T1130-09-61

Reliable HPLC Method for the Simultaneous Determination of Aspirin and Associated Related Substances in Drug Substance and Tablet Formulation

Margaret Maziarz, Paul Rainville

Waters Corporation, 34 Maple Street, Milford MA

CONTACT INFORMATION: Margaret_Maziarz@waters.com, Paul_Ranville@waters.com

aaps PharmSci 360

PURPOSE

To ensure compliance with the current good manufacturing practices (CGMP) regulations, the manufacturers must assure the identity, strength, quality, and purity of the pharmaceutical drug products. This requires establishment of robust analytical test methods that produce reliable and accurate results over the routine use in quality control (QC) laboratory. Aspirin is a common drug for relieving minor aches, pains, fevers, as well as prevention of heart attacks and mini strokes.

In this work, a single HPLC method was developed for the analysis of aspirin active pharmaceutical ingredient (API) and six related substances. Method performance characteristics including system suitability, linearity, accuracy, intraday and interday precisions were assessed in this study.

METHODS

Aspirin and related substances standard solutions

Individual stock standard solutions with related substances and aspirin API were prepared in diluent (60:40 water/acetonitrile with 0.1% formic acid) at 1.0 and 5.0 mg/mL, respectively. The API stock solution was diluted to 0.1 mg/mL and spiked with related substances at 10% level.

Aspirin sample solutions

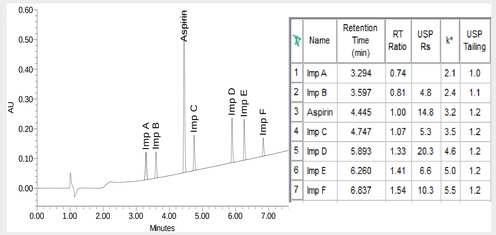
Drug tablets containing 81-mg of asprin tablets were crushed and dissolved in diluent (60:40 water/acetonitrile with 0.1% formic acid) at 1.6 mg/mL of aspirin by sonication for 10 minutes. After extraction, sample test solutions were centrifuged for 10 minutes at 3000 rpm and diluted to 0.1 mg/mL for aspirin assay and to 0.5 mg/mL for impurities analysis, respectively. Solutions were filtered through 0.2 µm Nylon syringe filter prior analysis.

Method conditions

LC System:	Alliance™ iS HPLC system with TUV detector
Column:	XSelect™ HSS T3 Column, 4.6 x 150 mm, 3.5 µm (P/N 186004786)
Column Temp.:	40°C
Mobile Phase:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Flow Rate:	1.8 mL/min
Gradient:	Time (min) / Flow (mL/min) / %A / %B / Curve Initial / 1.8 / 95.0 / 5.0 / 6 0.10 / 1.8 / 95.0 / 5.0 / 6 7.60 / 1.8 / 5.0 / 95.0 / 6 9.20 / 1.8 / 5.0 / 95.0 / 6 9.30 / 1.8 / 95.0 / 5.0 / 6 13.00 / 1.8 / 95.0 / 5.0 / 6
UV Detection:	237 nm
Injection Vol.:	15.0 µL
Sample Temp.:	10°C
Wash solvents:	Purge/Sample Wash: 60:40 water/acetonitrile Seal Wash: 90:10 water/acetonitrile

RESULTS

The XSelect HSS T3 column successfully separated all analytes, producing a USP Resolution (USP Rs) ≥ 4.9, peak tailing of 1.1 – 1.2, and retentivity factor (k*) ≥ 2.1.



	Name	Retention Time (min)	RT Ratio	USP Rs	k*	USP Tailing
1	Imp A	3.294	0.74		2.1	1.0
2	Imp B	3.597	0.81	4.8	2.4	1.1
3	Aspirin	4.445	1.00	14.8	3.2	1.2
4	Imp C	4.747	1.07	5.3	3.5	1.2
5	Imp D	5.893	1.33	20.3	4.6	1.2
6	Imp E	6.260	1.41	6.6	5.0	1.2
7	Imp F	6.837	1.54	10.3	5.5	1.2

Figure 1. Chromatographic separation achieved using XSelect HSS T3 column. UV at 237 nm.

