
CHAPTER- 2 REVIEW OF LITERATURE

Existing literature was thoroughly searched to understand all the aspects of SMEDDS and the drug selected for the study.

CARDIOVASCULAR DISORDERS AND THERAPY

Cardiovascular disorders are the world's most prevalent diseases. With the general aging of the world's population and rapid socio-economic changes in the developing world, cardiovascular diseases are expected to increase further in the future. Hence, there is a great need for adequate pharmacotherapy to provide symptomatic treatment and long-term protection. Among the most beneficial medications currently available are those that interfere with the actions of angiotensin II. Angiotensin II has a well-defined tropic effect on vascular and cardiac cells and the extracellular matrix. Over the last decade, several clinical trials have demonstrated the benefits of blocking angiotensin II. In particular, the angiotensin-receptor blockers (ARBs), originally indicated for hypertension, have shown themselves to have cardiovascular benefits beyond lowering blood pressure (1). An increased understanding of the renin-angiotensin system and the development of angiotensin-converting enzyme (ACE) inhibitors and angiotensin-II receptor antagonists (such as valsartan) has formed a major part in these accomplishments. Renin is released from the juxtaglomerular cells of the kidney and then cleaves its substrate, angiotensinogen, to form angiotensin-I. This is converted, by the angiotensin converting enzyme (ACE), to angiotensin-II. In the cardiovascular system the products of this pathway are important in both blood pressure regulation and sodium and volume homeostasis; angiotensin-II has a number of important and complex physiological actions, notable among which is vasoconstriction which results in blood pressure elevation. ACE inhibitors were first introduced for the treatment of hypertension but subsequent studies have shown that they are also able to reduce mortality and morbidity in congestive cardiac failure, decrease morbidity and mortality after myocardial infarction, prevent re-infarction, influence atherosclerosis and slow diabetic complications, including nephropathy. Nevertheless, there are disadvantages and, in particular, ACE inhibitors degrade bradykinin, resulting in a prolongation of its normally short half-life; this and related effects seem to be responsible for the cough and angioedema that are prominent adverse effects with the ACE inhibitors and can

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usually be avoided by the use of an angiotensin-II receptor antagonist, such as valsartan, olmesartan, irbesartan, losartan.

Valsartan is mildly hydroscopic and absorption is rapid with peak concentrations being shown in 1 to 2 hr following oral administration. Excretion is rapid and is mainly hepatic and fecal, there being a number of metabolic breakdown products. Valsartan is highly bound to serum proteins but metabolism does not appear to be dependent on any of the cytochrome 450 iso-enzymes. In summary, valsartan is an angiotensin receptor antagonist that binds in an insurmountable and specific manner to its receptor site. The half-life is long and provides for 24 hr blockade of the receptor site. The drug is mainly excreted unchanged into the bile so that dosage adjustments are seldom necessary, even in patients with renal insufficiency or the elderly. Valsartan effectively lowers blood pressure in all degrees of hypertension and exerts renoprotective effects. It has an important role in the management of cardiac failure and would appear to provide a valid alternative for the treatment of cardiac failure following myocardial infarction (2).

Olmesartan medoxomil is another drug falling under same class called an angiotensin II antagonist. It works by preventing the action of a hormone in the body called angiotensin II. Olmesartan blocks the receptors that angiotensin II acts on, and so prevents its actions. The main result of this is that the peripheral blood vessels are allowed to widen, which means that there is more space and less resistance in these blood vessels. This is the main mechanism by which the pressure in the blood vessels is lowered. Blocking the actions of angiotensin II also reduces the action of aldosterone on the kidneys. The result of this is an increase in the amount of fluid removed from the blood by the kidneys. This decreases the amount of fluid in the blood vessels, which also lessens the resistance and pressure in the blood vessels. The combined overall effect of these changes is to lower the blood pressure (3).

Olmesartan medoxomil administered orally as a prodrug, whereupon it undergoes rapid and complete bioactivation through ester hydrolysis by the enzyme arylesterase during absorption through the gastrointestinal tract into its active form, olmesartan (RNH- 6270). Once in the bloodstream, it is highly bound (99%) to plasma proteins. Olmesartan shows linear pharmacokinetics after oral

administration. Using the oral and intravenous area under the plasma concentration-time curve (AUC), the mean oral bioavailability in healthy adult volunteers was 29%. The median peak concentration (C_{\max}) occurred at two hours (range 1–4h) and the mean elimination half-life was approximately 14–16 hours, with duration of action of up to 24 hours (4).

