



Application Note #704055

## **Clenbuterol in Equine Plasma – A Study of Superior Ion Source Robustness & High Sensitivity on a New EVOQ™ Model LC Triple Quadrupole Mass Spectrometer**

### **Abstract**

This application note demonstrates the sustained high sensitivity performance of the EVOQ LCTQ under repeated exposure to equine plasma samples. Clenbuterol in crashed equine plasma was injected by dilute-and-shoot technique without the use of a divert valve. Four calibration curves were generated, and between each two adjacent calibration, 100 matrix samples were injected. The goal was to maintain a consistent response factor (<10% RSD) for all calibration curves. The results from EVOQ system exceeded the goal with the response factor RSD<5% over a period of two days and over 400 total injections of matrix samples.

### **Introduction**

Sustained high sensitivity and robust performance are crucial for success in a laboratory doing quantitative analysis. To test the ion source design of the EVOQ LC-TQ, equine plasma was used in a dilute-and-shoot manner, without the use of an in-line divert valve. The ion source was exposed to all matrix eluting off the column from every sample injection. Since the liquid eluent is de-solvated under the heated probe gas during the electrospray process, salts, lipids, sugars, proteins, and other contaminants are liable to coalesce within the ion source, causing sensitivity degradation over time. In designing the new ion source for EVOQ, the gas flow dynamics within the ion source chamber are carefully studied, and the design avoids areas of coalescence, cold spots, and eliminates gas recirculation. By using a gas entrainment exhaust (active exhaust) system, the ion source robustness was significantly improved, while maintaining high sensitivity. Crashed equine plasma spiked with clenbuterol was chosen as the matrix for the test of the source robustness.

## Experimental

### Sample Preparation

Equine plasma was crashed using cold acidified acetonitrile (3:1 v/v). The samples were centrifuged for 10mins and the supernatant dissolved 1:1 v/v with mobile phase A.

### Chromatography (Advance HPLC)

- Column: C18, 3 $\mu$ , 100x2.1mm (ACE®, Part Number: 111-1002)
- Injection volume: 30 $\mu$ L
- Flow rate: 0.45mL/min
- Mobile phase A: Water with 0.2% Formic acid
- Mobile phase B: Acetonitrile with 0.2% Formic acid
- Gradient conditions:
  - 0.0min 10% B
  - 0.3min 10% B
  - 2.5min 95% B
  - 2.8min 10% B
  - 4.5min 10% B

### Mass Spectrometry (EVOQ Elite)

- VIP Heated-ESI Temp: 300°C
- Heated probe gas: 68 units
- Nebulizer gas: 90 units
- Cone gas temp: 300°C
- Cone gas: 10 units
- Spray voltage: +3500 V
- Clenbuterol & d9-Clenbuterol MRM transitions:
  - Clenbuterol: m/z 277.1>168 (CE:25v)
  - d9-Clenbuterol: m/z 286.1>204 (CE:12v)

## Results and Discussion

The response factor is the area ratio (calibrant/IS) divided by the concentration across the calibration range. The RSD% of response factor is a stringent measure of sensitivity because it takes into consideration every calibration point change on the curve. Any decrease in sensitivity will be reflected by a higher %RSD response factor. Demonstrating ion source robustness and sustained high sensitivity together is practically important, and of higher value than a plot of repeated injections at high levels, commonly seen in vendors' marketing literature.

The %RSD of the response factors of the four calibration curves was less than 5%, which is well within the acceptance criteria of validated bioanalytical methods. Figure 1 shows the overlaid first and fourth calibration curves, indicating insignificant divergence, and high sensitivity across the calibration range.

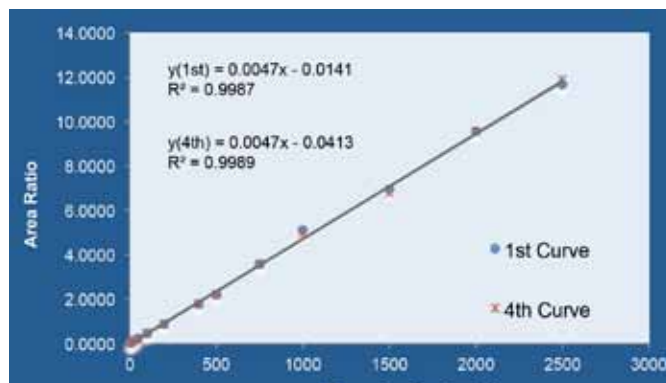


Figure 1: Overlay of curve 1 and curve 4, indicating sustained high sensitivity performance over the course of 400 plasma injections.

### Active Exhaust System

The Active Exhaust system evacuates the ion source housing by a slight pressure differential thereby eliminating recirculation of nebulized gases and contaminants.

