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IRON ASSAY KIT

Colorimetric Ferene S method.
Kit for measurement of Iron concentration in wine.
Reference: FER-COL-B

PRINCIPLE OF THE METHOD

Under acid environment, iron is ionized.

After reduction at ferrous state (Fe²⁺), iron react with Ferene S to form a stable coloured complex with a maximum of absorbance at 600nm.

The intensity of the colour measured is proportional to the quantity of iron presents in the sample.

METROLOGICAL CHARACTERISTICS

SPECIFICITY: The method is specific for the iron.
LINEARITY: 40 mg/L

SAMPLE

Wine.

REAGENTS - INITIAL CONCENTRATION

Reagent 1	Acetate Buffer pH 4.8, Hydroxylamine sulfate Accelerators and sulfatans.	62 mmol/L 65 mmol/L	2x100 mL
Reagent 2	Ferene S Detergents.	2.0 mmol/L	1x50 mL

REAGENTS – STORAGE AND STABILITY

Kit: Store at +2-8°C. Do not refrigerate.
Stable until the expiry date shown on the label.

Opened reagents: The Reagents are stable after opening until the expiry date shown on the bottles when are protected from direct light, tightly closed, and stored at reported temperature.

Working Reagent: Stable 30 days if stored at +2-8°C (20 days at +15-25°C), on board of the instruments (S300-S150)
"S300-S150-ENOCHEM-JOLLY-TEKNO"

REAGENTS PREPARATION MANUAL AND COBAS MIRA PROCEDURE

Reagents are liquid ready to use .

REAGENTS PREPARATION SATURNO 300 - SATURNO 150 - ENOCHEM - JOLLY - TEKNO PROCEDURE

Reagents are liquid ready to use .

Working Reagent
To prepare Working Reagent, mix 4 volume of Reagent 1 with 1 volume of Reagent 2 (4R1+1R2) depending on the number of sample, or add 10 mL of Reagent 2 to one bottle of Reagent 1.

REAGENTS – PRECAUTION AND WARNING

- This method describes the manual use of this kit. For use with automatic analyzer see the specific applications.
- Presence of particulate material, turbidity, indicates deterioration of the reagents.
- Quality control data sheet of the reagents are available upon request. Refer to the batch number on the label.

ADDITIONAL EQUIPMENT

Pipettes.
Spectrophotometer.
Cuvette (Lightpath = 1 cm).

SAFETY PRECAUTIONS

Reagent 1 Harmful



Irritating (Xi)
R36: Irritating to eyes.
S2: Keep out of the reach of children.
S25: Avoid contact with eyes.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Reagent 2

Referring to european law this product is not classified as a dangerous substance. The reagents contain inactive components such as detergent and preservatives. The total concentration of these components is lower than the limits reported by 67/548/CEE and 88/379/CEE directives and next modifications about classification, packaging and labelling of dangerous substances.
However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended (*).

Standard



Corrosive (not included in the kit FER-COL-B)

Risk phrases
- Causes severe burns (R 35)

Safety phrases
- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S 26)
- Wear suitable protective clothing (S 36)
- Wear suitable gloves (S 37)
- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible) (S 45)
- Wear eye/face protection (S 39)

* EU-Dir 1999/11 Commission Directive of March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.

WASTE MANAGEMENT

Please refer to local legal requirements.

ANALYTICAL PROCEDURE (MANUAL)

Wavelength $\lambda = 600$ (578) nm
Lightpath 1 cm
Temperature +15-25°C
Measurement Against distilled water.
Reaction end point (increase)

Allow reagents to reach working temperature before using.

	STANDARD	SAMPLE	
Reagent 1	2000	2000	μ L
Sample	-	250	μ L
Standard	250	-	μ L

Mix thoroughly and incubate for 5 minutes.
Measure the absorbance (A1) of the Sample and the Standard against the blank.
Then pipette:

	STANDARD	SAMPLE	
Reagent 2	500	500	μ L

Mix thoroughly and incubate for 5 minutes.
Measure the absorbance (A2) of the Sample and the Standard against the blank.

