EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 1
405-417
Year: 2017

DEVELOPMENT OF SHORT & ROBUST ANALYTICAL METHOD ON UPLC-MS FOR QUANTITATIVE ESTIMATION OF DIFFERENT API'S IN THEIR FORMULATED PRODUCTS – A GREEN ANALYTICAL CHEMISTRY APPROACH

Tapeesh Bharti* and Richa Singhal

Jubilant Chemsys Ltd., B-34, Sector 58, Noida, U P India.

***Corresponding Author: Tapeesh Bharti**
Jubilant Chemsys Ltd., B-34, Sector 58, Noida, U P India.

Article Received on 16/12/2016 **Article Revised on ……** **Article Accepted on 21/12/2016**

ABSTRACT
As over 70% of pharmaceutical compounds are bases, the analysis of these basic compounds by liquid chromatography (LC) continues to be of great value and interest. The novel approach towards Green Analytical Chemistry was carried out to develop and validate a rapid and selective analytical method by using Reverse Phase Ultra Performance Liquid Chromatography (RP-UPLC) technique for quantitative estimation of different API's in raw materials & their pharmaceutical dosage forms. The development was done by using a known solution which contain an equimolar ratio of Propanolol, Veraprmil, Niflumic acid and Diltiazem, in terms of resolution and then validate the method by analyzing Carbamazepine, Terbinafine HCl & Atorvastatin Calcium. The compounds were analyzed with a total run time of 2.5min. (in reverse phase) at compound wavelength. Optimum retention was achieved on Waters Acquity UPLC BEH C18 column (2.1 × 30mm, 1.7µm) using gradient elution with 0.1% Trifluoro Acetic Acid in water (volatile ion pair reagent) and Acetonitrile as mobile phase. The developed method was validated with respect to specificity, linearity, precision, accuracy, ruggedness (reproducibility), robustness and stability. The method is economical in terms of the time taken and the amount of solvent used, thus promoting green chemistry concept. To the best of our knowledge, a work on method development and validation of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium by using RP-UPLC technique, disclosed in this investigation, was not published elsewhere.

KEYWORDS: Carbamazepine, Terbinafine HCl, Atorvastatin Calcium, Ultra Performance Liquid Chromatography, Liquid Chromatography-Mass Spectrometry (LCMS).

INTRODUCTION
The earlier reported study of these drugs are mainly performed by RP-HPLC methods[1-11] using long columns with higher particle size, which were more time consuming. Also we need to change the method conditions accordingly while analyzing different API as per their polarities and elution order. The purpose of the present study is to develop versatile, rugged, and time saving method for quantitative estimation of different API's in their formulated products. The target is attained by selecting more advance technique of Waters Acquity UPLC-MS[11-13], which gives more accurate result in shorter run time. The experiments were planned using the 'Design of Experiments' (DoE) approach to select an appropriate LC–MS compatible ion-pair reagent. Different UPLC/DAD methods using lower concentration of volatile Ion Pair mobile phase were studied to improve retention, column efficiency and detector linearity optimization. We found that, in some cases, column efficiency may not be attained by using alcohol based mobile phase even with small particle size (< 2 µm) & with large range of flow rate. Therefore the strategy to shorten the analysis was achieved by using Acetonitrile based mobile phase. The method development was done by using an equimolar ratio of Propanolol, Veraprmil, Niflumic acid and Diltiazem as system suitability standard. The optimization of experimental conditions was done using mass compatible volatile buffer TFA (with 0.1%v/v solution in water) and Acetonitrile along with a shorter column having 1.7µm particle size.[14-17] In this study, Trifluoro Acetic Acid (TFA) has given a very good peak shape with a tailing factor of 1.4 and theoretical plates up to 15,000. Use of ion-pair volatile reagent has proved to be good replacement of sodium alkyl sulphonate modifiers.[18] TFA in a very lower concentration (i.e. 0.1% v/v) can also be used for isolation of impurities in semi-preparative work.

The developed method has been validated individually on Carbamazepine, Terbinafine HCl & Atorvastatin Calcium formulated drugs by following several

parameters as mentioned in ICH guideline[19-21] i.e. linearity, specificity, accuracy, precision, robustness, ruggedness & stability. The drugs and their formulated products were first dissolved in Acetonitrile and the final dilution was done by a mixture of Water: Acetonitrile (50:50) v/v as diluents. The method showed excellent recoveries for all drugs in bulk and can be used on several other API's having different polarities. The pure Active Pharmaceutical Ingredient (API), used in this project, is obtained from Jubilant Life Science Limited with a COA (Certificate of Analysis). The Tablet used was Terbinaforce (formulated by Mankind Pharmaceuticals Pvt. Ltd), Tegretal (formulated by Novartis) and Biotor, (formulated by United Biotech Pvt. Ltd.) for validation of Terbinafine HCl, Carbamazepine and Atorvastatin Calcium respectively.

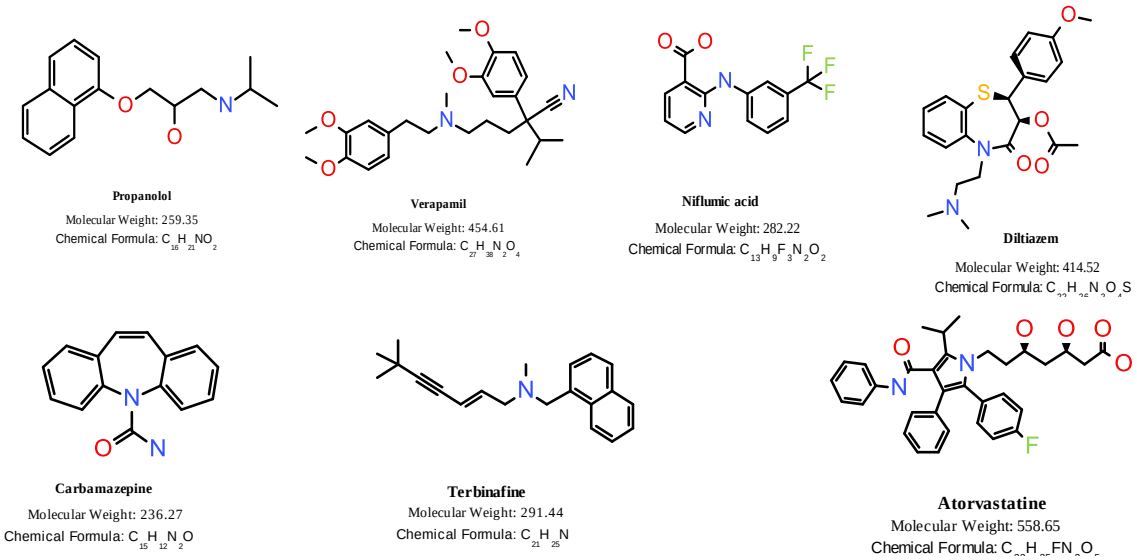


Figure 1. Structure of API's used in the analysis

2. MATERIAL AND METHOD

Materials

Apparatus

Chemicals used in this study included gradient grade Acetonitrile (Sigma Aldrich, USA) and MS grade Tri Fluoro Acetic Acid (Sigma Aldrich, USA). Water used for UPLC analysis was purified using Millipore Milli Q-Plus water purification system (Millipore SAS, France).

