EGE UNIVERSITY GRADUATE SCHOOL OF APPLIED AND NATURAL SCIENCES

(MASTER THESIS)

QUALITY CRITICS OF LIPID LOWERING CHEMICAL INGREDIENTS IN INDUSTRY

Melek İŞLEK

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Melek İŞLEK tarafından Yüksek Lisans tezi olarak sunulan “Endüstrideki Lipid Düşürücü Kimyasal Etken Maddelerin Kalite Değerlendirmesi” başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi’nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve .....tarihinde yapılan tez savunma sınavında aday oybirliği/oyçokluğu ile başarılı bulunmuştur.

	Jüri Üyeleri:	**İmza**
Jüri Başkanı	**: .....**	**.....**
Raportör Üye	**: .....**	**.....**
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ÖZET
ENDÜSTRİDEKİ LİPİD DÜŞÜRÜCÜ KİMYASAL ETKEN MADDELERİN KALİTE DEĞERLENDİRMESİ

İŞLEK, Melek

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Mayıs 2010, 109 sayfa

Atorvastatin Ca impürite metod validasyonunun incelenmesi olarak oluşturulan bu tez derlenen ve değerlendirilen deneysel bir çalışmanın sonuçlarından oluşturulmuştur.

Bu tez çalışmasında, lipid düşürücü etken madde türevi olan statin sınıfında bulunan Atorvastatin Ca etken maddeli ilaçların, statin grubu içindeki yeri ve önemi incelenmiştir.Sözkonusu ilaçların içinde bulunan safsızlıkların tayininde kullanılan UPLC (Ultra Performance Liquid Chromatography) jyardımıyla elde edilen sonuçların validasyon açısından değerlendirilmesi ve incelenmesi amaçlanmıştır.

Atorvastatin Ca tabletlerinde bulunan safsızlıkların analitik metod validasyonu, standart (International Conference on Harmonization) kurallar baz alınarak yapılmıştır. Atorvastatin Ca etken maddesine ait dokuz adet safsızlığın Ultra Performans Sıvı Kromatografisi cihazı ile analizlenmesi ve bu sayede bir seri ilaç analizinde harcanan süre 430 dakikadan 175 dakikaya, toplam tüketilen mobil faz 500 ml'den 70 ml'ye düştüğü gözlenmiştir. Bu analiz metodunun işletmeye daha az maliyetli ve çevreye daha duyarlı olunduğu saptanmıştır.

Bu ilaçların ekonomisini analizleyebilmek için, hastanenin izni ile alınan 3500 hastanın kandaki LDL-C (düşük yoğunluklu lipoprotein kolestrolü), HDL-C (yüksek yoğunluklu lipoprotein kolesterolü), toplam kolesterol değerleri, MatLab yardımıyla yaşa bağlı ve istatistiksel dağılım grafikleri çizildi.

Anahtar Kelimeler: Statin, Atorvastatin Ca safsızlıkları, UPLC, Kolesterol

ABSTRACT

QUALITY CRITICS OF LIPID LOWERING CHEMICAL INGREDIENTS IN INDUSTRY

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MSc in Chemical Engineering

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Atorvastatin Ca is the most widely prescribed drug throughout the world. In this thesis, the importance of Atorvastatin Ca, an active ingredient which is in the statin class of lipid lowering drugs, was investigated.

The impurity method validation of Atorvastatin Ca tablets was carried out according to ICH (International Conference on Harmonization) guidelines. Hence, with this analytical method which is employed using Ultra Performance Liquid Chromatography, the analysis of these nine impurities for Atorvastatin Ca drug were identified for the first time in literature. Also, the required time for the analysis of one batch drug reduced from 430 min to 175 min and the total mobile phase consumption for one batch analysis reduced from 500 ml to 70 ml.

Approximately 3500 female and male patients' blood cholesterol, LDL-C (low density lipoproptein cholesterol), HDL-C (high density lipoprotein cholesterol), and triglyceride test results were taken from a hospital with permission and evaluated statistically. Distribution and statistical graphs were drawn according to age and the results using Matlab 7.7.

For the economical analysis, cost analysis was carried out with 4 different statins. Atorvastatin, simvastatin rosuvastatin and pravastatin were used for this investigation.

Key words: Statins, Atorvastatin Ca impurities, UPLC, Cholesterol

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TABLE OF CONTENT

Page

TABLE OF CONTENT (continued)

Page

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Page

LIST OF FIGURES

Figure **Page**

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LIST OF FIGURES (continued)

Figure **Page**

LIST OF TABLES

Table **Page**

LIST OF TABLES (continued)

Table Page

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1. INTRODUCTION

1.1 The Objective of the Study

The objective of this study is to validate a new analytical method for the determination of impurities of Atorvastatin Ca tablets which is mostly prescribed lipid lowering drug throughout the world by using Ultra Performance Liquid Chromatography (UPLC).

Atorvastatin is used for the treatment of elevated total cholesterol, LDL, triglycerides and to elevate HDL cholesterol. The effectiveness of atorvastatin in lowering cholesterol is dose-related, meaning that higher doses reduce cholesterol more. Atorvastatin prevents angina, stroke, heart attack, hospitalization for congestive heart failure, and revascularization procedures in individuals with coronary heart disease.

Atorvastatin reduces the risk of myocardial infarction, stroke, angina and revascularization procedures in adults with multiple risk factors for coronary artery disease, also prevents heart attacks and strokes in patients with type 2 diabetes with multiple risk factors for coronary artery disease.

As a results of this analytical method validation, it was the first time of nine impurities of Atorvastatin Ca were identified in an analytical method. While doing this validation, International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Q2 Analytical Method Validation Procedure Guideline was followed

The second task of this thesis is to evaluate the cholesterol levels statistically which was reported from a hospital in Mediterranean region and used in this thesis with the permission of the hospital to be able to have an opinion about cholesterol levels in population. As a results of this evaluation the cost effectiveness of Atorvastatin Ca was investigated. Mat Lab 7.7 software was used to graph these data statistically. Age distribution, LDL-C and HDL-C and totatlcholesterol reference limit plots and distribution plots for male and female patients were given in the results.

1.2 General Information and Literature Review

1.2.1 Cholesterol

Cholesterol (Figure 1.1) is an important sterol in mammals in quantitative terms. It is an integral part of both plasma membrane and organelle membranes as well as a precursor for important molecules like bile salts and steroid hormones (Plösch et al., 2006)

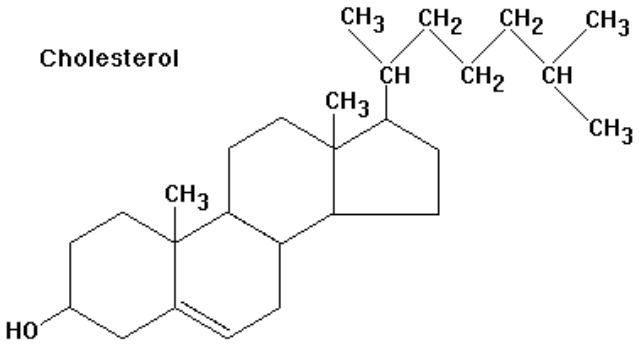


Figure 1.1 Structure of Cholesterol.

1.2.1.1 Biosythesis of cholesterol and its biological importance

Steroids are lipids that contain four carbon rings joined to form the steroid bucleus, cyclopentanoperhydropheanthrene as seen in Figure 1.2.

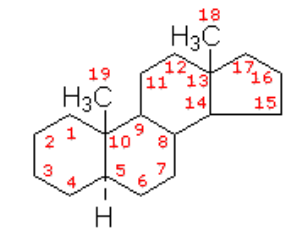


Figure 1.2 Structure of Cyclopentanoperhydropheanthrene.

Cholesterol (Figure 1.1) is a sterol compound and a sterol is a class of steroid characterized by a hydroxyl group at C-3, an aliphatic chain of at least eight carbons are C-17.

The liver is the major site of cholesterol biosynthesis, although other tissues are also active in this regard (i.e., the intestines, adrenals, gonads, skin, neural tissue and aorta)

All 27 carbon atoms of cholesterol are derived from the acetate moiety of acetyl coenzyme A (acetyl CoA), the enzyme system residing in the cytosolic and microsomal fractions.

Cholesterol biosynthesis can be thought of as occuring in five groups of reactions.

Formation of mevalonate from acetyl CoA

Formation of isoprenoid units

Formation of squalene

Cyclization of squalene to form lanosterol

Conversion of lanosterol to cholesterol

Most important step of cholesterol biosynthesis is formation of mevalonate from acetyl CoA and it is described as: β-Hydroxy-β-methylglutaryl CoA (HMG CoA) can be formed in the cytosol from acetyl CoA in two steps by thiolase and HMG CoA snythetase. The rate-time limiting step of cholesterol biosynthesis is the conversion of HMG CoA to mevalonate by HMG CoA reductase. This rate-limiting step is inhibited by dietary cholesterol as well as by endogenously synthesized cholesterol.

1.2.1.2 Control of cholesterol biosynthesis

The reduction of HMG CoA by HMG CoA reductase as the rate-limiting step in cholesterol biosynthesis. Although later steps may be affected by a prolonged stimulu (e.g., long term feding of cholesterol), their rates never become less than that of HMG CoA reductase.

The feeding of cholesterol reduces the hepatic biosynthesis of cholesterol by reducing the activity of HMG CoA reductase. Importantly, intestinal cholesterol biosythesis does not respond to the feding of high-cholesterol diets.

HMG CoA reductase activity is also reduced by fasting , which limits the availbaility of acetyl CoA and NADPH for cholesterol biosynthesis.

In contrast, the feding of diets high in fat or carbohydrate tends to increase hepatic cholesterol biosythesis.

HMG CoA reductase can undergo reversible phosphorylation-dephosphorilation; the phosphorylated enzyme is less active than the dephosphorlyated form. However, there is no evidence that the changes in enzyme activity that are observed with cholesterol feding or with fasting are accompained by changes in phosphorylation of HMG CoA reductase.

1.2.1.3. Esterification of cholesterol

The bulk of the cholesterol in tissues and about 65 % of plasma cholesterol is esterified with long-chain fatty acis at C-3 (hydroxyl group). The synthesis of cellular cholesterol ester requires ATP (adenoise triphospahte) in order to form fatty acyl CoA derivatives, which are then transferred to the 3-β-hydroxyl group of cholesterol. Cholesterol associated with plasma lipoproteins can be esterified by lecithin: cholesterol acyltransferase (LCAT) (Halkerston,1988).

1.2.1.4. Plasma lipids and transport of cholesterol

The plasma lipids are neutral lipid (triglycerides), phospholipids, cholesterol, cholesterol esters and free fatty acids.

Since lipids are not water-soluble, they are transported in blood bound to transport proteins. There are different types of these proteins which are called according to their separation in ultracentifugation and electrophoresis into chylomicrons, very low density lipoproteins (VLDL) or pre-β-lipoproteins, intermediate density lipoproteins (IDL), low density lipoproteins (LDP) or β-lipoproteins, high density lipoproteins (HDL) or α-lipoproteins

Chylomicrons are microscopically detectable lipid spheres with a diameter of approximately 1 μm. Their protein component is only 2 % by weight and the lipids are mainly triglycerides. During electrophoresis, they remain at the origin.

Very low density lipoproteins (VLDL) have a molecular weight of $5x10^6$ and migrate during electrophoresis before the β-globulins (hence, the name pre-β-lipoproteins). They transport mainly neutral lipids that are formed in the body.

Intermediate density lipoproteins (IDL) are VLDL particles that have released their triglyceride component into fat tissue or muscle.

Low density lipoproteins (LDL), with a molecular weight of $2\text{-}3x10^6$, contain cholesterol as the major component. They behave like β-globulins during electrophoresis.

High density lipoproteins (HDL) have a high protein content and low cholesterol content. They can take up cholesterol from vascular walls and transform in into IDL.

Chylomicrons are formed during fat absorption in the intestinal wall and retransported via the lymph to the blood. After cleavage of triglycerides by lipoprotein lipase (cleasring factor), the fatty acids are transported into fat tissue (for storage) and muscle (for energy). The remnants contain a large fraction of

cholesterol and are bound to a special receptor (which only occurs in the liver), allowing uptake into liver cells. In the liver, cholesterol is convered into bile acids which are secreted in to bile, or re-relased into the blood in the form of VLDL together with apoproteins, phospholipids and triglycerides. For VLDL, fat and muscle tissues take up the free fatty acids produced by lipoprotein lipase. This results in IDL particles which can attach to LDL receptors in the liver for rapid uptake into hepatocytes. The remaining portion of IDL is converted to LDL after cleavage of lipoprotein E. This can then bind to LDL receptors and enter hepatocytes and other cells by endocytosis. Intracellular enzymatic degradation of IDL and LDL releases cholesterol in the liver. Negative feedback inhibits the new synthesis of cholesterol by blocking the synthesis of hydroxymethylglutaryl-coenzyme-A reductase (see below) and stimulating formation of cholesterol esters by activation of acetyl-CoA-cholesterol acyltransferase. De novo synthesis of LDL receptor and intracellular cholesterol concentrations regulate the chlesterol levels in plasma.

HDL, formed in extrahepatic tissues, can take up cholesterol from cells and transfer them to IDL after esterification with lecithin-cholesterol acyltransferase which restarts the cycle (Mutscher and Herendorf, 1995) .

1.2.1.5 Clinical disorders of lipoprotein metabolism

Familial disorders of lipid metabolism include both hyper- and hypolipidemias.

Hyperlipidemias

Hyperlipoproteinamias, problems in lipid metabolism manifest mainly in the form of hyperlipoproteinemias, i.e. an incerase in one or more lipoprotein fractions. Depending on the type of the most utilized lipoproteins, there are different types listed in Table 1.1.

Table 1.1 Classification of hyperlipoproteinemias

Type	Changes in lipoproteins	Name	Synonym
I	Chylomicrons increased	Hyperchylomicronemia	Exogenoous, fat-induced, hypertriglyceridemia
IIa	LDL increased	Hyper-β-lipoproteinemia	Hereditatary xanthomatous
IIb	LDL and VLDL increased	Hyper-β- and pre-β-lipoproteinemia	Hypercholesterolemia and Hypertriglyceridemia
III	IDL and chylomicrons increased	"Broad- β-disease""	Hypercholesterolemia and Hypertriglyceridemia
IV	VLDL increased	Hyper-pre-β-lipoproteinemia	Endogenous (carbohydrate-induced) hypertriglyceridemia
V	VLDL and chylomicrons increased	Hyper-pre-β-lipoproteinemia with hyperchylomicronemia	Mixed endogenous-exogenous hypertriglyceridemia

Types IIa, IIb and IV represent 95 % of all cases. The differentiation is important not only because of the clinically different forms and pathophysiological relevance but also because there are different types of treatment. The reasons for hyperlipoproteinemia in the primary forms are genetic dysfunctions of lipid metabolism; in secondary forms, excess weight, poor diet, alcohol abuse and metabolic diseases (hypothyroidism, diabetes mellitus, gout) (Mutscher and Herendorf, 1995) .

If the hyperlipidemia is the principal manifastation of the diasease, it is called a primary hyperlipidemia. If the hyperlipidemia is due to an underlying disease process, as found in some cases of thyroid, liver or kidney disease, it is called secondary hyperlipidemia (Halkerston, 1988) .

Lipid, lipoprotein, and apolipoprotein results by hyperlipidemic status at baseline are presented in Table 1.2. By definition, mean triglyceride and cholesterol levels at baseline were higher among relatives with hyperlipidemia compared to those with normal lipids at baseline (Mc Neely, 2001) .

Table 1.2 Comparison of lipids, lipoproteins, and apolipoproteins at follow-up by hyperlipidemic status at baseline

	Normolipidemic relatives at baseline	Hyperlipidemic[a] relatives at baseline	*P* value[b], adjusted for age and sex	*P* value[b], adjusted for age, sex, medications, and baseline study
Baseline				
N	157	130		
Triglycerides (mg/dl)	82.7±44.0	179.8±194.4	-	-
Cholesterol (mg/dl)	180.8±29.7	240.8±51.4	-	-
Follow-up				
n	148	107		
BMI (kg/m^2)	26.9±5.6	27.2±5.5	0.684	0.483
ΔBMI(kg/m^2)[c]	4.6±4.8	4.8±5.4	0.813	0.660
Triglycerides (mg/dl)	157.1±103.7	207.4±163.0	0.003	<0.001
ΔTriglycerides (mg/dl)	77.5±97.0	42.6±197.2	0.785	0.975
Cholesterol (mg/dl)	197.8±30.6	233.6±42.2	<0.001	<0.001
ΔCholesterol (mg/dl)	19.8±33.4	-1.3±60.6	<0.001	<0.001
HDL-C (mg/dl)	47.0±14.0	48.9±19.8	0.443	0.732
LDL-C (mg/dl)	118.9±28.9	142.8±38.1	<0.001	<0.001
LDL size (Á)	265.7±9.1	264.1±9.4	0.200	0.099
apoA-I (mg/dl)	137.6±26.8	143.1±34.7	0.139	0.264
apoB (mg/dl)	102.6±20.6	123.7±26.5	<0.001	<0.001
Lp(a)(nmol/L)	9.7±11.0	8.3±11.9	0.099	0.179

Data are means ± S.D.

[a] Hyperlipidemia defined ≥ age –and sex- specific 90th percentile for triglycerides or cholesterol based Lipid Research Clinic data at baseline [30]. Excludes 32 individuals on lipid lowering medication at follow-up (nine were normolipidemic and 23 were hyperlipidemix at baseline).

[b] *P* values based on natural logarithm of triglycerides, Δtriglycerides, and Lp(a) values. *P* values for ΔBMI were further adjusted for baseline BMI; *P* values for Δtriglycerides were further adjusted for baseline triglycerides, and *P* values for Δcholesterol were further adjusted for baseline cholesterol.

[c] For ΔBMI, data missing for 11 individuals (seven missing baseline BMI, four missing follow-up BMI).

Hypolipidemias

Hypobetalipoproteinemia (Basen-Kornzweig syndrome) is a rare genetic disorder with a recessive inheritance characterized by neurologic symptoms, including ataxia and mental retardation.

A-lipoprotein deficiency (Tangier Disease) is a rare familial disorder characterized by recurrent polyneuropathy, lymphadenopathy, tonsillar hyperplasia, and hepatosplenomegaly (from storage of cholesterol in reticuloendothelial cells) (Halkerston, 1988) .

The basis for successful treatment of hyperlipoproteinemia is an adeqaute diet. It should normalize body weight, insure balanced nutrient composition (approximately 55 % carbohydrates, up to 30 % fat with 10 % each saturate, monounsaturated and polyunsaturated fatty acids, 10- 20 % proteins), contain bulk agents (vegetables, fruits, etc.) in at least 35 g, as well as, limit cholesterol uptake to less than 300 mg/day.

If the lipid blood level cannot be controlled with diet alone and there is also incerased risk for atherosclerosis, then lipid-lowering drugs should be used.

The target cholesterol level depends on other risk factors (Table 1.3) .

Table 1.3 Goals for different groups of people

	LDL	**TC**	**Triglyceride**
No other risk factors	< 155 mg/dl	< 215 mg/dl	< 200 mg/dl
Other risk factors HDL concentation < 35 mg /dl, male sex, smoking, stres, high blood pressure, diabetes mellitus, coronary disease	< 135 mg/dl	<200 mg/dl	< 200 mg /dl

In long term studies with lipid-lowering agents (e.g. anion exchange resins, gemfibrozil), a significant decrease in the number of heart attacks, but not in overall mortality, has been observed (Mutscher and Derendorf, 1995) .

1.2.1.6 C<u>ompounds that lower triglyceride and cholesterol blood levels</u>

Aryloxyalkane carnonic acids (clofibrate, clofibric acid derivatives, clofibrate analogs) and ***Nicotinic acid and analogs*** (nicotinyl alcohol, acipimox) are the compounds that lower triglyceride and cholesterol blood levels.

Anion exchange resins **(**colestramine), ***inhibitiors of hyroxymethylglutary-coA reductase (HMG-CoA Reductase Inhibitors***) are the compounds that mainly lower cholesterol levels.

A particularly effective way of blocking cholesterol synthesis is inhibition of HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. This decreases intravellualar cholesterol concentrations which results in increased formation of LDL receptors. Consequently, the concentration of LDL and total cholesterol in blood is decreased (Mutscher and Derendorf, 1995) .

Statins include natural (lovastatin), semi-synthetic (simvastatin and pravastatin) and synthetic compounds (fluvastatin, atorvastatin, cerivastatin, rosuvastatin and pitavastatin) and are potent, specific and competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. They are highly effective in reducing total cholesterol and low density lipoprotein (LDL) choletserol levels in the human body. HMG-CoA reductase is the key enzyme that catalyzes the conversion of HMG-CoA to mevalonate, which i an early rate-limiting step in the biosyntheic pathway of cholesterol.(Novakova et al, 2008)

Lovastatin, a highly active competitive inhibitor of HMG-CoA reductase, is isolated from Aspergillus terreus. It is an inactive lactone (prodrug) which is converted in the body into the active, open ring hydroxy acid. It is mainly used in patients with primary hypercholesterolemia.

The dose-dependent decrease in LDL and total cholesterol is between 20- 40 %. After oral administration, lovastatin is approximately 30 % absorbed and undergoes a significant fisrtpass effect. Elimination is both via renal and biliary mechanisms. The plasma half-life of the active hydroxy acid is 1-2 hours.

Probucol

Probucol is a diyhioether derivative that lowers LDL cholesterol levels, but also lowers HDL cholesterol levels. It seems to inhibit cholesterol biosynthesis and absorption, and incerases bile acid excretion in the intestine. The

bioavailability of probucol is low. The drug accumulates in adipose tissue and is eliminated very slowly. The plasma half life extends to several weeks. Probucol is primarily indicatedfor severe type IIa hyperlidemia.

Dextrothyroxine

Dextrothyroxine is the D-enantiomer of the naturally occuring L-thyroxine and has approximately 20 % of its cholesterol-lowering effect, but only a fraction of the other thyroid effects. (2.5- 10 %). Dextrothyroxin increases LDL degradation and increases oxidation of cholesterol to form bile acids. The full effect occurs only after several weeks. The dose is slowly increased from 1-2 mg daily to 4 mg dailiy after 2 weeks, then to 6-8 mg daily after another 2 weeks. Side effects are due to the thyroid activity and include sleeplessness, nervousness, tremor, tachycardia, angina pectoris, hyperhermia, etc. Due to these undesirable effects, dextrothyroxine should only be used in selected cases of hypercholesterolemia, if at all. Dextrothyroxine is contraindiated in heart attacks, arrhythmias, severe liver or kidney damage, and severe atherosclerotic changes.

Sitosterol

Some plant sterols which are related to cholesterol and poorly absorbed inhibit the absorption of cholesterol and estrification in the epithelia cells. They cause a modest decrease in cholesterol blood levels. Sitosterol is used in doses of 1-2 g several times a day. A reliable effect is only seen with doses of more than 10 g/ day. Side effects are gastrointestinal symptoms (flatulence, constipation, etc.) (Mutscher and Derendorf,1995) .

Although a change in life-style is often the method of first choice for lipid lowering, lipid-lowering drugs, in general, help to control elevated levels of different forms of lipids in patients with hyperlipidemia. While one group of drugs, statins, lowers cholesterol, the other group, fibrates, is known to take care of fatty acids and triglycerides. In addition, other drugs, such as ezetimibe, colesevelam, torcetrapib, avasimibe, implitapide, and niacin are also being considered to manage hyperlipidemia. As lipids are very critical for

cardiovascular diseases, these drugs reduce fatal and nonfatal cardiovascular abnormalities in the general population (Pahan,2006) .

Fibrates

In contrast to statins, this group of drugs does not inhibit cholesterol biosynthesis. However, these drugs stimulate β-oxidation of fatty acids mainly in peroxisomes and partly in mitochondria. Therefore, this group of drugs is known to lower plasma levels of fatty acid and triacylglycerol. Clofibrate was the first such drug, developed in Japan in the 1960s. Eventually, the discovery of several other fibrate drugs such as ciprofibrate, bezafibrate, fenofibrate, and gemfibrozil has revolutionized lipid-lowering research. However, the enthusiasm has been short-lived, because prolonged use of some of these drugs like clofibrate and ciprofibrate causes peroxisome proliferation leading to hepatomegaly and tumor formation in the liver of rodents. Therefore, there are concerns about widespread use of these drugs in humans. Only gemfibrozil and fenofibrate, due to their milder effect on peroxisome proliferation, are being used as lipid-lowering drugs in humans (Pahan, 2006)

Cholesterol researches are tabulated according to subject and results in Table 1.4.

Table 1.4 Literature search for cholesterol studies

CHOLESTEROL		
SUBJECT	**RESULT**	**REFERENCE**
Cholesterol homeostasis in neurons and glial cells	Cholesterol is excreted from the brain in the form of 24-hydroxycholesterol. Apolipoprotein E and cholesterol have been implicated in the formation of amyloid plaques in Alzheimer's Disease	Vance et al. (2005)
Molecular mechanisms of cholesterol absorption and transport in the intestine	Particular importance is the development of cholesterol absorption inhibitors that are effective in lowering plasma cholesterol level in humans	Hui and Howles (2005)

High cholesterol absorption efficiency and rapid biliary secretion of chylomicron remnant cholesterol enhance cholelithogenesis in gallstone-susceptible mice	Genetic variations in cholesterol absorption efficiency are associated with cholesterol gallstone formation in inbred mice and cholesterol absorbed from the intestine provides an important source for biliary hypersecretion.	Wang et al (2005)
LDL-cholesterol, HDL-cholesterol or triglycerides* which is the culprit?	Statins act primarily to reduce LDL-cholesterol; they also produce modest increases in HDL-cholesterol and moderate triglyceride reductions and appear to normalize lipoprotein particle composition.	Taskinen (2003)
LDL and HDL subclasses in acute ischemic stroke: Prediction of risk and short-term mortality	Acute ischemic stroke (AIS) is associated with adverse distributions of LDL and HDL subclasses. In addition, shortterm mortality after AIS is associated with increased sdLDL particles. Our results indicate that sdLDL is an independent predictor of both AIS onset and consecutive short-term mortality.	Zeljkovic et al. (2009)
Impaired HDL response to fat in men with coronary artery Disease	Thus men with premature coronary artery disease and a low HDL cholesterol appear to have an impaired elevation of HDL cholesterol in response to dietary fat, and insulin resistance may underlie this response.	Clifton and Noakes (2000)

1.2.2 Statins- The HMG CoA Reductase Inhibitors

1.2.2.1 Discovery and development of the statins

In 1971, a research for microbial products that would inhibit HMG-CoA reductase and that might therefore reduce levels of plasma cholesterol in humans. Some microorganisms would produce such novel compounds, and this possibility fascinated the scientists because such products had not been isolated previously. These studies led to the discovery of a potent, low toxicity reductase inhibitor, named mevastatin (formally called compactin or ML-236B) th prototype of the statins.

The first statin molecule-mevastatin- was discovered by Endo et. Al. İn 1976 as a fungal product extracted from Penicillium citrinum (Endo et al., 1976) .

Subsequently, the biochemical mechanisms of action of mevastatin, and by the end of the 1970s, they showed that mevastatin markedly reduced the levels of total and low-density lipoproptein (LDL) cholesterol in both experimental animals and patients with primary hypercholesterolemia. These findings apparently stimulated the worlwide development of mevastatin analogs (statins) in the 1980s, and by 1991, three statins – lovastatin, simvastatin and pravastatin-had been approved and marketed in the USA and many other countries. Since then, three statins that were chemically synthesized have also been introduced to the market. All of these statins have been well established as effective and safe cholesterol-lowering agents and have been used by miilons of patients (Gaw et al., 2000) .

1.2.2.2. Types of statins

Chemical structures and pharmacokinetic properties of the statins are shown in Table 1.5. Among all statins, lovastatin, simvastatin and pravastatin are derivatives of fungal products while other newly developed statins are completely synthetic. The fungal products lovastatin, simvastatin and pravastatin are structurally related and they have a hydronaphthalene ring in common.

Lovastatin and simvastatin are orally administered as inactive prodrugs in the lactone forms while pravastatin is given in the active open acid form. Other totally synthetic statins have different structures although they also have an open acid HMG-like moiety between the 4-fluorophenyl- and isopropyl- (or cyclopropyl-) groups. The structural difference may account for their solubility differences in water.

Pharmacokinetic drug-drug interactions influence drug efficacy, tolerability, and compliance, and such interactions are both common and of more clinical relevance than often appreciated. Therefore, the different pharmacokinetic profiles among the statins should be carefully considered in order to understand the different spectrum of drug interactions, which are important determinants of

safety in patients with hypercholesterolemia, especially in those requiring long-term therapy with drugs that are well-known CYP3A (metabolism) substrates and/or inhibitors (Corsinia et al, 1999) .

Table 1.5 Pharmacokinetic and chemical data of statins

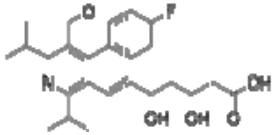
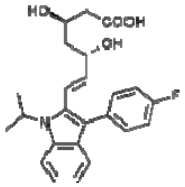
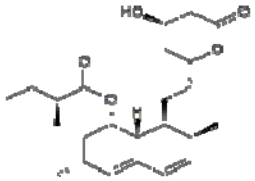
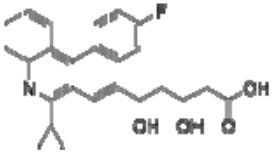
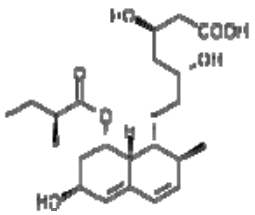
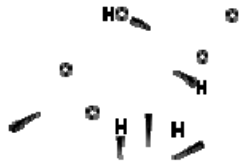
STATINS	IMAGE	CHEMICAL FORMULA MW (g/mol)	DERIVATION	PHARMACOKINETIC DATA			
				Bioavailability	Metabolism in liver	Half life	Excretion
Atorvastatin		$C_{33}H_{35}FN_2O_5$ **MW:** 558.64	Synthetic	12 %	CYP3A4	14 hours	Bile
Cerivastatin		$C_{26}H_{34}FNO_5$ **MW:** 459.55	Synthetic	60 %	CYP3A4 CYP2C8	2–3 hours	Bile
Fluvastatin		$C_{24}H_{26}FNO_4$ **MW:** 411.466	Synthetic	24 %	CYP2C9	0.5-3.1 hours	Bile
Lovastatin		$C_{24}H_{36}O_5$ **MW:** 404.54	Fermentation-derived.	< 5 %	CYP3A4	1.1-1.7 hours	negligible

Table 1.5 continued

Pitavastatin		$C_{25}H_{24}FNO_4$ **MW:** 421.461	Synthetic	60 %	Hepatic	11 hours	Biliary
Pravastatin		$C_{23}H_{36}O_7$ **MW:** 424.528	Fermentation-derived	17 %	Non-metabolic	77 hours	Urine / Faeces
Rosuvastatin		$C_{22}H_{28}FN_3O_6S$ **MW:** 481.539	Synthetic	20 %	CYP2C9 CYP2C19	19 hours	Urine / Faeces
Simvastatin		$C_{25}H_{38}O_5$ **MW:** 418.566	Fermentation-derived	5 %	CYP3A4	3 hours	Renal 13%, faecal 60%

Among the statins, rosuvastatin would appear to have the most favorable overall profile (McKenney,2003). Scientific researches related with statins are listed in Table 1.6.

