應用手冊

The Analysis of Total Homocysteine in Plasma using A Quattro Micro Tandem Mass Spectrometer

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For research use only. Not for use in diagnostic procedures.

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief highlights the analysis of total homocysteine in plasma using a Quattro Micro Tandem Mass Spectrometer.

Benefits

- This method has been shown to be rapid, precise and accurate for the measurement of total homocysteine
- The simplicity of the assay makes it ideal both for non-specialised staff and routine high throughput

Introduction

Total homocysteine (tHcy) is currently regarded as a risk factor for cardiovascular disease.[1] This increased interest has lead to a multitude of studies requiring the determination of total homocysteine in conjunction with other factors. The introduction of mass spectrometry now allows a single sample to be analysed for a large number of analytes.

There are various methods for measuring tHcy,[2] including HPLC, enzyme immunoassay (EIA) GC-MS and LC-MS/MS. A recent review[3] suggests that GC-MS should be considered as the reference method. A comparison of GC-MS with other laboratory methods drew the conclusion that the methods were not interchangeable and reference material was urgently required.[4]

The most recent method for measuring homocysteine uses a deuterium-labelled internal standard and tandem mass spectrometry.[5] This development requires no derivatization and therefore leads to an increase in sample throughput[3] compared to existing techniques. We have developed an LC-MS/MS method that requires minimal sample volume, no centrifugation or transfers and therefore allows the possibility of automated sample preparation together with high throughput capability.

Experimental

A Quattro Micro tandem benchtop mass spectrometer fitted with a Z SPRAY ion source was used for all analyses. The instrument was operated in electrospray positive ionisation mode and was coupled to a Waters 2790 Alliance HT HPLC System. All aspects of system operation and data acquisition were controlled using MassLynx NT v3.5 Software.

Results and Discussion

Achieving maximum sensitivity for homocysteine can be performed by measuring product ions from the fragmentation of the protonated molecule $[M+H]^+$. A solution of homocysteine (10 pmol/µL) was infused into the mass spectrometer and the cone voltage optimised to maximise the intensity of the $[M+H]^+$ precursor (parent) ion (*m/z* 136). The collision energy was then adjusted to optimise the signal for the most abundant product ion (*m/z* 90), using argon as the collision gas at a pressure 5.0×10^{-3} mbar. The product ion spectrum of homocysteine is shown in Figure 1. The process was repeated for the d4-homocysteine analogue, which was used as an internal standard for quantification and to correct for losses during sample processing.

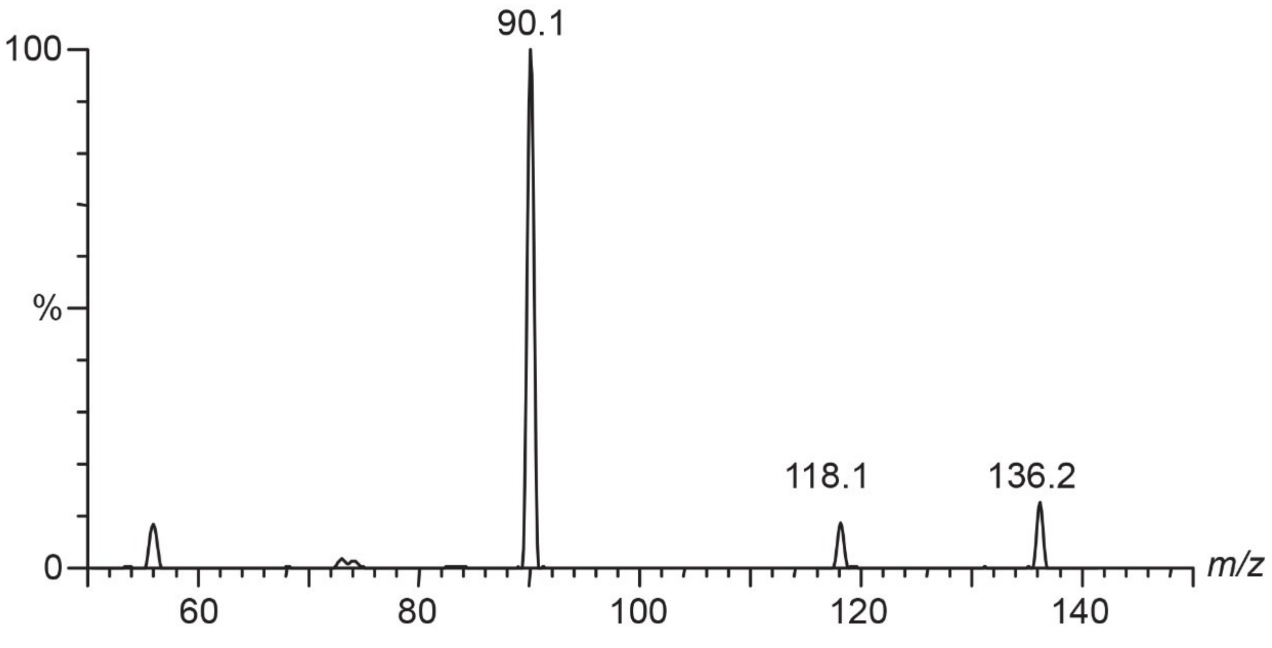

Figure 1. Product ion spectrum of Hhmocysteine.

