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Comparison of HPLC method and commercial ELISA assay for asymmetric dimethylarginine (ADMA) determination in human serum

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Abstract

The performance of a new ELISA assay kit (DLD Diagnostika GmbH, Hamburg, Germany) for the determination of asymmetric dimethylarginine (ADMA) was evaluated against a reversed phase HPLC method. ADMA concentrations of 55 serum samples were measured with both methods. The intra-assay CV for ADMA-ELISA was 19% ($n = 10$). Inter-assay CVs for ADMA-ELISA were 9% for kit control 1 (0.410 ± 0.037 μM) and 14% for kit control 2 (1.174 ± 0.165 μM). The intra- and inter-assay CVs for HPLC assay for ADMA were 2.5% (0.586 ± 0.015 μM) and 4.2% (0.664 ± 0.028 μM), respectively. There was no correlation between these two methods ($R^2 = 0.0972$). The effect of storage conditions of the samples on ADMA concentrations was investigated by HPLC. ADMA concentration was stable after four freezing and thawing cycles. Overall, the HPLC method offered better sensitivity, selectivity and, very importantly, simultaneous determination of ADMA, SDMA, L-homoarginine and L-arginine.

Keywords: ADMA; SDMA; L-Homoarginine; L-Arginine; ELISA; HPLC

1. Introduction

Asymmetric dimethylarginine (ADMA) has been demonstrated to be an endogenous competitive inhibitor of nitric oxide synthase (NOS) (EC 1.14.13.39) that competes with the binding of the natural substrate L-arginine [1]. ADMA is produced from methylated arginine residues in proteins by protein methyltransferases [2,3] and it is metabolized by the enzyme N^G,N^G-dimethylarginine dimethylaminohydrolase 1 and 2 (DDAH) [4,5]. ADMA has a stereoisomer, symmetric dimethylarginine (SDMA), which is not an NOS inhibitor. Therefore, the ADMA assay methods should be able to distinguish between these two isomers. Increased ADMA levels are associated with reduced nitric oxide (NO) synthesis as assessed by impaired endothelium-dependent vasodilatation. ADMA has been reported to accumulate in the plasma of patients with hypercholesterolemia [6], atherosclerosis [7], hypertension [8,9], diabetes [10–12], renal failure [1,13] and chronic heart failure [14,15]. In addition, ADMA might be an independent predictor of future vascular events [16].

The measurement of the ADMA concentrations in plasma has been of interest, since its association with NO metabolism was discovered in 1992 [1]. Several HPLC methods have been reported for the analysis of ADMA [9,17,18]. The HPLC methods utilize several detection techniques, but the most common method for ADMA analysis has been fluorescence detection with ortho-phthaldialdehyde (OPA) or naphthalene-2,3-dicarboxaldehyde (NDA) derivatization due to their high sensitivity. In addition, a few other methods for the determination of ADMA have been published such as capillary electrophoresis [19], liquid chromatography–mass spectrometry (LC–MS) [20,21] and gas chromatography coupled with mass spectrometry (GC–MS) [22] or tandem mass spectrometry (GC–MS–MS) [23]. Only a few commercial ELISA kits are available for ADMA measurement [24,25].

The aim of the present study was to evaluate the performance of a ADMA-ELISA kit (DLD Diagnostika GmbH, Hamburg, Germany) for the determination of ADMA against an optimized

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HPLC method for ADMA, SDMA and L-arginine according to the method described by Teerlink et al. [18]. ADMA concentrations in serum samples of 55 patients were measured with both methods. The effect of storage conditions of samples on ADMA concentration was also investigated.

2. Experimental

2.1. Reagents

L-NMMA (N^G-monomethyl-L-arginine), L-homoarginine and 3-mercaptopropionic acid were obtained from Fluka (Buchs, Switzerland). ADMA (N^G,N^G-dimethyl-L-arginine), boric acid and OPA were obtained from Sigma (St. Louis, MO, USA). L-Arginine was obtained from Calbiochem (Merck Biosciences, Darmstadt, Germany). Hydrochloric acid was obtained from Riedel-deHaën (Sigma–Aldrich Laborchemikalien GmbH, Seelze, Germany). Ammonium hydrochloride, methanol and HPLC-grade acetonitrile were obtained from Merck (Darmstadt, Germany). Potassium dihydrogenphosphate was obtained from FF-Chemicals (Yli-Ii, Finland). ADMA-ELISA kits were purchased from DLD Diagnostika GmbH (Hamburg, Germany).

