Therapeutic Drug Monitoring of 8 new anticancer agents by High-Performance Liquid Chromatography-Tandem Mass Spectrometry

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INTRODUCTION

The treatment of some cancers has shifted from conventional chemotherapy drugs to chronic treatment with molecular targeted therapies. Targeted therapies include drugs such as Tyrosine kinase inhibitors (eg: Imatinib, Dasatinib, Nilotinib, Sunitinib, Sorafenib, Vandetanib, Lapatinib, Vatalanib and Erlotinib) that present better efficiency and lower side effects than conventional anti cancer drugs.

GOAL

The goal was to develop and validate a specific and sensitive method for the quantitation of Tyrosine kinase inhibitors (eg: Imatinib, Dasatinib, Nilotinib, Sunitinib, Sorafenib, Vandetanib, Lapatinib, Vatalanib and Erlotinib) in plasma samples using liquid chromatography coupled to mass spectrometry.

ANALYTICAL CONDITIONS

- **Sample preparation:** 50µl of plasma samples were extracted with methanol containing internal standard and the organic layer diluted into the mobile phase.
- Analytical conditions are reported on table 1.
- For each analyte, 2 SRMs transitions are monitored: one is used for quantitation (Q_0) and the other one is used for confirmation (Q_1) (Table 2).
- 3 quality controls (CQI) at the following concentrations : 750, 1500 and 7500 ng/mL for each analyte have been used for validation.

Table 1: Analytical conditions

Internal standard	Imatinib-D_8
Injection volume	20µL
Flow rate	300 µL/min
Analytical column	C18 (100 mm x 2.1; 1.9 µm)
Mobile phase	Ammonium formate 10 mM with 0.1% formic acid+ acetonitrile containing 0.1% formic acid
Ionization mode	Positive mode using HESI source
Scan mode	SRM Mode (XCalibur®software)

Exemple: MSMS spectrum of Imatinib obtained by infusion

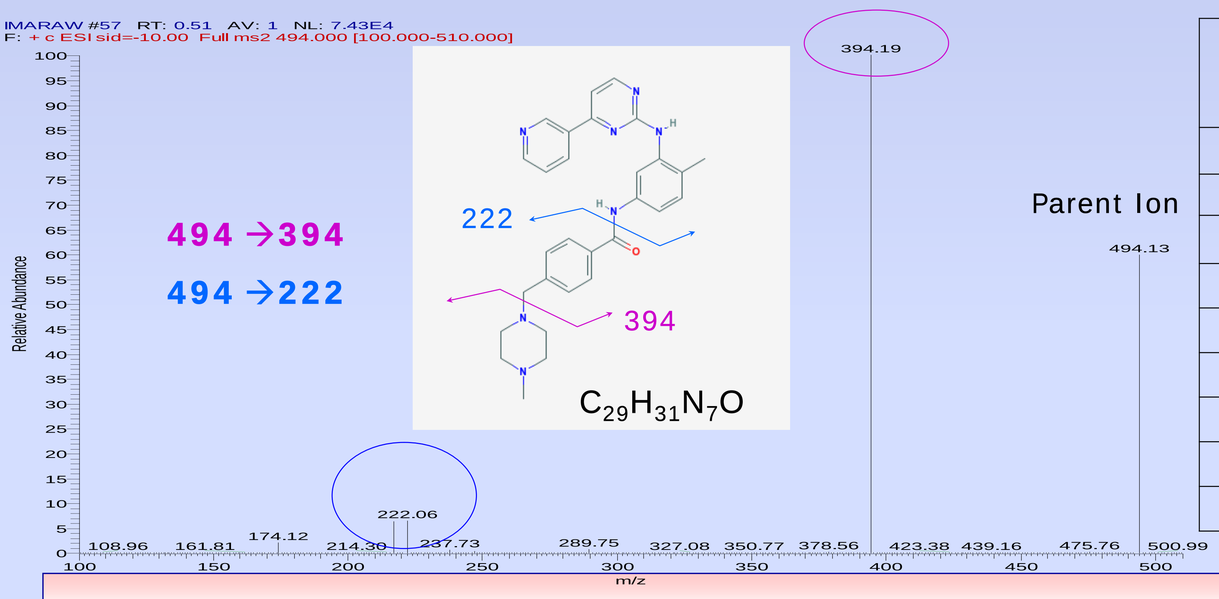

Table 2 : Retention time (RT) and SRMs transitions for each drug.

Molecules	RT(min)	Exact Mass	Parent Ion $[M+H]^+$ (m/z)	Q_0 Fragment ion (m/z)	Q_1 Fragment Ion (m/z)
Vatalanib	3,02	346,098856	347	92	127
Erlotinib	3,07	393,168856	394	278	336
Sunitinib	3,08	398,211804	399	283	326
Sorafenib	3,62	464,086303	465	252	270
Vandetanib	3,03	474,106667	475	83	112
Dasatinib	3,03	487,155722	488	401	232
Imatinib	**3,00**	**493,259009**	**494**	**394**	**222**
Nilotinib	3,33	529,183793	530	289	261
Lapatinib	3,21	580,134732	581	350	364

RESULTS AND DISCUSSION

1. Calibration curves were established from 100 to 10 000 ng/ml in human plasma, calculated and fitted by 1/x2 weighted linear regression . R^2 values were above 0.977 for all drugs (n = 10).
2. Intra-day (n=6) and inter-day (n=60) variabilities were evaluated by injecting the QCs. CVs were all below 15%
3. Precision was good with values below 15% and accuracy was close to 100% (n=60) for all quality controls.
4. Recoveries (Extraction recovery ER, ionization recovery IR and global recovery GR) have been studied at two levels of concentration : 500 and 5000 ng/mL. Results are reported in table 4 are those obtained for 5000 ng/ml. For the lower concentration (500 ng/ml), a higher matrix effect has been observed and for this reason, ionization recovery was above 100.
5. Specificity has been evaluated and no inteferences have been detected.
6. The limit of quantitation has been established to 50ng/ml for all the molecules.

Table 3 : Results obtained for CQI 1 (750 ng/ml)

Molecules	Intra-day precision CV(%)	Inter-day precision CV(%)	Accuracy (%)	Precision (%)
Vatalanib	4,00	12,24	109,84	9,84
Erlotinib	2,91	14,13	106,62	6,62
Sunitinib	3,90	17,44	92,51	-7,49
Sorafenib	3,29	10,17	87,78	-12,22
Vandetanib	5,72	13,67	100,93	0,93
Dasatinib	5,14	14,87	108,19	8,19
Imatinib	3,19	7,35	105,84	5,84
Nilotinib	2,46	11,54	103,27	3,27
Lapatinib	3,92	13,50	109,95	9,95

Table 4: Recoveries (GR,ER and IR) obtained at 5000ng/ml.

Molecules	GR (%)	ER (%)	IR (%)
Vatalanib	98,3	98,5	99,8
Erlotinib	112,2	102,4	109,5
Sunitinib	88,3	97,8	90,2
Sorafenib	99,3	105,0	94,6
Vandetanib	104,7	99,1	105,6
Dasatinib	114,3	94,8	120,5
Imatinib	107,4	98,4	109,1
Nilotinib	100,8	99,7	101,1
Lapatinib	102,6	97,1	105,7

CONCLUSION

We have developed and validated a method for the analysis of 8 anti cancer drugs using the LC/MSMS technology. It is simple, sensitive, spécific, reproducible, accurate, linear and fast (< 15 minutes)

It can be used for pharmacology and pharmacokintics studies for patients in hematology and oncology area.