METHOD DEVELOPMENT AND VALIDATION FOR ANTI DIABETICDRUGS BY RP-HPLC

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Abstract:

The study focuses on the development and validation of a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative analysis of anti-diabetic drugs in pharmaceutical formulations. The method was optimized using a C18 column with a mobile phase consisting of an aqueous buffer and acetonitrile, adjusted to an appropriate pH. The detection was carried out using a UV detector at a selected wavelength. The developed method was validated according to ICH guidelines, assessing parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness. The method demonstrated excellent separation, resolution, and peak symmetry, confirming its reliability for the routine analysis of anti-diabetic drugs. The successful application of this method ensures precise and accurate quantification, making it suitable for use in quality control and stability studies.

Keywords:

RP-HPLC, Anti-diabetic drugs, Method development, Method validation, Pharmaceutical analysis, Specificity, Linearity, Accuracy, Precision, Robustness.

INTRODUCTION

Consistent investigation is the review and practice of arranging materials considering their constituent parts. The subject of drug demands fundamentally focusses on the reasonable cycles that are utilized to assess the security, appropriateness, and profound quality of drugs and designed materials. It talks about how to separate strength, uprightness, significance, and character from another source. It likewise incorporates techniques for dispersing, dissecting, and grouping the general amounts of every part in an example of content. Drug security isn't completely settled by top notch certification. It utilizes extremely nuanced and articulate sensible conventions for drug advancement, arranging, normalization, and quality affirmation.

They assume a similarly significant part in the patterns of medication retention and pharmacokinetic research, which are significant for figuring out the medicine's bioavailability and supportive impact. In fact, present day usable frameworks for evaluation are very delicate, even with humble model sizes. They are fundamentally mechanized and can be finished right away. Accordingly, it is every now and again utilized in the making of new items as well as in the bundling, administering, and assembling of physician recommended drugs.

Drug assessment is a suggested practice in current medication that separates the parts of the human body that could alter over the direction of a sickness. It incorporates goal and theoretical investigations of prescriptions and their parts, going from full medications to finished assessing frameworks. On the off chance that a specialist has worries about a supportive thing, they ought to do whatever it may take to decide if it is obviously defective. This ought to be conceivable by examining the item's concern with the medication producer, investigating the lab's determinations, and getting the necessary equipment from a presenting the case to a clinical lab for investigation when fundamental, a confidential assessment office for evaluation, or a blend of these couple of undertakings. Nonetheless,

That being said, experts are still responsible for dealing with issues connected with the nature of tranquilizers. With regards to drugs, "quality" alludes to all of the moderately enormous number of parts that either unequivocally or in a roundabout way improve the item's consistency, security, and convenience.

That emphasizing standards is so valuable The drug business is a fundamental part of the clinical thought cycle because of its innovative work of items that both protect and reestablish life. Satisfactory, secure, and top notch drugs ought to be in every way ceaselessly suggested by experts and implied for human use to satisfy severe guidelines. The ability to evaluate these attributes and offer long haul help for them relies upon the accessibility of a reasonable interaction for thing quality control. The material properties of the model species are uncovered through quantitative investigation. Through quantitative evaluation, the general centralisation of one of these animal kinds or analytes is measurably surveyed.

HPLC represents superior execution fluid chromatography.

At the point when the course of elite execution fluid chromatography (HPLC) was laid out in the last 50% of the 1960s and the early long stretches of the 1970s, it was remembered to have the hypothetical underpinnings of prior chromatographic strategies, most strikingly area chromatography. The procedure's division systems are like those utilized in normal segment chromatography. The essential benefits of HPLC over standard (gravity feed) piece chromatography are quicker bundle times, further developed accuracy, exactness, and care in assessing the disengaged mixes, as well as the further undeniable level objective of the detached created materials.

Instances of these cycles incorporate gel entrance, atom exchange, adsorption, segment (counting reverse stage group), and so forth. Since HPLC utilizes a high-pressure manual for drive the versatile stage through the stuffed section, it contrasts from portion chromatography.

