International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 8s, 2025
https://www.theaspd.com/ijes.php

"Development And Validation Of An Rp-Hplc Method For Estimating Process-Related Impurities In Antidiabetic Drugs"

Sachin K. Hodgar[1*], Sumit R.Deore[2]

[1*,2]*School of Pharmaceutical Sciences, Sandip University, Nashik, Maharashtra, India.*
**Corresponding Author: Sachin K. Hodgar*
**Email Id-skh.vniprc@gmail.com,deoresumit@gmail.com*

ABSTRACT:
There has been ever increasing interest in impurities present in Active Pharmaceutical Ingredient's (API's). Nowadays, not only purity profile but also impurity profile has become mandatory according to the various regulatory authorities. The study aimed to develop and validate a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of process-related impurities in Dapagliflozin, Vildagliptin, and Sitagliptin. The method was optimized using various mobile phases and validated according to ICH guidelines for parameters including linearity, accuracy, precision, robustness, LOD, and LOQ. The optimized method employed an Agilent Poroshell C18 column (150 mm × 4.6 mm, 5 µm), with a mobile phase of acetonitrile and 0.05% orthophosphoric acid in water (30:70, v/v), at a flow rate of 1.0 mL/min, and detection at 210 nm. The method exhibited good linearity ($r^2 > 0.998$), accuracy (recovery within 98–102%), and precision (RSD < 2%). The method is suitable for routine quality control of impurities in antidiabetic formulations.
Regulatory bodies across the globe have established comprehensive guidelines to ensure that impurity profiling is rigorously conducted during the development and manufacturing of pharmaceuticals. These guidelines are designed to protect public health by setting acceptable limits for impurities and mandating robust analytical methods for their detection and quantification. Thus impurity profiling is a cornerstone of pharmaceutical development, ensuring that drug products are safe, effective, and of high quality. Regulatory guidelines such as FDA (Food and Drug Administration, USA), EMA (European Medicines Agency), Pharmacopoeias etc. The presence of impurities can accelerate the degradation of drug products, thereby reducing their shelf life and stability. Stability studies are conducted to understand how impurities develop over time and under various storage conditions, which is essential for establishing appropriate storage guidelines and expiration dates.

Keywords- *FDA, EMA, API, shelf life.*

INTRODUCTION

There has been ever increasing interest in impurities present in Active Pharmaceutical Ingredient's (API's). Nowadays, not only purity profile but also impurity profile has become mandatory according to the various regulatory authorities. In the pharmaceutical world, an impurity is considered as an inorganic or organic material, or residual solvents other than the drug substances, or ingredients, arising out of synthesis or unwanted chemicals that remain with APIs. Impurity profiling includes identification, structure elucidation and quantitative determination of impurities and degradation products in bulk drug materials and pharmaceutical formulation. The control of impurities in Formulated products and API's were regulated by various regulatory authorities like ICH, USFDA, Canadian Drug, and Health Agency. Impurity profiling is very important in the modern pharmaceutical analysis due to the fact that unidentified, potentially toxic impurities are hazardous to health and in order to increase the safety of drug therapy, impurities should be identified and determined by the selective method. Nowadays, it is a mandatory requirement in various pharmacopeias to know the impurities present in APIs and finished drug products. Thus, impurity profiling can act as a Quality Control tool.

It can provide crucial data regarding the toxicity, safety, various limits of detection and limits of quantitation of several organic and inorganic impurities, usually accompany with APIs and finished products. There is a strong requirement to have unique specifications/standards with regard to impurities. The major aim of bulk drug industries and pharmaceutical industries is to produce the best quality product. As drugs are meant for saving lives and even minute quantities of impurities are unacceptable. Hence, impurity profiling has become very important. Impurity can be defined as any substance that exists with the original drug; it can be starting material

