

## DETERMINATION OF CARBOFURAN ON HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY USING TSK GEL AMIDE 80 AS STATIONARY PHASE

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### ABSTRACT

The hydrophilic interaction liquid chromatography (HILIC) coupled to environmental friendly capillary liquid chromatography was employed to investigate retention behavior of carbofuran. Aim of this research is to investigate retention behavior of carbofuran using TSK gel amide 80 as stationary phase. Several condition was conducted to investigate retention behavior of carbofuran such as comparison study of TSK gel amide 80 with other polar column, comparison study retention behavior of carbofuran on various system wavelength, water content effect in HILIC mode, effect of buffer concentration on HILIC mode, and analytical performance. Results showed that TSK gel imidazole was superior compare to other polar stationary phase on determine carbofuran, wavelength 251 and 254 nm was resulting higher absorbance for carbofuran than others, increase of water content on mobile phase was decrease the retention time, also increase buffer salt concentration was decrease the retention time and according to analysis performance that is the accuracy was  $101 \pm 10,1$ , the LOD 0.66 ppm while LOQ 2.22 ppm. As conclusions that TSK gel amide 80 was offering good determine on carbofuran even using capillary liquid chromatography with 10 cm length of column.

Keywords : carbofuran, HILIC, TSK gel amide 80, capillary liquid chromatography

### INTRODUCTION

Most of determination of pesticides are on reverse phase chromatography [1-10]. The gradient elution in combination with binary solvent systems, i.e. with water and acetonitrile or methanol added with acetate or formate buffer. Reverse phase chromatography means using non polar compound as stationary phase and polar compound as mobile phase. The other hand, the non polar stationary phase on reverse phase mode still have disadvantages, e.g. low compatibility with high aqueous eluent and insufficient selectivity towards polar solutes [11-15].

Hydrophylic interaction chromatography (HILIC) has become an alternative technique for separation of polar and hydrophylic compound [16-28]. The term of HILIC was proposed by Alpert in 1990 as metode complementary both normal and reverse-phase chromatography which is combine an acetonitrile-rich and low aqueous mobile phase to elute the analytes in order of increasing polarity. Partitioning of the solutes from mobile phase into hydrophilic layer caused by the water-enriched liquid layer that is establish upon stationary phase. The selectivity of the separation is influenced by interactions such as ion-exchange, hydrogen bonding, and dipole-dipole. In addition, the use of high percentage of organic solvents (usually acetonitrile) enhanced the ionization and increase the sensitivity in mass spectrophotometry [29-34].

Carbofuran is the most toxic pesticides which is in carbamate group that widely used all over the world to eradicated the insect. Carbofuran has polar site on the

molecule in which possibly to bond and separate using polar stationary phase [35-40]

TSK Gel Amide 80 is silica bonded (covalent) carbamoyl groups that usually used in normal or Hilic mode, with the target saccharide, peptides, and polar compound. TSK Gel Amide 80 is superior stability over conventional amide bonded phase column. Carbamoyl group of TSK gel amide 80 is superior for determining organic polar compound as like carbofuran. HILIC using TSK gel amide 80 column offers better selectivity, retention, peak shape and reproducibility compare to reverse phase liquid chromatography column [41-43]. In addition, using TSK gel amide 80 as HILIC stationary phase to determine carbofuran propose easy process compare to Gas Chromatography (GC) that have to use derivatization [44]. Aim of this research is to investigate retention behavior of carbofuran using TSK gel amide 80 as stationary phase.

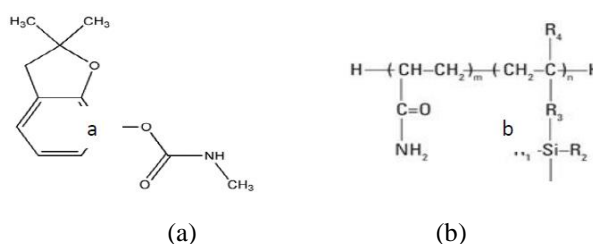


Fig 1. a). Chemical structure of carbofuran,  
b). Chemical structure TSK gel amide 80