2.2. Samples

Serum samples for ADMA and ELISA comparison test were collected from the subjects of Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) [26] or their relatives [27]. For method evaluation and sample type comparison, EDTA-plasma and serum samples were collected from healthy volunteers. All blood samples were drawn after a 12 h overnight fast. Serum was allowed to clot at room temperature for 30 min. The blood samples were centrifugated at 1500 × *g* for 10 min and plasma or serum were separated and stored frozen in −70 °C until analysis.

2.3. ELISA assay

2.3.1. Sample preparation

Acylation was conducted in the 96-well reaction plate supplied with the kit according to the instructions of the manufacturer. Standards, kit controls and samples (20 μl) were mixed with 25 μl acylation buffer and 25 μl equalizing reagent. Subsequently, 25 μl acylation reagent was added and the reaction plate was incubated for 30 min at room temperature on an orbital shaker. Diluted equalizing reagent (100 μl) was added and the incubation was continued for 45 min. After the incubation, the samples were ready for the ELISA analysis.

2.3.2. Performance of assay

The ADMA-ELISA kit consists of a split-type reaction plate (12 × 8) coated with ADMA, six standards (0–5 μM), rabbit anti-ADMA antiserum, goat anti-rabbit-IgG-peroxidase conjugate, TMB substrate solution, stop solution and wash buffer. Aliquots (50 μl) of the acylated standards, kit controls or samples were processed according to the instructions of the kit manufacturer. Absorbances were measured with a microplate reader (Tecan SPECTRAFluor, Tecan Group Ltd., Maennedorf, Switzerland) using a wavelength of 450 nm (reference wavelength 620 nm). All samples, kit controls and standards were analyzed in duplicate.

2.4. HPLC assay

2.4.1. Sample extraction and chromatographic conditions

ADMA, SDMA, L-homoarginine and L-arginine concentrations were determined by HPLC using precolumn derivatization with OPA according to the method described by Teerlink et al. [18] with small modifications. In order to optimize the HPLC method, we modified that of Teerlink et al. by using longer total run time (38 min versus 30 min) and 35 vol.% acetonitrile instead of 50 vol.% for column washing after elution of the last analyte. Our injection volume was two-fold compared to Teerlink's method and autosampler derivatized every sample just before each HPLC run instead of derivatization of all purified samples at the same time. Calibration standard containing L-arginine (21 μM), L-homoarginine (2 μM), ADMA (3 μM) and SDMA (2 μM). L-NMMA (6 μM) was used as an internal standard. Plasma pool (58.6 ± 4.3 μM for L-arginine, 1.2 ± 0.03 μM for L-homoarginine, 0.643 ± 0.029 μM for ADMA and 0.654 ± 0.028 μM for SDMA) was used as quality control. Prior to analysis standards, quality controls and samples were extracted on Oasis MCX solid phase extraction cartridges (Waters, Milford, MA, USA). Briefly, standards, quality controls and samples (200 μl) were mixed with internal standard (100 μl L-NMMA) and 700 μl phosphate buffered saline (PBS), pH 7.2 and then applicated onto the columns. The columns were washed with 1 ml of 100 mM HCl and 1 ml of methanol. Dimethylarginines were eluted with 1 ml ammonia–water–methanol (10:40:50, v/v). The eluents were dried under nitrogen (+55 °C) and dissolved in 100 μl ion exchanged water (Milli Q, Millipore, Billerica, MA, USA) for HPLC analysis.

