Analytica Chimica Acta 677 (2010) 169–175

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Simultaneous determination of guanidinosuccinic acid and guanidinoacetic acid in urine using high performance liquid chromatography/tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 2 March 2010
Received in revised form 4 August 2010
Accepted 8 August 2010
Available online 13 August 2010

Keywords:
Guanidinosuccinic acid
Guanidinoacetic acid
Creatinine
Arginine
Diabetes mellitus
Liquid chromatography/tandem mass spectrometry

ABSTRACT

We present a method for the simultaneous determination of guanidinosuccinic acid (GSA) and guanidinoacetic acid (GAA) from urine by protein precipitation and liquid chromatography/tandem mass spectrometry. The chromatographic separation was performed using a cation exchange column with an elution gradient of 0.1 mM and 20 mM ammonium acetate buffers. GSA was detected with the mass spectrometer in negative ion mode monitoring at *m/z* 174.1, and GAA, creatinine, arginine, and homoarginine were in positive ion mode monitoring at *m/z* 118.1, 114.1, 175.1, and 189.1, respectively. As an internal standard, L-arginine-$^{13}C_6$ hydrochloride and creatinine-d_3 (methyl-d_3) were used. The calibration ranges were 0.50–25.0 μg mL^{-1}, and good linearities were obtained for all compounds ($r > 0.999$). The intra- and inter-assay accuracies (expressed as recoveries) and precisions at three concentration levels (1.00, 5.00 and 25.0 μg mL^{-1}) were better than 83.8% and 7.41%, respectively. The analytical performance of the method was evaluated by determination of the compounds in urine from male C57BL/J Iar *db*/*db* diabetes mellitus (DM) mice. The values of GSA and GAA corrected by the ratios of the individual compounds to creatinine were significantly increased in DM mice compared with control mice. These results indicated that the newly developed method was useful for determining urinary guanidino compounds and metabolites of arginine.

1. Introduction

Guanidinosuccinic acid (GSA) and guanidinoacetic acid (GAA) are representative guanidino compounds (GCs) that are produced by the urea cycle and transamidination from arginine (Arg). The structures of GSA and GAA are shown in Fig. 1. The major pathway for GC synthesis was described in a previous report [1]. Certain GCs may be related to the pathological expression of uremic syndrome, cardiovascular disease and neurotoxins in the brain. The GSA concentration in the cerebrospinal fluid was reported to be higher in uremic patients than in control subjects [2]. In addition, the plasma GSA level was significantly higher in nephrectomized mice and the same tendency could be seen for most arginine metabolites (Args) [3]. The serum, urinary and renal cortex GAA levels were significantly decreased in diabetic rats. These observations suggest that high serum glucose levels may affect GAA synthesis in the renal cortex [4]. Another group reported that GSA injection led to significant dose-dependent effects on cognitive performance, activity and social exploratory behavior of mice [5]. Furthermore, the hippocampal volume was decreased after injection of GSA [5]. The authors suggested that nitrogen oxide (NO) and cyclic guanosine monophosphate (cGMP) production were involved in GSA neurotoxicity, and that the effects were caused via *N*-methyl-D-aspartate (NMDA) receptors. These observations may indicate that GSA and GAA contribute to the symptoms of patients with reduced renal functions. It is going to be suggested that the determination of GSA, GAA and Args in biological samples is important for elucidating the mechanism of renal dysfunction.

Analytical methods for the determination for GCs and Args have been developed using several techniques. GSA has been detected by the Sakaguchi reaction [6,7], colorimetric analysis and thin-layer chromatography [8,9]. However, these methods are unreliable

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doi:10.1016/j.aca.2010.08.005

Table 1
Analytical conditions of HPLC and MS systems for determining GSA, GAA, Cr, Arg and H-Arg.

HPLC system	NANOSPACE S1-2 (Shiseido)
Analytical column	CAPCELLPAKSCX UG80 (35 × 2 mm i.d., 5μm particle size)
Guard column	CAPCELLPA C18 MGII (10 × 2 mm i.d., 3μm particle size)
Mobile phase	Gradient A: 0.1 mM CH_3 $COONH_4$/MeOH = 90/10 (pH = 3) B: 20 mM CH_3 $COONH_4$/MeOH = 90/10 (pH = 3) Initial: B/A = 0/100, 0–4 min: 0/100, 4–5 min: 0/100 → 100/0, 5–11 min: 100/0, 11–11.1 min: 100/0 → 0/100, 11.1–13 min: 0/100
Flow rate	600 μL min^{-1}
Oven temperature	40 °C
Divert valve	0–1.3 min: waste, 1.3–11 min: detector, 11–13 min: waste
MS system	TSQ quantum ultra (Thermo Fisher Scientific)
Ionization	0–1.9 min: HESI (−), 1, 9–13 min: HESI (+)
Spray voltage	(−):2500 V, (+): 1000 V
Vaporizer temperature	380 °C
Sheath gas pressure	65 psi
Auxiliary gas pressure	20 psi
Capillary temperature	380 °C
Collision gas pressure	1.4 m Torr
Tube lens offset	GSA: 34, GAA: 55, Cr: 55, Arg: 54, H-Arg: 66, ISI: 54 and IS2: 55
Collision energy	GSA: 14 eV (*m*/*z* 174.1 > 132.0), GAA: 11 eV (*m*/*z* 118.1 > 101.1), Cr: 18 eV (*m*/*z* 114.1 > 44.3), Arg: 24 eV (*m*/*z* 175.1 > 70.1), H-Arg: 24 eV (*m*/*z* 189.1 > 84.1), IS1: 24 eV (*m*/*z* 181.1 > 74.1),ISI: 24 eV (*m*/*z* 117.1 > 47.3) and IS1: Arg-$^{13}C_6$, IS2:Cr-d_3

