

# Troubleshooting in Drug Compliance Monitoring by LCMS/MS

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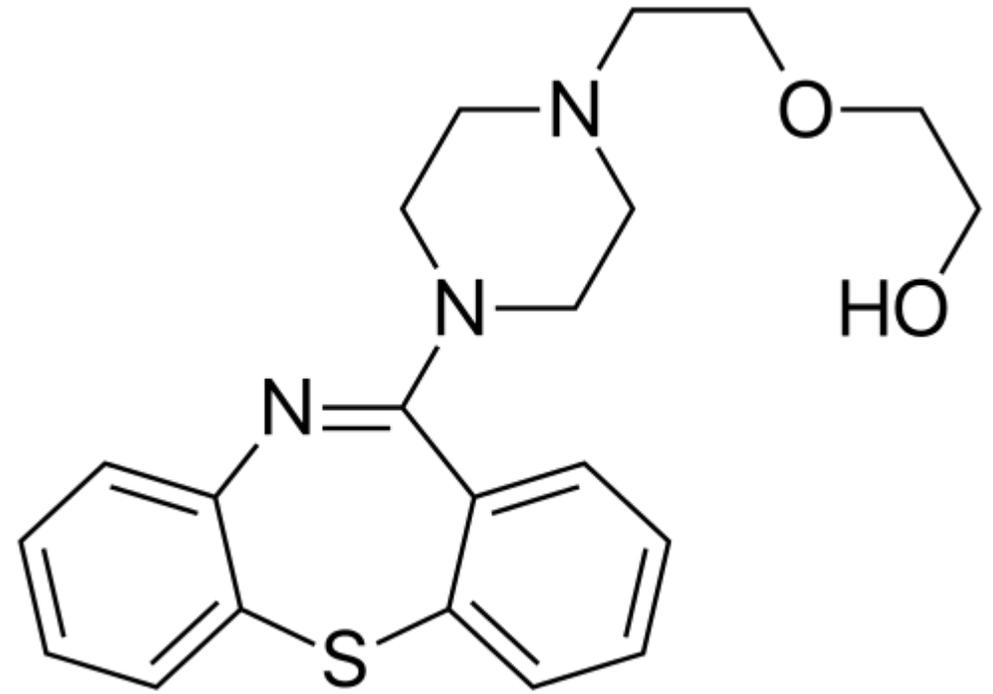
# Cases for Discussion Today

- Validation
- Proficiency
- Values
- Breakdown





# Validation Case: Quetiapine



When Your Results Don't Match the Reference Laboratory's Results

<http://www.nmslabs.com/tests/Quetiapine--Urine/4051U>

# Problem: Our Lab vs Their Lab

SAME



**Standards and Calibrators**

VS

DIFFERENT



**Patient Results**

# Possible Explanations

- a. Difference in  $m/z$  for detection
- b. Difference in calibrator material
- c. Difference in specimen preparation
- d. Difference in metabolism



# Poll: Why Different Patient Results?

Do you think that the difference in  $m/z$  for detection explains the results?

*Yes or No? Vote now!*



# What We Found:

A proficiency test was not available. The reference laboratory method added internal standards and did not hydrolyze with glucuronidase.

So the correct response was:



Because difference in specimen preparation was the real issue.

# Takeaways from this Case

- Validation of tests using another laboratory is an **accepted method** when no proficiency test is available.
- **BUT** not all metabolites are reported by big pharma.
- In this case, the glucuronide metabolite was **not reported** by the manufacturer.
- Make sure the methods are the same

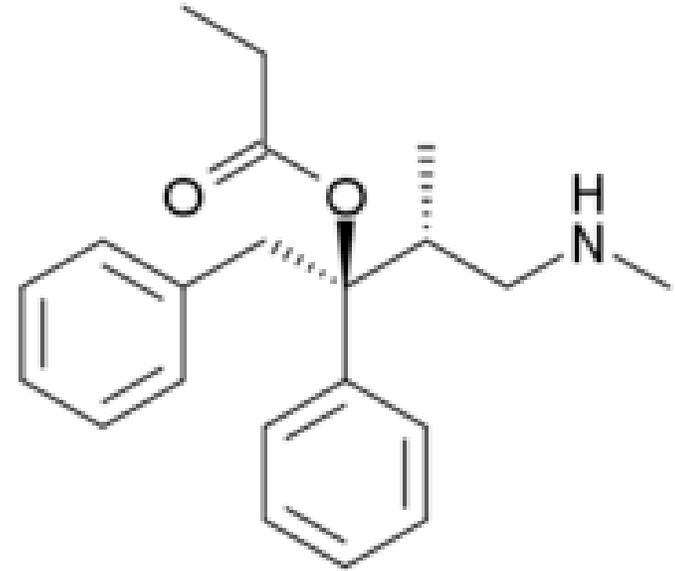




# Proficiency Case: Norpropoxyphene

When Your Results Don't Find the Drug

Crews et.al. Journal of Analytical Toxicology 2009; 33: 379-383



# Problem: Failed Proficiency Test

Our laboratory did not find the norpropoxyphene in the challenge test.

