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Development and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Olanzapine and Samidorphan in Bulk and Pharmaceutical Dosage Form

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Abstract:

Objective: The aim of the study was to develop and validate a stability indicating RP-HPLC Method for simultaneous estimation of Olanzapine and Samidorphan in bulk and formulation.

Materials and Methods: The chromatographic separation was achieved on Agilent C18 columns (4.6 × 150 mm, 5 µm) using a mixture of acetonitrile and 0.01% orthophosphoric acid in a ratio of 70:30 (v/v) as mobile phase. The flow rate was set at 1.0 mL/min and detection was performed at 268 nm. Retention time of Olanzapine and Samidorphan was found to be 2.211 min and 2.772 min respectively.

Results: The method was validated as per ICH Q2B. Linearity was observed in the range 5–30 µg/mL for Olanzapine and 2.5–15 µg/mL for Samidorphan ($R^2 = 0.999$). Average % recovery was found to be 99.83% for Olanzapine and 99.53% for Samidorphan. The %RSD of intra and interday precision studies were found to be < 0.5%. LOD and LOQ values were found to be 0.27 µg/mL and 0.82 µg/mL for Olanzapine, 0.08 µg/mL and 0.24 µg/mL for Samidorphan, respectively. Forced degradation studies under different stress conditions reveal that the method is stability indicating.

Conclusion: The developed and validated rapid, simple, sensitive and specific RP-HPLC method will be useful for routine quality control analysis and stability study of Olanzapine and Samidorphan in bulk drug and marketed formulations.

Keywords: Olanzapine, Samidorphan, RP-HPLC, Stability-Indicating, Method Validation, ICH Guidelines, Pharmaceutical Analysis, Quality Control

1. Introduction

The quality of a drug is crucial to assuring the safety and efficacy of pharmaceutical formulations [1]. To ensure that all pharmaceutical and chemical formulations can be guaranteed as safe and effective, quality assurance and quality control is required. Analytical testing of pure drug substances and their dosage forms is a key component in establishing suitability for patient use. The quality of the analytical data is also dependent on the method, so it is important stake holders develop rugged and robust analytical methods to achieve statutory certification and regulatory compliance [2].

Problematic issues in method development for pharmaceutical drug analysis are very well documented and arise from the range of differences in the nature and properties of drugs [3]. These problem statements require actions to be taken that meet the quality parameters of selectivity, speed, cost effective, simple, sensitive, reproducibility and accuracy [2]. This includes the example of Olanzapine and Samidorphan where co-formulation poses challenges due to numerous structural and functional differences [4]. The method needs to have the capability to precision quantify both compounds concurrently while working around formulation excipients and any degradation products [4].

Consequently, the current study aims to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for the simultaneous estimation of Olanzapine and Samidorphan in bulk and pharmaceutical dosage form [5]. This includes the development of a stability-indicating RP-HPLC method, validated according to ICH guidelines, suitable for regular quality control testing in pharmaceutical industries.

2. Materials and Methods

2.1 Chemicals and Reagents

The following materials and reagents were used in the study: Olanzapine and Samidorphan pure drugs (API), combination tablets (Lybalvi), distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen ortho phosphate buffer, and ortho-phosphoric acid. All chemicals and solvents were of HPLC grade and procured from Rankem [6].

2.2 Instrumentation and Chromatographic Conditions

- **HPLC System**: WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, PDA detector, autosampler, and integrated with Empower 2 Software.
- **UV-VIS Spectrophotometer**: PG Instruments T60 with matched quartz cells (2 mm and 10 mm), integrated with UV Win 6 Software.
- **Column**: Agilent C18 (4.6 × 150 mm, 5 µm)
- **Mobile Phase**: Acetonitrile and 0.01% orthophosphoric acid (70:30 v/v)
- **Flow Rate**: 1.0 mL/min
- **Detection Wavelength (λmax)**: 268 nm
- **Injection Volume**: 10 µL
- **Column Temperature**: 30°C
- **Diluent**: Acetonitrile and water (50:50 v/v)
- **Run Time**: 10 minutes
- **Retention Times**: 2.211 min for Olanzapine and 2.772 min for Samidorphan.

