Supelco®
Analytical Products

Application Note

Photometric Analysis of Iron in Pale Beer via Reaction with 1,10-Phenanthroline According to ASBC Standards

Introduction

The determination of iron content in beer is a critical analytical parameter due to its pronounced impact on product quality and stability. Iron levels critically affect the quality attributes of beer, such as its flavor and appearance. Elevated iron levels can catalyze oxidation reactions that accelerate beer staling and generate undesirable off-flavors, particularly metallic notes that compromise the intended sensory profile. Moreover, excess iron can interact with other beer components to form complexes responsible for color alteration, haze formation, and sedimentation. These visual imperfections not only affect consumer appeal but also signal potential stability issues in the product.[1]

To ensure product integrity, brewers strive to maintain minimal iron concentrations, thereby preventing oxidative and complexation reactions detrimental to shelf life and nutritional value. The American Society of Brewing Chemists (ASBC) recommends a photometric determination of iron using 1,10-phenanthroline as the chromogenic reagent.[2] The Spectroquant® Iron Test (**1.00796**) employs this same detection principle and offers a rapid, precise, and user-friendly alternative to the ASBC method. The test kit contains pre-prepared reagents and features pre-programmed calibration curves on compatible photometric instruments, minimizing analytical preparation time and potential sources of error.

A comparative analysis was conducted on nine beer samples, encompassing both pale and dark varieties, to evaluate the performance of the Spectroquant® Iron Test relative to the ASBC reference method. The results obtained demonstrated close agreement between the two approaches, confirming the suitability of the Spectroquant® test for routine iron determination in brewing applications.

Experimental

The following procedure describes the photometric determination of iron in pale beer.

Method

All iron ions present in the sample are quantitatively reduced to Fe(II) ions by ascorbic acid. In a buffered medium, the resulting Fe(II) ions react with 1,10-phenanthroline to form a stable red complex, the intensity of which is measured photometrically. In the absence of the ascorbic acid (reagent Fe-3) only the Fe(II) fraction is determined, allowing differentiation between total and ferrous iron content. The procedure follows the same analytical principle as described in APHA 3500-Fe B and DIN 38406-1, ensuring methodological equivalence with established standardized protocols.

Measuring range

Measuring range	Cell size
0.010 – 1.000 mg/L Fe	50 mm
0.05 – 2.50 mg/L Fe	20 mm
0.10 – 5.00 mg/L Fe	10 mm

Sample material

Light colored beer samples

Reagents, instruments and auxiliaries

Reagents

Spectroquant® Iron Test (**1.00796**)

Instrument

For the measurement one of the following Spectroquant® photometers is necessary:

- Spectroquant® VIS Spectrophotometer Prove 100 plus (**1.73026**)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (**1.73027**)

- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (**1.73028**)
- Spectroquant® Colorimeter Move 100 (**1.73632**)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® NOVA 60 and Prove series.

Instrument Accessories

Depending on the measuring range, at least one of the following cells is necessary:

- Rectangular cells 10 mm (**1.14946**)
- Rectangular cells 20 mm (**1.14947**)
- Rectangular cells 50 mm (**1.14944**)

Software for Data transfer

Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

Other Reagents and Accessories

- Ultrapure-water (e.g. Type 1 from a suitable Milli-Q® system) or water for analysis (**1.16754**)
- Pipettes for pipetting volumes of 0.50 and 8.0 mL

For AQA

- Spectroquant® CombiCheck 90 (**1.18700**)
- Iron standard solution, 0.0500 mg/L Fe (**1.33014**)
- Iron standard solution, 0.1000 mg/L Fe (**1.33018**)
- Iron standard solution, 0.300 mg/L Fe (**1.33019**)
- Iron standard solution, 1.00 mg/L Fe (**1.33020**)

Analytical Procedure

Sample Preparation

- Expel carbon dioxide from the beer sample and allow froth to dissipate completely (avoid filtration, if possible). For samples with high iron content, degas by gentle shaking only.
- If the sample is turbid, filter through an iron-free folded filter.

Preparation of Measurement Solutions

Sample Blank

- Pipette 8.0 mL of the pretreated sample into a test tube.
- Add 0.5 mL of ultrapure water and mix.
- Add 1 dose of reagent Fe-3 and shake vigorously until the reagent is completely dissolved.
- Allow to stand for 10 minutes.
- Transfer the solution to a photometric cell and measure.

Reagent Blank (recommended for measuring in 50 mm cell)

- Pipette 8.0 mL of ultrapure water into a test tube.
- Add 1 drop of reagent Fe-1 while holding the bottle vertically. Mix thoroughly.
- Add 0.5 mL of reagent Fe-2 with a pipette and mix.
- Add 1 dose of reagent Fe-3 and shake vigorously until the reagent is completely dissolved.
- Allow to stand for 10 minutes.
- Transfer the solution to a photometric cell and measure.

