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The use of laser-induced fluorescence or ultraviolet detectors for sensitive and selective analysis of tobramycin or erythropoietin in complex samples

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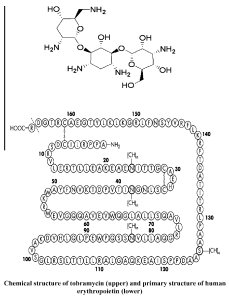
HIGHLIGHTS

- LIF detector is used for tobramycin analysis in human urine.
- Urine samples were injected directly without pretreatment.
- Erythropoietin was analyzed in the presence of albumin by CE-UV.
- EK and discontinuous buffer used to increase method sensitivity.

GRAPHICAL ABSTRACT

Chemical structure of tobramycin (upper) and primary structure of human erythropoietin (lower).



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ABSTRACT

Complex samples analysis is a challenge in pharmaceutical and biopharmaceutical analysis. In this work, tobramycin (TOB) analysis in human urine samples and recombinant human erythropoietin (rhEPO) analysis in the presence of similar protein were selected as representative examples of such samples analysis. Assays of TOB in urine samples are difficult because of poor detectability. Therefore laser induced fluorescence detector (LIF) was combined with a separation technique, micellar electrokinetic chromatography (MEKC), to determine TOB through derivatization with fluorescein isothiocyanate (FITC). Borate was used as background electrolyte (BGE) with negative-charged mixed micelles as additive. The method was successively applied to urine samples. The LOD and LOQ for Tobramycin in urine were 90 and 200 ng/ml respectively and recovery was >98% (n = 5). All urine samples were analyzed by direct injection without sample pre-treatment. Another use of hyphenated analytical technique, capillary zone electrophoresis (CZE) connected to ultraviolet (UV) detector was also used for sensitive analysis of rhEPO at low levels (2000 IU) in the presence of large amount of human serum albumin (HSA). Analysis of rhEPO was achieved by the use of the electrokinetic injection (EI) with discontinuous buffers. Phosphate buffer was used as BGE with metal ions as additive. The proposed method can be used for the estimation of large number of quality control rhEPO samples in a short period.

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Introduction

Pharmaceutical and biopharmaceutical analysis are based on qualitative and quantitative analysis of traditional and biotech

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drugs. However one of the most important challenges in analysis is the sensitivity of the analytical methods. This sensitivity is not needed only for the analysis of low detection substances but also for low concentrations. Therefore, the use of sensitive hyphenated analytical techniques, such as capillary electrophoresis techniques (CE), are increasingly in a wide range of applications. In general, CE separates the components of a sample on the bases of differences in their charge-to-size ratio, and then detects the separated components using UV or fluorescence based on their properties. However theses detectors cannot be used for the analysis of low detection substances. Also they are not sensitive enough for detection and quantitation of very minute concentrations. Therefore, derivatization reactions which give stable derivative are essential in the case of low detection substances. On the other hand electrokinetic injection (EK) coupled with discontinuous buffers are used for enhance sensitivity towards low analyte concentration. In this work, tobramycin (TOB) analysis in human urine samples and recombinant human erythropoietin (rhEPO) analysis in the presence of similar protein were selected as representative examples of such samples analysis. TOB is a member of aminoglycosides antibiotics (Fig. 1). It exhibits bactericidal activity against a broad spectrum of bacteria specially Pseudomonas-aeruginosa [1]. However when determination of the drug was required, particularly in biological fluids, its detection was complicated because of low detection sensitivity due to the poor chromophore effects and when chemical derivatization was used, poor stability of the determination was found.

The literature showed a mass spectrometric [2], spectrofluorimetric [3,4], spectrophotometric and colorimetric methods [5–7] for TOB analysis. But each of these methods is not ideal to efficiently detect TOB at trace level. Regarding to chromatographic analysis of TOB, it was analyzed by paper chromatography [8] and thin layer chromatography [9]. Gas liquid chromatography [10]. However, HPLC is the most common method of analysis of TOB [11,12] But the major drawback was the toxicity of the reagent and slowness of reaction. Also, the main disadvantages of the reported HPLC pre- and post-derivatizations were the instability of the derivatives or complicated procedures [13–18]. Few trials of separation of TOB by CE are reported [19–22]. However these methods were unlikely to give low detection sensitivities. Therefore, derivatization was done with OPA with 3-mercaptopropionic acid (MPA) and then separation of the derivatives by capillary zone electrophoresis (CZE) [23,24] or by MEKC [25] then direct UV detection. However, instability of the produced derivative was a problem.

