GC-MS and ESI-MS detection of catechol

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Abstract
The study aimed at the detection of catechol using GC-MS and ESI-MS. Catechol is an excellent starting material for oxidative polymerization since it contains the very reactive two hydroxyl groups in ortho-position, like other polyphenols in nature. Polymerization of catechol is known to happen under oxidative conditions or by use of a catalyst. Catechol was silylated with BSTFA-TMCS shortly before being analyzed by GC-MS.
The results obtained indicate that catechol polymerizes immediately on its own under ambient conditions (without oxygen saturation or pH adjustment) forming a dimer and trimer. Results from ESI-MS confirm the formation of dimers and trimers. The combination of these two techniques led to the proposal of plausible molecular structures. These structures are characterized by the presence of ether- and hydroxyl functional groups. Effect of two solvents; water and methanol was investigated whereby water yielded less mass fragments in the GC-MS analysis as compared to methanol. The results obtained are of great importance since catechol is a key reagent in most of the syntheses. Since polyphenols are present in the environment, it could be playing a vital role in dissolution, transport and complexation of metals.

Key words:
Catechol, derivatization, GC-MS, ESI-MS, silylation, BSTFA-TMCS.

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1. Introduction

Catechol, $C_6H_6O_2$ has been widely used as the starting molecule for the synthesis of fulvic/humic acid-like compounds, FALC/HALC (Dubey et al., 1998; Sanchez-Cortes et al., 2001; Aktas et al., 2003; Jung et al., 2005; Smejkalova et al., 2006). It is an excellent starting material for oxidative polymerization since it contains the very reactive two hydroxyl groups in ortho-position, like other polyphenols in nature (Sanchez-Cortes et al., 2001). Polymerization of catechol is known to happen under oxidative conditions or by use of a catalyst. Some studies have been reported about the detection of catechol by Gas chromatography-mass spectrometry (GC-MS) and/or Electrospray ionisation mass spectrometry (ESI-MS) (Šmejkalova *et al.*, 2006; Lourenço *et al.*, 2006; Moldoveanu and Kiser, 2007). However, in all these studies, silylation was undertaken while dissolution was in other solvents (methanol, pyridine, dichloromethane) but not in water.

Electrospray ionisation mass spectrometry (ESI-MS) and Gas chromatography-mass spectrometry (GC-MS) are among the most reliable analytical methods. ESI-MS as a method has a lot of advantages as it takes place at atmospheric pressure, ionizes a wide range of polar, hydrophilic molecules with both acidic and basic functional groups, and can be operated in the positive or negative ion mode. Since the samples for ESI are prepared in water or made up in a mixture of water and/or simple, low-molecular-weight organic solvents that evaporate during ESI, solvent-generated ions do not interfere with the mass spectral information generated for the substances being analyzed (Gaskell, 1997).

GC-MS has the synergistic combination of two powerful analytical techniques; the chromatograph that separates the components of a mixture as a function of time, and the mass spectrometer which provides information that aids in the structural identification of each component. In order to have high-resolution GC for the analysis of catechol, the sample must be derivatized. There are several reagents that can be used for derivatization, though silylation is the most widely used derivatization procedure for sample analysis by GC (Quintana *et al*., 2004; Zhang and Zuo, 2005; Šmejkalova *et al.*, 2006).

In this method, active hydrogen is replaced by an alkylsilyl group. Use of BSTFA-TMCS (99/1) (v/v) as silylation reagent is advantageous because of its fast reactivity with compounds containing hydroxyl groups, its high volatility resulting in non co-elution of early eluting peaks, low thermal degradation and good solubility in common organic solvents of the derivatized compounds. As a result, GC separation is improved and detection is enhanced. The molecular formula of BSTFA is $CF_3C{=}NSi(CH_3)_3OSi(CH_3)_3$, and for TMCS it is $ClSi(CH_3)_3$. However, certain functional groups can form some unexpected derivatives from silylation reagents and their by-products. Little (1999) reported artefacts in trimethylsilyl derivatization reactions. The mechanism of BSTFA-TMCS silylation is as shown in **Figure 1**.

