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METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ANALYSIS OF ASPIRIN AND SIMVASTATIN IN PHARMACEUTICALS BY RP- HPLC

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ABSTRACT

A simple, accurate and precise reverse phase high performance liquid chromatography (RPHPLC) method has been developed and validated for the simultaneous determination of aspirin and simvastatin in combined dosage form. Separation was performed on a C18 column [ODS column, 250mm x 4.5mm with particle size 5ìm. with a mobile phase consisting of acetonitrile: methanol: phosphate buffer (55:30:15) at a flow rate of 1ml/min and UV detection was carried out at 225nm. The developed method was validated for the parameters like system suitability, specificity, linearity, accuracy and robustness according to the ICH guidelines Q2B. Retention times of aspirin and simvastatin were found to be 3.4 and 7.44 respectively. Linearity was found in the range from 10-50µg/mL for aspirin and 2-10µg/mL for simvastatin with correlation coefficients 0.9998 and 0.9999 respectively. The % recovery for 100% spiked level was 99.44 and 101.20 for aspirin and simvastatin respectively. The developed method was accurate, robust, selective, linear and repeatable which could be used for routine analysis of aspirin and simvastatin in their combined dosage forms.

Key words: *Aspirin; Simvastatin; Simultaneous; RP-HPLC.*

INTRODUCTION

Aspirin (2-acetoxybenzoic acid) is analgesic and antipyretic. Its mode of action as an anti-inflammatory and anti-rheumatic agent may be due to inhibition of synthesis and release of prostaglandin[1]. Aspirin inhibit platelet aggregation by irreversible inhibition of platelet cyclooxygenase and thus inhibits the generation of thrombooxygenase A_2 a powerful inducer of platelet aggregation and vasoconstriction[2]. Simvastatin acts by inhibiting HMG CoA reductase and is used for the treatment of hypercholesterolemia. After oral administration, this prodrug is converted into á-hydroxy acid of Simvastatin, which is a potent inhibitor of HMG CoA reductase, a key enzyme required for the synthesis of cholesterol in liver[3-4]. Literature surveys suggest that there are analytical methods by HPLC for the estimation of aspirin and simvastatin individually but not simultaneously. The objective of this study was therefore to develop a simple, sensitive, and precise HPLC method for the simultaneous analysis of aspirin and simvastatin for use as a quality control tool in the development of simvastatin–aspirin.[5-7]

MATERIALS AND METHODS

Chemicals and Reagents

Reference standard samples of aspirin and simvastatin were procured from Aurobindo Pharma Ltd., Hyderabad. HPLC grade methanol and water procured from Merck specialties pvt ltd, Mumbai, India.

Equipment

Agilent 1120 compact LC chromatographic system, equipped with variable wavelength programmable UV detector and Rheodyne injector with 20 µL fixed loop was used for the chromatographic separation. The chromatogram was recorded and peaks quantified by means of Ezchrome software. Chromatographic separation was carried out on a C_{18} column [Agilent ODS 5 column, 250mm x 4.6mm].

Chromatographic Conditions

Mobile phase consisting of acetonitrile: methanol: buffer (55:30:15) with a flow rate of 1ml/min was used for chromatographic separation. The mobile phase was filtered through Millipore filter of 0.45ìm grade and sonicated for 15 min before use. The flow rate was maintained at 1 ml/min and the injection volume was 20ìL. UV detection was carried out at 235 nm and the separation was achieved at ambient temperature.

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EXPERIMENTAL

Preparation of standard stock solution

The stock solutions of aspirin and simvastatin were prepared by accurately weighing 10 mg each into a separate 10 ml volumetric flasks A and B and made up to the volume with mobile phase to get 1000µg/mL respectively. From the above standard stock solutions 1ml from volumetric flask A and 0.5 ml from volumetric flask B was transferred to a 10 ml volumetric flask and made up to the volume with same mobile phase to get 100µg/mL for aspirin and 50 µg/mL of simvastatin respectively. Aliquots of each standard solutions were transferred to a series of 10ml volumetric flasks and the volume was made up to mark with mobile phase to give binary mixtures of various concentrations ie.., 0, 20, 30, 40 and 50 ìg/ml for aspirin and 2,4,6,8,10ìg/ml for simvastatin.

Preparation of sample solution

Twenty tablets each containing 100mg of aspirin and 50mg of simvastatin were accurately weighed and finely powdered. The powder equivalent to 100 mg of aspirin and 50 mg of simvastatin were accurately weighed, mixed with 50 ml of mobile phase in 100 ml volumetric flask and sonicated for 5 min with occasional shaking and made up to volume with mobile phase. The solution was then filtered through 0.45µ membrane filter. Final stock containing 20µg/mL and 10µg/mL of aspirin and simvastatin respectively were prepared.