## EXPERIMENTAL

### Reagents and Materials

The commercial standard of carbofuran was purchased from chem service USA. Acetonitrile was of HPLC grade from merck (Darmstadt, Germany). Ultra pure water from simplicity UV system (Milipore, Bedford, MA, USA) with the electrical resistivity of  $18,2 \text{ M}\Omega \text{ cm}^{-1}$  was used in all experiments. Stock solution of carbofuran as well as their diluted mixture were prepared in acetonitrile. Ammonium acetate was purchased from Nacalai (Kyoto, Japan). All solutions were filtered through PTFE  $0.45 \mu\text{m}$  membrane filters (GL chromatodisc). Stationary phase is TSK gel amide 80 ( $5 \mu\text{m}$  size particle) and DEAE-2SW was purchased from Tosoh Corp. (Tokyo, Japan), polar imidazole and polar pyridine was purchased from Sepax Technologies, inc (USA).

### Instruments

Capillary LC system used in this work consists of an L.TEX 8301 micro-feeder equipped with an MS-GAN 050 gas-tight syringe (0.5 mL Ito, Fuji, Japan) as a pump, a model Rheodyne-7520 microinjection valve (Cotati, California, USA) with the injection volume of  $0.2 \mu\text{L}$  as an injector, a  $0.32 \text{ mm i.d} \times 0.45 \text{ mm o.d} \times 100 \text{ mm}$  micro-column, a model Jasco UV-2075 as UV Detector. The data were acquired by a CDS ver 5 data processor (copyright LAsoft, USA).

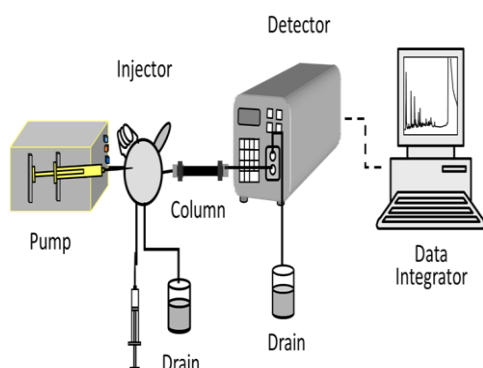


Fig 2. Capillary liquid chromatography system

### Column Packing

TSK gel amide 80 was packing as HPLC column ( $0.32 \text{ mm i.d} \times 0.45 \text{ mm o.d} \times 100 \text{ mm}$ ) by a slurry packing method. The column packing conditions were as follows :  $0.1 \text{ g}$  of TSK gel amide 80 were disperse in  $1 \text{ ml}$  methanol to form a suspension, which was slurry-packed into a fuse silica capillary column ( $0.32 \text{ mm i.d} \times 0.45 \text{ mm o.d} \times 100 \text{ mm}$ ) using syringe that equipped by a long teflon tube with  $0.2 \text{ ml}$  methanol as propelling solvent.



Fig 3. Capillary column

### Chromatographic condition

The chromatographic evaluation, the column temperature was as room temperature (about  $25^\circ\text{C}$ ). The flow rate was  $2 \mu\text{L min}^{-1}$  and injection volume  $0.2 \mu\text{L}$ . All HPLC analysis was carried out at least in duplicate.

- Comparison study of TSK gel amide 80 as stationary phase with other polar stationary phase.
- Comparison study of the system wavelength. The wavelength  $251, 254, 276, 280, 282 \text{ nm}$  was used in this experiment.
- Water content effect in HILIC mode : to study the effect of various concentration of water in HILIC mode on retention behavior. Binary mobile phase system is used that is acetonitrile and water with various concentration of water as follow  $10 \%, 20 \%, 30 \%$  and  $40 \%$ .
- Effect of buffer concentration on HILIC mode. To study effect of various concentration of buffer salt on retention behavior. The mobile phase is ACN with variation of ammonium acetate  $5, 10, 15$  and  $20 \text{ mM}$ .
- Analytical performance such as accuracy, %RSD, LOD and LOQ.

