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A NOVEL 6-COLUMN EXTRACTION SYSTEM FOR HIGH THROUGHPUT ANALYSIS

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Presented at ASMS, Montreal, Canada, 8th-12th June 2003

INTRODUCTION

Since the late 90's, high throughput methods that can breach the 1000-analysis-per-day barrier are gaining significant interest in many fields due to the high demands in combinatorial chemistry and drug discovery. Multiple approaches are available, such as high throughput screening (HTS) using 96-well plates, LC/MS/MS systems, cassette dosing, fast separations and on-line SPE[1,2,3]. Lately, an interest in developing "total solutions" has emerged.
Total solutions are platforms capable of achieving good robustness, high reliability, extended lifetime, sub ng/mL detection limits and rapid throughput. With these desired advantages, these platforms also require generic extraction protocols, which help reduce analytical costs. Furthermore, the availability of a wide range of chemistries and formats will ensure a high level of success, especially for difficult analyses while still maintaining throughput for extended period of time.
The system described here incorporates a parallel extraction protocol using 6 extraction columns and 2 analytical columns. The extraction protocol is comprised of 6 steps: a- **load**, b- **wash 1**, c- **wash 2**, d- **wash 3**, e- **elution** and f- **reconditioning**. Each extraction step was done simultaneously with the other steps during a short period of time, ranging from 1.5 to 4 minutes. The unit did not required additional software control.

Figure 1. On-line cartridge features:

- *Direct plasma injection*
- *> 200 injections per cartridge*
- *Compatible with rapid LC gradient*
- *Fast cycle time for MS*
- *Sorbent Oasis® HLB/MCX/MAX*
- *2.1 mm I.D. x 20 mm 25 µm particle*

Depending on sample volume availability, detection limits can range from 1.0 to 0.01 ng/mL. The lifetime of the extraction columns was determined to be at least 200 injections for crude plasma samples (simple 1:1 aqueous dilution and 200 mL injection volume) before the chromatography showed a degradation of the peak shape. However, the lifetime of the extraction columns largely depended on the complexity of the sample (i.e., $CaCO_2$, urine, plasma … etc), the injection volume and the desired limit of quantification (LOQ's). When using low injection volumes (i.e 5 mL), up to 1000 injections can be achieved per extraction column, which gives a potential of 6000 analyses for the entire system. There is a trade off between column lifetime and desired LOQ's, with lower LOQ's leading to lower column lifetime.

1- J. Zweigenbaum, J. Henion, *Anal. Chem.*, 2000, **72**, No 11, 2446
2- C.R. Mallet, J.R. Mazzeo, U.D. Neue, *Rapid Commun. Mass Spectrom.*, 2001, **15**, 1075
3- C.R. Mallet, Z. Lu, J.R. Mazzeo, U.D. Neue, *Rapid Commun. Mass Spectrom.*, 2002, **16**, 805

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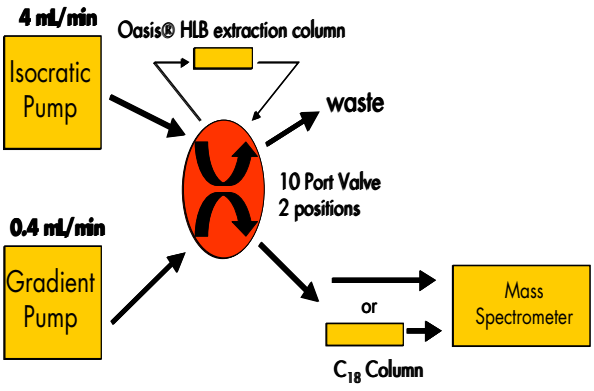

Figure 2. Common sequential valve setup for on-line SPE LC/MS/MS

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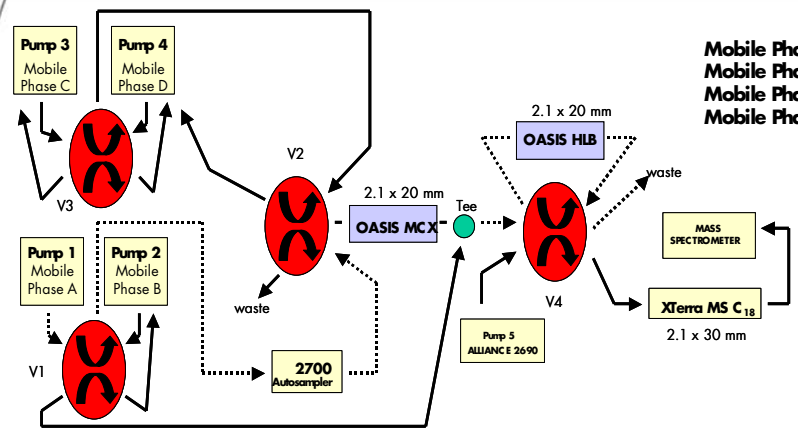

