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Application Note 00297

Evaluation of Varian 1200L LC/MS/MS for the Analysis of Cyclosporin A

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Introduction

The primary goal of the study was to evaluate the usefulness and performance of the 1200L system for analysis by LC/MS/MS. The compound Cyclosporin A was employed as a test compound for this study. In addition, the usefulness of automated on-line extraction was investigated and used to perform the analysis. All system components and reporting are controlled and fully automated through the Varian MS Workstation. The Multi Reaction Monitoring (MRM) capability of the system was used to prove the sensitivity and robustness of the LC/MS/MS system.

Instrumentation

The system comprises:

- 1 x Prostar™ 410 Varian Autosampler/Injector. The autosampler has the capability to dilute, derivatize and inject in 3 differing modes: full loop, partial loop or microliter pickup mode. In addition, the module has a built-in column oven
- 2 x Prostar 210 Series LC pumps with 5ml analytical pump heads delivering and controlling the high pressure flow for the analytical column
- 1 x Prostar 210 Series LC pumps with 5ml analytical pump heads delivering and controlling the high pressure flow for the on-line extraction capability with switching valve.
- 1 x API interface, ESI (APCI also available, but not required for this study)
- 1 x 1200L MS/MS triple quadrupole instrument with 180° collision cell

The entire modular system is fully integrated and controlled by the Varian MS Workstation software running on the latest Microsoft XP and Dell PC platform. A diagrammatic layout of the system is shown in Figure 1.

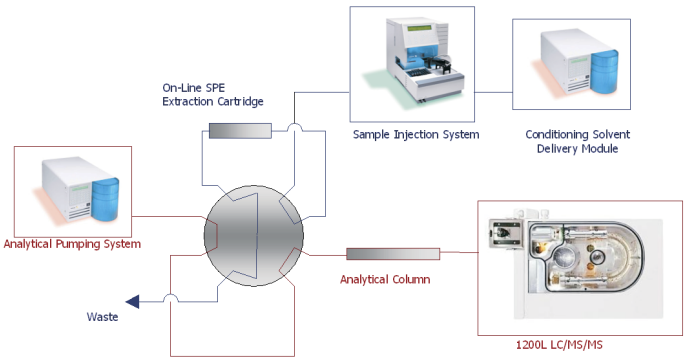

Figure 1 System Hardware

Materials and Reagents

The materials employed for analysis were obtained from Sigma-Aldrich and were as follows:

- Cyclosporin A (10 mg; p/n C-3662)
- Bovine Serum (100 mL; p/n B-9433)

Sample Preparation/Procedure

Standard solutions for Cyclosporin A were made up in Water/Acetonitrile (70:30) at various concentrations and used to produce a calibration curve.

To ascertain the robustness of the system and the analysis, cyclosporin A was spiked into a complex matrix (bovine serum), extracted and analyzed. The extraction consisted of taking a spiked bovine plasma (250 µL) adding acetonitrile (250 µl) and shaking. The sample was centrifuged at 13000 rpm for 10 minutes. A protein precipitate settled in the bottom of the vial. The meniscus was then transferred to a clean sample bottle and analyzed.

HPLC Conditions

Column	Pursuit® C18, 3 µm, 100 x 2 mm (Varian Part No. A3001100X020)
Mixer	250 µL static mixer
Solvent A	0.1% formic acid:2 mM NH_4 Ac in water (v/v)
Solvent B	Acetonitrile

The on-line extraction used iso-cratic method of 90:10 (A:B), at 0.35 mL/min

Further work using 10 or 20 µm particle size in the SPE column results in improved lifetime providing increased cost-savings.

The analytical gradient used is given below:

LC Program	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	75	25	0.35
	4:30	0	100	0.35
	7:00	0	100	0.35
	7:06	75	25	0.35
	11:00	75	25	0.35

Column Temp. 25˚C
Injection Volume 100 µL
Injection Solvent water
The cartridge was back flushed and switched to be in-line with analytical column after 1.0 minute and reversed after 9.0 minutes.

MS Conditions

The major MS parameters are listed below.

