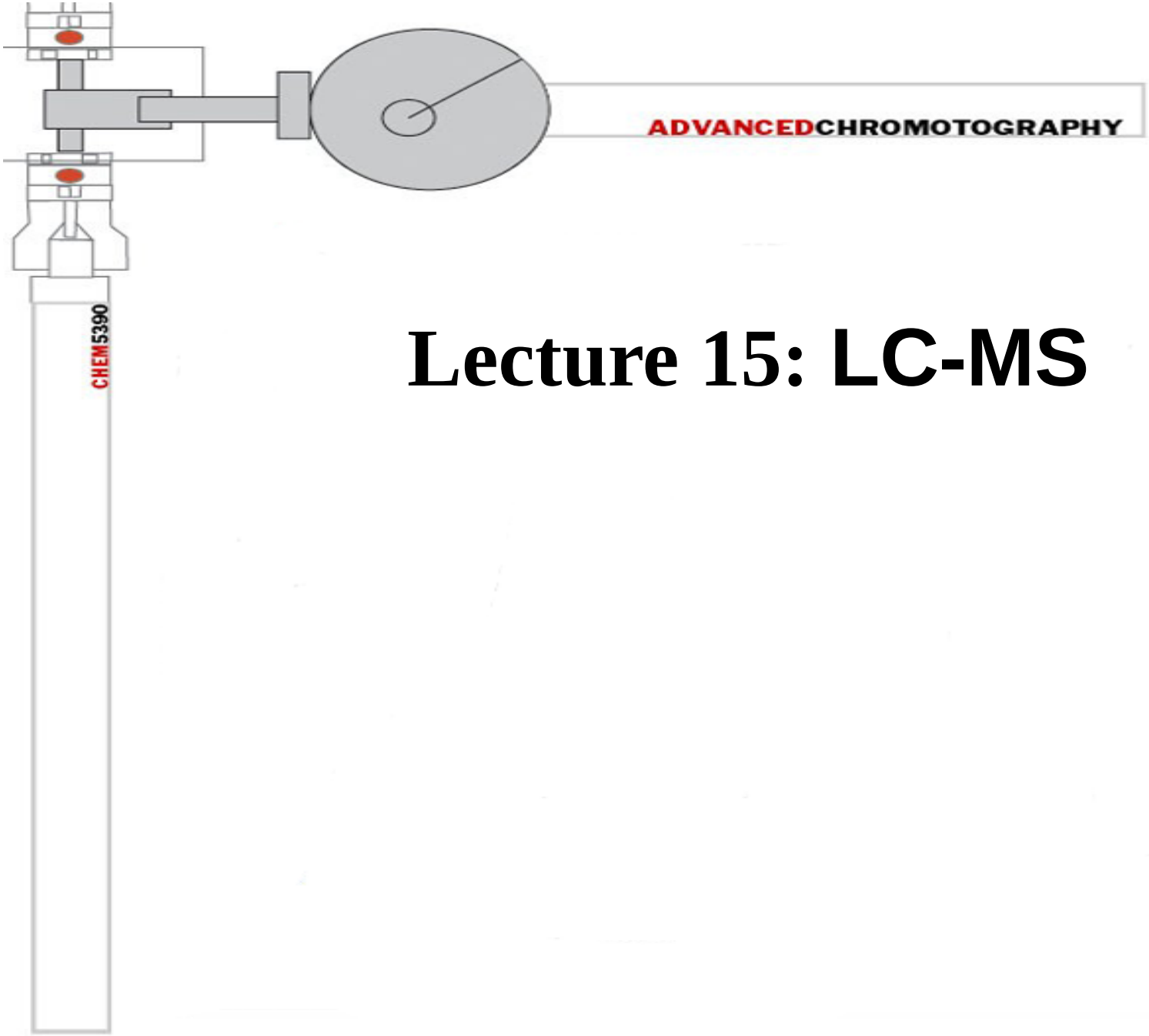
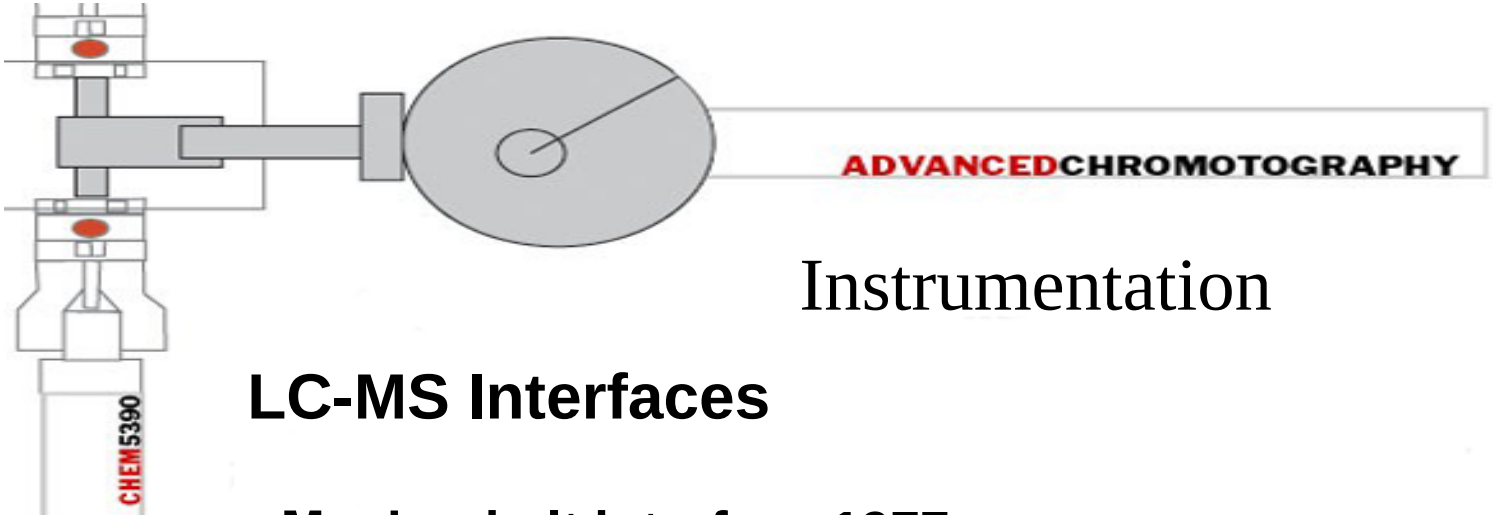


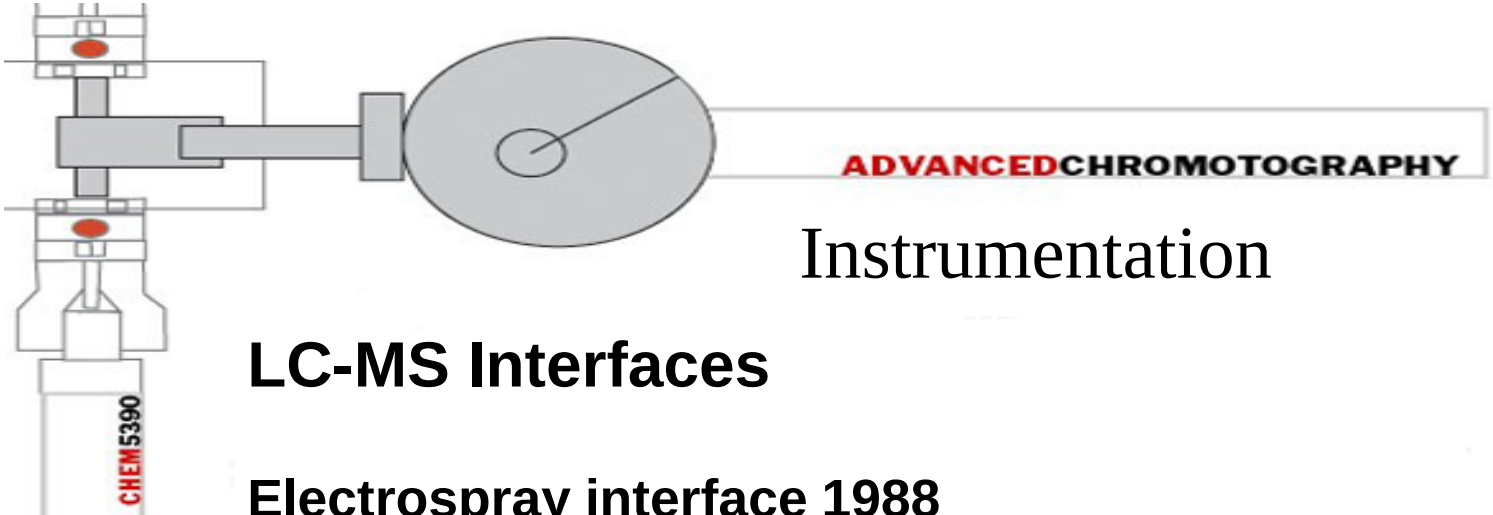
Lecture 15: LC-MS



Instrumentation

LC-MS Interfaces

- **Moving-belt interface 1977**
- **Direct-liquid-introduction interface 1980**
- **Thermospray interface 1983**
- **Frit FAB/continuous-flow FAB interface 1985/1986**
- **Atmospheric-pressure chemical ionization interface 1986**
- **Particle-beam interface 1988**
- **Electrospray interface 1988**



LC-MS Interfaces

Electrospray interface 1988

High performance liquid chromatography is an effective technique for the separation of compounds of high molecular weight. However, two major problems for the study of this type of molecule have severely limited the application of LC–MS. Specifically,:
(a) The inability to ionize, in an intact state, many of the labile and/or involatile molecules involved.
(b) Should ionization be possible, the lack of appropriate hardware to allow the mass analysis and efficient detection of the ions of high m/z ratio involved.

Electrospray is an ionization method that overcomes these problems.

Electrospray interface – 1st generation

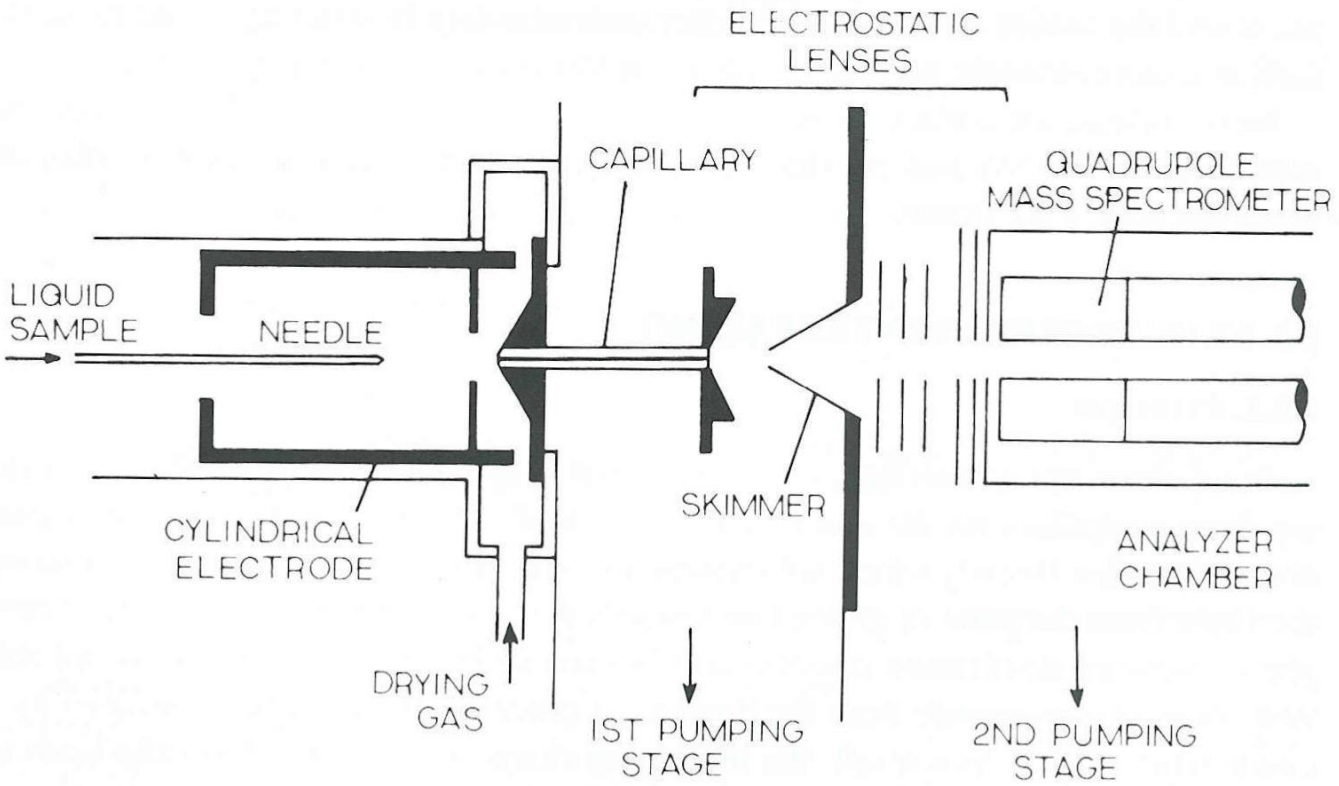
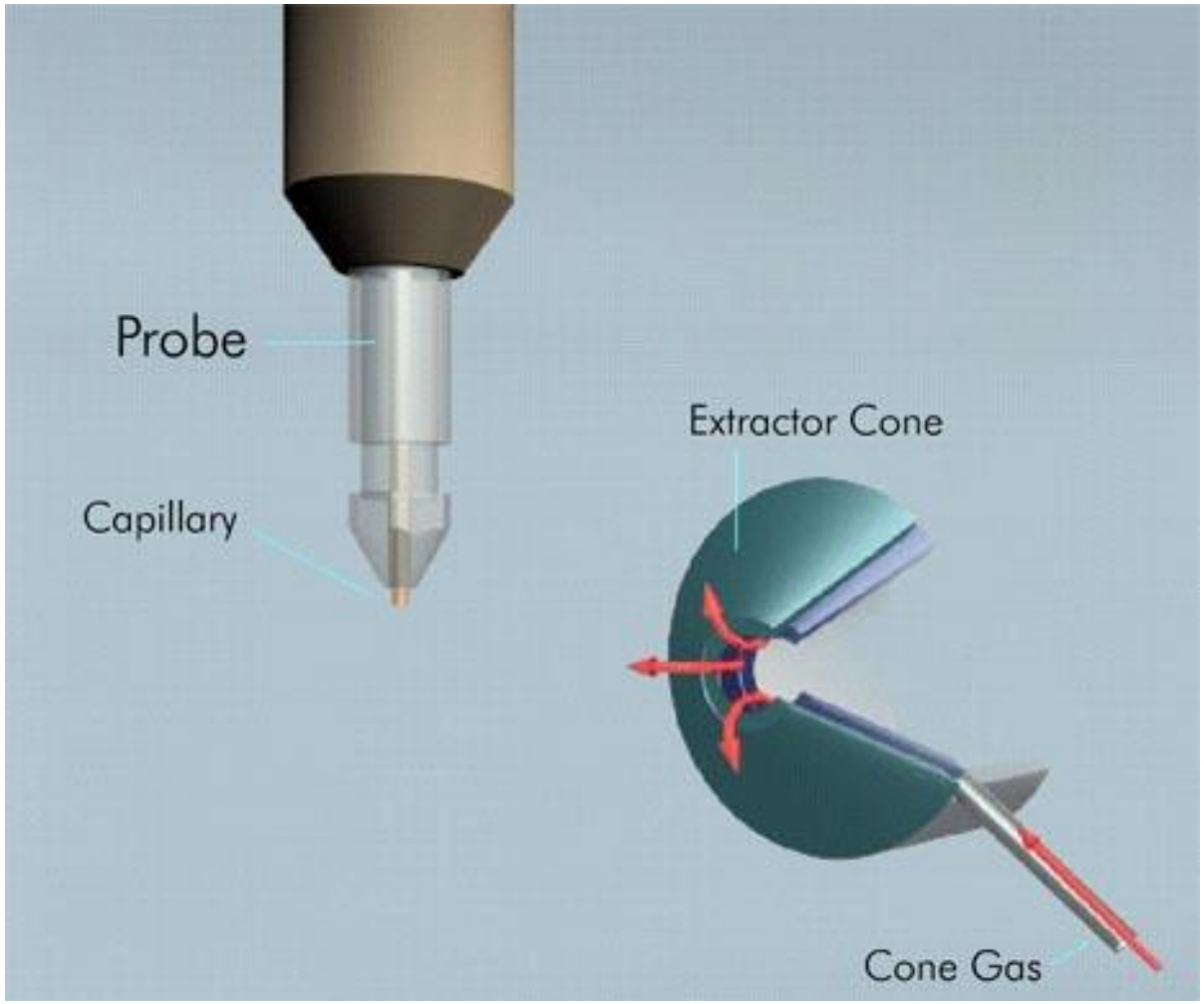


Fig. 1.13. Schematic diagram of the first-generation electrospray LC-MS interface as described by Whitehouse et al. [216]. Reproduced from Ref. [216] with permission. © 1985, American Chemical Society.

Electrospray interface - orthogonal



Instrumentation

LC-MS Interfaces

Electrospray interface

A liquid, in which the analyte(s) of interest have been dissolved, is passed through a capillary (typically stainless steel), at atmospheric pressure, maintained at high voltage (3 to 4 kV).

The liquid stream breaks up with the formation of highly charged droplets which are desolvated as they pass through the atmospheric-pressure region of the source towards a counter electrode.

Electrospray interface

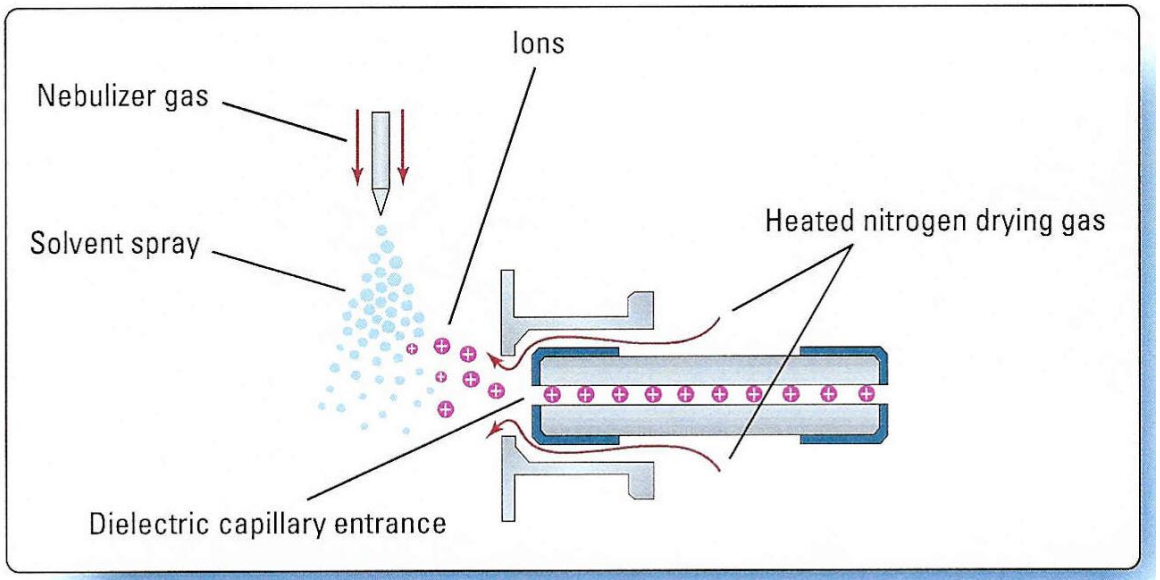


Figure 4. Electrospray ion source

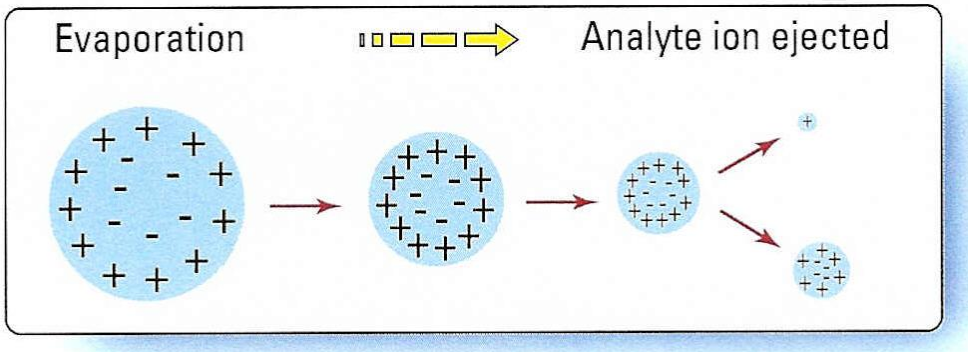


Figure 5. Desorption of ions from solution

Instrumentation

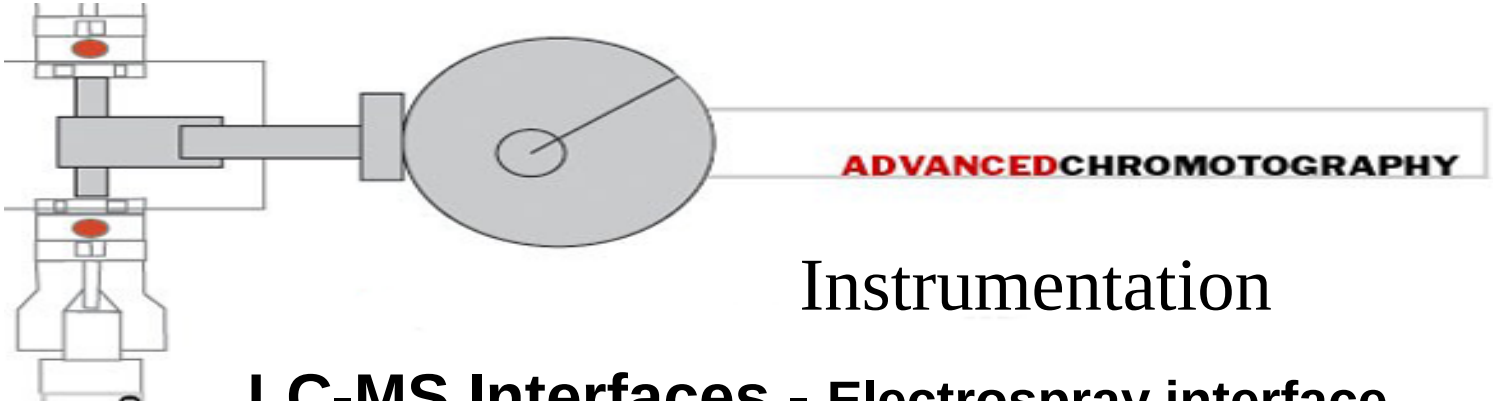
LC-MS Interfaces

Electrospray interface

Desolvation is assisted by a stream of a drying gas, usually nitrogen, being continually passed into the spraying region.

