High Resolution LC-MS Beta-Agonist Screening Using TurboFlow/Exactive

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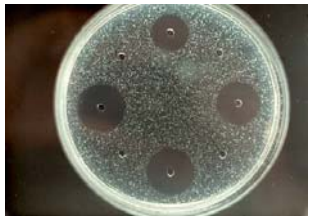
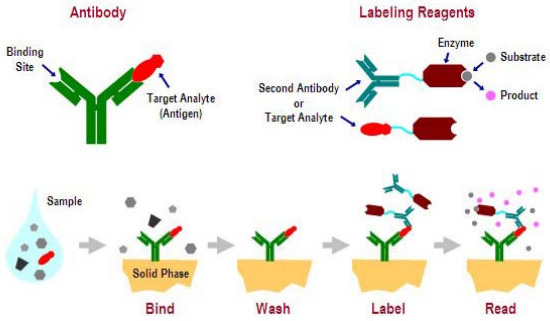
5th Thermo Scientific
High Resolution GC/MS Meeting on POPs
Barcelona, 29-30 April, 2010

Screening methods

- Biological tests



- ELISA



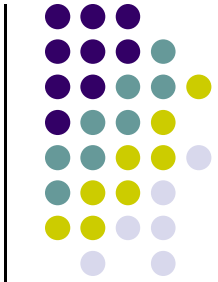
- GC-MS

- LC-MS, LC-MS/MS

- LC-(Q-)TOF/MS

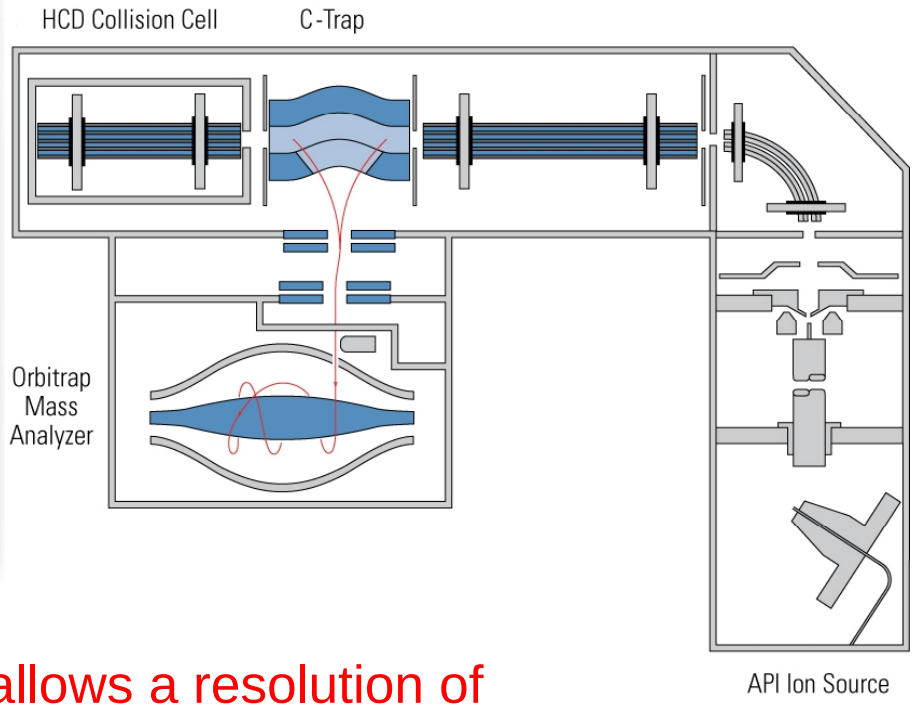
- LC-Orbitrap/MS

LC-MS/MS –
a good screening technique?



- Advantages especially in MRM mode
- However, MRM is target analysis
  ("You see only what you want to see")
  - Despite a large number of reference substances "other" compounds of interest which may occur in large concentrations can be easily overlooked
- For the optimization of the mass transitions and the accurate quantification the availability of the corresponding reference compounds is necessary

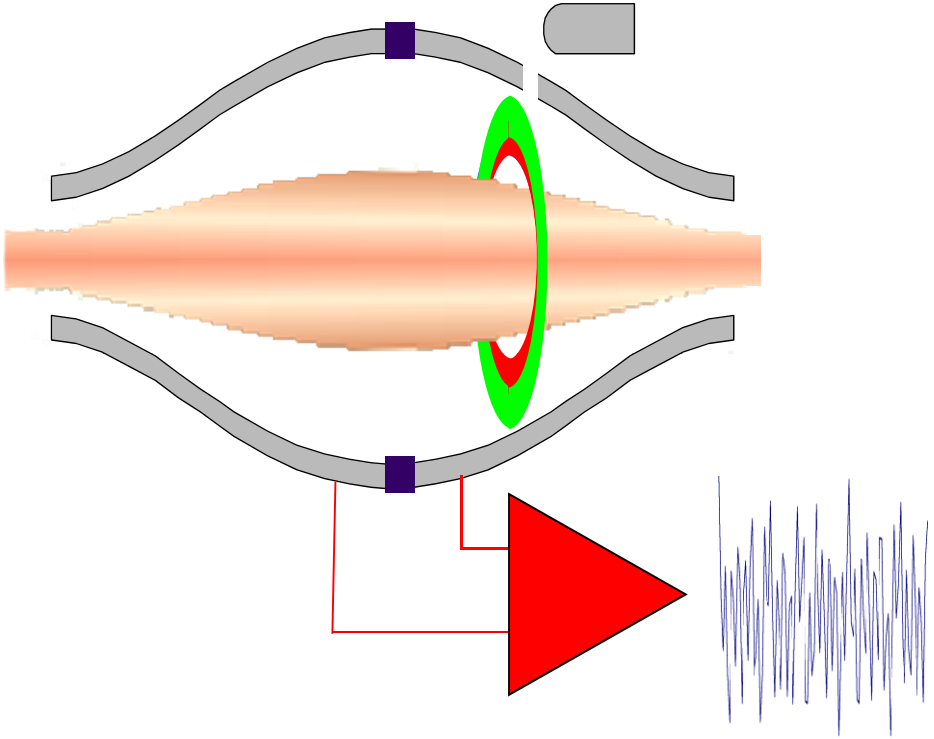
Exactive™ Bench-Top LC-MS-[MS]

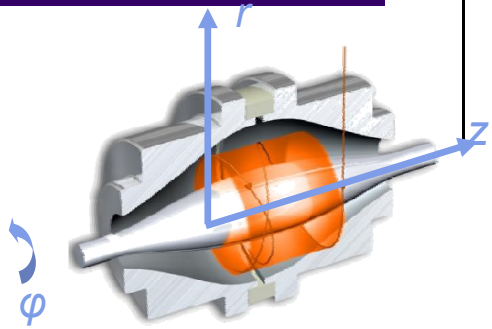


POWERED BY
Orbitrap™
Technology

The Exactive allows a resolution of 100,000 with a mass accurracy <1ppm

Orbitrap – Principle of Operation





Hyper-logarithmic potential distribution: "ideal Kingdon trap"

$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$

- Characteristic frequencies:
  - Frequency of rotation ω_φ
  - Frequency of radial oscillations ω_r
  - Frequency of axial oscillations ω_z

$$\omega_z = \sqrt{\frac{k}{m/q}}$$

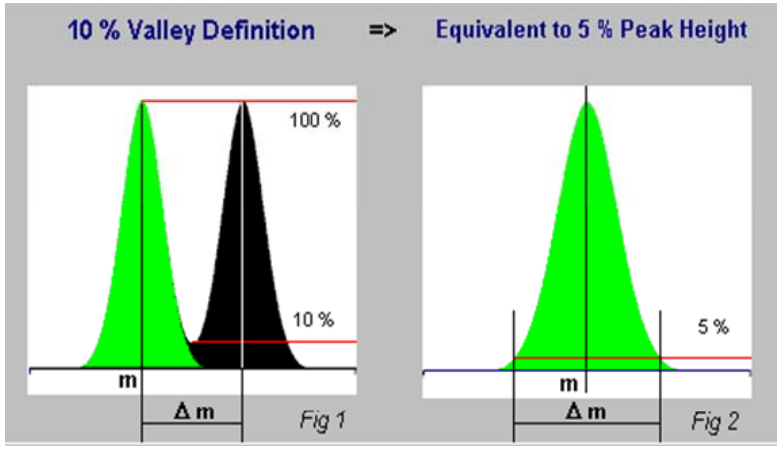
$$\omega_\varphi = \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1} \qquad \omega_r = \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2}$$

Makarov A. *Anal. Chem.* 2000, *72*, 1156-1162.

