Review on Simultaneous Estimation of Fixed Dose Combination: System Suitability Parameter of RP-HPLC Analysis

By

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A thesis submitted to the Department of Pharmacy in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

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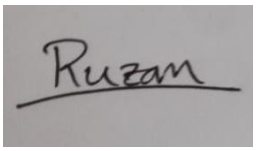

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Approval

The thesis titled "Review on Simultaneous Estimation of Fixed Dose Combination: System suitability parameter of RP-HPLC Analysis" submitted by Ruzan Islam (18346018), of Spring, 2022 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study does not involve any kind of animal or human trial. This material is the authors' own original work, which has not been previously published elsewhere.

Abstract

In modern pharmaceuticals, Reverse Phase- High performance Liquid Chromatography (RP-HPLC) is one of the most essential tools. When analyzing, attempting to separate, and identifying compounds from a complicated mixture, RP-HPLC is the most advantageous option because it permits purification of most classes of compound. For any chromatography system suitability is required and are designed to assess the analytical system's constituent parts and demonstrate that its performance satisfies the criteria set forth by the method. 12 articles were picked from number of reliable sources and journals where RP-HPLC was used to asses combination drugs in solid dosage form. In this review article, the system suitability of those selected studies is reviewed on the basis of USP guideline in order to criticize and evaluate the robustness of the method. If any incomplete or misleading information was identified, the possible cause and solution for those methodologies were explained in this review.

Keywords: Reverse Phase- High performance Liquid Chromatography (RP-HPLC), System suitability, Combination drugs.

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List of Acronyms

RP-HPLC: Reverse phase- High performance Liquid Chromatography

LC: Liquid Chromatography

SST: System Suitability Test

USP: United State Pharmacopeia

RSD Relative Standard Deviation

T_f : Tailing Factor

Rs: Resolution

PDA: Photo-diode array

ACN: Acetonitrile

UV: Ultra-violet

LOD: Limit of quantification

LOQ: Limit of quantification

CDER: Centre for Drug Evaluation and Research

SST: System suitability

CHAPTER 1: INTRODUCTION

1.1 REVERSE PHASE CHROMATOGRAPHY

Chromatography is most widely used and versatile analytical technique for compound separation, identification, qualification and quantification. Although, there are many types of chromatographic technique, Reverse phase- High performance Liquid Chromatography (RP-HPLC) is the most common in pharmaceutical industries. It separates substances that are dissolved in a liquid sample, allowing for qualitative and quantitative study of the components and amounts of each. It is a method of liquid chromatography that separates molecules based on hydrophobic interactions between the ligands bound to the stationary phase and the solute molecules in the mobile phase (Shaffer, n.d.). In RP-HPLC, the stationary phase is covalently bound to alkyl or aromatic ligands to create a hydrophobic surface. Here the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e., the stationary phase, is what drives the separation mechanism. (Eriksson, 2018, p. 06). A polar solvent that contains the solutes is the mobile phase which moves over the hydrophobic stationary phase. In this case, the basis of separation is usually formed by hydrophobic interactions between hydrophobic solutes in the mobile phase and the stationary phase. (Shaffer, 2021).

Reverse phase- High performance Liquid Chromatography (RP- HPLC) has two phases: Mobile phase, and stationary phase. The mobile phase here is polar whereas the stationary phase is non-polar.

In reversed phase chromatography, the mobile phase is usually aqueous, indicating a highly organized water structure surrounds both the solute molecule and the immobilized ligand. The amount of hydrophobic surface that is exposed to the solvent is reduced when the solute binds to the immobilized hydrophobic ligand. Consequently, the level of organized water structure is reduced with a corresponding favorable increase in system entropy. In this way, it is advantageous from an energy point of view for the hydrophobic moieties, i.e., solute and ligand, to associate (Eriksson, 2018, p. 06).

The solute mixture is first applied to the sorbent; the solutes are then eluted by passing the mobile phase. Two types of elution can be carried out. One is gradient elution, in which the proportion of organic solvent in mobile phase is raised gradually over time, and other one is isocratic elution, in which the concentration of organic solvent is constant. (Neha, 2021).

1.2 PHYSICOCHEMICAL PROPERTIES

In the review, the physicochemical properties of the compounds assayed by RP-HPLC methods are taken into consideration. The main parameters that are considered are logP & pKa. When developing an HPLC technique, an analyte's physicochemical characteristics are crucial. Studying the physical characteristics of the drug molecule, such as its solubility, polarity, pKa and logP, is necessary for the development of chromatography techniques.

The maximum amount of a material that may be completely dissolved in a given amount of solvent is known as its solubility. The range of possible intermolecular interactions and the structure of medicinal compounds can both be significantly explained by solubility (Gong & Brittain, 2007).

LogP indicates how readily an analyte will partition between an aqueous and organic phase. A molecule's or a molecule's moiety's affinity for a lipophilic environment is referred to as lipophilicity. The logarithmic form of a chemical's partition coefficient between octanol and water called log P, provides a measurement of its lipophilicity. log P value refers to the lipophilicity of unionized species. The logarithmic form of a chemical's partition coefficient between octanol and water, or log P, provides a measurement of its lipophilicity. Higher value of log P indicates higher lipophilicity (Supriyo & Dilipkumar, 2017).

A molecule's level of acidity is indicated by the pKa value. A proton's retention on a Bronsted acid is used to measure an acid's strength. The strength of the acid and its capacity to donate protons increase with decreasing pKa values. If pKa is used to assess an acid's or base's strength, the stronger the acid is the lower the pKa value; the stronger the base is the higher the pKa value (Watson, 2005). The stronger acid has greater the ionization. The lower the pKa will produce lower pH the compound in solution.

1.3 SYSTEM SUITABILITY PARAMETERS (SST) OF RP-HPLC

The system suitability test (SST) is a test that determines the compatibility and effectiveness of a chromatographic system prior to use. Any chromatographic system's performance is susceptible to ongoing change when used regularly, which might compromise the accuracy of the findings of analytical procedures. With carefully chosen SST combinations, the operation parameters of the entire chromatographic system can be verified. These combinations are used to determine common chromatographic parameters like limit of detection (LOD), limit of quantification (LOQ), resolution, capacity, tailing, and RSD as well as the number of

effective theoretical plates. The system is then deemed appropriate if the responses fall within predetermined limits (Tiryaki et al., 2009).

Before using a chromatographic system, its appropriateness and efficacy are tested via the System Suitability Test (SST). Any chromatographic system's performance is susceptible to ongoing change when used regularly, which might compromise the accuracy of the findings of analytical procedures. With carefully chosen SST combinations, the operation parameters of the entire chromatographic system may be verified. These mixtures are used to determine common chromatographic properties including resolution, asymmetry, detection limit, and selectivity. The system is then only considered appropriate if the results fall inside predetermined limits. In the review, I reviewed Reverse phase- high performance liquid chromatography (RP-HPLC) techniques that have been used for the analysis of tablet dosage forms.

I assessed the following system suitability parameters which are Repeatability (RSD- relative standard deviations), Capacity factor (k'), Resolution (R_s), Tailing factor (T) and Theoretical plate number (N).

These parameters are crucial because they illustrate system specificity, accuracy, and column stability.

The capacity factor is a measure of where the peak of interest is located with respect to the void volume, i.e., elution time of the non-retained components. In order to calculate or quantify how much interaction the solute (sample peak) has with the stationary phase material; chromatography uses a capacity factor or ratio (the relative time interacting with the support vs. the mobile phase). If this contact is too brief, no retention occurs (Dejaegher et al., 2021).