2.3 Method Development Strategy

The method development involved systematic trials with various mobile phases and buffer systems. Initial trials using methanol:water (50:50 v/v) and acetonitrile:water (50:50 v/v) did not produce satisfactory peak resolution. A trial with acetonitrile:0.01N KH_2PO_4 (50:50 v/v) resulted in Olanzapine eluting in the void range. The optimized conditions were achieved using acetonitrile and 0.01% OPA (70:30 v/v), which gave satisfactory resolution and retention times.

2.4 Method Validation (as per ICH Q2B Guidelines)

Linearity

Six concentrations ranging from 5–30 µg/mL for Olanzapine and 2.5–15 µg/mL for Samidorphan were injected in duplicate. Regression equations were:

- **Olanzapine**: $y = 52004x + 6377.4$ ($R^2 = 0.999$)
- **Samidorphan**: $y = 42606x + 1899.9$ ($R^2 = 0.999$).

Accuracy

Accuracy was assessed by standard addition at 50%, 100%, and 150% levels. The mean recovery values were:

- **Olanzapine**: 99.83%
- **Samidorphan**: 99.53%.

Precision

The %RSD values for both intra-day (repeatability) and inter-day (intermediate precision) studies were below 0.5% for both drugs, indicating excellent precision.

Specificity

No interfering peaks were observed in the blank and placebo at the retention times of the analytes, confirming specificity.

Robustness

Deliberate changes in flow rate, mobile phase composition, and temperature did not significantly affect the results. All %RSD values remained <2% under altered conditions, confirming robustness.

LOD and LOQ

- **Olanzapine**: LOD = 0.27 µg/mL, LOQ = 0.82 µg/mL
- **Samidorphan**: LOD = 0.08 µg/mL, LOQ = 0.24 µg/mL.

System Suitability

All system suitability parameters were within ICH-recommended limits:

- Tailing Factor (T) < 2
- Theoretical Plates > 2000
- Resolution (Rs) > 5.0
- %RSD for peak area < 2%.

3. Results and Discussion

3.1 Method Development

Method development was carried out by optimizing various chromatographic conditions including mobile phase composition, column selection, and buffer systems. Multiple trials were performed to obtain sharp, symmetrical peaks with acceptable retention, resolution, and system suitability parameters for both Olanzapine and Samidorphan. The optimized wavelength selected for detection was 268 nm [8].

Table: Method Development Trials

Trial No.	Mobile Phase (v/v)	Column	Observation
Trial 1	Methanol:Water (50:50)	BDS C18 (4.6 × 150 mm, 5 µm)	Peaks not eluted
Trial 2	Acetonitrile:Water (50:50)	BDS C18 (4.6 × 150 mm, 5 µm)	Peaks eluted; baseline disturbance and peak asymmetry observed

Trial 3	Acetonitrile:0.01N KH_2PO_4 (50:50)	Agilent C18 (4.6 × 150 mm, 5 µm)	Peaks eluted; Olanzapine eluted in void range (<2 min)
Optimized	Acetonitrile:0.01% OPA (70:30)	Agilent C18 (4.6 × 150 mm, 5 µm)	Good resolution, acceptable tailing factor and theoretical plates. Retention: 2.211 & 2.772 min

