Nitrosamines Analysis in Pharmaceuticals by LC-MS/MS

Confidently Detect and Quantify Mutagenic Impurities in APIs and Drug Products

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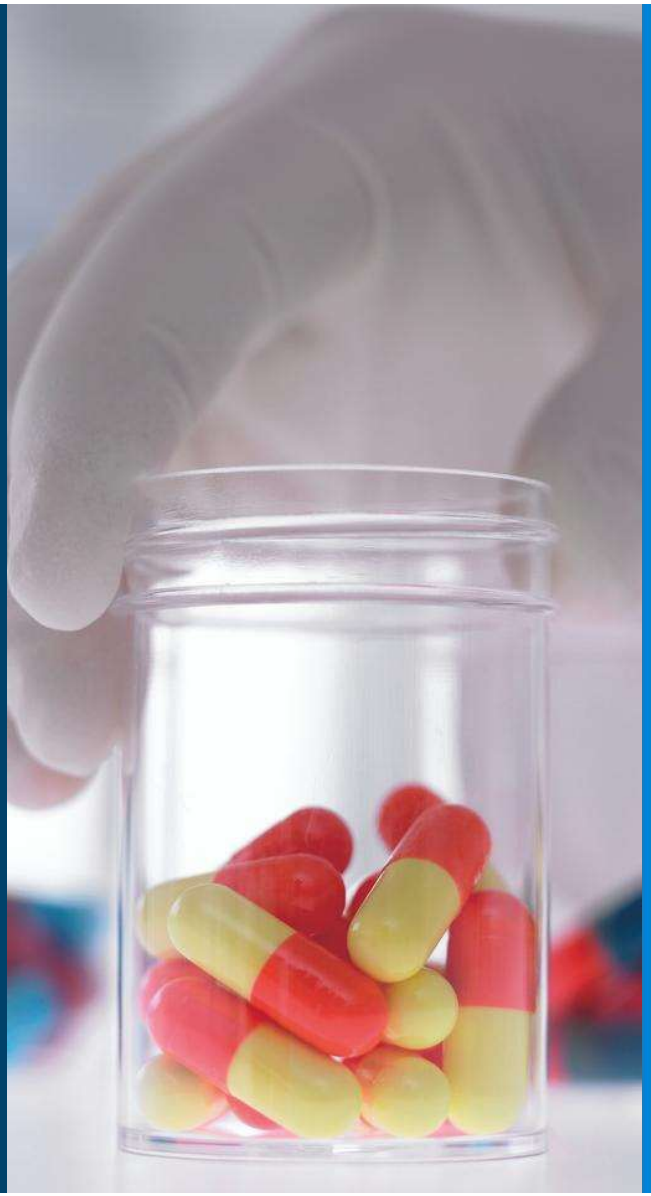
DE91188398

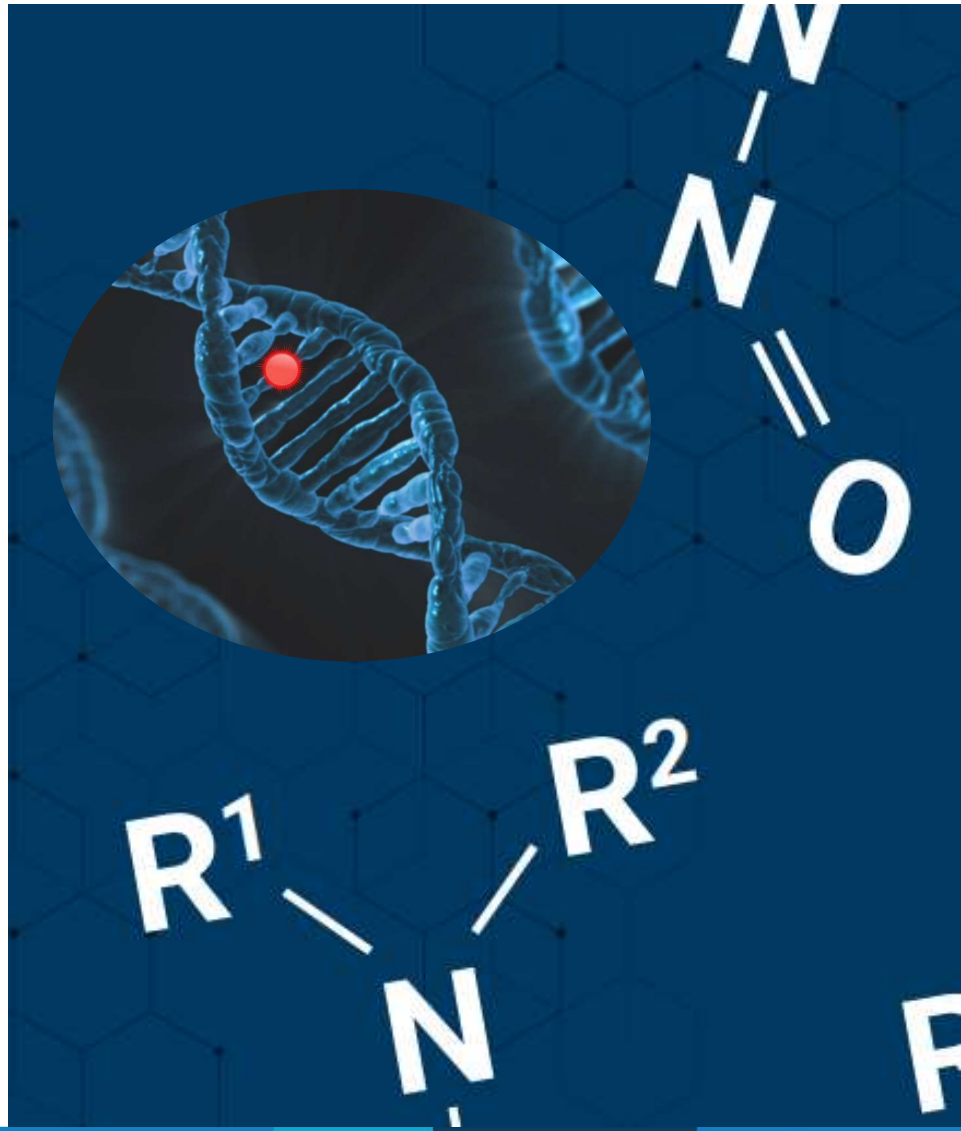


Agilent
Trusted Answers

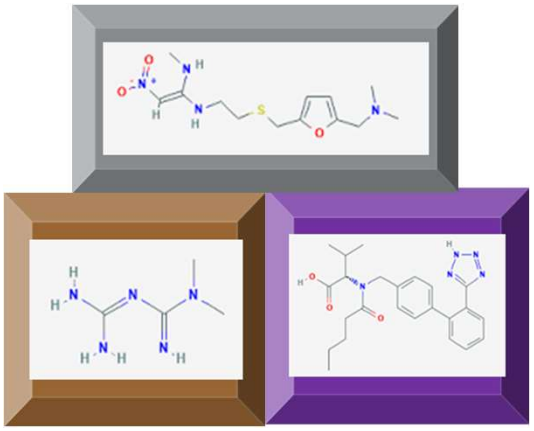
Mutagenic impurities

Mutagenic impurities in APIs and drug products pose a significant risk to health and safety—even in small quantities—and thus are a major concern for drug makers.

Mutagenic impurities can damage DNA, leading to mutations and potentially cancer. Efforts to address and control the presence of trace levels of mutagenic impurities is of special concern to global regulators.



Nitrosamines in Recent News!



July 2018

- FDA recalled valsartan DP due to NDMA contaminant

Oct 2018

- FDA expanded recall to other sartans, due to NDMA & NDEA

Feb 2019

- FDA expanded recall as new contaminant, NMBA identified

April 2019

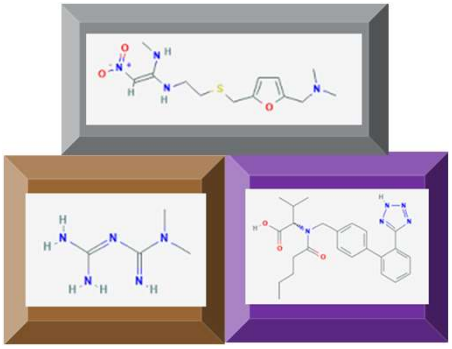
- FDA identified additional contaminants, EIPNA & DIPNA

Sep 2019

- FDA recalled ranitidine based drugs

May 2020

- FDA recalled metformin based drugs



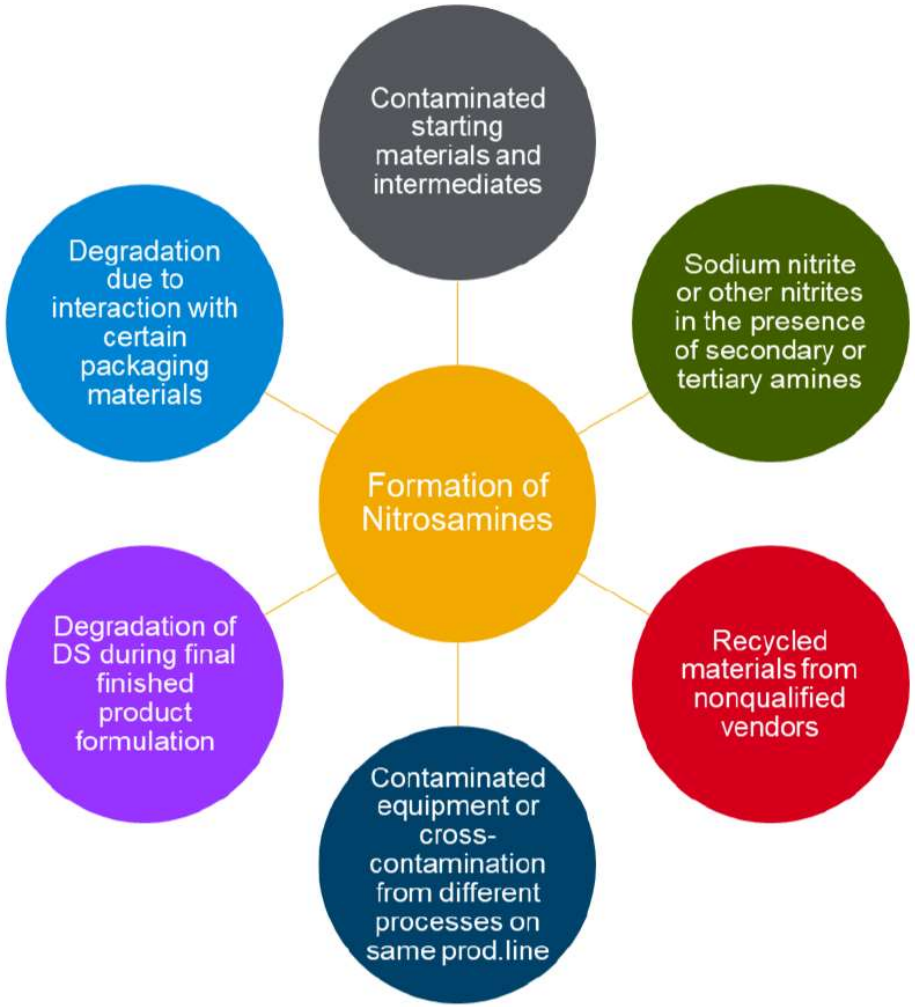
For detailed info, refer to: FDA Press Releases; EMA Press Releases

Formation of Nitrosamines

Nitrosamines are formed by chemical reactions that occur during API manufacturing whether from:

- starting materials
- Intermediates
- Reactants
- reuse of solvents
- and by products

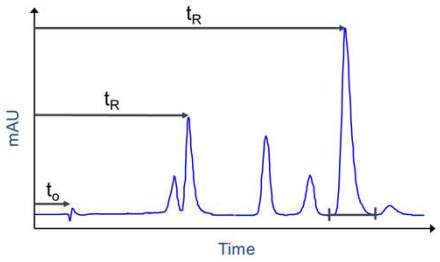
They can form through degradation products generated during formulation or storage or from environmental contaminants.



What is Chromatography-Mass Spectrometry?

Liquid Chromatography (LC)

Separates analytes based on chemical properties. Measures "Retention" on a separation column.



LC/MS is a powerful analytical technique

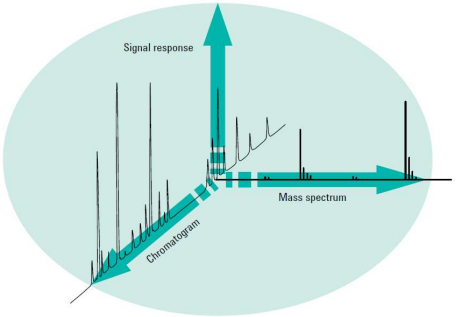
Separating power of High-Performance Liquid Chromatography (HPLC)	Detection power of Mass Spectrometry (MS) – charged ions (m/z)

Separation RT, molecular weight, Structure Identity and Quantity of specific components

Highly Selectivity and Very Sensitivity

LC/MS

Separates based on "mass" (m/z) of a molecule's ion. The analyte must be ionized in the gas phase in order to be detected.



