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Enzymatic and polarographic determination of Metation and its analogues after separation on silica gel thin layers

It is possible to determine polarographically a mixture of Metation (O,O-dimethyl-O-(4-nitro-3-methylphenyl)-phosphothioate) and its analogues (Metaoxon: O,O-dimethyl-O-(4-nitro-3-methylphenyl)-phosphate; S-methyl isomer, bis-(*p*-nitro-methylphenyl) isomers) after its separation on silica gel layers[1]. Moreover, various substrates and enzymatic sources of esterases have been used[2–8] for the estimation of the inhibition properties of cholinesterase inhibitors.

In order to make more effective use of the samples in the analysis of organophosphate insecticides we combined these two analytical steps, *viz.* the enzymatic determination of the inhibition effect of Metation on esterases and its polarographic determination.

Apparatus

All polarographic measurements were performed on a Polarograf LP-60 with an EZ-2 recorder (product of Laboratorní přístroje, Prague) connected to it. Kalousek's vessel with a saturated calomel electrode was used. When the height of the mercury reservoir was 70 cm, the capillary used had an out-flow velocity of 2.3 mg/sec and a drop time of 3.1 sec.

Solutions and reagents

A solution of $3.61 \cdot 10^{-3}$ *M* Metation (VÚAgT, Bratislava) and, $3.84 \cdot 10^{-3}$ *M* Metaoxon in chloroform were used. Sörensen's borate buffer was pH 9.2; the enzymatic source was a homogenate of beef liver[2]; the visualisation reagent was β-naphthyl acetate with Fast Blue B[2]. A 1 % solution of gelatine was also used.

Procedure and construction of the calibration curve

10–40 μl of a stock solution of Metation are applied onto a silica gel layer and developed in a mixture of light petroleum (b.p. 60–80°)–acetone (3:1. After finishing the development and drying the silica gel layer the chromatographic plates are sprayed with enzyme solution and are allowed to incubate at 37° in a water-saturated chamber for 30 min. The plates are oversprayed with the visualisation reagent and semiquantitative evaluation of the inhibition efficiency is planimetrically calculated from the areas corresponding to the spots (see Fig. 1)[6]. The spots containing the separated substances are transferred into the chromatographic columns and are eluted with 4 ml of acetone and collected in 10-ml volumetric flasks. 0.1 ml of gelatine solution is added and the volumetric flasks are made up with borate buffer. These solutions are transferred to a Kalousek's vessel and after desoxygenation with nitrogen the polarographic waves are recorded from —0.4 V to —0.8 V (see Fig. 2).

Discussion and conclusions

The advantage of the method described lies in the possibility of analysing even very small quantities of samples due to their better utilisation.

The contribution of each substance separated to the whole inhibition effect can

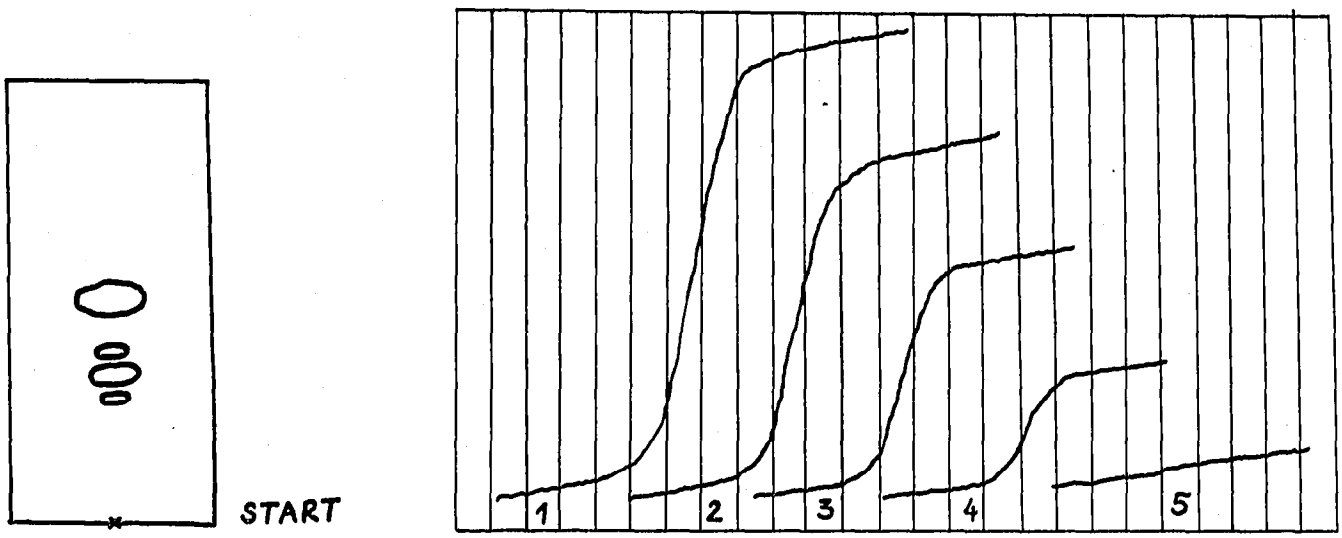

Fig. 1. Separation of Metation from its analogues. Silica gel/light petroleum (b.p. 60–80°)–acetone (3:1), enzymatic visualisation. From origin: spot 1, Metaoxon; spots 2 and 3, isomers of Metation; spot 4, Metation.

Fig. 2. Polarogram for the construction of the calibration curve for Metation after elution from thin layers. (1) 4 μg/ml; (2) 3 μg/ml; (3) 2 μg/ml; (4) 1 μg/ml; (5) 0 μg. Sensitivity 1:5, performed according to the working procedure described in the text.

be calculated after the chromatographic separation of Metation and its analogues and the planimetric semiquantitative evaluation of their inhibition efficiency. The actual percentage composition of the samples analysed is determined polarographically from the same plates.

The presence of unreacted β-naphthyl acetate and Fast Blue B and compounds arising from the coupling of enzymatically released β-naphthol with Fast Blue B does not disturb the subsequent polarographic determination of the substance to be analysed after its elution from the thin layer of silica gel.

The standard deviation of the semiquantitative enzymatic determination is ±20%, of the polarographic determination ±6%.

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