HPLC analysis was carried out on a Merck Hitachi liquid chromatography system (Hitachi, Tokyo, Japan) consisting of a gradient pump (D-6200), an autosampler (AS-4000) and a fluorescence detector (F1000). Standards, quality controls and samples (100 μl) were incubated for 2 min with the 100 μl OPA reagent (1 mg/ml OPA in borate buffer, pH 9.5, containing 0.1 vol.% 3-mercaptopropionic acid) before automatic injection (40 μl) into the HPLC. The OPA-derivatives of ADMA and internal standard were separated on Symmetry C18 column (4.6 mm × 150 mm, 5 μm, Waters, Milford, MA, USA) with fluorescence monitor set at λ^{ex} = 340 nm and λ^{em} = 455 nm. The column temperature was kept at +30 °C. Standards, quality controls and samples were eluted from the column with 50 mM K-phosphate buffer, pH 6.5 and 8.7 vol.% acetonitrile, at a flow rate of 1.1 ml/min. After elution of the last analyte, the column was washed with stronger solvent (35 vol.% acetonitrile from 24 to 29 min). After washing, the column was equilibrated for 8 min with separation buffer, resulting in a total run time of 38 min. L-Arginine was analyzed with the same method, but in that case the injection volume was 10 μl and the total run time was shorter (33 min). Data acquisition and analysis were performed

using D-7000 HPLC System Manager software (Hitachi, Tokyo, Japan).

2.4.2. Standard curves and recovery

Calibration standards (0.09, 0.18, 0.38, 0.74, 1.47, 2.96 and 7.5 μM) were prepared for ADMA and SDMA from the stock solutions (3.64 mM) diluted in PBS. Calibration standards for L-arginine were 1.5, 3.1, 6.3, 12.5, 25, 50, 100 and 200 μM. Calibration curves were calculated by graphing the ratio of peak heights of the standard to peak heights of the internal standards versus concentration. The recovery of ADMA, SDMA and L-arginine was studied by spiking three plasma samples from healthy volunteers with known quantities of standard, extracting and quantitating spiked analyte.

2.4.3. Sample type and storage stability

To test the influence of sample collection tube on the analysis, blood samples from healthy volunteers were collected into different Vacutainer tubes (Becton Dickinson Diagnostics, Plymouth, UK). Tubes with anticoagulant (heparin, LH 68 I.U., ref 368884 and LH PST II, ref 367374 or EDTA, K2E 5.4 mg, ref 368856) and tubes without anticoagulant were tested (serum, CAT, ref 369032 and SST II Advance, ref 367957). The results of plasma samples were compared with serum values. To study the storage stability of ADMA in frozen sample and the effect of variable freezing and thawing cycles, the plasma samples were divided into aliquots and were placed into a freezer (−20 °C). The aliquots were analyzed after one, two, three or four freezing and thawing cycles. Time between freezing and thawing cycles was 24 h.

2.5. Statistics

Data are presented as mean ± standard deviation (S.D.). Statistical analyses were performed with the regression analysis. A probability level of <0.05 was considered statistically significant.

3. Results and discussion

3.1. ELISA assay

Three different microtiter plates were tested, and standards, kit controls and samples were analyzed in duplicate. Quality controls from the ADMA-ELISA kit were analyzed in every plate. In ELISA test, the mean linearity from the dilutions is 90%, the mean recovery was 95% from spiked samples and sensitivity was 0.05 μM according to manufacturer kit instructions for use. Absorbance of the ADMA-ELISA standards was from 0.3 to 1.8 AU as reported by Schulze et al. [24]. Concentrations of the kit control 1 (0.410 ± 0.037 μM) were between the target range values (0.24–0.45 μM) in every analyzed plate, whereas the concentrations of the kit control 2 (1.174 ± 0.165 μM) were higher than the target range values (0.6–1 μM) in two of three plates. The intra-assay CV of the plasma pool (0.436 ± 0.083 μM, $n = 10$) was 19% in the ADMA-ELISA assay. The inter-assay CV of ADMA was 9% for kit control 1 and 14% for kit control 2 ($n = 3$).

3.2. HPLC assay

Method validation was done for the HPLC method and parameters including calibration linearity, recovery, reproducibility and stability were studied.

3.2.1. Sample preparation and recovery

Solid phase extraction was performed for the standards, quality controls and the samples before HPLC separation. For the ADMA, SDMA and L-homoarginine quantitation, only 0.2 ml of plasma or serum is needed in order to accurately determine normal low levels of the analyte. The mean recoveries for ADMA, SDMA, L-homoarginine and L-arginine from plasma were 95, 95, 100 and 113% (Table 1), respectively, which agrees well with other reports [18,28].

