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Article

Gas Chromatographic Determination of Guanidino Compounds in Uremic Patients Using Glyoxal as Derivatizing Reagent

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The guanidino compounds guanidine, methylguanidine, guanidinoacetic acid, guanidinopropionic acid, guanidinobutyric acid and guanidinosuccinic acid were eluted and separated after pre-column derivatization with glyoxal from an HP-5 column (30 m × 0.32 mm i.d.) with film thickness 0.25 μm at an initial column temperature of 100°C for 2 min, with ramping of 20°C/min up to 250°C and a nitrogen flow rate of 3 mL/min. Detection was by flame ionization detection. Linear calibrations were observed within 0.1–20.0 μmol/L, with limit of detection within 0.024–0.034 μmol/L for each compound. The separation was repeatable with relative standard deviation (RSD) ($n = 6$) within 1.2–1.8 and 1.1–1.6% in terms of retention time and peak height/peak area, respectively. The method was applied for the determination of the guanidino compounds from serum of uremic patients ($n = 7$) and healthy volunteers ($n = 8$), and amounts were observed within 1.33–11.71 and 0.07–0.39 μmol/L with RSD 1.1–3.5 and 1.1–3.0%, respectively. The results were further supported by the standard addition method.

Introduction

The guanidino compounds, which are present in serum, are involved in the urea and guanidine cycle (1). Many have the ability to interact with reactive species and are indicated as uremic toxins. The concentration of many guanidino compounds increases in the serum, urine and cerebrospinal fluids of nondialyzed and homodialyzed uremic patients (3–4). Methylguanidine (MG) is indicated to be related to uremic polyneuropathy found in uremia (5). Guanidinosuccinic acid (GSA) is related to uremic bleeding diathesis and could contribute to uremic encephalopathy (6). GSA, MG and guanidine (G) are considered to be responsible for generalized seizures after intracerebroventricular administration in mice (7–8). The guanidino compounds are small, water-soluble molecules like urea, but their kinetic behavior differs significantly on intradialysis from urea (9).

The determination of guanidino compounds at trace levels in biological fluids is of clinical importance. The guanidino compounds are small molecules, and derivatization with a suitable reagent is generally used for their sensitive detection. The analytical procedure for the determination of guanidino compounds is primarily based on high-performance liquid chromatography (HPLC) (10–17), gas chromatography (GC) (18–22) and capillary electrophoresis (CE) (23–24). The reagents for pre- and post-column derivatization with HPLC are primarily ninhydrin (16–17), benzoin (10–12), furoin (17), anisoin (14), 9, 10-phenanthrenequinone (25), 9, 10-phenanthrenequinone-3-sulphonate (26) and 2,2'-pyridoin (27).

GC potentially very useful for the separation of volatile organic compounds, because it uses a short analysis time and involves less expensive equipment with lower running cost. The guanidino compounds have been converted to volatile products by the reaction with hexafluoroacetylacetone (18) or hexafluroacetylacetone and trimethylsilyltrifluoroacetamide (19, 21), trifluoroacetic anhydride (20) or pentafluorobenzyl bromide (22). However, GC determination has only been performed for a limited number of guanidino compounds (two compounds). There is a need to develop a simple GC procedure that involves an inexpensive derivatizing reagent with short derivatization and elution times and with adequate sensitivity applicable to the biological fluids.

Methylglyoxal and glyoxal are known to be present in the biological fluids of uremic patients and are reported to react with the guanidino group in proteins (28–32). Methylglyoxal is reported to bind and reversibly modifies arginine residues (33–36). The reactions are reported to occur at physiological concentrations of methylglyoxal (32). The present work examines the reaction of glyoxal as derivatizing reagent for the possible GC elution and separation of guanidino compounds; G, MG, guanidinoacetic acid (GAA), guanidinopropionic acid (GPA), guanidinobutyric acid (GBA) and GSA. Reaction and GC operating conditions are optimized for the quantitative determination of the guanidino compounds from the serum of uremic patients and healthy volunteers.

