Analysis of non-volatile compounds by GC: Derivatization methods

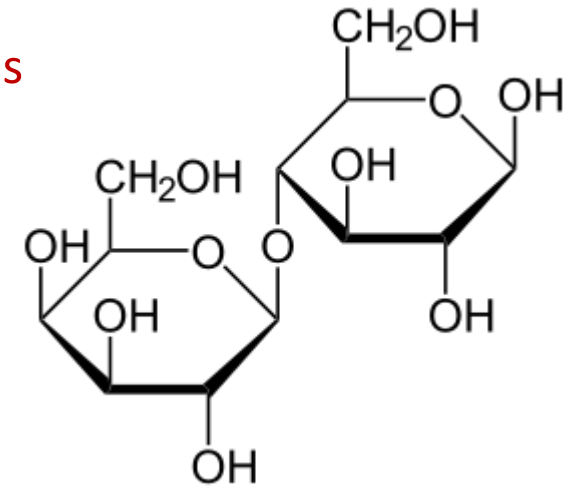
Who uses Derivatization Reagents

- Large Drug Testing Laboratories
- Forensic Laboratories
- Hospitals
- Small Testing Laboratories
- Environmental Laboratories
- Pharmaceutical Laboratories

Derivatization: process of chemically modifying a compound to produce a derivative with properties suitable for a specific analytical method.

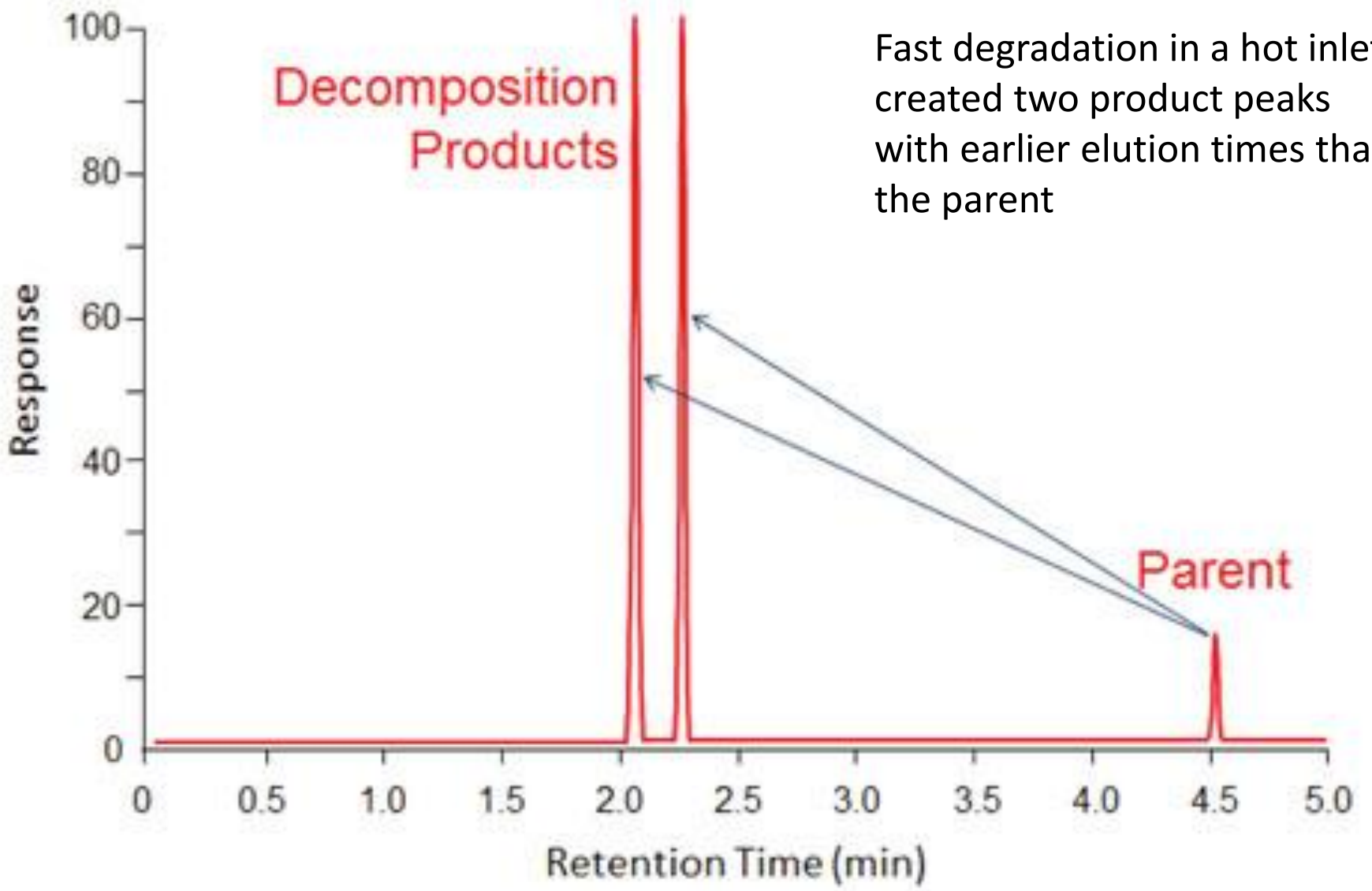
- It allows the analysis of compounds not directly amenable to GC analysis due to low volatility or thermal instability.
- It improves chromatographic behavior or detectability.
- Without derivatization, many compounds do not produce a useful chromatogram (i.e. multiple peaks, or one big “blob”).

Example: saccharides



lactose, b.p. 669 °C

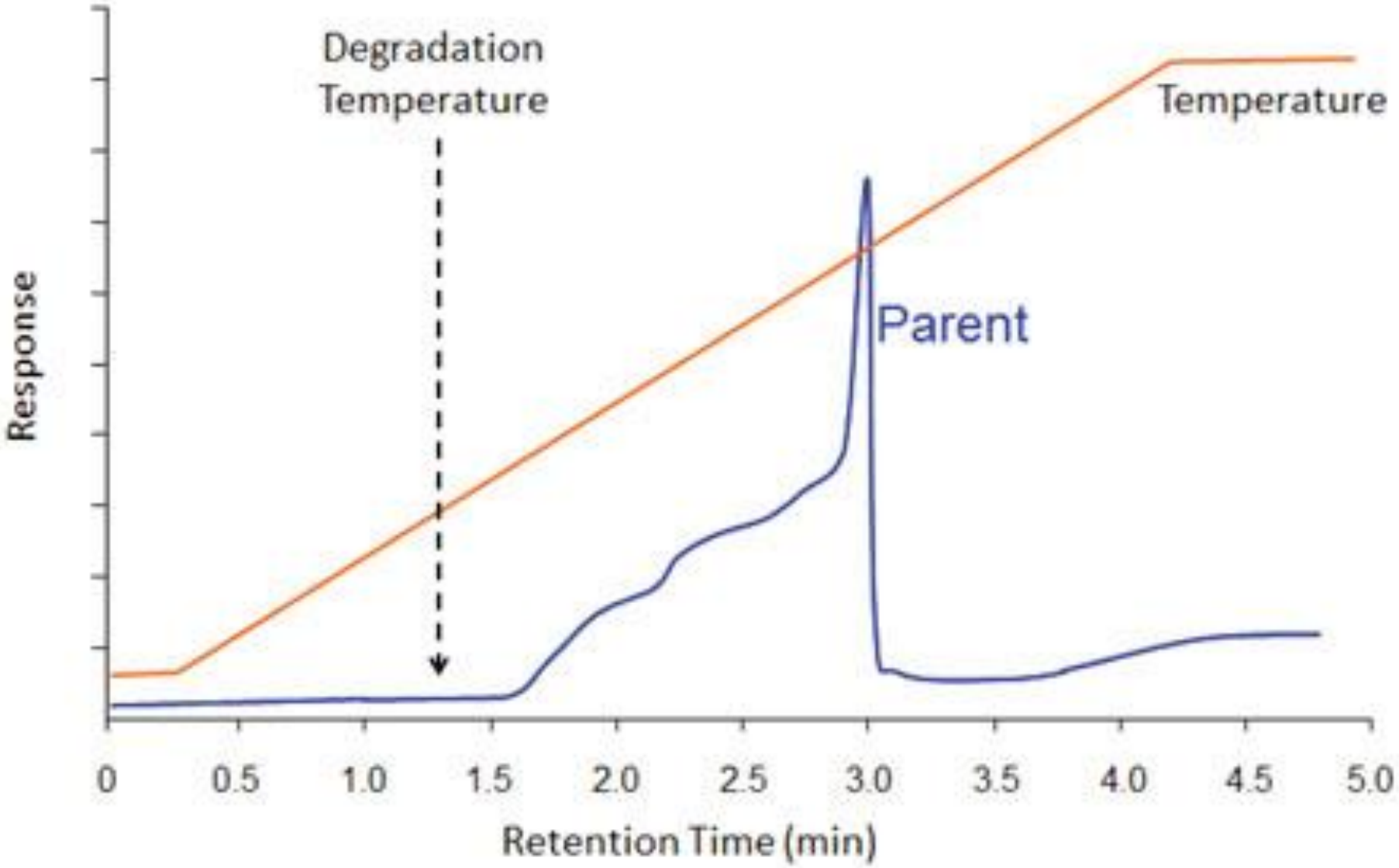
Cases where derivatization is needed (i)



