

A New HPLC Method for Reversed-Phase Separations with Multi-Solvent, Multi-Column Selectivity, and Multi-Wavelength Spectrophotometric UV Detection

Michel Lau and Eugene M. Fujinari

Department of Chemistry and Biochemistry, San Francisco State University, CA 94132

Abstract

A reversed-phase HPLC application is optimized using non-nitrogen containing mobile phases that are compatible with the element specific chemiluminescent nitrogen detector (CLND). In order to accelerate HPLC method development of nitrogen containing compounds, an advanced HPLC system is configured with multi-solvent, multi-column selectivity, and multi-wavelength spectrophotometric UV detection capability. This system coupled with a CLND can provide key analytical approach for the biotechnology, pharmaceutical, food, and chemical industries.

Introduction

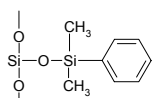
HPLC-CLND is a sensitive technique that is routinely used for quantitation of nitrogen-containing compounds in the low microgram range [1, 2]. A reversed-phase HPLC method using non-nitrogen containing mobile phases to study solvent selectivity, retention (k'), and column selectivity on C18 and PFP stationary phases was recently reported [3].

In this study, a HPLC system with online degassing and low pressure gradient capabilities was used to study the retention of several selected analytes on two different columns and two different mobile phase mixtures. The mobile phase was made by mixing solvent B: water with 0.1% trifluoroacetic acid (TFA) and solvent D: methanol with 0.08% TFA (Composition 1). A second mobile phase mixture (Composition 2) with an organic solvent (71% methanol: 29% isopropyl alcohol) having the same eluotropic value (ϵ^0) as acetonitrile on C18 columns was used to study solvent selectivity. Stationary phase selectivity was studied using Ultra Phenyl and Ultra pentafluorophenyl (PFP) columns. Detection was done by splitting the eluent flow to two detectors, a multi-wavelength UV (photodiode array) detector and the CLND.

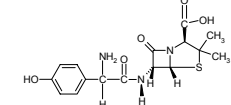
Using the current setup, time for method development was significantly reduced.

STATIONARY PHASES

ANALYTES



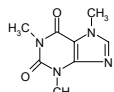
phenyl



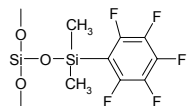
AM = amoxicillin



SA = saccharin



CA = caffeine



Pentafluorophenyl (PFP)

| EXPERIMENTAL | |
|---------------------|--|
| Column | Reversed-Phase HPLC with UV and CLND detection Dimensions: 150mm x 4.6mm ID Packing 1: Ultra Phenyl (Restek) Packing 2: Ultra PFP (Restek) Particle size: 5 μm Pore size: 100 Å Column Temp: Ambient |
| Mobile Phase | Composition 1: B: Water w/ 0.1% TFA D: Methanol w/ 0.08% TFA Composition 2: B: Water w/ 0.1% TFA D: 71%Methanol:29%IPA:Water w/ 0.08% TFA |
| Pump | Flow Rate: 1 mL/min (isocratic elution) |
| Detector | LPG system: Smartline 1000, Manager 5000 (Knauer) UV detector: Smartline 2600 (Knauer) 1 AUSF, τ=1s Wavelengths: 254, 280, 214nm CLND: Antek 8060 (PAC LP) |
| Injector | 20 or 10 μL sample loop |
| Sample size | 4μL partial-filled injection |

Results and Discussion

The capacity factor of three analytes was studied on two different columns. The mobile phase selectivity of compositions 1 and 2 are shown in Figs. 1a-1b and Figs. 1c-1d. The column selectivity on the Phenyl and PFP is represented in Figs. 1a-1c and Figs. 1b-1c, respectively. Sample chromatograms featuring column selectivity is shown in Figs. 2a and 2b., and mobile phase selectivity in Figs. 2a and 2c. Chromatographic separations can be optimized by changing columns, mobile phases and mobile phase composition.

