World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2025
Volume: 4; Issue: 3
Page: 1358-1366

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN, VILDAGLIPTIN, AND METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Article Received: 09 May 2025 | | Article Revised: 30 May 2025 | | Article Accepted: 23 June 2025

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DOI: https://doi.org/10.5281/zenodo.15775227

How to cite this Article: K. Gayathri Devi, Dr. B. V. Narasimha Rao, A. Udaya Krishna Veni, Ch. Naga Phani, K. Bhuvaneswari, N. Yahoshuva, S. Ramya, S. G. Sampath Rama Raju (2025) METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN, VILDAGLIPTIN, AND METFORMIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC. World Journal of Pharmaceutical Science and Research, 4(3), 1358-1366. https://doi.org/10.5281/zenodo.15775227

ABSTRACT

In the present study, a reverse phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of Dapagliflozin, Vildagliptin and Metformin in pharmaceutical formulations. To assess the effect of method parameters on chromatographic separation of the Dapagliflozin, Vildagliptin and Metformin statistically designed experiments were performed by varying different method parameters such as buffer concentration, pH of mobile phase, flow rate, and column temperature. The separation was performed on Prontosil ODS C18 Column (250 x 4.6 mm and 5µm) at room temperature using Methanol: acetonitrile and pH 5.6 phosphate buffer in the ratio of 55:25:20 (v/v) in isocratic condition at a flow rate of 1.0 mL/min. The detection was performed by a photo diode array (PDA) detector at 223 nm with total run time of 15 min. Calibration curves were linear in the concentration range of 2.0-12.0 µg/mL for Dapagliflozin, 20-120 µg/mL for Vildagliptin and 100-600µg/mL for Metformin. The LOD was noticed to be 0.15 µg/mL, 1.5 µg/mL and 7.5 µg/mL whereas the LOQ was calculated as 0.5 µg/mL, 5 µg/mL and 25 µg/mL respectively for Dapagliflozin, Vildagliptin and Metformin proves the sensitivity of the developed method. The %RSD was below 2 which confirms the ruggedness and robustness of the method. The % assay in formulation analysis was found to be 98.90, 98.40 and 99.67 for Dapagliflozin, Vildagliptin and Metformin respectively. Hence, the method was used for the routine analysis of Dapagliflozin, Vildagliptin and Metformin in bulk drug as well as in pharmaceutical formulations.

KEYWORDS: Dapagliflozin, Vildagliptin, Metformin, ruggedness and robustness, etc.

INTRODUCTION

SGLT2, a sodium-glucose cotransporter mostly found in the proximal tubule of the nephron, is inhibited by dapagliflozin. Since 90% of glucose reabsorption in the kidneys is facilitated by SGLT2, its blockage permits glucose excretion in the urine. Patients with type 2 diabetes mellitus can potentially lose weight and improve their glycemic control thanks to this excretion.[1-3] By specifically blocking dipeptidyl peptidase-4 (DPP-4), an enzyme that quickly truncates and inactivates GLP-1 and GIP following their release from the intestinal cells, vildagliptin lowers blood glucose. Following the second amino acid from the N-terminal end, DPP-4 cleaves oligopeptides. By significantly extending the half-life of GLP-1 and GIP, inhibition of DPP-4 raises the amounts of active incretin hormones in the blood.[4,5] Metformin decreases blood glucose levels by decreasing hepatic glucose production (also called gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral glucose uptake and utilization.[6] Till now, there has been no analytical method has been developed for the simultaneous estimation of these three combinations of drugs in bulk and pharmaceutical formulation.