ESI Parameters

Ionization Mode	ESI negative
Collision Gas	2.0 mTorr Argon
API Drying Gas	39 psi at 200˚C
API Nebulizing Gas	59 psi
Scan Time	1.0 sec
SIM Width	0.7 amu
Needle	5000V
Shield	600V
Capillary	Variable
Detector	1600V

Scan Parameters

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (-V)
Cyclosporin A	1200	1089	38

Results and Discussion

The analysis time was considered adequate for the study undertaken. Standards at five levels (5, 10, 50, 200 and 400 µg/L) were analyzed by injecting onto the on-line SPE cartridge and then switching and eluting the cartridge onto the analytical column. The results were then used to produce a 5-point external standard calibration curve. The overlaid MRM chromatogram results, for each level of standard, are shown in Figure 2.

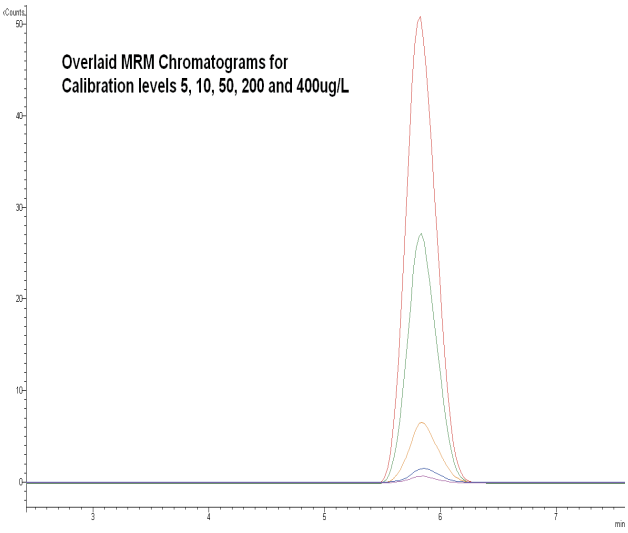

Figure 2 Overlaid MRM Chromatograms of Standards

The results indicate that the on-line extraction and analysis is a viable system for this type of analysis. A calibration curve was then produced for Cyclosporin A and this is given in Figure 3. Excellent linearity was found with an $r^2 = 0.999$.

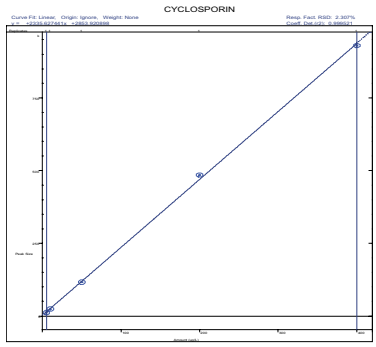

Figure 3 Five (5) Point External Standard Calibration Curve for Cyclosporin A

To give an indication of the Limit of Detection (LOD) and Limit of Quantitation (LOQ), the lowest standard (5 µg/L) was diluted by a factor of 10. This was then analyzed in MRM mode and the resulting chromatogram is shown in Figure 4.

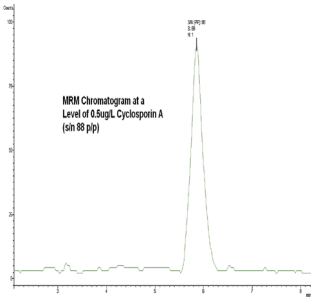

Figure 4 0.5 µg/L Cyclosporin A Standard

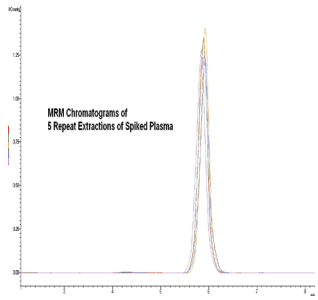

Figure 5 Repeatability results of spiked bovine plasma

The peak-to-peak signal/noise was calculated as 88. This gives a good indication of the LOD and LOQ that can be achieved.

The analytical system was investigated for reproducibility and robustness with a complex matrix. For this study Bovine plasma was used as matrix and spiked at a level of 20 µg/L with Cyclosporin A. The samples were protein precipitated and analyzed employing the on-line system. The results of five individually extracted spiked bovine plasmas is shown in Figure 5.

Conclusion

The concept behind this preliminary analysis has been largely proven. Further development is required to optimize the on-line extraction and reduce the total analysis time to aid throughput. However, these preliminary results indicate that the Varian 1200L triple quadrupole mass spectrometer when combined with the LC Prostar gives an integrated, high performance solution for the analysis of Cyclosporin A.

These data represent typical results.
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