

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

## Determination of iron limiting values according to PH. EUR. 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 8\*

M. HILP

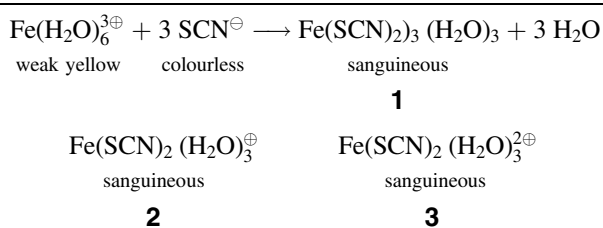
PH. EUR. 2002 uses elemental bromine performing iron limit tests for maleic acid (iron 5 ppm) and titanium dioxide (iron 200 ppm). 1,3-Dibromo-5,5-dimethylhydantoin (DBH) can replace bromine water. For the iron limit test of maleic acid bivalent iron is oxidized to trivalent iron by bromine resp. DBH, because the unsaturated, in high concentration existing acid reacts substantially slower. On the other hand maleic acid removes the excess of bromine. The bromine oxidation for the iron limiting values of titanium dioxide according to the pharmacopoeia is not required. Metallic iron as well as ferrous salts are converted to trivalent iron, when the titanium test solution is prepared by boiling with concentrated sulphuric acid in the presence of anhydrous sodium sulphate.

#### 1. Introduction

PH. EUR. 2002 generally uses the complex formation of thioglycollic acid for the iron limit tests. Bivalent as well as trivalent iron can be determined with this method [2, 3]. This seems not to be a recommendable analytical method, because thioglycollic acid is an extremely toxic, obnoxious-smelling compound, which has to be stored under inert gas [4–6].

Moreover, the determination using thioglycollic acid requires an ammoniacal medium and cannot be applied in the presence of high concentrations of organic resp. inorganic acids such as for the maleic acid and titanium dioxide test solution. Thus PH. EUR. 2002 uses the complex formation of trivalent iron with potassium thiocyanate [7–9] to the iron thiocyanate aquo complexes [10] **1**, **2**, **3**. A limiting value of 5 resp. 200 ppm iron is demanded and visually compared with a standard solution.

#### Scheme



The photometrical determination of iron with thiocyanate is described as very susceptible to failure [7–9]. The detection limit is found below 10 ppm [8]. Furthermore, the absorptivity depends on various factors such as temperature, waiting time after addition of reagents, ionic strength, presence of anions, light and others. However, this method should be sufficient, because the limit tests of PH. EUR. 2002 are only analysed semiquantitatively and visually.

Possibly existent bivalent iron has to be oxidized to trivalent iron with bromine water before the addition of potassium thiocyanate, because ferrous thiocyanate is a colourless compound [11]. According to PH. EUR. 2002 the excess of bromine is blown away with a current of air to avoid the formation of cyanogen bromide [12].

In contrast to elemental bromine 1,3-dibromo-5,5-dimethylhydantoin (DBH) is a stable and easy to handle crystalline compound, and has been qualified in many cases for pharmaceutical analysis [1, 12–18].

#### 2. Investigations and results

Photometrical measurements are carried out and calibration graphs are produced to check the possible application of DBH and the possible simplification of the analytical prescription of PH. EUR. A visual estimation is too imprecise for this purpose.

##### 2.1. Maleic acid (iron 5 ppm)

Maleic acid also consumes bromine and exists in a concentration of  $8.6 \cdot 10^{-3}$  mol per analysis corresponding to about 0.7 mol/l. Due to the solubility of 35.8 g bromine /l [19] a theoretical consumption of about 38 ml (!) of bromine water is required. Yet, maleic acid reacts very slowly with bromine at room temperature. Bromine water is discoloured by a solution of 0.1 M maleic acid at room temperature after about 12 h, whereas only 1–2 min are required at a boiling water bath. The results (Fig. 1) show, that bromine reacts substantially faster with iron(II) ions to trivalent iron than with maleic acid. The amount of bromine applied is sufficient. However, an excess of bromine must not to be removed, because it is bound from the high excess of maleic acid within a waiting time of 10 min. At first the brown coloured solution is discoloured after 5 min. Bromine can be detected only in traces after 5 min. After 10 min allowing to stand, the test with fluorescein paper [20] is negative.

DBH can replace bromine, as also shown in Fig. 1. An existing excess of DBH can also be bound by maleic acid. No more bromine can be detected already after a waiting time of 5 min with fluorescein paper, when DBH is applied.

