Preliminary extration of tannins by 1-butyl-3-methylimidazole bromide and its subsequent removal from *Galla chinensis* extract using macroporous resins

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Abstract

In recent years, Ionic Liquids have become increasingly attractive as “green solvents” used in the extraction of bioactive compounds from natural plant. However, the separation of ionic liquid from the target compounds was difficult, due to their low vapour pressure and high stabilities. In our study, ionic liquid-based ultrasonic and microwave-assisted extraction was used to obtain the crude tannins, then the macroporous resin adsorption technology was further employed to purify the tannins and remove the ionic liquid from crude extract. The results showed that XDA-6 had higher separation efficiency than other tested resins, and the equilibrium experimental data were well-fitted to Langmuir isotherms. Dynamic adsorption and desorption were performed on XDA-6 packed in glass columns to optimize the separation process. The optimum conditions as follows: the ratio of column height to diameter bed was 1:8, flow rate 1BV/h, 85% ethanol was used as eluant while the elution volume was 2BV. Under the optimized conditions, the adsorption and desoption rate of tannins in XDA-6 were 94.81% and 91.63% respectively. The content of tannins was increased from 70.24% in *Galla chinensis* extract to 85.12% with a recovery of 99.06%. The result of UPLC-MS/MS analysis showed that [bmim]Br could be removed from extract.

Keywords: Ionic liquid; Macroporous resins; *Galla chinensis*; Tannins

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1 Introduction

Galla chinensis is a traditional Chinese herb and has been commonly used as a medical plant in China for a long time. The gallotannins, one type of hydrolysable tannins, are the dominant components and account for 60%-77% in *G. chinensis* [1], having many different biological and pharmacological properties such as antimicrobial [2-3], antioxidant [2], anticancer [4-5], and anticariogenic activities [6]. Due to their biological effects, there has been growing studies focusing on the extraction of tannin from a *Galla chinensis* [7-9].

In previous study, ionic liquid-based ultrasonic and microwave-assisted extraction (IL-UMAE) was found as a simple, rapid, and effective preparation technique for extraction of tannins [10]. Moreover, there has been a growing interest in using ionic liquid as environment friendly solvents in the extraction of bioactive compounds from plant sources for their unique solvent properties [11-13]. However, The residue of ionic liquids in extracts may lead to extracts pollution. Therefore, it was indispensable for removing ionic liquid from the extracts. Due to their low vapour pressure, the removal of ionic liquid could not be realized by conventional vacuum concentration.

Macroporous resin is a durable polymer with high adsorption capacity and a wide range of polarity [14]. It can selectively adsorb the targeted constituents from aqueous and non-aqueous system by electrostatic force, hydrogen bonding interaction, complexation and size sieving action [15]. So far, macroporous resins have been widely used in the separation and enrichment of bioactive compounds from many

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plant extracts [16-20]. However, to the best of our knowledge, there are no reports about isolation and purification of tannins and removal of ionic liquid from *G. chinensis* extracts by macroporous resins. The aim of the present study, therefore, was to establish a simple and efficient method for the simultaneous purification of tannins and removal of ionic liquid from *G. chinensis* extracts by macroporous resin.

2 Materials and methods

2.1 Reagents and materials

The *Galla chinensis* was obtained from China Beijing Tongrentang Group Co., Ltd (Beijing, China). Gallic acid (with purity > 98%) was obtained from Kemiou Chemical Reagent Company (Shanghai, China). 1-butyl-3-methylimidazolium bromide ([bmim]Br, 99.5%) was purchased from Center for green chemistry and catalysis, LICP, CAC. UPLC grade methanol was purchased from Sigma-Aldrich (St.Louis, MO,USA). All the other reagents were of analytical grade.

Macroporous resins including HPD-100, AB-8, XDA-6, ADS-7, ADS-17, and LX-38 were provided by Chemical plant of Nankai University (Tianjin, China), Cangzhou Bon Co., Ltd (Hebei, China), and Sunresin Technology Co., Ltd (Xian, China). The characteristics of these resins are reported in Table 1. Prior to use, the resins were pretreated by soaking in 95% ethanol for 24 h sequentially to remove the monomers and porogenic agents trapped inside the pores during the synthesis process. Then they were washed with deionized water until the ethanol was thoroughly replaced by deionized water.

