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Therapeutic Drug Monitoring in Breast Cancer Therapy – Quantification of CDK4/6 Inhibitors by LC-MS/MS

Katharina Habler, Michael Vogeser, Daniel Teupser
Institute of Laboratory Medicine, University Hospital, LMU Munich, Germany.

Introduction

With 1.7 million new cases per year, breast cancer is the third most common cancer worldwide. The cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors palbociclib, ribociclib, and abemaciclib were approved in recent years by the U.S. Food and Drug Administration (FDA) and European Medicine Agency (EMA) for the treatment of breast cancer. Generally, a fixed dosing regimen of oral tumor therapeutics (OTT) is used. However, several described side effects and pharmacokinetic interactions (CYP3A4 inducers and inhibitors) may affect appropriate drug levels and therapeutic success. OTT extend the options for cancer therapy in the home environment, but also pose challenges for physicians and patients. Therapeutic drug monitoring (TDM) can help to verify appropriate drug levels, monitor adherence, and also to detect interactions. The aim of our work was the development of a robust semi-automated stable isotope liquid-chromatography tandem mass spectrometry (2D-ID-LC-MS/MS) method for all previously approved CDK4/6 inhibitors for perspective use in routine diagnostics.

Materials & Methods

Analytes/metabolites – internal standards:

- Abemaciclib/abemaciclib M2/abemaciclib M20 – abemaciclib-d8
- Palbociclib – palbociclib-d8
- Ribociclib – ribociclib-d6

Sample preparation:

- 50 µL calibrator/QC/sample + 200 µL internal standard
- Vortex (30 sec) + centrifuge (12,600 g, 5 min)
- 50 µL supernatant + 950 µL H_20-ACN (7:3; v/v)

2D-LC-MS/MS:

- Acquity UPLC system + Xevo TQ-S (Waters, USA)
- Online SPE: HLB Oasis® Direct Connect (2.1x30 mm, 20 µm; Waters, USA)
- Analytical column: Raptor® Biphenyl (2.1x50 mm, 2.7 µm; Restek, USA)
- Mobile phases: 0.1% formic acid in H_20 (A1/A2) + 0.1% formic acid in MeOH (B1/B2)

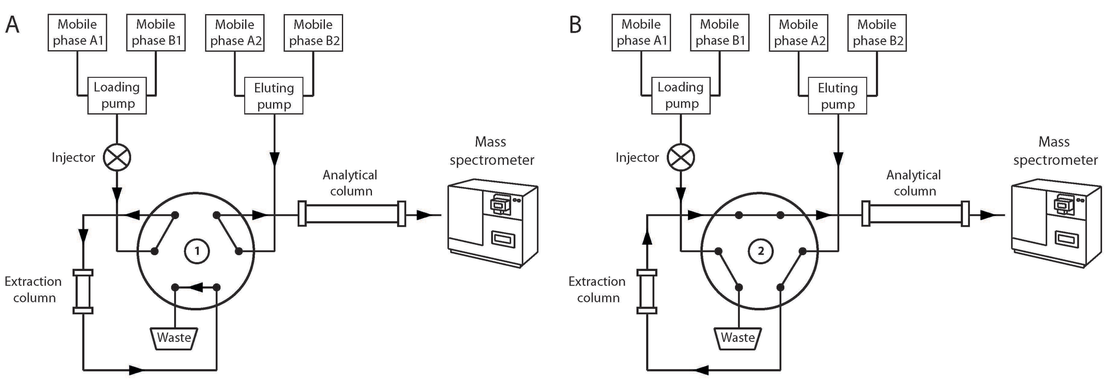

Figure 1. Principle of the 2D-chromatography via online SPE with A) online clean-up of sample extract and B) chromatographic separation.

Results

We present the first 2D-LC-MS/MS method for the simultaneous quantification of abemaciclib, palbociclib, ribociclib, and the relevant pharmacollogically active metabolites M2 and M20.

The validation of the method was based on the FDA "Bioanalytical Method Validation – Guidance for Industry" in terms of calibration, intraday and interday inaccuracy and imprecision, carryover, selectivity and sensitivity, matrix effect, recovery, process efficiency, dilution integrity, and stability.