The equation for capacity factor is $k' = \frac{tttt - tt0}{tt0}$ where tr is the retention time of the target and t_0 is the unretained peak time

When comparing a collection of data's standard deviation to the mean, relative standard deviation—also known as RSD or the coefficient of variation—is used to assess whether it is little or big. In other words, one can determine how accurate the average of the data is by looking at the relative standard deviation. The performance of the HPL chromatograph, which comprises the plumbing, column, and ambient conditions, as well as the time the

samples are analyzed, is shown by injection precision given as RSD (relative standard deviation). It should be noted that differences in production and sample preparation are not taken into account.

The equation for calculating RSD as per USP <621> is,

$$\%\,RRRRRR = \frac{100}{yy} \sqrt{\frac{\Sigma\Sigma(yy_{ii} - yy)^2}{nn - 1}}$$

Where, yy_{ii} = individual values expressed as peak area, peak height, or ratio of areas by the internal standardization method

y= mean of individual values

n = number of individual values

An indicator of how well two peaks is separated is called resolution (Rs). Well-separated peaks are necessary for quantitation in order to be accurate. If probable interference peak(s) are something to be concerned about, this metric is highly helpful.

The equation for resolution is,

$$RRRR = \frac{(\mathrm{tR2} - \mathrm{tR1})}{(\frac{1}{2})\,(\mathrm{tw1} + \mathrm{tw2})}$$

Where, tR2 and tR1 are the retention times of closely eluted compounds, and Wb1 and Wb2 are their peak widths at the base.

Peak tailing is a common occurrence that can affect how accurately a chromatographic system's accuracy is estimated because it can be challenging to integrate data depending on where the peak stops.

Knowing where the upslope and downslope are is essential; otherwise, accuracy will decrease. Due to difficulties the integrator encounters in determining where the peal ends and subsequently estimating the area beneath the peal, the accuracy of quantitation decreases as

peak tailing rises. For the most accurate estimation of the peak of interest area, the analyst specifies the integrator variables (SiliCycle, 2022).

The equation for tailing factor is, $TTTT = \frac{(aa+bb)}{2aa}$,

Where, a= distance between the leading edge of the peak and the peak midpoint (perpendicular from the peak highest point) (SiliCycle, 2022).

b= distance between the peak midpoint (perpendicular from the peak highest point) and the trailing edge of the peak (SiliCycle, 2022).

Theoretical plate number (N) is an index that represents column efficiency. It describes the number of plates as defined by plate theory and can be used to calculate column efficiency using a calculation where the sharper the peaks, the higher the theoretical plate number. Peak location, particle size in column, flow-rate of mobile phase, column temperature, viscosity of mobile phase, and analyte molecular weight are all variables that might impact N (Tiryaki et al., 2009).

The equation for Theoretical plate number is, $N= 16 (\frac{tttt}{ttt})^2 = \frac{LL}{HH}$

Where, H is the height equivalent of a theoretical plate and L is the column efficiency per unit length of the column (Dejaegher et al., 2021).

Table 1: System Suitability parameter recommendations as per USP guideline

System suitability parameter	Recommendations (USP)
Capacity factor (k')	> 2
RSD	<1% for (n ≥ 5)
Resolution (Rs)	> 2
Tailing factor (T)	≤ 2
Theoretical plate number (N)	> 2000

The USP validation process was used in the review since it is often used. The process of validating a new analytical procedure for compendial usage is addressed in USP general Chapter 1225 – "Validation of Compendial Procedures". Even with a completely validated

technique, the end-user might not be certain that it can be used with a specific component or product in a particular laboratory with a specific set of staff, tools, consumables, and reagents. By establishing a mechanism for evaluating the applicability of compendial processes, USP's proposed new general chapter 1226, titled "Verification of Compendial Procedures," aims to close the gap in the correct use of compendial procedures. (United States Pharmacopeial Convention., 2007)

Validation of Compendial Procedure <1225> of USP, defines analytical performance characteristics, recommends data for submission to USP-NF as well as provides guidance on which analytical performance characteristics are needed based on the type of test. Besides incorporates ICH guidelines Q2(R1) (Validation of Analytical Procedures: Text and Methodology) and Q2(R2) (Analytical Procedure Development and Revision of Q2R1 Analytical validation) (United States Pharmacopeial Convention., 2007). The aim of validating an analytical method is to show that it is appropriate for the purpose for which it is being used. Q2(R1) indicates the validation requirements needed for various analytical techniques (ICH Q2B, n.d.).

CHAPTER 2: METHODOLOGY

Each piece of information for this review study was gathered following a literature search. The material was gathered from a number of reliable sources, including peer-reviewed papers and an online scholarly database. The databases that were searched for this study are SpringerLink, Hindawi, Wiley, PubMed and ScienceDirect. Relevant papers were collected using appropriate keywords such as ‘simultaneous estimation’, ‘RP-HPLC’, ‘combination drug’ etc. Around 60 articles were collected based on their title and content. Then 12 papers that made up this review research were carefully selected and examined.

CHAPTER 3: LITERATURE REVIEW

In this review study, the use of RP-HPLC in the assay of drug combination and the system suitability parameters were reviewed. The products that were assessed in those articles are given below along with their physicochemical properties and uses.

Table 2: Class of the combinational drugs and uses

SL	Drug name	Drug type	Uses	References
1	Nitazoxanide	Antiprotozoal drug	Treats giardiasis and Cryptosporidium-related diarrhea.	(Gadapa & Tripathi, 2012)
	Ofloxacin	Fluoroquinolone ne antibiotics	Treats pneumonia, infections of the skin, bladder, reproductive organs, and prostate.	
2	Aliskiren Hemifumarate	Direct renin inhibitor	Treats hypertension, risk of heart attacks, renal issues, and strokes.	(Gajula et al., 2021)
	Amlodipine Besylate	Anti-hypertensive, calcium channel blocker	Treats angina, coronary artery disease, hypertension.	
3	Ofloxacin	Fluoroquinolone ne antibiotics	Treats pneumonia, infections of the skin, bladder, reproductive organs, and prostate.	(Dhandapani et al., 2010)
	Ornidazole	antibiotics	Treats post-operative infection, amoebiasis, chlamydiosis, giardia, and trichomonas infections	
4	Lansoprazole	Proton pump inhibitor	Treats gastroesophageal reflux disease, heartburn, acid reflux, and indigestion	(Patel et al., 2015)
	Domperidone	Antiemetic	Treats nausea, vomiting & stomach pain	

5	Aceclofenac	NSAID	Treat osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis	(K.A. Shaikh et al., 2008)
	Paracetamol	Antipyretic, analgesic	Treat mild pain, headaches, toothaches, and sprains & fevers	
	Chlorzoxazone	NSAID	Relieve the discomfort, painful muscle or bone conditions.	
6	Atorvastatin	HMG-CoA reductase inhibitors	reduce the risk of heart attack and stroke	(Ganeshbhai CHAUDHARI et al., 2007)
	Amlodipine	calcium channel blockers	lowers blood pressure, controls chest pain	
7	Paracetamol	Antipyretic, analgesic	Treat mild pain, headaches, toothaches, and sprains & fevers	(Pattan et al., 2015)
	Etoricoxib	NSAID	Treat osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and gout-related pain, swelling, and inflammation	
8	Atorvastatin Calcium	HMG-CoA reductase inhibitors	reduce the risk of heart attack and stroke	(Bhinge et al., 2012)
	Fenofibrate	antilipemic agents	lower and treat high blood triglyceride and cholesterol levels	
9	Gemcitabine Hydrochloride	Chemotherapeutic agent	Used in chemotherapy in breast cancer	(Rajesh et al., 2011)
	Capecitabine Hydrochloride	Chemotherapeutic agent	Used in chemotherapy in breast cancer and colorectal cancer	
10	Rosiglitazone	Antidiabetic agent	help regulate blood sugar levels.	(Rathinavel et al., 2009)
	Gliclazide	Antidiabetic agent	treat non-insulin-dependent diabetes	
11	Atorvastatin Calcium	HMG-CoA reductase	reduce the risk of heart attack and	(Suma et al., 2012)

		inhibitors	stroke	
	Aspirin	NSAID	treat of pain and fever	
12	Ramipril	Angiotensin-converting-enzyme inhibitors	treat high blood pressure and heart failure.	(Sharma et al., 2012)
	Aspirin	NSAID	treat of pain and fever	
	Atorvastatin	HMG-CoA reductase inhibitors	reduce the risk of heart attack and stroke	

The Following table 3 contains, physiochemical properties of drugs including their pKa and logP value.

