UHPLC Analysis of benzodiazepines and their metabolites by LC-MS/MS (using YMC-Triart C18)

Overview

The analysis of benzodiazepines in biological fluids is important to clinicians and forensic toxicologists because of their sedative, hypnotic, antianxiety, antiepileptic and muscle relaxant properties. It is challenging to analyse a mixture of etizolam, triazolam, and their metabolites because their chemical structures, molecular weights and fragmentation patterns during mass spectrometry are very similar. This application note, developed by Shimadzu (Japan)[1], shows a high-resolution separation using the robust YMC-Triart C18 for the simultaneous analysis of etizolam, triazolam, and their metabolites 8-ethylhydroxyetizolam, alpha-hydroxyetizolam, 4-hydroxytriazolam and alpha-hydroxytriazolam.

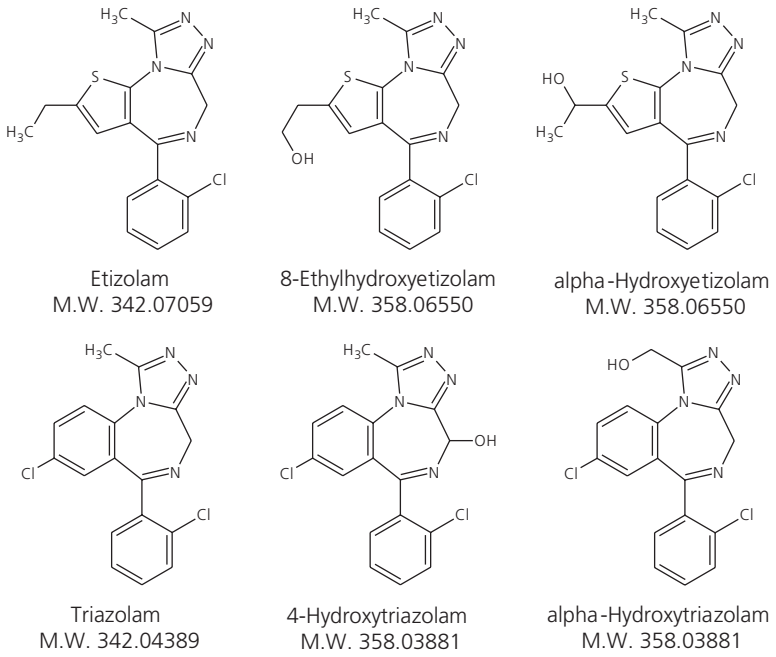
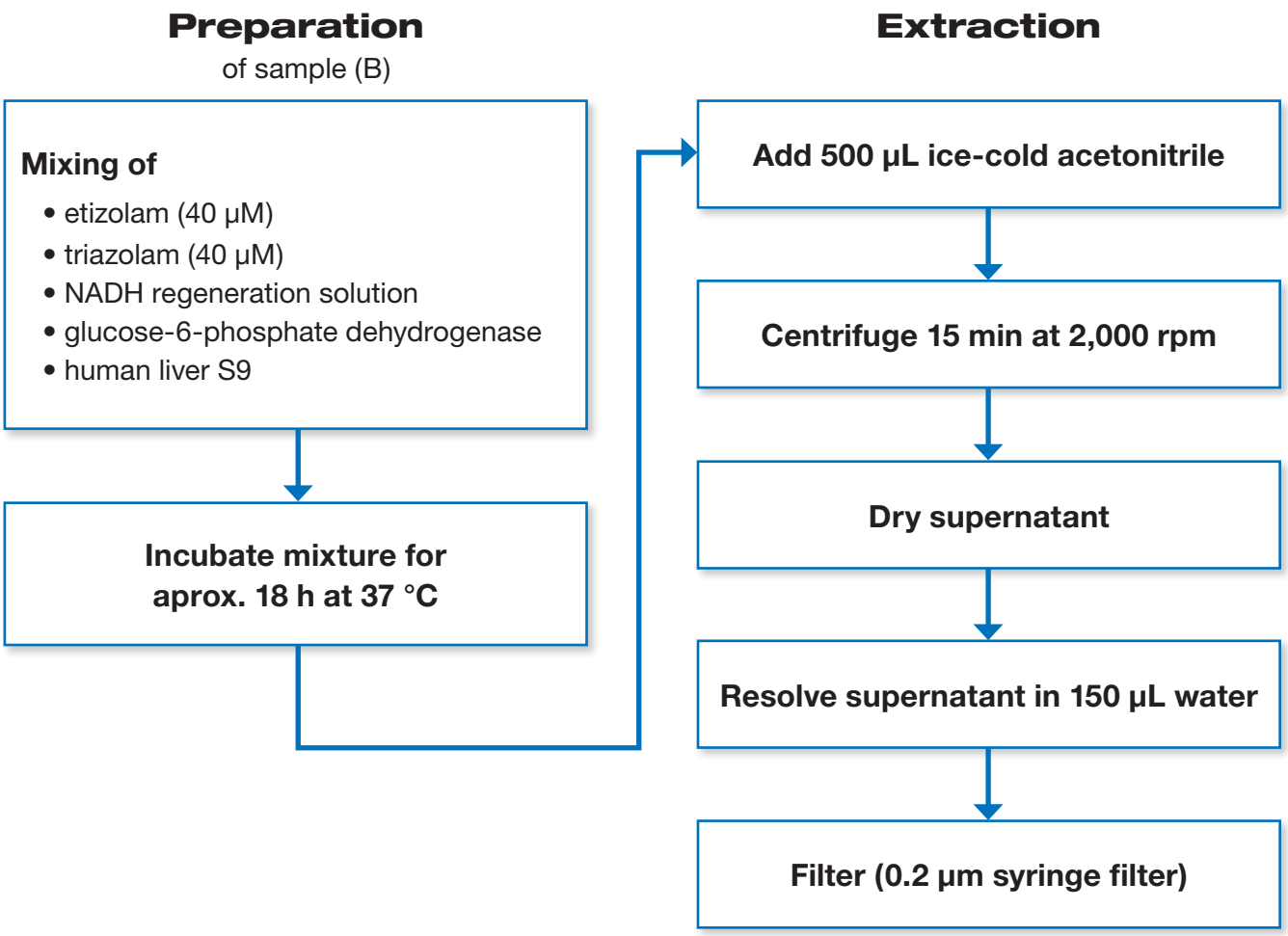


