

GCMS BASED STRUCTURE ASSIGNMENT FOR FURAN FATTY ACIDS ISOLATED FROM EUROPEAN CARP (*CYPRINUS CARPIO*)



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Introduction

The European carp (*Cyprinus carpio*) is an established pest species in Australian inland rivers and studies that investigate alternative routes to exploitation or bioregulation are of interest to river management plans. The species expresses a family of unusual furan substituted fatty acids that are of interest for their potential to be either anti-nutrients or useful therapeutic substances. We describe a multi-dimensional gas chromatographic approach to detecting the presence of structural variants for the family.

Discussion

The identification of known furan fatty acids as either their methyl ester or pyrrolidinamide derivatives is possible using the characteristic ions formed in EI-MS. The stability of the furylmethylene alkyl-pyrrolidinamide cations yield uncomplicated mass spectra that are ideal for the assignment of structural homologues (Fig 1). Using a series of correlation plots for retention time quartets, familial patterns were determined for furan acids of different substitution. The chromatographic relationships between compounds on different phases were used to interpolate the location in chromatographic space of homologous compounds and also to aid in the assignment of structure for cases where mass spectral data alone was inconclusive.

Correlation plots of retention time data against molecular weight (Fig 2) and for data pairs between columns and for each column set (Fig 3) were prepared. Using the known furan esters as a template, retention time and molecular weight could be used to predict retention time windows for minor furan acids. These could be detected by using selected ion monitoring or targeting areas of chromatographic space with full scan data. The stability of fragment ions associated with the furan allowed for ready detection of compounds containing unsaturation in the fatty acid tail ($m/z - 2$) and species in which the 4-furyl chain was seven rather than five carbons in length ($m/z + 28$).

Conclusion

Over 30 furan fatty acids were tentatively identified using this model (Table 1). The main structural variations were methyl substitution of the furan ring, chain length of the 2'-furyl substituent and degree of unsaturation in the 5'-furyl substituent. One example of unsaturation in the 2'-furyl substituent was also detected. The identification of the compounds is preliminary to determining their physiological significance. Orthogonality of separation was provided by the unique selectivity of the carborane (HT8) and biscyanopropyl (BPX90) phases relative to the 5 % phenyl substituted phases.