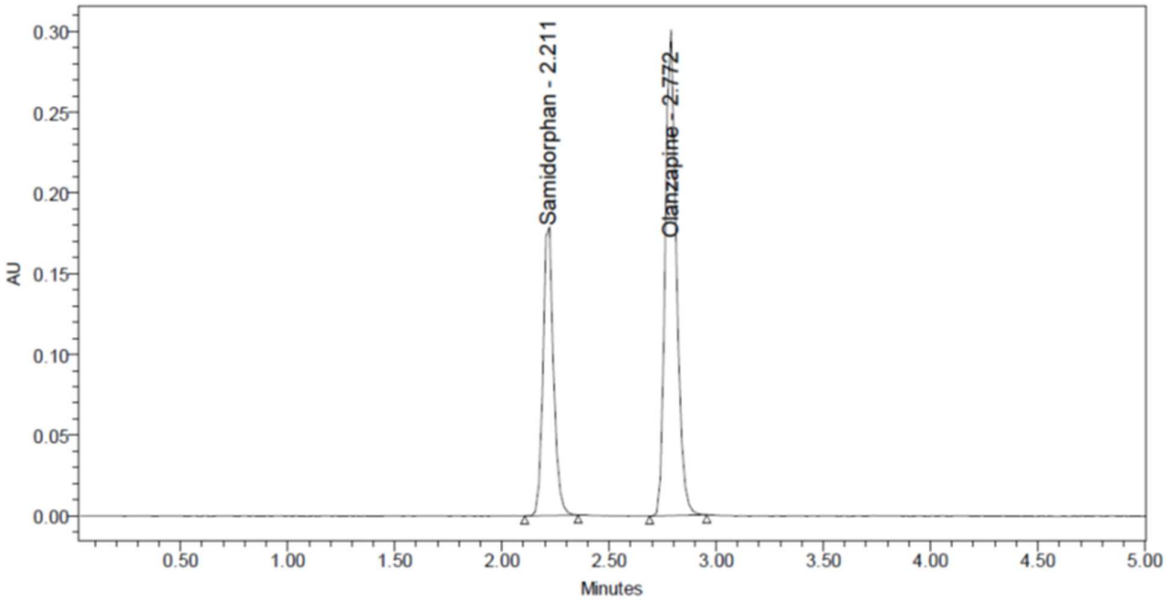

Fig : Optimized Chromatogram

Samidorphan and Olanzapine were eluted at 2.211 min and 2.772 min respectively, with satisfactory resolution, plate count, and tailing factors. Therefore, the method was finalized for validation.

3.2 System Suitability

System suitability tests were performed to confirm the suitability and performance of the chromatographic system before validation. Parameters evaluated include retention time (RT), USP plate count (theoretical plates), tailing factor, and resolution (Rs). All results complied with ICH Q2(R1) guidelines [9].

The retention time for Olanzapine ranged from 2.220 to 2.224 minutes and for Samidorphan from 2.784 to 2.794 minutes. The theoretical plates were consistently above 6000 for Olanzapine and above 13000 for Samidorphan, indicating good column efficiency. Tailing factors were well within the acceptable limit of ≤2.0, showing symmetrical peak shapes. Resolution (Rs) between the two drugs was consistently greater than 5.4, confirming adequate separation.

Table: System Suitability Parameters for Olanzapine and Samidorphan

S. No	Olanzapine			Samidorphan			Resolution (Rs)
	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	

1	2.220	6650	1.08	2.784	13479	1.20	5.4
2	2.220	6939	1.11	2.785	13522	1.19	5.5
3	2.221	6644	1.07	2.787	13391	1.19	5.4
4	2.223	6465	0.99	2.793	13528	1.11	5.5
5	2.224	6551	1.04	2.793	13418	1.11	5.5
6	2.224	6784	1.07	2.794	13699	1.11	5.6

3.3 Specificity

Specificity was established by comparing chromatograms of the blank, placebo, and standard drug solutions [10]. The retention times observed were 2.227 min for Olanzapine and 2.785 min for Samidorphan. No interfering peaks were detected at these retention times in either the blank or placebo chromatograms, indicating that the method is free from interference by excipients or other formulation components.

Hence, the method can be considered specific for the accurate estimation of both analytes in the presence of common excipients.

3.4 Linearity

Linearity was evaluated by preparing six concentrations of **Olanzapine** (5–30 µg/mL) and **Samidorphan** (2.5–15 µg/mL). Each concentration was injected in duplicate, and the corresponding peak areas were recorded. The average peak areas and concentrations are presented in the table below.

