DETERMINATION OF HISTIDINE IN HUMAN URINE UNDER THE NEW CONCEPT OF HIGH PRESSURE LIQUID CHROMATOGRAPHY – PULSED – POST COLUMN DERIVATIZATION

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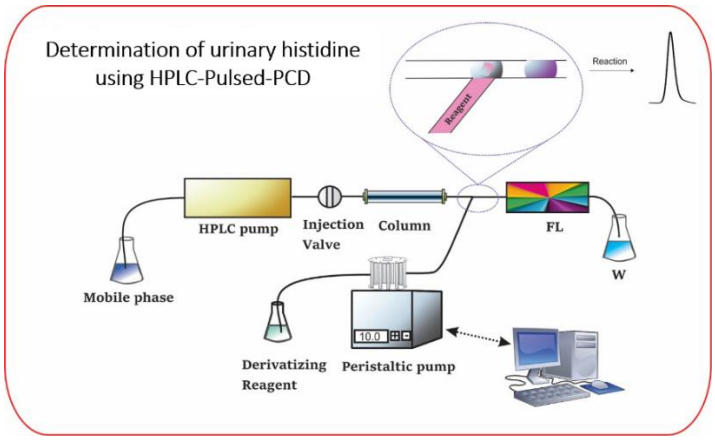
In the present work, we developed a method for the determination of urinary histidine under the new concept of Pulsed-post column derivatization (Pulsed-PCD). Histidine is an essential amino acid that has proven to play unique roles in human organism and its determination is associated with the diagnosis of histidine metabolism disorders. Pulsed-PCD is an alternative approach to minimize the consumption of the reagents in liquid chromatography coupled to on-line post column derivatization. In the proposed approach, the reagent is introduced as a well-defined pulse (at microliter levels) that overlaps the eluted zone of the analyte through precise tuning. The development of the new concept included the investigation of the tuning of the pulse with the analyte, the profile of the pulse and the robustness of the approach. Histidine was determined in eleven urine samples after separation by cation exchange chromatography and Pulsed-PCD derivatization with *o*-phthalaldehyde. The proposed method enabled the determination of the analyte in the range of 0.5 – 15 μmol L^{-1} (LOD = 0.1 μmol L^{-1}), with satisfactory precision (2-5 %) and accuracy (81 – 105 %), using a representative reagent pulse of 100 μL. The analytical results were within the normal levels and ranged between 110 and 1520 μmol L^{-1}.



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