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DIFFERENTIAL SCANNING CALORIMETRY AS A TOOL FOR OBTAINING QUANTITATIVE DATA ON THIN LAYER CHROMATOGRAPHY

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ABSTRACT

An analytical method for the quantitative determination of some aminoacids is proposed. The analysis is carried out in two steps. The first is based on a thin layer chromatographic separation (TLC) which is realized on alumina and allows the aminoacids to be separated. The second is based on an application of differential scanning calorimetry (DSC), by which it is possible to characterize and determine the amount of the compound contained in each spot, after having removed from the plate the layer corresponding to the spot. This is put in the sample capsule of the differential scanning calorimeter, while the reference capsule is filled with an identical weight of the sample-free aluminum oxide from the same TLC plate. The DSC signal is proportional to the quantity of the sample present in the spot.

INTRODUCTION

Thin layer chromatography (TLC) is a widely applied qualitative analytical technique. To obtain quantitative data, the layer corresponding to the spot under investigation must be cut off and then eluted with an appropriate solvent to complete the extraction. Quantitative analysis is then carried out by a suitable analytical technique.

To avoid elution and to simplify the procedure, we tried to use the differential scanning calorimetry (DSC) to quantify the sample in each spot. In the DSC technique, aluminum oxide is an inert material used to dilute the samples and to obtain a better thermal diffusivity with a consequent increase in the peak area, as shown by Barrall and Rogers [1]. Aluminum oxide is also often used in the preparation of plates for thin layer chromatography; after chromatographic elution, the samples present in each spot are perfectly diluted chemical systems in an inert material. The layer corresponding to the spot can be cut off, and the sample capsule filled with the aluminum oxide obtained, while the reference capsule is filled with an identical weight of sample-free aluminum oxide obtained from the same chromatographic plate.

The DSC signal is proportional to the enthalpic molar effect corresponding to any chemical, physical or physico-chemical change induced by the temperature increase in the sample and is then proportional to the quantity

of the sample present in the spot under investigation. The proposed analytical technique consists of two steps: chromatographic TLC separation, and quantitative analysis by DSC. A mixture of three aminoacids, L-alanin, L-leucin and L-valin, was analyzed in this way.

EXPERIMENTAL

Instrumentation

The DSC curves were obtained using a DuPont Model 990 DSC cell and console. The heating rate was 10°C min^{-1}, and the sensitivity 0.05 mcal sec^{-1} full scale deflection on the first channel and 0.10 mcal sec^{-1} full scale deflection on the second. The furnace atmosphere consisted of dry oxygen at a flow rate of 100 ml min^{-1}. The peak areas were measured by means of a Linseis series 2000 integrator. The TLC plates were prepared by a Shandon Unaplan apparatus.

Reagents

The amino acids, aluminum oxide neutral type T and all other reagents were supplied by Merk, Darmstaad.

RESULTS

Glass plates (20 × 5 and 20 × 20 cm) were prepared by the Shandon Unaplan apparatus using a mixture of 30 g aluminum oxide neutral type T and 60 ml water. The thickness of the layers ranged between 0.2 and 0.4 mm. After spreading, the plates were air-dried for 12 h and then stored in a desiccator. Before use, the plates were activated in an oven at 110°C for 45 min, and stored in a desiccator to reach room temperature.

The aqueous solutions of the amino acids contained 4 g amino acid l^{-1}. The solution containing the compound to be separated (volumes ranged between 10 and 50 μl, corresponding to 40 μg minimum and 200 μg maximum) was placed on the point of application and dried in a warm air stream; elution commenced soon after.

All three amino acids were developed using ninhydrin. After several experiments, the best mixture for obtaining a good separation was found to be *n*-butanol : ethanol : water (60 : 40 : 40). The optimum thickness of the alumina layer was found to be 0.2 mm. The elution was carried out in parallel on two identical plates and under the same conditions. One of the plates was used as reference and the other as sample. After elution, the reference plate was developed so that the position of each amino acid could be identified by comparison. Areas of the alumina on the sample plate corresponding to each amino acid (about 1 cm^2) were then scraped off and each placed in a DSC capsule.

