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Single strand hollow fiber membrane (SSHFM): An on-line sample preparation for the flow based colorimetric determination of free iron in fruit juices

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ABSTRACT

A single strand hollow fiber membrane (SSHFM) was developed for the on-line sample preparation for the flow based colorimetric determination of free iron levels in fruit juices. The SSHFM, as used, could separate Fe^{2+} from some spectrophotometric interfering agents in the fruit juice, such as pigments, solid suspensions and polysaccharides. The screening process was likely to have been primarily based on dialysis, wherein only ions or molecules that are smaller than the pores of the membrane can diffuse through while relatively larger molecules or particles could not. Two flow modes, a continuous and stopped flow, were studied. Factors that influenced the sensitivity (%dialysis) of the method, such as the flow rate, sample volume, flow direction and stopped flow time, were optimized. The stopped flow mode was found to be relatively more sensitive than the continuous flow mode and displayed a linear range of 1–30 mg L^{-1} Fe^{2+}, a limit of detection of 0.5 mg L^{-1}, and a % relative standard deviation of less than 2% ($n = 8$) for the analysis of 10 mg L^{-1} Fe^{2+} spiked grape juice samples. A sample throughput of 24 samples h^{-1}, was attained without any further sample treatment.

1. Introduction

Iron is an essential dietary mineral for humans, and is involved in the production of red blood cells and redox processes. The recommended daily intake of dietary iron are 1 mg iron per kg body mass per day for normal infants, and 10–15 mg per day for children, adolescents and adult men and women [1]. Iron deficiency may cause a decreased growth rate, poor pregnancy and lower working capacity in adults. Iron fortified foods, such as milk, rice and fruit juice, may be necessary to prevent iron deficiency. Among these iron-fortified foods, iron-fortified fruit juice is widely available because it is cost effective, of a low allergic potential and is acceptable as a dietary supplement in many countries. In addition, fruit juices contain a high level of vitamin C that can help adsorb and keep the iron in the bioavailable form (Fe^{2+}). Nevertheless, excess iron can cause a metallic taste, may promote cancer and increase the risk of cardiovascular diseases [2]. The typical level of iron that can be fortified without sensory changes in the fruit juice products is less than 30 mg L^{-1} [3]. Thus, a method for the quality control of the iron-fortified level is necessary.

There are many methods for the determination of iron in fruit juice available in the literature, such as the colorimetric method with 1,10-phenanthroline [4], atomic absorption spectroscopy [5,6] and HPLC with electrochemical and flame-AAS detection [7]. Since they all use a batch analysis approach and are relatively laborious, they are not convenient for handling a large number of samples. A continuous approach, such as flow-based methods, may be considered as suitable alternatives because they can afford high throughput analysis and automation [8–10]. However, when applied to samples with complex matrices, such as fruit juices, which contain pigments, sugars and suspended particulates, then typically sample preparation steps, such as wet-acid digestion [4,11], wet-ashing [12] and solid phase extraction (SPE) [13], are required for the elimination of these interfering agents that might otherwise cause a high background signal.

There are a few on-line sample preparation methods reported, such as SPE [14], digestion [15], extraction [16] and membrane dialysis [17,18], that can be incorporated with a flow based analysis method to provide a fully automated determination of analytes in samples with complex matrices. On-line membrane dialysis is of interest because it is simple, has low energy consumption and does not involve organic solvents. Typically, membrane dialysis differentiates between molecules based on their size compared to the pore size of the membrane. The membrane separates two solutions and allows the molecules whose sizes are smaller than the pores of the membrane to diffuse through the membrane from one solution (donor solution) to the other solution (acceptor solution), while the larger molecules are restricted. Membrane modules for on-line membrane dialysis have been available in two major types, a sandwich-type for flat sheet membranes and a tubular type for

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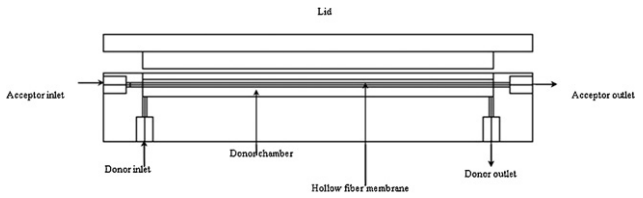

Fig. 1. Schematic drawing of the single strand hollow fiber membrane (SSHFM) module.

