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INNOVATIONacademy

TDM of Triazole Antifungals

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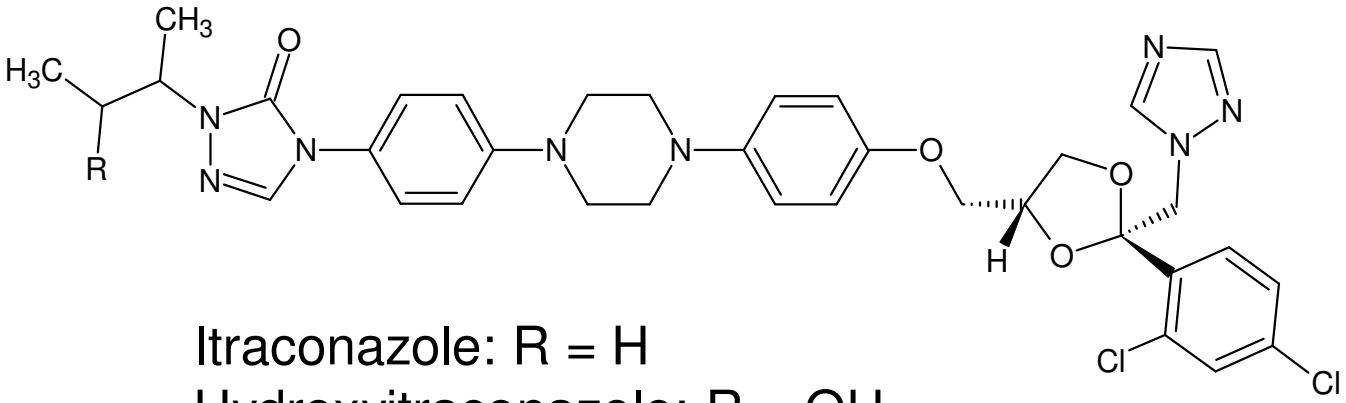
TDM of Triazoles

- Overview
  - Pharmacokinetics
  - Risk of drug-drug interactions
- Methodology for Therapeutic Drug Monitoring (TDM)
- Patient samples
  - Itraconazole
  - Posaconaxole
  - Voriconazole
- Internal calibration for LC/MS
- Conclusions

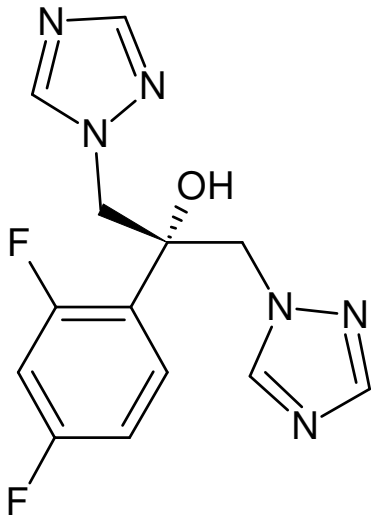
Overview

- Fungal pathogens recognised complication of organ transplantation & chemotherapeutic regimens
  - Can be associated with increased morbidity/mortality
- One option: Targeted prophylactic antifungal therapy
- Triazole antifungals act by inhibiting lanosterol 14-α-demethylase, giving rise to accumulation of toxic metabolites hence fungal cell death
- Treatment failure has been associated with lower average plasma triazole concentrations

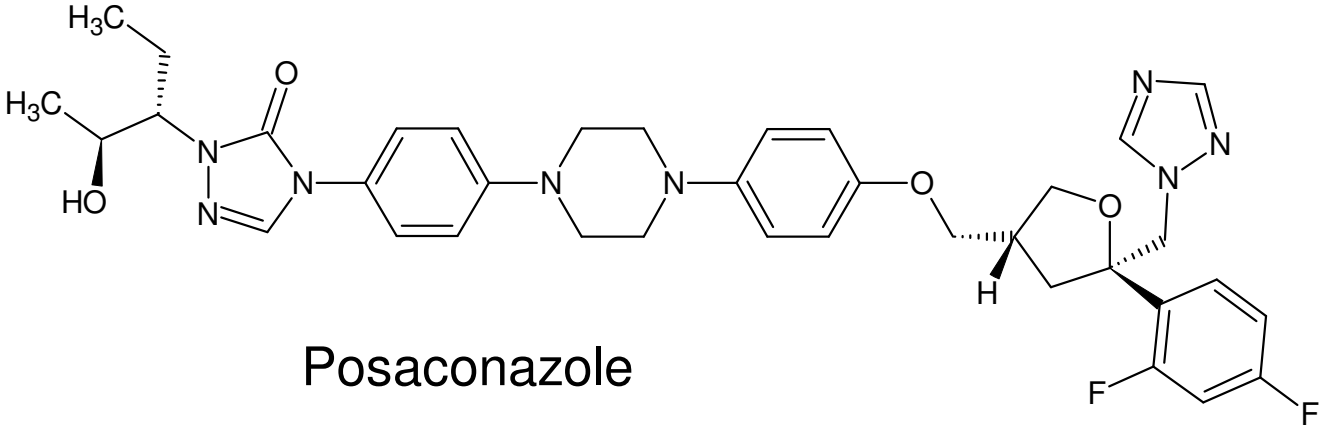
Triazole Antifungals



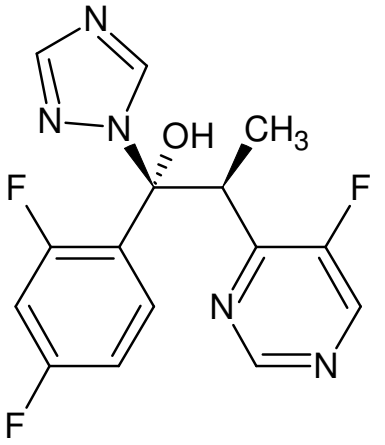
Itraconazole: R = H
Hydroxyitraconazole: R = OH



Fluconazole



Posaconazole



Voriconazole

Pharmacokinetics

- Variable bioavailability
  - Effect of food
    - Posaconazole: up to 4-fold increased absorption if taken with fatty meal
  - Effect of other drugs
    - Proton pump inhibitors: reduce posaconazole C_{max} by up to 50 %
    - Histamine receptor antagonists
- Variable metabolism
  - Genetic polymorphism: voriconazole (CYP 2C19)
    - Plasma concentrations in individuals prescribed the same dose may vary up to 100-fold
  - Drug-drug interactions

CYP Interaction Profiles of Triazoles

	CYP3A4		**CYP2C8/9**		**CYP2C19**	
	Inhibitor	Substrate	Inhibitor	Substrate	Inhibitor	Substrate
Fluconazole	**++**		**++**		**+**	**+**
Itraconazole	**+++**		**+++**		**+**	
Voriconazole	**+**	**+**	**++**	**+**	**++**	**+++**
Posaconazole	**++**					

