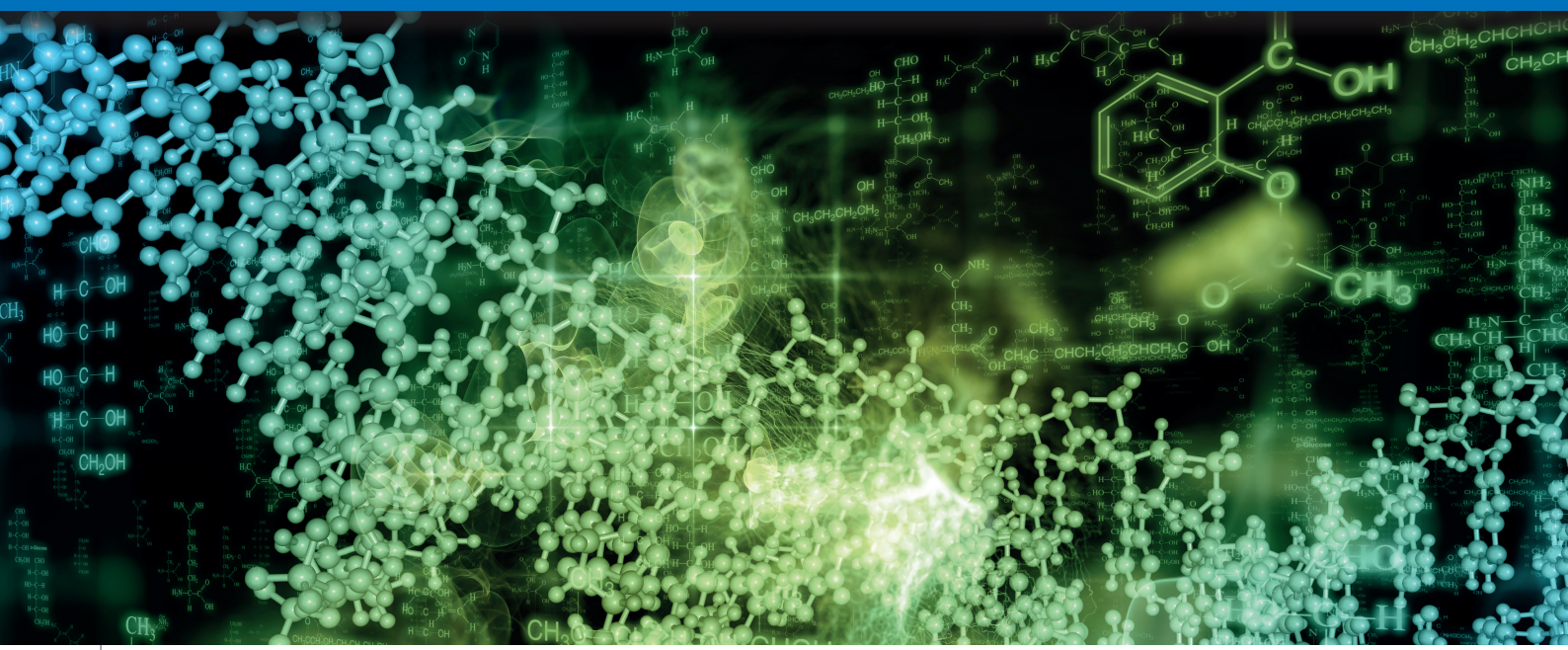

Electron Induced Dissociation for the Differentiation of Isomeric Metabolites of Diclofenac

Electron induced dissociation (EID) is applied for the differentiation of structural isomers of diclofenac metabolites. EID showed superior capability over collision induced dissociation (CID) by providing detailed structural information to locate the hydroxyl groups on different rings.

In the human body, metabolism of drugs is a detoxification process. In some cases, the metabolites formed are chemically or pharmacologically active and may play an important role in observed pharmacology and/or toxicology in humans[1]. Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) commonly used to reduce inflammation and pain. A major metabolic pathway for diclofenac is phenyl hydroxylation, resulting in two major metabolites, 4'-OH-diclofenac (4'-OHD) and 5-OH-diclofenac (5-OHD), catalyzed by cytochrome CYP2C9 and 3A4, respectively[2]. Determining the structures of these metabolites is critical for understanding the underlying biological activities and safety risk.

NMR is well-established as a powerful structural elucidation technique[3]; however, it requires a relatively large amount of purified material that requires labor intensive and time-consuming purification steps. Alternatively, low energy CID, one of the most commonly used MS/MS techniques, provides detailed structural information for molecules of interest. Unfortunately, low energy CID is unable to differentiate the structural isomers due to the lack of specific bond cleavages.

Keywords:
EID, solariX, Metabolomics

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Alternative fragmentation methods, including electron capture dissociation (ECD), have been developed and extensively studied in recent years[4-6], but are limited to multiply charged ions. Consequently, ECD cannot be used for the structure elucidation of small molecules since they typically form singly charged molecules. Recently, electron induced dissociation (EID) has emerged as a technique that can be used to dissociate singly charged molecules[5,7]. In this note, EID is to differentiate isomeric diclofenac metabolites and the EID fragmentation behavior is compared with that generated via traditional CID.

