

Simultaneous Liquid Chromatographic Determination of Two Co-Prescribed Anti-Cancer Drugs in Bulk Drug, Dosage Formulations and in Human Serum Using Multivariate Technique: Application to *in vitro* Drug Interaction

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Abstract

Present study describes the development and validation of a high-performance liquid chromatographic (HPLC) assay for the simultaneous quantification of atorvastatin and celecoxib in human serum and pharmaceutical formulations. The separation was carried out on Bondapak, C₁₈ (10 μm, 25×0.46 cm) column using mobile phase 80:20 methanol-water of pH 3.5, maintained with o-phosphoric acid. The method was optimized using multivariate technique and detector response was recorded at five different wavelengths. Chromatography was performed at ambient temperature by pumping the mobile phase at a flow rate of 1.0 mL min⁻¹. Calibration curves were found to be linear with correlation coefficient greater than 0.998 over the concentration range 2-60 and 2-70 ng mL⁻¹ with detection limits 0.024 and 0.019 ng mL⁻¹ in bulk drug and 0.04 and 0.01 ng mL⁻¹ in serum at isobestic point. The method was validated for specificity, selectivity, and linearity, accuracy, precision and intermediate precision. The proposed method is valid for the determination of drugs in pharmaceutical formulations without interference of excipients. Recovery values of both drugs were 99.33 to 101.65%. Moreover the *in vitro* interaction studies of atorvastatin in presence of celecoxib were carried out at physiological temperature (37°C), simulating stomach environment and the reactions were studied by RP-HPLC.

Keywords: Atorvastatin; Celecoxib; Anticancer drugs; HPLC; Multivariate, Drug interaction

Introduction

Atorvastatin (Figure 1a) belongs to lipid-lowering drug class statins, widely used for the treatment of hypercholesterolemia [1] to reduce total cholesterol, LDL in blood [2], apo-B which is responsible for carrying cholesterol to tissues [3], triglycerides levels [4] and C-reactive protein (CRP) [5] as well as increase HDL levels. Besides, they are potent anti-oxidative [6,7], antitumor [8], anti-inflammatory [9,10], immunomodulator [11,12], anti-malarial [13] and bone forming agents [14]. They also reduce the risk of total prostate cancer as well as more aggressive prostate cancer [15]. Moreover, patients who are taking statins, showed significantly lower pretreatment prostate-specific antigen level, percentage of positive biopsy cores and earlier clinical stage [16].

Celecoxib (Figure 1b) is a non-steroidal anti-inflammatory drug (NSAID), used to treat inflammatory conditions such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis [17]. NSAIDs are known to relieve symptoms of primary dysmenorrhea, fever and also possess mild antiplatelet effect. They are useful in sepsis-induced acute pneumonia [18] and in retarding metastases of mammary carcinoma [19]. Celecoxib may delay or help in preventing progression in prostate cancer patients [20].

Statins are administered together with NSAIDs to hypercholesterolemia patient also suffering from inflammations [21]. Atorvastatin and celecoxib are two widely co-prescribed drugs for clinical treatment of not only such problems but recent *in vivo* studies have showed that atorvastatin in combination with celecoxib inhibits prostate PC-3 tumors in immunodeficient mice [22], suppresses the incidence and multiplicity of colon adenocarcinoma in the azoxymethane-induced rat colon carcinogenesis model [23,24]. The

possible mechanisms by which statins and NSAIDs inhibit growth of cancer cell are described by Xiao and Yang [21]. Atorvastatin and celecoxib in combination has a potent inhibitory effect on the progression and growth of androgen-dependent prostate tumors to androgen independence in a xenograft model [25,26], also they have potency to prevent progression of atherosclerosis [27].

A variety of techniques have been reported for the analysis of atorvastatin alone or in combination with other drugs [28-32], similarly different analytical procedures have been reported for the determination of celecoxib [33-35] in bulk drug, human serum and in pharmaceutical formulation. To the best of our knowledge, there is no LC method yet reported for separation and simultaneous analysis of these two drugs in human serum and in pharmaceutical formulation with lowest detection limits.

