ORGANIC MATTER SULFURIZATION DURING EARLY DIAGENESIS IN FLORIDA BAY

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Abstract

Organic matter (OM) sulfurization is a significant sink for reduced sulfur in marine sediments. The formation of organic sulfur compounds (OSC) during diagenesis has been demonstrated to enhance biomarker preservation, thus affecting molecularly based paleoenvironmental reconstructions. Recent studies have focused on the pathways of OM sulfurization and suggested intermolecular polysulfide incorporation might be an important pathway of formation. This study analyzed several sediment cores from Florida Bay to determine whether OSC were formed in recent sediments. Polysulfide cleavage was performed on samples from different depths and sites within Florida Bay in order to break the S-S cross-linked bonds, if present. The results confirmed that organic sulfur compounds formed in shallow sediment, and that they had different profiles with depth among cores.

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Chapter 1: Introduction

Previous research has revealed that the incorporation of reduced sulfur into organic matter is a quantitatively significant sink in the ocean, second only to formation of pyrite (Werne et al., 2004 and references therein). Organic sulfur (OS) has been shown to influence petroleum formation and quality, the coupled global biogeochemical cycles of carbon, sulfur and oxygen, sedimentary microbial activity, and organic matter preservation and molecularly based paleoenvironmental reconstructions (Meyers 1997). For example, OS can influence both the molecular weight distribution of sedimentary organic matter and its subsequent fate, by reacting with certain molecules and preserving them against diagenetic decay (Brassell et al., 1986). Furthermore, the reactions of inorganic sulfur with organic matter may affect the hydrocarbon composition of sediments and oils (Sinninghe Damsté and de Leeuw, 1990; Orr and Sinninghe Damsté, 1990). Finally, reconstruction of paleoenvironments is based in part on the analysis of organic compounds preserved in marine and lacustrine sediments. In addition to well-known biomarkers such as n-alkanes, isoprenoids, steroids, and hopanoids, organic sulfur compounds (OSCs) have also drawn increasing attention for this purpose.

At present, it is generally thought that OSCs are mainly formed by incorporation of inorganic sulfur into organic matter during early diagenesis in the upper layers of sediments. This is apparent from the occurrence and structure of non-biogenic organic sulfur compounds in shallow sediments (Francois, 1987; Brassell et al., 1986, Werne et al., 2000; Kok et al., 2000). The sulfurization process involves structural

rearrangements that stabilize functionalized lipids and protect organic compounds against further degradation. For example, incorporation of sulfur into double bonds of steroid side chains leads to cyclic thiophenic moieties that are much more resistant to diagenetic alteration than the precursor double bonds (Kohnen et al., 1993).

There are four factors that determine the extent of formation of organic sulfur compounds. First, an anoxic environment is required. Inorganic sulfide is the ultimate source for all reduced sulfur which is incorporated into organic matter to form OSC, though it may proceed via other reactive intermediate sulfur compounds, such as polysulfides or thiosulfate. While sulfide can be oxidized via many pathways (e.g. the source(s) of oxidation could be diffusion from the overlying water column, nitrate, (iron) oxide or oxyhydroxide minerals, or even microbial activity), the most common is direct reaction with oxygen. Therefore, in order to avoid sulfide being re-oxidized into sulfate, a low-oxygen environment is required. In addition, with the presence of oxygen, organic matter will be mineralized by heterotrophic bacteria, leading to lower organic matter concentration in sediments, further reducing the potential for organic matter sulfurization (see below). Second, sufficient sulfate must be present to produce sulfide via (bacterial) sulfate reduction. As sulfate is the major sulfur-containing component in the ocean, sulfate availability is typically not a limiting factor for sulfate reduction in marine systems. In contrast, in lacustrine systems, sulfate is typically present in low concentrations and could be consumed rapidly, resulting in low amounts of sulfide produced. Third, sufficient reactive organic matter must be present, not only to react with inorganic sulfur, but organic matter is also the major substrate providing energy for

dissimilatory sulfate reduction by bacteria (Jorgensen, 1982; Canfield, 1989; Aizenshtat et al., 1999). Finally, reactive iron minerals have been shown to out-compete organic matter to react with sulfide (Gransch and Posthuma, 1974; Canfield et al., 1992, 1996; Jøgensen, 1982). Thus, organic sulfur compounds of high amount are not expected in iron-rich sediments, unless there is sufficient excess sulfide to react with both iron and organic matter (Aizenshtat 1983; Bates et al., 1995; Brüchert and Pratt, 1996; Urban et al., 1999; Filley et al., 2002).

The mechanism(s) of formation of organic sulfur remain(s) incompletely constrained. One reason is that various organic sulfur compounds are found in different environments, suggesting that multiple formation pathways exist, making it difficult to trace probable mechanisms (Werne et al., 2004). It is known that sulfur can be incorporated into organic matter intramolecularly to form low-molecular-weight OSC (Hartgers 1997; Wakeham 1995; Putschew et al. 1995), or via intermolecular reactions, resulting in macromolecular interconnected organic compounds linked by C-S_x-C bonds (x = 1 mono sulfur, x > 1, then polysulfide). Studies have shown that high-molecular-weight acyclic (poly)sulfides were formed by insertion of inorganic sulfur into functionalized lipids, cross-linking different molecules in an intermolecular mechanism (Filley 2002; Eglinton et al. 1994).

The presence of monosulfide and polysulfide-linked lipids in recent sediments is often determined through the use of strong chemical desulfurization reagents, such as activated Raney nickel (Wakeham et al., 1995; Filley et al., 1996) or nickel boride (Schouten et al., 1993), that cleave all C-S bonds in the sample. These procedures release sulfur-bound

biomarkers, which are then analyzed as saturated hydrocarbons, in some cases deuterating the site of sulfur attachment (Sinninghe Damsté et al., 1988; Schouten et al., 1993). Other types of reagent, including methyl lithium/methyl idiode (MeLi/MeI) (Kohnen et al., 1991; Schouten et al., 1995), $LiAlH_4$ (Adam et al., 1991; Schaeffer et al., 1995) and EtS-Na+/MeI (Adam et al., 2000), selectively cleave S-S bonds, leaving sulfur atoms attached to the biomarker. This reaction therefore not only provides direct molecular evidence of polysulfide linkages, it also allows the potential analysis of the sulfur isotope composition of the remnant sulfur atoms in the OSC released, potentially providing even more information about the pathway(s) of diagenetic sulfurization of organic matter.

The purpose of this study is to uncover the occurrence and distribution of organic sulfur compounds in recently deposited sediments. We present the results of laboratory polysulfide cleavage experiments on samples collected from five different sites in Florida Bay, identifying the pools of sedimentary organic matter that contain the highest concentrations of OSC. The focus of these experiments was on the formation of sulfur-containing high molecular weight organic matter and comparisons among different polar fractions of OM, in order to increase our understanding of the mechanism(s) of sulfurization.

Chapter 2: Methods

2.1 Site Description

Florida Bay (Fig. 1) is a shallow lagoon with an average depth of less than 3m, which covers approximately 1,100 square miles (2,850 square km) between the southern tip of Florida and the Florida Keys. It is located on a shallow shelf where freshwater from the Everglades mixes with the saltwater from the Gulf of Mexico. Inflow of fresh water into Florida Bay occurs through sheet flow across the southern Everglades.

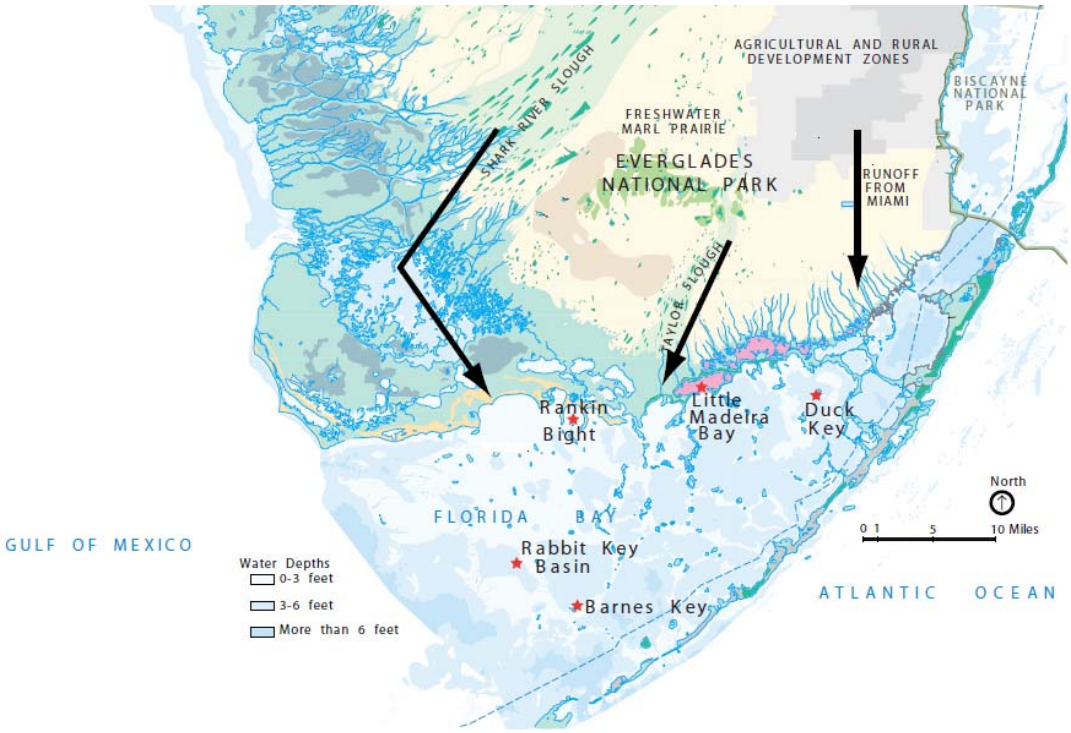

Fig.1. Location maps of five sites. Map was obtained from the web http://www.nps.gov/ever/planyourvisit/loader.cfm?csModule=security/getfile&PageID=324396.

Of particular interest to this effort, Florida Bay possesses favorable conditions for the formation of organic sulfur compounds. First, there is an abundant supply of reduced sulfur, in the form of dissolved sulfides. Up to several mM sulfide has been reported in pore waters (Lyons et. al, 2004). Second, this is a carbonate mud depositional system, so there is little iron present, with typical sediment concentrations of 70-100 ppm (Rude and Aller, 1991, Brown and Cohen, 1995). The low iron concentrations reduce the possibility of significant pyrite formation, thereby increasing the likelihood of abundant organic sulfur formation. Organic carbon concentrations from previous studies demonstrate a high level of organic carbon content in some Florida Bay sites, necessary to support bacterial sulfate reduction.

Samples were taken by push core from an array of sites representing different organic carbon and sulfide concentrations, as well as contrasting hydrological inputs and varying vegetation, primary productivity, and water depth. This was done not only to get an adequate sampling of the bay, but also to gain insight into the causes of potential variability found among cores. Samples were collected from five cores taken from Florida Bay (see Figure 1 for locations): Rankin Bight (FBT2-8-1, 0-40 cm), Duck Key (FBT2-25, 0-35 cm), Rabbit Key (RKB-2, 0-34 cm), Barnes Key (FBT2-18A-1, 1-40 cm) and Little Madeira Key (0-41 cm). Sixteen samples from the five cores were chosen to perform polysulfide cleavage based on OC% and total sulfur content (listed below in Table 1).

2.2 Sample Treatment

2.2.1 Carbon Concentration

Sediments were heated in an oven at 60 °C overnight to remove any remaining water and homogenized; aliquots of dry sediment were taken for total carbon (TC) and total inorganic carbon (TIC) analysis by UIC Model 5014 Carbon Coulometer. Total organic carbon content was determined by difference between TC and TIC (TOC = TC - TIC) (Marlow et al., 2001). Typically, 12 mg of each sample was placed in a vessel, weighed and the inorganic carbon was volatilized with 5 ml 2N HCl in an acidification module. For TC measurements, another 12 mg of each sample was placed into a small platinum boat which was then fired in a CM 5300 furnace apparatus at 950 °C to oxidize all the carbon present. In both cases, CO_2 was produced and measured by titration in a carbon coulometer equipped with an infra-red detector.

2.2.2 Sulfur Concentration

Total sulfur (TS) measurements were made with a UIC Model 3200 sulfur coulometer system. Samples were covered with vanadium pentoxide (V_2O_5) and combusted in the presence of oxygen at 1050 °C. Evolved gases pass through a column of reduced Cu to quantitatively convert all sulfur to SO_2^-. Inorganic sulfur (total sulfide, or CRS: chromium reducible sulfur) was performed using standard chromium reduction with iodometric titration on extracted samples selected from each of the five cores in the laboratory of Tim Lyons at the University of California Riverside following methods in

Werne et al. (2004). Total organic sulfur content (TOS) was determined by difference (TOS = TS - CRS).

2.2.3 Sulfur isotope measurements

Additional sample aliquots were extracted via chromium reduction, but were trapped as Ag_2S for isotope measurement (of reduced inorganic sulfur, i.e., pyrite) using ~30 ml 3% $AgNO_3$ with 10% NH_4OH (Newton et al., 1995). The residues after chromium reduction were filtered and dried in a dessicator in preparation for isotopic measurement of remaining sulfur, which was assumed to be total organic sulfur. The CRS and TOS were then placed in tin boats with V_2O_5 catalyst and analyzed for their sulfur isotope compositions by the continuous flow EA-IRMS method described in Werne et al. (2004) in the Lyons lab.

2.3 Total Lipid Extraction (TLE) and Fractionation

Bulk sediments were extracted using a mixture of DCM/MeOH (9:1 v/v) in a Soxhlet apparatus for 24 h to obtain the total lipid extract (TLE). After solvent removal by evaporation, the TLE was split into 3 aliquots, two of them (TLE-1 & TLE-2) were further separated for analysis, and the remainder (TLE-3) was archived.

Our separation scheme (Fig. 2) was designed to determine which pool of organic matter contained the greatest amount of organic sulfur, and to minimize the chance that organic sulfur was lost during sample work-up. The separation scheme resulted in four fractions, named F1, F2, F3 and F4. Each fraction contains one apolar, one polar I and one polar II

subfraction (separated using alumina column chromatography, see below). Apolar fractions were analyzed via gas chromatography directly, however, polar I fractions were first derivatized (see below) before running on GC and GC/MS, and in most cases, polar II subfractions were archived for future analysis. The only exception is F1-polar II, which was treated with MeLi/MeI for chemical degradation.

Fraction F1 came from TLE-1. It was first separated via alumina (Al_2O_3) column chromatography, eluting successively with hexane/DCM (9:1 v/v) to obtain the F1-apolar, DCM/MeOH (1:1 v/v) to obtain the F1-polar I, and MeOH to obtain the F1-polar II. F1-polar I was then split into two equal portions, F1-polar Ia and F1-polar Ib. After derivatization, F1-polar Ib was analyzed by GC, while F1-polar Ia samples were subjected to polysulfide cleavage as described below (Kok et al., 2000).

Fraction F2 came from TLE-2, which was treated with MeLi/MeI first to cleave polysulfide bonds in the TLE, and then separated into F2-apolar, F2-polar I, and F2-polar II by alumina column chromatograph as described above.

Fraction F3 came from F1-polar Ia. This fraction was treated in the same way as F2 (polysulfide cleavage followed by alumina column chromatography) and then resulted in F3-apolar, F3-polar I, and F3-polar II after the second alumina column chromatography separations.

