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A simple and sensitive method to analyze genotoxic impurity hydrazine in pharmaceutical materials

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ABSTRACT

Hydrazine (N_2H_4) is a known genotoxic impurity that typically needs to be controlled down to low ppm level in pharmaceutical development. Hydrazine, however, is a challenging molecule to analyze using conventional analytical techniques due to its physical and chemical properties (e.g. lack of chromophore, absence of any carbon atom, low molecular weight, high polarity and volatility). Additionally, analysis in pharmaceutical samples commonly encounters significant interference from matrix components that greatly overshadow the response of hydrazine. This work describes a simple, accurate and sensitive reversed-phase liquid chromatography—UV derivatization method for determination of trace amount hydrazine in pharmaceutical materials featuring three prominent strategies to address the problems associated with hydrazine analysis. First, the derivatization reaction attaches chromophores to hydrazine, which greatly increases its sensitivity by UV–vis detection. Secondly, the derivatization reaction generates a lambda max that is well-shifted away from the absorption wavelengths of pharmaceutical matrix interferences. Thirdly, from a separation standpoint, the derivatization further removes matrix interference effects through chromatography by achieving higher resolution of the derivative product from the active pharmaceutical ingredient (API) and its related impurities for accurate quantitation for trace level of genotoxic impurities (GTIs). 2-Hydroxy-1-Naphthalaldehyde (HNA) was chosen as the derivatizing reagent, and the resulting hydrazone product has a maximum UV absorbance at wavelength of 406/424 nm which is in the visible range. Since most drug substance and impurities have UV absorbance ranging from 190 to 380 nm, interference from the matrix was minimized and the appropriate selectivity was obtained, the detection limit is 0.25 ppm (0.25 μg/g API). This method was validated and applied as a generic method to determine hydrazine for pharmaceutical process control and drug material release.

1. Introduction

Hydrazine (N_2H_4) is a small molecule chemical used in the formation of indazoles [1,2] (a common moiety of many small molecule drugs), the Knorr Synthesis [3,4], the Gabriel Synthesis [5], and the Wolff-Kishner reaction [6], of which all are commonly used reactions in pharmaceutical drug API processes [1,2,4,7,8]. However, hydrazine is a known genotoxic impurity (GTI) with genotoxicity and carcinogenicity. Over the years, regulatory health agencies have steadily increased the level of scrutiny required for control of GTIs in drug substance materials, which in turn raises the bar on requirements for analytical methods to quantify hydrazine at low ppm levels. According to the recently published addendum to ICH M7 (R1) in June 2015, hazard assessment data for hydrazine showed it to be mutagenic/genotoxic in vitro and in vivo and possibly carcinogenic to humans [9], leading hydrazine to a Class 1 GTI classification, which needs be controlled at or below a compound specific acceptable limit. In general, most pharmaceutical drug substances have an acceptable intake of GTI that must be controlled to a daily dose of 1.5 μg per person for long-term treatment, which corresponds to a theoretical 1:100,000 carcinogenic risk [10]. This drives the industry to seek sensitive methods to accurately quantitate hydrazine in order to control its levels during the synthetic process and in the final drug substance.

Despite the need for accurate hydrazine analysis, hydrazine quantitation is particularly difficult from an analytical standpoint. It is highly reactive, which causes unwanted side reactions during sample preparation and ultimately leads to inconsistent results and a high frequency of false positives. Additionally, hydrazine has neither a chromaphore for UV based detection methods nor an ionizable group for sensitive analysis by mass spectrometry. Because of its lack of carbon atoms, hydrazine is also difficult to analyze by

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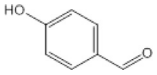
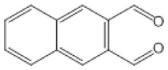
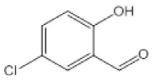
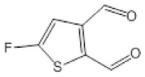
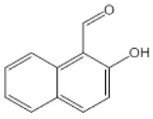
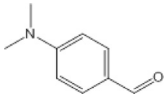
Table 1
Derivatization Reaction Screening.

