

Application Bulletin

 Metrohm

Of interest for:
Pharmaceuticals,
food analysis

No. 215/1 e

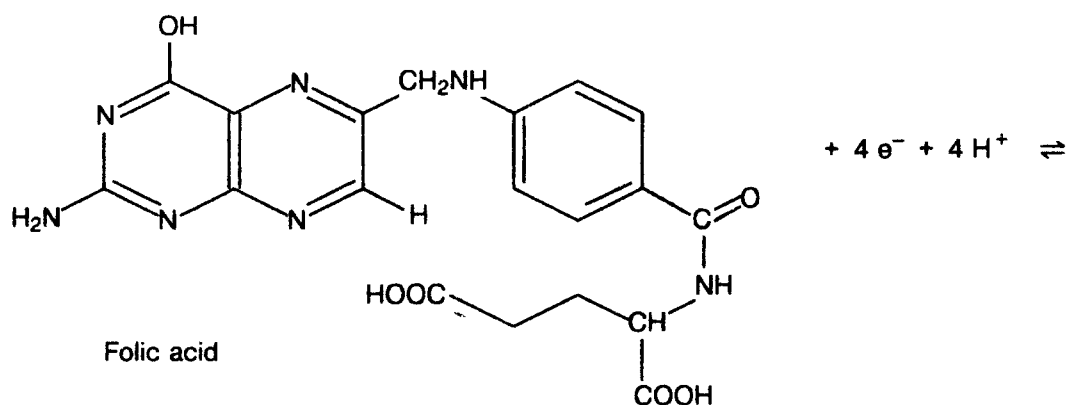
Polarographic determination of folic acid (vitamin B₉, vitamin B_C)

Summary

► This work is one of a series of Application Bulletins published at varying intervals for the polarographic determination of vitamins. The determination of folic acid, a vitamin of the B series (vitamin B₉, vitamin B_C) is described. The determination in monovitamin tablets is detailed. The linearity range of the determination is also given. The limit of determination is ca. 1.5 µg folic acid / 20 mL cell volume.

Theory

► According to Kretzschmar and Jaenicke [6], folic acid is reduced in alkaline solution (pH ≥ 9) at the DME to tetrahydrofolic acid following the below scheme:



This reaction has only limited reversibility.

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

Polarographic determination of folic acid (vitamin B₉, vitamin B_C)

| | |
|---------------------------|---|
| Reagents | <p>For preparation of the solutions, distilled or demineralised, distilled water is used. If need be, a pH value of 8.0 is set by addition of dilute NaOH.</p> <ul style="list-style-type: none"> ▶ Primary solution: $c(\text{boric acid}) = 0.1 \text{ mol/L} + c(\text{NaOH}) = 0.05 \text{ mol/L}$ To 6.2 g boric acid in a beaker are added 100 mL dist. water. After addition of 2 g NaOH, the mixture is stirred until everything has dissolved. After dilution with dist. water to 950 mL, the pH is adjusted to between 11.1 and 11.2 with $c(\text{NaOH}) = 2 \text{ mol/L}$. The solution is then made up to 1000 mL with dist. water. Use only CO₂-free dist. water! ▶ $c(\text{NaOH}) = 2 \text{ mol/L}$ 80 g/L NaOH in CO₂-free dist. water ▶ $c(\text{NaOH}) = 0.1 \text{ mol/L}$ 4 g/L NaOH in CO₂-free dist. water ▶ Standard, stock sol.: The stock solution of the standard is prepared with a concentration of 0.5 mg/mL. The actual content of the folic acid used must be taken into account here. Example: 127.5 mg 98% folic acid = 125 mg 100% folic acid are weighed into a beaker and 80 mL dist. water added. $c(\text{NaOH}) = 0.1 \text{ mol/L}$ is added with stirring until the solution becomes clear and a pH value of 8.0 has been reached. An additional 120 mL dist. water are added and the pH value re-adjusted to 8.0. The solution is added to a 250 mL volumetric flask, which is then filled to the mark with dist. water and the contents mixed. Store solution in a brown glass bottle in a refrigerator. It is best to prepare fresh solutions daily and used CO₂-free dist. water. ▶ Standard, working sol.: This is prepared when needed from the stock solution by dilution with dist. water pH = 8. |
| Sample preparation | <ul style="list-style-type: none"> ▶ Injection solutions These are adjusted to pH = 8 with NaOH and can then be used directly. ▶ Vitamin tablets (monovitamin tablets) 10 tablets are weighed out to obtain the average weight and then ground to a powder (grinder, mortar). 200 mg of the resulting powder are weighed into a beaker and 30 mL dist. water added. The pH value is adjusted to 8.0 by addition of $c(\text{NaOH}) = 0.1 \text{ mol/L}$ with stirring. The solution is stirred for a further 15 min and the pH value again adjusted to 8.0 if need be. After filtration of the solution through a paper filter into a dark glass bottle, the filter is washed four times with 5 mL aliquots of dist. water pH = 8.0. The filtrate is transferred to a 100 mL volumetric flask, which is filled to the mark with dist. water (pH = 8.0) and the contents mixed. Use solution for the analysis <u>immediately!</u> |
| Method | <ul style="list-style-type: none"> ▶ 0.50 mL of the solution obtained from the tablets are mixed in a polarographic vessel with 19.5 mL primary solution and deaerated with nitrogen. The DP polarogram (amplitude -50 mV) is then recorded at the DME between -0.7 V and -1.2 V. The peak potential of folic acid is at ca. -0.97 V. ▶ The content is determined by double standard addition. Ensure that the concentration of the folic acid in the polarographic vessel including that due to the standard additions does not exceed the linearity range (175 µg per 20 mL). ▶ Figs 1 to 5 show the parameter settings of the 646 VA Processor. |

