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Replacement of cyanogen bromide solution PH. EUR. with 1,3-dibromo-5,5-dimethylhydantoin (DBH)

Analytical methods of pharmacopoeias with DBH in respect to environmental and economical concern, part 13[1]

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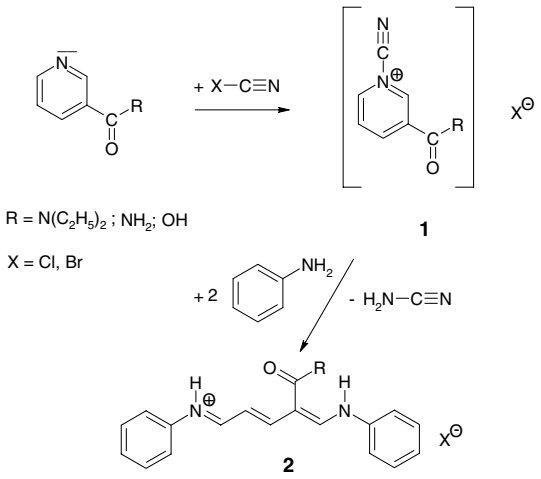
PH. EUR. 2002 identifies nicotinamid, nicotinic acid, and nikethamide according the reaction of König using cyanogen bromide solution prepared with bromine water and ammonium thiocyanate immediately before use. This colour reaction can be better performed with 1,3-dibromo-5,5-dimethylhydantoin (DBH) and sulphanilic acid as condensation component. The prescriptions of PH. EUR. have been improved in respect to environmental and economical concern.

1. Introduction

The reaction of König [2–4] mainly serves for the quantitative spectrophotometric determination of pyridine derivatives [5]. PH. EUR. 2002 uses this reaction for the identification of nicotinamide [6] nicotinic acid [7] and nikethamide [8]. A yellow colour results from the reaction with cyanogen bromide solution prepared from bromine water and ammonium thiocyanate immediately before use and a solution of aniline. A 1-cyanopyridinium halogenide **1** arises, which condenses with aniline to a polymethine dye **2** (Scheme 1) [6, 8].

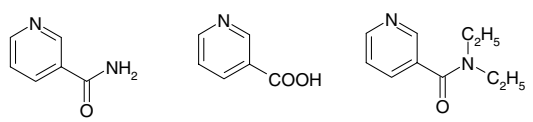
DBH is in contrast to elemental bromine a stable and easy to handle crystalline compound [1, 9–18] and contains about 110 % active bromine. It has been qualified in many cases for pharmaceutical analysis [1, 9–18].

Scheme 1



2. Investigations, results and discussion

The cyanogen bromide solution of PH. EUR. 2002 can be prepared with DBH/HAc and an ammonium thiocyanate solution in a simple manner when testing the sample. The addition of the test solution before or after the reaction of DBH with ammonium thiocyanate has no influence.



Nicotinamide (2002 : 0047) [2] **Nicotonic acid** (2002 : 0459) [2] **Nikethamide** (2002 : 0233) [2]

Aniline, which is quickly discoloured to brownish red by air oxidation, has to be distilled. Furthermore 4-aminophenol [6], benzidine [5], naphthylamine [5], sulphanilic acid [5], barbituric [5] acid and others are recommended as condensation components in the literature [5].

Whereas PH. EUR. 1 [19] produces the cyanogen bromide solution with potassium cyanide and bromine, the less toxic and less water hazardous ammonium thiocyanate is used since PH. EUR. 2 [20]. The reaction may correspond to Scheme 2. Sulphate can be detected in the solution with barium chloride. The required ammonium thiocyanate solution for decolorization of the bromine solution corresponds to the molar ratio of 1 : 4.

Scheme 2

$$NH_4SCN + 4\ Br_2 + 4\ H_2O \rightarrow BrCN + NH_4HSO_4 + 7\ HBr$$

Sulphanilic acid is more suitable than aniline, which is applied as condensation component [6–8] by the pharmacopoeia. Sulphanilic acid is a stable substance, if protected from daylight, and thus does not need any recrystallization before the performance of the analysis.

The reaction of chloramine T with potassium cyanide to generate cyanogen chloride, as described in the literature [5], has been investigated. *p*-Toluenesulphonate obtained by the reaction serves as condensation component. However, this colour reaction is weaker using ammonium thiocyanate instead of potassium cyanide and is therefore not recommended.

