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RESEARCH ARTICLE

Simultaneous Estimation of Aspirin and Vonoprazan in Active Pharmaceutical Ingredient and pharmaceutical formulation by RP-HPLC

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ABSTRACT

A reliable, precise, and sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Aspirin and Vonoprazan in bulk drug and pharmaceutical formulations. Chromatographic separation was achieved using a Phenomenex C18 column with a mobile phase consisting of 0.02 M potassium dihydrogen phosphate (KH_2PO_4), acetonitrile, and water in a 40:40:20 (v/v/v) ratios, adjusted to pH 4.0 with orthophosphoric acid. The method showed good linearity over concentration ranges of 10–50 µg/mL for Aspirin and 2–10 µg/mL for Vonoprazan with correlation coefficients greater than 0.999. Validation in accordance with ICH Q2 (R1) guidelines confirmed high specificity, accuracy, precision with %RSD < 2, robustness, and suitable limits of detection and quantification. The method was successfully applied to marketed combined dosage forms, proving its applicability for routine quality control.

Keywords: Aspirin, Vonoprazan, RP-HPLC, Simultaneous Estimation, ICH Validation,Chromatographic Method.

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Introduction

Aspirin, a cyclooxygenase inhibitor, is widely used for its analgesic, antipyretic, and antiplatelet properties, while Vonoprazan, a potassium-competitive acid blocker, efficiently suppresses gastric acid secretion. The combination of these drugs mitigates gastrointestinal side effects while preserving therapeutic benefits. Accurate simultaneous quantitation of these drugs is essential in combined pharmaceutical formulations. RP-HPLC techniques are preferred due to their sensitivity and specificity. This study aims to develop and validate a novel RP-HPLC method following regulatory guidelines for routine analysis of Aspirin and Vonoprazan.

Experimental

Materials

Active Pharmaceutical Ingredients of Asprin as a gift sample from Spark Lifesciences and Vonoprazan as a gift sample Bio-Synth

Chemicals Used

Chemical used in the research are Acetonitrile AR Grade (Fisher Scientific Ltd. India), Buffer Capsules,Methanol, Ortho Phosphoric Acid GR (Merk ,India),Potassium Di Hydrogen Phosphate GR (Merk ,India),DMSO. Acetonitrile HPLC Grade (Merk, India), Distilled Water HPLC Grade (Fisher Scientific Ltd. India), Methanol HPLC Grade (Merk ,India).

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Equipments

Analytical Balance(Model, UV-Vis Spectrophotometer 1800 (Shizmazu Corporation ,Kyoto, Japan), Syringe filter(vertipure Nylon syringe Filter,133 mm diameter,0.45µm pore size,Vertical,Thialand, Sonicator (Sonapros Ultrasonic Processor / Sonicator Oscar ultrasonics Pvt. Ltd Maharashtra, India), pH Meter (Instrument India, Mumbai), Indicator paper pH 1.0-14.0, High Performance liquid Chromatography (HPLC) (Shizmazu Corporation ,Kyoto , Japan) consists of with following Solvent delivery module LC-20AD Prominence. UV-VIS Detector SPD-20A/SPD-20AV Prominence. Phenomex C18 HPLC Column ,4.6X150mm,5µm,USA,

Microliter Syringe(Hamilton Bonaduz AG Switzerland).

Chromatographic Conditions

The chromatographic analysis was performed using a Phenomenex C18 column (250 × 4.6 mm, 5 µm particle size), pre-equilibrated with the mobile phase comprising 0.02 M potassium dihydrogen phosphate (KH_2PO_4): acetonitrile: water (40:40:20, v/v/v), adjusted to pH 4.0 with orthophosphoric acid.

- Flow rate: 1.0 mL/min
- Detection wavelength: 235 nm (isobestic point)
- Injection volume: 20 µL
- Run time: 15 minutes

Preparation of Standard Stock Solutions

Accurately weighed quantities of Aspirin (100 mg) and Vonoprazan (100 mg) were transferred to separate 100 ml volumetric flasks. Each was dissolved in 50 ml of acetonitrile and the volume was made up to the mark with water to obtain 1000 µg/ml stock solutions.