Reagents and Chemicals

A well-characterized working standard of Terbinafine HCl, Carbamazepine & Atorvastatin Calcium was procured from Jubilant Life Sciences Limited, India. Commercially available Terbinaforce (Mankind Pharmaceuticals Pvt. Ltd), Tegretal (NOVARTIS) and Biotor (United Biotech Pvt. Ltd.) was purchased from local pharmacy (Noida, India).

METHOD

Method Development and Optimization of Chromatographic Conditions.

Solubility

From the literature review, Propanolol, Verapamil, Niflumic acid, Diltiazem, Carbamazepine, Terbinafine HCl & Atorvastatin Calcium are soluble in Acetonitrile Water mixture.

Selection of chromatographic method

Proper selection of the method depends upon the nature of the sample (ionic / ionisable / neutral molecule) and solubility. The drugs selected in the present study are polar in nature and hence reverse phase, ion-pair or ion exchange chromatography might be used. The reverse phase UPLC was selected for the separation because of its simplicity and suitability. This method can be directly used for LC-MS analysis also, on need basis.

Selection of wavelength

The sensitivity of the any LC method which uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. Method development is done using Diode array (i.e.Plot Max) detection and Validations are performed with Compound wavelength which is 222nm, 237nm, 246nm for Terbinafine HCl, Carbamazepine & Atorvastatin Calcium analysis, respectively, as shown in Figure 2.

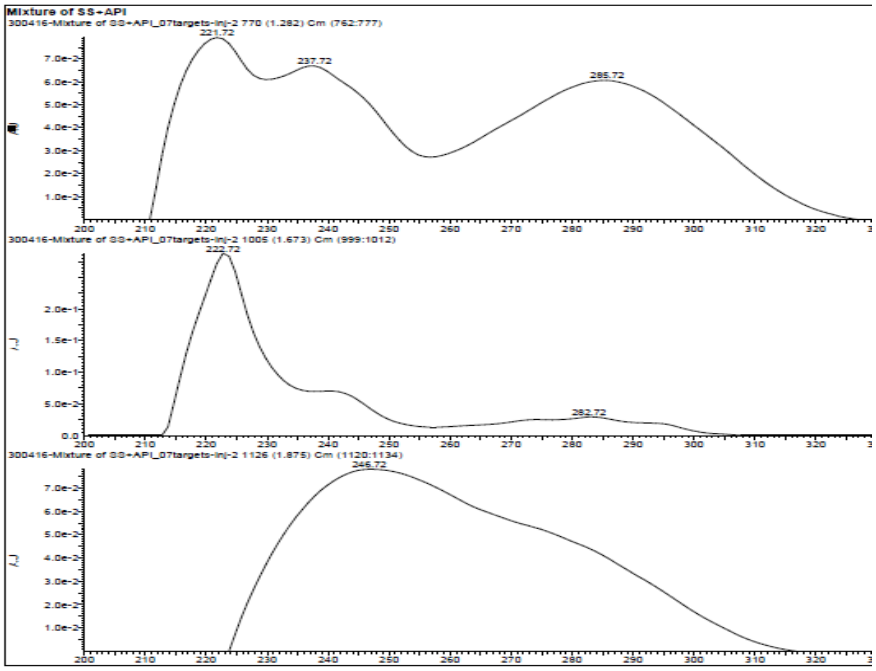


Figure 2. Wavelength pattern of Terbinafine, Carbamazepine & Atorvastatin

Selection of mobile phase

Initially Acetonitrile & Water mobile phase was tried using different gradient methods (Figure-3). 5mM Ammonium Acetete in water is also tried as buffer with ACN using different gradient methods (Figure-4), but separation was achieved by using Acetonitrile with 0.1% Trifluoro Acetic Acid in water as buffer (Figure 5).

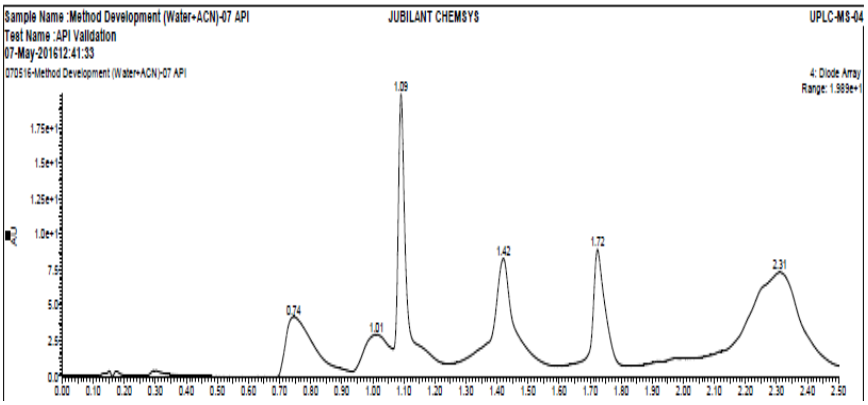


Figure 3. Chromatogram obtained- Water: ACN as mobile phase for Gradient elution

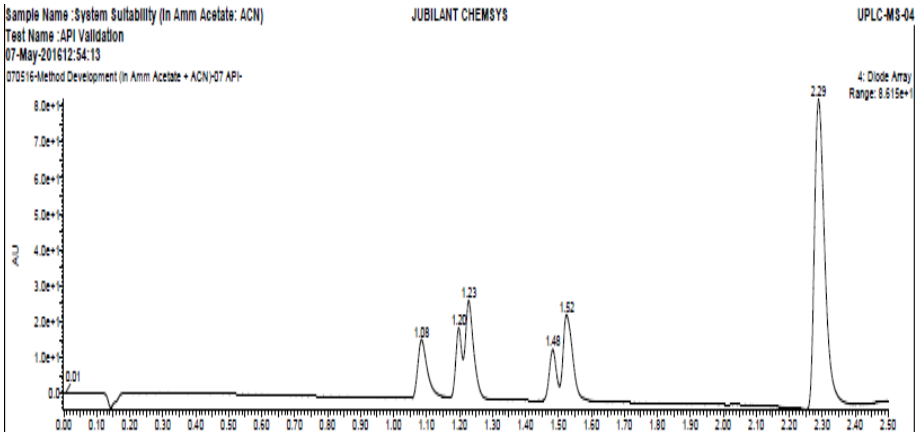


Figure 4. Chromatogram obtained- 5mM Ammonium Acetate in water: ACN as mobile phase for Gradient elution

