Determination of EDTA-2Na in pickles and canned mix congee by a fluorescent Zirconium-Quercetin complex

Di Jin
China Agricultural University
Can Wang
China Agricultural University
Xiaoqing Yue
China Agricultural University
Jun Wang (wangjun1@cau.edu.cn)
China Agricultural University

Research Article

Keywords: EDTA-2Na, Zirconium-Quercetin complex, fluorescence scanning, rapid detection

Posted Date: June 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1743644/v1

Abstract

In this study, we developed and validated a three-dimensional fluorescence method for the determination of Ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) in pickles and canned mix congee by forming a ternary fluorescent complex of zirconium-quercetin-EDTA system. The conditions of complexation reaction and fluorescence scanning parameters were optimized. During the fluorescent complex reaction, $NH_3 \cdot H_2O$ and anhydrous ethanol were used to enhance the fluorescence intensity. The analysis of EDTA-2Na by fluorescence scanning could be accomplished within 3 minutes. Under the condition, satisfactory linearities were obtained ($R^2 \geq 0.99$) with the limits of quantitation (LOQs) of 24.6 μg/g. The recoveries were 79.8-113.2%, with a relative standard deviation (RSD) below 9.7%. Compared with the HPLC-DAD method, the relative error of the two methods was less than 10%, which indicated the results of the two methods were consistent. A convenient and accurate method was established to analyze EDTA-2Na in pickles and canned mix congee, which provided a new option to rapidly monitor EDTA-2Na in foods.

Introduction

Disodium ethylene diamine tetra acetate, which is EDTA-2Na, as one of the allowed food additives is used in Codex General Standard for Food Additives (Codex Alimentarius). EDTA-2Na is the most widely used to form stable water-soluble chelate complexes with various metal ions such as iron, copper, calcium, magnesium, etc., due to its efficient complexing ability and high stability constant (Bortot et al. 2019), which can prevent food from discoloration, metamorphism, turbidity and oxidative loss of vitamin C caused by the metal ion (Evstatiev et al. 2021). It is usually applied in drinks, jams, pickles, canned foods and other processed foods as chelating agents, preservatives, antioxidants or color protection agents (Shen et al. 2020; Zhao et al. 2018).

Excessive EDTA-2Na intake can lead to the loss of trace elements for the human body, which may cause vomiting, diarrhea, acute abdominal pain or other symptoms, with consequent a spectrum of body damage (Lamas et al. 2013). Based on this situation, many countries and organizations have established the strict limits for the remaining amount of EDTA-2Na in foods. For instance, Japan mandates that the remaining amount of EDTA-2Na for canned food does not exceed 250 μg/g and beverages does not exceed 35 μg/g (Krokidis et al. 2005). Similarly, China the food additives health standards specifies that the remaining amount of EDTA-2Na for pickles and canned mix congee does not exceed 250 μg/g (GB2760-2014 n.d.). According to the Codex General Standard for Food Additives, EDTA-2Na in canned vegetables and food supplements should not exceed 365 μg/g and 150 μg/g respectively (Codex Alimentarius). Pickles and canned mix congee are the most commonly used foods in Asia, especially in breakfast. EDTA-2Na is often used in the manufacture of these foods, and improper addition can cause the content to exceed the standard, resulting in human damage. Consequently, a direct and rapid method for detecting EDTA-2Na in food is sorely required to guarantee food safety.

Currently, the detection methods of EDTA-2Na in foods can be divided into four main methods, include colorimetric analysis, titration analysis, high performance liquid chromatography and ion chromatography methods, etc (Chiumiento et at. 2015; Fingerhut et al. 2009). Among these methods, high performance liquid chromatography is the most commonly used and has relatively accurate measurement results. The extraction of samples occurred derivatization reaction with Cu^{2+} or Fe^{3+}, followed by high performance liquid chromatography with UV detection analysis (Choi et al. 2009; Jiménez. 2014). However, if the sample contained Fe^{3+} itself, it would lead to the measured results higher than normal results. Meanwhile, chromatographic methods generally need complicated pretreatments and longer analyzing time, which does not satisfy requirement of rapid detection for a large number of samples. Fluorescence spectrum are typically used as rapid analysis methods, due to its high sensitivity and fast detection speed. Therefore, it is very meaningful to establish an analytical method for the accurate analysis of EDTA-2Na in pickles and canned mix congee by fluorescence. Metals, quercetin, and EDTA-2Na can form ternary complexes with strong fluorescence, which are used to analyze metal elements, such as zirconium and terbium, etc (Rizk et al. 2022). However, there is no report on the use of this ternary complex to analyze EDTA-2Na in food. Based on these, the fluorescence method of ternary complexes of zirconium-quercetin-EDTA was researched to detect EDTA-2Na residues in pickles and canned mix congee. A sensitive and rapid method for EDTA-2Na was explored and evaluated.

Experimental

Materials

Five different brands of pickle samples and canned mix congee were purchased respectively at a supermarket in Beijing; Disodium Ethylenediaminetetraacetate acid (>99.0%, $C_{10}H_{16}N_2O_8$, J&K Technology Co., Ltd., Beijing); Zirconium oxychloride octahydrate (≥98%, $ZrOCl_2{\cdot}_8H_2O$, J&K Technology Co., Ltd., Beijing); Quercetin (≥99%, $C_{15}H_{14}O_9$, Yi Xiu Bogut Biological Technology Co., Ltd., Beijing); Hexadecyl trimethyl ammonium bromide (≥99%, $C_{19}H_{42}BrN$, blue-Yi Chemical Products Co., Ltd., Beijing); Ferric chloride ($FeCl_3$); ascorbic acid ($C_6H_8O_6$); tetrabutylammonium hydroxide ($C_{16}H_{39}NO_2$); Acetic acid (CH_3COOH); anhydrous ethanol (C_2H_6O); hydrochloric acid (HCL); chloroform ($CHCl_3$); n-butanol ($CH_3(CH_2)_3OH$); ammonia water ($NH_3{\cdot}H_2O$) used in this work were of analytical grade (Beijing chemical Works); Acetonitrile CH_3CN and Formic acid HCOOH (Chromatographic grade, J & K Technology Co., Ltd., Beijing).

