2D liquid chromatography mass spectrometry for multi-class antibiotic analysis

Robin S Wegh, Bram van de Kooi, Chiel GM Kaal, Bjorn JA Berendsen, Tina Zuidema

Introduction

Within the Dutch National Residue Control Plan (NP) a large variety of antibiotics have to be monitored in many different matrices. For all antibiotics included in the NP instrumental multi-analyte or multi-class methods are operational. However, no multi-class methods are available that include all relevant antibiotics including the aminoglycosides. Development of a multi-method for these different antibiotic groups is considered very challenging because of the wide variety of chemical properties. We studied the use of comprehensive 2-dimensional liquid chromatography (2D-LC) for analysis of tetracyclines, sulfonamides, macrolides, (fluoro)quinolones and aminoglycosides. The main challenge is to interface two orthogonal separation principles in order to optimise the peak capacity and to obtain retention for all compounds.

Method development

Tested stationary phases

Hydrophilic Interaction Liquid Ion Chromatography (HILIC)
- Syncronis HILIC 5µ 100x2.1mm

Reversed Phase (RP)
- Kinetex 5u C18 100A 100x4,6mm

Weak cation exchange (WCX)
- Dionex Ionpac CS19-4µm analytical column 2x250mm

Alpha-1-acid-glycoproteïn (AGP)
- ChromTech CHIRAL-AGP 2.0x150mm

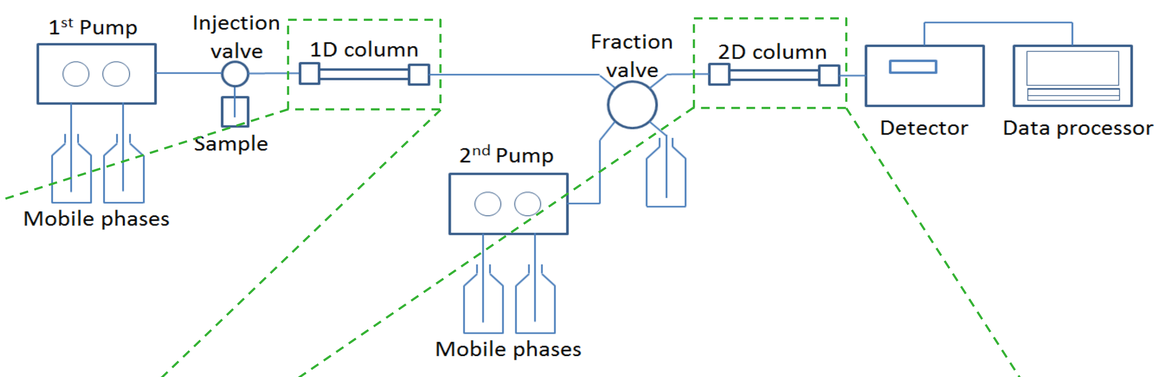
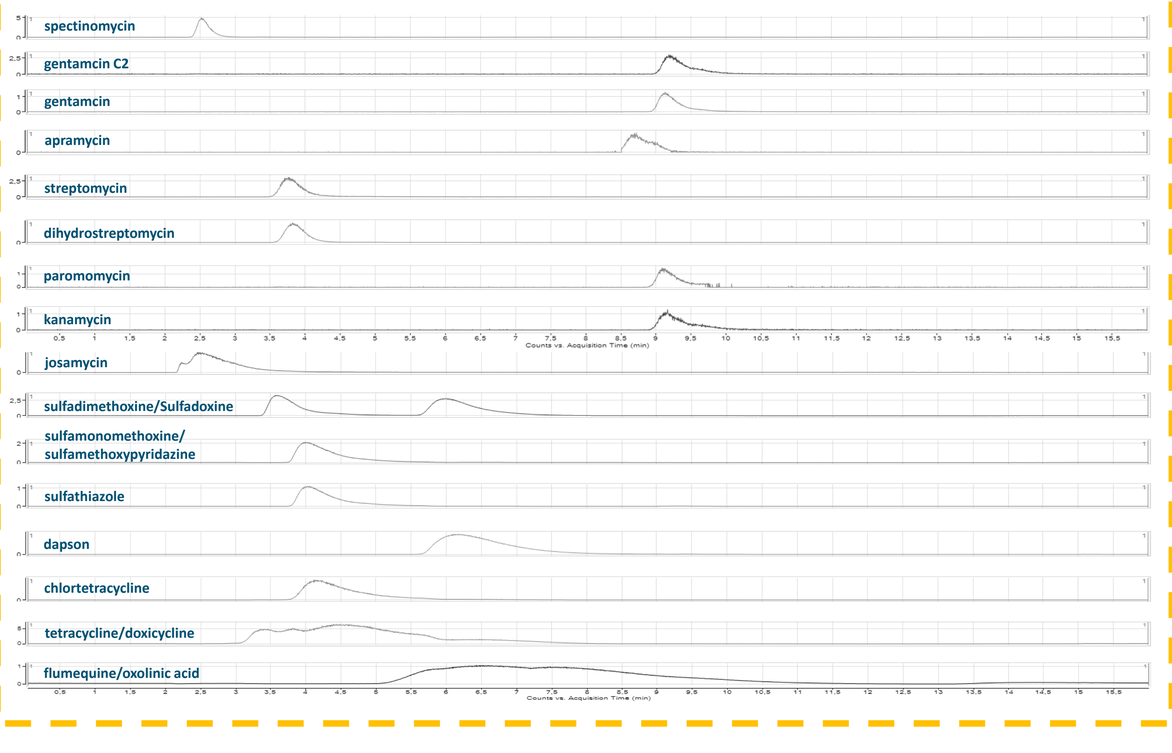


