VI. List of Conferences, Symposium, Seminars & Workshops
INTERNATIONAL CONFERENCES/SYMPOSIUM
2. International conference on “Advances in Formulation Development: Challenges and Applications” at Faculty of Pharmacy, Parul University, Baroda, 4th & 5th March 2016. (Poster Presentation)
3. GUJCOST and GSTBM Sponsored International Seminar on “Current Trends in Biological Sciences” at MG Science Institute, Ahmedabad, 26th & 27th December 2015. (Poster Presentation)

NATIONAL CONFERENCES/SEMINARS
1. National Symposium on “Recent Advances and Future Trends in Chemical Technology-2016” at Department of Chemical Engineering, Institute of Technology, Nirma University, Ahmedabad, 24th September 2016. (Poster Presentation)
2. National Seminar on “Recent Advances in Drug Discovery” at Institute of Pharmacy, Nirma University, Ahmedabad, 28th March 2016. (Poster Presentation)
7. GUJCOST and DST Sponsored National Conference on Innovative Approaches and Science Awareness “ at Sheth M.N. Science College, Hemchandracharya North Gujarat University, Patan, 28th February 2015. (Poster Presentation)

\textbf{STATE LEVEL SEMINARS}

1. DST and GUJCOST Sponsored One Day State Level Seminar on “Recent Green Trends in Chemical Sciences”, at The H.N.S.B. Ltd. Science College, Himmatnagar, 29th February 2016. \textit{(Poster Presentation)}


4. Science Excellence-2014 organized by Department of Botany, Gujarat University, Ahmedabad, 4th January 2014. \textit{(Oral Presentation)}

\textbf{WORKSHOPS}

1. The Faculty Empowerment Workshop entitled “National Workshop on Basic ICT Skills, e-Learning and MOOCs for Educators” organized and sponsored by Guru Angad Dev Teaching Learning Centre of MHRD, SGTB Khalsa college, University of Delhi at Gujarat University on 2nd & 3rd December 2016.

2. Short term training programme on “Instrumental Methods of Chemical Analysis” organized by Department of Science, School of Technology, Pandit Deendayal Petroleum University, Gandhinagar and Torrent Pharmaceutical Ltd. Dated 25th-29th July, 2016.

3. Workshop on “Hands-on training on computational methods in drug discovery” held at Department of Chemistry, Gujarat University, Ahmedabad dated 23rd & 24th June, 2016.

4. Participation in the “Regional Training Programme on URKUND (An Anti-Plagiarism Detection Tool)”, organized by Gujarat University and INFLIBNET Centre in collaboration with URKUND, Sweden & eGalactic-Pune at Gujarat University, Ahmedabad, 24th September 2015.


Awarded Certificates

National Symposium on

“Recent Advances and Future Trends in Chemical Technology-2016”

This is to certify that Mr./Ms. Priyanka A. Shah has presented poster as author/coauthor entitled “Monitoring of Plasma Phospholipids to assess Matrix effect in the analysis of Desmedosone and its active metabolite Desmedosone by LC-MS/MS” has been awarded as BEST POSTER in the National Symposium on “Recent Advances and Future Trends in Chemical Technology-2016” organized on Saturday, 24th September 2016 at Department of Chemical Engineering, Institute of Technology, Nirma University, Ahmedabad.

Convener
Organizing Secretary
Head of Department
Director
The H. N. S. B. Ltd. Science College, Himatnagar
(Managed by The Himatnagar Kelavani Mandal)
NAAC Accredited “B” Grade

DST & GUJCOST Sponsored One Day State Level Seminar on
Recent Green Trends in Chemical Sciences (RGTCS-2016)

Certificate

This is to certify that Prof./Dr./Mr./Ms. Priyanka A. Shelke
has attended State Level Seminar entitled "Recent Green Trends in Chemical Sciences
(RGTCS-2016)" organized by Chemistry Department of The H.N.S.B. Ltd. Science College,
Himmatnagar held on 29th February, 2016.
He/She has participated and obtained ....2nd.... Rank in Poster presentation.

Dr. S. S. Andher
Convener

Dr. B. S. Patel
Principal

Department of Chemistry, Gujarat University
2017
National Conference
ON
“INNOVATIVE APPROACHES AND SCIENCE AWARENESS”
Organized by
NORTH GUJARAT EDUCATION SOCIETY, MUMBAI MANAGED
SHETH M.N. SCIENCE COLLEGE, PATAN (N.G.)
(Affiliated to Hemchandracharya North Gujarat University, Patan)

CERTIFICATE

This is to certify that Dr./Mr./Miss Priyanka Shah has attended National Conference on “INNOVATIVE APPROACHES AND SCIENCE AWARENESS” and has presented a poster at UG/PG/Research level in chemical /life Mathematical & Physical Sciences. He/She has been awarded with First prize.

Date: 28th February, 2015

Dr. Kalpesh Parikh
Convener

Dr. P. J. Vyas
Organizing Secretary

Dr. N. K. Patel
Organizing Secretary
SCIENCE EXCELLENCE-2014

organized by

Department of Botany, Gujarat University, Ahmedabad

CERTIFICATE

Dr./Mr./Ms. Shah Priyanka A.

of Department of Chemistry, School of Sciences, Gujarat University

has actively participated in Science Excellence - 2014, Paper / Poster

presentation competition on Saturday, 4th January - 2014.

He/She presented a paper entitled UPLC-MS/MS assay for the simultaneous
quantification of carvedilol & its active metabolite 4'-hydroxyphenyl carvedilol in human
plasma to support a bioequivalence in the subject Chemistry under
Study in healthy volunteers

UG / PG / Research / Faculty category and awarded First Prize.

Dr. Archana U. Mankad
Secretary General - SCIXL - 2014
Gujarat University

Dr. Mukul I. Shah
Patron - SCIXL - 2014
Gujarat University
Short communication

LC–tandem mass spectrometry method for the simultaneous determination of metformin and sitagliptin in human plasma after ion-pair solid phase extraction

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A B S T R A C T

A reversed-phase liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) method was established for simultaneous determination of two oral hypoglycemic drugs metformin (MET) and sitagliptin (STG) in human plasma. The analytes were extracted from 50 μL human plasma by ion-pair solid phase extraction using sodium lauryl sulphate on Phenomenex Strata-X (30 mg/1 mL) cartridges. The chromatographic separation was accomplished on XSelect HSS CN (150 x 4.6 mm, 5 μm) column using mobile phase consisting of methanol-8.0 mM ammonium formate in water, pH 4.5 (80:20, v/v) under isocratic condition. Tandem MS detection was performed on a triple quadrupole spectrometer equipped with an electrospray ionization source, operated in the positive mode. Multiple reaction monitoring (MRM) was used to quantify the analytes following transitions, m/z 130.1 → 60.1 and m/z 408.3 → 235.1 for MET and STG respectively. The method displayed acceptable linearity in the concentration range of 4.00–3200 ng/mL for MET and 1.00–800 ng/mL for STG. The intra-batch and inter-batch precisions were ≤5.1% and accuracy ranged from 96.5 to 103.3% for both the drugs. The mean recovery of MET and STG obtained from spiked plasma samples was 82.5% and 90.4% respectively with minimal matrix interference. Both the drugs were found to be stable under all mandatory storage conditions. The validated method was successfully applied to a clinical pharmacokinetic study for a fixed-dose tablet formulation containing 500 mg MET and 50 mg STG in 16 healthy volunteers.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder involving resistance to secreted insulin. Oral hypoglycemic drugs belonging to four different classes namely sulfonylureas, biguanides, thiazolidinediones and alpha-glucosidase inhibitors have been used in the treatment of T2DM. T2DM is a result of abnormal β-cell function which is related to insulin deficiency, insulin resistance together with decreased glucose transport into muscle and fat tissues and increased hepatic glucose output [1,2]. A coherent oral antidiabetic drug (OAD) combination that entails both an insulin sensitizer and an insulin secretogogue is generally used to treat T2DM, as they address the twin defects of impaired β-cell function and insulin resistance which characterize T2DM [3].

Metformin (MET) is recognized as the first-line glucose lowering OAD in the management of T2DM that acts predominantly by inhibiting hepatic glucose release and has also been shown to exert several cardioprotective effects [1,4]. Moreover the gastrointestinal side effects associated with its use are normally tolerable and self limiting. MET is considered to be a key drug for combination therapy with other OAD (sulfonylureas, glitazones, gliptins) as there are no drug-drug interactions and is not metabolized through the hepatic CYP system [1,5]. This approach has enlarged the array of remedies for the management of T2DM.

