



# Combination of coagulant-assisted and SPE purification for the HPLC determination of lycorine and galanthamine in various foods

Jun Sugiura<sup>1</sup> · Tomoyuki Tsuchiyama<sup>1</sup> · Masahiro Nakajima<sup>1</sup>Received: 7 April 2019 / Accepted: 24 October 2019 / Published online: 30 October 2019  
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## Abstract

A simple high performance liquid chromatography (HPLC) method has been developed for the analysis of lycorine and galanthamine, typical alkaloids of narcissus, in food. Samples were extracted with sonication using water containing poly-aluminium chloride (PAC) and sodium hydroxide (NaOH) as a solvent followed by solid phase extraction (SPE) with hydrophilic-lipophilic balanced (HLB) cartridge. Elution of the analytes was achieved with 0.1 v/v% formic acid kept at 95 °C. Purification with PAC, which is normally used as a coagulant, was successfully combined with SPE, demonstrated the perfect strategy for the removal of interfering substances from the foods. Separation of the analytes was achieved on reversed phase C18 column. The analytes were monitored doubly at wavelengths of 240 and 290 nm for the detection. The method demonstrated excellent linearity with coefficients of determination ( $r^2$ ) higher than 0.999 over the range of 0.2–20  $\mu\text{g ml}^{-1}$  for both lycorine and galanthamine. Limits of quantification (LOQs) of the method were in the range of 0.15–0.52  $\mu\text{g ml}^{-1}$ . Method performance was evaluated, demonstrated to have quantitative recoveries of 92.8–98.9% with satisfactory intra-day precision of lower than 3.7% and inter-day precision of lower than 5.0%. The method was also applied to a wide variety of foods with complex matrices, indicated the potential of practical use in plant poisoning caused by narcissus contaminated foods.

**Keywords** Lycorine · Galanthamine · Narcissus · Plant poisoning · Poly-aluminium chloride · High performance liquid chromatography (HPLC)

## 1 Introduction

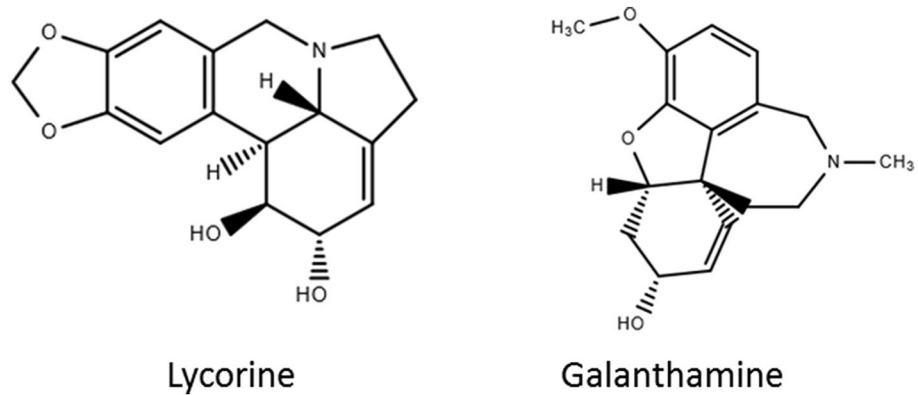
Plant poisoning has been one of the problems confronting Japan in terms of food safety, as vast majority of people enjoy going to natural places such as mountains where a wide variety of plants, both edible and poisonous ones, are grown. Although the number of plant poisoning is not large compared to viral or bacterial food poisonings, they sometimes can be fatal, and should be worth paying attention. Over the past fifty years from 1961 to 2010, growing number of food poisonings caused by higher plants had been reported to Ministry of Health, Labor and Welfare in Japan though the actual number was unknown because

of the difference on how to deal with the case depending on times [1]. Among the reported cases, ones caused by narcissus, also called “daffodil” was 31 cases, where a total of 137 people displayed symptoms during the same time period [1]. Plant poisonings caused by narcissus have been observed almost every year, and recently became the most frequently occurred ones of all plant poisonings through 2008 to 2017 [2]. Bulbs and aerial parts of narcissus are frequently mistaken as onions and Chinese chives because of their similarities with these foods in appearance. Plant poisonings caused by narcissus are also seen in other countries. For example, it was reported that a dozen school children in England had been hospitalized after

✉ Jun Sugiura, vice.city.2016@icloud.com | <sup>1</sup>Food Department, Nagoya City Public Health Research Institute, 1-11, Hagiya-ma-cho, Mizuho-ku, Nagoya 467-8615, Japan.



**Fig. 1** Chemical structures of lycorine and galanthamine



having a soup contaminated with a daffodil bulb in 2009 [3]. It was also a reported case of daffodil ingestion in 2012 that hospitalized 10 people in England [4].

