ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 81–86

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Determination of iodine values according to Hanuš using 1,3-dibromo-5,5-dimethylhydantoin (DBH) Analytical methods of pharmacopeias with DBH: part 7☆

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Received 23 April 2001; received in revised form 19 August 2001; accepted 8 September 2001

Abstract

USP/NF 2000 [The United States Pharmacopeia, Rockville USA, 24th ed., 2000, p. 1868, The National Formulary, 19th ed. 2000] and PH. EUR. 1997 [European Pharmacopoeia, third ed., Council of Europe, Strasbourg, 1997, pp. 63–64] determine the iodine values according to Hanuš with iodine monobromide in glacial acetic acid. This reagent can be replaced by a solution of 1,3-dibromo-5,5-dimethylhydantoin (DBH) and potassium iodide or iodine in the same solvent. Both reagents yield equivalent results by means of method comparison according to Passing and Bablok [J. Clin. Chem. Clin. Biochem. 21 (1983) 709; 22, (1984) 431] in relation to the official method of PH. EUR.

Keywords: Iodine value; DBH, dibromantin; 1,3-dibromo-5,5-dimethylhydantoin, 1,3-dibromo-5,5-dimethyl-2,4-imidazolidinedione [77-48-5]

1. Introduction

The iodine value is characteristic for the content of unsaturated fatty acids in fats, fixed oils, emulsifiers and solubilizers [1–4]. Halogen is added to the double bonds. After the addition of potassium iodide the excess of the halogenating agent reacts to iodine, which is titrated with thiosulfate. Errors can result by substitution of hydrogen atoms at the aliphatic carbon atoms through halogen. Therefore, chlorine is unsuitable for the determination, whereas bromine can be applied in diluted solutions only to avoid the substitution. On the other hand, iodine is too inactive. Several methods for the determination of the iodine values have been developed since more than 100 years [5,6]. Von Hübl has formulated and defined in his publications of 1884 for the first time the term 'iodine value' [5] (iodine degree [6]) as the amount of iodine absorbed of the fat calculated in units percent. The Hübl-method activates iodine by addition of mercuric chloride, which considerably accelerates the reaction.

☆ [Professorial dissertation (2000) Marburg; Fresenius J. Anal. Chem. 360 (1998) 184; Pharmazie 53 (1998) 321; 56 (2001) 548; J. Pharm. Biomed. Anal. 25 (2001) 363; Pharmeuropa 13 (2000) in print].

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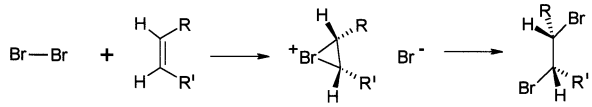
0731-7085/02/$ - see front matter
PII: S0731-7085(01)00632-X



Bromonium ion

The method according to Hanuš [7–10], using a solution of iodine monobromide in glacial acetic acid, is up to date the official pharmacopoeia determination in Europe since PH. EUR. 1 [11] and mostly applied by USP/NF 2000 [12]. Iodine monobromide is in equilibrium with iodine and bromine. The degree of dissociation of iodine monobromide to bromine and iodine amounts 8% in carbon tetrachloride at 25 °C [13].

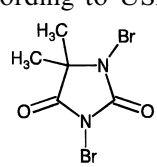
$$2IBr \rightleftarrows I_2 + Br_2$$

According to Suzuki and Koizumi [14,15], only bromine adducts are formed by the Hanuš method, so that the term 'bromine value' [7–9] instead of iodine value may be more correct.

PH. EUR. 1997 [16] and USP 2000 produce the halogenating reagent by dissolving iodine monobromide in glacial acetic acid, whereas USP 1995 [17] and AOAC [18] are mixing equivalent amounts of a standardized iodine solution and a standardized bromine solution in the same solvent.

USP/NF 2000 demands the determination of an iodine value in 40 monographs. PH. EUR. 1997 prescribes the determination of an iodine value in 28, with PH. EUR. Supplement 1998 [19] in 33 monographs.

