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Automated and simplified two Dimensional LC and High-Resolution MS approach for the identification and characterization of unknown impurity in Mirabegron tablets

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Introduction

Increase in regulatory demands of pharmaceutical industry requires the stringent levels of detection and identification of impurities in pharmaceutical drug substances and products. One dimensional (1D) liquid chromatography (LC) has been widely adopted in the separation and identification of pharmaceutical compounds. However, this technique has proven to have limitations in selectivity and peak capacity when analysing chemically complex samples. Two dimensional LC coupled with High resolution mass spectrometer has evolved as more automated and simplified tool for the characterization of unknown impurities in the recent times without changing the Non-volatile mobile phase conditions of HPLC method.

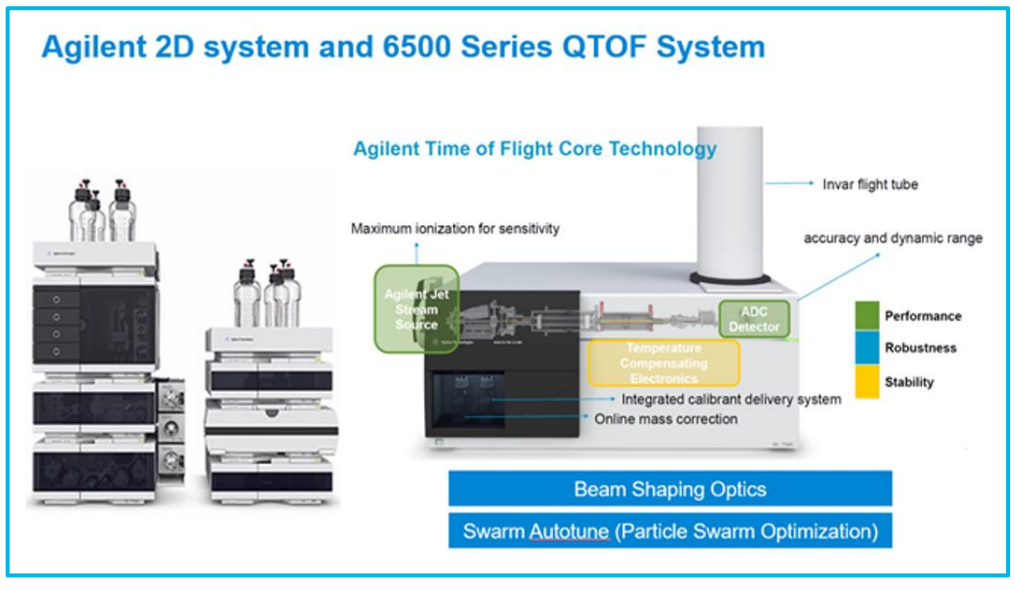
The use of 2D-LC/MS can serve as an additional analytical tool providing enhanced peak capacity and selectivity and thus superior assessment of peak purity and impurity identification. With the increasing complexity and regulatory expectations, . A novel and simple multiple heart cutting based 2DLC and High-Resolution Mass Spectrometry workflow used in the presented work for the identification and structure elucidation of unknown impurity in Mirabegron tablets.

Instrumentation

The Agilent 1290 Infinity II 2D-LC System coupled to an Agilent 6545 LC/Q-TOF was composed of the following modules:

1290 Infinity II high-speed pump (G7120A)
1290 Infinity II Flexible pump (G7104A)
1290 Infinity II Multi sampler (G7167B)
Two 1290 Infinity II multicolumn thermostat (G7116B)
1260 Infinity II Diode array detector WR (G7115A)
1290 Infinity II Diode array detector FS (G7117A)
Agilent 2D-LC Valve with multiple heart cutting set up

