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Review article

Current Analytical Methods and Their Limitations in Quantifying Antidiabetic Drug Nateglinide and Repaglinide in Pharmaceutical Formulations and Biological Fluids

SK Manirul Haque[1]

[1] Department of Chemical Engineering, Jubail Industrial College, Jubail Industrial City-31961, Saudi Arabia

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Abstract

Pharmaceutical growth dominated a transformation in human health. These drugs need to attend to their target only, so they must be free from impurities and appropriately controlled. Due to that, diverse instrumental techniques were advanced at steady intervals to accomplish their intention to quantify the limits per the regulatory. NTG and RPG pharmaceuticals might generate impurities during the development phases, packing, and shipping, which could be risky to administer. Hence, detecting and quantifying them using various analytical techniques at multiple stages is necessary. This review highlights the function of different analytical methods, including UV–Vis, HPLC, HPTLC, UPLC, HPLC/MS and UPLC/MS, in quantifying drugs, impurities and metabolites in bulk, pharmaceutical formulations and biological fluids. Also, it discussed the specific advantages and limitations of individual techniques. It compared them regarding sensitivity, specificity, cost, time consumption, efficacy, and the practical challenges of implementing these analytical techniques in real-world settings to determine pharmaceuticals.

1. INTRODUCTION

Type 2 diabetes cases are increasing worldwide, but it is promising to handle the number by exercising and diet control.[1] The study proved that hypoglycaemic drugs that belong to the Meglitinide class of short-acting insulin secretagogues could constrain blood glucose by accelerating the insulin release from the pancreas, which is ATP-supported potassium channels β cells.[2] The deadly illness needs regulation, and it could possibly cause lifelong difficulties like peripheral vascular disease due to probable disorders in blood circulation, stroke, which minimises blood flow towards the brain, blindness, cardiovascular disease, diabetic neuropathy that damages the nerve and renal failure because of kidney damage.[3-5] Diabetes is also considered to be a protein and carbohydrate reduction with the metabolism of fat due to a deficiency of insulin that causes hyperglycemia.[6]

The meglitinide agent, repaglinide (**Figure 1a**), is used for antidiabetic management of type 2 diabetes mellitus. It is chemically known as *S*(+)2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid. The other D-phenylalanine derivative, nateglinide (**Figure 1b**), also has been approved for treating diabetes mellitus. So, it is necessary to understand the drug development process, which is essential to support scientific and commercial success. Ensuring an active pharmaceutical ingredient (API) ‘s physical and chemical assets and pharmaceutical formulation is vital for regulatory agreement and therapeutic accomplishment. The major challenge for drug development is manufacturing and controlling components, ensuring that the compound and product’s chemical and physical properties are examined at all crucial phases of the pathway. Stability studies are necessary to verify and provide the identity, purity and potency of ingredients and formulated products. The analytical method’s limitations included controlled applicability to individual problems. These methods can be challenging to apply for complex issues and time-consuming, labour-intensive processes. Their applicability is also limited in real-world problems and difficulties when combined with external factors. These limitations make methods less versatile but provide adaptability to the approximate methods that could routinely solve complicated issues in various fields. As a result, analytical methods’ limitations can indicate increased reliance on approach methods, which could help the researchers understand the method’s applicability and select a suitable technique for the analysis. Due to its therapeutic importance, several methods, such as ultraviolet-visible spectrophotometry (UV–Vis), high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), ultra–pressure liquid chromatography (UPLC), high-performance liquid chromatography-mass spectrophotometry (HPLC-MS) and ul-

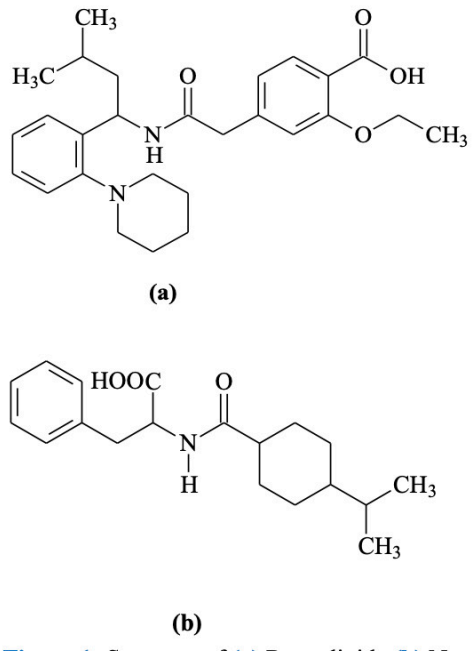


Figure 1. Structure of (a) Repaglinide (b) Nateglinide.

tra–pressure liquid chromatography-mass spectrophotometry (UPLC-MS) are based on different techniques had been reported in API, pharmaceutical formulations and biological fluids. The main aim of antidiabetic drugs is to serve humans and free them from promising illness and disease prevention. For medicines like NTG and RPG to provide their projected target,[7,8] they would be protected from contamination or other obstacles that harm humans.

The current review focuses on determining and quantifying NTG and RPG in API, pharmaceutical formulations and biological fluids. It also signified the introduction of analytical techniques and their advantages and limitations in quantifying the drugs. It also emphasizes advancing the reported practice from the older UV-Vis approach to innovative hyphenated methods. It might help new researchers from academia and industry to understand their functions and capabilities, which could open an option whether these methods are still the best or are being supplanted with more advanced techniques.

2. ANALYTICAL TECHNIQUES

2.1. SPECTROPHOTOMETRY

UV–Vis spectrophotometry is a valuable and easy technique for a drug's qualitative and quantitative determination due to its simple, low-cost technique, without pretreatment prior to analysis (**Scheme 1**). It also required less time and labour intake. This technique, mainly used in pharmaceutical formulation analysis, has increased based on the redox and complexation reaction.

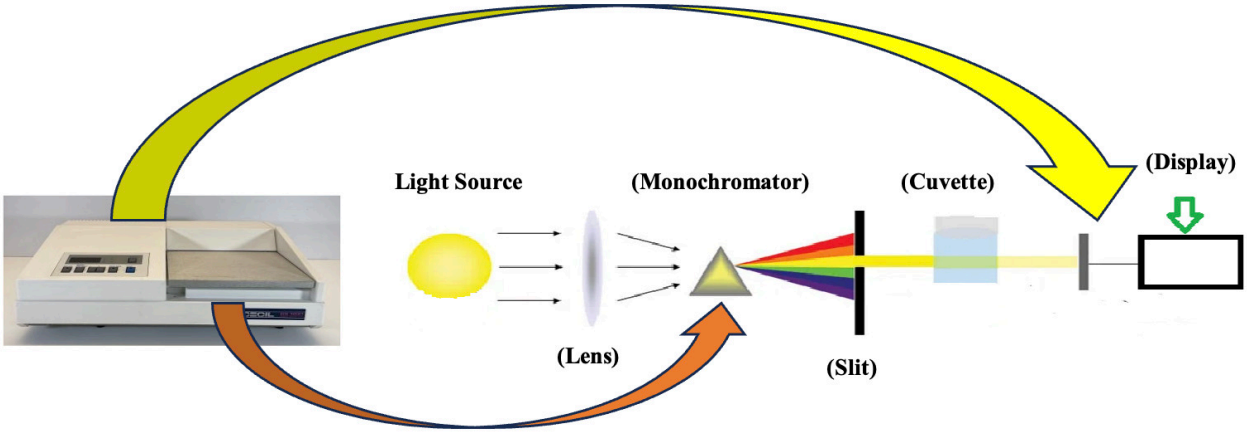
The detailed investigation based on the specificity, sensitivity, and broader range of each reported procedure were included in **Table 1**,[9-22] and studies elaborate on all reported procedures for both. Nearly all the analytical determinations used methanol as a diluent for RPG quantification[11-13,15,16] at different wavelengths. However, the procedure showed excellent linearity in bulk and pharmaceutical formulations. The precision results were within the acceptable limit. The developed method[9] is based on the kinetic spectrophotometry applied response surface methodology combined Box–Behnken design, which reduced the uses of reagents and solvents with the minimum number of trials and supported the eco-friendly green analysis. The % assay of the RPG in tablets was entirely satisfactory, about 98.24% as per the Indian Pharmacopoeia.[10] The degradation results also confirmed its value within the permissible range. The % recovery was 97.92–101.35%, and the % RSD was less than 2% (**Figure 2**). The literature continued to reveal the NTG quantification[17-22] with a wider dynamic range in bulk and pharmaceutical formulations (**Figure 3**). The determination was based on visible spectrophotometry.[17,19,22] It showed no interference from excipients due to higher wavelength in the visible region with an acceptable % recovery. However, minor positive interference was detected in the occurrence of a significant amount of excess excipients such as glucose, lactose, Arabic gum, sodium chloride, sucrose and starch. Few developed procedures have used[18,22] methanol as a diluent that focuses on precise results and confirms that all the reported procedures are susceptible to accessing the drug concentration in different matrices.