Iodine monobromide is a very toxic compound, whose vapors are irritant to the eyes and mucous membranes [20] and, therefore, it is difficult to weigh up. In contrast to iodine monobromide or elemental bromine dibromantin (DBH) is a stable and easy to handle crystalline compound [21–26], DBH has been qualified for the determination of iodide [22,23,26], of the iodine content of organic compounds according to Schöniger [21,26] and for identification tests [24–26]. Now it is shown, that DBH can also facilitate the determination of the iodine value according to USP and PH. EUR.



2. Experimental

2.1. Materials

Acetic acid [64-19-7] min 99.8% p.a., Riedel-de Haën art. 33209 = HAc; 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); iodine, Iodum PH. EUR. 1997, USP 2000 [7553-56-2], Riedel-de-Haën art. 3002; iodine monobromide, [7789-33-5] for synthesis, Merck art. 820378; potassium iodate [7758-05-6] p.a., volumetric standard, Merck art. 5053; potassium iodide [7681-11-0] $\geq$ 99.5%, p.a., Roth, D-76185 Karlsruhe, art. 6750; sodium acetate anhydrous [127-09-3] = NaAc, p.a., Merck art. 106268; sodium thiosulfate pentahydrate [7772-98-7] > 98.5%, Roth art. 8649; starch soluble [9005-84-9] extra pure Erg. B. 6, Merck art. 101253.

2.1.1. Fats and fixed oils

Almond oil [8007-69-0], *Amygdalae oleum*, H. Lamotte, Bremen; arachis oil, peanut oil [8002-03-7], *Arachidis oleum*, H. Lamotte, Bremen; avocado oil [8024-32-6], *Avocado oleum*, H. Lamotte, Bremen; Becel, diet-margarine, Union Deutsche Lebensmittelwerke, Hamburg; butter, Markenbutter, Süßrahmbutter, Hessenmilch Kassel; castor oil [8001-79-4], *Ricini oleum*, Mainland, Pharm. Fabrik, Frankfurt; cocoa butter [8002-31-1], *Cacao oleum*, Caelo, Hilden; cod-liver oil [8001-69-2], *Oleum jecoris*, H. Lamotte, Bremen; Flora soft, 80% fat, Union Deutsche Lebensmittelwerke, Hamburg; Lätta, semi-bold margarine 40% fat, Union Deutsche Lebensmittelwerke, Hamburg; lard, Adeps suillus, Mainland, Pharm. Fabrik, Frankfurt; Linseed oil [8001-26-1], *Oleum lini*, H. Lamotte, Bremen resp. Unimills, Hamburg; oleyl oleate, *Oleyis oleas*, Cetiol®, charge 1 and charge 2, Henkel, Düsseldorf; olive oil [8001-25-0], *Olivae oleum*, H. Lamotte, Bremen; Rama, margarine, breakfast quality, 80% fat, Union Deutsche Lebensmittelwerke, Hamburg; sesame oil [8008-74-0], *Sesami oleum*, Mainland, Pharm. Fabrik, Frankfurt; soya oil [8001-22-7], *Sojae oleum*, H. Lamotte, Bremen; sunflower oil [8001-21-6], *He-*

lianthi oleum, H. Lamotte, Bremen; wheat germ oil [8006-95-9], *Oleum tritici germinum*, Caelo, Hilden.

2.2. Solutions

DBH/I_2-solution, 3.57 g (1.25×10^{-2} mol) of DBH and 6.35 g (2.5×10^{-2} mol) of iodine are dissolved in 500 ml of glacial acetic acid. When heating the iodine in glacial acetic acid on a water bath, cooling to room temperature and dissolving DBH afterwards, the preparation time can be reduced. DBH/KI-solution, 7.15 g (2.5×10^{-2} mol) of DBH and 8.30 g (5×10^{-2} mol) of potassium iodide are dissolved in glacial acetic acid to 500 ml. 1/60 M KIO_3, 3.567 g of potassium iodate p.a., volumetric standard, are diluted to 1000.0 ml; 0.25 M NaAc/10 M HAc, pH-buffer about 3.0–20.5 g of anhydrous sodium acetate are dissolved in 570 ml of glacial acetic acid and diluted to 1000.0 ml with water; 0.1 M $Na_2S_2O_3$ is prepared according to PH. EUR. 1997 and standardized with 20.00 ml of 1/60 M KIO_3, 10.0 ml of 0.25 M NaAc/10 M HAc, 5.0 ml of 1 M KI and 0.5 ml of starch solution, iodide-free (PH. EUR. 1997); starch solution, iodide-free PH. EUR. 1997, without HgI_2, is stable at a temperature of about 4 °C for about 6 weeks. It is necessary to avoid a temperature below 0 °C.

