Talanta 218 (2020) 121163

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Design of an automatic spectrophotometric system

Diego Barzallo[a], Kaewta Danchana[a,b], Víctor Cerdà[a,b], Edwin Palacio[a,*]

[a] Department of Chemistry. University of the Balearic Islands, E-07122, Palma, Spain
[b] Sciware Systems, Bunyola, Spain

ARTICLE INFO

Keywords:
Fluorometric system
3D printed
CCD detector
MSFIA
Soft drinks
Quinine

ABSTRACT

A Multi-Syringe Flow Injection Analysis (MSFIA) fluorometric system based on a 3D printing device hosting a CCD detector has been designed for the determination of quinine in soft drinks. A LED controlled by an electronic circuit was used as a radiation source. The entire system is connected to two USB outputs of a computer. The AutoAnalysis program has been used for data acquisition and treatment. The results are more accurate and precise than those obtained with a manual method using a conventional spectrofluorometer. This developed system is a viable alternative to reduce the consumption of reagents, the impact on the environment and reduce measurement costs.

1. Introduction

Automated analytical methods have been developed using flow techniques that require less human intervention and provide better performances of the analytical parameters compared to manual or batch analytical methods [1–3]. However, measurement systems coupled to the main flow techniques usually require conventional optical instruments, therefore the complexity of the automated system increases and they occupy large spaces within the laboratory, which leads the analyst to take these considerations into account [4]. For these reasons, there are different investigations based on the development of miniaturized systems based on 3D printing for spectrometric measurements that require an excitation source like a LED [5], a holder created by 3D printing [6], and low cost detectors like: a webcam [7], a digital microscope [8,9], a smartphone [10] or an endoscope for manual analysis. However, the main limitation of these instruments that combine optics and electronics devices to carry out analytical measurements is that the images obtained by these detectors subsequently require their treatment for the quantification of the analyzed samples. Therefore, these kinds of detectors are not able to be used in automated systems based on flow techniques. The use of CCD detectors [11,12] allows solving these problems which are connected via USB to the computer without the need for an external power supply for its operation. In addition, these types of detectors work in the visible, ultraviolet and near-infrared range that allow real-time spectroscopic measurements (such as absorbance, reflectance or fluorescence), and also allow adjusting some parameters that improve the resolution of the displayed spectra and data obtained during experimentation.

On the other hand, quinine is a natural alkaloid used mainly as a flavoring in soft drinks due to its characteristic bitter taste. However, this alkaloid causes health problems when it is consuming in excessive doses such as headache, fever, nausea, abdominal pain, blindness, asthma and diarrhea [13]. For this reason, some countries have established maximum limits of quinine content in soft drinks. The U.S. Food and Drug Administration (FDA) and German Federal Institute for Risk Assessment (BfR) established that the maximum concentration of quinine in soft drinks must be 83 mg L^{-1} and 85 mg L^{-1} respectively [14,15]. In addition the European Union legislation established a maximum limit of quinine in soft drinks of 100 mg L^{-1} [16].

Taking these considerations into account, in the present work a fluorometric system based on 3D device containing a quartz flow cell was designed for the determination of quinine in soft drinks using Multisyringe Flow Injection Analysis (MSFIA) [17]. To validate the proposed method, the obtained results were compared with those of a conventional spectrofluorometer.

2. Experimental

2.1. Chemicals and samples

All reagents were analytical grade. Quinine sulfate dihydrate (MM 782.94 g mol^{-1}, CAS 6119-70-6) was purchased from Scharlau, Spain. Milli-Q water (Milli-Q plus, 18.2 MΩ cm^{-1}) was used to prepare a 0.05 M sulfuric acid solution (Scharlau, Spain). A stock solution of quinine sulfate 0.8 g/L^{-1} was prepared by dissolving 0.039 g of $(C_{20}H_{24}N_2O_2)_2{\cdot}H_2SO_4{\cdot}2H_2O$ in 0.05 M sulfuric acid solution (Scharlau,

* Corresponding author.
E-mail address: edwin.palacio@uib.es (E. Palacio).

https://doi.org/10.1016/j.talanta.2020.121163
Received 2 April 2020; Received in revised form 14 May 2020; Accepted 16 May 2020
Available online 27 May 2020
0039-9140/