Derivatization Reagent	Structure	Maxima Wavelength (nm)	Experimental Outcome
4-Hydroxylbenzaldehyde		340	Promising candidate, the derivatized product was difficult to separate from the API matrix components
Naphthalene-2,3-Dicarboxaldehyde		363	Costly reagent and not suited for repeated batch analysis in large scale quantities
5-Chloro-Salicylaldehyde		365	Chromatography screen showed inadequate resolution from API peak and matrix components
5-Fluoro-2,3-Thiophenedicarboxaldehyde		N/A	Chromatography screen showed inadequate resolution from API peak and matrix components
2-Hydroxy-1-Naphthaldehyde		406	Ideal candidate, simple reactions conditions; chromatography screen showed high selectivity in derivatized product
4-Dimethylaminobenzaldehyde		397	No reaction observed

flame ionization detection (FID) due to low sensitivity issues. Other newer technologies such as charged aerosol detection (CAD) have been explored [11], but the sensitivity is not high enough to detect free hydrazine at low levels due to its volatility. As such, derivatization becomes an attractive approach to achieve the necessary selectivity and sensitivity.

There have been many reported methods used to detect and quantify hydrazine [12–14], most of which use a derivatization approach coupled with analytical techniques ranging from GC [15–17], GC–MS [15,18], LC [19–21], LC–MS/MS [22–24], CAD [11], Capillary electrophoresis [25], electrochemical sensing [26–30] and ion chromatography (IC) [31]. The literature has demonstrated many different applications with a large portion of the reported literature focusing on environmental detection of hydrazine from drinking waters or bioanalytical applications [15,18,23]. In particular, the work done by Oh and associates details a method to achieve ultra-low limits of detection of hydrazine from drinking water samples [23]. Their study assessed an assortment of derivatization agents involving a Schiff base reaction with hydrazine. Naphthalene-2,3-dialdehyde was selected as the derivatization reagent to react with hydrazine in a 1:1 ratio in a controlled reaction allowing full conversion of the hydrazine into the derivatized product. A fast LC–MS/MS method was then developed to analyze the derivatized hydrazone product and quantified using a triple quadrupole mass spectrometer in SRM to reach part-per-billion level limits of detection with suitable accuracy and precision [23].

Hydrazine quantitation in a pharmaceutical setting, however, poses another very specific set of challenges due to the interference from the bulk drug matrix and related substances, and currently there is lack of good sensitive methods for the quantitation of hydrazine from pharmaceutical compounds. Currently there is a derivatization-headspace GC–MS approach detecting hydrazine from complex pharmaceutical samples using acetone and deuterated acetone as the derivatization agent [17]. From a good manufacturing practice (GMP) standpoint, methods using UV detection are preferred given its prevalent availability in GMP laboratories as opposed to other detection methods (i.e. mass spectrometry), especially during method transfer activities across worldwide different contract manufacturing and research facilities. Analytical methods must still be sensitive enough to quantitate low GTI levels of hydrazine in drug samples, but these samples contain enormous levels of matrix interferences stemming from the active pharmaceutical ingredient (API) itself and its related substances, which generally constitute about 97–99% (w/w) of the sample with hydrazine present in the sample at ppm (w/w) levels by mass. These matrix components are usually UV absorbing causing significant inferences to hydrazine even when analyzing the derivatized hydrazone product. Additionally, these matrix components factor into the chromatography, as most of these components are API or API related substances that retain well on reversed-phase chromatography and potentially coelute with the derivatized hydrazone product.

The goal of this study is to develop a sensitive, selective, accurate, reproducible and simple method to analyze hydrazine from pharmaceutical API and intermediates. The strategy to address the common problems associated with hydrazine analysis (e.g. sensitivity, matrix interferences, and challenging chromatography) is to use a derivatization reaction approach to meet pharmaceutical method requirements. Selection of the best derivatization agent is critical for the success of the method development. The resulting derivatized product must give strong UV absorption to satisfy the high sensitivity needs of a GTI method in the pharmaceutical industry. Additionally, the UV absorption lambda max must also be well-shifted away from the absorption regions of the drug API and its related substances matrix components, which are typically strong absorbing around 190–380 nm. Of course, the derivatization reaction should be selective to hydrazine and should be optimized so that there is nearly 100% conversion of hydrazine into the derivatized hydrazone product to be representative of true sample hydrazine content. From a separation point of view, the chromatography must be developed for high resolution of the derivatized hydrazone product from the rest of the API and its related impurities to ensure adequate sensitivity for low level GTI detection. In the ideal case, the method would elute all the matrix components from the sample early near the void volume and retain the hydrazine product of interest for analysis further along in the method. The method also must consider the fact that the API peak greatly overshadows the hydrazine peak, and that peak tailing associated with

such an API large peak will obscure the sensitive analysis of adjacent low level peaks from hydrazine.