METHOD VALIDATION

Linearity

Linearity of the method was determined by mean of calibration graph using an increasing amount. Linearity was evaluated by visual inspection of a calibration graph. The linearity of the method was determined in concentration range of 10-100µg/ml for aspirin and 2-10µg/ml for simvastatin. Each solution was injected in triplicate. The slope, intercept was reported as required by ICH. A calibration curve of concentration vs. peak area was plotted, shown in the Figure 1 and 2. Regression equations were established and the correlation coefficients were determined. The chromatogram showing the separation of aspirin and simvastatin is shown in the Figure 3. The results of regression analysis are given in the Table 1.

Table 1: Results for Linearity (n=3)

Parameters	Aspirin	simvastatin
Slope	636059	182219
Intercept	49876	34876
Correlation co-efficient	0.9998	0.9999
LOD and LOQ	0.61µg/ml and 0.33µg/ml	0.79µg/ml and 1.157µg/ml

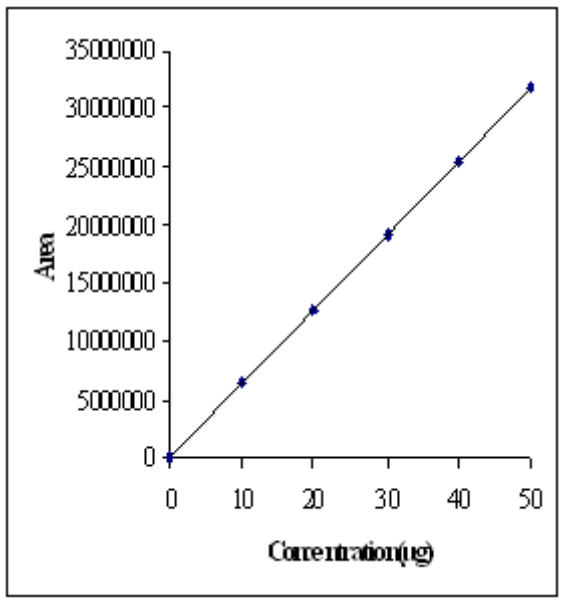

Fig. 1 : Calibration curve for aspirin

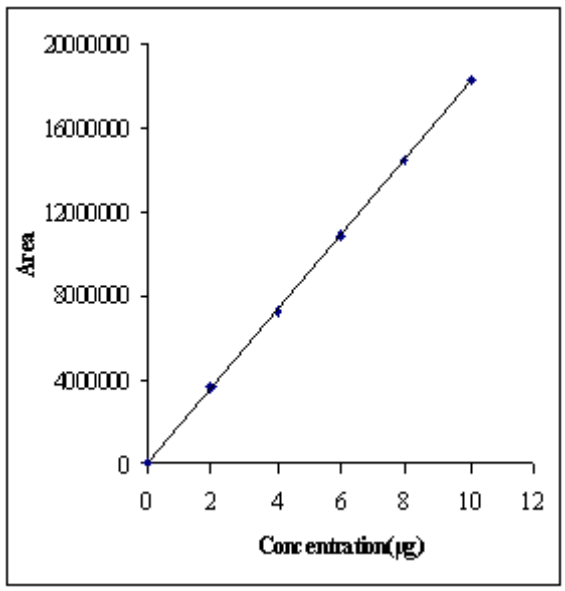

Fig. 2 : Calibration curve for simvastatin

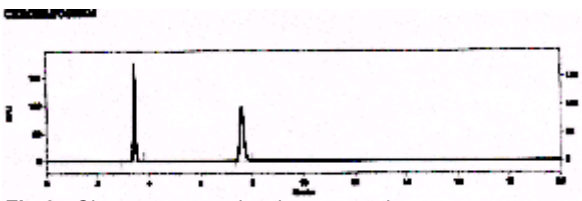

Fig 3 : Chromatogram showing separation

Precision

Precision was studied by measuring intra-day (repeatability which was carried out by analyzing the drug solutions within same day) and inter-day (by injecting of samples over two consecutive days)

variation of the method. Study was carried out by injecting six replicates of 100% concentration and the % RSD of the peak areas were determined. The results are given in Table 2.

Table 2: Results for Precision (n=6)

Drug	Intra day precision(%RSD)	Inter day precision(%RSD)
Aspirin	0.015	0.098
simvastatin	0.452	0.564

Accuracy

To determine the accuracy of the proposed method, recovery experiments were performed by standard addition technique. In this method a known quantity of pure drug was added at three different levels i.e. 80 %, 100%, and 120% to pre-analyzed sample solutions and calculating the recovery of aspirin and simvastatin for each concentration. The % recovery at each spike level was calculated and given in Table 3.

Table 3: Results for Accuracy (n=3)

Spiked level	Mean %recovery		Mean %RSD	
	Aspirin	simvastatin	Aspirin	simvastatin
80	99.16	98.46	0.61	0.64
100	99.44	101.2	0.63	0.28
120	100.26	99.58	0.56	0.82

System suitability

The system suitability test involves a comparison of the chromatogram trace with a standard trace. Alternatively these parameters can be calculated experimentally to provide a quantitative system suitability test report such as number of theoretical plates, capacity factor, separation (relative retention), resolution, tailing factor. System suitability was carried out by injecting 100% concentration of aspirin and simvastatin at different injection volumes in the range of 10-50 µL. The %RSD for tailing factor and theoretical plate number should be less than 1%. The results are tabulated in Table 4.