MATEIALS AND METHODS

Chemicals and Reagents

The working standard drug Dapagliflozin (98.75% purity), Vildagliptin (98.43% purity) and Metformin (98.50% purity) were obtained from Hetero Labs, Hyderabad, Telangana. The formulation dosage form having brand name Dopadax VGM containing 10 mg of Dapagliflozin, 100 mg of Vildagliptin and 500 mg of Metformin were purchased from local Pharmacy. HPLC grade Methanol, Water and Acetonitrile were purchased form Merk chemicals private limited, Mumbai. The buffer solutions used for the study were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of standard drug solution

An accurately weighed 100mg of Dapagliflozin standard drug was taken in a 100 ml volumetric flask. Then it was dissolved completely in 50 ml Methanol using ultrasonic Sonicator. The final volume in the volumetric flask was made up to the mark using same solvent and then the solution was filtered using 0.2 µ nylon membrane filter paper. Standard stock solution having 1000 µg/mL was obtained. The concentrations required in method development and validation parameters were prepared from the stock (1000 µg/mL) solution of Dapagliflozin. The same procedure was used for the preparation of Vildagliptin and Metformin solution. The combined solution having Dapagliflozin (10µg/mL), Vildagliptin (100µg/mL) and Metformin (500µg/mL) was prepared by mixing equal known volume of individual drug solution of known concentration. The combined drug solution was used for the separation and simultaneous quantification of Dapagliflozin, Vildagliptin and Metformin using HPLC method.

Preparation of formulation solution

20 Dapadax VGM containing 10 mg of Dapagliflozin, 100 mg of Vildagliptin and 500 mg of Metformin tablets were ground to fine uniform powder using clean, dry mortar and pestle. Tablet powder equivalent to 10 mg Dapagliflozin was weighed accurately and was taken in an air tight 100 mL volumetric flask. The tablet powder was dissolved in 10 mL Methanol, it was filtered using 0.2 µ nylon membrane filter paper and final volume was made up to 100 ml. The Dapagliflozin sample stock solution at a concentration of 100 µg/mL was obtained. Working sample solution of Dapagliflozin at a concentration of 10 µg/mL was prepared by accurately measuring 1mL sample stock solution and made up to 10 mL in a volumetric flask. Based on the formulation dosage, the solution contains 100µg/mL of

vildagliptin and 500 µg/mL of Metformin. The same solution was used for formulation analysis in the developed method.

METHOD DEVELOPMENT

Selection of Wavelength

To select a suitable wavelength, the standard solutions of 10 µg /mL was prepared and scanned in the UV-Vis spectrophotometer. The obtained wavelength maximum was selected as suitable wavelength for the detection.

Optimized Chromatographic Conditions

Parameter	Condition
Mobile Phase	Methanol: acetonitrile and phosphate buffer pH 5.6 in the ratio of 55:25:20 (v/v)
Column	Prontosil ODS C18 Column (250 x 4.6 mm and 5µm)
Flow Rate	1.0 ml/min
Wavelength	223nm
Injection Volume	20 µL
Temperature	Ambient
Run time	15min

METHOD VALIDATION

The method was validated with respect to specificity, system suitability, LOD & LOQ, linearity, accuracy, precision, ruggedness and robustness according to the ICH guidelines. Validation studies were carried out by replicate injections of the sample and standard solutions into the column.[7,8]

Specificity

Specificity of the method was checked by injecting the solution into the chromatograph. Specificity of the method was assessed by comparing the chromatogram of Dapagliflozin, Vildagliptin and Metformin individually, combined standard, blank and formulation solutions to those obtained for tablet solutions. Retention time of the Dapagliflozin, Vildagliptin and Metformin in individual, standard solution, and in the sample, solution was compared to determine the specificity of the method.

System suitability

The system suitability was determined by making six replicate injections of the standard solution and analyzing Dapagliflozin, Vildagliptin and Metformin for its peak area, peak USP tailing factor, number of theoretical plates and resolution. The proposed accepted criteria are not more than 2% for RSD%, not less than 2 for resolution, not more than 2 for USP tailing factor, and not less than 2000 for the number of theoretical plates.

LOD & LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were defined as the lowest concentration of analyte in a sample that can be detected and quantified. The standard solutions of Dapagliflozin, Vildagliptin and Metformin for evaluating LOD and LOQ were prepared by diluting them with suitable solvent. The LOD and LOQ were determined by the signal-to-noise (S/N) ratio for each compound through analyzing a series of diluted solutions until the S/N ratio yield 3 for LOD and 10 for LOQ, respectively.