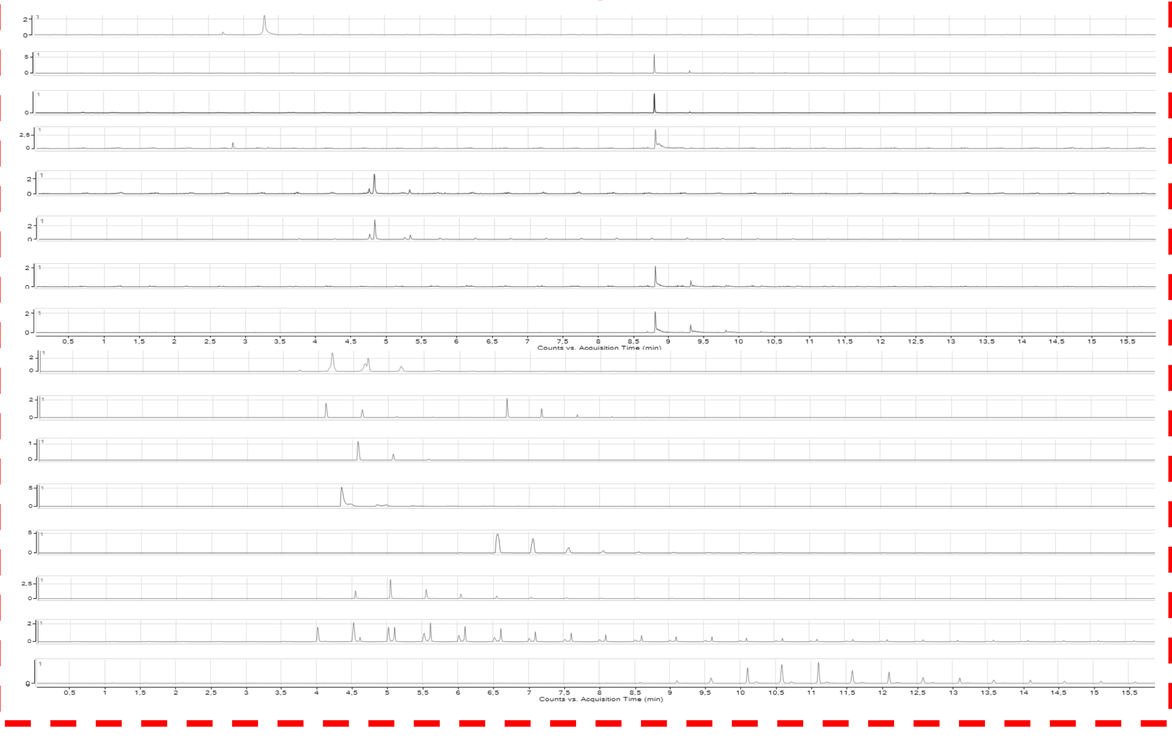
***Figure 1**: Schematic overview of the comprehensive 2D-LC system*

Results

1-D system: AGP



2-D system: RP



Conclusions

1D column	2D column	Conclusions
RP	HILIC	✓Conditioning time too short for HILIC in 2D
HILIC	RP	✓Aminoglycosides retained in 1D ✘1D solvent composition non compatible for injection on 2D gradient
WCX	RP	✓Aminoglycosides retained in 1D ✘No elution of some aminoglycosides
AGP	RP	✓All antibiotics can be retained ✓Highly compatible mobile phases ✘Broad peaks for tetracyclines and (fluoro)quinolones in 1D

RIKILT Wageningen UR
P.O. Box 230, 6700 AE Wageningen
Contact: robin.wegh@wur.nl
www.wageningenur.nl/rikilt

Acknowledgements

This research was funded by the Dutch Ministry of Economic Affairs under its statutory tasks.