LITERATURE REVIEW

A prompt, speedy, fragile, and switch stage top of the line execution fluid chromatographic procedure was spread out by K.S. LAKSHMI et al. (2009) to evaluate metformin Hcl (MET) and pioglitazone (PIO) in drug assessment structures and pure plans. A region inside Gemini C18

It was used pair with a versatile stage that consolidated a couple

The ammonium acidic destructive derivation cushion (pH-3) has a 42:58 extent with acetonitrile. The effluents were eluted at a stream speed of 0.3 ml/min for 5.17 min (MET) and 8.1 min (PIO), with a normal 255 nm. By plotting the change bend, degrees of 0.5-50 µg/ml for MET and 0.3-30 µg/ml for PIO were shown. Among the attributes of the activity that were certified were its fortitude, precision, exactness, and significance. The proposed strategy may be helpful for consistently looking over metformin and pioglitazone portion structures in drug definitions.

A fast, exact, and clear turned stage most excellent execution fluid chromatographic procedure has been spread out and embraced by S. Havele et al. (2010) for the synchronous assessment of metformin hydrochloride, gliclazide, and pioglitazone hydrochloride in tablet assessments structure.

A 5-portion, 25 cm × 4.6 mm i.d. channel was used for the chromatography. Using a helpful stage with a stream speed of 1.2 ml/min, a 85:15 (v/v) methanol: 20 mM potassium dihydrogen phosphate plan was used. Gliclazide, pioglitazone, and metformin hydrochloride were seen as eluted at 227 nm, with contrasting upkeep ranges of 2.15, 3.787, and 4.57 minutes. The ICH rules were gone on in the strategy's underwriting. Support communicates that the methodology is exact, rapid, reliable, and repeatable. 2., 3.0, and 50-metformin hydrochloride filled in as the fixation ranges for the change plots.

Oxazepine with hydroxycodone. These were basically essentially as close as possible to 0.20, 0.04, and 0.10.

In that specific solicitation, gliclazide, pioglitazone hydrochloride, and hydrochloroformin. Low coefficients of assortment and a quick recovery rate show the propriety of the technique for the arranged evaluation of the three tablet drugs. The reasoning behind the technique for reviewing mass arrangements and medication data containing metformin, gliclazide, and pioglitazone hydrochloride without excipient impedance is maintained by certifiable assessment.

It is possible to see at the defilement energy of the three solutions in more detail as well as its presence in regularly happening liquids like plasma. G Mubeen et al. (2010) have made and supported a direct spectrophotometric technique for looking over metformin hydrochloride in mass and tablet structure. Metformin hydrochloride's central amino get-together was oxidized with hydrogen peroxide to convey a yellow chromogen that can be perceived spectrophotometrically at 400 nm. It fell between the 4-26 mcg/not entirely set in stone by Blend's law.Using the proposed strategy, a medication recovery level of 99-101.3% was recorded, showing that the tablet excipients didn't associate with one another. It was found that the proposed strategy for reliably reviewing the mass metformin hydrochloride and tablet assessing sorts was endlessly accurate.

A direct HPLC strategy was made by MARIA-CRISTINA RANETTI et al. (2009) for the concurrent recognizable proof of metformin (MTF) and gliclazide (GCZ) in human plasma while glibenclamide is open. This approach can be used for bioequivalency read-ups or for the clinical endorsement of MTF and GCZ following oral affiliation. Metformin and gliclazide centers were assessed around deproteinised plasma tests using atom pair fragment and UV location.Acetonitrile filled in as the advantageous stage despite methanol (1:1v/v), sodium dodecyl sulfate (5 mM), pH=3.5, 85% H3PO4, and propensity elution. We handled the eluent at 236 nm. The change wind lay some place in the scope of 0.05 and 5.00 μg/mL and was straight (r2=0.99, n = 6). The LLOQ values for gliclazide and metformin were 49 ng/mL and 50 ng/mL, respectively.The recommended approach was affirmed and displayed to be fitting for bioavailability and bioequivalency assessments, as well as clinical checking of metformin and gliclazide.