**FAILED**



# Possible Explanations

- a. Our process was correct: repeat the sample.
- b. Our process was wrong; find the flaw.
- c. The material was not correct.



# Poll: How to Pass the Test?

Do you think finding the flaw in the test process would find the drug?

*Yes or No? Vote now!*



# Corrective action: What are the next steps?

- a. Send proficiency test sample to another laboratory
- b. Order new calibrators and controls
- c. Run full spectra scan of proficiency sample
- d. All of the above



# Corrective action: Next steps results

- a. **Send proficiency test sample to another laboratory:** norpropoxyphene found by GC-MS
- b. **Order new calibrators and controls:** Same results as before
- c. **Run full spectra scan of proficiency sample:** Norpropoxyphene not found

No evident resolution. Note the GC-MS parent drug m/z of 308 was the same as the LC-MS/MS m/z used to monitor the proficiency test sample.

# Corrective action: Next steps results

- Order new calibrator material from a different provider as a solid.
- Weigh out standards and make new solutions and rerun new calibrator:
  - a. Expected result find Norpropoxyphene
  - b. Observe same quantitation
  - c. Amount observed much lower than weighed in
  - d. Amount observed much higher than weighed in

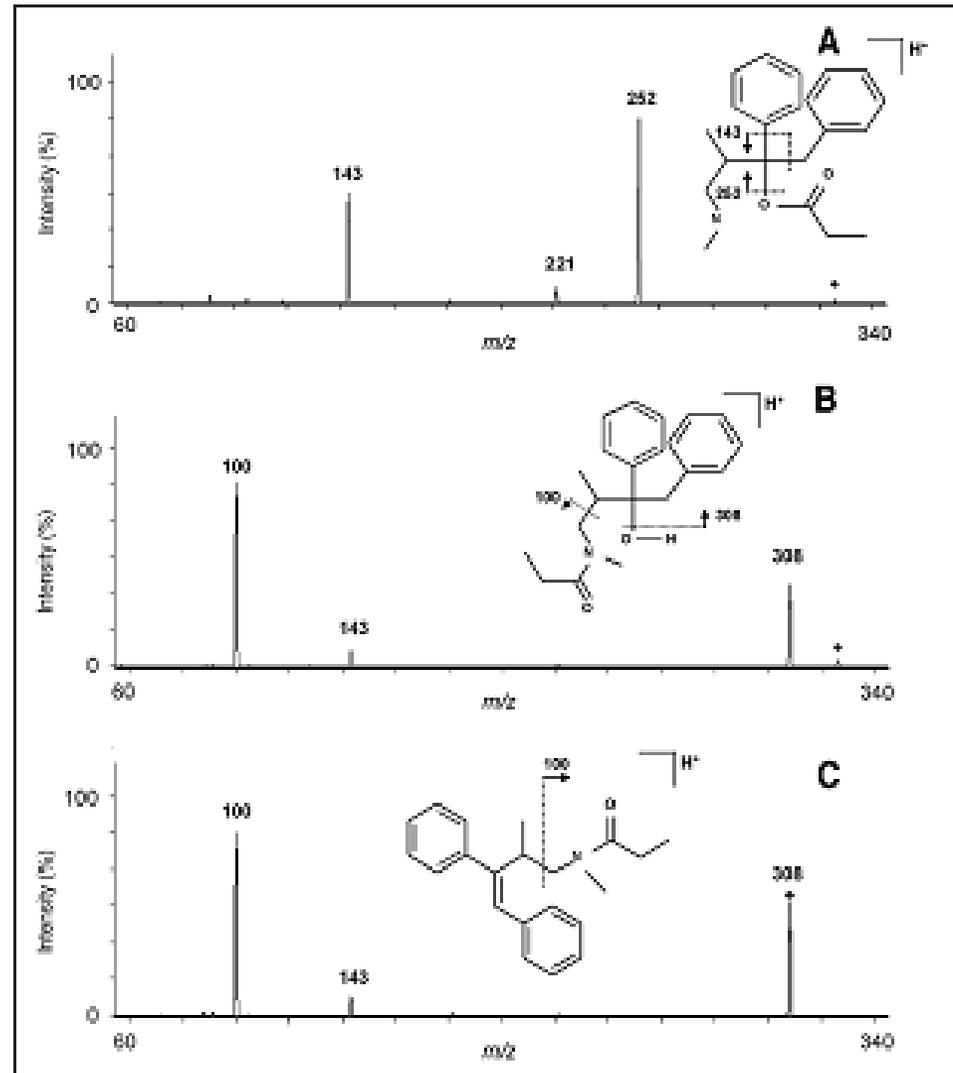
# Corrective action: Next steps results

- Run MS scans on new and old calibrators and controls
- Found old calibrators had parent peak at 308 m/z while those made from powder had peak at 326 m/z
