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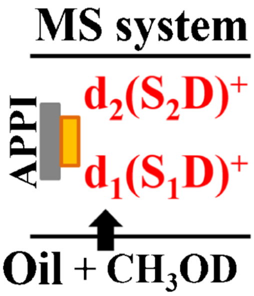
RESEARCH ARTICLE

Application of Atmospheric Pressure Photoionization H/D-exchange Mass Spectrometry for Speciation of Sulfur-containing Compounds

Thamina Acter,[1] Donghwi Kim,[1] Arif Ahmed,[1] Ji-Hyoung Ha,[2] Sunghwan Kim[1,3]

[1]Department of Chemistry, Kyungpook National University, Daegu, 702-701, Republic of Korea
[2]World Institute of Kimchi, Gwangju, 61755, Republic of Korea
[3]Department of Chemistry, Green Nano Center, Daegu, 702-701, Republic of Korea



Abstract. Herein we report the observation of atmospheric pressure in-source hydrogen–deuterium exchange (HDX) of thiol group for the first time. The HDX for thiol group was optimized for positive atmospheric pressure photoionization (APPI) mass spectrometry (MS). The optimized HDX-MS was applied for 31 model compounds (thiols, thiophenes, and sulfides) to demonstrate that exchanged peaks were observed only for thiols. The optimized method has been successfully applied to the isolated fractions of sulfur-rich oil samples. The exchange of one and two thiol hydrogens with deuterium was observed in the thiol fraction; no HDX was observed in the other fractions. Thus, the results presented in this study demonstrate that the HDX-MS method using APPI ionization source can be effective for speciation of sulfur compounds. This method has the potential to be used to access corrosion problems caused by thiol-containing compounds.

Keywords: Hydrogen/deuterium exchange, Mass spectrometry, Atmospheric-pressure photoionization

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Introduction

Hydrogen–deuterium exchange coupled to mass spectrometry (HDX-MS) is a powerful tool for identifying chemical structures. The HDX technique can be largely divided into three categories. The first is in-solution HDX, where HDX occurs in the solution phase by storing target molecules in the deuterated solution. The masses of the hydrogen/deuterium exchanged compounds are later measured with MS. Solution HDX has been used mainly to study the solution-phase structure of proteins and peptides [1–3]. The second category is HDX under vacuum or reduced pressure. This is performed while analyzing compounds by chemical ionization typically coupled to gas chromatography. Deuterated gas can be used to achieve HDX and chemical speciation is done by counting the number of exchanged peaks [4, 5]. The third and most recently reported method is atmospheric pressure in-source HDX, where HDX is achieved by ionizing compounds with atmospheric-pressure ionization (API). HDX occurs in the ionization source under atmospheric pressure. Electrospray ionization (ESI) [6–9], atmospheric-pressure chemical ionization (APCI) [10, 11], and atmospheric-pressure photo ionization (APPI) [12, 13] have been used for API-HDX. In API-HDX, the deuterated solvent is supplied by mixing the sample solution with a deuterated solvent [10, 12–14] or by generating vapor of the deuterated solvent in the ionization source [15–17]. In all the techniques described above, HDX has been performed only on nitrogen- or oxygen-containing compounds. ESI HDX has been used to study –OH and –NH_2 groups in proteins, peptides, metabolites, antibiotics, and humic substances [8, 9, 18, 19]. In another example, APPI and APCI HDX have been used to study nitrogen- or oxygen-containing aromatic compounds in crude oils [11, 13, 20, 21].

Recently, in-solution HDX with D_2O followed by NMR has been used for structural elucidation of thiols in peptides [22]. Wang and Schrader employed deuterium-labeled alkylating reagents to the alkylation of crude oil to distinguish the tagged sulfur species from that of the original crude oil [23]. Therefore, HDX-MS can potentially be used for the analysis of thiol compounds. However, there are very few reports on the use of HDX-

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Correspondence to: Sunghwan Kim; *e-mail:* sunghwank@knu.ac.kr

MS for thiol-containing compounds. There is only one report describing the use of chemical ionization (CI) [4] and none describing the use of API-HDX. There have been so few studies because detection of sulfur-containing compounds by ESI and CI coupled with gas chromatography (GC) is difficult [24–26].

Atmospheric-pressure photoionization (APPI) can efficiently ionize gas-phase nonpolar species (and polar species) through direct photon ionization or via proton transfer [27]. Therefore, the investigation of the HDX behavior of sulfur-containing compounds using APPI source could be worthwhile. Identification of sulfur-containing compounds is important because they are the major contributors to internal corrosion in petroleum refineries [28, 29]. In particular, thiols are responsible for sulfidic corrosion because they are considered to be the most reactive sulfur-containing species and their corrosivity changes with structures [30–32].