These drugs have found good therapeutic values in the treatment of hypertension, but due to poor water solubility they suffer with slow dissolution rate and low bioavailability. Many approaches are reported for improvement of solubility and bioavailability of such drugs which we will discuss in next section.

SOLUBILITY ENHANCEMENT APPROCHES FOR POORLY WATER SOLUBLE DRUGS

A drug molecule has to be water soluble to be readily delivered to the cellular membrane but needs to be hydrophobic to cross the membrane. Both properties can be utilized to develop various delivery systems that can deliver water insoluble drugs well in humans. Through years of diligent and intelligent research by pharmaceutical scientists, many techniques dealing with the formulation issues of water-insoluble drugs have been developed and accumulated in the pharmaceutical literature. Water-insoluble drugs with high permeability, drug absorption by the gastrointestinal (GI) tract are limited by drug dissolution rate. Therefore water-insoluble drug formulations for oral solid dosage forms should be focused on the enhancement of the dissolution rate of water-insoluble drugs (5). A various approaches adopted to improve solubility and thereby bioavailability of water-insoluble drugs like complexation with cyclodextrin, solid dispersion, nanoparticles, micronization, lipids based system and permeation enhancers (6). Among these systems the use of carrier systems, such as liposomes (7), mixed micelles (8), multiple emulsions (9), microemulsions (10), microspheres (11), has been shown to improve the gastro intestinal absorption of water-insoluble drugs. Indeed, in some selected cases, these approaches have been successful. In recent years, much attention has focused on lipid-based formulations to improve the oral bioavailability of poorly water soluble drug compounds. In fact, the most popular approach is the incorporation of the drug compound into inert lipid vehicles such as oils (12), surfactant dispersions (13, 14) self-emulsifying formulations (15–18),

emulsions (19, 20) and liposomes (21) with particular emphasis on self-emulsifying drug delivery systems (SEDDS). Microemulsion systems have received increasing attention during the past few years. Formulations based on microemulsions have several advantages over conventional formulations, namely thermodynamic stability, enhanced drug solubilization and ease of manufacturing (22). In addition, the presence of surfactant and in some case co-surfactant, for example, and medium chain diglycerides in many cases, serves to increase the membrane permeability, thereby increasing drug uptake (23). These microemulsions may some time suffers with the instability problem and to overcome this drawback advance microemulsion technique was developed terms as self-emulsifying drug delivery system (SEEDS) and self-microemulsifying drug delivery system (SMEDDS).

SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEMS (SMEDDS)

Self-emulsifying/microemulsifying drug delivery systems (SEDDS/SMEDDS) can be described as isotropic solutions of oil and surfactant, which form o/w (micro) emulsions on mild agitation in the presence of water. Self-emulsifying formulations spread readily in the GI tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification (17, 24). Several published studies describing modest to substantial increase in drug bioavailability from SMEDDS formulation, relative to conventional solid dosage forms are mentioned below:

- The utility of SEDDS has been investigated by Charman and coworkers who, although unable to show enhanced bioavailability of an investigational lipophilic drug WIN 54954, were able to demonstrate greatly improved pharmacodynamics using systems based on medium chain triglyceride (MCT) and ethoxylated glyceryl trioleate (Tagat TO) (25).
- Self-emulsifying w/o microemulsions based on MCTs such as Captex 355 and Captex 8000 have been reported. The systems contained a mixture of mono and diglycerides (Capmul MCM) in combination with Tween 80 as surfactant. The bioavailability of calcein, a water-soluble marker, and an RGD peptide were shown to be significantly increased using a

microemulsion concentrate and preformulated w/o microemulsions compared to the control aqueous formulation (26).