With these strategies in mind, the work from this study describes a simple derivatization method for hydrazine analysis from different drug substance samples using reversed-phase HPLC-UV. The study conducted a screen of different derivatization reactions to identify the best candidate that fits our needs; the reaction was then optimized to fully convert the hydrazine into a derivatized hydrazone product. Using the optimized derivatization reaction, the chromatography was then developed for the hydrazone product to achieve the resolution requirements needed for trace level GTI analysis. The final method was qualified for specificity, accuracy, sensitivity, linearity, and precision. An application of this analysis was demonstrated using two pharmaceutical materials with different physicochemical properties.

2. Experimental

2.1. Compounds and reagents

Hydrazine Monohydrate (>99%), 2-Hydroxy-1-Naphthaldehyde (>99%), 4-Hydroxylbenzaldehyde, 5-Fluoro-2,3-Thiophenedicarboxaldehyde, and Trifluoroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO). 5-Chloro-Salicylaldehyde and 4-Dimethylaminobenzaldehyde were obtained from Alfa Aesar (Ward Hill, MA), and Naphthalene-2,3-Dicarboxaldehyde was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Dimethyl Sulfoxide (>99.9%) was obtained from Alfa Aesar (Ward Hill, MA), and Acetonitrile, HPLC grade was from EMD Chemicals (Darmstadt, Germany). Methanol, HPLC grade was obtained from Burdick & Jackson (Morristown, NJ). De-ionized water was from an in-house Milli-Q water purification system (Millipore, Billerica, MA). All experimental compounds and drug samples were produced in-house at Genentech. GEN1 molecular weight is ~650 g mol^{-1} and pK_a ~6.0, while GNE2 molecular weight is ~200 g mol^{-1} and pK_a ~12. GNE1 is very hydrophobic while GNE2 is more hydrophilic, with the solubility of GEN2 in water is 10 times higher than that of GNE1.

2.2. Equipment

HPLC analysis was conducted on an Agilent 1290 Infinity Series HPLC-DAD system (Santa Clara, CA) equipped with a G4226A 1290 autosampler, G4220A 1290 binary pump, G1316C 1290 thermostatted column compartment, and G4212A 1290 photodiode array. An Eclipse XDB-C18 HPLC column (3.5 μm, 3.0 × 150 mm) from Agilent (Santa Clara, CA) was used for the analysis. A Mettler Toledo XP 205 Micro Balance (Columbus, OH), 8510 Branson Sonicator (Danbury, CT), and VWR Dyla-Dual Hot Plate/Stirrer (Bridgeport, NJ) were also used for the sample preparation and the derivatization reaction.

2.3. Sample preparation and derivatization reaction

For the derivatization screening experiments, each reagent was prepared at a 0.1 mg/mL concentration in DMSO. To each solution, a hydrazine spike was added to make a 200 μg/mL hydrazine final concentration standard solution. The 4-Hydroxybenzaldehyde was sonicated for 30 min at room temp. The 5-Chloro-Salicylaldehyde, 5-Fluoro-2,3-Thiophenedicarboxaldehyde, and Naphthalene-2,3-Dicarboxaldehyde reactions was sonicated for 20 min at room temperature. The 4-dimethylaminobenzaldehyde, 2-Hydroxy-1-Naphthaldehyde, and 4-Hydroxylbenzaldehyde reactions were sonicated for 30 min at room temperature. Samples were removed and directly injected onto the HPLC system.

For the finalized method conditions, a diluent solution was first prepared by adding 10 mg of derivatization reagent 2-Hydroxy-1-Naphthaldehyde (HNA) into 100 mL DMSO. This diluent solution was then used to prepare the API drug sample. Sample Solution was prepared by adding 20 mg drug material into a 10 mL volumetric flask, diluted with sample diluent to volume. The flask was then sealed with glass stopper, incubated at 100 °C in a water bath for 30 min for the derivatization to proceed through to completion. Samples were aliquotted out and directed injected onto the HPLC system.