Experimental

Chemicals and solutions

The compounds G, MG, GAA, GPA (Sigma, St. Louis, MO), GBA (Sigma, Switzerland), GSA (Sigma GmbH, Germany), glyoxal (40%) (Fluka, Switzerland) and methanol (RDH, Germany) were used. Guaranteed reagent grade hydrochloric acid (37%), potassium chloride, acetic acid, sodium acetate, ammonium acetate, sodium tetraborate, boric acid, sodium bicarbonate, sodium carbonate, ammonium chloride and ammonia solution were from E-Merck (Germany).

Stock solutions of guanidino compounds containing 1000 μmol/L were prepared in water. Further solutions were prepared by appropriate dilution. The derivatizing reagent glyoxal (2%) was prepared in methanol. Buffer solutions (0.1M) between pH 1 and10 at unit intervals were prepared as follows:

Table I
Quantitative Data of Guanidino Compounds by GC using glyoxal as derivatizing reagent

Compound	Calibration range (μmol/L)	LOD (μmol/L)	LOQ (μmol/L)	Coefficient of Determination (r^2)	Regression Equation using serum as matrix (± SD)
G	0.1-20	0.034	0.102	0.9989	Y = 3.17(±0.22)x-0.39 (±0.03)
MG	0.1-20	0.028	0.084	0.9985	Y = 2.34(±0.27)x-0.17 (±2.24)
GAA	0.1-20	0.026	0.078	0.9981	Y = 3.25(±0.04)x-0.65 (±0.35)
GBA	0.1-20	0.024	0.072	0.9923	Y = 1.99(±0.36)x + 0.83 (±3.04)
GPA	0.1-20	0.026	0.078	0.9984	Y = 1.21(±0.63)x-0.08 (±5.35)
GSA	0.1-20	0.034	0.102	0.9957	Y = 2.52(±0.19)x + 0.95 (±1.57)

pH 1 and 2 were prepared directly from HCl (0.1M) by dilution with KCl (0.1M), acetic acid and sodium acetate (pH 3–6), ammonium acetate (pH 7), boric acid and sodium tetraborate (pH 7.5–8.5), sodium bicarbonate and sodium carbonate (pH 9) and ammonium chloride and ammonia (pH 10).

Equipment

The pH measurements were made with an Orion 420A pH meter (Orion Research, Inc., Boston, MA) with combined glass electrode and reference internal electrode. The spectrophotometric study was carried out with a double beam Hitachi 220 spectrophotometer (Hitachi, Ltd., Tokyo, Japan) with dual 1-cm silica cuvettes. Fourier transform infrared spectroscopy (FTIR) spectrum of the internal standard was recorded on Nicolet Avatar 330 FT-IR (Thermo Nicolet Electron Corporation, USA) with attenuated total reflectance (ATR) accessory (Smart Partner) within 4000–600 cm^{-1}. GC-flame ionization detection (FID) studies were carried out on an Agilent model 6890 network GC system interfaced with FID and split injector (Agilent Technologies, Sanata Clara, CA); hydrogen generator (Parker Balston, Analytical Gas System, H2-90, Parker Hannifin, Havorhill, MA), pure nitrogen (99.9%) (British Oxygen Company, Karachi) and air for FID were from Fountain Air Compressor (Fountain Hills, AZ) connected with a molecular sieve. A computer with Chemstation software controlled the gas chromatograph. An HP-5 capillary column (30 m × 0.32 mm i.d.) with film thickness of 0.25 μm (J & W Scientific GC columns, USA) was used throughout the study.

Analytical procedure

To the solution (0.2–1.0 mL) containing an appropriate concentration of guanidino compounds was added sodium tetraborate buffer pH 7.5 (0.2 mL) glyoxal (2% in methanol *v*/*v*) (0.2 mL) and 2,3-dimethyl-5,6-diphennyl-5,6-dihydropyrazine (0.2 mL, 0.3 μmol/L) as internal standard. The contents were warmed in a water bath at 80°C for 20 min and allowed to cool at room temperature (5 min). Chloroform (1 mL) was added and contents were mixed well. The layers were allowed to separate and an aliquot of the organic layer was transferred to a septum-capped sample vial. The sample (1 μL) was analyzed on the HP-5 column using the following temperature program: 100°C for 2 min followed by a heating rate of 20°C/min up to 250°C with a total run time of 10.5 min. Nitrogen flow rate was 3 mL/min with split ratio 10:1. The injector and detector temperatures were fixed at 270°C. Flow rates for FID were fixed, hydrogen 40 mL/min, nitrogen 45 mL/min and air 450 mL/min.

