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Research Article

DEVELOPMENT AND VALIDATION OF ULTRA-FASTSTABILITY INDICATING UPLC METHOD FORQUANTIFICATION OF RELATED COMPOUNDS OF ACETYLSALICYLIC ACID IN IT`S SOLID DOSAGE FORM

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ABSTRACT

A simple, sensitive and specific UPLC method was developed for the identification and quantification of related compounds of aspirin from its solid dosage forms. The chromatographic separation employs the gradient elution using C_{18} column with the length of 100 mm x 2.1mm dimensions by using the solvent-A (containing mixture of water and orthophosphoric acid), solution-B (containing mixture of acetonitrile and methanol) with the flow rate of 0.4mL/min. All the analytes were detected and quantified at the wavelength of 237nm by using photo diode array detector. The method was validated as per ICH guidelines and found to be specific, precise, linear and accurate. This method has very lower limit of detection and limit of quantification. The peak purity obtained with all related compounds with the aid of photo diode array detector was satisfactory. Hence this method was concluded that stability indicating method.

Keywords: Aceylsalicylic acid, Aspirin, Stability indicating UPLC method.

INTRODUCTION

Acetylsalicylic acid[1] is chemically designed as 2-(Acetyloxy)benzoic acid baring the molecular weight of 180.2.Even though this molecule is very basic molecule, due to its several different effects on human body still dispensing to as alone and with combination with other drugs.

The literature survey reveals that, acetylsalicylic acid was reported in Ph.Eur [1].There are several methods were reported in different journals for estimating acetylsalicylic acid and its related compounds with different methods.All the reported methods were having very longer run times.

Therefore, here we developed new stability indicating UPLC method to quantify the related compounds present in the formulation of Acetylsalicylic acid tablets with very shorter runtime with good efficiency.

Regulatory agencies recommend the use of stability indicating methods [3] for the quantification of stability samples [4]. This requires stress studies in order to generate the potential related impurities under stressed conditions, method development and validation [5]. With the evident of the International Conference on Harmonization (ICH) guidelines [6], requirements for the establishment of stability indicating methods have become more clearly mandated. Environmental conditions including light, heat and the susceptibility of the drug product towards hydrolysis or oxidation can play an important role in the formation of potential impurities. Stress testing can help identifying degradation products and provide important information about intrinsic stability of the drug product.

Names of related compounds and those relative retention times were listed in Table-1 and chemical structures were shown in Figure-1.

MATERIALS AND METHODS

Acetylsalicylic acid active pharmaceutical ingredient and its related compounds kindly supplied by Dr.Reddy`s Laboratories, Hyderabad. Acetonitrile, methanoland orthophosphoric acid were obtained from Merck Limited, Mumbai. High purity de-ionized water was obtained from Millipore, Milli-Q purification system.

Instrumentation

Acquity UPLC System equipped with auto sampler and binary gradient pump with in-built degasser used. It was connected with photo diode array detector and operated with Empower software.

Chromatographic Conditions

ACQUITY UPLC BEH C18, 1.7µm, 2.1 x 100mm UPLC column was used as stationary phase maintained at 30°C. The mobile phase involved a variable composition, solvent-A containing mixture of water and orthophosphoric acid in the ratio of 1000:4 v/v, solution-B containing mixture of acetonitrile and methanol in the ratio of 80:20 v/v respectively. The mobile phase was pumped at the flow of 0.4 mL/min with gradient and the gradient programme was mentioned in Table-2.The optimum wavelength selected was 237nm which represents the wavelength of maximum response analyte in order to permit the optimum determination.

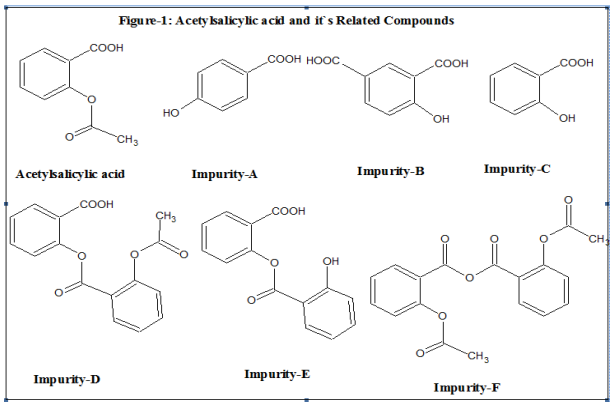

Fig.1

Table 1: List of Acetylsalicylic acid Related Compounds and its RRT`s

S. No	Related Compound Name	Relative Retention Time
1	Impurity-A	0.46
2	Impurity-B	0.64
3	Acetylsalicylic acid	1.00
4	Impurity-C	1.58
5	Impurity-D	2.53
6	Impurity-E	2.76
7	Impurity-F	3.08

Table 2: Gradient programme

Time (min)	Flow mL/min	Solution A (% v/v)	Solution B (% v/v)
0.0	0.4	70	30
2.0	0.4	70	30
5.0	0.4	30	70
6.0	0.4	30	70
6.2	0.4	70	30
8.0	0.4	70	30

Standard solution

Solution containing Acetylsalicylic acid8µg/mL

Sample solution

Solution containing Acetylsalicylic acid 800µg/mL

Forced degradation sample solutions for specificity study

Multiple stressed samples were prepared as indicated below and chromatographed along with un-stressed sample as control sample.

