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The separation and analysis of symmetric and asymmetric dimethylarginine and other hydrophilic isobaric compounds using aqueous normal phase chromatography

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ABSTRACT

Two biologically important compounds with clinical relevance, asymmetric dimethylarginine and symmetric dimethylarginine, are analyzed using aqueous normal phase chromatography on silica hydride-based columns. Two different stationary phases were tested, a commercially available Diamond Hydride™ and a 2-acrylamido-2-methylpropane sulfonic acid experimental column. Two types of analytical protocols were investigated: analysis of the compounds when separation was achieved and analysis of the compounds with partial chromatographic separation. Urine samples from tuberculosis patients were tested for levels of asymmetric and symmetric dimethylarginine. The mass spectrometric technique of in-source fragmentation that can provide data similar to a tandem mass analyzer was evaluated as a means of identification and quantitation of the two compounds when complete separation is not achieved. This same protocol was also evaluated for two other isobaric compounds, glucose-1 and glucose-6 phohsphate, and leucine and isoleucine.

1. Introduction

Both asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) plasma levels have been associated with various forms of renal and cardiac physiological conditions [1,2]. ADMA has been shown to be a potent inhibitor of the enzyme nitric oxide synthase (NOS) which regulates the conversion of arginine into the vasodilator nitric oxide [3–5]. ADMA levels in biological fluids have been shown to correlate closely with renal functions and could be used as a biomarker for certain diseases [6]. Also, it has been shown that above normal amounts of ADMA and SDMA in blood plasma can be used to predict the extent of detrimental effects from stroke [7]. Monitoring of ADMA has also been found to be of importance in acute critical conditions such as septic shock [8–10]. The extent of the clinical applications of ADMA and SDMA as well as other small polar physiological analytes makes the development of new approaches for their determination a topic of significant importance.

HPLC based methods have been the most successful to date for the analysis of ADMA and SDMA [11]. A number of approaches have been proposed in developing analytical schemes for these two compounds. Because of their polarity, in many cases the compounds are derivatized in order to be retained in reversed-phase and in some cases to also enhance detection. A very early method for the preparation of the two compounds utilized an ion-exchange column that required almost five hours for the separation to be completed [12]. Another early protocol described the analysis of underivatized ADMA/SDMA in plasma from renal failure patients that required considerable sample preparation and a 20 min analysis time using UV detection at 200 nm [13]. Typical examples of pre-column derivatization are methods for the determination of ADMA and SDMA in plasma samples that allow for reversed-phase retention and high sensitivity by fluorescence detection [14–17]. Another reversed-phase method with derivatization utilized MS/MS for detection and quantitation with the each compound having a daughter ion that was unique [18]. One other approach reported utilized hydrophilic liquid interaction chromatography (HILIC) to analyze underivatized ADMA and SDMA with MS/MS detection [19]. Some sensitivity that would have been gained at the high acetonitrile concentrations in the HILIC method may have been at least partially lost because the mobile phase con-

