Iranian Journal of Analytical Chemistry

Volume 9, Issue 1, March 2022 (56-62)

Original Research Article

مجله ایرانی شیمی تجزیه دوره ۹، شماره ۱، فروردین ۱۴۰۱ (۶۲–۵۶)

Determination of Free Amino Acids in Fertilizer Samples by Switchable Hydrophilic Solvent-Based Extraction (SHSE) Followed by HPLC-UV

Kobra Sadat Hasheminasab^{1*}, Karim Shahbazi²

 Assistant professor, Soil and Water Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran,
 Associate Professor, Soil and Water Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

> Received: 23 January 2022 Accepted: 16 March 2022 DOI: 10.30473/ijac.2022.63614.1233

Abstract

In the present work, switchable hydrophilic solvent-based extraction (SHSE) followed by high performance liquid chromatography with UV detection (HPLC-UV) was used to determine free amino acids in fertilizer samples. During the phase transformation of SHSE, the organic phase appeared to effectively capture the target analytes. In this extraction technique, 200 μ L of a water-immiscible solvent (dipropylamine) is used, which can be solubilized in the acidic aqueous phase. Phase separation is then brought about by the addition of sodium hydroxide. The variables affecting this method were optimized to achieve the best extraction efficiency. The optimized conditions included: volume of sample 25 mL, volume of extraction solvent 200 μ L, and extraction time 2 min. Under the optimal experimental conditions, good detection limits (0.0006-0.0021 μ mol ml⁻¹), linearities (R² > 0.997), and precision (relative standard deviation less than 5.0%) were obtained. Finally, the developed method was successfully applied to the determination of target analytes in different types of fertilizer samples and acceptable recoveries (> 97.2%) were obtained.

Keywords

Switchable Hydrophilic Solvent-Based Extraction; Free Amino Acids; Fertilizer; Dipropylamine; HPLC-UV.

1. INTRODUCTION

Amino acids are the building blocks of proteins. Like any living organism, plants synthesize amino acid sequences to form proteins. Amino acids and proteins play an important role in the genetic and physiological processes of organisms such as the synthesis of nucleic acids, proteins, enzymes, alkaloids, vitamins, terpenoids and chlorophyll, and the formation of vegetative tissues [1-3]. The use of amino acids in plant nutrition allows the plant to use them immediately without having to synthesize them, and can save this energy for other metabolic processes that allow the farmer to increase production [4]. Amino acids bind to nutrients such as iron, copper, magnesium, zinc, etc., protecting them from degradation or oxidation and faster and better uptake and transfer of these elements from the cell membrane. In fact, the supply of amino acids through the leaves leads to better absorption of microelements. When amino acids are co-administered with the plant, the synergistic effects lead to better absorption of the elements as well as faster growth and greater resistance to environmental stress [5]. The use of amino acids in agriculture is a tool that can be very useful to improve production and crop quality, to

overcome moments of stress due to low temperatures, droughts, etc. In view of these requirements and the need to control fertilizers containing amino acids used in agriculture and to prevent counterfeit fertilizers with unrealistic percentages from entering the market, an efficient method for determining the concentration of amino acids in fertilizer samples in laboratories is needed. There are many techniques for measuring the concentration of free amino acids in solution, which can be referred to as thin layer chromatography, gas chromatography, capillary electrophoresis, and high performance liquid chromatography [6-8].

Sample preparation is an essential part of the analytical procedure. The main objective of sample preparation is both clean up and enrich the analytes of interest from the sample and to convert the analytes into a form suitable for the analytical instrument. Conventional sample preparation techniques such as solid phase extraction (SPE) and liquid-liquid extraction (LLE) are considered time consuming and require large amounts of toxic and expensive organic solvents [9-10].

Homogeneous liquid-phase microextraction (HLPME) takes advantage of the phenomenon of

^{*}Corresponding Author: k.hasheminasab@areeo.ac.ir

phase separation from a homogeneous solution and extracts the target solutes simultaneously into a very small organic phase [11]. In this method, the initial condition is a homogeneous solution and there is no interface between the water phase and the extraction solvent phase. Therefore, it has the advantage of an extremely fast extraction rate, since the surface contact between the aqueous phase and the organic phase does not present any obstacles during the extraction process. In these cases, phase separation is based on the phenomenon of salting out, the change of temperature and pH, and the formation of ion pairs. This method has been mainly studied as a powerful enrichment method for separation of the desired component or instrumental analysis [12-14].