The aim of this present paper was to show the immediate polymerization of catechol whether in milli-Q water or methanol at ambient conditions, through detection by GC-MS and ESI-MS. The findings got reveal detection of more molecular ions as than has been reported in literature. The effect of water as a dissolving solvent (instead of methanol) for catechol was also tested. The results show that polymerization of catechol takes place without any form of catalysis and is spontaneous.

2. Experimentation

2.1. Reagents and standards

All chemicals were of analytical reagent grade and were used without any further purification. Catechol Reagent Plus ($\geq$ 99 %) was supplied from *Sigma Aldrich*. For the dilutions, we used methanol of HPLC isocratic grade, supplied from *VWR Prolabo,* and ultra pure water purified with a Milli-Q academic (18.2 MΩ.cm^{-1} Millipore *S.A.S*). For GC-MS analysis, we used a mixture of *N,*

O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (99:1, v/v), supplied by *Supelco* in a chloroform solvent as the silylation reagent. The standard solution used was a 10 ppm *n*-alkanes (C_8 to C_{40}) in a chloroform solvent supplied from *Accu Standard Inc*. For ESI-MS, the calibration standard used was cluster of Li formiate. 10 mM LiOH (hydrated from Aldrich) were diluted in isopropanol:water (50:50, v:v)

2.2. Gas chromatography-mass spectrometry (GC-MS) analysis

2.2.1. Sample preparation and derivatization

Each catechol sample was ground to fine powder and then put in clear vials. A solution of 2.21 $mg.mL^{-1}$ of catechol in methanol and a solution of 2.06 $mg.mL^{-1}$ of catechol in Milli-Q water were prepared. 100 µL of the previous solutions were drawn and evaporated to dryness under nitrogen flux, followed by addition of 100 µL of BSTFA-TMCS (99:1, v/v). The vials were closed and slightly heated at 60 °C for 15 minutes. Once the derivatization process was complete, 1 µL of the reaction mixture was injected into the GC-MS system. Blanks and BSTFA-TMCS reagent samples were also analyzed using the same protocol to help in the interpretation of the spectral data. All the sample preparations and analyzes were performed in triplicate to ensure good reproducibility of the data.

2.2.2. GC-MS analyses

The measurements of samples (derivatized catechol, blanks and BSTFA-TMCS solutions) were conducted on a Hewlett Packard HP6890 series GC-MS instruments. 1 µL of the sample was injected using the splitless injection mode, which was held at 300°C and 1.6 bars, and a capillary column (J & W DB-5 capillary 60 m length x 250 µm i.d x 0.10 µm film thickness) was used for analytical separation. Helium was used as a carrier gas at a flow rate of 1 $mL.min^{-1}$. The oven was temperature-programmed from 60 to 130°C at a rate of 15°$C.min^{-1}$, then ramping from 130 to 315°C at a rate of 3°$C.min^{-1}$ and finally held there for 15 minutes. The mass spectrometer operated in a full scan mode in the range of m/z 50-550 and by electron impact ionisation energy of 70 eV. It was calibrated by using a manual tune which determined the relative abundances and the isotopic ratio of the main ions coming from the PFTBA (perfluorotributylamine) fragmentation: m/z = 69, 219, 502. Before and after each series of analyses, the GC-MS instrument was calibrated by using a 10 ppm *n*-alkanes (C_8 to C_{40}) standard solution in a chloroform (or in a dichloromethane) solvent. Blanks were analysed, followed the same path of analyses as sample from the sampling point to the injection point in the GC-MS, under the same conditions throughout. In order to know the ions resulting from the silylation reagent, a BSTFA-TMCS *Supelco* (99/1) (v/v) mixture in chloroform was analyzed: 50 µL of BSTFA-TMCS (99-1) *Supelco* solution were diluted in 50 µL of chloroform solution ($CHCl_3$) and analyzed. Compounds were identified using the *Standalone software*.