Courtesy of Thermo Scientific

Resolution Definitions

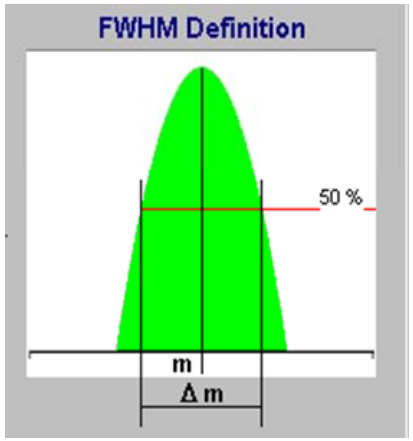
10% Valley



Magnetic Sector

$2\ R_{FWHM} = R_{10\%\ valley}$

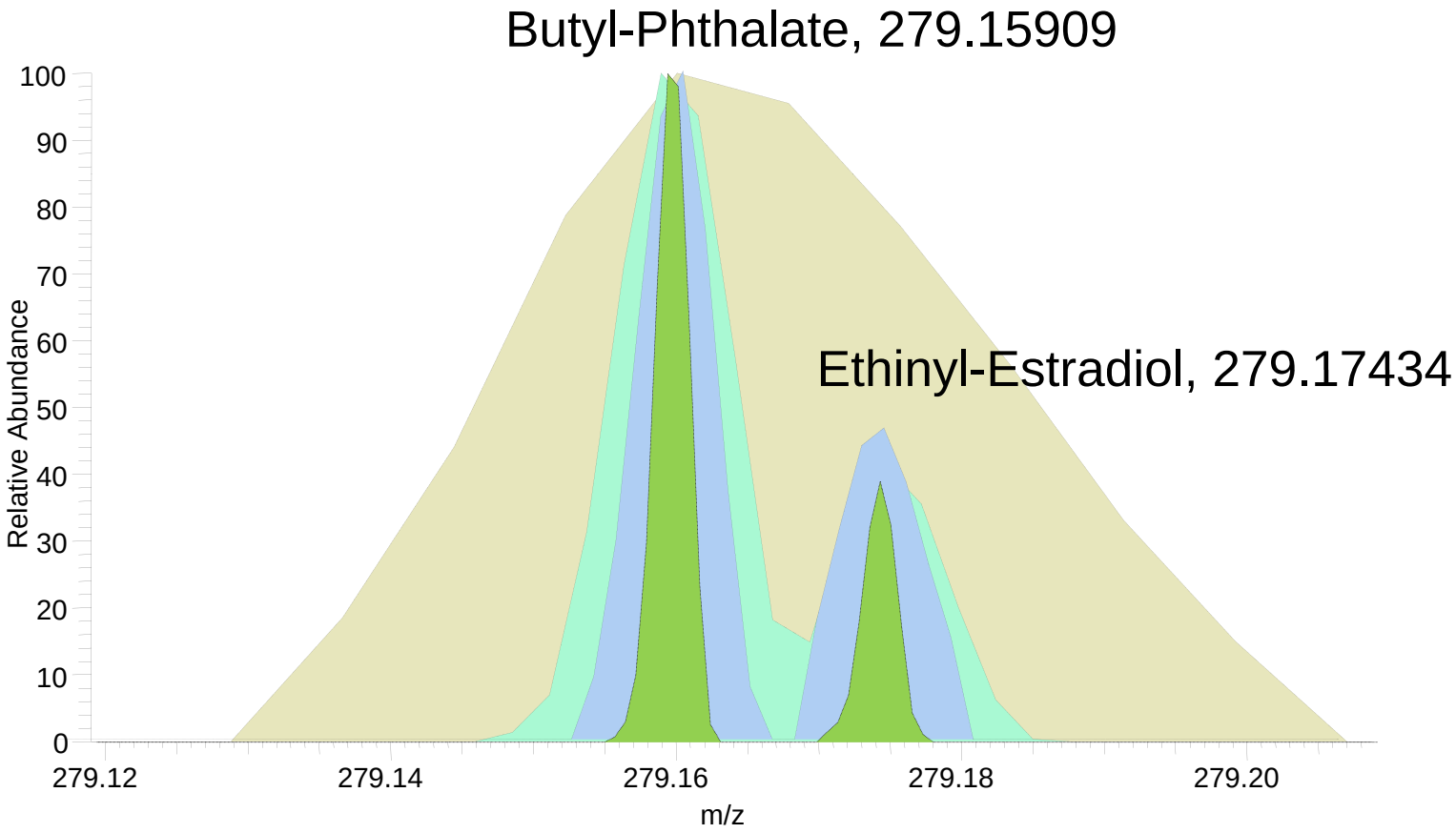
Full Width Half Maximum



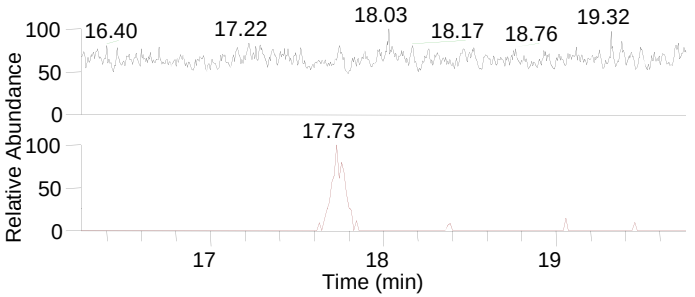
FT ICR
Orbitrap
ToF
Quadrupole

Influence of resolution on mass separation

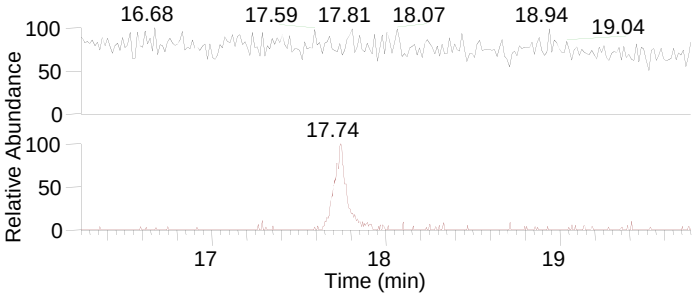
Resolution: 10k, 30k, 50k, 100k



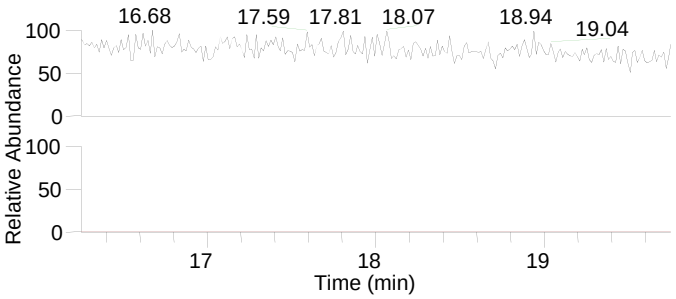
Ethinyl-Estradiol
(Standard: 100 ng/ml)



RP = 100,000

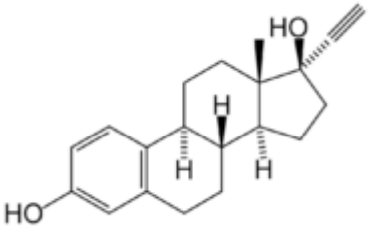


RP = 30,000



RP = 10,000

Ethinyl-Estradiol

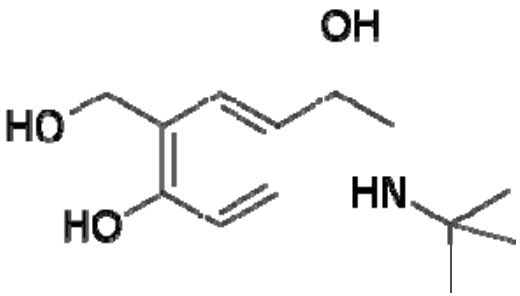


The isobaric phthalate background ion interferes with the Ethinyl-Estradiol ion.

