**Scholars Academic Journal of Biosciences (SAJB)**
***Abbreviated Key Title: Sch. Acad. J. Biosci.***
©Scholars Academic and Scientific Publisher
A Unit of Scholars Academic and Scientific Society, India
www.saspublisher.com

**ISSN 2347-9515 (Print)**
**ISSN 2321-6883 (Online)**

Chemistry

# Amino acid inoic liquid functionalized magnetic graphene oxide nanocomposite coupled with high performance liquid chromatography for the separation /analysis rutin

**Siyi Chen Xiashi Zhu***
College of Chemistry & Chemical Engineering, Yangzhou University, Yangzhou, PR China

**Original Research Article**

***Corresponding author**
*Siyi Chen Xiashi Zhu*

**Article History**
*Received: 08.12.2017*
*Accepted: 08.01.2018*
*Published:30.01.2018*

**DOI:**
10.36347/sajb.2018.v06i01.017

**Abstract:** Amino acid inoic liquid functionalized graphene oxide-based magnetic nanomaterials ($Fe_3O_4@SiO_2@GO@AAIL$) as a new extractant combined with HPLC for the separation/analysis rutin was established. This new extractant were characterized using FTIR, SEM and VSM. A comprehensive study of the parameters affecting the extraction/elution rate were optimized. Under the optimum conditions, the linear range, detection limit, correlation coefficient ($R^2$) and relative standard deviation (RSD) were found to be 2.5-5×$10^3$ ng $mL^{-1}$, 0.02 ng $mL^{-1}$ , 0.9996 and 1.90% (n =11, c=1.0μg $mL^{-1}$ ), respectively and the erichment factor in the proposed experiment was approximately 7.5. This assay is satisfactory for the application of the actual sample of rutin.

**Keywords:** Magnetic solid phase extraction High performance liquid chromatography.

## Highlights

- A novel amino acid inoic liquid functionalized magnetic graphene oxide $Fe_3O_4@ SiO_2 @GO@AAIL$ was prepared.
- $Fe_3O_4@ SiO_2 @GO@AAIL$ was used as an adsorbent for the extraction of rutin
- The adsorbent was effective for extraction of the analytes from real samples.
- This method was successfully applied for the determination of rutin in lily, rutin tablets and mouse serum.

## INTRODUCTION

Rutin,3’, 4’, 5, 7-tetrahydroxyflavone-3-β-D-rutinoside, is such a kind of natural flavonoids, also known as vitamin P( Fig-1).It is widely found in buckwheat, citrus and other plants. Studies have shown that rutin has a wide range of physiological activities, such as anti-tumor, anti-bacterial, anti-tumor, anti-oxidation, and lower blood pressure, dilute the blood, reduce capillary permeability, is commonly used in clinical treatment[1].Therefore, it is important to study the determination of rutin in plants or pharmaceutical preparations.

At present, Rutin in plants is analyzed by capillary electrophoresis [2], high-performance liquid chromatography [3], ultraviolet spectrophotometry [4], electrochemical [5] and voltammetry [6]. HPLC has the advantages of high separation efficiency and fast analysis speed. However, the actual sample matrix is complex and low concentration. To improve the method of selectivity and sensitivity, HPLC is usually integrated with separation/enrichment technology.

Separation/enrichment techniques commonly used include liquid-liquid extraction [7] and solid-phase extraction [8].MSPE is a kind of extraction technique using magnetic or magnetizable material as a solid-phase substrate, which has the good points of short adsorption time, simple separation and less organic solvent consumption [9]. MSPE extractant is mainly $Fe_3O_4$ nanoparticles (Nps) modified by specific functional groups to achieve selectivity, affinity and extraction ability to target analytes [10]. Many functional materials have been used to modify $Fe_3O_4$ Nps , such as $SiO_2$ [11], β-cyclodextrin [12], ionic liquid [13], graphene oxide [14] etc. The graphene oxide(GO) has a high specific surface area, rich surface functional groups, chemical stability and lasting corrosion resistance [15].Magnetic nanoparticles modified by graphene oxide have been successfully used for analysis of drug [16, 17]. Ionic liquids have been loaded into magnetic graphene oxide nanomaterials($Fe_3O_4@GO@IL$) to improve extraction selectivity and efficiency [18-20]. Amino acid ionic liquids(AAILs) containing amino acid cations or anions is synthesized from natural amino acids, which has the characteristics of low toxicity, good biocompatibility and good biodegradability [21]. However, $Fe_3O_4$@$SiO_2$@GO@AAIL composites has seemly not been reported for the separation/analysis of rutin.

In this paper, $Fe_3O_4$@$SiO_2$@GO@AAIL nanoparticles were synthesized and used as magnetic solid phase adsorbents in combination with HPLC.The synthesized extracting agent possesses not only some advantages of magnetic but also good adsorption of amino acid ionic liquid. This method shows the characteristics of rapid adsorption and easy separation. The factors affecting the extraction and elution conditions were explored in this paper. The method has been satisfactorily applied to determine rutin in actual sample.

## EXPERIMENTAL

### Apparatus and reagents

All chemicals and reagents were at least of analytical grade unless otherwise stated. $FeCl_3$, $FeSO_4 \cdot 7H_2O$, PEG, tetraethyl orthosilicate (TEOS),Ammonia, anhydrous ethanol, tetraethyl orthosilicate, isopropanol, N-hydroxysuccinimide (NHS),1-ethyl-(3-dimethylaminopropyl) phthalimide acetate(EDC), N, N-dimethylformamide (DMF), Hydrazine hydrate 85%, 3-aminopropyltrieth- oxysilane (APTES), Alanine, methyl imidazole, bromo-n-hexane , glacial acetic acid, methanol (high performance liquid chromatography grade) (the above reagents are produced by Sinopharm Group Chemical Reagent Co., Ltd.),Graphene oxide(Suzhou permanent graphene technology co., ltd), Rat（Yangzhou University.

### Instruments

LC1220 HPLC (Agilent, America), Tensor 27 fourier transform infraredspectroscopy (FTIR, Bruker Company, Germany), DZF-6020 vacuum oven (Shanghai, China),KQ-50E Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co., Ltd.),Centrifuge TDL80-2B (Shanghai Anting Science Instrument Factory)

### Sample preparation

For lily, 5.00g of lily powder sample was accurately weighed and taken into the 50mL beaker. The sample was ultrasonic extracted for 5 h after adding 10.0 mL of methanol. Then the extraction sample was filtered with 0.45μm filter and diluted with methanol to 25.0mL.

For rutin tablet, 20 tablets of rutin were crushed, and then 1.0000 g of sample powder was weighed and placed in a 50 mL of beaker. After adding 10.0 mL of ethanol the sample solution was ultrasonic extracted for 4 h. Then the extraction solution was filtered with 0.45um filter and diluted with methanol to 25 mL.

For rat serum, Nine healthy adult rats were fed with dose of 10 mg $Kg^{-1}$, and divided into 3 groups. The plasma was taken at 0.5 hour. Plasma was centrifuged at 4000 r/ min for 5 min, and supernatant was used as sample. To remove serum protein, 0.5mL of supernatant was added 2.5mL methanol, then centrifuged at 3000r/min for 10min. The final supernatant was analysised.

### Preparation of $Fe_3O_4$@$SiO_2$@GO@AAIL

AAILs synthesis was seen from reference [22]. $Fe_3O_4$NPs were prepared by the conventional co-precipitation method [23, 24]. $Fe_3O_4$@$SiO_2$@GO was prepared according to the literature method [25].

According to the literature [26], the ultrasonic coating method was used to synthesize the $Fe_3O_4$@$SiO_2$@GO@AAIL composite. 0.1g of ready-prepared AAIL were dissolved in 10mL methanol, then 0.1g$Fe_3O_4$@$SiO_2$@GO material was added was added into aqueous mixtures for further reaction. Putting the whole reaction device into ultrasonic bath, and ultrasonication for 2h intermittently. $Fe_3O_4$@$SiO_2$@GO@AAIL composite was washed with methanol 3 times, then dried in vacuum.

