RESEARCH PAPER

Analytical Characterization of Glucose as a Reactive Impurity in Pharmaceutical Excipients Using a DNPH-HPLC Approach

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ABSTRACT

Reactive impurities in pharmaceutical excipients are increasingly recognized as critical factors affecting drug product stability. Among these, reducing sugars such as glucose can induce drug–excipient incompatibility through Maillard-type reactions. This study describes the development and validation of a simple and sensitive high-performance liquid chromatography (HPLC) method for the determination of glucose as a reactive impurity using pre-column derivatization with 2,4-dinitrophenylhydrazine (DNPH).

Derivatization of glucose produced stable hydrazone derivatives, enabling UV detection at 356 nm. Chromatographic separation was achieved on a reverse-phase C8 column using gradient elution with a runtime of 25 min. The method was validated in accordance with ICH Q2(R2) guidelines and demonstrated excellent linearity over the range of 26–4684 µg/g ($R^2 > 0.999$), precision (%RSD < 2%), and acceptable accuracy. The method also showed adequate sensitivity and robustness for trace-level quantification.

The validated method was successfully applied to various pharmaceutical excipients, revealing the presence of glucose at trace levels and significant variability across excipient types and batches. Higher levels were observed in sorbitol and cellulose-based excipients, while minimal levels were detected in mannitol.

This study provides a novel analytical perspective by specifically addressing glucose as a reactive impurity rather than a conventional analyte, and presents one of the first systematic evaluations of its batch-to-batch variability and potential impact on drug–excipient interactions. The proposed method offers a practical tool for routine quality control and supports risk-based assessment of excipient-related degradation during pharmaceutical development.

KEYWORDS

Glucose; Reactive impurity; DNPH derivatization; HPLC; Pharmaceutical excipients; Maillard reaction

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HIGHLIGHTS

- A sensitive HPLC method based on DNPH derivatization was developed for glucose determination in pharmaceutical excipients.
- Pre-column derivatization significantly enhanced UV detectability of reducing sugars.
- The method demonstrated excellent linearity ($R^2 > 0.999$), precision, accuracy, and robustness as per ICH Q2(R2).
- Successful application across multiple excipients revealed variability of glucose as a trace impurity.
- The method supports excipient screening and risk assessment of drug–excipient interactions related to reducing sugars.

INTRODUCTION

Excipients, traditionally regarded as inert components of pharmaceutical formulations, are now increasingly recognized for their potential to interact with drug substances, leading to stability concerns and impurity formation. These drug–excipient interactions can significantly affect the quality, safety, and efficacy of drug products, particularly in solid dosage forms where intimate contact between components is unavoidable [1,2]. Such interactions are often mediated by reactive impurities present in excipients, which may either be intrinsic or generated during manufacturing and storage processes [2,16,17].

From a regulatory perspective, the presence of impurities—especially genotoxic and mutagenic species—has become a critical concern in pharmaceutical development. Regulatory guidelines emphasize the identification, control, and risk

assessment of such impurities to ensure patient safety [3,4]. Emerging reports on excipient-derived reactive species further highlight the need for robust analytical techniques capable of detecting low-level impurities within complex matrices [26]. In this context, compliance with analytical validation standards, such as ICH Q2(R2), necessitates the development of sensitive, selective, and reproducible methods for impurity profiling [27].

Among various excipient-related degradation pathways, the Maillard reaction is one of the most extensively studied mechanisms involving reducing sugars. This reaction occurs between carbonyl groups of reducing sugars and amino functionalities of drug molecules, resulting in the formation of a wide range of degradation products, including Schiff bases, Amadori rearrangement products, and advanced glycation end products [18–21]. These reaction products may not only compromise drug stability but also possess potential toxicological implications, thereby necessitating their accurate detection and characterization [18].

