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Methodical approaches to bioassay of substances containing phenolic hydroxyls in the structure

Alexander Leonidovich Khokhlov

The Yaroslavl State Medical University, Russia

Biography

Khokhlov Alexander Leonidovich - head of the Department of Clinical Pharmacology of The Yaroslavl State Medical University, corresponding member of The Russian scientific academy, Professor, doctor of Medical Sciences.

Abstract

The aim of the study is development of methodological approaches to bioassay of substances containing phenolic hydroxyls in the structure using mycophenolic acid and methyldopa which contains one and two phenolic hydroxyls, respectively, as examples. HPLC-MS method was used for measuring concentrations of mycophenolic acid in plasma in the range from 0.05 µg/ml to 30.00 µg/ml. Determination of methyldopa in stabilized plasma was performed by HPLC-MS/MS in the range from 0.02 µg/ml to 3.00 µg/ml. Sample preparation was carried out using protein precipitation for both analytes. Investigation of short-term stability and freeze/thaw stability of the samples showed the mycophenolic acid was stable in K3EDTA-plasma, and addition of antioxidant is not required. Methyldopa rapidly degradated in K3EDTA- and heparin-plasma. The selection of a combination of stabilizer and anticoagulant was performed by adding aqueous solutions of ascorbic acid, sodium sulfite, thiosulphate and metabisulphite in concentrations of 5 and 10% in different ratios to the plasma. Addition of 5% solution of ascorbinic acid to K3EDTA- plasma in the ratio of 1:5 (antioxidant/plasma, v/v) was optimal to prevent oxidation of methyldopa.

The method development for bioassay of potentially unstable compounds, such as phenolic substances, should begin with selection of anticoagulant based on the study of short-term stability and freeze/thaw stability. If an unsatisfactory result was obtained, the combination of an anticoagulant and the antioxidant solution, the concentration of the solution and volume ratio «antioxidant /plasma» should be investigated.

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