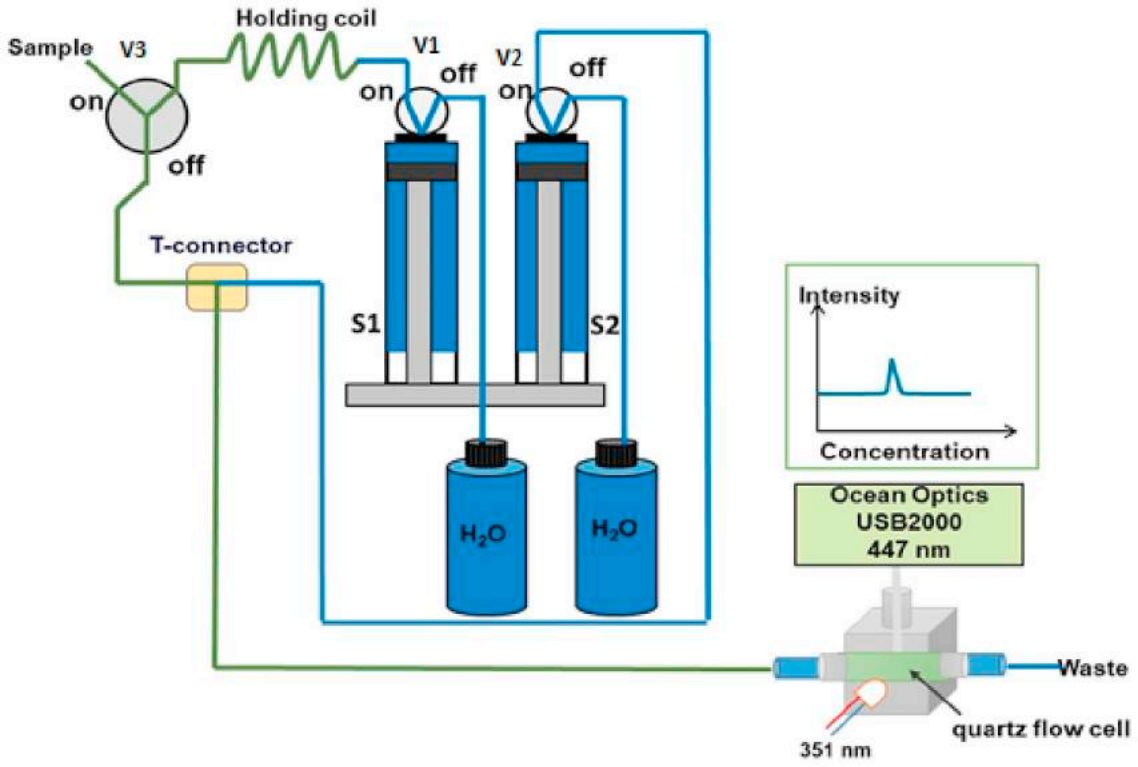

Fig. 1. Schematic representation of the MSFIA system using a CCD detector for determining of quinine in soft drinks.

Spain) and made up to 50 mL. The stock solution was stored in an amber bottle under refrigeration at 4 °C (for 3 days). Working standard solutions of 0–50 mg L^{-1} were prepared daily by dissolving an adequate amount of quinine sulfate stock solution in 0.05 M sulfuric acid (Scharlau, Spain).

Three soft drinks: Tónic schweppes, Tonic Water y Nordic Mix were purchased from a local supermarket (Majorca, Spain). The samples were degassed using an ultrasonic bath for 5 min and each sample was diluted (1: 2) with 0.05 M sulfuric acid solution. Three soft drinks were stored at room temperature.

A conventional spectrofluorometer (LS-50B., PerkinElmer® Inc.) was used as a reference method for the determination of quinine in different soft drinks. The scanning range was 300–700 nm, with excitation and emission wavelengths at 351 nm and 447 nm, respectively. The standard solutions were prepared in the linear range of 0–500 μg L^{-1}. Soft drinks were diluted (1: 200) with 0.05 M sulfuric acid solution (Scharlau, Spain).

