

# Journal of Food and Drug Analysis



Volume 30 | Issue 2

Article 9

2022

# Chemiluminescence of lucigenin-tetracycline and itsapplication for sensitive determination of procyanidin

Follow this and additional works at: https://www.jfda-online.com/journal

Part of the Food Science Commons, Medicinal Chemistry and Pharmaceutics Commons, Pharmacology Commons, and the Toxicology Commons

080

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License.

# **Recommended Citation**

Quan, Shuai; Ji, Kaixiang; Liu, Fangshuo; Barkae, Tesfaye Hailemariam; Halawa, Mohamed Ibrahim; Hanif, Saima; Lou, Baohua; Li, Jianping; and Xu, Guobao (2022) "Chemiluminescence of lucigenin-tetracycline and itsapplication for sensitive determination of procyanidin," *Journal of Food and Drug Analysis*: Vol. 30 : Iss. 2 , Article 9.

Available at: https://doi.org/10.38212/2224-6614.3411

This Original Article is brought to you for free and open access by Journal of Food and Drug Analysis. It has been accepted for inclusion in Journal of Food and Drug Analysis by an authorized editor of Journal of Food and Drug Analysis.

# Chemiluminescence of lucigenin-tetracycline and its application for sensitive determination of procyanidin

Shuai Quan <sup>a,b</sup>, Kaixiang Ji <sup>a,b</sup>, Fangshuo Liu <sup>b,c</sup>, Tesfaye Hailemariam Barkae <sup>b,c</sup>, Mohamed Ibrahim Halawa <sup>d,e</sup>, Saima Hanif <sup>f</sup>, Baohua Lou <sup>b,c,\*</sup>, Jianping Li <sup>a,\*\*</sup>, Guobao Xu <sup>a,b,c,\*\*\*</sup>

<sup>a</sup> College of Chemistry and Bioengineering, Guilin University of Technology, Guilin, 541004, China

<sup>b</sup> State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, PR China

<sup>c</sup> University of Science and Technology of China, Hefei, 230026, China

<sup>d</sup> Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

<sup>e</sup> Guangdong Laboratory of Artificial Intelligence & Digital Economy (SZ), Shenzhen 518060, PR China

<sup>f</sup> Department of Biological Sciences, National University of Medical Sciences (NUMS), The Mall, Abid Majeed Road Rawalpindi, Punjab, 46000, Pakistan

#### Abstract

Tetracycline was reported to trigger intense lucigenin chemiluminescence (CL). Based on its significant quenching to lucigenin-tetracycline CL, procyanidin was detected. The mechanism of the lucigenin-tetracycline CL and its quenching by procyanidin were proposed. Under the optimum conditions, the calibration curve is linear in the range of 10–1000 nM for procyanidin with a detection limit of 4.4 nM. The RSD for nine replicate determinations of procyanidin was less than 1.7%. Moreover, this method was satisfactorily applied to the determination of procyanidin in human urine samples and health food capsule of grape seed.

Keywords: Chemiluminescence, Lucigenin, Procyanidin, Tetracycline

# 1. Introduction

C hemiluminescence (CL) has been considered as a popular and powerful analytical technique and universally used in chemical and biological sensing fields owing to its simple and relatively cheap instrumentation, quick response, high sensitivity, wide linear range and low background noise [1,2]. Lucigenin (*N*, *N*'-dimethyl biacridinium dinitrate), one of the most extensively used chemiluminescent materials, has gained enormous concern due to its excellent CL properties and broad range of analytical applications, including drug analysis, metal ion detection, clinical diagnosis [3–5]. Hydrogen peroxide as a classical co-reactant generates strong CL signals with lucigenin in alkaline medium. However, the poor specificity and instability of the traditional lucigenin-H<sub>2</sub>O<sub>2</sub> CL system in the presence of common interferents including metal ions and their complexes result in limitations on its development of sensing analysis [6]. In recent years, metal nanoparticles or quantum dots (carbon dots and black phosphorus quantum dots) have been utilized as catalysts in lucigenin CL to stimulate the emission reaction [3,7–9]. Therefore, it is of great aspiration to explore new lucigenin CL systems to broaden the scope of CL applications.