Plant poisoning caused by narcissus usually occurs with symptoms of vomiting, stomachache and diarrhea triggered by alkaloids in the plant [5]. Typical alkaloids found in narcissus are lycorine and galanthamine with the chemical structures shown in Fig. 1. Lycorine have been studied for a long time in search of its beneficial bioactivities, indicated its anti-cancer and anti-virus potentials [6, 7], while galanthamine works as an inhibitor of acetylcholine esterase and have been used for the treatment of Alzheimer's disease [8]. Although there are such positive aspects of lycorine and galanthamine, they are also known as alkaloids triggering the symptoms above. In search of the culprit of plant poisoning, analytical method for the detection of alkaloids naturally contained in a certain plant is necessary. Although there are analytical methods developed for the detection of lycorine and galanthamine in the past [9–15], they are all targeted to analytes in plant materials, not intended for food analysis. However, in most cases, narcissus was consumed together with other food as a result of cooking. Thus, analytical method capable of detecting lycorine and galanthamine in food is highly desirable. In our previous study, poly-aluminium chloride (PAC), which is a commonly used coagulant for the purification of water, was used for the clean-up step of HPLC and LC–MS/MS methods for the detection of the both alkaloids from foods [16]. However, the methods developed were specialized only for aerial part of narcissus, and just one-step purification with PAC was considered to be insufficient and cannot be applicable to the food with extremely complex matrices. As a matter of fact, when the method was applied to sauce, which can be used for a wide variety of cuisine, determination of the compounds were practically impossible since interfering peaks were emerged at the same retention time of both lycorine and galanthamine in the chromatogram. For the reasons above, further purification is necessary for applying the method

to a wide range of foods. SPE has been one of the most commonly used purification step in sample preparation [17] and considered to be ideal for the further purification because of its applicability in various research fields and availability of a wide variety of packing materials. However, SPE has a shortcoming of having to use large amount of toxic organic solvent such as acetonitrile and methanol in preconditioning and elution steps. For this reason, SPE step with minimized consumption of organic solvent is preferable while maintaining its purification ability. Considering all the reasons above, the purpose of our research is to develop a simple HPLC method with combined purification steps of PAC and SPE with the minimized consumption of organic solvent for the determination of lycorine and galanthamine from various foods.

## 2 Materials and methods

### 2.1 Food samples and plant materials

A total of 6 food samples (curry, stew, miso soup, hashed beef, Japanese-style pancake and Chinese soup) were purchased from local supermarkets in Nagoya, Japan. Ten bulbs of *Narcissus tazetta* were purchased through the internet and the leaves were harvested at the site of our research institute. Food samples and plant materials were stored at 4 °C and –30 °C, respectively until being analyzed.

### 2.2 Chemicals and reagents

Lycorine hydrochloride was purchased from Sigma-Aldrich (MO, USA). Galanthamine hydrobromide was purchased from TOCRIS (Bristol, UK). PAC was purchased from Kishida Chemical. (Osaka, Japan). Sodium hydroxide (NaOH) and formic acid were purchased from Wako Pure Chemical (Osaka, Japan). Acetonitrile and methanol were purchased from Kanto Chemical. (Tokyo, Japan). All reagents used in

this study were of analytical or HPLC grade. Water used in all experiments was purified through a PURELAB Ultra Analytic system (ORGANO CORPORATION, Tokyo, Japan).

### 2.3 Chromatography

Chromatographic analyses were carried out using prominence HPLC system (Shimadzu, Kyoto, Japan) equipped with solvent delivery unit (LC-20AD), degasser (DGU-20A<sub>3</sub>), auto sampler (SIL-20AC HT), column oven (CTO-20AC) and Diode array detector (SPD-M20A). Separation of lycorine and galanthamine was performed on a XBridgeC18 (3.5  $\mu\text{m}$  particle size, 150 mm length, 4.6 mm i.d., Waters, MA, USA) using binary linear gradient elution. Two mobile phases were mixed by the binary pump. Mobile phase A: acetonitrile–water–formic acid (50:950:1, v/v/v) and mobile phase B: acetonitrile–formic acid (100:0.1, v/v). Elution program was a liner gradient from 100% A for the first 4 min, from 100 A to 0% A in 0.1 min, then held for 2 min, ramped up to 100% A in 0.1 min, held for the final 4.8 min. The total chromatographic run time was 11.00 min. The mobile phase flow rate was 1.0 ml min<sup>-1</sup>. The injection volume was 20  $\mu\text{L}$ . The column oven temperature was set at 40 °C. Detection were performed at wavelengths of 240 nm and 290 nm.

### 2.4 Preparation of standard solution

Stock standard solutions of lycorine and galanthamine (500  $\mu\text{g ml}^{-1}$  each) were prepared in methanol, stored at –20 °C before use. Working standard solutions ranging from 0.2 to 20  $\mu\text{g ml}^{-1}$  were prepared from stock solution and diluted step by step with water.

### 2.5 Sample preparation

Homogenized sample (1 g) was accurately weighed into a test tube. Ten ml water was added to the sample, followed by the addition of 0.5 ml 30 w/v% PAC and 0.5 ml 10 w/v% NaOH. After a brief shake, the solution were sonicated for 30 min. Then, the solution were vacuum filtered through a glass filter into a 20-ml glass beaker, followed by washing with 10 ml water. The filtrate was loaded on to a 60-mg hydrophilic-lipophilic balance (HLB) cartridge from Waters (MA, USA), which was conditioned with 6 ml water. Then, the beaker was washed with 3 ml water and the content was also added to the cartridge. After washing the cartridge with 6 ml water, 5 ml of 0.1 v/v% formic acid, which was kept at 95 °C, was instantly applied on the column under vacuum condition. After cooling the obtained solution down to a room temperature, water was added up to 5 ml before HPLC analysis.

