Development of the Method for Therapeutic Drug Monitoring of Selected Benzodiazepines by Liquid Chromatography

Kateřina Krátká[1], Pavel Šištík[1,2]

[1]*Institute of Laboratory Medicine, Faculty of Medicine, University of Ostrava, Syllabova 19, 703 00 Ostrava, Czech Republic*

[2]*Department of Clinical Pharmacology, Institute of Laboratory Medicine, University Hospital Ostrava, 17. listopadu 1790, 708 52 Ostrava, Czech Republic*

E-mail: katerina.kratka@fno.cz

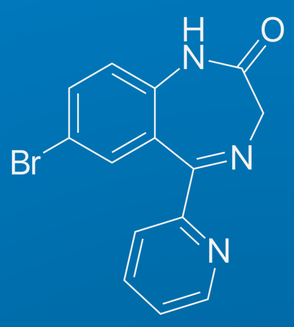
Introduction

Benzodiazepines are one of the most used psychopharmaceuticals today. There were about 48.84 defined drug doses per 1000 inhabitants in the Czech Republic between 2008–2010 [1]. However, these substances are also abused as illegal drugs [2]. For these reasons, we need fast and sensitive methods for their determination in biological samples. The aim of this work was to develop a UPLC/MS method for the determination of alprazolam (ALP), bromazepam (BRO), clobazam (CLOB), diazepam (DIA), clonazepam (CLON), norclobazam (N-CLOB) and nordiazepam (N-DIA) in patient serum.

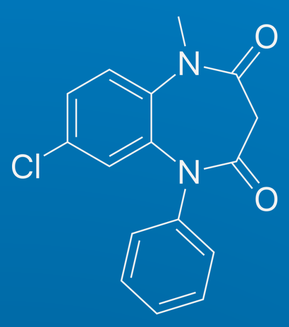
Structures

Alprazolam

Bromazepam

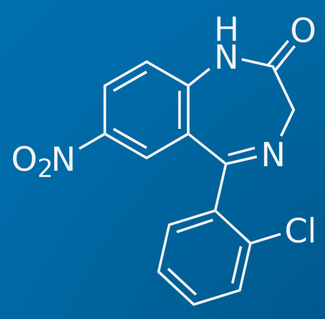


Clobazam

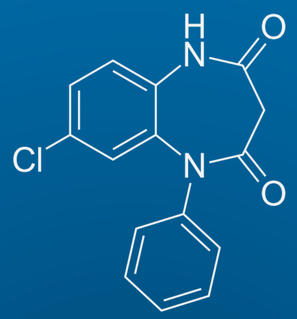


Diazepam

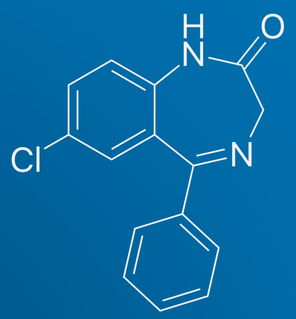
Clonazepam



Norclobazam



Nordiazepam



Experimental

Apparatus: Acquity UPLC with Acquity qDA detector (Waters, USA)
Column: Acquity UPLC C18 50×2.1 mm, 1.7 µm (Waters, USA), temperature 50 °C

Mobile phase: gradient elution, flow rate 0.5 ml/min, injection 10 µL
D: ammonium acetate (2 mmol/L) in 5% ACN + 0.1% HCOOH (pH 6.7)
E: ammonium acetate (2 mmol/L) in 95% ACN + 0.1% HCOOH (pH 8.9)

Time (min)	Flow rate (ml/min)	MF D (%)	MF E (%)
0.00	0.5	80	20
4.00	0.5	80	20
4.10	0.5	80	20
7.00	0.5	50	50
7.50	0.5	40	60
7.60	0.5	0	100
9.00	0.5	0	100
9.10	0.5	80	20
11.00	0.5	80	20