Instruments and Equipment

A 1260 high performance liquid chromatograph with a diode array detector (Agilent, USA); ODS-C18 liquid chromatography column (250 × 4.60 mm, 5 μm, Agilent, USA); F-7000 fluorescence spectrophotometer (Hitachi, Japan); QL861 vortex mixer (Kylin-bell Instrument Co., Ltd., Jiangsu, China); Feige TDL-5-A desktop centrifuge (Anke Scientific Instruments corp., Shanghai, China); Milli-Q ultrapure water machine (Millipore, USA); FA1104B electronic analytical balance (sensitivity: 1/10000, Vietnam Scientific

Instruments Ltd., Shanghai, China); KQ-500DE CNC heated ultrasonic cleaning instrument (Kunshan Ultrasonic Instrument Co., Ltd., Jiangsu, China); RE-52AA rotary evaporator (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China)

HPLC-DAD method

Preparation of calibration standard solutions

Disodium ethylene diamine tetra acetate standard 100 mg (accurate to 0.0001g) was dispersed in deionized water with a concentration of 1000 μg/mL. The 10.0, 20.0, 50.0, 100.0, 200.0, 400.0 and 600.0 μL solutions were added in 10mL volumetric flask respectively and mixed with 0.1 mol/L $FeCl_3$ (1 mL) and 0.23 mol/L Ascorbic acid solution (100 μL). All solutions were stored at room temperature for one night. Quantification was performed using an external calibration method. Calibration curves were prepared at concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 40.0, 60.0 μg/mL.

Pretreatment and Derivatization of Samples

The homogenized canned mix congee, pickled vegetables (5.0g, respectively) were added to a 50 mL Teflon screw centrifugal tube and 20 mL of deionized water was added. The samples were then extracted by ultrasonication (80W) for 30 min. Next, the solutions were centrifuged for 10 min at 3500 r/min. Then the residues were re-extracted twice by using the same methods. The filtered solution extractions were transferred into the volumetric flask (100 mL) and complemented with deionized water. The obtained filtrate (500 μL) was diluted with 0.1 mol/L $FeCl_3$ solution (100 μL) and 0.23 mol/L ascorbic acid (10 μL). Next, reaction mixture was made up to 1 mL with ultrapure water, vortexed for 10 s, and kept for overnight.

HPLC condition

The elution was isocratic with a flow rate of 1.0 mL/min using a mixture of 0.13% tetrabutylammonium hydroxide aqueous solution adjusted pH to 4 by formic acid (80%, V) and acetonitrile (20%, V) as the eluent. The target compound was determined at a wavelength of 254 nm.

Three-dimensional fluorescence method

Optimization of Derivative Conditions

In order to obtain the optimal reaction conditions for the derivatization of the ternary mixed complexation, the different volumes of $NH_3 \cdot H_2O$ (0.2, 0.4, 0.6, 0.8 and 1.0 mL) and absolute ethanol (0.5, 1.0, 1.5, 2.0, 2.25, 2.5, 2.75, 3.0 and 3.25 mL) were mixed with 2×10^{-3} mol/L Zr (IV) (1 mL), 1.0×10^{-2} mol/L quercetin (1 mL) and 1000.0 μg/mL EDTA-2Na solution (0.4 mL). Furthermore, hexadecyl trimethyl ammonium bromide (CTMAB) was also used to study the effect on the fluorescence intensity of the system. EDTA-2Na (10.0, 20.0, 50.0, 100.0, 200.0 and 400.0 μL), 2×10^{-3} mol/L Zr (IV) (1 mL), 1.0×10^{-2} mol/L quercetin (1 mL) were mixed with 1.0×10^{-2} mol/L CTMAB (3.5 mL) or without the solution. The all solutions were

made up to 10.0 mL with ultrapure water in a volumetric flask. After vortexing for 10 s, fluorescence analysis was performed within 3 min.

Preparation of calibration standards

2×10^{-3} mol/L Zr (IV) (1 mL), 1.0×10^{-2} mol/L quercetin (1 mL), different volume of EDTA-2Na solution, 2.75 mL of absolute ethanol and 0.6 mL of 0.5 mol/L $NH_3{\cdot}H_2O$ solution were mixed and diluted to 10.0 mL with ultrapure water in volumetric flask. After vortexing for 10 s, fluorescence analysis was carried out within 3 min. Quantification was performed using an external calibration method. Calibration curves were prepared at EDTA-2Na concentrations of 1.0, 2.0, 5.0, 10.0, 20.0, 30.0, 40.0 μg/mL.

Pretreatment and Derivatization of Samples

The extraction method was in the same way as mentioned in the section about quantification of EDTA-2Na by HPLC-DAD. For extremely complex canned mix congee samples, it is also necessary to remove sugar and protein. A 20.0 mL of the extract was placed into a 50.0 mL screw-top PTFE centrifuge tube while anhydrous ethanol (30.0 mL) was added. The tube was shaken rapidly, refrigerated at 4 °C for 1h, and then centrifuged at 5000 r/min for 5 min. The filtered mixture was evaporated by rotary evaporation under reduced pressure at 55 °C for 6 min. After removing ethanol, the volume was adjusted to 20.0 mL with ultrapure water. A 10.0 mL of the above solution was taken into a 15.0 mL screw-top PTFE centrifuge tube. Afterwards, 2 ml of Sevag reagent (chloroform and n-butanol at a ratio of 4:1/V: V) was used to deposit protein under centrifugation for 5 min at 3000 r/min. The supernatant was collected for derivatization. The above treatment was not required for pickles. Then 4.65 mL of the supernatant was derivatized in the same method as the calibration standard solution.

