

Enhanced Sensitivity and Quantitation by Obtaining Symmetrical Peak Shapes for Basic Pharmaceuticals

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Introduction

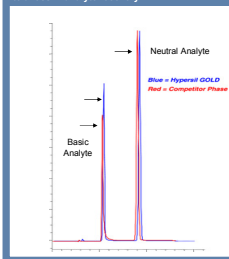
HPLC is widely used in the analysis of trace components, i.e. those contributing <0.1% in complex mixtures. The success of this technique in terms of determining accurate concentrations of impurities relies on gaining good separation and excellent peak shapes for all of the analytes involved. In order to achieve the best measurement accuracy, trace components of interest need to be completely resolved from adjacent peaks. The quality of the separation will be dependant upon system parameters such as sample preparation, column efficiency, sample injection and sample detection.

Quantitation of trace components can be performed by measurement of peak height or peak area; for either, the chromatographic separation needs to produce sharp, efficient peaks. The accuracy and sensitivity of the analysis is determined by the chromatographic parameters that affect separation, namely stationary phase and mobile phase.

By combining a new bonding technology with the very latest in silica template, exceptional peak shapes for basic compounds can be obtained, allowing the chromatographer to gain sensitivity and resolution. Figure 1 shows these new bonding technology improvements. An 'older' Type B silica is compared with Hypersil™ GOLD; it can clearly be seen that an improvement in peak shape for basic analytes leads to greater peak heights.

In this poster applications which highlight the advantages of gaining excellent peak shape are shown, including how this potentially affects the analysis of low levels of impurities.

FIGURE 1: Highly pure Type B silica (blue) and "older" Type B silica (red). The loss in peak height is due to secondary interactions with basic analytes, which lead to a loss in analyte recovery.



All calculations for tailing factor within this presentation use the USP designation as in equation 1:

$$\text{Equation 1: } T_f = a/b \text{ (5\% of peak height)}$$

Column Characterization

A multitude of HPLC column characterization tests are used world-wide, ranging from "in-house" tests in the pharmaceutical industry to academically tendered analysis. All of them suggest, in some form, an analyte generally affected by underlying silanols to measure peak shape. However, no single test has ever been globally accepted; therefore, we used several of these analyte probes to assess the overall performance of the new Hypersil GOLD media.

Figure 2 shows two of these tests. Two probes were chosen: amitriptyline, which is proposed as NIST 870 test (USP, 2003), and a Thermo Electron internal basic probe. Both of these bases interact in a different way with the stationary phase surface. This is significant because some phases appear better than others depending upon which probe is used. This is why a general agreement has never been used as to which test is most suitable. A target of 1 for any probe is always the asymmetry goal, as shown by the red point in Figure 2.

FIGURE 2. Tailing on two basic compounds, with a T_f target of 1.0 for both.

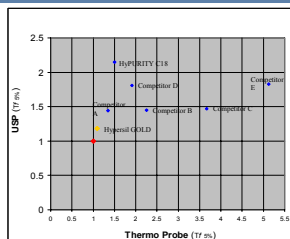
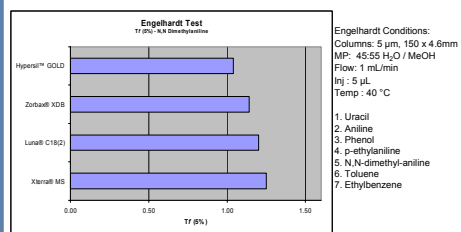


Figure 3 shows results of another suggested test, that of Engelhardt¹, which uses N,N-dimethylaniline to measure peak tailing. It can be seen again that Hypersil GOLD gave better peak shape for the basic probe compared with other market-leading columns in our analysis.

FIGURE 3. Engelhardt Test: tailing factor T_f of N,N-dimethylaniline.



Engelhardt Conditions:
Columns: 5 µm, 150 x 4.6mm
MP: 45:55 H₂O / MeOH
Flow: 1 mL/min
Inj: 5 µL
Temp: 40 °C

1. Uracil
2. Aniline
3. Phenol
4. p-ethylaniline
5. N,N-dimethyl-aniline
6. Toluene
7. Ethylbenzene

By using these probes when developing a new stationary phase, this ultimately leads to a stationary phase which has been optimized for the analysis of basic analytes. By achieving symmetrical peak shapes, the analyst can then obtain excellent peak heights and therefore gain sensitivity and resolution.