Tetracyclines belongs to a category of pharmaceutical compounds and are the first antimicrobial agents possessing a broad-spectrum activity against wide range of pathogenic gram-positive and gram-negative

https://doi.org/10.38212/2224-6614.3411 2224-6614/© 2022 Taiwan Food and Drug Administration. This is an open access article under the CC-BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Received 1 January 2022; revised 4 March 2022; accepted 29 March 2022. Available online 15 June 2022

<sup>\*</sup> Corresponding author at. State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, PR China.

<sup>\*\*</sup> Corresponding author at.

<sup>\*\*\*</sup> Corresponding author at. College of Chemistry and Bioengineering, Guilin University of Technology, Guilin, 541004, China. E-mail addresses: loubh@ciac.ac.cn (B. Lou), likianping@263.net (J. Li), guobaoxu@ciac.ac.cn (G. Xu).

bacteria by inhibiting the formation of proteins within bacteria. Such compounds, including tetracycline (TC), chlortetracycline (CTC) and oxytetracycline (OTC), are widely used to prevent different diseases and cure various bacterial infections in humans and animals. In addition, they have also been employed in applications such as feed additives, pest control and preservation of agricultural products [10]. Broad and strong antibacterial efficiency and relatively low cost of TCs make obvious contributions to its wide-ranging clinical benefits [11]. Based on previous reports, reactive oxygen species (ROS) can be generated by TCs involved in photochemical oxidation in aqueous solution and autoxidation [12,13]. In the presence of some metal ions, TCs are involved in the formation of oxygen free radicals [14]. It means that TCs may react with lucigenin to generate CL. However, there are few reports for the CL systems of lucigenin with TCs [15,16].

Procyanidin (PC), widely existing component in plants, is an important polyphenolic compound that plays a significant role in the physiological activities of human body. PC has potential biological activities, such as antioxidative, anti-tumor, anti-aging, and anti-inflammatory effects. Furthermore, PC has a promising anticarcinogenic agent, since it is capable of inhibiting the proliferation of tumor cells of various tumors such as colorectal, gastric and prostatic cancers via the upregulation and downregulation of mRNA expressions [17-20]. PC resists the fibril formation of human islet amyloid polypeptide and may have potential to treat Alzheimer Disease. PC is also widely used in health food, pharmaceutical industries and functional cosmetics, and is one of the available mature products in the plant extract market. Owing to its considerable clinical anticancer effect, it is absolutely necessary to monitor its biological levels in human beings. Moreover, the detection of PC in the different matrices is highly required to ensure the quality, safety and effectiveness of the manufactured product at the quality control test. In this context, several methods have been reported for the successful determination of PC, including high performance liquid chromatography (HPLC) [21], spectrophotometry [22], capillary electrophoresis [23] and spectrally sensitive pulse photometry [24]. However, these methods still have shown various limitations. For example, chromatographic methods require large amount of capital investment in testing instruments and the complicated procedures [17].

In this work, lucigenin-TC CL was investigated for the first time. TC was able to generate obvious CL signals upon its unique interaction with alkaline solution of lucigenin. Subsequently, the effect of PC on the developed lucigenin-TC system was firstly investigated. It turns out that PC can remarkably inhibit the CL of lucigenin-TC system. By utilizing the quenching effect, a new flow-injection analysis (FIA) for PC sensitive detection was designed, as shown in Scheme S1. Also, the possible mechanism of the lucigenin-TC CL was discussed. This CL approach for PC detection is distinguished by simplicity, fastness, and straightforwardness and shows excellent sensitivity and selectivity in real sample testing.

#### 2. Experimental section

## 2.1. Materials and reagents

Serine, proline, alanine, glycine, tyrosine, tryptophan, ascorbic acid and phenol were purchased from Beijing Chemical Reagent Company (Beijing, China). Cysteine was acquired from Shanghai Yuanju Biotechnology Co., Ltd. (Shanghai, China). Resorcinol was purchased from Shantou Xilong Chemical Plant Co., Ltd. (Shantou, China). Phloroglucin and 3aminophenol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Lucigenin, dopamine hydrochloride and glutathione (GSH) were purchased from Shanghai Aladdin Biochemical technology Co., Ltd. (Shanghai, China). Tetracycline hydrochloride and PC were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Lucigenin stock solution (5.0 mM) was prepared by dissolving 0.1021 g of lucigenin in 40 mL water. TC stock solution (5.0 mM) was prepared by dissolving 0.1111 g of TC in 50 mL NaOH (0.1 M) solution. Stock solution of PC (250 µM) was prepared by dissolving 0.0059 g solid PC in 40 mL water. All the chemicals were analytical-reagent grade and used without further purification. Deionized water was used during all the experiments.