Linearity

The calibration curve in the developed method was constructed from LOQ concentration. The Dapagliflozin, Vildagliptin and Metformin standard stock solution of 1 mg/mL was used for preparation of subsequent aliquots. Various aliquots were prepared by serial dilution and the solutions were loaded and 20µL was injected into column. All measurements were repeated for each concentration. The calibration curve of the area under curve versus concentration was recorded individually for Dapagliflozin, Vildagliptin and Metformin against its analyte strength. Form the calibration curve, correlation and regression values were calculated for Dapagliflozin, Vildagliptin and Metformin individually.

Precision

The precision studies were carried out by estimating response of Dapagliflozin, Vildagliptin and Metformin six times at a standard concentration and results are reported in terms of % RSD. The intra-day and inter-day precision studies were carried out by estimating the corresponding responses six times on same day for intraday and intraday for three different days and it was expressed as the percentage relative standard deviation (%RSD) which was calculated as per the following expression

%RSD = (standard deviation / mean) x 100.

Accuracy

Accuracy of method was observed by recovery result from two placebos preparations accurately spiked with different concentration of Dapagliflozin, Vildagliptin and Metformin. Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre a nalyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated by using the formula.

%RSD = (standard deviation / mean) x 100.

Ruggedness

Two laboratory analysts carried out the precision of Dapagliflozin, Vildagliptin and Metformin at a standard concentration by different analysts in the laboratory conditions, the prepared solution were analyzed in the optimized conditions. Peak area that obtained was used for the determination of ruggedness of the method. Ruggedness was expressed in terms of % RSD which must be less than 2.

Robustness

Robustness of the proposed method included six deliberate variations to some chromatographic parameters. The modifications include different mobile phase ratios and different detector wavelengths and different percentage in the mobile phase (in the range of ± 5 of the nominal value and the normal %). The % change in each of the changed condition was calculated.

Assay

This proposed method was applied to the determination of Dapagliflozin, Vildagliptin and Metformin in commercially combined tablets. The sample solution prepared was analyzed in the optimized conditions. Peak area of the resultant chromatogram was used for the estimation of assay using label clime recovery method. The % assay was calculated for Dapagliflozin, Vildagliptin and Metformin using its corresponding standard calibration values.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT

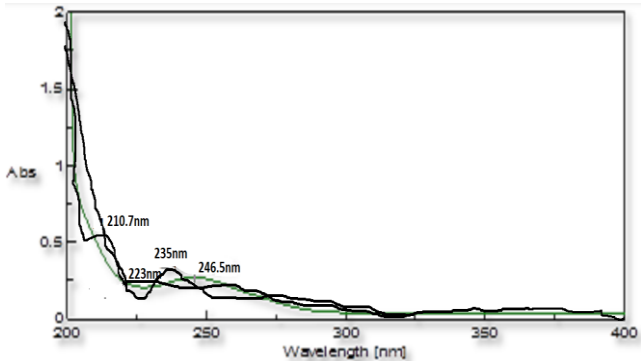

Figure 1: Overlay Spectra of Vidagliptin, Metformin and Dapagliflozin.

Table 1: Results for Optimized Chromatogram.

S.NO	Drug	Retention Time (min)	Resolution	Theoretical Plates	Tailing Factor
1	Dapagliflozin	10.57	8.71	9858	1.06
2	Vildagliptin	5.83	-	5281	1.04
3	Metformin	8.41	6.28	6957	0.95

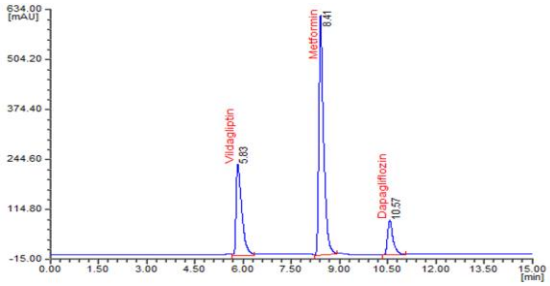

Figure 2: Optimized Chromatogram of Vidagliptin, Metformin and Dapagliflozin.

