Reliable HPLC Analysis of Aspirin and Associated Related Substances in Drug Substance and Tablet Formulation

Authors: Margaret Maziarz, Paul Rainville
Affiliations: Waters Corporation, 34 Maple Street, Milford MA 01757

INTRODUCTION

To ensure compliance with the current good manufacturing practices (CGMP) regulations, manufacturers must assure quality and purity of the pharmaceutical drug products using robust and reliable analytical test methods.

Aspirin is a common drug for relieving minor aches, pains, fevers, as well as prevention of heart attacks and mini strokes. Aspirin is available in tablets, with 81–650 mg of aspirin per tablet. In this work, a single HPLC method is presented for the analysis of aspirin active pharmaceutical ingredient (API) and six associated related substances listed by the European Pharmacopeia[1].

Name	Compound	Molecular formula	Monoisotopic mass (Da)	structure
Aspirin API	2-Acetoxybenzoic acid, O-Acetylsalicylic acid	$C_9H_8O_4$	180.04	
Impurity A	p-Salicylic Acid, 4-hydroxybenzoic acid	$C_7H_6O_3$	138.03	
Impurity B	4-Hydroxy-1,3-benzenedicarboxylic Acid, 4-Hydroxyisophthalic acid	$C_8H_6O_5$	182.02	
Impurity C	Salicylic Acid, 2-Hydroxybenzoic acid,	$C_7H_6O_3$	138.03	
Impurity D	Acetylsalicylsalicylic Acid, 2-(Acetyloxy)benzoic Acid	$C_{16}H_{12}O_6$	300.06	
Impurity E	2-((2-hydroxybenzoyl)oxy)benzoic acid, salsalate	$C_{14}H_{10}O_5$	258.05	
Impurity F	2-Acetoxybenzoic anhydride, O-acetylsalicylic anhydride,	$C_{18}H_{14}O_7$	342.07	

Table 1. Aspirin and related substances (impurities) used in the study.

METHODS

EXPERIMENTAL

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 with diluent to 0.1 mg/mL and spiked with related substances at 10% level.

Aspirin sample solutions

Crushed tablets were 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 analysis and to 0.5 mg/mL for impurities analysis, respectively. Solutions were filtered through 0.2 µm Nylon syringe filter prior analysis.

Method conditions

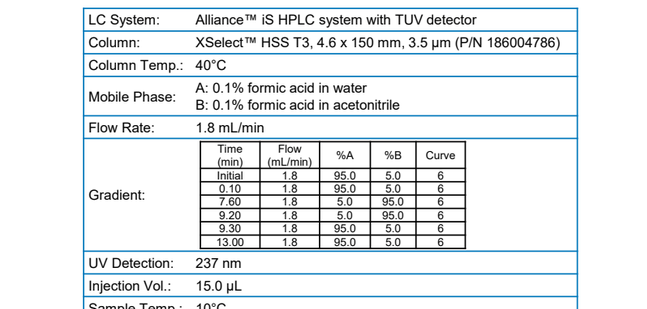
LC System:	Alliance™ iS HPLC system with TUV detector
Column:	XSelect™ HSS T3, 4.6 x 150 mm, 3.5 µm (P/N 186004786)
Column Temp.:	40°C
Mobile Phase:	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Flow Rate:	1.8 mL/min
Gradient:	Time (min) / Flow (mL/min) / %A / %B / Curve Initial 1.8 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

Table 2. Method conditions for the analysis of aspirin API and related substances.

RESULTS

Data acquisition and analysis performed using the Empower™ 3.6.0 Software. Summary reports with results generated using the report templates available in the Empower project.

The method run on the Alliance™ HPLC iS System successfully separated aspirin API and six associated related substances within 7.6 minutes, producing a minimum USP Resolution (USP Rs) ≥ 4.8, peak tailing of 1.0 – 1.2, and retentivity factor (k*) ≥ 2.1 (Figure 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

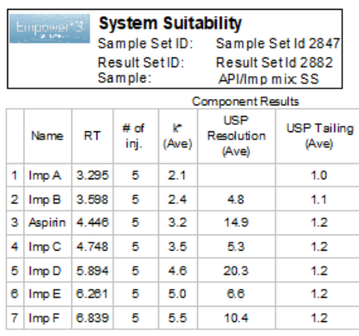
Figure 1. Representative chromatogram of the mixture standard solution of aspirin API and related substances run on Alliance iS HPLC System. UV at 237 nm.

System suitability

System suitability was assessed using five replicate injections of the mixture standard solution, assuring the suitability requirements specified in the USP monograph for aspirin tablets were met. The USP suitability requirements listed for the assay and impurities procedures include[2]:

- Assay: peak tailing of not more than (NMT) 2.0 and relative standard deviation (RSD) of NMT 2.0
- Impurities: resolution between salicyclic acid and aspirin peaks of not less than (NLT) 2.0; RSD for salicyclic acid of NMT 4.0%

Method demonstrated excellent system suitability with the RSD for peak areas and retention time (RT) ranging from 0.07 to 1.43% and 0.02 to 0.03%, respectively. Additionally, the USP suitability requirements for the assay and impurities procedures were met.