As evident from the progressive decline in the metabolic status of patients suffering from T2DM and undergoing treatment with conventional insulin secretagogues like sulfonylureas, new OAD which can target the so-called incretin hormone system can offer potential advantages over sulfonylureas [1,2]. In this regard, the intestinal hormone glucagon-like peptide-1 (GLP-1) stimulates insulin secretion and inhibits glucagon secretion in a glucose dependent manner, and thereby minimizes risk of hypoglycemia. This intestinal hormone is rapidly inactivated by
RESEARCH ARTICLE

LC-MS/MS analysis of metformin, saxagliptin and 5-hydroxy saxagliptin in human plasma and its pharmacokinetic study with a fixed-dose formulation in healthy Indian subjects

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Abstract
A specific and rapid liquid chromatography–tandem mass spectrometry method is proposed for the simultaneous determination of metformin (MET), saxagliptin (SAXA) and its active metabolite, 5-hydroxy saxagliptin (5-OH SAXA) in human plasma. Sample preparation was accomplished from 50 μL plasma sample by solid-phase extraction using sodium dodecyl sulfate as an ion-pair reagent. Reversed-phase chromatographic resolution of analytes was possible within 3.5 min on ACE 5C18 (150 × 4.6 mm, 5 μm) column using acetonitrile and 10.0 mM ammonium formate buffer, pH 5.0 (80:20, v/v) as the mobile phase. Triple quadrupole mass spectrometric detection was performed using electrospray ionization in the positive ionization mode. The calibration curves showed good linearity (r² ≥ 0.9992) over the established concentration range with limit of quantification of 1.50, 0.10 and 0.20 ng/mL for MET, SAXA and 5-OH SAXA respectively. The extraction recoveries obtained from spiked plasma samples were highly consistent for MET (75.12–77.84%), SAXA (85.90–87.84%) and 5-OH SAXA (80.32–82.69%) across quality controls. The validated method was successfully applied to a bioequivalence study with a fixed-dose formulation consisting of 5 mg SAXA and 500 mg MET in 18 healthy subjects. The reproducibility of the assay was demonstrated by reanalysis of 87 incurred samples.

KEYWORDS
5-hydroxy saxagliptin, human plasma, ion-pair solid phase extraction, LC-MS/MS, metformin, saxagliptin

1 | INTRODUCTION

The treatment of type 2 diabetes mellitus (T2DM) with fixed-dose combination (FDC) formulation containing two or more therapeutic agent having complementary mode of action has gained significant attention in clinical studies. T2DM commonly involves abnormal β-cell function that leads to insulin deficiency, resistance to insulin, decreased glucose transport into muscle and fat cells and an increase in hepatic glucose levels. To address these issues an FDC therapy comprising of an insulin sensitizer and an insulin secretagog presents a rational line of treatment for T2DM (Gumnesson et al., 2014; Howlett, Porte, Alavoine, Kuhn, & Nicholson, 2003; Scheen, 2010a, 2010b). The combination therapy of metformin (MET) an insulin sensitizer and saxagliptin (SAXA) which acts as an insulin secretagog can help in providing more efficacious and cost-effective treatment with reduced tablet burden than their monotherapy for impaired β-cell function.

MET is considered as a first-line glucose lowering agent in the management of T2DM. It acts by decreasing production of hepatic glucose and improves insulin sensitivity by increasing peripheral glucose uptake. Although gastrointestinal side effects are generally well tolerated, an important side effect associated with the use of MET monotherapy is lactic acidosis, which occurs mainly owing to renal insufficiency. Further, owing to progressive decline in the metabolic status of patients with T2DM as a result of reduced insulin secretion, combination of MET with other oral antidiabetic drugs can provide a new treatment option for patients with T2DM (Scheen, 2010a, 2010b). In this regard, drugs that can inhibit dipeptidyl peptidase-4 (DPP-4), an atypical serine protease can help in lowering fasting and post-prandial blood glucose in patients. DPP-4 rapidly inactivates intestinal hormone glucagon-like peptide-1, which stimulates insulin secretion and inhibits glucagon secretion and thereby reduces the risk of hypoglycemia (Drucker & Nauck, 2006). SAXA, which belongs to the
An improved LC–MS/MS method for the simultaneous determination of pyrazinamide, pyrazinoic acid and 5-hydroxy pyrazinoic acid in human plasma for a pharmacokinetic study

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A B S T R A C T

In the present work the plasma levels of PZA and its two active metabolites, pyrazinoic acid (PA) and 5-hydroxy pyrazinoic acid (5-OH PA) were determined by a sensitive and rapid LC–MS/MS method. The analytes and their labeled internal standards were extracted from 200μL plasma samples by liquid-liquid extraction with methyl tert-butyl ether: diethyl ether (90:10, v/v) under acidic conditions. Their separation was achieved on a Zorbax Eclipse XDB C18 (100 × 4.6 mm, 3.5 μm) column using methanol and 0.1% acetic acid (65:35, v/v) as the mobile phase within 4.0 min. Detection and quantitation were done by multiple reaction monitoring on a triple quadrupole mass spectrometer following the transitions, m/z 124.1 → 81.1, m/z 125.0 → 80.9 and m/z 141.0 → 81.0 for PZA, PA and 5-OH PA respectively in the positive ionization mode. All the analytes were baseline resolved with a resolution factor of 3.3 and 6.4 between PZA and its metabolites, PA and 5-OH PA respectively. The calibration curves were linear from 0.100–30.0 μg/mL, 0.03–9.00 μg/mL and 0.002–6.00 μg/mL for PZA, PA and 5-OH PA respectively with r² ≥ 0.9980 for all the analytes. The intra-batch and inter-batch accuracy and precision (% CV) across quality controls varied from 93.5–106.7% and 1.10–4.57 respectively for all the analytes. The mean extraction recovery of PZA, PA and 5-OH PA was 83.7%, 89.2% and 80.8% respectively, which was consistent at higher as well as lower concentration levels. The% change in the stability of analytes under different storage conditions ranged –6.7 to 7.1 for all the analytes. The method was applied to assess the comparative bioavailability of a 500 mg PZA test and reference formulation in healthy subjects. The assay reproducibility was also tested by reanalysis of 22 incurred subject samples.

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1. Introduction

PZA, an amide derivative of pyrazine-2-carboxylic acid is considered by World health Organization (WHO) to be a pivotal sterilizing drug for the treatment of TB in combination with other drugs for short-course chemotherapy [1]. The combination of PZA with isoniazid and rifampicin offers strong synergistic and accelerating effect for the treatment and therefore it is highly recommended in every combination therapy [2]. Due to the presence of PZA in the combination therapy there is significant reduction in the duration of current chemotherapy regimens [3]. Although the mode of action of PZA is not completely known, it is likely that during the early phase of inflammation PZA targets bacilli residing in acidified compartments of the lung [2]. PZA enters Mycobacterium tuberculosis by passive diffusion and through an ATP-dependent transport system [4]. This intracellular accumulation takes place because of an inefficient efflux system unique to M. tuberculosis [5].

PZA is a pro-drug and gets converted to its active form pyrazinoic acid (PA) by the enzyme pyrazinamidase. PZA is well absorbed orally and mainly metabolized by the liver. The plasma half-life of PZA is about 3–4 h. It is rapidly absorbed from the gastrointestinal tract to achieve peak serum concentration of 6–8 μg/mL at 1.5–2.0 h after oral administration [6]. Another important metabolite of PZA is 5-hydroxy pyrazinoic acid (5-OH PA) which is formed by the action of xanthine oxidase on PA via oxidation process. The same enzyme is also responsible for the conversion of PZA to 5-hydroxy pyrazinamide (5-OH PZA). One more but less significant metabolite pyrazinuric acid (PZU) is produced when PA combines with glycine. In the treatment involving PZA, measurement of PZA and PA in human plasma is essential to prevent or minimize the risk of...
Simultaneous analysis of losartan, its active metabolite, and hydrochlorothiazide in human plasma by a UPLC-MS/MS method

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Abstract: A selective and sensitive ultra performance liquid chromatography-tandem mass spectrometry method was developed for the simultaneous determination of losartan (LOS), EXP-3174, which is an active metabolite LOS carboxylic acid, and hydrochlorothiazide (HCTZ) in human plasma. Solid-phase extraction was carried out on Oasis HLB cartridges with 100 μL of plasma to give an extraction recovery in the range of 88.5%–102.5% for the three analytes. Chromatography on a BEH C18 column afforded baseline separation of all the analytes within 2.4 min using 1.0% formic acid in water and acetonitrile (15:85, v/v) as the mobile phase. Quantitation was performed with multiple reaction monitoring in the negative ionization mode. The response of the method was linear over a dynamic range of 0.5–500, 1.0–750, and 0.25–150 ng/mL for LOS, EXP-3174, and HCTZ, respectively. Extent of signal suppression/enhancement was examined through postcolumn infusion. The effect of matrix components was evaluated by postextraction spiking and calculation of the slope of calibration lines. The method was successfully applied to a bioequivalence study of 50 mg losartan and 12.5 mg hydrochlorothiazide tablet formulation in 65 healthy human subjects. Reproducibility of the method was shown by reanalysis of 213 incurred samples.