## 3 Results and discussion

### 3.1 Concentration and volume of PAC and NaOH solution

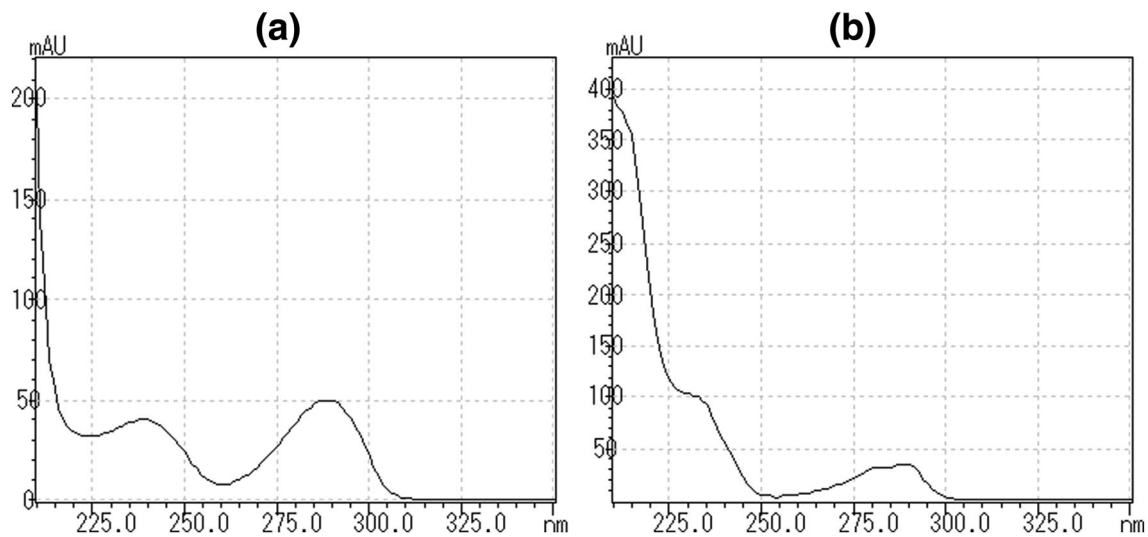
PAC is widely used for waste water treatment and its effect exceedingly depends on pH of the solution. Keeping pH of the solution at an appropriate value is essential to bring out potential of PAC. Our previous study demonstrated that mixing the same amount of 30 w/v% PAC and 10 w/v% NaOH makes the solution around pH 7 where the effect of PAC become biggest [16]. In the present study, 30 w/v% PAC and 10 w/v% NaOH were similarly used for clean-up process and their amount were set at 0.5 ml each as in our previous study.

### 3.2 Selection of wavelength for the detection

Lycorine and galanthamine are alkaloids and have absorbance peak at the wavelength of 290 nm as shown in Fig. 2. In our previous study for lycorine and galanthamine, wavelength of 290 nm was solely used for the UV detection. The wavelength of 290 nm is specific and therefore, could alleviate the effect of interfering substances. However, the analysis of standard solution revealed that the wavelength of 240 nm has stronger intensity in terms of signal to noise ratio. For this reason, both wavelengths of 290 and 240 nm were selected for the detection of lycorine and galanthamine. Monitoring targets at double wavelength was considered to be able to increase the reliability of the result compared to just one wavelength.

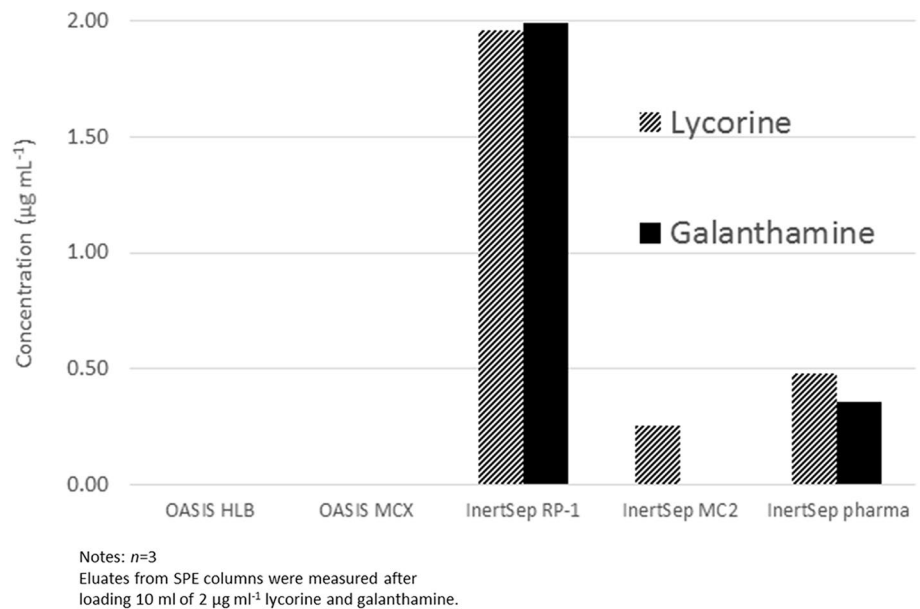
### 3.3 Selection of SPE cartridge column

Purification step using PAC can effectively remove interfering substances by taking them into precipitates as a result of coagulation. The PAC purification worked successfully for the determination of artificial sweeteners and preservatives, proved its effectiveness on clean-up process [17, 18]. However, their concentrations in food are relatively high when compared with potential interfering substances, making them easily detectable under appropriate analytical conditions. However, in plant poisoning cases of narcissus, alkaloid concentration is expected to fluctuate depending on parts of the plant, time and location of harvest and its content in food. Thus, detection of analytes in a wide range of concentration was highly desirable, even in the food with highly complex matrices such as sauce, stew and curry, which can be potential matrices for the analysis of lycorine and galanthamine in food. However, the extract obtained after the PAC purification which we



