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A Potential Rapid and Sensitive LC-MS/MS Application for Quantification of Amlodipine Besylate Plasma Concentrations in Patients with Hypertension

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AMLODIPINE BESYLATE (AML)

- Long-acting 1,4-dihydropyridine calcium channel blocker

- Acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels

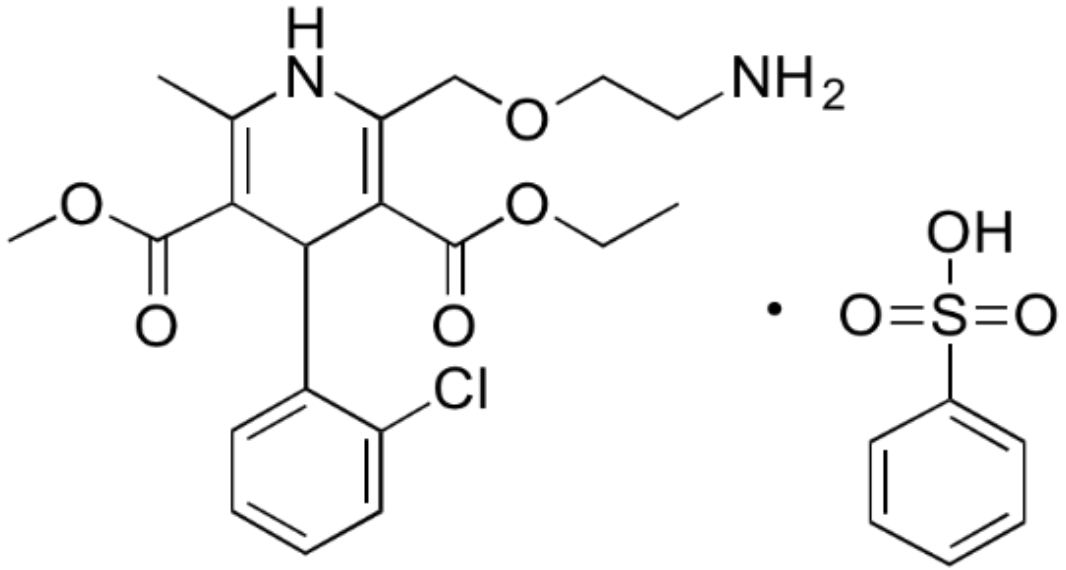

Figure 1: Chemical structure of Amlodipine Besylate (AML)

BURDEN OF HYPERTENSIVE DISEASE

NHMS 2015 HIGHLIGHTS

CARDIOVASCULAR DISEASES

DIABETES MELLITUS

- 17.5% (3.5 million) of adults 18 years and above have diabetes
- 8.3% are known diabetes
- 9.2% are previously undiagnosed with diabetes

HYPERTENSION

- 30.3% (6.1 million) of adults 18 years and above have hypertension
- 13.1% are known to have hypertension
- 17.2% are previously undiagnosed with hypertension

HYPERCHOLESTEROLEMIA

- 47.7% (9.6 million) of adults 18 years and above have hypercholesterolemia
- 9.1% are known to have hypercholesterolemia
- 38.6% are previously undiagnosed with hypercholesterolemia

