2.1 Literature review for analytical methodologies of aceclofenac and/or paracetamol

Analytical methods for determination of aceclofenac in pharmaceutical formulations and/or its degradation products or in presence of its metabolites in biological fluids.

Zawilla and coworkers developed and validated three sensitive and reproducible methods for quantitative determination of aceclofenac in pure form and in pharmaceutical formulation. The first method was based on the reaction between the drug via its secondary aromatic amino group and p-dimethylamino cinnamaldehyde (PDAC) in acidified methanol to give a stable coloured complex after heating at 75°C for 20 min. Absorption measurements were carried out at 665.5 nm. Beer's law was obeyed over concentration range 20–100 µg/mL. The other two methods are high performance liquid chromatography and densitometric methods by which the drug was determined in the presence of its degradation products over concentration range of 20–70 µg/mL and 1–10 µg/spot (Zawilla et al., 2002).

El-Saharty and coworkers developed and validated three methods for the determination of aceclofenac in the presence of its degradation product, diclofenac. The first method was third derivative spectrophotometry (D3) was measured at 283 nm where its hydrolytic degradation product, diclofenac does not interfere. Second method was based ratio-spectra first-derivative spectrophotometry (RSD1) at 252 nm for aceclofenac and at 248 nm for determination of degradation product over concentration ranges of 4-32 µg/mL for both aceclofenac and diclofenac. The third method was based on the quantitative determination of aceclofenac by thin layer chromatography using chloroform: methanol: ammonia (48:11.5:0.5 v/v/v) as mobile phase. Chromatograms were scanned at 274 and 283 nm for aceclofenac and diclofenac, respectively. The method determined aceclofenac and diclofenac in concentration ranges of 2-10 and 1-9 µg/spot (El-Saharty et al., 2002).

Hassan and coworkers developed and validated five new selective, precise and accurate methods for the determination of aceclofenac in the presence of its degradation product. Method A utilized third derivative spectrophotometry at 242 nm. Method B was RSD1 spectrophotometric
method based on absorption measurement at 245 nm. Method C pH induced difference spectrophotometry using UV measurement at 273 nm. Method D was spectrodensitometry based on quantitative determination of aceclofenac by thin layer chromatography at 275 nm. Regression analysis of Beer's plot showed good correlation in the concentration ranges 5–40, 10–40, 15–50, 50–200, 1–50 µg/mL for methods A, B, C, D and E respectively. These methods are suitable as stability indicating methods for the determination of aceclofenac in presence of its main degradation product, diclofenac (Hassan et al., 2003).

Kang and coworkers developed a new LC/MS/MS based method which allowed simultaneous determination of aceclofenac and its three metabolites (4′-OH-aceclofenac, diclofenac, and 4′-OH-diclofenac) in plasma. After acetonitrile-induced precipitation of proteins from the plasma samples, aceclofenac, 4′-OH-aceclofenac, diclofenac, 4′-OH-diclofenac, and flufenamic acid (an internal standard) were chromatographed on a reverse-phase C18 analytical column. The isocratic mobile phase of acetonitrile/0.1% formic acid (aq) [80:20 (v/v)] was eluted at 0.2 mL/min. Quantification was performed on a triple–quadrupole mass spectrometer employing electrospray ionization, and the ion transitions were monitored in multiple reaction-monitoring mode. The monitored transitions for aceclofenac, diclofenac, 4′-OH-diclofenac, 4′-OH-aceclofenac and flufenamic acid were m/z 352.9 → 74.9, 296.1 → 251.7, 311.8 → 267.7, 368.9 → 74.9, and 279.9 → 235.9, respectively. The limits of detection were 2 ng/mL for aceclofenac and 0.2 ng/mL for both diclofenac and 4′-OH-diclofenac. The method was used successfully to measure the concentrations of aceclofenac and its three metabolites in plasma from healthy subjects after administration of a single 100 mg dose of aceclofenac (Kang et al., 2008).

Celiz and coworkers analysed polar pharmaceuticals in waste water by LC-MS-MS. The pharmaceuticals investigated include aceclofenac, diclofenac, carbamazepine, and trimethoprim. Analysis was performed by liquid chromatograph with hybrid linear ion-trap mass spectrometer with a polar reversed phase column to achieve good separation and to minimize matrix effects (Celiz et al., 2009).

Hinz and coworkers developed and validated a liquid-liquid extraction-based reversed-phase HPLC method with UV detection for the analysis of aceclofenac and three of its metabolites (4′-hydroxy-aceclofenac, diclofenac, 4′-hydroxy-diclofenac) in human plasma. The analytes were
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separated using an acetonitrile-phosphate buffer gradient at a flow rate of 1 mL/min, and UV
detection at 282 nm. The retention times for aceclofenac, diclofenac, 4'-hydroxy-aceclofenac, 4'-
hydroxy-diclofenac and ketoprofen (internal standard) were 69.1, 60.9, 46.9, 28.4 and 21.2 min
respectively. The validated quantitation range of the method was 10-10,000 ng/mL for
aceclofenac, 4'-hydroxy-aceclofenac and diclofenac, and 25-10,000 ng/mL for 4'-hydroxy-
diclofenac. The developed procedure was applied to assess the pharmacokinetics of aceclofenac
and its metabolites (Hinz et al., 2003).

Lee and coworkers developed a fully automated narrowbore high performance liquid
chromatography (HPLC) with column-switching for the simultaneous determination of
aceclofenac and diclofenac from human plasma samples. Plasma sample (100 µL) was directly
introduced onto a Capcell Pak MF column (20×4 mm I.D.), where primary separation was
occurred to remove proteins and concentrate target substances using acetonitrile–potassium
phosphate (pH 7, 0.1 M) (14:86, v/v). The drug molecules eluted from MF column were focused
in an intermediate column (35×2 mm I.D.) by the valve switching step. The substances enriched
in intermediate column were eluted and separated on the narrowbore phenyl–hexyl column
(100×2 mm I.D.) using acetonitrile–potassium phosphate (pH 7, 0.02M) (33:67, v/v) with total
analysis time of 17 min. The response was linear over concentration range 50-10000 ng/mL. The
detection limit was 10 ng/mL (Lee et al., 2000).

