A Fast and Robust Method for the Quantification of Venetoclax by LC-MS/MS

Regula Steiner[1], Daniel Müller[1]

[1]Institute of Clinical Chemistry, University Hospital Zürich

Venetoclax

We aimed to develop a fast, simple and sensitive high-throughput LC-MS/MS method for the analysis of Venetoclax in clinical routine.

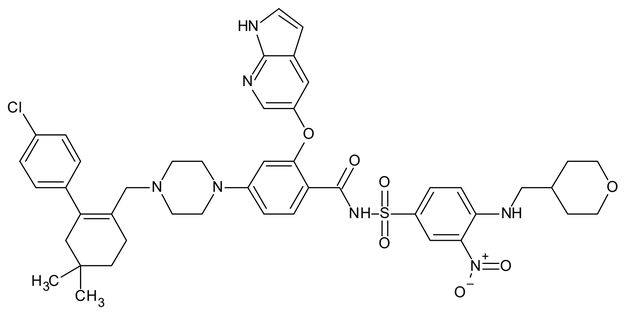


Figure 1: Structure of Venetoclax

Week	Dose per day
1	20 mg
2	50 mg
3	100 mg
4	200 mg
5 and after	400 mg

Table 1: Titration scheme for Venetoclax[1]

Venetoclax, a BCL-2-inhibitor for the treatment of B-cell lymphoma associated with chromosome 17p deletion, is used more and more in the clinics. Because of severe toxicity, Venetoclax has to be titrated at the beginning of the therapy. Usually it is administered once daily, with a titration period of five weeks.

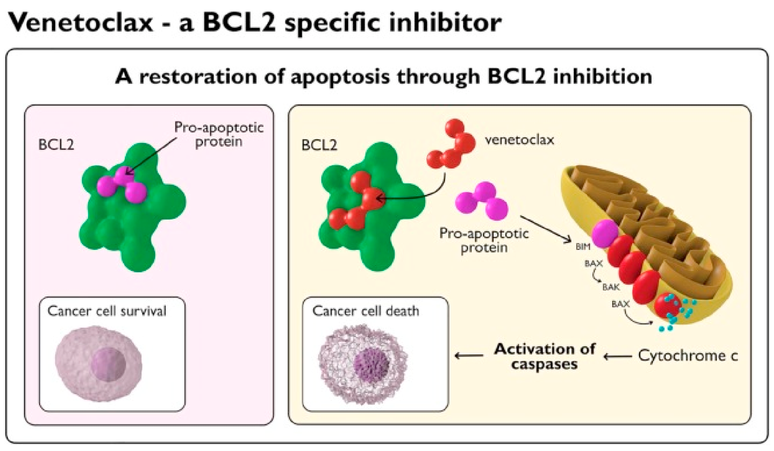


Figure 2: Mechanism of action of Venetoclax [2]

The peak plasma concentration of Venetoclax was reached after five to eight hours in steady state and the AUC is linearly increasing for doses between 150 and 800 mg. C_{max} in steady state after a daily dose of 400 mg was determined at 2,1 ± 1,11 mg/L.

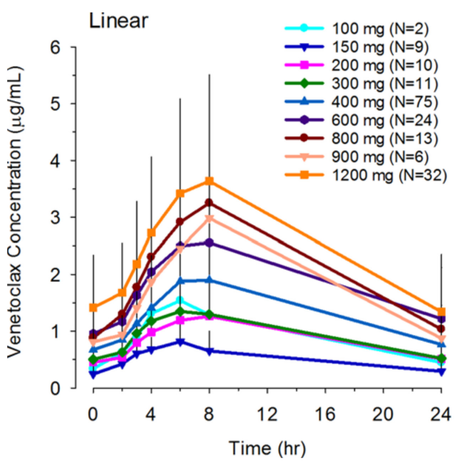


Figure 4: Pharmacokinetics of Venetoclax [4,5]

Dose per day	Peak concentration (5-7h post dose)
100mg	1.36-1.80 mg/L
150mg	0.66-1.16 mg/L
200mg	0.77-1.91 mg/L
300mg	0.75-2.27 mg/L
400mg	0.99-3.21 mg/L

Dose per day	Trough concentration
400mg	0.69-0.99 mg/L

Table 2: Plasma levels of Venetoclax [4,5]

Venetoclax is metabolized over CYP3A4 and therefore prone to interactions, which makes therapeutic drug monitoring highly recommended. Especially the combination with Azol-antimycotic drugs is potentially toxic and dose of Venetoclax has to be reduced because of interactions.

