

1. Introduction

Certified reference materials (CRMs) are widely used to guarantee accurate and comparable results in chemical analysis. Purity CRMs usually locate at the top of metrological traceability hierarchy, and are prerequisites for solutions and matrix CRMs. Mass balance (MB), and quantitative nuclear magnetic resonance (qNMR) are widely used primary measurement methods for the purity assessment of organic CRMs. In MB, the normalized peak area ignores the difference among different impurities. The response of structurally related impurities in chromatography will lead to a big bias. In qNMR, the selection of quantitative hydrogen or other nuclei determines the accuracy of results, because the overlap between the measureand and structurally related impurities may introduce a positive bias for mass fraction. From above all, impurity fingerprint decoding is vitally important for purity assignment in both MB and qNMR. However, structurally related impurities identification and quantification are significant and challenging work. In CCQM K55.a and K148.a comparisons, numerous trace level impurities were visibly present, but no consensus was reported for their identity [1]. Just as stated in ISO 33407:2024, this can be the most critical and challenging effort of CRM characterization due to the large number of possible impurity structures [2].

Neonicotinoids (NEOs) are the mostly widely used insecticides in the world market according an influential report in 2020 [3]. Among this family, imidacloprid (IMI), acetamiprid (ACE), thiacloprid (THI), thiamethoxam (TMX), and dinotefuran (DNT) are representatives of the first, second and third generations of NEOs. Due to their wide application, these three have been among the most frequently detected insecticides in food, environmental or clinical samples. With the wide and persistent contamination, they raises deep concern about.

To address this problem, NEOs CRMs are necessary. Here, a new strategy combining liquid chromatography-high resolution mass spectrometry (LC-HRMS) and de novo identification tool (SIRIUS) was developed for the identification of the structurally related impurities (Fig. 1). HRMS was adopted to acquire the MS and MS/MS spectra of impurities after chromatography separation. Then, isotope pattern analysis was used for MS data to find molecular formula, and fragmentation tree was used for MS/MS data to elucidate chemical structure. Simultaneously, the main component worked as an anchor for structure elucidation and impurity confirmation by the shared neutral loss and fragmented ions. This combined strategy improved identification accuracy and speed, and provided a new perspective and instructive method for impurity identification in chemicals.

