ASSAY OF RELATED SUBSTANCE IN ACETYLSALICILIC ACID

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Abstract
This paper present method for validation of assay determination of related substance from acetylsalicylic acid. As it is established by European Pharmacoeia. ed.3.Addendum 2000. for Salicylic Acid value for the concentration limit is stipulated to be 0.1 % and for related substance 0.25 %. For the detection and quantitation determination of Salicylic Acid in the Acetylsalicylic acid we have used a HPLC chromatographic method. known as a specific test for the control of related substance.

Keywords: Acid Salicylic. Acid acetylsalicylic. HPLC

INTRODUCTION

Salicylic acid is a white. crystalline. weakly acidic substance. with melting point 135^0C. Acetylsalicylic acid decomposes rapidly in solutions of ammonium acetate or of the acetates. carbonates. citrates or hydroxides of the alkali metals. Acetylsalicylic acid is stable in dry air. but gradually hydrolyses in contact with moisture to acetic and salicylic acids. In solution with alkalis. the hydrolysis proceeds rapidly and the clear solutions formed may consist entirely of acetate and salicylate[1].
Separation and detection of acetylsalicylic acid and salicylic acid is effectuated through HPLC – chromatographic method. using UV – detector. The content of salicylic acid is determined using as external standard a solution of salicylic acid with well – known concentration.

MATERIALS AND METHODS

Instrument – Chromatograph HPLC - ABLE & JASCO PU – 1580
Injector – manual –RHETODYNE
Detector – JASCO - 1575
Procesing data – BORWING
Column -l= 100 mm. d=4.6 mm
Stationary phase -NUCLEOSIL 100. C18. 5µ
Mobil phase -acetonitril:bidistilled water:phosphoric acid=200:800:2
Flow - 1 ml/min
Injection volume -20 µl
Temperature - ambient
Elution type - isocratic
Wavelength - 237 nm
Salicylic acid p.a.
Acetonitril supragrade HPLC grade
Phosphoric acid p.a.
Bidistilled water

Reference solution a:
50 mg salycilic acid is dissolved in mobil phase and completed at 50 ml with mobil phase 1 ml from this solution is diluated with mobil phase al 100 ml.
Reference solution b:
10 mg salicylic acid is dissolved in mobil phase and completed at 10 ml with mobil phase.1 ml from this solution and 0.2 ml from test solution is dissolved at 100 ml with mobil phase.
Test solution:
0.1 g acetylsalicylic acid is dissolved in acetonitril and completed at 10 ml with acetonitril.
Analysed batches: Acetylsalicylic Acid. 20 – 60 mesh. lot 29;
Acetylsalicylic Acid. needle cristals. lot 1002
After the stabilizing of beseline. inject from each reference solution seven times to obtained peak response for Salicylic Acid and Acetylsalicylic Acid. It will be recorded retention times and each peaaks area.

RESULTS AND DISCUSSION

Calculate the purity test result on the basis peak response /area/ considering each peak with exception of peak belong to solvent and main components.

Salicylic Acid assay. % = $[PR_{TS}]/[PR_{Rsa}]$

Total impurity assay. % = $[TPR_{TS}]/[PR_{Rsa}]$

Where : PR_{TS} = peak response /area/ of the salicylic acid in test solution

PR_{Rsa} = peak response /area/of the salicylic acid in reference solution a

TPR_{TS}= sum of peak response of any peaks in the chromatograms other than solvent peak and peak of main component obtained for test solution.

The chromatographic characteristics are:
Retention time: - Salicylic Acid – 12.49 min
Acetylsalicylic Acid – 7.43 min
Column plate number–12 460/m for Acetylsalicylic Acid
-25 280/m for salicylic Acid
For the validation of a method it is recommended the study of some parameters such as : precision. specification. exactity. linearity and range. The most important aspect of the validation strategy is the work experimental plan. so that suitable parameters can be studied simultaneously in the validation process so decreasing the number of determinations[3][4].
3.1. Precision study
Degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of homogeneous sample.
Precision when the error contributed by the entire method is considered.
Precision when only the error contributed by HPLC system is considered.
Precision evaluation is realised by calculatin of the relative standard deviation. RSD. for a certain number of determinations/ injections: for a value of RSD ‹ 2%. it is necessary to have 5 determinations. and for RSD › 2%. to have 6 determinations [2].
Prepare reference solution with 2 independent weighings as described in the Procedure description. and inject minim in tripicate each reference solution. Record the chromatograms and calculate the relativ standard deviation of the peak response.

