Powerful separation techniques applied in Ph. Eur. monographs

Maria Silvana Bellini Ph.D.

European Directorate for the Quality of Medicines & Healthcare (EDQM)

Council of Europe

Topics

- EDQM and Council of Europe
- Basis of the European Pharmacopoeia (Ph. Eur.)
- Techniques in Ph. Eur. monographs:
  - Liquid - Gas Chromatography/Mass Spectrometry
  - Nuclear Magnetic Resonance spectrometry
- Control of elemental impurities (ICH Q3D): impact on Ph. Eur. Monographs/Chapters

EDQM and Council of Europe

Council of Europe (CoE)

- International organisation (created in 1949) with legal personality recognised under public international law
- Serves 800 million Europeans in 47 member states
- Located in Strasbourg, France

Core values

human rights, democracy and the rule of law

Access to good medicines is a human right!

EDQM and Council of Europe

EDQM - Directorate of CoE created in 1964.

Mission is **protects public health** by:

- supporting development, implementation and monitoring the application of **quality standards** for safe medicines (and components) and their safe use by patients.

European Pharmacopoeia,
including its Reference Standards,

are the quality standards, **legally binding** in Member States

Basis of the European Pharmacopoeia

- Safety first! Products of proven safety
- Products evaluated and approved by Competent Authorities of Member States
- **Impurity profile** for existing and approved synthetic routes
- Robust validated **analytical methods** based on laboratory testing

PREFACE

"The European Pharmacopoeia currently contains more than 300 general chapters …the European Pharmacopoeia Commission has established a new working party (MG) …to ensure they keep pace with instrument and method innovation."

Techniques in Ph. Eur.

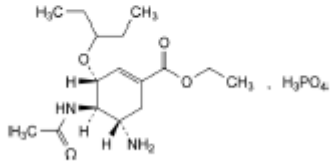
- Liquid chromatography (LC) Ph. Eur. 2.2.29.
  - Separation of a wide range of organic compounds (small-molecule to peptides/proteins)
  - Based on equilibrium partition between the mobile phase/stationary phase in LC column
  - Detectors: UV/Vis spectrophotometers, Fluorescence, Refractive Index, ELSD, etc.
- Gas chromatography (GC) Ph. Eur. 2.2.28.
  - Separation of volatile organic compounds
  - Based on equilibrium partition between the carrier gas /stationary phase in GC column
  - Detectors: FID, TCD, etc.
- Mass Spectrometry (MS) Ph. Eur. 2.2.43.
  - More sensitive & specific detector applied stand-alone or in tandem with LC or GC (hyphenated techniques)
  - Used for compounds with no chromophore or when structural elucidation needed
  - Analytes are ionised and identified based on mass/charge ratio

LC/MS

- Chapter 2.2.59 Glycan analysis of glycoproteins
- Interferon beta-1a concentrated solution (1639)
  - Production session: Identification using MS or LC/MS
- Meldonium dihydrate (2624)
  - Test of related substances
- Oseltamivir phosphate (2422)
  - Test of Impurity B
- Imatinib mesilate (2736)
  - Test of Impurity F

OSELTAMIVIR PHOSPHATE

Oseltamiviri phosphas



$C_{16}H_{31}N_2O_8P$ M_r 410.4
[204255-11-8]

Impurity B. Liquid chromatography (2.2.29) coupled with mass spectrometry (2.2.43).

Test solution. Dissolve 0.100 g of the substance to be examined in water for chromatography R and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 2.5 mg of oseltamivir impurity B CRS in 5.0 mL of anhydrous ethanol R and dilute to 50.0 mL with water for chromatography R. Dilute 2.0 mL of the solution to 100.0 mL with water for chromatography R.

Reference solution (b). Dissolve 50.0 mg of oseltamivir phosphate (impurity B-free) CRS in reference solution (a) and dilute to 5.0 mL with the same solution.