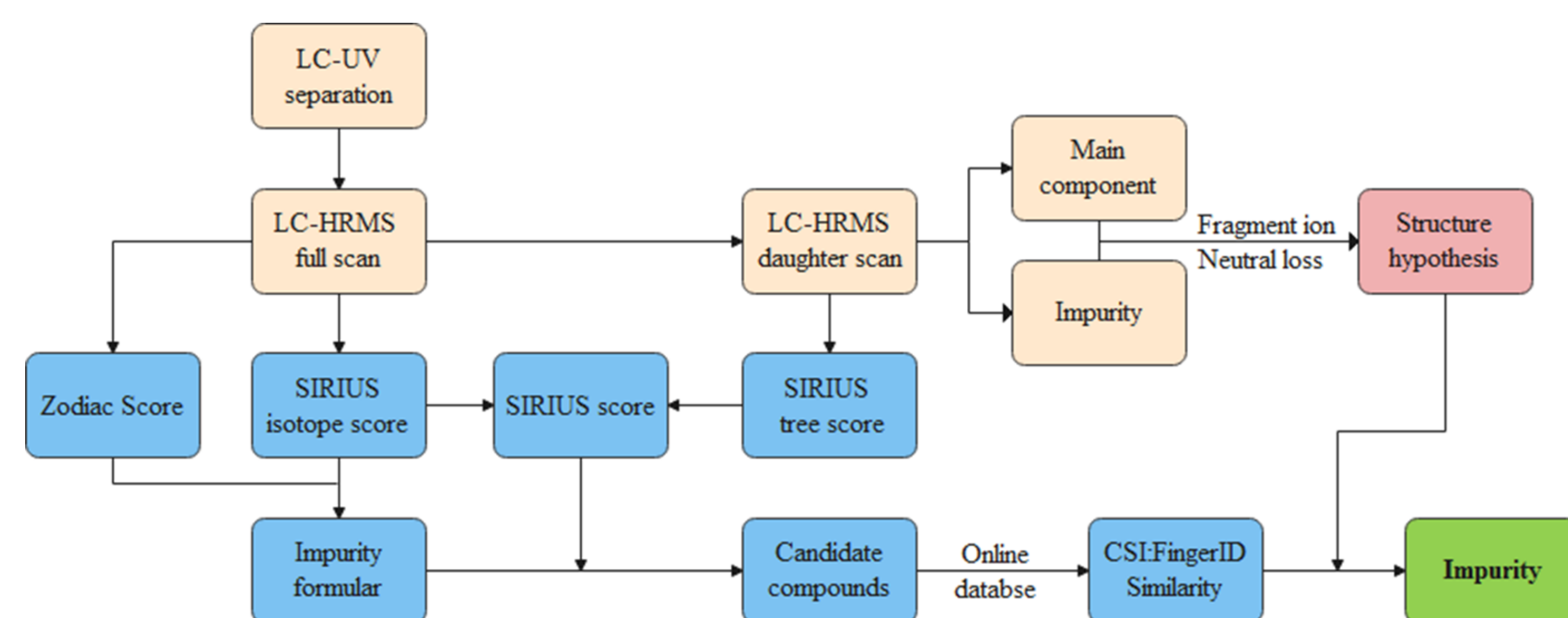


Fig. 1 Flowchart for impurity identification

2. Impurity fingerprint

The amount of structurally related impurities was closely relevant to their synthetic pathway. They are mainly from residual precursors, byproducts or degradation products that usually have similarly chemical structure. For structurally related impurity quantification, the big challenge was the chromatographic separation between main components and impurities. After careful optimization of the elution program on a Shimadzu LC-20A liquid chromatography, variation of water and acetonitrile was chosen to baseline separate impurities. Then, MS and MS/MS spectra of impurities were collected on a Thermo-Fisher Vanquish Q-Exactive Plus LC-MS.

MS data was identified through isotope pattern analysis in SIRIUS. The ideal molecular formula was selected according to the isotope score and with Zodiac score with human judgment. The calculated molecular formula was compared with main component to find the difference moiety. The MS/MS data was further identified through fragmentation trees in SIRIUS. Tree score was used for the fragmentation pattern analysis. Meanwhile, the MS/MS spectrum was compared with main component to find the same or different fragments. The chemical structure of impurity was selected with tree score and human judgment. SIRIUS score was a combination of isotope score and tree score, with the promising value of 100.000%. Moreover, the CSI:FingerID helped to elucidate the fragment mechanism and the ideal value was 0. CSI:FingerID depended on molecular structure databases, therefore it didn't work for unreported structure.