2.2. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

All the chromatography methods, from traditional to hyphenated, are more accurate and precise depending on the available analyte's characteristics and present stationary and mobile phases. Based on that, different chromatography techniques were established for pharmaceutical analysis. HPLC has recently shown much interest due to its ease of instrumentation, selectivity, and high specificity for drug analysis (**Scheme 2**). As usual, it is a wholly improved type of column chromatography, in which a solvent is constrained through a column at high pressures rather than allowed to move under gravity. As an outcome, it is significantly faster. Generally, using a non-polar stationary phase for polar analytes is called reverse phase chromatography, whereas for non-polar components, using a polar mobile phase is known as normal phase chromatography.

However, it always needs to be kept in mind during method development that solvents and reagents must be used less to be eco-friendly and use a white analytical methodology. The determination of RPG[23-28] and NTG[29-36] employed varied column types, and a combination of different mobile phases containing buffers and an organic modifier could be used to develop the method for their quantification **(Table 2)**.

The reported method was first applied to determine RPG instantaneously in the presence of remdesivir (RDV) and dexamethasone (DXM) using a lesser organic solvent in the mobile phase.[23] It was a prominent procedure to produce more precise results with a large number of quality control samples. The sample recoveries agreed with their respec-



Scheme 1. Instrumentation and figure of spectrophotometry.

Table 1. Determination based on the spectrophotometric analysis.

Diluent/Reagent	Detection (nm)	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Applications	References
1-chloro-2,4-dinitrobenzene	423	2–20	0.76	2.44	API, pharmaceutical and biological fluid	[9]
Acetonitrile: Water = 70:30 v/v	244	2–70	0.1109	0.336	Bulk and tablets	[10]
Methanol	294	1–35	0.0169	0.0512	Bulk and tablets	[11]
Methanol	308	5–50	0.568	1.821	Tablet	[12]
Methanol	237	10–90	5.4603	16.5465	Pharmaceutical dosage forms	[13]
Methanol, 0.1 N HCl and phosphate buffer (pH–6.8)	299, 283	1–6, 1–6	0.19, 0.21	0.58, 0.64	Tablet formulation	[14]
Methanol	287	5–40	1.01	3.13	Dosage forms	[15]
Methanol	241	5–30	1.15	3.48	Tablets	[16]
1-chloro-2,4-dinitrobenzene and DMSO	421	1–15	0.21	0.62	Pharmaceutical formulations	[17]
Methanol	258	50–250	4.2	12.5	Tablet dosage form	[18]
3-methyl-2-benzothiazolinone hydrazone	634	50–300	11.78	35.7	Tablets	[19]
0.1 M NaOH, 0.1 M HCl	210, 270	3–54, 4–72	0.91, 0.72	2.73, 2.16	Pharmaceuticals	[20]
0.1M piperazine	220	5–30	----	----	Bulk and pharmaceutical formulation	[21]
Methanol	465	2–10	0.625	1.101	Crude and marketed sample	[22]

tive label claims in the formulation with a lesser retention time,[24,25] and formulation excipients did not restrict RPG estimation, demonstrating appropriateness for identifying counterfeit drugs.[28]

Few analytical procedures are selective and can quantify RPG in biological fluids[26,27] with excellent accuracy, precision, and higher therapeutic stability range, including the liquid extraction practice that was sensitive and reproducible with enhanced extraction and development efficiency.[26]

However, the bioanalytical-based HPLC procedure that mingled with the fluorescence detector required 40°C as

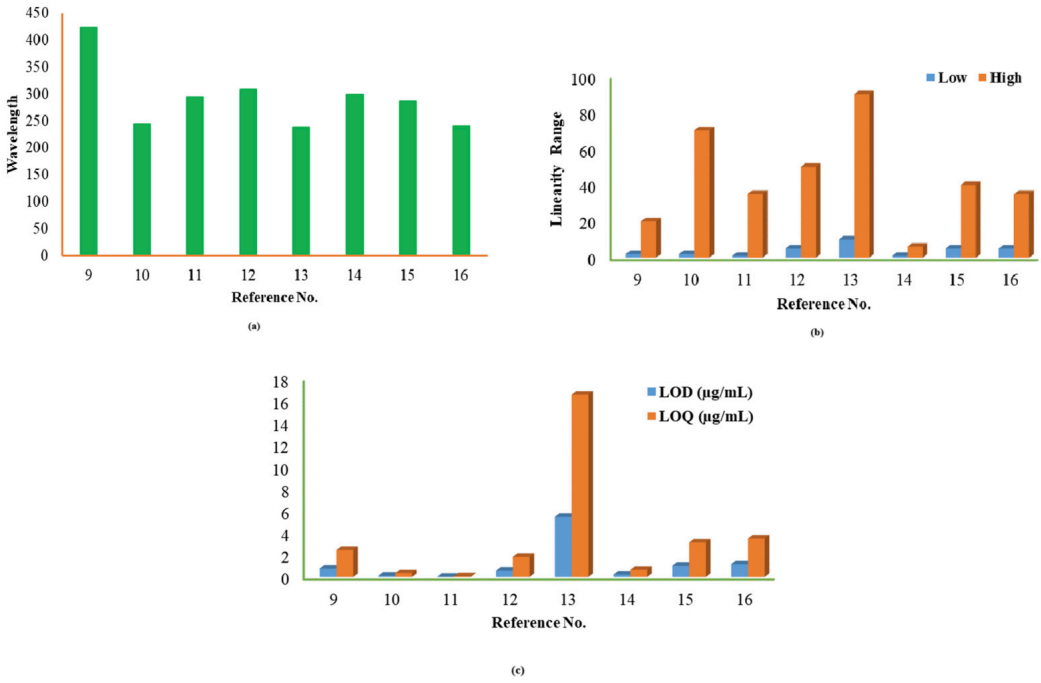


Figure 2. (a) Wavelength (b) Linearity range (c) LOD and LOQ determination respected to reference no. to quantify RPG using spectrophotometry.

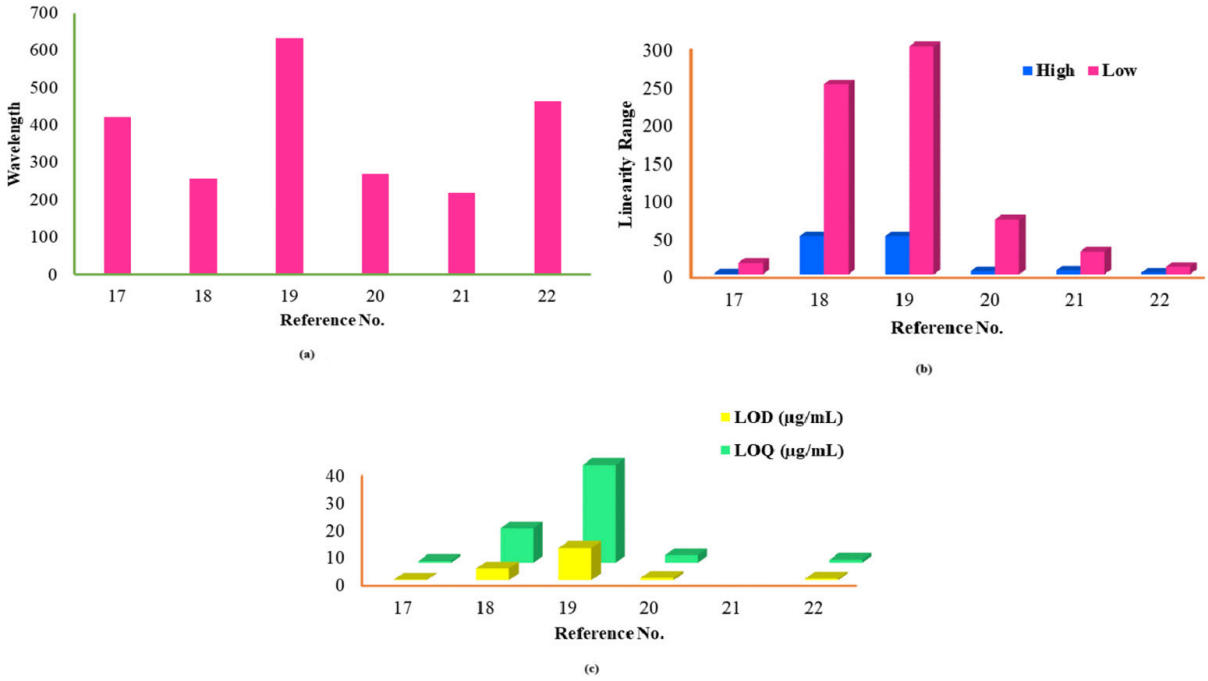
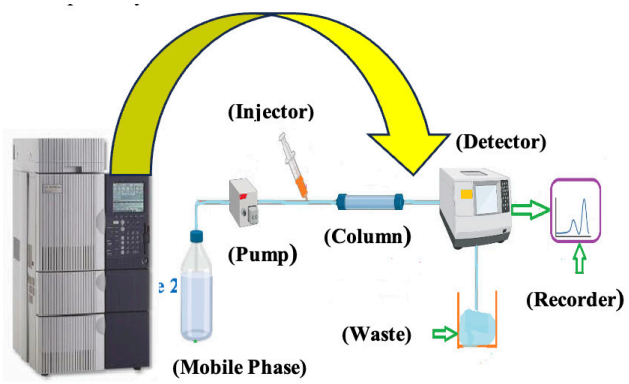


Figure 3. (a) Wavelength (b) Linearity range (c) LOD and LOQ determination respected to reference no. to quantify NTG using spectrophotometry.

column temperature and a run time of 23 min,[27] which also included a simple protein precipitation process for its determination and validation. The precision results were assessed based on the %RSD value and calculated as 8.30% and less.