METHOD VALIDATION

Specificity & System suitability

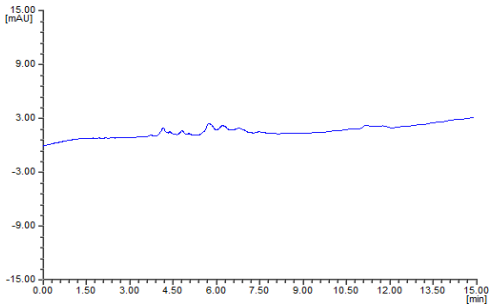

Figure 3: Chromatogram of Blank.

LOD & LOQ

The LOD was noticed to be 0.15 µg/mL, 1.5 µg/mL and 7.5 µg/mL respectively for whereas the LOQ was calculated as 0.5 µg/mL, 5 µg/mL and 25 µg/mL respectively for Dapagliflozin, Vildagliptin and Metformin. Results confirmed that the method was sensitive and can be useful for the detection and analysis of drugs at very lowest concentrations.

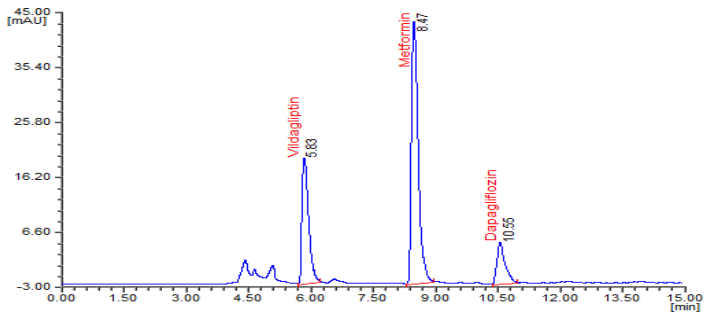

Figure 4: Chromatogram of LOD.

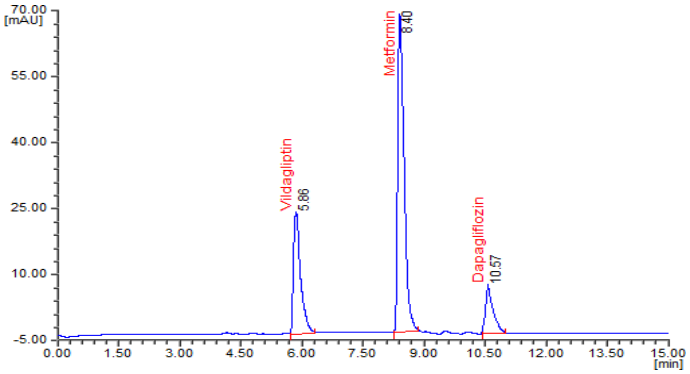

Figure 5: Chromatogram of LOQ.

Linearity

Table 2: Results for Linearity

S. No	Level	Dapagliflozin		Vildagliptin		Metformin	
		Concentration in µg/mL	Peak Area	Concentration in µg/mL	Peak Area	Concentration in µg/mL	Peak Area
1	Level 1	02	43585.7	20	93186.3	100	149985.3
2	Level 2	04	86514.9	40	189585.2	200	309810.9
3	Level 3	06	129986.1	60	297694.1	300	469051.5
4	Level 4	08	169874.2	80	400241.9	400	619898.8
5	Level 5	10	230118.3	100	509746.7	500	759974.1
6	Level 6	12	269491.7	120	620515.8	600	927968.7

Linearity was observed in the concentration range of 2-12 µg/mL for Dapagliflozin, 20-120 µg/mL for Vildagliptin and 100-600 µg/mL for Metformin with regression equation of y = 46368x - 3491.2 (R^2 = 0.9994), y = 10529x – 13872 (R^2 = 0.9995) and y = 3109.1x - 229.77 (R^2 = 0.9998) respectively for Dapagliflozin, Vildagliptin and Metformin.