Jessop et al. have synthesized a new type of solvent called "switchable" or "smart" solvents (SS) [15]. Switchable hydrophilic solvents (SHS) are defined as solvents that change their physical properties reversibly and abruptly [16]. The SHS can be mixed with water samples and can be easily separated from the aqueous phase by removing CO2 from the solution [17]. Alternatively, the hydrophilic switch can also be triggered by changing the charge of the SHS through pH shift [18], which is particularly useful in the context of microextraction. The main advantages of using SS are the extraction of analytes in a homogeneous phase without dispersive solvent and the ease of phase separation without additional equipment. SPs are environmentally friendly compounds [18-19].

In this work, switchable hydrophilic solvent-based extraction (SHSE), followed by HPLC-UV was proposed for the determination of free amino acid in fertilizer samples. The applicability of this method in real samples was evaluated by analyzing fertilizer samples.

2. EXPERIMENTAL

2.1 Reagents and standard solutions

All reagents were of analytical reagent grade unless otherwise stated. Phenyl isothiocyanate (PITC) and triethylamine (TEA) were purchased from Sigma. Hydrochloric acid and acetonitrile (HPLC grade) and ethanol (99%) were purchased from Merck. Double deionized water was used to prepare the solution and mobile phase.

The standard for amino acids is a milliliter ampoule (Termo Scientific, USA) containing 2.5 micromoles/milliliter of each amino acid in 0.1 N hydrochloric acid, including: Aspartic acid (Laspartic acid), glutamic acid. L-glutamic acid, Lserine, L-glycine, L-histidine, L-arginine, Lthreonine, L-alanine, L-proline, L-tyrosine, Lvaline, L-methionine, L-cysteine (1.25 µmol / ml), lileucine, L-leucine, L-phenylalanine and L-lysine. This standard was stored at -20 $^{\circ}$ C.

2.2 Instrument

A high- performance liquid chromatography (HPLC) instrument from KNAUER EuroChrom, model Germany, equipped with a four-channel pump, model 1001K- and UV-Vis detector was used.

The chromatographic conditions we optimized for PTH-amino acid separation were: Flow rate 0.5 mL/min, buffer A: 0.28 M sodium acetate, 0.05% TEA, and 5% ACN (pH 6.3810.04); B: 60% ACN; mobile phase isocratic at 0% B for 2 min; first linear gradient from 0 to 43% B in 7 min; second linear gradient from 43 to 50% in 4 min, finally changing to 100% B in 1 min. Wash step at 100% B isocratic flow for 8 min; return to initial conditions 0% B in 1 min and maintain at 0% B isocratic flow for 9 min until column equilibrium step.

2.3. Fertilizer samples.

Fertilizer samples were selected from fertilizer samples sent to Iran Soil and Water Research Institute for analysis.

2.4. Sample preparation

0.4 g of the solid sample and 2 mL of the liquid sample were transferred into a 25 ml balloon. About three quarters of the balloon volume was poured into a 0.1 N hydrochloric acid solution and stirred for 20 minutes.

2.5 Extraction procedure

First, 100 μ L of water immiscible solvent (DPA) and 200 μ L of HCl were added to the sample solution, which formed a single phase. Then, 2 mL of NaOH solution (5 M) was added as a trigger for phase separation. The turbid solution formed immediately and complete separation of water and DPA occurred after about 2 min. Finally, DPA (50 μ L) was collected from the surface of the sample solution and 20 μ L of the collected DPA was directly injected into the HPLC using a 25 μ L Hamilton syringe.

2.6. Chromatographic procedure.

Amino acid derivatives were prepared according to our previous research [20]. To obtain the amino acids, 20 μ l of the solution extracted from the manure was transferred to glass tubes and the contents were completely dried in a vacuum vessel. To each of the glass tubes containing the standard and dried samples, 20 to 30 μ l of the pH adjusting solution was added, stirred, and placed in a vacuum vessel to dry completely. The pH adjusting

solution contains a 1: 2: 2 ratio of water, ethanol and triethylamine, respectively, which was freshly prepared for each purpose and mixed in dark, clean This thoroughly containers. step triethylamine activates the environment and prepares the conditions for the derivative. Then 20 µl of derivative solution was added and vortexed. The derivative solution contains 1: 1: 1: 7 ratio of ethanol, water, triethylamine and phenyl isothiocyanate, respectively. After addition of the derivative solution, the samples and standards were stored in the dark at room temperature for 20 minutes to complete the reaction between PITC and amino acids. The tubes were then placed in a vacuum to dry (removal of solvent) and reconstituted in 100 µL diluent for analysis. The diluent was 0.005 M sodium phosphate buffer, pH 7.42, and 6% ACN. Finally, 20 µL was injected for analysis. These standards and dried samples can be stored down to -20 °C for the next few days.

