CHAPTER-II
REVIEW OF LITERATURE
This chapter deals with the review of literature on analytical procedures of pharmaceutical compounds *viz.* Riluzole, Febuxostat, Voriconazole and Exemestane.

**(a) Riluzole**

Riluzole is a member of the benzothiazole class. Chemically, riluzole is 2-amino-6-(trifluoromethoxy) benzothiazole. \( \text{C}_8\text{H}_5\text{F}_3\text{N}_2\text{OS} \) is the molecular formula and 234.2 is its molecular weight. It is a white to slightly yellow powder that is very soluble in dimethylformamide.

An isocratic, reversed-phase high-performance liquid chromatographic procedure (HPLC) was developed by *Milena Colovic et.al.*\(^{[1]}\) for determination of the neuroprotective agent riluzole in mice plasma, brain and spinal cord. The procedure is based on isolation of the compound and the internal standard from plasma and central nervous system tissues using a Bakerbond spe™ C8 cartridge, with satisfactory recovery and specificity. Separation was on a C18 column, coupled with an UV detector at 263 nm. The assay was linear over a wide range, with a lower limit of quantification of 100 ng ml\(^{-1}\) or g\(^{-1}\) using 0.1 ml of plasma and about 100 mg of brain tissue. The precision and accuracy were within the acceptable limits for an HPLC assay. The method is currently used to support pharmacological studies of the activity of riluzole when given in combination with other potential
neuroprotective agents in an animal model of familiar amyotrophic lateral sclerosis transgenic mice.

_H. J. M. van Kan et al._,[2] have developed a specific, accurate and precise high-performance liquid chromatographic assay for the determination of riluzole, a drug used to treat patients with amyotrophic lateral sclerosis. Samples were treated by extraction with dichloromethane followed by reversed-phase chromatography with ultraviolet detection at 260 nm. Preset validation criteria were met from 20 to 2000 ng/mL with a linear response curve. Extraction recovery of riluzole was 65--76%. The accuracy of the method was 102--103%. Intra- and inter-day coefficients of variation were in the ranges 2.8--4.9% and 1.8--9.7%. A detection limit of 5 ng/mL was found. Determination of concentrations in serum and plasma resulted in similar results below 500 ng/mL. At higher values a matrix effect cannot be excluded. The presented method can be used to monitor plasma or serum levels in ALS patients.

A simple method was developed by _Adriana Maltese et al._,[3] for separation and quantification of riluzole in rat brain. The analysis was performed by high-performance liquid chromatography using a C18 reversed-phase column (Hypersil ODS) with UV detection at 264 nm. The mobile phase consisted of methanol-water containing 1% triethylamine adjusted with orthophosphoric acid to pH 3.2. The retention time was 8.6 min. A simple liquid–liquid extraction with ethyl acetate was used to obtain riluzole from
brain samples. The limit of quantification was 10 ng/g. The recovery was about 80%. The relationship between peak areas and concentrations was linear over the range between 0.01 and 0.8 μg/g, with \( r^2 \) value over 0.99. The assay provided good reproducibility and accuracy and proved to be suitable for pharmacokinetic studies of riluzole.

The photo-degradation behavior of a pharmaceutical compound previously under development for treatment of overactive bladder have been studied by Lianming Wu et al.\textsuperscript{[4]} Samples of \{4-(4-chloro-3-fluorophenyl)-2-[4-(methyloxy)phenyl]-1,3-thiazol-5-yl\} acetic acid were stressed with visible light and were observed to degrade into a single primary photo-degradation product. This unknown product was analyzed by liquid chromatography tandem mass spectrometry (LC–MS/MS) with accurate mass measurement and hydrogen/deuterium exchange to determine its molecular weight and formula, isotope distribution patterns and exchangeable protons, and product ion structures. By comparison of the fragmentation pathways of the protonated and sodiated species, the charge was found to locate in the electron-rich part of the molecule after fragmentation. MS-derived structural information combined with stopped-flow \( ^1H \) LC-nuclear magnetic resonance (NMR) analysis suggested that the degradation product was 4-chloro-\(N\)-(4-methoxybenzoyl)-3-fluorobenzamide. This unique photo-degradation product was subsequently isolated using preparative-scale chromatography, and its structure was confirmed using 1D and 2D NMR techniques involving the \( ^1H \), \( ^{13}C \), \( ^{15}N \) and \( ^{19}F \) nuclei. The structure of this product suggests that \{4-(4-
chloro-3-fluorophenyl)-2-[4-(methyloxy)phenyl]-1,3-thiazol-5-yl) acetic acid has reacted with singlet oxygen ($^1\Delta_g$) via a [4 + 2] Diels-Alder cycloaddition upon photo-irradiation to cause photo-oxygenation in the solid-state (as is common in solution phase), resulting in an unstable endoperoxide that rearranges to the final degradation product structure. Photo-degradation of a structurally related thiazole, 4-(4-Chlorophenyl)thiazol-2-amine, proceeded via a similar process but in a less reactive manner. However, when exposed to the same conditions, sulfathiazole did not degrade, indicating that this photo-degradation process may only occur for thiazole-containing compounds with specific substituents, such as aryl rings.