The oxidation with bromine or DBH is unnecessary for the standard solution, which is prepared with ferric ammonium sulphate according to PH. EUR. An excess of oxidant has not to be removed. Addition of hydrochloric acid is required due to the dependence of the iron thiocyanate complex absorptivity on pH and on the anion.

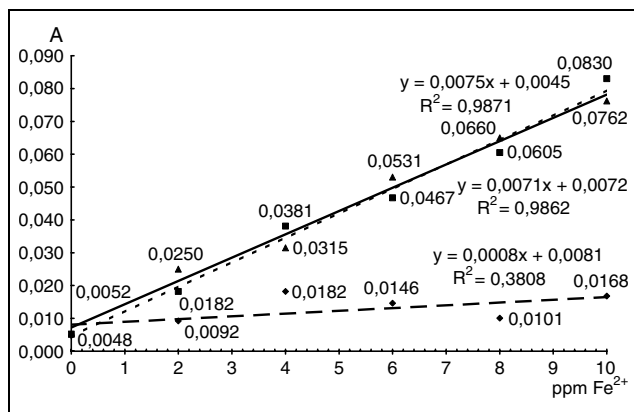


Fig. 1: Photometrical determination of Fe<sup>2+</sup> in presence of maleic acid (ppm referring to the weighed sample of 1 g of maleic acid) —◆— without ... ■... with the addition of bromine —▲— with the addition of DBH

Results of maleic acid samples performed with bromine water agree with those performed with DBH and are calculated by means of a calibration graph carried out with an iron standard solution (2 ppm Fe<sup>3+</sup>).

## 2.2. Titanium dioxide (iron 200 ppm)

Titanium dioxide has to be boiled in concentrated sulphuric acid in a Kjeldahl flask for the preparation of a test solution, because the analyte is insoluble in water, diluted acids and bases. Sodium sulphate has to be added for a boiling-point elevation. Titanium(IV) sulphate, Ti(SO<sub>4</sub>)<sub>2</sub> [21, 22] is formed, which is hydrolyzed on dilution with water to titanium oxide sulphates of various compositions. Possibly existent bivalent iron has to be oxidized to trivalent iron with bromine water as described by maleic acid. Likewise the excess of bromine is blown away with a current of air.

DBH proves significantly better than bromine water mainly for high iron(II) concentrations [12]. In sulphuric acidic solutions at a pH value of about 1.5 DBH is relatively inert to 5-sulphosalicylic acid. An excess of DBH can only be bound in the presence of bromide ions. 5-Sulphosalicylic acid forms a violet complex with ferric ions in acidic solution [23, 24]. However, the violet solution is completely discoloured in the presence of sulphate [24] at a pH value of about 1.5, if the concentration of sulphuric acid corresponds to the concentration of the titanium dioxide test solution. Furthermore, titanium(IV) salts exist in high concentrations in the test solution and result with 5-sulphosalicylic acid in a yellow coloured complex [25, 26] which disturbs the visual and photometrical interpretation [12]. Formic acid is chemically almost inert at a low pH value and inapplicable for the removal of an excess of bromine or DBH.

According to PH. EUR. 2002 0.500 g of titanium dioxide, 5 g of anh. sodium sulphate, glowed at a temperature of 600–700 °C, are boiled with 10 ml of water(!) and 10 ml of conc. sulphuric acid for the preparation of the test solution. The application of 10 g of sodium sulphate decahydrate with or without an addition of water is more advantageous. A blackish brown solid substance [27] temporarily arises, even if the prescription according to PH. EUR. is observed exactly. When boiling in pure sulphuric acid or with addition of only 5 g of sodium sulphate decahydrate the reached temperature is too low and titanium dioxide is not dissolved.

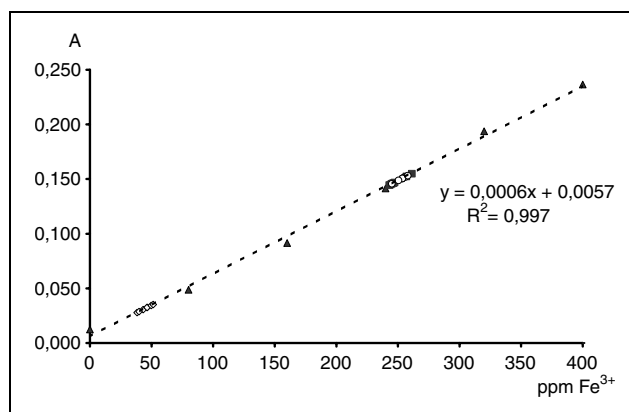


Fig. 2: Photometrical determination of Fe in titanium dioxide —▲— Fe<sup>3+</sup> calibration curve, ◇ TiO<sub>2</sub> about 45 ppm Fe ■ addition of 100 µg of Fe<sup>0</sup> without Br<sub>2</sub> ○ addition of 100 µg of Fe<sup>0</sup> with Br<sub>2</sub>