Table 1

The relativ starndard deviation of the peak response for the reference solution

Number	Peak response
1	880570
2	880637
3	881347
4	880354
5	879437
6	880329
7	881445
Average	880588
RSD.%	± 0.07

Prepare one standard solution as described in the Procedure description. and inject in triplicate each standard solution. Record the chromatograms and calculate the relative standard deviation of the peak responses.

Table 2

The relativ starndard deviation of the peak response for the reference solution

Number	Procedure		System	
	Lot 29. %	Lot 1002. %	Lot 29. %	Lot 1002. %
1	0.00909	0.0517	0.00919	0.0523
2	0.00908	0.0512	0.00920	0.0522
3	0.0092	0.0517	0.00923	0.0527
4	0.00912	0.0516	0.00918	0.0525
5	0.00913	0.0509	0.00921	0.0520
6	0.00919	0.0515	0.00925	0.0524
7	0.00913	0.0515	0.00920	0.0521
Average	0.00913	0.0515	0.00921	
RSD. %	± 0.71	± 0.53		

Table 3

Precision.method precision and system precision in house requirement

TYPE	IN HOUSE REQUIREMENT	RESULTS
PRECISION	Less than 0.7 %	± 0.08
METHOD PRECISION	Less than 2.0 %	± 0.71 / ± 0.53
SYSTEM PRECISION	Less than 1.0 %	

The RSD value indicates valid results from a statistical point of view.

Considering the in house requirement. the method provide acceptable precision results. and suitable for related substance assay in acetylsalicylic acid.

3.2 Accuracy

The accuracy of an analytical method is the closeness of the test result obtained with the method to the true value.

It is determined by applying the analysis method to a number of samplings with known concentrations. the analyt being used in the correct concentration in accordance with the standards for the decreasing the backround sonds [2].

Prepare test solution with 7 weighings as described in Procedure rescription. Record the chromatogram and calculate the salicylic acid assay. Compare the results obtained with HPLC and colorimetric method from the same solution.

Table 4

The results obtained with HPLC and colorimetric method from the same solution

No	In house requirement	RESULTS HPLC.%		RESULTS Colorimetric.%		Δ. %	
		29	1002	29	1002	29	1002
1		0.00909	0.0517	0.0098	0.06	0.00071	0.0083
2		0.00908	0.0512	0.0099	0.062	0.00082	0.0108
3		0.00920	0.0517	0.01	0.061	0.0008	0.0093
4		0.00912	0.0516	0.0098	0.060	0.00068	0.0084
5		0.00913	0.0509	0.01	0.062	0.00087	0.0111
6		0.00919	0.0515	0.0098	0.060	0.00064	0.0085
7		0.00913	0.0521	0.011	0.063	0.00187	0.0109
	Average RSD.%	0.00913 ± 0.71	0.0515 ± 0.53	0.01 ± 0.83	0.061 ± 0.75	0.00091	0.0096

From the calculations made it can be observed that statistical deviation is less than 1.5% at the trust level of 95% and the experimental error is less than 5%.Thus. the method is also valid from the accuracy point of view.

Considering the house requirements. the method provides acceptable accuracy results. and is suitable for determined assay of salicylic acid in acetylsalicylic acid.

3.3 Selectivity study

The selectivity is the ability of an analytical method to measure the analyte accutately and specifically in the presence of components that may be expected to be presente in the sample. It is a measure of degree of interference. or absence of these. in the analysis.

The method was optimized using several eluent combination. The selectivity of the method was accepted. when no new impurity peak should be identified on the chromatogram separating from the main peak.

Comparing the corresponding chromatograms obtained by changing the composition of the eluent the salicylic acid peak is considerated to be pure at given eluent composition because no new impurity peak appears.The method is considered as selective.and can be used for purity testing of acetylsalicylic acid. The resolutioin this case is 22.13. See the enclosure for the chromatograms obtained in the optimum eluent composition.

3.4 Peak assimetry

Peak asymetry expresses the degree of deviation of peak shape from the ideal.