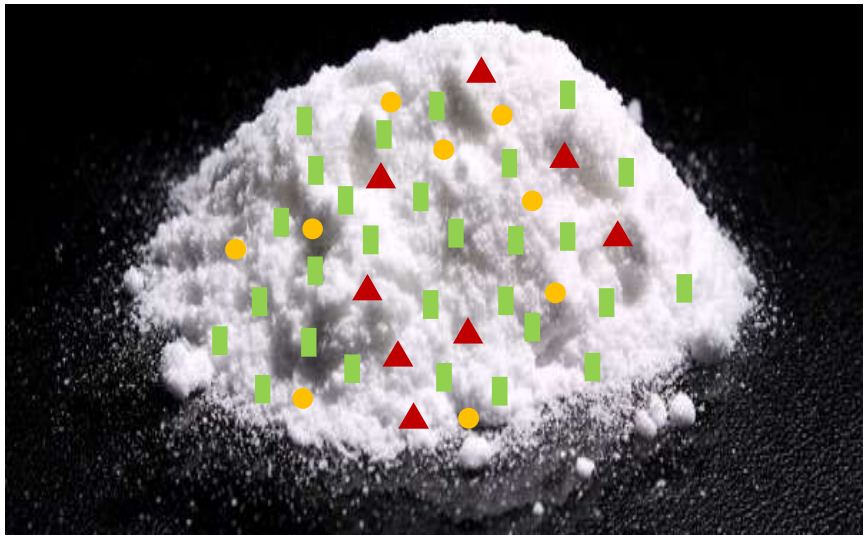
High Pressure Liquid Chromatography

Analysis of active pharmaceutical ingredients by High Pressure Liquid Chromatography (HPLC)

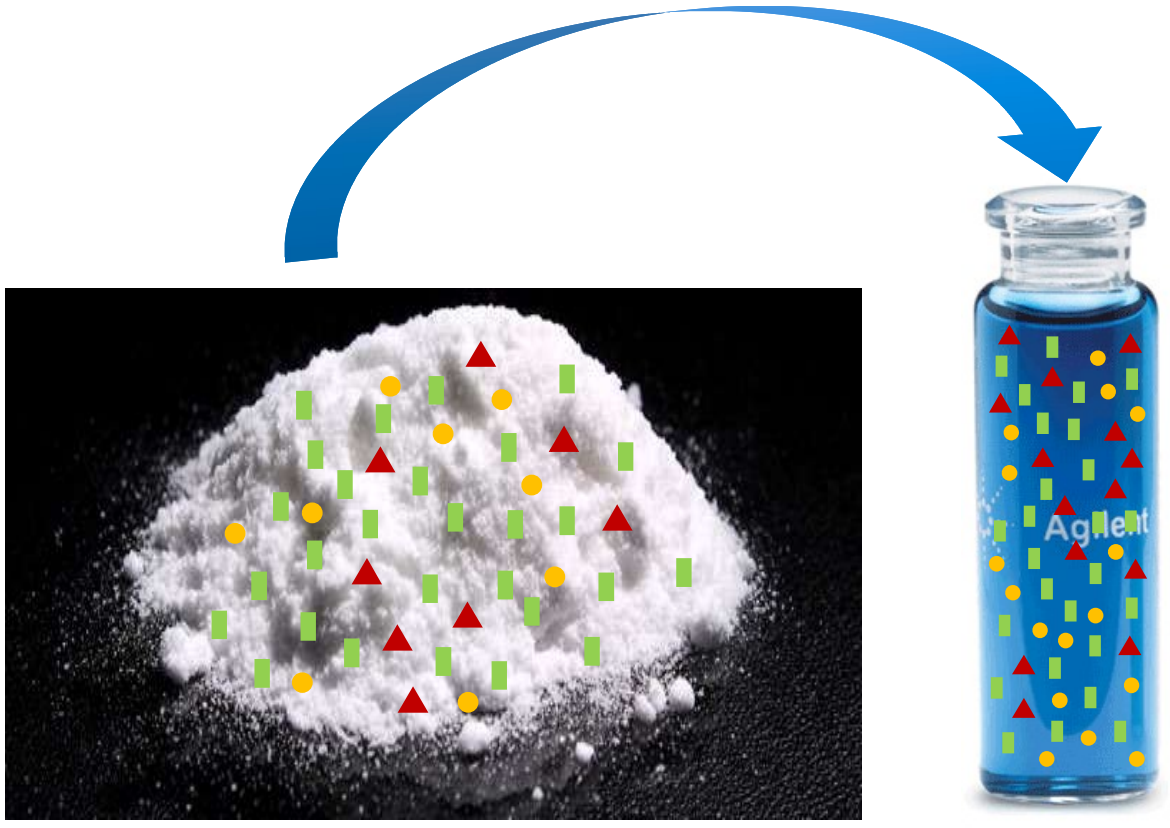
A typical requirement of the pharmaceutical industry is the quality control of active ingredients (▮) in drugs.

It is very important to identify drug impurities (● ▲) that may occur during synthesis or by decomposition of the active ingredient.

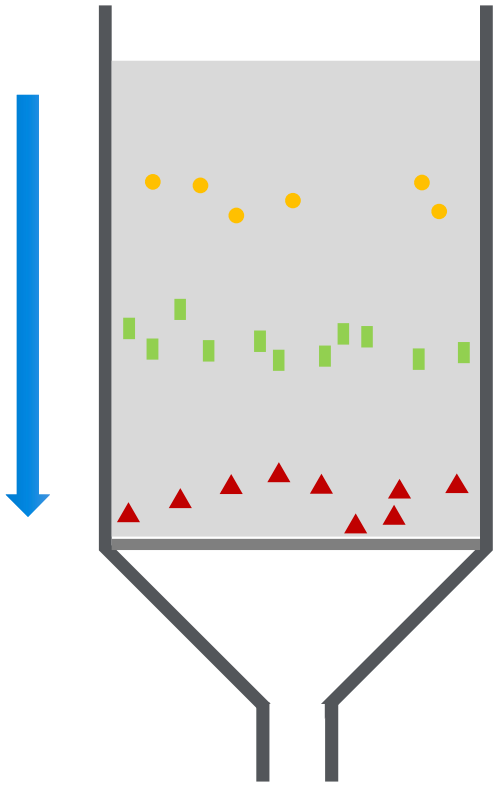
Quality control ensures patient safety.



Step 1: Tablet Dissolution and Substance Release



Step 2: Separation

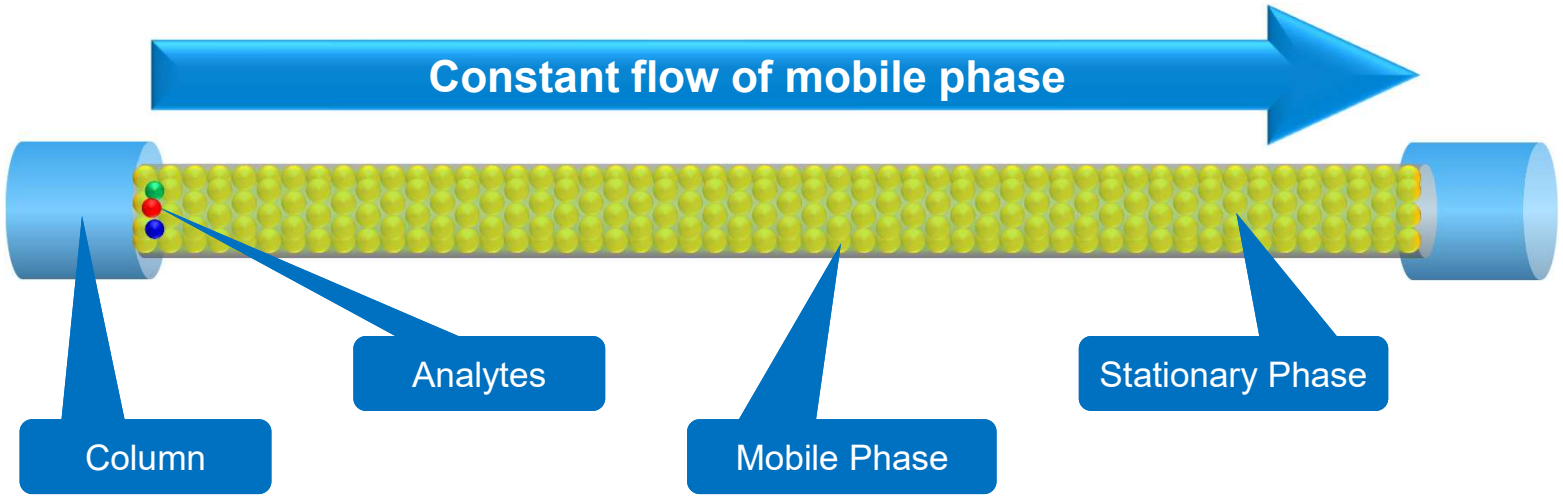


This means the substances reach the end of the column at different times.

Thereby the substances in the tablet are separated.

The Chromatographic Process

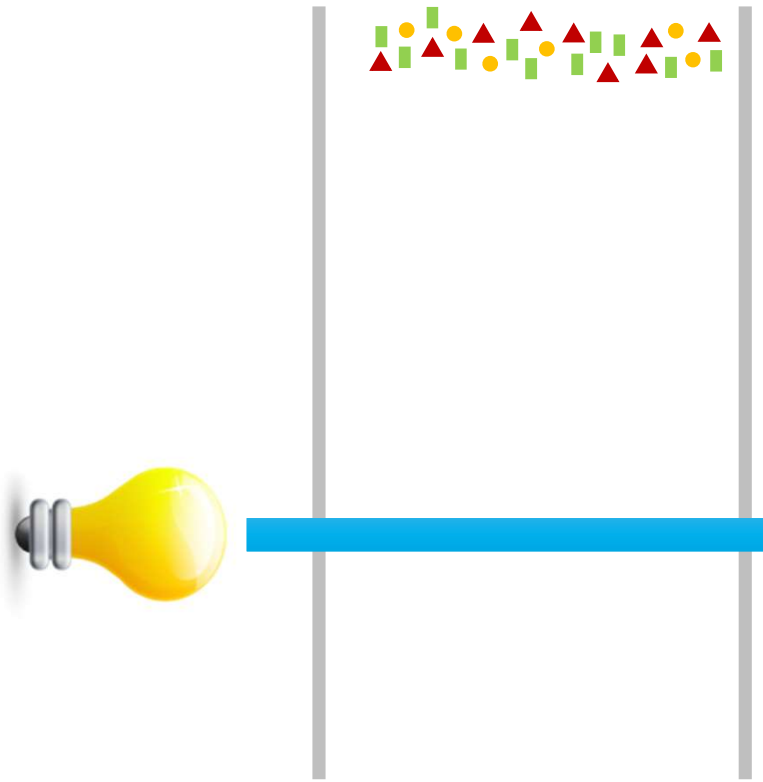
Introduction



The stationary phase retains analytes due to various interactions.

When different chemical components pass through the column at different rates they become separated in single zones.

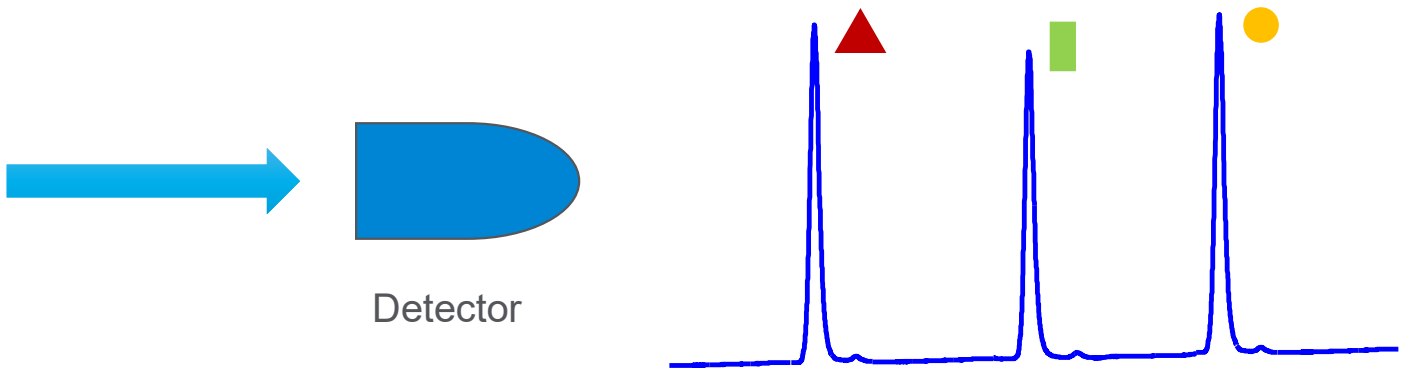
Step 3: Quantitative Determination of Substances



A detector measures the intensity of light. More light absorbed by the substance means less light is transmitted to the detector.