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2.2 Preparation of sample solutions

Dried *Galla chinensis* was ground into powder and passed through a sieve (60-mesh). *Galla chinensis* powder was extracted using the ionic liquid-based ultrasonic/microwave-assisted extraction (UMAE) technique as described by Lu et al [10] (2011). The extracts were combined, filtered, and then concentrated by a rotary evaporator under vacuum. The content of tannins in extracts was 70.24%. Deionized water was added to get tannins solutions of different concentrations.

2.3 Quantitative tannins determinations

The content of total phenolic was determined by the Folin–Ciocalteu method [21], The results were compared to a gallic acid calibration curve, and the total phenolics were expressed as gallic acid equivalents (GAE). The control used distilled, determination of each sample was performed in triplicate.

The tannins content was measured according to the method of Wang and Lu [22] (2004). Briefly, weighing 0.6 g casein into 25 mL of the sample solution, and the sample solution was then put in the shaking water bath at 30°C to precipitate tannins completely. After 1 h, the sample solution was filtered and the 2 mL filtrate was added to a 25 mL Volumetric flask, then 1 mL tungsten molybdophosphate solution and 10 mL distilled water were added. Finaly, adding 12 mL 29% sodium carbonate solution to Volumetric flask, and the mixture was kept at room temperature for 30 min. Then, the absorbance of mixture was measured at 760 nm. Measure the content of non-tannin phenols of the filtrate as mentioned above, the results were compared to a gallic acid calibration curve. The tannin content was calculated by the following formula:

Tannin content = total polyphenol content－non-tannin polyphenol content

2.4 Identification of tannins and ionic liquids in extracts

Analysis was done on a Waters Acquity UPLC^{TM} system coupled with a SYNAPT Mass spectrometer (Waters, USA). The Waters Acquity UPLC BEH C_{18} column (100mm×2.1mm, 1.7_m) was used for UPLC separation. The column oven temperature was 45℃. The eluents were: (A) 0.1% formic acid; (B) acetonitrile/methanol (20 : 80, v/v). The gradient program was as follows: 10-30% B (15 min) and 30-100% B (18 min) at a constant flow of 0.3 mL/min. The peaks of the tannins were monitored at 280 nm. The MS data were acquired with electrospray ionization in negative mode. The operating parameters were as follows: MS temperature, 100°C; capillary voltage, 3.0 kV and cone voltage, 20 V. Nitrogen was used as the desolvation gas at 500 L/h and 250°C. Spectra were scanned over a mass range of m/z 100–2000.

2.5 Static adsorption and desorption

In order to choose the most suitable macroporous resin to separate tannins from the crude extract, static adsorption and desorption tests were performed as follows: pre-weighed amounts of different hydrated adsorbent (equal to 5 g dry resin) and 20 mL of sample solution were added to an Erlenmeyer flask. The flasks were shaken in a shaker (120 rpm) for 1 h at 28°C to reach adsorption equilibrium. The solutions after adsorption were analyzed by section 2.3. Subsequently, the resin was washed three

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times with deionized water and then desorbed with 20 mL 95% (v/v) ethanol. The contents of tannins in desorption solution were monitored by section 2.3. The adsorption capacity, adsorption and desorption ratio for tannins were calculated on each resin by the following equation:

$$q_e = \frac{(C_0 - C_e)Vi}{W} \quad (1)$$

$$E(\%) = \frac{(C_0 - C_e)}{C_0} \times 100\% \quad (2)$$

$$D(\%) = \frac{C_d V_d}{(C_0 - C_e)V_i} \times 100\% \quad (3)$$

where q_e represented the adsorption capacity (mg/g) at adsorption equilibrium; C_0 and C_e are the initial and equilibrium concentrations of solute in the solution, respectively (mg/mL); V_i is the volume of the sample solution (mL); W is the weight of dry resin (g); C_d is the concentration of solute in the desorption solution (mg/mL); V_d is the volume of the desorption solution (mL); D is the desorption ratio (%); and E is the adsorption ratio (%).

