High efficiency of semi-preparative Supercritical Fluid Chromatography with Molecularly Imprinted Polymer as stationary phase (SFC-MIP). Application on urinary steroids purification for IRMS analysis.

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Introduction

In spite of numerous and significant advances in terms of analytical instrumentation during the past two decades, sample preparation still remain a key and a critical step that determines the final performances of the developed analytical process. Recently, the use of sorbents like Molecularly Imprinted Polymers (MIPs) has been considered as a relevant technique of sample purification since such material can present an adjusted selectivity for a specific class of compounds. Nevertheless, as shown later, MIPs sometimes behave like a reverse-phase sorbent and specific interactions are limited particularly when biological samples are loaded on it. In this context, the aim of the present study was to assess the capability of SFC-MIP to reach a better selectivity. The optimized sample preparation strategy was then implemented on urine samples in the scope of confirmation of anabolic steroid abuse in cattle by GC-C-IRMS. IRMS analysis was chosen because of the high degree of purification needed due to its poor specificity.

Material

SPE-MIP

MIP SPE: AffinIMIP®

- Estrogens, Polynit, Particles diameter range: 25–80 µm.

GC-MS

- Instrument: GC-MS-6897 Agilent
- Column: OptimaL-MS, 30 m x 0.25 mm i.d., 0.25 µm
- Injection: Full Scan (50 – 500 m/z)
- Oven: 60°C (1 min), 20°C/min to 220°C, 5°C/min to 270°C (2 min), 1°C/min to 290°C, 20°C/min to 320°C (3 min).

SFC-MIP

- Instrument: Investigator SFC system, Waters
- Column: AffinIMIP® Extract, Polynit, (450 = 2.6 mm i.d., 12-25 µm particle size)

SAMPLE PREPARATION

5 mL of urine from untreated pregnant cow was spiked with 12 natural steroids (11 androgens (A) and 17α-estradiol (E)) and then deconjugated by β-Glucuronidase E. Coli.

The use of MeCN in SFC-MIP increased specific recognition. Chromatographic separation of mono- (AM) and dihydroxylated (AD) androgens has been achieved. Nevertheless, estradiol was still retained on the column using these conditions. Addition of 5% of MeOH in MeCN allowed to elute estradiol while preserving specificity.

GC-MS: purity assessment

All purified extracts were analyzed by GC-MS and their chromatograms, as well the associated mass spectra and the absence of co-elution with targeted steroids, allowed us to assess their purity. Regarding the mass spectra obtained, the noise was divided by a factor of ten between SPE-MIP and SFC-MIP methods. Moreover, targeted steroids present less co-elution with SFC-MIP methods. To conclude, SFC-MIP technique was more efficient than the SPE MIP. Indeed, the presence of water prevents specific recognition to take place. Because of the higher purification of the fraction F(E) obtained with the gradient MeCN/MeOH, this method was applied to the selective isolation of urinary steroids in the further scope of IRMS analysis.

Application on urinary steroids for IRMS analysis

Sample preparation with SFC-MIP MeCN/MeOH method was applied on urine samples collected from treated cows with 17β-bolsterone (B), 17β-testosterone (T), 17β-estradiol (E2) and androstenedione (AED) (sampling between 1 and 3 days after injection)

All samples were analyzed in triplicate for MeCN/MeOH gradient with m/z 13 and 12 spectra. 2 µl was injected of the diluted fraction from SPE-MIP. Chromatograms showed the presence of the labeled steroid from SPE-MIP. Total ion chromatograms were compared to the labeled standard from SPE-MIP.

Conclusion

MIP-SFC showed a better selectivity compared to MIP-SPE method. Indeed, removal protic solvent allowed to increase specific recognition to estradiol by electrostatic interactions. Furthermore, chromatographic separation of estradiol, mono- and dihydroxylated steroids has been achieved with aprotic solvent. The purification obtained by the MIP-SFC method on the urine samples from treated cows was sufficient for IRMS analysis. Thus, with this strategy, differences of δ13C values between Endogenic Reference Compounds (ERC), bolosterone and AED metabolites from urine samples of treated cows were demonstrated for the first time. In this study, we proved the high potential of MIP when used as a stationary phase in SFC as purification technique. To conclude, this sample preparation technique can be used for small organic compounds purification.