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RESEARCH ARTICLE

Rapid Communications in Mass Spectrometry WILEY

The use of pseudo-MRM for a sensitive and selective detection and quantification of polycyclic aromatic compounds by tandem mass spectrometry

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Rationale: Multiple Reaction Monitoring (MRM) is a sensitive and selective detection mode for target trace-level analysis. However, it requires the fragmentation of labile bonds which are not present in molecules such as Polycyclic Aromatic Hydrocarbons (PAHs) and their heterocyclic derivatives (PANHs, PASHs).

Methods: We present the application of an alternative tandem mass spectrometry (MS/MS) mode called "pseudo-MRM" for the GCMS/MS analysis of Polycyclic Aromatic Compounds (PACs). This mode is based on the monitoring of transitions with no mass loss between the precursor and the product ion. Pseudo-MRM peak areas were compared with those of classic MRM on three different mass spectrometers: two triple quadrupoles and an ion trap.

Results: For all non-polar PACs studied here (PAHs, PANHs and PASHs), the pseudo-MRM transition was always the most intense. The classic MRM transitions exhibited peak areas 2 to 5 times lower. On the contrary, for the functionalized PACs (oxygenated and nitrated PAHs), classic MRM was favored over pseudo-MRM. These observations were confirmed on two triple quadrupoles (QqQs), and the real-world applicability of pseudo-MRM on QqQs was validated by the successful analysis of Diesel PM. However, a comparison with an ion trap showed that pseudo-MRM was never favored on that instrument, which caused fragmentation of non-polar PACs in MS/MS.

Conclusions: The results of this study show an important gain in sensitivity when using pseudo-MRM instead of MRM for non-polar PACs on QqQ instruments. The selectivity of MRM is preserved in pseudo-MRM by applying non-zero collision energies to which only these non-polar PACs are resistant, not the isobaric interferences. No interference issue was observed when analyzing Diesel PM, a complex matrix, with our pseudo-MRM method. Therefore, we advise for a broader use of this MS/MS mode for trace-level determination of non-polar PAHs.

1 | INTRODUCTION

The hyphenation of chromatography and tandem mass spectrometry (MS/MS) is a well-known technique for the target analysis of organic micropollutants in environmental matrices. In particular, the Multiple Reaction Monitoring (MRM) mode is generally the most interesting mode to implement for such applications.[1–3] It is based on the collision-induced dissociation (CID) of a precursor ion at a

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given collision energy and the monitoring of the resulting product ions which are specific to each compound, which is why MRM reduces the risk of isobaric interferences on the signal of the target compound. It can be applied on analyzers such as Triple Quadrupoles (QqQ), Ion Traps (IT), Quadrupole Time-Of-Flight (QTOF), etc.

However, MRM requires a good fragmentation yield when the Collision Energy is applied. This yield is related to the presence of labile bonds in the molecule. This is not the case for Polycyclic Aromatic Hydrocarbons (PAHs), which are very stable structures made up of conjugate aromatic systems, and therefore do not possess any labile chemical bonds.[4,5] This leads to a major issue concerning the trace-level determination of PAHs (typically in environmental samples) which is the loss of signal intensity due to the low fragmentation yield obtained for PAHs in MS/MS, and hence higher instrumental detection limits for these compounds.

To overcome this problem, a "pseudo-MRM" mode can be implemented. It relies on the monitoring of transitions with the same *m/z* value as the precursor and product ions, i.e. transitions with no mass loss between MS^1 and MS^2.[4,5] It was first presented in 2014 by Shang et al.[5] They described the use of this monitoring mode for the particular case of PAHs which are "difficult-to-fragment" compounds. According to their study, pseudo-MRM exhibits a higher sensitivity and a higher signal-to-noise ratio than Single Ion Monitoring (SIM) which is impacted by isobaric interferences and "classic" MRM which is impacted by the low response of product ions.[5] Since then, pseudo-MRM has been successfully applied for the trace-level determination of PAHs,[6,7] but, in most of the recent analytical methods targeting PAHs and their derivatives in environmental samples, classic MRM and SIM are still widely used despite their drawbacks described above.[8–12]

Gas chromatography/mass spectrometry (GC/MS) is the best possible coupling for the analysis of trace-level PAHs in various samples. Indeed, there is no atmospheric pressure ionization (API) source enabling an efficient ionization of PAHs with LC/MS couplings, apart from the uncommon Atmospheric Pressure Laser Ionization (APLI).[4,13] On the other hand, the well-known Electron Ionization (EI) has proved to be very adaptable to the ionization of Polycyclic Aromatic Compounds (PACs) for GC/MS analysis.[4,14,15]

In this communication, we present interpreted data arguing in favor of a broader use of pseudo-MRM mode instead of classic MRM mode for a more sensitive, but not less selective, detection of PAHs at trace-level using GC/MS/MS. These data were obtained from three different mass spectrometers, two QqQs and an IT. The different patterns observed for these instruments are discussed here.

