

# Application Bulletin

Of interest for:  
Pharmacy  
Food analysis

No. 224/1 e

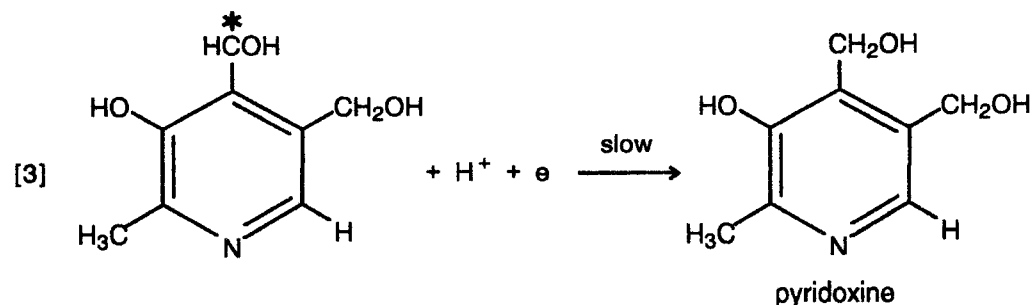
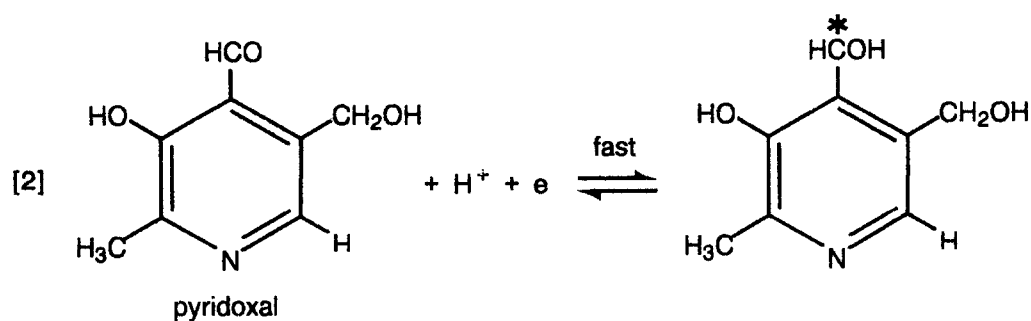
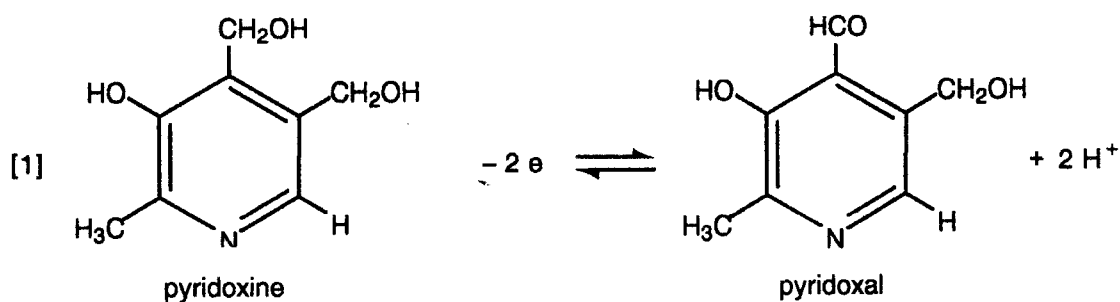
## Polarographic determination of pyridoxine (vitamin B<sub>6</sub>)

### Summary

► This work is one of a series of Application Bulletins published at varying intervals dealing with the polarographic determination of vitamins. It describes the determination of pyridoxine (vitamin B<sub>6</sub>). The method given allows determination in monovitamin and in some multivitamin preparations. The linearity range of the analysis is also specified. The limit of determination is ca. 2 µg pyridoxine · HCl / 20 mL cell volume.

### Theory

► Pyridoxine · HCl is oxidised to pyridoxal by treatment with "active" manganese dioxide in a phosphate buffer pH = 6.8...7.0 [1]. At pH ≈ 13, the electrochemical reduction at the DME or SMDE occurs in two steps. The first, reversible and fast reaction step leads to an intermediate and is used for the determination [2]. The second, slow and irreversible reaction step leads back to pyridoxine [3].