### MSPE of rutin

In the MPSE procedure, $Fe_3O_4$@$SiO_2$@GO@ AAIL was applied to the extraction of rutin, as shown i n Fig-2, 14mg of $Fe_3O_4$@$SiO_2$@GO@AAIL nanopartic les0.5mL of rutin standard solution (20μg $mL^{-1}$) were a dded into the 10ml centrifuge tube. The mixture was sh aking at room temperature for 15 min. Afterwards, the magnetic adsorbent was separated from the suspension by an external magnet. 8.0mL HAc were used to eluted the isolated MNPs. 20.0μL of elution was injected into t he HPLC system for detection of the target analytes.

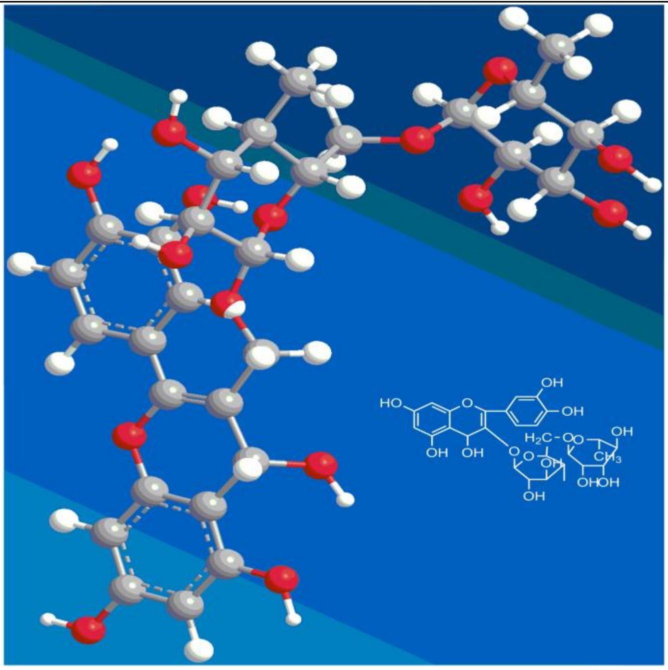

**Fig-1: Chemical structure of Rutin**

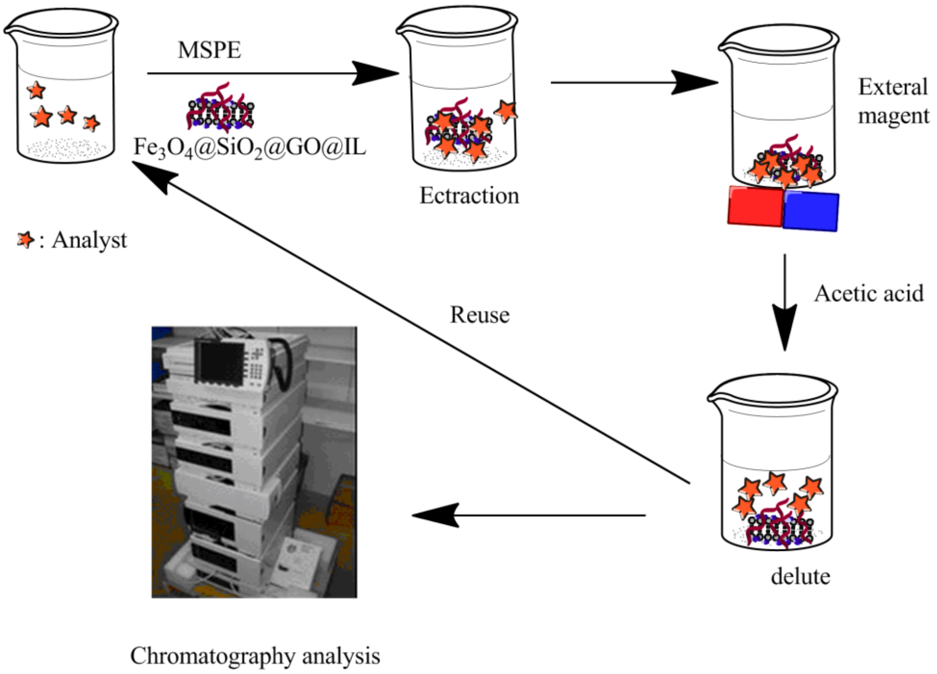

**Fig-2: The procedure for MSPE of the target rutin with $Fe_3O_4@SiO_2@GO@AAIL$**

### HPLC-UV analysis of actual samples

Three samples were assayed using LC1200 (HPLC, Agilent, USA). VP-ODS $C_{18}$Column (250mm×4.6mm) was used the analytical column. The mobile phase consisted of Methanol as solvent A and Phosphate buffer (0.1%) as solvent B, the ratio as follows 60:40(v/v). 0.8 mL $min^{-1}$ is used as flow rate. The UV detection wavelength was controlled at 254nm.

### Adsorption capacity

To study the adsorption capacity of $Fe_3O_4@SiO_2@GO@AAILs$ on rutin ($q_e$, mg $g^{-1}$), the maximum amount of rutin extracted by 1.0 g $Fe_3O_4@SiO_2@GO@AAIL$ can be calculated by the following formula [27]:

$$q_e = \frac{(C_o - C_e)\times V}{m}$$

Where $C_o$ represents the concentration of rutin before extraction, $C_e$ represents the concentration of rutin in the extraction equilibrium (μg $mL^{-1}$), and V is the volume of the solution (mL).

## RESULTS AND DISCUSSION

### Characterization of $Fe_3O_4$@$SiO_2$@GO@AAIL

The morphology of the as-prepared $Fe_3O_4$@$SiO_2$@GO@AAIL NPs was characterized by FT-IR, SEM and VSM.

### The characterization of FTIR

The FT-IR spectra of (curve a) $Fe_3O_4$@$SiO_2$@GO and (curve b)$Fe_3O_4$@$SiO_2$@GO@AAIL were shown in Fig-3. Fe-O bonds produce a peak at about 599 $cm^{-1}$.The peak at 1074 $cm^{-1}$ is generated by the Si-O bond stretching vibration. The characteristic peaks of GO are generated at 1073, 1599 and 1725 $cm^{-1}$ by C=O stretching vibration in C-O-C, C-C and -COOH, respectively.In the curve b, the C-N stretching vibration peaks at 1270$cm^{-1}$, 1725$cm^{-1}$ is significantly increased due to the C=O role of -COOH of amino acid ionic liquid. N-H stretching vibration peak was at 3109$cm^{-1}$. It is proved that $Fe_3O_4$ has been successfully modified by $SiO_2$, GO and AAIL.

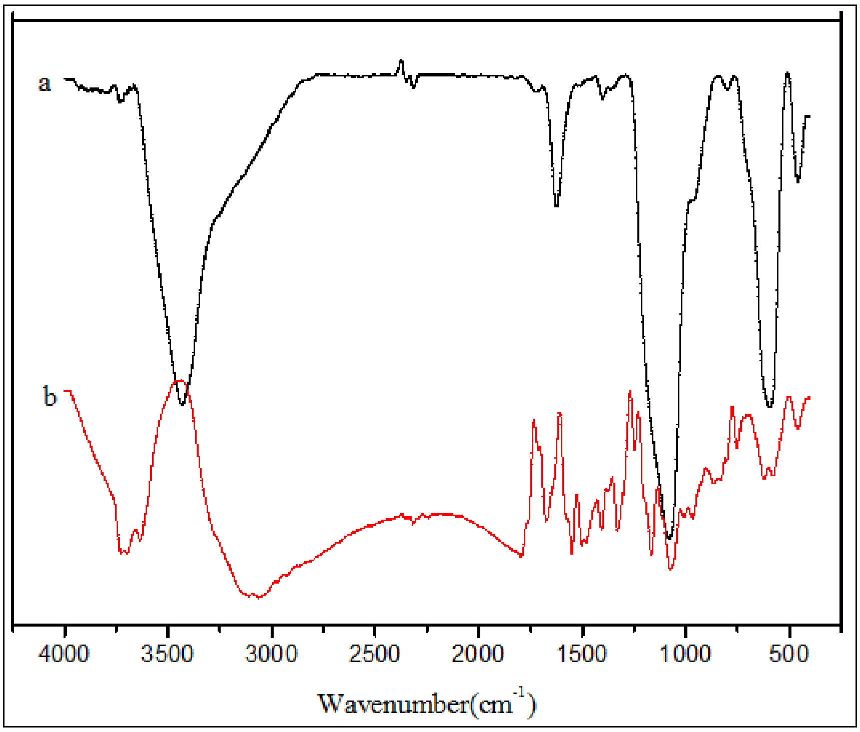

**Fig-3: Infrared spectrum: (a) $Fe_3O_4$@ $SiO_2$@GO (b) $Fe_3O_4$@$SiO_2$@GO@ AAIL**

The characterization of SEM and Magnetic properties analysis

### The characterization of SEM and Magnetic properties analysis are from reference [28]

### Optimization of adsorption conditions

### Selection of magnetic adsorbent

In this paper, four magnetic adsorbents were used to extract rutin, which were (a) $Fe_3O_4$, (b)$Fe_3O_4$@$SiO_2$, (c) $Fe_3O_4$@$SiO_2$@GO, (d)$Fe_3O_4$@$SiO_2$@GO@AAIL, as shown in Fig-6. It can be seen from the Fig-4 that the extraction rate of the $Fe_3O_4$@$SiO_2$@GO@AAIL is significantly higher than that of the other materials under the same pH conditions. So $Fe_3O_4$@$SiO_2$@GO@AAIL is selected as the extraction material.