- The bioavailability of a poorly water soluble 5 α -reductase inhibitor has similarly been shown to be improved in Beagle dogs (27).
- The most notable example of a SMEDDS relates to the oral delivery of cyclosporin A, in particular the commercial Neoral[®] formulation. The Neoral formulation uses an isotropic concentrated blend of surfactant based on medium chain length partial glycerides, a medium chain length triglyceride oil and drug. Exposure of this concentrate to water results in formation of initially a w/o microemulsion which on further mixing with water undergoes phase inversion to yield an o/w microemulsion. The delivery of cyclosporine A via microemulsion formulations has been considered in some detail (28, 29), and the superiority of the Neoral[®] microemulsion pre-concentrate over the original Sandimmune formulation has been demonstrated on several occasions (30, 31, 32). Noble and Markham concluded that Neoral offers more predictable and more extensive drug absorption than the standard Sandimmune formulation (33).
- Bioavailability of vasopressin and insulin from w/o microemulsions is higher when the oil phase is based on straight-chain rather than branched-chain fatty acid esters. Observed differences in bioavailability are related, at least in part, to the large reduction in lipolytic activity exhibited by lipases toward branched chain fatty acid substrates (34).
- After topical administration with an occlusive patch, water partitions into the SMEDD forming a microemulsion gel phase. As the extent of hydration increases, the drug solubility decreases resulting in a supersaturated system which the authors claim results in enhanced drug activity and improved pharmacodynamics (35).
- The formulation of a SMEDDS containing flurbiprofen and based on phospholipid, ethyl oleate and ethanol has also been reported. After parenteral administration, significant increases in flurbiprofen half-life and an altered biodistribution pattern were observed (36).

- The formulation and performance of SEDDS containing the anti-malarial halofantrine has also been reported. The SEDDS and SMEDDS were isotropic mixtures of medium or long chain triglyceride, monoglyceride (Capmul MCM), drug and ethanol. Six- to eight-fold improvements in bioavailability were observed relative to tablet formulations (37).
- Coenzyme Q10 (CoQ10), is a lipid soluble compound that is used as an antioxidant and in the treatment of cardiovascular disorders including angina pectoris, hypertension and congestive heart failure. The drug is poorly absorbed from the GI tract possibly due to its high molecular weight and water insolubility. SEDDS approach was evaluated for improved oral bioavailability of CoQ10. An optimized formulation determined on the basis of mean emulsion droplet diameter containing acetylated monoglycerides (Myvacet 9-45), Labrafac CM-10 and propylene glycol monolaurate (lauroglycol) was developed. The oral bioavailability studies carried out on dogs resulted in a two-fold higher bioavailability with CoQ10 SEDDS as compared to the powder formulation (38).
- Tipranavir (TPV), a potent anti-HIV drug of the new class of nonpeptidic protease inhibitors (NPPIs) was incorporated into a new SEDDS formulation. When compared to the initial formulation, which is a hard filled capsule (HFC), the new SEDDS formulation administered in a soft gelatin capsule, led to an approximately two-fold higher bioavailability (39).
- Saquinavir (SQV), a potent and well-tolerated anti-HIV drug is currently used as a protease inhibitor (PI) in highly active antiretroviral therapy (HAART) regimens. The significant improvement in bioavailability (331%) of SQV with SEDDS is attributed to capmul, a glyceride (medium chain mono- and diglycerides) type excipient used in the SGC formulation. Capmul dissolves the drug to a high extent, and the drug is rapidly released (40).
- The effect of structured triglycerides with varying intramolecular structures and chain lengths (medium chain and long chain fatty acids) incorporated into a SMEDDS on the intestinal lymphatic transport and absorption of

halofantrine into the blood was investigated (41, 42). Based on this study it was therefore hypothesized that medium chain fatty acid enhanced the absorption into the systemic blood circulation whereas long chain fatty acid enhanced the lymphatic transport. Thus, the absorption profile of a drug formulated into a SMEDDS could be manipulated by varying the medium and long chain triglyceride content in the formulation in order to improve the oral bioavailability of highly lipophilic drugs.

