



## Separation of $\beta$ -sitosterol by using three ionic liquid-based silicas with solid phase extraction

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### Abstract

Three ionic liquid-based silicas were synthesized, and variable adsorption conditions were optimized by RSM. Under the optimized condition, 0.1mg/mL of  $\beta$ -sitosterol in methanol: water (60:40, vol%) with 10 min adsorption, Sil-HIm was selected and was applied as the sorbent in SPE. Two samples such as peanut and corn were used and after washing by water and acetone and eluting by methanol-1% vol acetic acid, 0.0008 mg/g and 0.006mg/g of  $\beta$ -sitosterol were detected from them with satisfactory recoveries. The proposed Sil-IL-SPE could be potentially applied to separation, purification and determination of  $\beta$ -sitosterol from complicated natural products.

**Keywords:** ionic liquid, silica,  $\beta$ -sitosterol, SPE, RSM

### 1. Introduction

In recent years, phytosterols have become a research focus because they can reduce cholesterol levels in humans [1], also they possess anti-cardiovascular [2], anti-inflammatory [3], anti-cancer activities [4] and cardio-protective [5] activities. As one of the most abundant phytosterols in nature,  $\beta$ -sitosterol has several positive effects i.e., lowering blood fat, anti-inflammation and anti-cancer function and moreover [6], pharmacological activity of regulating the balance of bone metabolism, protecting the gastric mucosa and inhibiting the activity of harmful bacteria [7].

Cui *et al.* prepared a graphene oxide-based composite monolith column to on-line enrich  $\beta$ -sitosterol with the recovery in the range of 80.40–98.00% [8]. Alam *et al.* used HPTLC method to analysis  $\beta$ -sitosterol from three Hibiscus species and 11.85mg/g was obtained [9]. Delgado-Zamarreño *et al.* used QuEChERS-liquid chromatography to detect  $\beta$ -sitosterol in seeds and nuts, and the contents between 0.49mg/g and 4.27mg/g were founded [10].

In order to separation of  $\beta$ -sitosterol from natural plants, adsorption is one of the most suitable method. The sorbent in this method plays a decisive role. There are lots of sorbent can be used and ionic liquid-based sorbent was paid a great attention. Ionic liquids (ILs) are known as green reaction medias with excellent chemical properties. ILs are receiving a lot of attention in the fields of analytical chemistry, sample preparation, organic synthesis, extraction and separations [11, 12]. Hydrophobicity, miscibility with several inorganic/organic solvents,  $\pi$ - $\pi$  interactions between functional groups are the typical characteristics of ILs which were widely applied in solvent or sorbents modifications [13, 14]. Some studies related to preparation of IL modified silica and extraction of benzene series compounds by IL modified materials were already published [15, 16].

In order to investigate interaction and adsorption mechanism between IL groups and  $\beta$ -sitosterol, in this study three ILs with different polarities were modified on silica surface.

Adsorption capacities of all sorbents were evaluated by the assistance of response surface methodology (RSM) and solid-phase extraction (SPE) under different conditions such as concentration, adsorption time, ratio of solution.

### 2. Materials and methods

#### 2.1 Materials and instruments

Silica (40  $\mu$ m), (3-chloropropyl) trimethoxysilan, ethylimidazole, butylimidazole, hexylimidazole, and  $\beta$ -sitosterol were purchased from Aladdin Inc. (Shanghai, China) and all grades of purity were higher than 98.0%. Organic solvents such as acetone, methanol, toluene *et al.* were supported by Beilian Company (Tianjing, China) and purities were higher than 99.0 %. Ultrapure water was produced by a purification machine (UPH-I-5, Youpu, China) and all organic solvents should be filtered before use. The chromatographic conditions were: HPLC (LC3000, CXTH, Beijing, China) with a commercial Wondasil C<sub>18</sub> column (4.6×250 mm, 5  $\mu$ m, SHIMADZU, Japan), and methanol/water (80:20, v/v) was used as mobile phase. The flow rate, UV wavelength, and injection volume were 0.8 mL/min, 250 nm and 5.0  $\mu$ L, respectively.

#### 2.2 Preparation of IL-based silicas

Silica was activated by dipping in HCl aqueous solution for 24 hr and washed by water and acetone. Then other silica was synthesized by following steps shown in Fig. 1. Firstly, chlorine was modified on silica surface by adding (3-chloropropyl)trimethoxysilane as the coupling compounds. 50.0 g of activated silica, 65.0 mL of (3-chloropropyl) trimethoxysilane and 200.0 mL of toluene were well mixed in a flask and heated to 100°C for 12 hr. When the powder was washed by ethanol and fully dried, 3-chloropropyl based silica (Sil-Cl) was obtained. Then 5.0 g of Sil-Cl and 50.0 mL of toluene were prepared in each five flasks, 5.0 g of ethylimidazole, butylimidazole and hexylimidazole were added into the flasks respectively. After all flasks were heated

and refluxed for 12 hr, different IL group based silicas were obtained and recorded as Sil-EIm, Sil-BIm and Sil-HIm.

