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Arginine and methylated arginines in human plasma and urine measured by tandem mass spectrometry without the need for chromatography or sample derivatisation

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ABSTRACT

A method for the simultaneous analysis of asymmetric dimethylarginine, symmetric dimethylarginine, monomethylarginine and arginine in human plasma and urine, with short analysis time and isotopic internal standardisation for each analyte is described. The method requires neither sample derivatisation nor the need for chromatographic separation of analytes. The method described shows good precision and accuracy and is suited for both research purposes and implementation in the busy, routine clinical laboratory. In addition the synthesis and utilisation of isotopically labelled symmetric dimethylarginine and monomethylarginine is described for the first time, avoiding the use of surrogates such as homoarginine for internal standardisation.

1. Introduction

Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and monomethylarginine (MMA) are naturally occurring amino acids that circulate in plasma and are excreted in the urine. They are formed by the enzymatic methylation of arginine residues within proteins by protein methyl transferases (PRMT) [1]. A number of PRMTs have been identified and fall into two classes. PRMT 1 form MMA and ADMA whilst PRMT 2 form MMA and SDMA [1]. Upon proteolysis these methylated arginines are released into cells and subsequently into the circulation [1]. ADMA is metabolised by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) [2], of which there are two isoforms, and is also excreted via the kidney. SDMA is not metabolised by DDAH and it is thought that its only route of elimination is via the kidney.

ADMA and MMA are potent inhibitors of nitric oxide synthases (NOS) whilst SDMA has been shown to have no effect upon these enzymes [3]. NOS act upon arginine to produce nitric oxide (NO) and citrulline. The resultant NO induces vascular relaxation and also platelet adhesion and smooth muscle proliferation [4]. By inhibiting NO production it is thought that increased concentrations of ADMA may contribute towards the atherogenic process. The role of ADMA as a vascular risk factor has been reviewed elsewhere [5] and will not be considered further. A number of studies have also shown that increased plasma ADMA concentrations are associated with renal dysfunction, cardiovascular risk, diabetes, pre-eclampsia, pulmonary hypertension and insulin resistance [6–10] and that this increase in ADMA is probably due to impaired metabolism by DDAH [2]. Since SDMA is thought not to be metabolised, but only excreted via the kidney, interest has arisen in its possible use as a marker of renal dysfunction [11].

The growing interest in methylated arginines, both for research and possible clinical applications, means that there is a need to develop simple but reliable methods for their measurement. ADMA and SDMA are isomers, and therefore their measurement is problematic. A number of methods have been described, mostly focussing upon the analysis of ADMA, these include HPLC, LC–MS, LC–MS/MS, GC–MS, CZE and ELISA [12–18]. Problems with these methods include: the use of complicated chromotographic gradients to effect the separation of ADMA from SDMA, long run times, and lack of appropriate internal standardisation. Most methods are unable to measure all relevant analytes within one run and the ability to simultaneously measure ADMA and arginine offers particular advantages, as it has been shown that increases in the arginine/ADMA ratio are associated with increases in nitric oxide production [19]. With increasing interest in the clinical applications of measurements of arginine and methylated arginines there is a

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need for the development of simple and robust methods for their determination, which are suitable for implementation in a busy, routine clinical environment.

The work described here shows that it is possible to simultaneously measure ADMA, SDMA, MMA and arginine in plasma and urine without the need to derivatise these substances nor to separate them chromatographically. The ability to measure all four analytes within one run with short analysis time has advantages both for research into their inter-relationships and also for application of the method in the routine clinical laboratory. In addition the utilisation of isotopically labelled internal standards for each individual analyte adds robustness to the measurements, increasing their reliability in the clinical setting.

2. Experimental

2.1. Chemicals

N^G,N^G-dimethylarginine hydrochloride, N^G,$N^{G\prime}$-dimethyl-L-arginine di(*p*-hydroxyazobenzene-*p*′-sulphonate) salt, N^G-methyl-L-arginine acetate salt, arginine, copper carbonate, cyanogen bromide-activated Sepharose, dimethylamine, dimethylsulphate, 1,2-dimethyl-2-thiopseudourea hydroiodide, 1,3-dimethyl-2-thiourea, *N*,*N*′-dimethylurea and Supelclean LC-SCX solid phase extraction tubes were purchased from Sigma–Aldrich (Dorset, UK). Formic acid and 0.1 M HCl were obtained from VWR (Leicestershire, UK). Ammonia solution (35%) and HPLC grade methanol were purchased from Fisher Scientific (Leicestershire, UK). L-Arginine-d_7:HCl and L-ornithine-d_2:2HCl were supplied by CK Gas Products Ltd. (Hampshire, UK).