Table: Linearity Data for Olanzapine and Samidorphan

Olanzapine Concentration (µg/mL)	**Peak Area**	**Samidorphan Concentration (µg/mL)**	**Peak Area**
0	0	0	0
5	260,511	2.5	106,732
10	532,694	5.0	216,221
15	797,709	7.5	329,357
20	1,040,692	10.0	424,418
25	1,326,774	12.5	532,453
30	1,546,675	15.0	640,921

The calibration curves showed excellent linearity for both drugs. The linear regression equations were:

- **Olanzapine**: $y = 52004x + 6377.4$
- **Samidorphan**: $y = 42606x + 1899.9$

The correlation coefficient (R^2) was found to be **0.999** for both analytes, confirming a strong linear relationship between concentration and peak area.

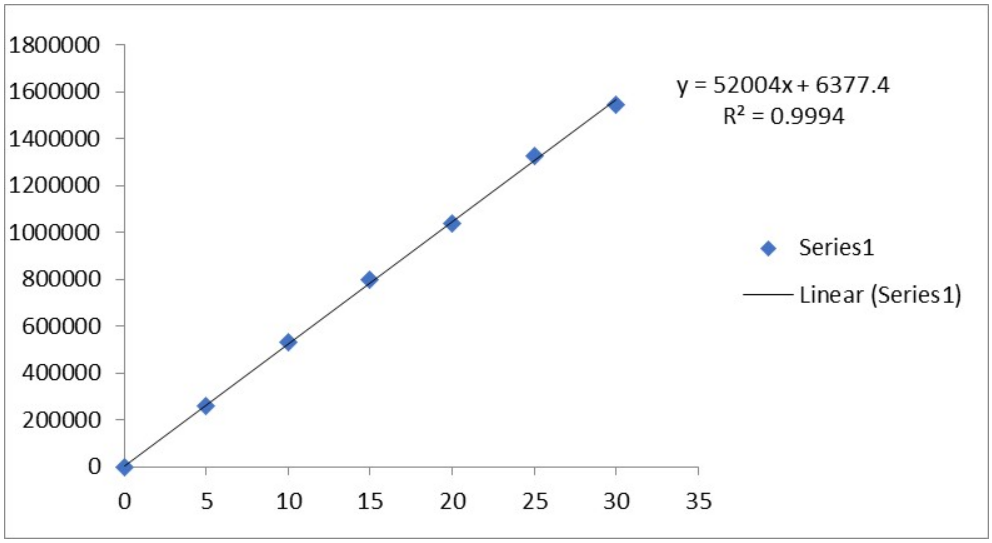

Fig : Calibration curve of Olanzapine

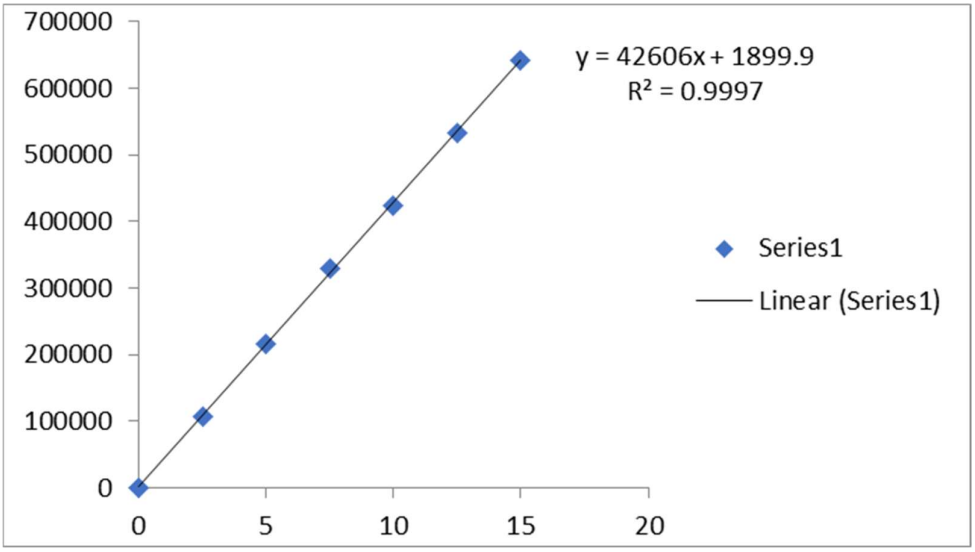

Fig : Calibration curve of Samidorphan

3.5 Precision

Three separate stages evaluated the precision of the method: System Precision, Method Precision (Repeatability), and Intermediate Precision (Day-to-Day Variation). In all cases, six replicate injections were relied upon, and all results are expressed as mean, standard deviation (SD), and percentage relative standard deviation (%RSD). The ICH reports that a %RSD of less than 2.0 is acceptable for precision [11].

System Precision

System precision was determined by injecting six replicates from the same volumetric flask containing the working standard solution. The %RSD values were 0.3%, and 0.2% for Olandzapine and Samidorphan, respectively, indicating very good repeatability of the system.

Method Precision (Repeatability)

Method precision was assessed using six independent working sample solutions prepared from a common stock. Each working sample was injected separately. The %RSD values reported here were 0.4% for Olanzapine and 0.2% for Samidorphan, confirming very good reproducibility of the overall method.

Intermediate Precision (Day-to-Day Precision)