Moreover, to supplement the current knowledge about pseudo-MRM application to PACs, we compared the behavior of PAHs with those of Polycyclic Aromatic Heterocycles such as azaarenes (PANHs) and thiaarenes (PASHs), and substituted PAHs such as nitrated PAHs (NPAHs) and oxygenated PAHs (OPAHs), therefore giving a larger perspective on MS/MS methods for the detection and quantitation of PACs.

2 | MATERIALS AND METHODS

2.1 | Chemicals

GC/MS/MS experiments were performed by injecting standard solutions of PACs at concentrations giving appropriately intense signals (from 0.1 to 5 $mg{\cdot}L^{-1}$). The pure standards were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France), LGC Standards (Molsheim, France), Cluzeau Info Labo (Sainte Foy la Grande, France) and BCP Instruments (Oullins, France).

Analytical grade ethyl acetate, which was the selected solvent for standard solutions, and acetonitrile and toluene, which were the extraction solvents for the application to a real sample, were purchased from VWR Chemicals (Fontenay-sous-Bois, France).

In our study of MRM and pseudo-MRM relative signal intensities, we selected ten different PACs, two per group: anthracene and benz(a)anthracene for PAHs, acridine and benz(c)acridine for PANHs, dibenzothiophene and benzonaphtho(2,1-d)thiophene for PASHs, anthraquinone and benzanthraquinone for OPAHs, 9-nitroanthracene and 7-nitrobenz(a)anthracene for NPAHs. The chemical structures of these compounds are reported in Figure 1.

2.2 | GC/MS/MS

The first set of GC/MS/MS injections was performed on a CP-3800 gas chromatograph coupled to a 320MS QqQ mass spectrometer (both from Varian Inc., Walnut Creek, CA, USA). The analytical column used here was a capillary Rxi-PAH column (60 m x 0.25 mm, 0.10 µm; Restek, Bellefonte, PA, USA). The injection was performed in splitless

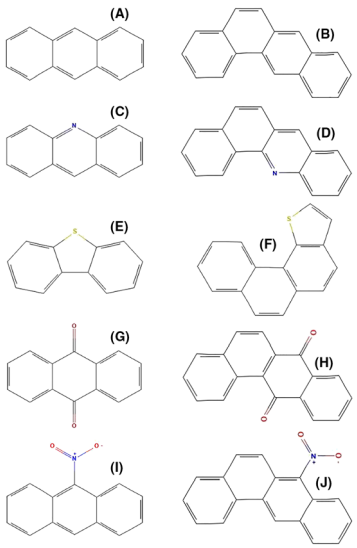

FIGURE 1 Molecular structures of the polycyclic aromatic compounds studied in this communication: (A) anthracene, (B) benz(a) anthracene, (C) acridine, (D) benz(c)acridine, (E) dibenzothiophene, (F) benzonaphtho(2,1-d)thiophene, (G) anthraquinone, (H) benzanthraquinone, (I) 9-nitroanthracene, and (J) 7-nitrobenz(a) anthracene [Color figure can be viewed at wileyonlinelibrary.com]

mode at 250°C. A temperature gradient was programmed from 60°C to 320°C in 41 min to separate the various PACs. The temperature of the transfer line was set at the final oven temperature, i.e. 320°C, and the source temperature was 250°C. Electron ionization (EI) was performed at 70 eV. Voltages applied in the collision cell ranged from 0 to 40 V, and argon was used as collision gas at a pressure of 1.5 mTorr.

The results obtained from this set of injections were compared with those of two different GC/MS/MS setups. The first one was another QqQ instrument, the TSQ Quantum (Thermo Fisher Scientific Inc., Waltham, MA, USA). Separation of PACs was performed on a Trace Ultra gas chromatograph (Thermo Fisher Scientific Inc.) equipped with a HP5-ms column (30 m x 0.25 mm, 0.10 µm). The on-column injection mode was used on a programmable temperature vaporization injector. EI was performed at 70 eV and 220°C. Argon pressure was 1.5 mTorr, and MS/MS collision energies were from 0 to 50 V.

