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Application News

Gas Chromatograph Mass Spectrometer

No. GCMS-1401

Analysis of Opioids Using Isotope Dilution with GCMS-TQ8030 GC/MS/MS

■ Introduction

Development of methods for analysis of drugs of abuse has become a high priority for both forensic toxicology and law enforcement. The large numbers of individual drugs and new "designer drugs" has made method development for these compounds a significant undertaking.

Gas chromatography mass spectrometry (GCMS) has been used extensively for analysis of drug residues and trace-level drugs in biological fluids. The most significant challenges have been matrix interference and achievement of meaningful detection limits for the compounds of interest. Triple quadrupole GC/MS/MS has emerged as a powerful technique for trace-level analysis in these complex biological matrices. Operation of the triple quadrupole GC/MS/MS in the Multiple Reaction Monitoring (MRM) mode provides exceptional sensitivity, selectivity, and specificity for detection and quantitation of targeted drugs in the presence of background interferences.

The isotope dilution technique, using isotopically-labeled analogs of target compounds as internal standards, is a widely used analytical approach for precise quantitation in drug assays. However, in many cases, when using deuterium-labeled analogs the mass spectra differ only slightly from the corresponding unlabeled compounds. The challenge is complicated when the native and labeled compounds completely or partially co-elute, as they often do, and the spectra overlap. Combining the specificity of unique MRM transitions for close-eluting native and labeled analogues, with the sensitivity of triple quadrupole MRM transitions is a powerful technique for unambiguous, quantitative determination of this important compound class.

This application note presents instrument configuration, operating parameters, and analytical results for analysis of a common narcotic, hydrocodone, using the isotope dilution technique paired with the specificity of the MRM analysis mode of the Shimadzu GCMS-TQ8030 triple quadrupole GC/MS/MS (Figure 1). Internal standard calibration of codeine and oxycodone was also included in the study.

Figure 1: Shimadzu GCMS-TQ8030 Triple Quadrupole GC/MS/MS

■ Experimental

The analyses were conducted using a Shimadzu GCMS-TQ8030 triple quadrupole GC/MS/MS operated in the multiple reaction monitoring (MRM) mode, with optimized collision energy (CE) for each MRM transition providing ultimate sensitivity. The instrument configuration and operating conditions are shown in Table 1.

Table 1: Instrument Configuration and Operating Conditions for Analysis of Opioid Drugs

Instrument	**GCMS-TQ8030**
Inlet	270 °C Splitless liner with glass wool (Shimadzu 221-48876-02) Splitless injection, sampling time 1 minute
Column	RXI-5MS, 30 m x 0.25 mm x 0.25 µm (Restek 13423) Helium carrier gas Constant linear velocity 37 cm/second
Oven Program	100 °C, hold 1.0 minute 20 °C/minute to 250 °C, hold 3.0 minutes 10 °C/minute to 300 °C, (no final hold) MS interface 250 °C Analysis time 20 minutes
Ion Source	200 °C Electron ionization (EI) mode, 70 eV
Operation Mode	Multiple Reaction Monitoring (MRM) Argon gas, 200 kPa
Detector	Electron multiplier 1.0 kV

Six MRM transitions were selected for both hydrocodone-d_3 and hydrocodone, most of which had unique precursor ions paired with common product ions. (Refer to Table 2.) This approach allowed evaluation of any potential mass spectral interference, or cross-talk, between the transition pairs of these two co-eluting compounds. Three transitions were selected for codeine and oxycodone, since they were chromatographically resolved from the other compounds, and there were no isotopically labeled internal standards used.

Table 2: MRM Transition Details with Optimized Collision Energies (CE)

Compound	**Transition #1 (CE)**	**Transition #2 (CE)**	**Transition #3 (CE)**	**Transition #4 (CE)**	**Transition #5 (CE)**	**Transition #6 (CE)**
Hydrocodone-d_3 (IS)	302 > 242 (11V)	302 > 214 (19V)	302 > 185 (27V)	302 > 273 (19V)	302 >245 (27V)	302 > 231 (27V)
Hydrocodone	299 > 242 (11V)	299 > 214 (19V)	299 > 185 (27V)	299 > 270 (19V)	299 > 242 (23V)	299 > 228 (23V)
Codeine	299 > 162 (11V)	299 > 229 (19V)	299 > 280 (15V)			
Oxycodone	315 > 258 (11V)	315 > 230 (19V)	315 > 201 (19V)			

Calibration standards were prepared in methanol, and data for a 5-point calibration were acquired over the range of 25-200 ng/mL (parts-per-billion, ppb). The calibration curve for hydrocodone was generated using the isotope dilution technique. The concentration of the internal standard, hydrocodone-d_3 was held constant at 100 ng/mL. The concentration range of the calibration was sufficient to satisfy the requirements of the specific application. The chromatographic conditions chosen were intended to fit into a larger scheme for analysis of numerous drug classes, so optimization of the chromatographic conditions for efficiency was not considered in this study.