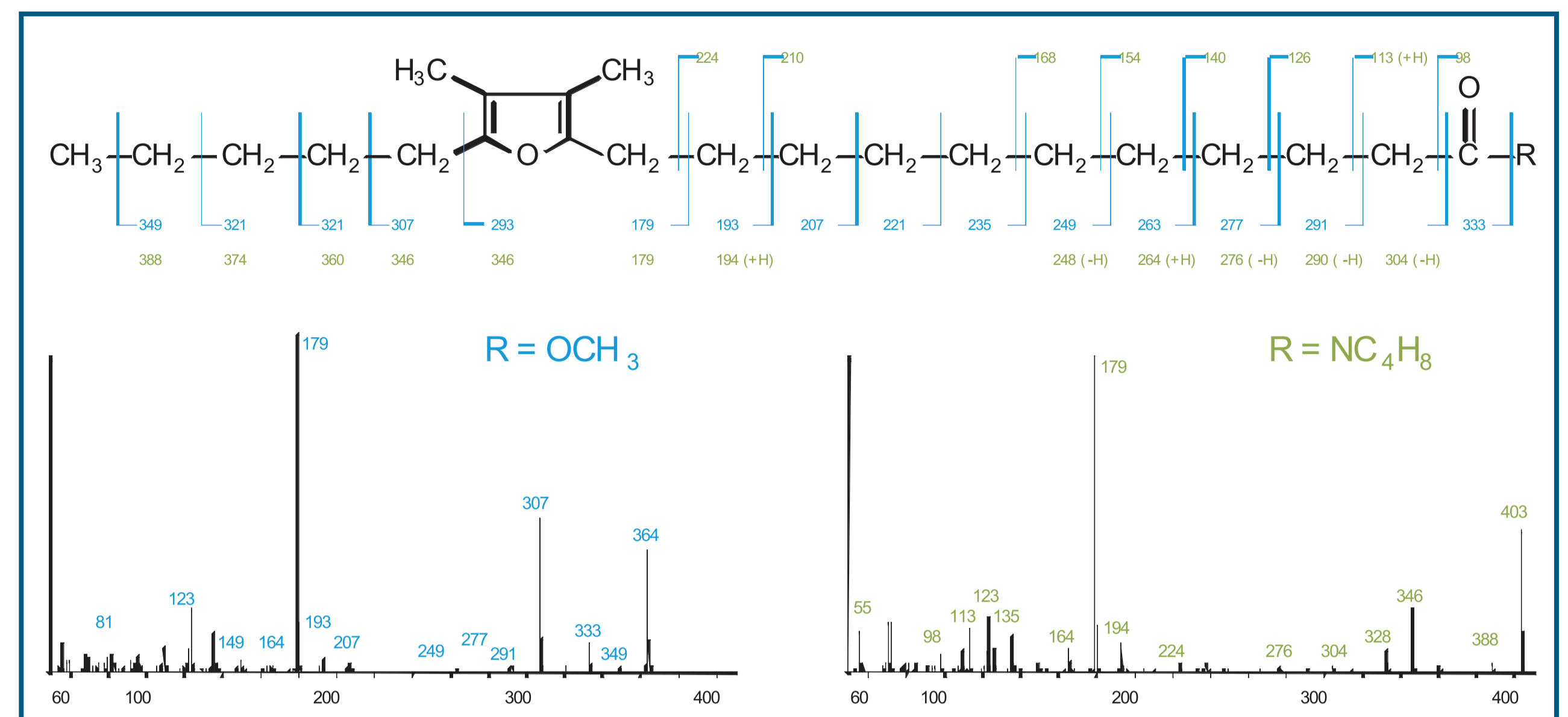


Figure 1: The rationalization of the structure for the major furan fatty acid 12, 15-epoxy-13, 14-dimethyleicosan-12, 14-dienoic acid by EI-MS of the methyl ester (left with mass assignments in blue) and the pyrrolidinamide (right with mass assignments in green).