- Paclitaxel is one of the most potent chemotherapeutic agents currently employed for the treatment of solid tumors. However, it is a very lyophobic compound; insoluble in most pharmaceutically acceptable solvents. There was a significant improvement in the relative bioavailability of the paclitaxel in SMEDDS as compared to those of Taxol[®] (43). A novel SMEDDS has been developed for the oral delivery of paclitaxel. The formulation contained vitamin E as an oil phase, DOC-Na, TPGS, and Cremophor RH 40 as surfactants to increase the solubility of paclitaxel. The surfactants might moderately inhibit the P-gp efflux system, leading to a slight improvement of paclitaxel oral absorption. The low amount of Cremophor and lymphatic transport of paclitaxel microemulsions in the gut might also be beneficial to the oral absorption of paclitaxel in SMEDDS (44).
- A supersaturable SEDDS (S-SEDDS) was developed for the oral delivery of paclitaxel. The fact that high content of surfactants in orally administered SEDDS could lead to adverse effects at the GI tract and a decrease in intestinal absorption of the drug as a result of a decrease in free drug, led to the development of this new SEDDS in which the surfactant concentration was lowered and a cellulose polymer, hydroxypropyl methylcellulose (HPMC), was used as a viscosity enhancer in an attempt to reduce/prevent drug precipitation upon GI fluid dilution. The resultant formulations were stable and contained paclitaxel at a temporarily supersaturated state. *In vivo* studies conducted on Sprague Dawley rats supported these results. Oral administration of a 10 mg/kg dose of paclitaxel as S-SEDDS containing 5% HPMC yielded almost five fold

higher AUC_{0-∞} values compared to the market formulation. These findings demonstrated that the presence of HPMC in the paclitaxel SEDDS prevented macroscopic precipitation of the drug from the formulation and thus provided a supersaturable formulation with improved oral bioavailability (45).

- A new self-emulsifying drug delivery system (SEDDS) and selfmicroemulsifying drug delivery system (SMEDDS) have been developed to increase the solubility, dissolution rate, and, ultimately, oral bioavailability of a poorly water soluble drug, carvedilol. The *in vitro* dissolution rate of carvedilol from SEDDS and SMEDDS was more than two-fold faster compared with that from tablets. The developed SEDDS formulations significantly improved the oral bioavailability of carvedilol significantly, and the relative oral bioavailability of SEDDS compared with commercially available tablets was 413% (46).
- Silymarin was found effective clinically to treat a variety of liver disorders, including acute and chronic viral hepatitis, toxin- and drug-induced hepatitis and cirrhosis, and alcoholic liver disease. It was also found effective in treating certain cancers, such as breast, prostate, and skin cancers. The effectiveness of silymarin as liver disease remedy was discounted by its poor water solubility and low bioavailability after oral administration. The phase behavior of silymarin SMEDDS composed of simply silymarin/ethyl linoleate/Tween 80/ethyl alcohol was studied. The bioavailability of orally administered silymarin SMEDDS was evaluated in rabbits, and implication of the effect of microemulsion particle size and release characteristics on absorption was discussed. Relative bioavailability of silymarin SMEDDS was dramatically enhanced approximately 1.88 and 48.82-fold that of silymarin PEG 400 solution and suspension, respectively (47).
- An optimized SMEDDS formulation consisting of Labrafac CM10 (31.5% wt/wt), Tween 80 (47.3% wt/wt), PEG 400 (12.7% wt/wt), and fenofibrate (8.5% wt/wt) was successfully developed with an increased dissolution rate, increased solubility, and, ultimately, increased bioavailability of a poorly water-soluble drug, fenofibrate. The developed formulation showed higher pharmacodynamic potential as compared with plain fenofibrate.

Results from stability studies confirmed the stability of the developed formulation. Study confirmed that the SMEDDS formulation can be used as a possible alternative to traditional oral formulations of fenofibrate to improve its bioavailability (48).