Selection of Scanning Scope, PMT Negative Voltage and Slit Width

In order to obtain the appropriate scanning scope, negative voltage of the photomultiplier tube and slit width, 40.0 μg/mL EDTA-2Na ternary mixed complex solution was used as the experimental mode. In the condition of excitation wavelengths of 350-500 nm and 5-nm intervals, the emission wavelengths of 450-600 nm and 5-nm intervals were scanned to determine optimal excitation and emission wavelengths to use for analysis. Moreover, the photomultiplier tube voltage and slit width were also optimized considering the resolution and intensity by comparing the results at 250 V, 400 V, and 700 V with the slit width (5 nm). Similarly, by controlling voltage, the suitable slit width can be obtained.

Method validation

According to the ICH Harmonized Tripartite Guidelines on Validation of Analytical Procedures: Methodology (ICH 2005), linearity, fortified recoveries, RSDs, and sensitivity were evaluated for the optimized method.

Linear range and sensitivity

Calibration curves were respectively diluted in solution at concentrations of 40, 30, 20, 10, 5, and 1 μg/mL, where y was the fluorescence response and x was the concentration. Three-dimensional fluorescence limits of detection (LOD) and quantification (LOQ) (μg/g) were calculated based on the standard deviation (σ, counts) of 11 readings of the standard solution blank, per Eq. 1:

$$LOD = \frac{3.3\sigma k}{slope} \ and \ LOQ = \frac{10\sigma k}{slope} \quad (1)$$

where slope was the sensitivity (counts mL/μg) and k was the dilution factor (mL/g)

Recovery and Repeatability

To evaluate the accuracy of the method, the samples of spiked pickle and canned mix congee were taken to analyze the recovery. The spiked concentrations were at three level of 40.0, 100.0, 160.0 μg/g, respectively. Each level was analyzed in triplicate. And the repeatability was evaluated via the relative standard deviation of the five replicates using canned mix congee and pickle samples.

Accuracy via comparison by the HPLC and fluorescence method

In order to further verify the accuracy of the results for the determination of EDTA-2Na in food by fluorescence method, the EDTA-2Na contents in the 10 samples were analyzed by HPLC-DAD and fluorophotometry simultaneously, and each level was determined in triplicate. Moreover, the accuracy was calculated as the relative error (RE %) of the two methods.

$$RE \ (\%) = \frac{A-B}{B} * 100\% \quad (2)$$

where A was the content by Fluorescence method (μg/g) and B was the content by HPLC-DAD method (μg/g).

Results And Discussion

Optimization of Fluorescence scanning parameters

Scope of wavelength

When performing fluorescence scanning, selecting an appropriate fluorescence scanning range can improve analysis efficiency (Bian C et al. 2021). In addition, the selection of excitation and emission wavelengths will directly affect the detection sensitivity (O' Farrell N et al. 2006). On the basis of results of three-dimensional scanning (Fig. 1), the EDTA-2Na ternary mixed complex can obtain the maximum fluorescence response value when the excitation wavelength is 440 nm and the emission wavelength is 525 nm. Furthermore, it can effectively avoid the influence of Raman and Rayleigh scattering on the samples under this condition. Finally, we chosen the mentioned wavelength for experiment.

Photomultiplier tube voltage and the slit width

Photomultiplier tube (PMT) is a photodetector of fluorescence spectrophotometer, whose role is to convert an optical signal into an electric signal, and then the signal is amplified by circuit (Zheng W et al. 2020; DA López-Mora. 2021). Within a certain range, the fluorescence intensity is proportional to the voltage at both ends of the photomultiplier tube. But the larger magnification, the noise also increases, which may result in reduced sensitivity (Cui et al. 2014). Therefore, on the premise of meeting the analytical requirements, the voltage of photomultiplier tube should not be set too high. On the other hand, the slit width is larger, the fluorescence intensity is stronger. But too much will reduce the slit width resolution (Xu et al. 2017). According to the fluorescence scanning contour map (Fig. 2), it can be seen that under the 5nm slit width, the contour map under the 250 V (Fig. 2a) was obviously sparser than that at 400 V (Fig. 2b), indicating that the signal intensity of the target with the same concentration was weaker at low voltage. However, when the voltage was increased to 700 V (Fig. 2c), the noise was too large, resulting in the generation of the invalid central area.

When the voltage of photomultiplier tube was selected to keep 400 V under the conditions of slit widths of 5 or10 nm, the fluorescence scanning contour lines were obtained separately by scanning the EDTA-2Na mixed complex solution with a concentration of 40.0 μg/mL. As shown in Fig. 3, the contour map of 10 nm slit width (Fig. 3b) was denser and more clearly than that under the slit width of 5 nm (Fig. 3a). In other words, the fluorescence intensity of 10 nm is stronger with a good resolution. To sum up, three-dimensional fluorescence scanning parameters were decided as follow: the excitation wavelength of 440 nm, the emission wavelength of 525 nm, the photomultiplier tube voltage of 400 V and the slit width of 10 nm.

