

Direct liquid chromatography with photodiode and fluorescence detection for simultaneous quantification of gonadotropin-releasing hormone (GnRH), its complex with Cu^{2+} , and catabolites.

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Rok wydania

2009

Czasopismo

Polish Journal of Chemistry

Numer woluminu

83

Strony

383-390

Kolekcja

Naukowa

Język

Angielski

Typ publikacji

Artykuł

Gonadotropin-releasing hormone (GnRH) and its complex with Cu^{2+} (Cu-GnRH) were separated on a Nova Pak C18 column (4 m, 150 3.9 mm I.D., Waters). Analyses of underivatized GnRH and Cu-GnRH were performed by a gradient elution program (HPLC method I), UV detection at 280 nm and fluorescence detection (excitation = 280 nm/emission = 360 nm). The mobile phases used were: acetonitrile with 0.08% trichloroacetic acid (w/w) and water with 0.1% trichloroacetic acid (w/w). Elutions were carried out in a binary gradient mode with a flow-rate of 1 mL/min and column temperature of 28°C. The proposed gradient elution program with UV and fluorescence detection allowed satisfactory fractionation of GnRH (at 31.5 $\pm$ 0.2 min) from Cu-GnRH (at 30.3 $\pm$ 0.2 min) and endogenous species present in samples of cytosol and subcellular organelles from the hypothalamus. The proposed reversed-phase HPLC method I with fluorescence detection provides a more sensitive analytical tool for routine and simultaneous quantification of GnRH, Cu-GnRH and their enzymatic degradation products (catabolites) in all biological samples as compared with HPLC method I with UV detection. To avoid problems due to overlapping peaks corresponding to GnRH, Cu-GnRH, and the respective enzymatic degradation products in samples of cytosol and subcellular organelles from the hypothalamus, we propose a very shallow binary gradient elution program (method I). The separation efficiency of GnRH and Cu-GnRH peaks in standards and biological samples was assessed based on purity analysis of UV spectra (250-300 nm) and on the values of ratios of the fluorescence response to UV response at 280 nm. Our second reversed-phase liquid chromatographic method (HPLC method II) with pre-column derivatization of aminoacids in catabolites of GnRH and Cu-GnRH enabled investigations of the degradation pattern as well as of the yield of enzymatic degradation of GnRH and its Complex with Cu^{2+} in pituitary cytosol and sub-cellular organelles.

Słowa kluczowe

gonadotropin-releasing hormone (GnRH), Cu-GnRH, GnRH and Cu-GnRH catabolites, reversed-phase HPLC, UV detection, fluorescence detection

Adres publiczny

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Plik został wygenerowany dnia 2026-10-05 15:07:07

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