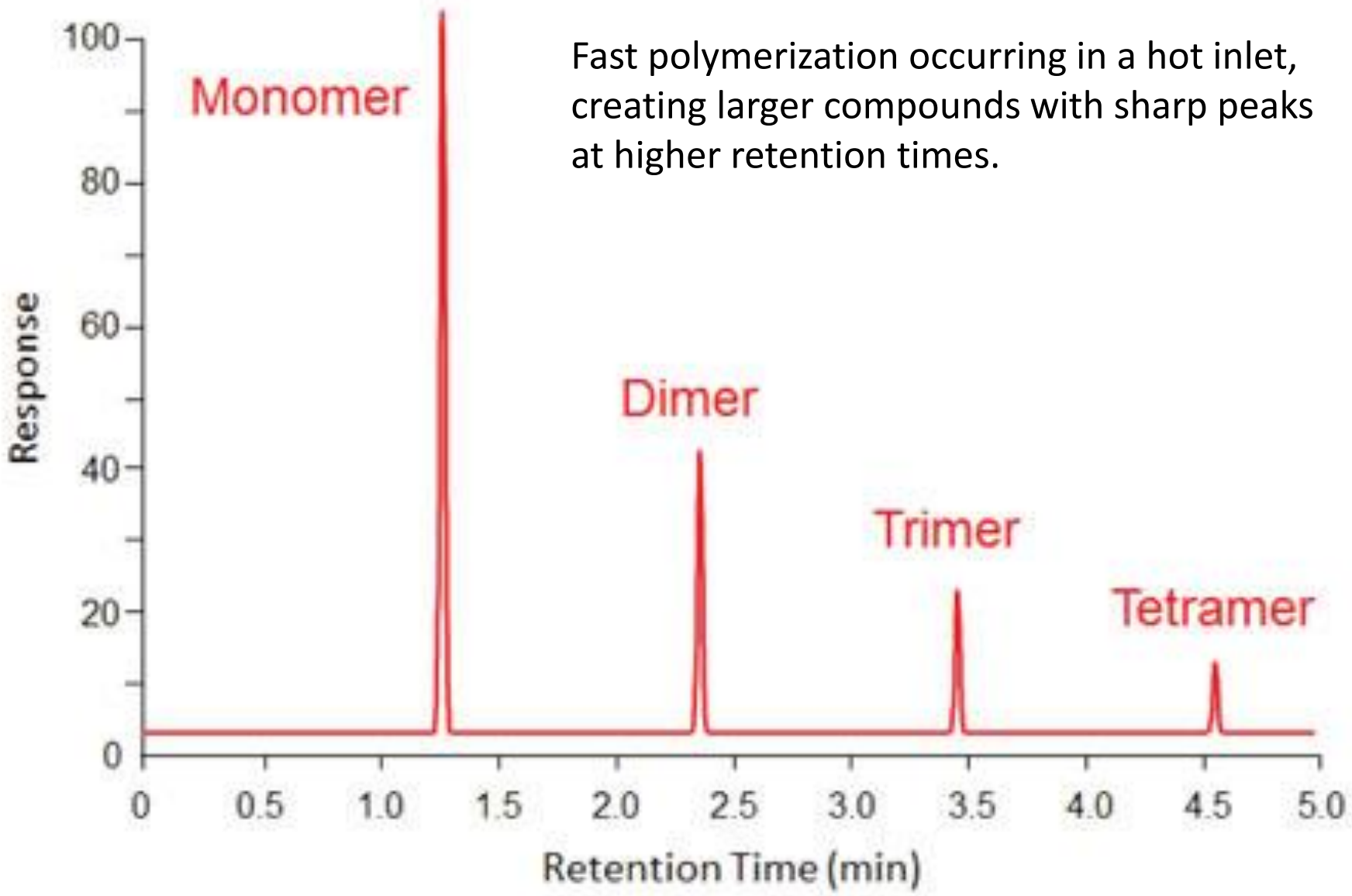
Fast degradation in a hot inlet created two product peaks with earlier elution times than the parent

Cases where derivatization is needed (ii)

Degradation to earlier eluting compounds when the degradation temperature is exceeded during the T program. Some intact parent compound still made it through the column.



Cases where derivatization is needed (iii)



Fast polymerization occurring in a hot inlet, creating larger compounds with sharp peaks at higher retention times.

Main Types of Derivatization

Silylation

– Most prevalent method in general. Replacement of the active hydrogen in R-COOH, R-OH, R-SH, R-NH, R-NH_2, R-$CONH_2$, R-CONH-R′ with a TMS (trimethylsilane) group.

Alkylation

– Replacement of the active hydrogen in R-OH, R-SH, R-NH, R-NH_2, R-$CONH_2$, R-CONH-R′ with an alkyl group, or sometimes an aryl group.

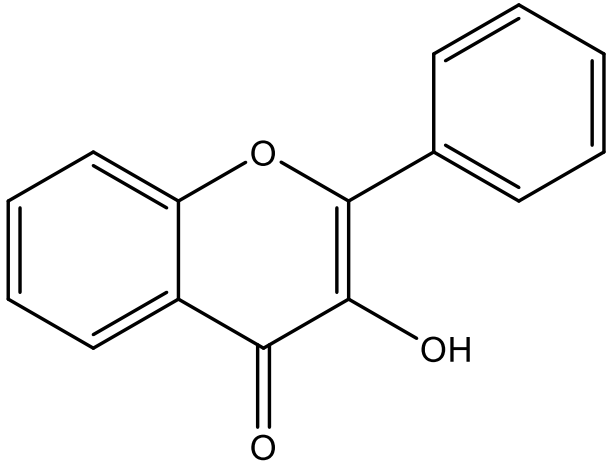
Esterification

- First choice for derivatization of carboxylic acids and other acidic functional groups. Acids are reactive compounds, too polar to be well separated by GC. Underivatized acids tend to tail because of adsorption and non-specific interactions with the column.

Acetylation

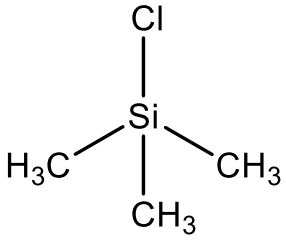
– Commonly used to add chlorinated or fluorinated groups (ECD)

Silylation

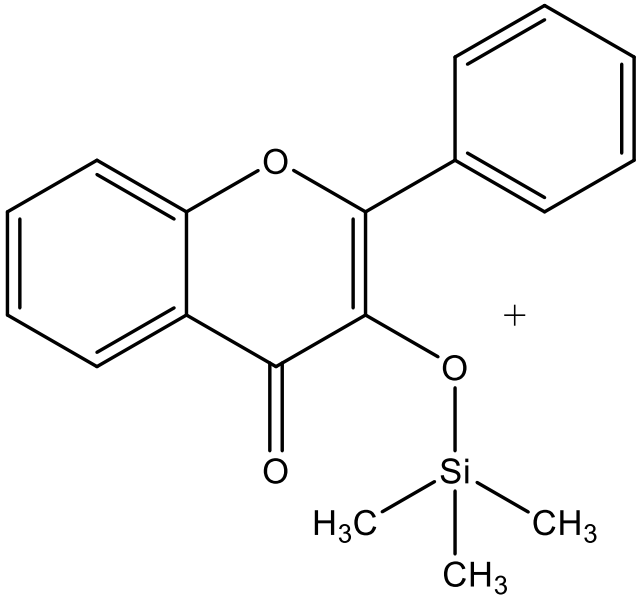


Flavonol (decomposes upon heating)

