Article

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Large-scale Enrichment of Sulfur-containing Acid in Acetonitrile Using Triazine Analog as the Derivatizing Reagent for Desorption from Humic-fraction-modified Silica Gel

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The analog of triazine is used as the derivatizing reagent for enriching large-scale acid (e.g. amino acid) containing a sulfur atom on the humic-fraction-modified silica gel in acetonitrile and desorbing from the adsorbent in hexane, respectively. The percent yield of the chemical derivatization under alkaline conditions, ranging from about 8 to almost 100%, is pH dependent, and varies significantly among these examined analytes, believed to be due to the structure of the analyte, not the derivatizing reagent. The percentage of enrichment, not reproducible under an aqueous environment and independent of the type of triazine analogs, reaches almost 100% in all cases. The force leading to the adsorption is the complexation between carboxyl groups on analyte and a humic-fraction-modified adsorbent based on the adsorption equilibrium results. Consequently, these results are not reproducible under ethyl ether or methanol environments due to the competition for binding sites from solvent molecules.

Keywords: Humic fraction; Enrichment; Desorption; Carboxyl group; Triazine analog; Sulfur-containing acids.

INTRODUCTION

Humic acid (HA) is a product of the microbial degradation of dead plant matter and can be found in soil nearly everywhere at variable content.[1] Its structural complexity, being a mixture of numerous acids with various components, such as quinone, phenol, catechol and sugar moieties, leads to many agricultural benefits and applications.[2-6] Other than that, the sorption-desorption of quaternary herbicides in vineyard-devoted soils, for example, has been described.[7-11] Applications that are adsorption-oriented, using humic-fraction-modified silica gel as the adsorbent, have been implemented recently in carboxyl-containing pesticides and biogenic amines in acetonitrile, organophosphorus pesticides, phthalate-based plasticizers, triazine analogs and pesticidal carboxylate esters in hexane.[12-15] In these studies, analytes are dissolved and adsorbed under suitable conditions. Additives that present in the matrix negatively affect the adsorption. No additive was involved in the process. Note, adsorption for analytes not soluble in acetonitrile or hexane will not occur.

In this work, a humic-fraction-modified silica gel was used as the adsorbent for enriching several sulfur-containing acids (e.g. cysteine) in large scale in acetonitrile after chemical derivatization with a triazine analog containing chlorine atom. These triazine analogs, along with those bearing no chlorine atom, are worldwide used pre-emergent weed herbicides for animal feed crops in the United States.[16-18] This is the first time they are used as the derivatizing reagents in an adsorption-oriented enrichment process. How the structure of the analyte and triazine analog affect both the chemical derivatization yield and the percentage of enrichment was explored, and discussed as well. Finally, the chemical derivatization reaction and adsorption mechanisms were carefully studied by examining the FTIR results, and by comparing the adsorption equilibrium data and the adsorption behavior in different liquid phases with those obtained in previous work.

EXPERIMENTAL PROCEDURES

Apparatus: The HPLC system used in this study was a Hitachi Model L-7100 coupled to a D-2500 Chromatopac data station and a UV detector. The detection wavelength for the adsorption evaluation, enrichment and derivatization yield measurements was set at 275 nm in all cases. A Hitachi spectrometer Model U-3900 was used to acquire the UV spectra. FTIR spectra with a resolution of 4 cm^{-1} were obtained by scanning samples 10 times on a Shimadzu Model FTIR-8400 system.

Chemicals: All chemicals employed in this study, including the organosilane reagent used as a linker in chemical immobilization reactions, the sulfur-containing acids, triazine analogs

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and the reagents used in enrichment evaluation and chemical derivatization reactions were purchased from Sigma (St. Louis, MO, USA) and the Aldrich Chemical Co. (Milwaukee, WI, USA), respectively. The silica gel (5 μm particle diameter, 100 Å porosity with a specific surface area of 400 m^2/g), used as the supporting matrix of the solid phase in the enrichment evaluation at ambient temperatures, was a product of Silicycle (Quebec City, QC, Canada) and was chemically modified with a humic fraction collected under acidic conditions before being used as the adsorbent according to the derivatization procedures reported previously.[19,20] The solvents, such as toluene, acetonitrile, methanol, triethylamine, methylene chloride, hexane and ethyl ether, were of an HPLC grade and purchased from Fisher Scientific (Pittsburgh, PA, USA) and Merck Taiwan Ltd. (Taiwan, ROC). They were used to wash the humic-fraction-modified silica gel before the adsorption evaluation, and the adsorption followed by the enrichment evaluation and also used as the mobile phase in HPLC analysis. In all cases, filtered (0.2 μm) and distilled water were used.