- Reran proficiency test sample and found norpropoxyphene at 326 m/z which was the literature value.

# Why was a different m/z observed?

- Different between the two norpropoxyphene standards was 326 – 308 or 18 amu. This is consistent with loss of a water molecule between the two standards.
- Cause of water loss is dehydration of the calibrators used to set up the method.
- Original calibrators sent in a methanol solution which caused dehydration.
- Proficiency test solution made from powder with no dehydration.

# Norpropoxyphene structures



# Poll: How to Pass the Test?

Do you think finding the flaw in the test process that is the LC-MS/MS SOP would find the drug?

*Yes or No? Vote now!*



# What We Found:

Repeat of test and review of procedures proved negative. The issue was ultimately found to be flawed material from the supplier.

So the correct response was:



Because the LC-MS/MS was working properly, the reference material was not correct.

# Problem solution: continued

- GC-MS method uses a preparation step which causes dehydration of the norpropoxyphene and the product has an  $m/z$  of 308 on analysis.
- In this case GC-MS and LC-MS/MS measure different products with different masses.
- Side note: The urinary excreted norpropoxyphene is present in both forms, cyclized (dehydrated) and non cyclized (hydrated).
- This is why the drug was found in patient samples.

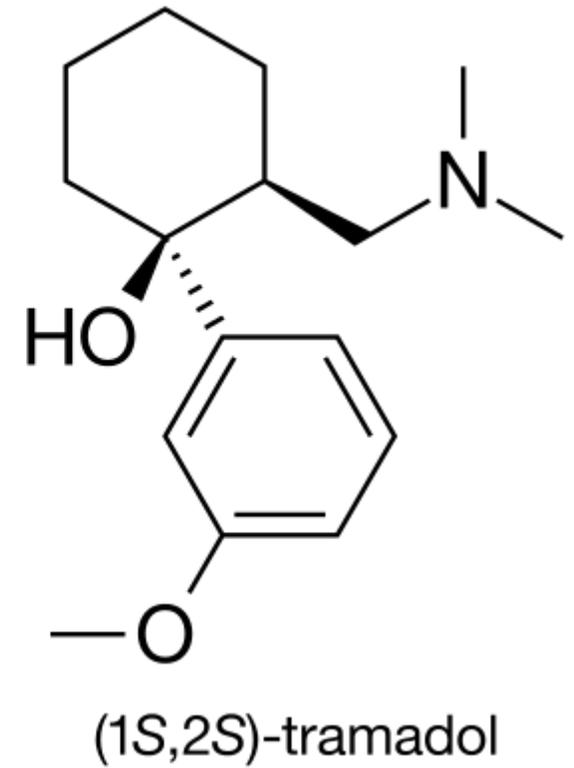
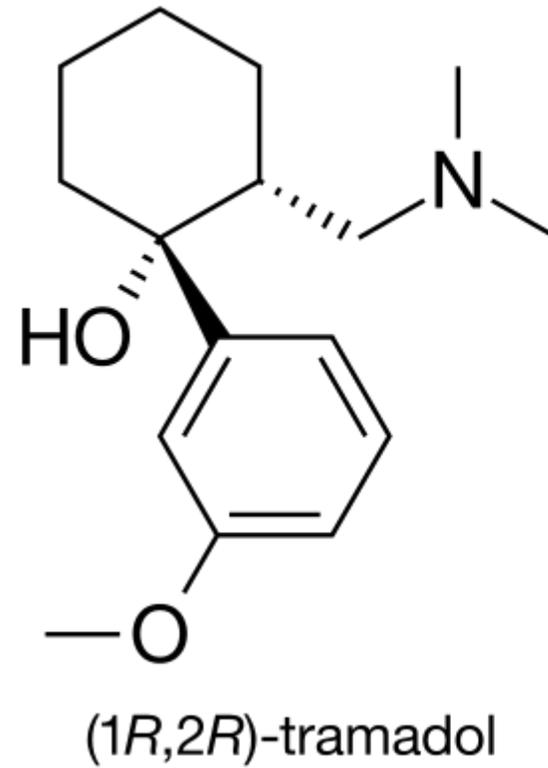
# Takeaways from this Case

- Never assume the material from your supplier is correct.
- Check the literature for articles on correct values.





