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Technical Note

Optimizing Liquid Electron Ionization Interface to Boost LC-MS Instrumental Efficiency

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Abstract: Liquid Electron Ionization (LEI) is a powerful and robust interface for the qualitative and quantitative analysis of medium-low-molecular-weight compounds, including numerous environmental pollutants and toxicological substances. Although the robustness and performance of this interface have already been demonstrated, research on its optimization can still improve instrumental performance in terms of detectability. In this study, different setups of the interface's vaporization micro-channel (VMC) made using different capillaries and various sizes were tested to evaluate the correspondent instrumental performance. The results show that a new combination of capillaries in the interface set up significantly improves instrumental detectability, reaching LOD values almost five times lower than those of the previous setup.

Keywords: LC-EI-MS; LEI; liquid chromatography; mass spectrometry

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1. Introduction

Liquid Electron Ionization (LEI) is an interface that enables the coupling of liquid nanoflows with electron ionization (EI) as a mass spectrometry (MS) ion source [1]. EI is performed with gas-phase samples, primarily in conjunction with gas chromatography (GC) as a hyphenated technique. It induces "hard" ionization, generating fragment ions with specific abundances and m/z values characteristic of the analytes, which allows for database recognition of the molecules. Liquid chromatography (LC), on the other hand, is typically coupled with ambient-pressure ion (API) sources, such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The LEI interface offers a viable alternative to these commonly used ion sources thanks to its low matrix effect and the extensive structural information provided by the fragmentation in the EI ion source, which is crucial for identifying unknown compounds [2]. Soft ionization methods used in LC often yield limited structural information, necessitating the use of high-resolution or tandem MS to obtain detailed information about unknown molecules. At the same time, the coupling of LC to EI enables the analysis of some GC-non-amenable molecules and does not require derivatization steps, minimizing sample treatment [3]. LEI, operating at nanoflows typically between 400 and 600 nL min^{-1}, is compatible with conventional UHPLC and HPLC (with flow splitting) and direct MS techniques [4]. LEI has shown good compatibility with both normal and reversed phase chromatography solvents, enabling the separation and MS analysis of a broader range of analytes [5]. However, while LEI provides valuable structural information due to its hard fragmentation, this also results in lower instrumental sensitivity compared to API techniques like ESI. Additionally, the need to split the flow under HPLC

conditions presents a further challenge in achieving a limit of detection (LOD) comparable to that of conventional HPLC-MS instruments, as only a small percentage of the mass injected reaches the MS detector. To address these limitations, researchers studying the LEI interface could enhance the transport and vaporization of analytes to and at the EI source. The LEI interface relies on the nebulization and vaporization of the flow in the VMC. This step is critical for the performance of LEI, making the efficient transfer of analytes to the ion source essential for the LEI's development, and the materials used for the interface can significantly affect the results [6]. In this work, the VMC setup was evaluated using flow injection analysis (FIA) and LC, testing different combinations of capillaries, utilizing for the first time a deactivated silica capillary as a VMC capillary to evaluate whether doing so could improve inertness and instrumental response compared to the previously used silica capillaries, focusing on instrumental detectability as the main parameter for comparison. The analytes used to test the different setups are low-molecular-weight PAHs and pesticides with physical–chemical properties suitable for LEI vaporization and analysis. Also, the three setups studied were evaluated using two different MS instruments, an EI-QqQ MS for FIA analyses and an EI-QTOF MS for LC-MS analyses. An investigation of instrumental performances using the two instruments was employed to evaluate the repeatability and roughness of the results, avoiding misleading interpretations and erroneous conclusions.

2. Materials and Methods

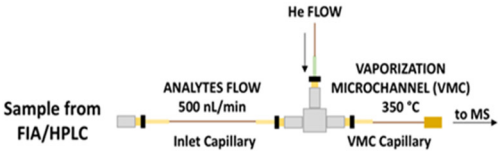
2.1. Samples, Chemicals, and Materials

Analytical standards of naphthalene, anthracene, and pyrene were purchased from Supelco Inc. (Bellefonte, PA, USA), and atrazine, chlorpyrifos, clorfenvinfos, dichlorvos, alachlor, and metalaxyl were purchased from Sigma Aldrich. HPLC-grade acetonitrile (ACN), used for the analysis and the preparation of standard solutions, was purchased from VWR. Ultrapure water was obtained with a Direct-Q 3 UV system from Millipore Corp. The deactivated silica capillary was purchased from Agilent (material number: CP805310), while the other capillaries used were purchased from Polymicro Molex (Phoenix, AZ, USA).

