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SACUBITRIL / VALSARTAN (ENTRESTO): A REVIEW ON DEVELOPMENT OF ANALYTICAL METHODS AND ITS ASSESSMENT IN PURE, BULK FORMULATION, BIOLOGICAL FLUIDS AND PHARMACEUTICAL PREPARATION

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Abstract
This Review focuses on the Recent Developments in Analytical Techniques for Estimation of Valsartan and Sacubitril Alone or in combination with other drugs in various Biological media like Human plasma and urine. The main Objective of this Review is to Unify and Interpret widely Scattered information of reported studies on Potential, Reliable and efficient Analytical Methodologies which can Estimate All the Major Components of Antihypertensive drugs. The information and suggested outlined below may facilitate and guide further Needed studies to optimise the use of Analytical Techniques like HPLC (High Performance Liquid Chromatography), HPTLC (High Performance Thin Layer Chromatography), GC (Gas Chromatography) etc. for determination of Antihypertensive Analytes in formulation. The presented work is focused on the use of different Analytical Methods for the estimation of Valsartan and Sacubitril in API as well as formulation. From the Reviewed Literature it is obvious that HPLC is a commonly Available method of testing in Pharmaceutical Laboratory.

Keywords: Valsartan, Sacubitril. HPLC, HPTLC, GC

1. Introduction
Heart Failure does not mean the heart has stopped working. Rather, it means that the heart works less efficiently than normal. Due to various possible causes, blood moves through the heart and body at a slower rate, and pressure in the heart increases. As a result, the heart cannot pump enough oxygen and nutrients to meet the body's needs. As a result, the kidneys may respond by causing the body to retain fluid (water) and salt. If fluid builds up in the arms, legs, ankles, feet, lungs, or other organs, the body becomes congested, and congestive heart failure is the term used to describe condition.

1.1 Causes of Heart failure
Heart failure is caused by many conditions that damage the heart muscle, including:

1. **Coronary artery disease (CAD),** a disease of the arteries that supply blood and oxygen to the heart, causes decreased blood flow to the heart muscle. If the arteries become blocked or severely narrowed, the heart becomes starved for oxygen and nutrients.
2. **Heart Attack** occurs when a coronary artery becomes suddenly blocked, stopping the flow of blood to the heart muscle. A heart attack damages the heart muscle, resulting in a scarred area that does not function properly.
3. **Cardiomyopathy,** damage to the heart muscle from causes other than artery or blood flow problems, such as from infections or alcohol or drug abuse.
4. **Condition that overwork the heart,** conditions including high blood pressure, valve disease, thyroid disease, kidney disease, diabetes, or heart defects present at birth can all cause heart failure. In addition, heart failure can occur when several disease or condition are present at once.

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1.2 Symptoms of Heart failure

1 You may not have any symptoms of heart failure, symptoms can be constant or can come and go. The symptoms can include:
2 **Congested Lungs,** fluid backup in the lungs can cause shortness of breath with exercise or difficulty breathing at rest or when lying flat in bed. Lung congestion can also cause a dry, hacking cough or wheezing.
3 **Fluid and water retention**, less blood to your kidneys causes fluid and water retention, resulting in swollen ankles, legs, abdomen (called edema), and weight gain. Symptoms may cause an increased need to urinate during the night. Bloating in your stomach may cause a loss of appetite or nausea.
4 **Dizziness, fatigue, and weakness**. Less blood to your major organs and muscles makes you feel tired and weak. Less blood to the brain can cause dizziness or confusion.
5 **Rapid or irregular heartbeats**. The heart beats faster to pump enough blood to the body. This can cause a rapid or irregular heartbeat.

1.3 Types of Heart Failure

1 **Systolic dysfunction** (or systolic heart failure) occurs when the heart muscle doesn't contract with enough force, so there is less oxygen-rich blood that is pumped throughout the body.
2 **Diastolic dysfunction** (or diastolic heart failure) occurs when the heart contracts normally, but the ventricles do not relax properly or are stiff, and less blood enters the heart during normal filling.

2. Epidemiology

Heart Failure affects nearly 6 million People; Roughly 670,000 Peoples are diagnosed with heart failure each year. It is the leading cause of hospitalization in peoples older than age 65. Globally, around 26 million people suffer from heart failure. Blocked of renin angiotensin aldosterone system using angiotensin – converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, mineralocorticoid receptor antagonists, etc. have been pivotal to treat heart failure and to reduce associated mortality. Chronic heart failure is a progressive condition characterized by elevated blood pressure (hypertension), reduced ejection fraction and decreased tissue oxygen delivery. Heart failure a very common syndrome and the leading cause of death worldwide. Approximately 30% of all deaths are due to heart failure, with reduced ejection fraction probably the most important modifiable risk factor.

3. Introduction to Drug [2- 6]

Table 1: Physicochemical Properties of Sacubitril and Valsartan (Drug Profile)

Parameter	Details of Valsartan	Details of Sacubitril
Name of Drug	Valsartan	Sacubitril
CAS No.	137862-53-4	1369773-39-6
IUPAC Name	4-[2-(2H-tetrazol-5-yl)phenyl]benzoic acid	4-[[(2*S*,4*R*)-5-ethoxy-4-methyl-5-oxo-1-(4-phenylphenyl)pentan-2-yl]amino]-4-oxobutanoic acid

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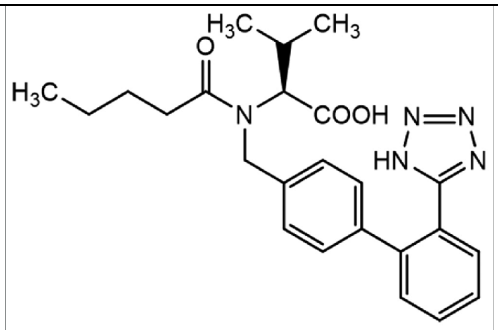
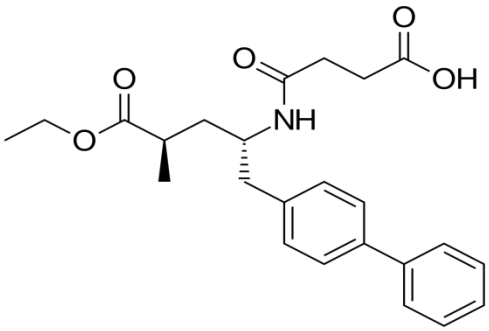
Molecular Formula	$C_{24}H_{29}N_5O_3$	$C_{24}H_{29}NO_5$
Structure		
Description	White Colored Powder	white powder consisting of thin hexagonal plates
Molecular weight	435.519g/mol	411.49 g/mol
Solubility	• Freely soluble in ethanol, methanol, acetonitrile and • sparingly soluble in water.	• Freely soluble in water
Category	Angiotensin Receptor Blocker (ARBs)	Neprilysin Inhibitor
Mechanism of Action	• Valsartan belongs to the angiotensin II receptor blocker (ARB) family of drugs, which selectively bind to angiotensin receptor 1 (AT1) and prevent angiotensin II from binding and exerting its hypertensive effects. • These include vasoconstriction, stimulation and synthesis of aldosterone and ADH, cardiac stimulation, and renal reabsorption of sodium among others. Overall, valsartan's physiologic effects lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium.	• Sacubitril is a pro-drug that upon activation acts as a neprilysin inhibitor. It works by blocking the action of neprilysin thus preventing the breakdown of natriuretic peptides. This leads to a prolonged duration of the favorable effects of these peptides. • The natriuretic peptide system consists of three ligands and three receptors. These peptides cause effects such as diuresis, natriuresis, vasodilation, and inhibition of aldosterone synthesis and renin secretion as a circulating hormone, and thereby play an important role in regulating blood pressure and blood volume • ANP and BNP are mainly synthesized in cardiac tissue: ANP in the atrium and BNP in the ventricle. CNP is mainly expressed in the central nervous system.

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Pka and Log P	• Acid (pKa=4.73) • carboxylic (pKa=3.9) • Log P : 1.499	• 4.6 • Log P : 1.29
Melting Point	• 105-110˚C	• 138 ºC
Pharmacokinetic Parameter		
Absorption	Valsartan is rapidly absorbed orally. After oral administration of valsartan 80 mg capsule and solution formulation in 12 healthy volunteers, maximum plasma concentration (C max) of valsartan (1.64 mg/l and 3.25 mg/l) were respectively reached in ~ 1-2 h. Plasma levels and the area under the plasma concentration time curve were not linearly related to dose, indicating a saturable first pass metabolism. According to the AUC value obtained, the bioavailability of capsule was 23 % and that of solution was 39 %. The deconvolution results of the plasma levels, measured after administration of the two oral formulations with the I.V bolus dose as three unit impulse response showed that valsartan was 24% absorbed from capsule and 41 % from the solution. Valsartan is absorbed rapidly, 50% of it in capsule being absorbed within 1.6 h and 90 % within 4.6 h. the absorption occurs by a passive diffusion process. Food has not been reported to affect the absorption of valsartan. Hence, it can be administered with or without food.	Peak plasma concentration of sacubitril and its metabolites, LBQ657 are reached in 0.5 hr and 2 hr respectively. Food does not clinically affect the systemic exposure of sacubitril or LBQ657. The oral bioavailability of sacubitril is >60%. It should be noted that the valsartan found in this combination is more bioavailable than other market available valsartan.
	Valsartan has only limited distribution outside the plasma compartment and is extensively bound to the plasma proteins (94-97%) and hence is only limited distributed outside plasma compartment. Because of the presence of carboxylic groups valsartan is soluble in neutral pH	103 L