Table 1.6 Researches on statins and their results

STATINS	SUBJECT	RESULTS	REFERENCE
Simvastatin	Effect of simvastatin on the oxidation of native and modified lipoproteins	The most pronounced inhibition of oxidation in presence of simvastatin was found for HDL	Sobal et al. (2005)
Simvastatin	Effect of simvastatin on vascular smooth muscle	The effect of simvastatin on vascular smooth muscle may involve both Ca_{2q} release from intracellular stores and blockade of extracellular Ca_{2q} entry.	Sotomayor and Perez (2009)
Lovastatin	Lovastatin alters the isoprenoid biosynthetic pathway in acute myelogenous leukemia cells in vivo	Lovastatin has long been used as an effective drug for lowering cholesterol levels by blocking hydroxymethylglutaryl coenzyme A reductase (HMGR) activity there by decreasing endogenous production of mevalonate	Lewis et al. (2005)
Lovastatin	Lovastatin potentiates the antidepressant efficacy of fluoxetine in rats	The possibility that statins could be used to facilitate the effects of antidepressants in humans.	Renshaw et al. (2009)
Pravastatin	Effects of Pravastatin Treatment on Lipoprotein Levels	In patients with type 2 diabetes mellitus and hypercholesterolemia, pravastatin improved the lipid profile and the atherogenic potential associated with abnormal lipoprotein composition.	Miccoli et al. (2000)
Pravastatin	Estrogen/progesterone replacement versus pravastatin	In menopausal patients with elevated C not responding to diet, pravastatin wasmost effective to decrease LDL-C, and oral estrogen–micronised progesterone most effective to increase HDL-C.	Lemay et al. (2001)

Pravastatin	The Influence of Pravastatin and Atorvastatin on Markers of Oxidative Stress in Hypercholesterolemic Humans	Statin therapy results in variable effects on oxidative stress markers in hypercholesterolemic subjects	Ky and Burke (2008)
Pravastatin	Early Improvements in insulin sensitivity and inflammatory markers are induced by pravastatin	insulin resistance was improved even in short-term treatment by pravastatin.	Lee et al. (2008)
Fluvastatin	The effect of fluvastatin on cardiac events in patients with symptomatic coronary artery disease during one year of treatment	Lipid-lowering therapy with fluvastatin significantly reduces the risk of cardiac events in hyperlipidaemic patients with symptomatic coronary heart disease (CHD) during 1 year of treatment.	Riegger et al. (1999)
Fluvastatin	Fluvastatin therapy improves microcirculation in patients with hyperlipidaemia	fluvastatin therapy improves microcirculation in hypercholesterolaemic patients within 6 weeks.	Haak et al.(2001)
Fluvastatin	The effect of different doses of fluvastatin on inflammatory markers in the early phase of acute coronary syndrome	The high-dose fluvastatin invention may play a stronger anti-inflammatory effect in ACS patients	Yang et al. (2006)
Cerivastatin	Long-Term Efficacy and Safety of Cerivastatin 0.8 mg in Patients with Primary Hypercholesterolemia	Cerivastatin 0.8 mg reduced LDL-C versus cerivastatin 0.4 mg or pravastatin 40 mg and brought 81.8% of all patients,and 54.1 % of patients with atherosclerotic disease, to National Cholesterol Education Program (NCEP) goals	Isaacsohn et al.(2001)
Cerivastatin	Molecular mechanism of the anti-cancer activity of cerivastatin,	Cerivastatin induces a potent decrease in cell proliferation.	Denoyelle et al. (2003)
Cerivastatin	Cerivastatin Versus Branded Pravastatin in the Treatment of Primary Hypercholesterolemia	A cost savings in favor of cerivastatin was a reflection of the lower drug acquisition cost of cerivastatin compared with branded pravastatin.	McPherson et al.(2001)

Literature search which is related to Atorvastatin Ca is given in Table 1.7.

Table 1.7 Studies with Atorvastatin Ca in literature

SUBJECT	RESULTS	REFERENCE
Extractive Spectrophotometric Determination of Atorvastatin in Bulk and Pharmaceutical Formulations	The proposed spectrophotometric methods are simple, sensitive, and suitable for the determination of atorvastatin in bulk and pharmaceutical dosage forms.	Erk et al. (2003)
Atorvastatin associated liver disease	atorvastatin can rarely cause significant acute hepatotoxicity.	Clarke et. al. (2006)
Quantification of atorvastatin calcium in tablets by FT-Raman spectroscopy	The proposed procedure can be a fast and accurate method of atorvastatin calcium quantification in commercial tablets, as well as a convenient way of simultaneous determination of other ingredients in the tablet mass.	Mazurek,and Szostak (2009)
Atorvastatin is more effective than pravastatin in preventing recurrent cardiac events	High-dose atorvastatin is more effective than Standard therapy for people with acute coronary syndrome.	Cannon et al.(2004)
52 week atorvastatşn and simvastatin study in reaching LDL and TG targets	Atorvastatin 20 or 40 mg/d for up to 1 year of treatment was significantly more effective than simvastatin 20 or 40 mg/d in reducing LDL-C and TG levels	Olsson,et al.(2002)
Effects of atorvastatin on inflammation and oxidative stress	Low dosage atorvastatin has several beneficial effects in hyperlipidemic patients: improvement of inflammation, oxidative stress, blood pressure, and LDLC	Ohashi et al.(2005)
Comparison of the Efficacy and Safety of Rosuvastatin Versus Atorvastatin,Simvastatin, and Pravastatin Across Doses	* Rosuvastatin 10 mg reduced LDL cholesterol significantly more than atorvastatin 10 mg * Rosuvastatin 20 mg reduced LDL cholesterol significantly more than atorvastatin 20 and 40 mg * Rosuvastatin 40 mg reduced LDL cholesterol significantly more than atorvastatin 40 mg	Jones et al. 2003

Table 1.7 continued

Comparative Effects of Rosuvastatin and Atorvastatin Across Their Dose Ranges in Patients With Hypercholesterolemia and Without Active Arterial Disease	Rosuvastatin was consistently more effective than atorvastatin across their respective dose ranges in reducing LDL cholesterol and improving other lipid measures in patients with hypercholesterolemia	Schneck et al.(2003)
Photochemical behavior of the drug atorvastatin in water	Experiments showed that the drug was recovered unchanged by keeping it in the dark in aqueous solution (at different pHs) even after 30 days.	Cermola et al. (2006)
Guidelines for Lowering Lipids to Reduce Coronary Artery Disease Risk: A Comparison of Rosuvastatin with Atorvastatin, Pravastatin, and Simvastatin for Achieving Lipid-Lowering Goals	A drug such as rosuvastatin, which has been shown to reduce LDL cholesterol levels sufficiently to reach the lower goal levels in a large number of hypercholesterolemic patients	Shepherd et al.(2003)
Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets	A novel UPLCmethodwas successfully developed and validated for simultaneous determination of ATO, FEN and impurities	Kadav and Vora (2008)
In vitro availability of atorvastatın in presence of losartan	Interactions of atorvastatin in presence of losartan revealed that the availability of atorvastatin either decreased or increased which reflects its complexation with this drug.	Arayne et al.(2006)

Rosuvastatin is the latest statin came up to cholesterol lowering class and the investigations related with this drug given in Table 1.8.

Table 1.8 Researches on effects of Rosuvastatin tablest

SUBJECT	RESULT	REFERENCE
Expanding Options with a Wider Range of Rosuvastatin Doses	Rosuvastatin 5 mg is well tolerated and has beneficial effects across the atherogenic lipid profile by reducing LDL-C and total cholesterol,	Olsson (2006)
Metabolism,Excretion, and Pharmacokinetics of Rosuvastatin in Healthy Adult Male Volunteers	That metabolism is a minor route of clearance for this agent.	Martin et al. (2003)
Impact of Rosuvastatin Use on Costs A Data Analysis	Increasing the use of rosuvastatin can result in cardiovascular event reduction and cost savings.	Huse et al. (2006)
Efficacy and Safety of Rosuvastatin inTaiwanese Patients	Rosuvastatin 10 mg/d is safe and effective in Taiwanese patients	Chiang et al. (2008)
Comparative pharmacology of rosuvastatin	Rosuvastatin has been shown to reduce LDL cholesterol	McTaggart (2003)
Pharmacokinetics and Tolerability of Multiple-Dose Rosuvastatin	Rosuvastatin systemic exposure appeared to be dose-proportional over the dosing range of 5 to 20 mg with multiple-dose administration.	Zhang and Li (2009)
The Dose Proportionality of Rosuvastatin in HealthyVolunteers	Rosuvastatin systemic exposure was dose proportional over the dose range of 10 to 80 mg.	Martin et al. (2003)
Rosuvastatin Versus Atorvastatin in Achieving Lipid Goals in Patients at High Risk for Cardiovascular Disease	In this study in patients with primary hypercholesterolemia in clinical practice, greater reductions in LDL-C levels were achieved	Binbrek et al.(2006)
Rosuvastatın ıs more effectıve than pravastatın or sımavastatın at ımprovıng thelıpıd profiles of hypercholesterolaemıc patıents	ROS 5 and 10 mg were more effective than PRA 20 mg and SIM 20 mg at improving the lipid profiles of hypercholesterolaemic patient.	Paoletti et al.(2001)

1.2.2.3 Pleitropic effects of statins

Statins are commonly used to treat several forms of hypercholesterolemia. They have potent cholesterol lowering effects and they could reduce morbidity and mortality assocaited with coronary heart disease significantly, as proved by many clinical trials (Novakova et al,2008) .

Statins affect many metabolic pathways and organ systems and they may positively impact multiple disease states independent of LDLc reduction (Almuti et al, 2006) .

Randomized controlled trials with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have consistently demonstrated significant reductions in cardiovascular morbidity and mortality. Statins are currently the most widely used drugs in many countries. The most important adverse effects are associated with muscle and liver toxicity. However, with increased use and dose of statins and their over-the-counter availability in some countries more cases of other rare side effects may be seen in clinical practice (Kiortsis et al., 2007) .

In Table 1.9 some effects of statins that may have potential therapeutic implications in acute neural trauma (stroke, spinal cord trauma, and head injury) and neurodegenerativediseases (Alzheimer disease, Parkinson disease, multiple sclerosis and primary brain tumors) were shown (Cucchiara and Kasner, 2001; Stuve et al., 2003) .

Table 1.9 Therapeutic effects of statins in animal models of acute neural trauma and neurodegenerative diseases

Neurological disorders	Stalin	Effect	References
Ischemia	Atorvastatin	Beneficial	Tuñón et a1.(2007)
Spinal cord trauma	Atorvastatin	Beneficial	Pannu el al. (2005)
	Simvastatin	Beneficial	Holmberg et al. (2006)
Head injury	Atorvastatin	Beneficial	Qu et al.. 2005; Wang et al, 2007
Alzheimer disease	Atorvastatin	Beneficial	Sparks et al (2005)
	Simvastatin	Beneficial	Petanceska et al. 2002; Eckert et al. 2005; Sjögren et al.. 2003
Multiple sclerosis	Atorvastatin	Beneficial	Neuhaus et al (2005)
EAE	Lovastatin	Beneficial	Paintlia et al, 2006; Stuve et al.. 2003a
Neurofibromatosis type 1	Lovastain	Beneficial	Li et al.. 2005b
Migraine	Statin	Beneficial	Liberopoulos and Mikhailidis (2006)
Parkinson disease	Statin	Beneficial	Lieberman et al. (2005)

Chuengsamarn et al., 2009 showed that statin effects the bone formation and bone resorption beyond lipid lowering effect (Figure 1.3) In conclusion, the lipophilic statin as moderate to high dose had beneficial positive effect to increasing bone mineral density and could be additive use for prevention of bone loss in hyperlipidemia patients (Chuengsamarn et al., 2009) .

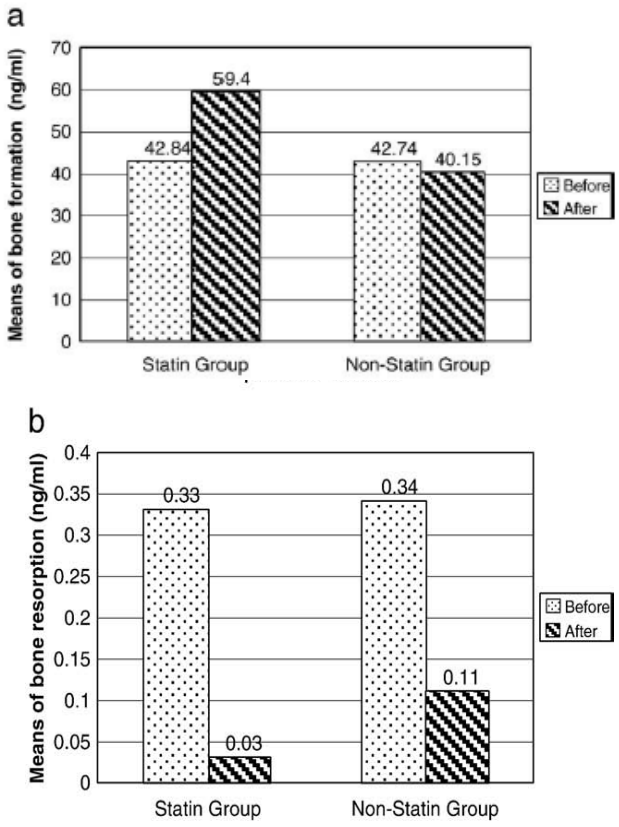


Figure 1.3 (a and b) Statin therapy effect on patients with hyperlipidemia

Some clinical evidence supports a statin antihypertensive effect. Statins are the lipid lowering agents with the largest preventive effect in patients at high risk for cardiovascular disease, and particularly in those showing a combination of high BP and hypercholesterolemia. The study of Prandin et al., 2009 was carried out with 284 patients with hypertension and hypercholesterolemia. All the patients were naive to statin therapy and had been on established antihypertensive treatment for at least 6 months. Actively smoking patients were excluded from the study. As a result of the study, statins have antihypertensive effect as shown in the Table 1.10 (Prandin et al.,2009) . Ref olarak yazılmamış

Table 1.10 Statin effect on hypertension research

Variable	All patients		Males		Females	
	B	End study	B	End study	B	End study
N.	284		129		155	
Age (years)	59.5±7		58.6 ±8		60.3 ± 16	
BMI (kg/m^2)	26.4 ±3		26.2 ±4		27.1 ±5	
SBP (mmHg)	149.6±15	142 ±12	148.2 ±14	140.6±14	150.7±15	142.9±13
DBP (mmHg)	90.1 ±8	84.8 ±7	91.1 ±8	85.3±8	89.4 ±8	84.3 ±7
HR (b/min)	68.7±10	67.4 ±11	67.9 ±9	67.1 ±10	69.3 ±12	67.6±10
TC (mg/dL)	266.0 ±48	216.0±44	258.5 ±42	212.2 ±47	273.2 ± 52	219.1 ±48
LDL-C (mg/dL)	179.2 ±36	131 ±24	174.4 ±35	129.7 ±28	182.8 ±37	132.1±35
HDL-C (mg/dL)	52.4±12	53.7 ± 14	49.8±11	51.3±12	54.6 ± 13	55.7±16
TG (mg/dL)	178.9±56	169.3 ±60	175.5 ±53	166.7 ±58	183.0 ±68	171.5±59
Glucose (mg/dL)	102.9 ±26		103.9±34		100.9 ±16	
Creatinine (mg/dL)	0.98 ±0.25		1.14 ±0.23		0.85 ±0.18	

BMI = body mass index, SBP = systolic blood pressure. DBP= diastolic blood pressure, HR= heart rate. TC= total cholesterol, LDL-C= low density lipoprotein cholesterol. HDL-C= high density lipoprotein cholesterol, TG = triglycerides.
*p<0.05; **p<0.01 compared to the parallel group.

According to Table 1.10, through the statin theraphy, systolic and diastolic blood pressure levels were decreased at the end of study, beyond the lipid lowering effect of statin. Also, LDL and TC levels were decreased considerably. HDL was effected positively, as expected (Prandin et al.,2009) . Ref olarak yazılmamış.

The study about the effect of long-term statin use on psychometric measures in an adult population with underlying coronary artery disease (CAD) was carried out. The results of this observational study suggest that longterm statin therapy consistently improves psychological well-being among a cohort of patients with CAD. A progressive, cumulative reduction in the levels of depression, anxiety, and hostility was observed over a prolonged period of statin use (Fig 1.4) . Figure 1.4 shows that, number of patients of each year on x axis, on y axis percentage change in Kellner questionaire score (Ravid et al., 2003) .

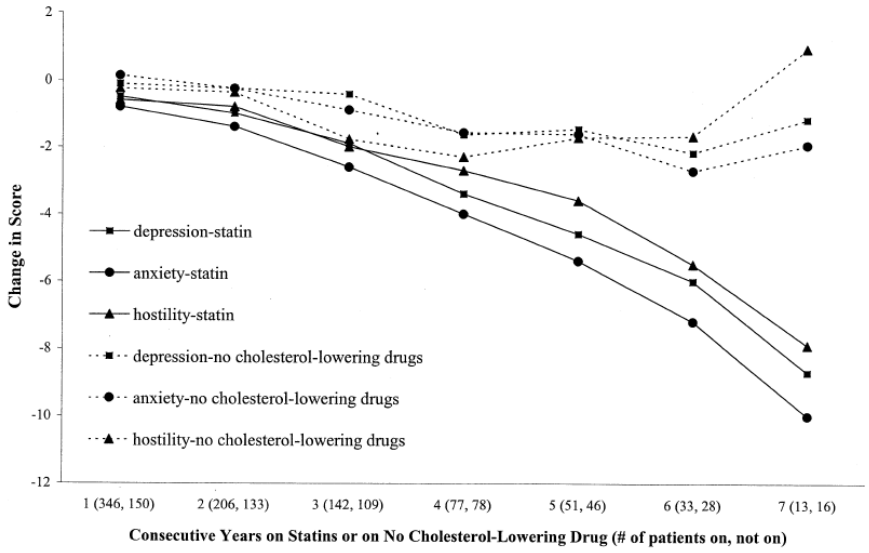


Figure 1.4 Psychological effects of statins compared with no-cholesterol lowering drug.

Although the statin safety profile on the whole is good, there are a number of statin-related events responsible for morbidity in older persons who are at increased risk of potential consequences. The benefit of drug therapy should always be balanced against the potential risks and the risks of adverse drug events minimized to the extent possible (Zarowitz, 2007) .

From a safety perspective, both research trial evidence and clinical practice experience have demonstrated that statins are generally well tolerated. However, as with all pharmaceuticals, safety considerations exist with both monotherapy and combination statin therapy, mainly involving potential adverse effects on muscle, liver, kidney, and the nervous system (Bays, 2006) .

Pleitropic effects of statins were investigated in literature and listed in Table 1.11.

Table 1.11 Pleitropic effects of statins

SUBJECT	RESULT	REFERENCE
Applications of statins in cardiothoracic surgery:more than just lipid-lowering	Statins may also play a role in heart valve surgery, lung transplantation, pulmonary resection and thoracic aortic aneurysm repair	Paraskevas (2008)
Statins—Treatment Option for Central Nervous System Autoimmune Disease?	Besides their lipid-lowering properties, statins exert pleiotropic immunomodulatory effects that may be of therapeutic benefit in CNS autoimmune disease.	Weber et al. (2007)
Statins in tumor suppression	There has been a dramatic influx of new information regarding the potential antitumor and chemopreventive effects of various statins.	Sassano and Platanias (2008)
Statins inhibit aminoglycoside accumulation and cytotoxicity to renal proximal tubule cells	The inhibition of the mevalonate pathway by statins may provide a potential therapeutic strategy to prevent AG-induced nephrotoxicity.	Antoine et al. (2010)
Pleiotropic effects of statin therapy:molecular mechanisms and clinical results	Statins effects include improving endothelial function,decreasing vascular inflammation, inhibiting smoothmuscle proliferation and immunomodulation.	Wang et al. (2007)
Statin-associated adverse effects beyond muscle and liver toxicity	Increased use and dose of statins and their over-the-counter availability in some countries more cases of other rare side effects may be seen in clinical practice.	Kiortsis et al.(2007)
Long-Term Statin Use and Psychological Well-Being	Long-term use of statins among patients with CAD appeared to be associated with reduced risk of anxiety, depression, and hostility.	Young-Xu et al. (2003)
Effects of statins vs. non-statin lipid-lowering therapy on bone formation	The lipophilic statin as moderate to high dose of simvastatin could be additive for the prevention of bone loss in hyperlipidemia patients.	Chuengsamarn et al.(2009)
Exploring new indications for statins beyond atherosclerosis: Successes and setbacks	Statins are effective, established therapy for the prevention of vascular events in patients at risk.	Water (2010)

Table 1.11 continued		
Comparison of biochemical effects of statins and fish oil in brain: The battle of the titans	Antioxidant, anti-inflammatory, and anti-apoptotic properties of statins and fish oil contribute to the clinical efficacy of treating neurological disorders with statins and fish oil	Farooqui et al. (2007)
Statin use and the risk of breast cancer	statin use was associated with a larger, significant increase in breast cancer risk in the subgroup of long-term HRT users.	Beck et al. (2003)
New insights into the pharmacodynamic and pharmacokinetic properties of statins	Statins may have direct effects on the arterial wall, which may contribute to their antiatherosclerotic actions.	Corsini et al. (1999)

1.2.2.4 The cost-effectiveness of statin use

The cost effectiveness of any intervention is defined by the ratio of the net health care resource use to the incremental health consequences.

Table 1.12 expresses the costs, according to the report of Heart Disease and Stroke statistics 2010 update of American Heart Association in USA.

Table 1.12 Estimated direct and indirect costs (in billions of dollars) of CVD and Stroke: US 2010

	Heart Diseases*	CHD	Stroke	Hypertensive Disease	HF	Total CVD†
Direct costs						
Hospital	$110.2	$56.6	$21.0	$8.5	$20.9	$1557
Nursing home	$24.7	$13.0	$17.1	$5.1	$47	$508
Physicians/other professionals	$24.7	$139	$3.8	$13.9	$2.5	$48.1
Drugs/other						
Medical durables	$21.5	$10.0	$1.3	$24.7	$3.2	$507
Home health care	$8.3	$2.5	$5.0	$2.7	$3.8	$18.8
Total expenditures†	$189.4	$96.0	$48.2	$54.9	$35.1	$324.1
Indirect costs						
Lost productivity/morbidity	$25.6	$11.3	$7.5	$9.0	...	$417
Lost productivity/ morbidity‡	$101.4	$69.8	$ 8.0	$12.7	$4.1	$137.4
Grand totals†	$3164	$177.1	$73.7	$76.6	$39 2	$503.2

*This category includes CHD, HF, part of hypertensive disease, cardiac dysrhythmias, rheumatic heart disease, cardiomyopathy, pulmonary heart disease, and other or ill-defined "heart" diseases.
† Totals do not add up because of rounding and overlap.
‡ Lost future earnings of persons who will die in 2010. discounted at 3%.

The total direct and indirect cost of cardio vascular diseases and stroke in the United States for 2010 is estimated at $ 503.2 billion as seen in Table 1.12. This table includes health expenditures (direct costs, which include the cost of physicians and other professionals, hospital and nursing home services, prescribed medications, home health care, and other medical durables) and lost productivity resulting from morbidity and mortality (indirect costs). Total hospital costs (inpatients, outpatients, and ED patients) projected for the year 2010 are estimated to be $155.7 billion. (Heart Disease and Stroke Statistics 2010 Update Report From the American Heart Association) .

All of the available statins are effective and safe, but they vary greatly in terms of cost-effectiveness. Fluvastatin has been determined to be a cost-effective therapeutic agent in the large proportion of the population with mild-to-moderate dyslipidemia who fit treatment guidelines of the National Cholesterol Education Program (NCEP). Atorvastatin and simvastatin are cost effective for the relatively smaller number of patients who require greater reductions in cholesterol (Farmer, 1998) .

According to result of the study carried out by American Heart Association in Dallas, 1997 cardiovascular disease-related expenditures remain a massive economic burden in the United States. Economic estimates have suggested that approximately $160 billion is expended in the United States on treatment and prevention of atherosclerosis-related complications (American Heart Assoc., Heart Disease and Stroke Statistics 1997 Update, Dallas).

Statin therapy compared with other lipid-lowering therapy statins are more cost-effective than bile acid resins and fibrates and equivalent to niacin. The cost-effectiveness of specific therapies varied according to patient population.

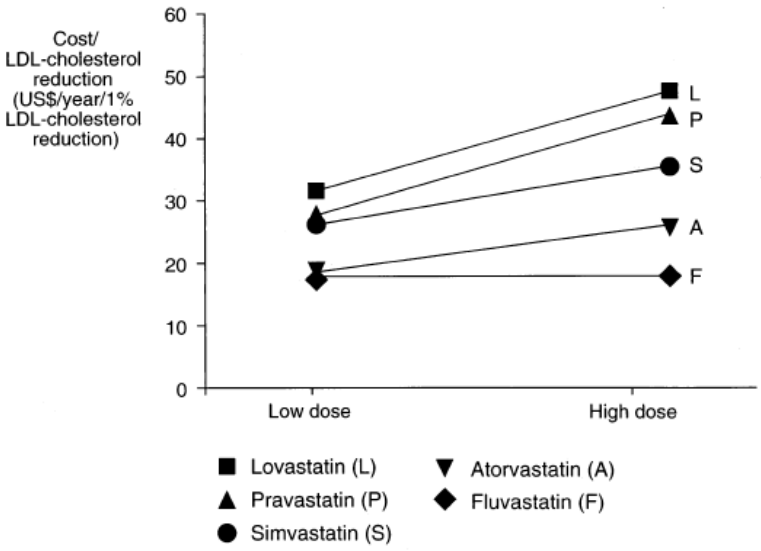


Figure 1.5 Effect of drug dosage on cost-effectiveness.

Usual low doses are 20 mg/day for fluvastatin, lovastatin, and pravastatin and 10 mg/day for simvastatin and atorvastatin. Higher dose represents doubling of the low dose. As seen in Figure 1.5 cost of 1 % LDL cholesterol reduction versus low and high doses of statins takes place.

Because of its lower acqusition cost (see in Table 1.13), fluvastatin is the most appoptriate choice for lipid lowering threaphy in these patients. Simvastatin and atorvastatin may be more appropriate in the relatively fewer number of patients who need greater reductions (30%) in LDL cholesterol (Figure 1.6). These important data can be helpful to managed care organizations and others in developing strategies to meet the NCEP goals among their patient populations and thus decreasing the burden of coronary artery disease (Farmer, 1998).

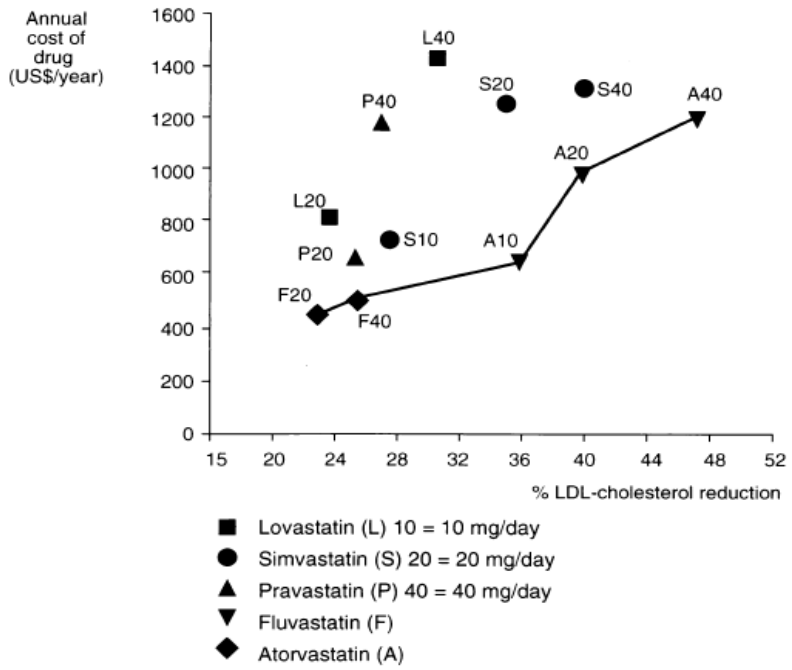


Figure 1.6 Cost-effectiveness of treatment with statins.

In Figure 1.6, y axis shows annual US dollar cost of drug versus at x-axis, expected low-density lipoprotein (LDL) cholesterol reduction. The line represents a cost efficiency frontier of the most cost-effective drug for a given level of desired LDL cholesterol reduction.

Table 1.13 Cost-effectiveness of treatment with statins

Drug/Dose (mg/day)	Annual Cost (US$/yr)	LDL-C Reduction (%)	Cost/LDL-C Reduction (US$/yr 1% LDL-C Reduction)
Fluvastatin			
20	439.20	23	19.09
40	489.60	26	16.83
Lovastatin			
20	610.00	24	33.75
40	1,458.00	30	48.60
80	2,916.00	40	72.90
Pravastatin			
20	709.20	25	28.37
40	1,195.20	27	44.27
Simvastatin			
10	730.80	28	26.10
20	1,274.40	35	36.41
40	1,324.80	40	33.12
Atorvastatin			
10	655.20	36	18.20
20	1,015.20	40	25.38
40	1,224.00	47	26.04

LDL-C= low-density lipoprotein cholesterol.

Costa 2008, investigated the cost effectiveness of rosuvastatin versus the other statins in patients with dislipidemia in British Columbia over a 1-year time horizon. As shown in Figure 1.7, x-axis is the percent LDL cholesterol reduction and the y-axis is the cost (Canadian $). According to this figure, the closer to the lower right corner, the greater the cost-effectiveness benefit (ie, the best efficacy at the lowest cost), since bigger LDL reduction smaller cost, that is the expected and wanted from a drug (Scharplatz, 2008).

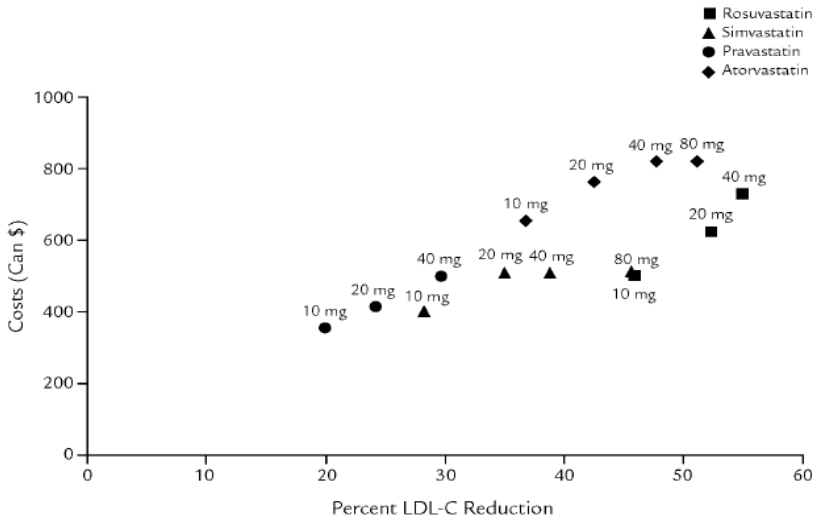


Figure 1.7 Mean annual costs and reductions in low-density lipoprotein cholesterol (LDL-C) per patient for all doses of the 4 most commonly used statins in British Columbia.

Figure 1.8 shows the CEAC (cost effectiveness acceptibility curve) for LDL-C reduction with 4 statins. The intersection of 2 lines denotes the monetary value at which the likelihood of being cost-effective passes from one statin to another. The threshold is interpreted as the point at which both therapies are optimal 50% of the time, based on a monetary value for each percent decrease in LDL-C. Rosuvastatin had the highest probability of being cost-effective compared with the other statins over a broad range of monetary values per unit of clinical effect (Scharplatz, 2008).

