CHEMISTRY
McMaster University

www.chemistry.mcmaster.ca

Department of Chemistry, McMaster University
1280 Main Street West, Hamilton, ON Canada L8S 4M1
(905) 525-9140 ext. 24504 (voice); (905) 522-2509 (fax)

27 September, 2018

Dr. Phillip Steindel
Review Editor, JoVE

Re: Revised Manuscript*:* "High Throughput and Comprehensive Drug Surveillance using Multisegment Injection-Capillary Electrophoresis-Mass Spectrometry (JoVE58986)"

Dear Dr. Steindel,

Please note that the editorial and reviewer's comments are in *italic*, whereas our responses are in normal font. All changes made to the revised manuscript based on the reviewer comments are highlighted using track changes to identify all of the edits.

Editorial Request for Formatting Changes:
We thank the editor for highlighting the changes required for our manuscript and the changes are highlighted (track change format) in the revised version of the manuscript.

-Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
-Please revise lines 157-159, 189-190, and 214-215 to avoid previously published text.
-Please define all abbreviations before use.

We have updated these changes in the revised manuscript

-JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by "(see Table of Materials)" to draw the readers' attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Surine™, Cerilliant, Round Rock, Polymicro Technologies Inc., MicroSolv, Agilent, Wavemetric Inc., Igor Pro 5.0, Kim wipe, Agilent MassHunter, OxycontinTM, CelexaTM, EffexorTM, etc.

We have removed these names from the revised manuscript, and we have included these commercial trade names or suppliers only in the Table of Materials as requested.

-Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.

We have updated the numbering of the protocol based on the recommendations above.

-Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

-Line 105: The meaning of this statement is unclear. If details are presented in lines 107-110, please combine these two steps.

We have combined lines 107-110 with line 105

-Line 109: Do you mean that sonicate and degas the solution at the same time? If not, please specify the time needed for each procedure.

We have updated the statement to "sonicate the solution for 15 min"

-Line 112: The meaning of this statement is unclear. If details are presented in lines 113-119, please combine these two steps.

We have combined lines 113-119 with line 112

-Lines 121-128: Please describe how to prepare standards in the imperative tense.
-Lines 130-142: Please describe how to prepare calibration curves in the imperative tense.
-Line 165: Please describe how capillary conditioning is actually done in the imperative tense.

We have described the protocols in the imperative tense.

-Line 183: What is the flow rate of BGE?

The BGE flow rate is approximately 10 nL/min but it is determined by the magnitude of the electroomostic flow and the applied pressure gradient. As a result, it is not a fixed flow rate setting like in conventional chromatography.

- Line 201: Where is sheath liquid delivered?

We have updated the requested information. It is being delivered to the CE-MS interface to provide a make-up flow for stable spray formation.

-Lines 205-206: Please move the equipment information to the Table of Materials.

Yes, we have moved the equipment information to the Table of Materials.

-Lines 218-225: Please describe how to actually perform data analysis. To be filmed, software must have a GUI (graphical user interface) and software steps must be more explicitly explained ('click', 'select', etc.). Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc.) to your protocol steps.

We have described data analysis section in details as requested.

-Lines 230-244: Please describe in the imperative tense the specific actions being performed here.

We have described the specific action in the imperative tense.

-Please include single-line spaces between all paragraphs, headings, steps, etc.
-After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
-Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.
-Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

We have updated the recommended changes and highlighted the section for filming.

-Discussion: As we are a methods journal, please also discuss critical steps within the protocol, any modifications and troubleshooting of the technique, and any limitations of the technique.

We have addressed the above recommendations.

-References: Please do not abbreviate journal titles.

We have made the requested changes in the revised manuscript.

-Reviewer #1:
Comments to the Author
This publication described the protocol for a high throughput and sensitive screening method for identification and quantification drugs of abuse in urine. Preparation of BGE and solution are clearly described.
-However, I think more information should be provided regarding compounds identification and quantification when no d-IS is available.

We thank **reviewer #1** for the critical comments concerning drug identification and quantification when matching deuterated internal standards are unavailable. Supporting information was provided in the long abstract and discussion sections. For instance, "detection of one or more bio-transformed metabolites of parent drug adds further confidence towards unambiguous identification" and "when a matching D-IS is unavailable for certain DoA, a surrogate IS is used for data normalization using a synthetic chemical standard not found in urine (F-Phe) as described previously [ref. 14]". The latter point is repeated both in results and discussion section of the revised manuscript, which we have also previously demonstrated in our previous work.

