Application Note: 310

Improved Quantitative Selectivity of Clenbuterol in Human Urine Using High Resolution on the TSQ Quantum Mass Spectrometer

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Key Words

- TSQ Quantum™
- High Resolution Analysis
- Improved Sensitivity
- Quantitation

The data presented here was acquired on a TSQ Quantum mass spectrometer.

Introduction

Clenbuterol (Figure 1) is a beta-2-adrenergic agonist, an effective bronchodilator drug used for the treatment of human asthma. It relieves bronchial airway smooth muscle contractions caused by Chronic Obstructive Pulmonary Disease (COPD) and allergy-induced respiratory distress.

Clenbuterol has significant anabolic effects and could be used as a drug of abuse in athletes and livestock for its muscle growth stimulant properties. It raises the body temperature and hence facilitates fat tissue catabolism. Due to Clenbuterol having these anabolic properties, it must be routinely monitored in biological samples by veterinary and human doping control laboratories.

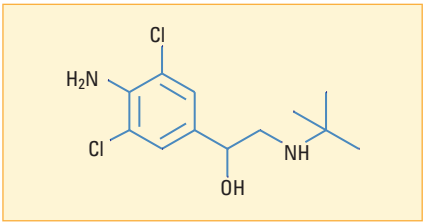

Figure 1: Chemical structure of Clenbuterol

Goal

One of the limitations to quantitation is the unequivocal identification of analytes in biological samples due to endogenous matrix interferents.

This report describes the use of high resolution on the Thermo Scientific TSQ Quantum to exploit the negative mass defect of a compound containing Chlorine, such as Clenbuterol, and hence improve the selectivity of the quantitative assay.

Clenbuterol ($C_{12}H_{18}Cl_2N_2O$, molecular weight 276.08 amu) was infused, 0.1 ng/µL, into the ESI source and the four most abundant product ions for the MS/MS breakdown were determined using the automated compound optimization procedure on the TSQ Quantum (Figure 2).

The transition yielding the most abundant product ion (*m/z* 203.0) was selected for the analysis of Clenbuterol.

Experimental Conditions

Sample Preparation: Human urine extracts were prepared using a C18 Solid Phase Extraction media. The extracted urine was spiked with Clenbuterol in the concentration range 0.1, 0.5, 1, 5, 10, 50 and 100 pg/µL for the calibration standards. No internal standard was used in this study.

Sample Analysis: The spiked urine extracts were chromatographed using a Thermo Scientific Surveyor™ LC on a C18 100 mm × 2.1 mm column at a flow rate of

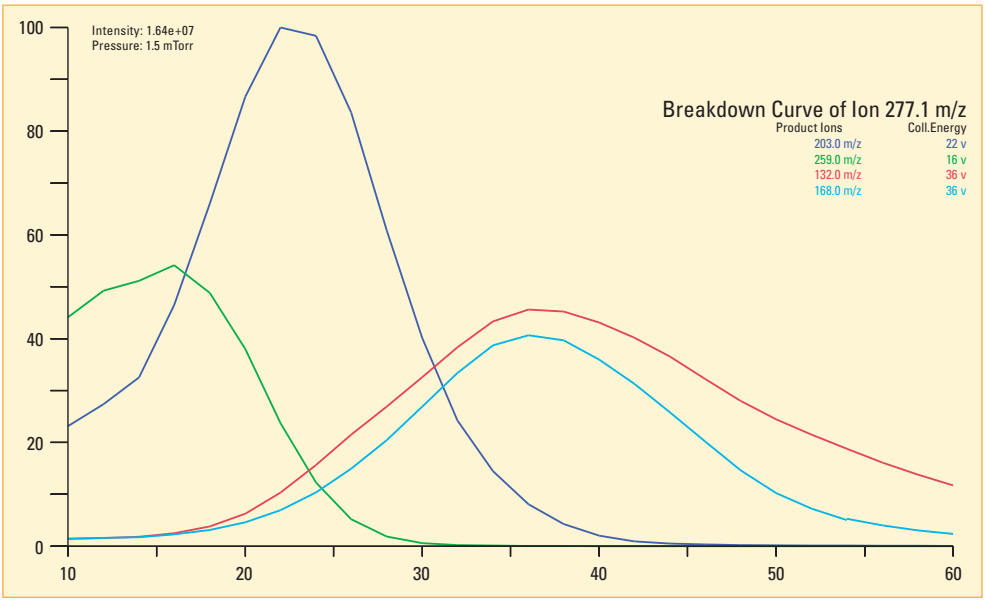

Figure 2: Automated optimization of MS/MS parameters for Clenbuterol

300 µL/min with a linear gradient of 10% solvent B (Methanol/Ammonium acetate [10 mM] 90/10 v/v) to 100% B over 5 minutes. Solvent A was Ammonium acetate (10 mM). The calibration standards were injected in duplicate at volumes of 10 µL.