Preparation of Working Standard Solutions

Aliquots from the stock solutions were appropriately diluted with water to obtain working standards of desired concentrations for method calibration and validation.

Selection of Detection Wavelength

UV scanning of the standard drug solutions (200–400 nm) revealed maximum absorbance at 225 nm for Aspirin and 247 nm for Vonoprazan. The isobestic point for the two drugs was observed at 235 nm (Fig. 1), which was selected for simultaneous estimation.

Preparation of Mixed Standard Solutions

Weigh accurately 100 mg of Aspirin and 10 mg of Vonoprazan. Transfer in 100 ml volumetric flask add 20 ml of Acetonitril and sonicate for 45 minutes, made up to 100 ml with diluent.

From this solution transfer 1 ml of the mixed stock solution into 10 ml volumetric flask. Add diluent up to volume; use this solution for further work. The chromatogram obtained is shown in Fig. 2. Retention time was found to be 7.79 min and 9.29 min for Aspirin and Vonoprazan respectively.

Linearity

The method was found linear over a concentration range 10-50µg/mL for Aspirin and 2 -10 µg/ml for Vonoprazan. The results shown in the Fig.3 and 4, Tables 1, 2 and 3.

Precision

The % RSD value of repeatability study was found to be 0.695 for Aspirin and 0.479 for Vonoprazan (Table 4 and 5).

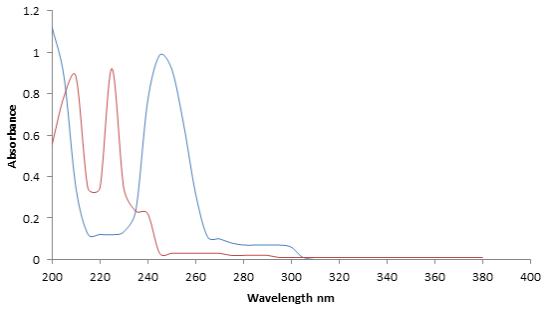

Fig. 1: Overlaps spectra of Aspirin and Vonoprazan

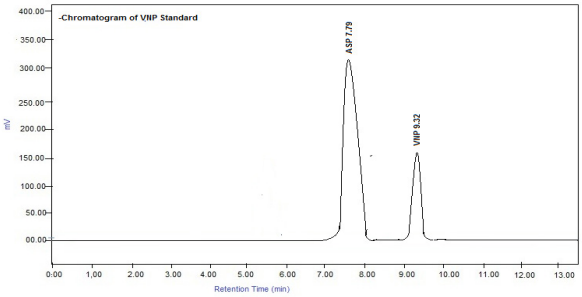

Fig. 2: Chromatogram of synthetic mixture of Aspirin and Vonoprazan

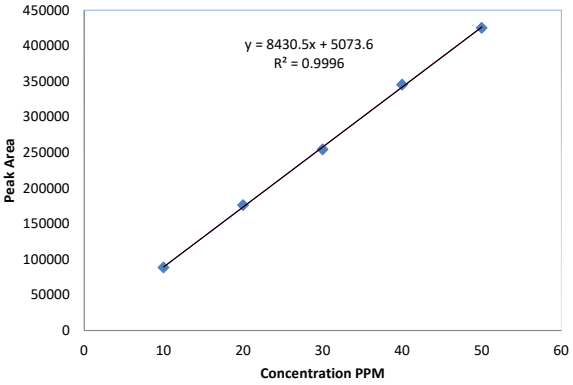

Fig. 3: Calibration curve of Aspirin

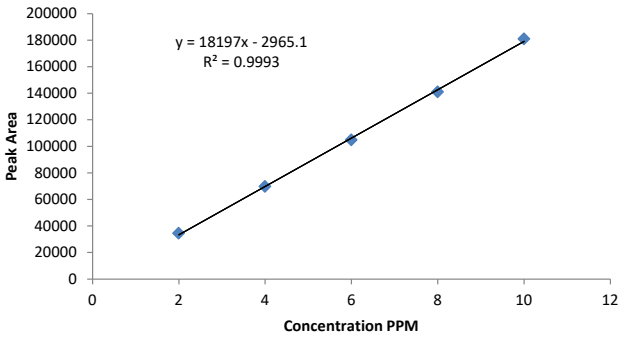

Fig. 4: Calibration curve of Vonoprazan

Table 1: Calibration data of Aspirin

S. No.	*Actual concentration PPM*	*Peak area*	*Concentration found PPM*
1	10	88544	9.90
2	20	176092	20.29
3	30	254433	29.58
4	40	345522	40.39
5	50	425355	49.86