Detector

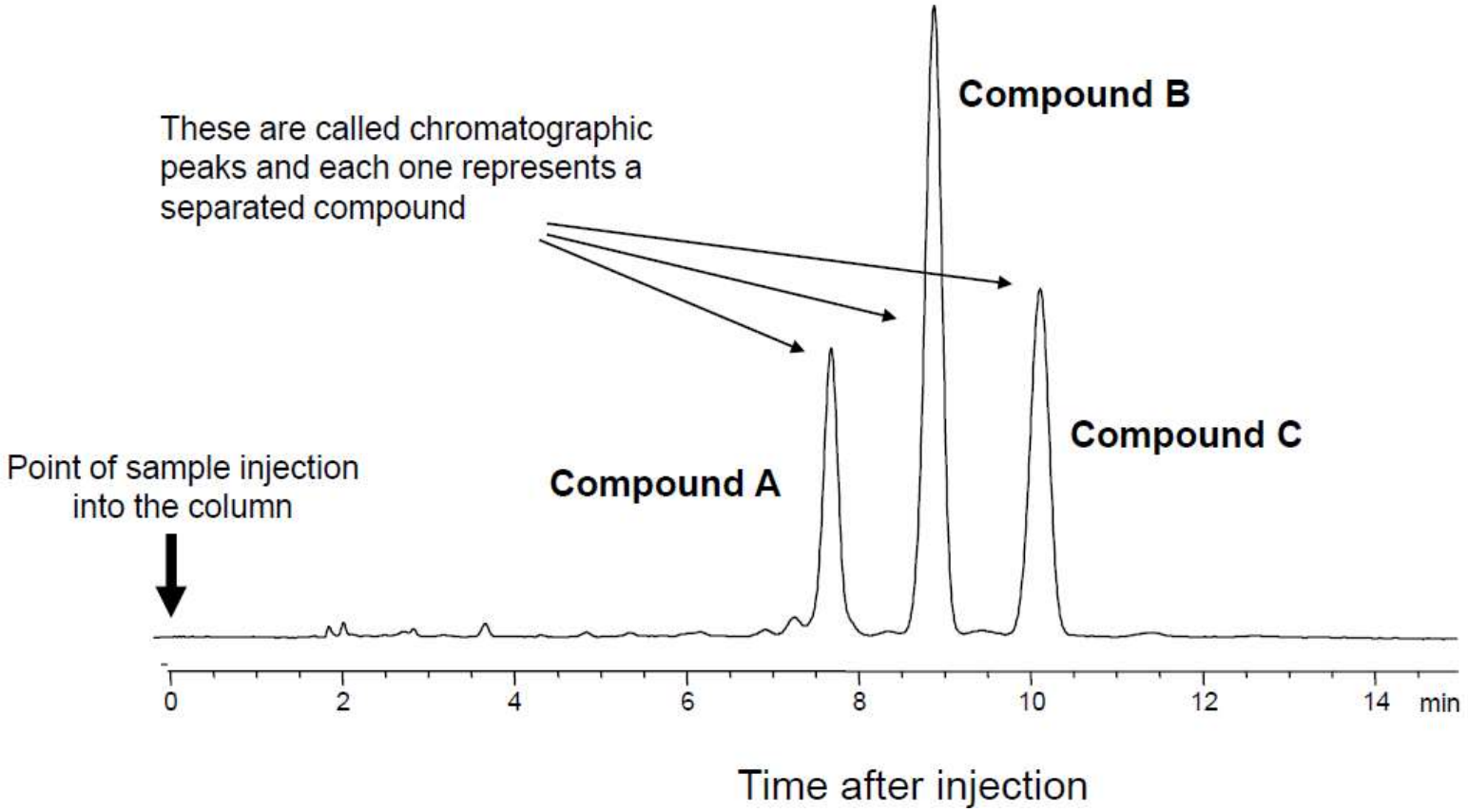
Step 3: Quantitative Determination of Substances



The detector records the light intensity. Reducing light intensity produces a detector response, a so-called „peak“.

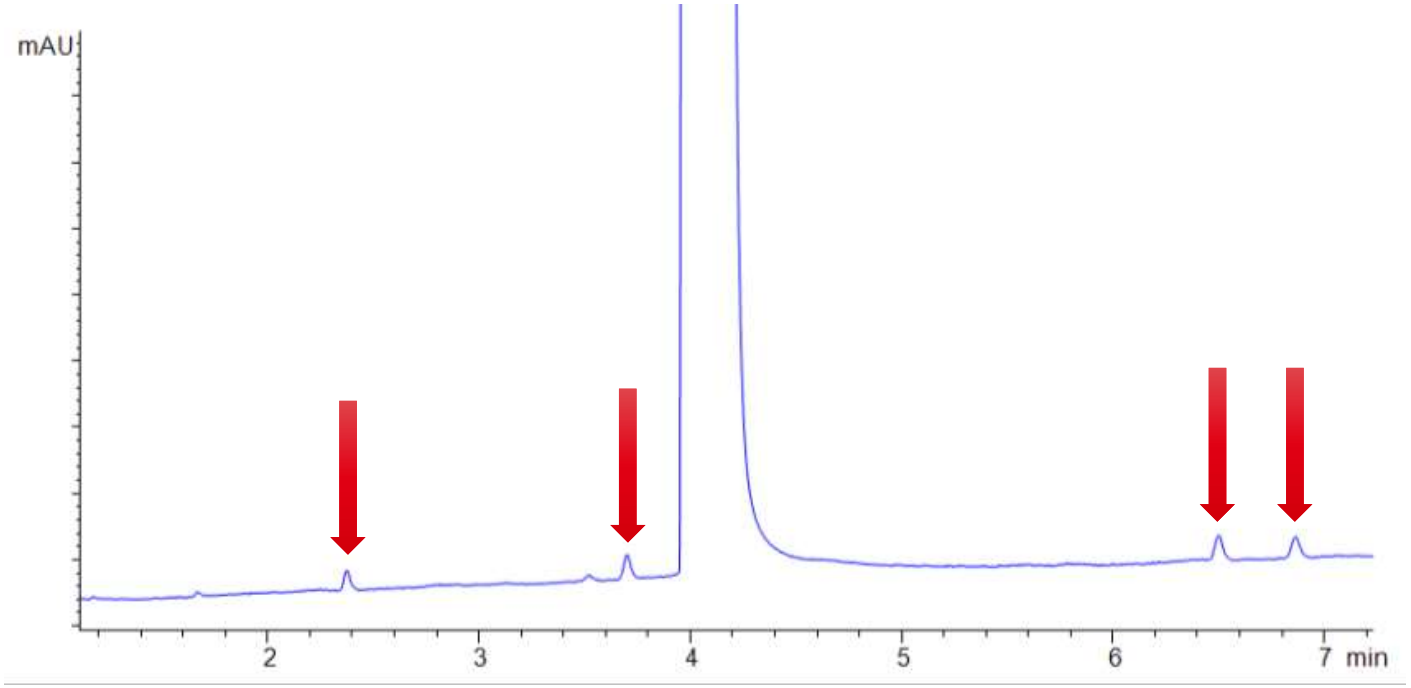
Peak height or area corresponds to the reduction of light intensity by absorption of the substance.

HPLC is an abbreviation for High Performance Liquid Chromatography



Example: Analysis of an Active Ingredient

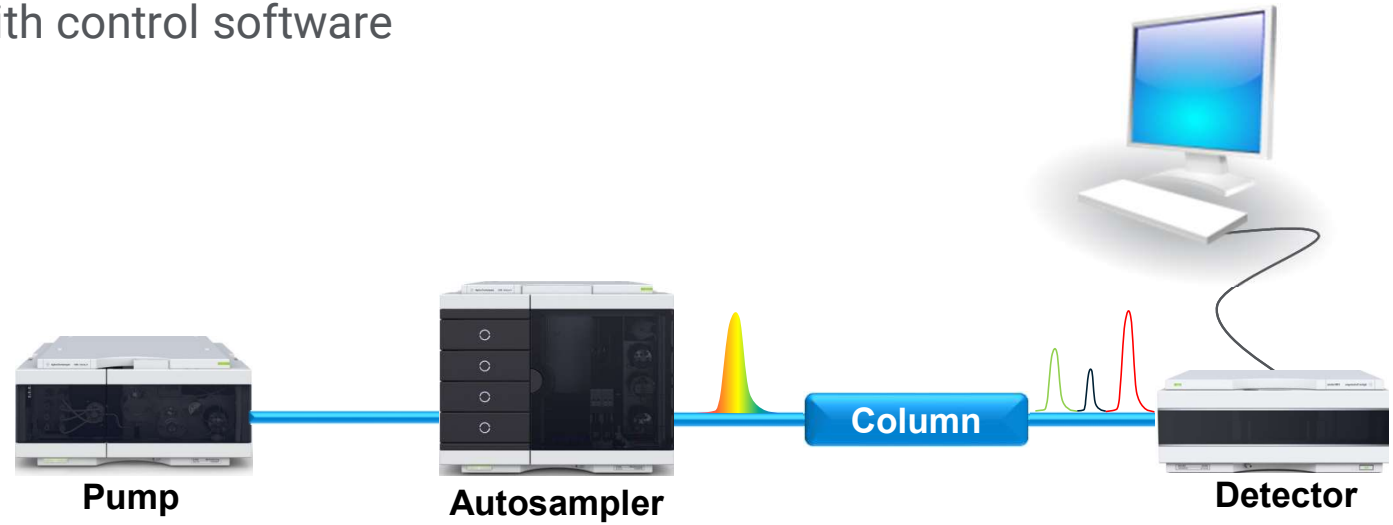
Impurities must not reach certain thresholds, to ensure patient safety.



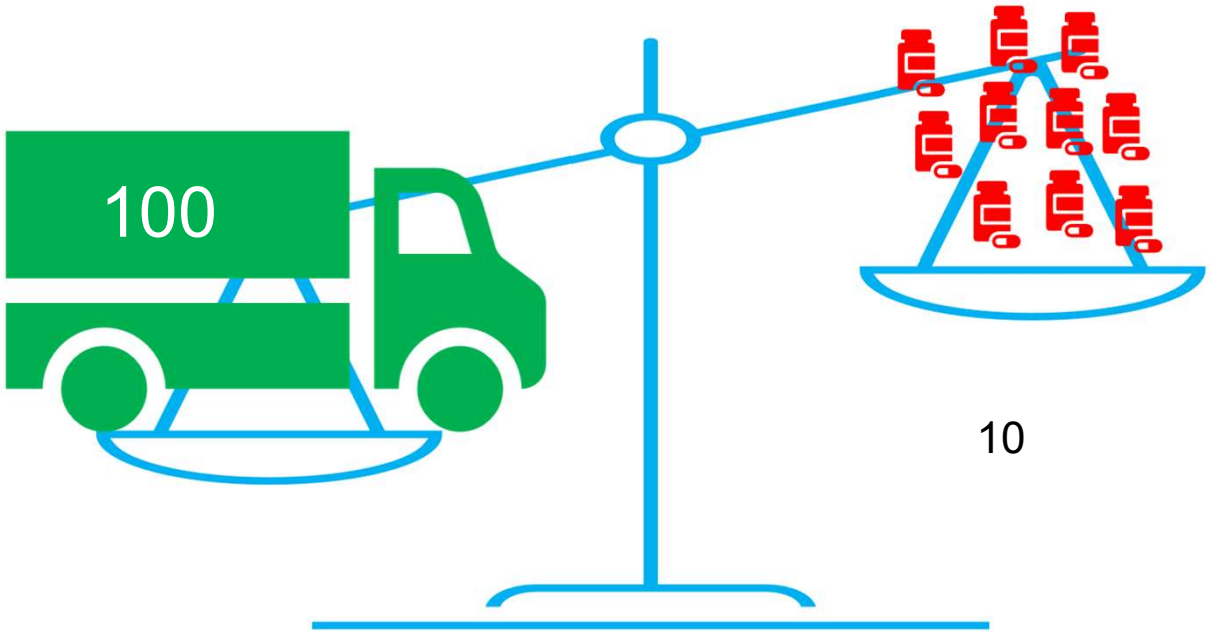
HPLC System General Design

Introduction

- Pump with Degasser : Deliver flow of solvents/buffers
- Autosampler : Inject sample
- Column (installed in a Column Compartment)
- Detector : To detect compound (Could be MS, DAD, UV, etc.)
- Computer with control software

