The Oscillopolarographic Determination of *Meprobamate* in Biological Material

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The oscillopolarographic properties of *Meprobamate* and some other drugs were studied. A method for quantitative determination of *Meprobamate* was elaborated and applied on biological material (urine, liver, kidneys, spleen and blood serum). *Luminal*, *Aprobarbital*, *Kalipnone*, and *Bromural* do not interfere, while *Doridene* and *Guajacurane* can be separated by paper chromatography.

The frequency of *Meprobamate* (2-methyl-2-*n*-propylpropandiol-dicarbamate) poisoning is rising in all countries and therefore the need of a specific method for detection and determination of this compound in biological material has become urgent.

A few colorimetric methods were presented in literature in the course of the last years [1—7]. Some of them were applied to biological material, but they are rather complicated. The toxicological practice needs a rapid and reliable method, but the claim on accuracy is not very high. Thus the oscillopolarographic method is very suitable for this purpose.

The present work is based on our preliminary note on the oscillopolarographic activity of certain ataractics, where we have established that *Meprobamate*, dissolved in 1 N-KOH in the concentration of 10^{-3} M supplies an incision of Q 0.55 [8, 9].

Experimental

Chemicals and Apparatus

1. HCl 37 % p. a.
2. KCl p. a.
3. KOH p. a.
4. Na_2SO_4 p. a.
5. $(NH_4)_2SO_4$ p. a.
6. Na_2CO_3 $10H_2O$.
7. Ethanol 96 % p. a.
8. Ether p. a.
9. Sephadex G 25.
10. Samples of *Meprobamate*, *Luminal*, *Aprobarbital*, *Kalipnone*, *Bromural*, *Doridene*, and *Guajacuran*.

Allmeasurings were carried out on Polaroscope Křižík P 576.

Oscillopolarographic properties of the pure *Meprobamate* and some other drugs (*Luminal*, *Aprobarbital*, *Kalipnone*, *Bromural*, *Doridene*, and *Guajacurane*), that can be present in the same portion of extract from biological material were examined in 1 N-HCl, 1 N-KCl, 1 N-KOH and 10 N-KOH aqueous solutions. Only *Guajacurane* and *Doridene* were found as interfering substances when determining *Meprobamate*. On Fig. 1 the incision positions of these three drugs in solutions mentioned above are demonstrated. (Dash marks on Fig. 1 express less significant incisions.) When the mixture of all three drugs is used, no incision in 1 N-HCl is obtained. *Meprobamate* in 10 N-KOH solution gives a very sharp incision compared with that in other mediums (Fig. 2). The sensitivity in this solution is about 5 times higher, but the incision appears only about 1 second after the fall of the previous drop. Thus the reproducibility of the incision depth is worse.

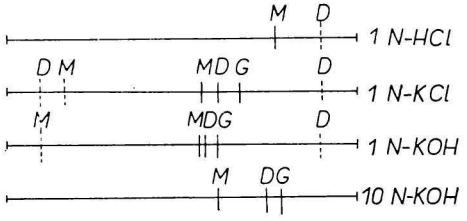

Fig. 1. Relative positions of cathodic incisions of *Meprobamate* (M), *Doridene* (D), and *Guajacurane* (G) in different mediums.

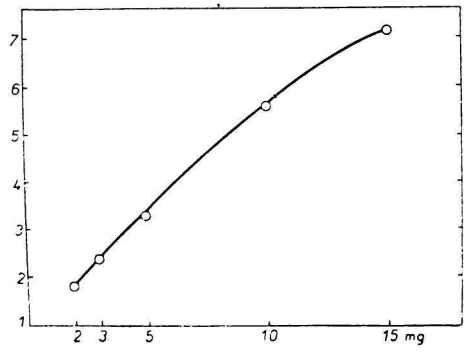

Fig. 3. Calibration curve of *Meprobamate* in 1 N-KOH.