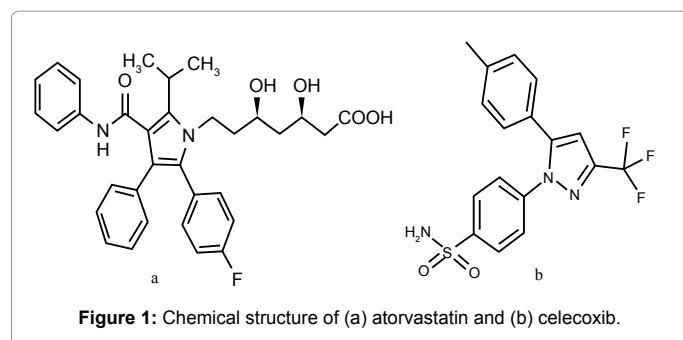
Chemometrics is the discipline that uses mathematical and statistical methods to extract the relevant information on material systems. Both descriptive and predictive problems, especially in chemistry are untied by applying chemometrics employing core data-

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analytic disciplines such as multivariate statistics, applied mathematics, and computer science. Evenly, multivariate calibration allows for accurate quantitative analysis in the presence of heavy interference by other analytes. In the present study, multivariate calibration technique has been applied for simultaneous LC determination of atorvastatin and celecoxib in order to minimize the limitations of single data processing. Under the optimized conditions, the proposed technique was successfully applied for the routine analysis of both drugs with high resolution and sensitivity. Mathematically;

$$A_{240} = a \cdot C_x + k_1 \quad (1)$$

$$A_{245} = b \cdot C_x + k_2 \quad (2)$$

$$A_{250} = c \cdot C_x + k_3 \quad (3)$$

$$A_{255} = d \cdot C_x + k_4 \quad (4)$$

$$A_{260} = e \cdot C_x + k_5 \quad (5)$$

where, A represents the area under curve of analyte, C_x is the concentration of analyte, a, b, c, d and e are the slope and k_1, k_2, k_3, k_4 and k_5 are the intercept of linear regression equation at different wavelengths. The sum of above equations is as follows; which can be rearranged as equation (7) to determine the concentration of analytes.

$$A_T = (a+b+c+d+e) C_x + K_T \quad (6)$$

$$C_x = \frac{A_T - K_T}{(a+b+c+d+e)} \quad (7)$$

where, A_T and K_T represents the sum of area and intercepts at different wavelength.

The method was found to be sensitive for the determination of these drugs in human serum and pharmaceutical formulation without interference of excipients. Moreover the *in vitro* interaction studies were carried out at physiological temperature (37°C) in different pH like pH 4, 6.8 and 9, simulating stomach environment (full stomach and intestinal pH) and the reactions were studied by RP-HPLC.

Experimental

Materials and reagents

Atorvastatin calcium was a classic gift by Genix Pharma Private Limited and celecoxib was obtained from Getz Pharma. Both raw substances were used as reference standards without any extra purification process. Pharmaceutical formulations named Genovax[®] (10 mg) and Celbexx[®] capsules (100 mg) were purchased from local pharmacy (Karachi). The human serum used for this research was voluntarily donated by a healthy person at Fatmid Foundation Karachi. Analytical grade methanol and 85% *o*-phosphoric acid were obtained from Merck, Darmstadt, Germany. Double distilled de-ionized water was used throughout the procedure. All solutions were filtered through 0.45 μ m filter and degassed by sonicator.

Instruments

Dissolution equipment manufactured according to B.P 2007 standard with slight modification. Shimadzu 1800 UV-visible spectrophotometer was used for the determination of isosbestic point and individual λ_{max} of analyte. The HPLC system consisted of Shimadzu LC-20-AT HPLC pump, rheodyne manual injector fitted with a 20 μ L loop and a variable wavelength programmable SPD-20A Shimadzu UV-visible detector. Chromatographic separation was achieved on a Bondapak, C_{18} (10 μ m, 25 \times 0.46 cm) column. The chromatographic and integrated data were recorded using a CBM-102 communication Bus Module Shimadzu. Data acquisition was performed by Shimadzu Class-GC 10 software (version 2).

Chromatographic conditions

A mixture of methanol and water (80:20 v/v) was used as mobile phase, the pH of which was adjusted to 3.5 using *o*-phosphoric acid (85% w/w). The mobile phase used throughout was freshly prepared and was filtered through a millipore vacuum filter system equipped with 0.45 μ m filter and degassed by a sonicator. Chromatography was performed at ambient temperature by pumping the mobile phase at flow rate of 1.0 mL min⁻¹. Column effluent was monitored at five different wavelengths including 240, 245, 250, 255 and 260 nm.