or intermediates that are formed due to any side reactions. Our aim is to provide details regarding impurity and its profiling, which is very important in the Pharmaceutical sector. As per the, International Conference on Harmonization (ICH), "any component of the drug product that is not the chemical entity defined as the drug substance or an excipients in the drug product is considered an impurity. During the manufacturing of drugs, the Active Pharmaceutical Ingredients (APIs) and excipients are rarely 100% pure. This lack of purity is due to the presence of various components introduced from sources such as the synthesis process, excipients themselves, residual solvents, or degradation products. These unintended and undesirable substances are collectively known as impurities. In any pharmaceutical product or drug substance, when impurities are expected to be present, it is essential to identify and characterize them using appropriate analytical methods—a process known as impurity profiling. Impurity profiling is a systematic approach designed to identify, isolate, and elucidate the structure of unknown impurities. This process is crucial for accurately determining and quantifying the impurities within a medicinal substance. Impurity profiling is a critical aspect of pharmaceutical quality control and drug development, ensuring that medications are safe, effective, and of high quality. Impurities in pharmaceutical formulations can arise from various sources during the manufacturing process, including raw materials, intermediates, manufacturing processes, degradation, and packaging. These impurities, even in trace amounts, can significantly impact the safety, efficacy, and stability of the final drug product. It ensures that these impurities remain within acceptable limits, preventing any toxicological effects on the human body. Therefore, producing high-quality and efficacious medicinal products requires stringent impurity reporting.

It is a crucial aspect of pharmaceutical development and quality control. It involves identifying, quantifying, and controlling the presence of impurities in drug substances and products to ensure their safety, efficacy, and quality. Some impurities can be toxic, mutagenic, or carcinogenic, posing serious health risks to patients. For instance, certain degradation products might form during the shelf life of a drug, potentially interacting with biological systems in unpredictable and harmful ways. Identifying and controlling these impurities is crucial to prevent adverse health outcomes Impurities can interfere with the therapeutic activity of the drug's API, leading to reduced efficacy. This is particularly concerning in drugs with a narrow therapeutic index, where even small variations in drug concentration can lead to suboptimal treatment outcomes or adverse effects.

MATERIALS and METHOD

DRUG-

Dapagliflozin, Vildagliptin and Sitagliptin
Methanol, Water
Method Development by RP – HPLC [37]

PRELIMINARY CHARACTERIZATION OF DRUG

Color, odor and appearance

Dapagliflozin, Vildagliptin and Sitagliptin was evaluated for parameters like color; odor & appearance are shown in result. [35]

Determination of solubility

The solubility was determined in Water & Methanol at a concentration of 3 mg/mL as follows and is given in results.
Water: Weighed approx. 30 mg of Dapagliflozin, Vildagliptin and Sitagliptin and sonicated for 5-10 minutes to dissolve in 10 ml of Water.
Methanol: Weighed approx. 30 mg of Dapagliflozin, Vildagliptin and Sitagliptin and sonicated for 5-10 minutes to dissolve in 10 ml of Methanol. [36]

Selection of analytical wavelength

Selection of solvent
Methanol was selected as the solvent for dissolving Dapagliflozin, Vildagliptin and Sitagliptin.

Preparation of standard stock solutions
Vildagliptin: In order to prepare stock solution, weighed accurately 10 mg Vildagliptin and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM).

Solutions for UV scan:
Further diluted 1 mL to 25 mL with methanol. (20 PPM)
Dapagliflozin: In order to prepare stock solution, weighed accurately 10 mg dapagliflozin and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM).

Solutions for UV scan:
Further diluted 1 mL to 25 mL with methanol. (20 PPM)
Sitagliptin: In order to prepare stock solution, weighed accurately 10 mg sitagliptin and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (500 PPM).

Solution for UV scan:
Further diluted 1 mL to 25 mL with methanol. (20 PPM)
Selection of analytical wavelength [3]
Methanol as a blank Dapagliflozin, Vildagliptin and Sitagliptin standard solution (20 PPM each) was scanned from 400 nm to 200 nm. An absorption maximum was determined for both drugs. Dapagliflozin, Vildagliptin and Sitagliptin showed Q-point at 210 nm shown in results.

Method Development by RP – HPLC [37]
Preparation of standard stock solution for Chromatographic development:
Vildagliptin: Weighed 20 mg Vildagliptin and transferred into a 20 mL of clean and dried volumetric flask, added about 15 mL of methanol, sonicated to dissolve it completely and made volume up to the mark with methanol (1000 PPM).
Further diluted 1 ml of stock solution to 10 mL with mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.
Dapagliflozin: Weighed 20 mg of Pioglitazone and transferred into a 20 mL of clean and dried volumetric flask, added about 15 mL of methanol, sonicated to dissolve it completely and made volume up to the mark with methanol (1000 PPM).
Further diluted 1 ml of stock solution to 10 mL with mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.
Sitagliptin: Weighed 20 mg of Pioglitazone and transferred into a 20 mL of clean and dried volumetric flask, added about 15 mL of methanol, sonicated to dissolve it completely and made volume up to the mark with methanol (1000 PPM).
Further diluted 1 ml of stock solution to 10 mL with mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.
Selection of analytical wavelength for HPLC method development: Analytical wavelength for the examination was selected from the Q-point from the spectrophotometric analysis and it was 210 nm.