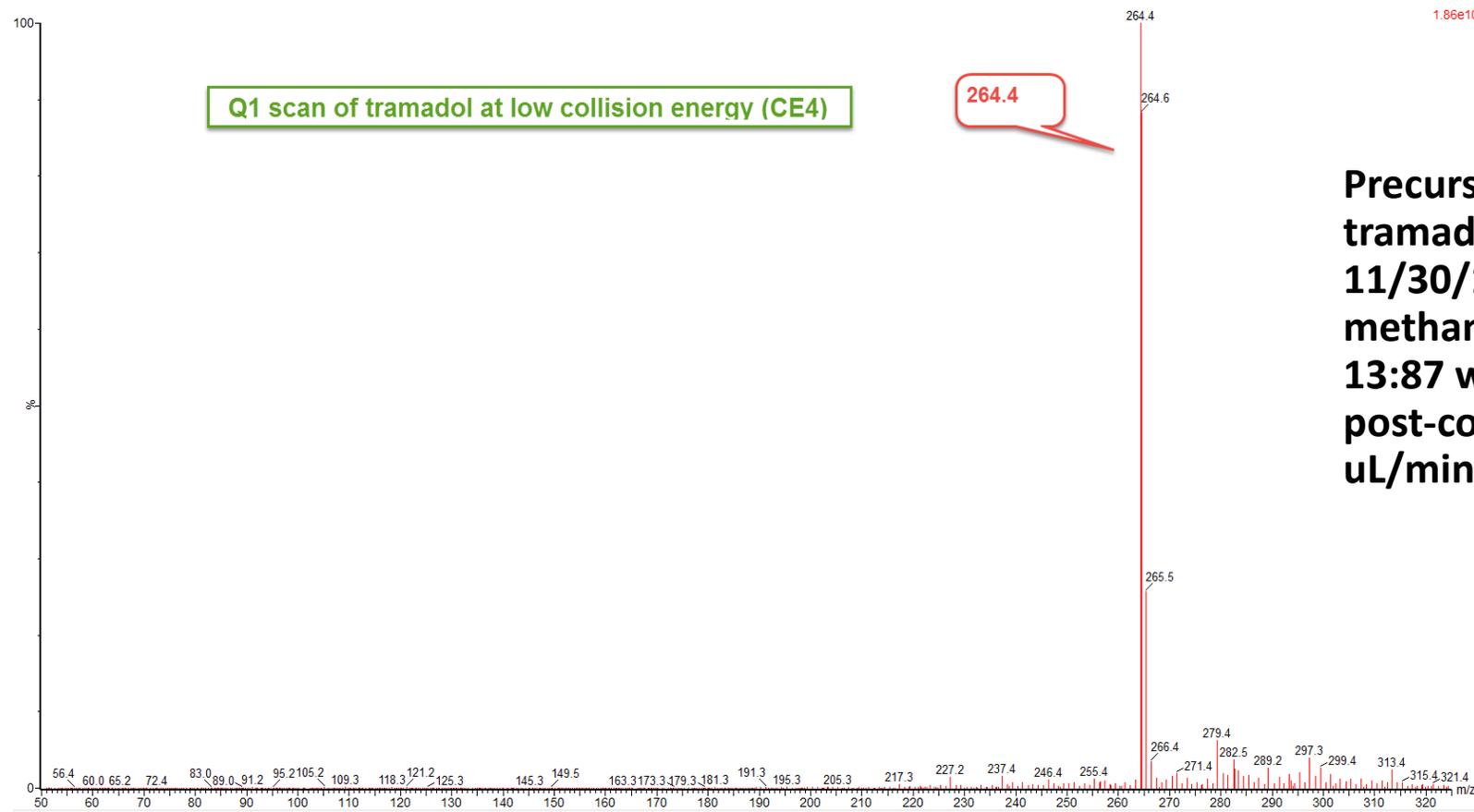
# Values Case: Tramadol



When Your Results are Incomplete

<http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/62595>

# Problem: Only One Ion Found



**Precursor and product ion scans of tramadol on Waters XEVO TQS 11/30/15 (1,000 ng/mL in methanol, combined flow, LC at 13:87 water:meoh, 0.4 mL/min and post-column syringe infusion at 2 uL/min)**



# Possible Solutions

- Assume there is only one ion.
- Do a pill count.
- Find a metabolite.
- Review prescription requisition.