2.6 Static adsorption isotherms

The adsorption isotherm on the selected macroporous resin was performed by adding a certain amount of hydrated resin (equal to 5 g dry resin) and of sample solutions (20 mL) at different initial concentrations (2.96, 5.93, 11.85, 18.96, and 23.70 mg/mL) into each flask. The sample solution of each concentration in the flask were shaken on an incubation shaker (120 rpm) for 1 h at three different temperatures (28°C, 33°C and 38°C), respectively. The equilibrium concentration (C_e) of tannin was determined by section 2.3, and the adsorption capacity (q_e) of resins was calculated by equation

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(1). The static adsorption isotherms was made by C_e as independent variable and q_e as dependent variable, and their degrees of fitness to Freundlich and Langmuir theoretical equations were evaluated. Langmuir isotherm is the simplest theoretical model used to describe monolayer adsorption [23]. It is expressed by the following formula:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{Kq_m} \quad (4)$$

where K is the adsorption equilibrium constant; q_m is the empirical constant, represent the theoretically calculated maximum adsorption capacity of the resin; C_e and q_e represent the same parameters as in formulae (1).

The Freundlich isotherm is an empirical equation for nonideal adsorptions [24]. It can be used to describe the adsorption behavior of monomolecular layer as well as that of the multimolecular layer. It is expressed by the formula below:

$$q_e = KC_e^{1/n} \quad (5)$$

where K is the Freundlich constant that is an indicator of adsorption capacity; and $1/n$ is an empirical constant, which is correlated with the adsorption intensity of the resin; C_e and q_e represent the same parameters as in formulae (1).

2.7 Dynamic adsorption and desorption

Dynamic adsorption and desorption experiments were carried out on glass columns (2.0×50 cm) wet-packed with the pretreated hydrated selected resin (equal to 26.31 g dry resin). The bed volume (BV) of the resin was 50 mL. The sample solution with a tannins concentration of 23.70 mg/mL was pumped through glass column at a

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prescribed flow rate at 28°C. Following adsorption, the adsorbate-laden columns were washed first by deionized water (2BV) at a flow rate of 1 BV/h, finally, tannins were eluted by different concentrations of ethanol solution at a flow rate of 1 BV/h. In experiment process, the effects of the ratio of column diameter to height, feeding flow rate, concentrations and volume of elution on the capability of adsorption and desorption were studied. The concentrations of tannins in effluent liquid and desorption solution were determined by section 2.3. Elution curves on the resins were obtained based on the concentrations of aqueous ethanol as elution solution and contents of tannins therein. Dynamic adsorption and desorption tests were repeated three times under optimal conditions.

3 Results and discussion

3.1 The choice of the resins

Our study shows that the adsorption and desorption capacities of different macroporous resins for tannins were distinct. As shown in Fig. 1, it could be clearly seen that the adsorption and desorption ratios of tannins on XDA-6 resin were considerably higher than those of other adsorbing resins. The reason was not only because of its similar polarity with the compounds tannins, but also because of its larger surface area. Therefore, XDA-6 resin with the high adsorptive effect was selected to do the following experiments.

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3.2 Adsorption isotherms

Equilibrium adsorption isotherms on XDA-6 resin at three different temperatures were obtained for tannins. As shown in Fig. 2, the adsorption capacity of tannins on XDA-6 resin increased with the increase of initial concentration and reached the saturation plateau when the initial concentration of tannins was 23.70 mg/mL. Thus, the concentration of tannins in the feed solution was selected at 23.70 mg/mL. In addition, at the same initial concentration, the adsorption capacity of tannins decreased with the increase of temperature from 28°C to 38°C. This indicated the adsorption was a thermopositive process. Therefore, 28°C was selected as adsorption temperature.

The Langmuir and Freundlich parameters at different temperatures were summarized in Table 2. As can be seen from Table 2, the correlation coefficients (R^2) of Langmuir equations for tannins were higher than that of Freundlich equation, which indicated that Langmuir equation can describe adsorption behavior better than Freundlich equation in the studied concentration range. The theoretical maximum adsorption capacity (q_m) increased with the decrease of temperature for tannins, and the maximum value accounted from the Langmuir equation was 99.01 mg/g when the temperature was 28°C.

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3.3 Dynamic adsorption and desorption

3.3.1 Effect of the ratio of column diameter to height on adsorption

The effect of bed height on dynamic adsorption capacity was conducted. The results shown that adsorption capacity of tannins increased with the increase of bed height at the same test sample conditions, when the ratio of column diameter to height was increased to 1:8, adsorption capacity showed a significant increase (data not shown). Furthermore, the dynamic adsorption capacity was almost constant regardless of increase of the ratio of column diameter to height. Moreover, too higher ratio indicated insufficient use of the bed, as well as the insufficient desorption leading to lower recovery of the products [25]. Thus, the ratio of column diameter to height was selected as 1: 8.