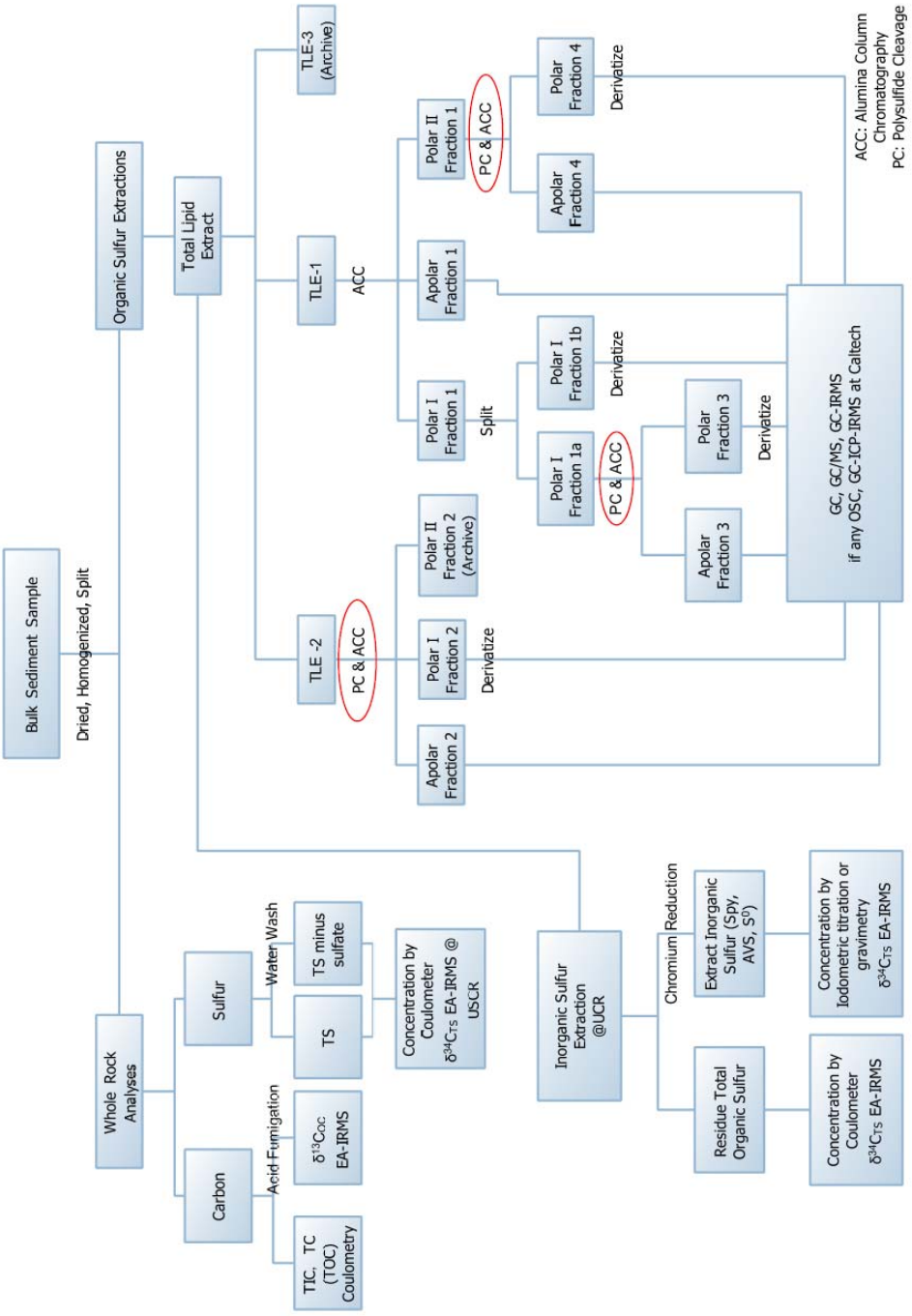

Fig. 2. Flow chart of the whole research project, which includes bulk sediment treatment, separation and analysis.

Fraction F4 came from F1-polar II, which was subjected to polysulfide cleavage and subsequent alumina column chromatography, resulting in F4-apolar, F4-polar I, and F4-polar II.

All of the polar I fractions (F1-F4) were derivatized to trimethylsilyl ethers before GC analysis to prevent GC column degradation. Normally, 100 µl of bis-(trimethylsilyl) trifluoroacetamide (BSTFA) and 100 µl of acetonitrile (stored over Na_2SO_4) were injected into each 4ml vial that containing dry sample (polar I) under N_2 flow. Vials were then placed in a heating block for 2 hours at 60-70 °C, after which they were cooled, blown dry under N_2, and redissolved in solvent prior to GC analysis.

2.4 Polysulfide Cleavage

In our study, MeLi/MeI was chosen as the polysulfide cleavage reagent, which protected carbon skeletons and left a sulfur atom attached to the compounds bound via sulfurization (see mechanism below). Typically, a glass tube was sealed and nitrogen gas (N_2) flow was equipped to remove air/H_2O. Approximately 20 mg polar I or polar II fraction was dissolved in 2 ml diethyl ether at room temperature, 3 ml MeLi was added into the solution while stirring, and 100 μl of MeI were added after 5 min. The tube was then transferred into an ice-water bath. After 15 min, the mixture was quenched with MilliQ water (3 ml) and extracted with hexane three times. The hexane extracts were combined and dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness under N_2. The cleavage products were separated using alumina column chromatography as described above. After transfer into GC vials (1 ml), separated fractions were dissolved in 100 μl ethyl acetate (EA), subsequently analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) as described below.

The mechanism of polysulfide cleavage is shown in Fig. 3, that described by some references (Kohnen et al, 1991; Adam et al, 2000), in polysulfide organic matter R1-C-S-Sn-S-C-R2 (n=0, 1, 2 …) (Scouten et al, 1993). Briefly, MeLi broke the S_n-S bond and a methyl group was attached to the R_1-C-S-S_n group, forming R_1-C-S-Sn-CH_3, while a lithium was added to R_2-C-S moiety and became R_2-C-S-Li. After each cleavage step, one -S-CH_3 and one -S-Li group were formed. Excess MeLi continued to split S-S bonds in R_1-C-S-S_n-CH_3 until no S-S bonds remained, with the major products being R_1-C-S-CH_3, R_2-C-S-Li and CH_3-S-CH_3. When MeI was added, lithium was replaced by a methyl

group, resulting in the formation of R_2-C-S-CH_3 and LiI. Thus, the final products were R_1-C-S-CH_3, R_2-C-S-CH_3, CH_3-CH_3 and LiI. LiI was removed by cold water and alumina column chromatography, and CH_3-S-CH_3 (dimethyl sulfide) was removed by N_2 evaporation. Thus only R_1-C-S-CH_3 and R_2-C-S-CH_3 remained for analysis via GC-FPD and GC/MS, and the original carbon skeletons of the sedimentary organic compounds were maintained.

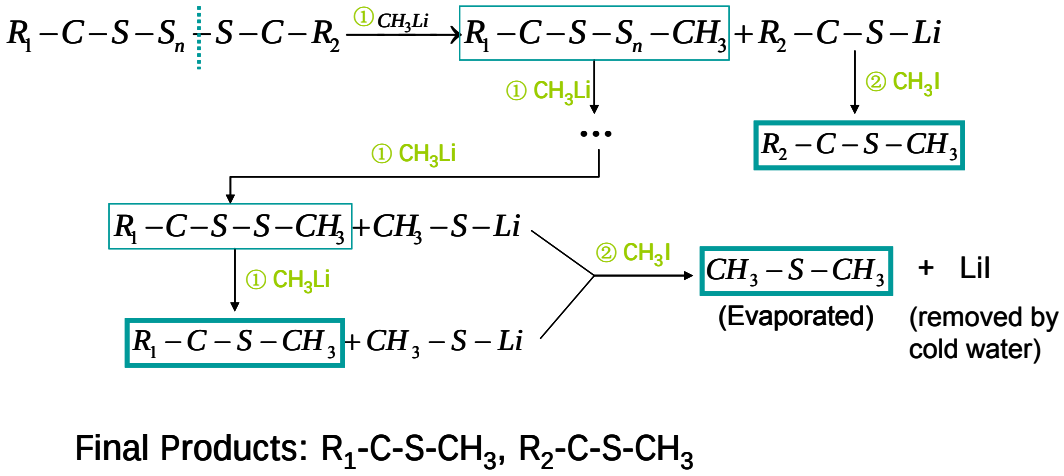

Fig. 3. Mechanism of the polysulfide bond cleavage using MeLi/MeI

2.5 Gas Chromatography and Mass-Spectrometry

2.5.1 Gas Chromatography (GC)

GC analyses were carried out on an Agilent 6890 gas chromatograph equipped with a split-splitless injector, a flame ionization detector (FID), a sulfur-selective flame photometric detector (FPD) and an HP1 fused silica capillary column (30m x 320μm, 0.25 μm film thickness). Helium was used as carrier gas (2.6 ml/min). The samples

(dissolved in ethyl acetate) were injected at 50 °C and subsequently the oven was programmed to 130 °C at 10 °C/min and then at 4 °C/min to 320 °C at which it was held for 10 min. For quantification an internal standard (5α-androstane) was injected into fractions. All organic sulfur compounds were assumed to have a similar response ratio relative to the internal standard. The concentrations of individual compounds were determined by quantification of the GC peak area (by FID) relative to that of the internal standard.

2.5.2 Elemental Sulfur Removal

After GC analyses, the total lipid extracts were treated with activated copper to remove elemental sulfur in order to avoid interference with GC-MS analysis. Copper beads were activated by adding small amount of 2N HCl, then decanted and rinsed with MilliQ water, and finally washed three times each with DCM, acetone and hexane. Excess activated and cleaned copper beads were added to the vials containing the extracts in DCM, and kept overnight. Blackening of the copper indicated that elemental sulfur was present in the sample. Each extract dissolved in DCM was then filtered over a cotton-wool plugged pipette to remove the copper and was subsequently evaporated to dryness under N_2.

2.5.3 Gas Chromatography-Mass Spectrometry (GC-MS)

Structures of organic sulfur compounds were determined using an Agilent 6890 gas chromatograph interfaced to an Agilent 5973 mass spectrometer operated at 70 eV with a mass range m/z 50-650. GC conditions were as above, except the carrier gas flow rate

was 2.0 ml/min. OSC were identified where possible based on retention time and comparison of mass spectra with the published literature.

Chapter 3: Results

3.1 Bulk Sediment Carbon and Sulfur

Table. 1. Bulk total organic carbon (TOC) and total sulfur concentration (TS), OSC abundance (mg/g TLE) and elemental sulfur (S^o) in F1-apolar (AP), F1-polar I (PI TMS) and F2-apolar samples.

Florida Bay	Depth/cm	Label	TOC%	TS%	F1 AP		F1 PI (TMS)		F2 AP
					OSC (mg/g)	S°	OSC (mg/g)	S°	OSC (mg/g)
Duck Key FBT2-25	5-6	DK(1)	2.62	0.198		√			
	25-26	DK(2)	4.04	0.274		√		√	194.7
	34-35	DK(3)	37.3	0.973		√	2.1		6
Rabbit Key RKB-2	5-6	RK(1)	4.52	0.205		√			
	15-16	RK(2)	4.49	0.306		√			0.6
	25-26	RK(3)	2.34	0.410		√			10.1
Barnes Key FBT-2 18A-1	5-6	BK(1)	2.10	0.571*		√			
	19-20	BK(2)	1.36	0.507*		√		√	2.9
	34-35	BK(3)	2.66	0.490*		√		√	14.9
Rankin Bight FBT-2-8-1	6-8	RB(1)	4.01	0.662*		√		√	53.4
	16-18	RB(2)	3.98	n/d	51.3	√			n/d
	22-24	RB(3)	2.40	0.556*	16.7	√			43.6
	34-36	RB(4)	2.47	0.526*	**	√	**	√	99.9
Little Madeira	5-6	LM(1)	2.05	0.202		√			**
	20-21	LM(2)	1.15	0.160		√		√	
	30-31	LM(3)	1.02	0.130		√			

√ Sulfur signal was detected by FPD.
* Total Sulfur Data was measured at another depth but close to the one we were looking at.
** OSC could be detected in this samples but was too low be quantified by FID.
n/d: not determined.

According to TOC profiles (Fig. 4), these five cores analyzed could be generally classified into two groups: Rabbit Key, Rankin Bight and Little Madeira have the same TOC profile with the maximum value at the surface (RKB-2 5.23%, FBT-2-8-1 4.62%

and Little Madeira 3.10%), decreasing with depth. In contrast Duck Key and Barnes Key had constant TOC values (FBT2-25 1.40-2.70% and FBT-2 18A-1 1.90-3.10% respectively) with little fluctuations, though it should be noted that Duck Key TOC and TS (detailed in next section) increased significantly below 20 cm. The Rankin Bight core was taken in approximately three feet of water in a region covered with seagrass.

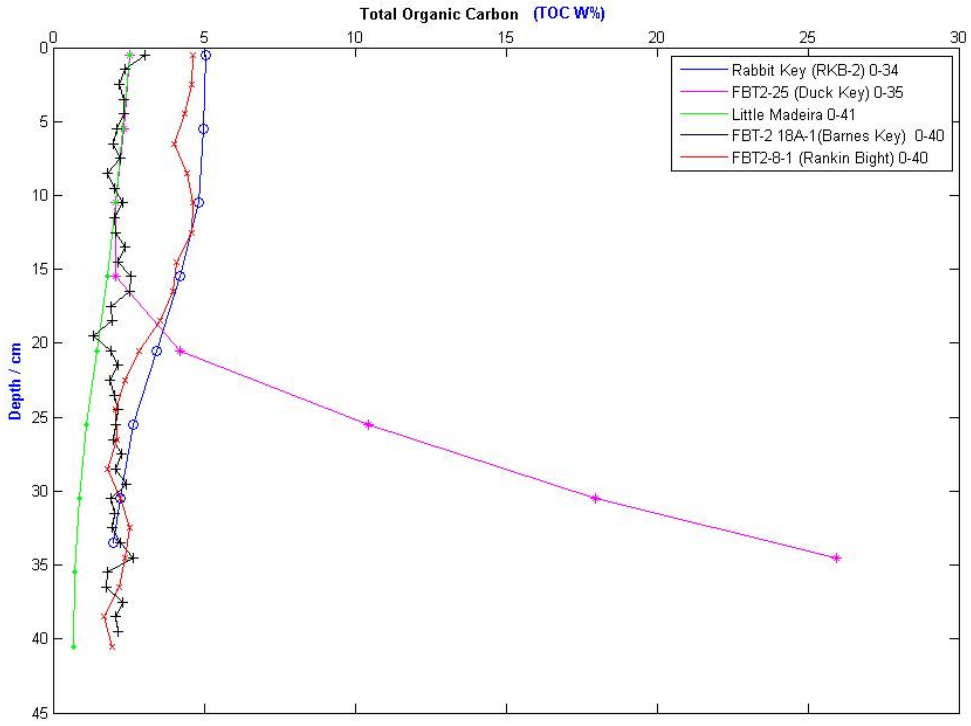

Fig. 4. Plot of TOC values from five cores of Florida Bay

Compared with TOC% values, TS% (Fig. 5) had more remarkable shifts. TS% varies between 0.10% and 0.97%. At the Duck Key site, TS and TOC shared similar curves and both had larger values than that of other cores at depth. Little Madeira had minimum sulfur concentration compared with other cores.