3. RESULT AND DISCUSSION

In order to obtain the best extraction performance, various parameters affecting the extraction procedures were studied and optimized. All optimization studies were performed using 500 μ g L-1 standards and the optimal values were selected based on the highest average of triplicate measurements based on peak areas.

Optimization of parameters was performed using the one-at-a-time method. All experiments were performed at room temperature.

3.1 Optimization of conditions3.1.1 Selection of extraction solvent

For quantitative recoveries, the selection of appropriate switchable solvents in SS-LPME methods is of immense importance. The extraction solvent should have the following properties (1) ability to extract the target compounds, (2) ability to transform from hydrophilic form to hydrophobic form and vice versa by pH shift, (3) high solubility in water for the hydrophilic form and low solubility in water for the hydrophobic form of the switchable solvent.

Based on these criteria, two switchable solvents, dipropylamine (DPA) and triethylamine (TEA), were investigated in this section. Therefore, extraction was performed using 150 μ L of each solvent. The results are shown in Fig. 1. According to the extraction results, DPA showed better extraction efficiency compared to TEA under the same conditions. Therefore, based on the quantitative recoveries, DPA was selected as the extraction solvent for further work.



Fig. 1. The influence of extraction solvent on the extraction efficiency of amino acids obtained from SHSE. Extraction conditions: Volume of fertilizer sample 25 mL, volume of extraction solvent 150 μ L, analyte concentration 500 μ g L⁻¹, room temperature.

3.1.2. Influence of the volume of the extraction solvent (acceptor phase)

In HLLME, the volume of extraction solvent is an important parameter because it affects EF. Therefore, in order to determine the optimal volume of extraction solvent, different volumes of DPA (100, 150, 200, 250, 300 μ L) were studied while keeping the other experimental conditions constant. At volumes less than 200 μ L of DPA, no cloudy condition forms due to the partial dissolution of DPA in water. The extraction efficiency of the analytes decreased at higher volumes of extraction solvent. Therefore, based on the experimental data shown in Fig. 2, 200 μ L of DPA was used in all subsequent experiments.



Fig. 2. The influence of the volume of extraction solvent on the extraction efficiency of amino acids. Extraction conditions: DPA as extraction solvent, volume of fertilizer sample 25 mL, concentration at 500 μ g L⁻¹ and room temperature.

3.1.3 Effect of sample volume (donor phase).

The effect of sample volume on analytical signals was investigated in the range of 15-30 mL to obtain the best results. As the results show, the analytical signals increased with increasing sample volume but remained constant after 25 mL. Therefore, the sample volume of 25 mL was chosen as the optimal volume for subsequent extractions.

3.1.4 Effect of the volume of the NaOH solution

NaOH is necessary for the separation of the phases in the presented microextraction system [21]. Hydrophilic protonated DPA can be converted to hydrophobic form by adding NaOH solution. To investigate the influence of volume on analytical signals, some experiments were performed by

adding different volumes of NaOH solution (10 M) ranging from 2 to 6 mL. The other experimental conditions were kept constant. The results showed that the analytical signals were enhanced up to 2 mL of NaOH solution, while phase separation did not occur at lower volumes of NaOH solution. Further increase in the volume of NaOH solution resulted in a decrease in extraction efficiency as hydrophobic DPA did not occur. Therefore, 4 mL of NaOH solution was used in all subsequent experiments.

3.1.5. Effect of extraction time

In this work, the extraction time was defined as the time interval between the injection of a mixture of DPA/HCl and just before the start of the collection of DPA. When not stirred or centrifuged, phase separation takes at least 2.0 minutes after the addition of NaOH. Therefore, the effect of extraction time was investigated in the range of 2.0-10 minutes, and the results showed that the extraction time had no significant effect on the analytical signal. Therefore, 2.0 minutes was selected as the optimal extraction time. On the other hand, at SHS-ME, the extraction solvent is completely dissolved in the sample solution and the contact area between extraction solvent and sample solution is infinite. Therefore, the transfer

of analytes from the sample solution to the extraction solvent is very fast and independent of time.

