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Letter to the Editor

Determination of dimethylated arginines in human plasma by high-performance liquid chromatography

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Dimethylarginines (DMA), i.e., N^G,N^G-dimethyl-L-arginine (asymmetric DMA, ADMA) and N^G,N'^G-dimethyl-L-arginine (symmetric DMA, SDMA) (Fig. 1) are endogenous compounds occurring in several tissue proteins, plasma and urine of humans [1,2]. ADMA is a potent inhibitor of nitric oxide (NO) synthesis and this is believed to be, at least in part, the mechanism responsible for hypertension occurring in renal failure [2]. The stereoisomer SDMA has been shown to be biologically inactive [2]. The interest in the hypertensive action of ADMA led to the development of various analytical methods among which high-performance liquid chromatography (HPLC) with fluorescence detection using *o*-phthaldialdehyde (OPA) for pre-column derivatization is the most efficient [3–6].

The main problem in the HPLC analysis of dimethylarginines in plasma is the difficult separation of the OPA-derivatives of the two isomers rather than their separation from other amino acids or lack in sensitivity. In recent years, several attempts have been undertaken to optimize separation of the OPA derivatives of ADMA and SDMA from one another and from interfering amino acids. A useful procedure represents the elimination of acidic and neutral amino acids by using solid-phase extraction cartridges with cation or anion exchangers [2–4]. It has been shown that simple protein precipitation by 5-sulfosalicylic acid is satisfactory for the HPLC determination of ADMA in human plasma with a retention time of 13 min and a total analysis time of 35 min on HPLC columns packed with 5 μm octadecylsilica (ODS) [5]. However, in this work no data were presented for SDMA. Using similar HPLC conditions Park et al. [6] could not separate at all the OPA derivatives of the two isomers ADMA and SDMA. It cannot, therefore, be excluded that in the study of Chen et al. [5] ADMA has not been chromatographically separated from SDMA. Almost

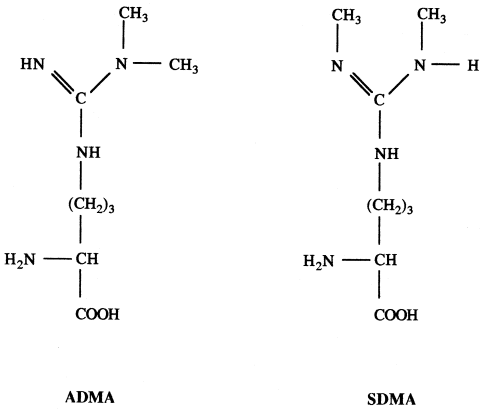

Fig. 1. Chemical structures of N^G,N^G-dimethyl-L-arginine (ADMA) and N^G,N'^G-dimethyl-L-arginine (SDMA).

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baseline-resolution of the OPA-derivatives of ADMA and SDMA can be achieved by using analytical columns packed with 3 μm ODS with retention times of about 50 min and a total analysis time of about 70 min [3]. Baseline separation of ADMA–OPA and SDMA–OPA derivatives (retention times 12 and 11 min, respectively) can be achieved by using 7 μm phenyl (C_6H_5) silica within a total analysis time of 20 min (Fig. 2) [4]. Remarkably, ADMA– and SDMA–OPA derivatives elute on phenylsilica columns in the opposite order as on columns packed with ODS [4]. This HPLC system offers furthermore the opportunity to simultaneously determine L-arginine (retention time about 6 min), since the ratio of L-arginine to ADMA plasma levels may be of importance in arteriosklerosis as has been shown for peripheral arterial occlusive disease and hypercholesterolemia [4,7]. The whole HPLC method will be reported shortly by our group.

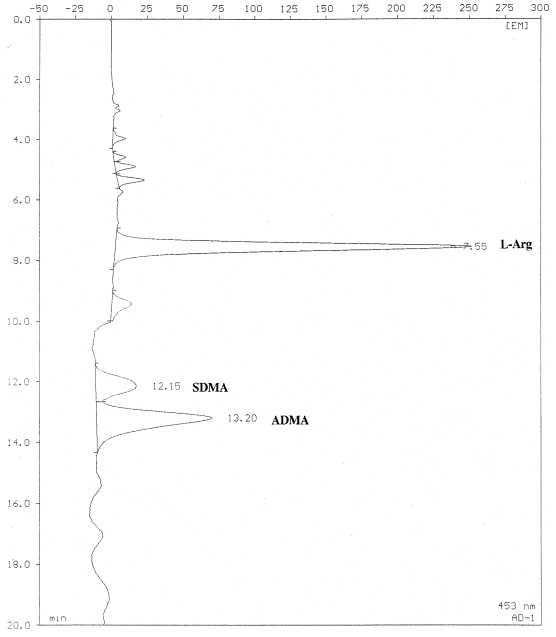

Fig. 2. Typical chromatogram from the high-performance liquid chromatographic determination of N^G,N^G-dimethyl-L-arginine (ADMA), N^G,N'^G-dimethyl-L-arginine (SDMA) and L-arginine (L-Arg) in plasma of a healthy human by pre-column derivatization with *o*-phthaldialdehyde and separation on a phenyl silica column by using a similar chromatographic system described in [4]. Note that the sensitivity of the fluorescence detector was increased after 10 min.

Pettersson et al. [3] have reported ADMA and SDMA mean plasma levels in healthy humans of 0.58 and 0.56 μ*M*, respectively, with a mean ADMA to SDMA ratio of 1.04 (range 0.8 to 1.4). In patients with end stage renal failure ADMA and SDMA have been measured in plasma at elevated mean levels of 1.25 and 3.1 μ*M*, respectively. Interestingly, the mean ratio ADMA to SDMA in the patients was determined as 0.4 which is significantly lower than that in healthy humans. In 16 patients with end stage renal failure we also measured comparably elevated plasma concentrations (mean±S.D.) for ADMA (1.81±0.82 μ*M* vs. 0.52±0.21 μ*M* in healthy volunteers) and SDMA (3.32±0.91 μ*M* vs. 0.55±0.23 μ*M*), and an ADMA to SDMA ratio of 0.54±0.17 vs. 0.95±0.19. It has been suggested that the decreased ADMA to SDMA ratio in the patients is due to a higher susceptibility of SDMA to renal excretory function while ADMA undergoes metabolic clearance by N^G,N^G-dimethyl-L-arginine dimethylaminohydrolase [3].

Unlike SDMA, ADMA, a potent inhibitor of the synthesis of the endogenous regulator of vascular tone NO, is most probably responsible for renal hypertension [2]. Increase of SDMA and ADMA plasma levels in favor of SDMA, and differences in metabolism and susceptibility to renal excretion underline the absolute necessity of satisfactory chromatographic separation of ADMA from SDMA prior to detection. On the basis of currently available data, accurate and rapid quantitation of ADMA, SDMA and of their precursor L-arginine is best carried out by a combination of solid-phase extraction from plasma using ion-exchangers and HPLC separation of the OPA-derivatives on phenylsilica columns [4].

With respect to the nomenclature used by Pettersson et al. [3] we point out that the term enantiomer is incorrect for the stereoisomeric SDMA and ADMA.

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