Calibration curves

To obtain the calibration curves corresponding to each amino acid, an identical amount (18—22 mg) of the alumina used to prepare the TLC plates was weighed in two capsules. Different volumes (between 10 and 30 μl) of the standard solution of each amino acid were added to one of the two capsules by a microsyringe. To the second capsule was added the same volume of water. The two capsules were then placed in an oven at 60°C until all the water had been removed and then analyzed by DSC. The DSC curves in Fig. 1 show that each amino acid decomposes giving an exothermic peak between 240 and 450°C. The calibration curves were then constructed plotting peak areas vs. amino acid weight.

Many samples of each weight were prepared in order to obtain a series of statistically useful data. The following conditions were choosen: atmosphere: air; heating rate: 10°C min^{-1}; sensitivity: channel 1 = 0.05 (mcal sec^{-1}) in.$^{-1}$; channel 2 = 0.10 (mcal sec^{-1}) in.$^{-1}$: scale: 50°C in.$^{-1}$.

Using an atmosphere of air, the peak areas and baseline were not satisfactory and a dynamic oxygen atmosphere was therefore used. The peak area, which in air was about 0.010 in.^{2} μg^{-1}, was about 0.027 in.^{2} μg^{-1} in oxygen. In this way, the three calibration curves reported in Fig. 2 were obtained. The corresponding equations and correlation coefficients are summarized in Table 1.

To check if the calibration curves so obtained were reproducible when the amino acid was applied to the TLC plate and then eluted, the following procedure was used. Increasing volumes (10—50 μl) of the standard solutions of each amino acid were applied to two identical TLC plates, one used as reference and the other as sample. After elution, the reference plate was developed, the positions of the spots identified, and the corresponding areas on the sample plate scraped and each collected in a DSC capsule. The

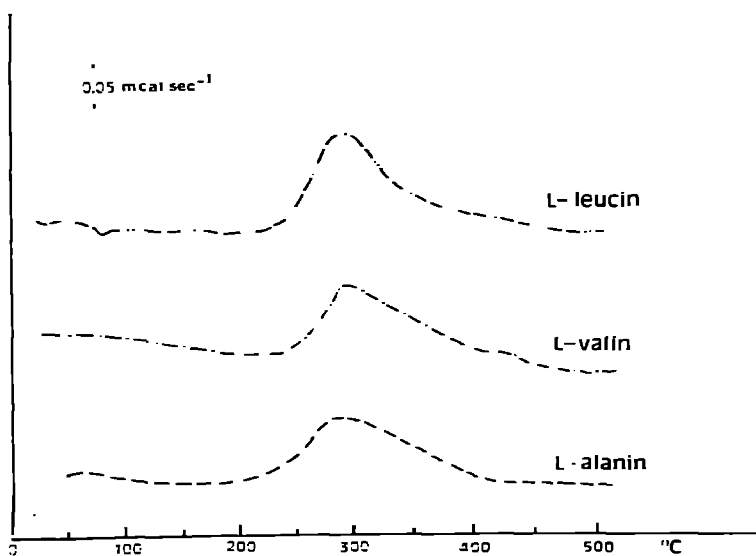

Fig. 1. DSC curves. Heating rate: 10°C min^{-1}; atmosphere: dynamic oxygen.