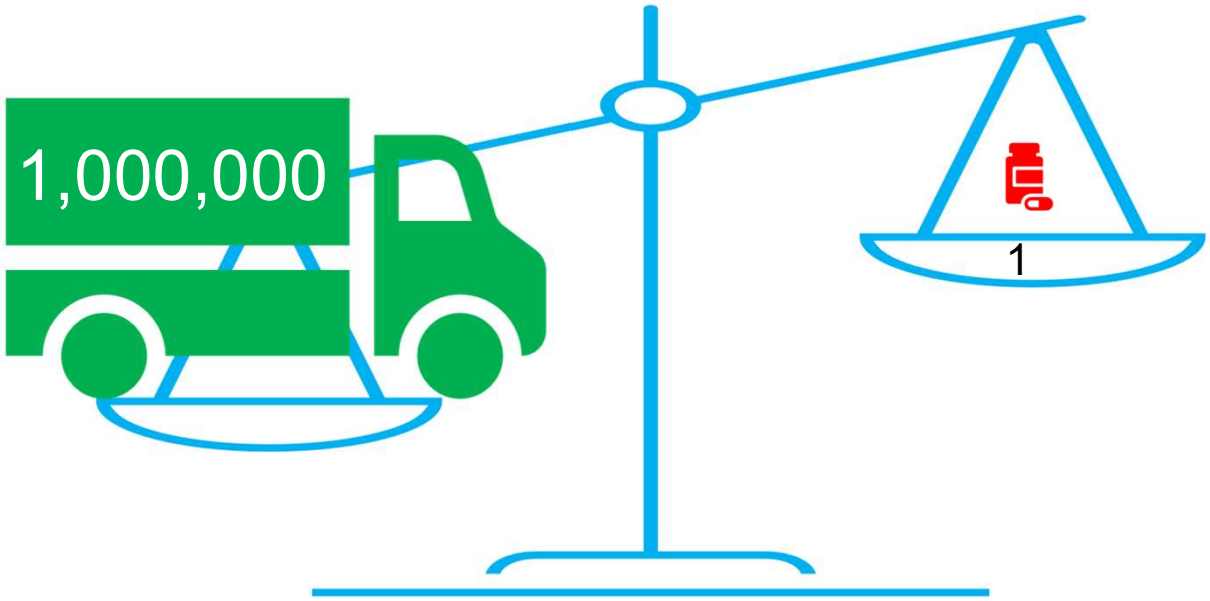


Percent concentration



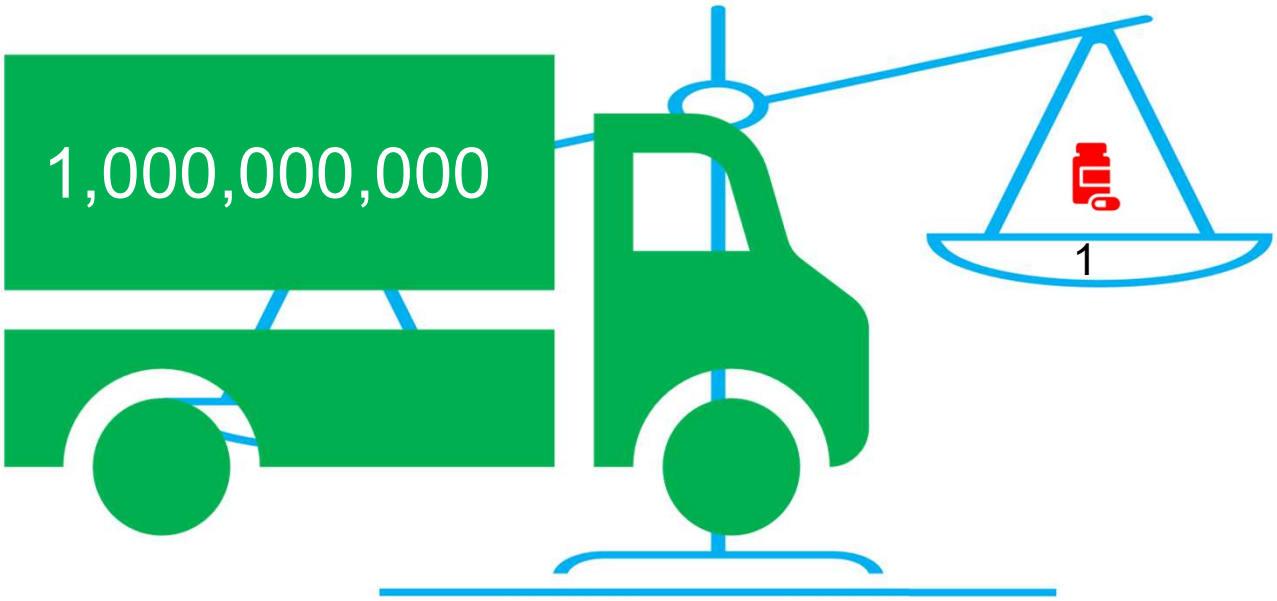
$$\frac{10}{100}$$

PPM – Part per Million



$$1\ \text{ppm} = \frac{1}{1{,}000{,}000}$$

PPB – Part per Billion



$$1 \text{ ppb} = \frac{1}{1{,}000{,}000{,}000}$$

Next-Gen LC/TQ

Agilent's new high-end LC/TQ platform

Intelligent Instrumentation

AI-Powered Tuning,
Early Maintenance Feedback
Intelligent Reflex

Workflows: "Discovery" to "Routine Analysis"

Scientific insights in routine analysis, translational medicine, targeted omics

Performance Improvements

2-3x sensitivity improvements,
Enhanced iFunnel speed
High precision at low dwell times
Production-ready robustness

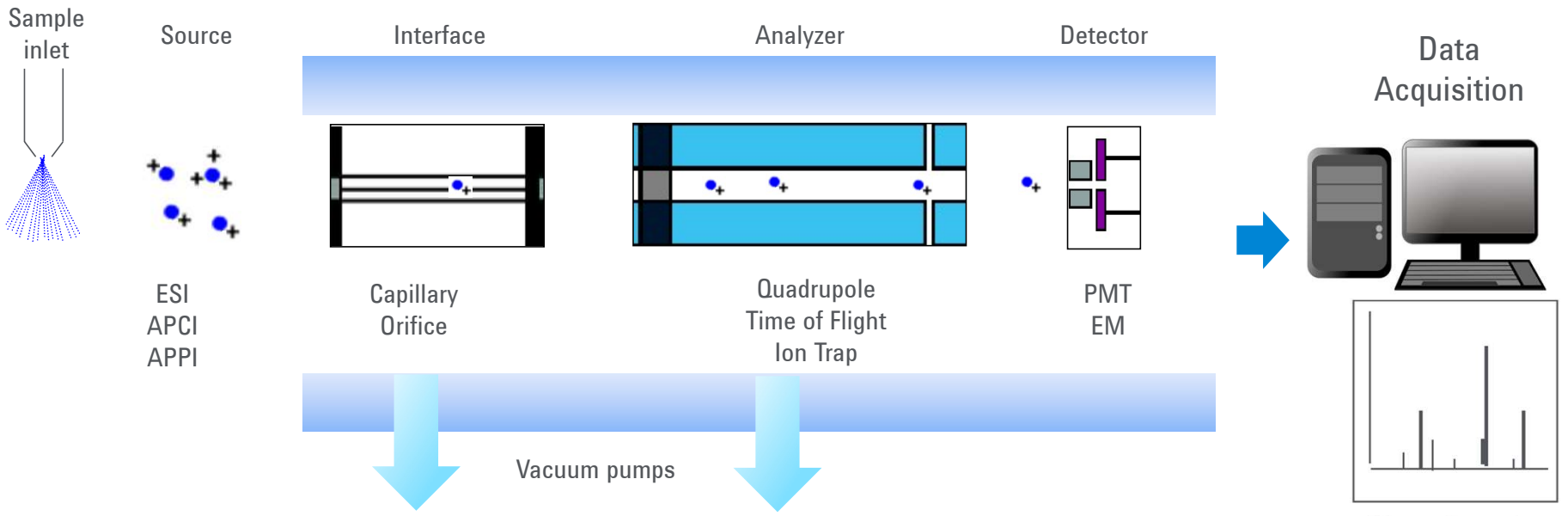
Basic components of a Mass Spectrometry (MS) system

(A) SOURCE: ionizes sample into "flight"

(B) ANALYZER: separates sample based on m/z

(C) DETECTOR: detects ions

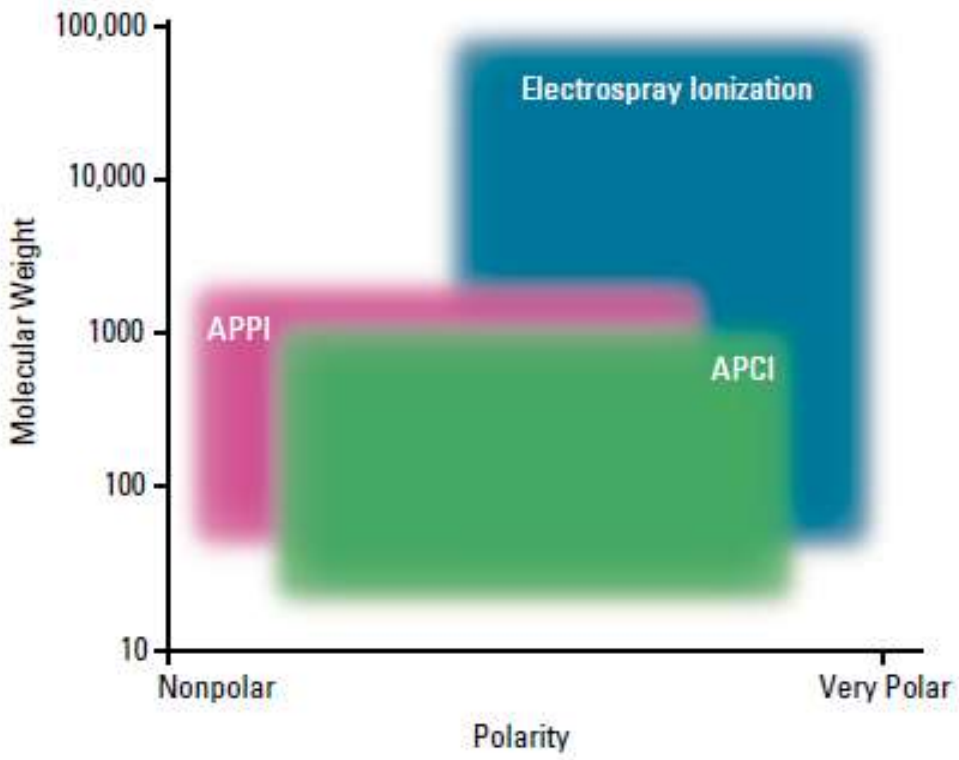
(D) COMPUTER: deconvolutes data & plots as *Abundance vs m/z*



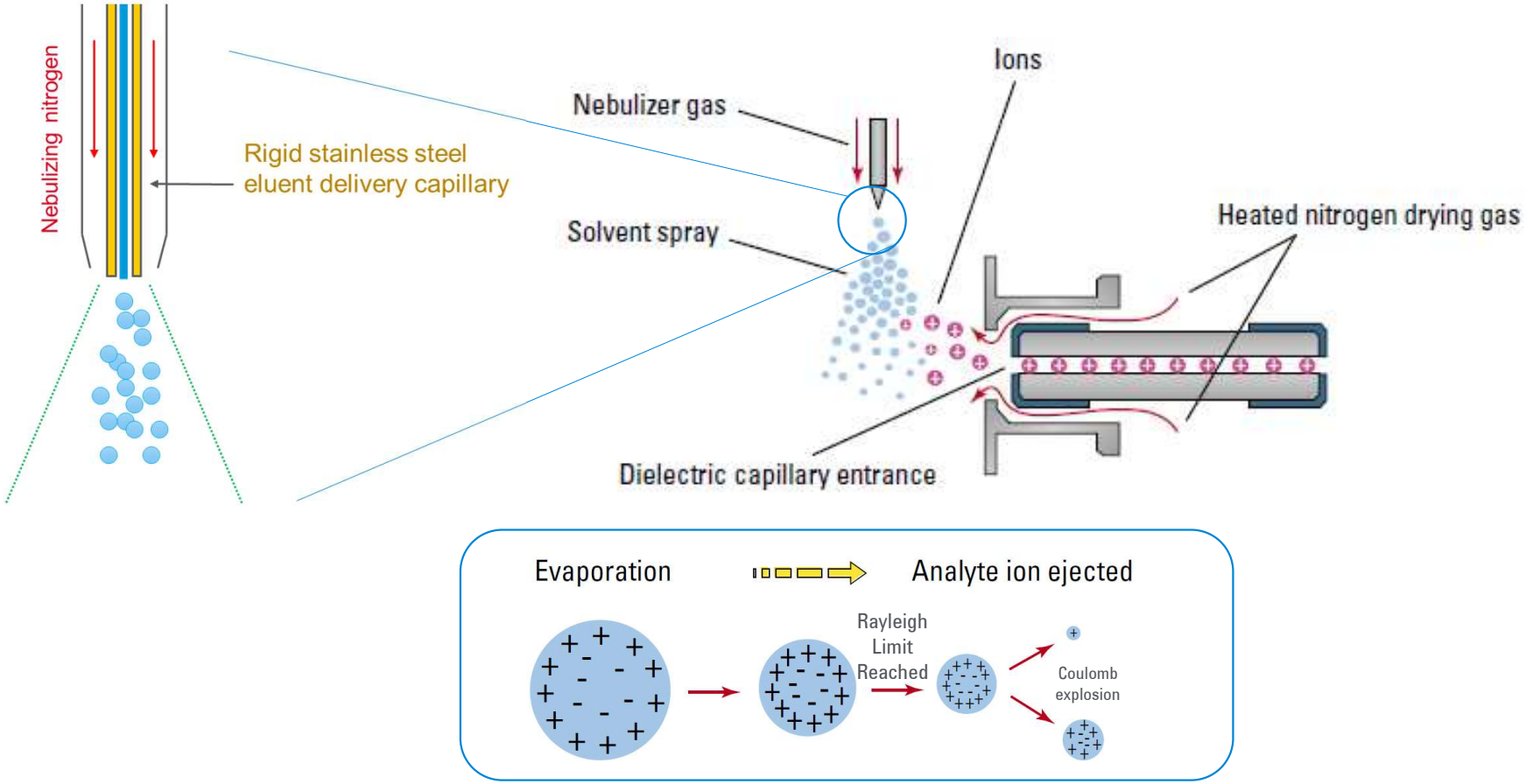
Generalized diagrammatical representation of a mass spectrometer.

Relative Applicability of LC/MS Techniques

- Several different types of ion sources
- Suitable for different classes of compounds
- Atmospheric pressure ionization (API) technique
  - Electrospray ionization (ESI)
  - Atmospheric pressure chemical ionization (APCI)
  - Atmospheric pressure photoionization (APPI)



Electrospray ionization (ESI)