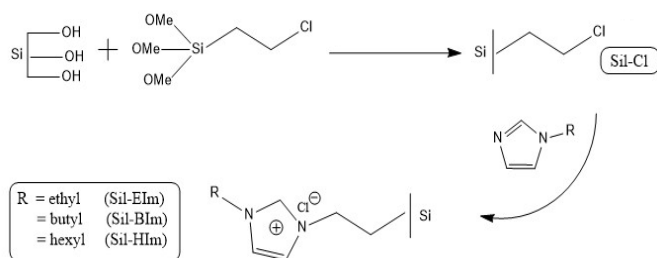


Fig 1: Synthesis process of ionic liquid-based silicas.

### 2.3 Experimental design

According to the CCD method from RSM using Design-Expert software (v.8.0.6, Stat-Ease, Inc, Minneapolis, USA), three major factors such as methanol/water ratio, adsorption time and concentration, which can significantly affect the maximum adsorption amount, were selected. Three factors of the CCD were designated X1, X2, and X3 and divided into three levels in Table 1. The codes -1, 0, and +1 were assigned as low, intermediate, and high values, respectively.

Table 1: Levels of independent variables used in CCD

Variables	Level		
	-1	0	+1
Methanol/water (v:v) ( $X_1$ )	20	60	100
Adsorption time (min) ( $X_2$ )	5	17.5	30
Concentration (mg/mL) ( $X_3$ )	0.005	0.05	0.1

### 2.4 Procedure of SPE

0.1 g of Sil-IL were packed into empty SPE cartridge (60 mm×10 mm) which was capped at both ends with polyethylene frits to prevent particles from leaking. The Sil-IL-SPE cartridge was conditioned with 2.0 mL methanol. Then a small volume of sample solution was loaded on the cartridge and 2.0 mL of water, acetone, acetonitrile and methanol were used as washing solvents for 5 times. The elution was carried out with 2.0 mL methanol-acetic acid (9:1, v/v) mixture. The solvents collected from the washing and elution steps were analyzed by HPLC.

## 3. Results & Discussion

### 3.1 Characterization

The characteristics of all obtained IL based silicas were analyzed by FT-IR (Nicolet 6700, Thermo Fisher, Waltham, USA) in the range of 400-4000  $\text{cm}^{-1}$  with a scan rate of 20 scans/min. In Fig. 2, silica exhibits a conspicuous peak in the range of 3200-3250  $\text{cm}^{-1}$  and it belongs to the finger print region of the -OH group. In modified silica, -OH peak is obviously decreased. Sil-Cl exhibits a conspicuous peak at 699.3  $\text{cm}^{-1}$  and the finger print region of the C-Cl group ranges from 690 to 704  $\text{cm}^{-1}$ . In ionic liquid-based silica, C-Cl peak disappeared and a new peak emerged at 1565.9  $\text{cm}^{-1}$ . The characteristic region of the amide bands ranged from 1500 to 1600  $\text{cm}^{-1}$ , indicating the replacement of C-Cl groups by imidazole groups. The above results showed that IL-based silicas were successfully created.

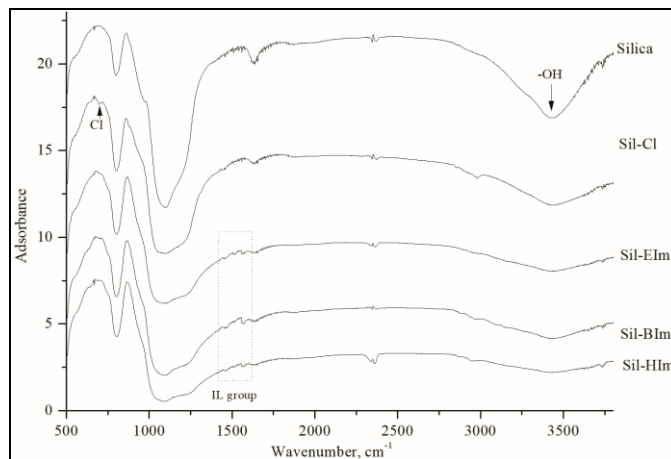


Fig 2: FT-IR of silica, Sil-Cl and three Sil-ILs.

