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DETERMINATION OF ACETYLCHOLINESTERASE ACTIVITY BY GAS-LIQUID CHROMATOGRAPHY

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Under ordinary conditions of work of the gas-liquid chromatograph the temperature of the column and vaporizer is always kept above the boiling point of the least volatile component of the test mixture, and in that way all components of the mixture are converted into the vapor phase. Under these conditions, however, it is impossible to introduce a large volume of the test mixture into the chromatograph, because the chromatograph column will cease to function normally due to overloading. If a small volume of sample is introduced into the chromatograph, however, the presence of a trace component cannot be detected. This drawback is not found in Deans' method, the essence of which is that the column and vaporizer temperature is lower than the boiling point of the principal component (in our case – water). Under these conditions of work by introducing an adequate volume of the sample, it is possible to fill virtually the whole of the column with test liquid, and in that case, when the flow of carrier gas passes through the column, the liquid sample to be tested will perform the role of stationary phase, whereas the trace component will be concentrated toward the "tail" of the main component.

Deans studied this method in detail and used two columns, connected together in sequence, the first of which was fitted with a thermal conductivity detector (katharometer), the second with a flame-ionization detector (FID) [3, 4]. He found that the peak of the trace component always comes out in the "tail" of the principal component, and its intensity is proportional to the concentration of the trace component, given equal volumes of sample. The characteristics of the columns, the material with which they are packed, and the nature of the stationary phase of the solid carrier and gas carrier do not play an essential role. A change of column temperature, as usual, affects only the retention time.

We have modified Deans' method and have used it to determine acetylcholinesterase (AChE) activity.

EXPERIMENTAL METHOD

The proposed method is based on the reaction of enzymic hydrolysis of acetyl-β-methylcholine by AChE with the formation of acetic acid:

$$[CH_3{=}COO{=}(CH)CH_3{=}CH_2{=}N^+(CH_3)_3]Cl^- \xrightarrow{-H_2O}$$
$$\longrightarrow CH_3COOH + [HO{=}(CH)CH_3){=}CH_2{=}N^+(CH_3)_3]Cl^-.$$

Acetic acid (the trace component) is determined on a chromatograph fitted with an FID. Since the column and vaporizer temperature must be below the boiling point of the principal component (water), we chose 90°C. Lowering the temperature below 90°C leads to an increase in retention time of the trace component (acetic acid). Unlike Deans, we used one column

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with an FID insensitive to water,so that only the acetic acid peak is proportional to its concentration in the sample and to the size of the sample. Having calibrated the chromatograph beforehand relative to known standard mixtures, the concentration of the trace components in the test mixtures is determined [2].

The reagents and conditions of the enzymic hydrolysis experiments were similar to those described by Murav'eva [1], with certain modifications.

Reagents: 1) a standard solution of mecholine (acetyl-β-methylcholine) 0.48% (0.02M); 2) standard solutions of glacial acetic acid in dilutions from 10^{-2} to 10^{-6} M; 3) 1/15 M phosphate buffer, pH 7.8.

Course of Determination. Phosphate buffer (2 ml) was measured out into the control (Nos. 1 and 2) and experimental test tubes, after which 2 ml of distilled water was added to the control tubes and 1 ml to the experimental tube. Next, 1 ml of blood was added to the experimental tube and control tube No. 1, whereas 1 ml of 0.48% mecholine solution was added to control tube No. 2; the contents were mixed and the tubes covered and incubated at 37°C. After incubation for 60 min the tubes were removed from the incubator and, by means of a special syringe, 1 ml of the test fluid was introduced into the chromatograph. The conditions of operation of the LKhM-9MD gas-liquid chromatograph were: the temperature in the column and vaporizer was 90°C, the rate of flow of helium 20 ml/min. The stainless steel column was 150 cm long, with an internal diameter of 3 mm, and was filled with 7% PEYS (diethyleneglycol succinate) on LAC-3R728 (solid phase).

Before work began bidistilled water was introduced into the chromatograph to stabilize the zero line of the instrument. From 1 to 2 μg of acetic acid or more could be determined. A characteristic calibration curve for quantitative determination of acetic acid is given below [sic]. The quantity of CH_3COOH in the test sample is found from the height of the peak on the chromatogram.

CONCLUSIONS

1. A modified Deans' method for quantitative gas-chromatographic determination of trace components in a large volume of sample of test mixture introduced into the apparatus was developed and used.

2. In the suggested modification this method can be used for gas-chromatographic determination of microquantities of acetic acid.

3. Deans' method can be used for quantitative differential determination of acetylcholinesterase and pseudocholinesterase in the blood, based on determination of end products of hydrolysis of specific substrates.

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