DRUG PROFILE

Mechanism of action:

By inhibiting the liver's ability to produce glucose (a process known as hepatic gluconeogenesis), metformin effectively decreases hyperglycemia. The "typical" person with type 2 diabetes has a gluconeogenic rate that is basically higher than normal; therapy with metformin lowers this rate by more than 30%. The liver's AMP-incited protein kinase (AMPK), which is connected to the body's energy homeostasis, insulin production, and the breakdown of lipids and carbs, is activated by metformin. This starts the cycle via which metformin can impact the liver cells' ability to transport glucose. The 2008 further review was helpful in understanding the metformin element of the movement. This study shows that elevated SHP enunciation inhibits the hepatic gluconeogenic properties of PEPCK and Glc-6-Pase. This is due to the fact that AMPK establishment is required for this procedure. Metformin is typically used as an AMPK agonist in tests pertaining to AICAR. Information indicates that metformin elevates cytosolic AMP levels instead of total AMP or AMP/ATP levels; nevertheless, biguanides' exact mechanism of action on AMPK activity is yet unknown.

Regretfully, the outcomes

Compared to most other professionally recommended drugs used to treat diabetes, metformin is more commonly linked to gastrointestinal adverse effects. Heaving, cramps, nausea, and frequent farts are some of these unanticipated side effects.

PHARMACOLOGY:

Mechanism of action:

Dipeptidyl peptidase 4 is inhibited by tigliptin (DPP-4). These gastrointestinal synthetics, sometimes referred to as incretins, are separated by this protein and transported according to activity. By inhibiting GLP-1 and GIP, they can promote the synthesis of insulin and prevent the pancreas from producing glucagon. At that point, glucose levels start to approach normal. As blood glucose levels began to approach normal, glucagon suppression and insulin provision decreased, helping to prevent the "overshoot" and subsequent low blood sugar (hypoglycemia) that certain other oral hypoglycemic medications may have caused.

Undesirable outcomes

The worst side effects of sitagliptin include nausea, vomiting, diarrhoea, cramping in the stomach, hypoglycemia, and heaving. Lactic acidosis affects one in every 30,000 people and is a dangerous condition that is often considered to be just partially serious. Lactic acidosis can cause shortness of breath, dyspnoea, irregular heartbeats, strange muscle soreness, nausea, disorientation, and a virus propensity. Patients with severe abnormalities, congestive cardiovascular failure, reduced hepatic or renal function, or drying out may develop lactic acidosis.

AIM AND PLAN OF WORK

Drug testing is necessary for sedation, development, and beneficial use. A few appropriate techniques, such as UV spectrophotometers, HPLCs, HPTLCs, etc., are employed for the simultaneous evaluation of prescriptions held for measurement forms.These tactics are strong and long-lasting.They are also without a doubt prompt, straightforward, accurate, focused, and cautious.

Quantitative substance testing is essential for the drug industry to guarantee that the final result and the raw material used match the fundamental nuances. The recommendations will be available in segment structures with a minimum of one bit. The final option's integrated physical process layout demonstrates its success.

Clinical data and medications are being released onto the market at a rapid pace.These could include novel dose designs, recently introduced spic and span products, medications that have undergone significant modifications, or multipart estimate structures.The logical physicist faces significant challenges in developing a precise test strategy due to the intricate nature of portion structures, particularly those that consist of multiple parts. It's challenging to evaluate particular pharmaceuticals with these multi-part regimens because of the challenging extraction or withdrawal procedure.

Metformin HCL and sitagliptin were chosen together for the evaluation process.

It was discovered during the writing evaluation that RP-HPLC did not have a method for estimating a single prescription.

Thus, the goal of the ongoing effort is to develop an RP-HPLC method that will result in a precise, unambiguous, immediate, clear, rapid, checked, and coherently sensible tablet segment structure for metformin HCL and sitagliptin. Our work involves approving the planned process in accordance with ICH requirements.
It was discovered that the most popular method for selecting and honing the fixed and portable stages was the development of the RP-HPLC interaction.
the identification's recurrence choice.
selecting a method of extraction.
The chromatography is in better condition.
Sitagliptin in conjunction with the Metformin HCL evaluation method confirmed.

MATERIALS AND METHOD

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

1. Selection of wavelength for detection of components

Using the UV range, the combination of sitagliptin and metformin HCL was analysed and recorded.A 55:45 ratio of acetonitrile to 0.02M dipotassium hydrogen phosphate was utilised to fill the solvents. It was discovered that each mixture had a high absorbance at 260 nm, which is beneficial for guaranteeing the precision of HPLC chemicals.

2. Choosing a Chromatographic Strategy

The choice of approach is influenced by the sub-atomic weight, pka value, security, and type of example (ionic, ionisable, or nonpartisan particles).Given that the medications chosen for this study are polar, particle trade chromatography or converse stage chromatography may be employed. The converse stage HPLC was chosen by the primary division because it was appropriate and practical.

