Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 695–701
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical Methods

Determination of total phenols in tea infusions, tomato and apple juice by terbium sensitized fluorescence method as an alternative approach to the Folin–Ciocalteu spectrophotometric method

Masoomeh Shaghaghi [a], Jamshid L. Manzoori [a,*], Abolghasem Jouyban [b]

[a] Department of Analytical Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran
[b] Faculty of Pharmacy and Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Received 17 July 2007; received in revised form 25 October 2007; accepted 8 November 2007

Abstract

A fast screening of total phenols in tea infusions, tomato and apple juice samples using terbium sensitized fluorescence is described. The proposed method is based on the fluorescence sensitization of terbium (Tb^{3+}) by complexation with flavonols (quercein as a reference standard) (at pH 7.0), which fluoresces intensely with an emission maximum at 545 nm when excited at 310 nm. Quercetin and terbium cations (at pH 7.0) form a stable complex and the resulted emission at 545 nm can be used for the determination of the total phenols concentration expressed in terms of "quercetin equivalent". Based on the obtained results, a sensitive, simple and rapid spectrofluorimetric method was developed for the determination of total phenols. In the optimum conditions, the calibration graph was linear from 0.01 to 2 μg mL^{-1}, with the limit of detection of 0.002 μg mL^{-1}. The relative standard deviation values were in the range of 0.75–2.3%. The total concentrations of quercetin equivalent in five tested samples were found in the range of 6.6–27.9 μg mL^{-1} and the results compare favorably with those obtained by spectrophotometric method ($r = 0.999$).

Keywords: Sensitized fluorescence; Flavonols; Total phenols; Terbium

1. Introduction

Flavonoids are a group of polyphenolic compounds widely distributed in the medicinal plants, vegetables, fruit juices and a variety of beverages (tea, coffee, wines and fruit drinks). Flavonoids, and particularly quercetin derivatives, have received more attention as dietary constituents during the last few years. Experimental studies demonstrated that they possess numerous beneficial effects on human health, including cardiovascular protection, anticancer activity, antiulcer effects, and antiallergic, antiviral, and anti-inflammatory properties. This health-promoting activity seems to be related to the natural antioxidant (free-radical scavenging) activity of flavonoids (Chu, Chang, & Hsu, 2000; Hollman & Katan, 1999).

Flavonoids have strong coordination ability towards a wide range of cations (Leopoldini, Russo, Chiodo, & Toscano, 2006; Zhou et al., 1999). Their multiple hydroxyl groups and the carbonyl group on ring C, have several available sites for metal complexation. The experimental (Kaldas, Walle, Van der Woude, McMillan, & Walle, 2005) and theoretical (Cornard, Dangleterre, & Lapouge, 2005; Satterfield & Brodbelt, 2000) studies indicated that the preferred complexation site for flavonoids involves the hydroxyl group on carbon 3 or 5 and the adjacent 4-carbonyl group (Fig. 1).

Several analytical methods have been proposed for the determination of flavonoids including reversed-phase high-performance liquid chromatography (RP-HPLC) (Bilbao, Lacueva, Jauregui, & Lamuela-Raventos, 2007;

* Corresponding author. Fax: +98 411 3340191.
E-mail address: manzoori@tabrizu.ac.ir (J.L. Manzoori).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.11.008

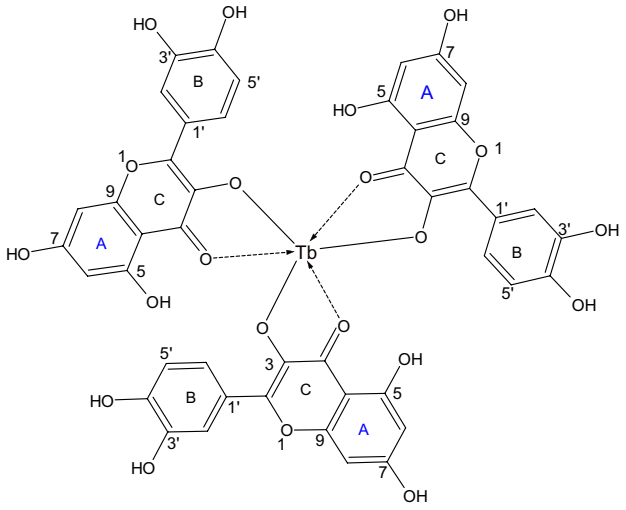

Fig. 1. The structure of Tb–quercetin complex tentatively proposed.