Figure 3. Oasis® MCX/HLB/XTerra® Sequential on-line SPE LC/MS/MS configuration with multiple washes

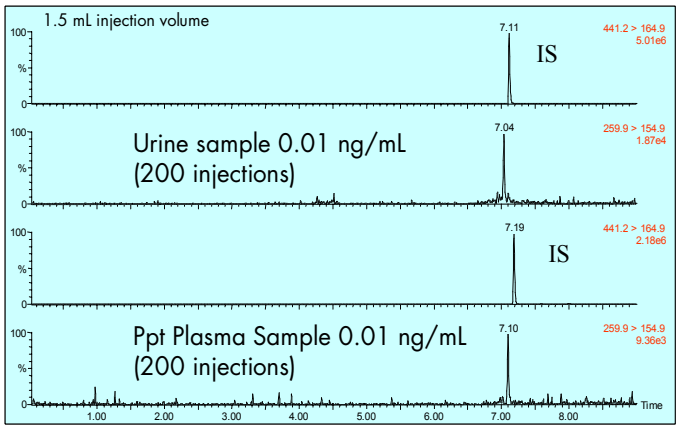

Figure 4. Analysis of propranolol in rat plasma and human urine using Oasis® MCX/HLB/XTerra® sequential configuration

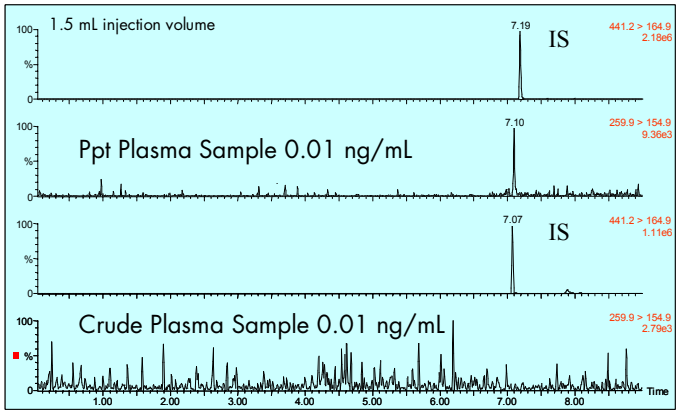

Figure 5. Comparison of protein precipitation samples vs crude plasma samples at 0.01 ng/mL LOQ

EXPERIMENTAL

As it can be seen from the chromatograms (Figures 4 & 5), a rigorous extraction protocol removes more interferences, thus giving lower sensitivity. In fact, the sub ng/mL LOQ's is attributed to a pre concentration effect. However, a drawback of sequential methodology is longer run time per sample. Using a parallel system can drastically reduce the run time below 3 minutes. Parallel analysis uses an extraction column for each of the extraction steps. Each step is done simultaneously. After each injection, each column is switched toward the next step using a single pulse logic activation. An extraction cycle ends at the sixth injection and can be kept in a continuous loop.