As the droplets shrink, the charge concentration in the droplets increases. The repulsive force between ions with like charges exceeds the cohesive forces and ions are ejected (desorbed) into the gas phase.

Analyte ions are obtained from these droplets which then pass through two differentially pumped regions into the source of the mass spectrometer.

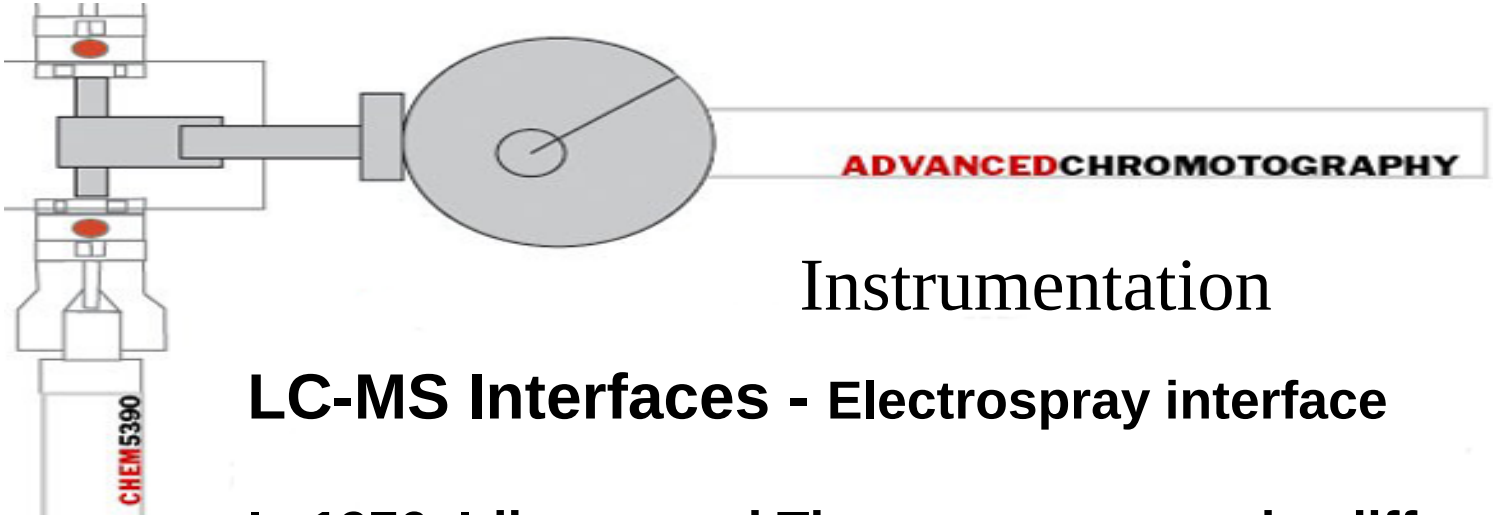


Instrumentation

LC-MS Interfaces - Electrospray interface

The mechanism by which potential transfers from the liquid to the analyte, creating ions, remains a topic of controversy.

In 1968, Malcolm Dole first proposed the charge residue mechanism in which he hypothesized that as a droplet evaporates, its charge remains unchanged.
The droplet's surface tension, ultimately unable to oppose the repulsive forces from the imposed charge, explodes into many smaller droplets.
These Coulombic fissions occur until droplets containing a single analyte ion remain. When the solvent evaporates from the last droplet, a gas-phase ion forms.

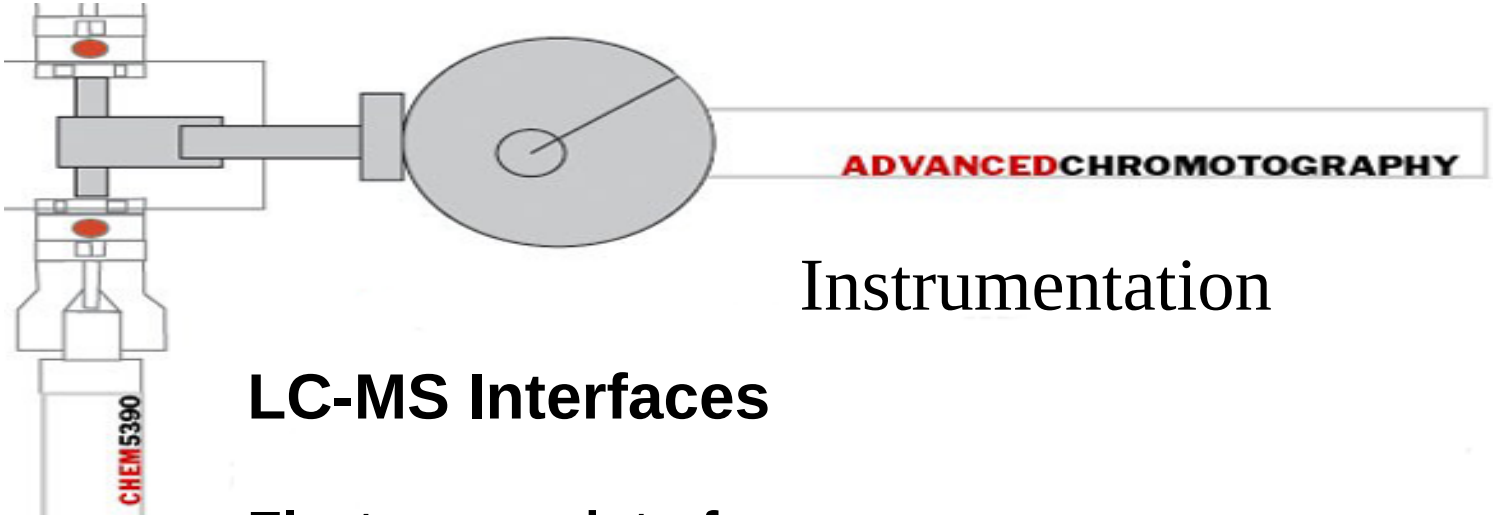


Instrumentation

LC-MS Interfaces - Electrospray interface

In 1976, Iribarne and Thomson proposed a different model, the ion evaporation mechanism, in which small droplets form by Coulombic fission, similar to Dole's model.
However, according to ion evaporation theory, the electric field strength at the surface of the droplet is high enough to make leaving the droplet surface and transferring directly into the gas phase energetically favorable for solvated ions.

It is possible that the two mechanisms may actually work in concert: the charge residue mechanism dominant for masses higher than 3000 Da while ion evaporation dominant for lower masses.



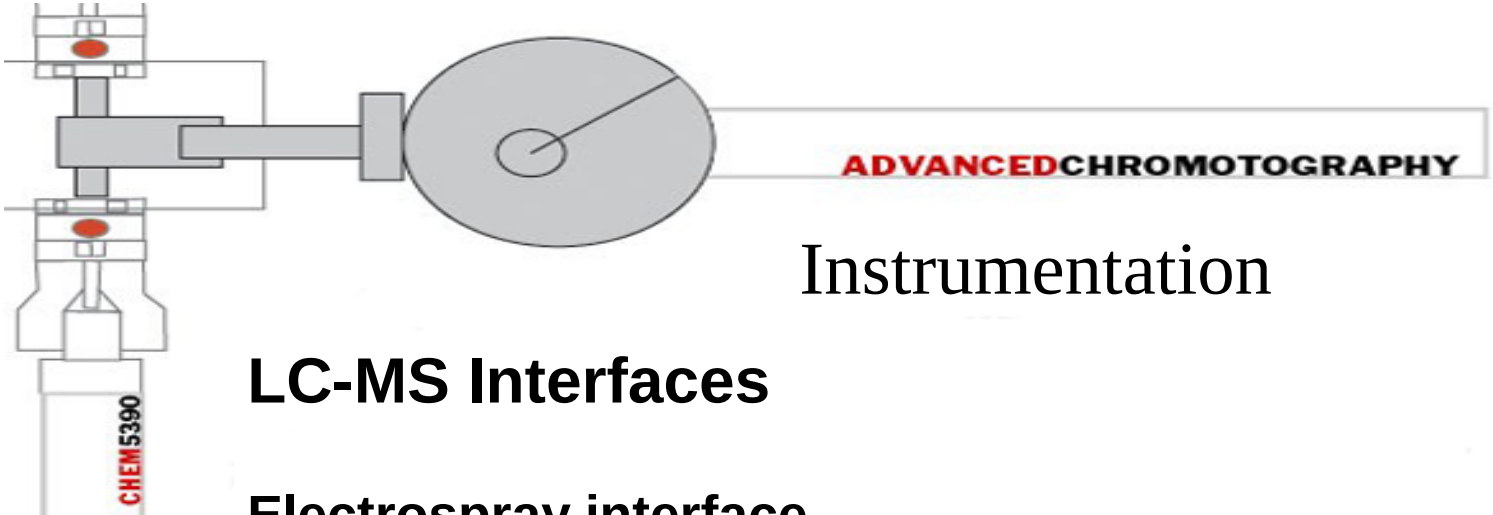
LC-MS Interfaces

Electrospray interface

Since ionization takes place directly from solution, thermally labile molecules may be ionized without degradation.

In contrast to most other ionization methods, the majority of ions produced by electrospray are multiply charged.

Electrospray is useful for large biomolecules such as proteins, peptides, etc… while still able to analyze smaller molecules.

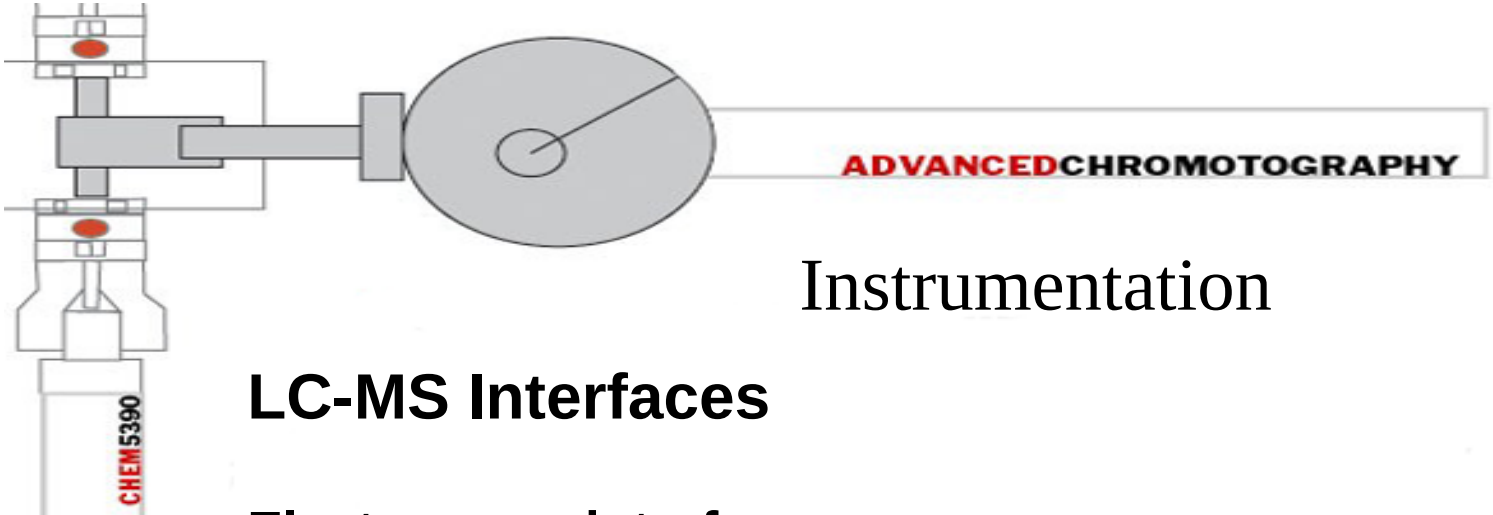


LC-MS Interfaces

Electrospray interface

In positive ionization mode:
- a trace of formic acid is often added to aid protonation of the sample molecules.

In negative ionization mode:
- a trace of ammonia solution or a volatile amine is added to aid deprotonation of the sample molecules.



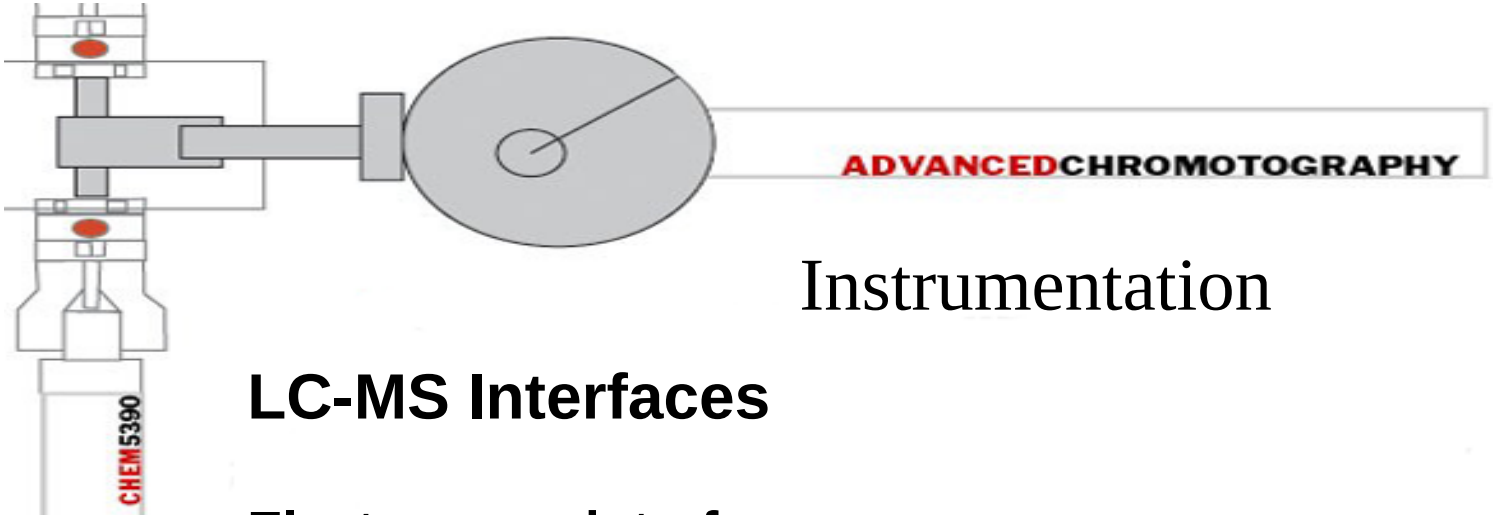
LC-MS Interfaces

Electrospray interface

Proteins and peptides are usually analyzed under positive ionization conditions.

Saccharides and oligonucleotides are usually analyzed under negative ionization conditions.

In all cases, the m/z scale must be calibrated by analyzing a standard sample of a similar type to the sample being analyzed (e.g. a protein calibrant for a protein sample), and then applying a mass correction.



LC-MS Interfaces

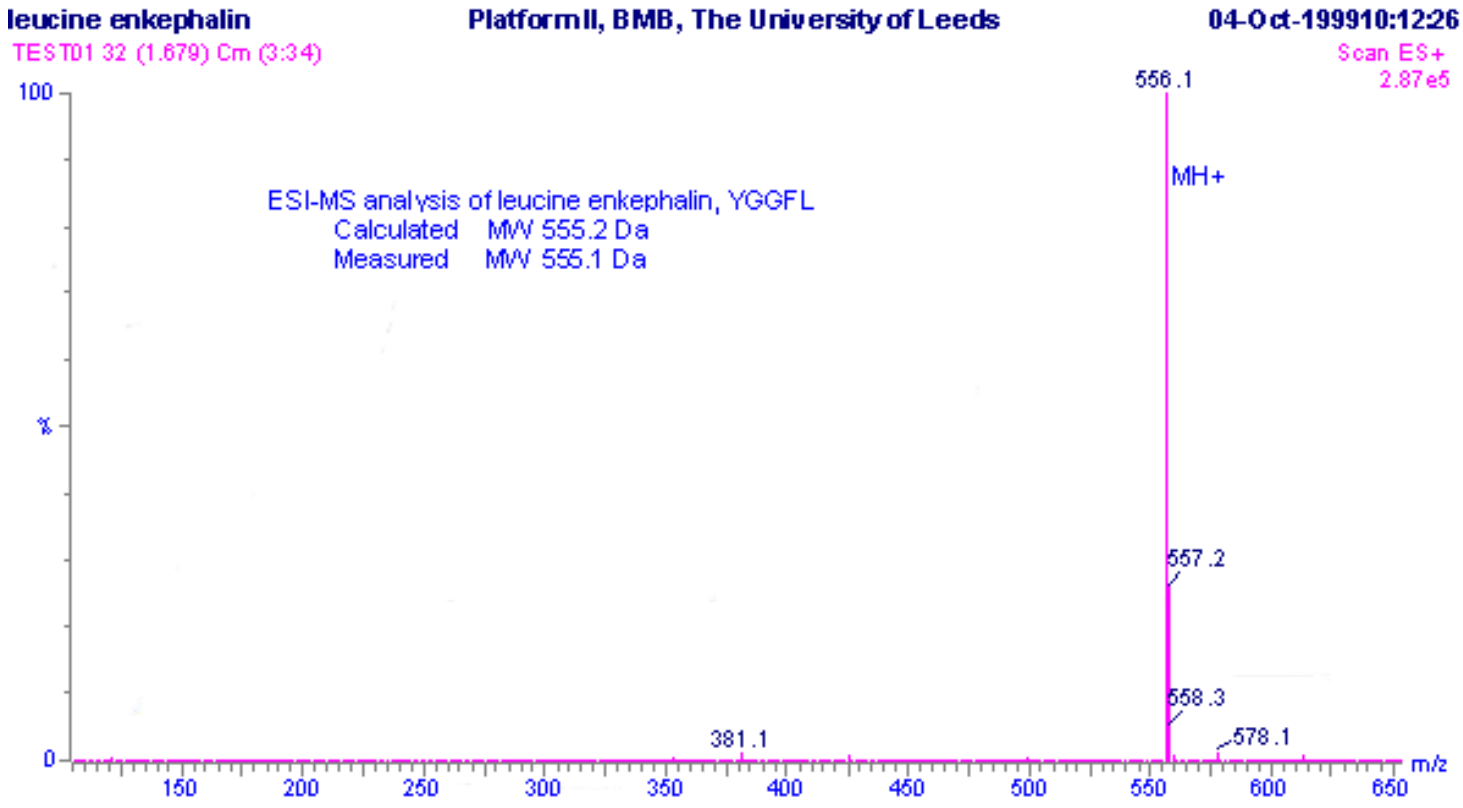
Electrospray interface

In ESI, samples (M) with molecular masses up to ~1200 Da give rise to singly charged molecular-related ions.

Protonated molecular ions of the formula (M+H)+ in positive ionization mode.

Deprotonated molecular ions of the formula (M-H)- in negative ionization mode.

Example of this type of sample analysis is shown in the m/z spectrum of the pentapeptide leucine enkephalin, YGGFL.
The molecular formula is C28H37N5O7 and the calculated molecular weight is 555.2692 Da.



The m/z spectrum shows dominant ions at m/z 556.1, which are consistent with the expected protonated molecular ions, (M+H+) under positive ionization conditions.

Instrumentation

LC-MS Interfaces

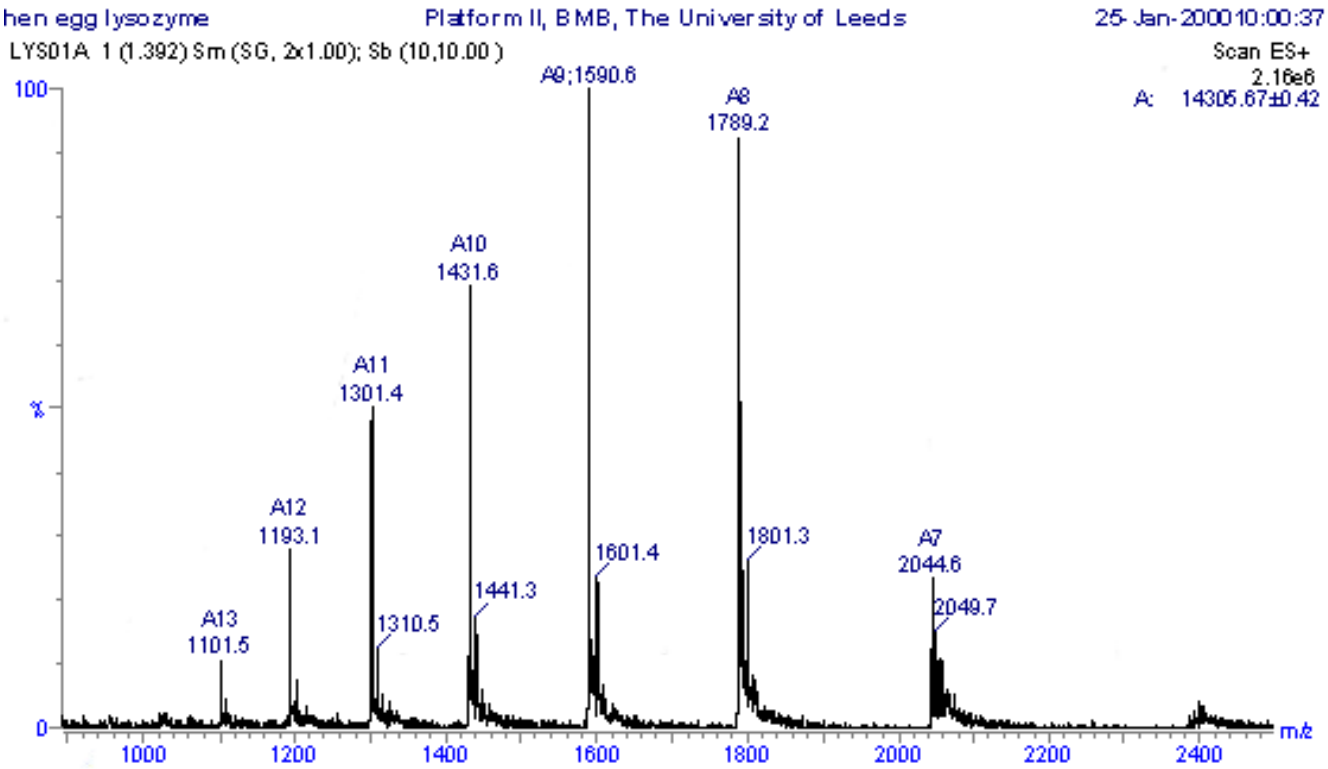
Electrospray interface

Samples (M) with molecular weights greater than ~1200 Da give rise to multiply charged molecular-related ions.