Intermediate precision was completed by assessing the method precision the next day with fresh preparations. The %RSD values reported here were 0.5 for Olanzapine and 0.2% for Samidorphan, confirming that the overall method has been consistent over different days.

Table: Precision Studies for Olanzapine and Samidorphan

Precision Type	Drug	Mean Area	Standard Deviation (SD)	%RSD
System Precision	Olanzapine	1,067,583	3,386.5	0.3
	Samidorphan	430,238	773.3	0.2
Method Precision *(Repeatability)*	Olanzapine	1,059,723	3,809.6	0.4
	Samidorphan	429,970	968.6	0.2
Intermediate Precision *(Day-to-Day)*	Olanzapine	1,038,184	5,460.0	0.5
	Samidorphan	424,473	940.1	0.2

3.6 Accuracy

Accuracy of the method was determined using the standard addition method at three concentration levels: 50%, 100%, and 150% of the target concentration. For each level, three replicate samples were prepared and analyzed. The percentage recovery was calculated for both Olanzapine and Samidorphan, and the mean % recovery at each level was found to be within acceptable limits, confirming the accuracy of the method.

Table: Accuracy Data for Olanzapine and Samidorphan

% Concentration (at Specification Level)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean Recovery
Olanzapine – 50%	10.0	10.0067	99.72–100.65	**99.83%**
Olanzapine – 100%	20.0	20.0133	99.77–100.30	

Olanzapine – 150%	30.0	29.8100	98.76–99.68	
Samidorphan – 50%	5.0	5.0000	99.65–100.69	**99.53%**
Samidorphan – 100%	10.0	9.9533	99.35–99.63	
Samidorphan – 150%	15.0	14.8500	98.93–99.09	

3.7 Sensitivity (LOD & LOQ)

The sensitivity of the method was assessed by determining the **Limit of Detection (LOD)** and **Limit of Quantitation (LOQ)** for both **Olanzapine** and **Samidorphan**. These values were calculated based on the standard deviation of the response and the slope of the calibration curve, as per ICH guidelines.

The results demonstrate that the developed method is highly sensitive, capable of detecting and quantifying even trace amounts of the analytes.

