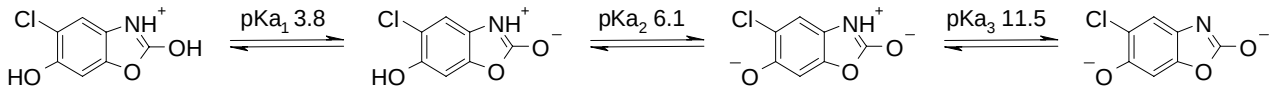
Supporting information

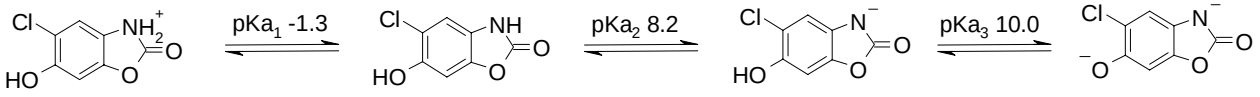
The dissociation constants for the (A) enol- and (B) keto- tautomeric isomers of 6-hydroxychlorzoxazone [a]

A



pH < 3.8 | 3.8 < pH < 6.1 | 6.1 < pH < 11.4 | 11.5 < pH

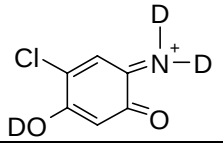
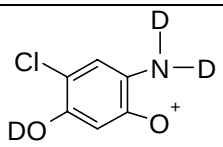
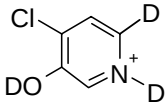
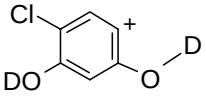
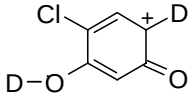
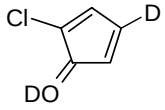
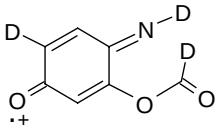
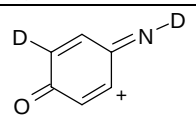
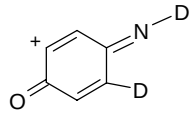
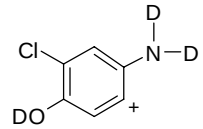
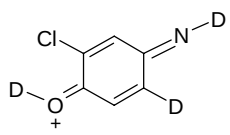
B



pH < -1.3 | -1.3 < pH < 8.2 | 8.2 < pH < 10.0 | 10.0 < pH

[a] The dissociation constants ± 95% confidence limits was estimated using the ACD/pKa DB software. The pKa values were estimated for the acid form of the 2- and 6-hydroxy functional groups and the conjugated acid form of the basic nitrogen in benzoxazole ring, i.e., pKa3 for the enol and pKa1 for the keto tautomers. The preliminary simulation of the apparent dissociation constants for various HCZ functional groups indicated that over wide ranges of pH commonly used for robust liquid chromatographic separations (i.e., pH 3 to 8), the enol- form was highly ionized (LogD -0.99, ratio of ionized/unionized ~ 9.8), while the keto- tautomer was mainly in its un-ionized form (LogD 1.59, ratio of ionized/unionized ~ 0.025). Therefore, the benzoxazole nitrogen appeared to be highly protonated in acidic mobile phases (0.1% formic acid, pH ~ 3), which could enhance the ionization and detection of HCZ under (+) ESI.

The calculated heats of formation for tentative fragment structures of deuterated HCZ [a]

Structure	*m/z*	Heat of Formation (eV)
	161	5.35
	161	5.74
	133	5.94
	145	7.65
	145	6.80
	117	8.13
	154	6.09
	108	10.81
	108	12.72
	145	10.18
	145	8.05

[a] The theoretical estimation of heats of formation were performed using semiempirical PM3 quantum mechanic approximation, using the HyperChem v 7.0 (Hypercube, Inc., Gainesville, FL) software. Geometry optimization was completed using the Polak-Ribiere conjugate gradient algorithm method, with a RMS gradient of 10^{-3}.