In comparison, the accuracy was estimated in terms of % recovery within 98.6–112% for the sample concentration in the range of 10–2000 ng/mL in rat plasma, which was considered accurate, precise and reproducible as per the FDA guidelines. The NTG quantification enhanced with Box–Behnken helped to reduce the analysis time and solvent consumption.[29] The retention time was decreased with higher reproducibility, increasing the method's quantification capability.[30-35] Waters Nova Pak eluted the NTG



Scheme 2. Instrumentation and figure of HPLC.

within 1.4 minutes at room temperature.[35] Parent drug molecule detection could be possible at temperatures over 30°C and higher run time.[36] The graphical representation of the valuable data (**Figures 4 and 5**) demonstrates its respective quantitative perspective to quantify RPG and NTG, respectively.

2.3. HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

High-performance thin-layer chromatography (HPTLC) is the most convincing and cumulative form of thin-layer chromatography (**Scheme 3**). It concerns about the chromatographic layers based on different functionalities, and these principles were applied to develop advanced equipment for all phases in the method development. It includes a standardized process based on efficient qualitative and quantitative testing approaches depending on scientific evidence. It also encounters all the quality needs of a current analytical research laboratory. The progress in HPTLC aims to increase the compound's resolution to be separated and facilitate the substance's quantitative analysis.

The developments could be with the stationary phase, likely higher-quality chromatography plates that contain significantly thinner particle sizes that permit improved resolution with lower LODs, higher sensitivity, and low cost.[37] The reported method was applied to quantify NTG and its present impurities.[38] The advantage of the procedure was to use fewer organic solvents, which could be eco-friendly and cost-effective due to reduced methanol consumption with a linearity range of 100–600 µg/band. The procedure comprehensively determines its impurities 1 and 2 in bulk and pharmaceutical formulation with LOD and LOQ 4.17 (ng/band), 0.28 (ng/band), 6.21 (ng/band) and 1.81 (ng/band), respectively. Applying the preparative liquid chromatography method also showed no excipient interference from the formulation during impurity isolation.

The NTG determination could be quantified with other drug combinations like with metformin applying ethyl acetate, chloroform and acetic acid (6:4:0.1, v/v/v) as a mobile phase with pre-coated silica gel (60 F–254) on aluminium plates as a stationary phase which detected within the range of 200–2400 ng/band at 216 nm.[39] The procedure had a higher recovery percentage of 99.72, with the potential to quantify simultaneously from formulation without any intervention from the excipients. However, a similar stationary phase could be used to determine RPG from other combinations like metformin and melamine with different mobile phase mixtures of methanol, chloroform and acetic acid (1: 8.5: 0.5, v/v/v), which detected at 240 nm[40]

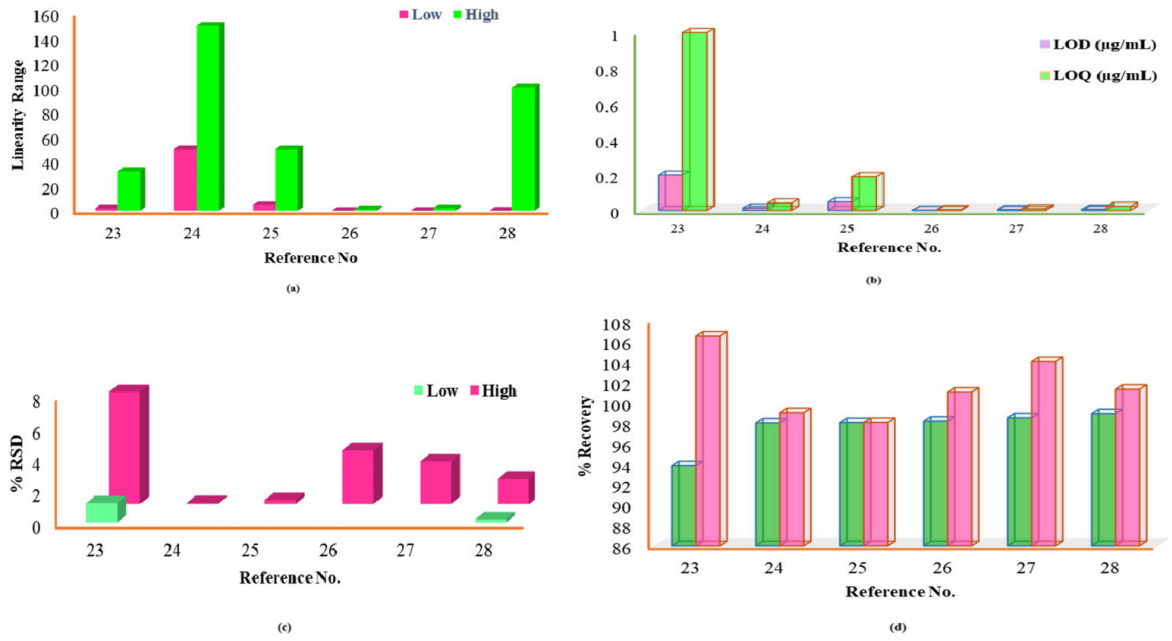


Figure 4. (a) Linearity range (b) LOD and LOQ (c) % RSD (d) % Recovery against reference no. to quantify RPG applying HPLC.

Table 2. Important parameters for the determination of drugs using HPLC.

Chromatographic conditions	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Recovery (%)	RSD (%)	Applications	References
Column: Purospher RP-C_{18} (150x4.6 mm, 5 µm) Mobile phase: TFA in water (0.1%): acetonitrile = 70:30, v/v Detection: 235 nm Retention time: 7.61 min	2–32	0.2	1	93.83 –106.49	1.22 – 7.09	Human plasma	23
Column: C_{18} (150×4.6mm, 5µm) Mobile phase: Methanol and water mixture (gradient elution) Flow rate: 0.6 mL/min Wavelength: 245.5 nm Retention time: 2.564 min Run time: 10 min	50.02–150.06	0.01436	0.04353	98–99	0.0643	Bulk and dosage form	24
Column: C_{18} (4.6×150mm, 3.5 µm) Mobile phase: KH_2PO_4 (pH–2.2): Acetonitrile = 35:65% v/v Flow rate: 0.6 mL/min Wavelength: 240 nm Retention time: 3.825 min Run time: 7 min	5–50	0.05	0.19	98.03–98.05	0.26	Pure and pharmaceutical formulation	25
Column: C_{18} (250×4.6 mm, 5 µm) Mobile phase: Acetonitrile: trifluoroacetic acid (0.05%) in water = 55:45, v/v Flow rate: 1 mL/min Wavelength: 285 nm Retention time: 4.3 min	0.01–1	0.00173	0.00523	98.17–101.02	3.39	Rabbit plasma	26
Column: C_{18} (250×4.6 mm, 5 µm, 100 Å) Mobile phase: 10 mM phosphate buffer (pH 6.0) and ACN = 53.6:46.4, v/v Flow rate: 1 mL/min Fluorescence Detector: Excitation–240 nm and Emission–380 nm Temperature: 40°C Run time: 23 min	0.01–2	___	LLOQ–2.69	98.5–104	2.69	Rat plasma	27
Column: Kromasil 100-C_{18} (30×0.4 cm, 10 µm) Mobile phase: Gradient elution of ACN, KH_2PO_4 (0.05) and sodium octane sulphonate (0.01 M) with pH 3.55 Flow rate: 0.85 mL/min Wavelength: 220 nm Retention time: 14.32 min Run time: 20 min	0.1–100	0.007	0.024	98.9–101.3	0.2–1.6	Pharmaceutical samples	28
Column: C_{18} ODS (250×4.6mm, 5µm) Mobile phase: Phosphate buffer : Acetonitrile: Methanol =	10–120	0.754	2.28	100.39	0.259	Tablet dosage form	29

