RAPID DEVELOPMENT OF THERAPEUTIC DRUG MONITORING FOR IMMUNOSUPPRESSANT DRUGS

Author Details:
O. Tribut
Laboratory of Pharmacology
Rennes's Hospital, France
Tel: +33 2 99 28 42 80
Fax: +33 2 99 28 41 84
Email: olivier.tribut@chu-rennes.fr

Human organ transplantation is a relatively new area of application for Chromatography and Mass Spectroscopy to be associated with. The need to accurately monitor with short analysis times and high specificity the levels of immunosuppressant drugs in the patients' body from blood samples can occasionally be a 'life-or-death' situation. For many years the Instrument companies who manufacture mass spectrometers paid little attention to the clinical area when other areas within life sciences, environmental and then the –omics group received much attention. It was left to those scientists usually working in laboratories within Hospitals and clinics to forge a path (1) and develop the potential that initially LC and then LC/MS and now LC/MS/MS can add to the clinicians' toolbox. In the last 3 years we have seen the Instrument companies recognise the potential within this area and coupled with opportunities to improve their sales of Informatics systems and related services, most major mass spectroscopy manufacturers have a dedicated Business segment with specific responsibilities for clinical applications.

This article shows a potential template for a major Hospital to use LC/MS/MS for such an area with the measurement of immunosuppressant drugs in a reproducible highly specific manner with good accuracy and speed at reasonable cost/analysis.

Spectroscopy Focus

Combined immunosuppressant therapy using cyclosporine, everolimus, sirolimus or tacrolimus has demonstrated benefits. Immunosuppressant drugs (IS) have been commonly quantified in several laboratories for transplanted patients. The recent evolution of tandem mass spectrometry allows scientists to quantify more than four drugs within a single blood spot. Several analytical methods do exist at the moment such as immunoassay, classic HPLC with UV detection, and LC/MS/MS. There are drawbacks however with the first option mentioned being expensive and the second time consuming. Only LC/MS/MS permits simultaneously, rapid and precise determination of the different immunosuppressant drugs. New developments on LC/MS/MS technology now permit routine quantification on a routine and simple basis. There remains still a problem with the LC method, which demands the preparation of calibration samples daily. This note exposes the different parameters of LC/MS/MS and conditions for an immediately useable routine assay for any mass spectrometry or HPLC developer whose work involves assaying IS concentrations in human whole blood.

GOAL

Develop a practical method to quantify four immunosuppressant drugs for therapeutic drug monitoring. To analyze the large numbers of samples, the Finnigan TSQ Quantum Discovery (Thermo Fisher Scientific, France) due to its specificity, is a mass spectrometer capable of simultaneously determining a number of compounds. Simultaneous chromatographic monitoring of whole blood is useful for reducing turnaround time.

Conventional LC methods generally require extensive optimization but Chromsystems GmbH (Munich, Germany) provides a multilevel calibrator and quality control set made up of lyophilized samples on the basis of human whole blood. The calibrator set covers the therapeutically relevant concentration ranges. This removes the need for the manual preparation of stock and standard solutions any longer.

EXPERIMENTAL CONDITIONS

Chemicals and reagents: Water, acetonitrile were both HPLC grade and purchased from Fisher, France. The internal standard (ascomycin) was purchased from Sigma, France. Calibrator and quality control (QC) standards were purchased from Chromsystems GmbH.

Samples: Lyophilised samples were reconstituted and an aliquot (50µL) directly transferred into glass tubes. These glass tubes were frozen at -20°C with a stock solution of internal standard (prepared by dissolving ascomycin (12ng/mL) in acetonitrile: water (50:50)). The daily routine involved the thawing of one set of calibrator, QC and internal standard before extraction.

An ammonium-adduct based liquid chromatography-tandem mass spectrometry method was developed for the simultaneous determination of four immunosuppressant drugs in human whole blood. These compounds and the internal standard were extracted from the biological matrix by a salting-out procedure.