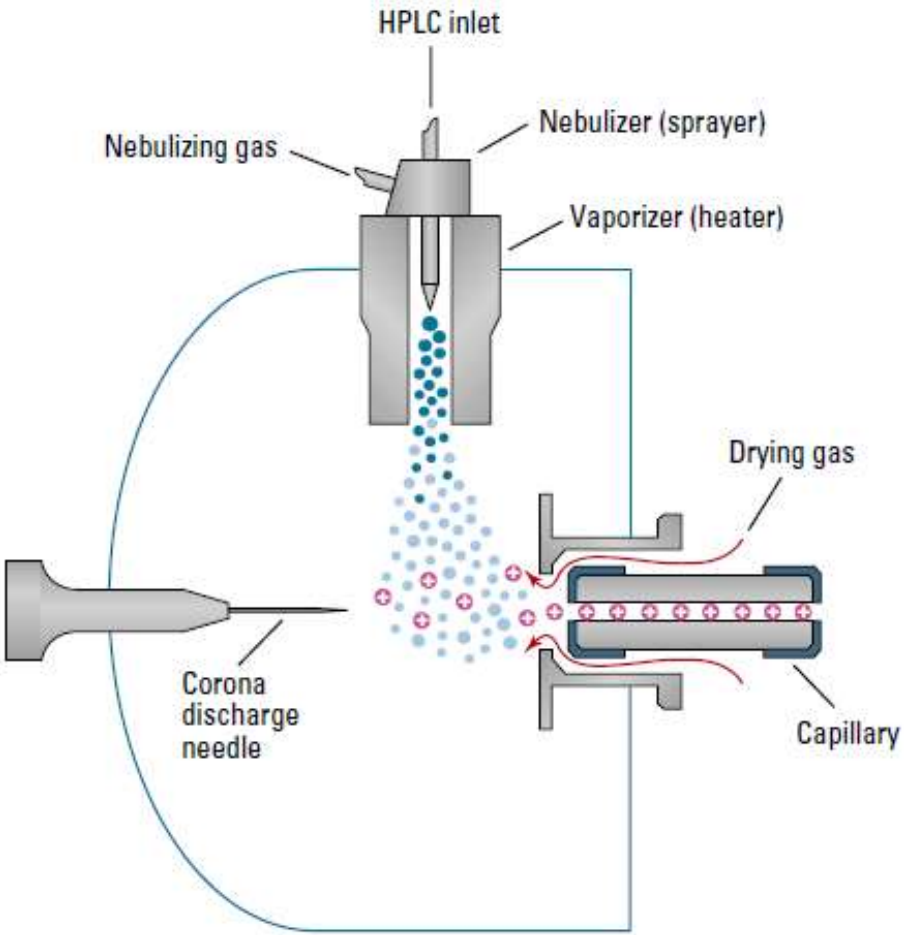
Electrospray Considerations

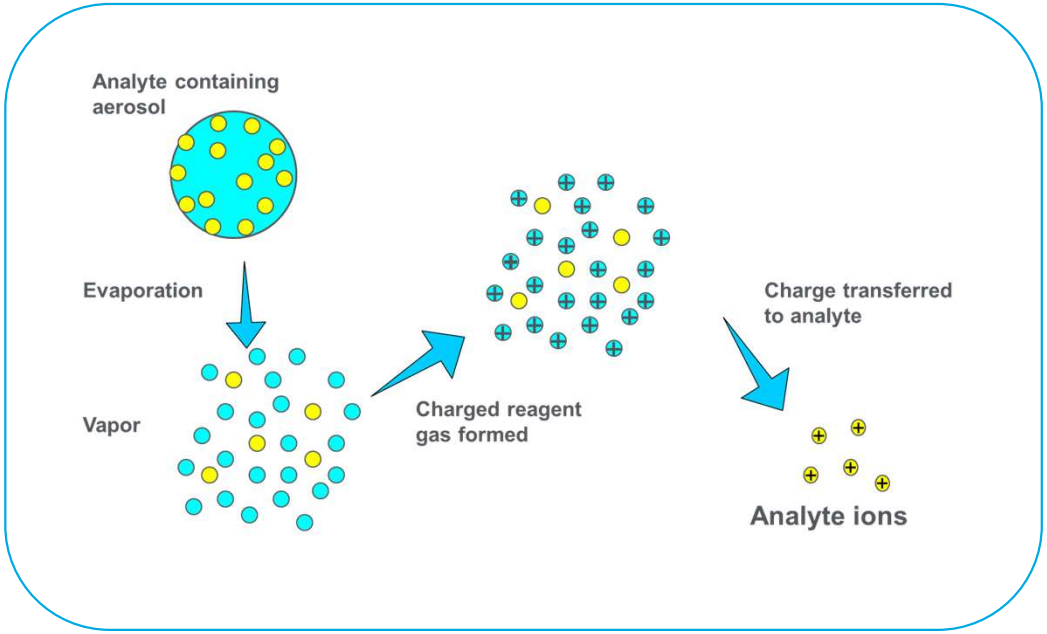
- **Samples**
- Ions in solution: catecholamines, sulfate conjugates, quaternary amines
- Compounds that can have a charge induced: menthol
- Compounds containing heteroatoms: carbamates, benzodiazepines
- Multiply charged in solution: proteins, peptides, oligonucleotides

- **Solution Chemistry Parameters**
- Flow rate
- Sample pK, solution pH
- Solution conductivity

- **Samples to Avoid**
- Extremely non-polar samples: PAHs, PCBs

APCI Interface





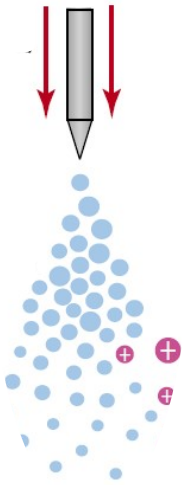
APCI Considerations

- **Samples**
- Compounds of intermediate MW and polarity: PAHs, PCBs, fatty acids, phthalates.
- Compounds that don't contain acidic or basic sites (e.g. hydrocarbons, alcohols, aldehydes, ketones, and esters
- Samples containing heteroatoms: ureas, benzodiazepines, carbamates
- Samples that exhibit a poor electrospray response

- **Solution Chemistry Parameters**
- Less sensitive to solution chemistry effects than ES
- Tolerates higher flow rates than ES
- Accommodates some solvents not compatible with ES

- **Samples to Avoid**
- Thermally labile compounds due to vaporization process

Atmospheric Pressure Ionization Techniques



Electrospray (ESI)	APCI
Volatility not required	Some volatility required
Preferred technique for thermally labile analytes	Analyte must be thermally stable
Ions formed in solution	Ions formed in gas phase
Can form multiply charged ions	Forms singly charged ions only

Available Ion Sources

Extensive ion source portfolio lets you choose the ideal match for your application

Agilent Jet Stream (AJS)

With the aid of superheated N_2 sheath gas for ultra-high sensitivity. Drastically increase ion formation by enhanced droplet desolvation.

Electrospray Ionization (ESI)

Analyze the broadest array of molecules in a concentration dependent manner. Suitable for standard flow, capillary flow, and nanoflow regimes

Nano Electrospray (Nano ESI)

Analyze the broadest array of molecules in a concentration dependent manner. Suitable for standard flow, capillary flow, and nanoflow regimes

Atmospheric Pressure Chemical Ionization

Atmospheric Pressure Chemical Ionization (APCI)

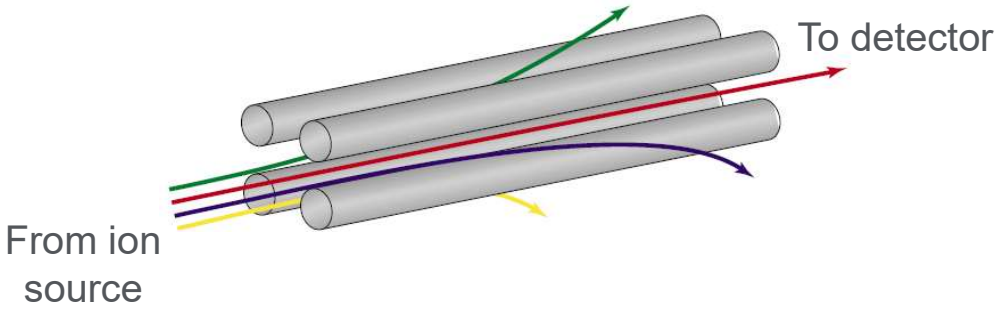
Complement your standard Electrospray Ionization analysis. You can detect difficult to ionize polar and nonpolar analytes with APCI

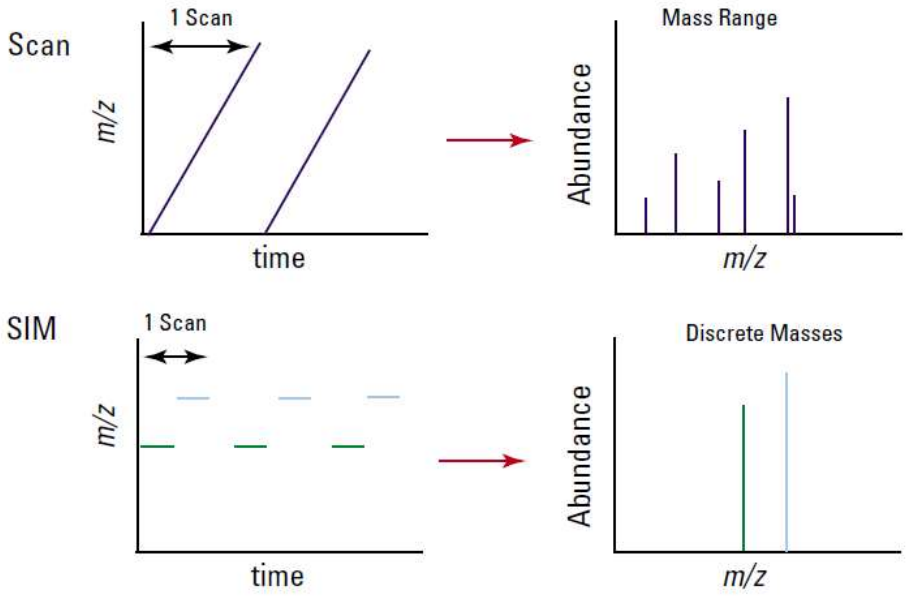
Multimode Ionization (MMI)

Deliver simultaneous ESI and APCI with high ionization efficiency. Provide added coverage across a wider range of analyte properties. Maximize throughput by eliminating the need to run samples twice.

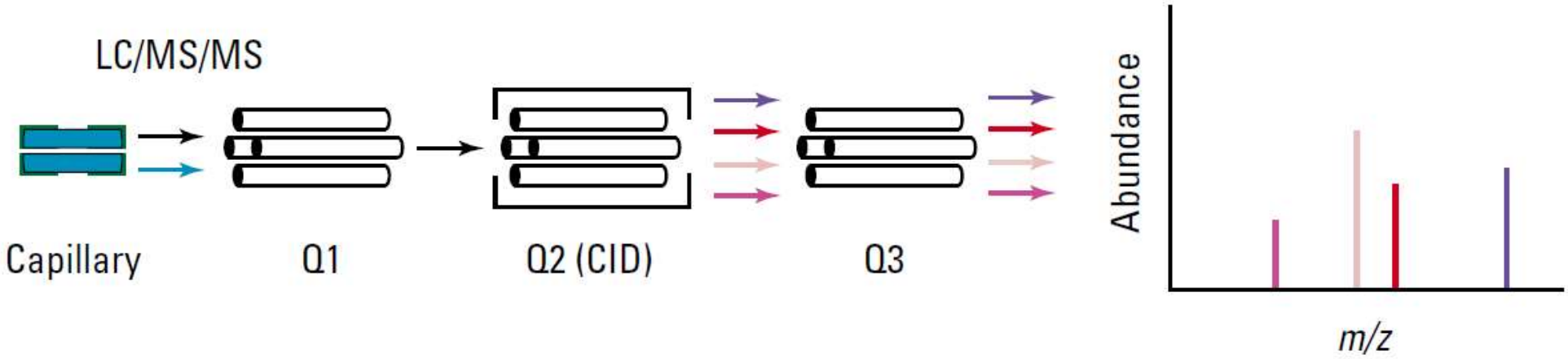
Quadruple mass analyzer

- Scanning (scan) mode : Range of m/z ratios
- Selected ion monitoring (SIM) mode : only a few m/z ratios





Collision-Induced Dissociation and Multiple-Stage MS



- To obtain structural information
- Analyte ion are fragmented by colliding them with neutral molecules
- Voltages are applied to the analyte ion to add energy to the collisions and create more fragmentation

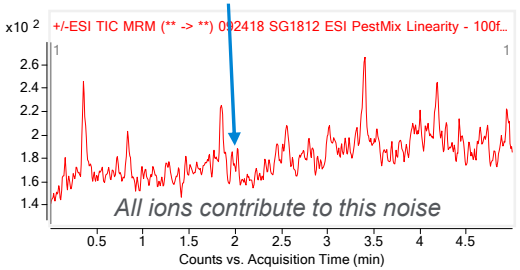
Quadrupole Mass Spectrometry: Quantitative Modes of Operation

Specifically in the context of Quantitation

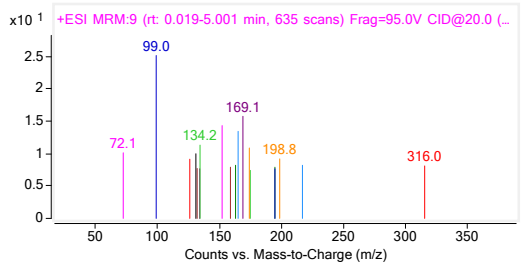
SQ Fullscan

All ions pass through

Analyte peak is ambiguous



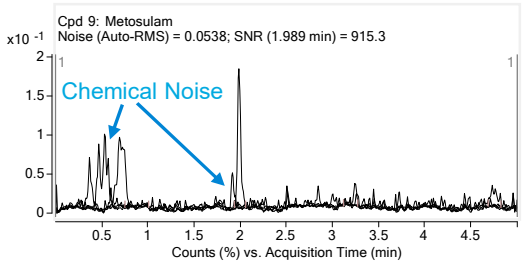
Complex Mass Spectrum

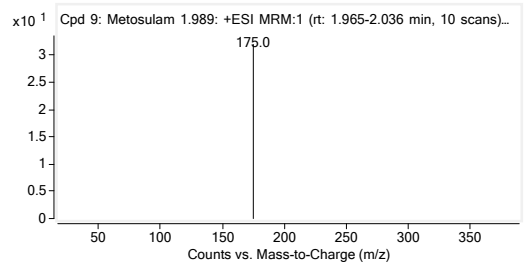


SQ Selected Ion Monitoring (SIM)

Specific m/z is selected

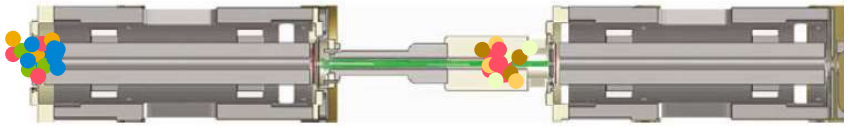
Analyte peak is detected but still contaminated (Sensitive)





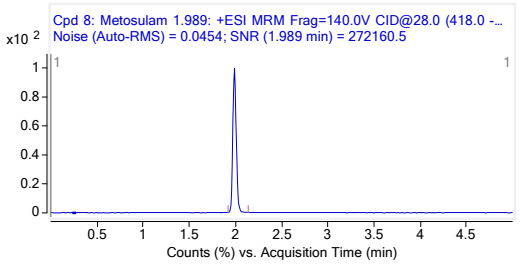
TQ Multiple Reaction Monitoring (MRM)

