

Review Article

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HPLC and LC-MS/MS measurement methods for the quantification of asymmetric dimethylarginine (ADMA) and related metabolites

[ADMA ve ilişkili metabolitlerin kantitasyonu için HPLC ve LC-MS/MS ölçüm yöntemleri]

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Abstract: Methyl arginine derivatives such as asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-N-monomethyl arginine (L-NMMA) are formed by proteolytic catalysis following methylation of arginine residues in proteins. These metabolites reduce NO production. Methylated arginines are an important biomarker for various diseases such as cardiovascular and renal diseases. Therefore, many methods have been developed to reliably and accurately measure the levels of these metabolites. This review, HPLC and LC-MS/MS methods developed for the measurement of methylarginine derivatives are discussed. In HPLC methods, solid phase extraction, derivatization and subsequent separation by reverse phase chromatography were performed. Since these metabolites are polar, they are difficult to retain in conventional reverse phase columns. In addition, as serum levels of these metabolites are low, sensitivity problems have been observed in HPLC methods. Derivatization has been applied to eliminate these problems. However, there

have been problems with the stability of derivatives formed. Another important problem is that the separation of stereoisomer ADMA and SDMA can only be achieved chromatographically. Tandem mass spectrometric methods are accurate, selective, sensitive and rapid since analytes are separated depending on m/z ratios rather than chromatographic separation. Therefore, tandem mass spectrometry methods might be considered as the goal standard for these analytes.

Keywords: chromatography; methodology; methylated arginine; nitric oxide; tandem mass spectrometry.

Öz: Asimetrik dimetilarjinin (ADMA), simetrik dimetilarjinin (SDMA), L-N-monometil arjinin (L-NMMA) gibi metil arjinin türevleri, proteinlerdeki arjinin rezidülerinin metilasyonunu takiben proteolitik katalizleri ile oluşmaktadır. Bu metabolitler NO üretimini azaltır. Metillenmiş arjininler kardiyovasküler ve renal hastalıklar gibi çeşitli hastalıklar için önemli bir biyobelirteçtir. Bu nedenle, bu metabolitlerin seviyelerini güvenilir ve doğru bir şekilde ölçmek için birçok yöntem geliştirilmiştir. Bu derlemede, metilarjinin türevlerinin ölçümü için geliştirilen HPLC ve LC-MS / MS yöntemleri tartışılmıştır. HPLC yöntemlerinde katı faz ekstraksiyonu, türevlendirme ve bunları takiben ters faz kromatografisi ile ayırım uygulanmıştır. Bu metabolitler polar olduğundan, geleneksel ters faz kolonlarında tutulması zordur. Ayrıca, bu metabolitlerin serum seviyeleri düşük olduğundan, HPLC yöntemlerinde duyarlılık problemleri gözlenmiştir. Bu sorunları gidermek için türevlendirme uygulanmıştır. Bununla birlikte, oluşan türevlerin stabilitesi ile ilgili sorunlarla karşılaşmıştır. Bir

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başka önemli problem, stereoizomer ADMA ve SDMA'nın ayrılmasının sadece kromatografik olarak gerçekleştirilebilmesidir. Tandem kütle spektrometrik yöntemler doğru, selektif, hassas ve hızlıdır, çünkü analitlerin ayrımı kromatografik ayırma yerine m / z oranlarına bağlı olarak gerçekleşmektedir. Bu nedenle, tandem kütle spektrometrisi yöntemleri bu analitler için altın standart olarak kabul edilebilir.

Anahtar Kelimeler: kromatografi; metillenmiş arjininler; metodoloji; nitrik oksit; tandem kütle spektrometri.

Introduction

The formation, metabolism and elimination of methylated arginines

Arginine is a conditional essential amino acid and is involved in many important biological processes. Main metabolic pathways involved arginine: (1) protein synthesis; (2) nitric oxide (NO) and citrulline synthesis via the nitric oxide synthase (NOS) pathway; (3) ornithine and urea synthesis through arginase 1 in the urea cycle; (4) the formation of methylated arginine derivatives; (5) synthesis of ornithine, proline, glutamate through the arginase 2; (6) synthesis of polyamines and creatine [1]. Methylation of arginine residues in proteins is one of the post-translational modification mechanisms of proteins that affect important cellular functions in eukaryotic cells [2]. These reactions are catalyzed by the transfer of the methyl group from S-adenosine methionine (SAM) to the guanidine groups of arginine residues through enzymes called protein-arginine methyl transferase (PRMTs) [3]. Asymmetric dimethyl arginine (ADMA), symmetrical dimethyl arginine (SDMA) and N-monomethyl-L-arginine (L-NMMA) are released to cytosol by proteolytic catalysis of methylated proteins [4]. The molecular structures of arginine, ADMA, SDMA and L-NMMA are shown in Figure 1 [5].

To date, nine human PRMT genes have been cloned, and these enzymes have been classified as those with type 1, type 2 and type 3 activity. Type 1 PRMTs are responsible for the production of ADMA and L-NMMA, while Type 2 PRMTs are responsible for the production of SDMA and L-NMMA [6]. The plasma concentrations of ADMA are variable and are associated with cardiovascular diseases or many other conditions. In a healthy person, 60 mg of ADMA is produced daily, and other methylarginine derivatives are 20–50% of ADMA [7]. ADMA is removed from the body through catabolism and renal excretion. Two catabolic pathways have been defined for methylarginines.

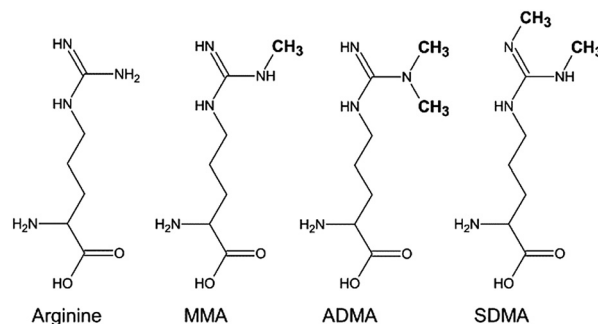


Figure 1: The molecular structures of arginine, ADMA, SDMA and L-NMMA [5].

The first is the pathway catalyzed by the dimethylamino-hydrolase (DDAH) enzyme, which is specific for L-NMMA and ADMA, and the second is the transamination pathway catalyzed by alanineglyoxylate aminotransferase 2 (AGXT2), which contributes to asymmetric as well as symmetric methylarginines [8]. While ADMA and L-NMMA are mainly metabolized to citrulline and monomethylamine or dimethylamine by DDAH, a small amount is undergoing renal elimination [9]. The DDAH enzyme has two isoforms, DDAH1 and DDAH2, and the ADMA metabolism rate of DDAH2 is 70 times less than DDAH1 [10]. SDMA is almost exclusively removed by renal excretion, so it is considered an endogenous indicator of renal function [11]. The metabolism of ADMA, SDMA and L-NMMA, which are derivatives of methylarginine, are shown in Figure 2.

The role of methyl arginine derivatives in the regulation of NO production

Vallance et al. showed for the first time in 1992 that ADMA inhibits NO synthesis and is higher in end-stage kidney patients than the control group. This study has been a milestone and after these findings, many studies on ADMA and NO pathways have been conducted [12]. Endothelium-derived NO is synthesized from L-arginine through the endothelial isoform of the NOS (eNOS) enzyme. eNO relaxes vascular smooth muscles and regulates blood pressure, suppresses vascular disease processes involving leukocyte adhesion and platelet aggregation and it is important in vascular regeneration [13]. ADMA and L-NMMA are endogenous competitive inhibitors of the eNOS enzyme. SDMA decreases NO production by competing indirectly with the uptake of arginine into endothelial cells and re-absorption in the kidney without directly inhibiting NOS [14]. Plasma L-NMMA concentrations are approximately 10% of ADMA, so ADMA is considered a major endogenous NOS inhibitor, therefore ADMA is a strong

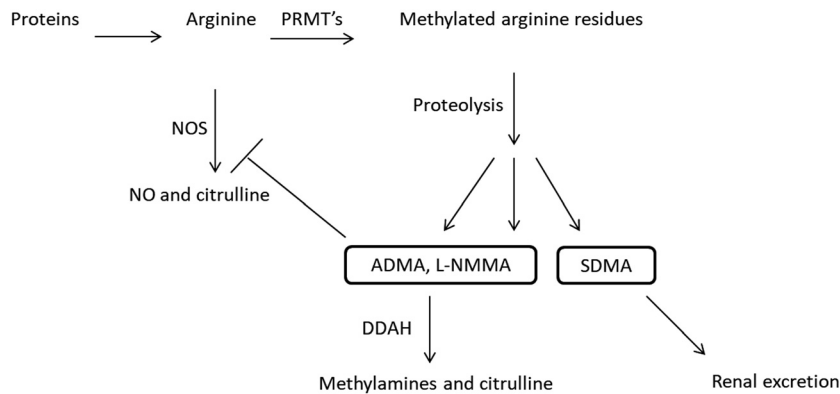


Figure 2: The metabolism of ADMA, SDMA and L-NMMA, which are derivatives of methyl-arginine.

and independent risk factor for cardiovascular diseases and in the presence of cardiovascular risk factors [15]. In addition to eNOS, ADMA inhibits two other isoforms of NOS, neuronal and inducible NOS (nNOS and iNOS). Although the effect of ADMA on NO synthesis inhibition has been well studied in cells, data on purified enzymes are few and contradictory. Komori reported that ADMA has a weak inhibitory effect on iNOS and nNOS ($K_i > 300 \mu\text{mol/L}$ in both cases) [16]. However, later studies have revealed that ADMA strongly inhibits the nNOS enzyme with $0.4 \mu\text{mol/L}$ K_i and $1.5 \mu\text{mol/L}$ IC_{50} values. It has been shown to be a weak competitive inhibitor of eNOS enzyme with $3.9 \mu\text{mol/L}$ K_i and $12 \mu\text{mol/L}$ IC_{50} values [17]. Its experimental application as a tool to modulate NOS activity is very limited.

As mentioned above, ADMA is known to damage vascular integrity through NOS inhibition. However, it is known that NOS activity is also influenced by the concentration of extracellular arginine, the substrate of NOS. As a result, the level of arginine can modulate the inhibitory effect of ADMA. Therefore, the plasma Arginine/ADMA ratio may be a better indicator for the risk of atherosclerosis. In various studies, Arginine/ADMA ratio has been shown to be related to impaired organ function, cardio, cerebrovascular diseases and mortality [18].

Relationship between diseases and methylated arginines

Many studies on ADMA have been conducted and increased ADMA levels have been reported in many diseases such as atherosclerosis, hypertension, diabetes, insulin resistance, preeclampsia, pulmonary hypertension, stroke, chronic kidney diseases, chronic heart failure, fibrotic disorders and Alzheimer [19, 20].

Nitric oxide is a major endothelium-derived vasoactive substance and plays an important role in the regulation of endothelial homeostasis. Low NO levels are associated with

impaired endothelial function. ADMA is a non-selective inhibitor of NO production. Thus, increased ADMA levels have been reported in cardiovascular diseases such as coronary artery disease, peripheral arterial disease, chronic heart failure, pulmonary hypertension, stroke, hypertrophic cardiomyopathy, pre-eclampsia and in the presence of cardiovascular risk factors such as hypercholesterolemia, hypertension, hyperhomocysteinemia, tobacco exposure, hyperglycemia and insulin resistance [21]. Increased methylated arginine levels have been found in masked hypertension [22, 23]. In addition to that poor sleep quality has been associated with increased ADMA levels in normotensive patients with type 2 diabetes mellitus [24].

Approximately 20% of ADMA and L-NMMA produced daily are undergoing renal elimination with urine. The remaining part is metabolized by DDAH and AGXT2 enzymes. Both enzymes are expressed in the kidneys. In summary, the kidneys have an important role in the excretion of ADMA and L-NMMA through urine and their metabolism through these enzymes. Almost all of the SDMA is undergoing renal elimination. Thus, elevated methylarginine levels have been reported in chronic kidney disease [25].