2.3.1. Method validation

Samples of hydrazine in DMSO were prepared at 2, 4, 10, 20, 40, 100, 200 μg/L concentrations for the linearity and range determination. For the LOQ, sample at a concentration of 2 μg/L was prepared in DMSO and measured for 6 replicated injections to determine the average signal-to-noise ratio of the lowest concentration.

Accuracy and precision experiments consisted of 6 replicates for 1 ppm (μg/g) hydrazine concentration and 3 replicates for 10 and 100 ppm (ug/g) in 2 mg/mL pharmaceutical compounds GNE1 and GNE2. Unspiked preparations of the pharmaceutical materials were used as a control to determine the specificity of the method.

3. Results and discussion

3.1. Derivatization reagent screening and optimization

The selection of a suitable derivatization agent is key to designing this method for hydrazine analysis. To evaluate the sensitivity, selectivity, reaction conditions, efficiency, cost and commercial availability, six derivatizing agents, 4-Dimethylaminobenzaldehyde, 4-Hydroxybenzaldehyde, 5-Chloro-Salicylaldehyde, 5-Fluoro-2,3-Thiophenedicarboxaldehyde, Naphthalene-2,3-Dicarboxaldehyde, and 2-Hydroxy-1-Naphthaldehyde were screened. Table 1 summarizes the results of the derivatization agent screening. As mentioned previously, the derivatization agent should maximize conversion of free hydrazine to the derivatized hydrazone with high selectivity. More importantly, the derivatized hydrazone product should have a strong UV maximum that is well shifted away from conventional 190–380 nm wavelengths of drug sample matrix absorption and meet our chromatography needs as well. Clearly, the 2-Hydroxy-1-Naphthaldehyde (HNA) derivatized hydrazone product stands out given that it exhibits the largest shift in UV maxima, at 406 nm, which is away from the matrix interferences of the API and well into the visible spectrum range. This shift can be attributed to a significantly higher degree of conjugation, which generates a much stronger chromaphore in the derivatized product as compared to the other tested derivatization products. To reinforce this point, the UV absorbance spectrum of the derivatized HNA-hydrazone product seen in Fig. 1 shows multiple UV maxima at 406 and 424 nm that had molar absorptivities of 2.8 × 10^4 and 2.3 × 10^4 L Mol^{-1} cm^{-1}, respectively. Also in terms of the chromatography, the HNA-hydrazine reaction is the best choice because the resultant hydrazone product is hydrophobic and can be strongly retained on reversed-phase LC, a point we will further discuss later in this paper. HNA is also reasonably priced and a commercially-available reagent considering the fact that this method will be used repeatedly for multiple batch analysis across multiple manufacturing sites worldwide.

Fig. 2 illustrates the proposed reaction of two HNA molecules to one hydrazine molecule to form a 2,2′-dihydroxy-1-Naphthaldazine or hydrazone product [30]. Dimethyl Sulfoxide (DMSO) served as an excellent strong solvent to dissolve the pharmaceutical compounds, derivatizing reagent HNA, and yellow

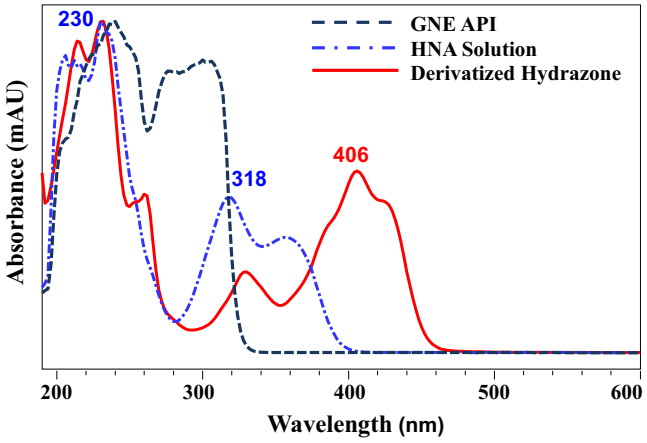


Fig. 1. UV absorbance spectrum of the HNA derivatized product showing a maxima at 406 nm.