Table 1: Instrumentation detail



Experimental

First-dimension pump	
Mobile Phase A	Ammonium acetate Buffer with non volatile Ion pairing reagent ; pH 6.4
Mobile Phase B	Acetonitrile: Methanol (100:900)v/v
Mode	Gradient
Flow rate	1.5 ml/min
Second- dimension pump	
Solvent A	10mM ammonium acetate (pH 6.4)
Solvent B	Acetonitrile
Flow rate	0.5ml/min
2D-Gradient	T 0 2 4 6 8 8.1 9 %B 10 10 25 45 45 10 10
1D run time	70 minutes
2D gradient stop time	9 min (each cut)
2D cycle time	10 min (each cut)
Multisampler	
Injection volume	10µL
Thermostatted column compartment	
1D	45°C
2D	55°C
Multiple heart-cutting	
Mode	Time-based multiple heart-cutting
Detector	
1D & 2D	UV, 235nm

Table 2: 2D-LC Method Conditions

MS Conditions

System	6545 LC/QTOF System
Gas Temp	250 °C
Drying Gas	14 L/min
Nebulizer	35 psi
Sheath Gas Temp	375°C
Sheath Gas Flow	12 L/min
Capillary	3500 V
Nozzle Voltage	300 V
Fragmentor	200 V

Table 3: MS conditions

Results and Discussion

1. • Methanol based sample extraction of an impurity used for sample preparation.

2. • Impurities in the tablet were separated on 1290 Infinity II UHPLC and 2D- LC/MS analysis was performed

3. • Multiple hearting cutting valve used to divert the entire impurity peak from 1st dimension column to 2nd dimension.

4. • Accurate mass MS and MSMS data acquired for impurity using 6545 LC/Q-Tof MS.

5. • Identification of impurities using Agilent Mass Hunter software-based algorithms like molecular feature extraction to extract the molecules from raw data

6. • Molecular formula generation tool was used to generate the molecular formula and Data processing using Molecular Structure Correlation software.

- Molecular formula generated for impurity with mass accuracy less than 2ppm and optimized MSMS data with abundant fragment ions followed by data processing with Molecular structure correlator (MSC) software enabled the more accurate prediction of structure.

- Molecular structure correlator software used along with chemspider database to identify and correlate the possible structure with the help of experimental fragmentation spectra

- By employing 2D-LC/MS approach, we obtain accurate mass and empirical formulae of unknown impurities and can further propose possible chemical structures which can be confirmed by other techniques like NMR.