■ Results and Discussion

Chromatography

The total ion chromatogram (TIC) acquired in the MRM mode for the opioid drug mix is shown in Figure 2. The chromatographic peaks for hydrocodone-d_3 and hydrocodone partially overlap, with the deuterium labeled analog eluting first. In the MRM mode, the TIC is the sum of the signal for each MRM transition for that particular analyte, so the appearance of the chromatogram is slightly different than the typical TIC chromatogram from full scan analysis.

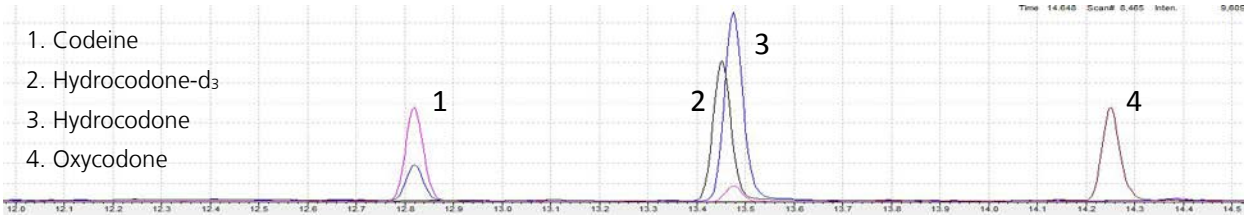


Figure 2: Total Ion Chromatogram (TIC) of Opioid Standard

Mass Spectral Results in the Full Scan ("Q3 Scan") Mode

The full scan mass spectra of hydrocodone-d_3 and hydrocodone are shown in Figures 3A and 3B. Notable features of these mass spectra are the prominent molecular ions for the labeled and unlabeled compounds at *m/z* 302 and 299, respectively, with the difference of 3 *m/z* units associated with the isotopically labeled n-methyl group on hydrocodone-d_3.

Common fragment ions are present in both spectra at *m/z* 242, 214, 199, 185, and 115 (indicated with an ↓ in figures 3A and 3B). These fragments represent loss of a fragment which includes the labeled n-methyl group from hydrocodone-d_3, and the corresponding unlabeled n-methyl group from hydrocodone, to form identical fragment ions from the two compounds.

Fragment ion pairs in the spectra for the labeled/unlabeled compounds can be seen at *m/z* 287 and 284, 273 and 270, 231 and 228, 99 and 96, 62 and 59 (indicated with an * in figures 3A and 3B). In this case, the corresponding fragments are offset by a difference of 3 *m/z* units (e.g. 287 and 284), and represent the loss of the same non-labeled group from hydrocodone-d_3 and hydrocodone, respectively.