Optimization of HPLC method
Following trials are taken for estimation of Dapagliflozin, Vildagliptin and Sitagliptin. [38]
Principle: Reversed Phase Liquid Chromatography with Isocratic elution and UV detection.
Trial 1:

Chromatographic Conditions:
Standard solution: Dapagliflozin 100PPM, Vildagliptin 100PPM and Sitagliptin 100 PPM
Detector: U.V. Detector
Column: Agilent, Poroshell C18,
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20µl
Wavelength: 210 nm
Mobile phase: Methanol: water (70:30)
Flow Rate: 1.0 ml/min
Observation: The trial 1 results are shown in results.

Trial 2:
Chromatographic Conditions:
Standard solution: Dapagliflozin 100PPM, Vildagliptin 100PPM and Sitagliptin 100 PPM
Detector: U.V. Detector
Column: Agilent, Poroshell C18,
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20µl
Wavelength: 210 nm
Mobile phase: Acetonitrile: Water (70:30)
Flow Rate: 1.0 ml/min
Observation: The trial 2 results are shown in results.

Trial 3:
Chromatographic Conditions:
Standard solution: Dapagliflozin 100PPM, Vildagliptin 100PPM and Sitagliptin 100 PPM
Detector: U.V. Detector
Column: Agilent, Poroshell C18,
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20µl
Wavelength: 210 nm
Mobile phase: Acetonitrile: Water (50:50)
Flow Rate: 1.0 ml/min
Observation: The trial 3 results are shown in results.

Trial 4:
Chromatographic Conditions:
Standard solution: Dapagliflozin 100PPM, Vildagliptin 100PPM and Sitagliptin 100 PPM
Detector: U.V. Detector
Column: Agilent, Poroshell C18,
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20µl
Wavelength: 210 nm
Mobile phase: Acetonitrile: 0.05% OPA in Water (70:30)
Flow Rate: 1.0 ml/min
Observation: The trial 4 results are shown in results.
Trial 5:

Chromatographic Conditions:
Standard solution: Dapagliflozin 100PPM, Vildagliptin 100PPM and Sitagliptin 100 PPM
Detector: U.V. Detector
Column: Agilent, Poroshell C18,
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20µl
Wavelength: 210 nm
Mobile phase: Acetonitrile: 0.05% OPA in Water (50:50)
Flow Rate: 1.0 ml/min
Observation: The trial 5 results are shown in results.

Trial 6:
Chromatographic Conditions:
Standard solution: Dapagliflozin 100PPM, Vildagliptin 100PPM and Sitagliptin 100 PPM
Detector: U.V. Detector
Column: Agilent, Poroshell C18,
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20µl
Wavelength: 210 nm
Mobile phase: Acetonitrile: 0.1% OPA in Water (30:70)
Flow Rate: 1.0 ml/min
Observation: The trial 6 results are shown in results
Optimized Chromatographic condition: Trial no. 6 considered as optimized chromatography which is as follows:

Chromatographic Conditions:
Detector: U.V. Detector
Column: Agilent, Poroshell C18,
Column Dimension: (150 mm X 4.6 mm i.d.) 5µm
Column Oven temperature: 40°C
Injection Volume: 20µl
Wavelength: 210 nm
Mobile phase: Acetonitrile: 0.05% OPA in water (30:70)
Flow Rate: 1.0 ml/min.

4.4.2. Preparation of System suitability stock solutions:
Vildagliptin: Weighed 20 mg of Vildagliptin and transferred in 20 mL volumetric flask, added 15 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol. (1000 PPM)
Dapagliflozin: Weighed 15 mg of dapagliflozin and transferred in 50 mL volumetric flask, added 35 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol. (300 PPM)
Sitagliptin: Weighed 15 mg of sitagliptin and transferred in 50 mL volumetric flask, added 35 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol. (300 PPM)
System suitability standard mixture solution:

Pipette out 1.0 mL of Vildagliptin standard stock solution, 1.0 mL of dapagliflozin and 1.0 ml of sitagliptin standard stock solution and transferred in 20 mL volumetric flask, made volume up to the mark with mobile phase. (Vildagliptin = 50 PPM Dapagliflozin = 15 PPM & Sitagliptin = 15 PPM)
50 PPM of Vildagliptin, 15 PPM of dapagliflozin and sitagliptin are the working concentration.