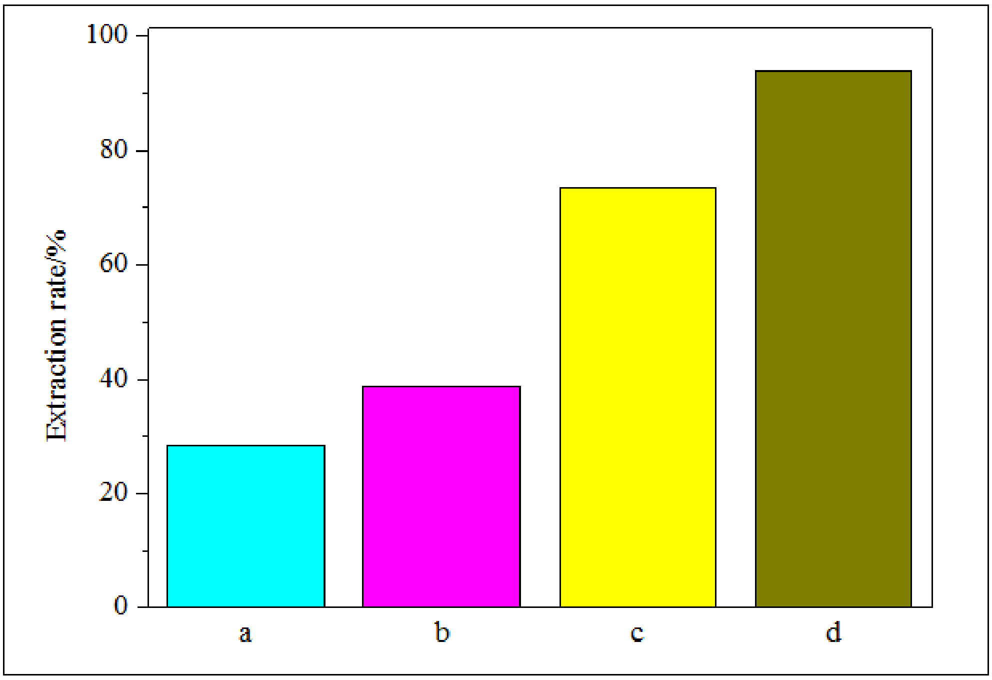

**Fig-4: Selection of extraction materials: (a)$Fe_3O_4$ (b)$Fe_3O_4$@$SiO_2$ (c)$Fe_3O_4$@$SiO_2$@GO (d) $Fe_3O_4$@$SiO_2$@GO@AAIL**

**The effect of pH**

Different pH (pH = 3.5~12.0) had showed an vital effect in the extraction of target analytes. As shown in Fig-5, the extraction rate of rutin was gradually increased when the pH value of the solution is 3.5~6.0, remained at around 94.0% in the range of pH 6.0~7.0 and reduced when the pH value exceeds 7.0. The reason may be that rutin is a polyphenolic compound, the neutral molecular structure is always maintained at pH 6.0~7.0, and the form of ions in solution at pH>7.0 [29]. So rutin in neutral form is more easily extracted. Therefore, the best solution for the sample pH is 6.0.

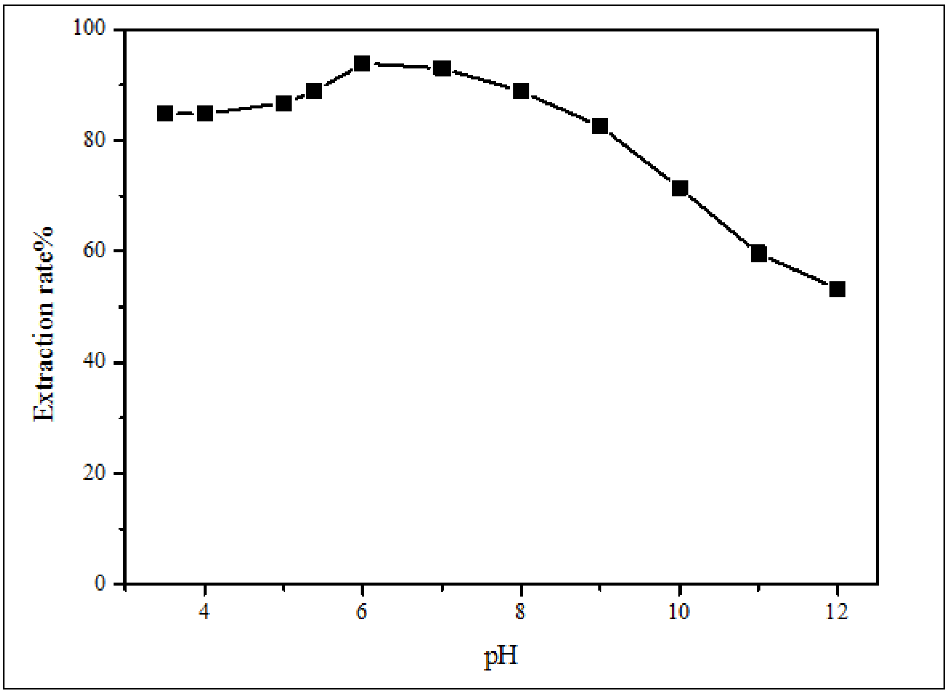

**Fig-5: Effect of pH on extraction rate**

### Effect of amount of adsorbent

The amount of adsorbents in MSPE would directly affect the extraction efficiency of analyte, different amounts of $Fe_3O_4$@$SiO_2$@GO@AAIL nanoparticles were examined in the rang of 2.0~18.0mg. Results as shown in Fig-6, the extraction rate of $Fe_3O_4$@$SiO_2$@GO@AAIL nanoparticles on rutin was the highest at 14.0mg, reaching 94.0%, then remained unchanged. In this paper, the mass of $Fe_3O_4$@$SiO_2$@GO@AAIL nanoparticles is 14.0mg.

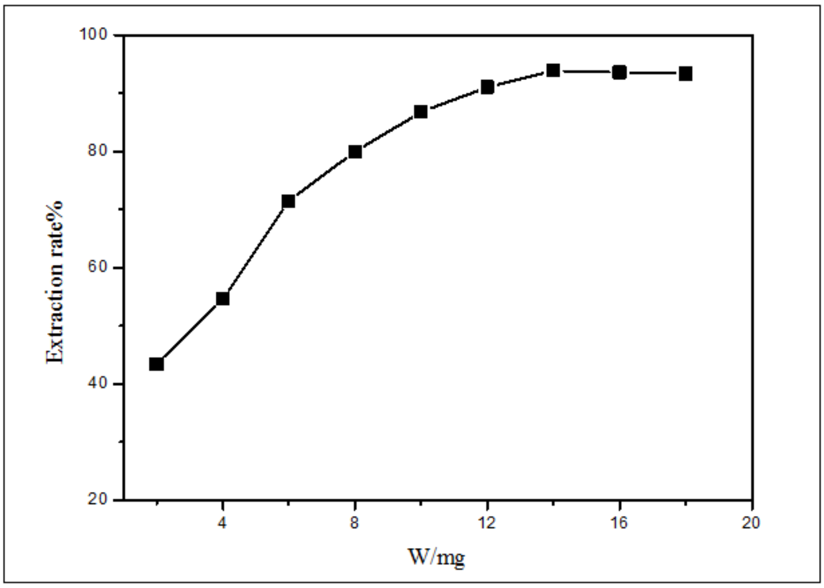

**Fig-6: Effect of the amount of adsorbent on the extraction rate**

### The effect of temperature

The extraction rate of $Fe_3O_4$@$SiO_2$@GO@AAIL on rutin was studied at 0.0-50.0℃.The temperature has little effect on the extraction rate, and the extraction rate is kept above 90.0%. Therefore, this experiment is selected at room temperature.