Column:

- *size*: *l* = 0.05 m, Ø = 3.0 mm;
- *stationary phase*: end-capped octadecylsilyl silica gel for chromatography R (5 µm);
- *temperature*: 40 °C.

Mobile phase: mix 10 volumes of a 1.54 g/L solution of ammonium acetate R in water for chromatography R, 30 volumes of acetonitrile R1 and 60 volumes of water for chromatography R.

Flow rate: 1.5 mL/min.

Post-column split ratio: use a split ratio suitable for the mass detector (e.g. 1:3).

Detection:

- mass detector: the following settings have been found to be suitable and are given as examples; if the detector has different setting parameters, adjust the detector settings so as to comply with the system suitability criterion:
  - ionisation: ESI-positive;
  - detection *m/z*: 356.2;
  - dwell: 580 ms;
  - gain EMV: 1;
  - fragmentator voltage: 120 V;
  - gas temperature: 350 °C;
  - drying gas flow: 13 L/min,
  - nebuliser pressure: 345 kPa;
  - capillary voltage (Vcap): 3 kV.

Injection: 1 µL of the test solution and reference solution (b).

Run time: 3 min.

System suitability: reference solution (b):

- *repeatability*: maximum relative standard deviation of 15 per cent determined on 6 injections.

Limit:

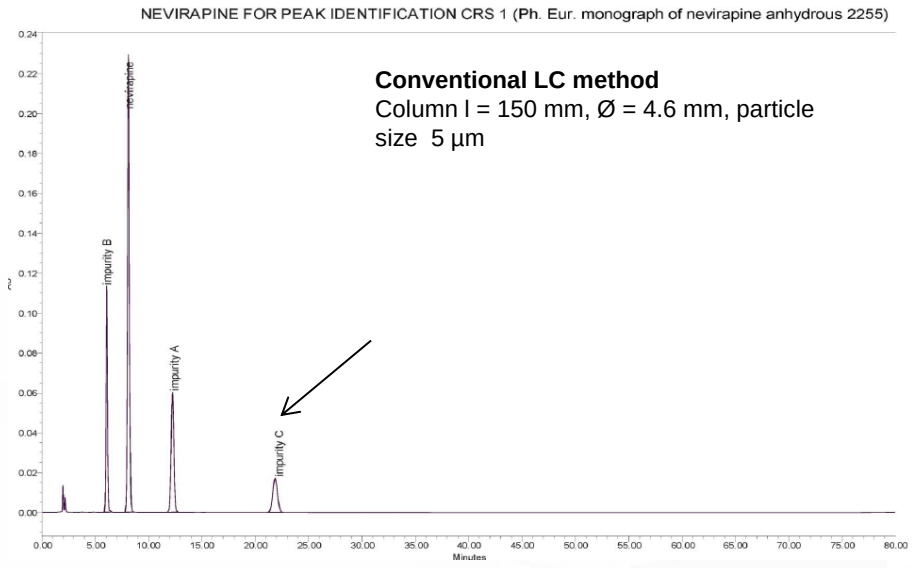
- *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (100 ppm).

LC - narrow bore columns

- Current LC trend is to use narrow bore column (i.e. 2.1 mm i.d.) with small particles (sub-2 µm) in high pressure system.

