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Determination of Acetylglutamine in Pharmaceutical Preparations

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A colorimetric method for the quantitative determination of acetylglutamine, after acid hydrolysis, is proposed. An ion-exchange chromatographic procedure is described for the elimination of interfering substances in pharmaceutical preparations.

ACETYLGLUTAMINE, like glutamine and glutamic acid, is used for treating metabolic disorders of the central nervous system and has the advantage of being much more stable than glutamine in aqueous solution.[1] There are few references to acetylglutamine in the literature and, as far as is known, no method is given for its determination. The method described here is based on the liberation of ammonia from acetylglutamine by boiling with acid and colorimetric measurement of the ammonia with Nessler's reagent.

In formulated preparations it is necessary first to eliminate degradation products of acetylglutamine that interfere; such products are glutamine and ammonium salts of acetylglutamic acid, glutamic acid and 5-pyrrolidone-2-carboxylic acid. This can be achieved by passing a solution of the preparation through a suitable cation-exchange resin. The acetylglutamine is not retained and is determined in the eluent after hydrolysis. The Nessler determination is liable to interference from many organic substances and inorganic ions, which give rise to colours or precipitates. It is necessary, therefore, to isolate the ammonium ions before the colorimetric reaction. Again, the method used is ion-exchange.

The Kjeldahl distillation method is not suitable, as volatile interfering substances may be present (*e.g.*, alcohols or aldehydes), and others may liberate ammonia under alkaline conditions.

Experimental

Reagents—

Hydrochloric acid, 2 N.
Sulphuric acid, 3 N.
Gum acacia solution, 1 *per cent. w/v, freshly prepared.*
Nessler's reagent—Prepare according to the British Pharmacopoeia 1963, p. 961.
*Amberlite CG*120 (200 *mesh*).

Preparation of each column—

Wash 1·0 g of the resin with 2 N hydrochloric acid until clean, and prepare a column (1 cm in diameter) between two plugs of absorbent cotton-wool. Pass ammonia-free water through the column until the effluent is free from chloride ions. Prepare two columns in this manner.

Procedure—

Prepare an aqueous solution of the sample, so that the concentration of acetylglutamine is about 1 mg per ml. Pass 25 ml of this solution through the resin column. Collect the effluent in a 50-ml graduated flask and pass water through the column until the graduation mark is reached. Mix 10·0 ml of the solution with 0·5 ml of 3 N sulphuric acid and boil under reflux for 5 hours. Cool the solution, neutralise with dilute aqueous sodium hydroxide and adjust to 50 ml with water. Pass 20·0 ml of this solution through the second resin column. Wash with ammonia-free water until the effluent gives no reaction from interfering substances

with Nessler's reagent. Elute the column with three 5-ml portions of 2 N hydrochloric acid, neutralise the eluate with aqueous sodium hydroxide and dilute to volume in a 20-ml graduated flask. To 5·00 ml of the neutral eluate add 2 ml of fresh gum acacia solution, swirl it, add 2 ml of Nessler's reagent and, after 1 minute, read the colour with a blue - green filter against water in 1-cm cells.

Prepare a calibration graph by similarly treating 5 ml of standard aqueous solutions containing 100 and 200 μg of ammonium chloride per 5 ml. Carry out a blank determination on 5 ml of ammonia-free water and deduct the extinction value from those of sample and standards. Calculate the acetylglutamine content of the sample (1 μg of ammonium chloride $\equiv$ 3·52 μg of acetylglutamine).

Discussion

The amount of resin in the first column is adjusted according to the amount of impurities to be removed.

Glutamine, ammonium ions, various basic organic compounds, some of which may be coloured, and inorganic cations are retained by this first column. The acetylglutamine and other classes of substances pass through in the effluent.

The hydrolysis is carried out in weakly acidic solution, so that after neutralisation a weak solution of cations is obtained. This aids the subsequent chromatography.

The ammonium ions produced by the hydrolysis of the acetylglutamine are retained in the second column, while other interfering substances, inorganic anions and organic compounds not held on the first column, are not retained.

Recovery of acetylglutamine in the presence of degradation products was shown to be quantitative for each stage of the analysis, and an over-all recovery of 97 $\pm$ 1 per cent. was obtained by comparison with the ammonium chloride standards, the optical densities of which follow the Lambert - Beer law.

A few classes of substances (*e.g.*, amides and ureides) interfere with the determination, but they can usually be eliminated by solvent extraction before hydrolysis. Sedative substances, such as bromural, carbromal, diethylbromoacetamide or meprobamate, which may be formulated with acetylglutamine, can be extracted from aqueous solutions with diethyl ether or chloroform. The method also gives good results with mixtures containing 60 per cent. of sugar.

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Reference

1. Sekules, G., and Guadagnini, G., *Farmaco, Ed. Prat.*, 1966, **21**, 22.

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