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Application of 1,3-dibromo-5,5-dimethylhydantoin (DBH) instead of bromine gas or bromine water decolorization for drug identification according to PH. EUR.

Analytical methods of pharmacopoeias with DBH in respect to environmental and economical concern, part 14[1]

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PH. EUR. 2002 identifies biotin, flucytosine, polysorbate 80 and sorbic acid using the decolorization of bromine water. These tests can be better performed with 1,3-dibromo-5,5-dimethylhydantoin (DBH) in combination of the reaction with fluorescein sodium resp. sodium bromide. Also fluorescein sodium PH. EUR. Suppl 2002 can be identified with DBH.

1. Introduction

As recently published [1–6], DBH can replace elemental bromine and hypohalogenite solutions performing colour reactions according to PH. EUR. 2002 and supplements.
Identification tests have been improved in respect to environmental and economical concern. In contrast to elemental bromine resp. sodium hypochlorite solution resp. sodium hypobromi-le solution, DBH is a stable and easy to handle crystalline compound [1–6].

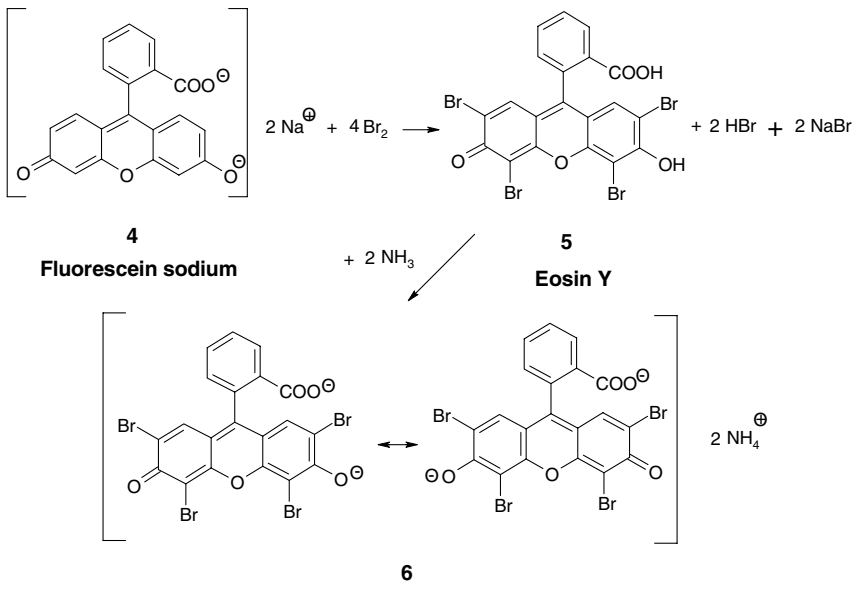
2. Investigations, results and discussion

2.1. Replacement of bromine gas with DBH

2.1.1. Fluorescein sodium PH. EUR. 2002

Fluorescein sodium of PH. EUR. 2002 mainly serves as diagnostic reagent in ophthalmology [7]. As identification test the bromination to eosin Y [8, 9] is applied (Scheme 1). The bromination leads to a bathochromic shift of the absorption maximum in the visible range [10]. The position of the absorption maximum, however, depends on the pH value, because fluorescein as well as eosin Y can exist as cation, as neutral molecule resp. zwitterion, as monoanion and as dianion in relation to the hydrogen ion concentration [10].
For the identification according to PH. EUR. 2002 [11] a piece of filter paper is impregnated with a solution of fluorescein sodium, which is hold over a flask with bromine in an exhaust hood. A violet colour arises immediately. The waiting time of 1 min and a following exposing to ammonia vapour, as described in the pharmacopoeia, is not necessary.
In my opinion the application of elemental bromine for the identification of fluorescein sodium according to PH. EUR. 2002 is unacceptable. The test of Ph. Helv. 8 (1997) is performed more easily. The bromine liberated from a bromide/bromate solution in strong hydrochloric acid solution reacts immediately with fluorescein sodium. Afterwards the test solution is alkalized with sodium hydroxide solution.

