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Identification of lactate

Analytical methods of pharmacopoeias with DBH in respect to environmental and economical concern, part 4[1]

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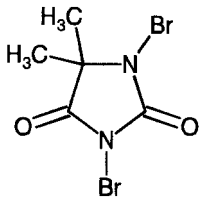
The identification of lactate according to Ph. Eur. 1997 and DAB 2000 uses the oxidation of lactic acid to pyruvic acid by boiling with bromine water in sulphuric acid. Acetaldehyde arising by decarboxylation is detected according to Legal applying a time consuming and troublesome procedure. 1,3-Dibromo-5,5-dimethylhydantoin (DBH) as well as potassium bromate can replace elemental bromine. Lactic acid and all lactates of Ph. Eur. 1997 and DAB 2000 can be identified better and faster using lactate oxidase (LOD, test strip system Accusport®). According to DAB 2000 the base of ethacridine lactate has to be separated. This is no longer necessary, if an enzymatic identification is applied.

1. Introduction

Ph. Eur. 1997 includes the monographs calcium lactate pentahydrate, calcium lactate trihydrat, lactic acid and sodium lactate solution, DAB 2000 ethacridine lactate monohydrate and potassium lactate solution. All monographs similarly identify lactate according to the prescription 2.3.1. of Ph. Eur. 1997 (identification reactions of ions and groups). The liberated lactic acid is oxidized to pyruvic acid by boiling with bromine water in sulphuric acid solution [2, 3]. Acetaldehyde arising by decarboxylation is detected as colour complex according to Legal [7, 8]. Bromine water has to be removed in the heat, then solid ammonium sulphate has to be dissolved and afterwards a solution of sodium pentacyanonitrosylferrate in diluted sulphuric acid has to be added drop by drop without mixing. Now the reaction mixture is covered with a layer of concentrated ammonia. After 30 min a dark green ring appears at the junction of the two liquids. The test is very time consuming and troublesome to perform. Working in an exhaust hood is required.

DBH, an easy to handle, crystalline, organic compound can replace elemental bromine [1, 2, 9, 10]. The application of DBH for the identification of lactate to simplify this test is investigated.



2. Investigations, results and discussion

2.1. Oxidation with DBH and potassium bromate

DBH as well as a solution of potassium bromate and potassium bromide resp. potassium bromate and hydrochloric acid can replace elemental bromine for the identification of lactate (see Table 1 und Table 2). It can be demonstrated, that lactic acid is only oxidized to pyruvic acid, if the acidified test solution is boiled with liberated bromine. Also the concentrations of DBH resp. potassium bromate and potassium bromide significantly affect the test of lactate. Using 5-sulphosalicylic acid (SSS) [1, 2, 9–11] the excess of bromine can be removed without interferences. Heating of the lactate test solution with alkaline DBH solution and the following acidification leads to a negative result. On the other hand a positive test is found, if sodium pyruvate is added under the same reaction conditions. A 1% solution of sodium pentacyanonitrosylferrate is more advantageous than the 10% solution of the pharmacopoeia. The recognition of the formed green ring is improved.

2.2. Identification of lactate with lactat-oxidase

As the lactate test of Ph. Eur. 1997 cannot be improved significantly by the application of DBH resp. potassium bromate, a biochemical test system to identify lactate is investigated. The monograph glucose, liquid, of Ph. Eur. Suppl. 1999 applies e. g. test sticks containing glucose oxidase and peroxidase for the identification of glucose. Test strip systems with lactate oxidase for the quantitative determination of lactate in blood by reflection photometry are commercially available. This determination is applied mainly in sports medicine for training control and training management. For the investigation the test strip system Accusport® of Boehringer Mannheim/Hestia (Roche Diagnostics) [2, 4–6] is used (Fig.).

