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Development and Validation of HPTLC Method for Studying Stress Degradation of Aspirin in Fulvic Acid Inclusion Complex

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MKA and MMA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MJA and MFA managed the analyses of the study. Author MAM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A new, precise high performance thin layer chromatographic (HPTLC) method for the analysis aspirin (ASP) in inclusion complexes with HPβCD and fulvic acid (FA) was developed and validated as per ICH guidelines. A precoated silica gel aluminium plate 60F-254 and a mixture of solvents, toluene: ethylacetate: formic acid (5:4:1 v/v) were used as stationary and mobile phase, respectively. This developed method was found to give an excellent defined sharp peak at a retention factor (RF) value of 0.52 ± 0.001. The LOQ and LOD values were found 35.29 and 123.54 ng / spot, respectively. The spray dried inclusion complexes of ASP/HPβCD and ASP/FA in the molar ratio 1:1, were subjected for forced degradation under stress conditions, and a significant reduction of ASP degradation were noted in complexed ASP as compared to ASP alone.

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Keywords: Aspirin; HPβCD; fulvic acid; inclusion complex; stability.

1. INTRODUCTION

Aspirin (ASP) is chemically acetyl salicylic acid, a very old medication with high medical values, which possess various activities viz, anti-inflammartory, analgesic, and anti-aggregatory activity [1,2]. The carboxyl and ester groups are present in the structure of acetyl salicylic acid, the ester group of ASP is sensitive to hydrolysis in the presence of moisture, which reduces its bioavailability and causes ulceration effects on the human stomach [3-6]. A strategy must to develop to reduce/inhibit the hydrolysis of ASP.

Hydroxy propyl-β-cyclodextrin (HPβCD) is a cyclic oligosaccharide containing seven D-(+)-glucopyranose units, widely used in the pharmaceutical field for their ability of improving the solubility and stability of drugs. The chemical structures of HPβCD suggest that these have structures exhibiting a hydrophilic exterior and a hydrophobic interior. The interior of this HPβCD is thus capable of forming inclusion complexes with non-polar solutes and drug molecules with low bioavailability. These drug molecules can be entrapped in the hydrophobic interior so as to increase their solubility and dissolution rate, thereby enhancing their bioavailability. Such entrapment is also capable of enhancing the stability of the drug molecules [7,8].

Shilajit (syn: salajit, shilajatu, mumie or mummiyo) is a pale brown to blackish brown exudation coming out in variable consistency from the layer of rocks in various mountain ranges in the world, especially in Himalayan mountain ranges [9-14]. The shilajit contains fulvic (FA) constituent, which are a family of organic acids, natural compounds, and components of the humus. The interior of FA is hydrophobic in nature similar to HPβCD, which are capable of forming inclusion complexes with non-polar drugs with poor solubility/stability. The drug molecules can be entrapped inside the hydrophobic cavity of FA extracted from shilajit, to increase their solubility, dissolution, stability and bioavailability [15-18].

As ASP is well known and established drug, hence, literature search revealed various methods (UV, HPLC and HPTLC) reported for the analysis of ASP [17,19-21]. HPTLC offer many advantages compared to other analytical techniques in terms of reliability, accuracy, robustness and simultaneous analysis of compound [22,23]. The inclusion complexes of ASP with cyclodextrins has been already reported [4,5], this study explored the potential of FA as complexing agent to address the stability problem of ASP in comparison to HPβCD and analyzed by developed HPTLC method.

2. MATERIALS AND METHOD

2.1 Material

Shilajit was received from Dabur Research Foundation, India. Asprin and HPβCD were purchased from "Sigma Aldrich, USA". All other chemicals/solvent used in the study were of analytical grade.

2.1.1 Preparation of stock solution and ASP calibration curve

A stock solution (1mg/mL) was prepared by dissolving 100 mg of ASP and dissolved in 100 mL of methyl alcohol. Different volumes of the prepared stock, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 and 2µl were spotted two times on TLC plate to acquire concentrations of 100, 200, 300……2000 ng per spot of ASP. The analyzed peak area versus drug concentration were plotted to get calibration plot.

