APPLICATION BRIEF

Liquid Chromatography

# Analysis of Water-Soluble Vitamins Using Quasar SPP HILIC Column

## Introduction

In 1906, English biochemist Sir Frederick Gowland Hopkins also discovered that certain food factors were important to health. All the vitamins we recognise today were discovered during the early 20th century.[1] There are two groups of vitamins, fat soluble and water soluble. Both types are regarded as essential for normal growth and our overall well being.

Vitamin $B_2$ (Riboflavin) and vitamin $B_3$ (Niacinamide) are water soluble vitamins, naturally occurring in a number of food stuffs including milk and eggs. Both vitamins are used as dietary supplements and can help lower cholesterol, prevent migraines.[2]

Hydrophilic interaction liquid chromatography (HILIC), as first suggested by Alpert in 1990 provides an alternative approach to effectively separate small polar compounds, on polar stationary phases, which are not well retained under reversed phase conditions.[3] Water is the strongest solvent in HILIC and as mobile phases typically contain low levels of water the resulting methods are ideally suited for MS applications.

This application brief illustrates the efficient separation of vitamin $B_2$ and $B_3$, Figure 1, applying the HILIC mode of separation using the Quasar SPP HILIC column.

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## Experimental Conditions

### Method Parameters

All HPLC method parameters are shown in Table 1.

*Table 1.* HPLC Method Parameters.

| Quasar SPP HILIC | 150 mm | 4.6 mm | 5 µm | N9308960 |
|---|---|---|---|---|
| **Mobile Phase** | $H_20$: ACN, 5:95 | | | |
| **Flow Rate** | 1mL/min | | | |
| **Temp** | 30 °C | | | |
| **Wavelength** | 254nm | | | |
| **Injection Vol.** | 5 µl | | | |
| **Analyte** | 1. Vitamin $B_2$<br>2. Vitamin $B_3$ | | | |

### Solvents and Samples

All solvents were HPLC grade and samples were filtered using a 0.45 µm nylon filter, P/N 02542880.

## Results and Discussion

Any polar chromatographic surface can be used for HILIC separations. Typical HILIC stationary phases consist of classical bare silica or silica modified with polar functional groups.

HILIC is a hybrid of normal phase (NP), reverse phase (RP) and ion chromatography techniques, (Figure 2). The eluents of RP combined with the stationary phases of NP and charged analytes of ion chromatography yield the basis of HILIC. The mechanism of separation has been the subject of much discussion in the literature however it is generally agreed that a water-rich layer forms on the surface of the polar stationary phase vs. the water-deficient mobile phase, creating liquid/liquid partitioning. However, the separation mechanism is more complex than partitioning alone, with dipole-dipole and electrostatic interactions also contributing to retention.

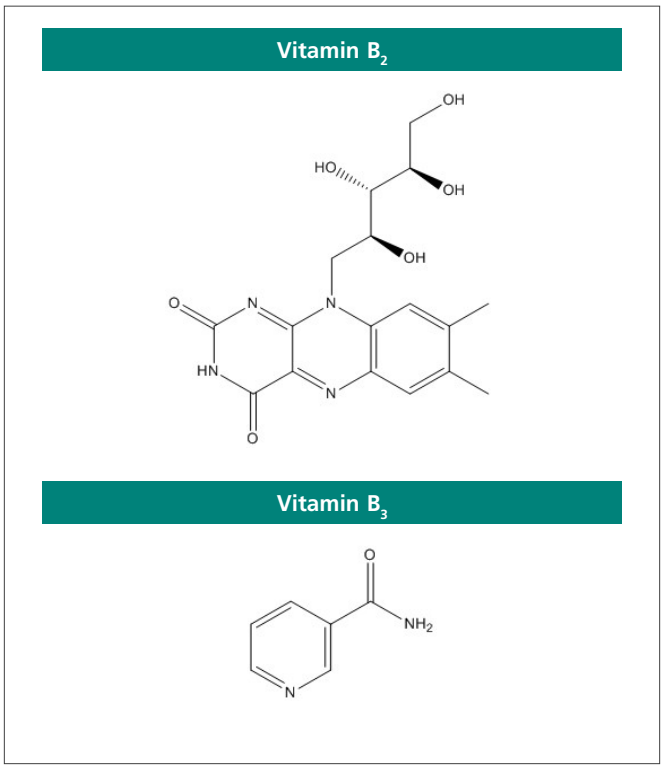


*Figure 1.* Chemical structures of vitamin $B_2$ and $B_3$.