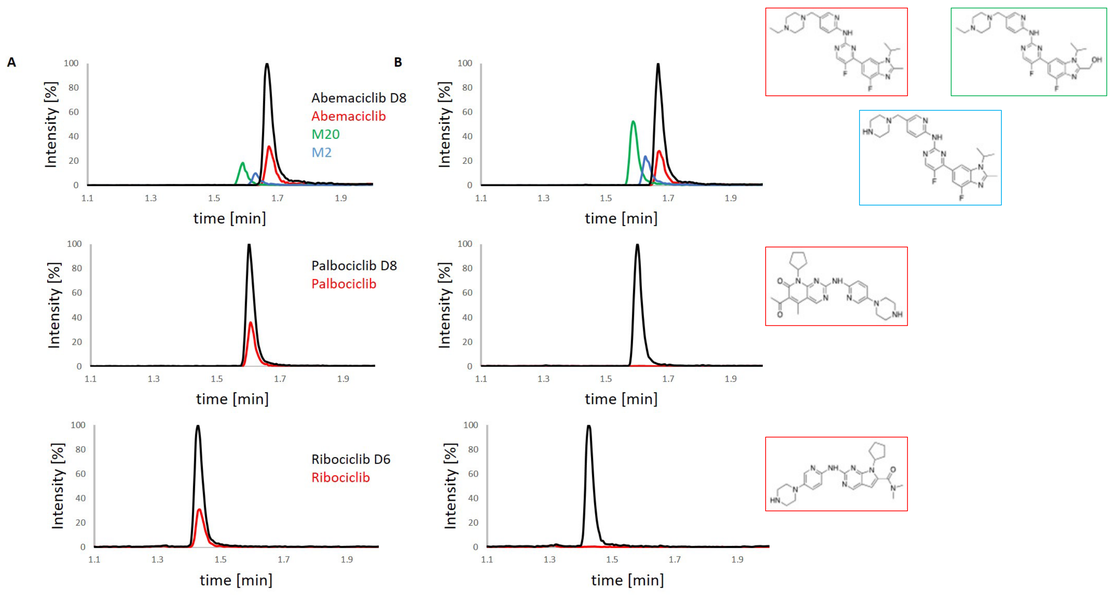

Figure 2. Chromatogram of A) QC I with 20 ng/mL abemaciclib, 10 ng/mL M2, 15 ng/mL M20, 10 ng/mL palbociclib, 100 ng/mL ribociclib and B) authentic sample with 40.2 ng/mL abemaciclib, 47.2 ng/mL M2, 95.5 ng/mL M20.

As compassionate use in an individual treated with abemaciclib trough values of abemaciclib, M2, and M20 as well as a concentration time profile over 12 h were analyzed. The pharmacokinetic parameters of abemaciclib in serum were an $AUC_{0\text{-}12h}$ of 673 ng*h/mL, a T_{min} at 12 h with a C_{min} of 42.3 ng/mL, and a T_{max} at 4h with a C_{max} of 66.9 ng/mL.

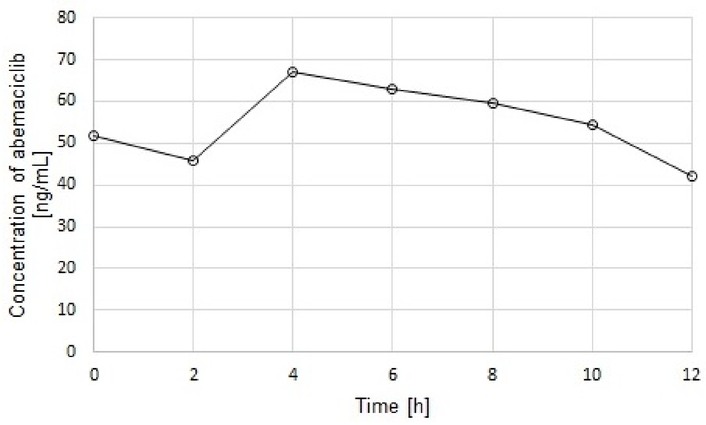

Figure 3. Concentration time profile of abemaciclib.

Conclusion

TDM offers the opportunity to support this new emerging, but also challenging form of therapy in the home environment: The valuable puzzle piece durg concentration can be combined with dosing regimens and side effects, among other factors, to increase adherence, therapy safety, and treatment success. TDM of OTT in routine diagnostics provides an option that can pave the way for 'total drug monitoring' and make personalized medicine more broadly available.

Literature
An UHPLC-MS/MS method for quantification of the CDK4/6 inhibitor abemaciclib in human serum. Katharina Habler, Michael Vogeser, Daniel Teupser, Journal of Mass Spectrometry & Advances in the Clinical Lab (2022), 24, 15-21.
Therapeutic Drug Monitoring in Breast Cancer Therapy – LC-MS/MS method for quantification of the CDK4/6 inhibitors abemaciclib, palbociclib, ribociclib, and major metabolites abemaciclib M20 and M2 in human serum. Katharina Habler, Anne-Sophie Kalla, Michael Rychlik, Michael Vogeser, Daniel Teupser, Journal of Pharmaceutical and Biomedical Analysis (2023), 225, 1-9.