Bhinge and developed a stability-indicating assay method for the determination of aceclofenac
after being subjected to different International Conference on Harmonization prescribed stress
conditions, such as hydrolysis, oxidation, heat, and photolysis. Aceclofenac was decomposed
under hydrolytic stress (neutral, acidic, and alkaline) and also on exposure to light (in solution
form). The compound was stable to oxidative stress, heat, and photolytic stress (in solid form).
The major degradation product was diclofenac, which was confirmed through comparison with
the standard. Separation of the drug from major and minor degradation products was achieved on
a C_{18} column using methanol and 0.02% of ortho phosphoric acid in a ratio of 70:30. The method
was linear over the concentration range of 17-100 µg/mL. The detection wavelength was 275 nm
(Bhinge et al., 2008).
Musmade and coworkers developed and validated a simple and sensitive high-performance liquid chromatographic (HPLC) method for quantification of aceclofenac in rat plasma. Ibuprofen was used as an internal standard (IS). The present method used protein precipitation for extraction of aceclofenac from rat plasma. Separation was carried out on reversed-phase C$_{18}$ column (250 mm × 4.6 mm, 5 µ) and the column effluent was monitored by UV detector at 282 nm. The mobile phase used was methanol-triethylamine (pH 7.0; 0.3% v/v in Milli-Q water) (60:40%, v/v) at a flow rate of 1.0 mL/min. This method was linear over the range of 50.0–3500.0 ng/mL. The method was successfully applied for pharmacokinetic study of aceclofenac in rats (Musmade et al., 2007).

Gowda and coworkers evaluated the bioequivalence of two formulations containing 100 mg of aceclofenac in plasma by a validated HPLC method with UV detection (Gowda et al., 2006).

**Analytical methods for determination of paracetamol in pharmaceutical formulations and/or its degradation products or in presence of its metabolites in biological fluids.**

Morelli proposed a spectrophotometric method for the determination of paracetamol in pure form and in tablets. The method was based on reaction of the drug with ammonium molybdate in strongly acidic medium to produce molybdenum blue. Beer’s law was followed for concentrations up to 6 µg/mL of paracetamol and the detection limit was 0.10µg/mL. The molar absorptivity at 670 nm was 2.6 × 10$^4$ L/mol/cm (Morelli, 1989).

Vilchez and coworkers developed a spectrofluorimetric method for the determination of paracetamol. The method was based on the oxidation of the analyte to give the fluorophore 2, 2'-dihydroxy-5, 5'-diacetyldiaminebiphenyl. Sodium hypochlorite was used as an oxidizing reagent and the optimum pH was found to be 10.0 (sodium carbonate-boric acid buffer solution). Linear concentration range of application was 0.1-100.0 µg/mL of paracetamol, the detection limit 0.01 µg/mL. The method has been satisfactorily applied to the determination of paracetamol in pharmaceutical formulations and biological fluids (Vilchez et al., 1995).
Afshari and coworkers described a novel spectrophotometric method for rapid quantification of acetaminophen in serum. Free unconjugated acetaminophen was separated from other endogenous interferents by extracting the drug into ethyl acetate and hydrolysis to \( p \)-aminophenol by treatment with heat and acid. The later compound was capable of undergoing an oxidative coupling reaction with \( p \)-xylenol (2, 5-dimethylphenol) catalyzed by sodium periodate. The resultant indophenol derivative formed was measured spectrophotometrically at 635 nm. The proposed method had a linearity range from 25-600 µg/mL. The proposed method for acetaminophen determination was simple, rapid and was especially suitable for screening for drug overdose in an emergency situation (Afshari et al., 2001).

Yesilada and coworkers described a second derivative spectroscopic method for the determination of \( p \)-aminophenol in paracetamol powder. Second derivative absorbance (\( d^2A/d\lambda^2 \)) values were measured at 223.8 nm (\( \Delta = 4.2 \) nm) where \( p \)-aminophenol showed derivative responses obeying Beer's law but paracetamol had negligible derivative absorption (Yesilada et al. 1991).

Monser and coworkers developed a simple, rapid and convenient high performance liquid chromatographic method for the simultaneous determination of paracetamol, 4-aminophenol and 4-chloracetanilide in pharmaceutical preparation. The chromatographic separation was achieved on porous graphitized carbon (PGC) column using an isocratic mixture of 80/20 (v/v) acetonitrile/0.05 M potassium phosphate buffer (pH 5.5) and ultraviolet detection at 244 nm. The linear ranges were 1-50 µg/mL for paracetamol, 5-40 µg/mL for 4-aminophenol and 4-chloracetanilide. The sensitivity of detection was 0.1 µg/mL for paracetamol and 0.5 µg/mL for 4-aminophenol and 4-chloracetanilide. The proposed method was successfully applied to the analysis of commercially available paracetamol dosage forms. The retention selectivity of these compounds on PGC was compared with those of octadecylsilica (ODS) packing materials in RP-HPLC. The ODS column gave little separation for the degradation product (4-aminophenol) from paracetamol, whereas PGC column provided better separation in much shorter time (Monser et al., 2002).

Mohamed and coworkers developed a specific spectrophotometric method for the determination of \( p \)-aminophenol and acetaminophen. The method was based on the reaction of \( p \)-aminophenol
at ambient temperature with sodium sulphide in presence of an oxidant to produce a methylene blue-like dye. Different oxidizing agents were tried, e.g., Ce (IV) and Fe (III). The colour developed within 10 min and remained stable for at least 3 h. The method was applied successfully to the determination of \( p \)-aminophenol in the presence of acetaminophen without prior separation (Mohamed et al., 1997).

Hewala described two stability-indicating methods, HPLC and derivative difference spectrophotometry for the determination of acetaminophen as well as its degradation product, 4-aminophenol. The former method was based on the selection of two chromatographic systems using an octadecylsilane (C\(_{18}\)) stationary phase. The later method involved the use of derivative (first and second) difference spectrophotometric technique. Interference from the coformulated excipients (colorant, flavour, and preservative) of liquid formulations was completely eliminated. The proposed methods were applied for determination of acetaminophen and 4-aminophenol in pharmaceutical formulations (Hewala, 1994).

Kamberi and coworkers developed and validated a sensitive HPLC method for simultaneous determination of trace impurities such as \( n \)-propionyl-\( p \)-aminophenol, 3-chloro-4-hydroxyacetanilide, 4'-hydroxyacetophenone, 4-hydroxyacetophenone oxime, 4-acetoxyacetanilide and 4'-chloroacetanilide in acetaminophen drug substance. The chromatographic separation was achieved on an Eclipse XDB C\(_{18}\) column using a gradient elution with methanol and 0.01 M phosphate buffer at pH 3.0. The limit of quantitation was 0.1 µg/mL for each impurity. The proposed method was successfully applied to the analyses of different lots and different manufacturers of acetaminophen drug substance (Kamberi et al., 2004).