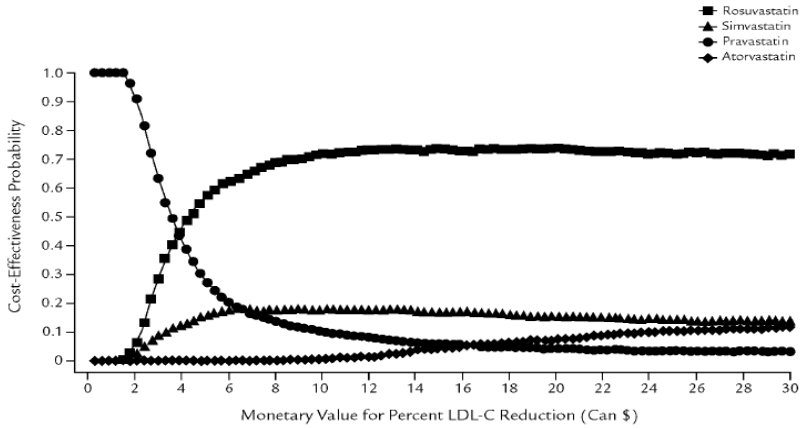


Figure 1.8. Cost-effectiveness acceptability curves for the 4 statins.

1.2.3 Pharmaceutical impurities and drug quality

Controlling and minimizing the side effects of drugs are the key issues in assuring the safety of drug therapy. Since side effects are inherent properties of the drug material, these cannot be influenced by drug analysts.

At the same time drug analysts play a predominant role in assuring the quality of bulk drug materials and drug formulations and this is also closely related to the safety issue. The three main attributes of drug quality are identity, strength and purity. Of these, in the case of bulk drug materials, purity is of prominent importance: by the identification (structure elucidation) and quantitative determination of the impurities and degradation products, the risk of their contribution to the side effect profile of the drug materials can be avoided or at least controlled/minimized (Görög, 2008).

References on drug quality were investigated in literature and given in Table 1.14 which is listed in terms of subject and the analytical methods used in the study. Impurity analysis and profiles effecting drug quality are carried out at different eqipments such as UV, HPLC or LC-MS.

Table 1.14 Drug quality studies in literature

SUBJECT	METHOD	RESULTS	REFERENCE
Active drug substance impurity profiling Part I. LC:UV diode array spectral matching	UV	The technique investigated, UV spectral matching, was found to be extremely powerful for monitoring the impurity profile of a drug substance.	Nicolas et al. (1998)
Determination of cholesterol-lowering statin drugs in aqueous samples using liquid chromatography–electrospray ionization tandem mass spectrometry	LC-MS	All of the statins were detected in an untreated sewage sample at 4–117 ng/ l and in a treated sewage sample at 1–59 ng/ l; but only atorvastatin was detected in a surface water sample at 1 ng/ l.	Miao and Metcalfe (2003)
Assuring quality of drugs by monitoring impurities	HPLC	New impurities may be observed as changes are made in the synthesis, formulation, or production procedures, albeit for improving them.	Ahuja (2007)
Impurity profile tracking for active pharmaceutical ingredients: Case reports	LC-MS	Impurity profile method based on screening various stationary phases and changing the pH of the mobile phase and elucidation of impurity structures through the utilization of LC–MS, preparative-LC and NMR is demonstrated	Zhou et al. (2007)
Pharmaceutical Impurities	UV	Different types and origins of impurities in relation to International Conference on Harmonization (ICH) guide-lines and, degradation routes, including specific ex-amples, are presented.	Roy (2002)
Quantitative analysis of lovastatin in capsule of Chinese medicine Monascus by capillary zone electrophoresis with UV–vis detector	UV	Capillary zone Electrophoresis (CE) is a powerful technique to determine lovastatin in the complex extract of the drug and fermentation.	Li et al. (2006)

Solubility of lovastatin in a family of six alcohols: Ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, and 1-octanol	HPLC	Solubility of lovastatin was butanol > 1-propanol > 1-pentanol > 1-hexanol > 1-octanol.	Chmielowski et al. (2008)
Fast analysis of pravastatin in production media	HPLC Capillary electrophoresis	The most sensitive was the method using particle column, followed by the system using monolithic column and the MECK method.	Kocijan et al. (2005)

1.2.3.1 Definition of pharmaceutical impurity

According to EMEA (European Medicine Agency) , any component of the new drug substance that is not the chemical entity defined as a new drug substance. Similarly, an impurity in a drug product is any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product. Monitoring of drug substance impurities is routinely accomplished using HPLC(Nicolas, 1998).

1.2.3.2. Classification of impurities

Medicines are the formulated forms of active pharmaceutical ingredients. There are 2 types of impurities in medicines:(1) Impurities associated in with active pharmaceutical ingredients and (2) Impurities that form are created during formulation and or with aging or that are related to the formulated forms. (http://www.aapspharmscitech.org)

The safety and quality of the drug substance and drug product in a generic product can be impacted by the presence of impurities. The nature and the quantity of these impurities is governed by a number of different factors, including synthetic route of the drug substance, reaction conditions, quality of the starting material of the drug substance, reagents, solvents, purification steps, excipients, drug product manufacturing processes, packaging, and storage of the end product (Basak et al., 2007) .

According to ICH (International Conference on Harmonization) guidelines, impurities associated with APIs (Active Pharmaceutical Ingredients) are classified into the following categories (Table 1.15) .

Table 1.15 Classification of impurities

Type of Impurity	Examples	Source
Organic	Starting materials, by-products, intermediates, degradation products, reagents, ligands, catalysts,	Organic impurities which cn arise during the manufacturincg process and / storage of the new drug substance.
Inorganic	Heavy metals, residual metals, inorganic salts, and other materials (e.g charcoal, filter aids)	Inorganic impurities which can result from synthetic process for the API.
Residual Solvent	ICH Class 1 Solvents e.g benzene, 1,2-dichloromethane, ICH Class 2 Solvents e.g. cyclohexane, methanol ICH Class 3 Solvents e.g acetone, ethanol. etc.	Inorganic and organic liquids which are used as vehicles for the preparation of solutions or suspensions in the synthesis of a new drug substance.

Impurities can also be formed by degradation of the end product during manufacturing of bulk drugs. However, degradation products resulting from storage or formulation to different dosage forms or aging are common impurities in the medicines (Swarbrick and Boylan, 1993). The structures, formulas and determination techniques of Atorvastatin Ca impurities are given in Appendix 1.

1.2.3.3 Effects of impurity control on drug quality

The control of pharmaceutical impurities is currently a critical issue to the pharmaceutical industry. Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during formulation, or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now getting important critical attention from regulatory

authorities. The different pharmacopeias such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly in corporating limits to allowable levels of impurities present in the Active Pharmaceutical Ingredients or formulations (Roy, 2002).

The safety of a drug product is dependent not only on the toxicological properties of the active drug substance itself, but also on the impurities that it contains. Therefore, identification, quantification, and control of impurities in the drug substance and drug product, are an important part of drug development and regulatory assessment

ICH Q3A (Impurities in New Drug Substances) and Q3B (Impurities in New Drug Products) address issues relevant to the regulation of impurities in the drug substance and drug product. While many of the concepts and principles outlined in these documents are applicable to Abbreviated New Drug Applications (ANDAs), certain additional or modified restraints need to be considered. When FDA receives an ANDA, a monograph defining certain key attributes of the drug substance and drug product is frequently available in the United States Pharmacopeia (USP). Sometimes, literature information on drug product impurities may also be available. These public standards and literature data play a significant role in the regulatory assessment process of an ANDA.

Identified specified impurities should be included in the list of impurities along with specified unidentified impurities that are estimated to be present at a level greater than the identification threshold given in Table 1.16. For impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the quantitation and/or detection limit of the analytical procedures should correspond to the level at which the impurities are expected to be controlled (Basak et al., 2007).

Table 1.16 Drug substance impurities thresholds

Maximum daily dose[a]	Reporting threshold[b c]	Identification threshold[c]	Qualification threshold[c]
≤2 g/day >2 g/day	0.053% 0.03%	0.10% or 1.0 mg/day intake (whichever is less)	0.15% or 1.0 mg/day intake (whichever is less) 0.05%

[a] The amount of drug substance administered per day.
[b] Higher reporting threshold should be scientifically justified.
[c] Lower threshold can be appropriate if the impurities are unusually toxic.

The acceptance criterion for impurities in the drug substance should be set no higher than the qualified level. The drug product specisification includes, where applicable, a list of the following types of degradation products: each specified identifed degradation product, each specified unidentified degradation product, any unspecified degradation product with an acceptance criterion of not more than the identification threshold in Table 1.17 and the total degaradation products (Basak et al., 2007).

Table 1.17 Thresholds for degradation products in drug products

Maximum daily dose[a]	Reporting threshold[b c]
≤1 g >1 g Maximum daily dose[a]	0.1% 0.005% Identification threshold[b c]
< 1 mg 1 mg 10 mg >10mg-2g >2g Maximum daily dose[a]	1.0% or 5 µg TDI, whichever lower 0.5% or 20 µg TDI. whichever is lower 0.2% or 2 mg TDI, whichever is lower 0.10% Qualification threshold[b c]
<10 mg 10 mg-100 mg >100 mg-2 g >2 g	1.0% or 50 µg TDI, whichever is lower 0.5% or 200 µg TDI, whichever is lower 0.2% or 3 mg TDI, whichever is lower 0.15%

[a] The amount of drug substance administered per day.
[b] Thresholds for degradation products are expressed either as a percentage of the drug substance or as total daily intake (TDI) of the degradation product Lower thresholds can be appropriate if the degradation product is unusually toxic.
[c] Higher thresholds should be scientifically justified.

1.2.3.4. Analytical methods

The safety and quality of the generic drug product/drug substance can be impacted by the presence of impurities. The nature and quantity of these impurities is governed by a number of factors, including the synthetic route of drug substance, reaction conditions, quality of the starting material, reagents, solvents, purification steps, and storage of the end product. As the structure of impurities are sometimes unknown, several spectroscopic and micro-chemical techniques have been developed which require minute quantities of material and readily enable the structural elucidation of the impurity. Versatile analytical methods are also available for the detection and monitoring of impurities in drug substances and drug products. The primary criterion of analytical methodology is the ability to differentiate the compounds of interests. The commonly used methods are separation (isolation) and detection and quantification (spectroscopic) in tandem.

The separation methods include thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE). HPLC is the most commonly used method for impurity monitoring in an inexpensive way. TLC can be used to separate a broad range of compounds. The primary difficulties related to the TLC method are limited resolution, detection, and ease of quantitation. Gas chromatography can provide the desired resolution, selectivity, and quantitation, unless the sample is not volatile. Capillary electrophoresis is a useful technique when very low quantities of samples are available and high resolution is required. Based upon these developments, it is now possible to replace all non-specific assay methods with highly specific and precise separation methods for assay and detection of impurities, thus greatly improving the value of the analytical determination in bulk drug materials.

The spectroscopic methods include ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS). Ultraviolet spectroscopy at a single wavelength provides minimal selectivity of analysis while the availability of diode array detectors offers much more information at various

wavelengths to ensure greater selectivity. Infrared spectroscopy provides specific information on some functional groups that may allow quantitation and selectivity. Nuclear magnetic resonance spectroscopy offers fairly detailed structural information on molecules and is a very useful method for characterization of desired product and associated impurities. Mass spectrometry which requires minute amounts of sample, provides excellent structural information based upon mass ion fragmentation patterns. Thus, UV, IR, NMR, and MS are excellent techniques for characterization and analysis of pharmaceutical compounds and impurities (Basak et al., 2007).

International Conference on Harmonization (ICH) introduced a number of guidelines on validation of analytical procedures in order to assist the pharmaceutical industry. These guidelines were written with separation methods such as HPLC chromatography in mind, and not spectroscopic ones such as NIR (Near Infrared). Since the introduction of these guidelines, NIR spectroscopy has been increasingly used in quality and process control in the pharmaceutical industry. For that reason, the Note for Guidance of NIR spectroscopy was rewritten in order to make it more appropriate to spectroscopic techniques. The ICH guidelines list seven validation characteristics that must be considered for all analytical methods: specificity, linearity, range, accuracy, precision (repeatability, intermediate precision and reproducibility), detection limit, quantification limit and robustness (Cruz et al., 2009).

Monitoring of drug substance impurities is routinely accomplished using HPLC. However, HPLC retention times can vary, resulting in uncertainty as to whether a peak at a new retention time is a new impurity. Because standards of the minor impurities (less than 0.1 % by area) are not usually available, some method is needed to characterize each of these peaks without isolating them. This on-line characterization might be accomplished using UV diode array spectral matching. This work sought to assess the sensitivity and selectivity of UV spectral matching for monitoring the impurity profile of drugs, using as an illustration DuP 941, an anti-cancer drug under development (Nicolas and Scholz,1998).

In order to detect accurately and to quantify (i.e., ‘‘profile’’) DRIs (Degradation Related Impurities), a stability indicating analytical method is needed. Ideally, such a method should resolve all DRIs from the parent and from each other, and should detect and accurately quantify all DRIs. The overall strategy for developing a stability-indicating method using stress testing has been expressed simply (Fig. 1.9). This strategy involves:

identifying likely or ‘‘potential’’ DRIs (Degradation Related Impurities) through stress-testing studies using highly-resolving or ‘‘discriminating’’ methods;

determining which of the potential DRIs (Degradation Related Impurities) are relevant (i.e., those DRI’s that form during accelerated stability and longterm stability studies);

developing ‘‘focused’’ stability indicating impurity methods (methods that resolve and detect the DRIs (Degradation Related Impurities) relevant to real-world handling and storage conditions); and,

quantifying DRI (Degradation Related Impuritiy) levels via long-term, formal stability studies, and establishing specifications, storage conditions, and shelf-life.

These studies are typically performed on both the drug substance and the formulated product (Baertschi,2006).

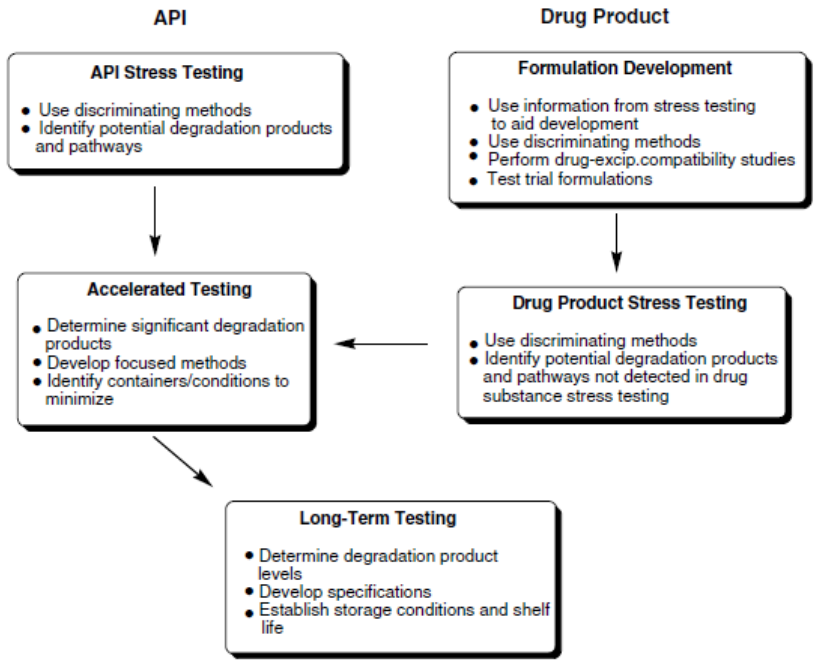


Figure 1.9 Overall strategy for the prediction, identification and control of stability-related issues.

The use of chromatographic methods such as thin-layer chromatography (TLC), gas-chromatography (GC), analytical and preparative high-performance liquid chromatography (HPLC), spectroscopic methods such as mass spectrometry (MS) and NMR spectroscopy as well as hyphenated techniques (HPLC/diode-array UV, GC/MS and HPLC/MS) might be used for impurity profile (Görög et al.,1996).

A simple high-performance liquid chromatographic (HPLC) method was developed for the analysis of atorvastatin (AT) and its impurities in bulk drug and tablets. This method has shown good resolution for AT, desfluoro-atorvastatin (DFAT), diastereomer-atorvastatin (DSAT), unknown impurities and formulation excipients of tablets. A gradient reverse-phase HPLC assay was used with UV detection.

Drug quality in Atorvastatin Ca tablets was investigated in some references previoulsy, in Table 1.18.

Table 1.18 Drug quality researchs on Atorvastatin Ca tablets

SUBJECT	METHOD	RESULT	REFERENCE
Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets	UPLC	Determination of six compounds (ATO, FEN, FEN-A, FEN-B, FEN-C and A-LCT) in tablets with excellent selectivity, precision and accuracy.	Kadav and Vora (2008)
An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets	HPLC	AT, desfluoro-atorvastatin (DFAT), diastereomer-atorvastatin (DSAT), unknown impurities and formulation excipients of bulk drug and the tablets.	Erturk et al. (2003)
Isolation and structure determination of oxidative degradation products of atorvastatin	HPLC-UV LC-MS	Partially degraded substance atorvastatin calcium four main oxidative degradation products were detected.	Kracun et al.(2009)

1.2.3.5. HPLC

High-performance liquid chromatography (HPLC), together with its derivative techniques, is today the dominant analytical separation tool in many industries. It is used extensively in the pharmaceutical industry in applications ranging from content uniformity assays to pharmacokinetic studies. The chemical industry relies on HPLC in the quality control of raw materials, intermediates and finished products.

In liquid chromatography, the mobile phase is a liquid, while the stationary phase can be a solid or a liquid immobilizer on a solid. High-performance liquid chromatography comprises all liquid chromatographic techniquess that require the use of elevated pressures to force the liquid through a packed bed of the stationary phase. It is therefore also often called high-pressure liquid chromatography (Neue, 1997).

The traditional form of liquid chromatography employed a polar adsorbent such as silica or alumina, and a nonpolar mobile phase based on hydrocarbons such as petrol ether or chlorinated hydrocarbons such as chloroform. This type of chromatography is known as normal-phase chromatography, in contrast to reversed-phase chromatography. It is also offen referred to as adsorption chromatography. It is based on the interaction of the polar functional groups of the analytes wtih polar sites on the surface of the packing. Normal-phase chromatography has lost its initial importance and has been eclipsed by reversed-phase chromatography.

In reversed-phase chromatography, a nonpolar stationary phase is used in conjunction with polar, largely aqueous mobile phases. Between 70 and 80 % of all HPLC applications utilize this technique. Its popularity is based largely on its ease of use; equilibraion is fast, retention times are reproducible, and the basic principles of the retention mechanism can be understood easily. Most stationary phases are silice-based bonded phases, but polymeric phases, phases based on inorganic substrates other than silica, and graphitized carbon have found their place as well. Some applications, such as protein separations, require a surface less hydrophobic than tat of reversed-phase packings. Proteins are denatured by the aqueous-organic mobile phases commonly used in reversed-phase chromatography. If the hydrophobicity of the stationary phase is reduced significantly, proteins can be eluted with water or dilute buffer as eluent. In this technique, termed hydrophobic interactioon chromatography, the analytes are typically adsorbed onto the packing in a buffer with a high salt concentration, and eluted with a buffer of low ionic strength. This technique can be viewed as an extension of reversed-phase chromatography.

In ion-exchange chromatography, the interaction of charged analytes with oppositely charged functional groups on the stationary phase is utilized. Elution is effected by either an increase of the ionic strength of the buffer, thus increasing the concentration of competing counterions, or through the change of pH, which can modify the charge of the analyte or of the ion exchanger. Strong and weak cation and anion exchangers are employed. The application range of ion-exchange chromatoghraphy is broad, covering organic and inorganic analytes. A large

application area is the separation of biopolymers, spcifically, proteins and nucleic acids.

In size-exclusion chromatography, the separation is based on the partial exclusion of analytes from the pores of the packing, due to the size of the analyte. It is used largely for the analysis or characterization of industrial polymers and biopolymers, but its separation range extends all the way down to oligomers. Known also as gel-permeation chromatography, it is one of the parent techniques of today's instrumental HPLC.

Hydrophilic interaction chromatography can be viewed as an extension of normal-phase chromatography to th realm of very polar analytes and aqueous mobile phases. Suitable stationary phases are the same as used in normal-phase chromatography. The most important application is the separation of sugars, oligosccharides, and complex carbohydrates.

True liquid-liquid partition chromatography is seldom used today. In this technique, the pores of a packing are filled with a liquid that is immiscible with the mobile phase. The preparation of the column is complicated, and it is difficult to maintain precise equilibrium conditions. The use of bonded phases has completely displaced classic partition chromatography. One can argue that reversed-phase chromatography or hydrophilic interaction chromatography rare versions of partition chromatography that use a very thin layer of stationary phase. True liquid-liquid partioning is briefly discussed as a special technique of normal-phase chromatography (Neue, 1997).

The use of a commercially available elevated pressure HPLC system has been shown to be beneficial in the pharmaceutical development area. In our hands we have been able to obtain faster analysis than that achieved with conventional HPLC, and without sacrificing separating power. Elevated pressure HPLC was shown to be capable of giving good retention time and peakarea precision, and has given comparable data to that from conventional HPLC (Wren, 2006).

1.2.3.6 UPLC- fast and accurate chromatography

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about ninefold decrease in analysis time as compared to the conventional HPLC system using 5 μm particle size analytical columns, and about threefold decrease in analysis time in comparison with 3 μm particle size analytical columns without compromise on overall separation (Kadav and Vora, 2008).

UPLC has been evaluated in terms of practical gain in speed and efficiency that can be achieved compared to current HPLC systems. (Villiers et al., 2004)

At Kadav, 2008 study, a stability indicating UPLC method was developed and validated for the simultaneous determination of atorvastatin, fenofibrate and their impurities in tablets. The chromatographic separation was performed on acquity UPLC^{TM} BEH C18 column (1.7 micron, 2.1mm×100mm) using gradient elution of acetonitrile and ammonium acetate buffer (pH 4.7; 0.01M) at flow rate of 0.5 ml/min. UV detection was performed at 247 nm. Total run time was 3 min within which main compounds and six other known and major unknown impurities were separated. Stability indicating capability was established by forced degradation experiments and separation of known degradation products. The method was validated for accuracy, repeatability, reproducibility and robustness. Linearity, LOD and LOQ was established for atorvastatin, fenofibrate and their known impurities. The results of method validation are given in Table 1.19

Table 1.19 Method validation results for assay of individual compound

Parameter	ATO	FEN
System precision[a] (%R.S.D.)	0.52	0.60
Tailing factor	1.15	1.18
Repeatability[b] (%assay)	99.9	100.4
Repeatability1 (% R.S.D.)	0.5	0.4
Intermediate precision[b] (%assay)	100J	100.1
Intermediate precision[c] (%R.S.D.)	0.6	0.7
Linearity[d] {correlation coefficient)	0.9992	0.9998
Linearity[d] (equation)	Y=3104x+385	Y= 2205x+9758
LOQ(µg/ml)	0.003	0.04
Accuracy[e] (%R.S.D.)	0.8	0.8
Accuracy[e] (%recovery)	100.4	100.0
Selectivity[f]	No interference	No interference
Stabi1ity-24h (%)[g]	99.9	100.1

[a] Determined on five replicate injections.
[b] Average of six determinations.
[c] Determined on six values.
[d] Six levels, from 50% to 150% of standard concentration.
e Determined at three levels (50%, 100% and 150%) with triplicate determination at each level.
[f] Demonstrated by forced degradation and separation of known degradation products.
[g] Correlation with freshly prepared sample (%).

A novel UPLC method was successfully developed and validated for simultaneous determination of ATO (atorvastatin), FEN (Fenofibrate) and impurities. The total run time was 3 min, within which both the drugs and their degradation products were separated. Method validation results have proved the method to be selective, precise, accurate, robust and stability indicating. Sample solution stability was established for determination of assay as well as impurities. As a result of this research, new method could be successfully applied for the routine analysis as well as stability study (Kadav and Vora.,2008).

1.2.3.7 Advantages of application of UPLC in pharmaceutical analysis

The advantages of UPLC are clearly obvious. The separation mechanisms is still the same, chromatographic principles are maintained while speed, sensitivity and resolution is improved. This all supports easier method transfer from HPLC to UPLC and its revalidation.

The main advantage was particularly a significant reduction of analysis time, which meant also reduction in solvent consumption. From this point of view, UPLC is more convenient for complex analytical determination of

pharmaceutical preparations. Analysis duration, solvent consumption and consequently analysis cost is a very important aspect in many analytical laboratories (Novakova,2005).

The UPLC method developed for the quantification of emodin, rhein, aloe-emodin, chrysophanol and physcion was found to be capable of giving faster analysis with good resolution than that achieved with conventional HPLC. The basic chromatographic conditions like stationary phase, solvents and UV detection, employed in HPLC were taken into account when developing the new UPLC method. The detection wavelength, type of stationary phase, buffer and solvent used in HPLC were kept constant (Wang et al., 2008).

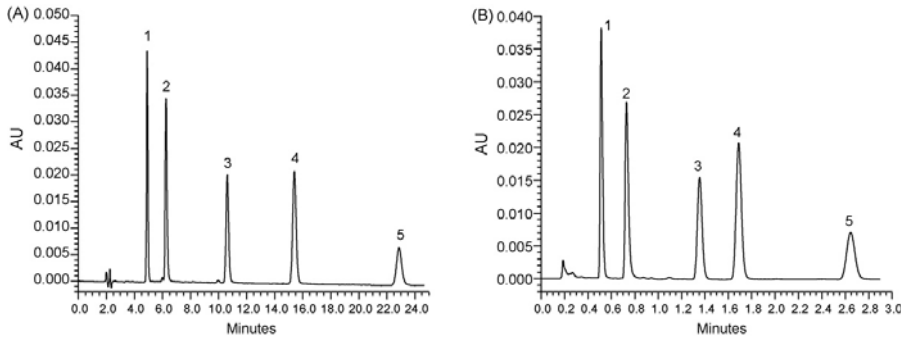


Figure 1.10 Comparison of chromatograms of mixed standard obtained from (A) HPLC and (B) UPLC. (1) aloe-emodin; (2) rhein; (3) emodin; (4) chrysophanol; and (5) physcion.

As a conclusion of Novakova's study in 2005, UPLC advantages are obvious. The comparison of data was made for four pharmaceutical analytical methods transferred between HPLC and UPLC. The separation mechanisms are still the same, chromatographic principles are maintained while speed, sensitivity and resolution is improved. This all supports easier method transfer from HPLC to UPLC and its revalidation. The main advantage was particularly a significant reduction of analysis time, which meant also reduction in solvent consumption. From this point of view, UPLC is more convenient for complex analytical determination of pharmaceutical preparations.

2. EXPERIMENTAL

The investigation of the impurity method validation of Atorvastatin Ca is a result of experimental data that was collected and compiled to form this thesis.

2.1. Materials and Methods

2.1.1 Standards and reactives

Standards

All standards were supplied from Ranbaxy Laboratories Limited.

Table 2.1 Standards used in the study

Atorvastatin Ca working standard
Atorvastatin Amid impurity standard
Desfloro Atorvastatin Ca impurity standard
Oxo Atorvastatin Ca impurity standard
Atorvastatin Methyl Esther impurity standard
Diastereomer Atorvastatin Ca impurity standard
3'-Deshydroksy Atorvastatin Ca impurity standard
O-methyl impurity standard
Atorvastatin lactone working standard
Ketal Atorvastatin Ca impurity standards

Reactives

Reactives used in the study are given in Table 2.2.

Table 2.2 Reactives used in the study

Water R (HPLC grade)
Acetonitrile R (HPLC grade, JT Baker)
Tetrahydrofouran (HPLC grade, Merck)
Methanol (HPLC grade, JT Baker)
Ammonium dihydrogen phosphate (HPLC grade, Merck)

2.1.2. Equipment used in method validation

Acquity UPLC pump, Acquity UPLC Tunable UV (TUV) detector, Shimadzu LC-2010C degassifier, Acquity UPLC Sample Organizer, Column manager, heater/cooler, binary solvent manager, and sample manager system and 2 µl loop, Acquity C18 100 mm x2.1 mm dimensions and 1.7 µm packed column used in Waters Acquity UPLC system. Mobile phases and samples were filtered by using Milipore Albet Schott Duran 0,2 µm filters before using at UPLC system.

Mettler Toledo ax105 Delta Range model balance used for weighing, Metrohm 654 pH meter and electrode used for pH measuring. Isolab A type pipets, Maxwell model ultrasound bath were used for preparation of mobile phases and samples. While preparing the solutions Labor branded magnetic stirers were used.

Equipment	: UPLC (Waters Acquity)
Detector	: TUV
Column	: Acquity C18 100 mm x2.1 mm, 1.7 µm
Column Temperature	: 30 ºC ± 1 ºC
Tray Temperature	: 5 ºC
Flow	: Gradient (0.375 ml/min)
Wavelength	: 246 nm
Injection Volume	: 1.7 µl
Injection technique	: Partial loop with needle overfill (PLUNO)
Duration	: 25 min
Solvent	: ACN: Water (60:40) (h / h)
Mobile Phase	:Mobile Phase A = ACN:THF (v/v) (925:75) Mobile Phase B = Phosphate Buff: Mobile Phase A (v/v) (580:420)

	Mobile Phase C= Phosphate Buffer: Mobile Phase A: Methanol (100:100:300)
Blank	: 10 mL methanol in a 100.0 mL volumetric flask with solvent.

Photographs of Acquity UPLC which is used in this analytical method validation study are shown in Figure 2.1 to Figure 2.10

Figure 2.1. Picture of Waters Acquity UPLC 1 (Ultra Performance Liquid Chromatography)

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Figure 2.2. Binary Solvent Manager equipment on Acquity UPLC

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Figure 2.3. UV Tunable Detector Part of Acquity UPLC

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Figure 2.4. Column Manager out and detector in

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2.1.3. Solutions used in impurity method of Atorvastatin Ca tablets

Gradient Reverse Phase UPLC method was used for determination of impurities in Atorvastatin Calcium Tablets. Impurity method validation is carried out according to ICH Q2(R1) Validation of Analytical Procedures guideline. Solutions and the preparation of these solutions in the method are tabulated below.

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2.1.3.1. Preparation of system suitability solution

Table 2.3 Preparation of System Suitability Solution

Used Standards	Amount (mg)	Volume (mL)
Atorvastatin Ca	25	50 ml Volumetric flask + dissolve with 5 mL methanol in an ultrasound bath, add solvent up to volume line.
Diastereomer Atorvastatin Ca Impurity	2.0	
Atorvastatin Lacton	2.0	

2.1.3.2. Preparation of standard solution (0.005 mg Atorvastatin/ mL)

Table 2.4 Preparation of Standard Solution

Used Standards	Amount (mg)	Volume (mL)
Atorvastatin Ca	50	100 ml Volumetric flask + dissolve with 10 mL methanol in an ultrasound bath, add solvent up to volume line. Dilute 5 ml of this soultion into 50 ml two times.

2.1.3.3. Preparation of sample solution (0.5 mg Atorvastatin Ca /mL)

Table 2.5 Preparation of System Suitability Solution

Used Sample	Amount (mg)	Volume (mL)
Atorvastatin Ca Tablets (powder of 20 weighed tablets)	775 mg (= 50 mg Atorvastatin	100 ml Volumetric flask + dissolve with 10 mL methanol in an ultrasound bath, add solvent up

	Ca)	to volume line.

2.1.3.4. Preparation of plasebo solution

Table 2.6 Preparation of Plasebo Solution

Used Plasebo	Amount (mg)	Volume (mL)
Atorvastatin Ca Tablets Plasebo	725 mg (= amount included one tablet)	100 ml Volumetric flask + dissolve with 10 mL methanol in an ultrasound bath, add solvent up to volume line.