#### 2.2. Instruments

The CL signals were measured by utilizing a FIA based CL system which consists of Biophysics CL (BPCL) ultra-weak luminescence analyzer (the Institute of Biophysics, Chinese Academic of Sciences), an intelligent flow injection sampler (IFIS–C mode) (ReMax Inc., Xi'an, China) and a home-made flow cell. The flow cell was put in a light-tight box of the luminescent analyzer. The loop injector was equipped with an injection loop of 50  $\mu$ L. Unless specifically mentioned, the photomultiplier tube (PMT) voltage was set at 1100 V. Electron paramagnetic resonance (EPR) spectra were measured using a Bruker EMX Plus EPR Spectrometer.

#### 2.3. CL measurement of lucigenin-TC system

Scheme S2 shows the schematic diagram of the FIA system for the CL measurement. In brief, channel 1 and 2 were respectively used to deliver an aqueous solution of 0.5 mM TC and 0.1 M NaOH solution into the flow cell at a flow rate of 2.0 mL/ min. Then 200  $\mu$ L of 0.1 mM lucigenin solution was injected through the loop injector. Finally, the CL signals were measured.

# 2.4. Procedure for PC detection

0.5 mM TC in water and 0.1 M NaOH solution were pumped into the flow cell through channel 1 and 2 at a flow rate of 2.0 mL/min, respectively. Different concentrations of PC were mixed with 0.1 mM lucigenin first, and then 200  $\mu$ L of mixture solution was injected through the loop injector, and the CL signals were measured.

# 2.5. Procedure for PC detection in real samples

Lucigenin-TC system was further applied for the assay of PC in human urine samples and commercially available grape seed extract capsule with as PC main component (GloryFeel GmbH, Germany). The human urine samples were collected from healthy individual. The human urine samples and grape seed extract samples were diluted with double distilled water to minimize the interference of other components and to bring PC concentrations into the linear calibration range. 0.5 mM TC in water and 0.1 M NaOH solution were pumped into the flow cell through channel 1 and 2 at a flow rate of 2.0 mL/min, respectively. Diluted samples were mixed with 0.1 mM lucigenin first, and then 200 µL of mixture solution was injected through the loop injector, and the CL signals were measured. Standard addition method was used for evaluate the percentage recoveries.

# 3. Results and discussion

# 3.1. CL of Lucigenin-TC system

It is well known that lucigenin solution generates weak CL signal owing to its reaction with dissolved oxygen. The addition of TC to lucigenin solution leads to a great CL enhancement as illustrated in Fig. S1. This obvious CL signal obtained after the addition of TC to lucigenin solution indicates that TC is a nice coreactant for lucigenin CL. As shown in Fig. 1, the CL spectrum was recorded by measuring CL intensity versus different wavelength filters ranging from 425 to 575 nm. The maximum CL emission wavelength is obtained at 490 nm, which is in accordance with the characteristic wavelength of emission for the excited state of *N*-methylacridone [25]. Therefore, it also confirms that lucigenin acts as a luminophor in this CL reaction, while TC is the main coreactant.