(M+nH)n+ in positive ionization mode

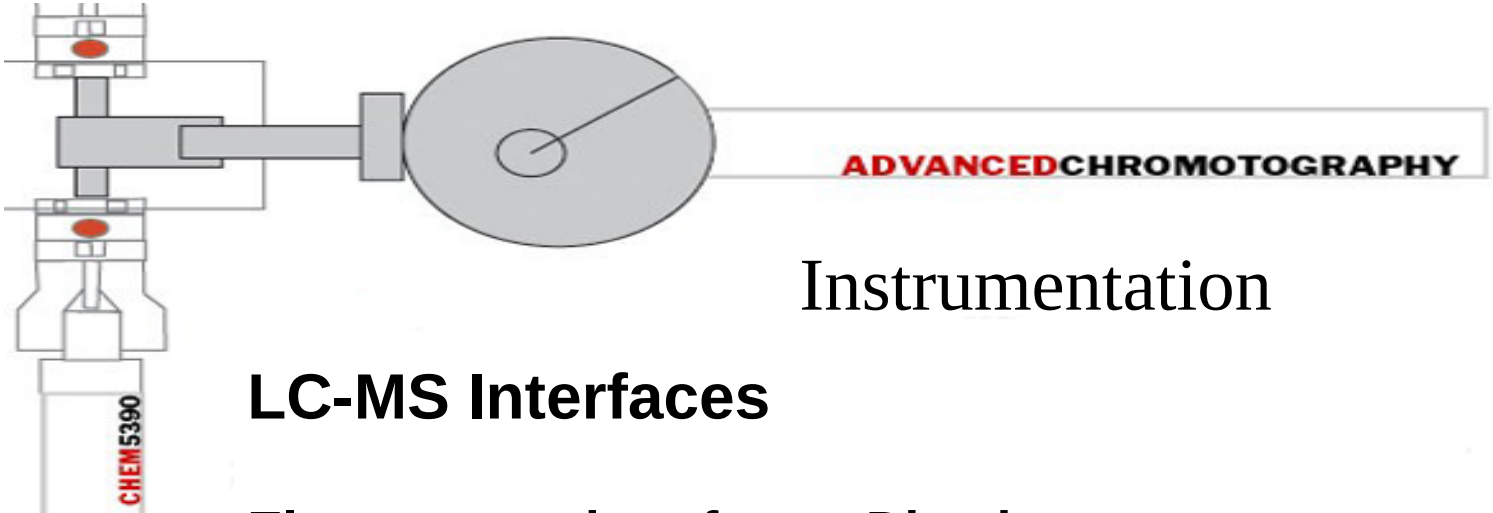
(M-nH)n- in negative ionization mode

Example is presented in the positive ionization m/z spectrum of the protein hen egg white lysozyme.



The m/z values can be expressed as follows: $m/z = (MW + nH+)/n$

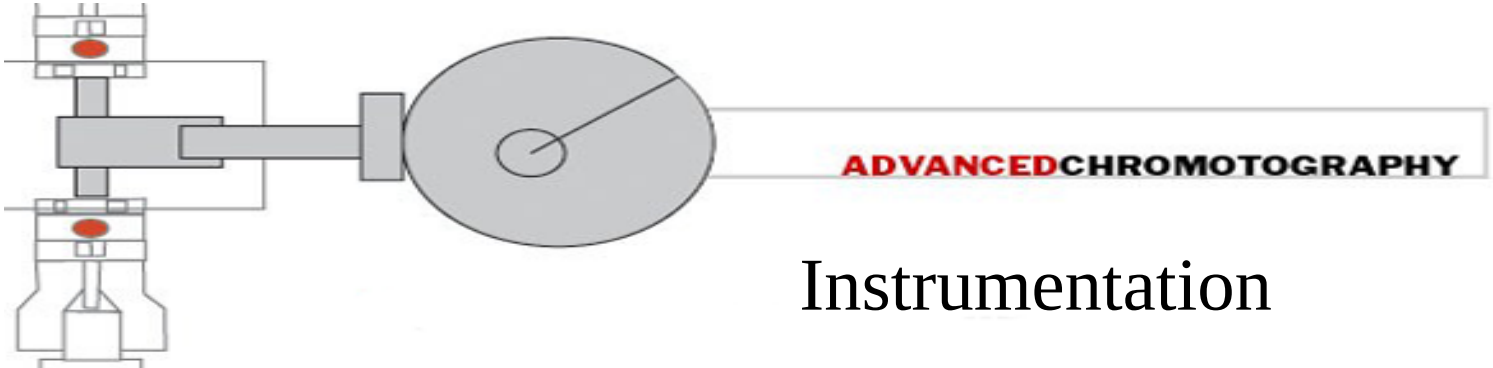
where: m/z = the mass-to-charge ratio MW = the molecular mass of the sample
n = the integer number of charges on the ions H = the mass of a proton = 1.008 Da



LC-MS Interfaces

Electrospray interface – Disadvantages

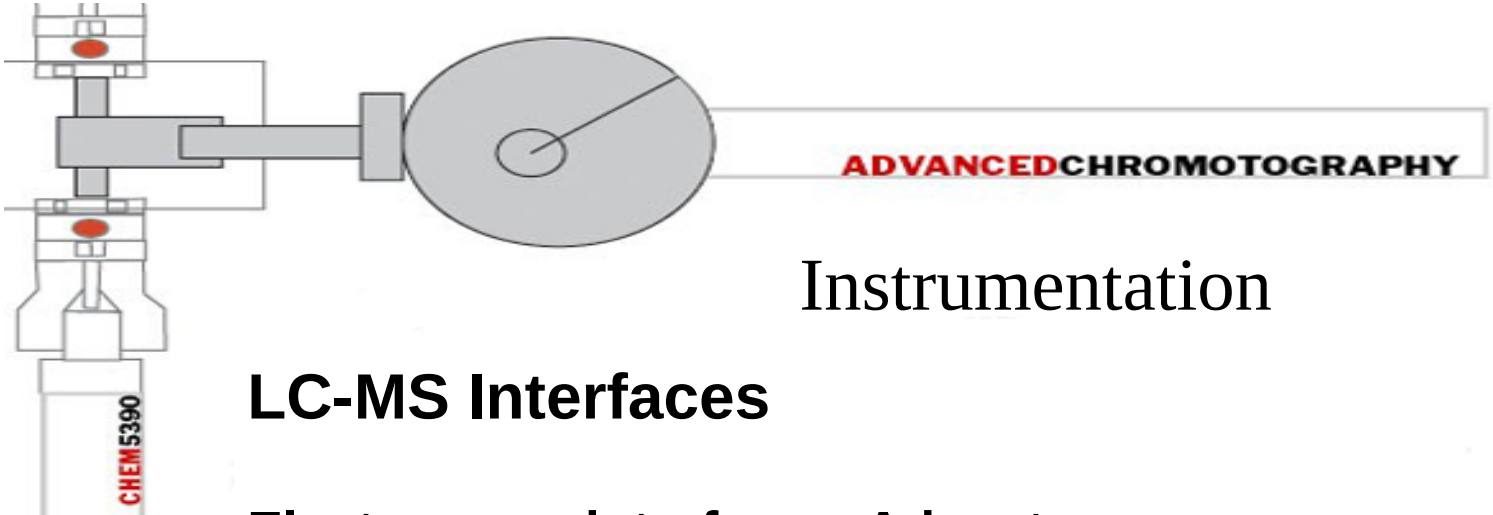
- **Electrospray is not applicable to non-polar or low-polarity compounds.**
- **The mass spectrum produced from an analyte depends upon a number of factors and spectra obtained using different experimental conditions may therefore differ considerably in appearance.**
- **Suppression effects may be observed and the direct analysis of mixtures is not always possible. This has potential implications for co-eluting analytes in LC–MS.**



LC-MS Interfaces

Electrospray interface – Disadvantages

• Electrospray is a soft-ionization method producing intact molecular species and structural information is not usually available.
Electrospray sources are capable of producing structural information from cone-voltage fragmentation but these spectra are not always easily interpretable. Experimentally, the best solution is to use a mass spectrometer capable of MS–MS operation but this has financial implications.

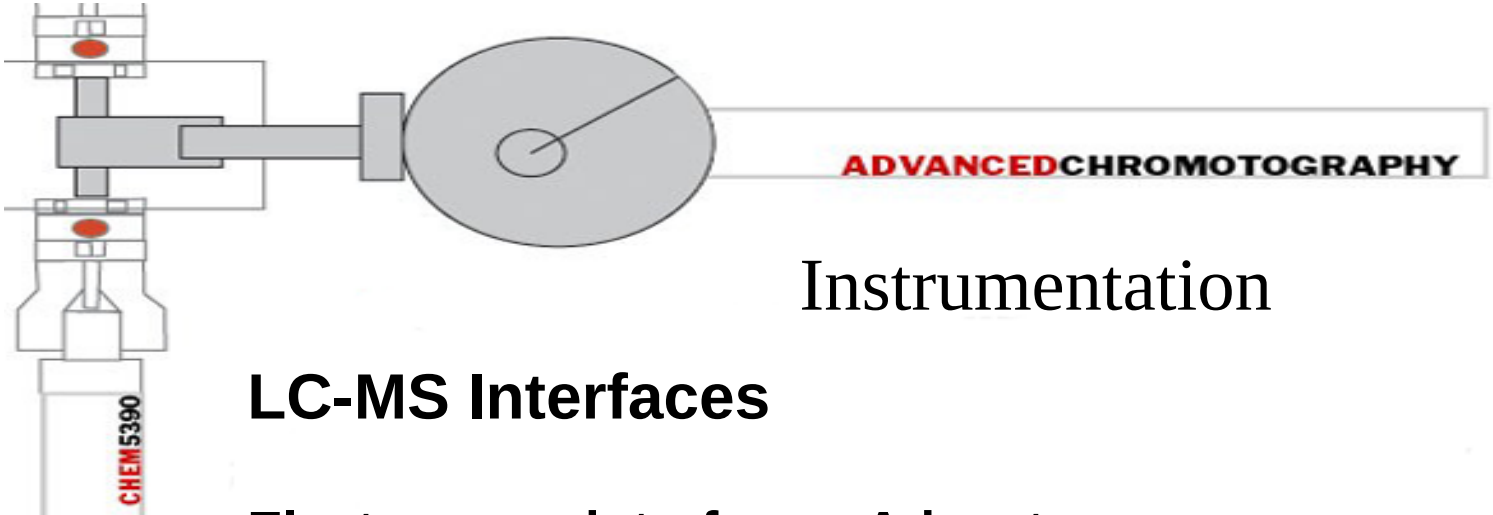


LC-MS Interfaces

Electrospray interface – Advantages

- **Ionization occurs directly from solution and consequently allows ionic and thermally labile compounds to be studied.**

- **Mobile phase flow rates from nl min^{-1} to in excess of 1 ml min^{-1} can be used with appropriate hardware, thus allowing conventional and microbore columns to be employed.**

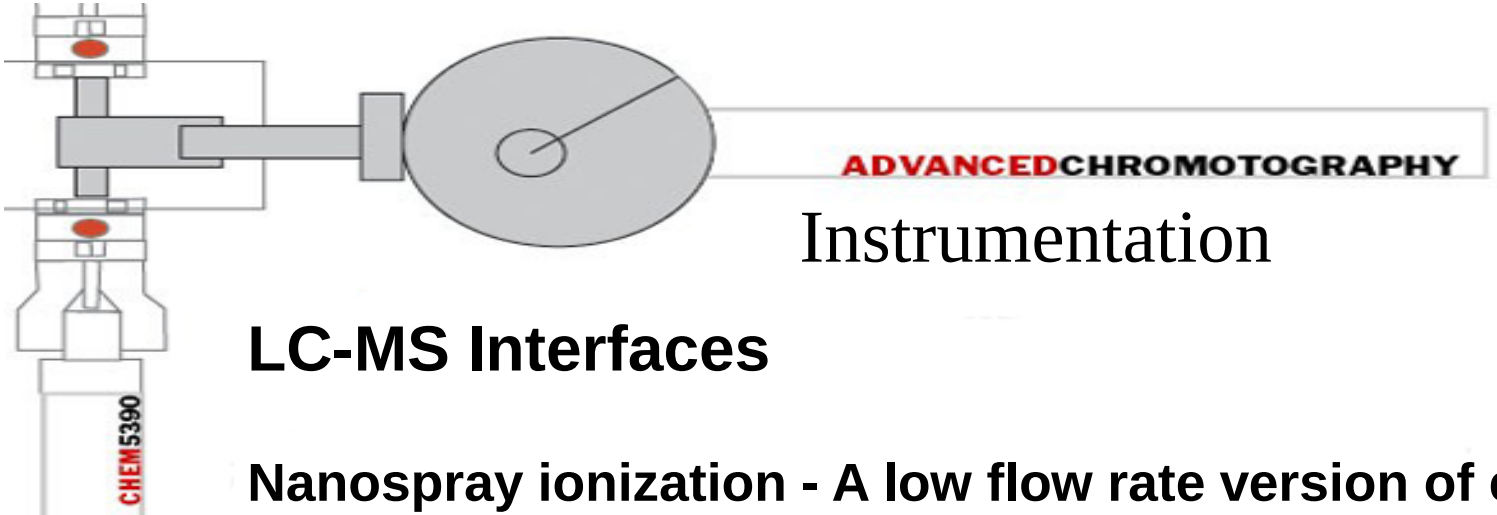


LC-MS Interfaces

Electrospray interface – Advantages

• Electrospray ionization, in contrast to the majority of other ionization methods, produces predominantly multiply charged ions of the intact solute molecule. This effectively extends the mass range of the mass spectrometer and allows the study of molecules with molecular weights well outside its normal range.

• For high-molecular-weight materials, an electrospray spectrum provides a number of independent molecular weight determinations from a single spectrum and thus increased precision.



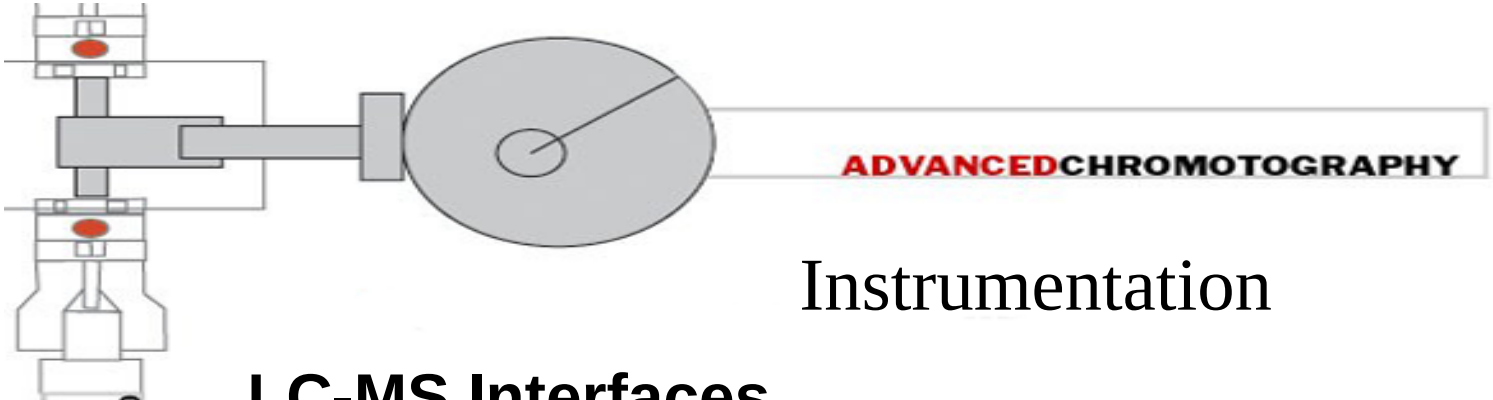
Instrumentation

LC-MS Interfaces

Nanospray ionization - A low flow rate version of electrospray.

The flow rate of solute and solvent using this procedure is very low, 30 - 1000 nL/min, and so far less sample is consumed than with the standard electrospray ionization technique.

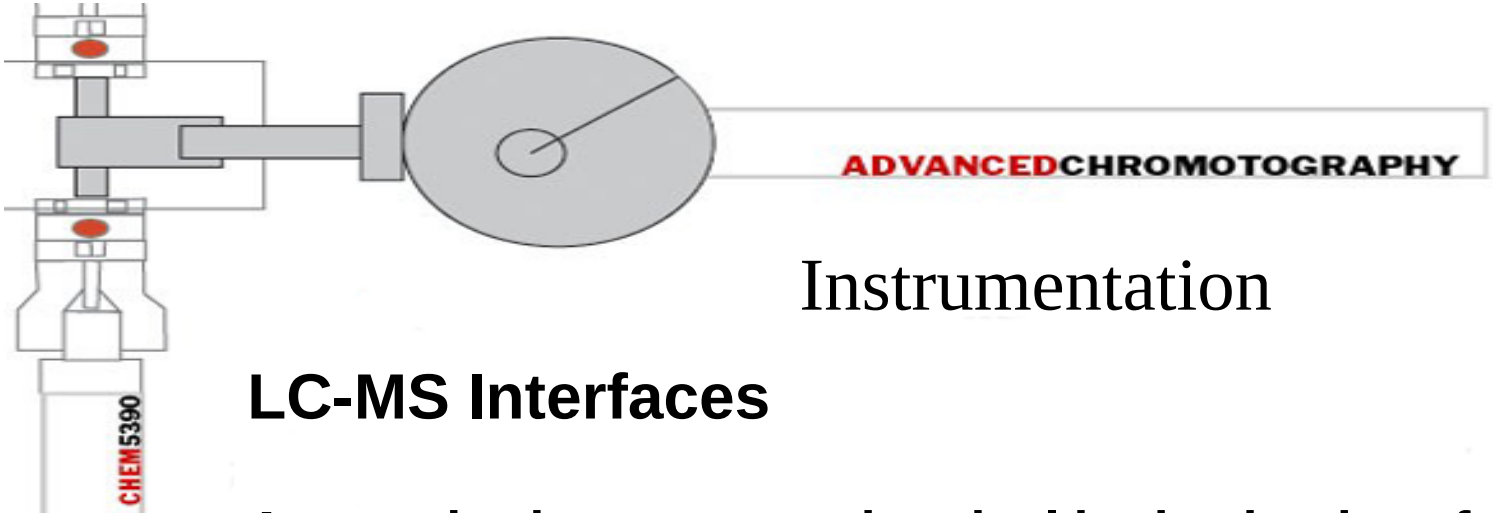
A common application of this technique is for a protein digest mixture to be analyzed to generate a list of molecular masses for the components present, and then each component to be analyzed further by tandem mass spectrometric (MS-MS) amino acid sequencing techniques



Instrumentation

LC-MS Interfaces

- **Moving-belt interface 1977**
- **Direct-liquid-introduction interface 1980**
- **Thermospray interface 1983**
- **Frit FAB/continuous-flow FAB interface 1985/1986**
- **Atmospheric-pressure chemical ionization interface 1986**
- **Particle-beam interface 1988**
- **Electrospray interface 1988**

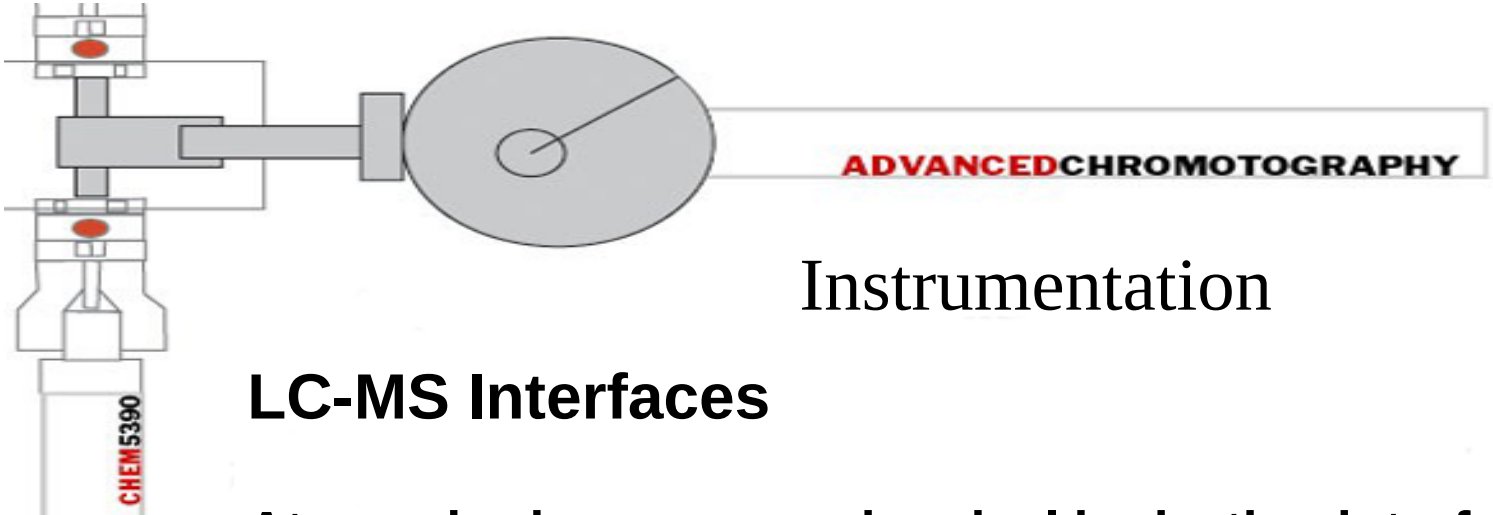


Instrumentation

LC-MS Interfaces

Atmospheric-pressure chemical ionization interface 1986

Although work demonstrating APCI was published in parallel with that demonstrating ESI, APCI was not widely adopted until ESI was commercialized, which occurred in the wake of Fenn's work.



Instrumentation

LC-MS Interfaces

Atmospheric-pressure chemical ionization interface 1986

Atmospheric-pressure chemical ionization (APCI) is another of the techniques in which the stream of liquid emerging from an HPLC column is dispersed into small droplets, in this case by the combination of heat and a nebulizing gas.

As such, APCI shares many common features with ESI and thermospray which have been discussed previously.

The differences between the techniques are the methods used for droplet generation and the mechanism of subsequent ion formation.