*Bollikonda Satyanarayana et.al.,* [5] were observed nine unknown peaks during the process development of riluzole, in the range of 0.05 to 0.15%. These impurity samples were analyzed by LC-MS and the respective peaks were identified at $m/z$ 177, 177, 177, 234, 234, 312, 255, 396 and 333. Among the nine impurities, six impurities were identified as process-related impurities and the remaining three impurities were assigned to the starting material and its isomers. The six process-related impurities were synthesized and characterized based on their spectral data (IR, NMR and MS).

A novel stability indicating LC method was developed and validated for the quantitative determination of riluzole by *K. Siva Kumari et.al.,* [6] in bulk drugs and in pharmaceutical dosage forms in the presence of its isomers, related substances and degradation products. The drug was...
subjected to stress conditions of hydrolysis (acid, base and neutral), oxidation, photolysis and thermal degradation. Considerable degradation was observed under base hydrolysis and oxidation. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.5%. The developed method was validated with respect to linearity, accuracy, precision, specificity, ruggedness and robustness.

A novel simple, sensitive, selective, and rapid high-performance liquid chromatography coupled with tandem mass spectrometry method was developed and validated by Babu Rao Chandu et al.\textsuperscript{[7]} for quantification of riluzole in human plasma. The chromatography was performed by using a Zorbax-SB-C18 (4.6×75 mm, 3.5 μm) column, isocratic mobile phase 0.1% formic acid/acetonitrile (10:90 v/v), and an isotope-labeled internal standard (IS), [¹³C,¹⁵N₂] riluzole. The extraction of drug and internal standard was performed by liquid–liquid extraction and analyzed by MS in the multiple reaction monitoring (MRM) mode using the respective [M+H]\textsuperscript{+} ions, m/z 235.0/165.9 for riluzole and m/z 238.1/169.0 for the IS. The calibration curve was linear over the concentration range 0.5–500.0 ng/ml for riluzole in human plasma. The limit of quantification (LOQ) was demonstrated at 0.5 ng/ml. The within-batch and between-batch precision were 0.6–2.3% and 1.4–5.7%, and accuracy was 97.1–101.1% and 98.8–101.2% for riluzole respectively. Drug and IS were eluted within 3.0 min. The validated method was successfully applied in a bioequivalence study of riluzole in human plasma.
Telekone R.S et al.\textsuperscript{[8]} have developed a simple, precise and economical UV spectrophotometric method for the estimation of riluzole in bulk and pharmaceutical formulations. Riluzole has absorbance maxima at 261 nm in zero order spectrum method (Method A), in the first order derivative spectra, showed sharp peak at 251 nm when $n = 1$ (Method B). Method C is based on calculation of area under curve (AUC) for analysis of riluzole in the wavelength range of 251 nm - 271 nm. The method obeys Beer-Lambert’s law in the concentration range of 2-20 $\mu$g/ml in all three methods. Results of the analysis, validated statistically and by recovery studies were found to be satisfactory.

Clinical trials have shown that Riluzole extends the survival or time before tracheostomy for patients with ALS. Due to its speed, sensitivity, and selectivity, liquid chromatography/tandem mass spectrometry (LC-MS/MS) has become the method of choice for analyzing drug candidates in biofluids. However, there are no published LC-MS/MS methods available for the determination of riluzole in biological samples. Weimin Wang et al.\textsuperscript{[9]} have described the development and validation of a specific and robust LC-MS/MS method for accurate quantification of riluzole in human serum.

The literature survey on Riluzole reveals that no UPLC method has been introduced for the impurity profiling of related compounds.
Uloric (febuxostat) is a xanthine oxidase inhibitor. The active ingredient in Uloric is 2-[3-cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid, with a molecular weight of 316.38. The empirical formula is C_{16}H_{16}N_{2}O_{3}S. Febuxostat is a non-hygroscopic, white crystalline powder that is freely soluble in dimethylformamide; soluble in dimethylsulfoxide; sparingly soluble in ethanol; slightly soluble in methanol and acetonitrile; and practically insoluble in water. The melting range is 205°C to 208°C.

A simple HPLC method was developed and validated by Nancy Cooper et al.,\textsuperscript{10} for the determination of uric acid (UA), xanthine (X) and hypoxanthine (HX) concentrations in human serum to support pharmacodynamic (PD) studies of a novel xanthine oxidase inhibitor during its clinical development. Serum proteins were removed by ultrafiltration. The hydrophilic analytes and the I.S. were eluted by 100% aqueous phosphate buffer mobile phase. The hydrophobic matrix components (late peaks) were eluted with a step gradient of a higher organic mobile phase. Validation on linearity, sensitivity, precision, accuracy, stability, and robustness of the method for PD biomarkers (UA, X, and HX) was carried out in a similar manner to that for pharmacokinetic (PK) data where applicable. Issues of selectivity for endogenous biomarker analytes and individual concentration variations were addressed during method validation. Standards were prepared in analyte-free phosphate buffer. Quality control samples were prepared in
control serum from individuals not dosed with the xanthine oxidase inhibitor. The method was simple and robust with good accuracy and precision for the measurement of serum UA, X, and HX concentrations.