2.1.2 Preparation and characterization of ASP/ Hpβcd and ASP/FA complexes

Inclusion complexes of aspirin with HPβCD and fulvic acid (FA) extracted from shilajit were prepared using spray drying method in the molar ratio of 1:1. Inclusion complexation of ASP in FA was confirmed by fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD) and *in vitro* release studies. We reported formulation development data in our previous publications [4,5].

2.1.3 Sample preparation and HPTLC analysis of aspirin complexes

Accurately weighed inclusion complex samples (equivalent to 100 mg ASP) were dissolved in methanol and diluted suitably with mobile phase to get a 50 mL of stock solution. The stock solution was filtered to remove insoluble material and sonicated for 15 min. 1 ml of above stock solution was further diluted to 10 ml and analyzed by developed HPTLC method.

2.1.4 HPTLC instrumentation condition

The sample were spotted in the form of bands of width 3 mm with a "Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm wit 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland)". The sample application rate on plate was constant as 150 nl/s and kept a space of 5.5 mm between two bands. The dimension of slit and scanning speed was set to 4 × 0.1 mm and 20 mm/s, respectively. The mobile phase composed of toluene: ethylacetate: formic acid (5:4:1 v/v). The TLC plate was saturated with mobile phase in twin trough glass chamber to obtain ascending linear development. The length of chromatogram was carried out to 75 mm. After development, TLC plate was kept in hot air oven to dry. Dried TLC plate was scanned on "Camag TLC scanner IV" at wavelength 237 nm.

2.2 Method Validation

The developed HPTLC method for the analysis of ASP were validated in terms of "linearity, precision, accuracy, robustness, sensitivity and specificity" according to ICH guidelines [2]. The linearity of ASP was determined by plotting the concentration of ASP against HPTLC peak area of ASP. Linearity range of ASP was measured in the range of 100-2000 ng/spot. The accuracy of the method was measured in percent recovery (% recovery) at four different % analytes of ASP (0, 50, 100 & 150%). The precision of the method was studied in terms of repeatability/intra-day and intermediate/inter-day precision. The repeatability/intra-day precision was determined at three sample concentrations six replicates (100, 200 and 300 ng/spot) on the same day same lab. and intermediate/ inter-day precision. However, intermediate/inter-day precision was measured at three concentrations of six replicates (100, 200 and 300 ng/spot) on different days. As per ICH guidelines, precision easurement should be at three concentration levels in the linearity range [2]. The limit of detection (LOD) and limit of quantification (LOQ) were measured based on a 'signal and noise ratio'. The concentration of sample that giving three and ten signal to noise was fixed for LOD and LOQ respectively.

$$LOD = 3.3 * SD/S$$

$$LOQ = 10 * SD/S$$

2.2.1 Force degradation studies of ASP and complexes

2.2.1.1 Acid and base induced degradation

ASP and prepared spray dried complexes of ASP/FA and ASP-HPβCD (equivalent to10 mg ASP) were dissolved separately in 10 mL acid (0.1M HCl) and base (0.1M NaOH), and were heated under reflux condition for 4h at temperature 75°C, then applied on TLC plate (200 ng/spot) three times [24,25]. Chromatographic analysis was performed as described above.

2.2.1.2 Hydrogen peroxide (H_2O_2) induced degradation

The pure drug ASP and prepared complexes (ASP/FA and ASP-HPβCD) were added in 25 mL of hydrogen peroxide (30%, v/v) to get a concentration (2 mg/mL) equivalent to ASP. The prepared samples (200 ng/spot) were applied on TLC plate three times and chromatographic analysis was performed as described above.

2.2.1.3 UV light induced degradation

ASP and their complexes of ASP/FA and ASP-HPβCD (equivalent 10 mg of ASP) were added separately in methyl alcohol and exposed to UV at 254 nm in a UV chamber for 24 h. The solutions obtained were applied (200 ng per spot) to TLC plates and chromatography was performed as described above.