ADMA has also been reported to be involved in the pathogenesis of neurodegenerative diseases. Studies have reported that ADMA levels increase in diseases such as Alzheimer [19] and Parkinson [26]. Although this condition is thought to be related to impaired cerebral circulation and vascular endothelium dysfunction, its exact mechanism has not been elucidated yet.

In addition, studies have shown that plasma ADMA levels increase with age. Although this is thought to be associated with decreased DDAH activity with age, there is no study to evaluate DDAH activity in the elderly yet. Moreover, ADMA clearance may be secondary to a decrease in GFR and/or the number of tubular cells containing DDAH as a result of renal tissue involvement with age [27].

It is thought that dysregulation of NO metabolism may initiate coagulation anomalies, platelet activation, local

expression of adhesion molecules and inflammatory mediators by promoting endothelial damage. Therefore, increased ADMA, which play a role in NO metabolism, are also associated with inflammatory diseases such as ankylosing spondylitis [28], rheumatoid arthritis [29], ulcerative colitis and Crohn's disease [30]. However it has been also suggested by increasing in ADMA levels by age plays a role in the decrease of inflammatory attacks in Behçet disease [31].

Thyroid hormones play an essential role in the regulation of cardiac function and cardiovascular hemodynamics [32]. Even minor changes in the reference range of thyroid hormones are associated with an increased risk of cardiovascular disease [33]. ADMA and L-NMMA is an independent risk factor for cardiovascular diseases [34]. In studies based on these findings, increased ADMA levels have been reported in both hypothyroidism and hyperthyroidism [35, 36].

Recent studies have shown that plasma ADMA levels are elevated in various types of cancer, such as lung, cancer, gastric cancer, breast cancer, colon cancer, hematological malignancies. ADMA is mainly metabolized through DDAH and increased expression of DDAH-1 has been shown to promote tumor growth in *in vivo* studies. Therefore, although it is thought that dysregulation in the DDAH/ADMA/NO pathway may be related to tumor development, its mechanism has not been fully elucidated yet [37].

Increased levels of methylated arginines were also shown associated with cardiovascular disease in the environmental toxicology studies. Increased methylated arginines have been found in manganese exposed welders [38], cadmium exposed people [39] and in trichloroethylene exposure [40].

Analysis of methylated arginines

To date, many methods have been developed that allow the measurement of methyl arginines in urine, serum, plasma, tissues. These methods include high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), capillary electrophoresis (CE), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS) [41]. Although ELISA methods allow a large number of samples to be run in a short time, the risk of cross reactivity is high and ADMA values are approximately 20–50% higher than chromatographic methods. Although GC-MS methods provide good separation between ADMA and

SDMA, it requires various extraction and derivatization steps, and pretreatment steps take approximately 2.5–3 h [42]. HPLC and LC-MS/MS based methods frequently used biological fluid samples such as plasma, serum and urine as the biological material for the measurement of methyl-arginine derivatives, these methods have also been applied successfully in tissue extracts, cell culture supernatants.

HPLC and LC-MS or LC-MS/MS measurement methods for methylated arginines

Traditionally, amino acid analyses were performed after post-column derivatization with ion-exchange chromatography. Apart from amino acids, this technique has also been used for post translational modified amino acids such as ADMA and SDMA. However, the disadvantage of this method is that long analysis times of up to 40 min are required to provide sufficient resolution [5, 43].

Although many measurement methods have been developed for ADMA and related metabolites in the process until today, various difficulties have been encountered in the measurement of these analytes. These difficulties are result from not well retained in conventional RP columns due to being polar compounds, difficult to separate due to their physicochemical and structural similarities, differences in plasma concentrations, lack of chromophores that enable their specific determination, thermally unstable and non-volatile compounds. HPLC and LC-MS/MS are among the most widely used among these methods. HPLC and LC-MS/MS methods published for measurement of ADMA and related metabolites are summarized in Tables 1 and 2, respectively.

Measurement methods have been developed using UV, fluorescent and mass detectors for the determination of ADMA and related metabolites. UV detectors provide measurement based on the absorbance of the molecules at a certain wavelength. Often the analysis of amino acid levels is based on the light absorbance of the carboxyl group at a wavelength of 200–210 nm. Amino acids with benzene rings can be determined in the wavelength range of 250–280 nm. In addition to amino acids, most organic molecules provide strong UV absorbance at wavelengths less than 210 nm. Therefore, determination of amino acids with sufficient sensitivity and selectivity in UV detectors is very difficult, and it is not possible to specifically define ADMA, SDMA, arginine with HPLC-UV detector. Therefore, fluorescent detectors have been widely used to provide more specific and sensitive determination of the derivatives of methylarginine. However, methylarginines do not have an endogenous fluorescence character. Therefore,

Table 1: HPLC methods published for measurement of ADMA and related metabolites.

Method	Analyte	Extraction	Derivatization	Device	CV%	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Ex/Em	Ref.
HPLC-FD	ARG, ADMA, SDMA	SPE	NDA	Rainin Dinamax SD 200 liquid chromatograph (Varian, Walnut Creek, CA) with fluorescence detector	1.7–2.0	0.01 µmol/L NA	0.03–16.0 µmol/L	Waters Spherisorb S/P (250 × 4.6 mm, i.d. column, 5 µm) (Waters Corp., Milford, MA, USA)	Plasma	25 mM sodium acetate buffer containing 38% v/v MeOH (pH: 6.8)	30 min	NPA	420/ 483 nm	Marra et al., 2003 [44]
HPLC-FD	GLN, NOHA, CIT, ARG, NMMA, HARG, ADMA, SDMA, ORN, PUT, AGM, SPERM, SPERM	SPE, phosphate buffer at pH: 6.81 (PP)	OPA/MPA	Merck-Hitachi (Darmstadt, Germany) liquid chromatograph equipped with L-4500A diode array (DAD) or L-7480 fluorescence detector	0.73–3.33	0.54/ 1.8 pmol	0.1–12 nmol/mL	Merck LiChro-CART® (Pur-osphere® STAR RP-18 endcapped, 250 × 4 mm i.d.; 5 µm particle size; 120 Å pore size)	Urine	A: buffer (disodium hydrogen phosphate/tassium hydrogen phosphate, pH 6.88); B: ACN; C: MeOH	60 min	NA	338/ 455 nm	Markowski et al., 2007 [45]
HPLC-FD	ARG, HARG, ADMA, SDMA	SPE	OPA/MPA	Alliance 2690 XE separations module and a Model 474 fluorescence detector from Waters	1.2–2.0	NA/0.01 µmol/L	0.1–20.0 µmol/L	Symmetry C18 column (3.9 × 150 mm; 5 µmol/L particle size; 100 Å pore size)	Serum, plasma	A: 50 mM potassium phosphate buffer (pH: 6.5), containing 8.7% ACN; B: ACN/water (50/50, v/v)	30 min	MMA	340/ 455 nm	Teerlink et al., 2001 [46]
HPLC-FD	ARG, NMMA, ADMA, SDMA, NOHA, ORN, AGM, CIT	EtOH(PP)	OPA/MPA	Waters 1525 HPLC system with a 2475 multi λ fluorescence detector	<10%	NA/1.5 nM	0.1–10.0 µmol/L	Nova-Pak C18 column (3.9 × 150 mm; 4 µm particle size; 60 Å pore size)	Plasma	A: 20 mM potassium phosphate buffer (pH: 6.0); B: ACN/MeOH/water (4/3/3, v/v/v)	35 min	HARG	340/ 455 nm	Zhang et al., 2003 [47]
HPLC-FD	ARG, ADMA, SDMA, HARG	SPE	OPA/MPA	Chemstation system series 1100 (Agilent, Palo Alto, CA, U.S.A.) with fluorescence detector (Merck-Hitachi, LaChrom L7480, Darmstadt, Germany).	1.4–3.1	0.05/0.1 µmol/L	0.1–1.25 µmol/L	Waters Agilent Zorbax SB C18 (4.6 × 250 mm, 5 µmol/L).	Plasma, serum	50 mM KH ₂ PO ₄ (pH = 6.5) and 9% (v/v) ACN	25 min	MMA	340/ 455 nm	Meinitzer et al., 2007 [48]
HPLC-FD	ARG, MMA, HARG, ADMA, SDMA	SPE	OPA/MPA	Waters 1525 HPLC system with a 2475 multi λ fluorescence detector	1.9–2.6	0.001/ 0.004 µmol/L	0.125–4 µmol/L	C18 column (Symmetry, 4 µmol/L, 4.6 × 150 mm)	Plasma	50 mM acetate buffer (pH 6.3) containing 9% (v/v) ACN	25 min	MEA	340/ 455 nm	Blackwell et al., 2009 [49]
HPLC-FD	ADMA, SDMA	SPE	OPA/MPA	Agilent 1100 (Agilent Technologies, California, USA) and a model RF-10AXL fluorescence detector from Shimadzu (Japan)	<5.2%	0.015/0.05 µmol/L	0.25–2.00 µmol/L	Chromolith Performance RP-18 (100 × 4.6 mm)	Plasma	25 mM of phosphate buffer (pH 6.5) containing 7% ACN	25 min	MMA	340/ 455 nm	Ivanova et al., 2010 [50]

Table 1: (continued)

Method	Analyte	Extraction	Derivatization	Device	CV%	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Ex/Em	Ref.
HPLC-FD	L-NMMA, ADMA, SDMA	SPE	NBD-F	Chromatographic system was composed of Pump L-7100 (Hitachi, Tokyo, Japan), 655A-52 Column Oven (Hitachi) and FP-920S intelligent fluorescence detector (Jasco, Tokyo, Japan)	5.56–6.73	20/NA fmol	50–5,000 fmol	Unison UK-C18 (150 × 4.6 mm I.D., Imtakt, Kyoto, Japan)	Plasma	A: 50 mM sodium phosphate buffer (pH 3.2)–ACN (91:9, v/v); B: ACN	25 min	NPA	470/530 nm	Nonaka et al., 2005 [51]
HPLC-FD	ADMA, SDMA, HARG, ARG	SPE	AccQ-Fluor™	Shimadzu VP series HPLC system and fluorescence detector	2.06–2.69	0.04/0.20 µmol/L	0.25–6 µmol/L	C18 Gemini-NX analytical column (150 × 4.6 mm, 3 µmol/L)	Plasma	A: 20 mM borate buffer pH 9; B: ACN; C: water	40 min	NPA	250/395 nm	Jones et al., 2010 [52]
HPLC-FD	ARG, L-NMMA, ADMA, SDMA	SPE	OPA/ME	Shimadzu (a SCL-6A system with Shimadzu RF 550) spectrofluorometric detector (Kyoto, Japan)	NA	NA/0.18 mM	0.25–4 µmol/L	ODS column (wide-pore size, 300 Å)	Human plasma, rat urine, rat kidney cytosol	50 mM citrate buffer (pH 6.8)–MeOH–ACN (920:72:153, v/v/v)	20 min	HARG	340/455 nm	Pl et al., 2000 [53]
HPLC-FD	ADMA, SDMA	SPE	OPA/ME	HP-1050 series liquid chromatograph with Waters 474 scanning fluorescence detector (Millipore, Milford, MA, USA)	NA	0.015/NA µmol/L	0.05–7.5 µmol/L	3 µmol/L Ultracarb 30DS(20) (150 × 4.6 mm O.D.) column (Phenomenex, Torrance, CA, USA)	Plasma	A: 0.015 mol/L phosphate buffer–MeOH tetrahydrofuran (99:0.5:0.5, v/v/v); B: 0.015 mol/L phosphate buffer–MeOH–ACN (40:45:15, v/v/v)	50 min	NA	338/425 nm	Pettersson et al., 1995 [54]
HPLC-FD	ARG, HARG, ADMA	SPE	AccQ-Fluor™	Waters liquid chromatograph with Perkin-Elmer (Melbourne, Australia) LS40 fluorescence detector	0.48–2.67	0.1/NA µmol/L	0.05–1.5 µg/mL	5 µmol/L Luna C18(2) column (150 × 3.0 mm) (Phenomenex, Torrance, CA, USA)	Plasma	A and B were 4 and 30% ACN in 0.1 M sodium acetate pH: 6, respectively.	35 min	MMA	250/395 nm	Heresztyn et al., 2004 [55]
HPLC-FD	ADMA, SDMA, HARG, ARG	SPE	OPA/MPA	Merck Hitachi liquid chromatography system (Hitachi, Tokyo, Japan) with fluorescence detector (F1000)	2.5–4.2	0.1/0.3 µmol/L	0.1–7.5 µmol/L	Symmetry C18 column (4.6 × 150 mm, 5 µm, Waters, Milford, MA, USA)	Serum	50 mM K-phosphate buffer, pH 6.5 and 8.7 vol % ACN	38 min	MMA	340/455 nm	Valtonen et al., 2005 [56]