## RESULTS AND DISCUSSION

### Comparison study of TSK gel amide 80 as stationary phase with other polar stationary phase

The purpose of the comparison is to investigate retention behavior of carbofuran on different polar stationary phase. The comparison stationary phase was used that are polar pyridine, polar imidazole, and DEAE-2SW. Investigation of retention behavior of carbofuran was conducted using  $10 \text{ ppm}$  concentration of carbofuran and  $85\%$  concentration of acetonitrile. Fig 1. showed that peak height of using TSK gel amide 80 is two time bigger than other stationary phase. It showed that using TSK gel amide 80 more sensitive than others. TSK Gel Amide 80 is superior stability over conventional amide bonded phase column. Carbamoyl group of TSK gel amide 80 is superior for determining organic polar compound as like carbofuran. The result indicated that pesticide compound is suitable to be detected by HILIC due to their hydrophilicity and polarity. Carbofuran has amine functional group as secondary amine whereas very active due to have nitrogen which is as donor a proton (positive

charge) to hydrogen, so it can interacted with the surface of amide functional group of TSK gel amide 80. This compound also has carbonyl functional group that bonding with amine forming amide that also as polar site by available of oxygen double bonding, also it can interact with the surface of amide functional group of TSK gel amide 80

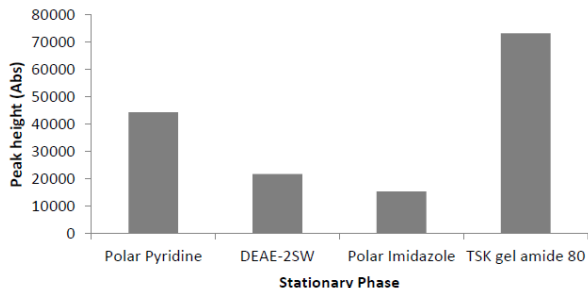


Fig.4 The peak height of carbofuran on different concentration of carbofuran using various stationary phase.

**Comparison study of system wavelength.**

The wavelength 251, 254, 276, 280, 282 nm was used in this experiment which is this wavelength according to other research results. Fig 2. Showed that wavelength of 251 and 254 nm give better peak height than others. It showed that wavelength of 251 until 254 nm was optimum wavelength for carbofuran.

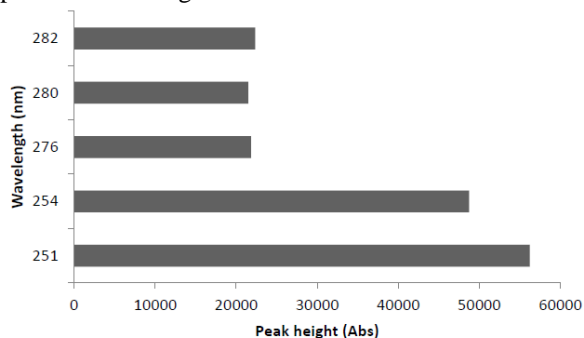


Fig.5 The peak height of carbofuran on different wavelength.

**Water content effect in mobile phase on HILIC mode.**

The mobile phase composition has big effect on the retention in HILIC mode and the large amount of organic solvent give more benefit the compound with low aqueous solubility. In HILIC, retention of the solution in the column is influenced by many factors such trials water content, pH, salt concentration and temperature of the column buffer. In HILIC, A water-rich layer on the surface of stationary phase formed by mobile phase vs the water-deficient mobile phase, created a liquid-liquid extraction system which is the analyte distributed between these two layers.

The various concentration of water was changed from 10 to 40%. Fig 3. Showed that the retention behavior of carbofuran at various concentration of water in the mobile phase. The retention time was decreasing trend upon the

increasing of water content in the mobile phase, which indicated typical HILIC retention mechanism. The stationary phase TSK gel amide 80 exhibited pronounced retention for carbofuran in HILIC mode.

