Institut für Pharmazeutische Chemie der Philipps-Universität, Marburg, Germany

Colour reactions of PH. EUR. for identification of drugs using 1,3-dibromo-5,5-dimethylhydantoin (DBH) instead of elemental bromine

Analytical methods of pharmacopoeias with DBH in respect to environmental and economical concern, part 10[1]

M. HILP

PH. EUR. 2002 and supplements identify aloes (Rosenthaler reaction), amiloride hydrochloride, chlorhexidine, dienestrol, quinidine sulphate, quinine hydrochloride and quinine sulphate (Thalleioquine reaction) and trifluoperazine hydrochloride using elemental bromine. This colour reaction can be performed better with 1,3-dibromo-5,5-dimethylhydantoin (DBH). Some prescriptions of PH. EUR. have been improved in respect to environmental and economical concern. The identification of amiloride hydrochloride with bromine water according to PH. EUR. 2002 or with DBH shows no UV fluorescence as reported in the pharmacopoeia.

1. Introduction

Colour reactions are still relevant for the analysis of drugs and therefore are often applied in PH. EUR. Expensive equipment such as IR, NMR, UV-VIS, MS and others is not necessary. These identification tests are therefore suitable for laboratories of pharmacies and for quality control of drugs in developing countries [2]. According to the guidelines of the WHO [3] the tests should be easy and fast, if a fully equipped laboratory is not available. The reagents and the laboratory equipment required should be restricted to a minimum. Unstable, corrosive, expensive or difficult to obtain reagents should be excluded. Therefore, the application of elemental bromine is not recommendable for colour reactions. DBH is in contrast to elemental bromine a stable and easy to handle crystalline compound [1, 4–11] and contains about 110% active bromine. DBH has been qualified for the determination of iodide [5–7], of the iodine content of organic compounds according to Schöniger [4], iodine value according to Hanuš [10], phenol, resorcin [11] and propylthiouracil [1] Bromine can be replaced by DBH for the identification of lactate [8], fumaric, maleic acid and maleates [9].

2. Investigations, results and discussion

2.1. Identification of aloes according to the Rosenthaler reaction

PH. EUR. 2002 applies the Rosenthaler reaction [12–14] for the identification and differentiation of aloes, barbados, and aloes, cape. An aqueous extract of aloes, barbados, forms a brownish yellow precipitate with bromine water, whereas the supernatant liquid is coloured violet. The aqueous extract of aloes, cape, shows a yellow precipitate with a yellowish orange supernatant. Talc, prescribed by the pharmacopoeia for clarification of the aqueous extract is practically ineffective. The addition of colloidal anhydrous silica (Aerosil® 200, Degussa) is, however, very effective.

Bromine water can be replaced by DBH [1, 4–11]. DBH solutions in acetic acid with sodium acetate buffered or not buffered [5] prove better than alkaline DBH solutions. The latter solutions require acidification with hydrochloric acid, whereby a significantly lower amount of precipitate is formed in comparison to bromine water. The colour shift of the filtrate, which can be observed by addition of DBH/NaOH, does not depend on DBH, but on the NaOH solution (aloes, barbados: dark reddish brown, aloes, cape: pale brownish orange). Using DBH in glacial acetic acid the filtrate of aloes, barbados, yields an explicit violet colouring without precipitation, while the filtrate of aloes, cape, shows only a slightly yellow to weak brownish colour. Aloes, cape leads to a yellow colouring with a yellow precipitate using an acetate buffered DBH solution in acetic acid, aloes, barbados, to a dark brown colouring with a violet cast and a dark brown precipitate. Aloes, barbados, (brownish red) and aloes, cape, (yellow) can be distinguished only by means of the filtrate colour without addition of bromine water or DBH.