Experimental

Sample Preparation

Diclofenac, 4'-OHD and 5-OHD were generously provided by Ian S. Mcintosh (MRL). Each compound was directly infused into the mass spectrometer by a TriVersa NanoMate robot (Advion, Inc., Ithaca, NY, USA) at a concentration of 20 pmol/µL in a spray solution of 50:50 acetonitrile:water with 0.1% formic acid.

Mass Spectrometry

EID experiments were performed on a 9.4T Solarix qQq-Fourier transform ion cyclotron resonance (ICR) mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Mass spectra were collected with 4 M data points, and summed over 100-200 scans depending on signal quality. The transient length was 0.84 s, and the estimated resolving power was ~150,000 at m/z 400. The signal-to-noise ratio (S/N) threshold was set to 3, and signals below that threshold were ignored. Data were analyzed using DataAnalysis 4.4 (Bruker Daltonics) with a mass accuracy of < 3 parts per million (ppm).

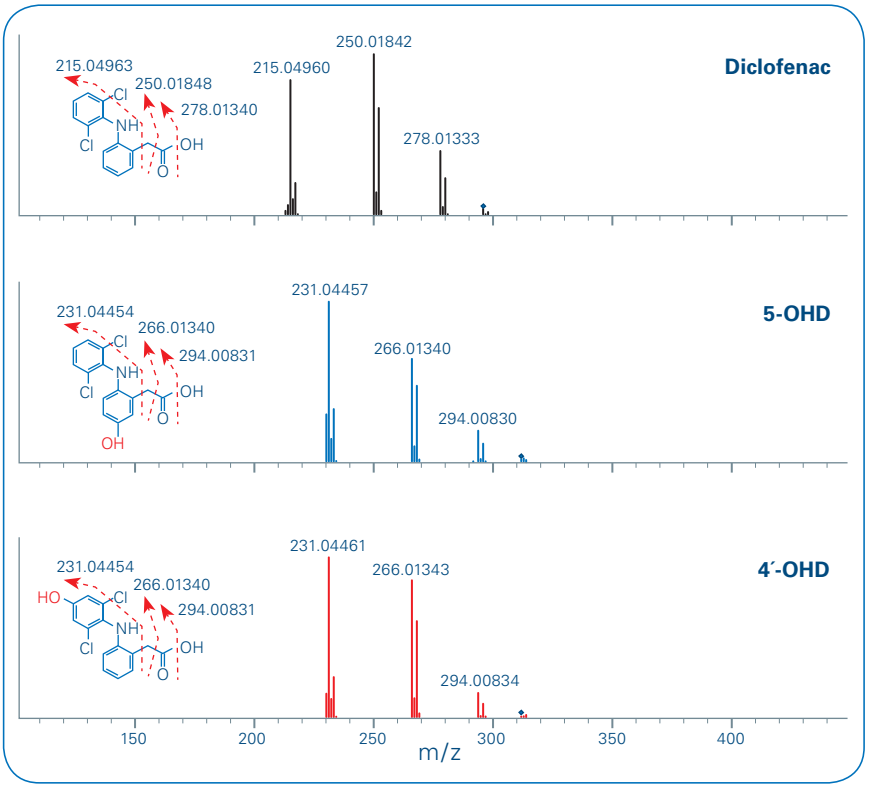

Figure 1: CID spectra of 4'-OHD and 5-OHD

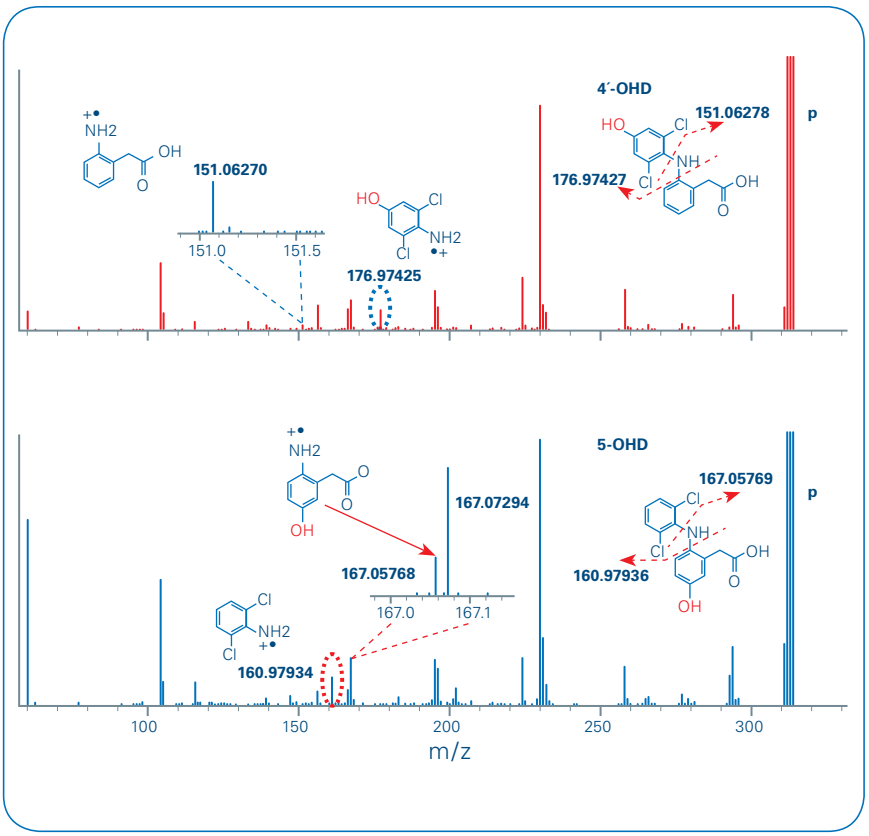

Figure 2: EID spectra of 4'-OHD and 5-OHD

Results and Discussion

CID Mass Spectrometry

Figure 1 shows the CID spectra of diclofenac, 4'-OHD, and 5-OHD. CID of all three compounds exhibited the same fragmentation behavior, always leading to chemical bond cleavage at identical locations. CID fragmentation of these compounds could not generate diagnostic fragments to differentiate which of the phenyl rings underwent hydroxylation.

EID Mass Spectrometry

The EID spectra of diclofenac, 4'-OHD and 5-OHD are shown in Figure 2. A greater number and variety of fragments were detected under EID, especially fragment ions from the cleavage of the two aromatic ring systems. The most abundant fragments observed in the EID spectrum are summarized in Table 1. Diagnostic ions for the pair of isomers were at m/z 176.97425 and 151.06270 for 4'-OHD; m/z at 160.97934 and 167.0575 for 5-OHD under EID fragmentation. The resulting fragments preserved the substituents on the phenyl rings, including the chlorine and the hydroxyl. These fragments enabled the differentiation of the two structural isomers with hydroxyl groups on the two different phenyl rings. In particular, the diagnostic ion at m/z 167.05756 for 5-OHD was very close to another fragment with m/z 167.07295, and was fully resolved by FT-ICR (Magnetic Resonance Mass Spectrometry, MRMS) at the resolving power of ~150,000.