Key words: Losartan, EXP-3174, hydrochlorothiazide, solid phase extraction, UPLC-MS/MS, human plasma

1. Introduction

Losartan (LOS) is a nonpeptide, orally active, and selective angiotensin II Type 1 (AT1) receptor antagonist drug used mainly to treat hypertension associated with heart failure or renal impairment. It differs from angiotensin-converting enzyme (ACE) inhibitors by producing direct antagonism II receptors.1,2 LOS is well absorbed following oral administration with an oral bioavailability of about 33% and reaches peak serum levels in 1.0 h. It undergoes significant first-pass metabolism to produce an active 5-carboxylic acid metabolite, designated as EXP-3174, which is mediated by cytochrome P450 enzymes CYP3A4 and CYP2C9. This metabolite is a long-acting (up to 24 h), noncompetitive antagonist at the AT1 receptor and contributes to the pharmacological effects of LOS. It is 10–40 times more potent in blocking AT1 receptors than the parent drug.3

Hydrochlorothiazide (HCTZ) is a popular diuretic of the thiazide class that reduces plasma volume by increasing the excretion of sodium, chloride, and water. The decrease in plasma volume results in counter-regulatory stimulation of the rennin-angiotensin system and the sympathetic nervous system.4 Thus, the complimentary action of an angiotensin II receptor antagonist and a thiazide has led to their extensive use in the treatment of...
ORIGINAL ARTICLE

COMPLEXATION STUDY OF GLIMEPIRIDE WITH Mg2+, Ca2+, Cu2+ AND Zn2+ CATIONS IN METHANOL BY CONDUCTOMETRY, SPECTROPHOTOMETRY AND LC-MS

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ABSTRACT

Objective: The present work describes complexation study of sulfonylurea based drug, glimepiride with divalent metal ions (Mg2+, Ca2+, Cu2+ and Zn2+) in methanol by conductometry, spectrophotometry and liquid chromatography-mass spectrometry.

Methods: The stoichiometry of resulting metal ion-glimepiride complexes were ascertained by molar conductance vs. mole ratio of glimepiride/metal ion plots, Job's method of continuous variation and liquid chromatography-mass spectrometric analysis. The values of enthalpy and entropy of complexation reactions in methanol were obtained from van't Hoff plots.

Results: The formation constants of 1:1 (M2+: glimepiride) complexes at different temperatures followed the order Mg2+>Cu2+>Zn2+>Ca2+ by conductometry as well as spectrophotometry. High molar conductivities were observed for all the complexes indicating formation of charged complex and the results were supported by the presence of protonated precursor complex ions in the mass spectral study.

Conclusion: The stability of complexes increased with temperature suggesting endothermic nature of complexation reactions. The thermodynamic data showed that all the complexes formed were entropy stabilized and enthalpy destabilized. A good linear relationship between ∆H and T∆S values suggests existence of entropy-enthalpy compensation in the complexation of these four cations with glimepiride.

Keywords: Glimepiride, Conductometry, Spectrophotometry, Liquid chromatography-mass spectrometry, Formation constant, Thermodynamic parameters.

INTRODUCTION

Diabetes mellitus is one of the most serious metabolic disorders characterized by high blood glucose (blood sugar) and disrupted insulin secretion. Diabetic patients have an increased prevalence of hypertension, sexual dysfunction, atherosclerotic cardiovascular, peripheral arterial, cerebrovascular and kidney diseases [1]. Glimepiride (GMP), a second generation sulfonylurea is well tolerated and offers an efficacious option for the treatment and management of Type 2 (non insulin dependent) diabetes [2]. It reduces blood glucose levels without deleterious alterations in plasma lipoproteins in Type 2 diabetic patients. GMP increases insulin levels in blood by binding to sulfonylurea receptor on the adenosine triphosphate (ATP) sensitive potassium ion channel of pancreatic β-cell and thus facilitates outflow of potassium ions and influx of calcium ions to promote insulin secretion from pancreas [3]. Its binding with 65-kDa protein regulates release of insulin and lowers threat of hypoglycaemia [4]. GMP is administered orally in a once daily dose of 1-2 mg which can extend up to 8 mg. It gets completely absorbed after 1 h, showing maximum glucose lowering effects within 4 h after administration [5].

Metal ions play a vital role in growth, overall health and maintenance of human body but at the same time can induce various diseases due to their imbalance. Electron rich ligands or drugs effectively bind and interact with metal ions to give so-called “metalodrugs” and “metallo pharmaceuticals”. They offer promising and unique therapeutic applications as they possess multi-functional properties without the threat of drug-resistant strain [6]. Several drugs and potential pharmaceuticals possess metal-binding sites and can influence their biological activity and sometimes can inflict damage to the target biomolecules [7]. As GMP has an extensive use with fewer side effects for long term therapy, co-administration with number of other drugs and various dietary supplements may enhance or reduce its effects. For instance, magnesium oxide is taken as an antacid that increases GMP absorption rate throughout the body while lithium supplement with GMP reduces blood glucose level leading to increased risk of hypoglycaemia [8]. Chromium supplements reduce blood glucose level effectively however; effect on glucose metabolism has not been observed [9]. Further, higher intake of zinc is associated with somewhat lower risk of Type 2 diabetes especially in women [10]. In a 24 weeks therapy, GMP taken with insulin glargine (containing zinc chloride) showed improved glycaemic control without any incidence of hypoglycaemic episodes [11]. In this context it is essential and useful to investigate the effect of metal ion on the therapeutic efficacy and mode of action of GMP.

Literature survey revealed few studies on metal ion-GMP binding with different transition and inner transition metal ions. GMP complexation with mercury and lanthanum metal ions have been studied by spectral and thermogravimetric analysis [12, 13] have synthesized lanthanide (Nd3+, Tb3+ and Er3+) -GMP complexes and evaluated their antimicrobial activity. Metal complexes of Mn2+ and Co2+ with GMP have been synthesized and characterized by IR spectra, electronic spectra and molecular conductance data [14]. In another report, GMP complexes with Cu2+, Mg2+, Ni2+ and Cd2+metal ions were characterized by their physical and analytical data [15]. Similarly, spectral studies have been carried for GMP complexes with Hg2+ and molybdenum [16, 17]. Furthermore, Nd3+ and Sm3+ complexes of GMP are also studied for their hypoglycemic activity [18, 19].

Conductometry and spectrophotometry are two versatile and widely used tools in the field of co-ordination chemistry to investigate the phenomena of complexation [20, 21]. In the present study, binding of GMP with bivalent metal ions Mg2+, Ca2+, Cu2+ and Zn2+ has been investigated by conductometry and spectrophotometry. Formation constants of the complexes have been evaluated by both these techniques. The stoichiometry of complexes is verified through molar conductance-mole ratio plots, Job’s method of continuous variation and confirmed by liquid chromatography-mass spectrometric (LC-MS) analysis. Thermodynamic parameters of
Fast and sensitive LC-MS/MS method for the simultaneous determination of lisinopril and hydrochlorothiazide in human plasma

Jaivik V. Shah, Priyanka A. Shah, Priya V. Shah, Mallika Sanyal, Pranav S. Shrivastav

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Short Communication

SPE–UPLC–MS/MS assay for determination of letrozole in human plasma and its application to bioequivalence study in healthy postmenopausal Indian women

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Bioequivalence study

A B S T R A C T

A rapid and sensitive ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method is described for determination of letrozole in human plasma. Following solid phase extraction (SPE) of letrozole and letrozole-d4 on Orochem DVB-LP cartridges, chromatography was performed on Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm) column using methanol-0.1% formic acid in water (85:15, v/v) as the mobile phase. Detection was carried out on a triple quadrupole mass spectrometer with an electrospray source, operated under positive ionization mode. Quantitation of letrozole and letrozole-d4 was done using multiple reaction monitoring (MRM) following the transitions at m/z 286.2→217.0 and m/z 290.2→221.0, respectively. The calibration plots were linear through the concentration range of 0.10→100 ng/mL (r² ≥ 0.9990) using 100 μL human plasma. The extraction recovery of letrozole ranged from 94.3% to 96.2% and the intra-batch and inter-batch precision was ≤ 5.2%. The method was successfully applied to a bioequivalence study of letrozole after oral administration of 2.5 mg tablet formulation to 16 healthy postmenopausal Indian women. The assay reproducibility was also established through incurred sample reanalysis (ISR) of 74 subject samples.

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1. Introduction

Letrozole (LTZ) is a third-generation, potent and selective non-steroidal aromatase inhibitor. It is approved for anti-hormonal therapy in postmenopausal women having estrogen receptor positive breast cancer. Aromatases (cytochrome P-450 [CYP 19]) are enzymes that convert adrenal androgens into estrogens in the peripheral tissues, which are responsible for the promotion and progression of breast cancer in postmenopausal women. LTZ effectively suppresses the production of estrogen by preventing the aromatase enzyme from producing estrogens through competitive binding to the heme moiety of cytochrome P-450 subunit 1,2.

LTZ is rapidly and completely absorbed after oral administration with mean absolute bioavailability of 99.5%. It is weakly bound to proteins (60%), primarily to albumin and has 1.9 L/kg volume of distribution at steady state. It is metabolized by cytochrome P450 isoenzymes (CYP 3A4 and CYP 2A6) into a pharmacologically inactive carbinol metabolite, which further undergoes glucuronide conjugation [1,2]. Approximately 90% of LTZ is recovered in urine, with a major part of about 75% corresponding to the glucuronide conjugate of the carbinol metabolite. The terminal elimination half-life of LTZ is about two days [2]. However, due to its low therapeutic dose (2.5 mg) and wide distribution in tissues, the plasma concentration of LTZ is rather low. Thus, it is essential to establish a reliable, selective and sensitive analytical method for the quantitation of LTZ, especially for pharmacokinetic applications.