Stock standard solutions

Accurately weighed 10 mg of standard atorvastatin and celecoxib were transferred in to two separate 100 mL volumetric flasks and volumes were made up with 80:20 methanol-water diluent to bring the final concentration of 100 μ g mL⁻¹. These solutions were further used for preparation of working solutions of drugs. The stock solutions were prepared once and stored at 4°C protected from light.

Buffer of pH 4 was prepared by taking 0.1 M potassium chloride and its pH was adjusted with 0.1 N hydrochloric acid. For preparing pH 6.8 buffer, potassium dihydrogen orthophosphate (0.6 g), disodium hydrogen orthophosphate (6.4 g) and sodium chloride (5.85 g) were dissolved in 1000 mL deionized water and pH was adjusted with 17% ammonium hydroxide. pH 9 buffer was prepared by dissolving 17.4 g of potassium dihydrogen orthophosphate in 800 mL of deionized water and pH was adjusted with 17% ammonium hydroxide.

Analytical procedure

Aliquots of each solution were accordingly diluted with 80:20 methanol-water diluent to working standard solutions of concentration range 2-60 and 2-70 ng mL⁻¹ for atorvastatin and celecoxib respectively. 20 μ L of these solutions were injected into the LC system (n=5). Before analysis, all the solutions were filtered through a 0.45 μ m vacuumed filter and degassed by sonicator.

Pharmaceutical preparations

Ten tablets of Genovax and ten Celbexx capsules were separately powdered and amount equivalent to 10mg of atorvastatin and 10 mg of celecoxib were dissolved in mobile phase in separate 100 mL volumetric flasks. The solutions were subjected to vigorous shaking and then allowed to stand for 1 h with intermittent sonication for complete extraction of the drug. Both the solutions were then filtered and volume was brought to mark with mobile phase and then treated as above.

Drug serum solution

The drug serum solution was prepared by adding 1.0 mL of serum in

9 mL of acetonitrile. The mixture was vortexed for one minute and then centrifuged for 10 minutes at 10,000 rpm. The obtained supernatant was filtered by 0.45 μm membrane filter. The clear solution obtained was spiked with drug solutions to produce desired concentrations of atorvastatin (4, 8 and 16 ng mL^{-1}) and celecoxib (4, 8 and 16 ng mL^{-1}) in human serum. 20 μL of these solutions were injected into the LC system.

Method validation

The method was validated following the ICH 2006 guidelines by reporting its linearity, limit of detection and quantification as well as precision and recovery of atorvastatin and celecoxib in its formulation and human serum. Also the specificity and selectivity, system suitability and robustness were measured.

The system suitability was evaluated on each day of method validation after equilibrating the HPLC system with initial mobile phase composition, by injecting ten replicates of same standard. The detector response was observed. For specificity studies, chromatograms of pure solvent, reference standards, solution of pharmaceutical formulation and blank and spiked serum sample were recorded and interference due to excipients of tablet and endogenous components of serum were observed. Linearity was evaluated by plotting calibration curve between concentration and peak area for API and serum at five different wavelengths 240, 245, 250, 255 and 260 nm at six concentration level for each component and regression characteristics including intercept, slope and correlation coefficient were calculated. Accuracy was determined by calculating the found concentration and percentage of recovery of analytes in pharmaceutical formulation and human serum at all the wavelengths in the concentration range of 2-60 and 2-70 ng mL^{-1} for atorvastatin and celecoxib respectively. Five replicates at six concentration levels for reference standard and three samples of human serum were analyzed for the determination of precision of the proposed method. It is reported as percent relative standard deviation. The LOD and LOQ were determined at the concentration where signal to noise ratio was three times and ten times to the baseline noise respectively. Some of the chromatographic parameters like pH and composition of mobile phase, flow rate and wavelength were intentionally varied and the subsequent effect on analytical results was observed.