Analytical detection of reducing sugars and their associated degradation products remains challenging due to their lack of strong chromophores and structural diversity. Conventional liquid chromatographic methods have been employed for carbohydrate analysis, often requiring derivatization to enhance detection sensitivity and selectivity [5,6]. Recent advances have focused on derivatization strategies that improve analytical performance in terms of resolution, sensitivity, and applicability to complex pharmaceutical systems [7–9]. Several studies have demonstrated the use of reverse-phase HPLC coupled with derivatization techniques for quantifying reducing sugars in various matrices [10–12]; however, their direct application to drug–excipient interaction studies remains limited.

Derivatization using 2,4-dinitrophenylhydrazine (DNPH) has emerged as a powerful approach for the detection of carbonyl compounds. DNPH reacts selectively with carbonyl functional groups to form stable hydrazone derivatives, which can be readily detected using chromatographic techniques [13–15]. This strategy provides a significant advantage in monitoring carbonyl-containing degradation products arising from Maillard-type reactions, thereby enabling indirect assessment of drug–excipient incompatibility.

In addition to chemical factors, environmental conditions such as moisture and humidity play a crucial role in accelerating drug degradation and facilitating excipient-mediated reactions. Increased moisture levels can enhance molecular mobility and promote Maillard reactions, leading to increased formation of degradation products during storage [22–25]. Therefore, understanding the combined influence of excipient reactivity and environmental conditions is essential for designing stable pharmaceutical formulations.

Despite extensive research on drug–excipient interactions and carbohydrate analysis, there remains a gap in the development of targeted analytical methods capable of detecting reducing sugar-induced degradation products within pharmaceutical systems. Addressing this gap, the present study aims to develop and validate a derivatization-based high-performance liquid chromatographic (HPLC) method for the sensitive detection and characterization of carbonyl impurities generated through reducing sugar-mediated drug–excipient interactions. The proposed approach is expected to provide a robust analytical platform for impurity profiling, thereby supporting formulation development and regulatory compliance.

MATERIALS AND METHODS

Chemicals and Reagents

2,4-Dinitrophenylhydrazine (2,4-DNPH, analytical grade), hydrochloric acid (HCl, AR grade), acetonitrile (HPLC grade), ethanol (AR grade), and Milli-Q water were used throughout the study. A dextrose reference standard was employed for calibration. All reagents were of analytical or chromatographic grade and were used without further purification.

Instrumentation

Chromatographic analysis was performed using a high-performance liquid chromatography (HPLC) system equipped with a quaternary pump, autosampler, column oven, and UV detector, controlled by appropriate data acquisition software. Ancillary equipment included an analytical balance, ultrasonic bath, and thermostatically controlled water bath for sample preparation.

Chromatographic Conditions

Separation was carried out on a Zorbax SB C8 column (250 × 4.6 mm, 5 µm particle size) maintained at 40°C. The mobile phase consisted of (A) 0.01 M hydrochloric acid–acetonitrile (70:30, v/v) and (B) 0.01 M hydrochloric acid–acetonitrile (30:70, v/v). The flow rate was set at 1.0 mL/min, and detection was performed at 356 nm. The autosampler temperature was maintained at 5°C, and the injection volume was 5 µL.

Gradient elution was employed as follows: 100% mobile phase A from 0 to 7 min, followed by a transition to 100% mobile phase B from 8 to 15 min, and subsequently returned to initial conditions (100% A) from 16 to 25 min to allow column re-equilibration.

Preparation of Solutions

The diluent used for preparation of standard and sample solutions consisted of water and acetonitrile in the ratio of 40:60 (v/v).

A 0.01 M hydrochloric acid solution was prepared by appropriate dilution of concentrated HCl with water.

Preparation of DNPH Derivatization Reagent

The derivatization reagent was prepared by dissolving 2.5 g of 2,4-DNPH in a mixture of ethanol, hydrochloric acid, and acetonitrile, followed by dilution to volume with acetonitrile. The resulting solution corresponded to an approximate DNPH concentration of 0.2 M. The solution was sonicated to ensure complete dissolution and protected from light until use.