The benefit of this is shown in the analysis of tricyclic antidepressants in Figure 4. If full resolution of all of the peaks is not achieved, then peak shapes cannot be accurately measured. The Hypersil GOLD column resolves all of the analytes and improves the peak shapes compared to other alkyl chain stationary phases.

Methods

Figure 4:

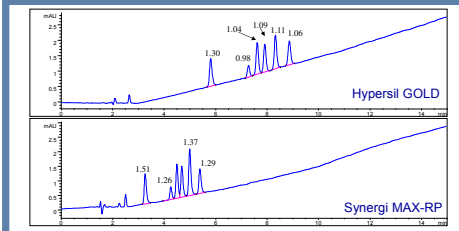
150x4.6mm, 5µm Hypersil GOLD and
150x4.6mm, 5µm Synergi™ Max-RP
A: H₂O 0.1% Formic acid
B: ACN + 0.1% Formic acid
Flow rate: 1 mL/min
Gradient: 30 to 50% B in 15 minutes
Temperature: 30 °C

Figure 5 & 6:

50x2.1mm, 5µm Hypersil GOLD and
50x2.1mm, 5µm Hypersil BDS C18
A: H₂O 0.1% Formic acid
B: ACN + 0.1% Formic acid
Flow rate: 0.2 mL/min
Gradient: 5 to 100% B in 10 minutes
Temperature: 30 °C
LCMS: Finnigan™ Surveyor™ and Finnigan Surveyor MSQ™
+ve ESI 450 °C/0.0 kV/40 V (scan from m/z 400 to 600)

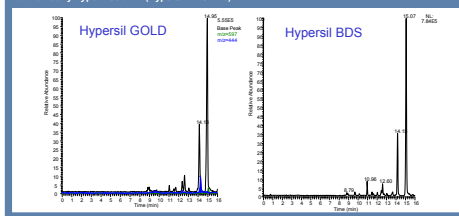
Results

FIGURE 4. Tailing factors for 6 tricyclic antidepressants



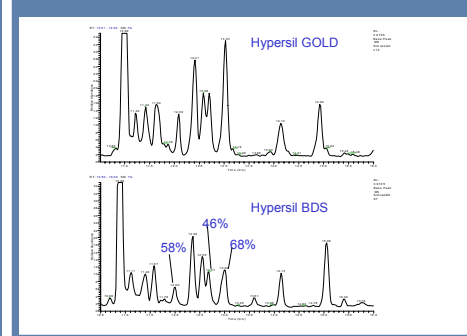
To the analyst trying to identify impurities within their sample to adhere to FDA guidelines², validation of peaks of 0.1% of the parent drug must be achieved. If sharp peak shapes are not achieved and the analyst is basing calculations on peak height, then the sample may well appear to contain very few impurities above this threshold. As instrumentation improves and allows more accurate quantification³ at trace levels, the HPLC column should not be responsible for losing this gain.

FIGURE 5. Simvastatin analysis comparing a latest technology Type B column (Hypersil GOLD) and an early Type B column (Hypersil BDS C18)



In the analysis of simvastatin (Figure 5) when a drug formulation is run and there is an abundant peak, the chromatography achieved is very similar regardless of column choice. However, once the compound has been degraded and other peaks appear, some at very low concentrations, the analysis is now enhanced by obtaining very symmetrical, well-resolved peaks (Figure 6). This aids greatly in achieving the target of accurate quantification of trace components of 0.1% or less in a complex mixture.

FIGURE 6. Degraded simvastatin after 23 days in acetonitrile at 40 °C. The Y-axis is normalized to Hypersil GOLD. Percentages equal loss in peak height.



Conclusions

In this poster we have demonstrated the use of a new stationary phase, Hypersil GOLD, and the improvement in peak shapes that can be achieved.

Using columns with the latest in column bonding technology allows the analyst to achieve better sensitivity and resolution of their target sample. Peak shape, and therefore sensitivity, improvements are of particular interest when trace analysis of compounds is necessary. It is shown that obtaining the sharpest peak shapes leads to excellent peak heights, with gains of up to 68% achievable.

References

1. A Chromatographic Test Procedure for RP-HPLC Columns, LC'GC December 1997
 2. ICH Q3A – Impurities in a new drug substance
 3. ICH Q3B(r) – Impurities in a new drug product
- Optimising Sensitivity in Trace Analysis. L. Pereira; M. Woodruff; P. DeLand; M. Euerby; Poster presented at IMSC 2003

Additional Information

For additional information, please visit our website: www.thermo.com/columns

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Simvastatin is sold under the name Zocor by Merck. Zocor is a trademark of Merck. P020125, E020105