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Development and validation of fast LC-MS/MS method for the determination of folic acid in human plasma

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Folic acid, the synthetic form of folate vitamin, is converted when ingested into forms of reduced folate that are identical to those arising from ingestion of naturally occurring folate in food. Several reports have suggested that high levels of unmetabolised folic acid in plasma are potentially harmful and may promote the growth of pre-existing cancers or malignant lesions. A fast, sensitive, and robust isocratic reversed phase HPLC–tandem mass spectrometry (LC–MS–MS) method was developed for routine quantification of unmetabolised folic acid in human plasma. The developed method was capable of producing a baseline separation of free folic acid from similar endogenous components in plasma, and detection was achieved in 3 minutes only. The fast LC-MS/MS quantification was preceded by a rapid sample preparation step of protein precipitation producing a complete quick and simple procedure for the quantification of large number of samples obtained in pharmacokinetic and epidemiological studies. Other methods required laborious solid-phase extraction, solvent–solvent extraction or lengthy gradient reversed phase or online hydrophilic interaction chromatography. The folic acid and its deuterated internal standard were detected using negative ion electrospray-tandem mass spectrometry with multiple reaction monitoring of the diagnostic fragment ions of each deprotonated molecule. The recovery of folic acid from spiked plasma was >77% over a concentration range from 13.17 ng/mL to 3657.5 ng/mL with intraday precision within run of 2.2-19.8% and between run precision of 3.1-13.2%. Stability studies were carried out for spiked samples in order to define storage and handling conditions.

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