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A highly sensitive switch-on spectrofluorometric method for determination of ascorbic acid using a selective eco-friendly approach

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A highly sensitive spectrofluorometric method was developed for determination of ascorbic acid in tablets. The reducing characteristics of ascorbic acid were employed to restore fluorescence of salicylate in presence of iron (III). The method showed excellent green characteristics according to the analytical Eco-scale and the green analytical procedure index. The method was found simple, inexpensive and rapid with high selectivity and sensitivity.

Abstract
Ascorbic acid has recently been extensively used due to its role in the management of COVID-19 infections by stimulating the immune system and triggering phagocytosis of the corona virus. The currently used spectrofluorometric methods for determination of ascorbic acid require using derivatizing agents or fluorescent probes and suffer from a number of limitations, including slow reaction rates, low yield, limited sensitivity, long reaction times and high temperatures. In this work, we present a highly sensitive spectrofluorometric method for determination of ascorbic acid by switching-on the fluorescence of salicylate in presence of iron (III) due to a reduction of the cation to iron (II). The addition of ascorbic acid resulted in a corresponding enhancement in the fluorescence intensity of iron (III)-salicylate complex at emission wavelength = 411 nm. The method was found linear in the range of 1–8 μg/mL with a correlation coefficient of 0.9997. The limits of detection and quantitation were 0.035 μg/mL and 0.106 μg/mL, respectively. The developed method was applied for the determination of ascorbic acid in the commercially available dosage form; Ruta C60 tablets. The obtained results were compared with those obtained by a reported liquid chromatographic method at 95% confidence interval, no statistically significant differences were found between the developed and the reported methods. Yet, the developed spectrofluorometric method was found markedly greener than the reference method, based on the analytical Eco-scale and the green analytical procedure index. This work presents a simple, rapid and sensitive method that can possibly be applied for determination of ascorbic acid in pharmaceuticals, biological fluids and food samples.

1. Introduction
The sales and the interest in ascorbic acid (ASC) has increased in the last two years to unprecedented levels due to its role in the
treatment and prevention of COVID-19 infections [1]. ASC promotes cellular immunity by increasing the number of immune cells, including lymphocytes and neutrophils. Moreover, ASC accumulates intracellularly to trigger the chemotaxis process and phagocytosis of the virus. The scavenging ability of ASC helps protect neutrophils and phagocytes from oxidative damage [2]. On the other hand, ASC is essential for human health due to its biological activity in metabolic processes [3]. ASC prevents oxidative damage to proteins, lipids, and DNA, which has been implicated as a major factor in the development of chronic diseases such as cataract, cancer, and cardiovascular diseases [4]. This vitamin is also considered as an indirect antioxidant by regenerating other biologically important antioxidants such as vitamin E and glutathione to their active state [5]. ASC is included in the biosynthesis of collagen and acts as a co-factor in the biosynthesis of cholesterol, L-carnitine, catecholamines, amino acids, and some peptide hormones [6,7]. ASC deficiency reduces resistance to bacterial, viral and fungal infections, and is associated with symptoms of scurvy such as muscle weakness, tooth loss, rash, tiredness, and joint pain [8].

A number of techniques have been utilized for determination of ASC in different matrices, including UV/visible spectroscopy [9–11], spectrofluorometry [12–18], high performance liquid chromatography [19,20], and electrochemistry [21–23]. Compared with other techniques, spectrofluorometry is more sensitive and more selective than UV/visible spectroscopy and does not require sophisticated instruments such as HPLC and LC/MS [24,25]. Direct spectrofluorometric detection of ASC is not possible due to lack of fluorophoric agents. Chemical derivatization with fluorogenic or fluorophoric agents is essential. However, these chemicals are expensive, and the reactions require time, catalysis or energy.

In this work, a rapid and simple spectrofluorometric method for determination of ASC was developed. The proposed method depends on the ability of ASC to reduce iron (III) in iron(III)–salicylate complex, which abolishes the quenching effect of iron(III) and switches on the fluorescence of salicylate. The intensity of salicylate emission was found proportional to the concentration of ASC. To the best of our knowledge, this redox reaction has never been employed for spectrofluorometric detection of non-fluorescent drugs. The developed method is simple, rapid, does not need expensive reagents or sophisticated procedures and is more suitable for routine analysis of ASC.