- Self-microemulsifying drug delivery system (SMEDDS) was developed to enhance the oral bioavailability of the poorly water-soluble drug, oridonin. The influence of the oil, surfactant and co-surfactant types on the drug solubility and their ratios on forming efficient and stable SMEDDS were investigated in detail. The SMEDDS were characterized by morphological observation, droplet size and zeta potential determination, cloud point measurement and *in vitro* release study. The optimum formulation consisted of 30% mixture of Maisine 35-1 and Labrafac CC (1:1), 46.7% Cremophor EL, and 23.3% Transcutol P. *In vitro* release test showed a complete release of oridonin from SMEDDS in an approximately 12 h. The absorption of oridonin from SMEDDS showed a 2.2-fold increase in relative bioavailability compared with that of the suspension. Our studies demonstrated the promising use of SMEDDS for the delivery of oridonin by the oral route (49).
- The investigation was done to design a thermodynamically stable and dilutable self-nanoemulsion formulation of Ramipril, with minimum surfactant concentration that could improve its solubility, stability and oral bioavailability. Based on higher drug release, optimum globule size, minimum polydispersity, lower viscosity, lower surfactant concentration, higher solubility as well as higher bioavailability without variable absorption has been optimized as nanoemulsion formulation of ramipril containing Sefsol 218 (20% w/w), Tween 80 (18% w/w) and Carbitol (18% w/w) as oil, surfactant and cosurfactant, respectively. The *in vivo* studies revealed significantly greater extent of absorption than the conventional capsule formulation. The absorption of ramiprilat from ramipril nanoemulsion resulted in 2.94-fold increase in bioavailability as compared to conventional capsule and 5.4-fold to that of drug suspension. Studies illustrated the potential use of ramipril formulated as nanoemulsions, can be

used as a liquid formulation for pediatric and geriatric patients as well can be formulated as SNEDDS using soft gelatin capsules as unit dosage form. Studies also showed how nanoemulsion formulation can be optimized for the delivery of hydrophobic compounds with higher drug loading, minimum surfactant concentration and proper infinite dilution can be achieved without drug precipitation (50).

- Currently, several formulations have been developed to produce modified emulsified formulations as alternatives to conventional SEDDS. These include, but are not limited to self-microemulsion formulations, surfactant dispersions (51), preformulated freeze dried emulsions (52), microencapsulated emulsions (53), lipid/cross-linked polymeric matrices (54), self-emulsifiable pellets (55), and solid self-emulsifying systems (self-emulsifying tablets) (56). All these formulations will produce fine oil droplets or micelle dispersions upon aqueous dilution.

2.3 PATENTS

- As valsartan is very potent molecule, many formulations containing valsartan are available in the market and as well numbers of patents are approved for different dosage forms of it. Presently valsartan tablets are marketed by Novartis as DIOVAN® in doses of 40, 80, 160 and 320 mg and it is used to treat hypertension. WO 9524901 A1 (CIBA-GEIGY AG) is directed to the use of valsartan for the treatment of diabetic nephropathy. WO 9749394 A2 (NOVARTIS AG), discloses compressed solid oral dosage forms, e.g., by compaction of valsartan (optionally in salt form) optionally combined with HCTZ. In patent no having WO 0038676 A1, it has been found surprisingly that it is possible to improve the bioavailability characteristics of known solid formulation of valsartan by increasing the proportion of microcrystalline cellulose (57).
- The valsartan has low water solubility and hence all formulation suffers with low dissolution profile. One of the investor tried to overcome this problem by formulation tablet containing valsartan particles having size (D_{50}) 150 μm or below (58).

- The combination therapy of valsartan with other antihypertensive agent is also patented. Solid oral dosage form containing valsartan was patented optionally in combination with hydrochlorothiazide (HCTZ). It has been reported that valsartan combined in a dose range from about 10 to 250 mg with hydrochlorothiazide in a dose range from about 6 to 60 mg, is more suitable for efficient treatment of hypertension. This combination is proved to have grater efficiency in reducing elevated blood pressure to normal levels than it would have if used at the same dose range in monotherapy. More preferred unit dose of about 80 mg valsartan and 12.5 mg or 25 mg of hydrochlorothiazide and 160 mg valsartan and 12.5 mg or 25 mg of hydrochlorothiazide. (59).
- As we discussed utility of SMEDDS above, patents are granted on this lipid based formulation. Self-emulsifying formulation of CETP inhibitors are patented by Sheth et al. (60). A liquid formulation for oral administration of the CETP inhibitor has improved bioavailability compared with conventional solid formulations. The formulation comprises the CETP inhibitor, or a pharmaceutically acceptable salt thereof; an oil; and one or more nonionic surfactants having a hydrophilic balance (HLB)>10. The oil phase comprises one of more caprylate and caprate mono- and diglycerides or a mixture thereof; the nonionic surfactant is polysorbate 80; and the formulation does not include a triglyceride.
- Another invention relates to pharmaceutical formulation of (3R, 3aR, 3aS)-hexahydrofuro[2,3-b]furyl(1S,2R)-3-[[[(4-aminophenyl)](isobutyl)amino]-1-benzyl-2-hydroxypropylcarbamate, salts, esters, polymorphic and pseudopolymorphic forms thereof, which are self-microemulsifying drug delivery systems and comprise as carrier a lipophilic phase, one or more surfactants, a hydrophilic solvent and nucleation inhibitor (61).
- Microemulsion was discussed as potential source of water insoluble drugs delivery systems. Microemulsion have previously been delivered only in the form of soft or hard gelatin capsules, or as a liquid dispensed directly into the patient's mouth. The microemulsions of the present invention are administered in the form of solid particles which may be further formulated