2.2. Samples

Blood from apparently healthy individuals was collected by venepuncture into tubes containing lithium heparin as anticoagulant. After centrifugation at $1000 \times g$ for 10 min plasma was removed from the samples and stored at −20 °C until analysis. Portions of 24 h urine collections, obtained from individuals undergoing routine biochemical investigations but with no history of vascular disease, were stored at −20 °C until analysis.

2.3. Instrumentation

Mass spectrometric analysis was performed using a Perkin-Elmer HPLC autosampler and micro-pump (series 200) coupled to a Perkin-Elmer Sciex API 2000 triple quadropole mass spectrometer. The signals were processed using Analyst-NT software supplied by Perkin-Elmer. The mobile phase consisted of 0.1% formic acid pumped at a flow rate of 50 μl/min. A dwell time of 500 ms was used for each analyte with a total run time of 3 min.

2.4. Internal standard synthesis

An ornithine-d_2 copper complex was used as the starting point for synthesis of all internal standards. The preparation of the complex was based upon that described by Albsmeier et al. [20]:

(a) For each synthesis ornithine-d_2 copper complex was prepared as follows: 20.6 mg ornithine-d_2:2HCl was dissolved in 1 ml water. Small amounts of copper carbonate were added, with mixing, resulting in the development of a blue colour. Copper carbonate continued to be added until saturation was achieved and the intensity of the colour no longer increased. The ornithine-d_2 copper complex preparation was centrifuged and the supernatant removed and stored at 4 °C for later conversion into ADMA-d_2, SDMA-d_2 or MMA-d_2.

(b) Synthesis of ADMA-d_2: this was based upon the procedure described by Albsmeier et al. [20]. In brief 0.5 g bromcyan-agarose was suspended in 10 ml of 1 mM HCl in methanol and mixed for 30 min. The bromcyan-agarose was then washed with 10 ml of 1 mM HCl and three times with 10 ml water. The ornithine-d_2 complex prepared above was added to the bromcyan-agarose and mixed overnight at 4 °C. The mixture was then centrifuged, the supernatant removed and discarded and the bromcyan-agarose washed with 10 ml 1 M HCl and then three times with 10 ml water. The ornithine-d_2 bromcyan-agarose complex was suspended in 20 ml 20% dimethylamine and the mixture incubated at 50 °C for 24 h. The supernatant was then removed and dried under vacuum, reconstituted in methanol:water 50:50, centrifuged and the supernatant again dried under vacuum. The residue was dissolved in 10 ml 0.1 M HCl to yield a stock solution. Analysis of the product by mass spectrometry confirmed the formation of ADMA-d_2; no formation of ADMA, SDMA, MMA or arginine was detected.

(c) Synthesis of SDMA-d_2: this was based upon a procedure described by Kakimoto and Akazawa [21] for the synthesis of SDMA from ornithine. Dimethylsulphate (50 μl) was added to dimethylthiopseudourea (54 mg). The container was sealed and heated to 110 °C for 30 min. The ornithine-d_2 copper complex prepared above was added along with 100 μl 5 M NaOH and 9 ml water. The mixture was then incubated at 30 °C for 3 days. The mixture was centrifuged and the supernatant removed and dried under vacuum, reconstituted in methanol:water 50:50, centrifuged and the supernatant again dried under vacuum. The residue was dissolved in 10 ml 0.1 M HCl to yield a stock solution. Analysis of the product by mass spectrometry confirmed the formation of SDMA-d_2; no formation of ADMA, SDMA, MMA or arginine was detected.