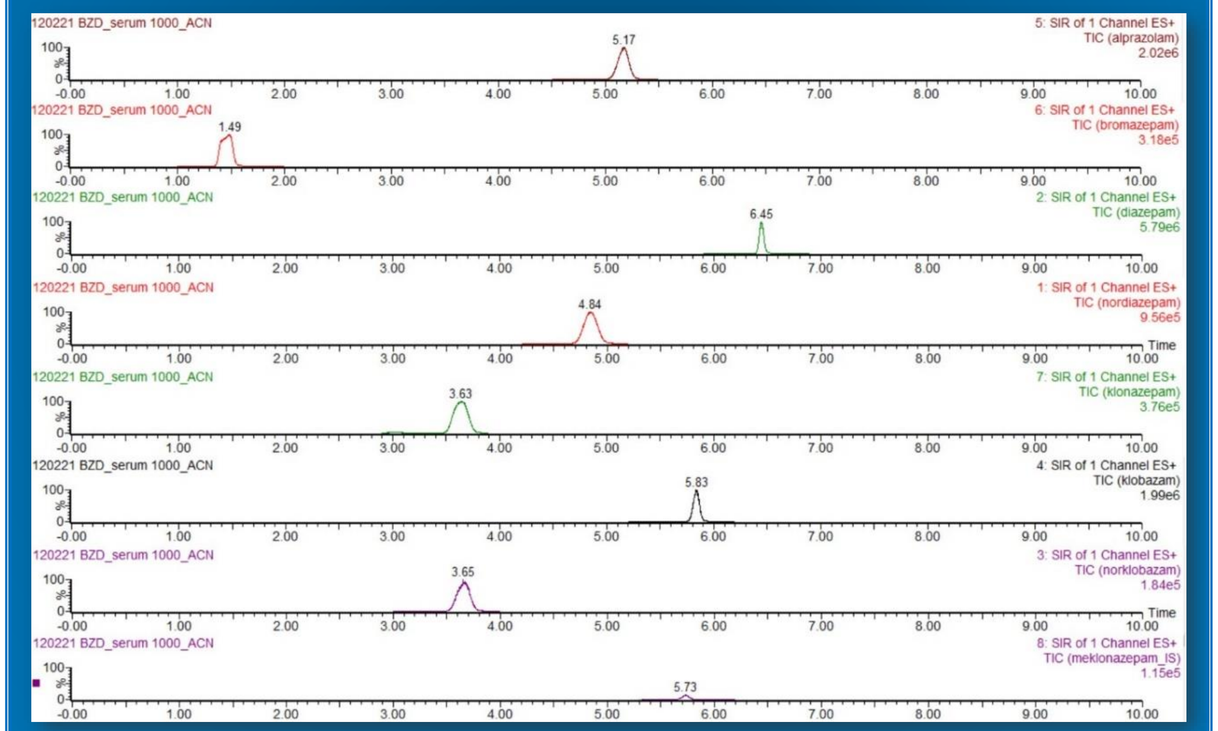
Results

The detection of all 7 mentioned benzodiazepines in a single run was achieved on UPLC system with qDA mass spectrometer detector. The MS detection was based on SIR mode with ESI+ ionization. Patient serum samples were prepared using the protein precipitation method. The developed method was validated according to the FDA criteria (variation coefficient was in the range 0.10–7.86% and recovery was 97.5–109.6% for all analytes). The limit of quantification is 1 ng/mL for all analytes except clobazam, which has LOQ 5 ng/mL).

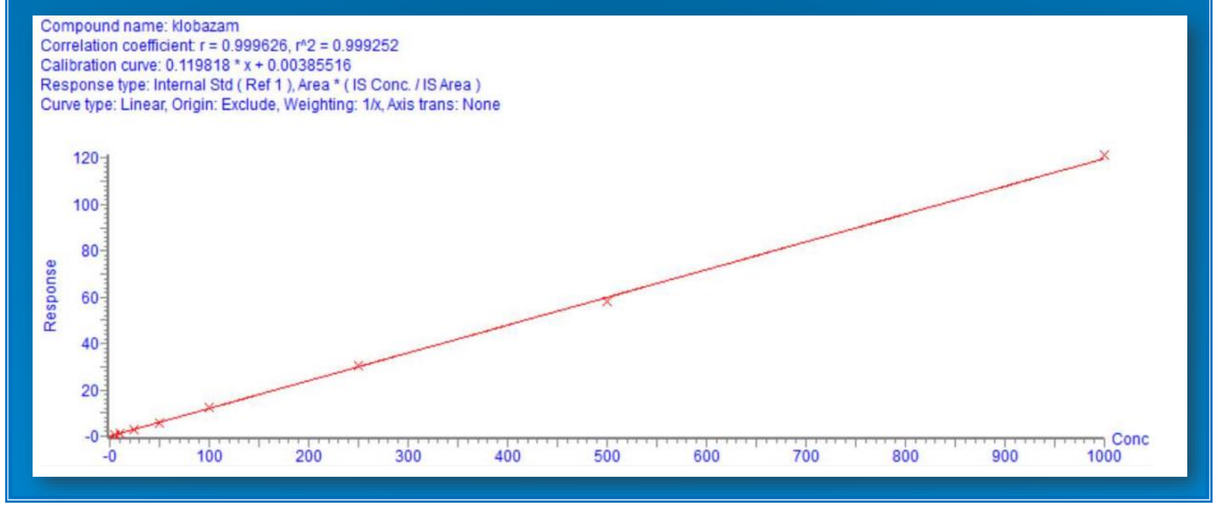
Parent ions of searched benzodiazepines in MS detection

Analyte	ALP	BRO	CLOB	DIA	CLON	N-CLOB	N-DIA
Parent ion (m/z)	309.1	316.0	301.1	285.1	316.0	287.1	271.1

UPLC/MS chromatogram of 7 benzodiazepines in serum (1000 ng/mL)



Calibration dependence of clobazam measured by the UPLC/MS method



Conclusion

A new UPLC/MS method was developed for simultaneous determination of seven benzodiazepines in serum after protein precipitation. This method is based on gradient elution of mobile phases consisting of ammonium acetate and ACN, with C18 column, ESI+ ionization and qDA detection. The developed method was validated according to the FDA criteria and was successfully applied for benzodiazepines detection in patient serum at the department of clinical pharmacology of University Hospital Ostrava.

References

1. [Psychotropic Substances] - Part three. International Narcotics Control Board [online]. Vídeň: Vienna International Centre International Narcotics Control Board, [2020] (accesed 05.05.2024).https://www.incb.org/documents/Psychotropics/technicalpublications/2011/Table_IV_levels_of_consumption_PSY_2011english.pdf.
2. I. I. Papoutsis, et al., J. Pharm. Biomed. Anal. 52 (2010) 609–614.

Acknowledgements

Authors thank the University hospital Ostrava for technical and financial support provided during the development of the new method.