Colorimetric assay for the determination of iron in wine and food analysis
4 x 100 ml (200 assays)

For in vitro use only
Store between +2 and +8°C

Principle

The Iron is dissociated from proteins in particular conditions of ionic strength, and reduced to a bivalent state through ascorbic acid. It will then react with the chromogenic reagent FERENE-S and build a stable complex, giving a blue colour which is proportional to the concentration of Iron in the sample.

Assay specifications

Wavelength:	582 nm (575 – 582 nm)
Light path:	1.00 cm (glass; plastic)
Temperature:	25 to 37°C
Method:	end point
Reaction:	15 minutes
Measurement:	against air or against water
Linearity:	2 – 40 mg/L

Reagents

1:   Buffer, 4 vials with approx. 84 ml each (Buffer > 0.1 mol/L)

2:   Ferene-S, 4 vials with approx. 16 ml each (Ferene-S > 0.1 mmol/L; Ascorbic acid > 0.1 mol/L).

3:   Calibrator, 1 x 5 ml (Iron = 20 mg/l).

All reagents are ready for use. They are stable at 2-8 °C up to the expiry date shown on the package if not contaminated during handling.

Let the reagents reach the laboratory temperature (20 – 25 °C) before use. Mix kindly before pipeting. Close immediately after handling.

The reagents are not hazardous. The general safety rules for the work in chemical laboratories should be applied. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

- Wine can be used directly.
- Use colorless, clear and neutral liquid samples directly if Iron conc. is between 2 – 40 mg/L; otherwise, dilute with water to reduce it in this range.
- Strongly coloured samples have to be treated with PVPP (polyvinylpolypyrrolidone e.g. 1 g/100 mL sample)
- For application on biochemistry analysers, it is recommended to add PVP (polyvinylpyrrolidone) at a final concentration of 5 g/l into R1 (2.1 ml of a mother solution 200 g/l in each vial)
- Turbid solutions have to be filtered or centrifuged
- Samples containing carbon dioxide have to be degassed

Procedure

Pipette into cuvettes:	Reagent blank (RB)	Calibrator	Samples
Reagent 1 (buffer)	1680 µl	1680 µl	1680 µl
Distilled water	100 µl	-	-
Standard (vial 3)	-	100 µl	-
Sample	-	-	100 µl
Mix carefully. Read the absorbance A_1 after 5 minutes at 25 to 37 °C, then add:			
Reagent 2 (Ferene-S)	320 µl	320 µl	320 µl
Mix carefully. Read the absorbance A_2 after 10 min. at 25 to 37 °C. The color is stable 30 min. at room temperature.			

Calculation

$\Delta A = (A_2 - df \times A_1)_{sample\ or\ standard} - (A_2 - df \times A_1)_{RB}$

with df = dilution factor of the optical densities by reagent volumes:
df = (sample volume + R1) / (sample volume + R1 + R2) = 0.848

and $\quad C_{sample}[mg/L] \quad = \dfrac{c_{standard}[mg/L]}{\Delta A_{standard}} \times \Delta A_{sample}$

Since the concentration of the standard is fixed at 20 mg/L, this gives the following calculation formula depending on the unit that is preferred:

$C_{sample}[mg/L] = (\Delta A_{sample} / \Delta A_{standard}) \times 20$

or $C_{sample}[\mu mol/L] = (\Delta A_{sample} / \Delta A_{standard}) \times 358$

Notes

1. A proportional variation of the reaction volumes does not change the result.
2. Use one way cuvettes or very clean tubes washed with diluted HCl and distilled water.
3. Specificity: this test is specific for Iron, no interferences were detected.
4. The sample volume is low compared to other tests, because the Ferene-S chromogen is very sensitive; the reduced sample volume has the advantage of reducing interferences by the sample matrix
5. The test takes place under acidic pH conditions, so in many cases no pH adjustment of the sample is needed (for instance for wine samples or after perchloric acid treatment)

Additional application examples

- Determination of iron in flour:
    - weigh precisely 0.8 grams of flour
    - resuspend in 6 – 7 ml of 7% (w/v) perchloric acid
    - shake and mix for 10 min.
    - fill to the mark at 10 ml with 7 % (w/v) perchloric acid
    - mix a few times by inversion
    - centrifuge at 1,700 - 2,000 x g for 20 min; alternatively, filtration is also possible (e.g. Macherey Nagel ref. 531012, MN 615 1/4, diameter 125 mm)
    - use the supernatant solution as sample for Iron determination

The content in iron is calculated with the formula:

$Content\ [g/100\ g] = \dfrac{C_{iron}\ [g/l]}{weight_{sample}\ [g/l]} \times 100$

(weight$_{sample}$ = 80 g/L if initial weight is 0.8 g in 10 ml as in our example)

Literature

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).