It is well-known that ROS usually play a critical role in the CL system of lucigenin [9]. Three main ROS, including singlet oxygen  $({}^{1}O_{2})$ , hydroxide radical (HO<sup>•</sup>) and superoxide anion radical  $(O_2^{\bullet-})$ , are commonly incorporated in CL triggering from lucigenin species. So the effect of radical scavengers including NaN<sub>3</sub>, thiourea, and superoxide dismutase enzyme (SOD) for  ${}^{1}O_{2}$ , HO<sup>•</sup>, and  $O_{2}^{\bullet-}$  on the CL intensity were investigated respectively. Fig. S2 shows the effect of radical scavengers on lucigenin-TC CL. NaN<sub>3</sub> and thiourea have little influence on CL intensity; while SOD inhibits CL intensity much. This clearly implies that  $O_2^{\bullet-}$  plays pivotal role in lucigenin-TC CL. To further explore the mechanism, the effect of oxygen on this CL system was investigated. Before the measurement, nitrogen gas was bubbled through the reacting solution for 30 min to ensure the removal of dissolved oxygen. As illustrated in Fig. S3, the CL intensity decreased about 43% after the dissolved oxygen was removed from the reactant solutions. Moreover, electron paramagnetic resonance (EPR) spectrum was measured to confirm the formation of  $O_2^{\bullet-}$  in the lucigenin-TC system. 5,5'-Dimethyl-1-pyrroline-Noxide (DMPO) can capture  $O_2^{\bullet-}$  to form the spin adduct of DMPO-O<sup>•-</sup><sub>2</sub>. Fig. S4 shows the characteristic peak of DMPO- $O_2^{\bullet-}$  spin adduct with the addition of DMPO on this system. These results



Fig. 1. CL spectrum of lucigenin-TC system. The wavelengths of the filters are 425 nm, 440 nm, 460 nm, 490 nm, 535 nm, 555 nm and 575 nm, respectively. C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(NaOH), 0.1 M.

Taking all these experiments into consideration, the possible reaction mechanism is proposed as shown in Scheme S3. The 4-dimethylamino group of TC is dissociated in alkaline solutions, leading to the generation of TC anionic radical and secondary amine radical (Eq 1 in Scheme S3). These intermediate radicals are converted to guinone and imine products via an oxidation reaction, accompanied by the formation of  $O_2^{\bullet-}$  (Eq 2 in Scheme S3) [13,26]. Lucigenin cation radical (Luc<sup>•+</sup>) can be produced through one electron reduction reaction in alkaline conditions. And then,  $Luc^{\bullet+}$  reacts with  $O_2^{\bullet-}$  to form highly unstable intermediate lucigenin dioxetane  $(LucO_2)$  (Eq 3 in Scheme S3), which subsequently decomposes to the electronically excited N-methylacridone. Finally, the excited N-methylacridone is spontaneously returned to its ground state, resulting in strong CL emission (Eq 4 in Scheme S3) [27,28].

# 3.2. PC-induced CL quenching of lucigenin-TC system

Figure 2 shows the effect of PC on lucigenin-TC CL. The lucigenin-TC system shows much stronger CL than the lucigenin-PC system. However, the CL intensity of the lucigenin-TC system was notably decreased with the addition of PC. The inhibition on the lucigenin-TC CL in the presence of PC may be caused by the consumption of  $O_2^{\bullet-}$  and its effect on reaction process. There are some reports that PC has strong antioxidant activity and is an efficient free radical scavenger even at low concentrations [29].



Fig. 2. Comparison diagram of different CL with PC. C(lucigenin), 0.1 mM; C(PC), 1 μM; C(TC), 0.5 mM; C(NaOH), 0.1 M.

Therefore, the lucigenin-TC CL system could be used for the determination of PC.

## 3.3. Optimization of experimental conditions

It is reported that alkaline conditions are advantageous to the CL emission with great intensity. Therefore, the CL intensity of the lucigenin-TC system in different concentrations of NaOH solutions was measured. Figure 3A shows the influence of NaOH concentration on the CL inhibition efficiency.  $(I_0 - I)/I_0$  is the CL inhibition efficiency,  $I_0$  is the CL intensity in the absence of PC, and I is the CL intensity in the presence of PC. The inhibition efficiency increased rapidly in the NaOH concentration range 0.05-0.1 M and gradually decreased as the NaOH concentration increased further. With the increase of NaOH concentrations, the background CL intensity of lucigenin in the presence of TC enhanced continually due to the faster deprotonation and decomposition of reactant. While the concentration of NaOH was above 0.1 M, the higher concentration probably caused more side reactions in the more alkaline environment, leading to the decrease of CL inhibition efficiencies. Therefore, 0.1 M NaOH was used for the subsequent experiments. In addition, the effect of TC concentrations on the CL system was studied. As shown in Fig. 3B, the CL intensities keep increasing as TC concentrations increase because of the generation of more reactive species. The CL inhibition efficiency of PC increased with the concentration of TC rising to 0.5 mM and then decreased slightly as its concentration was more than 0.5 mM, resulting from synergistic effect of PC and TC [30]. Consequently, 0.5 mM was selected as the optimal TC concentration in the following experiments.