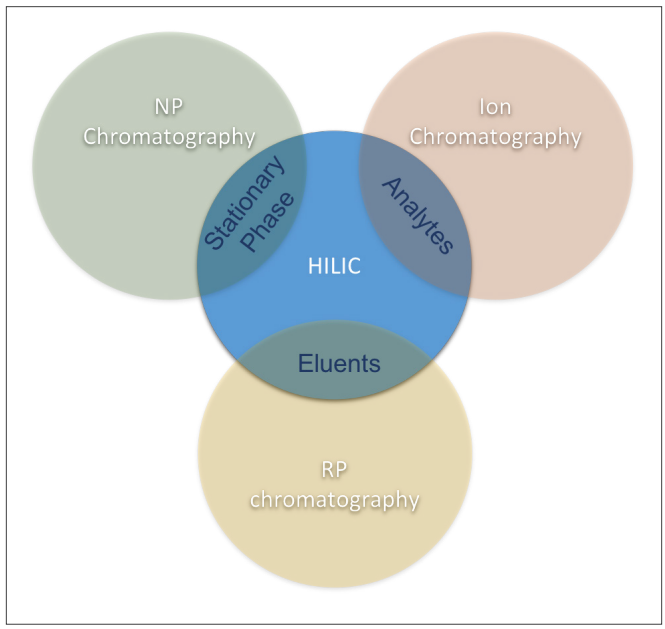


*Figure 2.* HILIC a hybrid of techniques.

The more polar compounds will have a stronger interaction with the stationary aqueous layer and are therefore retained longer than the less polar compounds. The elution order opposite to that observed in reverse phase HPLC.

Vitamins $B_2$ and $B_3$ contain several polar functionalities, which can also be in a charged state, and results in little retention using an alkyl chain bonded phase and traditional RP separations. So they are ideal candidates for HILIC separations. As Figure 3 illustrates, a good retention of these polar compounds is readily achieved in the HILIC mode, with vitamin $B_2$ eluting after vitamin $B_3$, as it is more polar.

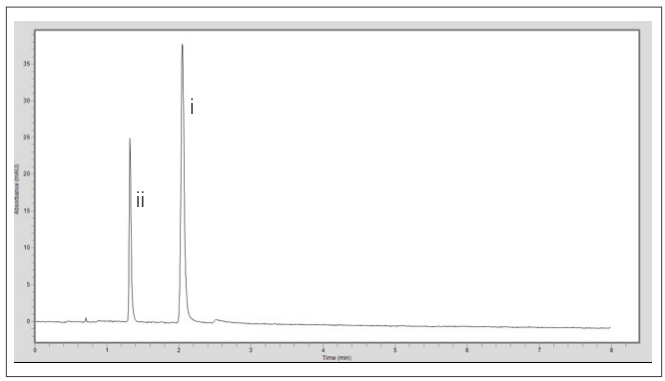


*Figure 3.* HPLC Analysis of (i) vitamin $B_2$ and (ii) vitamin $B_3$.

## Conclusion

- Quasar SPP HILIC phase is an unbonded silica phase for use in the HILIC mode of separation.
- It offers increased retention of polar compounds over RP separations and vitamins $B_2$ and $B_3$ are well retained.
- The HILIC mode offers enhanced MS sensitivity due to the high organic content of the mobile phase.
- With a solid core and outer porous silica coating of the SPP phase you can realise up to a 50% improvement in cost and efficiency over traditional porous silica columns.

## References


1. https://www.vitamins-nutrition.org/vitamins/history-vitamins.html
2. "Riboflavin". Drugs.com, The American Society of Health-System Pharmacists. 1 August 2018. Archived from the original on 30 December 2016. Retrieved 7 November 2018.
3. Alpert AJ. J Chromatogr A. 1990;499:177–196


## Consumables

| Phase | Length (mm) | I.D. (mm) | µm | Part |
|---|---|---|---|---|
| Quasar SPP HILIC | 150 | 4.6 | 2.6 | N9308919 |
| Quasar SPP HILIC | 100 | 4.6 | 2.6 | N9308920 |
| Quasar SPP HILIC | 50 | 4.6 | 2.6 | N9308921 |
| Quasar SPP HILIC | 150 | 3 | 2.6 | N9308922 |
| Quasar SPP HILIC | 100 | 3 | 2.6 | N9308923 |
| Quasar SPP HILIC | 50 | 3 | 2.6 | N9308924 |
| Quasar SPP HILIC | 150 | 2.1 | 2.6 | N9308925 |
| Quasar SPP HILIC | 100 | 2.1 | 2.6 | N9308926 |
| Quasar SPP HILIC | 50 | 2.1 | 2.6 | N9308927 |
| Quasar SPP HILIC | 150 | 4.6 | 5 | N9308960 |
| Quasar SPP HILIC | 100 | 4.6 | 5 | N9308961 |
| Quasar SPP HILIC | 50 | 4.6 | 5 | N9308962 |
| Quasar SSP HILIC Guard Cartridge (3/pack) | 10 | 3 | 2.6 | N9308994 |
| Quasar SSP HILIC Guard Cartridge (3/pack) | 10 | 3 | 5 | N9308995 |
| Quasar Guard Cartridge Holder | - | - | - | N9306876 |
| Nylon Filters | - | - | - | 02542880 |


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