The second comparative GC/MS/MS study was performed on an IT (ITQ 900; Thermo Fisher Scientific Inc.) with a Trace 1300 gas chromatograph (Thermo Fisher Scientific Inc.) and a TurboMatrix 350 thermal desorber (PerkinElmer, Waltham, MA, USA) used as injector after thermal desorption at 300°C and cryo-focusing at −20°C. EI was performed at 70 eV and 210°C. In an IT such as this one, it is the helium present in the trap which serves as the collision gas. Its flow rate was 0.3 mL/min and the pressure in the ITQ was 40 mTorr. Collision amplitudes ranged from 0.0 to 2.0 arb.

2.3 | Preparation of Standard Reference Material (SRM)

In order to demonstrate the applicability of pseudo-MRM for the determination of PAHs, PANHs and PASHs in complex samples, we analyzed a SRM from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). This SRM was Diesel Particulate Matter (PM) from an industrial forklift, registered as SRM 2975.

SRM 2975 (0.06 g) was weighed and deposited on a glass fiber filter (GFF) (Whatman, GE Healthcare, Little Chalfont, UK).

The extraction of Diesel PM was performed by pressurized liquid extraction (PLE) on an ASE 300 instrument (Dionex Corp., Sunnyvale, CA, USA) with a mixture of acetonitrile and toluene (1:1, v/v), in a 33 mL cell at 110°C.

The extract was concentrated to 1 mL with a TurboVap II concentrator (Biotage, Uppsala, Sweden) and then diluted 4-fold with ethyl acetate for a better compatibility with the GC/MS/MS analysis.

3 | RESULTS AND DISCUSSION

3.1 | MRM and pseudo-MRM behavior of various PACs

The data presented here correspond to the signal intensities obtained for the main *m/z* transitions of ten PACs from five different classes at various collision energies.

The peak areas measured on the Varian 320MS QqQ for the three most intense mass transitions are represented in Figure 2, with applied collision voltages from 0 to 40 V. One of these transitions shows no mass loss, a second transition exhibits a slight mass loss, and the third corresponds to a more important mass loss. The peak areas are normalized by the highest peak area obtained for a given compound so that relative response factors of the less intense transitions can be better observed.

This representation enables to identify for each class of compounds which MS/MS mode gives the most intense signal: the pseudo-MRM (dark grey circles) or the classic MRM (blue diamonds and orange triangles). When comparing the results obtained for the various PACs, we clearly distinguished two main patterns. For PAHs, PANHs and PASHs, the pseudo-MRM transition was highly favored. For the six molecules of these families, the highest possible response of the other MRM transitions relative to the pseudo-MRM transition varied from 20% for benzonaphtho(2,1-d)thiophene (see Figure 2F) to 61% for benz(c)acridine (see Figure 2D). It expresses a signal enhancement from 2 to 5 times when one monitors the pseudo-MRM transition instead of classic MRM transitions.

On the other hand, when polar PAH derivatives such as OPAHs and NPAHs were studied, the pseudo-MRM mode gave lower intensities than MRM transitions with mass losses: from 51% of the highest obtained peak area in the case of 9,10-anthraquinone (see Figure 2G) to 86% for 9-nitroanthracene (see Figure 2I).

This difference in fragmentation ability between the compounds studied here can easily be explained by their chemical structure: while OPAHs and NPAHs possess labile chemical bonds with electron-withdrawing groups which can be fragmented with the application of low voltages, this is not the case for the aromatic structures of PAHs, PANHs and PASHs where no labile bond can be found (see Figure 1). Therefore, mass losses were only obtained at higher voltages applied and at lower yields.

According to these results, we can argue in favor of the monitoring of such pseudo-MRM transitions. Due to their higher peak areas than those of the comparative MRM transitions, they can lead to a more sensitive detection and quantification of PAHs, PANHs and PASHs when trace and ultra-trace levels of these compounds are expected, in environmental samples for instance.

If we focus only on the pseudo-MRM traces (dark grey circles), another major observation is to be drawn from Figure 2. One could expect the fragmentation yield to increase with the collision energy, and therefore the pseudo-MRM peak area to drop down continuously with the increase in collision voltage. Instead, we noticed an enhancement of the pseudo-MRM signal from 0 V, where it is practically nil, to about 15 V applied, where it reaches a maximum. This pattern was effective for all the PAHs, PANHs and PASHs involved in this study (see Figures 2A–2F).