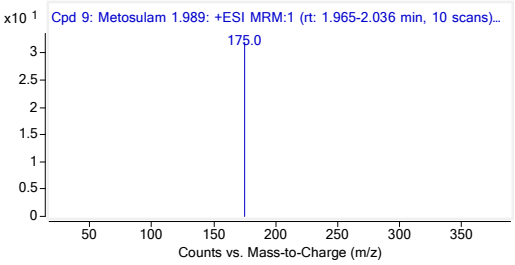
Also known as: Selected Reaction Monitoring (SRM)



Specific precursor and product m/z's are selected

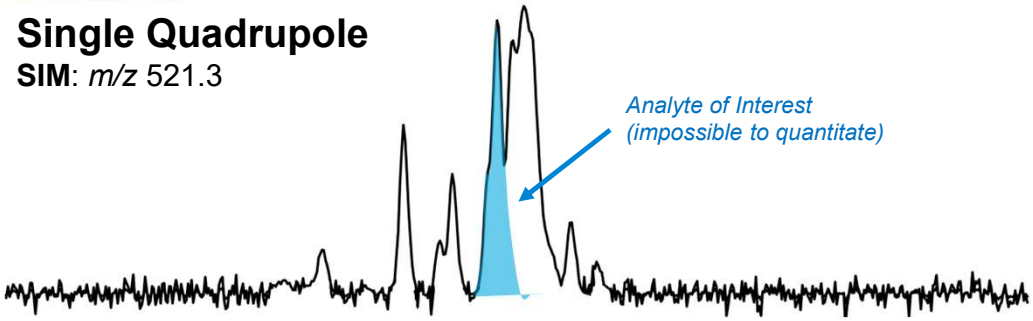
Undesired chemical noise is filtered out (Sensitive & Selective)

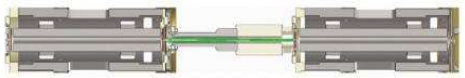




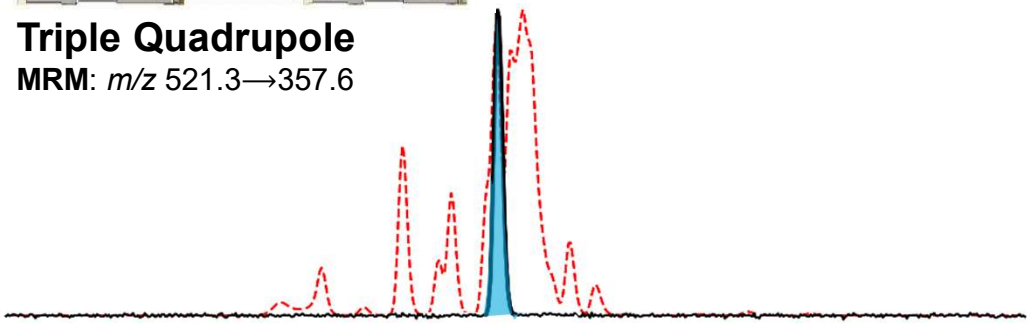
Why use a Triple Quadrupole LC/MS?

Single Quadrupole
SIM: *m/z* 521.3





Triple Quadrupole
MRM: *m/z* 521.3⟶357.6



Single Ion Monitoring (SIM)

All analytes with specified m/z are detected

- Compound of interest may coelute with other analytes of the same m/z.
- Baseline noise may be much higher due to background ions of the same m/z.

Multiple Reaction Monitoring (MRM)

Only analytes producing a specific "reaction" are detected

- Uses two stages of filtering. Basically, two SIMs operated at each quadrupole.
- Higher *specificity* of analyte ions.
- Drastic reduction of chemical noise, improving detection limit of analytes.

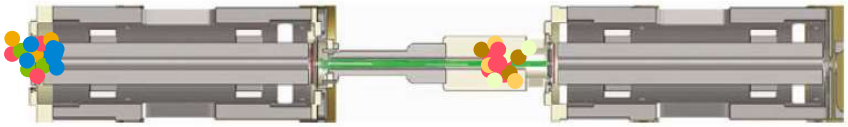
GOLD STANDARD IN QUANTITATIVE CHEMICAL ANALYSIS

Targeted screening of compound of interest

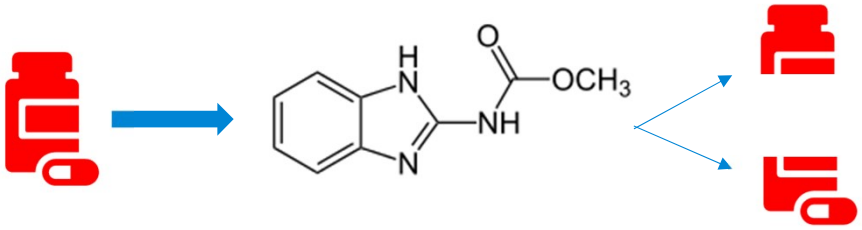
Optimum mass transition and collision energy

Multiple Reaction Monitoring (MRM)

Also known as: Selected Reaction Monitoring (SRM)



Specific precursor and product m/z's are selected



Compound	Q1 Mass (Precursor)	Collision energy (v)	Q3 Mass (Fragment)	Dwell Time
NDMA	75.1	12	58.0	10 ms
	75.1	18	43.1	10 ms

- Pros:
  - Highly selective
  - High sensitivity
  - High multiplexity
  - Short assay time

Analytical Technologies For Nitrosamine Impurity Analysis In Drug Substances and Drug Products

GC/MS Based Screening and Targeted Quantitation

Columns & Accessories

8890 GC/7697A HSS/ 5977B GC/MSD

8890 GC/7693 LS/ 7010B GC/TQ

High Throughput MS Based Screening

RapidFire- LC/TQ

MassHunter Software

LC/MS Based Screening, Targeted Quantitation

Infinity II LC

Columns & Accessories

6470 LC/TQ

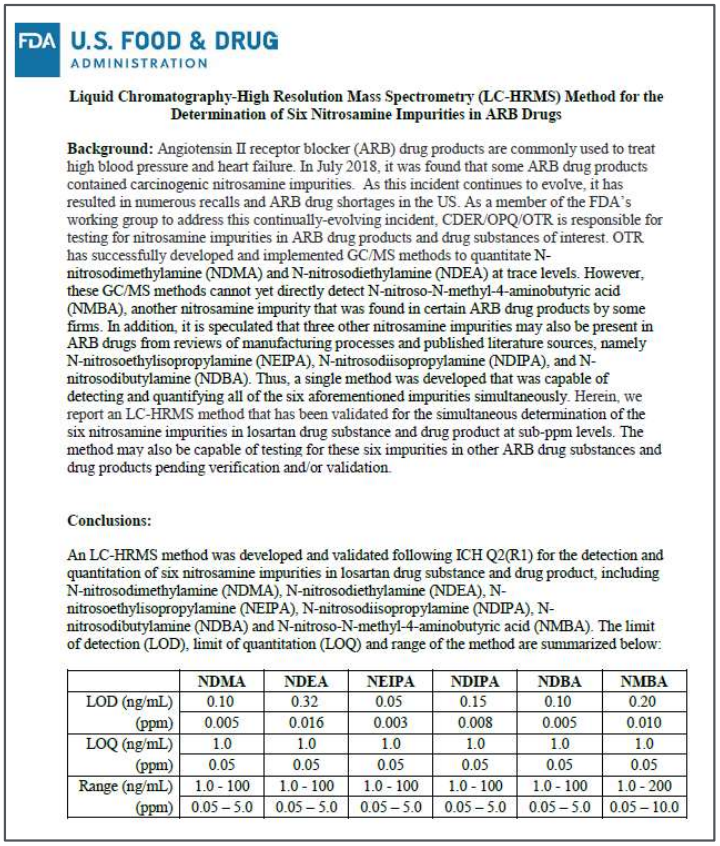
Ultivo LC/TQ

6546 LC/Q-TOF

Nitrosamine Analysis in Sartans

6470 LC/TQ

USFDA on LCMS Based Methods on ARB Drugs



FDA U.S. FOOD & DRUG ADMINISTRATION

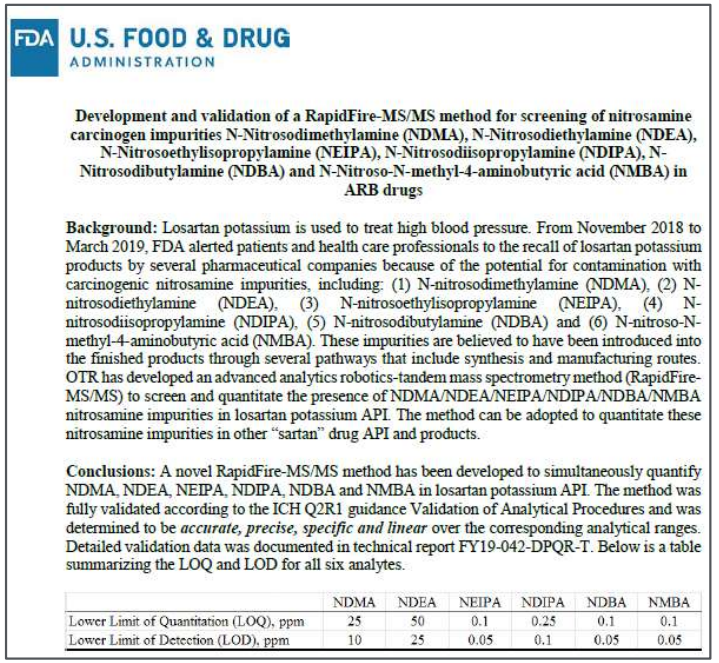
Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs

Background: Angiotensin II receptor blocker (ARB) drug products are commonly used to treat high blood pressure and heart failure. In July 2018, it was found that some ARB drug products contained carcinogenic nitrosamine impurities. As this incident continues to evolve, it has resulted in numerous recalls and ARB drug shortages in the US. As a member of the FDA's working group to address this continually-evolving incident, CDER/OPQ/OTR is responsible for testing for nitrosamine impurities in ARB drug products and drug substances of interest. OTR has successfully developed and implemented GC/MS methods to quantitate N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) at trace levels. However, these GC/MS methods cannot yet directly detect N-nitroso-N-methyl-4-aminobutyric acid (NMBA), another nitrosamine impurity that was found in certain ARB drug products by some firms. In addition, it is speculated that three other nitrosamine impurities may also be present in ARB drugs from reviews of manufacturing processes and published literature sources, namely N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), and N-nitrosodibutylamine (NDBA). Thus, a single method was developed that was capable of detecting and quantifying all of the six aforementioned impurities simultaneously. Herein, we report an LC-HRMS method that has been validated for the simultaneous determination of the six nitrosamine impurities in losartan drug substance and drug product at sub-ppm levels. The method may also be capable of testing for these six impurities in other ARB drug substances and drug products pending verification and/or validation.

Conclusions:

An LC-HRMS method was developed and validated following ICH Q2(R1) for the detection and quantitation of six nitrosamine impurities in losartan drug substance and drug product, including N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosodiisopropylamine (NDIPA), N-nitrosodibutylamine (NDBA) and N-nitroso-N-methyl-4-aminobutyric acid (NMBA). The limit of detection (LOD), limit of quantitation (LOQ) and range of the method are summarized below:

	NDMA	NDEA	NEIPA	NDIPA	NDBA	NMBA
LOD (ng/mL)	0.10	0.32	0.05	0.15	0.10	0.20
(ppm)	0.005	0.016	0.003	0.008	0.005	0.010
LOQ (ng/mL)	1.0	1.0	1.0	1.0	1.0	1.0
(ppm)	0.05	0.05	0.05	0.05	0.05	0.05
Range (ng/mL)	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 200
(ppm)	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 10.0



FDA U.S. FOOD & DRUG ADMINISTRATION

Development and validation of a RapidFire-MS/MS method for screening of nitrosamine carcinogen impurities N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA), N-Nitrosoethylisopropylamine (NEIPA), N-Nitrosodiisopropylamine (NDIPA), N-Nitrosodibutylamine (NDBA) and N-Nitroso-N-methyl-4-aminobutyric acid (NMBA) in ARB drugs

Background: Losartan potassium is used to treat high blood pressure. From November 2018 to March 2019, FDA alerted patients and health care professionals to the recall of losartan potassium products by several pharmaceutical companies because of the potential for contamination with carcinogenic nitrosamine impurities, including: (1) N-nitrosodimethylamine (NDMA), (2) N-nitrosodiethylamine (NDEA), (3) N-nitrosoethylisopropylamine (NEIPA), (4) N-nitrosodiisopropylamine (NDIPA), (5) N-nitrosodibutylamine (NDBA) and (6) N-nitroso-N-methyl-4-aminobutyric acid (NMBA). These impurities are believed to have been introduced into the finished products through several pathways that include synthesis and manufacturing routes. OTR has developed an advanced analytics robotics-tandem mass spectrometry method (RapidFire-MS/MS) to screen and quantitate the presence of NDMA/NDEA/NEIPA/NDIPA/NDBA/NMBA nitrosamine impurities in losartan potassium API. The method can be adopted to quantitate these nitrosamine impurities in other "sartan" drug API and products.

Conclusions: A novel RapidFire-MS/MS method has been developed to simultaneously quantify NDMA, NDEA, NEIPA, NDIPA, NDBA and NMBA in losartan potassium API. The method was fully validated according to the ICH Q2R1 guidance Validation of Analytical Procedures and was determined to be *accurate, precise, specific and linear* over the corresponding analytical ranges. Detailed validation data was documented in technical report FY19-042-DPQR-T. Below is a table summarizing the LOQ and LOD for all six analytes.

