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RECENT ADVANCES IN DOPING ANALYSIS (5)

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CAPILLARY ELECTROPHORESIS (CE) FOR THE SCREENING AND QUANTITATIVE DETERMINATION OF SOME DIURETICS AND β-BLOCKERS

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Micellar Electrokinetic Capillary Chromatography (MEKC) and Capillary Zone Electrophoresis (CZE) have been evaluated for the analysis of some banned drugs in sports: including a screening and determination of some diuretics using MEKC and a validated quantitative determination of the β-blocker atenolol by CZE.

MEKC was applied to the screening of some diuretics: Furosemide, Piretanide, Torasemide, Etacrinic acid and Clopamide, in spiked urine samples. A good separation between the compounds and the biological matrix was achieved in less than 10 minutes. Two of these compounds: Furosemide and Piretanide were determined, in urine samples obtained from healthy volunteers after the ingestion of a therapeutic dose. A clean-up step was used involving liquid-liquid extraction with chloroform at a pH value of 2.5. The organic layer was evaporated to dryness and reconstituted with electrolyte (borate buffer, pH 9 and SDS 150mM). The sample was introduced hydrodinamically during 10 seconds, the applied voltage was 30KV and a photometric detector (diode array) was used at a 230nm wavelength. Recoveries of around 80%, very good reproducibilities (RSD below 3.7%), and a L.O.D of 25 ppb were obtained.

On the other hand, a simple Capillary Zone Electrophoresis method was developed for the quantitation of the β-blocker atenolol in human urine samples. Urine samples were collected from healthy volunteers after the intake of a therapeutic dose and patients suffering from hypertension and under treatment with atenolol. The samples were collected from 0 to 24 hours, and the compound was easily detected and quantified at all time intervals. The found concentrations were in agreement with the pharmacokinetic data. The electrophoretic separation was performed using a fused silica capillary. The electrolyte consisted on a buffer NA_2HPO_4(25mM)/$Na_2B_4O_7$ (25mM); (50/50; v/v). The sample was introduced hydrostatically for 25 s, and the running voltage was 20KV at the injector end of the capillary, photometric detection was used at a wavelength of 214 nm. Using a simple solid-phase extraction, a recovery of 70.69±3.27% and a very good separation from the urine matrix were achieved, aswell as a very good separation from other posible interfering substances present in the pharmaceutical forms (Hydrochlorothiazide, Chorthalidone, Hydralazine). A good reproducibility, linearity and accuracy are obtained, and a quantitation limit of 0.1 μg/ml, allows the method to be applied to pharmacokinetic studies.

The ability to separate a large number of closely related materials in a very short time is one of the major benefits of CE. In a single run there is essentially zero solvent comsumption and the sample injection volumes are on the order of 5 to 10 nl.[1-2]
The main drawbacks of CE are the poor concentration sensitivity and the fact that the separation buffer and the sample diluent have to be similar. For most applications the concentration sensitivity can be overcome by preconcentration of the samples, as only a very low volume is needed for the injection. As with all separation methods, it is frecuently desirable to identify the separated species. The coupling of Mass Spectrometry (MS) to CE provides sensitive mass selective detection, ultimately, structural information[3-4].

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