Conditions for Percent Yield Measurement and Enrichment Evaluation: A standard solution of a triazine analog was prepared at a concentration of 2.57×10^{-3} M, and used as the reference for estimating the percent yield of derivative in the 3-h chemical derivatization reaction by comparison based on the chromatographic peak area. The UV spectra for atrazine and its cysteine derivative, purified through an adsorption procedure described in this work, are shown in Figure 1. As can be seen, a similarity between the two spectra was observed. The molar absorptivity of the atrazine derivative of cysteine was calculated to be 2.13×10^5 L/mol cm and found to be reasonably close to that for atrazine.[21] As a result, the estimation by comparison based on the chromatographic peak area was acceptable.

An HNO_3 concentrate solution was diluted to obtain the desired pH, which in turn was used in the 3-h derivatization reaction to evaluate the pH effect on the percent yield of a derivatization reaction.

In the enrichment evaluation, a matrix solution was sampled for HPLC analysis first before a weighed amount of humic-fraction-modified adsorbent (10 mg) was added to it for a 3-h adsorption after the derivatization reaction under the alkaline conditions was completed. The adsorbent was collected by decanting the liquid phase, and it was washed several times with acetonitrile before being left to dry under a reduced pressure and lowered temperature. 100 μL of fresh hexane was then added to the dried adsorbent to release the derivative. The resulting solution was sampled immediately without further treatment for the HPLC analysis with a C_{18} column (150 × 4.6 mm i.d.; 5-μm particle diameter) under the elution of an acetonitrile solvent at a flow rate of 1.0 ml/min. The percentage of enrichment was calculated in triplicate to obtain an average in all cases, based on the difference in peak areas obtained before and after the enrichment evaluation. The enrichment was also evaluated in the liquid phase of acetonitrile and ethyl ether, respectively. These data were then used in the discussion of a mechanism involved in the adsorption process.

The chemical derivatization reaction was carried out according to the procedure in previous work.[22] The amount of derivatizing reagent used in the reaction was deliberately in excess of 2.00×10^{-3} M, the concentration of analyte, in order to drive the reaction in the right direction for completeness.

RESULTS AND DISCUSSION

The derivatizing reagents dominating the solid-phase peptide synthesis strategies for many years for amino protection are acid chloride typed. The most widely used of them include *N*-*tert*-butyloxycarbonyl (*t*-BOC) and fluorenylmethyloxycarbonyl (FMOC) chlorides.[23-26] As described previously, the generated reagent cations, due to the leaving of highly electronegative chlorine anions, will react with the nucleophilic side chains of amino acids to form a peptide bond under alkaline conditions.[27-31] This mechanism for chemical reaction is also applicable to the other class of derivatization reagents, such as isocyanate and isothiocyanate.[32-37] However, these compounds were mainly used to create additional attractive (e.g. cyanate group) or repulsive forces (e.g. sulfur atom) for enantioresolving analyte containing amino groups through the

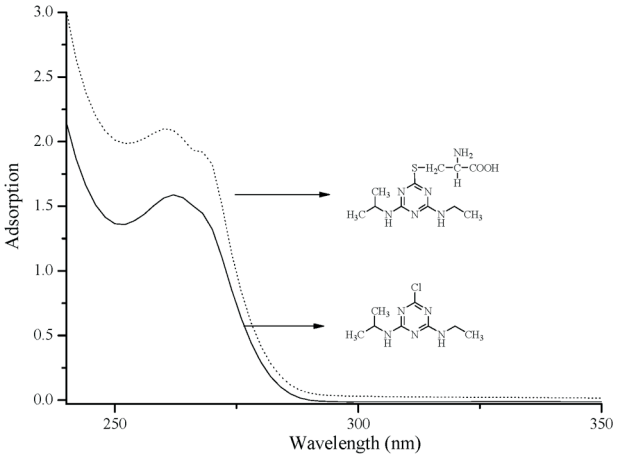

Fig. 1. The UV spectra of derivatizing reagent atrazine and cysteine derivative of atrazine obtained by purification method described in this study.

