

TABLE 565

CC AUTOMATED SEPARATION OF GUANIDINES

(D. J. DURZAN, *Can. J. Biochem.*, 47 (1969) 660)

Column:

Three chromatographic columns have been used to separate monosubstituted guanidines. Two of them are identical with those used for separation of amino acids in biological fluids (see J. V. BENSON AND J. A. PATTERSON, *Anal. Biochem.*, 13 (1965) 265). Column diameter 0.9 cm in all cases, length specified in the figures. Column 21.5 × 0.9 cm for basic derivatives.

Ion exchanger:

Beckman Custom Research Resin PA-28, 7.5% cross-linked with a spherical particle diameter of $16 \pm 6 \mu$. For the separation of basic guanidine compounds the PA-35 resin nominally 7.5% cross-linked was used. Spherical particle diameter was $13 \pm 6 \mu$ in this case.

Buffers:

Composition indicated in the table.

Operating conditions:

Buffers were applied at a rate of 50 ml/h to the ion-exchange column containing the sample to be analysed. Column temperature was regulated as indicated in the figures. Effluent from the column entered 0.075 in. diameter tubing via the proportioning pump (Technicon) and was mixed with air at a rate 48 ml/h together with Sakaguchi's reagent A. Flow-rate 2 ml/h. After mixing in a glass coil, Sakaguchi's reagent B was introduced in the stream via 0.056 in. diameter Solvaflex tubing at a rate of 64 ml/h. Before entering a second jacketed mixing coil the mixture was cooled to 5°. Colours with monosubstituted guanidines developed immediately and absorbance at 495 nm was recorded.

Notes:

In a separate table the constants C_{HW} for multiplying the integrated peak areas of colour produced at 495 nm with Sakaguchi's reagent to convert areas to μ moles of monosubstituted guanidine are summarized.

Buffer composition	pH				
	2.2 ± 0.03	3.25 ± 0.005	4.25 ± 0.02	4.26 ± 0.02	5.28 ± 0.02
Sodium concentration (N)	0.20	0.20	0.20	0.38	0.35
Sodium citrate · 2H ₂ O (g)	19.6	78.43	78.43	149.0	137.26
Conc. HCl (ml)	16.5	49.3	33.5	60.9	26.0
Thiodiglycol (ml)	5	20.0	20.0	—	—
Brij 35 solution 50 g/100 ml (ml)	2	8	8	8	8
Pentachlorophenol (ml)	0.1	0.4	0.4	0.4	0.4
Final volume (l)	1	4	4	4	4

Column	C_{HW}
<i>Acidic and neutral column</i>	
Taurocyamine	7.55 ± 0.32
Lombricine	6.58 ± 0.43
α -Keto- δ -guanidinovaleric acid	4.14 ± 0.23
Guanidinosuccinic acid	11.27 ± 0.25
Octopine	7.14 ± 0.10
Homooctopine	6.27 ± 0.08
Guanidinoacetic acid	10.37 ± 0.47
α -Guanidinopropionic acid	8.95 ± 0.46
<i>Basic column</i>	
Guanidinoacetic acid	8.18 ± 0.90
α -Guanidinopropionic acid	7.03 ± 0.58
N-Acetyl-L-arginine	8.62 ± 0.40
L-Argininic acid	4.20 ± 0.01
β -Guanidinopropionic acid	21.70 ± 0.27
L-2-Amino-3-guanidinopropionic acid	3.24 ± 0.25
γ -Guanidinobutyric acid	8.74 ± 0.33
L-Arginine	21.61 ± 0.77
δ -Guanidinovaleric acid	10.18 ± 0.49
ϵ -Guanidinocaproic acid	12.52 ± 0.35

TABLE 565 (continued)