2.2. System description

Fig. 1 shows a schematic representation of the system used for the determination of quinine in soft drinks by Multi-Syringe Flow Injection Analysis (MSFIA) with spectrofluorometric detection. To aspirate and dispense the liquids into the fluorometric system, a multi-syringe module (Crison Instruments S.A., Barcelona, Spain) was used together with an additional solenoid valve (V3) which was connected to the rear of the multi-syringe module. The module was equipped with two 5 mL glass syringes. The top of each syringe is connected to a three-way solenoid valve (V1, V2) that allows the connection with the manifold (position ON, activated) or with the reagent container (position OFF, deactivate). The solutions in the syringes were Milli-Q water. V3 was used for loading sample to a 2.5 mL holding coil 0.8 mm i.d. which was connected to syringe 1. S1 allows dispensing sample to the T-connector PMMA (polymethylmethacrylate) that retains the amount of sample to be transported by the carrier of the syringe 2. The use of two syringes allows increasing the analysis frequency, while S1 fills the holding coil with sample avoiding to contaminate S1; S2 is filled with water to transport the sample to the detector, thus avoiding to aspirate and dispense liquids a greater number of times through a single syringe.

Furthermore, a 3D printing holder device was used to host a LED (λexc = 351 nm) as a radiation source, and a flow cell constituted by a quartz tube of 1.8 i.d. and 3.0 mm o.d. This device was connected to a small USB2000 CCD detector (Ocean Optics Inc., Dunedin, FL, USA) located 90° respect to the radiation source to avoid reflected or transmitted incident light reaching the detector. Finally, the operation of the entire system has been carried out using the Autoanalysis 5.0. Program (Sciware Systems, Bunyola, Spain), which also allows obtaining the analytical signal and quantifying the analyzed samples.

2.3. Operation of the MSFIA system

The procedure started by lowering the pistons of the syringes to pick water through the OFF position of V1 and V2. Then the valve V3 was activated in order to pick a certain volume of diluted sample with sulfuric acid, which was stocked it in the holding coil, while the other valves were deactivated.

Once the sample and the carrier were loaded to the system, V1 was activated and V3 deactivated, which allowed dispensing the sample to the T-connector. The appropriate amount of sample was dispensed to the fluorometric flow cell by using the water carrier (V2 - ON), and the emitted intensity of the sample was measured at 447 nm. The real system MSFIA used in this research is shown in Fig. S1.

2.4. Fluorometric system designed

Fig. 2. 3D support design made with Rhinoceros program.

The LED used provides monochromatic UV light of 351 nm which is powered with the electronic circuit of Fig. 3, allowing to control the intensity of the exciting light. The current voltage applied to the LED was 5 V (high current intensity). The photographs of several components for making the designed fluorometric system are shown in Fig. S2.

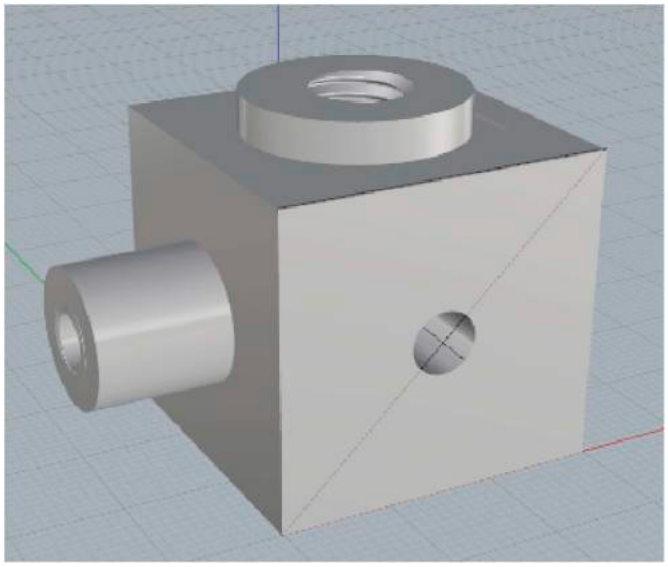

Fig. 2. Shows the holding support of the LED and quartz flow cell device was created by 3D printing with black methacrylate resin. This holder was designed using the Rhinoceros program and printed using FormLabs 2+ stereolithographic printer.