A reverse phase HPLC method for determining the content of febuxostat and its related substances, on a C18 column was performed by ZHANG Cong et al. The mobile phase was methanol-acetonitrile-0.05% (w) phosphoric acid (V: V: V=24:46:30). The flow rate was 1.0 mL·min⁻¹. Ultraviolet absorption detector was set at 315 nm and column temperature at 35 °C. The linear range of febuxostat was between 15.7 and 94.3 mg·L⁻¹ (r² = 0.9998). The average recovery was 100.5% with RSD of 1.0%. The related substances of febuxostat could be completely separated from febuxostat. The limit of detection (LOD) was 0.5 ng. It concludes that the method is simple and accurate for quality control of the febuxostat.

Dongmei Zhou et al. have developed a LC/MS/MS bioanalytical method. The method involves addition of [D7] febuxostat as internal standard and protein precipitation with acetonitrile. An API 4000 triple quadrupole mass spectrometer, operated in positive turbo ion spray mode, is used to monitor the precursor - product ion transitions of m/z 317 - 261 (febuxostat) and m/z 324 - 262 ([D7]febuxostat). This method is applicable for the determination of febuxostat in human plasma over the concentration range of 2.00 to 1000 ng/mL with a validated lower limit of quantification (LLOQ) of
2.00 ng/mL. A dilution factor of 40 was validated with a dilution QC of 20,000 ng/mL.

A validated HPLC method with fluorescence detection for Plasma concentrations of naproxen were determined by Reza Khosravan\cite{3} to evaluate the effect of febuxostat on the pharmacokinetics of indomethacin and naproxen and vice versa, 2 multiple-dose, 3-period crossover studies were performed in healthy subjects. In study 1, subjects received febuxostat 80 mg once daily, indomethacin 50 mg twice daily, or both. In study 2, subjects received febuxostat 80 mg, naproxen 500 mg twice daily, or both. Twenty-four-hour blood samples were collected on day 5 in study 1 and day 7 in study 2. In study 1, 90% confidence intervals of geometric mean ratios for maximum plasma concentration (C\text{max}) and area under the curve (AUC) were within the 0.80 to 1.25 no-effect ranges for febuxostat and indomethacin. In study 2, 90% confidence intervals for febuxostat C\text{max} and AUC extended above that range, with increases of 28% and 40% in C\text{max} and AUC\text{24}, respectively. However, 90% confidence intervals for naproxen C\text{max} and AUC were within the 0.80 to 1.25 range. Febuxostat had no effect on the plasma pharmacokinetics of indomethacin and naproxen. Similarly, indomethacin had no effect on the plasma pharmacokinetics of febuxostat.

A very few methods have been listed for the purity of Febuxostat by HPLC; impurity profiling of febuxostat has not been described by UPLC.
Voriconazole is designated chemically as (2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)-2butanol with an empirical formula of C\textsubscript{16}H\textsubscript{14}F\textsubscript{3}N\textsubscript{5}O and a molecular weight of 349.3. Voriconazole drug substance is a white to light-colored powder.

*R. Ferretti et al.,*\textsuperscript{[14]} have developed a coupled achiral-chiral high-performance liquid chromatography (HPLC) with an achiral amino-based column coupled with a chiral amylose-based column has been used for qualitative and quantitative determination of the potential chiral and achiral impurities of Voriconazole (UK-109,496), a new antifungal agent with two stereogenic centres. The effect of the organic mobile-phase modifier, ethanol, was studied. The assay response was linearly dependent on concentration over the range 1.2 – 40.4 µg for Voriconazole and 2.5–104.0 ng for the impurities. The limit of detection was 2.5 ng for each analyte.

*Richard Gage et al.,*\textsuperscript{[15]} have described a simple, rapid and reproducible method with a calibration range of 0.2–10 µg ml\textsuperscript{-1} voriconazole in human plasma which is more appropriate for routine clinical use than the authors previously published method. The method utilises protein precipitation with acetonitrile as the only sample preparation involved prior to reverse phase HPLC. No internal standard was required.

A novel method based on liquid chromatography-mass spectrometry with electrospray ionization, have been developed by *Lei Zhou et al.,*\textsuperscript{[16]} for
analysis of voriconazole in aqueous humor. The separation was achieved on a reversed-phase C-18 column eluted by 70% acetonitrile-30% water-0.01% TFA. The correlation between the concentration of voriconazole to peak area was linear (r(2)=0.9990) between 0.04 and 60 ng, with a coefficient of variance of less than 3%. Limit of quantitation (LOQ) was estimated to be 5 ng/ml voriconazole with an injection volume of 2 µl of aqueous humor. Both infra-day and inter-day imprecision were less than 3% over the whole analytical range. Parallel analyses of voriconazole samples by LC-MS and by high-performance liquid chromatography (HPLC)-UV showed that the two methods were highly correlated (r(2)=0.9985). LC-MS was used to determine voriconazole levels achieved in the aqueous humor of the rabbit eye, following topical application of 5 or 10 µg voriconazole in the form of eyedrops for 11 days b.i.d. The lower dosage produced an aqueous humor concentration of 7.29 +/- 5.84 µg/ml, while the higher dosage produced a concentration of 14.56 +/- 12.90 µg/ml.