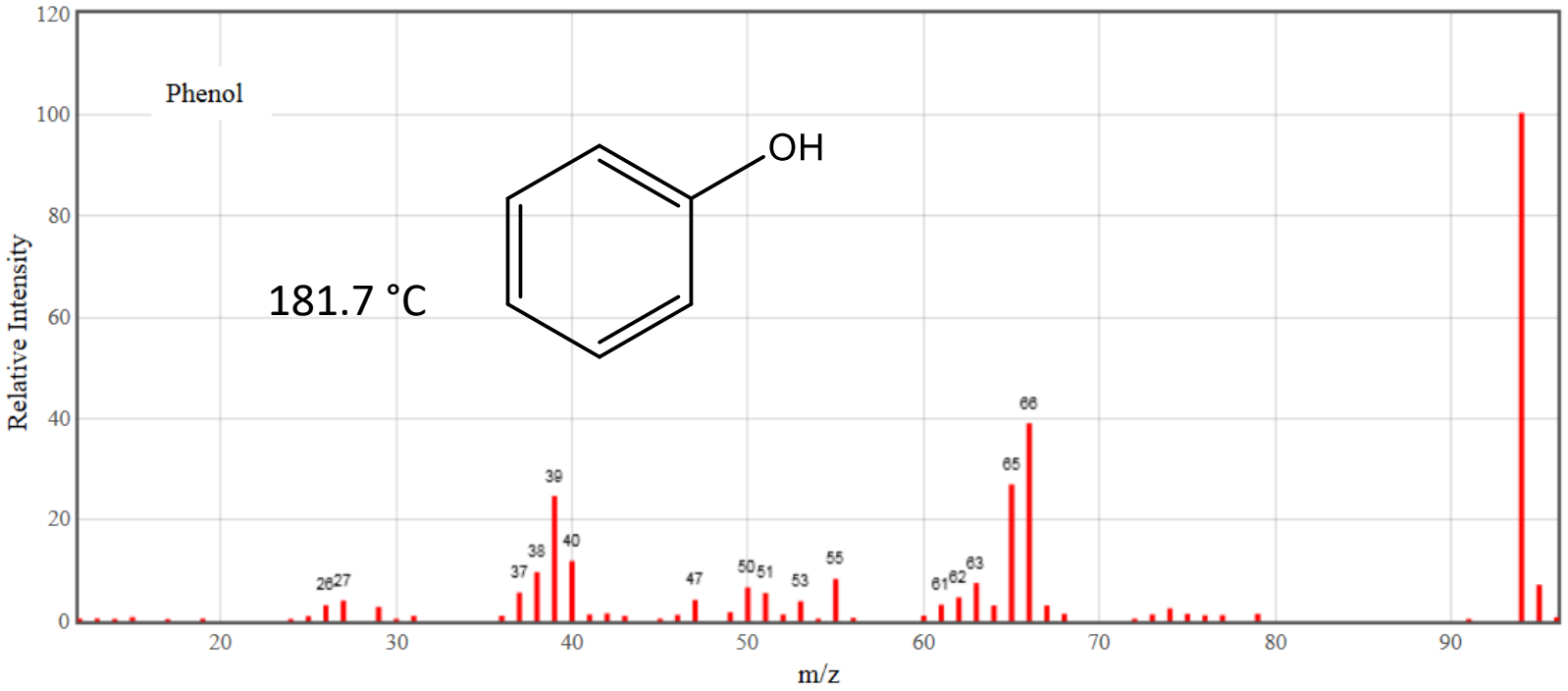
+

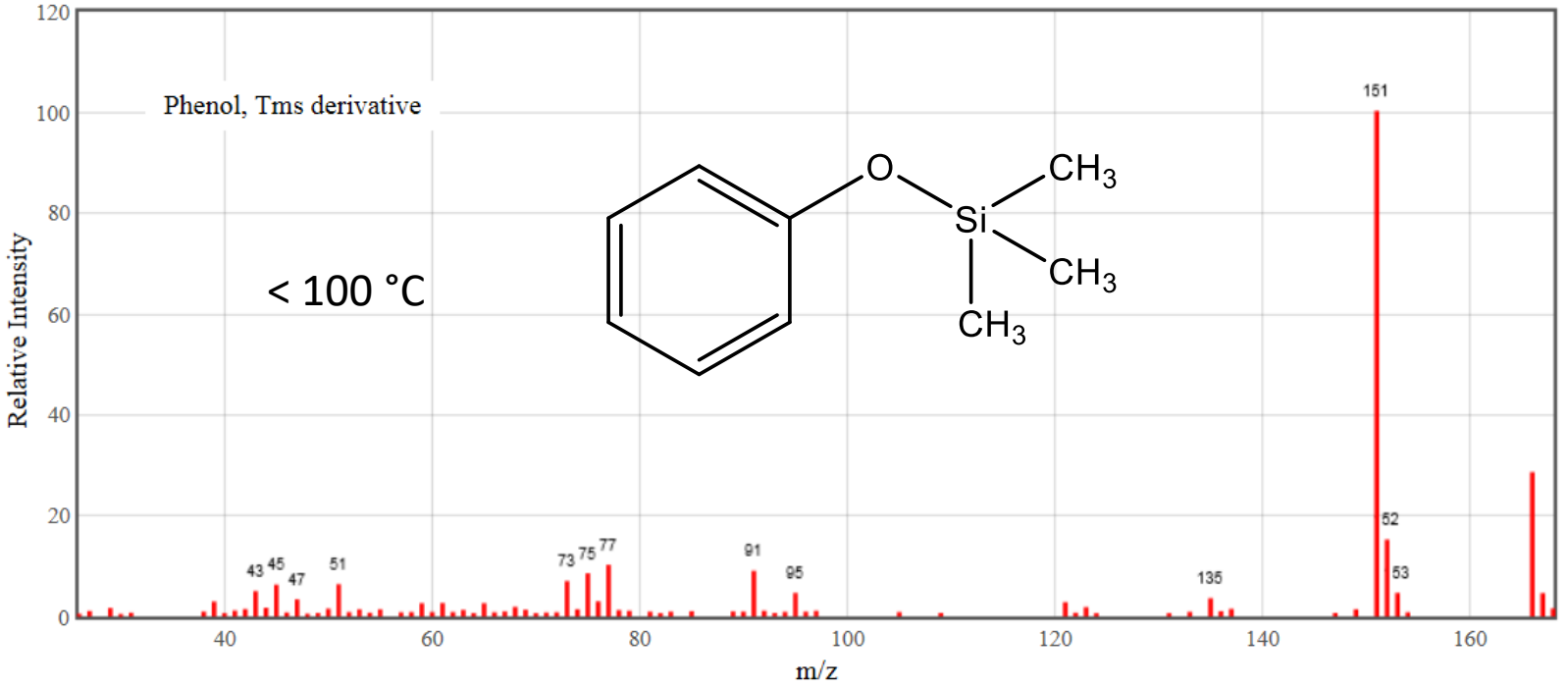


Chlorotrimethylsilane



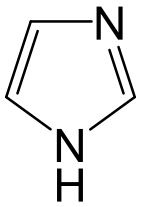
Flavonol TMS derivative


Phenol
OH
181.7 °C
Relative Intensity
m/z
120
100
80
60
40
20
0
20
30
40
50
60
70
80
90
26
27
37
38
39
40
47
50
51
53
55
61
62
63
65
66


Phenol, Tms derivative
O
Si
CH3
CH3
CH3
< 100 °C
Relative Intensity
m/z
120
100
80
60
40
20
0
40
60
80
100
120
140
160
43
45
47
51
73
75
77
91
95
135
151
52
53

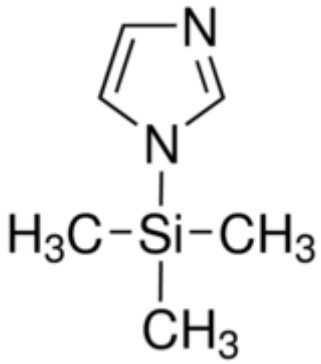
Caution: derivatives may also be derivatizing agents!!