Table 1: (continued)

Method	Analyte	Extraction	Derivatization	Device	CV%	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Ex/Em	Ref.
HPLC-FD	ADMA, L-NMMA	NA	NBD-F	Merck Hitachi liquid chromatography system (Hitachi, Tokyo, Japan) with fluorescence detector (F1000)	5.1–7.2	36/140 nM	140 nM - 140 μ mol/L	Chromolith™ Performance RP-18e (100 $\times$ 4.6 mm I.D., Merck, Darmstadt, Germany)	Plasma	50 mM phosphate buffer (pH 3.2) and ACN (91:9, v/v)	15 min	MMA	470/530 nm	Nonaka et al., 2006 [57]
HPLC-FD	ADMA, SDMA, ARG	TCA(PP)	OPA/ME	Dionex Ultimate 3000 (ThermoScientific, Waltham, MA, USA) coupled to a Dionex fluorescence detector	<5	NA/0.01 μ mol/L	12 nM - 0.5 μ mol/L	C18 Gemini-NX analytical columns (150 $\times$ 4.6 mm, 3 μ m)	Plasma	50 mM potassium phosphate (pH 6.5) containing 14% (v/v) ACN	20 min	NA	340/455 nm	Schou-Pedersen et al., 2018 [58]
HPLC-FD	ADMA, SDMA, L-NMMA	SPE	NBD-F	Hitachi (Tokyo, Japan) LC system and fluorescence detector (FP-920S, Jasco).	1.79–4.5	20 fmol/NA	50–5,000 fmol	Unison UK-C18 (150 $\times$ 4.6 mm I.D., Imtakt, Kyoto, Japan)	Plasma	A: sodium phosphate buffer (pH 3.2); ACN (91:9, v/v); B: ACN	42 min	HARG	470/530 nm	Tsunoda et al., 2005 [59]
HPLC-FD and ECD	MMA, ADMA, SDMA, HARG, ARG	SPE	OPA/ME for FD FHT for ECD	Shimadzu LC system ED 605 pulse ECD detector (GL Science, Tokyo, Japan) and Shimadzu RF-535 fluorescence detector	<8%	LOD: 0.3–0.5 pmol ECD; 1–2 pmol FD	0.5 pmol up to 150 μ mol/L for ECD; 1.5 pmol - 250 μ M for FD	Wakopak Combi ODS(4.6 mm i.d. 50 mm, 5 mm, Wako Pure Chemicals)	Plasma, urine	ACN - 100 mM sodium phosphate buffer (pH 7.0, 1:1, v/v)	50 min	NPA	340/455 nm	Suzuki et al., 2012 [60]
HPLC-FD	ADMA, SDMA, L-NMMA, ARG	SPE/PCA(PP)	OPA/MPA	Agilent Technologies (Santa Clara, CA, USA) with G1321B fluorescence detector	$\leq$ 5%	0.007 μ mol/L/NA	0.007–150 μ mol/L	1.0 $\times$ 100 mm Luna® C18(2) column with 3 μ mol/L particles and 100 Å pore size (Phenomenex, Torrance, CA, USA)	Plasma	A: sodium phosphate and sodium phosphate monobasic and 5% v/v HPLC-grade ACN in ultrapure water; B: 50% ultrapure water and 50%	20 min	MEA	340/455 nm	Alkaitis et al., 2014 [61]
HPLC-FD	ADMA, CIT, ARG	5-SSA, MeOH, EtOH(PP)	OPA/ME	Shimadzu (Kyoto, Japan) LC-20A HPLC system, a RF-10AXL fluorescence detector	<7%	0.05 mM/NA	0.007–150 μ mol/L	150 $\times$ 4.6 mm Novapak C18 column with a particle size of 5 μ m	Plasma	A: 50 mM sodium acetate (pH 6.8) water; B: MeOH and THF in a ratio 97:3(v/v)	66 min	NA	338/425 nm	Rao et al., 2017 [62]

Table 1: (continued)

Method	Analyte	Extraction	Derivatization	Device	CV%	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Ex/Em	Ref.
HPLC-FD	ADMA, HARG, SDMA	ACN(PP)	Ninhydrin	Waters (Milford, MA, USA) HPLC system model Alliance 2695 equipped with a Waters 474 fluorescence detector	<8%	4/12 nmol/L	0.125– μmol/L	4.6 mm × 15 cm Waters XBridge C18 5 μmol/L column	Plasma	Aqueous solution of 13 mmol/L NH ₄ HCO ₃ containing 10% v/v THF	12 min	HARG	390/ 497 nm	Soglia et al., 2008 [63]
HPLC-PDA	ARG, HARG, ADMA, SDMA, HCY	TCA(PP)	PITC	Waters series liquid chromatograph with Waters 2996 photodiode array detector	1.37–9.67	17.6/ 53.3 pmol	0.3– 3.75 mM	C18 column of 250 × 4.6 mm I.D, with a particle size of 5 μmol/L	Plasma, serum	A: sodium acetate buffer; B: 60% ACN (60:40, CH ₃ CN/H ₂ O, v/v)	30 min	NA	269 nm	Ravula et al., 2018 [64]
HPLC-FD	ARG, ADMA, SDMA, L-SDMA, L-NMMA	SPE, MeOH(PP)	NDA-CN	Pump L-7100 (Hitachi, Tokyo, Japan), 655A-52 Column Oven (Hitachi) and FP-920S intelligent fluorescence detector (Jasco, Tokyo, Japan)	<4.5%	3.6 pg/NA	0.048– 4.8 ng	250 × 0.4 mm Nucleosil 100 CN column with a particle size of 5 μm (Tracer Anal., Barcelona, Spain).	Plasma	Tri-sodium citrate dihydrate and tetramethylammonium-bromide buffer (pH 2.8) – ACN-water (80:12:8)	25 min	NA	420/480 nm	Unceta et al., 2002 [65]

ARG, arginine; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; L-NMMA, N-monomethyl-L-arginine; HARG, homoarginine; CIT, citrulline; 5-SSA, 5-sulfosalicylic acid; NPA, N⁶-propyl-L-arginine; MEA, Monoethylarginine; NDA, naphthalene-2,3-dicarboxaldehyde; OPA, o-phthalaldehyde; MPA, 3-mercaptopropionic acid; ME, mercaptoethanol; NBD-F, 4-fluoro-7-nitro-2,1,3-benzoxadiazole; GLN, glutamine; NOHA, N⁶-hydroxy-L-arginine; ORN, ornithine; PUT, putrescine; AGM, agmatine; SPERM, spermidine; SPERM, spermine; HCY, homocysteine; OPA, o-phthalaldehyde; FHT, 6-ferrocenyl-1-hexanethiol; PITC, phenylisothiocyanate; THF, tetrahydrofuran; PCA, perchloric acid; TCA, trichloroacetic acid; ACN, acetonitrile; MeOH, methanol; EtOH, ethanol; FD, fluorescence detector; ECD, electron capture detector; PP, protein precipitation; SPE, solid phase extraction; IS, internal standard; CV, variation coefficient; time, run time. CV%, LOD, LOQ, linearity values and IS explained for ADMA.

Table 2: LC-MS/MS methods published for measurement of ADMA and related metabolites.

Method	Analyte	Extraction	Derivatization	Device	CV %	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Q1/Q3 (ADMA, SDMA)	Ref.
APCI- HPLC-MS	ARG, ADMA SDMA	5-SSA(PP)	NA	Shimadzu (Kyoto, Japan) LC system coupled with Shi- madzu LCMS-2010 mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an API interface	1.6–6.8	0.012/0.2 µmol/L	0.2–110 µmol/L	2.0 × 150 mm Shimadzu VP-ODS column with a particle size of 5 µmol/L	Urine	Water (95%), ACN (5%) and TFA (0.4%)	6.5 min	HARG	203 and 203 for SIM mode	Huang et al., 2003 [66]
ESI-LC- MS/MS	ARG, ADMA, SDMA, HARG, CIT	MeOH(PP)	n-butanol and acetyl chloride	Acquity UPLC Waters (Manchester, En- gland) coupled with a Micromass Quattro Ultima triple quadru- pole mass spec- trometer (Waters Co., Milford, MA, USA) equipped with an ESI positive mode	5.6–14.7	0.01/0.05 µmol/L for serum and plasma; 0.1/0.1 µmol/L for urine	0.05–4 µmol/L for serum and plasma; 0.1–8 µmol/L for urine	UPLC BEH C18 (1.7 m, 2.1 × 50 mm) from Waters Co.	Serum, urine, plasma	A: water with 0.1% formic acid; B: MeOH with 0.1% formic acid	1.9 min	D6-ADMA	ADMA: 259.3 → 214.0 and SDMA: 259.3 → 228.0	Gangi et al., 2010 [67]
ESI-LC- MS/MS	ADMA, SDMA, HARG and 37 physiologic amines	ACN(PP)	APDS	A Waters® ACQUITY UPLC® system (Wa- ters Corporation, Milford, MA) coupled to a 5500 QTRAP® mass spectrometer (AB Sciex® Redwood City, CA)	2.53–8.05	NA/0.25 µmol/L	0.05–2 µmol/L	Inertil ODS-3 col- umn (2.0 µm, 2.1 mm i.d., 100 mm; GL Sciences, Tokyo, Japan)	Plasma	A: APDSTAGWako Eluent Buffer; B: distilled deion- ized water (Milli-Q)/ACN (40:60)	19 min	D6-ADMA	ADMA: 323 → 203 and SDMA: 323 → 203	Arashida et al., 2017 [68]
ESI-LC- MS/MS	ARG, ADMA, SDMA, MMA, ORN, CIT	ACN, MeOH(PP)	NA	Accela LC system (Thermo Fisher Sci- entific, San Jose, CA, USA) TSQ Quantum Access (Thermo Fisher Scientific) coupled to a triple quadrupole mass spectrometer equip- ped with ESI inter- face operated in positive mode	1.3–14.6	0.001– 0.003 µmol/L	0–0.2 µmol/L	Luna HILIC (hydro- philic interac- tion liquid chromatog- raphy) analyt- ical column (50 × 2.0 mm, 3 µm, Phenom- enex, Torrance, CA, USA).	Erythrocytes	A: 1.5 mM ammo- nium formate (pH 3.2); B: 1.5 mM ammo- nium formate in ACN/MeOH (95:5:0.5, v/v) (pH 3.2)	25 min	D7-ADMA	ADMA: 203.1 → 46.4 and SDMA: 203.2 → 172.1	Squellierio et al., 2008 [69]
ESI-LC- MS/MS	ARG, MMA, HARG, ADMA, SDMA, CIT	NA	DEPC	Waters system model Acquity UPLC equip- ped with a Waters tandem quadrupole mass spectrometer (TQD) (Waters Italia, Milan, Italy).	3.11–3.88	0.01–0.03 µmol/L	0.31–5 µmol/L	100 × 4.6 mm Zorbax Eclipse Plus C18 3.5 µmol/L column	Plasma	Aqueous solution of 0.4% v/v for- mic acid and ACN (95:5, v/v)	10 min	D6-ADMA	ADMA: 275.33 → 46 and SDMA: 275.33 → 70	Sorigia et al., 2019 [70]