HPLC approach under polar-organic elution instead of being the group protector for solid-phase peptide synthesis. In this study, the reaction mechanism for the derivative product of sulfur-containing acid by reacting with atrazine analogs remains the same, as shown in Figure 2. It was believed that a positively charged carbon atom at Position 1 was induced as a result of strong electronegativity of the chlorine atom attached to it under alkaline conditions and was subject to the attack of a nucleophile-containing electron-rich functional group with the elimination of one HCl molecule. This proposed mechanism is also supported by the FTIR spectrum in Figure 3. The characteristic stretching frequency centered at 2547.39 cm^{-1} for S-H of cysteine (top) and for C-Cl of atrazine at 990.09 cm^{-1} (middle) was disappeared. However, the vibrational frequency centered at 805.89 cm^{-1} for C-S-C of atrazine derivative of cysteine (bottom) was observed. These FTIR results, along with the percent yield results summarized in Table 1, clearly indicated that it was the sulfur, not the nitrogen or oxygen atom on the analyte, that conducted the nucleophilic attack. This happened because the nitrogen or oxygen atom in the amino or hydroxyl group attracts electrons more tightly making these groups weaker nucleophiles than the thio group in an aqueous phase.[28-30] Furthermore, the nitrogen in an α-amino acid was not ready to donate its lone pair of electrons due to the nearby electron withdrawn carboxylic group. As a result, the percentage yield of derivatization for those analytes bearing no amino groups, such as 3-mercaptopropionic acid in Table 1, was affected negatively. In addition, the percentage yield of derivatization for an analyte with a methylated thio group, such as methionine, was also expected to be low because the nearby hydrogen atom for producing HCl molecules in the derivatization reaction was absent. The aforementioned description clearly accounts for the significantly varied percent yield of derivatization under alkaline conditions ranging from about 8 to almost 100%, as shown in Table 1.

This mechanism of derivatization involving a triazine analog was further investigated by changing the pH value