into solid dosage forms. The drug-containing microemulsions are adsorbed onto a solid particulate (i.e., powder). Although drug is in a solid form, it is maintained as a microemulsion, or in the case of self-microemulsifying drug delivery system (SMEDDS), in a state readily converted to a microemulsion in vivo, which thereby enhances the dissolution. Microemulsion in solid dosage form increases the substance surface area of the drug-containing microemulsion. The adsorbent increases the area available for interaction with gastro-intestinal fluid and/or with the site of absorption to thereby promote absorption of the drug. This invention is suitable for the oral administration of active substances that display poor bioavailability, slow absorption or long T_{max} . This technique offers advantage for the drugs which are degraded during passage through the gastrointestinal system. As drug contained within internal oil phase of a microemulsion makes this system suitable for proteins and peptides. This is also useful for the delivery of small molecule drugs and nutritional supplements, such as vitamins and minerals (62).

- Non-steroidal anti-inflammatory drugs, commonly abbreviated NSAIDs are well-known drugs for the treatment of pain and inflammation. One of major drawbacks with NSAIDs is that they have severe gastro-intestinal side-effects. They are lipophilic compounds with poor aqueous solubility. A biopharmaceutical problem with these compounds is that their absorption from the gastro-intestinal tract (GIT) may be dissolution rate limited, resulting in poor bioavailability upon oral administration. The problem mentioned above has been solved by providing a novel Self-Emulsifying Drug Delivery System, commonly known as SEDDS, suitable for oral administration. The present invention comprises of drug, one or more surfactant. This composition will form an in situ oil-in-water emulsion of small droplets of nanometer to micron size upon contact with gastrointestinal fluids, the droplets being constituted of drug, forming a core of droplet which is covered by one or several layers of surfactant. The in situ formed oil-in water emulsion will provide a good bioavailability of the drug upon oral administration. Storage stability of

emulsion is not a concern since the emulsion is not formed until the pre-concentrate has been taken by the patient, i.e. first at the moment of administration. This invention is particularly useful in the treatment of pain and inflammation (63).

2.4 ANALYTICAL METHODS

Many analytical methods are important tools to determine the plasma concentration of drug. Literature shows various techniques like spectroscopic methods, chromatography i.e. HPLC and LCMS are used for plasma analysis of drug.

- Daneshtalab and co-workers (64) developed an easy assay for the quantitation of the angiotensin II receptor antagonist valsartan in human plasma using a liquid extraction procedure. The method involves acid extraction from 1 ml human plasma with methyl-*tert.*-butyl ether followed by back-extraction into a basic medium. An isocratic HPLC equipped with reverse phase column and a fluorescence detector was used at room temperature.
- Chiral drug can not be easily separated by simple silica column and such enantiomers being considered as an impurity. Valsartan is also one of such drugs and contains one asymmetric centre. The quantitative method by chiral HPLC of the optical purity of valsartan and its valinbenzyl ester tosylate precursor was developed and validated (65).
- A selective, accurate and precise high-performance liquid chromatographic assay coupled to fluorescence detection was developed for the detection of some angiotensin II receptor antagonists (ARA II): Losartan, Irbesartan, Valsartan, Candesartan cilexetil and its metabolite Candesartan M1. The analytes and the internal standard (bumetanide, a high-ceiling diuretic) were extracted from plasma under acidic conditions by means of solid-phase extraction using C8 cartridges. This procedure allowed recoveries close to 80% for all these drugs excluding Candesartan cilexetil (70%) which presented adsorption processes on glass and plastic walls. The analytes and potential interferences were separated on a reversed-phase column, μ Bondapak C18, at room temperature. This assay method has been