2. Experimental

2.1. Apparatus and software

A JASCO model FP-6300 spectrophotometer (Tokyo, Japan), equipped with PC powered 150 W xenon lamp, holographic grating with 1500 grooves/mm, modified Rowland mount excitation and emission monochromators and Spectra Manager software V1.53.01. The scanning parameters include an excitation slit width of 10 nm, an emission slit width of 5 nm, a scanning speed of 1000 nm/min, at low sensitivity medium response. The wavelength of maximum excitation (λ<sub>ex</sub>) was 290 nm, and the wavelength of maximum emission (λ<sub>em</sub>) was 411 nm. Digital analytical balance (Sartorius AG, Germany) and Automatic Water Still (Sci Finetech, Seoul, South Korea) were used. HANNA pH 211 Microprocessor pH-meter with double junction glass electrode calibrated with standard buffers was used for adjusting the pH of the solutions.

2.2. Materials

ASC (99.00 %purity; Sigma-Aldrich, Hamburg, Germany), ammonium iron (III) sulfate (98.50% purity; Alpha Chemia Lab reagent, Mumbai, India), sodium salicylate (98.80 %purity; DOP, Organic Kimyasan, Istanbul, Turkey), nitric acid (70 %, density = 1.431 g/mL; Merck, Darmstadt, Germany) and double distilled water using Automatic Water Still (Sci Finetech, Seoul, South Korea) were used. The commercially available Ruta C60<sup>®</sup> tablets (Batch No. 1911053) were purchased from a local market. Each tablet contains 60 mg of rutin and 160 mg of ASC.

2.3. Reagents and solutions

Ammonium iron (III) sulfate (Mol. Wt. 482.1 g) and sodium salicylate (Mol. Wt. 160.1 g) were used to prepare 1 mM iron (III) salicylate. A concentration of 20 mM sodium salicylate solution was prepared by weighing 0.3202 g, dissolving in distilled water, and completing to volume in a 100-mL volumetric flask. A concentration of 20 mM ammonium iron (III) sulfate was prepared by weighing 0.964 g, which is then completed to 100 mL using 0.5 mM nitric acid solution. The role of nitric acid as a diluent is to increase the stability of iron (III) salicylate complex [26,27]. Iron (III) salicylate in a concentration of 10 mM was prepared by mixing equal volumes of each of the previously prepared solutions, then the former solution was diluted with water to prepare a working solution containing 1 mM iron (III) salicylate. The ASC stock solution was prepared by dissolving 0.1 g of ASC (Mol. Wt. 176.12 g/mol) in 100 mL distilled water, then 0.1 mg/mL ASC working solution was prepared by taking 10 mL from 1 mg/mL ASC stock solution completed to 100 mL distilled water. ASC solution should be freshly prepared and kept in a dark bottle until use.

2.4. Reaction procedures and calibration curve

In a set of 10 mL volumetric flasks, 1 mL of 1 mM iron (III) salicylate reagent was added to different aliquots of ASC working standard solution (100 µg/mL) to prepare reaction mixtures containing ASC in the range of 1–8 µg/mL, mixed well and then completed to volume with distilled water. The fluorescence intensity was measured immediately at 411 nm (λ<sub>em</sub>). Reagent blank was also prepared, and its emission spectrum was recorded. The relative fluorescence spectra were calculated by subtracting the blank emission spectrum from that of the ASC solutions using Spectra Manager Software. The calibration curve was prepared by plotting the relative difference in fluorescence intensity at 411 nm against ASC concentrations (µg/mL) and the regression equation was computed.

2.5. Dosage form analysis

The dosage form was analyzed by weighing ten tablets of Ruta C60<sup>®</sup> then powdering in a mortar. For preparing 1.6 mg/mL, an amount equivalent to one tablet (0.47 g) was dissolved in 50 mL water, sonicated for 20 min, then completed to 100 mL distilled water in a 100 mL volumetric flask and finally filtered. Several dilutions were performed using distilled water. A tablet assay solution containing 5 µg/mL ASC was prepared by taking 500 µL from the stock solution to make the reaction with iron (III) salicylate. The results of the relative fluorescence intensity of the tablet assay solution (5 µg/mL) were compared to a standard 5 µg/mL ASC solution and then percent recoveries ± standard deviation (SD) were calculated.