## 3.4. Detection of PC

Figure 4 shows that effect of PC concentrations on the CL intensities of the lucigenin-TC system. It was obvious that the CL intensities decreased with increasing PC concentrations. The CL inhibition efficiency is linear with the concentrations of PC in the range 10–1000 nM. The linear equation is  $(I_0 - I)/I_0 = 0.097 + 4.27 \times 10^{-4}$  C (where C is the PC concentration in nM) (R = 0.996). The limit of detection (LOD) was calculated to be 4.4 nM at a signal-tonoise ratio of 3. The RSD for nine successive detections at the concentration 1  $\mu$ M is about 1.7% (Fig. S5), indicating the good reproducibility of this detection method. By comparison, our method has the advantages of simple instrument, fast response and high sensitivity, as shown in Table S1.



Fig. 3. (A) Effects of NaOH concentrations on the CL inhibition efficiencies. C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(PC), 1  $\mu$ M; PMT voltage, 900 V. (B) Effects of TC concentrations on the CL inhibition efficiencies (red line) and CL intensity (black line). C(lucigenin), 0.1 mM; C(NaOH), 0.1 M; C(PC), 1  $\mu$ M.

## 3.5. Selectivity of this method for PC

The effect of the common interfering substances on PC detection was evaluated including dopamine, phenol, resorcinol, phloroglucin, 3-aminophenol, ascorbic acid, cysteine, glutathione, serine, proline, alanine, glycine, tyrosine, tryptophan, Ni<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>, and Ag<sup>+</sup>. As shown in Fig. S6, the CL intensity of lucigenin-TC system decreases significantly in the presence of PC. Other substances have much smaller effects on the CL intensities. By comparison, PC has stronger reducing power and more reducing groups than others, resulting in fine selectivity.

#### 3.6. Determination of PC in real samples

In order to study the applicability of the proposed method, we have determined PC in human urine samples and commercially available grape seed extract capsule. To evaluate the recoveries, the human urine samples were diluted 100-fold with double distilled water to minimize interference from potential interferents. The diluted urine samples were respectively spiked with different known concentrations of PC. In addition, 6.0 mg of grape seed extract samples was added in 10 mL deionized water. The solutions were then diluted with deionized water to bring the calculated concentration of PC to 500 nM based on the production information. To evaluate the recoveries, the diluted sample solutions were respectively spiked different known concentrations of PC. As shown in Table S2, the recoveries for PC determination in human urine and grape seed extract capsule were in the range of 94.9-100.2% and 101.6-104.1%, respectively. Moreover, we detected PC in grape seed extract capsule using HPLC-UV. As shown in Table S3, the measured values by our method and HPLC-UV method are similar and are also similar to that (95.1%) stated by the manufacturer. It suggests that this method is promising for the detection of PC in real samples.



Fig. 4. (A) CL emission—time curves in different concentrations of PC from 10 to 1000 nM and (B) PC calibration curve. C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(NaOH), 0.1 M.

# 4. Conclusion

In conclusion, TC was employed as an efficient coreactant for lucigenin CL in the absence of specific catalysts or luminescent materials. Notably, PC was found to significantly suppress CL emission of lucigenin-TC system for the first time. This PCinduced CL quenching for lucigenin-TC system was mainly ascribed to its antioxidant effect. A new strategy in combination with flow-injection analysis for sensitive and selective PC determination was established based on this quenching effect on the lucigenin-TC CL. Moreover, the proposed method exhibiting good reproducibility has been successfully applied for the determination of PC in human urine samples and health food capsule of grape seed. With the merits of biocompatibility, simplicity and robustness, our newly developed lucigenin-TC system provides a new way for the biological

detection of PC and thus holds great promise in health food, pharmaceutical industries and functional cosmetics.