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2.1.3.5. Preparation of impurity stock solutions used in validation

Atorvastatin Amide, Desfloro Atorvastatin Ca, Oxo Atorvastatin Ca, Diastereomer Atorvastatin Ca, O-metil Atorvastatin Ca, 3'-Deshydroxy Atorvastatin Ca, Atorvastatin Lactone, Atorvastatim Methyl Ester, Ketal Atorvastatin impurity standards were weighed approximately 5 mg and prepared separately into 100 mL volumetric flask and dissolved with 10 mL methanol in an ultrasound bath, added solvent up to volume line.

2.1.3.6. Preparation of spike sample solution used in validation

775 mg (= 50 mg Atorvastatin Ca) Atorvastatin Ca Tablets (powder of 20 weighed tablets) were wieghed and put into 100 ml Volumetric flask and dissolved with 10 mL methanol in ultrasound bath, and 5 ml of each impurity stock solution was added into the flask and solvent was added up to volume line. This solution was filtered using 0.2 µm filters.

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2.1.3.7. Preparation of impurity solutions at limit concentration

5 mL of (each impurity stock solutions) Atorvastatin Amide, Desfloro Atorvastatin Ca, Oxo Atorvastatin Ca, Diastereomer Atorvastatin Ca, O-metil Atorvastatin Ca, 3'-Deshydroxy Atorvastatin Ca, Atorvastatin Lactone, Atorvastatin Methyl Ester, Ketal Atorvastatin impurity stock solutions diluted separately with solvent in 100 mL volumetric flasks. Filtered using 0.2 µm filters.

2.2. Impurity Validation Method

Gradient Reverse Phase UPLC method was used for determination of impurities in Atorvastatin Calcium Tablets. Impurity method validation is carried out according to ICH Q2(R1) Validation of Analytical Procedures guideline as shown in Figure 2.5.

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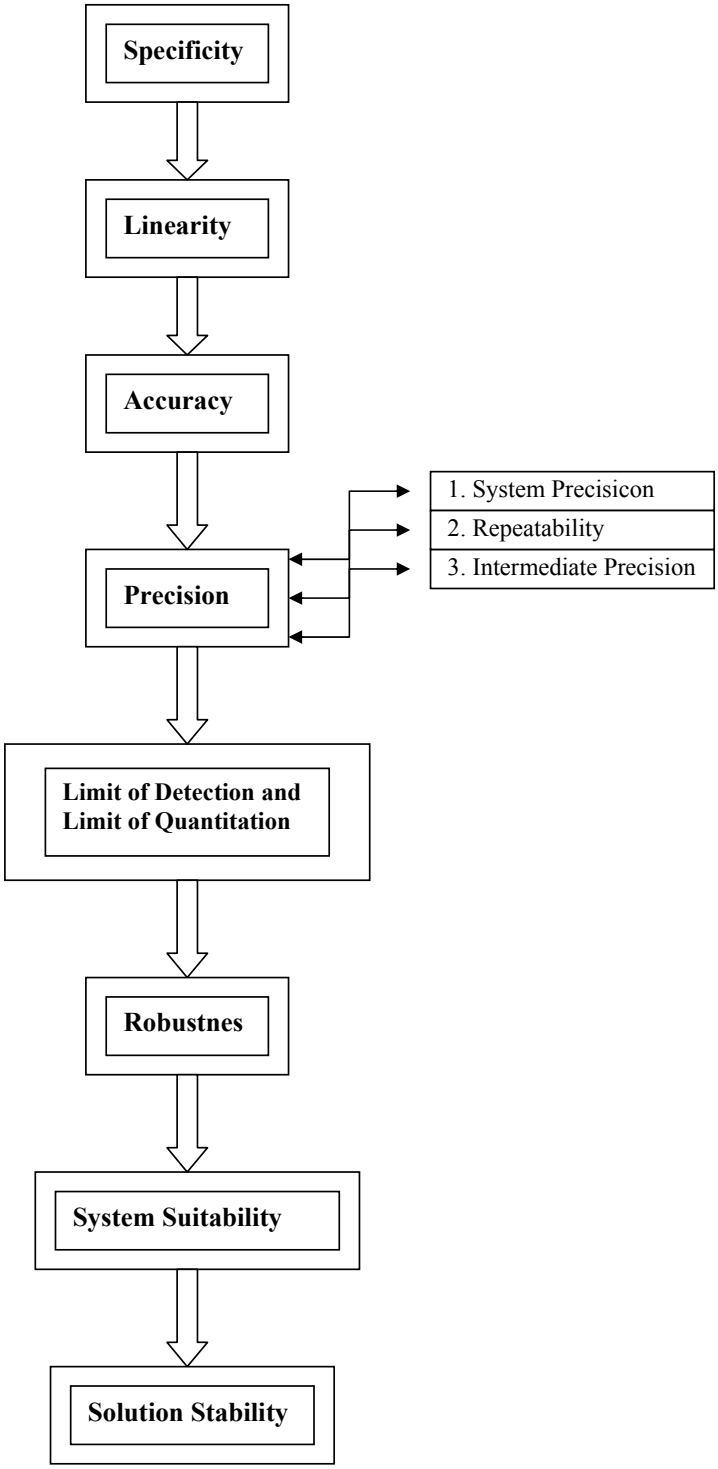
Specificity
Linearity
Accuracy
Precision
1. System Precisicon
2. Repeatability
3. Intermediate Precision
Limit of Detection and
Limit of Quantitation
Robustnes
System Suitability
Solution Stability

Figure 2.5 Analytical Method Validation Procedure according to International Conference on Harmonization (ICH)

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2.2.1 Specificity

Experimental explanation: Impurity Method was created in Empower software and uploaded to UPLC system. After pressure, temperature and flow controls were done, if conditioning and equilibration were achieved, analysis could be started.

System suitability solution, blank, plasebo solution, standard solution, impurity standard solutions at limit concentration, sample solution, spike sample solution injections were done according to impurity method.

Acceptance Criteria:

System suitability test should be successful. Second step, impurity peaks should be seperated from one another, Atorvastatin Ca, plasebo, blank peaks. Also, peak purity investigated by UPLC software.

2.2.2 Linearity

Experimental explanation: Nine Atorvastatin Ca standard solutions at different concentration which are in the concentration range between 0.1 % and 1.2 % are prepared for unknown impurities. Six Atorvastatin Ca standard solutions at different concentration which are in the concentration range between 0.1 % and 1.2 % are prepared for known impurities. 100 % Standard solution is injected three times and system precision must be evaluated. By using linearity data results, correlation coeffients for known impurities are also calculated.

*: Known impurities (Atorvastatin Amid, Desfloro Atorvastatin Ca, Oxo Atorvastatin Ca, Diastereomer Atorvastatin Ca, O-metil Atorvastatin Ca, 3'-Deshidroksi Atorvastatin Ca, Atorvastatin lakton, Atorvastatin Metil Ester, Ketal Atorvastatin Ca).

Acceptance criteria: Correlation coefficient (r) must be greater than 0.975 according to ICH.

Residual sum of squares (RSS) should be reported and ratio of intercept point on y axis to area at 100 % concentration should be lower than 5.0 %.

2.2.3 Accuracy

Experimental explanation: Sample preparation is applied for three concentrations which are 100 %, 120 % and LOQ level concentrations. Three samples at each concentration have to be prepared and injected only one time, and percentage recovery should be calculated.

Samle preparations: 725 mg plasebo was weighed into 100 ml volumetric flask, solved by 10 ml methanol, then 10 mL of Atorvastatin Ca stock solution was added onto the plasebo, completed with solvent up to volume line and filtered by using 0.2 µm filter. (0.005 mg Atorvastatin /mL). The same procedure is applied once again but not 10 mL, 12 ml of Atorvastatin Ca stock solution added. (0.006 mg Atorvastatin /mL).

50 mg Atorvastatin Ca and 725 mg plasebo should be weighed in a volumetric flask, solved with 10 ml methanol then 5 ml from each impurity stock solution is taken into the flask. (0.5 mg Atorvastatin/mL, 0.0025 mg Impurity /mL). This operation is done once again taking by 6 ml from each impurity stock solution. (0.5 mg Atorvastatin/mL, 0.003 mg Impurity /mL).All samples should be prepared also at the LOQ concentration.

Acceptance Criteria:

Recovery for each sample should be in the range of 90.0 % - 110.0 % and for at LOQ concentrations, recovery should be in the range of 70.0 % -130.0 %.

2.2.4 Precision

2.2.4.1 <u>System precision</u>

Experimental explanation: Atorvastatin Ca standard solution must be injected three times consecutively.

Acceptance criteria: Relative standard deviation must be lower than 5.0 % (RSD ≤ 5.0 %)

2.2.4.2 Repeatability

At 100 % concentration, six Atorvastatin Ca sample solutions should be prepared and one injection of each solution must be done. Impurities at limit concentration must be added to sample which does not include impurity. The amount of impurity must be calculated in the sample that includes impurities, and afterwards impurity must be added into the solution to be able to achieve the limit concentration. Also, a drug product sample not including impurity should be prepared. Known impurities are calculated by using percentage recovery. Unknown impurities are calculated by the help of standard solution as mentioned in the calculation section of method.

Acceptance criteria: Relative standard deviation must be lower than 5.0 % (RSD ≤ 5.0 %)

2.2.4.3 Intermediate precision

Experimental explanation: At 100 % concentration six different solutions should be prepared and analysed by two analysts by injecting one time. Impurities at limit concentration must be added to sample which does not include impurity. The amount of impurity must be calculated in the sample that includes impurities, and afterwards impurity must be added into the solution to be able to achieve the limit concentration. Also, a drug product sample not including impurity should be prepared. Known impurities are calculated by using percentage recovery. Unknown impurities are calculated by the help of standard solution as mentioned in the calculation section of method.

Acceptance Criteria: The difference between the averages of two different studies;

- % Each impurity and Total Impurity — Difference

<0,1	ignore
0,10 – 0,20	≤±0,05
>0,20 – 0,50	≤±0,10
>0,50 – 1,0	≤±0,20
>1,0	≤±20%

2.2.5 Limit of detection (LOD) and limit of quantitation (LOQ)

Experimental explanation: Determination curve obtained by linearity study is used to calculated LOD and LOQ. Calculation method is given below. After the determination of LOQ, it should be validated by analysing three sample solutions.

Acceptance Criteria:

LOQ must be higher than reporing level fort he impurity or degradation product.

Percentage recovery must be in the range of 70 %- 130 % for LOQ .

$$\mathbf{LOD} = \frac{3.3 * \sigma}{S} \qquad \mathbf{LOQ} = \frac{10 * \sigma}{S}$$

σ = Standard deviation (deviation of y-axis interception point at regression curve)

S = Slope of regression curve

2.2.6 Robustness

Experimental explanation: One injection from system suitability solution, consecutive three injection of standard solution must be done and system suitability parameters investigated.

- Column temperature 25 °C and 35 °C were adjusted, and system suitability parameters were investigated.

- Flow was changed at ± %10.0 and system suitability parameters were investigated

- Wave length changed to 241 nm and 251 nm and system suitability parameters were investigated.

- The percentage of organic solvent in mobile phase was changed ± % 2.0 and system suitability parameters were investigated.

Acceptance criteria: Resolution between Atorvastatin Ca and Diastereomer Atorvastatin should be higher than 1.5.

Column efficiency for Atorvastatin Ca peak, must be higher than 1000 theoretical plate and tailing factor can be maximum 1.5.

2.2.7 Solution stability

Experimental explanation: Atovastatin Ca standard solution must be prapared fresh and analysed according to certain time intervals. Drug product sample is prepared by adding impurities at limit concentrations. Samples must be compared freshly prepared standard and be calculated according to standard.

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3. RESULTS AND DISCUSSION

The investigation of the impurity method validation of Atorvastatin Ca is a result of experimental data that was collected and compiled to form this thesis. The discussion is the result of this data.

3.1 Method Transfer from HPLC to UPLC

The main target of the chromatographic method was to achieve separation of impurities and main component Atorvastatin Ca. Some impurities of atorvastatin are degradation products and exists in bulk drug sample. During LC developments study, it was observed that Diastereomer Atorvastatin Ca impurity closely eluting with atorvastatin, while the others have bigger relative retention time. The response of impurities and atorvastatin ca was found to be adequate at 246 nm.

The chromatographic separation was achieved ACE C18, 250 x 4.6 mm, 5 µm column maintained at 25 ^{o}C. In an isocratic mode using a mobile phase consisting of buffer (phosphate buffer pH=3 and acetonitrile in the ratio (53:47 , v/v) , there was a good separation between the atorvastatin lactone, atorvastatin amide, atorvastatin methyl ester , 3'-deshydroxy Atorvastatin Ca, Ketal Atorvastatin Ca impurities, while the analysis time was 60 min. To reduce the run time and achieve beter peak shape, it was decided to switchover to gradient mode at UPLC. The basic chromatographic conditions like stationary phase, solvents and UV detection, employed in HPLC were taken into account while developing the new UPLC method. The detection wavelength and solvent used in HPLC were kept constant. The stationary phase C18 was chosen in order to have similar chemistry as that used in the HPLC. Acquity BEH C18, 100x2.1 mm, 1.7 µm column was employed for the separation. The injection volume was scaled to 1.7 µl from 20 µl as used in HPLC. The flow rate was scaled to 375 µl while HPLC

analysis flow rate was 1ml/min with approximayely 3000 psi backpressure. Taking in to account the capability of high operating pressure of UPLC, flow rate was chosen 0.375 ml/min with a back pressure of 13,500 psi. At this flow rate, the run time was decreased to 25 min without affecting the separation of atorvastatin and its impurities. (Table 3.1)

Table 3.1 Comparison of HPLC and UPLC Methods

Parameters	**HPLC Method**	**UPLC Method**
Column Type	ACE C18 5 µm, 250 x4.6 mm	Acquity C18 1.7 µm (100x2.1 mm)
Column Temperature	25 ^{o}C	30 ^{o}C
Tray Temperature	At room temperature	5 ^{o}C
Injection Volume	20 µl	1.7 µl
Wavelength	246 nm	246 nm
Run Time	60 min	25 min
Retention Time for Atorvastatin	18 min	6 min
Flow rate	1 ml/min	0.375 ml/ min
Equipment back pressure	3000 psi	13 000 psi
Mobile Phase	Isocratic Flow (MF) Mobile Phase (MF) = Phosphate Buffer pH = 3: ACN (v/v) (53:47)	Gradient Flow (MF B+ MF C) Mobile Phase B (MF B) = Phosphate Buffer: Mobile Phase A (580:420) (h/h) Mobile Phase C (MF C) = Phosphate Buffer: Mobile Phase A: Methanol (100:100:300) (v/v/v) Mobile Phase A = ACN: THF (v/v) (925:75)
Impurities	Atorvastatin Lactone Atorvastatin Amide Atorvastatin Methyl ester 3'-Deshydroxy Atorvastatin Ca Ketal Atorvastatin Ca	Atorvastatin Amide Desfluoro Atorvastatin Oxo Atorvastatin Ca Diastereomer Atorvastatin Ca O-Methyl Atorvastatin Ca 3'-Deshydroxy Atorvastatin Ca Atorvastatin Methyl ester Atorvastatin Lactone Ketal Atorvastatin Ca
Total Analysis Time for one batch	**430 min**	**175 min**

Total Mobile Phase Consumption for one batch analysis	**500 ml**	**70 mL**

3.2 Comparison Study of Chromatographic Performance

A comparative data on chromatographic performance of HPLC and UPLC has been obtained by injecting a solution of Atorvastatin Ca and impurities (impurities 50 µg/ml each, Atorvastatin Ca 5 µg/mL). The performance parameters are shown in Table 3.2. It is observed that the elution time of Atorvastatin Ca in UPLC was reduced by 3-fold to that of ioscratic mode of HPLC. The resolution and theoretical plates obtained for Atorvastatin Ca in UPLC showed comparatively better separation efficiency than HPLC. The new UPLC method has some advantages compared with the HPLC impurity method which was developed by S. Ertürk,et al., 2003. S. Ertürk and colleauges developed a method for the analysis of atorvastatin (AT) and its impurities in bulk drug and tablets, and this method had shown good resolution for AT, desfluoro atorvastatin (DFAT), diasteromer atorvastatin (DSAT), unknown impurities and formulation excipients of tablets. According to this method, retention time of Atorvastatin Ca is 30 minutes. It is observed that the elution time of Atorvastatin Ca in new UPLC method was reduced by 5-fold to that of Ertürk, et al.'s HPLC method. and also by the new UPLC method allow to identify and calculate nine of Atorvastatin Calcium impurities (Figure 3.1 and Figure 3.2).

Table 3.2 A comparison of system performance of HPLC and UPLC

Component	**Elution Time**		**Resolution**		**Tailing Factor**		**USP Plate count**	
	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC
Atorvastatin Ca	17.5	6.2	1.5	1.5	1.084	1.1	17383	20395

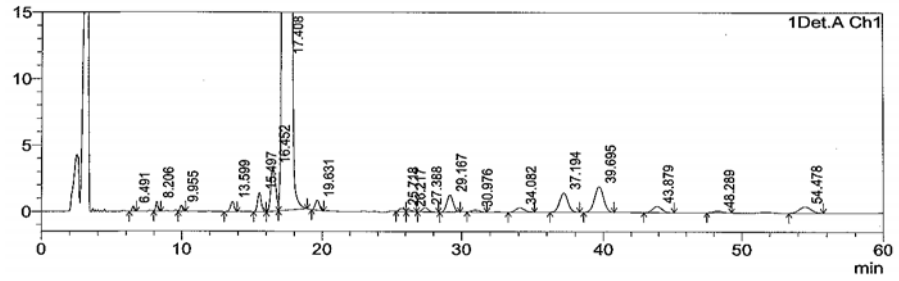


Figure 3.1 HPLC chromatogram of Atorvastatin Ca

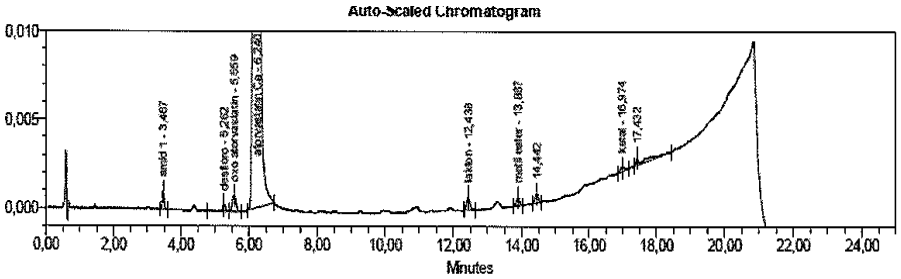


Figure 3.2 Atorvastatin Ca tablet analysis with UPLC method

3.3. UPLC Method Validation

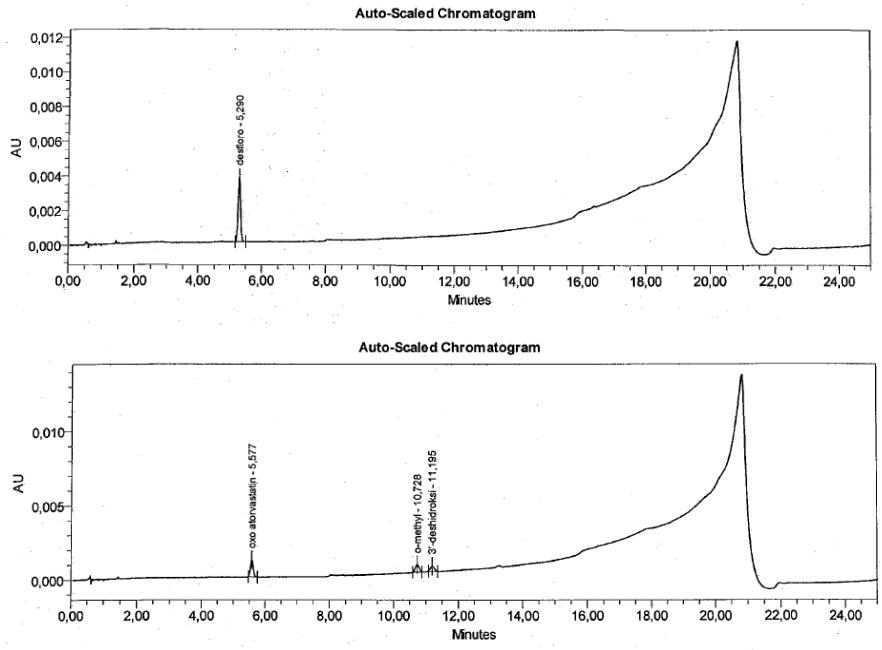
The validation study allowed the evaluation of the method for its sutability for routine analysis.

3.3.1 Specificity

It is the ability of analytical method to measure the analyte response in the presence of its potential impurities and degradents. The specificity of the UPLC method was determined by injecting individual impurity samples, wherein no interference was observed for any of the components (Figure 3.4) and Table 3.3. represents the relative retention times and purity results. Detailed results obtanined from analytical metod validation were expressed in tables and graphs in Appendix 2.

Table 3.3 Specificity parameters of spiked sample

	Retention Time (RT) min	Relative Retention Time (RRT)	Resolution	Purity
Atorvastatin Amid	3.5	0.56	-	Passed
Desfloro Atorvastatin Ca	5.3	0.85	13.8	Passed
Oxo Atovastatin Ca	5.6	0.90	1.8	Passed
Diastereomer Atorvastatin Ca	5.9	0.95	2.3	Passed
Atorvastatin Ca	6.2	1.00	1.8	Passed
O-Methyl Atorvastatin Ca	9.9	0.80	18.1	Passed
3'-Deshydroksy Atorvastatin Ca	11.7	0.94	2.6	Passed
Atorvastatin Lactone	12.4	1.00	3.3	Passed
Atorvastatin Methyl Ester	13.9	1.12	2.4	Passed
Ketal Atorvastatin	16.9	1.36	16.9	Passed



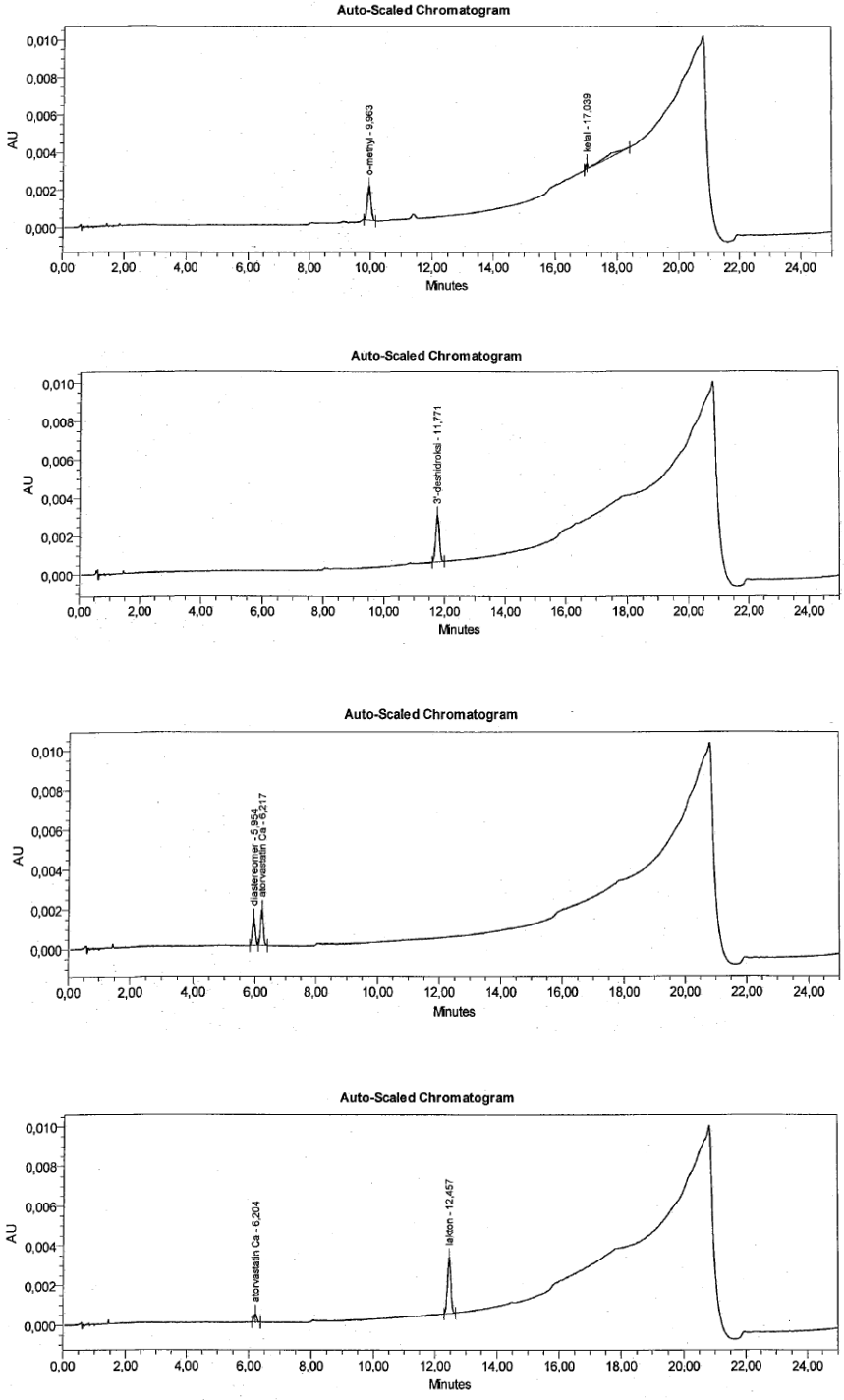
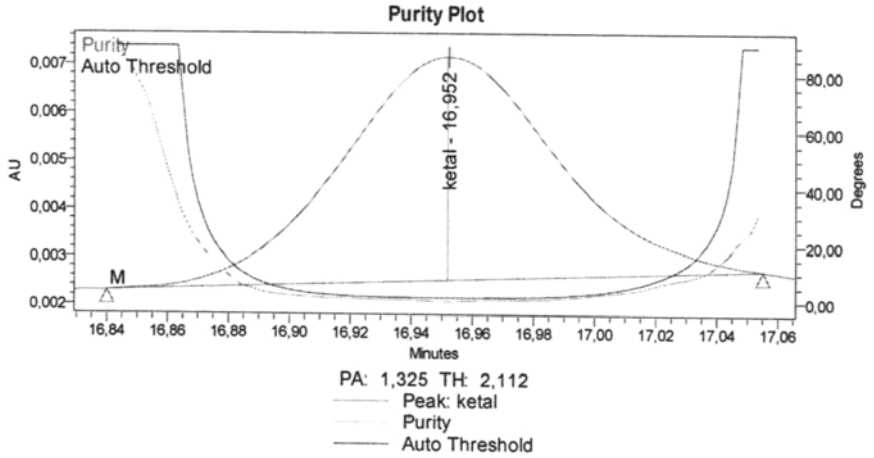


Figure 3.3 UPLC chromatograms of atorvastatin ca and its impurities as a specifity study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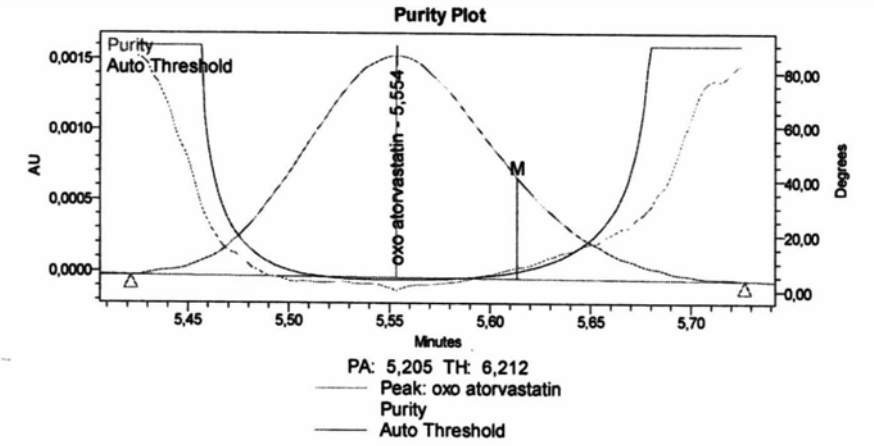
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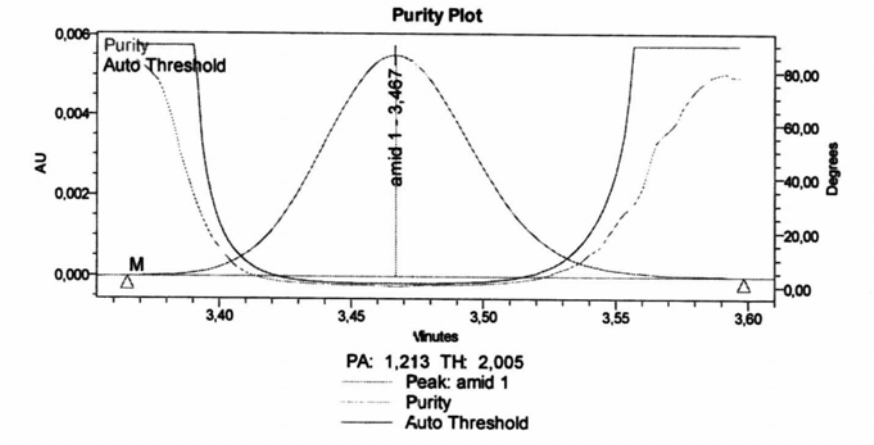
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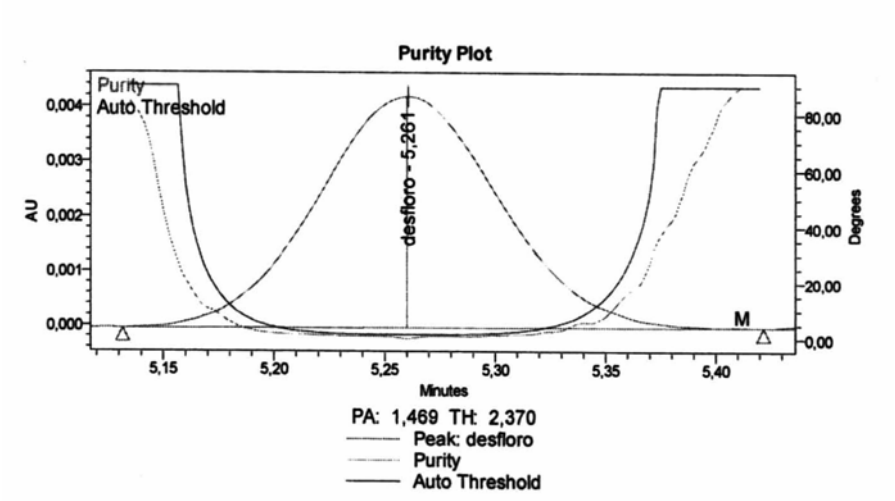
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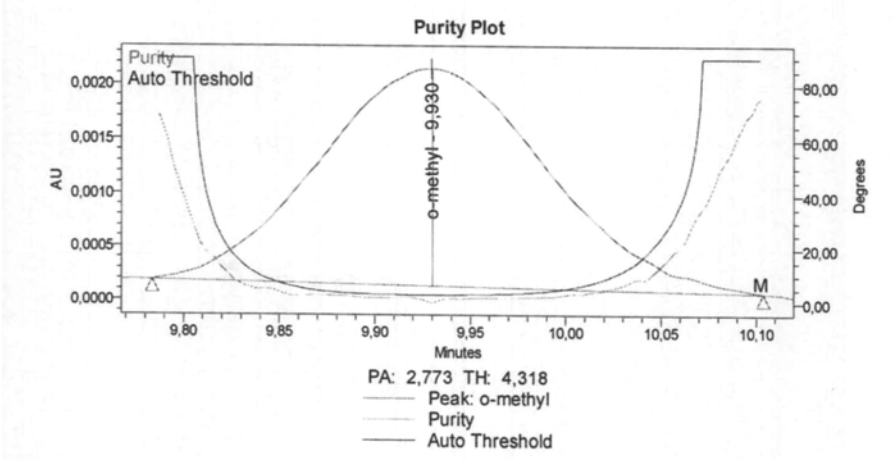
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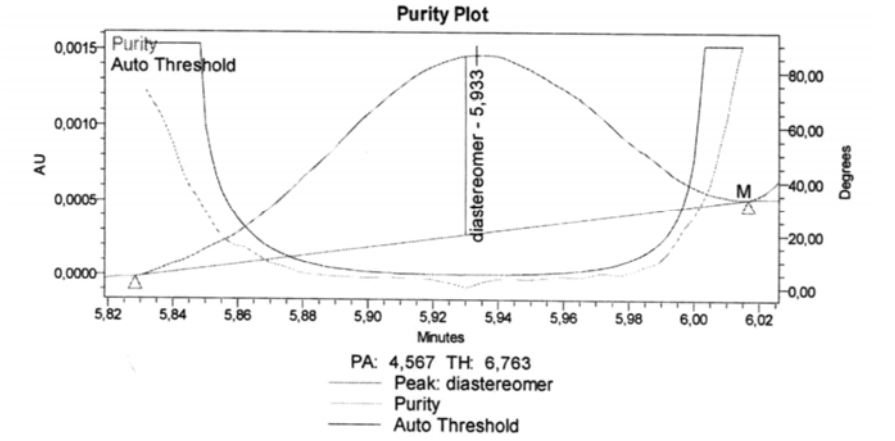
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Peak: diastereomer
Purity
Auto Threshold