3. Results and discussion

3.1. Theory

The enediol functional group in ASC is a weak chromophore with low molar absorptivity. The determination of ASC using direct
spectrofluorometry is not possible due to lack of conjugated rigid structure. Spectrofluorometric determination of compounds generally has the advantages of selectivity and high sensitivity [28,29]. In this work, a spectrofluorometric determination of ASC, using iron (III)-salicylate was developed. In this reaction, the properties of iron (III) salicylate were exploited in the determination of ASC. Iron (III) salicylate is a purple-colored reagent formed by a complexation reaction between iron (III) and salicylate. Upon complexation with iron (III) (Fig. 1), salicylate ion loses its strong native fluorescence ($\lambda_{ex}$ 290 nm, $\lambda_{em}$ 411 nm). Formation constants control displacement reaction in metal-complexes [30,31]. Ferric salicylate has a formation constant in the range of $1.90 \times 10^3$ to $2.61 \times 10^4$ [32,33]. Although these formation constants allow ascorbate to displace salicylate in the less stable ferric salicylate complex, redox reaction in which ASC acts a reducing agent for ferric ions is another possible mechanism. The reducing properties of ascorbic acid could convert ferric to ferrous salicylate. Unlike iron (III), the produced iron (II) does not quench the fluorescence of salicylate. This could be ascribed to the electron configuration of iron (II) in which the 3d orbital is full, while the vacant 3d orbital of iron (III) could induce intersystem crossing and non-radiative relaxation of the excited electrons. Fig. 2 shows the redox reaction of ASC and iron (III)-salicylate. The proposed sensing mechanism of ASC is confirmed through supporting iron (III)-salicylate complex formation in a stoichiometric ratio of 1:1 with LC-MS/MS experiment (Fig. S1) and augmenting redox based sensing of ASC performing experiments with 1,10-phenanthroline reagent (Fig. S2). The formation of a characteristic yellow-to-orange colored ferroin at $\lambda_{max} = 510$ nm indicated that ASC could reduce ferric ions to ferrous ions.

3.2. Method optimization

Several factors that may affect the reaction were studied; these factors included iron (III) salicylate concentration, solvent type, solvent pH and reaction time. The emission intensity at 411 nm were measured in the presence and in the absence of ASC. The optimization was performed to achieve the maximum difference ($\Delta F_{411nm}$) in the presence of ASC in order to attain the maximum possible sensitivity. In a previous work, different salts of iron (III) were tried with sodium salicylate [27]: iron (III) chloride, iron (III) nitrate and ammonium iron (III) sulfate. The maximum quenching effect and the highest repeatability were obtained when ammonium iron (III) sulfate was used, so that iron (III) salicylate complex was prepared using iron (III) ammonium sulfate and sodium salicylate.

3.2.1. Effect of iron (III) salicylate concentration

The effect of iron (III) salicylate concentration was studied by varying the concentrations of reagent from 40 to 110 $\mu$M and measuring $\Delta F$ at 411 nm (Fig. 3a). It was observed that the fluorescence intensity markedly increased with the increase in iron (III)-salicylate concentration from 40 to 50 $\mu$M, then a slight increase was observed over the concentration range of 50–80 $\mu$M. Increas-
ing iron (III)-salicylate concentration from 80 to 90 μM was associated with another marked increase in ΔF<sub>411nm</sub> followed by a plateau. The concentration of 100 μM iron (III) salicylate was selected as the optimum level due to the high response and the relatively stable emission.

3.2.2. Effect of pH

Studying the pH effect is essential due to its potential role in the reaction yield and rate. The effect of pH on the reaction was studied out by completing the reaction flasks to volume using the proper diluent solutions prepared at different pH values ranging from 3 to 11 using 0.1 N HCl or 0.1 N NaOH for pH adjustment. It was found that the highest response was observed in the pH range of 4–8, with maximum ΔF<sub>411nm</sub> obtained at pH 5 (Fig. 3b). The diminished response at high pH could be due to precipitation of iron(III) in alkaline media [34]. The reduced intensity at low pH could be due to the low stability of iron (III) -salicylate in acidic media. It is here worth mentioning that comparable results were obtained using distilled water (pH 5.5), so no pH adjustment was performed.

3.2.3. Effect of the solvent type

Different solvents were used to study the effect of diluent type on the reaction. Water, acetonitrile, ethanol, and methanol were studied (Fig. 3c). The maximum ΔF<sub>411nm</sub> value was attained when water was used as a diluting solvent. The lower ΔF<sub>411nm</sub> obtained when an organic solvent was used as a diluent could be due to solubility effect, effect of solvent on iron (III) salicylate formation constant, or the effect of solvent on salicylate emission. Water was selected as a solvent not only because of the high ΔF<sub>411nm</sub> but also because of its high safety and eco-friendliness compared with other organic solvents.