Table 2: (continued)

Method	Analyte	Extraction	Derivatization	Device	CV %	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Q1/Q3 (ADMA, SDMA)	Ref.
ESI-LC-MS/MS	ARG, ADMA, SDMA	MeOH, ACN(PP)	NA	(Alliance 2695, Waters – USA) HPLC with Triple Quadrupole Mass Spectrometer equipped with an electrospray ion source (ESI) operating in positive mode (Micromass Quattro micro, Waters-USA)	5.7–8.4	NA	0.22–1.29 µmol/L	Luna silica column, 3 µmol/L, 100 × 2 mm i.d., Phenomenex-USA	Plasma	90% Solution A (ACN/TFA): acetic acid; 1.000: 0.25:10 and of 10% Solution B (water/TFA): acetic acid; 1.000: 0.25:10	6 min	¹³ C ₆ -L-Arg	ADMA: 203 → 46 and SDMA: 203 → 172	D'Apolito et al., 2008 [71]
ESI-LC-MS/MS	ARG, ADMA, SDMA	ACN, ammonium formate buffer solution (PP)	NA	Agilent 1100 system (Waldbronn, Germany) many equipped with Thermo Fisher Scientific (Waltham, MA, USA) TSQ Discovery Max triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) ion source	2.12–3.77	0.003/0.15 µmol/L	0.15–3 µmol/L	Atlantis HILIC silica column with 5 µm particle size and with the dimensions 150 × 2.1 mm (Waters, Eschborn, Germany)	Plasma	20% of 0.025% TFA and 1% propionic acid solution in water (resulting pH = 1.5) and 80% ACN	8 min	D7-ADMA	ADMA: 203.1 → 46.1 and SDMA: 203.1 → 172.1	Martens-Loben-hoffer et al., 2012 [72]
ESI-LC-MS	ARG, ADMA, SDMA, CIT	ACN(PP)	OPA/ME	Agilent 1100 system (Waldbronn, Germany) comprising a ThermoFinnigan LCQ ion trap mass spectrometer (San Jose, USA) with an electrospray ionization (ESI) ion source	<8	NA	0.4–8 µmol/L for serum 2.5–50 µmol/L for urine	Merck Superspher 100 RP18 250 × 4 mm column	Plasma, urine	A: 1 ml formic acid (0.1%) and 1 g ammonium formate in 1 L water, resulting in a pH of 3.5; B: MeOH	21 min	¹³ C ₆ -arginine and HARG	379.2 and 379.2	Martens-Loben-hoffer et al., 2003 [73]
ESI-LC-MS/MS	ARG, ADMA, SDMA	MeOH	HCl and 1-butanol	Varian 1200L Triple Quadrupole MS equipped with two Varian ProStar model 210 HPLC pumps	1.9–5.4	0.5 nM/NA	0–4 µmol/L	Chirobiotic T, 20 × 1.0 mm i.d., microbore guard column packed with tetracoplanin covalently bonded to 5 µm spherical silica (Astec, Whippany, NJ, USA)	Plasma	ACN–water (60:40, v/v, pH 4) containing 0.1 wt% ammonium formate	1.6 min	D6-ADMA	ADMA: 259 → 214 and SDMA: 259 → 228	Schwedhelm et al., 2007 [74]

Table 2: (continued)

Method	Analyte	Extraction	Derivatization	Device	CV %	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Q1/Q3 (ADMA, SDMA)	Ref.
ESI-LC- MS/MS	ADMA, ARG	Filtration	NA	Waters (Milford, MA, USA) 2695 high-performance liquid chromatograph and A Waters Quattro-micro tandem mass spectrometer	NA	0.06/0.20 $\mu\text{mol/L}$	0–2 $\mu\text{mol/L}$	Mixed-mode ion exchange analytical column SIELC Technologies (Prospect Heights, IL, USA)	Plasma	A: 0.01% TFA in de-ionized water (MPA); B: 0.25% TFA in ACN	1.6 min	D ₄ LVS and N ₁₅ ARG	ADMA: 203.1 → 46.1 and SDMA: 203.1 → 172.1	Bishop et al., 2007 [75]
ESI-LC- MS/MS	ARG, ADMA, MMA, SDMA	SPE	NA	Perkin-Elmer HPLC autosampler and micro-pump (series 200) coupled to a Perkin-Elmer Sciex API 2000triple quadrupole mass spectrometer	0.9–4.1	0.023/0.077 $\mu\text{mol/L}$	5–25 $\mu\text{mol/L}$	NA	Plasma, urine	0.1% formic acid	3 min	ADMA-d2	ADMA: 203.01 → 46.32 and SDMA: 203.01 → 172.03	Weaving et al., 2008 [76]
ESI-LC- MS/MS	ARG, CIT, ADMA, SDMA	ACN containing 0.5% acetic acid and 0.025% TFA(PP)	NA	Shimadzu LC-20AD delivery pump, SIL-20AC autosampler and CBM-20A system controller (Shimadzu Scientific Instruments; Columbia, MD) and ABI/Sciex API3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA)	2.09–14.4	NA/0.025 $\mu\text{mol/L}$	0.025–1.000 $\mu\text{mol/L}$	150 × 2.1 mm Alltima HP HILIC 3 $\mu\text{mol/L}$ column	Rat plasma, urine, cell lysate	Isocratic elution with 15% mobile phase A (water containing 0.5% acetic acid and 0.025% TFA) and 85% mobile phase B (ACN containing 0.5% acetic acid and 0.025% TFA)	6 min	¹⁵ N ₆ -ARG	ADMA: 203.2 → 46.2 and SDMA: 203.2 → 172.2	Shin et al., 2011 [77]
ESI-LC- MS/MS	MMA, ADMA, SDMA, HARG	SPE	HCl and 1-butanol	Perkin Elmer Series 200 HPLC system (Perkin Elmer Inc., Shelton, CT, USA)AB Sciex API 3000 triple quadrupole mass spectrometer	<9.6%	0.2/0.8 nM	0.1–5 $\mu\text{mol/L}$	Waters XTerra MS C18 column (3.5 μm , 3.9 × 100 mm)	Plasma	Ammonium formate in water - ACN (95.5:4.5, v/v) containing 0.1 vol % formic acid	7 min	D7-ADMA	ADMA: 259 → 214 and SDMA: 259 → 228	Daivids et al., 2011 [78]
ESI-LC- MS/MS	ADMA, HCY	0.1% formic acid in MeOH	NA	Waters Alliance (Waters Co., Milford, MA, USA) system Micro-mass Quattro micro triple quadrupole mass spectrometer (Micromass UK Limited, Manchester, England)	2.4–4.2	0.01–0.69 $\mu\text{mol/L}$	0.69–131.47 $\mu\text{mol/L}$	Atlantis HILIC silica (100 × 2.1 mm, 5 $\mu\text{mol/L}$, Waters)	Urine	Water/MeOH/ACN (35/35/30, v/v) containing 0.2% formic acid	4.5 min	cystamine dihydrochloride	ADMA: 203 → 45.7	Gopu et al., 2011 [79]

Table 2: (continued)

Method	Analyte	Extraction	Derivatization	Device	CV %	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Q1/Q3 (ADMA, SDMA)	Ref.
ESI-LC-MS/MS	ADMA, SDMA, ARG	ACN, TFA propionic acid (PP)	NA	Agilent 1100 system (Waldbronn) with Thermo Finnigan TSQ Quantum Discovery Max triple quadrupole mass spectrometer	<8	0.02–0.15 $\mu\text{mol/L}$ for plasma 0.02–0.075 $\mu\text{mol/L}$ for cell culture 0.02–0.15 $\mu\text{mol/L}$ for urine	0–100 $\mu\text{mol/L}$ for urine 0–3 $\mu\text{mol/L}$ for plasma, cell culture	125 $\times$ 3 mm Nucleosil 100-5 silica column (Macherey-Nagel)	Plasma, urine, cell culture	Water-ACN-TFA-propionic acid (10:90:0.025:1 by volume)	7 min	D6-ADMA	ADMA: 203.2 $\rightarrow$ 46.1 and SDMA: 203.2 $\rightarrow$ 172.1	Martens-Loben-hoffer et al., 2006 [80]
ESI-LC-MS	ADMA, SDMA, ARG	ACN	HCl and n-butanol	Shimadzu LC-10 system (Shimadzu, Kyoto, Japan) with LC/MS-2010 single quadrupole mass spectrometer (Shimadzu, Kyoto, Japan)	5.9–7.8	NA–0.08 $\mu\text{mol/L}$	0.08–5 $\mu\text{mol/L}$	Thermo Hypersil-Keystone Hypurity C18 (150 mm 2.1 mm, 5 $\mu\text{mol/L}$) analytical column	Plasma	Ammonium acetate (0.25 mmol/L) and MeOH (93:7, v/v)	10 min	HARG	259 and 259	Yi et al., 2011 [81]
ESI-LC-MS/MS	ADMA, SDMA, ARG	Acetone	HCl and n-butanol	Varian ProStar HPLC systems with Varian 1200L Triple Quadrupole mass spectrometer	2.6–4.1	3–50 nmol/L	0.025–4 $\mu\text{mol/L}$	Varian analytical column [50 $\times$ 2.0 mm (i.d.)] packed with Polaris C18-Ether (3 $\mu\text{mol/L}$ bead size)	Plasma	A: MeOH containing 0.1 formic acid; B: water containing 0.1 formic acid	4 min	D6-ADMA	ADMA: 259.3 $\rightarrow$ 214 and SDMA: 259.3 $\rightarrow$ 228	Schwedhelm et al., 2005 [82]
HESI-LC-MS/MS	ADMA, SDMA, ARG creatinine	0.1% formic acid in ACN	NA	NANOSPACE SI-2 (Shiseido, Tokyo, Japan) HPLC system with Thermo Fisher Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer	0.19–6.32	0.742–2.47 $\mu\text{mol/L}$	0.50–50.0 $\mu\text{g/mL}$	Mightysil SI 60 (250 $\times$ 3 mm I.D., 5 $\mu\text{mol/L}$ particle size)	Plasma, urine, tissue	10 mM ammonium acetate buffer H ₂ O/MeOH/ACN (20/35/45, v/v) at pH 4	15 min	Arg- ¹³ C ₆ and Cr-d3	ADMA: 203.1 $\rightarrow$ 70.1 and SDMA: 175.1 $\rightarrow$ 70.1	Saigusa et al., 2011 [83]
LC-QTOF-MS	ADMA, SDMA, methionine, glycine, ARG, HCY, SAM, SAH, betaine, creatine, creatinine, DMG, cysteine, glutathione	ACN(PP) and SPE	NA	Agilent 1200 Series HPLC system coupled to a hybrid quadrupole time-of-flight mass spectrometer Agilent 6530 Series from Agilent Technologies (Santa Clara, CA, USA)	1–12.4	NA	159 ng/mL - 1.4 $\mu\text{g/mL}$	Poroshell EC-C18 120 Å (4.6 $\times$ 100 mm, 2.7 $\mu\text{mol/L}$) from Agilent Technologies (Santa Clara, CA, USA)	Plasma	A: 0.5 Mm perfluorheptanoic acid and 5 mM ammonium formate; B: ACN	19 min	Glutathione- ¹³ C ₂ ¹⁵ N, Creatinine-d3, SDMA-d6	ADMA: 203.15 $\rightarrow$ 46.06 and SDMA: 203.15 $\rightarrow$ 172.1	Benito et al., 2015 [84]

