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Extraction, Purification and Detection by Liquid Chromatography?Electrospray-Ionization Mass Spect

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Abstarc :

The interconversions of C1-substituted folates form the core of plant one carbon metabolism. An analytical method for extraction, purification and liquid chromatography?electrospray-ionization mass spectrometry detection of low level, labile tetrahydrofolate monoglutamates from *Arabidopsis thaliana* was developed in this study. Under gold fluorescent light, folates were extracted by homogenizing the leaves in extraction buffer and heating the homogenate. A portion of leaf homogenate was incubated with crude preparation of rat plasma conjugase to remove the polyglutamate tails in order to simplify the mixture for analysis. The resulting extract containing the monoglutamyl folates was then applied to an agarose affinity column containing folate binding protein which was purified from milk whey. After elution from the affinity column, the folates were separated and identified by liquid chromatography?electrospray-ionization mass spectrometry on a 150×0.32 mm Symmetry 300 C18 column using isocratic elution with mobile phase (0.1% formic acid/95% acetonitrile, containing 0.1% formic acid, 88:12) at a flow rate of 0.3 ?l/min. In positive ion mode, ions representing 5-methyl-tetrahydrofolic acid, 5-formyl-tetrahydrofolic acid and 5,10-methenyl-tetrahydrofolic acid were detected and identified. It is concluded that this folate extraction procedure, combined with folate binding protein affinity column purification is applicable to liquid chromatography?electrospray-ionization mass spectrometry and this method is appropriate for analysis of tetrahydrofolate metabolites in plant leaf tissues. [Nature and Science 2003;1(1):32-36].

Key Word :
liquid chromatography?electrospray-ionization mass spectrometry; rat plasma conjugase; folate binding protein; affinity column; tetrahydrofolate metabolites; Arabidopsis thaliana

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