In this study, APPI-HDX of sulfur-containing compounds was investigated and reported. To the best of our knowledge, this is the first attempt to perform API-HDX MS for unambiguous speciation of sulfur-containing compounds having diverse structures. In order to determine their structures, the present study covers (1) optimization of ionization to maximize the HDX efficiency, (2) observation of the HDX of standard sulfur-containing compounds, and (3) application of the current method for the analysis of oil samples.

Experiment

Preparation of Standards and Samples All the chemicals and HPLC-grade solvents were purchased from J.T. Baker (Center Valley, PA, USA) and Sigma-Aldrich (St. Louis, MO, USA). The detailed information of the 31 standard compounds and crude oil properties are listed in the Supporting Information (Supplementary Tables S-1 and S-2). Solid-phase extraction (SPE) cartridges, with 1 g propyl sulfonic acid silica in a 6 mL tube (Bond Elute PRS, part no. 12256010), were used. The standard compounds were dissolved in toluene to produce 1 mM stock solution, which was diluted to obtain a final concentration of 10 μM in toluene.

Soroosh crude oil from Iran was used in this study. The oil was chosen because it has high sulfur (3.99%) and mercaptan contents (30 ppm). The crude oil was separated into its three fractions by ligand exchange chromatography using modified Ag+-SCX (strong cation exchange) SPE cartridges according to previously reported procedure [33]. Briefly, at first the modified Ag+-SCX SPE cartridges were conditioned with 6 mL of dichloromethane:acetone (DCM:ACE) (90:10) solvents. Then the oil sample was loaded. The first and second fractions were eluted with 18 mL of DCM:ACE (90:10) and 18 mL of acetonitrile, ACN:DCM (50:50), respectively. The retained oil compounds on the Ag+-SCX sorbent were recovered by the addition of 6 mL of concentrated hydrochloric acid:methanol (HCl:MeOH) (50:50) to the cartridge followed by 12 mL of toluene:methanol (TOL:MeOH) (50:50). These steps were repeated and an additional 12 mL of toluene was percolated through the cartridge in the end. Finally, the third fraction of combined eluates (top and bottom layer) was collected. The top layer was the organic phase, which was isolated and dried with sodium sulfate. The separated three fractions were dissolved in perdeuterated toluene at 1.0 mg/mL and diluted to a final concentration of 0.5 mg/mL.

Mass Spectrometry In order to perform HDX, standard compounds were dissolved with 10:90 (v:v) deuterated methanol:toluene solvent to the final concentration of 10 μM just before the MS analysis. All the experiments were performed with the APPI source. The analyte solution was directly infused at 100 μL/min. Q Exactive quadrupole Orbitrap mass spectrometer (Thermo Fisher Scientific Inc., Rockford, IL, USA) was used to obtain the mass spectra of all the standard compounds. For the delivery of standard solution to the APPI source, a Harvard stainless steel syringe pump model 11 (Harvard, Holliston, MA, USA) was utilized. Typical APPI (+) conditions were as follows: tube lens radio frequency (rf) level, 20 Hz; tube lens voltage, 25 V; skimmer voltage, 15 V; C-Trap rf, 550 V; sweep gas flow, 0 (arbitrary units); sheath gas flow, 10 (arbitrary units), and auxiliary gas flow, 5 (arbitrary units). High-purity (99%) nitrogen was obtained by evaporation of liquid nitrogen and used as the source gas. External positive calibration was done using (+) Pierce Velos solution (Thermo Fisher Scientific) into the ESI source. The data acquisition parameters were as follows: *m*/*z* range 50–750; maximum injection time 60 ms, 1 micro scan, automatic gain control (AGC) ON, and resolution of 140,000.

7T Fourier transform ion cyclotron resonance mass spectrometer (FTICR MS; Bruker Daltonics, Billerica, MA, USA) was used for the crude oil sample analysis. Experimental conditions for (+) APPI FTICR MS were as follows: solution flow rate, 1800 μL/h; nebulizing gas temperature, 200 °C and flow rate, 3.3 L/min; drying gas temperature, 220 °C and flow rate 2.2 L/min; capillary voltage 200 and 3000 V; collision cell rf voltage 1200 V and energy −1.5 V; ion accumulation time, 0.05 s for the first fraction and 0.5 s each for the second and third fractions. Spectra were acquired with 8 MW transient size and summed over 200 time-domain transients. Internal recalibration was performed using the radical cation of S_1 series for the first and second fractions, and those of the N_1 and N_1D_1 series for the third fraction.