An analytical method for the determination of voriconazole (UK-109,496; Pfizer) in plasma was developed and validated by Gennethel J. Pennick et al. The method utilizes solid-phase extraction technology and high-performance liquid chromatography. The lower limit of quantitation is 0.2 µg/ml, and the range of linearity tested was 0.2 to 10 µg/ml.

A I H Adams and A M Bergol have described the validation of an isocratic HPLC method for the assay of voriconazole in tablets. The method
employs a Merck LiChrospher® 100 RP-8 (125 × 4.6 mm I.D., 5 μm particle size) column, with a mobile phase of methanol : triethylamine solutions 0.6 %, pH 6.0 (50:50, v/v) and UV detection at 255 nm. A linear response ($r^2 > 0.9999$) was observed in the range of 20.0–100.0 μg mL$^{-1}$. The method showed good recoveries (average 100.4%) and the relative standard deviation intra and inter-day were ≤ 1.0 %. Validation parameters as specificity and robustness were also determined. The method can be used for both quality control assay of voriconazole in tablets and for stability studies as the method separates voriconazole from its degradation products and tablet excipients.

A rapid and sensitive RP-HPLC method with UV detection (260nm) for routine analysis of voriconazole in a pharmaceutical formulation was developed by G. Srinubabu et al. Chromatography was performed with mobile phase containing a mixture of acetonitrile and water (50:50, v/v) with flow rate of 1.0 ml/min. Quantitation was accomplished with internal standard method. The procedure was validated for linearity (correlation coefficient=0.9999), accuracy, robustness and intermediate precision. Experimental design was used for validation of robustness and intermediate precision. To test robustness, three factors were considered. Percentage of acetonitrile in mobile phase, flow rate and pH; an increase in the flow rate results in a decrease of the drug found concentration, while the percentage of organic modifier and pH have no important effect on the response. For intermediate precision measure the variables considered were: analyst, equipment and number of days. The R.S.D. value (0.45%, n=24) indicated a
good precision of the analytical method. The proposed method was simple, highly sensitive, precise and accurate and retention time less than 4 min indicating that the method is useful for routine quality control.

A simple liquid chromatographic method was developed for the separation and quantification of voriconazole and its enantiomer in drug substance by A. Nagarjuna et al.\textsuperscript{[20]} The separation was achieved on Chiralcel-OD (250 mm × 4.6 mm × 10 μm) using mobile phase consisting of n-hexane and ethanol in the ratio 9:1 (v/v) with a flow rate of 1.0 mL min\(^{-1}\), at 27 °C column temperature and detection at 254 nm with an injection volume of 20 μL. Ethanol was used as diluent. The method is capable of detecting the (25, 3\(R\)) enantiomer down to 0.0075% and can quantify down to 0.021% with respect to sample concentration. The method is rapid and the resolution achieved was about 3.0. This method can be employed for the quantification of (2S, 3\(R\)) enantiomer in voriconazole drug substance.

Zak K. Shihabi et al.\textsuperscript{[21]} have described a simple method for analysis of the antifungal drug voriconazole in serum. It utilizes a short cyano cartridge column, isocratic separation, and direct serum deproteinization with a mixture of trichloroacetic acid (TCA) and methanol. The advantages of this method are the simplicity and the low concentration of the organic solvent in the mobile phase.

Simple, sensitive, and selective high-performance liquid chromatographic method for the simultaneous determination of voriconazole
and posaconazole concentrations in human plasma was developed and validated by Kathrin Kahle et al. [22] Quantitative recovery following liquid-liquid extraction with diethyl ether was achieved. Linearity ranged from 0.10 to 20.0 µg/ml for voriconazole and from 0.05 to 10.0 µg/ml for posaconazole. The intra- and interday coefficients of variation were less than 8.5%, and the lower limits of quantitation were <0.05 µg/ml.

Gu, Ping et al. [23] have developed and validated an isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method and validated for the determination of voriconazole and its related substances. The drug substance was subjected to stress conditions of UV light, water hydrolysis, acid, base, oxidation, and deoxidization to observe the degradation products. The successful separation of voriconazole from its synthetic impurities and degradation products formed under stress conditions was achieved using an Agilent Zorbax SB-C18 (250mm × 4.6 mm i.d., 5 µm) column maintained at 25°C with a mobile phase of a mixture of ammonium phosphate dibasic buffer (pH adjusted to 6.0 using diluted orthophosphoric acid; 50 mM)-acetonitrile (52:48, v/v). The mobile phase flow rate was 1.0 mL/min, and the detection wavelength was 250 nm. The stress sample solutions were assayed against the qualified reference standard of voriconazole and the mass balance in each case was close to 99.7%, confirming its stability-indication capacity. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity, and robustness. The developed HPLC method to determine the related substances
and assay determination of voriconazole can be used to evaluate the quality of regular production samples. It can be also used to test the stability samples of voriconazole.