successfully applied to plasma samples obtained from hypertensive patients under clinical studies after oral administration of a therapeutic dose of some of these ARA II compounds (66).

- Capillary electrophoresis (CE) offers an alternative technique for determination of concentration of ARA-II compounds (67).
- A capillary zone electrophoretic method is reported to separate and identify six ARA-IIs: candesartan, eprosartan mesylate, irbesartan, losartan potassium, telmisartan, and valsartan. In advanced to this, introduction of micellar electrokinetic capillary chromatography (MEKC) has overcome the difficulty of separating neutral analytes using CE, and has increased the selectivity in the separation of charged molecules. Compounds having the same charges and similar structures often migrate at almost the same velocity in CE, whereas differences in their distribution constants in the micellar phase lead to baseline separations. A selective MEKC method that was capable of separating and quantifying six ARA-II (68).
- A high-performance liquid chromatographic (HPLC) method for the determination of valsartan in human plasma is reported. The assay is based on protein precipitation with methanol and reversed-phase chromatography with fluorimetric detection. The overall speed of analysis can be improved by the elimination of tedious extraction steps and optimization of chromatographic conditions. The validated method allows determination of valsartan in the 98–10200 ng/ml range. The assay is rapid, the analysis time is only 2.8 min. The precision and accuracy of the method are well within the limits required for bioanalytical assays. The limit of quantification 98 ng/ml permits the use of the method for pharmacokinetic studies (69).
- Two simple and sensitive spectrophotometric and liquid chromatographic (LC) methods are described for the determination of ramipril and olmesartan medoxomil. The one method was based on the absorption factor. Ramipril and olmesartan medoxomil exhibit λ_{max} at 210 nm and 256 nm respectively. Olmesartan medoxomil has some interference at 210nm, while ramipril do not show any absorption at 256 nm. Quantitative estimation of ramipril was carried out by subtracting the absorption due to

olmesartan medoxomil at 210 nm using experimentally calculated absorption factor. Beer's law was obeyed for ramipril and olmesartan medoxomil at 2-6 $\mu\text{g/mL}$ and 8-24 $\mu\text{g/mL}$ respectively. The other method, high-performance liquid chromatographic method was developed for the determination of ramipril and olmesartan medoxomil using sodium perchlorate: acetonitrile (60:40 v/v) as the mobile phase and measuring the response at λ_{max} 210 nm. The analysis was performed on a Phenomenex C8 (250 X 4.0 mm), 5 μm column. The calibration curve was found linear for ramipril and olmesartan (70).

- A rapid, sensitive, and specific method for quantification of olmesartan, the prodrug of olmesartan medoxomil, in human plasma, using zidovudine as internal standard, is developed. Sample preparation involved a simple solid-phase extraction procedure. The extract was analyzed by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry (LC-MS-MS). Chromatography was performed isocratically on a 5 μm C18 analytical column (50 mm \times 4.6 mm i.d.) with water-acetonitrile-formic acid 20:80:0.1 (v/v) as mobile phase. The response to olmesartan was a linear function of concentration over the range 4.82–1,928 ng/mL. The method was successfully applied in a bioequivalence study of an olmesartan formulation after administration as a single oral dose (71).
- A specific, sensitive and fast method based on high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was developed for the determination of olmesartan in human plasma and urine. Solid-phase extraction (SPE) was used to isolate the compounds from biological matrix followed by injection of the extracts onto a C18 column with isocratic elution. The method was validated over the concentration range of 0.2–1000 and 5–10,000 ng/mL for olmesartan in human plasma and urine, respectively. The method was applied to the pharmacokinetic study of olmesartan medoxomil in healthy Chinese male and female subjects (72).

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