Nemeth and coworkers developed a micellar electrokinetic chromatographic (MEKC) method for determination of paracetamol and its main impurity 4-aminophenol in pharamaceutical preparations. Phosphate buffer (pH 9.0) containing sodium dodecyl sulphate (75mM) was found as the ideal running buffer for the separation. The analysis time was 10 min. The limit of quantitation for 4-aminophenol was 6 µg/mL. The linearity of the method was studied in the concentration ranges 20-260 µg/mL for paracetamol and 20-150 µg/mL for 4-aminophenol. The method was successfully applied for the quality control of paracetamol containing products (Nemeth et al., 2008).
Sena and coworkers described a stability-indicating analytical method for determination of acetaminophen and its degradation product, \( p \)-aminophenol, in an effervescent tablet. Tablets assayed by ion-pair high-performance reversed-phase liquid partition chromatography required no sample cleanup. As little as 0.005% \( p \)-aminophenol could be detected (Sena et al., 1979).

Milch and Szabo developed second derivative UV spectrophotometry for the determination of acetaminophen, even in the presence of high amounts of the impurity, 4-aminophenol. The fluorimetric method used for the estimation of 4-aminophenol impurity was less sensitive than the colorimetric methods used in the pharmacopeias (Milch and Szabo, 1991).

Rao and Narasaraju developed a simple and rapid gradient reversed phase HPLC method for simultaneous determination of paracetamol and its related compounds in bulk drugs and pharmaceutical formulations. As many as nine process impurities and one degradation product of paracetamol have been separated on a Symmetry C\(_{18}\) column (4.6 \( \times \) 250 mm i.d., particle size 5 \( \mu \)m) with gradient elution using 0.01 M potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile as mobile phase and photo diode array detection at 215 nm. The proposed RP-HPLC method was successfully applied to the analysis of commercial formulations (Rao and Narasaraju, 2006).

Cekic developed a simple and specific method for simultaneous determination of paracetamol and \( p \)-aminophenol in pharmaceutical preparations. The method was based on the hydrolysis of paracetamol to \( p \)-aminophenol, which, using dissolved oxygen as an oxidant in the alkaline region was further transformed in to benzoquinoneimine, capable of reacting with tiron to produce a green indophenol dye. The absorbance was measured at 601nm in alkaline medium. Paracetamol and \( p \)-aminophenol were determined in pharmaceutical products in the 1.5-15 mg/L. The developed method can be applied to the determination of \( p \)-aminophenol in the presence of paracetamol without prior separation (Cekic et al., 2005).

Filik and Tavman, developed a new cloud-point preconcentration approach for the spectrophotometric determination of \( p \)-aminophenol in the presence of paracetamol in pharmaceutical preparations. The determination was based on the reaction of \( p \)-aminophenol with 2-(2-hydroxyphenyl)-1H-benzimidazole (HPBI) at room temperature in the presence of an
oxidant to produce indophenol blue dye. The absorbance was measured at 650 nm (Filik and Tavman, 2007).

Bloomfield developed a sensitive and rapid assay method for 4-aminophenol in paracetamol drug and tablet formulation, by flow injection analysis with spectrophotometric detection. The method was based on reaction of 4-aminophenol present in paracetamol with alkaline sodium nitroprusside reagent to form specific blue indophenol derivative. The colored derivative was then detected spectrophotometrically at 710 nm. The method was fully validated and linear down to 0.01 µg/mL (Bloomfield, 2002).

Safavi and Moradlou, developed a H-point standard addition method (HPSAM) for simultaneous determination of paracetamol and p-aminophenol in pharmaceutical formulations. Paracetamol and p-aminophenol react with Fe (III)/1, 10-phenantroline complex and result in the formation of a colored complex, i.e., Fe (II)/1, 10-phenantroline (ferroin). The colored complex was measured at 510 nm. The linear ranges of paracetamol and p-aminophenol were 0.8-20.0 µg/mL and 0.2-10.0 µg/mL, respectively. The detection limits for paracetamol and p-aminophenol were 0.21 and 0.04 µg/mL, respectively. Limit of quantification was also obtained as 0.40 and 0.32 µg/mL for paracetamol and p-aminophenol, respectively. The proposed method was successfully applied for the simultaneous determination of paracetamol and p-aminophenol in paracetamol formulations (Safavi and Moradlou, 2004).

Chen and coworkers developed and validated a rapid and sensitive method for the simultaneous determination of paracetamol and guaifenesin in human plasma, using liquid chromatography-tandem mass spectrometry. After extraction from plasma samples by diethyl ether-dichloromethane (3:2, v/v), the analytes and internal standard osalmide were chromatographed on a C18 column. Detection was performed on a triple quadrupole tandem mass spectrometer by selected reaction monitoring (SRM) mode via atmospheric pressure chemical ionization (APCI). The method was linear in the concentration range of 0.05-20.0 µg/mL for paracetamol and 5.0-2000.0 ng/mL for guaifenesin (Chen et al., 2005).

Wang and coworkers developed and validated a rapid, selective and convenient liquid chromatography-mass spectrometric method for the simultaneous determination of paracetamol
and caffeine in human plasma. Analytes and theophylline (internal standard) were extracted from plasma samples with diethyl ether-dichloromethane (3:2, v/v) and separated on a C\textsubscript{18} column (150 × 4.6 mm ID, 5 µ particle size, 100 Å pore size). The mobile phase consisted of 0.2% formic acid-methanol (60:40, v/v). The assay was linear in the concentration range between 0.05 and 25 µg/mL for paracetamol and 10-5000 ng/mL for caffeine (Wang et al., 2008).

Hu and coworkers developed a highly sensitive liquid chromatographic-atmospheric pressure chemical ionization-tandem mass spectrometric method to quantitate phenacetin and its metabolite paracetamol in rabbit plasma. The analytes and internal standard oxazepam were extracted from plasma by liquid–liquid extraction using ethyl acetate, and separated on a Zorbax C\textsubscript{18} column using acetonitrile–0.1% formic acid in water (40:60 v/v) at a flow of 0.4 mL/min. Detection was carried out by multiple reaction monitoring on a iontrap LC-MS-MS system with an atmospheric pressure chemical ionization interface. The assay was linear over the range 4–1,600 ng/mL for phenacetin and 3–2,000 ng/mL for paracetamol, with a lower limit of quantitation of 4 ng/mL for phenacetin and 3 ng/mL for paracetamol (Hu et al. 2009).

Johnson and Pumb investigated the human metabolism of acetaminophen using UPLC and exact mass TOF MS. The performance of two high throughput LC/MS approaches, namely monolith columns and sub-2 µm particle ultra performance liquid chromatography (UPLC) columns was compared for the detection and identification of the human metabolites of acetaminophen in urine. The UPLC system produced approximately three times the sensitivity and detected more metabolites than the monolithic column approach. The sharp peaks produced by UPLC seem to be particularly advantageous when coupled to electrospray mass spectrometry, apparently reducing ion suppression leading to superior sensitivity and hence lower limits of detection (Johnson and Pumb, 2005).