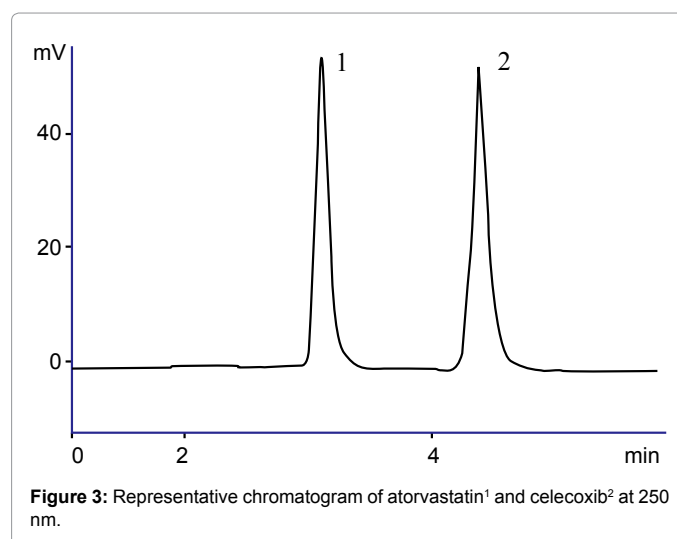
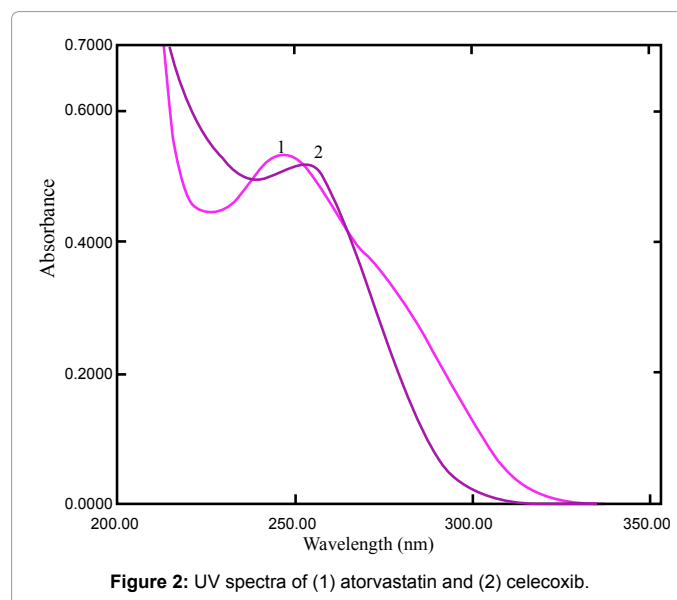
In vitro interaction studies by RP-HPLC

100 mL stock solution of 100 $\mu\text{g mL}^{-1}$ atorvastatin and celecoxib were separately prepared in simulated gastric juice buffers of pH 4, 6.8 and 9 individually. Equal volume of each drug solution for each pH was mixed together in reaction flask to get the final concentration of 50 $\mu\text{g mL}^{-1}$. These flasks were kept at water bath at 37°C for two hours for interaction with constant stirring. 2 mL of these solutions were withdrawn at 0 min and periodically after every 15 min for 2 h, filtered through 0.45 μm membrane filter and analyzed by HPLC employed the proposed method. The volume of dissolution fluid was maintained by adding an equal amount of dissolution fluid withdrawn, which had previously been maintained at the same temperature in the same bath.

Results and Discussion

UV scan of atorvastatin and celecoxib showed maximum absorbance at 248 and 254 nm respectively (Figure 2); therefore in order to minimize error and investigate the optimum conditions, a well known multivariate technique is applied for the determination of these drugs in binary mixture which provides significant improvements in precision over methods that are restricted to a small number of

wavelengths. The five linear regression functions at wavelengths of 240, 245, 250, 255 and 260 nm for individual active compounds were calculated by using relationships between the multivariate HPLC data and linearity range. Along with the multivariate analysis, a fixed suitable wavelength of 250 nm, selected from UV spectra of individual drug, was chosen for the liquid chromatographic analysis in API, pharmaceutical formulation and human serum and then method was validated. The representative showing the retention times of 3.08 and 4.34 min for atorvastatin and celecoxib respectively is given in figure 3. Multivariate calibration technique applied to the pharmaceutical formulations is expressed in table 1 and the precision of method at different wavelengths are given in table 2. The regression characteristics including slope, intercept, standard error, standard error estimate and correlation coefficient for both drugs at different wavelengths were determined by plotting calibration curves between concentrations of drug against peak area. Table 3 represents the regression characteristics and detection and quantitation limits at each wavelength. Multivariate chromatogram of atorvastatin and celecoxib is represented in figure 4.