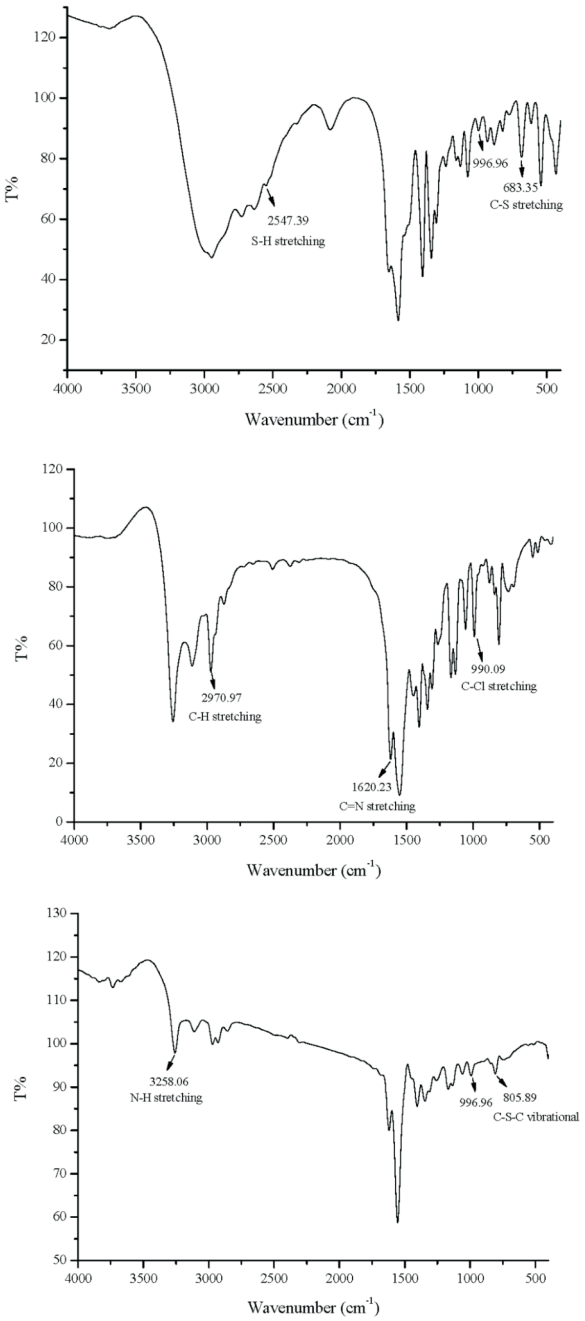

Fig. 3. FTIR spectra for cysteine (top), atrazine (middle) and atrazine derivative of cysteine (bottom).

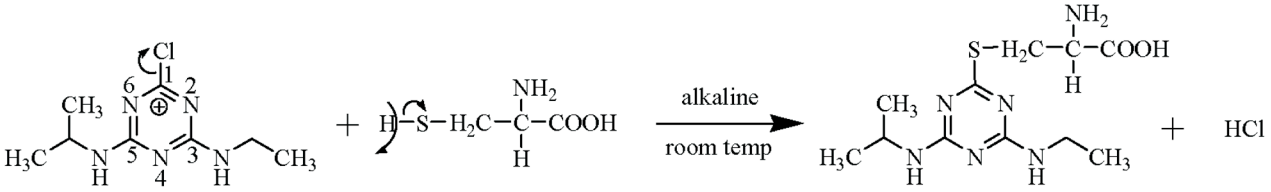

Fig. 2. The proposed mechanism for the derivatization reaction involving in atrazine and cysteine under the alkaline conditions.

Table 1. The percent yield of derivative resulted from the reaction of sulfur-containing acids with three triazine-type derivatizing reagents under alkaline conditions[a]

Analyte, reagent Structures *vs.* percent yield[b], Enrichment (%)	Atrazine	Propazine	Trietazine
Cysteine HS−H$_2$C−CH(NH$_2$)−COOH	88.18% (~100)	~100% (~100)	90.01% (~100)
Homocysteine HS−H$_2$C−H$_2$C−CH(NH$_2$)−COOH	~100% (~100)	~100% (~100)	~100% (~100)
Methionine H$_3$C−S−H$_2$C−H$_2$C−CH(NH$_2$)−COOH	7.11%	6.05%	7.89%
Penicillamine H$_3$C−C(SH)(CH$_3$)−CH(NH$_2$)−COOH	15.29%	1.22%	13.31%
Thiolactic acid HS−CH(CH$_3$)−COOH	48.01% (~100)	51.00% (~100)	47.31% (~100)
3-Mercaptopropionic acid HS−CH$_2$−CH$_2$−COOH	8.50%	8.80%	7.66%

[a] The saturated Borax solution was used as the liquid phase for dissolving analyte in all cases. The data were an average of three measurements. The chemical derivatization reaction was carried out for a 3-h time period.
[b] The data in parentheses represent the percentage of enrichment. Note that only the derivatives with sizable percent yields were measured. All the data were an average of three measurements.

under a chemical reaction. Results summarized in Figure 4 show the effect of pH change on the percent yield for the derivatization of cysteine using atrazine as the derivatizing reagent. As can be seen, lower percent yields were observed as the pH values were lowered. This is because the concentration of hydrogen ions at a lower pH is expected to be high, and thus, hinder the production of HCl in the derivatization process, which in turn would lower the percent yield of derivatization reaction.