Several methods are reported for the determination of LTZ as a single analyte [3–12], together with its inactive metabolites [13,14] and with other drugs like tamoxifen and anastrozole [15] in different biological matrices. Mainly liquid chromatography with UV [4,5,12], fluorescence [3,14] or mass spectrometry [6–11,13,15] detection has been used for the quantification of LTZ in various matrices like human urine, rat plasma and human plasma. All previous methods based on liquid chromatography with mass
Original Article

Determination of lercanidipine in human plasma by an improved UPLC–MS/MS method for a bioequivalence study

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ABSTRACT

An improved and reliable ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) method has been developed and validated for the determination of lercanidipine in human plasma. Plasma samples with lercanidipine-d3 as an internal standard (IS) were prepared by solid phase extraction on Phenomenex Strata-X cartridges using 100 μL of human plasma. Chromatographic analysis was performed on UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm) column under isocratic conditions. Linear calibration curves were obtained over a wide dynamic concentration range of 0.010–20.0 ng/mL. Matrix effect was assessed by post-column infusion, post-extraction spiking and standard–line slope methods. The mean extraction recovery was > 94% for the analyte and IS. Inter-batch and intra-batch precision (% CV) across five quality controls was < 5.8%. Bioequivalence study was performed with 36 healthy subjects after oral administration of 10 mg of lercanidipine and the assay reproducibility was evaluated by reanalysis of 133 incurred samples.

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1. Introduction

Hypertension, one of the major causes of cardiovascular diseases, has affected young and elderly population in the world. Antihypertensive drugs have been proven agents to prevent cardiovascular morbidity and mortality since long ago [1,2]. Dihydropyridine calcium channel blockers are a potent class of antihypertensive drugs and work primarily as vasodilators. They are widely used for the treatment and management of hypertension and coronary artery diseases. Lercanidipine (LER), a third-generation dihydropyridine calcium channel blocker, helps in peripheral vasodilation by preventing the entrance of calcium ions through L-type calcium channels in cell membrane [3], and has shown high efficacy for patients with high cardiovascular risk and diffuse atherosclerosis. LER has high lipophilicity, which is responsible for smooth onset and prolonged therapeutic action compared with the first- and the second-generation calcium channel blockers. It has shown lower incidence of adverse events such as lack of activation of heart rate [4–6]. LER has a plasma half life of 8–10 h which, however, does not relate to its duration of antihypertensive activity. After oral administration, LER is almost completely absorbed from the gastrointestinal tract and reaches peak plasma concentration within 1–3 h. It is approximately 98% protein bound and has a distribution volume of 2–2.5 L/kg. LER gets extensively metabolized by cytochrome P450 3A4 to inactive pyridine derivatives which are eliminated in urine and feces [4,6].

To optimize drug therapy, reduce drug accumulation, and lessen the frequency of adverse effects, it is essential to develop reliable, rapid and sensitive bioanalytical methods. There are several methods described in the literature for the determination of LER as a single analyte [7–13] or in combination with other antihypertensive drugs [14–17] in biological matrices. Enantioselective determination of LER in human plasma has been described for pharmacokinetic studies using chiral columns [7,8]. Racemic LER has been estimated using different analytical techniques like voltammetry [9,10], high performance liquid chromatography–ultraviolet detection (HPLC–UV) [11], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [12] and ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) [13] in human serum [9,10], human urine [10], rabbit serum [11] and human plasma [12,13]. Salem et al. [12] presented a selective and rapid method to determine LER in the concentration range of 0.1–16 ng/mL within 10 min.

UPLC is a rapid separation technique with enhanced chromatographic efficiency compared with conventional HPLC. Unlike HPLC,
SENSITIVE AND RAPID ESTIMATION OF LAPATINIB, AN ANTICANCER DRUG IN SPIKED HUMAN PLASMA BY LC-MS/MS

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ABSTRACT
Objective: The work presents a sensitive, selective and rapid determination of lapatinib, a potent anticancer drug in human plasma by liquid chromatography-tandem mass spectrometry.

Methods: Liquid-liquid extraction of lapatinib and lapatinib-d4, added as an internal standard (IS) was carried out from 100 µl plasma sample. Chromatographic analysis was performed on ACE C18 (100 mm × 4.6 mm, 5 µm) column using 10 mm mol ammonium formate buffer (pH 3.5) and acetonitrile (10:90, v/v) as the mobile phase. The precursor ion → product ion transitions for lapatinib (m/z 581.1 → 365.2) and IS (m/z 585.1 → 365.0) were monitored on a triple quadrupole mass spectrometer in the positive electrospray ionization mode. The method was validated in accordance with the US FDA guidelines.

Results: A linear concentration range was established from 2.50-2500 ng/ml for lapatinib. The intra-batch and inter-batch precision were ≤ 4.81 %. The recovery of lapatinib and IS from plasma samples ranged from 88.7 to 95.8 % and 85.9 to 96.5 % respectively. The accuracy and precision (% CV) for the stability of lapatinib under different storage conditions showed a variation from 95.2 to 102.2 % and 1.19 to 4.35 % respectively at low and high QC levels. Under optimized chromatographic conditions, the retention time for lapatinib was 1.406 min with a total run time of 2.5 min for each sample.

Conclusion: The validation results demonstrate that the method is simple, accurate, precise and reproducible. The developed method can be readily used for pharmacokinetics/bioequivalence studies in patients as well as healthy subjects.

Keywords: Lapatinib, Lapatinib-d4, Liquid chromatography-tandem mass spectrometry, Human plasma, Sensitive, High-throughput.

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INTRODUCTION

Lapatinib is an orally active and a dual tyrosine kinase (TK) reversible inhibitor of epidermal growth factor receptor (ErbB1/EGFR) and human epidermal growth factor receptor 2 (ErbB2/HER2). These ErbB receptors play a major role in tumor cell growth and survival and are therefore key therapeutic targets [1]. Lapatinib acts intracellularly by reversibly binding to the cytoplasmic ATP-binding site of the kinase and at the same time blocking receptor phosphorylation and activation. As a result, it prevents ensuing downstream signaling events.

The ability of lapatinib to specifically inhibit ErbB1 and ErbB2 receptors is unique compared to other small molecule TK inhibitors like erlotinib and gefitinib [2, 3]. Two effective combinations of lapatinib with capecitabine/letrozole have been approved by the US Food and Drug Administration for the treatment of metastatic HER2 positive breast cancer and for postmenopausal women with HER2 & estrogen receptor positive breast cancer respectively [4].

Currently, lapatinib is available as a 250 mg tablet formulation and is recommended as a single dose at least 1.0 h before or after a meal [1]. However, it has been demonstrated that food increases the bioavailability of the drug after oral administration [5]. Further, the peak plasma concentration reaches within 3-6 h post dosing. Lapatinib is mainly bound to plasma albumin (>99%) and has a high volume of distribution (>2200 L), suggesting good drug distribution [6]. Lapatinib exhibits large inter-individual variation in pharmacokinetics due to the contribution of several metabolizing enzymes and transporters involved in the process of absorption and disposition. Thus to support efficient lapatinib therapeutic drug monitoring and clinical pharmacokinetics it is essential to develop highly sensitive, rapid and reliable methods to monitor the plasma concentration of lapatinib in patient samples.

Several methods are described in the literature for the individual [7-10] as well as the simultaneous determination of lapatinib [11-18] with other TK inhibitors in human plasma [7-10, 12-18] or cellular samples [11]. These methods have mainly used liquid chromatography with mass spectrometry detection for the analysis of lapatinib except one report which utilizes UV as the detector [9]. Some of these developed methods are either less sensitive [8 50 ng/ml] [7, 15, 17, 18], have higher retention time for lapatinib (>3.0 min) [8, 10, 12, 15-18] or require large plasma volume for processing (>250 µl) [8, 10, 13]. Moreover, it has been emphasized that the use of isotope-labeled IS is essential to minimize variation in the recovery of anticancer drugs [8]. The comparative features of liquid chromatography-mass spectrometric (LC-MS/MS) method developed for lapatinib in human plasma is presented in table 1. Thus, the primary objective of the work was to develop a simple extraction procedure with reduced sample volume for processing using a deuterated IS for precise and quantitative recovery. This was essential as lapatinib is an anticancer drug and is estimated clinically mainly in patients and therefore, it is important to minimize blood loss during subject sample analysis. The other aspect was to optimize chromatographic run time with adequate retention on a reversed phase column to maximize throughput. The developed method was fully validated as per current regulatory guidelines especially for matrix effect and stability of lapatinib under different conditions.