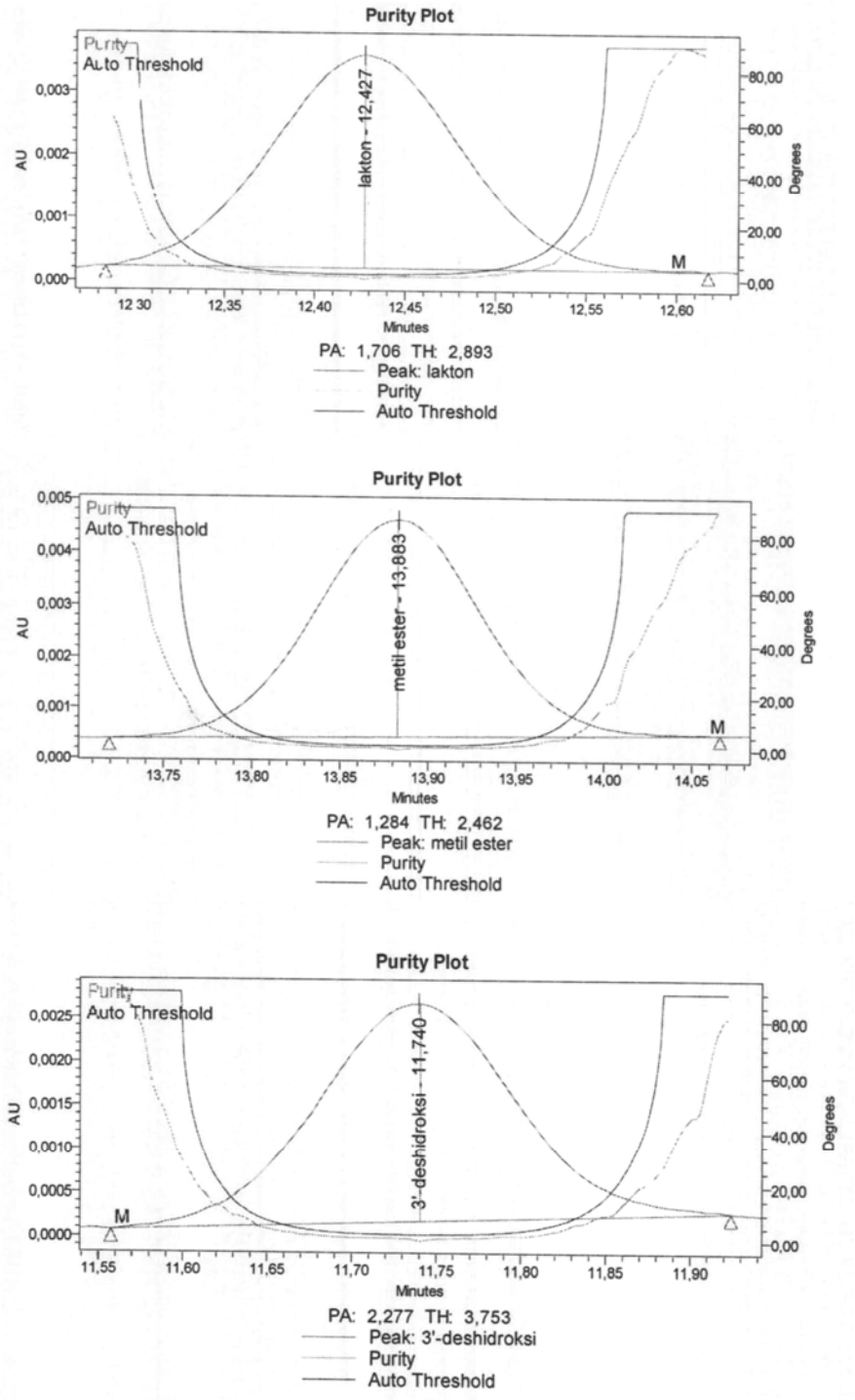


Figure 3.4 PDA Peak Purity Graphs for specifity study

The chromatograms were checked for the appearance of ay extra peak. Peak purity of these samples was verified using a PDA detector.(Figure 3.4) The purity of the principle and other chromatographic peaks was found to be satisfactory.

3.3.2 Linearity

Linear calibration plots for the related substance method were obtained over the calibration range (mimimum reporting limit to 120 %) at six concentratin levels in triplicate. All linearity study results and regression graphs are given in Appendix 2.

Table 3.4 Regression equations obtained from linearity study

Solutions	**Regression equation**	***Correlation Coefficient (R^2)***
Atorvastatin Ca	y= 10351881x- 405	*0.9999*
3'Deshydroxy Atorvastatin	y= 8425921x- 87.87	*0.9999*
Diastereomer Atorvastatin	y= 5507836x-10,	*0.9997*
Atorvastatin Amide	y=7418367x-1	*1.0000*
Desfluoro Atorvastatin	y= 8334978x-290	*0.9967*
Ketal Atorvastatin	y= 978978x+23	*0.9999*
Atorvastatin Lactone	y= 10499529x +76	*0.9999*
Atorvastatin methyl ester	y= 10770813x- 24	*1.0000*
O-methyl Atorvastatin	y= 7954946x-27	*0.9999*
Oxo-Atorvastatin	*y= 1254125x -129*	*0.9984*

The results showed excellent correlation between the peak area and concentration of impurities.

3.3.3 Accuracy

The accuracy of the method was determined for the related substance by spiking known amount of impurities in atorvastatin bulk sample (test preparation) in triplicate at levels, LOQ, 100%, 120 % of the specified limit. The recoveries of 3' Deshydroxy Atorvastatin Ca was calculated and given as an example, in Table

3.5 and the list of obtained recoveries for all impurities of Atorvastatin were tabulated in Table 3.6.

Table 3.5 3' Deshydroxy Atorvastatin Ca Recovery

Level (%)	**Theoretical concentration (mg/ml)**	**Experimental concentration (mg/ml) *10^{-6}**	**Recovery (%)**	**Recovery Average (%)**
LOQ	0.000242	256	105.8	101.7
	0.000242	226	93.4	
	0.000242	256	105.8	
100	0,002417	248.1	102,7	102,5
	0,002417	247.2	102,3	
	0,002417	248.1	102,7	
120	0,002900	294.9	101,7	101,8
	0,002900	295.4	101,9	
	0,002900	294.9	101,7	

Table 3.6 Recovery results of impurities

Level	**Impurity**	**Amount added (mg/ml) *10^{-6}**	**Amount recovered (mg/ml) *10^{-6}**	***Recovery %***
LOQ	Atorvastatin Amid	374	375	*100.8*
	Desfloro Atorvastatin	340	335	*97.6*
	Oxo Atovastatin	476	493	*100.9*
	Diastereomer Atorvastatin	323	351	*96.2*
	Ketal Atorvastatin	414	422	*102.9*
	O-Methyl Atorvastatin	393	487	*112.5*
	Methyl ester Atorvastatin	286	275	*97.3*
	3'-Deshydroksy Atorvastatin	242	256	*101.7*
	Atorvastatin Lactone	254	239	*99.7*
100 %	Atorvastatin Amid	242.7	244.1	*100.3*
	Desfloro Atorvastatin	231.2	267.3	*112.8*
	Oxo Atovastatin	235.1	231.6	*100.9*
	Ketal Atorvastatin	229.9	233.4	*101.6*
	O-Methyl Atorvastatin	196.7	201.2	*101.6*
	Methyl ester Atorvastatin	238.1	242.8	*101.7*
	3'-Deshydroksy Atorvastatin	241.7	248.1	*102.5*
	Atorvastatin Lactone	211.6	211.8	*99.7*

120 %	Atorvastatin Amid	291.2	244.8	*100.3*
	Desfloro Atorvastatin	277.5	314.3	*113.7*
	Oxo Atovastatin	282.1	281.2	*99.6*
	Diastereomer Atorvastatin	251.6	246.9	*97.7*
	Ketal Atorvastatin	275.8	276.5	*100.1*
	O-Methyl Atorvastatin	236	236.3	*100.3*
	Methyl ester Atorvastatin	285.7	286.6	*100.3*
	3'-Deshydroksy Atorvastatin	290	294.9	*101.8*
	Atorvastatin Lactone	*2539*	*0.002525*	*99,0*

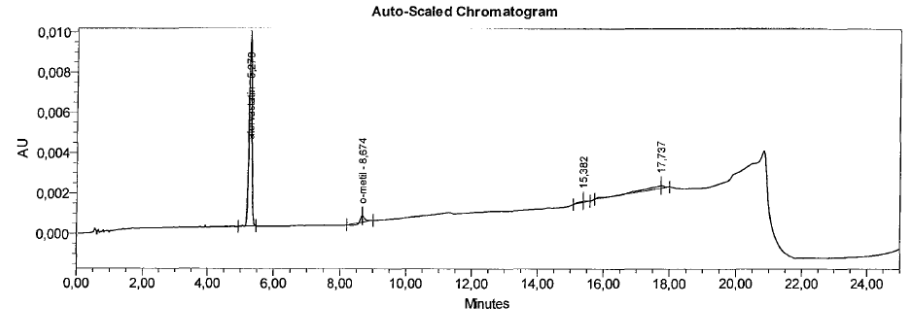
3.3.4 Precision

The precision (repeatability) of the impurity method was evaluated by carrying out six independent assays. System precision was evaluated with triplicate standard injections which were given in Figure 3.5 according to the area of the Standard injections RSD % (relative standard deviation) between these injections must be within the acceptable limit 5 %. (Table 3.7)

3.3.4.1 System precision

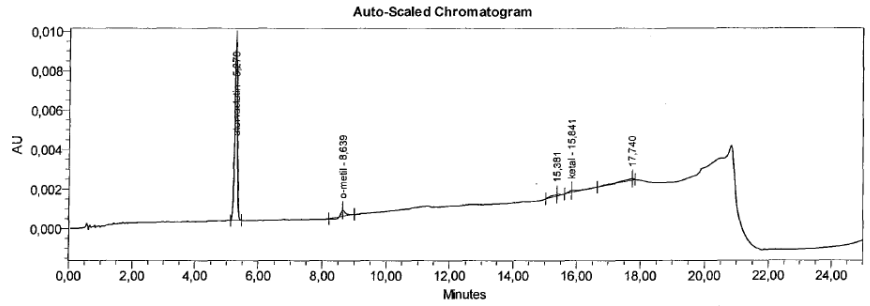
Table 3.7 System precision results

Injection No	***Atorvastatin Ca Standard Area***
1	*53860*
2	*53388*
3	*53127*
Average	*53459*
%RSD	*0.7*



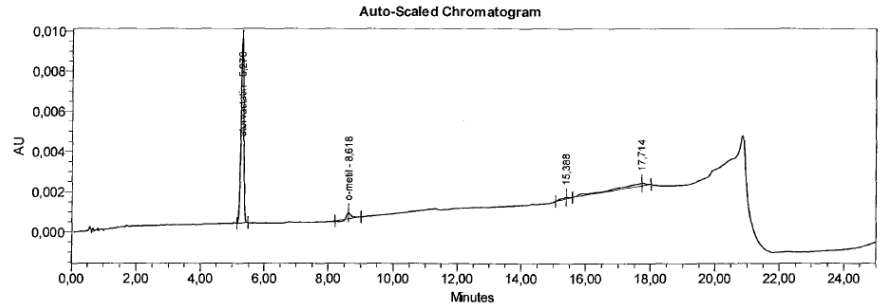
Peak Results

	Vial	Name	RT	Area	% Area	Height	Total Area
1	1:A,5	atorvastatin	5,278	53860	80,681	9271	66757



Peak Results

	Vial	Name	RT	Area	% Area	Height	Total Area
1	1:A,5	atorvastatin	5,278	53388	83,173	9202	64189



Peak Results

	Vial	Name	RT	Area	% Area	Height	Total Area
1	1:A,5	atorvastatin	5,278	53127	77,366	9154	68670

Figure 3.5 Triplicate standard injection for the evaluation of system precision

3.3.4.2 Repeatability

Repeatability results for six samples of each impurity are tabulated in Table 3.8 below.

Table 3.8 Repeatability results in terms of Relative standard deviation (RSD) %

		Samples (% concentration)					
% RSD	**Impurities**	**1**	**2**	**3**	**4**	**5**	**6**
2.6	Atorvastatin Amid	0.43	0.46	0.44	0.45	0.45	0.46
2.2	Desfloro Atorvastatin	0.46	0.49	0.47	0.48	0.47	0.48
5.0	Oxo Atorvastatin	0.40	0.46	0.42	0.44	0.44	0.45
2.7	Diastereomer Atorvastatin	0.20	0.21	0.20	0.21	0.21	0.20
4.4	O-Methyl Atorvastatin	0.39	0.44	0.43	0.43	0.43	0.44
1.7	3'Deshydroxy Atorvastatin	0.42	0.44	0.43	0.43	0.43	0.44
2.9	Atorvastatin Lactone	0.39	0.42	0.39	0.39	0.40	0.40
2.5	Methyl Ester Atorvastatin	0.41	0.44	0.43	0.43	0.43	0.44
2.9	Ketal Atorvastatin	0.34	0.37	0.35	0.36	0.36	0.36
3.5	Highest Unknown Impurity	0.17	0.19	0.18	0.18	0.18	0.18
7.1	Total Unknown	0.43	0.48	0.45	0.44	0.50	0.51

3.3.4.3 Intermediate precision

The results of intermediate precision study are given in Table 3.9 and detailed data are given in Appendix 2. The general method validation results inluding peak purity results, slope of regression equations, precision and intermediate precision for all impurities are summarized in Table 3.10.

Table 3.9 Intermediate Precision Results

Impurities	Difference between results averages of two analysts
Atorvastatin Amid (%)	0.001
Desfloro Atorvastatin (%)	0.001
Oxo Atorvastatin (%)	0.004
Diastereomer Atorvastatin (%)	0.001
O-Methyl Atorvastatin (%)	0.000
3'Deshydroxy Atorvastatin (%)	0.005
Atorvastatin Lactone (%)	0.001
Methyl Ester Atorvastatin (%)	0.002
Ketal Atorvastatin (%)	0.004
Highest Unknown Impurity (%)	0.007
Total Unknown (%)	% 12.8

Table 3.10 UPLC method validation results of Atorvastatin Ca and impurities

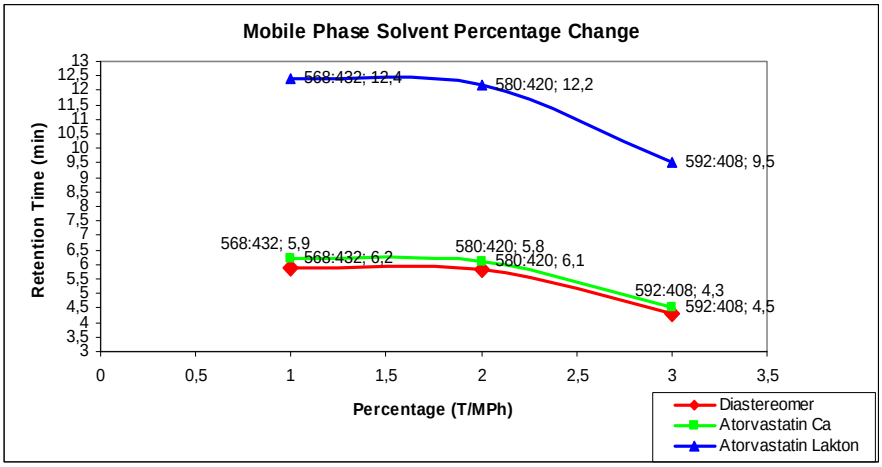
	ACa	**3'Deshy**	**DA**	**AA**	**DFA**	**KA**	**LA**	**AME**	**OMA**	**OXA**
Peak Purity	pure	pure	pure	pure	pure	Pure	pure	pure	pure	pure
Regression equation (y)										
Slope (b)	10351881	8425921	5507836	7418367	8334978	978978	10499529	10770813	7954946	1254125
Intercept (a)	-405	-87.87	- 10	-1	-290	23	76	-24	-27	-129
Correlation Coefficient	0.9999	0.9999	0.9997	1.0000	0.9967	0.9999	0.9999	1.000	0.9999	0.9984
Precision (% RSD)	0.82	0.43	0.08	0.055	1.52	0.1	0.34	0.09	0.17	4.68
Intermediate precision (%)	0.7	1.7	2.7	2.6	2.2	2.9	2.9	2.5	4.4	5.0

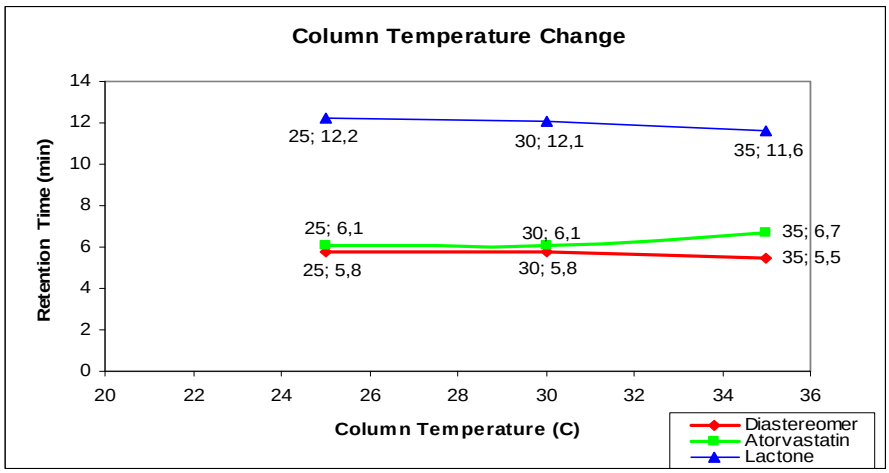
3.3.5 LOD (Limit of Detection) and LOQ (Limit of Quantiation)

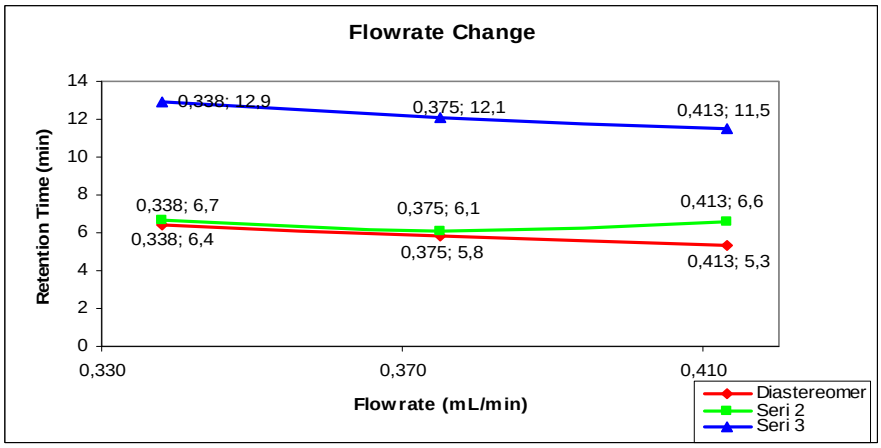
LOD values were determined with a signal to noise ratio 3. limit of quantification (LOQ) values were calculated three times of LOD. LOQ values for impurities were found to be 0.03 % each of analyte concentration. The LOD values were found 0.01 % of each of analyte concentration.

3.3.6 Robustness

In all the deliberately varied chromatographic conditions, the chromatogram for system suitability solution for related substance showed satisfactory resolution between atorvastatin and its impurities. As mentioned in materials and methods section, mobile phase solvent percentage change, wave length change, column temperature change and flow rate change parameters were evaluated. As a result, the impurity method was found robust. Figure 3.6 expresses the change in robustness parameters and as seen in the figure, little deviations from method set-up values are ignorable, method is robust and there will be no effect of these deviations, as expected.







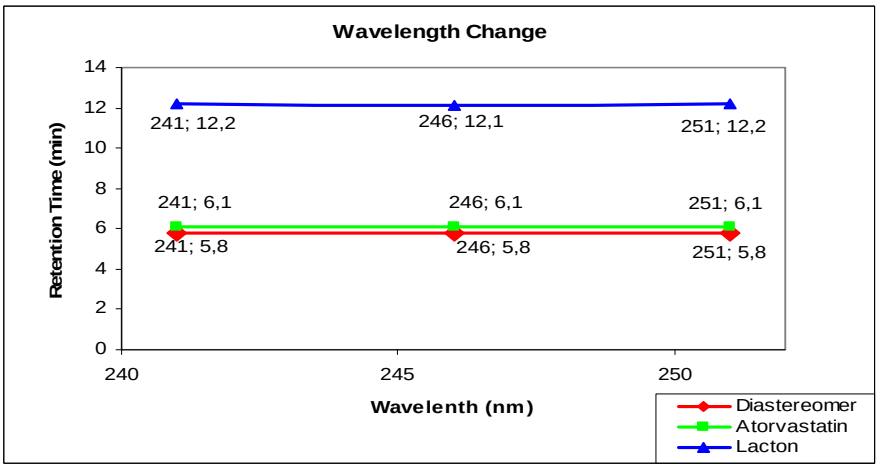


Figure 3.6 Robustness Parameters evaluated in the method

3.3.7 Solution stability

The results obtained from stability test, atorvastatin standard solution is stabile for 8 hours at 5 ^{o}C. However, oxo and o-methyl atorvastatin ca impurity solutions must be fresh. The solution stability test results and graphs of each impurity at +5 ^{o}C were given in Appendix 2.

3.4 Statistical Cholesterol Levels Investigation

All cholesterol data which is used in statistical analysis were obtained from a hospital in Mediterranean region with publishing permit. These data only investigated from statistical point of view. The objective of this investigation is to maintain an opinion about cholesterol levels in local population in Mediterranean region. As a results of this evaluation the cost effectiveness of Atorvastatin Ca was investigated.

Serum Total cholesterol, LDL, HDL and triglyceride concentrations were measured in patients and distribution of these measured values are graphed and the limit values for LDL, HDL, total cholesterol and triglyceride were considered when evaluating the data obtained from investigation.

HDL-C was measured in 1385 female and 1080 male, LDL-C was measured in 1438 female and 1154 male, total cholesterol was measured in 1384 male and 1708 female, triglyceride was measured in 1698 female, 1377 male patients.

While investigating the data Normal Distribution was used in MatLab.The normal distribution is a two-parameter family of curves. The first parameter, μ, is the mean. The second, σ, is the standard deviation. The standard normal distribution , $\Phi(x)$, sets μ to 0 and σ to 1.

$$y = f(x,\mu,\sigma) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(x-\mu)^2}{2\sigma^2}}$$

$$erf(x) = 2 \times \Phi(x\sqrt{2}) - 1$$

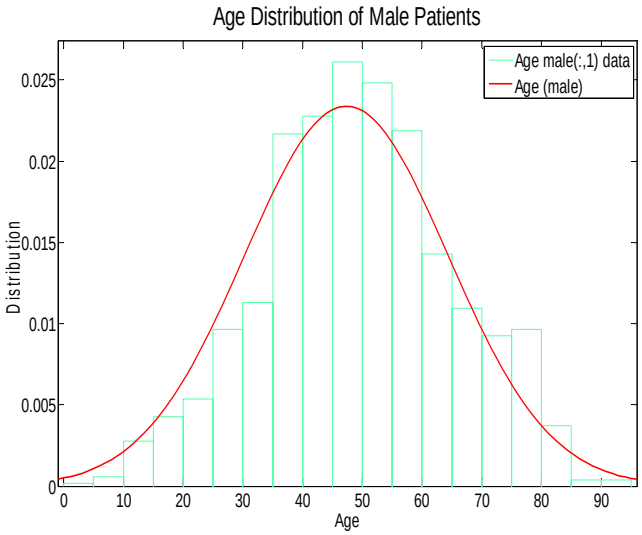
$$\sigma = \left(\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2\right)^{1/2}$$

$$where \quad \bar{x} = \frac{1}{n}\sum x_i$$

The results were shown using MatLab 7.7.0 (R2008B) software from Figure 3.7 to Figure 3.11

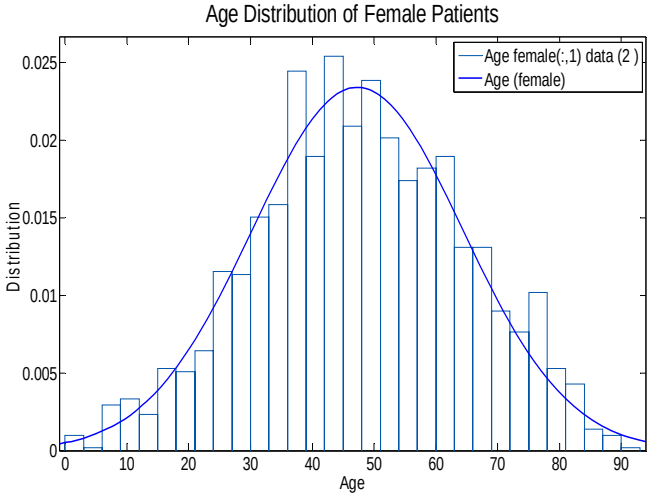
Table 3.11 Normal Distribution Parameters

	Variance	**Sigma**	**Std error (mu/sigma)**	**Mean**
HDL female	274.1	16.556	0.445/0.315	59.836
HDL male	435.2	20.862	0.635/0.444	55.809
LDL female	912.1	30.201	0.796/0.563	122.159
LDL male	888.8	29.814	0.878 /0.621	118.744
Triglyceride female	7780.7	80.208	2.141/ 1.515	141.516
Triglyceride male	35060.6	187.240	5.045/ 3.571	181.956
Cholesterol female	2357.1	48.550	1.175 /0.831	209.262
Cholesterol male	2632.5	51.307	1.380 / 0.976	203.611
Age distribution female	291.1	17.060	0.412/ 0.292	43.318
Age distribution male	274.6	16.569	0.445/0.315	48.6276



Variance	Sigma	Std error (mu/sigma)	Mean
291.074	17.06	0.4129/0.292	47.31

(a)



Variance	Sigma	Std error (mu/sigma)	Mean
274.557	16.5697	0.4456/0.3152	48.62

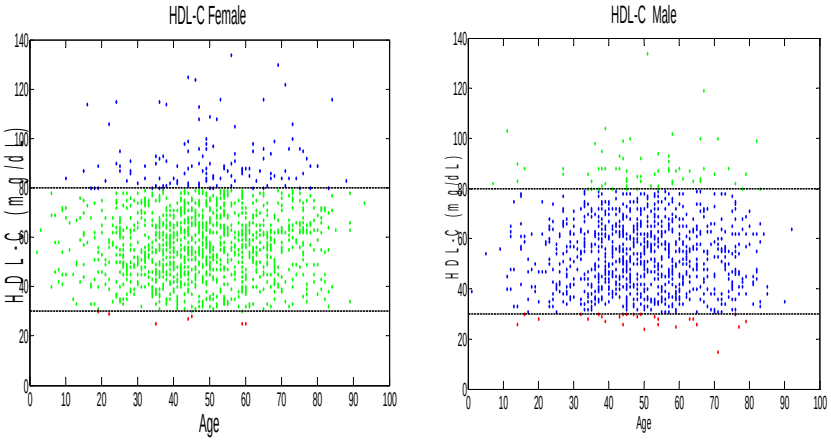
(b)

Figure 3.7 Age distributions of male (a) and female (b) patients

Age distribution graphs were drawn according to patients in total cholesterol measurement test. Approximately the same age distributions were obtained from male and female patients. Maximum peak points are different, female patients have maximum block values at 36-38 and 44-46 years range. However, male patients have maximum values 45-50 years range. National Cholesterol Education Program Limits were taken in Table 3.12 as reference range because there is no published or announced reference range for Turkey.

Table 3.12 NCEP (national cholesterol education program) reference limits

LDL	< 100 mg/dL, OPTIMAL 100-129 mg/dl, NEAR OPTIMAL 130-159 mg/dL, BORDER LINE 160-189 mg/dl, HIGH > 190 mg/dl, VERY HIGH
TRIGLYCERIDE	<150 mg/dl, OPTIMAL 150–199 mg/dL BORDER LINE 200–499 mg/dL HIGH >500 mg/dL VERY HIGH
TOTAL CHOLESTEROL	< 200 mg /dl, DESIRABLE 200-239 mg/dl, BORDER LINE >240 mg/dl, HIGH
HDL	< 40 mg/dl, LOW > 60 mg/dl, HIGH

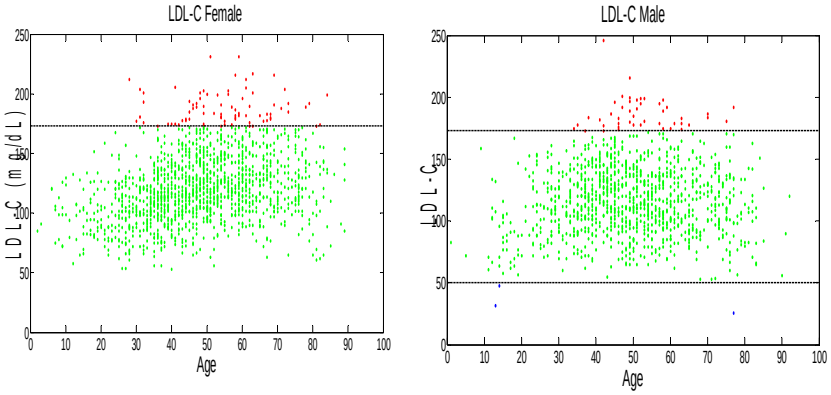


(a) (b)

Reference range: 30-80 mg/dl

Figure 3.8 HDL-C levels of male (a) and female (b) patients

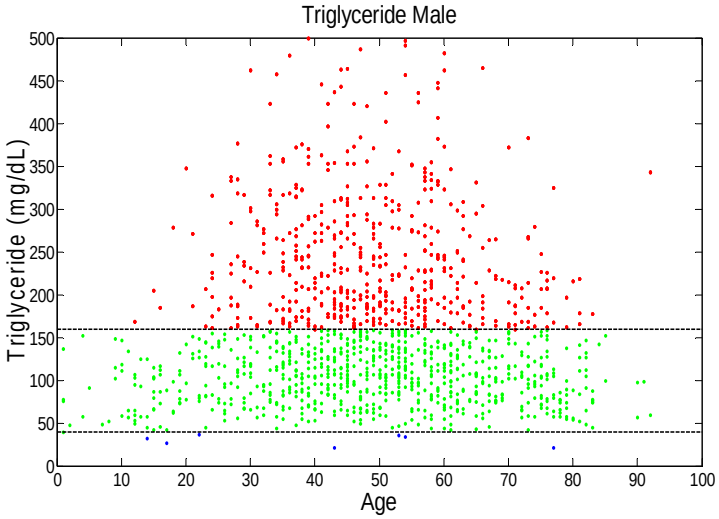
As shown in Figure 3.7 (a) and (b) HDL cholesterol levels were expressed for both male and female patients. The acceptable limit for HDL is between 30 mg/dL and 80 mg/dL. Under the limit of 30 mg/dL, much more male patients exist than female patients. Besides, upper limit was exceeded by more female patients. In conclusion, female patients have better HDL-C profile than that male patients have.



