The present program uses three cards for the standard analysis using either the height–width or the height method—which is only valid where small sample volumes less than 0.2 ml are used (MONDINO[3])—and two for summing the nitrogen values and calculating the nitrogen recovery, and a single card for expressing the results on a percentage basis. Thus six cards suffice for the twenty-three cards of MONDINO's program while the results are always retained in the computer for presentation in the other ways shown in Fig. 2.

Copies of these programmes (to calculate either in the range 0–1 or 0–2 $\mu$moles) are available from the author.

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1 A. MONDINO, *J. Chromatog.*, 41 (1969) 156.
2 L. W. NAUMAN, *J. Chromatog.*, 41 (1969) 456.
3 A. MONDINO, *J. Chromatog.*, 39 (1969) 262.

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## Quantitative gas chromatographic determination of glutamic acid, 2-pyrrolidone-5-carboxylic acid and 2-pyrrolidone*

Work in our institute on the thermal and catalytic lactamization of glutamic acid (Glu) and on photochemical reactivity of 2-pyrrolidone-5-carboxylic acid (PCA) has made it necessary to develop analytical methods for the simultaneous quantitative determination of the two substances in the presence of their degradation products, such as 2-pyrrolidone (PYR).

Previous research has revealed the presence of PCA in many vegetable extracts and in biological fluids[1–3]. According to some authors, this acid is partially or totally formed from Glu as a result of the analytical methods used, particularly when they involve heat treatment[4–7]. A useful method for the qualitative and quantitative determination of the two substances is therefore of general interest.

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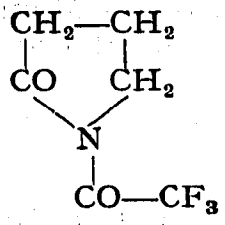
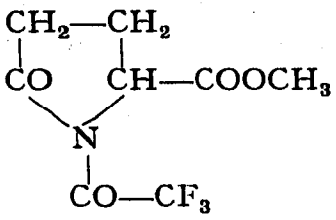
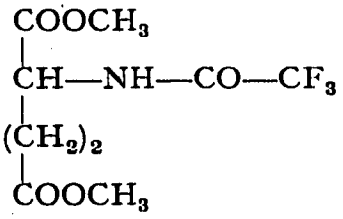
The procedure used by us involves the preparation of volatile derivatives by esterification of the carboxyl groups with methanol and their subsequent N-trifluoroacetylation. Thus the following derivatives are obtained from the three compounds analyzed:







Particular attention has been given to the study of the conditions that result in a higher yield of volatile derivatives and prevent the conversion of Glu to PCA and *vice versa*.

In this connection, preliminary research carried out by us has shown that the conditions for the lactamization of Glu are much more severe, as regards heating temperatures and times, than those reported by other authors[6].

*Experimental*

*Lactamization of glutamic acid*

Glutamic acid either in the solid state or dissolved in water or methanol is heated for 1 h at temperatures from 40° to 150°.

A rapid analysis for PCA, if any, in the presence of Glu is carried out on plates of Silica Gel G (Merck). A solvent system consisting of butanol–acetic acid–water (120:30:50) is used for the development. The plates are then sprayed with ninhydrin reagent for the detection of Glu and with RYDON–SMITH reagent[8] for PCA.

*Preparation of volatile derivatives*

Solution 1:0.4 *M* aq. Glu.

Solution 2:0.04 *M* aq. PCA*.

Solution 3:0.04 *M* aq. PYR.

Solution 4:0.04 *M* methyl stearate in methylene chloride.

Solution 5:0.75 *N* anydrous HCl in methanol, prepared by passing dry HCl gas into absolute methanol.

Five ml each of solutions 1, 2, 3 and 4 are placed in a 50-ml calibrated flask; the solvent is then removed with a rotary evaporator under reduced pressure, while maintaining the temperature below 45°.

After addition of 10 ml of solution 5, the flask is connected to a reflux condenser and placed for 1 h in a water bath at 55°. The HCl–methanol is then removed by evaporation under reduced pressure, as described above. The residue is taken up in 2 ml of trifluoroacetic anhydride and dissolved by stirring. After standing at room temperature for 40 min, the solution is ready for the gas chromatographic analysis.

* PCA is prepared by heating glutamic acid at 180° for 1 h. Purification is carried out by dissolving the reaction product in boiling 95% ethanol. The solution is decolorized with charcoal and the PCA is precipitated by cooling in an ice bath. The precipitate is filtered off, repeatedly washed with cold 95% ethanol and dried at 90° in an oven. A crystalline white solid, m.p. 170°, is obtained.

The thin-layer chromatographic analysis of the product, carried out as described above, shows that there is no remaining Glu.

*Gas chromatographic procedure*

*Apparatus.* A Varian-Aerograph 1520 B gas chromatograph equipped with a flame-ionization detector is used.

*Operating conditions.* They are as follows:

Columns: 3 mm × 1.5 m stainless steel

Carrier gas: $N_2$, 20 ml/min

Support: 80/100 mesh silanized Chromosorb W

Stationary phase: 0.65% OV-17 + 0.75% EAS

Injector temperature: 200°

Detector temperature: 250°

Oven temperature: programmed: initial value 70°; isothermal at 70° for 4 min; 8°/min for 4 min, up to 102°; isothermal at 102° for 6 min; 15°/min for 4 min, up to 160°; isothermal at 160° for 5 min

Analysis time: 23 min

Amount of sample injected: 2 $\mu$l

*Quantitative determination.* The "internal standard" method is used, with methyl stearate as the standard.