2.3. Assays

General information: the sample weight depends on the estimated iodine value and the corresponding details of PH. EUR. 1997. Samples of about 0.15–0.5 g are put into a cut off micro test tube of about 1 cm in length and about 0.6 cm in diameter on a micro balance (e.g. Mettler M5). If a larger amount of the sample is required, the iodine flasks are tared on an analytical balance (e.g. Mettler B5) and the samples are put into the flask directly. Also 0.5 ml of starch solution, iodide-free, (PH. EUR. 1997) are added at the end of the titration with 0.1 M $Na_2S_2O_3$.

The iodine value determinations of fixed oils and fats are performed according to the exact prescription of PH. EUR. 1997. Then 25.00 ml of iodine monobromide solution is replaced with 25.00 ml DBH/KI resp. 25.00 ml DBH/I_2 for the recommended method.

For the determination of the iodine value of the margarine Becel and Lätta with iodine monobromide resp. DBH/KI about 0.5 g of the samples are accurately weighed. In deviation from the prescription according to PH. EUR. 1997, the sample is dissolved in 15 ml of chloroform and 20 ml of glacial acetic acid. The margarine Flora soft yields a turbid solution with chloroform, which is used for the determination. The margarine Lätta forms due to its additives such as skimmed milk and edible gelatin a flocculent, partly a threadlike precipitate, which persists until the end of the titration and absorbs iodine.

2.3.1. Iodine value determination of the margarine Lätta after extraction with chloroform

About 0.5 g of the margarine Lätta are accurately weighed on a glass boat and extracted five times each with 2.5 ml of chloroform and 2.5 ml of water. Two milliliters of ethanol 1% denaturized with petroleum ether are added for a better phase separation. In order to determine the iodine value according to PH. EUR. 1997 the combined chloroform layers are used.

2.3.2. Stabilities of the halogenating reagents for the determination of the iodine value at room temperature under light protection

Iodine monobromide, decrease% (days): 1.6 (30); 1.8 (60); 1.5 (90); 2.2 (120); 2.3 (150). DBH/KI, decrease% (days): 1.4 (30); 3.4 (60); 4.2 (90); 4.9 (120); 5.1 (150). DBH/I_2, decrease% (days): 18 (30); 22 (60); 25 (90); 26 (120); 28 (150).

2.4. Statistical methods

Evaluations and graphics have been performed with EXCEL 97 on an IBM-compatible PC running under WINDOWS 95. The built-in *F*- and *t*-test routines of EXCEL 97 have been used. The transformation of the Passing–Bablok algorithm [27–29] to an EXCEL-macro according to Dr Martin Holz, D79395 Neuenburg has been applied.

3. Results and discussion

As shown in Table 1, DBH and potassium iodide or DBH and iodine can substitute iodine

Table 1
Determination of the iodine value of fixed oils and fats using DBH/KI resp. DBH/I_2 in comparison with iodine monobromide according to PH. EUR. 1997

