Use Of Noise Parameters to Optimize Data Collection Rates in LC-MS

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OBJECTIVES

- Determine and quantify system noise as a function of instrument dwell time
- Demonstrate precision limits of quantification due to instrument noise
- Examine the relationship between dwell time and instrument contamination

METHODOLOGY

- Model analyte for all experiments was paclitaxel, M.W. 854 Da, MRM transition 854>286 Da, HPLC mobile phase ACN/water/formic acid
- Data collected on Micromass Quattro Ultima and Sciex 5000 platforms

RESULTS

- Chromatographic noise is a function of dwell time
- As absolute peak height decreases, noise begins to dominate the signal (Figure 1)

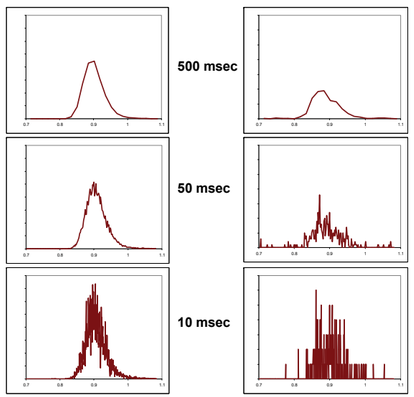

Figure 1. Effect of dwell time on peak shape for a 40 ng/mL (left) and 200 pg/mL (right) injection.

- With all other factors constant, there is an irreducible uncertainty placed on the signal by instrument noise
- The only way to reduce this noise is by increasing the dwell time

System Noise

- Time – intensity data were collected for background (no analyte) at different dwell times (Figure 2).

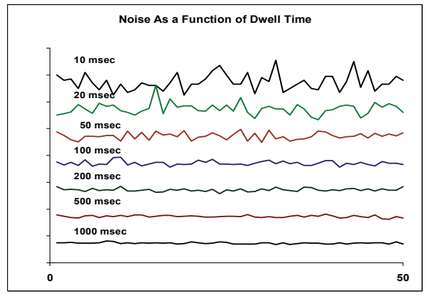

Figure 2. Samples of instrument noise at different dwell times. Scale same for all traces.

- The mean signal showed no change as a function of dwell
- The signal standard deviation (SD) showed a rapidly increasing value with decreasing dwell time (Figure 3)

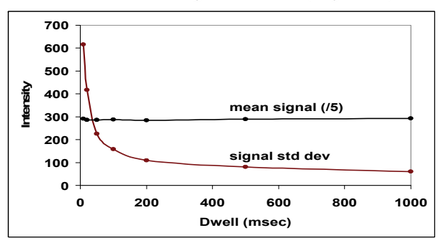

Figure 3. Mean background signal and the standard deviation of the noise.

- Signal SD vs. the reciprocal of the dwell shows characteristic 1/f noise (Figure 4)

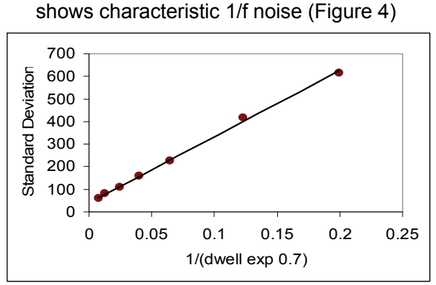

Figure 4. Inverse relationship of noise SD and dwell time.

- Precision is limited by instrument noise
- There is a minimum dwell time associated with maximum precision

Precision Limits

- Multiple injections (n = 6 for all points) of analyte were made at decreasing dwell times to determine the effect of noise on precision of quantification
- The %CV (peak area) tracked the standard deviation of the noise (Figure 5)

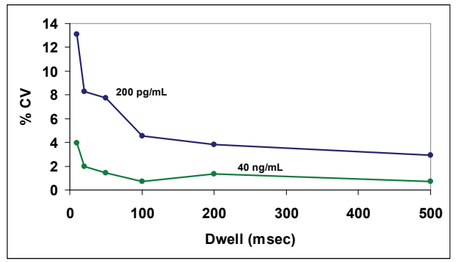

Figure 5. Effect on %CV for multiple injections of analyte with varying dwell time.