Data Analysis Xcalibur 2.2 SP1.48 software (Thermo Fisher Scientific) was used to process the mass spectra of the standard compounds obtained from HDX-MS experiments. The mass spectra of the isolated crude oil fractions obtained from FTICR HDX-MS experiments were processed using the in-house-developed software of Statistical Tool for Organic Mixtures' Spectra for Hydrogen/Deuterium eXchange (STORMS-HDX) [34, 35] for more reliable and faster results.

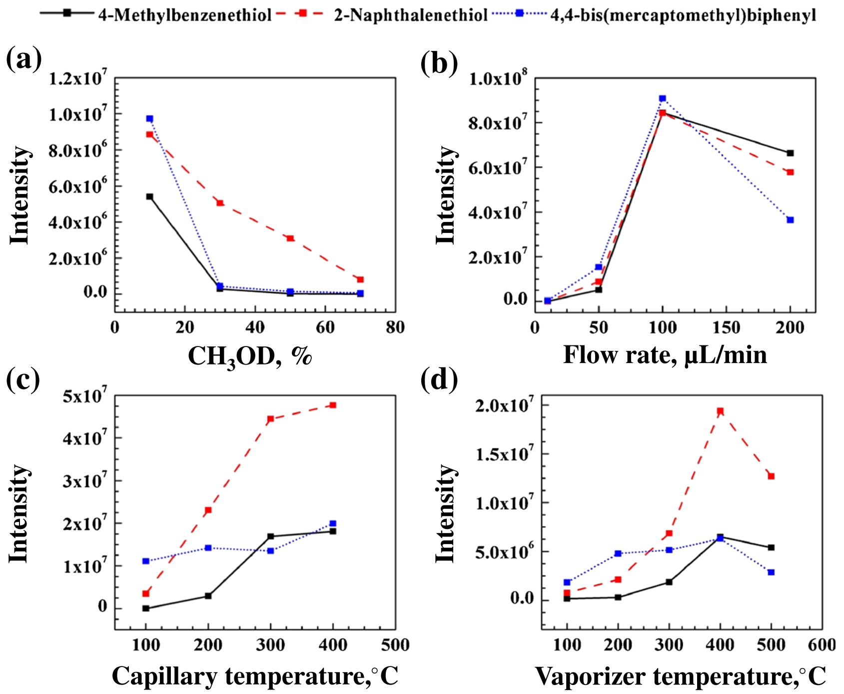

Figure 1. Influence of major experimental parameters of (+) APPI HDX-MS, i.e., (**a**) concentration of CH_3OD in Toluene solvent, (**b**) flow rate of analyte solution, (**c**) capillary temperature, and (**d**) vaporizer temperature on the HDX efficiency of three standard sulfur compounds. The black line represents for 4-methylbenzenethiol ($d_1M^{+\cdot}$), red line for 2-naphthalenethiol ($d_1M^{+\cdot}$), and blue line for 4,4´-bis(mercaptomethyl)biphenyl ($d_2M^{+\cdot}$)

Notation for Exchanged Peaks As mentioned in our previous studies [20], the typical ions observed in (+) HDX MS analysis and their notations are as follows:

$$d_n(M^{+\cdot},\ MD^{+}) \tag{1}$$

$$M^{+\cdot} \tag{2}$$

where, (1) $d_nM^{+\cdot}$ is the exchanged radical ion and d_nMD^+ is the exchanged ion, where d_n is the number of deuterium atoms incorporated in the ion and D represents an additional deuterium ion (deuteron) attached during ionization (n = 0, 1, 2, 3…); (2) $M^{+\cdot}$ is the positive molecular ion.

Results and Discussion

APPI H/D-Exchange MS Optimization

The potential operating parameters influencing the H/D-exchange efficiency of the (+) APPI-MS conditions were investigated. The optimization was performed to increase HDX efficiency for the analysis of sulfur-containing compounds.

The parameters were evaluated according to the H/D-exchange ion abundance of the compounds. Three thiol compounds — 4-methylbenzenethiol ($d_1M^{+\cdot}$), 2-naphthalenethiol ($d_1M^{+\cdot}$), and 4,4'-bis(mercaptomethyl)biphenyl ($d_2M^{+\cdot}$) — were used for optimization of HDX-MS methods. The obtained data are summarized in Figure 1. The intensity of the most abundant HDX peak in each compound was used in the plots. The raw data to generate Figure 1 are presented in Supplementary Table S-3.

Similar to our previous studies [11, 20], CH_3OD was chosen as the deuterating reagent for HDX due to its effectiveness to exchange isotopes in CI, ESI, and APPI techniques [12, 36, 37]. Toluene is the most popular dopant for APPI because it increases the ion yield efficiency by proton transfer and/or by charge exchange reactions [38, 39]. For example, Robb et al. demonstrated the increasing ion-formation efficiency of sulfur-containing compounds (diphenyl sulfide) by using toluene in APPI [27]. In addition, the signal response of both low- and high-proton affinity compounds can be enhanced using toluene in the APPI source [27]. Hence, toluene was used as the co-solvent for APPI HDX in this study.