**Fig. 2** UV spectra of **a** lycorine and **b** galanthamine

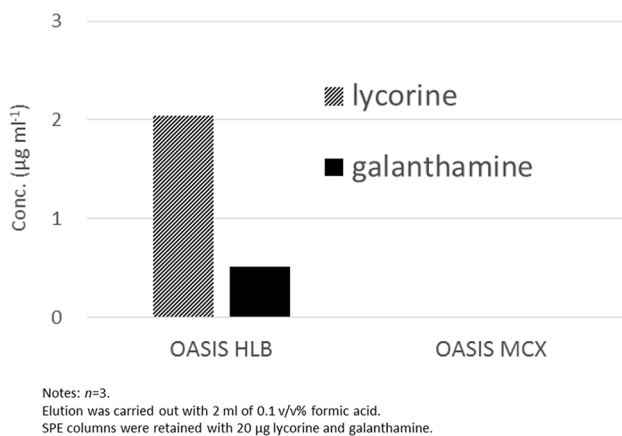
**Fig. 3** Retention behaviour among the five SPE cartridges



reported on our previous study [16] contains only 0.02 g sample per milliliter, leading higher limits of detection. Also, when our previously reported method was applied to sauce, interfering peaks were appeared on the chromatogram, made it hard to determining the compounds. What is more, PAC derived metal ions dissolved in the final solution could have negative impact on HPLC column and analytical instrument [17].

For the reasons above, further purification using SPE was considered to be necessary. In this study, five SPE cartridge columns, namely OASIS HLB (Waters, MA, USA), OASIS MCX (Waters, MA, USA), InertSep RP-1 (GL Science, Tokyo, Japan), InertSep pharma (GL Science, Tokyo, Japan)

and InertSep MC-2 (GL Science, Tokyo, Japan), were all prepared in a same size (60-mg, 3-ml) for investigating their retaining capacity of lycorine and galanthamine under a designated condition. Each column was first conditioned with 6 ml of water and loaded with 10 ml of lycorine and galanthamine solution at a concentration of 2 µg ml<sup>-1</sup>. Then, the solution passing through the column were subjected to HPLC analysis for determination. As shown in Fig. 3, both OASIS HLB and OASIS MCX showed strong retention capacity of lycorine and galanthamine as they were not detected from the solution passing through the columns. Tertiary amine in the structure of packing material was considered to play an important role because of



**Fig. 4** Elution of lycorine and galanthamine with 0.1 v/v% formic acid

the same functional group found in the structures of both lycorine and galanthamine.

For the elution of lycorine and galanthamine with solution containing minimum amount of organic solvent, OASIS HLB was suitable because certain amount of both analytes were eluted from the column using 0.1 v/v% formic acid as shown in Fig. 4. OASIS MCX still showed strong retention possibly because of the ionic bonding between benzene sulfonate in the SPE column and quaternary ammonium both in lycorine and galanthamine under acidic condition. Although it was possible to elute with organic solvent such as methanol and acetonitrile in basic condition, it's not preferable in terms of the recent trend of green analytical chemistry [19]. For these reasons above, OASIS HLB was finally chosen and used for further study.

### 3.4 Elution from SPE cartridge column

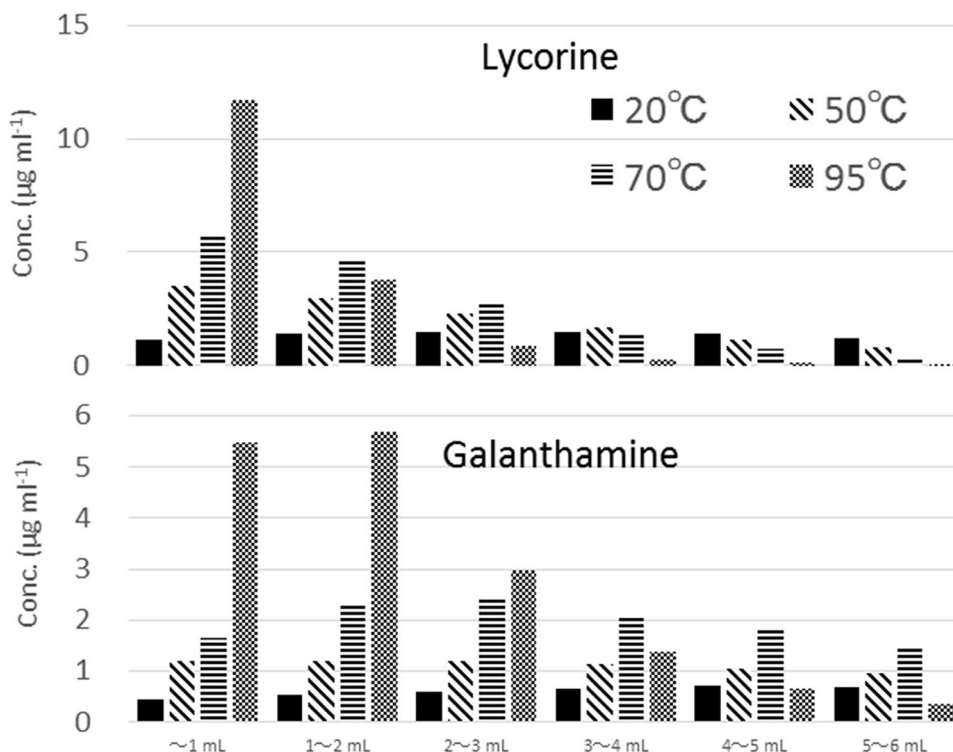
Although 0.1 v/v% formic acid has shown its ability to elute analytes from SPE columns, it was necessary to evaluate the pH of eluting solution as the elution of lycorine and galanthamine are based on their ratio between ionic and molecular form. Reported pKa of lycorine was 6.4 [20], while that of galanthamine was reported to be 8.2 [21]. For determining the appropriate concentration of the eluting solution, the pH measurement of formic acid at concentrations of 0.01, 0.1 and 1 v/v% were carried out with a pH meter, indicated that their pH values were 3.3, 2.7 and 2.2, respectively. All the pH values measured were well below the pKa of lycorine and galanthamine, meaning the majority of analytes are existing as ionic form which has weaker interactions with column packing material. However, a concentration of 0.1 v/v% was thought to be appropriate and chosen because of the same concentration of HPLC mobile phase.