2.3. Electrospray ionization-mass spectrometry (ESI-MS) analysis

Sample solutions were diluted in milli-Q water or in methanol with a 10^{-2} molarity. They were analyzed using a Bruker micrOTOF-Q equipped with an ESI source operating in negative mode, with a capillary voltage of 4.5 kV. Desolvation gas flow and temperature were set at 240 $L.h^{-1}$ and at 190°C respectively. The acquisition range was 50–3000 Da. The sample was injected via a syringe pump with a flow rate of 180 $\mu L.h^{-1}$. ESI interface tuning and mass calibration were accomplished in negative modes by using cluster of LiFormiate.

3. Results and Discussion

3.1. GC-MS analyses

GC-MS analysis of intermediates/products was based on the detection limit of m/z 550.

3.1.1. Analysis of BSTFA-TMCS solution in a chloroform solvent

Ions observed resulting from contaminants were identified in blank runs and were discarded in the interpretation of the chromatograms. The total ion chromatogram of the BSTFA-TMCS solution show seven peaks with the following retention time; 7.2, 7.8, 7.9, 8.4, 9.1, 10.6, and 20.2 minutes with molecular ion masses m/z = 187, 309, 336, 355, 369, and 443 (**Figure 2a & b**). This reagent solution yielded ions at m/z = 69 [CF_3^+], 73 [$Si^+(CH_3)_3$], 77 [$Si^+(CH_3)_2F$], 100, 103, 135, 147 [$(CH_3)_2Si = O^+Si(CH_3)_3$], 207, 221, 251, 281, 295, 309, and 369 (**Table 1**). There are no reported mass spectra in the commercial database for BSTFA-TMCS reagent. Little (1999) listed the mass spectra of major ions for different trimethylsilyl derivatives. In the case of TMS derivative of disilicic acid, we have six fragments (m/z 73, 147, 207, 221, 281, and 295) in common as seen in **Table 2**.

Several artefacts caused by the derivatization reagent were observed in the GC-MS chromatograms. This observation has earlier been noted by Little (1999). BSTFA used by itself does not generate artefacts with carboxylic acids, but it does with phenol. It cannot derivatize totally the phenol functional group hence producing artefacts. Incomplete silylation of compounds lead to multiple peaks hence affecting the determination of the number of components present in a sample. Little (1999) suggested that BSTFA should be used with DMF to have complete derivatization for phenol.

3.1.2. Effect of solvent on the analysis of catechol

3.1.2.1. Catechol diluted in methanol prior to silylation reaction

Five molecular ions with m/z values of 187, 309, 336, 355, and 369 were observed to be common in both the samples of silylated catechol and BSTFA-TMCS solution, hence were not considered in interpreting the catechol spectra. The total ion chromatogram show two main peaks at the retention time 10 and 20 minutes. The later is due to BSTFA-TMCS, with the same mass spectrum as that observed in BSTFA-TMCS reagent. The former gives a mass spectrum which has two peaks at m/z = 73 and 254 whereby m/z = 73 is trimethylsilane, TMS [$Si(CH_3)_3$] while m/z = 254 the molecular ion corresponding to the catechol monomer coupled with two TMS (bis(trimethylsilylated)catechol), see **Figure 2e**.

Šmejkalova *et al.* (2006), Lourenço *et al.* (2006) and Moldoveanu and Kiser (2007) studied catechol using GC-MS and reported the molecular ion m/z 254 at the retention time of 10.20 (Lourenço *et al.*, 2006) and 13.88 (Moldoveanu and Kiser, 2007) minutes. Šmejkalova *et al.* (2006) observed two ions specific of the silylation with m/z 73 and 147. Indeed, all derivatized substrates analysed by GC-MS yielded ions at m/z 50, 69, 73, 75, 77, 100 and 147 corresponding to CH_3Cl^+, CF_3^+, $Si^+(CH_3)_3$, $HO^+=Si(CH_3)_2$, $Si^+H(CH_3)_2F$, not resolved, and $(CH_3)_2Si=O^+Si(CH_3)_3$ fragments respectively. Moreover, they suggested that ions at m/z 136, 151, and 166 resulted from the molecular rearrangement within the fragmentation process. An observation of these fragments and other fragments was made in our samples.