Chromatographic conditions	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Recovery (%)	RSD (%)	Applications	References
30:55:15, v/v Flow rate: 1 mL/min Wavelength: 221 nm Retention time: 5.261 min Run time: 10 min Column temperature: 30°C							
Column: Sunfire C_{18} (4.6×250mm, 5µm) Mobile phase: Water: Acetonitrile = 60:40%, v/v Flow rate: 0.9 mL/min Wavelength: 220 nm Retention time: 3 min Run time: 6 min Column temperature: 35°C	20–100	—	—	100.17	0.65–0.89	Bulk and pharmaceutical formulations	30
Column: C_{18} (25cm×4.6mm, 5µm) Mobile phase: Potassium dihydrogen phosphate (20 mM) and Methanol = 15: 85, v/v Flow rate: 1.2 mL/min Wavelength: 227 nm Retention time: 3.78 min	3–15	0.22	0.67	99.19	0.8 – 1.72	Formulation	31
Column: Kinetex pentafluorophenyl (4.6 x 150 mm, 5µm) Mobile phase: 0.05 M Na_2HPO_4 adjusted pH=2 with H_3PO_4 and methanol = 30:70, v/v Flow rate: 1 mL/min Wavelength: 215 nm Column temperature: 22°C Retention time: 4.779 min	2.54–40.70	1.07	2.54	98.78–100.76	0.84	Tablet dosage form	32
Column: ACE C_{18} (150 x 4.6 mm , 5 µm) Mobile phase: Acetonitrile: Trifluoroacetic acid (0.05%) = 25:25, v/v Flow rate: 1.5 mL/min Wavelength: 210 nm Retention time: 4.21 min	284.6–1012.5	—	—	101.10	0.33	Drug substance	33
Column: Waters Nova-Pak C_{18} (150×3.9 mm, 4 µm) Mobile phase: KH_2PO_4 (10 mM): Acetonitrile = 40: 60, v/v adjusted pH 3.5 with 10% H_3PO_4 Flow rate: 1.5 mL/min Wavelength: 210 nm Column temperature: Ambient temperature Retention time: 1.4 min	10–200	0.0151	0.05	90	0.216 – 3.936	Rat plasma	34
Column: Inertsil C_{18} (250×4.6 mm, 5 µm)	14.4–33.2	1.55	4.69	98.43–101.27	1.35 – 1.83	Bulk and dosage forms	35

Chromatographic conditions	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Recovery (%)	RSD (%)	Applications	References
Mobile phase: Phosphate buffer (pH–4): Acetonitrile: Methanol = 30: 60: 10, v/v/v Flow rate: 1 mL/min Wavelength: 221 nm Retention time: 4.21 min							
Column: Hypersil BDS C_{18} (250×4.6 mm, 5 µm) Mobile phase: KH_2PO_4 (10 mM) buffer of pH 2.5 and Acetonitrile = 35:65, v/v Flow rate: 1 mL/min Wavelength: 210 nm Column temperature: 30±2°C Retention time: 9.261 min Run time: 16 min	0.10074 –3.32983	—	—	98.19–99.83	4.95 – 10.72	Rabbit plasma	36

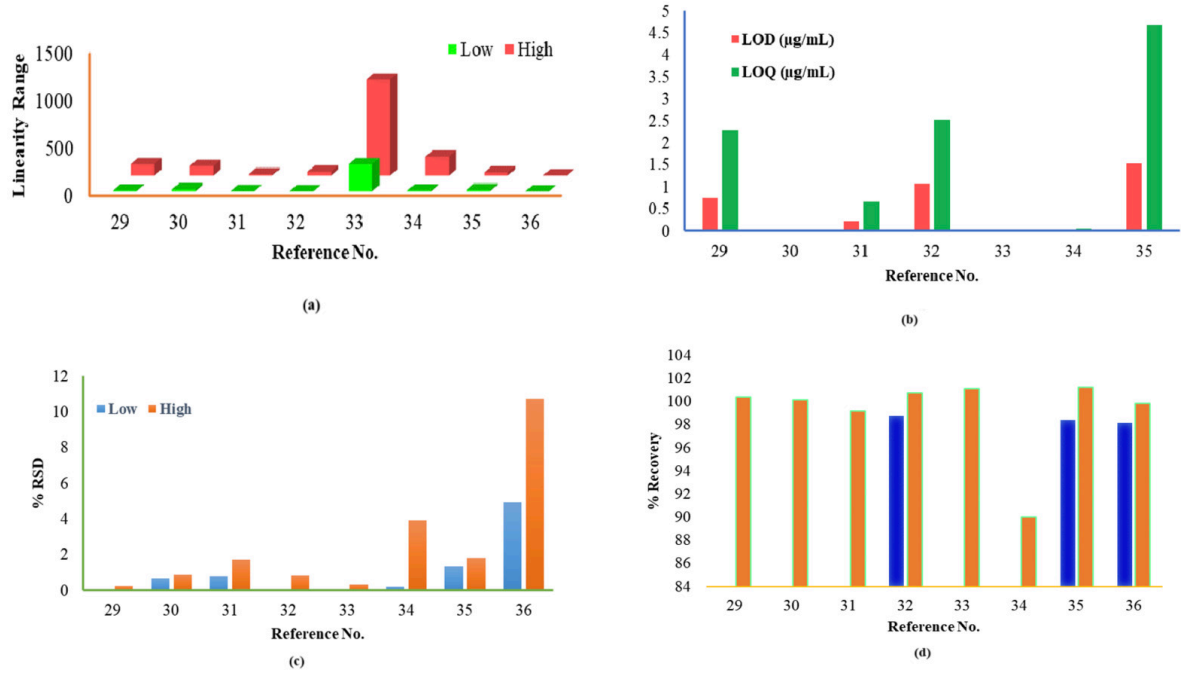


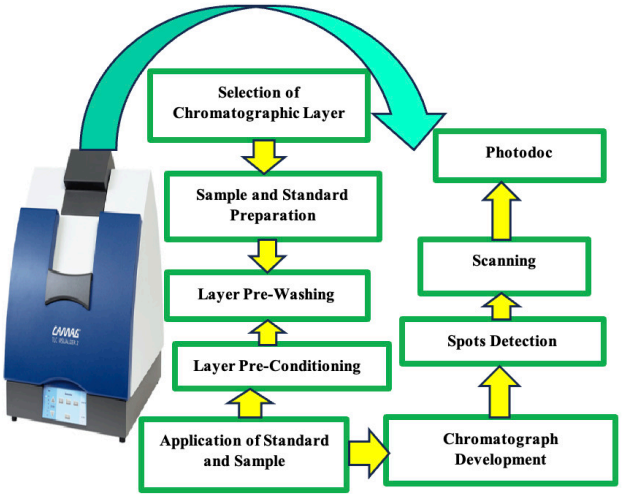
Figure 5. (a) Linearity range (b) LOD and LOQ (c) % RSD (d) % Recovery against reference no. to quantify NTG applying HPLC.

within the range of 0.2–1.5 µg/band. Whereas the other solvent system combined with methanol, ammonia, chloroform and acetic acid (1.5: 0.9: 7.5: 0.1, v/v/v/v) could able to determine its purity and related impurities with a wider dynamic range of 50–800 ng/spot. The percentage accuracy showed a higher % recovery between 98.79 and 99.61.[41] Therefore, HPTLC methods had many benefits compared to LC, which included one standard plate that could analyse simultaneous samples with reduced equilibrium time and higher mobile phase pH. It could also hold larger samples and consume less solution with decreased run time without solvent pretreatment. The stability-indicating assets indicated that the substances could possibly be assessed in the occurrence of their degradation product. Thus, it can simultaneously evaluate their degradation products in stability samples in the industry.

2.4. ULTRA-PRESSURE LIQUID CHROMATOGRAPHY (UPLC)

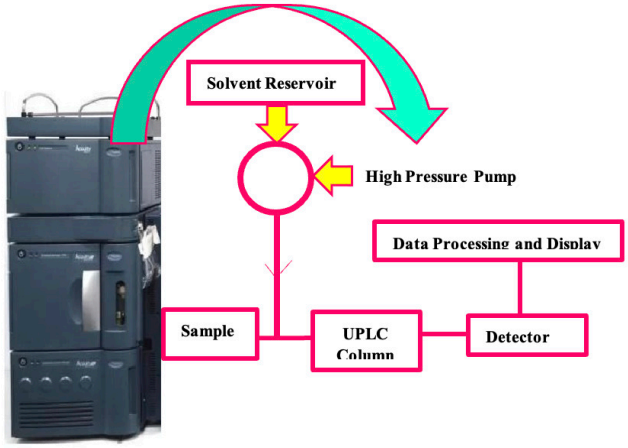
Ultra-pressure liquid chromatography (UPLC) is a new practice that creates new potential, specifically involving reduced time and solvent utilization. The UPLC chromatographical system uniquely resists the system's high back pressures by connecting particular types of analytical columns (**Scheme 4**). In conventional HPLC, the particle size selection should be an agreement in which the smaller the particle size, the higher the influence of increasing the column back pressure, which could be a restraint of employing such columns.

The column diameters of 1 and 2.1 mm could cause similar difficulties and reduce usage under normal settings. A sensitive and consistent UPLC procedure was built to quantify NTG in the presence of another drug, metformin, with the help of Phenomenox C_{18} column (50×2.1 mm, 3.5 µm) using ammonium formate buffer of pH-3 and acetonitrile (75: 25, v/v) as mobile phase, eluted within 3 minutes at 260 nm. The linearity range of the reported method was in the range of 7.5–45 µg/mL with LOD and LOQ values of 0.09 and 0.3 µg/mL, respectively. The precision values were within the acceptable limit regarding % RSD between 0.45 and 0.58%.[42]



Scheme 3. Instrumentation and figure of HPTLC.

Whereas Waters Acquity UPLC SB C_{18} followed a slightly higher flow rate and column temperature of 30°C to elute NTG at 1.71 min with a broader linearity range of 15–90



Scheme 4. Instrumentation and figure of UPLC.