	NDMA	NDEA	NEIPA	NDIPA	NDBA	NMBA
Lower Limit of Quantitation (LOQ), ppm	25	50	0.1	0.25	0.1	0.1
Lower Limit of Detection (LOD), ppm	10	25	0.05	0.1	0.05	0.05

USFDA Guidance Document

U.S. FOOD & DRUG ADMINISTRATION

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) Method for the Determination of Six Nitrosamine Impurities in ARB Drugs

	NDMA	NDEA	NEIPA	NDIPA	NDBA	NMBA
LOD (ng/mL)	0.10	0.32	0.05	0.15	0.10	0.20
(ppm)	0.005	0.016	0.003	0.008	0.005	0.010
LOQ (ng/mL)	1.0	1.0	1.0	1.0	1.0	1.0
(ppm)	0.05	0.05	0.05	0.05	0.05	0.05
Range (ng/mL)	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 100	1.0 - 200
(ppm)	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 5.0	0.05 – 10.0

Conversion of ng/mL into ppm

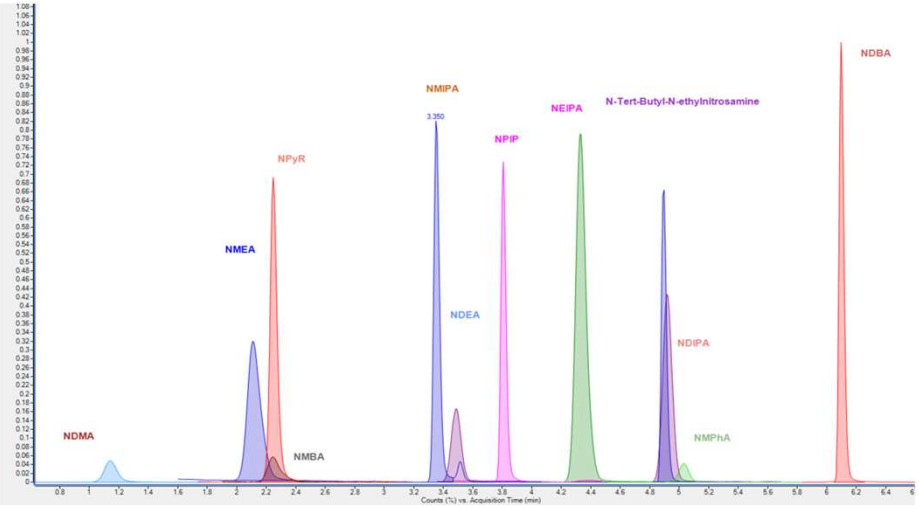
mg/kg=µg/g= **ppm**= [Value in ng/mL]/ [weight of sample in mg] * [Volume of sample diluent in mL]

For example if we get value of **1ng/mL** from calibration curve for a sample size of **20mg/mL** then

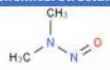
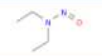
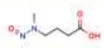
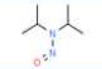
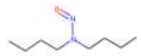
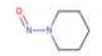
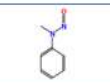
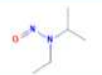
mg/kg=µg/g= **ppm**= [**1ng/mL**]/[**20mg**] * [**1mL**] = **0.05**

Nitrosamine Analysis Using 6470 LC/TQ

Compound	LOD (ng/mL)	LOD (S/N)	LOQ (ng/mL)	LOQ (S/N)	Linearity Range(ng/mL)
NDMA	0.05	7.40	**0.1**	17.92	0.075-100
NDEA	0.025	28.4	**0.05**	33.8	0.05-100
NMBA	0.025	10.58	**0.05**	30.98	0.025-100
NEIPA	0.025	45.16	**0.05**	61.23	0.05-100
NDIPA	0.025	8.16	**0.05**	17.89	0.05-100
NDBA	0.05	266.46	**0.1**	463.84	0.05-100
NMEA	0.075	7.85	**0.1**	12.82	0.1-100
NPyR	0.075	21.74	**0.1**	31.24	0.075-100
NPIP	0.05	23.99	**0.1**	27.31	0.075-100
NMPhA	0.075	16.51	**0.1**	27.38	0.1-100
NMIPA	0.05	25.67	**0.075**	64.09	0.075-100
N-Tert-Butyl-N-ethylnitrosamine	0.05	32.76	**0.1**	85.99	0.075-100



12 Nitrosamine Impurities

Nitrosamine Compounds	Chemical Structure
N-nitrosodimethylamine (NDMA)	
N-nitrosodiethylamine (NDEA)	
N-nitroso-4-methyl-4-aminobutyric acid (NMBA)	
N-nitrosoethylisopropylamine (NEIPA)	
N-nitrosodiisopropylamine (NDIPA)	
N-nitrosodibutylamine (NDBA)	
N-nitrosoethylmethylamine (NMEA)	
N-nitrosopyrrolidine (NPyR)	
N-nitrosopiperidine (NPIP)	
N-methyl-N-nitrosoaniline (NMPhA)	
N-isopropylmethyl nitrosamine (NMIPA)	
N-Tert-Butyl-N-ethylnitrosamine	

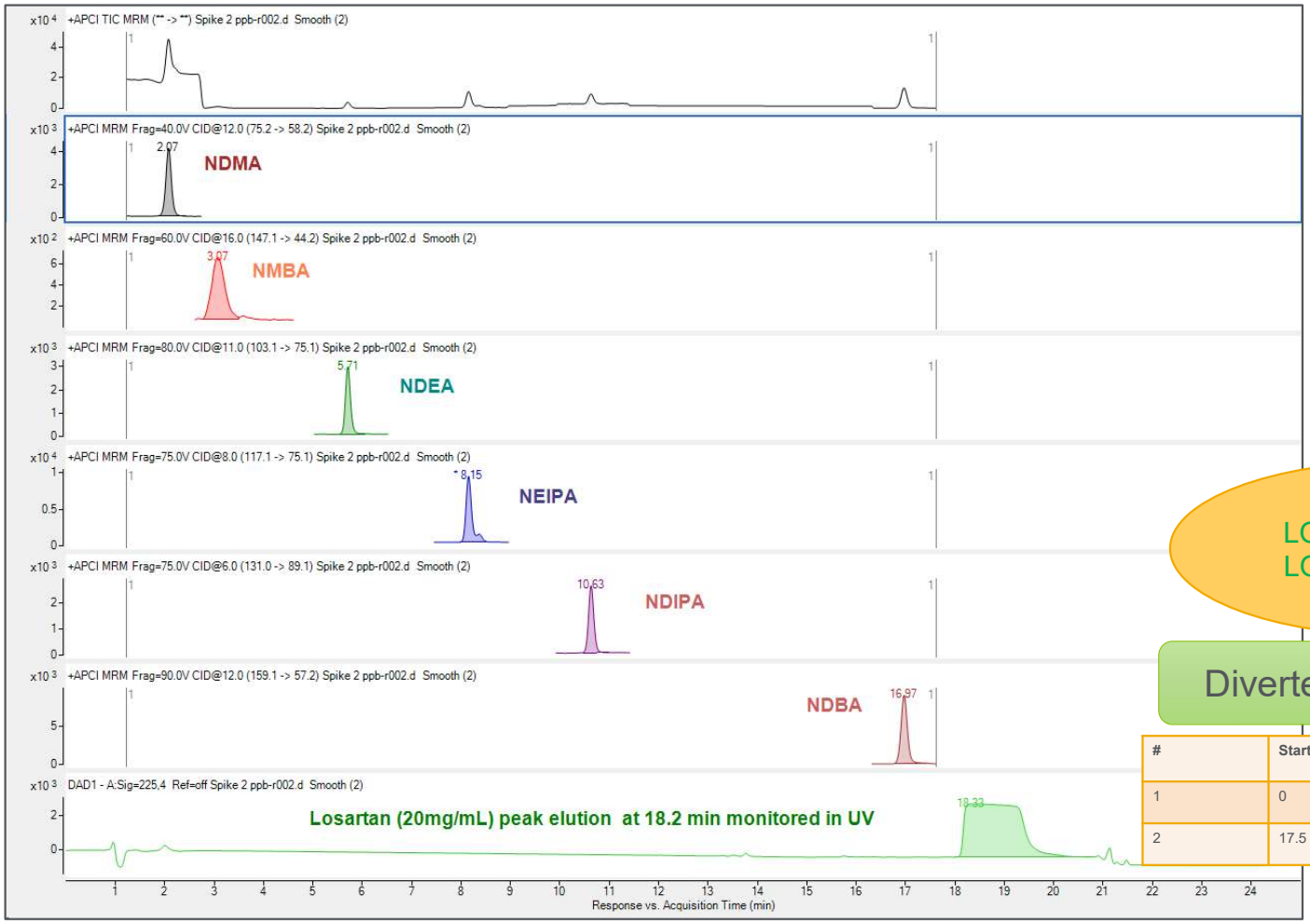
Nitrosamine Analysis Using 6470 LC/TQ

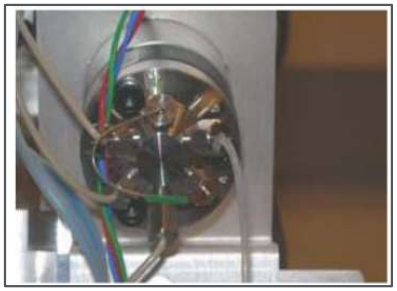
Compound	Precursor Ion (m/z)	Product Ion (m/z)	Retention Time(min)	Retention Time Window (Min)	Fragmentor (V)	Collision Energy (V)	CAV (V)	Polarity
NDEA	103.1	75.1	3.484	1.5	80	9	3	+
NDEA	103.1	47.1	3.484	1.5	80	17	3	+
NDMA	75.1	58	1.143	1.24	75	12	3	+
NDMA	75.1	43.1	1.143	1.24	110	18	3	+
NMBA	147.1	44.2	2.247	1.2	60	16	3	+
NMBA	147.1	87.2	2.247	1.2	60	10	3	+
NEIPA	117.1	75.1	4.325	1.0	75	8	3	+
NEIPA	117.1	47.1	4.325	1.0	75	18	8	+
NDIPA	131.1	89.1	4.916	1.0	75	6	3	+
NDIPA	131.1	43.1	4.916	1.0	75	12	8	+
NDBA	159.1	57.2	6.096	1.0	90	12	1	+
NDBA	159.1	41.1	6.096	1.0	90	22	3	+
NMEA	89.1	61.1	2.109	1.37	75	10	3	+
NMEA	89.1	43.1	2.109	1.37	75	12	3	+
NPyR	101.1	55.1	2.248	1.43	90	24	3	+
NPyR	101.1	41	2.248	1.43	90	19	3	+
NPIP	115.1	69.1	3.809	1.0	90	12	3	+
NPIP	115.1	41.2	3.809	1.0	90	24	3	+
NMPhA	137	66.1	5.029	1.32	45	26	3	+
NMPhA	137	107	5.029	1.32	45	12	3	+
NMIPA	103.1	61	3.358	1.0	60	8	7	+
NMIPA	103.1	43	3.358	1.0	60	8	5	+
N-Tert-Butyl-N-ethylnitrosamine	131	75.1	4.897	1.0	40	4	5	+
N-Tert-Butyl-N-ethylnitrosamine	131	57.1	4.897	1.0	40	6	5	+

Triple quadrupole mass spectrometer configuration and parameters

Parameter	Value
Instrument	Agilent Ultivo triple quadrupole mass spectrometer
Ion Source	Atmospheric pressure chemical ionization (APCI)
MS/MS Mode	Dynamic MRM (dMRM)
Ion Mode	Positive
Drying Gas Temperature	300 °C
Drying Gas Flow	6 L/min
Nebulizer Pressure	55 psi
APCI Heater	350 °C
APCI Needle Positive	4 µA
Capillary Voltage, Positive	3,000 V
MS1/MS2 Resolution	0.7/0.7 (unit/unit)
Dwell Time	Variable

Nitrosamines in Losartan





Integrated diverter valve used to divert API to waste

LOQ @ 0.1 ng/mL
LOQ @ 0.005 ppm

Diverter Valve Program

#	Start Time(Min)	Scan Type	Diverter Valve
1	0	dMRM	MS
2	17.5	dMRM	Waste

Recovery Study in Losartan drug substance

Nitrosamine Impurity	Concentration (ng/mL)	Recovery %
NMBA	0.4	92
	1	113
	2	115

Nitrosamine Impurity	Concentration (ng/mL)	Recovery %
NDEA	0.4	103
	1	103
	2	101

Nitrosamine Impurity	Concentration (ng/mL)	Recovery %
NEIPA	0.4	100
	1	100
	2	101

Nitrosamine Impurity	Concentration (ng/mL)	Recovery %
NDIPA	0.4	107
	1	98
	2	99

Recovery Study in Losartan

Nitrosamine Impurity	Concentration (ng/mL)	Recovery %
NDMA	2	110

Nitrosamine Impurity	Concentration (ng/mL)	Recovery %
NDBA	2	91

- Excellent Recovery for each nitrosamines
- Recovery study performed at different conc. levels due to presence of NDMA and NDBA in the drug substance

Reproducibility Data

#	Conc. (ng/mL)	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDBA
1	1	2436	4844	9962	34563	13899	16452
2	1	2442	4937	10067	32146	13871	16342
3	1	2435	4827	10066	32805	14375	16942
4	1	2578	4996	10182	32838	13822	16670
5	1	2442	4987	10145	33254	14335	16706
6	1	2434	4966	10193	33108	13868	16691
Average		2461	4926	10103	33119	14028	16634
SD		57.34	73.30	87.92	803.46	254.55	211.18
RSD (%)		**2.33**	**1.49**	**0.87**	**2.43**	**1.81**	**1.27**

Reproducibility Data with Bracketing Standards

#	Conc. (ng/mL)	NDMA	NMBA	NDEA	NEIPA	NDIPA	NDBA
1	1	2556	5484	10530	36010	14023	18686
2	1	2409	5609	10727	36593	13478	18853
3	1	2436	4844	9962	34563	13899	16452
4	1	2442	4937	10067	32146	13871	16342
5	1	2435	4827	10066	32805	14375	16942
6	1	2578	4996	10182	32838	13822	16670
7(Bracketing Std)	1	2442	4987	10145	33254	14335	16706
8(Bracketing Std)	1	2434	4966	10193	33108	13868	16691
Average		2467	5081	10234	33915	13959	17168
SD		63.16	295.66	259.96	1629.64	289.90	1005.59
RSD (%)		**2.56**	**5.82**	**2.54**	**4.81**	**2.08**	**5.86**

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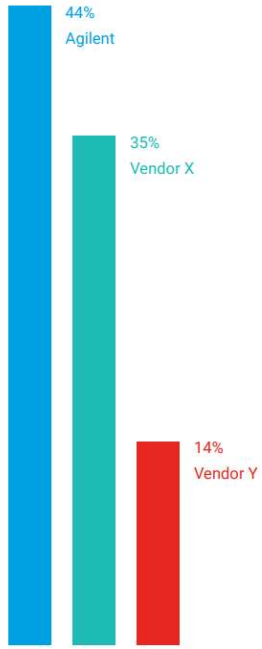

Agilent LC instrumentation cited as most reliable

Regular LCGC reader surveys since 2011 show Agilent LC instrumentation most frequently cited as most reliable (graph shows data from 2018 survey).