The voltage needed to activate the additional solenoid valve (V3) was taken from the rear panel of the multisyringe burette.

An Ocean Optics USB2000 CCD spectrometer connected via USB to the computer was used in this work (Fig. S1). This detector did not need any other external power supply for its operation. Once the light source excited the sample, the emitted light was measured by the spectrometer and the acquired data treated with the AutoAnalysis program. The advantages of this type of detector are its small size, robust optoelectronics, selectable sensitivity and the possibility to measure its dark current which could be removed during the experimentation allowing a better instrument response.

The proposed MSFIA method has been executed with the Autoanalysis program, which makes the data acquisition, treatment and provides a numerical value based on the height or area of the peak. The analyst may choose what is the best data to build the calibration curves taking into account the accuracy and precision of the method. Fig. S3 includes a display of the emission spectra obtained during data acquisition.

3. Results and discussions

3.1. Optimization of experimental conditions

For the automation of the proposed manual analytical method, the flow rate and volume of sample used must be taken into account. The optimization of these parameters was carried out by means of a univariate experimental analysis for the correct operation of the MSFIA system in fluorometric measurements. For both parameters, a standard solution of quinine sulfate 40 mg L^{-1} diluted in 0.05 M sulfuric acid was used. Fig. 4A indicates that the optimal flow rate to transport the sample to the detector was 2.5 mL min^{-1}, which provided a higher intensity in the analytical signal and an increase in the analysis frequency compared to using lower flow rates. Fig. 4B shows that the fluorescence intensity increases with sample volume due to a greater amount of sample will be excited by the radiation source [18]. However, the quinine in soft drinks is at levels of mg·L^{-1}, therefore, the proposed system not required to obtain a high sensitivity compared to other samples where the target analytes are in very low concentrations. In the present work, 0.8 mL of sample was used for the analysis, which also allows a greater reducing the reagents used.

3.2. Analytical parameters

Fig. 5 shows the emission spectra obtained from standard solutions of quinine sulfate 0–500 μg L^{-1}. The fluorescence intensity from quinine was observed at 447 nm.

Once the data were obtained using the Autoanalysis program, these were compared with a manual method using a conventional spectrofluorometer. Fig. 6 shows the calibration curves obtained using the proposed MSFIA system and a conventional method. Both calibration curves were obtained from standard solutions of quinine sulfate. In the MSFIA system, the linearity range was evaluated through spiking of quinine sulfate 0–50 mg L^{-1} in 0.05 M sulfuric acid, while the linearity range of the conventional spectrofluorometer was 0–500 μg L^{-1}.

Table 1 shows the results obtained of both calibration curves such as intercept, slope, correlation coefficient and linearity range. LOD y LOQ were calculated based on the standard deviation of the blanks and the slope, taking into account the established in the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) [19]. Repeatability (intra-day) and reproducibility (inter-day) were evaluated based on the relative standard deviation (% RSD) by analyzing triplicate samples.

The results obtained using an MSFIA fluorometric system coupled to a CCD detector, which provides satisfactory values of LOD (2.9 mg L^{-1}) and LOQ (9.7 mg L^{-1}). These values are lower compared to the conventional spectrofluorometer due to the linearity range used. The linearity range in the automatic method is in the mg·L^{-1} range, whereas the manual spectrofluorometric method is in μg·L^{-1}. The automatic method has the advantage that only requires diluting each sample of soft drink with 0.05 M sulfuric acid (in a proportion of 1: 2), this dilution avoids saturation of the analytical signal of the CCD detector. In the conventional spectrofluorometer requires diluting each sample of soft drink in a proportion of (1: 200) with 0.05 M sulfuric acid. Therefore, the spectrofluorometer needs a higher dilution to the μg L^{-1} levels to quantify quinine in soft drinks. Furthermore, the proposed system requires less analyst intervention and less time for analysis of