### The effect of extraction time

The extraction time of $Fe_3O_4$@ $SiO_2$@GO@AAIL on rutin at pH=6.0 at room temperature was studied. As shown in Fig-7, the extraction rate is more than 92.0% in 15 min with time (1-50 min) and remains stable. The extraction process takes some time to achieve the extraction equilibrium in order to obtain higher extraction rate. Therefore, the extraction time chosen in this experiment is 15min.

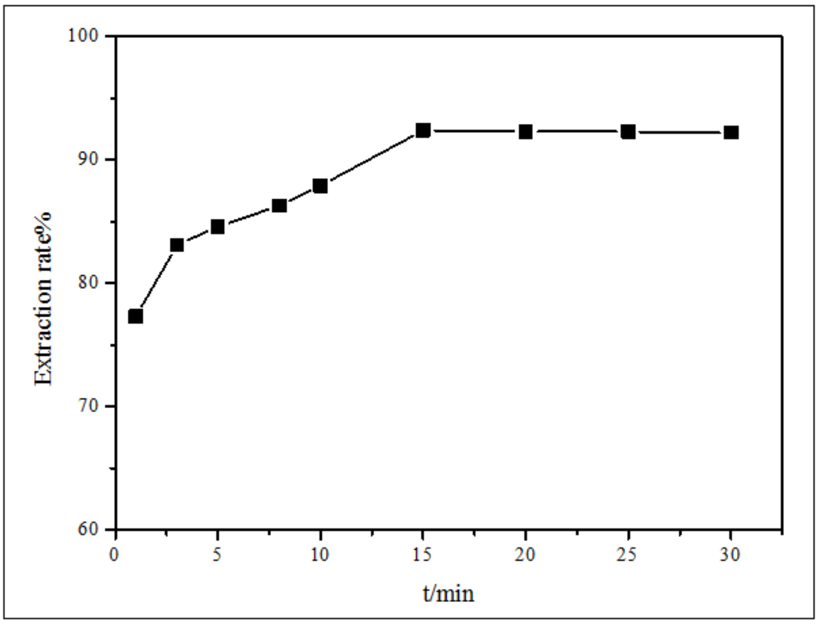

**Fig-7: Effect of adsorption time on the extraction rate**

The adsorption mechanism can be discussed by using the adsorption kinetic model to explore the extraction mechanism. The general adsorption kinetic model has a first-order kinetic model, a second-order kinetic model and a Weber-Morris model [30]. Among them, the second-order kinetic model is a chemical adsorption model, which is mainly through the extraction agent and analyte between the chemical force, including: electrostatic force, intermolecular force, hydrogen bonds, hydrophobic effects [31] . The second-order adsorption kinetic model is fitted and the formula is as follows [32]:

$$\frac{t}{q_t}=\frac{1}{k_2q_e^2}+\frac{t}{q_e}$$

Where $q_e$ is the adsorption amount of the analyte at the time of adsorption equilibrium, and $q_t$ is the adsorption amount of the analyte at time t adsorption. Usually by $t/q_t$ on t mapping, linear regression and fitting to get the corresponding kinetic parameters, if the fitting equation shows that the adsorption process in line with the secondary adsorption dynamic model. In this study, Fe3O4@SiO2@GO@AAI L adsorption of rutin, with this method to fit (Fig-8). The results show that the linear relationship is good, $R^2$=0.9998, demonstrating that the extraction process is in accordance with the secondary adsorption dynamic model. The adsorption process is a chemical adsorption process. Some principles are observed that the interaction between the ionic liquid and the rutin on the surface of the extractant is mainly hydrophobic.The method has good extraction effect on rutin, and can obtain higher extraction rate and shorter extraction time.

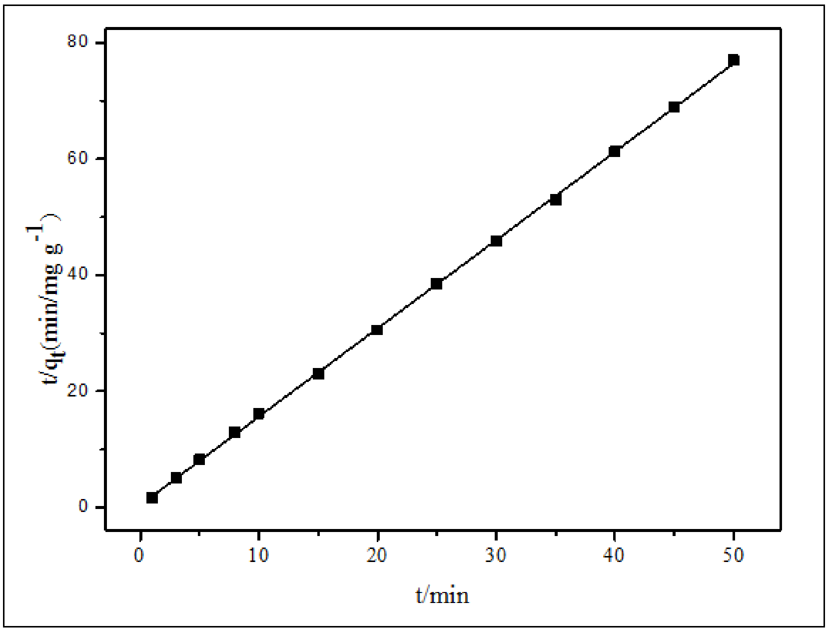

**Fig-8: Adsorption kinetic model**

**Effect of ionic strength**

Taking into account the role of ionic strength, this experiment used 0-25% (w/v) sodium chloride as the electrolyte model, as shown in Fig-9. In the absence of NaCl solution, the extraction rate of rutin was the highest. $Na^+$ competes with $[C_6ALA]^+$ for the $Fe_3O_4$@$SiO_2$@GO matrix [21], leading to a decrease in the extraction rate. So this experiment didn’t chose to add NaCl solution.

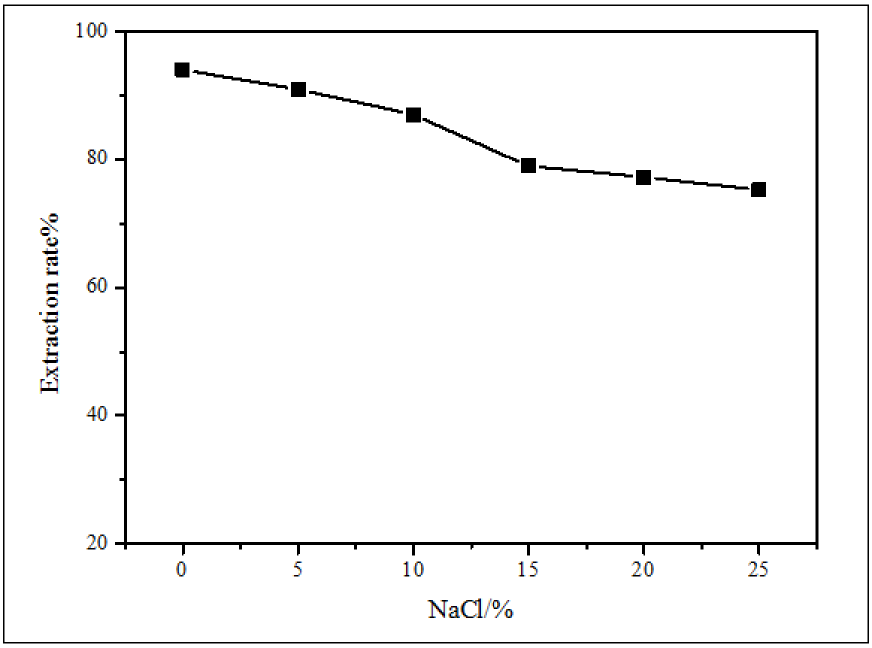

Fig-9: Effect of ionic strength on extraction rate

**Effect of sample volume**

When the rutin content in the immobilized solution is 10.00 μg, the sample volume sample (10.0 to 100.0 mL) was discussed. The results are shown in Fig-10. As the volume of the solution increased, the extraction rate of $Fe_3O_4$@$SiO_2$@GO@AAIL does not change much, but when the solution volume is above 60.0mL, the extraction rate is less than 85%, so the solution allows the maximum adsorption volume for 60.0mL.