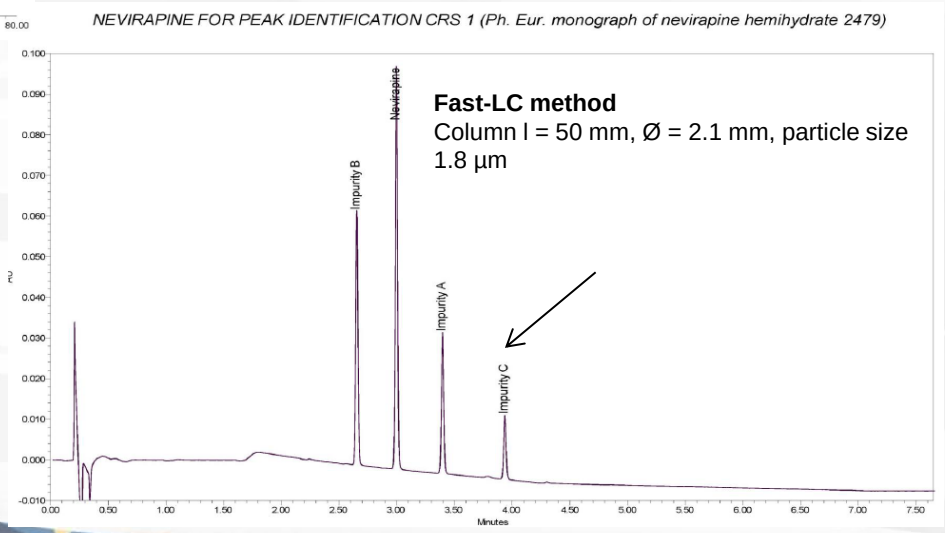
- Applied in following Ph. Eur. monographs (test for related substances):
  - Hydroxychloroquine sulfate (2849)
  - Indapamide (1108) (test for Impurity C)
  - Levocabastine Hydrochloride (1484)
  - Nevirapine hemihydrate (2479)
  - Quetiapine fumarate (2541)
  - Solifenacin succinate (2779)
  - Thiocolchicoside crystalline from ethanol (2896) and hydrate (2814)

Advantages..cont'd



Conventional LC method
Column l = 150 mm, Ø = 4.6 mm, particle size 5 µm

- ✓ Improvement of separation speed (shorter run),
- ✓ Lower solvent consumption
- ✓ Resolution maintained
- ✓ Sensitivity



Fast-LC method
Column l = 50 mm, Ø = 2.1 mm, particle size 1.8 µm

GC/MS

- Chapter 2.5.37 -> 2.5.41 Determination of methane-toluene-benzene sulfonic acid esters (known genotoxicity, limited at ppm level)
  - 2.5.37, Methyl ethyl and isopropyl methanesulfonate in methanesulfonic acid
  - 2.5.38, Methyl ethyl and isopropyl methanesulfonate in active substances
  - 2.5.39, Methanesulfonyl chloride in methanesulfonic acid
  - 2.5.40, Methyl ethyl and isopropyl toluenesulfonate in active substances
  - 2.5.41, Methyl ethyl and isopropyl benzenesulfonate in active substances
- Norflurane (2257): Identification and test for related substances

NORFLURANE

Norfluranum

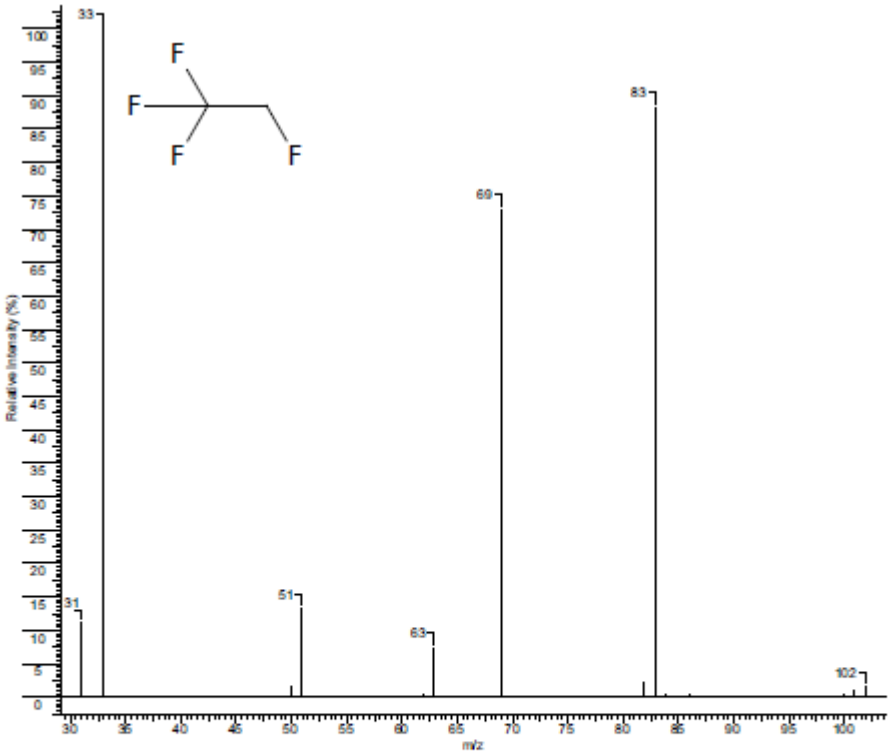
$C_3H_2F_4$ M_r 102.0
[811-97-2]