# Poll: How to Validate Results if only one Daughter ion is used?

Do you think that looking for a metabolite would validate positive tramadol results?

*Yes or No? Vote Now!*





# What We Found:

A review of the literature found a metabolite formed in sufficient amounts, with standards.

So the correct response was:



Because a metabolite existed to complete the test.



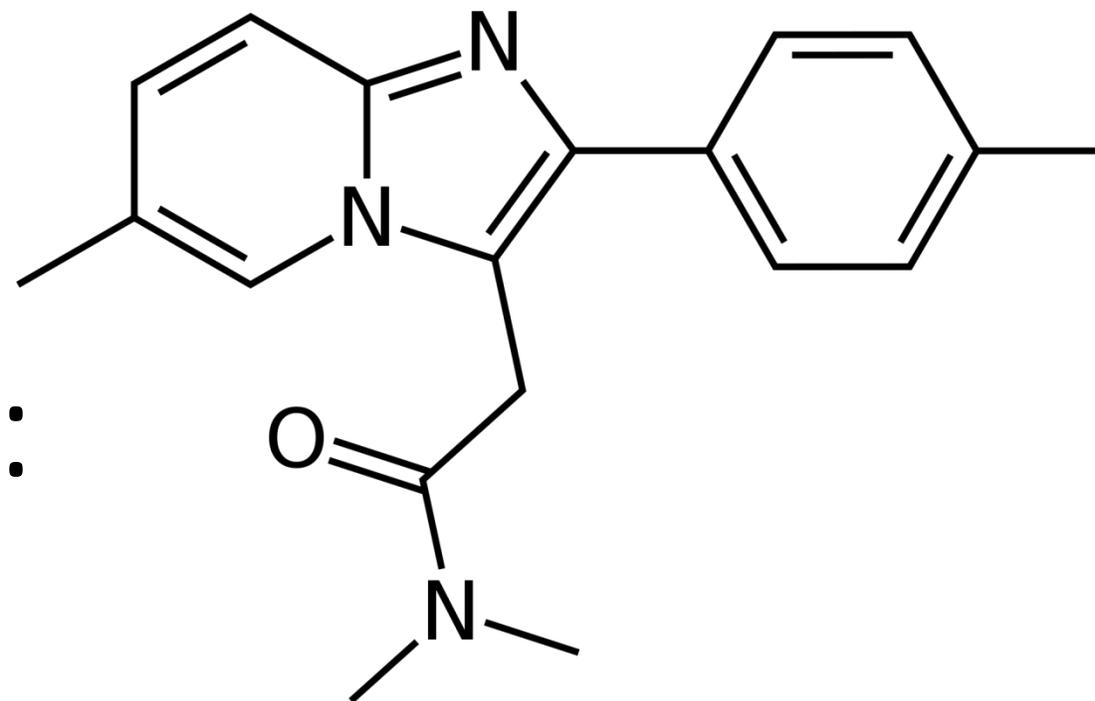
# Takeaways from this Case

- Fragment patterns are not always useable.
- Read the literature!
- If you can't measure the parent drug, then measure the metabolite.





# Breakdown Case: Zolpidem



When Your Results Don't Find the Parent or the Metabolite

[Schwoppe DM et al J Anal Toxicol.](#) 2014 Oct;38(8):513-8

# Problem: Parent Drug Not Found

Our laboratory could not find the parent drug in patient specimens and there were no known metabolites available from our usual supplier.





# Possible Explanations

- There is no parent drug.
- Find controls and standards for metabolites.
- If you can't find it, you can't measure it.
- Find a metabolite.



# Poll: How to respond to negative results?

Do you think a review of this drug's metabolism would be useful?

*Yes or No? Vote Now!*



# What We Found:

A review of the literature for metabolic products revealed that a metabolite had been synthesized in sufficient amounts by another lab.

So the correct response was:



Because another lab reported successfully synthesizing the metabolite for this drug.

# Takeaways from this Case

- Not all drugs have some parent drug excreted into urine.
- Literature search is **essential** to complete the test.
- If your lab synthesizes it, be sure to publish your results!



# Wrap-Up

- Drug monitoring by LC-MS/MS is a targeted analysis
- Drug metabolism is not always known
- Calibrators can be incorrect
- Drug may not fragment in a way that meets standards for identification
- Parent drug may not be in urine



# Questions?

Please type your questions in the chat area

# Thank You!

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