Table 2: Calibration data of Vonoprazan

S. No.	*Actual concentration PPM*	*Peak area*	*Concentration Found PPM*
1	2	34525	1.73
2	4	69788	3.67
3	6	104877	5.60
4	8	140993	7.59
5	10	180888	9.78

Table 3: Regression analysis Aspirin and Vonoprazan

S. No.	*Parameters*	*Aspirin*	*Vonoprazan*
1	Limit of linearity (µg/ml)	10-50	2-10
2	Regression equation	8430.5x+5070	18197x+2965
3	Correlation coefficient (r2)	0.9996	0.9993
4	LOD (µg/ml)	1.2	0.317
5	LOQ (µg/ml)	3.8	0.960

Table 4: Repeatability for Aspirin

S. No.	*Actual concentration µg/ml*	*Peak area*	*Concentration found µg/ml*
1	10	88544	9.90
2	10	88544	9.90
3	10	89555	10.02
4	10	89662	10.03
5	10	88676	9.92
6	10	88277	9.87
	Mean	88876	9.941
	STDV	582.8	0.069
	%RSD	0.007	0.695

Table 5: Repeatability for Vonoprazan

S. No.	*Actual concentration µg/ml*	*Peak area*	*Concentration found µg/ml*
1	4	69788	3.67
2	4	70322	3.70
3	4	70442	3.71
4	4	70562	3.71
5	4	69883	3.68
6	4	69883	3.68
	Mean	70147	3.692
	STDV	334.11	0.018
	%RSD	0.00476	0.497

Table 6: Intraday precision of Aspirin

S. No.	*10 µg/ml*	*20 µg/ml*	*30 µg/ml*
1	88544	179883	278883
2	88544	179555	278833
3	89555	179666	278874
4	89662	179777	278442
5	88676	179666	276736
SD	562.8	124.81	922.68
MEAN	88996.2	179709.4	278353.6
%RSD	0.632	0.069	0.331
Mean % RSD	0.344		

Table 7: Intraday precision of Vonoprazan

S. No.	*4 µg/ml*	*6 µg/ml*	*8 µg/ml*
1	69788	104877	140993
2	70322	104737	141235
3	70442	107766	140998
4	70562	107647	140938
5	69883	104877	141098
SD	344.50	1576.94	117.26
MEAN	70199.4	105980.8	141052.4
%RSD	0.491	1.488	0.083
Mean % RSD	0.687		

The % RSD value for intraday study was found to be 0.344 for Aspirin and 0.687 for Vonoprazan (Table 6 and 7).
The % RSD value for intraday study was found to be 0.294 for Aspirin and 0.559 for Vonoprazan by different analyst (Table 8 and 9).
The % RSD value for interday was found to be 0.004 for Aspirin and 0.058 for Vonoprazan. (Table 10 and 11)

The %RSD values indicate the repeatability of this method is satisfactory which are below 2% follows ICH guideline.

Table 8: Intraday precision of Aspirin

S. No.	*Analyst I*			*Analyst I*		
	10 µg/ml	*20 µg/ml*	*30 µg/ml*	*10 µg/ml*	*20 µg/ml*	*30 µg/ml*
1	88544	179883	278883	86673	176882	227377
2	88544	179555	278833	86637	176888	226773
3	89555	179666	278874	86732	177733	226777
4	89662	179777	278442	86722	178773	226777
5	88676	179666	276736	86781	176788	227663
SD	562.8	124.81	922.68	55.6	851.50	420.04
MEAN	88996.2	179709.4	278353.6	86709.0	177412.8	227073.4
%RSD	0.632	0.069	0.331	0.064	0.480	0.185
Mean % RSD	0.344			0.243		