Our main hypothesis to explain this phenomenon, which can be rather startling at first glance, is related not to the fragmentation of the ions, but to their transmission in the QqQ. Indeed, it is possible that the voltages applied in the second quadrupole (Q2) serve not only as collision energies, but also as ion guides from the first

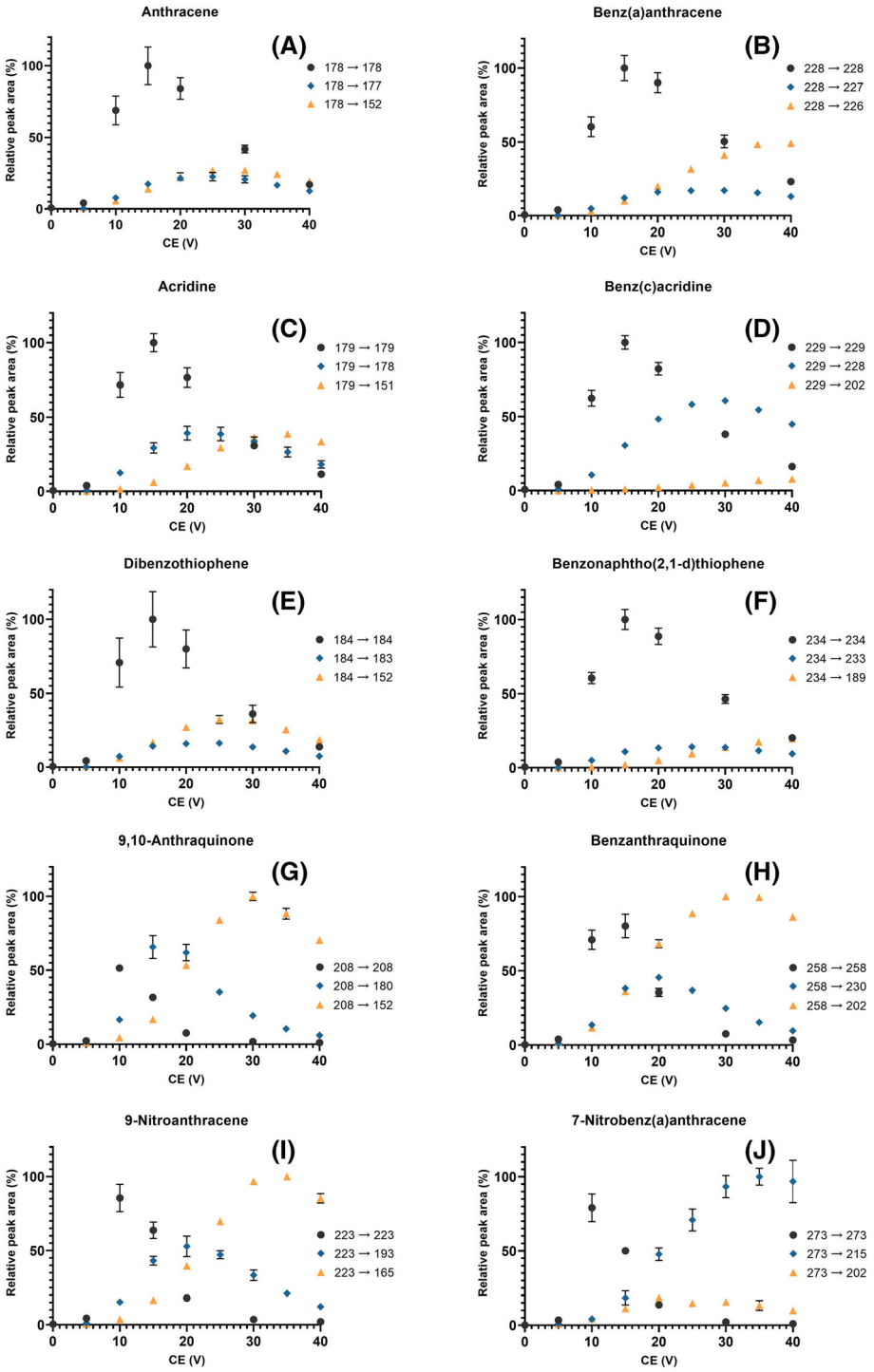

FIGURE 2 For each PAC studied, peak areas of the three main MS/MS transitions relative to the highest peak area obtained for a given compound, depending on the collision energy applied. The dark grey circles correspond to the pseudo-MRM transition, the blue diamonds to the MRM transition with the smaller mass loss, and the orange triangles to the MRM transition with the higher mass loss. The error bars express standard deviations on triplicate analyses [Color figure can be viewed at wileyonlinelibrary.com]

quadrupole (Q1) towards the third (Q3). In particular, the Varian 320MS used here is a U-shaped QqQ. This geometry could increase ion losses from Q1 to Q3 when very low voltages are applied in Q2.