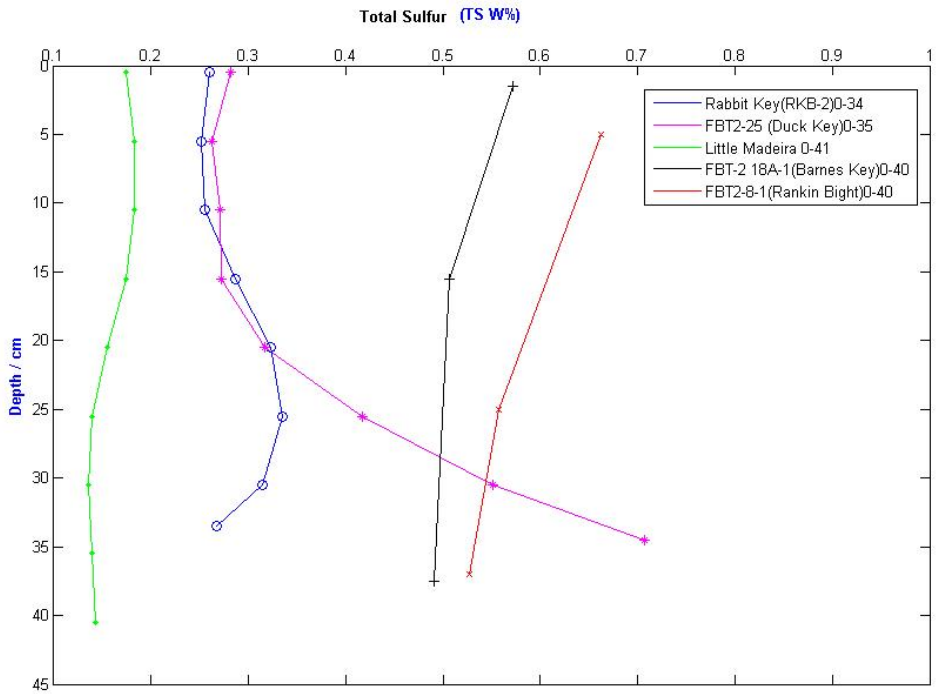

Fig. 5. Plot of TS values from five cores of Florida Bay

3.2 Organic Sulfur Compounds

Several samples from F1-apolar, F1-polar I and F2-apolar showed evidence of organic sulfur compounds on the GC-FPD (Table. 2). Most sulfur signals in the F1-apolar and F1-polar I fractions indicated to elemental sulfur (Fig. 6). In F2-apolar samples, there are three prominent peaks eluting before 40 min, 44 min and 47 min with sulfur responses on the FPD (Fig. 7). Most OSCs were present in deeper sediments at higher abundances, except in Little Madeira F2-apolar samples, where organic sulfur was detected in surface sediment (5-6 cm), but not at greater depths (20-21 cm and 30-31 cm). However, this

observation was in accordance with the Little Madeira TS profile, showing higher sulfur concentration at 5-6 cm than at 20-21 and 30-31 cm. At Duck Key (FBT2-25), the F2-apolar sample at 25-26 cm had the strongest sulfur signals, while sulfur signals in samples at 5-6 and 34-35 cm were both negligible according to the abundance (Fig. 8-10).

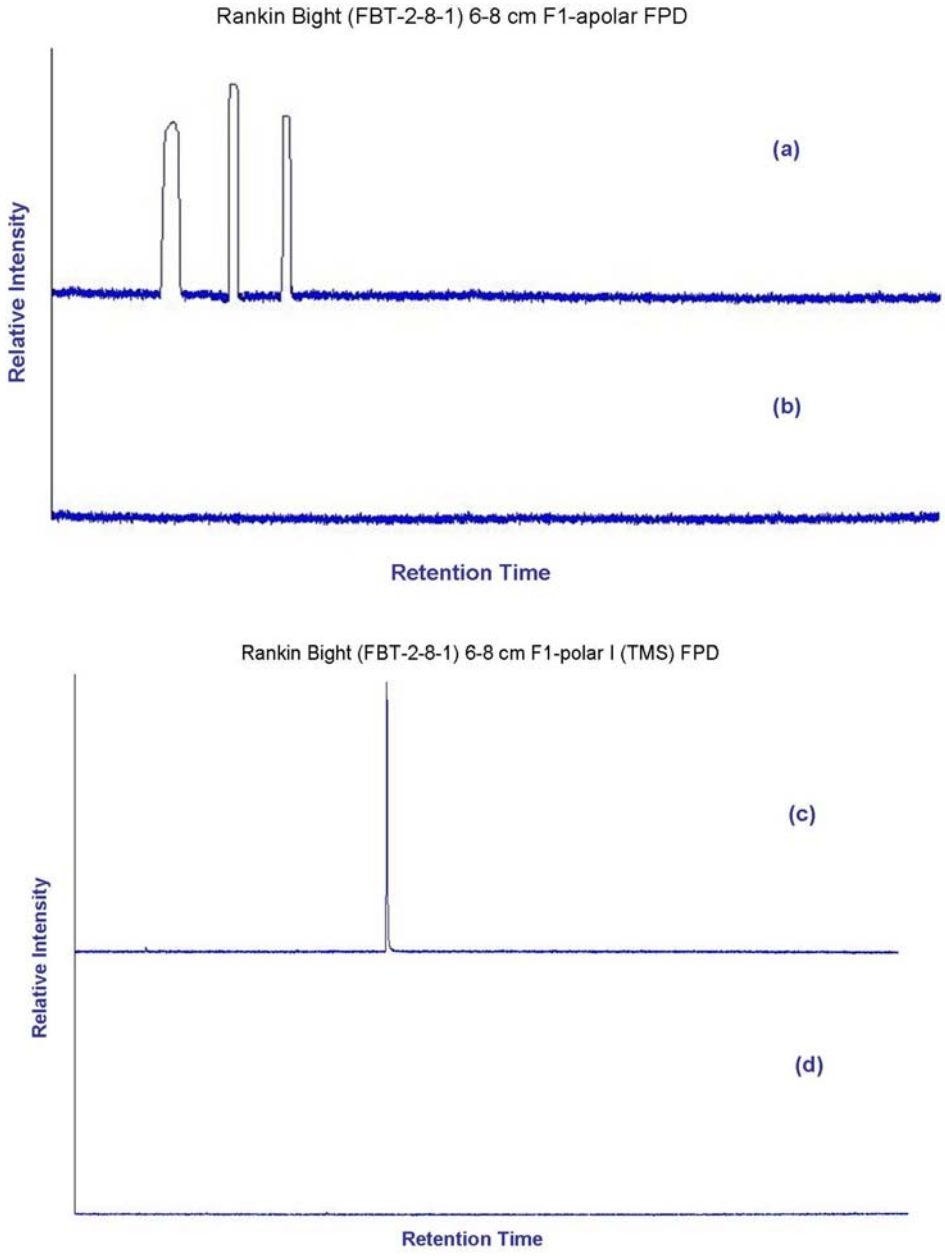

Fig. 6. Chromatogram from FPD Detector of Rankin Bight (FBT-2-8-1) 6-8 cm F1-apolar and F1-polar I (TMS), (a)&(c): F1-apolar and F1-polar I (TMS) before elemental sulfur removal, (b)&(d): F1-apolar and F1-polar I (TMS)after elemental sulfur removal.

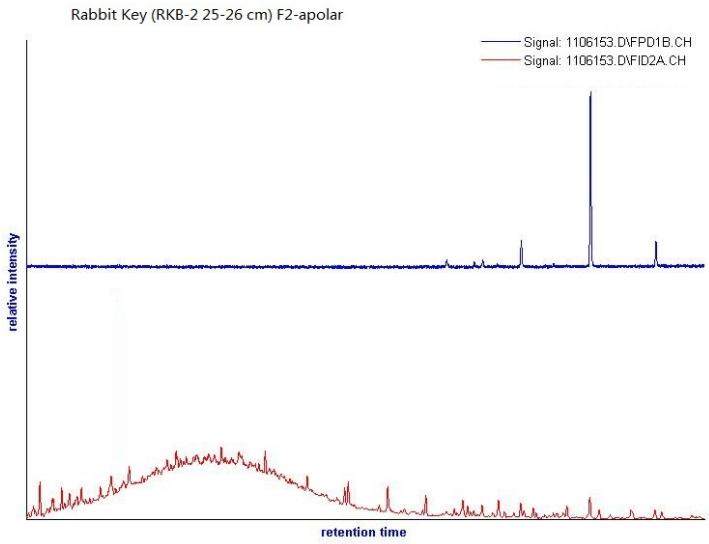

Fig. 7. GC-FID/FPD chromatograph of Rabbit Key (RKB-2 25-26 cm) F2-apolar, three prominent peaks were detected by FPD at 40, 44 and 47 min.

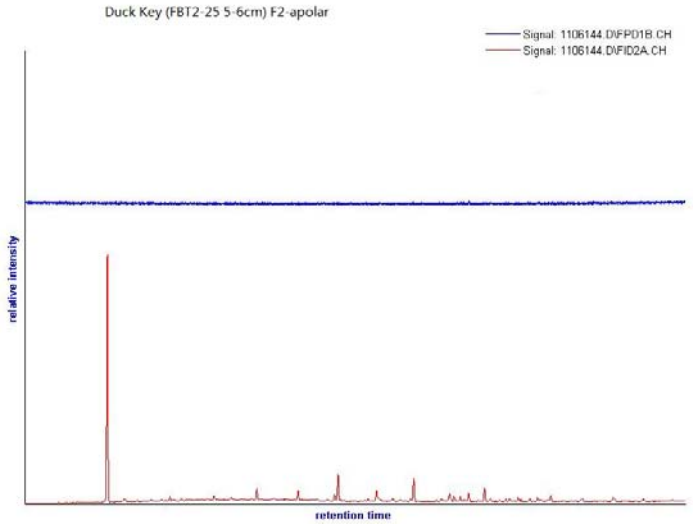

Fig. 8. GC-FID/FPD chromatograph of Duck Key (FBT2-25 5-6 cm) F2-apolar, no obvious peak was detected by FPD.

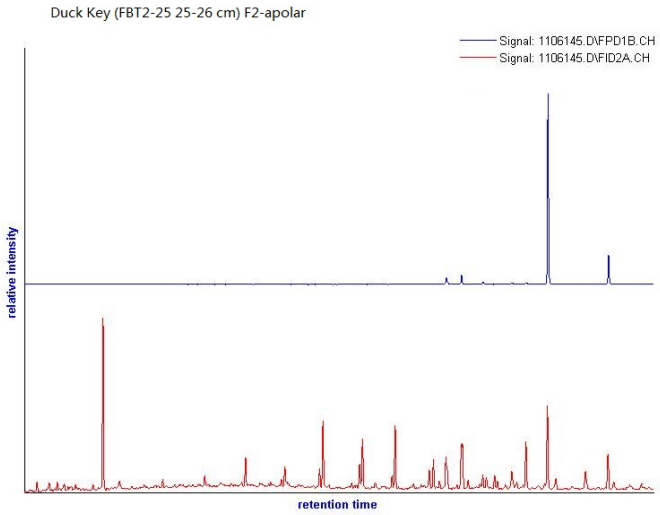

Fig. 9. GC-FID/FPD chromatograph of Duck Key (FBT2-25 25-26 cm) F2-apolar, which has the strongest sulfur signals and more peaks than that of 5-6 cm from the same key.

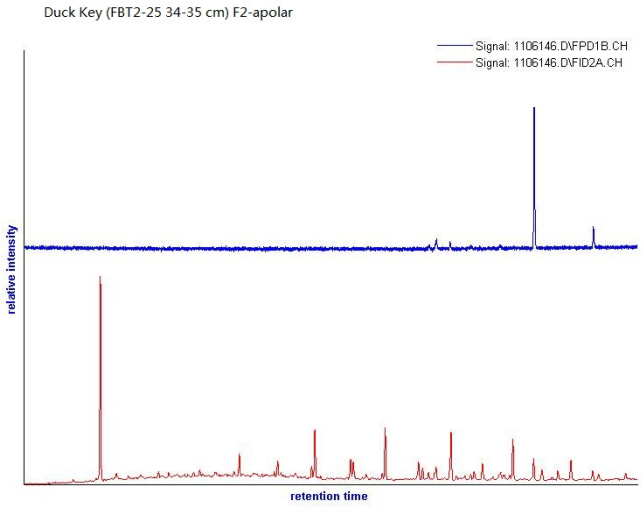

Fig. 10. GC-FID/FPD chromatograph of Duck Key (FBT2-25 34-35 cm) F2-apolar, whose peaks were found at similar retention time to that of 25-26 cm, but with weaker abundance.

Most organic sulfur compounds were quantified, except F2-apolar 5-6 cm from Little Madeira, F1-apolar 34-36cm from Rankin Bight (FBT2-8-1) and F1-polar I 34-36cm from Rankin Bight (FBT2-8-1), which we were unable to quantify due to their low abundance of OSC (less than 0.1 mg/g). In F2-apolar samples, the OSC abundance appears to be increasing with depth in all cores except Duck Key (FBT2-25) in which the abundance reached its maximum at 25-26 cm and then decreased significantly.

The most common organic sulfur compound released upon polysulfide cleavage eluted in GC-FPD analysis at about 43.77 min, which corresponded to 44.1 min in the GC/MS total ion chromatogram (TIC). This is the most abundant OSC in Rabbit Key (RKB-2) and Barnes Key (FBT-2 18A-1). In Duck Key (FBT2-25) and Rankin Bight (FBT-2-8-1), the most abundant OSC was detected at 39.6 min on FPD (corresponding to 39.9 min on GC/MS). The third most frequent peaks were at 46.75 min (47.1 min on GC/MS) in every core. Additional, but minor, OSC at 38.84 min were present in three cores: Duck Key (FBT2-25 34-35cm), Rabbit Key (RKB-2 25-26cm) and Rankin Bight (FBT-2-8-1 6-8, 22-24cm). No OSC were quantified from Little Madeira samples according to their poor abundances.

Table. 2. F2-apolar OSC Abundance at different retention time.

	Retention Time/min	39.59	43.77	42.72	38.84	38.78	46.75	42.01	40.59	38.46	37.33	Total
Duck Key FBT2-25	**25-26**	51.81	47.99	28.68		27.50	22.03	9.76	6.96			194.74
	34-35	3.00	1.20		1.05		0.75					6.00
Rabbit Key RKB-2	**15-16**		0.61									0.61
	25-26		4.16		2.40		1.98			38.459		47.00
Barnes Key FBT-2 18A-1	**19-20**		2.91									2.91
	34-35		5.84				4.59		4.43			14.86
Rankin Bight FBT-2-8-1	**6-8**	18.42	13.06		9.21		6.14		6.52			53.36
	22-24		13.63		7.82		7.93		11.43	2.81		43.63
	34-36	33.08	31.03				17.59		11.56		6.66	99.92

3.3 Organic Sulfur Compound Identification

OSC at 39.9, 44.1 and 47.1 min (in the TIC) were chosen for GC/MS identification because they were present in the highest abundance, and most consistently among the cores.

The prominent OSC at 39.9 min (Fig. 11) was difficult to identify, we refer to it as "unknown OSC". It has a base peak presented at m/z 57, followed by fragment ions at m/z 71, 85. Other prominent peaks were at m/z 145, 213, 255, 275 and 396.

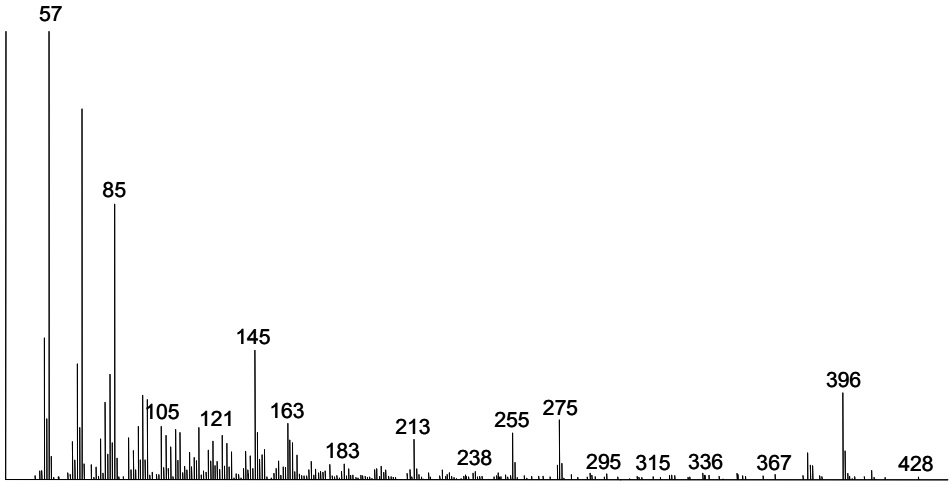

Fig. 11. Mass spectra of OSC frequently found in F2-apolar samples at 39.9 cm.