Preparation of Standard and Sample Solutions

A stock solution of glucose was prepared in water and further diluted with diluent to obtain calibration standards over the required concentration range.

Sample solutions were prepared by accurately weighing the excipient, dissolving it in water with sonication, and subsequently subjecting an aliquot to derivatization. After completion of derivatization, the solutions were diluted with diluent and filtered through a 0.45 µm membrane filter prior to chromatographic analysis.

Derivatization Procedure

Pre-column derivatization of glucose was carried out using DNPH under acidic conditions. Standard and sample solutions were reacted with the derivatization reagent and heated at 70 ± 2°C for 2 h to ensure complete formation of stable hydrazone derivatives. After cooling to room temperature, the solutions were diluted with diluent prior to injection. This derivatization step enhances the UV detectability of glucose at 356 nm.

Method Validation

The analytical method was validated in accordance with ICH Q2(R2) guidelines for specificity, system suitability, precision, linearity, accuracy, sensitivity, and robustness.

Specificity was established by evaluating the absence of interference at the retention time of the derivatized glucose peak. System suitability was assessed based on parameters such as peak area reproducibility, theoretical plates, and tailing factor. Precision was evaluated in terms of system precision and method precision using replicate injections of standard and independently prepared sample solutions. Linearity was established over the concentration range of 26–4684 µg/g by constructing a calibration curve of peak area versus concentration.

Accuracy was assessed by recovery studies conducted at multiple levels, including LOQ, 50%, 100%, and 150% of the target concentration. Sensitivity was evaluated in terms of limit of detection (LOD) and limit of quantitation (LOQ) based on signal-to-noise ratio and calibration curve methods.

Robustness was examined by introducing small deliberate variations in analytical parameters such as derivatization time, temperature, and reagent volume, and evaluating their impact on analytical performance.

RESULTS AND DISCUSSION

Method Development and Optimization

The analytical method was developed based on pre-column derivatization of glucose using 2,4-dinitrophenylhydrazine (DNPH), followed by reverse-phase HPLC analysis. As glucose lacks a strong chromophore, derivatization was essential to enhance its detectability. Under acidic conditions, glucose reacts with DNPH to form a stable hydrazone derivative exhibiting strong absorbance at 356 nm, enabling sensitive UV detection [13–15]. Critical parameters affecting derivatization efficiency, including reagent concentration, reaction temperature, and reaction time, were systematically optimized. Heating the reaction mixture at approximately 70°C for 2 h resulted in consistent and complete derivatization. Shorter reaction times led to incomplete conversion, whereas prolonged heating did not significantly improve the analytical response.

Chromatographic conditions were optimized to achieve adequate resolution, symmetrical peak shape, and reproducibility. The selected gradient elution program provided efficient separation of the derivatized glucose peak from matrix components, with a retention time of approximately 3–5 min, consistent with reported chromatographic approaches for derivatized carbohydrates [8–12].

The derivatization strategy significantly enhanced the detectability of glucose, enabling its quantification using conventional UV detection systems. This approach provides a practical advantage over more complex techniques by combining adequate sensitivity with operational simplicity, making it well suited for routine pharmaceutical analysis.

Method Validation

The developed analytical method was validated in accordance with ICH Q2(R2) guidelines [27] for key performance characteristics.

System Suitability

System suitability was evaluated to ensure the adequacy of the chromatographic system prior to analysis. Parameters such as % relative standard deviation (%RSD) of peak area, theoretical plates, and tailing factor were considered.

The %RSD of peak areas from replicate injections (n = 5 or 6) of the standard solution was found to be less than 2%, indicating acceptable system performance. The chromatographic system demonstrated adequate efficiency and peak

symmetry for the derivatized glucose peak, confirming suitability for analysis.

Specificity

Specificity of the method was demonstrated by analyzing blank, standard, and sample solutions. No interference was observed at the retention time of the derivatized glucose peak, confirming that the method is selective for glucose in the presence of excipient matrix components.