As speculated from Fig. 4, the total volume of the solution necessary for complete elution was considered to be large, which could decrease the sensitivity of the method. In hope of minimizing the solution needed for the elution, enhancing its elution strength by adjusting the temperature of the solution was thought to be ideal because of unnecessary to add extra organic solvent to the solution. For the purpose of assessing the temperature effect on elution strength, temperatures of 20, 50, 70 and 95 °C were investigated by fractionally analyzing eluates from SPE columns. For maintaining the designated temperatures above, the solution was kept in a water bath with a thermometer. Handlings on the experiments were carried out in a prompt way for the prevention of temperature drop of the solution while in operation. Vacuum strength in a manifold was also increased for the same purpose. As can be seen in Fig. 5, elution strength dramatically increased along with the temperature rise. Temperatures of 50 °C seemed to be enough for the complete elution of lycorine, but large amount of galanthamine still remained on the column. On the other hand, the highest temperature of 95 °C showed the ideal elution pattern because over 95% of lycorine and galanthamine were eluted within 5 ml. Therefore, in further study, the eluting solution of 0.1 v/v% formic acid was kept at a temperature of 95 °C before the elution.

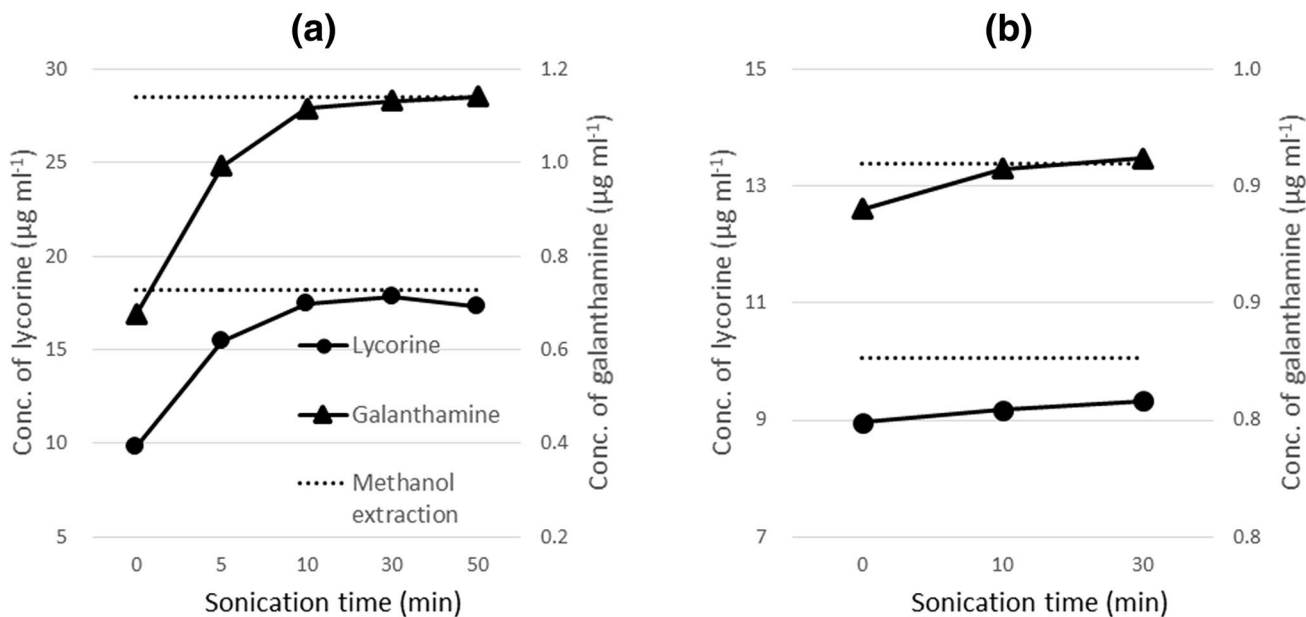
### 3.5 Influence of sonication time

For the optimization of sonication time, extraction efficiency was investigated using both bulb and aerial of narcissus. Homogenized sample (1 g) was weighed into a test tube followed by the addition of 10 ml water, 0.5 ml 30 w/v% PAC and 0.5 ml 10 w/v% NaOH solutions. Then, the tube was sonicated for a designated duration. After that, solution in the tube was vacuum filtered followed by washing with 10 ml water. Finally, the solution was filled up to 20 ml with water before HPLC analysis. Sonication times of 0, 5, 10, 30 and 50 min were applied to the bulbs and that of 0, 10 and 30 min were applied to the leaves. At the same time, an extraction method with methanol and homogenization [12] with slight modification was also performed for comparing the efficacy of extraction between the two methods. Briefly, 1 g sample was added with 25 ml methanol followed by homogenization (7500 rpm, 5 min) and vacuum filtration. Then, filter was washed three times with 25 ml methanol each and finally, the solution was evaporated to dryness followed by reconstituting with 20 ml water. As can be seen in Fig. 6, concentrations of lycorine and galanthamine were increased along with the time of sonication in bulb and reached to the level obtained from the other method at around 30 min of sonication. On the other hand, concentration of lycorine was at a standstill