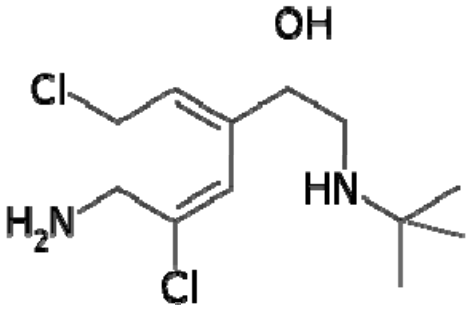
At resolution of 10,000 the steroid mass is shifted because the isobaric ions are not resolved.

beta-agonists

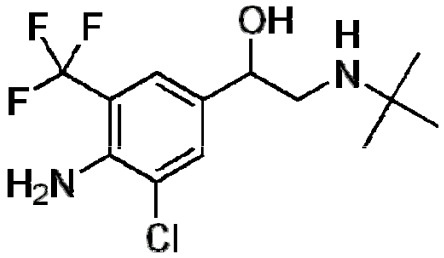
Salbutamol



Clenbuterol

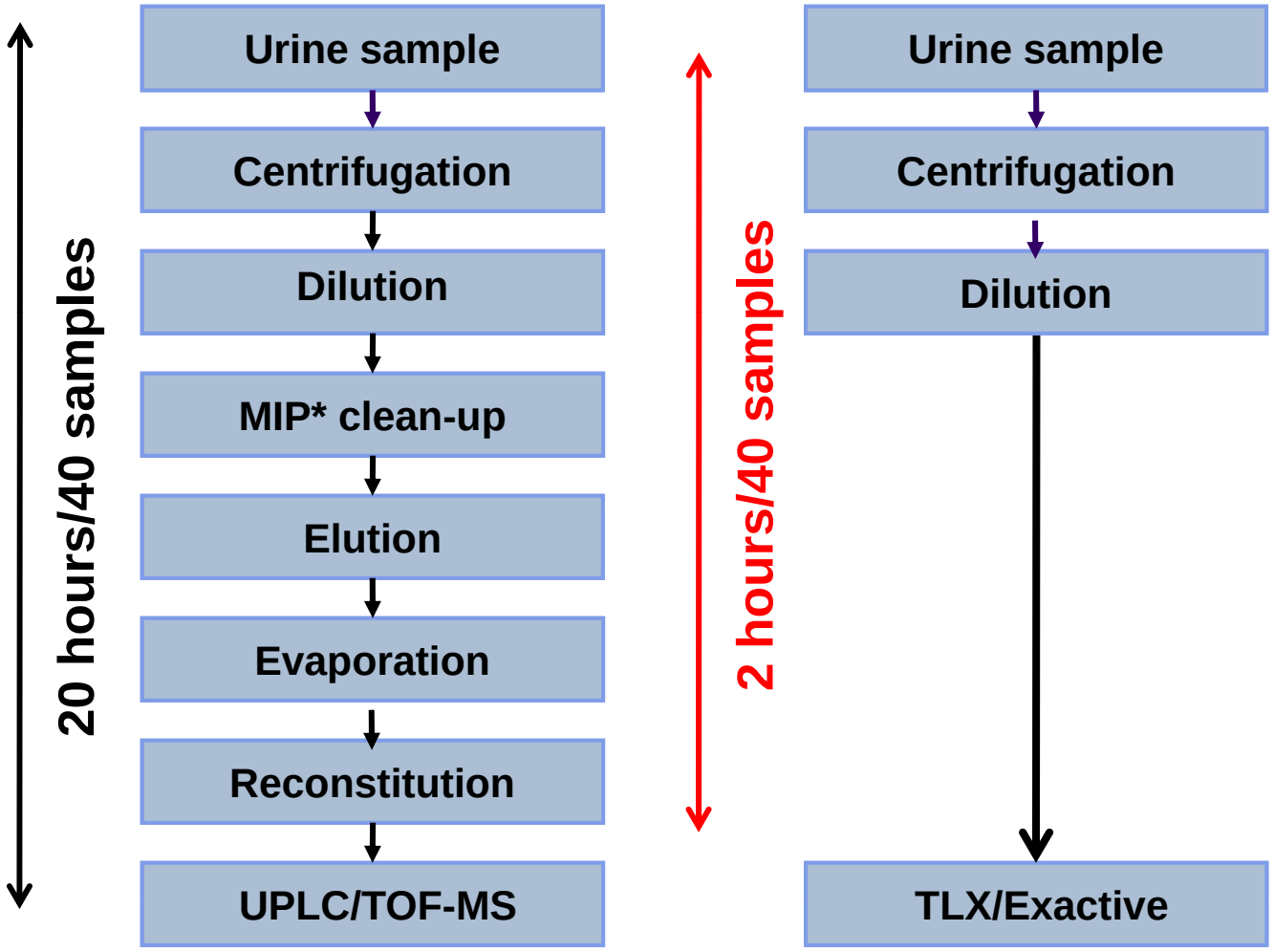


Mabuterol



- are a class of drugs used to treat asthma and other pulmonary diseases
- for growth-promoting purposes in farm animals (illegal)
- different chemical properties e.g. amphoteric
- 24 substances are mentioned