ng mL ⁻¹	ngmL ⁻¹					Multi Calibr
	240 nm	245 nm	250 nm	255 nm	260 nm	
Atorvastatin						
2	1.95	1.99	2.00	2.00	2.00	2.00
4	3.93	3.99	4.00	4.01	3.98	4.02
8	7.84	7.99	8.00	7.97	7.94	7.96
16	15.16	14.88	14.71	15.33	14.77	15.95
30	29.68	29.67	29.77	29.87	29.96	30.04
60	58.82	59.59	60.00	59.19	59.41	59.98
Celecoxib						
2	2.01	2.00	1.99	2.01	2.00	2.00
4	4.01	4.01	3.94	3.99	4.00	4.00
8	8.03	8.00	8.06	7.98	8.00	16.01
16	15.63	16.29	16.04	16.21	15.71	34.93
35	35.21	35.15	35.04	35.61	35.05	50.29
70	70.07	69.80	70.11	69.94	70.06	69.97

Table 1: Accuracy of the method at different wavelengths.

ng mL ⁻¹	% RSD				
	240 nm	245 nm	250 nm	255 nm	260 nm
Atorvastatin					
2	1.37	0.93	0.28	1.32	1.57
4	0.21	0.58	1.19	0.11	0.17
8	0.73	0.09	0.80	0.50	1.55
16	1.51	0.10	1.02	1.11	1.55
30	0.72	0.29	1.27	0.45	0.35
60	0.30	0.68	1.69	0.89	0.80
Celecoxib					
2	0.59	0.92	2.20	0.35	0.60
4	0.56	0.05	1.14	0.09	0.19
8	0.36	0.20	0.09	0.24	0.29
16	0.62	0.14	2.38	0.86	0.21
35	1.41	0.27	1.36	0.10	0.21
70	0.73	0.58	2.32	0.26	0.29

Table 2: Precision of the method at different wavelengths.

λ_{max}	Intercept	R ²	SE ^a	SEE ^b	LOD (ng mL ⁻¹)	LOQ (ng mL ⁻¹)
Atorvastatin						
240	Y = 4958x + 23174	0.9980	1.22	1.89	0.048	0.144
245	Y = 5020x + 23137	0.9980	1.12	1.74	0.038	0.116
250	Y = 5102x + 23068	0.9999	1.08	1.68	0.024	0.073
255	Y = 5057x + 23818	0.9986	1.16	1.80	0.046	0.140
260	Y = 5052x + 22993	0.9982	1.10	1.71	0.056	0.169
Serum	Y = 4692x + 52131	0.9985	1.55	1.94	0.040	0.110
Celecoxib						
240	Y = 4815x + 20851	0.9980	0.96	1.32	0.050	0.153
245	Y = 4887x + 20996	0.9985	0.73	1.00	0.031	0.093
250	Y = 4849x + 20827	0.9982	0.83	1.14	0.019	0.057
255	Y = 4958x + 19862	0.9985	0.73	1.01	0.010	0.030
260	Y = 4839x + 20380	0.9980	1.01	1.40	0.049	0.149
Serum	Y = 4601x + 70072	0.9979	0.50	0.33	0.010	0.030

^aStandard error, ^bStandard error of estimate

Table 3: Regression characteristics.

Method development and optimization

The parameters were initially optimized; like mobile phase and pH were varied and preliminary tests were performed to establish the optimum conditions necessary for the analysis of drugs in pharmaceutical formulations and human serum. Separation was

achieved on Bondapak, C₁₈ (10 μ m, 25 \times 0.46 cm) column. Different solvent system like acetonitrile-water and methanol-water in various ratios (80:20, 70:30, 60:40) were checked with varying pH (2.8-3.6) in order to study system suitability. Mobile phase 80:20 methanol-water with pH adjusted at 3.5 produced high resolution sharp peaks for both drug. The method was then applied for *in vitro* interaction studies of these drugs simulating human body environment. The % availability of drugs was calculated alone and in presence of each other. The proposed method is simple and does not involve much time to prepare samples.