Briefly, 50 µL of whole blood was mixed with 50 µL dilution of an internal standard comprised of 1 mL of acetonitrile containing 50% of zinc sulphate solution (20g/L) and saturated with 150 µL of ammonium sulphate solution (400g/L). The mixture was centrifuged for 10 min at 3000 g and 100µL of supernatant was transferred into another vial for direct injection into LC *(Figure 1).*

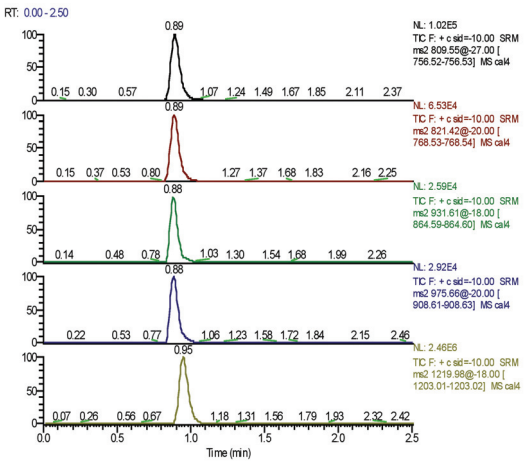

Figure 1.Chromatogram of calibrator 3 (respectively: ascomycin, tacrolimus, sirolimus, everolimus and cyclosporine).

Liquid Chromatography

Analytical column: 2.1x30 mm, 1.9 µm Hypersil® Gold C18 (Thermo Fisher Scientific)

Time (min)	Flow Rate (µL)
0	100
1	200
1.5	600
2	600
2.5	200

Flow Rate: see Table below

Injection volume: 12 µL (partial loop)

Flush volume: 2000 µL (acetonitrile water 75/25(v/v))

Wash volume: 2000 µL (acetonitrile water 75/25(v/v))

Column oven temperature: 60 °C

Mobile phase: acetonitrile and 10mM ammonium acetate 95/5 (v/v)

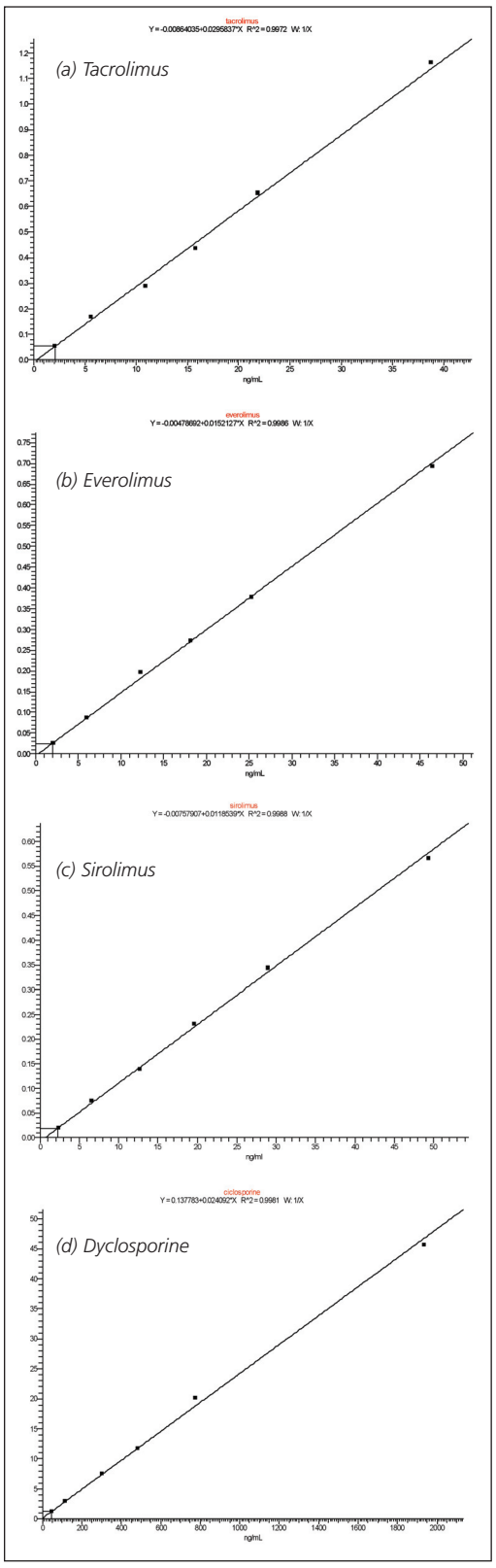

Figure 2: Calibration curves: a) tacrolimus, b) everolimus, c) sirolimus and d) dyclosporine