+(L)-Lactate is oxidized stereospecifically to pyruvate in the presence of lactate oxidase, whereby the compound **1** serves as a mediator and transfers 2,18-phosphomolybdic acid to molybdenblue. Lactic acid and the lactate of the pharmacopoeias exist mainly as racemates. Physiologic

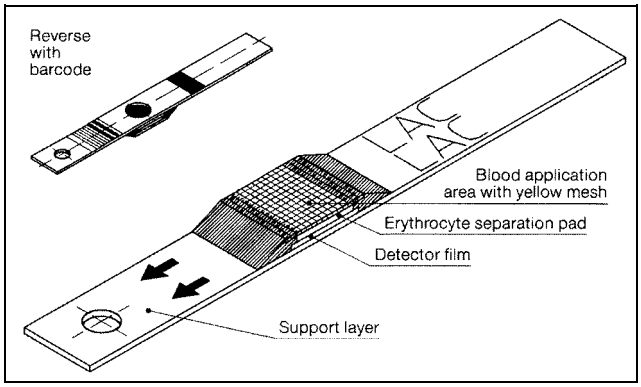


Fig.: Construction of the lactate test strip system Accusport® of Boehringer Mannheim GmbH/Hestia (Roche Diagnostics)

Table 1: Identification of lactate with DBH modified according to Ph. Eur. 1997 (1% sodium pentacyanonitrosylferrate in 1 M H_2SO_4)

DBH	KBr	Acid	Boiling	Sodium pyruvate	Test
86 mg $3 \cdot 10^{-4}$ mol	71 mg $6 \cdot 10^{-4}$ mol	0.5 ml 1 M H_2SO_4	+		−
29 mg 10^{-4} mol	24 mg $2 \cdot 10^{-4}$ mol	0.5 ml 1 M H_2SO_4	+		−
14 mg $5 \cdot 10^{-5}$ mol	12 mg 10^{-4} mol	0.5 ml 1 M H_2SO_4	+		+
7 mg $2.5 \cdot 10^{-5}$ mol	6 mg $5 \cdot 10^{-5}$ mol	0.5 ml 1 M H_2SO_4	+		(+)
14 mg $5 \cdot 10^{-5}$ mol		0.5 ml 1 M H_2SO_4	+		−
14 mg		0.5 ml 32% HCl	+		(+)
14 mg		1.0 ml 32% HCl	+		−
14 mg		1.0 ml 10% HCl	+		+
14 mg		0.5 ml 10% HCl	+		−
1 ml 0.05 M DBH−0.5 M NaOH		0.5 ml 32% HCl	+		1–2 min +
1 ml 0.05 M DBH−0.5 M NaOH		1 ml 32% HCl	+		15 min (+)
1 ml 0.05 M DBH−0.5 M NaOH		after 5 min 1 ml 10% H_2SO_4 (pH 1.3) + 5 ml 1 M SSS	−		− brown ring
1 ml 0.05 M DBH−0.5 M NaOH		after 5 min 1 ml 10% H_2SO_4 (pH 1.3)	−	5 mg	+
0.5 ml 0.05 M DBH−0.5 M NaOH		after 5 min 1 ml glacial HAc +3 ml 1 M SSS (pH 1.3)	−		−
0.5 ml 0.05 M DBH−0.5 M NaOH		after 5 min 1 ml glacial HAc + 3 ml 1 M SSS	−	5 mg	+
0.5 ml 0.05 M DBH−0.5 M NaOH		after 5 min + 0.5 ml glacial HAc (pH 2.9) + 1.5 ml 1 M SSS	−		−
0.5 ml 0.05 M DBH−0.5 M NaOH		after cooling to room temperature 0.5 ml glacial HAc (pH 2.9) + 1.5 ml 1 M SSS	+		−
0.5 ml 0.05 M DBH−0.5 M NaOH		after cooling to room temperature 1 ml 10% H_2SO_4 + 1.5 ml 1 M SSS	+		−
0.5 ml 0.05 M DBH−0.5 M NaOH		after cooling to room temperature 0.5 ml glacial HAc (pH 2.9) + 1.5 ml 1 M SSS	+	5 mg	+
0.5 ml 0.05 M DBH−0.5 M NaOH		after cooling to room temperature + 1 ml 10% H_2SO_4 + 1.5 ml 1 M SSS	+	5 mg	+