The large exhaust opening ensures rapid removal of the nebulized components, keeping the area in front of the ion sampling orifice swept and clean.

The nebulized gases are entrained in the air-flow of the Active Exhaust system which removes them from the LC-MS system.



Table 1. Calibration Curve no. 1				Table 2. Calibration Curve no. 4			
Sample ID	Concentration (pg/mL)	Area Ratio	Response Factor	Sample ID	Concentration (pg/mL)	Area Ratio	Response Factor
Plasma C1	5	0.0229	0.00459	Plasma C1	5	0.0227	0.0045
Plasma C2	10	0.0468	0.00468	Plasma C2	10	0.0477	0.0048
Plasma C3	25	0.1130	0.00452	Plasma C3	25	0.1117	0.0045
Plasma C4	50	0.2162	0.00432	Plasma C4	50	0.2144	0.0043
Plasma C5	100	0.4556	0.00456	Plasma C5	100	0.4462	0.0045
Plasma C6	200	0.8706	0.00435	Plasma C6	200	0.8549	0.0043
Plasma C7	400	1.7639	0.00441	Plasma C7	400	1.7819	0.0045
Plasma C8	500	2.2001	0.00440	Plasma C8	500	2.1869	0.0044
Plasma C9	750	3.5731	0.00476	Plasma C9	750	3.5756	0.0048
Plasma C10	1000	5.0905	0.00509	Plasma C10	1000	4.9443	0.0049
Plasma C11	1500	6.9408	0.00463	Plasma C11	1500	6.7704	0.0045
Plasma C12	2000	9.5574	0.00478	Plasma C12	2000	9.5426	0.0048
Plasma C13	2500	11.6480	0.00466	Plasma C13	2500	11.8610	0.0047
		Average	0.004596			Average	0.004568
		Std. Dev	0.0002104			Std. Dev	0.0002111
%RSD of Response Factor			4.58%	%RSD of Response Factor			4.62%

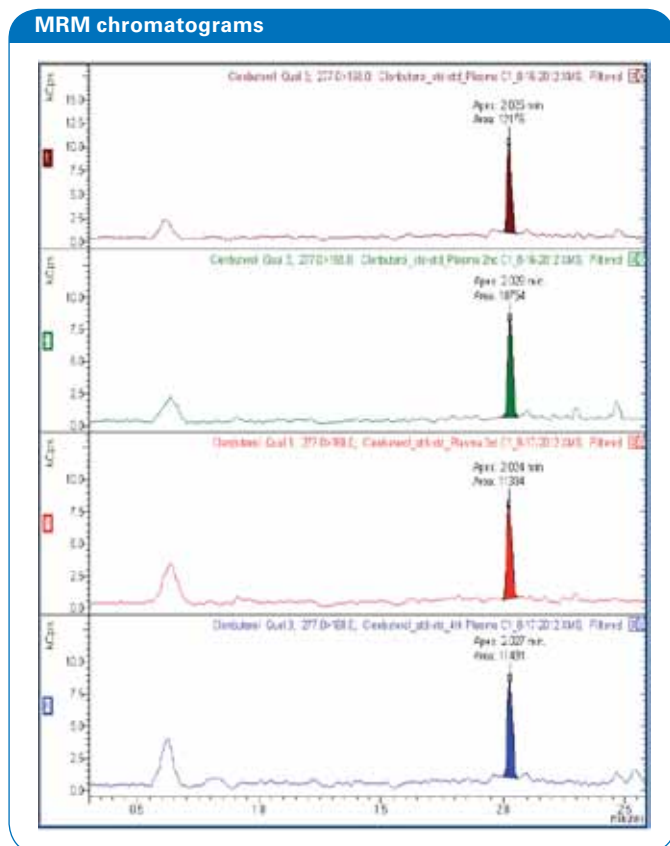


Figure 3: Representative MRM chromatograms of the 5 ppt Clenbuterol in equine plasma (150 fg on-column) from the first to fourth calibrations, illustrating consistent peak areas, and thus response factors.

Tables 1 and 2 indicate the sustained high sensitivity performance of the ion source after repeated exposure to plasma samples, a key requirement for the harsh environment in a routine bioanalytical laboratory. Figure 3 shows the chromatograms for the first calibration level for all four calibration curves.

### Conclusion

This experiment was designed to show the EVOQ LC-TQ running for over 48hrs (the weekend), and being able to successfully analyze 400 matrix samples with negligible loss in sensitivity. The Active Exhaust design allows the ion source on the EVOQ LC-TQ to perform at the highest sensitivity level despite repeated exposure to matrix, ensuring a peace of mind in the unforgiving conditions of a quantitative analyses laboratory.

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