+ weak; ++ moderate; +++ potent

Worth et al., Intern Med J 2008; 38: 521–37

Anal Bioanal Chem
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ORIGINAL PAPER

An automated method for the simultaneous measurement of azole antifungal drugs in human plasma or serum using turbulent flow liquid chromatography-tandem mass spectrometry

L. Couchman · S. L. Buckner · P. E. Morgan · M. M. Ceesay · A. Pagliuca · R. J. Flanagan

Analytical and Bioanalytical Chemistry 2012; 404: 513-23

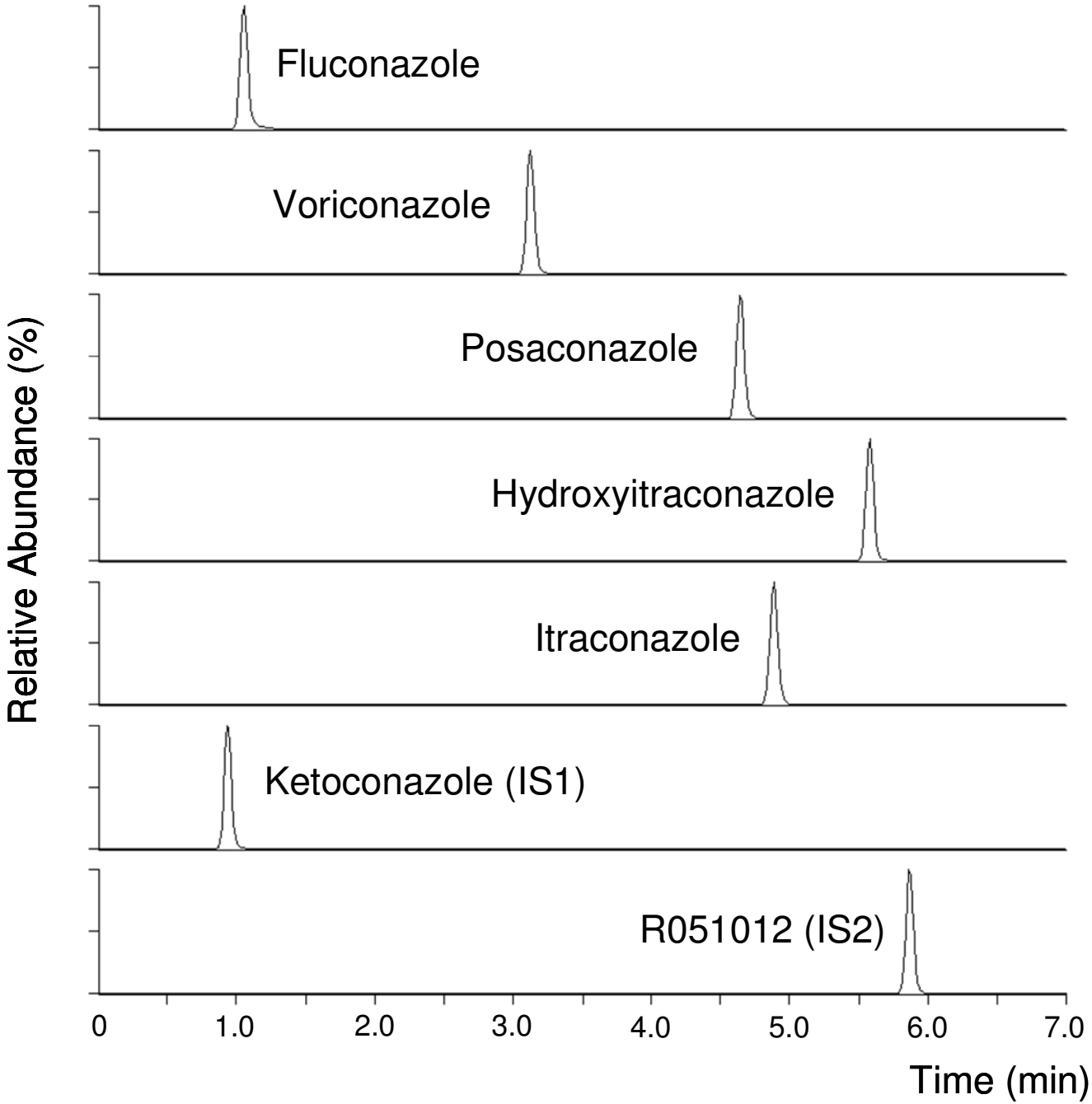
Triazoles: Methodology

- Aria Transcend TLX-II: 4 x Accela 600 Quaternary Pumps
- TSQ Vantage MS/MS (APCI, positive mode SRM)
- TurboFlow column: Cyclone C18-P-XL (50 x 0.5 mm i.d., ~ 50 µm, ambient temperature)
- Analytical column: Gemini C6 Phenyl (100 x 3 mm i.d., 3 µm, 40 °C)
- Total analysis time 12 min, data collected for 7 min per injection during multiplexing