3.2.4. Effect of time

Studying the reaction time is important to determine the optimum time of measurement and to avoid any variability in results due to the reaction rate. The effect of reaction time was studied along 25 min and it was observed that no change in fluorescence intensity was attained at the selected reaction times, so the fluorescence intensity was measured instantaneously after completing the reaction. The fluorescence measured was stable during 25 min as shown in Fig. 3d, which improves the method robustness against small changes in time between solution preparation and measurement. Moreover, the instantaneous reaction between ASC and iron (III)-salicylate helps save time, energy and catalysis.

3.3. Method validation

The method was validated according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. The different validation parameters were studied, including linearity, range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), specificity and robustness. The results were compared with the acceptance limits to guarantee that the developed method is suitable for routine analysis of ASC in pharmaceutical tablets.

3.3.1. Linearity and range

The iron (III) salicylate fluorescence intensity was measured at 411 nm before and after reaction with increasing concentrations of ASC, under optimum conditions. The linearity was determined by plotting the difference in fluorescence intensity ΔF on the y-axis against ascorbic acid concentrations on the x-axis. As shown in Fig. 4, ΔF<sub>411nm</sub> was found to increase proportionally with the concentration of ASC. The calibration curve was linear in the range 1–8 μg/mL with a correlation coefficient of 0.9997.
3.3.2. Accuracy and precision

Assessing the method’s accuracy and precision is essential to confirm the trueness and the reliability of the results. The accuracy of the proposed method was determined by measuring three replicates of three different concentrations within the linearity range and calculating the percent recovery as shown in Table 1. The results showed that the calculated %recoveries were in the range 98–102 %, which was deemed acceptable according to the adopted ICH guidelines. The precision of the method was assessed by measuring three replicates of three different concentrations in the same day (Intraday precision) and in three consecutive days (inter-day precision) and by calculating the standard deviation (SD) and the percent relative standard deviation (%RSD) as shown in Table S1. The within-day and the between-day results showed that the %RSD was always less than 2%, which proves that the method is sufficiently precise.

3.3.3. Specificity

According to the ICH criteria, the developed method is specific if there is no interference from co-formulated active ingredients and excipients. The specificity of this developed method was evaluated by studying the influence of potential interfering substances such as glucose, lactose, starch, avicel, KCl, NaCl, Mg stearate and citric acid. The results showed that the method could tolerate high concentrations of each substance excipient before the measured emission being affected (Table S2). This proved that this method has a good selectivity for determination of ASC.

3.3.4. LOD and LOQ

Both limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to ICH guideline Q2 (R1), adopting the approach based on the standard deviation of the response of blank samples (σ) and the slope of the calibration curve (S). The calculations were performed using the following equations.

\[
\text{LOD} = 3.3\sigma/S \\
\text{LOQ} = 10\sigma/S
\]

The calculated LOD and LOQ were found to be 0.035 and 0.106 μg/mL, which indicates that the method has sufficient sensitivity for the determination of ASC in bulk, diluted solutions and pharmaceutical dosage forms.

3.3.5. Robustness

The method is deemed robust if it can withstand small deliberate variations in measurement conditions. The robustness of the proposed fluorometric method was studied by small changes in the concentration of iron (III) salicylate (µM), pH and wavelength (nm). The values of the SD and %RSD were calculated for each parameter as shown in Table S1. The %RSD indicated that the method results were not affected by ±5 µM changes in iron (III) salicylate concentration, ±0.3 pH units and ±2 nm in the wavelength of detection. These results indicate that the method is adequately robust for routine ASC determination in quality control laboratories.