(d) Synthesis of methylarginine-d_2: this was based upon a procedure described by Corbin [22] for the synthesis of methylarginine from ornithine. To the ornithine-d_2 copper complex prepared above, 2 ml of 35% ammonia solution and 23.2 mg 1,2-dimethyl-2-thiopseudourea was added. The mixture was then incubated at 25 °C for 24 h. The mixture was then centrifuged and the supernatant removed and dried under vacuum, reconstituted in methanol:water (50:50), centrifuged and the supernatant again dried under vacuum. The residue was dissolved in 10 ml 0.1 M HCl to yield a stock solution. Analysis of the product by mass spectrometry confirmed the formation of MMA-d_2; no formation of ADMA, SDMA, MMA or arginine was detected.

2.5. Mass spectrometry

The parent/daughter ions for each analyte, along with the optimal potential settings for their production, were determined by continuous infusion of a 10 μM solution of each analyte in 0.1% formic acid and utilisation of the Autotune facility provided with the Analyst-NT software.

Optimal nebulizer conditions were determined by repeat injection of 10 μl of a 10 μM solution of ADMA and stepwise variation of individual gas flows and temperature.

2.6. Working standards for sample analysis

Four standards solutions, in 0.1 M HCl, were prepared with concentrations of 0.5, 1.0, 2.0 and 5.0 μM for ADMA, SDMA and MMA and 20, 40, 80 and 200 μM for arginine. Standards were

stored frozen at −20 °C and analysed with each batch of samples.

2.7. Sample preparation and analysis

To 200 μl plasma or diluted urine (1:9 with deionised water) was added 50 μl internal standard (containing approximately 5 μM of each of ADMA-d_2, SDMA-d_2, methylarginine-d_2 and 50 μM arginine-d_7) and 1 ml 0.1 M HCl. SPE columns were conditioned with two column reservoirs of methanol (approximately 1.2 ml each) followed by two reservoirs of 0.1 M HCl. The prepared sample was then applied to the column and drawn through under vacuum. The column was washed with two reservoirs of water and then two reservoirs of methanol. The analytes were then eluted with 1 ml of 35% ammonia solution:methanol (1:4) and the eluate dried under vacuum. Finally the residue was reconstituted in 100 μl 0.1% formic acid and transferred to autosampler vials and 20 μl injected into the mass spectrometer. Results were calculated automatically from the standards using weighted linear regression (weighting: 1/(concentration)^2).

2.8. Analytical method validation

To assess linearity five aqueous standards were prepared (ADMA and SDMA: 5, 10, 15, 20 and 25 μM; methylarginine: 10, 20, 30, 40 and 50 μM and arginine: 50, 100, 150, 200 and 250 μM). Each standard was analysed in triplicate in random order.

Within- and between-batch precision was assessed by analysing 10 samples. For low concentrations pooled plasma or pooled urine was used. For intermediate and high concentrations the low concentration pools were spiked with aqueous standard (pool:standard::19:1).

Recovery experiments were performed by spiking 10 individual plasma or urine samples with either 0.1 M HCl or aqueous standard at two different concentrations (sample:standard::19:1).

The limit of detection and limit of quantitation were determined by analysing 10 sample blanks (water in place of sample).

3. Results

Unique parent/daughter combinations for the individual analytes were found to be *m/z* ADMA: 203.01 → 46.32, ADMA-d_2: 205.01 → 46.32, SDMA: 203.01 → 172.03, SDMA-d_2: 205.01 → 174.03, MMA: 189.04 → 70.10, MMA-d_2: 191.04 → 72.10, arginine: 175.05 → 70.13, arginine-d_7: 182.05 → 77.13. The fragmentation patterns of ADMA and SDMA are shown in Figs. 1 and 2. Further analysis of the unique daughter ion for ADMA showed that its production did not arise from the loss of formic acid (HCOOH) but was rather due to molecular rearrangement, with the subsequent loss of $CH(NH_2)OH$ [23]. The probable mechanisms for the production of unique daughter ions for ADMA and SDMA