Imidazole



b.p. 256°C

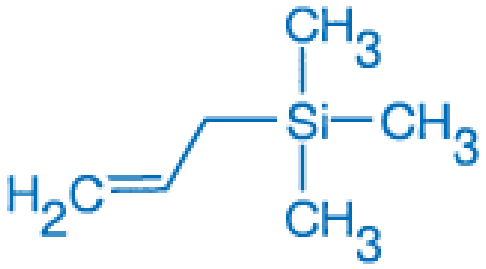
Trimethysilyl imidazole



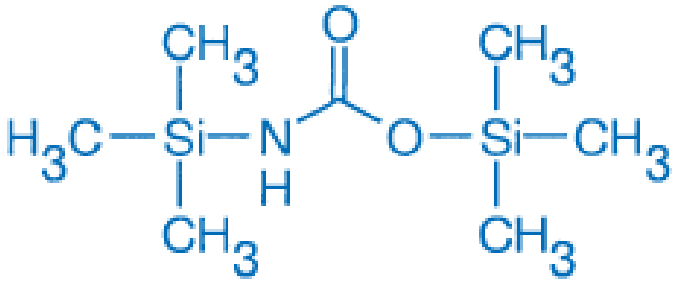
Sold by Sigma-Aldrich as GC derivatization reagent

Examples of other reagents for silylation

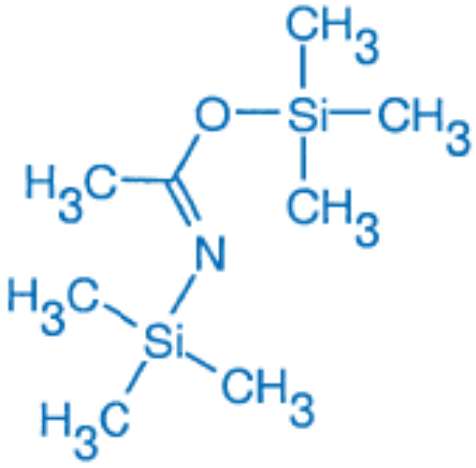
Allyltrimethylsilane



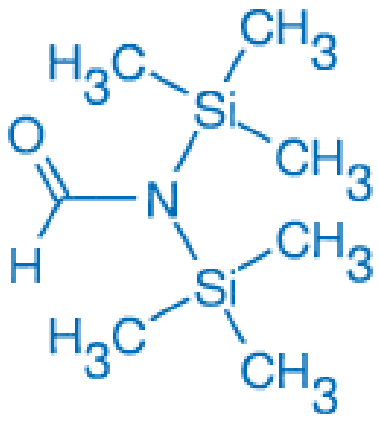
N,O-Bis(trimethylsilyl)carbamate, BSC



N,O-Bis(trimethylsilyl)acetamide, BSA

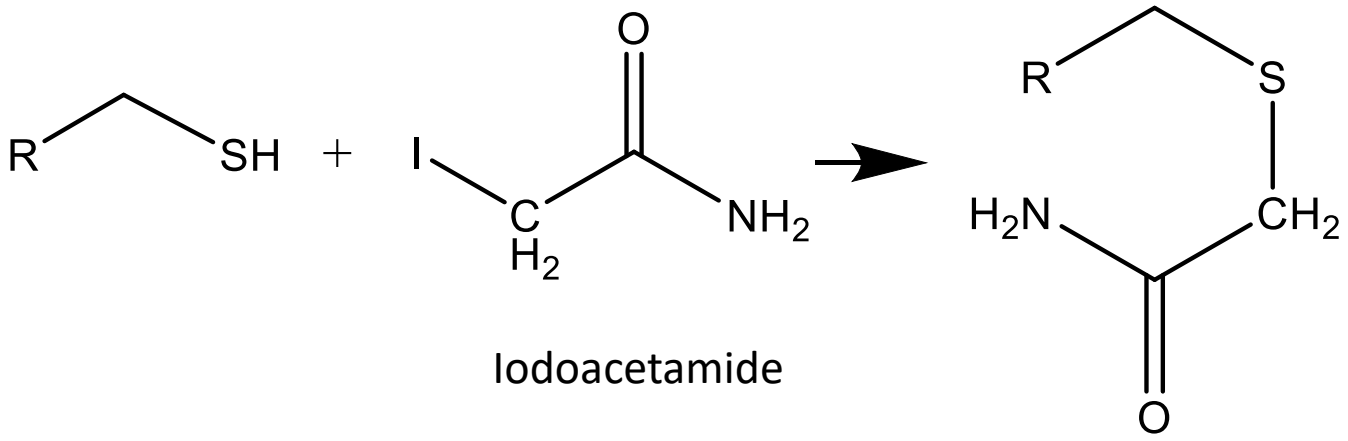


N,N-Bis(trimethylsilyl)formamide, BSF



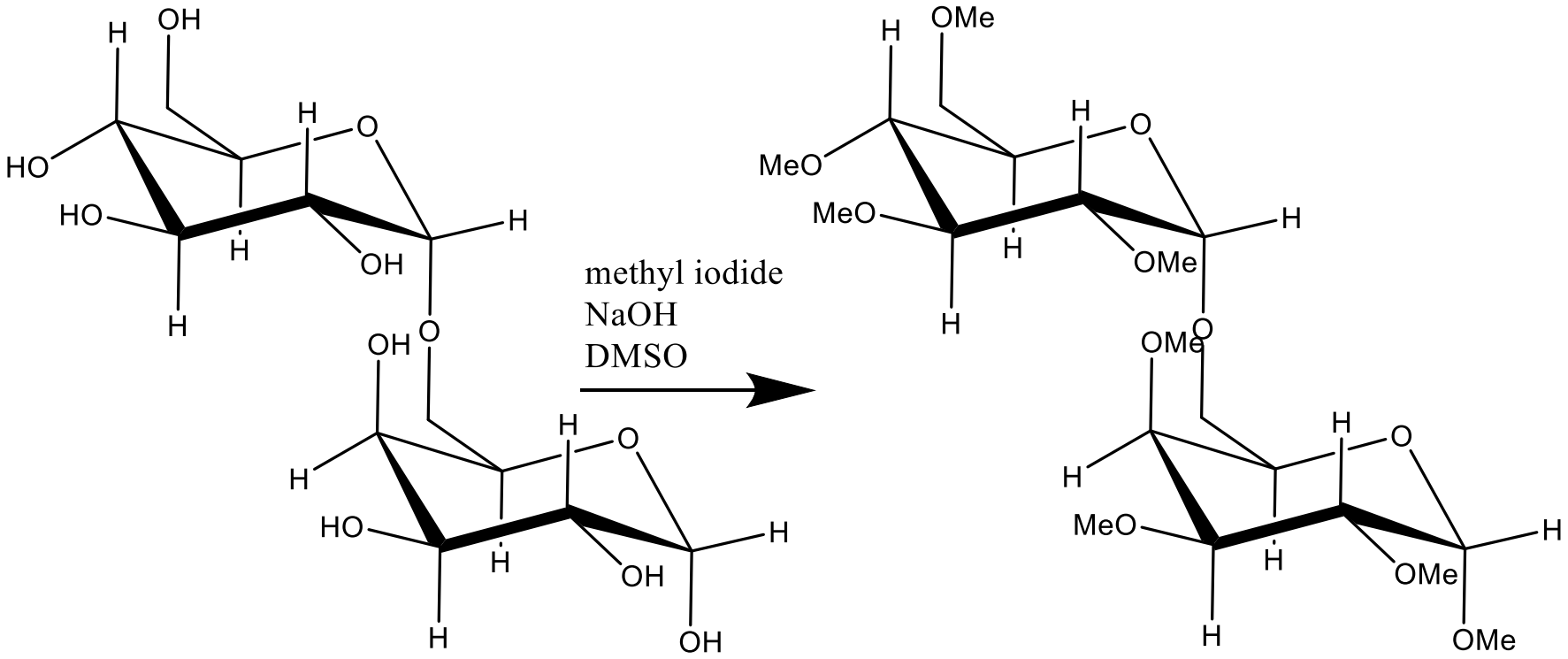
Alkylation

- **Methylation (-OH, -NH_2)**
- **Ethylation (-OH)**
- **Iodoacetamidation (-SH)**