Method performance

The performance of the method was assessed by measuring system suitability of five replicate injections of the standard mixture for intraday and interday assay analysis. The intraday and interday performance demonstrated excellent repeatability of replicate injections with relative standard deviation (RSD) of the peak areas and retention times ranging from 0.03 to 0.30% and 0.01 to 0.03%, respectively.

Intraday method performance measured against the USP suitability criteria defined in the USP monograph for aspirin tablets[2]. Method met the USP criteria across four sets of data for a one-day analysis. (salicyclic acid: impurity C)

Parameter	USP Requirement[2]	System Suitability 1 Intraday	System Suitability 2 Intraday	System Suitability 3 Intraday	System Suitability 4 Intraday
Aspirin Assay	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Tailing factor for aspirin	Not more than (NMT) 2.0	1.2	1.2	1.2	1.2
Relative standard deviation (RSD) for aspirin	Not more than (NMT) 2.0%	• RSD of areas: 0.07% • RSD of RT: 0.03%	• RSD of areas: 0.08% • RSD of RT: 0.02%	• RSD of areas: 0.03% • RSD of RT: 0.03%	• RSD of areas: 0.03% • RSD of RT: 0.02%
Impurities	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Resolution between salicyclic acid and aspirin	Not less than (NLT) 2.0	5.3	5.3	5.3	5.3
RSD for salicyclic acid	Not more than (NMT) 4.0%	• RSD of areas: 0.17% • RSD of RT: 0.02%	• RSD of areas: 0.07% • RSD of RT: 0.02%	• RSD of areas: 0.25% • RSD of RT: 0.02%	• RSD of areas: 0.13% • RSD of RT: 0.02%

Table1. Intraday method performance.

Interday method performance measured on different days met the USP suitability criteria defined in the USP monograph for aspirin tablets[2]. (salicyclic acid: impurity C).

Parameter	USP Requirement[2]	Day 1	Day 2	Day 5
Aspirin Assay	*N/A*	*N/A*	*N/A*	*N/A*
Tailing factor for aspirin	Not more than (NMT) 2.0	1.2	1.2	1.2
Relative standard deviation (RSD) for aspirin	Not more than (NMT) 2.0%	• RSD of areas: 0.07% • RSD of RT: 0.02%	• RSD of areas: 0.03% • RSD of RT: 0.03%	• RSD of areas: 0.07% • RSD of RT: 0.02%
Impurities	*N/A*	*N/A*	*N/A*	*N/A*
Resolution between salicyclic acid and aspirin	Not less than (NLT) 2.0	5.3	5.3	5.3
RSD for salicyclic acid	Not more than (NMT) 4.0%	• RSD of areas: 0.14% • RSD of RT: 0.02%	• RSD of areas: 0.24% • RSD of RT: 0.03%	• RSD of areas: 0.15% • RSD of RT: 0.02%

Table 2. Interday method performance.

Linearity

The calibration plot for aspirin API in the range of 80 to 120% with respect to the API working concentration in the sample preparation of 0.1 mg/mL. The calibration curve of the concentration versus the peak area at each level produced a correlation coefficient (R^2) ≥ 0.999. In addition, the percent deviation of the calculated X values or concentrations ranged from –0.35 to 0.81% .

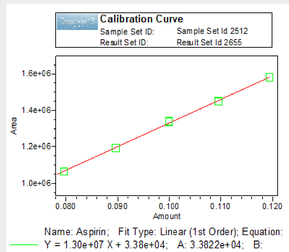


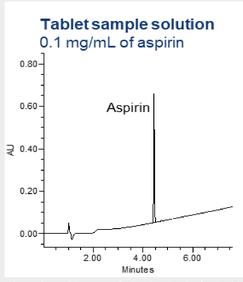
Figure 2. Calibration curve for aspirin API.