**Fig. 5** Elution of analytes with 0.1 v/v% formic acid in various temperature



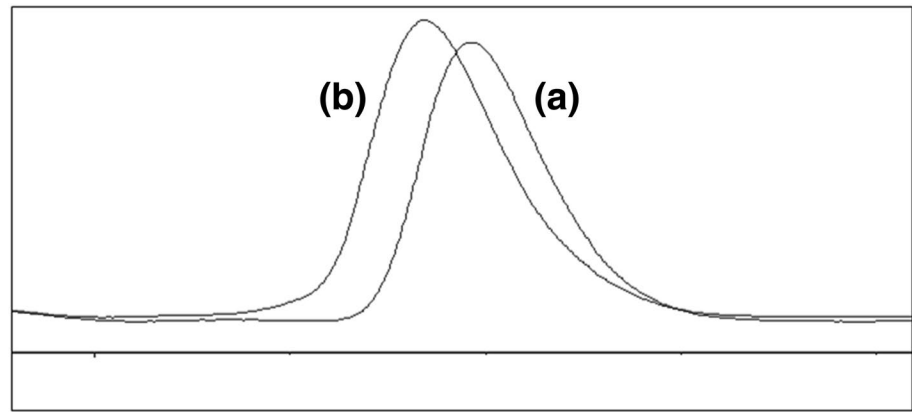
Notes:  $n=3$ .  
 Lycorine and galanthamine were fractionally eluted with 0.1 v/v% formic acid.  
 SPE columns were retained with 15 µg lycorine and galanthamine.  
 0.1 v/v% formic acid was kept in water at a fixed temperature.



Notes:  $n=3$ .  
 1 g sample was added with 10 ml water followed by the addition of poly-aluminium chloride (PAC) and NaOH solutions before sonication.

**Fig. 6** Extraction from **a** a bulb and **b** leaves of narcissus with different time of sonication and comparison with the methanol extraction method

**Fig. 7** Chromatograms of narcissus leaf extract after performing (a) the proposed extraction and (b) the methanol extraction



Notes: Sonication time was 30 min for the proposed extraction.  
Detection wavelength was set at 240 nm.

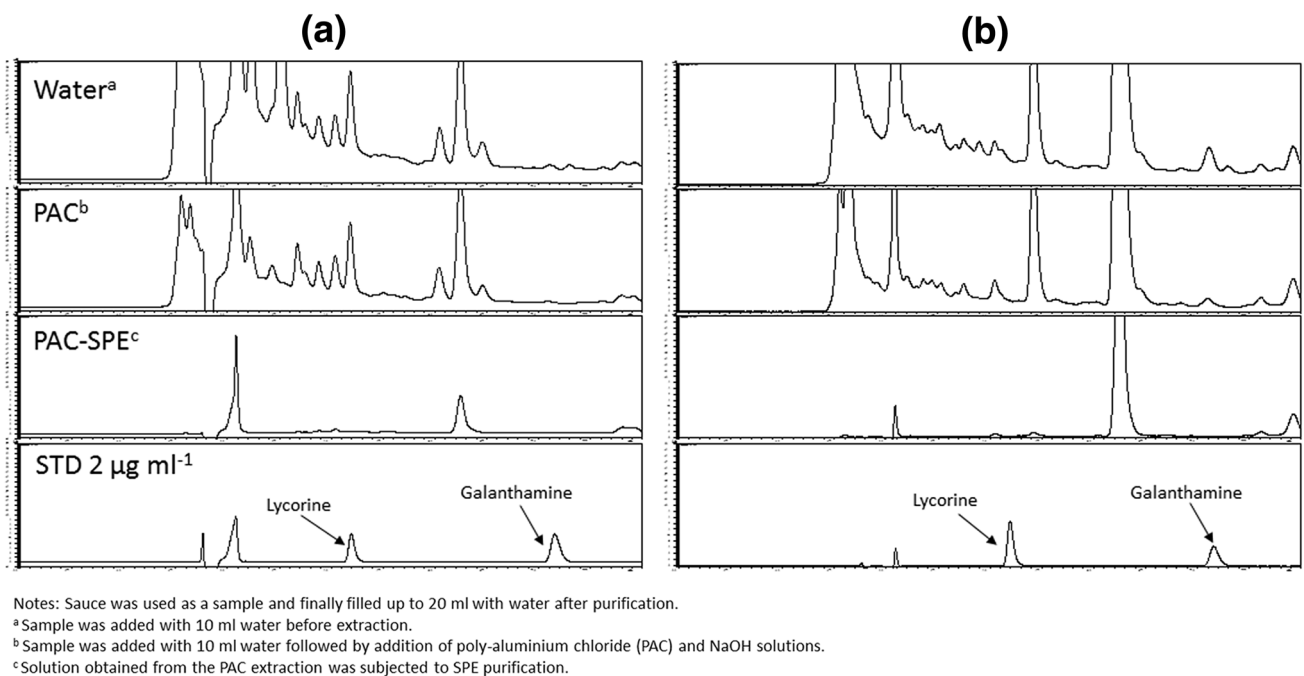
and slight below the level of the other method even in the 30 min of sonication. A possible reason for the gap of concentration between the two methods could be explained by the existence of interfering peak appearing at almost the same retention time as lycorine. When comparing the chromatograms between the two methods, lycorine peak in the chromatogram obtained from the methanol extraction had wider peak width than the other as in Fig. 7. Spectral patterns at the both front and rear edges of the peak were different from the standard spectrum of lycorine, indicating the presence of interference over lycorine peak. Judging from the reason above-mentioned, lycorine in leaves was also considered to be sufficiently extracted with 30 min of sonication and therefore, sonication time was set at 30 min for further study.

