

CHROM. 18 715

## Note

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### Rapid determination of arginine by high-performance liquid chromatography\*

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During research in this laboratory, a need arose for the determination of arginine in plasma from dairy cows. Since arginine is the last peak in amino acid analysis, a shortened procedure was developed.

#### METHOD

##### *Sample preparation*

Plasma samples (1 ml) were deproteinized by the addition of 50  $\mu$ l of 100% (w/v) salicylsulphonic acid and centrifugation at 10 000 g for 10 min. A 50- $\mu$ l volume of the supernatant was injected into the column.

##### *Chromatographic conditions*

Details of the apparatus are given elsewhere<sup>1</sup>. Column: 20 mm  $\times$  2 mm (an AAA pre-column, Pickering, Mountain View, CA, U.S.A.), or an identical column packed in the laboratory with Dionex DC-4A or a similar resin. Eluent: 0.64 M Li<sup>+</sup>, pH 7.5 (Pickering). Column temperature: 65°C. Ninhydrin flow-rate: 0.2 ml/min. Reaction bath temperature: 135°C. Linear flow-rate gradient from 0.05 to 0.5 ml/min in 5 min, 0.5 ml/min from 5 to 7 min and 0.4 ml/min from 7 to 14 min. At 6 min, 0.3 M lithium hydroxide was pumped through the column for 1 min, followed by equilibration with pH 7.5 buffer for the remaining 7 min.

Detector settings were: signal A, 405  $\pm$  5 nm; signal B, 570  $\pm$  5 nm (both wavelengths of absorption maxima of Ruhemann purple<sup>1</sup>). The two channels were used to confirm peak identification and quantity.

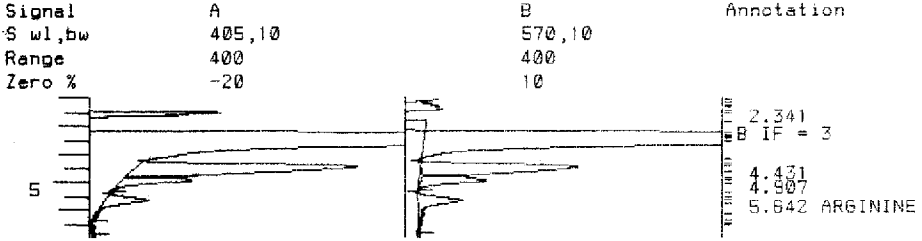
#### RESULTS

The arginine peak was eluted at 5.65 min (Fig. 1). The peak width at half height was about 15 s as compared with 75 s for a regular protein hydrolysate<sup>1</sup>. It seems that, when using lithium buffer, the wavelength of 570 nm is preferable to that

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Offline plot from DPU memory



ESTD

#	NAME	GR	SI	TIME [min]	TYPE	REF	AMOUNT [ NANOMOLES ]	WIDTH [min]	dTIME [min]	QUOTIENT [amount]
1	ARGININE	A		5.642	TPB	R	1.73020	0.270	0.007	A/B 0.982

MUL FACTOR = 1

ESTD FOR SIGNAL A

#	NAME	GR	TIME [min]	HEIGHT [MAU]	TYPE	REF	AMOUNT [ NANOMOLES ]	WIDTH [min]	SYM
1	ARGININE		5.642	54.977	TPB	R	1.73020	0.270	0.725

MUL FACTOR = 1

ESTD FOR SIGNAL B

#	NAME	GR	TIME [min]	HEIGHT [MAU]	TYPE	REF	AMOUNT [ NANOMOLES ]	WIDTH [min]	SYM
1	ARGININE		5.648	57.524	BB	R	1.75240	0.310	0.622

MUL FACTOR = 1

Fig. 1. Chromatogram of venal plasma. Arginine level: 1.73 nmol.

of 405 nm, due to the straight baseline obtained in spite of the column flow-rate gradient (Fig. 1); a tangential-skim mode had to be used at 405 nm due to the nature of the baseline. The average amount of arginine determined from six consecutive injections of a calibration mixture containing 12.5 nmol arginine was  $12.56 \pm 0.26$  nmol, *i.e.*, a relative standard deviation of 2.1% at both wavelengths. The response at both wavelengths was linear over the concentration range from 1.56 to 12.5 nmol (Fig. 2). Amounts of 1 nmol (equivalent to 30  $\mu\text{g/ml}$ ) could easily be quantitated in blood plasma from the subabdominal vein of lactating cows.

Calibration curve

$$\text{height} = a2 * \text{amount}^2 + a1 * \text{amount} + a0$$

for

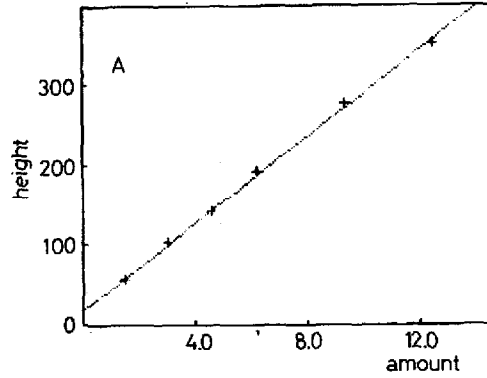
PEAK # = 1            SIGNAL = A

Single points

#	amount	height
1	12.5	349.984131
2	9.375	277.755371
3	6.25	191.053833
4	4.6875	143.514771
5	3.125	102.125183
6	1.5625	55.52121

Calibration coefficients

a0 = 17.11573  
a1 = 27.426936  
a2 = 0

Calibration curve

$$\text{height} = a2 * \text{amount}^2 + a1 * \text{amount} + a0$$

for

PEAK # = 1            SIGNAL = B

Single points

#	amount	height
1	12.5	359.893311
2	9.375	284.656494
3	6.25	192.76062
4	4.6875	149.41528
5	3.125	102.365211
6	1.5625	55.748993

Calibration coefficients

a0 = 16.009071  
a1 = 27.980999  
a2 = 0

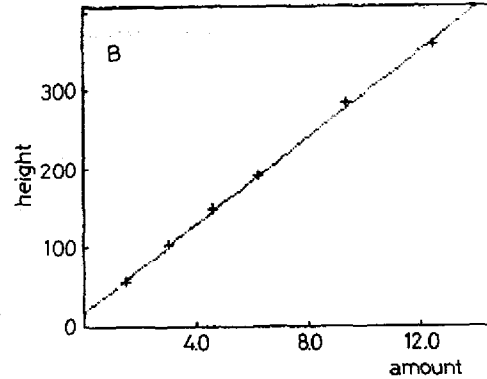


Fig. 2. Plots of arginine peak height (mAu = milli absorption units) against amount (nmol): multilevel calibration was performed using single determinations of each standard concentration; the regression coefficients for signals A and B were 0.9992 and 0.9991, respectively.

## REFERENCE

- 1 Z. Harduf, *J. Chromatogr.*, 363 (1986) 261-266.