According to Milbauer [28] iron(II) sulphate is oxidized with sulphuric acid to iron(III) sulphate under the conditions of the Kjeldahl determination at about 200 °C forming sulphur dioxide. Therefore the question arises, whether bivalent iron is oxidized quantitatively to trivalent iron on preparing the titanium dioxide test solution. The oxidation with bromine water according to PH. EUR. should be consequently unnecessary. When about 100 µg of metallic iron equivalent to about 200 ppm Fe (total content about 250 ppm Fe) are added, the results do not differ significantly (F-Test:  $\alpha = 0.22$ ; t-Test:  $\alpha = 0.21$ ) after the digestion with and without bromine water (see Fig. 2). Thus it can be concluded, that an oxidation is not required.

## 3. Discussion

The application of DBH instead of bromine water can be recommended for the iron limit test of maleic acid. However, an excess of bromine or DBH is bound to maleic acid. For titanium dioxide an oxidation of elemental iron and iron(II) is not necessary. The tests can be simplified in comparison to PH. EUR.

A visual determination of a limit test should only be performed if the value found differs explicitly from the demanded limiting value. Otherwise a photometrical determination with a statistical evaluation according to the guidelines of good laboratory practice (GLP, ChemG, append. 1 to § 19 a sect.1) [29] is demanded.

## 4. Experimental

### 4.1. Reagents

Acetic acid [64-19-7], min. 99.8% p. a., Riedel-de Haën art. 33209 = HAC; ammonium iron(III) disulphate dodecahydrate [7783-83-7], ferric ammonium sulphate dodecahydrate, extra pure, Riedel-de Haën art. 12303; bromine [7726-95-6], extra pure DAB 6, Merck art. 1945; diammonium iron (II) disulphate hexahydrate, [10045-89-3], ferric ammonium sulphate p.a., Merck art. 103793; 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); fluorescein sodium, Fluoresceinum natrium PH. EUR. 2002, USP 2000 [518-47-8], extra pure, Merck art. 3992; formic acid [64-18-6], 98–100%, extra pure, Merck art. 263; hydrochloric acid [7647-01-0], Acidum hydrochloricum concentratum PH. EUR. 2002, fuming, 37%, extra pure Merck art. 100314; iron, metallic [7439-89-6], fine powder, Riedel-de Haën art. 12310; maleic acid, Acidum maleicum PH. EUR. 2002 [110-16-7], extra pure, Riedel-de Haën art. 27709; potassium thiocyanate 333-20-0 pure, Merck art. 5124; sodium sulphate [7757-82-6], anhydrous, Natrii sulfas anhydricus PH. EUR. 2002, fine powder, extra pure, Merck, art. 6645; sodium sulphate decahydrate [7727-73-3], Natrii sulfas decahydricus PH. EUR. 2002, extra pure, Merck

art. 6642; 5-sulphosalicylic acid dihydrate [5965-83-3], extra pure, Merck, art. 689 = SSS; sulphuric acid [7664-93-9], p. a. conc., 95–97%, Riedel-de Haën art. 30743; titanium dioxide, Titanii dioxidum PH. EUR. 2002, USP 2000 [13463-67-7], extra pure Merck art. 805.

#### 4.2. Solutions

0.02 M DBH/HAc: 0.57 g (0.002 mol) of DBH are dissolved in glacial acetic acid with stirring to 100 ml; iron standard solution (2 ppm  $Fe^{2+}$ ) is prepared by dissolution of iron standard solution (200 ppm  $Fe^{2+}$ ) with  $H_2O$  immediately before use; iron standard solution (2 ppm  $Fe^{3+}$ ) and iron standard solution (4 ppm  $Fe^{3+}$ ) are prepared by dissolution of iron standard solution (20 ppm Fe) R; iron standard solution (200 ppm  $Fe^{2+}$ ): 0.702 g of diammonium iron (II) disulphate hexahydrate are dissolved in 25 ml of 1 M sulfuric acid and  $H_2O$  to 500 ml; maleic test solution (200 g/l): 50 g of maleic acid are dissolved in about 100 ml of  $H_2O$  by heating. The solution is filtered and then diluted to 250 ml with  $H_2O$ ; titanium dioxide test solution II modified: 0.500 g of titanium dioxide and 10 g of sodium sulphate decahydrate and 10.0 ml of sulphuric acid are heated to boiling with the usual precautions. After the interim formation of a brownish black precipitate [27] and after a waiting time of about 10 to 15 min a clear, a yellow coloured solution is obtained. Cool to room temperature and add slowly 30.0 ml of water and 10.0 ml of sulphuric acid R. Cool again and dilute with  $H_2O$  to 100.0 ml.