Draves & Walker, 2000; Fang, Li, Pan, & Huang, 2007; Ruckert et al., 2004), gas chromatography–mass spectrometry (GC–MS) (Luan, Li, & Zhang, 2000), LC with tandem mass spectrometry (LC–MS–MS) detection (Riedel et al., 2004), LC with ionization electrospray mass spectrometry (LC–ESI–MS) (Brito, Araujo, Lin, & Harnly, 2007) and capillary electrophoresis with electrochemical detection (Xu, Yu, & Chen, 2006). Some of these methods (LC–MS) are very expensive, complicated, time consuming, need extraction (sample preparation) or derivatization and also suffer from high consumption of samples (Brito et al., 2007; Luan et al., 2000), consequently not widely used in routine laboratories.

As the biological activity of flavonoids belongs to their mixtures and the amount of individual flavonoids in fruits and plants is usually low, they have often been recorded unspecifically as 'total phenolics'. Various enzymatic, spectrophotometric and electrochemical methods have been reported for this purpose (Benzie & Strain, 1999; Cliffe, Fawer, Maier, Takata, & Ritter, 1994; Roura, Andres-Lacueva, Estruch, & Lamuela-Raventos, 2006; Volikakis & Efstathiou, 2005).

It is well known that some trivalent lanthanide ions in an aqueous medium at ambient temperature have fluorescence properties due to, principally, the transition within the 4f-shell but are weakly fluorescesing species due to their low molar absorptivities and poor quantum yields (Sinha, 1971). However, when these ions, in particular Eu^{3+} and Tb^{3+} are chelated with ligands that have a broad intense absorption band their fluorescence can be dramatically enhanced (Lis, 2002). Intramolecular transfer of energy from the excited organic molecule to a resonance level of the lanthanide followed by the lanthanide emission is responsible for excitation of lanthanide ion in the formed complex and also fluorescent enhancement. If there is efficient intramolecular energy transfer, the upper emitting levels of the lanthanides are more effectively excited by this technique than by the direct form, producing an enhanced fluorescence of the lanthanide by several orders of magnitude. This phenomenon has been named the lanthanide sensitized fluorescence that is remarkable interest for the trace determination of lanthanide ions and a variety of medically important compounds (Van Uitert & Iida, 1962). The main features of the process are large Stokes shift, a narrow-emission bands and long fluorescence lifetimes, and hence avoid potential background fluorescent emission interferences from the biological matrix, so selectivity and detection limits are improved (Van Uitert & Iida, 1962).

Due to the importance of flavonoids as contributors of beneficial health effects of fruit and vegetables consumption, the purpose of this work was to develop and validate a rapid, simple and reliable fluorimetric method, as an alternative approach to the widely used Folin–Ciocalteu (FC) spectrophotometric method (Benzie & Strain, 1999; Singleton, Orthofer, & Lamuela-Raventos, 1999) for the determination of total phenols (quercetin equivalent) based on terbium sensitized fluorescence (TSF). To the best of our knowledge, there was no report on flourimetric method for the determination of total phenol of plants and fruit juices until now. It seems that terbium sensitized fluorescence method is particularly appealing for this purpose, because it combine a very simple and relatively low-cost instrumentation with high sensitivity and selectivity.

Obtained studies using UV–vis spectrophotometry indicated that quercetin can be complexated with terbium cations (at pH 7.0) to form a stable product and can be used for the determination of total phenols in real samples in terms of quercetin equivalent (QE). QE determined in this way is satisfactorily correlated to a weighted sum of the individual concentrations of all flavonols. The expression in terms of QE is justified by the fact that quercetin is one of the major flavonols found in beverages such as wines, tea infusions, and also in tomato juice (Fang et al., 2007) and always present flavonol and is the most powerful antioxidant in the flavonoid family.

2. Experimental

2.1. Reagents

All reagents and solvents used were of analytical grade and were used without further purification. Doubly distilled water was used throughout.

A 10^{-2} M terbium(III) solution was prepared by dissolving the appropriate amount of terbium(III) chloride hexahydrate ($TbCl_3 \cdot 6H_2O$) (Acros Organics, USA) in doubly distilled water and stored in a polyethylene containers to avoid memory effects of terbium adsorbed on glass vessels.

A stock solution (100 μg mL^{-1} and 10^{-3} M) of quercetin (Sigma) was prepared in ethanol and doubly distilled water and for experiments freshly diluted in water in order to have less than 2% of ethanol. Aqueous solutions of quercetin were found to be stable for about 18 h. After this per-

iod, substantial decrease of absorbance and fluorescence was observed reaching 50% of starting value after 20 h. Since there were no shifts of maxima and complete UV/vis spectra decreased proportionally, this phenomenon could be caused by slow precipitation of quercetin. Therefore, freshly prepared quercetin solution was used in each experiment.