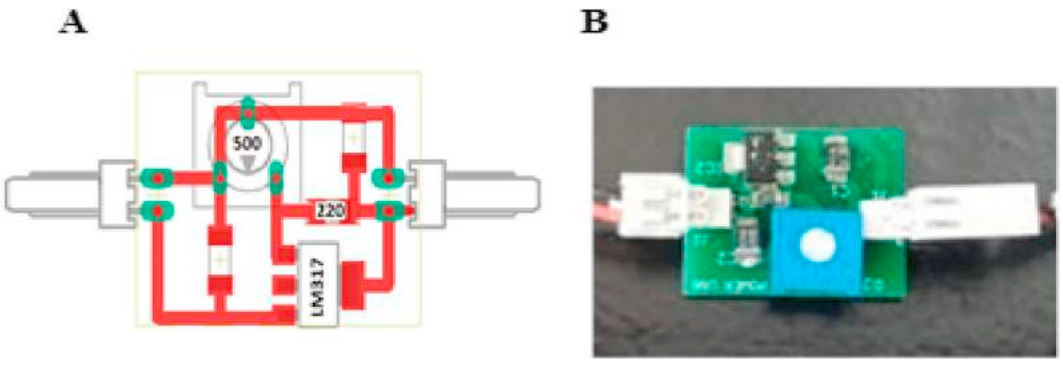

Fig. 3. A) Schematic representation of the regulated voltage power supply for the LED made with the Eagle program. B) Photo of its corresponding smd PCB card.

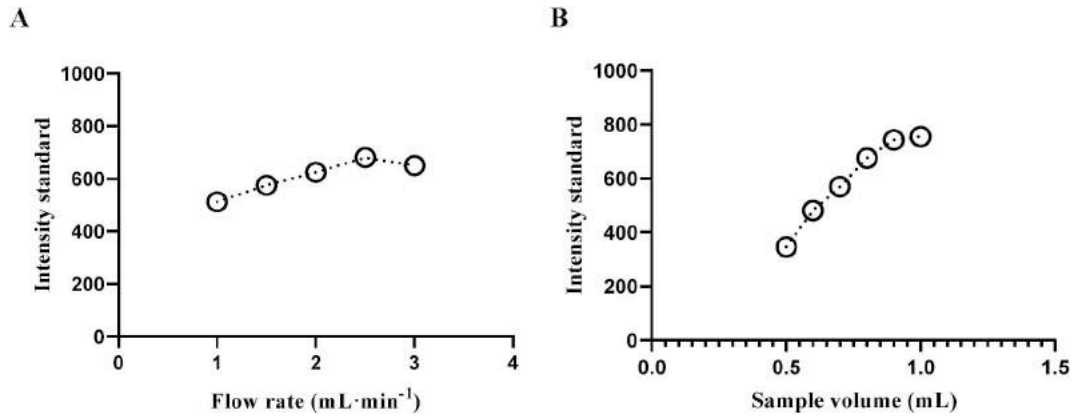

Fig. 4. A) Effect of the flow rate using 0.8 mL sample, B) Effect of the sample volume. Working conditions: 4 mg L^{-1} quinine sulfate diluted in 0.05 M acid sulfuric.

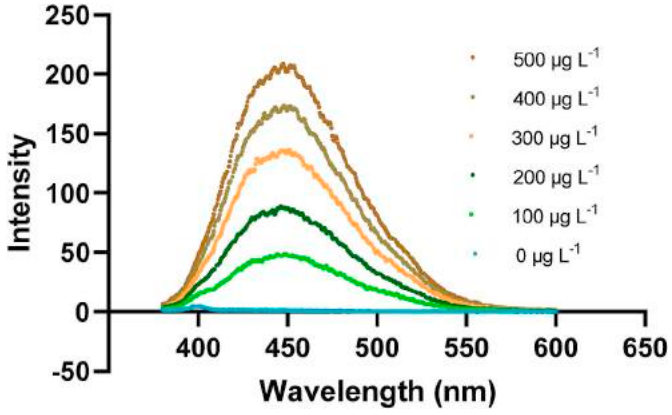

Fig. 5. Emission spectra obtained using different concentrations of quinine sulfate.

quinine in soft drinks with an analysis frequency of 40 injections·h^{-1}. On the other hand, the sample volumes used were not at the same for the two methods. In the MSFIA system the sample volume used was 0.8 mL while the volume used in the manual method was of 3.0 mL.