#### **Conflict of interest**

The authors declare no conflicts of interest.

#### Acknowledgements

This project was kindly supported by the National Natural Science Foundation of China [No. 21804127 and 21874126], and the Chinese Academy of Sciences (CAS)-the Academy of Sciences for the Developing World (TWAS) President's Fellowship Programme.

# Appendix



Scheme S1. Schematic of TC induced CL of lucigenin for PC detection.



Scheme S2. Diagram of flow injection analysis for lucigenin-TC CL system.



Scheme S3. Mechanism of lucigenin-TC CL system.



Fig. S1. Comparison diagram of different CL. C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(NaOH), 0.1 M.



Fig. S2. CL intensity of lucigenin-TC system in the absence (a) and presence of radical scavengers (b) 0.5 mM NaN<sub>3</sub>, (c) 1.0 mM thiourea, (d) 1  $\mu$ g/mL SOD. C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(NaOH), 0.1 M.

ORIGINAL ARTICLE



Fig. S3. CL emission-time curves recorded in the presence (red line) and absence of oxygen (black line). C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(NaOH), 0.1 M; PMT voltage, 700 V.



Fig. S4. The EPR spectra of the adduct DMPO- $O_2^{\bullet-}$  in the proposed CL system. C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(NaOH), 0.1 M.



Fig. S5. CL intensity of lucigenin-TC system at the PC concentration of 1  $\mu$ M under 9 consecutive detections. C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(NaOH), 0.1 M.



Fig. S6. Interference study of PC detection. The concentrations of PC, dopamine (DA), phenol (Phe), resorcinol (Res), phloroglucin (Phl), 3aminophenol (3-Amp), ascorbic acid (AA), cysteine (Cys) and glutathione (GSH) are 1  $\mu$ M. The concentrations of serine (Ser), proline (Pro), alanine (Ala), glycine (Gly), tryptophan (Try), tyrosine (Tyr), Ni<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>, and Ag<sup>+</sup> are 10  $\mu$ M. C(lucigenin), 0.1 mM; C(TC), 0.5 mM; C(NaOH), 0.1 M.

Table S1. Comparison of some previously reported assays for PC detection.

Method	Linear range	LOD	Reference
RP-LC-UV	34.6 – 259.3 μM	5.0 µM	[31]
MIPs-SPE	$10.8 - 172.9 \ \mu M$	5.2 nM	[32]
LC-MS	$2.3 - 777.9 \ \mu M$	103.7 nM	[33]
RP- HPLC-DAD	$0.2 - 34.6 \ \mu M$	43.4 nM	[34]
Nano-LC-TOF-MS	$0.2 - 3.6 \ \mu M$	13.5 nM	[35]
UHPLC-MS/MS	$0.2 - 10.7 \ \mu M$	1.0 nM	[36]
CL	10 – 1000 nM	4.4 nM	This work

Table S2. Results of the detection of PC in real samples.

Samples	Spiked	Found	Recovery	RSD
	(nM)	(nM)	(%)	(n = 3, %)
Urine	0.0	-	_	2.4
	200.0	199.1	99.6	2.8
	500.0	474.5	94.9	1.5
	700.0	701.7	100.2	4.8
Grape seed extract capsule	0.0 100.0 200.0 400.0	485.2 609.4 696.3 907.9	 104.1 101.6 102.6	4.1 1.7 2.4 1.4

Table S3. Comparison of PC content in grape seed extract capsule detected by HPLC-UV and CL.