2.1 Impurities in TMX

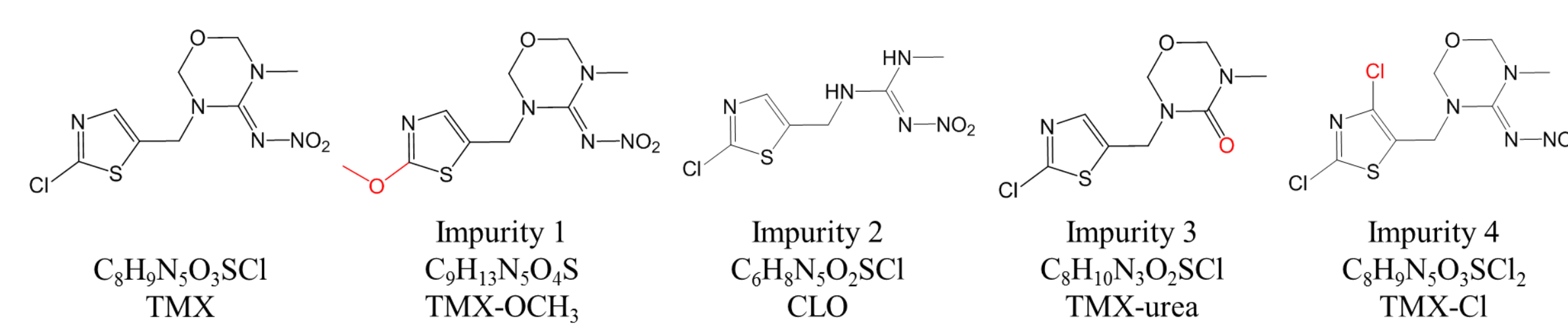
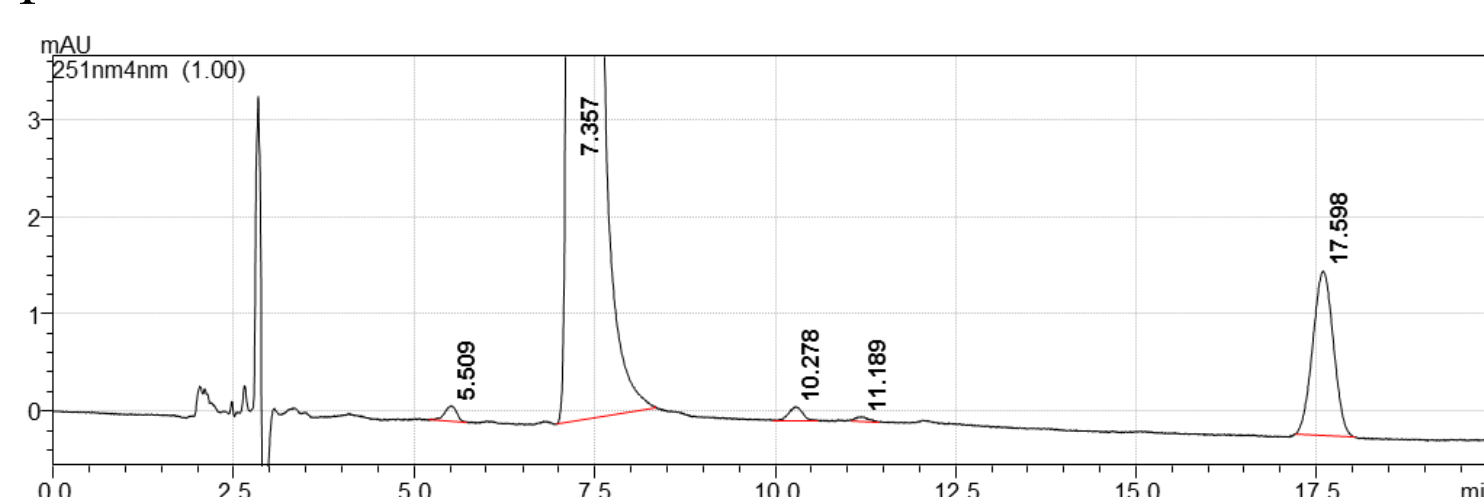


Fig. 2 A-Chromatogram of TMX; B-Structure of impurities

With Eclipse plus C18 column (250×4.6 mm, 5 μm) at isocratic elution (H₂O:acetonitrile=80%:20%), four impurities in TMX were separated as Fig. 2A. After calculation, these four impurities were two byproducts (TMX-OCH₃ and TMX-Cl) and two metabolites (clothianidin and TMX-urea) as Fig. 2B. Two metabolites were further verified with commercial standard by retention time, UV spectrum and mass spectrum [4]. While, two byproducts were from side reaction

has no commercial standards right now. From the UV spectrum, impurity 4 (TMX-Cl) was the main impurity. For qNMR analysis, TMX-Cl had one less hydrogen atom than TMX, so this hydrogen was a good choice for qNMR quantification to avoid peak overlapping with impurities [5].

2.2 Impurities in THI and DNT

With an Inertsil ODS-SP column (250×4.6 mm, 5 μm) at gradient elution, 18 impurities were totally found as Fig. 3 [6]. With an Eclipse plus C8 column (250×4.6 mm, 5 μm) at gradient elution, four peaks were separated. For peak 3, this was an isomer of DNT. Therefore, only two impurities were found as Fig. 3 [7]. Interchange of the E and Z isomer happened at room temperature, therefore the peak broadened.