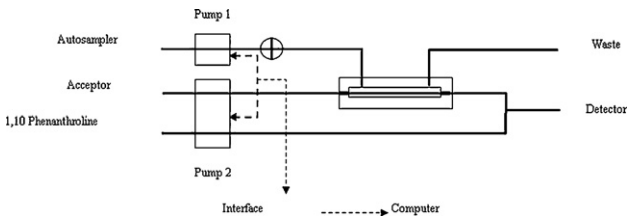

Fig. 2. Schematic drawing of the on-line SSHFM flow based system for the colorimetric determination of Fe^{2+}.

hollow fiber membranes [19]. Generally, the tubular type-hollow fiber membrane module is available in bundles and has been widely used in ultrafiltration [20–22], desalination [23,24] and reversed osmosis [25,26], while the single strand hollow fiber membrane has mostly been applied to liquid phase microextraction [27]. Hollow fiber membranes offer a relatively high surface area and low cost per unit strand. However, there are few reports on the application of single strand hollow fiber membrane to on-line dialysis. Nordmeyer and Hansen [28] developed the dialyzing-injection system using single strand Cuprophane dialysis fiber for screening of proteins in human blood serum prior to analysis of free calcium ion by liquid chromatography. In this work, a single strand hollow fiber membrane (SSHFM) based system was developed for the on-line sample preparation with a flow based analysis and applied for the colorimetric determination of iron in fruit juices.

2. Experimental

2.1. Reagents and standards

All chemicals and reagents used were analytical grade. Deionized water was used for dilution and preparation of standard solutions. A standard stock solution of 1 g L^{-1} Fe^{2+} was prepared daily from ferrous (II) sulfate (Ajax chemical). A 2 mmol L^{-1} 1,10 phenanthrolien (Merck, USA) solution was used as a complexing agent. A 10% (w/v) hydroxylamine HCL (Ajax chemical, USA) solution was used as the reducing agent and was prepared daily. Concentrated HNO_3 and H_2SO_4 were obtained from Merck, USA. Ferric ammonium sulfate (Carlo Erba Reagents, France) was used for recovery study. Calcium sulfate (Baker Analyzed, USA), magnesium sulfate (BDH lab supplies, UK), zinc sulfate (Carlo Erba Reagents, France), potassium sulfate (Merck, USA) and sodium sulfate (Ajax chemical, USA) were used for interference study. Red grape juice samples were purchased from local stores.

2.2. Single strand hollow fiber membrane (SSHFM)

A single strand of polyethersulfone hollow fiber membrane (0.7 mm ID, 0.3 mm thickness, Membrana, GmbH, Germany) was installed in a tubular type module made of polyacrylic, as illustrated schematically in Fig. 1. The module was drilled for the donor chamber (3 mm width × 10 mm length × 5 mm depth) and its inlet and outlet channels. The acceptor inlet and outlet channels were drilled to the donor chamber in order to fit the hollow fiber membrane in the donor chamber. The donor chamber was closed and sealed by the polyacrylic lid.

2.3. On-line SSHFM flow based system for the colorimetric determination of Fe^{2+} in fruit juices

The SSHFM was installed as a part of the flow system for the on-line determination of Fe^{2+}, as illustrated schematically in Fig. 2, which consisted of peristaltic pumps (Masterflex L/S), SSHFM module, six-port valve, cuvette based configuration flow cell, spectrophotometer (LW scientific model V325XS) and data acquisition card (DAQ card model USB-6009 from National instrument). Deionized water was used as the carrier stream in both the donor and acceptor solutions. The sample was loaded into the carrier stream via the six-port valve and carried to the donor chamber of the SSHFM. The acceptor solution was pumped into the lumen of the SSHFM to receive the Fe^{2+} from the donor solution, and on exiting was then mixed with the 1,10-phenanthroline yielding a pink color of Fe^{2+}-1,10-phenanthroline complex, which was subsequently detected spectrophotometrically at 505 nm.

Two flow modes were studied. In the continuous flow mode, the acceptor solution was continuously fed through the lumen of the SSHFM, while in the stopped flow mode, the acceptor solution was stopped for a certain period of time, after which it was pumped out. The flow rates of donor and acceptor solutions were optimized as detailed later.