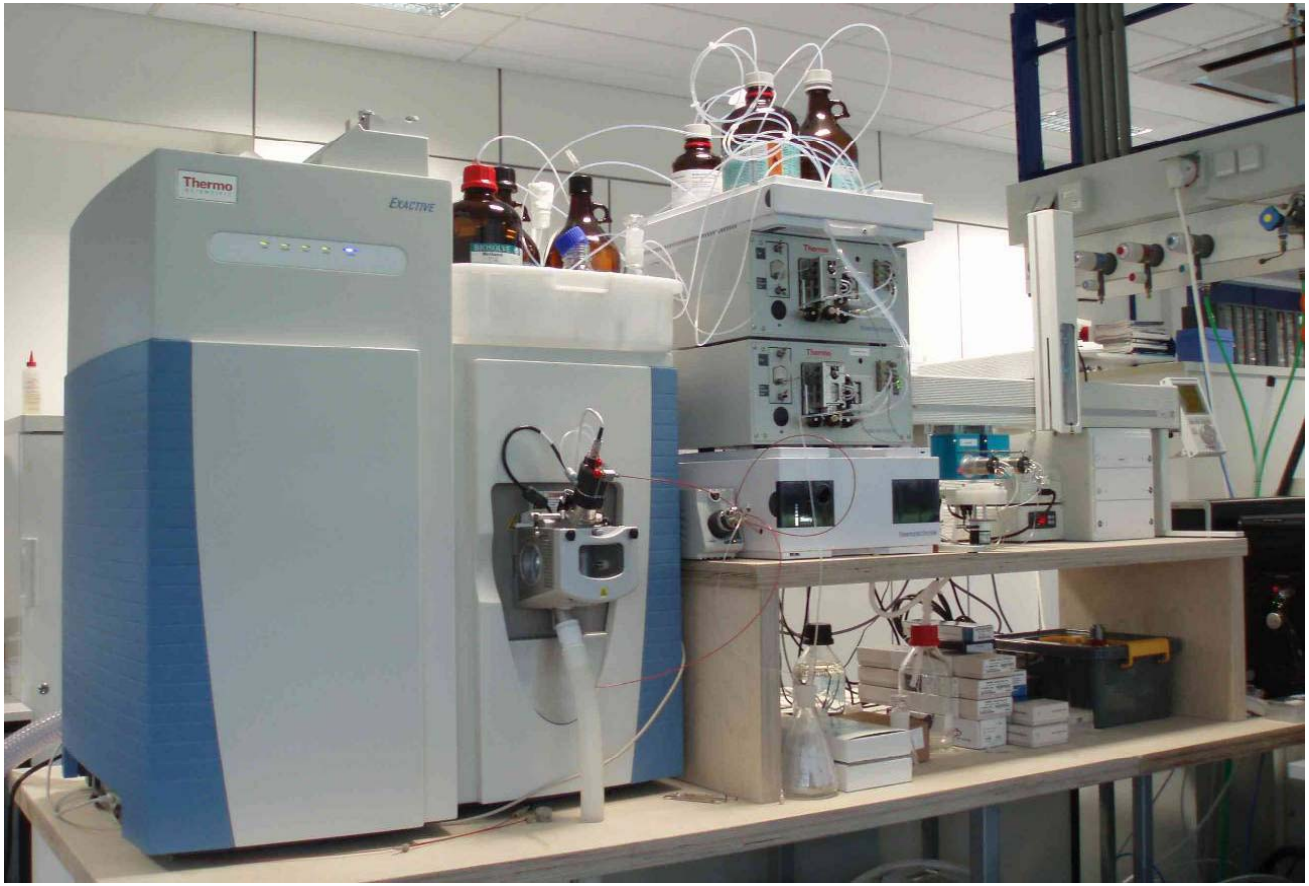
Sample preparation



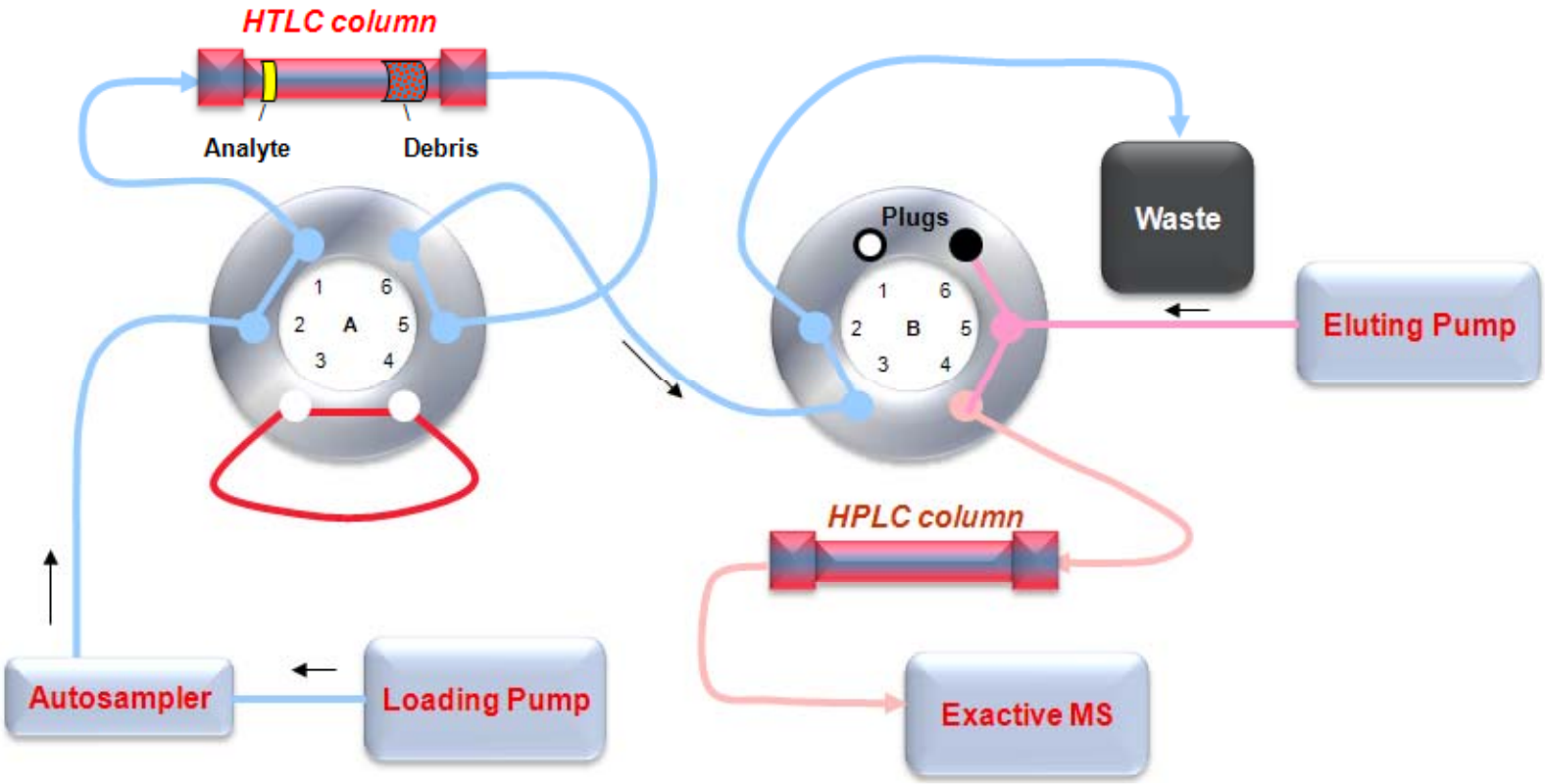
** Molecularly imprinted polymer SPE column*

CVUAs analytical set up

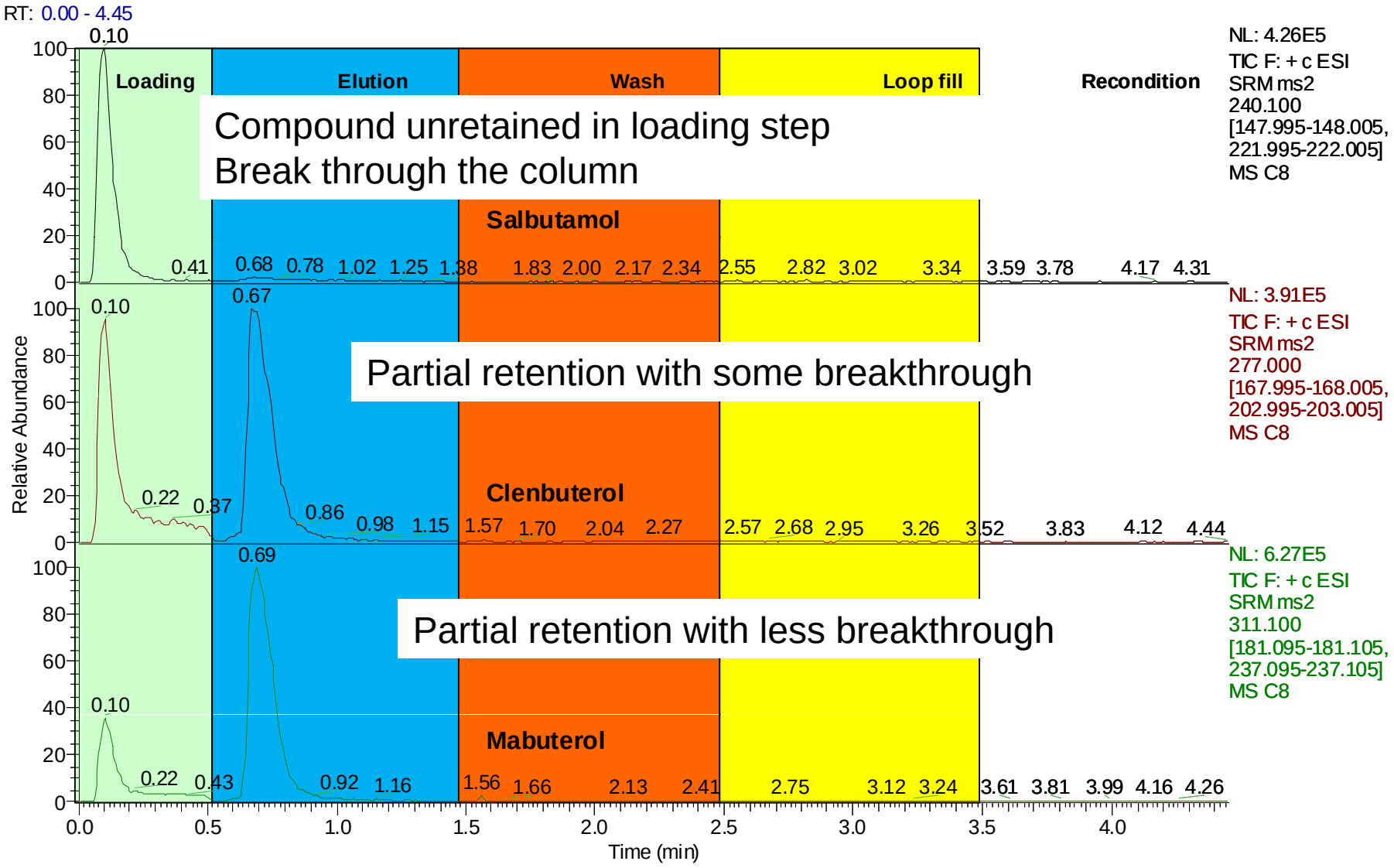
Thermo TurboFlow® coupled with Exactive™ High Resolution LC-MS/MS



