CAPILLARY ELECTROPHORESIS (CE) AND FRET AS TOOLS FOR TESTING INHIBITORS OF HUMAN PROTEINKINASE CK2

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Protein kinase CK2 is of increasing impact as a target for treating neoplastic diseases. Upregulated CK2-activity can be found in a variety of tumors. Until now, the common *in vitro* assay to quantify compound-driven CK2-inhibition needs the use of radioactive isotopes. We present two novel CK2-inhibition assays that could complete or even replace the standard radiometric assay.

The first assay is based on Förster-resonance-energy-transfer (FRET) between the donor-fluorophor EDANS and the acceptor DABCYL within the CK2 substrate peptide [DABCYL]-RRRDDDSDDD-[EDANS]. This peptide possesses an elastase cleavage site adjacent to the phosphate-acceptor serine. The non-phosphorylated peptide can be cleaved by elastase and consequently FRET is hampered. Upon phosphorylation the elastase recognition site within the peptide is masked, cannot be cleaved and FRET is retained. The degree of phosphorylation is measured as donor-fluorescence intensity that develops during the loss of FRET. Thus fluorescence intensity is inversely correlated with CK2-activity.

The second assay is based on a direct product quantification of a CK2-reaction by capillary electrophoresis. The acquisition of a phosphate moiety leads to a difference in electric charge between substrate and product and enables their electrophoretic separation (“mobility-shift”). Quantification is performed by calculating the area of the product peptide peaks, recorded by UV-absorption. The IC_{50}-values of Emodin and TBB that were determined by this assay showed a good agreement with published data [1]. Subsequently, new inhibitors of human CK2 with IC_{50} values in the nanomolar range could be identified.

[1] Gratz A, Götz C, Jose J (2010) *Electrophoresis* 31:634-40.

TRACING CHEATERS IN SPORTS –
MASS SPECTROMETRY IN ANTI-DOPING RESEARCH

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In sports the (mis-)use of drugs is regulated by the World Anti-Doping Agency (WADA). Its list of prohibited substances classifies the use of several classes of substances and prohibited methods in sports as doping (Tab. 1). The analyses in doping control are mainly performed utilizing GC-MS(/MS), LC-MS/MS and isotope ratio mass spectrometry (IRMS).
Current research mainly focuses on the identification of metabolites suitable for elongated detection of classical doping agents, the discrimination between endogenously produced and synthetic congeners, the detection of new performance enhancing substances and the identification of their metabolites. This also includes the characterization of new designer steroids that are marketed as dietary supplements. Additionally the manipulation of doping control samples is detectable by the help of mass spectrometric techniques.

Tab 1: Classes of prohibited substances and methods in sports

Anabolic Agents	Peptide Hormones, Growth Factors and and related Substances	Beta-2 Agonists	Hormone Antagonists and Modulators	Diuretics and other Masking Agents
Stimulants	Narcotics	Cannabinoids	Glucocorticoids	
Enhancement of Oxygen Transfer	Chemical and Physical Manipulation	Gene Doping	Alcohol *)	Beta-Blockers *)

*) only prohibited in particular sports

CE IN PHARMACEUTICAL ANALYSIS – APPLICATION TO DRUG IMPURITY PROFILING

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CE has been recognized as a suitable technique for the determination of the stereoisomeric purity as well as for the analysis of related substances of drugs, for the determination of organic and inorganic counterions, for the analysis of peptides and proteins, etc. [1]. This is also reflected by the fact that CE has been included as a general monograph in the European Pharmacopoeia as well as the United States Pharmacopeia several years ago. The technique is applied in several monographs of the pharmacopeias for drug identification and/or tests.

Traditionally, the stereoisomeric composition of a drug is determined by optical rotation which is not very accurate. Thus, current research efforts aim at the development of new CE methods for the determination of the stereoisomer composition of drugs. The studies include methods for the simultaneous determination of related substances of the drugs besides the stereoisomers. Such analyses are typically performed in separate tests in the pharmacopeias. Examples for the method development in cyclodextrin-mediated separations in EKC and MEEKC will be discussed.

[1] Capillary Electrophoresis Methods for Pharmaceutical Analysis, S. Ahuja, M. I. Jimidar, eds., Elsevier, Amsterdam, The Netherlands 2008.

Preparation of monolithic columns for LC-MS/MS analysis of proteins and drugs

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During the past few years monolithic supports have been used for an increasing variety of applications. The examples for applications of monoliths presented show that the chromatographic performance of bioreactors and affinity media prepared from monolithic media is superior compared to that of conventional particle-based systems. The ease of fabrication and modification combined with a long life time of the prepared columns and their potential to be used in fully automated analytical systems make them attractive tools for yet an increasing number of applications.