Table 4: System suitability parameters

Parameters	Aspirin	Simvastatin
Retention time (min)	3.42	7.06
Theoretical plates	9812	11472
Tailing factor	0.4	0.3
Resolution	13.40	

Selectivity/Specificity

A method is said to be specific when it produces a responses only for a single analyte. Selectivity is the ability of the method to produce a response for the analyte in the presence of other interferences, in order to prove that the method chosen was specific and selective, the parameters like retention time (Rt), resolution (RS) capacity factor, tailing factor were calculated.

LOD and LOQ

The LOD and LOQ values were determined by the formulae LOD = 3.3 ó/S and LOQ = 10 ó/S (Where, ó is the standard deviation of the responses and S is mean of the slopes of the calibration curves)

Robustness

The robustness of the method was investigated under a variety of conditions including changes in the mobile phase composition, flow rate and detection wavelength and the % RSD was determined. The results are tabulated in Table 5.

Table 5: Results for Robustness

Parameters	SD		%RSD	
	Aspirin	simvastatin	Aspirin	simvastatin
Change in nm 232	5009.139	8562.220	0.015	0.452
Change in nm 237	60853.5	38032.9	0.42	0.43
Change in flow rate 0.9	98654.78	38395.98	0.65	0.46
Change in flow rate 1.1	107689.7	67456.8	0.76	0.78

RESULTS AND DISCUSSION

Spectrophotometric determination of aspirin and simvastatin in overlay mode shows that both the drugs absorb appreciably at 235nm, hence 235 nm is selected as the detection wavelength. Several different mobile phases were used for the initial trials in the simultaneous quantitation of both the drugs, but optimum results were attained with acetonitrile: methanol: phosphate buffer (55:30:15). The two peaks were symmetric and sufficiently resolved. An optimized chromatogram showing the retention times is given in the Figure 1.

The peak areas corresponding to the concentration range of aspirin 10-50 µg/ml and simvastatin12-10 µg/mL represented a correlation of 0.9998 and 0.9999 respectively. The regression analysis was given in the Table 1. The method was precise with a %RSD of 0.015 and 0.452 for aspirin and simvastatin respectively. The calibration curves were shown in the Figures 2 and 3. Accuracy of the method was examined by performing recovery studies by standard addition method for drug product. The recovery of the added standard to the drug product sample was calculated and it was found to be 99.44 and 101.20 w/w for aspirin and simvastatin respectively and the % RSD was less than 2 for both the drugs which indicates a good accuracy of the method to that of the label claim. The Results are tabulated in Table 2 and 3. Limit of detection of aspirin and simvastatin were 0.61µg/ml and 0.33µg/ml respectively. Limit of quantification of aspirin and simvastatin were 0.79µg/ml and 1.157µg/ml respectively.

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatogram confirms the presence

of aspirin and simvastatin at 3.4min and 7.4min respectively without any interference. The results are given in Table 4.

Robustness was carried out by change in the flow rate (±1ml/min) and variation in wavelength (± 2 nm). Solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D was calculated for each condition. The results are given in Table 5.

CONCLUSION

The developed RP-HPLC method for simultaneous assay of aspirin and simvastatin in combined tablets dosage forms is simple, precise, specific and highly accurate. The method can be employed in the routine analysis for simultaneous estimation and for quality control of raw materials and in formulations.

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REFERENCES

1. Sneader W. The Discovery of Aspirin: A Reappraisal. BMJ (Clinical research ed.). 2000; 321:1591–1594.
2. Julian D G, Chamberlain D A, Pocock S J. A comparison of aspirin and anticoagulation following thrombolysis for myocardial infarction (the AFTER study): A multicentre unblinded randomised clinical trial. British Medical Journal. 1996; 313: 1429–1431.
3. Maggon K. Best-selling human medicines 2002-2004". Drug Discovery Today. 2005; 11: 739–42.
4. Eggertsen R, Andreasson A, Andren L. Effects of treatment with a commercially available St John's Wort product (Movina) on cholesterol levels in patients with hypercholesterolemia treated with simvastatin. Scand J Prim Health Care. 2007; 25:154-9.
5. Suresh Kumar S, Latif D, Krishnamurthy B, Prashant B. Analytical method development and validation for aspirin. International Journal of Chem Tech Research. 2010, 2, 389-399.
6. Bonde P, Sharma S, Kourav N. Development and validated UV spectrophotometric and RP-HPLC methods for the estimation of simvastatin and ezetimibe in combined pharmaceutical dosage form. Inter J Curr Trends Sci Tech. 2010; 1(3): 135–142.
7. Pawar AKM, Nageswara Rao ABN, Sreekanth K, Palavan C, Gowri Sankar D. Simultaneous estimation of aspirin ramipril, metaprolol and atervostatin in bulk and its polypill using isocratic RP-HPLC technique. Journal of Pharmaceutical Research. 2012; 5(1): 26-29.