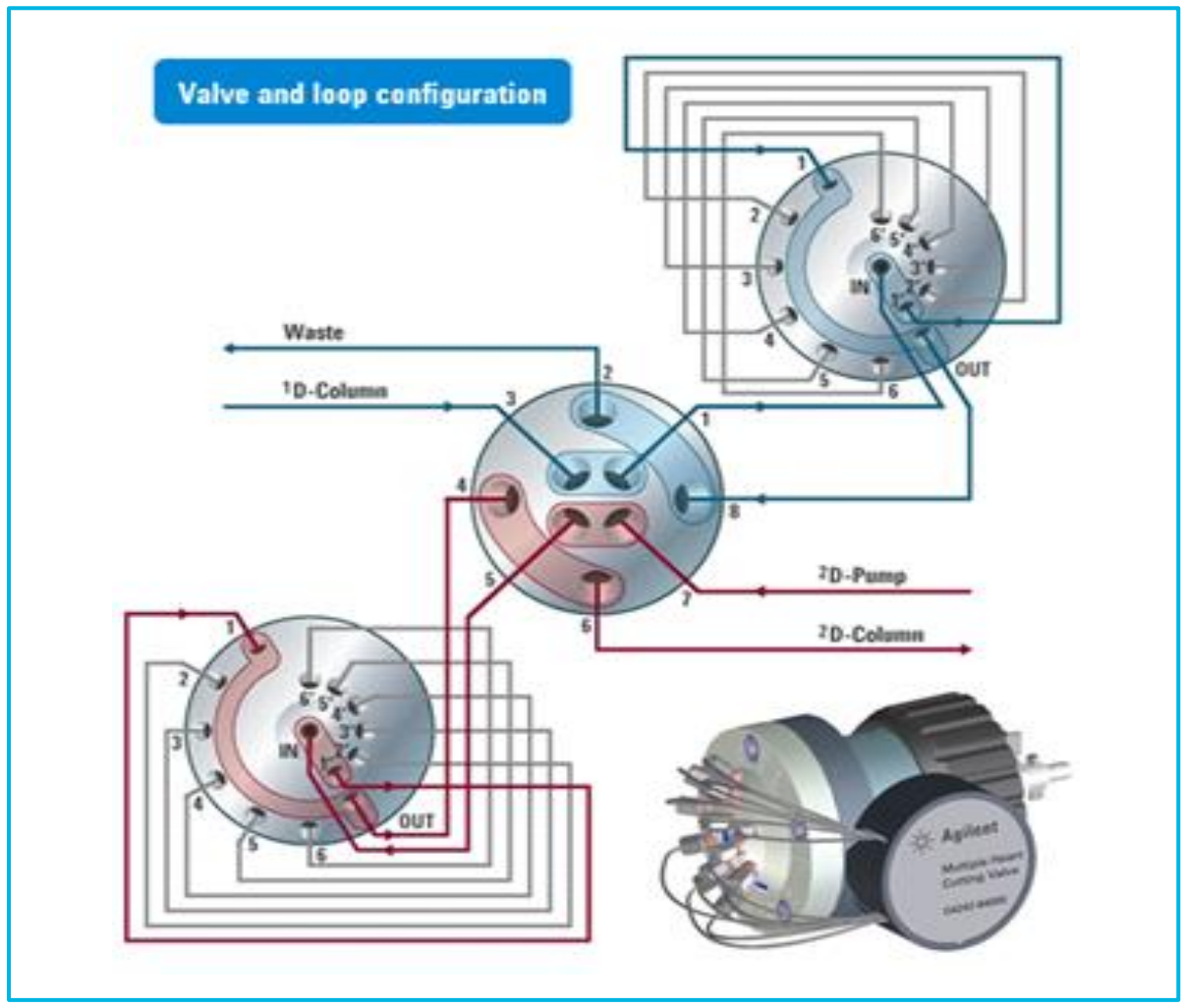


Figure 2: 2D-LC Valve with Multiple Heart Cutting Decks

2D-LC UV Data:

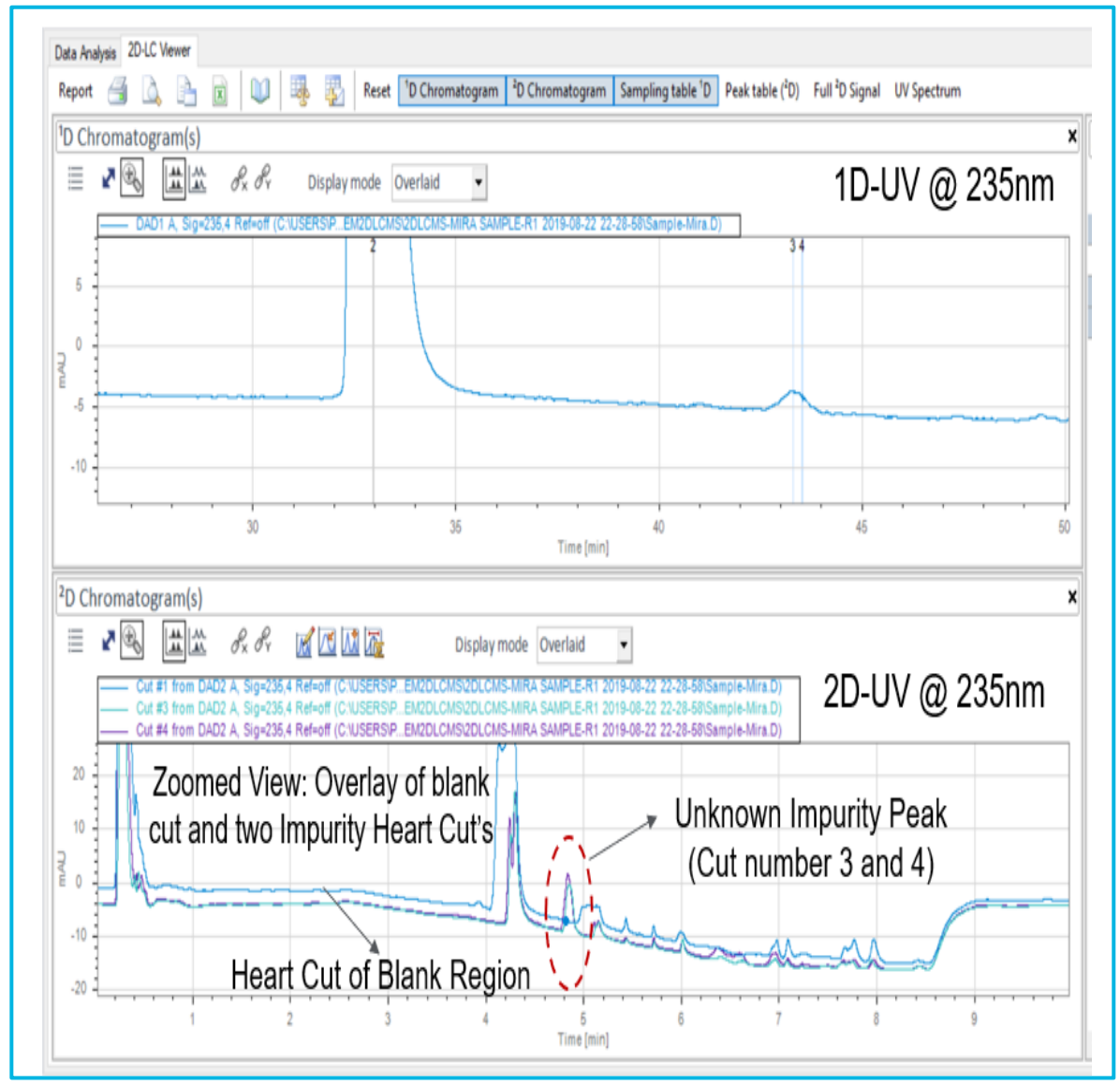


Figure 3: 1D & 2D UV Chromatograms

Results and Discussion

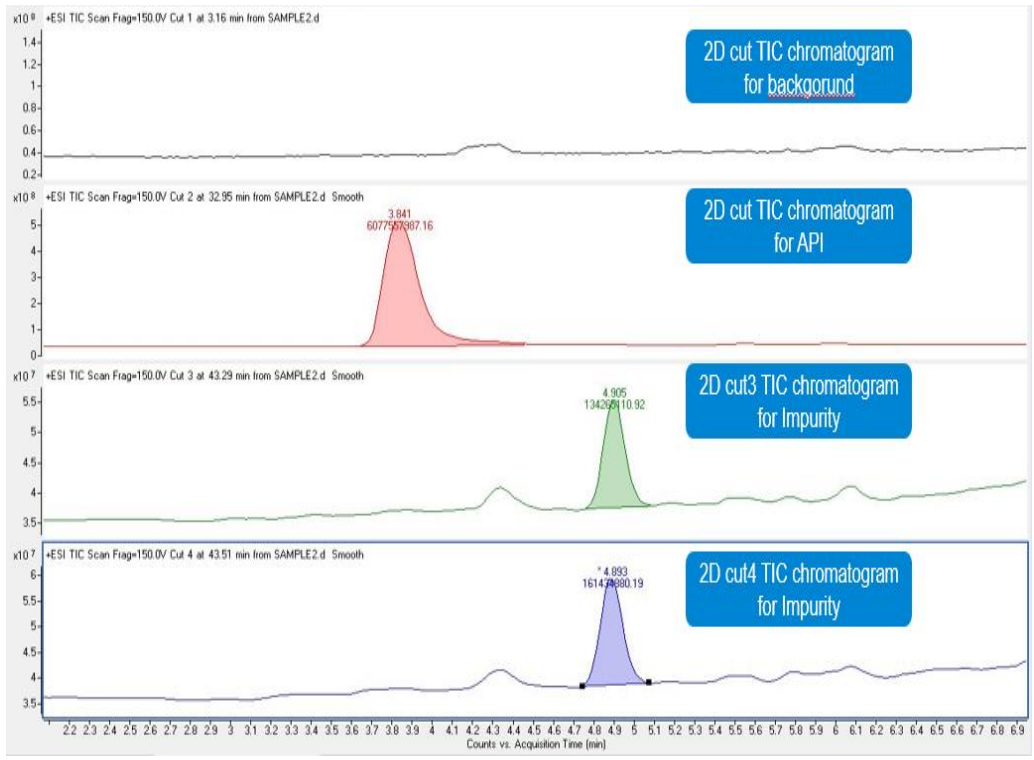
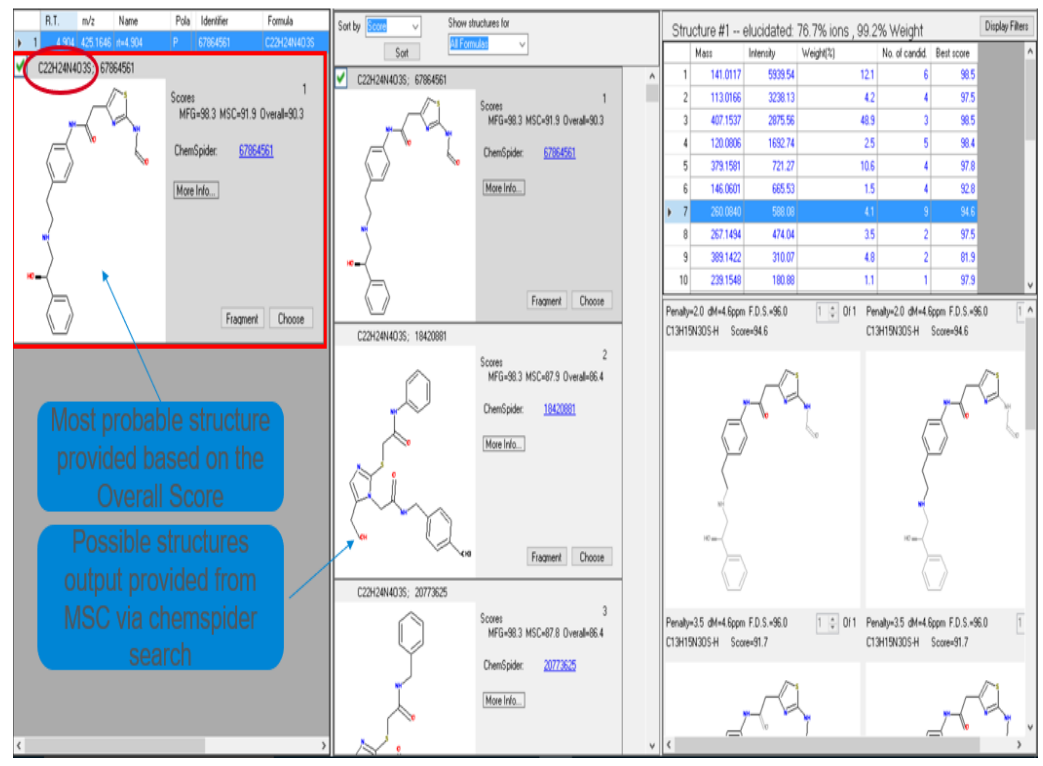


Figure 4: MS Total Ion chromatogram in ESI positive mode



MSC proposes possible matches by correlating accurate mass/formula of experimental MS/MS with in-silico fragment ions from a structural database (e.g.chemspider).

Figure 6: Proposed Impurity Structure using Molecular structure correlator (MSC) software