3.2. Validation of the method

The optimized SHSE method was used for the determination of free amino acids in fertilizer samples. The main analytical figures of merit are summarized in Table 1. The detection limit (LOD) was determined based on a signal-to-noise (S/N) ratio of 3. Method repeatability was evaluated and RSDs (n=5) ranged from 3.2-5.0%.

The enrichment factor (EF) was defined as the ratio between the final analyte concentration in the organic phase ($C_{org, final}$) and the initial concentration of the analyte in the sample solution ($C_{s, initial}$):

$$EF = \frac{C_{\rm org,final}}{C_{\rm s,initial}} \tag{1}$$

Extraction recovery (R %) was calculated for each analyte using the following equation:

$$R\% = \left(\frac{V_0}{V_s}\right) \left(\frac{C_{\text{org,final}}}{C_{\text{s,initial}}}\right) \times 100$$
$$= EF\left(\frac{V_0}{V_s}\right) \times 100 \tag{2}$$

Where V_0 is the volume of the organic phase, V_s is the volume of the sample.

Amino acids	R ²	LDR ^a (µmol ml ⁻¹)	LOD ^b (µmol ml ⁻¹)	RSD ^c (n=3, %)	Recovery %
Asp	0.998	0.005-0.50	0.0021	3.6	98.5
Glu	0.997	0.005-0.50	0.0021	3.9	99.9
Ser	0.999	0.005-0.50	0.0021	4.2	97.5
Gly	0.999	0.005-0.50	0.0021	4.3	98.2
His	0.999	0.005-0.50	0.0021	5.0	97.5
Arg	0.997	0.005-0.50	0.0021	3.8	97.8
Thr	1.000	0.005-0.50	0.0021	4.2	98.5
Ala	1.000	0.005-0.50	0.0021	4.4	99.8
Pro	0.997	0.005-0.50	0.0021	5.0	97.2
Tyr	0.999	0.005-0.50	0.0021	4.3	98.4
Val	0.999	0.005-0.50	0.0021	4.9	97.8
Met	1.000	0.005-0.50	0.0021	3.5	97.2
Cys	0.998	0.002-0.025	0.0006	4.1	98.5
Ile	0.998	0.005-0.50	0.0021	3.2	98.9
Leu	1.000	0.005-0.50	0.0021	3.5	99.8
Phe	0.999	0.005-0.50	0.0021	4.8	97.6
Lys	0.999	0.005-0.50	0.0021	5.0	98.9

 Table 1. Some analytical performance data of SHSs method for free amino acids.

^aLinear dynamic range

^bLimit of detection (signal-to-noise = 3)

°Relative standard deviations

3.3. Analysis of the real sample

The proposed method was successfully applied for the determination of free amino acids in different fertilizer samples and acceptable recoveries (> 90.1%) were obtained. Fig. 3 shows the obtained chromatograms of free amino acids extracted from a fertilizer using SHSE-HPLC-UV.



Fig. 3. Chromatogram of free amino acids extracted from one fertilizer using SHSE-HPLC-UV.

4. CONCLUSION

The SHSE method using switchable hydrophilic solvents (SHS) was applied for the simultaneous determination of 17 amino acids in fertilizer HPLC-UV. phase samples by Liquid microextraction using switchable solvents is an environmentally friendly, simple and rapid method. The main advantages of using SHSs are extraction of analytes in a homogeneous phase without dispersing solvent and easy phase separation without additional equipment. It is simple and fast and does not require special laboratory equipment for phase separation. This method was successfully applied to the determination of amino acids in various fertilizer samples. SHSs-HPLC-UV method was compared with Direct-HPLC-UV method in Table 2. The figures of merit of SHSs method is better than the direct determination. In addition, the use of the SHSs method eliminates the interferences and increases the life time of the HPLC column.

Acknowledgements

We gratefully acknowledge financial support from the Soil and Water Research Institute (SWRI, Project No: 12-10-10-068-96068-961637).