2.4. Method optimization

Parameters concerning the sensitivity of the method such as sample loading volume, donor flow rate, acceptor flow rate, flow mode and flow direction were optimized using standard Fe^{2+} solution. The signals of the Fe^{2+} after dialyzed with SSHFM and complexed with 1,10-phenanthroline were measured and compared. The method performance was evaluated for linear range and limit of detection. The method repeatability and recovery was determined using known concentration Fe^{2+} spiked grape juice samples. Major mineral ions that are present in the fruit juice such as Na^+, K^+, Ca^{2+}, Mg^{2+} and Zn^{2+} were also studied for interference.

2.5. Conventional method for the determination of Fe^{2+} in fruit juices

A 2 mL aliquot of the sample was digested by the addition of 2 mL of a 1:1 (v/v) concentrated HNO_3: concentrated H_2SO_4 and heated on a hotplate for 30 min until the sample turned into a white clear solution. Then, 2 mL of 10% (w/v) hydroxylamine HCL (Ajax chemical) and 2 mL of 1,10-phenanthroline were added to the cooled solution. The solution was adjusted to pH 6–8 by ammonium hydroxide (QRëC), and the absorbance of the pink or red color solution that formed was measured at 505 nm.

3. Results and discussion

3.1. SSHFM for screening of Fe^{2+} from the background colored interferences

Since the detection method of Fe^{2+} is based on the spectrophotometric determination of the formation of the pink color of the Fe^{2+}-1,10-phenanthroline complex, then the background color of the fruit juice or particulate matter in suspension would interfere with its detection and so must be eliminated or separated from the Fe^{2+} prior to forming the colored complex for measurement. Fig. 3 illustrates the screening feature of the SSHFM for separating Fe^{2+} from the colored background of red grape juice. The screen-

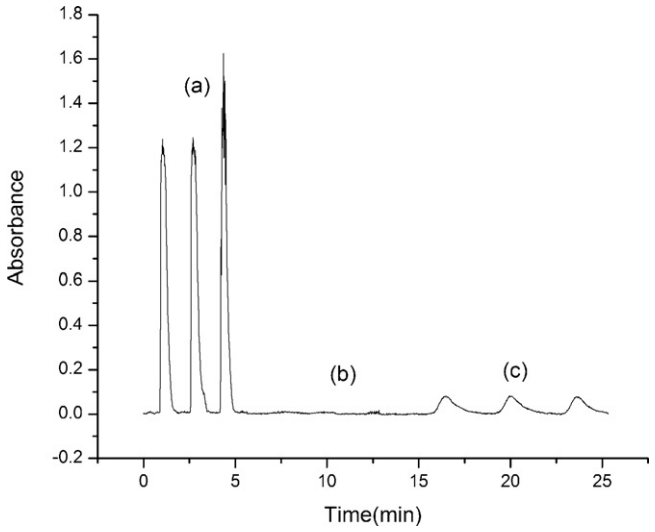

Fig. 3. The screening ability of the SSHFM flow based system. The signal of (a) the red grape juice sample directly injected to the detector; (b) the acceptor solution after screening the grape juice by the SSHFM and (c) the Fe^{2+}-1,10-phenanthroline complex in the acceptor solution after screening the grape juice by the SSHFM. Data shown are representative of 3 replicate injections.

ing process was likely to have been primarily based on dialysis and probably accompanied by an ultrafiltration process. According to the pore size of the membrane, only ions or molecules that are smaller than the pores of the membrane could diffuse through, while relatively larger molecules or particles, such as pigments, solids in suspension and polysaccharides in the fruit juice, could not.

3.2. Method optimization

3.2.1. Sample loading volume

The sample loading volume corresponds to the mass of the analytes entering the system that contribute to the signal. However, at a constant flow rate, the higher the loading volume is the longer the required loading and cycle times are. Fig. 4 shows the absorbance values at 505 nm obtained from a loading volume of 0.5 mL and 1.5 mL of a 20 mg L^{-1} standard Fe^{2+} solution using a sample flow rate of 2.7 mL min^{-1} and a slow acceptor flow rate of 0.6 mL min^{-1}.