3. RESULTS and DISCUSSION

3.1 Method Development

HPTLC method was developed for ASP under chamber saturation condition using solvent composition toluene: ethylacetate: formic acid (5:4:1 v/v) as a mobile phase. The saturation time (15 minutes) was optimized and densitometric analysis was performed at 237 nm. A very sharp, symmetrical with high resolution peak of ASP were found at RF of 0.52 ± 0.001 (Fig. 1). A stability-indicating HPTLC method of ASP was developed to quantify the ASP and its complex with ASP/HPβCD and ASP/FA. A simple, rapid, precise, cost-effective HPTLC method has been developed for the analysis of pure ASP and its formulations.

3.2 Method Validation

The linearity of the ASP was validated by the least square linear regression equation and

correlation coefficient (R^2). The six concentrations point calibration curve was found to linear in the range of 100-2000 ng/spot with good linearity response. The linear regression equation and coefficient of correlation (R^2) for the ASP were obtained as y = 3.623x + 595.9 and 0.9919, respectively, where y is the peak area response and x is the ASP drug concentration (Table 1). The mean values of slope and intercept with SD were found as 3.623 ± 0.200 and 595.9 ± 191.73, respectively. The repeatability and intermediate precision experiment of sample the were carried out by measuring intra-day and inter-day variation for ASP determination at three different levels (100, 200 and 300 ng per spot), three times, respectively. Thee RSD values for inter-day and intra-day precision were found in the range of 0.90 – 1.35% and 0.63 – 1.49%, respectively (Table 2). The low values of % RSD are indicative of the high repeatability of the method. The accuracy of the developed method was judged by recovery analysis, and it was found maximum recovery (99.39 – 99.96%) with low RSD values (1.02 – 1.23%), indicated accuracy of method (Table 3). The values of LOD and LOQ were calculated as 35.29 and 123.54 ng / spot, respectively. This indicated method could be used for wide range of detection of ASP.

3.2.1 Force degradation studies of ASP and complexes

The chromatograms and amount of ASP degraded under forced conditions (acidic, basic, photolytic and oxidising degradation condition) were used to assess the stability of ASP in complexes. The ASP alone was degraded as 74.9%, 64.2%, 64% and 85.4% after acidic, basic, UV light and H_2O_2 exposure (Table 3). A significant protection of ASP was noted, when ASP was complexed with HPβCD and FA. The ASP drug alone showed a significant decrease in peak area after acidic degradation condition (Fig. 2), However, ASP degradation was significantly reduced in the inclusion complex of ASP/FA as shown in Fig. 3. this proved that developed HPTLC method can be successfully used for the stability studies of ASP in formulation.

Table 1. Linear regression data for the calibration curve (n=6) ASPIRIN

Parameters	ASP
Linearity range ng/spot	100-2000
Regression equation	Y = 3.623*X + 595.9
R^2	0.9919
Slope ± SD	3.623 ± 0.200
Intercept ± SD	595.9 ± 191.73
Standard error of slope	0.08207
Standard error of slope	78.29
95% Confidence Intervals of slope	3.449 to 3.79
95% Confidence Intervals intercept	429.9 to 761.9
P value	<0.0001

Table 2. Intra-day and inter-day precision data (n=3)

Conc. (ng/spot)	Repeatability (Intra-day precision)			Inter-day precision		
	Area ± SD	SE	%RSD	Area ± SD	SE	%RSD
100	659.90±5.03	2.90	0.76	659.89±6.00	3.46	0.90
200	1083.77±6.85	3.95	0.63	1075.07±14.54	8.39	1.35
300	1528.17±22.83	9.32	1.49	1514.17±15.80	6.45	1.04

SD- standard deviation; RSD- relative standard deviation; SE- standard error

Table 3. Accuracy data for HPTLC method of ASP

% Analyte	Theoretical content (ng)	Exp. Content (ng) ± SD	% Recovery	% RSD
0	300	298.17±3.66	99.39	1.23
50	450	448.00±4.56	99.96	1.02
100	600	597.17±6.55	99.53	1.10
150	750	746.33±8.14	99.51	1.09