Table: Sensitivity Data for Olanzapine and Samidorphan

Molecule	**LOD (µg/mL)**	**LOQ (µg/mL)**
Olanzapine	0.27	0.82
Samidorphan	0.08	0.24

3.8 Robustness

The robustness of the RP-HPLC method was examined by introducing small purposeful changes in the chromatographic conditions. The parameters that were modified included: flow rate, the composition of the mobile phase and temperature of the column. Each condition was replicated, and these changes enabled us to assess the robustness and reliability of the method to various conditions.

The results demonstrated that for both Olanzapine and Samidorphan, the %RSD values were acceptable (≤2.0%). This confirms that the method is robust and is unaffected by some minor deviations in analytical parameters.

Table : Robustness Data for Olanzapine and Samidorphan

S. No	**Condition**	**%RSD of Olanzapine**	**%RSD of Samidorphan**
1	Flow rate (−) 0.9 mL/min	0.2	0.3
2	Flow rate (+) 1.1 mL/min	0.2	0.9
3	Mobile phase (−) 75B:25A	1.0	0.5

4	Mobile phase (+) 65B:35A	0.6	0.4
5	Temperature (−) 25°C	0.8	0.5
6	Temperature (+) 35°C	0.9	0.9

3.9 Assay

The assay was conducted on the marketed pharmaceutical formulation, Lybalvi, labelled to contain Olanzapine 20 mg and Samidorphan 10 mg per tablet. The standards and sample solutions were prepared and analysed in six replicates under the optimized chromatographic conditions.

The average % assay values for Olanzapine and Samidorphan were:

- Olanzapine: 99.07%
- Samidorphan: 99.74%

These results confirm that the method is accurate and suitable for regular quality control determination of the drug formulation.

Table: Assay Data of Olanzapine and Samidorphan

S. No	**Standard Area**		**Sample Area**		**% Assay**	
	Olanzapine	**Samidorphan**	**Olanzapine**	**Samidorphan**	**Olanzapine**	**Samidorphan**
1	1,069,769	429,897	1,058,799	428,371	98.98	99.37
2	1,066,669	430,788	1,059,483	430,009	99.04	99.75
3	1,069,600	429,977	1,055,214	430,592	98.64	99.88
4	1,069,588	429,590	1,058,369	430,529	98.94	99.87
5	1,068,783	431,546	1,059,715	430,985	99.06	99.97
6	1,061,087	429,628	1,066,758	429,335	99.72	99.59
Average	**1,067,583**	**430,238**	**1,059,723**	**429,970**	**99.07**	**99.74**
SD	3,386.5	773.3	3,809.6	968.6	0.40	0.22
%RSD	0.3	0.2	0.4	0.2	**0.4**	**0.2**

The assay results are within acceptable limits of 98–102% as per regulatory requirements. Low %RSD values for both drugs indicate excellent reproducibility and consistency of the method.

3.10 Forced Degradation Studies

Forced degradation studies for the pharmaceutical formulation were performed to evaluate whether the developed RP-HPLC method was stability-indicating. The stress conditions applied to the degraded formulation included acidic, alkaline, oxidative, thermal, photolytic (UV), and hydrolytic (water) environments. After degradation, the samples were analyzed and the percentage of degraded drug and undegraded drug was determined.

The method showed effective separation of degradation products, as well as the parent drugs with no interference with each other. All samples were in the acceptable range of degradation, which established both the method specificity and stability-indicating ability.

Table: Degradation Data of Olanzapine and Samidorphan

S. No	Degradation Condition	Area		% Drug Degraded		% Drug Undegraded	
		Olanzapine	Samidorphan	Olanzapine	Samidorphan	Olanzapine	Samidorphan
1	Acid	997,037	409,001	93.21	94.87	6.79	5.13
2	Alkali	1,016,956	400,589	95.07	92.92	4.93	7.08
3	Oxidation	1,016,013	412,903	94.98	95.78	5.02	4.22
4	Thermal	1,026,013	420,903	95.91	97.63	4.09	2.37
5	UV	1,046,756	425,717	97.85	98.75	2.15	1.25
6	Water	1,066,110	427,836	99.66	99.24	0.34	0.76

Minor changes in retention times were seen during acid and base degradation studies due to pH changes in the mobile phase. However, the acid samples were neutralized by utilizing 2N NaOH while the alkali samples samples were neutralized using 2N HCl, therefore no significant retention time difference was observed in the final chromatograms. The method was successfully developed to selectively and accurately quantify the analytes in the presence of any degradation products confirming the stability-indicating property of the method.