3.3.2 Dynamic leakage curve on XDA-6 resin

In order to calculate the quantity of resin, the feed volume of sample solution and the proper sample flow rate, dynamic leakage curve for tannins on XDA-6 resin was constructed and shown in Fig. 3. Breakthrough point was defined as 1% ratio of the exit to the inlet solute concentration [26]. As shown in Fig. 3, breakthrough volume of tannins on XDA-6 resin were 2.5, 2.0, 1.5 BV at flow rates of 1.0, 2.0, 3.0 BV/h, respectively. Therefore, the loading volume of 2.0 BV and the flow rate of 1.0 BV/h were selected in further experiments.

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3.3.3 Effect of concentration of ethanol solution on desorption

Different concentrations (75%, 85% and 95%) of ethanol solutions were used to perform desorption tests in order to choose a proper desorption solution. Initially, with the increase of ethanol concentration, the desorption ratios of tannins increased accordingly and reached their peak value at the concentration of 85%, and then decreased with the increase of ethanol concentration (data not shown). The reason of this phenomenon perhaps can be explained as follows: when the active ingredient was eluted from resin by high concentration ethanol (95%), the partial impurities would be eluted, which decrease the content of the tannins in *Galla chinensis* extracts. Therefore, 85% ethanol was selected as the appropriate desorption solution for tannins and was used in the following tests.

3.3.4 Dynamic desorption curve on XDA-6 resin

The dynamic desorption curve on XAD-6 resin was obtained based on the volume of desorption solution and the tannins concentration in the desorption solution. It was shown that about 100 mL of the desorption solution could completely desorbed tannins from XDA-6 resin when the flow rate was set at 1.0 BV/h (data not shown). Therefore, the eluting volume of 2.0 BV was selected as the proper desorption volume in consideration of the efficiency.

Our results revealed that the optimum parameters for the enrichment and separation of tannins with XDA-6 resin were confirmed as follows, for adsorption: tannins concentration in sample solution 23.70 mg/mL, adsorption temperature 28℃,

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the ratio of column diameter to height 1:8, feed volume 2.0 BV, flow rate 1.0 BV/h; for desorption: elution solvent aqueous-ethanol (15:85, v/v), volume 2.0 BV, flow rate1.0 BV/h.

3.3.5 Validation of the separation process of tannins

The dynamic adsorption and desorption tests were repeated for three times under optimal conditions. The desorption solution was analyzed by section 2.3 and UPLC-MS/MS, and the RSD for recovery yield was calculated for tannins. As shown in Table 3, after separation on XDA-6 resin by 85% elution, the content of tannins was increased from 70.24% in crude extract to 85.12% with a recovery of 99.06%. The chromatograms of the test samples before and after separation with XDA-6 resin are shown in Fig. 4. By comparison, It can be seen that the [bmim]Br has been removed form the crude extract of *Galla chinensis*, and the content of [bmim]Br in crude extract was decreased from 2.5 mol/L to 0 mol/L.

4 Conclusions

In this study, the preparative purification process of tannins and removal of ionic liquid from extract with macroporous resin has been successfully developed. Among six resins tested, XDA-6 resin offers the best separation efficiency for tannins and its adsorption data fit best to the Langmuir isotherm. Several parameters in the separation process, such as the concentration and volume of the feeding sample, the ratio of column diameter to height, flow rate, the concentration and volume of the eluent,

were optimized for most effective enrichment and preparative separation. Under optimal conditions, the tannins content in the final product was increased from 70.24% to 85.12% with a recovery of 99.06%. Simultaneously, the [bmim]Br in the crude extract was removed. In conclusion, this study provided an effective method for enrichment of tannins and removal of ionic liquid from *Galla chinensis* extracts.