Two methylthioether compounds were identified from their mass spectra in the TIC. The one eluting at 44.1 min is identified as 1, 2-bis(methylthio)pentacosane ($C_{27}H_{56}S_2$, molecular weight (MW) 444). This OSC was the most abundant compound, and was present in all samples that contained polysulfides. It was identified based on a base peak at m/z 383. This OSC showed a small the molecular ion, and minor fragments at m/z 57 and 87.

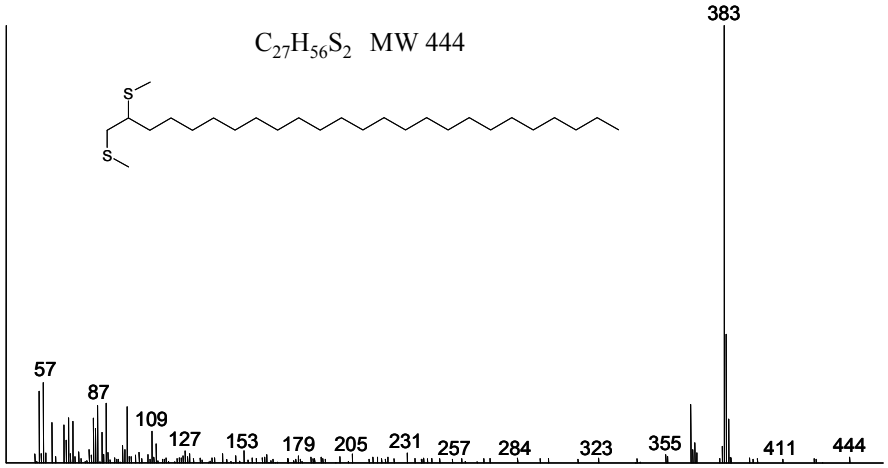

Fig. 12. Mass spectra of OSC frequently found in F2-apolar samples at 44.1 min and its structure.

The OSC eluting at 47.2 min is tentatively identified as 1, 2-bis(methylthio) heptacosene ($C_{29}H_{56}S_2$, MW 470). At this point, we have not been able to identify the positions of the two double bonds. This compound was the third most abundant OSC in our samples. The most significant peak presented at m/z 411 in the mass spectra; besides, there are no other significant peaks including its molecular ion (M^+ 470).

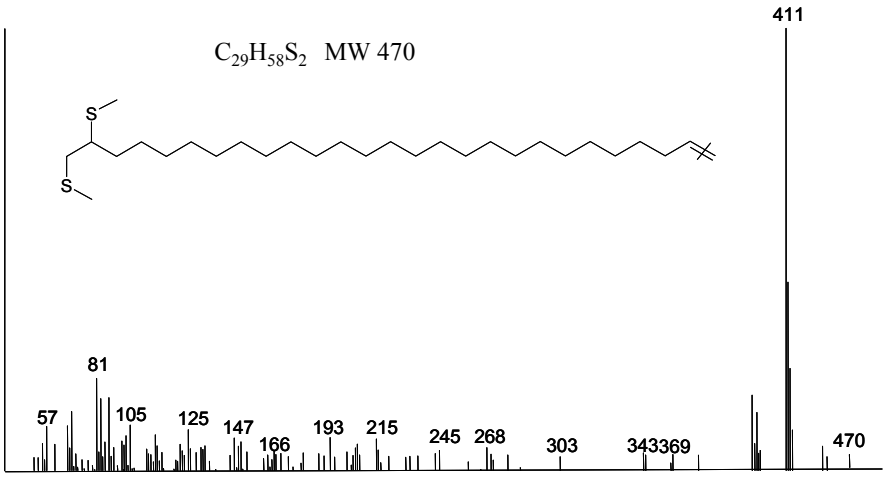

Fig. 13. Mass spectra of OSC frequently found in F2-apolar samples at 47.2 min and its expected structure. There is a double bond in this compound, but we don't know the exact position.

Chapter 4: Discussion

The presence of plant matter is likely the cause of considerably higher organic carbon values near the surface. In contrast, the Barnes Key core (FBT-2 18A-1) was taken in less than one foot water depth, and is thus likely to be very well mixed. Additionally, this core was taken from a region in which there was no seagrass growing (Lyons, 2004).

4.1 Organic sulfur compound occurrence:

FPD signals in some samples from apolar and polar I fractions from F1 and F2 indicated that organic sulfur compounds were generated in early stage diagenesis, since the samples were collected from shallow cores (less than 45 cm) and OSC were observed in the surface samples, such as Barnes Key (FBT 2-8-1) 6-8 cm.

There are some possibilities to explain why no sulfur was detected in many samples. First, it is possible that sulfur incorporation into the compounds in some fractions only occurs on much longer timescales. Thus, in very surface samples sulfur hasn't been incorporated into organic matter yet. Second, MeLi/MeI was utilized as cleavage reagent, which only breaks S-S bonds. This reagent would not release OSC from macromolecules containing a single sulfur atom bridge, and therefore some sulfur-containing molecules might have remained out of the analytical window of our procedure. Third, our objective was to investigate the organic sulfur compounds in total lipid extract. It is quite possible that there are some organic sulfur compounds in the kerogen portion, which was not included in this study.

F1 samples were fractionated by alumina column chromatography first, and then went through polysulfide cleavage, whereas F2 samples were fractionated with alumina column chromatography after S-S bond breakage (Fig. 14). GC-FID/FPD chromatograms indicated that organic sulfur compounds were mostly found in F2-apolar samples, while in most F1 apolar and polar I samples primarily elemental sulfur was detected. This indicates that most of the organic sulfur compounds were released from macromolecules in the total lipid extract that are lost in alumina column chromatography. Furthermore, these compounds are clearly linked by polysulfide bonds. Elemental sulfur in these samples was most likely through (partial) oxidation of the abundant sulfide present, particularly as these are shallow sediments, with strong potential for periodic oxidation during storms.

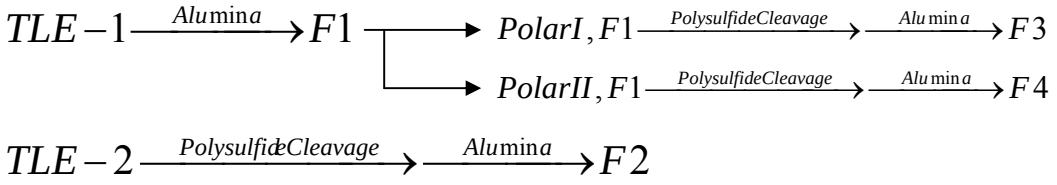

Fig. 14. Comparison of F1-F4. F1 was separated from TLE-1, while F2 was got from TLE-2. F3 and F4 were separated from F1-polar I and F1-polar II after polysulfide cleavage and another alumina column chromatography.

Fractions F3 and F4 were generated from F1-polar I and F1-polar II, after polysulfide cleavage and a second alumina column chromatographic separation. No sulfur was detected in these fractions. As the results of F2-apolar confirmed that S-S bonds in organic compounds were able to be broken and detected by GC, this result indicates that

no detectable organic sulfur compounds with polysulfides were present in F1-polar I or F1-polar II fractions.

Thus, we conclude that the polysulfide bound OSC in these samples were bound into a macromolecular matrix which was not amenable to alumina column chromatography, but that these compounds are released upon polysulfide cleavage of the total lipid extract. In our study, iron hadn't been included into our consideration, beyond the fact that Florida Bay is an Fe-limited system (Lyons, 2004), which is generally thought to enhance the potential for OSC formation. We identified fewer OSC in Florida Bay sediments compared with Filley et al's (2002) results in Mud Lake, Florida. The lower abundance of polysulfide bound OSC in Florida Bay supports their conclusion that the availability of reactive iron can in some systems stimulate polysulfide formation, thereby enhancing formation of OSC (Filley et al., 2002). Furthermore, we suggest that additional OSC are most likely present in the kerogen (i.e. macromolecular organic matter that is not extractable by organic solvents), and that these OSC may be released through polysulfide cleavage of the extracted residues of our sediment samples.

4.2 Variability among Florida Bay sampling sites:

The concentration of OSC in Little Madeira samples was too low to be quantified. In contrast with other cores, OSC from Little Madeira appeared in a very early stage at a depth of 5-6 cm, while no sulfur peaks were detected at 20-21 and 30-31 cm. In its TOC and TS profiles, the concentration of both TOC and TS at the surface (2.05%, 0.202%) is higher than 20-21 (1.15%, 0.160%) and 30-31 (1.02%, 0.130%). We suspect that this site

may have been disturbed (e.g. by storms) in the recent past, resulting in sediment mixing and oxygenation, which would have removed all inorganic sulfides and enhanced organic matter degradation, leading to low concentrations of both OC and TS.

No OSC were identified in the surface samples from Rabbit Key (RKB-2), Barnes Key (FBT-2 18A-1) or Rankin Bight (FBT-2-8-1), but concentration of OSC increased with depth at these sites. OSC appeared first at 15-16 cm at Rabbit Key, where both TOC and TS concentrations began to increase, suggesting that higher concentrations of TOC and TS lead to increasing OSC abundance. Due to insufficient TS data for Barnes Key and Rankin Bight, it was only expected that the amount and abundance of OSC would also increase with depth. It should be pointed out that while most of OSC were detected from F2-apolar samples, F1-apolar samples at 16-18 cm and 22-24 cm of Rankin Bight samples also released OSC. Considering F1-apolar OSC abundance at 16-18 cm was 51.3 mg/g TLE, we expected more OSC to be detected in F2-apolar at the same depth, as all the OSC in F1-apolar should also be detected in F2-apolar; however, this was not observed. At Barnes Key the sulfurization process started perhaps shallower than 19-20 cm, while OSC were produced during an earlier stage at Rankin Bight (at 6-8 cm), which was similar to Little Madeira (5-6 cm).

In Duck Key (FBT2-25) samples, both TOC and TS had minimum values (2.62%, 1.198%) at 5-6 cm depth, which could explain why no sulfur was incorporated into organic matter in the surface. From 0 to 15 cm, TOC and TS had almost constant low values (1.83-2.68%, 0.198-0.330%). Below 15 cm both TOC and TS increased in

concentration substantially (TOC: 1.45-37.31%, TS: 0.227-0.973%). At 20-21 cm, the abundance of OSC from F2-apolar fractions reached 194.7 mg/g TLE, while at 30-31 cm there was only 6 mg/g OSC in F2-apolar, and about 2.1 mg/g OSC was detected in F1-polar I. According to a large concentration difference between OSC at 30-31 cm and that of 20-21 cm, we expect that at 30-31 cm, sulfide was largely incorporated into kerogen instead of lipids.

Based on the location of methylthioethers on the tentatively identified OSC, we can speculate about the structures of the original OSC before polysulfide cleavage, though the number of sulfur atoms was not sure. And we also consider that the precursor of OSCs were aliphatic alkane/alkene (C_{25}, C_{27}). Notice that both identified OSCs were odd-number while even-number homologues of long chain n-alkanes/alkene were conspicuously absent in detectable amount, thus indicated of a terrestrial higher plant origin according to Eglinton (1962).

For example, 1, 2-bis(methylthio)pentacosane ($C_{27}H_{56}S_2$, MW 444, Fig. 15), could have been derived from cleavage of an intramolecular polysulfide chain (e.g., Fig. 16) or an intermolecular polysulfide chain (Fig. 17), and the precursor that got polysulfides incorporated might be pentacosadiene $C_{25}H_{48}$ (Fig. 18).

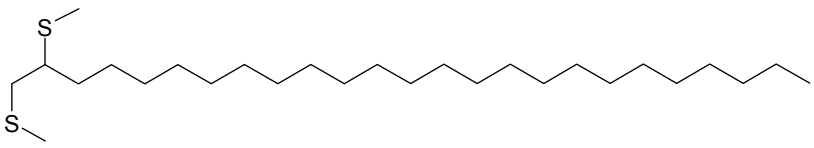

Fig. 15 Structure of 1, 2-bis(methylthio) pentacosane ($C_{27}H_{56}S_2$, MW 444) at 44 min.

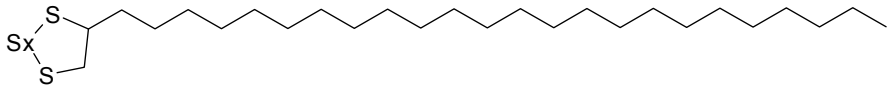

Fig. 16. Intramolecular polysulfide incorporation

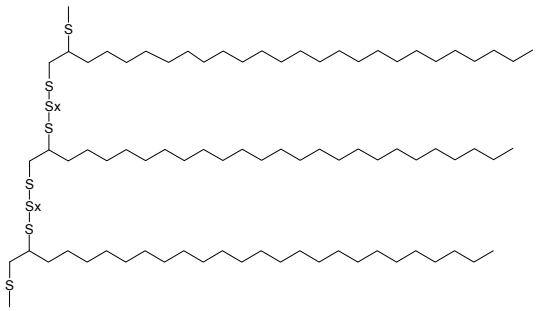

Fig. 17. Intermolecular polysulfide incorporation.

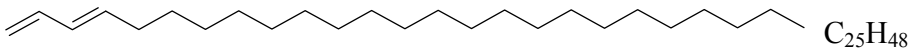

Fig. 18. Precursor of polysulfide incorporation.

Chapter 5: Conclusion

In Florida Bay, TOC and TS generally decrease steadily with depth, with the exception of Duck Key (FBT2-25). The OSC profiles had the two values reached up to 37.3% and 0.973% at the bottom. And the abundance of OSC was increasing as TOC and TS declined.

Experimental results indicate that there are polysulfide bound organic compounds formed during early diagenesis, since OSC were detected in the surface sediments. Three OSC appeared in most cores that had higher abundances than other sulfur compounds, two OSC were identified and indicated the precursor of sulfurization might be long-chain alkene with an odd Carbon number (C25, 27), their presence suggested a terrigenous input (Eglinton and Hamilton, 1963; Schaeffer et al, 1995).

Compared with others' studies (e.g., Filley, 2002), low abundances of polysulfide bound OSC were formed in Florida Bay, which may be due in part to lack of reactive iron to stimulate polysulfide formation. Furthermore, we suggest that additional OSC are most likely present in the kerogen (i.e. macromolecular organic matter that is not extractable by organic solvents), and that these OSC may be released through polysulfide cleavage of the extracted residues of our sediment samples.