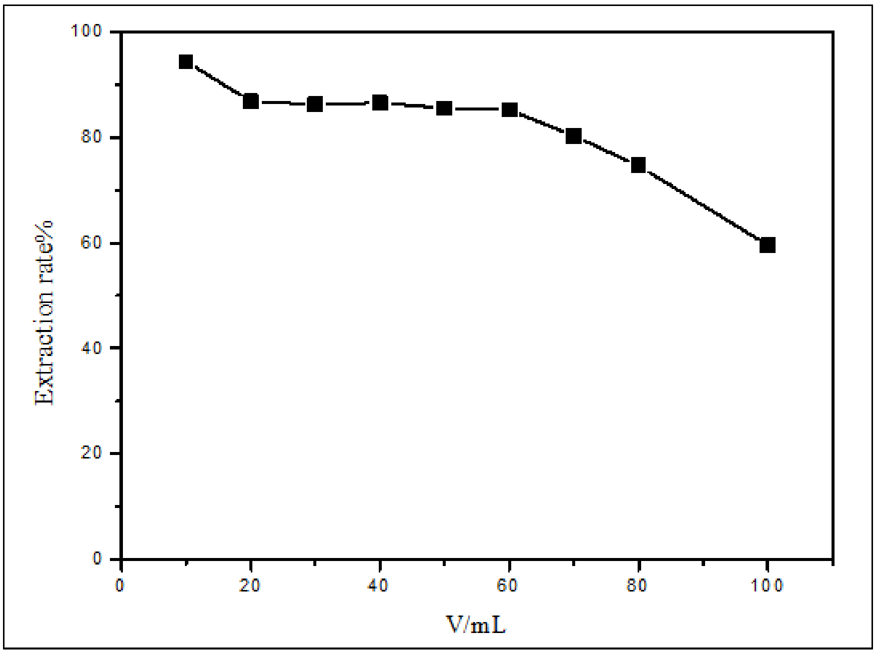

Fig-10: Effect of solution volume on adsorption rate

In this study, the adsorption capacity of $Fe_3O_4$@$SiO_2$@GO@ AAIL for rutin was achieved by changing the concentration of rutin during the extraction process. The results are shown in Fig-11. The results showed that the adsorption capacity of AAIL-based magnetic graphene oxide was 4.09mg $g^{-1}$ when the concentration of rutonium reached 4.2 g $mL^{-1}$.

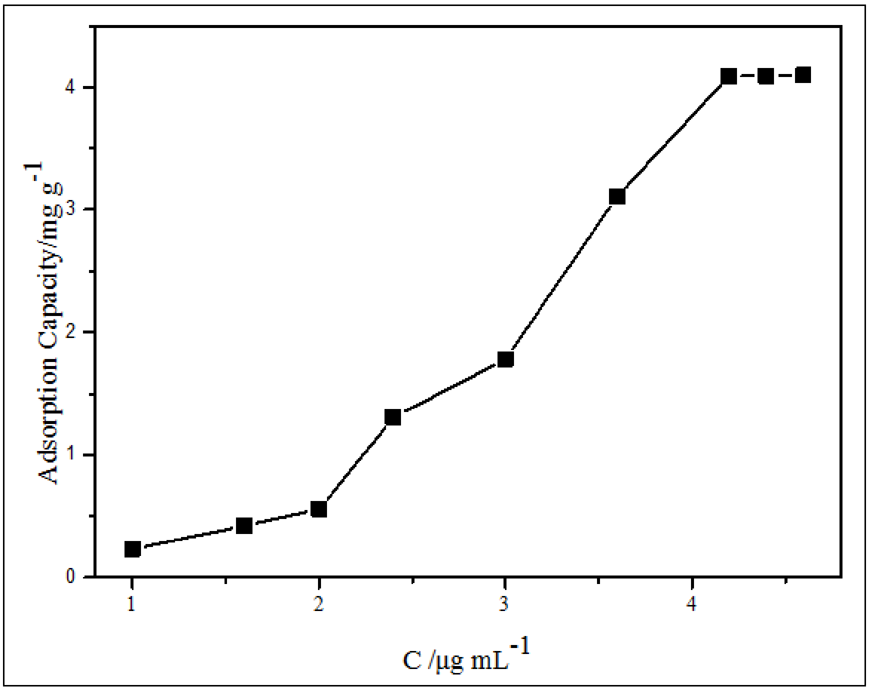

**Fig-11: Adsorption capacity of $Fe_3O_4$@$SiO_2$@AAIL for rutin**

## Optimization of elution conditions

### Eluent selection

The adsorbent was eluted with different eluents in order to achieve the reuse of the $Fe_3O_4$@$SiO_2$@GO@ AAIL, as shown in Fig-12. As can be seen from the Fig-13: (1) the three strong acids eluents(2.0mol $L^{-1}H_2SO_4$, 2.0mol $L^{-1}$HCl and 2.0mol $L^{-1}HNO_3$) is almost useless for the elution of the rutin; (2) it is proved that the acetic acid molecule[HAc] plays a major role in elution(comparing extraction rates of 0.5mol $L^{-1}$ sodium acetate[$Ac^-$] and 0.5mol $L^{-1}$ acetic acid HAc); (3) the elution of acetic acid for rutin was related to [HAc] concentration. So HAc is chosen as the eluent.

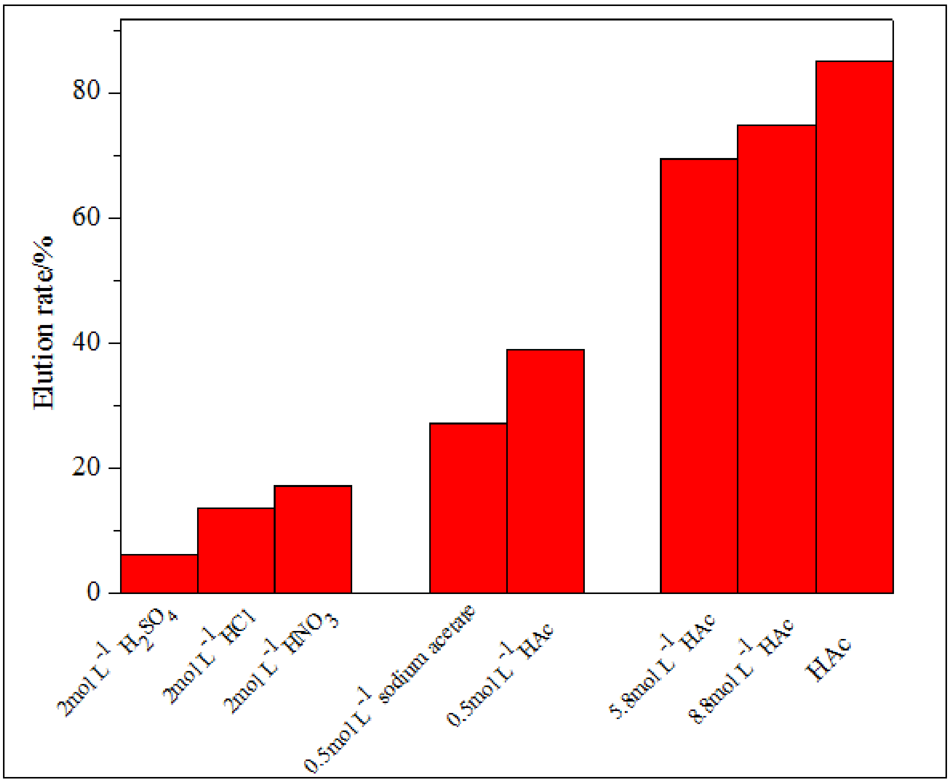

**Fig-12: The elution rates of different eluent**

### The effect of eluent volume

When the eluent volume is between 1.0-9.0 mL, as shown in Fig-13, the eluent volume is in the range of 8.0-9.0 mL and the elution rate remains approximately 95.0%. Therefore, the optimal elution volume is 8.0mL and the pre-enrichment factor is 7.5 (the quotient of volume before extraction and after elution).

