

Assay for Methotrexate with Simultaneous Detection of Citrovorum Factor and Vincristine

by

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Abstract

An assay procedure has been developed for methotrexate (MTX) detection at increased sensitivity, which also allows for simultaneous detection of citrovorum factor (CVF) and vincristine (VCR). The assay employs high-pressure liquid chromatography (HPLC) with continuous fluorescence and UV detection of the column eluate. Batches of samples can be prepared in 45 minutes, and eluted in 30 minutes.

To achieve the desired sensitivity, MTX is reductively cleaved into a more fluorescent product, tentatively identified as 2,4-diamino-6-methylpteridine (DAMP). Initial attempts to form the reduction product tetrahydromethotrexate (THMTX) were abandoned when the procedure could not be adapted to plasma samples.

The reductive cleavage procedure utilizes $\text{Na}_2\text{S}_2\text{O}_4$ as the reducing agent and is carried out at 100°C and pH 6, conditions determined to optimize product formation. 2-hydroxyfolic acid (2-OH-FA), which is added as an internal standard, and CVF are also cleaved in the reaction. The MTX, CVF, and 2-OH-FA products can all be detected fluorometrically with excitation at 367 nm and emission at 463 nm. VCR, which is not affected by the reaction procedure, can be measured by UV absorption at 254 nm. Hydroquinone (HQN) is added after the reaction as an internal standard for the UV measurements.

MTX concentrations as low as 5×10^{-9} M in spiked plasma samples have been detected. The fluorescence intensity is directly proportional to initial MTX concentration at least up to 10^{-6} M. The HPLC column gives the assay selectivity by separating other folates and fluorescent plasma components from the MTX peak.

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