One of the major operating parameters in HDX-MS was the amount of deuterating agent used in the analyte solution (Figure 1a). Since we used CH_3OD in our previous HDX-experiments, we already noticed that its concentration should be minimal for increasing the exchange efficiency. Reduction of APCI and APPI signal responses by methanol addition has also been previously reported [40, 41]. Here, HDX signal intensities of different sulfur-containing compounds in the positive mode were studied to investigate the effect of the relative amount of deuterated solvent in the analyte solution on HDX efficiency. HDX efficiency was found to decrease with increasing CH_3OD concentration. Thus, 10% CH_3OD

Table 1. Major HDX Product Ions of Standard Sulfur-Containing Compounds Analyzed in (+) APPI HDX-MS

Group	Category	ID	Standard compounds	Relative abundances						
				$M^{+\cdot}$	d_0MD^+	$d_1M^{+\cdot}$	d_1MD^+	$d_2M^{+\cdot}$	d_2MD^+	d_3MD^+
I	Linear non-aromatic thiols	1	1-Butanethiol	-	-	-	100	-	99	-
		2	1-Decanethiol	-	-	100	-	-	-	-
		3	1-Undecanethiol	-	-	-	100	-	21	-
		4	1-Dodecanethiol	-	-	-	100	-	31	-
		5	1-Octadecanethiol	-	-	100	-	16	-	-
		6	1,3-Propanedithiol	-	-	-	-	100	-	-
		7	1,4-Butanedithiol	-	-	-	-	-	-	-
		8	1,8-Octanedithiol	-	-	-	-	-	100	-
	Cyclic non-aromatic thiols	9	Cyclohexanethiol	-	-	-	100	-	56	46
		10	Cyclopentanethiol	-	-	-	100	-	-	-
	Aromatic mono/di/tri-thiols	11	Benzylmercaptan	-	-	-	-	-	-	-
		12	4-Methylbenzenethiol	-	-	100	-	-	-	-
		13	4-tert-Butylbenzylmercaptan	-	-	-	100	-	53	12
		14	p-Xylene-alpha-thiol	-	-	-	100	-	32	16
		15	2-Naphthalenethiol	-	-	100	-	-	-	-
		16	1,4-Benzenedimethanethiol	-	-	-	-	-	-	-
		17	Benzene-1,2-dithiol	-	-	21	-	100	-	-
		18	4,4'-bis(mercaptomethyl)biphenyl	-	-	64	-	100	-	-
II	Thiophenes	19	Dibenzothiophene	100	-	-	-	-	-	-
		20	4,6-Dimethyldibenzothiophene	100	-	-	-	-	-	-
		21	Tetra-phenylthiophene	33	100	-	-	-	-	-
		22	Tetrahydrothiophene	-	100	-	-	-	-	-
		23	2-Methylthiophene	-	100	-	-	-	-	-
		24	3-Hexylthiophene	100	25	-	-	-	-	-
	Sulphides	25	Dibutyl Sulfide	-	100	-	-	-	-	-
		26	Ethyl phenyl sulfide	100	-	-	-	-	-	-
		27	Phenyl vinyl sulfide	100	26	-	-	-	-	-
		28	1,2-Benzenediphenylene Sulfide	100	-	-	-	-	-	-
	Disulphides	29	Dipropyl disulfide	100	-	-	-	-	-	-
		30	Dibenzyl disulfide	100	31	-	-	-	-	-
		31	Phenyl disulfide	100	-	-	-	-	-	-

was selected in this study to observe the exchanged peak with minimum signal reduction in (+) APPI-HDX MS.

In order to understand the effect of flow rate of the analyte solution on the signal intensities, four different flow rates (between 10 and 200 μL/min) were used (Figure 1b). The signal intensities increased sharply up to a flow rate of 100 μL/min, beyond which they decreased gradually. Therefore, 100 μL/min was chosen as the best analyte solution flow rate for all sulfur-containing compounds.

To establish the optimum capillary temperature for the analysis of sulfur-containing compounds, the capillary temperature was varied in the range of 100–400 °C. It was observed that increasing the capillary temperature can increase the HDX efficiency (Figure 1c), which agreed well with previously reported HDX data [42, 43]. All the test compounds showed increased HDX efficiency when the temperature was raised from 100 °C, and thus 300 °C was considered as optimum.