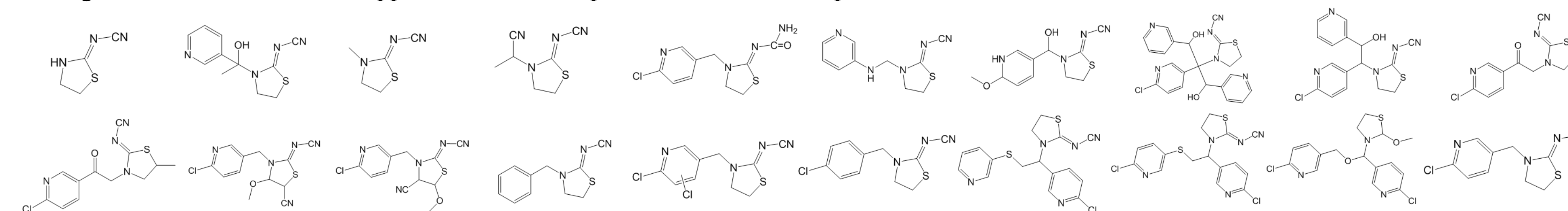


Fig. 3 Structure of impurities in THI

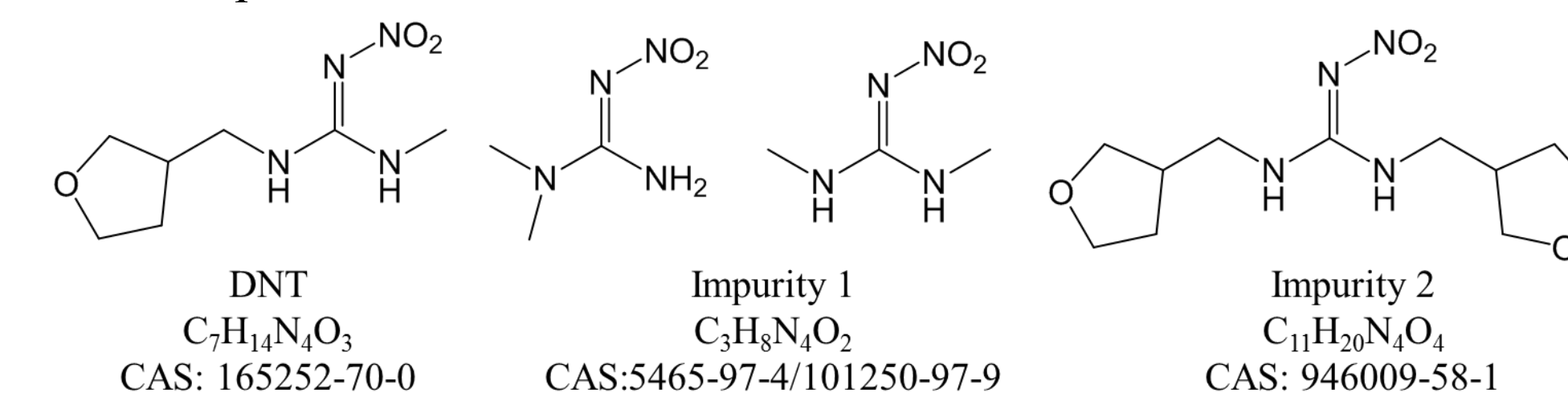
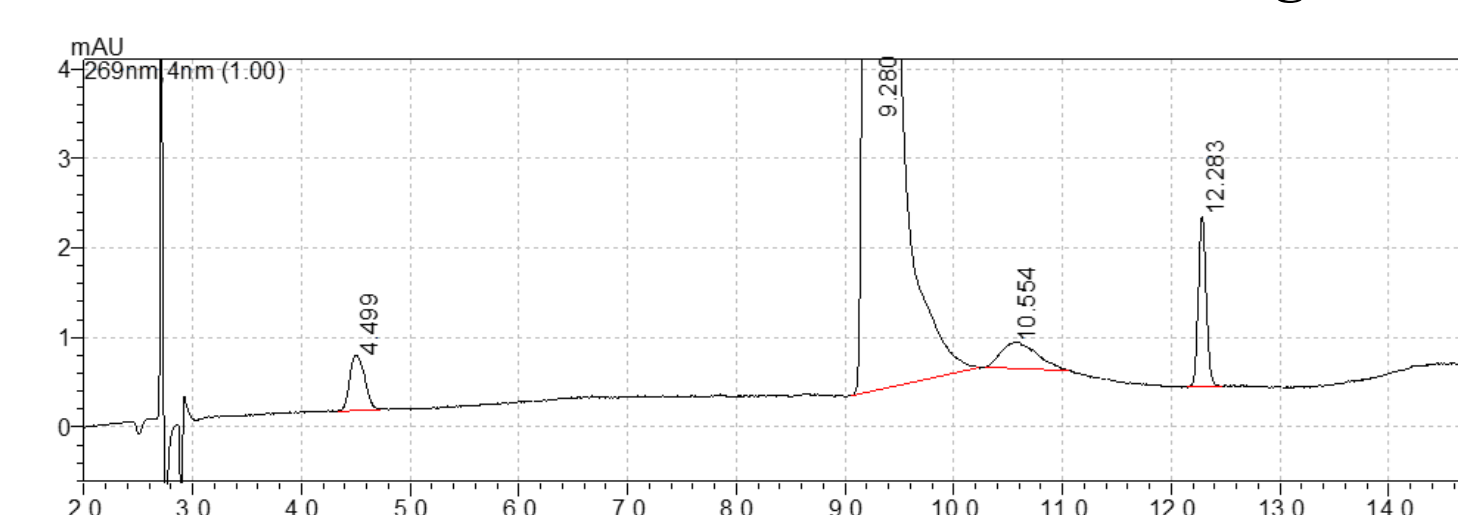