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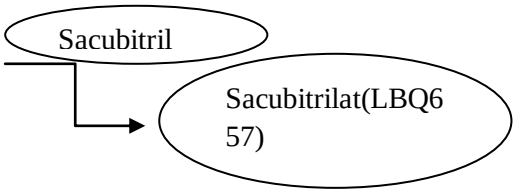
Distribution	range and is mainly present in the ionized form at physiological pH. The volume of distribution at steady is about 171.	
Metabolism	Valsartan does not require any metabolism in the body to become active. After the oral administration of 80 mg of [^{14} C] radiolabelled valsartan. Only one pharmacologically inactive metabolites was found in plasma nearly about 11 %. The primary metabolites was identified as valeryl 4- hydroxyl valsartan (M1) accounted for about 9 % of the dose and is inactive in hypertension. M1 has about 200 fold lower affinity for the AT1 receptor than valsartan.	Sacubitril is metabolized to LBQ657 by esterase. A low Concentration (<10%) of hydroxyl metabolite has been identified in plasma. 
Protein Binding	Valsartan is highly bound to serum proteins (95 %), mainly serum albumin.	Sacubitril and its metabolite, LBQ657 are highly bound to plasma protein (94-97%).
Elimination	Valsartan is mainly excreted in faeces via biliary excreation and hence it is not recommended for patient with hepatic dysfunction and biliary cirrhosis. After the administration of an I.V. dose in healthy volunteers, plasma clearance of valsartan was found to be ~ 2 1/h. renal clearance (0.62 1/h) was found to be only 30% of total plasma clearance. Hence it is clear that, vaslartan is eliminated mostly by non-renal routes. It is only slightly metabolized and excreted mainly unchanged in bile (80%) and urine (20%). M1 is formed by oxidative biotransformation and accounts for 9±3 % of the dose in excreta.	52% to 68% of sacubitril (primarily as the active metabolite LBQ657) is excreted in urine. 37% to 48% of sacubitril (primarily as LBQ657) is excreted in feces.

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4. Introduction to Sacubitril / Valsartan [3]

Sacubitril/ valsartan (SAC/VAL) is a novel acting, first in a new class of drugs approved for the treatment of heart failure. It consist of neprilysin inhibitor prodrug " sacubitril " (SAC) and angiotensin receptor blocker " valsartan " (VAL) in their anionic forms along with sodium cations and water in the molar ratio of 1:1:3:2.5, respectively.

THE COMBINATION IS SOLD UNDER THE BRAND NAME "ENTRESTO" BY NOVARTIS AND HAS BEEN APPROVED IN MORE THAN 57 COUNTRIES, INCLUDING INDIA.

In July 2015 "US food and drug administration (FDA)"approved ENTRESTO tablets as a combined formulation of sacubitril and valsartan for the treatment of heart failure. The combination drug sacubitril /valsartan (ENTRESTO) is used in place of an ACE inhibitor or ARB.

ENTRESTO was approved by the US food and drug administration (FDA) in July 7, 2015 to reduce the risk of death and hospitalization in heart patient classified under the new York heart association (NYHA) class II- IV, approved to reduce to decrease the risk of heart failure in those with reduced ejection fraction (outbound pumping of blood by heart). The recommended starting dose of ENTRESTO IS 49/51 Mg (sacubitril/valsartan) twice daily. Double the dose of ENTRESTO after 2 to 4 weeks to the target maintenance dose 97/103 mg (sacubitril/valsartan) twice daily, as tolerated by the patient. Reduce the starting dose to 24/26 mg (sacubitril/valsartan) twice daily for: Patient no currently taking an angiotensin-converting enzyme inhibitor (ACEi) or an angiotensin II receptor blocker (ARB) or previously taking a low dose of these agents. Patient with severe renal impairment, Patient with moderate hepatic impairment, Double the dose of ENTRESTO every 2 to 4 weeks to the target maintenance dose of 97/103 mg (sacubitril/valsartan) twice daily, as tolerated by the patient.

4.1 Mechanism of Action [3]

Sacubitril (AHU-377), neprilysin inhibitor, is a prodrug that is activated to the active metabolite 'Sacubitrilat' (LBQ657) by de-ethylation via esterases. Sacubitril, thus, increases the levels of these peptides, promoting natriuresis, vasodilation and reduction of ECF volume via sodium excretion; eventually reducing preload and ventricular remodeling.

Valsartan inhibits the effects of angiotensin-II by selectively blocking the receptor type-1 (AT1), and concomitantly inhibiting angiotensin-II-dependent aldosterone release. Blockade of AT1 thus reduces vasoconstriction, sodium and water retention and myocardial hypertrophy. In experimental studies, sacubitril/ valsartan have shown to attenuate angiotensin-II-mediated cardio-renal fibrosis and cardiac remodeling and dysfunction after experimental myocardial infarction; attributed to superior inhibition by sacubitril/valsartan on cardiac fibrosis and cardiac hypertrophy than either stand-alone neprilysin inhibitor or ARB.

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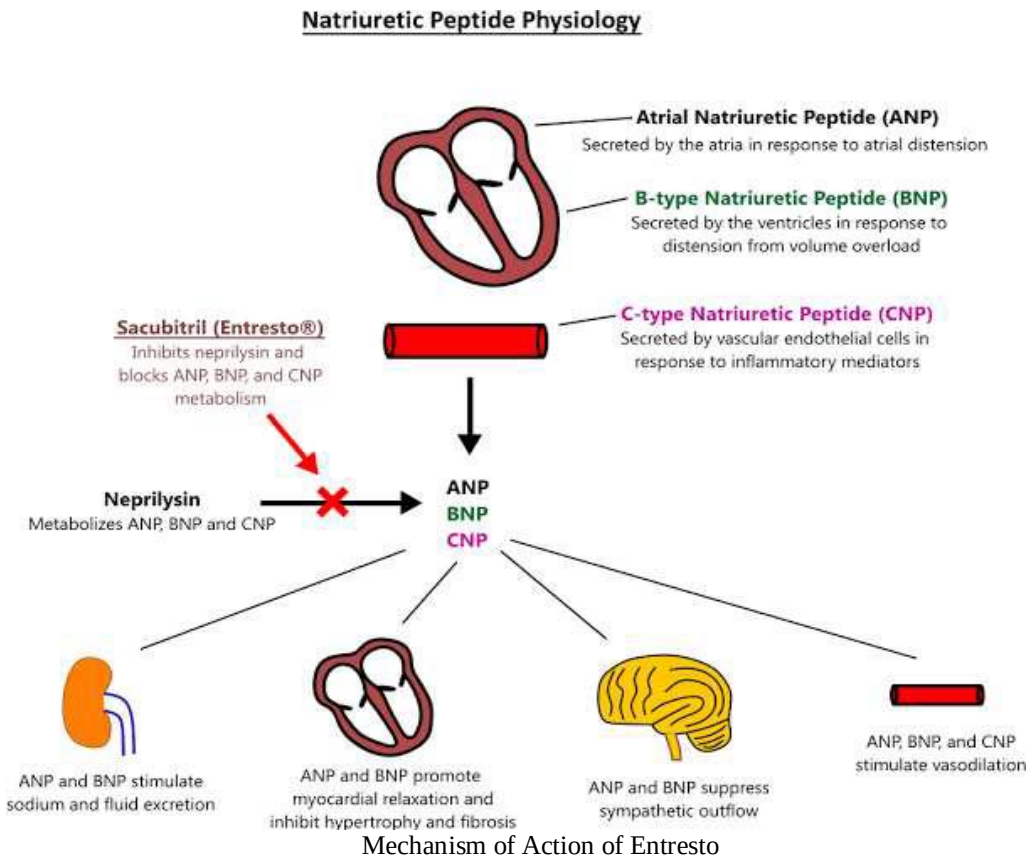

Mechanism of Action of Entresto

4.2 Pharmacology [14]

ENTRESTO contains a neprilysin inhibitor, sacubitril, and an angiotensin receptor blocker, valsartan. ENTRESTO inhibit neprilysin (neutral endopeptide; NEP) via LBQ657, the active metabolites of the prodrug sacubitril, and blocks the angiotensin II type-1 (AT1) receptor via valsartan. The cardiovascular and renal effect of ENTRESTO in heart failure patient are attributed to the increased levels of peptide that are degraded by neprilysin, such as natriuretic peptides, by LBQ657, and the simultaneous inhibition of the effect of angiotensin II by valsartan. Valsartan inhibits the effect of angiotensin II by selectively blocking the AT1 receptor, and also inhibits angiotensin II- dependent aldosterone release.

5. Pharmacokinetic

5.1 Absorption

Following oral administration, ENTRESTO dissociate into sacubitril and valsartan. Sacubitril is further metabolized to LBQ657. The peak plasma concentration of sacubitril, LBQ657, and valsartan are reached in 0.5 hours, 2 hours, and 1.5 hours, respectively. The oral absolute bioavailability of sacubitril is estimated to be ≥ 60 %. The valsartan in ENTRESTO is more bioavailable than the valsartan in other marketed tablet formulation; 26mg, 51mg, and 103mg of valsartan in ENTRESTO is equivalent to 40mg, 80mg, and 160mg of valsartan in other marketed tablet formulation respectively. Following twice-daily dosing of ENTRESTO,

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steady state level of sacubitril, LBQ657, and valsartan are reached in 3 days. At steady state, sacubitril and valsartan do not accumulate significantly, whereas LBQ657 accumulates by 1.6- fold. ENTRESTO administration with food has no clinically significant effect on the systemic exposure of sacubitril, LBQ657, or valsartan. Although there is a decrease in exposure to valsartan when ENTRESTO is administered with food, this decrease is not accompanied by a clinically significant reduction in the therapeutic effect. ENTRESTO can therefore be administered with or without food.

5.2 Distribution

Sacubitril, LBQ657 and valsartan are highly bound to plasma protein (94% to 97%). Based on the comparison of plasma and CSF exposure, LBQ657 crosses the blood brain barrier to a limited extent (0.28%) the average apparent volume of distribution of valsartan and sacubitril are 75 and 103 L, respectively.

5.3 Metabolism

Sacubitril is readily converted to LBQ657 by esterase; LBQ657 is not further metabolized to a significant extent. Valsartan is minimally metabolized; only about 20% of the dose is recovered as metabolites. A hydroxyl metabolites has been identified in plasma at low concentration (<10%).