Table 2: (continued)

Method	Analyte	Extraction	Derivatization	Device	CV %	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Q1/Q3 (ADMA, SDMA)	Ref.
ESI-LC- QTOF-MS	ARG, CIT, ADMA, SDMA	ACN	PFBC	NanoAcquity UPLC system with Xevo G2 Q-TOF MS (Waters)	2.23–12.4	0.03–0.1 $\mu\text{mol/L}$	0.05–2.5 $\mu\text{mol/L}$	Acquity HSS T3 column (50 $\times$ 1.0 mm, 1.75 $\mu\text{mol/L}$) with 0.22 $\mu\text{mol/L}$ membrane inline filter (Waters)	Serum	A: consisted of 0.1% FA in water; B consisted of 0.1% FA in MeOH	14.5 min	D7-ADMA	397.1296 and 397.1298	Wiśniewski et al., 2017 [85]
ESI-LC- MS/MS	ARG, MMA, ADMA SDMA	Filtration	NA	Agilent Technologies 1100 series HPLC with Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies, Madrid, Spain)	2.5–3.6	0.019–0.064 $\mu\text{mol/L}$	0–150 $\mu\text{mol/L}$ urine 0–3 $\mu\text{mol/L}$ plasma	Nucleosil C18 (4.0 $\times$ 150 mm) with 5 μm particles (Teknokroma)	Plasma, urine	100% of aqueous mobile phase of TFA 0.2%	9.5 min	D7-ADMA	ADMA: m/z 203.2 $\rightarrow$ 70.1 and SDMA: m/z 203.2 $\rightarrow$ 70.1	Andrade et al., 2015 [86]
ESI-UPLC- MS/MS	ADMA, SDMA	Filtration	HCl and n-butanol	Acquity UPLC System and a Quattro Micro triple quadrupole mass spectrometer (Milford, MA, USA)	2–7.79	7.9–23.7 nM	0.1–4.94 $\mu\text{mol/L}$	Acquity UPLC BEH C18 column (1.7 $\mu\text{mol/L}$, 2.1 $\times$ 100 mm)	Serum	A: 0.1% acetic acid in MeOH; B: 0.1% acetic acid in 5 mM ammonium acetate (pH 4.3)	10 min	D7-ADMA	ADMA: m/z 259 $\rightarrow$ 214 and SDMA: m/z 259 $\rightarrow$ 228	Boelaert et al., 2016 [87]
ESI-LC- MS/MS	ADMA, CML	ACN	NA	Shimadzu LC-10 system (Shimadzu, Kyoto, Japan) with mass spectrometer comprised an AB SCIEX (Concord, CA, USA) model API 3000 triple quadrupole	3.81–8.81	NA–20 ng/mL plasma NA–50 ng/mL for urine	20–1000 ng/mL for plasma 50–2,000 ng/mL for urine	Welch Ultimate® XB-NH ₂ column (150 $\times$ 2.1 mm, 5 $\mu\text{mol/L}$)	Plasma, urine	A: ACN; B: 5 mM ammonium formate buffer pH 4.0	7 min	CML-d2	ADMA: m/z 203 $\rightarrow$ 46	Jing et al., 2015 [88]
ESI-LC- MS/MS	ADMA, SDMA, ARG	1% ammonium acetate in MeOH and 1% formic acid in ACN	NA	Thermo Fisher TSQ Quantum Access with a TLX-4 HPLC system and tandem mass spectrometer	3.2–7.7	NA	0.55–4.43 $\mu\text{mol/L}$	Polaris Si-A analytical column	Plasma	A: 25 mM ammonium formate in water with 1% formic acid; B: MeOH	5 min	¹³ C-ARG and d7-ADMA	ADMA: m/z 203.15 $\rightarrow$ 46.6 and SDMA: m/z 203.1 $\rightarrow$ 172.2	El-Khouly et al., 2012 [89]
ESI-LC- MS/MS	ADMA, SDMA	TCA(PP)/SPE	NDA/ME	Varian 1200L LCMS/MS system (Agilent Technologies, Palo Alto, CA, USA) with a triple quadrupole mass analyzer	2.7–6.8	2.6–8.7 nM	0.05–2.5 $\mu\text{mol/L}$	Kinetex 75 $\times$ 3.0 mm, 2.6 μm C18 column from Phenomenex (Torrance, CA, USA)	Plasma	A: ultrapure water; B: HPLC-grade MeOH	23 min	N-propyl-ARG	ADMA: m/z 369.3 $\rightarrow$ 282.0 and SDMA: m/z 369.3 $\rightarrow$ 282.0	Hui et al., 2012 [90]
LC-ESI- QTOF	ARG, ADMA, SDMA, CIT	ACN(PP)	benzoyl chloride	Nano-Acquity UPLC system equipped with Xevo G2 XS Quadrupole TOF MS (waters)	3.07–14.9	0.03–0.08 $\mu\text{mol/L}$	0.05–2.5 $\mu\text{mol/L}$	Acquity HSS T3 column (50 $\times$ 1.0 mm, 1.75 $\mu\text{mol/L}$) from Waters	Serum	A: 0.1% formic acid (FA) in water; B: 0.1% FA in MeOH	10 min	D7-ADMA	307.1717 and 307.1717	Fleszar et al., 2018 [91]

Table 2: (continued)

Method	Analyte	Extraction	Derivatization	Device	CV %	LOD/LOQ	Linearity	Column	Sample	Mobile phase	Time	IS	Q1/Q3 (ADMA, SDMA)	Ref.
APCI-LC-MS	ARG, ADMA, MMA, SDMA	5-SSA(PP)	NA	Shimadzu LC system with LCMS-2010 quadrupole mass spectrometer (Shi- madzu Kyoto, Japan)	<5	0.010–0.2 μmol/L	0.2–60.0 μmol/L	2.0 × 150 mm Shimadzu VP-ODS column with a particle size of 5 μm	Plasma	Water/ACN (90/ 10, v/v) con- taining 0.5% TFA	6.5 min	HARG	203 and 203 SDMA	Huang et al., 2004 [92]
ESI-LC- MS/MS	HARG, ARG, ADMA, SDMA	MeOH(PP)	NA	HPLC Agilent 1100 se- ries was equipped with an Agilent 1100 LC/MSD SL quadru- pole ion trap	NA	NA–0.06 μmol/L	0.1–25 mg/L	Supelcosil™ LC-Si 3.3 cm × 4.6 mm i.d., 3 μmol/L particle size	Urine	A: 0.1% formic acid in water; B: 100 mM ammo- nium formate in water titrated to pH 4.5 with for- mic acid	20 min	NMMA	ADMA: m/z 203.2 → 158 SDMA: m/z: 203.2 → 172	Servillo et al., 2013 [93]
ESI-LC- MS/MS	Methionine, methionine sulfoxide, choline, betaine, DMG, ARG, ADMA, SDMA, HARG, creati- nine, cys- tathionine trimethyllysine	TCA(PP)	NA	1100 HPLC system (Agilent Technolo- gies) equipped with API 3,000 triple- quadrupole tandem mass spectrometer (AB Sciex)	5.2–7.8	0.08–0.16 μmol/L	0.08–5 μmol/L	Fortis Phenyl col- umn (150 × 4.6 mm; particle size, 3 μmol/L) from Fortis Technologies	Plasma	2.5% MeOH in 150 mmol/L acetic acid (pH 5.2)	4.2 min	D7-ADMA	ADMA: m/z 203.2 → 46.1 SDMA: m/z: 203.2 → 172.1	Middtun et al., 2013 [94]
ESI-LC-MS	ARG, ORN, CIT, SDMA, ADMA	ACN(PP)	NA	Agilent series 1100 LC- MSD system (Agi- lent, USA)	3.2–13	NA–0.1 nmol/mL	0.5–2.13 nmol/mL	Atlantis dC18 col- umn (4.6 × 100 mm, 3.0 μmol/L)	Plasma	0.03% TFA in water	5 min	D7-ADMA	203.1 and 203.1	Wang et al., 2010 [95]

SAM, S-Adenosyl-L-methionine; SAH, S-Adenosyl-L-homocysteine; DMG, dimethylglycine, cysteine; CML, ε-N-carboxymethyl-L-lysine; TFA, trifluoroacetic acid; APDS, 3-aminopyridyl-N-succinimidyl carbamate; DEPC, diethylpyrocarbonate; PFBC, 2,3,4,5,6-pentafluorobenzoyl chloride. CV%, LOD, LOQ, linearity values and IS explained for ADMA.

the use of a fluorescent chromophore will allow these analytes to be detected in the fluorescence detector. Analysis of low levels of molecules can be facilitated by derivatization. So this step is a feature that increases sensitivity. However, the formation of similar derivatives of methylarginines has led to the problem of specificity in fluorescent and UV detectors. On the other hand, the use of mass spectrometers increased the analysis specificity by allowing analytes to be determined according to mass to charge (m/z) ratios and characteristic fragmentation patterns, if any [43]. However, difficulties were encountered in mass spectrometers in the separation of strict isobaric molecules with the same molecular weight and chemical formula. Succinic and methylmalonic acid are strict isobars with the same chemical formula ($C_4H_6O_4$) and molecular weight (11,809 g/mol). Such compounds can not only be distinguished by MS, often MS is combined with a separation method or tandem MS is used [96].

In HPLC-based methods derivatization was performed in the pretreatment steps due to the poor retain of polar compounds in reverse phase columns and lack of intrinsic chromophores for these analytes. Various methods have been developed for the analysis of these analytes without derivatization to shorten the time required for sample preparation. However, these polar analytes are difficult to retain on RP columns, the separation of ADMA and SDMA is insufficient, they have poor peak shapes, high background noises and questionable quantitations [79]. The start of the analysis process under high pressure with UPLC technology and narrow diameter columns were able to solve some of these problems. Derivatization increases the column retention of the analytes, detectability and sensitivity of analysis. Pre-column derivatization is commonly used in chromatographic methods. For this purpose, agents such as *o*-phthalaldehyde (OPA), naphthalene-2,3-dicarboxaldehyde (NDA), *o*-phenylenediamine, AccQ-Fluor™ (6-aminoquinolyl-N-hydroxysuccinimidy carbamate), 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), ninhydrin, phenylisothiocyanate have been used [44, 45, 51, 52, 63, 64]. Among these derivatives, OPA is the most widely used. OPA has advantages such as its ease of use, rapid derivatization reaction at room temperature, giving clean chromatograms since itself is non-fluorescent and it is a cost-effective reagent however, it has some disadvantages [97]. OPA-based methods require complex gradient systems to provide can separate analytes due to their poor selectivity. Therefore, the elution time is long. Moreover, since the derivatives formed with OPA are rapid decomposed, strictly standardized injection times are required [44, 66]. The reaction between OPA

and amino acids depends on the presence of thiol groups. For this purpose, both 2-mercaptoethanol (ME) and 3-mercaptopropionic acid (MPA) are used. In addition, ME and MPA were added to increase the stability of methylarginine OPA derivatives. However, OPA-ME derivatives are quite unstable, and OPA-MPA derivatives are much more stable. It has been shown that the peak area of the OPA derivatives still decreases by 5–10% in 20–25 h and by 35% in 72 h. These reductions can affect the precision and recovery of the method. Derivatives formed with NDA are more stable than OPA. OPMA-ME and OPA-MPA derivatives of ADMA are shown in Figure 3 [5].