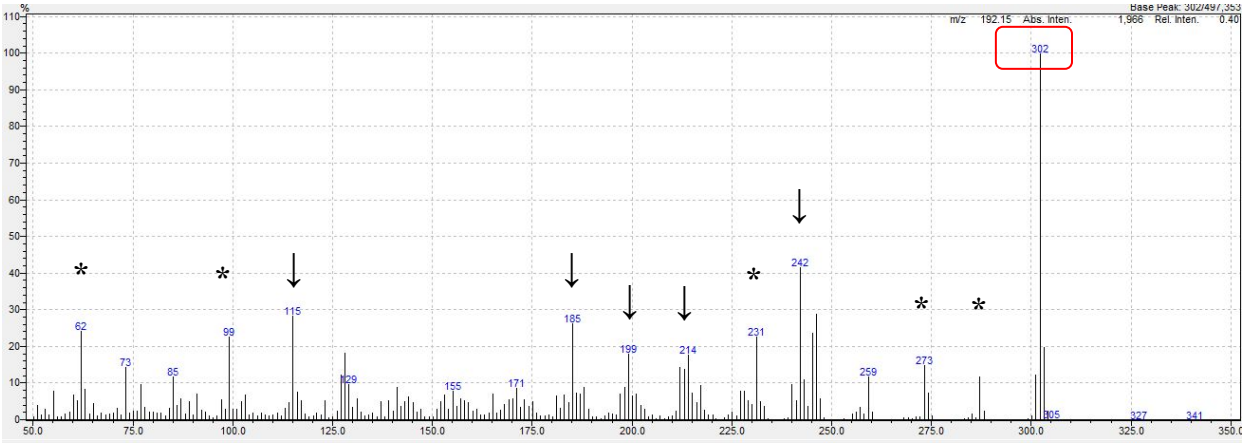


Figure 3A: Total Ion Mass Spectrum of Hydrocodone-d_3

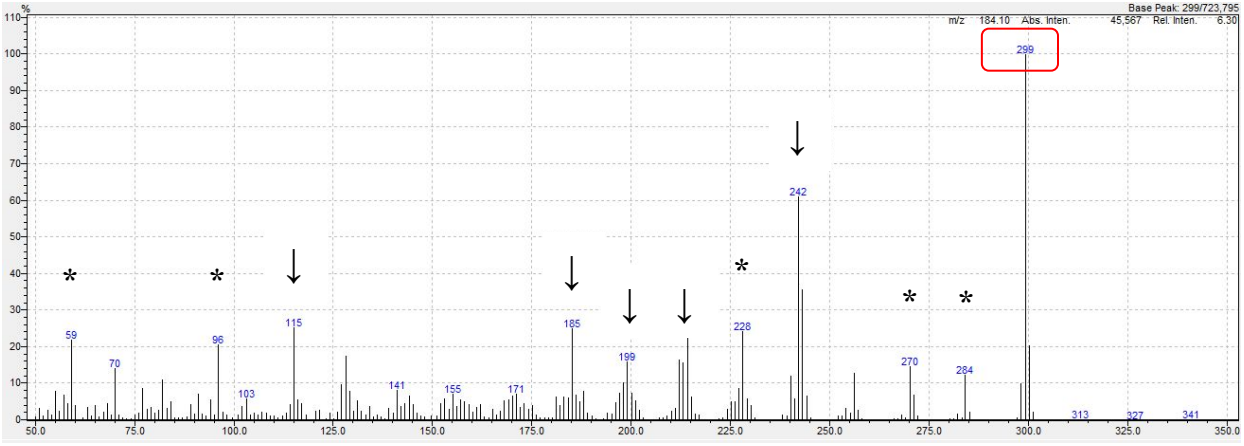


Figure 3B: Total Ion Mass Spectrum of Hydrocodone

The full scan spectra of hydrocodone-d_3 and hydrocodone were used to select precursor and product ions for the MRM transitions. Three transitions were selected for each compound based on their unique molecular ions and common product ions (e.g. 302 → 242 and 299 → 242). To illustrate the unique specificity of the MRM mode, a second set of three transitions was defined using the molecular ions and unique product ions for hydrocodone-d_3 and hydrocodone (e.g. 302 → 273 and 299 → 270). The ions selected for MRM transitions are tabulated in Table 2 above.

Mass Spectral Results in the Multiple Reaction Monitoring (MRM) Mode

Operation of the GCMS-TQ8030 in the MRM mode provides enhanced selectivity for analysis of trace-level contaminants in complex matrices, such as drugs of abuse in biological fluids, because the co-extracted matrix interferences are significantly minimized. The compound specificity that can be achieved by using unique MRM transitions for each compound, even when they have common product ions, is illustrated in Figure 4. Figure 4 includes six overlaid MRM chromatograms for hydrocodone-d_3 and six for hydrocodone, as described above. Note that the chromatograms corresponding to the MRM transitions for hydrocodone-d_3 and hydrocodone are uniquely defined for each of the analytes and do not interfere with one another, even for the three transitions that have common MRM product ions. The non-interfering chromatograms illustrate the power of the MRM mode, and the specificity that can be achieved when unique transitions are selected for close-eluting compounds with similar mass spectra.