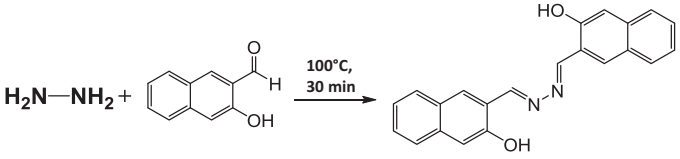


Fig. 2. Reaction scheme of 2-Hydroxy-1-Naphthaldehyde with hydrazine.

derivatized product. After selecting HNA as the derivatization reagent, the reaction was optimized to ensure maximum conversion of hydrazine. A number of reaction conditions, such as pH, reaction temperature, reaction time, and the mole ratio of HNA/hydrazine, were optimized through separate studies, the results of which are collectively summarized in Fig. 3. Fig. 3a shows the effect of pH and temperature on the derivatization reaction where there was no observable difference in reaction efficiency between acidic pHs (achieved by spiking 0.5 mM and 0.1 mM acetic acid in DMSO) and blank DMSO. However, basic pHs of 8.3 and 9.3 had a detrimental effect on the reaction efficiency; therefore, blank DMSO with no additive was chosen as the diluent solvent. In addition, increased temperatures at 100 °C facilitated the reaction in forming the derivatized hydrazone product. Reaction kinetics at 100 °C was monitored from 15 min to 4 h. (Fig. 3b) showing that 20 min was sufficient to reach completion of the reaction. The mole ratio of HNA/hydrazine was also optimized to ensure the derivatizing agent is in excess to hydrazine. Fig. 3c shows that the percent difference of the hydrazone peak response within a range of 2:1 to 22:1 molar ratio of HNA/hydrazine was less than 2%. The excess amount of HNA in solution did not interfere with the chromatography and analysis of hydrazine. Additionally, solution stability of the reaction product was assessed where three replicate derivatization preps showed ≤1.0% difference over a 3 day period stored in ambient room temperature. Based on these studies, the optimal reaction conditions were chosen to be 100 °C in a water bath for 30 min using 0.1 mg/mL of HNA in DMSO as the derivatizing solution.

3.2. HPLC method development

A reversed-phase LC method was developed to resolve and analyze the derivatized hydrazone product. A number of columns with different stationary phase chemistries were screened in the method development process such as Acentis Express C18, Waters Symmetry C18, Xbridge Phenyl, Xselect Phenyl-hexyl, Zorbax SB-Aq, and Zorbax Eclipse XDB-C18 (data not shown). As mentioned previously, the ideal scenario for the chromatography is where

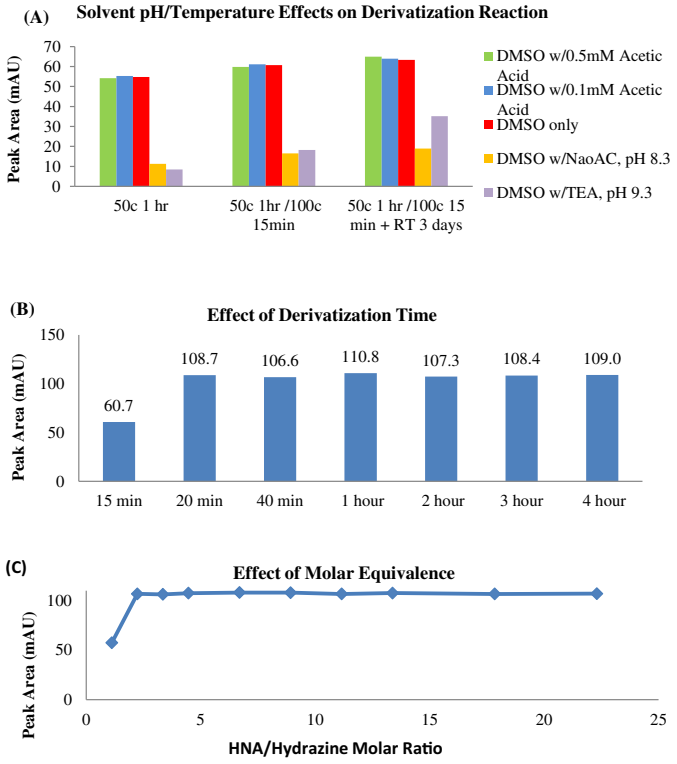


Fig. 3. Derivatization optimization experiments where (A) Temperature/Solvent pH study performed concurrently using incremental amounts of acetic acid in DMSO. Basic pHs were achieved using sodium acetate (NaOAc) and trimethylamine (TEA) in DMSO. (B) Reaction kinetics study where temperature was set constant at 100 °C. (C) Reagent concentration with increasing quantities of mole to hydrazine at 100 °C.