μg/mL.[43] The method's novelty was verifying the specificity test by forced degradation study. The UPLC method can quantify RPG in pharmaceutical formulations with precise and validated procedures within 2.1 minutes and reveals exceptional performance in terms of sensitivity and speed, which could be used as a routine RPG analysis. The method is stability-indicating for RPG assay in pure and formulations. Also, the method's degradation study reveals it is precarious in acidic and basic forms.[44]

The product's quality, as assessed by UPLC, would be better with less time. Still, the problem is related to column life because it requires high pressure, possibly damaging the column competence. Innovative and selective approaches were advanced to establish whether drugs with commercial formulations were single- or combined-dosage form

2.5. HPLC AND UPLC COMBINED MASS SPECTROPHOTOMETRY

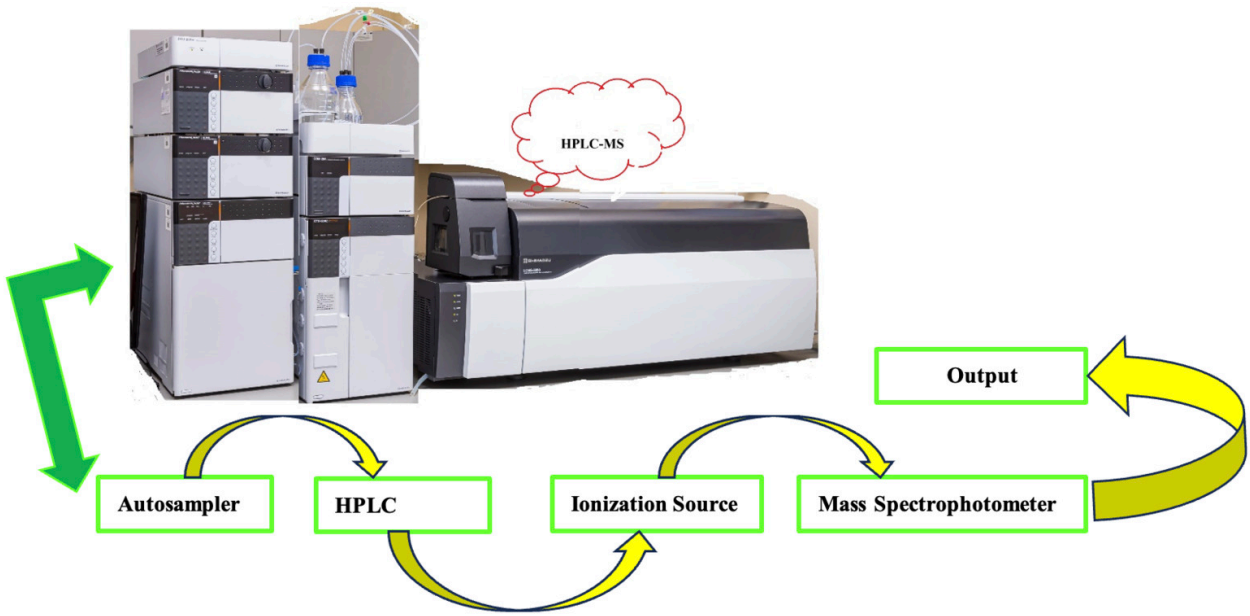
LC is liquid chromatography combined with mass spectrometry (MS), either HPLC–MS (**Scheme 5**) or UPLC–MS (**Scheme 6**). The chromatography method's separation ability and mass spectrophotometry identification facility are combined. MS is a perfect technique that can confirm a compound's structure using its mass spectrum, and no standard is needed. Whereas LC-MS is a hyphenated practice, its fragmentation pattern could provide an idea of the structure of the compound with high selectivity and sensitivity.[45] Also, the isotope peaks show the availability of oxygen, bromine and chlorine peaks.

These techniques are rapid and reliable for determining NTG and RPG as single or combined formulations with other drug substances or in the presence of their active metabolites. The LCMS determination extensively included lower amounts of biological samples[46] to quantify NTG and RPG (**Table 3**). Additionally, fabric phase sorptive extraction (FPSE) enhanced the extraction process with a versatile and convenient practice that ensures lower LOQs, streamlining the method to a significant level.

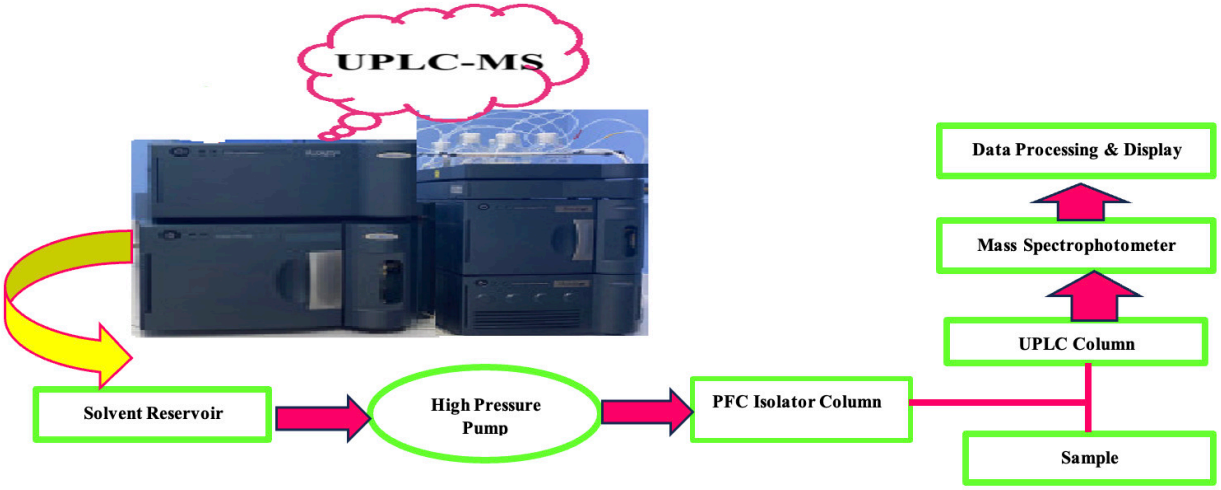
The NTG's degradation products were confirmed with LCMS and characterized as 2-amino-3-phenylpropanoic acid, 2-(cyclohexanecarboxamido)-3-phenylpropanoic acid and 4-isopropyl-N-phenethylcyclohexanecarboxamide. They can be done in acid, base, hydrolysis, and oxidative conditions.[47] The technique was also enhanced by applying a C_{18} gold column, reducing its runtime and boosting the method's ability for excellent sample throughput. The hydrophilic interaction liquid chromatography combined mass spectrophotometry-based assay was developed and validated for 13 antihyperglycaemic drugs, including NTG and RPG,[48] in human urine samples (**Figure 6**). The HILIC instrumentation applied high-quality solvents as a mobile, which enhanced the assay method's sensitivity and claimed to be the first simultaneous quantitative determination of 13 oral antihyperglycaemic drugs. The rapid method confirmed that no procedure was reported in any biological matrix for simultaneous quantification of NTG in a combined form with metformin.[49] The kinetic parameters were determined in solutions, and nine degradation products of RPG were identified[50] that could be beneficial for further findings to count them as new related substances (RS) in the pharmacopoeial profile (**Figure 7**). The UPLC/MS method was quantified with a broader analysis range of 500–4000 ng/mL with a 0.6 mL/min flow rate in plasma samples,[51] including a fast and reproducible solution preparation technique to implement a simple protein precipitation method to study the bioequivalence, pharmacokinetic, therapeutic monitoring and bioavailability studies NTG, an antidiabetic drug agent. However, the parallel artificial liquid membrane extraction technique could be combined with UPLC electrospray ionization tandem mass spectrometry to estimate therapeutic RPG's drug monitoring in diabetic patients efficiently.[52] The developed PALME technique offered a straightforward sample preparation protocol for direct extraction from human plasma. The Analytical Green Calculator (AGREE) tool scrutinised PALME's welfare and ecological impact, and the outcome was an eco-friendly assessment. All the results of the reported methods highlighted the advantage of the developed method in quantitating NTG and RPG from different sources in terms of sample throughput, reagents/solvent consumption, % recovery, selectivity and sensitivity.

3. METHOD DEVELOPMENT, ADVANTAGES AND LIMITATIONS OF THE PROPOSED TECHNIQUES

The drug development process begins with innovating a drug molecule with medicinal value in fighting, examining and curing diseases. It is necessary to characterize the synthesized molecule to initiate preliminary safety and therapeutic effectiveness by analyzing them, which are required to discover drug applicants for further investigations. Pharmaceutical development evidence requires scientific justification for formulation progress and a correct explanation of the dosage form. Regulatory assistance specifies limited information on the data constraints correlated with pharmaceutical development.



Scheme 5. Instrumentation and figure of HPLC-MS.



Scheme 6. Instrumentation and figure of UPLC-MS.

However, additional, comprehensive data is accessible for the toxicological estimation of excipients, which are a significant portion in the solid dosage forms, which assist as diluents to permit the formulation with correctly sized coverings from unwanted organoleptic abilities of the drug substance. The most common excipients, such as lactose, sucrose, cellulose, magnesium stearate, talc, calcium phosphate, starch, etc, are used in pharmaceutical formulations. In specific cases, excipients do not intermingle chemically but could help drug molecule degradation. It is also crucial to understand their chemical stability, impurity identification and quantification, which are beyond the recognised threshold vital to assess the impurities toxicity profiles to discriminate these from its active pharmaceutical ingredients (API) and pharmaceutical formulations.