Sample	Found by HPLC-UV [37] (%)	Found by CL method (%)
Grape seed extract capsule	96.8	90.2

## References

- Mendenhall GD. Analytical applications of chemiluminescence. Angew Chem Int Ed 1977;16:225–32.
- [2] Safavi A, Absalan G, Bamdad F. Determination of iproniazid by flow injection analysis with chemiluminescence detection based on the qxidation of luminol by trichloroisocyanuric acid and sodium dichloroisocyanurate. J Food Drug Anal 2009;17:17–21.
- [3] Jia J, Zhang Y, Yin H, Wei N, Dong Y, Chu X. Black phosphorus quantum dots sensitized lucigenin chemiluminescence in mild alkaline condition and its application in sensitive detection of Co<sup>2+</sup>. Microchem J 2020;153:104506.
  [4] Mostafa IM, Gilani MRHS, Chen YQ, Lou BH, Li JP, Xu GB.
- [4] Mostafa IM, Gilani MRHS, Chen YQ, Lou BH, Li JP, Xu GB. Lucigenin-pyrogallol chemiluminescence for the multiple detection of pyrogallol, cobalt ion, and tyrosinase. J Food Drug Anal 2021;29:510–20.
- [5] Yao HC, Xu EJ, Zeng WY, Zeng XY, Zhang M, Chen J. Determination of doxorubicin in pharmaceutical preparation and rat plasma with luminol-K<sub>3</sub>Fe(CN)<sub>6</sub> chemiluminescence system. J Food Drug Anal 2013;21:279–85.
- [6] He Y, Cui H. Synthesis of dendritic platinum nanoparticles/ lucigenin/reduced graphene oxide hybrid with chemiluminescence activity. Chem-Eur J 2012;18:4823–6.
- [7] He Y, Cui H. Label free and homogeneous histone sensing based on chemiluminescence resonance energy transfer between lucigenin and gold nanoparticles. Biosens Bioelectron 2013;47:313–7.
- [8] Sieliwoniuk EN, Malejko J, Topczewska A, Kojło A, Żyłkiewicz BG. A comparison study of chemiluminescence systems for the flow injection determination of silver nanoparticles. Microchem J 2019;144:221–8.

- [9] Wang C, Lan Y, Yuan F, Fereja TH, Lou B, Han S, et al. Chemiluminescent determination of L-cysteine with the lucigenin-carbon dot system. Microchim Acta 2019;187:50.
- [10] Hernández M, Borrull F, Calull M. Analysis of antibiotics in biological samples by capillary electrophoresis. TRAC-Trend Anal Chem 2003;22:416–27.
- [11] Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, et al. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. Lancet Infect Dis 2014;14:742–50.
- [12] Davies AK, McKellar JF, Phillips GO, Reid AG. Photochemical oxidation of tetracycline in aqueous solution. J Chem Soc Perk T 1979;2:369–75.
- [13] Kruk I, Lichszteld K, Michalska T, Nizinkiewicz K, Wrońska J. The extra-weak chemiluminescence generated during oxidation of some tetracycline antibiotics 1. Autoxidation J Photoch Photobio B 1992;14:329–43.
- [14] Quinlan GJ, Gutteridge JMC. Hydrixyl radical generation by the tetracycline antibiotics with free radical damage to DNA, lipids and carbohydrate in the presence of iron and copper salts. Free Radical Bio Med 1988;5:341–8.
- [15] Syropoulos AB, Calokerinos AC. Continuous-flow chemiluminometric determination of some tetracyclines. Anal Chim Acta 1991;255:403–11.
- [16] Petrenko IU, Titov VI, Vladimirov IA. [Generation of active forms of oxygen by antibiotics of the tetracycline series during tetracycline catalysis of oxidation of ferrous iron]. Antibiot Khimioter 1995;40:3–8.
- [17] Shi L, Wang J, Lv Y. Research progress on analysis methods of procyanidins. IOP Conf Ser: Earth Environ Sci 2021;705:012006.
- [18] Sakurai T, Kitadate K, Nishioka H, Fujii H, Kizaki T, Kondoh Y, et al. Oligomerized grape seed polyphenols attenuate inflammatory changes due to antioxidative properties in coculture of adipocytes and macrophages. J Nutr Biochem 2010;21:47–54.
- [19] Costa AGV, Garcia-Diaz DF, Jimenez P, Silva PI. Bioactive compounds and health benefits of exotic tropical red-black berries. J Funct Foods 2013;5:539–49.
- [20] Clifton PM. Effect of grape seed extract and quercetin on cardiovascular and endothelial parameters in high-risk subjects. J Biomed Biotechnol 2004;2004:272-8.
- [21] Gao C, Cunningham DG, Liu H, Khoo C, Gu L. Development of a thiolysis HPLC method for the analysis of procyanidins in cranberry products. J Agric Food Chem 2018;66: 2159–67.
- [22] Grabber JH, Zeller WE. Direct versus sequential analysis of procyanidin- and prodelphinidin-based condensed tannins by the HCl-butanol-acetone-iron assay. J Agric Food Chem 2020;68:2906–16.
- [23] Urbonavičiūtė A, Jakštas V, Kornyšova O, Janulis V, Maruška A. Capillary electrophoretic analysis of flavonoids in single-styled hawthorn (Crataegus monogyna Jacq.) ethanolic extracts. J Chromatography A 2006;1112:339–44.
- [24] Ricci A, Iaccheri E, Benelli A, Parpinello GP, Versari A, Ragni L. Rapid optical method for procyanidins estimation in red wines. Food Control 2020;118:107439.
- [25] Lan Y, Yuan F, Fereja TH, Wang C, Lou B, Li J, et al. Chemiluminescence of lucigenin/riboflavin and its application for selective and sensitive dopamine detection. Anal Chem 2019;91:2135–9.
- [26] Imani-Nabiyyi A, Sorouraddin MH, Amjadi M, Naseri A. Luminol/CdTe quantum dots/sodium periodate system in conjunction with response-surface methodology for chemiluminometric determination of some tetracyclines. J Lumin 2014;151:57–65.
- [27] Maeda K, Kashiwabara T, Tokuyama M. Mechanism of the chemiluminescence of lucigenin. II. The charge-transfer structure of lucigenin and reduction of 10,10'-dimethyl-9,9'biacridinium dication by electron transfer from nucleophiles. B Chem Soc Jpn 1977;50:473–81.
- [28] Maskiewicz R, Sogah D, Bruice TC. Chemiluminescent reactions of lucigenin. 1. Reactions of lucigenin with hydrogen peroxide. J Am Chem Soc 1979;101:5347–54.