MATERIALS AND METHODS

Chemicals and reagents

Working standards of lapatinib (99.5%) and lapatinib-d4 (IS, 98.9%) were purchased from TLC Pharmachem (Toronto, Canada). HPLC grade acetonitrile and methanol were obtained from J. T. Baker (Mumbai, India). Guaranteed reagent grade formic acid, ammonium...
Simultaneous quantification of atenolol and chlorthalidone in human plasma by ultra-performance liquid chromatography–tandem mass spectrometry

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ABSTRACT: A simple, sensitive and reproducible ultra-performance liquid chromatography–tandem mass spectrometry method has been developed for the simultaneous determination of atenolol, a $\beta_1$-adrenergic receptor-blocker and chlorthalidone, a monosulfonamyl diuretic in human plasma, using atenolol-d7 and chlorthalidone-d4 as the internal standards (ISs). Following solid-phase extraction on Phenomenex Strata-X cartridges using 100 $\mu$L human plasma sample, the analytes and ISs were separated on an Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 $\mu$m) column using a mobile phase consisting of 0.1% formic acid–acetonitrile (25:75, v/v). A tandem mass spectrometer equipped with electrospray ionization was used as a detector in the positive ionization mode for both analytes. The linear concentration range was established as 0.50–500 ng/mL for atenolol and 0.25–150 ng/mL for chlorthalidone. Extraction recoveries were within 95–103% and ion suppression/enhancement, expressed as IS-normalized matrix factors, ranged from 0.95 to 1.06 for both the analytes. Intra-batch and inter-batch precision (CV) and accuracy values were 2.37–5.91 and 96.1–103.2%, respectively. Stability of analytes in plasma was evaluated under different conditions, such as bench-top, freeze–thaw, dry and wet extract and long-term. The developed method was superior to the existing methods for the simultaneous determination of atenolol and chlorthalidone in human plasma with respect to the sensitivity, chromatographic analysis time and plasma volume for processing. Further, it was successfully applied to support a bioequivalence study of 50 mg atenolol + 12.5 mg chlorthalidone in 28 healthy Indian subjects. Copyright © 2015 John Wiley & Sons, Ltd.

Introduction

Hypertension is one of the leading causes for cardiovascular diseases worldwide. Several mono and combination therapies are recommended for the treatment and management of mild to moderate and acute cases of hypertension. Nevertheless, majority of the patients require two or more antihypertensive agents to achieve the target blood pressure of <140/90 mmHg to minimize cardiovascular mortality and morbidity. The atenolol and chlorthalidone drug combination has shown better efficacy, tolerance and improved compliance in mild to moderate hypertension owing to their complimentary mechanisms of action (Pareek et al., 2008; Sweetman, 2002; TENORETIC®, ).

Atenolol (ATN), a hydrophilic $\beta_1$ cardioselective $\beta$-adrenergic receptor-blocking agent, is widely used in the treatment of high blood pressure, arrhythmias and angina pectoris. Like other antihypertensive agents, ATN lowers the systolic and diastolic blood pressure by 15–20% in a single drug treatment and reduces cardiovascular mortality. Following oral administration, the absorption of ATN from the gastrointestinal tract is about 50%, but is consistent. The peak plasma concentration occurs within 2–4 h after dosing. The atenolol blood levels are consistent and subject to little variability. There is no significant hepatic metabolism of atenolol and >90% of that absorbed reaches the systemic circulation unaltered. The plasma half-life is about 6–7 h and protein binding is very low (6–16%) (Sweetman, 2002; Shrivastav et al., 2010). Chlorthalidone (CHL) is a monosulfonamyl diuretic, which increases the excretion of sodium and chloride. Although the mechanism of action for reducing blood pressure is not fully known, it is related to the excretion and redistribution of body sodium. Like ATN, CHL is also not completely absorbed (~60%) after oral administration. The plasma

Abbreviations used: ATN, atenolol; CHL, chlorthalidone; CS, calibration curve standard; MF, matrix factor.

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Challenges in optimizing sample preparation and LC-MS/MS conditions for the analysis of carglumic acid, an N-acetyl glutamate derivative in human plasma

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This paper describes a systematic approach to overcoming challenges in developing a robust and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for reliable and precise determination of carglumic acid in human plasma. Sample extraction was tested on several reversed-phase solid-phase extraction (SPE) sorbents with different chemistries, such as hydrophobic C18, hydrophilic-lipophilic balance, and mixed-mode cation and anion exchange. The best recovery under the optimized extraction conditions was obtained with Oasis MAX (30 mg, 1cc) mixed-mode anion exchange (~ 50%) cartridge, compared to other sorbents from 100 μL plasma sample. Complete analytical separation of carglumic acid and carglumic acid-13C5 15N as an internal standard (IS) from endogenous plasma components was achieved on ACE 5CN (150 × 4.6 mm, 5 μm) column under isocratic conditions using acetonitrile:methanol (50:50, v/v) 0.1% acetic acid in water [80:20, v/v] as the mobile phase. The deprotonated precursor → product ion transitions for carglumic acid (189/146) and IS (195/152) were monitored in the negative ionization mode on a triple quadrupole mass spectrometer. The regression curves were linear over a concentration range of 6.00-6000 ng/mL (r² ≥ 0.9987). Matrix effect was evaluated in terms of IS-normalized matrix factors, which ranged from 0.95 to 1.01 across four quality control levels. Intra- and inter-batch accuracy and precision, and the stability of carglumic acid in spiked plasma samples were assessed under different conditions. The method was applied to assess the pharmacokinetics of 100 mg/kg body weight carglumic acid in a healthy Indian subject. Copyright © 2015 John Wiley & Sons, Ltd.

Additional supporting information may be found in the online version of this article at the publisher’s web site.

Keywords: carglumic acid; LC-MS/MS; Oasis MAX sorbent; matrix effect; human plasma

Introduction

N-acetylglutamate synthetase (NAGS) deficiency, first reported in 1981, is caused by the deficiency of N-acetylglutamate synthetase enzyme, which catalyzes the synthesis of N-acetylglutamate (NAG) from acetyl CoA and glutamate.1,2 NAG is an essential allosteric activator of mitochondrial carbamyl phosphate synthetase I (CPS I), which is the first step in the urea cycle.3 The deficiency of NAGS results in the accumulation of ammonia, causing hyperammonemia, which can lead to brain damage or even death depending upon the severity of the case. Carglumic acid (N-carbamyl glutamate or N-carbamyl-L-glutamate) has shown promising results for several conditions associated with hyperammonemia.4,5 It is a synthetic analogue of NAG that activates the CPS I enzyme and is used in the first rate limiting step of the urea cycle stimulating ureagenesis.5,6 As the deficiency of NAGS is an extremely rare disorder, there are few reports on the use of carglumic acid, and these are mainly based on single patients. In case of acute hyperammonemia, the drug is found to be very effective, precluding the need for detoxification of ammonia in the urea cycle. For long-term treatment, carglumic acid is effective in maintaining standard plasma ammonia levels and thus circumvents the need for additional drug therapy.3

Carglumic acid is sold as 200 mg dispersible tablets for enteral use under the brand name Carbaglu® since 2003 in Europe and from 2010 in the USA.7 A pharmacokinetic study in 12 healthy subjects who received 100 mg carglumic acid per kg body-weight showed maximum plasma concentration of 2.6 μg/mL after 3 h. The drug is estimated to be absorbed to the extent of 30%, with an elimination half life of 5.6 h.8 A literature review revealed limited information on bioanalytical methods for the analysis of carglumic acid in biological fluids. Thus, in the present work a fully validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method is described for the determination of carglumic acid in human plasma. All three stages of quantitative bioanalysis, namely sample preparation, chromatography, and MS-MS detection were concurrently optimized. The method was successfully used to analyze carglumic acid concentration in plasma samples obtained from a healthy subject after oral administration of a single oral dose of 100 mg/kg body weight Carbaglu® formulation.
Application of a UPLC-MS/MS method for the analysis of alosetron in human plasma to support a bioequivalence study in healthy males and females

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ABSTRACT: A simple, rapid and sensitive ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method has been developed and validated for the determination of alosetron (ALO) in human plasma. The assay method involved solid-phase extraction of ALO and ALO 13C-d3 as internal standard (IS) on a LichroSep DVB-HL (30 mg, 1 cm\textsuperscript{3}) cartridge. The chromatography was performed on an Acquity UPLC BEH C\textsubscript{18} (50 × 2.1 mm, 1.7 \textmu m) column using acetonitrile and 2.0 mM ammonium formate, pH 3.0 adjusted with 0.1% formic acid (80:20, v/v) as the mobile phase in an isocratic mode. For quantitative analysis, the multiple reaction monitoring transitions studied were m/z 295.1/201.0 for ALO and m/z 299.1/205.1 for IS in the positive ionization mode. The method was validated over a concentration range of 0.01–10.0 ng/mL for ALO. Post-column infusion experiment showed no positive or negative peaks in the elution range of the analyte and IS after injection of extracted blank plasma. The extent of ion-suppression/enhancement, expressed as IS-normalized matrix factor, varied from 0.96 to 1.04. The assay recovery was within 97–103\% for ALO and IS. The method was successfully applied to support a bioequivalence study of 1.0 mg alosetron tablets in 28 healthy Indian male and female subjects. Copyright © 2015 John Wiley & Sons, Ltd.

