### RESULTS AND DISCUSSION

The current spectral library contains 12 designer benzodiazepines and three prescriptions of conventional benzodiazepines most common in Germany, allowing screening for 53 benzodiazepine-type substances. The method can easily be extended into one new designer benzodiazepines emerge on the drug market or according to specific needs of the user. The limit of detection was 5 ng/ml for the majority of the analytes, whereas nine compounds could only be detected at concentrations above 10 ng/ml. No interference, being highly stable in serum or during alkaline extraction, was the only compound that could not be detected at practically relevant concentrations. In serum, linear ranges of recently marketed metabolites or degradation products could not be detected in MS².

For each analyte, the slope of the calibration curve was determined and calculated concentrations within this range were reported as semi-quantitative results. The results of this study not only MS² analysis could be extended by compounds that are precursors (e.g. diclazepam) was enlarged by compounds that are precursors (e.g. diclazepam). Considering the fact that patents and scientific literature describe the synthesis and detailed results of clinical model studies, more than a hundred different benzodiazepines, it can be assumed that this sub-group of NPS will extend quite possibly in the future. Several applications demonstrate that semi-quantitative information can be obtained from ion trap screening data using single linear range, the obtained accuracy allows to distinguish therapeutic, sub-therapeutic and potentially toxic serum levels.

### CONCLUSIONS

The present method allows automated identification and semi-quantitative determination of designer benzodiazepines, including 11 designer benzodiazepines. Limits of detection of the assay allow the detection of sub-therapeutic concentrations or concentrations in the low linear range for the majority of medical benzodiazepines, making the screening applicable for clinical and forensic analysis. This study provides a valid tool for forensic laboratories.

### REFERENCES


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**OVERVIEW**

- Screening for benzodiazepines and designer benzodiazepines
- Spectra recording and library building

**INTRODUCTION**

In 2012 the group of Neubauer et al. described a new method for the LC-MS² screening of benzodiazepine-type substances. The method is developed to be able to detect compounds with LC-MS² analysis. The linear range was limited to 250 ng/ml. To set up the semi-quantitative part of the screening linear range of each compound has to be evaluated. The limit of detection (LOD) was set at the lowest concentration still leading to a positive identification by the DataMatrix in replica determination.

**METHODS**

Sample Preparation (Alkaline liquid-liquid extraction) Assay

- Preparation of 1 ml urine sample from 40 mg ketamine (500 ng/ml) and 1 ml urine after administration of 500 ng/ml of each compound. The sample was diluted to 1 ml with water and 1 ml of serum. The sample was diluted to 1 ml with water.

**LC Systems**

- Dionex Ultimate 3000 LC System

**Eluent**

- Water: 0.1% ammonium formate, 0.1% formic acid, 1% acetonitrile

**Eluent B**

- Acetonitrile: 0.1% ammonium formate, 0.1% formic acid, 1% water

**Gradient**

- LC²/m/z (150 ng/ml - 500 ng/ml)

**MS - Settings**

- Bransky standard A² is triplicate; positive mode
- Eluent B: 5% - 95% in 10 min

**Data Evaluation and Reporting**

- Data was evaluated and processed by ABI Sciex Mass Biosoftware Data Interchange 2.0 (DAI²). The data was processed and report according to the forensic laboratory.

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