Optimization of Conditions for Ternary Mixed Complexation Reaction

In this process of Zirconium-quercetin-EDTA ternary complex reaction, $NH_3 \cdot H_2O$ provided the weak alkaline system of pH 8.0-9.0 required for the reaction (Burilova et al.2021), and anhydrous ethanol could enhance the fluorescence intensity due to solvent effect (Kim et al.2019). When 0.5 mol/L $NH_3 \cdot H_2O$ addition was less than 0.6 mL (Fig. 4a), or anhydrous ethanol addition was less than 2.75 mL (Fig. 4b), the fluorescence response of EDTA-2Na ternary complex solution under the same concentration was strengthened with an increase in the amounts of two agents. But when the addition amount of $NH_3 \cdot H_2O$ was greater than 0.6 mL (Fig. 4a) or anhydrous ethanol was greater than 2.75 mL (Fig. 4b), the fluorescence intensity did not change significantly ($P>0.05$) with the increase of the amount. Therefore, it was decided that the addition of 0.5 mol/L $NH_3 \cdot H_2O$ and the anhydrous ethanol were 0.6 mL and 2.75 mL respectively in the ternary complex system.

Throughout this reaction system, CTMAB as a stabilizer can extend the fluorescence stabilization time (Feng et al.2020), but also has some of the fluorescence quenching effects. As observed in Fig. 4c, compared to the samples without CTMAB, samples' fluorescence response with CTMAB were all decreased obviously. For example, it was reduced by 16 times when the concentration of EDTA-2Na was

2 μg/mL. Thus, in this ternary complex reaction, the stabilizer CTMAB was not applied in order to ensure the fluorescence sensitive.

Method Validation

To ensure the effectiveness of the method for the determination of EDTA-2Na in pickles and canned mix congees, the analytical performance parameters were evaluated including fortified recoveries at three spiking levels, linearity, RSDs, LOD and LOQ.

Calibration

Preparation of different concentration EDTA-2Na complexing solutions was followed by three-dimensional fluorescence analysis. The curve equation was , and the linear range was 1.0-40.0 μg/mL for EDTA-2Na with a correlation coefficient (R^2) of 0.9945. The limits of detection and quantification of EDTA-2Na calculated by the method were 8.2 μg/g (LOD) and 24.6 μg/g (LOQ) respectively, which were lower than 22.8 μg/g (LOD) and 75.2 μg/g (LOQ) of HPLC-DAD method. It showed that the method has the better sensitivity than that of HPLC-DAD method.

Accuracy and repeatability of methods

Recovery and RSD were used to assess the accuracy and repeatability of the methods described above. The repeatability in this method obtained by samples were below 1.6% (Table 1). Among three different spiked concentrations, the average recoveries of EDTA-2Na was 79.8–85.8% and 105.9–113.2% in canned mix congee and pickles, respectively (Table 2). The RSD of these recoveries were below 10%. In summary, the recovery and RSD in the method were adequate for detecting EDTA-2Na in food samples.

Sample analysis and validation

The data of sample detection were showed in Table 3. All REs were in the range of 4.4 to 13.4%. Notably, compared to HPLC-DAD method, the content of EDTA-2Na in pickles analyzed by fluorescence (106.0-141.3 μg/g) had relative errors less than 10%. There was relatively weak interference from matrix of samples to target peaks. For the canned mix congees which were subjected to remove sugar and protein, the relative error of the detection results of the two methods could also be controlled within 13.5%. The results of the two methods were consistent, which showed that the developed fluorescence method was accurate and convenient to determine content of EDTA-2Na in the samples.

Conclusion

We developed a method for determining the content of EDTA-2Na in pickles and canned mix congee. The reaction conditions and fluorescence detection conditions of Zirconium-Quercetin-EDTA ternary systems were successfully selected and optimized. The method was verified as having satisfactory linearity ($R^2 \geq 0.99$), good recoveries (79.8-113.2%), adequate repeatability (RSD $\leq$ 9.7%), and high sensitivity (LOQ = 24.6 μg/g). Compared with the HPLC method, the relative error was less than 13.5%, indicating that the

accuracy and sensitive of the method was better. A sensitive and convenient method of Zirconium-Quercetin-EDTA ternary system by fluorescence was established to detect EDTA-2Na of pickles and canned mix congee, which was useful for evaluating and ensuring food safety.

Declarations

Data Availability

The authors declare that all data supporting the findings of this study are available within the article and its supplementary information file.

CRediT authorship contribution statement

Di Jin: Methodology, Investigation, Formal analysis, Writing- Original draft preparation; *Can Wang:* Visualization, Investigation, Writing- Original draft preparation, Data Curation; *Xiaoqing Yue:* Validation, Resources; *Jun Wang:* Conceptualization, Supervision, Writing- Reviewing and Editing.

Funding

This work was financially supported by the National Natural Science Foundation of China (31671860) and the Center for Food Evaluation, State Administration for Market Regulation, China (No. 20184831).

