

PW8 High sensitivity capillary LC-MS/MS measurement of dityrosine in human urine

Martin P. Bucknall^{1, 2}, Michael J. Davies³, Alicia J. Jenkins⁴, and George A. Smythe^{1, 2}

1. Bioanalytical Mass Spectrometry Facility, Analytical Centre, UNSW, Sydney, Australia
2. School of Medical Sciences, UNSW, Sydney, Australia
3. The Heart Research Institute, Camperdown, Sydney, Australia
4. Department of Medicine, St Vincent's Hospital, University of Melbourne, Australia.

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Increased dityrosine levels in acute inflammatory, but not chronic disease states, nor with age.

o,o'-Dityrosine (DT), an oxidation product of tyrosine produced by reaction between tyrosyl radicals, is a potential biomarker of free radical oxidative protein damage *in vivo* [1]. Attempts to measure DT concentrations in various physiological and pathological systems have produced inconsistent results [2, 3 & 4]. DT concentrations in comparable biological matrices varying over three orders of magnitude have been reported, together with contradictory claims of significant DT elevation in several ageing-related pathologies.

A highly specific assay for the quantification of urinary DT was developed using capillary HPLC with electrospray tandem quadrupole mass spectrometry (HPLC-MS/MS). The assay has the highest absolute sensitivity for DT of any method reported to date, with a detection limit of 3 fmol on column. Urine samples from healthy volunteers and patients with various pathologies were analysed. DT was present in normal human urine at 5-25 $\mu\text{mol/mol}$ creatinine (or 20-200 nM), and does not correlate with age. Urine DT levels were elevated in states of acute infection and inflammation ($p=0.010$ 1-tailed T-test), but not in stable chronic disease. Assay specificity may have affected the results of other studies involving measurement of modified amino acids as markers of oxidative stress. The utility of DT measurement as an index of oxidative damage is discussed.

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