

# Application Bulletin

Of interest to: Pharmacy, Foodstuffs

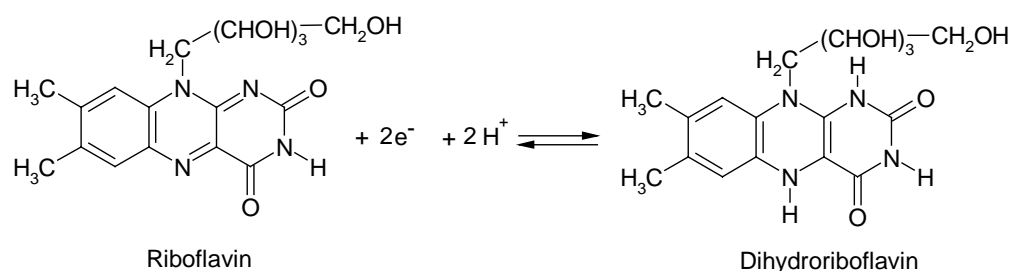
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## Polarographic determination of riboflavin

### Summary

This Application Bulletin describes the polarographic determination of riboflavin (vitamin B<sub>2</sub>). The procedure allows an analysis in monovitamin preparations. The limit of determination is approx. 100 µg/L.

### Theory



### Apparatus and accessories

- 746 VA Trace Analyzer with 747 VA Stand or
- 757 VA Computrace

### Reagents

All of the used reagents must be of purest quality possible (p.a. or suprapur). Only oxygen-free high purity water should be used

- Potassium chloride, puriss p.a., CAS 7447-40-7
- Potassium carbonate, puriss p.a., CAS 584-08-7
- Potassium hydroxide, puriss p.a., CAS 1310-58-3
- Riboflavin, CAS 83-88-5

### Ready to use solutions

- Supporting electrolyte:  $c(\text{KCl}) = 0.05 \text{ mol/L} + c(\text{K}_2\text{CO}_3) = 0.1 \text{ mol/L}$   
6.9 g potassium carbonate and 1.86 g potassium chloride are weighed in a 500 mL calibrated flask and made up to the mark with oxygen-free high purity water.
- $c(\text{KOH}) = 0.2 \text{ mol/L}$   
KOH (1.12 g) is weighed into a beaker and dissolved in 100 mL deaerated ultrapure water. After transfer to a plastic bottle, the solution is deaerated for 3 min with nitrogen and the bottle closed. Before use, deaeration is performed with nitrogen for 5 min.
- Riboflavin standard solution:  $\beta(\text{Riboflavin}) = 1 \text{ g/L}$   
Only 100 mL of standard solution are prepared at once. The solution must be prepared freshly every day. The vitamin content of the starting material must be taken into account. Riboflavin (100 mg, or a correspondingly greater amount, example: content of vitamin B2 98.8 %  $\rightarrow$  sample weight 100 : 0988 = 101.2 mg) is weighed into a beaker and 8 mL KOH solution is added. Nitrogen is passed through the mixture, which is then stirred to dissolve the vitamin. After addition of 50 mL oxygen-free ultrapure water, the solution is rinsed into a 100 mL volumetric flask, which is then filled to the mark with ultrapure water. The flask is wrapped in aluminum foil and stored in a cool place in the dark.

## Sample preparation

### Solutions

These can be used directly.

### Tablets

10 tablets are weighed exactly to obtain the average weight and then ground to a powder as quickly as possible (mortar, grinder). An amount corresponding to the average weight of a tablet is weighed into a 125 mL conical flask, 8 mL  $c(\text{KOH}) = 0.2 \text{ mol/L}$  added and extraction performed for 15 min in the dark with stirring and bubbling through nitrogen. Towards the end of the extraction time, 50 mL oxygen-free high purity water are added. After the solid fraction has settled, the mixture is filtered through a paper filter into 100 mL volumetric flask. The filter is washed three times with 3 mL aliquots of KOH solution and the filtrate plus washings made up to 100 mL with oxygen-free high purity water. The volumetric flask is wrapped in aluminum foil and stored in a cool place in the dark. Analyse as quickly as possible!

## Analysis

### Measuring solution:

10 mL (diluted) sample  
+ 10 mL supporting electrolyte

The polarogram is recorded with following parameters:

working electrode	DME
stirrer speed (rpm)	2000
mode	DP
purge time	300 s
equilibration time	10 s
pulse amplitude	50 mV
start potential	-350 mV
end potential	-900 mV
voltage step	6 mV
voltage step time	0.6 s
sweep rate	10 mV/s
peak potential	-525 mV

The concentration is determined by standard addition.

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**Remarks**

- Riboflavin solutions - especially alkaline solutions- are rapidly destroyed by atmospheric oxygen, light and heat. It is thus absolutely essential to work with darkened vessels under nitrogen. Samples and standards must be analysed rapidly and stored in a cool place.
- Fe(II) ions do not interfere with the determination.
- The SMDE can not be used for this determination.
- Very small amounts of riboflavin can be determined by adsorptive voltammetry using the method described by Wang.
- The determination is linear until approx. 10 mg/L.