Conflict of Interest The authors declare no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the author.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Bian C, Ge D, Wang G et al (2021). Enhancement of waste activated sludge dewaterability by ultrasound-activated persulfate oxidation: Operation condition, sludge properties, and mechanisms. Chemosphere, 262, https://doi.org/10.1016/j.chemosphere.2020.128385
2. Bortot Coelho, F. E., Gionco, C., Paganini, M. C., Calza, P., & Magnacca, G. (2019). Control of Membrane Fouling in Organics Filtration Using Ce-Doped Zirconia and Visible Light. Nanomaterials (Basel, Switzerland), 9(4), 534. https://doi.org/10.3390/nano9040534
3. Burilova, E., Solodov, A., Shayimova, J., Zhuravleva, J., Shurtakova, D., Evtjugin, V., Zhiltsova, E., Zakharova, L., Kashapov, R., & Amirov, R. (2021). Design of High-Relaxivity Polyelectrolyte Nanocapsules Based on Citrate Complexes of Gadolinium (III) of Unusual Composition. International journal of molecular sciences, 22(21), 11590.
4. https://doi.org/10.3390/ijms222111590

5. Chiumiento, F., D'Aloise, A., Marchegiani, F., & Melai, V. (2015). Determination of EDTA in feed and premix formulations by HPLC-DAD. Food chemistry, 175, 452–456. https://doi.org/10.1016/j.foodchem.2014.11.159
6. Choi J H, Mamun M, El-Aty A et al (2009). Inert matrix and Na4EDTA improve the supercritical fluid extraction efficiency of fluoroquinolones for HPLC determination in pig tissues. Talanta, 78(2), 348–357. https://doi.org/10.1016/j.talanta.2008.11.021
7. Codex Alimentarius (1995) General standard for food additives - Codex Stan 192-1995. http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/gsfa/en/.
8. Cui, G., Jun, S. B., Jin, X., Luo, G., Pham, M. D., Lovinger, D. M., Vogel, S. S., & Costa, R. M. (2014). Deep brain optical measurements of cell type-specific neural activity in behaving mice. Nature protocols, 9(6), 1213–1228. https://doi.org/10.1038/nprot.2014.080
9. DA López-Mora, Md Phd I C, Md Phd AF (2021). Digital PET vs Analog PET: Clinical Implications? Seminars in Nuclear Medicine, 52(3), https://doi.org/10.1053/j.semnuclmed.2021.10.004
10. Evstatiev, R., Cervenka, A., Austerlitz, T., Deim, G., Baumgartner, M., Beer, A., Krnjic, A., Gmainer, C., Lang, M., Frick, A., Schachner, H., Khare, V., & Gasche, C. (2021). The food additive EDTA aggravates colitis and colon carcinogenesis in mouse models. Scientific reports, 11(1), 5188. https://doi.org/10.1038/s41598-021-84571-5
11. Feng, X., Zhang, Y., Zhang, C., Lai, X., Zhang, Y., Wu, J., Hu, C., & Shao, L. (2020). Nanomaterial-mediated autophagy: coexisting hazard and health benefits in biomedicine. Particle and fibre toxicology, 17(1), 53. https://doi.org/10.1186/s12989-020-00372-0
12. Fingerhut, R., Dame, T., & Olgemöller, B. (2009). Determination of EDTA in dried blood samples by tandem mass spectrometry avoids serious errors in newborn screening. European journal of pediatrics, 168(5), 553–558. https://doi.org/10.1007/s00431-008-0788-9
13. GB/T 21970–2008 (n.d.) National Health and Family Planning Commission of the People's Republic of China. National Standard for Food Safety - Standard for the Use of Food Additives
14. ICH (2005) ICH harmonized tripartite guidelines on validation of analytical procedures: text and methodology Q2 (R1) Ministry of Health, Canada https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf Assessed on 22 August 2020
15. Jiménez J J (2014). Determination of calcium disodium ethylenediaminetetraacetate (E385) in marketed bottled legumes, artichokes and emulsified sauces by gas chromatography with mass spectrometric detection. Food Chemistry, 152, 81–87. https://doi.org/10.1016/j.foodchem.2013.11.134
16. Kim, N. H., Lee, J., Park, S., Jung, J., & Kim, D. (2019). A Schiff Base Fluorescence Enhancement Probe for Fe (III) and Its Sensing Applications in Cancer Cells. Sensors (Basel, Switzerland), 19(11), 2500. https://doi.org/10.3390/s19112500
17. Krokidis, A. A., Megoulas, N. C., & Koupparis, M. A. (2005). EDTA determination in pharmaceutical formulations and canned foods based on ion chromatography with suppressed conductimetric

detection. Analytica Chimica Acta, 535(1-2), 57-63. https://doi.org/10.1016/j.aca.2004.12.011

18. Lamas, G. A., Goertz, C., Boineau, R., Mark, D. B., Rozema, T., Nahin, R. L., Lindblad, L., Lewis, E. F., Drisko, J., Lee, K. L., & TACT Investigators (2013). Effect of disodium EDTA chelation regimen on cardiovascular events in patients with previous myocardial infarction: the TACT randomized trial. JAMA, 309(12), 1241–1250. https://doi.org/10.1001/jama.2013.2107
19. O' Farrell N, Houlton A, & Horrocks, B. R. (2006). Silicon nanoparticles: applications in cell biology and medicine. International journal of nanomedicine, 1(4), 451–472. https://doi.org/10.2147/nano.2006.1.4.451
20. Rizk, M., Sultan, M. A., Tawfik, B. M., & El-Eryan, R. T. (2022). Reliable and sensitive procedure for quantification of antiseptic chlorhexidine di-gluconate via quenching the fluorescence of lanthanide-EDTA-probe. Microchemical Journal, 172, 106817. https://doi.org/10.1016/j.microc.2021.106817
21. Shen C, Xu H, Liu L et al (2020). EDTA-2Na assisted dynamic hydrothermal synthesis of orthorhombic LiMnO2 for lithium ion battery. Journal of Alloys and Compounds, 830, 154599. https://doi.org/10.1016/j.jallcom.2020.154599
22. Xu, D., Zhou, W., & Peng, L. (2017). Three-dimensional live multi-label light-sheet imaging with synchronous excitation-multiplexed structured illumination. Optics express, 25(25), 31159–31173. https://doi.org/10.1364/OE.25.031159
23. Zhao Q, Piao J, Peng W et al (2018). A Metal Chelator as a Plasmonic Signal-Generation Superregulator for Ultrasensitive Colorimetric Bioassays of Disease Biomarkers. Advanced Science, 5(7), 1800295. https://doi.org/10.1002/advs.201800295
24. Zheng W, Jia L, Huang F (2020). Vacuum-Ultraviolet Photon Detections. iScience, 23(6), https://doi.org/10.1016/j.isci.2020.101145