Analysis of guanidino compounds from serum

A blood sample (10 mL) collected from healthy volunteers was kept at room temperature (30°C) for 1 h and centrifuged at 3000 g for 30 min. The supernatant layer of the serum was separated and twice the volume of methanol was added (10 mL). The contents were mixed well and again centrifuged at 3000 g for 20 min. The supernatant layer was collected and total serum was treated as described previously, but the addition of the derivatizing reagent glyoxal (2% *v*/*v* in methanol) was increased to 2 mL and the addition of internal standard was omitted. The derivatized guanidino compounds were extracted in chloroform (4 mL). The layers were separated with a separating funnel and the aqueous layer was collected. Ten aliquots (0.5 mL each) of serum from aqueous phase was added ($n = 10$) to calibrators for all six guanidino compounds within linear calibration range (Table I) and 0.2 mL (2.0 μmol/L) internal standard (2,3-dimethyl-5,6-diphenyl-5,6-dihydropyrazine). Each solution was further processed as described previously. Calibration curves were recorded from ratio of average peak height ($n = 4$) of analyte and internal standard against the concentration of guanidino compounds.

The blood samples (5 mL) collected from healthy volunteers and uremic patients were kept at room temperature (30°C) for 1 h and centrifuged at 3000 g for 30 min. The supernatant layer of the serum (2.5 mL) was separated and then added to 5.0 mL of methanol. The contents were mixed well and again centrifuged at 3000 g for 30 min. The supernatant layer was collected and 0.5 mL of solution was transferred to a screw-capped sample vial. The solution was added to 0.2 mL (2.0 μmol/L) of internal standard and the previously described analytical procecure was followed. Quantitation was carried out from the linear regression equation Y = ax + b prepared from the calibration curve using internal standard.

Analysis of guanidino compounds from serum using linear calibration with spiked samples

A blood sample (5 mL) from a uremic patient and a healthy volunteer was treated as described in the preceding section. Two portions of 0.5 mL of serum were taken from each after deproteinization with methanol. To each portion was added a solution of a mixture of guanidino compounds (0.5 mL, 5 μmol/L each) and both the solutions were processed as described previously. The quantitation was carried out by calibration curve from the increase in response with added standards.

Blood samples of uremic patients with verbal consent were collected by vein puncture in sterilized EDTA tubes from the medical wards at Liaquat University of Medicine and Health Sciences Hospital (Jamshoro and Hyderabad, Pakistan). The

blood samples of healthy volunteers who had not been taking any medicine for at least one precedent week were collected from Institute of Advanced Research Studies in Chemical Sciences, University of Sindh (Jamshoro, Pakistan). The samples were collected with verbal consent. The uremic patients and healthy volunteers were informed about the objectives of the project. The collected samples were quickly analyzed for guanidino compounds as they were received. The internal standard, 2,3-dimethyl-5.6-diphenyl-5,6-dihydropyrazine, was prepared by heating together an equimolar (0.002M) solution of dimethylglyoxal and meso-stilbenediamine in methanol. The precipitates obtained were recrystallized from methanol; M.P = 105°C. The FTIR-indicated primary peaks in cm^{-1} were found at 1611 (s), 1570 (w), 1500 (m), 1341 (w), 1305 (w), 1266 (w), 1232 (m), 1160 (m), 840 (m), 755 (s) and 706 (w).

Results and Discussion

The guanidino compounds react with glyoxal to form cyclic imidazolone compounds as reported for methylglyoxal (32) (Figure 1). GC of the guanidino compounds was examined before and after derivatization with glyoxal. Guanidino compounds without derivatization did not elute from the HP-5GC column, but eluted after derivatization. Each compound eluted as a single peak. Therefore, reaction conditions and GC elution and separation of the guanidino compounds were optimized. The effect of pH, amount of derivatizing reagent added per analysis, effect of time and temperature and the order of addition of the reagents on derivatization was examined. The effects of

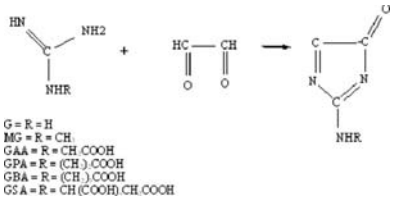

Figure 1. Schematic diagram of reaction of guanidino compounds with glyoxal.