3.2.2. Sample derivatization and chromatography

For the HPLC method, ADMA, SDMA, L-homoarginine and L-arginine were derivatized into a fluorescent derivative by reaction of their α-amino group with OPA reagent. Mercaptopropionic acid was used for the derivatization because of its higher stability with the OPA reagent compared to mercaptoethanol. The precolumn derivatization procedure was performed automatically by the autosampler giving repeatable results. Under isocratic conditions, ADMA and SDMA were almost completely separated. The retention times were as follows: L-arginine (10.52 ± 0.13 min), internal standard (14.87 ± 0.22 min), L-homoarginine (16.85 ± 0.25 min), ADMA (19.76 ± 0.29 min) and SDMA (20.89 ± 0.30 min). Representative chromatograms of a plasma sample with and without internal standard are presented in Fig. 1.

3.2.3. Linearity and detection limit

Analyte concentrations of the samples were calculated from the calibration standard, which was extracted and chro-

Table 1
Recovery of ADMA SDMA, L-arginine and L-homoarginine (μM) from human plasma ($n = 3$)

	ADMA		SDMA		L-Homoarginine		L-Arginine	
	Mean ± S.D.	Recovery (%)	Mean ± S.D.	Recovery (%)	Mean ± S.D.	Recovery (%)	Mean ± S.D.	Recovery (%)
Plasma	0.390 ± 0.010		0.379 ± 0.021		5.07 ± 0.07		106.4 ± 2.6	
Spiked plasma	2.252 ± 0.101	95	2.246 ± 0.092	95	8.73 ± 0.139	100	129.0 ± 2.9	113

Spike added ADMA 1.97 μM, SDMA 1.97 μM, L-homoarginine 3.65 μM and L-arginine 19.95 μM.

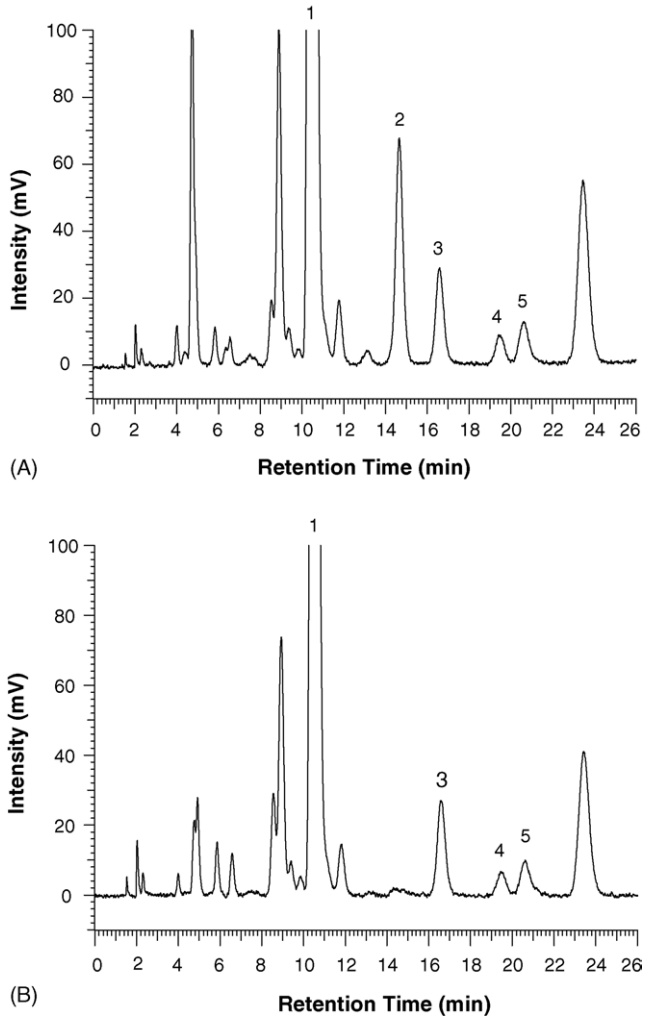

Fig. 1. Typical chromatogram of normal plasma with internal standard (L-NMMA) (A), and without internal standard (B). Peak identity: (1) L-arginine; (2) L-NMMA; (3) L-homoarginine; (4) ADMA; (5) SDMA. HPLC conditions are described in Section 2.