A 0.05 M Tris-(hydroxymethyl)aminomethan–hydrochloric acid (Tris–HCl) buffer solution was prepared by dissolving a desired amount of Tris–base (Merck) in 90 mL of water, adjusting the pH to 7.0 with HCl and making up the volume to 100 mL with water.

A Folin–Ciocalteu reagent and Na_2CO_3 were obtained from Merck. Tea bags were purchased from a local store with the trade marks of Ahmed, Alghazalen and Golestan. The net weights of the tea powder in the bags were 2.35 ± 0.01, 1.91 ± 0.01 and 1.98 ± 0.01 g, respectively.

2.2. Apparatus

Fluorescence spectra and intensity measurements were performed using a Shimadzu RF-540 spectrofluorimeter (Kyoto, Japan) equipped with a 150 W xenon lamp, using 1.0 cm quarts cell. The excitation and emission monochromator bandwidths were 10 nm. The excitation wavelength was set at 310 nm and the fluorescence was measured using the peak height 545 nm. All measurements were performed at 25 ± 0.1 °C using a thermostated cell holder and a thermostatically controlled water bath (Rikakika, Japan). UV measurements (230–500 nm) were carried out at room temperature by means of a Shimadzu 1650 PC spectrophotometer. The pH of solutions was measured with Metrohm model 654 pH meter (Herisau, Switzerland).

2.3. Sample preparation

Tea infusion was prepared by immersing a tea bag in 250 mL of boiling water for 5 min. The mixture was further diluted to 500 mL with water. A 5.00 mL portion of fresh tomato and apple juices (prepared by filtering) was transferred in a 100 mL flask and diluted with water. Finally, 0.1 mL (for fluorescence measurements) or 0.5 mL (for spectrophotometry measurements) of prepared sample was used.

2.4. Methods

All measurements were corrected for the background fluorescence of blank which was taken as the solution containing all reagents except the quercetin. Optimization of terbium sensitized fluorescence was investigated by varying the pH or the concentration of one of the components while the rest remained constant.

2.4.1. Experimental procedure

For the terbium sensitized fluorescence determination, the analytical procedure used to construct the calibration graphs was follows: to 5 mL volumetric flasks, 2 mL of 1×10^{-2} M Tb^{3+} solution, 0.25 mL of 0.05 M Tris–HCl buffer (pH 7.0) solution and aliquots of working quercetin solutions were added. The final quercetin concentrations were in the range of 0.01–2 μg mL^{-1}. The solutions were thermostated at 25 ± 0.1 °C and the fluorescence intensity was measured at 545 nm (after 5 min) using an excitation wavelength of 310 nm against a blank solution. Both emission and excitation slits were set at 10 nm.

2.4.2. Determination of total phenols using fluorimetric method

The proposed method was applied to the determination of total phenols in tea infusions, tomato and apple juices. The multiple standard addition method (four additions) was used as the quantification technique, using a quercetin standard solution and the results were expressed in terms of QE.

2.4.3. Determination of total phenols using spectrophotometric method

Total phenolic contents of fruit juices and tea infusion samples were determined by the Folin–Ciocalteu method (Singleton et al., 1999). Briefly, aliquots of 0.5 mL of samples and standards were mixed with 2.4 mL of deionized water, 2 mL of 2% sodium carbonate (Na_2CO_3), and 0.1 mL of Folin–Ciocalteau reagent. After incubation at room temperature for 60 min, the absorbance of the reaction mixture was measured at 750 nm against a deionized water blank on a spectrophotometer (Shimadzu, model 1650 PC). Using a seven point standard curve quercetin solutions (1–10 μg mL^{-1}), the total phenolic contents of samples were determined in triplicates. The data were expressed as QE (μg mL^{-1}).

3. Results and discussion

3.1. Interaction between quercetin and Tb(III) (UV–vis studies)

The UV–vis absorbance spectra of 5×10^{-5} M quercetin and Tb(III)–quercetin system (with increasing concentration) in the Tris–HCl buffer solution (pH 7.0) are shown in Fig. 2. The UV–vis spectrum of quercetin, like most flavones and flavonols, shows an intense absorbance at 368 nm (band I) and at 255 nm (band II) (spectrum (a) of Fig. 2). Band I is related to ring B (cinnamoyl system) and band II to ring A (benzoyl system) (Fig. 1) (Kang et al., 2004; Zhou, Wang, Wang, & Tang, 2001). The spectra are related to the $\pi \rightarrow \pi^*$ transitions within the aromatic ring of the ligand molecules. When Tb^{3+} was added to the solution, in comparison of with quercetin absorption spectrum, that of the complex is shifted to the long-wavelength region as shown in Fig. 2. Such bathochromic shift can be explained by the extension of the conjugated system with the complexation. Band I red shifts by ca. 80 nm and a new stronger intense absorbance peak appear at 447 nm. Band II