3.3. Interferences

Fluorescence quenching is an important parameter that must to be taken into account in any developed fluorimetric method. Samanidou et al. [20] found that other food additives present in soft drinks such as citric acid, glucose, sugar, saccharin, sodium benzoate (main preservative); these do not interfere in the determination of quinine.

The only interference in the determination of quinine in soft drinks is chloride ion, which can quench the quinine fluorescence, either due to a static quenching forming a non-fluorescente complex with the quinine or a dynamic quenching where excite fluorophore is deactivated by collision [21]. However, the chloride ion does not interfere in the determination of quinine at concentrations below 0.4 mM, which generally is the content in a tonic drink [22].

Table 1
Comparison of the results obtained using the automatic MSFIA method (Table S1) with the manual conventional spectrofluorometer.

Analytical Parameters	MSFIA	Spectrofluorometer
Calibration curve	I = 27.5 + 16.4 [quinine] mg L^{-1}	I = 7.0 + 0.4 [quinine] µg L^{-1}
Correlation coefficient (r^2)	0.995	0.996
Linearity range	0–50 mg L^{-1}	0–500 µg L^{-1}
LOD (3σ)	2.9 mg L^{-1}	1.4 µg L^{-1}
LOQ (10σ)	9.7 mg L^{-1}	4.7 µg L^{-1}
Repeatability (RSD, %)	0.4–0.7	0.6–1.7
Reproducibility (RSD, %)	0.6–1.7	1.5–2.6
Analysis frequency	40 injections·h^{-1}	17 samples·h^{-1}
Sample volume	0.8 mL	3.0 mL

3.4. Determination of quinine in soft drinks

Table 2 shows the concentrations of quinine obtained for some soft drinks. The samples analyzed with both methods were compared with a F-test (for a 95% confidence) presented *p*-value (0.28) > α (0.05) which indicates the samples have the same variance. Therefore, the t-test was used at a 95% confidence level to compare the results obtained with both methods, presented a *t*stat = 0.35 y *t*crit = 2.12, which means that the proposed system reports very similar results to those obtained with the spectrofluorometer.

The concentration of quinine in the analyzed soft drinks is below the maximum limit allowed by the European Union legislation, which is

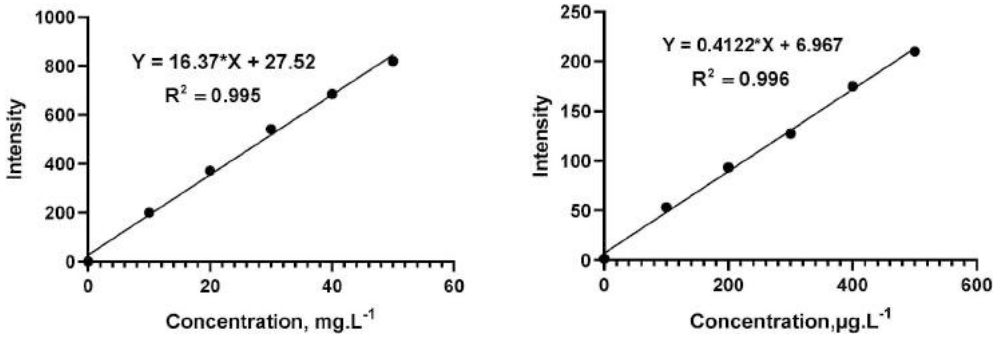

Fig. 6. A) Calibration curve using the MSFIA fluorometric system., B) Calibration curve using a conventional spectrofluorometer.

Table 2
Quinine concentrations in soft drinks.

Sample	MSFIA mg L^{-1}	Spectrofluorometer mg L^{-1}
Tónica schweppes	83.2 ± 1.4	81.5 ± 1.2
Tonic water	65.4 ± 1.1	66.8 ± 1.8
Nordic Mix	64.0 ± 1.3	63.5 ± 1.5

100 mg L^{-1}. In addition, the system proposed was compared with data reported by other works. Samanidou et al. [20] proposed a HPLC method with fluorescence detection for quinine determination in soft drinks. This method was able to obtain good results regarding its sensitivity without any interference effect. Buleandra et al. [24] determined quinine in soft drinks using a voltammetric method with an electrochemically pretreated pencil graphite electrode. However, both methods are manual and require further intervention by the analyst. Tzanavaras et al. [23] presented an automated method by coupling zone fluids (ZF) to liquid chromatography which allowed the determination of quinine in beverages, obtaining good sensitivity to levels of mg·L-1. However, the proposed new method offers several important advantages: it is a simpler method, it also allows a direct analysis of quinine in soft drinks and the detector is only a 3D device that contains an LED as the radiation source and a USB 2000 CCD which collects the intensity emitted by quinine in soft drinks.