The stability of NDA derivatives has been shown to decrease by approximately 6% in 24 h and 13% in 72 h. Also, in derivatization with NDA, ADMA and SDMA could be separated without the risk of overlap. However, since the chemical reaction is slower in derivatization with NDA (20 min), longer incubation time is required than OPA (1 min). [44, 64]. NBD-F is another fluorescent derivative used in amino acid analysis. L-NMMA concentration is lower than SDMA and ADMA. In previous studies, plasma arginine, ADMA and SDMA concentrations of healthy volunteers were reported to be approximately 65, 0.4 and 0.5 μ M, while L-NMMA levels were reported to be approximately 10% of ADMA [43]. Another problem with derivatives with OPA is that OPA derivative intensities are insufficient to detect methylarginines simultaneously. NBD-F based methods have higher sensitivity and stability than OPA. It has been shown that the peak area of the OPA-mercaptopropionic acid derivatives still decreases by 5–10% in 20–25 h and by 35% in 72 h. NBD-F derivatives have been shown to be stable for at least three days at room temperature. Therefore, NBD-F derivatives are more stable than others. At the same time, derivatization with NBD-F is much more sensitive. In the method developed by Nonaka et al. in derivatization with NBD-F, LOD values for L-NMMA, SDMA, ADMA were 12, 20, 2 fmol respectively,

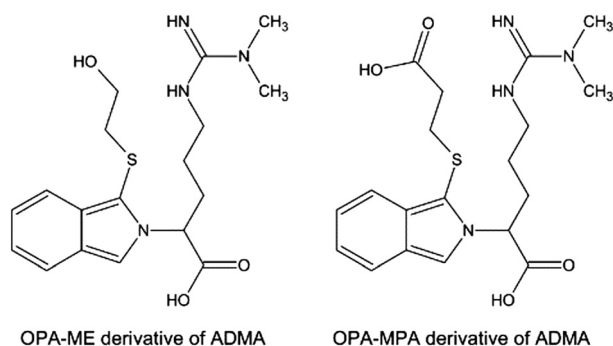


Figure 3: OPMA-ME and OPA-MPA derivatives of ADMA [5].

while for OPA derivatization these values were reported as 50–200 fmol [51]. Derivatives formed with AccQ-Fluor™ have been shown to be stable for up to one week at room temperature. However, the disadvantage of this method is that AccQ-Fluor™ derivatization leads to a long chromatography of up to 50 min [52].

In mass spectrometric methods, agents such as OPA, NDA, butanol, dansyl chloride were used as derivatives. OPA derivatives have also been used in mass spectrometric analysis, but SDMA and ADMA have been found to have the same fragmentation pattern in ESI ionization. The most popular approach in mass spectrometric methods is butylation of amino acids to form butyl esters of the acid residue [67]. Schwedhelm et al. published the first LC-MS based method to separate butylated metabolites of methylated arginine's by specific fragmentation pattern instead of chromatographic separation [74]. Butylation and fragmentation pattern of Arginine, ADMA and SDMA are shown in Figure 4.

Chromatographic separation of the derivatives of methylarginine was generally performed using an isocratic or gradient system using reverse phase chromatography. Separation of methylarginine derivatives can be achieved under reverse phase chromatography conditions with analytical columns packed with octadecyl silica or phenyl silica [40]. Reasonable retention of non-derivatized amino acids in phenyl reverse phase columns, as in C18 columns, can only be achieved with high aqueous content in the mobile phase. Phenyl-type columns provide complementary selectivity by providing pi-pi interaction [99]. These columns have been used especially for the separation of positional isomers tocopherols, flavonoids, polynuclear

aromatics and nitroaromatic compounds, active pharmaceutical ingredients (APIs) and related compounds [100]. In C18 columns, the OPA derivative of ADMA is eluted before SDMA, whereas in the phenyl column this order is inverted. The NDA derivatives showed the same elution sequence in the phenyl column. Silica-based monolithic columns have high permeability and can be operated at higher flow rates. Comparing conventional RP columns with monolithic columns, the retention time of OPA derivatives was reduced by two times without loss of resolution [41]. Hydrophilic interaction liquid chromatography (HILIC) column technology has also been tried as the separation and retention of analytes in traditional RP columns is poor. HILIC column is a variation of normal phase chromatography. The main difference between normal phase and HILIC is the separation mechanism and mobile phase. While 100% organic mobile phase is used in normal phase, HILIC uses organic mobile phases that can be mixed with water. However, HILIC methods generally require longer column equilibration, and sometimes poor retention and peak shape can be seen in this method [70].

Complex gradient systems have been implemented that can extend the elution time up to 70 min in order to enable separation of analytes. In addition to derivatization in the pretreatment steps of HPLC and LC-MS/MS methods, processes such as SPE, protein precipitation, evaporation and ultrafiltration were applied to prevent the interfering effect of other components in the sample matrix, such as proteins. Precipitation was generally achieved with 5-sulfo salicylic acid, methanol, ethanol, acetonitrile.

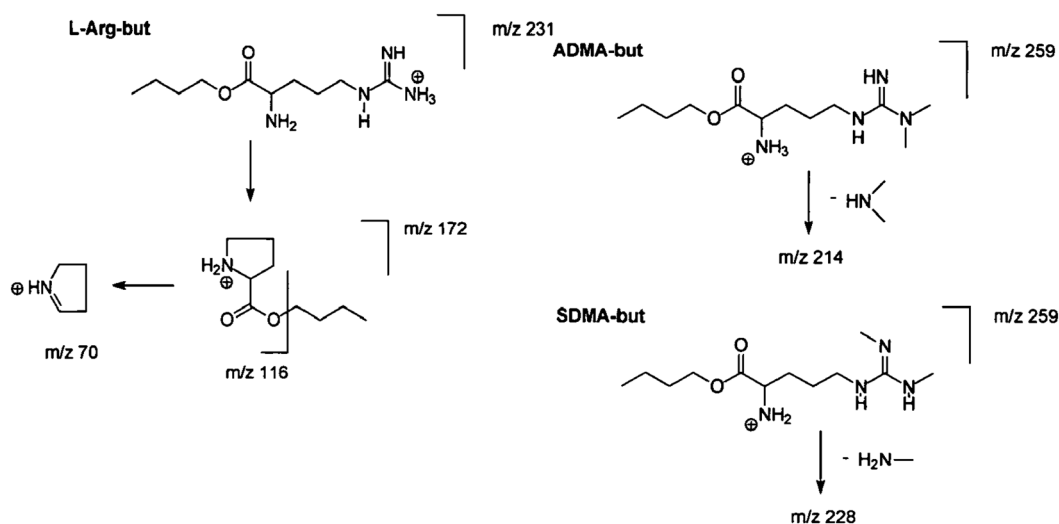


Figure 4: Butylation and fragmentation pattern of Arginine, ADMA and SDMA. Butylation and fragmentation pattern of Arginine, ADMA and SDMA showed in Figure 4 [98].

Another problem in the correct quantitation of methylarginines is the internal standard selection. It is widely used in HPLC methods as the internal standard of homoarginine. However, the use of homoarginine an endogenous compound as an internal standard may lead to a systematic error in the measurement. Again, the use of homologs as an internal standard is another problem. When these molecules that are chemically different from analytes are used, they possess potentially different behaviors during the sample preparation stages. These differences are matrix dependent and may also lead to systematic error. The most correct approach to eliminate such problems is the use of stable-isotope labeled analogs as internal standards [43].

A critical point in the HPLC method is the separation of ADMA and SDMA. Schwedhelm et al. and Martens-Lobenhoffer et al. overcame this problem with the use of tandem mass spectrometry. Schwedhelm et al. has enabled the separation of methyl arginine derivatives through a specific fragmentation pattern instead of chromatographic separation. In this method, specific ion transitions of m/z 259.3 $\rightarrow$ 214 and m/z 259.3 $\rightarrow$ 228 were used for the ester derivatives of ADMA and SDMA, respectively. These fragment ions related to the neutral loss of dimethylamine and methylamine, respectively, Martens-Lobenhoffer et al. used 46.1 fragment ions formed by the loss of dimethylammonium ion for ADMA, and 172.1 fragment ions formed due to the neutral loss of methylamine for SDMA in the fragmentation pattern [72,74].

Conclusion

ADMA and L-NMMA reduce the production of NO by inhibiting the NOS enzyme, while SDMA inhibits the uptake of arginine, the substrate of this enzyme, into cells. Therefore, ADMA and related metabolites are an important risk factor for inflammatory, renal and neurodegenerative disorders, especially cardiovascular diseases. Various HPLC, GC-MS, CE, ELISA, LC-MS, LC-MS/MS methods have been developed to measure the level of these metabolites in serum, plasma, urine, tissue, and cell culture samples. Among these methods, HPLC methods are common. Although many measurement methods have been developed for ADMA and related metabolites, various difficulties have been encountered in the measurement of these analytes. These difficulties are result from not well retained in conventional RP columns due to being polar compounds, difficult to separate due to their physicochemical and structural similarities, differences in plasma concentrations, lack of chromophores that enable their specific determination, thermally unstable and non-volatile

compounds. In order to increase the sensitivity of the analysis, to ensure the retention of the compounds in conventional reverse phase columns, to increase detectability, derivatization with agents such as OPA, NDA, NBD-F, AccQ-FluorTM was performed in HPLC methods. However, in these methods, complex gradient systems that require long elution times have been used to ensure the separation of ADMA and SDMA. The stability problems due to the derivatives formed have disrupted the precision and recovery of the analysis. In studies conducted with HPLC methods, it has been reported that there are stability problems in OPA derivatives, NDA derivatization incubation time is relatively long, and AccQ-FluorTM causes long chromatogram. It has been reported that the derivatives formed with NBD-F are stable for at least three days at room temperature, the incubation time is short (about 3 min), and the sensitivity of NBD-F based methods is higher than other derivatives. When HPLC methods applied to date are evaluated, separation with conventional C18 columns may be ideal following solid phase extraction followed by pre-column derivatization with NBD-F. However, LC-MS/MS is considered the gold standard in the analysis of these metabolites. The sensitivity and selectivity of LC-MS/MS is very good compared to HPLC methods. In LC-MS/MS based methods, these metabolites could be separated by HILIC column technology following protein precipitation without derivatization using specific fragmentation pattern. However, HILIC columns often require long column equilibration and problems have been observed due to poor retention of analytes, peak shapes, poor separation of ADMA and SDMA. In LC-MS / MS based methods, derivatization has been widely applied to further increase the sensitivity of analysis, to facilitate separation of ADMA and SDMA, and to increase column retention of analytes. In LC-MS / MS based methods, butanol derivatization has been widely applied following protein precipitation. With butanol derivatization, it was possible to sensitively and economically analyze a large number of metabolites such as ADMA, SDMA, L-NMMA, arginine, ornithine, citrulline, with an average run time of as little as 5 min after an incubation of 20 min.