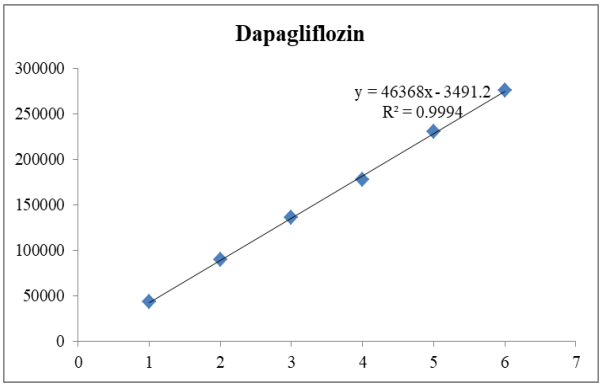

Figure 6: Linearity graph for Dapagliflozin.

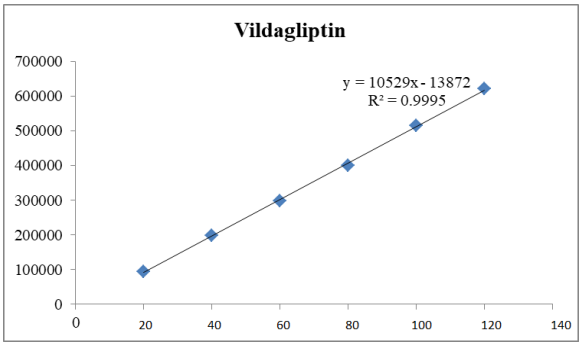

Figure 7: Linearity graph for Vildagliptin.

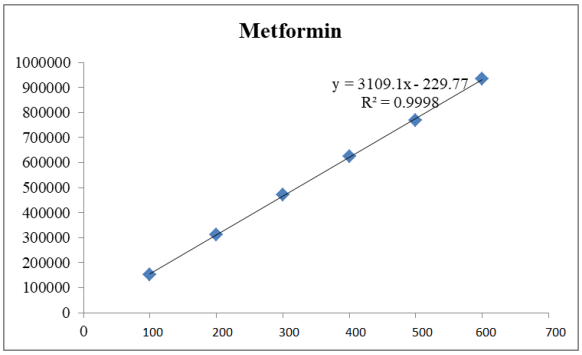

Figure 8: Linearity graph for Metformin.

Precision

The % RSD was found to be 0.33, 1.03 and 0.19 respectively for Dapagliflozin, Vildagliptin and Metformin in intraday precision whereas 0.37, 0.89 and 0.34 respectively for Dapagliflozin, Vildagliptin and Metformin in intraday precision. The % RSD was found to be with in the acceptance limit of less than 2 for the three analytes in both intraday and interday precision. Hence the developed method was found to be precise.

Table 3: Results for Intraday Precision.

S. No	Peak area response		
	Dapagliflozin	Vildagliptin	Metformin
1	178598.8	399582.4	620251.3
2	177154.6	395487.1	621585.9
3	177520.3	396956.8	623451.5
4	177959.3	405857.4	622159.4
5	177858.2	404143.5	621514.3
6	177006.5	402989.3	620508.9
% RSD	**0.33**	**1.03**	**0.19**

Table 4: Results for Interday Precision.

S. No	Peak area response		
	Dapagliflozin	Vildagliptin	Metformin
1	178776.7	399901.0	621489.1
2	177312.6	394610.2	621582.0
3	177875.1	395365.7	624115.4
4	177440.8	404113.6	619038.7
5	177303.1	400996.4	623309.3
6	176897.5	399366.9	618893.1
% RSD	**0.37**	**0.89**	**0.34**

Accuracy

The % recovery was fond to be within the range of 98.15 - 100.25, 98.37 - 99.63 and 98.41 - 99.46 for Dapagliflozin, Vildagliptin and Metformin respectively. The % RSD in each spiked level was found to be with in the acceptable level for Dapagliflozin, Vildagliptin and Metformin in 50 %, 100 % and 150 % spiked level. The results found to be with in the acceptance limit of 98-102 and % RSD of <2 which sense to conformation that the proposed method was accurate.

Ruggedness

Ruggedness was expressed in terms of % RSD which must be less than 2. The % RSD was found to be 0.41, 1.21 and 0.17 respectively for Dapagliflozin, Vildagliptin and Metformin in the developed method. Results found to be with in the acceptance limit confirms that the method is rugged.

