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Impurity Profiling of Aspirin in Tablet Dosage Forms by Reverse Phase High Performance Liquid Chromatography

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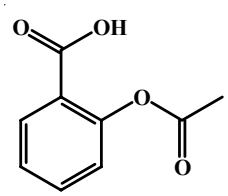
An efficient reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for quantification of aspirin impurities which may coexist in bulk drugs and solid dosage forms. The separation was achieved on C18 column (100 mm × 4.6 mm, 5 µm) using a mobile phase of acetonitrile, isopropyl alcohol and sodium perchlorate. Flow rate was 1.5 mL/min. The photo diode array detector was operated at 275 nm. Forced degradation studies were performed on tablets powder which contain aspirin using acid hydrolysis, base, peroxide, water and UV, thermal, sunlight, humidity degradations. The method was validated for specificity, linearity, precision, accuracy and limit of quantification. The degree of linearity of the calibration curves, the recoveries of aspirin impurities, the limit of detection and quantification, for the HPLC method were determined. The method was found to be simple, specific, precise, accurate and reproducible. This method was applicable for the quality control of commercial aspirin tablets to quantify the drug and its related substances and to check the formulation content uniformity.

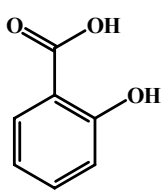
Key Words: Aspirin, Impurity profiling, Reversed phase HPLC.

INTRODUCTION

Aspirin[1] is chemically, 2-acetoxy benzoic acid (acetosol) is salicylate drug often used as an analgesic (to relieve minor aches and pains), antipyretic (to reduce fever) and as an antiinflammatory. It also has an antiplatelet (blood-thinning) effect and is used in long-term, low doses to prevent heart attacks and blood clot formation in people at high risk for developing blood clots. The active ingredient aspirin has one impurity i.e., salicylic acid (Fig. 1) in this process. This impurity may be present in small quantities and reduce the quality of aspirin. Therefore separation quantification of aspirin and its impurity was quiet important not only for quality assurance but also for monitoring reactions involved in process development. Literature survey revealed that different HPLC[2-6], spectrophotometry[7,8], spectroflourimetry[9], UV[10], near IR[11], FT-Raman spectroscopy[12], non-ionic resin chromatography[13], TLC[14] methods for determination of impurity of aspirin was reported. The proposed method is simple, fast, accurate and precise for estimation of aspirin impurity in tablets.



2-Acetoxybenzoic acid (aspirin)



2-Hydroxybenzoic acid (salicylic acid)

Fig. 1. Structures of aspirin and salicylic Acid

EXPERIMENTAL

The waters LC system with a photo diode array detector was used for method development and forced degradation studies. The output signal was monitored and processed using EMPOWER software.

The waters LC system, used for method validation was water alliance HPLC consisted of 2695 separation module, variable wavelength programmable UV detector waters 2996, EMPOWER software and Xterra RP 18 column (100 mm × 4.6 mm, 5 µm particle size) was used.

Chromatographic conditions: The mobile phase was made by first preparing a buffer solution of sodium perchlorate (0.7 g of sodium perchlorate in 1000 mL of water, pH adjusted to 2.5 with perchloric acid). 850 volumes of this buffer solution was then mixed with 140 volumes of acetonitrile and 10 volumes of isopropyl alcohol to yield mobile phase.

Separations were performed on a Xterra RP 18 column (100 × 4.6) mm, 5 µm particle size). The mobile phase flow rate was 1.5 mL/min (isocratic) with 50 °C column temperature, 20 µL injection volume and UV detection at 275 nm. Run time of 10 min.

Procedure

Diluent preparation: Mixed 0.1 % phosphoric acid (prepared in acetonitrile) and phosphate buffer (dissolve 0.68 g of potassium dihydrogen phosphate and 0.71 g of disodium hydrogen orthophosphate in 1000 mL milli Q water) in the ratio of 50:50. Add 0.6 mL of 50 % w/v solution of sodium hydroxide. The final pH of the diluent is about 3.8.

Salicylic acid stock solution: Weighed accurately 45 mg of salicylic acid in to a 100 mL volumetric flask. Dissolved and diluted to volume with diluent.

Reference solution: Transfer 10 mL of salicylic acid stock solution in to a 100 mL volumetric flask and dilute to volume with diluent.

System suitability preparation: Transfer 10 mL of salicylic acid stock solution in to a 50 mL volumetric flask. Added about 75 mg of aspirin in to a same flask. Dissolved and diluted to volume with diluent.

Sample preparation: The test solution was prepared by taking 10 tablets (750 mg) in 500 mL diluent to get 1.5 mg/mL of aspirin.

Method validation: The precision of test method was evaluated by analyzing six samples prepared by spiking test preparation with aspirin impurity solution of 6.0 % with respect to test concentration. The relative standard deviation was calculated for the response of each impurity.