Table 2. Major Type of HDX Product Ions Summarized for Sulfur-Containing Compounds in (+) APPI HDX-MS

Group I (Thiols/mercaptans)			Group II (Thiophenes, sulfides, disulfides)
Aliphatics (linear)	Aliphatics (cyclic)	Aromatics	
$d_nM^{+\cdot}/d_nMD^+$			$M^{+\cdot}/d_0MD^+$

The influence of the vaporizer temperature was screened for the HDX efficiency in the range of 100–500 °C (Figure 1d). Similar to APCI, APPI occurs in the vapor state [44]; therefore, the vaporizer temperature should be as high as possible to vaporize the analyte more effectively. The HDX efficiency increased significantly with increasing vaporizer temperature from 100 °C to 400 °C and then dropped sharply at 500 °C, which matched well with our previous studies [11, 20]. Therefore, 400 °C was used as the optimum vaporizer temperature for all compounds in the (+) APPI interface.

The optimized experimental conditions for the (+) APPI-HDX MS analysis of standard compounds are summarized and presented in Supplementary Table S-4.

Analysis of Standard Compounds

Thirty-one standard sulfur-containing compounds were analyzed under the optimized conditions of (+) APPI HDX-MS. All the compounds were divided into two categories according to their functional groups (Supplementary Table S-1) and each compound was given an identification number (ID)

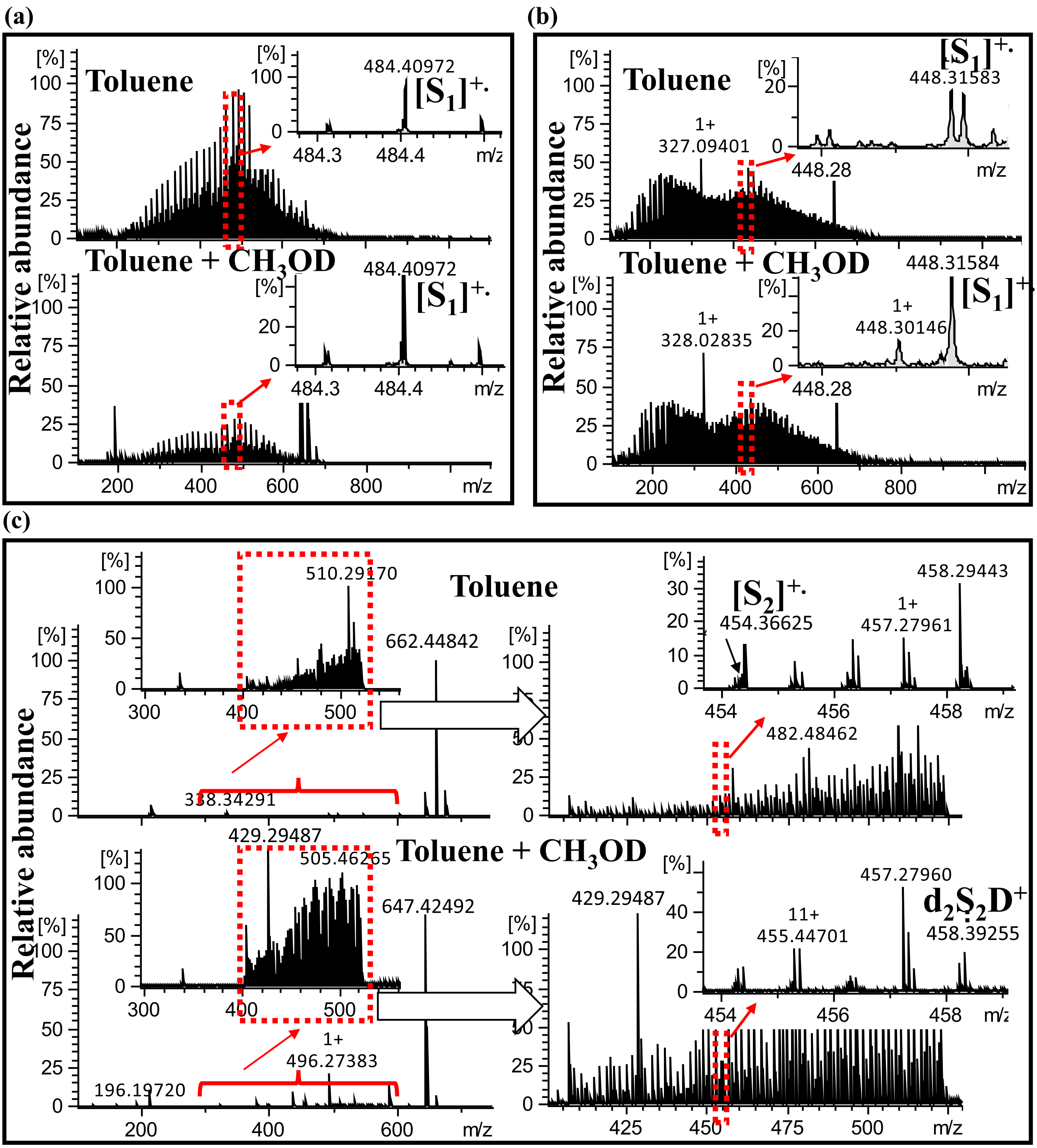

Figure 2. (+) FTICR APPI HDX-MS broadband and narrowband (inset) spectra of isolated oil fractions, where (**a**) first fraction, (**b**) second fraction, and (**c**) third fraction analyzed in toluene (top) and toluene with CH_3OD solvents (bottom)