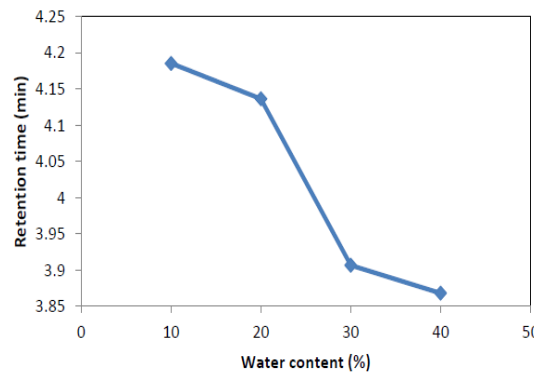


Fig 6. Plot of retention time and water content in the mobile phase for carbofuran.

**Effect of buffer salt concentration**

To control pH and ion strength mobile are usually used ion additives whereas they can also affecting to the polarity of the analyte that is making different in retention. The effect of buffer salt concentration was studied by employing different concentration of ammonium acetate (5,10,15, and 20 mM) in the mobile phase. The plot of retention factor to the concentration of ammonium acetate was shown in Fig 7. The retention time was decreasing trend upon increasing concentration of ammonium acetate. Higher salt concentration resulted in decrease in the retention time of carbofuran. Hence, repulsive electrostatic interaction may be activated between carbofuran and stationary phase.

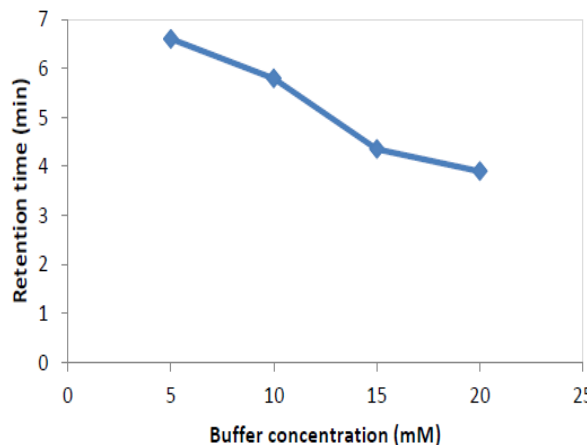


Fig 7. Plot of retention time and buffer concentration in the mobile phase for carbofuran.

### Analytical Performance

The various concentration of the carbofuran was conducted under HILIC mode. According to that chromatogram can calculated accuracy, %RSD, LOD and LOQ value. The value are  $101 \pm 10.1$ , 10.01 %, 0.66 mg/L, and 2.22 mg/L respectively.

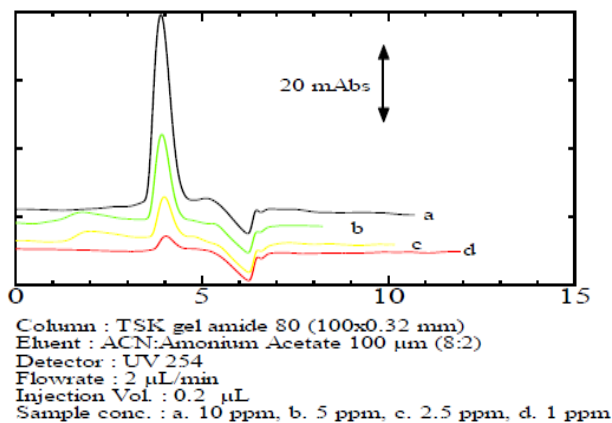


Fig.8. Retention behavior of carbofuran on various concentration using TSK gel amide 80 as stationary phase

### CONCLUSIONS

The use of TSK gel amide 80 as the stationary phase showed good ability in the determination of carbofuran. Retention behavior that occurs indicates the existence of a mechanism in the determination of carbofuran HILIC using TSK gel amide 80 as the stationary phase. TSK gel amide 80 was offering good determine on carbofuran even using capillary liquid chromatography with 10 cm length of column

### ACKNOWLEDGEMENT

The author would like to thank all those who have helped smooth this study primarily to Takeuchi Sensei and Lim Sensei as well as all members of the laboratory. Authors thanks also for IAARD who has given me the opportunity to continue studies