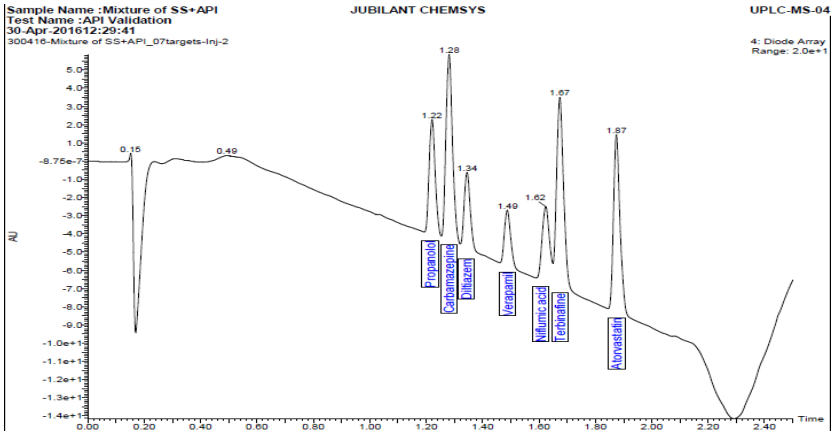
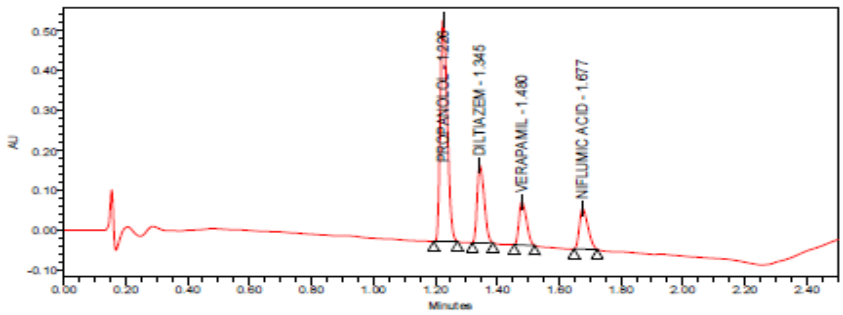


Figure 5. Chromatogram obtained- 0.1% TFA in Water: ACN as mobile phase for Gradient elution

Optimized chromatographic method conditions

Chromatographic analysis was performed on Waters Acquity UPLC system (Waters, USA) equipped with a binary solvent delivery system, an auto-sampler and a photodiode array detector. Gradient separation method was applied in the analysis using a Waters Acquity UPLC BEH C18 column (30 × 2.1 mm, 1.7 µm) at 30°C, with a flow rate of 0.6 mL/min. The mobile phase consisted of (A) 0.1% Trifluoro Acetic Acid in water and (B) Acetonitrile. A linear gradient elution method was applied as follows with respect to A: 0 min: 90%; 0.2min: 90%; 1.30min: 55%; 1.90 min: 35%; 2.0min: 10%; 2.40min: 90%; 2.50min: 90% with curve 6. The auto sampler was maintained at 10°C and the sample injection volume was 1µL. In this study, Trifluoro Acetic Acid (TFA) has given a very good peak shape with a tailing factor of 1.4 and theoretical plates up to 15,000 (Figure 6).



Peak Results

	Name	RT	USP Plate Count	USP Tailing
1	PROPANOLOL	1.23	13676.99	1.32
2	DILTIAZEM	1.34	15131.03	1.33
3	VERAPAMIL	1.48	15982.86	1.36
4	NIFLUMIC ACID	1.68	16057.81	1.37

Figure 6. Chromatogram obtained: System Suitability solution in developed UPLC method

The TIC also shows separated APIs (Figure 7) evident from individual mass data (Figure 8).

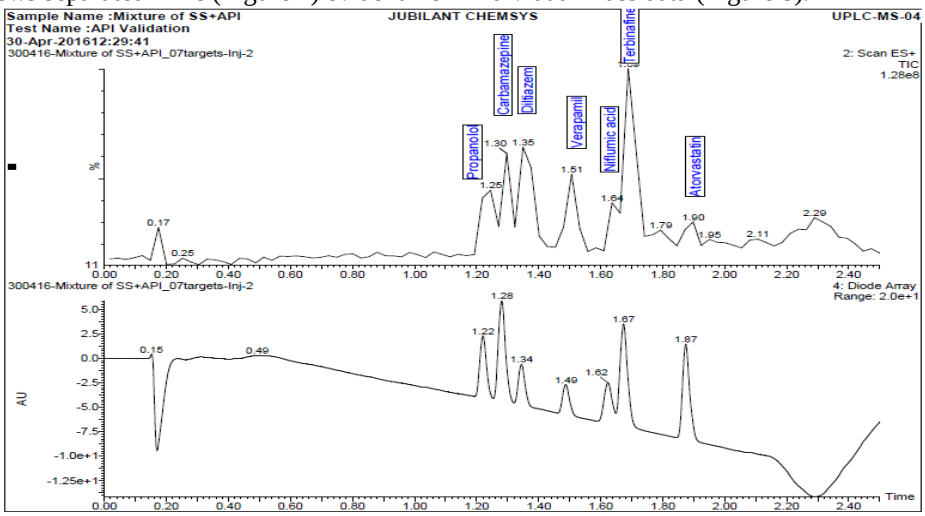


Figure 7. UPLC-MS chromatogram with TIC: All APIs in developed UPLC method

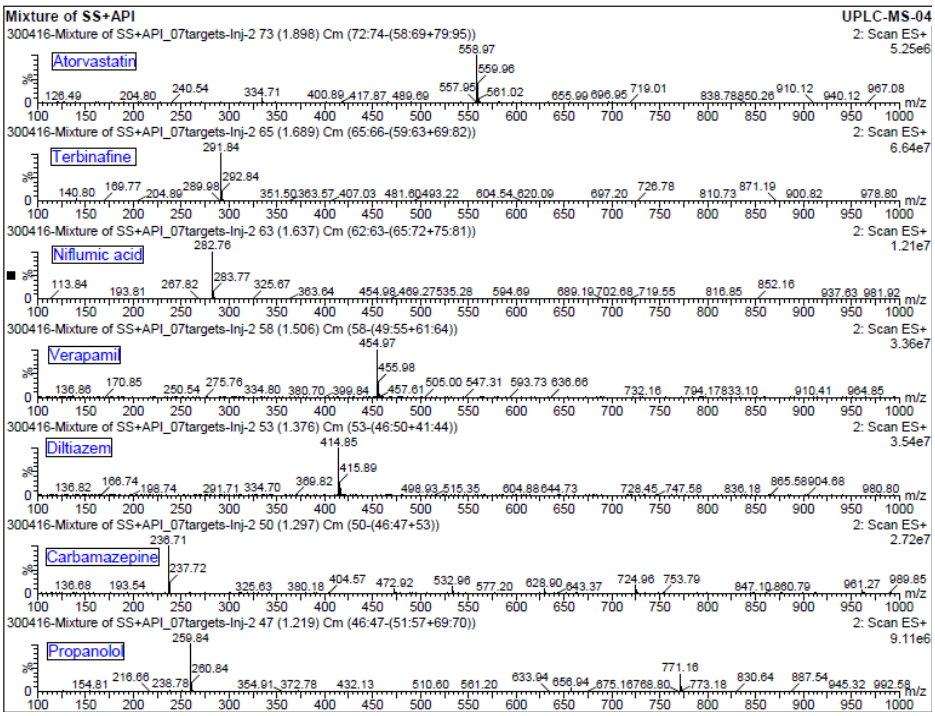


Figure 8. Mass spectral data: All APIs in developed UPLC method.