Plasma (10 µL) was aliquoted into a 96 well plate and d_8-homocystine, 10 µM, (10 µL) added and mixed for 1 minute. Dithiothreitol (DTT) 500 mM (20 µL) was added and mixed for 10 minutes at room temperature.

Finally, water/0.1% formic acid/0.025% trifluoroacetic acid (100 µL) was added as a diluent and mixed for 1 minute. The resultant solution was then sampled (4 µL) directly from the microtitre well.

The method uses an isocratic elution using a Waters Symmetry C_8 Column (2.1 x 100 mm, 3.5 µm) with aqueous 30% methanol /0.1% formic acid, at 250 µL/min. The cycle time (injection-to-injection) is ~2 minutes.

The MRM chromatograms for homocysteine and d_4- homocysteine are shown in Figure 2.

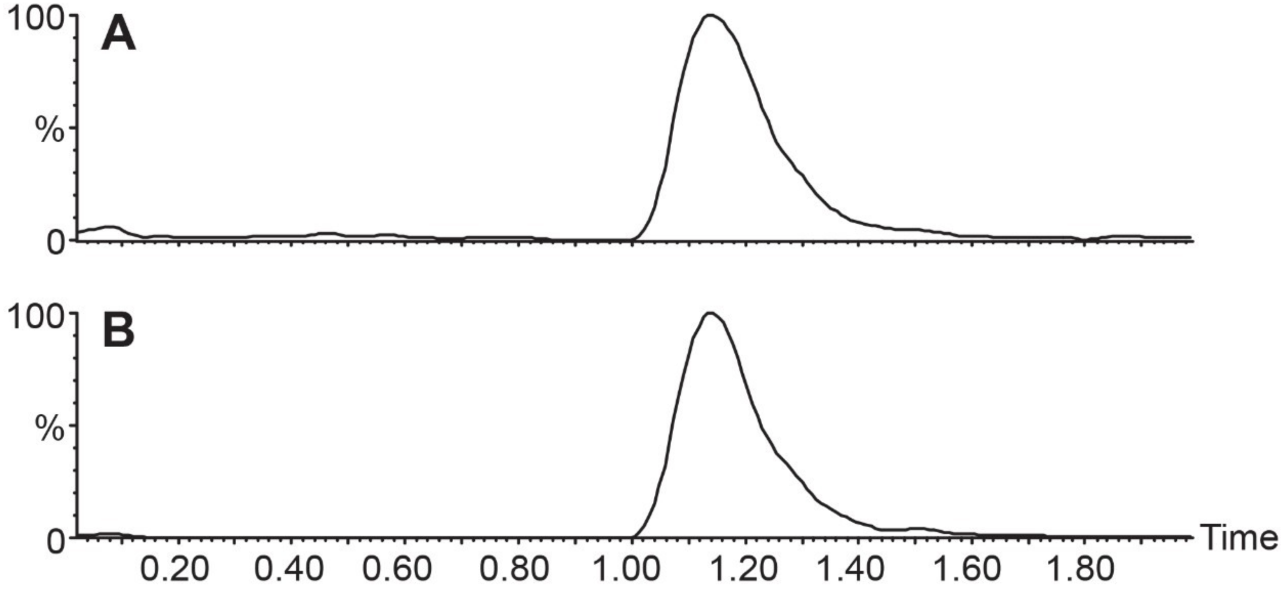

Figure 2. MRM chromatograms of d4-homocysteine (A) and homocysteine (B) at a concentration of (10 mM) using the appropriate transition(s).

Plasma containing endogenous Hcy was spiked with a range of known Hcy concentrations (0 5, 10, 15, 25, and 50 µmol/L). The slope and positive intercept, calculated in QuanLynx, are used to calculate the endogenous value (7.96 µmol/L), as shown in Figure 3. The total homocysteine corrected for endogenous content is shown in Figure 4.

Homocysteine	15	15	136>90
d_4-Homocysteine	15	15	140>94

Table 1. Tune parameters for both homocysteine and d4-homocysteine.

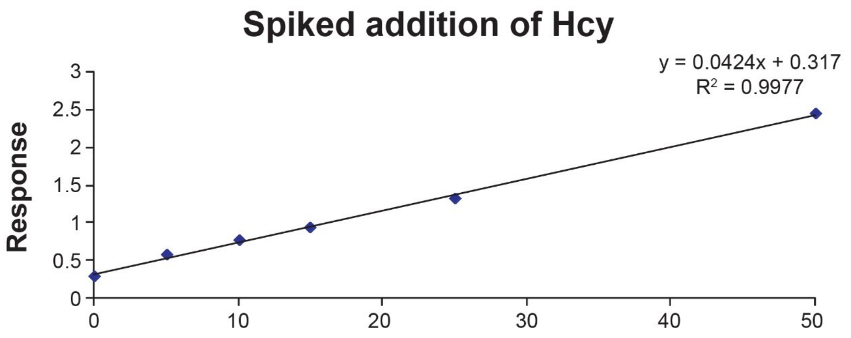

Figure 3. Calibration curve for the spiked addition of Hcy in plasma.