2.2. LC-LEI-MS/MS Apparatus and Working Conditions

An Agilent 1290 Infinity II HPLC (Agilent Technologies, Palo Alto, CA, USA) was coupled to a triple-quadrupole MS (Agilent 7010B, Agilent Technologies) operating in EI via an LEI interface. The mobile phase was 100% ACN at a 10 µL min^{-1} flow rate. An HPLC column (Agilent Zorbax XDB-C18, 0.3 × 150 mm, 3.5 µm particle size) was installed between the pump exit and the injector to guarantee sufficient backpressure for working under stable conditions and not for chromatographic purposes. The 10 µL min^{-1} flow was split using a passive flow splitter (PFS) to set the injection flow rate to 500 nL min^{-1}, tuning the length and internal diameter of the capillaries connected to a T-junction (part number: U-428, IDEX, Northbrook, IL, USA) of the PFS properly by utilizing a Sensirion flowmeter (SLG-0075, Sensirion AG, Stäfa, Switzerland) with 100% H_2O as the mobile phase (in accordance with producer indications) to measure the flow exiting the inlet capillary, monitoring the vacuum parameters of the MS instrument for additional confirmation. Standard solutions were analyzed through FIA using an external injector equipped with a 100 nL internal sample loop (VICI AG International, Schenkon, Switzerland). LEI interface was used as described elsewhere [1,2]. Briefly, the inlet capillary coming from the PFS was connected to a T junction (part number U-428, IDEX, Northbrook, IL, USA) with an internal hole measuring 0.5 mm to allow the passage of the inlet capillaries used in all the setups and inserted inside the VMC capillary connected to the EI ion source, positioning the inlet capillary at the beginning of the hot zone. In the third transversal connection, helium gas was provided at a constant flow rate of 1.2 mL min^{-1}. Three setups were tested

using different combinations of capillaries for the VMC and the inlet capillary, as described in Figure 1. VMC temperature was set to 350 °C after preliminary optimization of the acquisitions. The MS analyses were performed in selected ion monitoring (SIM) mode for PAH analysis and MRM mode for the pesticides. The instrumental detectability was checked in triplicate for the 3 setups studied.



	Inlet Capillary	VMC Capillary
Setup 1	Silica 150 µm O.D., 30 µm I.D.	Silica 800 µm O.D., 400 µm I.D.
Setup 2	Silica 150 µm O.D., 30 µm I.D.	De-activated Silica 700 µm O.D., 500 µm I.D.
Setup 3	Silica 360 µm O.D., 25 µm I.D.	De-activated Silica 700 µm O.D., 500 µm I.D.

Figure 1. Liquid Electron Ionization (LEI) interface design and capillary combinations for the 3 setups studied while varying the dimensions of inlet and VMC capillaries.

2.3. LC-LEI-HRMS Method

LEI interface was installed on an Agilent 7250 Q-TOF to perform LC-MS analyses. Chromatographic separation was performed with an Agilent 1290 Infinity II HPLC using a Kinetex 1.7 µm XB-C18 150 × 2.1 mm column with the following gradient: from 5% of Solvent B at 0 min to 100% of solvent B at 19 min, which was then kept constant until 26 min (Solvent A: H_2O with 0.1% formic acid; Solvent B: ACN with 0.1% formic acid). Helium at a flow rate of 1.2 mL min^{-1} was delivered to the LEI interface. A total of 8 µL of sample was injected, and the LC flow rate was set to 0.2 mL min^{-1}, which was successively split 1:400 times with a passive flow splitter to regulate the flow at 500 nL min^{-1} and then conveyed in the LEI-MS system. MS acquisitions were performed in full-scan mode in a mass range of 83–600 m/z.

3. Results and Discussion

3.1. Flow Injection Analysis with LEI-QqQ

The LEI interface performs its function by vaporizing the liquid flow and the analytes. This step is crucial, as peak shape and instrumental response highly depend on their vaporization and transport from the LEI interface to the MS ion source. The use of a more inert material was considered to reduce gas phase interactions between the analytes and the VMC capillary [6], which might result in a longer elution time, the loss of gaussian peak shape, and, for high-boiling-temperature molecules, analyte deposition, resulting in carryover, loss of sensitivity, and an increase in background noise. Silica is currently the best option for use as a VMC capillary material for its ease of use.