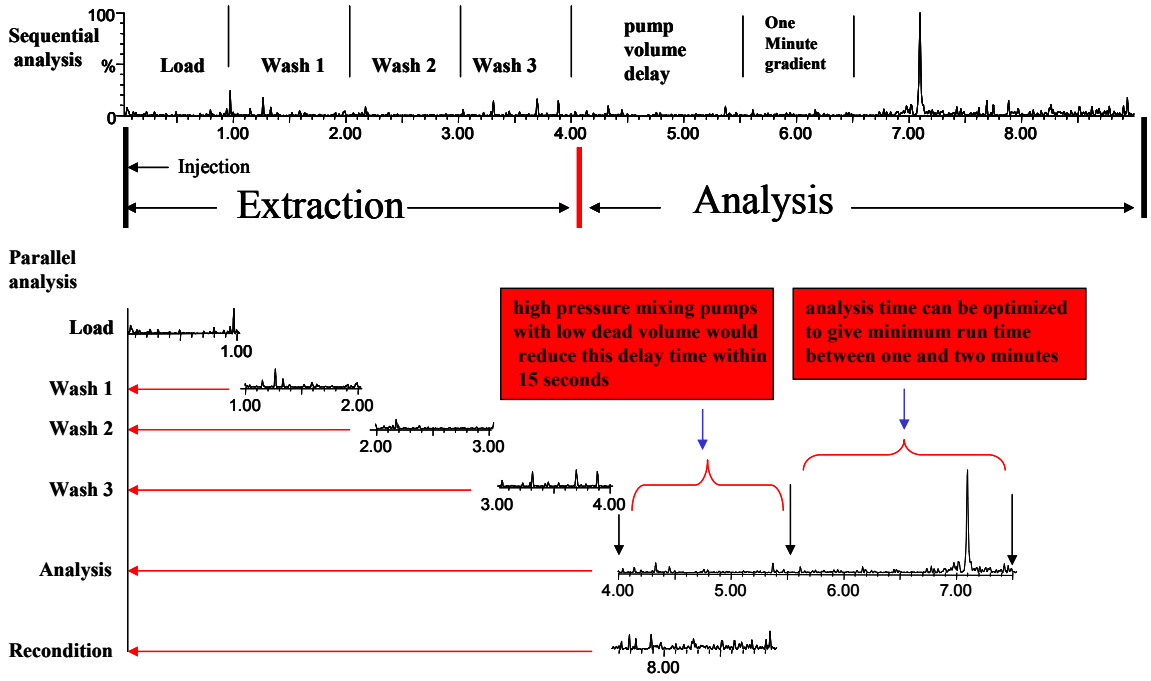

Figure 6. Sequential versus parallel analysis summary.

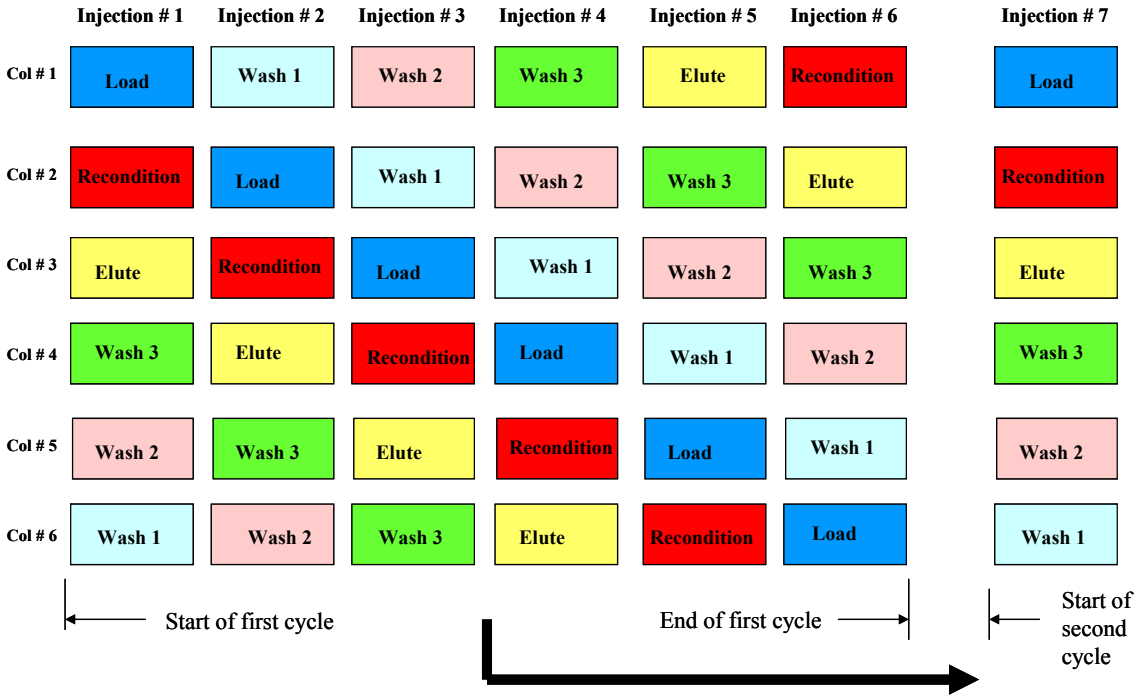

Figure 7. Cycle time during parallel analysis.