Method validation

System suitability test: System suitability was evaluated by calculating the capacity factors (*k'*), theoretical plates (N), tailing factor (T) and resolution (Rs). The column efficiency expressed by number of theoretical plates was greater than 2000 and tailing factor was in the range of 1.06-1.23. The data is summarized in table 4.

Specificity: Specificity of developed method was demonstrated by injecting placebo like starch, magnesium stearate, lactose and hydroxymethylpropylcellulose individually under the same experimental conditions. Similarly the blank serum sample was injected to study the possible interference for the selectivity of atorvastatin and celecoxib. Both the tested samples were found to be free from interfering components or excipients at the retention times of analyte.

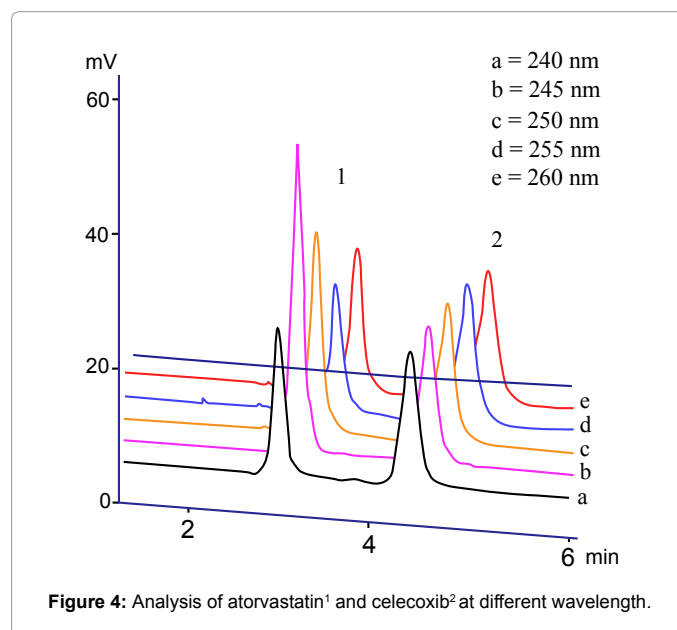


Figure 4: Analysis of atorvastatin¹ and celecoxib² at different wavelength.

Parameter	λ_{max}	k' ^a	N ^b	T ^c	Res ^d
Atorvastatin	240	0.30	3827	1.18	0.76
	245	0.70	3149	1.15	1.64
	250	0.69	3147	1.15	1.78
	255	0.68	3139	1.23	1.82
	260	0.65	3137	1.23	1.87
Celecoxib	240	0.82	3609	1.09	1.53
	245	1.38	3351	1.06	1.48
	250	1.37	3372	1.07	1.48
	255	1.36	3405	1.06	1.48
	260	1.30	3410	1.06	1.49

^aCapacity factor, ^bTheoretical plates, ^cTailing factor, ^dResolution

Table 4: System suitability parameters.

Representative chromatogram of solvent, reference standards, solution of pharmaceutical formulation and blank and spiked serum sample is shown in figure 5.

Precision and repeatability: The precision of the method was assessed with respect to repeatability and is expressed in terms of relative standard deviation. The intraday precision (%RSD) values obtained for repeatability studies at isobestic point of 250 nm were in the range of 0.13-1.90 for atorvastatin and celecoxib for API and human serum respectively. The data is presented in table 5.

Accuracy: Accuracy of the proposed method was evaluated by performing the recovery tests of pharmaceutical formulation and human serum by spiking a known amount of standard solution followed by triplicate analysis using proposed method. The percent recovery values calculated were in the range of 99.33-101.65 and 99.20-101.44% in pharmaceutical formulations and human serum respectively (Table 5).

Linearity: Linearity of calibration graphs was found to be 2-60 and 2-70 ng mL⁻¹ for atorvastatin and celecoxib respectively. The correlation coefficient (R²) obtained from regression line at each wavelength for bulk drug and human serum was greater than 0.998. Slope, intercept, standard error and standard error estimate are given in table 3.