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Table

Table 1 Physical properties of different resins

Resin series	Surface area (m^2/g)	Particle diameter (mm)	Pore diameter (Å)	Moisture content (%)	Polarity
HPD-100	650-700	0.3-1.25	85-90	65-75	Nonpolar
AB-8	480-520	0.3-1.25	130-140	33.11	Weak polar
XDA-6	450-500	0.3-1.25	120-160	40.12	Moderate polar
ADS-17	250-300	0.3-1.25	90-150	38.57	Moderate polar
ADS-7	100	0.3-1.25	25-30	50-60	Polar
LX-38	500-600	0.3-1.25	60-90	50-60	Polar

Table 2 Langmuir and Freundlich parameters of tannins adsorption on XDA-6 resin

Compound	Temperatue (°C)	Langmuir equation	R^2	q_m(mg/g)	Freundlich equation	R^2	1/n
Tannins	28	C_e/q_e=0.0101 C_e+0.0085	0.9978	99.01	q_e=47.75+ $C_e^{0.5857}$	0.9852	0.5857
	33	C_e/q_e=0.0115 C_e+0.0109	0.9943	86.96	q_e=38.25+ $C_e^{0.6647}$	0.9874	0.6647
	38	C_e/q_e=0.0131 C_e+0.0152	0.9993	76.34	q_e=30.17+ $C_e^{0.6158}$	0.9905	0.6158

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Table 3 Purification results of tannins on XDA-6 resin by optimized conditions (*n*=3)

Compound	Adsorption ration (%)	Desorption ration (%)	Initial content in crude extracts (%)	Content in final product (%)	Recovery yield (%)
Tannins	94.81	91.63	70.24	85.12	99.06
RSD(%)	1.59	1.21	2.35	1.73	2.17

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Fig. 1 Adsorption capacity, adsorption and desorption ration of tannins on different resins.

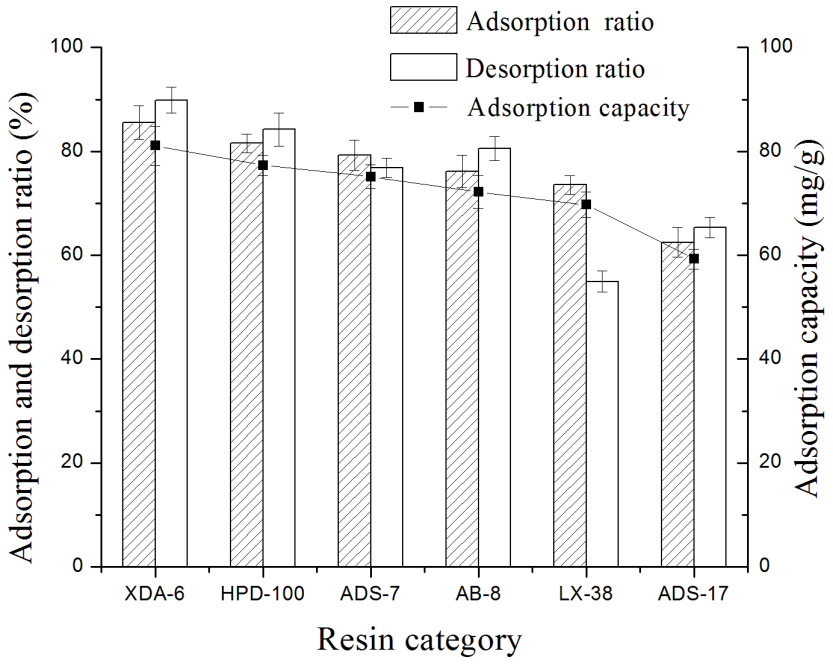

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Fig. 2 Adsorption isotherms of tannins on XDA-6 resin at different temperatures.