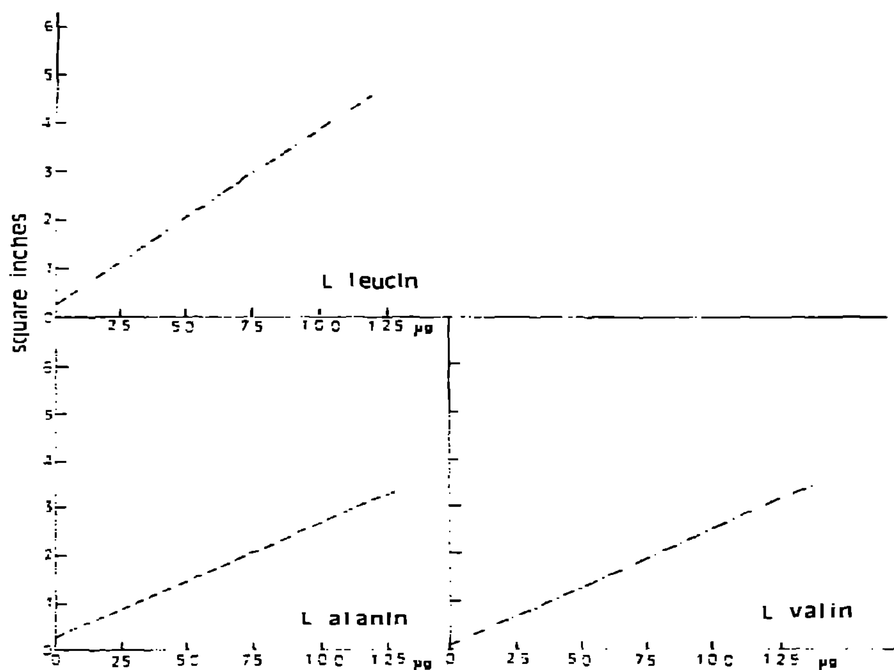

Fig. 2. Calibration curves.

reference capsule was filled with an identical weight of aluminum oxide obtained from an area of the same amino acid-free chromatographic plate.

A new series of calibration curves was obtained by plotting peak areas vs. amino acid weight. The correlation between the first and second series was very good, with a correlation coefficient $r = 0.99$. Finally, a control was carried out by applying 40 µl of solutions containing a mixture of the three amino acids to the TLC plates. The concentration of each amino acid in each solution was variable and ranged between 40 and 200 µg in 40 µl. After elution and separation, the aluminum oxide corresponding to each spot area was scraped and collected in a DSC capsule. The reference capsule was prepared as previously described. A third series of calibration curves was obtained which reproduces the other two series within the limits of experimental error.

TABLE 1

Equations and correlation coefficients for the amino acids

Amino acid	Straight line equation	Correlation coefficient, r
L-Alanin	$y = 0.072 + 0.025x$	0.99
L-Leucin	$y = 0.176 + 0.03545x$	0.99
L-Valin	$y = 0.2986 + 0.01967x$	0.99

DISCUSSION

The analytical methods proposed above for obtaining quantitative data by TLC separation suggest the cutting off of the layer corresponding to the spot under investigation, eluting the obtained material with a suitable solvent, and then analyzing the chemical species of interest present in the solvent. Each step of the analytical procedure can introduce errors. In particular, elution cannot be quantitative.

Obviously, any direct analysis of the sample present in the spot will reduce the possibility of error and at the same time will simplify the analytical technique and reduce the operation time. The proposed analytical method requires only the removal of the portion of the layer under investigation, after which analysis is carried out directly by differential scanning calorimetry. The instrumental signal is proportional to the molar enthalpy of the thermal process and this is directly proportional to the quantity of sample present in the spot. The sensitivity is quite good. The minimum sample mass that can be analyzed is about 40 μg, but it can be dramatically reduced, to about 5 μg, using a more sophisticated instrument such as a computerized Perkin-Elmer DSC-2. The technique, here applied to aluminum oxide plates, can be applied to any kind of material used to prepare the TLC plates. DSC is, in fact, a differential technique; by weighing exactly the same amount of chromatographic layer in the sample and reference capsule, opposing signals derived from the support will be obtained and there will be no peak. The only limiting parameter is the difference in weight between sample and reference due to the reduced weighed mass.

ACKNOWLEDGEMENT

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REFERENCE

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