A previous study showed how ceramic can be a highly valuable material for its inertness [7], but its fragility makes it difficult to use, and it requires more frequent maintenance as it becomes dirty faster than silica. Stainless steel was also used initially for direct-EI analysis, but it has lower inertness and can significantly interact with polar and high-boiling-temperature molecules. For this reason, the evaluation of a deactivated silica capillary represents an interesting compromise between inertness and ease of use. Table 1 details the instrumental limit-of-detection (LOD) values obtained. The use of a 500 µm I.D. capillary as a VMC capillary in setup 2, rather than the 400 µm I.D. capillary in setup 1, resulted in increased instrumental detectability, as the LODs were lower for most of the tested analytes, such as chlorpyrifos, atrazine, metalaxyl, naphthalene, pyrene, and anthracene. The elution time and peak shape were consistent between setups 1 and 2, indicating minimal difference in the speed of mass transport between the inlet capillary and the MS ion source. Setup 3—wherein a 360 µm O.D. capillary was used as the inlet capillary, with a reduced free volume in the tee junction and the VMC due to the presence

of a thicker capillary compared to that in setups 1 and 2, in which a 150 µm O.D. capillary was used—was tested with the hypothesis of improving the mass transfer to the MS thanks to a faster flow of helium in the LEI interface. However, setup 3 did not show a significant change in the peak shape or elution time. Nevertheless, even if the elution time did not improve significantly, setup 3 produced the best results in terms of detectability, typically increasing this parameter 4- to 5-fold compared to the original LEI setup, as shown in Table 1. The enhanced detectability of setup 3 compared to setup 2 for certain analytes highlights the correlation between the reduction in free volume within the helium gas path at the interface and the instrumental response. This improvement may result from the increased gas velocity around the inlet capillary, which could enhance analyte transport in the gas phase while reducing gas-phase interactions.

Table 1. Instrumental LOD values obtained in Flow Injection Analysis (FIA) in which 100 nL of standard solutions was injected at the desired concentration utilizing an EI-QqQ mass spectrometer.

Analytes	**Molecular Weight**	**MRM Transitions and SIM Ions Monitored (*m*/*z*)**	**Setup 1 LOD Concentration/Mass Injected**	**Setup 2 LOD Concentration/Mass Injected**	**Setup 3 LOD Concentration/Mass Injected**
Chlorpyrifos	350.6	197–169	1 ppm/100 pg	500 ppb/50 pg	250 ppb/25 pg
Clorfenvinfos	359.6	267–159	250 ppb/25 pg	250 ppb/25 pg	50 ppb/5 pg
Atrazine	215.7	200–122	1 ppm/100 pg	250 ppb/25 pg	250 ppb/25 pg
Metalaxyl	279.3	206–132	1 ppm/100 pg	250 ppb/25 pg	250 ppb/25 pg
Diclorvos	221.0	109–79	250 ppb/25 pg	250 ppb/25 pg	50 ppb/5 pg
Alachlor	269.8	188–132	1 ppm/100 pg	500 ppb/50 pg	500 ppb/50 pg
Naphthalene	128.2	128	250 ppb/25 pg	5 ppb/0.5 pg	5 ppb/0.5 pg
Anthracene	178.2	178	25 ppb/2.5 pg	5 ppb/0.5 pg	5 ppb/0.5 pg
Pyrene	202.2	202	25 ppb/2.5 pg	5 ppb/0.5 pg	5 ppb/0.5 pg

3.2. LC-LEI.QTOF MS Analysis

The results gathered via Flow Injection Analysis (FIA) were further evaluated under chromatographic conditions with the objective of assessing the repeatability of the instrumental performances of the three setups studied with different ion sources, MS instruments, and operative conditions. The results of the chromatographic evaluation demonstrated a trend across different instrument configurations that was consistent with the outcomes obtained under FIA conditions. For illustrative purposes, Figure 2 displays chromatograms for atrazine and clorfenvinfos at an injected mass of 20 pg for each analyte. In the case of atrazine, the results indicate that setup 1 was unable to provide a sufficient response for detecting the analyte at this low concentration, as no significant signal was observed over the background noise. However, setups 2 and 3 were successful in detecting atrazine, with a signal-to-noise ratio of 3, indicating an enhanced detectability relative to setup 1. The extracted ion chromatogram of atrazine for setup 1 exhibited a higher noise baseline compared to setup 3, masking the analyte signal and reducing the overall signal-to-noise ratio. A different result was observed for clorfenvinfos, for which the EIC noise levels were relatively consistent across all three setups, indicating that the baseline noise for this analyte was not strongly affected by instrument configuration. Nevertheless, the peak intensity for clorfenvinfos was significantly higher in setup 3 than in setup 2, with setup 1 showing the lowest response. The increased peak intensity observed for setup 3 reflects an improvement in signal strength corresponding to an enhanced detection capability for clorfenvinfos. These results highlight the robustness of setup 3 in terms of achieving better limits of detection across different analytes.