CALCULATIONS (MANUAL)

$$\text{Iron (mg/L)} = \frac{A2 - (A1 \times 0,818) \text{ Sample}}{A2 - (A1 \times 0,818) \text{ Standard}} \times 20$$

Note:
For concentrations higher than 40 mg/L dilute the sample with distilled water, repeat the determination and multiply the result by the dilution factor.

ANALYTICAL PROCEDURE (COBAS MIRA)

GENERAL
MEASUREMENT MODE : ABSORB
REACTION MODE : R-S-SR1
CALIBRATION MODE : SLOPE AVG
REAGENT BLANK : REAG/DIL
CLEANER : NO
WAVELENGTH : 600 nm
DECIMAL POSITION : 3
UNIT : mg/L

ANALYSIS
POST DIL. FACTOR : 2.00
CONC. FACTOR : NO
SAMPLE CYCLE : 1
VOLUME : 25.0 μ L
DILUTION NAME : H2O
VOLUME : 5.0 μ L
REAGENT CYCLE : 1
VOLUME : 200 μ L
START R1 CYCLE : 12
VOLUME : 50.0 μ L
DILUTION NAME : H2O
VOLUME : 5.0 μ L

REAGENT 1

REAGENT 2

CALCULATION
SAMPLE LIMIT : NO
REAC. DIRECTION : INCREASE
CHECK : ON
CONVERS. FACTOR : 1.00000
OFFSET : 0.00000
TEST RANGE LOW : 0
TEST RANGE HIGH : 40.000
NORM. RANGE LOW : NO
NORM. RANGE HIGH : NO
NUMBER OF STEPS : 1
CALC. STEP A : ENDPOINT
READINGS FIRST: 11 LAST: 16

CALIBRATION
CALIB. INTERVAL : *
BLANK : *
REAG. RANGE LOW : NO
REAG. RANGE HIGH : NO
BLANK RANGE LOW : NO
BLANK RANGE HIGH : NO
STANDARD POS : *

1 : 20.00 mg/L 2 : NO
3 : NO 4 : NO
5 : NO 6 : NO
7 : NO 8 : NO
REPLICATE : DUPL
DEVIATION : 10%
CORRECTION STD : NO

CONTROL
CS1 POS : *
CS2 POS : *
CS3 POS : *

*Data entry by the user.
*Data inserito dall'utilizzatore.

ANALYTICAL PROCEDURE (SATURNO 300 - SATURNO 150 - ENOCHEM - JOLLY - TEKNO PROCEDURE)

Wavelength $\lambda = 600$ (578) nm
Lightpath 1 cm
Temperature +15-25°C
Measurement Against distilled water.
Reaction end point (increase)

Allow reagents to reach working temperature before using.

	STANDARD	SAMPLE	SAMPLE BLANK	REAGENT BLANK	
Reagent 1	-	-	2000	-	μL
Working Reagent	2000	2000	-	2000	μL
Distilled water	-	-	-	200	μL
Sample	-	200	200	-	μL
Standard	200	-	-	-	μL

Mix thoroughly and incubate for 5 minutes.

Measure the absorbance (ABS) of the Sample Blank, Sample, Standard and Reagent Blank against distilled water.

CALCULATIONS

(SATURNO 300 - SATURNO 150 - ENOCHEM - JOLLY - TEKNO PROCEDURE)

$$\text{Iron (mg/L)} = \frac{(\text{ABSSample} - \text{ABSReag.Blank}) - \text{ABSSample Blank}}{(\text{ABSStandard} - \text{ABSReag.Blank})} \times 20$$

Note:

For concentrations higher than 40 mg/L dilute the sample with distilled water, repeat the determination and multiply the result by the dilution factor.

QUALITY CONTROL

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

BIBLIOGRAPHY

Stokey, L.L., Anal. Chem., 42; 779-81 (1970).

Carter, P., Anal. Biochem., 40; 450-8 (1971).

Lauber, K.J., Clin. Chem. Clin. Biochem., 18; 147-148 (1980).

Kaplan, L.A., Pesce, A.J.: Clinical Chemistry, Mosby Ed. (1996).