Finally, twelve ions for trimethylsilyl derivatives of catechol were identified, with m/z values of 182, 240, 254, 314, 318, 328, 402, 420, 434, 476, 506, and 550 (**Table 2**). Their mass spectra and their respective structures suggested are shown in **Figure 2c-i.** Šmejkalova *et al.* (2006) reported the molecular masses of 434 and 506 Da corresponding to C-O and C-C dimers of catechol respectively. However, we suggest one other plausible structure for m/z 434 (**Figure 2g**). Lourenço *et al*. (2006) reported also the molecular ion with m/z 240, but did not suggest any structure.

Contrary to the sample preparation followed by Šmejkalova *et al.* (2006), our dried residues were not redissolved in pyridine, but directly silylated by adding BSTFA/TMCS.

3.1.2.2. Catechol diluted in milli-Q water prior to silylation reaction

In order to know if there was any reaction between catechol and methanol, a sample of catechol in water as a solvent was prepared. The same procedure as that of methanol was followed. The total ion chromatogram of the sample with water seems to be less noisy than that of methanol. It has six molecular ions with m/z values of 187, 205, 254, 258, 328, and 506. Molecular ions with m/z = 254, 328, and 506 are in common with those of the methanol solvent.

3.2. ESI-MS analyses

Catechol was diluted at 10^{-2}M in milli-Q water. The peaks analyzed are some of those peaks whose m/z values were within the GC-MS mass range of detection (**Table 3**). For these analyzed peaks we have proposed structures. Several peaks were observed: the majority is due to some clusters with water, but we focus on peaks at m/z = 109, 217, and 325. The ESI-MS spectra of pure catechol confirmed detection of catechol as a monomer (m/z = 109) and the formation of dimers and trimers with m/z values; 217 and 325 respectively (**Figure 3**). The proposed structures have molecular weight, MW = 110 (catechol), 218 (dimer of polycatechol), and 326 (trimer of polycatechol) for ions at m/z = 109, 217, and 325 respectively. Some of the proposed structures are in agreement with what has been published by Šmejkalova et al. (2006). ESI-MS spectra indicated presence of oligomers whose structures we are still working on.

4. Conclusions

GC-MS and ESI-MS analytical techniques are important for analyzing small molecules with good precision. GC-MS provides reliable results when samples are silylated before analysis. Sample preparation is crucial in order to have good results. The combination of these two techniques led to the proposal of plausible molecular structures. These structures are characterized by the presence of ether-, ester-, and hydroxyl functional groups. Different solvents need to be used in order to have better interpretation of results obtained. Our study shows that catechol polymerization is spontaneous. The case of catechol polymerization with other reagents would be investigated in detail in our forthcoming paper.

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Literature cited

Aktas N., Sahiner N., Kantoslu Ö., Salih B. and Tanyolaç A. (2003). Biosynthesis and Characterization of Laccase Catalyzed Poly(catechol). *Journal of Polymers and the Environment*, **11**, 123-128.

Dubey S., Singh D. and Misra R.A. (1998). Enzymatic synthesis and various properties of poly(catechol). *Enzyme and Microbial Technology*, **23**, 432-437.

Gaskell S.J. (1997). Electrospray: principles and practice. *Journal of Mass spectrometry*, **32**, 677-688.

Jung A.-V., Frochot C., Parant S., Lartiges B.S., Selve C., Viriot M.-L. and Bersillon J.-L. (2005). Synthesis of amino-phenolic humic-like substances and comparison with natural aquatic

humic acids: A multi-analytical techniques approach. *Organic Geochemistry*, **36**, 1252-1271.

Little J.L. (1999). "Artifacts in trimethylsilyl derivatization reactions and ways to avoid them." *Journal of Chromatography A*, **844**, 1-22.