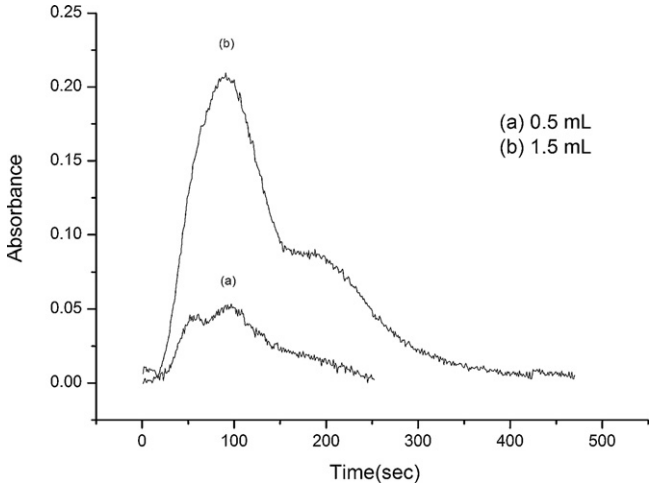

Fig. 4. The signal of the Fe^{2+}-1,10-phenanthroline complex in the acceptor solution after SSHFM screening of a 20 mg L^{-1} standard Fe^{2+} solution at loading volumes of 0.5 and 1.5 mL. The donor and acceptor flow rates were 2.7 and 0.6 mL min^{-1}, respectively.

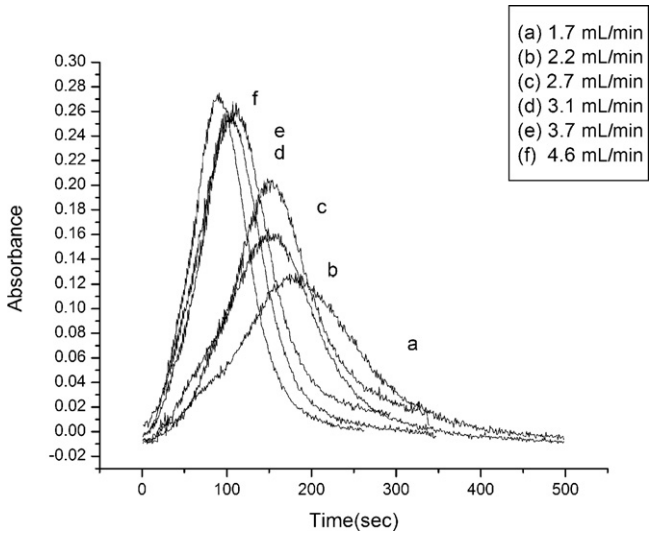

Fig. 5. The signal of the Fe^{2+}-1,10-phenanthroline complex in the acceptor solution after SSHFM screening of a 1.5 mL loading of a 20 mg L^{-1} standard Fe^{2+} solution at various donor flow rates and an acceptor flow rate of 0.6 mL min^{-1}.

Both solutions were continuously flowed in the same direction. The signal obtained from the 1.5 mL loading volume gave a much higher signal while the cycle time, which was estimated from the peak width, was within 5 min.

3.2.2. Donor flow rate

The donor flow rate is responsible for the feeding rate of the analyte to the donor chamber where the analyte is subsequently transferred to the acceptor solution. Using a loading of 1.5 mL of a 20 mg L^{-1} standard Fe^{2+} solution into the donor chamber, the flow rates were varied from 1.7 to 4.6 mL min^{-1}, with a constant acceptor flow rate of 0.6 mL min^{-1}. The higher flow rates exhibited a higher signal and smaller peak width (Fig. 5). As the analyte entered the chamber, the analyte concentration in the chamber increased and the mass transfer across the membrane proceeded according to the concentration gradient, which was higher as the donor flow rate was increased. However, too high a donor flow rate would cause the sample to leave the donor chamber too fast so that the time for the analyte to be transferred to the acceptor solution would not be sufficient. For these reasons, a donor flow rate of 3.1 mL min^{-1} was used for further study.

3.2.3. Stopped flow mode

Since the mass transfer of the analyte across the membrane is driven by the concentration gradient between the donor and acceptor solutions, it would be better to fill the donor chamber with the sample and let the mass transfer proceeded for a certain period of time. Indeed, the signal obtained from the stopped flow mode appeared to be much better than the continuous flow mode, with a %mass transfer of the stopped flow mode of 31% compared to only 14% in the continuous flow mode. The absorbance signal of the Fe^{2+}-1,10-phenanthroline complex increased as the stopped time increased within the range of 10–30 s, but did not increase any further as the stop time was increased up to 300 s (Fig. 6), presumably since equilibrium had already been reached by 30 s. Therefore, a 30 s stopped time was selected for further study.

