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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.
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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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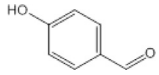
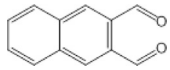
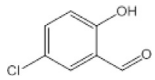
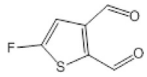
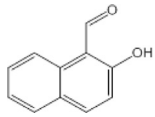
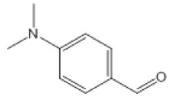
Table 1
Derivatization Reaction Screening.

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

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