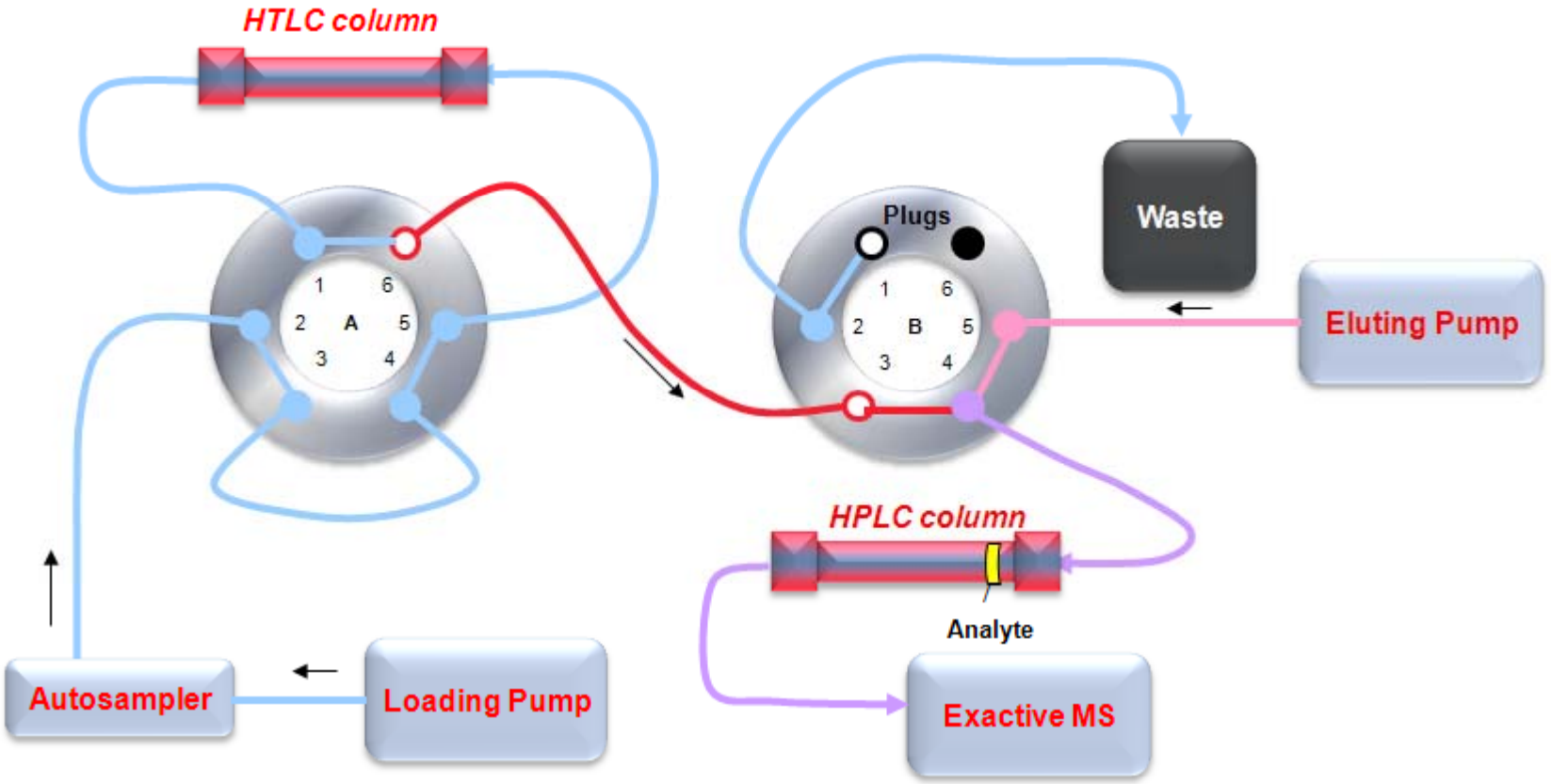
TurboFlow: Loading Step



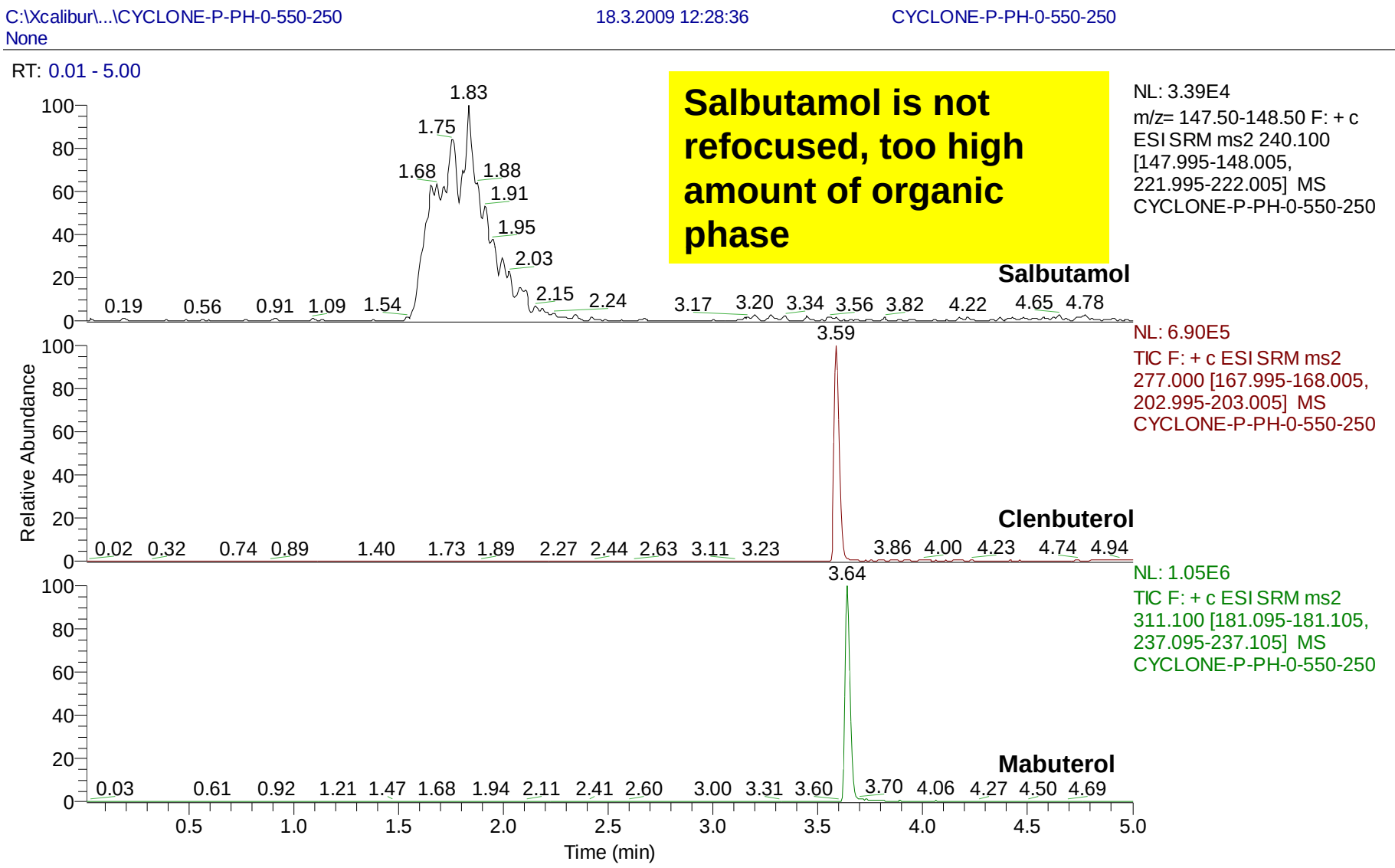
Loading step:
Example of the chromatogram