#### 4.3. Limit tests

Comparisons according to PH. EUR. and under other conditions see Hilp [12]. Solutions marked with R correspond to PH. EUR. In contrast to PH. EUR. molar concentrations are used preferably.

##### 4.3.1. Maleic acid; iron limit test (5 ppm)

Maleic acid test solution (5.00 ml, 200 g/l) is mixed with 2.00 ml of 2 M HCl and 1.00 ml of 0.02 M DBH/HAc. After 5 min 2.50 ml of 1 M KSCN R are added. As standard 5.00 ml of iron standard solution (1 ppm  $Fe^{3+}$ ) R, 2.00 ml of 2 M HCl R and 1.00 ml of HAc are mixed the same time and then 2.50 ml of 1 M KSCN R are added. The test solution should not be coloured more intensely than the standard solution. The absorptivity of the test solution, measured at 480 nm, should be less or equal that of the standard solution, if the visual estimation is ambiguous.

##### 4.3.2. Decolorization of bromine water by maleic acid

23 mg ( $2 \times 10^{-4}$  mol) of maleic are dissolved in 2.0 ml of  $H_2O$  and 0.5 ml of bromine water are added. The solution is discoloured after about 12 h at room temperature or 1–2 min in a boiling water bath.

##### 4.3.3. Oxidation of iron(II) ions by bromine water resp. DBH in presence of maleic acid

Maleic acid test solution (5.00 ml, 200 g/l) is mixed with a ml of iron standard solution (2 ppm  $Fe^{2+}$ ), b ml of  $H_2O$  and 1.00 ml of HAc, 2.00 ml of 2 M HCl R with or without the addition of 0.05 ml of bromine water resp. 1.0 ml of 0.02 M DBH/HAc. After 5 min the excess of bromine is removed with a stream of nitrogen. A filter paper, which is impregnated with 0.001 M fluorescein sodium solution [20], serves for testing bromine traces. 2.5 ml of 1 M KSCN are added, after 15 min the transmission is measured at 480 nm and the absorptivity ( $A = 2 - \log T\%$ ) is calculated. The ppm Fe contents relate to the weight of maleic acid.

Without oxidant: 0 ppm Fe: b = 6.50, A = 0.0048; 2 ppm Fe: a = 1.00, b = 5.50, A = 0.0092; 4 ppm Fe: a = 2.00, b = 4.50, A = 0.0182; 6 ppm Fe: a = 3.00, b = 3.50, A = 0.0146; 8 ppm Fe: a = 4.00, b = 2.50, A = 0.0101; 10 ppm Fe: a = 5.00, b = 1.50, A = 0.0168.

With 0.05 ml of bromine water: 0 ppm Fe: b = 6.45, A = 0.0052; 2 ppm Fe: a = 1.00, b = 5.45, A = 0.0182; 4 ppm Fe: a = 2.00, b = 4.45, A = 0.0381; 6 ppm Fe: a = 3.00, b = 3.45, A = 0.0467; 8 ppm Fe: a = 4.00, b = 2.45, A = 0.0605; 10 ppm Fe: a = 5.00, b = 1.45, A = 0.0830.

With 1.0 ml of 0.02 M DBH/HAc: 0 ppm Fe: b = 5.50, A = 0.0052; 2 ppm Fe: a = 1.00, b = 4.50, A = 0.0250; 4 ppm Fe: a = 2.00, b = 3.50, A = 0.0315; 6 ppm Fe: a = 3.00, b = 2.50, A = 0.0531; 8 ppm Fe: a = 4.00, b = 1.50, A = 0.0650; 10 ppm Fe: a = 5.00, b = 0.50, A = 0.0762.