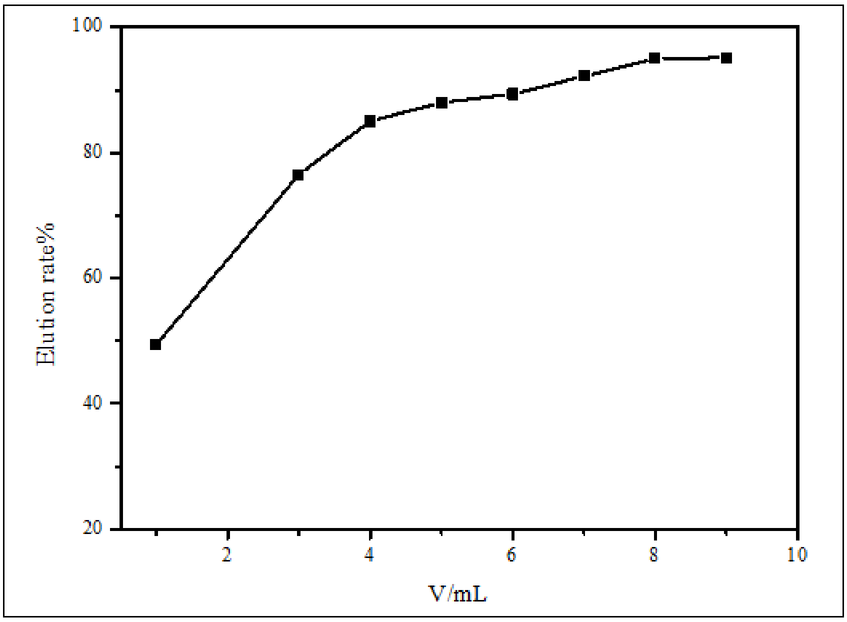

**Fig-13: Effect of eluent volume on elution rate**

### The effect of elution time

The elution of $Fe_3O_4$@$SiO_2$@GO@AAIL extracted with rutin was carried out as shown in Fig-14. Elution was carried out at the optimum eluent volume. As the elution time increased (1-50 min), the elution process completed within 30 min, the elution rate of 95.0% or more, and remained stable within 50 min, so the best elution time is 30min.

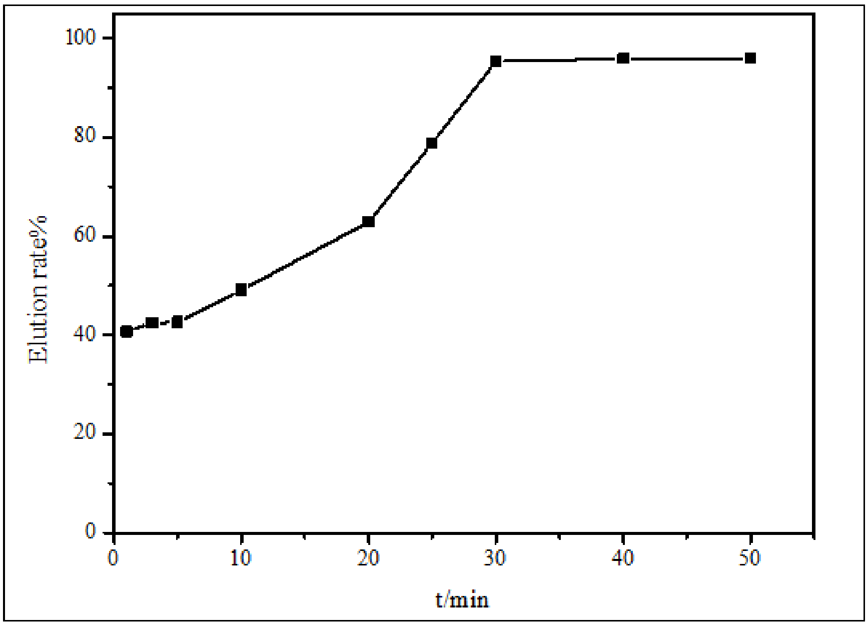

**Fig-14: Effect of elution time on elution rate**

### The effect of elution temperature

The effects of elution temperature on the elution rate of rutin (5.0-50.0℃) were studied. As shown in Fig-15, the elution rate was decreased with the rise of elution temperature, and the elution rate is the highest at 5.0°C, reaching 100.0%. The reason may be that the acetic acid molecule (HAc) plays a major role in the elution process (Fig-14) and (HAc) was getting smaller due to the ionization constant of acetic acid bigger with the increase of temperature [33], the elution rate was decreased with the rise of elution temperature. So the experiment selected 5.0 ℃ best elution temperature.

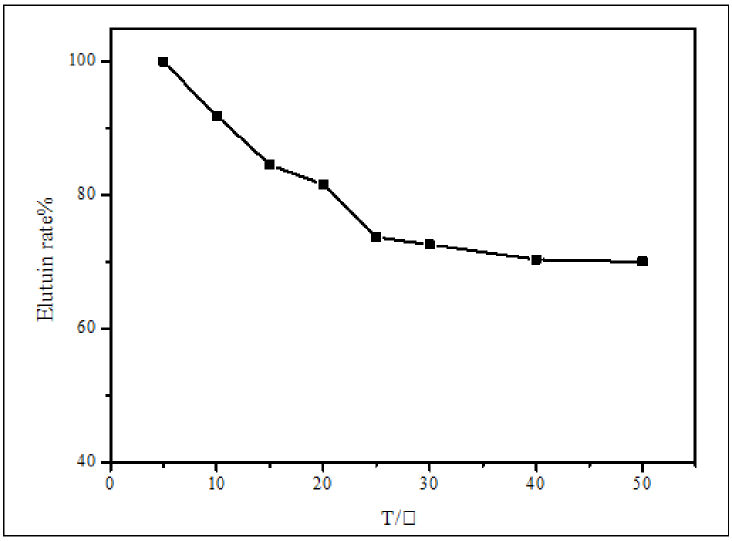

**Fig-15: Effect of elution temperature on elution rate**

### The repeated times of $Fe_3O_4@SiO_2@GO@AAIL$

In order to repeat the use of $Fe_3O_4@SiO_2@GO@AAIL$, each MSPE use 8.0mL glacial acetic acid elution, and then re-used in the MSPE process, the experimental results shown in Fig-16. The results clearly show that after 4 times elution, the extraction rate was below 85.0%. As a result of repeated elution, Rutin's fine residue affects the next extraction process, and $Fe_3O_4@SiO_2@GO@AAIL$ material may be lost.

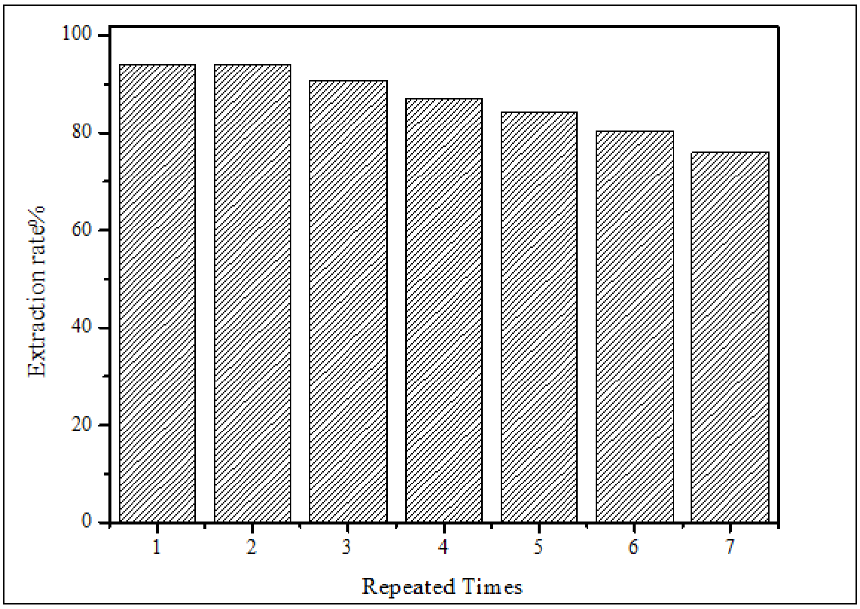

**Fig-16: Number of reuse of $Fe_3O_4@SiO_2@GO@AAIL$**

### Interference experiment

Real samples contain particularly different kinds of ionic species and compounds, which may have negative effect on the extraction process, since their compositions are usually complex. The effect of interfering substance on the extraction of rutin (c=1.00 μg $mL^{-1}$) was studied. The allowable error range was ≤±5%. The results are shown in Table-1.