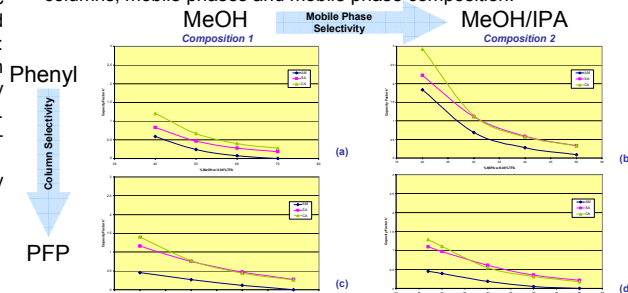


Fig. 1. Retention of AM,SA,CA on different columns and with different mobile phases: (a) Phenyl column with MeOH/H₂O. (b) Phenyl column with MeOH-IPA/H₂O. (c) PFP column with MeOH/H₂O. (d) PFP column with MeOH-IPA/H₂O.

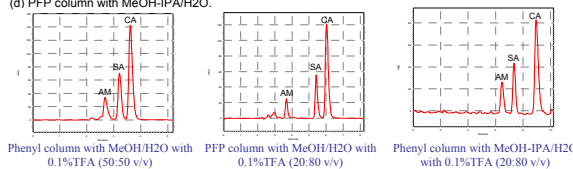


Fig. 2. Sample chromatograms showing column selectivity and mobile phase selectivity.

Multi-wavelength UV detection and CLND

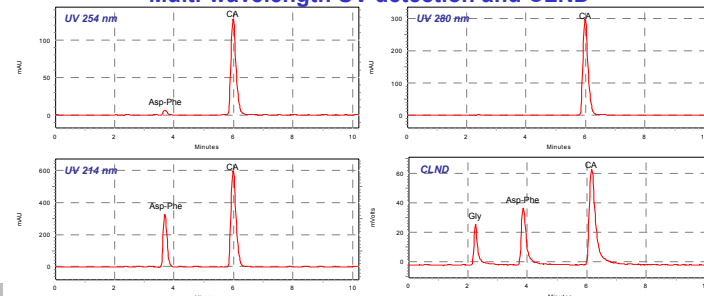


Fig. 3. Chromatograms with simultaneous UV detection at 254nm, 280nm, 214nm, and CLND.

Simultaneous UV detection

CLND allow for monitoring N-containing compounds without UV chromophores, such as glycine (Fig. 3). The linear response of the CLND correlates to the amount of nitrogen in the compound, offers solution to analyses without multiple calibration curves (Fig. 4).

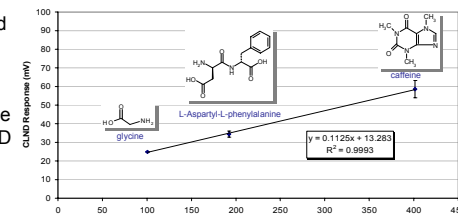


Fig. 4. CLND response is linear based on nanograms of nitrogen in the compounds.

Conclusion

An important application of RP-HPLC for pharmaceutical compounds is the separations and detection of nitrogen-containing analytes containing primary, secondary, and tertiary amine groups. The retention, solvent and column selectivity for amoxicillin, saccharin, and caffeine were studied with phenyl and PFP columns and provided a shorter analysis time. A multi-wavelength UV detector, coupled with the CLND can provide an efficient and accurate chromatographic profiles of biologically important molecules. The CLND shows important information about nitrogen containing compounds that may not be detected by UV methods alone.

REFERENCES

- [1] Taylor, E. W.; Qian, M. G.; Dollinger, G. D. *Anal. Chem.* **1998**, *70*, 3339-3347.
- [2] Popa-Burke, I. G., et al. *Anal. Chem.* **2004**, *76*, 7278-7287.
- [3] Chan, K.M.; Fujinari, E. M. *Am. Lab.* **2004**, *36*(24):18-22.

ACKNOWLEDGEMENTS

The Smartline HPLC system was loaned by **KNAUER - Advanced Scientific Instruments**. Ultra Phenyl and Ultra PFP columns were provided by **Restek Corporation**. Loan of the ANTEK 8060 CLND was possible by Dr. Mark E. Homan, **PAC LP**. Their generous support for this research is greatly appreciated.