This hypothesis is strengthened by the observation of Shang et al, who reported a decrease in intensity for pseudo-MRM transitions right from the very low collision energies. Their study was performed on a linear triple quadrupole; therefore, it is very likely that, on this instrument, no voltage was required to transfer the ions from Q1 to Q3.[5]

Actually, it represents an interesting opportunity for trace-level analysis of these compounds in complex matrices. Indeed, if pseudo-MRM was only effective at collision energies close to zero, it would lack specificity, because, in this case, isobaric interferences would also respond on the monitored transition, and pseudo-MRM would present no benefit over MS^1 and Single Ion Monitoring (SIM).

On the contrary, our results (see Figures 2 and 3) showed that pseudo-MRM is applicable at collision voltages up to 15–20 V in QqQ instruments. At such non-zero collision energies, isobaric

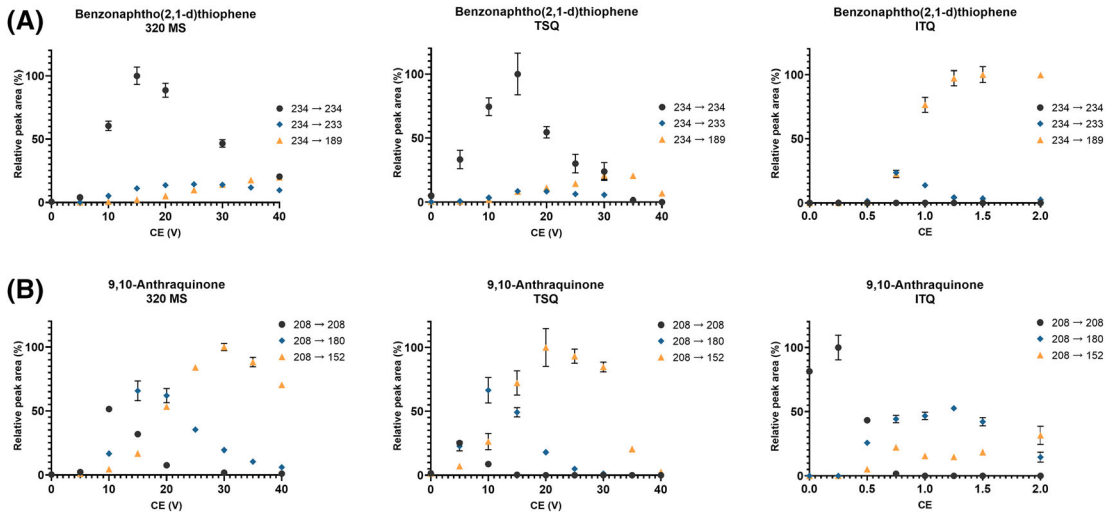

FIGURE 3 Peak areas of the three main MS/MS transitions relative to the highest peak area obtained for (A) benzonaphtho(2,1-d)thiophene and (B) anthraquinone. From left to right, the graphs obtained on the Varian 320MS (QqQ), on the Thermo TSQ quantum (QqQ), and on the Thermo ITQ 900 (IT). The dark grey circles correspond to the pseudo-MRM transition, the blue diamonds to the MRM transition with the smaller mass loss, and the orange triangles to the MRM transition with the higher mass loss. The error bars express standard deviations on triplicate analyses [Color figure can be viewed at wileyonlinelibrary.com]

interferences which possess chemical structures other than those of PAHs and their heterocyclic derivatives are likely to be fragmented, thus their ability to exhibit pseudo-MRM signal will be severely decreased. Hence, the higher sensitivity obtained with the monitoring of pseudo-MRM transitions does not go along with a lack of selectivity, if non-zero collision energies are applied.

3.2 | Similarities and differences between different mass spectrometers

3.2.1 | Comparison of two triple quadrupoles

The results described in section 3.1, which were obtained with the Varian 320MS instrument, were compared with the exact same MS/MS transitions monitored on a Thermo TSQ Quantum instrument. The goal was to determine whether the pseudo-MRM pattern we observed was specific to our mass spectrometer or reproducible on other instruments.

Briefly, the comparison of results obtained on both instruments clearly indicated the same tendency of a higher peak area when transitions with no mass loss were monitored for PAHs, PANHs and PASHs. Even though the relative peak areas of the less intense transitions and the optimal collision energies can slightly differ from one QqQ to another, the most intense transition remained the same for these compounds, i.e. the pseudo-MRM (see Figure 3A).