Material	Expected iodine value	Iodine value according to PH. EUR. 1997 with								
		1Br			DBH/KI			DBH/I_2		
		n	Mean (%)	R.S.D. (%)	*n*	Mean (%)	R.S.D. (%)	*n*	Mean (%)	R.S.D. (%)
Almond oil, *Amygdalae oleum*	USP/NF 2000: 95–105	7	98.8	0.33	7	99.1	0.59	7	99.1	1.05
Arachis oil, peanut oil, *Arachidis oleum*	USP/NF 2000: 84–100	7	92.8	0.22	7	93.2	0.45	7	92.6	0.49
Avocado oil, *Avocado oleum*	DAC 1986 [30]: 80–90	7	85.7	0.17	7	85.4	0.24	7	85.9	0.32
Becel diet margarine 80% fat	90.4[a]	7	93.6	0.37	7	93.8	0.29			
Butter 'Süßrahmbutter'	21–29[b]	7	26.4	1.12	7	26.2	0.41			
Castor oil, *Ricini oleum*	PH. EUR. 1997: 82–90	7	85.8	0.27	7	85.4	0.62	7	85.4	0.54
Cocoa butter, *Cacao oleum*	DAB 2000 [31]: 33–42	7	35.4	0.63	7	35.0	0.49			
Cod-liver oil, *Oleum jecoris*	DAB 7 [32]: 150–180	7	163.1	0.23	7	163.4	0.20	7	162.7	0.49
Flora soft 80% fat	79.3[a]	7	64.4	0.27	7	64.5	0.13			
Lätta, semi-bold margarine 40% fat	29.2[a]	7	31.6	0.97	7	31.5	1.46			
		7	31.1[c]	1.01						
Lard, *Adeps suillus*	DAB 2000 [31]:46–60	7	57.5	0.34	7	57.6	0.19	7	57.9	0.59
Linseed oil, H. Lamotte, *Oleum lini*	DAB 7 [32]: 165–190	7	192.4	0.18	7	191.8	0.35	7	192.0	0.31
Linseed oil, Unimills *Oleum lini*	DAB 7: 165–190	7	180.8	0.07	7	181.1	0.21	7	180.9	0.36
Oleyl oleate, *Oleyis oleas*, Cetiol®, charge 1	DAB 2000: 85–105	7	98.4	0.27	7	98.1	0.21	7	98.8	0.10
Oleyl oleate, *Oleyis oleas*, Cetiol®, charge 2	DAB 2000: 85–105	7	96.6	0.16	7	96.8	0.13	7	96.3	0.22
Olive oil, *Olivae oleum*	USP/NF 2000: 79–88	7	81.5	0.37	7	81.9	0.63	7	81.8	0.30
Rama, margarine breakfast quality 80% fat	59.2[a]	7	59.6	0.50	7	60.0	0.41			
Sesame oil, *Sesami oleum*	USP/NF 2000: 103–116	7	111.1	0.32	7	111.4	0.29	7	111.9	0.57
Soya oil, *Sojae oleum*	USP/NF 2000: 126–140	3	132.5	0.27	7	133.4	0.14	–		
Sunflower oil, *Helianthi oleum*	DAC 1986: 120–140	7	134.3	0.27	7	134.3	0.32	7	134.2	0.40
Wheat germ oil, *Oleum tritici germinum*	128[d]	7	128.1	0.28	7	128.1	0.33	7	128.2	0.34

[a] Calculated to the found iodine value of the fatty phase according to Wijs and the fat content reported by the Union Deutsche Lebensmittelwerke GmbH.
[b] Calculated to the fat content of 82–84% [33] of butter and the iodine value of milk fat with 26–35 [34].
[c] Extraction with chloroform.
[d] As reported from the company Caelo GmbH.

monobromide under the same conditions of PH. EUR. 1997. Due to additives the margarines Becel and Lätta are not completely soluble in chloroform. In deviation of PH. EUR. 1997 a mixture of chloroform and acetic acid is used for the determination with iodine monobromide and DBH/KI. As Lätta forms shortly after dissolution a flocculent, partly a thread-like precipitate, which is caused by the ingredients skimmed milk and edible gelatin, the determination of the iodine value with chloroform extracted fatty phase is performed. No significantly different results are obtained.

A solution of DBH/KI, if possible, is preferable compared with a solution of DBH/I_2. Iodine is more difficult to weigh due to its volatility and irritating vapors. It dissolves much slower in glacial acetic acid at room temperature [16,17] than potassium iodide. During 1 month DBH/KI has a similar stability as the iodine monobromide solution, if protected from light. The efficiency of DBH/I_2 decreases fast.