Scheme 1

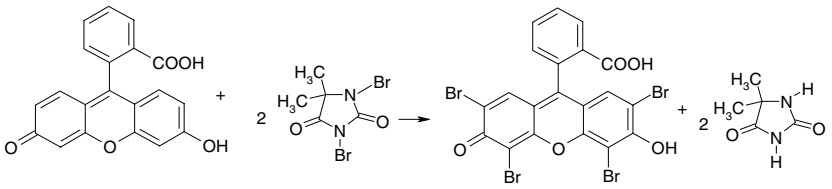


4 **Fluorescein sodium**

5 **Eosin Y**

6 **Eosin Y dianion**

Scheme 2

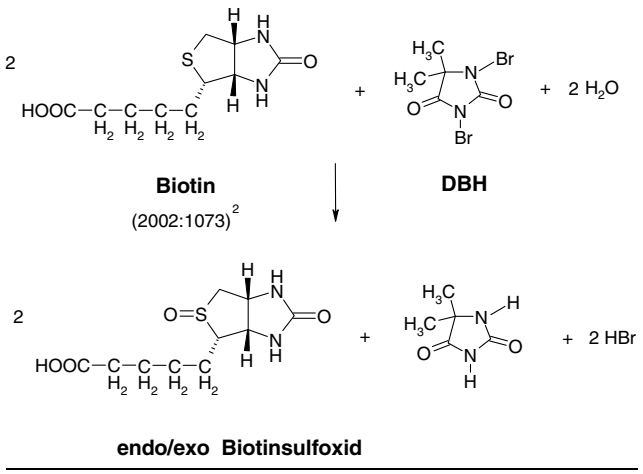


The identification of fluorescein sodium can also be performed with DBH in a simple manner. With DBH/HAc and a fluorescein sodium solution an orange colour appears and the characteristic fluorescence is quenched. By slow addition of sodium hydroxide solution a bathochromic effect to brownish red can be observed. A quenching of the fluorescence in acetic acid solution does not occur in the absence of DBH and presence of bromide ions.

2.2. Identification using the consumption of DBH instead of bromine water decolorization

PH. EUR. 2002 applies the decolorization of bromine water for the identification of biotin, flucytosine, polysorbate 80 and sorbic acid. Bromine water can be replaced by solutions of DBH in glacial acetic acid. However, it is disadvantageous to expell elemental bromine using DBH and bromide ions to observe the decolorization of the test solution. It is substantially simpler to use the failure of the bromination of fluorescein to eosin (Scheme 2) as the basis of a test [2, 8, 9]. Fluorescein shows the above mentioned fluorescence at 366 nm, which is quenched by bromination to eosin. Performing a blank test with water instead of the test solution a brownish orange coloured solution due to the bromination of fluorescein is obtained. This identification test cannot be used concerning flucytosine. It is quite evident that this brominated test material eliminates bromine again and reacts with fluorescein. Polysorbate leads to a fluorescence quenching of fluorescein in UV. However, the analysis and the blank test of polysorbate differ significantly in UV and in the visible region. Also the absence of a brownish yellow colour after the reaction of the test solution with DBH and the addition of a sodium bromide solution is evident for a positive test. The application of sodium iodide instead of sodium bromide is unsuitable because it depends e. g. on the concentration. A bluish colour after the addition of starch solution is only obtained at a definite concentration of iodide and this reaction is too susceptible to failure [2].

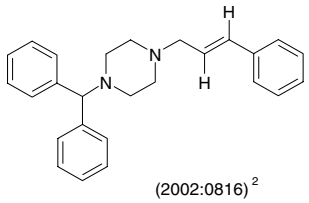
Scheme 3



2.2.1. Biotin

Biotin [12] may be oxidized with DBH analogous to the reaction of *N*-bromosuccinmide [13] resp. calcium hypochlorite [14] to endo/exo biotin sulfoxide [13]. The identification test of biotin can be performed with fluorescein as well as with sodium bromide solution.

2.2.2. Cinnarizine



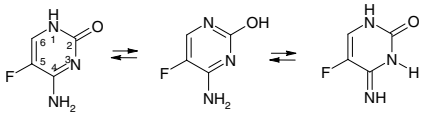
(2002:0816)[2]

According to Mayer and Rücker [15] the decolorization with bromine water is reversible. This could not be confirmed. According to my investigations a yellowish brown colourant is formed, which precipitates when the acetone containing solution is diluted with water. The yellowish brown colourant can be extracted with diethyl ether. As cinnarizine is practically insoluble in water, PH. EUR. 1997 applies acetone for the test solution. However, acetone reacts with bromine to bromoacetone [16]. Therefore, acetone is unsuitable and obviously responsible for the formation of the colourant. If cinnarizine is dissolved in glacial acetic acid, bromine water is discoloured, but in comparison to other compounds of the group the reaction is slow and requires about 5 min. The reaction solution remains colourless even after a longer waiting period. Since the test with acetone as solvent cannot be performed, PH. EUR. 2002 [15] has eliminated the identification by decolorization of bromine water without substitution. The addition of bromine to the phenylogous double bound is the reason, why bromine water is discoloured in acetic acid [15].
DBH/HAc is also applicable for cinnarizine under the above mentioned conditions. A waiting time of at least 5 min is necessary.