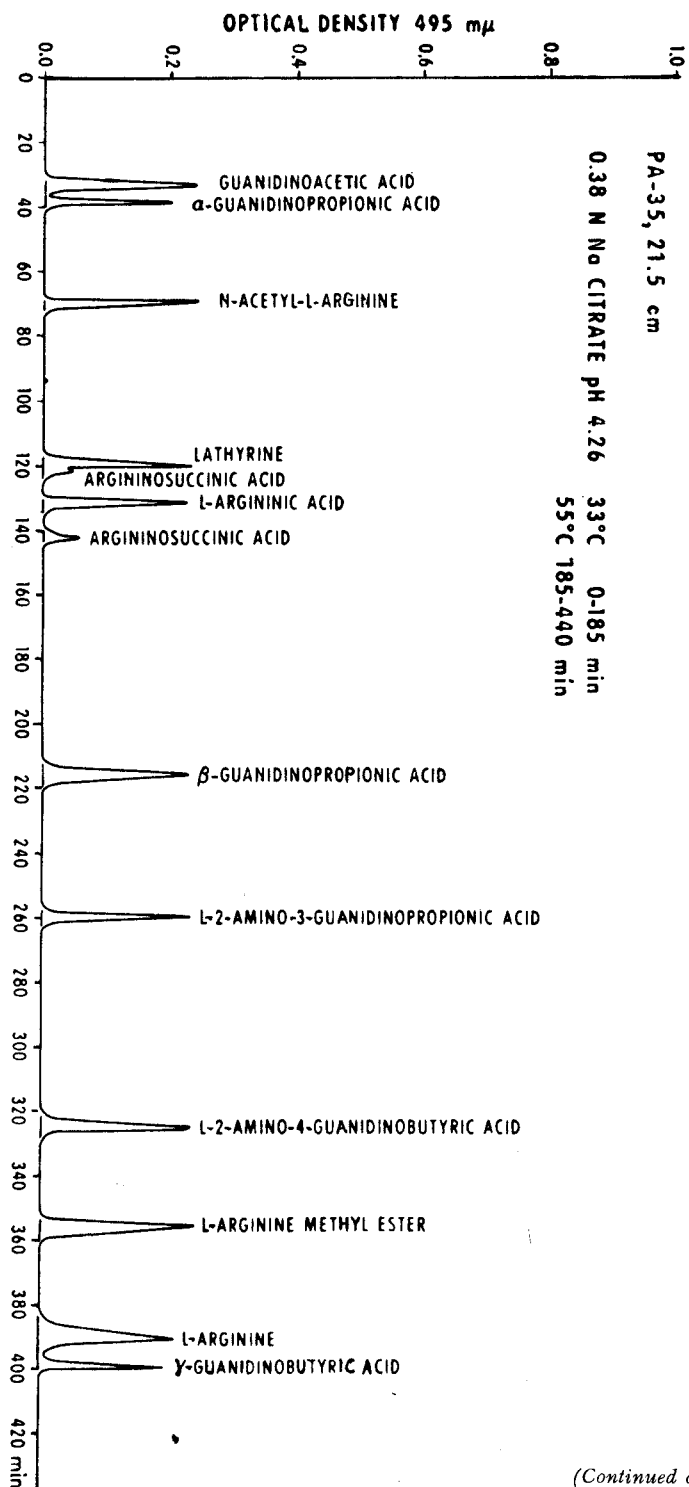


Fig. 1

(Continued on p. D148)

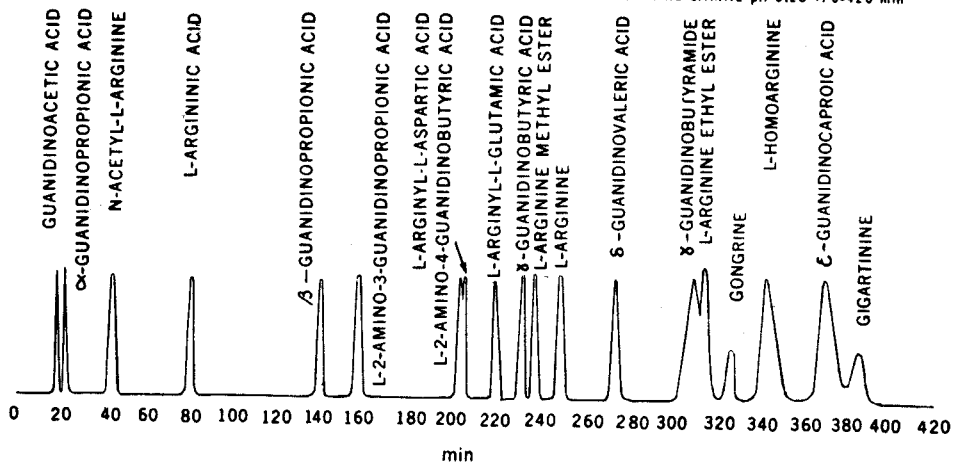


Fig. 2

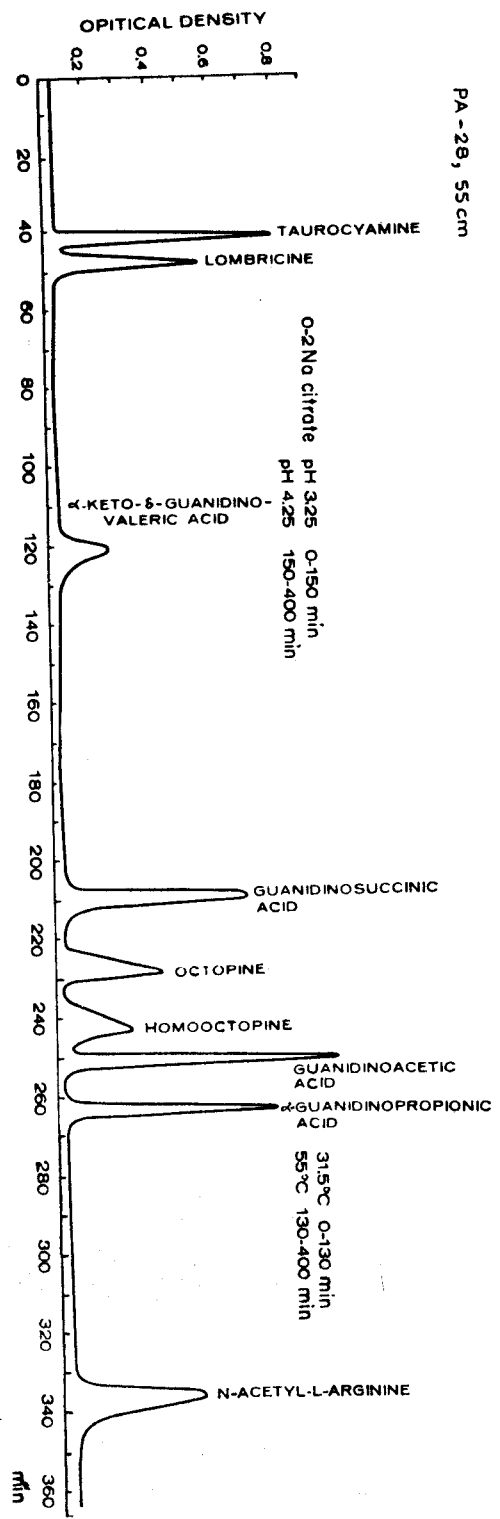


Fig. 3