The geometry of the TSQ is neither linear nor U-shaped but three-dimensional L-shaped. Similarly to the U-shape of the 320MS, and in contrast with the linear QqQ from the work of Shang et al,[5] this means that the ion path is not direct towards the third quadrupole and the detector. Therefore, here again, voltages in the collision cell (Q2) are required for the pseudo-MRM to be efficient, so that the molecular ion can be quantitatively transferred from Q1 to Q3. Even if the apex of the pseudo-MRM transition breakdown curve was a bit shifted towards lower collision energies in comparison with the results obtained on the 320MS (see Figure 3A), pseudo-MRM still required collision energies around 10 or 15 V on the TSQ instrument. It enables the specificity of this detection mode to be maintained. According to these results obtained on two different QqQs, pseudo-MRM seems to be very efficient for the determination of PAHs and their heterocyclic derivatives with triple quadrupole instruments.

3.2.2 | Comparison of the triple quadrupoles with an ion trap

We also wanted to compare these results with what would give the same MRM and pseudo-MRM experiments on an IT. Indeed, it is known that MS/MS fragmentation on these analyzers generally shows major differences. While triple quadrupoles perform simultaneously precursor ion selection (Q1), fragmentation (Q2) and product ion monitoring (Q3) in three spatially distinct areas of the mass spectrometer, all these processes happen in the same space in the ion trap.[16]

For instance, when Kang et al performed MS/MS fragmentation studies on the same molecules (isoflavones) with both a QqQ and an IT, they observed significant differences between the obtained product ion spectra.[17] Similarly, Bristow et al reported the difficulty of creating "universal" MS/MS CID product ion spectral libraries, because depending on the mass spectrometer, even if the generated product ions are generally the same, their relative abundance can vary greatly.[18]

Consistently with this prior knowledge, we observed completely different MRM trends on our target compounds with the IT compared to those of the QqQs (Figure 3).

Indeed, for PAHs, PANHs and PASHs, the monitoring of the pseudo-MRM transitions gave almost no signal. For instance, in Figure 3A, we report peak areas obtained for the benzonaphtho (2,1-d)thiophene which showed the most pronounced pseudo-MRM pattern on the 320MS (see section 3.1) as well as on the TSQ (see Figure 3A). On the contrary, on the ITQ 900, no signal above background noise was obtained, while the transition with the higher mass loss was highly favored (see Figure 3A).

This suggests a higher fragmentation ability of the IT where the resonance energies applied, and maybe more importantly the time spent in resonance in the IT, are sufficient to break up stable aromatic structures.[19] Moreover, the pressure in the space where the CID takes place is also much higher in the ITQ (40 mTorr) than in QqQs (1.5 mTorr), which could lead to more collisions happening. This higher fragmentation ability was confirmed by the fact that when one looks at the two MRM transitions for PAHs, PANHs and PASHs, the higher mass loss (-C_2H_2, -S or -CHS for instance) is always favored over the loss of only one hydrogen atom (−1 Da). The pattern observed for benzonaphtho(2,1-d)thiophene (presented in Figure 3A) was repetitive for all the PAHs, PANHs and PASHs studied here (data not shown).

Conversely and surprisingly, the other group of PACs we distinguished in this study, i.e. polar derivatives (OPAHs and NPAHs), exhibited with the IT a pseudo-MRM prevalence trend which was not found with QqQs (see Figure 3B). In Figure 3B, we present the MRM and pseudo-MRM breakdown curve of 9,10-anthraquinone for which the pseudo-MRM relative peak area was the lowest on the 320MS (see section 3.1) and on the TSQ (see Figure 3A). On the ITQ, however, the maximal peak area was obtained for the 208 → 208 *m/z* transition, and the second highest peak area only reached about 53% for the 280 → 180 *m/z* transition. We could argue that maybe the two selected MRM transitions were not the most intense possible if the fragmentation in the ITQ is really different from the fragmentation in the QqQs, but anyway, pseudo-MRM still gave very high absolute peak areas, around 10^7.

However, here again, there was a major difference with the pseudo-MRM profile obtained on the QqQs. The pseudo-MRM intensity already decreased from very low fragmentation amplitudes: from 0.5 in the case of anthraquinone (see Figure 3B), and even steadily from 0 for the three other polar PACs (data not shown).