(+) = slightly positive

Table 2: Identification of lactate with potassium bromate modified according to Ph. Eur. 1997 (1% Sodium pentacyanonitrosylferrate in 1 M H_2SO_4)

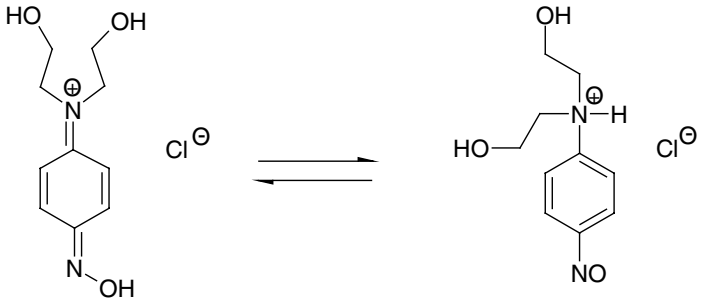
$KBrO_3$	KBr	Acid	Boiling	Test
32 mg $1.9 \cdot 10^{-4}$ mol	115 mg $9.7 \cdot 10^{-4}$ mol	0.5 ml 10% H_2SO_4	+	_
10 mg $6 \cdot 10^{-5}$ mol	36 mg $3 \cdot 10^{-4}$ mol	0.5 ml 10% H_2SO_4	+	_
5 mg $3 \cdot 10^{-5}$ mol	18 mg $1.5 \cdot 10^{-4}$ mol	0.5 ml 10% H_2SO_4	+	+
2.5 mg $1.5 \cdot 10^{-5}$ mol	9 mg $7.5 \cdot 10^{-5}$ mol	0.5 ml 10% H_2SO_4	+	(+)
1 ml 0.03 M $KBrO_3$−0.045 M KBr		0.5 ml 10% H_2SO_4	+	+
1 ml 0.03 M $KBrO_3$−0.045 M KBr		1.0 ml 10% H_2SO_4	+	+
0.5 ml 0.03 M $KBrO_3$−0.15 M KBr		0.5 ml 10% H_2SO_4	+	+
0.5 ml 0.03 M $KBrO_3$−0.015 M KBr		1.0 ml 10% H_2SO_4	+	+
0.5 ml 0.015 M $KBrO_3$−0.075 M KBr		0.5 ml 10% H_2SO_4	+	(+)
0.5 ml 0.015 M KBrO3−0.075 M KBr		1.0 ml 10% H_2SO_4	+	+
0.2 ml 0.015 M $KBrO_3$−0.075 M KBr		0.5 ml 10% H_2SO_4	+	−
0.2 ml 0.015 M $KBrO_3$−0.075 M KBr		1.0 ml 10% H_2SO_4	+	(+)
1 ml 0.01 M $KBrO_3$−0.01 M KBr		0.5 ml 10% H_2SO_4	+	(+)
1 ml 0.01 M $KBrO_3$−0.01 M KBr		1.0 ml 10% H_2SO_4	+	+
0.5 ml 0.01 M $KBrO_3$−0.01 M KBr		0.5 ml 10% H_2SO_4	+	(+)

Table 2 (continued)

$KBrO_3$	KBr	Acid	Boiling	Test
0.5 ml 0.01 M $KBrO_3$	−0.01 M KBr	1.0 ml 10% H_2SO_4	+	(+)
0.5 ml 0.01 M $KBrO_3$	−0.01 M KBr	1.0 ml 10% H_2SO_4	−	− brown ring
1 ml 0.01 M $KBrO_3$	−0.01 M KBr	1.0 ml 10% H_2SO_4	−	− brown ring
1 ml 0.01 M $KBrO_3$	−0.01 M KBr	1.0 ml 10% H_2SO_4 after 5 min 200 µl 0.2 M SSS	−	− brown ring
1 ml 0.01 M $KBrO_3$	−0.01 M KBr	1.0 ml 10% H_2SO_4 after heating 200 µl 0.2 M SSS	+	+
1 ml 0.01 M $KBrO_3$	−0.005 M KBr	1.0 ml 10% H_2SO_4	+	+
1 ml 0.01 M $KBrO_3$	−0.001 M KBr	1.0 ml 10% H_2SO_4	+	+
0.5 ml 0.01 M $KBrO_3$		0.5 ml 32% HCl	+	+