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Appendix I: Bulk carbon and sulfur concentration of five cores in Florida Bay

Coulometry data from Rabbit Key (RKB-2) 0-34 cm

Depth/cm	Weight % T0C	Weight % TIC	Weight % TC	TS Sample/mg	TS Weight/mg	TS%
0.5	5.23	6.47	11.70	215.1	0.6398	0.2974
5.5	4.52	8.21	12.73	200.6	0.4680	0.2333
10.5	5.63	8.36	13.99	159.0	0.3254	0.2047
15.5	4.49	8.84	13.33	153.3	0.4694	0.3062
20.5	3.12	9.67	12.79	164.9	0.5004	0.3035
25.5	2.34	9.73	12.07	162.5	0.6663	0.4100
30.5	2.17	9.56	11.73	180.7	0.6453	0.3571
33.5	1.75	10.13	11.88	104.9	0.1689	0.1610

Coulometry data from Duck Key (FBT2-25) 0-35 cm

Depth/cm	Weight % T0C	Weight % TIC	Weight % TC	TS Sample/mg	TS Weight/mg	TS%
0.5	2.68	9.02	11.70	199.9	0.6595	0.3299
5.5	2.62	9.7	12.32	202.9	0.4012	0.1977
10.5	1.85	10.16	12.01	199.4	0.6141	0.3080
15.5	1.83	10.17	12.00	197.8	0.4498	0.2274
20.5	1.45	10.27	11.72	205.8	0.6964	0.3384
25.5	4.04	9.13	13.17	198.6	0.5449	0.2744
30.5	19.89	5.12	25.01	200.5	1.0517	0.5245
34.5	37.31	0.83	38.14	199.7	1.9428	0.9729

Coulometry data from Little Madeira 0-41 cm

Depth/cm	Weight % T0C	Weight % TIC	weight % TC	TS Sample/mg	TS Weight/mg	TS%
0.5	3.10	9.39	12.49	203.8	0.3139	0.1540
5.5	2.05	10.34	12.39	196.1	0.3965	0.2022
10.5	1.85	10.26	12.11	202.1	0.3614	0.1788
15.5	2.39	9.67	12.06	198.6	0.3839	0.1933
20.5	1.15	10.59	11.74	200.2	0.3197	0.1597
25.5	0.92	10.57	11.49	201.6	0.2181	0.1082
30.5	1.02	10.35	11.37	199.2	0.2582	0.1296
35.5	0.57	10.85	11.42	200.7	0.3173	0.1581
40.5	0.61	10.84	11.45	199.5	0.2713	0.1360

Coulometry data from FBT-2 18A-1(Barnes Key) 0-40 cm

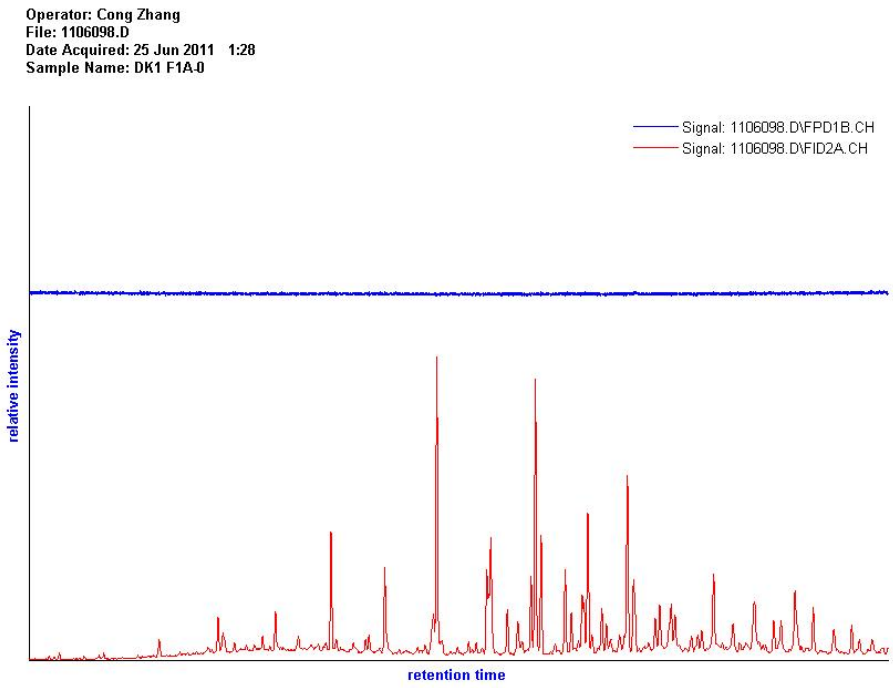
Depth/cm	Weight % T0C	Weight % TIC	Weight % TC
0.5	3.03	9.21	12.24
1.5	2.39	9.51	11.9
2.5	2.19	9.74	11.93
3.5	2.33	9.77	12.1
4.5	2.35	9.9	12.25
5.5	2.1	9.84	11.94
6.5	2.01	10.06	12.07
7.5	2.21	9.92	12.13
8.5	1.82	10.11	11.93
9.5	2.02	10.05	12.07
10.5	2.31	9.93	12.24
11.5	2.04	10.12	12.16
12.5	2.07	9.96	12.03
13.5	2.37	9.88	12.25
14.5	2.14	9.95	12.09
15.5	2.59	9.74	12.33
16.5	2.54	10.02	12.56
17.5	1.91	10.27	12.18
18.5	1.94	10.22	12.16
19.5	1.35	10.54	11.89
20.5	1.92	10.13	12.05
21.5	2.14	9.99	12.13
22.5	1.87	10.33	12.2
23.5	2.04	10.22	12.26
24.5	2.16	10.11	12.27
25.5	2.08	10.15	12.23
26.5	1.99	10.37	12.36
27.5	2.25	9.91	12.16
28.5	2.07	10.05	12.12
29.5	2.41	9.72	12.13
30.5	1.92	10.15	12.07
31.5	2.02	10.08	12.1
32.5	1.97	10.15	12.12
33.5	2.22	9.96	12.18
34.5	2.66	9.82	12.48
35.5	1.8	10.25	12.05
36.5	1.75	10.41	12.16
37.5	2.3	9.9	12.2
38.5	2.09	10.07	12.16
39.5	2.17	10.1	12.27

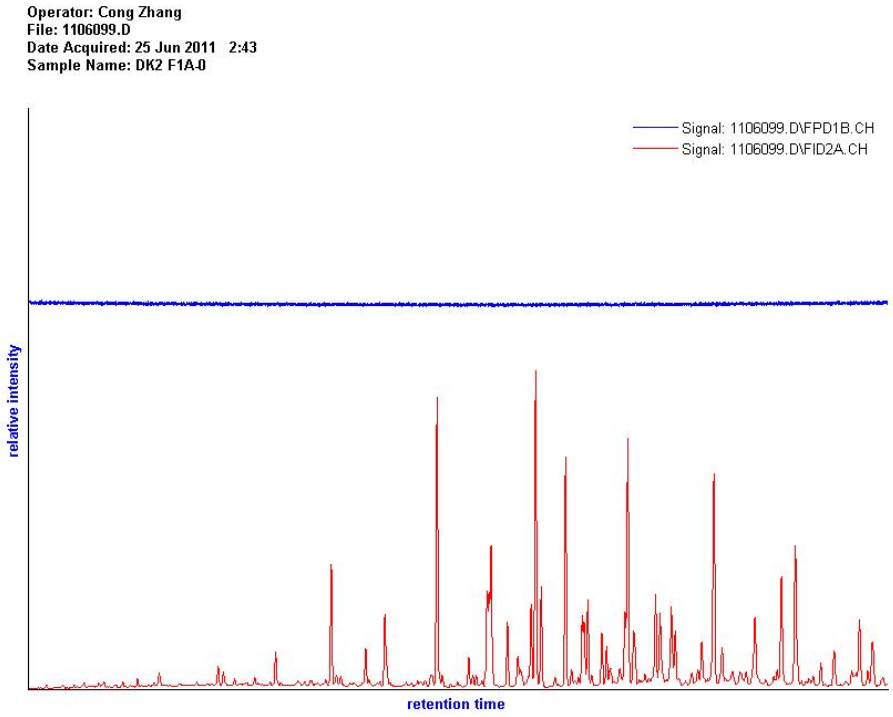
Coulometry data from FBT2-8-1 (Rankin Bight) 0-40 cm

Depth/cm	Weight % T0C	Weight % TIC	weight % TC
0.5	4.62	7.34	11.96
2.5	4.61	7.63	12.24
4.5	4.36	7.94	12.3
6.5	4.01	8.4	12.41
8.5	4.45	8.12	12.57
10.5	4.65	7.68	12.33
12.5	4.59	7.85	12.44
14.5	4.08	8.28	12.36
16.5	3.98	8.29	12.27
18.5	3.55	8.15	11.7
20.5	2.85	8.59	11.44
22.5	2.4	8.5	10.9
24.5	2.07	8.72	10.79
26.5	2.11	8.56	10.67
28.5	1.79	9.02	10.81
30.5	2.23	8.76	10.99
32.5	2.52	8.61	11.13
34.5	2.37	8.67	11.04
36.5	2.18	9.11	11.29
38.5	1.67	9.09	10.76
40.5	1.95	8.85	10.8

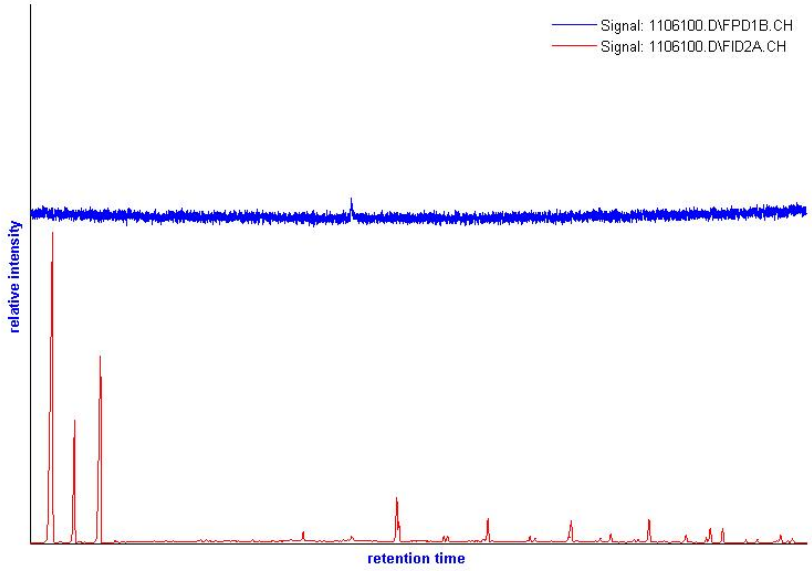
Appendix II: GC-FID/FPD Chromatograph

F1-apolar:

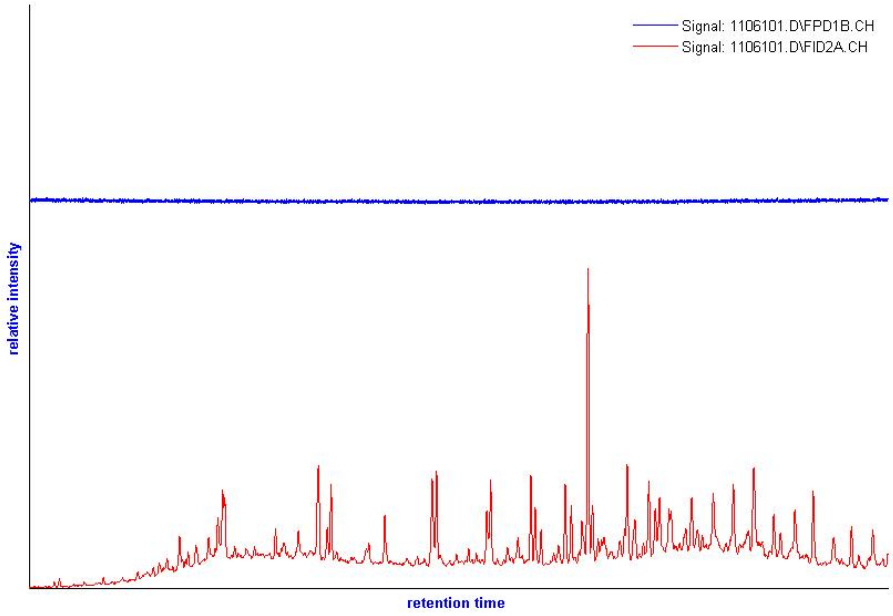




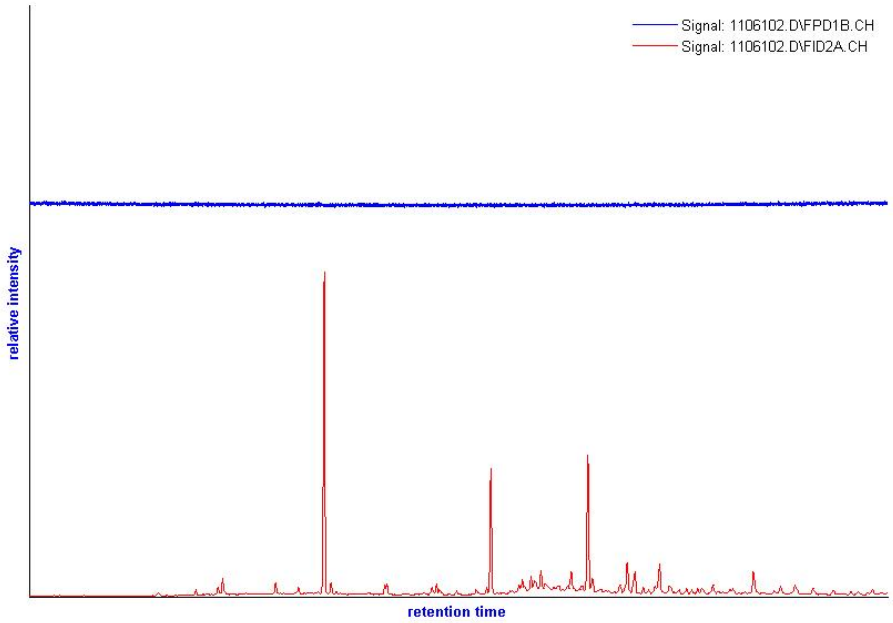
Operator: Cong Zhang
File: 1106100.D
Date Acquired: 25 Jun 2011 3:57
Sample Name: DK3 F1A-0



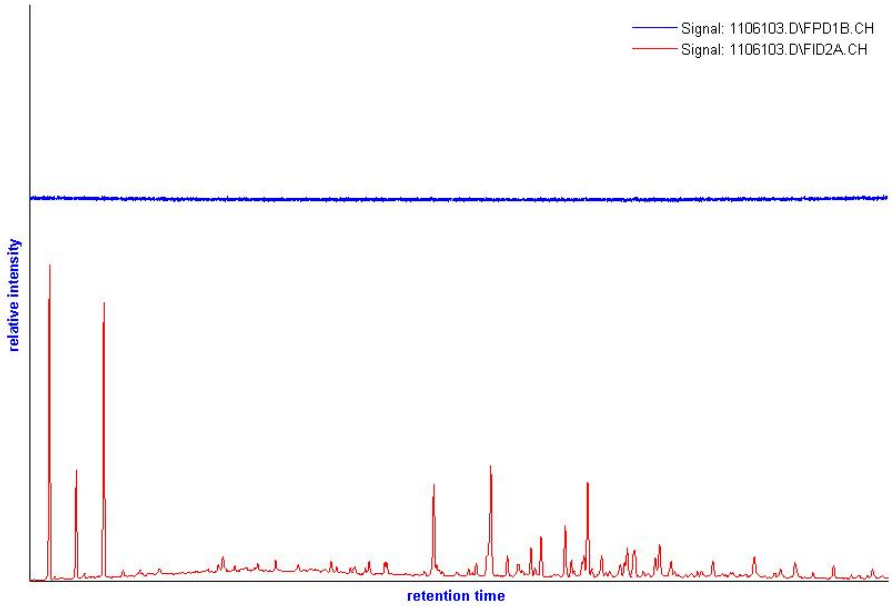
Operator: Cong Zhang
File: 1106101.D
Date Acquired: 25 Jun 2011 5:12
Sample Name: RK1 F1A-0



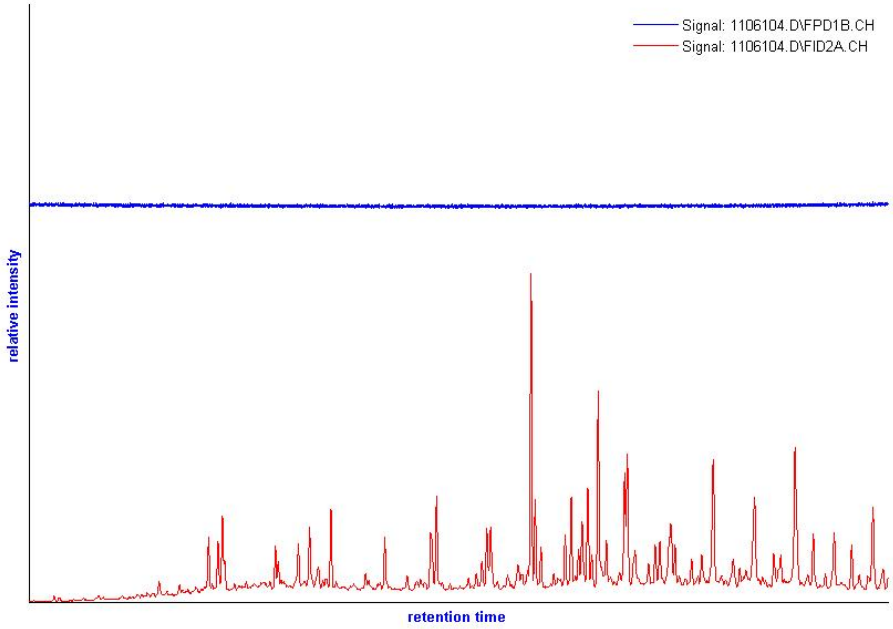
Operator: Cong Zhang
File: 1106102.D
Date Acquired: 25 Jun 2011 6:27
Sample Name: RK2 F1A-0



