On the detection of mebezonium iodide

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Zum Nachweis von Mebezoniumiodid

Mebezonium iodide [4,4'-methylene-bis(cyclohexyl-trimethyl)-ammonium di-iodide] is employed as a muscle relaxant for euthanasia of animals in the agent T 61 in addition to embutramide and tetracaine. Suicide as committed by a laboratory assistant by i.v. injection of T61 called for the determination of the substance in biological material.

In the literature we were hitherto solely acquainted with work by Bertol et al. [1] which likewise deals with the detection of mebezonium iodide. The authors removed protein from blood and urine with trichloro-acetic acid, applied parts of this deproteinated solution to a TLC glass plate and used chloroform/ethanol 80/20 as the solvent. Mebezonium iodide remains at the starting point. It was extracted here with water and the solution subjected to measurement in UV. Due to the degree of putrefaction in our case this procedure was not applicable so that it was necessary to develop a new method.

Quaternary ammonium compounds frequently fail to be detected in chemico-toxicological routine tests since they are not immediately extractable from aqueous solutions. In addition to the use of ion exchangers, ion-pair extraction has above all proved efficacious in such circumstances for the separation of biological material.

Giebelmann [2] compiled a list of the commonest systems for the relevant organic compounds in 1981. Examination of the counter ions which appeared suitable showed that the best results were obtained with bromothymol blue or polyiodide ions and methylene chloride as the solvent. For splitting purposes, high tension electrophoresis [4] was employed because of the speed of the method and its sensitivity. Dragendorff's reagent gives sensitive coloration. Semi-quantitative determination was achieved by comparison of the size of the spot and the intensity of coloration with known quantities. Gas-chromatographic identification gives results with adequate sensitivity by use of an OV-1 glass column and an N-FID. Preliminary de-alkylation, as is usually carried out in the case of gas-chromatographic determination of quaternary ammonium compounds, is not necessary if the iodide or polyiodides are used.

Procedure

A Treatment of blood and organs [3]. 10 ml of blood or 10 g of liver substance are homogenised with 70 ml of acetone and 5 ml of 10% tartaric acid and subjected to ultrasonic treatment. The acetone is carefully removed in vacuo after addition of a 16% solution of ammonium chloride (5 ml) and the remaining aqueous solution filtered after having been briefly heated in a water bath.

B Extraction with bromothymol blue modified after Stevens and Moffat [6]. The solution, after having been brought to pH 10, is first agitated with 20 ml of methylene chloride as a preliminary method of washing. It is then treated with 10 ml of a 0.15% bromothymol blue solution (0.3 g of bromothymol blue are dissolved under heat in 10 ml of methanol and diluted to 20 ml with water). The mixture is extracted twice with 30 ml of methylene chloride in each case. After filtering, the extract is evaporated carefully until dry and approx. 0.3 ml of methanol is added. Parts of this solution are split up by electrophoresis.

C Extraction with polyiodides [5]. The solution, after having been brought to pH 7 with ammonium hydroxide, is first extracted with 20 ml of methylene chloride which is rejected. Thereupon 0.2 ml of potassium iodide solution is added (1 g of iodine + 2 g of potassium iodide in 20 ml of water). This solution is then extracted twice with 20 ml of methylene chloride. The solvent is evaporated over a water bath and a residue is (if necessary) stored over potassium hydroxide in vacuo for 24 h to remove any excessive iodine. The residue is treated with methanol and likewise split up by electrophoresis.

D High-tension electrophoresis. Medium: Acetic acid/formic acid/water (15/5/80) pH 1.9; paper 2043 B Schleicher & Schüll Quick-flow. 4000 V, 50 mA. Running time 15 min.

Relative distance 1.7 (petidine 1.0, quinine 1.45, doxylamine 1.7).

The yield in the case of the processes described amounts to approximately 50% (bromotymol blue) to 70% (polyiodide). Amounts down to 2 µg of mebezonium iodide can still be detected by electrophoresis.

E Gas chromatography. The methanolic solution as obtained after the extraction described in C is employed. GC: Perkin Elmer 8300. Column: 2.5% OV-1 Chrom G. Column temperature 270°C, injector 200°C, detector 270°C, RI after Kovats: 2190.

References

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