5.4 Elimination

Following oral administration, 52% to 68% of sacubitril (primarily as LBQ657) and ~13% of valsartan and its metabolites are excreted in urine; 37% to 48% of sacubitril (primarily as LBQ657), and 86% of valsartan and its metabolites are excreted in feces. Sacubitril, LBQ657, and valsartan are eliminated from plasma with a mean eliminated half-life (T½) of approximately 1.4 hours, and 9.9 hours, respectively.
Linearity/nonlinearity: The pharmacokinetics of sacubitril, LBQ657, and valsartan were linear over an ENTRESTO dose range of 24mg sacubitril/26mg valsartan to 194mg sacubitril/206mg valsartan.

6. Case studies December 2018 [15-16]

2018 – 12– 8 17:00:00
Devon Giannelli, PharmD candidate and Craig
I. Coleman, PharmD

Case 1

KG is 55-year-old man presenting to the outpatient heart failure (HF) clinic for his scheduled 6 month follow-up appointment. He notes feeling short of breath when walking up 1 flight of stairs and has had difficulty dressing himself on occasion. KG also complains of some dyspnea on exertion and has had a persistent, nonproductive cough. He says that these symptoms have worsened over the past two weeks, though he denies any chest pain or neurological deficit. KG has a medical history of HF with reduced ejection fraction (HFrEF), classified as New York heart association class III, with an ejection fraction 30%, hyperlipidemia, and hypertension. Today his physical exam indicates a blood pressure of 130/85 mm Hg and bilateral 2+ pitting edema in his legs, and previsit laboratory testing shows a B- type natriuretic peptide level of 255 pg/dL (normal= <120). KG's home medication include atorvastatin 40mg once daily, carvedilol 12.5 mg twice daily, enalpril 10 mg twice daily, and frusemide 20 mg once daily. What changes to his medication might you suggest to decrease morbidity and mortality associated with HFrEF?

Answer

Current guideline suggest that changing KG's enalpril therapy to sacubitril/valsartan (ENTRESTO) can further decrease morbidity and mortality in patient with class III HFrEF like him who have previously tolerated angiotensin – converting enzyme inhibitor (ACEIs) or angiotensin receptor blockers (ARBs). Sacubitril/valsartan is a neprilysin inhibitor/ARB combination drug that lowers mortality risk more than enalpril alone.

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Neprilysin is an enzyme that breaks down natriuretic peptides, resulting in a decrease in sodium and water retention, systemic vascular resistance, and ventricular hypertrophy.
Initiation of sacubitril/valsartan 49mg/51mg twice daily would be reason – able after enalpril is discontinued for 36 hours. The purpose of delaying the initiation of sacubitril/valsartan when switching from an ACEI is to lower the risk of developing angioedema. An eventual target dose of 97/103 mg twice daily should be recommended.

Case 2

This case study looks at the use of sacubitril/valsartan (brand name: ENTRESTO), which is recommended as replacement for ACEi or ARB in patient with reduced ejection fraction heart failure who remain symptomatic on current best therapy.
Mr. H, a 62-year-old Maori man, is at your clinic for repeat of his usual medication for hypertension and heart failure.
You are reviewing your notes ahead of the appointment. Mr. H was seen last month by cardiology for follow-up of his longstanding heart failure, and clinic letter mentions that he may be a candidate for new medication, entresto.
Mr. H also has mildly impaired renal function and type II diabetes for which he takes metformin 500mg BD. He is an ex-smoker and he drinks 1-2 cans of beer per night. His father died at age 58 from a heart attack, and his mother died in her 70s from emphysema. His younger brother is also hypertensive.
Mr. H is compliant with his medication. He currently takes frusemide 40mg, cilzapril 5mg, bisoprolol 10mg, and spironolactone 25 daily.
Mr. H tells you he became tired and short of breath doing of supermarket shopping, and on his daily walks.

- He can walk approximately 100 meters on the flat before needing to stop. He is not breathless doing his usual daily tasks such as showering or doing laundry.
- He does not have chest pain.
- You assess him as having NYHA class III symptoms.

So, it seems he may benefits from this medication……..but are there any contraindication? Mr. H's blood pressure today is 134/76, and a quick search of medTech shows his systolic blood Pressure (SBP) Usually Ranges 120 – 140 mmHg.

- His blood test from two weeks earlier show eGFR 55ml/min and potassium 4.8mmol/L.
- His liver function tests are normal.
- He has tolerated cilzapril for the past six years with no angioedema.
- You assess him as appropriate for sacubitril/valsartan.
- But, he has been stable on his current regimen for some time…….is it worth changing?

You consider the evidence and decide to start Mr. H on sacubitril/valsartan.
How will you start this medication, and what monitoring is required?

6.1 How to start sacubitril/valsartan

It is recommended that you consider starting this drug in close consultation with your local heart failure service.

1) Firstly, patients must stop their ACEi two days before starting the new medication. (patient switching from an ARB can take their last ARB dose then start sacubitril/ valsartan when the next dose is due).
2) Determine the appropriate start dose:
    - Patient on maximum ACEi / ARB dose start sacubitril/valsartan at 49/51mg BD
    - Patient on less than maximum ACEi/ARB dose start
    - Sacubitril/valsartan at the lower dose of 24/26mgBD
    - Patient at high risk of hypotension, including those aged ≥ 75 years and those with SBP 100-110mmHg, start sacubitril/valsartan at 24/26mg BD

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3) Titrate up to the target dose of 97/103mg BD with clinical review at each dose titration step. Sacubitril/valsartan causes both hypotension and vasodilation, so symptoms, blood pressure (including standing), renal function and electrolytes should be assessed at each visit.
4) Once a stable dose, regularly review patient's symptoms and monitor for symptomatic hypotension and worsening renal function.

6.2 Potential side effect

Symptomatic hypotension, especially in older patient
Angioedema (0.4% Vs 0.2%)
Diarrhea
Headache
Gastritis
Worsening renal function

Important interaction

There are several interactions to be aware of, and full information can be found on NZ formulary. Some key drug classes to consider are:

1) Statins: sacubitril/valsartan may increase the effect of statins.
2) Drugs may increase potassium and thereby increase the risk of hyperkalaemia: potassium – sparing diuretics.
3) NSAIDs: risk of worsening renal function with coadministration, especially in elderly and volume-depleted patient (think: triple whammy).
4) Sildenafil: risk of hypotension even with a single dose of sildenafil.
5) Frusemide: theoretical risk of reduced effect of frusemide.
6) Metformin: theoretical risk of reduced effect of metformin but clinical significance uncertain in the trails.

7. Patent [16-17]

ENTRESTO (valsartan +sacubitril) is a drug marketed by Novartis pharms corp and is included in one NDA. There are six patents protecting this drug. This drug has ninety – two patent family members in thirty – seven countries.

The generic ingredient in ENTRESTO is sacubitril; valsartan. There are eleven drug master file entries for this Compound.

Mumbai – Hyderabad based NATCO pharma said it has launched in India valsartan – sacubitril, a drug used for heart ailment, under its brand name VALSAC. In doing so, the homegrown drug maker has set the stage for a patent challenges from swiss drug maker NOVARTIS, which is rapidly building on VYMADA, its patented brand in India. According to patent experts, Novartis will most likely file a patent challenge case against NATCO's launch.

THE EARLIEST PATENT EXPIRATION OF VALSARTAN – SACUBITRIL IS EXPECTED TO BE IN 2023.

Summary of Entresto:-

INTERNATIONAL PATENTs	**92**
US PATENTs	**6**
APPLICANTs	**1**
NDAs	**1**

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8. ADVERSE EFFECT:

Clinically significant adverse reactions include:

1) Angioedema
2) Hypotension
3) Impaired renal function
4) Hyperkalemia

1) Angioedema: angioedema is an area of swelling of the lower layer of skin and tissue just under the skin or mucous membrane. The swelling may occur in the face, tongue, larynx, abdomen or arms and legs. ENTRESTO may cause angioedema in the double blind period of PARADIGM- HF, 0.05% of patient treated with ENTRESTO and 0.2% of patient treated with enalpril had angioedema . if angioedema occurs, discontinue entresto immediately.
2) Hypotension: ENTRESTO lowers blood pressure and may cause symptomatic hypotension.
3) Impaired renal function: as a consequences of inhibiting the renin- angiotensin- aldosterone system, (RAAS), decrease in renal function may be anticipated in susceptible individuals treated with ENTRESTO.
5) Hyperkalemia: through its action on the RAAS hyperkalemia may occur with ENTRESTO. In the double blind period of PARADIGM- HF, 12% of patient treated with ENTRESTO and 14% of patient treated with enalpril hyperkalemia as an adverse event.

9. TOXICITY:

1) Fetal toxicity
2) Carcinogenesis and mutagenesis
3) Impairment of fertility
4) Animal toxicology and or/ pharmacology

1) **Fetal toxicity: -** when pregnancy is detected, discontinue ENTRESTO as soon as possible. Drugs that act directly on the renin angiotensin system can cause injury and death to the developing fetus.

2) **Carcinogenesis and mutagenesis**: - carcinogenicity studies conducted in mice and rats with sacubitril and valsartan did not identify any carcinogenic for ENTRESTO.
The LBQ657 Cmax at the high dose (HD) of 1200mg/kg/day in male and female mice was respectively 14 and 16 times that in human at the MRHD.
The LBQ657 Cmax in male and female rats at the HD of 400mg/kg/day was respectively, 1.7 and 3.5 times that at the MRHD.
The doses of valsartan studied (high dose of 160 and 200mg/kg/day in mice and rats respectively) were about 4 and 10 times respectively, the MRHD on a mg/m^2 basis.
Mutagenicity and clastogenicity studies conducted with ENTRESTO, sacubitril and valsartan did not reveal any effect at either the gene or chromosome level.