### 3.6 Effect of each purification step

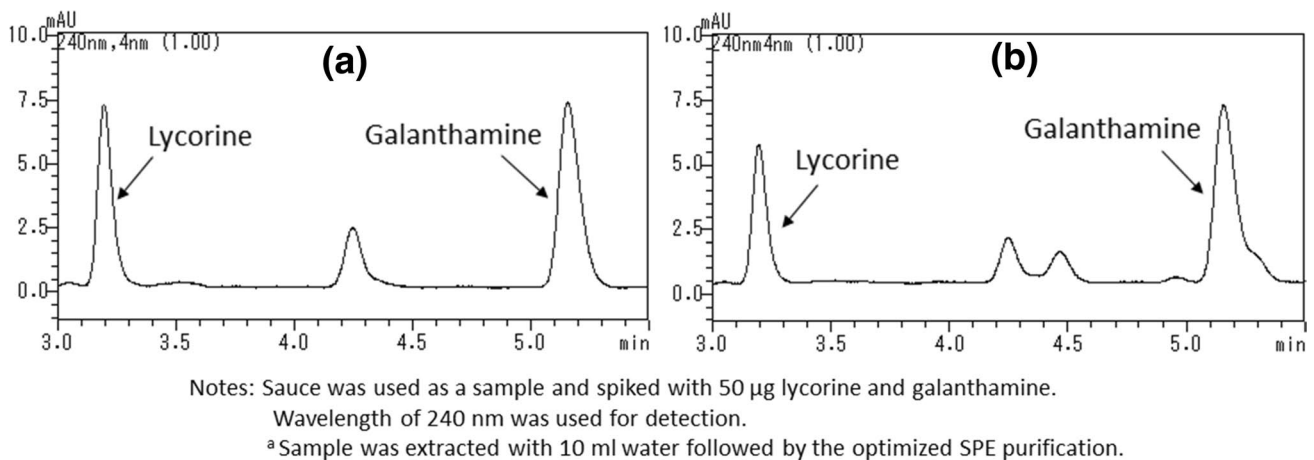
To confirm the necessity and effect of each purification step, experiments had to be carried out in a sample with complex matrices. As a typical sample with highly complex matrices, sauce was selected for the study because it is a mixture of a wide variety of food with dark color, making it relatively easy to visualize the deference in effect among each purification step. More importantly, sauce could be potential matrix for narcissus' food poisoning because of its wide applicability to cooked food. There were three patterns investigated and the first one was water extraction only. In the water extraction, 1 g sample was added with 10 ml water followed by 30 min of sonication. Then, the solution was vacuum filtered with a glass filter, finally filled up to 20 ml with water. The second one was extraction using PAC. In the second pattern, 0.5 ml 30 w/v% PAC and 0.5 ml 10 w/v% NaOH were added after 10 ml water addition. The last one was extraction using PAC followed by SPE clean-up, the proposed method. In the last one, the solution obtained in

the second pattern were subjected to the optimized SPE process. But the final solution were filled up to 20 ml with water for making sample concentration in the final solution even. Visual investigation between the first and second patterns revealed that PAC coagulation perfectly worked for the removal of pigments as the first and second patterns yielded blown colored and transparent solutions, respectively. As can be seen in Fig. 8, even only the PAC purification made a deference in the chromatogram when compared with the water extraction. Specifically, the removal of interfering substances in an early retention time was successfully achieved. However, there were still interfering peaks potentially becoming obstacle for the determination of lycorine and galanthamine. After additional purification step with SPE, the interfering peaks were completely disappeared. Although water extraction followed by SPE purification also demonstrated huge effect in the chromatogram, loss of lycorine from the SPE column and a minor interfering peak over galanthamine's were observed as in Fig. 9. The recovery loss was possibly caused by acid condition of the extract, weakening the affinity of lycorine to the solid phase because of the surge of ionic form. On the other hand, PAC extract could maintain its pH value neutral, indicating successful removal of acids from samples. The PAC clean-up process was also preferable in a way that it could cut the time of filtration by almost half compared with the water extraction. It was speculated that particles in the water extract are small enough to be caught in a filter network, gradually accumulated to the level in which water cannot easily pass through. In contrast, the PAC extract forms aggregation of particles large enough to be on top of the filter paper, making it easier for water penetration through it.

In this study, once again, the PAC extraction followed by SPE clean-up demonstrated its necessity and effectiveness.