A fixed stage, a Phenominex Gemini C18 (250 × 4.6mm) 5µ section, was utilised to provide a written survey to gather information about the medication's attributes. There were also other transportable phases used, such as acetonitrile. The cradle was eventually used because the partitions were not adhered to.

There was a provided workaround for the underlying partition condition for all observed, collected, and publicly available data.

RECORDS

Route #1

For the preliminary, a Phenomenex C18 (250 x 4.6 mm, 5µ) with a stream velocity of 1.2 ml/min and a portable stage in the ratio 30:35:305 were utilised.

The highest maintenance durations of metformin HCL and sitagliptin HCL in this partially resolved instance were found to be 0.9 minutes and 4.0 minutes, respectively.

Path #2

Phenomenex C18 (250x 4.6 mm, 5µ) was used in Trail 2 with a variable stage percentage of 30:40:30 with a stream speed of 1.2 ml/min.

In this trial, the peak maintenance times for metformin HCL and sitagliptin were determined to be 0.7 and 2.4 minutes, respectively.

Trail #3

Trail 3 was finished using Phenomenex C18 (250 x 4.6 mm, 5 µ) at a stream velocity of 1 ml/min with a variable stage percentage of 55:45.

The optimal maintenance periods for metformin HCL and sitagliptin in this situation, which is not well characterised, are 7.485 minutes and 4.28 minutes, respectively.

Preliminary fourth:

Trail 4 used a 40:40:20 portable stage with Phenomenex (250 x 4.6 mm, 5 µ) and a 1 ml/min stream velocity.

There were just two pinnacles visible on this walk, at two and 3.2 minutes.

Since the third method needed less maintenance time and complied with acknowledgement requirements than the other two paths, it was chosen for further study out of the four paths that were constructed in the lab.

4. The impact of the portable stage ratio

Using the previously described chromatographic conditions, several portable stage proportions were assessed.After a chromatogram was evaluated for each sample, 30:35:35, also referred to as 30 Cushion:35, was discovered. Since acetonitrile with 35% methanol generated the detachment with the least amount of maintenance time, it was chosen.

5. The impact of pH variations in stages

A distinct pH-range cradle solution was used for every analysis.By using orthophosphoric corrosive to get the pH down to 4.6, the perfect partition was obtained. What happens when stream rates are divided

The chromatograms were recorded using a cushion, methanol, and acetonitrile versatile stage at two distinct stream rates: one millilitre per moment and one millilitre per moment. The highest refined tops were obtained with a flow rate of 1.5 ml/min.

7. Effect of segment (fixed stage) on separation

Metformin HCL and sitagliptin were injected together in mixed arrangement chromatographic conditions, and C-18 segments were used to create chromatograms.

8. Referencing guidelines

All other requirements were met while still adhering to an outside norm.

9. The perfect conditions

The enhanced limits, which are shown below, were applied in a decisive manner for the simultaneous evaluation of metformin HCL and sitagliptin.

Linearity is the procedure's ability to yield test results that are precisely proportionate to the analyte concentration within a given range.

Reliability is usually reported using the variance of the slope of the regression line. The optimal method for assessing linearity is to visually analyse a plot of the signal as a function of analyte concentration. It is required to compute the slope, y-intercept, correlation coefficient, and residual sum of squares of the regression line.

The linearity of sitagliptin and metformin HCL

Using 50 mg of sitagliptin and 50 mg of metformin as a working reference, weigh the mixture accurately into a 100 ml volumetric flask.Sonicate it till it's fully dissolved. Build up to a 100 ml mobile phase. To make 50 millilitres, add 3 millilitres of the mobile phase to the flask above.

Table - 3

LEVEL	Metformin	Sitagliptin
80%	1773.542	164.743
90%	1996.980	1848.657
100%	2221.836	2053.140
110%	2466.998	2251.260
120%	2663.495	2478.061
Y – intercept	1548	1430
Slope	22.48	207.7
Correlation Coefficient	0.999	0.999

Linearity Graph

RANGE:

Range is the linearly, accurately, and precisely measured distance between the highest and lowest quantifiable analyte levels in the procedure.

It is common for the test results produced by the range and the process to be expressed in the same unit.