Spectrophotometry is the numerical measurement of a substance's reflection or transmission assets against wavelength, requiring minimal time and labour consumption. Due to its excellent precision, it enhanced its application in pharmaceuticals. However, the homogeneous solution necessary with optical density must not be low or high. Some components can dissolve only in nonpolar solvents and cannot be used in plastic cuvettes. Also, many components with close characteristic wavelengths in a sample could possibly obstruct each other, and pH needs to be verified cautiously since coloured components are pH-dependent.

Meanwhile, the advanced liquid chromatography technique, which is liable to HPLC, likely helps separate molecules in a complex chemical and biological matrix. The

Table 3. Hyphenated techniques to determine NTG and RPG in pharmaceutical formulations and biological fluids.

Stationary Phase	Mobile Phase	Flow rate (mL/ min)	Linearity (ng/mL)	Applications	References
ZIC-Chilic (150×2.1 mm, 3 µm)	Ammonium formate solution (10 mM) of pH = 6.5 and acetonitrile = 10: 90, v/v	0.2	125–10000	Human plasma	46
YMC-Triart Diol-HILIC column (50×3.0 mm., 3 µm) with C_{18} guard column (4×3.0 mm)	Gradient elution of ammonium formate (1 mM) of pH 5 and acetonitrile	0.35	5–400	Human urine	48
Hypersil Gold C_{18}	Gradient elution of ammonium formate (5 mM) and acetonitrile	0.5	0.01–200	Human plasma	49
ZIC-Chilic (150×2.1 mm, 3 µm)	Ammonium formate solution (10 mM) of pH = 6.5 and acetonitrile = 10: 90, v/v	0.2	6.25–500	Human plasma	46
YMC-Triart Diol-HILIC column (50×3.0 mm., 3 µm) with C_{18} guard column (4×3.0 mm)	Gradient elution of ammonium formate (1 mM) of pH 5 and acetonitrile	0.35	2–300	Human urine	48
Gemini C_{18} 110A (100×2.0 mm, 3 µm)	Solvent A: 10mM ammonium formate (0.1%) and acetonitrile-water (1:99, v/v); Solvent B: 10mM ammonium formate (0.1%) and acetonitrile-water (95:5, v/v)	0.4	—	Tablets	50
Acquity HSS Cyano (100x2.1 mm, 1.8 µm)	Gradient elution of water 0.1% formic acid) and acetonitrile (0.1% formic acid)	0.6	500–4000	Human plasma	51
C_{18} Eclipse-Plus-RRHD (50 × 2.1 mm, 1.8 µm)	Water (0.1 % formic acid) and acetonitrile (0.1 % formic acid) = 62:38, v/v	0.4	0.1–100	Tablet	52

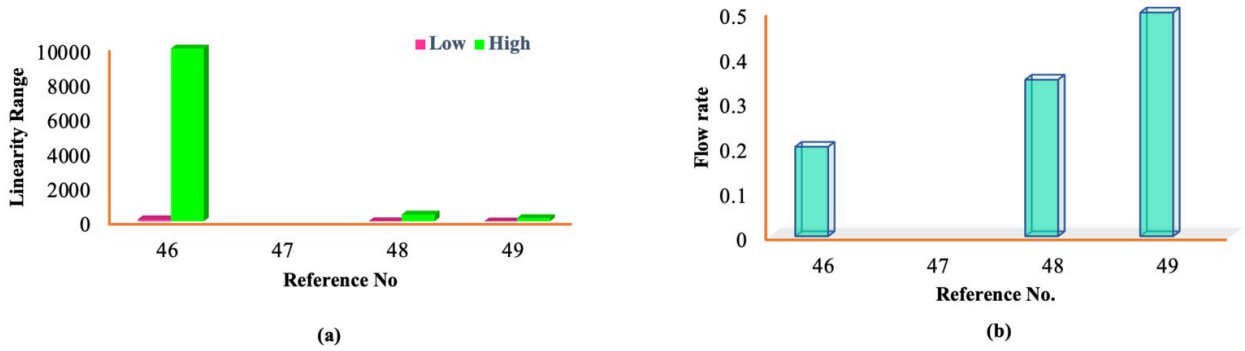


Figure 6. (a) Linearity range (b) Flow rate against reference no. to quantify NTG applying HPLC-MS.

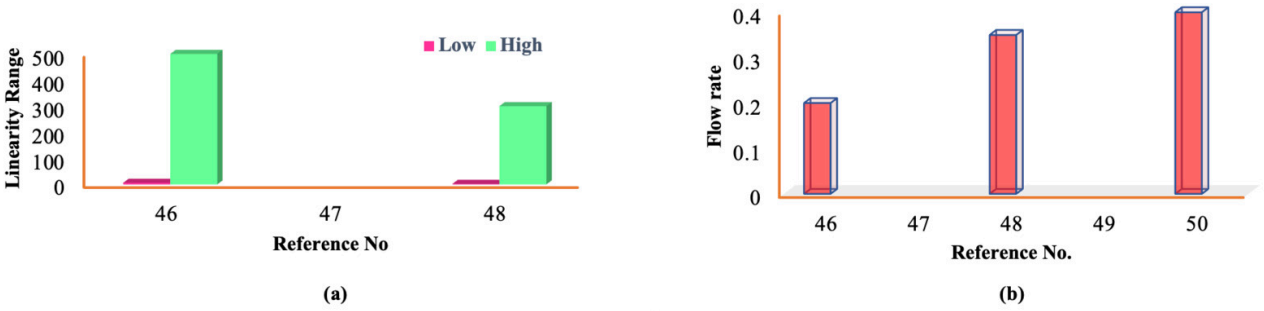


Figure 7. (a) Linearity range (b) Flow rate against reference no. to quantify RPG applying HPLC-MS.

specificity, accuracy and precision results are excellent. However, it also stated that these parameters are only attainable when suitability tests are performed with a wide range. In HPLC, the detection is crucial to ensure all the components are detected. HPLC-UV detectors are widely used and can monitor a range of wavelengths simultaneously. Over a certain period, researchers mostly used UV absorbance with the reversed-phase mode whenever suitable. This offered reliability, sensitivity, repeatability, and analysis time but was restricted by column price, solvents, and column packing materials, ultimately affecting long-term reproducibility. HPTLC is an advanced form of thin-layer chromatography (TLC). Several amendments were made to the primary method of TLC to automate the discrete steps to achieve the enhanced resolution and limit of detection. However, it required a large volume of expensive organics, stringent operation conditions, mainly dust-free environment and temperature control and highly skilled and trained people to run the system. It is also limited to plate length and is indirectly restricted to a specific length for component separation. The developed UPLC promotes advancement in three main areas: sensitivity, resolution, and speed. This technique could work with less than 2 µm fine particles.

Consequently, it reduced the column length and ultimately decreased solvent consumption and run time. However, increased column pressure minimises column life and is necessary for more maintenance. The separation technique's coupling with another leads to the development of hyphenated techniques like LC-MS and UPLC-MS. The LC-MS method is rapid analysis. Quantifying several analytes requires a short time and quickly determining parent drug and their metabolites. However, highly trained and skilled people must apply high-cost reagents, sample pretreatment before each analysis, and complex sample preparation. The UPLC-MS technique is robust, proficient in bioequivalence study, and straightforward for biological fluids with lower LOD and LOQ values. It needs sample derivatization and multi-reaction monitoring with high-cost equipment.

These drugs are noteworthy and are one of the fastest-growing products in the pharmaceutical industry due to their increasing demand. The spectrophotometers are simple to supervise and available everywhere due to their low cost. The biological fluids and trace analysis can be used for susceptible instruments like HPLC, HPTLC, and UPLC combined with the mass spectrophotometer. Every technique has advantages and limitations (**Table 4**), which is also essential to selecting the correct methods for particular samples, depending on the capacity and capability, but never compromising the valuable data directly related to the efficacy and safety of drug products and human health. The innovations of nanotechnology, artificial intelligence, and green analytical chemistry drastically improve the precision and productivity of pharmaceutical analysis to safeguard that drugs execute their expected intent. Various instrumental methods have been built over time for drug evaluation. Therefore, analytical instrumentation and techniques are necessary for identifying and quantifying these drug impurities. Pharmaceutical manufacturing is constantly changing, and there is a considerable attempt to translate new regulatory requirements into practice by implementing new technologies.

4. CONCLUSION

The present review discussed the summary of the reported method as per the literature for estimating RPG and NTG, not only in bulk and pharmaceutical formulations but also in biological matrices. Analytical techniques such as UV-Vis, HPLC, HPTLC, UPLC, HPLC/MS and UPLC/MS were employed to determine drugs for quality control samples. The main objective is to provide academicians, researchers, and scientists with appropriate information about glitinide agents. After studying each technique's capabilities and limitations, people may understand the drug's behaviour and easily enhance new technology that could benefit human health and provide a better, greener methodology. These drugs are critical due to their mounting demands in our daily lives, which is the crucial reason for manufacturing more products in the pharmaceutical industry. HPLC and UV-Vis technology are low-cost, easy to handle, and available, boosting their application for pure and pharmaceutical dosage forms. The biological fluids and trace analysis would go for susceptible instruments combined with a mass spectrophotometer. Therefore, the present review discusses various analytical methods and compares efficacy when applied to NTG and RPG. Also, ideally, it provides insights into which strategies are most effective un-

Table 4. Advantages and Limitations of the proposed techniques to quantify NTG and RPG.