Table 3: Physiochemical property

SL	Drug name	pKa	logP	References
1	Nitazoxanide	9.98	10.62	(Gadapa & Tripathi, 2012)
	Ofloxacin	9.99	0.65	
2	Aliskiren Hemifumarate	9.100	3.12	(Gajula et al., 2021)
	Amlodipine Besylate	9.101	1.64	
3	Ofloxacin	9.96	0.65	(Dhandapani et al., 2010)
	Ornidazole	9.97	0.26	
4	Lansoprazole	9.109	3.03	(Patel et al., 2015)
	Domperidone	9.110	2.9	
5	Aceclofenac	9.104	3.88	(K.A. Shaikh et al., 2008)
	Paracetamol	9.105	0.91	
	Chlorzoxazone	9.106	1.94	
6	Atorvastatin	9.102	5.39	(Ganeshbhai CHAUDHARI et al., 2007)
	Amlodipine	9.103	1.64	
7	Paracetamol	9.111	0.91	(Pattan et al., 2015)

	Etoricoxib	9.112	2.79	
8	Atorvastatin Calcium	4.46	6.98	(Bhinge et al., 2012)
	Fenofibrate	-4.9	4.86	
9	Gemcitabine Hydrochloride	5.38	0.28	(Rajesh et al., 2011)
	Capecitabine Hydrochloride	8.63	1.17	
10	Rosiglitazone	6.84	2.95	(Rathinavel et al., 2009)
	Gliclazide	5.8	1.52	
11	Atorvastatin Calcium	4.46	4.24	(Suma et al., 2012)
	Aspirin	3.41	1.24	
12	Ramipril	3.75	0.92	(Sharma et al., 2012)
	Aspirin	3.41	1.24	
	Atorvastatin	4.46	4.24	

In table 4, the chromatographic conditions are given including drug name, the HPLC name, the column & solvent used in the method development.

Table 4: Chromatographic Conditions

SL	Drug	HPLC System	Column	Solvent	References
1	Nitazoxanide	Schimadzu LC-2010 CHT	Luna C18 (250mm x 4.6mm, 5µm)	Orthophosphoric acid buffer & Acetonitrile (40:60)	(Gadapa & Tripathi, 2012)
	Ofloxacin				
2	Aliskiren Hemifumarate	Waters 2695 HPLC system	Inertsil ODS C-8 (150 × 4.6 mm,5 µm)	phosphate buffer and acetonitrile in the ration of40: 60%	(Gajula et al., 2021)
	Amlodipine Besylate				
3	Ofloxacin	Shimadzu LC-20 AT	Phenomenex C18 (250 mm x 4.6 mm i.d, 5 µm)	phosphate buffer and Acetonitrile ration o 70: 30, (pH- 3.5)	(Dhandapani et al., 2010)
	Ornidazole				
4	Lansoprazole	Isocratic HPLC (Perkin Elmer, USA) pump.	RP-C18 (2.27µm size, 250 mm´4.6 mm i.d.)	Acetonitrile: Methanol (81:19)	(Patel et al., 2015)
	Domperidone				

5	Aceclofenac Paracetamol Chlorzoxazone	A Jasco HPLC	Zorbax SB C18 (250 x 4.6 mm, 5 µm)	acetonitrile and buffer (40:60, v/v)	(K.A. Shaikh et al., 2008)
6	Atorvastatin Amlodipine	HPLC Hitachi pump L-7110	Lichrospher® 100 C18 (250mm x 4.0 mm i.d.,5µm)	(Isocratic) Acetonitrile and 50mM potassium dihydrogen phosphate buffer (60: 40, v/v)	(Ganeshbhai CHAUDHARI et al., 2007)
7	Paracetamol Etoricoxib	Waters Alliance HPLC system 2695	ODS, C8-3 (250 mm x 4.6 mm i.d, 5 µ)	Methanol: acetonitrile: phosphate buffer pH 3.5 (40:20:40 v/v)	(Pattan et al., 2015)
8	Atorvastatin Calcium Fenofibrate	Cyberlab-chrom-HPLC V 4.0	capcellpak C8 DDS5 column (4.6 mmLD x 250 mm i.d. µm	(Isocratic) acetonitrile: KH_2PO_4 (72:28 v/v)	(Bhinge et al., 2012)
9	Gemcitabine Hydrochloride Capecitabine Hydrochloride	Water HPLC 2695	Inertsil ODS-3 C-18column (250× 4.6 mm 5µm)	Acetonitrile: water: triethylamine in the ratio of (70: 28: 2v/v)	(Rajesh et al., 2011)
10	Rosiglitazone Gliclazide	Water HPLC Milli-Q	Phenomenix Gemini C-18 (250 x 4.6 mm 5 µ)	Acetonitrile, phosphate buffer (pH 4.5) and methanol (50:35:15 v/v)	(Rathinavel et al., 2009
11	Atorvastatin Calcium Aspirin	HPLC SHIMADZU - SPD10A	C 18 column (250x 4.6mm 5 µm)	Acetonitrile: Ammonium Acetate buffer 0.02M (68:32) pH 4.5 (Isocratic)	(Suma et al., 2012)
12	Ramipril Aspirine Atovastatine	Shimadzu HPLC (LC-10 AT VP) system	Luna C18 (5µM, 25cm×4.6mm i.d) phenomenex	(A)acetonitrile methanol (65:35) and, (B) 10 mM sodium	(Sharma et al., 2012)

This table represents the system suitability parameters of each assay.

Table 5: System suitability parameters

SL	Drug	RSD	Theoretical plate	Resolution	Capacity factor	Tailing factor
1	Nitazoxanide	0.440 %	10049	14.75	-	1.09
	Ofloxacin	0.440%	1676	-	-	1.49
2	Aliskiren Hemifumarate	0.497% (n=6)	3545	-	-	1.33
	Amlodipine Besylate	1.265%(n=6)	4743	-	-	1.25
3	Ofloxacin	0.326% (n=6)	3059	-	-	1.14
	Ornidazole	0.425% (n=6)	12500	19.61	0.33	1.14
4	Lansoprazole	0.050% (n=5)	784	2.05	-	1.50
	Domperidone	0.110% (n=5)	985	-	-	1.25
5	Aceclofenac	1.590% (n=6)	6426	9.10	-	0.95
	Paracetamol	1.210% (n=6)	5069	0.00	-	1.50
	Chlorzoxazone	1.46 (n=6)	10798	9.96	-	1.14
6	Atorvastatin	-	1677	> 2	-	1.28
	Amlodipine	-	727	-	-	1.20
7	Paracetamol	<1.5%	6475	good resolution	2.81	1.28
	Etoricoxib	< 1.5%	8174	-	5.33	1.11
8	Atorvastatin Calcium	0.100% (n=5)	6280	good resolution	-	1.33
	Fenofibrate	0.200% (n=5)	12252	-	-	1.12
9	Gemcitabine Hydrochloride	0.460% (n=6)	4000	-	-	1.30
	Capecitabine Hydrochloride	0.450% (n=6)	3009	2.70	-	1.10
10	Rosiglitazone	1.200%	-	-	-	-
	Gliclazide	0.190%	-	-	-	-
11	Atorvastatin Calcium	0.058%	9865	4.44	-	1.26
	Aspirin	0.513%	8070	0.79		1.25
12	Ramipril	1.000%	6535	-	0.00	1.36
	Aspirin	0.470%	10353	6.05	0.30	1.24
	Atorvastatin	0.780%	3334	13.65	2.14	1.06