(+) = slightly positive

Scheme: Enzymatic identification of lactate with test strip system Accusport® of Boehringer Mannheim GmbH/Hestia (Roche Diagnostics)



1 a

Bis-(2-hydroxyethyl)-4-hydroxyliminocyclo= hexa-2,5,-diethylidene ammoniumchloride

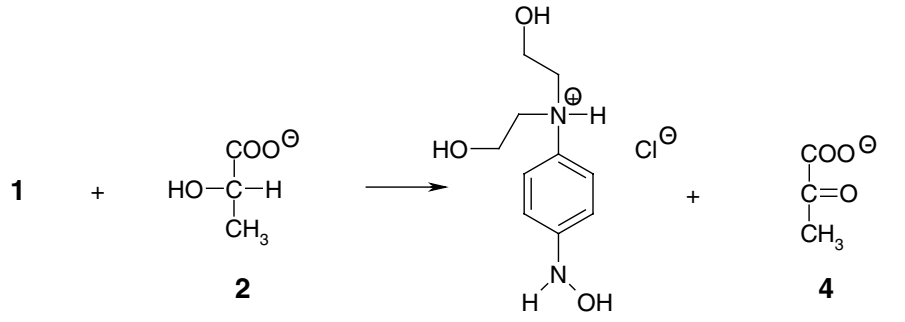
1b

N,N-bis(2-hydroxyethyl)-4-nitro= soaniline hydrochloride

Ethanol, 2,2´- [(4-nitrosophenyl)imino]bis-monohydrochloride

[53743-87-6]

1



2

(+) L-Lactate

(+)-(S)-Lactate

3

N,N-bis(2-hydroxyethyl)-4-hydro= xylaminoaniline hydrochloride

4

Pyruvate

3 + 2,18-Phosphomolybdic acid $[P_2Mo_{18}O_{62}]^{6-}$ → Molybdenblue + 1

conditions must be observed in the identification of lactate with an enzymatic test. As the pH-optimum of lactate oxidase lies at 6–7 [12], the test solution of lactic acid must be neutralized e.g. with 0.33 M phosphate buffer solution pH 7.5 Ph. Eur. 1997. Concerning the lactates of the pharmacopoeias a pH-regulation is not necessary. Likewise false negative results are obtained with high concentrations of lactates. A concentration of about 0.05 M proves suitably. Whereas identifying lactate of ethacridine lactate according to Ph. Eur. 1997 resp. DAB 2000 the base of the salt must be separated. This can be avoided, if the developed enzymatic identification is applied. As expected the result with ethacridine hydrochloride, prepared according to the literature 13, is negative.

3. Experimental

3.1. Materials

Acetic acid, min. 99.8% p.a. [64-19-7], Riedel-de Haën, Art. 33209 = HAc; ammonium concentrated solution [1336-21-6] about 25% NH_3, for analysis, reag. ISO, reag. Ph. Eur., Riedel-de Haën, Art. 3051; ammonium sulphate [7783-20-2], Riedel-de Haën, extra pure, Art. 11225; bromine [7726-95-6], extra pure DAB 6, Merck Art. 1945; calcium lactate pentahydrate [63690-56-2], Boehringer Ingelheim KG; 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); disodium hydrogen phosphate [7778-77-0] R Ph. Eur. 1997, p.a. Merck Art. 6580; ethacridine lactate monohydrate [1837-57-6] Vetter Pharma-Fertigung GmbH & Co. KG./Ravensburg; hydrochloric acid [7647-01-0] 32% p.a., Merck Art. 1.00319; lactic acid [50-21-5], 90% m/m, extra pure, Merck, Art. 366; potassium bromate [7758-01-2] extra pure FCC, Merck Art.104914; potassium bromide [7758-02-3], extra pure DAB, Ph. Eur, BP, Merck Art.104900; potassium dihydrogen phosphate [7778-77-0] R Ph. Eur. 1997, p.a., ISO, Merck Art. 4873; potassium lactate solution [996-31-6], 60% (m/m), Purac biochem bv, Gorinchem/The Netherlands; sodium hydroxide [1310-73-2] Rotipuran, 99%, Roth Art. 9356; sodium lactate solution [72-17-3] 50% (m/m), Purac biochem bv, Gorinchem/The Netherlands; sodium pyruvate [113-24-6], Boehringer Mannheim; sodium pentacyanonitrosylferrate, sodium nitroprusside [13755-38-9] reag. Ph. Eur., Merck Art. 159332; 5-sulphosalicylic acid dihydrate [5965-83-3], extra pure, Merck Art. 689 = SSS, sulphuric acid [7664-93-9], p.a., conc., 95–97%, Riedel-deHaën Art. 30743.