2.2. Amiloride hydrochloride

According to PH. EUR. 2002 amiloride hydrochloride gives a greenish yellow colouring after addition of a 20% cetrimide R solution, sodium hydroxide solution and bromine water. After acidification with hydrochloric acid the colour changes to dark yellow, whereby the solution shall show a blue UV fluorescence at 365 nm. The mechanism of the colour reaction is unknown [15]. Using bromine water the formation of a greenish yellow colouring and a change to dark yellow after acidification with hydrochloric acid can be observed according to the pharmacopoeia. An UV fluorescence cannot be detected. The yellow colouring which arises after acidification is partially caused by liberated bromine in the alkaline solution. Also the application of a smaller amount of bromine water and the replacement of hydrochloric acid by dilute sulphuric acid does not cause an UV fluorescence. Cetrimide causes a slight bathochromic shift in alkaline and acidic solution, which is produced partially by the bromide anion of the quaternary salt. The addition of a sodium bromide solution shows the same effect. The addition of cetrimide seems not to be necessary. Using an alkaline DBH-solution the addition of a sodium hydroxide and cetrimide solution is not required. The colouring according to PH. EUR. 2002 can be observed as well in alkaline as in acidic media, but an UV fluorescence cannot be seen.

2.3. Chlorhexidine

PH. EUR. 2002 describes chlorhexidine as diacetate, digluconate solution and dihydrochloride. These biguanidine

derivatives can be identified according to the Sakaguchi-reaction [6]. However, chlorhexidine salts yield a reddish orange colouring with bromine water in alkaline medium and without the addition of 1-naphthol. A dissolution of these substances in a warm 1% solution of cetrimide according to PH. EUR. 2002 is necessary. The mechanism of the colour reaction is unknown [16].

The identification test can be simplified significantly using 0.01 M DBH/0.1 M NaOH. The solubility of the salts for the preparation of a 0.001 M solution is sufficient [16–18] and thus the addition of cetrimide as solubilizer is not required. The addition of sodium hydroxide solution prescribed in PH. EUR. 2002 can also be omitted.

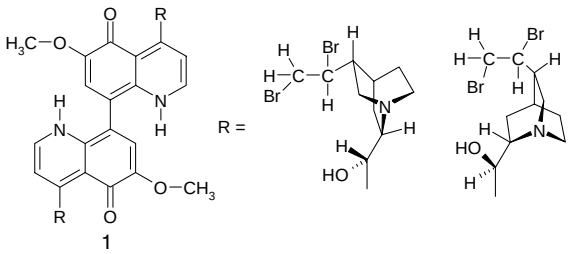
2.4. Dienestrol

PH. EUR. 2002 applies a 1% solution of bromine in acetic acid for the identification of dienestrol [19–21]. The test of the pharmacopoeia is time-consuming and uses hepatotoxic and the environmentally hazardous chloroform. Heating for 2 min on a water bath a colourant is generated and exists as colloid solution. Due to various particle sizes the colour can vary from purple to violet. A part of the obtained solution is mixed with ethanol in order to avoid the coagulation of the colourant particles. After extraction with chloroform the aqueous ethanolic phase is colourless and the organic phase is red coloured. A positive result is also found when testing diethylstilbestrol, whereas diethylstilbestrol dipropionate and diacetate as well as hexestrol are negative [19].

Heating is not necessary when using 0.05 M DBH/HAc. It is possible to replace chloroform by ethyl acetate. The test described can be performed more rapidly, simply and safely in respect to environment.