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References

- Hsu CN, Tain YL. Impact of arginine nutrition and metabolism during pregnancy on offspring outcomes. *Nutrients* 2019;11:1452.
- Wang YC, Peterson SE, Loring JF. Protein post-translational modifications and regulation of pluripotency in human stem cells. *Cell Res* 2014;24:143–60.
- Fulton MD, Brown T, Zheng YG. The biological axis of protein arginine methylation and asymmetric dimethylarginine. *Int J Mol Sci* 2019;20:3322.
- Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethylarginine: dimethylarginine dimethylaminohydrolase pathway. *Arterioscler Thromb Vasc Biol* 2004;24:1023–30.
- Teerlink T. HPLC analysis of ADMA and other methylated L-arginine analogs in biological fluids. *J Chromatogr B Anal Technol Biomed Life Sci* 2007;851:21–9.
- Tain YL, Hsu CN. Toxic dimethylarginines: asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA). *Toxins* 2017;9:92.
- Tain YL, Huang LT. Asymmetric dimethylarginine: clinical applications in pediatric medicine. *J Formos Med Assoc* 2011;110:70–7.
- Jarzebska N, Mangoni AA, Martens-Lobenhoffer J, Bode-Böger SM, Rodionov RN. The second life of methylarginines as cardiovascular targets. *Int J Mol Sci* 2019;20:4592.
- Liu X, Fassett J, Wei Y, Chen Y. Regulation of DDAH1 as a potential therapeutic target for treating cardiovascular diseases. *Evid base Compl Alternative Med* 2013;2013:619207.
- Pope AJ, Karupiah K, Cardounel AJ. Role of the PRMT-DDAH-ADMA axis in the regulation of endothelial nitric oxide production. *Pharmacol Res* 2009;60:461–5.
- Oliva-Damaso E, Oliva-Damaso N, Rodriguez-Esparragon F, Payan J, Baamonde-Laborda E, Gonzalez-Cabrera F, et al. Asymmetric (ADMA) and symmetric (SDMA) dimethylarginines in chronic kidney disease: a clinical approach. *Int J Mol Sci* 2019;20:3668.
- Tousoulis D, Georgakis MK, Oikonomou E, Papageorgiou N, Zaromitidou M, Latsios G, et al. Asymmetric dimethylarginine: clinical significance and novel therapeutic approaches. *Curr Med Chem* 2016;22:2871–901.
- Kielstein JT, Fliser D. The past, presence and future of ADMA in nephrology. *Néphrol Thérapeutique* 2007;3:47–54.
- Caplin B, Leiper J. Endogenous nitric oxide synthase inhibitors in the biology of disease: markers, mediators, and regulators?. *Arterioscler Thromb Vasc Biol* 2012;32:1343–53.
- Tousoulis D, Georgakis MK, Oikonomou E, Papageorgiou N, Zaromitidou M, Latsios G, et al. Asymmetric dimethylarginine: clinical significance and novel therapeutic approaches. *Curr Med Chem* 2015;22:2871–901.
- Víteček J, Lojek A, Valacchi G, Kubala L. Arginine-based inhibitors of nitric oxide synthase: therapeutic potential and challenges. *Mediat Inflamm* 2012;2012:318087.
- Kielstein A, Tsikas D, Galloway GP, Mendelson JE. Asymmetric dimethylarginine (ADMA) – a modulator of nociception in opiate tolerance and addiction?. *Nitric Oxide* 2007;17:55–9.
- Notsu Y, Yano S, Shibata H, Nagai A, Nabika T. Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: shimane CoHRE study. *Atherosclerosis* 2015;239:61–6.
- Liu X, Xu X, Shang R, Chen Y. Asymmetric dimethylarginine (ADMA) as an important risk factor for the increased cardiovascular diseases and heart failure in chronic kidney disease. *Nitric Oxide* 2018;78:113–20.
- Selley ML. Increased concentrations of homocysteine and asymmetric dimethylarginine and decreased concentrations of nitric oxide in the plasma of patients with Alzheimer's disease. *Neurobiol Aging* 2003;24:903–7.
- Sibal L, Agarwal SC, Home PD, Boger RH. The role of asymmetric dimethylarginine (adma) in endothelial dysfunction and cardiovascular disease. *Curr Cardiol Rev* 2010;6:82–90.
- Hosaf M, Abusoglu S, Avci A, Demir K, Unlu A, Eryavuz D, et al. Total methylated arginine load as a risk parameter in subjects with masked hypertension. *Clin Exp Hypertens* 2020;42:126–30.
- Taner A, Unlu A, Kayrak M, Tekinalp M, Ayhan SS, Arıbaş A, et al. The value of serum asymmetric dimethylarginine levels for the determination of masked hypertension in patients with diabetes mellitus. *Atherosclerosis* 2013;228:432–7.
- Aribas A, Kayrak M, Tekinalp M, Akilli H, Alibasic H, Yildirim S, et al. The relationship between serum asymmetric dimethylarginine levels and subjective sleep quality in normotensive patients with type 2 diabetes mellitus. *Korean J Intern Med* 2015;30:316–24.
- Mihout F, Shweke N, Bigé N, Jouanneau C, Dussault JC, Ronco P, et al. Asymmetric dimethylarginine (ADMA) induces chronic kidney disease through a mechanism involving collagen and TGF- β 1 synthesis. *J Pathol* 2011;223:37–45.
- Kirbas S, Kirbas A, Tufekci A, Cumhuri Cure M, Cakmak S, Yazici T, et al. Serum levels of homocysteine, asymmetric dimethylarginine and nitric oxide in patients with Parkinson's disease. *Acta Clin Belg* 2016;71:71–5.
- Kielstein JT, Bode-Böger SM, Frölich JC, Ritz E, Haller H, Fliser D. Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. *Circulation* 2003;107:1891–5.
- Kemény-Beke Á, Gesztelyi R, Bodnár N, Zsuga J, Kerekes G, Zsuga M, et al. Increased production of asymmetric dimethylarginine (ADMA) in ankylosing spondylitis: association with other clinical and laboratory parameters. *Joint Bone Spine* 2011;78:184–7.
- Vatansev H, Öztürk B, Yılmaz S, Sivrikaya A, Dağlı M, Kıyıcı A, et al. Asymmetric dimethylarginine and arginine levels in patients with rheumatoid arthritis. *Turk J Biochem* 2013;38:169–75.
- Owczarek D, Cibor D, Mach T. Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), arginine, and 8-iso-prostaglandin F2 α (8-iso-PGF2 α) level in patients with inflammatory bowel diseases. *Inflamm Bowel Dis* 2010;16:52–7.
- Öztürk B, Vatansev H, Taner A, Yılmaz S, Dağlı M, Unlu A. Asymmetric dimethylarginine levels in Behçet's disease. *Turk J Biochem* 2014;39:362–7.
- Danzi S, Klein I. Thyroid hormone and the cardiovascular system. *Minerva Endocrinol* 2004;29:139–50.
- Jabbar A, Pingitore A, Pearce SH, Zaman A, Lervasi G, Razvi S. Thyroid hormones and cardiovascular disease. *Nat Rev Cardiol* 2017;14:39–55.
- Liu X, Xu X, Shang R, Chen Y. Asymmetric dimethylarginine (ADMA) as an important risk factor for the increased

- cardiovascular diseases and heart failure in chronic kidney disease. *Nitric Oxide* 2018;1:113–20.
35. Abdel-Messeih PL. Asymmetric dimethyl arginine in hypothyroid patients. *Isot Radiat Res* 2012;44:725–36.
 36. Hermenegildo C, Medina P, Peiró M, Segarra G, Vila JM, Ortega J, et al. Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in hyperthyroid patients. *J Clin Endocrinol Metab* 2002;87: 5636–40.
 37. Li H, Zhou Y, Zhao A, Qiu Y, Xie G, Jiang Q, et al. Asymmetric dimethylarginine attenuates serum starvation-induced apoptosis via suppression of the Fas (APO-1/CD95)/JNK (SAPK) pathway. *Cell Death Dis* 2013;4:e830.
 38. Iritas SB, Akturk T, Tutkun L, Gündüzöz M, Abuşoğlu S, Celik S, et al. Elevated asymmetric dimethylarginine levels in manganese-exposed welders. *Trace Elem Electrolytes* 2019;36: 190–6.
 39. Tutkun L, Gunduzoz M, Turksoy VA, Deniz S, Oztan O, Cetintepe SP, et al. Assessment of endothelial dysfunction with methylated arginines and L-arginine in cadmium-exposed people: a pilot study. *Clin Lab* 2019;65. <https://doi.org/10.7754/clin.lab.2019.181249>.
 40. Iritas SB, Dip A, Gunduzoz M, Tutkun L, Turksoy VA, Deniz S, et al. Assessment of potential cardiovascular risk in trichloroethylene exposure by serum methylated arginine levels. *Int J Environ Health Res* 2019;24:1–12.
 41. Schwedhelm E. Quantification of ADMA: analytical approaches. *Vasc Med* 2005;10:S89–95.
 42. Németh Z, Ajtay L, Hejjel T, Ferenci Z, Ábrám E, Murányi E, et al. The issue of plasma asymmetric dimethylarginine reference range - a systematic review and meta-analysis. *PLoS One* 2017; 12:e0177493.
 43. Martens-Lobenhoffer J, Bode-Böger SM. Chromatographic-mass spectrometric methods for the quantification of L-arginine and its methylated metabolites in biological fluids. *J Chromatogr B Anal Technol Biomed Life Sci* 2007;851:30–41.
 44. Marra M, Bonfigli AR, Testa R, Testa I, Gambini A, Coppa G, et al. High-performance liquid chromatographic assay of asymmetric dimethylarginine, symmetric dimethylarginine, and arginine in human plasma by derivatization with naphthalene-2,3-dicarboxaldehyde. *Anal Biochem* 2003;318:13–7.
 45. Markowski P, Baranowska I, Baranowski J. Simultaneous determination of L-arginine and 12 molecules participating in its metabolic cycle by gradient RP-HPLC method: application to human urine samples. *Anal Chim Acta* 2007;605:205–17.
 46. Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PA. Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. *Anal Biochem* 2002;303:131–7.
 47. Zhang WZ, Kaye DM. Simultaneous determination of arginine and seven metabolites in plasma by reversed-phase liquid chromatography with a time-controlled ortho-phthalaldehyde precolumn derivatization. *Anal Biochem* 2004;326:87–92.
 48. Meinitzer A, Puchinger M, Winklhofer-Roob BM, Rock E, Ribalta J, Roob JM, et al. Reference values for plasma concentrations of asymmetrical dimethylarginine (ADMA) and other arginine metabolites in men after validation of a chromatographic method. *Clin Chim Acta* 2007;384:141–8.
 49. Blackwell S, O'Reilly D, Talwar D. HPLC analysis of asymmetric dimethylarginine (ADMA) and related arginine metabolites in human plasma using a novel non-endogenous internal standard. *Clin Chim Acta* 2009;401:14–9.
 50. Ivanova M, Artusi C, Boffa GM, Zaninotto M, Plebani M. HPLC determination of plasma dimethylarginines: method validation and preliminary clinical application. *Clin Chim Acta* 2010;411: 1632–6.
 51. Nonaka S, Tsunoda M, Imai K, Funatsu T. High-performance liquid chromatographic assay of NG-monomethyl-L-arginine, N⁶,N⁶-dimethyl-L-arginine, and N⁶,N^{6'}-dimethyl-L-arginine using 4-fluoro-7-nitro-2,1,3-benzoxadiazole as a fluorescent reagent. *J Chromatogr A* 2005;1066:41–5.
 52. Jones CE, Darcy CJ, Woodberry T, Anstey NM, McNeil YR. HPLC analysis of asymmetric dimethylarginine, symmetric dimethylarginine, homoarginine and arginine in small plasma volumes using a Gemini-NX column at high pH. *J Chromatogr B Anal Technol Biomed Life Sci* 2010;878:8–12.
 53. Pi J, Kumagai Y, Sun G, Shimojo N. Improved method for simultaneous determination of L-arginine and its mono- and dimethylated metabolites in biological samples by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 2000;742:199–203.
 54. Pettersson A, Ugglä L, Backman V. Determination of dimethylated arginines in human plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1997;692: 257–62.
 55. Heresztyn T, Worthley MI, Horowitz JD. Determination of L-arginine and N⁶, N⁶ - and N⁶, N^{6'} -dimethyl-L-arginine in plasma by liquid chromatography as AccQ-Fluor fluorescent derivatives. *J Chromatogr B Anal Technol Biomed Life Sci* 2004;805:325–9.
 56. Valtonen P, Karppi J, Nyyssonen K, Valkonen VP, Halonen T, Punnonen K, et al. Comparison of HPLC method and commercial ELISA assay for asymmetric dimethylarginine (ADMA) determination in human serum. *J Chromatogr B Anal Technol Biomed Life Sci* 2005;828:97–102.
 57. Nonaka S, Tsunoda M, Aoyama C, Funatsu T. Determination of N⁶,N⁶-dimethyl-L-arginine in rat plasma and dimethylarginine dimethylaminohydrolase activity in rat kidney using a monolithic silica column. *J Chromatogr B Anal Technol Biomed Life Sci* 2006; 843:170–4.
 58. Schou-Pedersen AMV, Lykkesfeldt J. Comparison of three sample preparation procedures for the quantification of L-arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma using HPLC-FLD. *J Anal Methods Chem* 2018; 2018:6148515.
 59. Tsunoda M, Nonaka S, Funatsu T. Determination of methylated arginines by column-switching high-performance liquid chromatography-fluorescence detection. *Analyst* 2005;130: 1410–3.
 60. Suzuki K, Katayama M, Takamatsu K, Kaneko S, Ishikawa H, Tsukimura T, et al. The stable o-phthalaldehyde-ferrocene/6-ferrocenyl-1-hexanethiol pre-column derivatization high-performance liquid chromatography of dimethylarginine by novel dual fluorescence and electrochemical detector. *Biomed Chromatogr* 2013;27:535–8.
 61. Alkaitis MS, Nardone G, Chertow JH, Ackerman HC. Resolution and quantification of arginine, monomethylarginine, asymmetric dimethylarginine, and symmetric dimethylarginine in plasma