Mass spectrometry

Finnigan TSQ Quantum (Thermo Fisher Scientific)
Ionization mode: positive ion ESI
Spray voltage: 4500
Sheath gas pressure: 35
Auxiliary gas pressure: 5
Ion Transfer Tube temperature: 200 °C
Source CID: -10
Collision pressure: 1.5
SRM transition: see Table 2
Scan width: 0.01
Scan time: 0.03
Q1 and Q3 resolution: 0.7 Da FWHM

Sample analysis: One segment allowed the acquisition of the five SRM transitions *(Table 1)*. A calibration curve of peak area ratio versus concentration was used for quantification *(Figure 2)*. The linearity of calibration curves had a correlation coefficient of >/=0.99 *(Table 2)*.

Precision of the method was <10% within batch and <12% between batches (Table 3). The lower limits of quantification, reproducible with a precision and accuracy <20% were 1 ng/mL for everolimus, sirolimus and tacrolimus and 10 ng/mL for cyclosporine.

A high flow-rate regime was used after component elution just for the time needed for the column clean up and the assay took 2.5 minutes per sample injection.

CONCLUSION

An LC/MS/MS method to monitor 4 immunosuppressant drugs was developed using the Finnigan TSQ Quantum Discovery. The use of the Chromsystems multilevel calibrator and quality control set shows to be fast and simple.

The authors suggest that this LC/MS/MS method with lyophilized calibrator and control set is effective for the routine monitoring of immunosuppressant drugs in medical labs.

The required time for the entire analytical procedure permits routine therapeutic drug monitoring of forty samples in a morning and this method has been successfully used to analyze more than 8000 whole blood samples from transplant recipients in a year.

REFERENCES

(1) Challenges Involved in Running MS Based Assays in Clinical Environments. Neil Lever, Jane Tiller, Harefield Hospital, UK, International Labmate January 2006

Table 1: SRM Transitions

Compound	Precursor (m/z)	Product (m/z)	Collision energy	Tube lens
ascomycin	809.55	756.52	27	129
cyclosporin	1219.98	1203.10	18	185
everolimus	975.66	908.62	20	140
sirolimus	931.61	864.59	18	150
tacrolimus	821.42	768.53	20	135

Table 2: Calibrator

Component	Curve	Weighting	Origin	Equation	
ascomycin	Average response factor	equal	ignore	%RSD=23	
cyclosporin	Linear	1/X	Ignore	Y = 0.336992+0.0312439*X	R^2 = 0.9929
everolimus	Linear	1/X	Ignore	Y = -0.00349751+0.0101604*X	R^2 = 0.9935
sirolimus	Linear	1/X	Ignore	Y = -0.00566645+0.00860854*X	R^2 = 0.9914
tacrolimus	Linear	1/X	Ignore	Y = -0.00190807+0.0247685*X	R^2 = 0.9983