Detection and quantization limits: The detection and quantization limits were calculated according to ICH guidelines using formulae: LOD=3.3 S/N and LOQ=10 S/N, where S is the standard deviation of the peak area of the sample and N is the slope of calibration curve. At isobestic point, the LOD was found to be 0.024 and 0.019 ng mL⁻¹ (in API) and 0.04 and 0.01 ng mL⁻¹ (in serum) and LOQ was found to be 0.073 and 0.057 ng mL⁻¹ (in API) and 0.11 and 0.03 ng mL⁻¹ (in serum) for atorvastatin and celecoxib respectively (Table 3).

Ruggedness: In the ruggedness study, the operating parameters were deliberately changed and subsequent effect was examined. Under the same chromatographic conditions, one parameter was altered, while keeping others constant. It was observed that the proposed method is able to withstand relatively minor variations in parameters like pH and composition of mobile phase, flow rate etc (Table 6).

In vitro interaction studies by RP-HPLC

Patients with multiple number of medical complain are treated with a large number of medication, or most of the time more than one medicine is prescribed to patient for a single complain to accelerate the effect of each other. It may happen that these drugs interact with each other and suppress the potency of other leading to the increase or decrease in their bioavailability. It is therefore necessary to be aware of drug-drug interaction of simultaneously prescribed medicines in order to suggest the suitable drug therapy. Atorvastatin and celecoxib are two simultaneously prescribed anticancer drugs, also having potency to prevent the progression of atherosclerosis [27].

In the present study, the *in vitro* interaction of atorvastatin and celecoxib has been studied by RP-HPLC technique in buffers of pH 4, 6.8 and 9 at physiological temperature simulating the pH of different areas of GI tract and blood pH and % availability was investigated in presence of each other. Prior to this, the availability of both the drugs were individually investigated under the same experimental conditions. Results of *in vitro* interaction studies of drugs at different pH is given in table 7 and representative chromatogram showing separation of atorvastatin and celecoxib after interaction at different pH is presented in figure 6. It was illustrated that up to 3 hrs, both drugs are available

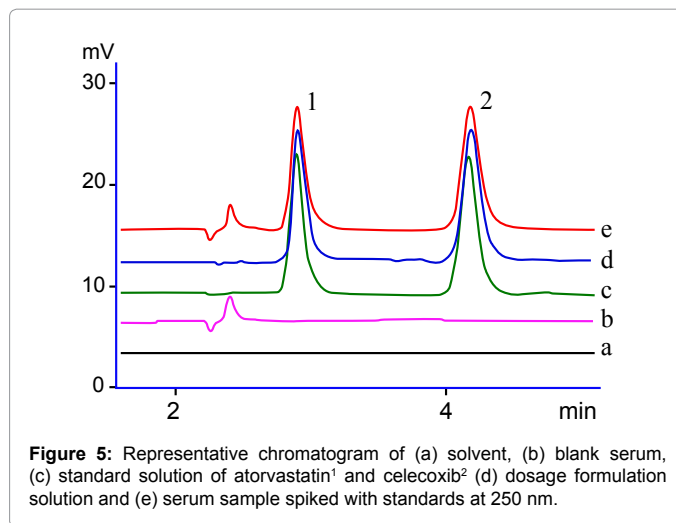


Figure 5: Representative chromatogram of (a) solvent, (b) blank serum, (c) standard solution of atorvastatin¹ and celecoxib² (d) dosage formulation solution and (e) serum sample spiked with standards at 250 nm.

ng mL ⁻¹	%RSD	%Rec		% Er	ng mL ⁻¹	%RSD	%Rec		% Er
		Ator	Genovax				Cele	Celbexx	
2	0.28	100.34	-0.34		2	0.58	101.65	-1.65	
4	1.19	99.33	0.67		4	1.51	99.44	0.56	
8	0.80	99.90	0.10		8	0.29	101.47	-1.47	
16	1.02	100.04	-0.04		16	1.44	101.00	-1.00	
30	1.27	99.85	0.15		35	0.74	99.99	0.01	
60	1.69	100.06	-0.06		70	1.90	100.04	-0.04	
Serum									
4	0.15	100.11	-0.11		4	0.43	101.15	-1.76	
8	0.32	99.37	0.63		8	0.13	100.92	-1.10	
16	1.39	99.20	0.80		16	0.27	101.44	-1.06	

Table 5: Accuracy and precision of atorvastatin and celecoxib in pharmaceutical formulation and in human serum.