Fig. 4 A-Chromatogram of TMX; B-Structure of impurities

2.3 Impurities in ACE and IMI

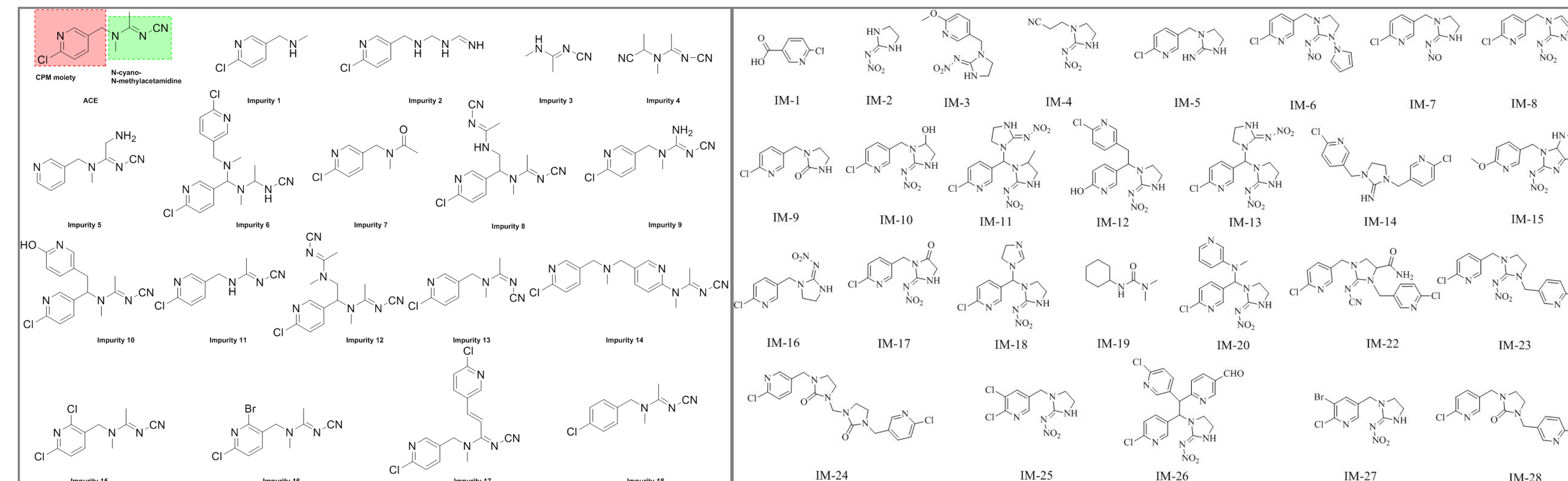


Fig. 5 Structure of impurities A-ACE [8]; B-IMI

4. Conclusion

By this strategy, 4, 18, 2, 28 and 28 impurities were elucidated in thiamethoxam, thiacloprid and dinotefuran, imidacloprid and acetamiprid materials respectively. These impurities were from intermediate residue, side reaction or degradation products. Among them, a few impurities were further verified with chemical standards but most impurities were reported for the first time even didn't have CAS numbers. This work provides a new perspective and instructive method for impurity identification in purity assignment in MB and qNMR.

References

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Acknowledgements

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