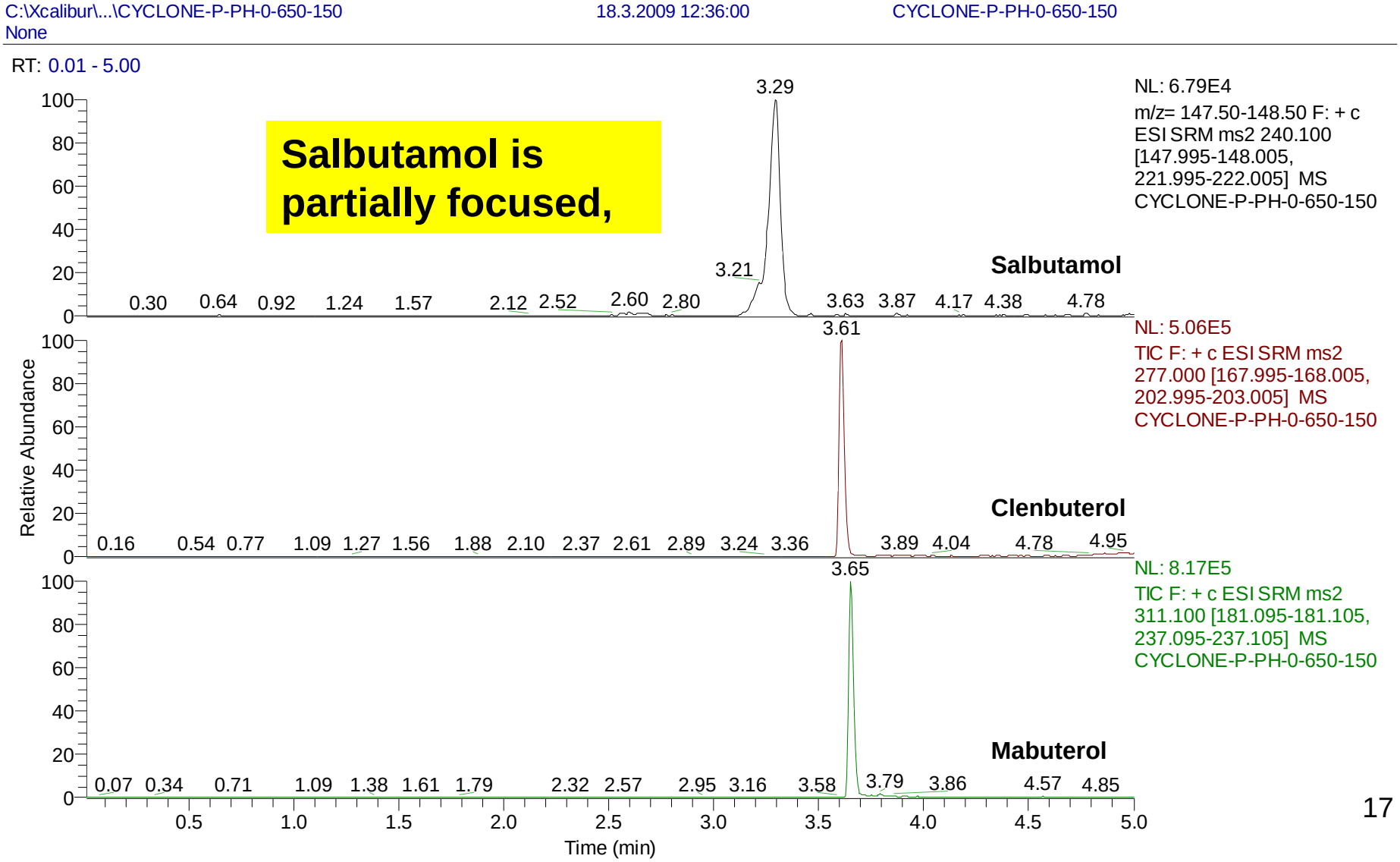
TurboFlow: Transfer/Focusing Step



Transfer to analytical column – 250:550, 0% Methanol



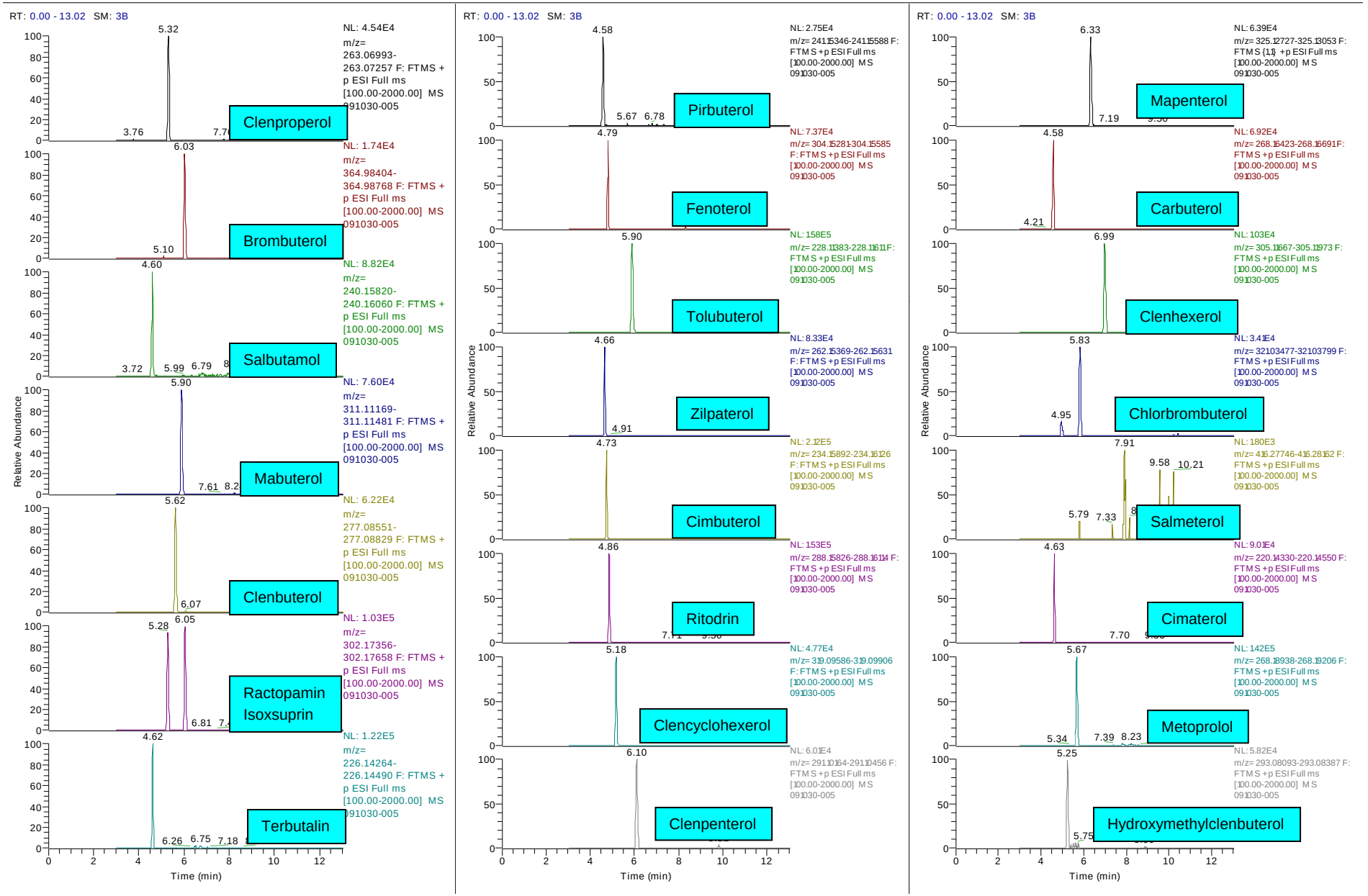
Transfer to analytical column – 100:600, 0% Methanol



