Improving Quality to Improve Throughput in Quantitative Neurotransmitter Analysis

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Biomarkers are endogenous compounds whose quantities are indicative of disease / symptom / treatment state and/or change in that state. We are specifically interested in analysis of neurotransmitter biomarkers associated with central nervous system disorders such monoamines, amino acids, acetylcholine and neuropeptides. Many of these neurotransmitters are present at low pg/mL concentrations in biological samples and therefore the greatest attention to detail in all aspects of the sample collection and in the analytical techniques utilized must be practiced in order to successfully analyse them.

Monoamine analyses were performed on a Waters Alliance 2795 attached to an Antec Leyden electrochemical detector. Amino acid analyses were performed on a Waters Acquity equipped with a fluorescence detector utilizing OPA derivatization. Acetylcholine was analysed on a Waters Acquity equipped with a Waters Quattro Ultima (triple quad mass spectrometer). Neuropeptides were analysed using an Eksigent 2D nano-LC attached to a Waters Quattro Ultima. In vivo microdialysis samples were supplied by our neuroscience group.

Miniaturization of the components of the Alliance autosampler was performed to increase the sensitivity, robustness and throughput of the monoamine analyses (norepinephrine, dopamine and serotonin). The sample loop was reduced down to either 2 or 5µL which along with other modifications facilitated lower sample consumption.

High throughput analysis of glutamate has been achieved using the Acquity UPLC setup. Sample usage is minimal (<5µL) and therefore this analysis can be readily performed along side the monoamine analysis. Similarly a high throughput analysis of acetylcholine has been implemented requiring <5µL of sample.

Neuropeptides (angiotensin II, met and leu-enkephaline, vasopressin, oxytocin, ACTH and NPY) are presently under examination utilizing a nanoLC setup attached to a triple quad mass spectrometer. To date we have successfully measured low pg/mL (<10pg/mL) levels of angiotensin II and the enkephalins in plasma and CSF samples. Work is underway to observe all the indicated neuropeptides in microdialysis samples.

In parallel to the methods described above efforts are underway to further improve the monoamine analysis by utilizing LC/MS/MS methodologies. However, one of the immediately recognizable benefits achieved to date is the ability to measure multiple analytes from one sample which was previously not possible.