- using HPLC with internal calibration. *Biomed Chromatogr* 2016; 30:294–300.
62. Rao P, Bitla AR. Simultaneous determination of arginine, citrulline, and asymmetric dimethylarginine in plasma by reverse-phase high-performance liquid chromatography. *J Lab Phys* 2017;9:243–8.
 63. Sotgia S, Zinella A, Pinna GA, Deiana L, Carru C. A new selective pre-column ninhydrin-based derivatization for a RP-HPLC determination of plasma asymmetric dimethyl-L-arginine (ADMA) by fluorescence detection. *Amino Acids* 2008;34:677–82.
 64. Ravula V, Annapurna Singh S. A simple reversed-phase HPLC method for the estimation of dimethylarginines and homocysteine in plasma and serum. *SSC Plus* 2018;1:404–10.
 65. Unceta N, San Vicente A, Goicolea MA, Sallés J, Barrio RJ. Determination of methylarginines in human plasma by HPLC with pre-column derivatization using naphthalenedicarboxaldehyde as fluorogenic agent. *J Separ Sci* 2002;25:665–70.
 66. Huang LF, Guo FQ, Liang YZ, Hu QN, Cheng BM. Rapid simultaneous determination of arginine and methylated arginines in human urine by high-performance liquid chromatography–mass spectrometry. *Anal Chim Acta* 2003;487: 145–53.
 67. Di Gangi IM, Chiandetti L, Gucciardi A, Moret V, Naturale M, Giordano G. Simultaneous quantitative determination of N(G),N(G)-dimethyl-L-arginine or asymmetric dimethylarginine and related pathway's metabolites in biological fluids by ultrahigh-performance liquid chromatography/electrospray ionization-tandem mass spectrometry. *Anal Chim Acta* 2010;677: 140–8.
 68. Arashida N, Nishimoto R, Harada M, Shimbo K, Yamada N. Highly sensitive quantification for human plasma-targeted metabolomics using an amine derivatization reagent. *Anal Chim Acta* 2017;954:77–87.
 69. Squellerio I, Tremoli E, Cavalca V. Quantification of arginine and its metabolites in human erythrocytes using liquid chromatography-tandem mass spectrometry. *Anal Biochem* 2011;412:108–10.
 70. Sotgia S, Zinella A, Paliogiannis P, Pinna GA, Mangoni AA, Milanesi L, et al. A diethylpyrocarbonate-based derivatization method for the LC-MS/MS measurement of plasma arginine and its chemically related metabolites and analogs. *Clin Chim Acta* 2019;492:29–36.
 71. D'Apolito O, Paglia G, Tricarico F, Garofalo D, Pilotti A, Lamacchia O, et al. Development and validation of a fast quantitative method for plasma dimethylarginines analysis using liquid chromatography-tandem mass spectrometry. *Clin Biochem* 2008;41:1391–5.
 72. Martens-Lobenhoffer J, Bode-Boger SM. Quantification of L-arginine, asymmetric dimethylarginine and symmetric dimethylarginine in human plasma: a step improvement in precision by stable isotope dilution mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 2012;904:140–3.
 73. Martens-Lobenhoffer J, Bode-Boger SM. Simultaneous detection of arginine, asymmetric dimethylarginine, symmetric dimethylarginine and citrulline in human plasma and urine applying liquid chromatography-mass spectrometry with very straightforward sample preparation. *J Chromatogr B Anal Technol Biomed Life Sci* 2003;798:231–9.
 74. Schwedhelm E, Maas R, Tan-Andresen J, Schulze F, Riederer U, Böger RH. High-throughput liquid chromatographic-tandem mass spectrometric determination of arginine and dimethylated arginine derivatives in human and mouse plasma. *J Chromatogr B Anal Technol Biomed Life Sci* 2007;851:211–9.
 75. Bishop MJ, Crow B, Norton D, Paliakov E, George J, Bralley JA. Direct analysis of un-derivatized asymmetric dimethylarginine (ADMA) and L-arginine from plasma using mixed-mode ion-exchange liquid chromatography-tandem mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 2007;859:164–9.
 76. Weaving G, Rocks BF, Bailey MP, Titheradge MA. Arginine and methylated arginines in human plasma and urine measured by tandem mass spectrometry without the need for chromatography or sample derivatisation. *J Chromatogr B Anal Technol Biomed Life Sci* 2008;874:27–32.
 77. Shin S, Fung SM, Mohan S, Fung HL. Simultaneous bioanalysis of L-arginine, L-citrulline, and dimethylarginines by LC-MS/MS. *J Chromatogr B Anal Technol Biomed Life Sci* 2011;879:467–74.
 78. Davids M, Swieringa E, Palm F, Smith DE, Smulders YM, Scheffer PG, et al. Simultaneous determination of asymmetric and symmetric dimethylarginine, L-monomethylarginine, L-arginine, and L-homoarginine in biological samples using stable isotope dilution liquid chromatography tandem mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 2012;900:38–47.
 79. Gopu CL, Hari PR, George R, Hari Krishnan S, Sreenivasan K. Simultaneous determination of homocysteine and asymmetric dimethylarginine in human urine by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 2013;939:32–7.
 80. Martens-Lobenhoffer J, Bode-Boger SM. Fast and efficient determination of arginine, symmetric dimethylarginine, and asymmetric dimethylarginine in biological fluids by hydrophilic-interaction liquid chromatography-electrospray tandem mass spectrometry. *Clin Chem* 2006;52:488–93.
 81. Yi L, Zhang P, Ji X, Liang Y, Wang Y, Zhou Z, et al. Quantitation of L-arginine and asymmetric dimethylarginine in human plasma by LC-selective ion mode-MS for Type 2 diabetes mellitus study. *Chem Pharm Bull (Tokyo)* 2011;59:839–43.
 82. Schwedhelm E, Tan-Andresen J, Maas R, Riederer U, Schulze F, Böger RH. Liquid chromatography-tandem mass spectrometry method for the analysis of asymmetric dimethylarginine in human plasma. *Clin Chem* 2005;51:1268–71.
 83. Saigusa D, Takahashi M, Kanemitsu Y, Ishida A, Abe T, Yamakuni T, et al. Determination of asymmetric dimethylarginine and symmetric dimethylarginine in biological samples of mice using LC/MS/MS. *Am J Anal Chem* 2011;02:303–13.
 84. Benito S, Sanchez A, Unceta N, Andrade F, Aldamiz-Echevarria L, Goicolea MA, et al. LC-QTOF-MS-based targeted metabolomics of arginine-creatine metabolic pathway-related compounds in plasma: application to identify potential biomarkers in pediatric chronic kidney disease. *Anal Bioanal Chem* 2016;408:747–60.
 85. Wisniewski J, Fleszar MG, Piechowicz J, Krzystek-Korpaczka M, Chachaj A, Szuba A, et al. A novel mass spectrometry-based method for simultaneous determination of asymmetric and symmetric dimethylarginine, L-arginine and L-citrulline optimized for LC-MS-TOF and LC-MS/MS. *Biomed Chromatogr* 2017;31:e3994.
 86. Andrade F, Llarena M, Lage S, Aldamiz-Echevarria L. Quantification of arginine and its methylated derivatives in healthy children by liquid chromatography-tandem mass spectrometry. *J Chromatogr Sci* 2015;53:787–92.
 87. Boelaert J, Schepers E, Glorieux G, Elout S, Vanholder R, Lynen F. Determination of asymmetric and symmetric dimethylarginine in

- serum from patients with chronic kidney disease: UPLC-MS/MS versus ELISA. *Toxins* 2016;8. <https://doi.org/10.3390/toxins8050149>.
88. Jing Z, Kuang L, Liu N, Yang J. LC-MS/MS for the simultaneous determination of polar endogenous ADMA and CML in plasma and urine from diabetics. *Bioanalysis* 2015;7:1261–71.
89. El-Khoury JM, Bunch DR, Reineks E, Jackson R, Steinle R, Wang S. A simple and fast liquid chromatography-tandem mass spectrometry method for measurement of underivatized L-arginine, symmetric dimethylarginine, and asymmetric dimethylarginine and establishment of the reference ranges. *Anal Bioanal Chem* 2012;402:771–9.
90. Hui Y, Wong M, Kim JO, Love J, Ansley DM, Chen DD A new derivatization method coupled with LC-MS/MS to enable baseline separation and quantification of dimethylarginines in human plasma from patients to receive on-pump CABG surgery. *Electrophoresis* 2012;33:1911–20.
91. Fleszar MG, Wisniewski J, Krzystek-Korpacka M, Misiak B, Frydecka D, Piechowicz J, et al. Quantitative analysis of L-arginine, dimethylated arginine derivatives, L-citrulline, and dimethylamine in human serum using liquid chromatography-mass spectrometric method. *Chromatographia* 2018;81: 911–21.
92. Huang LF, Guo FQ, Liang YZ, Li BY, Cheng BM. Simultaneous determination of L-arginine and its mono- and dimethylated metabolites in human plasma by high-performance liquid chromatography-mass spectrometry. *Anal Bioanal Chem* 2004; 380:643–9.
93. Servillo L, Giovane A, D'Onofrio N, Casale R, Cautela D, Castaldo D, et al. Determination of homoarginine, arginine, NMMA, ADMA, and SDMA in biological samples by HPLC-ESI-mass spectrometry. *Int J Mol Sci* 2013;14:20131–8.
94. Midttun O, Kvalheim G, Ueland PM. High-throughput, low-volume, multianalyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS. *Anal Bioanal Chem* 2013;405:2009–17.
95. Wang HY, Hu P, Jiang J. Rapid determination of underivatized arginine, ornithine, citrulline and symmetric/asymmetric dimethylarginine in human plasma by LC-MS. *Chromatographia* 2010;71:933–9.
96. Kushnir MM, Nelson GJ, Frank EL, Rockwood AL. High-throughput analysis of methylmalonic acid in serum, plasma, and urine by LC-MS/MS. Method for analyzing isomers without chromatographic separation. *Methods Mol Biol* 2016;1378: 159–73.
97. Cardinael P, Casabianca H., Peulon-Agasse V, Berthod A. Sample derivatization in separation science. *Anal Separ Sci* 2015;5. <https://doi.org/10.1002/9783527678129.assep063>.
98. Boeger R, Schwedhelm E, Maas R, Riederer U. Method for determination of arginine, methylated arginines and derivatives thereof. European patent office publ. of application with search report EP20040026164, 2004.
99. Yang M, Fazio S, Munch D, Drumm P. Impact of methanol and acetonitrile on separations based on pi–pi interactions with a reversed-phase phenyl column. *J Chromatogr A* 2005;1097: 124–9.
100. Zhou L, Wu Y, Johnson B, Thompson R, Wyvratt J. Chromatographic separation of 3,4-difluorophenylacetic acid and its positional isomers using five different techniques. *J Chromatogr A* 2000;866:281–92.