Lourenço E.L.B.; Ferreira A.; Pinto E.; Yonamine M. and Farsky S.H.P. (2006) On-fiber derivatization of SPME extracts of phenol, hydroquinone and catechol with GC-MS Detection. *Chromatographia*, **63**, 175-179.

Moldoveanu S.C. and Kiser M. (2007) Gas chromatography/mass spectrometry versus liquid chromatography/fluorescence detection in the analysis of phenols in mainstream cigarette smoke. *Journal of Chromatography A*, **1141**, 90-97.

Quintana J.B., Carpinteiro J., Rodriguez I., Lorenzo R.A., Carro A.M., Cela R. (2004). Determination of natural and synthetic estrogens in water by gas chromatography with mass spectrometric detection. *Journal of Chromatography A*, **1024**, 177–185.

Sánchez-Cortés S., Francioso O., García-Ramos J.V., Ciavatta C. and Gessa C. (2001). Catechol polymerization in the presence of silver surface. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **176**, 177-184.

Šmejkalová D., Piccolo A. and Spiteller M. (2006). Oligomerization of humic phenolic monomers by oxidative coupling under biomimetic catalysis. *Environ. Sci. Technol*., **40**, 6955-6962.

Zhang K. and Zuo Y. (2005). Pitfalls and solution for simultaneous determination of estrone and 17 α-ethinylestradiol by gas chromatography–mass spectrometry after derivatization with *N,O*-bis(trimethylsilyl)trifluoroacetamide, *Analytica Chimica Acta* **554**, 190–196.

FIGURES AND TABLES CAPTION

Table 1: List of some major fragments and molecular ions found in the BSTFA-TMCS solutions

Our study Fragments m/z BSTFA-TMCS	Ion	**Little (1999)** Fragments m/z TMS derivative of disilicic acid
69	CF_3^+	/
73	$(CH_3)_3Si^+$ (or TMS^+)	73
77	$(CH_3)_2FSi^+$ (or TMS^+)	/
100	$CF_3C{=}O\text{-}N^+H$	/
103		/
135		/
147	$(CH_3)_2Si{=}O^+\text{-}TMS$	147
207		207
221		221
251		/
281		281
295		295
309		/
/		327
/		341
369		/
/		399
/		415
/		529
/		503
/		591

Table 2: List of the major fragments and molecular ions of catechol in comparison with those in literature.

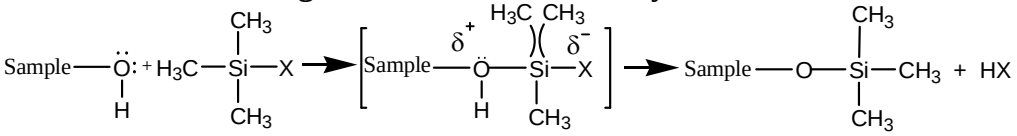
This GC-MS study		Smejkalova *et al.*, 2006	Lourenço *et al.*, 2006
in methanol	in water		
136	/	136	/
151	/	151	/
166	/	166	/
/	/	181	/
182	/	/	/
/	**187**	/	/
/	**205**	/	/
225	/	/	/
/	/	239	239
240	/	/	**240**
/		255	/
254	**254**	**254**	**254**
/	**258**	/	/
299	/	/	/
314	/	/	/
318	/	/	/
328	**328**	/	/
402	/	/	/
420	/	/	/
434	/	**434**	/
476	/	/	/
506	**506**	**506**	/
550	/	/	/

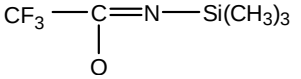
in bold: molecular ion

Table 3. Comparison of GC-MS and ESI-MS molecular ion data.