- [29] Gaulejac NS-CD, Provost C, Vivas N. Comparative study of polyphenol scavenging activities assessed by different methods. J Agric Food Chem 1999;47:425–31.
- [30] Gaulejac NS-CD, Vivas N, Freitas VD, Bourgeois G. The influence of various phenolic compounds on scavenging activity assessed by an enzymatic method. J Sci Food Agr 1999;79:1081–90.
- [31] Lopes GC, Bruschi ML, de Mello JCP. RP-LC-UV determination of proanthocyanidins in guazuma ulmifolia. Chromatographia 2009;69:175–81.
- [32] Jia M, Yang J, Zhao Y, Liu Z, Aisa HA. A strategy of improving the imprinting effect of molecularly imprinted polymer: Effect of heterogeneous macromolecule crowding. Talanta 2017;175:488–94.
- [33] Redeuil K, Befxlrtholet R, Kussmann M, Steiling H, Rezzi S, Nagy K. Quantification of flavan-3-ols and phenolic acids in milk-based food products by reversed-phase liquid chromatography-tandem mass spectrometry. J Chromatography A 2009;1216:8362–870.

- [34] Hadad GM, Abdel Salam RA, Soliman RM, Mesbah MK. Rapid and simultaneous determination of antioxidant markers and caffeine in commercial teas and dietary supplements by HPLC-DAD. Talanta 2012;101:38–44.
- [35] Contreras MDM, Arráez-Román D, Fernández-Gutiérrez A, Segura-Carretero A. Nano-liquid chromatography coupled to time-of-flight mass spectrometry for phenolic profiling: A case study in cranberry syrups. Talanta 2015;132:929–38.
- [36] Yin F, Li L, Chen Y, Lu T, Li W, Cai B, et al. Quality control of processed Crataegi Fructus and its medicinal parts by ultra high performance liquid chromatography with electrospray ionization tandem mass spectrometry. J Sep Sci 2015;38: 2630–9.
- [37] Wu J, Zhou X, Hu JN, Yu LD, Zhu XB, Zhu LQ, et al. Rapid determination of proanthocyanidin and vitamin E in grape seed vitamin E soft capsule. Zhongguo Shi Pin Tian Jia Ji 2020;31:31–7 [In Chinese, English abstract].