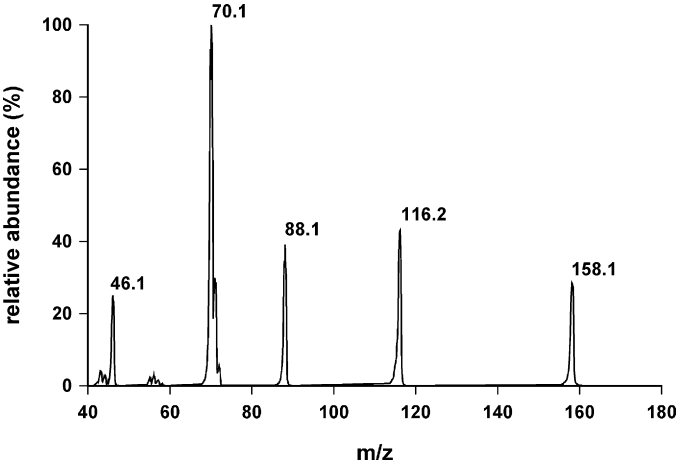

Fig. 1. ADMA fragmentation pattern.

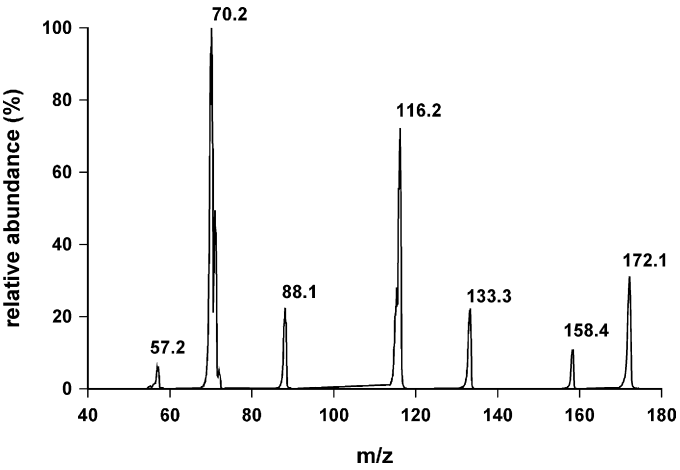

Fig. 2. SDMA fragmentation pattern.

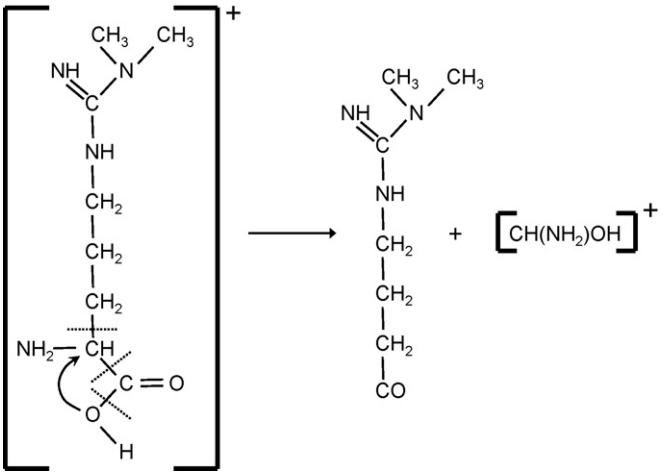

Fig. 3. Fragmentation mechanism for ADMA.

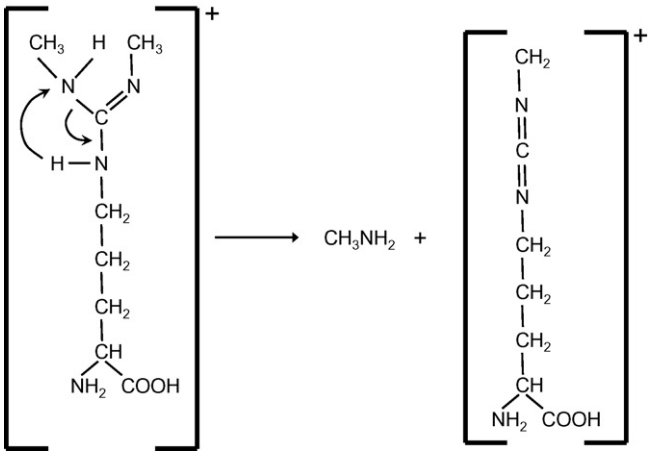

Fig. 4. Fragmentation mechanism for SDMA.