MS Conditions

Mass spectrometer: TSQ Quantum

Ionization mode: Electrospray (ESI), positive ion

SRM: Clenbuterol 277.1 → 203.0 ± 0.3 Da, 22 eV
Collision energy Resolution

Experiment 1: 0.7 Da FWHM on Q1 and Q3

Experiment 2: 0.1 Da FWHM on Q1, 0.7 Da FWHM on Q3

Two separate quantitative analyses were performed at peak widths of 0.1 Da and 0.7 Da Full Width Half Maximum (FWHM) on Q1 in SRM mode. A peak width of 0.7 Da FWHM was used on Q3 for all analyses.

Results

The chromatogram of a pure standard of Clenbuterol in aqueous solvent demonstrates the retention time at 5.8 minutes (Figure 3).

Experiment 1: Quantitative Analysis Performed at 0.7 Da FWHM

The data below shows the quantitative analysis of Clenbuterol in Human urine at peak width settings of 0.7 Da FWHM on Q1 and Q3. Chromatograms are shown for blank urine (Figure 4) and urine containing Clenbuterol at 0.1 pg/µL (Figure 5).

A calibration curve of Clenbuterol analyzed at 0.7 Da FWHM was constructed using linear fit of peak area plotted against concentration, weighted 1/x (Figure 6). A correlation coefficient of r^2=0.9990 with an equation of Y=8496.82+266143*X was obtained for the curve.

The peak area, back-calculated values and precision of all calibration standards are shown in Table 1.