Marketed formulation contains Sitagliptin and Dapagliflozin (15 mg) and Vildagliptin (50 mg).

System suitability is a Pharmacopeial requirement and is used to verify, whether the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from five replicate injection of standard drug solution and the results are recorded.

Acceptance criteria [39, 40, 41]
1. RSD should not be more than 2.0 % for five replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.

EXPERIMENTAL DATA:
The developed method for estimation of Dapagliflozin, Vildagliptin and Sitagliptin was validated as per ICH guidelines for following parameters.
Placebo Sample solution preparation:
Weighed 221.4 mg of placebo material (Which is equivalent to 50 mg of Vildagliptin , 15 mg of Sitagliptin and 15 mg of Dapagliflozin) and transferred to clean and dried 100 mL of volumetric flask. Added 70 mL of methanol, sonicated for 15 minutes with intermittent shaking. After 15 minutes allow cooling the solution to room temperature and made volume up to the mark with methanol. Filtered the solution through suitable 0.45 µ PVDF syringe filter discarding 3-5 mL of initial filtrate. Further dilute 2.0 ml of filtered stock solution to 20 ml with mobile phase, injected the resultant solution and chromatograms were recorded

Preparation of linearity solution
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
5 levels of Linearity were performed from 50% to 150% of working concentration.
Vildagliptin: Weighed 25 mg Vildagliptin and transferred in 50 mL volumetric flask, added 35 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol. (500 PPM)
Dapagliflozin: Weighed 15 mg of Dapagliflozin and transferred in 100 mL volumetric flask, added 70-75 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol (150 PPM). [46, 47]

Table No. 2 Linearity levels prepared as follows

Level	Vildagliptin Stock solution (mL)	Dapagliflozin Stock solution (mL)	Diluted to	Vildagliptin Conc (µg/mL)	Dapagliflozin Conc (µg/mL)	Sitagliptin Conc (µg/mL)
50%	1.0	1.0	20	25.00	7.50	7.50
75%	1.5	1.5	20	37.50	11.25	11.25
100%	2.0	2.0	20	50.00	15.00	15.00
125%	2.5	2.5	20	62.50	18.75	18.75
150%	3.0	3.0	20	75.00	22.50	22.50

Determination
Each level injected in triplicate and mean area calculated. Calibration curve was plotted graphically as a function of analyte concentration in µg/mL on X-axis Vs mean area on y-Axis as given in results.

Acceptance criteria
Correlation Coefficient: NLT 0.98
Intercept: To be report
Slope: To be report
Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Detection limit:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value

Quantitation limit:
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.
As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and

LOQ
Procedure for preparation of Accuracy sample solution:
Take clean and dried 9 volumetric flasks of 100 mL. Weighed 221.4 mg of placebo and transferred in each 100 mL volumetric flask. Weighed Vildagliptin and Dapagliflozin
API as per accuracy level and transferred in same 100 ml volumetric flask. Added 70 mL of methanol and sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through 0.45 µ PVDF syringe filter discarding 3-5 mL of filtrate. Further dilute 2.0 ml of filtrate to 20 ml with mobile phase.

Table No. 3 Accuracy levels details

Level (%)	Vildagliptin API (mg)	Dapagliflozin API (mg)	Wt of Placebo (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)	Vildagliptin Added Conc (µg/mL)	Dapagliflozin Added Conc (µg/mL)
50	25.2	7.8	221.8	100	2.0	20	25.20	7.80
	25.0	7.6	221.3	100	2.0	20	25.00	7.60
	25.1	7.7	222.2	100	2.0	20	25.10	7.70
100	50.1	15.2	221.5	100	2.0	20	50.10	15.20
	50.2	15.1	221.7	100	2.0	20	50.20	15.10
	50.1	15.3	220.9	100	2.0	20	50.10	15.30
150	75.1	22.6	222.4	100	2.0	20	75.10	22.60
	75.1	22.5	221.9	100	2.0	20	75.10	22.50
	75.2	22.6	221.7	100	2.0	20	75.20	22.60