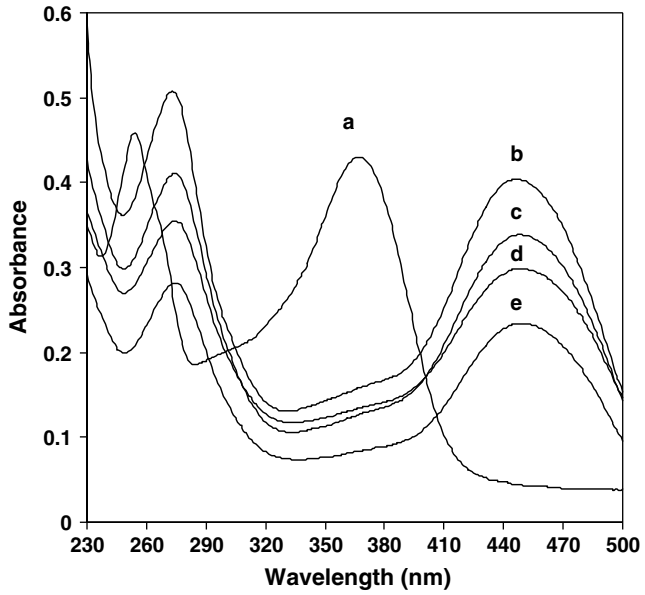

Fig. 2. UV/vis absorption spectra of (a) free quercetin (5×10^{-5} M) and the Tb(III)–quercetin mixtures at different concentration of Tb^{3+}, (4×10^{-3} M (b), 4×10^{-4} M (c), 2×10^{-4} M (d) and 1×10^{-4} M (e)) in Tris–HCl (pH 7.0) buffer solution.

shows a stronger intense absorbance at higher wavelength (273 nm) (spectrum (b) of Fig. 2). The results indicate that quercetin could form a stable complex with Tb^{3+}. There are three possible groupings on quercetin that can interact with metal ions: (I) the 3- and 4-hydroxyls of ring B; (II) the 3- or 5-hydroxyls, and (III) the 4-carbonyl of ring C. As the 3-hydroxy group has a more acidic proton, the 3-OH and 4-oxo groups are the first sites to be involved in the complexation process (Jovanovic, Steenken, Tosic, Marjanovic, & Simic, 1994). The peak at 368 nm red shifted, and a new peak at higher wavelength emerged suggesting that Tb^{3+} has bonded with ring B. The new peak at 447 nm can be explained by binding of Tb^{3+} with the 3-OH group of quercetin bring electronic redistribution between the quercetin molecule and Tb^{3+} to come into being a big extended π bound system. On this base, electron transition of quercetin changed from $n \rightarrow \pi^*$ transition to $\pi \rightarrow \pi^*$ transition and the decrease of energy transition lead to the appearance of a new peak at the longer wavelength (447 nm). A proposed structure of the complex is shown in Fig. 1.

3.2. Fluorescence characteristics of quercetin complex with terbium(III) in aqueous solution

The addition of terbium ions to aqueous solutions of quercetin (pH 7.0) results in the appearance of the narrower and stronger new emission bands at the range of 450–600 nm ($\lambda_{max} = 545$ nm), characteristic of terbium ion fluorescence which is due to the complex formation of quercetin with terbium(III) (Fig. 3).

The additional evidences proving the formation of the Tb–quercetin complex are: (a) the free Tb^{3+} aqueous solutions under the same conditions fluoresces too weakly to be observed and (b) in the presence of Tb^{3+}, the emission band of Tb^{3+} appears whose peak intensity is much greater than that of uncomplexed quercetin. Therefore, it was decided to test whether quercetin can be determined by the terbium sensitized fluorescence method.

As an example, the fluorescence excitation and emission spectra of Tb–quercetin (a), quercetin (b) and Tb^{3+} (c), at pH 7.0 are shown in Fig. 3. From spectra b and c in Fig. 3A, it can be seen that only the Tb^{3+} and quercetin solutions have little or nearly no peak. Under the same conditions, after addition of the quercetin into Tb^{3+} solution, quercetin can form a ternary complex with Tb^{3+} ion, which leads to energy transfer from quercetin to Tb^{3+}, so two strong characteristic peaks of Tb^{3+} ion appear at 490 and 545 nm. The most intense band is the $^5D_4 \rightarrow {}^7F_5$ transition at 545 nm, thus 545 nm was selected for measurement (see Fig. 3B).