REFERENCES

 F.A. Abo Sedera, A. Amany, A. El-Latif, L. A.A. Bader, and S. M. Rezk, Effect of NPK mineral fertilizer levels and foliar application with humic and amino acids on yield and quality of strawberry, *Egypt. J. Appl. Sci.*, 25 (2010) 154–169.

- [2] S. A. Faten, A. M. Shaheen, A. A. Ahmed, and R. M. Asmaa, The effect of foliar application of urea and amino acids mixtures as antioxidants on the growth and yield and characteristics of squash, *Res. J. Agric. Biol. Sci.*, 6 (2010) 583–588.
- [3] S. A. El-Desouky, F. H. Ismaeil, A. L. Wanas, E. S. L. Fathy, M. M. Abd El-All, and M. M. Abd, Effect of yeast extract, amino acids and citric acid on physioanatomical aspects and productivity of tomato plants grown in late summer season, *Minufiya, J. Agric. Res.*, 36 (2011) 859–884.
- [4] J. Li, J. Ma, Q. Li, S. Fan, L. Fan, H. Ma, Y. Zhang and L. Zheng. "Determination of 35 Free Amino Acids in Tea Using Ultra-Performance Liquid Chromatography Coupled With Quadrupole Time-of-Flight Mass Spectrometry." *Front. nutr.* 8 (2021) 1-14.
- [5] M. Kazem Souri, Aminochelate fertilizers: the new approach to the old problem; a review, *Open Agric.*, 1 (2016) 118-123.
- [6] H. Liu, Measurement of blood plasma amino Acids in ultrafiltrates by high-performance liquid chromatography with automatic precolumn O-phthaldialdehyde derivatization, *Methods mol. biol.*, 159 (2000) 123-140.
- [7] M. Friedman, Applications of the ninhydrin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences, J. Agric. Food Chem., 52 (2004) 385-406.
- [8] M.J.G.Castro, J.L. Hernández, J.S. Lozano and M.J.O. Concha, Determination of amino acids in green beans by derivatization with phenylisothiocianate and high-performance liquid chromatography with ultraviolet detection, J. Chromatogr. Scien, 35 (1997) 181-185.
- [9] P. Liang, J. Xu, L. Guo and F. Song, Dynamic liquid-phase microextraction with HPLC for the determination of phoxim in water samples, *J. Sep. Sci.* 29(2006) 366–370.

Table 2. Comparison of the proposed method (SHSs) with direct method for amino acid determination in fertilizer

Method	LDR ^a (µmol ml ⁻¹)	LOD ^b (µmol ml ⁻¹)	RSD ^c (n=3, %)	Recovery %	Reference
Direct-HPLC-UV	0.013-0.125	0.0047-0.0094	< 6.1	97-102	[20]
SHSs-HPLC-UV	0.005-0.50	0.0006-0.0021	< 5	97.2-99.9	-

K.S. Hasheminasab, K. Shahbazi/ Determination of free amino acids in fertilizer samples | 61

- [10] A.R. Fontana, M. M. de Toro, J. C. Altamirano, One-step derivatization and preconcentration microextraction technique for determination of bisphenol A in beverage samples by gas chromatography-mass spectrometry, J. Agric. Food Chem. 59 (2011) 3559-3565.
- [11] S. Igarashi and T. Yottuyanagi, Homogeneous liquid–liquid extraction by pH dependent phase separation with a fluorocarbon ionic surfactant and its application to the preconcentration of porphyrin compounds. *Microchim. Acta*, 106 (1992) 37-44.
- [12] T. Sudo and S. Igarashi, Homogeneous liquid–liquid extraction method for spectrofluorimetric determination of chlorophyll a, *Talanta*, 43 (1996) 233–237.
- [13] S.Berijani, M. Sadigh and E. Pournamdari, J. Chromatogr. Sci., 54 (2016) 1061–1067.
- [14] P.G. Jessop, D.J. Heldebrant, X. Li, C.A. Eckert and C.L. Liotta, Green chemistry: reversible nonpolar-to-polar solvent, *Nature*, 436 (2005) 1102.
- [15] P. Pollet, C.A. Eckert and C.L. Liotta, Switchable solvents, *Chem. Sci.*, 2 (2011) 609–614.
- [16] G. Lasarte-Aragones, R. Lucena, S. Cardenas and M. Valcarcel, *Talanta*, 131 (2015) 645– 649.
- [17] J. Rameshgar, K.S. Hasheminasab, L. Adlnasab and H. Ahmar, Switchablehydrophilicity solvent-based microextraction combined with gas chromatography for the determination of nitroaromatic compounds in water samples, J. Sep. Sci. 40 (2017) 3114– 3119.
- [18] L. Phan, D. Chiu, D.J. Heldebrant, H. Huttenhower, E. John, X. Li, P. Pollet, R. Wang, C.A. Eckert, C.L. Liotta and P.G. Jessop, Switchable solvents consisting of amidine/alcohol or guanidine/alcohol mixtures, *Ind. Eng. Chem. Res.* 47 (2008) 539-545.
- [19] P.G. Jessop, S.M. Mercer, D.J. Heldebrant, CO2 -triggered switchable solvents, surfactants, and other materials, *Energy. Environ. Sci.* 5 (2012) 7240-7253.

