GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF CAPTOPRIL AND ITS APPLICATION IN PHARMACOKINETIC STUDIES IN HEALTHY SUBJECTS

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ABSTRACT

Captopril is a highly specific competitive inhibitor of the angiotensin-converting enzyme (ACE). This enzyme converts angiotensin I, a relatively inactive decapeptide, to angiotensin II, a potent endogenous vasoconstrictor substance. Captopril is widely used for the treatment of hypertension and congestive heart failure. This drug, which contains a sulphydryl group binds readily to albumin and to other plasma proteins. Captopril is unstable in blood and plasma ex vivo because of the reactivity of sulphydryl group which results in oxidation and rapid formation of disulphides. Therefore a fixative or a stabiliser must be added to each blood sample immediately following collection.

In this assay, N-ethylmaleimide (NEM) was used as a stabilizer in the collection tubes, prior to a rapid extraction technique by solid phase extraction (SPE). An enhanced and sensitive Gas Chromatography-Mass Spectrometry (GCMS) assay was developed for the quantitation of captopril. A commercially available internal standard, thiosalicylic acid (TSA) was used to minimise error in quantitation by GCMS. To enhance the volatility and gas chromatography elution properties, both captopril and TSA were derivatised to ester products by using pentafluorobenzylbromide. Captopril and TSA are quantitated as their bis-pentafluorobenzyl derivatives. The assay was linear from 1 to 160 ng/ml with mean recoveries of 104% and 99% for captopril and TSA, respectively, when the assay was carried out at 1.5, 75 and 150 ng/ml captopril . At the three concentrations of captopril mentioned, the coefficient of variation (CV) for inter–assay precision was 7.4, 9.5, and 4.4% respectively while accuracy was 6.1, 7.4, and 5.7%, respectively.

Four sets of bioequivalence studies were conducted for four generic captopril products with three products containing 25 mg captopril and one product containing 12.5 mg captopril. Bioequivalence studies were carried out in accordance with the Malaysian guideline on Good Clinical Practice (GCP) (adopted from ICH) which incorporates the Declaration of Helsinki as well as being accordance with standard operating procedures (SOP). All the generic products were found to be bioequivalent with the reference products based on statistical assessment of three parameters, namely T_{max}, C_{max} and AUC.

The ratio test over reference (T/R) for T_{max}, C_{max} and AUC were very close to 1 with the values ranging from 0.9 to 1.4, 0.9 to 1.1 and 1.0 to 1.1 for T_{max}, C_{max} and AUC, respectively. The mean values for T_{max}, C_{max} and AUC for 25 mg dose of brand leader and generic products was very similar. The values were 0.7, 117.1 and 212.6 for brand leader and 0.7, 120.5 and 212.6 for generic products. This is the same with 12.5 mg dose, where the mean value for T_{max}, C_{max} and AUC were 0.8, 55.6 and 115.7 for Brand leader, while the generic products were 0.8, 48.9, and 106.4, respectively.

A standard two-stage analysis of pharmacokinetic parameters obtained from the oequivalence studies carried out, indicated that the clearance (CL), half-life ($t_{1/2}$) and elimination rate constant (K) in the 66 subjects studied, were within the range obtained from published data.

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ABBREVIATIONS

ACE	angiotensin converting enzyme
RAAS	renin – angiotensin-aldosterone system
FDA	food and drug administration
SH	sulphydryl
CHF	congestive heart failure
CONSENSUS	cooperative north Scandinavian enalapril survival study
SOLVD	studies of left ventricular dysfunction
MI	myocardial infarction
GCMS	gas chromatography-mass spectrometry
HPLC	high performance liquid chromatography
LOQ	limit of quantitation
SD	standard deviation
CV	coefficient of variation
SEM	standard error of the mean
MRT	mean residence time
C_{max}	maximum plasma concentration
T_{max}	time to achieve peak plasma concentration
AUC	area under the plasma concentration-time curve
AUC_{0-t}	area under the plasma concentration-time curve from time zero to the last measurable concentration
$AUC_{0-\infty}$	area under the plasma concentration curve from time zero to infinity
K	elimination rate constant

$t_{1/2}$	elimination/terminal half-life
V_d	Volume of distributioin
CL	clearance
BE	bioequivalence
DCA	drug control authority
T	test
R	reference
ANOVA	analysis of variance
CI	confidence interval
α	significance level
CIC	clinical investigation centre
SUCXeS	Shimadzu UMMC centre for xenobiotic studies
GMP	good manufacturing practice
ICH	International Conference of Harmonisation
GCP	good clinical practice
MESC	University Malaya Medical Ethics Sub-Committee
SOP	standard operating procedure
COA	certificate of analysis
CRFs	case record form