-Do you think this approach could also be used for instance for highly polar substances, such as pesticides screening?

Indeed, our methodology is applicable for high throughout screening of other classes of polar/ionic chemicals in complex sample matrices, such as anionic pesticides (e.g., glyphosate, glufosinate, ethephon etc.).

-I think this manuscript could gain in clarity with a table describing all the steps of the injection process.

We have updated the description of the serial injection process then in the revised manuscript, but have kept the format requested by the journal for the protocol. This process will be much clearer as well during the recoding session of our protocol by video.

-I think description of the procedure for Matrix effect identification and correction should be discussed. (line 309-311), especially when no d-IS is available.

Please refer to our responses above. Additionally, we have also mentioned in the discussion and protocol sections that the mass calibrant ions added to the sheath liquid also serve as an effective way to monitor for potential ion suppression or enhancement effects in "real-time" during separation, which was also described extensively in our previous manuscript [ref. 14].

-As shown highlighted in figure 1B, you have a diminution of the isobaric resolution, probably due to the gradient pressure used (2mbar/min). How do you manage pic integration to get reliable quantification in such circumstance?

Indeed, Figure 1B is an extreme (yet impressive) example of simultaneous resolution of three isomeric opioids (equimolar concentrations of standards in synthetic urine) from 10 independent samples injected within a single run when using MSI-CE-MS. It is true that baseline resolution is not fully achieved for the later migrating peaks due to increased longitudinal diffusion; however, this is still sufficient for reliable drug screening purposes which often requires semi-quantitative performance to detect drugs above a minimum threshold/cutoff level. Moreover, detection of all three opioid isomers in all urine samples analyzed is also not likely to occur under most circumstances. The reviewer is reminded that drug screening by immunoassays has no ability to discriminate among such complex mixtures of opioid isomers with adequate selectivity.
-Reference 1 and 7: for internet references, please add the visiting date.

-Reference 10, the complete title is "Rapid analysis of metabolites and drugs of abuse from urine samples by desorption electrospray ionization-mass spectrometry" instead of "Rapid analysis of metabolites and drugs of abuse from urine samples"
-Reference 13, the right title should be "The Sweat Metabolome of Screen-Positive Cystic Fibrosis Infants: Revealing Mechanisms beyond Impaired Chloride Transport". Also correct the referenced page of the publication (p904-913)
-Reference 14, the right title should be "High Throughput Screening Method for Systematic Surveillance of Drugs of Abuse by Multisegment Injection-Capillary Electrophoresis-Mass Spectrometry" instead of "A high throughput screening method for systematic surveillance of drugs of abuse by multisegment injection capillary electrophoresis-mass spectrometry"

We have updated all the references cited above as recommended by the reviewer.

-Line 392: this work is not the first introduction of MSI-CE-MS based on electrokinetic plug. This approach was already described and used in another publication (DOI: 10.1016/j.jpba.2018.06.029). This should be cited and reference added in the list.

We have updated the recently published reference [ref 16] in the introduction. In fact, as this manuscript was primarily focused on optimization of electroextraction preconcentration, we were not aware of the use of MSI-CE-MS as it is only briefly described in the methodology section. Also, there were no explicit comments on the rationale of using the electrokinetic spacer plugs for sample, which we have elaborated in the current manuscript.

-Figure 2C is referenced twice (lines 290 and 298). According to your manuscript the referenced figure in line 298 should be Figure 2D.

We have updated the figure number correctly as requested

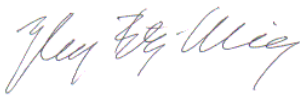
-Reviewer #2:

-This is an excellent manuscript showing the utility of multi-segment injection CE-MS for the quantitative analysis of various drugs of abuse in patient urine samples. The experimental procedures are described clearly and in detail. Given the opioid epidemic or opioids crisis in various countries, I expect that the proposed analytical tool will have a great impact in the screening of DoAs.

We thank reviewer # 2 for the positive comments about our manuscript.

We thank the editor and both external reviewers for their constructive feedback, and hope that our revised manuscript is deemed acceptable for publication.

With best regards,



Philip Britz-McKibbin
Professor and Cystic Fibrosis Canada Researcher
Department of Chemistry and Chemical Biology
McMaster University
E-mail: britz@mcmaster.ca