Electron-Induced Dissociation (EID) as a complementary tool to collision induced dissociation (CID) for structural characterization of pesticides photo-oxidation products

High resolution mass spectrometry (HR-MS) and tandem mass spectrometry (MS/MS) are essential tools for structural elucidation of unknown compounds such as pollutant degradation compounds.

Abstract

In some cases, differentiation of isomers is challenging. It has been shown that electron induced dissociation (EID) is complementary to collision induced dissociation (CID). In this work, CID and EID have been examined. We demonstrate the effectiveness of EID for better characterization of degradation products of pesticides.

Keywords: Tandem mass spectrometry, environmental application, pesticides, collision induced dissociation (CID), electron induced dissociation (EID), structural characterisation

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Introduction

Pesticide analysis has recently become one of the major focus in the environmental field. All the common water treatment processes for pesticides, such as hydrolysis, biodegradation, chemical oxidation and UV irradiation, are still under development. These treatments do not lead to full mineralization of pollutants and produce transformation products which can contaminate the environment. Today, most of the pesticide transformation products (TPs) remain unknown and many studies aim to identify them to understand their impact on the environment [1]. Indeed, the knowledge of the structure of TPs, through the use for example of ab initio calculations, allows to predict their toxicity. If one compound has been proven to be toxic, it is important to be able to search for it in the environment. Some of these TPs may result from direct photolysis. We have studied the fragmentation pathways of the protonated molecular ion of metolachlor with EID and CID and then used these results for the elucidation of possible TPsOH structures.

Experimental

Sample preparation

All chemicals except metolachlor-D_6 (98% atom D) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France) and used as received (98% purity). Metolachlor-D_6 was purchased from C.I.L. Cluzeau Info Labo (Sainte-Foy-La-Grande, France). The chemical structures of metolachlor (Meto) and metolachlor-D_6 (MetoD_6) are displayed in Table 1. Reference solutions of Meto and MetoD_6 were diluted to 2 μM in water/ acetonitrile / formic acid (50/50/0.1) and infused in the ESI source for structural characterisation. Aqueous solutions of reference compounds (10 ppm) were photolysed for 1 h using a high-pressure mercury lamp (200 – 1100 nm). These solutions were then diluted in H_2O/CH_3CN (50/50) and acidified with formic acid (FA) (0.1%) before LC-MS analysis. TPs were separated by LC using a C18 column (Atlantis 3 μm, 150 x 2.1 mm, Waters). Mobile phases were (A) 20/80 CH_3CN 0.1% FA and (B) H_2O 0.1% FA. Flow rate was 0.2 mL/min. The elution gradient was: 20% (A) from 0 to 11 min; 50% (A) from 11.1 min to 20 min. The following fractions of interest were collected for MS/MS experiments: F2c (8.39 min); F2d (9.39 min): F2e (10.06 min) and F4 (22.58 min).