*CN Patel et.al., [24]* have developed and validated reversed phase high performance liquid chromatographic method for the estimation of voriconazole in bulk and formulation using prominence diode array detector. Selected mobile phase was a combination of water: acetonitrile (35:65 % v/v) and wavelength selected was 256 nm. Retention time of voriconazole was 3.95 min. Linearity of the method was found to be 0.1 to 2 μg/ml, with the regression coefficient of 0.999. This method was validated according to ICH guidelines. Quantification was done by calculating area of the peak and the detection limit and quantitation limit were 0.026 μg/ml and 0.1 μg/ml, respectively. There was no significant difference in the intra day and inter day analysis of voriconazole determined for three different concentrations using this method. Present method can be applied for the determination of voriconazole in quality control of formulation without interference of the excipients.

*A. B. Khetre et.al., [25]* have studied the development and validation of stability indicating HPLC method for voriconazole, an antifungal drug. Voriconazole was subjected to stress degradation under different conditions recommended by International Conference on Harmonization. The sample so generated was used to develop a stability-indicating high performance liquid
chromatographic method for voriconazole. The peak for voriconazole was well resolved from peaks of degradation products, using a Hypersil C18 (250×4.6 mm) column and a mobile phase comprising of acetonitrile: water (40:60, v/v), at flow rate of 1 ml/min. Detection was carried out using photodiode array detector. A linear response ($r > 0.99$) was observed in the range of 5-25 μg/ml. The method showed good recoveries (average 100.06%) and relative standard deviation for intra and inter-day were $\leq 1.5 \%$. The method was validated for specificity and robustness also.

V.V. Wamorkar et al., [26] have developed and validated RP-HPLC method for the determination of voriconazole in bulk drug. The developed method is found to be specific, reproducible, and stability indicating. The Hypersil, C18 (250 X 4.6 mm) 5μ column was used and mobile phase consisting of water:acetonitrile to achieve good resolution and retention of the analyte and its impurities. The detector linearity was established from concentrations ranging from 5-100 μg/ml. The method was tested at different levels of specificity and accuracy as per requirements given in ICH guidelines. The molecule was exposed to the stress conditions such as acid, base, oxidation, heat and light as per the recommendations of ICH guidelines. The method was proved to be robust with respect to changes in flow rate, mobile phase composition and allied columns. The proposed method is found to be sensitive, precise, rapid, reproducible, and offers good column life.
A simple, sensitive and accurate stability indicating analytical method for voriconazole have developed and validated by Ahmed B. Eldin et.al.,[27] using RP-HPLC techniques and applying the proposed method in the assay of voriconazole tablets (Vfend), since there is no official monograph. The procedure was developed and validated under acidic, basic, oxidative and photo-irradiated conditions. Chromatography was performed with mobile phase containing a mixture of acetonitrile and 0.05M disodium hydrogen phosphate buffer, pH5.5 (1:1, v/v) with flow rate of 1.0 ml per min., C18 column and UV detection at 255 nm. The developed method satisfies the system suitability criteria, peak integrity, and resolution for the parent drug and its degradants. The method was validated for linearity (correlation coefficient = 0.9999), accuracy, robustness and precision. The proposed method was simple, highly sensitive, precise and accurate and the run time less than 15 minutes indicating that the method is useful for routine quality control analysis and stability testing. Voriconazole was determined to be more sensitive in the basic conditions, Photo degradation is observed only under severe conditions of light exposure.

Voriconazole is a very potent antifungal agent used to treat serious fungal infections (candidiasis) it is also the therapy of choice for aspergillosis. After standard dosing, several factors affect exposure of voriconazole, resulting in large variability and demanding further elucidation of drug distribution. For measurements at the site of action, microdialysis is considered to be an outstanding minimally invasive method.
determination of voriconazole in microdialysate and human plasma a new, efficient, reliable, and robust HPLC assay using UV detection at 254 nm have developed and validated by Franziska Simmel et al. After a simple sample preparation using acetonitrile for plasma and for microdialysate, 20 μL were injected and separated on an RP-18 column. The chromatographic run time was less than 4 min. Overall, the assay showed high precision (CV 93.9 to 99.5%) and accuracy (RE −96.7 to +107%) for both matrices. Of the 36 drug products typically co-administered with voriconazole, none except ambroxol interfered with its peak signal, and this interference was successfully managed. In summary, the method is highly suitable for application in (pre)clinical microdialysis studies, e.g., of critically ill patients with invasive mycoses.

A chiral capillary electrophoresis (CE) method have developed by Owens PK et al., for the direct separation of the four stereoisomers of a new broad spectrum antifungal agent, voriconazole. Cyclodextrin (CD) modified micellar electrokinetic chromatography employing, alpha-CD, beta-CD, gamma-CD, hydroxyl propyl-beta-CD and hydroxyl ethyl-beta-CD was not sufficiently selective for the four neutral stereoisomers. Three anionic sulpho butyl-ether-beta-CD (SBE-beta-CD) electrolyte additives, each having a defined degree of substitution (DS) (6.5, 4.5 and 1.0) were subsequently examined. The complete CE separation of all four stereoisomers was obtained when using the medium substituted additive DS = 4.5. In liquid chromatography (LC), two approaches were examined for the direct chiral
separation of the stereoisomers of voriconazole: (a) use of the neutral and anionic CD mobile phase additives and (b) a vancomycin chiral stationary phase. The CD additives were shown to be extremely selective for two stereoisomers of voriconazole (active drug and its enantiomer) but unable to discriminate between the opposite two stereoisomers. The converse was observed, however, when the vancomycin chiral stationary phase was employed.