Tables

Table 1 Sample contents for reproducibility by the fluorescence method (μg/g) (n=5)

Samples	No.1	No.2	No.3	No.4	No.5	Average content	RSD (%)
Pickle	107.3	109.1	107.9	104.5	106.8	107.1	1.6
Canned mix congee	117.9	120.3	119.1	120.8	121.1	119.8	1.1

Table 2 The recovery results of EDTA-2Na in samples (n=3)

Sample type	Initial content (μg/g)	Spike (μg/g)	Average content (μg/g)	Recovery (%)	RSD (%)
Mixed Congee	138.1	40.0	170.0	79.8	9.7
		100.0	225.4	87.3	5.4
		160.0	275.4	85.8	7.9
pickles	120.8	40.0	166.1	113.2	6.1
		100.0	229.4	108.6	3.2
		160.0	290.2	105.9	2.8

Table 3 The comparisons of Fluorescence and HPLC-DAD methods (n=3)

Samples	Sample No.	Content by HPLC-DAD (μg/g)	RSD (%)	Content by Fluorescence (μg/g)	RSD (%)	RE (%)
Pickles	1	96.8	1.5	106.0	2.9	9.5
	2	103.5	2.3	109.0	7.4	5.3
	3	136.9	1.8	142.9	2.6	4.4
	4	101.7	2.7	110.0	9.2	8.2
	5	133.8	6.3	141.3	4.3	5.6
Mixed Congee	1	105.3	3.1	119.4	5.0	13.4
	2	102.7	4.2	110.3	4.1	7.4
	3	123.8	2.4	136.1	7.3	9.9
	4	102.3	1.6	113.3	5.1	10.8
	5	95.1	1.9	107.5	3.0	13.0