### REFERENCES

1. Saito-Shida, Shizuka; Nemoto, Satoru; Teshima, (2015), Reiko Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes. 50(11), 760-776
2. Wu, Lijie; Hu, Mingzhu; Li, Zhanchao; Song, Ying; Yu, Cui; Zhang, Hanqi; Yu, Aimin; Ma, Qiang; Wang, Ziming (2016), Food Chemistry , 192, 596-602.
3. Costa, Anna I. G.; Queiroz, Maria E. L. R.; Neves, Antonio A.; de Sousa, Flaviane A.; Zambolim, Laercio (2015) Food Chemistry , 181, 64-71.
4. Besil, Natalia; Ucles, Samanta; Mezcuca, Milagros; Heinzen, Horacio; Fernandez-Alba, Amadeo R. (2015) Analytical and Bioanalytical Chemistry, 407(21), 6327-6343.

5. Hanot, V.; Goscinny, S.; Deridder, M. (2015) Journal of Chromatography A , 1384, 53-66.
6. Tuzimski, Tomasz; Rejczak, Tomasz (2014) Journal of AOAC International, 97(4), 1012-1020.
7. Xia, Ning; Gao, Yanping (2015) International Journal of Electrochemical Science, 10(1), 713-724, 12 pp
8. Khin, L. Z, and Somporn, C., 2007., Chiang Mai J. Sci.; 34(2) : 227-234
9. K. Vinod Kumar and N. V. S. Naidu, Der Pharma Chemica, 2013, 5(1):313-319
10. Lijun He, Mingliang Zhang, Wenjie Zhao, Jie Liu, Xiuming Jiang, Shusheng Zhang, Lingbo Qu. Talanta 89 (2012) 433– 440
11. Nzoughet, J. Kouassi; Gallart-Ayala, H.; Biancotto, G.; Hennig, K.; Dervilly-Pinel, G.; Le Bizec, B., Metabolomics (2015), 11(6), 1884-1895.
12. Hernandez-Hernandez, Oswaldo; Quintanilla-Lopez, Jesus Eduardo; Lebron-Aguilar, Rosa; Sanz, Maria Luz; Moreno, F. Javier, Journal of Chromatography A (2015),
13. Contrepolis, Kevin; Jiang, Lihua; Snyder, Michael, Molecular & Cellular Proteomics (2015), 14(6), 1684-1695.
14. Zhou, L.; Wu, Y.; Johnson, B. D.; Thompson, R.; Wyvratt, J. M, (2000), Journal of Chromatography A, 866(2), 281-292.
15. Sanchez, R., Vazquez, A., Villen-Altamirano, J., and Villen, J., 2005. Journal of the Science of Food and Agriculture, Volume Date 2006, 86(1), 129-134.
16. Sentkowska, A., Biesaga, M., and Pyrzynska, K., 2013. Talanta. 115, p284-290.
17. Tu Liang, Qing Fu, Aijin Shen, Hui Wang, Yu Jin, Huaxia Xin, Yanxiong Ke, Zhimou Guo, Xinmiao Liang. 2015. Journal of Chromatography A. 1388 (2015) 110-118.
18. Lizhen Qiao, Abou Dou, Xianzhe Shi, Hua Li, Yuanhong Shan, Xin Lu, Guowang Xu, 2013. Journal of Chromatography A. 1286 (2013) 137-145
19. Jovanovic, Marko; Rakic, Tijana; Ivanovic, Darko; Jancic-Stojanovic, Biljana, Instrumentation Science & Technology (2015), 43(2), 156-169.
20. Zuo, Yuegang; Zhou, Si; Zuo, Ruiting; Shi, Tian; Yang, Yang; Henegan, Patricia., High-Performance Liquid Chromatography (HPLC) (2014), 1-21
21. Jovanovic, Marko; Rakic, Tijana; Jancic-Stojanovic, Biljana; Ivanovic, Darko; Medenica, Mirjana., Analytical and Bioanalytical Chemistry (2014), 406(17), 4217-4232
22. Willemse, Chandre M.; Stander, Maria A.; de Villiers, Andre., Journal of Chromatography A (2013), 1319, 127-140.
23. Kahsay, Getu; Song, Huiying; Van Schepdael, Ann; Cabooter, Deirdre; Adams, Erwin, Journal