CLINICAL PRACTICE GUIDELINES

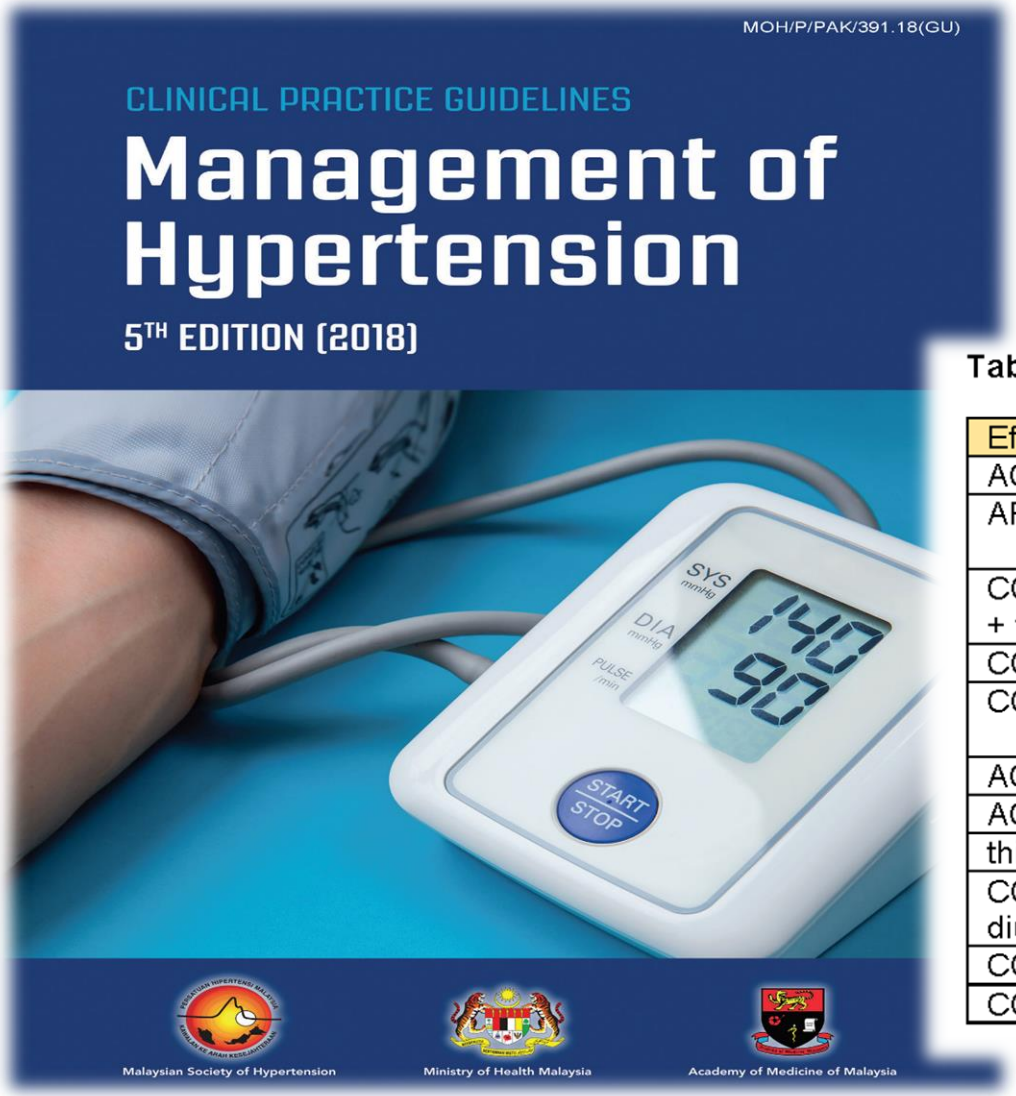

Table 5-A. Effective Anti-Hypertensive Combinations Used in Outcome Trials

Effective combination	Patients studied
ACEI + thiazide-like diuretics [79]	Post stroke
ARB + thiazide[82,112]	Hypertensive with Left Ventricular Hypertrophy. High risk hypertensives
CCB + ACEIs or ß-blocker + thiazide[80]	Patients with Coronary Artery Disease
CCB + thiazide[82]	High risk hypertensives
CCB + ACEI[110]	Medium risk hypertensives with no overt vascular diseases
ACEI + thiazide-like diuretics[83]	High risk hypertensives with diabetes
ACEI + CCB[84]	High risk hypertensives
thiazide-like diuretics + ACEI[113]	Very elderly (>80 years old)
CCB + thiazide or thiazide-like diuretics[114]	Medium risk hypertensives
CCB + ARB [114]	Medium risk hypertensives
CCB + ß – blocker[114]	Medium risk hypertensives

CLINICAL UTILITY

- 83.3% of the patients were prescribed on maximum dose of AML

- However, blood pressure control is still not satisfactory.