(Supplementary Table S-1 and Table 1). All the mass spectra with calculated signal-to noise (S/N) ratios of the major HDX product ions obtained from (+) APPI HDX MS are presented in the supporting information (Supplementary Figure S-1). The relative abundances of the major HDX product ions are tabulated in Table 1.

Group I The thiols or mercaptans listed from #1 to #18 in Table 1 belong to group I. They contain the R-SH functional group, where R is an alkyl, cycloalkyl, or aryl group connected to the S-atom directly or through alkyl groups. This group comprises aliphatic (including linear and cyclic) and aromatic thiols, all of which possess one or multiple –SH functionalities.

Most of the aliphatic linear thiols with one –SH group were ionizable by (+) APPI HDX-MS (compounds #1–5 in Table 1 and Supplementary Figure S-1). As a result, the major product ion was $d_1M^{+\cdot}$ or d_1MD^+. For example, compound #3 or 1-undecanethiol (exact mass: 188.1598) produced mainly d_1MD^+ ion at *m/z* 191.1794. Conversely, linear thiols with two –SH groups (compounds #6–8 in Table 1 and Supplementary Figure S-1) produced d_2M^+ or d_2MD^+ ion.

Cyclic aliphatic thiols having one thiol group were also characterized by a dominant d_1MD^+ species (compounds #9 and #10 in Table 1 and Supplementary Figure S-1). The single strong exchanged peak indicated the presence of one –SH group. Aromatic thiols having one –SH group showed intense peaks corresponding to $d_1M^{+\cdot}$ or d_1MD^+ ion in (+) HDX (compounds #12–15 in Supplementary Figure S-1 and Table 1). Thus 2-naphthalenethiol (compound #15 in Supplementary Figure S-1; exact mass: 160.034668) showed abundant $d_1M^{+\cdot}$ at *m/z* 161.0393, indicating one HDX at the –SH group. No signal response was found for compound #11.

Aromatic thiols having two –SH groups contain two exchangeable hydrogens (compounds #16–18 in Supplementary Figure S-1 and Table 1). Thus, these compounds produced $d_2M^{+\cdot}$ ion, which exhibits HDX on all the –SH groups in (+) HDX. Compound #18 produced abundant $d_2M^{+\cdot}$ ion denoting two HDX steps in positive mode (Supplementary Figure S-1 and Table 1). The only exception was compound #16, which was not ionized by (+) APPI-HDX. For most compounds listed in Table 1, the number of exchanged peaks agreed well with the number of –SH groups.

It should be noted that the exchange of non-labile hydrogen was noticed for linear, cyclic, and aromatic thiols (Table 1). Linear thiols showed one additional exchange (d_2MD^+ ion for #1, #3, and #4; $d_2M^{+\cdot}$ ion for #5 in Table 1), when cyclic and aromatic thiols showed two additional exchanges (d_2MD^+ and d_3MD^+ ions for #9, #13, and #14 in Table 1). Although there were some exchanges of non-labile hydrogens for a number of thiols, the effect could be negligible considering their lower abundances compared with the major HDX ions obtained from the –SH groups. However, compound #1 was exceptional as it showed d_2MD^+ ion as abundant as the d_1MD^+ ion.

Group II The non-acidic sulfur compounds containing the RSR or RSSR functional groups (#19 to #31 in Table 1 and Supplementary Figure S-1) belong to group II. This group includes thiophenes or cyclic sulfides, dialkyl sulfide, alkyl aryl sulfides, and diaryl sulfide molecules, which contain no exchangeable hydrogen in their structures. $M^{+\cdot}$ ion was mainly observed from these compounds (Table 1).

The molecular ion was formed by photoionization or charge exchange reaction, similar to previous studies of thiophene compounds [45]. Although these compounds were diluted with deuterating agents during the analysis, the appearance of a dominant positive molecular ion confirmed no HDX occurred, even at the non-labile site of their structures.

Instead of the molecular ion, abundant d_0MD^+ ion was observed for compounds #21, #22, #23, and #25 (Supplementary Figure S-1 and Table 1). In particular, the addition of more aromatic rings to the thiophene structures increased the sensitivity of the thiophene compounds (compounds #19–#21 in Supplementary Figure S-1).