3) **Impairment of fertility**: - ENTRESTO did not show any effect on fertility in rats up to a dose of 73mg sacubitril / 77 mg valsartan/kg/day ($\leq$ 1.0 – fold and $\leq$ 0.18- fold the MRHD on the basis of the AUCs of valsartan and LBQ657 respectively.

4) **Animal toxicology and /or pharmacology**: - The effects of ENTRESTO on amyloid- β concentration in CSF and brain tissue were assessed in young (2 to 4 years old) cynomolgus monkeys treated with ENTRESTO (24mg sacubitril / 26mg valsartan/kg/day) for 2 weeks.
In this study ENTRESTO affected CSF Aβ clearance, increasing CSF Aβ 1-40, 1-42 and 1-38 level in CSF, there was no corresponding increase in Aβ level in the brain. In addition, in a toxicology

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study in cynomolgus monkeys treated with ENTRESTO at 146mg sacubitril / 154mg valsartan /kg/day for 39 weeks, there was no amyloid β accumulation in the brain.

DOSAGE FORM AND STRENGTH:-

ENTRESTO is supplied as unscored, ovaloid, film- coated tablet in the following strength:

- ENTRESTO 24/26 mg, (sacubitril 24 mg and valsartan 26 mg) are violet white and debossed with "NVR" on one side and "LZ" On the other side.
- ENTRESTO 49/51 mg, (sacubitril 49 mg and valsartan 51 mg) are pale yellow and debossed with "NVR" on one side and "L1" on the other side.
- ENTRESTO 97/103 mg, (sacubitril 97 mg and 103 mg) are light pink and debossed with "NVR" on one side and "L11" on the other side.

10. ANALYTICAL METHODs

The main purpose of analytical method development and validation is to prove that the proposed analytical method is accurate, specific, precise, and robust for the particular drug. [7] Analytical method validation parameter as per USP and ICH are as follow:

1) Specificity
2) Linearity
3) Precision
4) Accuracy
5) Range
6) Limit of detection
7) Limit of quantification
8) Robustness
9) System suitability test
10) Ruggedness

Literature survey reveals that various analytical method have been developed to estimate valsartan and sacubitril in bulk, tablet dosage form, synthetic mixture and in biological sample. The method consist of UV Spectrophotometric Analysis, Stability indicating RP-HPLC Method, LC/MS/MS, HPTLC, DSC, NMR, Spectrofluorimetry.

10.1 UV Spectroscopy [8]

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near-infrared ranges and it is based on Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length. Thus, for a fixed path length, it can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration, UV-VIS spectroscopy has been in general use for the last 37 years and over this period its become the most important analytical instrument in the modern day laboratory. In many application, other techniques could be employed but none rival UV-VIS spectroscopy for its simplicity, versatility, accuracy, speed, and cost-effectiveness.

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Table 1 UV spectroscopic method

Sr No.	Title	Method	Description	Reference no.
1.	UV Spectrophotometric method for estimation of valsartan in bulk and tablet dosage form.	UV method	**λmax:** 220nm to 250nm **linearity:** 5-30µg/ml **solvent:** phosphate buffer 6.8 **Accuracy (%recovery):** at 250nm (99.8%), at 220nm (99.52%) **LOD**: 0.0598µg/ml **LOQ:** 0.192µg/ml **Correlation coefficient:** 0.995	28
2.	Development and validation of new UV-spectroscopic assay method for valsartan in pure and in formulation	UV method	**λmax:** 250.80nm **linearity**: 5 to 30µg/ml **solvent:** methanol **accuracy(% recovery):** 99.26% - 100.7% **LOD:** 1.79µg/ml **LOQ:** 5.95µg/ml	27
3.	UV spectrophotometric method for estimation of sacubitril in synthetic mixture.	UV method	**λmax:** 242 nm **linearity:** 2-12µg/ml **solvent:** methanol and water 25:75 v/v correlation coefficient: 0.999	20
4.	Simultaneous spectrophotometric determination of sacubitril and valsartan in their recently approved p'ceutical preparation.	UV spectrophotometric ,simultaneous equation method	**λmax:** 220 to 250 nm **linearity:** 1-15µg/ml **Solvent:** Methanol **For valsartan** **accuracy(%recovery):** 100.06 **LODµg/ml at 220nm:** 0.276 **LODµg/ml at 250nm**: 0.294 **LOQµg/ml at 220nm:** 0.836 **LOQµg/ml at 250nm:** 0.891 **r^2 at 220nm**: 0.9996 **r^2 at 250nm:** 0.9996 **For sacubitril** **Accuracy (% recovery):** 99.60 **LODµg/ml at 220nm:** 0.115 **LODµg/ml at 250nm:** 0.131 **LOQµg/ml at 220nm:** 0.348 **LOQµg/ml at 250nm**: 0.398 **r^2 at 220nm:** 0.9999 **r^2 at 250nm:** 0.9999	22

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Spectrofluorimetry Method of Analysis				
1	Development of hybrid spectrofluorimetric method for simultaneous determination of Valsartan and Sacubitril in LCZ696 tablets.	Spectrofluorimetry with derivative ratio mathematical treatment	**First derivative ratio synchronous fluorescence:** 258-295 (peak-to-peak) **Detection Wavelength:** 204nm **Linearity for VAL**: 60-200 ng mL^{-1} **Linearity for SAC**: 20-200 ng mL^{-1}	59
2	First derivative emission spectrofluorimetric method for the determination of LCZ696, a newly approved FDA supramolecular complex of valsartan and sacubitril in tablets	First Derivative Emmission Spectrofluorimetric Analysis	**VAL** **Fluorescence: 416 nm** **Linearity range (µg/mL):** 0.2–3 **LOQ (µg/mL):** 0.1339 **LOD (µg/mL):** 0.0442 **Correlation coefficient:** 0.9996 **SAC** **Fluorescence:** 314 nm **Linearity range (µg/mL):** 0.04–0.8 **LOQ (µg/mL):** 0.0138 **LOD (µg/mL):** 0.0045 **Correlation coefficient:** 0.9999	60

From the Literature survey it has been conclude that…

- Most commonly used λmax in UV- Spectrophotometric Analysis for the estimation of Valsartan/Sacubitril is, in between 220nm to 250nm
- And mostly used Solvent for spectrophotometric Analysis of Sacubitril and Valsartan alone or in combination with other drugs is " Methanol"

10.2 HPLC methods (LC, RP-HPLC, UPLC)

Liquid Chromatography is the most widely used analytical tool in the pharmaceutical industry and reversed-phase is the most frequently used mode. During the drug development process, liquid chromatographic methods are used to determine the quality of the drug substance (Active Pharmaceutical Ingredient) and drug product. [10]

Table 2 HPLC, LC, UPLC Methods

1.	Development and validation of HPLC method to determine valsartan in nanoparticles.	HPLC method	**Column** : phenomenex C18, 250mm x 4.6 mm id, 5µ **Wavelength:** 250nm **Mobile phase**: ammonium formate and acetonitrile (57:43 v/v)	31
2.	RP- HPLC method for estimation of valsartan in solid oral dosage form.	HPLC methods Isocratic mode	**Column:** ODS C18 (250mm x 4.6 mm, 5µm **Wavelength:** 248nm	28

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			Mobile phase: acetate buffer(PH 4.6): acetonitrile : methanol (38:24:38% v/v) **Flow rate:** 1.2 ml/min **Retention time:** 4.6 ± 0.06 min **Accuracy(% recovery):** 99.95% and 99.57% **LODµg/ml:** 0.17 **LOQµg/ml:** 0.56 **Diode array detector**	
3.	New method development and validation for the simultaneous estimation of sacubitril and valsartan in a bulk and p'ceutical dosage form.	LC method	**Column:** C18 **Wavelength:** 241nm **Linearity**: 60 - 140µg/ml **Solvent :** 80 volume of methanol and 20 volume of water **Correlation coefficient :** Val(0.997), sac(0.997)	18
4.	Simultaneous estimation of sacubitril and valsartan in synthetic mixture by RP-HPLC method.	RP-HPLC method	**Column:** C18 (250*4.6mm, 5µm) **Wavelength:** 276nm **Mobile phase:** acetonitrile: methanol: water(30:50:20%v/v) **Flow rate:** 1ml/min **For valsartan** **Retention time:** 3.264min **Accuracy (%recovery):** 99.59-101.05% **LOD:** 23.85µg/ml **LOQ:** 72.27µg/ml **Linearity and concentration range:** 50-250µg/ml **For sacubitril** **Retention time:** 2.464min **Accuracy (% recovery):** 99.25-100.90% **LOD:** 19.28µg/ml **LOQ:** 58.45µg/ml **Linearity and concentration range:** 50-250µg/ml	21
5.	HPLC and HPTLC coupled with photodiode array and fluorescence detectors for analysis of valsartan and sacubitril in their supramolecular complex with quantitation of sacubitril related substance in raw material and tablets.	HPLC – DAD/FLD (diode array detector/fluorescence detector)	First trials involve the use of different **reversed phase column :** Zorbax SB-C8(250 x 4.6mm, particle size 5µ),Zorbax eclips plus- C18 (150 x 4.6mm, 3.5µm) and Zorbax eclips plus-C18 (250 x 4.6mm,5µ) **Wavelength :** DAD(diode array detector) which was set at 255nm **Mobile phase:** acetonitrile 25 mM phosphate buffer of pH 3 (sodium dihydrogen phosphate monohydrate adjusted with orthophosphoric acid) in	25