**Analytical methods for simultaneous determination of aceclofenac and paracetamol in pharmaceutical formulations and/or in presence of its degradation products.**

Srinivasan and coworkers developed and validated ultraviolet spectrometric methods for simultaneous estimation of aceclofenac and paracetamol in solid dosage form. The methods were based on solving simultaneous equation and Q-value analysis on measurement of absorptivity at
276, 244 and 266 nm, respectively. The linearity lies between 2-20 µg/mL for aceclofenac and 2-24 µg/mL for paracetamol (Srinivasan et al., 2006).

Nikam and coworkers developed and validated ultraviolet spectrometric method for simultaneous estimation of aceclofenac and paracetamol in pharmaceutical formulation. The methods employed were simultaneous equation method, first order derivative method, area under curve method and absorbance ratio method for simultaneous estimation of paracetamol and aceclofenac. The first method employed the formation and solving of simultaneous equation using 244 and 273 nm as the two wavelengths for forming equation. The second method employed first order derivative spectroscopy to eliminate spectral interference. The third method employed selection of area under curve in wavelength region of 242-246 and 271-275 nm and solving the equation. The fourth method employed 266 nm as $\lambda_1$ (isobestic point) and 244 nm as $\lambda_2$, which is the $\lambda_{\text{max}}$ of paracetamol (Nikam et al., 2007).

Momin and coworkers developed and validated a RP-HPLC method for determination of aceclofenac and paracetamol in tablets by reverse phase C$_{18}$ column (Intersil 4.6 mm × 25 cm, 10 µm) using acetonitrile: 50 mM NaH$_2$PO$_4$ in a ratio of 65:35 (pH adjusted to 3.0 with orthophosphoric acid) as a mobile phase at a flow rate of 1.5 mL/min and detection at 276 nm. The retention time for aceclofenac and paracetamol was found to be 1.58 and 4.01 min, respectively (Momin et al., 2006).

Gopinath and coworkers developed and validated a RP-HPLC method for simultaneous determination of aceclofenac and paracetamol in tablets. The estimation was carried out on a Hichrom C$_{18}$ (25 cm × 4.6 mm i.d. 5µ) column with a mobile phase consisting of acetonitrile: 20 mM phosphate buffer (pH 5.0) (60:40v/v) at a flow rate of 0.8 mL/min. Detection was carried out at 265 nm. Etoricoxib was used as an internal standard. The retention time of paracetamol, aceclofenac and etoricoxib was 4.75, 6.44 and 8.83 min, respectively (Gopinath et al., 2007).

Natarajan and coworkers developed and validated a stability-indicating isocratic HPLC method for simultaneous estimation of acetaminophen and aceclofenac in tablets. The stability indicating capability of the method was proven by subjecting the drugs to ICH stress conditions of alkaline and acidic hydrolysis, oxidation, photolysis and thermal degradation and resolution of the
degradation products formed therein. The separation was obtained using a mobile phase of mixture of pH 7.0 buffer, methanol and acetonitrile in the ratio 200:120:135 with final pH 7.0 on a ODS column (4.6 mm × 250 mm, 5µ) with UV detection at 275 nm at flow rate of 1 mL/min. For stress studies, a diode array detector was used. The elution order was acetaminophen followed by aceclofenac. The linear range was 600-1400 µg/mL and 120-280 µg/mL for acetaminophen and aceclofenac respectively (Natarajan et al., 2007).

Jamil and coworkers developed and validated a stability-indicating LC method for simultaneous analysis of aceclofenac and paracetamol in conventional tablets and in microsphere formulations. The mobile phase was 80:20 (v/v) methanol-phosphate buffer (10 mM at pH 2.5 ± 0.02). UV detection was at 276 nm. The method was linear over the concentration ranges of 16–24 and 80–120 µg/mL for aceclofenac and paracetamol, respectively. The limits of detection and quantitation for aceclofenac were 0.0369 and 0.1120 µg/mL, respectively; those for paracetamol were 0.0631 and 0.1911 µg/mL respectively (Jamil et al., 2008).

Gandhi and coworkers developed and validated a densitometric method for analysis of aceclofenac and paracetamol as the bulk drugs and in combined tablet dosage forms. The method used aluminium plates coated with silica gel 60 F254 as stationary phase and ethyl acetate-n-butanol-glacial acetic acid 7.5: 2.5: 0.005 (v/v) as mobile phase. The two drugs were resolved with Rf values 0.29 ±0.019 and 0.74 ±0.025 for aceclofenac and paracetamol, respectively. The respective calibration plots were linear over the ranges 50–1000 and 200–1500 ng/band (Gandhi et al., 2008).

Garg and coworkers developed and validated a spectrophotometric method for simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone in tablets. The method was based on the additivity of absorbances, for the determination of aceclofenac, paracetamol and chlorzoxazone in tablet formulation. The absorption maxima of the drugs found to be at 276, 282, and 248 nm respectively for aceclofenac, paracetamol and chlorzoxazone in methanol (Garg et al., 2007).

Shaikh and coworkers developed and validated a RP-HPLC method for simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone in tablets. This method used a Zorbax C_{18} (250 × 4.6 mm, 5 µm) analytical column. Mobile phase was acetonitrile and buffer
(40:60, v/v), buffer containing 50mM orthophosphoric acid; pH of the buffer is adjusted to 6 with 10% w/v sodium hydroxide solution. The instrumental settings were at a flow rate of 1 mL/min; the column temperature was 25°C and detector wavelength was 270 nm (Shaikh et al., 2008).

Joshi and coworkers developed and validated a RP-HPLC method for simultaneous estimation of three component tablet formulation containing aceclofenac, acetaminophen, and chlorzoxazone. The estimation was carried out on a Luna C18 (5µm × 25 cm × 4.6 mm i.d.) column using a mixture of buffer, methanol, and acetonitrile in the ratio 215:130:155 with final pH of 6.5 as a mobile phase, at a flow rate of 1.5 mL/min. Ultraviolet (UV) detection was performed at 275 nm. Total run time was 10 min; the acetaminophen, chlorzoxazone, and aceclofenac were eluted at the retention times of 2.055, 5.096, and 7.605 min, respectively. Calibration curves were linear over the concentration ranges of 5-50 µg/mL for acetaminophen and chlorzoxazone, and 5-30 µg/mL for aceclofenac. The limits of detection were 16.2, 14.6, and 4.8 ng/mL, and limit of quantitation were 49.0, 46.5, and 14.5 ng/mL for acetaminophen, chlorzoxazone, and aceclofenac respectively (Joshi et al., 2008).