3.3. Optimization of analytical signal

The fluorescence intensity of Tb–quercetin is strongly dependent on pH. Solutions containing reagents and their corresponding blank solutions with various pH values from 2 to 9 were prepared using HCl or NaOH. The results indicated that the fluorescence reached maximum intensity at pH 7.0. This is in accordance with the fact that the ligand will coordinate with terbium ions more efficiently in its ionic forms. Results showed that 0.25 mL of Tris–HCl (0.05 M, pH 7.0), in a final 5 mL, was the most suitable buffer.

Another important parameter influencing the fluorescence intensity is the terbium concentration which was studied in the range of $(0–5) \times 10^{-3}$ M for complex. The intensity is enhanced with an increase in the Tb^{3+} concentration and reached the maximum value for concentrations of 4×10^{-3} M.

It is well known that the lanthanide sensitized fluorescence can be influenced by using the second ligand, solvents and various surfactants (Georges, 1995). Thus, the influences of different concentrations of EDTA and Phen (10^{-5}–10^{-4} M), as co-ligands, and several concentrations of various surfactants such as Triton X-114 (0.01–0.05%), Brij-35 (0.01–0.4%), SDS, CTAB and AOT (5×10^{-4}–10^{-3} M) were investigated. In all cases, the presence of a micellar medium and a co-ligand caused a slight decrease in the fluorescence intensity of the system. The results showed that in the presence of the concentrations of 5–10% of organic solvents, such as methanol, ethanol, propan-2-ol, dimethylformamide, 1,4-dioxane, acetone and dimethylsulfoxide, the fluorescence intensity was quenched and the maximum fluorescence intensity was observed in Tris–buffered solution. According to these different results, the use of a synergistic agent, a surfactant or an organic solvent was discarded. This is a major advantage compared to some lanthanide sensitized fluorescence based methods because the addition of the enhancer always elongates the

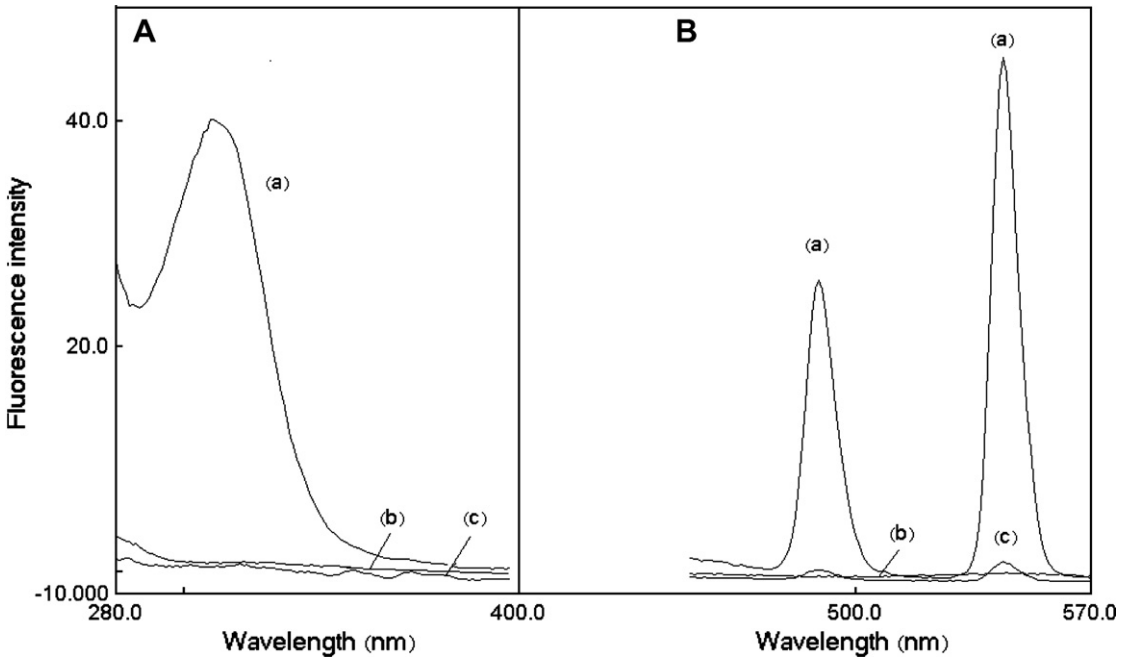

Fig. 3. Terbium sensitized fluorescence excitation ($\lambda_{em} = 545$ nm) (A) and emission ($\lambda_{ex} = 310$ nm) (B) spectra of the Tb–quercetin complex: (a) Tb–quercetin, (b) quercetin and (c) Tb^{3+}. Conditions: $[Tb^{3+}] = 4 \times 10^{-3}$ M, [quercetin] = 1 μg mL^{-1}, pH 7.0.

time for the determination of the sample and causes higher costs for the additional reagent.

