Reviewer’s report

Title: Accidental intoxications in toddlers: lack of cross-reactivity of vilazodone and its urinary metabolite M17 with drug of abuse screening immunoassays

Version: 0 Date: 09 Oct 2018

Reviewer: Reviewer 2

Reviewer’s report:

PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?

No - there are minor issues

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

Yes - the approach is appropriate

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?

Yes - experiments and analyses were performed appropriately

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Could an appropriately REVISED version of this work represent a technically sound contribution?

Probably - with minor revisions

PEER REVIEWER COMMENTS:
GENERAL COMMENTS: The paper describes a computer-based test to discover metabolites of vilazodone that resemble the structure of amphetamines. Furthermore, the authors describe in detail the synthesis of a metabolite of the antidepressant drug vilazodone that has structural similarity to amphetamines. Finally, the cross-reactivity of vilazodone and its metabolite is tested in immunoassays from Roche Diagnostics.

The paper is well written, and are easy to comprehend. The objectives of the study are mostly well explained and the methods for 2D similarity testing and synthesis of the metabolite M17 are presented in detail. The figures and tables are presented in a well-organized manner.

The discussion is mostly repeating results, and should be rewritten, focusing on possible explanations why no cross-reactivity was found, in what way one could further examine possible cross-reactivity of vilazodone (urine samples from patients administered vilazodone, discovering other metabolites and testing cross-reactivity of these). The authors should also consider discussing potential consequences of false positive amphetamine screen in patients administered vilazodone. If former case reports with false positive amphetamine results originate from other amphetamine assays than the one used in the present study (Roche), this should also be discussed, and testing cross-reactivity of vilazodone and metabolites in other amphetamine assays should be encouraged.

The conclusion of the paper is almost identical to the conclusion in the abstract, and should also be rewritten.

REQUESTED REVISIONS:

Abstract:

Line 52: Hyphen "cross-reactivity"

Background:

Line 94-96: The authors could consider removing this sentence. QT-prolongation is not further referred to in the manuscript.

Line 116: "Reports" and "reported" in the same sentence, please consider alternatives.

Line 119-124: Since immunoassays from different companies have different characteristics, the authors should present which immunoassay they are referring to here. In reference 18 it is the same as the one tested in the article. I have unfortunately not been able to access reference 17.

Methods:

Line 152: The first "or" should be "of".
The presentation of 2D molecular similarity analysis and chemical synthesis of M17 should switch place, presented in the same order as the study actually was performed.

The description of the synthesis of M17 is comprehensive. Depending on the scope of the journal, this could be a part of supplementary material, and presented in a short form in the main manuscript.

Line 257-258: "Drug of abuse assays" and "drug abuse assays" are both used in this manuscript. The authors could choose one writing style.

Line 261: Screeningscreening…

Line 273-279: Characteristics of different immunoassays could rather be a subject of the discussion.

Results:

The first paragraph describes an interesting retrospective analysis, summarized in table 2. It is not obvious for me if this retrospective analysis is the same as the one referred to in "Background" from ref 17 and 18, though the legend of table 1 describes in more detail in what way the results are created (a combination of results from ref 17 and 18, with supplemental data from a new chart review?). The objective of doing a retrospective analysis should be described in "Background" since it is an important part of the work as well. More details of how it is performed (the combination of former results and a new chart review) should be incorporated in the first paragraph of "Method". Are the same immunoassay methods used in both ref 17, 18 and in the new chart review? Table 1 should be referred to in "Background", and not under the banner "Results", since this is not part of the present study.

Line 316-318: This sentence should not be a part of the results section.

Discussion:

The first two paraphrases are mostly repeating information from "Background" and could be shortened/removed from the manuscript. Some of the text in paragraph two could be incorporated in a discussion on possible clinical consequences of false positive amphetamine results in patients with intake of vilazodone. The authors should consider discussing if a false positive amphetamine assay could result in different patient treatment or follow-up. The authors could also discuss if positive amphetamine immunoassay results in children routinely should be confirmed with specific analytical methods.

Line 416-427: This paragraph is repeating information from the results section and could be shortened.
Line 431: Cross-reactivity is tested up to a concentration of 100,000 ng/mL. This is a common upper concentration for cross-reactivity testing. Do the authors have any reason to believe that the concentration of vilazodone and/or metabolites could exceed this concentration in urine from patients with vilazodone overdose? This could be addressed in the discussion.

Conclusion:

The conclusion of the paper is almost identical to the conclusion in the abstract. Studies that could further enlighten the question of cross-reactivity of vilazodone and its metabolites on amphetamine assays could be emphasized.

Note: This reviewer report can be downloaded - see attached pdf file.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable

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