Arun M. Prajapati et al., [30] have described a validated reversed-phase high-performance column liquid chromatographic (RP-HPLC) and first-derivative UV spectrophotometric methods for the estimation of voriconazole (VOR) in oral suspension powder. The RP-HPLC separation was achieved on Phenomenex C18 column (250 × 4.6 mm id, 5 μm particle size) using water–acetonitrile (40 + 60, v/v; pH adjusted to 4.5 ± 0.02 with acetic acid) as the mobile phase at a flow rate of 1.4 mL/min and ambient temperature. Quantification was achieved with photodiode array detection at 255 nm over the concentration range of 0.1–1 μg/mL with mean recovery of 99.49 ± 0.83% for VOR by the RP-HPLC method. Quantification was achieved with UV detection at 266 nm over the concentration range of 8–20 μg/mL with mean recovery of 99.74 ± 0.664% for VOR by the first-derivative UV spectrophotometric method. These methods are simple, precise, and sensitive, and they are applicable for the determination of VOR in oral suspension powder.
Chhun et al., [31], have developed and validated a sensitive and selective high-performance liquid chromatographic (HPLC) method with ultra-violet detection for the simultaneous determination of posaconazole and voriconazole, two systemic anti-fungal agents. An internal standard diazepam was added to 100 µL of human plasma followed by 3 mL of hexane-methylene chloride (70:30, v/v). The organic layer was evaporated to dryness and the residue was reconstituted with 100 µL of mobile phase before being injected in the chromatographic system. The compounds were separated on a C8 column using sodium potassium phosphate buffer (0.04 M, pH 6.0): acetonitrile ultrapure water (45:52.5:2.5, v/v/v) as mobile phase. All compounds were detected at a wavelength of 255 nm. The assay was linear and validated over the range 0.2–10.0 mg/L for voriconazole and 0.05–10.0 mg/L for posaconazole. The biases were comprised between –3 and 5% for voriconazole and –2 and 8% for posaconazole. The intra- and inter-day precisions of the method were lower than 8% for the routine quality control (QC). The mean recovery was 98% for voriconazole and 108% for posaconazole. This method provides a useful tool for therapeutic drug monitoring.

It is revealed that most of the methods have been given on pharmacokinetic estimations by HPLC or by LC-MS. Impurity profiling has not been described for Voriconazole by UPLC.
Exemestane is chemically described as 6-methylenandrosta-1,4-diene-3,17-dione. Its molecular formula is C_{20}H_{24}O_{2} and its structural formula is as follows: The active ingredient is a white to slightly yellow crystalline powder with a molecular weight of 296.41. Exemestane is freely soluble in N, N-dimethylformamide, soluble in methanol, and practically insoluble in water.

C. Alliev et al.,\textsuperscript{[32]} have developed a high performance liquid chromatographic mass spectrometric method with thermospray ionization for the determination of concentrations of exemestane (6-methylenandrosta-1,4-diene-3,17-dione, FCE 24304) in human plasma. The sensitivity of the method allowed the determination of exemestane concentrations as low as 1 ng ml\textsuperscript{-1}. A suitable internal standard was used for quantification. The intra-day precision (relative standard deviation, R.S.D.) ranged from 16.3\% near to the lower limit of quantification to 0.3\% close to the upper end of the calibration graph. The mean accuracy of the method was found to be 97.8\% (R.S.D.=7\%). Unlike a previously developed gas chromatographic/mass spectrometric method, the new method proved to be suitable for the determination of unchanged drug plasma levels in exemestane-treated subjects.