This fluorescence behavior of the system could be ascribed to the fact that quercetin are two dentates ligand where two strong donor from 3-hydroxyl, and the 4-carbonyl of ring C, giving a better stability to the system, so that it is less affected by non-radiative processes than other Tb^{3+} chelates.

The experimental results showed that the order of the addition of the various components has small effects on the fluorescence intensity of system. Considering the stability of the system along with the fluorescence intensity enhancement, the following order was chosen for further study: Tb^{3+} solution, Tris–HCl buffer and quercetin standard solution.

3.4. Analytical figures of merit

By using the optimized conditions described above, a spectrofluorimetric method was developed for the determination of total phenols in QE. The calibration graphs ($n = 18$) were found to be linear in the range of 0.01 and 2 μg mL^{-1} for quercetin and its equation was $F = 34.24(\pm 0.38)C + 3.69(\pm 0.28)$, where F is the fluorescence intensity and C is the concentration of quercetin expressed in μg mL^{-1}. The limit of detection was calculated as $3S_b/m$ (where S_b is standard deviation of the blank and m is slope of the calibration graph) was found to be 0.002 μg mL^{-1}.

In order to study the precision of the proposed method, series of six solutions of 0.2 and 0.1 μg mL^{-1}of quercetin were measured on the same day. By applying the IUPAC definition, the relative standard deviation (RSD) for six analyses was 0.75–2.3%.

3.5. Determination of quercetin equivalent (QE) in real samples

To examine the applicability of the method as a rapid, simple, reliable and sensitive alternative approach to the widely used FC method, the proposed method was applied to the determination of the total phenols, expressed in QE in tea infusions, tomato and apple juice samples using the standard addition method. For this, fluorescence spectrum of background signal and the analytical signal (sample-Tb complex) before and after spiking of a tea infusion sample with known amounts of quercetin was studied (Fig. 4) Comparing this spectrum with fluorescence emission spectrum of the quercetin standard solution (Fig. 3), show that emission peak of Tb^{3+}–quercetin complex at 545 nm can be quantified and expressed in terms of QE which can be used as a valuable index of the flavonol content.

For performing the correlation study, FC method was used as a reference method for the determination of total phenols. To evaluate the linearity of the FC method, we prepared calibration curves with quercetin as an index for total phenols. The FC method was linear over working range between 1 and 10 μg mL^{-1}. Least-squares regression analysis gave the following results for the quercetin calibration curves: $y = 0.04x + 0.557$, $r = 0.999$, RSD values 4.6–5.8% and LOD 0.82 μg mL^{-1}. Table 1 presents the results obtained for the determination of total phenols in real samples. The results obtained by the TSF and FC methods are in good agreement (Regression equation: $y = 0.997x + 0.70$

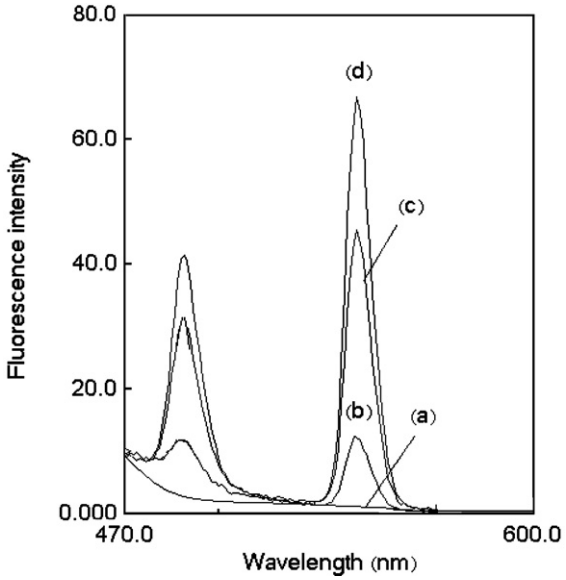

Fig. 4. Typical fluorescence emission spectra of tea infusion (a) background signal, (b) tea infusions in the prescence of Tb^{3+} and (c,d) the same solution after the addition of quercetin standard solution (0.25 and 0.5 μg mL^{-1}, respectively).