### Apparatus

► 2.646.003X VA Processor with 2.647.0020 VA Stand or  
2.506.0010 Polarecord or 2.626.0010 Polarecord with 2.663.002X VA Stand

<b>Reagents</b>	<p>For preparation of the solutions, only reagents of analytical purity grade and ultrapure water should be used.</p> <ul style="list-style-type: none"> <li>▶ <b>Active manganese dioxide:</b> Manganese(IV)oxide, active e.g. Merck No. 6037</li> <li>▶ <b>c(NaOH) = 2.5 mol/L:</b> NaOH (25 g) is dissolved in ultrapure water and after cooling the solution made up to 250 mL.</li> <li>▶ <b>Phosphate buffer: pH = 6.8 ... 7.0</b> Na<sub>2</sub>HPO<sub>4</sub> · 7H<sub>2</sub>O (7.16 g) and 2.8 g KH<sub>2</sub>PO<sub>4</sub> are weighed into a beaker and dissolved in 950 mL ultrapure water. The pH value is then adjusted to 6.8...7.0 by addition of 2 mol/L H<sub>3</sub>PO<sub>4</sub> or 2.5 mol/L NaOH and the solution made up to 1 liter with ultrapure water.</li> <li>▶ <b>Standard solutions:</b> <p><b>ρ(pyridoxine · HCl) = 3000 mg/L</b>  The stock solution is freshly prepared every day in quantities not exceeding 50 mL. The content of the starting material must be taken into account. Pyridoxine · HCl (150 mg or correspondingly more) is dissolved in 30 mL phosphate buffer, the solution made up to 50 mL with ultrapure water and stored in a refrigerator.</p> <p><b>ρ(pyridoxal) = 180 mg/L</b>  An aliquot (3.00 mL) of the above pyridoxine standard is pipetted into a 50 mL conical flask and 17 mL phosphate buffer added. After addition of 2.1 g MnO<sub>2</sub>, the flask is stoppered and shaken for 1 h. The contents are then filtered through a filter paper into a 50 mL volumetric flask and the conical flask and filter paper washed 6 times with 3 mL aliquots of phosphate buffer. The purified filtrates are made up to the mark (50 mL) with phosphate buffer. This standard must also be freshly prepared every day and stored in a refrigerator.  1 mL = 180 µg pyridoxine · HCl als pyridoxal</p> </li> </ul>
<b>Sample preparation</b>	<ul style="list-style-type: none"> <li>▶ <b>Tablets and capsules</b>  10 tablets or the contents of 10 capsules are weighed exactly to determine the average weight and then pulverised (mortar or grinder). The average weight of one tablet or the content of a capsule is weighed into a centrifuge tube, which can be stoppered, and 20.0 mL phosphate buffer added. After addition of 2.1 g manganese dioxide, the tube is stoppered and shaken for one hour. MnO<sub>2</sub> is separated out in a centrifuge at 5000 rpm.</li> </ul>
<b>Method</b>	<ul style="list-style-type: none"> <li>▶ To 14.5 mL phosphate buffer in a polarographic vessel are added 500 µL of the clear sample solution and 5 mL c(NaOH) = 2.5 mol/L and deaeration performed for 5 min with nitrogen. The DP polarogram is then recorded (amplitude -50 mV) at the DME or SMDE between -1.0 V and -1.5 V.</li> <li>▶ The peak potential of pyridoxal is at ca. -1.25 V.</li> <li>▶ The content is determined by double or triple standard addition, each with 200 µL standard.</li> <li>▶ <b>Figs 1 to 5</b> show the parameter settings of the 646 VA Processor.</li> </ul>

**Polarographic determination of pyridoxine (vitamin B<sub>6</sub>)**

<b>Linearity</b>	▶ <b>Fig. 8</b> shows a graph of nA/μg pyridoxine. It is not intended to represent a calibration curve, but solely to illustrate the approximate slope of the curve. The curve is linear up to 1.7 mg/20 mL.
<b>Remarks</b>	▶ After the determination of pyridoxine, nicotinamide could also be analyzed in the same solution. (Sweep between -1.4 V and -1.9 V with appropriate standard additions.)  ▶ Not only tablet constituents such as methyl cellulose or polyvinyl pyrrolidone, but also the actual tablet coatings can adversely affect the sensitivity of the determination. In several cases it is advisable to remove the tablet coatings before pulverising.
<b>Literature</b>	▶ Jacobsen, E. / Tommelstad, T.M. <i>Differential pulse polarographic determination of pyridoxine in multivitamin tablets.</i> <i>Anal. Chim. Acta</i> <b>162</b> , (1984) 379-383  ▶ Göbbeler, K.H. / Breinlich, J. <i>Quantitative wechselstrompolarographische Simultanbestimmung von Vitaminen der B-Gruppe.</i> <i>Pharm. Ztg.</i> <b>48</b> , (1972) 1859-1862  ▶ Söderhjelm, P. / Lindquist, J. <i>Electrochemical assay of thiamine, riboflavine, pyridoxine, nicotinamide and ascorbic acid in pharmaceutical preparations.</i> <i>Acta Pharm. Suec.</i> <b>13</b> , (1976) 201-212