- Options:
  - Add other antihypertensive drugs
  - Assess patients compliance + decide to add antihypertensive drugs

High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine an

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Ravi Damani,[1] Joanne Hepworth[3] Nil
Webster Madir

ABSTRACT

Objectives Non-a
cause of suboptimal
practical tools exist
We used a simple u
prevalence of antihy

Conclusions Non-adherence to blood pressure lowering therapy is common, particularly in patients with suboptimal blood pressure control and those referred for

Biochemical Screening for Nonadherence Is Associated With Blood Pressure Reduction and Improvement in Adherence

Pankaj Gupta, Prashanth Patel, Branislav Štrauch, Florence Y. Lai, Artur Akbarov, Gaurav S. Gulsin, Alison Beech, Věra Marešová, Peter S. Topham, Adrian Stanley, Herbert Thurston, Paul R. Smith, Robert Horne, Jiří Widimský, Bernard Keavney, Anthony Heagerty, Nilesh J. Samani, Bryan Williams, Maciej Tomaszewski

Abstract—We hypothesized that screening
tandem mass spectrometry–based bioch
hypertensive patients. A retrospective a
conducted in 2 European countries (Unit
was diagnosed using biochemical anal

improvement in adherence and a clinically meaningful BP drop. We further show that a majority of initially nonadherent patients can successfully improve their adherence through repeated LC-MS/MS–based analysis and achieve BP targets similar to those who have been persistently adherent to treatment.