Summary of results obtained with standard compounds

In general, the exchanged ion ($d_nM^{+\cdot}$/d_nMD^+), molecular ion ($M^{+\cdot}$), and/or deuterated ion (d_0MD^+) produced during HDX-MS analysis are referred to as HDX product ions. The major HDX product ions obtained from (+) APPI-HDX MS are summarized in Table 2. For nonaromatic and aromatic compounds with n –SH groups (group I), the d_nM^+/d_nMD^+ ions can be used as indicators for the number of –SH groups in the molecules (Table 2). Therefore, the d_nM^+/d_nMD^+ ions can effectively identify the number of –SH groups in nonaromatic and aromatic thiols.

Application of API-HDX for Sulfur Speciation of Crude Oil

Three fractions, collected after the separation of high-S-content oil samples by ligand exchange chromatography, were analyzed by (+) APPI-HDX MS. These three fractions mostly contain thiophenes, sulfides, and mercaptans [33]. The time and *m/z* domain spectra with and without added CH_3OD are shown in Figure 2. Figure 2a and b represent the broad band and expanded spectra of first and second fractions. After switching from toluene to toluene + CH_3OD), no increase of mass (refer to expanded spectra in Figure 2a and b) in sulfur-compounds was observed in the overall spectra. Thus, the results demonstrate that H/D-exchange does not take place during ionization of the sulfur compounds present in the first and second fractions. For the third fraction (Figure 2c), the selected *m/z* range ($400 < m/z < 530$) was focused to increase the dynamic range of analysis [46], which was necessary to detect compounds with thiol functionalities. In the expanded spectra of Figure 2c, the difference between the mass for $M^{+\cdot}$ ion (without added CH_3OD) and d_2MD^+ ion (with added CH_3OD) ion was 4.0263, which was assumed to be the exchange of two H-atoms by two D-atoms (2×1.0063) followed by the addition of one D-atom. Thus the H/D-exchange was confirmed by the calculation of non-exchanged mass and their presence in the without added CH_3OD spectra.

Figure 3 shows the major HDX ions produced in the isolated fractions. The most abundant ion observed in the first and second fractions was the $[S_1]^{+\cdot}$ molecular ion (Figure 3a). Thus, the absence of abundant exchanged peaks ($[d_nS]^{+\cdot}$ or $[d_nSD]^+$) clearly indicated that no HDX occurred (refer to Figure 2a and b). According to Table 1, the $[S_1]^{+\cdot}$ molecular ion was produced mainly from group II compounds, which do not have

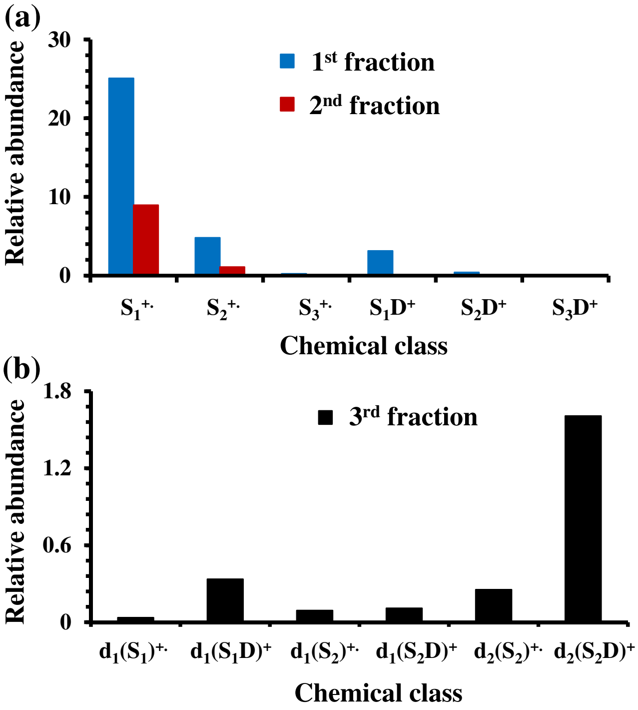

Figure 3. Major chemical classes observed in the isolated oil fractions, (**a**) first fraction (blue), second fraction (red), and (**b**) third fraction (black), analyzed by (+) APPI HDX FT-ICR MS

exchangeable hydrogen (e.g., thiophenes, sulfides, and disulfides).