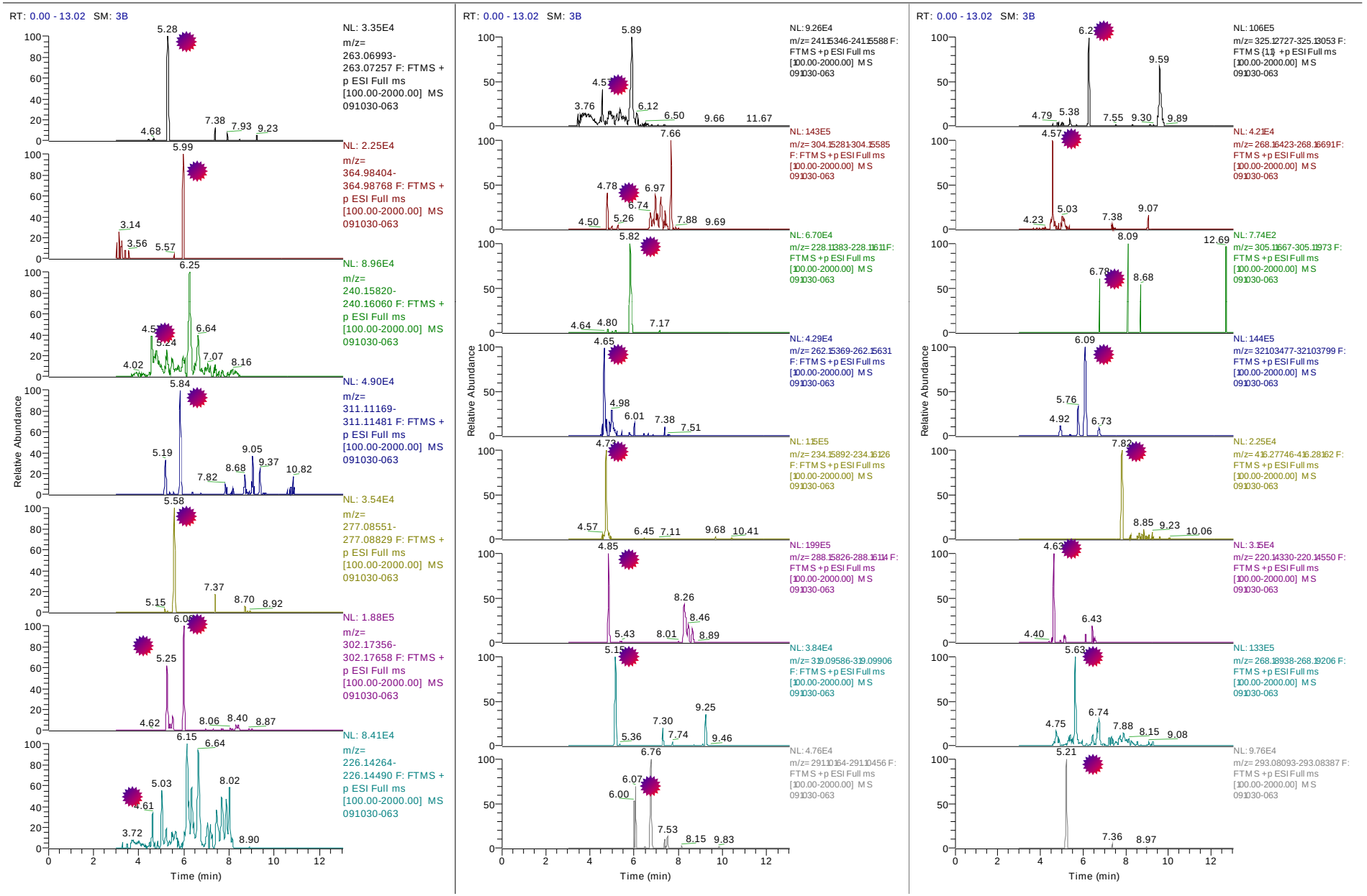
Analytical method

TLX Column	Cyclone-P, 0.5 mm x 50 mm
Loading	10 mM NH_4Ac in Water, pH 8, 1.5 ml/min, 30 s
Elution	Methanol/0.1% acetic acid, 1/1, 0.1 ml/min, 120 s
Wash	Methanol/0.1% acetic acid, 8/2, 1.5 ml/min, 60 s Acetonitrile/acetone/isopropanol, 3/3/4
HPLC	Column: Phenyl Column 4.6 x 100 mm Gradient: A: Methanol, B: 0.1% acetic acid from 0% B to 95 % B in 300 s

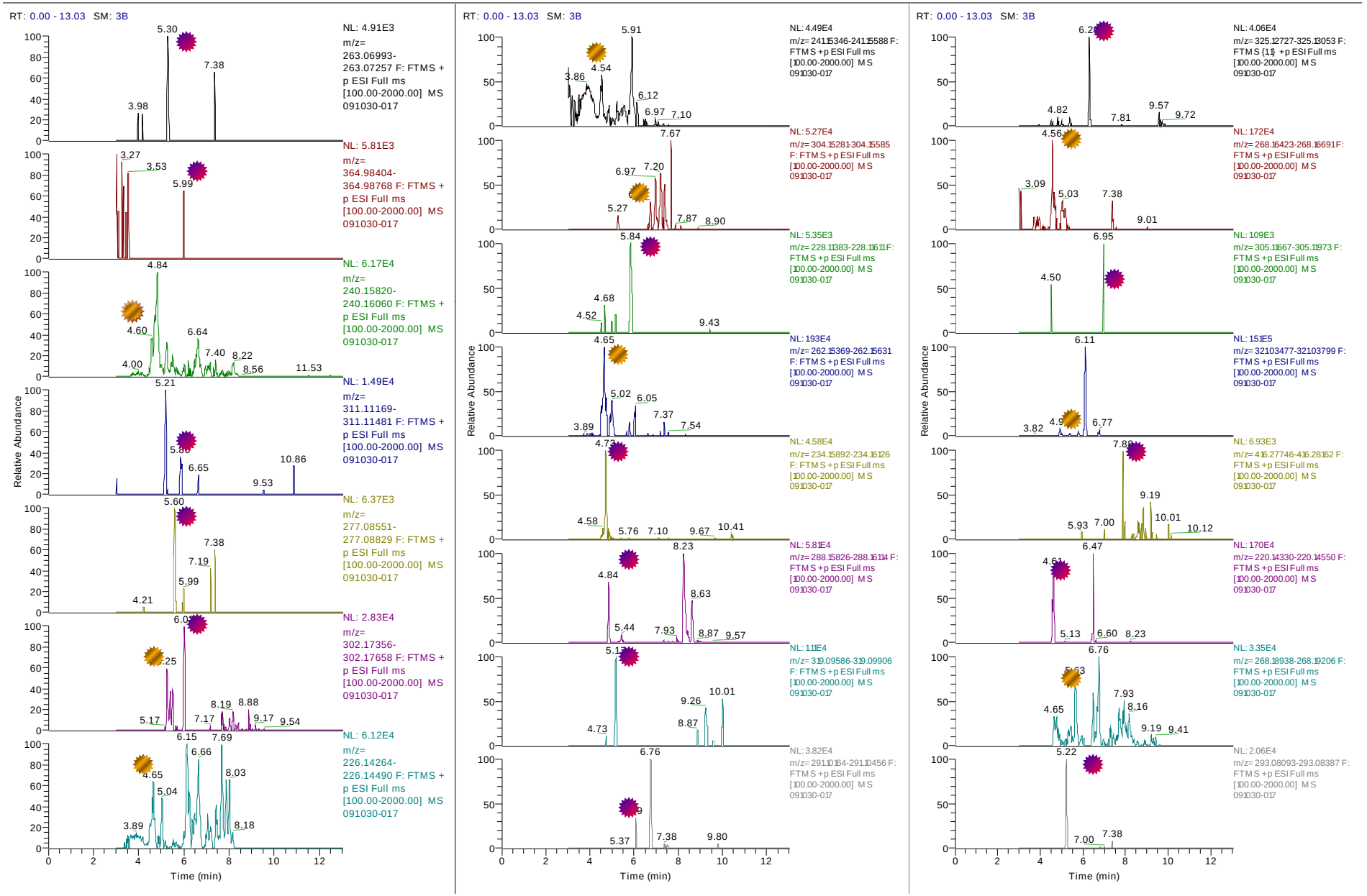
24 beta-agonists (standard; each 0.25 ng/ml)



beta-agonists in urine (spike: 1.0 ng/ml)



beta-agonists in urine (spike: 0.25 ng/ml)