2.2.3. Flucytosine

Flucytosine can exist in three tautomeric forms **1a**–**1c** (Scheme 4). The amino-oxo-tautomer **1a** is predominant

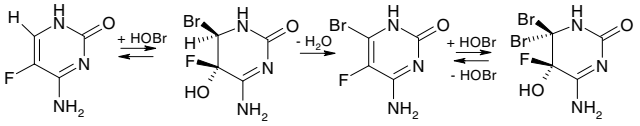
Scheme 4



(2002:0766)[2]

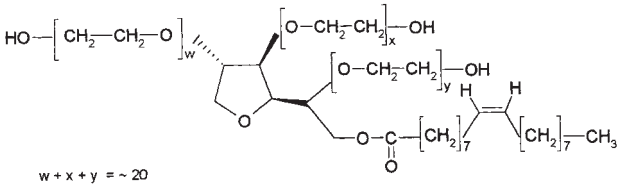
in crystalline form and in polar solvents [17]. From apolar solvents the amino-hydroxy-tautomer **1b** can be isolated.
6-Bromoflucytosine (**3**) according to an addition-elimination mechanism [18] and the unstable products **2** and **4** should be formed as brominated products (Scheme 5). DBH in glacial acetic acid reacts with flucytosine. After the addition of a sodium bromide solution the test solution remains colourless, whereas a blank test with water shows a brownish yellow colouring. However, the identification of flucytosine cannot be performed with fluorescein. The formation of orange coloured eosin and a fluorescence quenching in UV can be observed, even if a waiting time of 30 min and a low concentration of DBH is used for testing. Obviously bromine is only unstably bounded in the brominated products of flucytosine. This may explain the observed conversion to eosin.

Scheme 5



2.2.4. Polysorbate 80

Polysorbate 80 is a mixture of partial esters and polyethylene glycol ethers of sorbitol and its anhydrides with a relative molar mass of about 1286. The non-ionic emulsifier contains oleic acid, which accounts for the decolorization of bromine water by addition of bromine to the unsaturated fatty acid. The amount of 0.5 ml of bromine water according to PH. EUR. 2002 is too high in comparison to the prescribed concentration of polysorbate 80 (about 79 µmol). Therefore a total decolorization cannot be observed. A concentration of about 0.22 mmol of Br_2 results due to the solubility of 35.8 g bromine/l [20]. Consequently only about 0.36 ml of bromine water can be applied for a total decolorization. This agrees with the experimental results. Using 0.4 ml of bromine water the test solution shows a weak yellow colour, whereas with 0.35 ml total decolorization can be observed.



(2002:0428)[2]

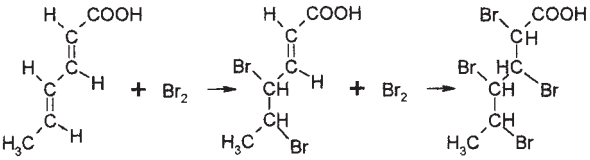
formula see [19]

Applying DBH/HAc the identification test can be performed with fluorescein as well as with sodium bromide. It should be mentioned that in the presence of the non-ionic emulsifier polysorbate 80 the intensity of the typical greenish yellow colour of fluorescein is diminished and a fluorescence in UV cannot be observed. However, analysis and blank test differ significantly at daylight and in UV.
The identification test with bromine water resp. DBH is not necessary on determining the iodine value of 18–24 demanded from PH. EUR. 1997 and Suppl. 2000 (no more in PH. EUR 2002) for polysorbate 80.

2.2.5. Sorbic acid

For the identification test of sorbic acid by decolorization of bromine water the test material is applied in fortyfold molar excess in comparison with bromine. As sorbic acid is sparingly soluble in water the reaction is performed in 96% ethanol. Sorbic acid reacts with bromine in apolar solvents to 4,5-dibrom-2-hexenic acid [21–23] and 2,3,4,5-tetrabromhexanic acid [22] (Scheme 6). According to Font et al. [24, 25] (*E*)-(4*RS*,5*SR*)-5-bromo-4-hydroxy-2-hexenic acid is formed at first with bromine in aqueous solutions.