Sample Preparation

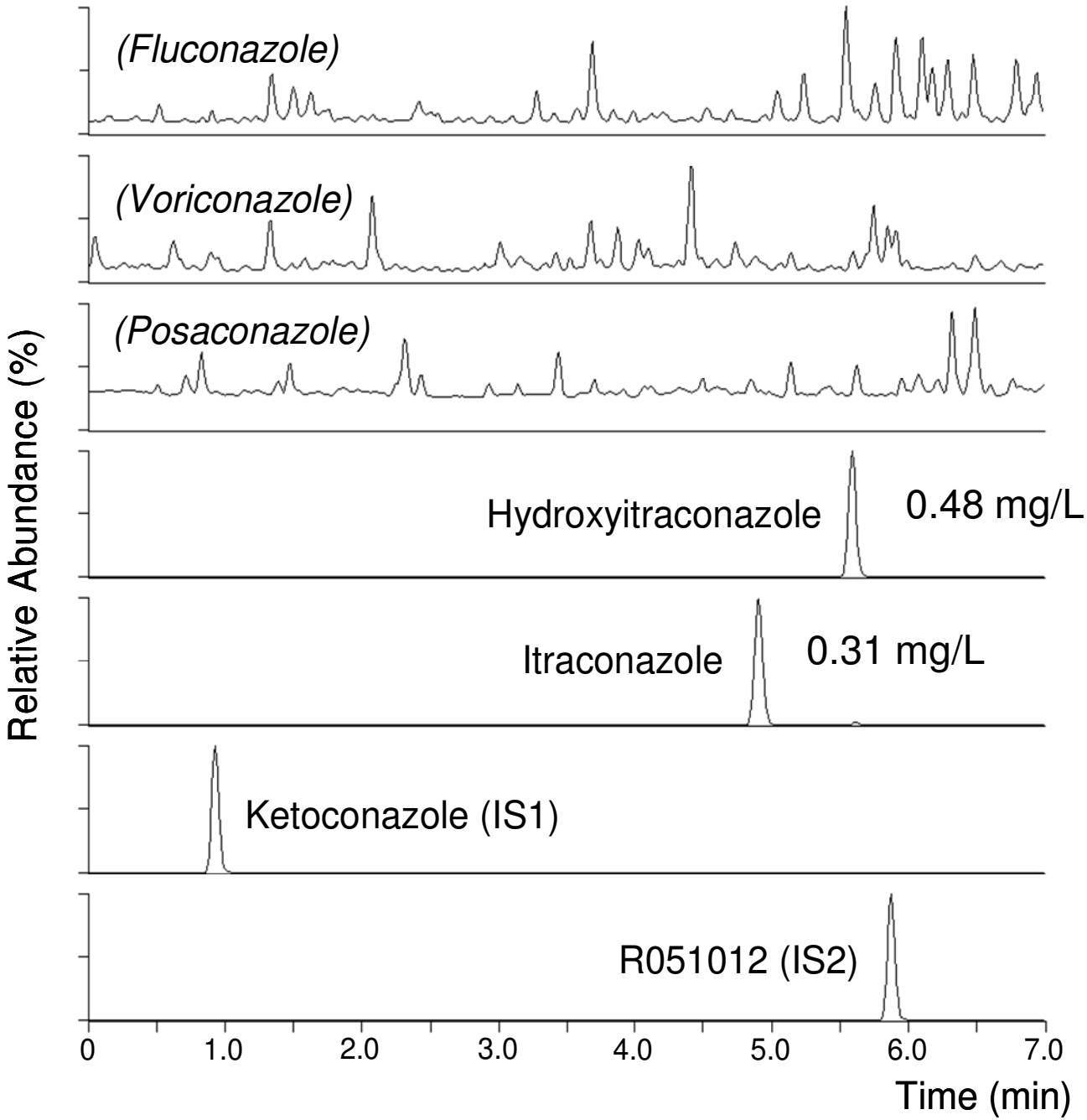
- 25 µL centrifuged plasma/serum
- 975 µL aqueous internal standard solution
- Mixed directly into autosampler vial using automated dilutor (Hamilton 530C)
- Inject 30 µL prepared sample

Azole Calibration Standard 5 (1.0 mg/L all analytes)



Typical patient sample

(400 mg/d itraconazole)



TurboFlow Technology

What does it offer?

- Direct injection of matrix after some pre-treatment
  - Serum/plasma
  - Whole blood
  - Saliva
  - Urine
- Reduced sample preparation time
- Minimise matrix effects
- Less operator/human error
- High throughput, especially with multiplexing

Practical Considerations

- Large sample dilution increases TurboFlow column life/assay reproducibility cf. direct injection of plasma/serum
- Dilution ensures disruption of plasma protein binding
- TurboFlow + HPLC to remove interference from metabolites (voriconazole *N*-oxide, posaconazole glucuronide, etc.)
- Method easily adapted for new compounds/active metabolites (e.g. isavuconazole, ravuconazole)
- Multiplexing maximises throughput, reduces solvent consumption/instrument time

Summary Clinical Results
(N = 373, 100 patients)

Analyte	Samples (patients)	Serum concentration (mg/L)	
		Median	Range
Fluconazole	60 (26)	6.12	0.85 – 18.0
Itraconazole	163 (47)*	0.58	0.02 – 5.31
OH-Itraconazole		0.85	0.02 – 4.42
Posaconazole	57 (12)	0.43	0.02 – 2.47
Voriconazole	45 (15)	0.92	0.04 – 3.99

* 2 samples (2 patients) OH-itraconazole detected, but itraconazole < 0.01 mg/L

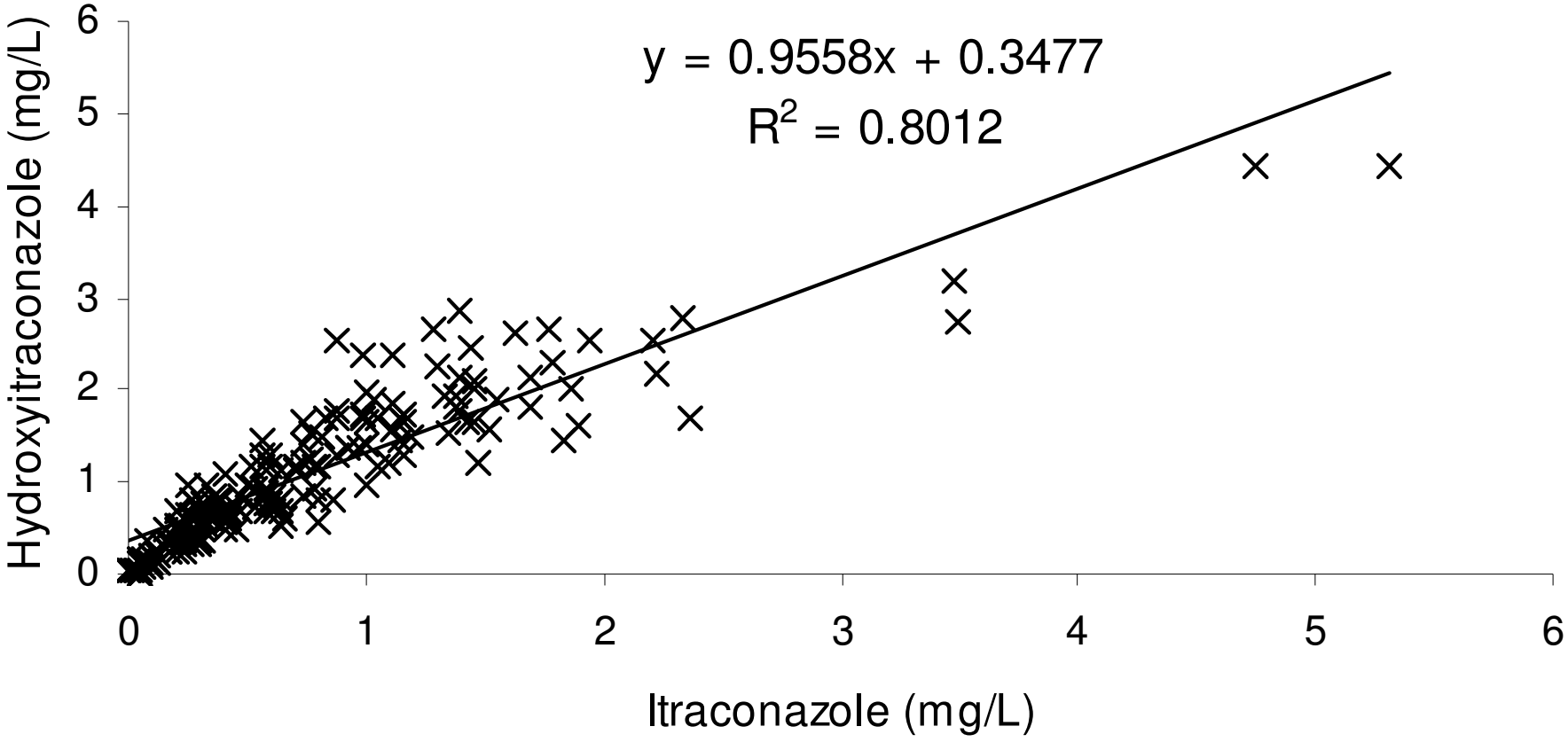
Exclusions: 24 samples (20 patients): more than one analyte > 0.01 mg/L (switching between drugs);