- of Pharmaceutical and Biomedical Analysis (2014), 87, 142-154.
24. Kozlik, Petr; Simova, Veronika; Kalikova, Kveta; Bosakova, Zuzana; Armstrong, Daniel W.; Tesarova, Eva., *Journal of Chromatography A* (2012), 1257, 58-65.
  25. Danaceau, Jonathan P.; Chambers, Erin E.; Fountain, Kenneth J., *Bioanalysis* (2012), 4(7), 783-794.
  26. Onorato, Joelle M.; Langish, Robert; Bellamine, Aouatef; Shipkova, Petia., *Journal of Separation Science* (2010), 33(6-7), 923-929.
  27. Olsen, B. A., *Journal of Chromatography A* (2001), 913(1-2), 113-122.
  28. Noel S. Quiming, Nerissa L. Denola, Yoshihiro Saito, Alicia P. Catabay, Kiyokatsu Jinno, *Chromatographia* 2008, 67, April (No. 7/8)
  29. M. B. Sorde, B. N. Poul, A. Y. Ghodke, O. G. Bhusnure, *Int. Res J Pharm. App Sci.*, 2013; 3(2):137-142
  30. Bogusław Buszewski & Sylwia Noga *Anal Bioanal Chem* (2012) 402:231–247
  31. C. F. Poole, (2000). *Chromatography*, Wayne State University, Detroit, MI, USA. Academic Press
  32. E. Kenndler, (2004). *Introduction to Chromatography*. Institute for Analytical Chemistry, University of Vienna, Version 19/01/2004
  33. Raquel G. C. Silva, Carla B. G. Bottoli and Carol H. Collins, *Journal of Chromatographic Science*, 2012;50:649–657
  34. Stefan Kittlaus, Julia Schimanke, Günther Kempe, Karl Speer, *Journal of Chromatography A*, 1283 (2013) 98– 109
  35. Alexander L.N. van Nuijs, Isabela Tarcomnicu, Adrian Covaci, *Journal of Chromatography A*, 1218 (2011) 5964– 5974
  36. FAO/WHO (1985) Data Sheet on Pesticides No. 56 — Carbofuran. Food and Agriculture Organization of the United Nations and World Health Organization (VBC/PDS/DS/85.56).
  37. Food and Agriculture Organization of the United Nations (2002), International Code of Conduct on the Distribution and Use of Pesticides. Retrieved on 2007
  38. Chin-Pampillo, J.S., Hidalgo, K. R., Masís-Mora, M., Carazo-Rojas, E., and Rodríguez, C.E., 2015, *Environ Sci Pollut Res* DOI 10.1007/s11356-015-5093-3
  39. Wang, Jixia; Guo, Zhimou; Shen, Aijin; Yu, Long; Xiao, Yuansheng; Xue, Xingya; Zhang, Xiuli; Liang, Xinmiao, *Journal of Chromatography A* (2015), 1398, 29-46.
  40. Inoue, Koichi; Tsuchiya, Hirofumi; Takayama, Takahiro; Akatsu, Hiroyasu; Hashizume, Yoshio; Yamamoto, Takayuki; Matsukawa, Noriyuki; Toyo'oka, Toshimasa, *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* (2015), 974, 24-34.
  41. <http://www.separations.eu.tosohbioscience.com/Products/HPLCColumns/ByMode/NormalHydrophilic/TSKgel+Amide-80.htm>
  42. Feng, Bing; Cai, Youqiong; Zhang, Xiaoling; Yu, Huijuan, *Applied Mechanics and Materials* (2013), 395-396(Advanced Materials and Processes III), 633-636.
  43. Guo, Xiujie; Zhang, Xiuli; Guo, Zhimou; Liu, Yanfang; Shen, Aijin; Jin, Gaowa; Liang, Xinmiao, *Journal of Chromatography A* (2014), 1325, 121-128.
  44. Coburn JA, Ripley BD, Chau AS, 1976 Journal - Association of Official Analytical Chemists [59(1):188-196]
  45. Fabrizio Bruner, 1993. *Gas Chromatographic Environmental Analysis. Principles, Techniques, Instrumentation*. Wiley VCH Publisher, Inc. p79.