Molecularly imprinted polymer for solid-phase extraction of diazepam and its metabolites from hair samples

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BACKGROUND AND AIM: Molecularly imprinted polymers (MIPs) are synthetic crosslinked polymers with strategically positioned binding sites for a selected analyte, creating the potential for MIPs to function as selective solid phase extraction (SPE) sorbents. An MIP is prepared in-situ by mixing the template, functional monomer and cross-linkers in a suitable porogen. After polymerization, the template is removed, leaving behind imprinted binding cavities within the polymer network. The aim of this study was to develop an anti-diazepam MIP to be used as a specific sorbent for SPE of benzodiazepines from hair.

METHODS: An anti-diazepam MIP was synthesized based on work done by Vlatakis et. al. [G. Vlatakis, L. I. Andersson, R. Muller and K. Mosbach, Nature, 361 (1993) 645-647]. The resulting MIP was ground, sieved and packed in SPE cartridges. A molecularly imprinted solid-phase extraction (MISPE) procedure followed by LC-MS-MS analysis was optimised in the same manner as conventional SPE. The selectivity of the MIP sorbent was studied, using non-imprinted polymer as a control sorbent. The method demonstrated excellent accuracy and precision and good selectivity for diazepam. HPLC separation was carried out using a Gemini C18 column with gradient elution of 3 mM ammonium formate + 0.001% aqueous formic acid and acetonitrile at a flow rate of 0.3 ml min-1. Ionization of analytes was performed using electrospray ionisation (ESI) in the positive ion mode. The nitrogen sheath and auxiliary gas flow rates and collision energy were set at the optimized conditions for each analyte.

RESULTS: The recoveries of diazepam standards at concentrations of 20 and 50ng per 30 mg blank hair were 90.3% (RSD = 10.2%) and 93% (RSD = 1.5%), respectively compared to the non-imprinted polymer, which gave recoveries of 11.25 (RSD = 24.9%) and 16.3% (RSD = 17.1%) respectively. The LOD of diazepam in spiked hair samples was 0.09 ng per 30 mg hair. Also, extracts were cleaner than with conventional SPE, showing fewer interferences in LC-MS-MS. The method was also found to be applicable to the analysis of the diazepam metabolites nordiazepam, temazepam and oxazepam and other benzodiazepine drugs, including 7-aminoflunitrazepam, lorazepam, chlordiazepoxide, flunitrazepam and nitrazepam. The recoveries of 50 ng of standards of these drugs spiked into blank hair were between 39-102.9 % (RSD ≤ 17 %) and LODs ranged from 0.03 to 0.78 ng/mg. The MISPE procedure was applied to the screening of post-mortem hair samples from drug related deaths and gave results which were in good agreement with those from enzyme linked immunosorbent assay (ELISA) data and also with the results of analysis of the corresponding autopsy blood samples using a validated in-house LC-MS method.

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CONCLUSIONS: The performance characteristics of the method incorporating MISPE, in terms of sensitivity, selectivity, accuracy and precision indicate that it is applicable to the analysis of trace concentrations of diazepam and its metabolites in hair samples.

KEYWORDS: *Molecular imprinted polymer, Benzodiazepines, Hair samples analysis*

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