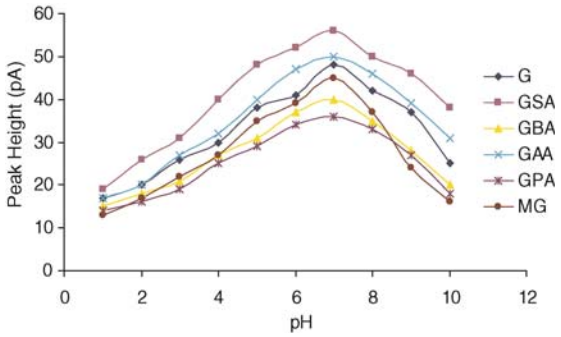

Figure 2. Effect of pH on GC response (average peak height) ($n = 3$) of the guanidino compounds as derivatives of glyoxal from the HP-5 column (30 m × 0.32 mm i.d.) with film thickness of 0.25 μm at column temperature 100°C for 2 min, followed by heating rate 20°C/min up to 250°C for 2 min; nitrogen flow rate was fixed at 3 mL/min with split ratio 10:1, and the injector and detector temperatures were fixed at 270°C.

pH was examined between 1 and 10 with unit intervals and then at 0.5 unit. The maximum response [average peak height ($n = 3$)] was observed within pH 6–8, and pH 7.5 was selected (Figure 2). The volume of buffer added per analysis was varied from 0.2 to 1.0 mL at an interval of 0.2 mL, and change in the volume of added buffer did not have any effect on the response, so volume of the added buffer was fixed at 0.2 mL. The percentage of derivatizing reagent and volume added were varied from 1 to 5% in methanol at an interval of 1% and 0.1–1.0 mL at an interval of 0.1 mL, respectively. The addition of the derivatizing reagent was not critical as long as excess of the reagent was present during derivatization. The addition of 0.2 mL of 2% v/v of derivatizing reagent was considered to be optimum. Warming time and temperature were varied from 10–50 min at an interval of 10 min and 40–100°C at an interval of 10°C, and the optimal response was observed by warming at 80°C for 20 min, so this temperature and time were selected. The order of addition of analyte, buffer and derivatizing reagent was varied and no effect was observed on average response [peak height ($n = 3$)]. Chloroform, tertiary butanol, 1, 2-dichloroethane and ethyl acetate were examined as solvents for the extraction of the derivatives. Chloroform indicated better extraction and was selected for the study.

The conditions for GC elution and separation of the guanidino compounds from the HP-5 column were examined. Different temperature elution programs and nitrogen flow rates were investigated with initial temperature 80–100°C for 1–2 min, followed by heating rate 10–25°C/min up to 200–250°C with nitrogen flow rate 1.0–4.0 mL/min. The elution program was selected that gave the best sensitivity, with complete separation between derivatizing reagent, six guanidino compounds and internal standard within 10 min had an initial temperature of 100°C for 2 min followed by a heating rate of 20°C/min up to 250°C for 2 min with a nitrogen flow rate of 3 mL/min . The order of elution for the derivatives of the compounds was (1) derivatizing reagent, (2) G, (3) MG, (4) GAA, (5) GPA, (6) GBA, Internal standard 2,3-dimethyl-5,6-diphenyl-5,6-dihydropyrazine and (7) GSA with capacity factor (k') 2.4, 3.5, 4.4, 5.1, 5.9 6.6 and 6.8, respectively (Figure 3). The order of elution followed the natural process, with retention time increasing with increase in the molecular mass within the homologous series of guanidino compounds.

Repeatability of the derivatization and GC elution was examined in terms of retention time and peak height ($n = 5$). The relative standard deviations (RSDs) were obtained within 1.2–1.8 and 1.1–1.6%, respectively.