LCMS/MS -
Plasma Drug Level Quantification

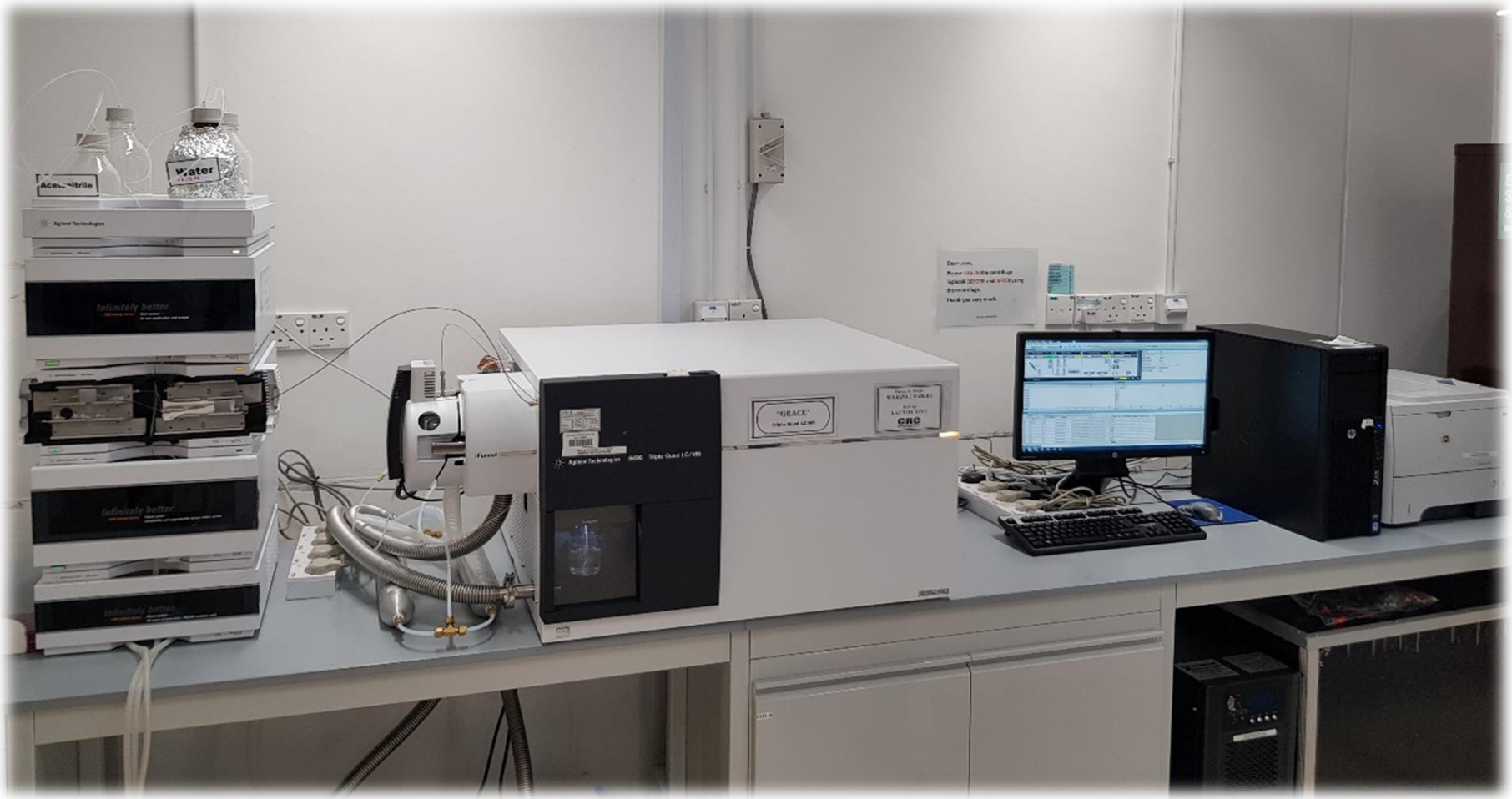


Figure 3: UHPLC Triple Quad LCMS System

OBJECTIVE

- To develop & validate an analytical method
- For rapid and sensitive determination of AML in human plasma, using LC-MS/MS for clinical application

METHODOLOGY (1)

Optimisation:

- Analytical standard AML (HPLC assay 99.9%)
- Carbamazepine (HPLC assay 99.0%) as internal standard (IS) at 5ng/mL.

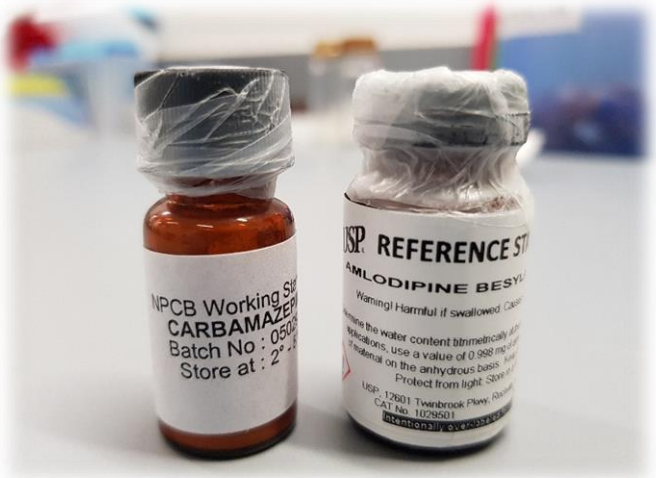


Figure 4: Internal standard & reference standard

METHODOLOGY (2)

- Column: Poroshell 120EC-C18, 2.1 x 50mm i.d., 2.7µm particle size
- Agilent 1290 Infinity Binary Liquid Chromatography System couple with a mass spectrometer (Agilent 6490 Triple Quadrupole LC/MS system)
- Assisted by:
  - 0.1% formic acid in ultrapure water
  - Pure acetonitrile
- Mobile phase: Isocratic elution
- Flow rate: 0.25mL/min

Figure 5: LC MS/MS column

METHODOLOGY (3)

Mass spectrometry conditions:

- MRM, positive ion mode
- Mass transition:

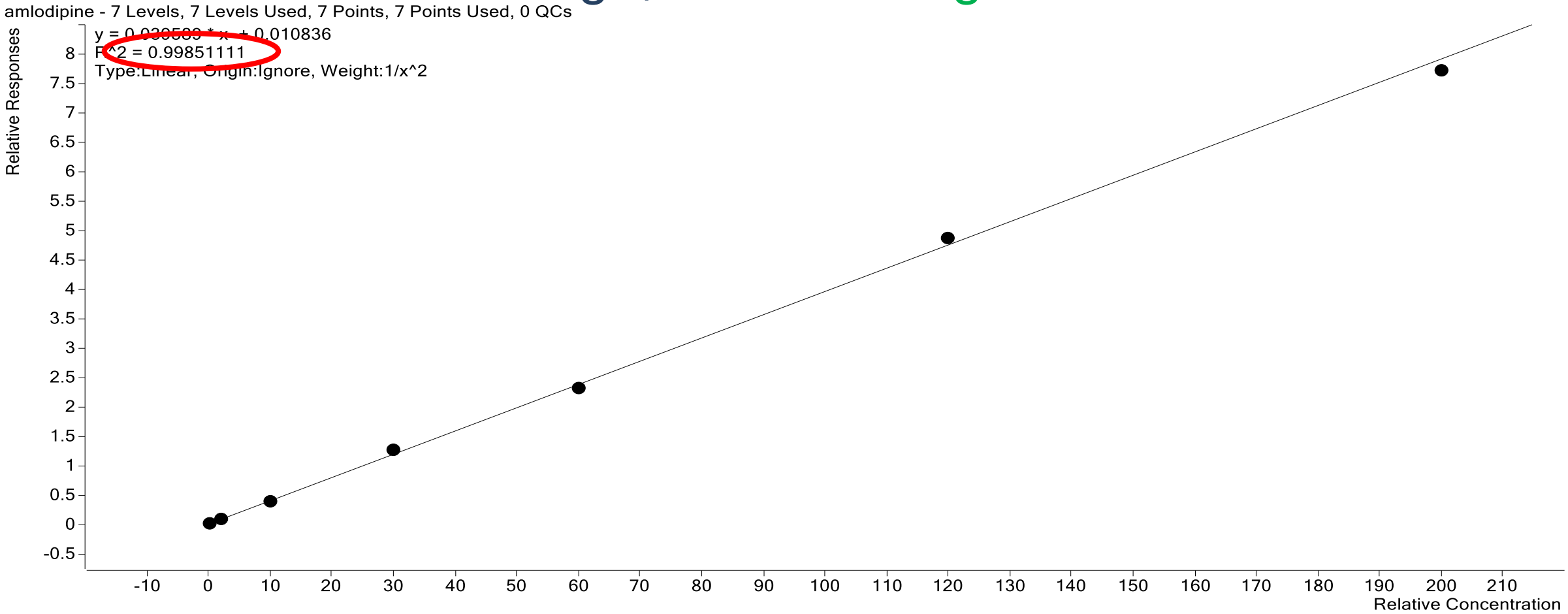
  AML m/z 409.1 → 294.0, 409.1 → 238.0

  Carbamazepine m/z 237.0 → 194.1, 237.0 → 179.0

- Sample preparation: Protein precipitation with acetonitrile
- Retention time: (0.965 min) ~1 minutes AML
- Total run time: ~3.5 minutes

METHOD VALIDATION (1)

- Concentration range, 0.5-500.0 ng/mL



METHOD VALIDATION (2)

		Amlodipine			
Nominal Concentration (ng/mL)	Day	Intra-Day		Inter-Day	
		% RE	%CV	% RE	%CV
LLOQ (0.5)	1	-0.836	5.47	-2.92	5.74
	2	-3.48	4.41		
	3	-4.45	7.22		
LQC (3)	1	2.53	6.81	-0.771	8.56
	2	-2.21	10.9		
	3	-2.63	8.16		
MQC (200)	1	-0.820	1.20	-6.93	5.53
	2	-10.0	2.15		
	3	-9.90	4.67		
HQC (400)	1	0.520	4.19	-1.49	5.54
	2	1.88	3.31		
	3	-6.87	4.69		
ULOQ (500)	1	5.77	2.06	-2.87	6.06
	2	-3.64	3.56		
	3	-10.7	3.34		