Mahajan and coworkers developed a densitometric TLC method for analysis of aceclofenac, paracetamol and chlorzoxazone in tablets. The identification and quantification were performed on 10 cm × 10 cm aluminium backed TLC plates coated with 0.2 mm layers of silica gel 60 F254, previously washed with methanol, and using toluene-2-propanol-ammonia 4:4:0.4 (v/v) as mobile phase. Detection was performed at 274 nm. The RF values of aceclofenac, paracetamol, and chlorzoxazone were 0.28 ± 0.01, 0.72 ± 0.02, and 0.51 ± 0.02, respectively. Calibration plots were linear in the range 400–1400 ng/band for aceclofenac, 2000–7000 ng/band for paracetamol, and 1000–3500 ng/band for chlorzoxazone (Mahajan et al., 2008).

2.2 Literature review for analytical methodologies of telmisartan and/or hydrochlorothiazide

Analytical methods for determination of telmisartan in pharmaceutical formulations and/or its degradation products or in biological fluids.

Torrealday and coworkers developed a HPLC-fluorimetric method for the quantitation of the angiotensin II receptor antagonist telmisartan in urine using a Novapak C18 column 3.9 ×150 mm, 4 µm. The mobile phase consisted of a mixture acetonitrile–phosphate buffer (pH 6.0, 5
mM) (45:55, v/v) pumped at a flow rate of 0.5 mL/min. Effluent was monitored at excitation and emission wavelengths of 305 and 365 nm, respectively. Separation was carried out at room temperature. The method was sensitive enough with LOQ was 1 µg/mL (Torrealday et al., 2003).

Palled and coworkers developed a simple, fast and precise reverse phase high performance liquid chromatographic method for the determination of telmisartan from tablet dosage forms. A hypersil BDS C_{18} (250 mm × 4.6 mm). In isocratic mode, mobile phase acetonitrile: methanol (60:40) was used. The flow rate was 1.2 mL/min, and eluent monitored at 245 nm (Palled et al., 2005).

Prabhu and coworkers developed and validated a stability-indicating HPTLC method for determination of telmisartan in pharmaceutical formulations (Prabhu et al., 2007).

Patil and coworkers developed a stability-indicating LC method for the simultaneous determination of telmisartan and ramipril in dosage form. A chromatographic separation of the two drugs was achieved with an ACE 5 C_{18}, 25 cm analytical column using buffer–acetonitrile (55:45 v/v). The buffer used in mobile phase contained 0.1 M sodium perchlorate monohydrate in double distilled water and adjusted to pH 3.0 with trifluoroacetic acid. The instrumental settings were a flow rate of 1.5 mL/min, column temperature at 30 °C, and detector wavelength of 215 nm using a photodiode array detector. The resolution between ramipril and telmisartan were found to be more than 5. Telmisartan, ramipril and their combination drug product were exposed to thermal, photolytic, hydrolytic and oxidative stress conditions, and the stressed samples were analysed by the proposed method. Peak homogeneity data of telmisartan and ramipril was obtained using photodiode array detector, in the stressed sample chromatograms, demonstrated the specificity of the method for their estimation in presence of degradants. The proposed method was found to be suitable and accurate for quantitative determination and the stability study of telmisartan and ramipril in pharmaceutical preparations (Patil et al., 2007).

Londhe and coworkers developed and validated a stability-indicating RP-HPLC method for determination of telmisartan in pharmaceutical formulations. The drug was separated from its degradation products on a C_{18} column at ambient temperature with methanol-water 80:20 (v/v),
pH 4.0 (adjusted by addition of orthophosphoric acid), as mobile phase at a flow rate of 1.0 mL/min. Under these conditions the retention time of telmisartan was 4.85 ± 0.05 min. Quantification on the basis of peak area was achieved by UV detection at 225 nm; calibration plots were linear in the concentration ranges of 10–60 µg/mL. The drug was subjected to acidic and alkaline hydrolysis, and oxidising, dry heat, wet heat, and photodegrading conditions. Because the method could effectively separate the drug from its degradation products, it can be regarded as stability indicating (Londhe et al., 2010).

Shah and coworkers identified and characterized a photolytic degradation product of telmisartan using LC–MS/TOF, LC–MS², LC–NMR and on-line H/D exchange mass studies. Telmisartan was subjected to stress studies under ICH prescribed conditions of hydrolysis (acidic, neutral and basic), photolysis, oxidation and thermal stress. The drug showed labiality under only photo-acidic condition by forming a single degradation product. HPLC separation of the drug and the degradation product was achieved on C₈ column using gradient method. To characterize the product, a complete mass fragmentation pathway of the drug was initially established. Subsequently, the degradation product peak was subjected to LC–MS/TOF and on-line H/D exchange mass studies. Based on these studies, a tentative structure was assigned to the product as 3-((1′, 7′-dimethyl-2′-propyl-1H, 3′H-2, 5′-bibenzo[d]imidazol-3′-yl) methyl)-6H-benzo[c]chromen-6-one, which was verified through ¹H LC–NMR experiments (Shah et al., 2010).

Chen and coworkers developed and validated liquid chromatographic–electrospray ionization mass spectrometric method for fast determination of telmisartan in human plasma. Plasma of 0.1 mL was deprotenated with methanol, centrifugation, evaporation to dryness and dissolving in mobile phase, samples were separated using a Hypersil-Keystone C₁₈ reversed-phase column (150 × 2.1 mm i.d., 5 µm), together with a mobile phase containing of acetonitrile–10 mM ammonium acetate (42:58, v/v), 0.2% acetic acid and was isocratically eluted at a flow rate of 0.2 mL/min. Telmisartan and its internal standard, valsartan, were measured by electrospray ion source in positive selective ion monitoring mode. The method demonstrated linearity from 1 to 2000 ng/mL (r = 0.9988). The limit of quantification for telmisartan in plasma was 1 ng/mL with good accuracy and precision (Chen et al., 2005).
Li and coworkers developed rapid, selective and sensitive method for determination of telmisartan in human plasma by liquid chromatography–tandem mass spectrometry. Telmisartan and the internal standard, diphenhydramine, were extracted from plasma using diethyl ether–dichloromethane (60:40, v/v), and separated on a Zorbax extend C$_{18}$ column using methanol–10 mM ammonium acetate (85:15, v/v) adjusted to pH 4.5 after mixing with formic acid as mobile phase. Detection was carried out by multiple reactions monitoring on a Q-trap™ LC–MS/MS system with an ESI interface. The assay was linear over the range 0.5–600.0 ng/mL with a limit of quantitation of 0.5 ng/mL and a limit of detection of 0.05 ng/mL. The assay was applied to a pharmacokinetic study of telmisartan given as a single dose (80 mg) to healthy volunteers (Li et al. 2005).