Table 1
Optimal potential settings (V)

	Declustering potential	Focussing potential	Entrance potential	Collision energy	Collision entrance potential	Collision exit potential
ADMA	26	360	−8.0	37	13.6	0
ADMA-d_2	26	360	−8.0	37	13.6	0
SDMA	31	220	−6.5	17	12.0	30
SDMA-d_2	31	220	−6.5	17	12.0	30
Methylarginine	41	210	−6.5	33	13.6	0
Methylarginine-d_2	41	210	−6.5	33	13.6	0
Arginine	31	60	−8.5	31	12.0	2
Arginine-d_7	31	60	−8.5	31	12.0	2

Table 2
Regression data for linearity studies

	Slope	S.D. slope	Intercept	S.D. intercept	S.E.
ADMA	0.1722	0.0019	0.0406	0.0312	0.0516
SDMA	0.1955	0.0014	0.0670	0.0237	0.0391
MMA	0.1823	0.0017	0.0639	0.0568	0.0938
Arg	0.0183	0.0001	0.0235	0.0230	0.0379

are shown in Figs. 3 and 4. Optimal potential settings are shown in Table 1.

Optimal nebulizer conditions were: curtain gas (air) 40 psi, ion source gas 1 (nitrogen) 10 psi, ion source gas 2 (nitrogen) 0 psi, collision gas (nitrogen) 4 psi and temperature 500 °C.

The method was shown to be linear to at least 25 μM for both ADMA and SDMA, 50 μM for MMA and 250 μM for arginine. Regression data are shown in Table 2.

Data for precision and recovery studies are shown in Tables 3 and 4.

The limit of detection (3× blank S.D.) for ADMA, SDMA, MMA and arginine was 0.023, 0.011, 0.009 and 0.258 μM respectively, whilst the limit of quantitation (10× blank S.D.) was 0.077, 0.037, 0.030 and 0.860 μM respectively.

Table 3
Precision data

	Within-batch		Between-batch	
	Mean (μmol/l)	%CV	Mean (μmol/l)	%CV
Plasma				
ADMA	0.497	1.7	0.499	4.1
	1.411	2.1	1.486	2.6
	5.637	0.9	5.693	1.8
SDMA	0.547	2.2	0.515	7.6
	1.338	2.4	1.335	3.0
	4.931	1.7	4.825	1.8
Methylarginine	0.158	3.0	0.153	13.5
	2.280	0.9	2.403	2.5
	11.560	0.6	11.950	3.0
Arginine	109	1.1	114	1.4
	125	1.1	133	2.2
	237	1.2	239	1.9
Urine				
ADMA	86.2	2.0	85.6	2.1
	102.4	2.0	102.2	2.6
	185.7	2.0	182.9	2.2
SDMA	68.8	2.7	69.8	3.2
	84.9	2.0	87.0	3.0
	163.7	2.2	166.9	3.3
Methylarginine	2.1	2.9	1.7	10.8
	22.5	1.9	22.9	2.0
	104.8	2.0	106.5	2.4
Arginine	48.2	5.6	39.0	12.7
	72.5	2.5	62.7	6.0
	169.0	3.1	165.5	7.1

Table 4
Recovery data

	Added (μmol/l)	Mean recovery (%)	%CV
Plasma			
ADMA	1	91.7	10.3
	5	91.4	2.2
SDMA	1	91.7	11.8
	5	91.4	4.0
Methylarginine	2	90.3	5.8
	10	91.6	1.5
Arginine	20	98.2	23.2
	100	90.5	4.3
Urine			
ADMA	20	91.7	19.5
	100	92.0	5.6
SDMA	20	98.0	23.2
	100	93.5	5.9
Methylarginine	20	98.3	6.1
	100	98.3	4.6
Arginine	20	89.4	14.3
	100	92.6	4.3

The results of the analysis of lithium heparin plasma from nine healthy volunteers are shown in Table 5 and the results of analysis of five 24 h urine collections are shown in Table 6.

Fig. 5 shows the peaks corresponding to ADMA, SDMA, MMA and their respective internal standards obtained for the first plasma sample in Table 5. The peaks associated with arginine and arginine-d_7 are much larger than the others and have been omitted from the figure for purposes of clarity.