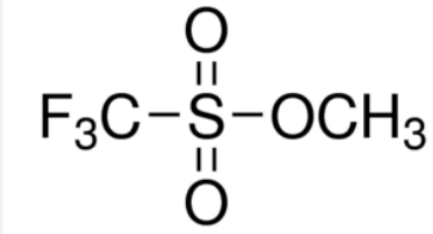
Iodoacetamide

Example of methylation with CH_3I: disaccharide

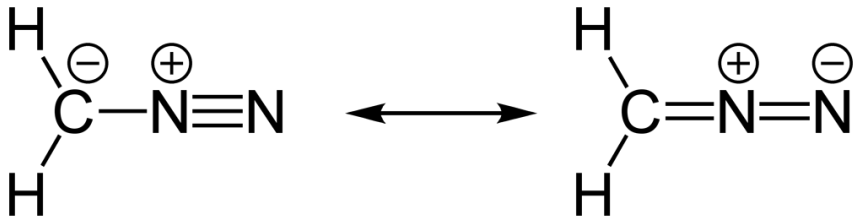


Examples of other reagents for methylation

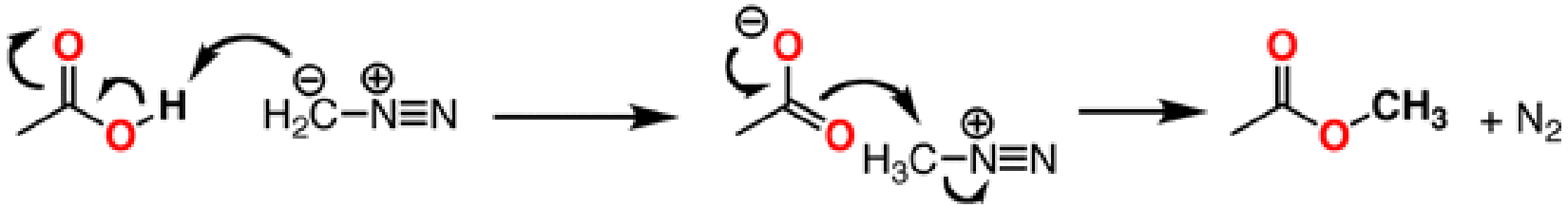
Methyl trifluoromethanesulfonate



diazomethane



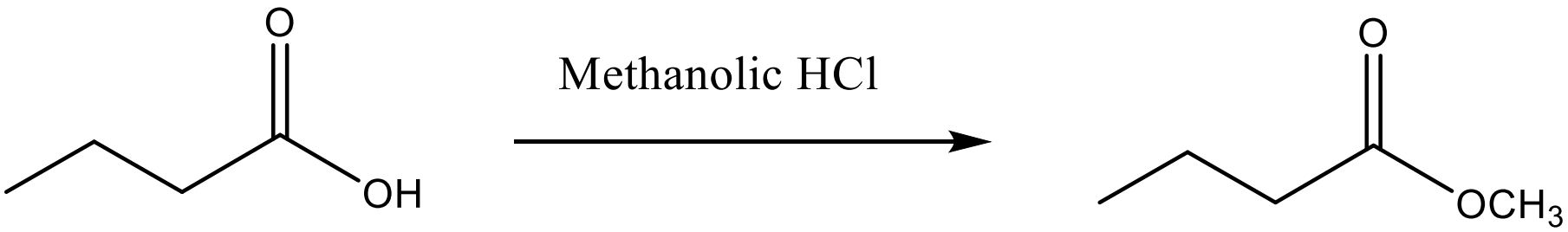
How it works: *Formation of methyl esters*

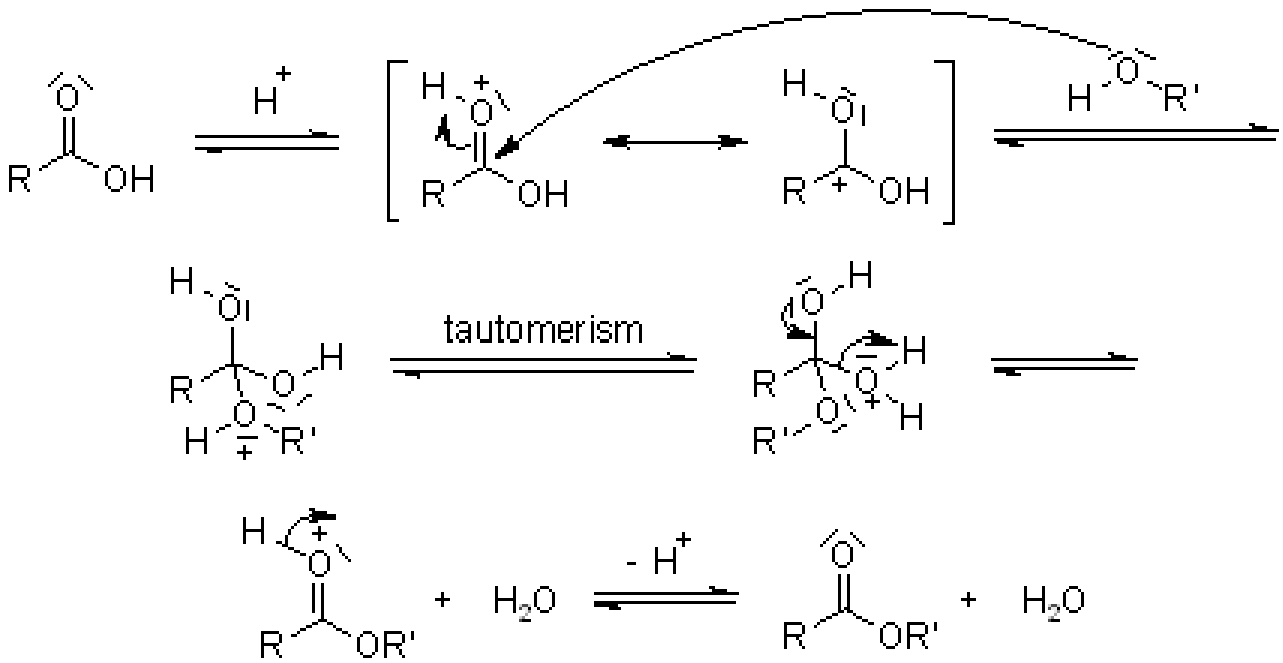


Diazomethane is protonated by the carboxylic acid, forming a carboxylate

The carboxylate then attacks the methyl group, displacing N_2

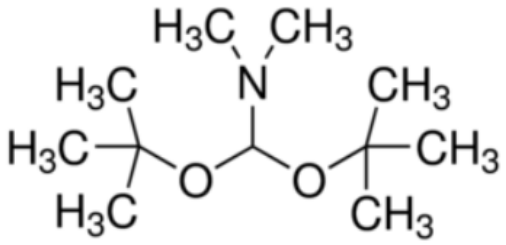
Fisher esterification: e.g. C(O)OH to C(O)OR



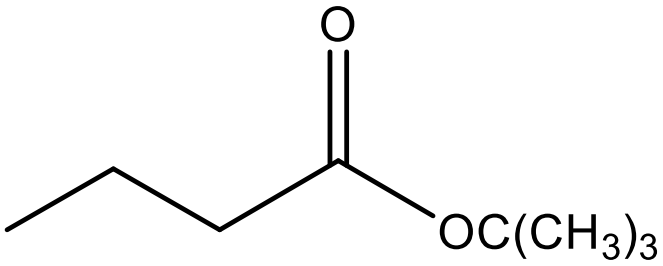


Another reagent for esterification of carboxylic acids:

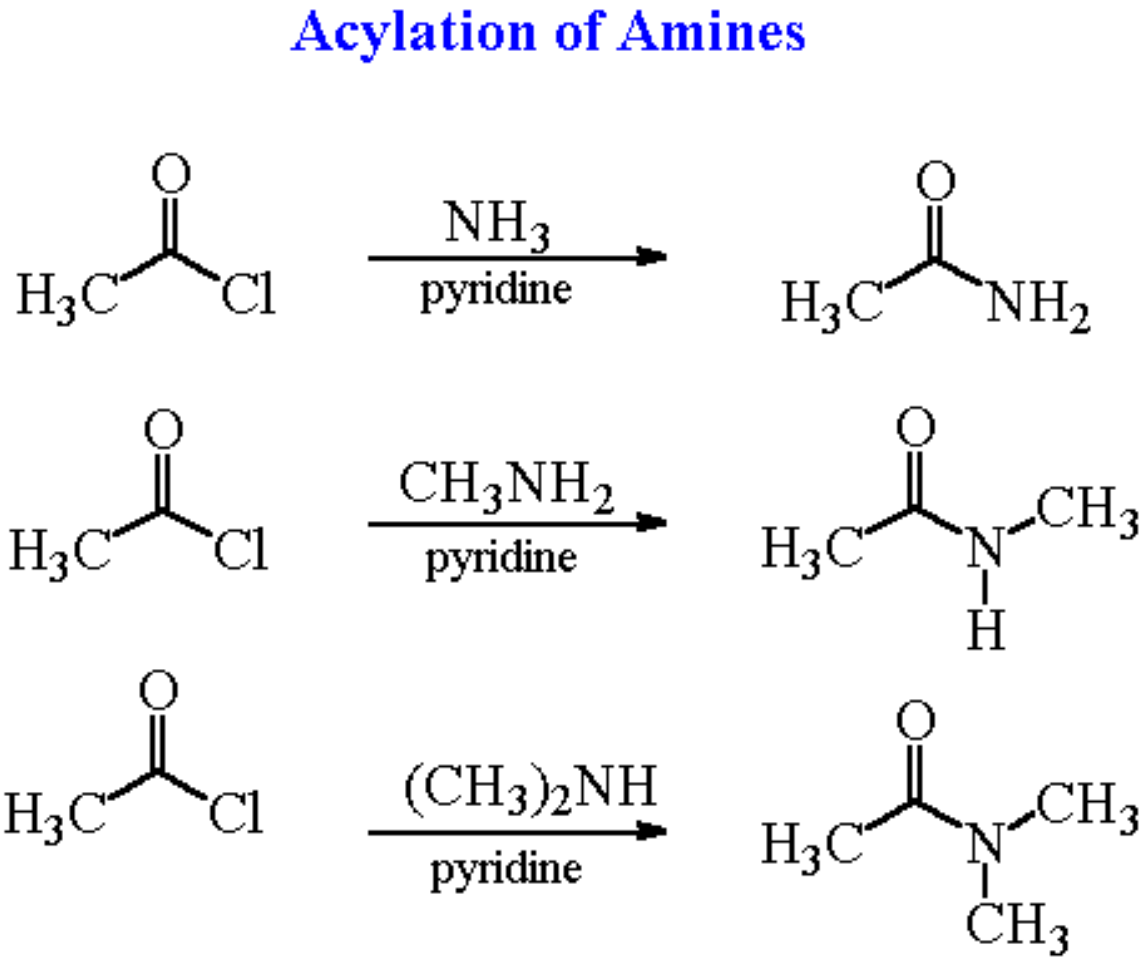
***N*,*N*-Dimethylformamide di-*tert*-butyl acetal**



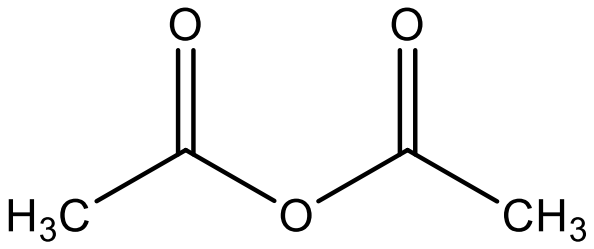
Product:



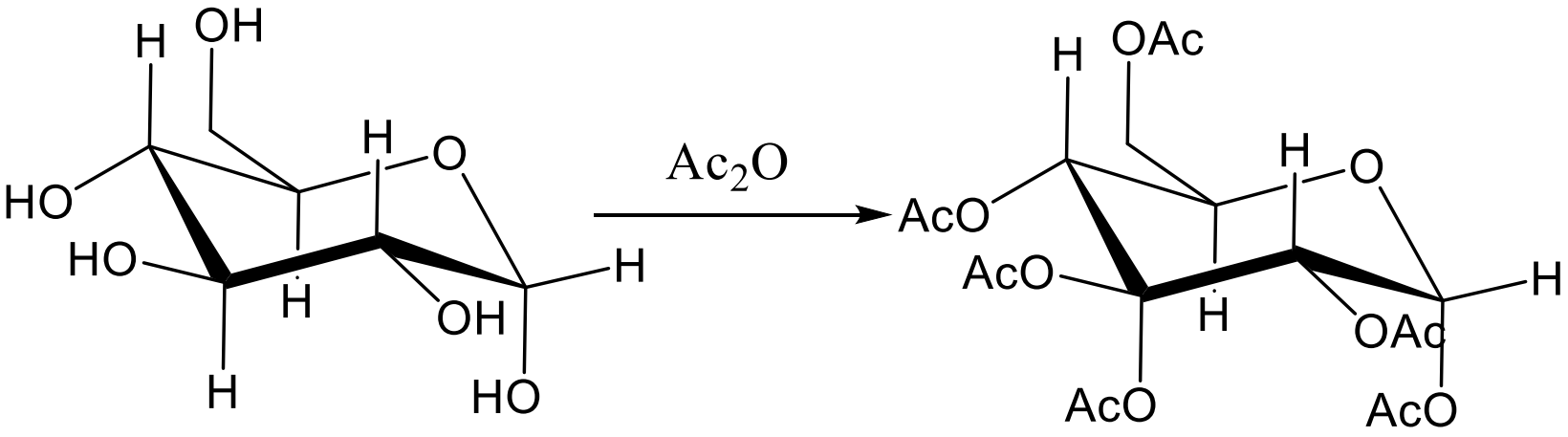
Acetylation or acylation with acetyl chloride



Another reagent for acetylation: acetic anhydride



Ac_2O



US EPA METHOD 8041
PHENOLS BY GAS CHROMATOGRAPHY (ALKYLATION)

https://www.o2si.com/docs/epa-method-8041.pdf

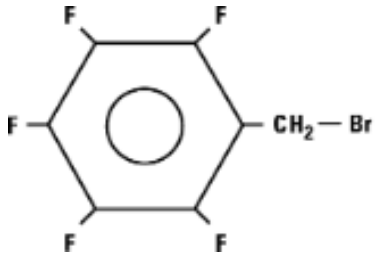
SUMMARY OF METHOD:

Samples are extracted using an appropriate sample preparation method.

Prior to analysis, the extracts are cleaned up, as necessary, and the solvent exchanged to 2-propanol.

Underivatized phenols may be analyzed by FID.

The target phenols also may be derivatized with diazomethane or pentafluorobenzyl bromide (PFBBr) and analyzed by gas chromatography.



PFBBr
MW 260.9
bp 174-175°C
d_4^{20} 1.86

APPARATUS AND MATERIALS

Gas chromatograph - An analytical system complete with a gas chromatograph suitable for on-column injection, and all required accessories, including syringes, analytical columns, gases, flame ionization detector (FID), **electron capture detector (ECD),** and a data system.

Column: 30 m x 0.53 mm ID fused-silica open- tubular column, cross-linked and chemically bonded with 95 percent dimethyl and 5 percent diphenyl-polysiloxane (**DB-5, RT-5, SPB-5**, or equivalent), 0.83 µm or 1.5 µm film thickness.

Temperature programming: not described,

ACTA CHROMATOGRAPHICA, NO. 16, 2006

DETERMINATION OF CONDITIONS FOR DERIVATIZATION AND CHROMATOGRAPHIC ANALYSIS BEFORE SIMULTANEOUS ANALYSIS OF CHLOROVERATROLES AND PENTAFLUOROBENZYL DERIVATIVES OF CHLOROCATECHOLS AND CHLOROGUAIACOLS IN ENVIRONMENTAL AND FOOD SAMPLES

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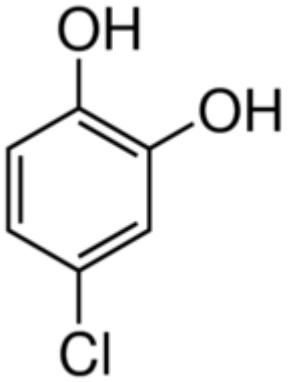
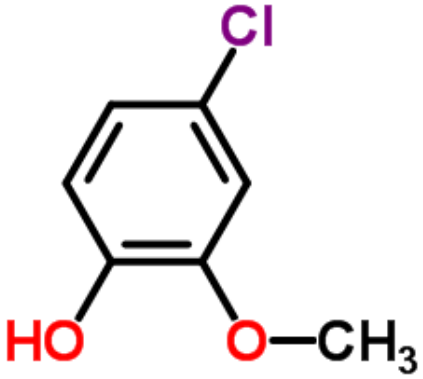
A range of chlorinated aromatic compounds, for example chlorocatechols, chloroveratroles, and chloroguaiacols are known components of pulp bleaching effluents.

Some of these compounds, for example chlorinated guaiacols have been shown to be toxic at concentrations of 1 ppm or less, so persistence of such compounds in the aquatic environment, sediments, or soil may be a matter of serious concern.