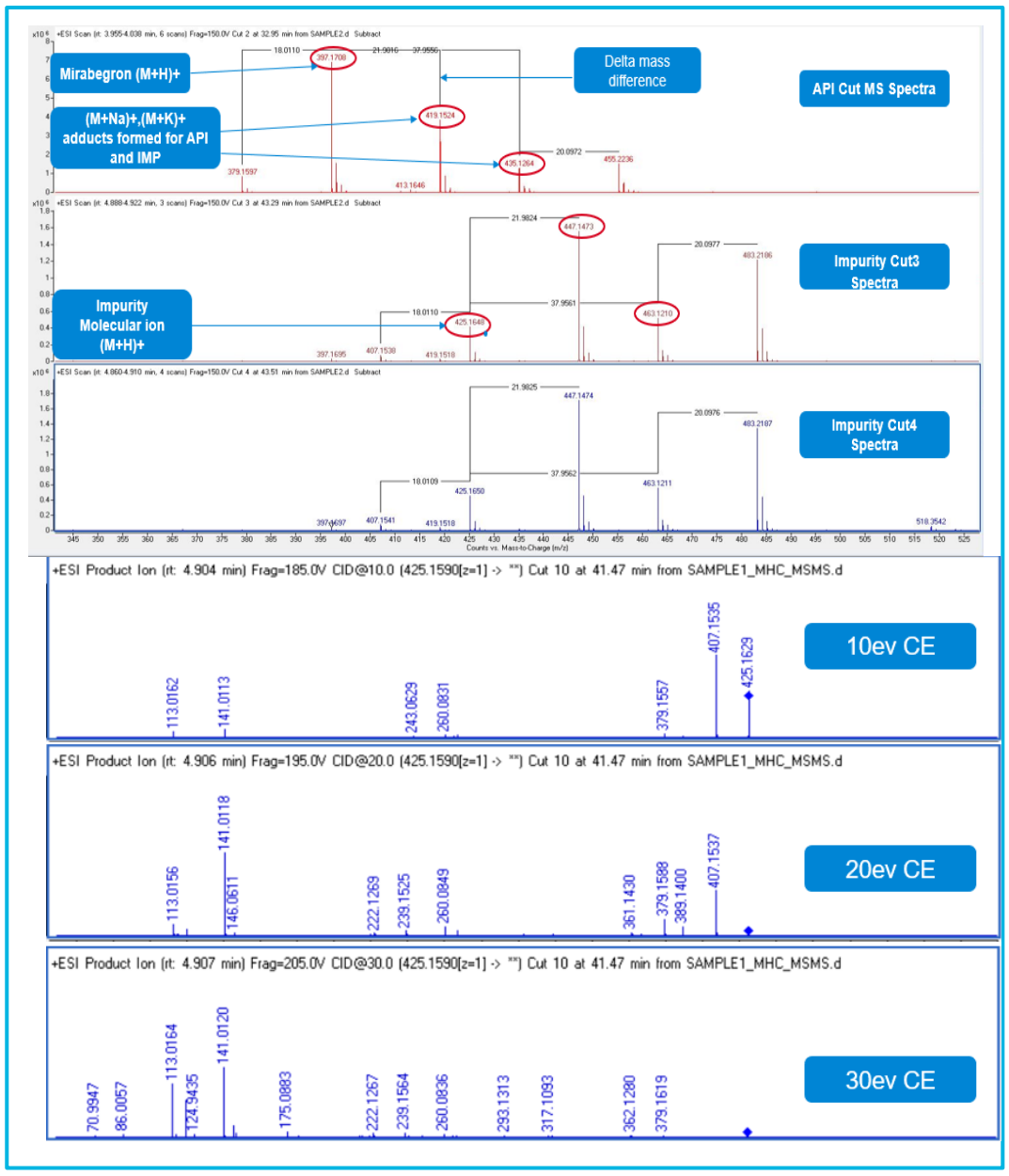


Figure 5: High Resolution MS Spectra of API and Impurity cuts of 2D LC and optimized High resolution MS/MS Spectra of Impurity using different Collison energies in ESI Positive mode

Conclusions

- 2D-LC/MS is proven to be a useful technique for Peak Purity Analysis especially when coupled to a MS and using an orthogonal method on the 2nd Dimension. It provides enhanced peak capacity and selectivity. It can provide mass spectral information for methods that are not MS compatible with a single dimension.
- 2D-LC MS/MS workflow presented here is simple and automated and can be efficiently used by Non expert users during their routine challenges of unknown impurity identification.

References

- Heart-cut 2D-LC/MS approach for pharmaceutical impurity identification using an Agilent 6540 Q-TOF LC/MS System .Agilent Technologies application Note:5991-1873 EN
- Two-Dimensional Liquid Chromatography (2D-LC) in Pharmaceutical Analysis: Applications Beyond Increasing Peak Capacity. Chromatographia (2018) 81:401–418

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