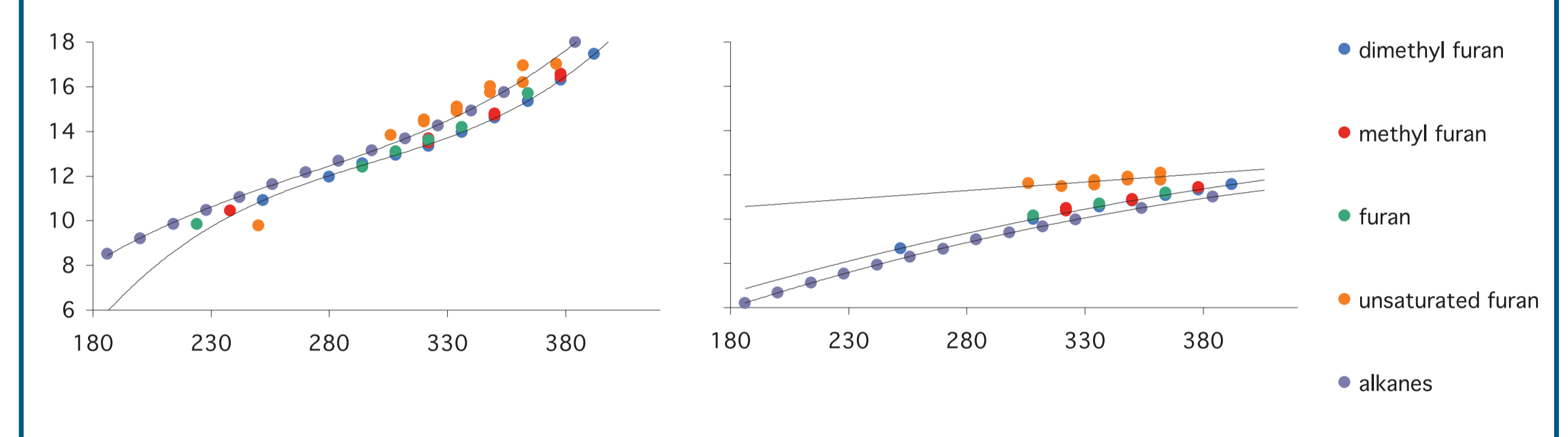


Figure 2: Correlation of retention times for different groups of fatty acid methyl esters on HT8 (left) and BPX90 (right) phases with molecular weight.

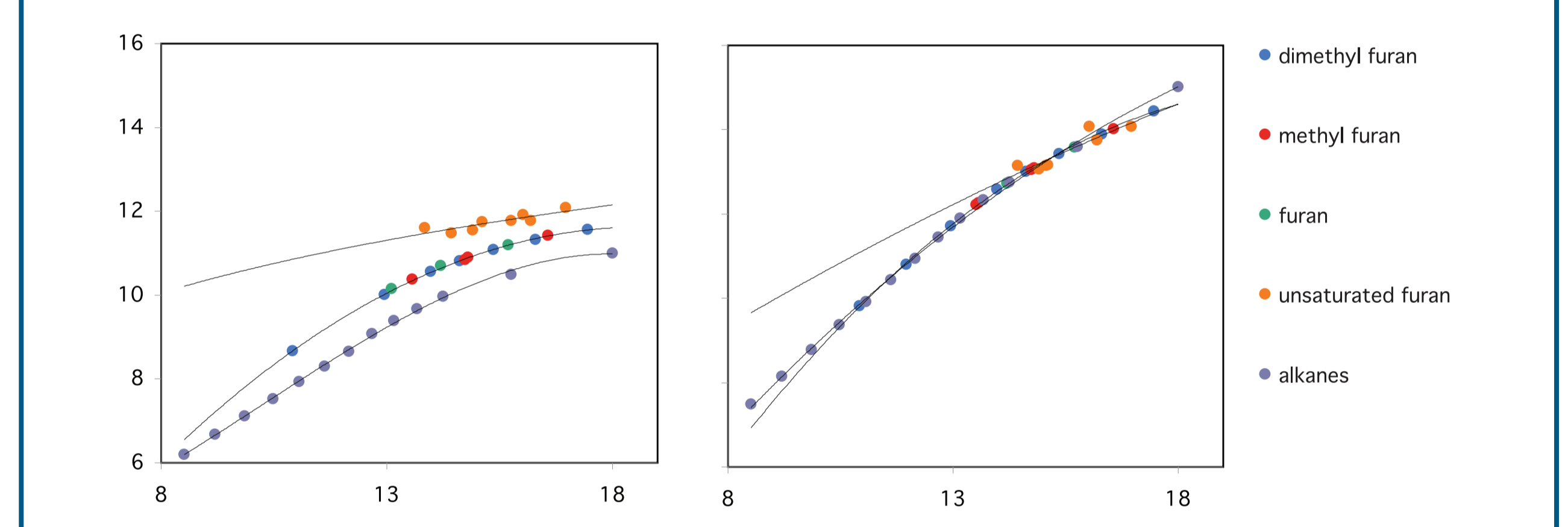


Figure 3: Correlation of retention times for different groups of fatty acid methyl esters on HT8 with retention times on BP5 (left) and BPX90 (right).

