Connecting photorespiration and AOX1a expression in Arabidopsis plants under water deficit

Cristina Cruz2, Dave Prinxton1, Ana B. da Silva1, Ana R. Matos1, Jorge Marques da Silva1, Maria C. Arrabaça1 and João D. Arrabaça1

1 Plant Molecular Biology and Biotechnology Lab, BioFig, Faculdade de Ciências, Universidade de Lisboa, Portugal; 2 Universidade de Lisboa, Faculdade de Ciências, Centro de Biologia Ambiental (CBA);

Arabidopsis thaliana plants with altered expression of aox1a (AOX1-A) and wild-type (Col-0) were grown under well watered conditions or under increasing water deficit for 2 weeks. Photorespiration rate was assessed based on the assumption that changes in the glycine/serine ratio immediately after the beginning of a dark period should reflect the photorespiratory activity. Accordingly leaves were collected under light conditions and 30 sec after dark. Photorespiratory rates were determined as the difference between the glycine/serine 30 sec after dark and under light conditions. Amino acids were extracted from frozen (-80°C) leaves with HCl (0.1 M). The extracts were centrifuged (1600 g, 4°C, 10 min), and an internal standard (α-aminon-butyric acid) was added. Samples were centrifuged again and filtered (0.2 µm) into the HPLC vials. Standards of glycine and serine were prepared in 0.1 M HCl.

The leaf content of glycine and serine determined in illuminated leaves was similar for the Col-0 and for AOX1-a plants grown under good water availability. The same was observed for the glycine/serine ratio. However water deficit conditions differently affected glycine and serine pools of Col-0 and AOX1-a. The Col-0 glycine/serine was not affected by the water deficit, while that of the AOX1-a increased. Altogether the results show no differences in the photorespiratory rate of Col-0 and AOX1-a in well-watered plants. But a decrease was observed in the mutant under water deficit conditions. The results confirmed those obtained using infra-red gas analysis and strengthen a connection between photorespiration and AOX1a expression in Arabidopsis plants.

Analytical approach for the determination of antiepileptic drugs in human plasma

Geraldes L.1, Barroso M.2, Queiroz J. A.1, Gallardo E1

1 CICS - Centro de Investigação em Ciências da Saúde - UBI, Covilhã; 2 Instituto Nacional de Medicina Legal - Delegação do Sul, Lisboa

Nowadays, in most developed countries, epilepsy’s incidence rate is of about 40-70 in 100000. Epileptic seizures are controlled by means of antiepileptic drugs, and a number of therapeutic agents are available. The possibility of drug interactions between these agents, the advent of internet and the growing problem of self-medication may lead to an increased number of intoxications. Therefore, there is the need of rapid and sensitive analytical methods for the reliable quantitation of these compounds in human biological samples to help and assist emergency services in the treatment of intoxicated individuals.

The goal of this work was the development and validation of a method for the quantitative determination of four selected antiepileptic drugs (primidone, carbamazepine, phenytoin and phenobarbital) and metabolite (10,11-epoxycarbamazepine) in human plasma, using solid-phase extraction and liquid-chromatography-diode array detector (HPLC-DAD). The method used a sample amount as low as 0.2 mL, and was fully validated according to internationally accepted guidelines for bioanalytical method validation. The studied parameters were selectivity, linearity, limits of detection (LOD) and quantitation (LLOQ), precision and accuracy, bench top and freeze/thaw stability, and extraction efficiency.

The method was selective, as no interfering compounds were detected by analysis of blank plasma samples of 20 different origins, and linear from 0.5-25 µg/mL for all compounds, except for carbamazepine (0.1-25 µg/mL) and primidone (1-25 µg/mL). Intra- and interday precision and accuracy were in conformity with the above mentioned criteria, i.e., coefficients of variation of less than 15% and bias within a ±15% interval from the nominal value (excepted at the LLOQ, for which 20% was accepted). Extraction efficiency was higher than 90% for