Table 1 also lists the percentages of enrichment for several selected derivatives through a 3-h adsorption on humic-fraction-modified silica gel in acetonitrile. In the enrichment evaluation, sulfur-containing acids were first derivatized with triazine analogs to obtain chromophore for easy detection in HPLC analysis. The other purpose for using triazine analogs as the derivatizing reagent was their specific desorptive characteristic in acetonitrile on humic-fraction-modified silica gel.[12,15] This controllable adsorption/desorption process by simply switching liquid phases on the same adsorbent is the key for this method to be applicable to enrich sulfur-containing acids in a large scale. In other words, selective adsorption/desorption for a particular component (e.g. derivatizing reagent or derivative) and the removal of residual reagents (e.g. salt from the alkaline Borax solution, and the remaining, unreacted analyte) in the derivatization reaction can be easily achieved just by changing the liquid phase. As can be seen, the percentage of enrichment in Table 1 for these selected derivatives

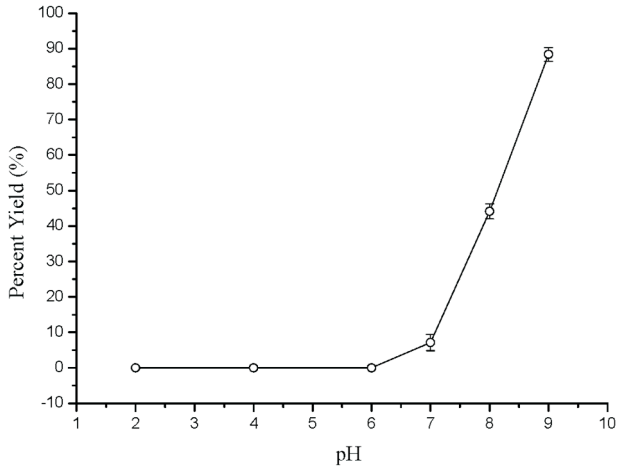

Fig. 4. The dependence of the percent yield of derivative on the pH for a 3-h derivatization reaction.

reached almost 100% in all cases. A typical chromatogram showing the enrichment results for an atrazine-cysteine derivative is shown in Figure 5. Since the supernate was only decanted after a 3-h adsorption in acetonitrile, the chromatographic peak for atrazine still existed. However, the peak intensity was reduced dramatically revealing the importance of washing the adsorbent with fresh acetonitrile to fully remove the residual atrazine before dissolving the adsorbent in hexane to release the adsorbed derivative for sequential HPLC analysis. The enrichment was also examined in other liquid phases, such as ethyl ether and methanol, under the same conditions. Unfortunately, reproducibility of the aforementioned results was not observed owing to the competition for the limited number of binding sites on the adsorbent from solvent molecules.[12-15]

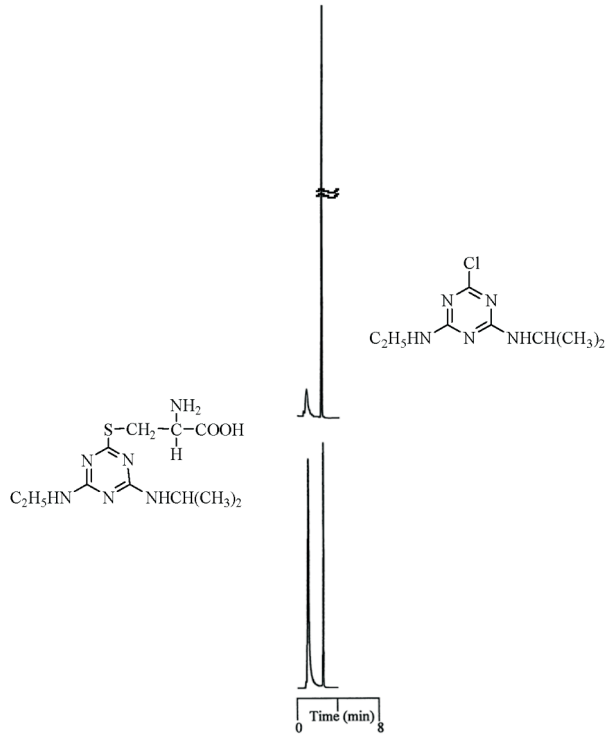

Fig. 5. The HPLC chromatograms with UV detection showing the enrichment of atrazine-cysteine derivative before and after the process.