Scheme 6



(2002:0592)[2]

Using DBH/HAc problems arise due to the small solubility of sorbic acid in water. Thus, DBH does not react with sorbic acid in a test solution with 96% ethanol. The test cannot be performed with fluorescein also when waiting 15 min. With a sodium bromide solution bromine is expelled in the test solution after a waiting time of about 15 min. Sorbic acid is likewise soluble in glacial acetic acid. On the application of this solvent the same problems arise. Apparently elemental bromine must be liberated from DBH at first for the addition to the double bonds of sorbic acid. Therefore, a definite portion of water is necessary. Tests with fluorescein as well as with sodium bromide are possible, if 50% ethanol for the dissolution of the sorbic acid is applied. The best results are obtained, when sorbic acid is dissolved in glacial acetic acid and then diluted with water to 1 M concentration.
In conclusions, aggressive elemental bromine can be replaced advantageously by easy to handle DBH for identification reactions of PH. EUR [1–6]. Cinnarizine can be identified with bromine resp. DBH, if glacial acetic acid instead of acetone as solvent is applied. Lower amounts of test material are required when using DBH. A lower consumption of chemicals is of economic benefit and is also a contribution to environmental protection.

3. Experimental

3.1. Chemicals and drugs

Acetic acid [64-19-7], min. 99.8% p.a., Riedel-de Haën art. 33209 = HAc; acetone [67-64-1], p.a., Riedel-de Haën art. 32201; D (+) biotin [58-85-5],

PH. EUR. 2002, USP 2000, (vitamin H), for biochemical purpose, Merck art. 1643; bromine [7726-95-6], extra pure DAB 6, Merck art. 1945; cinnarizine [298-57-7] PH. EUR. 2002, ratiopharm-Merckle; 1,3-dibromo-5,5-dimethylhydantoin = 1,3-dibromo-5,5-dimethyl-2,4-imidazolidinedione [77-48-5], for synthesis Merck art. 803600 = DBH (for analytical purpose qualified); flucytosine PH. EUR. 2002, USP 2000 [2022-85-7] Hoffmann-La Roche; fluorescein sodium, PH. EUR. 2002, USP 2000 [518-47-8], extra pure, Merck art. 3992; polysorbate 80 [9005-65-6] PH. EUR. 2002, USP/NF 2000, Tween® 80, ICI; sodium bromide [7647-15-6], extra pure, Merck art. 6360; sodium iodide PH. EUR. 2002 [7681-82-5], extra pure, Riedel-deHaën art. 03129; sorbic acid [110-44-1] PH. EUR., Merck art. 100662.

3.2. Solutions

0.01 M DBH/HAc: 0.29 g (0.001 mol) of DBH are dissolved in glacial acetic acid with stirring to 100 ml.

3.3. Identification tests

For comparisons according to PH. EUR. and under other conditions see Hilp [2]. Solutions marked with R correspond to PH. EUR. In contrast to PH. EUR. molar concentrations are used preferably.

3.3.1. Identification of fluorescein sodium

Test solution (0.5 ml) is mixed with 0.5 ml of 0.01 M DBH/HAc. An orange colour arises and no UV fluorescence is to be observed. A colour shift appears to brownish red when 2.0 ml of 5 M NaOH is added slowly.
Test solution (0.005 M): About 19 mg (50 μmol) of fluorescein sodium are dissolved with H_2O to 100 ml.

3.3.2. Identification of biotin, cinnarizine, polysorbate 80 and sorbic acid using the consumption of DBH and the detection with fluorescein sodium

2.5 ml of 0.0025 M test solution are mixed with 0.1 ml of 0.01 M DBH/HAc. Add 0.1 ml of 0.005 M fluorescein sodium solution. A greenish yellow coloured, turbid solution with an intensive fluorescence at 366 nm is obtained. A fluorescence cannot be observed when testing polysorbate 80. A blank test performed with 2.5 ml of water shows an orange colouring without any fluorescence in UV. Cinnarizine requires a waiting time of 5 min after the addition of DBH.

3.3.3. Identification of biotin, cinnarizine, flucytosine, polysorbate 80 and sorbic acid using the consumption of DBH and the detection with sodium bromide

2.5 ml of 0.0025 M test solution are mixed with 0.1 ml of 0.01 M DBH/HAc. Add 0.1 ml of 0.1 M NaBr. The test solution remains colourless. A blank test performed with 2.5 ml of water shows a yellow colouring. Cinnarizine requires a waiting time of 5 min after the addition of DBH.
Test solution (0.0025 M): About 1.5 mg of biotin resp. about 0.81 mg of flucytosine resp. about 8.04 mg of polysorbate 80 are dissolved in 2.5 ml of water, resp. about 2.3 mg of cinnarizine in 2.5 ml of HAc resp. about 0.70 mg of sorbic acid in 2.5 ml of 1 M HAc.

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[1] Part 13: [1]
[2] Year of the monograph published in PH. EUR.: Monograph reference number

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