Atmospheric-pressure chemical ionization interface 1st generation

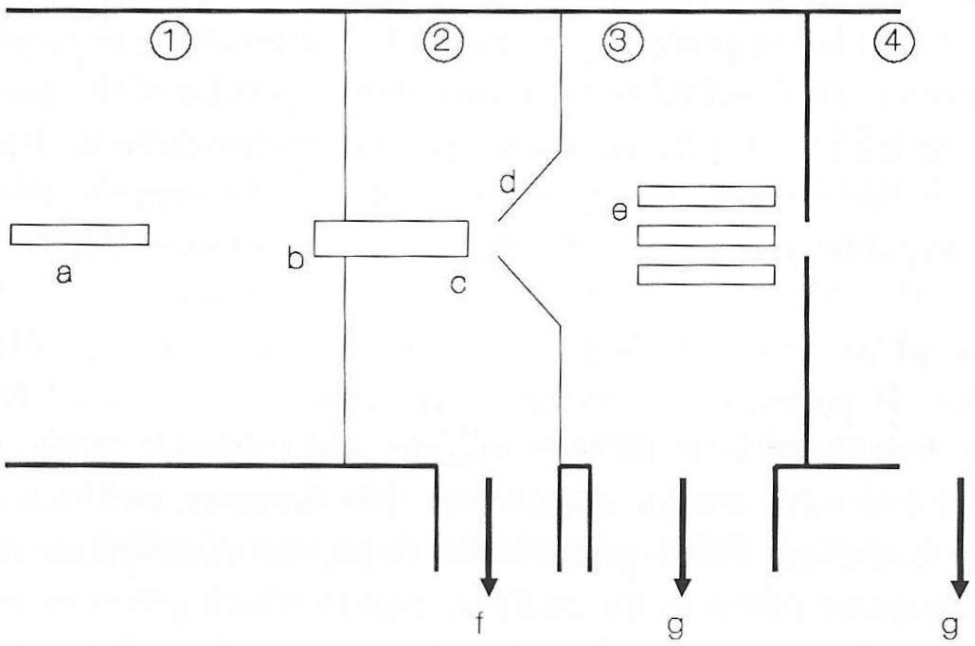


Fig. 1.15. Schematic drawing of the atmospheric-pressure ion source as a molecular beam apparatus. (1) Atmospheric-pressure ionization source region, (2) first pumping stage, (3) second pumping region, and (4) mass analyzer region. (a) Transfer tube from LC (either electrospray needle assembly or APCI heated nebulizer), (b) sampling orifice, (c) nozzle, (d) skimmer, (e) quadrupole, hexapole, or octapole ion collection and focusing device, (f) high through-put mechanical pump, and (g) turbomolecular pumps.

Instrumentation

LC-MS Interfaces

Atmospheric-pressure chemical ionization interface 1986

The LC eluent is sprayed through a heated (250-400 ^{o}C) vaporizer at atmospheric pressure.

The heat vaporizes the liquid, and the solvent molecules are ionized by electrons discharged from a corona needle.

The solvent ions transfer charge to the analyte molecules through chemical reactions (chemical ionization).

The analyte ions then pass through a capillary sampling orifice into the mass analyzer.

Atmospheric-pressure chemical ionization interface

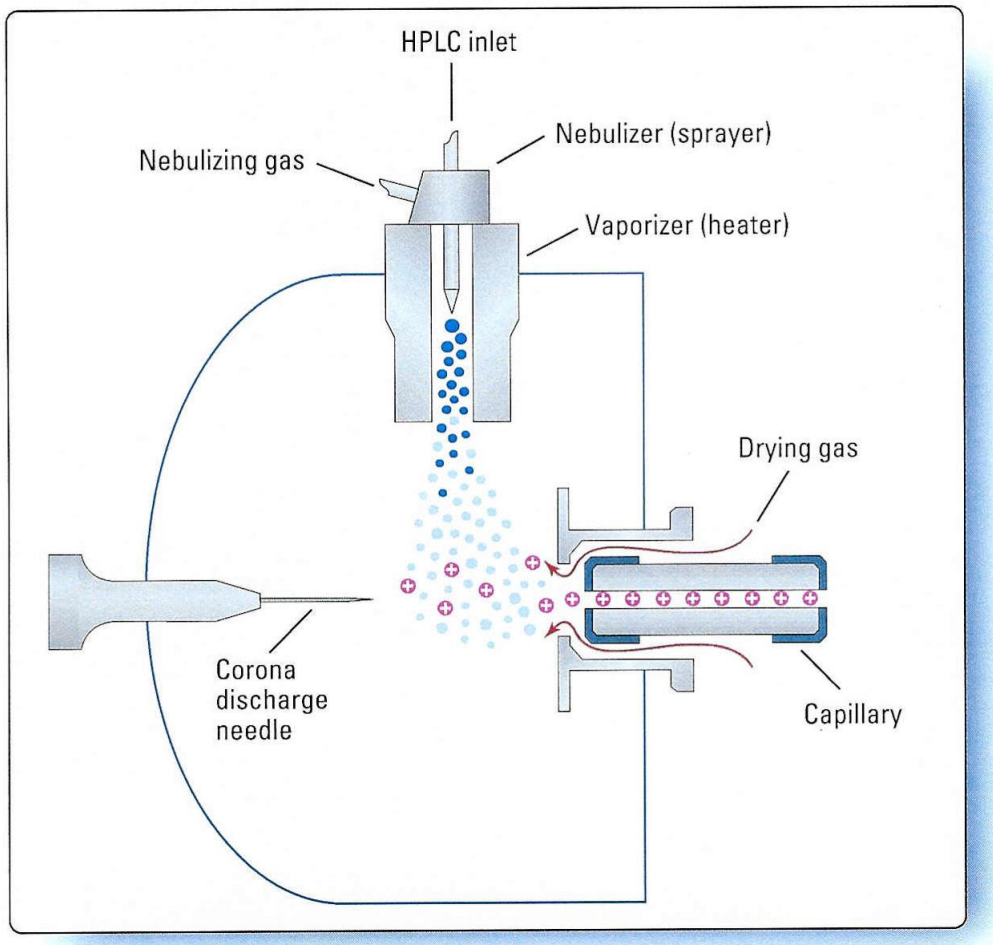
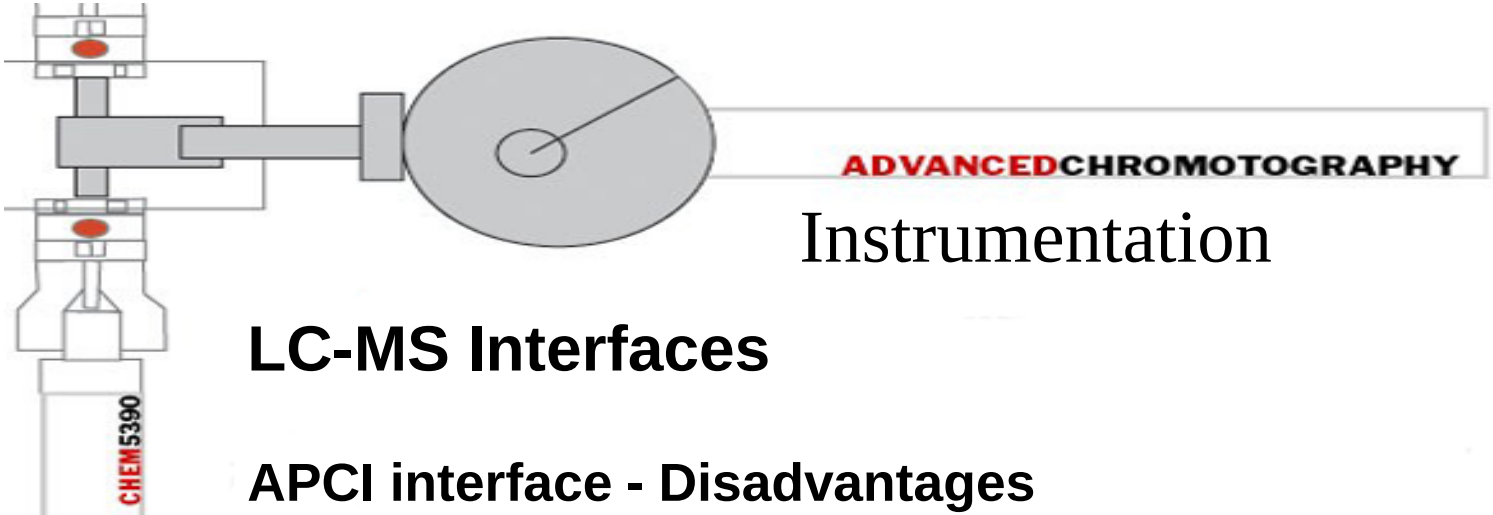


Figure 6. APCI ion source



LC-MS Interfaces

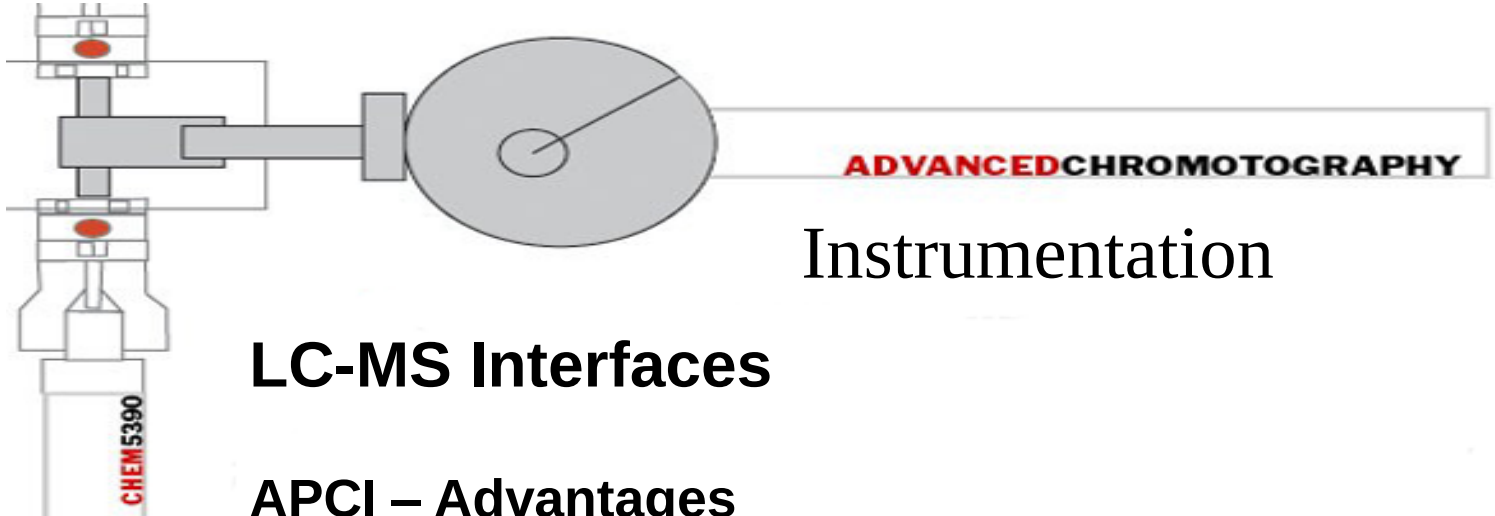
APCI interface - Disadvantages

- APCI spectra can contain ions from adducts of the analyte with the HPLC mobile phase or organic modifiers, such as ammonium acetate, that may be present. The presence of ions such as $(M + NH_4)+$ and $(M + CH_3COO)-$ may hinder interpretation of the spectra obtained.

- Structural information is not usually available unless cone-voltage fragmentation or MS–MS is used.

- APCI is not able to function effectively at very low flow rates.

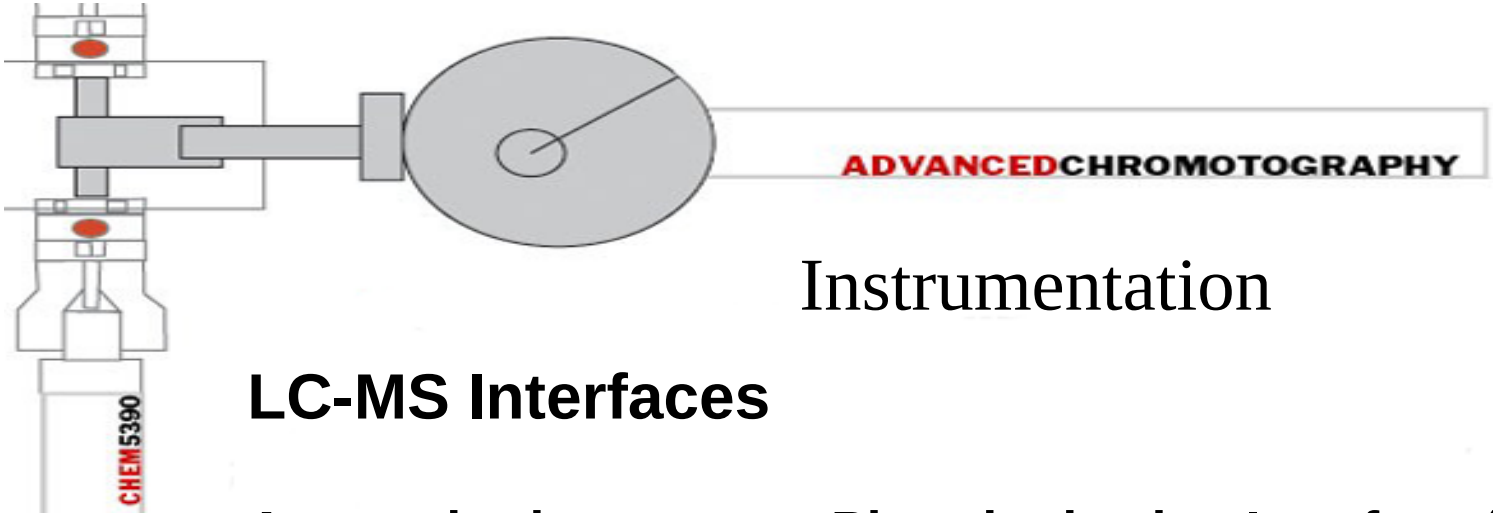
- APCI is not suitable for analytes that are charged in solution.



LC-MS Interfaces

APCI – Advantages

- APCI produces ions from solution and compounds with a degree of thermal instability may be studied without their decomposition.
- APCI is best applied to compounds with low to moderately high polarities.
- APCI is a soft ionization technique which usually enables the molecular weight of the analyte under study to be determined.
- APCI is able to deal with flow rates up to 2 $mlmin^{-1}$ and is, consequently, directly compatible with 4.6 mm HPLC columns.
- APCI is more tolerant to the presence of buffers in the mobile phase stream than is ESI.
- APCI is more tolerant to changes in experimental conditions than ESI, including gradient elution.



Instrumentation

LC-MS Interfaces

Atmospheric-pressure Photoionization Interface (APPI)

Atmospheric-pressure photoionization (APPI) is a relatively new technique. Just as in APCI, a vaporizer converts the LC eluent to the gas phase.

A discharge lamp generates photons in a narrow range of ionization energies. The range of energies is chosen to ionize analyte molecules while minimizing ionization of solvent molecules.

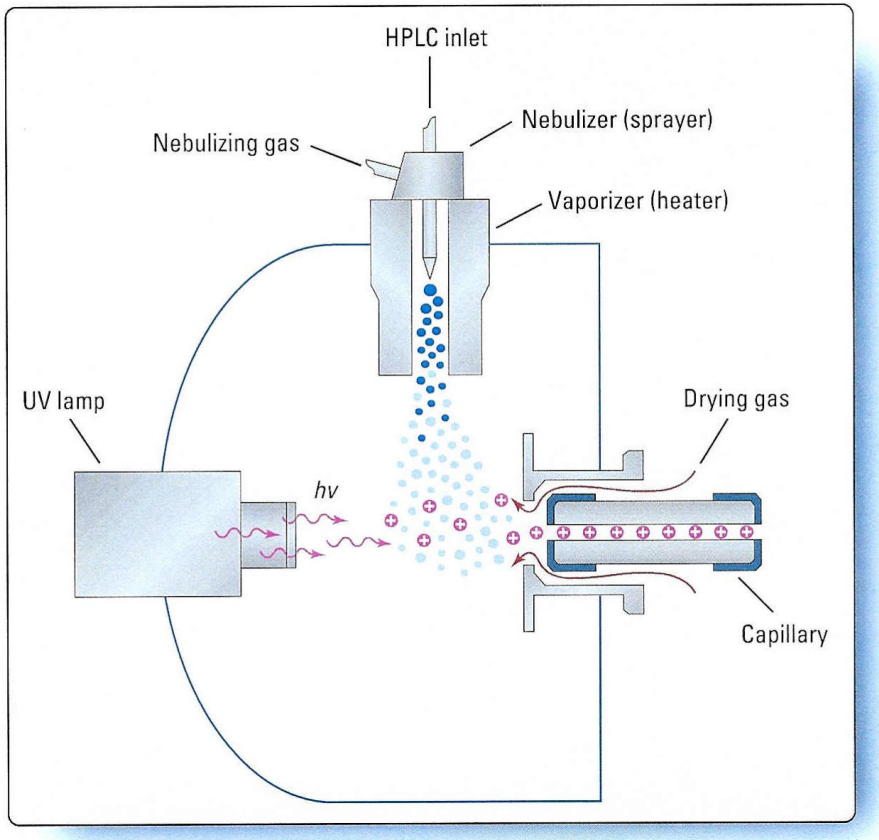
The resulting ions pass through a capillary sampling orifice into the mass analyzer.

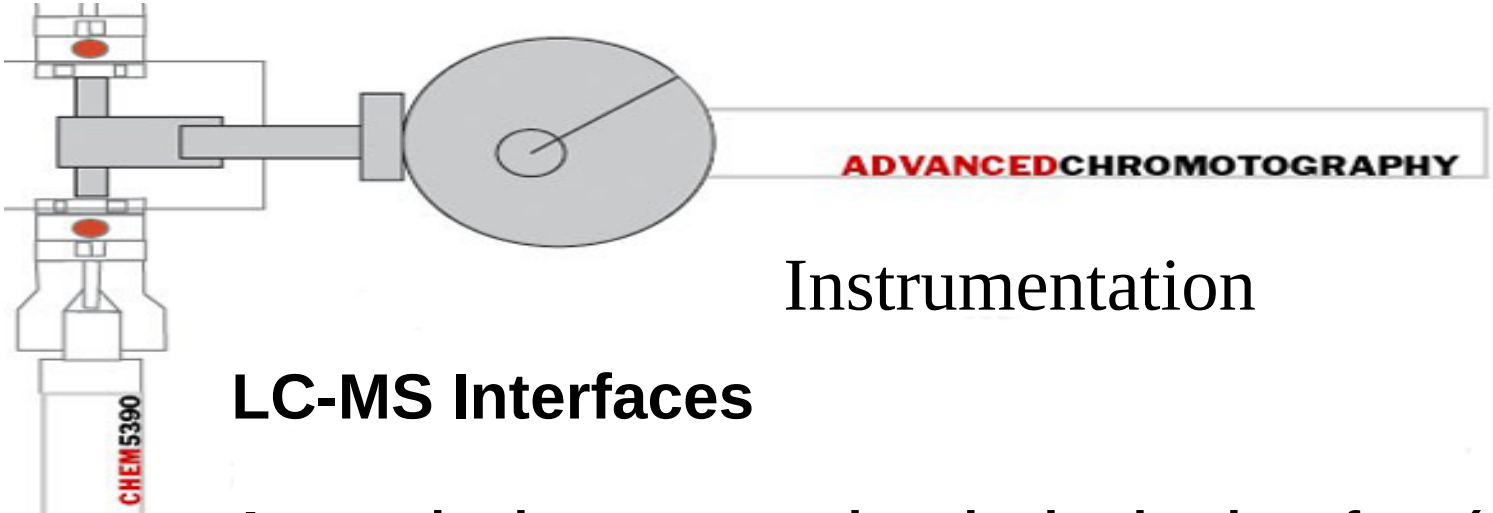
Instrumentation

LC-MS Interfaces

Atmospheric-pressure photoionization interface (APPI)

Figure 7. APPI ion source



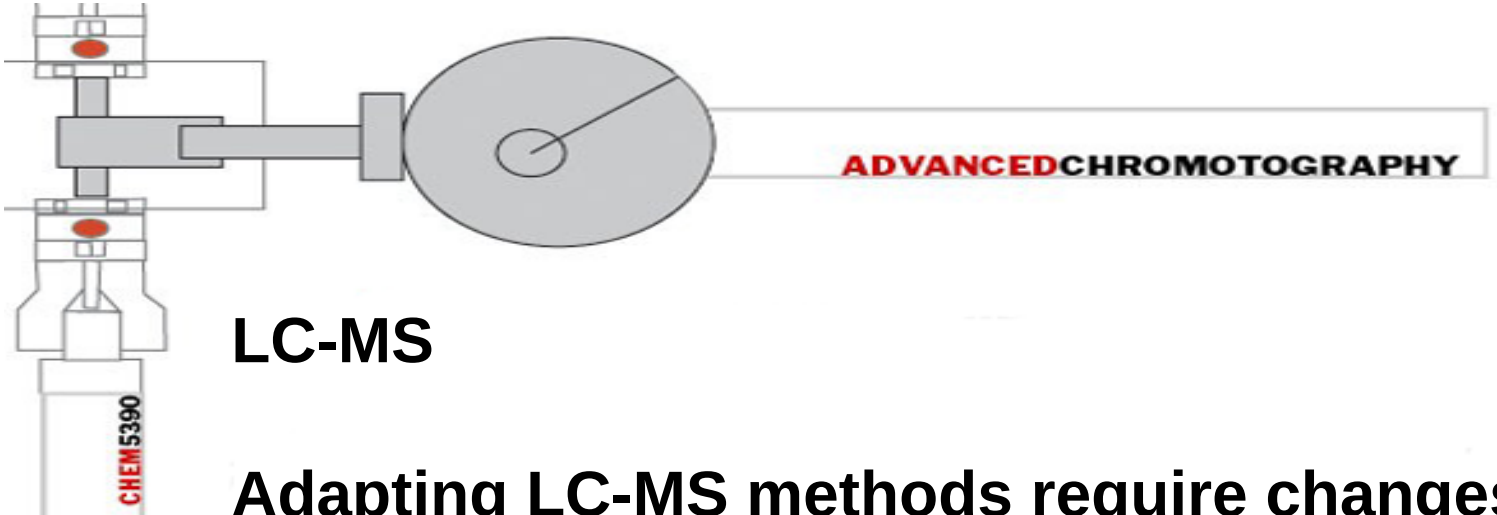


Instrumentation

LC-MS Interfaces

Atmospheric-pressure photoionization interface (APPI)

Atmospheric-pressure photoionization is applicable to the same compounds as APCI, however it responds better to highly nonpolar compounds and low flow rates (<100 uL/min).