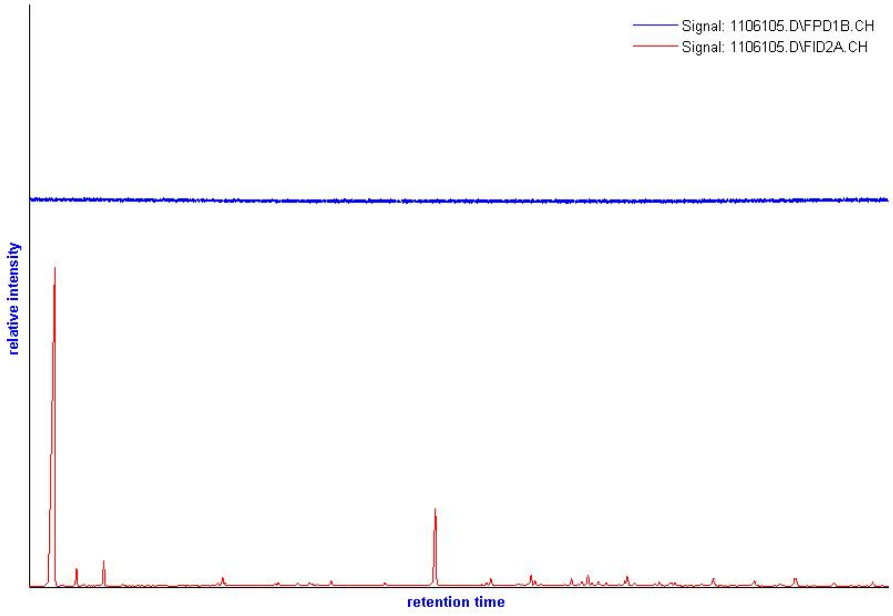
Operator: Cong Zhang
File: 1106103.D
Date Acquired: 25 Jun 2011 7:41
Sample Name: RK3 F1A-0



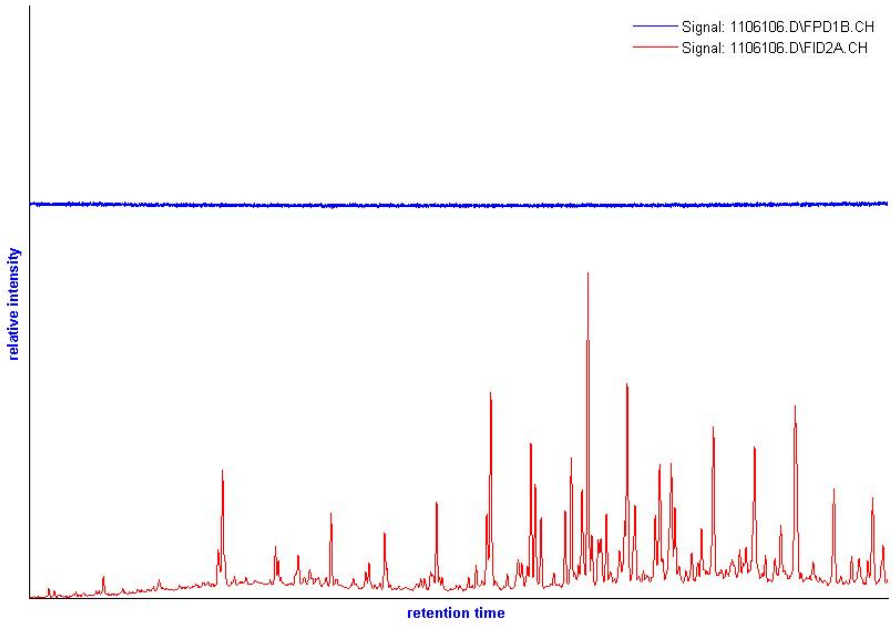
Operator: Cong Zhang
File: 1106104.D
Date Acquired: 25 Jun 2011 8:56
Sample Name: BK1 F1A-0



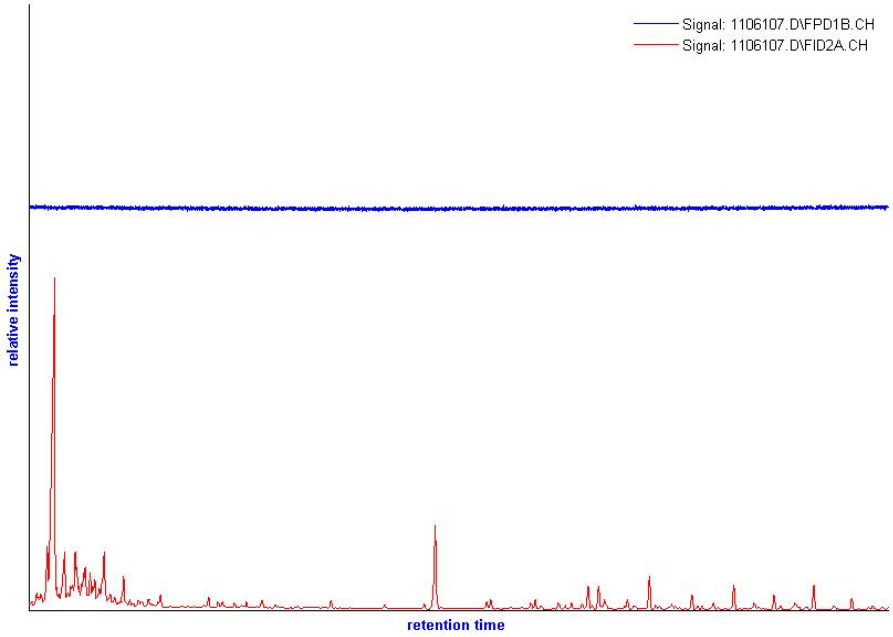
Operator: Cong Zhang
File: 1106105.D
Date Acquired: 25 Jun 2011 10:11
Sample Name: BK2 F1A-0



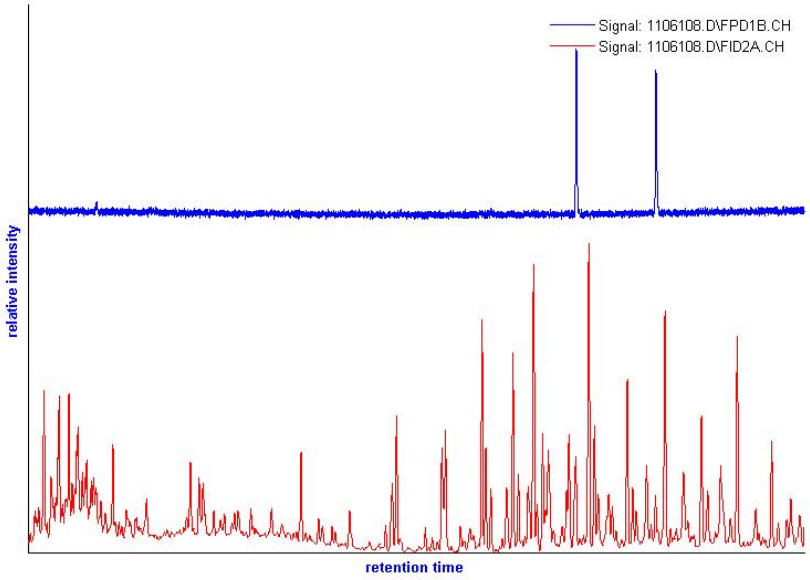
Operator: Cong Zhang
File: 1106106.D
Date Acquired: 25 Jun 2011 11:26
Sample Name: BK3 F1A-0



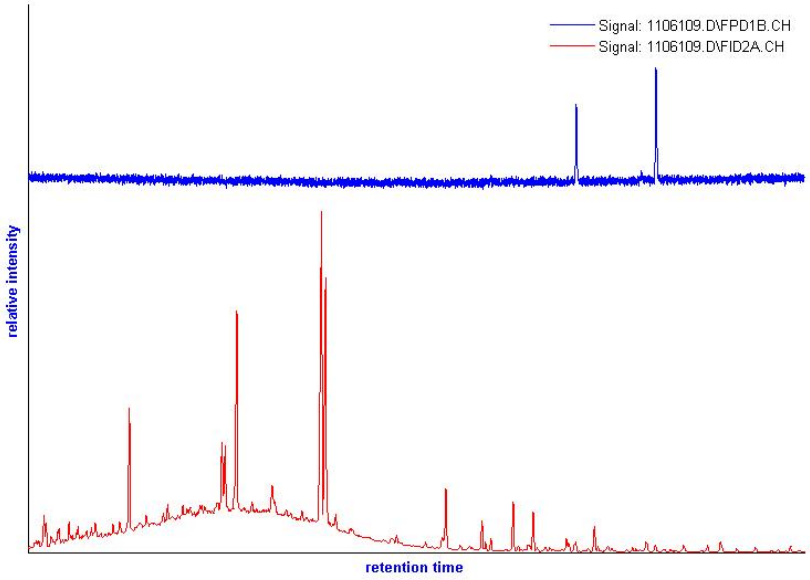
Operator: Cong Zhang
File: 1106107.D
Date Acquired: 25 Jun 2011 12:41
Sample Name: RB1 F1A-0



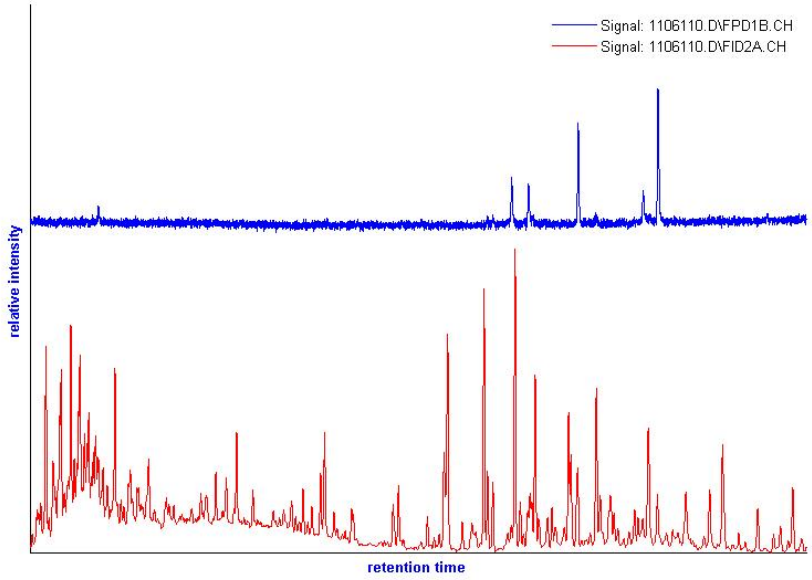
Operator: Cong Zhang
File: 1106108.D
Date Acquired: 25 Jun 2011 13:56
Sample Name: RB2 F1A-0



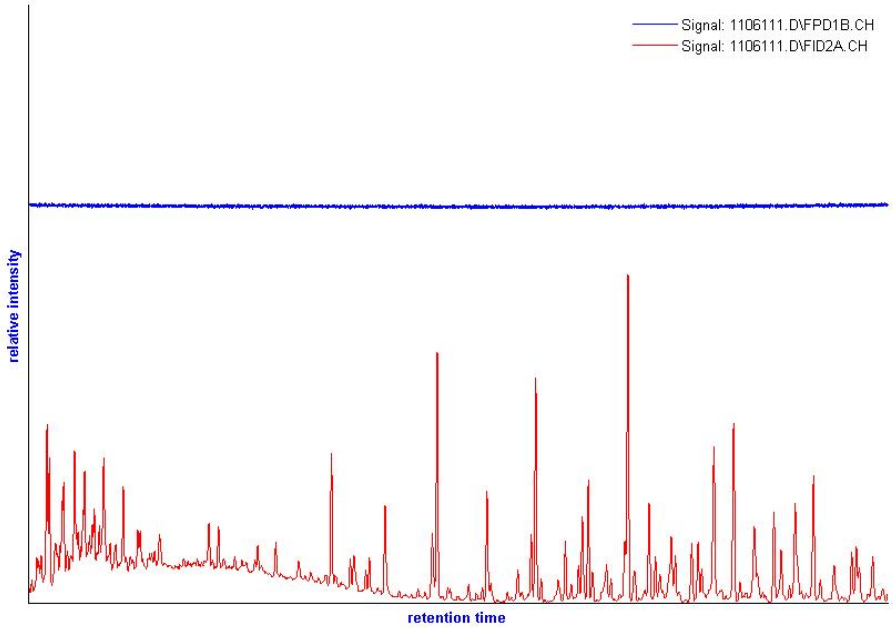
Operator: Cong Zhang
File: 1106109.D
Date Acquired: 25 Jun 2011 15:10
Sample Name: RB3 F1A-0



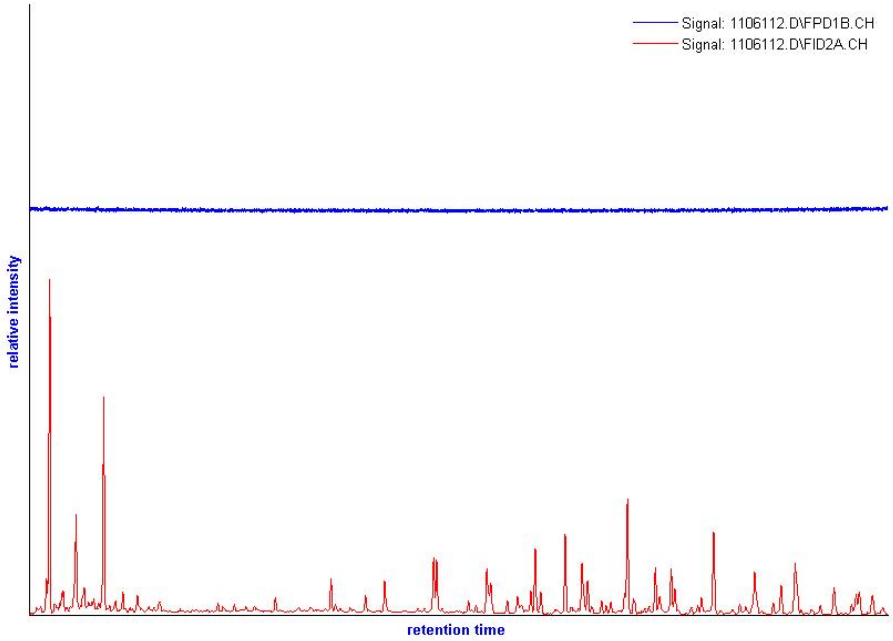
Operator: Cong Zhang
File: 1106110.D
Date Acquired: 25 Jun 2011 16:25
Sample Name: RB4 F1A-0