Techniques	Advantages	Limitations
Spectrophotometry	• Relatively simple to use • Accurate • Required a relatively small volume • Wide analysis range • Low cost • Less electric power consumption	• The other components with similar wavelengths in the sample can absorb light as the interset analyte, leading to interference that could affect the results. • Samples preparation with extraction or dilution added additional steps could be an error source. • It provides the compound's total concentration without any structural or chemical data of the analyte. • It may not be sensitive enough to detect analyte's very low concentrations, particularly in complex matrices. • Imperfect equipment designs due to stray light, detectors affected signal measurement and reduced sensitivity. • RPG and NTG are practically insoluble in water and primarily soluble in methanol/organic solvents and acids, which could deviate from green analysis and possibly reduce the technique's efficacy.
HPLC	• Automated and fast technique • Excellent sensitivity and specificity • Identifying specific components in a sample mixture • Wide range of flow rates • Gradient solvent system applied for specific analyses • Quick and precise quantitative analysis • High resolution and repeatability • It has improved and innovative separation efficacy and detection limits.	• It required expensive organics that increased analysis costs. • Troubleshooting could be a problem when developing new methods. • Pump process consistency depends on sample cleanliness and mobile phase degassing. • It is challenging to use coelution and adsorbed analytes. • It could have reproducibility challenges with column-to-column and batch-to-batch samples. • Higher electricity consumption • Necessary high-skilled analyst
HPTLC	• Higher detection and separation efficiency • Appropriate for matrix sample • Acceptable sensitivity • There are no requirements for sample clean-up • Efficacy with nanogram samples and quantified with higher efficiency. • Mobile phase filtration is not necessary.	• It is a fragile and heavy-weight glass at a high cost. • Plate length is constrained for the separation. • Aluminium plate loses their shape at high temperatures, likely more than 120°C. • It required rigorous operating conditions. • Inadequacy of standardization • Lack of reproducibility • Lack of sensitivity and poor precision. • It required a skilled analyst to run the system.
UPLC	• It showed higher sensitivity, selectivity and dynamic range. • It provides a speedy analysis to quantify parent drug and their products. • Less solvent consumption. • The method's efficacy offers a broad flow rate range, significantly reducing analysis time.	• Low particle size developed high pressure that reduced column life. • Regular maintenance is required. • High cost. • Necessary skilled and trained analyst. • Higher electric consumption • Silica-based particles have terrific mechanical strength but demonstrated peak tailing with basic components at a limited pH range. Polymeric columns could help to overcome this problem, but they showed reduced efficacy and limited capability.
HPLC-MS	• It is the most robust method with higher sensitivity and selectivity. • It required a smaller run time to quantify parent drugs and their metabolites. • A higher degree of automation. • Better reproducibility.	• Complex sample preparation and procedure. • Analyte's pretreatment before each analysis • Expensive reagent cost and chromatograph. • High maintenance cost. • Phosphate buffer is not friendly with LCMS but is regularly used in LC progress. • Higher electric consumption • Operating and data analysis requires a skilled and trained person.

Techniques	Advantages	Limitations
	• The method's efficacy is in quantifying drugs and their impurities with precise molecular weight and a wider range of samples.	
UPLC-MS	• Fast and accurate • Better detection level • Robust and sensitive • Competent bioequivalence study • Low LOD and LOQ value • Low noise and high sensitivity • Solvent consumption • Very little run time could be 1 minute • Therapeutic drug monitoring is the most reliable method for assessing drug safety and efficacy.	• High equipment cost • Multi reaction monitoring • Higher electric consumption • Required sample derivatization • Higher electric consumption • Operating and data analysis requires a skilled and trained person.

der specific conditions for determination and quantification in API, pharmaceutical tablets, and biological fluids.

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REFERENCES

1. Bassin SR, Srinath R. The impact of physical activity in patients with type 2 diabetes. *American Journal of Lifestyle Medicine*. Published online 2023. doi:10.1177/15598276231180541

2. DeMarsilis A, Reddy N, Boutari C, et al. Pharmacotherapy of type 2 diabetes: An update and future directions. *Metabolism*. 2022;137:155332. doi:10.1016/j.metabol.2022.155332

3. Yachmaneni A Jr, Jajoo S, Mahakalkar C, Kshirsagar S, Dhole S. A comprehensive review of the vascular consequences of diabetes in the lower extremities: current approaches to management and evaluation of clinical outcomes. *Cureus*. 2023;15(10):e47525. doi:10.7759/cureus.47525

4. Chen R, Ovbiagele B, Feng W. Diabetes and stroke: epidemiology, pathophysiology, pharmaceuticals and outcomes. *The American Journal of the Medical Sciences*. 2016;351(4):380-386. doi:10.1016/j.amjms.2016.01.011

5. Shukla V, Shakya AK, Perez-Pinzon MA, Dave KR. Cerebral ischemic damage in diabetes: an inflammatory perspective. *Journal of Neuroinflammation*. 2017;14:21. doi:10.1186/s12974-016-0774-5

6. Dilworth L, Facey A, Omoruyi F. Diabetes mellitus and its metabolic complications: the role of adipose tissues. *International Journal of Molecular Sciences*. 2021;22(14):7644. doi:10.3390/ijms22147644

7. Li Y, Chen L, Xu Y, et al. Helical-like assembly of nateglinide as coating for oral delivery of insulin and their synergistic prevention of diabetes mellitus. *Advanced Science*. 2023;10:2301879. doi:10.1002/advs.202301879

8. Wang LC, Fang FS, Gong YP, Yang G, Li CL. Characteristics of repaglinide and its mechanism of action on insulin secretion in patients with newly diagnosed type-2 diabetes mellitus. *Medicine (Baltimore)*. 2018;97(38):e12476. doi:10.1097/MD.0000000000012476

9. Haque SM. Validated kinetic spectrophotometric methods to optimize robustness study with Youden factorial combinations to determine repaglinide using response surface methodology via Box–Behnken design. *Arabian Journal of Science Engineering*. 2023;48:129-144. doi:10.1007/s13369-022-06782-w

10. Bhavyasri K, Begum S, Sowjanya D, Sumakanth M. Spectrophotometric method and its validation for repaglinide in its bulk and formulation dissolution samples including stress studies. *International Journal of Pharmaceutical Sciences and Clinical Research*. 2022;2(3):85-90.

11. Kharbade S, Asnani A, Pratyush K. Development and validation of UV spectrophotometric method for simultaneous estimation of metformin HCl and repaglinide in pharmaceutical formulation. *Journal of Drug Delivery and Therapeutics*. 2019;9(3):344-347. doi:10.22270/jddt.v9i3.2676

12. Serap SA, Berna Y. Derivative spectrophotometric and isocratic high performance liquid chromatographic methods for simultaneous determination of repaglinide and metformin hydrochloride in pharmaceutical preparations. *American Journal of Analytical Chemistry*. 2017;8:541-552. doi:10.4236/ajac.2017.89039

13. Mishra J, Nayak SK, Sahoo SK. Development, validation and stability study of UV spectrophotometric method for determination of repaglinide in bulk and pharmaceutical dosage forms. *Journal of Innovations in Applied Pharmaceutical Sciences*. 2016;1(3):10-16.

14. Nishith P, Kanu RP. Development and validation of UV spectrophotometric method for simultaneous estimation of metformin HCl and repaglinide in bilayer tablet. *Journal of Pharmaceutical Science and Bioscientific Research*. 2015;5(1):104-109.

15. Manal MF, Noha SR. Development and validation of chromatographic and spectroscopic methods for estimation of repaglinide and metformin HCl in combined dosage form. *Journal of Global Trends in Pharmaceutical Sciences*. 2014;5(3):1844-1848.

16. Dhole SM, Khedekar PB, Amnerkar ND. Comparison of UV spectrophotometry and high performance liquid chromatography methods for the determination of repaglinide in tablets. *Pharm Methods*. 2012;3:68-72. doi:10.4103/2229-4708.103875

17. Haque SM. Optimized Box–Behnken experimental design based response surface methodology and Youden's robustness test to develop and validate methods to determine nateglinide using kinetic spectrophotometry. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2022;268:120712. doi:10.1016/j.saa.2021.120712

18. Venkatesh S, Jyothi S, Chiluka R, Padmavathi Y. Development of UV- spectrophotometric method for the simultaneous estimation of nateglinide and piperine in combined tablet dosage form. *Asian Journal of Pharmaceutical Analysis*. 2020;10(3):134-140. doi:10.5958/2231-5675.2020.00024.1

19. Babu NR, Padmavathi Y, Babu RS, Unnisa A, Bhavana M, Kumar PR. Estimation of nateglinide by using MBTH as a chromogenic reagent. *Asian Journal of Pharmaceutical Analysis*. 2019;9(4):199-204. doi:10.5958/2231-5675.2019.00033.4

20. Basavaiah K, Rajendraprasad N. Stability indicating UV spectrophotometric methods for the determination of nateglinide in pharmaceuticals. *Austin Journal of Analytical and Pharmaceutical Chemistry*. 2018;5(1):1096.