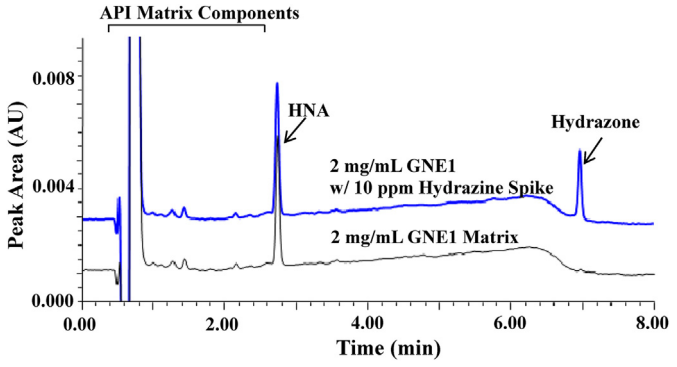


Fig. 4. Chromatogram overlay showing specificity of the derivatized HNA-hydrazone product and its separation from the pharmaceutical material matrix components.

the API matrix components elute early near the void volume and far from the derivatized hydrazone product. The Zorbax Eclipse XDB-C18 was selected because it achieved the strongest retention of the hydrazone product and highest resolution from the API matrix components as seen in Fig. 4. This can be attributed to the addition of two hydrophobic, conjugated moieties onto the hydrazine molecule, which significantly increases its retention on a C18 column and is ideal for separation from the more polar API matrix components. It is also important to note that the Zorbax Eclipse XDB-C18 column was chosen over the Waters Symmetry C18 column because of a more stable baseline. The peak shape and resolution of the hydrazone product was excellent with this column.

The finalized reversed-phase LC method was developed to analyze the derivatized hydrazone from drug API samples. A 0.05% TFA

Table 2
Summary of final HPLC method.

Parameters	Conditions	
HPLC Column	Eclipse XDB-C18, 3.5 μm, 3.0 × 150 mm	
Mobile Phase	A: 0.05% TFA in water	
	B: 0.05% TFA in Acetonitrile	
Injection Volume	10 μL	
Column Temperature	30 °C	
Gradient	Time (min)	% B
	0	50
	5	90
	10	90
Flow Rate	1.0 mL/min	
UV Wavelength	406 nm	

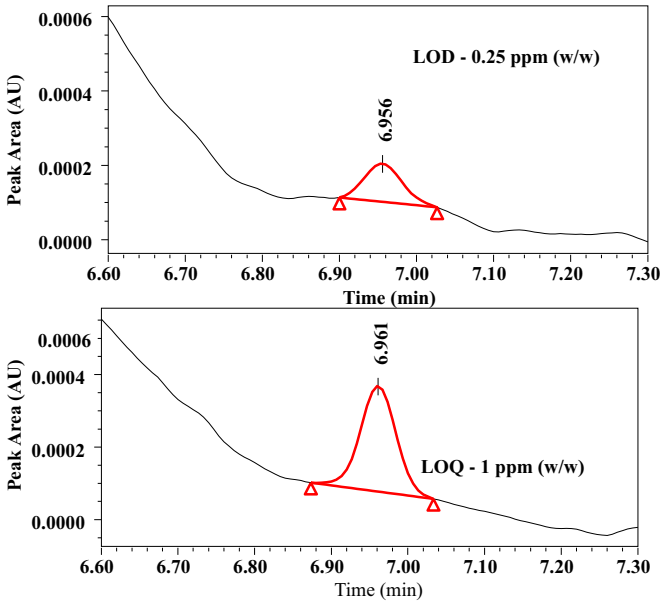


Fig. 5. Magnified-view of hydrazone standard peaks at the LOD (0.5 μg/L or 0.25 ppm (w/w) in 2 mg/mL API) and LOQ (2 μg/L or 1 ppm (w/w) in 2 mg/mL API) levels.

in water solution was used for mobile phase A (MPA), while 0.05% TFA in acetonitrile was used for mobile phase B (MPB). A flow rate of 1 mL/min was used with an injection volume of 10 μL while the column temperature was maintained at 30 °C. For the final method, a UV detection wavelength of 406 nm was selected for detection of derivatized hydrazone. Total run time was 10 min. The binary gradient started with MPB at 50% the gradient was increased to 90% MPB in 5 min. A wash step was added after the gradient for 5 min at 90% MPB, and then re-equilibrated at 50% MPB for 5 min. The HPLC method parameters are summarized in Table 2.