2.5. Thalleioquine reaction

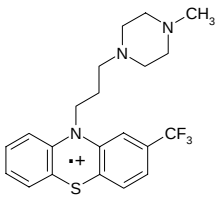
PH. EUR. 2002 describes the Thalleioquine reaction for the identification of quinidine sulphate, quinine hydrochloride and quinine sulphate [22]. Cinchona alkaloids develop an emerald green colouring with bromine water in ammoniacal solution. If bromine water is added and alkalized with ammonia after about 1 min a red colouring is observed. The red colour changes over grey to a greyish green. After 2 min before alkalization a reddish colour arises, which changes to pale green. The addition of ammonia after 30 min leads to a weaker coloured solution. All reaction solutions show a green fluorescence at 366 nm at the surface. According to Takada et al. [23] the red colourant should have the structure **1**. A stable blue radical of high relative molar mass with an unknown structure should be responsible for the green colouring of the Thalleioquine reaction. By the addition of a few amount of ammonia the above described red colour is obtained, which turns by further addition of ammonia over brownish red and grey to green. For the colour test DBH/HAc is necessary. A test solution with a two-fold concentration in comparison to PH. EUR. yields an emerald green colouring with a green UV fluorescence. In order to increase the solubility quinidine sulphate resp. quinine hydrochloride resp. quinine sulphate are dissolved in 0.1 M NaCl. Test solutions with lower concentrations require longer waiting times until a green colouring can be observed. With acetate buffered DBH solution the identification can be characterized better. After the addition of ammonia with various concentrations a red colour appears, which changes over brownish red and grey to greyish green.



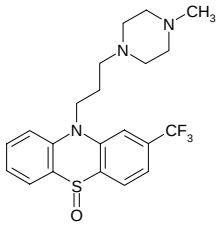
1

2.6. Trifluoperazine hydrochloride

For the identification of trifluoperazine hydrochloride according to PH. EUR. 2002 the phenothiazine is shaken with bromine water. After addition of conc. sulphuric acid drop by drop a reddish violet colouring arises [24–29]. According to Bosch and Kochi [30] a radical cation **2** is formed by the oxidation of trifluoperazine, which is further oxidized to the pale brownish yellow trifluoperazine sulfoxide **3** catalysed by NO resp. NO_2 or nitrite. The colour test using DBH can be improved by the addition of a sodium nitrite solution, whereby the red colour changes immediately to a pale brownish yellow. DBH/HAc does not prove as a suitable reagent for the identification of trifluoperazine hydrochloride. A positive test is only received by heating to get a pale orange colouring. The addition of conc. sulphuric acid drop by drop now leads to a dark violet solution. Without previous heating only a yellowish orange colouring with an extremely weak violet cast can be observed. It is quite evident, that elemental bromine is liberated by heating the DBH solution and is required for the formation of the violet radical cation. Such a colour reaction does not correspond to the demand that tests should be performed simply and fast. However, if bromide ions containing DBH/NaOH is applied, no previous heating is necessary. By cautious addition of conc. sulphuric acid to the mixture of the test solution and DBH/NaBr/NaOH an upper yellow phase and at the junction of the two phases a dark violet colouring arises. After shaking an uniform dark violet solution can be observed.