Robustness

% Change was found to be in the range of within the acceptable limit of less than 2 for Dapagliflozin, Vildagliptin and Metformin in the developed method. This conforms that the small change in the analytical conditions doesn't influence the results and hence the proposed method was found to be suitable for the analysis of Dapagliflozin, Vildagliptin and Metformin when small change in the analytical conditions.

Table 5: Results for Robustness

S. No	Condition	Dapagliflozin		Vildagliptin		Metformin	
		Area	% Change	Area	% Change	Area	% Change
1	Standard	177974.2	--	400251.9	--	623521.8	--
2	MP+ **(60:20:20)**	178776.7	0.45	397666.2	0.65	621489.1	0.33
3	MP- **(50:25:25)**	177312.6	0.37	394610.2	1.41	621582.0	0.31
4	WL (+5nm) 228	177875.1	0.06	395365.7	1.22	624115.4	0.10
5	WL (-5nm) 218	177440.8	0.30	403992.0	0.93	619038.7	0.72
6	pH+ 5.7	177303.1	0.38	402568.8	0.58	623309.3	0.03
7	pH- 5.8	176897.5	0.60	399769.3	0.12	618893.1	0.74

Assay

The % assay in formulation analysis was found to be 98.90, 98.40 and 99.67 for Dapagliflozin, Vildagliptin and Metformin respectively. More than 98% assay was observed in the developed method for Dapagliflozin, Vildagliptin and Metformin. Hence the method was found to be suitable for the routine analysis of Dapagliflozin, Vildagliptin and Metformin in bulk drug as well as formulations.

Table 6: Results for Formulation.

S. No	Drug	Brand	Label claim	Concentration Prepared	Concentration Found	% Assay
1	Dapagliflozin	Dopadax VGM	10 mg	10µg/mL	9.89 µg/Ml	98.90
2	Vildagliptin		100 mg	100 µg/mL	98.40 µg/mL	98.40
3	Metformin		500 mg	500 µg/mL	498.37 µg/mL	99.67

CONCLUSION

Three anti-diabetic medications—Dapagliflozin, Vildagliptin, and Metformin—were separated and their simultaneous quantification in bulk drug and pharmaceutical formulations was accomplished using the straightforward, sensitive, and affordable isocratic RP-HPLC method. The ICH guidelines were followed in the effective development and validation of the approach. The validation results demonstrated the specificity, sensitivity, linearity, accuracy, precision, and

robustness of this approach. The developed approach is specific for determining the formulation of Dapagliflozin, Vildagliptin, and Metformin tablets and does not have any other co-eluting peaks with the primary peaks. In routine analysis, the comparatively short run time (15 min) allows for the quick measurement of numerous samples. Thus, it can be said that the established RP-HPLC method may be successfully used to quantify the dose forms of metformin, dapagliflozin, and vildagliptin in tablets.

REFERENCES

1. "Farxiga- dapagliflozin tablet, film coated". DailyMed. National Institutes of Health, National Library of Medicine, U.S. Department of Health & Human Services. 3 February 2020. Archived from the original on 30 October 2020. Retrieved 5 May 2020
2. https://go.drugbank.com/drugs/DB06292
3. The EMPA-KIDNEY Collaborative Group (12 January 2023). "Empagliflozin in Patients with Chronic Kidney Disease". New England Journal of Medicine. 388 (2): 117–127. doi:10.1056/NEJMoa2204233. ISSN 0028-4793. PMC 7614055. PMID 36331190.
4. Ahren B: DPP-4 inhibitors. Best Pract Res Clin Endocrinol Metab. 2007 Dec;21(4):517-33. doi: 10.1016/j.beem.2007.07.005. [Article]
5. "Galvus EPAR". European Medicines Agency (EMA). 26 September 2007. Retrieved 20 February 2025.
6. "Metformin Hydrochloride". The American Society of Health-System Pharmacists. Archived from the original on 24 December 2016. Retrieved 2 January 2017.
7. Chowdary K.P.R., Devala G, Himabindo G., Validation of analytical methods. Eastern Pharmacist, 1999, 39-41.
8. Validation of analytical procedures methodology ICH harmonized tripartite guide lines 1996, 1-8.