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder, which results from dysregulation of intestinal motor, sensory and central nervous system function. It is characterized by abdominal pain, bloating and disturbed bowel habits (Balfour et al., 2000; Olden, 2012). The diagnoses for IBS is classified based on three symptom patterns: diarrhea-predominant IBS (IBS-D); constipation-predominant IBS (IBS-C); and an alternating pattern of IBS-D and IBS-C (IBS-A). According to the American College of Gastroenterology IBS Task Force, three classes of medications, that is, antibiotics, tricyclic antidepressants and the 5-HT\textsubscript{3} antagonist, have shown greater efficacy in the treatment of IBS-D, which is thought to be predominant (American College of Gastroenterology Task Force on Irritable Bowel Syndrome, 2009). Alosetron (ALO) is a selective and highly potent serotonin 5-HT\textsubscript{3} antagonist that is used in the treatment and management of IBS. It is approved by US Food and Drug Administration for the treatment of severe cases of IBS-D, specifically in women. The efficacy of ALO is associated with selective antagonism of 5-HT\textsubscript{3} receptor, leading to normalization of GI motility, intestinal secretion and abdominal pain (Andersen and Hollerbach, 2004; Balfour et al., 2000; Olden, 2012). It is rapidly absorbed after oral administration (1.0 mg once or twice daily) with a mean bioavailability of ~50–60\% and plasma protein binding of about 82\% (Prometheus Laboratories Inc, 2010). The peak plasma concentration occurs in about 1 h and is rapidly eliminated with a half-life of 1.5 h. ALO is extensively metabolized (94\%) in the liver through the human microsomal cytochrome P450 system into several metabolites (Andersen and Hollerbach, 2004; Balfour et al., 2000; Koch et al., 2004). Clinical trials in patients have shown gender-related difference in the pharmacokinetics of ALO with about 27\% lower plasma concentration in men compared with women (Andersen and Hollerbach, 2004; Koch et al., 2002).

There are few methods available in the literature for the determination of ALO in biological fluids. A liquid chromatography method with fluorescence detection was used for the determination of ALO in plasma or serum employing a fully automated procedure (Lloyd et al., 1996). This method was established over a concentration range of 0.1–20 ng/mL to support pharmacokinetic/biopharmaceutical studies. In another method, ALO pharmacokinetics was studied after repeated dosing (Koch et al., 2004). In this method serum concentra-

Abbreviations used: ALO, alosetron; CS, calibration standard; GI, gastrointestinal; IBS, irritable bowel syndrome; MRM, multiple reaction monitoring.

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Keywords: Alosetron; UPLC-MS/MS; gender effects on pharmacokinetics; solid phase extraction; high throughput
Conductometric studies on complexation of Ag\(^+\) cation by C-thiophene calix[4]resorcinarene in pure and mixed non-aqueous solvent systems

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Abstract The present work relates to the complexation reaction between a new ionophore C-thiophene calix[4]resorcinarene (C-TC[4]R) with Ag\(^+\) cation in pure acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), dimethylformamide (DMF) and dimethylsulfoxide (DMSO) and their binary mixtures by conductometric method. The conductance data showed that the stoichiometry of the complexes formed in pure as well as binary solvent mixtures was 1:1 (Ag\(^+\): C-TC[4]R). The stability of C-TC[4]R complexes with Ag\(^+\) metal ion was observed to be sensitive to the nature of the solvent system. The results obtained show that the selectivity of C-TC[4]R for Ag\(^+\) cation in pure solvents followed the order MeOH > DMF > EtOH > DMSO > ACN. In binary solvent systems, there was a linear change in log \(K_f\) values for all the binary mixtures (ACN–DMF, ACN–DMSO, MeOH–DMF, EtOH–DMF and EtOH–DMSO) except MeOH–DMSO. The results show that the complexation reactions were spontaneous and entropy (\(\Delta S^o\)) as well as enthalpy (\(\Delta H^o\)) stabilized in all the solvent systems except EtOH–DMSO, in which the complexes were entropy destabilized. A linear relationship between \(\Delta H^o\) and \(\Delta S^o\) values indicate the existence of entropy–enthalpy compensation in the formation of [Ag–C-TC[4]R]\(^+\) complex in all the solvent systems studied.

Keywords C-Thiophene calix[4]resorcinarene • Ag\(^+\) cation • Conductometry • Solvent effect • Formation constant • Entropy–enthalpy compensation

Introduction Calixarenes and their structurally related analogs, calix[4]resorcinarenes are a versatile group of supramolecular hosts with a concave binding cavity which has high affinity towards various cations like alkali and alkaline earth metal ions, transition metal ions, ammonium ions, different anions and also small organic molecules [1–3]. The structure of calix[4]resorcinarenes consists of four resorcinol units, which are usually in a bowl-like conformation with intramolecular hydrogen bonds between the hydroxyl groups at the upper rim. This conformation of resorcinarenes enables it to adapt to the size and shape of the incoming guest leading to a variety of complexes in solution as well as in solid state [4, 5]. In basic aqueous media the phenolic hydroxyl groups can deprotonate and are rendered highly soluble. When calix[4]resorcinarenes are dissolved in polar solvents like alcohols, they exist in three types of self-assembled aggregates, monomeric, dimeric and hexameric species [6]. There are several reports on the complexation of metal ions like Ag\(^+\), Cs\(^+\), Au\(^+\), and Pd\(^{2+}\) by various resorcinarenes derivatives [7–11]. These complexes have been investigated using different analytical techniques like X-ray crystallography, \(^1\)H NMR and \(^{13}\)C NMR techniques. These studies are mainly focused on the complexation and crystallization of resorcinarene derivatives with metal ions in different solvent systems. In addition to these techniques,
Selective and rapid determination of raltegravir in human plasma by liquid chromatography–tandem mass spectrometry in the negative ionization mode

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KEYWORDS
Raltegravir; LC–ESI–MS/MS; Negative ionization mode; Human plasma; Bioequivalence study

Abstract A selective and rapid high-performance liquid chromatography–tandem mass spectrometry method was developed and validated for the quantification of raltegravir using raltegravir-d\textsubscript{3} as an internal standard (IS). The analyte and IS were extracted with methylene chloride and n-hexane solvent mixture from 100\textsubscript{\mu}L human plasma. The chromatographic separation was achieved on a Chromolith RP-18e endcapped C\textsubscript{18} (100 mm x 4.6 mm) column in a run time of 2.0 min. Quantitation was performed in the negative ionization mode using the transitions of m/z 443.1 \rightarrow 316.1 for raltegravir and m/z 446.1 \rightarrow 319.0 for IS. The linearity of the method was established in the concentration range of 2.0–6000 ng/mL. The mean extraction recovery for raltegravir and IS was 92.6\% and 91.8\%, respectively, and the IS-normalized matrix factors for raltegravir ranged from 0.992 to 0.999. The application of this method was demonstrated by a bioequivalence study on 18 healthy subjects.

1. Introduction

Raltegravir (RAL), a hydroxypyrimidinone carboxamide derivative, is an integrase strand-transfer inhibitor (INSTI) used in the treatment and management of human immunodeficiency virus (HIV) infection [1]. It was first approved by USFDA in 2007 for
DETERMINATION OF CAPECITABINE-AN ANTICANCER DRUG IN DRIED BLOOD SPOT BY LC-ESI-MS/MS

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ABSTRACT

Objective: Capecitabine (Cape), the first oral prodrug which belongs to the group of fluoro pyrimidines is the most frequently prescribed anticancer drug for the treatment of metastatic breast and colorectal cancers. The article describes a selective and robust method for determination of Cape in dried blood spots (DBS) by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Cape fortified DBS was punched and extracted with ethyl acetate using capecitabine-d11 as the internal standard (IS). Chromatographic separation of Cape and IS from endogenous matrix was performed on Phenomenex Gemini C18 (150 × 4.6 mm, 5 μm) column under isocratic condition using acetonitrile: 2 mmol ammonium formate (pH 3.0, adjusted with 0.1 % formic acid) (80:20, v/v) as the mobile phase. Detection and quantification were carried on a triple quadrupole mass spectrometer, using electro spray ionization technique in the positive ionization mode.

Results: The method was established over a concentration range of 10-10000 ng/ml. Accuracy, precision, selectivity, recovery, matrix effect and stability of the analyte were also estimated and the results were within the acceptance criteria. Further, precise results were obtained using an optimum spot volume of 10 μl with good spot homogeneity. Blood samples with hematocrit values varying from 24 % to 45 % gave acceptable results with good accuracy and precision.

Conclusion: The efficiency of dried blood spot sample preparation, short analysis time and high selectivity permits estimation of Cape in a small blood volume. The validation results suggest that the method is precise, accurate, and reproducible and can be useful in therapeutic drug monitoring of Cape.

Keywords: Capecitabine, Capecitabine-d11, Dried blood spot, Selective, LC-MS/MS, Post-column infusion.

