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ROLE OF LC-MS IN PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES IN BIOANALYSIS: A REVIEW

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ABSTRACT

Bioanalysis is a subdiscipline of analytical chemistry for the quantitative measurement of xenobiotics (chemically synthesized or naturally extracted drug candidates and genetically produced biological molecules and their metabolites or post-translational modified products, biotics (macromolecules, proteins, DNA, large molecule drugs, metabolites) in biological systems. LC-MS is analytical tool of choice for selective and sensitive detection of compound of interest in difficult matrices. The focus of LC-MS bio analysis in the pharmaceutical industry is to provide a quantitative measurement of active drug and/orits metabolite for the accurate assessment of pharmacokinetics, toxicokinetics, bioequivalence (BE) and exposure response relationships. Clinical pharmacokinetics is the science of rate of movement of the drugs within biological system whereas pharmacodynamics is the study of biochemical and physiologic processes underlying drug action. The quality of these studies, which are often used to support regulatory filings and other evaluations, is directly related to the conduct of underlying bio analysis.

INTRODUCTION

Bioanalysis is a sub discipline of analytical chemistry for the quantitative measurement of xenobiotics (chemically synthesized or naturally extracted drug candidates and genetically produced biological molecules and their metabolites or post-translation ally modified products, biotics (macromolecules, proteins, DNA, large molecule drugs, metabolites) in biological systems..LC-MS is analytical tool of choice for selective and sensitive detection of compound of interest in difficult matrices. Clinical pharmacokinetics is the science of rate of movement of drugs within biological system. Pharmacodynamics is the study of biochemical and physiologic processes underlying drug action. The focus of LC-MS bioanalysis in the pharmaceutical industry is to provide a quantitative measurement of the active drug and/or its metabolite(s) for the accurate assessment of pharmacokinetics, toxic kinetics, bioequivalence (BE),andexposure–response(pharmacokinetics/pharmacodynamics) relationships. The quality of these studies, which are often used to support regulatory filings and other evaluations, is directly related to the conduct of the underlying bioanalysis.

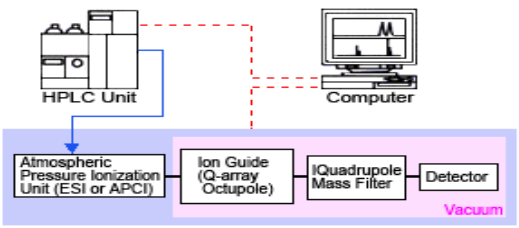

Fig 1: LC –MS schematic

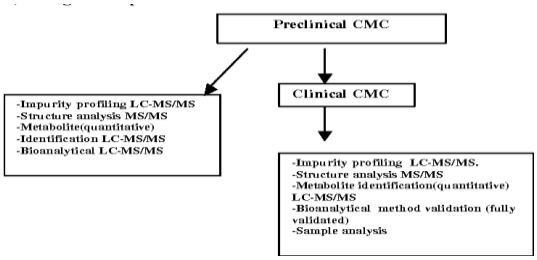

Fig 2: Role of LC/MS at different stages of drug development

CASE STUDY:

To explain pharmacokinetic interactions, a pharmacokinetic study was designed. A sensitive bio-analytical method was therefore developed and validated for 5fluorouracil and methotrexate in mouse plasma, brain and urine with liquid chromatography coupled to a single quadrupole mass spectrometer. Chromatographic separation was accomplished with isocratic elution (5 mM ammonium acetate and methanol, 70:30, %v/v) at a flow rate of 300 µL/min. The limit of quantitation for both drugs was 15.6 ng/mL (plasma and brain) and 78.1 ng/mL (urine), with interday and intraday precision and accuracy ≤15% and a total run time of 6 min. However, there are very few sensitive analytical methods reported for a truly simultaneous determination of 5-FU and MTX in complex biological matrices such as the plasma, brain and urine. This sensitive bio-analytical method was applied in the PK analysis for 5-FU and MTX in male Swiss–Webster mice (n = 3). This method allowed characterization of the brain concentrations of 5-FU over a period of 24 h, which has not been reported extensively in the past.