Calibration curve_%Deviation

	Name	X Value	Response	Calc. Value	% Deviation
1	Aspirin	0.079600	1064112	0.079447	-0.192
2	Aspirin	0.079600	1064893	0.079507	-0.117
3	Aspirin	0.079600	1064518	0.079478	-0.153
4	Aspirin	0.089600	1192734	0.089365	-0.262
5	Aspirin	0.089600	1193229	0.089403	-0.220
6	Aspirin	0.089600	1193677	0.089438	-0.181
7	Aspirin	0.099900	1339905	0.100713	0.814
8	Aspirin	0.099900	1337305	0.100513	0.614
9	Aspirin	0.099900	1339095	0.100651	0.752
10	Aspirin	0.109500	1450279	0.109225	-0.252
11	Aspirin	0.109500	1448917	0.109120	-0.347
12	Aspirin	0.109500	1449131	0.109136	-0.332
13	Aspirin	0.119400	1580940	0.119300	-0.084
14	Aspirin	0.119400	1581148	0.119316	-0.070
15	Aspirin	0.119400	1582096	0.119389	-0.009

Aspirin assay in tablet formulation

The assay results for six samples ranged from 93.4 to 93.6%, meeting the USP acceptance criteria of not less than (NLT) 90.0 and not more than (NMT) 110.0% of the labeled amount of aspirin[2].



%Recovery results

Sample Analysis
Sample Set Id: 1753
Result Set Id: 1893

	SampleName	Channel	Aspirin
1	Asp Tab 0.1mg	Absorbance A	93.5
2	Asp Tab 0.1mg	Absorbance A	93.6
3	Asp Tab 0.1mg	Absorbance A	93.4
4	Asp Tab 0.1mg	Absorbance A	93.4
5	Asp Tab 0.1mg	Absorbance A	93.4
6	Asp Tab 0.1mg	Absorbance A	93.4
Mean			93.451
Std. Dev.			0.084
% RSD			0.09

Figure 3. Aspirin assay determination in drug tablet formulation.

Related substances analysis

The related substances content (% impurity) was determined by comparing peak areas of each related substance to the aspirin API peak area. For related substances testing, the drug tablets.

Results met the USP acceptance criteria for salicyclic acid (imp. C): NMT 0.3% and for coated tablets of NMT 3.0%[2].

Sample Analysis
Sample Set Id: 1753
Result Set Id: 1926

% impurity
Impurity Response Summarized by Name

	SampleName	Imp C	Imp D
1	Asp Tab 0.5mg	0.906	0.050
2	Asp Tab 0.5mg	0.899	0.047
3	Asp Tab 0.5mg	0.908	0.044
4	Asp Tab 0.5mg	0.907	0.045
5	Asp Tab 0.5mg	0.906	0.045
6	Asp Tab 0.5mg	0.909	0.048
Mean		0.91	0.05
Std. Dev.		0.004	0.002
% RSD		0.40	4.42

Sample Analysis
Sample Set Id: 1753
Result Set Id: 1926

% impurity
Impurity Response Summarized by Name

	SampleName	Imp C
1	Asp Drug Sub 0.5mg	0.140
2	Asp Drug Sub 0.5mg	0.145
3	Asp Drug Sub 0.5mg	0.149
4	Asp Drug Sub 0.5mg	0.149
5	Asp Drug Sub 0.5mg	0.149
6	Asp Drug Sub 0.5mg	0.161
Mean		0.15
Std. Dev.		0.007
% RSD		4.61

Figure 4. Percent (%) impurity determination in drug tablet and drug substance formulation.

CONCLUSIONS

- A single LC method run on the Alliance iS HPLC System was successfully developed for the simultaneous analysis of aspirin active ingredient and six associated related substances.
- Method exhibited excellent system suitability results, linearity, accuracy, intraday and interday performance.
  - Relative standard deviations (RSD) of peak areas and retention times for intraday and interday studies were ≤ 0.25% and ≤ 0.03%, respectively.
- Method demonstrated a reliable determination of aspirin assay and related substances (impurities) content in the drug substance and tablet formulations.

REFERENCES

1. 1. Ph. Eur. Monograph. Acetylsalicylic Acid. The European Pharmacopeia 10.0. 01/2017:0309
2. USP Monograph for Aspirin Tablets. United States Pharmacopeia USP43-NF38, official 1-May-2020

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™