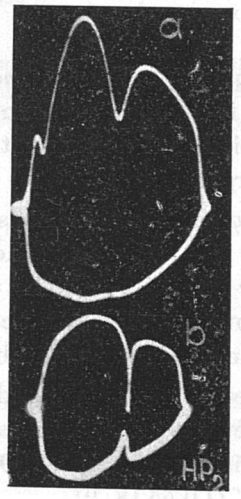

Fig. 2. Oscillopolarograms of *Meprobamate*. *a*) 10 mg% in 1 N-KOH; *b*) 3 mg% in 10 N-KOH.

When elevating the reservoir the incision of *Meprobamate* rises a little. By increasing the temperature the incision diminishes. The prolonged heating in KOH solution causes desintegration of *Meprobamate*, accompanied by disappearance of the incision of 0.55 and by formation of two others of Q 0.10 and 0.40, the former of which disappears earlier than the latter. In neutral medium *Meprobamate* is relatively stable, thus KOH should be added just before measuring.

The calibration curve in 1 N-KOH (Fig. 3) was prepared in ranges of 2—15 mg%. The average absolute error was lower than 0.5 mg%.

In view of the fact that *Meprobamate* cannot be determined in the presence of *Guajacurane* and *Doridene* and that we did not reach a sufficient partition by other methods, we tried to separate the three compounds mentioned by paper chromatography and determine *Meprobamate* after elution of spots. The ethanolic eluate from paper contains oscillopolarographically active bodies. When the chromatographic paper was treated for 30 min. with ethanol and then dried before chromatographic development the process was successful. The system ligroine—butanol—H_2O (100 : 20 50) is convenient for good separation. Standards of drugs examined were spotted in one starting point and the *Meprobamate*-containing sample was applied to the strip. After detection of standards the *Meprobamate* spot was cut out, eluted by ethanol and after evaporating dissolved in water and determined oscillopolarographically. However the combined intoxication by *Meprobamate* and one of the two other drugs is very rare, and therefore this procedure need not be used in a great majority of cases.

When working with biological material, *Meprobamate* must be separated. For urine the following procedure is recommended [10]. After slight alcalisation of urine by sodium carbonate *Meprobamate* is extracted by diethylether, the ether layer is evaporated on water bath and the product dissolved in water. The same procedure is repeated with another portion of urine with a known standard addition of *Meprobamate*. Both the samples are diluted 1 1 by 2 N-KOH and measured. The result is calculated using calibration curve. The addition of standard is necessary for incomplete extraction of *Meprobamate*. The present physiological bodies do not disturbed the determination.

Then the extraction from spleen, kidneys and liver was studied by the old Stass Otto method and by that of A. Dressler [11]. Better results were obtained by the latter. The homogenised organs are mixed with dry sodium sulphate and extracted by ethanol. The ethanolic solution is transferred into a vessel containing ammonium sulphate and evaporated. The residue is dissolved in water and treated by a similar procedure, as the urine. The standard addition is to be added into the homogenised organs.

The isolation of *Meprobamate* from blood serum was realised according to R. Kalvoda [12] by gel filtration using Sephadex G 25 column 8×60 mm. 2 ml of serum containing up to 20 mg% of *Meprobamate* was applied, eluted by water and 0.5 ml fractions were collected. The desired protein free compound was found in the 9th—11th fractions.

The method was applied in 5 cases of *Meprobamate* intoxication and the urine levels found were 10—35 mg%.

The autors would like to express their thanks to Dr. R. Kalvoda from Polarographic Institute, Prague, for valuable advices.

OSCILOPOLAROGRAFICKÉ STANOVENIE MEPROBAMÁTU V BIOLOGICKOM MATERIÁLI

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Opisuje sa oscilopolarografické stanovenie meprobamátu po predchádzajúcej extrakcii z biologického materiálu. Metóda bola vypracovaná pre účely súdnej analytiky.

ОСЦИЛЛОПОЛЯРОГРАФИЧЕСКОЕ ОПРЕДЕЛЕНИЕ *МЕПРОБАМАТА* В БИОЛОГИЧЕСКОМ МАТЕРИАЛЕ

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Разработан метод осциллополярографического определения *мепробамата* после его экстракции из биологического материала для целей судебного анализа.

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Received September 18, 1963