This supports our prior hypothesis that, in the case of QqQs, the increase in pseudo-MRM intensity from 0 to about 15 V is due to the necessity of sufficient voltages to transfer the ions from Q1 to Q3, whereas, for the IT, the selection, fragmentation and ejection processes take place in the same space, so the only criterion ruling pseudo-MRM intensity is the bond breaking.

Hence, the pseudo-MRM mode is probably not the most suitable MS/MS mode to use on an IT, because, in this case, there is some incompatibility between the necessity of using non-zero collision energies to have a better specificity than at the MS^1 level and the rapid decrease in peak area with the increase in collision energy.

A more global outcome of this instrumental comparative study is the confirmation that in the course of a MS/MS method development, the search of the most intense *m/z* transitions is to be performed with great care. It is always instrument-dependent, so it is not sufficient to reproduce transitions from the literature, especially if it is not the exact same mass spectrometer used.

3.3 | Application to a complex sample: Diesel Particulate Matter standard reference material

As described in section 2.3, the applicability of pseudo-MRM to complex matrices was demonstrated thanks to a Diesel PM reference material, SRM 2975. The latter was certified by NIST against its content of PAHs and NPAHs,[20] but previous works also reported the presence of several OPAHs and PASHs in this SRM and their concentrations.[21–25]

The GC/MS/MS instrument selected for this experiment was the Varian 320MS QqQ. It was demonstrated that this instrument showed particularly enhanced responses in the pseudo-MRM mode (see sections 3.1 and 3.2), in contrast with the ITQ for which pseudo-MRM was less efficient than MRM. According to the results from section 3.2, fragmentation patterns of PACs were equivalent on the Varian 320MS and on the TSQ Quantum.

Among the PACs which exhibit an important pseudo-MRM prevalence, anthracene was present in SRM 2975. The GC/MS/MS chromatogram of anthracene (retention time (t_R) = 16.7 min) and its isomer phenanthrene (t_R = 16.5 min) in SRM 2975 is shown in Figure 4. The grey trace corresponds to the pseudo-MRM transition (*m/z* 178 → 178) and the orange trace corresponds to the most intense MRM transition (*m/z* 178 → 152) (see Figure 2A). The higher signal obtained for phenanthrene conforms with the respective concentrations reported for these two compounds in SRM 2975.[20,22,25]

The objective was to evaluate typical indicators of MS/MS method performance and matrix effects: signal-to-noise (S/N) ratio and the ratio between quantitation and confirmation transitions. These indicators are reported for both compounds in Table 1. In this case, pseudo-MRM is the most intense transition, so it is considered as the quantitation transition, and MRM is used only as confirmation for a reliable identification of the target PACs.

The MRM/pseudo-MRM ratios obtained with the injection of the SRM 2975 extract are compared with the mean of the MRM/pseudo-MRM ratios obtained with standard solutions at three different concentrations in Table 1. The relative difference between these identification ratios in SRM 2975 and in the neat standards is only +2.1% for phenanthrene and +13.3% for anthracene. Thus, both values are below the commonly accepted criterion of ±20% required for a decisive identification of organic micropollutants in environmental samples.

The comparison of S/N ratios for phenanthrene and anthracene in SRM 2975 is in favor of their pseudo-MRM transition. Indeed, the S/N ratios of the 178 → 178 transition are about two times higher