**Table-1: Effects of Interference Substances**

| Tested substances | Tested substances to analyte ratio (w/w) |
|---|---|
| $Na^{+}$、$Ca^{2+}$、$SO_4^{2-}$、$Cl^{-}$ | 500 |
| Citrate、Glucose | 400 |
| $Mg^{2+}$、$Al^{3+}$、Tartaric acid、vitamin C | 200 |
| Emodin | 5 |
| Quercetin | 2 |

## Analysis performance

The linear range of the rutin was 2.5-5×$10^{3}$ ng mL$^{-1}$, the linear equation was I (peak area) = 2688c+698358 (μg mL$^{-1}$), the linear correlation coefficient ($R^2$) was 0.9996, the relative standard deviation was 1.90% (N=11, c=1.0 μg mL$^{-1}$). The detection limit was 0.02 ng mL$^{-1}$ and the enrichment factor was 7.5(the quotient of volume before extraction and after elution).

## Compared with other methods

Comparing this method with other methods, it can be seen from Table-2 that the method has the advantages of low detection limit, high sensitivity and simple operation.

**Table-2: Comparison of the results with other literatures**

| Method | RSD (%) | LOD | LR | Ref |
|---|---|---|---|---|
| CZE | 3.00 | 0.333 μg mL$^{-1}$ | 6.25 - 200 μg mL$^{-1}$ | [2] |
| SPE-HPLC | 3.04 | 8.3 μg L$^{-1}$ | 0.001–0.1 mg mL $^{-1}$ | [3] |
| SPE- UV–visibLe | 2.9 | 17.0 ng mL$^{-1}$ | 0.050–8.0 μg mL$^{-1}$ | [4] |
| IL-DMC-PE | 2.5 | 1.17nmol L$^{-1}$ | 0.008–4.0 μmol L$^{-1}$ | [5] |
| SMWCNT-PEDOT-IL-Adv | 1.5 | 7.7×$10^{-8}$ mol L$^{-1}$ | 1.0×$10^{-6}$ - 1.4×$10^{-5}$ mol L$^{-1}$ | [6] |
| MSPE- HPLC | 1.90 | 0.02ng mL$^{-1}$ | 2.5-5×$10^{3}$ ng mL$^{-1}$ | This method |

## Sample determination

In the optimization experimental conditions, the extraction chromatogram of the sample is shown in Fig-17. There is almost no interference in the actual sample. It can be observed that: (1) the retention time of rutin was 5.3min (curve a); (2) the signal of rutin in actual sample was significantly enhanced after enrichment (curve c、 e、 g).The recovery results was shown in Table-3. The recoveries of lily, rutin tablets and mouse serum samples are in rang of 96.0%-102.2%, 94.7%-105%, 97.3%-101.7%, respectively. The determination of three samples was shown in Table-4. The results are satisfactory.

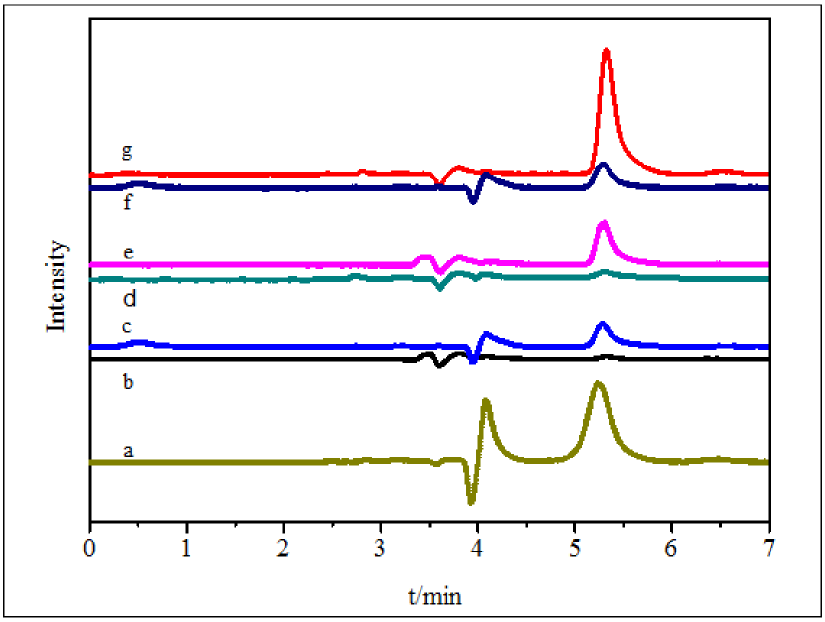

**Fig-17: HPLC–UV chromatograms of samples with MSPE procedure (a) Rutin standard solution (b) lily sample (c) the lily sample after enrichment (d) serum sample after taking 0.5h (e) serum sample after taking 0.5h of enrichment (f) Rutin tablet sample (g) Rutin tablet sample after enrichment**

**Table-3: The recorvies of rutin in real samples**

| Sample | Added($\mu g\ mL^{-1}$) | Found ($\mu g\ mL^{-1}$) | Recovery (%) | RSD intra-day(%) | RSD inter-day(%) |
|---|---|---|---|---|---|
| Lily | 0.00 | 0.76±0.12 | — | 2.7 | 2.9 |
| | 0.50 | 1.24±0.54 | 96.0 | 3.7 | 7.9 |
| | 2.00 | 2.78±1.02 | 102.2 | 2.5 | 8.5 |
| | 3.00 | 3.71±0.62 | 98.5 | 2.9 | 4.8 |
| Rutin tablet | 0.00 | 0.97±0.77 | — | 3.2 | 4.1 |
| | 0.50 | 1.44±0.19 | 94.7 | 3.5 | 9.3 |
| | 2.00 | 2.96±0.80 | 99.4 | 5.6 | 5.8 |
| | 3.00 | 4.12±0.23 | 105.0 | 8.2 | 5.9 |
| Mouse serum[a] | 0.00 | 1.3±0.76 | — | 2.0 | 9.1 |
| | 0.50 | 1.81±1.89 | 101.7 | 5.0 | 9.0 |
| | 2.00 | 3.26±0.91 | 98.2 | 4.4 | 7.3 |
| | 3.00 | 4.22±1.82 | 97.3 | 4.1 | 6.4 |

a: At the dose of 10mg $kg^{-1}$, the serum samples were taken at 0.5h.

**Table-4: Sample measurement results**

| Sample | Label value | Intragastric dose | Collected time(h) | Found |
|---|---|---|---|---|
| Lily | / | / | / | 3.02$\mu g\ g^{-1}$ |
| Rutin tablet | 20.00mg $tablet^{-1}$ | / | / | 19.45mg $tablet^{-1}$ |
| Mice serum | / | 10mg $Kg^{-1}$ | 0.5 | 2.6$\mu g\ mL^{-1}$ |
| | | | 1.0 | 1.4$\mu g\ mL^{-1}$ |
| | | | 1.5 | 0.7$\mu g\ mL^{-1}$ |

## CONCLUSION

In this paper, $Fe_3O_4@SiO_2@GO@AAIL$, a magnetic solid phase extraction material was synthesized successfully and analyzed in combination with HPLC. This study shows that the newly nanomaterials have many advantages of simple operation, separation and analysis time and high extraction efficiency in enrichment of rutin. The method is simple, safe, rapid and stable, and the result of sample determination is satisfactory.

