique that is analogous to traditional liquid liquid extraction (LLE). In SLE, the extraction interface occurs between the buffered sample absorbed onto an inert solid support (diatomaceous earth) and a water immiscible organic solvent. This provides excellent extraction efficiency, while alleviating many of the liquid handling issues associated with traditional LLE.

Benzodiazepines are a widely prescribed class of drugs known primarily for their sedative and hypnotic effects. Because of these effects they have found use as muscle relaxants, anticonvulsants, anxiolytics and for the treatment of various sleep disorders. This widespread use, along with various forms of misuse, has led to the necessity of rapid and reliable methods for their analysis and quantitation. This poster will show the application of supported liquid extraction in the analysis of benzodiazepines from various human biological fluids.

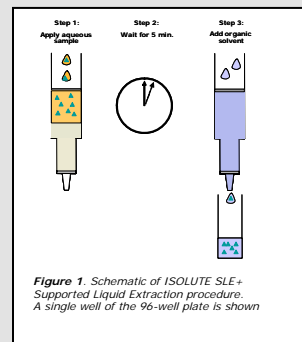


Figure 1. Schematic of ISOLUTE SLE+ Supported Liquid Extraction procedure. A single well of the 96-well plate is shown.

## Experimental Procedure

### Reagents

All benzodiazepines (alprazolam,  $\alpha$ -hydroxy alprazolam, bromazepam, diazepam, nordiazepam, estazolam, flunitrazepam, flurazepam, lorazepam, midazolam, nitrazepam, oxazepam, temazepam and triazolam), ammonium acetate, formic and acetic acids were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained from the Welsh Blood Service (Pontyclun, UK). Human urine was kindly donated from a healthy human volunteer. Control human blood was purchased from Sera Laboratories International (West Sussex, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q water was used throughout.

### Sample Preparation

#### Supported Liquid Extraction Procedure

**Plate:** ISOLUTE SLE+ Supported Liquid Extraction Plate 200 mg, part number 820-0200-P01

**Sample Pre-treatment:** Blank matrix (100  $\mu$ L) was spiked with the benzodiazepines at 50 ng/mL. The matrix was then diluted 1:1 v/v with 1% formic acid aq, H<sub>2</sub>O or 0.5M NH<sub>4</sub>OH prior to loading. This sample dilution results in approximate loading pH's of 3.2, 8.0 and 10.4, respectively.

**Whole Blood Cell Lysis:** Sonication for 10 minutes in buffer followed by centrifugation at 11,000 rpm for 10 minutes. Cellular debris (pellet) was discarded.



**Sample Application:** The pre-treated plasma was loaded onto the plate, a pulse of vacuum applied to initiate flow and the samples left to absorb for 5 minutes.

**Analyte Elution:** Addition of 1 mL of various water immiscible extraction solvents. The extraction solvents evaluated were DCM, 95:5 (v/v) DCM/IPA, MTBE and EtOAc.

**Post Extraction:** The eluate was evaporated to dryness and the analytes reconstituted in 500  $\mu$ L of 80:20 (v/v) H<sub>2</sub>O/MeOH prior to analysis.

### HPLC Conditions

**Instrument:** Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA).

**Column:** Luna Phenyl-Hexyl 5  $\mu$ m analytical column (50 x 2.0 mm id) (Phenomenex, Cheshire UK).

**Guard Column:** Luna Phenyl-Hexyl security guard column (Phenomenex, Cheshire, UK).

**Mobile Phase:** 0.1% formic acid aq and MeCN at a flow rate of 0.3 mL/min.

**Gradient:** The gradient conditions were set to 70%, 0.1% (v/v) formic acid aq and 30% MeCN increasing to 45% MeCN over 5 minutes. Initial starting conditions were resumed at 5.1 minutes.