The CID technique tends to cleave the weakest bonds first. EID induces more cleavages while sparing the more labile chloro and the hydroxyl groups, which were predominantly cleaved under CID. A direct comparison of EID to CID fragmentation demonstrates the valuable application of EID technique in the structural characterization of isomers.

Table 1

Measured m/z			Proposed formula
Diclofenac	4'-OHD	5-OHD	
	312.01899	312.01899	$[C_{14}H_{12}Cl_2NO_3]^+$
296.02412			$[C_{14}H_{12}Cl_2NO_2]^+$
	294.00840	294.00840	$[C_{14}H_9Cl_2NO_2]^{\bullet+}$
278.01357			$[C_{14}H_{10}Cl_2NO]^+$
	277.05028	277.05012	$[C_{14}H_{12}ClNO_3]^{\bullet+}$
	266.01345	266.01389	$[C_{13}H_{10}Cl_2NO]^+$
	258.03183	258.03169	$[C_{14}H_9ClNO_2]^+$
250.01862			$[C_{13}H_{11}Cl_2N]^{\bullet+}$
242.03697			$[C_{14}H_9ClNO]^+$
	230.03672	230.03672	$[C_{13}H_9ClNO]^+$
214.04186	214.04174	214.04164	$[C_{13}H_9ClN]^+$
208.07578			$[C_{14}H_{10}NO]^{\bullet+}$
	195.06789	195.06781	$[C_{13}H_9NO]^{\bullet+}$
180.08059			$[C_{13}H_{10}N]^+$
	183.06782	183.06788	$[C_{12}H_9NO]^{\bullet+}$
179.07298			$[C_{13}H_9N]^{\bullet+}$
	176.97419		$[C_6H_5Cl_2NO]^{\bullet+}$
	167.07289	167.07289	$[C_{12}H_9N]^{\bullet+}$
		167.05756	$[C_8H_9NO_3]^{\bullet+}$
160.97941		160.97930	$[C_6H_5Cl_2N]^{\bullet+}$
151.06269	151.06265		$[C_8H_9NO_2]^{\bullet+}$

Conclusions

The combination of high accuracy, high resolution MRMS mass spectrometry, and EID fragmentation has been successfully applied to distinguish a pair of structural isomers (4'-OHD and 5-OHD). By utilizing high electron energy (>10 eV), EID accessed more high energy fragmentation pathways than CID while retaining critical low energy bonds, thus providing complementary structural information and in some cases even additional information.

In general, an EID-based mass spectrometry method has shown great capability for structural characterization of small molecules carrying a single charge and exhibited great potential in differentiation of isomeric compounds.

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