1290 Infinity II Multicolumn Thermostat

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Adheres to compliance guidelines under FDA 21 CFR Part 11, EU Annex 11, and GAMP 5

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- Approvals and eSignatures
- User management of compliant systems
- Secure storage of active records (methods, worklists, data files)
- Secure storage of non-Agilent data (ECM XT)
- Secure archival of past data

Data Acquisition

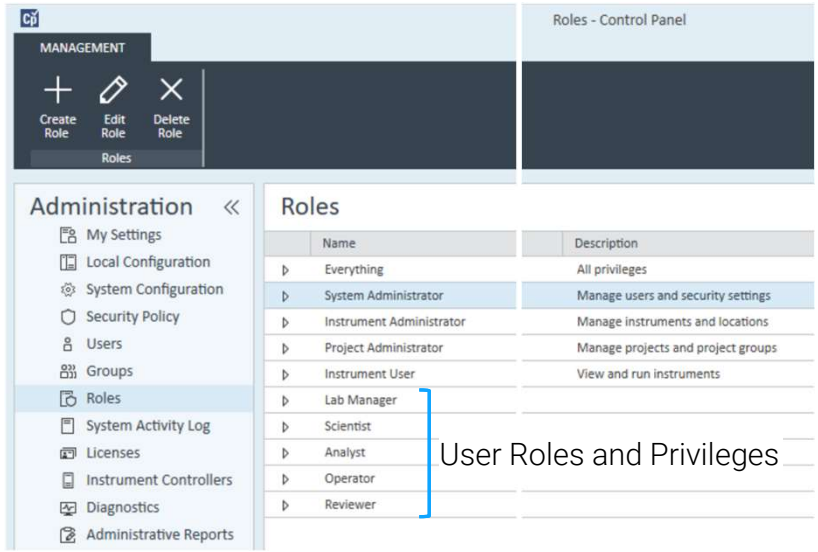
- MassHunter Acquisition
- Setup Instruments / Methods
- User Access Control

Data Processing

- MassHunter Quantitative Analysis
- Reporting

MassHunter 12 supports technical controls, audit trails, and data integrity

Adheres to compliance guidelines under FDA 21 CFR Part 11, EU Annex 11, and GAMP 5



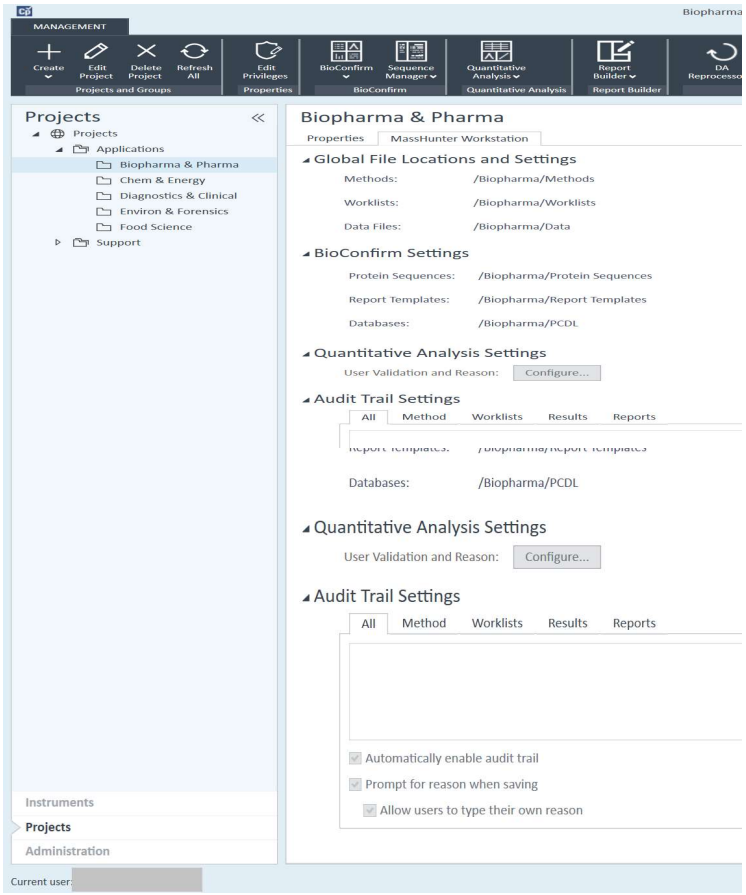
Lab Manager – *Administrative privileges*

Scientist – *Mass spectrometry expert*

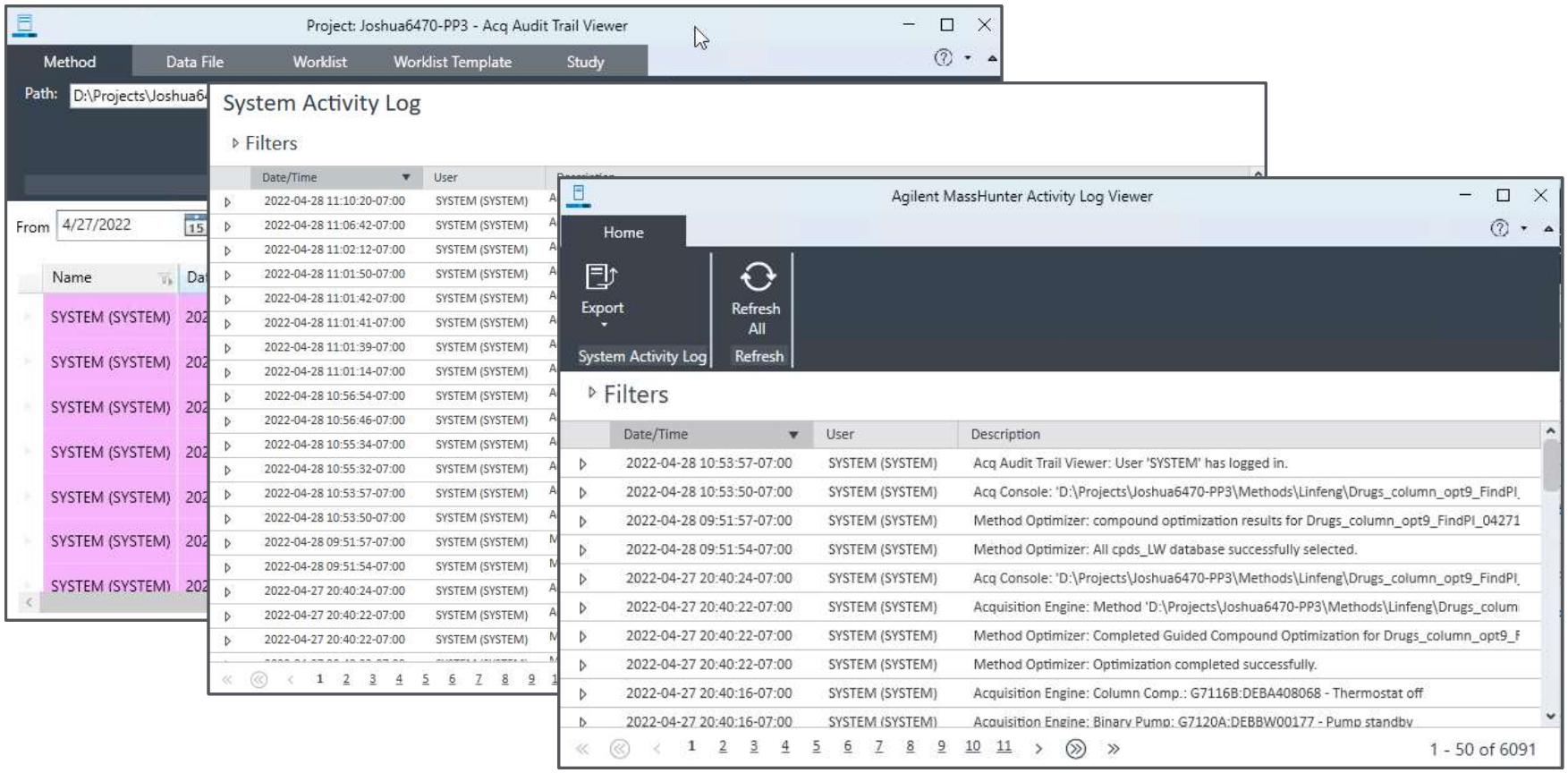
Analyst – *Protein Scientist* – Sample information and workflow selection

Operator – *Lab Technician* – Open method, run only

Reviewer – *File and audit trail reviewer* – No data processing or method editing



MassHunter 12 Supports Document Audit Trail Reviews



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- Instrument sensitivity is far better than required both on LC/TQ and LC/Q-TOF platforms
- Results reproducibility is just excellent whereas we have seen %RSD issues with competition at LOQ levels
- Agilent APCI source is a very stable ionization source, bringing incremental value to the data quality
- Multisampler feature like Multi wash just makes sure that there is absolutely no carry over issues
- Complete solution available in terms of handling issues of Ranitidine, Metformin etc. using LCMS or GCMS platforms under the common umbrella of Masshunter software

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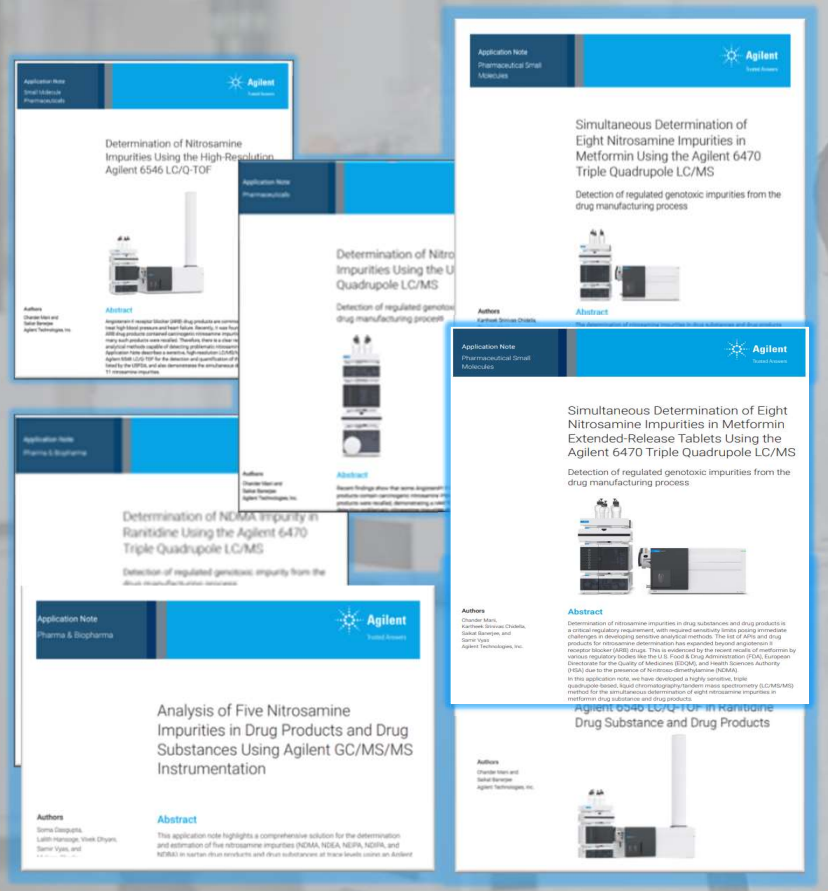
Application note



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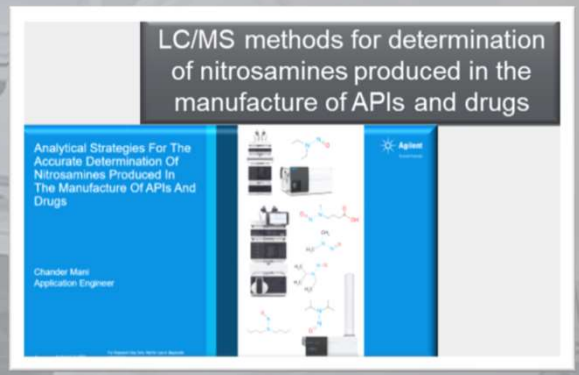

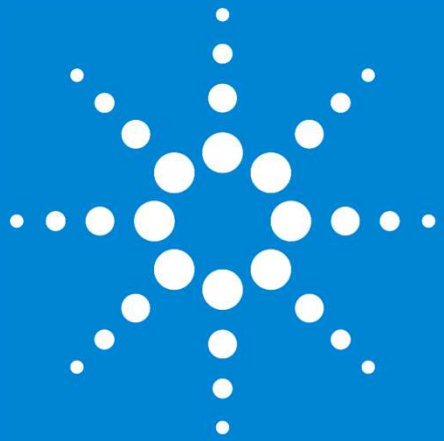

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