21. Mastanamma S, Sukeerthi K. UV-spectrophotometric determination of nateglinide in bulk and pharmaceutical dosage form using hydrotropic solubilization technique. *International Journal of Pharmaceutical Sciences and Drug Research*. 2016;8(3):153-156. doi:10.25004/IJPSDR.2016.080305

22. Babu GR, Rao AL, Surekha PSL, Kalapraveen T, Rao PS. Quantitative estimation of nateglinide in pharmaceutical dosage forms by visible spectrophotometry. *International Journal of Research in Pharmacy and Chemistry*. 2013;3(4):803-807.

23. Smerikarova M, Bozhanov S, Mateeva A, Maslarska V. Development of simple HPLC-UV method for the simultaneous determination of repaglinide, dexamethasone, and remdesivir, and its application to synthetic mixture and human plasma. *Current Pharmaceutical Analysis*. 2023;19:662-672. doi:10.2174/0115734129263384230928052923

24. Bhavyasri K, Begum S, Sumakanth M. Novel RP-HPLC method development and validation of metformin HCl and repaglinide in bulk and tablet dosage form. *International Journal of Pharmaceutical and Phytopharmacological Research*. 2023;13(1):7-12. doi:10.51847/Xc9UhmnyiY

25. Patan A, Basha SR, Ketha RK, Cheriyan BV, Muthukumar VA. Development and validation of new RP- HPLC method for the simultaneous estimation of metformin hydrochloride and repaglinide in pure and pharmaceutical formulations. *Research Journal of Pharmacy and Technology*. 2021;14(3):1323-1328. doi:10.5958/0974-360X.2021.00235.3

26. Navamanisubramanian R, Panchagiri S, Nerella R, Duraipandian C, Seetharaman S. Stability indicating RP-HPLC method for estimation of repaglinide in rabbit plasma. *International Journal of Applied Pharmaceutics*. 2019;11(3):206-210. doi:10.22159/ijap.2019v11i3.33675

27. Han DG, Kwak J, Seo SW, et al. Pharmacokinetic evaluation of metabolic drug interactions between repaglinide and celecoxib by a bioanalytical HPLC method for their simultaneous determination with fluorescence detection. *Pharmaceutics*. 2019;11(8):382. doi:10.3390/pharmaceutics11080382

28. Elkady EF, El-Zaher AA, Elwy HM, Saleh MA. Validated liquid chromatographic method for simultaneous determination of metformin, pioglitazone, sitagliptin, repaglinide, glibenclamide and gliclazide-application for counterfeit drug analysis. *Journal of Analytical & Bioanalytical Techniques*. 2015;S13:007. doi:10.4172/2155-9872.S13-007

29. Krishna BS, Yamineswari P, Pravallika JB, et al. Analytical method development and validation of nateglinide and metformin by RP-HPLC. *Indo American Journal of Pharmaceutical Sciences*. 2023;10(4):68-77.

30. Bhabad AA, Jadhav PB. RP-HPLC method development and validation for simultaneous estimation of nateglinide and metformin. *International Journal of Creative Research Thoughts*. 2022;10(7):b1-b16.

31. Rana K, Sharma P. Development and validation of a HPLC method for the determination of metformin hydrochloride, nateglinide and pioglitazone hydrochloride in multicomponent formulation. *Asian Journal of Research in Chemistry*. 2021;14(1):7-12. doi:10.5958/0974-4150.2021.00002.X

32. Ozkurt C, Unal A, Killic O, Yilmaz DC. Determination of nateglinide in tablet formulation by HPLC using a pentafluorophenyl core-shell column. *Journal of the Institute of Science and Technology*. 2021;11(1):413-419.

33. Dey S, Basak S, De A, et al. Simultaneous pharmacokinetics estimation of nateglinide and pioglitazone by RP-HPLC: computational study to unlock the synergism. *Journal of Chromatographic Science*. 2020;58(4):309-322. doi:10.1093/chromsci/bmz116

34. Chengalva P, Angala Parameswari SA, Aruna G. Development and validation of RP-HPLC method for metformin hydrochloride and nateglinide in bulk and combined dosage form. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2016;8(4):267-271.

35. Hacioglu A, Citlak A, Karakus S. Development and validation of an HPLC method for determination of nateglinide in drug substances. *Marmara Pharmaceutical Journal*. 2015;19:103-108. doi:10.12991/mpj.2015199640

36. Pani NR, Nath L, Singh AV, Mahapatra SK. Development and validation of analytical method for the estimation of nateglinide in rabbit plasma. *Journal of Pharmaceutical Analysis*. 2012;2(6):492-498. doi:10.1016/j.jpha.2012.05.002

37. Haque SM, Ratemi ES. Drug development and analysis review. *Pharmaceutical Chemistry Journal*. 2017;50:837-850. doi:10.1007/s11094-017-1543-1

38. Patil PM, Tekade M, Agarkar S, Taleuzzaman M. Isolation and characterisation of nateglinide and its impurity in bulk and marketed formulation by HPTLC method. *Research Journal of Pharmacy and Technology*. 2023;16(5):2343-2346. doi:10.52711/0974-360X.2023.00385

39. Abdelhamid NS, Elsaady MT, Ali NW, Abuelazem WG. Simultaneous determination of repaglinide, metformin hydrochloride and melamine by new HPLC and HPTLC chromatographic methods. *Analytical Chemistry Letters*. 2019;9(3):418-429. doi:10.1080/22297928.2019.1622450

40. Akhtar J, Fareed S, Aqil M. Stability-indicating assay of repaglinide in bulk and optimized nanoemulsion by validated high performance thin layer chromatography technique. *Journal of Pharmacy & Bioallied Sciences*. 2013;5(3):184-190. doi:10.4103/0975-7406.116800

41. Thomas AB, Patil SD, Nanda RK, Kothapalli LP, Bhosle SS, Deshpande AD. Stability-indicating HPTLC method for simultaneous determination of nateglinide and metformin hydrochloride in pharmaceutical dosage form. *Saudi Pharmaceutical Journal*. 2011;19(4):221-231. doi:10.1016/j.jsps.2011.06.005

42. Narikimalli A, Galla R. A stability indicating UPLC method development and validation for the simultaneous estimation of nateglinide and metformin hydrochloride in bulk and tablet dosage form. *Future Journal of Pharmaceutical Sciences*. 2023;9:55. doi:10.1186/s43094-023-00503-2

43. Rani AP, Madhavi S, Rao BT, Reddy CHS. Stability indicating UPLC method for estimation of metformin hydrochloride and nateglinide simultaneously in the presence of stress degradation products. *Indian Drugs*. 2020;57(5):56-64. doi:10.53879/id.57.05.11332

44. Xavier CM, Basavaiah K. Development and validation of a simple stability indicating UPLC method for the determination of repaglinide in pharmaceuticals. *Thai Journal of Pharmaceutical Sciences*. 2013;37:84-94.

45. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. *Arabian Journal of Chemistry*. 2017;10(Supplement 1):S1409-S1421. doi:10.1016/j.arabjc.2013.04.016

46. Stamou P, Parla A, Kabir A, et al. Hydrophilic interaction liquid chromatography–electrospray ionization mass spectrometry combined with fabric phase sorptive extraction for therapeutic drug monitoring of pioglitazone, repaglinide, and nateglinide in human plasma. *Journal of Chromatography B*. 2023;1217:123628. doi:10.1016/j.jchromb.2023.123628

47. Kumari P, Babbar R, Singh R, Saini B. Identification and characterization of degradation products of nateglinide. *International Journal of Health Sciences*. 2022;6(S2):12894-12904. doi:10.53730/ijhs.v6nS2.8413

48. Truong QK, Mai XL, Lee JY, et al. Simultaneous determination of 14 oral antihyperglycaemic drugs in human urine by liquid chromatography–tandem mass spectrometry. *Archives of Pharmacal Research*. 2018;41:530-543. doi:10.1007/s12272-018-1011-9

49. Chunduri RHB, Dannana GS. Development and validation of LC-MS/MS method for simultaneous quantification of metformin and nateglinide in human plasma and its application to a pharmacokinetic study. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2016;5(2):651-667.

50. Gumieniczek A, Berecka-Rycerz A, Mroczek T, Wojtanowski K. Determination of chemical stability of two oral antidiabetics, metformin and repaglinide in the solid state and solutions using LC-UV, LC-MS, and FT-IR methods. *Molecules*. 2019;24(24):4430. doi:10.3390/molecules24244430

51. Fachi MM, Cerqueira LB, Leonart LP, Francisco TMGd, Pontarolo R. Simultaneous quantification of antidiabetic agents in human plasma by a UPLC-QToF-MS method. *PloS One*. 2016;11(12):e0167107. doi:10.1371/journal.pone.0167107

52. Sameh AA, Nehad AA, Mohammed A, Yaser MA. Parallel artificial liquid membrane extraction coupled with UPLC-ESI-MS/MS method for high-throughput quantitation of repaglinide in diabetic patients. *Talanta*. 2024;269:12598. doi:10.1016/j.talanta.2023.125498