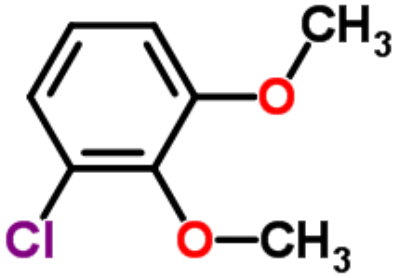
chlorocatechols



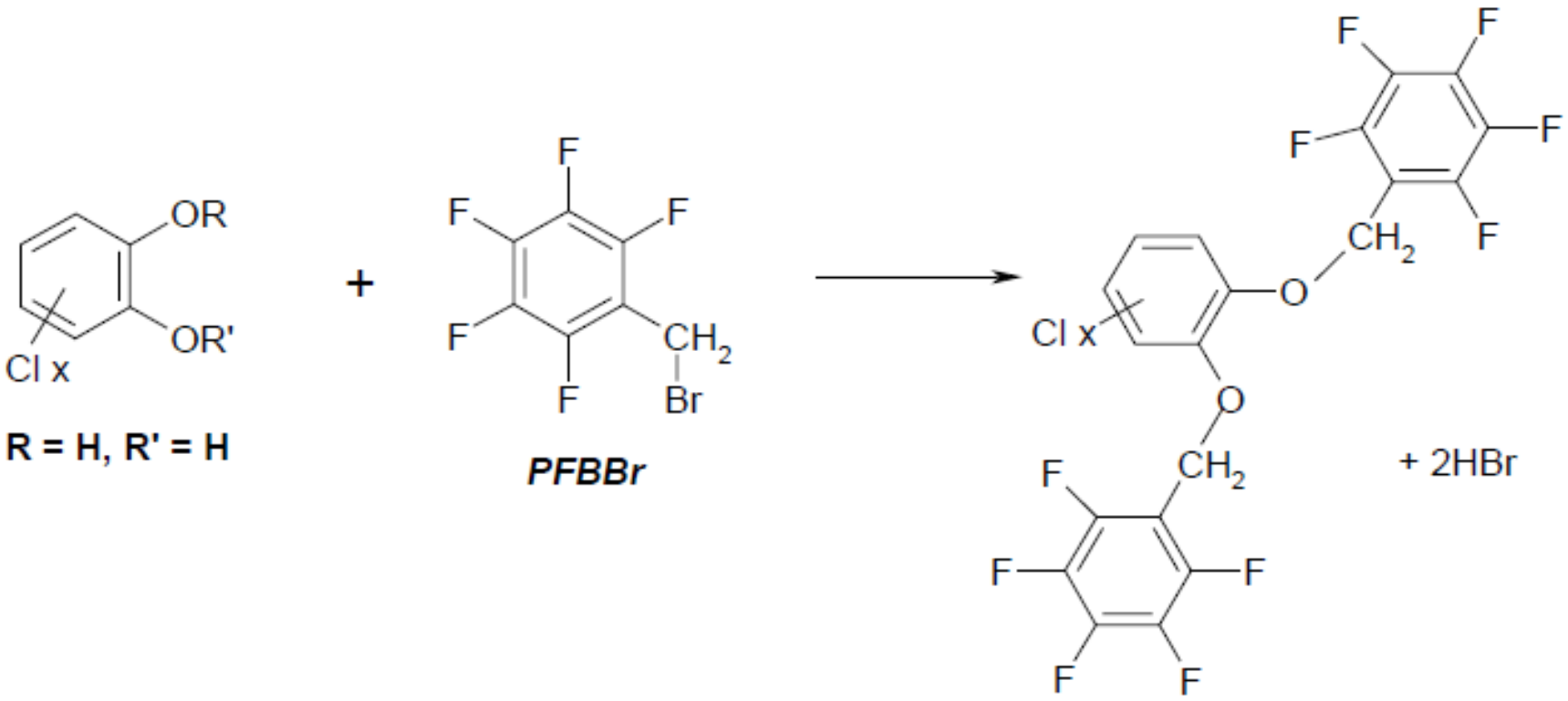
chloroguaiacols



chloroveratroles



Derivatization with PFBBr



http://www.us.edu.pl/uniwersytet/jednostki/wydzialy/chemia/acta/ac16/zrodla/03_AC16.pdf

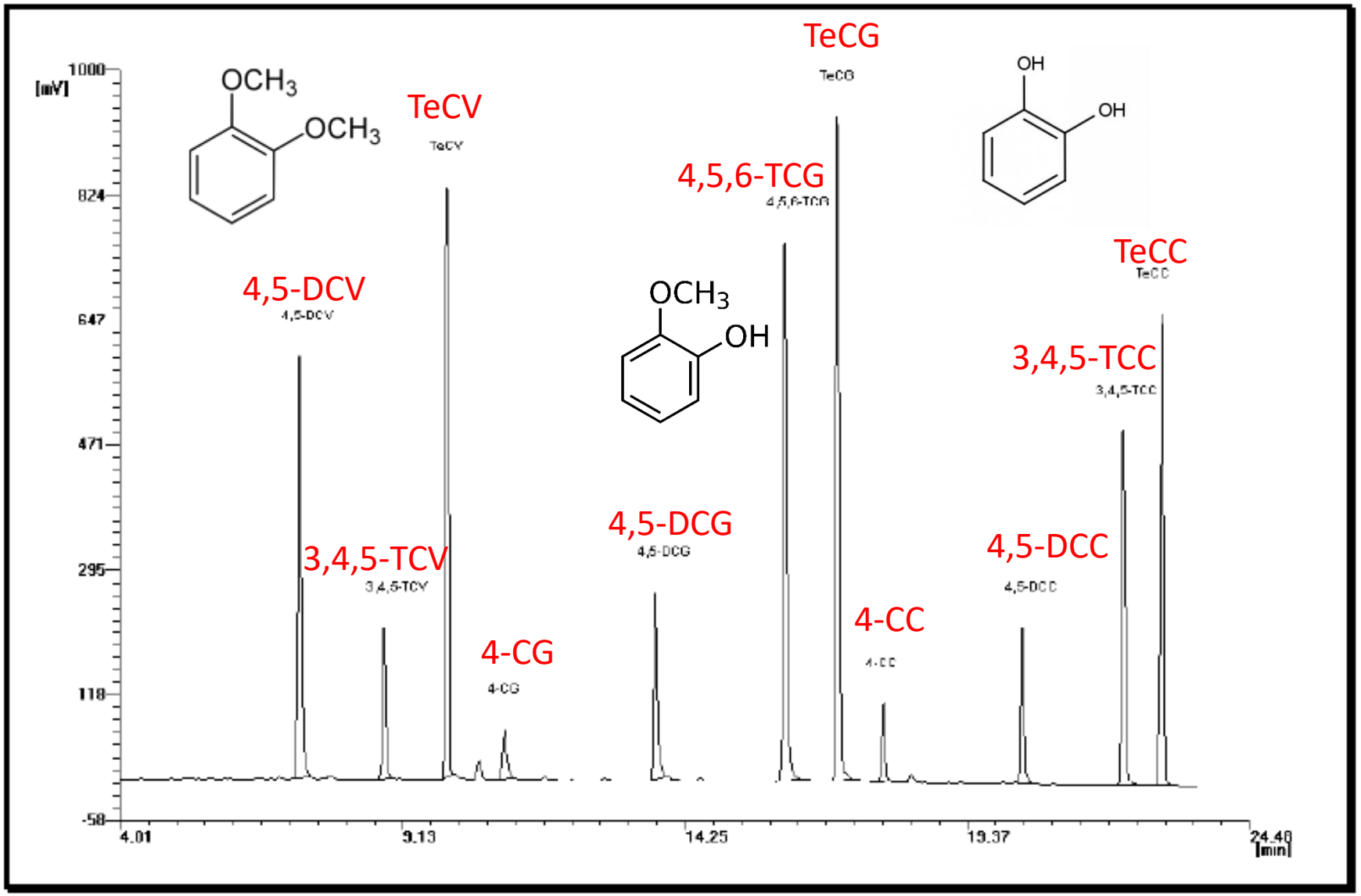


Fig. 2

GC–ECD chromatogram obtained from chlorinated underivatized veratroles and penta-fluorobenzyl derivatives of chlorocatechols and chloroguaiacols