Parameter	Atorvastatin				Celecoxib		
	t _r	N	T	t _r	N	T	
pH	3.4	2.97	4048	1.51	3.96	5148	1.41
	3.5	3.08	3451	1.30	4.35	4235	1.16
	3.6	3.15	4156	1.41	4.60	5272	1.26
Mobile phase	82:18	2.74	2914	1.47	3.68	3534	1.27
	80:20	3.00	3115	1.46	4.03	3836	1.27
	78:22	3.33	3300	1.53	4.46	4125	1.35
Flow rate	0.9	3.33	3341	1.52	4.47	4197	1.34
	1.0	3.15	3615	1.44	4.17	4323	1.29
	1.1	2.74	2908	1.48	3.68	3522	1.28

Table 6: Ruggedness.

Time (min)	Atorvastatin	Celecoxib	Atorvastatin	Celecoxib	Atorvastatin	Celecoxib
	pH 4		pH 6.8		pH 9	
0	115.67	101.47	123.96	127.59	108.63	101.74
30	98.42	98.00	99.07	98.85	99.78	99.04
60	98.87	99.79	98.96	99.23	98.94	98.76
90	98.38	100.64	99.58	98.80	99.36	98.42
120	98.76	100.46	99.32	98.19	99.57	98.67
150	98.52	100.38	99.10	98.84	98.36	98.12
180	98.31	99.45	98.69	98.42	99.17	98.63

Table 7: Interaction studies by RP-HPLC.

98-100% at all the studied pH and no as such reduction in availability of drugs was observed indicating that no interaction occurred between atorvastatin and celecoxib. At the end of experiment, the availability

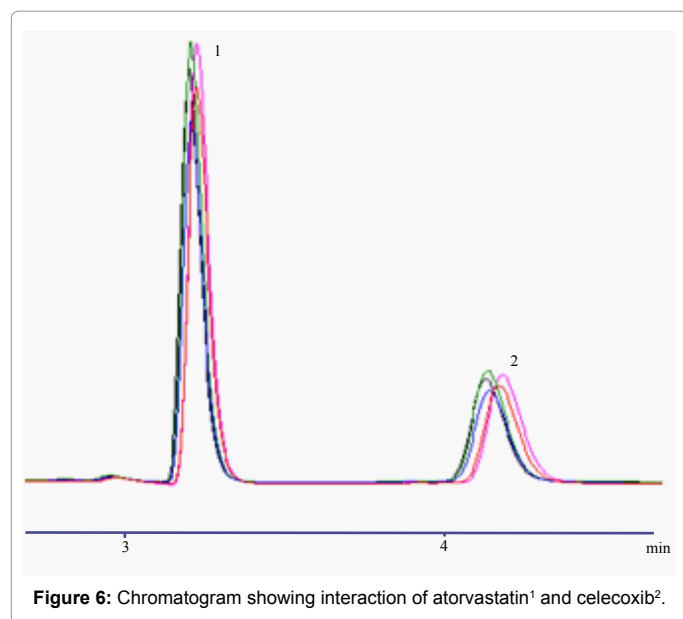


Figure 6: Chromatogram showing interaction of atorvastatin¹ and celecoxib².

of atorvastatin and celecoxib was 98.31, 98.69 and 99.17% and 99.45, 98.42 and 98.63% at pH 4, 6.8 and 9 respectively.

Conclusion

In the present study, multivariate calibration technique based on regression analysis has been established for two widely co-prescribed anticancer drugs atorvastatin and celecoxib. This technique was subjected to high performance liquid chromatography for simultaneous quantitative analysis of binary mixtures with lowest detection limits.

It was found that the technique is fast, economic and involves non-destructive analytical measurements to estimate sample properties which would otherwise require time-consuming, expensive or destructive techniques. Good separation and resolution of the chromatographic peaks was achieved without interference of excipients and with good percent recoveries. All calibration curves were found to be linear with correlation coefficient greater than 0.997. Moreover, the method showed excellent accuracy, precision, selectivity and reproducibility for simultaneous determination of both drugs in pharmaceutical formulations and in human serum. Also the *in vitro* interaction studies have been investigated for atorvastatin and celecoxib at 37°C in simulated stomach environment.

Acknowledgement

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