COPYRIGHTS

- [20] B. Ahmadi Abd, K.S. Hasheminasab, M. payehghadr and K. Shahbazi, Measurement of free amino acids in soil and agricultural fertilizer samples by optimization of high performance liquid chromatography equipped with ultraviolet detector, J. Appl. Chem., 16 (2021) 21-32
- [21] P.G. Jessop, D.J. Heldebrant, X. Li, C.A. Eckert and C.L. Liotta, Green chemistry: reversible nonpolar-to-polar solvent, *Nature*, 436 (2005) 1102-1102.

© 2022 by the authors. Lisensee PNU, Tehran, Iran. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution 4.0 International (CC BY4.0) (http://creativecommons.org/licenses/by/4.0)

Iranian Journal of Analytical Chemistry 9 (2022) 56-62 | 62

تعیین اسیدهای آمینه آزاد در نمونه های کود با استفاده از استخراج مبتنی بر حلال آب دوستی قابل تغییر جفت شده با کروماتوگرافی مایع با کارایی بالا مجهز به آشکارساز فرابنفش

کبرا سادات هاشمی نسب*۱، کریم شهبازی^۲

۱. استادیار موسسه تحقیقات خاک و آب، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران
 ۲. دانشیار موسسه تحقیقات خاک و آب، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران
 ۲. دانشیار موسسه تحقیقات خاک و آب، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران

چکیدہ

در پژوهش حاضر، از استخراج مبتنی بر حلال آبدوست قابل تعویض جفت شده با کروماتوگرافی مایع با کارایی بالا مجهز به أشکارساز فرابنفش برای تعیین اسیدهای آمینه آزاد در نمونههای کود استفاده شد. هنگام تبدیل فاز، حلال آلی به طور موثر آنالیت های هدف را جذب می کند. در این روش استخراج، ۲۰۰ میکرولیتر از یک حلال غیر قابل امتزاج با آب (دی پروپیلامین) استفاده می شود که می تواند در فاز آبی اسیدی حل شود. سپس جداسازی فاز با افزودن هیدروکسید سدیم انجام می شود. منغیرهای موثر بر این روش برای دستیابی به بهترین بازده استخراج بهینه شدند. شرایط بهینه شامل: حجم نمونه ۲۵ میلی لیتر، حجم حلال استخراج ۲۰۰ میکرولیتر و زمان استخراج ۲ دقیقه بود. تحت شرایط بهینه، حدود تشخیص خوب (۲۰۰۱–۲۰۰۰۶ میکرومول بر میلیلیتر)، خطی (۹۲۷–82) ، دقت (انحراف استاندارد نسبی کمتر از ۵٪) به دست آمد. در نهایت، روش توسعهیافته با موفقیت برای تعیین آنالیتهای هدف در انواع مختلف نمونههای کود به کار گرفته شد و بازیابی قابل قبولی قابل قبولی ۲/۹۲ دست آمد. در نهایت، روش توسعهیافته با موفقیت برای تعیین آنالیتهای هدف در انواع مختلف نمونههای کود به کار گرفته شد و بازیابی قابل قبولی قابل قبولی قابل قبولی قابل قدر ا

واژەھاي كليدى

استخراج مبتنى بر حلال أبدوست قابل تعويض، أمينو اسيدهاى أزاد؛ كود، دى پروپيل أمين؛ كروماتوگرافى مايع با كارايى بالا مجهز به أشكارساز فرابنفش.