Table 2
Linearity and correlation coefficients of GSA, GAA, Cr, Arg and H-Arg.

	Calibration range	Equation[a]	Correlation coefficient
GSA	0.500–25.0 μg mL^{-1} (2.86–143 μmol L^{-1})	$y = 0.0117x - 0.0005$	$r = 0.999$
GAA	0.500–25.0 μg mL^{-1} (4.27–214 μmol L^{-1})	$y = 0.271x + 0.0137$	$r = 0.999$
Cr	0.500–25.0 μg mL^{-1} (4.42–221 μmol L^{-1})	$y = 0.776x - 0.0048$	$r = 0.999$
Arg	0.500–25.0 μg mL^{-1} (2.87–244 μmol L^{-1})	$y = 0.142x + 0.0002$	$r = 0.999$
H-Arg	0.500–25.0 μg mL^{-1} (2.66–133 μmol L^{-1})	$y = 0.087x + 0.0016$	$r = 0.999$

[a] x, analyte concentration (μmol L^{-1}); y, peak area ratio.

because of cross-reactions and unfit for trace analyses. From 1980 to 1989, high performance liquid chromatography (HPLC) methods were developed for determining GSA [10–12]. However, these methods require much time for the chromatographic separation and derivatization. On the other hand, GAA has been determined by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) since 1990 [13–19]. GC/MS is sensitive and selective, but requires much time for the sample preparation that involves solid-phase extraction and derivatization. In 2008, Carling et al. [20] reported the development of a rapid, sensitive and reliable method for GAA using LC/ESI-MS/MS. This method is useful for quantitative determination of GAA and Args, but has not been described for determination of GSA. Consequently, simultaneous methods for detecting GSA and GAA by LC/MS/MS have not been reported.

A kinetic study of GCs and Args was reported by Eloot et al. [21]. These compounds were found to be distributed in the plasma, urine, kidneys and cerebrospinal fluid and were taken up by the choroid plexus and distributed in the cerebrum [22–25]. Urine samples are useful biological samples for clinical diagnosis to detect patient diseases, and can be collected in a convenient, non-invasive and chronologic manner. The purpose of the present study was to

Table 3
Accuracy of determination method for GSA, GAA, Cr, Arg and H-Arg.

Added per sample (μg mL^{-1})		Intra-day (n=5)			Inter-day (n = 3)
		Day 1	Day 2	Day 3	
GSA	1.00	1.03 ± 0.05	1.00 ± 0.04	0.98 ± 0.07	1.00 ± 0.03
	5.00	5.25 ± 0.15	5.15 ± 0.10	5.20 ± 0.15	5.20 ± 0.05
	25.0	27.9 ± 1.05	25.1 ± 0.95	26.8 ± 0.20	27.2 ± 0.60
GAA	1.00	1.04 ± 0.05	1.01 ± 0.02	1.00 ± 0.04	1.00 ± 0.02
	5.00	5.00 ± 0.05	5.00 ± 0.10	4.90 ± 0.05	4.95 ± 0.05
	25.0	24.2 ± 0.95	21.9 ± 1.05	21.0 ± 0.70	22.4 ± 1.65
Cr	1.00	1.10 ± 0.20	1.10 ± 0.05	0.90 ± 0.25	1.05 ± 0.30
	5.00	4.70 ± 0.35	4.35 ± 0.15	4.30 ± 0.10	4.45 ± 0.45
	25.0	23.1 ± 0.40	24.9 ± 0.65	23.3 ± 0.45	23.8 ± 0.70
Arg	1.00	1.05 ± 0.05	0.95 ± 0.10	1.05 ± 0.05	1.02 ± 0.05
	5.00	5.20 ± 0.15	4.65 ± 0.05	4.70 ± 0.20	4.80 ± 0.35
	25.0	28.2 ± 0.90	25.2 ± 1.45	25.3 ± 0.55	26.2 ± 1.75
H-Arg	1.00	1.03 ± 0.03	1.03 ± 0.04	1.00 ± 0.03	1.00 ± 0.02
	5.00	5.15 ± 0.10	4.85 ± 0.15	4.85 ± 0.10	4.95 ± 0.15
	25.0	27.8 ± 1.50	25.7 ± 1.05	25.5 ± 0.50	26.3 ± 1.10