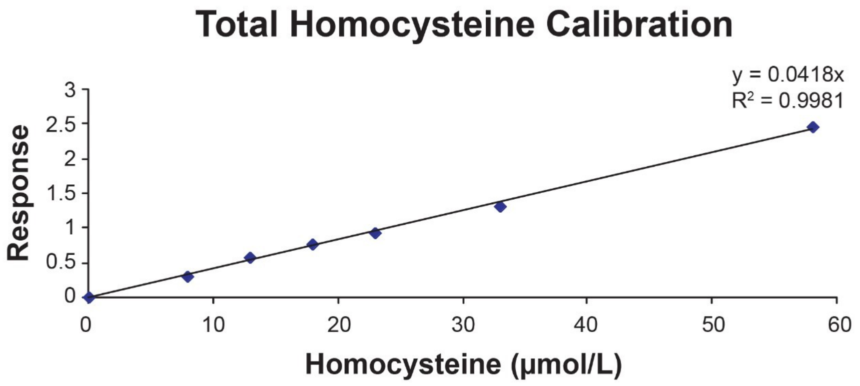

Figure 4. Corrected calibration curve for tHcy in plasma.

The performance characteristics of the assay were examined using two samples with homocysteine concentrations of 14.6 and 37.7 μm. Inter-assay results gave CV's of 5% and 8%, respectively (n=5) and the intra-assay (n=10) CV's for both samples was <2%. Validation of the method was performed using patient samples (n=50) kindly provided by Wythenshawe Hospital, Manchester, which were analysed using both LC-MS/MS and an Abbot TDx Total Homocysteine kit. The LC-MS/MS method showed excellent correlation (r^2= 0.9149) and slight positive bias (0.44) towards to LC-MS/MS method as shown in Figure 5.

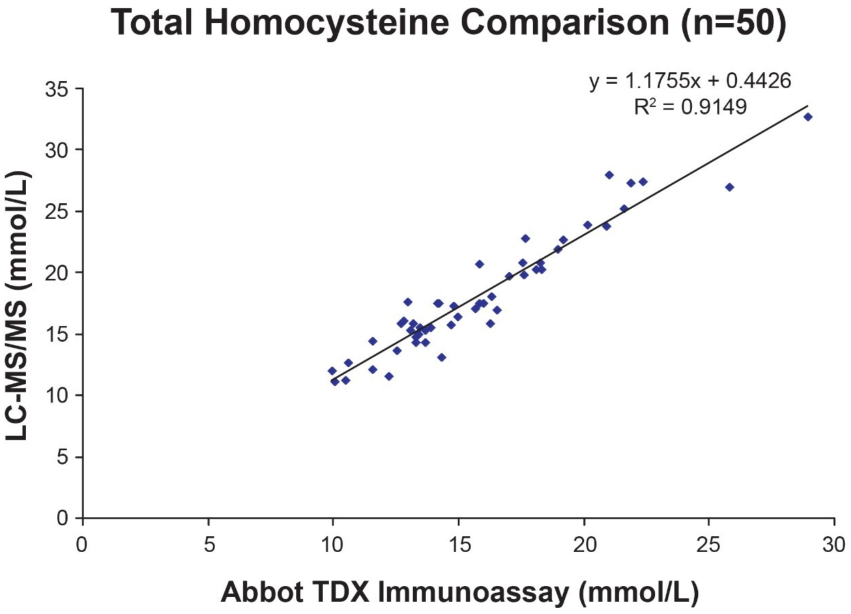

Figure 5. tHcy comparison using LC-MS/MS and an Abbot TDx kit.

Conclusion

This method has been shown to be rapid, precise and accurate for the measurement of total homocysteine.

The simplicity of the assay makes it ideal both for non-specialised staff and routine high throughput.

References

1. Boushey CJ. Beresford SA. Omenn GS. Motulsky AG. A Quantitative Assessment of Plasma Homocysteine as a Risk Factor for Vascular Disease. Probable Benefits of Increasing Folic Acid Intakes. *J Am. Med. Assoc.* 274 (1995) 1049–1057.

2. Pfeiffer CM. Huff DL. Smith SJ. Miller DT. Gunter EW. Comparison of Plasma Total Homocysteine Measurements in 14 Laboratories: An International Study. *Clin. Chem.* 45 (1999) 1261–1268.

3. Rasmussen K. Moller J. Total homocysteine measurement in clinical practice. *Ann. Clin. Biochem.* 37 (2000) 627–648.

4. Ubbink JB. Delport R. Riezler R. Vermaak WJ. Comparison of Three Different Plasma Homocysteine Assays

with Gas Chromatography-Mass Spectrometry. *Clin. Chem.* 45 (1999) 670–675.

5. Magera MJ. Lacey JM. Casetta B. Rinaldo P. Method for the Determination of Total Homocysteine in Plasma and Urine by Stable Isotope Dilution and Electrospray Tandem Mass Spectrometry. *Clin. Chem.* 45 (1999) 1517–1522.

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WAB34, February 2002

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