Table 4. Forced degradation of ASP and their complexes

Samples	% ASP degradation			
	Acidic	Basic	UV light	H_2O_2
ASP alone	74.9±4.2	64.2±3.5	64.0±4.1	85.4±5.3
Spray dried ASP-FA (1:1)	24.3±2.1	47.9±3.7	35.2±2.0	69.0±4.6
Spray dried ASP-HPβCD (1:1) (1:1)	12.6±3.2	37.8±3.5	27.9±1.9	68.0±2.5

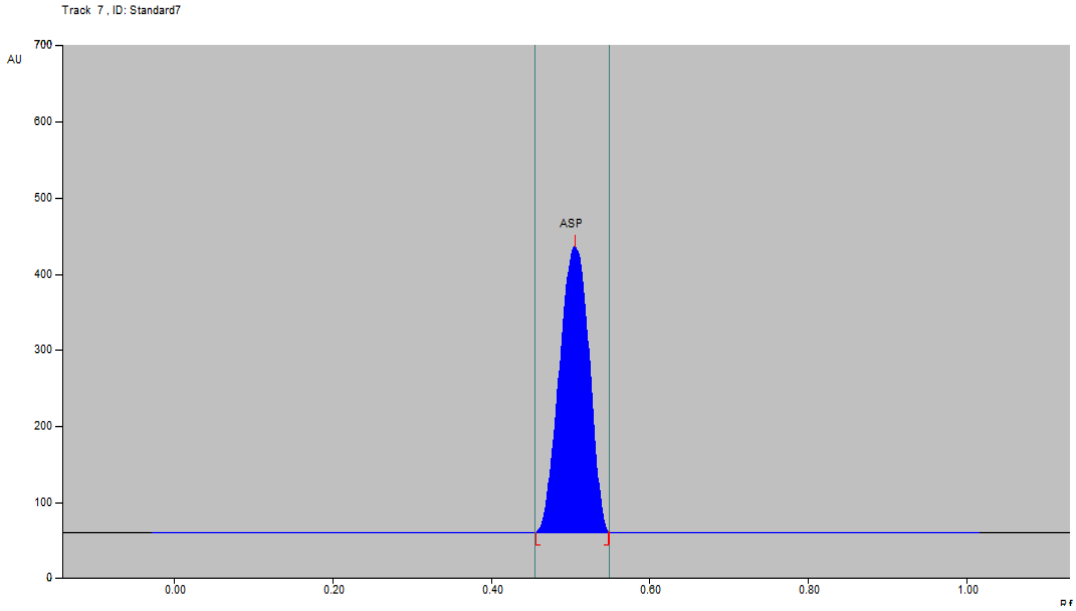

Fig. 1. A typical HPTLC chromatogram of ASP (RF-0.52)