**Analytical methods for determination of hydrochlorothiazide in pharmaceutical formulations and/or its degradation products or in biological fluids.**

Tamat and Moore investigated the photolytic decomposition of hydrochlorothiazide. Hydrochlorothiazide decomposed upon irradiation with near-UV light (λ>310 nm) both in methanol and aqueous solutions. In the photolysis the chlorine substituent was removed to be replaced by either –H or –OR from the solvent ROH. Hydrolysis of the thiadiazine ring was superimposed upon the dechlorination. The presence of oxygen inhibits the decomposition. The mechanism of the photolysis was suggested to involve cation radical which facilitates the hydrolysis step. 5-Chloro-2, 4-disulphonamido-aniline, the normal hydrolysis product from hydrochlorothiazide, was also susceptible to photolytic dechlorination by a similar mechanism (Tamat and Moore, 1982).

Rehm and Smith developed a photometric method for determination of hydrochlorothiazide and its hydrolysis product, 4-amino-6-chloro- m-benzenedisulfonamide and formaldehyde. Ultraviolet absorption measurements at 271 mµ were suitable for the determination of hydrochlorothiazide in the absence of the disulfonamide and for the determination of total hydrochlorothiazide (nonhydrolyzed plus hydrolyzed) where the extent of hydrolysis was less than 10%. The disulfonamide resulting from the hydrolysis of hydrochlorothiazide was determined by reaction with nitrous acid and coupling of the diazonium compound formed with chromotropic acid. The resulting red colour which was read at 500 mµ (Rehm and Smith, 1959).
Daniels and coworkers developed a stability-indicating assay for hydrochlorothiazide in tablet formulations and in the bulk form. Hydrochlorothiazide was dissolved or extracted using methanol. An aliquot of the solution, containing sulfadiazine as an internal standard, was chromatographed on a 10-µm C₁₈ column with an aqueous mobile phase containing 5% methanol as the modifier. The pH was adjusted to about 4.5 with acetic acid. The method gave accurate results for nine lots (four different suppliers) of tablets and two bulk drug lots (two different suppliers). The method can also be used as a test for impurities in hydrochlorothiazide (Daniels et al., 1981).

Desai and coworkers investigated povidone and poloxamer mediated degradation of hydrclo-rothiazide in an antihypertensive combination tablet product. It was hypothesized that the mechanism of degradation of HCTZ in the presence of povidone and/or poloxamer was due to solubilization of the HCTZ by these excipients in the moisture present in tablets, followed by its hydrolysis (Desai et al., 1996).

Fang and coworkers purified and identified an impurity in bulk hydrochlorothiazide. The high-pressure liquid chromatographic method was developed that was selective and sensitive to the subject impurity. The impurity was concentrated and purified using a combination of solid phase extraction and reverse-phase high-pressure liquid chromatography. Subsequently, the impurity had been identified as a specific HCTZ-CH₂-HCTZ isomer utilizing a variety of analytical techniques, including hydrolysis, ultraviolet spectroscopy, liquid chromatography/mass spectrometry, and ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy. The data resulting from the application of these analytical techniques confirmed the identity of the impurity as a methylene bridged pair of HCTZ molecules (Fang et al., 2001).

Franolic and coworkers isolated and characterized a 2:1 hydrochlorothiazide-formaldehyde adduct impurity in hydrochlorothiazide drug substance. The impurities were analyzed by gradient elution chromatographic system, with acetonitrile-water as the mobile phase. Two known impurities of hydrochlorothiazide, 4-amino-6-chloro-1,3-benzenedisulfonamide and chlorothiazide, were separated, as well as a late-eluting, unknown recurring impurity. The unknown impurity was isolated by electrospray ionization LC-MS as a 2:1 hydrochlorothiazide-formaldehyde adduct of the parent drug substance. The adduct was believed to form through the
double condensation reaction of hydrochlorothiazide with excess formaldehyde during the parent compounds synthesis. The concentration of this impurity ranged from 0.02 to 1.1% (area %), and was 0.1% USP specification limit (Franolic et al., 2001).

Deventer and coworkers investigated the stability of selected chlorinated thiazide diuretics. A common property observed for thiazides was hydrolysis in aqueous media resulting in the formation of the degradation product aminobenzenedisulphonamide. The effect of pH, temperature and light were investigated for chlorothiazide, hydrochlorothiazide and altizide. For all three compounds the degradation product could be detected after incubation at pH 9.5 for 48 h at 60°C. At lower pH and temperature the degradation product could not be detected for all compounds. When samples were exposed to UV light altizide and hydrochlorothiazide were photodegraded to chlorothiazide (Deventer et al., 2009).

Papadoyannis and coworkers developed a HPLC method for determination of hydrochlorothiazide in pharmaceutical preparations and human serum after solid phase extraction. A Nucleosil C_{18} (100 × 4.6 mm, 5 µm) column was used with a mixture of CH_{3}CN-1% acetic acid, at a volume ratio 20:80. Detection was performed with a variable wavelength UV-visible detector at 270 nm, resulting in detection limits of 0.2 ng per 20 µL injection. The method was applied to the direct determination of HCT in pharmaceutical preparations (tablets) and biological fluids (human serum) (Papadoyannis et al., 1998).

Niopas and coworkers developed and validated a simple, specific, sensitive, precise, and accurate high performance liquid chromatographic procedure for the analysis of hydrochlorothiazide in human plasma. After addition of hydroflumethiazide as internal standard, the analytes were isolated from human plasma by double liquid-liquid extraction and separated on a reversed phase column with acetonitrile: water 20: 80, v/v as the eluent. Peaks were monitored at 271 nm. The method was found to be linear in the 5 to 80 ng/mL concentration range (r>0.999). The limit of quantification was found to be 5 ng/mL for 1 mL plasma samples. The method was applied to estimate the pharmacokinetics of hydrochlorothiazide after administration of a single oral dose containing 20 mg enalapril and 12.5 mg hydrochlorothiazide to 24 healthy volunteers (Niopas et al., 2002).
Fang and coworkers developed two sensitive and selective methods for the determination of hydrochlorothiazide (HCTZ) in human plasma and urine. Both methods were based on high performance liquid chromatography with tandem mass spectrometric (MS/MS) detection. The MS/MS detection was performed in the negative ionization mode. The developed methods were validated in the concentration ranges of 1-100 ng/mL and 0.05-20 µg/mL in human plasma and urine, respectively. The methods provided sufficient sensitivity and high sample throughput to map out the pharmacokinetics of HCTZ in human (Fang et al., 2005).