DEFINITION
1,1,1,2-Tetrafluoroethane (HFC 134a).

IDENTIFICATION
Carry out either test A or test B.

A. Infrared absorption spectrophotometry (2.2.24). *Carry out the test at atmospheric pressure.*
Preparation: dilute the gas to be examined in *nitrogen R* (approximately 20:80 *V/V* depending on the sensitivity of the spectrophotometer).
Comparison: *Ph. Eur. reference spectrum of norflurane.*

B. Mass spectrometry (2.2.43).
Results: the mass spectrum obtained with the gas to be examined is similar to the mass spectrum of norflurane shown in Figure 2257.-1.



m/z	RI (%)	ion	m/z	RI (%)	ion
31	11.1	$[CF]^+$	82	2.1	$[CF_2\text{-}CHF]^{+\cdot}$
33	100.0	$[CH_2F]^+$	83	88.2	$[CF_2\text{-}CH_2F]^+$
50	1.5	$[CF_2]^{+\cdot}$	100	0.3	$[CF_2\text{-}CF]^{+\cdot}$
51	13.2	$[CHF_2]^+$	101	0.9	$[CF_2\text{-}CHF]^+$
63	7.4	$[CF{=}CHF]^+$	102	1.5	$[CF_2\text{-}CH_2F]^{+\cdot}$
69	72.9	$[CF_3]^+$			

Figure 2257.-1. – *Mass spectrum of norflurane*

Nuclear Magnetic Resonance (NMR)

- Based on a physical phenomenon in which nuclei under a magnetic field absorb and re-emit electromagnetic radiation at a specific frequency, depending on the strength of the magnetic field and the magnetic properties of the isotopes.
- Suitable for the elucidation of the chemical structure by interpretation of their spectra, arising from, for example, ^{1}H or ^{13}C, ^{19}F, ^{15}N, ^{31}P.
- Intensities of the signals (integrals) are directly proportional to the number of nuclear spins in quantitative analysis.

NMR

- 2.2.64 Peptide identification by nuclear magnetic resonance
- Haemophilus type b and meningococcal group C conjugate vaccine (2622) and Haemophilus type b conjugate vaccine (1219)
- Meningococcal group C conjugate vaccine (2112) and Pneumococcal polysaccharide conjugate vaccine (adsorbed) (2150)
- Lauromacrogol 400 (2046)
- Pemetrexed disodium heptahydrate (2637)
- Poloxamers (1463)
- Medronic acid for radiopharmaceutical preparations (2350)
- Tetra-O-acetyl-mannose triflate for radiochemical preparations (2294)
- Heparin calcium (0332), Heparin Sodium (0333), Heparin low-molecular-mass (0828)
- Cod-liver oil (2398) and Salmon oil farmed (1910)
- Hydroxypropylbetadex (1804)
- Starch hydroxypropyl (2165) and Starch hydroxypropyl pregelatinised (2645)
- Tobramycin (0645)

LAUROMACROGOL 400

Lauromacrogolum 400

DEFINITION

Mixture of lauryl alcohol (dodecanol) monoethers of mixed macrogols. It may contain some free macrogols and it contains various amounts of free lauryl alcohol. The number of moles of ethylene oxide reacted per mole of lauryl alcohol is 9. The name of the substance is followed by a number (400) corresponding approximately to the average molecular mass of the macrogol portion.