3.4. Assessment of the method greenness

The greenness of the developed method was evaluated using the analytical Eco-scale and the green analytical procedure index (GAPI). The analytical Eco-scale is a semi-quantitative tool for the determination of the greenness of analytical methods. This scale depends on subtracting penalty points (PPs) for each parameter that does not comply with ideal green analysis [35,36] from a total score of 100. The method is considered inadequately green if the score was less than 50, acceptable if the score was 50–75 and excellent if the score was > 75. As shown in Table 3, our proposed

Table 1
Evaluation of accuracy for determination of ascorbic acid (ASC) by the proposed method.

| Concentration taken (µg/mL) | Concentration found (µg/mL) | Mean concentration found (µg/mL) | %Recovery | Mean %recovery ± standard deviation |
|---------------------------|-----------------------------|---------------------------------|-----------|-----------------------------------|
| 2                         | 2.036                       | 2.038                           | 1.985     | 2.02                              | 100.98 | 99.99 ± 1.03                     |
| 5                         | 4.950                       | 4.927                           | 4.908     | 4.93                              | 98.57  |                                  |
| 7                         | 7.030                       | 7.017                           | 7.041     | 7.03                              | 100.42 |                                  |
The method was found excellent compared with the reference method that was found to be only acceptable. GAPI is a five-pentagram representation for evaluating the effect of each step of the analytical process on the environment, from initial sampling to final measurement [37]. This index is a visual depiction of greenness that could be used to compare and evaluate different analytical approaches. Each section of the pentagram represents a step in the analytical process and is colored according to the greenness rating. The green, yellow, and red represent minimal, medium, and significant environmental effects, respectively. Sampling, shipping, storage, preparation, and analysis are all presented in the GAPI scale. The greenness of the developed spectrofluorometric method and the reference HPLC/UV method were compared using the GAPI evaluation. Because our method was organic solvent-free and the chemicals employed are markedly safe, the developed spectrofluorometric method was greener, according to the GAPI scale during sample preparation, as shown in Fig. 5.

### 3.5. Analytical application of the developed method to pharmaceutical dosage forms (Ruta C60°/C210° tablet)

The developed method was applied for the analysis of ASC in Ruta C60°/C210° tablets. The relative standard deviation (RSD), which is the % of the ratio between the standard deviation and the mean % recovery was calculated. Accepted values are attained when % RSD is ≤ 2. The results were compared with those of the reference HPLC/UV method [38] using Student’s t-test and F-test. The t-test compares the average %recovery to evaluate accuracies, while the F-test compares variances to assess precision. Theoretical values at 95% confidence level (p = 0.05) for each test were obtained from statistical tables at the corresponding degrees of freedom. No significant differences were assumed between the developed and the reference methods if the calculated t- and F- values were smaller than the tabulated values. The assay results given in Table 4 showed no statistically significant differences between the developed spectrofluorometric method and the reference HPLC/UV method in terms of accuracy and precision. Yet, our proposed method is easier, faster, cheaper, and greener.

### 3.6. Comparison with other reported methods

Determination of ASC by spectrofluorometry requires chemical derivatization or the addition of a fluorescent probe due to lack of

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**Table 2**

| Parameter                      | Conditions | %Recovery | Mean %Recovery | SD  | %RSD |
|--------------------------------|------------|-----------|----------------|-----|------|
| Conc. of iron (III) salicylate (μM) | 95         | 99.47     | 99.51          | 0.148 | 0.149 |
|                                | 100*       | 99.68     |                |     |      |
|                                | 105        | 99.39     |                |     |      |
| pH                             | 4.7        | 99.78     |                | 0.180 | 0.180 |
|                                | 5.0*       | 99.84     |                |     |      |
|                                | 5.3        | 100.12    |                |     |      |
| Wavelength (nm)                | 409        | 100.51    |                | 0.406 | 0.406 |
|                                | 411*       | 99.84     |                |     |      |
|                                | 413        | 99.78     |                |     |      |

*Optimum condition.

**Table 3**

| Reagents                      | PPs | The developed method | Instruments | PPs |
|-------------------------------|-----|----------------------|-------------|-----|
| Ammonium iron (III) sulfate   | 1   |                      | Spectrofluorometer | 0   |
| Sodium salicylate             | 1   |                      | HPLC        | 0   |
| Nitric acid                   | 4   |                      | Occupational hazards | 0 |
|                               | 56  |                      | Waste       | 8   |
| Total PPs: 12 score: 88       |     |                      |             |     |

| Reagents                      | PPs | The reference method | Instruments | PPs |
|-------------------------------|-----|----------------------|-------------|-----|
| Acetonitrile                  | 12  |                      |             |     |
| Methanol                      | 12  |                      |             |     |
| Acetic acid                   | 4   |                      |             |     |
| Total PPs: 28 score: 64       |     |                      |             |     |

