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Preparation and physical-chemical characterization of benzylamide stationary phase for High Performance Liquid Chromatography

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Abstract

This research project is focused on the development of an innovative methodology for the preparation benzylamide stationary phase, bonded onto silica surface. It involves the preparation of the stationary phase with benzoyl chloride and 3-aminopropyltrimethoxysilane and also the characterization by NMR-²⁹Si, NMR-¹³C, IR and Elementary Analysis. The NMR-²⁹Si spectrum of modified silica indicated the formation of siloxane bonding of the trialkoxysilane group with the silica, the IR spectra of stationary phase showed bands typical of aromatics and secondary amides. Analysing the NMR-¹³C spectra was possible to recognize all the carbons from the structure of the benzylamide stationary phase.

Key words:

Benzylamide stationary phase, preparation, characterization

Introduction

The stationary phase is of a great importance to the success of the separation of components of a mixture by High Performance Liquid Chromatography (HPLC), since it provides physical-chemical interactions with the analytes. The separation of polar and basic compounds, using conventional stationary phases is still problematic and, for this reason, it is interesting that new stationary phases be developed and tested to be used in this technique. This research project is focused on an innovative methodology for the preparation of a phenyl type stationary phase with embedded amide group (benzylamide stationary phase), bonded onto silica surface^[1]. Benzylamide stationary phase has been prepared by Nomura *et al.*^[2] using a heterogeneous phase. In this project, a new strategy was proposed in order to acquire a homogeneous phase, since it is expected to be more reproducible, avoiding parallel interactions of analytes with remaining silanol groups. In this methodology, the benzylamide silylating agent is first obtained and then chemically bonded onto the silica surface. By adding the characteristics of phenyl and amide groups, it is possible to have a phase that provides π - π interaction with the analytes, and at the same time, mainly through hydrogen bond, prevent it from interacting strongly with the residual silanols (acid characteristic) which could cause asymmetric peak

Results and Discussion

The silylating agent was prepared by adding the benzoyl chloride to the 3-aminopropyltrimethoxysilane in dichloromethane as solvent. The mixture was stirred for 24 h and then triethylamine was added and stirred for 2 h. The next step was to add 5 μ m Kromasil silica to the silylating agent in dry toluene and then reflux it by 72 hours. The prepared stationary phase was washed with solvents – isopropanol, dichloromethane, acetone and water/methanol 50/50- and dried at vacuum oven for 12 h and submitted to physical-chemical characterization to confirm the presence of the benzylamide group.

The results indicated that the benzylamide silylating agent was successfully prepared and was bonded onto the silica, since it was observed differences between the Infrared and NMR spectra of the pure and

bonded silica. In the NMR-²⁹Si spectrum of modified silica, the peak Q² was diminished as well as the ratio of Q³/Q⁴. The T² and T³ peaks were also observed at -55 and -65 ppm indicating the formation of siloxane bonding of the trialkoxysilane group with the silica. Also the IR spectra of stationary phase showed bands around 1540 and 1580 cm⁻¹ typical of aromatics and secondary amides. Analyzing the NMR-¹³C spectra in the image bellow, it was possible to recognize all the carbons from the structure of the benzylamide stationary phase.

Image 1. Analysis of NMR-¹³C spectra with the structure of the benzylamide phase

Finally, the elementary analysis showed an increase of carbon and nitrogen due to the incorporation of benzylamide group onto silica, and the theoretical (8.57) and experimental (8.36) ratios of C/N were similar: .

Table 1. Elementary Analysis

Carbon	Hydrogen	Nitrogen
19.74	1.99	2.36

Conclusions

The results showed that the benzylamide silylating agent was successfully prepared and bonded onto the silica. The next step will be the evaluations of the chromatographic performance with well established test-mixtures containing compounds of different polarities. The applicability of this phase will be evaluated using two mixtures: one of beta-blockers and other containing hydrophilic vitamins

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