Figures

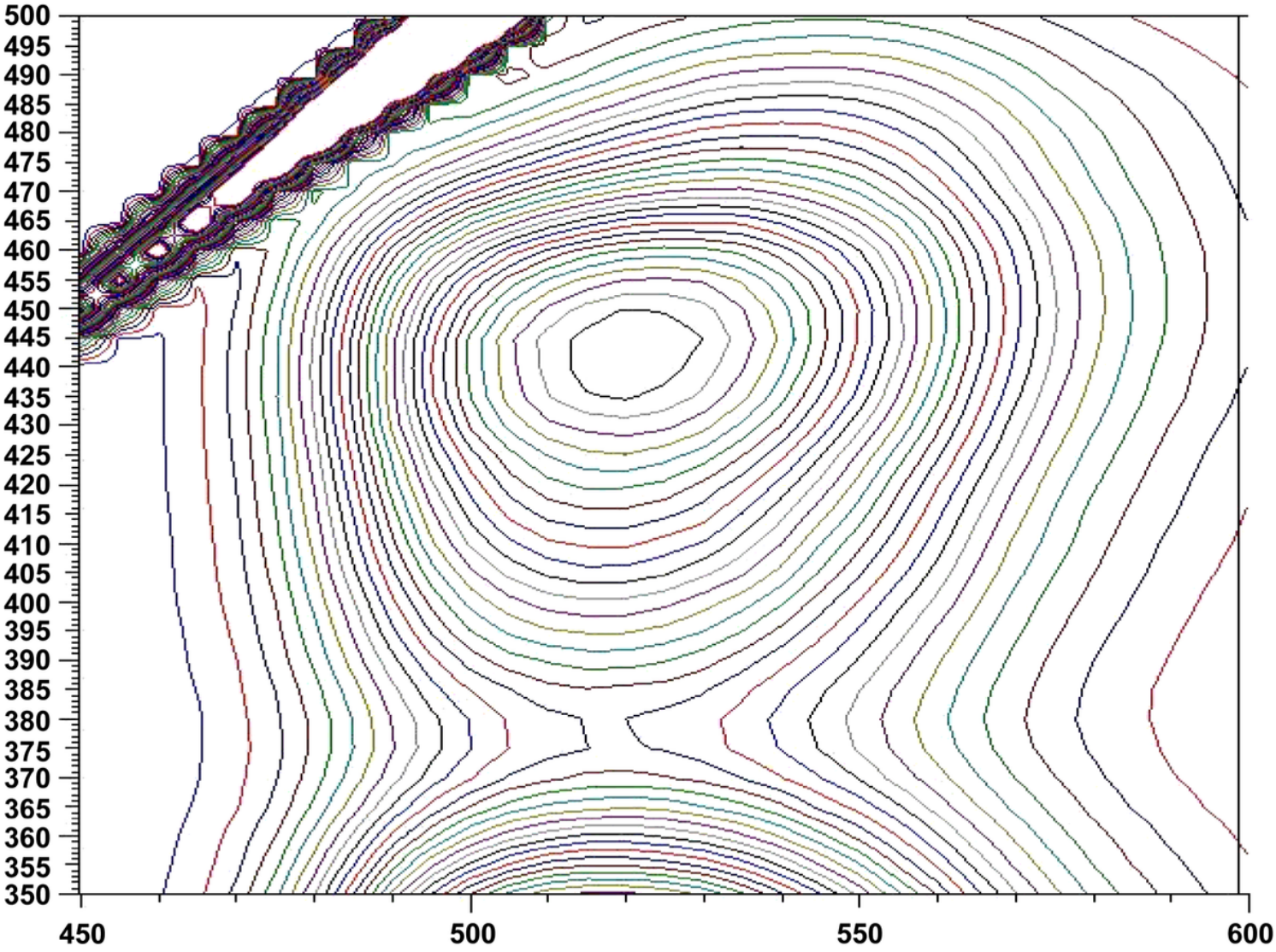

Figure 1

The contour plot of three-dimensional fluorescence spectrum for EDTA-2Na complex

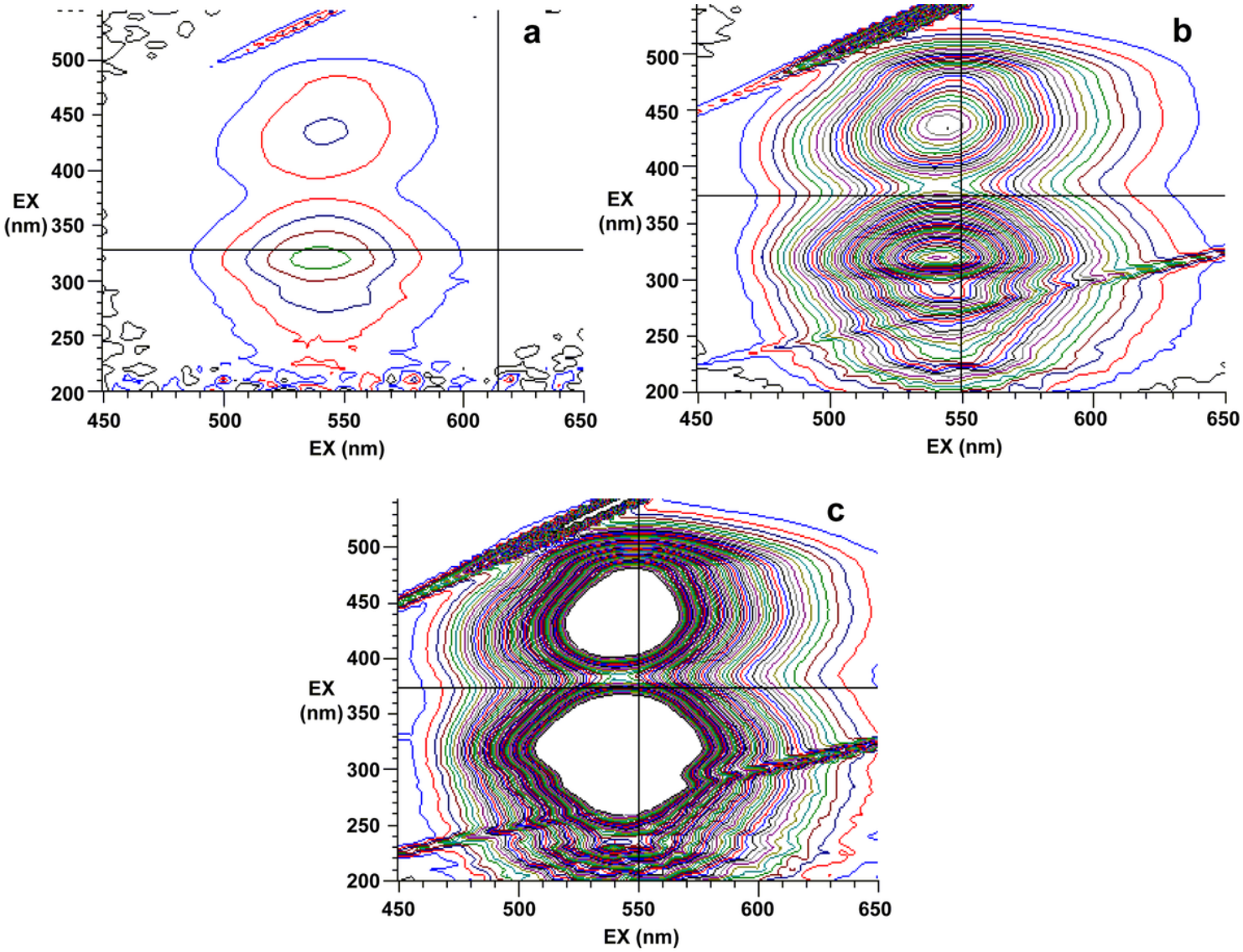

Figure 2

The fluorescence contour spectrum of different PMT voltage (a) 250V; (b) 400V; (c) 700V