4. Conclusion

The proposed MSFIA system is suitable for the determination of quinine in soft drinks, which reports results very similar to those obtained with a conventional spectrofluorometer through the t-test carried out with a 95% confidence level. It has several advantages such as an increase in the frequency of analysis, less intervention by the analyst and a significant decrease in samples and reagents amounts compared to the conventional spectrofluorometer. In addition, this proposed system requires a simple dilution of the samples to be analyzed, which reduces the sample preparation time. The results obtained were very good in terms of accuracy and precision, however, its sensitivity is less than the manual method, which can be corrected by working in a lower linearity range and increasing the integration time of the CCD detector in certain samples where its concentrations are below the order of mg L^{-1}. This developed system allows future research to analyse other non-alcoholic beverages to assess the number of flavours or dyes found within these drinks.

Credit author statement

Diego Barzallo: Research, writing, Kaewta Danchana: Supervision, Víctor Cerdà; : Conceptualization, Methodology, Supervision, Edwin Palacio: Reviewing.

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.

Acknowledgments

The authors acknowledge financial support from Spanish Ministry of Economy and Competitiveness (MINECO) through Project CTQ-2016-77155-R co-financed by FEDER funds. Diego Barzallo acknowledges the financial support from Carolina Foundation for his master degree. Kaewta Danchana acknowledges the financial support from the Conselleria d'Innovació, Recerca I Turisme of the Govern de les Illes Balears and of the Fons Social Europea (FPI/1867/2016).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121163.