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			a ratio 65:35 v/v **Flow rate:** 0.9ml/min **Injection volume:** 20µl **For valsartan** **LODµg/ml :** HPLC- DAD(0.067), HPLC- FD(0.0083) **LOQµg/ml :** HPLC- DAD(0.20), HPLC- FD(0.025) **Correlation coefficient :** HPLC-DAD(0.9999),HPLC- FD(0.998) **Linearity :** HPLC- DAD(0.2-20µg/ml), HPLC- FD(0.025- 10µg/ml) **For sacubitril** **LOD µg/ml :** HPLC-DAD (0.05), HPLC-FD(0.0033) **LOQ µg/ml :** HPLC-DAD(0.15), HPLC-FD(0.01) **Correlation coefficient**: HPLC-DAD(0.999), HPLC-FD(0.9998) **Linearity :** HPLC-DAD(0.2-20µg/ml), HPLC-FD(0.01-3µg/ml)	
6.	Experimental design approach in HPLC method development: application for the simultaneous determination of sacubitril and valsartan in presence of their impurities and investigation of degradation kinetics.	RP-HPLC method	**Column:** C18 cyano column (150mm,4.6mm,5µm) **Wavelength:** 254nm **Mobile phase:** ammonium acetate buffer (0.02m adjusted to PH 4 with acetic acid : ACN(55:45,v/v) **Flow rate:** 1ml/min **For valsartan** **Retention time:** 2.53min **For sacubitril** **Retention time:** 4.91min **Degradation studies:** a completed forced degradation of sacubitril was achieved and degradation kinetic under basic condition was studied and was found to follow first order kinetics.	33
7.	Development of assay method and forced degradation study of valsartan and sacubitril by RP-HPLC in tablet formulation.	RP-HPLC method	**Column:** C18 column (x terra, 250 x 4.6mm,5µ **Wavelength:** 263nm **Mobile phase:** ACN: methanol: potassium dihydrogen phosphate(30:50:20 v/v) **Flow rate:** 1ml/min **For valsartan** **Retention time:** 4.22min **Accuracy (%recovery):** 99.20-99.54% % RSD Precision: 0.27 **LODµg/ml:** 2.80	34

			LOQµg/ml: 8.50 **Correlation coefficient:** 0.998 **For sacubitril** **Retention time :** 3.01 min **Accuracy(% recovery) :** 99.85-100.90% % RSD precision: 0.31 **LODµg/ml :** 1.54 **LOQµg/ml :** 4.68 **Correlation coefficient :** 0.999 **linearity : 20 to 160µg/ml**	
8.	Quantification of Sacubitril and Valsartan in Tablet Formulation By RP-HPLC Method	RP-HPLC method	**Column:** Enable C18 G (250 mm x 4.6 mm x 5 µm) column using isocratic mode **Mobile Phase:** Methanol : Water(60:40 v/v) **Detection Wavelength**: 245 nm **Flow rate**: 1ml/min **Injection Volume**: 20µl	46
9.	Assay of Valsartan and Sacubitril in Combined Dosage Form by RP-HPLC (Method Development and Validation)	RP-HPLC Method	**Column:** C18 column [Inertsil ODS, 250 x 4.6 mm, 5µ] **Mobile Phase:** mixture of buffer (pH-2.7), acetonitrile and methanol in the ratio of 25:60:15 % v/v/v in the ratio of 30:50:20 %v/v **Flow Rate:** 1ml/min **Detection Wavelength:** 245 nm **For Valsartan** **Retention Time:** 4.280 min **Correlation Coefficient:** 0.9987 **% Recovery:** 99.92 to 99.84 % **% RSD:** 0.0478 and 0.158% **For Sacubitril** **Retention Time:** 3.407 **Correlation Coefficient:** 0.9988 **% Recovery:** 101.14 % to 101.28 % **% RSD:** 0.0355 and 0.154%	47
10.	Separation and Quantitation of Sacubitril – Valsartan Combination in tablet by new Ion-Pair HPLC.	Ion – Pair HPLC Method	**Column**: Standard C18 **Mobile Phase:** 45 % of 10-3 M of cetyltrimethylammonium bromide (Cetrimide) + 55 % ACN (PH -10) **Mean Recovery:** 95.0-105.0 % **Detector:** UV- Detector and a Shimadzu LC-20 AT with diode array detector. **Detection Wavelength:** 254 nm **Flow Rate:** 1 ml/min	26

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Bio – Analytical Method for determination of VAL and SAC (Experiment done on Biological Fluids)				
11.	Development and validation of RP-HPLC method for the estimation of valsartan and sacubitril in rat plasma.	RP-HPLC	**Column:** inertsil C18 (250 x 4.6mm,5µm **Wavelength:** 371nm **Mobile phase:** acetonitrile: dipotassium hydrogen phosphate(30:70 v/v) **Flow rate:** 0.8ml/min **Injection volume:** 10µl **For valsartan** **Retention time:** 15.366min **For sacubitril** **Retention time**: 10.725min **Column temperature: ambient** **Accuracy(% recovery): 98-102%**	24
12.	Validated for eco-friendly chromatographic methods for simultaneous determination of sacubitril and valsartan in spiked human plasma and in p'ceutical formulation	HPLC method	**Column:** reversed phase inertsil C18 (5µm, 150mm x 4.0mm,id) **Wavelength :** 267nm **Mobile phase :** green mobile phase consisting of methanol: ethanol : water (40:30:30,) by volume) +0.1% triethyl amine PH 3.5 **Flow rate :** 0.8ml/min **Injection volume :** 20µl **For valsartan** **% recovery :** 1)pure sample : 100.12±0.535 2)human plasma : 99.98±0.677 **Precision:-** **Repeatability :** 1)pure sample :0.425-0.234-0.255 2)human plasma : 0.895-0.566-0.356 **Reproducibility :** 1)pure sample : 0.402-0.325-0.457 2)human plasma : 0.872-0.765-0.467 **Robustness :** 1)pure sample : 99.95±0.68 2)human plasma : 100.35±0.97 **LOD :** 1)pure sample : 243ng.spot^{-1} 2)human plasma: 29ng.spot^{-1} **LOQ :** 1)pure sample : 737ng.spot^{-1} 2)human plasma : 88.2ng.spot^{-1} **Correlation coefficient :** 1)pure sample : 0.999 2)human plasma : 0.999 **Linearity :** 1)pure sample : 1-300µg/ml 2)human plasma : 0.25-50µg/ml **For sacubitril** **% recovery:** 1)pure sample :	13

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			100.15±0.420 2)human plasma : 100.56±0.588 **Precision:-** **Repeatability:** 1)pure sample 0.325-0.832-0.523 2)human plasma : 0.475-0.345-0.678 **Reproducibility:** 1)pure sample : 0.797-0.564-0.345 2)human plasma : 1.015-0.567-0.789 **Robustness:** 1)pure sample : 100.25±1.20 2)human plasma : 99.85±1.05 **LOD:** 1)pure sample : 308.6ng.spot^{-1} 2)human plasma : 74.9ng.spot^{-1} **LOQ:** 1)pure sample : 935ng.spot^{-1} 2)human plasma : 226.8ng.spot^{-1} **Correlation coefficient:** 1)pure sample: 0.999 2)human plasma : 0.999 **Linearity:** 1)pure sample : 1-300µg/ml 2)human plasma : 0.25-50µ/ml	
13	Development and Validation of a Bioanalytical HPLC Method for Quantification of Valsartan in Human Plasma and its Application in Pharmacokinetics Studies.	HPLC method coupled with fluorescence detector	**Column:** Waters, Xterra- C18 (150 x 4.6 mm, 5 µm) column **Mobile Phase**: acetonitrile: phosphate buffer containing 1 g/L sodium salt of 1-Hexane sulphonic acid with pH adjusted to 2.5 with orthophosphoric acid (52:48, v/v) **Flow Rate:** 1ml/min **Detection Wavelength:** 255 nm and 374 nm **Concentration Range:** 5.0-4000.0 ng/ mL (Diclofenac as internal standard (IS) in plasma) **% Recovery:** 87.22 % (Val), 99.33 % (IS)	62
14	Chromatographic method development and validation for the determination of valsartan in biological fluid.	HPLC Method Coupled with UV for detection	**Column:** Zorbax SB-C18 (5µm, 4.6mm × 15cm) column. **Mobile Phase:** Acetonitrile, water and glacial acetic acid (40:59:1 v/v) **Flow Rate:** Isocratic mode at 1mL/min **Detection Wavelength:** 264 nm **Concentration Range:** 0.06 to 8µg/mL **Regression Value:** 0.999	63
15	Validation of an analytical method for the determination of valsartan in human plasma by HPLC/UV with addition standard using losartan as an	HPLC Method	**Column:** C18 reversed phase column **Mobile Phase:** 45% acetonitrile and 55% phosphate buffer (adjusted to pH 2.7 ± 0.1 with phosphoric acid) **Detection Wavelength:** 265 nm	64

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	internal standard.		**Concentration Range:** 0.05 to 20 µg/ml **LOQ:** 1.485 µg/ml	
16	Development and Validation of HPLC Method for Simultaneous Determination of Amlodipine, Valsartan, Hydrochlorothiazide in Dosage Form and Spiked Human Plasma	HPLC Method	**Column:** RP-C18 chromatographic column, Phenomenex Kinetex (150 mm × 4.6 mm i.d) **Mobile Phase:** Acetonitrile-phosphate buffer (0.05 M) with pH 2.8 in the proportion of (40/60, v/v) **Flow Rate:** 0.8 ml/min **Detection Wavelength:** 227 nm **Retention Time:** 2.26 min (HCT), 3.16 min (AML), 11.19 min (VAL) **Concentration Range:** 4-28 µg /ml (AML), 5-40 µg /ml (VAL) and 1-12 µg /ml (HCT) **% Recovery:** 99.94% (AML) 99.96% (VAL) and 99.78% (HCT)	65
17	Rapid Quantification of Valsartan in Human Plasma by Liquid Chromatography using a Monolithic Column and Fluorescence Detection: Application for Pharmacokinetic Studies.	HPLC Method coupled with Fluorescence Detector	**Column:** Reversed-phase conditions using a Chromolith Performance (RP-18e, 100×4.6 mm) column **Mobile Phase:** Isocratic mobile phase consisting of 0.01 M disodium hydrogen phosphate buffer-acetonitrile (60:40 v/v) adjusted to pH 3.5 with diluted phosphoric acid. **Flow Rate:** 2 ml/min **Detector:** Model K 2600 fluorescence detector **Detection Wavelength:** 230 nm and 295 nm **Concentration Range:** 20 -2000 µg/ml	66
Stability Indicating Method and Determination of Degradation Kinetics of VAL & SAC				
18	Stability-indicating UPLC method for determination of Valsartan and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms.	RP-UPLC Method	**Column:** Waters Aquity BEH C18 (100 mm x 2.1 mm, 1.7 microm) column **Mobile Phase**: The solvent A contains a mixture of 1.0% acetic acid buffer, Acetonitrile in the ratio 90:10 (v/v); and the solvent B contains a mixture of 1.0% acetic acid buffer and acetonitrile in the ratio 10:90 (v/v), respectively. **Flow Rate:** 03 ml/min **Detection Wavelength:** 225 nm **Injection Volume:** 1.0 µl	67
19	A validated stability indicating HPLC method for the determination of valsartan in tablet dosage form.	HPLC Method	**Column:** C18 column (250 x 4.6 mm) **Wavelength:** 265nm **Mobile phase:** ammonium dihydrogen phosphate: methanol (33.5:66.5%)	30