In this literature review, 12 articles were selected from which system suitability parameters (SST) were reviewed. Although it is necessary to include all the SST parameters for validating and to ensure method robustness, not all the articles provided every parameter in their article. The parameters mentioned in the selected articles are given in table 5. Out of selected12, articles 3 of them mentioned all relevant SST parameters.

Capacity factor was least mentioned parameter in the selected papers, 3 out of 12 papers (25%). Out of these 3, only one method developed by Patttan meets SST as per USP<1225>. Article by Dhandapani and Rajesh sharma did not meet the required specification. So, it can be said that there is insufficient retention in the methods.

In the assay of ofloxacin and ornidazole developed by Dhandapani, and in assay of ramipril, aspirin, atorvastatin developed by Rajesh Sharma, the capacity factor was less than recommended value. The reason may be, the solvent was stronger than necessary or, the analyte ionization (polarity) was not suitable or perhaps the used column, was not strong enough.

In assay of ornidazole its mentioned retention factor was 0.33. Ornidazole having pKa of 9.97 is a weak base. Here phosphate buffer and Acetonitrile ration of 70: 30 was used, where pH was 3.5. The reason behind poor retention is the drug which is a weak base gets ionized in pH 3.5 which is a weak acid. As a result, the ionized drug in the buffer, the non-polar stationary phase of RP-HPLC could not retain the drug properly.

In assay of ramipril & aspirin the retention factor for ramipril was 0.00, and aspirin was 0.30, which is unsatisfactory. Ramipril pKa is 3.75 and aspirin pKa is 3.41. Both of these drugs are weak acids and the buffer used here was sodium dihydrogen phosphate monohydrate which has pH of 3. In this scenario, both the drug and buffer are acidic but still the retention is poor. The reason behind that may be, the choice of buffer. The buffer used here is not stable in such low pH. In such low pH the buffer did not have good buffering capacity thus the drugs did not retain properly.

Generally, to increase retention factor, if the drug is ionized (polar) column in RP-HPLC cannot retain the mobile phase effectively because column is non-polar. Non-polar compounds tend to bind with non-polar compounds. To retain the polar drug in column it needs to be converted into non-polar. For instance, if the drug is acidic, acidic buffer need to be used because acid is unionized in acidic medium. When drug and stationary phase both becomes non-polar it results in good retention.

To get better capacity, weaker solvent can be used by changing the polarity of the solvent. Besides, changing the pH of the analyte can be useful to increase the capacity factor or by changing the entire column with greater polarity will give better results.

RSD was included in 11 out of 12 (92%) of article. The value of RSD was insufficient in 5 out of 12 articles. In the assay of paracetamol and etoricoxib, by Pattan did not mention RSD precisely although the RP-HPLC method was considered precise, which can be misleading.

The absolute value of the coefficient of variation is the relative standard deviation, also known as RSD. When the amount of solute placed into the column exceeds the column's storage capacity, retention times typically shorten. The RSD value may differ if the RSD is calculated as over a period of days (interday). All matrices that fall inside the method's application's purview should have their accuracy determined. Due to the potential for significant matrix influence in LC procedures, this basic principle becomes even more crucial (sample preparation, ionization suppression, etc.)

To minimize the variation the analytical runtime should be within one day, the same reagents, equipment should be used and the operator should be the same.

Resolution was mentioned in 10 out of 12 articles (84%). Although in the assay of paracetamol and etoricoxib developed by Pattan, the resolution was not mentioned exactly. It only mentioned that the method gave ‘good resolution’ which can be misleading.

Sufficient resolution was found in the mentioned article expect the article developed by Suma where atorvastatin and aspirin were assayed, the resolution were less than 1. The reasons behind poor resolution are decrease in column efficacy, system contamination, sample deterioration which result in peak shape broadening, tailing or front delay, or interference of spurious peaks. Besides column temperature and dead volume may also worsen the resolution (Watson, 2005)

To improve the resolution, increasing column length, decreasing particle size, reducing peak tailing, increasing temperature & reducing system extra-column volume may be considered. Besides change in polarity, column diameter and film thickness can give better resolution (Watson, 2005).

Compounds with comparable polarity have stronger affinity for one another. Strong contacts will cause molecules with polarity similar to the stationary phase material to be retained longer, and vice versa.

The mobile phase inside the column becomes less viscous and moves more quickly as the column temperature rises, which reduces the retention times but results in poor peak separation. The temperature of the column should be higher than the sample components' highest boiling points without being excessively high to allow all of the components enough time to interact with the stationary phase and elute resolved peaks.

Reduction in column diameter can be another way to improve resolution. The selection of column diameter is determined by sample concentration. If sample loading exceeds column capacity, peak distortions, poor repeatability, and loss of resolution follow. Although narrow columns enhance resolution, their lower loading capacity is a trade-off. (Merck KGaA, Darmstadt, 2022).

Theoretical plate number (N) was mentioned by 11 out of 12 articles (92%). In the assay of Lansoprazole and Domperidone developed by Patel and the in the assay of amlodipine developed by Ganeshbhai, the plate count was 784, 986 and 727 which is quite lower than recommended value.

Theoretical plates in column chromatography are directly related to the efficiency of its column. Columns having greater number of plates is considered as more efficient in HPLC separation than the columns having a smaller number of plates. A more efficient column will have narrower peak than less efficient column which results in better resolution in chromatogram. Theoretical plates imply the better quality of separations as more equilibriums can be attained between stationary and mobile phases. (Watson, 2005)

Factors that can affect theoretical plate are size of the particle, inter-connecting tubing’s dead volume, profile of gradient, temperature of column, length of column, composition of stationary and mobile phase, particle shape and uniformity.

To increase theoretical plate count column length can be increased. By increasing the length plate number can be increased though it may have some shortcomings like increase in analysis time. To get good resolution column length need to be increased 4 times to double the resolution.

Optimizing the flow rate can also be effective for column efficiency. A flow rate that is greater than the ideal one to speed up the analysis. The efficiency gains from slowing the flow rate might be sufficient to separate two partially resolved compounds.

Use of smaller and spherical particle can also improve theoretical plate count. Smaller particles will have less interstitial volume and less widening of the mobile phase mass transport. Smaller particles will also lessen the broadening effects of eddy diffusion, provided that they are tightly packed.

The pore size of the stationary phase is a further element associated with the column and affecting column efficiency. Greater surface area is offered from small pores, whereas separation of bigger molecules is favored by higher pore sizes. To have a decent peak shape, it's crucial to select the right pore size. Peak broadening and poor resolution are caused by pore sizes that are either too tiny or too big.

Tailing factor T_f is mentioned by 11 out of 12 (92%) of the articles. Except in the article by Rathinavel where rosiglitazone and gliclazide were assayed, all article has mentioned tailing factor and were under recommended value given by USP.