Measurement Sample

- Pipette 8.0 mL of the pretreated sample into a test tube.
- Add 1 drop of reagent Fe-1 while holding the bottle vertically. Mix thoroughly.
- Add 0.5 ml of reagent Fe-2 with a pipette and mix.
- Add 1 dose of reagent Fe-3 and shake vigorously until the reagent is completely dissolved.
- Allow to stand for 10 minutes.
- Transfer the solution to a photometric cell and measure.

Measurement

- It is recommended to perform zero adjustment at the start of each new working day. To do this, open the method by scanning the barcode, tap the <Settings> button and select <ZERO ADJUSTMENT> menu item. Fill the same cell to be used for sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment and allow the instrument to automatically adjust. Confirm zero adjustment by selecting <OK>.
- When using a 50 mm cell, perform reagent blank once for each measurement series. To do this, tap on the <Settings> button and select <REAGENT BLANK> menu item. Fill the corresponding rectangular cell with the reagent blank and insert the cell into the cell compartment. The measurement starts automatically. Accept the reagent blank by activating <User RB> field and confirm with <OK>.
- Perform the sample blank for each sample measurement. To do this, tap <Settings> button and select <SAMPLE BLANK> menu item. Fill the corresponding rectangular cell with the sample blank and insert the cell into the cell compartment. The measurement starts automatically. Confirm with <OK>.
- After the sample blank has been measured, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read and record the result in mg/L Fe from the display.

Note: The procedure above applies to the Spectroquant® Prove series photometer. For Nova 60A or Move 100 instruments, refer to the respective user manual.

Notes on the measurement

- Turbid solutions yield false-high readings.
- The pH of the measurement solution must be maintained between 3.5 - 4.0.
- The color of the measurement solution remains stable for at least 60 min after completion of the respective reactions stated above.

Analytical quality assurance (AQA)

AQA is recommended before each measurement series. To check the photometric measurement system (test reagents, measurement device, handling) and the mode of working, the iron standard solutions (see section "Reagents, instruments and auxiliaries") or Spectroquant® CombiCheck 90 can be used. Besides a standard solution with 1.00 mg/L Fe, the Combi Check 90 also contains an additional solution (spike sample) for determining sample-dependent interferences (matrix effects).

Additional notes see under **www.qa-test-kits.com**.

Quality and batch certificates for Spectroquant® Iron test (**1.00796**), are available on the product website, including production control data per ISO 8466-1 and DIN 38402 A51.

Study Results and Discussion

Method validation, was performed using nine beer samples, including six pale and three dark varieties, to determine iron content. Results obtained with the Spectroquant® Iron Test were compared to those derived from the ASBC Beer-18 reference method.[1] In addition, spiking experiments were performed for both analytical procedures using three iron concentration levels (0.10, 0.50 and 1.00 mg/L). Recovery rates were calculated for pale and dark beer samples and are presented in **Tables 1** and **2**, respectively.

All measurements were carried out using 50-mm cells and a Spectroquant® Prove 600 plus spectrophotometer. The comparative results demonstrated concordance between the Spectroquant® and ASBC methods, confirming the analytical accuracy and robustness of the Spectroquant® Iron Test for determining iron in beer matrices.

Table 1. Iron content in unspiked and spiked pale beer samples analyzed using the SQ & ASBC method

Beer sample	Fe spike (mg/L)	SQ		ASBC	
		Measured conc. (mg/L)	Recovery rate	Measured conc. (mg/L)	Recovery rate
Pale beer 1 (Pilsner)	0.00	0.00	-	0.00	-
	0.10	0.09	89%	0.08	78%
	0.50	0.46	93%	0.46	93%
	1.00	0.93	93%	0.96	96%
Pale beer 2 (Lager)	0.00	0.00	-	0.00	-
	0.10	0.09	95%	0.10	98%
	0.50	0.47	95%	0.46	92%
	1.00	0.93	93%	0.94	94%
Pale beer 3 (Lager)	0.00	0.00	-	0.00	-
	0.10	0.08	78%	0.10	98%
	0.50	0.45	90%	0.44	87%
	1.00	0.92	92%	0.93	93%
Pale beer 4 (Lager)	0.00	0.00	-	0.00	-
	0.10	0.07	73%	0.12	120%
	0.50	0.50	99%	0.47	94%
	1.00	0.96	96%	0.92	92%
Pale beer 5 (Lager)	0.00	0.00	-	0.00	-
	0.10	0.08	78%	0.08	82%
	0.50	0.45	90%	0.43	85%
	1.00	0.93	93%	0.88	88%
Pale beer 6 (Lager)	0.00	0.00	-	0.00	-
	0.10	0.07	67%	0.09	87%
	0.50	0.41	83%	0.40	81%
	1.00	0.92	92%	0.90	90%