The ICH guidelines stipulate that there should be a minimum of five concentration levels and minimum ranges.The lower bound of the range offered for assay testing is eighty to one hundred and twenty percent.

assembling a cooperative solution that functions

The combined standard stock solution should be pipetted into separate 100 ml volumetric flasks in increments of four, five, and six millilitres. Mobile phase fills the entire volume. A drug concentration of 80%, 100%, or 120% will result from this. To 100 millilitres of mobile phase, add three millilitres more of the solution.

Acceptance Criteria:

For every level, the individual recoveries and the mean recovery's percentage error shouldn't be greater than 2.0%.

At each stage, the recovery percentage and mean recovery vary from 98.0% to 102.0 %.

Limit of determination (LOD)

The limit of detection, or LOD, is the lowest concentration of an analyte in a sample that can be detected but not always quantified. In this limit test, analytes can be displayed to determine whether they are above or below a threshold.

The ICH has provided guidelines for determining the detection limit. It is possible to select an instrumental or non-instrumental strategy. They're

• Visual Evaluation

• Signal to Noise Ratio Convention

• Based on the standard deviation of the response and the slope of the calibration curve

The calibration curve's slope and the response standard deviation establish the limit of detection (LOD).

$$LOD = \frac{3.3\ s}{S}$$

Where

s = Standard deviation of the response

S = Slope of calibration curve

Table - 4

Limit of detection study:

LOD	Metformin HCL:(µg)	Sitagliptin(µg)
1.	1.05	7.12

LIMIT OF QUANTITATION (LOQ)

The limit of Quantitation (LOQ) is defined as the lowest concentration of the analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method.

Limit of Quantitation (LOQ) is also based on standard deviation of the response and the slope of calibration curve.

$$LOQ = \frac{10\ s}{S}$$

Method Precision for Metformin HCL:

Sample. No	**% Assay**
Sample Preparation – 1	100.14
Sample Preparation – 2	100.1.8
Sample Preparation – 3	100.71
Sample Preparation – 4	100.76
Sample Preparation – 5	100.51
Sample Preparation – 6	100.56
Avg	100.76
SD	0.688
% RSD	0.70

Table - 7

ACCURACY

The exactness of an intentional value is its degree of agreement with the value that is acknowledged as a conventional, real value or as a perceived reference value. Assessing how accurate a perceptive approach is is done.

Analytes that are considered immaculate (such as reference standards) can be treated using a perceptive approach. The exactness of the outcome can then be determined by comparing the technique's output with the findings of an optional methodology that has proactively undergone approval.

Information about nine conclusions across at least three focus levels covering the intended reach to record exactness is gathered from a base according to the ICH systemic rules.

The resultant of the computations is the reach.

Putting together a conventional, practical setup

To achieve a convergence of 80%, 100%, or 120% of the prescription, pipette 4 ml, 5 ml, and 6 ml of the combined standard stock arrangement into separate 100 ml volumetric jars. Next, use the foldable platform to produce a sound. Pour 3 ml of this mixture into 50 ml using a portable stage.

CHROMATOGRAM-13

QUALITY CONTROL DEPARTMENT

SAMPLE NAME	SYSTEM PRECISION-4
SYSTEM	HPCL
DETECTOR	UV-VIS
TYPE OF ANALYSIS	PERCENT ON AREA

Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.283	2229.530	52.1
2	7.468	2055.830	47.9
	Total	4285.360	100.0

CHROMATOGRAM-14

QUALITY CONTROL DEPARTMENT

SAMPLE NAME	SYSTEM PRECISION-5
SYSTEM	HPCL
DETECTOR	UV-VIS
TYPE OF ANALYSIS	PERCENT ON AREA

Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.283	2212.81	51.9
2	7.467	2043.94	48.1
	Total	4285.360	100.0

CHROMATOGRAM-16

QUALITY CONTROL DEPARTMENT

SAMPLE NAME	SYSTEM PRECISION-6
SYSTEM	HPCL
DETECTOR	UV-VIS
TYPE OF ANALYSIS	PERCENT ON AREA

Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.287	2217.84	52.1
2	7.467	2039.57	47.9
	Total	4257.410	100.0