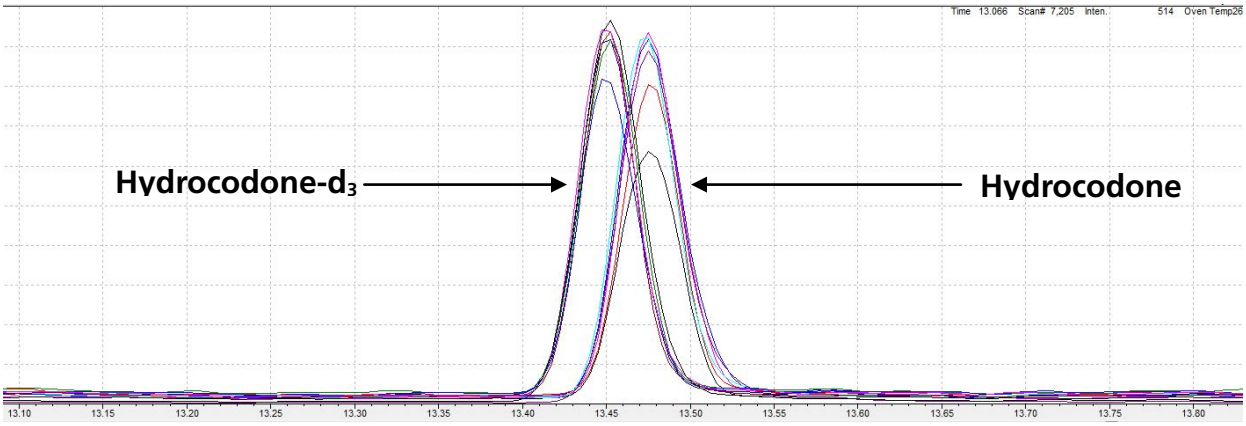


Figure 4: Six Overlaid MRM Chromatograms for Hydrocodone-d_3 and Six for Hydrocodone

Cross-Talk

"Cross-talk" is a phenomenon unique to triple quadrupole mass spectrometry. It occurs when residual ion fragments are not fully swept from the collision cell at the end of a cycle; they remain in the collision cell and are detected as "ghost fragments" in subsequent transitions. Cross-talk is depicted graphically in Figures 5A and 5B below. Figure 5A depicts slowing down of product ions in the collision cell, which results from interactions with the CID gas. In some cases, a small portion of the residual product ions have slowed down, and may not be completely swept from the collision cell during the transition, resulting in cross-talk. Figure 5B illustrates the results of cross-talk as "ghost" mass spectral fragment peaks that can appear in subsequent transitions.