matographed under the same conditions as the samples. Excellent linearity was observed for ADMA and SDMA over the concentration range 0.1–7.5 μM (correlation coefficients $R^2 = 0.999$, $y = 0.2848x + 0.0141$ for ADMA and $R^2 = 0.999$, $y = 0.2620x + 0.0153$ for SDMA, respectively). The calibration curve for ADMA and SDMA was linear up to 20 μM and it confirmed the results of Teerlink et al. [18]. The correlation coefficients for L-homoarginine and L-arginine calibration curve were $R^2 = 0.992$ ($y = 0.1810x + 0.0113$) and $R^2 = 0.999$ ($y = 0.4432x + 0.0903$), respectively. The calibration curve for L-arginine was linear up to 200 μM. For the analysis of samples, single-point calibration was performed because of the good linearity of the calibration curve. The linearity of serum samples was good ($n = 17$, $y = 0.2005x - 0.0005$, $R^2 = 0.9996$, $P < 0.001$). The lower limit of detection (LOD) of the HPLC assay based on a signal/noise ratio of 3 was below 0.1 μM for ADMA, SDMA, L-homoarginine and below 3 μM for L-arginine. The lower limit of quantitation (LOQ) was below 0.3 μM for ADMA, SDMA, L-homoarginine and below 10 μM for L-arginine.

3.2.4. Reproducibility

The intra-assay CVs of the plasma pool for ADMA (0.643 μM), SDMA (0.654 μM), L-homoarginine (1.2 μM) and L-arginine (58.6 μM) were 2.5, 5.6, 1.4 and 2.5% ($n = 9$), respectively. Inter-assay CVs of 10 series of samples for ADMA, SDMA, L-homoarginine and L-arginine were 4.2, 3.7, 2.9 and 2.8%, respectively, when two quality controls were analyzed in the same sequence.

3.2.5. Sample type and storage stability

In order to test the influence of sample collection tube on the analysis, blood samples from healthy volunteers ($n = 3$) were collected into five different Vacutainer tube types with and without anticoagulant. There were no significant differences between the ADMA (0.5364 ± 0.015, CV = 2.7%) or SDMA (0.402 ± 0.010, CV = 2.6%) concentrations collected to all different test tubes.

Mean concentration measurements of plasma (0.607 ± 0.064 μM) versus serum (0.603 ± 0.072 μM) samples gave significantly similar results for ADMA ($n = 15$, correlation coefficient = 0.907, $P < 0.001$, $y = 1.0276x - 0.0205$). Finally, the stability of ADMA concentration during the storage of samples was evaluated. ADMA (0.479 ± 0.011 μM, CV = 2.4%) and SDMA (0.462 ± 0.003 μM, CV = 0.7%) concentrations were stable in EDTA-plasma sample after four freezing and thawing cycles (Fig. 2). The storage stability of ADMA was tested by analyzing aliquots of EDTA-plasma seven times during 7 months storage in −20 °C and the mean concentration of ADMA was 0.661 ± 0.008 and the CV was 1.2%.

3.3. Method comparison

Fifty-five patient samples were measured with ELISA and HPLC methods. Both assays can be used for the measurement of ADMA concentrations from serum and plasma samples. The analytical sensitivity was 0.1 μM for the HPLC assay and 0.05 μM for the ELISA assay [24]. The reproducibility of the ELISA assay was worse than that of the HPLC assay and there was no correlation between these assays over the 0.39–0.909 μM