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| Linearity | <p>▶ Fig. 8 shows a graph nA/μg folic acid. It is not intended as a calibration curve, but simply to illustrate the approximate slope of the curve. The linearity range lies between 1.5 μg ... 175 μg folic acid per 20 mL.</p> |
| Remarks | <p>▶ The presence of ascorbic acid, ascorbates and iron(II) compounds (e.g. iron(II) fumarate) do not disturb the determination of folic acid.</p> <p>▶ Folic acid has to be extracted from foodstuffs and fodder.</p> |
| Literature | <p>[1] Ben-Bassat, A.H.I. / Frydam-Kupfer, G. et al. <i>Polarographic rapid microdetermination of folic acid with hanging drop electrode and rapid sweep.</i> <i>Polarography</i> <u>1964</u>, (1966) 993</p> <p>[2] Chen, H.Y. / Han, J.L. et al. <i>Study of alternating current adsorptive stripping voltammetry in flowing systems for determination of ultratrace amounts of folic acid.</i> <i>Fresenius, Z. Anal. Chem.</i> <u>334/7</u>, (1989) 621</p> <p>[3] El Maali, N.A. / Vire, J.C. et al. <i>Cathodic adsorptive stripping square wave voltammetry of folic acid (Vitamin B-9).</i> <i>Analisis</i> <u>17</u>, (1989) 213</p> <p>[4] Göbbler, K.H. / Breinlich, J. <i>Quantitative wechselstrompolarographische Simultanbestimmung von Vitaminen der B-Reihe.</i> <i>Pharm. Ztg.</i> <u>48</u>, (1972) 1859</p> <p>[5] Jacobsen, E. / Bjørnsen, M.W. <i>Polarographic determination of folic acid in pharmaceutical preparations.</i> <i>Anal. Chim. Acta</i> <u>96</u>, (1978) 345</p> <p>[6] Kretzschmar, K. / Jaenicke, W. <i>The redox mechanism of the system folic acid - dihydrofolic acid - tetrahydrofolic acid (II).</i> <i>Z. Naturforsch.</i> <u>26/10</u>, (1971) 999</p> <p>[7] Kruze, J.E. <i>Polarographic determination of ascorbic acid, folic acid and riboflavin.</i> <i>Farmatsiya</i> <u>18/4</u>, (1969) 59</p> <p>[8] Luo, D.B. <i>Determination of folic acid by adsorptive stripping voltammetry at the static mercury drop electrode.</i> <i>Anal. Chim. Acta</i> <u>189</u>, (1986) 277</p> <p>[9] Rozanski, L. <i>Polarographic determination of folic acid in tablets containing iron(II) sulphate.</i> <i>Analyst</i> <u>103</u>, (1978) 950</p> <p>[10] Solunina, L.A. / Dewjatinin, V.A. et al. <i>Polarographic determination of folic acid in pharmaceuticals.</i> <i>Farmatsiya</i> <u>17/2</u>, (1968) 45</p> |

Polarographic determination of folic acid (vitamin B₉, vitamin B_c)

Fig. 1 Example program page 2 of 646 VA Processor

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1 Detn. of Folic Acid (Vit. Bc) in Monovitamin Tab.  METHOD 4 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         5

RECOGNITION
7 SPIKE THRESH    4
8 H.THRESH        3
9 U.TOL           7
10 W.TOL          7
11 ASYM.TOL       9

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Fig. 2 Example program page 3 of 646 VA Processor

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Detn. of Folic Acid (Vit. Bc) in Monovitamin Tab.  METHOD 4 PAGE 3
MPL 1          EL.TYPE MME                      OPERATION SEQUENCE

OPERATIONS/PARAMETERS                      OPERATIONS/PARAMETERS
1 PURGE ;STIR ;          5 s
2 [ADDL ;OPURGE;OSTIR ; 5 s
3 (REP ;
4 DME ;MEAS ;          5 s
4a M.MODE      DPN    -50 mV
4b T.STEP      800 ms
4c U.SET       -700 mV
5 SWP 0 ;      100 s
5a U.END       -1.200 V
5b U.STEP      4 mV
   SW.RATE     5.0 mV/ s
6 OMEAS ;
7 REP) 1;
8 OMEAS ;PURGE ;STIR ;
9 BEEP ;ADD1]2;        30 s
10 OMEAS ;OPURGE;OSTIR ;
11 BEEP ;END ;