**Fig. 8** Effect of each purification step on chromatogram at wavelength of **a** 240 nm and **b** 290 nm



**Fig. 9** Difference on chromatograms of sample solution processed with **a** the proposed method and **b** water extraction-SPE<sup>a</sup>

### 3.7 Method validation

The method validation, including linearity, LOQs, recovery and precision was carried out. Recovery and precision tests were performed using sauce as a sample in duplicate successively for five days. Spiked levels of the sample were 5 and 50 μg g<sup>-1</sup>. Although a study reported the concentrations of lycorine and galanthamin are varying from 49.2 to 462 μg g<sup>-1</sup> and from 12.0 to 70.6 μg g<sup>-1</sup>, respectively in bulb [12], the selected spiked concentrations were determined after considering the degree of dilution through

cooking process. As in Table 1, result of the experiments were satisfactory, demonstrating the recoveries ranging from 92.8 to 98.9% with intra-day precision of less than 3.7% and inter-day precision of less than 5.0%. Linearity was tested by injecting standard solutions containing varying levels of the target compounds. In both lycorine and galanthamine, at both wavelengths of 240 nm and 290 nm, excellent linearity and coefficients of determination over 0.999 were obtained in the range of 0.2 to 20 μg ml<sup>-1</sup>. The LOQs of the method were evaluated by continuously injecting standard solution of 0.2 μg ml<sup>-1</sup> for



**Table 1** Repeatability and reproducibility of the method at two spiked levels

Detection wave-length (nm)	Analyte	Spiked level ( $\mu\text{g}$ )	Mean recovery (%)	Intra-day precision (%)	Inter-day precision (%)
240	Lycorine	5	98.4	3.7	5.0
		50	98.5	1.6	2.1
	Galanthamine	5	92.8	2.6	2.6
		50	98.9	2.1	2.1
290	Lycorine	5	97.2	2.8	2.9
		50	96.8	1.8	1.9
	Galanthamine	5	96.5	2.0	2.0
		50	97.7	2.3	2.3

Sauce was used as a sample

Experiments were performed in duplicate successively for five days

**Table 2** Result of the spike and recovery tests on six foods

	Lycorine		Galanthamine	
	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)
Curry	99.8	4.0	102.4	3.1
Stew	101.5	0.2	95.5	1.3
Miso soup	105.0	1.5	103.4	1.2
Hashed beef	105.1	2.3	93.2	3.4
Japanese-style pancake	102.9	0.8	95.7	1.9
Chinese soup	103.4	1.3	100.0	1.9

$n=3$

1 g sample was spiked with 5  $\mu\text{g}$  lycorine and galanthamine

twenty times. Then, concentrations equivalent to 10 times of standard deviation were calculated. As a result, LOQs of lycorine and galanthamine at 290 nm were 0.26 and 0.52  $\mu\text{g g}^{-1}$ , respectively and that at 240 nm were 0.15 and 0.27  $\mu\text{g g}^{-1}$ , respectively.

### 3.8 Application of the method to various foods

To confirm the applicability of the method to a wide variety of foods, six foods were prepared and analyzed with the proposed method. Foods prepared were curry, stew, miso soup, hashed beef, Japanese-style pancake and Chinese soup, all considered to have a variation of matrices and to be potential cause of narcissus' food poisoning. Every food was divided into two portions. One was spiked with 5  $\mu\text{g}$  lycorine and galanthamine for performing spiked and recovery tests and the investigation of interfering originated from the food. The recovery tests were performed in triplicate. The other portion was intentionally contaminated with narcissus for producing the actual situation of food poisoning. As shown in Table 2, results of spiked and

**Table 3** Result of the tests on foods contaminated with narcissus

	Lycorine		Galanthamine	
	Conc. ( $\mu\text{g g}^{-1}$ )	S.D.	Conc. ( $\mu\text{g g}^{-1}$ )	S.D.
Curry	57.4	0.67	3.51	0.07
Stew	80.2	0.85	5.24	0.11
Miso soup	66.0	0.57	3.80	0.08
Hashed beef	80.7	0.99	3.68	0.02
Japanese-style pancake	19.5	0.17	1.98	0.05
Chinese soup	13.9	0.12	1.15	0.03

$n=3$

Curry, stew, miso soup and hashed beef were homogenized together with a bulb of narcissus

Japanese-style pancake and Chinese soup were homogenized together with leaves of narcissus

recovery tests were satisfactory, demonstrating the recovery range of 99.8–105.1% for lycorine and 93.2–103.4% for galanthamine. As shown in Table 3, result of analysis of the foods contaminated with narcissus was also successful of being able to detect both analytes in all foods, proving the method applicability on actual contamination. The detected levels of lycorine and galanthamine were ranging 13.9–80.7  $\mu\text{g/g}$  and 1.15–5.24  $\mu\text{g/g}$ , respectively. Though minimal dose of lycorine and galanthamine that cause the symptoms to human is still not clearly known, there is a study experimenting effects of lycorine in beagle dogs, proved that emesis starts at 0.5 mg/kg [22]. The tendency is not directly applicable to human, however, the detected levels can be toxic depending on the amount of ingestion. More importantly, there are a variety of alkaloids found in narcissus [23] except for lycorine and galanthamine, indicating that much smaller amount can cause serious symptoms.

## 4 Conclusions

The proposed method demonstrated successful combination of PAC-assisted and SPE purification for the removal of interfering substances while concentrating lycorine and galanthamine. The method also took advantage of hot water in SPE elution, successfully minimizing the consumption of organic solvent. Satisfactory results for linearity, LOQs, recovery and precision were obtained on the developed method. Furthermore, applicability of the method to a wide variety of foods was also demonstrated. The method showed its possible usage on actual food poisoning or accidental ingestion of narcissus which is one of the most frequent cause of plant poisoning in Japan. It's especially useful in case where there are only processed samples left, contributing the accurate determination of the target compounds and the faster result of cause investigation.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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