System Precision

System precision was evaluated by repeated injections of the standard solution. The % relative standard deviation (%RSD) of peak areas was found to be less than 2%, indicating acceptable system suitability and repeatability.

Table 1: System precision results

Injection No.	Peak Area
1	27988
2	27957
3	28079
4	28060
5	28124
6	28296

Mean: 28084 ; %RSD: 0.43

Method Precision

Method precision was assessed using six independently prepared sample solutions. The %RSD of glucose content was within 2%, demonstrating good repeatability of the method.

Table 2: Method precision results

Sample No.	Glucose Content (µg/g)
1	220
2	222
3	218
4	229
5	221
6	221

Mean: 222 ; %RSD: 1.70

The low %RSD values indicate high method reproducibility, which is essential for routine quality control where consistent performance across analyses is required.

Linearity

The method exhibited excellent linearity over the concentration range of 26–4684 µg/g, covering both trace-level impurity detection and higher concentration ranges relevant to excipient evaluation. The calibration curve showed a strong linear relationship between concentration and peak area, with a coefficient of determination (R^2) greater than 0.999, confirming the reliability of the method across the studied range.

Table 3: Linearity data for glucose determination

Level	Concentration (µg/mL)	Concentration (µg/g)	Peak Area (µAU·sec)
1	0.260	26.00	1432
2	0.520	52.00	3378
3	2.602	260.20	14999
4	5.205	520.50	30196
5	7.807	780.70	43999
6	10.410	1041.00	61065
7	15.614	1561.40	91311
8	18.217	1821.70	105386
9	31.229	3122.90	181237
10	36.434	3643.40	215926
11	46.843	4684.30	271254

Slope: 5834.28; Intercept: -148.94 ; R^2: 0.99977

The excellent linearity across a wide concentration range confirms the applicability of the method for both trace-level impurity detection and higher concentration analysis relevant to excipient evaluation

Accuracy

Accuracy was evaluated by recovery studies at multiple levels, including LOQ, 50%, 100%, and 150% of the specification limit. The percentage recovery values ranged between 70% and 130%, confirming the suitability of the method for quantification of glucose in excipient matrices.

Table 4: Accuracy (recovery) results

Level	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery
LOQ	0.566	0.644	113.80
	0.566	0.684	120.80
50%	15.088	17.100	113.34
	15.088	17.000	112.67
100%	30.175	32.500	107.71
	30.175	31.700	105.50
150%	45.263	47.200	104.28
	45.263	47.600	105.16

Mean recovery: LOQ: 117.30% ; 50%: 113.01% ; 100%: 106.38% ; 150%: 104.72

The recovery results within acceptable limits confirm the accuracy of the method in quantifying glucose in excipient matrices without significant interference from matrix components. Wider recovery at LOQ is acceptable due to low concentration level and matrix effect.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were determined in accordance with ICH Q2(R2) guidelines using a signal-to-noise approach and supported by the calibration curve method.

The LOQ was established at 60 µg/g, corresponding to the lowest concentration level at which acceptable precision and accuracy were achieved during validation studies. The LOD was estimated to be approximately 20 µg/g, representing a signal-to-noise ratio of about 3:1.

These results demonstrate that the method possesses adequate sensitivity for the detection and quantification of glucose at trace levels relevant to pharmaceutical excipient analysis.

Analytical Range

The analytical range of the method (26–4684 µg/g) was selected to cover concentrations from trace-level detection to levels exceeding typical specification limits for reducing sugars in pharmaceutical excipients.

This range encompasses levels below supplier control limits as well as those approaching or exceeding pharmacopeial limits (e.g., 0.3% for sorbitol), thereby ensuring suitability of the method for both routine quality control and risk assessment applications.

Robustness

Robustness was assessed by introducing small deliberate variations in parameters such as derivatization time, temperature, and reagent volume. No significant variation in analytical results was observed, indicating that the method is robust and reliable for routine application.