Liquid Chromatography and Mass Spectrometry parameter

The liquid chromatography (LC) system used was an Agilent 1100 series HPLC system (Agilent® Technologies). For chromatographic separation an Agilent® Zorbax® SB-C18 (3.5 µm, 150 × 3 mm) analytical column coupled with a C18 guard cartridge (4 × 2.0 mm; Phenomenex, Torrance, CA, USA) was used.The mass spectrometry (MS) system used was an Agilent MSD SL-G1946D (Agilent® Technologies).These parameters were applicable for all matrices (plasma, brain, urine) and were as follows: fragmentor voltage, 100 V; drying gas flow rate, 8 L/min; gas temperature, 250°C; nebulizer pressure, 40 psig; capillary voltage, 2500 V (±). Analysis for 5-FU and 5-BU was done in a negative ion mode with single ion monitoring (SIM) values of 129 and 189, respectively. The ionization source used for the method was electrospray ionization.

Application to PK study

Noncompartmental analysis (NCA) for 5-FU and MTX was used to obtain primary and secondary PK parameter. Average (n = 3) plasma and tissue concentration data were used for the NCA performed by WinNonlin® version 6.0 (Phoenix).Area under the curve .(AUC0–∞) and area under the first moment curve (AUMC0–∞) were obtained by integrating concentration–time (C–t) data in plasma and brain from time zero to infinity. . Plasma and brain C–t profiles of animals receiving single i.v. bolus doses of 75 mg/kg 5-FU and 32 mg/kg MTX are shown in Fig. 1. Characterization of the profile of 5-FU in the brain opens up new avenues for the study of 5-FU-related neurotoxicity with or without other drugs such as MTX. Such a drugdrug interaction (DDI) study will be extremely crucial in providing a novel perspective to explain increased cognitive deficits observed in mice receiving a combination of 5-FU and MTX. A simple and sensitive high performance liquid chromatography-electrospray ionization mass spectrometry method has been evaluated for the assignment of clonidine hydrochloride in human plasma

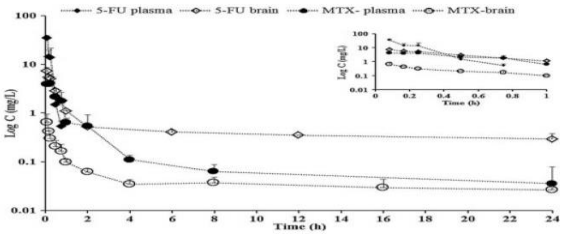

Fig 3: Concentration–time profiles for 5-FU and MTX in mouse plasma and brain.

CASE STUDY 2: LC-MS Method for Studying the Pharmacokinetics and Bioequivalence of Clonidine Hydrochloride in Healthy Male Volunteers

Instrumentation and operating condition: The analytical column used was a C18 column (Company, ZORBAX- XDB-ODS, USA) (2.1 mm□30 mm, 3.5 □) and was operated at 250ºC. The mobile phase consisted of acetonitrile-water 60:40 (v/v) and 0.2% formic acid was set at a flow rate of 0.2 ml/min. Mass Spectrometric (MS) detection was performed using a triple-quadruple mass spectrometer (Agilent Technologies, model LCMS-6410) with an Electrospray Ionization (ESI) interface. The ESI source was set at positive ionization mode. The (M +H)+ m/z 230 for clonidine was selected as detecting ions (m/z 230.0 →213).

Pharmacokinetic study: The pharmacokinetic parameters for clonidine were designed using standard non-compartmental methods. The peak serum concentration (Cmax) and the time to reach it (Tmax) were evaluated from visual examination of the data and used as criteria of the rate of absorption. The apparent elimination rate constant (β) was determined by linear regression of log-transformed data in the terminal phase of the serum concentrationtime profile. The pharmacokinetic profiles of clonidine from two tablet formulations were compared and the comparative bioavailability of test/reference product was calculated using the ratio of AUC0-f (test)/AUC0-C (reference).

CASE STUDY 3: Simultaneous pharmacokinetic and pharmacodynamic analysis of 5α-reductase inhibitors and androgens by liquid chromatography tandem mass spectrometry**.** Benign prostatic hyperplasia and prostate cancer can be treated with the 5α-reductase inhibitors, finasteride and dutasteride, when pharmacodynamic biomarkers are useful in assessing response. A novel method was developed to measure the substrates and products of 5α-reductases (testosterone, 5α-dihydrotestosterone (DHT), androstenedione) and finasteride and dutasteride simultaneously by liquid chromatography tandem mass spectrometry

Mass spectrometric conditions and fragmentation of analytes: All analytes and internal standards in solution ionised to form their protonated molecular ions in both positive electrospray (ESI) and APCI modes

Chromatographic conditions: Reported methods for detection of individual analytes predominantly use C18 columns, though these varied in length from 50 mm [15–17] to 150 mm. Acetonitrile has often been selected as the organic component of the mobile for analysis of dutasteride and finasteride [18–20], and methanol for androgens.Ionisation was improved when formic acid was added as a modifier, with 0.1% yielding maximum responses, while still retaining consistency in chromatographic separation.

