Poster n° W5167

Simultaneous analysis of Metformin and Gliclazide as an application in a broadened elution window by serially coupled RP-LC and HILIC

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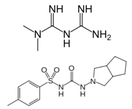
INTRODUCTION & SCOPE

Combination products share an increasing contribution to the new pharmaceutical products on the market as the therapeutic administration of multiple active pharmaceutical ingredients (API's) to patients can be facilitated in this manner. This approach can typically be applied for chronic illnesses (diabetes, hypertension, viral infections, etc.) whereby the combination of different API's brings a synergistic effect.

Case study: *anti-diabetic (Type II) drug compounds*

- *Metformin (biguanide, polar compound)*
- *Gliclazide (sulfonurea, hydrophobic compound)*



Simultaneous HPLC analysis of these 2 compounds and their related substances (present in one fixed-dose combination drug product) in one run…

…by only HILIC (suitable for Metformin) or only RP-LC (suitable for Gliclazide) ??

Expected **incompatibility**: lack of retention and separation for one of the two compounds and its related compounds

- Overlapping peaks: lack of selectivity/specificity
- Not suitable for assay/purity analysis (e.g. stability studies, release, …)

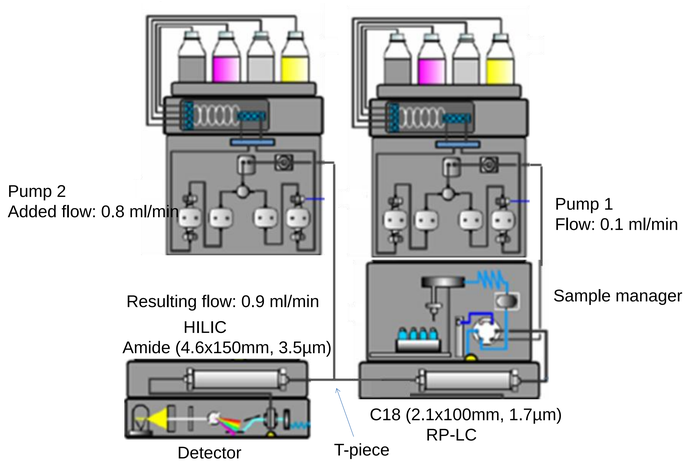
To achieve acceptable retention and separation with a single analysis, a set-up of HILIC serially coupled to RP-LC [1] was applied and investigated.

EXPERIMENTAL & METHODOLOGY I

- *Columns:* - Acquity UPLC® CSH™ C18 (2.1x100mm, 1.7 µm)
  - XBridge™ Amide (4.6x150mm, 3.5 µm)
- *Instrumentation:* Waters H-Class UPLC® (extended with 1 extra QSM pump and a column manager)
- *Data acquisition*: Empower® 3
- *Column Temperature:* 40 °C (for both columns)
- *Detection*: UV at 230 nm
- *Mobile Phase*: A: 25 mM Amm. formate (Aq) + 0.02% (v/v) FA (pH 4.4)
  B: ACN

EXPERIMENTAL & METHODOLOGY II

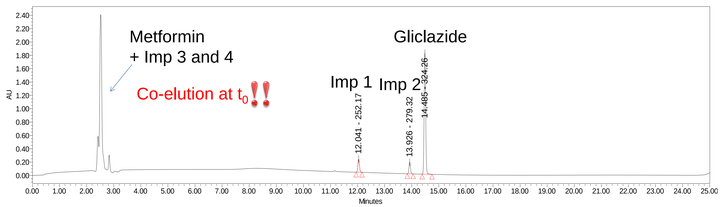
•*Schematic set-up*:



Reference:
[1] S. Louw, A. Pereira, F. Lynen, M. Hanna-Brown, P. Sandra, Journal of Chromatography A, 2008, 1208: p. 90-94

RESULTS & DISCUSSION I

RP-LC only

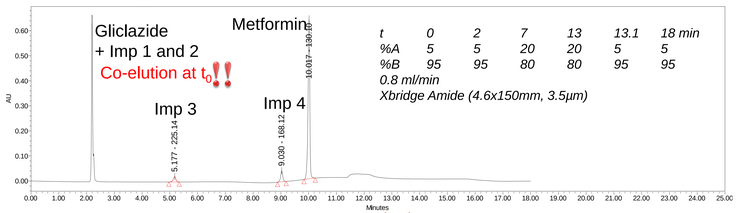


t	0	2	12	20	20.1	25 min	*0.1 ml/min*
%A	95	95	5	5	95	95	*Acquity UPLC CSH C18 (2.1x100mm, 1.7µm)*
%B	5	5	95	95	5	5	

- Gliclazide + Imp 1 and Imp 2
- Metformin + Imp 3 and Imp 4

RESULTS & DISCUSSION II

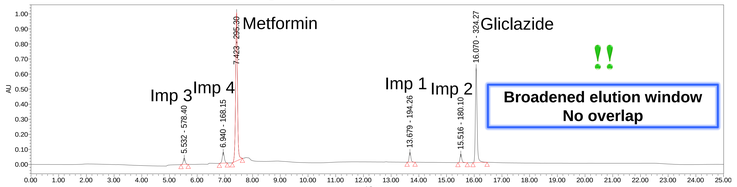
HILIC only



t	0	2	7	13	13.1	18 min	*0.8 ml/min*
%A	5	5	20	20	5	5	*Xbridge Amide (4.6x150mm, 3.5µm)*
%B	95	95	80	80	95	95	

- Gliclazide + Imp 1 and Imp 2
- Metformin + Imp 3 and Imp 4

RP-LC – HILIC serially coupled

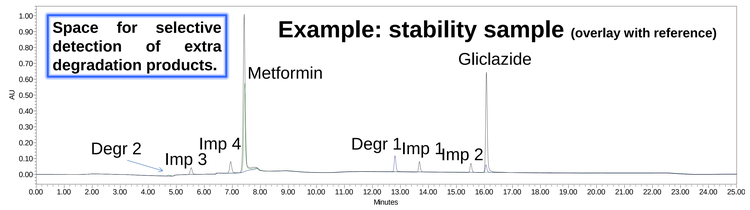


Pump 1	*t*	0	2	12	20	20.1	25 min	*0.1 ml/min*
	%A	95	95	5	5	95	95	
	%B	5	5	95	95	5	5	
Pump 2	*t*	0	2	8	20	20.1	25 min	*0.8 ml/min*
	%A	2	2	20	20	2	2	
	%B	98	98	80	80	98	98	

- Gliclazide + Imp 1 and Imp 2
- Metformin + Imp 3 and Imp 4

The 'Pump 2' flow is inserted after the first (RP-LC) column and before the second (HILIC) column via a T-piece.

- **HILIC conditions on the 2nd column without disturbing the RP-LC separation on the 1st column.**



CONCLUSION & REMARKS

- Serial tandem RP-HILIC offers broadened elution window for selective simultaneous analysis of polar and hydrophobic compounds
- Suitable platform for assay/purity analysis during e.g. stability studies of combination products
- 1 chromatographic run for simultaneous analysis of present APIs and related substances
- Lower probability of interferences and overlapping peaks
- Resulting output: a single conventional 1D chromatogram, comprehending all analytes of interest (If #peaks < 30, no need for advanced 2D-LC)