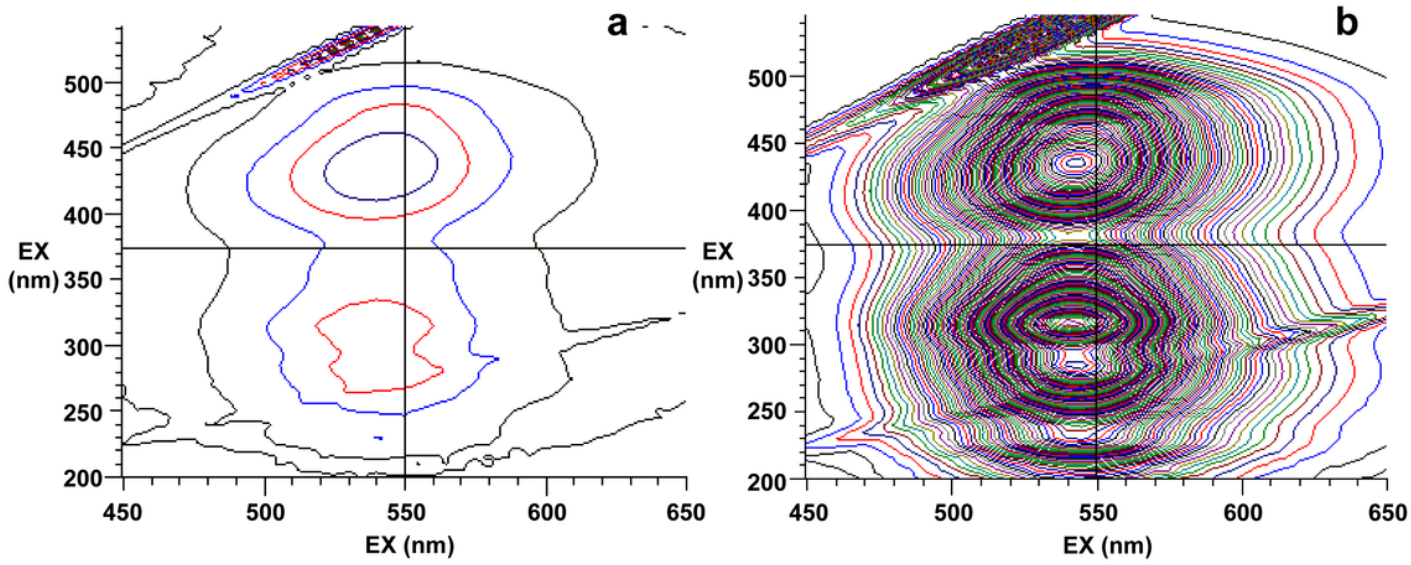

Figure 3

The fluorescence contour spectrum of different slit width (a) 5 nm; (b) 10 nm

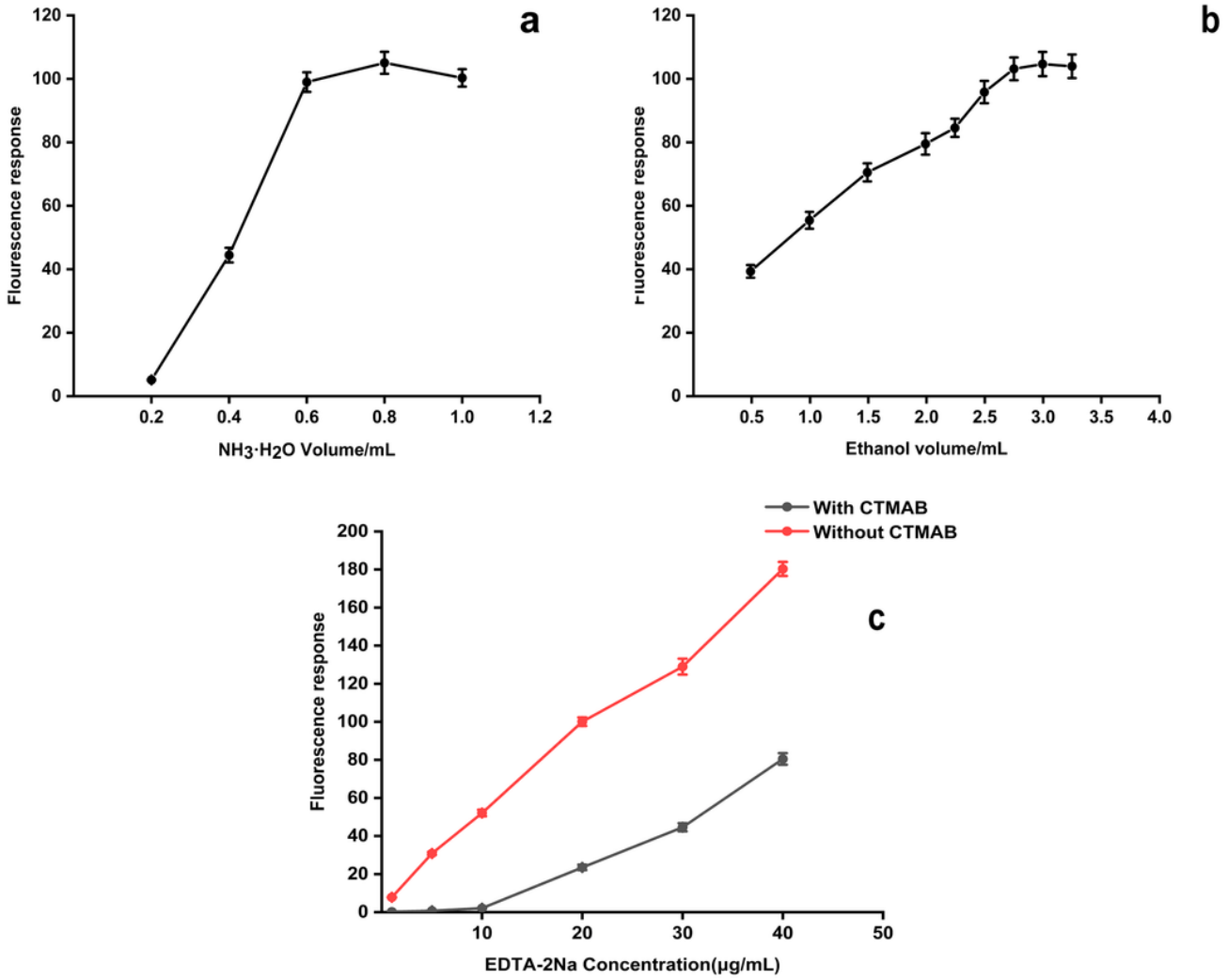

Figure 4

The effects of different conditions on the fluorescence intensity (n=3)

(a) Volume of NH3·H2O; (b) Volume of Ethanol; (c) CTMAB