**Table 4**

| Mean % recovery ± SD          | Developed method | Reported HPLC method [38] |
|-------------------------------|------------------|--------------------------|
| 100.75 ± 0.39                | 101.05 ± 0.55    |

* Ruta-C60° tablets labeled to contain 160 mg of ASC and 60 mg of rutin.  
b Mean percentage recovery of three different sample solutions.  
Ruta C60° tablet.

c % RSD: percentage relative standard deviation.

d T-test (2.776) 

e F-test (19)

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**Fig. 5.** GAPI assessment of the proposed spectrofluorometric method (b), and the reference method (b).
fluorophore. The reported spectrofluorometric methods employed fluorescent dyes, or quantum dots as shown in Table 5. These methods suffered from a number of limitations such as the complicated procedures [14,39,40], the long reaction time [15], the high temperature required [18,41,42] and the necessity for catalyst addition. Compared with these reported methods, our proposed method is faster, simpler, and does not require heating or catalysis due to the instantaneous reaction and the high yield. In addition, our proposed method is more sensitive than the other reported methods which could have more applications in a trace analysis, cleaning validation or adaptation to biological fluids.

4. Conclusions
A highly sensitive method has been developed for determination of ascorbic acid in dosage forms based on its redox properties. The reducing characteristics of ascorbic acid have been exploited to restore the native fluorescence of salicylate in presence of iron (III), which was converted to iron (II) upon addition of ascorbic acid. The method showed excellent green characteristics according to the analytical Eco-scale and the green analytical procedure index. The method gave results, comparable to the reported HPLC method in terms of accuracy and precision. The method could also overcome the limitations in current spectrofluorometric methods such as the slow reaction time, the low yield, the low sensitivity and the required heating step or catalysis. The developed method could be applied to determine ascorbic acid in pharmaceutical preparations. The method was found good, simple, inexpensive and rapid with high selectivity and sensitivity. This work could be extended in the future for more applications in other matrices such as biological fluids and food products. This reaction can also be applied for spectrofluorometric determination of other analytes with reducing properties.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material
Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2021.120802.

References
[1] R.Z. Cheng, Can early and high intravenous dose of vitamin C prevent and treat coronavirus disease 2019 (COVID-19), Med Drug Discov. 5 (2020) 100028, https://doi.org/10.1016/j.medidmed.2020.100028.
[2] G.P. Milani, M. Macchi, A. Guz-Mark, Vitamin c in the treatment of covid-19, Nutrients. 13 (2021) 1–10, https://doi.org/10.3390/nu13041172.
[3] M.J. Gonzalez, Intravenous Vitamin C and metabolic correction as adjuvant therapy for prostate cancer: a case report, J. Cancer Prev. Curr. Res. 5 (2016) 2018–2020, https://doi.org/10.15460/jjpcrc.2016.05.00164.