Limit of detection (LOD) for aspirin impurity was established by identifying the concentration, which gives signal to noise ratio of about 3. Limit of quantification (LOQ) for aspirin impurity was established by identifying the concentration, which gives signal to noise ratio of about 10. Precision and accuracy studies were also carried at the LOQ level by injecting six individual preparations of impurities and calculated the % RSD of the area.

Linearity test solution for related substance method was prepared from the impurities stock % of the specification. Plotting the peak areas solution at 10 concentration levels from LOQ to 150 of impurities versus its corresponding concentration. Calculated the slope, Y-intercept and correlation coefficient for each impurity.

An accurate study of aspirin impurity from spiked samples of aspirin test preparation was conducted. Sample were prepared in triplicate by spiking test preparation with 25, 50, 100, 150 and 200 % to the target concentration of aspirin impurity. % Recoveries of individual impurity was calculated by external standard method.

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. According to ICH guidelines forced degradation studies were performed on aspirin tablets powder to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light, heat, acid, base, oxidation, water, sunlight and humidity to evaluate the ability of the proposed method to separate aspirin from its degradation products.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, fast, accurate and precise HPLC method for analysis of aspirin impurity in tablets. In this process salicylic acid is a potential impurity for aspirin. The chromatographic separation was achieved by following isocratic flow using mobile phase containing acetonitrile, pH 2.5 buffer and isopropyl alcohol in the ratio of 14:85:1. The retention time for aspirin and salicylic acid are about 3.5 and 5.3 min, respectively. System suitability was established as the aspirin and salicylic acid were eluting closely. The resolution between aspirin and salicylic acid peaks found to be more than 2.0. The system suitability results are given Table-1. The relative retention time for salicylic acid is about 0.66.

Linearity results shows that good correlation existed between the peak area and concentration of impurity. The results are summarized in Table-2.

The % RSD of response of salicylic acid during precision and intermediate precision was found to be less than 15.0 %.The results are summarized in Table-3.

The recovery studies were performed from 25 to 200 % of target concentration (6.0 %).

TABLE-1
SYSTEM SUITABILITY

System suitability	Observed value	Acceptance criteria
Tailing factor for salicylic acid in standard	1.0	NMT 2.0
% Relative standard deviation for peak area of salicylic acid from 6 replicate injections of standard	0.5	NMT 2.0
Resolution between salicylic acid and aspirin	4.1	NLT 4.0

TABLE-2
LINEARITY DATA OF SALICYLIC ACID

Name of the impurity	Concentration range (µg/mL)	Coefficient of correlation (r)	Slope (b)	Intercept (a)
Salicylic acid	1.610 – 134.190	0.999	5150.65	951.562

y = bx+a, y is peak area of the substance, x is concentration of the substance, b is slope and a is intercept.

TABLE-3
PRECISION OF THE PROPOSED HPLC METHOD

Sample No.	Aspirin tablets % Salicylic acid
01	6.250
02	6.128
03	6.436
04	6.224
05	6.251
06	6.238
Average	6.255
% RSD	1.600

The % mean recoveries of aspirin in aspirin tablets are satisfactory. The results are summarized in Table-4.

TABLE-4
ACCURACY OF HPLC METHOD FOR DETERMINATION OF ASPIRIN IMPURITY IN TABLETS

Spike level (%)	Average 'µg/mL' added	Average 'µg/mL' found	Mean % recovery
25	22.41	24.00	107.1
50	44.81	46.20	103.1
100	67.21	69.11	102.7
150	89.62	90.86	101.4
200	134.43	136.00	101.1

The limit of detection and limit of quantification for all impurities were established by signal to noise method and precision and accuracy as verified at limit of quantification level. The results are summarized in Table-5.

TABLE-5
LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ) OF ASPIRIN IMPURITY

Name of the impurity	Test name		Signal to noise ratio		% Impurity at LOQ level
	Limit of detection	Limit of quantification	LOD	LOQ	
Salicylic acid	0.390 µg/mL	1.365 µg/mL	3	10	0.091

From forced degradation studies peak purity has been verified and purity angle is found to be less than purity threshold. The results are summarized in Table-6.

TABLE-6
FORCED DEGRADATION STUDIES

Stress condition	Aspirin		
	% Degradation	Purity angle	Purity threshold
Acid degradation	10.52	4.086	10.683
Base degradation	10.86	4.301	8.706
Peroxide degradation	8.30	3.940	9.589
Water degradation	6.49	3.709	9.252
UV degradation	0.03	4.357	10.802
Thermal degradation	12.20	2.225	6.520
Sunlight degradation	0.04	4.483	12.227
Humidity degradation	0.09	3.772	10.489

Conclusion

The RP-LC method developed for related substance determination of aspirin in aspirin tablets is precise, accurate, specific and selective. The method was validated shown satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be used for assessing the aspirin impurity in aspirin tablets.

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