Table 9: Intraday precision of Vonoprazan

S. No.	*Analyst I*			*Analyst II*		
	4 µg/ml	*6 µg/ml*	*8 µg/ml*	*4 µg/ml*	*6 µg/ml*	*8 µg/ml*
1	69788	104877	140993	676633	104888	139983
2	70322	104737	141235	674764	104999	138982
3	70442	107766	140998	676474	104393	137887
4	70562	107647	140938	678477	104566	137827
5	69883	104877	141098	673883	104335	137474
SD	344.50	1576.94	117.26	1786.03	295.73	1035.48
MEAN	70199.4	105980.8	141052.4	676046.2	104636.2	138430.6
%RSD	0.491	1.488	0.083	0.264	0.283	0.748
Mean % RSD	0.687			0.432		

Table 10: Interday precision Aspirin

Day 1				*Day 2*			*Day 3*		
S. No.	*10 µg/ml*	*20 µg/ml*	*30 µg/ml*	*10 µg/ml*	*20 µg/ml*	*30 µg/ml*	*10 µg/ml*	*20 µg/ml*	*30 µg/ml*
1	88544	179883	278883	88536	178938	278883	88833	178783	276663
2	88544	179555	278833	87790	179838	278838	88376	179555	276648
3	89555	179666	278874	87998	179983	278821	87363	178883	276872
4	89662	179777	278442	87988	179888	276636	87663	178988	278387
5	88676	179666	276736	88877	179883	278837	88773	178748	278737
MEAN	88996	179709	278353	88238	179706	278403	88202	178991	277461
STDV	562.82	124.81	922.67	452.19	432.56	988	661	329	1016
%RSD	0.006	0.001	0.003	0.005	0.002	0.004	0.007	0.002	0.004
Mean % RSD	0.003			0.004			0.004		

Table 11: Interday precision Vonoprazan

Day 1				*Day 2*			*Day 3*		
S. No.	*4 µg/ml*	*6 µg/ml*	*8 µg/ml*	*4 µg/ml*	*6 µg/ml*	*8 µg/ml*	*4 µg/ml*	*6 µg/ml*	*8 µg/ml*
1	69788	104877	140993	67738	108366	140837	67877	104673	138999
2	70322	104737	141235	673782	108387	140398	67884	104553	138988
3	70442	107766	140998	678483	108983	139092	67829	103983	138928
4	70562	107647	140938	676488	108773	139882	67982	103474	138982
5	69883	104877	141098	673883	108838	139882	67838	103488	138743
MEAN	70199	105981	141052	554075	108669	140018	67882	104034	138928
STDV	344.50	1576.939	117.257	271877	278.076	653.764	60.77	568.33	106.98
%RSD	0.005	0.015	0.001	0.491	0.003	0.005	0.001	0.005	0.001
Mean % RSD	0.007			0.166			0.002		

Table 12: Robustness study

Parameter	*Sample*	*Aspirin*	*Vonoprazan*
		%RSD	*%RSD*
Flow rate ml./min	Flow Rate: 0.9 ml/min (Decreased by 10%)	0.677	0.455
	Flow Rate: 1.0 ml/min	0.356	0.422
	Flow Rate: 1.1 ml/min (Increased by 10%)	0.655	0.786
Mobile Phase 0.02 M potassium dihydrogen phosphate (KH_2PO_4): acetonitrile: water	40:40:20	0.230	0.564
	42:38:20	0.564	0.454
	40:35:25	0.430	0.622
Colum Temperature	Column Temperature: 360C (Decreased by 10%)	0.565	0.432
	Column Temperature: 400C	0.450	0.543
	Column Temperature: 440C (Increased by 10%)	0.650	0.644
Detector Temperature	Detector Temperature: 360C (Decreased by 10%)	0.675	0.644
	Detector Temperature: 400C	0.345	0.544
	Detector Temperature: 440C (Increased by 10%)	0.655	0.544

Mean of three values

Table 13: Recovery study of Aspirin

Concentration taken 18 ppm (80%)		*Concentration taken 20 ppm (100%)*		*Concentration taken 22 ppm (120%)*	
Peak Area	*Conc. found ppm*	*Peak Area*	*Conc. found ppm*	*Peak Area*	*Conc. found ppm*
158988	18.26	176092	20.29	189888	21.92
156773	18.00	178883	20.62	190422	21.99
159882	18.36	176034	20.28	190433	21.99
Mean Con	18.21		20.40		21.97
SD of Con.	0.189		0.19		0.036
% Recovery	101.14		101.97		99.846
Mean % Recovery		100.98			