Validation of the quantitative determination

The linear calibration curves were constructed by recording average ratio of peak height ($n = 5$) of guanidino compound and internal standard versus concentration at the optimized operating conditions. The linear calibrations were observed within 0.1–20.0 μmol/L with coefficient of determination (r^2) with 10 calibrators within 0.9923–0.9989. The limits of detection (LODs) measured as signal-to-noise ratio (S/N) 3:1 were calculated within 0.024–0.034 μmol/L. The limits of quantitation (LOQs) were calculated as S/N 10:1 within 0.072–0.102 μmol/L (Table I). The reproducibility in terms of retention time and peak height was examined for all six guandino

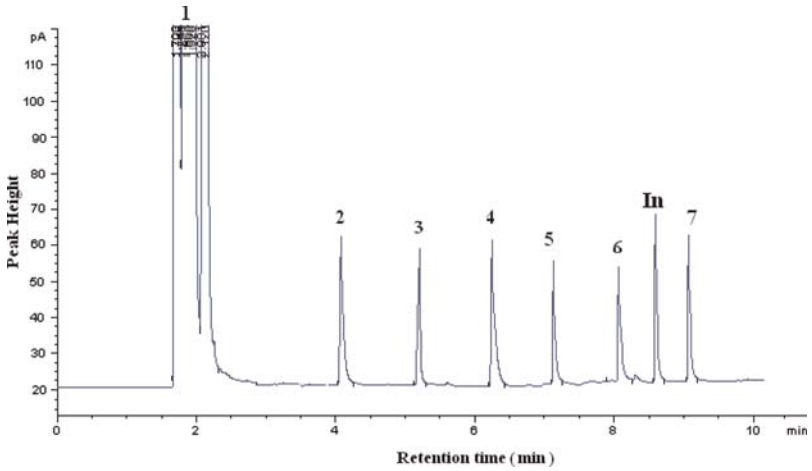

Figure 3. Separation of guanidino compounds and internal standard: (1) derivatizing reagent, (2) G, (3) MG, (4) GAA, (5) GPA, (6) GBA, (In) internal standard (2,3-dimethyl-5,6-diphenyl-5,6-dihydropyrazine) and (7) GSA; separation conditions are the same as described in Figure 2.

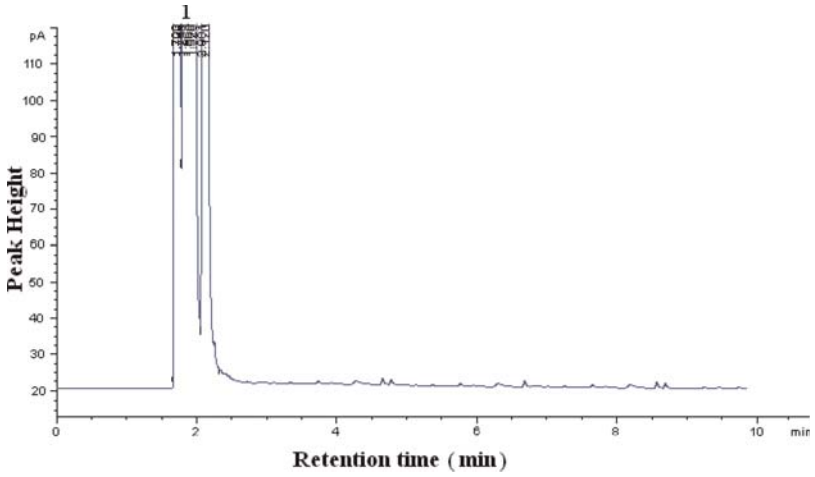

Figure 4. Chromatogram of blank from the blood serum of a uremic patient, following the analytical procedure, but without the addition of derivatizing reagent glyoxal.

compounds at inter-day ($n = 4$) and intra-day ($n = 4$) variations at three different concentrations 0.5, 4.0 and 15.0 μmol/L. The inter-day and intra-day changes in peak heights were observed with RSD 1.6–2.3 and 1.1–2.0%, and corresponding RSDs in retention times were 1.4–2.1 and 2.0–2.7%, respectively.