The mechanism for adsorbing pesticidal compounds bearing the single carboxyl functional group by humic-fraction-immobilized silica gel in acetonitrile has been reported.[12] Complexation interaction between carboxylic groups of the analyte and the adsorbent was the force resulting in the adsorption. However, the triazine analogs, used as the derivatizing reagents in this work, were adsorbed in hexane through a dipole-dipole oriented mechanism characterized by a faster adsorption equilibrium.[15] In the present work, an atrazine-cysteine derivative was first enriched through the proposed method, and then, used in the adsorption equilibrium study in acetonitrile. Upon comparison, a high similarity in the profile was observed in the 3-h adsorption evaluation as shown in Figure 6. This clearly indicated that the resulting adsorption was due to the interaction between the carboxylic groups of analyte, not the triazine moiety, and the adsorbent molecule.

CONCLUSIONS

Enriching derivative of the sulfur-containing acid in

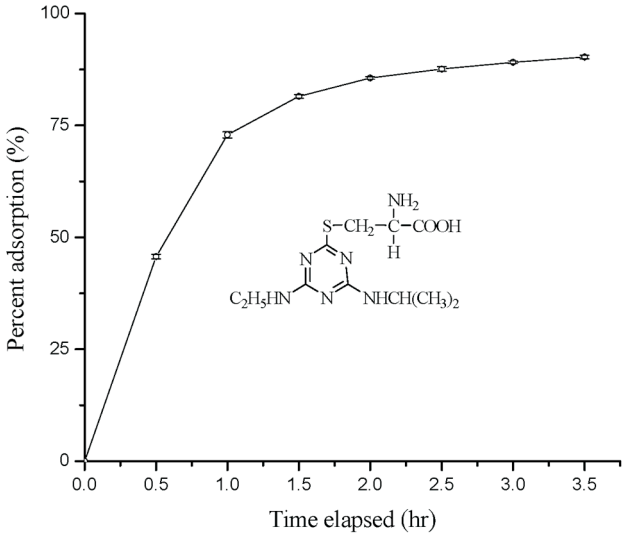

Fig. 6. The 3-h adsorption equilibrium of cysteine derivative of atrazine in acetonitrile on the humic-fraction-modified adsorbent.

large scale through the adsorption/desorption process accompanied with the switching of liquid phases on the humic-fraction-modified silica gel is demonstrated to be plausible. The chemical derivatization is pH dependent and the corresponding percent yield varies significantly among these examined analytes under the alkaline conditions by ranging from about 8 to almost 100%, and is believed to be due to the structure of the analyte, not the derivatizing reagent. The percentage of enrichment as a result of the complexation between carboxyl groups on analyte and an adsorbent reaches almost 100% in all selected cases, and is not reproducible under aqueous, ethyl ether and methanol environments.