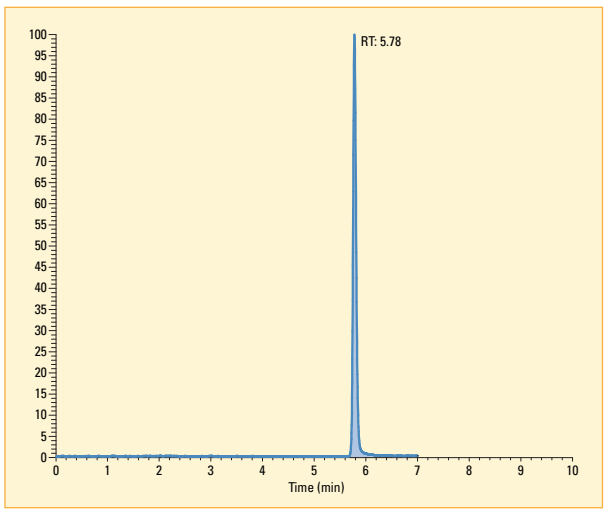

Figure 3: Determination of Clenbuterol retention time

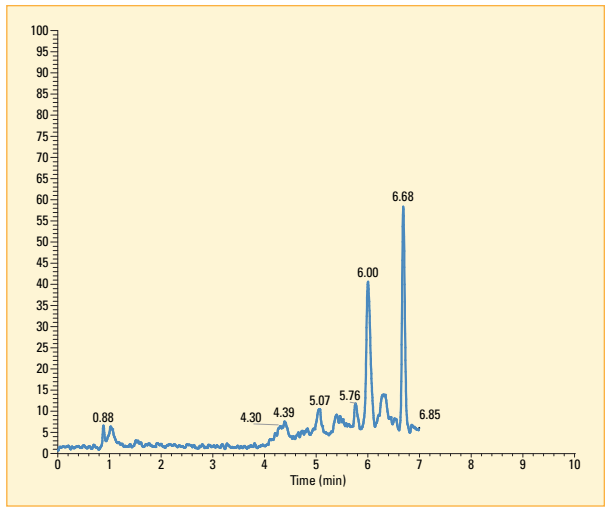

Figure 4: Urine blank, 0.7 Da FWHM

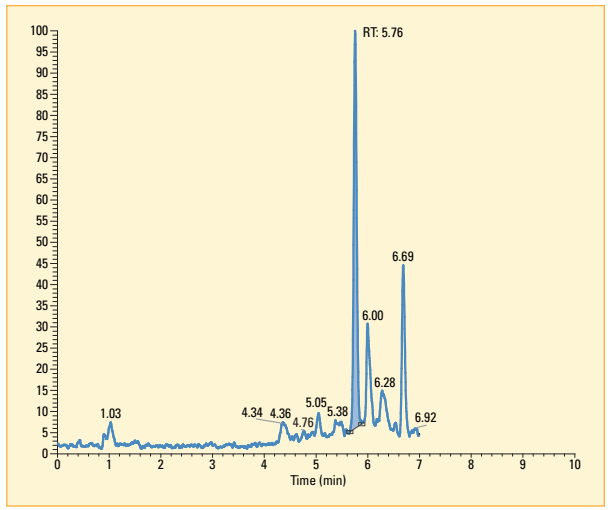

Figure 5: Clenbuterol, 0.1 pg/µL in urine, 0.7 Da FWHM

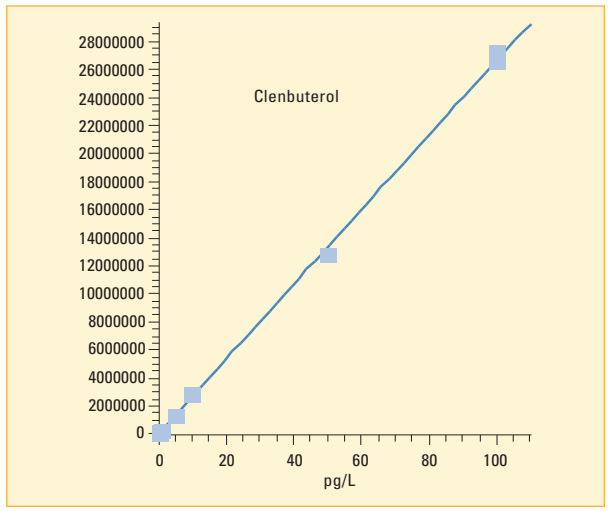

Figure 6: Clenbuterol curve at 0.7 Da FWHM

Experiment 2: Quantitative Analysis Performed at 0.1 Da FWHM

The data below shows the quantitative analysis of Clenbuterol in Human urine at peak width settings of 0.1 Da FWHM on Q1 and 0.7 Da FWHM on Q3. Chromatograms are shown for blank urine (Figure 7) and urine containing Clenbuterol at 0.1 pg/µL (Figure 8).

A calibration curve of Clenbuterol analyzed at 0.1 Da FWHM was constructed using linear fit of peak area plotted against concentration, weighted 1/x (Figure 9). A correlation coefficient of r^2=0.9994 with an equation of Y=2661.76+85951.1*X was obtained for the curve.

The peak area, back-calculated values and precision of all calibration standards are shown in Table 2.

SAMPLE NAME	AREA	CALC AMT	UNITS	%RSD
Urine blank	0.00	0.00	pg/L	
Urine blank	0.00	0.00	pg/L	
Cal 0.1 pg/L	33516.83	0.09	pg/L	4.5%
Cal 0.1 pg/L	31977.14	0.09	pg/L	4.5%
Cal 0.5 pg/L	136967.28	0.48	pg/L	0.6%
Cal 0.5 pg/L	137996.57	0.49	pg/L	0.6%
Cal 1 pg/L	289917.16	1.05	pg/L	1.3%
Cal 1 pg/L	295117.95	1.07	pg/L	1.3%
Cal 5 pg/L	1353210.91	5.05	pg/L	0.8%
Cal 5 pg/L	1338935.79	4.99	pg/L	0.8%
Cal 10 pg/L	2856289.00	10.70	pg/L	0.5%
Cal 10 pg/L	2877525.09	10.78	pg/L	0.5%
Cal 50 pg/L	12837781.41	48.20	pg/L	0.2%
Cal 50 pg/L	12797548.82	48.05	pg/L	0.2%
Cal 100 pg/L	27232776.65	102.29	pg/L	1.7%
Cal 100 pg/L	26578332.48	99.83	pg/L	1.7%

Table 1: Calculated standards at 0.7 Da FWHM

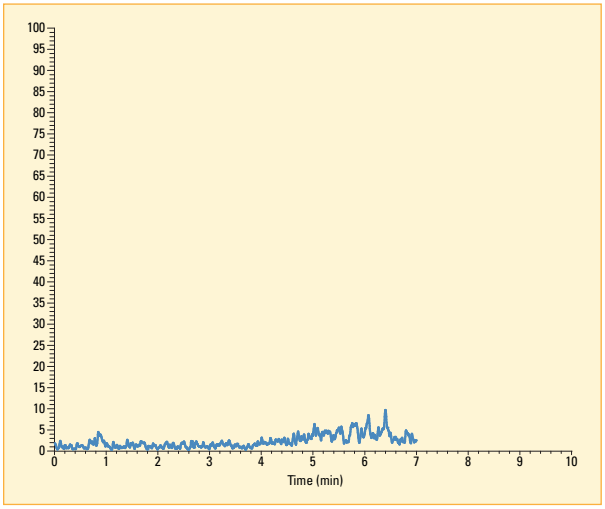

Figure 7: Urine blank, 0.1 Da FWHM

Discussion

Analysis, in SRM mode, of the spiked urine samples at a resolution setting of 0.7 Da FWHM resulted in a Clenbuterol peak eluting from the column upon a broad chemical noise background signal containing interferent peaks from the urine.