LC-MS

Adapting LC-MS methods require changes in sample preparation and solution chemistry to:

- **ensure adequate analyte concentration**
- **maximize ionization through careful selection of solvents and buffers**
- **minimize the presence of compounds that compete for ionization or suppress signal through gas-phase reactions**

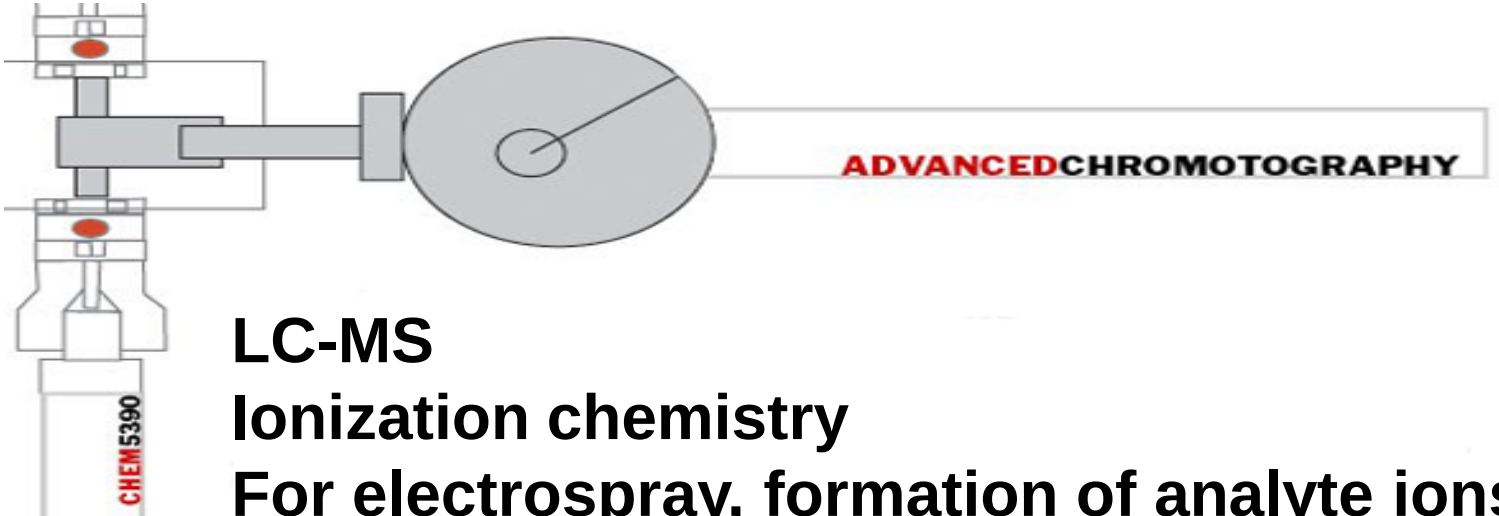
LC-MS
Sample Preparation

Generally consist of:

- **on-column concentration to increase analyte concentration**

- **desalting to reduce the Na and K adduct formation that commonly occurs especially in electrospray**

- **filtration to separate a low-molecular weight compounds from the matrix, i.e. drug from proteins in plasma, milk, or tissue**



LC-MS

Ionization chemistry

For electrospray, formation of analyte ions in solution is essential to achieving good results.

Techniques to help ion formation include:

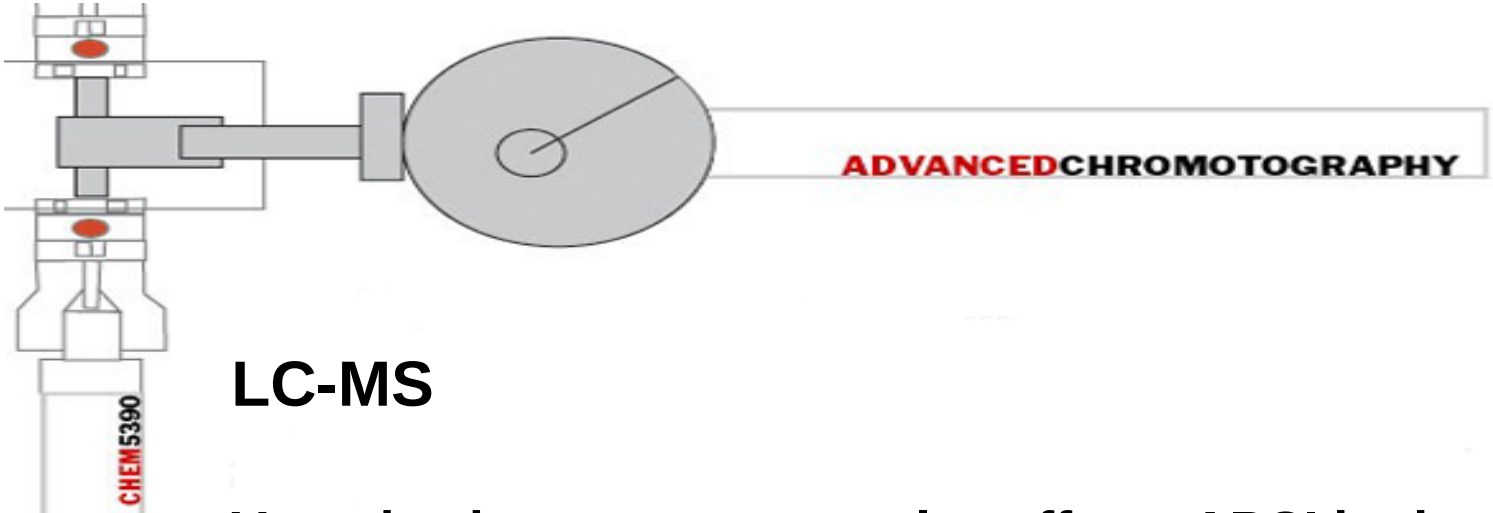
- **select more volatile buffers to reduce the buildup of salts in the ion source**
- **adjust solvent pH according to the polarity of ions desired and the pH of the sample**
- **use solvents that have low heats of vaporization and low surface tensions to enhance ion desorption**
- **make sure that gas-phase reactions do not neutralize ions through proton transfer or ion pair reactions**

LC-MS

Solution chemistry is less critical for APCI because ionization occurs in the gas phase, not the liquid phase.

Solvent selection can still have an effect on signal response, so need to:

- **select more volatile solvents**
- **select solvents with a lower charge affinity than the analyte**
- **protic solvents generally work better than nonprotic solvents for positive ion mode**
- **for negative ionization, solvents that readily capture an electron must be used**



LC-MS

Vaporization temperature also affects APCI ionization results.

The temperature must be hot enough to vaporize the solvent but not so hot as to cause thermal degradation of the analyte molecules.

LC-MS

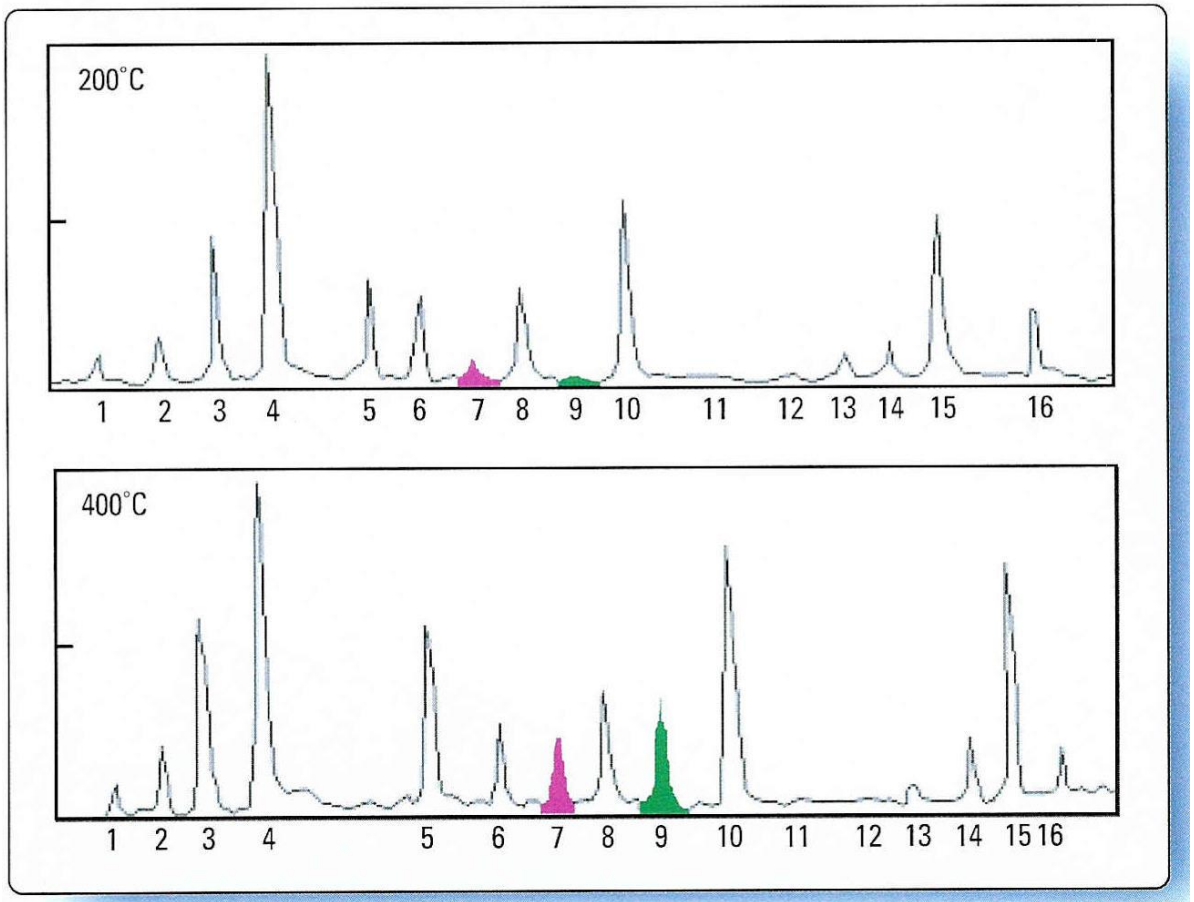
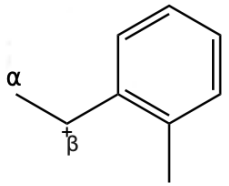
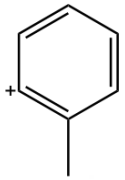


Figure 20. APCI analysis with an inadequate vaporizer temperature (200°C) yields poor results for some compounds compared to a more typical vaporizer temperature (400°C)

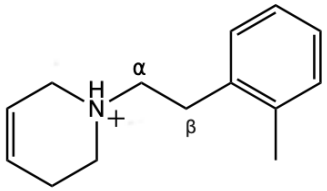
APCI – Example with Fentanyl and Fentanyl Derivatives

Chemical Formula: $C_9H_{11}^+$
Exact Mass: 119.086

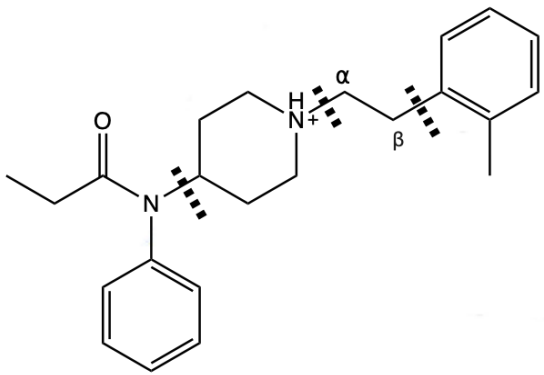




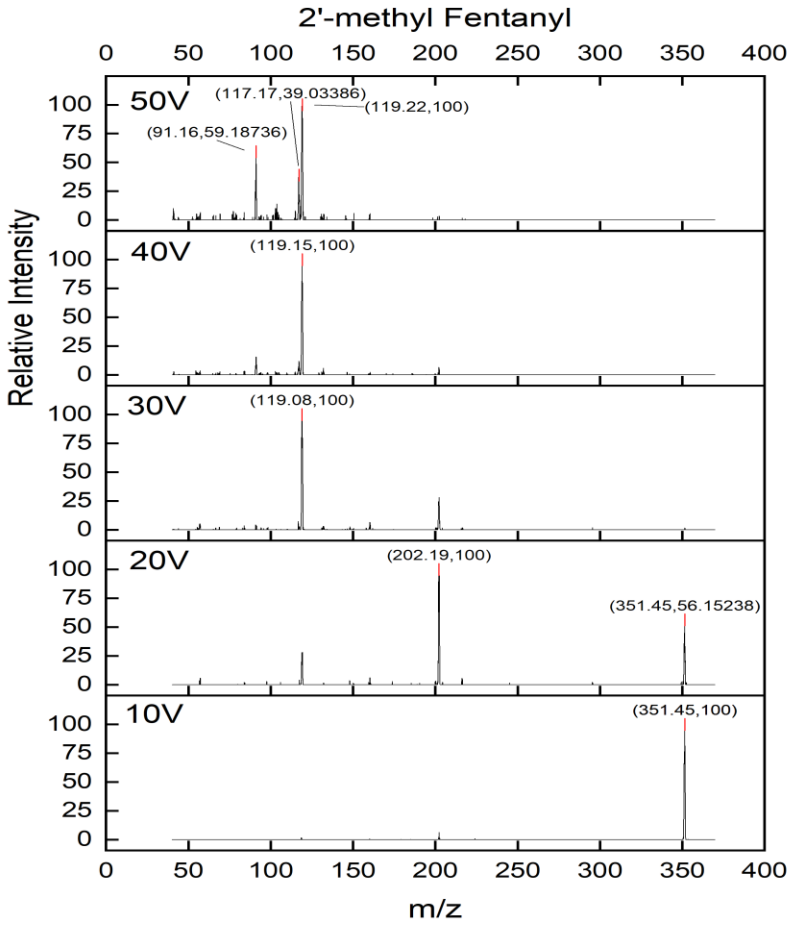
Chemical Formula: $C_7H_7^+$
Exact Mass: 91.054



Chemical Formula: $C_{14}H_{20}N^+$
Exact Mass: 202.159

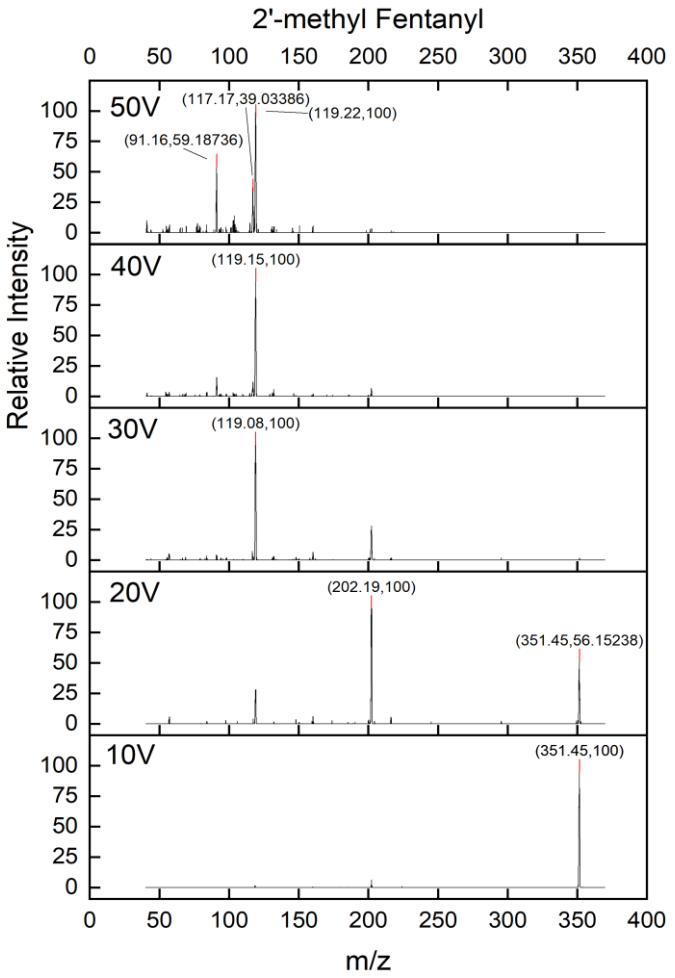


Chemical Formula: $C_{23}H_{31}N_2O^+$
Exact Mass: 351.243

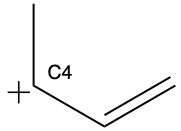


APCI – Example with Fentanyl and Fentanyl Derivatives

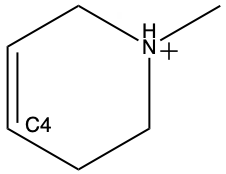
Volts	Bond Breaks
50V	1:0.59 ratio of N-a carbon bond: B carbon-benzene bond
40V	Breakage at the N-a Carbon bond
30V	Breakage at the N-a Carbon bond
20V	1:0.56 ratio of N C4 bond:intact parent molecule
10V	no fragmentation



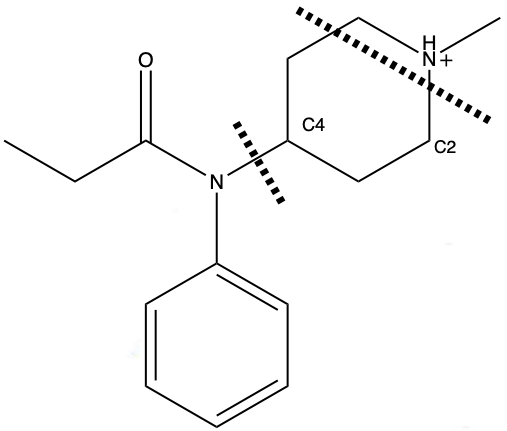
APCI – Example with Fentanyl and Fentanyl Derivatives



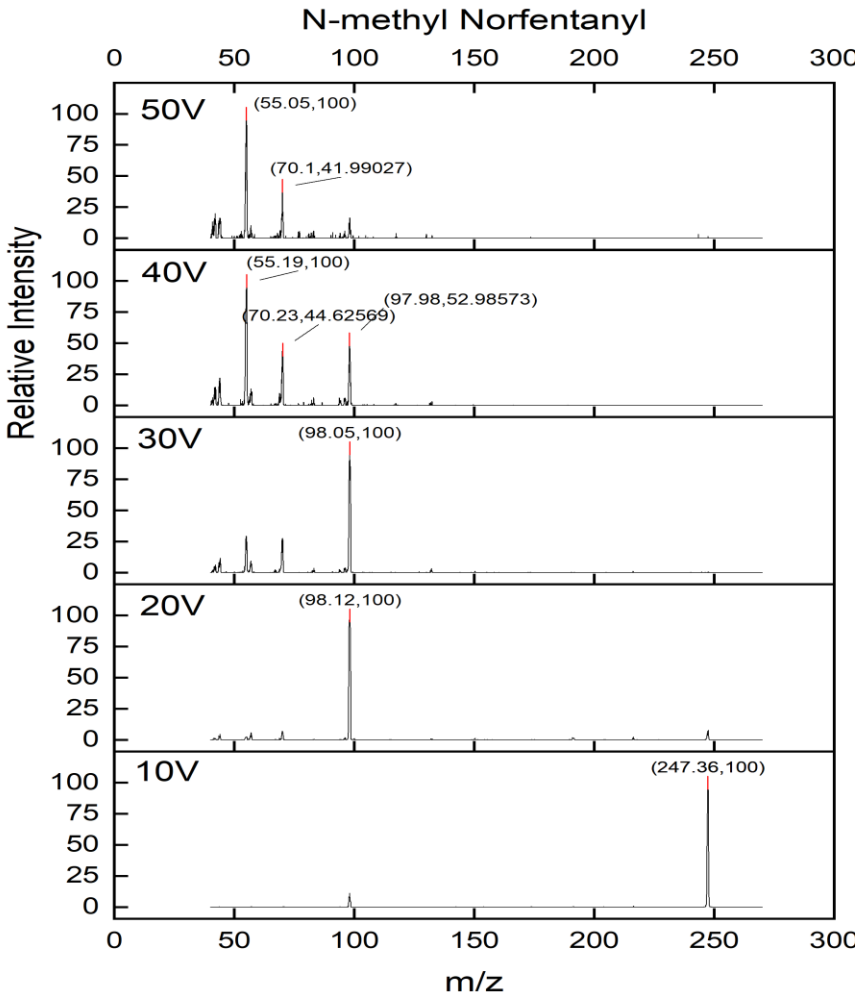
Chemical Formula: $C_4H_7^+$
Exact Mass: 55.054



Chemical Formula: $C_6H_{12}N^+$
Exact Mass: 98.096

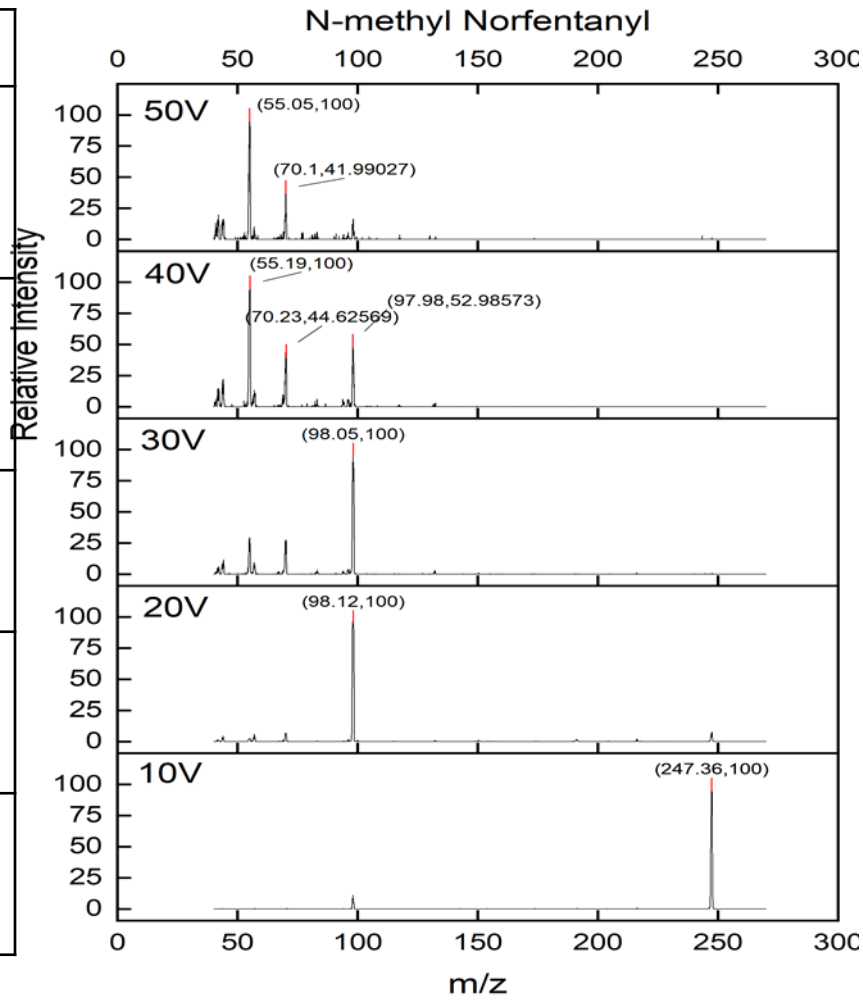


Chemical Formula: $C_{15}H_{23}N_2O^+$
Exact Mass: 247.180

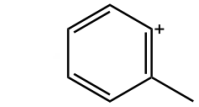


APCI – Example with Fentanyl and Fentanyl Derivatives

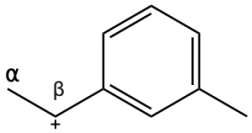
Volts	Bond Breaks
50V	Ring break at N-C2 and C5-C6 bond
40V	1:0.53 ratio of N-C2 and C5-C6 bond:N-C4 bond
30V	breakage of N-C4 bond
20V	breakage of N-C4 bond
10V	no fragmentation