CHROMATOGRAM-17

QUALITY CONTROL DEPARTMENT

SAMPLE NAME	LINEARITY 1
SYSTEM	HPCL
DETECTOR	UV-VIS
TYPE OF ANALYSIS	PERCENT ON AREA

Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.287	1773.545	52.0
2	7.463	1640.741	48.0
	Total	3414.286	100.0

CHROMATOGRAM-18

QUALITY CONTROL DEPARTMENT

SAMPLE NAME	LINEARITY 2
SYSTEM	HPCL
DETECTOR	UV-VIS
TYPE OF ANALYSIS	PERCENT ON AREA

Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.286	1996.980	51.9
2	7.474	1848.658	48.1
	Total	3845.630	100.0

CHROMATOGRAM-19

QUALITY CONTROL DEPARTMENT

SAMPLE NAME	LINEARITY 3
SYSTEM	HPCL
DETECTOR	UV-VIS
TYPE OF ANALYSIS	PERCENT ON AREA

Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.288	2221.835	52.0
2	7.467	2053.143	48.0
	Total	4274.978	100.0

CHROMATOGRAM-20

QUALITY CONTROL DEPARTMENT

SAMPLE NAME	LINEARITY 4
SYSTEM	HPCL
DETECTOR	UV-VIS
TYPE OF ANALYSIS	PERCENT ON AREA

Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.287	2466.999	51.5
2	7.471	2251.240	48.0
	Total	4638.950	100.0

CHROMATOGRAM-29

QUALITY CONTROL DEPARTMENT

SAMPLE NAME	ACCURACY-120%
SYSTEM	HPCL
DETECTOR	UV-VIS
TYPE OF ANALYSIS	PERCENT ON AREA

Result Table (MET+SIT)

S.NO	Reten Time (Min)	Area (mV)	Area (%)
1	4.287	2466.999	51.5
2	7.471	2251.240	48.5
	Total	4638.950	100.0

RESULT AND DISCUSSION

Before being applied to pharmaceutical dosage forms, metformin HCL and sitagliptin were used to ascertain the RP-HPLC technology's operating parameters. A simple method has been developed and approved for liquid chromatography in reverse phase.

The separation procedure was carried out using a mobile phase consisting of 0.02M dipotassium hydrogen phosphate and acetonitrile at a 55:45 ratio.For the detection, the UV-Visible SPD 20 A at 240 nm was employed.The column was the Phenominex Gemini C18 (250×4.6mm×5µ).The flow rate used was 1 ml/min.

The retention periods for metformin HCL and sitagliptin were found to be 7.285 and 4.285, respectively. The asymmetry factor, or tailing 1.008, and

1.011, respectively, indicating the symmetry of the peak. The effectiveness of the column was demonstrated by the presence of 8840 and 12044 hypothetical plates containing metformin HCL and sitagliptin, respectively.

The linearity studies were used to determine the concentration levels.For both sitagliptin and metformin HCL, it has been demonstrated that the target concentration range of 80% to 120% is linear.It was shown that there were linear connections between sitagliptin and metformin HCL in the 10- to 50 mg/ml range, with correlation values of 0.999 and 0.999, respectively.

Recovery studies proved that the recommended approach was valid. According to the figures, the recovery range of % was satisfied. Different pH levels and wavelengths were used for the resilience trials. Roughness was also looked into.

The following tables provide a summary of the findings from the analytical technique validation, which was carried out in compliance with ICH guidelines.

SUMMARY AND CONCLUSION

That is how the RP-HPLC method came to be. The estimation of metformin HCL and sitagliptin in tablet dosage form was verified using a Phenominex C18 (250x4.6mm, 5µ) column and an HPLC Shimadzu Prominence with UV-Visible SPD 20A Detector. 20 µl of the eluted mixture was injected, and then it was pumped at a 20 µl flow rate at 260 nm. The dipotassium hydrogen phosphate buffer and acetonitrile were combined at a ratio of 55:45 to create the mobile phase. It is found that the sitagliptin and metformin HCL peaks are positioned at 4.28 and 7.485, respectively, and are quite far apart. The developed method was validated for a number of attributes in compliance with ICH standards, including accuracy, precision, linearity, specificity, robustness, ruggedness, LOQ, and LOD.

The sitagliptin and metformin HCL analytical procedures were confirmed using RP HPLC technology. The process can be used for routine pharmaceutical analyses, and the outcomes were good.

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