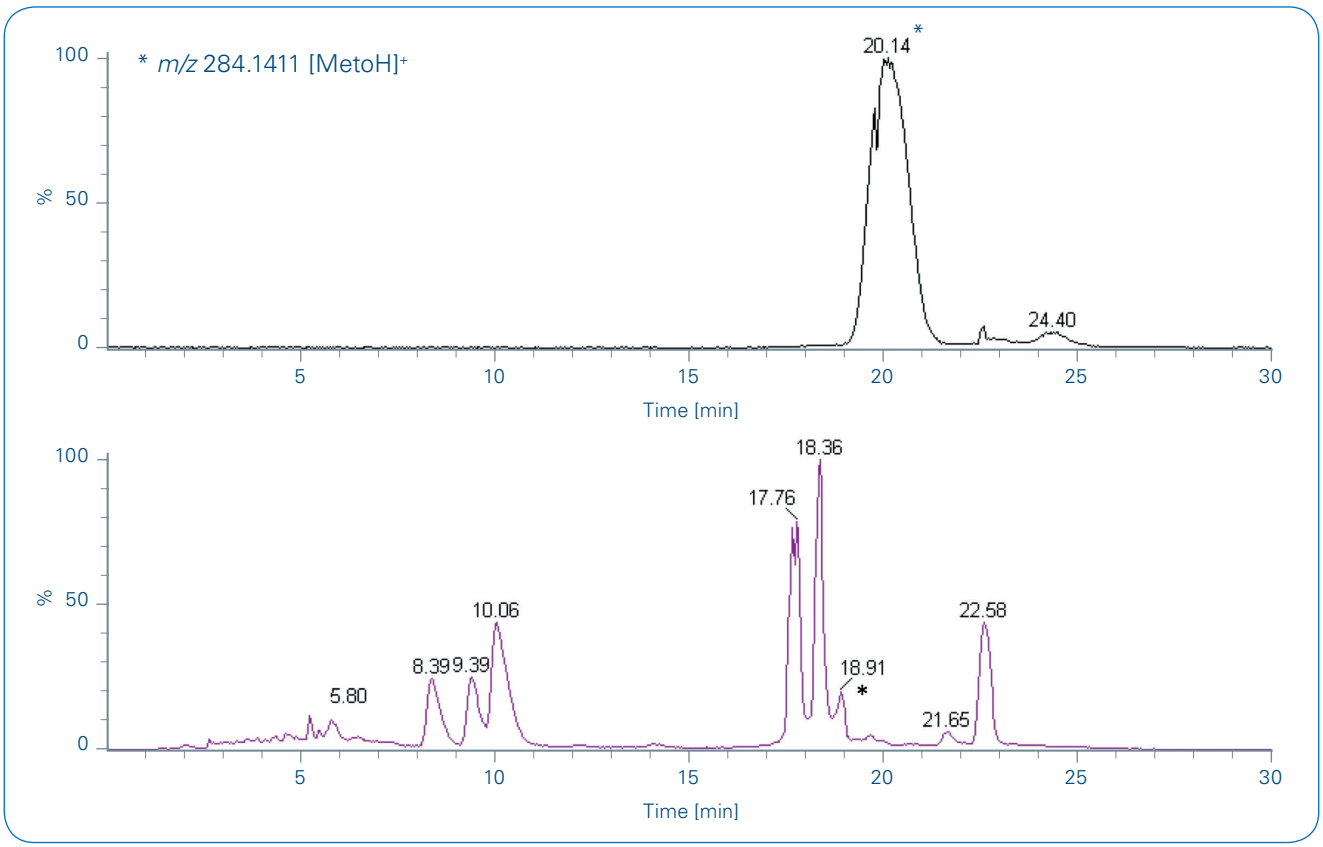
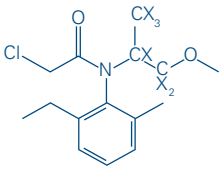


Figure 1. Extracted ion chromatograms (EIC) of the sum of m/z 284.1411 [MetoH]+ + m/z 266.1750 [TPOH+H]+ before (top) and after 60 min of irradiation (bottom) of aqueous metolachlor solution.

Mass spectrometry

All experiments were performed on a solariX MRMS instrument (Bruker Daltonics GmbH Co. & KG., Bremen, Germany) equipped with a 7T superconducting magnet (Bruker, Wissembourg, France). Samples were directly infused and ionized in ESI positive ion mode at 2 μL/min. Ion detection was set to 4M datapoints with a starting mass of *m/z* 57.7 resulting in a transient time of 0.839 s and a mass resolution of 350,000 at *m/z* 200. The protonated molecule was isolated with the quadrupole and either fragmented in the collision cell with CID (E_{col}=10 V and 20 V) or fragmented via electron irradiation time in the ICR cell (EID 13 eV). For the EID experiments, electron irradiation in the ICR cell was set between 0.2 s to 2 s. An ECD hollow cathode allows successive irradiation of ions with electrons. Under EID conditions, we ensured to avoid introducing fragment ions originating from unwanted collision dissociation prior to introduction into the ICR cell. NaI cluster ions were used for mass calibration and elemental compositions were calculated with a mass tolerance below 3 ppm.

Table 1. Names and chemical structures of the studied compounds

Structure	Chemicals	Formula	X	MW
	Metolachlor (Meto)	$C_{15}H_{22}NO_2Cl$	H	283.1334
	Metolachlor D_6 (MetoD_6)	$C_{15}H_{16}D_6NO_2Cl$	D	289.1710

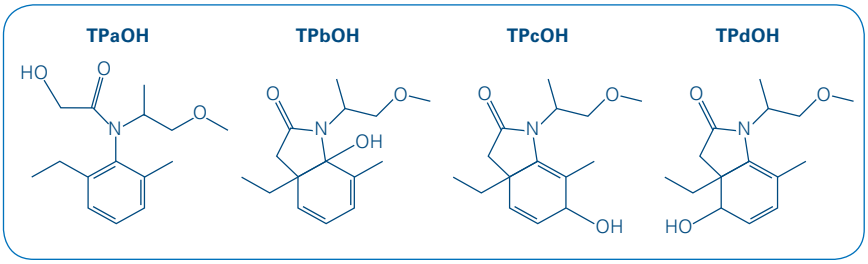


Figure 2. Proposed structures for monohydroxylated species (TPsOH) formed by photodegradation of metolachlor in water.