### 3.2 Optimization RSM and adsorption

Three variable conditions were optimized according to experimental design. As the result, the model proposed for the response of the adsorbed amount was determined as Eq. (1)

$$Y = 0.16 - 0.19X_1 - 0.053X_2 + 0.29X_3 + 0.11X_1X_2 - 0.24X_1X_3 + 0.011X_2X_3 - 0.049X_1^2 + 0.03X_2^2 + 0.15X_3^2 \quad (1)$$

It was found that the model was statistically significant, and the values of C.V. and Adeq. Precision for above regression equation were 51.14 and 12.53. The Model F-value of 11.82 and Prob > F less than 0.050 implies the model is significant. And the optimized conditions were: 0.1mg/mL of  $\beta$ -sitosterol in methanol:water (60:40, vol%) with 10 min adsorption.

Then under the optimized conditions, 0.1 g of each three IL-based silicas were mixed with 5 mL of 0.1mg/mL of  $\beta$ -sitosterol solution. After 10 min, the adsorption amounts on Sil-EIm, Sil-BIm and Sil-HIm were 0.53 mg/g, 0.76 mg/g and 0.83mg/g, respectively. Hence, Sil-HIm was selected as the best sorbent for further experiments.

### 3.3 Optimization of the Sil-IL-SPE Procedures

To evaluate the applicability of the Sil-HIm-SPE for separation and purification efficiency, the general loading, washing and elution procedures of SPE were optimized. Firstly, 1 mL of 0.1mg/mL of  $\beta$ -sitosterol solution was loaded into SPE. Then the Sil-HIm in cartridge was washed by different solvent (Table 2). Washing can reduce the amount of matrix interference during analyte separation. Water can remove hydrophilic interferences, acetone and acetonitrile removed organic interferences however acetone can remove more. For these three solvent, none of them could wash the  $\beta$ -sitosterol out. Methanol also can remove higher amount of interferences than acetonitrile, but a little amount of  $\beta$ -sitosterol was washed out. Therefore, water and acetone were considered optimal for washing.

The elution solvent should eliminate any interactions of the target compound that would prevent its elution. Ionic interactions can be eliminated using an aqueous acid. In this case, methanol-1% vol acetic acid with strong elution ability was considered. Within 3 times, all loaded  $\beta$ -sitosterol was eluted out.

**Table 2:** Eluted amount of  $\beta$ -sitosterol from SPE by different solvents.

Times	Amount of $\beta$ -sitosterol (mg)				
	Water	Acetone	Acetonitrile	Methanol	Methanol-1% vol acetic acid
1	0	0		0.0	0.04
2	0	0		0.0	0.05
3	0	0		0.0006	0.01
4	0	0		0.0006	0.0
5	0	0		0.0008	0.0
Total	0	0	0	0.002	0.1
Interferences content	19.5%	52.8%	7.9%	13.4%	0.3%

### 3.4 Application of Sil-IL-SPE to real samples

In order to validate the Sil-IL-SPE method, calibration curve was constructed using the areas of the chromatographic peaks measured at nine increasing spiking levels in the range of 0.0001-0.1 mg/g. Good linearity was obtained with correlation coefficients of  $R^2 > 0.9994$ .

To evaluate the performance of the proposed method, peanut and corn purchased from local market were extracted by methanol:water (60:40, vol%) and analyzed under the optimum SPE conditions. In Table 3, 0.0008 mg/g and 0.006 mg/g of  $\beta$ -sitosterol were founded in peanut and corn, respectively. Satisfactory recoveries were obtained with a low RSD.

**Table 3:**  $\beta$ -sitosterol amount in peanut and corn with RSD and recovery.

Sample	$\beta$ -sitosterol amount	RSD, %	Recovery, %
Peanut	0.0008 mg/g	8.6	87.5
Corn	0.006 mg/g	4.1	91.6

### 4. Conclusions

Three ionic liquid-based silicas were successfully synthesized, and the adsorption conditions were optimized by RSM. Initial concentration, methanol/water ratio and time were considered, and 0.1mg/mL of  $\beta$ -sitosterol in methanol:water (60:40, vol%) with 10 min were optimized. With the highest adsorption amount, Sil-HIm was selected and was applied as the sorbent in SPE. 10.0 mL of Water and acetone were used as washing solvents and 6.0 mL of methanol-1% vol acetic acid as elution solvent. With the SPE conditions, 0.0008 mg/g and 0.006mg/g of  $\beta$ -sitosterol were detected from peanut and corn with satisfactory recoveries.

### 5. Acknowledgments

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