and correlation coefficient, $r = 0.999$). A comparison of the proposed fluorimetric method with FC method, shows that this new TSF method, is very rapid (measurement was occurred within 5 min vs. 60 min) and very sensitive (about 850-fold). It is easily carried out, gives good precision and accuracy and provides the detection limit about 400-fold lower than that of FC method. Also the results showed that in samples that phenol concentrations are very low, FC method can not be used but TSF method was very suitable and allows analysis of samples with very low concentration. Therefore, it can be used as an alternative approach to FC method for the determination of total phenols (in QE). This method successfully applied to the determination of total phenols in tea infusions, tomato and apple juice samples directly after filtering, without needing a separation technique in analysis (solvent extraction, liquid extraction and solid phase extraction), due to high sensitivity and selectivity obtained with this system.

Table 1
Comparison of results of the determination of total phenols (in quercetin equivalent, QE) in real samples obtained by fluorimetry and spectrophotometry methods

Sample type	Total phenols (in QE, μg mL^{-1})[a]	
	Terbium sensitized fluorescence method	Spectrophotometric method
Tea infusion 1 (Ceylon, Ahmad tea)	27.9 ± 0.2	28.1 ± 1.1
Tea infusion 2 (Ceylon, Alghazalen tea)	21.2 ± 0.2	22.1 ± 0.3
Tea infusion 3 (Golestan)	22.4 ± 0.4	23.0 ± 1.0
Tomato juice (Refresh)	18.5 ± 0.2	19.6 ± 0.3
Apple juice (Refresh)	6.6 ± 0.2	7.0 ± 0.3
Diluted tea infusion 1 (dilution factor is 200-fold)	0.142 ± 0.001	Not detectable

[a] Average of three determinations ± SD.

Table 2
Comparison of results of the analytical characteristics of the proposed method with those of other methods for the determination of total phenols

Features	TSF method	FC method	HPLC–DAD[a]	Modified FC method[b]	FI–AdSV[c]
Linear range (μg mL^{-1})	0.01–2	1–10	0.27–0.97	1–14	Data was not provided
Detection limit (μg mL^{-1})	0.002	0.82	0.10	0.90	0.009
Correlation coefficient (r)	0.999	0.999	>0.990	0.998	0.970
RSD% range	0.8–2.3	4.6–5.8	9.6	0.6–2.7	3–6

[a] Bilbao et al. (2007).
[b] Roura et al. (2006).
[c] Volikakis and Efstathiou (2005).

Table 2 compares the analytical characteristics of the proposed TSF method with some published methods for the determination of total phenols. As can be seen, the TSF method has better detection limit, precision and sensitivity than other methods. This method is also simpler and faster than other methods and possesses wider determination range and good correlation coefficient.

4. Conclusions

A new spectrofluorimetric method was developed for the determination of total phenols (in QE) in real samples based on terbium sensitized fluorescence. The proposed method is very simple, precise, rapid and more sensitive than the other methods that have been used for the determination of total phenols until now (Benzie & Strain, 1999; Roura et al., 2006). The developed method was easily applied to the determination of total phenols in real samples of tea infusions, tomato and apple juice with excellent reproducibility. This method doses not require previous separation techniques probably, due to the fact that the most valuable feature of terbium sensitized cause selectivity is enhanced. Hence, flavonols can be determined in real samples without any significant interference from the abundant.

Acknowledgment

The authors would like to thank the research affairs of the University of Tabriz for the financial support.

References

Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antiox-

idant power and ascorbic acid concentration. *Methods Enzymology, 299*, 15–27.

Bilbao, M. D. L. M., Lacueva, C. A., Jauregui, O., & Lamuela-Raventos, R. M. (2007). Determination of flavonoids in a citrus fruit extract by LC–DAD and LC–MS. *Food Chemistry, 101*, 1742–1747.

Brito, E. S. D., Araujo, M. C. P. D., Lin, L. Z., & Harnly, J. (2007). Determination of the flavonoid components of cashew apple (Anacardium occidentale) by LC–DAD–ESI/MS. *Food Chemistry, 105*, 1112–1118.

Chu, Y. H., Chang, C. L., & Hsu, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*, 561–566.

Cliffe, S., Fawer, M. S., Maier, G., Takata, K., & Ritter, G. (1994). Enzyme assays for the phenolic content of natural juices. *Journal of Agriculture and Food Chemistry, 42*, 1824–1828.

Cornard, J. P., Dangleterre, L., & Lapouge, C. (2005). Computational and spectroscopic characterization of the molecular and electronic structure of the Pb(II)–quercetin complex. *Journal of Physical Chemistry A, 109*, 10044–10051.