Mobile phase preparation

Buffer was prepared by mixing 500 µL Tri Fluoro Acetic Acid in 500 mL Milli-Q water. It was then degassed by using vacuum filtration through 0.2µ (6,6-Nylon) membrane filter paper. Gradient grade Acetonitrile was used for gradient elution purpose.

Standard stock solutions Preparation

Accurately weighed and transferred 100 mg of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium standard in a 100 mL volumetric flask, each individually and added a little quantity of Acetonitrile. It was then sonicated to dissolve and dilute to volume with the diluents (Water: Acetonitrile; 50: 50).

Standard preparations

Transferred appropriate volume of standard stock solution into 20mL volumetric flask and diluted to volume with the same diluents, as above. The concentrations of standard solutions, used in analysis, are 250 µg/mL for Carbamazepine, 250 µg/mL for Terbinafine HCl and 100 µg/mL for Atorvastatin Calcium standard.

Sample Preparation

Finely grounded pre weighed 20 Tablets of Tegretal, Terbinaforce & Biotor tablets individually. Transferred approx. weight of each grounded sample, equivalent to standard concentration of API, to 20.0 mL volumetric flasks, separately. Then added a little quantity of Acetonitrile in each flask. It was then sonicated to dissolve, diluted to volume with the diluents (Water: Acetonitrile; 50: 50) and filtered through 0.2µm nylon filters. The final concentrations of sample solutions used in analysis are 250 µg/mL for Carbamazepine, 250 µg/mL for Terbinafine HCl and 100 µg/mL for Atorvastatin Calcium.

Assay procedure

Injected 1µL of the standard and sample solutions in the UPLC system. The chromatograms were recorded and measured the areas for Carbamazepine, Terbinafine HCl & Atorvastatin Calcium peaks and calculated the % Assay individually, by using following formula.

% Assay = (At/As) x (Ws/Ds) x (Dt/Wt) x (P/100) x (Avg. weight/Label Claim) x 100.

Where,

At = average area counts of sample preparation, As = average area counts of standard preparation

Ws= Weight of working standard taken in mg, Wt = Weight of sample taken in mg

Dt = sample dilution

Ds= standard dilution

P= Purity of Standard

In RP-UPLC method, chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds with shorter run time, less consumption of solvent and mass compatible method for further studies.

Method Validation

Linearity

A calibration curve was generated to confirm the linear relationship between the peak area and concentrations of six samples. The serial dilutions were prepared individually by accurately weighing and dissolving Carbamazepine, Terbinafine HCl & Atorvastatin Calcium pure API in Acetonitrile and water (50:50) v/v to obtain the final concentration 62.5-375 µg/mL for Carbamazepine, Terbinafine HCl & 25-150 µg/mL for

Atorvastatin Calcium. These solutions were injected in chromatographic system in replicate. The Linear detector response of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium is demonstrated by concentration versus Area curve.

Precision and stability

The standard solutions were analyzed for six replicates on the same day to assess intra-day variation and once again on the next day for 6 consecutive samples to assess inter-day variation. The same sample was subjected to UPLC analysis immediately and after 24hrs. to detect the peak area of the standard sample for the evaluation of stability.

Reproducibility

This was performed by analyzing an assay of six samples of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium against qualified reference standard. The %RSD for assay obtained from six precision samples & six standard samples were calculated to evaluate the reproducibility of the method.

Recovery

Recovery of the assay method for Carbamazepine, Terbinafine HCl & Atorvastatin Calcium were established by three determinations of test sample using Tablets at 50%, 100% and 150% of concentration. Each solution was injected thrice (n=3) in UPLC system and the average peak area was calculated to obtain percentage recoveries after calculation.

Robustness

This was performed by analyzing standard solution & sample solution by changing the flow rate (±0.10 mL/min), buffer concentration (±10%) and temperature (±5ºC).

Validation of developed UPLC method

Different chromatographic conditions such as mobile phase, wavelength, column and column temperature were experimented to achieve efficiency of the chromatographic system. Different gradients were checked in order to attain optimum retention of the API's. Minimizations of run time and cost were the major tasks while developing the method. Based on International Conference on Harmonization (ICH) guidelines, the method was validated with regard to precision, specificity, reproducibility, accuracy, linearity, stability of solution, robustness, limit of detection and quantification.

Linearity

Linearity was assessed in the range of 25%, 50%, 75%, 100%, 125% and 150% of working concentration. Injections of all concentrations were carried out in replicate. Calibration curve was constructed by plotting the mean peak area versus concentration, which was observed to be linear. The Linearity co-efficient of mean response, which was plotted against respective concentration, was calculated. The results are summarized in Table-1 and Figure 9.

Table-1: Linearity Data

Level (%)	Carbamazepine Area count			Terbinafine HCl Area count			Atorvastatin Ca Area count		
	Area-1	Area-2	Average	Area-1	Area-2	Average	Area-1	Area-2	Average
25	6358.01	6270.61	6314.31	5492.78	5473.02	5482.90	3668.99	3651.45	3660.22
50	12543.95	12587.39	12565.67	10892.15	10912.96	10902.56	6982.14	6903.49	6942.81
75	18876.01	18871.54	18873.78	16086.33	16145.32	16115.83	10315.79	10278.43	10297.11
100	25026.91	25088.36	25057.64	21781.52	21660.82	21721.17	13587.69	13637.61	13612.65
125	31346.58	31021.36	31183.97	27039.83	26904.81	26972.32	16901.42	16881.14	16891.28
150	37277.51	37039.89	37158.70	32069.71	32373.28	32221.50	19986.51	19999.25	19992.88

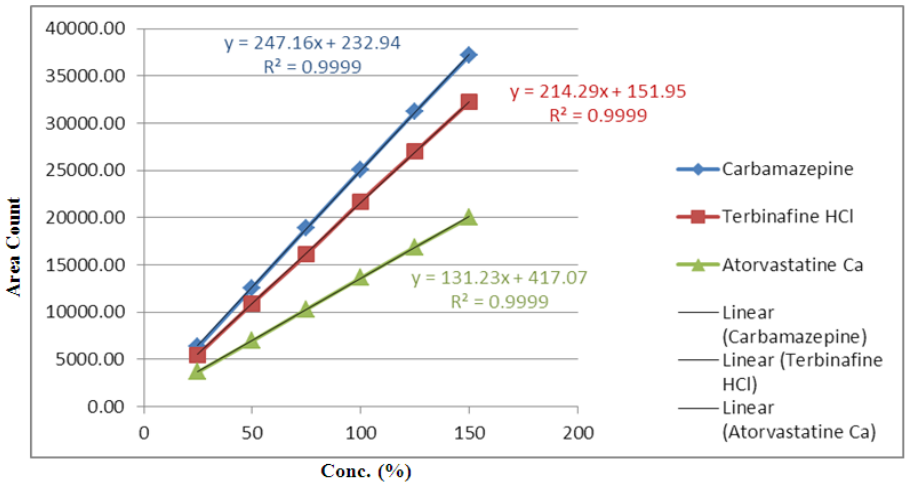


Figure 9. Calibration graph of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium

Accuracy

Recovery of the assay method for Carbamazepine, Terbinafine HCl & Atorvastatin Calcium was established by three determinations of test samples using Tablets at 50%, 100% and 150% concentrations, individually. Each solution was injected in triplicate (n=3) in UPLC system and the average peak area was calculated to obtain percentage recoveries. All the individual recoveries were found to be between 99.03 to 100.42%. All individual recovery levels were found to be within 0.10 to 0.31% (%RSD). The results are summarized in Table-2, 3, 4 & 5.

