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Introduction

Application of a new electrode material, Magic Diamond has been investigated for the analysis of (di-)sulfides cystine (CSSC), cysteine (CSH), glutathione (GSH), homocysteine (HCSH) and diglutathione (GSSG). A comparison was made with a conventional method using 2 flow cells in series for pre-reduction of disulfides. The new electrode material offers several advantages:

- Analysis of mono and disulfides all in 1 single run
- Diamond surface is compatible with extremely high potential
- Single cell analysis (no pre-reduction of disulfides)
- Highly reproducible even at high concentrations (2 μ M)



Fig. 1. Flexcell™ with working electrode holder and magic diamond electrode disk.

Method

HPLC ALEXYS MD disulfides analyser
Column ALF-115, 150 x 1 mm (ID), 3 μ m
Flow rate 50 μ L/min



Results

Conventional analysis using a dual flow cell (cell 1: reduction of disulfides to thiols, cell 2: oxidative detection of thiols) was compared with the new magic diamond single cell configuration.

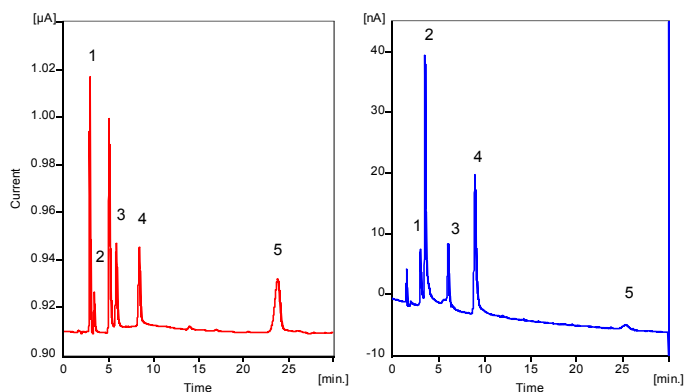


Fig. 2. Analysis of a mixture of Cystine (1), Cysteine (2), Glutathione (3), Homocysteine (4) and Diglutathione (5), 2 μ L, 2 μ M. Left: Magic Diamond, right: conventional method.

Linearity

The linearity has been studied in the range 0.15 - 50 μ M. Correlation coefficient r is better than 0.997 for Cysteine and better than 0.999 for all other compounds. Reproducibility in peak height (h) and area (a) is improved by a factor of 2 and more using Magic Diamond.

Reproducibility

Table 1. Reproducibility was determined with 2 μ L injections of an 1 μ M standard mixture in mobile phase, $n = 10$.

	conventional h (nA) RSD %	Magic Diamond h (nA) RSD %	conventional A (nA.s) RSD %	Magic Diamond A (nA.s) RSD %
CSSC	22.8 3.2	98.6 1.1	168.9 2.7	672 1.8
CSH	24.7 3.1	16.3 1.2	195.4 3.6	131 2.4
GSH	6.8 6.5	32.3 1.4	69.6 6	400.1 2.3
HCSH	18.8 2.7	30.3 0.8	233.4 2.8	463.2 1.2
GSSG	2.2 5.2	20.5 1.8	55.5 5	824.3 1.9

Response stability

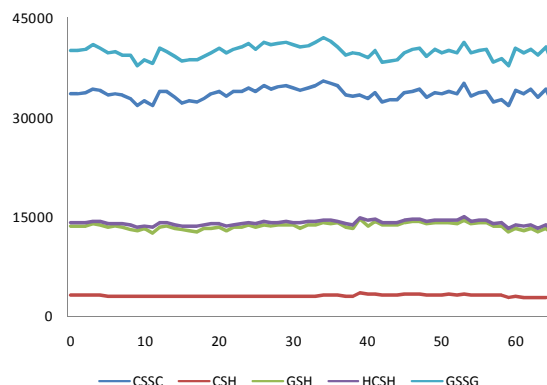


Fig. 3. Peak area's of 66 measurements of 2 μ L injections of an 50 μ M mixture of CSSC, CSH, GSH, HCSH and GSSG in mobile phase.

Conclusion

By using the new Magic Diamond electrode material in conjunction with the ALEXYS HPLC system the analysis of sulphur containing compounds including disulfides has become reliable with the following advantages:

- Single cell set-up
- 2 to 3 times better reproducibility (peak area, peak height)
- No cleaning pulse between injections
- Fast equilibration
- Robust

Outlook

The superior performance of the Magic Diamond electrode material and its applicability at higher potentials opens new opportunities the routine analysis of sulphur containing compounds by HPLC-EC. Other electroactive compounds are currently under investigation.