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Application of size exclusion chromatography and time-of-flight mass spectrometry to screening of polysaccharide-based plasma expanders

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Abstract

Dextran (DEX) and hydroxyethyl starch (HES) are polysaccharide-based glucose containing plasma expanders and are prohibited in sports as masking agents at all times. In DEX glucose molecules are mainly α-1,6 linked and in HES α-1,4 linked. In addition, in HES a part of glucose molecules are hydroxyethylated at positions C2, C3 and C6. The common pharmaceutical products include DEX with molecular weight of 40 or 70 kDa whereas HES has a molecular weight of 130 kDa and an average molar substitution of 0.4. After intravenous infusion both compounds are enzymatically cleaved in the body into appropriate sized fragments (10-40 kDa for DEX and 30-40 kDa for HES) and excreted in urine. The molecular distribution profile of the polymer excreted in urine varies and is dependent on e.g. molecular weight, substitution and branching degree of the drug as well as dosage and sample collection time.

At the moment, several techniques have been applied to screen HES and DEX in urine. Most common are chromatographic (GC and LC) mass spectrometric methods even though other such as enzymatic or colorimetric methods has been applied. The disadvantage of enzymatic and colorimetric methods is the lack of adequate specificity for DEX and HES. The chromatographic methods are specific for HES, but normal and abnormal urinary glucose and glucose-containing oligosaccharide content interferes with the detection of DEX and has lead to a suggestion of a threshold level of 500 µg/ml. Due to specificity issues unnecessary confirmatory analyses have to be performed wasting laboratory resources.

Here we present a novel approach for the screening of DEX and HES in human urine. The method is based on size exclusion chromatography – in-source collision induced dissociation - time-of-flight mass spectrometry (SEC-ISCID-TOFMS). Specificity is increased by separating interfering lower molecular weight matrix compounds from larger sized target molecules. The detection of the target analytes is based on three specific ISCID produced fragments with accurate mass for each fragment. With a straightforward sample preparation and short analysis time using solely a guard column, a high throughput method for screening of DEX and HES is achieved.

For the complete paper, please, see the following reference:

Kolmonen M, Leinonen A, Kuuranne T, Pelander A, Deventer K, Ojanperä I. (2011) Specific screening method for dextran and hydroxyethyl starch in human urine by size exclusion chromatography – in-source collision-induced dissociation – time-of-flight mass spectrometry. *Anal Bioanal Chem*, Published online 17 March, doi 10.1007/s00216-011-4838-1