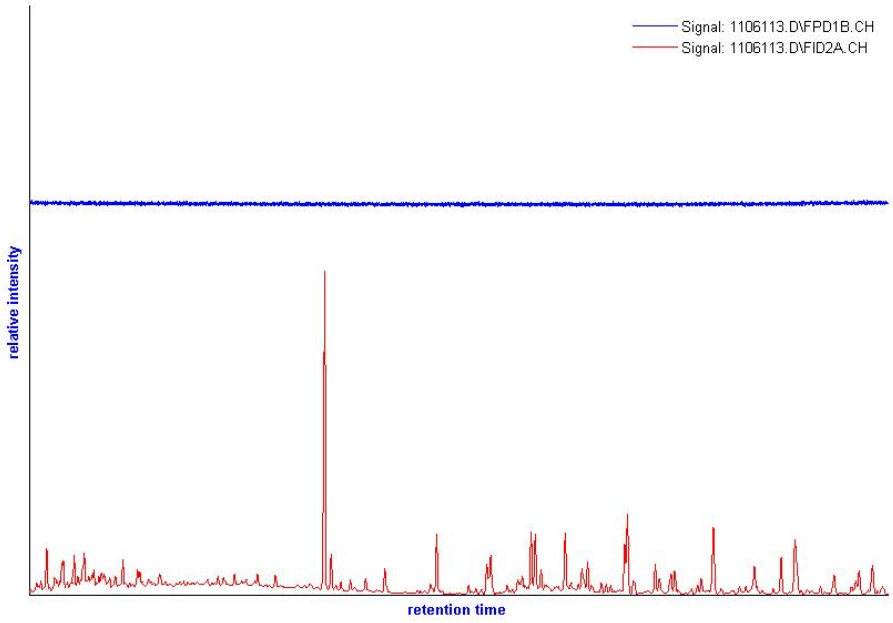
Operator: Cong Zhang
File: 1106111.D
Date Acquired: 25 Jun 2011 17:40
Sample Name: LM1 F1A-0



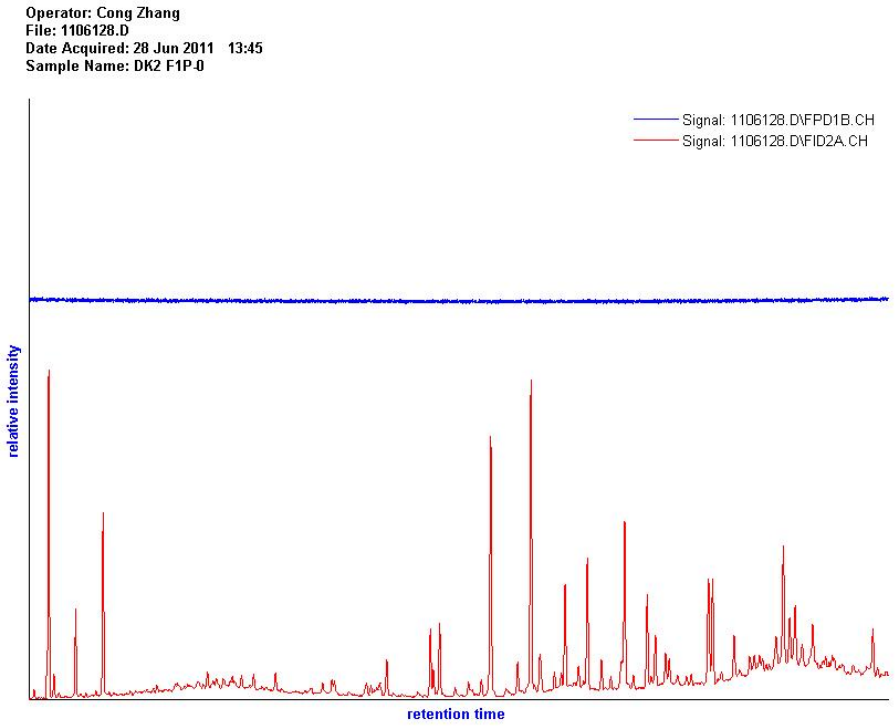
Operator: Cong Zhang
File: 1106112.D
Date Acquired: 25 Jun 2011 18:55
Sample Name: LM2 F1A-0

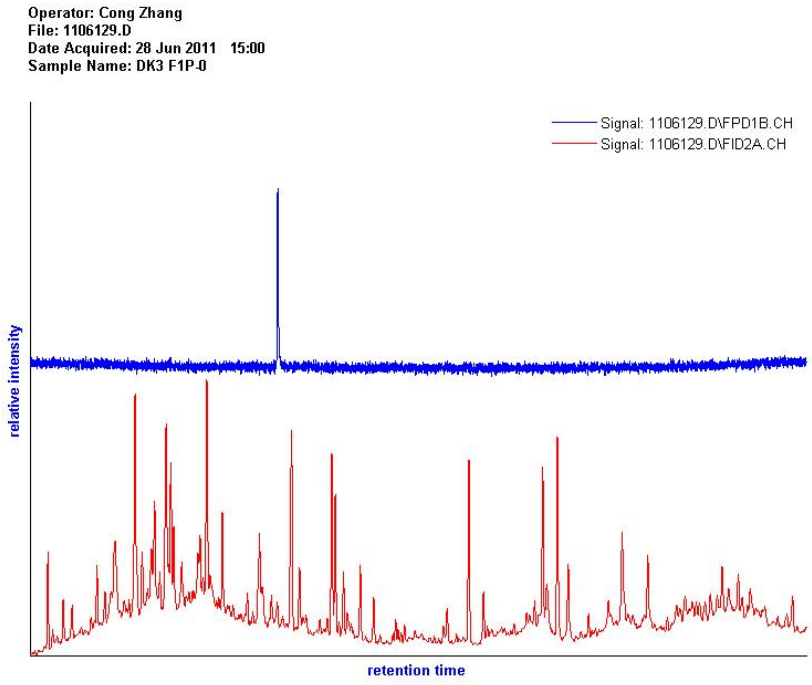


Operator: Cong Zhang
File: 1106113.D
Date Acquired: 25 Jun 2011 20:10
Sample Name: LM3 F1A-0

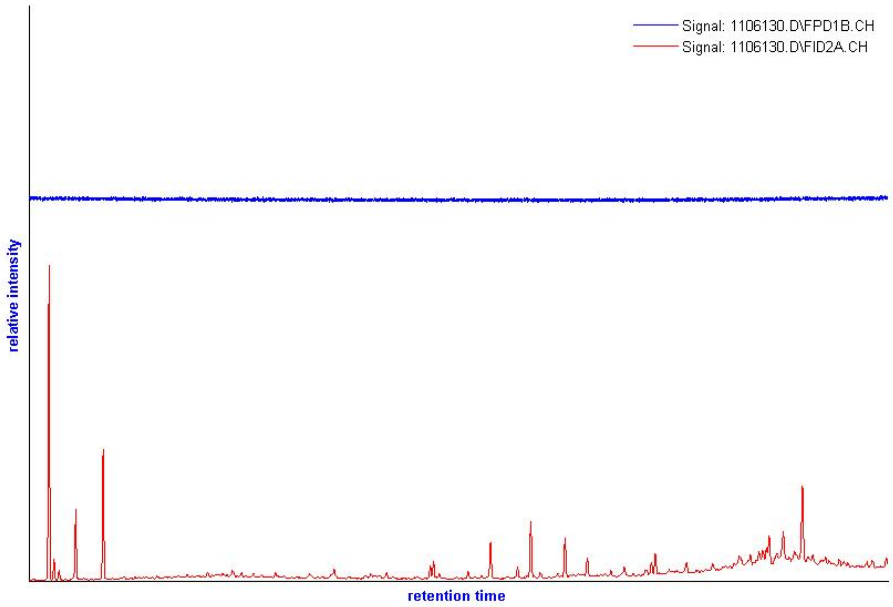


F1-polar I (TMS)

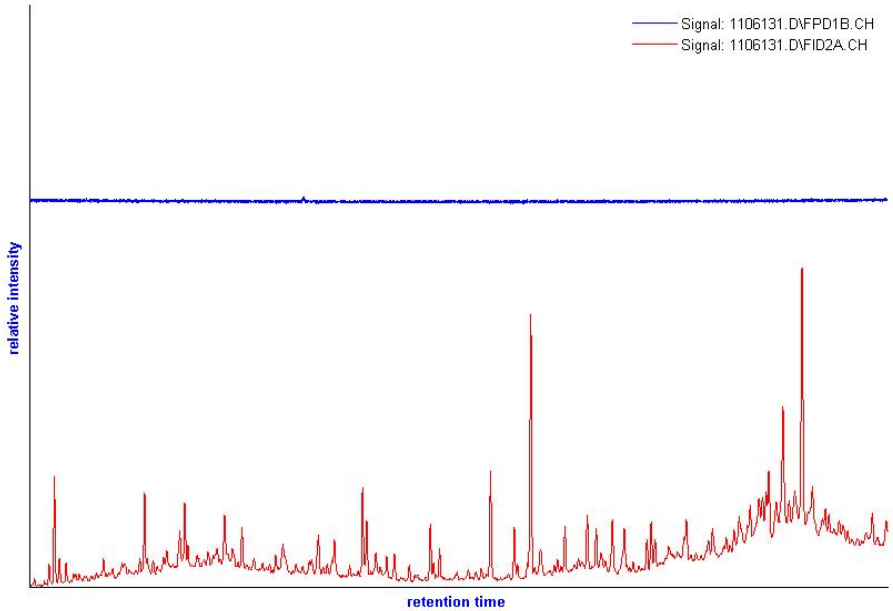




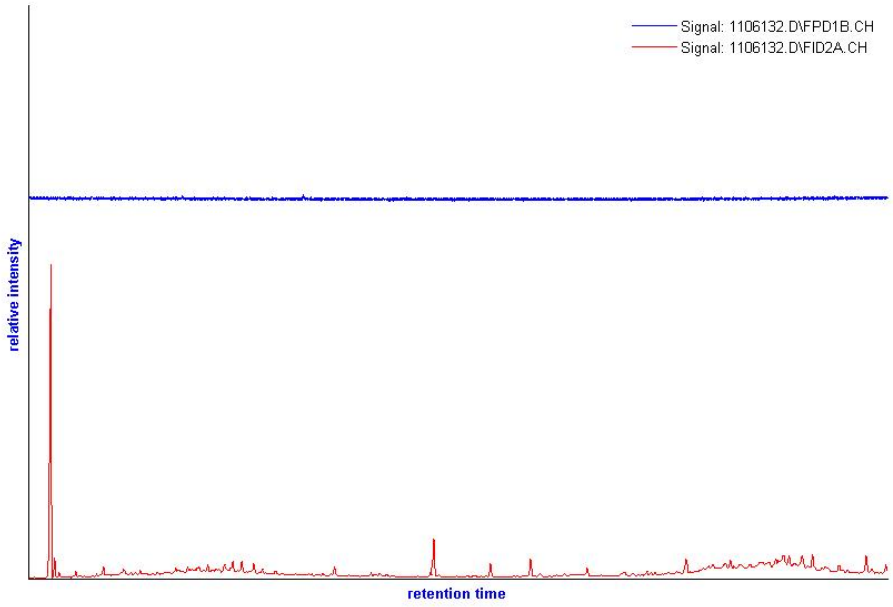
Operator: Cong Zhang
File: 1106130.D
Date Acquired: 28 Jun 2011 16:15
Sample Name: BK2 F1P-0



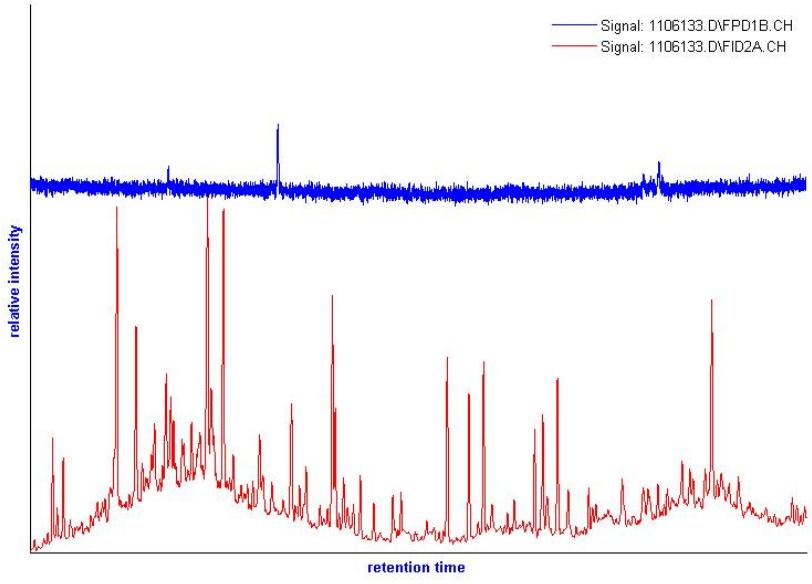
Operator: Cong Zhang
File: 1106131.D
Date Acquired: 28 Jun 2011 17:30
Sample Name: BK3 F1P-0



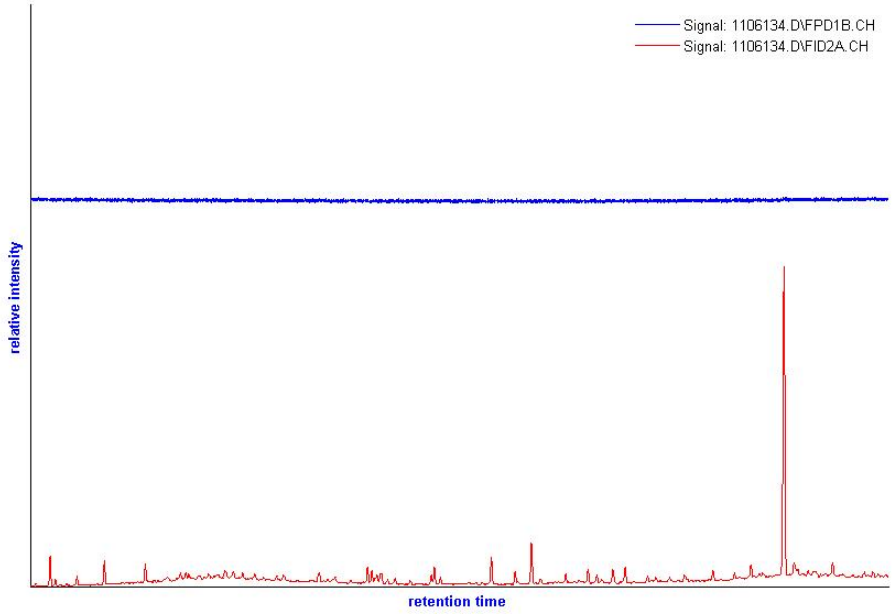
Operator: Cong Zhang
File: 1106132.D
Date Acquired: 28 Jun 2011 18:45
Sample Name: RB1 F1P-0



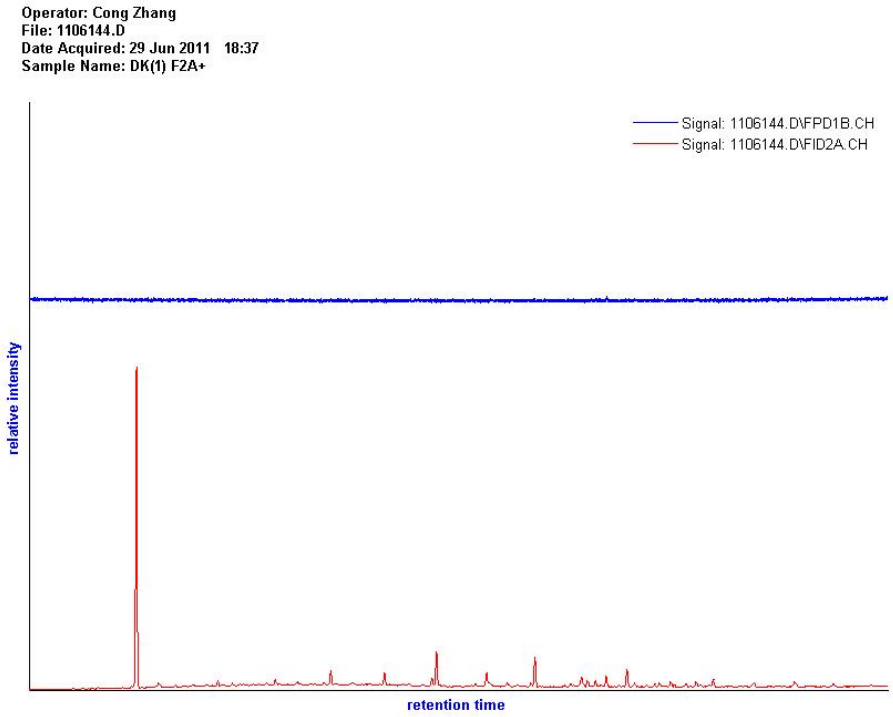
Operator: Cong Zhang
File: 1106133.D
Date Acquired: 28 Jun 2011 19:59
Sample Name: RB4 F1P-0