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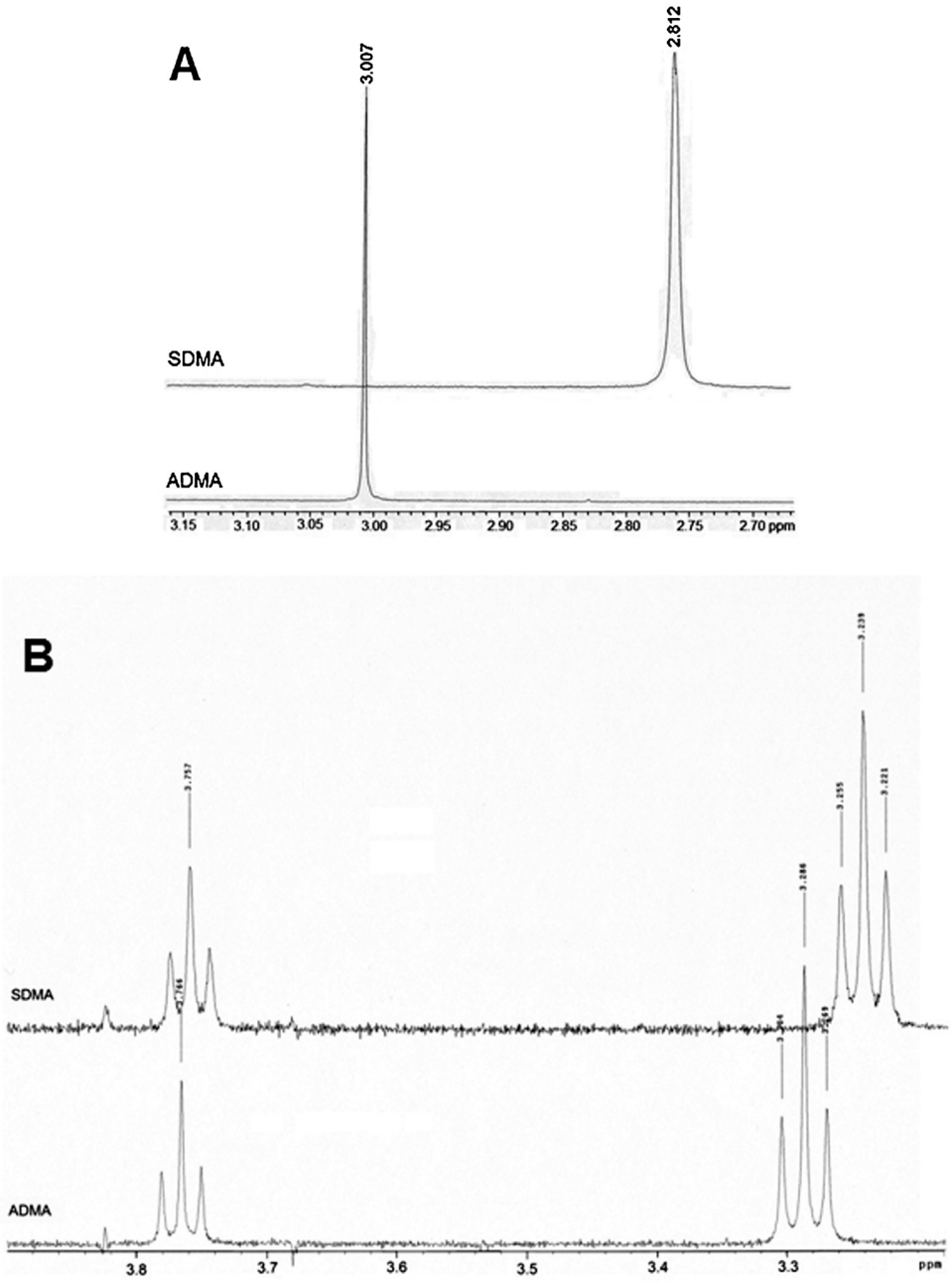

Fig. 1. Overlayed 400 Mz proton magnetic resonance spectra of ADMA and SDMA standards used for chromatographic evaluation. (A) methyl region and (B) methylene region.

tained 0.025% trifluoroacetic acid, a known suppressor of MS signal intensity.

Aqueous normal phase (ANP) chromatography utilizing silica hydride-based stationary phases is a versatile technique that can be applied to a broad range of analytical separations [20–22]. It versatility is based on the fact that all columns packed with silica hydride phases can operate in both the reversed-phase and normal-phase modes. There are a number of crucial differences between silica hydride and ordinary silica that lead to chromatographic advantages for the hydride material. These features include the dual retention capabilities cited above. In addition, the surface of silica hydride is slightly hydrophobic rather than highly polar due to silanols (as in HILIC phases) and thus often result in better peak shape for certain analytes, particularly bases. Other notable features of silica hydride include a very thin water shell (~0.5 monolayer) on the surface [23], rapid equilibration of the surface solvation layer after gradients and highly reproducible retention for polar compounds in the aqueous normal-phase mode. These properties have been used advantageously for the analysis of metabolites [24–28], nucleotides [29,30], pharmaceuticals [31–34], food and beverage components [35–39], lipophilic interferences [40] and chiral compounds [41].

Considering the proven capabilities of the silica hydride-based stationary phase for the analysis of hydrophilic compounds, the objective of this study was to determine if underivatized ADMA and SDMA could be better separated in a reasonable analysis time than in previous studies thus eliminating the need for extensive sample preparation or additional on-line procedures. In addition, the use of a newer and simpler mass spectrometric detection method, in-source fragmentation, for the detection of unique fragment ions