The coefficients for the quantitative determination of Glu, PCA and PYR are calculated from the mean molar responses relative to the internal standard ($RMR_{i.s.}$), on the basis of the ratios between peak areas.

*Results and discussion*

Preliminary experiments carried out to determine the conditions under which Glu is converted into PCA have shown that the lactamization occurs only at temperatures above 100°. The method described above, consistently employing much lower temperatures, prevents the formation of PCA, which would alter the results of the quantitative analyses. Fig. 1 shows a chromatogram obtained under the experimental conditions described.

The separation of the four substances is seen to be complete. The responses of the flame-ionization detector to PYR, PCA and Glu are very similar, whereas the

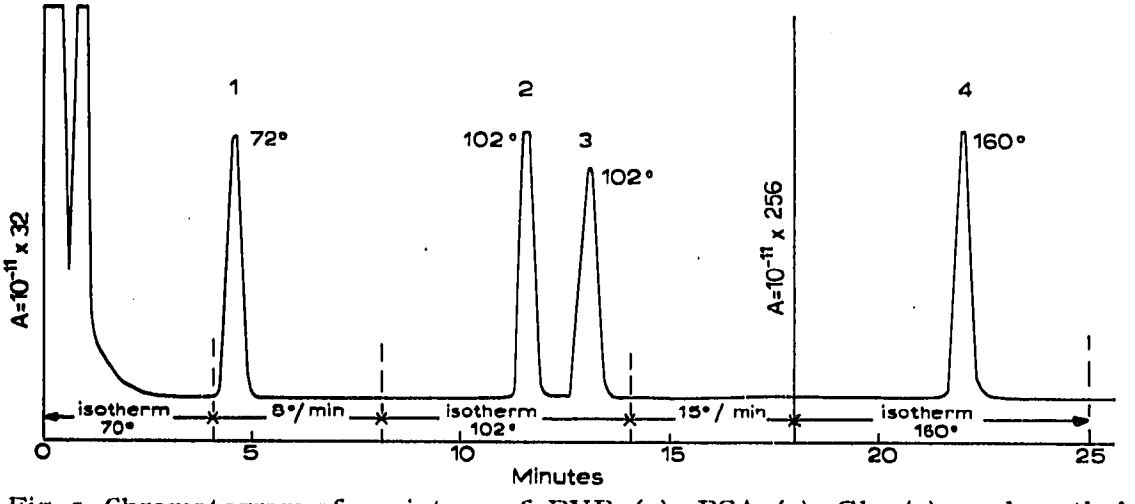



Fig. 1. Chromatogram of a mixture of PYR (1), PCA (2), Glu (3), and methyl stearate (4); 1.5 m × 3 mm I.D. stainless-steel columns packed with 0.65% OV-17 + 0.75% E.A.S. on 80–100 mesh silanized Chromosorb W.

TABLE I

RETENTION TIMES AND $RMR_{t.s.}$ FOR 2-PYRROLIDONE, 2-PYROLLIDONE-5-CARBOXYLIC AND GLUTAMIC ACIDS

| *Compound* | *Retention time (min)* | *Retention time relative to methyl stearate* | $RMR_{t.s.} \times 100$ |
|---|---|---|---|
| PYR | 4.5 | 0.22 | 13.7 |
| PCA | 11.5 | 0.52 | 11.5 |
| Glu | 13.0 | 0.59 | 14.5 |
| Methyl stearate (internal standard) | 22.0 | 1.00 | 100 |

response to methyl stearate is about 8 times greater, so that it is necessary, for equimolar samples, to reduce the sensitivity of the instrument after the Glu has emerged from the column.

Table I shows the retention times and the $RMR_{t.s.}$ of the three substances, calculated from the repeatedly checked results of a series of analyses.

The values contained in Table I are reproducible with an error of 5%.

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1 F. B. JOHNSTON AND M. M. HAMMIL, *Can. Inst. Food Technol. J.*, 1 (1968) 3.
2 S. FURUKAWA AND R. UEDA, *Hakko Kogaku Zasshi*, 41 (1963) 14; *C.A.*, 62 (1965) 15, 352c.
3 F. G. PRIOR FERRAZ AND M. E. RELVES, *Clin. Chim. Acta*, 11 (1965) 244.
4 J. H. SCHWARTZ, R. B. GREENSPUN AND W. L. PORTER, *J. Agr. Food Chem.*, 10 (1962) 43.
5 J. H. SCHWARTZ, R. B. GREENSPUN AND W. L. PORTER, *Food Technol.*, 15 (1961) 364.
6 R. S. SHALLENBERGER, H. R. PALLESEN AND J. C. MOYER, *Food Technol.*, 13 (1959) 92.
7 C. F. BAXTER, *J. Chromatog.*, 24 (1966) 270.
8 M. N. RYDON AND P. W. SMITH, *Nature*, 169 (1952) 922.

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