[4] S.J. Darihe, A. Ma, M.A. Ross, A.R. Collins, Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes, Cancer Res. 56 (1996) 1291–1295.
[5] R.A. Jacob, The integrated antioxidant system, Nutr. Res. 15 (5) (1995) 755–766, https://doi.org/10.1016/0271-5317(95)00041-G.
[6] G. Grosso, R. Bei, A. Mistretta, S. Marventano, G. Calabrese, L. Masuelli, et al., Effects of vitamin C on health: A review of evidence, Front. Biosci. 18 (2013) 1017–1029, https://doi.org/10.2741/b460.
[7] F.R. Mansour, W. Wei, N.D. Danielson, Separation of caffeine and acetylcarnitine in biological samples: a review, Biomed. Chromatogr. 27 (2013) 1339–1353, https://doi.org/10.1002/bmc.2995.
[8] H. Hemila, Vitamin C and infections, Nutrients. 9 (2017) 2020–2022, https://doi.org/10.3390/nu9060433.
[9] S. Georgé, P. Brat, P. Alter, M.J. Amiot, Rapid determination of polyphenols and vitamin C in plant-derived products, J. Agric. Food Chem. 53 (5) (2005) 1370–1373, https://doi.org/10.1021/jf04396b.
[10] H. Khajehsharifi, E. Pourbashir, H. Tavallali, S. Sarvi, M. Sadegh, The comparison of partial least squares and principal component regression in simultaneous spectrophotometric determination of ascorbic acid, dopamine and uric acid in real samples, Arab. J. Chem. 10 (2017) S3451–S3458, https://doi.org/10.1016/j.arabjc.2014.02.006.
[11] C. Mu, H. Lu, J. Bao, Q. Zhang, Visual colorimetric ‘turn-off’ biosensor for ascorbic acid detection based on hypochlorite–3,3′,5,5′-tetramethylbenzidine system, Spectrochim. Acta – Part A Mol. Biomol. Spectrosc. 201 (2018) 61–66, https://doi.org/10.1016/j.saa.2018.04.059.
[12] X. Wu, Y. Diao, C. Sun, J. Yang, W. Sun, Fluorimetric determination of ascorbic acid with o-phenylenediamine, Talanta. 59 (2003) 95–99, https://doi.org/10.1016/S0039-9140(02)00475-7.
[13] Y. Dilgin, G. Nisli, Fluorimetric determination of ascorbic acid in vitamin C tablets using methylene blue, Chem. Pharm. Bull. 53 (10) (2005) 1251–1254, https://doi.org/10.1248/cpb.53.1251.
[14] C.M. Durán, A.M. Conteño, Á. Rios, A continuous method incorporating p-cyclodextrin modified CdSe/ZnS quantum dots for determination of ascorbic acid, Anal. Methods. 7 (8) (2015) 3472–3479.
[15] J. Zhu, Z.J. Zhao, J.J. Li, J.W. Zhao, Fluorescent detection of ascorbic acid based on the emission wavelength shift of CdTe quantum dots, J. Lumin. 192 (2017) 47–55, https://doi.org/10.1016/j.jlumin.2017.06.015.
[16] B. Rezaei, A.A. Ensaﬁ, S. Nouroozi, Flow-injection determination of ascorbic acid and cysteine simultaneously with spectrofluorometric detection, Anal. Sci. 21 (9) (2005) 1067–1071, https://doi.org/10.2116/analsci.21.1067.
[17] L.I. Abd Ali, A.F. Qader, M.I. Salih, H.Y. Aboul-Enein, Sensitive spectrofluorometric method for the determination of ascorbic acid in pharmaceutical nutritional supplements using acriflavine as a fluorescent reagent, Luminescence. 34 (2) (2019) 168–174, https://doi.org/10.1002/bio.3589.
[18] F. Ma, J. Luo, X. Li, S. Liu, M. Yang, X. Chen, A “switch-on” fluorescence assay based on silicon quantum dots for determination of ascorbic acid,
S.F. El-Malla, R.H. Elattar, A.H. Kamal et al. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 270 (2022) 120802