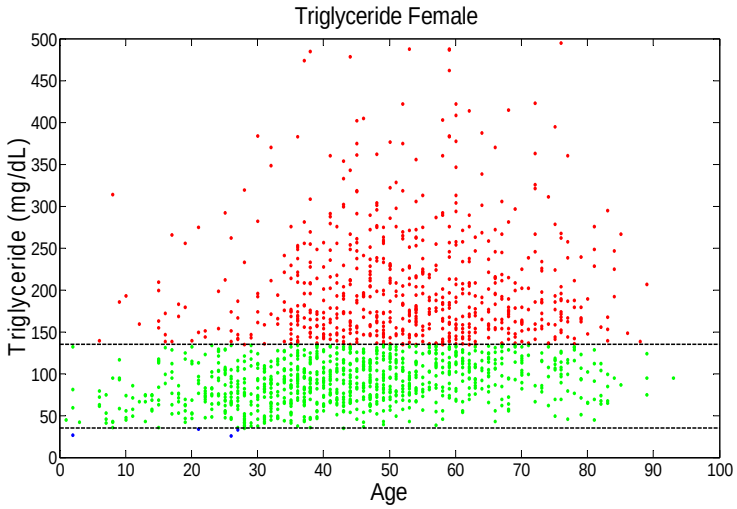
(a) (b)

Figure 3.9 LDL-C levels for male (a) and female (b) patients

Reference range for LDL-C is 50- 173 mg/dL. Patients who have measured values higher than the upper limit are in the risk category for coronary artery disease and atherosclerosis. As expressed in Figure 3.9 (a) and (b), most of patients are in the limit of acceptance, however if a patient representing in the limit, have other risk factors, such as, smoking, atherosclerosis etc, maximum acceptance limit would be lower.



(a)

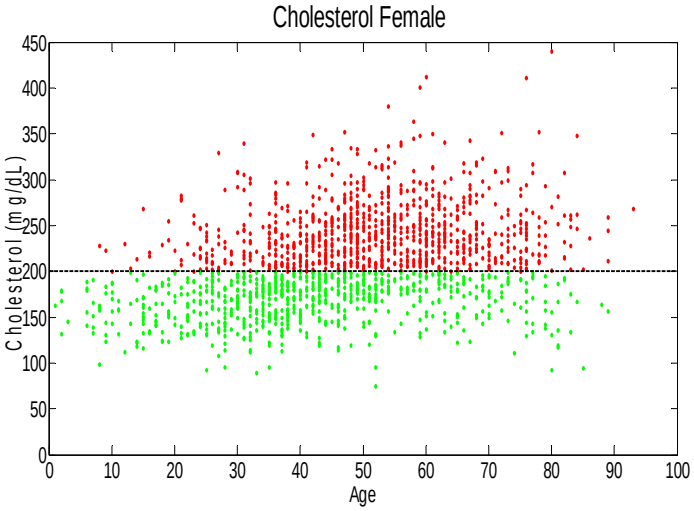


(b)

Figure 3.10 Triglyceride levels of male (a) and female (b) patients

As seen in Figure 3.10, male patients have higher levels of triglyceride than female patients. Reference range is between 35-135 mg/dl which is valid for hospital reports and quietely high amount of patients' triglyceride level is out of limit.

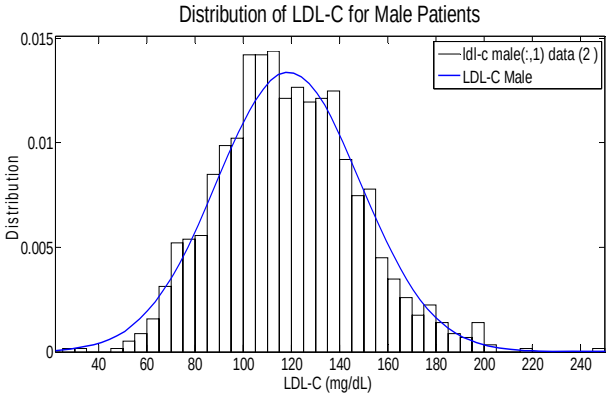
(a)



(b)

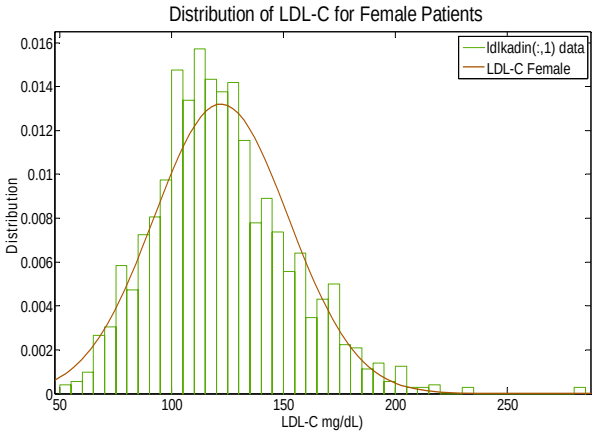
Figure 3.11 Total cholesterol level for male (a) anf female (b) patients

In comparison of female and male patients cholesterol levels from Figure 3.11, female patients have higher cholesterol levels in their blood than male patients. In this study, patients did not checked whether they have any disease or there was no special control, so these values express the spontaneously cholesterol measurement in the Mediterrenaen area in 2009.



(a)

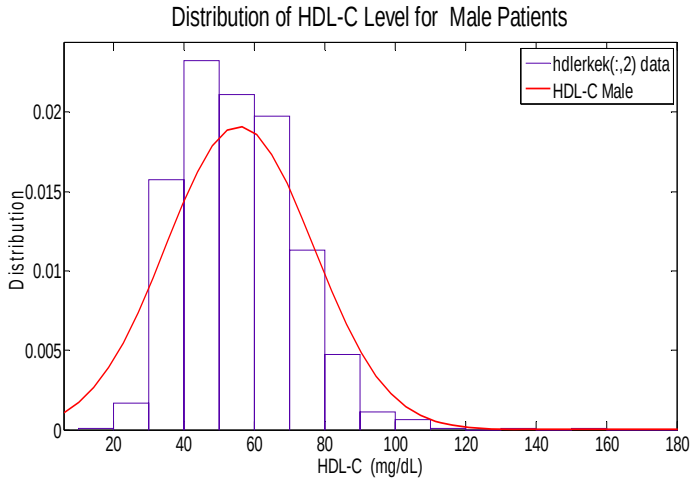
Variance	Sigma	Std error (mu/sigma)	Mean (50-173) mg/dl
912.141	30.20	0.7967/0.563	122.1



(b)

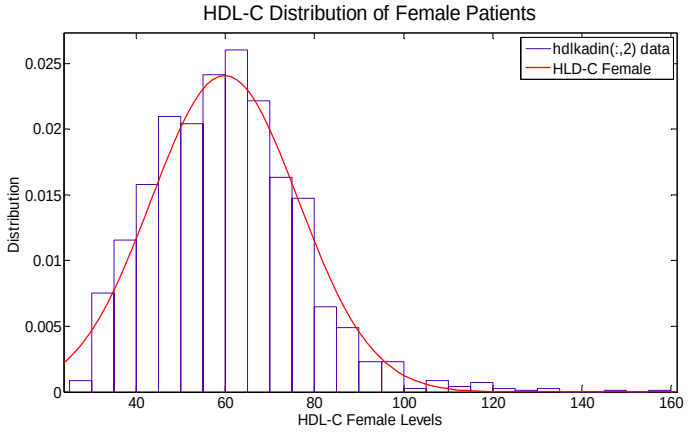
Variance	Sigma	Std error (mu/sigma)	Mean (50-173) mg/dl
888.895	29.8143	0.8780 / 0.621	118.7

Figure 3.12 LDL-C Distribution male (a) and female (b) patients



Variance	Sigma	Std error (mu/sigma)	Mean
274.125	16.5567	0.44537/0.315095	59.83

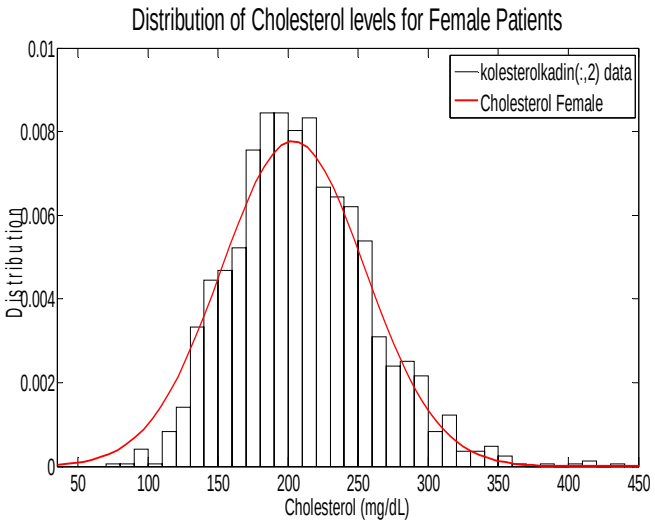
(a)



Variance	Sigma	Std error (mu/sigma)	Mean
435.255	20.8628	0.6352/0.4442	55.80

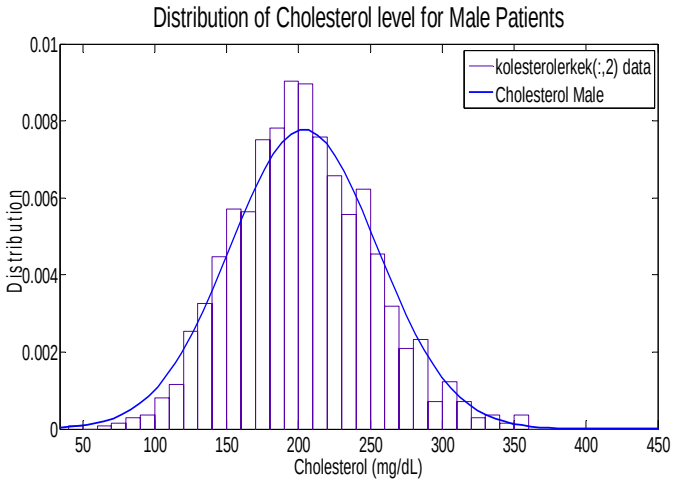
(b)

Figure 3.13 HDL-C Distribution male (a) and female (b) patients



Variance	Sigma	Std error (mu/sigma)	Mean (< 200 mg/dl)
2357.15	48.550	1.175 /0.831	209.2

(a)



Variance	Sigma	Std error (mu/sigma)	Mean (<200 mg/dl)
2632.5	51.307	1.380 / 0.976	203.6

(b)

Figure 3.14 Cholesterol Distribution male (a) and female (b) patients

107 types of Atorvastatin Ca drugs exist in the pharmaceutical market in Turkey. Atorvastatin Ca tablets are in the form of 10, 20, 40, 80 mg and 30 and 90 film tablets. If a patient who is suffered from high LDL-C level has to consume minimum 10 mg, maximum 80 mg during a day. Usage dosage changes depending on the risk level of LDL-C. At minimum risk grup, 10 mg atorvastatin for a day has enough affect to lower cholesterol.

1708 female and 1384 male patients blood cholesterol were measured, total cholesterol (TC) level of 929 female and 694 male patients are outside of the limit(200 mg/dl). According to this result, different costs are tabulated in Table 3.13.

Table 3.13 Cost of Atorvastatin Ca consumption for patients suffered from high Total Cholesterol

Drug	Price (TL)	Female Cost (TL)	Male Cost (TL)	Total Cost (TL)
Ateroz 10 mg Film Tablet	29,32	27238,28	20348,1	47586,36
Ateroz 20 mg Film Tablet	49,63	46106,27	34443,2	80549,49
Ateroz 40 mg Film Tablet	63,87	59335,23	44325,8	103661,01
Ateroz 80 mg Film Tablet	64,81	60208,49	44978,1	105186,63
Lipitor 10 mg Film Tablet	32,98	30638,42	22888,1	53526,54
Lipitor 20 mg Film Tablet	53,59	49785,11	37191,5	86976,57
Lipitor 40 mg Film Tablet	73,32	68114,28	50884,1	118998,36
Lipitor 80 mg Film Tablet	79,01	73400,29	54832,9	128233,23

Lipitor 80 mg FT- Ateroz 80 mg FT= 23,000 TL

The statistical analysis of cholesterol levels was carried out with approximately 3500 patients and their statistical distribution graphs were presented. The percentage of females patients whose LDL-C levels are out of limit is more than the percentage of male patients. Tables showing the percentages of the measured levels are given in Appendix 2.

Approximately the same age distributions were obtained for male and female patients, only the maximum peak points are different. The female patients have a density in the 36-38 and 44-46 year range. However, male patients have a maximum age category at the range of 45-50 years.

Economical analysis of the Atorvastatin drug consumption of these patients was carried out using two options. First, if a generic drug (Ateroz) or a second original (Lipitor) was used at the same dosages, a comparison of costs of these drugs was accomplished. The difference between the treatment costs for the original and a generic pharmaceutical for this study was 23,000 for only 1618 patients and for a month. If 10 mg of Atorvastatin Ca is required and is enough for treatment the total cost for only 1618 patients suffering from high total cholesterol is 47586,36 TL and if 80 mg was chosen for treatment, then 105186,63 TL would be the cost of the drug consumption. Table 3.13 includes the detailed costs of treatment with different dosages and secondly, for results that could not ensure the acceptance criteria, cost analysis was carried out with 4 different statins. Atorvastatin, simvastatin rosuvastatin and pravastatin were used for this investigation and are given in Table 3.14 and Figure 3.15.

Table 3.14 Prices of different statins in the local market

	PRICE (TL)			
Dosage	**Rosuvastatin**	**Atorvastatin**	**Simvastatin**	**Prasvastatin**
10 mg	30,98	25, 41	5,91	6,47
20 mg	46,41	43,32	5,38	10,16
40 mg	46,41	53,49	10,77	29,06
80 mg	----	53,49	----	----

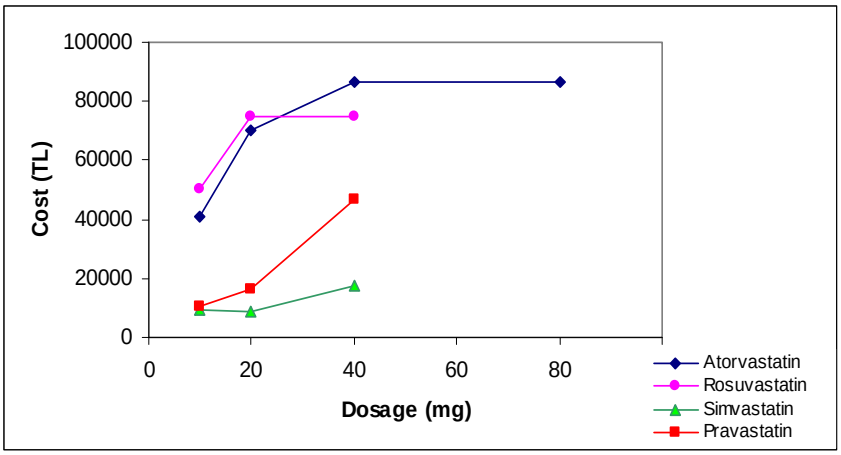


Figure 3.15 Cost comparison of statin treatment for the same population

4.CONCLUSIONS

In this study, the importance of Atorvastatin Ca, an active ingredient which is in the statin class of lipid lowering drugs, was investigated. Literature review for statins and especially atorvastatin was implemented on a large scale.

The investigation of the impurity method validation of Atorvastatin Ca is a result of experimental data that was collected and compiled to form this thesis

On the experimantal side of the study, the first and newly developed UPLC method for determining the nine impurities of Atorvastatin Ca was found to be capable of giving faster retention times along with maintaining good resolution than that achieved with conventional HPLC.

The method was completely validated showing satisfactory data for all the parameters tested. This method exhibited an excellent performance in terms of sensitivity and speed. Total analysis time for a one batch series decreased from 430 to 175 minutes and the total mobile phase consumption was lowered from 500 ml to 70 ml.

The statistical analysis of cholesterol levels was carried out with approximately 3500 patients and their statistical distribution graphs were presented. The percentage of females patients whose LDL-C levels are out of limit is more than the percentage of male patients.

The economical analysis was evaluated using four different statins including Atorvastatin, Simvastatin, Rosuvastatin, Pravastatin. In conclusion, the cost analysis for 10 and 20 mg dosage treatments, the cost of the drugs are sequentially: rosuvastatin>atorvastatin>pravastatin> simvastatin, and for 40 or 80 mg, atorvastatin has the maximum cost. Only the atorvastatin has the 80 mg dosage of these statins.

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Zhou L., Mao B., Reamer R., Novak T., Ge Z. , 2007, Impurity profile tracking for active pharmaceutical ingredients:Case reports, *Journal of Pharmaceutical and Biomedical Analysis* ,44 , 421–429

CV

M E L E K İ Ş L E K

PERSONAL INFORMATION

- **Marital Status:** Single
- **Birth Date:** 12 November 1980

WORK EXPERIENCE

- **March,2009- …, Regulatory Affairs Specialist**

Bristol-Myers Squibb Pharmaceuticals, İstanbul

- **February, 2008 – March, 2009 Regulatory Affairs Specialist**

Bilim Pharmaceuticals Co., İSTANBUL

- Written and verbal communication with all departmants of the company
- Planning and Organizing operation participants (QA/Production/QC/R&D etc.)
- Preparation of CTD format dossier (Module 1/Module 2/ Module 3/)
- Official correspondences related with regulatory affairs and marketing authorization subjects between Market Authorisation Holder and Ministry of Health
- Literature search of medical documents, PILs and articles
- PIL/SmPC writing and reformatting
- Launching, Pricing and Reimbursement
- Preparation of Package artworks
- Type variations (NTA/CTD) (e-variation through IEGM)
- Variation applications beside type variations
- Preparation of Package artworks

- **July, 2006 – November, 2007, Process Validation Engineer**

Sanovel Pharmaceuticals Co., İSTANBUL

- Process Validations of 60 types of drug products in consequence of company's replacement from Şişli to Silivri
- Worked as analyst in the quality control laboratory to carry out the physical and chemical tests of more than 30 different types of drug samples branded Sanovel Pharmaceuticals products

- Executed various instrumental analyses with and specialized in UPLC, HPLC (Agilent 1100 series/Chemstation software, Waters/Empower software), GC, FT-NIR Analyzer and UV-visible spectrophotometer.

- **November, 2004 – July, 2006, Drug Product Specialist (QC)**

Nobel Pharmaceuticals Co., DÜZCE

- Responsible from release of drug products branded including Nobelpharma, Nobel and other leading pharmaceutical companies, as well as the raw materials, more than 250, purchased for production.

SEMINARS ATTENDED

- CTD Dossier preparation and Modules, Augsut,2008, Bilim Pharmaceuticals.
- e-CTD application in the European Markets, February,2009, Bilim Pharmaceuticals
- Focusing to Objectives via Team Working, June, 2007, Sanovel
- Problem Solving and Making Decision, May, 2007, Sanovel
- UPLC Technology and Software, February, 2007, Sanovel
- MS Applications at Pharmaceutical Industry, Mövenpick Hotel, Istanbul / May 2006 – Likrom&Waters
- Stability, Nobel Pharmaceuticals, Duzce, 2006
- GMP and GLP, Nobelpharma Pharmaceuticals Co., Quality Control Manager, Duzce 2005
- Dissolution Profile Comparisons, F2 Similarity Factor, Fargem Co., Duzce, 2006

EDUCATION

- **September, 2008- January, 2010**

Master of Science in Chemical Engineering (ENG) (thesis stage)
Ege University, Izmir

Master Thesis: Quality Critics of active chemical ingredients of lipid lowering drugs in pharmaceutical industry.

- **September, 1998- July, 2002**

Bachelor of Science in Chemical Engineering (ENG)
Ege University, Izmir

A four-year Chemical Engineering education caused to win engineering sight of view and analythical thinking. Enthusiasim and superior characteristics are obtained by use of methods and practices of engineering and Industrial Technology, Process Design, Instrumentation, Project Planning, Organic and Inorganic Technology, Enviroment and Safety Engineering projects.

- **September, 1994 –July, 1998**

Selma Yiğitalp High School, İZMİR

A four-year education with one year dedicated English preparation class

CERTIFICATES

- Business English and Conversation

British Side, December,2009-January,2010 , İstanbul

- Personal Leadership Workshop

Bilim Pharmaceuticals, 18 – 20 September 2009, İstanbul

- First Aid Specialist,

Kurtaran First Aid Education Center, September 2009, Istanbul

- English Language Course, 5/7 Level

Callan School of English, 2008 London, UK

- Driving Safely Certificate

Autodrom, June 2007, Istanbul

- E-Trade and Import/Export Procedure and Applications (b2b, customs, letters)

Yorktrade Foreign Trade Institute, Izmir, 2004

- German, Intermediate Level Certificate

TÖMER, Izmir, Turkey,

- TOEFL and GMAT Course

Turkish-American Association, Izmir, 2004

INTEREST AND SKILLS

Language Skills

English, upper-intermediate level at reading and writing (Engineering instruction language) and lower advanced level at speaking.

German, Pre-Intermediate Level

Technical Skills

UPLC, HPLC, FT-NIR, GC, UV-visible spectro, Laboratory Equipments

Computer Skills

MS Office, Win 9x / NT / 2000 / XP, Chemstation , (UPLC/GC softwares)

Empower, IBM AS/400 System, PictureProject

Interests

Development of global and domestic pharmaceutical sector, regulatory affairs subjects and launching, new generation pharmaceuticals, marketing communications

Hobbies

Travel to new places, books, photography and swimming.

Appendix 1: Atorvastatin Ca as a Lipid Lowering Drug

Properties of Atorvastatin Ca

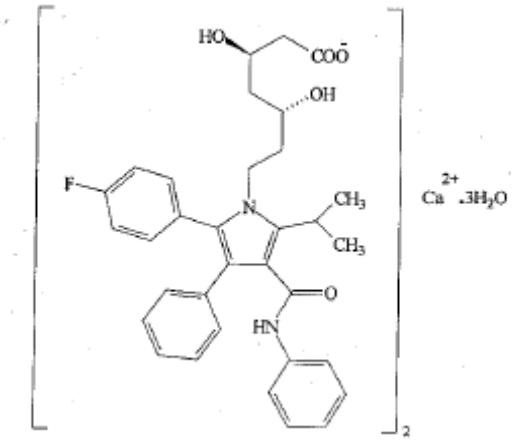


Figure 1: Atorvastatin Calcium

Chemical Name

[*R-(R*-R*)]*-2-(4-Fluorophenyl)-β,δ-dihyrdroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihiydrate

Molecular Formula

$(C_{33}H_{34}FN_2O_5)_2Ca.3H_2O$

Physical Form

White to off-white crystalline powder

Therapeutic Category

Anti-hypercholesterolemic agent

1.3.3.2 Elucidation of Atorvastatin structure

Infrared Spectrum

Infrared absorption spectrum of potassium bromide sipersion of Atorvastatin Calcium (crystalline) sample exhibits maxima only at the wavelengths as that of a similar preparation of Atorvastatin Calcium working standart (lot no: 16991) IR spectra was given in Figure 1 and Figure 2 in Appendix 1.

Ultraviolet Spectrum

Ultraviolet (UV) absorption spectra of a methanolic solution (0.01 % w/v) of Atorvastatin Calcium (crystalline) sample and that of Atorvastatin Calcium working standart recorded from 200-400 nm exhibit maxima at about 206 nm and 244 nm.

Uv spectra of the sample was shown in Figure 3 in Appendix 1.

Nuclear Magnetic Resonance Spectrum

The proton magnetic resonance spectra of Atorvastatin Calcium and that of Atorvastatin Calcium working standart are recorded in DMSO-d_6 using tetramethylsilane as an internal Standard on 300 Mhz instrument. The assignments of protons and their multiplicity patterns are provided Figure 4 and Figure 5 in Appendix 1..

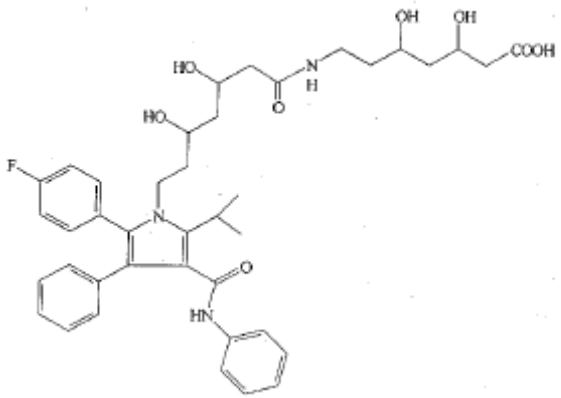
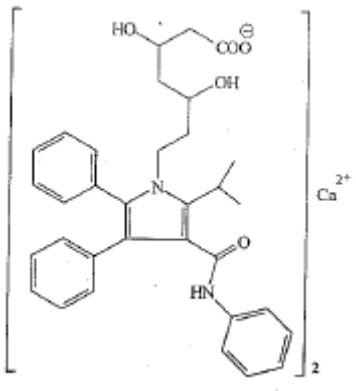
Mass spectrum

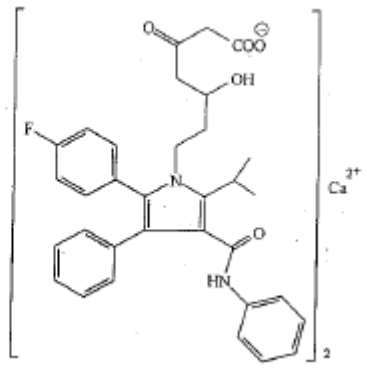
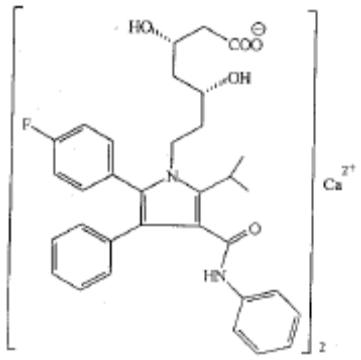
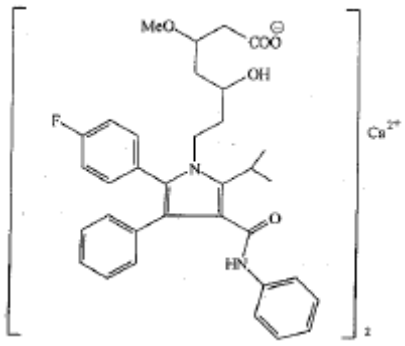
The mass spectra of Atorvastatin Calcium and that of working standart of Atorvastatin Calcium recorded in pozitive ion spray mode exhibit the same fragmentation pattern and at m/z=558.9 corresponds atorvastatin acid. The mass spectrum data is shown in Figure 6 in Appendix 1.

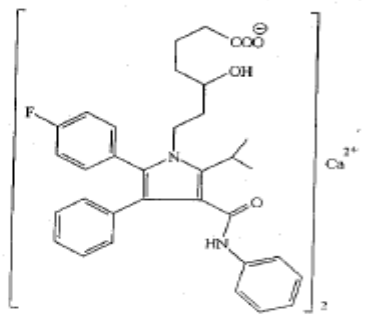
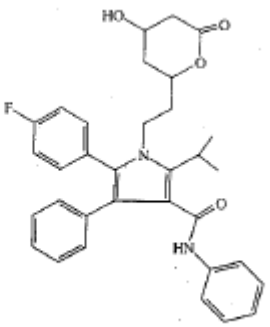
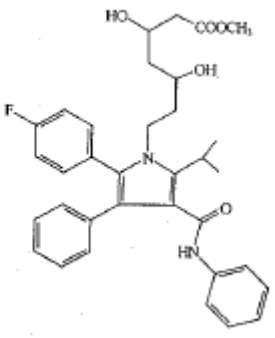
Brief Description of Atorvastatin Calcium Impurities

Atorvastatin Ca impurity standarts were ordered from Ranbaxy and described briefly in Table 1.

Table 1. Atorvastatin Ca impurities and their sources

Name of Atorvastatin Ca Impurity	Source
Atorvastatin Amide Impurity	a process related impurity formed during manufacture of Atorvastatin Calcium.
Amide impurity of Atorvastatin 	
Desfluoro Atorvastatin Calcium	process related impurity and may be formed durinf manufacturing process of Atorvastatin Calcium.
Desfluoro Atorvastatin calcium 	
Oxo-Atorvastatin Calcium	process related impurity as well as degradation product of Atorvastatin calcium. It may be formed by oxidation of Atorvastatin calcium.