further 24 samples (13 patients) all analytes < 0.01 mg/L

Itraconazole

- No drug detected in 3 samples (3 patients) prescribed 400 mg/d, one of whom switched to i.v. itraconazole
- 163 samples (47 patients)
  - 70 (43 %) itraconazole concentrations < 0.5 mg/L
  - 158 (97 %) samples from patients prescribed 400 mg/d by mouth

Itraconazole/Hydroxyitraconazole



Posaconazole

- No drug detected in a sample from patient prescribed 800 mg/d (previous serum posaconazole 0.83 mg/L on same dose)
- 57 samples (12 patients)
  - 30 (53 %) posaconazole concentrations < 0.5 mg/L
  - 43 (75 %) posaconazole concentrations < 0.7 mg/L
  - 46 (81 %) samples from patients prescribed 600 mg/d by mouth
- Oral dosage unlikely to give plasma concentrations considered appropriate for antifungal activity in most patients

Itraconazole/Posaconazole in Children

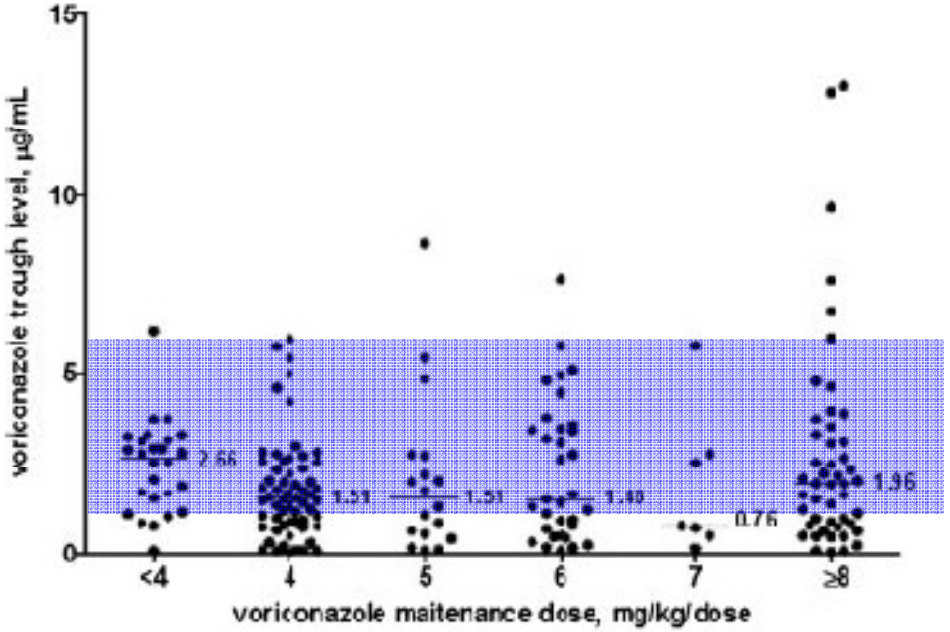
- Itraconazole
  - Possible that doses < 8 mg/kg too low for effective prophylaxis
- Posaconazole
  - Some case reports detailing efficacy & safety in children
  - Little known of Pk profile in children < 8 yr (limited data 8–16 yr)

Voriconazole

- 45 samples (45 patients) all prescribed 400 mg/d
  - 21 (47 %) voriconazole concentrations < 1 mg/L
  - 34 (76 %) voriconazole concentrations < 2 mg/L
  - No sample > 4 mg/L
- Suggested therapeutic ranges for voriconazole:
  - Matsumoto *et al.* (2009): 2 – 4 mg/L
  - Pascual *et al*. (2008): 1 – 6 mg/L

Voriconazole in Children

- Voriconazole
  - Linear PK in children
  - Walsh *et al.* (2004): standard adult dose (i.v.) → 3-fold lower plasma concentration
  - Oral bioavailability 45 % (cf. 96 % in adults)
- Trough concentration < 1 mg/L associated with 2.6 fold increased odds of mortality in children (Neely *et al.*, 2010)



Choi *et al.* Pediatr Blood Cancer 2013; 60: 82-7

Anal Bioanal Chem
DOI 10.1007/s00216-013-7361-8

RESEARCH PAPER

A novel approach to quantitative LC-MS/MS: therapeutic drug monitoring of clozapine and norclozapine using isotopic internal calibration

Lewis Couchman · Sarah L. Belsey · Simon A. Handley · Robert J. Flanagan

Assay Calibration

- FDA guidelines (batch processing):
  - Calibration curve ‘should consist of blank sample, zero sample, and 6–8 non-zero samples covering the expected range, including the LLoQ’
  - Calibrators analysed at the beginning and end of each batch, IQCs at regular intervals
  - Lengthy/expensive process not ideally suited to high-throughput applications
- One-point calibration
  - Compromise between desirable calibration and time/ workload
  - Best to have single calibrator in the mid-range
  - Peters & Maurer (2007): Large bias/imprecision observed for some drugs