Repeatability:
Preparation of sample solution (6 Samples prepared):
Weighed 20 tablets transferred in mortar pestle and crushed to fine powder. Mixed the contents with butter paper uniformly. Weighed the powder material (286.4 mg) equivalent to 50 mg of Vildagliptin, 15 mg of Sitagliptin and 15 mg of Dapagliflozin and transferred to clean and dried 100 mL of volumetric flask. Added 70 mL of methanol, sonicated for 15 minutes with intermittent shaking. After 15 minutes allow to cool the solution to room temperature and made volume up to the mark with methanol. Filtered the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of initial filtrate. Further diluted 2.0 ml of filtered stock solution to 20 ml with mobile phase. (50 ppm of Vildagliptin and 15 ppm of Dapagliflozin), injected the resultant solution and chromatograms were recorded and results are recorded. Six samples prepared.

Table No. 4 Precision (Repeatability) Sample details are as follows

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	286.1	100	2	20
2	286.9	100	2	20
3	285.8	100	2	20
4	286.4	100	2	20
5	287.2	100	2	20
6	286.3	100	2	20

Acceptance criteria:

% Assay: 90-110% for each sample and mean assay value
% RSD for % assay value of 6 samples: NMT 2%

Intermediate precision
It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of Repeatability parameter (6 Samples prepared).

Table No. 5 Intermediate Precision Sample details are as follows

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	286.7	100	2	20
2	286.2	100	2	20
3	287.1	100	2	20
4	287.3	100	2	20
5	286.4	100	2	20
6	286.8	100	2	20

Acceptance criteria:
% Assay: 90-110% for each sample and mean assay value
% RSD for % assay of 6 samples of Intermediate precision: NMT 2
% RSD for Total 12 samples: NMT 2% for test results (6 of Repeatability and 6 of Intermediate precision)

ROBUSTNESS
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

RESULT:
Determination**:** Blank and Standard solution were injected under different chromatographic conditions as shown below.
a) Changes in flow rate by ±10%. (±0.10 ml/min)
b) Change in column oven temperature. (± 2^{o}C)
c) Change in wavelength (± 2 nm)

REFERENCES
1. Ramesh Chaughule, Biomed J Sci & Tech Res, 2024, 60(1), 52185-52191.
2. Godge et al, Biological Forum: An International Journal, 15(3), 2023, 471-474.
3. Lalit K Sahu et al, Eur. Chem. Bull. 12(Special Issue 6) 2023, 5400-5408.
4. P Priya, P Janki et al, IJCRT, 11(3), 2023, 696-706.
5. Deepak M et al, Eur. Chem. Bull. 12(1) 2023, 2713-2729.
6. Maher Ali Almaqtari et al, Ecletica Quimica, 2023, 48(1), 72-94.
7. Maronesi et al, Drug Anal. Res., Porto Alegre, 2022, 6(2), 27-37.
8. Hari Naga Prasada Reddy Chittireddy et al, Mass Spectrometry Molecules 2022, 27, (8581), 1-12.
9. Patel R. V et al, IJNRD, 2022, 7 (4), 930-964.
10. Stefan Balkanski et al, Pharmacia,2021, 68(3): 591–596.
11. Nusrat K Shaikh et al, J. Global Trends Pharm Sci, 2021; 12 (2): 9237 – 9248.
12. Naseem Ahmad Charoo et al, Sci. Pharm. 2021, 90, 1-10.
13. Nikita S. Patel et al. Int. J. Pharm. Sci. Drug Res, 2020,12 (6), 660-667.
14. Matej Sova et al, ACS Omega 2020, 5, 5356−5364.
15. Enas Al-Qudah et al , J. Excipients and Food Chem, 2020, 11 (2), 29-41.
16. S. Arar et al, JLCR, 2020, 3 (2), 1-12.
17. Mendhule et al., IJPSR, 2020; Vol. 12(4): 2289-2295.
18. Grace et al. Asian J Pharm Clin Res, 2019, 12(3), 447-453.
19. Vaghela Yavantika V et al, IJRAR, 2019, 6 (2), 217-225.
20. Sonu A.Varma et al, JETIR, 2019, 6 (5), 1173-1183.