Oxo Atorvastatin calcium 	
Diastereomer Atorvastatin Calcium	is a process related impurity and may be formed during manufacturing process of Atorvastatin Calcium
Diastereomer Atorvastatin calcium 	
O-Methyl Atorvastatin Calcium	is a process related impurity formed during manufacturing process of Atorvastatin calcium
O-Methyl Atorvastatin calcium 	
3-Deshydroxy Atorvastatin Calcium	a process related impurity and may be formed during manufacturing process of Atorvastatin Calcium

3'-Deshydroxy Atorvastatin calcium 	
Atorvastatin Lactone	Process related impurity as well as possible degradation product of Atorvastatin calcium. It may be formed under acidic conditions
Atorvastatin Lactone 	
Methyl ester of Atorvastatin	process related impurity and may be formed during manufacturing process of Atorvastatin Calcium
Methyl ester of Atorvastatin 	
Ketal Atorvastatin Calcium	process related impurity and may be formed during manufacturing process of Atorvastatin Calcium

Ketal Atorvastatin calcium

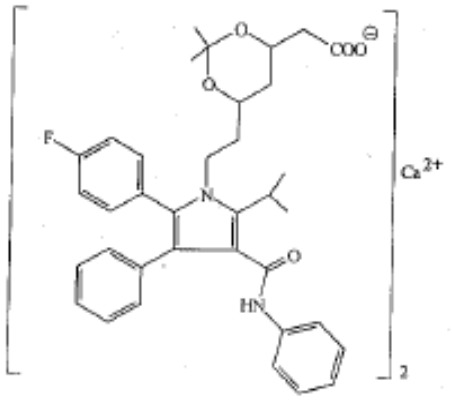


Figure 1. IR Spectrum of Atorvastatin Calcium Working standard

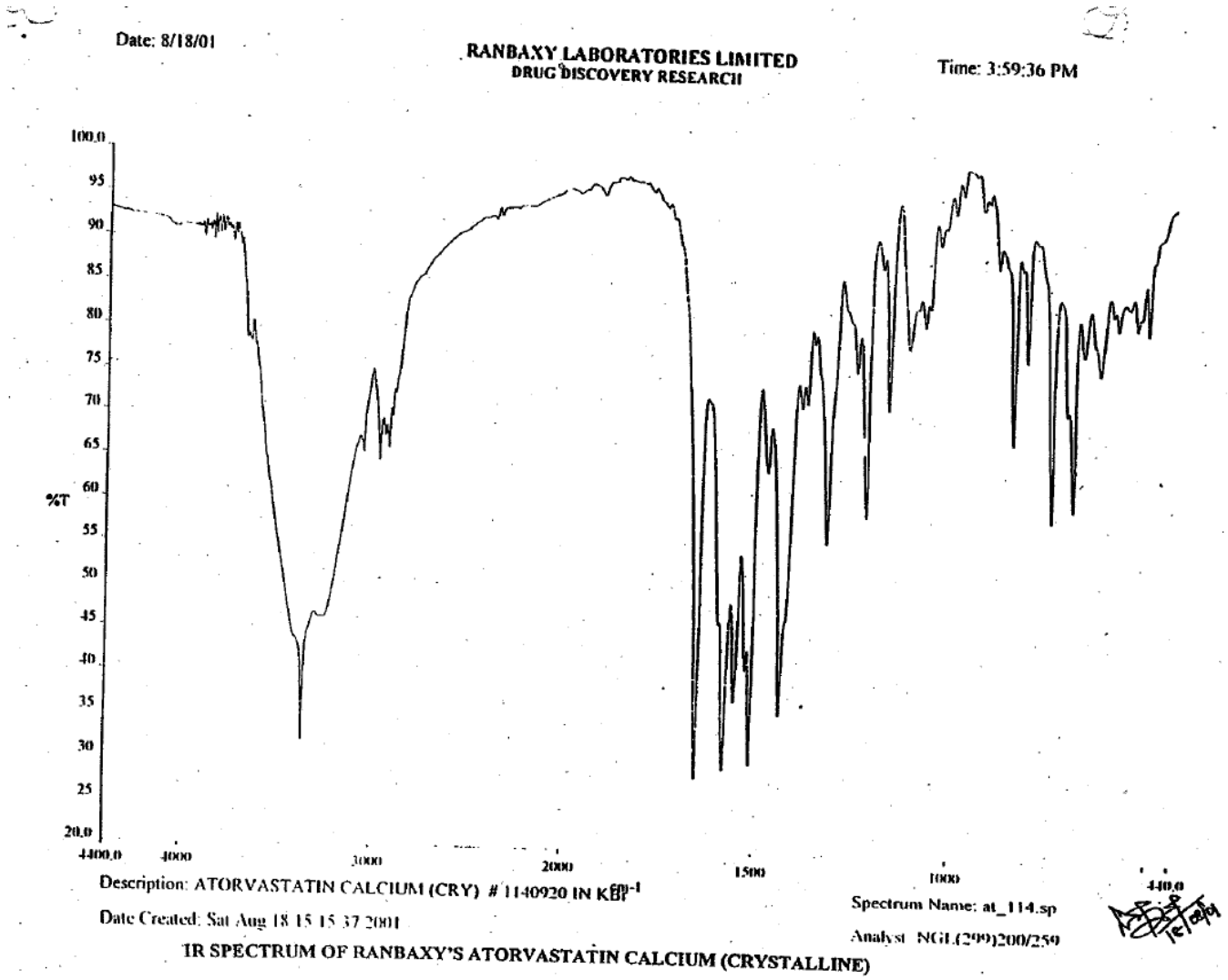
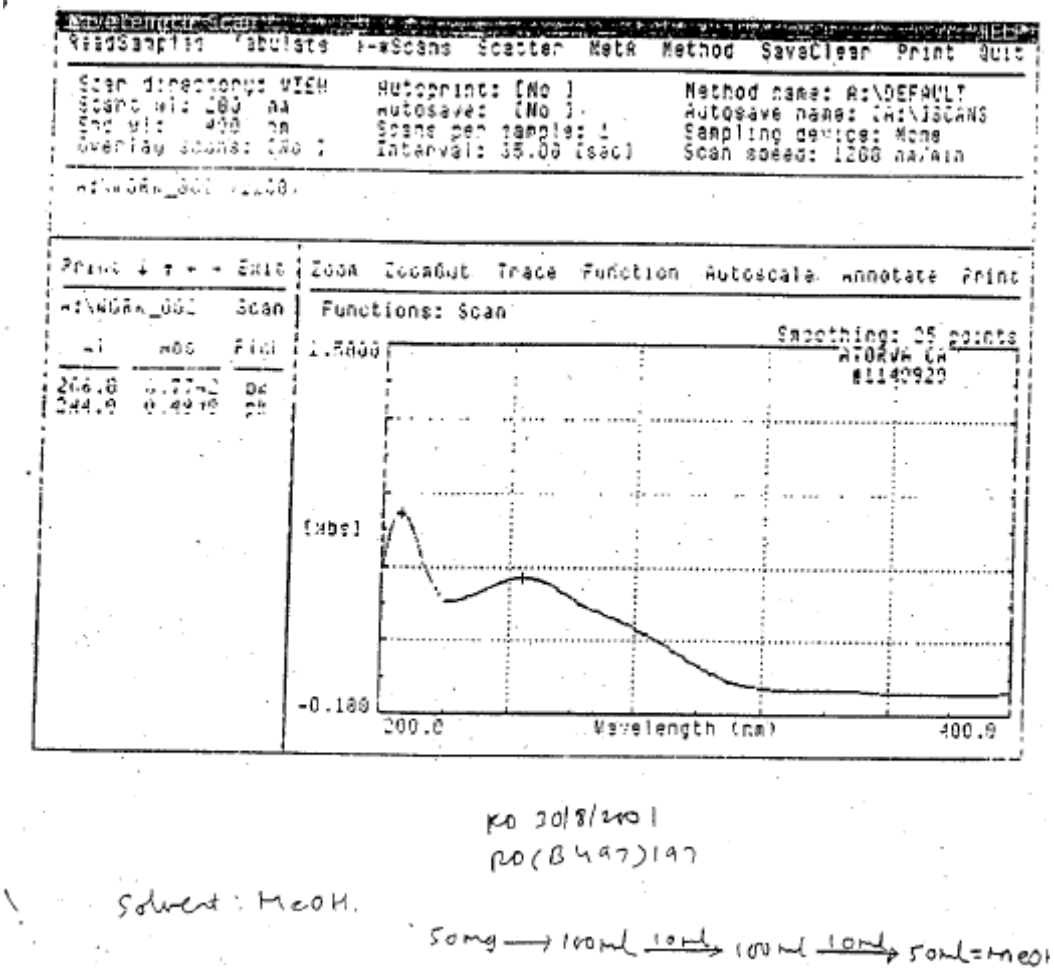


Figure 2. IR Spectrum of Atorvastatin Calcium Ranbaxy standard



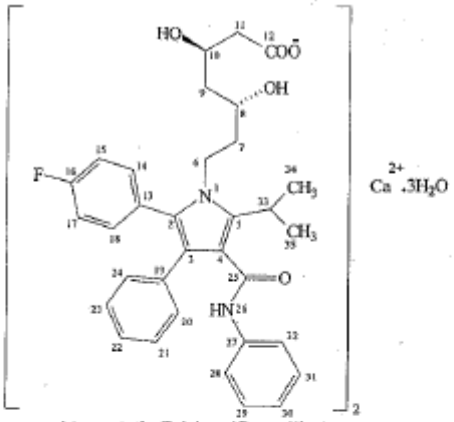
RO 20/8/2001
RO(B497)197

Solvent : MeOH.

50mg → 100mL 10mL → 100mL 10mL → 50mL=MeOH

UV SPECTRUM OF RANBAXY'S ATORVASTATIN CALCIUM (CRYSTALLINE)

Figure 3 : UV Spetrum of Atorvastatin Ca crystalline



Atorvastatin Calcium (Crystalline)

NMR Proton Assignment and Multiplicity Pattern for Atorvastatin Calcium (Crystalline) [Batch No. 1140920]

Chemical Shift (ppm)	Multiplicity	Assignment
1.21-1.42	m, 8H	C_{34}-H_3 & C_{35}-H_3, C_7-H_2
1.53-1.58	m, 2H	C_9-H_2
1.90-2.11	m, 2H	C_{11}-H_2
3.21-3.25	m, 1H	C_{33}-H
3.53	m, 1H	C_8-H
3.75	m, 2H	C_6-H_2
3.92-3.95	m, 1H	C_{10}-H
4.77	brs, 1H	-NH
6.96-7.53	m, 14H	Aromatic protons
9.84	s, 1H	-COOH

m = multiplet; brs = broad singlet; s = singlet

^{1}H NMR spectra of Ranbaxy's Atorvastatin Calcium (Crystalline) and Atorvastatin Calcium (Crystalline) working standard are provided on the following page.

Figure 4: NMR proton assignment and Multiplicity Pattern for Atorvastatin Ca

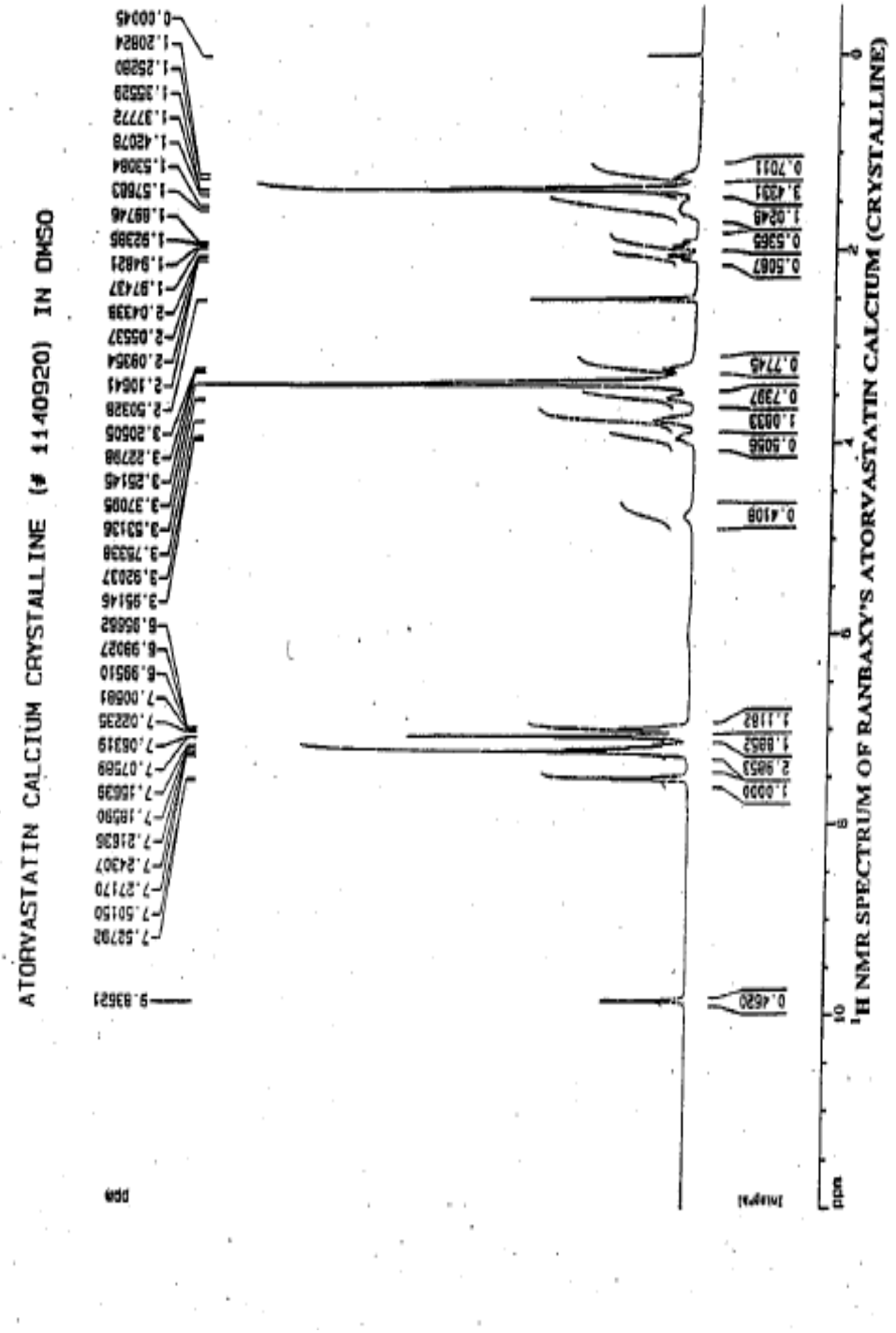
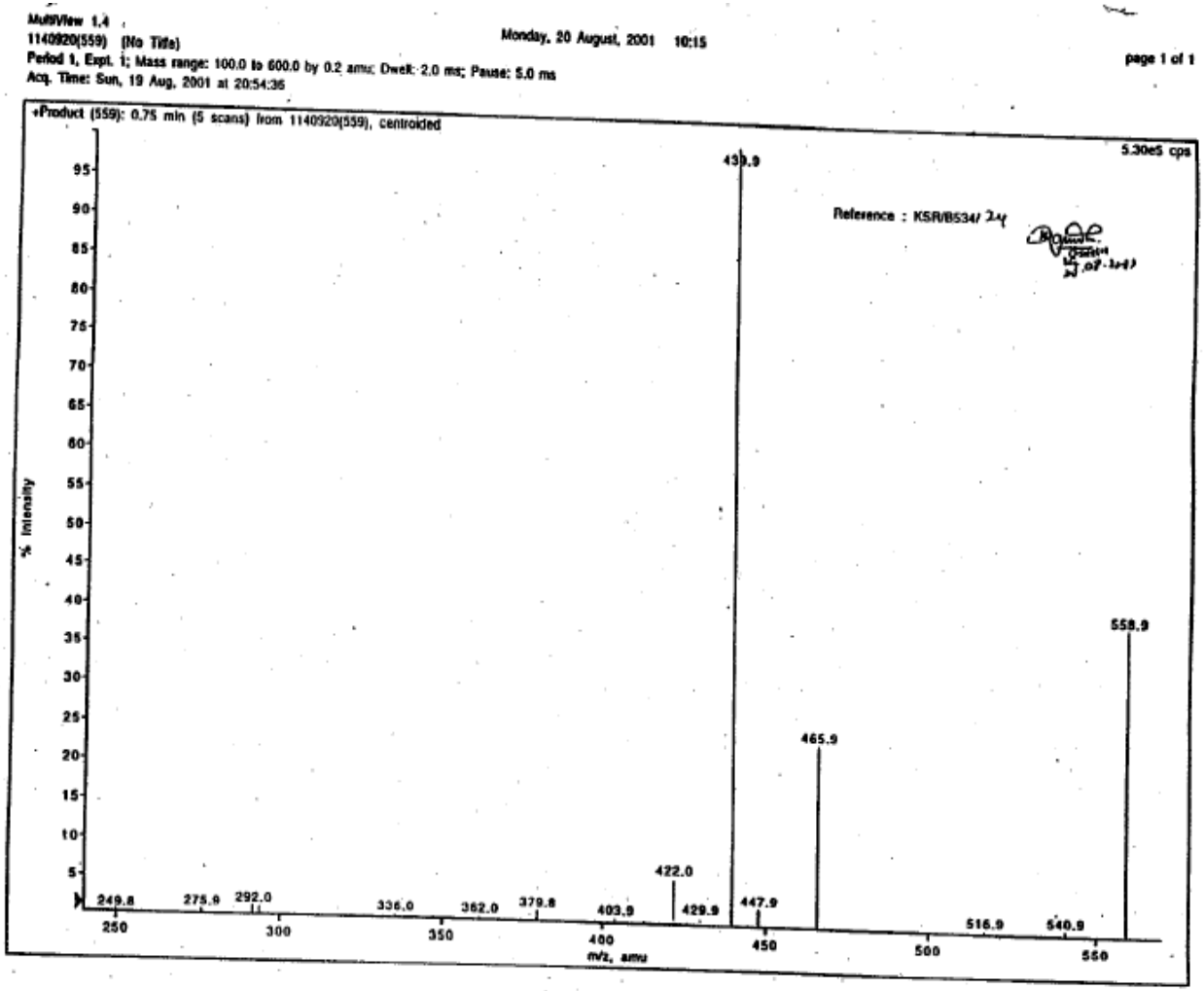


Figure 5: NMR spectrum of Atorvasttin Ca crystalline

Figure 6 : Mass Spectrum of Atorvastatin Ca



MASS SPECTRUM OF RANBAXY'S ATORVASTATIN CALCIUM (CRYSTALLINE)

APPENDIX 2

Table 1. Atorvastatin Ca Linearity Study Results

Standard Concentration (%)	Prepared Standard Concentration (mg/ml)*e-4	Area mAU	Calculated Area mAU
0.1	4.835	4595	4600
		4794	4600
		4882	4600
0.2	9.670	9687	9605
		9672	9605
		9300	9605
0.3	14.505	14730	14610
		14872	14610
		14888	14610
0.4	19.340	19655	19615
		19495	19615
		19669	19615
0.5	24.175	24544	24621
		24362	24621
		24543	24621
0.6	29.010	29010	29626
		28976	29626
		29092	29626
0.8	38.680	39896	39636
		40067	39636
		40080	39636
1.0	48.350	49656	49646
		49575	49646
		49619	49646
1.2	58.020	59746	59657
		59815	59657
		59629	59657

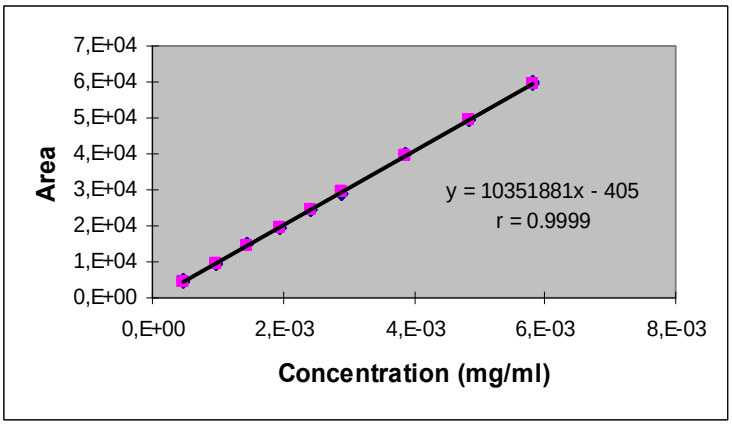


Figure 1. Atorvastatin Ca Linearity Study calibration graph

Table 2. 3'Deshydroxy Atorvastatin Ca Linearity results

Standard Concentration (%)	Prepared Standard Concentration (mg/ml)*e-4	Area (mAU)	Calculated Area (mAU)
0.1	4.833	3909	3984
		3953	3984
		4017	3984
0.2	9.666	8025	8057
		7982	8057
		8011	8057
0.3	14.499	12033	12129
		12192	12129
		12282	12129
0.4	19.332	16296	16201
		16199	16201
		16302	16201
0.5	24.165	20413	20273
		20263	20273
		20374	20273
0.6	28.998	24212	24346
		24255	24346
		24252	24346

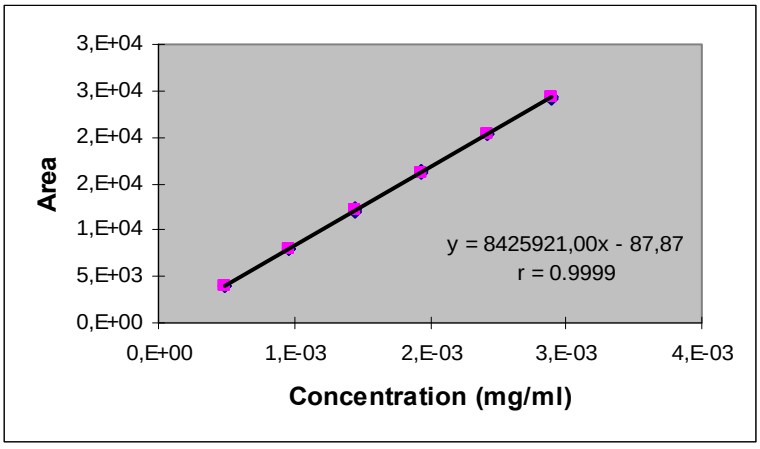


Figure 2. 3'Deshydroxy Atorvastatin Ca Linearity Study regression graph

Table 3. Diastereomer Atorvastatin Ca linearity study results

Standard Concentration (%)	Prepared Standard Concentration (mg/ml)*e-4	Area (mAU)	Calculated Area (mAU)
0.1	4.193	2244	2300
		2349	2300
		2293	2300
0.2	8.386	4641	4609
		4532	4609
		4574	4609
0.3	12.579	6753	6919
		7025	6919
		7024	6919
0.4	16.773	9158	9228
		9311	9228
		9189	9228
0.5	20.966	11628	11538
		11636	11538
		11646	11538
0.6	25.159	13711	13847
		13918	13847
		13688	13847

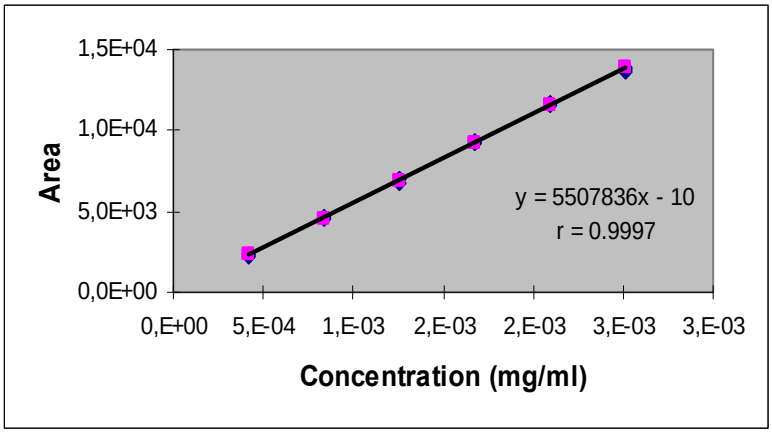


Figure 3. Diastereomer Atorvastatin Ca linearity study regression graph

Table 4. Atorvastatin Amid linearity study results

Standard Concentration (%)	Prepared Standard Concentration (mg/ml)*e-4	Area mAU	Calculated Area mAU
0.1	4.853	3585	3599
		3572	3599
		3590	3599
0.2	9.706	7200	7199
		7204	7199
		7217	7199
0.3	14.559	10824	10799
		10846	10799
		10817	10799
0.4	19.412	14357	14400
		14399	14400
		14368	14400
0.5	24.265	18001	18000
		18014	18000
		18025	18000
0.6	29.118	21576	21600
		21555	21600
		21640	21600

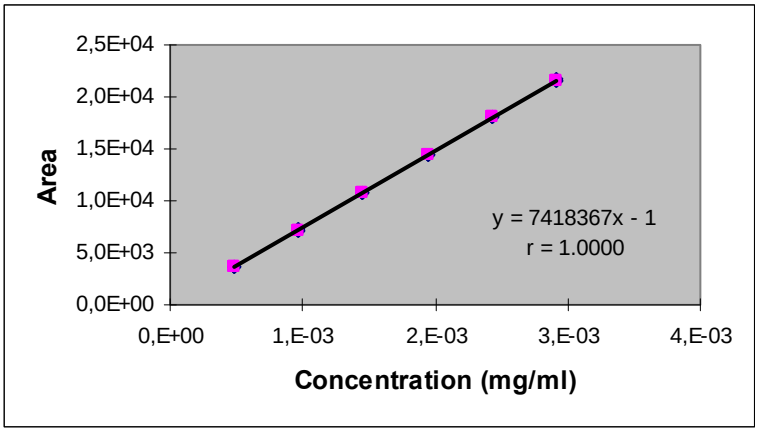


Figure 4. Atorvastatin Amid linearity study regression graph

Table 5. Defloro Atorvastatin linearity study results

Standard Concentration (%)	Prepared Standard Concentration (mg/ml)*e-4	Area mAU	Calculated Area mAU
0.1	4.624	3666	3564
		3659	3564
		3606	3564
0.2	9.249	7994	7418
		8039	7418
		8035	7418
0.3	13.873	10855	11273
		10929	11273
		10948	11273
0.4	18.498	14439	15127
		14525	15127
		14487	15127
0.5	23.122	18513	18982
		18551	18982
		18553	18982
0.6	27.747	23546	22836
		23598	22836
		23658	22836

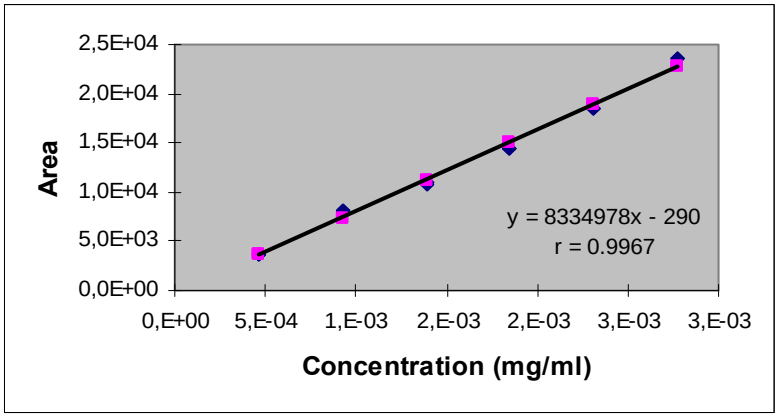


Figure 5. Defloro Atorvastatin linearity study regression graph

Table 6. Ketal Atorvastatin linearity study results

Standard Concentration (%)	Prepared Standard Concentration (mg/ml)*e-4	Area (mAU)	Calculated Area (mAU)
0.1	4.597	4404	4483
		4675	4483
		4531	4483
0.2	9.194	8983	8994
		9064	8994
		8968	8994
0.3	13.791	13552	13506
		13459	13506
		13476	13506
0.4	18.388	17966	18017
		17958	18017
		18083	18017
0.5	22.985	22637	22529
		22649	22529
		22710	22529
0.6	27.582	26903	27040
		26894	27040
		27028	27040

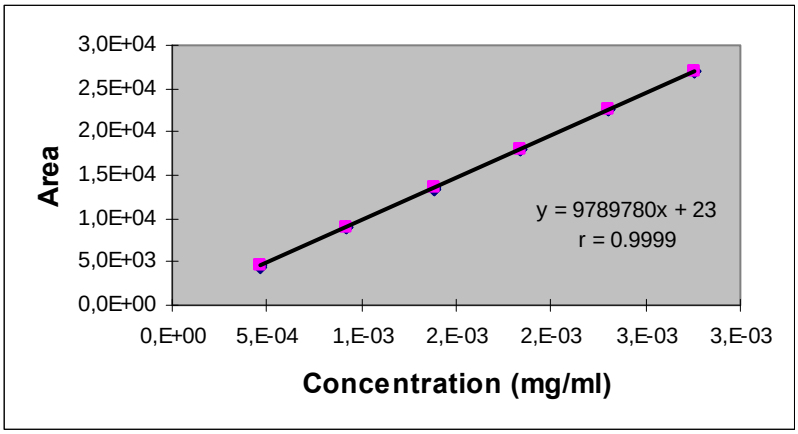


Figure 6. Ketal Atorvastatin linearity study graph

Table 7. Atorvastatin Lactone linearity study results

Standard Concentration (%)	Prepared Standard Concentration (mg/ml)*e-4	Area (mAU)	Calculated Area (mAU)
0.1	4.232	4552	4520
		4551	4520
		4579	4520
0.2	8.465	8848	8964
		8845	8964
		8839	8964
0.3	12.697	13435	13408
		13411	13408
		13632	13408
0.4	16.929	17819	17851
		17885	17851
		17867	17851
0.5	21.162	22235	22295
		22330	22295
		22350	22295
0.6	25.394	26762	26739
		26647	26739
		26744	26739

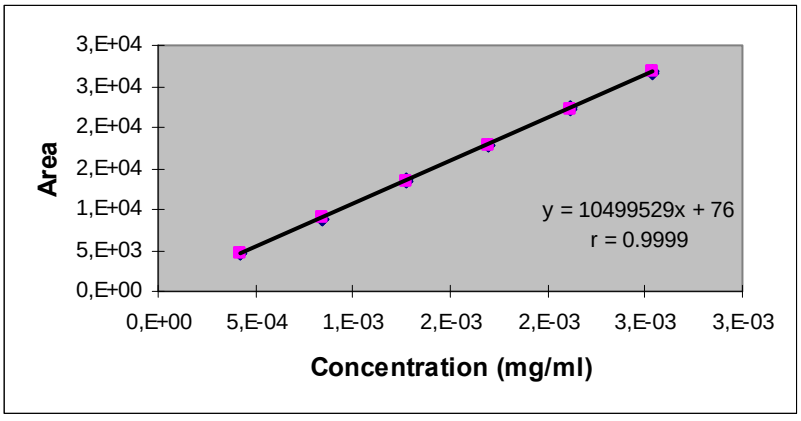


Figure 7.Atorvastatin Lactone linearity regression graph

Table 8. Atorvastatin Ca Methyl Ester linearity study results

Standart Concentration (%)	Prepared Standart Concentration (mg/ml)	Area mAU	Calculated Area (mAU)
0.1	4.762	5097	5105
		5070	5105
		5107	5105
0.2	9.524	10238	10234
		10249	10234
		10263	10234
0.3	14.286	15322	15363
		15375	15363
		15418	15363
0.4	19.048	20496	20492
		20494	20492
		20538	20492
0.5	23.810	25553	25621
		25517	25621
		25618	25621
0.6	28.572	30778	30750
		30709	30750
		30852	30750

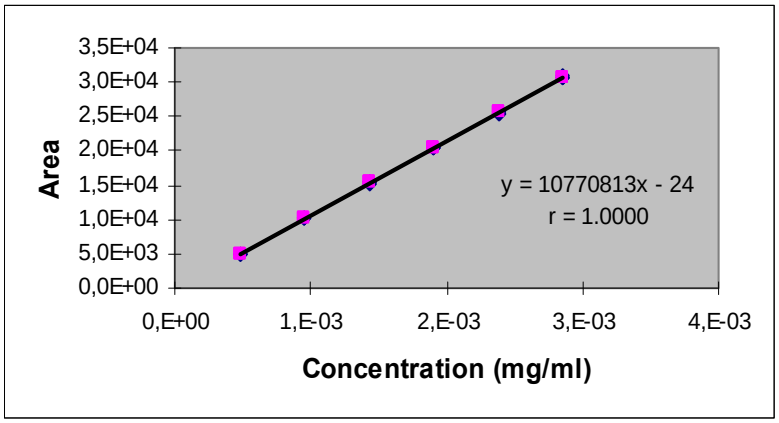


Figure 8. Atorvastatin Ca Methyl Ester linearity study regression graph

Table 9. O-methyl Atorvastatin Ca linearity study results

Standart Concentration (%)	Prepared Standart Concentration (mg/ml)	Area mAU	Calculated Area mAU
0.1	0.0003933	3111	3102
		3032	3102
		3036	3102
0.2	0.0007867	6437	6231
		6204	6231
		6125	6231
0.3	0.0011800	9368	9360
		9412	9360
		9404	9360
0.4	0.0015733	12471	12489
		12471	12489
		12527	12489
0.5	0.0019666	15591	15618
		15654	15618
		15634	15618
0.6	0.0023600	18751	18747
		18700	18747
		18710	18747