4. Discussion

The fact that ADMA and SDMA are isomers has always presented a challenge for their analysis and the vast majority of methods,

Table 5
Concentrations of arg and methylated arginines in plasma of nine healthy male volunteers

	Age (years)	ADMA (μM)	SDMA (μM)	MMA (μM)	Arg (μM)
	24	0.512	0.472	0.182	132
	18	0.177	0.448	0.201	249
	24	0.265	0.504	0.157	167
	20	0.290	0.414	0.332	316
	24	0.387	0.371	0.158	125
	20	0.484	0.523	0.167	125
	18	0.443	0.547	0.195	150
	19	0.627	0.456	0.190	133
	21	0.372	0.499	0.176	64
Mean ± 1 S.D.	20.9 ± 2.5	0.395 ± 0.139	0.470 ± 0.055	0.195 ± 0.054	162 ± 76

Table 6
Concentrations of arg and methylated arginines in five 24 h urine collections

Age (years)	Urine volume (ml)	ADMA (μmol/24 h)	SDMA (μmol/24 h)	MMA (μmol/24 h)	Arg (μmol/24 h)
59	1233	48.8	42.5	1.55	20.8
25	2594	57.6	62.3	1.85	6.0
41	2466	52.3	59.7	1.18	22.9
52	2714	34.2	58.1	1.35	5.6
35	2150	46.2	40.0	1.34	7.9
Mean ± 1 S.D.		47.8 ± 8.7	52.5 ± 10.4	1.5 ± 0.3	12.7 ± 8.5

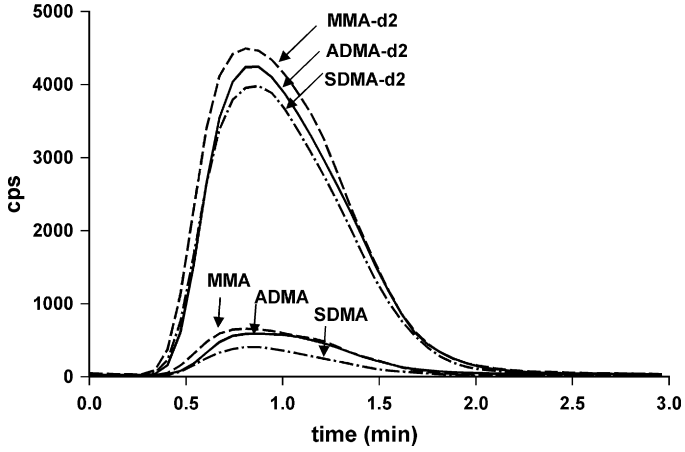

Fig. 5. Analysis of a typical plasma sample.

including LC–MS/MS, utilise chromatographic separation, often involving the use of complicated gradient systems. Here we show that unique daughter ions formed by ADMA and SDMA, without derivatisation, may be used for their determination. Although the unique daughter ions are not the fragments formed with greatest abundance they are still produced in sufficient abundance to allow their reliable quantitation.

The concentrations of plasma ADMA and SDMA obtained for nine healthy volunteers are consistent with data published elsewhere [12,15,17] as are the results of the analysis of five 24 h urine collections [18].

Internal standardisation of methylated arginine assays has also proven difficult and has not always been applied in assays so far described. Where this procedure has been implemented, the most commonly used internal standard has been homoarginine. Since homoarginine occurs naturally in human plasma its use as an internal standard, even though endogenous plasma concentrations are low, is far from ideal. In addition signal production in mass spectrometry can be subject to ion-suppression effects, where non-volatile co-eluting matrix components reduce the production of ions for the analyte of interest [24]. Suppression effects vary. In mass spectrometry in general, methods in which there is an extraction procedure remove much of the background matrix and produce cleaner preparations, they are therefore less subject to suppression effects than methods in which there is little or no sample cleanup. Where chromatography is used in conjunction with mass spectrometry ion-suppression can occur to different extents in different regions of the chromatogram and therefore an internal standard that does not co-elute with the analyte of interest (i.e. homoarginine and ADMA) may not compensate for its signal suppression. The ideal internal standard is an isotopically labelled form of the analyte but in the case of methylated arginines these are not commercially available. The synthesis and utilisation of isotopically labelled ADMA has been described by Albsmeier et al. [20], who also raised the issue of the necessity for the development of an isotopically labelled SDMA internal standard. We describe, for the first time, relatively simple procedures for the synthesis of SDMA-d_2 and MMA-d_2 from ornithine-d_2 which has allowed the development of a method with isotopically labelled internal standards for each, individual analyte. Although the yields for the synthesis of SDMA-d_2 and MMA-d_2 were low (each about 5%) sufficient isotopically labelled material was produced for many sample analyses.