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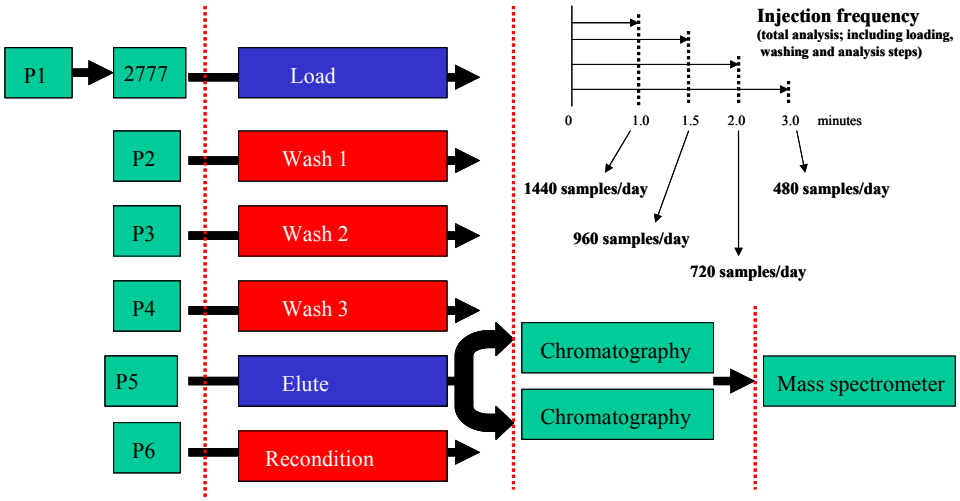

Figure 8. Total solution SPE-LC-MS/MS

CONCLUSION:

The results shown (Figures 9 & 10) clearly demonstrate that using a parallel configuration can reduce a typical run time below 2 minutes in comparison to 9 minutes total time with a sequential configuration. Faster analysis speed below one minute will demand gradient pumps and switching valves with smaller system volume. In future work, the total solution environment will require each individual elements to be constructed with a plug and play platform and connected to a central command structure.

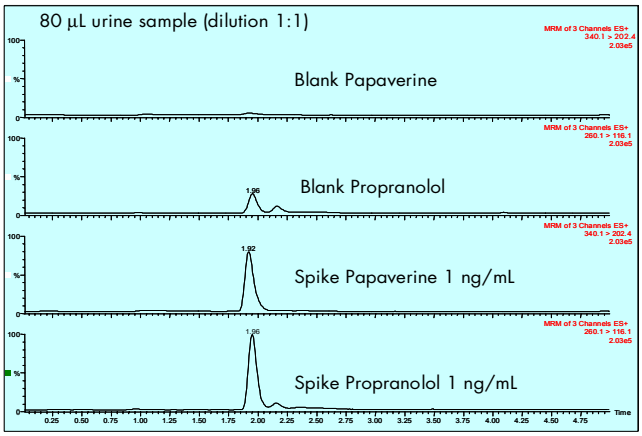

Figure 9. Parallel analysis—blank human urine using Oasis® 2D extraction protocol

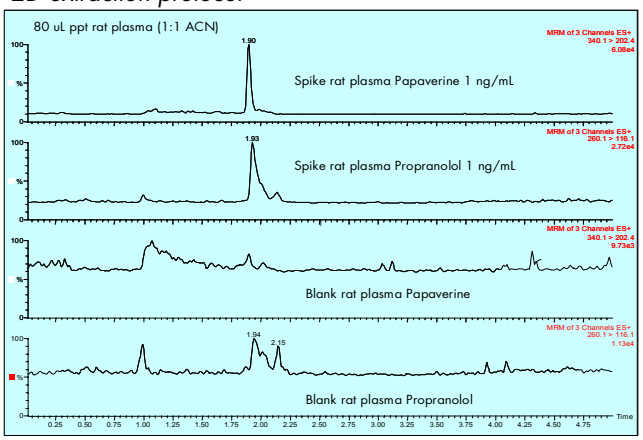

Figure 10. Parallel analysis—spiked ppt rat plasma using Oasis® HLB 2D extraction protocol

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