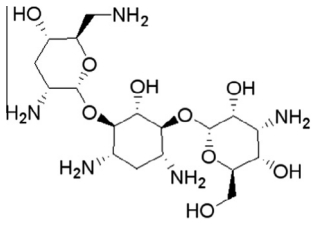

Fig. 1. Chemical Structure of TOB.

The other example used in this work is rhEPO (Fig. 2) which is a glycoprotein consisting of 165 amino acid residues. rhEPO is used as erythropoiesis-stimulating agents for renal anemia during dialysis, anemia of prematurity, and cancer related anemia worldwide. rEPO innovator and biosimilar products have been marketed in the USA, Japan, the EU and Egypt [26]. For clinical use, highly efficient methods are required to analyze recombinant proteins [27]. CE has been established as an effective analytical separation tool for a wide variety of analytes, ranging from small inorganic ions to biological macromolecules [28–31]. Separation and detection of erythropoietin by CE and CE–MS [32–36]. However, rhEPO either was alone or formulated with polysorbate 80. Albumin is used as rhEPO stabilizer and both were good separated by CE however without good sensitivity [37]. A trial to increase sensitivity was done by immunochromatographic removal of albumin in erythropoietin biopharmaceutical formulations for its analysis by CE [38]. However, this method was complex, expensive and time consuming. The European Pharmacopoeia (Ph. Eur.) monograph for Erythropoietin Concentrated Solution [39] describes a CZE method for identification of rhEPO and separation of its glycoforms. However, this method has shown poor reproducibility due to inadequate capillary conditioning [33,40]. In CE, EK is a highly controversial sampling technique. It is a simple mode of sample introduction which is suitable for on-line preconcentration of the analytes [41]. The main advantage of EK injection is that sensitivity of the methods can be by several orders of magnitude higher, and consequently, the limit of detection (LOD) correspondingly lower than using conventional hydrodynamic (HD) injection [41]. EK sampling can be exploited primarily for the separation of components of low diffusion coefficient, e.g., proteins, where the number of theoretical plates is in the order of millions [42]. The presence of salt, problematic to traditional CE methods and overly abundant in protein samples. In this work, desalting of samples followed by the

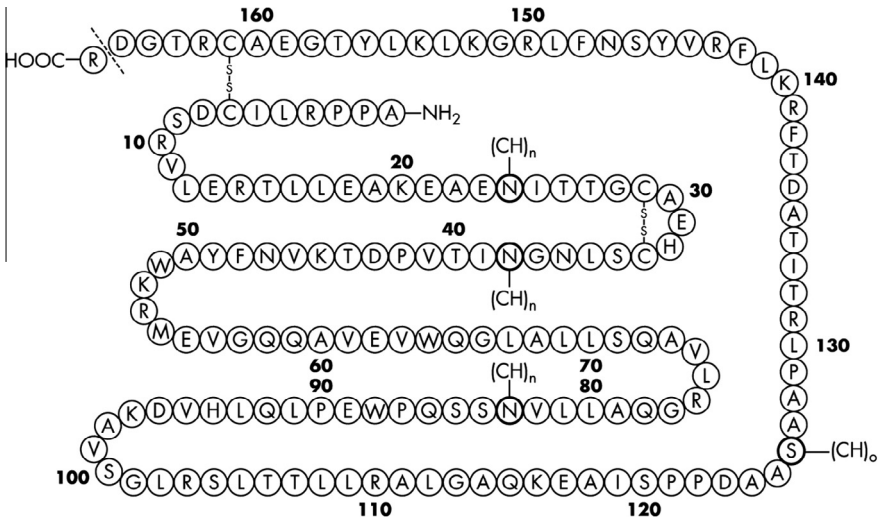

Fig. 2. Primary structure of human erythropoietin (mature hormone).

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