2



3

2.7. Conclusions

DBH specified for synthesis and delivered by the Merck company, has qualified for all the analytical reactions investigated [1, 4–11]. Storage, transport and weighing of DBH – even the smallest amounts are not problematic. A bromine odour of DBH can be observed scarcely. In contrast to PH. EUR qualitative tests can be performed in the semimicro range, as spot-tests analysis in micro range. Several prescriptions of the pharmacopoeia can been improved. 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; aloes, barbados, Aloe barbadensis PH. EUR. 2002, USP 2000 [8001-97-6], follower of Finzelberg, January 1965; aloes, cape, Aloe capensis PH. EUR. 2002, USP 2000 [8001-97-6], Anzag on 3. 5. 62; amiloride hydrochloride, Amiloridi hydrochloridum PH. EUR. 2002, USP 2000 [2016-88-8], Hennig Arzneimittel; ammonia solution [1336-21-6], approx. 25% NH_3, p. a., Riedel-deHaën art. 30501; bromine [7726-95-6], extra pure DAB 6, Merck art. 1945; cetrimide, Cetrimidum PH. EUR. 2002 [505-86-2], cetrimide 14, trimethyltetradecylammonium bromide, Serva, art. 35868; chlorhexidine diacetate, Chlorhexidini diacetas PH. EUR. 2002 [56-95-1], Sigma art. C-6143; chlorhexidine digluconate solution, Chlorhexidini digluconatis solutio PH. EUR. 2002 [18472-51-0], Novartis Pharma; chlorhexidine dihydrochloride, Chlorhexidini dihydrochloridum PH. EUR. 2002 [3697-42-5], Sigma art. C-8527; chloroform, trichloromethane [67-66-3], extra pure, DAB 9, Merck art. 159129; 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); dienestrol, Dienestrolum PH. EUR. 2002, USP 2000 [84-17-3], Cilag; ethyl acetate, [141-78-6], extra pure, Riedel-deHaën art. 27227; hydrochloric acid [7647-01-0], fuming, 37%, extra pure Merck, art. 100314; quinidine sulphate, Chinidini sulfas PH. EUR. 2002, USP 2000 [6591-63-5], Buchler; quinine hydrochloride, Chinini hydrochloridum PH. EUR. 2002 [130-89-2], Boehringer Mannheim; quinine sulphate, Chinini sulfas PH. EUR. 2002, USP 2000 [6119-70-6], Buchler; silica, colloidal anhydrous, Silica colloidalis anhydrica PH. EUR. 2002, USP 2000 [7631-86-9], Aerosil® Degussa; sodium acetate anhydrous [127-09-3], p. a., Merck art. 106268 = NaAc; sodium bromide [7647-15-6], extra pure, Merck art. 6360; sodium chloride, Natrii chloridum PH. EUR. 2002, USP 2000 [7647-14-5], extra pure, Merck art. 106400; sodium hydroxide, Rotipuran 99% [1310-73-2], Roth, art. 9356; sodium nitrite [7632-00-0], extra pure, Merck art. 106544; sulphuric acid [7664-93-9], p. a. conc., 95–97%, Riedel-deHaën art. 30743; talc, Talcum PH. EUR. 2002 [14807-96-6], Riedel-deHaën art. 18654; trifluoperazine hydrochloride, Trifluoperazini hydrochloridum PH. EUR. 2002, USP 2000 [440-17-5], Röhm Pharma.

3.2. Solutions

0.05 M DBH/HAc: 1.43 g (0.005 mol) of DBH are dissolved in glacial acetic acid with stirring to 100 ml; 0.01 M DBH/HAc is prepared analogously; 0.02 M DBH/6 M HAc/0.2 M NaAc: 2.85 g (0.01 mol) of DBH are dissolved with 170 ml glacial acetic acid and stirring in an amber coloured flask. Under further stirring 250 ml of 0.4 M NaAc and 80 ml of water are added; 0.05 M DBH/0.5 M NaOH: 1.43 g (0.005 mol) of DBH are dissolved with stirring in 0.5 M NaOH to 100.0 ml; 0.01 M DBH/0.1 M NaOH is prepared analogously; 0.01 M DBH/1 M NaBr/0.1 M NaOH: 29 mg (10^{-4} mol) of DBH and 1.03 g (10^{-2} mol) of sodium bromide are dissolved with 0.1 M NaOH to 10 ml; 6 M HAc/0.2 M NaAc: 1.6 g of anhydrous sodium acetate are dissolved in 35 ml glacial acetic acid and water to 100 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.

3.3.1. Identification of aloes according to the Rosenthaler reaction

Powdered aloes (0.1 g) is shaken with 10 ml of boiling water as described in PH. EUR. 2002. After cooling to room temperature 0.05 g of colloidal anhydrous silica PH. EUR. 2002, USP 2000 (Aerosil® 200, Degussa) is added and a clear dark reddish brown (aloes, barbados) resp. yellow (aloes, cape) filtrate is obtained.
If 1.0 ml of the filtrate is mixed with 1 ml of 0.01 M DBH/HAc; a reddish violet with aloes, cape, a yellow to pale brownish colour with aloes, barbados without formation of a precipitate is observed.
If 1 ml of the filtrate is mixed with 1 ml of 0.02 M DBH/6 M HAc/0.2 M NaAc; a dark brown colouring with a violet cast and a dark brown precipitate is observed with aloes, barbados. aloes, cape gives a yellow colour with a yellow precipitate. No reaction results with 6 M HAc/0.2 M NaAc.
If 1 ml of filtrate is mixed with 1 ml of 0.05 M NaOH, aloes, barbados, shows a dark reddish brown, aloes, cape, a pale orange to brown colour without forming a precipitate.