##### 4.3.4. Determination of the iron limiting value of maleic acid using bromine water resp. DBH on the basis of a calibration curve with an iron standard solution (2 ppm $Fe^{3+}$ )

Calibration: a ml of iron standard solution (2 ppm  $Fe^{3+}$ )R, b ml of  $H_2O$ , 1.00 ml of HAc and 2.00 ml of 2 M HCl R are mixed and 2.5 ml of 1 M KSCN R are added. After 15 min the transmission is measured at 480 nm. b = 10.00, A = 0.0035; a = 1.00, b = 9.00, A = 0.0044; a = 2.00, b = 8.00, A = 0.0155; a = 3.00, b = 7.00, A = 0.0325; a = 4.00, b = 6.00, A = 0.0438; a = 5.00, b = 5.00, A = 0.0680.

Iron limiting value: For the determination of the iron limiting value 5.00 ml of the maleic acid test solution (200 g/l) are mixed on application of 0.05 ml of bromine water with 4.95 ml of  $H_2O$ , 1.00 ml of HAc and 2.00 ml of 2 M HCl R, on application of 1.00 ml of 0.02 M DBH/HAc with 5.00 ml of  $H_2O$ , 2.00 ml of 2 M HCl R. Wait 5 min and then add 2.5 ml of 1 M KSCN R. After 15 min the transmission is measured at 480 nm The Fe content of maleic acid results on the basis of the calibration curve or can be calculated from equation generated by Excel with the correlation straight line ( $y = 0.0065x - 0.0048$ ).

Maleic acid using bromine water: A = 0.0159, 0.0141, 0.0123; found: 3.2, 2.9, 2.6 ppm Fe.

Maleic acid using DBH: A = 0.0177, 0.0155, 0.0141; found: 3.5, 3.1, 2.9 ppm Fe.

##### 4.3.5. Titanium dioxide; iron limit test (200 ppm)

Titanium dioxide test solution II (10.00 ml) modified according to PH. EUR. 2002 is mixed with 2.50 ml of 1 M KSCN R. As standard 5.00 ml of iron standard solution (2 ppm  $Fe^{3+}$ ) R, 5.00 ml of 7 M sulphuric acid and 2.50 ml of 1 M KSCN R are mixed at the same time. The test solution should not be coloured more intensely than the standard solution. The absorptivity of the test solution, measured at 480 nm, should be less or equal that of the standard solution, if the visual estimation is ambiguous.

##### 4.3.6. Photometrical determination of the iron limiting value for titanium dioxide on addition of metallic iron (ca. 100 $\mu$ g) with and without bromine oxidation

Calibration: a ml of the iron standard solution (4 ppm  $Fe^{3+}$ ) are mixed with b ml of  $H_2O$ , 5.00 ml of 7 M sulphuric acid and 2.50 ml of 1 M KSCN R. After 15 min the transmission is measured at 480 nm and the absorptivity is calculated ( $A = 2 - \log T\%$ ).

Calibration: b = 5.05 ml, A = 0.0123; ~80 ppm: a = 1.00 ml, b = 4.05, A = 0.0487; ~160 ppm: a = 2.00 ml, b = 3.05, A = 0.0915; ~240 ppm: a = 3.00 ml, b = 2.05, A = 0.142; ~320 ppm: a = 4.00 ml, b = 1.05, A = 0.194; ~400 ppm: a = 5.00 ml, b = 0.05 ml, A = 0.237.

Fe-content of titanium dioxide without bromine oxidation: 10.0 ml of the titanium dioxide standard solution II modified according to PH. EUR. 2002 are mixed with 0.05 ml of  $H_2O$  and 2.50 ml of 1 M KSCN R. After 15 min T% is measured at 480 nm.

Found.: 43; 46; 46; 50; 38; 40; 51 ppm Fe;  $\bar{x}$  = 45 ppm Fe;  $s_{rel}$  = 10.8%.

Addition of 205.0  $\mu$ g  $Fe^0$  to 1.000 g of titanium dioxide:

Calc.: ca. 250 ppm Fe; found.: 261; 257; 247; 245; 243; 246; 244 ppm Fe;  $\bar{x}$  = 249 ppm Fe;  $s_{rel}$  = 7.07%.

Fe-content of titanium dioxide with bromine oxidation and addition of 205.0  $\mu$ g  $Fe^0$  to 1.000 g of titanium dioxide: 0.05 ml of  $H_2O$  are replaced with 0.05 ml of bromine water. After 5 min the excess of bromine is removed with a current of nitrogen and worked as described above.