A procedure for the determination of exemestane, a new aromatase inhibitor, in biological fluids was described by S. Persiani et al.,\textsuperscript{[33]} Exemestane is extracted from human plasma and urine by solid-phase and liquid-liquid extraction, respectively. The test compound is then isolated from
endogenous steroids, its metabolites and/or degradation products by HPLC. The exemestane-containing fraction is collected and its exemestane content measured by radioimmunoassay (RIA). The automated HPLC system allowed a high specificity and reproducibility of retention times, and eliminated almost all manual operations. The RIA allowed the accurate and precise measurement of 12 pg of exemestane/ml in plasma (inter- and intra-assay RSD=17.7 and 13.4%, respectively) and 25 pg/ml in urine (inter- and intra-assay RSD=14.5% and 8.7%, respectively). The recovery of the whole procedure was evaluated by comparison of the RIA calibration curve obtained in plasma or urine (after extraction and HPLC) with that obtained directly in RIA buffer (without extraction and HPLC). The calibration curves were practically superimposable, indicating that the recovery of the whole procedure was excellent. The method was validated in terms of reproducibility, recovery and precision in the range 10–500 pg of exemestane/ml of plasma and 20–1000 pg/ml of urine. Finally the plasma levels of exemestane in a postmenopausal healthy volunteer treated daily for 7 days with oral exemestane at a dose of 1 mg (the lowest dose administered in clinical trials) were monitored using the method here described. Exemestane was detectable in all plasma samples collected (up to 24 h after drug intake). Therefore the analytical method described here should be sufficiently sensitive and specific for the determination of exemestane in plasma and urine from clinical trials in which therapeutic doses of the drug (10–25 mg/day) are administered.
Cenacchi, et.al., 134 have developed a sensitive, specific and rapid analytical method for the quantitation of exemestane (EXE) in human plasma. 6-Methylen-androsta-1,4-diene-3,17-dione(EXE), is an orally active irreversible steroidal aromatase inhibitor used for the therapy of metastatic postmenopausal breast cancer, with estrogen-dependent pathological conditions. The method involves extraction of EXE from human plasma by solid phase extraction using C2 end capped sorbent in the 96 well plate format (50 mg/2 ml). After conditioning of the sorbent with 1 ml of acetonitrile (x2) the plates were rinsed with 1 ml of water (x2). The prepared samples (0.5 ml plasma, spiked with [13C3] EXE as internal standard (IS) and diluted with 0.5 ml water were loaded and drawn through the plate with a minimum of vacuum. The plates were then washed with 1 ml acetonitrile: water (10:90) followed by a drying step for 30 min at full vacuum. Elution was by 0.15 ml of 0.1% trifluoracetic acid in acetonitrile (x2) under a minimum of vacuum. Aliquots of 80 micro were finally injected into the LC-MS-MS system. A Zorbax SB C8 column (4.6 x 150 mm, 5 micron) was used to perform the chromatographic separation; the mobile phase was 100% acetonitrile. MS detection used the heated nebulizer interface, with multiple reaction monitoring (MRM)(297-->121 m/z for EXE and 300-->123 m/z for IS) operated in positive ion mode. A weighed linear regression analysis (weighing factor 1/x2) was used to calculate EXE concentration in standard and unknown samples. The method was fully validated in the concentration range 0.05-25 ng ml(-1).
Politi, Lucia et.al.,[35] have reviewed liquid chromatographic-mass spectrometric (LC–MS) procedures for the screening, identification and quantification of doping agents in urine and other biological samples and devoted to drug testing in sports. Reviewed methods published approximately within the last five years and cited in the PubMed database have been divided into groups using the same classification of the 2004 World Anti-Doping Agency (WADA) Prohibited List. Together with procedures specifically developed for anti-doping analysis, LC–MS applications used in other fields (e.g., therapeutic drug monitoring, clinical and forensic toxicology, and detection of drugs illicitly used in livestock production) have been included when considered as potentially extensible to doping control. Information on the reasons for potential abuse by athletes, on the requirements established by WADA for analysis, and on the WADA rules for the interpretation of analytical findings are provided for the different classes of drugs.

A simple and sensitive liquid chromatography-tandem mass spectrometry (LC–MS/MS) method have developed and validated by Giuseppe Corona et.al.,[36] for the quantitation of exemestane (Exe) and its main metabolite 17-dihydroexemestane (DhExe) in human plasma. The analytes were extracted by protein precipitation with acetonitrile, containing stable $^{13}$C-labelled Exe ($^{13}$C$_3$-Exe) as internal standard, and measured by LC-MS/MS. The best chromatographic separation of the analytes from the interferences was achieved by using a Phenyl column operating under
isocratic regime conditions. The total chromatographic runtime was 5.0 min and the elution of Exe and DhExe occurred at 2.5 min and 2.9 min, respectively. Quantitation was performed by employing the positive electro spray ionization (ESI) technique and multiple reactions monitoring mode (MRM). The monitored precursor to product-ion transitions for Exe, DhExe and $^{13}$C$_3$-Exe internal standard were $m/z$ 297.0 $\rightarrow$ 120.8, $m/z$ 299.1 $\rightarrow$ 134.9 and $m/z$ 300.0 $\rightarrow$ 123.2, respectively. The lower limit of quantitation (LLOQ) was 0.1 ng/ml for DhExe and 0.2 ng/ml for Exe. The method was linear up to 36–51 ng/ml with $r^2 \geq 0.998$. The intra- and inter-assay precision were $\leq$ 7.7% and 5.1% for Exe and $\leq$ 8.1 and 4.9% for DhExe while deviations from nominal values were in the 1.5–13.2% and −9.0–5.8% ranges for Exe and DhExe, respectively. The analytical method resulted robust and suitable for pharmacokinetic monitoring of Exe and its main metabolite during adjuvant therapy in patients with breast cancer.