4. Conclusion

From the experimental results, it's clear that Olanzapine and Samidorphan can be estimated simultaneously by the newly developed RP-HPLC method. The method was found to be simple, rapid, accurate and sensitive and provided better results when compared with other analytical methods. Under optimized chromatographic conditions, both analytes were well separated and were not affected by excipients or degradation products. The method met the validation parameters according to the ICH guidelines, making the method reliable for the simultaneous analysis of Olanzapine and Samidorphan. This method may be used as a method to perform quality assurance and stability testing for olanzapine and samidorphan as bulk drugs and in marketed formulations.

References

1. Sachin Chalge, Vinod Chaware, Sharma T, Dr. Shivshankar D. Mhaske, Sanket Kakade. Quality assurance in pharmaceutical manufacturing : Ensuring consistency and safety. *International Journal of Pharmaceutical Sciences*. 2025;03. doi:https://doi.org/10.5281/zenodo.14690961

2. FDA. Analytical procedures and methods validation for drugs and biologics guidance for industry. Published 2015. https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf

3. Singh N, Vayer P, Tanwar S, Poyet JL, Tsaioun K, Villoutreix BO. Drug discovery and development: introduction to the general public and patient groups. *Frontiers in drug discovery*. 2023;3. doi:https://doi.org/10.3389/fddsv.2023.1201419

4. Haddad HW, Boardman E, Williams B, Mouhaffel R, Kaye AM, Kaye AD. Combination olanzapine and samidorphan for the management of schizophrenia and bipolar 1 disorder in adults: A narrative review. *Health Psychology Research*. 2022;10. doi:https://doi.org/10.52965/001c.34224

5. Krishnaphanisri Ponnekanti, Ramreddy Godela. A validated RP-UPLC method for estimation of Samidorphan and Olanzapine in mixed powder and combined tablets. *Future Journal of Pharmaceutical Sciences*. 2023;9. doi:https://doi.org/10.1186/s43094-023-00562-5

6. Menda J, Chintala V, Prasad KL, Surya P, Raja KP, Kumar KN. Quality by design tool evaluated green stability-indicating UPLC content determination method for the Olanzapine and Samidorphan dosage form. *Microchemical Journal*. 2024;197:109835. doi:https://doi.org/10.1016/j.microc.2023.109835

7. Rácz N, Imre Molnár, Zöldhegyi A, Rieger HJ, Róbert Kormány. Simultaneous optimization of mobile phase composition and pH using retention modeling and experimental design. *Journal of Pharmaceutical and Biomedical Analysis*. 2018;160:336-343. doi:https://doi.org/10.1016/j.jpba.2018.07.054

8. Lakka NS, Kuppan C. Principles of Chromatography Method Development. *Biochemical Analysis Tools - Methods for Bio-molecules Studies*. Published online October 26, 2019. doi:https://doi.org/10.5772/intechopen.89501

9. *ICH Guideline Q2(R2) on Validation of Analytical Procedures*.; 2022. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r2-validation-analytical-procedures-step-2b_en.pdf

10. Bhujbal S, Rupenthal ID, Agarwal P. Development and validation of a stability-indicating HPLC method for assay of tonabersat in pharmaceutical formulations. *Methods*. 2024;231:178-185. doi:https://doi.org/10.1016/j.ymeth.2024.10.001

11. Belouafa S, Habti F, Benhar S, et al. Statistical tools and approaches to validate analytical methods: methodology and practical examples★. *International Journal of Metrology and Quality Engineering*. 2017;8(9):9. doi:https://doi.org/10.1051/ijmqe/2016030