3.3. Sensitivity

The method was evaluated for its limit of detection (LOD) based on a signal-to-noise (S/N) ratio ≥3 and was determined to be 0.5 μg/L, which is equivalent to 250 ppb (w/w) of hydrazine in a 2 mg/mL solution of pharmaceutical material. The limit of quantification (LOQ) was based on a signal-to-noise (S/N) ratio ≥10 and determined to be 2 μg/L, which is equivalent to 1 ppm (w/w) of hydrazine in 2 mg/mL of pharmaceutical material (shown in Fig. 5). This LOQ is adequate to quantify trace hydrazine at the limits outlined by ICH and regulatory health guidelines [10]. Notably, the high method sensitivity reported from this work convincingly demonstrates the advantages of three previously discussed strategies to reduce matrix interferences. The derivatization reaction serves to

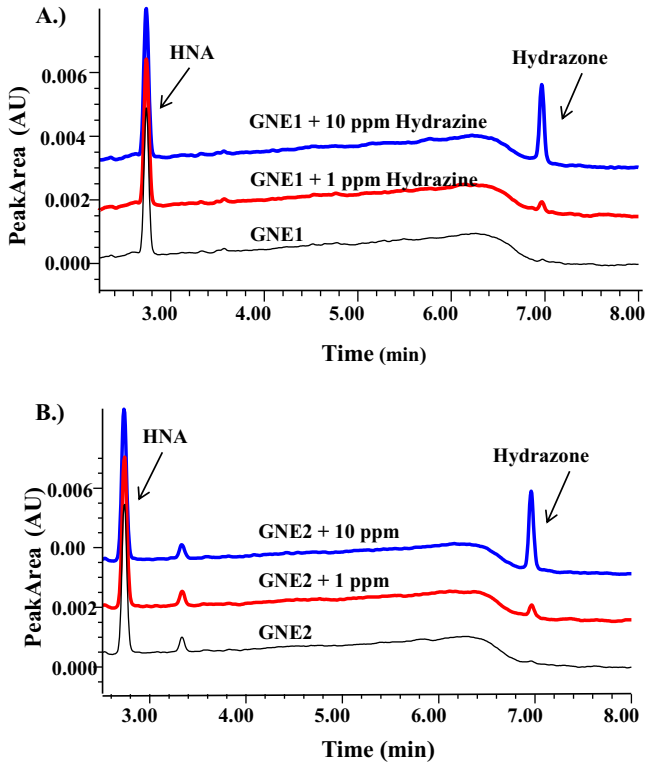


Fig. 6. Chromatogram overlays showing derivatized pharmaceutical compound with no hydrazine spiked, 1 ppm hydrazine spike, and 10 ppm hydrazine spike for both (A) GNE1 and (B) GNE2.

shift the UV maxima of the derivatization product away from 190 to 380 nm by increasing its pi-pi conjugation through aromatic rings and thus increase its molar extinction coefficient. The derivatization reaction also gives greater selectivity from a chromatography standpoint on a C18 reversed phase column, which allows for the derivatization product to be well separated from the API matrix components. Additionally, the method was performed using UV detection, an attractive benefit when considering its ease of use in a Good Manufacturing Practice (GMP) setting and ease of method transfer across external GMP facilities.

3.4. Method validation results

The method was then validated for its specificity, linearity and range, accuracy, and precision to demonstrate that the method is suitable for its intended use per ICH Q2(R1) guideline [32]. The full results of the method validation experiments are summarized in Table 3.

The specificity of this method was demonstrated by separation of two GNE drug compounds. The resolution of the hydrazone derivative from other matrix components is greater than 2.5. The representative chromatograms were showed in Fig. 4.