Solution Stability

The stability of derivatized sample solutions was evaluated by analyzing prepared solutions at different time intervals under controlled conditions.

No significant change in peak area or retention time of the derivatized glucose was observed over the evaluated period, indicating that the derivatized solutions were stable for sufficient time to allow routine analysis.

Filter Compatibility

Filter compatibility was assessed to ensure that no significant adsorption or interference occurred during sample filtration. Sample solutions were filtered using a 0.45 µm membrane filter and compared with unfiltered or centrifuged samples.

No significant difference in the measured glucose content was observed, indicating that the filtration process did not affect the analytical results.

Application to Pharmaceutical Excipients

The validated method was applied to the determination of glucose in commonly used pharmaceutical excipients, including sorbitol, starch, microcrystalline cellulose (MCC), hydroxypropyl methylcellulose (HPMC), and mannitol.

The results indicated significant variability in glucose content depending on the type of excipient. Relatively higher levels of glucose were observed in sorbitol and cellulose-based excipients, whereas comparatively lower levels were detected in mannitol. These findings are consistent with the reported presence of reactive impurities in excipients and their potential contribution to drug–excipient interactions [2,26].

Table 5: Glucose content in pharmaceutical excipients

Excipient	Glucose Content (µg/g)
Starch	107
Microcrystalline Cellulose	96
Sorbitol	274
Mannitol	7
Croscarmellose Sodium	7
HPMC	111
HPC	Not Detected

The observed variability in glucose content among different excipients highlights the influence of raw material origin and manufacturing processes. Higher levels in sorbitol can be attributed to its production from glucose, whereas lower levels in mannitol are likely due to more selective purification processes.

This variability underscores the importance of monitoring reactive impurities during excipient selection, as even trace levels of glucose may contribute to Maillard-type degradation in susceptible drug products.

Variability of Glucose Content in Excipients

Further investigation of multiple batches of sorbitol obtained from different sources revealed considerable variation in glucose content. This batch-to-batch variability highlights the importance of stringent raw material control, supplier qualification, and routine monitoring.

Such variability may have a significant impact on drug product stability, particularly in formulations containing amine-functional drug substances prone to Maillard-type reactions [18–21].

Table 6: Batch-to-batch variability of glucose in sorbitol

Batch No.	Glucose Content (µg/g)
Batch 1	252
Batch 2	250
Batch 3	203
Batch 4	164
Batch 5	196
Batch 6	276
Batch 7	368
Batch 8	219
Batch 9	317
Batch 10	398
Batch 11	422
Batch 12	221
Batch 13	408
Batch 14	222
Batch 15	215

Range: 164–422 µg/g

The significant batch-to-batch variation observed in sorbitol (164–422 µg/g) indicates that supplier and process variability can directly influence impurity levels. Such variation may not be adequately

controlled by compendial specifications alone and highlights the need for more stringent analytical monitoring.

Preliminary Assessment of Glucose-Induced Degradation

To establish the relevance of glucose as a reactive impurity, a preliminary stress study was performed by exposing a binary mixture of drug substance and glucose under accelerated stability conditions (40°C/75% RH). A gradual increase in impurity levels was observed over time, indicating the formation of degradation products associated with glucose interaction. The impurity level increased significantly upon storage, confirming the susceptibility of the drug substance to reducing sugar-mediated degradation.

Further observations indicated that environmental factors, particularly humidity, played a critical role in facilitating the interaction. Increased moisture conditions accelerated impurity formation, suggesting that water-mediated mobility enhances the reactivity of glucose with drug substances. These findings are consistent with reported Maillard-type reaction mechanisms and highlight the importance of monitoring reducing sugar impurities in excipients. This observation confirms that the presence of glucose, even at trace levels in excipients, can contribute to drug degradation, thereby justifying the need for its accurate quantification.