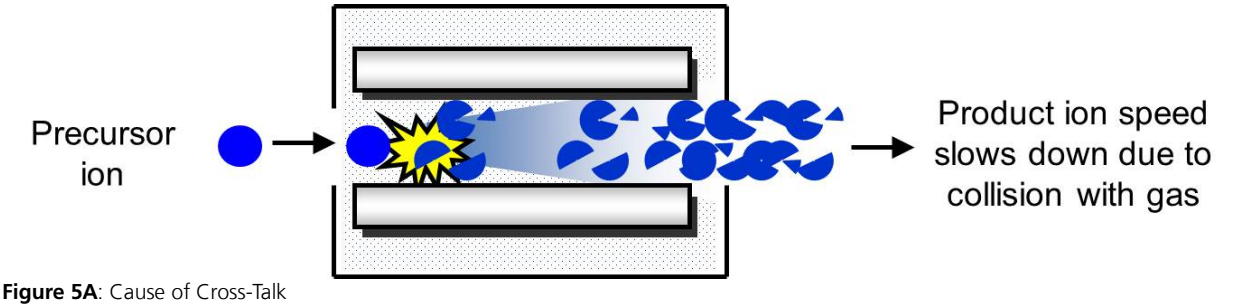


Figure 5A: Cause of Cross-Talk

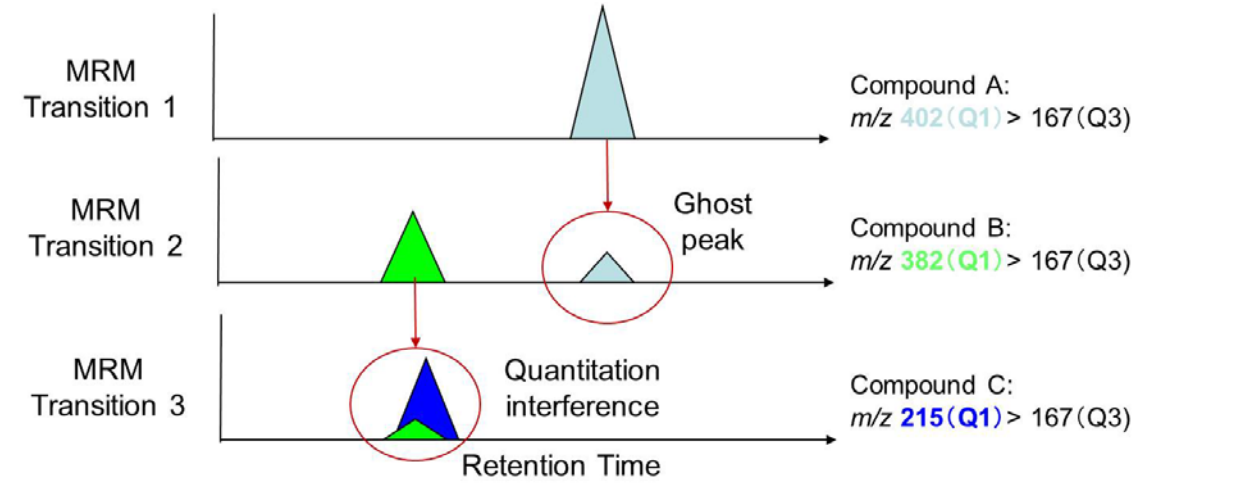
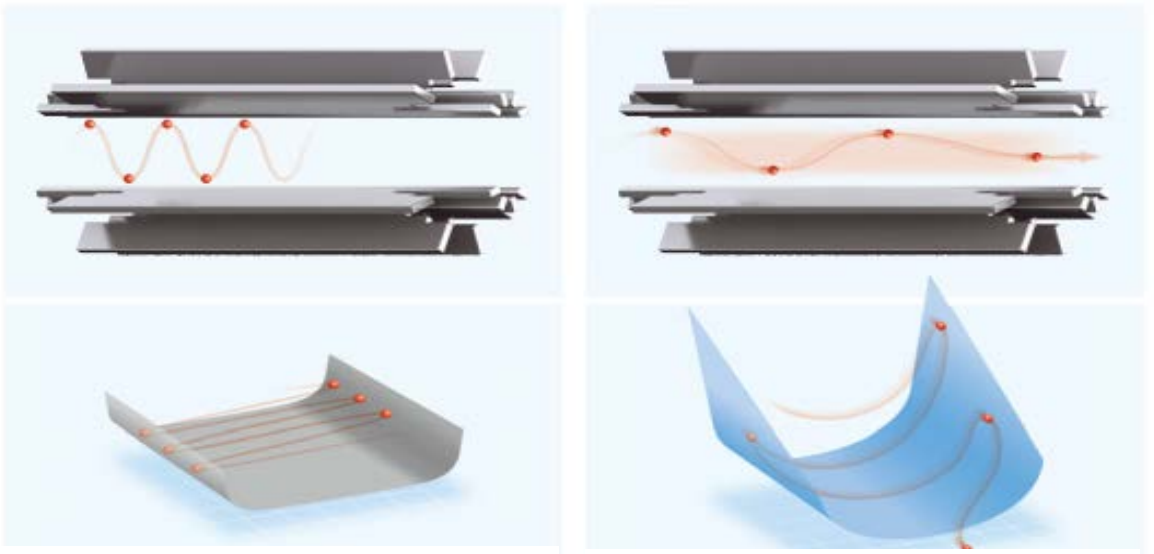


Figure 5B: Results of Cross-Talk

Cross-talk is virtually eliminated in the GCMS-TQ8030 using UFsweeper® technology. The UFSweeper design alters the pseudo-potential surface within the collision cell, shortening the path which ions must travel and accelerating them through the cell and toward Q3. This process completely clears the collision cell with each transition, and eliminates cross-talk from one transition to the next. The pseudo-potential surface of the GCMS-TQ8030 UFsweeper® technology is illustrated in Figure 6 below. The overlaid chromatograms in Figure 4 clearly show that there was no indication of cross-talk present.



With conventional technology, ions lose momentum and have a longer travel path due to collision with CID gas

The UFSweeper® pseudo-potential surface shortens the pathway and accelerates ions through the collision cell without losing momentum

Figure 6: Graphic Depiction of UFSweeper® Technology

Calibration Results

Five calibration standards were prepared for the opioids over the range of 25-500 ng/mL (ppb) and transferred to autosampler vials with limited-volume inserts for analysis; hydrocodone-d_3 was used as the the internal standard and was held at a constant concentration of 100 ng/mL. The calibration standards were analyzed using the instrument conditions outlined above. The electron multiplier was adjusted to give acceptable response at the lowest calibration level and avoid saturation at the highest calibration level.