3.3.2. Amiloride hydrochloride

2.5 ml of 0.01 M test solution are mixed with 0.25 ml of 0.05 M DBH/0.5 M NaOH. A greenish yellow colour is arising. After the addition of 0.5 ml of diluted HCl R the solution changes to a brownish yellow colour. No fluorescence in UV can be observed. A blind test performed with 2.5 ml of H_2O shows no colouring. However, a pale brownish yellow colouring can be observed due to the elimination of elemental bromine, which is less intensive in relation to the test solution.
0.01 M Test solution: 30 mg (10^{-3} mol) of amiloride hydrochloride are dissolved with H_2O to 10 ml.

3.3.3. Chlorhexidine diacetate, chlorhexidine digluconate solution, chlorhexidine dihydrochloride

Test solution of the salts of chlorhexidine (0.5 ml, 0.001 M) mixed with 0.2 ml of 0.01 M DBH/0.1 M NaOH result in a reddish orange colour.
0.001 M Test solution: 63 mg of chlorhexidine diacetate resp. 0.45 ml chlorhexidine digluconate solution PH. EUR. 2002 resp. 58 mg chlorhexidine dihydrochloride are dissolved with H_2O to 100 ml.

3.3.4. Dienestrol

Test solution (0.5 ml, 0.01 M) mixed with 0.2 ml of 0.05 M DBH/HAc gives a dark red colour. Dilute with 5 ml of H_2O and shake with 2.5 ml of ethyl acetate. The upper organic phase is dark red coloured, the lower, aqueous phase appears colourless.
0.01 M test solution: 2.7 mg (10^{-5} mol) dienestrol are dissolved in 10 ml of glacial acetic acid.

3.3.5. Thalleioquine reaction (quinidine sulphate, quinine hydrochloride, quinine sulphate)

If 2.5 ml of 0.005 M (corresponding to the base) test solution in 0.1 M NaCl of quinidine sulphate resp. quinine hydrochloride, resp. quinine sulphate is mixed with 0.5 ml 0.02 M DBH/6 M HAc/0.2 M NaAc and 0.4 ml of conc. NH_3 R, a dark red colour arises. Add further 0.1 ml conc. NH_3 R. The colour of the solution changes to brownish red and after about 1 min to green with formation of a green precipitate. A green fluorescence at the surface of the test tube at 366 nm can be observed. A blind test shows no colour.
If 2.5 ml of 0.005 M (corresponding to the base) test solution in 0.1 M NaCl of quinidine sulphate resp. quinine hydrochloride resp. quinine sulphate is mixed with 1 ml of 0.05 M DBH/HAc and 1 ml conc. NH_3 R, an intensive, green colour is arising, which increases during 15 min. A green fluorescence can be observed at 366 nm. A blind test shows no colouring.
0.005 M (corresponding to the base) test solution: 20 mg ($2.5 \cdot 10^{-3}$ mol) of quinidine sulphate resp. quinine sulphate resp. 20 mg ($5 \cdot 10^{-3}$ mol) quinine hydrochloride are dissolved in 0.1 M NaCl solution to 10 ml.

3.3.6. Trifluoperazine hydrochloride

An aqueous 0.001 M test solution (1 ml) is mixed with 0.1 ml 0.01 M DBH/1 M NaBr/0.1 M NaOH. A white turbidity is arising. Add drop by drop 0.5 ml of conc. H_2SO_4 R. A yellow upper phase and at the junction to the sulphuric acid a dark reddish violet colouring appears. After shaking a uniform, dark reddish violet solution can be observed. On adding 0.2 ml of 0.1 M sodium nitrite solution the colour changes immediately to pale brownish yellow, whereas the violet colour remains after dilution with 0.2 ml of H_2O.