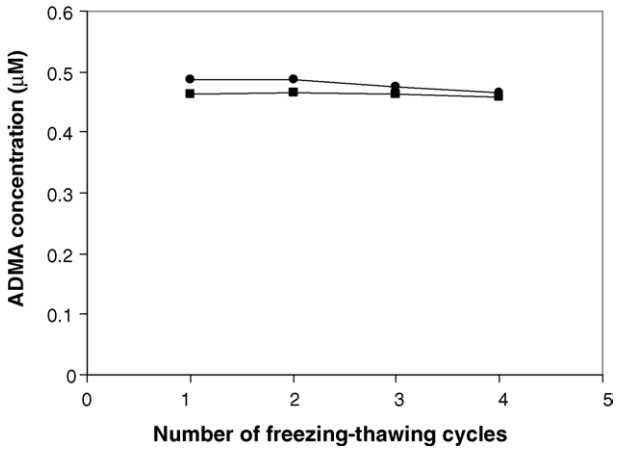

Fig. 2. The storage stability of ADMA (●) and SDMA (■) in frozen sample after one to four freezing and thawing cycles (24 h). ADMA concentration (μM) is presented mean concentration of four samples.

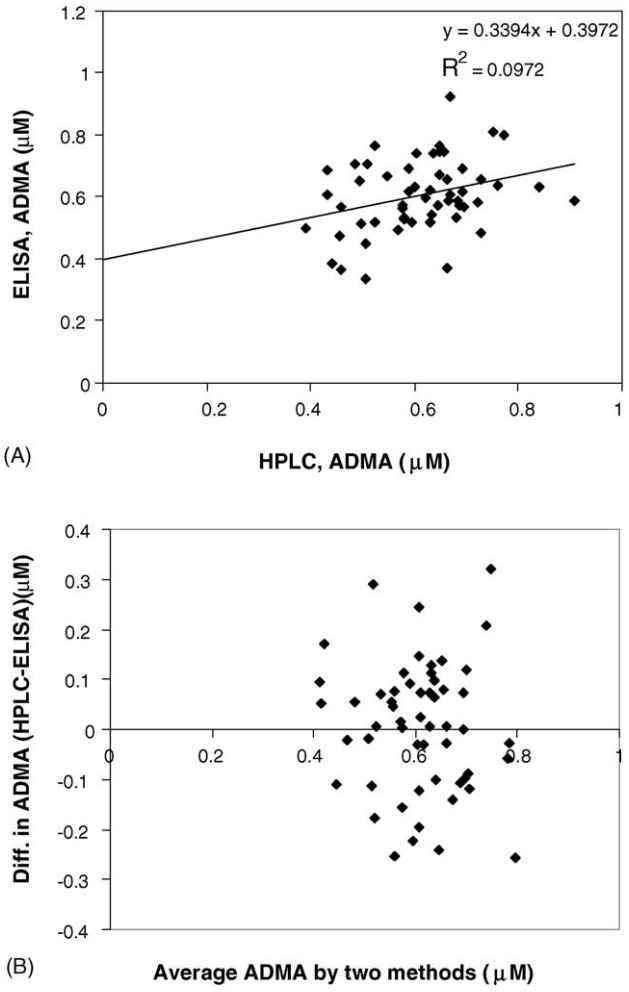

Fig. 3. (A) The correlation between the ELISA and HPLC ADMA methods in human serum samples ($n = 55$, $P = 0.796$). (B) Difference (HPLC-ELISA, μM) against mean for two ADMA methods.

concentration range ($R^2 = 0.0972$, $P = 0.796$, Fig. 3A). In addition, a plot of the difference between the methods against their mean shows that there is lack of agreement between these two ADMA methods (Fig. 3B). However, Schulze et al. have found good correlation between this new ELISA assay and GC–MS ($R^2 = 0.991$, $n = 9$) and LC–MS ($R^2 = 0.984$, $n = 29$) assays [24]. There are some important differences in the study settings which might at least partly explain these conflicting results. First, in the study of Schulze et al., the sample number was remarkably low as there were only two to three samples which had ADMA concentration below 1 μM. Second, in our study, we used authentic patient samples whereas the work of Schulze et al. [24], spiked samples were used for method comparison. Third, we do not know if the antibody lot used for kit evaluation of kit manufacturer is the same lot we used in our testing kits. One potential explanation would be non-specific binding of the antibodies in the ELISA assays; however, the specificity of ELISA assay was demonstrated to be good with 1.2% cross reactivity. In the HPLC assay, the reproducibility was excellent which suggests that the sample handling and derivatization procedures are unlikely sources of error. In our hands, the reproducibility for ADMA-ELISA kit was poor, although we followed strictly the instructions for use of the kit.