			Flow rate: 1ml/min **Injection volume:** 20µl **Retention time:** 11.9min **Accuracy(%recovery):** 100.8% **LODµg/ml**: 6 **LOQµg/ml:** 18	
20	Stability indicating RP-HPLC method for the simultaneous estimation of sacubitril and valsartan in drug products.	RP-HPLC Method	**Column:** C8 column (Luna C8 150*4.6, 3µm **Wavelength:** 267nm **Mobile phase:** 0.1% v/v trifluoroacetic acid in water: methanol (25:75) **Flow rate:** 1ml/min **For valsartan** **Retention time:** 3.049 min **Accuracy(% recovery):** level(50%, 100%, 150%) 99.3666 **LOD:** 0.006mg **LOQ:** 0.018mg **For sacubitril** **Retention time:** 3.99 min **Accuracy (% recovery):** level (50%, 100%, 150%) 99.566 **LOD:** 0.006mg **LOQ:** 0.017mg	19
21	Stability indicating analytical method development and validation for estimation of sacubitril and valsartan in bulk and p'ceutical dosage form using RP-HPLC.	RP-HPLC Method	**Column:** inertsil ODS (250 x 4.6mm,5µm **Wavelength:** 241nm **Mobile phase:** phosphate buffer: methanol: ACN(30:50:20&v/v) **Sample volume:** 20µl **Flow rate:** 1ml/min **For valsartan** **Retention time**: 4.003min **Accuracy(%recovery):** 98.08 **LOD:** 1.56µg/ml **LOQ:** 4.74µg/ml **Correlation coefficient :** 0.997 **Linearity:** 61.2-142µg/ml **For sacubitril** **Retention time:** 2.927min **Accuracy (% recovery):** 99.60 **LOD:** 0.72µg/ml **LOQ:** 2.20µg/ml **Correlation coefficient:** 0.997 **Linearity:** 58.8-137.2µg/ml	23
22	Development and validation of a stability indicating UHPLC method for sacubitril/valsartan	UHPLC Method	**Column:** XL C8, (100 x 4.6)mm; 3µm **Wavelength:** 240nm **Mobile phase:** tetrahydrofuran(THF)	12

	complex in the presence of impurities and degradation products.		and 0.1% perchloric acid in water (8:92,v/v) as a mobile phase A , THF: water: ACN(5:15:80,%,v/v) as a mobile phase in a gradient mode **Flow rate:** 0.6ml/min **Injection volume:** 2µl	
23	Development and Validation of a Specific Stability Indicating High Performance Liquid Chromatographic Method for Valsartan.	HPLC Method	**Column:** Waters 2695 using Symmetry C18 (250 mm × 4.6 mm × 5 µ) **Mobile Phase:** 0.02 mM sodium dihydrogen ortho-phosphate, pH adjusted to 2.5 using ortho-phosphoric acid (solvent A), and acetonitrile (solvent B) in the ratio of 58:42 %v/v. **Flow Rate:** 1 ml/min **Detection Wavelength:** 250 nm **Detector**: PDA Detector **Injection Volume:** 10 µl **Concentration Range:** 1 -200 µg/ml	68
24	Determination and validation of valsartan and its degradation products by isocratic HPLC.	Isocratic HPLC	**Column:** Isocratic RP-HPLC Separation C18 Column (250 mm Length x 4.6 mm Internal Diameter and 5 µm Particle size **Mobile Phase:** Methanol: Water (70:30v/v, pH 7.2 **Stability indicating assay and the Mobile Phase** comprising of methanol:water (60:40 v/v, pH 7.2) **Flow Rate:** 1.2 ml/min **Detection Wavelength:** 250 nm **Retention Time:** 0.40 min and 0.27 min for acid hydrolysis and oxidation of Valsartan	69
25	Determination of Inherent Stability of Valsartan by Stress Degradation and Its Validation by HPLC.	HPLC Method	**Column:** Kromasil C18 column **Mobile Phase:** Water: acetonitrile in the ratio of 60:40 with 0.5 % of ortho phosphoric acid (OPA) **Flow Rate:** 1 ml/min **Detection Wavelength:** 265 nm. **Concentration Range:** 20-320 µg/ml **Correlation coefficient:** 0.9996 **% Recovery:** 98.76 to 100.9%	70
26	Identification and Characterization of Degradation Products of Valsartan by UPLC/Q-TOF-MS Technique.	UPLC/Q-TOF-MS (Ultra-performance liquid chromatographic/quadrupole time-of-flight mass spectrometric) technique	**Column:** Acquity UPLCTM BEH C18 (100.0 × 2.1 mm, 1.7µm) column **Mobile Phase:** Isocratic mobile phase consisting of acetonitrile-2mM ammonium acetate (50:50, v/v) **Flow Rate:** 0.25 ml/min	71
27	Quality by Design Approach to Develop Stability Indicating	QbD Assisted HPLC	**Column: Phenyl hexyl Column** **Mobile Phase:** 10 mM KH2PO4 as a	72

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	Method to Quantify Related Substances and Degradation Products of Sacubitril by High Performance Liquid Chromatography.		Mobile Phase- A and the pH adjusted to 2.1 Methanol:acetonitrile (70:30 v/v) solvent mixture was employed as the mobile phase –B in a gradient Mode **Flow Rate:** 0.8 ml/min **Detection Wavelength:** 254 nm **Correlation Coefficient:** 09989 %	

10.3 TLC and HPTLC method

High Performance Thin Layer Chromatography (HPTLC) is a sophisticated and automated form of the thin – layer chromatography (TLC) with better and advanced separation efficiency and detection limit. It is also known as high pressure thin layer chromatography/ planar chromatography or flat bed chromatography. It is powerful analytical method equally suitable for qualitative and quantitative analytical task. Separation may result due to absorption or partition or by both, phenomenon's depending upon the nature of adsorbent used on plates and solvent system used for development. [9]

Table 3 HPTLC, TLC Methods

1.	Application of TLC densitometric method for simultaneous determination of sacubitril and valsartan in their newly approved p'ceutical formulation.	TLC method	**Stationary phase:** precoated silica gel (60 GF 254) **Mobile phase:** (toluene: ethyl acetate: methanol),4:4:2 **Wavelength(densitometrically):** 260nm **Linearity:** 0.1- 0.6 µg/band	45
2.	HPLC and HPTLC coupled with photo diode array and fluorescence detectors for analysis of valsartan and sacubitril in their supramolecular complex with quantitation of sacubitril related substance in raw material and tablet.	HPTLC method	**HPTLC plate :** (20 x 10 cm, aluminium plate with 250- µm thickness precoated with silica gel 60 F 254) **Wavelength :** densitometric scanning was performed at 255nm **Mobile phase :** chloroform – ethyl acetate – glacial acetic acid (10:10:0.1 , v/v) **For valsartan** **LODµg/ml :** 2.87 **LOQµg/ml :** 8.22 **Correlation coefficient** : 0.994 **For sacubitril** **LODµg/ml** : 2.27 **LOQµg/ml** : 7.56 **Correlation coefficient :** 0.993 **Linearity : 10-100µg/ml for valsartan and sacubitril**	25

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3.	Validated Eco- friendly chromatographic method for simultaneous determination of sacubitril and valsartan in spiked human plasma an in pharmaceutical formulation.	Densitometric HPTLC method	**Column :** HPTLC plates (10cm x 10cm), 0.1mm nano silica gel with particle size 6-9 µm F254 (merck) **Wavelength :** 260nm **Mobile phase :** (ethyl acetate : methanol : glacial acetic acid (9:1:0.1,by volume)as a green mobile phase **For valsartan** **Rf :** 0.61 **% Recovery for Val** 1) pure sample : 100.05± 0.902 2) human plasma: 100.24± 0.702 **Precision :-** **Repeatability :** 1)pure sample: 0.568-0.897-0.580 2)human plasma: 0.722-0.770-0.554 **Reproducibility:** 1) pure sample: 0.558-0.989-0.655 2) human plasma: 1.032-0.897-0.655 **Robustness :** 1)pure sample : 100.45±0.88 2)human plasma : 99.88±1.08 **LOD :** 1)pure sample: 202ng.spot^{-1} 2)human plasma : 2.45ng.spot^{-1} **LOQ :** 1)pure sample : 613ng.spot^{-1} 2)human plasma : 7.42ng.spot^{-1} **Correlation coefficient** : 1)pure sample : 0.9997 2)human plasma : 0.9996 **Linearity :** 1)pure sample : 0.8-4.5µg.spot^{-1} 2) human plasma : 9-75µg.spot^{-1} **For sacubitril** **Rf:** 0.84 **% Recovery:** 1)pure sample : 99.95± 0.645 2) human plasma : 99.89± 0.535 **Precision:-** **Repeatability:** 1)pure sample: 0.538-0.323-0.458 2)human plasma : 0.718-0.745-0.678 **Reproducibility:** 1)pure sample : 0.466-0.445-0.356 2)human plasma : 0.875-0.338-0.558 **Robustness:** 1)pure sample : 99.95±0.65 2)human plasma : 99.78±0.94 **LOD:** 1)pure sample: 262ng.spot^{-1} 2)human plasma : 2.02ng.spot^{-1} **LOQ:** 1)pure sample : 794ng.spot^{-1} 2)human plasma : 6.13ng.spot^{-1} **Correlation coefficient:** 1)pure sample: 0.9996 (2)human plasma: 0.9992 **Linearity:** 1)pure sample : 1.5-4.5µg.spot^{-1} 2) human plasma : 9-75µg.spot^{-1}	13

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There are three Reported HPTLC methods on Sacubitril and Valsartan, in first case they are using Toluene : ethyle acetate : methanol (4:4:2), in second case Chloroform :ethyle acetate: glacial acetic acid (10:10:0.1 v/v) and in third case ethyl acetate: methanol: glacial acetic acid(green mobile phase) (9:1:0.1 v/v) was used as a mobile phase for the estimation.