Acknowledgements: The author acknowledges Mrs. H. Yanardag for experimental performance and the companies Hennig Arzneimittel GmbH, Flörsheim and Novartis Pharma, Nuremberg for delivery of drugs.

[1] Part 9 [1]

References

1 Hilp, M.: J. Pharm. Biomed. Anal. in print.
2 German Pharma Health Fund e.V. (GPHF): Pharm. Unserer Zeit **28**, 36 (1999)
3 World Health Organization: Basic Tests for Pharmaceutical Substances pp. 1, Geneva 1986
4 Hilp, M.: Freseniuś J. Anal. Chem. **360**, 184 (1998)
5 Hilp, M.: Pharmazie **53**, 321 (1998)
6 Hilp, M.: Habil. thesis, Marburg (2000)
7 Hilp M.; Senjuk, S.: J. Pharm. Biomed. Anal. **25**, 363 (2001)
8 Hilp, M.: Pharmazie **56**, 548 (2001)
9 Hilp, M.: Pharmeuropa **13**, 715 (English version), 697 (French version) (2001)
10 Hilp, M.: J. Pharm. Biomed. Anal. in print
11 Hilp, M.: Pharmazie **56**, 777 (2001)
12 Rosenthaler, L.: Schweiz. Apoth. Ztg. **61**, 640 (1923)
13 Rosenthaler, L.: Pharm. Zentralh. **64**, 507 (1923)
14 Hartke, K.; Hartke, H.; Mutschler, E.; Rücker, G.; Wichtl, M.: Arzneibuch-Kommentar, Wissenschaftliche Erläuterungen zum Europäischen Arzneibuch, 11 suppl. A21 p. 2, Wissenschaftliche Verlagsgesellschaft mbH Stuttgart, Govi-Verlag – Pharmazeutischer Verlag GmbH Eschborn 1999
15 Hartke et al. [14], 1. suppl. A31 p. 2, 1993
16 Hartke et al. [14], 9. suppl. C55 p. 2, 1998

17 Martindale: The complete drug reference, Parfitt, K. (editor), 32. ed. p. 1107, Pharmaceutical Press, London 1999
18 Merck & Co., Inc.: The Merck Index, 12. ed. p. 348, no. 2140, Rahway, N. J., USA. 1996
19 Cocking, T. T.: Analyst **68**, 144 (1943)
20 Cocking, T. T.: Analyst **71**, 66 (1946)
21 Hartke et al. [14], 2. suppl. D22 p. 2, 1993
22 Hartke et al. [14], 11. suppl. C45 p. 2, 1999
23 Takada, A.; Negishi, H.; Ueda T.: Chem. Pharm. Bull. **31**, 2718 (1983)
24 Hartke et al. [14], 5. suppl. T68 p. 3, 1955
25 Kakác, B; Vejdelek, Z. J.: Handbuch der photometrischen Analyse organischer Verbindungen, vol. 2, p. 818, Verlag Chemie Weinheim 1974
26 Lucas G. H. W.; Fabierkiewicz, C.: J. Forensic Sci. **8**, 462 (1963), C. A. **59**, 8545a
27 Marcali, K.: Anal. Chem. **27**, 1586 (1955)
28 Moussa, A. F. A.: Pharmazie **32**, 540 (1977)
29 Post, A.; Warren, R.; Zarembo, J. J. E.; in Florey, K.: Analytical Profiles of Drug Substances vol. 9. p. 543, Academic Press, New York and others 1980
30 Bosch, E.; Kochi, J. K.: J. Chem. Soc. Perkin Trans. 1, 1057 (1995)

Received July 24, 2001
Accepted August 14, 2001

Dr. Manfred Hilp
Institut für Pharmazeutische Chemie
der Philipps-Universität
Marbacher Weg 6
D-35032 Marburg
Hilp@mailer.uni-marburg.de