[23] T.R.L.C. Paixão, M. Bertotti, FIA determination of ascorbic acid at low potential
[24] S.F. El-Malla, E.A. Elshenawy, S.F. Hammad, F.R. Mansour, N-doped carbon dots
[20] A. Mazurek, J. Jamroz, Precision of dehydroascorbic acid quantitation with the
[22] Z. Chang, Y. Zhou, L. Hao, Y. Hao, X. Xu, Z. Hu, M. Xu, Simultaneous determination
[26] F.R. Mansour, N.D. Danielson, Ligand exchange spectrophotometric method for
[25] M. Mabrouk, S.F. Hammad, A.A. Abdella, F.R. Mansour, A novel strategy for
[28] A. Kumar, A. Pandith, H.-S. Kim, Pyrene-appended imidazolium probes as 3,5-
[32] W.A.E. McBryde, J.L. Rohr, J.S. Penciner, J.A. Page, Stability constants of three
[31] A. Pandith, C. Dasagrandhi, H.-R. Kim, H.-S. Kim, Selective discrimination of
doubly charged nanosensor for determination of rutin and ascorbic acid mixture in
[35] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical Eco-Scale
[39] Huiyuan Sun, Xueliang Liu, Xinhuan Wang, Qiusen Han, Cui Qi, Yanmei Li,
[41] B. Szpikowska-Sroka, J. Połedniok, Spectrophotometric determination of L-
[42] Milton K. Sasaki, Mario A. Feres, Elias A.G. Zagatto, Flow systems with MnO2-
[38] T.Z. Attia, Simultaneous determination of rutin and ascorbic acid mixture in their
[37] J. Hofta-Wasyka, A new tool for the evaluation of the analytical procedure; Green
[36] M. Tobiszewski, M. Marz, A. Galuszka, J. Namieśnik, Green chemistry metrics with
[34] A. Nejati-Yazdinejad, Indirect determination of ascorbic acid (vitamin C) by spectrophotometric method, Int. J. Food Sci. Technol. 42 (2007) 1402–1407, https://doi.org/10.1111/j.1365-2621.2006.01353.x.
[33] B. Szpikowska-Sroka, J. Poledniok, Spectrophotometric determination of L-
[35] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Green chemistry metrics with special reference to green analytical chemistry, Molecules. 20 (2015) 10928–10946, https://doi.org/10.3390/molecules200610928.
[32] W.A.E. McBryde, J.L. Rohr, J.S. Penciner, J.A. Page, Stability constants of three
[31] A. Pandith, C. Dasagrandhi, H.-R. Kim, H.-S. Kim, Selective discrimination of
doubly charged nanosensor for determination of rutin and ascorbic acid mixture in
[30] A. Nejati-Yazdinejad, Indirect determination of ascorbic acid (vitamin C) by spectrophotometric method, Int. J. Food Sci. Technol. 42 (2007) 1402–1407, https://doi.org/10.1111/j.1365-2621.2006.01353.x.
[29] S.F. El-Malla, F.R. Mansour, A simple innovative spectrofluorometric method for
[28] A. Kumar, A. Pandith, H.-S. Kim, Pyrene-appended imidazolium probes as 3,5-
dinitrosalicylic acid sensors in 10% aqueous media, Dye. Pigment. 122 (2015) 351–358, https://doi.org/10.1016/j.dyepig.2015.07.008.
[27] S.F. Elmalla, F.R. Mansour, A simple innovative spectrofluorometric method for
determination of alendronate in bulk and in pharmaceutical tablets, Luminescence. 34 (3) (2019) 375–381, https://doi.org/10.1002/bio.3622.
[26] M. Mabrouk, S.F. Hammad, A.A. Abdella, F.R. Mansour, A novel strategy for
tetoralor detection based on turn-on plasmonic enhanced FRET synchronous
fluorometric sensor employing micellized chitosan/AgNPs nanocomposites: Preparation and mechanism investigation, Colloids Surfaces A Physicochem. Eng. Asp. 614 (2021) 126182, https://doi.org/10.1016/j.colsurfa.2021.126182.
[25] M. Mabrouk, S.F. Hammad, A.A. Abdella, F.R. Mansour, A novel strategy for
tetoralor detection based on turn-on plasmonic enhanced FRET synchronous
fluorometric sensor employing micellized chitosan/AgNPs nanocomposites: Preparation and mechanism investigation, Colloids Surfaces A Physicochem. Eng. Asp. 614 (2021) 126182, https://doi.org/10.1016/j.colsurfa.2021.126182.
[24] S.F. El-Malla, E.A. Elishenawy, S.F. Hammad, F.R. Mansour, N-doped carbon dots as a fluorescent nanosensor for determination of colchicine based on inner filter effect, J. Fluoresc. 31 (3) (2021) 675–684, https://doi.org/10.1007/s10895-021-02698-0.
[23] T.R.L.C. Paixão, M. Bertotti, FIA determination of ascorbic acid at low potential
using a ruthenium oxide hexacyanoferrate modified carbon electrode, J. Pharm. Biomed. Anal. 46 (3) (2008) 528–533, https://doi.org/10.1016/j.jpba.2007.10.033.
[22] Z. Chang, Y. Zhou, L. Hao, Y. Hao, X. Xu, Z. Hu, M. Xu, Simultaneous determination
of dopamine and ascorbic acid using β-cyclodextrin/Au nanoparticles/graphene-modified electrodes, Anal. Methods. 9 (4) (2017) 664–671.
[21] A. Mazurek, J. Jamroz, Precision of dehydroascorbic acid quantitation with the
use of the subtraction method - Validation of HPLC-DAD method for determination of total vitamin C in food, Food Chem. 173 (2015) 543–550, https://doi.org/10.1016/j.foodchem.2014.10.065.
[20] A. Mazurek, J. Jamroz, Precision of dehydroascorbic acid quantitation with the
use of the subtraction method - Validation of HPLC-DAD method for determination of total vitamin C in food, Food Chem. 173 (2015) 543–550, https://doi.org/10.1016/j.foodchem.2014.10.065.
[19] H. Iwase, Routine high-performance liquid chromatographic determination of
ascorbic acid in foods using L-methionine for the pre-analysis sample stabilization, Talanta. 60 (2003) 1011–1021, https://doi.org/10.1016/S0039-1409(03)00180-2.