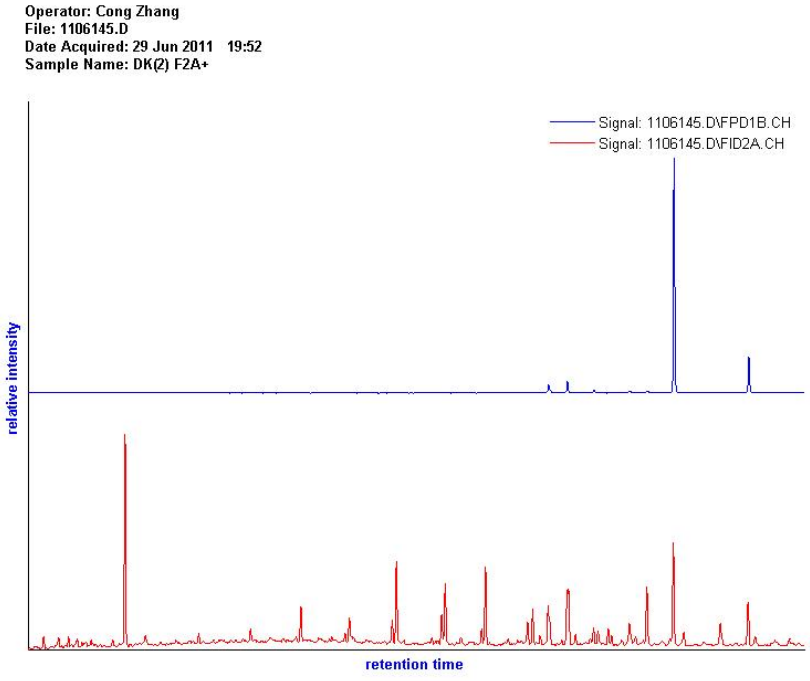


Operator: Cong Zhang
File: 1106134.D
Date Acquired: 28 Jun 2011 21:14
Sample Name: LM2 F1P-0

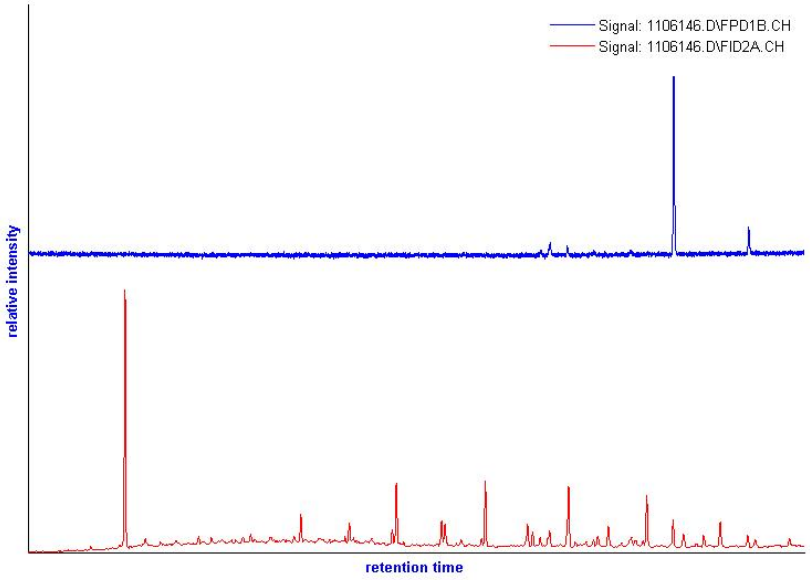


F2-apolar with 5α-androstane:

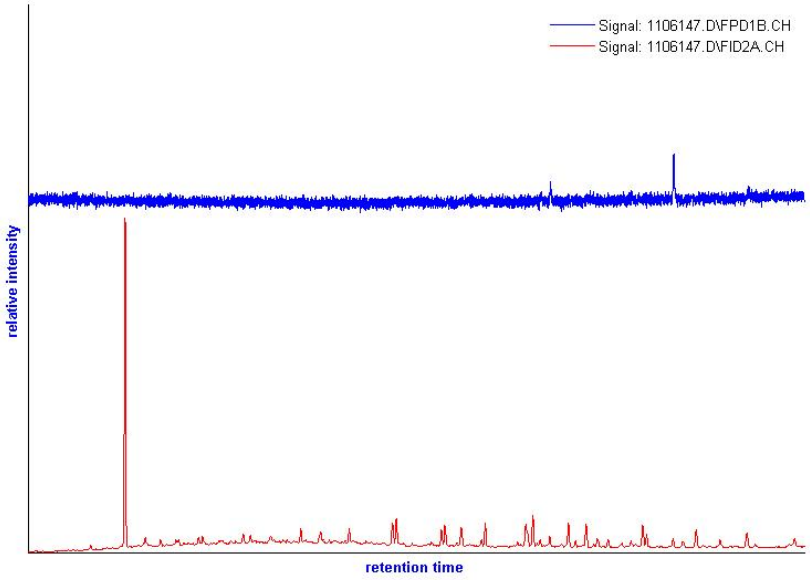




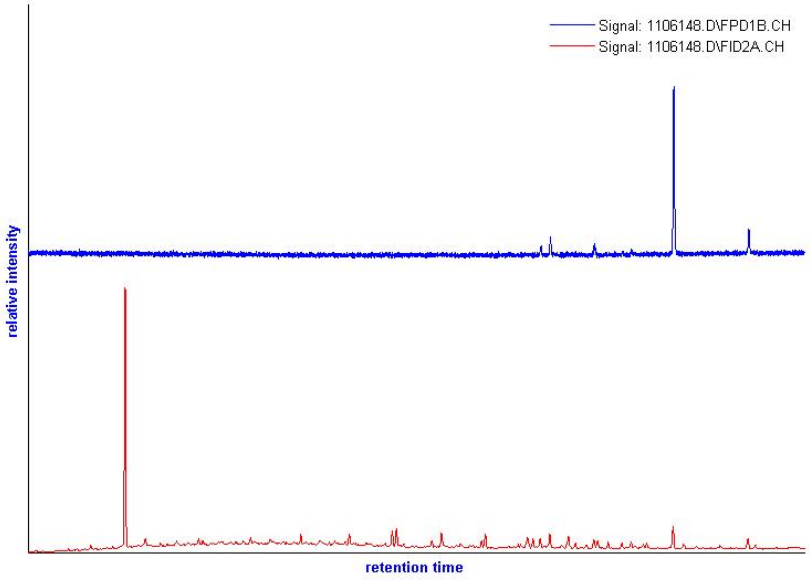
Operator: Cong Zhang
File: 1106146.D
Date Acquired: 29 Jun 2011 21:06
Sample Name: DK(3) F2A+



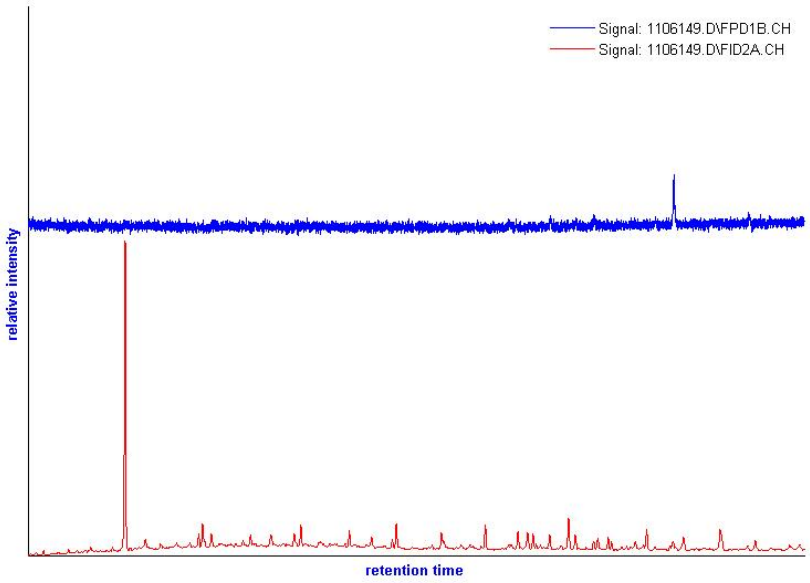
Operator: Cong Zhang
File: 1106147.D
Date Acquired: 29 Jun 2011 22:21
Sample Name: RK(2) F2A+



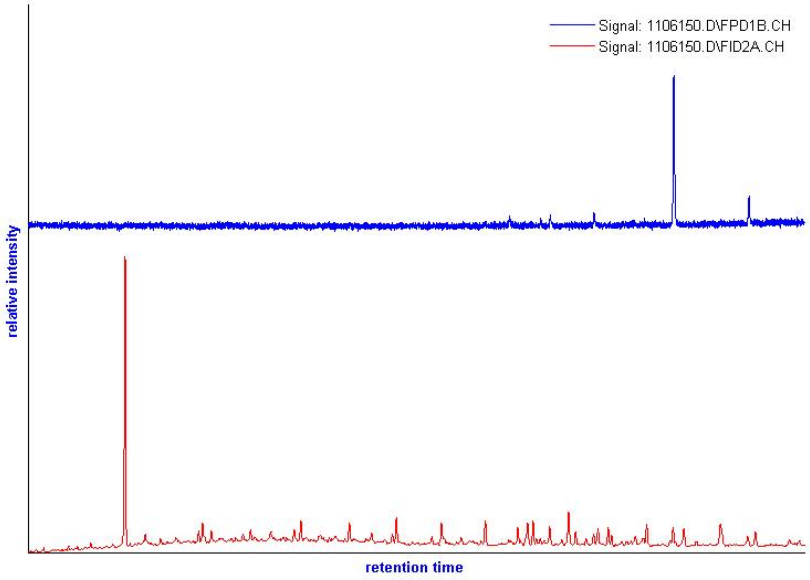
Operator: Cong Zhang
File: 1106148.D
Date Acquired: 29 Jun 2011 23:35
Sample Name: RK(3) F2A+



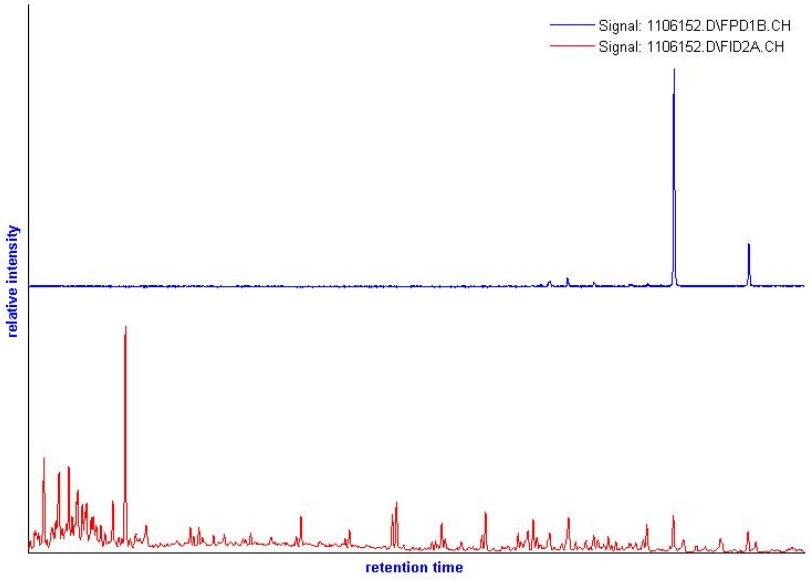
Operator: Cong Zhang
File: 1106149.D
Date Acquired: 30 Jun 2011 00:49
Sample Name: BK(2) F2A+



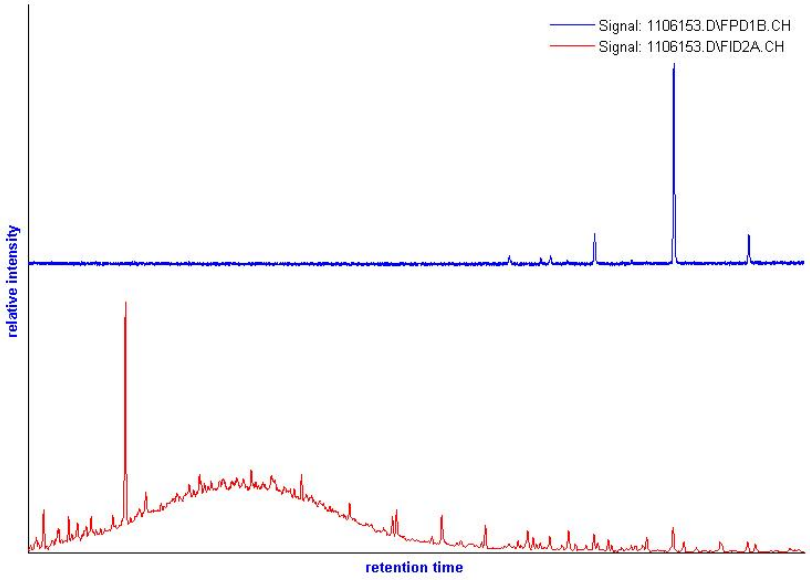
Operator: Cong Zhang
File: 1106150.D
Date Acquired: 30 Jun 2011 2:04
Sample Name: BK(3) F2A+



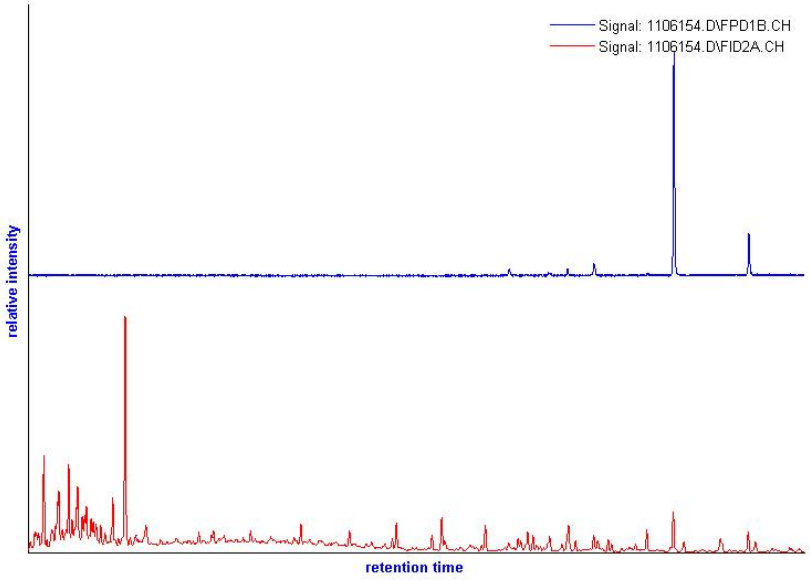
Operator: Cong Zhang
File: 1106152.D
Date Acquired: 30 Jun 2011 4:32
Sample Name: RB(1) F2A+



Operator: Cong Zhang
File: 1106153.D
Date Acquired: 30 Jun 2011 5:46
Sample Name: RB(3) F2A+



Operator: Cong Zhang
File: 1106154.D
Date Acquired: 30 Jun 2011 7:01
Sample Name: RB(4) F2A+



Operator: Cong Zhang
File: 1106155.D
Date Acquired: 30 Jun 2011 8:15
Sample Name: LM(3) F2A+