10.4 LC- MS, LC- MS/MS method

Liquid chromatography/ Mass Spectrometry (LC/MS) is fast becoming the preferred tool of liquid chromatographers. It is a powerful analytical technique that combines the resolving power of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography (LC) separates the sample components and then introduce them to the mass spectrometer (MS). The MS creates and detects charged ions. The LC/MS data may be used to provide information about the molecular weight, structure, identity and quantity of specific sample components. [10]

Table 4 LC-MS, LC-MS/MS Methods

1.	Development and validation of a reliable and rapid LC- MS/MS method for simultaneous quantification of sacubitril and valsartan in rat plasma and its application to a pharmacokinetic study.	LC- MS/MS liquid chromatographic method with electrospray ionization tandem mass spectrometric detection.	**Column:** Hypersil gold C18 column **Mobile phase :** 0.1% formic acid in mili Q water , 0.1% formic acid in acetonitrile in gradient elution mode **Accuracy(% recovery) :** 95.96% to 103.61% **Precision value :** 1.31 to 6.45% **Intraday precision :** 0.86- 5.00% **Interday precision :** 0.87- 10.20%	32
2.	Bioanalytical Method development and validation for Quantitative Estimation of Valsartan by LC-MS/MS in Human Plasma.	LC-MS/MS in human plasma	**Column:** Zorbax SB-C18, 4.6 × 50 mm, 5 µm (Make: Agilent technologies) column at 40 °C **Mobile Phase**: methanol: 0.1 % formic acid (80:20, v/v) **Flow Rate:** 1ml/min **Run Time:** 2.5 min **Retention Time:** 1.40 min **Concentration Range:** 50.85 to 12046.60 ng/mL. **% Recoveries:** 75.39-79.44 %.	61

10.5 Thermal method (DSC, TGA, DTG, TMDSC) [58]

Thermal analysis is a general term defining a technique used to analyze the time and temperature at which physical changes occur when a substance is heated or cooled. Each technique is defined according to the types of physical changes being analyzed. When evaluating material characteristic, it is necessary to use different technique or a combination of multiple techniques depending on the purpose.

Thermal changes
DSC (Differential scanning calorimeter), DTA (Differential Thermal Analysis)
Weight Change
TGA (Thermo Gravimetric Analyzer)
Dimensional Change
TMA (Thermo Mechanical Analyzer)
Evolved Gas
EGA (Evolved Gas Analyzer)

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Table 5 Thermal Methods (DSC, TGA, DTG, TMDSC)

1.	Investigation on thermal stability and purity determination of two anti-hypertensive drugs, valsartan and losartan potassium.	TGA (Thermogravimetry analysis) DTG (derivative thermogravimetry) DSC (differential scanning calorimetry)	**TGA/DTG :-** **Nitrogen flow rate :** 30ml/min **Heating rate :** 10°C/min **Temperature range :** 900°C **DSC:-** **Nitrogen flow rate :** 30ml/min **Heating rate :** 5°C **Temperature range :** 300°C	42
2.	Thermal behavior of valsartan active substance and in p'ceutical products.	TG/ DTG/ DTA(differential thermal analysis	**Nitrogen flow rate** : 20ml/min **Heating rate :** 10°C **Temperature range :** 20- 1000°C	38
3.	Thermal behavior and phase identification of valsartan by standard and temperature modulated differential scanning calorimetry.	TGA/ DSC/ TMDSC(temperature modulated differential scanning calorimetry)	**TGA:-** **Nitrogen flow rate :** 60ml/min **Heating rate :** 10°C/min **Temperature range :** 25 to 600°C **DSC:-** **Nitrogen flow rate :** 50- 60 ml/min **Heating rate :** 10°C/min **Temperature range :** 25 to 140°C **TMDSC:-** **Nitrogen flow rate** : 50ml/min **Heating rate :** 1°C/min **Temperature range :** 25 to 140°C	39
4.	Bisoprolol and bisoprolol- valsartan compatibility studied by differential scanning calorimetry, nuclear magnetic resonance and X- ray powder diffractometry.	TGA DSC TMDSC NMR Powder X- ray diffractometry(Bruker D8 ADVANCE instrument using a graphite bent –crystal monochromator(Cu Kα) NMR(Bruker MAS probe using 1.3 mm diameter zirconia rotors was employed)	**TGA:-** **Nitrogen flow rate :** 60ml/min **Heating rate :** 10°C/min **Temperature range :** 25 to 600°C **DSC:-** **Nitrogen flow rate :** 50 and 60 ml/min **Heating rate :** 1, 5 ,10°C /min **Temperature range :** 50 to 140°C **TMDSC:-** **Heating rate :** 1°C/min **Temperature range :** 50 to 140°C **NMR:-** **Spectra:** 1H And C13 frequencies of 699.73 and 175.97 MHz **Sample:** in D_20 and DMSO **IS:** Tetramethylesilane X-ray Diffractometry **Volatge/ Current:** 10-40° (2θ) Temperature Range: 0 to 120°C **(Discussion)** The crystalline and amorphous form of bisoprolol and its compatibility with two	43

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			amorphous form of valsartan were analyzed. Amorphous bisoprolol has low stability and recrystallizes above the glass transition. The compatibility study has shown that simple blending of the APIs to produce a fixed dose formulation of bisoprolol and valsartan is unsuitable due to physical and chemical reaction which causes amorphism into a new bisoprolol/valsartan material at elevated temperature, and potentially also under long –term storage. Thus, formulation of an FDC or polypill containing bisoprolol and valsartan would require physical separation of the ingredient to ensure a stable product. Similar problem might be expected with excipient or APIs containing carboxylic groups; for example with aspirin or folic acid. It was demonstrated that thermal methods play a pivotal role in early detection of API-API interaction leading to incompatibilities. Solution and solid state NMR and XRPD provide information about the molecular nature of these interaction. Variable temperature NMR and XRPD experiments are seen to be an ideal complement to thermal method in the investigation of drug- drug interaction may be too slow to be detected at ambient temperature.	

11. Valsartan Impurity

FDA Statement on Valsartan Impurity [73]

The FDA learned and reported that some generic versions of the angiotensin II receptor blocker (ARB) medicines contain **nitrosamine impurities** that don't meet the agency's safety standards. ARBs, including valsartan, irbesartan, losartan and others, are a class of medicines used to treat high blood pressure and heart failure. **Nitrosamineimpurities, including N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine(NDEA), are probable human carcinogens.** These two substances are known environmental contaminants and found in water and foods, including meats, dairy products and vegetables. But their presence in drug products is not acceptable.

We were deeply concerned when we learned about the presence of these impurities. We immediately undertook a major operation to investigate and to identify the root causes for the presence of these impurities in some ARB drugs, and to work with companies to address the risks that the impurities pose to patients. Our ultimate goal is to ensure that these impurities are not present in finished drug products, or their components (including active pharmaceutical ingredients, or API). There remains a great deal of public interest in this matter. Today, we want to provide an update on this ongoing investigation and outline the steps we've taken to identify the root causes of the nitrosamine impurities and to prevent a recurrence of this episode in the future.

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This continues to be an exhaustive effort led by a multidisciplinary team of chemists, toxicologists, physicians, pharmacists, communication specialists, investigators and analytical laboratory staff from across the FDA and in collaboration with global regulators.

While we're still investigating the root causes of the impurities, our ongoing effort has determined that the impurities may be generated when specific chemicals and reaction conditions are present in the manufacturing process of the drug's API, and may also result from the reuse of materials, such as solvents. This issue surfaced in the summer of 2018, when the FDA was informed that API manufactured by Zhejiang Huahai Pharmaceutical Co. Ltd. (ZHP), in Linhai, Taizhou Zhejiang China for some generic valsartan-containing medicines contained NDMA, posing a potential safety concern.

We've also worked with manufacturers of all ARB medicines to recall any product that poses a risk to patients. Because of the way API is distributed in the supply chain, one source of contaminated API can impact multiple products. As part of this continuing process, last week, we alerted patients and health care professionals to a voluntary recall of one lot of irbesartan and seven lots of irbesartan and hydrochlorothiazide (HCTZ) combination tablet distributed by Solco Healthcare LLC, a Prinston Pharmaceutical Inc. subsidiary. The recall is due to unacceptable amounts of NDEA in the irbesartan API manufactured by ZHP. We will continue to keep the public updated via our website
(/drugs/drug-safety-and-availability/information-about-nitrosamine-impurities medications)

In addition to our policy work, the FDA inspects manufacturing facilities worldwide.

Generally during CGMP inspections, we review the records that manufacturers must maintain regarding required impurity testing.