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Fig. 3 Example program page 4 of 646 VA Processor

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Detn. of Folic Acid (Vit. Bc) in Monovitamin Tab.  METHOD 4 PAGE 4
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 Fol.ac      -970 mV         1          c 50 uL      500.0 mg/L   0.000 g
2
3
4
5
6
7
8

9 SUPP.ELEC   Borate buff.11.2pH
10 V.MEAS     20.000 mL
11 ALIQUOT    1.000
12 DATE       91-05-25
13 TIME       14:32

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Polarographic determination of folic acid (vitamin B₉, vitamin B_C)

Fig. 4 Example program page 5 of 646 VA Processor

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Detn. of Folic Acid (Vit. Bc) in Monovitamin Tab.  METHOD 4 PAGE 5
MPL 1      EL.TYPE MME                          DATA OUTPUT
          a      b      c      d      e
SEGMENT    Y.AXIS/L  Y.AXIS/R  X.AXIS/DIV
1 SWP 0     0.00 A   -140 nA   100 mV
2
3
4
5
6
7
8

9 RECORD    SXXX      FR
10
11 SEND
12
    
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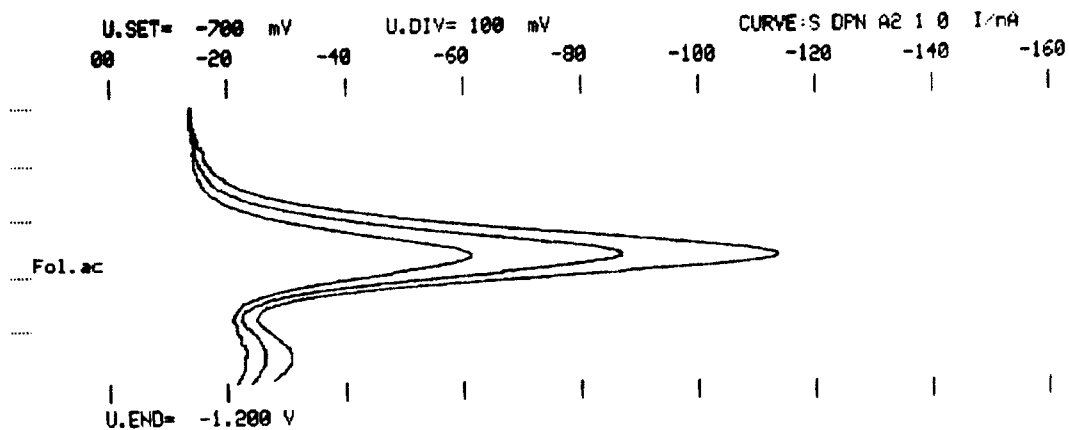
Fig. 5 Example program page 6 of 646 VA Processor

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Detn. of Folic Acid (Vit. Bc) in Monovitamin Tab.  METHOD 4 PAGE 6
MPL 1      EL.TYPE MME                          RES.CALCULATION
          a      b      c      d
ANALYTE    EVAL    R.QUANT  R.UNIT  SIGNIF.DIG
1 FOL.AC    N      rho(FOL.AC) mg/g      4
2
3
4
5
6
7
8

          (EV.QUANT  + ADDEND)  * FACTOR  / DIVISOR
11 FOL.AC    A      0.00000    108.500  1.00000
12
13
14
15
16
17
18
    
```

Fig. 6 Curve examples: Determination of folic acid in tablets with a declared content of 5 mg/tablet.



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Fig. 7 Result block of determination from Fig. 6

METROHM 648 VA-PROCESSOR (5.846.6041)
 Detn. of Folic Acid (Vit. Bc) in Monovitamin Tab. METHOD
 MPL 1 EL.TYPE MME

SUPP.ELEC Borate buff.11.2pH
 V.MEAS 20.000 mL
 ALIQUOT 1.000

REMARK Detn.folic acid in monovitamin tablet (5mg/tab)
 Ag/AgCl (3M KCl) reference electrode
 Prof.J.G.Dick

NAME
 RUN# 1

| ANALYTE | L | R | S | U.SUBST | EV.VALUE | DELTA | m.ANALYTE |
|---------|-------|---|---|----------|----------|-------------|-----------|
| Niamde | A0 | 0 | 0 | -968 mV | 36.79 nA | | |
| | A0 | 1 | 0 | -970 mV | 36.97 nA | | |
| | A1 | 0 | 0 | -970 mV | 59.18 nA | | |
| | A1 | 1 | 0 | -971 mV | 58.54 nA | 21.97 nA | |
| | A2 | 0 | 0 | -971 mV | 79.78 nA | | |
| | A2 | 1 | 0 | -972 mV | 79.04 nA | 20.54 nA | |
| | m.STD | | | 25.00 ug | SLOPE | 1.175 mg/uA | |

rho(fol.ac = 4.735 mg/g

SMPL.V,m 1.00000 mg IDENT APO folic ac.5mg/tab
 DATE 91-05-25 TIME 14:31

Fig. 8 Linearity curve 1.5 ... 175 µg folic acid / 20 mL