CHAPTER 4: CONCLUSION

In pharmaceuticals, RP-HPLC is a major tool for drug analysis. This is due to the ease, adaptability, and range of the reversed-phase approach, which can handle substances with various polarities and molecular masses. To properly operate RP-HPLC the concept of system suitability is very important. System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Data reliability can be increased with thorough validation and stringent chromatographic performance (system suitability) standards. In this review, USP reviewer guidance for chromatography, given by Centre for Drug Evaluation and Research (CDER) was followed. Data on release, stability, and pharmacokinetics can only be generated with good, trustworthy and established methodologies.

Although the selected 12 articles were collected from reputable and reliable publications like SpringerLink, Hindawi, Wiley, PubMed and Science direct the amount of database was relatively short. Due to access denial in many articles and publication significant number of articles had to be abandoned. Use of those paid and restricted article and journal would be ideal for this review as those were published by well renowned publications and more sophisticated journals.

This review article would be helpful in understanding and performing RP-HPLC for any type of drug. This could also contribute in development in method validation for chromatography which ultimately serves the food and drug industry.

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APPENDIX

1. RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of two Drugs Nitazoxanide, Ofloxacin and its Pharmaceutical Dosage Forms.

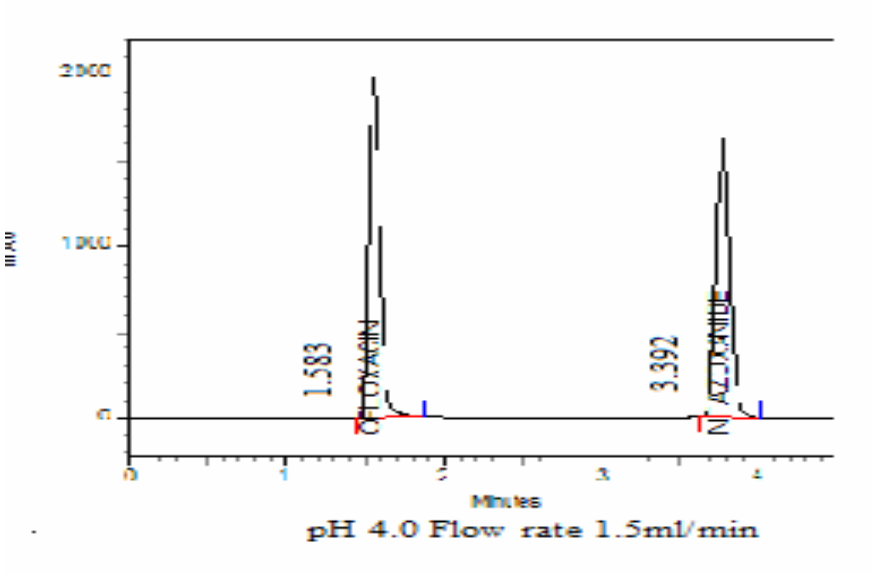

Fig: Chromatogram of Nitazoxanide and Ofloxacin (Gadapa & Tripathi, 2012)

2. A Validated Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Aliskiren Hemifumarate and Amlodipine Besylate in Pharmaceutical Dosage Form.

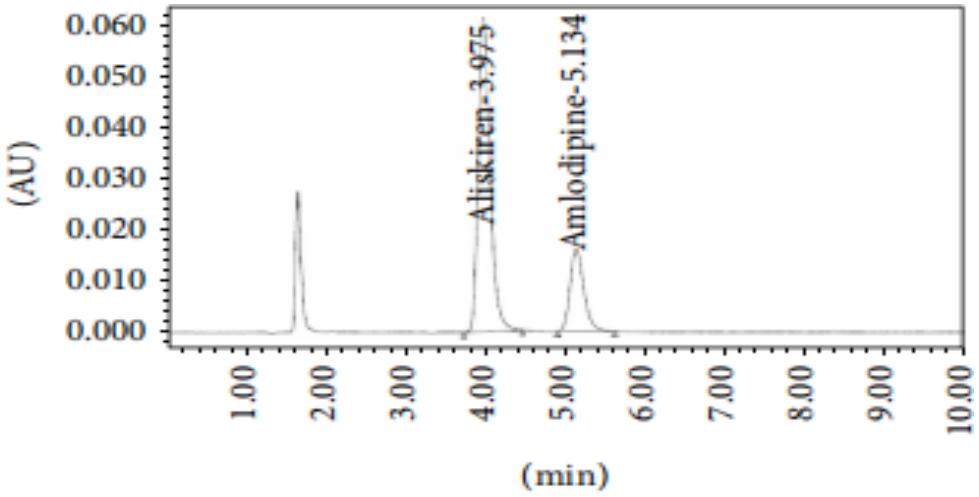

Fig: Chromatogram of Aliskiren Hemifumarate & Amlodipine Besylate (Gajula et al., 2021)

3. Method Development and validation for the simultaneous estimation of Ofloxacin and Ornidazole in tablet dosage form by RP-HPLC.

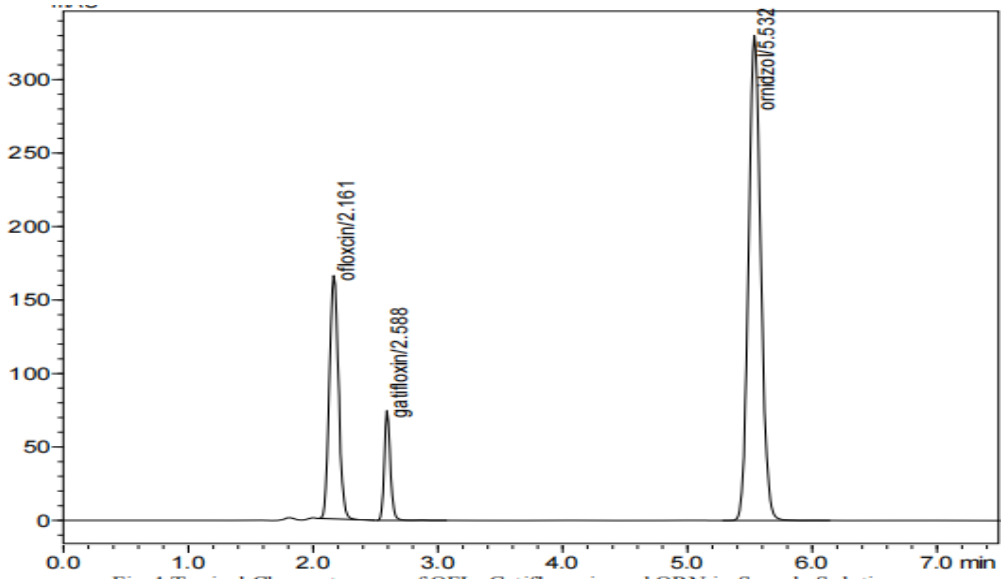

Fig: Chromatogram of Ofloxacin and Ornidazole (Dhandapani et al., 2010)