Tagliari and coworkers developed and validated a stability-indicating RP-HPLC method for determination of hydrochlorothiazide in pharmaceutical formulations. Isocratic chromatography was performed on a C18 column with 0.1 M sodium phosphate buffer pH 3.0/ acetonitrile (70:30 v/v) as mobile phase, at a flow rate of 1.3 mL/min and detection at 254 nm. The method was successfully applied for the quality control analysis of pharmaceutical formulation of HCTZ for pediatrics (Tagliari et al., 2008).

Ramakrishna and coworkers developed and validated a simple, sensitive and specific liquid chromatography-tandem mass spectrometric method for quantification of hydrochlorothiazide (I), a common diuretic and antihypertensive agent. The analyte and internal standard tamsulosin (II) were extracted by liquid liquid extraction with diethyl ether- dichloromethane (70:30, v/v). The chromatographic separation was performed on a reversed phase column (Waters symmetry C18) with a mobile phase of 10 mM ammonium acetate and methanol (15:85, v/v). The protonated analyte was quantitated in negative ionization by multiple reaction monitoring with a mass spectrometer. The mass transitions m/z 296.1→205.0 and m/z 407.2→184.9 were used to measure I and II, respectively. The assay exhibited a linear dynamic range of 0.5-200 ng/mL for hydrochlorothiazide in human plasma. The lower limit of quantification was 500 pg/mL. The total run time was 2.5 min for each sample. The validated method was successfully used to analyze human plasma samples for application in pharmacokinetic study (Ramakrishna et al., 2005).

Li and coworkers developed and validated a rapid and sensitive liquid chromatography-electrospray ionization-mass spectrometry method for quantification of hydrochlorothiazide in human plasma. The chromatographic separation was achieved on a C18 column by using water-
acetonitrile (68:32, v/v) as mobile phase. The method was linear within the range of 2.5-200 ng/mL. The lower limit of quantification was 1.0 ng/mL. The validated method was successfully applied for the evaluation of the pharmacokinetic profiles of hydrochlorothiazide in human volunteers (Li et al., 2006).

Liu and coworkers developed and validated a fast and sensitive liquid chromatography/tandem mass spectrometry method for determination of hydrochlorothiazide in human plasma the analyte and irbesartan, used as the internal standard, were precipitated and extracted from plasma using methanol. Analysis was performed on a Phenomenex Kromasil C8 column with water and methanol (27:73, v/v) as the mobile phase. Linearity was assessed from 0.78 to 200 ng/mL in plasma. The analytical method proved to be applicable in a pharmacokinetic study after oral administration of 12 mg hydrochlorothiazide tablets to human volunteers (Liu et al., 2007).

Takubo and coworkers developed a sensitive and selective method for the determination of hydrochlorothiazide (HCTZ) concentrations in rat plasma using high performance liquid chromatography–electrospray ionization tandem mass spectrometry (LC–MS/MS). An aliquot of plasma (50 µL) was mixed with the solution of internal standard, hydrofluorothiazide (HFTZ), and extracted with tert-butyl methyl ether. The reconstituted extract was applied to the LC–MS/MS system with a reversed phase C8 column and eluted with distilled water/acetonitrile (85/15, v/v). To enhance negative ionization of HCTZ and HFTZ in the multiple reaction monitor (MRM), the solution consisting of acetonitrile/1% (v/v) ammonia solution (95/5, v/v) was delivered after column separation. This additional technique, so-called the post-column addition, increased sensitivity of HCTZ and HFTZ about 500- and 200-fold, respectively. The calibration curve showed good linearity ($r=0.999$) over the range of 4–1000 ng/mL. This LC–MS/MS method was useful for pharmacokinetic studies of HCTZ in small animals, because it enabled the serial determination of plasma level of HCTZ in rats (Takubo et al., 2004).

**Analytical methods for simultaneous determination of telmisartan and hydrochlorothiazide in pharmaceutical formulations and/or in biological fluids.**

Wankhede and coworkers developed and validated a RP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet formulation. Chromatography was
performed on a ODS Hypersil C\textsubscript{18} (25 cm $\times$ 4.6 mm I.D.) column in isocratic mode with mobile phase containing acetonitrile: 0.05 M KH\textsubscript{2}PO\textsubscript{4} pH 3.0 (60:40). The flow rate was 1.0 ml/min and the eluent was monitored at 271 nm. The selected chromatographic conditions were found effectively separate telmisartan (RT-5.19 min) and hydrochlorothiazide (RT-2.97 min). Linearity for telmisartan and hydrochlorothiazide were found in the range of 4.1-20.48 µg/mL and 1.28-6.4 µg/mL (Wankhede et al., 2007).

Bhat and coworkers developed and validated a RP-HPLC method for simultaneous determination of telmisartan and hydrochlorothiazide from pharmaceutical formulation. The mobile phase consisted of methanol and acetonitrile (70:30 v/v) at a flow rate of 1 mL/min and the wavelength of detection was 270 nm. Rabeprazole was used as an internal standard. The retention times of telmisartan, hydrochlorothiazide and rabeprazole were 1.79 ± 0.01, 2.80 ± 0.01, and 3.19 ± 0.01 min, respectively (Bhat et al., 2007).

Rane and coworkers developed and validated a HPLC method for simultaneous determination of telmisartan and hydrochlorothiazide in combined pharmaceutical dosage forms. Chromatographic separation of two drugs was achieved on an ACE 5 C\textsubscript{18} 25 cm analytical column using buffer–acetonitrile (60:40, v/v) of pH 5.5, adjusted with acetic acid. The buffer used in mobile phase contains 50 mM ammonium acetate in double distilled water. The instrumental settings were: flow rate, 1 mL/min; column temperature, 30°C; and detector wavelength, 260 nm. Methyl paraben was used as an internal standard. The calibration curve shows excellent linearity over the concentration range for telmisartan and hydrochlorothiazide were 10–150 and 5–75 µg/mL, respectively (Rane et al., 2008).

Yan and coworkers developed a rapid and sensitive method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of telmisartan and hydrochlorothiazide in human plasma. Sample preparation involved liquid-liquid extraction with diethyl ether-dichloromethane (60:40, v/v). The analytes and internal standard, probenecid, were separated on a Venusil XBP C\textsubscript{8} column using gradient elution with acetonitrile-10 mM ammonium acetate-formic acid at a flow rate of 1.2 mL/min. Detection was carried out by electrospray negative ionization mass spectrometry using multiple reaction monitoring of the transitions at m/z 513.0 $\rightarrow$ 469.4 for telmisartan, m/z 295.9 $\rightarrow$ 268.9 for hydrochlorothiazide, and m/z 283.9 $\rightarrow$ 239.9 for probenecid. For both the analytes, the method was linear in the range...
1.00-600 ng/mL. The assay was successfully applied to a pharmacokinetic study in human volunteers given a single dose of a combination tablet containing telmisartan 80 mg and hydrochlorothiazide 12.5 mg (Yan et al., 2008).