Effect of additives

The interfering effect of some amino acids and additives was examined during the determination of the guanidino compounds. The compounds glycine, serine, alanine, phenylalanine, glutamic acid, aspartic acid, ascorbic acid, glucose and fructose were added at twice the concentration of MG and their effect on separation, retention time and peak height ($n = 4$) was examined. The responses were compared with standard solutions of the guanidino compound derivatives. The presence of amino acids and additives did not affect the determination and the relative error was observed within $\pm 2.9\%$. Four different test solutions covering the calibration range of G, MG, GAA, GPA, GBA and GSA were prepared and analyzed following analytical procedure and relative error was observed within $\pm 2.4\%$.

Analysis of serum

To examine the effect of matrix (serum) on the extraction and recovery of guanidino compounds, the serum of a healthy volunteer after deproteinization was treated with derivatizing reagent glyoxal following the analytical procedure to remove the naturally occurring guanidino compounds from serum. To 10 aliquots (0.5 mL each) from the serum remaining in aqueous phase were added different calibrators ($n = 10$) of all six guanidino compounds and the internal standard. The analysis of the solutions was carried out following the analytical procedure. Linear calibration curves were constructed by recording the ratio of average peak height ($n = 4$) of analyte and internal standard against the concentration of guanidino compounds. The r^2 values for G, MG, GAA, GPA, GBA and GSA were 0.9989, 0.9985, 0.9981, 0.9984, 0.9923 and 0.9957,

respectively. The linear regression equations calculated using internal standard are indicated in Table I with standard deviations for slope and intercept.

Calibrations were examined for the analysis of the guanidino compounds from the serum of uremic patients and healthy volunteers. Post-deproteinized serum was analyzed for the contents of G, MG, GAA, GPA, GBA and GSA. Glyoxal is also

Table II
The Analysis of Serum of Healthy Volunteers for the Guanidino Compounds Using Glyoxal as Derivatizing Reagent. The Concentrations are μmol/L (%RSD)

S.No	Age/Sex	G	GBA	GAA	GPA	GSA
1	19M	0.14 (2.8)	0.11 (3.0)	0.29 (1.2)	0.15 (1.8)	0.30 (2.8)
2	24M	0.14 (2.8)	0.09 (2.9)	0.27 (1.4)	0.14 (2.4)	0.32 (2.8)
3	26F	0.17 (1.9)	0.14 (1.8)	0.30 (2.4)	0.09 (2.4)	0.30 (1.4)
4	24F	0.18 (1.8)	0.13 (1.4)	0.36 (2.3)	0.07 (2.8)	0.38 (1.4)
5	30M	0.14 (2.8)	0.15 (2.4)	0.29 (2.8)	0.16 (2.8)	0.29 (2.4)
6	22F	0.16 (2.1)	0.09 (1.7)	0.28 (2.9)	0.14 (2.1)	0.28 (2.3)
7	21F	0.19 (1.8)	0.14 (1.8)	0.26 (2.1)	0.15 (1.8)	0.31 (1.8)
8	20F	0.12 (1.9)	0.15 (1.9)	0.25 (2.9)	0.13 (1.9)	0.39 (1.9)
9	25M	0.21 (1.5)	0.12 (2.0)	0.34 (2.3)	0.15 (2.7)	0.33 (2.1)
9*		0.24 (1.4)	0.11 (1.6)	0.30 (2.0)	0.11 (2.1)	0.32 (2.3)

* Spiked Sample, M = male, F = female.

Table III
The Analysis of Serum of Uremic Patients for the Guanidino Compounds Using Glyoxal as Derivatizing Reagent. The Concentrations are μmol/L (%RSD)