Found.: 256; 254; 254; 245; 258; 254; 250 ppm Fe;  $\bar{x}$  = 253 ppm Fe;  $s_{rel}$  = 4.15%.

Comparison without and with bromine oxidation: F-Test:  $\alpha$  = 0.220; t-Test:  $\alpha$  = 0.205.

#### 4.4. Statistical methods

Diagrams and evaluations were performed with Excel 97 on an IBM-compatible PC running under Windows 95. The built-in F- and t-test routine of Excel 97 has been used.

Acknowledgement: The author acknowledges Mrs. H. Yanardag for experimental performance.

\* Part 7 [1].

#### References

- Hilp, M.: J. Pharm. Biomed. Anal. in print
- Hartke, K.; Hartke, H.; Mutschler, E.; Rucker, G.; Wichtl, M.: Arzneibuch-Kommentar, Wissenschaftliche Erläuterungen zum Europäischen Arzneibuch, 10. suppl., 2.4. Grenzprüfungen, p. 7., Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Govi-Verlag – Pharmazeutischer Verlag GmbH, Eschborn, 1999
- Geffken, D.; Surborg, K. H.: Dtsch. Apoth. Ztg. **128**, 1235 (1988)
- <http://www.chemie.fu-berlin.de/chemistry/safety/chemsafety.html>
- Kühn-Birett, Merkblätter Gefährliche Arbeitsstoffe, ecomed, Landsberg/Lech, vol. 8, 13. suppl. 11/80 p T 03, 1980
- Falbe, J.; Regitz, M.: Römpp Chemie Lexikon, 9. ed., vol. 6, p. 4586, G. Thieme, Stuttgart, New York 1992,
- Kolthoff, I. M.; Elving, P. J.: Treatise on Analytical Chemistry, part. II, vol. 2, p. 286, Interscience Publishers, New York, London 1965
- Holzbecher, Z.; Divis, L.; Král, M.; Sucha, L.; Vlacil, F.: Handbook of Organic Reagents in Inorganic Analysis, p. 558, Ellis Horwood Limited, Chichester 1976

- 9 Lange, B.: Kolorimetrische Analyse, 5. ed., p. 145, Verlag Chemie, Weinheim 1956
- 10 Holleman-Wiberg: Lehrbuch der Anorganischen Chemie, 101. ed., p. 1521, de Gruyter, Berlin, New York, 1995
- 11 Meyer, R. J., Deutsche Chemische Gesellschaft (editor), Gmelins Handbuch der anorganischen Chemie, Eisen part B, system-No. 59, 8. ed., p. 743, Verlag Chemie, Berlin 1932
- 12 Hilp, M.: Habil. thesis, Marburg 2000
- 13 Hilp, M.: Fresenius' J. Anal. Chem. **360**, 184 (1998)
- 14 Hilp, M.: Pharmazie **53**, 321 (1998)
- 15 Hilp M.; Senjuk, S.: J. Pharm. Biomed. Anal. **25**, 363 (2001)
- 16 Hilp M.: Pharmazie **56**, 548 (2001)
- 17 Hilp M.: Pharmeuropa **13**, 715 (English version), 697 (French version) (2001)
- 18 Hilp M.: Pharmazie **56**, 777 (2001)
- 19 Weast, R. C.: Handbook of Chemistry and Physics, 57. ed., p. B-96, CRC Press, Cleveland 1976
- 20 Okác, A.: Qualitative Analytische Chemie, p. 512, Akademische Verlagsgesellschaft Geest & Portig K.-G., Leipzig 1960
- 21 Lit. 2: 5. suppl., T 49 p. 239 1995
- 22 Holleman-Wiberg [10], p. 1064
- 23 Fries, J.; Getrost, H.: Organische Reagenzien für die Spurenanalyse, p. 140, E. Merck Darmstadt 1975
- 24 Lange [9], p. 150
- 25 Lange [9], p. 151
- 26 Lange [9], p. 362
- 27 Williamson, W. O.: Mineralog. Mag. **25**, 513 (1940), C. A. **34**, 5348<sup>2</sup> (1940)
- 28 Milbauer, J., Bull. soc. chim. [5] **3**, 218 (1936)
- 29 Kühn-Birett [5], vol. 2, III-1.1, 131. suppl. 10/2000, ChemG. from 25.7.94, p. 34, 2000

Received June 6, 2001  
Accepted August 1, 2001

Dr. Manfred Hilp  
Institut für Pharmazeutische Chemie  
Marbacher Weg 6  
D-35032 Marburg  
Hilp@mail.uni-marburg.de