	Name	RT	# of inj.	k* (Ave)	USP Resolution (Ave)	USP Tailing (Ave)	%RSD of Peak Areas	%RSD of RT
1	Imp A	3.295	5	2.1		1.0	0.20	0.02
2	Imp B	3.598	5	2.4	4.8	1.1	0.13	0.02
3	Aspirin	4.446	5	3.2	14.9	1.2	0.07	0.03
4	Imp C	4.748	5	3.5	5.3	1.2	0.17	0.02
5	Imp D	5.894	5	4.6	20.3	1.2	0.09	0.02
6	Imp E	6.261	5	5.0	6.6	1.2	0.02	0.02
7	Imp F	6.839	5	5.5	10.4	1.2	1.43	0.02

Figure 2. System suitability results for five replicate injections of the mixture standard solution acquired on the Alliance iS HPLC System. UV at 237 nm.

Intraday and interday performance

The intraday method performance on the Alliance iS HPLC System was evaluated by analyzing five replicate injections of the mixture standard solution in four different sets in a one-day analysis. The results were assessed against the USP suitability requirements for aspirin tablet[2]. The intraday method performance was excellent, meeting the USP suitability requirements defined for aspirin assay and impurities procedures (Table 3). The RSD of aspirin peak areas and retention times ranged from 0.03% to 0.08% and 0.02 to 0.03% over four sets of data. The RSD for salicyclic acid (impurity C) peak areas ranged from 0.07 to 0.25%.

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%

Table 3. 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).

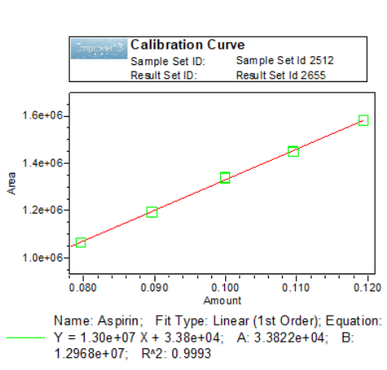
For interday performance of the method, a mixture standard solution was analyzed over different days including day 1, 2 and 5. Method exhibited excellent performance, generating comparable results across the tested days and meeting all the USP suitability criteria (Table 4).

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 4. 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).

Linearity and range

The calibration plot for aspirin API was constructed 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 (Figure 3). In addition, the percent deviation of the calculated X values or concentrations ranged from –0.35 to 0.81% .



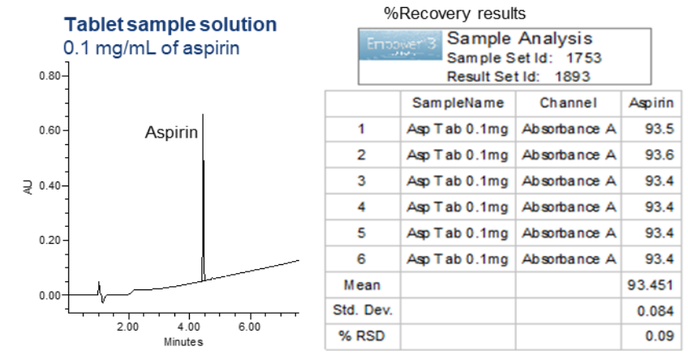
Calibration curve_%Deviation

	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

Figure 3. Calibration curve for aspirin API in the range of 80 - 120% with respect to the working concentration of 0.1 mg/mL and % deviation for the measurements. UV at 237 nm.

Aspirin assay in tablet formulation

For assay testing, the drug tablets containing 81-mg of aspirin were prepared at 0.1 mg/mL in diluent (60:40 water/acetonitrile with 0.1% formic acid). To calculate % recovery, the sample solutions were quantified against the calibration curve. The assay results for six samples ranged from 93.4 to 93.6% (Figure 4), 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].



	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 4. Aspirin assay determination in drug tablet formulation. The assay results met the USP criteria for aspirin tablets of 90.0 - 110.0% of the labeled amount of aspirin[2].

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 containing 81-mg of aspirin and drug substance were prepared at 0.5 mg/mL in diluent (60:40 water/acetonitrile with 0.1% formic acid), Figure 5.

The analysis revealed presence of two impurities in the aspirin tablet samples (impurity C and D at 0.91% and 0.05%) and presence of impurity C at 0.15% level in the drug substance sample (Figure 6). Results met the USP acceptance criteria for salicyclic acid (imp. C): NMT 0.3% and for coated tablets of NMT 3.0%[2].

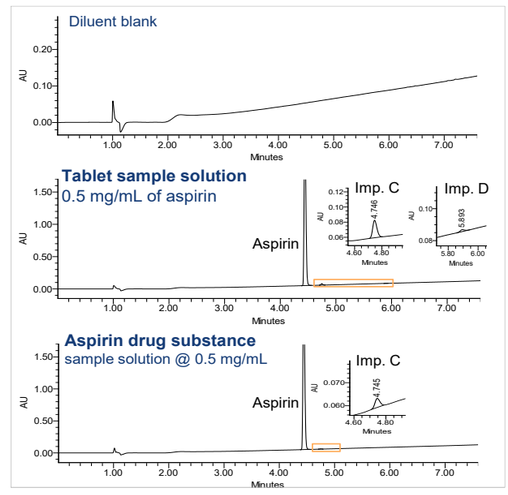


Figure 5. Sample solutions for analysis of related substances. UV at 237 nm.

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 6. Related substances (% impurities) results in aspirin tablet formulations and drug substance. UV at 237 nm.

CONCLUSION

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

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