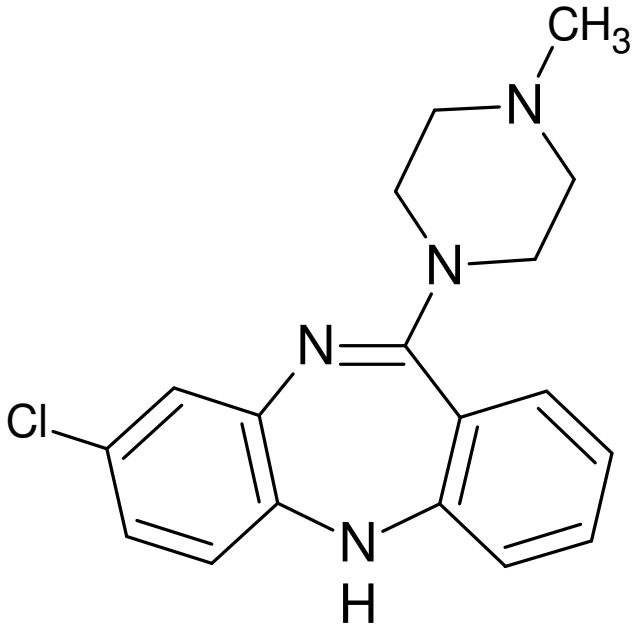
Internal Calibration (ICAL)

- Mixture of calibrators (isotopically-labelled/structurally similar analogues) at varying concentrations added to each sample
- Each injection: individual, multiple-point calibration curve produced
  - Based on the response of each of the chosen internal calibrators
  - Analyte concentrations calculated by interpolation

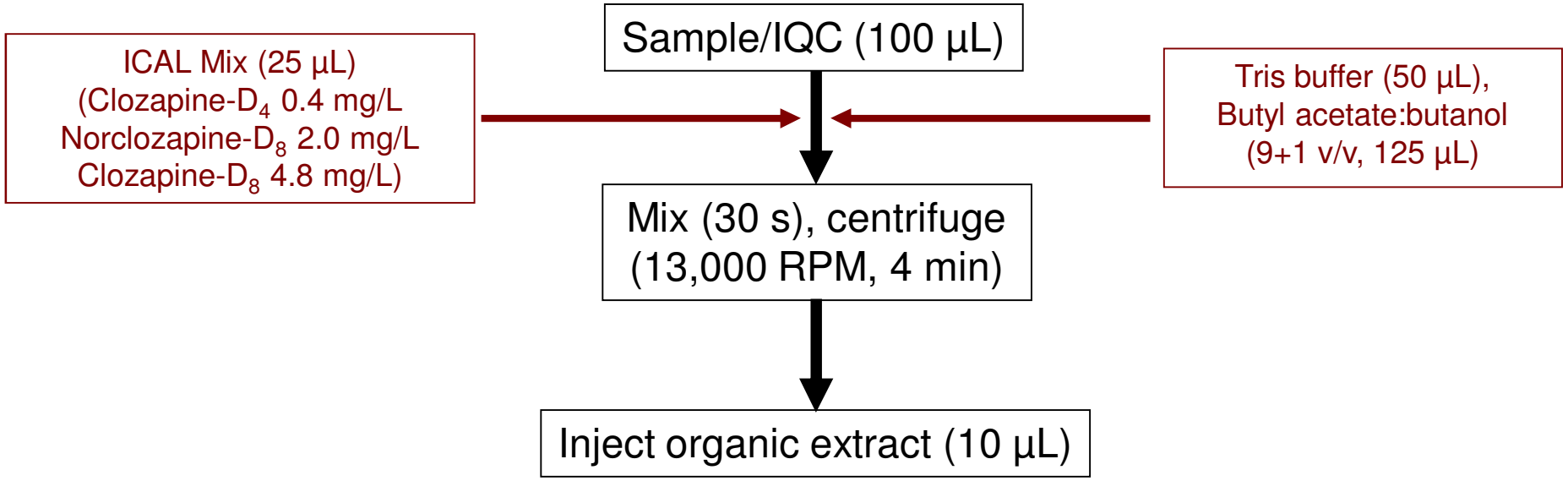
Assay Analytes & Calibrators

- Clozapine
- Norclozapine

- Clozapine-D_4 0.1 mg/L
- Norclozapine-D_8 0.5 mg/L
- Clozapine-D_8 1.2 mg/L



Sample Preparation



- Start of the day
  - IQCs
  - EQCs
- Prepare samples as they arrive in the laboratory
- IQCs repeated after every 20 samples

LC-MS/MS

- LC
  - Column: 100 x 2.1 mm i.d. S5SCX
  - Eluent: 35 mmol/L NH_4OAc/MeOH, pH 6.0
  - Flow rate: 0.5 mL/min

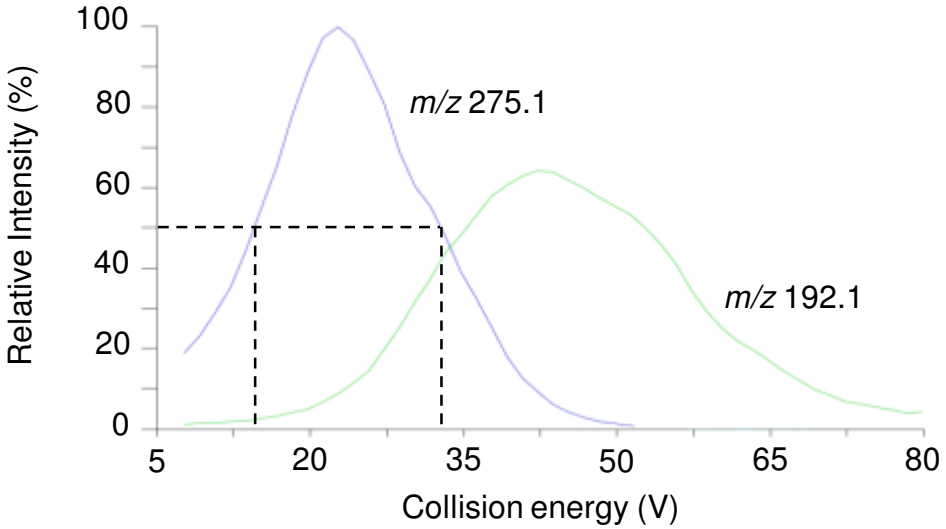
- MS
  - TSQ Quantum Access
  - APCI Positive Ionisation Mode
  - SRM transitions (quantifier and qualifier)

