



Guanidinoacetic acid (GAA) and Creatine (Cr) GC-MS Analysis Kit

Guanidinoacetic acid (GAA) is an important metabolite formed from glycine and arginine in the first step of the creatine synthesis pathway. Creatine (Cr) and phosphocreatine play an important role in the storage and transmission of energy in various tissues. Two enzymes are involved in Cr biosynthesis: arginine glycine amidinotransferase (AGAT, EC 2.1.4.1), which converts arginine and glycine to ornithine and guanidinoacetate (GAA), and S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase (GAMT, EC 2.1.1.2) and converts GAA to Cr. Cr is then transported to tissues via creatine transporters (SLC6A8), where it is converted non-enzymatically to creatinine. Creatine deficiency syndrome (CDS) consists of three inborn errors of metabolism: AGAT deficiency (OMIM 602360), GAMT deficiency (OMIM 601240) and SLC6A8 deficiency (OMIM 3000036).

Diagnosis is possible by measuring guanidinoacetate, creatine in plasma and urine. Patients with GAMT deficiency typically exhibit normal to low creatinine, very elevated GAA, and low Cr. Patients with AGAT deficiency typically exhibit normal to low creatinine, low GAA, and normal to low Cr. In comparison, elevated creatinine, normal GAA, normal to low Cr, and an elevated creatinine: creatine ratio characterizes patients with creatine transporter defect.

Highlights of the Analysis Kit



Consuming small volume of sample



Total run time is 14.85 min.



Long life span of GC column

Parameters

Guanidinoacetic acid and creatine

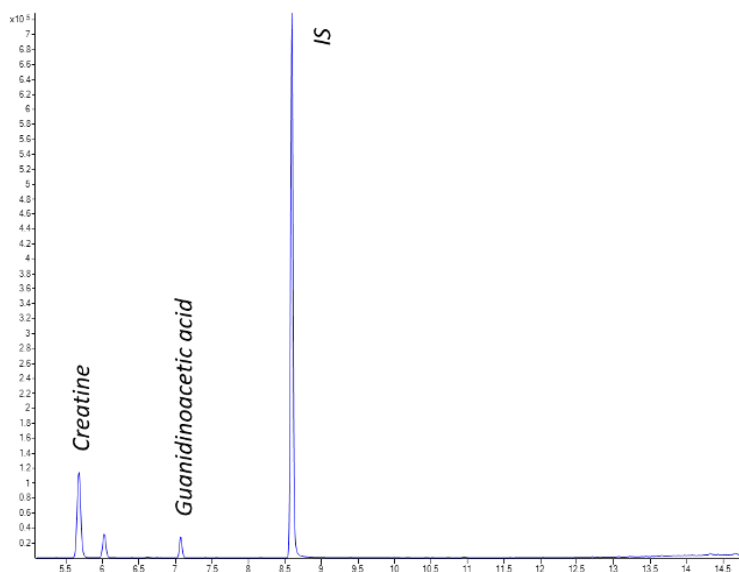
Sample Type

Serum, Urine

Sample Preparation

1	Take 100 µl serum and urine sample into screw cap glass tube.
2	Add 100 µl internal standard, 500 µl distilled water and 50 µl Reagent-1
3	Immediately close the cap of tubes and vortex for 10 sec.
4	Incubate 120 min. 70°C in the water bath or heater block
5	After cooling to room temperature, add 3 ml Reagent-2 and vortex for 10 sec.
6	Centrifuge at 4500 rpm 10 min.
7	2.5 ml of the upper phase is transferred to a new screw cap tube and evaporated under nitrogen stream.
8	The tube cap is closed by adding 50 µl Reagent-3 and 50 µl Reagent-4.
9	Incubate 30 min. 60°C in the water bath or heater block
10	Inject to GC system.

Example Chromatogram



Example chromatogram of guanidinoacetic acid and creatine

Method Performance

Serum/Plasma Validation Parameters

All results were obtained using Agilent 8890 GC-5977B MSD system

Analytes	LOQ (nmol/ml)	Linearity (nmol/ml)	Recovery		Repeatability			
			LLQC* (%)	HLQC** (%)	intra-day		inter-day	
					LLQC (%CV)	HLQC (%CV)	LLQC (%CV)	HLQC (%CV)
GAA	0.10	1.0 – 100.0	104	99	1.60	1.50	2.10	1.50
Cr	0.13	5.0 – 500.0	109	98	0.80	0.30	1.90	0.60

* LLQC: Low-level quality control

** HLQC: High-level quality control

Urine Validation Parameters

Analytes	LOQ (nmol/ml)	Linearity (nmol/ml)	Recovery		Repeatability			
			LLQC* (%)	HLQC** (%)	intra-day		inter-day	
					LLQC (%CV)	HLQC (%CV)	LLQC (%CV)	HLQC (%CV)
GAA	1.58	12.5 – 2500.0	95	95	1.80	0.20	2.00	0.30
Cr	2.56	25.0 – 5000.0	110	92	1.40	0.50	1.70	1.40

* LLQC: Low-level quality control

** HLQC: High-level quality control



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