3.2.4. Acceptor flow rate

The acceptor flow rate affects the peak shape of the signal, which corresponds to the sensitivity of the system. Thus, the acceptor flow rate was varied from 0.7 to 2.2 mL min^{-1}. The absorbance signal of the Fe^{2+}-1,10-phenanthroline complex improved as the acceptor

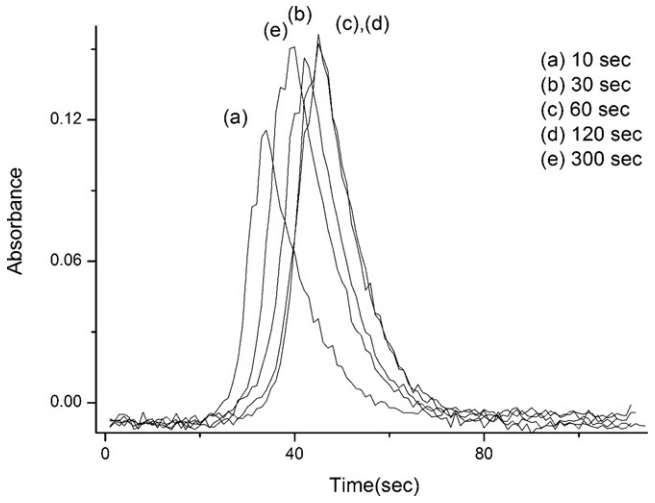

Fig. 6. The signal of the Fe^{2+}-1,10-phenanthroline complex in the acceptor solution after SSHFM screening of a 1.5 mL loading of a 10 mg L^{-1} standard Fe^{2+} solution at 10–300 s stop times and with an acceptor flow rate of 0.6 mL min^{-1}.

flow rate increased (Fig. 7), due to the narrower sample plug proceeding to the detector. However, the change in the absorbance signal obtained as the flow rate changed from 1.7 to 2.2 ml min^{-1} was negligible and so a flow rate of 1.7 mL min^{-1} was selected for further study.

3.2.5. Flow direction

No difference in the sensitivity of detection of Fe^{2+} (as the Fe^{2+}-1,10-phenanthrolien complex absorbance signal) was observed when the concurrent flow direction of the donor and acceptor solutions was changed to the countercurrent flow direction. Therefore, the concurrent flow direction was chosen because it generated a lower pressure.

3.3. Method evaluation

The optimized method was evaluated and summarized in Table 1. The recovery of Fe^{2+} from known concentration spiked grape juice samples was about 90%, and was highly reproducible with a relative standard deviation of eight repeated analyses being less than 2%. The method running time was 2.5 min per sample

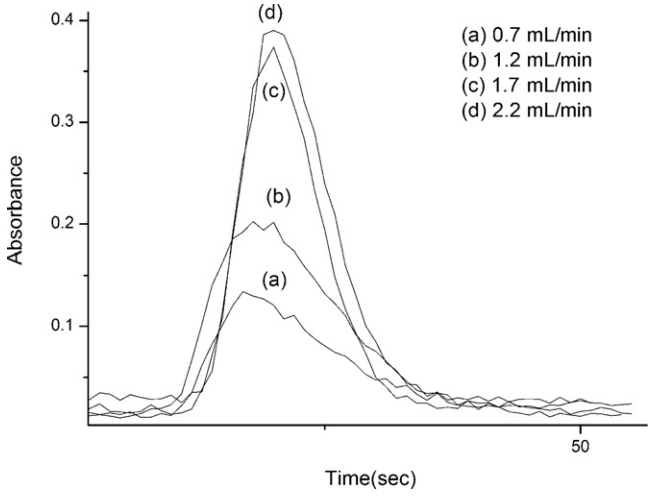

Fig. 7. The signal of the Fe^{2+}-1,10-phenanthroline complex in the acceptor solutions at various acceptor flow rates after SSHFM screening of a 1.5 mL loading of a 10 mg L^{-1} standard Fe^{2+} solution for with a 30 s stopped time.