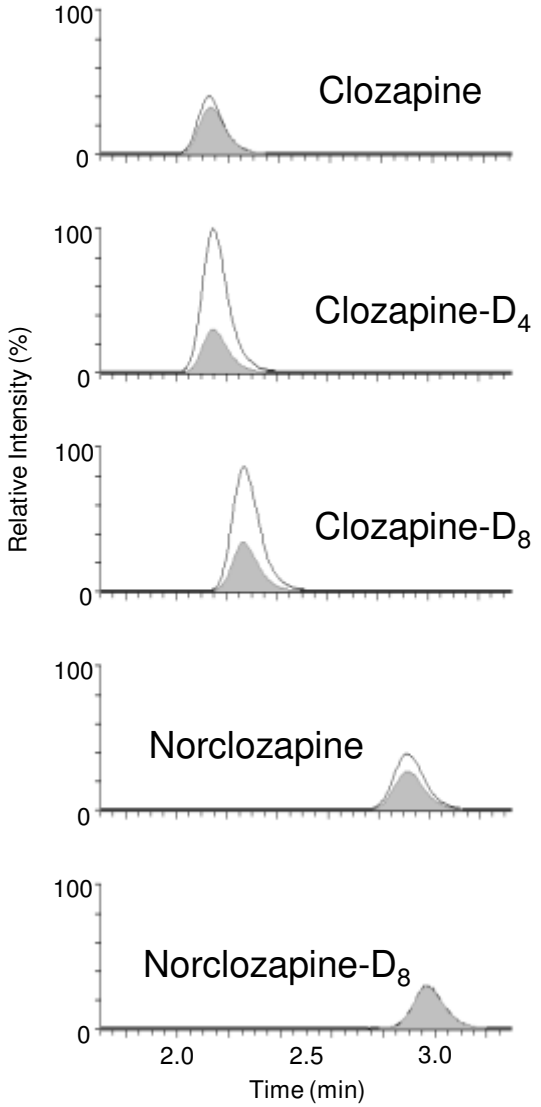
Method Development

- Equivalent instrument response at equal concentration
- Solution containing analytes and internal calibrators (all 1 mg/L)
- Different fragmentation characteristics
  - Mean response not equal for any compound
- Collision energy profiles for each compound
  - Adjusted to give equal responses
  - ‘Down-tune’ some analytes
  - Facilitated by isocratic LC system

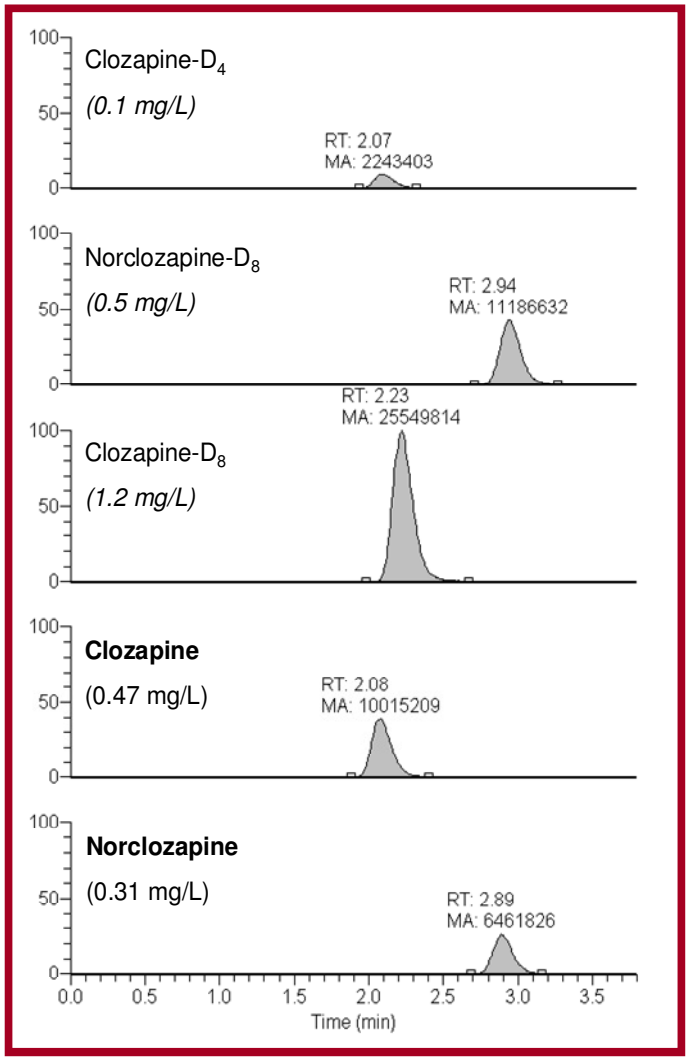
Adjusting Collision Energies

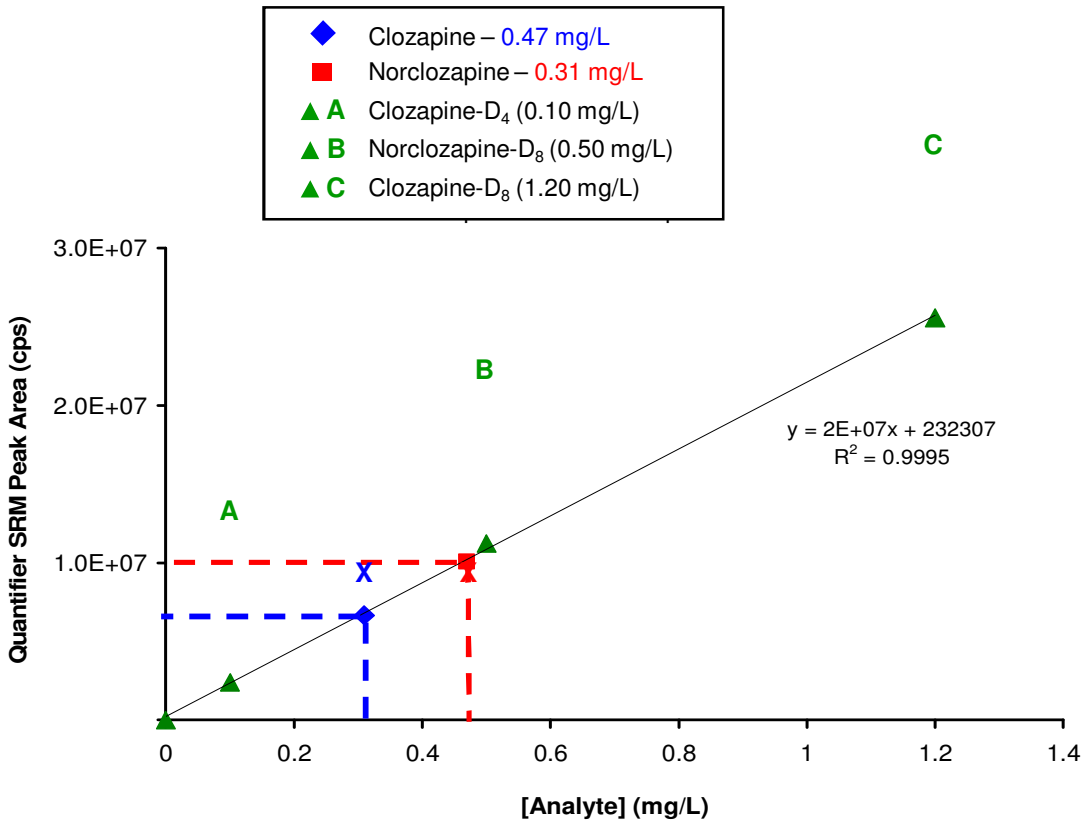
SRM fragmentation of clozapine-D8 (*m/z* 335.2 to product ions *m/z* 275.1 and 192.1)