Table 14: Recovery study of Vonoprazan

Concentration taken 18 ppm (80%)		*Concentration taken 20 ppm (100%)*		*Concentration taken 22 ppm (120%)*	
Peak area	*Conc. found ppm*	*Peak Area*	*Conc. found ppm*	*Peak Area*	*Conc. found ppm*
327772	17.85	367828	20.05	398877	21.76
332221	18.09	367277	20.02	401321	21.89
327711	17.85	367233	20.02	408838	22.31
Mean Con	17.93		20.03		21.99
SD of Con.	0.1421		0.0182		0.2853
% Recovery	99.62		100.15		99.93
Mean % Recovery	99.90				

Table 15: Analysis of market formulation

Tablet	*Drug labeled claim mg/ml*	*% Drug obtained*
Sample	Asprin 100mg	101.5
	Vonoprazan 10 mg	100.5

*Mean of three values

Table 16: System suitability parameters for Asprin and Vonoprazan

Parameters	*Asprin*	*Vonoprazan*
Theoretical plate	6762	8784
Retention Time(min)	7.79	9.32
Tailing factor	0.31	0.47
Resolution	2.03	

Table 17: Specificity for Aspirin

Sample type	*Retention Time (min)*	*Interfering Peaks*	*Peak Purity Pass/Fail*
Blank	—	None	—
Placebo	—	None	—
Standard	7.79	No	Pass
Sample	7.79 No		Pass

Table 18: Specificity for Vonoprazan

Sample Type	*Retention Time (min)*	*Interfering Peaks*	*Peak Purity Pass/Fail*
Blank	—	None	—
Placebo	—	None	—
Standard	9.32	No	Pass
Sample	9.32	No	Pass

Accuracy

The mean recovery of the added standard was 100.98 and 99.90 for Aspirin and Vonoprazan, respectively. These mean recovery values are well within the 98-102% indicating the method is accurate. (Table 13 and 14)

Detection Limit and Quantitation Limit

The detection limits were found to be 1.2µg/ml and 0.37µg/ml for Aspirin and Vonoprazan respectively.

The quantitation limits were found to be 3.8µg/ml and 0.96µg/ml for Aspirin and Vonoprazan respectively. These values indicate that the method is sensitive. (Table 3)

Analysis of marketed formulation

The assay results obtained by using the proposed method for the analysis of marketed tablet formulation containing 100 mg of Aspirin and 10 mg of Vonoprazan equivalent to per tablet were in good agreement with the labelled amounts of Aspirin and Vonoprazan. The average contents of Aspirin and Vonoprazan were 100 mg per tablet (101.5%) and 10 mg (100.5) per tablet respectively. This indicates that present method can be successfully used for the estimation of Aspirin and Vonoprazan in a combined tablet dosage form without interference of any impurity or excipient. (Table 15)

Robustness

The %RSD of mean assay values was found to be 0.677 for Aspirin and 0.455 for Vonoprazan with a flow rate of 0.9 mL/min.

The % RSD of mean assay values was found to be 0.655 for Aspirin and 0.786 for Vonoprazan with a flow rate of 1.1 mL/min. Also, % RSD of mean assay values was found to be 0.564 and 0.454 for Aspirin and Vonoprazan with a mobile phase ration 42:38:20. %RSD of mean assay values was found to be 0.430 and 0.622 for Aspirin and Vonoprazan with a mobile phase ration 40:35:25.The %RSD values of the data obtained are well below 2% indicating that method is robust i.e it is reliable and can be used for routine analysis of the drugs ((Table 12).

System Suitability Parameters

System suitability tests were carried out on freshly prepared standard stock solutions of containing Aspirin and Vonoprazan .System suitability parameters obtained with 20µl injection volumes are summarized in (Table 16).

Specificity

Good resolution was obtained between the drugs and excipients showing complete separation of Aspirin and Vonoprazan. No interference from excipients impurities, or degradation products ensured that the peak response was due to Aspirin and Vonoprazan only. (Table 17 & 18)

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