4. Simultaneous Estimation of Lansoprazole and Domperidone in Combined Dosage Form by RP-HPLC.

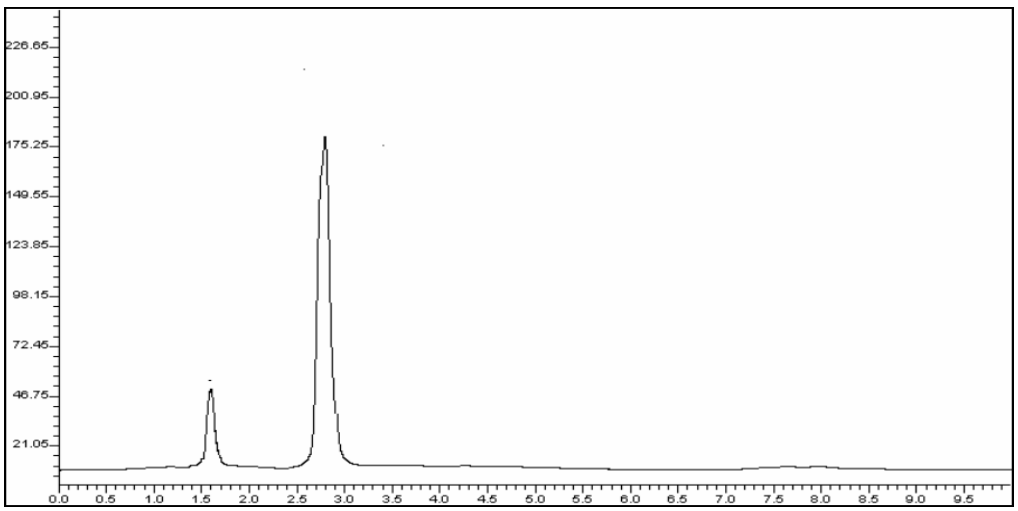

Fig: Chromatogram of Lansoprazole and Domperidone (Patel et al., 2015)

5. Simultaneous Determination of Aceclofenac, Paracetamol, and Chlorzoxazone by RP HPLC in Pharmaceutical Dosage Form.

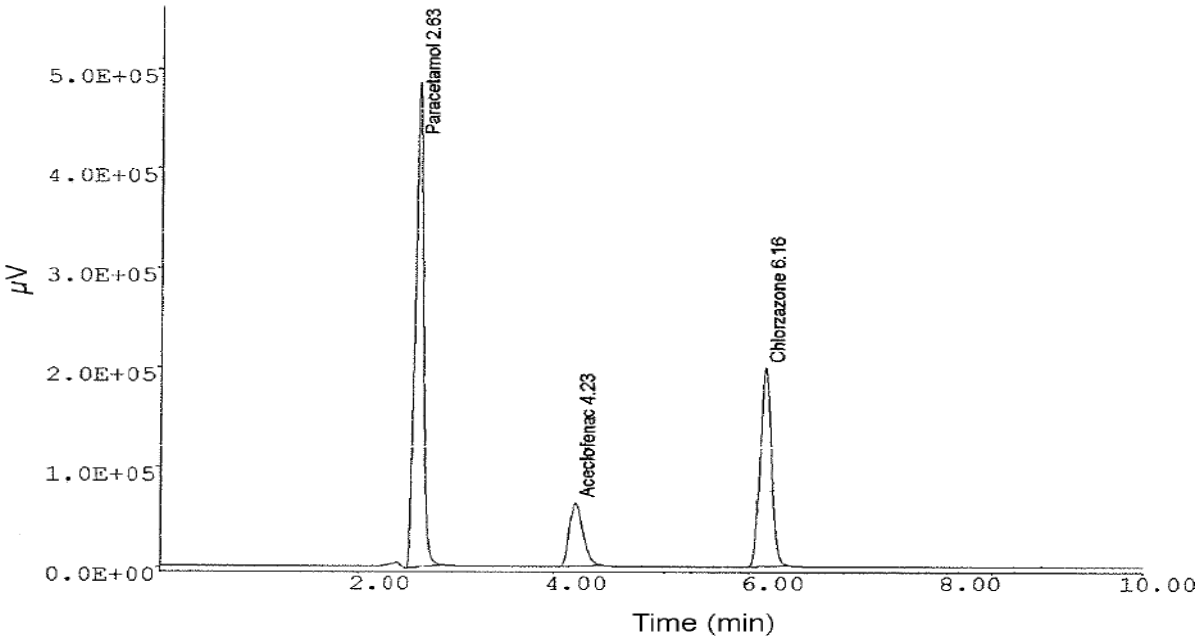

Fig: Chromatogram of Aceclofenac, Paracetamol, and Chlorzoxazone (K.A. Shaikh et al., 2008)

6. Stability Indicating RP-HPLC Method for Simultaneous Determination of Atorvastatin and Amlodipine from Their Combination Drug Products.

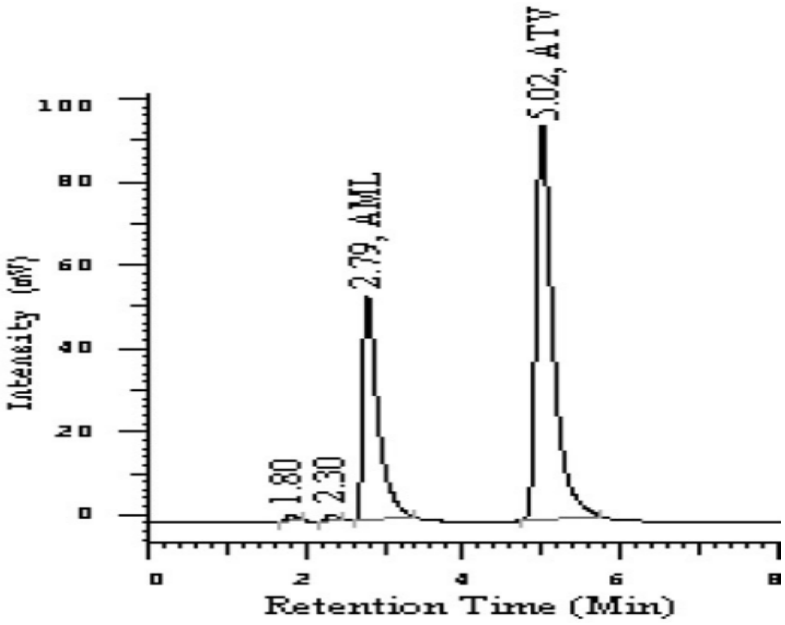

Fig: Chromatogram of Atorvastatin and Amlodipine (Ganeshbhai CHAUDHARI et al., 2007)

7. RP- HPLC Method for Simultaneous Estimation of Paracetamol and Etoricoxib from Bulk and Tablets.

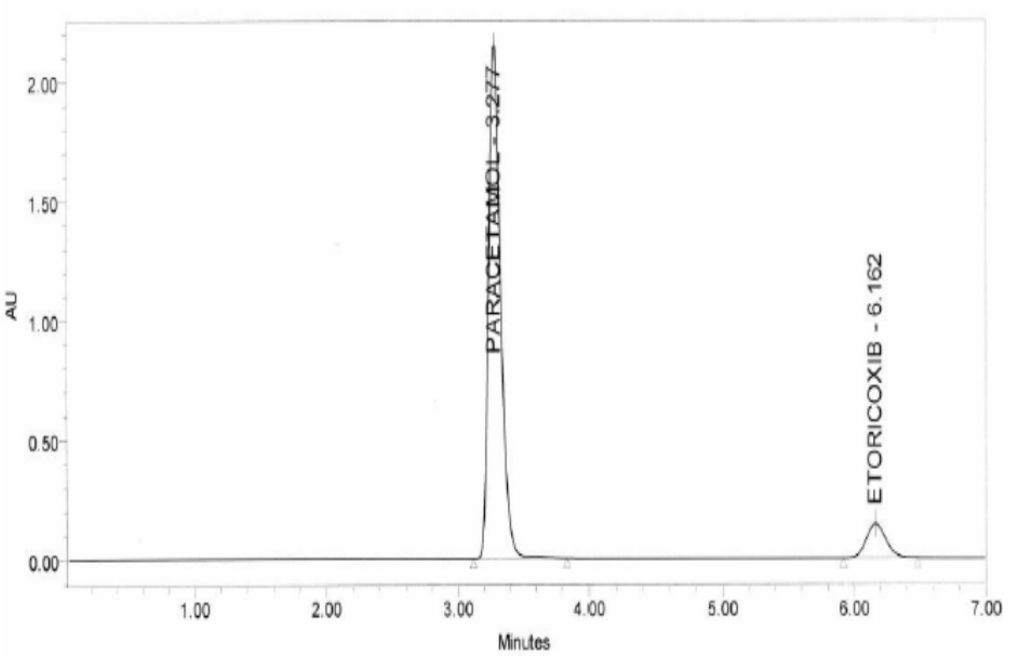

Fig: Chromatogram of Paracetamol and Etoricoxib (Pattan et al., 2015).