Draves, D. H., & Walker, S. E. (2000). Determination of hypericin and pseudohypericin in pharmaceutical preparations by liquid chromatography with fluorescence detection. *Journal of chromatography B: Biomedical Sciences and Applications, 749*, 57–66.

Fang, F., Li, J. M., Pan, Q. H., & Huang, W. D. (2007). Determination of red wine flavonoids by HPLC and effect of aging. *Food Chemistry, 101*, 428–433.

Georges, J. (1995). Investigation of fluorescence efficiency in the europium-thenoyltrifluoroacetone chelate in aqueous and ethanolic solutions by laser-induced fluorescence and photothermal spectroscopic methods. *Analytica Chimica Acta, 317*, 343–357.

Hollman, P. C. H., & Katan, M. B. (1999). Dietary flavonoids: Intake, health effects and bioavailability. *Food and Chemical Toxicology, 37*, 937–942.

Jovanovic, S. V., Steenken, S., Tosic, M., Marjanovic, B., & Simic, M. G. (1994). Flavonoids as antioxidants. *Journal of American Chemical Society, 116*, 4846–4851.

Kaldas, M. L., Walle, U. K., Van der Woude, H., McMillan, J. M., & Walle, T. (2005). Covalent binding of the flavonoid quercetin to human serum albumin. *Journal of Agriculture and Food Chemistry, 53*, 4194–4197.

Kang, J., Zhuo, L., Lu, X., Liu, H., Zhang, M., & Wu, H. (2004). Electrochemical investigation on interaction between DNA with quercetin and Eu–Qu_3 complex. *Journal of Inorganic Biochemistry, 98*, 79–86.

Leopoldini, M., Russo, N., Chiodo, S., & Toscano, M. (2006). Iron chelation by the powerful antioxidant flavonoid quercetin. *Journal of Agriculture and Food Chemistry, 54*, 6343–6351.

Lis, S. (2002). Luminescence spectroscopy of lanthanide(III) ions in solution. *Journal of Alloys and Compounds, 341*, 45–50.

Luan, T., Li, G., & Zhang, Z. (2000). Gas-phase postderivatization following solid-phase microextraction for rapid determination of trans-resveratrol in wine by gas chromatography–mass spectrometry. *Analytica Chimica Acta, 424*, 19–25.

Riedel, K. D., Rieger, K., Martin-Facklam, M., Mikus, G., Haefeli, W. E., & Burhenne, J. (2004). Simultaneous determination of hypericin and hyperforin in human plasma with liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B, 813*, 27–33.

Roura, E., Andres-Lacueva, C., Estruch, R. M., & Lamuela-Raventos, R. (2006). Total polyphenol intake estimated by a modified Folin–Ciocalteu assay of urine. *Clinical Chemistry, 52*, 749–752.

Ruckert, U., Eggenreich, K., Wintersteiger, R., Wurglics, M., Likussar, W., & Michelitsch, A. (2004). Development of a high-performance liquid chromatographic method with electrochemical detection for the determination of hyperforin. *Journal of Chromatography A, 1041*, 181–185.

Satterfield, M., & Brodbelt, J. S. (2000). Enhanced detection of flavonoids by metal complexation and electrospray ionization mass spectrometry. *Analytical Chemistry, 72*, 5898–5906.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Sinha, A. P. B. (1971). *Spectroscopy in inorganic chemistry*. New York: Academic Press.

Van Uitert, L. G., & Iida, S. (1962). Quenching interactions between rare-earth ions. *Journal of Chemical Physics, 37*, 986–992.

Volikakis, G. J., & Efstathiou, E. C. (2005). Fast screening of total flavonols in wines, tea-infusions and tomato juice by flow injection/adsorptive stripping voltammetry. *Analytica Chimica Acta, 551*, 124–131.

Xu, X., Yu, L., & Chen, G. (2006). Determination of flavonoids in *Portulaca oleracea* L. by capillary electrophoresis with electrochemical detection. *Journal of Pharmaceutical and Biomedical Analysis, 41*, 493–499.

Zhou, J., Gong, G. Q., Zhang, Y. N., Qu, J. Q., Wang, L. F., & Xu, J. W. (1999). Quercetin–La(III) complex for the fuorimetric determination of nucleic acids. *Analytica Chimica Acta, 381*, 17–22.

Zhou, J., Wang, L. F., Wang, J. Y., & Tang, N. (2001). Synthesis, characterization, antioxidative and antitumor activities of solid quercetin rare earth(III) complexes. *Journal of Inorganic Biochemistry, 83*, 41–47.