**Injection Volume:** 10  $\mu$ L

**Temperature:** Ambient

### Mass Spectrometry

**Instrument:** Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring mode (MRM).

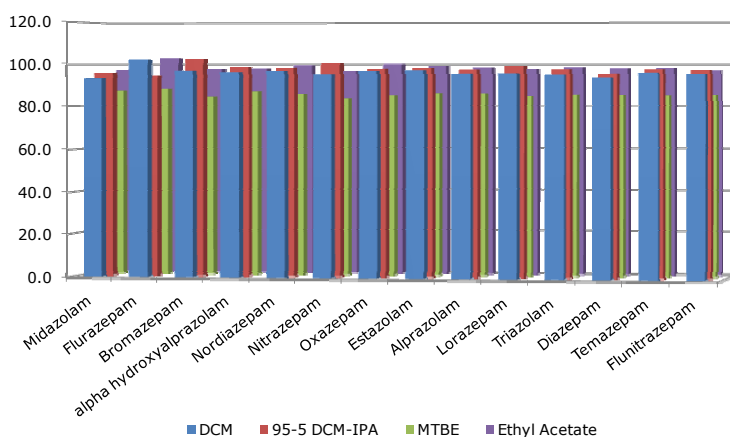
**Desolvation Temperature:** 350 °C

**Ion Source Temperature:** 100 °C

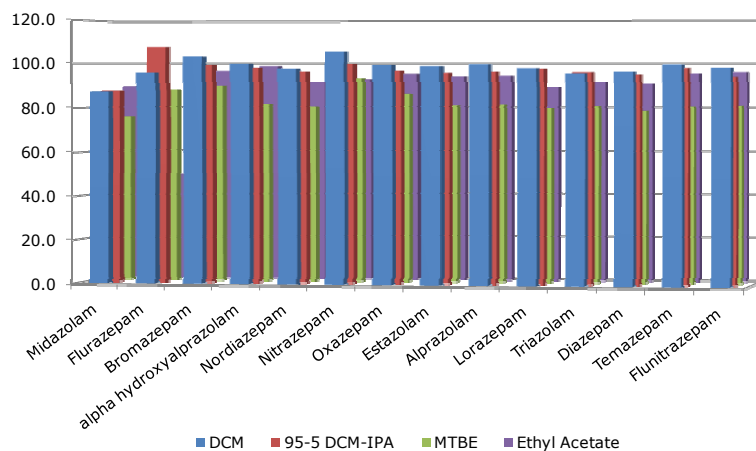
**Collision Gas Pressure:**  $2.6 \times 10^{-3}$  mbar

### Results

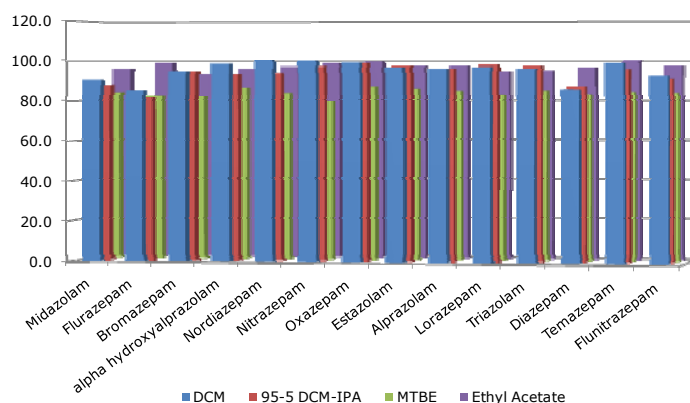
**Figures 1-3.** show the recovery charts for extraction of various benzodiazepines from plasma, urine and whole blood, respectively. Recoveries > 80% were observed for the majority of analytes from all three matrices using various extraction solvents. **Figures 4 and 5.** show the recovery charts for plasma using a 1% formic acid pre-treatment and urine with 0.5M NH<sub>4</sub>OH pre-treatment. All RSDs were below 10%, demonstrating reproducible extraction and recoveries.



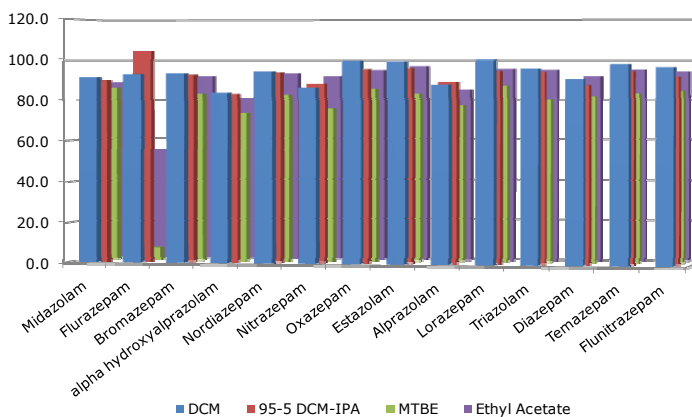
**Figure 1.** Benzodiazepine recovery profile from plasma using H<sub>2</sub>O pre-treatment



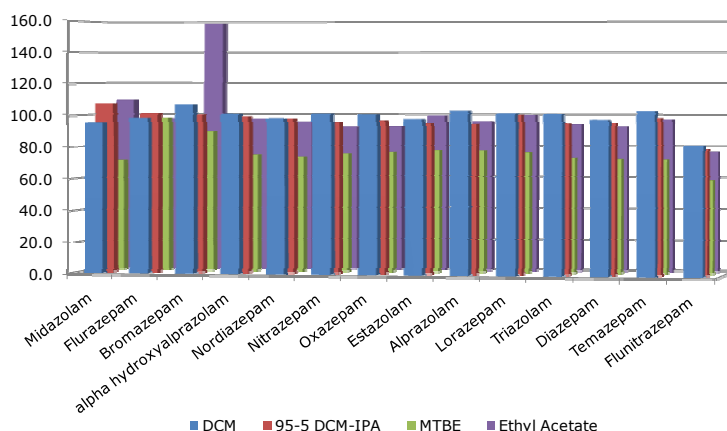
**Figure 2.** Benzodiazepine recovery profile from urine using  $H_2O$  pre-treatment



**Figure 3.** Benzodiazepine recovery profile from whole blood using  $H_2O$  pre-treatment



**Figure 4.** Benzodiazepine recovery profile from plasma using 1% formic acid aq pre-treatment



**Figure 5.** Benzodiazepine recovery profile from urine using a 0.5M NH<sub>4</sub>OH pre-treatment

## Overall Conclusions

- Pre-treatment was investigated using 1% formic acid, 0.1% formic acid, H<sub>2</sub>O and 0.5M NH<sub>4</sub>OH for plasma and urine but only 0.1% formic acid and H<sub>2</sub>O for whole blood (not all data included due to space limitation).
- The best extraction conditions were observed using sample pre-treatment with H<sub>2</sub>O.
- MTBE generally showed slightly lower recoveries than other extraction solvents for all conditions.
- High reproducible recoveries were observed using various extraction combinations.

This poster shows the applicability of SLE+ to benzodiazepine extraction. A variety of sample pre-treatment/extraction solvent combinations are possible leading to the possibility of tailoring the extraction

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