chain	furan acid	base	M	R ₁	R ₂	R ₃	n	m	
C12	4,7-epoxy-5,6-dimethylididecanosa-4,6-dienoic acid	179	252	C ₉ H ₁₁	CH ₃	CH ₃	1	0	
	4,7-epoxy-5 or 6-dimethylididecanosa-4,6-dienoic acid	165	238	C ₉ H ₁₁	CH ₃	H	1	0	
	4,7-epoxy-didecanosa-4,6-dienoic acid	151	224	C ₉ H ₁₁	H	H	1	0	
	4,7-epoxy-5,6-dimethylididecanosa-4,6,9-trienoic acid	177	250	C ₉ H ₉	CH ₃	CH ₃	1	0	
C14	6,9-epoxy-7,8-dimethylididecanosa-6,8-dienoic acid	179	280	C ₉ H ₁₁	CH ₃	CH ₃	3	0	
C15	7,10-epoxy-8,9-dimethylididecanosa-7,9-dienoic acid	179	294	C ₉ H ₁₁	CH ₃	CH ₃	4	0	
C16	8,11-epoxy-9,10-dimethylhexadecanosa-8,10-dienoic acid	179	308	C ₉ H ₁₁	CH ₃	CH ₃	5	0	
C17	9,12-epoxy-10,11-dimethylheptadecanosa-9,11-dienoic acid	179	322	C ₉ H ₁₁	CH ₃	CH ₃	6	0	
	9,12-epoxy-heptadecanosa-9,11-dienoic acid	151	294	C ₉ H ₁₁	H	H	6	0	
C18	10,13-epoxy-11,12-dimethyloctadecanosa-10,12-dienoic acid (major)	179	336	C ₉ H ₁₁	CH ₃	CH ₃	7	0	
	10,13-epoxy-11 or 12-methyloctadecanosa-10,12-dienoic acid	165	322	C ₉ H ₁₁	CH ₃	H	7	0	
	10,13-epoxy-11 or 12-methyloctadecanosa-10,12-dienoic acid	165	322	C ₉ H ₁₁	CH ₃	H	7	0	
	10,13-epoxy-octadecanosa-10,12-dienoic acid (major)	151	308	C ₉ H ₁₁	H	H	7	0	
	10,13-epoxy-11,12-dimethyloctadecanosa-10,12,16-trienoic acid	177	334	C ₉ H ₉	CH ₃	CH ₃	7	0	
	10,13-epoxy-11 or 12-dimethyloctadecanosa-10,12,16-trienoic acid	163	320	C ₉ H ₉	CH ₃	CH ₃	7	0	
	10,13-epoxy-octadecanosa-10,12,16-trienoic acid	149	306	C ₉ H ₉	H	H	7	0	
	8,11-epoxy-9,10-dimethyloctadecanosa-8,10,16-trienoic acid	205	334	C ₇ H ₁₁	CH ₃	CH ₃	5	0	
8,11-epoxy-9 or 10-dimethyloctadecanosa-8,10,16-trienoic acid	191	320	C ₇ H ₁₁	CH ₃	CH ₃	5	0		
C19	11,14-epoxy-12,13-dimethylnonadecan-11,13-dienoic acid	179	350	C ₉ H ₁₁	CH ₃	CH ₃	8	0	
	12,15-epoxy-nonadecan-12,14-dienoic acid	151	322	C ₉ H ₁₁	H	H	8	0	
	C20	12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid	179	364	C ₉ H ₁₁	CH ₃	CH ₃	9	0
		12,15-epoxy-13 or 14-methyleicosa-12,14-dienoic acid	165	350	C ₉ H ₁₁	H	CH ₃	9	0
C20	12,15-epoxy-eicosa-12,14-dienoic acid	151	336	C ₉ H ₁₁	H	H	9	0	
	12,15-epoxy-13,14-dimethyleicosa-12,14,7-trienoic acid	179	362	C ₉ H ₁₁	CH ₃	CH ₃	9	1	
	12,15-epoxy-13,14-dimethyleicosa-12,14,18-trienoic acid	177	362	C ₉ H ₉	CH ₃	CH ₃	9	0	
	10,13-epoxy-11,12-dimethyleicosa-10,12,18-trienoic acid	205	362	C ₇ H ₁₁	CH ₃	CH ₃	7	0	
	10,13-epoxy-11 or 12-dimethyleicosa-10,12,18-trienoic acid	191	348	C ₇ H ₁₁	CH ₃	CH ₃	7	0	
	C21	12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid	179	378	C ₉ H ₁₁	CH ₃	CH ₃	10	0
C22	14,17-epoxy-15,16-dimethyldocosa-14,16-dienoic acid (minor)	179	392	C ₉ H ₁₁	CH ₃	CH ₃	11	0	
	14,17-epoxy-15 or 16-methyldocosa-14,16-dienoic acid (minor)	165	378	C ₉ H ₁₁	H	CH ₃	11	0	
	14,17-epoxy-15 or 16-methyldocosa-14,16-dienoic acid (minor)	165	378	C ₉ H ₁₁	CH ₃	H	11	0	
	14,17-epoxy-docosa-14,16-dienoic acid (minor)	151	364	C ₉ H ₁₁	H	H	11	0	