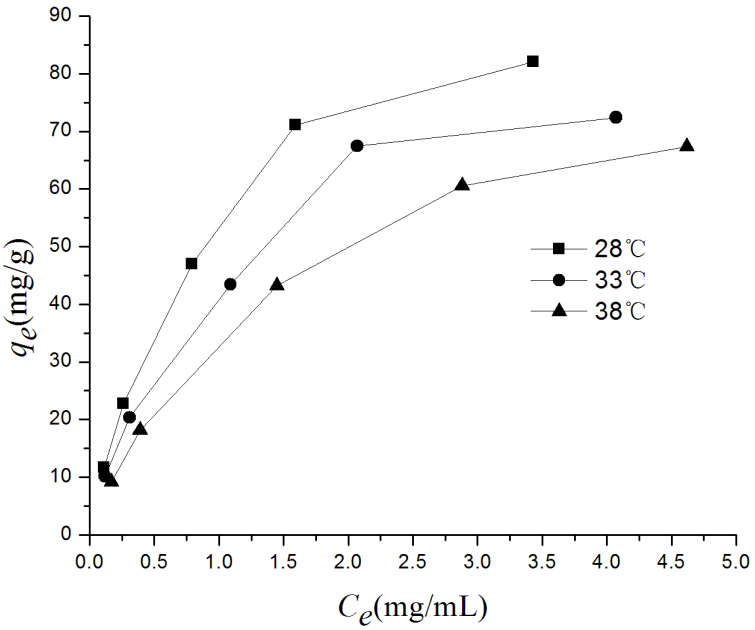

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Fig. 3 Dynamic leakage curve of tannins on XDA-6 resin at different flow rates. q_e is the adsorption capacity at adsorption equilibrium (mg/g); C_e is the equilibrium concentrations of solute in the solution (mg/mL); BV is bed volume.

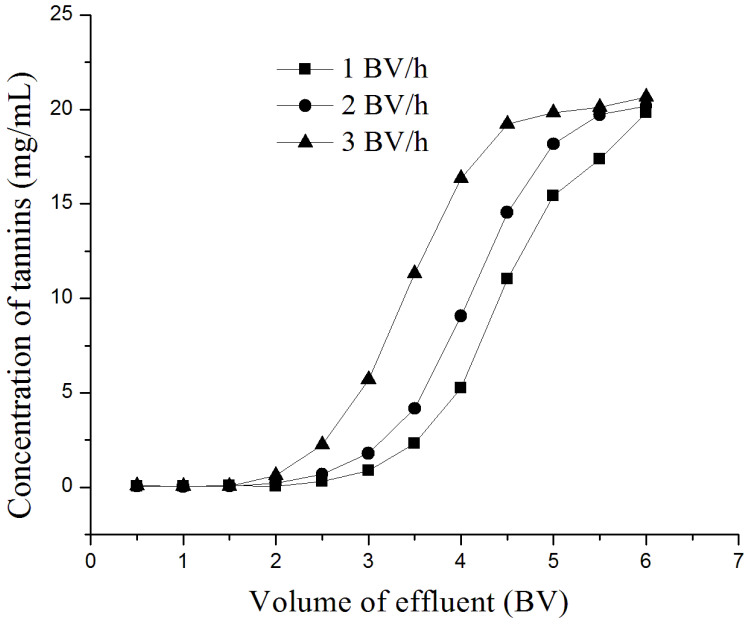

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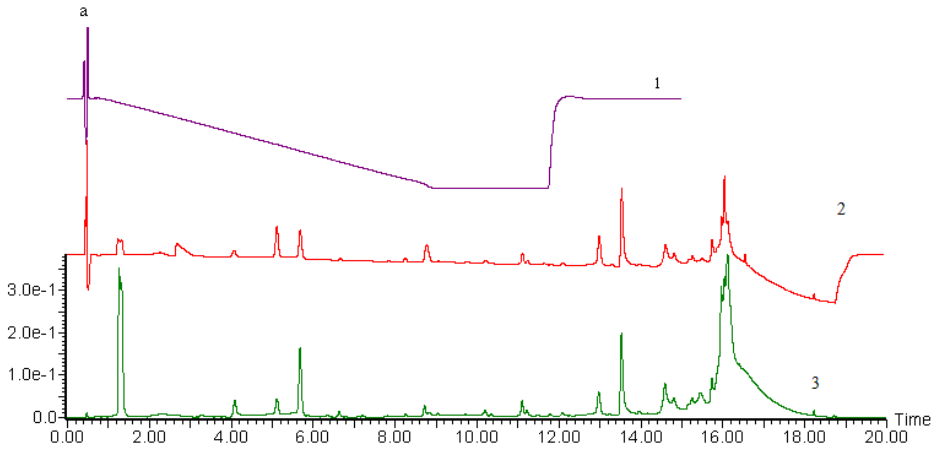
Fig. 4 Chromatograms of the [bmim]Br (1), crude feed (2) and products (3) after adsorption and desorption process with XDA-6 resin at the detection wavelength of 280 nm. a. [bmim]Br.



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