Bebawy and coworkers described four methods for direct determination of telmisartan (TELM) and hydrochlorothiazide (HCT) in combined dosage forms without prior separation. The first method was derivative spectrophotometry (1D) using a zero crossing technique of measurement at 241.6 and 227.6 nm for TELM and HCT, respectively. The second method was the first derivative of ratio spectrophotometry (1DD) where the amplitudes were measured at 242.7 nm for TELM and 274.9 nm for HCT. The third method was based on TLC separation of the two drugs followed by the densitometric measurements of their spots at 295 and 225 nm for TELM and HCT, respectively. The separation was carried out on silica gel 60 F254 using butanol: ammonia 25% (8:2 v/v) as mobile phase. The fourth method was spectrofluorimetric determination of TELM, depending on measuring the native fluorescence of the drug in 1 M sodium hydroxide using excitation wavelength at 230 nm and emission at 365 nm. The proposed methods were applied successfully for the determination of two drugs in bulk powder and in pharmaceutical formulations. The spectrofluorimetric method was utilized for the analysis of TELM in human plasma (Bebawy et al., 2005).

Shah and coworkers developed and validated a HPTLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage forms. The stationay phase used was precoated silica gel 60 F254. The mobile phase used was a mixture of chloroform: methanol: toluene (2:5:5 v/v/v). The detection of spots was carried out at 272 nm. The calibration curve was found to be linear between 250 to 500 ng/spot for telmisartan and 200 to 700 ng/spot for hydrochlorothiazide. The limit of detection and the limit of quantification for the telmisartan were found to be 75 and 190 ng/spot, respectively, and for hydrochlorothiazide 55 and 150 ng/spot, respectively (Shah et al., 2007).

Maheswari and coworkers developed and validated a HPTLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet formulations. The separation was achieved on precoated silica gel plate 60 F254 using ethyl acetate: chloroform: methanol (10:3:1 v/v/v) as mobile phase. Quantification was carried out by the use of densitometer in absorbance mode at 270 nm (Maheswari et al., 2007).
Methods for determination of hydrochlorothiazide with other antihypertensives in pharmaceutical formulations and/or its degradation products or in biological fluids.

Hertzog and coworkers developed and validated a stability-indicating HPLC method for the simultaneous determination of losartan potassium, hydrochlorothiazide, and their degradation products. The ultimate goal of this work was to develop and validate a single HPLC method selective for eight main components in tablets. The method demonstrated to be suitable for quantification to 0.1% levels of all the degradants as well as 100% levels of respective drug substances (Hertzog et al., 2002).

Suhagia and coworkers developed and validated a RP-HPLC method for simultaneous determination of losartan potassium and hydrochlorothiazide in tablet dosage forms. A Lichrospher 100 C18 column (20×4.6 mm i.d., 5 µm particle size) with isocratic mobile phase containing 20 mM KH2PO4 buffer (pH 3): acetonitrile: tetrahydrofuran in 60:30:10 were used. The flow rate was 1.0 mL/min and effluent was monitored at 215 nm. The retention time of losartan potassium and hydrochlorothiazide were 7.94 min and 3.26 min, respectively. Linearity for losartan potassium and hydrochlorothiazide were in the range of 4-40 µg/mL and 1-10 µg/mL, respectively (Suhagia et al., 2005).

Lusina and coworkers investigated the stablity of losartan/hydrochlorothiazide tablets. The stability study was demonstrated by stability-indicating HPLC method. An HPLC system equipped with a diode array detector set at 220 nm, was used to perform assays. HPLC analysis was performed using Hypersil ODS C18 column (200×4.6mm, particle size 5 µm). Mobile phase, a mixture of acetonitrile, water, orthophosphoric acid and triethylamine in the ratio 400:600:1:1 was used, at flow rate 1 mL/min. Elution was isocratic and run time was 10 min (Lusina et al., 2005).

Ivanovic and coworkers developed and validated a HPLC method for simultaneous determination of hydrochlrothiazide (HCTZ), lisinopril, and their impurities in pharmaceuticals. Chlorothiazide (CTZ) and disulfonamide (DSA), as potential impurities in hydrochlorothiazide,
and diketopiperazine (DKP), as an impurity of lisinopril, were analyzed. The optimum separation was achieved by gradient elution on a 4.6 × 20 mm, 3.5 µm particle size, C₁₈ column. The mobile phase was a gradient prepared by mixing 7:93 (v/v) acetonitrile-25 M M potassium dihydrogen phosphate, pH 5, and 50:50 (v/v) acetonitrile-25 M M potassium dihydrogen phosphate pH 5 in different ratios. The flow rate was 1.0 mL/min. UV detection was performed at 215 nm (Ivanovic et al., 2007).

Ivanovic and coworkers developed and validated a reversed–phase high performance liquid chromatographic method (RP–HPLC) for the determination of valsartan and hydrochlorothiazide, as well as their impurities level. The chromatograms were recorded using the Agilent 1100 Series chromatographic system with DAD detector. Separations were performed on a Hypersil ODS column (250 mm × 4.6 mm; 5 mm particle size) at 258°C column temperature. The gradient high performance liquid chromatographic system was developed, and the following mobile phases were used (A) the mixture acetonitrile–water (10:90 V/V); pH of the mobile phase was adjusted to 2.5 with 85% orthophosphoric acid, and (B) mixture acetonitrile–water (90:10 V/V); pH of the mobile phase was adjusted to 2.5 with 85% orthophosphoric acid. Injection volume was 50 mL, flow rate 1 mL/min and UV detection was performed at 256 nm. Methyl parahydroxybenzoate was used as an internal standard and acetonitrile–water (40:60 V/V) as a solvent (Ivanovic et al., 2007).

Liu and coworkers developed and validated a rapid and specific liquid chromatography-tandem mass spectrometry method for the simultaneous determination of hydrochlorothiazide and valsartan in human plasma. After extraction from plasma using methanol, hydrochlorothiazide, valsartan and hydroflumethiazide, irbesartan, used as the internal standard, respectively, were chromatographically analyzed on a Phenomenex Kromasil C₈ column with water and methanol (27:73, v/v) as the mobile phase. Selected reaction monitoring was specific for mass detection employing negative electrospray ionization. The calibration standards were linear over the concentration range (3.13–800 ng/mL for hydrochlorothiazide and 11.72–3000 ng/mL for valsartan). The method was found to be suitable for application to a pharmacokinetic study after oral administration of dispersible tablet containing 12.5 mg hydrochlorothiazide and 80 mg valsartan to human volunteers (Liu et al., 2008).