Table 1
SSHFM method performance for determination of Fe^{2+} in grape juice samples.

Parameter	Performance value	
Linear range	3–30 mg L^{-1}	
Linear least squares equation	0.049x + 0.020	
Coefficient of determination (R^2)	0.9965	
Limit of detection (S/N = 3)	0.6 mg L^{-1}	
Sample throughput	24 samples h^{-1}	
Repeatability (spiked Fe^{2+} 10 mg L^{-1})	2% (N = 8)	
Recovery of spiked grape juice samples	Found ± SD (mg L^{-1}), %RSD	%Recovery
Spiked Fe^{2+} 3 mg L^{-}	2.8 ± 0.04, 1.5% (N = 3)	91.7%
Spiked Fe^{2+} 5 mg L^{-1}	4.4 ± 0.09, 2.0% (N = 3)	88.5%
Real sample analysis (grape juice samples)	Found ± SD (mg L^{-1}), %RSD	
Conventional wet acid digestion	4.5 ± 0.25, 5.6% (N = 4)	
SSHFM	2.4 ± 0.19, 7.7% (N = 4)	
SSHFM with hydroxylamine	3.8 ± 0.31, 8.2% (N = 4)	

Table 2
Recovery study of spiked grape juice samples.

Spiked	Found ± SD (mg L^{-1}), %RSD	%Recovery
Fe^{2+} 10 mg L^{-1}	10.0 ± 0.83, 8.3% (N = 3)	99.8
Fe^{2+} 10 mg L^{-1} + hydroxylamine	10.0 ± 0.33, 3.2% (N = 3)	100.2
Fe^{3+} 10 mg L^{-1}	Not found	–
Fe^{3+} 10 mg L^{-1} + hydroxylamine	10.0 ± 0.61, 6.1% (N = 3)	100.5

Table 3
Interference study of some major mineral ions from grape juice samples spiked with Fe^{2+} 10 mg L^{-1}.

Interfered ion	Additional level (mg L^{-1})	Fe^{2+} found (mg L^{-1} ± SD, %RSD)	%Recovery
Na^+	500	10.1 ± 1.01, 10.0% (N = 3)	101.4
	1000	12.9 ± 0.09, 0.7% (N = 3)	128.5
K^+	500	9.7 ± 0.24, 2.5% (N = 3)	96.6
	1000	9.4 ± 0.51, 5.5% (N = 3)	94.2
Ca^{2+}	500	10.2 ± 0.57, 5.6% (N = 3)	102.5
	1000	10.7 ± 0.28, 2.6% (N = 3)	107.4
Mg^{2+}	500	9.9 ± 0.95, 9.5% (N = 3)	99.3
	1000	10.8 ± 0.42, 4.0% (N = 3)	107.5
Zn^{2+}	100	9.7 ± 0.35, 3.6% (N = 3)	96.8
	500	7.9 ± 0.70, 8.8% (N = 3)	79.3

providing a sample throughput of 24 samples h^{-1}. When compared with the conventional method (wet acid digestion) for the determination of Fe^{2+} in grape juice samples, our method apparently yielded a lower iron content level. Since the method of detection (complexation with 1,10-phenanthroline) is selective to Fe^{2+}, Fe^{3+} that may also be present in the grape juice would not be detected. The iron content was significantly improved when the acceptor solution was treated with reducing agent (hydroxylamine) prior to detection. This hypothesis was verified by the recovery of Fe^{2+} from the spiked Fe^{3+} grape juice samples when the acceptor solution was treated with hydroxylamine prior to detection (Table 2). Moreover, the presence of major mineral ions in the fruit juice samples did not significantly interfere with our method of analysis even though very high concentrations of such ions were added (Table 3). Nevertheless, the iron content found in the grape juice by our method was still somewhat less than that obtained by the conventional method, probably due to the fact that the SSHFM allows only free iron ions to transfer across, and so iron that is insoluble, such as that which is bound to organic components, might not be detected.

4. Conclusion

A simple and cost effective on-line sample preparation using SSHFM is proposed for flow based analysis systems. The method

is here applied for the colorimetric determination of free iron (as Fe^{2+}) content directly in grape juice samples without any sample pretreatments providing a high throughput analysis. Despite the matrix screening feature of the SSHFM, that only free ion species are determined may be its disadvantage.

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