APCI – Example with Fentanyl and Fentanyl Derivatives

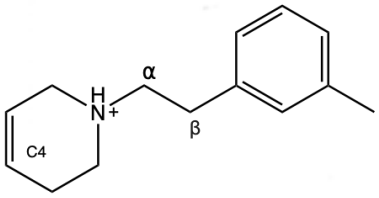


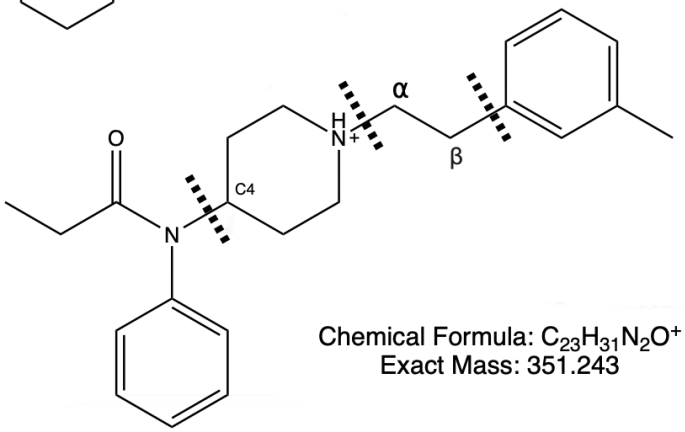
Chemical Formula: $C_7H_7^+$
Exact Mass: 91.054



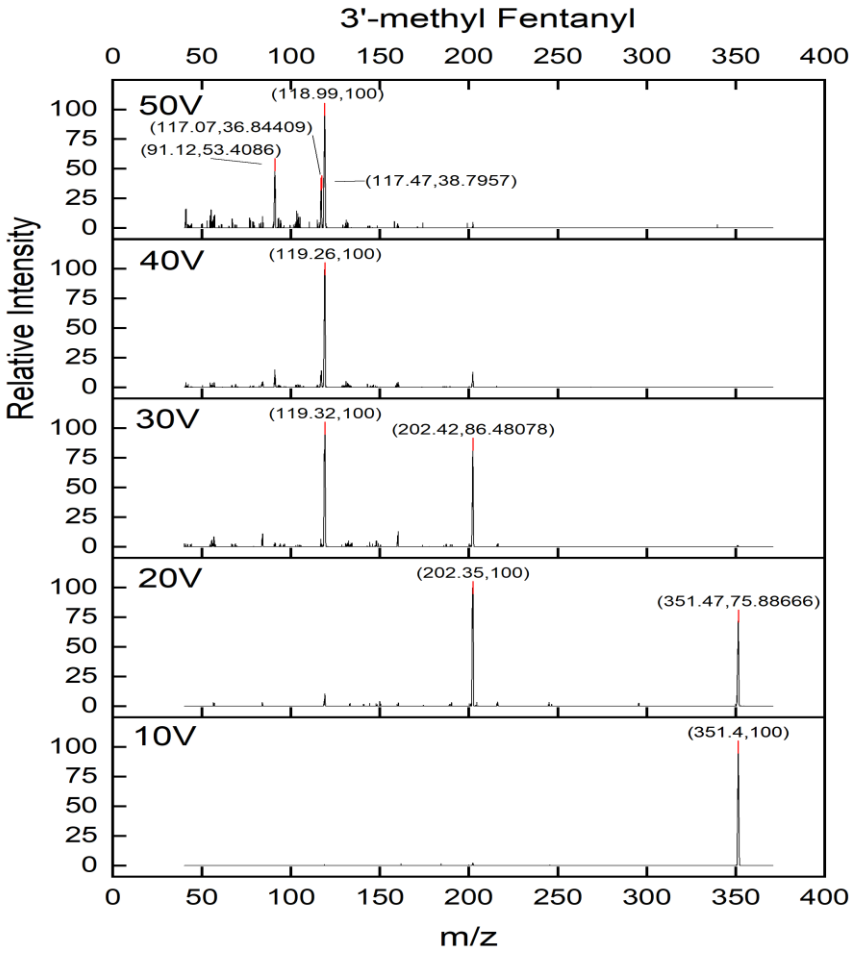
Chemical Formula: $C_9H_{11}^+$
Exact Mass: 119.086

Chemical Formula: $C_{14}H_{20}N^+$
Exact Mass: 202.159



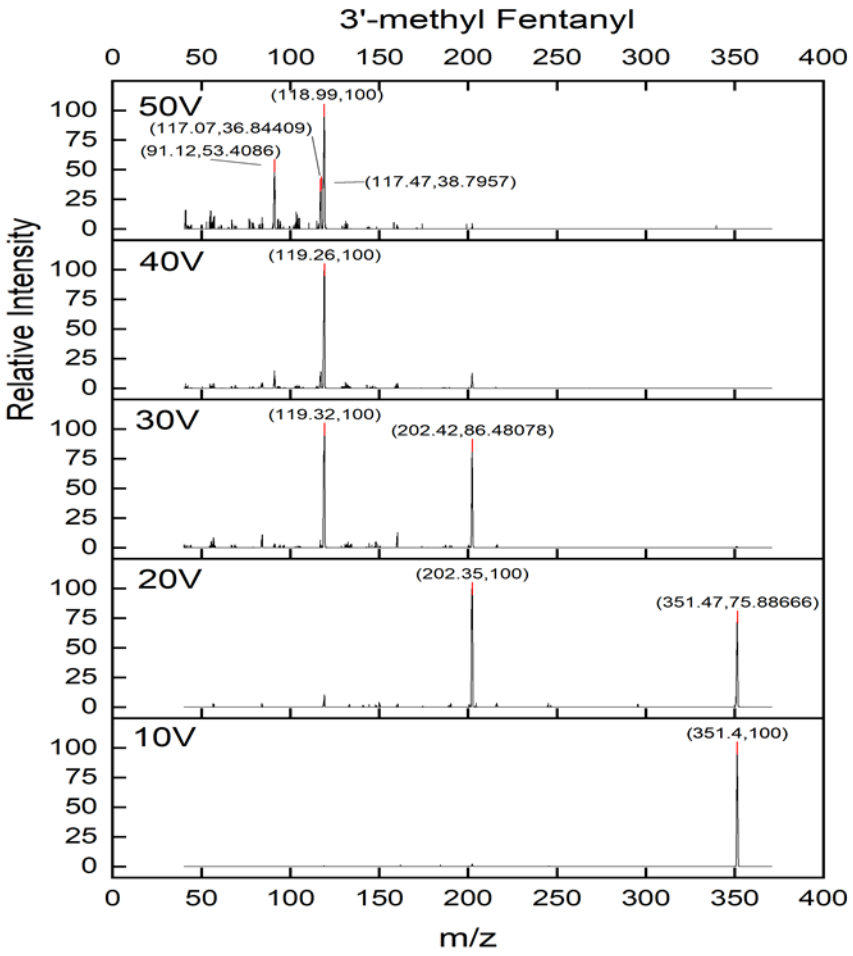


Chemical Formula: $C_{23}H_{31}N_2O^+$
Exact Mass: 351.243



APCI – Example with Fentanyl and Fentanyl Derivatives

Volts	Bond Breaks
50V	1:0.53 ratio of the N-a carbon bond: B carbon- benzene bond
40V	A break at the N-a carbon bond
30V	1:0.86 ratio of the N-a carbon bond:N-C4 bond
20V	1:0.76 ratio of the N-C4 bond: intact parent molecule
10V	no fragmentation



Instrumentation

LC-MS Interfaces

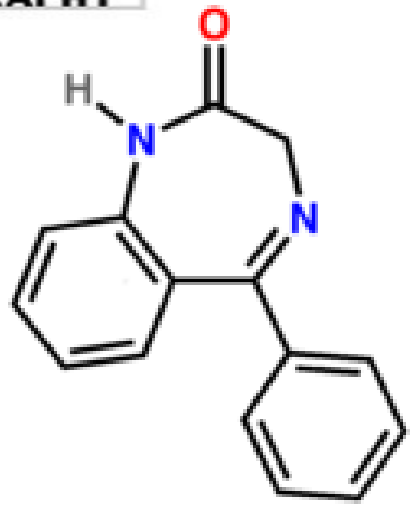
Other Electrospray Examples

LC-MS Applications

Pharmaceutical Applications

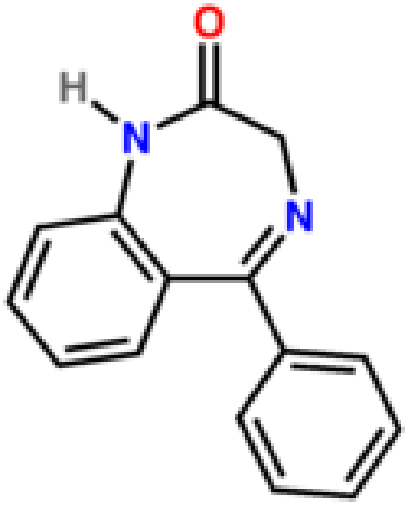
Information from the MS can be used to determine even compounds that are not separated by LC.

LC-MS Applications



Pharmaceutical Applications

Benzodiazepines are among the most commonly prescribed depressant medications in the United States today. More than 15 different types of benzodiazepine medications exist to treat a wide array of both psychological and physical maladies based on dosage and implications.
Commonly prescribed benzodiazepines include Xanax© (alprazolam), Librium© (chlordiazepoxide), Valium© (diazepam), and Ativan© (lorazepam).



LC-MS Applications

Pharmaceutical Applications

Example:
A series of benzodiazepines are analyzed using both UV and MS detectors. The UV trace does not give a separated mixture but the extracted ions in the MS can be identified.

LC-MS Applications

Pharmaceutical Applications

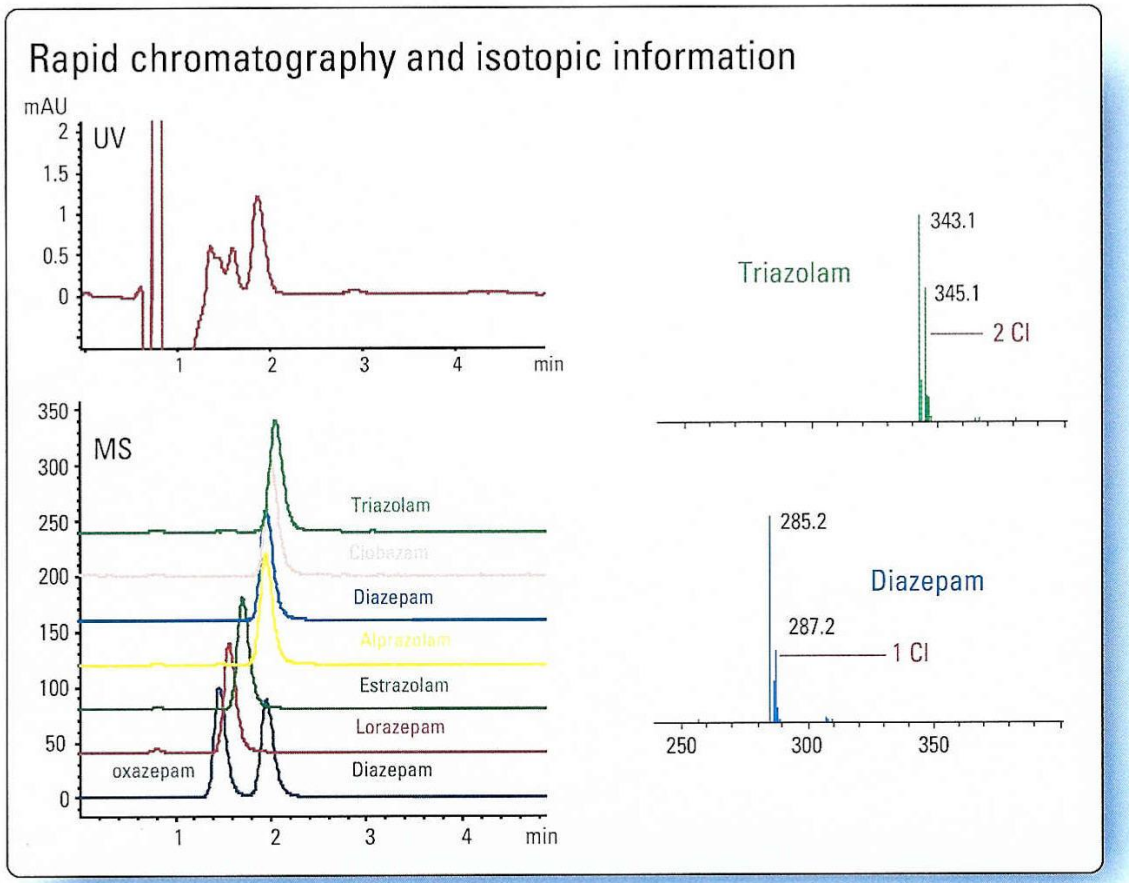


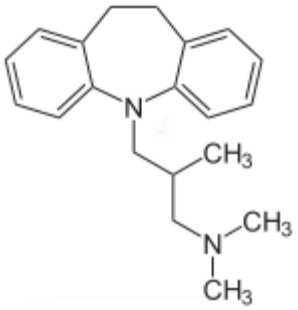
Figure 27. MS identification and quantification of individual benzodiazepines from an incompletely resolved mixture

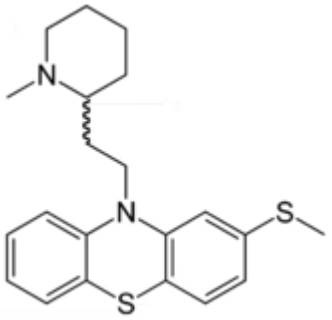
LC-MS Applications

Clinical Applications

Trimipramine (a tricyclic antidepressant) and Thioridazine (a tranquilizer) are at levels in urine that do not typically show up in the LC-UV.

However if the analytes from the LC are transferred to the MS, a single quad has enough sensitivity to detect the compounds.





LC-MS Applications

Clinical Applications

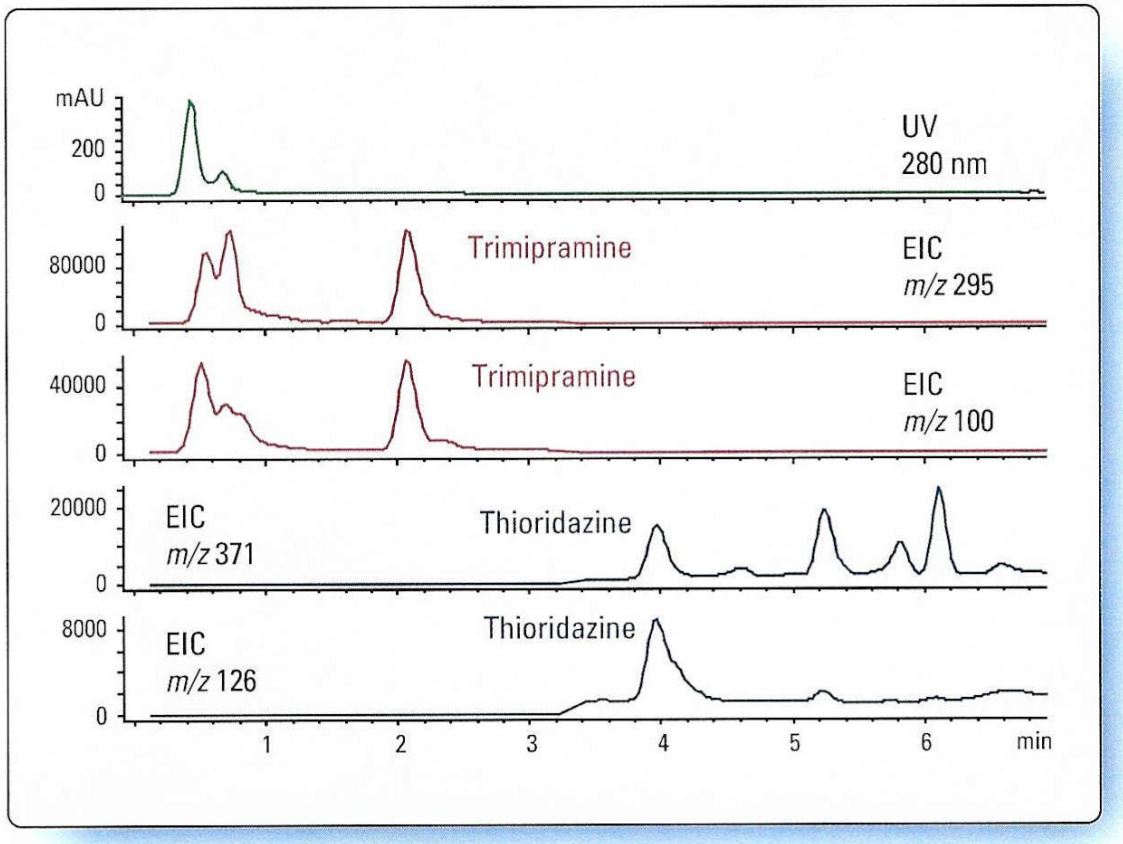


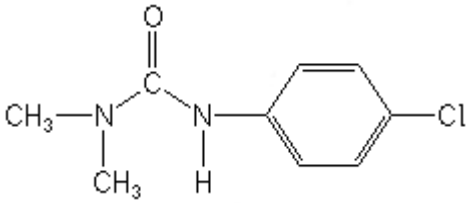
Figure 31. Trimipramine and thioridazine in a urine extract

LC-MS Applications

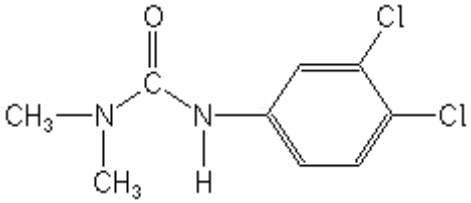
Environmental Applications

Phenylurea herbicides are used for pre- and post-emergence weed control in a wide variety of crops and are widely applied throughout the world. In general, these herbicides have long lifetimes in the environment.

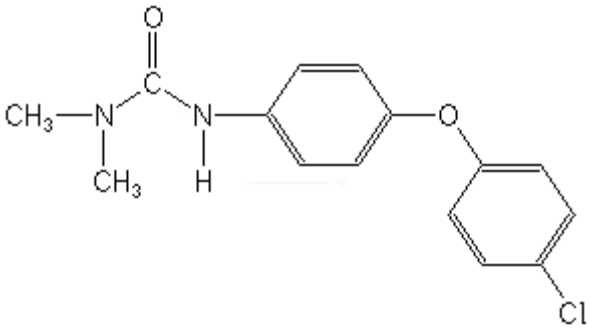
monuron



diuron



chloroxouron



LC-MS Applications

Environmental Applications

Phenylurea herbicides are very similar in structure and difficult to separate completely in LC, so UV detection is hard to interpret.

The monuron and diuron have one benzene ring and differ by a single chlorine giving very similar UV-vis spectra.

When analyzed using electrospray ionization on an LC-MS system, the compounds are easily identified.