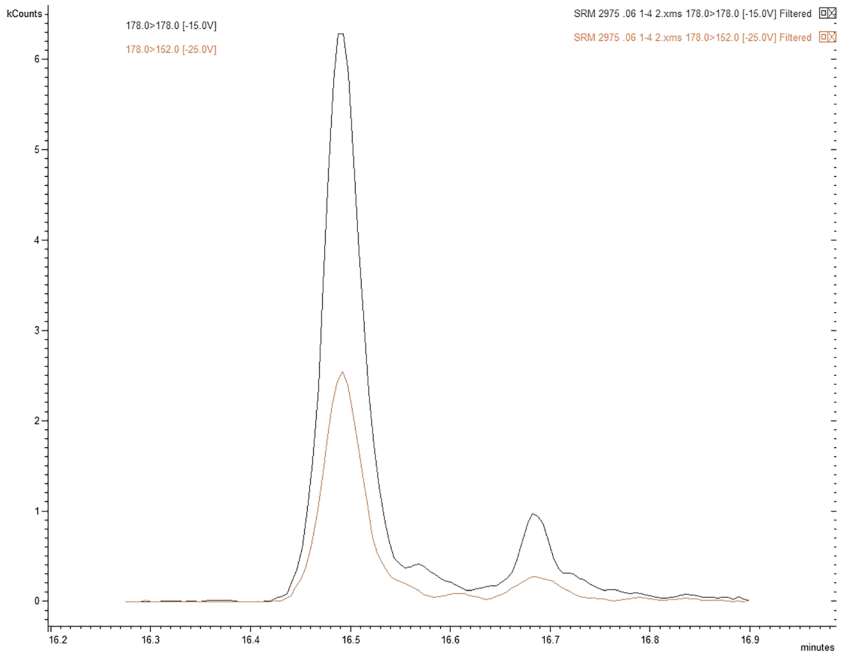

FIGURE 4 Pseudo-MRM (*m/z*178 → 178, grey trace) and MRM (*m/z*178 → 152, orange trace) chromatograms of phenanthrene (t_R = 16.5 min) and anthracene (t_R = 16.7 min) in Diesel PM SRM 2975 [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 For phenanthrene and anthracene (PAHs, 178 Da), comparison of MRM/pseudo-MRM peak area ratios in standard solutions (mean value over three standards at different concentrations) and in SRM 2975, and comparison of signal-to-noise ratios of the pseudo-MRM and MRM transitions in SRM 2975

	Phenanthrene	Anthracene
MRM/pseudo-MRM ratio (standards)	0.404	0.319
MRM/pseudo-MRM ratio (SRM 2975)	0.412	0.361
Absolute ratio deviation (SRM 2975)	+0.009	+0.042
Relative ratio deviation (SRM 2975)	+2.1%	+13.3%
pseudo-MRM S/N(SRM 2975)	405	27
MRM S/N(SRM 2975)	208	12

than the S/N ratio of the 178 → 152 transition for both peaks (405 vs. 208 and 27 vs. 12). This suggests that, even in the presence of a complex matrix such as Diesel PM, the sensitivity of pseudo-MRM is higher than MRM, and its specificity is preserved, perhaps due to the non-zero collision energies used, as described in section 3.1.

The same study of pseudo-MRM performance for the analysis of Diesel PM was also performed on a N- and S-heterocyclic compound, benzothiazole, and on two heavier PAH isomers, benzo(e)pyrene and benzo(a)pyrene. Their chromatograms and specificity indicators are reported in Figures S1 and S2 and Table S1 (supporting information). Briefly, all identification ratios of SRM 2975 were in agreement with those of the standard solutions, supporting the specificity of the pseudo-MRM transition. In the case of benzopyrene isomers, S/N ratios were equivalent for the MRM and pseudo-MRM transitions. In the case of benzothiazole, however, the S/N ratio was higher for the MRM transition even if the absolute peak area was higher for the pseudo-MRM transition, so the choice of the most appropriate quantitation transition would have to be carefully evaluated for each PAC of interest.

These results confirm the applicability of pseudo-MRM mode for the quantification of trace-level PAHs, PANHs and PASHs in complex environmental samples, with the possibility of enhancing their detection and thus reducing their limits of quantification.

4 | CONCLUSIONS

In this communication, we have presented data supporting the interest of using pseudo-MRM as a trustworthy alternative to MRM for the trace-level determination of unsubstituted PACs such as PAHs and heterocyclic derivatives, as the latter is generally less sensitive for these compounds. The signal enhancement with pseudo-MRM, and therefore the detection limit decrease, can be from 2 to 5 times according to our results. With the application of collision voltages around 15 V on U-shaped or L-shaped QqQs, the specificity of MRM can be preserved, because isobaric interferences will exhibit a far smaller response on pseudo-MRM transitions with such collision energies applied.

These statements were confirmed by the analysis of a Diesel PM reference material with both MRM and pseudo-MRM. Pseudo-MRM generated signals with higher S/N ratios than MRM for PAHs such as phenanthrene and anthracene, and the MRM/pseudo-MRM ratios for these compounds were consistent in neat standards and in the complex material.

However, these observations are only valid for triple quadrupoles. The different fragmentation properties of ion traps seem to make them less appropriate to pseudo-MRM.

Anyway, an extensive optimization of the monitored MS/MS transitions should be performed for each instrument when an analytical method is developed, because, depending on the mass spectrometer involved, the optimal mass transitions and the optimal collision energies differ.

Finally, pseudo-MRM seems very interesting and applicable to a broader scope of compounds, as long as they have a stable structure with no major labile bond. Such is the case, for instance, of fullerenes, which are compounds for which fragmentation is difficult to obtain; therefore, pseudo-MRM has already been successfully applied to the determination of these molecules.[26,27]

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/rcm.9307.

DATA AVAILABILITY STATEMENT

The datasets generated during this study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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