On the determination of iodine values large deviations of the results are tolerated. The values with iodine monobromide according to PH. EUR. 1997 agree with those using DBH/KI resp. DBH/I_2. The statistical evaluations according to the *F*-test (precision) and to the *t*-test (accuracy) are unsuitable for the comparison of the determination with the three different reagents. Relative small differences in the analytical values lead in some cases to significant disagreements in both directions. This may be particularly based on the fact, that the analytical procedures are performed during different days (inter-assay) with different room temperature and different light intensity. Furthermore, high precisions of small varying results as shown in Table 1 lead to false outliers [35]. If the statistical evaluations are performed with the application of DBH/KI, as well as of DBH/I_2 by means of the method comparison according to Passing and Bablock [27–29], no significant differences to the method of PH. EUR. using iodine monobromide are found (see Figs. 1 and 2).

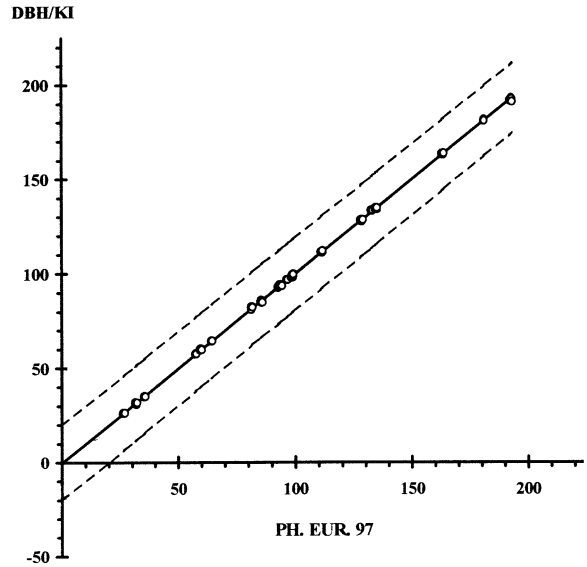


Fig. 1. Method comparison according to Passing–Bablok of the iodine value determination-using DBH/KI in relation to iodine monobromide according to PH. EUR. 1997.

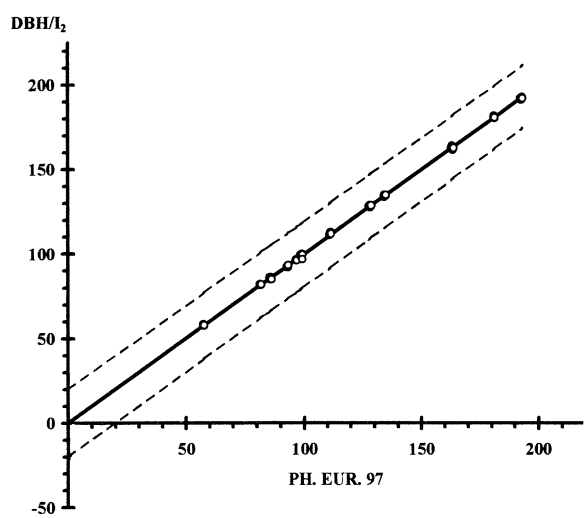


Fig. 2. Method comparison according to Passing–Bablok of the iodine value determination-using DBH/I_2 in relation to iodine monobromide according to PH. EUR. 1997.

4. Conclusions

The iodine value serves for the characterization of fats and fixed oils. The preparation of the halogenating reagent for iodine value determination using DBH and potassium iodide instead of very toxic and difficult to handle iodine monobromide can facilitate significantly the method of USP 2000 and PH. EUR. 1997. Furthermore, DBH is low-priced in comparison to iodine monobromide. The price reduction accounts about 70% for IBr, reagent PH. EUR. [36] resp. about 60% for IBr for synthesis [36].

Acknowledgements

The author acknowledges Professor Dr. A. Fahr for the statistical evaluation according to Passing–Bablok, C. Hegenbart and B. Reitz for experimental performance and the company H. Lamotte, Bremen for delivery of fatty oils.

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