

# Detection of Small Peptide Hormones for Anti-Doping Purpose via a Molecularly Imprinted Polymer-Based SPR Assay <sup>†</sup>

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Currently, there is a lack of low-cost, prompt and robust bioanalytical methods to detect small peptide hormones (e.g., gonadorelin, busorelin, leuprorelin, etc.) in the routine anti-doping protocol. These peptides are improperly used by male athletes to improve their sports performances by stimulating the endogenous secretion of testosterone in the bloodstream via the hypothalamic-pituitary-gonadal axis. For this reason, low molecular weight peptide hormones (<2000 Da) were banned by the World Anti-Doping Agency (WADA) and represent a new frontier in antidoping research. Our study aimed to design a molecularly imprinted polymer (MIP)-based assay to detect gonadorelin (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>) levels in urine samples. The process of molecular imprinting involves the synthesis of a polynorepinephrine (PNE) network structure with imprinted template binding sites. The interaction between gonadorelin and the “tailor-made” synthetic receptor has been investigated and optimized through a surface plasmon resonance (SPR) platform. Afterward, a competitive biomimetic assay was set by employing biotinylated gonadorelin tethered to streptavidin as signal-enhancer competitor molecule. This type of assay is suitable for the detection of small molecules that lack multiple epitopes. Encouraging results were recorded for gonadorelin in buffer and spiked artificial urine samples (av%recovery = 96.30%) in the low-ppb range, perfectly in line with the Minimum Required Level (MRPL = 2 ppb) at which all WADA-accredited laboratories must operate in routine daily operations.

Moreover, we intend to provide a strategy to detect gonadorelin which can be easily miniaturized to set-up a sensing device for in-situ athletes’ monitoring.

## References

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