S.No	Age/ Sex	G	MG	GAA	GBA	GPA	GSA
1	52M	3.61 (1.4)	1.44 (1.9)	5.03 (3.1)	1.77 (1.3)	1.34 (1.4)	11.20 (1.3)
2	55M	2.98 (1.8)	1.36 (1.8)	4.99 (2.0)	1.52 (1.3)	1.43 (2.9)	10.05 (2.6)
3	60F	3.41 (2.4)	1.91 (2.9)	4.96 (2.4)	1.60 (1.4)	1.33 (2.6)	9.63 (2.1)
4	53F	2.99 (2.3)	1.69 (2.9)	4.89 (2.9)	1.53 (2.9)	1.36 (2.4)	8.45 (2.8)
5	47M	2.99 (1.9)	1.82 (2.3)	6.30 (1.9)	1.44 (2.4)	1.56 (2.9)	8.64 (3.0)
6	51F	2.89 (2.3)	1.68 (2.1)	4.56 (1.6)	1.58 (2.9)	1.45 (2.3)	11.71 (3.1)
7	54F	2.79 (2.9)	1.88 (1.2)	5.33 (1.6)	1.63 (1.8)	1.43 (2.4)	8.36 (2.8)
8	56 M	3.09 (2.0)	1.79 (1.5)	5.65 (2.6)	1.67 (2.8)	1.50 (1.5)	9.87 (2.7)
8*		3.29 (1.3)	1.91 (1.8)	6.01 (2.4)	1.90 (2.7)	1.31 (2.1)	10.11 (2.5)

* Spiked Sample, M = male, F = female

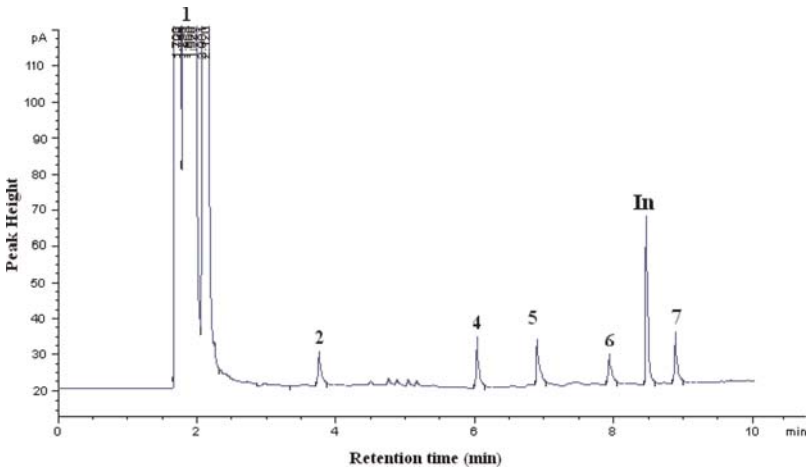

Figure 5. GC separation of guanidino compounds in serum of healthy volunteers: (1) derivatizing reagent, (2) G, (4) GAA, (5) GPA, (6) GBA, (In) internal standard and (7) GSA; separation conditions are the same as described in Figure 2.

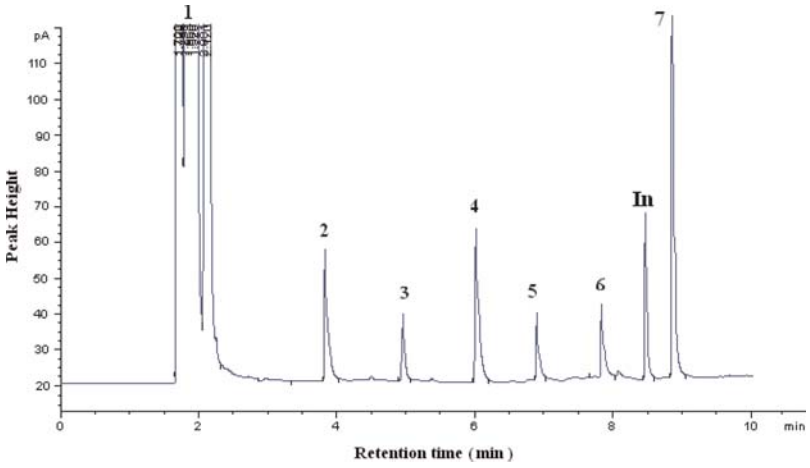

Figure 6. GC separation of guanidino compounds in serum of uremic patients: (1) derivatizing reagent, (2) G, (3) MG, (4) GAA, (5) GPA, (6) GBA (In) internal standard and (7) GSA; separation conditions are the same as described in Fig 2.