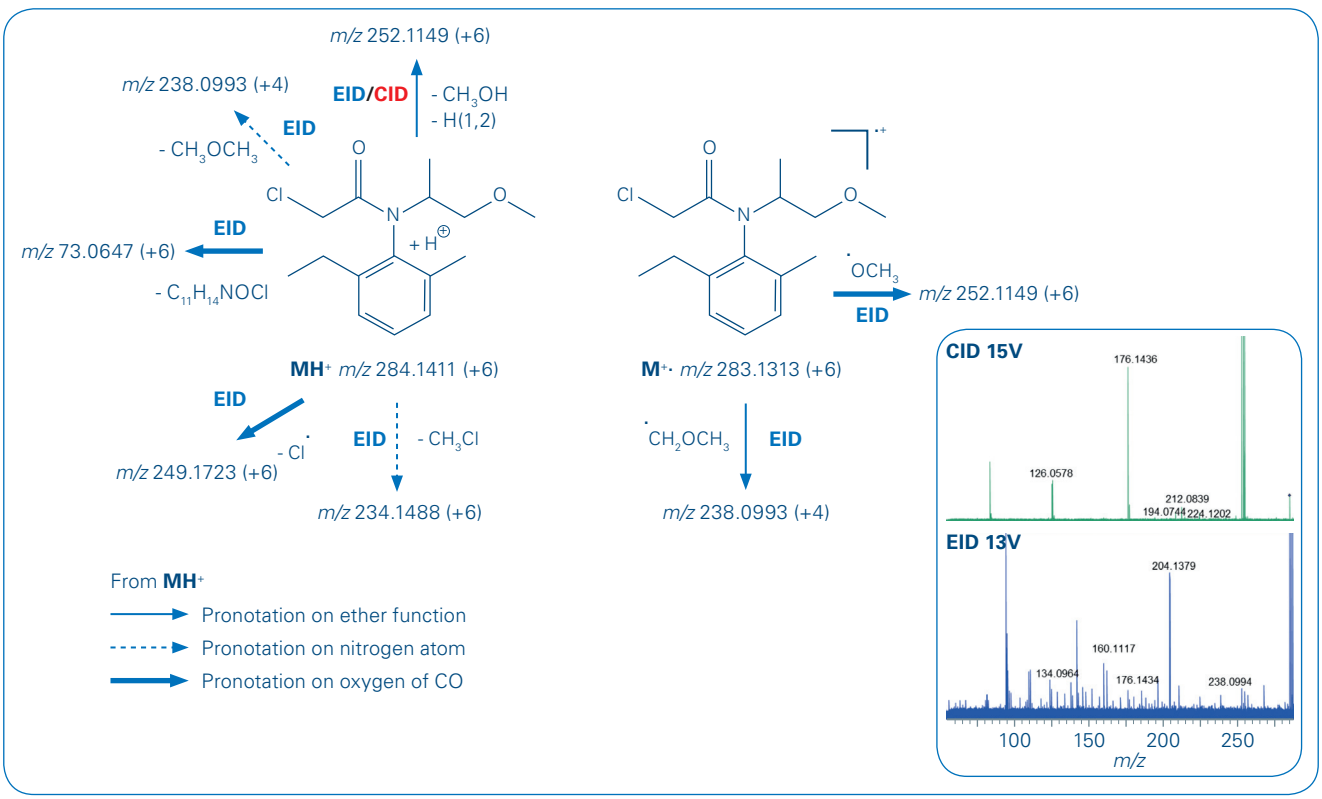


Figure 3. Fragmentation pathways of molecular ions MH^+ and $M^{+\cdot}$ for metolachlor (shift for metolachlorD_6) obtained in CID and EID (spectrum of fragmentation of protonated metolachlor on the right side, green: CID; blue: EID). Only fragment ions obtained by primary cleavage from molecular ions are reported.