GC-MS (m/z)	ESI-MS (m/z)	Molecular Weight (bond types)
182 (mono-trimethylsilylated)	109	110
240	109	110
254 (di-trimethylsilylated)	109	110
258	109	110
434 (tri-trimethylsilylated)	215 - 217	218 (C-O-C)
506 (tetra-trimethylsilylated)	215	218 (C-C)
506 (mono-trimethylsilylated)	433	434 (both C-C & C-O-C)
550	325	326 (both C-C & C-O-C)

Figure 1: Mechanism of silylation

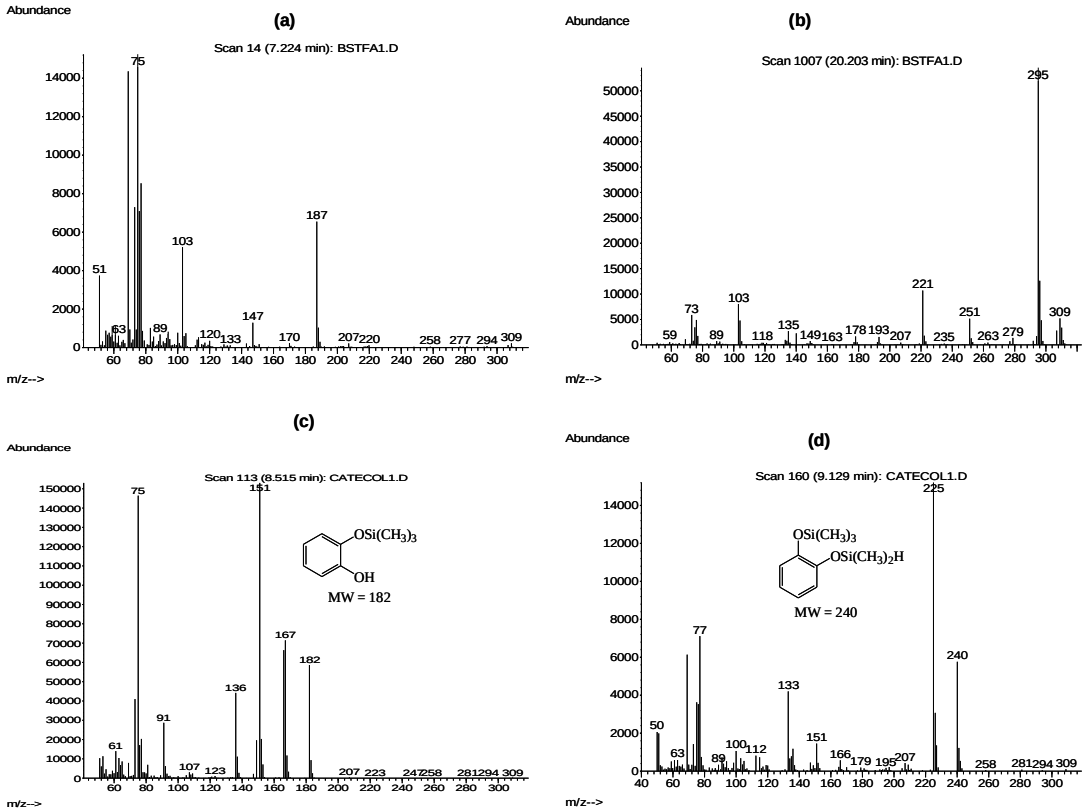


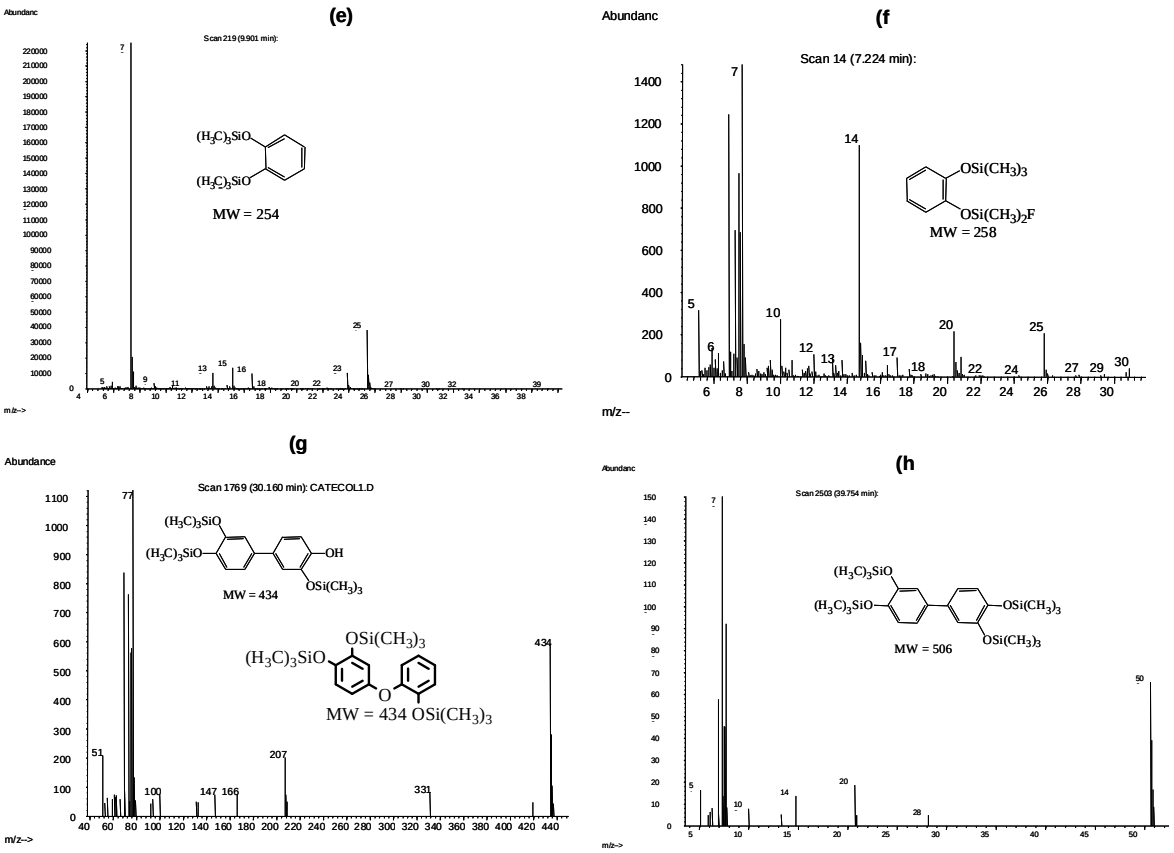
For BSTFA, 

For TMCS,

X = Cl

Figure 2: Some of the mass spectra for BSTFA-TMCS and catechol derivatives




(e)
Abundanc
Scan 219 (9.901 min):
(H3C)3SiO
(H3C)3SiO
MW = 254
m/z-->
(f
Abundanc
Scan 14 (7.224 min):
OSi(CH3)3
OSi(CH3)2F
MW = 258
m/z--
(g
Abundance
Scan 1769 (30.160 min): CATECOL1.D
(H3C)3SiO
(H3C)3SiO
OH
OSi(CH3)3
MW = 434
OSi(CH3)3
(H3C)3SiO
O
MW = 434 OSi(CH3)3
m/z-->
(h
Abundanc
Scan 2503 (39.754 min):
(H3C)3SiO
(H3C)3SiO
OSi(CH3)3
OSi(CH3)3
MW = 506
m/z-->