The same urine samples analyzed at a peak resolution setting of 0.1 Da FWHM resulted in elimination of the interfering isobaric mass peaks and the broad background chemical noise previously seen in the analysis at a peak width setting of 0.7 Da FWHM. The selected reaction monitoring analysis performed at a higher resolution setting of 0.1 Da FWHM resulted in increased selectivity of the assay and hence an increase in the precision that could be achieved.

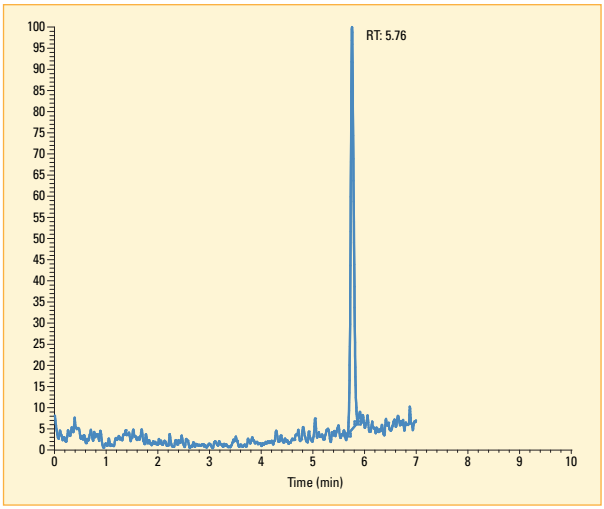

Figure 8: Clenbuterol, 0.1 pg/µL in urine, 0.1 Da FWHM

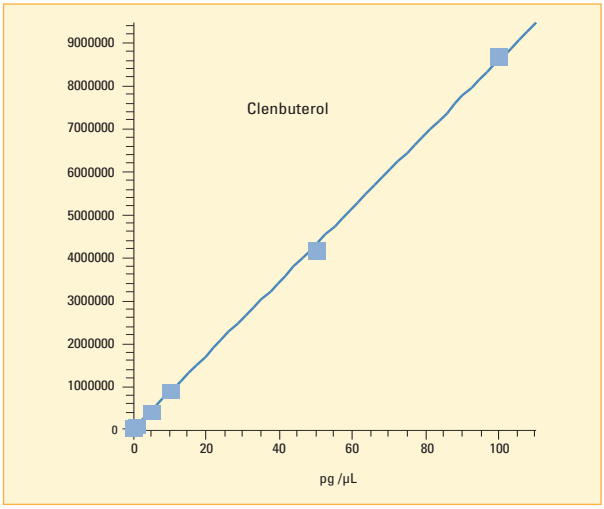

Figure 9: Clenbuterol curve at 0.1 Da FWHM

SAMPLE NAME	AREA	CALC AMT	UNITS	%RSD
Urine blank	0.00	0.00	pg/L	
Urine blank	0.00	0.00	pg/L	
Cal 0.1 pg/L	11245.02	0.10	pg/L	0.2%
Cal 0.1 pg/L	11272.54	0.10	pg/L	0.2%
Cal 0.5 pg/L	41960.02	0.46	pg/L	1.1%
Cal 0.5 pg/L	42592.84	0.46	pg/L	1.1%
Cal 1 pg/L	90353.60	1.02	pg/L	3.4%
Cal 1 pg/L	94633.92	1.07	pg/L	3.4%
Cal 5 pg/L	435920.49	5.04	pg/L	0.4%
Cal 5 pg/L	438538.32	5.07	pg/L	0.4%
Cal 10 pg/L	893656.24	10.36	pg/L	0.9%
Cal 10 pg/L	904758.00	10.49	pg/L	0.9%
Cal 50 pg/L	4120496.02	47.90	pg/L	1.3%
Cal 50 pg/L	4195902.58	48.78	pg/L	.3%
Cal 100 pg/L	8667429.70	100.81	pg/L	0.5%
Cal 100 pg/L	8727427.54	101.50	pg/L	0.5%

Table 2: Calculated standards at 0.1 Da FWHM

The increase in selectivity at a peak width setting of 0.1 Da FWHM is due to the fact that Clenbuterol is a chlorinated compound and thus the negative mass deficiency can be used to eliminate interferents from the urine matrix in SRM mode. This increased selectivity can be achieved without detrimental loss of transmission. Typically only a factor of two to three fold decrease in peak area is observed between analyses performed at 0.7 and 0.1 Da FWHM, however, greater selectivity could then be achieved.

The calibration curves for Clenbuterol concentrations of between 0.1 to 100 pg/µL at resolution settings of 0.1 and 0.7 Da FWHM both demonstrate excellent linearity. The calibration line at 0.7 Da FWHM showed a high intercept due to chemical background in the urine blank. This was significantly reduced by the use of high resolution.

The use of higher resolution to increase selectivity and precision could enable the limit of quantitation of an assay to be lowered and achieves a higher degree of confidence in identification of analytes in biological matrices.

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