

“Erythropoietin Receptor Beads: Comprehensive isolation and analysis of erythropoiesis-stimulating agents (ESAs)”

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Project overview:

In the past years, the number of reports on novel and innovative pharmaceuticals in relation to erythropoietin mimetic agents (EMA) increased considerably. The future perspective, to generate structures with a prolonged effect in patients by retaining the benefits of erythropoietin, led to significant changes in the molecular structure in emerging drug candidates. As a consequence for doping controls, the detection of an illicit intake of such compounds by athletes has continued to represent a complex analytical challenge.

The use of monoclonal antibodies for every variation and new structure is possible; however, anti-doping laboratories can profit from one single feature of all EMA – the affinity for the erythropoietin receptor (EPOR).

1. A modern cost- and time-effective tool ensuring low consumption of sample material and consumables particularly in peptide analytics, is the application of antibody-coated magnetic beads. A substitution of the antibody with the EPOR provides the platform for a new type of easy-to-use screening assay.
2. With regard to their widespread nature (i.e. from all forms of recombinant Epoetins to pegylated dimerized peptide molecules such as Peginesatide), an EMA-enriched extract is obtained that can be subjected to established as well as future detection and identification methods for prohibited erythropoiesis-stimulating and EPOR activating agents.
3. Due to the unification of urine pre-purification steps, cost intensive processes can be diminished and simplified; enabling at the same time a faster screening procedure.

The enrichment and isolation of low-concentrated proteins from complex matrices such as human urine is a valuable workup for further analysis and optimizes the exploitation of sample volumes provided. By using EPOR-coated magnetic nanoparticles, a pre-selection of potent erythropoiesis stimulating agents and potential non-hematological EPOR activators is accomplished, facilitating and expanding initial testing procedures for protein and peptide drugs.

Results and Conclusions:

For several years, the number of reports about developments regarding novel and innovative pharmaceuticals concerning erythropoietin (EPO) receptor-activating compounds and EPO mimetic agents (EMA) increased considerably. This class of compounds includes, amongst others,

biotechnologically derived forms of erythropoietin and their modified derivatives (Darbepoetin alfa, C.E.R.A.). The latter were developed to improve the pharmacokinetic characteristics by simultaneously maintaining their intrinsic activity. The development of small EPO-mimetic peptides (EMPs), which share no sequence homology with the primary structure of erythropoietin, introduced additional opportunities for the treatment of anemia. Contrary to the therapeutic benefits of expanding the group of EMA, these erythropoiesis-stimulating and endurance performance-enhancing drugs represent a new analytical challenge for doping control laboratories. The increasing complexity of the class of compounds has amplified the relevance of comprehensive and flexible detection methods and thus the development of a generic assay for the detection of new erythropoietin receptor (EPOR)-activating agents was established, exploiting the common feature of these compounds – their ability to bind to the EPOR. The application of magnetic beads equipped with a modified form of a monoclonal antibody proved to provide an effective sample extraction/preparation tool, ensuring reproducible results and low sample consumption. An EPOR-IgG1 fusion protein was coupled to Protein A/G-coated magnetic beads, providing the basis for the developed straight-forward receptor-affinity purification strategy for EMA. The receptor/ligand-enriched extract obtained from (doping control) urine samples is subsequently analyzed by means of established gel electrophoretic (SAR-PAGE or IEF) analytical methods or, complementary, by bottom-up liquid chromatography – (tandem) mass spectrometry. Proof-of-concept data were obtained from native and/or spiked urine specimens containing different ESAs, including darbepoetin alpha and C.E.R.A. as well as the PEGylated dimerized EMP molecule peginesatide. The use of a generic pre-purification protocol to isolate ESAs from human urine can improve the comprehensiveness of routine doping controls and allows to accelerate sample analyses while reducing costs associated with sample preparation steps. Overall, the principle of target protein isolation and enrichment by receptor-affinity purification has been shown to represent a valuable contribution to modern doping control analytical strategies, which warrants further investigation regarding the breadth of drugs possibly covered with this methodology.