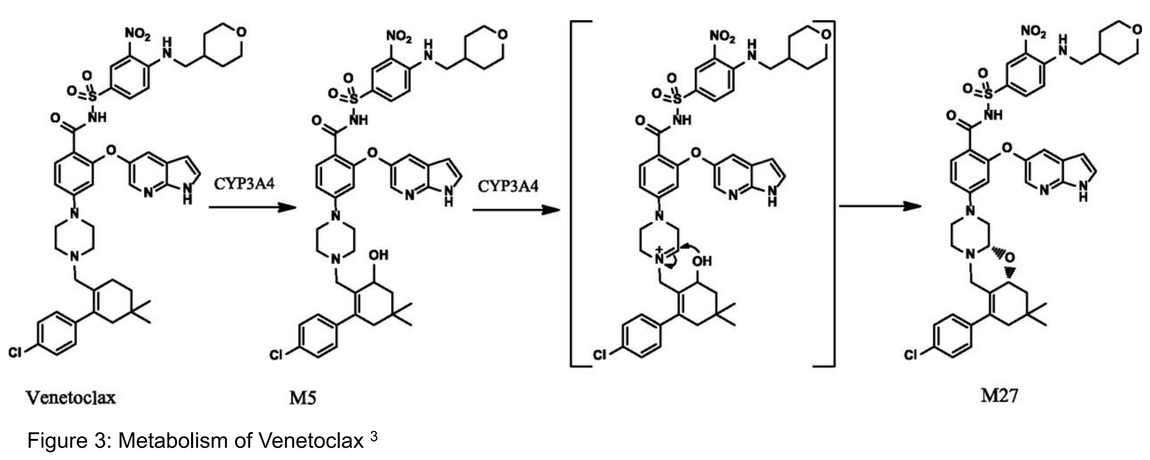
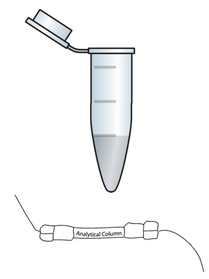


Figure 3: Metabolism of Venetoclax [3]

In combination with a strong CYP3A4 inhibitor, C_{max} of Venetoclax increased up to 2.3 times. Therefore, the combination of Venetoclax and CYP3A4 inhibitors is contraindicated during the titration phase and has to be closely monitored in steady state, because of the risk to develop a tumor-lysis syndrome. It is recommended to reduce the Venetoclax dose in steady state by 75% of normal dose if combined with CYP3A4 inhibitors.

LC-MS/MS Method



- 50µL Plasma + precipitation solution (containing Venetoclax-D7)
- Vortexed and centrifuged
- 20µL of supernatant injected

- Reversed phase chromatography with C8 column
- Isocratic conditions with 20:80 H2O/ACN 10mM ammonium acetate and 0.1% formic acid

- ESI ionization in positive mode

	Parent Mass	Quantifier	Qualifier
Venetoclax	868.2	636.3	321.1
Venetoclax-D7	875.3	643.3	321.1

Table 3: Parent and product masses

The method has been fully validated according to EMA and US-FDA guidelines.

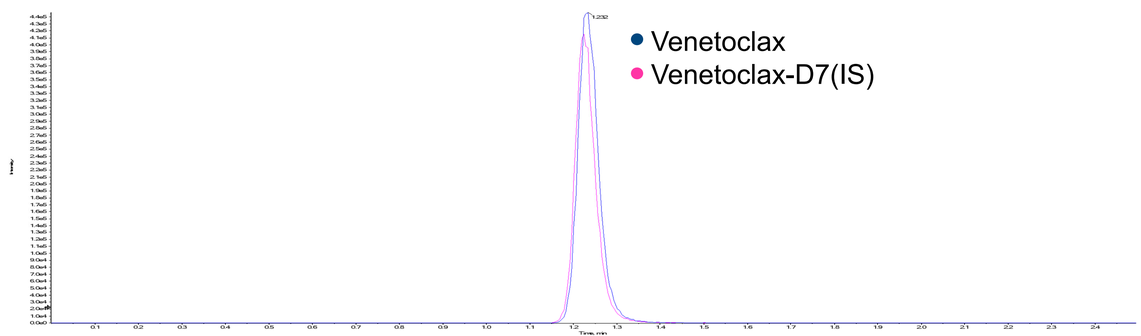


Figure 5: Chromatogram of Venetoclax and Venetoclax-D7 (Retention time 1.2 min)

Venetoclax was shown to be linear over the entire calibration range (0.01-5.0 mg/L) with a lower limit of quantification at 0.01 mg/L. The linear range spans over the whole therapeutic range of Venetoclax.