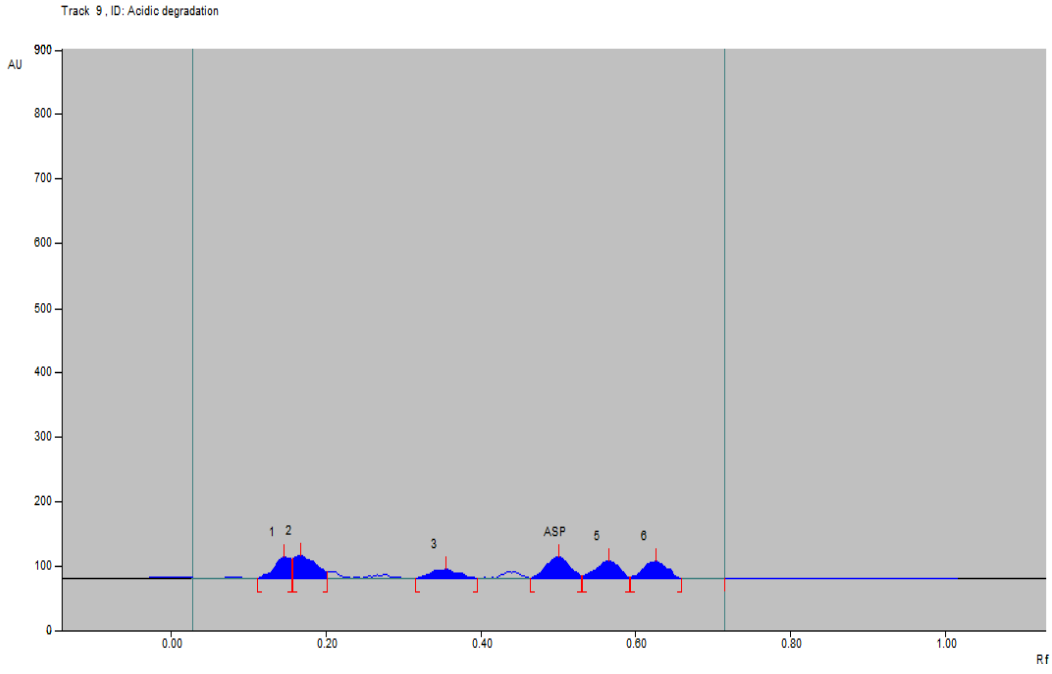

Fig. 2. HPTLC chromatogram of pure ASP after acidic degradation

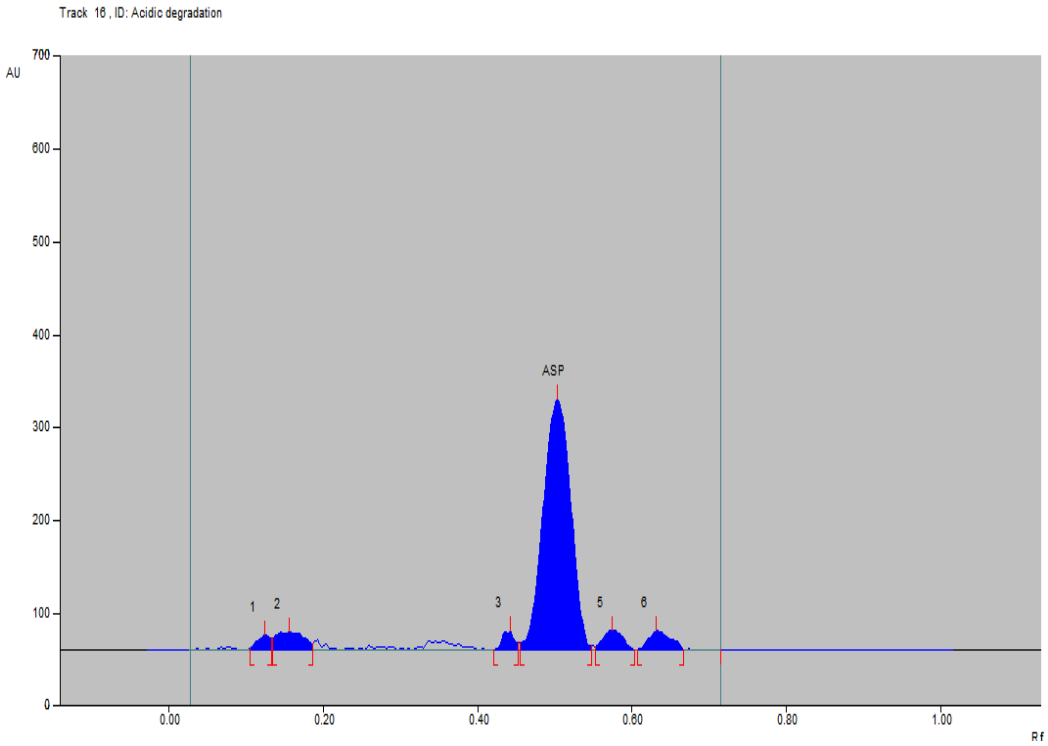

Fig. 3. HPTLC chromatogram of ASP in inclusion complex of ASP/FA after acidic degradation

4. CONCLUSION

The proposed HPTLC method is quite simple, accurate, precise, sensitive and economical. This method can be used for routine analysis of aspirin in low cost and in short time. In this method, no interference ASP peaks were observed by FA and HPβCD in complex analysis. The HPTLC peak of ASP at RF value of 0.52 was well resolved in complexes. The proposed validated HPTLC method was successfully applied for the analysis of pure ASP and in complexes (ASP/FA and ASP-HPβCD).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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