Figure 1: Structure of etizolam, triazolam and their metabolites.[1]

Three samples were analysed: a mixture of standards including etizolam, triazolam, alpha-hydroxytriazolam and 4-hydroxytriazolam (A), metabolised etizolam and triazolam using human liver S9 (mix of liver enzymes) (B) and blank metabolised matrix (C).

Sample Preparation



NADH regeneration solution:
1.6 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 3.3 mM magnesium chloride

glucose-6-phosphate dehydrogenase:
in citrate buffer, 0.4 U/mL

human liver S9:
in phosphate buffer (100 mM, pH 7.4)

The control sample (C) was prepared in the same way, but without etizolam and triazolam.

Table 1: Chromatographic conditions

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 150 x 2.0 mm ID
Part.no.:	TA12SP9-1502PT
Eluent:	A) 10 mM formic acid
	B) 10 mM formic acid in acetonitrile
Gradient:	40 % B (0 min), 65 % B (40 min), 40% B (40-60 min)
Flow rate:	0.3 mL/min
Injection volume:	1 µL
Column Temperature:	40 °C
Detection:	LCMS-8030 (Shimadzu Corporation), ESI-MS, positive

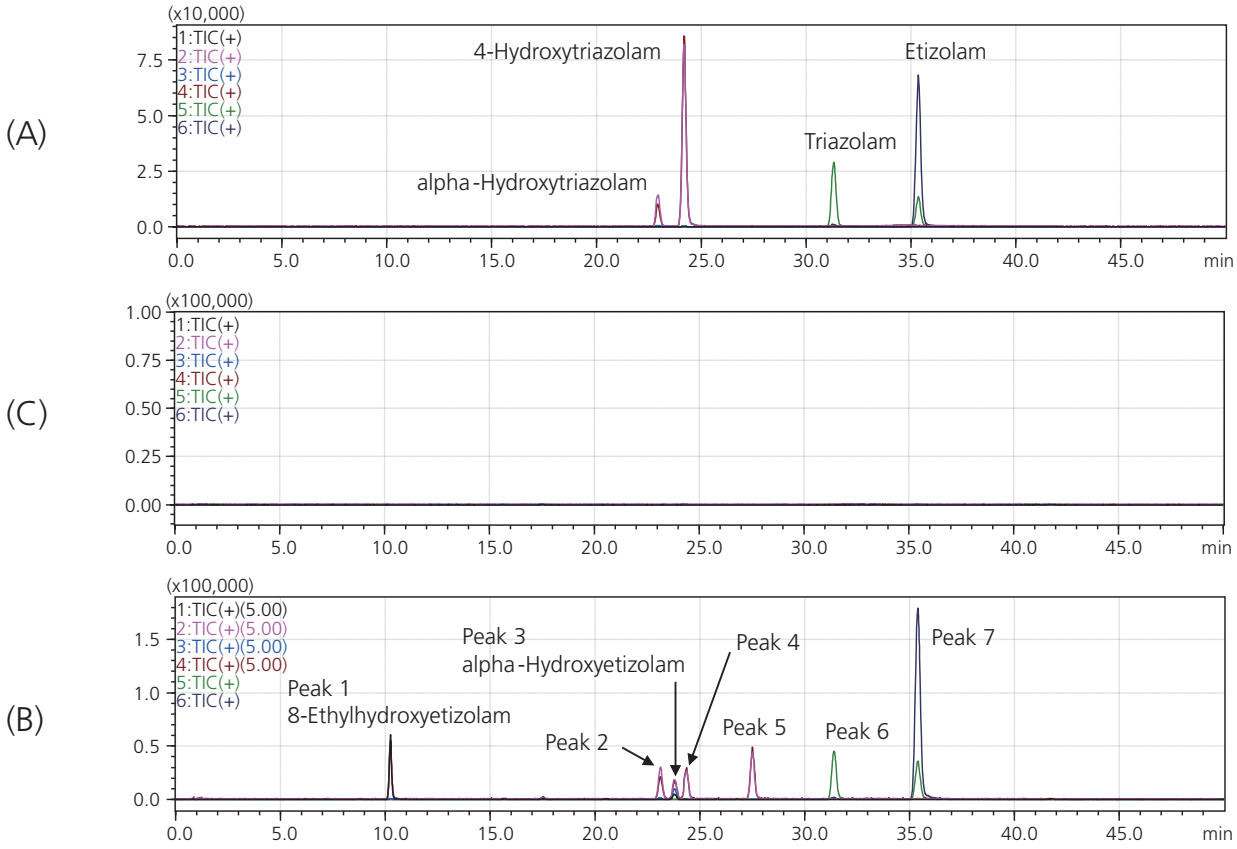


Figure 2: Chromatographic results of the 3 samples.)[1]

Results

Figure 2 shows the chromatographic results of the three samples analysed. Sample (A) was separated into 4 peaks which could be assigned to the 4 standards used. As expected, the blank matrix sample (C) showed no signal. In sample (B) the metabolised matrix of etizolam and triazolam showed 3 additional signals compared to sample (A). Two of these additional signals can be allocated to 8-ethylhydroxyetizolam (peak 1) and alpha-hydroxyetizolam (peak 3). The other signals can be allocated as follows: peak 2 to alpha-hydroxytriazolam, peak 4 to 4-hydroxytriazolam, peak 6 to triazolam and peak 7 can be allocated to etizolam.

Summary

In this application the separation of etizolam, triazolam and their metabolites using a YMC-Triart C18 column coupled to characterisation via MS is shown.
Furthermore, instructions for the sample preparation are given.

YMC-Triart C18 is an ideal choice for LC/MS-separations because it provides:

- High resolution
- Superior reproducibility
- Low bleeding

[1] M. Matsui, T. Minohata, N. Shoji, N. Kuriyama, C. Yokoyama, K. Matsumoto, J. Watanabe, J. Iida, “Identification of triazolam, etizolam and their metabolites in biological samples by liquid chromatography tandem mass spectrometry”, Application Note Shimadzu Corporation, Kyoto, Japan, Sep. 2012