LC-MS Applications

Environmental Applications

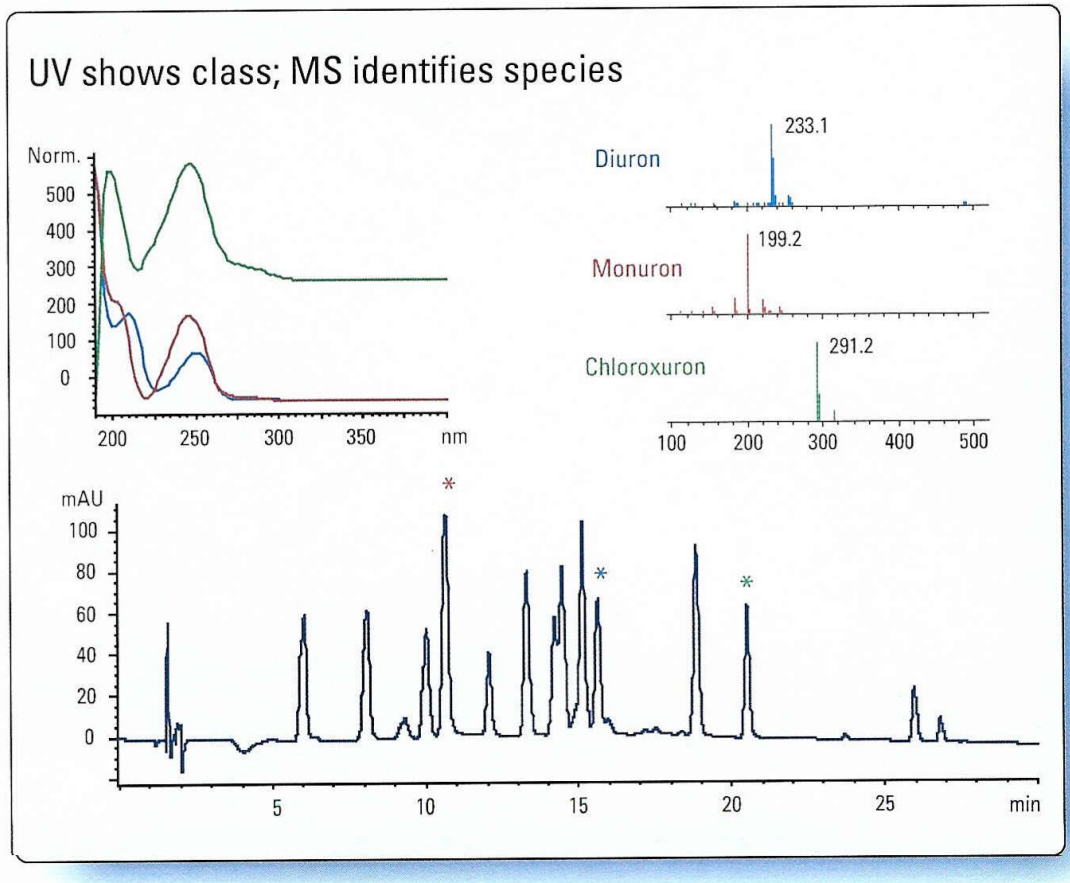


Figure 36. Chromatogram of phenylurea herbicide with UV and MS spectra

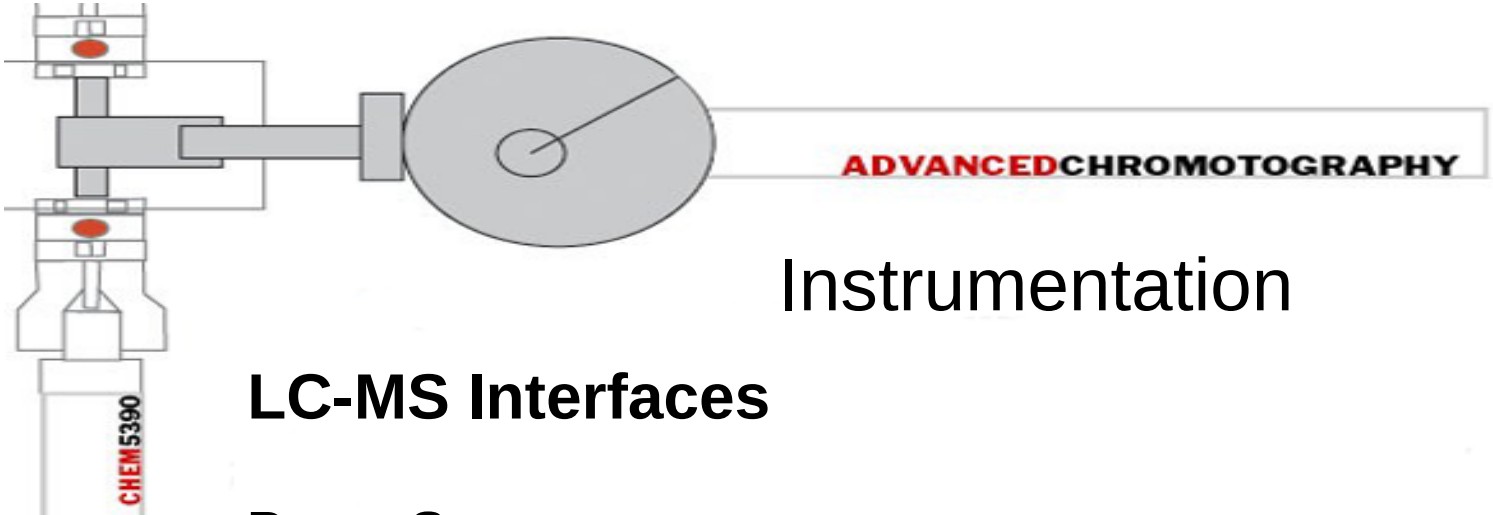
Instrumentation

LC-MS Interfaces

Paper Spray

Ambient ionization method as an alternative to traditional sample preparation.

Capable of ionizing a wide range of molecules and has shown to be especially useful in analyzing analytes within complex biofluids.



Instrumentation

LC-MS Interfaces

Paper Spray

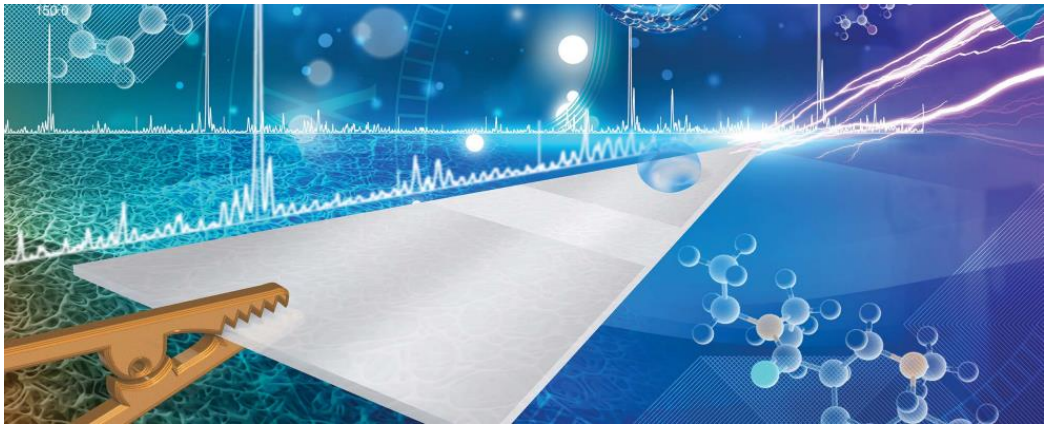
Apply an electric current to the paper which contains the sample (with compatible spray solvent).

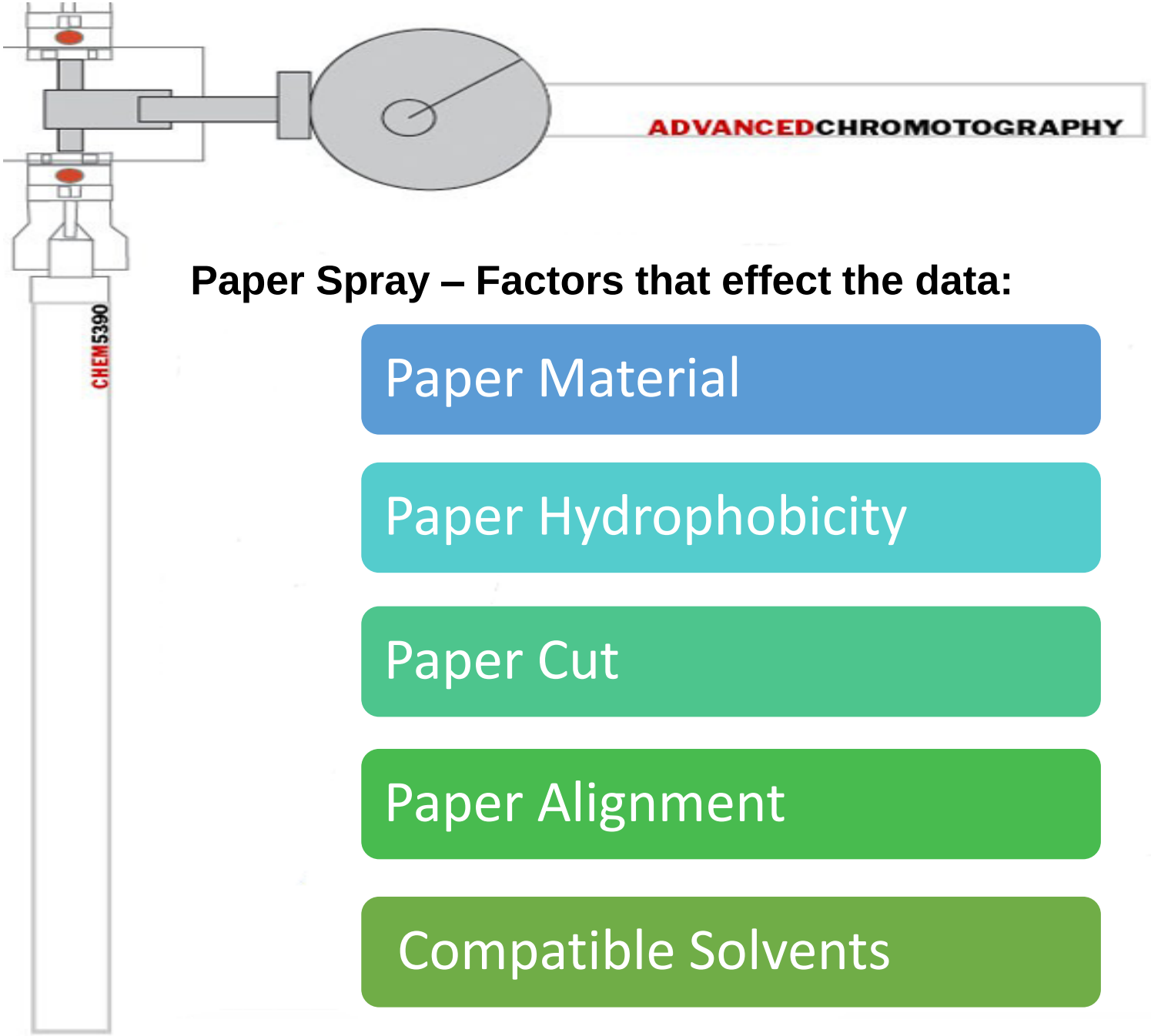
Analyte is ionize and introduces into the instrument for MS analysis.

Samples can be solids or liquids (as long as the analytes are volatile).

Instrumentation

Paper Spray





Paper Spray – Factors that effect the data:

- Paper Material
- Paper Hydrophobicity
- Paper Cut
- Paper Alignment
- Compatible Solvents

Paper Spray – Factors that effect the data

Paper Material

Teslin vs Cellulose Substrate

Teslin

- Uniform Pores
- Hydrophobic
- Synthetic polyolefin-based material with micropores that allows it to absorb and create strong interlocking bonds with chemistry

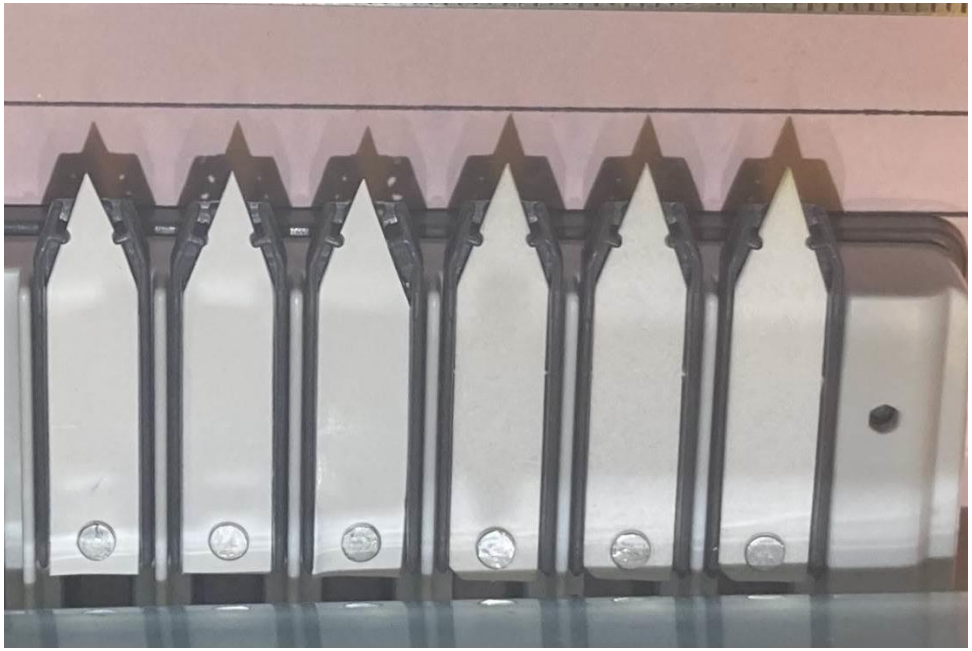
Cellulose Substrate

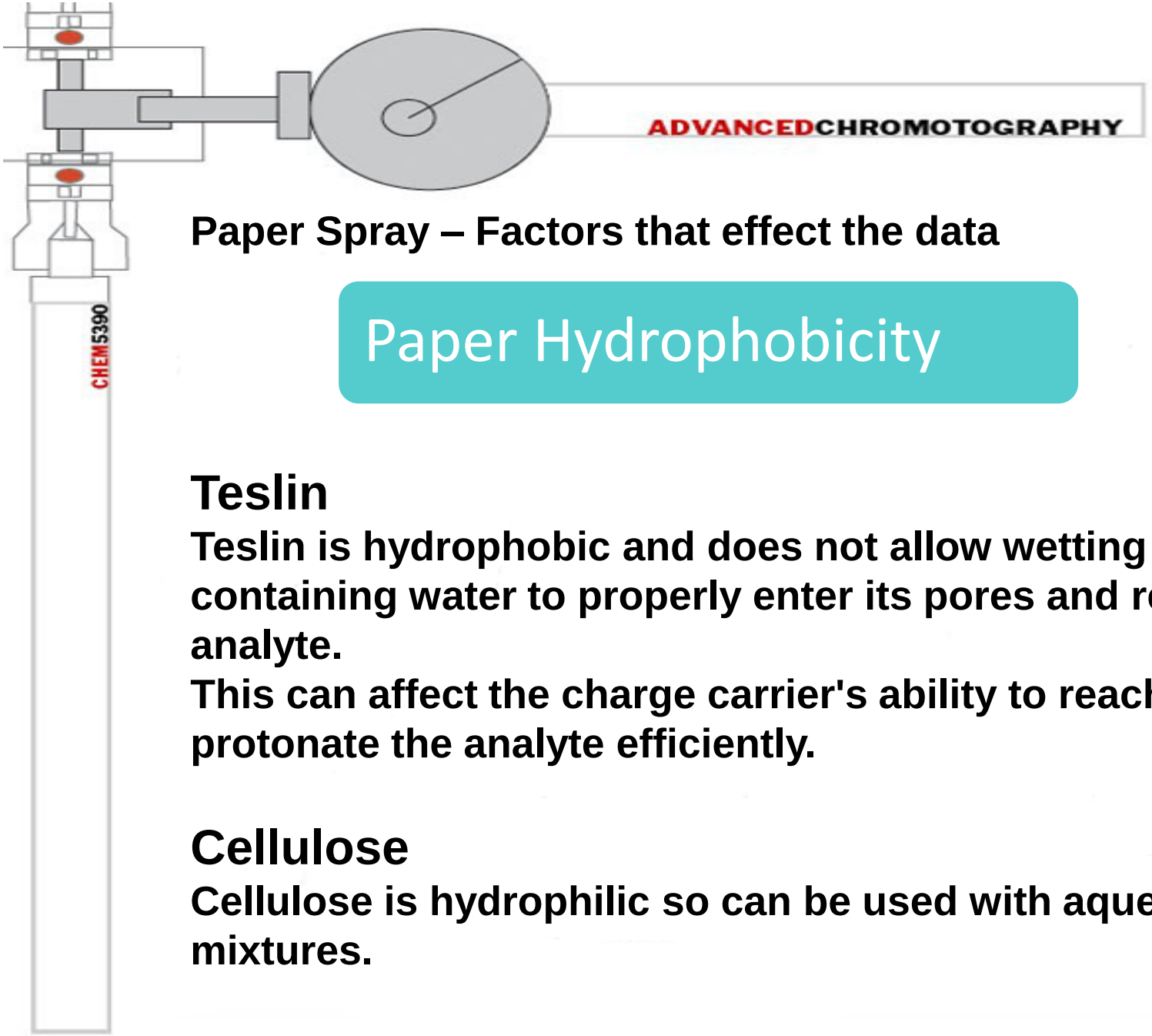
- Non-Uniform Pores
- Hydrophilic
- Made of cellulose with the ability to absorb high quantities of chemistry.

Paper Spray – Factors that effect the data

Paper Material

Teslin vs Cellulose Substrate.





Paper Spray – Factors that effect the data

Paper Hydrophobicity

Teslin

Teslin is hydrophobic and does not allow wetting mixtures containing water to properly enter its pores and reach the analyte.

This can affect the charge carrier's ability to reach and protonate the analyte efficiently.

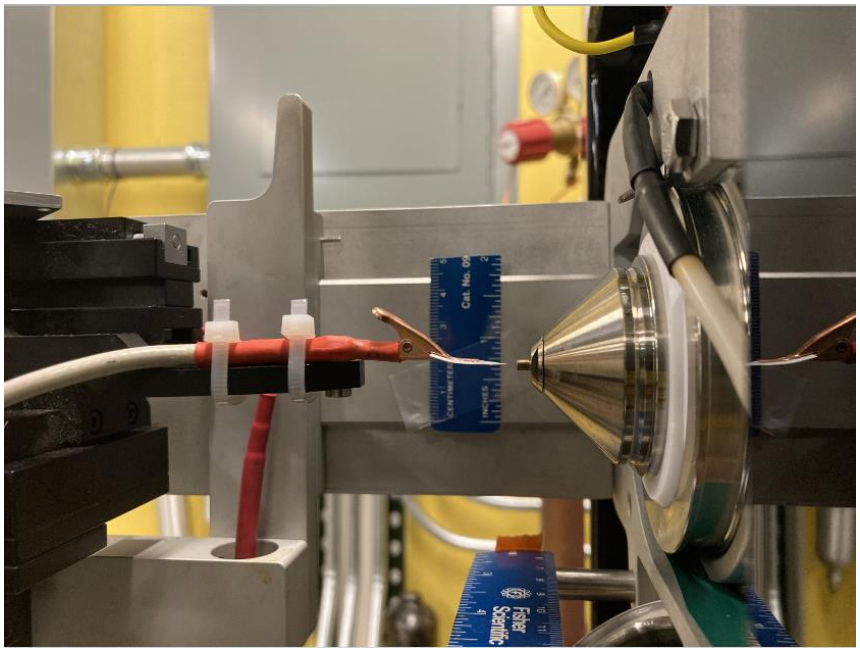
Cellulose

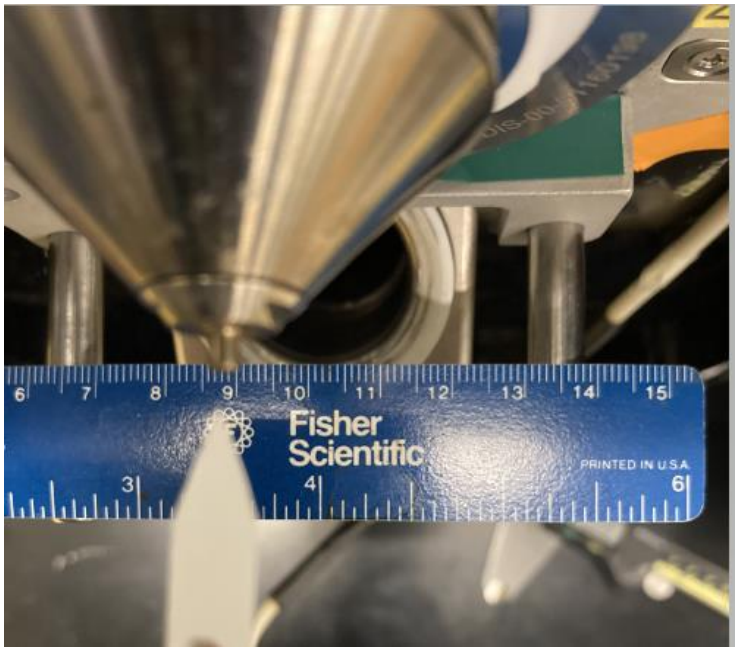
Cellulose is hydrophilic so can be used with aqueous mixtures.

Paper Spray – Factors that effect the data

Paper Alignment

Distance Optimization-The distance between the paper tip and the ion source greatly affects analyte intensity observed during analysis.