## Polarographic determination of pyridoxine (vitamin B<sub>6</sub>)

**Fig. 1** Example program page 2, 646 VA Processor

1	Detn. of Pyridoxine.HCl in Vitamin Preparations	METHOD	PAGE 2
2	MPL 1 EL.TYPE MME	GEN.SPECIFICATIONS	
PARAMETERS			
3	IR.MODE		N
4	SPEED		5
5	D.SIZE		5
6	N.DROPS		4
RECOGNITION			
7	SPIKE THRESH		3
8	H.THRESH		2
9	U.TOL		7
10	W.TOL		7
11	ASYM.TOL		8

**Fig. 2** Example program page 3, 646 VA Processor

1	Detn. of Pyridoxine.HCl in Vitamin Preparations	METHOD	PAGE 3
2	MPL 1 EL.TYPE MME	OPERATION SEQUENCE	
OPERATIONS/PARAMETERS			
1	PURGE ;STIR ;		5 s
2	[ADDL ;OPURGE;OSTIR ;		5 s
3	DME ;		
4	MEAS ;		5 s
4a	M.MODE DPN		-50 mV
4b	T.STEP		800 ms
4c	U.SET		-1.000 V
5	SWP 0 ;		100 s
5a	U.END		-1.500 V
5b	U.STEP		4 mV
	SW.RATE		5.0 mV/ s
6	OMEAS ;PURGE ;STIR ;		
7	BEEP ;ADD1]3;		25 s
8	OMEAS ;OPURGE;OSTIR ;		
9	BEEP ;END ;		

**Fig. 3** Example program page 4, 646 VA Processor

1	Detn. of Pyridoxine.HCl in Vitamin Preparations	METHOD	PAGE 4			
2	MPL 1 EL.TYPE MME	ALLOCATIONS				
	a	b	c	d	e	f
	SOLUTE	U.VERIF	DOS	V.SOLN	m.CONC	m.BLANK
	Subst	Ux	Soln	c, v	rho.x	bx
1	Vit.B6	-1.250 V	1	c 200 uL	180.0 mg/L	0.000 g
2						
3						
4						
5						
6						
7						
8						
9	SUPP.ELEC	Buffer 6.8-7 adj.13				
10	V.MEAS	20.000 mL				
11	ALIQOT	1.000				
12	DATE	91-11-25				
13	TIME	16:42				



**Polarographic determination of pyridoxine (vitamin B<sub>6</sub>)**

**Fig. 7** Result block of the previous determination (Fig. 6)

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METROHM 648 VA-PROCESSOR (5.646.6041)
Detn. of Pyridoxine.HCl in Vitamin Preparations      METHOD
MPL 1          EL.TYPE  MME

SUPP.ELEC      Buffer 6.8-7 adj.13
V.MEAS        20.000 mL
ALIQOT        1.000

REMARK         Pyridoxine.HCl (Vitamin B6) in Monovitamin Prepn.
                Ag/AgCl (3M KCl) reference electrode
NAME          Prof.J.G.Dick
RUN#          3

ANALYTE       L R S      U.SUBST      EV.VALUE      DELTA          m.ANALYTE
Vit.B1        A0 0 0      -1.247 V     72.06 nA
                A1 0 0      -1.246 V     108.1 nA      36.07 nA
                A2 0 0      -1.246 V     143.8 nA      35.67 nA
                A3 0 0      -1.246 V     179.5 nA      35.78 nA
                m.STD    36.00 ug     SLOPE        1.004 mg/uA   72.51 ug

rho(vitB6) =   3.098      mg/g

SMPL.V,m      7.02600 mg      IDENT B6 Multi.Vit.cap.bas
DATE 91-11-25 TIME 17:07
    
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**Fig. 8** Linearity curve 0 .... 648 µg pyridoxine · HCl / 20 mL

