A novel method for plasma metanephrine analysis using the Waters Unispray Ionisation technique.

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Measurement of plasma metanephrine and normetanephrine is useful in the diagnosis of phaeochromocytomas and paragangliomas, but many assays require a large volume of plasma due to poor assay sensitivity, and often require lengthy sample preparation. The current method in use in our laboratory involves dilution of sample 1:1 with aqueous internal standard, then deproteinisation with a 10 KDa centrifugal filter followed by online solid phase extraction using WCX cartridges[1]. This uses only 150 µL of sample, but requires a 20 minute centrifugation step for the sample filtration. Our aim was to develop a method for measurement of plasma metanephrines using a small sample volume with minimal hands-on preparation, suitable for use in a busy Clinical Biochemistry Laboratory.

We chose to deproteinise 50 µL of sample by precipitation with 150 µl of acetonitrile. Solid phase extraction was then carried out using a Waters Online Solid Phase Extraction Manager with WCX cartridges. We compensated for the increased sample dilution by using a Waters Unispray source, which gave us a 3-fold increase in ionisation efficiency compared to a conventional electrospray source. In the Unispray source, a high velocity spray from a grounded nebuliser is arranged to impact on a streamlined rod target that is positioned in close proximity and perpendicular to the spray axis. The stainless steel target is positioned upstream of the ion inlet aperture of the MS and typically held at a potential of 1kV with respect to the nebuliser and inlet. The mechanism of improved sensitivity is not fully understood, but is thought to involve spray electrification, the Coanda effect and surface gas flow microvorticity.

Full method validation was carried out for this assay, according to FDA guidelines[2]. The lower limit of quantification was 18.75 pmol/L for metanephrine and 20.2 pmol/L for normetanephrine. Inter and intra-assay precision was carried out using plasma pools at low medium and high concentrations, and the CVs were acceptable at all concentrations (<11% for intra-assay and < 7% for inter-assay). Recovery of metanephrine was calculated by spiking three concentrations of analyte (175, 600 and 3050 pmol/L for metanephrine and 171, 710 and 3100 pmol/L for normetanephrine) into three different plasma pools. Mean recovery was 101% (96.6-110%) for metanephrine and 93% (81-115%) for normetanephrine. The assay was linear up to 30000 pmol/L for metanephrine and 32300 pmol/L for normetanephrine.

We have developed a method for plasma metanephrine and normetanephrine which uses minimal sample volume and is suitable for use in a busy clinical laboratory. Further work will be carried out on the addition of 3-methoxytyramine into this assay.

References

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2. Guidance for Industry. Bioanalytical Method Validation. US Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Vetinary Medicine (CVM) May 2001.