

## APPLICATION OF LC-MS/MS METHODS IN THE QUANTIFICATION OF OXIDATIVE STRESS PARAMETERS

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Oxidative stress is a phenomenon that occurs due to the disturbance in the balance between the production of reactive oxygen species and the ability of biological systems to remove the resulting compounds. Oxidative stress is involved in the pathogenesis of many disorders in the human organism. This indicates the importance of quantification of oxidative stress parameters in biological samples. Traditionally, these parameters are determined by biochemical tests. Although these tests are routinely performed, they have many drawbacks. To determine the exact concentration of selected compounds, more sensitive analytical methods are becoming more important. In the modern scientific literature, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is increasingly mentioned (1). This method, with adequate selection of stationary and mobile phases, enables quantification of very low concentrations of selected parameters. In addition, these methods can simultaneously determine the concentration of many selected components. However, it is necessary to take into account that LC-MS/MS methods require a very purified biological sample from which the proteins have been maximally removed. In this work, we will present the results of determination of cysteine, cystine, reduced and oxidized glutathione in patients with microcellular lung cancer. The use of LC-MS/MS methods is becoming increasingly common for the analysis of oxidative stress markers in biological fluids. In the future, we are expected to move to even more modern, fully automated methods, which simultaneously purify and analyze samples.

### References

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## PRIMENA LC-MS/MS METODA U ISPITIVANJU PARAMETARA OKSIDATIVNOG STRESA

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Oksidativni stres je pojava koja nastaje usled narušavanja ravnoteže između proizvodnje i nagomilavanja reaktivnih jedinjenja kiseonika u organizmu i sposobnosti bioloških sistema da ukloni nastala jedinjenja. Oksidativni stres učestvuje u patogenezi mnogih poremećaja kao što su kardiovaskularne bolesti, dijabetes i bolesti bubrega. Ovo ukazuje na značaj određivanja odabranih parametara oksidativnog stresa u uzorcima biološkog materijala. Tradicionalno, najveći broj parametara se određuje biohemijskim testovima. Iako se ovi testovi rutinski izvode, oni imaju mnoge nedostatke. Da bi se odredila tačna koncentracija izabranih parametara, koji se u uzorcima nalaze u veoma niskim koncentracijama, sve veću prednost imaju osetljivije analitičke metode. U savremenoj naučnoj literaturi se u te svrhe sve više spominje tačna hromatografija spregnuta sa masenim detektorom (LC-MS/MS) (1). Ova metoda, uz adekvatan izbor stacionarne i mobilne faze, omogućava kvantifikaciju veoma niskih koncentracija odabranih jedinjenja. Sem toga, ovim metodama se istovremeno, u jednom uzorku, može odrediti koncentracija većeg broja odabranih komponenti. Ipak, neophodno je voditi računa o tome da tačna hromatografija spregnuta sa masenim detektorom zahteva dobro prečišćen uzorak biološkog materijala iz kog su maksimalno uklonjeni proteini. U ovom radu biće prikazani rezultati određivanja cisteina, cistina, redukovano i oksidovano glutationa kod pacijenata sa mikrocelularnim karcinomom pluća. Upotreba LC-MS/MS metoda je sve uobičajenija za analizu markera oksidativnog stresa u biološkim tečnostima zbog svoje osetljivosti. U budućnosti se očekuje razvoj ka još savremenijim, potpuno automatizovanim metodama, kojima se istovremeno uzorci prečišćavaju i analiziraju.

### Literatura

1. Winnik WM, Kitchin KT. Measurement of oxidative stress parameters using liquid chromatography–tandem mass spectroscopy (LC–MS/MS). Toxicol. Appl. Pharm 2008;233, 100-106.