Table 2. Recovery studies: Standard weight and area's calculation

Standards	Carbamazepine	Terbinafine HCl	Atorvaststin Ca	Standards Area	Carbamazepine	Terbinafine HCl	Atorvaststin Ca
Std. Weight (mg)	100.50	100.50	50.40	**Area Injection-1**	24752.37	22095.91	13505.60
Purity of Std. (%)	100.00	99.70	99.80	**Area Injection-2**	24910.23	22316.39	13472.03
Conc. (µg/mL)	250.00	250.00	100.00	**Area Injection-3**	24940.08	22463.10	13465.23
				Area Injection-4	24874.50	22154.70	13452.19
				Area Injection-5	24951.59	22101.78	13394.16
Sample Tablets	**Tegretal**	**Terbinaforce**	**Biotor**	**Area Injection-6**	24741.62	22067.49	13403.70
Label Claim (mg)	400.00	250.00	20.00	**Average Area**	**24861.73**	**22199.90**	**13448.82**
Average Wt. (mg)	559.89	427.99	186.45	**SD**	**92.86**	**156.76**	**42.58**
Conc. (µg/mL)	250.00	250.00	100.00	**% RSD**	**0.37**	**0.71**	**0.32**

Table 3. Recovery studies: for Carbamazepine

Level (%)	Sample area (per Inj.)	Average area	Sample Wt.(mg)	Sample Conc.(mg)	Amount Spiked (µg)	Amount Recovered (µg)	% Recovery	Average % Recovery	SD	% RSD
50	12493.26	12482.87	17.50	**12.56**	**125.63**	126.26	100.50	**100.42**	0.10	**0.10**
	12469.39					126.01	100.31			
	12485.95					126.18	100.44			
100	24594.48	24650.23	34.99	**25.13**	**251.25**	248.55	98.93	**99.15**	0.25	**0.25**
	24640.93					249.02	99.11			
	24715.29					249.77	99.41			
150	37025.34	36997.62	52.49	**37.69**	**376.88**	374.17	99.28	**99.21**	0.24	**0.24**
	37068.42					374.61	99.40			
	36899.11					372.90	98.94			

Table 4. Recovery studies for Terbinafine HCl

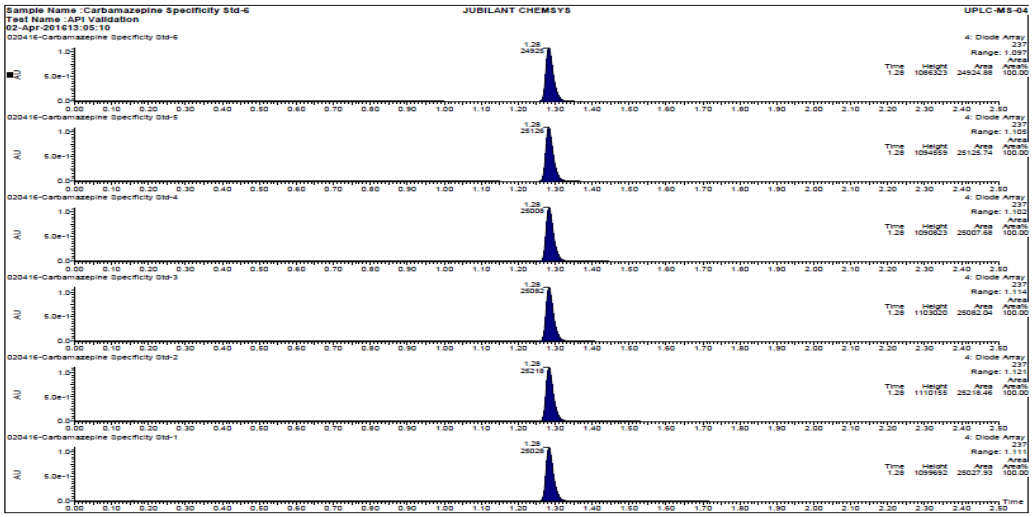
Level (%)	Sample area (per Inj.)	Average area	Sample Wt.(mg)	Sample Conc.(mg)	Amount Spiked (µg)	Amount Recovered (µg)	% Recovery	Average % Recovery	SD	% RSD
50	11010.45	10993.47	21.40	**12.56**	**125.63**	124.61	99.19	**99.04**	0.18	**0.18**
	10998.16					124.47	99.08			
	10971.79					124.17	98.85			
100	21947.68	22002.64	42.80	**25.13**	**251.25**	248.40	98.86	**99.11**	0.25	**0.26**
	22060.26					249.67	99.37			
	21999.99					248.99	99.10			
150	33152.75	33212.05	64.20	**37.69**	**376.88**	375.21	99.56	**99.74**	0.20	**0.20**
	33282.25					376.68	99.95			
	33201.14					375.76	99.70			

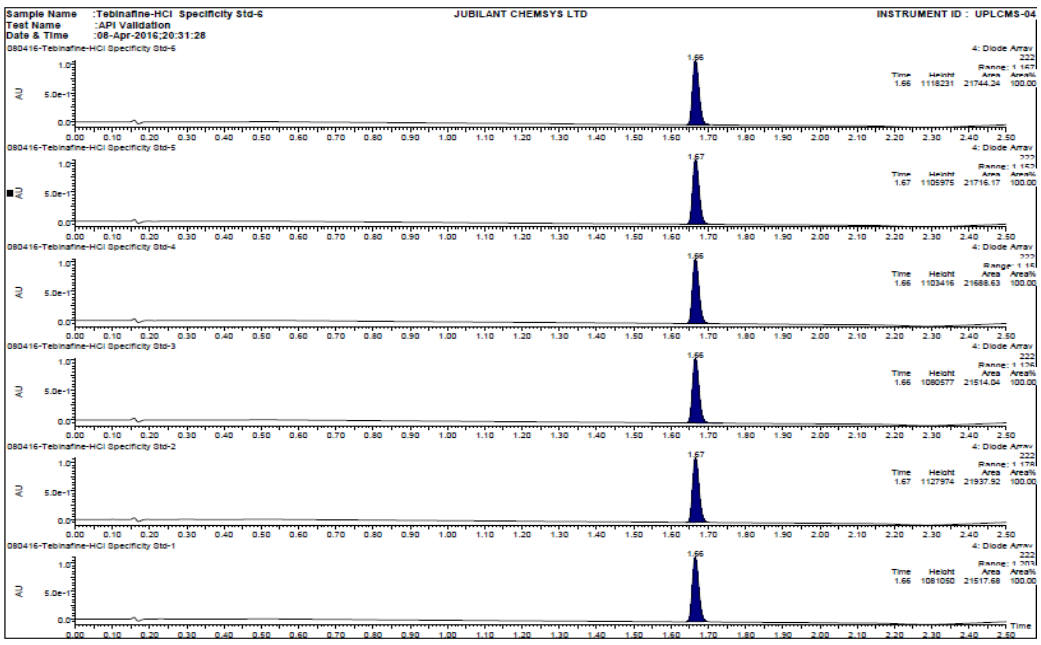
Table 5. Recovery studies for Atorvastatin Calcium