References

[1] J.M. Estela, V. Cerdà, Flow analysis techniques for phosphorus: an overview, Talanta 66 (2005) 307–331, https://doi.org/10.1016/j.talanta.2004.12.029.
[2] O. Chailapakul, P. Ngamukot, A. Yoosamran, W. Siangproh, N. Wangfuengkanagul, Recent electrochemical and optical sensors in flow-based analysis, Sensors 6 (2006) 1383–1410, https://doi.org/10.3390/s6101383.
[3] A. Rainelli, R. Stratz, K. Schweizer, P.C. Hauser, Miniature flow-injection analysis manifold created by micromilling, Talanta 61 (2003) 659–665, https://doi.org/10.1016/S0039-9140(03)00330-8.
[4] F.S. De Oliveira, M. Korn, Spectrophotometric determination of sulphate in automotive fuel ethanol by sequential injection analysis using dimethylsulphonazo(III) reaction, Talanta 68 (2006) 992–999, https://doi.org/10.1016/j.talanta.2005.06.055.
[5] P.K. Dasgupta, I.Y. Eom, K.J. Morris, J. Li, Light Emitting Diode-Based Detectors: Absorbance, Fluorescence and Spectroelectrochemical Measurements in a Planar Flow-Through Cell, in: Acta Anal Chim (Ed.), Elsevier, 2003, pp. 337–364, , https://doi.org/10.1016/S0003-2670(03)00575-0.
[6] K. Danchana, P. Phansi, C.T. de Souza, S.L.C. Ferreira, V. Cerdà, Spectrophotometric system based on a device created by 3D printing for the accommodation of a webcam chamber as a detection system, Talanta 206 (2020) 120250, https://doi.org/10.1016/j.talanta.2019.120250.
[7] W. Wongwilai, S. Lapanantnoppakhun, S. Grudpan, K. Grudpan, Webcam camera as a detector for a simple lab-on-chip time based approach, Talanta 81 (2010) 1137–1141, https://doi.org/10.1016/j.talanta.2010.01.058.
[8] K. Danchana, V. Cerdà, Design of a portable spectrophotometric system part II: using a digital microscope as detector, Talanta 216 (2020) 120977, , https://doi.org/10.1016/j.talanta.2020.120977.
[9] Y. Suzuki, M. Endo, J. Jin, K. Iwase, M. Iwatsuki, Tristimulus colorimetry using a digital still camera and its application to determination of iron and residual chlorine in water samples, Anal. Sci. 22 (2006) 411–414, https://doi.org/10.2116/analsci.22.411.
[10] H. Yu, Y. Tan, B.T. Cunningham, Smartphone fluorescence spectroscopy, Anal. Chem. 86 (2014) 8805–8813, https://doi.org/10.1021/ac502080t.
[11] C. Huang, G. Xia, S. Jin, M. Hu, S. Wu, J. Xing, Denoising analysis of compact CCD-based spectrometer, Optik 157 (2018) 693–706, https://doi.org/10.1016/j.ijleo.2017.11.170.
[12] G. Zonios, Noise and stray light characterization of a compact CCD spectrophotometer used in biomedical applications, Appl. Optic. 49 (2010) 163–169, https://doi.org/10.1364/AO.49.000163.
[13] I. Joint, FAO/WHO Expert Committee on Food Additives. Meeting (44th : 1995 : Rome, International Program on Chemical Safety., Toxicological Evaluation of Certain Food Additives and Contaminants in Food, World Health Organization, 1996.
[14] CFR, Code of federal regulations title 21 (n.d.), https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.575 , Accessed date: 8 May 2020.
[15] U. Bfr, H. Assessment, Quinine-containing Beverages May Cause Health Problems - Updated BfR Health Assessment No 020/2008, 9 May 2008, (2008).
[16] EUR-Lex, 32012R0872 - EN - EUR-Lex (n.d.), https://eur-lex.europa.eu/legal-content/es/TXT/?uri=CELEX%3A32012R0872 , Accessed date: 8 May 2020.
[17] K. Danchana, F. Maya, P. Wilairat, K. Uraisin, V. Cerdà, Spectrophotometric determination of bromide in water using the multisyringe flow injection analysis technique coupled to a gas-diffusion unit, Anal. Methods. 7 (2015) 4202–4208, https://doi.org/10.1039/c5ay00202h.
[18] M. Michalec, R. Koncki, Ł. Tymecki, Optoelectronic detectors for flow analysis systems manufactured by means of rapid prototyping technology, Talanta 198 (2019) 169–178, https://doi.org/10.1016/j.talanta.2019.01.092.
[19] ICH Q2(R1), Validation of analytical procedures: text and methodology - ECA academy (n.d.), https://www.gmp-compliance.org/guidelines/gmp-guideline/ich-q2r1-validation-of-analytical-procedures-text-and-methodology , Accessed date: 7 May 2020.
[20] V.F. Samanidou, E.N. Evaggelopoulou, I.N. Papadoyannis, Simple and rapid HPLC method for the determination of quinine in soft drinks using fluorescence detection, J. Liq. Chromatogr. Relat. Technol. 27 (2004) 2397–2406, https://doi.org/10.1081/JLC-200028156.
[21] T.M. Cheon, B.S. Cheong, H.G. Cho, J.H. Kim, K.S. Kim, Quinine assay with home-built UV-LED fluorometer: quantitative analysis, photo-bleaching, fluorescence quenching, and urine analysis, J. Kor. Chem. Soc. 56 (2012) 577–582, https://doi.org/10.5012/jkcs.2012.56.5.577.
[22] J.E. O'Reilly, Fluorescence experiments with quinine, J. Chem. Educ. 52 (1975) 610, https://doi.org/10.1021/ed052p610.
[23] P.D. Tzanavaras, C.K. Zacharis, T.D. Karakosta, A. Zotou, D.G. Themelis, High-throughput determination of quinine in beverages and soft drinks based on zone-fluidics coupled to monolithic liquid chromatography, Anal. Lett. 46 (2013) 1718–1731, https://doi.org/10.1080/00032719.2012.719248.
[24] M. Buleandra, A.A. Rabinca, M.C. Cheregi, A.A. Ciucu, Rapid voltammetric method for quinine determination in soft drinks, Food Chem. 253 (2018) 1–4, https://doi.org/10.1016/j.foodchem.2018.01.130.