Table 6 Methods for Determination of Valsartan Impurity

Sr No.	Title	Method	Description	Ref. No.
1.	NDMA impurity in valsartan and other pharmaceutical products: Analytical methods for the determination of *N*-nitrosamines.	1) GC 2) Liquid Chromatographic based Method 3) Non – Chromatographic Method 4) Solid Phase Extraction (SPE) 5) Liquid – Liquid Extraction (LLE) 6) Direct Liquid Extraction (DLE) 7) Distillation	**1) GC** More generally, most of the reported methods for NDMA analyses in scientific literature utilize GC separation. Moderate to high polarity stationary phases are used for separation with carbowax or analogous material being most popular. This material was also used in the recently published method of the FDA. In contrast, the United States Environmental Protection Agency (EPA) method for N-nitrosoamines (NAs) in drinking water recommends a polyphenylmethylsilicone column. In this column type the surface is modified by polyethyleneglycol residues to allow for additional dipol-dipol and hydrogen bonding of analyte and stationary	74

			phase. This may lead to improved Separation of polar analytes. **2) Liquid Chromatographic Method** Alternatively, high performance liquid chromatography (HPLC) on reversed phase columns (mainly RP, C-8 or C-18) is performed for NDMA determination. Detection by ultraviolet (UV) was reported by Al-Kaseem et al. and Li et al. using diode array detection (DAD) at a wavelength of 230 - 233 nm, respectively. The recently published method for the detection of NDMA in valsartan API and tablets uses a detection wave length 228 nm. **3) Non –Chromatographic Method** Several methods for the analysis of NAs were reported that do not apply chromatographic separation. They are generally considered as less selective and often used for screening or for determination of the sum of NAs. **Polarography**, initially reported for successful NA determination, later turned out to lack selectivity and its use seems to be discontinued in NA analyses. **colorimetric assay** is based on the Eisenbrand Preussman reaction. After cleavage of the nitrosoamine using HBr in acetic acid the resulting nitrite is detected by colorimetry. Nitrite was also reported to be liberated from NAs by photolysis and combined with a colorimetric assay. **4) SPE** SPE for NDMA concentration in combination with isotope dilution is mainly used in the field of water analysis. Especially relevant for ultra-	

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			trace analysis of NAs in ground and drinking water very high enrichment factors may be achieved. Thus, the EPA method 521 implies the use of SPE for concentration of water samples prior to GC-CI-MS/MS. In combination with HPLC based methods SPE appears the method of choice for NA extraction and various methods are reported for LC-MS/MS. **5) LLE** Dichloromethane (DCM, methylene chloride) is the most prominent extraction solvent for NDMA and other NAs. **6) DLE** Analysis of thirteen nitrosamines in cosmetic products via GC-MS was accomplished after direct liquid extraction (DLE) with water and acetonitrile. A pressurized liquid extraction of house dust with ethyl acetate to detect nitrosamines from third hand tobacco smoke was performed prior to subsequent analysis either by GC-MS or GC x GC-NCD. Hot water pressurized liquid extraction was utilized for the extraction of nitrosamines from sewage sludge prior to HS-SPME and subsequent GC-MS/MS detection with positive chemical ionization using methanol. **7) Distillation** Steam or vacuum distillation of volatile NAs commonly followed by LLE as sample preparation step was often used in food analysis. Analysis of NDMA in food products and air by a colorimetric approach was realized after several	

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			extraction steps and distillation.	
2.	Rapid and efficient high performance Liquid chromatography analysis of *N*-nitrosodimethylamine impurity in valsartan drug substance and its products.	HPLC Method	**Column:** Inertsil ODS-3 Column (150 mm X 4.6 mm X 5 µm, GL Science, Tokyo, Japan) **Mobile Phase:** Water containing 0.1 % formic acid and ACN containing 0.1 % formic acid **Flow Rate:** 0.8 ml/min **Detection Wavelength:** 235 nm **Retention Time:** 7.8 min (NDMA), 16.3 min (VAL) Correlation Coefficient: 0.999 **LOD:** 0.0085 µg/ml **LOQ:** 0.0285 µg/ml	75
3.	Identification and characterization of potential impurities of valsartan, AT1 receptor antagonist.	HPLC, LC-MS, Mass Spectrometry, NMR Spectrometry, Melting point determination, FT-IR Spectroscopy	**HPLC Method** **Column:** Shield-RP18, 250mm×4.6mm, 5_m (Waters, Milford, MA, USA) **Mobile Phase:** A: 0.01MKH2PO4 and 0.005MK2HPO4 (pH 3.0, adjusted with diluted phosphoric acid), B: water and acetonitrile (1:4, v/v) **Flow Rate**: 0.8 ml/min **Detection Wavelength:** 210 nm **LC-MS Method** **Column:** Shield-RP18, 250mm×4.6mm, 5_m (Waters, Milford, MA, USA) **Mobile Phase:** A: 0.01M ammonium acetate (pH 3.0, adjusted with trifluoroacetic acid), B: water and acetonitrile (1:4,v/v) **Flow Rate:** 0.8ml/min **Detection Wavelength:** 210 nm **NMR Spectrometry** The 1H NMR were recorded on Varian Mercury plus 400MHz FT-NMR spectrometer using CDCl3 for valsartan and impurity II, using DMSO-*d*6 for impurities I, III and IV and using mixture of DMSO-*d*6 and CDCl3 for impurity V.	76

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			Melting Point Determination Melting points of all impurities were determined in a Polmon digital melting point apparatus model no. MP96 (Polmon, Hyderabad, India). **FT –IR Spectroscopy** The IR spectra were recorded in the solid state as KBr (Make: Merck and grade: IR) dispersion medium using PerkinElmer Spectrum One FT-IR spectrophotometer (PerkinElmer, Boston, MA, USA).	
4.	Monitoring of Impurity Level of Valsartan and Hydrochlorothiazide Employing an RP–HPLC Gradient Mode.	RP-HPLC (Gradient Mode)	**Column:** Hypersil 120–5 ODS column (250 mm _ 4.6 mm; 5 mm particle size) **Mobile Phase:** A) 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:** 50 µl **Flow Rate:** 1 ml/min **Detection Wavelength:** 256 nm **Detector:** Agilent 1100 Series chromatographic system with DAD detector	77

12. Valsartan recall: global regulatory overview and future challenges [78]

Valsartan is an orally active antihypertensive drug developed in the 1990s and is a selective angiotensin II receptor blocker (ARB) which relaxes the blood vessels and thus reduces blood pressure; it is also used for treating patients with congestive heart failure and post myocardial infarction. There are eight other ARBs that patients may be switched to if they discontinue their valsartan therapy. Valsartan was patented by Novartis Pharmaceuticals in 1996 on the US market under the name Diovan. The patent was taken off in the USA in 2012, when valsartan was distributed as a generic. Valsartan alone and in combination with other drugs is sold by 30 companies in the US market.

On 5 July 2018 the European Medicines Agency (EMA) reviewed medicines containing valsartan following detection of an impurity, N-nitrosodimethylamine (NDMA), a probable human carcinogen, in medicines from Zhejiang Huahai Pharmaceutical Co Ltd, Linhai, China. Since the batches manufactured from this valsartan-active substance have been administered to many patients, the EMA's review focused on investigating the

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levels of NDMA in the products and the potential impact on patients who have been taking them. The agency further issued advisory notices on their website for patients not to stop taking their medicine.

NDMA is an organic chemical that forms in both industrial and natural processes, and has been used to make liquid rocket fuel, softeners, and lubricants. NDMA has been studied in animals and found to increase the occurrence of cancer. The US Environmental Protection Agency found an association between NDMA and liver toxicity, which could lead to liver cancer: NDMA exposure may be associated with bladder, renal, pancreatic, intestinal, colon, and stomach cancers. Immediately following the EMA's review, 24 countries, Germany, Norway, Finland, Sweden, Hungary, The Netherlands, Austria, Ireland, Bulgaria, Italy, Spain, Portugal, Belgium, France, Poland, Croatia, Lithuania, Greece, Canada, Bosnia and Herzegovina, Bahrain, and Malta, recalled approximately 2300 batches of valsartan products,8 while Hong Kong recalled 5 products of 2 companies9 and Canada recalled drug products of 5 companies.10 The Drug Regulatory Authority of Pakistan on 12 July recalled valsartan-containing drugs of nine manufacturers becoming the first developing country to announce separately the recall as a precautionary measure to protect patient health.11 The US Food and Drug Administration (FDA) on 13 July announced the voluntary recall of five valsartan-containing products.

The EMA, FDA, and World Health Organization issued updates on 17 and 18 July declaring that NDMA was not detected by routine tests and that some changes in the manufacturing process introduced by Zhejiang Huahai in 2012 were believed to have produced this impurity as a side product and that this impurity poses an unnecessary risk to patients, therefore, they should use medicines made with drug substances from other sources or consider other available treatment options. Immediately after these updates India and South Africa also recalled different valsartan products supplied by Zhejiang Huahai. The EMA update on 2 August revealed that the average level of NDMA detected was 60 parts per million which could result in one extra case of cancer for every 5000 patients taking the affected medicines at the highest dose (320 mg) every day for 7 years.

13. Conclusion

The improvement of quality of life has stimulated considerable research in drug design, bioavailability and safety, thus, in order to achieve these targets, highly sensitive and specific methods of analysis are necessary. An analytical method dedicated to the analysis of a drug compound in a given matrix should be developed in a critical manner, presented work is focused on the use of different analytical methods like HPLC (High Performance Liquid Chromatography), HPTLC (High Performance Thin Layer Chromatography), UHPLC (Ultra High Performance Liquid Chromatography), GC (Gas Chromatography), LC/MS/MS etc. for determination Valsartan and Sacubitril analytes in formulation as well as in API. From the reviewed literature it is obvious that HPLC is commonly available method of testing in pharmaceutical laboratory, so this method should be choice for complete determination of all compounds. For analysis of Valsartan and Sacubitril in pharmaceuticals, HPLC with UV detection is applicable because this method provides accurate results and low cost compared to more advanced detection techniques. This review carried out an overview of the current state-of art analytical methods for the determination of Valsartan and Sacubitril. The review would help analytical chemists in knowing the key solvents and their combinations for their available set of instrument in the analytical laboratory.

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