This monograph applies to lauromacrogol 400 used as active substance.

Average chain length of the fatty alcohol and average number of moles of ethylene oxide. Nuclear magnetic resonance spectrometry (2.2.33).

Test solution. If the substance is in the solid state at room temperature, heat gently before sampling. Dissolve 0.4 mL of the substance to be examined in 0.3 mL of a mixture of 1 volume of *deuterated methanol R* and 2 volumes of *deuterated chloroform R*, containing 0.1 mol/L of *chromium(III) acetylacetonate R* as a relaxation aid.

Apparatus: high resolution FT-NMR spectrometer operating at minimum 300 MHz.

Acquisition of ^{13}C NMR spectra. The following parameters may be used:

- *sweep width*: 250 ppm (– 15 ppm to 235 ppm);
- *irradiation frequency offset*: 110 ppm;
- *time domain*: 64 K;
- *pulse delay*: 3 s;
- *pulse program*: zgig 30 (inverse gated, 30° excitation pulse);
- *dummy scans*: 4;
- *number of scans*: 2048.

System suitability:

- *signal-to-noise ratio*: minimum 150, for the smallest relevant peak (CH_2 at 73.1 ppm);
- *peak width at half-height*: maximum 0.05 ppm, for the central $CDCl_3$ signal (at δ 78.6 ppm).

Calculation of the average chain length of the fatty alcohol and the average number of moles of ethylene oxide: define the signal at 23.2 ppm as 1.000 and normalise the integrals of the other signals listed in Table 2046.-1.

Limits:

- *average chain length of the fatty alcohol*: 10.0 to 14.0;
- *average number of moles of ethylene oxide*: 7.0 to 11.0.

Table 2046.-1. – *Shift values*

Signal	Shift (ppm)	Normalised integrals
CH_3	14.4	0.989
CH_2 (alkyl chain)	23.2	1.000
CH_2 (alkyl chain)	25.5	1.001
CH_2's (alkyl chain)	30	7.410
CH_2 (alkyl chain)	32.5	0.963
CH_2 (-CH_2-OH) (end CH_2-group of macrogol)	61.6	1.001
CH_2's (macrogol)	70.7	16.25
CH_2 (R-CH-O-macrogol) (CH_2 in alpha position)	72.6	0.998
CH_2 (macrogol)	73.1	0.929