Hydrolytic conditions (Acid, base-induced degradation)

Product sample was treated with 0.1N HCl and 0.1N NaOH respectively. The solutions wereneutralized as needed (with 0.1N NaOH or 0.1N HCl).

Oxidative condition: with hydrogen peroxide

Required sample was treated with 30% H_2O_2 under dark condition.

Thermal degradation study

Product sample was subjected to 105°C for 24 hrs.

Photolytic degradation study

As per ICH guidelines Sample was exposed to 1.2 million lux of visible and 200Wh/m^2 of ultra violet energy.

OPTIMIZATION OF METHOD

Selection of stationary phase

As aetylsalicylic acid and it`s related compounds were non polar in nature, reverse phase chromatography was selected. Initially both C_8 and C_{18} stationary phases were tested with the particle size of 5µm and lower. But comparatively adequate separation was attained with C_{18} stationary phase with 1.7µm particle size with shorter runtimes. But the stationary phase is not only the parameter which can give better separation among all impurities. Mobile phase, pH and organic modifies also plays very important role which leads to the best separation.

Selection of mobile phase

Initially started with sodium and potassium phosphatebuffer and then followed by with acetate buffer. But good separation with very shorter run time was obtained with water with phosphoric acid with lesser back column pressure.Whenacetonitrile was used as organic phase, good peak shape was observed but few impurities werecoming closely. But combination of acetonitrile and methanol yields good resolution.

After extensive and repeated experimental studies, the method finalized withACQUITY UPLC BEH C18, 1.7µm, 2.1 x 100mm column using variable compositions of solvent-A: Mixture of Milli-Q water and orthophosphoric acid, solvent-B: mixture of acetonitrile and methanol. The mobile phase pumped through column at the 0.4 mL/min flow rate. The column compartment maintained at the temperature of 30°C. Good detector response for detecting all the compounds found at 237nm. The typical LC chromatogram (Figure-2) represents the separation of all related components from each other.

METHOD VALIDATION

The developed method was validated according to ICH guidelines [6], with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range and robustness. System suitability features also assessed.

System suitability

The system suitability test performed according to USP 30 [7] and BP 2007 [8] indications. The observed RSD values for standard peaks of acetylsalicylic acid meets it`sacceptance criteria. Theoretical plates and USP tailing factor (Tf) also monitored. The results obtained are all within acceptable limits.

Specificity

Specificity is the ability of the method to unequivocally assess the analytes response in the presence of its other impurities.The peak purity indices for the analytes in stressed solutions were determined with PDA detectorunder optimized chromatographicconditions found to be better (purity angle < purity threshold with empower software) indicating that no additional peaks were co-eluting with the analytes and evidencing the ability of the method to assessunequivocally the analyte of interest in the presence of potential interference.

All thepeaks meet this specification, visibly confirmed in Figure-2 and Figure-3.

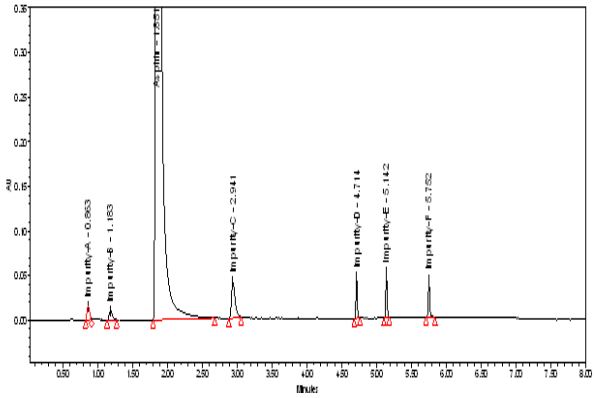

Fig.3: Specimen chromatogram of spiked test.

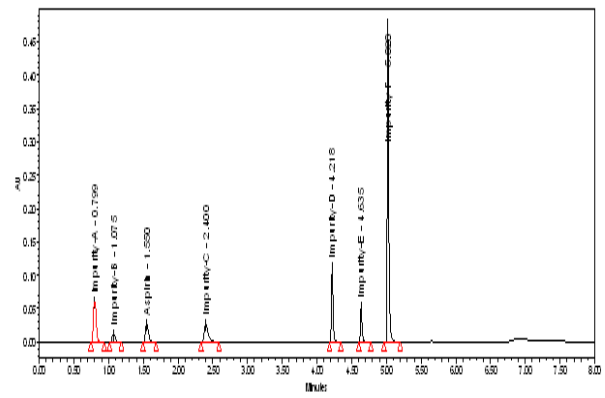

Fig.3: Impurity Mix

Stress study of sample

Stress studies were performed on samples to provide the stability-indicating property and specificity of the proposed method. Degradation study was attempted at the stress conditions of UV light (200Wh/m2),visible light of to 1.2 million lux, heat (105°C), acid (0.1N HCl), base (0.1N NaOH) and oxidation (30% H_2O_2) to evaluatethe ability of the proposed method to separate acetylsalicylic acid and its related compounds. All degradent impurities formed were completely separated from each otherwith acceptable peak purity. The details of the stress studyin Table-3, indicates that the method is stability indicating.