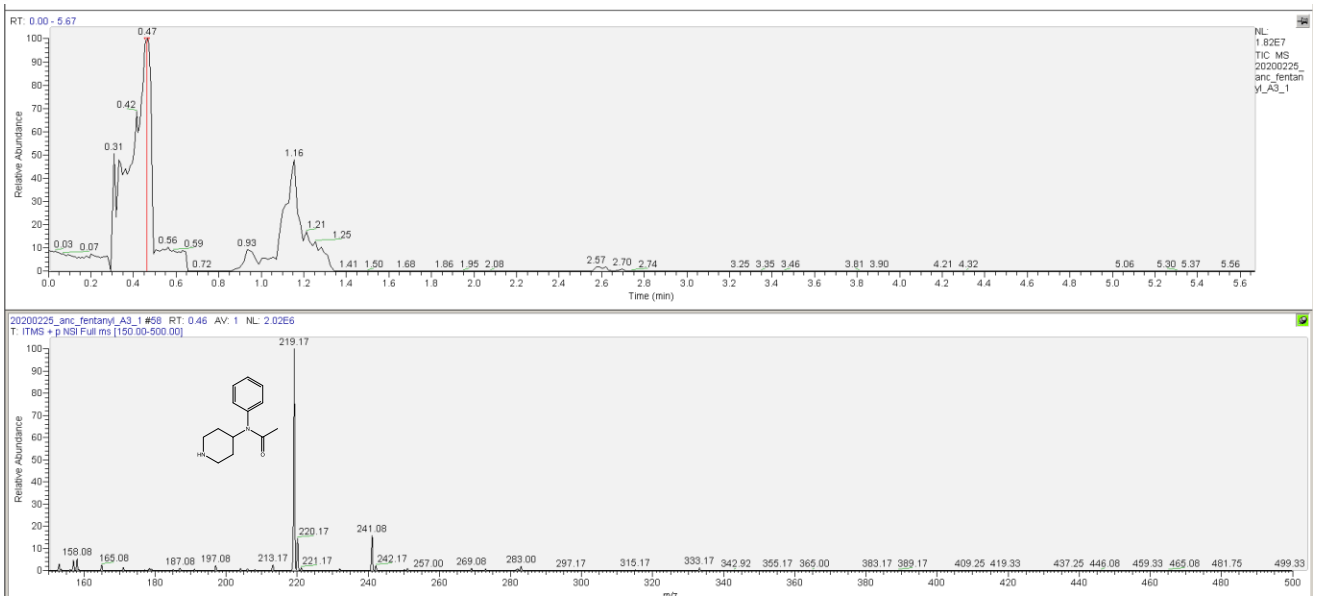




Paper Spray – Factors that effect the data

Compatible Solvents

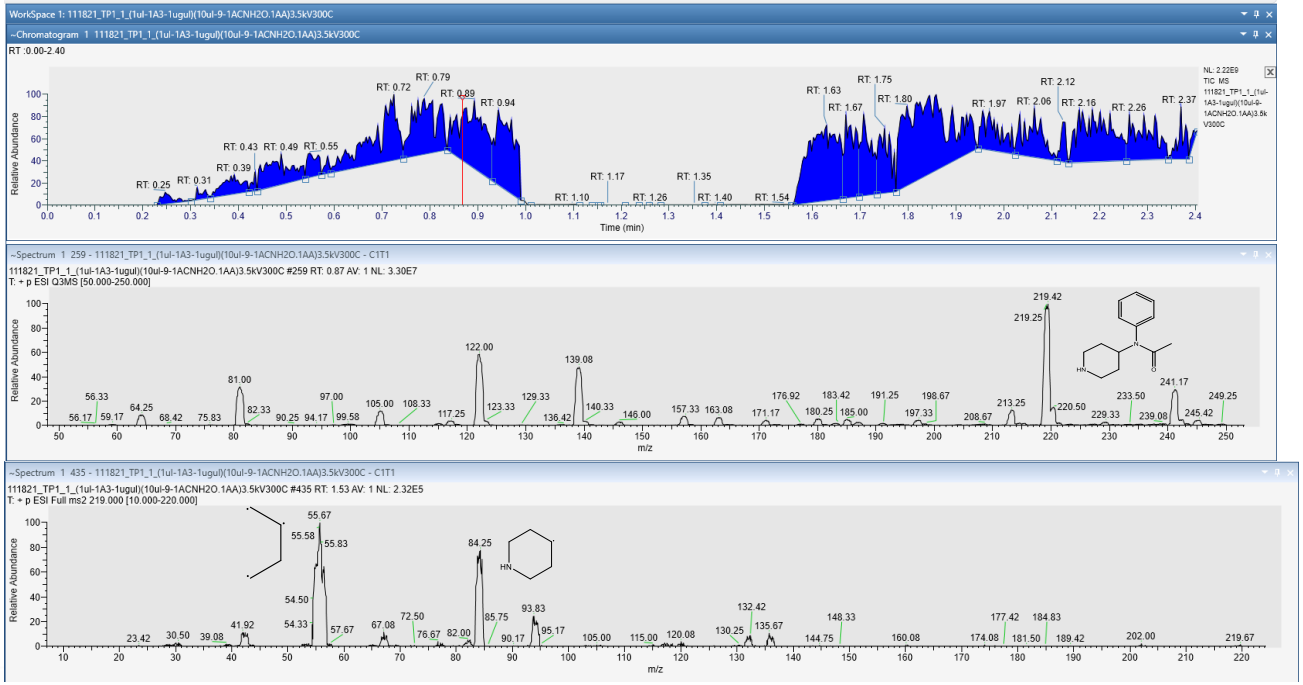
Sample: 1ul of 1mg/ml Solvent: 3-1 ACN/H2O 0.1 % Acetic Acid
Fentanyl Intensity: 2.02 E6 (Unstable peak, mostly E5 throughout whole acquisition)

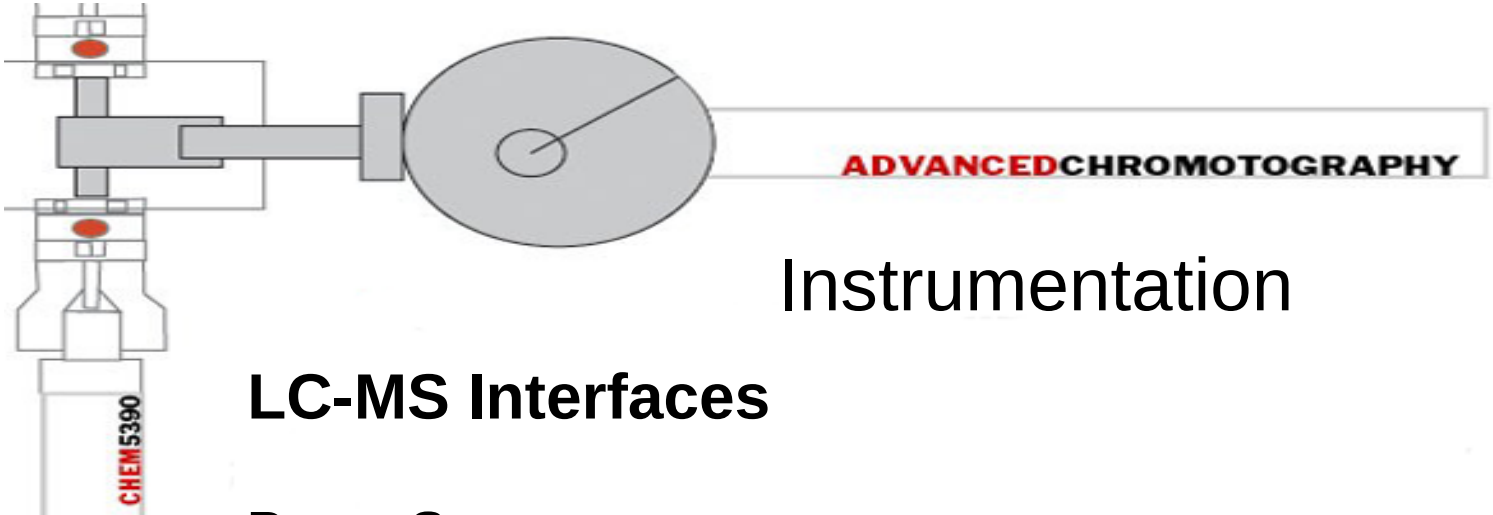


Paper Spray – Factors that effect the data

Compatible Solvents

Sample: 1ul of 1mg/ml Solvent: 9-1 ACN/H2O 0.1 % Acetic Acid
Fentanyl Intensity: 3.30 E7 (stable peak throughout whole acquisition)





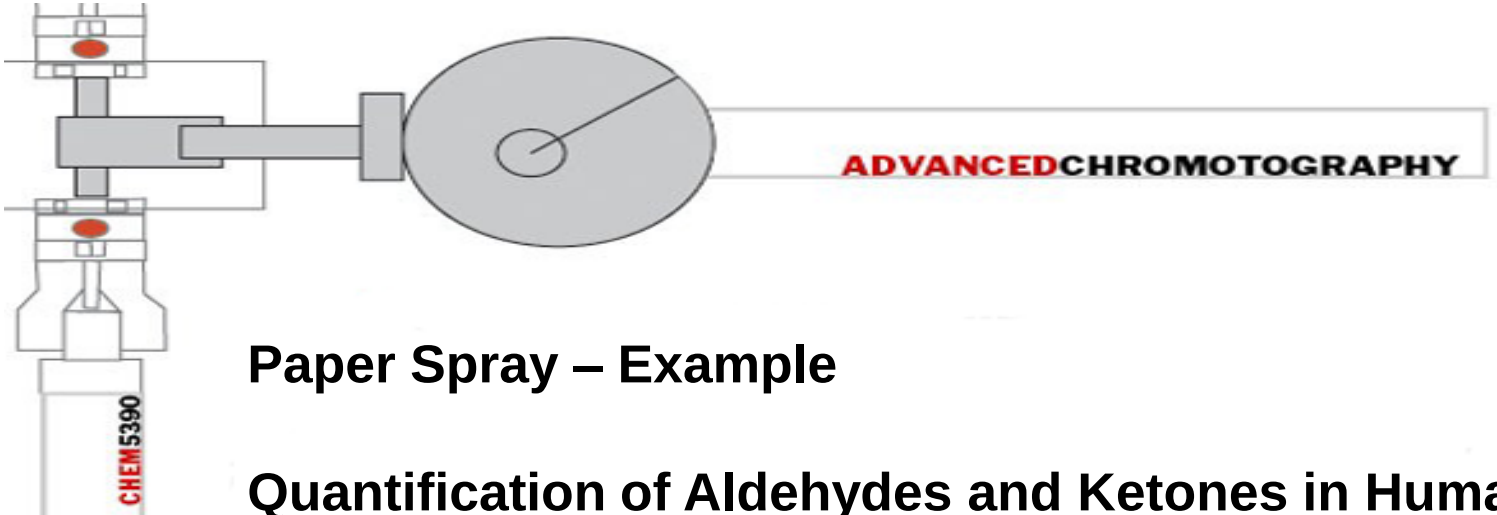
Instrumentation

LC-MS Interfaces

Paper Spray

Advantages:

It's ease of use and rapid analysis time make it a good alternative analytical method.
Samples compatible with PS = Solid and liquid (as long as the analytes are volatile)
Useful in analyzing analytes within complex biofluids.



Paper Spray – Example

Quantification of Aldehydes and Ketones in Human Serum

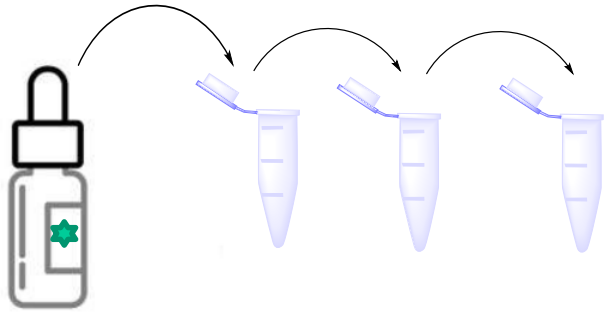
Aldehydes and Ketones of interest

- Acetaldehyde-DNPH
- Acetone-DNPH
- Benzaldehyde-DNAcrolein-DNPH
- n-Butyraldehyde-DNPH
- Crotonaldehyde-DNPH
- 2,5-Dimethylbenzaldehyde-DNPH
- Formaldehyde-DNPH
- Hexaldehyde-DNPH
- Isovaleraldehyde-DNPH
- Propionaldehyde-DNPH
- m, Tolualdehyde-DNPH
- Valeraldehyde-DNPH

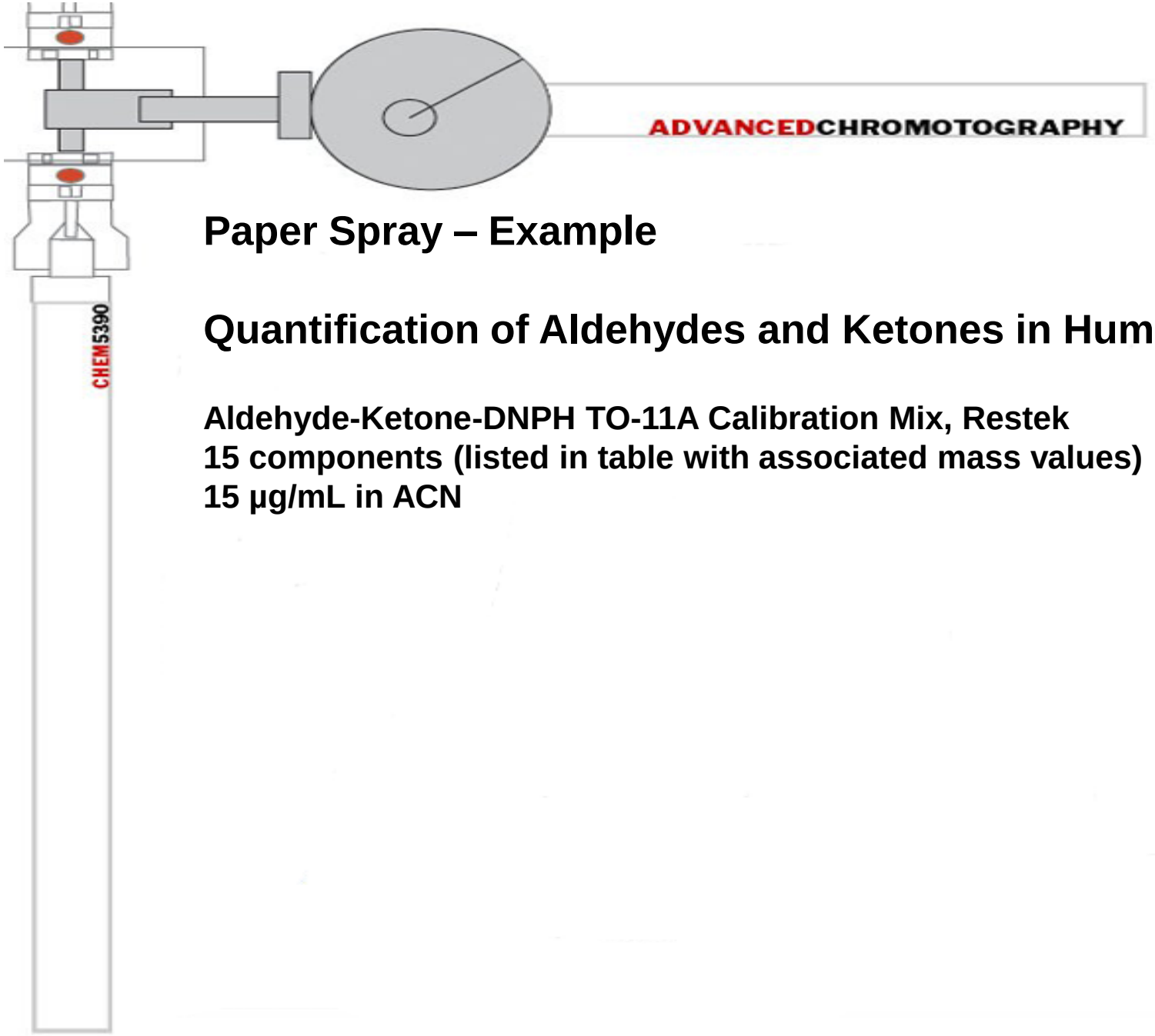
Paper Spray – Example

Quantification of Aldehydes and Ketones in Human Serum

Aldehydes and Ketones of interest



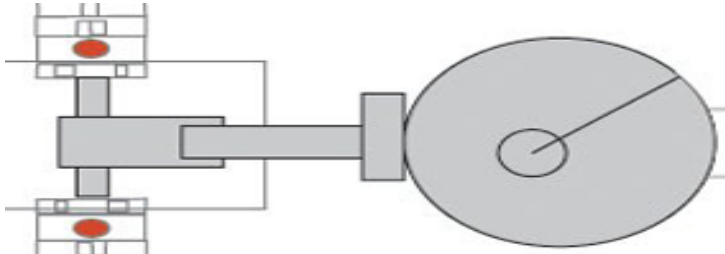
Standard serum solutions will be spiked with aldehyde-ketone standard concentrations of **100 nM, 500 nM, 1 uM, 5 uM and 20 uM** and analyzed on Teslin® substrate.



Paper Spray – Example

Quantification of Aldehydes and Ketones in Human Serum

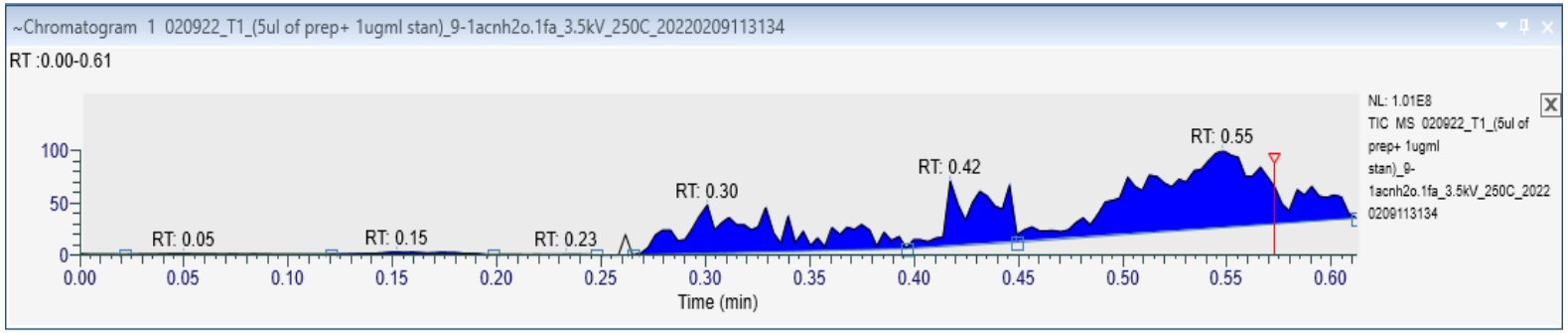
Aldehyde-Ketone-DNPH TO-11A Calibration Mix, Restek
15 components (listed in table with associated mass values)
15 µg/mL in ACN

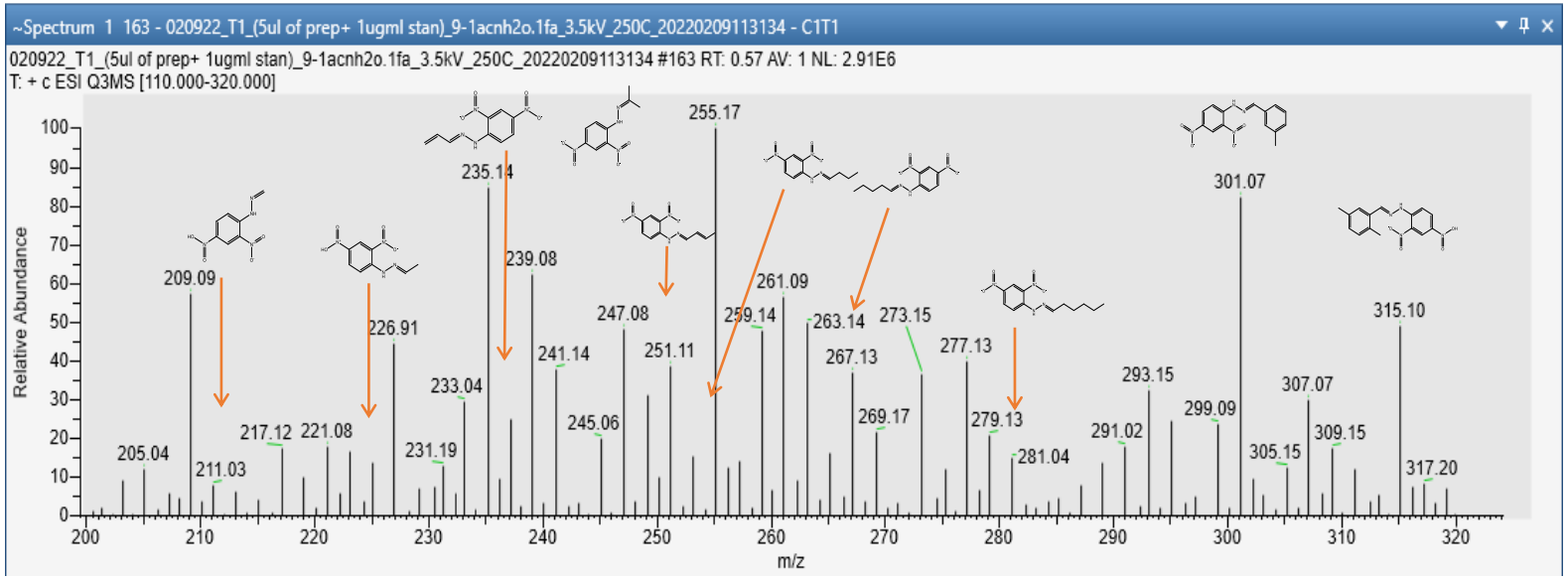


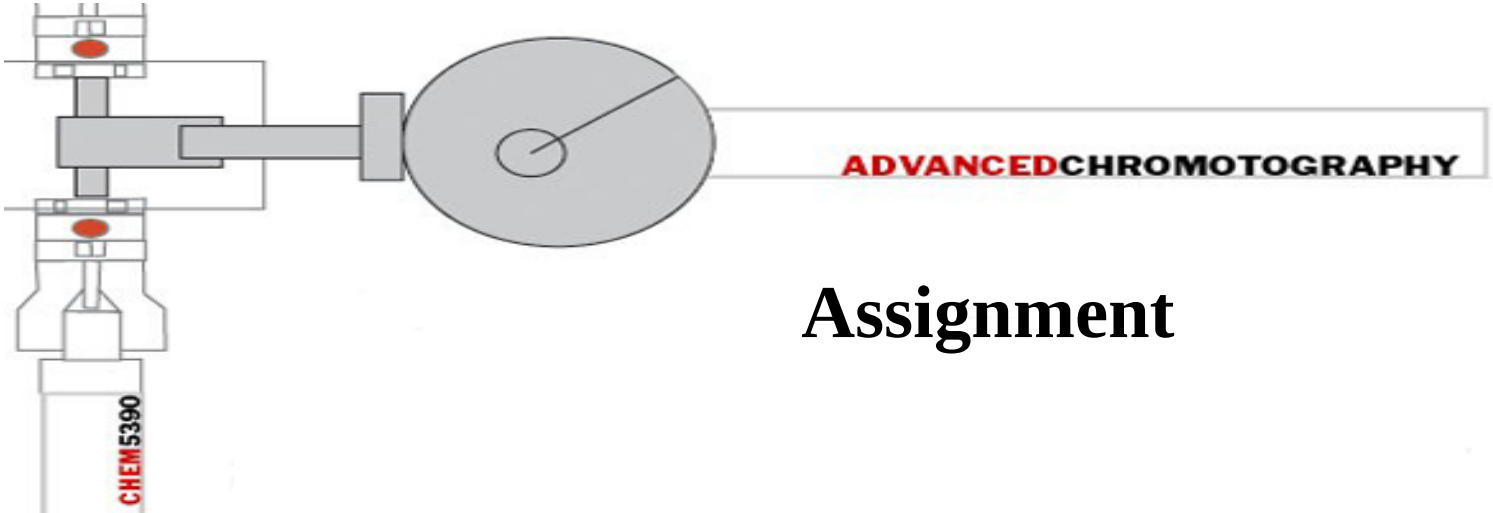
Paper Spray – Example
Quantification of Aldehydes and Ketones in Human Serum

Mixture Compound	Sigma Cat No.	Mass	M+H	$[M+H-OH]^+$
Formaldehyde-DNPH	(1081-15-8)	210.0	211.0	194.0
Acetaldehyde-DNPH	(1019-57-4)	224.1	225.1	208 .1
Acrolein-DNPH	(888-54-0)	236.1	237.1	220.1
Acetone-DNPH	(1567-89-1)	238.1	239.1	222.1
Propionaldehyde-DNPH	(725-00-8)	238.1	239.1	222.1
Crotonaldehyde-DNPH	(1527-96-4)	250.1	251.1	234.1
n-Butyraldehyde-DNPH	(1527-98-6)	252.1	253.1	236.1
Valeraldehyde-DNPH	(2057-84-3)	266.1	267.1	250.1
Isovaleraldehyde-DNPH	(2256-01-1)	266.3	267.3	250.1
Hexaldehyde-DNPH	(1527-97-5)	280.1	281.1	264.1
Benzaldehyde-DNPH	(1157-84-2)	286.1	287.1	270.1
m-Tolualdehyde-DNPH	(2880-05-9)	300.1	301.1	284.1
o-Tolualdehyde-DNPH	(1773-44-0)	300.1	301.1	284.1
p-Tolualdehyde-DNPH	(2571-00-8)	300.1	301.1	284.1

Paper Spray – Example
Quantification of Aldehydes and Ketones in Human Serum







Assignment

- Read Chapter 6: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.