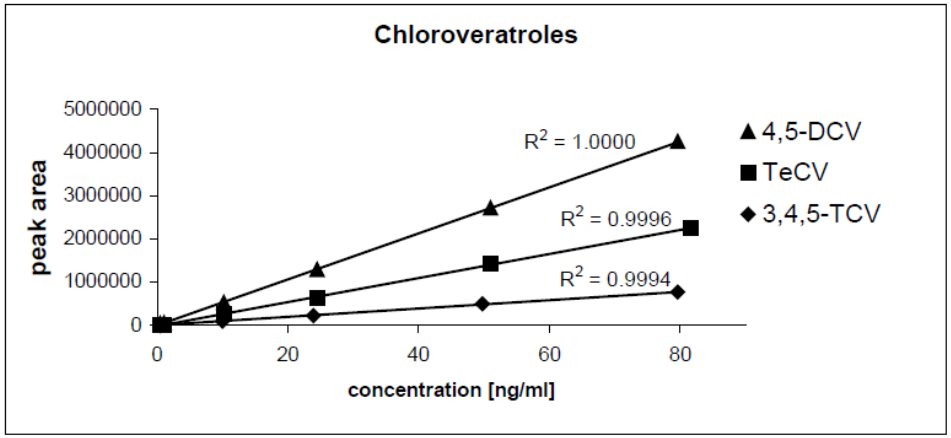


Fig. 3

Calibration plots for chloroveratroles

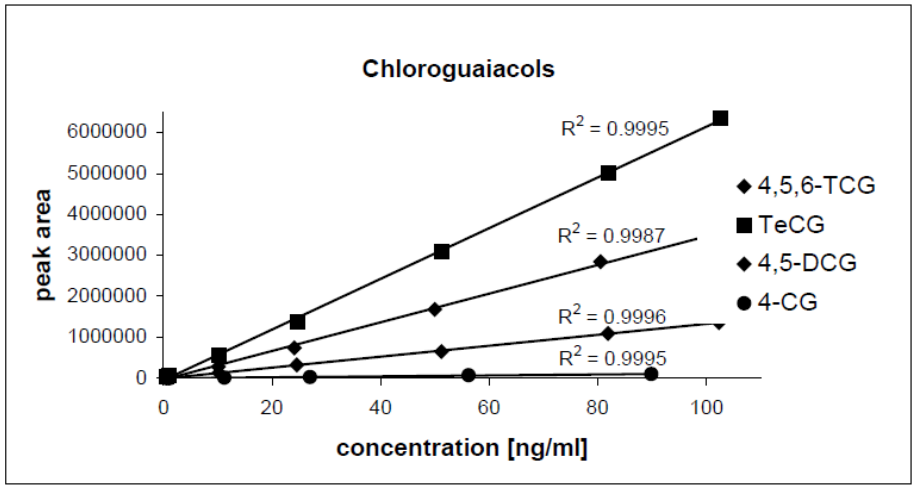


Fig. 5

Calibration plots for derivatized chloroguaiacols

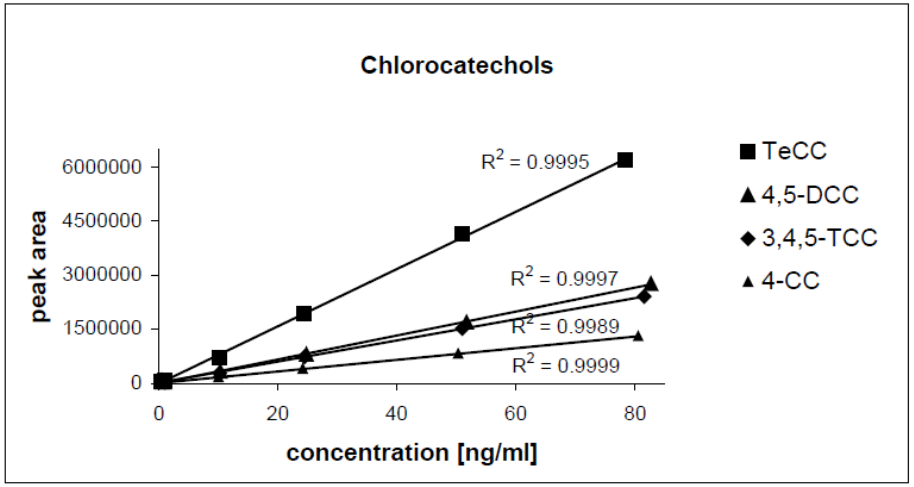


Fig. 4

Calibration plots for derivatized chlorocatechols

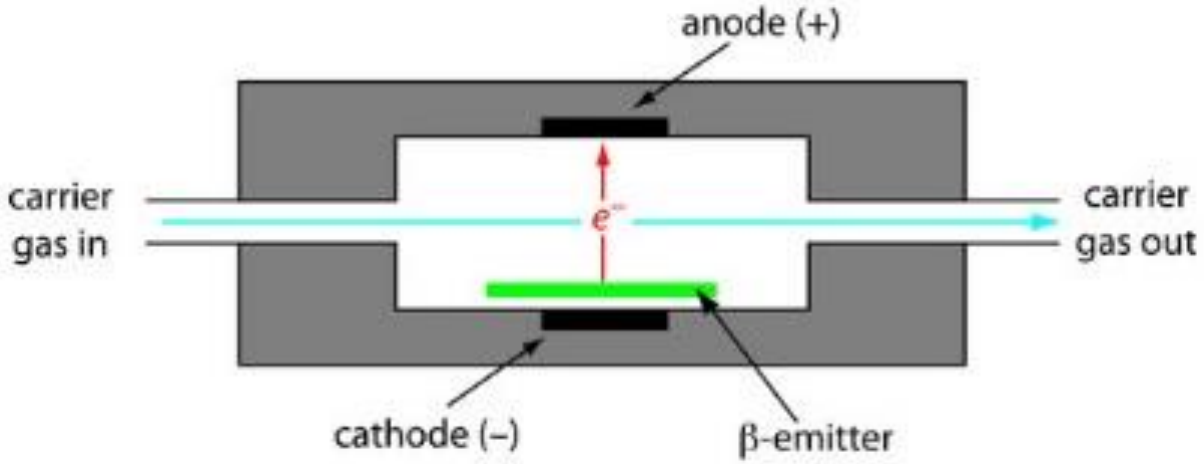
Table II

Linearity, detection limit (*DL*), and precision in GC–ECD analysis

Analyte	RT (min)	Linear range (ng mL^{-1})	*RSD* (%)	*DL* (ng mL^{-1})
4-CV	–	–	–	–
4,5-DCV	7.28	0.51–81.60	0.30	0.28
3,4,5-TCV	8.81	0.50–79.59	2.37	0.58
TeCV	9.95	0.51–81.60	0.11	0.65
4-CG	10.97	0.56–90.40	2.26	2.72
4,5-DCG	13.71	0.51–102.34	2.03	0.47
4,5,6-TCG	16.07	0.50–100.64	1.61	1.74
TeCG	17.02	0.51–102.61	0.14	0.99
4-CC	17.85	0.50–80.51	1.86	1.01
4,5-DCC	20.33	0.52–82.68	1.93	0.41
3,4,5-TCC	22.20	0.51–81.60	1.53	1.14
TeCC	22.90	0.51–81.60	0.16	0.18

Electron-Capture Detectors (ECD)

Composed of a radioactive source which emits electrons, a cathode which repels the electrons, an anode which collects the electrons.



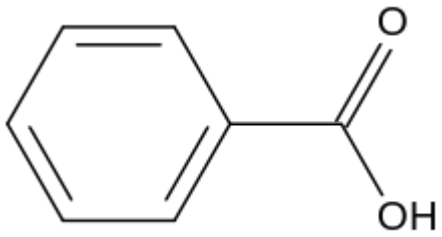
The detector consists of a β-emitter (^{63}Ni). The emitted e^- generate a standing current between the pair of electrodes. When a solute with a high affinity for electron capture elutes from the column, the current decreases. This serves as the signal.

Summary: GC of non volatiles

- Non-volatile compounds need to be derivatized into volatile analogs for GC
- Reagents sold by many companies as kits: conditions already established
- Reagents can be selective to one class of compounds or universal
- Reactions are often reversible if starting material is needed
- Sample loss may occur
- HPLC of non-derivatized materials preferable is instrument is available

Questions

1. Suggest a derivatization reaction that could be used for the analysis of benzoic acid by gas chromatography with an electron capture detector.



2. Methanolic HCl is good for methylating (esterifying) carboxylic acids, but not sugars. Suggest a method applicable to a mixture of carboxylic acids and monosaccharides for GC analysis with a FID detector.