Xevo TQ-XS

ACQUITY UUPLC I-Class System (SM-FTN)?

Supplier	Waters
Year of acquisition	2018
Price	12.5 milion CZK
Financing	OP VVV CORE FACILITIES CZ.02.1.01/0.0/0.0/16_017/0002515
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The last decade has seen a huge boom in pharmaceutical and biomedical research, but this has put a lot of pressure on instrumentation and big data processing. In the field of separation techniques and mass spectrometry, we can observe a trend of continuous innovation, modernisation and optimisation of instrumentation to meet these demands, especially in terms of miniaturisation, efficiency and speed of analysis.
Our analytical chemistry laboratory is engaged in the development of new methods for the determination of xenobiotics and endogenous substances in biological material with a relatively wide scope. Most often these are compounds from the category of drugs, metabolites, or endogenous substances as markers of various pathological conditions. However, with the technique available to us so far, we have quite often started to encounter physical limits of the instrumentation used and some projects could not be satisfactorily addressed at the level of current knowledge.
Nevertheless, after many years of great effort, it has been possible to obtain the financial resources to ensure the purchase of instrumentation up to the standards of today that can be found in the research laboratories of developed countries.
The instrument we have been waiting for is a modern Mass Detector (QQQ MS) and UHPLC system.

This instrumentation will form the instrumental basis of our Analytical Laboratory and will be used primarily for targeted, highly sensitive quantification of substances. The cell consists of two main modules. The Acquity ultra performance liquid chromatograph (UPLC) module and the Waters Xevo TQ-XS tandem mass spectrometer (MS).

The UPLC has several basic components found in most contemporary liquid chromatographs. It is a binary pump capable of forming a high pressure gradient up to 1300 Bar at a flow rate of 0.010 - 2.000 mL/min. Furthermore, it is a versatile autosampler allowing to work not only with classical chromatographic vials (2x 48 positions), but also, for example, with 96 and 384 well microtiter plates or other user-defined formats. The volume of the injected sample can be selected from 0.1 - 10.0 ?L. Samples can be tempered in a temperature range of 4 - 40 °C. Another part of the UPLC is a column thermostat with space for two columns up to 150 mm in length. This allows independent switching and heating over a temperature range of 20 - 90 °C. The column thermostat also contains an in-line mobile phase preheater to prevent temperature gradients during separation.

The detector part of the UPLC/MS set-up consists of the Xevo TQ-XS mass spectrometer, which belongs to the advanced triple quadrupole tandem mass spectrometers. At the time of acquisition (2018), the instrument was one of the most sensitive instruments of its kind available on the market. The spectrometer is equipped with a combined ESI/APCI ion source in a "Z-spray" arrangement that limits the entry of uncharged particles into the vacuum section of the instrument. The ion optics in the inlet section uses "StepWave" technology to further limit the passage of uncharged particles to the mass analyzer while optimally focusing the ion beam arriving at the first quadrupole.

The instrument's greatest advantages are undoubtedly its very high sensitivity and, thanks to the use of UPLC technology in the separation, the significantly shorter analysis times compared to conventional HPLC instrumentation.

Other advantages worth mentioning include the ability to quickly switch between ESI and APCI mode without having to change the ion source probe. Furthermore, due to the appropriate design of the instrument inlet, the input of uncharged particles to the mass analyzer is minimized. This actively reduces random chemical noise on the detector. The use of a photomultiplier in the incident ion detector provides the advantage of a longer instrument lifetime compared to a conventional electro-multiplier.

Finally, we would like to demonstrate the advantages of the new instrumentation with a concrete example. In 2017, a method for the determination of 17 bile acids in rat and human bile was developed and implemented in our laboratory. The analysis time per sample was 40 minutes. Due to this time, only about 30 samples could be analyzed per day, including a 7-point calibration curve. This is not really much. The limit of quantification of this method was around 200 nM, which unfortunately did not allow the use of the method for the analysis of individual bile acids in plasma, where we find concentrations much lower than in bile.

Using the new instrumentation, not only was the time per analysis reduced to 11 minutes, but the high sensitivity of the instrument also allowed us to move the limit of quantification by two orders of magnitude compared to the original method. Thus, we can now reach low plasma concentrations of the analysed bile acids with sensitivity. For illustration, a comparison of the separation using the original and new instrumentation is shown below. In order to make the chromatograms comparable, the time axis is the same in both cases from 0 to 40 min.

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Methods