Level (%)	Sample area (per Inj.)	Average area	Sample Wt.(mg)	Sample Conc.(mg)	Amount Spiked (µg)	Amount Recovered (µg)	% Recovery	Average % Recovery	SD	% RSD
50	6660.78	6659.46	46.61	**2.52**	**25.20**	24.96	99.05	**99.03**	0.31	**0.31**
	6679.31					25.03	99.33			
	6638.28					24.88	98.72			
100	13318.06	13356.07	93.23	**5.04**	**50.40**	49.91	99.03	**99.31**	0.28	**0.28**
	13394.18					50.20	99.59			
	13355.98					50.05	99.31			
150	19999.63	20008.11	139.84	**7.56**	**75.60**	74.95	99.14	**99.18**	0.28	**0.28**
	19956.38					74.79	98.93			
	20068.32					75.21	99.48			

Precision

The precision of the method was evaluated by carrying out six independent injection of test sample against a qualified reference standard. The % RSD of peak area of the test was found to be **0.33, 0.94 & 0.35** for Carbamazepine, Terbinafine HCl & Atorvastatin Calcium, respectively**.** The results are summarized in Table 6 & 7 and Figure 10.





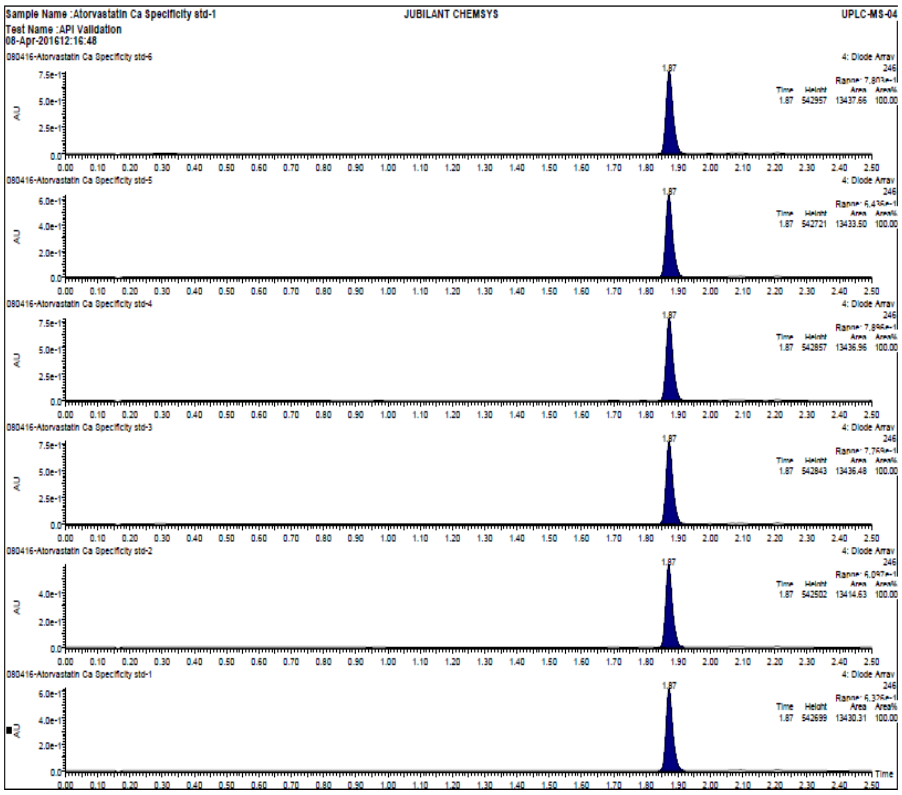


Figure 10. Chromatogram of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium Standard solution

Table 6. Results of Method Precision (Test area count)

Method Precision	Carbamazepine Area count			Terbinafine HCl Area count			Atorvastatine Ca Area count		
	Injection-1	Injection-2	Average	Injection-1	Injection-2	Average	Injection-1	Injection-2	Average
M-Precision-1	23986.38	24106.02	24046.20	21523.93	21853.42	21688.68	13165.37	13110.18	13137.78
M-Precision-2	23942.51	24018.80	23980.66	21565.07	21690.45	21627.76	13217.04	13251.42	13234.23
M-Precision-3	23897.27	23853.30	23875.29	21079.78	21474.63	21277.21	13282.60	13138.68	13210.64
M-Precision-4	24145.43	24038.71	24092.07	21703.13	21568.47	21635.80	13188.37	13154.08	13171.23
M-Precision-5	23967.30	24185.25	24076.28	21791.54	21297.23	21544.39	13219.60	13119.00	13169.30
M-Precision-6	23910.54	24154.53	24032.54	21865.52	21932.48	21899.00	13325.30	13199.36	13262.33
Average			**24017.17**			**21612.14**			**13197.58**
SD			79.56			202.84			46.43
% RSD			**0.33**			**0.94**			**0.35**

Table 7. Results of System Precision (Std. area count in specificity)

System Precision	Carbamazepine Area count	Terbinafine HCl Area count	Atorvastatine Ca Area count
S-Precision-1	25027.93	21517.68	13430.31
S-Precision-2	25218.46	21937.92	13414.63
S-Precision-3	25082.04	21514.04	13436.48
S-Precision-4	25007.68	21688.63	13436.96
S-Precision-5	25125.74	21716.17	13433.50
S-Precision-6	24924.88	21744.24	13437.66
Average	**25064.46**	**21686.45**	**13431.59**
SD	101.80	158.54	8.75
% RSD	**0.41**	**0.73**	**0.07**

Reproducibility (Intermediate Precision or Ruggedness)

An assay was performed by analyzing six samples of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium against qualified reference standard. The %RSD obtained from these samples was observed to be **0.19, 0.56 & 0.38** and %RSD of peak area of reference standard was observed to be **0.18, 0.45 & 0.50,** respectively. The results are summarized in Table 8 & 9.