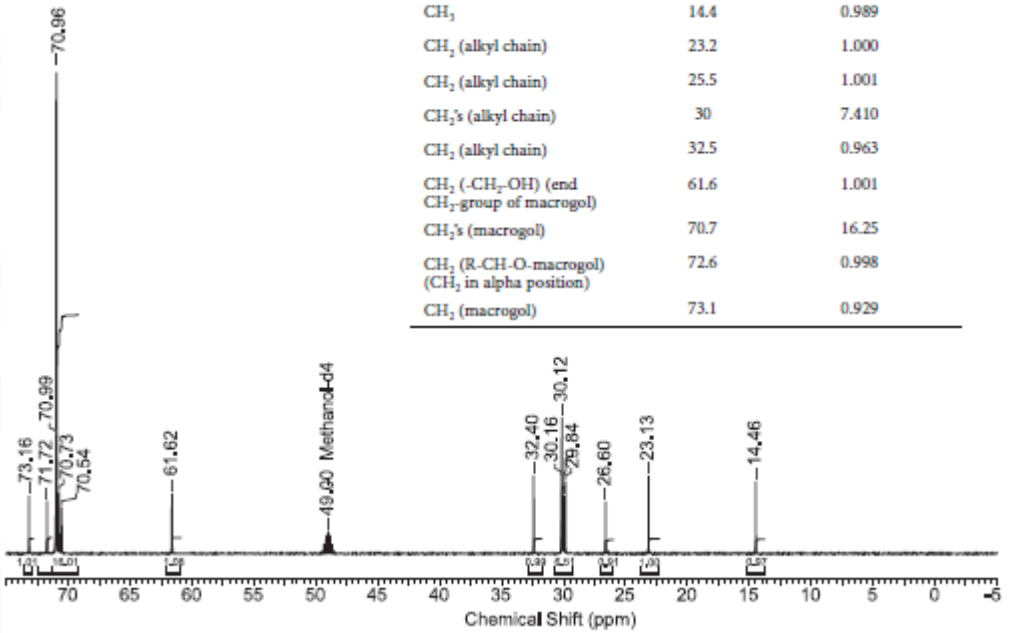
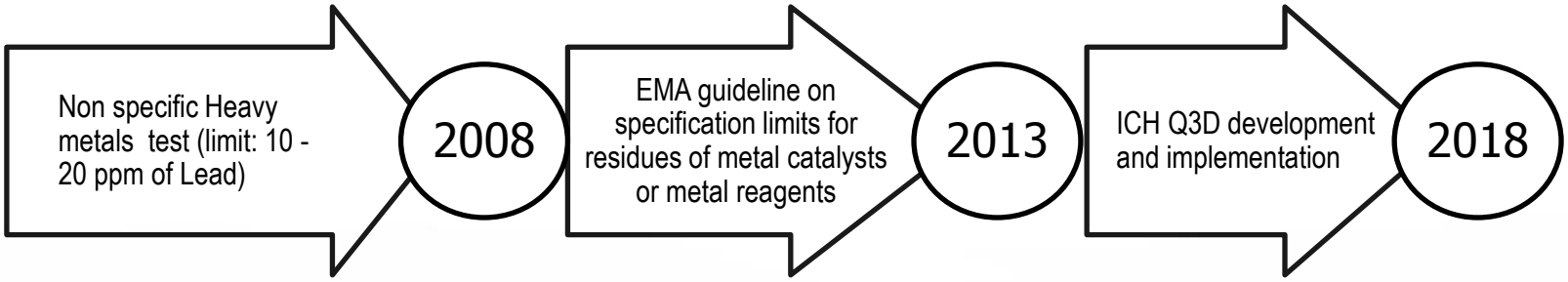


Figure 2046.-1. – *^{13}C NMR spectrum of lauromacrogol 400*

Control of elemental impurities



ICH Q3D Guideline

- elaborated considering human toxicity, route of administration and relying on principles of risk management
- only applies to drug products for human use
- Unless otherwise justified, test procedure should be specific for each elemental impurity identified for control during the risk assessment

Specificity of test procedure

Heavy metals test (2.4.8.) - UNSPECIFIC

Based on precipitation reaction with thioacetamide or sodium sulfide and a visual comparison with a reference containing known amount of lead.

Inductively Coupled Plasma-MS and Atomic Absorption Spectrometry techniques:

- specific to detect and quantify each element
- sensitive i.e. ppb level

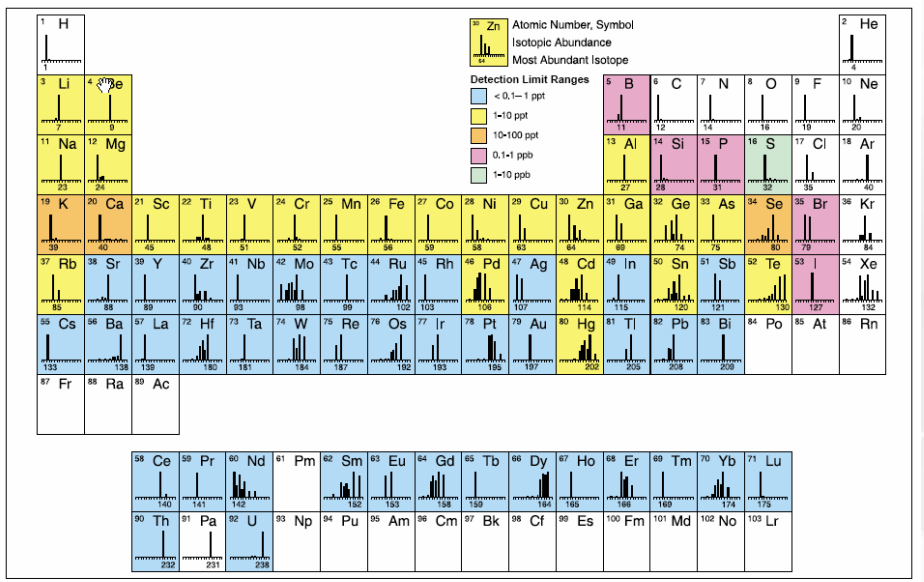


Figure 7. Approximate detection capabilities of the ELAN 6000/6100 quadrupole ICP-MS. (Courtesy of PerkinElmer, Inc.)

Control of elemental impurities cont'd

- Impact on Ph. Eur. Monographs/Chapters:
  - Individual monographs: Heavy metals deleted (except for substances for veterinary use only).
  - Chapter 5.20. "Metal catalyst or metal reagent residues": to be revised to reproduce the principles of ICH Q3D.
  - General method 2.4.20. "Determination of metal catalyst or metal reagent residues" : discussion ongoing for Pharmacopoeial Harmonisation within Pharmacopoeial Discussion Group.
  - General monograph 01/2013: 2619 "Pharmaceutical Preparations" addition of cross reference to Chapter 5.20. to be legally binding for medicinal products in scope of Q3D.

Conclusions

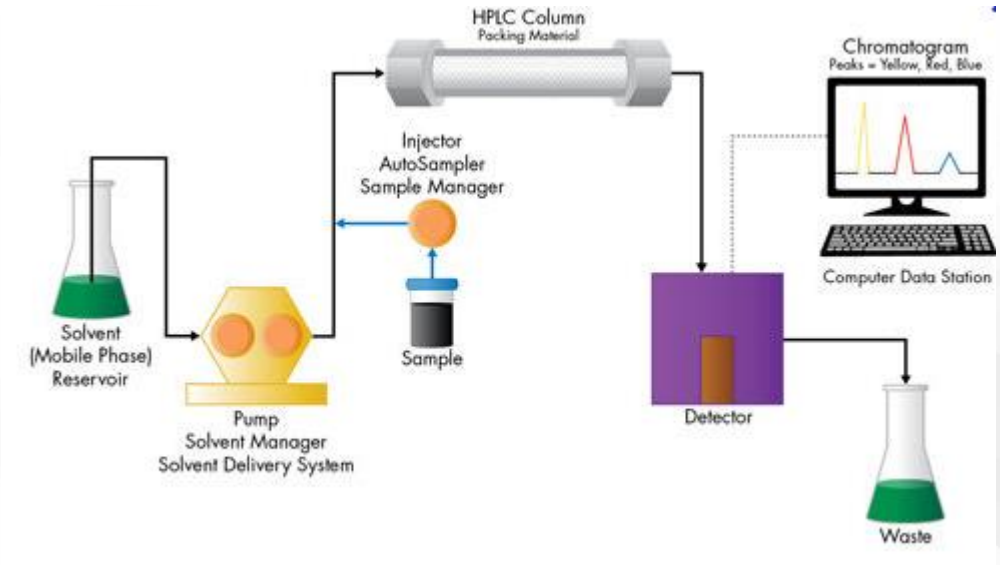
- European Pharmacopoeia and its Reference standards are the *quality standards* legally binding in Member States.
- New and revised monographs are elaborated to reflect recent *innovations* and the '*state-of-the-art'* in analytical methods/techniques.
- Ph. Eur. implementation strategy for the ICH Q3D guideline is under implementation, especially for mined excipients.

GRAZIE PER LA VOSTRA ATTENZIONE

DOMANDE?

BACK-UP SLIDE

Liquid chromatography



Courtesy of Waters ® – Bands, Peaks and Band Spreading