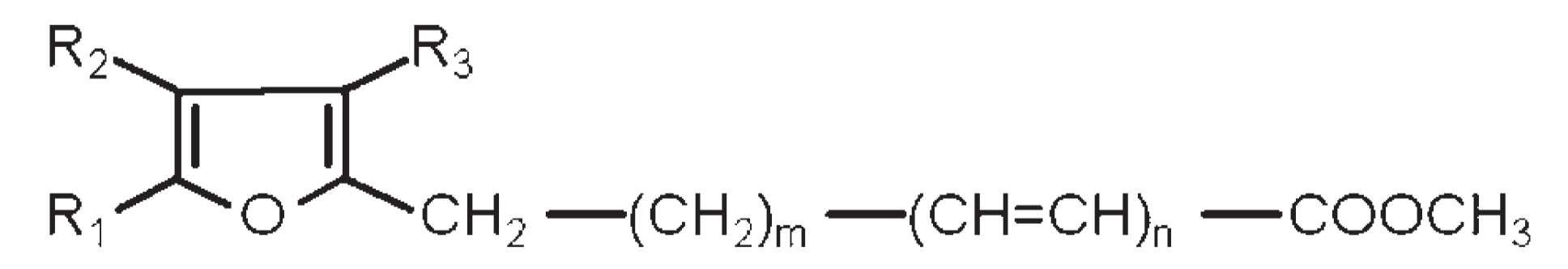


Table 1: The identification of furan fatty acids from carp gonad tissue on the basis of mass spectral characteristics and retention time

Experimental conditions:

Free and bound fatty acids were isolated from head and gonad tissues of the carp and converted to the corresponding methyl ester and pyrrolidinamide derivatives. The mixtures were analyzed by GCMS using capillary columns of identical dimensions but containing different phases (HT8, BPX90 and BP5) operated under identical conditions. All columns were 30 m x 0.25 mm i.d. with a 0.25 μm film thickness. Samples were 2-10 % solutions in hexane. Analysis was performed on a 6890 GC-5973N MSD (Agilent Technologies) fitted with an ETP 14642 electron multiplier. Injection was split 50:1 with a split flow of 65 mL/min at

a temperature of 250 °C. The carrier gas was helium with a nominal flowrate of 1.3 mL/min in constant flowrate mode and a nominal inlet pressure of 10.8 psi. The oven temperature was programmed from 50 °C (held for 2 minutes) to 270 °C (held for 15 minutes) at 20 °C/min. The transfer line was at 280 °C. MS scanned from 50-550 Da at 2.9 scan/sec.

Major furan acids and the saturated fatty acids were located by their characteristic base peaks (m/z 179, 165 and 151 for furan esters and m/z 74 for saturated esters). Individual compounds were identified on the basis of their EI mass spectra and retention times were determined for each compound on the three different columns.