Table 8. Results of reproducibility (Intermediate precision)

Method Precision	Carbamazepine Area count			Terbinafine HCl Area count			Atorvastatin Ca Area count		
	Injection-1	Injection-2	Average	Injection-1	Injection-2	Average	Injection-1	Injection-2	Average
M-Precision-1	24267.59	24229.69	24248.64	22668.48	22553.18	22610.83	13264.45	13279.30	13271.88
M-Precision-2	24399.00	24364.39	24381.70	22689.25	22895.19	22792.22	13258.85	13109.31	13184.08
M-Precision-3	24213.21	24333.16	24273.19	22260.99	22730.23	22495.61	13308.09	13350.40	13329.25
M-Precision-4	24217.20	24365.44	24291.32	22845.10	22710.89	22778.00	13315.82	13292.04	13303.93
M-Precision-5	24283.68	24312.93	24298.31	22126.51	22908.64	22517.58	13284.18	13292.75	13288.47
M-Precision-6	24247.93	24287.37	24267.65	22668.48	22553.18	22610.83	13306.09	13209.26	13257.68
Average			**24293.47**			**22634.18**			**13272.55**
SD			46.69			126.13			50.00
% RSD			**0.19**			**0.56**			**0.38**

Table 9. Results of reproducibility (Intermediate precision)

System Precision	Carbamazepine Area count	Terbinafine HCl Area count	Atorvastatin Ca Area count
S-Precision-1	24268.85	21503.73	13458.71
S-Precision-2	24383.94	21625.45	13313.35
S-Precision-3	24311.92	21634.59	13346.06
S-Precision-4	24375.35	21628.54	13465.87
S-Precision-5	24342.40	21777.48	13457.97
S-Precision-6	24354.18	21521.96	13442.78
Average	**24339.44**	**21615.29**	**13414.12**
SD	42.98	98.09	66.63
% RSD	**0.18**	**0.45**	**0.50**

Stability of Solution

The % Cumulative RSD for area and % assay of sample at initial hour and after 24 hours (at room temperature) was found to be **0.32, 0.47 & 0.29** for Carbamazepine, Terbinafine HCl & Atorvastatin Ca, respectively**.** The Assay method is also reproducible and shows stability up to 24 hrs. The results are summarized in Table 10, 11 & 12.

Table 10. Stability Data of Carbamazepine

Hours	Inj.	RT	Test Area	Average Area	% Assay
Initial Hour	Inj-1	1.28	24705.78	24627.76	98.26
	Inj-2	1.28	24549.73		
After 24 Hours	Inj-1	1.27	24781.37	24737.96	98.70
	Inj-2	1.28	24694.55		
Average		**1.28**		**24682.86**	**98.48**
SD		0.01		77.93	0.31
% RSD		**0.39**		**0.32**	**0.32**

Table 11. Stability Data of Terbinafine HCl

Hours	Inj.	RT	Test Area	Average Area	% Assay
Initial Hour	Inj-1	1.65	21970.22	21698.61	100.06
	Inj-2	1.66	21426.99		
After 24 Hours	Inj-1	1.65	21450.64	21554.13	99.39
	Inj-2	1.66	21657.61		
Average		**1.66**		**21626.37**	**99.72**
SD		0.01		102.16	0.47
% RSD		**0.35**		**0.47**	**0.47**

Table 12. Stability Data of Atorvastatin Calcium

Hours	Inj.	RT	Area	Average Area	% Assay
Initial Hour	Inj-1	1.87	13467.20	13470.25	100.29
	Inj-2	1.86	13473.29		
After 24 Hours	Inj-1	1.87	13521.60	13526.51	100.71
	Inj-2	1.87	13531.41		
Average		**1.87**		**13498.38**	**100.50**
SD		0.01		39.78	0.30
% RSD		**0.27**		**0.29**	**0.29**

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the sample containing Carbamazepine, Terbinafine HCl & Atorvastatin Calcium standard stock with those of the test sample. The specificity study reveals the absence of interference of impurities with the drug, since no extra peak appeared at the same retention time. The % RSD for six replicate measurements of peak area of standard preparation was found to be **0.41, 0.73 & 0.07** for

Carbamazepine, Terbinafine HCl & Atorvastatin Calcium, respectively. The results are summarized in Table 13.

Table 13. Specificity studies: Standard weight and Label Claim

Standards	Carbamazepine	Terbinafine HCl	Atorvastatin Ca
Std. Weight (mg)	100.50	100.50	50.40
Purity of Std. (%)	100.00	99.70	99.80
Conc. (µg/mL)	250.00	250.00	100.00
Sample Tablets	**Carbamazepine**	**Terbinafine HCl**	**Atorvastatin Ca**
Label Claim (mg)	400.00	250.00	20.00
Average Wt. (mg)	559.89	427.99	186.45
Sample Weight (mg)	136.20	178.20	462.20

Table 14. Recovery studies: Area's calculation

Standard Sol. & Sample Sol.	Carbamazepine Area count		Terbinafine HCl Area count		Atorvastatin Ca Area count	
	Standard Area	Sample Area	Standard Area	Sample Area	Standard Area	Sample Area
Replicate-1	25027.93	24074.21	21517.68	22402.11	13430.31	13269.68
Replicate-2	25218.46	24095.34	21937.92	22578.80	13414.63	13217.88
Replicate-3	25082.04		21514.04		13436.48	
Replicate-4	25007.68		21688.63		13436.96	
Replicate-5	25125.74		21716.17		13433.50	
Replicate-6	24924.88		21744.24		13437.66	
Average	25064.46	24084.78	21686.45	22490.46	13431.59	13243.78
SD	101.80	14.94	158.54	124.94	8.75	36.63
% RSD	**0.41**	0.06	**0.73**	0.56	**0.07**	0.28
% Assay (Sample)	99.25		99.83		100.03	

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (±0.10 mL/min), buffer concentration (±10%), column temperature (±5°C). In all the above varied conditions, the component of mobile phase was held constant, but no marked changes were observed in the chromatograms, which confirmed that the developed UPLC method is robust.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ studies were carried out to evaluate the detection and quantization limits of the method to determine the presence of any impurities by using the following equation:

LOD = 3.3 σ/S and LOQ = 10 σ/S.

Where σ is the standard deviation and S is the slope of the curve.

LOQ and LOD can be determined based on visual evaluation, signal-to-noise approach or standard deviation of the response and slope (calibration curve method). Limit of detection of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium was found to be **0.15 µg/mL, 0.16 µg/mL** & **0.14 µg/mL** and the limit of quantification of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium was determined to be **0.45 µg/mL, 0.48 µg/mL & 0.42 µg/mL** using signal-to-noise approach for determination.

