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Development of a chromatographic method for simultaneous analysis of glutathione forms

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Reduced glutathione (GSH)is the most abundant low molecular weight thiol-containing tripeptide (glycine, cysteine, and glutamate) which is synthesized in the cells. GSHplays critical roles in protecting cells from oxidative damage and the toxicity of xenobiotics. Besides, it is also involved in the regulation of intracellular redox homeostasis, which leads to its oxidation into oxidized glutathione (GSSG). Determining the ratio of GSH/GSSG in different biological samples is a major procedure for the evaluation of an individual's oxidative status and can be a potential biomarker of oxidative stress.
The aim of the present study was to developa modified HPLC-DAD that allows simultaneous quantification of both glutathione forms.
All experiments were performed on a HPLC system and separation was carried out using a RP-18 column. Throughout the experiments, the influence of the following parameters was evaluated: 1) mobile phase composition, 2) wavelength settings, 3) pH, 4) temperature and 5) flow rate. Different protocols for sample preparation was also assessed in blood cells and plasma samples.
From all the protocols tested, the best results were obtained using a mobile phase composed by sodium perchlorate acidized with orto-phosporic acid, and a flow rate of 1.5 mL/min at 40ºC. The meta-phosphoric acid was the one that showed better results in the sample preparation.
The proposed method, which was successfully developed for GSH and GSSG quantification, is simple, cheap and easy to perform. It would be interesting to assess whether this method is suitable for the quantification of other biological specimens.

Keywords: Reduced glutathione-GSH, oxidized glutathione-GSSG, HPLC-DAD, oxidative stress