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REFERENCES

1. Stevenson, F. J. *Humus Chemistry: Genesis, Composition, Reactions*; John Wiley & Sons: New York, 1994.
2. Baigorri, R.; Fuentes, M.; González-Gaitano, G.; García-Mina J. M.; Almendros, G.; González-Vila, F. J. *J. Agric. Food Chem.* **2009**, *57*(8), 3266–3272.
3. Alvarez-Puebla, R. A.; Valenzuela-Calahorro, R. A. C.; Garrido, J. J. *Sci. Total Environ.* **2006**, *358*, 243–254.
4. Piccolo, A. *Adv Agron.* **2002**, *75*, 57–134.
5. Ghabbour, E. A.; Davies, G. *Humic Substances: Structures, Models and Functions*; RSC publishing: Cambridge, 2001.
6. Fernández-Calviño, D.; Rodríguez-Suárez, J. A.; López-Periago, E.; Arias-Estévez, M.; Simal-Gándara, J. *Geoderma* **2008**, *145*(1-2), 91–97.
7. Pateiro-Moure, M.; Pérez-Novo, C.; Arias-Estévez, M.; López-Periago, E.; Martínez-Carballo, E.; Simal-Gándara, J. *J. Agric. Food Chem.* **2007**, *55*(15), 6219–6226.
8. Pateiro-Moure, M.; Arias-Estévez, M.; López-Periago, E.; Martínez-Carballo, E.; Simal-Gándara, J. *Bull. Environ. Contam. Tox.* **2008**, *80*(5), 407–411.
9. Pateiro-Moure, M.; Pérez-Novo, C.; Arias-Estévez, M.; Rial-Otero, R.; Simal-Gándara, J. *J. Colloid Interf. Sci.* **2009**, *333*(2), 431–438.
10. Pateiro-Moure, M.; Nóvo-Muñoz, J. C.; Arias-Estévez, M.; López-Periago, E.; Martínez-Carballo, E.; Simal-Gándara, J. *J. Hazard. Mater.* **2009**, *164*(2-3), 769–775.
11. Pateiro-Moure, M.; Arias-Estévez, M.; Simal-Gándara, J. *J. Hazard. Materi.* **2010**, *178*(1-3), 194–201.
12. Chen, C.; Chen, S. *J. Agric. Food Chem.* **2013**, *61*(15), 3600–3610.
13. Lai, Y.-S.; Chen, S. *J. Chem. Eng. Data* **2013**, *58*(8), 2290–2301.
14. Lai, Y.-S.; Chen, C.; Chen, S. *J. Agric. Food Chem.* **2013**, *61*(22), 5286–5290.
15. Hu, S. W.; Chen, S. *J. Agric. Food Chem.* **2013**, *61*(36), 8524–8532.
16. Rippen, G. *Handbuch Limueltchemikalien*, 3rd ed.; 16 Erg Lfg.; Ecomed: Landsberg/Lech: Germany, 1992.
17. Environmental Protection Agency. Cyanazine; Notice of Final Determination to Terminate Special Review of Cyanazine; Notice of Voluntary Cancellation and Cancellation Order of Cyanazine Product Registrations. *Federal Register.* **1996**, *61*, 39024–39029.
18. Buser, H. R. *Environ. Sci. Technol*. **1990**, *24*, 1049–1058.
19. Armstrong, D. W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill, C.; Chen, J. R. *Anal. Chem.* **1994**, *66*, 1473–1484.
20. Hsiao, Y.; Chen, S. *Chromatographia* **2009**, *70*, 1031–1038.
21. Tamrakar, U.; Mathew, S. B.; Gupta, V. K.; Pillai, A. K. *J. Anal. Chem.* **2009**, *64*(4), 386–389.
22. Chen, S. *Amino Acids* **2004**, *26*, 291–298.
23. Lundt, B. F.; Johansen, N. L.; Vølund, A.; Markussen, J. *Int. J. Pept. Protein Res.* **1978**, *12*(5), 258–268.
24. Andrew, B. H. *Protection Reactions. Amino Acids, Peptides and Proteins in Organic Chemistry: Protection Reactions, Medicinal Chemistry, Combinatorial Synthesis, Volume 4*. Wiley-VCH: Germany, 2011.
25. McKay, F. C.; Albertson, N. F. *J. Am. Chem. Soc.* **1957**, *79*(17), 4686–4690.
26. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*(22), 3404–3409.
27. Edman, P. *Acta Chem. Scand.* **1950**, *4*, 283–293.
28. Capriel, P.; Haisch, A.; Khan, S. U. *J. Agric. Food Chem.* **1985**, *33*, 567–569.
29. Acosta, E. J.; Steffensen, M. B.; Tichy, S. E.; Simanek, E. E. *J. Agric. Food Chem.* **2004**, *52*, 545–549.
30. Wells, P. R. *Chem. Rev.* **1963**, *63*, 171–219.
31. Dec, J.; Bollag, J. M. *J. Environ. Qual.* **2000**, *29*, 665–676.
32. Chen, S. *Biomed. Chromatogr.* **2006**, *20*(8), 718–728.
33. Chen, S. *Chromatographia* **2006**, *63*(1-2), 97–102.
34. Chen, S. *Amino Acids* **2004**, *26*(3), 291–298.
35. Chen, S. *J. Liq. Chromatogr. Relat. Technol.* **2003**, *26*(20), 3475–3495.
36. Chen, S. *J. Liq. Chromatogr. Relat. Technol.* **1994**, *17*(3), 483–497.
37. Pawlowska, M.; Chen, S.; Armstrong, D. W. *J. Chromatogr., A* **1993**, *641*(2), 257–265.