5. Conclusions

The work described here shows that it is possible to simultaneously measure ADMA, SDMA, MMA and arginine in both plasma and urine with a short run time (3 min). The use of unique daughter ions for the identification and quantitation of ADMA and SDMA alleviates the necessity for chromatographic separation of these isomers with complicated gradients and long run times, rendering it suitable for the busy, routine, clinical laboratory and research applications. In addition the synthesis of isotopically labelled ADMA, SDMA and MMA, by simple chemical procedures is described, the utilisation of which adds to the precision and robustness of the method.

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References

[1] S. Pahlich, R.P. Zakaryan, H. Gehring, Biochim. Biophys. Acta 1764 (2006) 1890.
[2] C.T.L. Tran, J.M. Leiper, P. Vallance, Atherosclerosis (Suppl. 4) (2003) 33.
[3] J. Leiper, P. Vallance, Cardiovasc. Res. 43 (1999) 542.
[4] R.H. Boger, Cardiovas. Res. 59 (2003) 824.
[5] V. Valkonen, R. Laaksonen, Clin. Chim. Acta 348 (2004) 9.
[6] P. Vallance, J. Leiper, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1023.
[7] R.H. Boger, C. Zoccali, Atherosclerosis (Suppl. 4) (2003) 23.
[8] F. Abbasi, T. Asagmi, J.P. Cooke, C. Lamendola, T. McLaughlin, G.M. Reaven, M. Stuehlinger, P.S. Tsao, Am. J. Cardiol. 88 (2001) 1201.
[9] D.P. Holden, S.A. Fickling, G.J. St. Whitley, S.S. Nussey, Am. J. Obstet. Gynecol. 178 (1998) 551.
[10] M.C. Stuhlinger, F. Abbasi, J.W. Chu, C. Lamendola, T.L. Mclaughlin, J.P. Cooke, G.M. Reaven, P.S. Tsao, JAMA 287 (2002) 1420.
[11] J.T. Kielstein, S.R. Salpeter, S.M. Bode-Boeger, J.P. Cooke, D. Fliser, Nephrol. Dial. Transplant. 21 (2006) 2446.
[12] A. Pettersson, L. Uggla, V. Backman, J. Chromatogr. B 692 (1997) 257.
[13] B. Chen, L. Xia, R. Zhao, J. Chromatogr. B 692 (1997) 467.
[14] K. Vishwanathan, R.L. Tackett, J.T. Stewart, M.G. Bartlett, J. Chromatogr. B 748 (2000) 157.
[15] E. Causse, N. Siri, J.F. Arnal, C. Bayle, P. Malatray, P. Valdiguie, R. Salvayre, F. Couderc, J. Chromatogr. B 741 (2000) 77.
[16] M. Marra, A.R. Bonfigli, R. Testa, I. Testa, A. Gambini, G. Coppa, Anal. Biochem. 318 (2003) 13.
[17] D. Tsikas, B. Schubert, F. Gutzki, J. Sandmann, J.C. Frolich, J. Chromatogr. B 798 (2003) 87.

[18] L. Huang, F. Guo, Y. Liang, Q. Hu, B. Cheng, Anal. Chim. Acta 487 (2003) 145.
[19] S.M. Bode-Boger, R.H. Boger, S. Kienke, W. Junker, J.C. Frolich, Biochem. Biophys. Res. Commun. 219 (1996) 598.
[20] J. Albsmeier, E. Schwedhelm, F. Schulze, M. Kastner, R.H. Boger, J. Chromatogr. B 809 (2004) 59.
[21] Y. Kakimoto, S. Akazawa, J. Biol. Chem. 245 (1970) 5751.
[22] J.L. Corbin, Anal. Biochem. 57 (1974) 310.
[23] G. Junk, H. Svec, J. Am. Chem. Soc. 85 (1963) 839.
[24] T.M. Annesley, Clin. Chem. 49 (2003) 1041.