

4-(5,6-ジメトキシ-2-フタルイミジニル)-2-メトキシフェニルスルホニルクロライドを用いる人血清中N-末端プロリルジペプチド、プロリン及びヒドロキシプロリンのHPLC-蛍光分析

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Fluorometric determination of N-terminal prolyl dipeptides, proline and hydroxyproline in human serum by pre-column high-performance liquid chromatography using 4-(5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride

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ABSTRACT: A highly sensitive HPLC method for the determination of prolyl dipeptides, Pro and Hyp in serum was developed. After deproteinization of serum and pretreatment with *o*-phthalaldehyde, the analytes were derivatized with 4-(5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride at 70°C for 10 min.

The fluorescent derivatives of prolyl dipeptides, Pro and Hyp, were separated on tandem reversed-phase columns by a gradient elution at 55°C and detected by fluorescence measured at 318 nm (excitation) and 392 nm (emission). The detection limits for prolyl dipeptides were 2-5 fmol/injection (S/N = 3). Pro-Hyp, Pro-Gly and Pro-Pro were identified as serum prolyl dipeptides. The within-day and between-day relative standard deviations were 1.5-7.9 and 2.4-10.8 %, respectively. The recoveries were in the range of 90.8-97.3 %. The concentrations of Pro-Hyp, Pro-Gly, Pro-Pro, Pro and Hyp in normal human serum (n=10) were 0.64 ± 0.35 , 0.078 ± 0.047 , 0.022 ± 0.016 , 177.0 ± 43.0 and 11.1 ± 3.5 μ M, respectively. The concentrations of Pro-Hyp and Pro-Pro in serum of a patient with bone metastases of prostatic cancer were about 3 times and 50 times, respectively, higher than those in normal human serum.

抄録 血清中プロリルジペプチド、Pro 及び Hyp の高感度プレラベル HPLC 分析法を開発した。血清を除タンパクし、*o*-フタルアルデヒドで処理したのち、プロリルジペプチド、Pro 及び Hyp は4-(5,6-ジメトキシ-2-フタルイミジニル)-2-メトキシフェニルスルホニルクロライドにより誘導体化された。誘導体は逆相系 HPLC により分離検出され、検出限界は2~5 fmol/injection (S/N=3)であった。血清中のプロリルジペプチドとして Pro-Hyp、Pro-Gly 及び Pro-Pro が同定された。日内及び日差変動はそれぞれ1.5~7.9及

び2.4～10.8%で、添加回収率は90.8-97.3%であった。骨転移のある前立腺ガン患者の血清中のPro-Hyp及びPro-Proの濃度は健常人に比べてそれぞれ約3倍及び約50倍高い値を示した。

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