Table 3: Quality Control

		Intraassay (n=10)		Interassay (n=10)	
Compound	level	Mean +/- SD	CV (%)	Mean +/- SD	CV (%)
cyclosporin	97.3	91.6+-2.42	2.6	101.7+-7.87	7.7
	240.0	247.2+-7.7	3.1	262.1+-15.4	5.9
	483.0	554.6+-16.16	2.9	523.3+-30.0	5.7
	1725.0	1947.1+-48.45	2.5	1700.5+-120.3	7.1
everolimus	2.63	2.62+-0.22	8.3	2.7+-0.32	12.0
	4.80	4.68+-0.37	7.9	4.9+-0.40	8.3
	10.0	9.76+-0.54	5.6	10.1+-0.78	7.8
	35.8	38.54+-1.33	3.5	36.0+-1.57	4.4
sirolimus	2.65	2.8+-0.28	9.9	2.8+-0.34	11.6
	9.62	9.1+-0.49	5.4	10.0+-0.87	8.8
	19.40	19.8+-0.41	2.1	20.4+-1.76	8.6
	39.60	42.5+-1.68	4.0	40.9+-2.95	7.2
tacrolimus	2.47	2.4+-0.19	8.1	2.6+-0.30	11.7
	6.48	6.4+-0.21	9.2	6.5+-0.39	6.0
	13.92	13.7+-0.57	4.2	13.5+-0.82	6.1
	30.60	31.8+-0.67	2.1	28.9+-1.41	4.9

> ***The required time for the entire analytical procedure permits routine therapeutic drug monitoring of forty samples in a morning and this method has been successfully used to analyze more than 8000 whole blood samples from transplant recipients in a year.***

Separation Science / Spectroscopy Meetings Calender 2008/09

MEETING	VENUE	DATES	CONTACT
2008			
EAPCCT (European Association of Poisons Centers & Clinical Toxicology)	Seville, Spain	6th-9th May	www.eapcct.org
HPLC 2008	Baltimore, USA	10th-16th May	www.hplc2008.org
Focus (Association of Clinical Biochemists)	Birmingham, UK	18th-22nd May	www.focus-acb.org.uk
Advances in Stationary Phases for Liquid Chromatography	Reading, UK	21st-22nd May	www.chromsoc.com
32nd International Symposium on Capillary Chromatography and 5th GCxGC Symposium	Riva Del Garda, Italy	26th-28th May	www.richrom.com
Chromatographie Haute Résolution - Chromatographie Haute Vitesse	Lyon, France	29th May	www.afsep.com
56th ASMS Conference	Denver, USA	1st-5th June	www.asms.org
Forum Labo	Paris, France	3rd-6th June	www.forumlabo.com
19th International Symposium on Pharaceutical & Biomedical Analysis	Gdansk, Poland	8th-12th June	www.pba2008.com
International Symposium on HPTLC	Helsinki, Finland	11th-13th June	www.hptlc.com
ADME and Applications of Laboratory Automation	Beerse, Belgium	12th June	www.elrig.org
20th International Symposium on Chirality	Geneva, Switzerland	6th-9th July	www.chirality2008.org
BMSS Annual 3 day meeting	York, UK	7-10th September	www.bmss.org.uk
27th International Symposium on Chromatography (ISC2008)	Munster, Germany	21st-25th September	www.isc2008.de
Drug Discovery 2008	Bournemouth, England	23rd-24th September	www.elrig.org
2nd International Symposium on Green Chemistry	Zurich, Switzerland	1st-2nd October	www.greenchemistrygroup.org
Détecteurs - Nouveautés Technologiques	Lyon, France	9th October	www.afsep.com
MipTec 2008	Basel, Switzerland	14th-16th October	www.miptec.com
APCE 2008	Kaohsiung, Taiwan	2nd-5th November	www.tl.ntu.edu.tw/apce2008
25th Montreux International LCMS Symposium	Montreux, Switzerland	12th-14th November	www.iaeac.ch
Advances in BioSeparations	Harlow, Engalnd	13th Novemebr	www.chromsoc.com
Separations Science - State of the Nation	Liverpool, England	27th November	www.chromsoc.com
2009			
Pittsburgh Conference	Chicago, USA	8th-13th March	www,pittcon.org
57th ASMS	Philadelphia, USA	30th May-June 4th	www.asms.org
HPLC2009	Dresden, Germany	28th June - 2nd July	www.hplc2009.com
Euroanalysis 2009	Innsbruck, Austria	6th-10th September	www.euroanalysis2009.at