*R. Suresh Kumar et al., [37]* have developed and validated a selective stability indicating HPLC method for quantification of impurities (process related and degradants) and assay determination of Exemestane. Stability indicating power of the method was established by forced degradation experiments and mass balance study. The chromatographic separation was achieved with Hypersil BDS-C-18 using gradient elution. The developed method is validated for parameters like accuracy, linearity, LOD, LOQ, ruggedness. Box–Behnken experimental design was applied to check the robustness of the method.
E. N. Semenistaya et al. [38] have developed for determining and confirming the presence of exemestane and its metabolite 17-hydroxyexemestane in urine. It is based on the application of high-performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC-MS/MS) and atmospheric pressure chemical ionization high-resolution mass spectrometry (HPLC-HRMS). To detect hydroxyexemestane, the analysis of the hydrolyzed fraction of urine is preferable. The recovery rates of exemestane and 17-hydroxyexemestane were 83 and 91%, respectively. The detection limits were 1 ng/mL for HPLC-MS/MS and 2.5 ng/mL for HPLC-HRMS. In spite of a considerable effect of ionization suppression, the sensitivity and selectivity of the determination are affected by the selection of the optimal detection conditions in HPLC-MS/MS and by the high accuracy of mass determination in mass spectrometry with orbitrap detection, enabling resolution at a level of 5 ppm. The procedures can be used for screening and confirmatory analysis.

Anastrozole (2,2'-[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methylpropionitrile)) and exemestane (6-methyleneandrostan-1,4-diene-3,17-dione) are therapeutically used to treat hormone-sensitive breast cancer in postmenopausal women. For doping purposes they may be used to counteract adverse effects of an extensive abuse of anabolic androgenic steroids (gynaecomastia) and to increase plasma testosterone concentrations. Excretion study urine samples and spot urine samples from women suffering from metastatic breast cancer, being treated with anastrozole or exemestane,
were collected and analyzed to develop/optimize a detection system for anastrozole and exemestane to allow the identification of athletes who do not comply with the internationally prohibited use of these cancer drugs. The assay was based on liquid-liquid extraction after enzymatic hydrolysis following liquid chromatography/tandem mass spectrometry (LC/MS/MS). Anastrozole, exemestane and its main metabolite (17-dihydroexemestane) were identified in urine by comparison of mass spectra and retention times with respective reference substances. Ute Mareck et al.,[39] have performed an assay validation for the analysis of anastrozole and exemestane regarding lower limits of detection (anastrozole: 0.02 ng/mL; exemestane: 3.1 ng/mL; dihydroexemestane: 0.5 ng/mL), interday precisions (6.6–11.1%, 4.9–9.1% and 5.6–8.3% for low [10 ng/mL], medium [50 ng/mL] and high [100 ng/mL] concentration) and recoveries (ranged from 85–97).

Analytical HPLC methods for derivatized amylose chiral stationary phases were developed by C. Danel et al.,[40] for the direct enantioseparation of substituted [1-(imidazol-1-yl)-1-phenylmethyl)] benzothiazolinone and benzoxazolinone derivatives with one stereogenic center. These analogues of fadrozole constitute new potent non steroidal inhibitors of aromatase (P450 arom.). The separations were made using normal phase methodology with mobile phase consisting of n-hexane-alcohol (ethanol, 1-propanol or 2-propanol) in various proportions, and a silica-based amylose tris-3,5-dimethylphenylcarbamate (Chiralpak AD), or tris-(S)-1-phenylethylcarbamate (Chiralpak AS). The effects of concentration of various aliphatic alcohols in
the mobile phase were studied. Baseline separation \((R_s > 1.5)\) was easily obtained in all cases, ethanol being often the more interesting modifier. The effects of structural features of the solutes along with the temperature of the column on the discrimination between the enantiomers were examined for different mobile phase compositions.

A rapid high-performance liquid chromatography (HPLC) method was developed by Paul \textit{et al.},\cite{41} for the quantitation of hydroxyl testosterone metabolites. The method combines a Hypersil BDS C\textsubscript{18} analytical column (10 cm\texttimes 0.46 cm) and a linear mobile phase (1.25 ml/min) gradient of tetrahydrofuran–acetonitrile–water (10:10:80, v/v) changing to tetrahydrofuran–acetonitrile–water (14:14:72, v/v) over 10 min then remaining isocratic for 3 min. The total run time for the chromatographic separation of eight metabolites of testosterone is 15 min. Detection by UV is linear between 300 ng/ml and 10 \(\mu g/ml\) with a limit of detection on column of 300 ng/ml.

Literature survey reveals that method of analysis for exemestane has been given by HPLC only. UPLC methods for the determination of impurity profiling were not described.
References


7. Babu Rao Chandu, Sreekanth Nama, Kanchanamala Kanala, Balasekhar Reddy Challa, Rihana Parveen Shaik and Mukkanti Khagga, Quantitative estimation of riluzole in human plasma by LC-


12. Dongmei Zhou, David Wilson, Sonny Gunawan, Zancong Shen, Chun Yang, Jennifer Yang, Li-Tain Yeh and Virginia Borges, A New Method for the Quantitation of Febuxostat in Human Plasma by LC/MS/MS, *Abstract session viewer*, MP16 Number 384.


22. Kathrin Kahle, Peter Langmann, Diana Schirmer, Ulrike Lenker, Daniela Keller, Annegret Helle, Hartwig Klinker, and Werner J. Heinz, Simultaneous Determination of Voriconazole and Posaconazole in


