On-line extraction of benzodiazepines using restricted access media and monolithic silica support with a column-switching approach for LC-MS analysis

A. BUGEY and C. STAUB

Institute of Forensic Medicine, University of Geneva, Av. de Champel 9, 1211 Geneva 4, Switzerland

AIMS: Since their introduction, silica monolithic supports are in the forefront, because of their properties, which allow fast separations at elevated flow rates without generating a high back pressure and without any loss of chromatographic properties. Consequently, these media have found widespread applicability in reversed-phase HPLC for performing very fast and highly efficient separations. Thanks to these features, a fast LC-MS method, which combines on-line sample extraction through a restricted access precolumn and separation with a silica monolithic support, has been developed for the simultaneous determination of eight benzodiazepines in whole blood.

METHODS: Proteins of blood sample (200 µL) were precipitated with 400 µL of acetonitrile containing the internal standard. After vortex mixing and centrifugation (5 min, 5000 rpm), 300 µL of the supernatant were evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 100 µL of washing mobile phase and 50 µL was then injected onto a restricted access media (Lichrospher® RP-8 ADS, 25 × 4.6 mm i.d., Merck). Macromolecules were washed off with aqueous washing mobile phase (5 mM ammonium adjusted at pH 3 with formic acid – acetonitrile, 98:2), while small compounds were retained onto the support. After extraction time of 2 minutes, the valve was switched to elute target compounds in the backflush mode to the analytical monolithic support (Chromolith™ Performance RP-18, 100 x 4.6 mm i.d., Merck) with eluting mobile phase (5mM ammonium formate adjusted at pH 3 with formic acid – acetonitrile, 60:40).

All experiments were performed on an Agilent Series 1100 system (Agilent, Waldbronn, Germany), equipped with an autosampler, degasser, and quaternary pump. MS detection was conducted using an Agilent Series 1100 MSD single quadrupole, VL version, equipped with an APCI interface. For quantification, molecular target ions were used in the positive selected ion monitoring (SIM) mode.

RESULTS: Column-switching conditions have been optimised for extraction of blood samples containing eight benzodiazepines amongst the most prescribed in Switzerland namely clonazepam, diazepam, flunitrazepam, lorazepam, midazolam, N-desalkylflurazepam, nordiazepam, and oxazepam.
The LC-MS procedure was validated according to the guidelines of the “Société Française des Sciences et Techniques Pharmaceutiques” (SFST) in the concentration range of 5 to 500 ng/mL. The limit of quantification was 5 to 10 ng/mL (depending on compound analysed). Validation data, including linearity, precision, and trueness were obtained, allowing subtherapeutic quantification of frequently low-dosed benzodiazepines.

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CONCLUSIONS: This column-switching device based on monolithic separation appeared a powerful tool for fast determination of drugs in biofluids. Indeed, the present procedure allowed a total analysis time of 7 minutes to be achieved.
Moreover, the method has been proven suitable for the determination of benzodiazepines in post-mortem blood samples. Finally, this column-switching device allowed to reduce sample handling with potentially infectious blood sample.

KEYWORDS: *Benzodiazepines, Column-switching, Monolithic Silica, HPLC-MS*

Corresponding author: christian.staub@hcuge.ch