In conclusion, DBH cannot only replace bromine water when preparing a cyanogen bromide solution but can also be generated during the performance of the test. No preparation of a separate cyanogen bromide solution is necessary. Sulphanilic acid is more stable and therefore more suitable than aniline used by the pharmacopoeia as condensation component. The test according to König can be greatly simplified in comparison with the pharmacopoeial method and performed with a lower consumption of chemicals. It can be tested in a semimicro range, as spot-test analysis in a micro range. A lower consumption of chemicals is of economic benefit and is also a contribution to environmental protection. This identification test with DBH is also suitable in developing countries to detect fal-

sified or qualitative low-graded drugs [21, 22]. According to the guidelines of the WHO [22] the tests should be performed easily and fast, if a fully equipped laboratory is not available. The required reagents and the laboratory equipment should be restricted to a minimum. Unstable, corrosive, expensive or difficult to obtain reagents should be excluded. Therefore, the application of DBH in contrast to elemental bromine is recommended.

3. Experimental

3.1. Chemicals and drugs

Acetic acid [64-19-7], min. 99.8% p.a., Riedel-de Haën art. 33209 = HAc; ammonium thiocyanate [1762-95-4], extra pure, Merck art. 1212; aniline [62-53-3], extra pure, Merck art. 1260; barium chloride [10326-27-9], p.a. Riedel-de Haën art. 31125; bromine [7726-95-6], extra pure DAB 6, Merck art. 1945; chloramine, chloraminum PH. EUR. 2002, [7080-50-4], N-chloro-p-toluenesulphonamide trihydrate, extra pure, Merck art. 102424; 1,3-dibromo-5,5-dimethylhydantoin = 1,3-dibromo-5,5-dimethyl-2,4-imidaolidinedione [77-48-5], for synthesis Merck art. 803600 = DBH (for analytical purpose qualified); nicotinamide, nicotinamidum PH. EUR. 2002, USP 2000, [98-92-0], for biochemistry, Merck art. 6818; nicotinic acid, acidum nicotinicum PH. EUR. 2002, USP 2000 [59-67-6], extra pure, Merck art. 500005; 1-naphthylamine [134-32-7], for synthesis, Merck art. 822291, ($1 \times H_2O$); nikethamide, nicethamidum PH. EUR. 2002 [59-26-7], Schwarzhaupt; sodium acetate anhydrous [127-09-3], p.a., Merck art. 106268 = NaAc; sodium hydroxide, Rotipuran 99% [1310-73-2], Roth, art. 9356; sulphanilic acid [121-57-3], reag. PH. EUR., Merck art. 159443.

3.2. Solutions

0.1 M Chloramine T/1 M NaOH: 2.8 g (0.01 mol) of chloramine T are dissolved with stirring in 100 ml 1 M NaOH; 0.05 M DBH/HAc: 1.43 g (5 mmol) of DBH are dissolved in glacial acetic acid with stirring to 100 ml; 0.25 M sulphanilic acid/1 M NaOH: 433 mg (2.5 mmol) of sulphanilic acid are dissolved in 1 M NaOH to 10 ml.

3.3. Identification tests

Comparisons according to PH. EUR. and under other conditions see Hilp [6]. Solutions marked with R correspond to PH. EUR. In contrast to PH. EUR. molar concentrations are used preferably.
Reaction according to König (nicotinamide, nicotinic acid, nikethamide): 0.5 ml of 0.01 M test solution of nicotinamide resp. nicotinic acid resp. nikethamide are mixed with 0.4 ml of 0.05 M DBH/HAc and 0.2 ml of 0.1 M NH_4SCN. The arising yellow colour turns to colourless when shaking. Add 0.5 ml of 0.25 M sulphanilic acid/1 M NaOH. After 2 min a yellow colour appears and after a short waiting period the colour turns to an intensive yellowish orange, which is stable for about 4 h. A blank test remains colourless.

0.01 M test solution: 12.2 mg (0.1 mmol) of nicotinamide resp. 12.3 mg (0.1 mmol) nicotinic acid resp. 17.8 mg (0.1 mmol) nikethamide are dissolved with H_2O to 10 ml.

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[1] Part 12 [1]
[2] Year of the monograph published in PH. EUR.: Monograph reference number

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