8. A New Approach to the RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Fenofibrate in Pharmaceutical Dosage Forms.

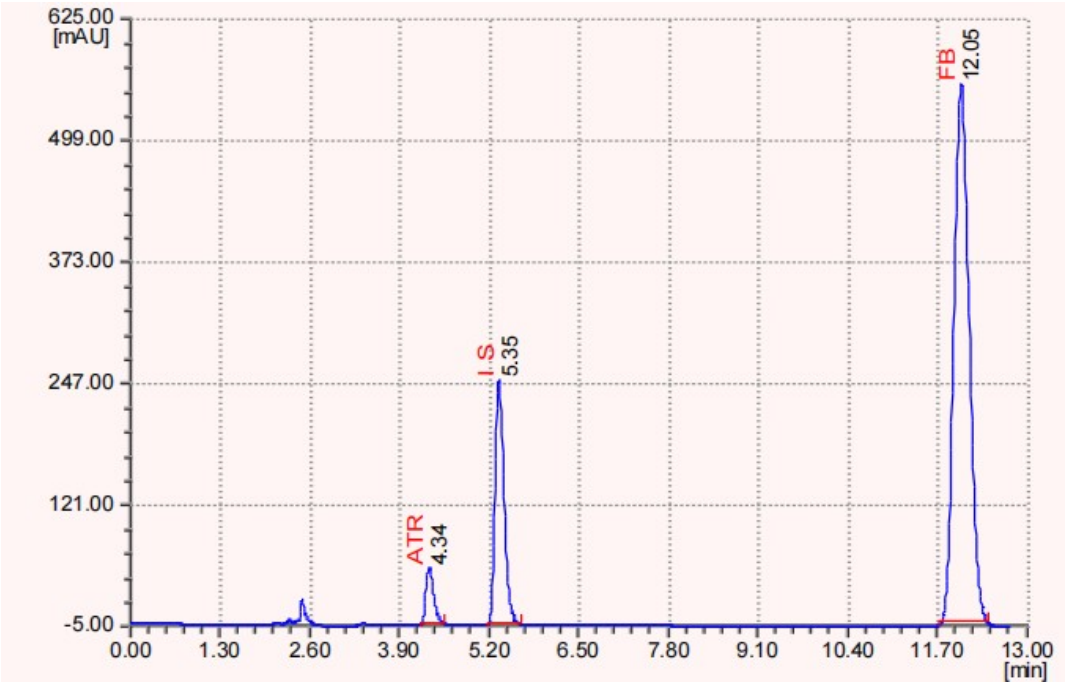

Fig: Chromatogram of Atorvastatin Calcium and Fenofibrate (Bhinge et al., 2012)

9. Simultaneous Estimation of Gemcitabine Hydrochloride and Capecitabine Hydrochloride in Combined Tablet Dosage Form by RP-HPLC Method

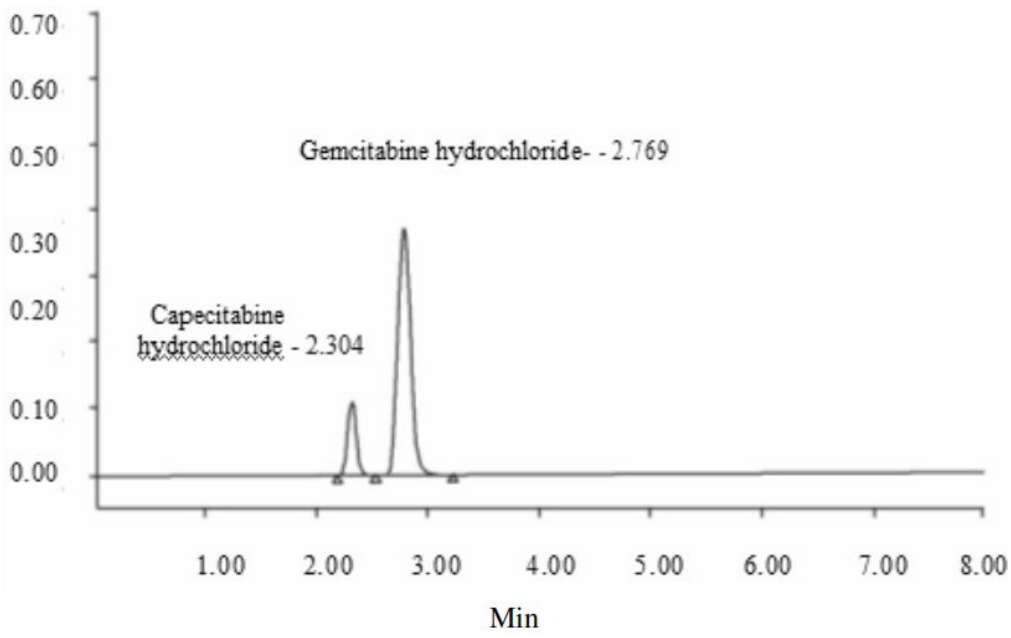

Fig: Chromatogram of Gemcitabine Hydrochloride and Capecitabine Hydrochloride (Rajesh et al., 2011)

10. RP-HPLC Method for the Simultaneous Estimation of Rosiglitazone and Gliclazide in Tablets.

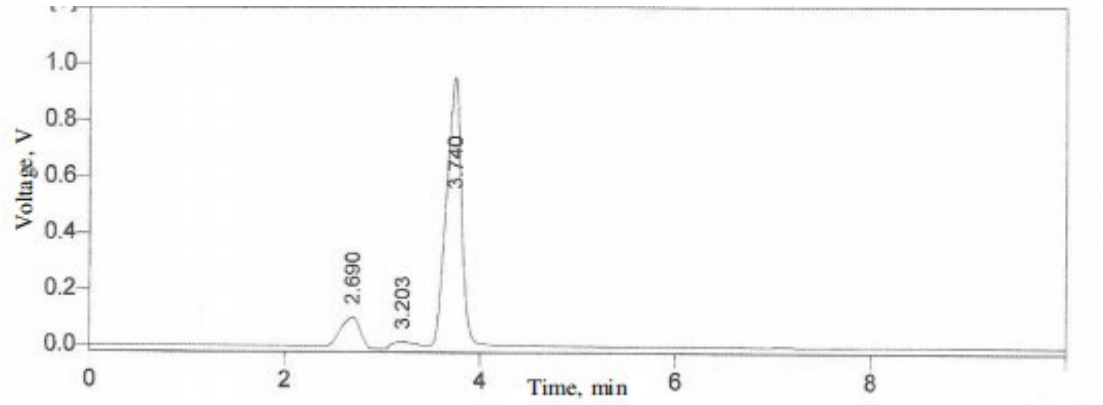

Fig: Chromatogram of Rosiglitazone (Rathinavel et al., 2009)