Calculating Analyte Concentration

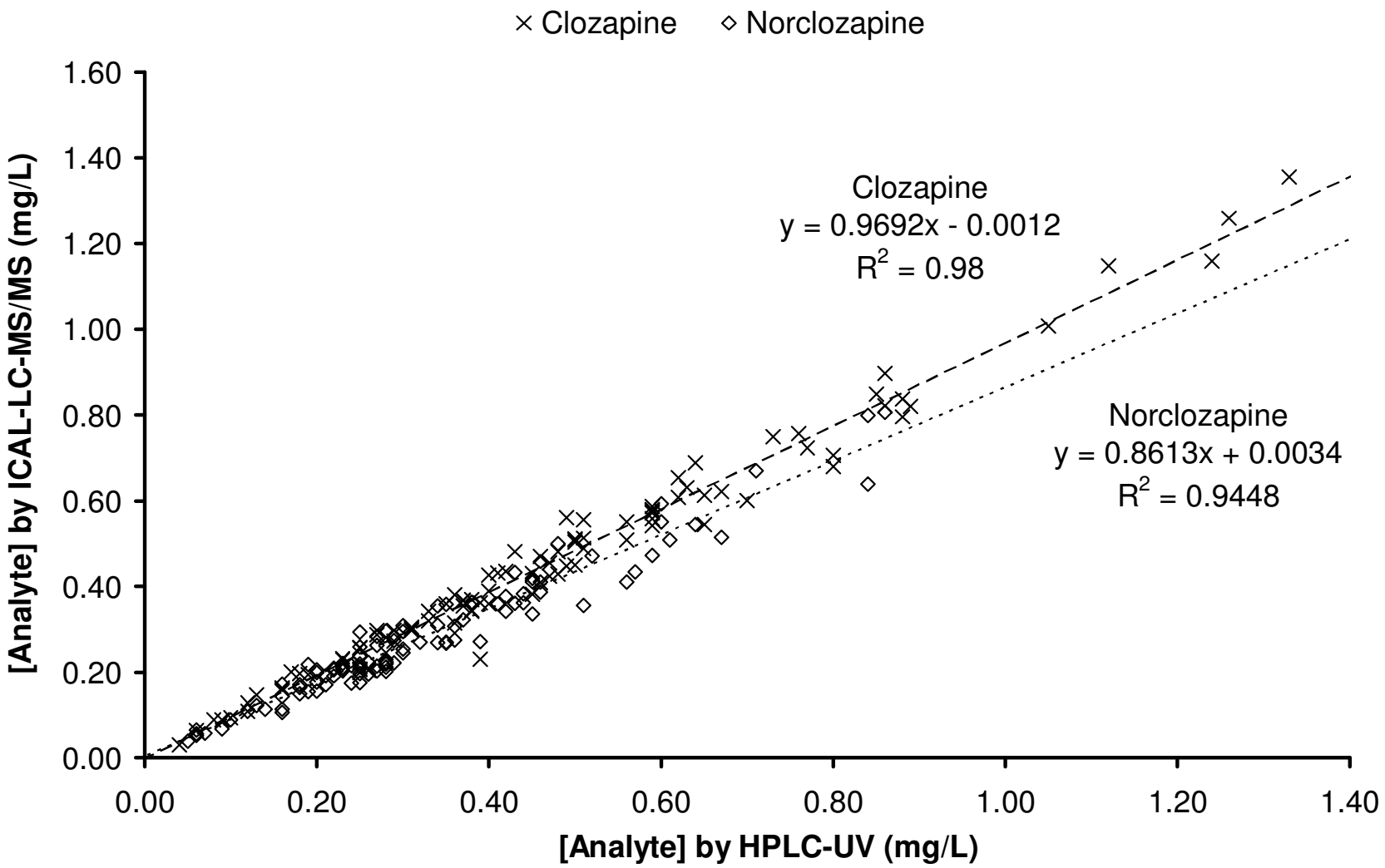




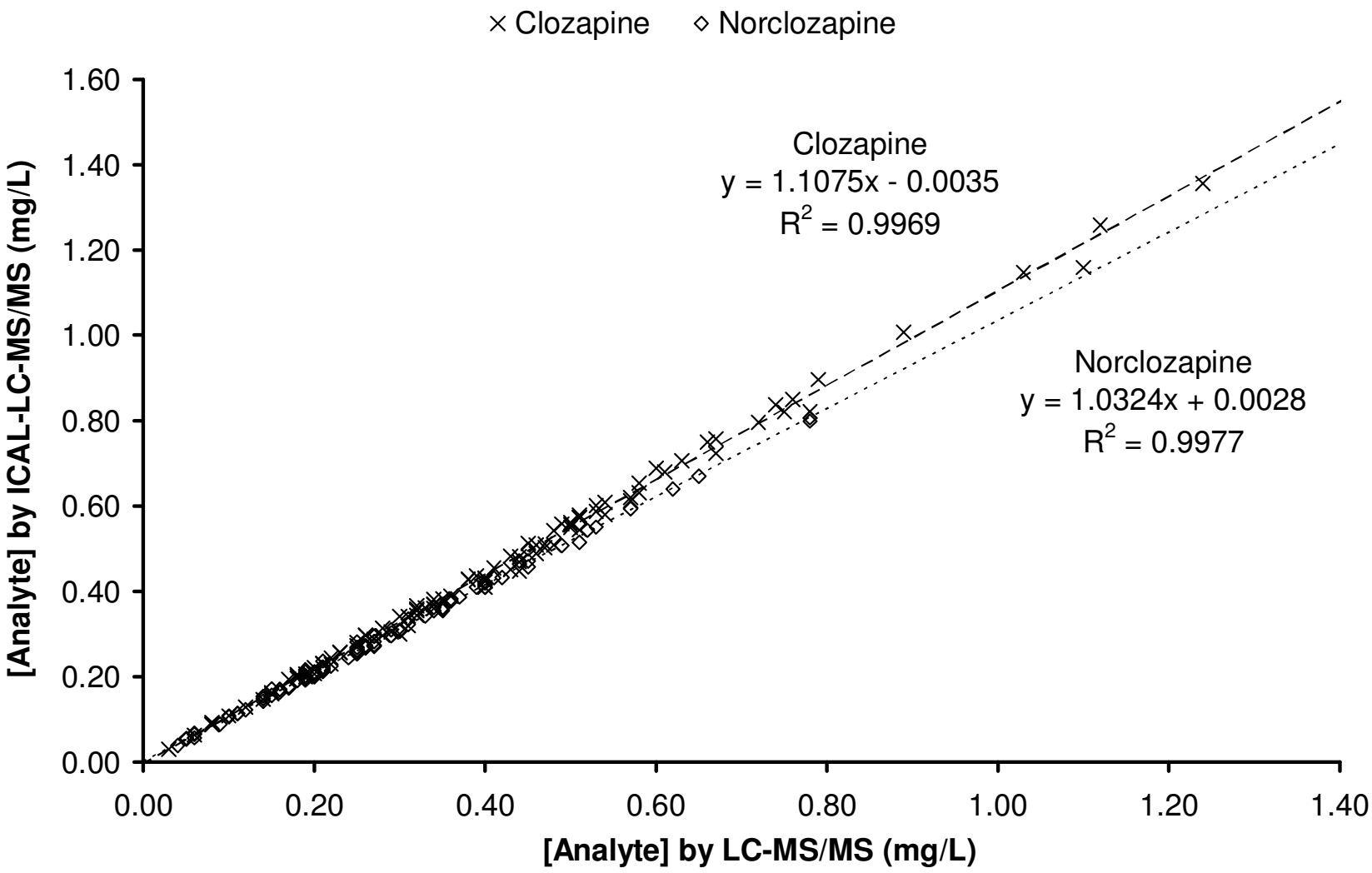
Method Validation

- Accuracy & Precision
  - Precision < 5 %
  - Accuracy 104–112 %
- LOD: 0.01 mg/L
  - 3.5 and 5.5 % for clozapine and norclozapine
- Recovery
  - Analytes & internal calibrators 90–106 %
- Matrix effects
  - None observed

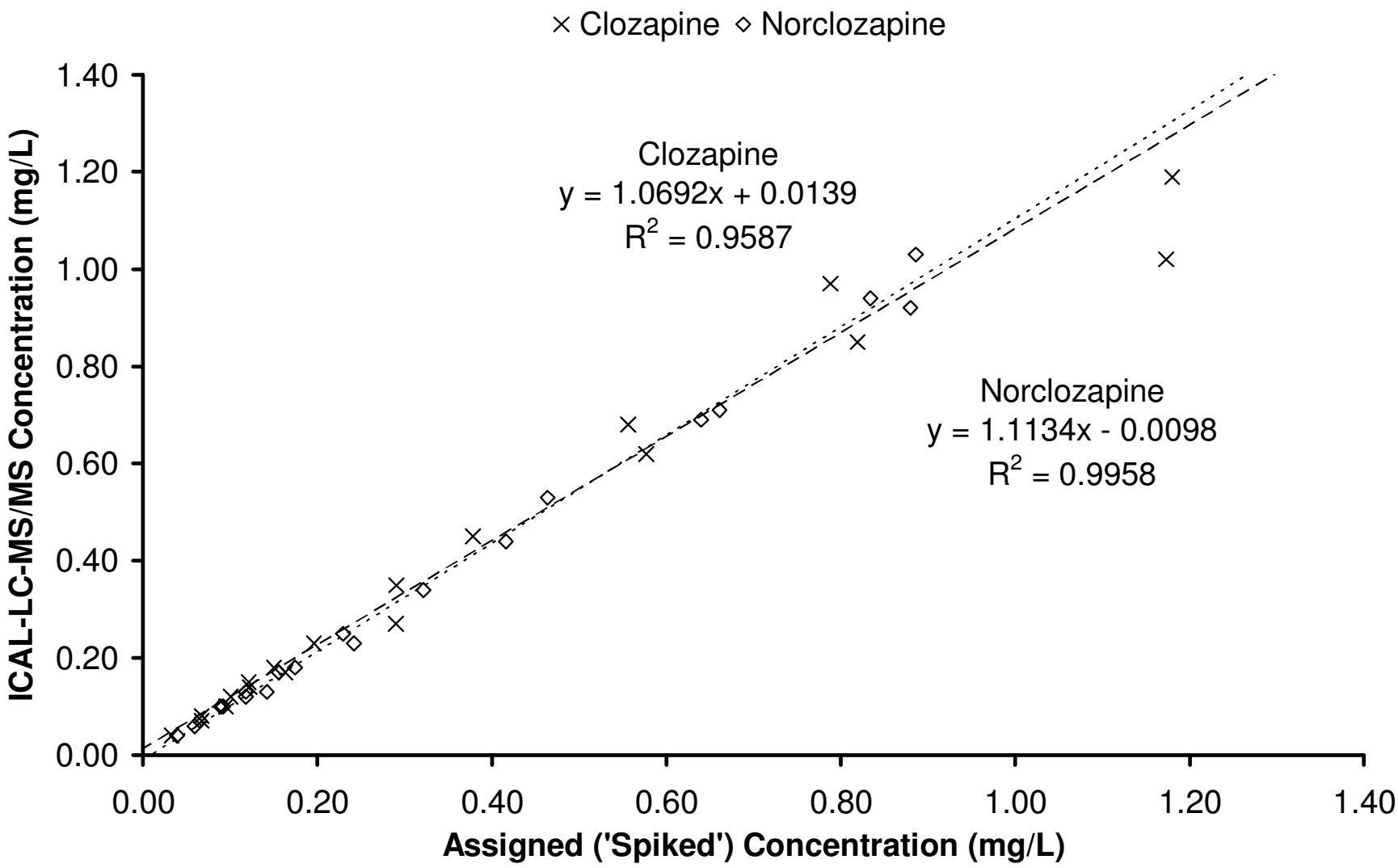
Patient Sample Comparison (N = 100)



Patient Sample Comparison (N = 100)



Heathcontrol Comparison (N = 22)



ICAL Summary

- Patient and EQA comparison: excellent agreement with existing LC-MS/MS and LC-UV methods
- Easily modified to include other analytes
- Standards do not need to be made up
  - Saves time & money
- Matrix effects minimised
  - Calibrators are in **exactly** the same matrix as the samples
- Same day reporting
- **Allows quantitative LC-MS/MS to become more akin to random-access clinical chemistry analyser**
- Caution: Stability of deuterated analogues

Conclusions – TDM Azole Antifungals

- TDM triazoles may be beneficial
  - Assessing adherence
  - Dose adjustment
  - Minimising the risk of dose-related toxicity (large inter-individual variability in clearance)
- Simple method
  - Realistic cost
  - Clinically useful turn-round time (3 days, less if more demand)
- TDM may become a valuable adjunct in the economic use of these agents

Acknowledgements

- Sarah Belsey, Lewis Couchman (KCH)
- Mansour Ceesay, Tony Pagliuca (Haematology, KCH)
- ThermoFisher Scientific