

# 1D/2D-UHPLC-MS System and its use in the Field of Drug Metabolism and Pharmacokinetics

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## INTRODUCTION

Drug metabolism and pharmacokinetics (DMPK) plays a crucial role in the development of new active pharmaceutical ingredients. The elucidation of metabolites has a great importance for the identification of metabolic liabilities of pharmacologically active compounds and cross species comparison for validation of toxicological animal models. Even very low concentrations of metabolites may play a major role. Separation of the biodegradation products by Ultra-High Performance Liquid Chromatography (UHPLC) coupled with mass spectrometry (MS) detection is the technology of choice, with high importance of well synchronized hyphenation.

Thus, a very flexible system to separate a high diversity of molecules in a wide range of operation modes is required. The Thermo Scientific™ Vanquish™ Flex UHPLC system consists of two binary pumps, DADs and column ovens equipped with four 2-position 6-port valves and one autosampler controlled by Thermo Scientific™ Chromeleon™ Chromatography Data system. The arrangement of the individual modules allows analysis in 1D-as well as 2D-LC mode without hardware reassembling.



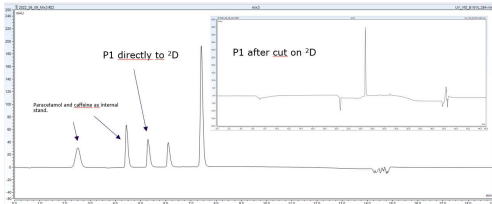
Collaboration of Thermo Fisher Scientific and Sanofi

New options in 2D-LC with a customized Vanquish UHPLC configuration



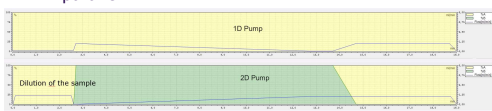
## Highlights

The recovery of the 1<sup>st</sup> dimension (1D) fraction in 2<sup>nd</sup> dimension (2D) is defined by the transfer factor. It is necessary to be able to determine the transfer factor between 1D and 2D.

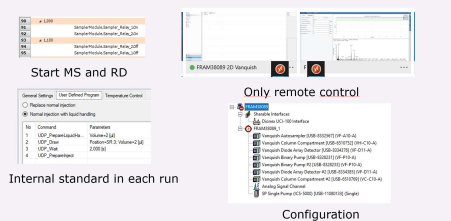


(Quantity 2D / Quantity 1D) x 100 = % transfer  
Transfer factor (UV signal of DAD2 was used): near 98 %

High flexibility for dilution of 1D flow or alternative elution from 2D column by 1D pump, 2D pump or even simulation of tertiary gradients by using 1D pump and 2D pump in parallel



Operation in detail



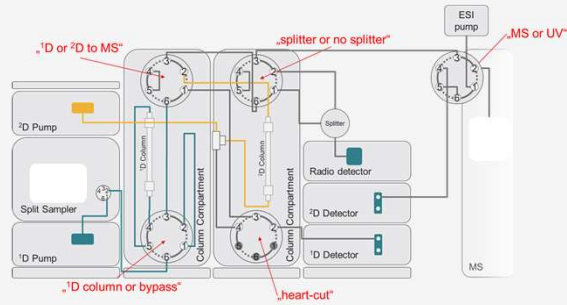
## Summary

- large organic volume injection possible
- easy method development
- high flexibility in operation mode
- wide range of buffers for elution and MS detection
- RD and MS available in either dimension

## REFERENCES

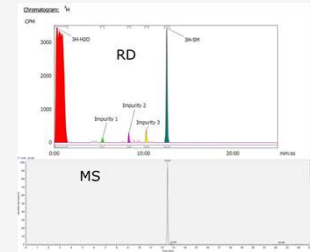
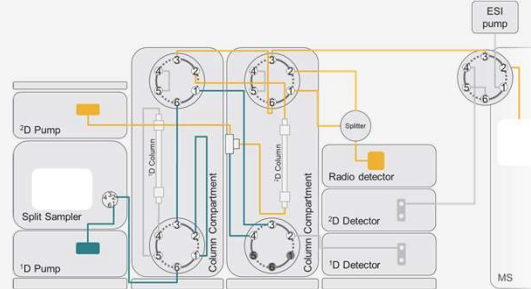
1. B. Koshel, R. Birdsall, W. Chen, Two-dimensional liquid chromatography coupled to mass spectrometry for impurity analysis of dye-conjugated oligonucleotides, J. Chromatogr. B 1137 (2020) 121906.
2. Sonja Krieger, Automated Switching Between 1D-LC and Comprehensive 2D-LC Analysis

Figure 1: Configuration of the 1D/2D-UHPLC-MS-RD system



The heart: 4 independent valves

Figure 2: 2D with splitter (MS and RD detection)

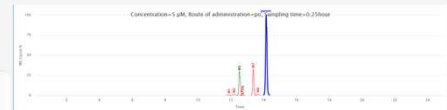
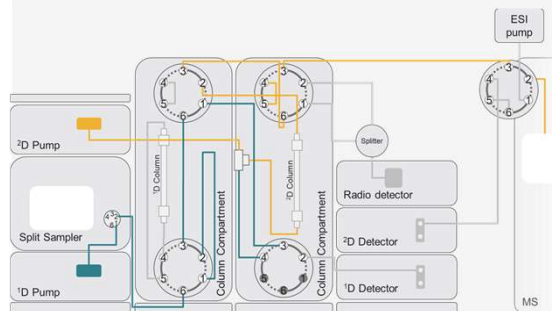


Sample loading (see figure 2)  
1D pump: 0,1 ml/min  
2D pump: 1 ml/min

Elution  
2D: 3 x 100 mm; 1ml/min  
Splitter: 1/3

Motivation: loading of high organic sample

Figure 3: 2D without splitter (MS detection only)

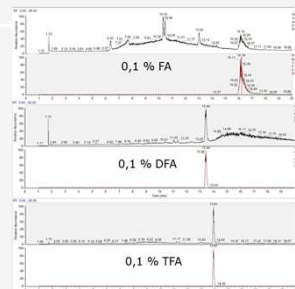
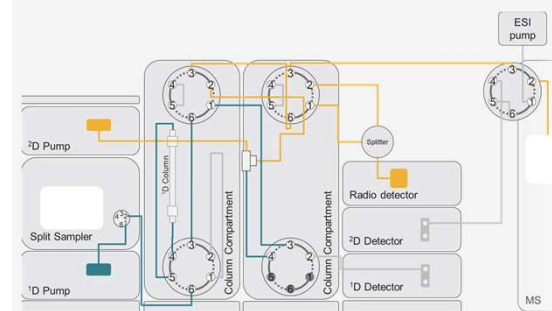


Sample loading (see figure 3)  
1D pump: 0,1 ml/min  
2D pump: 1 ml/min

Elution  
2D: 3 x 100 mm; 0,6ml/min

Motivation: focusing of high organic and large volume sample for sharp peaks

Figure 4: 1D with dilution before MS



1D pump 0,2 ml/min with 0,1 % TFA  
2D pump 0,8 ml/min 0,1 % FA in 75 % organic  
dilution factor is 5 before MS

Motivation: best separation in 1D without loss in ESI signal