Thermo LCquan - 090902

File Zoom View Apps Options Help

Instruments
Acquisition
Explore
Quantitate

Method
Sequence
Survey
Review All
Reports

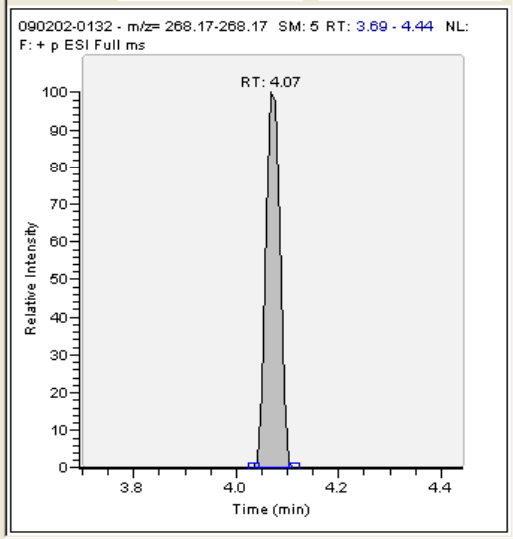
Review All Results - review standards, QCs, blanks and unknowns

	FileName	Sample ID	Sample Typ	Integration	Respo	ISTD Response	Response Ratio	Specified Conc	Calculated Conc	% Diff	% RSD
1	090202-0132	Urin 0.1-01	Standard	Method S	10966	NA	10966.217	0.100	0.085	-15.30	20.79
2	090202-0133	Urin 0.1-02	Standard	Method S	10771	NA	10771.123	0.100	0.083	-16.65	20.79
3	090202-0134	Urin 0.1-03	Standard	Method S	16427	NA	16427.022	0.100	0.123	22.53	20.79
4	090202-0135	Urin 0.1-04	Standard	Method S	13485	NA	13484.827	0.100	0.102	2.15	20.79
5	090202-0136	Urin 0.1-05	Standard	Method S	7714	NA	7713.508	0.100	0.062	-37.83	20.79
6	090202-0137	Urin 0.1-06	Standard	Method S	15336	NA	15335.605	0.100	0.115	14.97	20.79
7	090202-0138	Urin 0.1-07	Standard	Method S	16971	NA	16970.564	0.100	0.126	26.29	20.79
8	090202-0139	Urin 0.1-08	Standard	Method S	14080	NA	14079.536	0.100	0.106	6.27	20.79
9	090202-0140	Urin 0.1-09	Standard	Method S	18227	NA	18226.540	0.100	0.135	34.99	20.79
10	090202-0141	Urin 0.1-10	Standard	Method S	13845	NA	13844.663	0.100	0.105	4.64	20.79
11	090202-0142	Urin 0.1-11	Standard	Method S	14340	NA	14339.739	0.100	0.108	8.07	20.79
12	090202-0143	Urin 0.1-12	Standard	Method S	12592	NA	12591.644	0.100	0.096	-4.04	20.79
13	090202-0144	Urin 0.1-13	Standard	Method S	16801	NA	16800.781	0.100	0.125	25.12	20.79
14	090202-0145	Urin 0.1-14	Standard	Method S	16594	NA	16594.055	0.100	0.124	23.69	20.79
15	090202-0146	Urin 0.1-15	Standard	Method S	14749	NA	14749.050	0.100	0.111	10.91	20.79
16	090202-0147	Urin 0.1-16	Standard	Method S	10619	NA	10619.373	0.100	0.082	-17.70	20.79
17	090202-0148	Urin 0.1-17	Standard	Method S	10040	NA	10039.813	0.100	0.078	-21.71	20.79
18	090202-0149	Urin 0.1-18	Standard	Method S	8218	NA	8217.683	0.100	0.066	-34.34	20.79
19	090202-0150	Urin 0.1-19	Standard	Method S	14366	NA	14366.350	0.100	0.108	8.25	20.79
20	090202-0151	Urin 0.1-20	Standard	Method S	17730	NA	17729.568	0.100	0.132	31.55	20.79
21	090202-0153	Urin 0.25-01	Standard	Method S	36718	NA	36718.130	0.250	0.263	5.23	8.61
22	090202-0154	Urin 0.25-02	Standard	Method S	28963	NA	28963.414	0.250	0.209	-16.25	8.61
23	090202-0155	Urin 0.25-03	Standard	Method S	32929	NA	32929.079	0.250	0.237	-5.26	8.61
24	090202-0156	Urin 0.25-04	Standard	Method S	34352	NA	34352.264	0.250	0.247	-1.32	8.61
25	090202-0157	Urin 0.25-05	Standard	Method S	35579	NA	35579.479	0.250	0.255	2.08	8.61

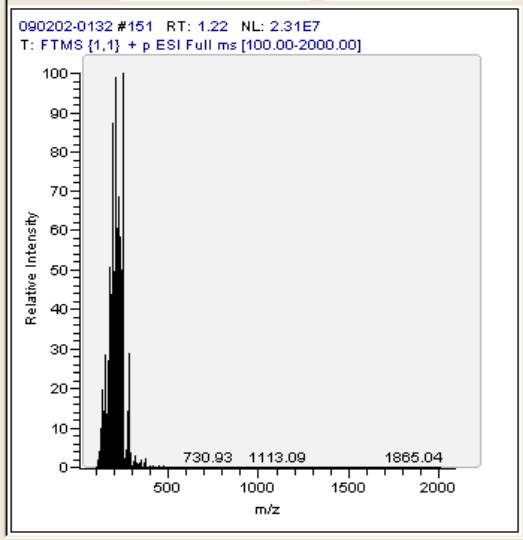
All | Standards | QCs | Blanks | Unknowns

- Carbuterol
- Pirbuterol
- Salbutamol
- Terbutalin
- Cimaterol
- Zilpaterol
- Cimbuterol
- Fenoterol
- Ritodrin
- Clencyclohexerol
- Isoxsuprine
- Hydroxymethylclenbuterol
- Clenproperol
- Clenbuterol
- Netoprolol
- Chlorbrombuterol
- Mabuterol
- Tulobuterol
- Ractopamin
- Brombuterol
- Clenpenterol
- Mapenterol
- Clenhexerol
- Salmeterol

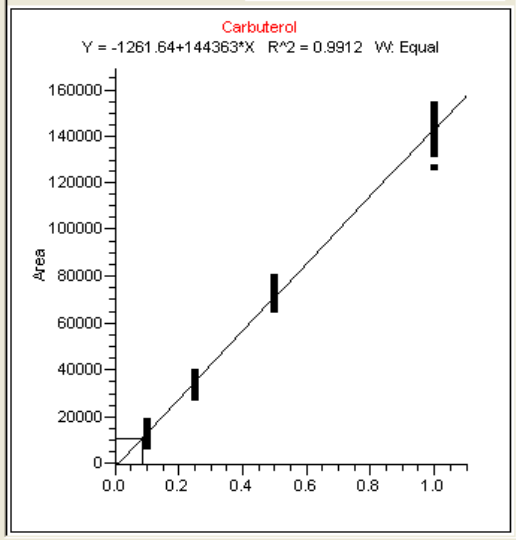
Selected | Chromatogram



Component | Spectrum



Calibration Curve



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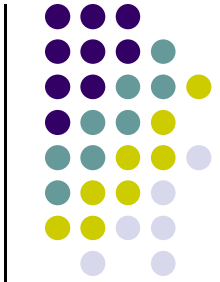
9/11/2009 2:30 PM

Validation parameters



Compound name	CCα (µg/l)	CCβ (µg/l)	Repeatability RSD (%) (0.5ppb)	In-house repro-ducibility RSD (%) (0.5ppb)	Recovery (%)
Brombuterol	0.19	0.46	20	24	97
Carbuterol	0.22	0.58	25	27	100
Chlorbrombuterol	0.14	0.38	14	26	99
Cimaterol	0.13	0.35	15	19	100,0
Cimbuterol	0.08	0.20	9	13	100
Clenbuterol	0.14	0.33	13	25	98
Clencyclohexerol	0.08	0.21	9	12	100
Clenhexerol	0.22	0.49	22	23	98
Clenpenterol	0.20	0.49	22	25	99
Clenproperol	0.10	0.24	11	15	100
Fenoterol	0.11	0.24	11	14	99
Hydroxymethylclenbuterol	0.09	0.21	9	14	100
Isoxsuprine	0.10	0.25	11	18	100
Mabuterol	0.09	0.23	9	17	100

Many thanks to

Michal Godula
Thermo Fisher Scientific

and our team

Thank you for your attention