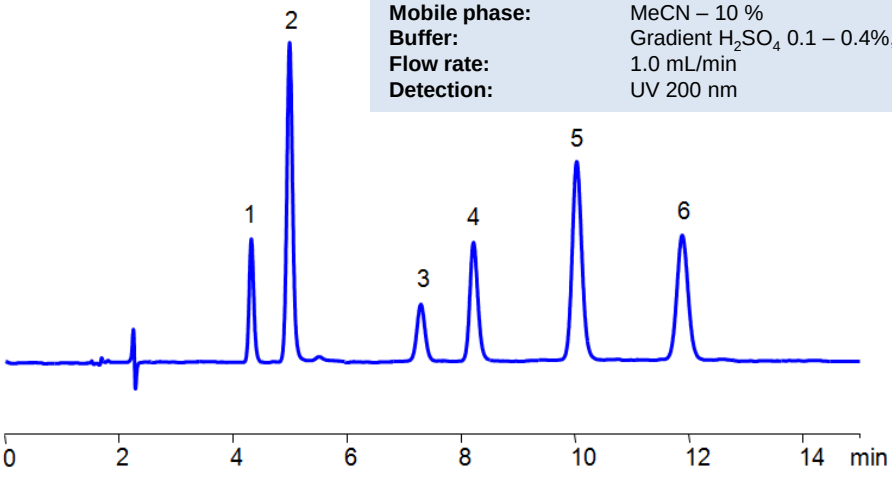
CoAp # 2023-1

Cool Applications

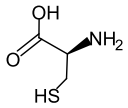
"Making Tough LC Applications Look Cool"

HPLC Method for Separation of Sulfur-containing Biomolecules

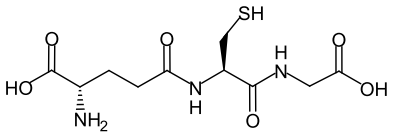
Column:	**Primesep 100**
Column size:	4.6 × 150 mm, 5 µm
Column part number:	100-46.150.0510
Mobile phase:	MeCN – 10 %
Buffer:	Gradient H_2SO_4 0.1 – 0.4%, 15 min
Flow rate:	1.0 mL/min
Detection:	UV 200 nm



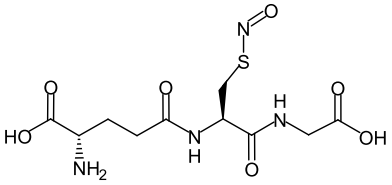
1. Cysteine (Cys)



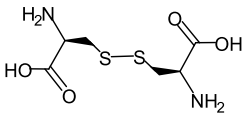
2. Glutathione, reduced



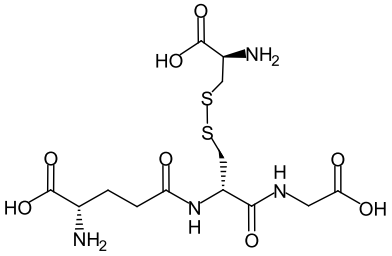
3. S-Nitroso-L-glutathione



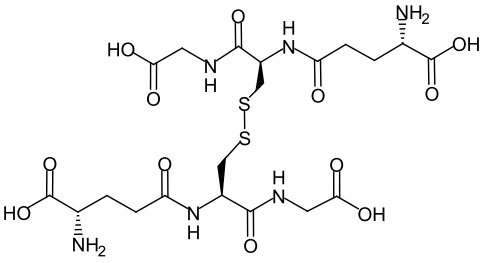
4. Cystine



5. Cysteine-glutathione disulfide



6. Glutathione, oxidized



Application Comments

Glutathione, a tripeptide prevalent in nearly all cells, plays numerous roles in biological systems. Comprised of three amino acids - L-glutamic acid, L-cysteine, and glycine - natural glutathione molecules are highly polar. Their retention and separation necessitate a specialized column and/or mobile phase. We are showing a very simple approach with UV detection that allows quick and efficient separation of most common glutathiones.

Primesep 100 column that was used in this method is one of the most popular mixed-mode columns that provides both a strong cation exchange interaction and a reverse phase interaction.

Having a small amount of sulfuric acid as the only ionic modifier in the mobile phase simplifies mobile phase preparation.

If speed of analysis needs to be further increased, smaller particles and a shorter column can be used. This separation material is also available in a solid core particle format.

The method can be used for many other polar molecules with amino groups.

Visit www.sielc.com to learn more about Primesep 100 column.