Table 15. Result data of Validation Summary

S.No.	Parameter		Carbamazepine	Terbinafine HCl	Atorvastatin Calcium	ICH acceptance limit
1	Accuracy		99.15 - 100.42	99.04 - 99.74	99.03 - 99.31	98-102%
2	Precision		0.33	0.94	0.35	% RSD<2
3	Linearity		0.9999	0.9999	0.9999	R^2 <0.999
4	Stability		0.32	0.47	0.29	% RSD<2
5	Specificity		0.41	0.73	0.07	% RSD<2
6	LOD		0.15 µg/mL	0.16 µg/mL	0.14 µg/mL	S/N=3
7	LOQ		0.45 µg/mL	0.48 µg/mL	0.42 µg/mL	S/N=10
8	Ruggedness (Reproducibility)		0.18	0.45	0.50	% RSD<2
9	Robustness	Buffer Conc.	NO remarkable changes observed	NO remarkable changes observed	NO remarkable changes observed	% RSD<2
		Flow Rate				
		Column Temp.				

CONCLUSION

We have developed a Reverse phase UPLC-MS method to determine Carbamazepine, Terbinafine HCl & Atorvastatin Calcium efficiently and accurately within a relatively short period. The new method showed a good precision (RSD<0.29%) and recovery (98.82% - 99.76%). The new gradient RP-UPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method is capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. Short run time allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. This method can be directly used for LC-MS analysis on need basis. It is suitable for rapid and accurate quality control of Carbamazepine, Terbinafine HCl & Atorvastatin Calcium in Tablet formulations.

ACKNOWLEDGMENT

We would like to sincerely thank Jubilant Chemsys management in facilitating this work and also the Analytical team for generating the spectral data.

REFERENCES

1. Ana Fortuna, Joana Sousa, Gilberto Alves, Amílcar Falcão, Patrício Soares-da-Silva: Development and validation of an HPLC-UV method for the simultaneous quantification of carbamazepine, oxcarbazepine, eslicarbazepine acetate and their main metabolites in human plasma, Analytical and Bioanalytical Chemistry, 2010; 397: 1605-1615.
2. Hammam A. Mowafya, e, Fars K. Alanazia*, Gamal M. El Maghraby: Development and validation of an HPLC–UV method for the quantification of carbamazepine in rabbit plasma, Saudi Pharmaceutical Journal, 2012; 20: 29–34.
3. Ana Serralheiroa, Gilberto Alvesb, Ana Fortunaa, Marília Rochab, Amílcar Falcãoa: First HPLC–UV method for rapid and simultaneous quantification of phenobarbital, primidone, phenytoin, carbamazepine, carbamazepine-10,11-epoxide, 10,11-trans-dihydroxy-10,11-dihydrocarbamazepine, lamotrigine, oxcarbazepine and licarbazepine in human plasma, Journal of Chromatography B, 2013; 925: 1–9.
4. R.Ramesh Raju*, N. BujjiBabu: Simultaneous analysis of RPHPLC method development and validation of Terbinafine and Bezafibrate drugs in pharmaceutical dosage form, Pharmacophore, 2011; 2: 232-238.
5. Irrena Baranowska, Piotr Markowski, Jacek Baranowshi: Development and Validation of an HPLC method for the simultaneous analysis of 23 selected drugs belonging to different therapeutic groups in Human Urine samples, Analytical Sciences, 2009; 25: 1307-1313.
6. L. Matysová, P. Solich , P. Marek, L. Havlíková, L. Nováková, J. Šícha: Separation and determination of terbinafine and its four impurities of similar structure using simple RP-HPLC method, Talanta, 2006; 68: 713-720.
7. Stanisz B, Kania L; Validation of HPLC method for determination of Atorvastatin tablets and for monitoring stability in solid phase. Acta Pol. Pharm, 2006; 63: 471-476.
8. Novakova L, Satinsky D, Solich P.: HPLC methods for the determination of simvastatin and Atorvastatin. Trends Anal. Chem., 2008; 27: 352-367.
9. Gupta KR, Wadodkar AR, Wadodkar SG: Validated Reverse Phase HPLC method for simultaneous estimation of Atorvastatin and Atenolol in tablets. Pharm. Lett., 2011; 3: 393-403.
10. Manzoor A, Manohara YN, Ravi MC.: RP-HPLC method development and validation for simultaneous estimation of Atorvastatin Calcium and amlodipine besylate, Int. J. Chem. Tech. Res., 2012; 4: 337-345.
11. N. Brignol, R. Bakhtiar, L. Dou, T. Majumdar, F. L. S. Tse: Quantitative analysis of terbinafine (Lamisil®) in human and minipig plasma by liquid chromatography tandem mass spectrometry, Rapid Commun. Mass Spectrom, 2000; 14(3): 141-9.
12. Yannis Dostikas, Constantinos Apostolou, Constantinos Kousoulos, Georgia Tsatsou, Yannis L. Loukas: An improved high-throughput liquid chromatographic/ tandem Mass spectrometric method for Terbinafine quantification in human plasma, using automated liquid-liquid extraction based on 96-well format plate, Biomedical Chromatography, 2007; 21: 201-208.
13. Kim KB, Seo KA, Kim SE, Bae SK, Kim DH, Shin JG: Simple and accurate quantitative analysis of ten antiepileptic drugs in human plasma by liquid chromatography/tandem mass spectrometry, J Pharm Biomed Anal., 2011; 56(4): 771-7.
14. Moh'd Salemaldajeha, Eyad Mallaha*, Riad Awada, Wael Abu Dayyiha, Feras Darwish Elhajjib, Israa H. Al-anic, Basmah A. Othmana and Tawfiq Arafata: Determination of carbamazepine in rat plasma in the presence of licorice juice by using HPLC and its pharmacokinetic applications, Journal of Chemical and Pharmaceutical Research, 2015; 7(12): 116-126.
15. Mallikarjuna et al.: Development and Validation of Stability-Indicating RP-UPLC Method for Simultaneous Estimation of Amlodipine Besylate and Atorvastatin Calcium in Pharmaceutical Dosage Forms, J Chromatograph Separat Techniq, 2013; 4(6).
16. Sinsinwar, R. P. S. Rathore, T. Bharti, S. S. Sisodia: Analytical Method Development and Validation for Assay Determination of Secnidazole in Formulated Product by RP-UPLC, Current Research in Biological and Pharmaceutical Sciences, 2014; 3: 1-7.
17. N. Khandelwal, T. Bharti, C. S. Rajput, R. P. S. Rathore: Analytical Method Development and

Validation of Olanzapine in Formulated Product, World J. Pharma research, 2015; 4: 1690-1699.
18. Girish R. Deshpande, Amrendra K. Roy, N. Someswara Rao, B. Mallikarjuna Rao, J. Rudraprasad Reddy: Rapid Screening of Volatile Ion-Pair Reagents Using UHPLC and Robust Analytical Method Development Using DoE for an Acetyl Cholinesterase Inhibitor: Galantamine HBr, Chromatographia, 2011; 73: 639-648.
19. Guidance for industry-Q2B Validation of Analytical Procedures: Methodology, International Conference of Harmonization, 1996; 1-9.
20. ICH guidelines, Q2 (R1) step 4, Validation of Analytical Procedures: Text and Methodology, 2005; 1-13.
21. S. Walfish: Analytical Methods: A Statistical perspective on the ICH Q2A and Q2B Guidelines for Validation of Analytics Methods, BIOPHARM International, 2006; 1-6.