(i

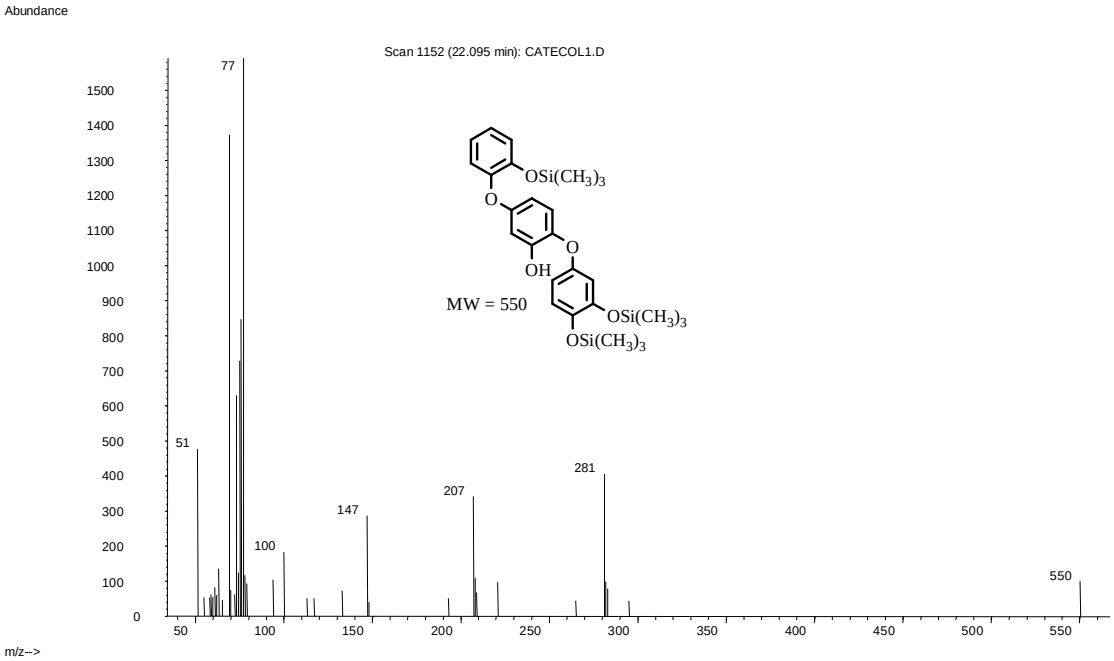


Figure 3: MS spectra of the pure catechol diluted in Milli-Q water (Negative mode, 50-3000 Da)

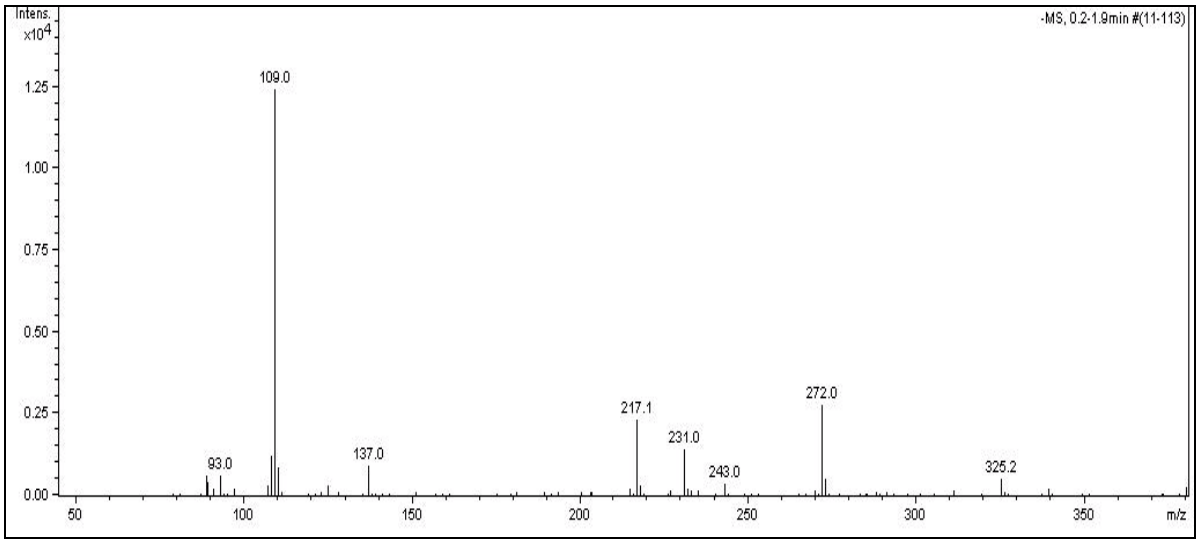


Figure 4: MS spectra of the pure catechol diluted in methanol (Negative mode, 50-3000 Da)