INTRODUCTION

Capecitabine (Cape, N4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine) is an orally administered fluoropyrimidine carbamate used in the treatment of metastatic breast and colorectal cancers [1]. The Food and Drug Administration (FDA) had approved Cape (Xeloda®; Hoffman-LaRoche, Nutley, NJ) in 2005 as an oral prodrug of 5-fluorouracil (5-FU) for use as monotherapy in the first line treatment of advanced colorectal cancer, adjuvant treatment of patients with stage III colon cancer and locally advanced or metastatic breast cancer [2]. Moreover, Cape is gradually replacing 5-FU in several indications, including gastric cancer. The principle mechanism of action of Cape is the inhibition of thymidylate synthase (TS) and incorporation into RNA and DNA. After oral administration and subsequent absorption across the digestive tract, it is converted to 5-FU through three sequential enzymatic reactions. It is first metabolized to 5-deoxy-5-fluorocytidine (5-DFCR) in the liver by the enzyme, carboxylesterase and then to 5-deoxy-5-fluorouridine (5-DFUR) in the liver and tumor tissue by cytidine deaminase. Finally, it is converted intracellularly to 5-FU by thymidine phosphorylase, an enzyme that is found in tumor tissue [3-5]. Cape is mainly eliminated as metabolites (>95 % of the dose) and the elimination half-life of the parent drug and its metabolites is around 1.0 h [5]. The bioavailability of Cape is nearly 100 % and its oral pharmacokinetics is linear, dependent on dose strength. The plasma protein binding (mainly to albumin) is 54 % for Cape and about 10 %, 62 %, and 10 % for its metabolites 5-DFCR, 5-DFUR and 5-FU respectively [2].

In the last decade, dried blood spot (DBS) technique has proved to be a superior alternative micro sampling approach for quantitative bio analysis of drugs in pharmaceutical research and development. Blood micro-collection technique using filter paper has challenged the conventional, invasive blood sampling by venepuncture [6, 7]. DBS technique offers distinct benefits like reduced sample volume collection (usually 10–25 μl) simplified sample collection and processing procedures, lack of post-collection processing, lower costs of biological sample storage and transport, improved (bio)chemical drug stability compared with frozen samples, reduced biohazard risk with minimum facility for storage and shipment and many more [7-9]. Due to such wide range of advantages it has shown considerable promise for toxicokinetics and pharmacokinetic analysis and becomes one of the popular micro sampling techniques.

Several assays have been reported for the determination of Cape alone [10, 11], along with its active metabolites in different biological matrices such as mouse plasma [12, 13], mouse serum and rabbit bile [13] and human plasma [14-23]. In two other reports, Cape has been determined together with some multi-cytostatic compounds [24, 25]. Mainly, liquid chromatography with UV [10, 11, 13, 16] and mass spectrometry [12, 14, 15, 17-25] detection has been used for the quantification of Cape and/or its active metabolites in different matrices.

In order to derive the benefits of DBS, the analyses must be sensitive enough to quantify the target analyte concentration in a few microlitres of blood present in a punched DBS disk. Indeed, sensitivity may sometimes contribute as one of the major challenges for DBS analysis. This issue can be circumvented by using sensitive mass spectrometers such as triple quadrupole mass spectrometers through which sufficient sensitivity and selectivity can be obtained with adequate confidence. A review of the literature revealed no DBS methods for the quantitation of Cape using LC-MS/MS and as such bioanalytical methods, illustrating the quantitative analysis of anticancer drugs in DBS are very limited. Thus, the aim of the present study was to develop and validate an LC-MS/MS method for the quantification of Cape in DBS. The method was fully validated based on the current regulatory guidelines [26]. The current method provides the simplicity and convenience inherent to the DBS technique, faster run time (2.5 min) and specificity through MS/MS detection.
Extractive spectrophotometric determination of five selected drugs by ion-pair complex formation with bromothymol blue in pure form and pharmaceutical preparations

Sneha G. Nair¹, Jaivik V. Shah¹, Priyanka A. Shah¹, Mallika Sanyal² and Pranav S. Shrivastav¹*

Abstract: Simple, precise, selective, and expeditious spectrophotometric methods have been developed for the determination of itopride (ITO), midodrine (MID), diclofenac (DIC), mesalamine (MES), and sumatriptan (SUM) in their pure form as well as in pharmaceutical preparations. The method was based on ion-pair complex formation between the drugs and anionic dye, bromothymol blue in an acidic medium (pH 2.0–4.0). The yellow colored complexes formed were quantitatively extracted into chloroform and measured at 411, 410, 413, 412, and 414 nm wavelength for ITO, MID, DIC, MES, and SUM, respectively. Beer’s law was obeyed in the concentration range of 3.0–30 µg/mL for ITO, 1.0–20 µg/mL for MID, 1.5–40 µg/mL for DIC, 1.2–12 µg/mL for MES, and 0.5–15 µg/mL for SUM. The stoichiometry of the complexes formed between the drugs and the dye was 1:1 as determined by Job’s method of continuous variation. The association constant (K₁ᵦ) of the ion-pair complexes formed was evaluated using Benesi–Hildebrand equation. Limit of detection, limit of quantification, and Sandell’s sensitivity of the methods were also estimated. The proposed methods were successfully employed for the determination of these drugs in their pharmaceutical dosage forms.

ABOUT THE AUTHORS
Our research group belongs to the Analytical Chemistry Division of the Department of Chemistry, Gujarat University, Ahmedabad, Gujarat, India, with strong interest in the area of pharmaceutical analysis and bioanalysis for more than a decade. We have active collaboration with many pharmaceutical industries and contract research organizations in Ahmedabad. Currently, the main research domains include (i) development and validation of LC-MS/MS-, UPLC-, HPLC-, HPTLC-based methods for the estimation of drugs and their metabolites in biological matrices, (ii) optimization of sample extraction protocols for efficient extraction of drugs and their metabolites from complex biological fluids, (iii) assessment of matrix effects and drug stability in biological fluids, (iv) application of validated protocols for bioequivalence study in healthy volunteers with new formulations, (v) reinforcing confidence in developed bioanalytical methods through incurred sample reanalysis as per standard guidelines.

PUBLIC INTEREST STATEMENT
The work described in this article highlights the importance of one of the most widely used analytical technique like spectrophotometry. This has been the technique of choice for analysts and researchers to estimate drugs in quality control labs as it is simple, economical, and gives acceptable results. Five drugs, namely itopride, midodrine, diclofenac, mesalamine, and sumatriptan have been reliably determined using spectrophotometry by utilizing a simple phenomenon of ion-pair complex formation between the drugs and a commonly used dye, bromothymol blue. The results were extensively validated as per standard guidelines and the method was successfully used to analyze these drugs in their commercial formulations with acceptable accuracy and precision.
Spectrophotometric Determination of Five Commercial Drugs in Pure Form and Pharmaceutical Formulations by Ion-Pair Complexation with Alizarin Red S

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Abstract

Simple, accurate, selective and rapid spectrophotometric methods have been developed for the determination of itopride, midodrine, diclofenac, mesalamine and sumatriptan in their pure as well as in pharmaceutical dosage forms. The method was based on ion-pair complex formation between the drugs and anionic dye, alizarin red S in an acidic medium (pH 2.0-4.0). The complexes formed were quantitatively extracted into chloroform and measured at 284, 287, 279, 285 and 280 nm wavelength for itopride, midodrine, diclofenac, mesalamine and sumatriptan respectively. Beer’s law was obeyed in the concentration range of 3.5-35 µg mL⁻¹ for itopride and diclofenac, 2.5-180 µg mL⁻¹ for midodrine, 3.0-80 µg mL⁻¹ for mesalamine and 4.0-200 µg mL⁻¹ for sumatriptan. The stoichiometry of the complexes formed between the drugs and the dye was 1:1 as determined by Job’s method of continuous variation. The association constant (K_{IP}) of the ion-pair complexes formed was evaluated using Benesi-Hildebrand equation. Limit of detection, limit of quantification and Sandell’s sensitivity of the methods were also estimated. The proposed methods were successfully employed for the determination of these drugs in their pharmaceutical dosage forms.

Keywords: Drugs, Alizarin Red S, Ion-pair complex, Spectrophotometry

1. Introduction

Methods based on spectrophotometry have been successfully used for the analysis of pharmaceuticals, especially for bulk drugs and in the quality control of marketed product as they are much simpler, cost-effective and less time consuming compared to other methods. These methods provide accurate and precise results and find widespread use, especially where expensive equipments like HPLC, HPTLC, GC and electrophoresis are not easily available [1-3]. Determination of drugs by spectrophotometry is accomplished either by direct measurement of UV light (zero order or derivative spectra) or through some chemical reactions like complex formation (ion-pair, charge-transfer) with specific reagents, oxidation-reduction process and catalysis [1].

Itopride (ITO) hydrochloride, a benzamide derivative is novel gastroprokinetic agent possessing acetylcholine esterase inhibitory and dopamine D2 receptor antagonist effects. It is
Highly Sensitive Determination of Colchicine in Human Plasma by UPLC-MS/MS for a Clinical Study in Healthy Subjects

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Abstract

A rapid and sensitive method is described using solid-phase extraction and ultra performance liquid chromatography-tandem mass spectrometry (UPLC-ESI-MS/MS) for the determination of colchicine in human plasma. Chromatography was performed on Waters Acquity UPLC BEH C8 (50 × 2.1 mm, 1.7 µm) column for the analysis of colchicine and colchicine-d₆ using acetonitrile-4.0 mM ammonium formate in water (90:10, v/v) as the mobile phase. Detection and quantitation was done by multiple reaction monitoring for colchicine (m/z 400.3 → 358.3) and IS (m/z 406.3 → 362.3) on a triple quadrupole mass spectrometer in the positive ionization mode. A linear range from 0.010-10.0 ng/mL with correlation coefficient, \( r^2 > 0.9996 \) was established for colchicine using 100µL plasma. Highly precise and quantitative recovery ranging from 100.2 to 101.1 % was obtained across four quality control samples. Matrix effect was assessed by post-column infusion, standard line slope and post extraction spike methods. Stability of colchicine in plasma was determined for different storage conditions like bench top, processed sample, freeze-thaw and long term. The method was applied to a bioequivalence study with 0.6 mg colchicine in 28 healthy volunteers. Assay reproducibility was ascertained by reanalysis of 129 subject samples.