AFTER METHOD SUCCESSFULLY DEVELOPED AND VALIDATED

6 Hypertensive patients prescribed on AML therapy

READY FOR PLASMA QUANTITATION

RESULT (1) – Baseline Characteristics

Characteristic(s)		Mean (Std. Dev.) / Percentage
Age		51.80 (9.87)
Gender	Male Female	50% 50%
Race	Chinese Malay Iban	50% 33.3% 16.7%
Risk Factor	Dyslipidemia Diabetes	100% 66.7%

RESULT (2) – Blood Pressure & Plasma Concentration

Characteristic(s)		Mean (Std. Dev.)
Blood Pressure	SYSTOLIC	142.5 (12.3)
	DIASTOLIC	82.5 (14.7)
AML Plasma Concentration		14.92 ng/mL (8.70)
Range: 6.72 to 29.11 ng/mL		

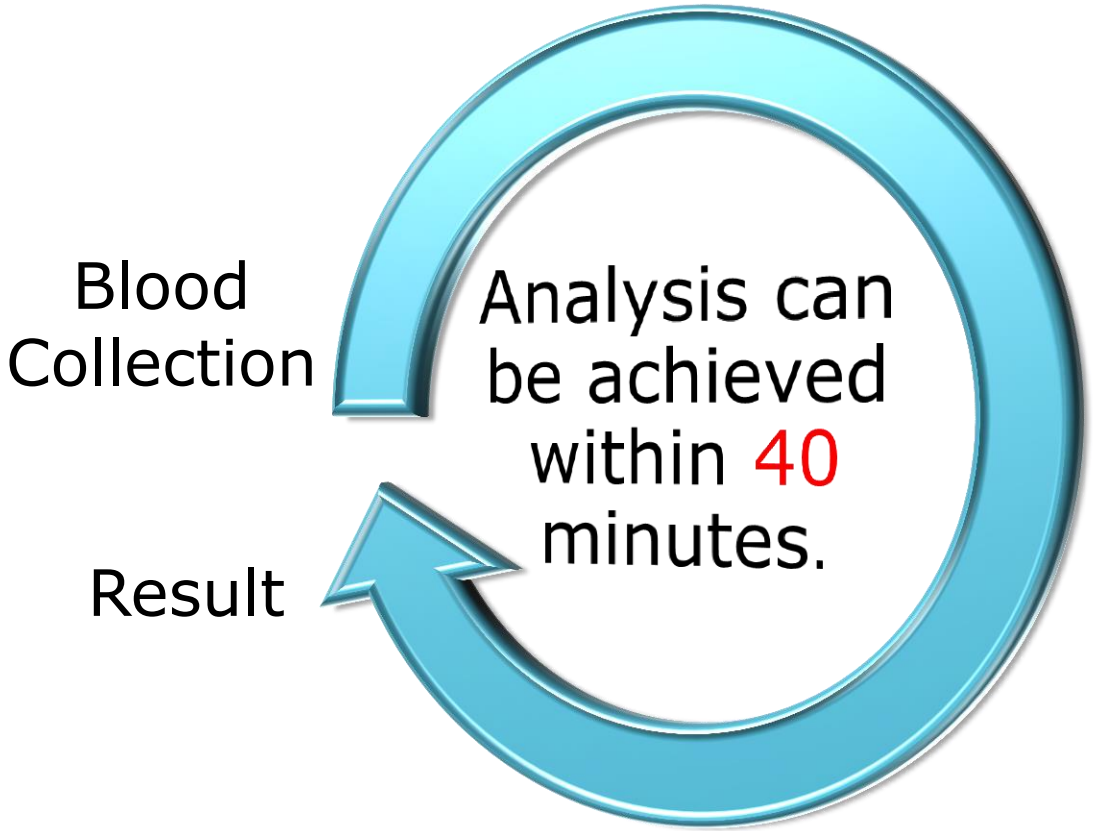
RESULT (3) – Inverse Relationship

Higher Plasma Concentration Of AML

Associated With

Lower Systolic and Diastolic BP

CONCLUSION (1)



- FAST
- SENSITIVE
- ACCURATE
- REPRODUCIBLE

CONCLUSION (2)

An inverse relationship was observed between plasma concentrations of AML and BP control.


THANK YOU