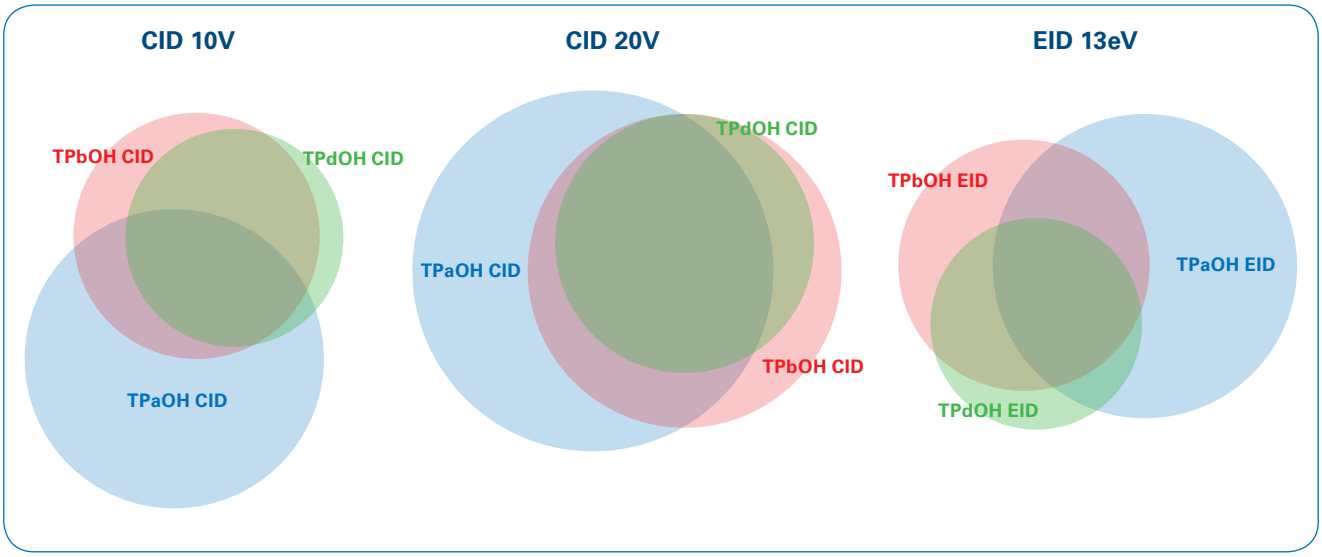


Figure 4. Venn diagrams of CID and EID mass spectra with precursor of three protonated isomers [TPsOH+H]⁺ at m/z 266.1750 ($C_{15}H_{24}NO_3$), Figure 2, showing diversity of fragment spectra.

Results and Discussion

TPs identification was performed by comparing the chromatograms of irradiated and non-irradiated solutions of metolachlor (and metolachlor-D_6). After 60 min of irradiation, only 5% of the initial amount of metolachlor could still be detected. The protonated products after photo irradiation were easily identified by mass spectrometry and confirmed by the presence of a sodium ion adduct $TPsNa^+$. It was observed that the chlorine atom was removed from all photo products. Among them several isomers at *m/z* 266.1750 ($C_{15}H_{24}NO_3$) were formed: after irradiation the chlorine atom was replaced by OH [1]. In Figure 1 the extracted ion chromatograms of *m/z* 284.1411 $[MH]^+$ and *m/z* 266.1750 $[TPOH+H]^+$ before and after 60 min of irradiation of metolachlor aqueous solution are shown.

Eliminiation of radical chlorine atom [1] occurs through homolytic cleavage of the C–Cl bond in the presence of water. Four TPs can be formed by direct addition of hydroxy radical ($OH^{\cdot}$) or after isomerisation (Figure 2).

For the elucidation of degradation product structures, we focused on the fragmentation mechanisms of metolachlor molecular ions. Figure 3 compares the primary degradation pathways of the molecular ions obtained from CID and EID mass spectra. Fragment ions were assigned in accordance with both, accurate mass and mass shift measurements obtained for the deuterated compound (MetoD_6). As already described in the literature [2-4], EID results in different fragmentation pathways and more extensive fragmentation compared to CID.

In the case of the CID mass spectra, fragment ions result only from protonation of ether function. While in addition to the ions observed in the CID mass spectra, many other fragment ions were obtained in EID, formed from protonation of all ionisation sites (heteroatoms and double bonds). From the protonation of the nitrogen atom, the 4 center-eliminations provide information on nitrogen substitutes.

The expected fragmentation for molecular ions of TPaOH and metolachlor are very similar. In CID, the presence of $[(TPaOH+H\text{-}CH_3OH) – CHOHCO]^+$ at *m/z* 176.1433 allows the localization of the hydroxy group. CID and EID mass spectra were compared with Venn diagrams (Figure 4) for three TPsOH isomers using two collision energies in CID (10 and 20 V) and 13 eV in EID. According to TPs structure, TPaOH can be identified easier by CID. TPbOH and TPdOH have very similar structures which can't be differentiated by CID using 20 V. However, EID at 13 eV provides higher number of fragment ions than CID at 10 V.