Keywords: Colchicine; Colchicine-d₆; UPLC-ESI-MS/MS; Sensitive; Human plasma; Bioequivalence

1. Introduction

Gout is a painful and progressive disease which can lead to joint destruction and deformity if not treated adequately. It is a medical condition characterized by recurrent attacks of acute inflammatory arthritis due to impaired metabolism of purines. This leads to hyperuricaemia, leading to accumulation of the metabolic end product urate in joints (Richette and Bardin, 2010).
As a First Author:


As a Co-Author:


27. Vivek Upadhyay, Vikas Trivedi, **Priyanka A. Shah**, Jaivik V. Shah, Pranav S. Shrivastav. Liquid-liquid extraction of everolimus an immunosuppressant from
human whole blood and its sensitive determination by UHPLC-MS/MS. *Journal of Advancement in Medical and Life Sciences* 1(3) (2014) 1-11. DOI: 10.15297/JALS.V1I3.06


Absolute Bioavailability: It is the extent or fraction of drug absorbed upon extravascular administration in comparison to the dosage size administered.

Absorption: Absorption of drugs is the process of uptake of the compound from the site of administration into the systemic circulation. A prerequisite for absorption is that the drug should be in aqueous solution. The only relatively rare exception is absorption by pinocytosis.

Accuracy: The degree of closeness of the determined value to the nominal or known true value under prescribed conditions. This is sometimes termed trueness.

Analyte: A specific chemical moiety being measured, this can be intact drug, biomolecule or its derivative, metabolite, and/or degradation product in a biologic matrix.

Analytical run (or batch): A complete set of analytical and study samples with appropriate number of standards and QCs for their validation. Several runs (or batches) may be completed in one day, or one run (or batch) may take several days to complete.

Bioavailability: It is the rate and extent to which a drug is absorbed or it otherwise available to the treatment site in body.

Bioequivalence: It is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent i.e. their plasma concentration-time profiles will be identical without significant statistical differences.

Bioequivalence Requirement: It is a requirement imposed by the Food and Drug Administration for in vitro and/or in vivo testing of specified drug products which must be satisfied as a condition of marketing.

Biological matrix: A discrete material of biological origin that can be sampled and processed in a reproducible manner. Examples are blood, serum, urine, feces, saliva, sputum, and various discrete tissues.

Blank: A sample of a biological matrix to which no analytes have been added that is used to assess the specificity of the bioanalytical method.

Blood: It consist of cellular material (99% red blood cells, with white blood cells and platelets making up the remainder), water, amino acids, proteins, carbohydrates, lipids, hormones, vitamins, electrolytes, dissolved gases, and cellular wastes. Each red blood cell is about 1/3 hemoglobin, by volume. The primary blood gases are oxygen, carbon dioxide, and nitrogen.

Blood-, Plasma-, or Serum-Levels: Demonstrate the drug concentration in blood, plasma or serum upon administration of a dosage form through various routes of administration. Blood, plasma, or serum-level curves are plots of drug concentration versus time on numeric or semi-log graph paper. These levels are obtained from blood samples by venopuncture in certain time intervals after administration of the drug product and chemical or microbiological analysis of the drug in the biological fluid.
**Calibration standard:** A biological matrix to which a known amount of analyte has been added or spiked. Calibration standards are used to construct calibration curves from which the concentration of analytes in QC samples and in unknown study samples.

**Calibration curve:** Plot of analyte response vs. known concentration obtained by analyzing a set of standard samples and measuring the response. The curve may use the response for the sample (height or area) or the ratio of the response to that of an internal standard.

**Clearance:** It is the hypothetical volume of distribution in mL of the un-metabolized drug which is cleared per unit time (mL/min or mL/h) by any pathway of drug removal (renal, hepatic and other pathways of elimination).

**Carry over:** It is a detector response observed in the blank, injected immediately after the highest calibration standard.

**Drug:** It is a chemical compound of synthetic, semi synthetic, natural or biological origin which interacts with human or animal cells. The interactions may be quantified, whereby these resulting actions are intended to prevent, to cure or to reduce ill effects in the human or animal body, or to detect disease-causing manifestations.

**Drug Product or Dosage Form:** It is the gross pharmaceutical form containing the active ingredient(s) [drug(s)] and vehicle substance necessary in formulating a medicament of desired dosage, desired volume and desired application form, ready for administration.

**Excretion:** It is the final elimination of the drug from the body's systemic circulation via the kidney into urine, via bile into intestines and saliva into feces, via sweat, via skin and via milk.

**First-pass Effect:** It is the phenomenon that some drugs are already metabolized between the site of absorption and reaching systemic circulation. First-pass effect may occur in the gut wall, in the mesenteric blood and/or in the liver. First-pass effect may occur upon peroral and deep rectal administration.

**Incurred sample reanalysis:** Reanalysis of selected study samples taken during a clinical or nonclinical study to determine the reproducibility compared with the initial results.

**Internal standard:** Test compound(s) (e.g. structurally similar analog, stable labeled compound) added to both calibration standards and samples at known and constant concentration to facilitate quantitation of the target analyte(s).

**Lower limit of quantitation (LLOQ):** The lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

**Matrix effect:** The direct or indirect alteration or interference in response due to the presence of unintended analytes (for analysis) or other interfering substances in the sample.

**Metabolism:** It is the sum of all the chemical reactions for biotransformation of endogenous and exogenous substances which take place in the living cell.

**Method:** A comprehensive description of all procedures used in sample analysis.
**Pharmaceutic Equivalence:** This term implies that two or more drug products are identical in strength, quality, purity, content uniformity and disintegration and dissolution characteristics; they may however differ in containing different excipients.

**Pharmacokinetics:** It deals with the changes of drug concentration in the drug product and changes of concentration of a drug and/or its metabolite(s) in the human or animal body following administration, i.e., the changes in drug concentration in different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage, binding, metabolism, and excretion.

**Plasma:** It consists of about 92% water, with plasma proteins as the most abundant solutes. Plasma appearance is transparent with a faint straw colour. It is mainly composed of water, blood proteins (albumins, globulins, and fibrinogens), and inorganic electrolytes. It serves as transport medium for glucose, lipids, amino acids, hormones, metabolic end products, carbon dioxide and oxygen. Plasma is the largest single component of blood, making up about 55% of total blood volume.

**Precision:** The closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.

**Processed/Extracted:** The final extract (prior to instrumental analysis) of a sample that has been subjected to various manipulations e.g. extraction, dilution, concentration.

**Quantitation range:** The range of concentration, including ULOQ and LLOQ that can be reliably and reproducibly quantified with accuracy and precision through the use of a concentration-response relationship.

**Quality control (QC) sample:** A spiked sample used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch.

**Recovery:** The extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method.

**Reference standard:** It is an established chemical form of a substance of known purity used as a standard in bioanalysis.

**Selectivity:** The ability of the bioanalytical method to measure and differentiate the analytes in the presence of components that may be expected to be present. These could include metabolites, impurities, degradents or matrix components.

**Serum:** It refers to blood plasma in which clotting factors (such as fibrin) have been removed.

**Stability in matrix:** The chemical stability of an analyte in a given matrix under specific conditions for given time intervals. Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system.
Stability evaluation is done to show that the concentration of analyte at the time of analysis corresponds to the concentration of the analyte at the time of sampling.

- **Solution stability:** The stability test for the standard stock solution of analyte done at the same temperature (room or refrigerated), container and solvent as that used in the analysis.

- **Bench top stability:** It is the stability of the analyte in matrix at working temperature conditions over a short period covering the sample time, when all precautions are taken to avoid specifically known stability problems of the analyte (e.g. light sensitivity).

- **Post preparative stability (Autosampler and/or dry state stability):** It is evaluated over the maximum time from completion of sample work-up to completion of data collection, with allowance also for potential delay in analysis due to equipment failure.

- **Freeze and Thaw stability:** This stability test is done to ensure that the sample remains stable after it is subjected to multiple freeze-thaw cycles in the process of the study.

- **Long term stability:** This is done to assess whether the analyte is stable in the plasma matrix under the sample storage conditions for the time period required for the samples generated in a clinical study to the last date of analysis.

**Therapeutic Equivalence:** This term indicates that two more drug products that contain the same therapeutically active ingredient elicit identical pharmacologic effects and can control the disease to the same extent.

**UPLC or UHPLC:** A rapid liquid chromatographic technique which operates at high pressure, generally more than 400 bar using particle sizes less than 2 µm packed in a narrow column.

**Upper limit of quantitation (ULOQ):** The highest amount of an analyte in a sample that can be quantitatively determined with precision and accuracy.

**Validation:** It is a documented evidence of performance characteristics of a method, satisfying the requirements for the intended application.

**Working standard:** It is an established chemical form of a substance which is characterized against reference standard used as a standard in bioanalysis.