21. Sen *et. al.,* Am. J. PharmTech Res. 2018; 8(5), 323-336.
22. C. F. Alves Giordani et al, Pharmazie, 2018, 73: 442-446.
23. P. B. N. Prasad et al, IJSR, 2018, 7 (2), 6-11.
24. Dattatray S. Metil et al, Medicinal Chemistry & Drug Discovery, 2018, 3, 2723 – 2729.
25. Yehia et al, Sep Sci plus 2018; 1–9.
26. Giordani et al. BMC Pharmacology and Toxicology 2018, 20(Suppl 1):82, 1-9.
27. R. Suresh et al, AJCPR, 2017, 5(2): 64–69.
28. Vuyyuru et al, Asian J. Chem, 2017,29, (9), 1941-1947.
29. Cholleti Vijay Kumara *et al Der Pharmacia Lettre, 2016, 8 (9):349-356.*
30. N. Kumar et al, Journal of Pharmaceutical and Biomedical Analysis, 2016, 119, 114–121.
31. Mohsin Kazia , Abdulmohsen Alqahtani et al, Development and optimization of sitagliptin and dapagliflozin loaded oral self-nanoemulsifying formulation against type 2 diabetes mellitus, Drug Delivery 2021, 28(1), 100–114.
32. Camila F A Giordani , Sarah Campanharo et al, In vitro toxic evaluation of two gliptins and their main impurities of synthesis, BMC Pharmacol Toxicol, 2019, 19;20(Suppl 1):82.
33. Anna Gumieniczek et al, Comprehensive Insight into Chemical Stability of Important Antidiabetic Drug Vildagliptin Using Chromatography (LC-UV and UHPLC-DAD-MS) and Spectroscopy (Mid-IR and NIR with PCA), Molecules 2021, *26*(18), 5632
34. Mahesh M. Deshpande et al, A Review on Impurity Profiling, Degradation Studies, and Bioanalytical Methods of Anti-diabetic Drugs, JPRI, 2022;34(34B): 43-71.
35. Rivera A, Farıas T, Ruiz-Salvador AR, De Ménorval LC. Preliminary characterization of drug support systems based on natural clinoptilolite. Microporous and Mesoporous Materials. 2003 Jul 18; 61(1-3):249-59.
36. Hefter GT, Tomkins RP, editors. The experimental determination of solubilities. John Wiley & Sons; 2003 Aug 1.
37. Frans SD, Harris JM. Selection of analytical wavelengths for multicomponent spectrophotometric determinations. Analytical Chemistry. 1985 Nov 1; 57(13):2680-4.
38. Kumar SD, Kumar DH. Importance of RP-HPLC in analytical method development: a review. International journal of pharmaceutical sciences and research. 2012 Dec 1; 3(12):4626.
39. Kirthi A, Shanmugam R, Prathyusha MS, Basha DJ. A review on bioanalytical method development and validation by RP-HPLC. Journal of global trends in pharmaceutical sciences. 2014; 5(4):2265-71.
40. Babu GR, Rao AL, Rao JV. A rapid RP-HPLC method development and validation for the quantitative estimation ribavirin in tablets. Int J Pharm Sci. 2015; 7:60-3.
41. Prathap B, Dey A, Johnson P, Arthanariswaran P. A review-importance of RP-HPLC in analytical method development. International Journal of Novel Trends in Pharmaceutical Sciences. 2013 Jan 10; 3(1):15-23.
42. Milost F, Čepar Ž. Could the suitability of the existing accounting system be argued?. Management (18544223). 2018 Sep 1;13(3).
43. Bhatt DA, Rane SI. QbD approach to analytical RP-HPLC method development and its validation. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3(1):179-87.
44. Ivanovic D, Medenica M, Malenovic A, Jancic B. Validation of the RP–HPLC method for analysis of hydrochlorothiazide and captopril in tablets. Accreditation and quality assurance. 2004 Jan;9:76-81.
45. Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic science international. 2007 Jan 17;165(2-3):216-24.
46. Jenke DR. Chromatographic Method Validation: A Review of Current Practices and Procedures. II. Guidelines for Primary Validation Parameters. Journal of liquid chromatography & related technologies. 1996 Mar 1;19(5):737-57.
47. Hills RG. Model validation: model parameter and measurement uncertainty.