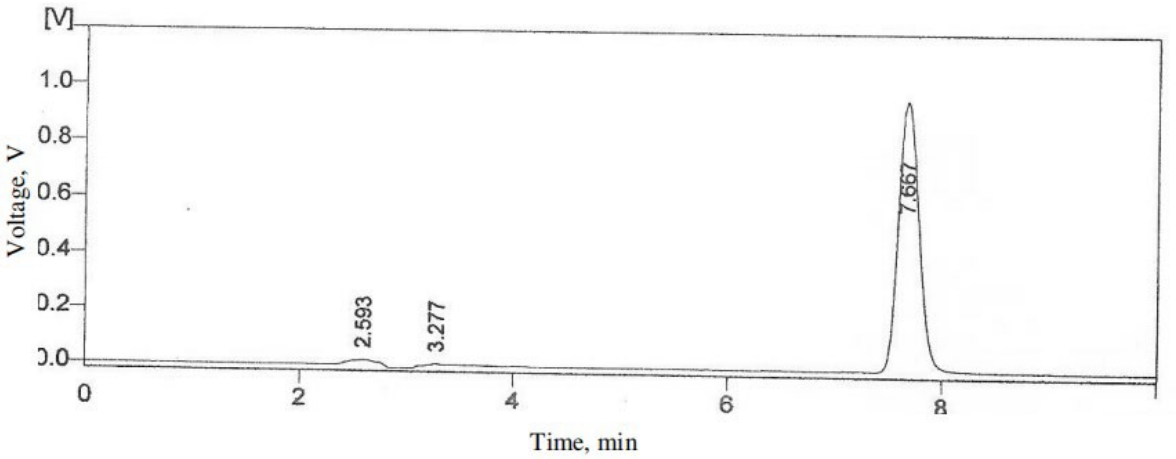

Fig: Chromatogram of Gliclazide (Rathinavel et al., 2009)

11. Simultaneous Estimation and Validation of Atorvastatin Calcium and Aspirin in Combined Capsule Dosage Form by RP HPLC Method.

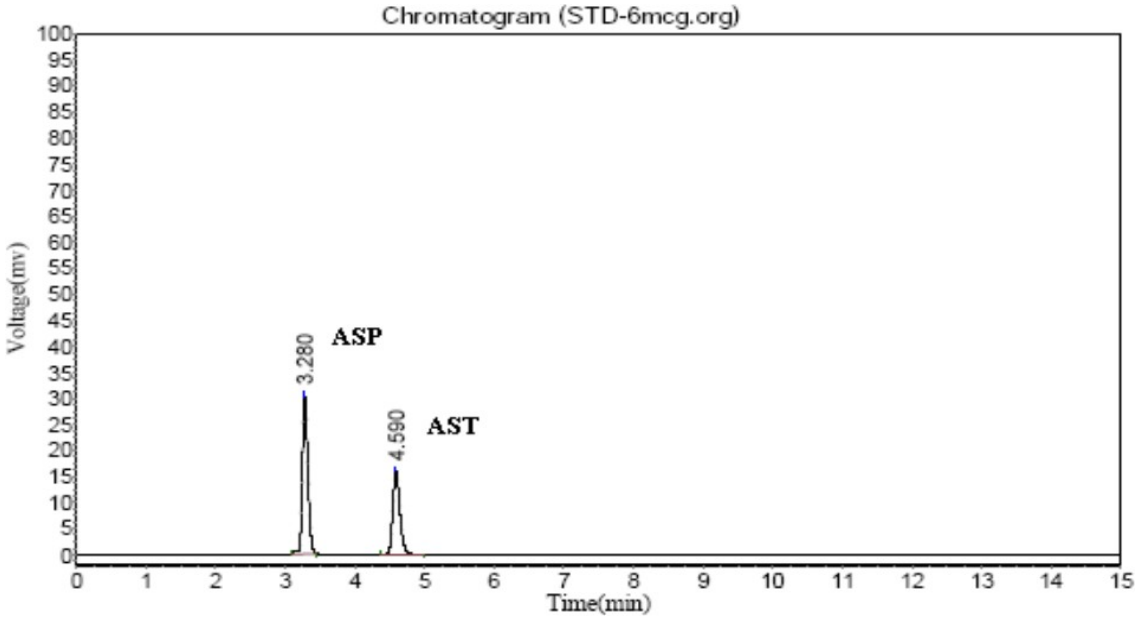

Fig: Chromatogram of Atorvastatin Calcium and Aspirin (Suma et al., 2012).

12. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ramipril, Aspirin and Atorvastatin in Pharmaceutical Preparations.

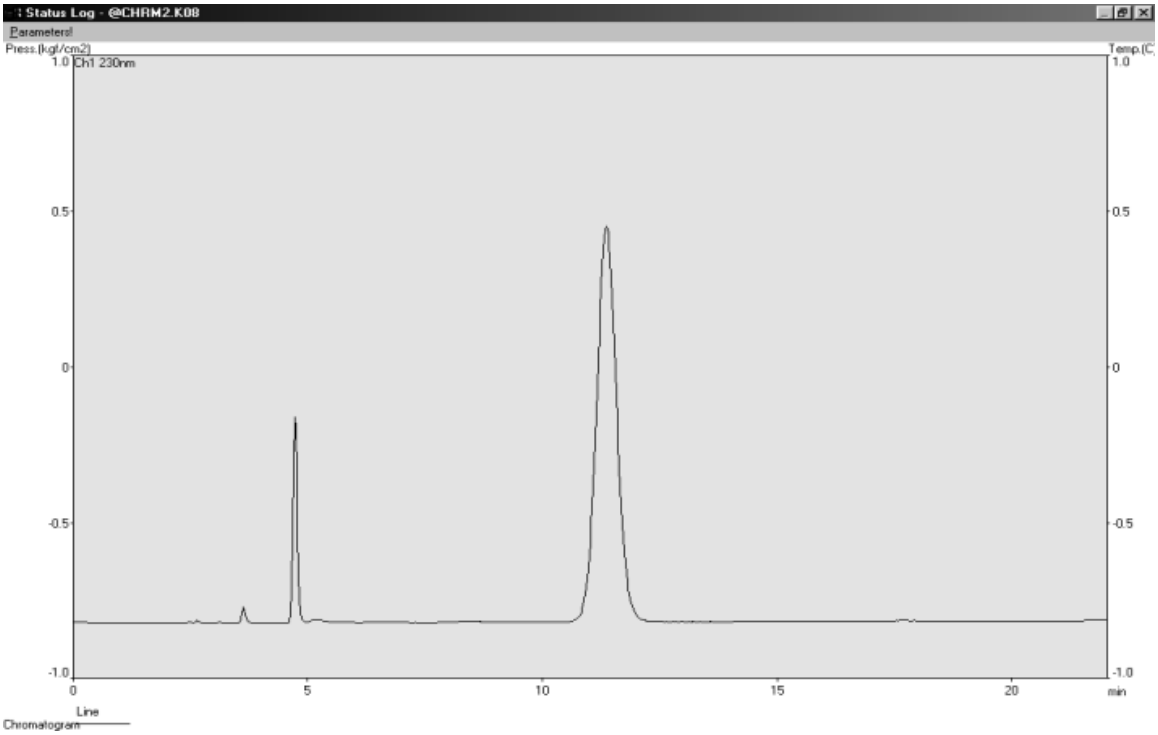

Fig: Chromatogram of Ramipril, Aspirin and Atorvastatin (Sharma et al., 2012)