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**Literature**

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Eine lichtabsorptiometrische und eine polarographische Bestimmungsmethode für Riboflavin.  
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**Figures**

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===== METROHM 746 VA TRACE ANALYZER (5.746.0101) =====
Method: AB219 .mth OPERATION SEQUENCE
Title : Determination of Riboflavin. AB219
    
```

	Instructions	t/s	Main parameters	Auxiliary parameters
1	SMPL>M		V.fraction mL	V.total L
2	DOS>M		Soln.name electrol	V.add 10.000 mL
3	STIR		Rot.speed 2000 /min	
4	PURGE	300.0		
5	(ADD			
6	STIR	10.0	Rot.speed 2000 /min	
7	OSTIR			
8	OPURGE	10.0		
9	(REP			
10	SEGMENT		Segm.name pol	
11	REP)1			
12	PURGE			
13	ADD>M		Soln.name VitB2Std	V.add 0.050 mL
14	ADD)2			
15	END			

```

Method: AB219 SEGMENT
                pol
    
```

	Instructions	t/s	Main parameters	Auxiliary parameters
1	DME			
2	DPMODE		U.ampl -50 mV	t.meas 20.0 ms
			t.step 0.60 s	t.pulse 40.0 ms
3	SWEEP	57.0	U.start -350 mV	U.step 6 mV
			U.end -900 mV	Sweep rate 10 mV/s
4	OMEAS		U.standby mV	
5	END			

```

Method: AB219 CALCULATION
                max. 15 lines
    
```

Quantity	Formula (R##, C##, A##)	Res.unit	Sig.dig.
VitB2	R1000=MC:VitB2	#g/g	5

**Fig. 1** Method for the determination of riboflavin with the 746 VA Trace Analyzer

```
===== METROHM 746 VA TRACE ANALYZER (5.746.0101) =====
Determ.      : 09081452          User:          Date: 1995-09-08
Modified     : 1995-09-12 10:47:18  Run : 0        Time: 14:52:22
Sample table: -
```

```
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Pos.  Ident.1/S1  Ident.2/S2  Ident.3/S3  Method.call  Sample size/S0
VitTab  B2          1.0
```

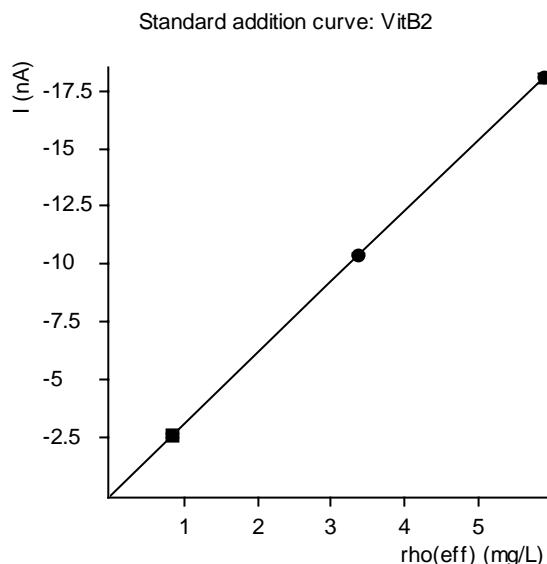
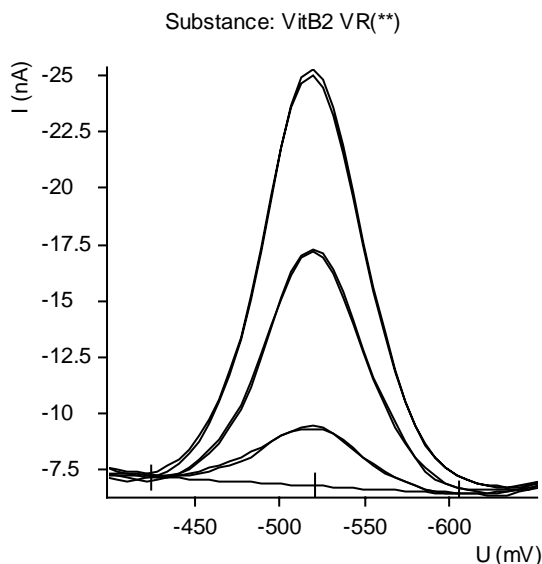
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Method : VitaminB
Title  : Determination of Vitamin B2
Remark1: Determination of Vitamin B2 in vitamin tablets
Remark2: 10 mL sample solution + 10 mL supporting electrolyte
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```
Substance : VitB2
Mass conc.: 1.708 mg/L          Mass      : 17.08 ug
MC.dev.   : 0.079 mg/L (4.64%)  Add.mass  : 50.35 ug
Cal.dev.  : -                  V0.sample: 10 mL
Comments  : -----
```

VR	U/mV	I/nA	I.mean	Std.dev.	I.delta	Comments
00	-520	-2.661	-2.591	0.0998		
01	-521	-2.520				
10	-519	-10.41	-10.38	0.0425	-7.794	
11	-519	-10.35				
20	-518	-18.11	-17.95	0.2245	-7.569	
21	-518	-17.80				

Substance	Techn.	Y.reg/offset	Slope	Nonlin.	Mean deviat.
VitB2	std.add.	-2.622e-09	-3.069e-06		1.361e-10

```
Final results
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VitB2 = 352.03 ug/g          +/- Res.dev.  %      Comments
                               16.3   4.64
```



**Fig. 2** Example of a determination of riboflavin with the 746 VA Trace Analyzer