Table-3: Results of Stress study

Mode of Degradation	Condition	% Degradation	Purity1 Angle	Purity 1 Threshold
Control	Un-stressed Sample	-	0.135	1.684
Thermal	105°C / 24 Hrs	10.2	0.254	1.487
Photolytic	1.2 million lux and 200Wh/m^2 of UV	6.1	0.325	1.958
Humidity	90%RH/25°C/ 120Hrs	2.3	0.198	1.685
Acidic	0.1 N HCl/25°C	15.2	0.484	1.451
Basic	0.1 N NaOH/25°C	8.2	0.321	1.658
Oxidation	30% H_2O_2/2 hrs	11.5	0.421	1.587

Determination of limit of quantification and detection (LOQ and LOD)

Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of acetylsalicylic acid and its related compounds.

Relative standard deviation (σ) method was applied, the LOQ and LOD valueswere predicted using following formulas (a) and (b).Precision was established at these predicted levels and the results are tabulated in Table-4.

(a) $LOQ = 10\sigma / S$

(b) $LOD = 3.3\ \sigma / S$

Whereσ= residual standard deviation of response

S = slope ofthe calibration curve.

Linearity

The linearity of an analytical procedure is its ability(within a given range) to obtain test results which are directly proportional to the concentration of analytepresent in the sample preparation. Response function was determined by preparing standard solution of each component at different concentration levels ranging from lower limit of quantification to 150% of impurity tolerance level.

Linearity was established by plotting graph to concentration versus corresponding response of analyte and determined the correlation coefficient. The correlation coefficients (r) exceed 0.999, the acceptable threshold suggested for linearity procedures to determine the impurity content in bulk drug [6]. The regression statistics areshown in Table-4.

Table-4: LOQ, LOD, RRF andRegression Statistics

S. No	Impurity Name	LOD (µg)	LOQ (µg)	RRF	Correlation Coefficient(r)
1	Impurity-A	0.048	0.146	0.83	0.99999
2	Impurity-B	0.021	0.064	0.31	0.99966
3	Acetylsalicylic acid	0.028	0.085	-	0.99992
4	Impurity-C	0.015	0.044	1.07	0.99998
5	Impurity-D	0.015	0.045	1.17	0.99998
6	Impurity-E	0.015	0.045	1.34	0.99996
7	Impurity-F	0.014	0.044	1.15	0.99996

Accuracy

Accuracy was evaluated by the simultaneous determination ofanalytes in sample prepared by standard addition method. Theexperiment was carried out by adding known amount of each related compound corresponding to three concentrationsat 50%, 100% and 150% of the specification level in sample solution.The samples were prepared in triplicate at each level. The quantificationof added analyte (%wt/wt) was carried out byusing an external standard of corresponding main drug prepared atthe analytical concentration. Relative response factors (Table-4) of all relatedimpurities were used to calculate the weight percentage of relatedimpurities in drug product

The experimental results revealed that approximately 90–110% recoveries were obtained for all the investigated related compounds. Therefore, based on the recovery data the estimation of related compounds that are prescribed in this report has been demonstrated to be accurate for intended purpose and is adequate for routine analysis.

Precision and ruggedness

The precision of an analytical procedure expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The repeatability (intra-day precision) refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment. Intermediate precision (inter-day precision) involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts. The results obtained %RSD values were less than 2%.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in chromatographic conditions, i.e. change in flow rate by ±0.05 mL/min, change in pH of the buffer by ±0.2 units, change in column oven temperature by ±5°C and change in organic composition of mobile phase by ±2% absolute. The sample spiked with all known impurities at impurity tolerance level was injected and the resolution among the impurities was monitored. The method was demonstrated to be robust over an acceptable working range of its HPLC operational conditions.

CONCLUSION

NovelUPLC method wasdeveloped and validated for the quantification of related substances of Acetylsalicylic acid from it`s drug product. Validation experiments provided proof that the UPLC analytical method is linear in the proposed working range as well as accurate, precise (repeatability and intermediate precision levels) and specific, being able to separate the main drug from its degradation products. The proposed method is also found to be robust with respect to flow rate, pH of buffer, wavelength organic and column temperature. Due to these characteristics, the method has stability indicating properties being fit for its intended purpose, it may find application for the routine analysis of the related compounds of Acetylsalicylic acid from solid dosage forms.

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