In the last (third) fraction, although thiols are present in low concentrations, mostly H/D-exchanged $d_1[S_1D]^+$ and $d_2[S_2D]^+$ ions were detected. The $d_1[S_1D]^+$ ion means one HDX with the addition of one deuterium atom and $d_2[S_2D]^+$ ion means two HDXs with the addition of one deuterium atom. Thus the observation of the $d_1[S_1D]^+$ ion indicates the presence of one exchangeable H atom attached to one –SH group, and the $d_2[S_2D]^+$ ion represents two exchangeable H atoms attached to two –SH groups (Figure 3b). The intensity of the $d_2[S_2D]^+$ ion peak was much higher than that of the $d_1[S_1D]^+$ ion peak. The *m*/*z*, mass accuracy, and signal-to-noise ratio of the peaks assigned to $d_1[S_1D]^+$ and $d_2[S_2D]^+$ ions are listed in Supplementary Tables S-5 and S-6)

The double bond equivalents (DBE) distributions of intense sulfur-containing peaks observed in each fraction are presented in Figure 4. The structures of the sulfur-containing compounds can be constructed based on the DBE distribution and characteristics of fractions. The examples of the possible structures are suggested based on the data presented in Figure 4 and previous studies [33, 47–49]. In the first fraction, abundant DBE starts from 6, indicating benzothiophenic structures [33], and DBE 8, 9, and 10 were most abundant in the second fraction, corresponding to the presence of diphenyl sulfide structures [49] (Figure 4a). In Figure 4b, higher DBE values 9 and 10 are dominant for $d_1[S_1D]^+$ ions, suggesting highly aromatic thiols (i.e., biphenyl-type structures). Lower DBE values 0, 1, and 2 are dominant for $d_2[S_2D]^+$ ions, suggesting nonaromatic dithiols. The DBE 0 should correspond to alkyl thiols. From literature studies, DBE 1 and 2 could be assigned to examples of the alkenyl or to one-ring and two-ring naphthenic thiols, respectively [47].

Conclusion

In this report, thiol-type compounds underwent exchange of the active hydrogen on the –SH group by optimized (+) APPI HDX-MS. This method was tested by the analysis of 31

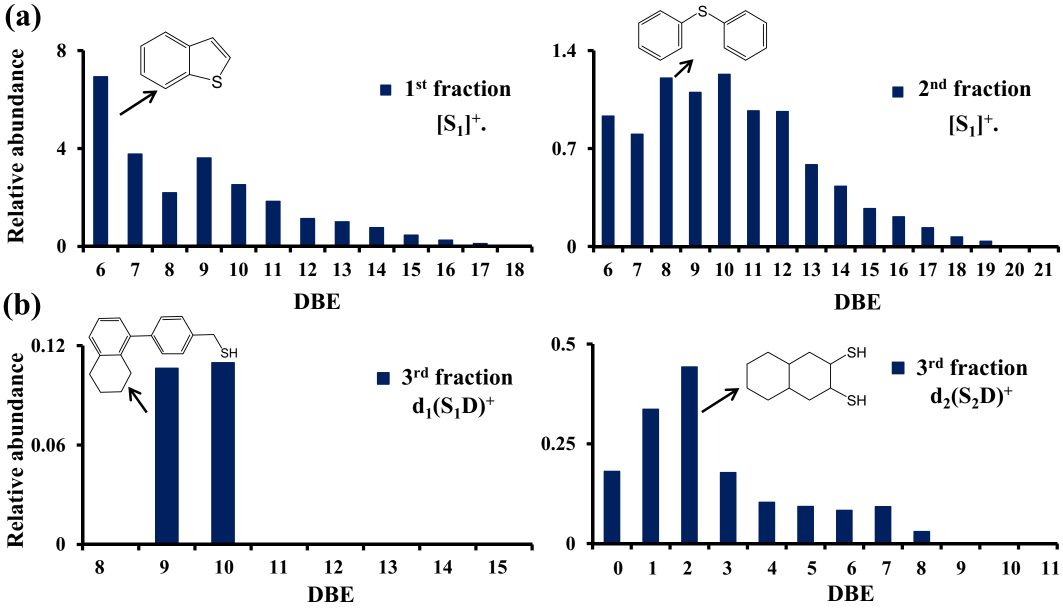

Figure 4. DBE distributions plots (along with the examples of possible structures) with abundant HDX product ions obtained from three different fractions of oil, (**a**) $[S1]^{+\cdot}$ ion for first (left) and second (right) fraction; (**b**) $d_1(S_1D)^+$ (left) and $d_2(S_2D)^+$ (right) ion for third fraction

standard sulfur-containing compounds with different structures. Using 10% deuterated methanol, H/D-exchanges were observed for thiols when the other compounds did not show any H/D-exchange. Based on the major H/D-exchanged ions produced, it has been demonstrated that thiols can be differentiated from other types of sulfur-containing compounds like thiophenes and sulfides. The information provided in this study could be used for speciation of sulfur-containing compounds in crude oils. We expect that the method described in this study can be used to resolve the problem of internal corrosion in refineries or oil transportation, caused by mercaptans.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no competing financial interest.

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