Table 2. Iron content in unspiked and spiked dark beer samples analyzed using the SQ & ASBC method

Beer sample	Fe spike (mg/L)	SQ		ASBC	
		Measured conc. (mg/L)	Recovery rate	Measured conc. (mg/L)	Recovery rate
Dark beer 1 (Export beer)	0.00	0.01	-	0.00	-
	0.10	0.06	60%	0.07	65%
	0.50	0.33	65%	0.32	63%
	1.00	0.65	65%	0.65	65%
Dark beer 2 (Bock beer)	0.00	0.00	-	0.00	-
	0.10	0.00	0%	0.07	71%
	0.50	0.23	46%	0.22	45%
	1.00	0.61	61%	0.61	61%
Dark beer 3	0.00	0.00	-	0.00	-
	0.10	0.00	0%	0.09	93%
	0.50	0.22	45%	0.23	46%
	1.00	0.61	61%	0.62	62%

No detectable or only minimal levels of iron were observed in any of the analyzed beer samples when measured using either the Spectroquant® test or the ASBC method. For the spiking experiments, a clear difference in method performance was found between pale and dark beers. While the amount of iron added to the pale beers could be recovered well in all samples, this was not the case for the dark beer samples. Both the Spectroquant® and ASBC methods yielded recovery values within an acceptable range of approximately 70–120%, indicating reliable quantification in these lighter matrices. In contrast, the dark beer samples exhibited significant matrix interferences that adversely affected iron recovery. For these samples, both analytical methods produced recoveries predominantly below 70%. At the lower spiking concentrations in particular, the Spectroquant® Iron Test showed markedly reduced recoveries, suggesting that the method is not suitable for reliable iron determination in dark beer matrices. Similarly, the ASBC method demonstrated inconsistent and low recoveries under the same conditions, further indicating that matrix effects in dark beers hinder accurate photometric determination of iron.

Conclusion

The Spectroquant® Iron Test (**1.00796**), using 1,10-phenanthroline, was demonstrated to be a quick and easy procedure for determining iron content in light-colored beers. The results obtained were comparable to those achieved using the ASBC Beer-18 reference method, which employs the same detection chemistry. Unlike the ASBC procedure, the Spectroquant® test does not require reagent preparation or calibration, resulting in reduced analysis time and lower operational costs.

However, the method was found unsuitable for dark beer samples due to low and inconsistent recovery values, which were also observed with the ASBC method. These findings suggest that both methods are influenced by matrix components present in dark beers, which interfere with the photometric detection of iron.

References

1. Van Mieghem T, Delvaux F, Dekleermaeker S, Britton SJ. Top of the ferrous wheel – the influence of iron ions on flavor deterioration in beer. Journal of the American Society of Brewing Chemists. American Society of Brewing Chemists. 2022:1–11. DOI: **10.1080/03610470.2022.2124363**
2. ASBC Methods of Analysis, online. Beer-18, Iron, A. Analysis by Colorimetry [Release date 1958, revised 1975 and reviewed 2015]. American society of brewing Chemists, St. Paul, Mn, U.S.A. DOI: **10.1094/ASBCMOA-Beer-18**

Related Products

Description	Cat. No.
Test kit & Instruments	
Spectroquant® Iron Test	**1.00796**
Spectroquant® VIS Spectrophotometer Prove 100 plus	**1.73026**
Spectroquant® UV/VIS Spectrophotometer Prove 300 plus	**1.73027**
Spectroquant® UV/VIS Spectrophotometer Prove 600 plus	**1.73028**
Spectroquant® Colorimeter Move 100	**1.73632**
AQA Products	
Spectroquant® CombiCheck 90	**1.18700**
Iron standard solution, traceable to SRM from NIST 0.0500 mg/l Fe(III) in HNO_3	**1.33014**
Iron standard solution, traceable to SRM from NIST 0.1000 mg/l Fe(III) in HNO_3	**1.33018**
Iron standard solution, traceable to SRM from NIST 0.300 mg/l Fe(III) in HNO_3	**1.33019**
Iron standard solution, traceable to SRM from NIST 1.00 mg/l Fe(III) in HNO_3	**1.33020**
Reagents & Accessories	
Water for analysis EMSURE®	**1.16754**
Rectangular cells 10 mm	**1.14946**
Rectangular cells 20 mm	**1.14947**
Rectangular cells 50 mm	**1.14944**
Spectroquant® Prove Connect to LIMS software package	**Y.11086**

For more information on spectrophotometric applications for beer, request or download the **Manual Analysis Methods for the Brewery Industry**

For more information, please explore the following links:

- Spectroquant® Photometric Analysis: **SigmaAldrich.com/spectroquant**
- Spectroquant® Prove Connect: **SigmaAldrich.com/spectroquant-prove-connect**
- Other Application Notes: **SigmaAldrich.com/wfa-applications**

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