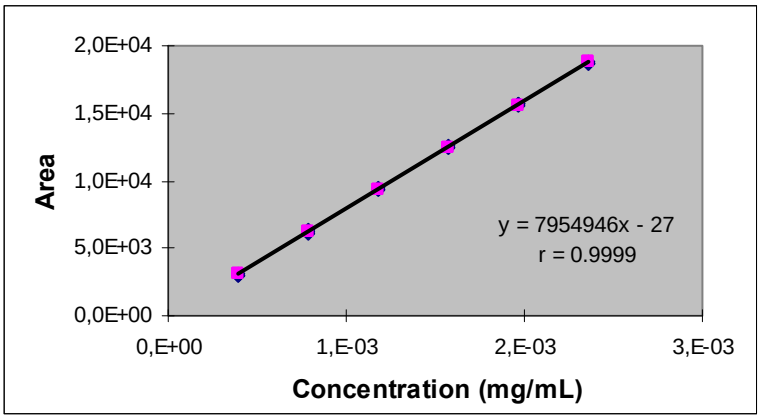


Figure 9. O-methyl Atorvastatin Ca linearity study regression graph

Table 10: Oxo-atorvastatin Ca linearity study results

Standart Concentration (%)	Prepared Standart Concentration (mg/ml)*e-4	Area mAU	Calculated Area mAU
0.1	4.702	507	461
		499	461
		413	461
0.2	9.404	1026	1051
		1064	1051
		1122	1051
0.3	14.106	1582	1640
		1593	1640
		1599	1640
0.4	18.808	2327	2230
		2293	2230
		2178	2230
0.5	23.510	2722	2820
		2774	2820
		2764	2820
0.6	28.212	3496	3409
		3430	3409
		3443	3409

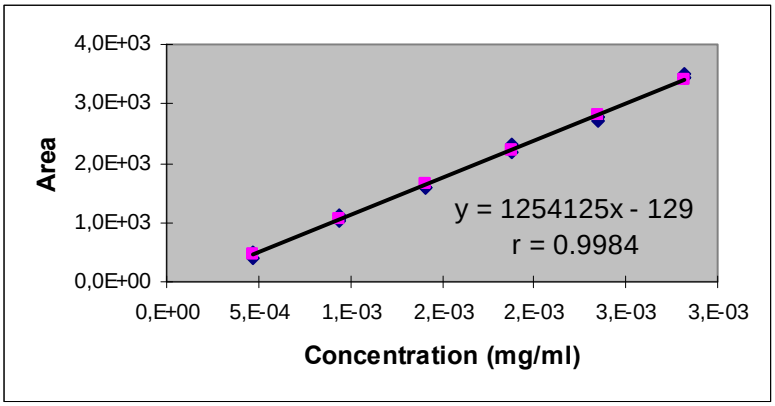


Figure 10. Oxo-atorvastatin Ca linearity study regression graph

Table 11. 3’ Deshydroxy atorvastatin accuracy study results

Level	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000242	0.000256	105.8	101.7
	0.000242	0.000226	93.4	
	0.000242	0.000256	105.8	
100	0,002417	0,002481	102,7	102,5
	0,002417	0,002472	102,3	
	0,002417	0,002481	102,7	
120	0,002900	0,002949	101,7	101,8
	0,002900	0,002954	101,9	
	0,002900	0,002949	101,7	

Table 12. Diastereomer atorvastatin accuracy study results

Level (%)	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000323	0.000316	97.8	96.2
	0.000323	0.000351	108.7	
	0.000323	0.000265	82.0	
100	0,002097	0,002043	97,5	96,6
	0,002097	0,002019	96,3	
	0,002097	0,002012	96,0	
120	0,002516	0,002469	98,1	97,7
	0,002516	0,002449	97,3	
	0,002516	0,002453	97,5	

Table 13. Atorvastatin Amid accuracy study results

Level	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000374	0.000375	100.3	100.8
	0.000374	0.000384	102.7	
	0.000374	0.000372	99.5	
100	0,002427	0,002411	99,4	100,3
	0,002427	0,002441	100,6	
	0,002427	0,002448	100,9	
120	0,002912	0,002897	99,5	99,1
	0,002912	0,002888	99,2	
	0,002912	0,002873	98,7	

Table 14. Desfloro Atorvastatin accuracy study results

Level	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000340	0.000335	98.5	97.6
	0.000340	0.000342	100.6	
	0.000340	0.000319	93.8	
100	0,002312	0,002640	114,2	112,8
	0,002312	0,002512	108,6	
	0,002312	0,002673	115,6	
120	0,002775	0,003160	113,9	113,7
	0,002775	0,003164	114,0	
	0,002775	0,003143	113,3	

Table 15: Ketal Atorvastatin accuracy study results

Level	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000414	0.000434	104.8	102.9
	0.000414	0.000422	101.9	
	0.000414	0.000422	101.9	
100	0,002299	0,002298	100,0	101,6
	0,002299	0,002375	103,3	
	0,002299	0,002334	101,6	
120	0,002758	0,002765	100,3	100,1
	0,002758	0,002768	100,3	
	0,002758	0,002747	99,6	

Table 16. Atorvastatin Lactone accuracy study results

Level	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000254	0.000239	94.1	99.7
	0.000254	0.000222	87.4	
	0.000254	0.000299	117.7	
100	0,002116	0,002082	98,4	99,7
	0,002116	0,002131	100,7	
	0,002116	0,002118	100,1	
120	0,002539	0,002521	99,3	99,0
	0,002539	0,002525	99,4	
	0,002539	0,002498	98,4	

Table 17. Atorvastatin Methyl ester accuracy study results

Level	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000286	0.000274	95.8	97.3
	0.000286	0.000275	96.2	
	0.000286	0.000286	100.0	
100	0,002381	0,002391	100,4	101,7
	0,002381	0,002428	102,0	
	0,002381	0,002447	102,8	
120	0,002857	0,002873	100,5	100,3
	0,002857	0,002866	100,3	
	0,002857	0,002858	100,0	

Table18. O-methyl atorvastatin accuracy study results

Level	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000393	0.000417	106.1	112.5
	0.000393	0.000487	123.9	
	0.000393	0.000423	107.6	
100	0,001967	0,001970	100,2	101,6
	0,001967	0,002012	102,3	
	0,001967	0,002015	102,5	
120	0,002360	0,002373	100,6	100,3
	0,002360	0,002363	100,1	
	0,002360	0,002363	100,1	

Table 19. Oxo- atorvastatin accuracy study results

Level	Theoretical concentration (mg/ml)	Experimental concentration (mg/ml)	Recovery (%)	Average (%)
LOQ	0.000476	0.000493	103.6	100.9
	0.000476	0.000452	95.0	
	0.000476	0.000496	104.2	
100	0,002351	0,002316	98,5	100,9
	0,002351	0,006988	297,2	
	0,002351	0,002428	103,3	
120	0,002821	0,002810	99,6	99,6
	0,002821	0,002812	99,7	
	0,002821	0,002806	99,5	

Table 20. Repeability study results

Sample	Atorvastatin Amid (%)	Desfloro Atorvastatin Ca (%)	Oxo Atorvastatin Ca (%)	Diastereomer Atorvastatin Ca (%)	O-Methyl Atorvastatin Ca (%)
1.	0.43	0.46	0.40	0.20	0.39
2.	0.46	0.49	0.46	0.21	0.44
3.	0.44	0.47	0.42	0.20	0.43
4.	0.45	0.48	0.44	0.21	0.43
5.	0.45	0.47	0.44	0.21	0.43
6.	0.46	0.48	0.45	0.20	0.44
ORT.	**0.45**	**0.48**	**0.44**	**0.21**	**0.43**
SD	**0.012**	**0.010**	**0.022**	**0.005**	**0.019**
% RSD	**2.6**	**2.2**	**5.0**	**2.7**	**4.4**

Table 21. Repeability study results (continued)

Sample	3'Deshydroxy Atorvastatin Ca (%)	Atorvastatin Lactone (%)	Methyl Ester Atorvastatin Ca (%)	Ketal Atorvastatin (%)	Maximum Unknown impurity (%)	Total Unknown impurity (%)
1.	0.42	0.39	0.41	0.34	0.17	0.43
2.	0.44	0.42	0.44	0.37	0.19	0.48
3.	0.43	0.39	0.43	0.35	0.18	0.45
4.	0.43	0.39	0.43	0.36	0.18	0.44
5.	0.43	0.40	0.43	0.36	0.18	0.50
6.	0.44	0.40	0.44	0.36	0.18	0.51
ORT.	**0.43**	**0.40**	**0.43**	**0.36**	**0.18**	**0.47**
SD	**0.008**	**0.012**	**0.011**	**0.010**	**0.006**	**0.033**
% RSD	**1.7**	**2.9**	**2.5**	**2.9**	**3.5**	**7.1**

Table 22. Atorvastatin Ca solution stability test results at +5^{O}C

Time ()	Atorvastatin		
	Concentration (%)	Change (%)	Area
0.	100.0	-	53191
2.	98.8	1.2	52543
4.	97.3	2.7	51749
6.	96.3	3.7	51200
8.	95.5	4.5	50811
10.	94.9	5.1	50469
12.	94.5	5.5	50268
16.	93.5	6.5	49753
20.	93.5	6.5	49730
24.	93.2	6.8	49559
28.	93.1	6.9	49520
32.	93.3	6.7	49652
36.	93.1	6.9	49528
40.	93.6	6.4	49802
44.	94.2	5.8	50118
48.	94.8	5.2	50430

Table 23. 3’ Deshydroxy solution stability test results at +5^{O}C

3'DESHİDROKSİ			
Time (hr)	Area (-1896)	Result	%Change
0.	20613	0,42	-4,76
5.	21797	0,44	-4,76
7.	21609	0,44	-4,76
9.	20910	0,43	-2,38
11.	20986	0,43	-2,38
13.	21757	0,44	-4,76
15.	20271	0,41	2,38
17.	21039	0,43	-2,38
19.	20646	0,42	0,00
21.	20959	0,43	-2,38
23.	20576	0,42	0,00

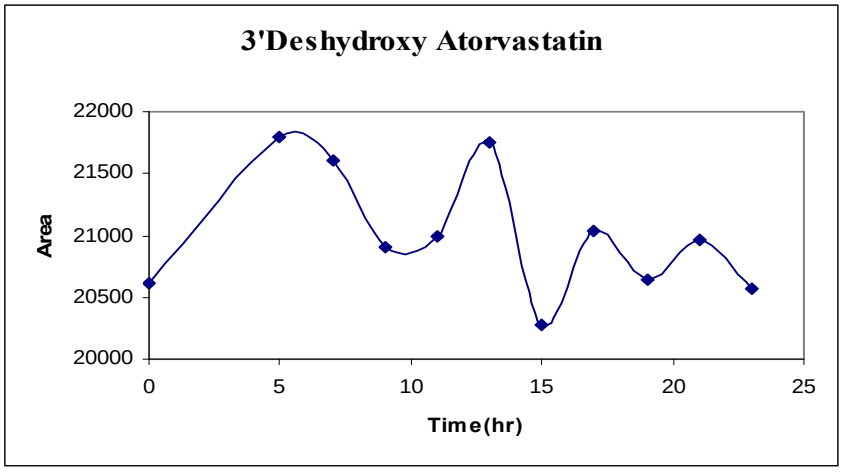


Figure 11. 3' Deshydroxy solution stability test results at +5^{O}C

Table 24. Diastereomer solution stability test results at +5^{O}C

Diastereomer Atorvastatin Ca			
Time(hr)	**Area**	**Result**	**%Change**
0.	5811	0,20	-5,00
5.	6009	0,21	-5,00
7.	6015	0,21	-5,00
9.	5903	0,20	0,00
11.	5866	0,20	0,00
13.	6010	0,21	-5,00
15.	5665	0,19	5,00
17.	5900	0,20	0,00
19.	5884	0,20	0,00
21.	5910	0,20	0,00
23.	5850	0,20	0,00

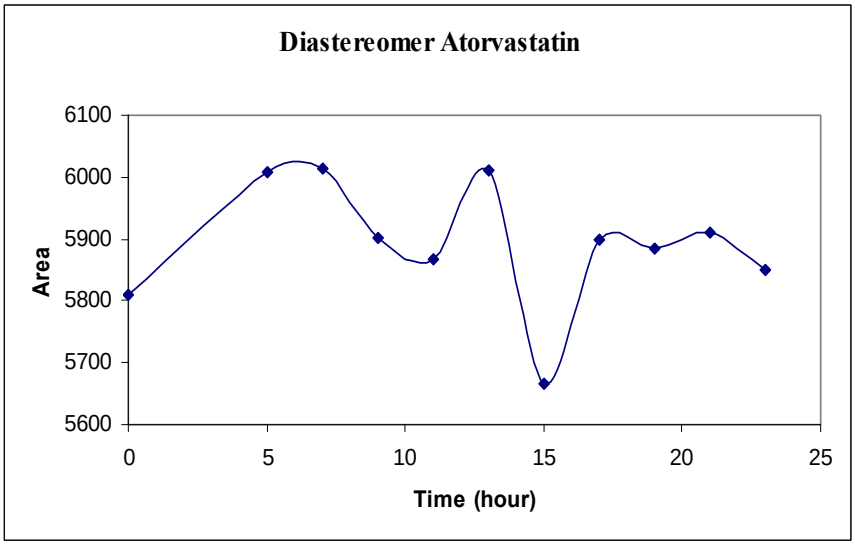


Figure 12. Diastereomer solution stability test graph at +5^{O}C

Table 25. Atorvastatin Amide solution stability test results at +5^{O}C

Atorvastatin Amide			
Time (hr)	**Area (-3632)**	**Result**	**Change %**
0.	20463	0,43	-4,7
5.	21452	0,45	-4,7
7.	21563	0,45	-4,7
9.	21128	0,44	-2,3
11.	21029	0,44	-2,3
13.	21669	0,46	-7,0
15.	20242	0,42	2,3
17.	21130	0,44	-2,3
19.	21011	0,44	-2,3
21.	21094	0,44	-2,3
23.	20878	0,44	-2,3

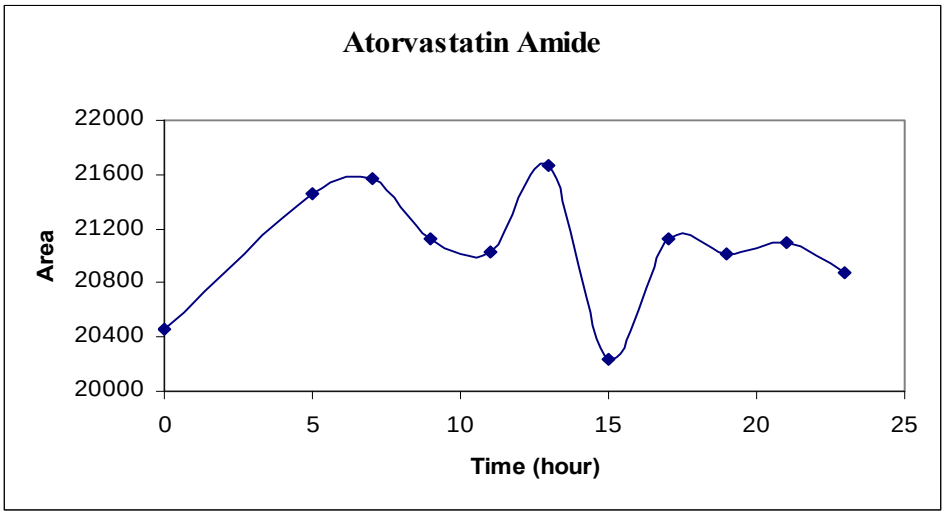


Figure 13. Atorvastatin Amide solution stability test graph at +5^{O}C

Table 26. Desfloro solution stability test results at +5^{O}C

Desfluoro Atorvastatin Ca			
Time (hr)	**Area (-1800)**	**Result**	**%Change**
0.	22390	0,46	-6,5
5.	23580	0,49	-6,5
7.	23702	0,49	-6,5
9.	23177	0,48	-4,3
11.	23074	0,48	-4,3
13.	23829	0,49	-6,5
15.	22212	0,46	0,0
17.	23188	0,48	-4,3
19.	23090	0,48	-4,2
21.	23186	0,48	-4,3
23.	22912	0,47	-2,2

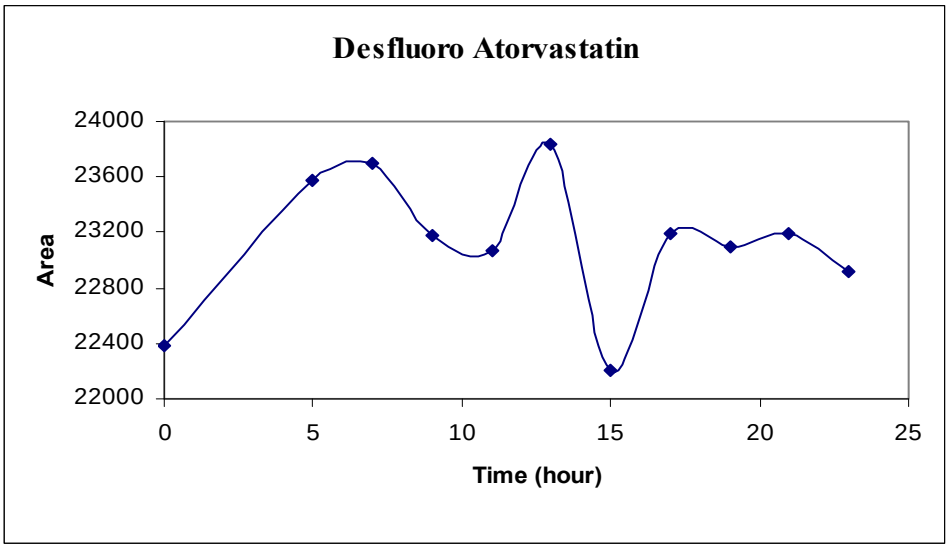


Figure 14. Desfluoro Atorvastatin solution stability graph at +5^{O}C

Table 27.Ketal Atorvastatin solution stability test results at +5^{O}C

Ketal Atorvastatin			
Time (hr)	**Area (-492)**	**Result**	**Change %**
0.	18545	0,34	-5,9
5.	19317	0,36	-5,9
7.	19446	0,36	-5,9
9.	19008	0,35	-2,9
11.	18996	0,35	-2,9
13.	19553	0,36	-5,9
15.	18260	0,34	0,0
17.	19111	0,36	-5,9
19.	19164	0,36	-5,9
21.	19130	0,36	-5,9
23.	18971	0,35	-2,9

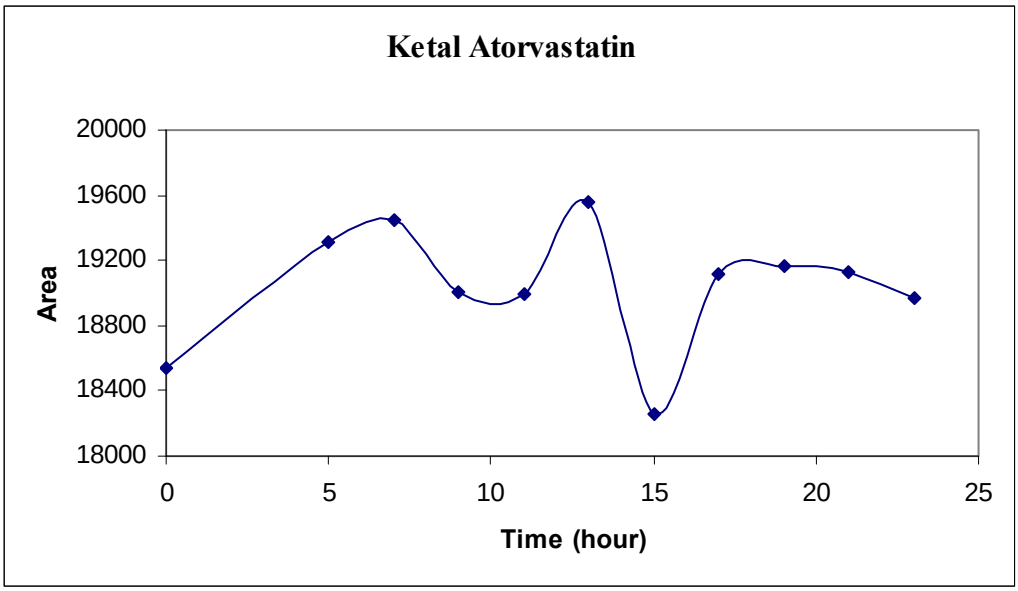


Figure 15. Ketal Atorvastatin solution stability graph at +5^{O}C

Table 28. Atorvastatin Lactone solution stability test results at +5^{O}C

Atorvastatin Lactone			
Time (hr)	**Area (-6255)**	**Result**	**Change %**
0.	27730	0,39	-2,6
5.	28652	0,40	-2,6
7.	28899	0,41	-5,1
9.	28079	0,39	0,0
11.	28087	0,39	0,0
13.	29030	0,41	-5,1
15.	26913	0,37	5,1
17.	28101	0,39	0,0
19.	27913	0,39	0,0
21.	28134	0,39	0,0
23.	27753	0,39	0,0

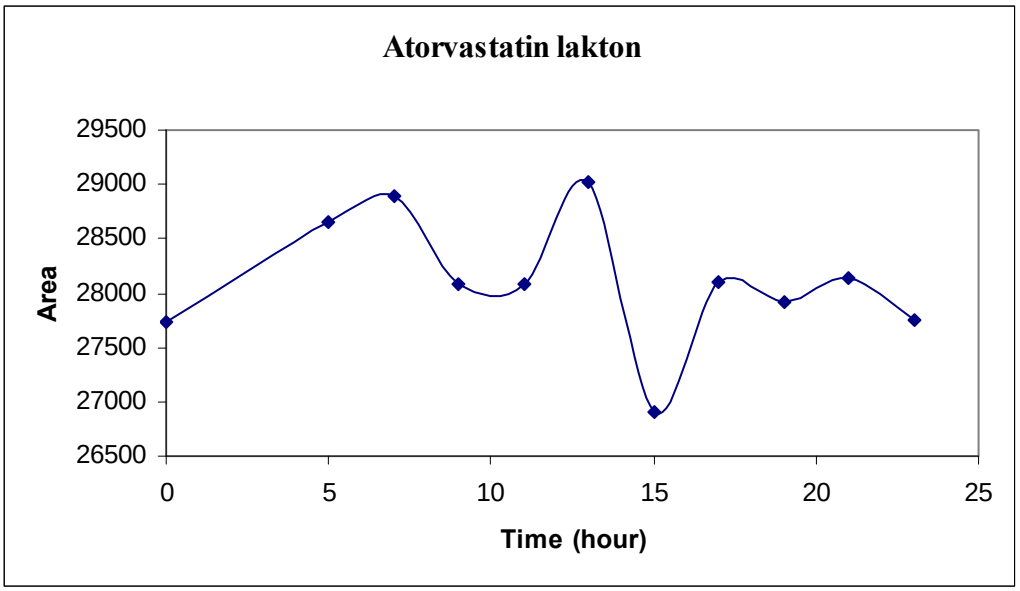


Figure 16. Atorvastatin Lactone solution stability graph at +5^{O}C

Table 29. Methyl Ester solution stability test results at +5^{O}C

Atorvastatin Methyl ester			
Time (hr)	**Area (-2503)**	**Result**	**Change %**
0.	26234	0,41	-7,3
5.	27501	0,44	-7,3
7.	27770	0,44	-7,3
9.	27115	0,43	-4,9
11.	27041	0,43	-4,9
13.	27943	0,44	-7,3
15.	26012	0,41	0,0
17.	27248	0,43	-4,9
19.	27099	0,43	-4,9
21.	27199	0,43	-4,9
23.	26898	0,43	-4,9

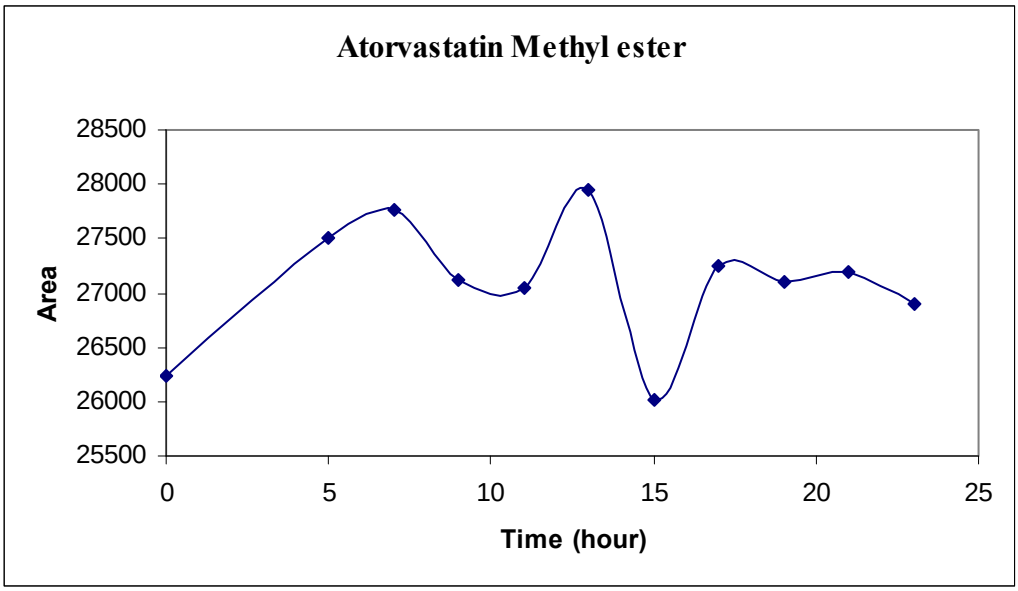


Figure 17. Atorvastatin merthyl ester solution stability graph at +5^{O}C

Table 30. O-methyl Atorvastatin solution stability test results at +5^{O}C

O-methyl Atorvastatin			
Time (hr)	**Area (-5742)**	**Result**	**Change %**
0.	22370	0,39	-12,8
5.	24308	0,44	-12,8
7.	24619	0,45	-15,4
9.	24134	0,43	-10,3
11.	24004	0,43	-10,3
13.	24818	0,45	-15,4
15.	23196	0,41	-5,1
17.	24058	0,43	-10,3
19.	24352	0,44	-12,8
21.	24417	0,44	-12,8
23.	23841	0,43	-10,3

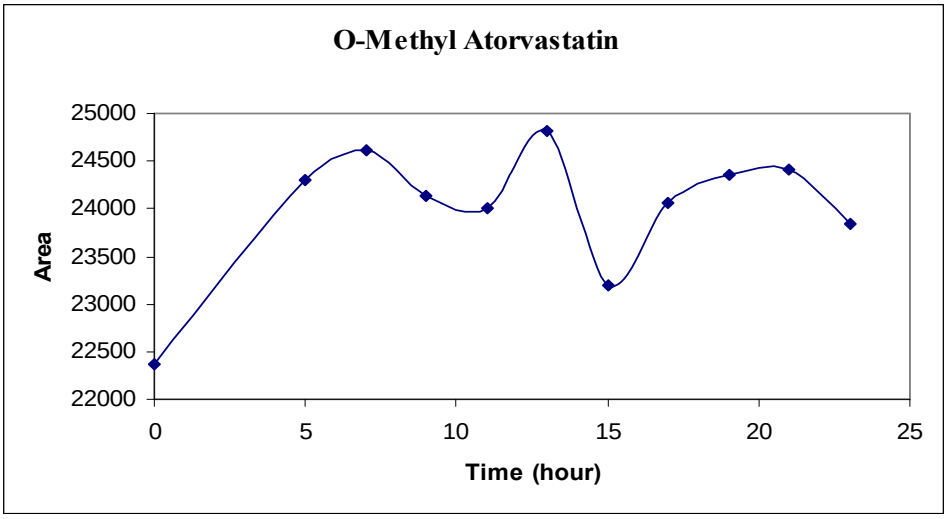


Figure 18. O-methyl Atorvastatin solution stability graph at +5^{O}C

Table 31. Oxo-atorvastatin Ca solution stability test results at +5^{O}C

Oxo-atorvastatin Ca			
Time (hr)	**Area (-3504)**	**Result**	**%Change**
0.	6173	0,40	-22,5
5.	6740	0,49	-22,5
7.	6837	0,50	-25,0
9.	6712	0,48	-20,0
11.	6752	0,49	-22,5
13.	7014	0,53	-32,5
15.	6631	0,47	-17,5
17.	6977	0,52	-30,0
19.	7039	0,53	-32,5
21.	7129	0,55	-37,5
23.	7095	0,54	-35,0

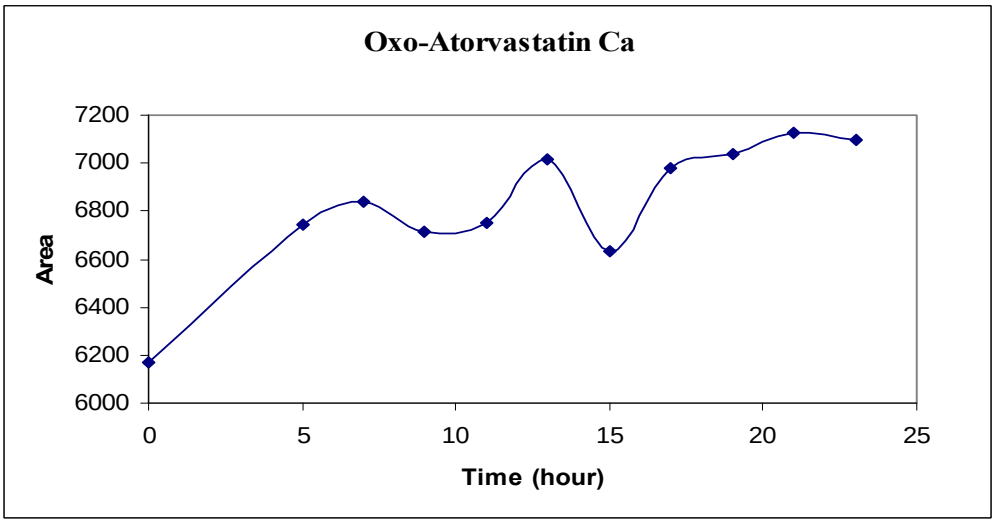


Figure 19. Oxo-atorvastatin Ca solution stability graph at +5^{O}C

Table 32. The percentage of the patients according to reference limit values

FEMALE					
HDL	**Range**	**0-30**	**30-80**	**80-∞**	**Total**
	Number of Patients	5	1258	121	1384
	Percentage	0,361	90,896	8,743	
LDL	**Range**	**0-50**	**50-173**	**173-∞**	**Total**
	Number of Patients	0	1360	77	1437
	Percentage	0	94,642	5,358	
Cholesterol	**Range**	**0-200**	**200-∞**		**Total**
	Number of Patients	779	928		1707
	Percentage	45,636	54,364		
Triglyceride	**Range**	**0-35**	**35-135**	**135-∞**	**Total**
	Number of Patients	6	996	695	1697
	Percentage	0,354	58,692	40,955	
MALE					
HDL	**Range**	**0-30**	**30-80**	**80-∞**	**Total**
	Number of Patients	28	984	67	1079
	Percentage	2.59	91.19	5.75	
LDL	**Range**	**0-50**	**50-173**	**173-∞**	**Total**
	Number of Patients	1	1105	47	1153
	Percentage	0.086	91.066	4.07	
Cholesterol	**Range**	**0-200**	**200-∞**		**Total**
	Number of Patients	693	690		1383
	Percentage	50.11	49.89		
Triglyceride	**Range**	**0-40**	**40-160**	**160-∞**	**Total**
	Number of Patients	8	801	567	1376
	Percentage	0,581	58,212	41,206	