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GAS CHROMATOGRAPHIC DETERMINATION OF SUCCINODINITRILE IN PHARMACEUTICALS*

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SUMMARY

A gas chromatographic determination of succinodinitrile was worked out which can be used for the analysis of aqueous solutions and mixtures with other therapeutically active substances. The aqueous solution of succinodinitrile is saturated with NaCl, extracted with chloroform, the extract evaporated, dissolved in acetone and chromatographed on silanised Chromosorb W impregnated with diethylene glycol succinate at 205° in a Perkin-Elmer F 11 chromatograph fitted with a flame ionisation detector.

Succinodinitrile is now widely used in medicine to restore cellular nucleoproteins and to re-establish balanced neuropsychological functions. It was therefore thought profitable to investigate the feasibility of a gas chromatographic determination of this substance.

Succinodinitrile has so far been identified by hydrolysing the cyano groups and characterising the resulting succinic acid, while the quantitative analysis has relied on the formation of an insoluble complex with cuprous chloride[1,2] $(CH_2CN)_2Cu_2Cl_2$, or on the conventional determination of nitrogen.

The first report[3] on the separation and identification of nitriles by gas chromatography appeared in 1960, but the results were not always reproducible. Later MUGNAINI AND GAMBELLI[4] reported good separations and quantitative determinations of some nitriles including succinodinitrile. These authors used Apiezon L mixed with sodium caproate as stationary phase on Celite support. They injected 5 μl samples and their chromatograph was equipped with a thermal conductivity detector. In the same year, ARAD-TALMI *et al.*[5] separated a mixture composed of acrylonitrile, propionitrile, butyronitrile, succinonitrile, and adiponitrile in an aqueous acidic solution. The latter was saturated with KCl, extracted with *o*-dichlorobenzene, the extract was dried and then injected into a gas chromatograph in 10–15 μl samples.

The object of this investigation was to devise a gas chromatographic technique for the identification and quantitative determination of succinodinitrile in an aqueous solution, singly or in mixtures with various therapeutically active substances such as

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calcium pantothenate, vitamins B_1, B_2, B_6, B_{12}, and PP, reserpine, dihydroisoandrosterone, benzyl alcohol, sucrose, and natural essences.

In the present method, the sample in an aqueous solution is saturated with NaCl, extracted with chloroform, the extract is concentrated, and the residue is dissolved in acetone and then chromatographed directly. The succinonitrile content is calculated with the aid of the calibration curve shown in Fig. 1, obtained by chromatography of known quantities of succinonitrile processed in the same way.

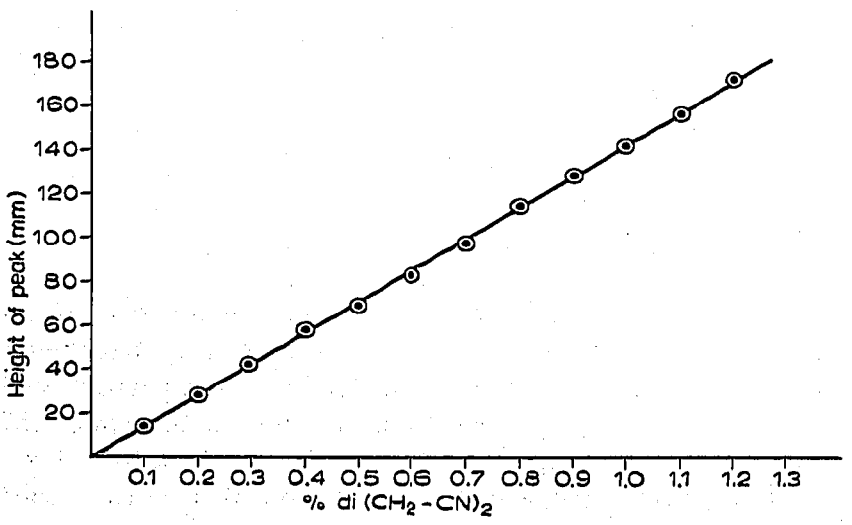

Fig. 1. Chromatographic calibration curve for succinonitrile.

This method is suitable for analysis, in that the standard error is $\pm$ 5 %, which is acceptable in the gas chromatographic determination of quantities of about 3–12 μg. Under experimental conditions, less than 0.1 μg of succinonitrile has been detected, but for ideal reproducibility, the best concentration is 0.1–1.2 mg/ml, since the calibration curve shows a linear relationship in this region.

EXPERIMENTAL

A Perkin-Elmer F 11 chromatograph fitted with a flame ionisation detector (hydrogen flame) was used. The Leeds-Northrup potentiometric recorder (Speedomax G, type S) had a full-scale deflection of 5 mV. The column had a length of 1.60 m and an internal diameter of 1/16 in., and was filled with 80–100 mesh silanised Chromosorb W impregnated with 15 wt. % of diethylene glycol succinate (DEGS). The temperatures of the column and the glass-lined vaporiser were 205 and 300°, respectively. The flow rate of carrier nitrogen was 32 ml/min, the chart speed was 0.5 in./min, and the attenuation factor was 500. Under these conditions the retention time of succinonitrile is 3 min 55 sec.

To construct the calibration curve, aqueous solutions of pure succinonitrile were saturated with NaCl and extracted five times with chloroform. The extracts were evaporated at 80° and the residue was redissolved in acetone, so as to obtain succinonitrile concentrations of 3–12 mg/ml. We then injected 1 μl samples of these solutions, measured the heights of the resulting peaks, and plotted these against the

succinonitrile concentration of the solution. This work was carried out in an isothermal regime.

In the determination of succinonitrile in the presence of other therapeutically active substances, an aqueous solution of the sample was saturated with NaCl and then subjected to the same treatment as described for the calibration samples; the volume of solution was 10 ml and 1 μl samples were injected. The sample was chromatographed under the conditions specified above, and the amount of succinonitrile present in the sample (in %) was determined by measuring the peak height and comparing it with the calibration curve.

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