In previous studies, there have been large deviations noted in ADMA concentrations between the different ADMA methods. Böger and Zoccali have found that ADMA and SDMA are bound to plasma proteins and it may explain the varying results from diverse methods due to different sample preparation techniques [29]. Most importantly, the choice of internal standard is a demanding task. We have found that the concentration of a naturally occurring amino acid, L-homoarginine, which is widely used as an internal standard in ADMA analysis [30–35], can vary substantially in normal serum and plasma samples (1.2–6.2 μM, $n = 61$, unpublished results). Recently, it has been reported that L-homoarginine is not present in biological samples [35], but in earlier studies, L-homoarginine has been found in small amounts also in other body fluids and organs, e.g. urine, cerebrospinal fluid, liver, kidney and brain [36–38], although its function is not yet fully resolved. Our choice of L-NMMA as internal standard was based on the lower concentration of L-NMMA (below 0.5 μM) [18,21,32] in human blood samples compared to the concentration of L-homoarginine (1.2–6.2 μM). Other possible choice for internal standard in ADMA measurement is N^G-propyl-L-arginine which has been used by Nonaka et al. [39].

4. Conclusions

We compared the commercial ELISA kit for the quantitative analysis of ADMA concentrations with HPLC method. The correlation between these assays appeared to be poor. In addition, ADMA concentrations between the methods differed widely. Therefore, a commercially available standard reference material with traceability is needed against which the calibration of different methods could be checked reliably. Overall, compared to the commercial ELISA-ADMA kit, our HPLC method offered better sensitivity, selectivity and, very importantly, simultaneous determination of ADMA, SDMA, L-homoarginine and L-arginine, although a good ELISA method has a higher sample throughput than other methods.

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References

[1] P. Vallance, A. Leone, A. Calver, J. Collier, S. Moncada, Lancet 339 (1992) 572.
[2] S. Clarke, Curr. Opin. Cell Biol. 5 (1993) 977.
[3] A.E. McBride, P.A. Silver, Cell 106 (2001) 5.
[4] T. Ogawa, M. Kimoto, K. Sasaoka, J. Biol. Chem. 264 (1989) 10205.
[5] M. Kimoto, G.S. Whitley, H. Tsuji, T. Ogawa, J. Biochem. (Tokyo) 117 (1995) 237.
[6] R.H. Böger, S.M. Bode-Böger, A. Szuba, P.S. Tsao, J.R. Chan, O. Tangphao, T.F. Blaschke, J.P. Cooke, Circulation 98 (1998) 1842.