Pharmacodynamic assessments in clinical samples: The assay presented was applied to a clinical research study with male volunteers studied prior to and following three months of finasteride (5mg daily) or dutasteride (0.5mg daily) [12]. Dutasteride and finasteride treatment resulted in a 46.5% and 47.1% suppression of DHT concentrations respectively. The concentrations of dutasteride achieved would be anticipated to inhibit both isozymes of 5αreducatse effectively. The reduction in DHT was accompanied by an increase in the concentrations of the enzyme substrate, testosterone, following treatment with both finasteride and dutasteride, of a similar magnitude to previously report.

CASE STUDY 4: Pharmacokinetic Evaluation of Empagliflozin in Healthy Volunteers Using LC-MS/MS and Comparison with Other Ethnic Populations. The present study considered the pharmacokinetic evaluation of empagliflozin after administration to Egyptian volunteers, and the results were compared with other ethnic populations. The FDA recognizes that standard methods of defining racial

subgroups are necessary to compare results across pharmacokinetic studies and to assess potential subgroup differences. A fully validated bioanalytical method is a prerequisite to perform a successful pharmacokinetic study.

Chromatographic and mass spectrometric conditions: A mixture of deionized water and acetonitrile in the ratio of (10:90, v/v) was used as the mobile phase. The column temperature was kept at 25 °C, the injection volume used was 10 µL, and the flow rate was 0.3 mL/min with 1.5 min as the run time. The main pharmacokinetic parameters of the study, Cmax, Tmax, t1/2, elimination rate constant, AUC0-t and AUC0-inf, were estimated using validated Excel software. For the extraction procedure, liquid-liquid extraction was tried using ethyl acetate, dichloromethane, and diethyl ether, and the best results were obtained with TBME. Molecular ions of 449.01 and 407.00 were observed for EG and DG, respectively, on the full scan mass spectra . The optimized collision energy produced significant fragments. The MS/MS transition of 449.01 → 371.21 and 407.00 → 328.81 for EG and the IS, respectively, were selected.

Pharmacokinetic evaluation of EG:

Development of correlations between drug concentrations and their pharmacologic responses enable clinicians to apply pharmacokinetic principles to actual patient situations. Pharmacokinetic studies are necessary for the submission of a new drug application (NDA) to the FDA and for re-examination of approved drugs. For extrapolation of clinical data from other countries, ethnic differences in pharmacokinetics must be discussed. The insulin-independent mechanism of action of EG contributes to a low risk of hypoglycemia that was proved by monitoring of blood glucose level of all volunteers while carrying out the study, and the results were in the normal range. Pharmacokinetic parameters can vary between different races, and the present analysis was the first study carried out on Egyptian volunteers and compared with the results obtained from other ethnic populations. There is no significant difference was observed between the studied group and the compared ethnic group which suggests that no dose adjustment should be considered with administration of 25 mg EG to Egyptian population.

Pharmacokinetic parameters	Empagliflozin
C_{max} (nMol/L), Mean ± S.D. (% C.V.)	576 ± 86 (14.93)
T_{max} (hours), Median (range)	1.5, (1–2)
$t_{1/2}$ (hours), Mean ± S.D. (% C.V.)	6.1 ± 1.2 (19.67)
Elimination rate constant (h^{-1}), Mean ± S.D. (% C.V.)	0.10012 ± 0.02156 (19.25)
$AUC_{0-t(12)}$ (nMol.h/L), Mean ± S.D. (% C.V.)	2806 ± 234 (8.34)
AUC_{0-inf} (nMol.h/L), Mean ± S.D. (% C.V.)	4103 ± 427 (10.41)

Table 1: Pharmacokinetic parameters of empagliflozin (EG) following oral administration of one JARDIANCE tablet nominally containing 25 mg of EG. Abbreviations: AUC = area under the curve; % C.V. = percent coefficient of variation; S.D. = standard deviation.

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