Determination of Glutathione in Yeasts by Hydrophilic Interaction Chromatography Coupled to Post Column Derivatization

T.D. Karakosta, P.D. Tzanavaras and D.G. Themelis
Aristotelian University of Thessaloniki, Laboratory of Analytical Chemistry, Department of Chemistry, GR-54124 Thessaloniki, Greece
ptzanava@chem.auth.gr

Glutathione (GSH) is a tri-peptide that contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. It has an important biological role since – as an antioxidant – it helps protect cells from reactive oxygen species such as free radicals and peroxides [1].

The present work reports a new method for the determination of GSH in yeast samples. The analyte was separated from the sample matrix by Hydrophilic Interaction Chromatography (ZIC-HILIC, 150 x 4.6 mm i.d., 5 μm, Merck) using a mixture of Acetonitrile (65 %) and Ammonium acetate (35 %, 15 mmol L\(^{-1}\), pH = 2.5) as mobile phase. Detection was carried out through on-line post column derivatization (PCD) by methyl propiolate (20 mmol L\(^{-1}\)) in alkaline medium (100 mmol L\(^{-1}\) borate buffer, pH = 12.0). The MP-GSH thioacrylate derivative was monitored at 285 nm.

The proposed method was validated in terms of linearity (up to 200 μmol L\(^{-1}\)), limits of detection (0.6 μmol L\(^{-1}\)) and quantitation (2.0 μmol L\(^{-1}\)), precision and accuracy. The developed HPLC-PCD method was successfully applied to the analysis of various yeast samples. The percent recoveries ranged between 91.2 and 105.6 %.

KEYWORDS: glutathione, hydrophilic interaction chromatography, post column derivatization, yeast

Figure 1. Representative HILIC-PCD chromatogram for the analysis of a real yeast sample.

REFERENCES