## Acknowledgments

The authors acknowledge the financial support from the National Natural Science Foundation of China (21375117) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

## REFERENCES

1. Pang P, Li H, Liu Y, Zhang Y, Feng L, Wang H, Wu Z, Yang W. One-pot facile synthesis of platinum nanoparticle decorated reduced graphene oxide composites and their application in electrochemical detection of rutin. Analytical Methods. 2015;7(8):3581-6.
2. Memon AF, Solangi AR, Memon SQ, Mallah A, Memon N, Memon AA. Simultaneous determination of quercetin, rutin, naringin, and naringenin in different fruits by capillary zone electrophoresis. Food Analytical Methods. 2017 Jan 1;10(1):83-91.
3. Zeng H, Wang Y, Liu X, Kong J, Nie C. Preparation of molecular imprinted polymers using bi-functional monomer and bi-crosslinker for solid-phase extraction of rutin. Talanta. 2012 May 15;93:172-81.
4. Gong A, Ping W, Wang J, Zhu X. Cyclodextrin polymer/$Fe_3O_4$ nanocomposites as solid phase extraction material coupled with UV–vis spectrometry for the analysis of rutin. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014 Mar 25;122:331-6.
5. Mohammadi N, Najafi M, Adeh NB. Highly defective mesoporous carbon–ionic liquid paste electrode as sensitive voltammetric sensor for determination of chlorogenic acid in herbal extracts. Sensors and Actuators B: Chemical. 2017 May 1;243:838-46.
6. Nagles E, García-Beltrán O. Determination of Rutin in Black Tea by Adsorption Voltammetry (AdV) in the Presence of Morin and Quercetin. Food Analytical Methods. 2016 Dec 1;9(12):3420-7.
7. Gong A, Zhu X. Dispersive solvent-free ultrasound-assisted ionic liquid dispersive liquid–liquid microextraction coupled with HPLC for determination of ulipristal acetate. Talanta. 2015 Jan 1;131:603-8.
8. Qin X, Zhu X. Determination of allura red in food by ionic liquid ß-cyclodextrin-cross-linked polymer solid phase extraction and high-performance liquid chromatography. Analytical Letters. 2016 Jan 22;49(2):189-99.
9. Jiang HM, Yang T, Wang YH, Lian HZ, Hu X. Magnetic solid-phase extraction combined with graphite furnace atomic absorption spectrometry for speciation of Cr (III) and Cr (VI) in

environmental waters. Talanta. 2013 Nov 15;116:361-7.

10. Li QL, Wang LL, Wang X, Wang ML, Zhao RS. Magnetic metal-organic nanotubes: An adsorbent for magnetic solid-phase extraction of polychlorinated biphenyls from environmental and biological samples. Journal of Chromatography A. 2016 Jun 3;1449:39-47.
11. Wang P, Wang X, Yu S, Zou Y, Wang J, Chen Z, Alharbi NS, Alsaedi A, Hayat T, Chen Y, Wang X. Silica coated Fe3O4 magnetic nanospheres for high removal of organic pollutants from wastewater. Chemical Engineering Journal. 2016 Dec 15;306:280-8.
12. Li N, Chen J, Shi YP. Magnetic reduced graphene oxide functionalized with β-cyclodextrin as magnetic solid-phase extraction adsorbents for the determination of phytohormones in tomatoes coupled with high performance liquid chromatography. Journal of Chromatography A. 2016 Apr 8;1441:24-33.
13. Chen J, Zhu X. Magnetic solid phase extraction using ionic liquid-coated core–shell magnetic nanoparticles followed by high-performance liquid chromatography for determination of Rhodamine B in food samples. Food chemistry. 2016 Jun 1;200:10-5.
14. Taghvimi A, Hamishehkar H, Ebrahimi M. The application of magnetic nano graphene oxide in determination of methamphetamine by high performance liquid chromatography of urine samples. Journal of the Iranian Chemical Society. 2016 Aug 1;13(8):1471-80.
15. Zhong Z, Li G, Luo Z, Liu Z, Shao Y, He W, Deng J, Luo X. Carboxylated graphene oxide/polyvinyl chloride as solid-phase extraction sorbent combined with ion chromatography for the determination of sulfonamides in cosmetics. Analytica chimica acta. 2015 Aug 12;888:75-84.
16. Wu J, Xiao D, Zhao H, He H, Peng J, Wang C, Zhang C, He J. A nanocomposite consisting of graphene oxide and Fe3O4 magnetic nanoparticles for the extraction of flavonoids from tea, wine and urine samples. Microchimica Acta. 2015 Oct 1;182(13-14):2299-306.
17. Abdolmohammad-Zadeh H, Talleb Z. Magnetic solid phase extraction of gemfibrozil from human serum and pharmaceutical wastewater samples utilizing a β-cyclodextrin grafted graphene oxide-magnetite nano-hybrid. Talanta. 2015 Mar 1;134:387-93.
18. Ding X, Wang Y, Wang Y, Pan Q, Chen J, Huang Y, Xu K. Preparation of magnetic chitosan and graphene oxide-functional guanidinium ionic liquid composite for the solid-phase extraction of protein. Analytica chimica acta. 2015 Feb 25;861:36-46.
19. Wen Q, Wang Y, Xu K, Li N, Zhang H, Yang Q, Zhou Y. Magnetic solid-phase extraction of protein by ionic liquid-coated Fe@ graphene oxide. Talanta. 2016 Nov 1;160:481-8.
20. Wu J, Zhao H, Xiao D, Chuong PH, He J, He H. Mixed hemimicelles solid-phase extraction of cephalosporins in biological samples with ionic liquid-coated magnetic graphene oxide nanoparticles coupled with high-performance liquid chromatographic analysis. Journal of Chromatography A. 2016 Jul 8;1454:1-8.
21. Chang-Zeng WU, Gong-Chun LI. Preparation and applications of amino aicd ionic liquids, Chemical Reagents 2012;34 933- 936.
22. Fukumoto K, Yoshizawa M, Ohno H. Room temperature ionic liquids from 20 natural amino acids. Journal of the American Chemical Society. 2005 Mar 2;127(8):2398-9.
23. Huang R, Fang Z, Yan X, Cheng W. Heterogeneous sono-Fenton catalytic degradation of bisphenol A by Fe3O4 magnetic nanoparticles under neutral condition. Chemical Engineering Journal. 2012 Jul 15;197:242-9.
24. Hong RY, Zhang SZ, Han YP, Li HZ, Ding J, Zheng Y. Preparation, characterization and application of bilayer surfactant-stabilized ferrofluids. Powder Technology. 2006 Nov 30;170(1):1-1.
25. Ma X, Wang J, Sun M, Wang W, Wu Q, Wang C, Wang Z. Magnetic solid-phase extraction of neonicotinoid pesticides from pear and tomato samples using graphene grafted silica-coated Fe 3 O 4 as the magnetic adsorbent. Analytical Methods. 2013;5(11):2809-15.
26. Huang Y, Wang Y, Pan Q, Wang Y, Ding X, Xu K, Li N, Wen Q. Magnetic graphene oxide modified with choline chloride-based deep eutectic solvent for the solid-phase extraction of protein. Analytica chimica acta. 2015 Jun 2;877:90-9.
27. Cheng Q, Qu F, Li NB, Luo HQ. Mixed hemimicelles solid-phase extraction of chlorophenols in environmental water samples with 1-hexadecyl-3-methylimidazolium bromide-coated Fe3O4 magnetic nanoparticles with high-performance liquid chromatographic analysis. Analytica chimica acta. 2012 Feb 17;715:113-9.
28. Gu W, Zhu X. Nanoparticles of type Fe 3 O 4-SiO 2-graphene oxide and coated with an amino acid-derived ionic liquid for extraction of Al (III), Cr (III), Cu (II), Pb (II) prior to their determination by ICP-OES. Microchimica Acta. 2017 Nov 1;184(11):4279-86.
29. Zhao J, Lin DQ, Yao SJ. Adsorption of rutin with a novel β-cyclodextrin polymer adsorbent: thermodynamic and kinetic study. Carbohydrate polymers. 2012 Nov 6;90(4):1764-70.
30. Russo V, Tesser R, Trifuoggi M, Giugni M, Di Serio M. A dynamic intraparticle model for fluid–solid adsorption kinetics. Computers & Chemical Engineering. 2015 Mar 4;74:66-74.
31. González-Centeno MR, Comas-Serra F, Femenia A, Rosselló C, Simal S. Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape

pomace (Vitis vinifera L.): experimental kinetics and modeling. Ultrasonics sonochemistry. 2015 Jan 1;22:506-14.

32. Zhang Y, Cheng Y, Chen N, Zhou Y, Li B, Gu W, Shi X, Xian Y. Recyclable removal of bisphenol A from aqueous solution by reduced graphene oxide–magnetic nanoparticles: adsorption and desorption. Journal of colloid and interface science. 2014 May 1;421:85-92.
33. Ferra MI, Graça JR, Marques AM. Ionization of Acetic Acid in Aqueous Potassium Chloride Solutions. Journal of Chemical & Engineering Data. 2011 Aug 22;56(9):3673-8.