2. Duffy J. R., Gaudin J., Clin. Biochem. 10, 122 (1977).

3. Higgins T., Clin. Chem. 27, 1619 (1981).

Applications on biochemistry analysers

General application features (all analyzers)

Temperature	37°C (25°C is also possible)
Wavelength	575 - 600 nm (primary) / 700 nm (secondary)
Test type and sequence	End-point test with 2 reagents test sequence: – pipeting R1 (5 Vol.) + sample (20 – 50 µl) – pre-incubation 2 – 3 min – measurement A1 (before addition of R2) – pipeting R2 (1 Vol.) – incubation >= 5 min at 37 °C – measurement A2 – calculation A2-A1 against calibration curve
Calibration	2 - 5 calibrators from 0 to 10 mg/l Linear calibration curve
Reagent blank	Yes
Reagents	R1 and R2 in the ratio approx. 5 to 1
Sample volume	20 µl for low sensitivity (0 – 20 mg/l) 50 µl for high sensitivity (0 – 10 mg/l)

Example Konelab / Arena

Test Definition:

	Low	High	Unit
Full name	Enzytec Iron		
Online name	#		
Test used	yes		
Test type	Photometric		
Result unit	mg/l		
Number of decimals	1		
Temperature	37 °C		
Test limit	0	20	mg/l
Initial absorbance	0.000	2.000	E
Dilution limit	0	20	mg/l
Secondary dilution 1 +	#	#	
Acceptance	Automatic		
Dilution 1 +	0.0		
Sample type	Wine (Other)		
Correction factor	1.00		
Correction bias	0.00		

Calibration parameter

Calibration type	Linear		
Curve direction	Ascending		
Repeat time (d)	1	Err. (mA)	#
Points/calibrator	simple	Err. (%)	#
Acceptance	Automatic		
Response limit min/max	# / #		
Bias correction in use	No		
Type of calibrators	Series	(separate is also possible)	
Calibrator identification	Iron calibrator (20 mg/l)		
Concentrations	0 to 10 mg/l		

Distribution

Additional blank	YES (or fixed time)	Antigen excess	No
Reagent 1	ENZYT Iron R1		
volume (µl)	150		
dispense with	Water	Volume (µl)	10
wash reagent	None		
Sample volume (µl)	15	(adjust to sensitivity needs)	
dispense with	Water	Volume (µl)	10
wash reagent	None		
Incubation (sec)	180		
Measurement BLANK	End-point		
response min./max	# / #		
Reagent 2	ENZYT Iron R2		
volume (µl)	25		
wash reagent	None		
dispense with	Water	Volume (µl)	10
Incubation (sec)	300		
Measurement	End-point		
λ 1 / λ 2 (nm)	600 / 700	575 / 700 is also possible	
measurement type	Fixed timing		

Example Lisa 200

Full Test name	Enzytec Iron Ferene
Short name	Iron
Units	mg/l
Type of test	End-point calibration
Filter (nm)	580
1st reading = zero	NO (A1 must be measured for each sample)
Waiting time 1 (tours before addition of R2)	4 (4 tours = 4 x 24 sec.)
Number of measurements (= incubation time)	15 (15 tours x 24 sec = 6 min)
Reagent 1 VOL (µl) DIL (µl) POS	250 0 #
Reagent 2 VOL (µl) DIL (µl) POS	45 0 #
Sample. VOL (µl) DIL	20 - 50 µl (adjust to sensitivity needs) 0
Starter (of reaction)	REAGENT 2
Waiting time 2 (tours after addition of R2)	0 (measurements start immediately after addition of R2)
Calibration	1 degree (= linear calibration; 2 degrees = non-linear calibration is also possible)
Blank = Standard	YES (water used for blank is used as 1st point of the calibration curve)
Number of standards	3
Standard 1 VAL (mg/l) POS	2 (= example with 2 mg/l) # (position on the instrument)
Standard 2 VAL (mg/l) POS	5 (= example with 5 mg/l) #
Standard 3 VAL (mg/l) POS	10 (= example with 10 mg/l) #
Nb repeat Sample/Control	1 (number of repetitions ; it is possible to choose 2)
Control VAL (mg/l) POS DEV	# (concentration of QC sample) # (position on the instrument) # (deviation accepted)
Pre-dilution	1
Post-dilution	4
Sample diluents	Physiologic solution
Type rincing	3
Normal values UPPER LOWER	20 (in mg/l) 0
Limit linearity	20 (in mg/l)
Blanc OD limit LOWER UPPER	0 500

value entered by the operator

Example Hitachi 717

TEST	[IRON]
ASSAY CODE	[2POINTS] - [24] – [50]
SAMPLE VOLUME (µl)	[30] –[5] (adjust to sensitivity needs)
REAGENT VOLUME R1	[250] – [50] – [NO]
REAGENT VOLUME R2	[40] – [20] – [NO] (can be lower)
WAVELENGTH	[700] [600]
CALIBRATION TYPE	[LIN] [1] [4]
STD.(1) CONC. POS.	[0] – [1] (example)
STD.(2) CONC. POS.	[2] – [2] (2 mg/l =example)
STD.(3) CONC. POS	[5] – [3] (5 mg/l =example)
STD.(4) CONC. POS	[10] – [4] (10 mg/l =example)
SD LIMIT	[0.1]
DUPLICATE LIMIT	[#]
SENSITIVITY LIMIT	0
LIMITE D.O (CINET)	[0] – [INCR.]
PROZONE CHECK	[32000] – [1]
EXPECTED VALUE	[#] – [#]
PANIC VALUE	[#] – [#]
INSTRUMENT FACTOR	[1.0]

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