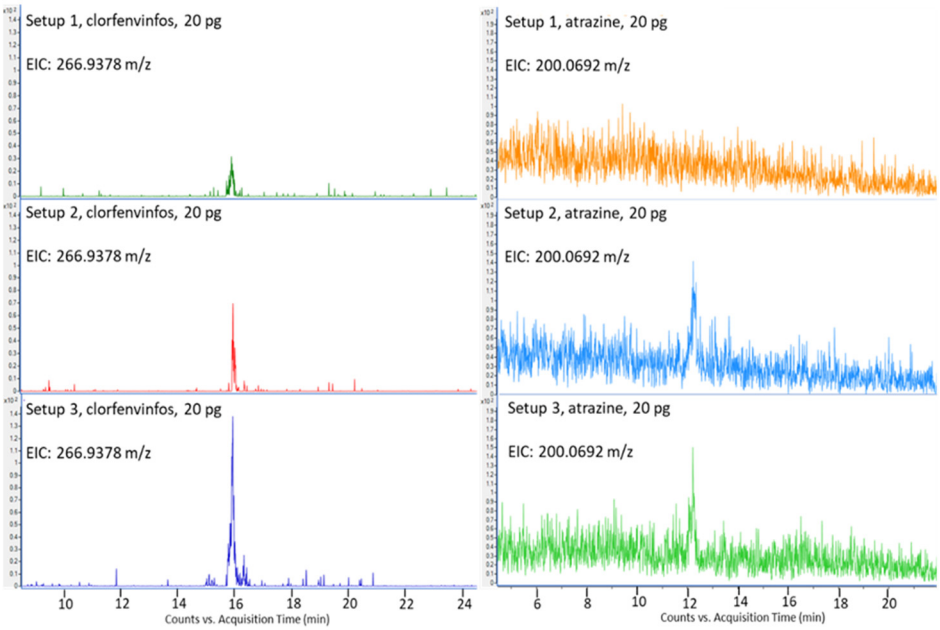


Figure 2. Clorfenvinfos and atrazine analysis with LC-LEI-QTOF with the 3 setups studied.

4. Conclusions

LEI is a useful interface that offers a valuable alternative to the conventional instruments used for routine analysis. This work represents significant progress in the design of an optimal LEI interface, as the instrumental detection limits obtained utilizing setup 3 increased 4 to 5-fold compared to the previous design. The results shown in this work emphasize that the optimal design for an LEI interface has not yet been achieved, as instrumental performance can still be improved. Further development and research could enhance the detection of high-boiling-point and thermolabile compounds. However, at the current stage, this improvement in detectability marks an important step toward achieving sensitivity levels comparable to those of commonly used instruments for the LC-MS analysis of small molecules, such as environmental pollutants or metabolites of biological or toxicological interest.

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References

1. Termopoli, V.; Famiglini, G.; Palma, P.; Piergiovanni, M.; Cappiello, A. Atmospheric pressure vaporization mechanism for coupling a liquid phase with electron ionization mass spectrometry. *Anal. Chem.* **2017**, *89*, 2049–2056. [CrossRef] [PubMed]
2. Rigano, F.; Tranchida, P.; Dugo, P.; Mondello, L. High-performance liquid chromatography combined with electron ionization mass spectrometry: A review. *Trends Anal. Chem.* **2019**, *118*, 112–122. [CrossRef]

3. Tsizin, S.; Fialkov, A.B.; Amirav, A. Electron Ionization Mass Spectrometry for Both Liquid and Gas Chromatography in One System without the Need for Hardware Adjustments. *J. Am. Soc. Mass Spectrom.* **2020**, *31*, 1713–1721. [CrossRef] [PubMed]
4. Termopoli, V.; Torrisi, E.; Famiglini, G.; Palma, P.; Zappia, G.; Cappiello, A.; Vandergrift, G.; Zvekic, M.; Krogh, E.; Gill, G. Mass Spectrometry Based Approach for Organic Synthesis Monitoring. *Anal. Chem.* **2019**, *91*, 11916–11922. [CrossRef] [PubMed]
5. Marittimo, N.; Grasselli, G.; Arigò, A.; Famiglini, G.; Agostini, M.; Renzoni, C.; Palma, P.; Cappiello, A. Liquid electron ionization-mass spectrometry as a novel strategy for integrating normal-phase liquid chromatography with low and high-resolution mass spectrometry. *Analyst* **2024**, *149*, 2664–2670. [CrossRef] [PubMed]
6. Luong, J.; Gras, R.; Jennings, W. An advanced solventless column test for capillary GC columns. *J. Sep. Sci.* **2007**, *30*, 2480–2492. [CrossRef] [PubMed]
7. Magrini, L.; Famiglini, G.; Palma, P.; Termopoli, V.; Cappiello, A. Boosting the Detection Potential of Liquid Chromatography-Electron Ionization Mass Spectrometry Using a Ceramic Coated Ion Source. *J. Am. Soc. Mass Spectrom.* **2016**, *27*, 153–160. [CrossRef] [PubMed]