Response factors were calculated and percent relative standard deviation (%RSD) determined using the GCMSsolution software. The precision of the calibration is evaluated using the %RSD of the response factors and the correlation coefficient (r) for each of the calibration analytes. The %RSD and correlation coefficient values for the multi-point calibration are shown in Table 3. The linear, multi-point calibration curve for hydrocodone is illustrated in Figure 7. Calibration results demonstrate linearity for each of the analytes.

Table 3: Results of the 5-Point Calibration for Three Opioids From 25 to 200 ng/mL using the MRM Analysis Mode

Compound	Calibration Type	Mean RF	RF %RSD	r
Codeine	Internal Standard	0.643	12.1	0.9995
Hydrocodone	Isotope Dilution	1.011	15.6	0.9999
Oxycodone	Internal Standard	0.376	14.8	0.9999

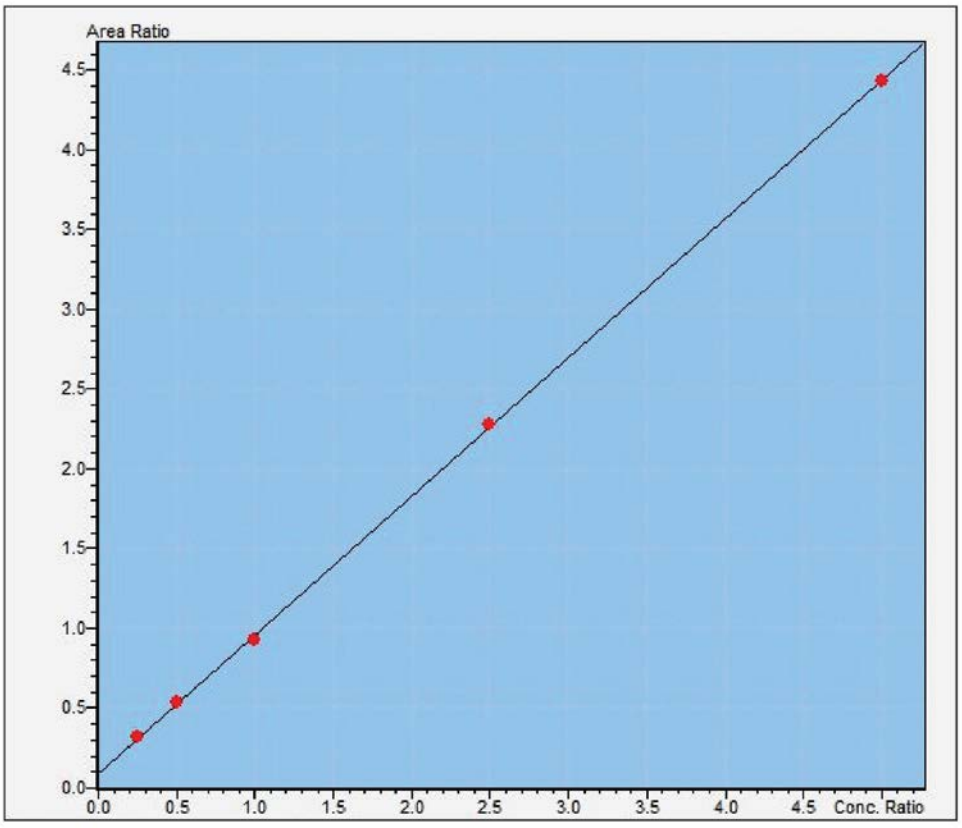


Figure 7: Linear, Multi-point Calibration for Hydrocodone from 25 to 200 ng/mL

■ Summary and Conclusion

The results demonstrate the power and specificity of the MRM analysis mode when using unique transitions for close-eluting compounds such as hydrocodone-d_3 and hydrocodone, even when they have similar mass spectra and common product ions. This experiment also illustrates the effectiveness of the Shimadzu GCMS-TQ8030 fast scanning and UFsweeper technologies for completely clearing the collision cell with each transition and eliminating any cross-talk. The multi-point calibration for hydrocodone was linear and passes thru zero, further supporting that there was no interference from cross-talk or the close-eluting deuterium-labeled internal standard.

■ Acknowledgement

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