The method exhibits good linearity and range with a linear regression fit of $R^2 = 1.000$ with a best fit equation of $y = 0.54x - 0.21$. The method has been demonstrated to be linear in a range of at least two orders of magnitude from 2 μg/L to 200 μg/L.

The detection and quantitation limits were validated based on an S/N ratio of ≥3 and 10, respectively. The limit of detection of 0.5 μg/L showed an S/N ratio range from 3 to 4, while a range of 11–20 of S/N ratio was observed for quantitation limit of 2 μg/L, of which the percent of relative standard deviation (RSD) of peak area was 7.0% for six replicate injections. The quantitation limit was also validated with sample matrix where 2 μg/L of hydrazine was spiked in 2 mg/mL concentration of drug material, which is

Table 3
Summary of method validation data.

Parameter	Experiment	Results
Linearity	Hydrazine standard at 7 levels: 2, 4, 10, 20, 40, 100, 200 μg/L	Correlation coefficient, R^2 = 1.00, Slope = 0.54, Intercept = −0.21
Specificity	Method Blank, 2 GNE compounds and spiked compounds with hydrazine	Absent target peak from blank, The resolution of the target and closest peak is more than 2.5
Detection limit	6 replicated injection of 0.5 μg/L hydrazine standard	Signal-to-noise ratio was 3, 4, 4, 5, 3, 3
Limit of Quantitation	6 replicated injection of 2 μg/L hydrazine standard	Signal-to-noise ratio was 11, 12, 12, 20, 11, 12 %RSD of peak area is 7.0%
Accuracy/Precision	6 replicate sample solutions (2 mg/mL) were spiked with hydrazine at 1 ppm 3 replicate sample solutions (2 mg/mL) were spiked with hydrazine at 10 and 100 ppm	GNE 1 | 1 ppm (n=6) | 10 ppm (n=3) | 100 ppm (n=3) Average %Recovery | 97.1 | 98.5 | 99.6 %RSD | 2.4 | 2.3 | 1.4
Solution Stability	Spiked sample solutions were stored at ambient conditions for 3 days	The %change in assay at t3 day was less than 1.0% from initial t0 day

equivalent to 1 ppm (w/w) of hydrazine. The average percent of recovery of six replicate injections at this level is 97.1% with a %RSD of 2.4%.

Accuracy and precision was validated on a pharmaceutical drug material spiked with hydrazine at three concentration levels covering the specified range with 6 replicates for 1 ppm hydrazine concentration and 3 replicates for 10 and 100 ppm. The pharmaceutical material was prepared at a concentration of 2 mg/mL. The percent recovery was calculated by the assay of spiked hydrazine in drug materials. The individual percent recoveries for all preparations were from 94.6–101.2% and the %RSD for all injections was 2.3%.

The developed hydrazine method was tested on a pharmaceutical drug substance GNE1 and a pharmaceutical intermediate compound GNE2. The samples were taken and subjected to the derivatization reaction; spiked sample solutions of 1 ppm and 10 ppm also were prepared and measured. Fig. 6 shows the chromatogram overlay of these experiments where the GNE compounds without hydrazine spiked were compared against 1 ppm and 10 ppm (w/w) spiked samples demonstrating that hydrazine was detected and accurately quantified.

4. Conclusion

We have developed a method for the sensitive and accurate quantitation of hydrazine in pharmaceutical materials using a simple derivatization reaction and RPLC-UV. Selection of the derivatization agent, 2-Hydroxy-1-Naphthaldehyde, was a key step toward this analytical approach, which generates a derivatized product that meets the specific requirements of our analytical strategies. The derivatization effectively shifts the resultant hydrazone product away to higher wavelengths in the UV spectrum where API matrix components do not interfere with the analysis. Secondly, the derivatization reaction generates a product that can be separated on a reversed-phase LC method with high resolution from the rest of the API matrix. A specific LC-UV method using an Eclipse XDB-C18 column was tailored to achieve the desired chromatography with the HNA-hydrazone product and was demonstrated for suitable specificity, linearity/range, accuracy and precision. The LOQ of the method was determined to be 1 ppm (w/w) based on the average signal-to-noise ratio of 6 replicate injections of a 2 mg/mL API and was adequate for sensitive quantification of hydrazine in pharmaceutical materials.

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