Peak asymetry can be determined by dropping a perpendicular line from the peak maximum to the baseline and calculation the ratio of rear **B** to the front **A** baseline segment at 10 % of the peak height.

$$Asf = B/A$$

General requirement : Asf max 1.8

Table 5

Peak assimetry for salicylicacid and acetylsalicilic acid

	Asf salicylic acid	**Asf acetylsalicylic acid**
Reference solution b	0.00	0.85

Based on the results obtain the method is suitable for determined content of salicylic acid in acetylsalicylic acid.

3.5 Linearity and range

The linearity of an analytical method is its ability to elicit test results either directly. or by a well defined mathematical transformation. that are proportional to the concentration of the analyte in a sample. within a given range. The range of analytical

method is the interval between the upper and lower levels of the analyte. within that acceptable precision. accuracy and linearity are demonstrated.

Prepare 3 reference stock solution with individual weighings. Dilute with eluent.to obtain reference solution with C= 0.05% ; 0.1% ; 0.2 % salicylic acid. Inject tripicate from each reference solution.Record the chromatograms. Prepare a calibration curve and statistically evaluate the results obtained.

Table 6

The relativ starndard deviation and average of the peak response for the reference solution with impurity level 0.05%. 0.1% and 0.2%

Impurity level	Average peak response	RSD %
0.05	635321	0.73
0.1	805932	0.43
0.2	1778595	0.58

Based on the results obtained the method is suitable for the determination of related substances in acetylsalicylic acid. with respect to linearity and range.

3.6 Reproductibility study

Reproductibility is generally defined as the precision of one or more operations when the given test is run with the same or different equipment.

It is the precisoion of single operator repeating the same analysis on the same day under the same experimental conditions.

It relates to the precision of a number of operators running the same test under the same or similar experimental conditions.

Prepare reference and standard solutions with 3 independent weighings as described in Procedure description minim three times in same day. Inject each solution triplicate. Record the chromatograms and calculate the average of the assay results for each individual test solution.

Table 7

The relativ starndard deviation and average of the peak response for the reference solution for three times in same day

Test	Results.%		RSD. %	
	29	1002	29	1002
1	0.00914	0.0514	±0.76	±0.56
2	0.00928	0.0517	±0.83	±0.6
3	0.00925	0.0513	±0.89	±0.63
Average RSD.%	0.00922 0.82	0.0514 1.3		

Prepare reference solution and test solution with 3 independent weighings as described in Procedure description at three different days. Inject each solution triplicate. Record the chromatograms for each individual test solution.

Table 8

The relativ starndard deviation and average of the peak response for the reference solution for three different days

Test	Results. %		RSD. %	
	29	1002	29	1002
1	0.00914	0.0514	±0.76	±0.56
2	0.00925	0.0520	±0.83	±0.6
3	0.00931	0.0526	±0.89	±0.63
Average RSD.%	0.00923 0.94	0.0520 1.15		

3.7 Sample stability

Sample stability is a measure of bias in the assay results within a preselected time interval using a single sample solution.

From the reference solutions study one is injected into the liquid chromatograph after 1. 2. 3. 5. hours elapsed from sample preparation. The ratios of peak response are statistically evaluated.

Table 9

Measure of sample stability

Time elapsed from sample preparation	Peak response
Zero time	880570
1 hour	880711
2 hours	881536
3 hours	881223
5 hours	879911
Average RSD. %	880790 ±0.64

Sample stored at room temperature for one day was examined for purity testing. No additional impurity or increased amount of any impurities were experienced.

Based on the data obtained no problem occurs with the sample.if its is stored at room temperature for one day.

Table 10

Based on the method validation data. the following criteria are included into the system suitability test

ANALYTICAL PERFORMANCE PARAMETER	CRITERION
Approx. retention time	12.5 min
Precision	Max.1.0%
Peak asymmetry	Max.1.8
Column efficacy	Min. 15000

CONCLUSIONS

The main aim of this study was to validate the developed HPLC method for purity testing of acetylsalicylic acid.

Based on the validation test results it can be stated that the developed methods satisfy the all validation criteria. precision. accuracy. selectivity. of the method are good. on the method used for purity testing are suitable for acetylsalicylic acid.

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