[7] H. Miyazaki, H. Matsuoka, J.P. Cooke, M. Usui, S. Ueda, S. Okuda, T. Imaizumi, Circulation 99 (1999) 1141.
[8] C.D. Goonasekera, D.D. Rees, P. Woolard, A. Frend, V. Shah, M.J. Dillon, J. Hypertens. 15 (1997) 901.
[9] B.M. Chen, L.W. Xia, R.Q. Zhao, J. Chromatogr. B 692 (1997) 467.
[10] 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.
[11] L. Tarnow, P. Hovind, T. Teerlink, C.D. Stehouwer, H.H. Parving, Diab. Care 27 (2004) 765.
[12] H. Päivä, T. Lehtimäki, J. Laakso, I. Ruokonen, V. Rantalaiho, O. Wirta, A. Pasternack, R. Laaksonen, Metabolism 52 (2003) 303.
[13] C. Zoccali, S. Bode-Böger, F. Mallamaci, F. Benedetto, G. Tripepi, L. Malatino, A. Cataliotti, I. Bellanuova, I. Fermo, J. Frölich, R. Böger, Lancet 358 (2001) 2113.
[14] M. Usui, H. Matsuoka, H. Miyazaki, S. Ueda, S. Okuda, T. Imaizumi, Life Sci. 62 (1998) 2425.
[15] Q. Feng, X. Lu, A.J. Fortin, A. Pettersson, T. Hedner, R.L. Kline, J.M. Arnold, Cardiovasc. Res. 37 (1998) 667.
[16] V.-P. Valkonen, H. Päivä, J.T. Salonen, T.A. Lakka, T. Lehtimäki, J. Laakso, R. Laaksonen, Lancet 358 (2001) 2127.
[17] Y. Dobashi, T. Santa, K. Nakagomi, K. Imai, Analyst 127 (2002) 54.
[18] T. Teerlink, R.J. Nijveldt, S. de Jong, P.A. van Leeuwen, Anal. Biochem. 303 (2002) 131.
[19] E. Caussé, N. Siri, J.F. Arnal, C. Bayle, P. Malatray, P. Valdiguié, R. Salvayre, F. Couderc, J. Chromatogr. B 741 (2000) 77.
[20] K. Vishwanathan, R.L. Tackett, J.T. Stewart, M.G. Bartlett, J. Chromatogr. B 748 (2000) 157.
[21] L.F. Huang, F.Q. Guo, Y.Z. Liang, B.Y. Li, B.M. Cheng, Anal. Bioanal. Chem. 380 (2004) 643.
[22] J. Albsmeier, E. Schwedhelm, F. Schulze, M. Kastner, R.H. Böger, J. Chromatogr. B 809 (2004) 59.
[23] D. Tsikas, B. Schubert, F.M. Gutzki, J. Sandmann, J.C. Frölich, J. Chromatogr. B 798 (2003) 87.
[24] F. Schulze, R. Wesemann, E. Schwedhelm, K. Sydow, J. Albsmeier, J.P. Cooke, R.H. Böger, Clin. Chem. Lab. Med. 42 (2004) 1377.
[25] N. Tyagi, K. Collins, M. Steed, A.V. Ovechkin, K.S. Moshal, S.C. Tyagi, Am. J. Physiol. Heart Circ. Physiol., in press.
[26] J.T. Salonen, Ann. Clin. Res. 20 (1988) 46.
[27] V.-P. Valkonen, J. Leiper, M. Pirskanen, T.-P. Tuomainen, F. Yunus, R. Hurtado-Guerrero, M. Tomás, M. Sentí, J. Laakso, P. Valtonen, J. Marrugat, R. Laaksonen, P. Vallance, J.T. Salonen, J. Am. Coll. Cardiol., submitted for publication.
[28] T. Heresztyn, M.I. Worthley, J.D. Horowitz, J. Chromatogr. B 805 (2004) 325.
[29] J.T. Kielstein, R.H. Böger, S.M. Bode-Böger, J. Martens-Lobenhoffer, G. Lonnemann, J.C. Frölich, H. Haller, D. Fliser, Clin. Nephrol. 62 (2004) 295.
[30] J.R. Chan, R.H. Böger, S.M. Bode-Böger, O. Tangphao, P.S. Tsao, T.F. Blaschke, J.P. Cooke, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1040.
[31] R.H. Böger, S.R. Lentz, S.M. Bode-Böger, H.R. Knapp, W.G. Haynes, Clin. Sci. (Lond.) 100 (2001) 161.
[32] J. Pi, Y. Kumagai, G. Sun, N. Shimojo, J. Chromatogr. B 742 (2000) 199.
[33] M.D. Savvidou, A.D. Hingorani, D. Tsikas, J.C. Frölich, P. Vallance, K.H. Nicolaides, Lancet 361 (2003) 1511.
[34] N. Wahbi, R.N. Dalton, C. Turner, M. Denton, I. Abbs, R. Swaminathan, J. Clin. Pathol. 54 (2001) 470.
[35] G. Trapp, K. Sydow, M.T. Dulay, T. Chou, J.P. Cooke, R.N. Zare, J. Sep. Sci. 27 (2004) 1483.
[36] A. Mori, Y. Watanabe, N. Fujimoto, J. Neurochem. 38 (1982) 448.
[37] B. Marescau, D.R. Deshmukh, M. Kockx, I. Possemiers, I.A. Qureshi, P. Wiechert, P.P. De Deyn, Metabolism 41 (1992) 526.
[38] W. Derave, B. Marescau, E. Vanden Eede, B.O. Eijnde, P.P. De Deyn, P. Hespel, J. Appl. Physiol. 97 (2004) 852.
[39] S. Nonaka, M. Tsunada, K. Imai, T. Funatsu, J. Chromatogr. A 1066 (2005) 41.