- Every instrument has an inherent minimum dwell time for data collection below which precision rapidly degrades

Contamination

- Injections of analyte were made at decreasing dwell times on two of the same model instrument, one of which was known to be badly contaminated (Figure 6)

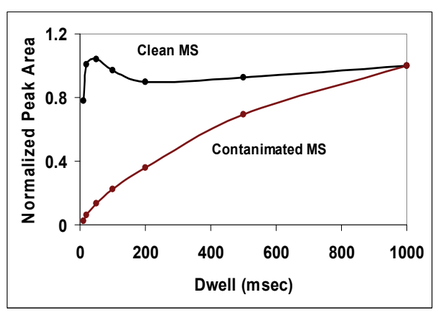

Figure 6. Normalized peak area response in clean vs. contaminated MS as a function of dwell time

- A plot of peak area vs. dwell is a sensitive indicator of internal contamination

- Replacement of the collision cell and ion optics in the high vacuum region resulted in a significant recovery of sensitivity (Figure 7)

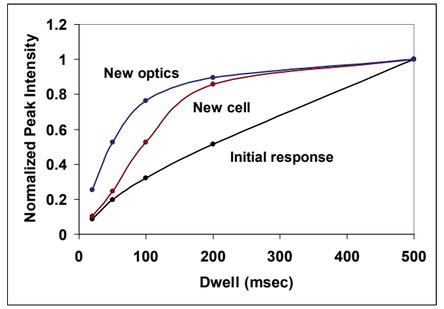

Figure 7. Effect of replacing collision cell and entire high-vacuum optics assembly in contaminated MS.

- A plot of sensitivity vs. dwell was performed on an instrument before and after a cleaning of the source only (Figure 8)

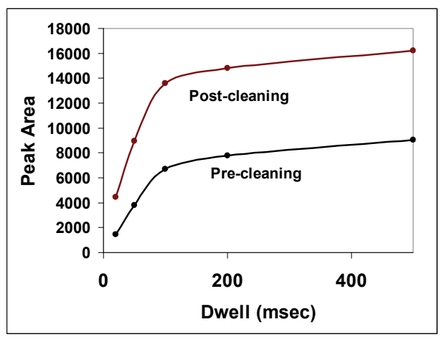

Figure 8. Effect of source cleaning on instrument response as a function of dwell time

- Cleaning the source resulted in an increase in absolute response with no effect on the curve shape
- The shape of the curve is affected by contamination in the high-vacuum region; the vertical displacement of the curve to contamination in the source

Peak Width

- Two instruments were tested for response to a narrow HPLC peak (3 sec) to determine suitability for use in UPLC (Figure 9)

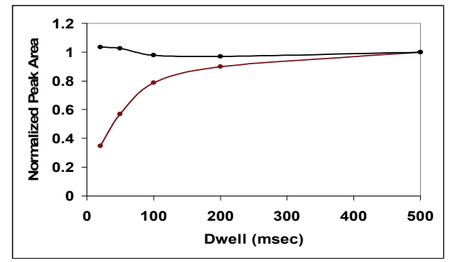

Figure 9. Peak area as a function of dwell time for a 3 sec wide peak on two instruments.

- Loss of response with decreasing dwell time indicates the MS in red would be unsuitable for use at short dwell times

CONCLUSIONS

- Precision of quantification is limited by inherent instrument noise
- Instrument noise is determined by dwell time
- Instrument sensitivity (hence cleanliness) can be estimated by a series of injections with different dwell times
- The shape of the dwell vs. response curve gives information about the condition of the ion optics. Pre and post cleaning curves indicate if the sensitivity problem is in the source or the high-vacuum region of the MS
- For narrow peaks (such as UPLC), the need for a minimum number of points to accurately determine peak shape may require reducing the dwell time to an interval that does not allow maximum precision