These results show that EID mass spectra of the protonated molecule include more fragment ions than those obtained in CID. Among these fragment ions, for each compound analyzed, there are characteristic ions of a collision method or of a

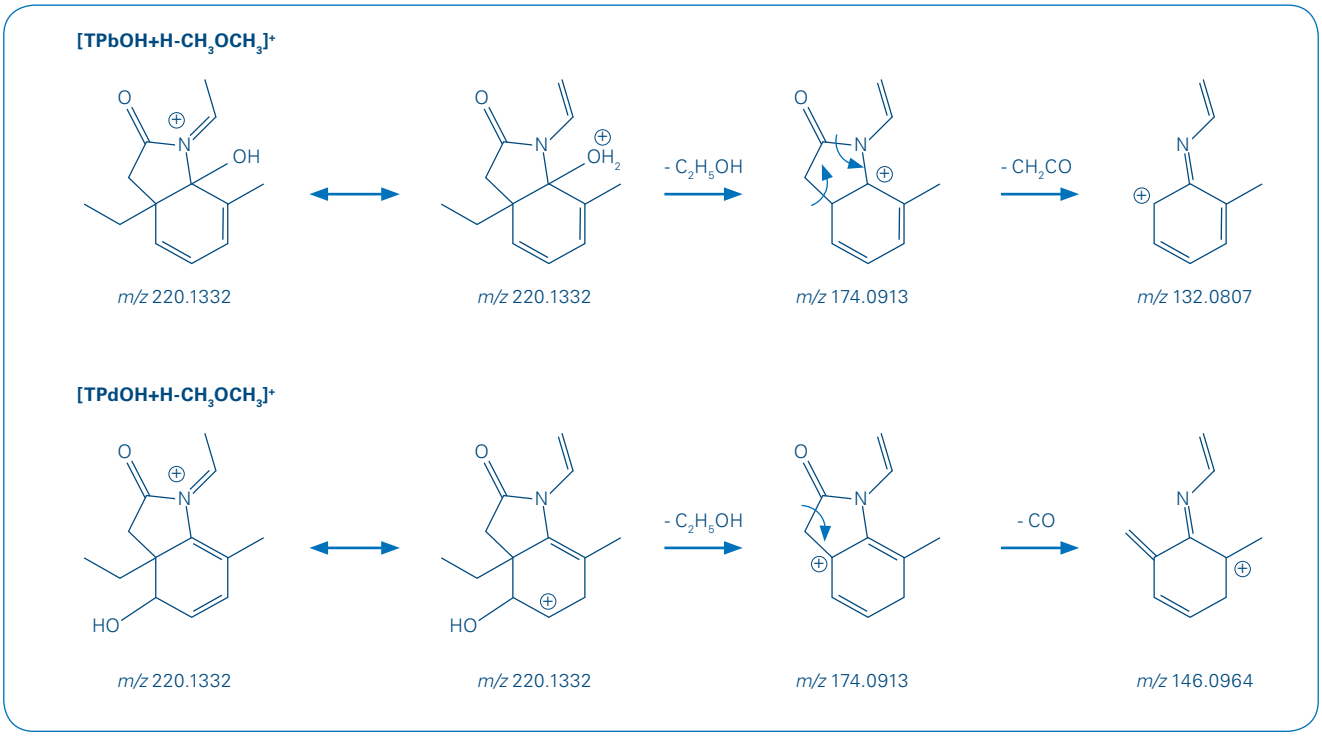


Figure 5. Proposed mechanisms for the formation of m/z 174.0913 observed in EID MS/MS spectrum of $[TPsOH+H]^+$ from F2d and F2e fractions.

single isomer. The complementarity of these different fragmentation techniques allows higher confidence in structural determination for each compound.

The EID MS/MS mass spectra obtained for compounds present in the fractions F2d and F2e have a fragment ion at *m/z* 174.0913 with a molecular formula of $C_{11}H_{12}NO$. This ion could be obtained from $[TPOH+H]^+$ by two consecutive eliminations of C_2H_6O, therefore allows to eliminate the TPcOH structure for which this mechanism can't be observed in contrast to the other two ions as represented in Figure 5.

The ion at *m/z* 132.0807 formed by elimination of CH_2CO from the fragment ion at *m/z* 174.0913 can only be formed from the protonated TPbOH precursor.

Conclusion

- EID mass spectra resulted in a higher number of fragment ions compared to CID and provided improved structural identification and elucidation of the examined analytes.
- Moreover, EID provided detailed structure information from both, protonated and radical cation precursor ions.
- Fragmentation using EID implies 4 center-eliminations and homolytic cleavages in addition to fragments observed in CID which is mainly induced by charge.
- EID and CID are complementary fragmentation techniques especially suitable for structural elucidation of unknown products.
- In this study differences obtained in CID and EID mass spectra were used to confirm the structure of isomeric compounds.

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