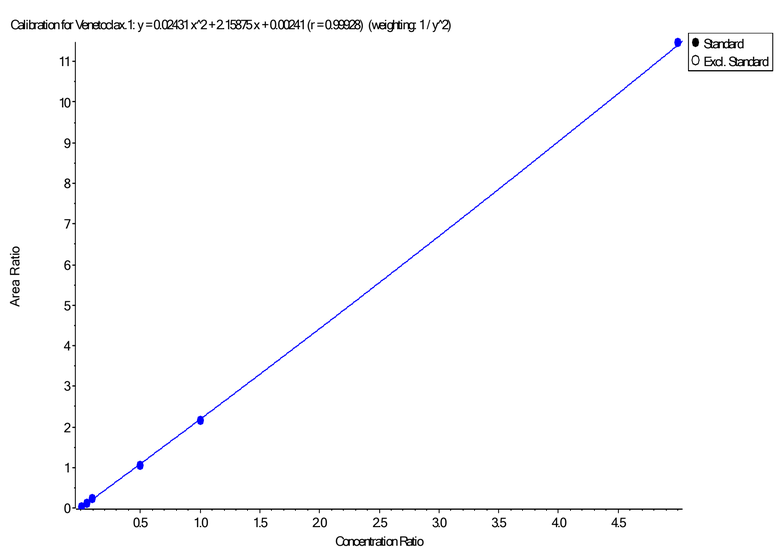


Figure 6: Calibration Curve for Venetoclax in the range of 0.01-5.0 mg/L with an linear correlation r= 0.9993

The method was demonstrated to be free of matrix effects for serum, heparin-, and EDTA-plasma.

	Interday Accuracy [%]	Interday Precision CV[%]	Intraday Accuracy [%]	Intraday Precision CV[%]
QC 0.08 mg/L	95.3	3.5	94.8	2.5
QC 0.8 mg/L	97.8	2.0	97.9	1.3
QC 3.0 mg/L	99.0	4.8	98.9	1.7

Table 4: Precision and accuracy of low, medium and high control samples

Plasma Levels in Patients

Patient	Venetoclax dose [mg/d]	Venetoclax plasma level [mg/L]	CYP Inhibitor Posaconazole [mg/L]	Comment
1	100	1.04	1.77	300 mg Posaconazole
2	100	0.94	2.79	300 mg Posaconazole
3	100	0.45	2.87	300 mg Posaconazole
4	400	1.66	-	
5	100	1.81	-	Stopped because of complications

Table 5: Trough plasma concentration of Venetoclax in five patients

The presented LC-MS/MS method allows a fast, simple and reliable determination of Venetoclax. Patients can be closely monitored in titration phase and in steady state. Especially, if a antimycotic prophylaxis therapy with a strong CYP3A4 inhibitor has to be combined with Venetoclax, plasma levels can be monitored to reduce the risk of tumor-lysis syndrome and other complications.

USZ Universitäts Spital Zürich

References

1. Fachinformation des Arzneimittel-Kompendium der Schweiz, Venclyxto
2. Mihalyova, J., et al., Venetoclax: A new wave in hematooncology. Exp Hematol, 2018. 61: p. 10-25.
3. Liu, H., et al., Metabolism and Disposition of a Novel B-Cell Lymphoma-2 Inhibitor Venetoclax in Humans and Characterization of Its Unusual Metabolites. Drug Metab Dispos, 2017. 45(3): p. 294-305.
4. Salem, A.H., et al., Pharmacokinetics of Venetoclax, a Novel BCL-2 Inhibitor, in Patients With Relapsed or Refractory Chronic Lymphocytic Leukemia or Non-Hodgkin Lymphoma. J Clin Pharmacol, 2017. 57(4): p. 484-492.
5. Salem, A.H., M. Dunbar, and S.K. Agarwal, Pharmacokinetics of venetoclax in patients with 17p deletion chronic lymphocytic leukemia. Anticancer Drugs, 2017. 28(8): p. 911-914.