3.2. Solutions

0.025 M Calcium lactate, pH = 6.5: About 68 mg ($2.5 \cdot 10^{-4}$ mol) of calcium lactate trihydrate resp. 77 mg ($2.5 \cdot 10^{-4}$ mol) of calcium lactate pentahydrate are dissolved in 10 ml of H_2O. 0.05 M DBH–0.5 M NaOH: 1.43 g ($5 \cdot 10^{-3}$ mol) of DBH are dissolved under stirring in 0.5 M NaOH to 100.0 ml. 0.05 M Ethacridine hydrochloride: 316 mg ($1.25 \cdot 10^{-5}$ mol) of ethacridine base [13] are dissolved in 25 ml of 0.05 M hydrochloric acid. The pH-value is increased from 2.9 to 7.8 with 350 µl of 0.1 M NaOH. A performed lactat test is negative. 0.05 M Ethacridine lactate: 452 mg ($1.25 \cdot 10^{-3}$ mol) of ethacridine lactate monohydrate are dissolved with heating in 25 ml of H_2O. The enzymatic test for lactate is positive. Increasing the pH-value to 8.1 by addition of 50 µl of 0.1 M NaOH, the lactate test is also positive. 0.03 M $KBrO_3$ – 0.045 M KBr: 501 mg ($3 \cdot 10^{-3}$ mol) of potassium bromate and 536 mg ($4.5 \cdot 10^{-3}$ mol) of potassium bromide are dissolved in H_2O to 100 ml. The $KBrO_3$–KBr solutions of Table 2 are produced in the same manner. 0.05 M Potassium lactate, pH = 6.5: About 107 mg ($5 \cdot 10^{-4}$ mol) potassium lactate solution 60% (m/m) are mixed with H_2O to 10 ml. 0.05 M Lactic acid, pH = ~6.6: About 50 mg ($5 \cdot 10^{-4}$ mol) of lactic acid Ph. Eur. 90% (m/m) are mixed with H_2O to 10 ml and neutralized with 0.5 ml of 0.33 M phosphate buffer solution pH 7.5 Ph. Eur. 1997 to a pH-value between 6–7. 0.05 M Sodium lactate solution, pH = 6.5: About 112 mg ($5 \cdot 10^{-4}$ mol) of sodium lactate solution 50% (m/m) are mixed with H_2O to 10 ml. 0.2 M SSS: 5.1 g (0.1 mol) of 5-sulfosalicylic acid dihydrate are dissolved with H_2O to 100 ml. 1 M SSS: 25.4 g (0.1 mol) of 5-sulfosalicylic acid dihydrate are dissolved with H_2O to 100 ml.

3.3. Identification of lactate with lactate oxidase (recommended)

1–2 Drops (about 25 µl) of 0.05 M lactate are brought on the top side of the lactate test strip system Accusport® of the company Boehringer Mannheim GmbH/Hestia (Roche Diagnostics). Within 1 min the yellow colour of the circular detection zone on the bottom side changes to deep green.

Lactic acid has to be neutralized to a pH-value between 6–7. In order to get a pH value of about 6.6, it is necessary to add 0.5 ml of 0.33 M phosphate buffer solution pH 7.5 R Ph. Eur. 1997 to 5 ml of 0.05 M lactic acid.

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[1] Part 3 [1].

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