Significance of the Developed Method

The developed method offers several advantages, including high sensitivity due to derivatization-based detection, good selectivity in complex excipient matrices, and a wide linearity range suitable for regulatory applications.

Furthermore, the method demonstrates broad applicability across different excipient types and can serve as a valuable tool for excipient screening during formulation development, routine quality control testing, and risk assessment of reducing sugar-mediated degradation pathways.

Overall, the results demonstrate that glucose is a relevant and variable impurity in commonly used pharmaceutical excipients, with potential implications for drug stability. The developed method provides a practical tool for identifying and quantifying such impurities, supporting a risk-based approach to excipient selection and formulation development.

Unlike conventional carbohydrate quantification methods reported in literature [10–12], the present method is specifically designed to address glucose as a reactive impurity within pharmaceutical excipients, providing improved relevance for stability assessment.

CONCLUSION

A simple, sensitive, and robust HPLC method based on pre-column derivatization using 2,4-dinitrophenylhydrazine (2,4-DNPH) was successfully developed and validated for the quantification of glucose in pharmaceutical excipients. The derivatization approach significantly enhanced the detectability of glucose, enabling reliable determination using conventional UV detection systems.

The developed method demonstrated excellent analytical performance, including high linearity, precision, accuracy, and robustness in accordance with ICH Q2(R2) guidelines [27]. The absence of interference from excipient matrices confirmed the specificity of the method, supporting its suitability for routine analytical applications.

Application of the method to a range of commonly used pharmaceutical excipients revealed the presence and variability of glucose as a trace impurity, highlighting that excipients cannot always be considered chemically inert. The observed variability across excipient types and batches underscores the importance of monitoring reducing sugars during excipient selection, qualification, and procurement processes.

Overall, the proposed method provides a practical and efficient analytical approach for the determination of glucose in pharmaceutical excipients. It can be effectively applied for excipient screening, routine quality control testing, and risk assessment of reducing sugar-mediated degradation, thereby supporting improved pharmaceutical product stability and quality.

The study reinforces the importance of considering excipients as potential sources of reactive impurities and highlights the need for their systematic evaluation during pharmaceutical development.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

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Ethical Approval

This study did not involve any human or animal subjects.

Author Contributions

Prakash Ashok Deshinge: Conceptualization, Method development, Investigation, Writing – original draft.

Co-authors: Supervision, review and editing, technical guidance

FIGURES

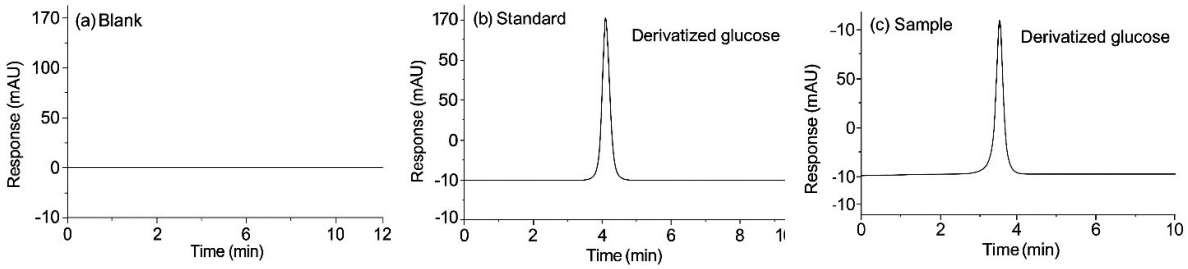

Figure 1a Figure 1b Figure 1c

Figure 1: Representative chromatograms of (a) blank, (b) derivatized glucose standard, and (c) excipient sample

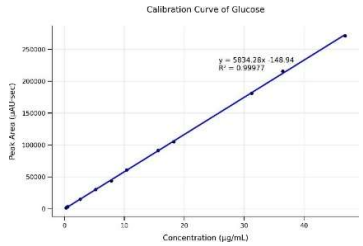

Figure 2: Calibration curve of glucose showing linearity over the concentration range

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