Table IV
Comparative data for Analytical Procedures for the Determination of Guanidino Compounds.

SNo	Analytical Method	Derivatizing reagent	Calibration range	Optimal reaction time of derivatization	Limit of detection	Retention time	Detection device	Ref
1	MEKC	Benzoin	0.057-14.11 μM/L	5 min	0.019-0.03 μM/L	6 min for 7 guanidino compounds	Photo diode array detection	24
2	HPLC	Anisoin	0.45-1310.8 μM/L	5 min	2-155 fM	40 min with gradient elution for 9 guanidino compounds	Fluorescence	14
3	GC-MS	Hexafluoroacetylacetone and pentafluorobenzylbromide	5-100 n mol	2.25 hr	10 nM/L	6.5 min for 1 guanidino compound (GAA)	GC-MS	22
4	GC-MS	Hexafluoroacetylacetone and Monotrimethylsilyltrifluoroacetamide	38-7325 μM/L	2 hr	1.22-1.54 μM/L	5 min for 2 guanidino compounds (GAA & CRT)	GC-MS	19
5	GC	Glyoxal	0.06-7.5 μM/L	20 min	0.024-0.034 μM/L	10 min	FID	Present method

reported to be present in blood serum (37, 38); therefore, a blank determination without addition of derivatizing reagent was simultaneously performed, but the elution of the guanidino compounds was not detected (Figure 4). The identification of the analytes was carried out by comparing chromatographic retention time (tR) with those of standard guanidino compounds and spiking each of the compounds in sequence. The average amounts ($n = 3$) from nine healthy volunteers within the ages of 19 and30 years indicated G within 0.12–0.21 μmol/L, GAA within 0.25–0.36 μmol/L, GPA within 0.07–0.16 μmol/L, GBA within 0.09–0.15 μmol/L and GSA within 0.28–0.39 μmol/L with RSDs within 1.1–3.0% (Table II; Figure 5). Similarly, the analysis of the serum of eight uremic patients within the ages of 47 and60 years indicated concentrations (μmol/L) of G within 2.79–3.61, MG within 1.36–1.91, GAA within 4.56–6.30, GPA within 1.33–1.56, GBA within 1.44–1.77 and GSA within 8.36–11.71 with RSDs within 1.0–3.5% (Table III; Figure 6). A serum sample of a uremic patient and a healthy volunteer was spiked with 5 μmol/L of guanidino compounds. Increases in the responses (peak height/peak area) were observed without change in the peak shape, and the results agreed with the observed results by calibration, with average percent recovery within 73.3–114% ($n = 3$) with RSDs within 1.9–2.7%, respectively (Tables II and III).

The average response ($n = 4$) of the guanidino compounds standards obtained from serum was compared with that of aqueous solutions, and the recovery from the serum was calculated to 96, 97, 95, 95, 96 and 97% for G, MG, GAA, GPA, GBA and GSA with RSD 1.6, 2.7, 2.5, 1.5, 2.6 and 2.7%, respectively.

The amounts observed for guanidino compounds from serum agreed with the reported values for uremic patients (17, 39).

The results of the present study are compared with reported Micellar electrokinetic chromatography (MEKC), HPLC and GC methods (Table IV). The results with reported GC procedures (18–22) required warming at 80–100°C for 2–3 h with derivatizing reagent hexafluoroacetylacetone following a second derivatization with trimethylsilyltrifluoroacetamide (19, 21) or pentafluorobenzyl bromide (22) for the determination of one to two guanidino compounds, but the present method indicated an optimal response with warming time of 20 min at 80°C for the analysis of six guanidino compounds. A previously reported HPLC procedure (14) using anisoin as derivatizing reagent is based on the separation of nine guanidino compounds by gradient elution within 40 min using spectrofluorimetric detection. The present method reports the separation and determination of six guanidino compounds within 10 min. The use of a simple derivatizing reagent is an added advantage of the present procedure.

Conclusion

This study work reports a new GC procedure for guanidino compounds using glyoxal as derivatizing reagent. The separation was repeatable and indicated adequate sensitivity for the determination of six guanidino compounds from the deproteinized serum of uremic patients.

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