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Short communication

A generic approach for the determination of trace hydrazine in drug substances using *in situ* derivatization-headspace GC–MS

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ABSTRACT

In situ derivatization-headspace GC–MS methodology has been developed for the determination of hydrazine in drug substance at low ppm levels. This general method uses acetone or acetone-d_6 as the derivatization reagent. The resulting acetone azine or acetone azine-d_{12} can then be analyzed by headspace GC–MS. The method gives excellent sensitivity with a limit of quantitation (LOQ) as low as 0.1 ppm when the API (active pharmaceutical ingredient) samples are prepared at 10 mg per headspace injection vial. The spike recoveries of hydrazine at the 1 ppm level were between 79% and 117% in various APIs tested. The precisions (%RSD) of six preparations of the hydrazine standards at the concentration of 1 ppm level were typically between 2.7 and 5.6%. A linear range of concentrations from 0.1 to 10 ppm has been demonstrated with $R^2 \geq 0.999$. This general method has been tested in a number of API matrices and successfully applied to the determination of hydrazine in support of API batch releases and process chemistry at GlaxoSmithKline.

1. Introduction

Hydrazine is a common building block and reagent for the manufacture of drug substance [1–4]. However, hydrazine is a known genotoxic compound [5]. According to EMEA's guidelines, the intake of hydrazine impurity must be controlled to no greater than 1.5 μg/day when a drug is taken for more than 12 months [6]. Thus depending on the daily dose, the level of hydrazine in drug substance must be controlled at low ppm levels. For instance, hydrazine is controlled and monitored at 1 ppm in Povidone and Copovidone [5]. Such a low limit poses tremendous challenges not only to the manufacturing process but also to the analytical method.

Many methods have been developed for the analysis of hydrazine. Direct analysis of hydrazine in an investigational drug was reported using ion chromatography coupled with electrochemical detector with a limit of quantitation (LOQ) of 100 ppm [4]. The majority of the analytical methods, however, are based on detection of hydrazine derivatives which are analyzed by TLC [5], HPLC [5,7–12], GC [13–15], or GC–MS [16] techniques. Kean et al. recently gave an excellent review on the subject [5], thus the reported analytical methods will not be discussed in details herein. Detecting trace hydrazine in drug substances is challenging because of the potential interferences caused by large amounts of drug substance and its impurities. Furthermore, the large amount of drug substance makes the analysis of the derivatives by direct injection GC impractical because very often methods suffer from robustness issues due to contamination, i.e., limited number of injections can be made and frequent instrument cleaning are required. For HPLC related methods, on the other hand, the separation of drug substance (or impurities) and the hydrazine derivative may have to be optimized for each individual drug substance which can be difficult to achieve sometimes. As Kean et al. alluded to in the article, there is an obvious need to develop a generic method that can be used to detect hydrazine in various drug substances [5].

Headspace GC is a common technique in pharmaceutical analysis and has been routinely used for the analysis of volatiles in drug substance [17]. Headspace is discriminatory in nature since only the volatiles in the injection vials can be transferred to the GC system while the non-volatile interferences (drug substance and impurities) will remain in the headspace injection vials. Thus higher sample loading is possible without significant adverse effects to the analysis. It is well known that hydrazine reacts with acetone producing acetone azine (Scheme 1a) [15], of which the boiling point is 133 °C/763 mmHg. Thus it is possible to analyze acetone azine with a headspace GC technique. In this report, hydrazine was analyzed by headspace GC–MS after *in situ* derivatization with acetone. A low LOQ of 0.1 ppm (w/w) was achieved. Since acetone is sometimes used as a solvent in the manufacture process of drug substances, the derivatization product, acetone azine, may be present in drug substance prior to derivatization. In an effort to differentiate hydrazine from pre-existing acetone azine,

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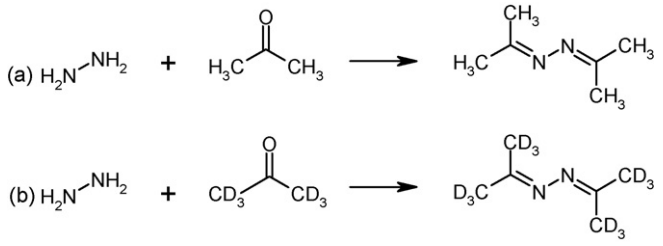

Scheme 1. Illustration of derivatization reactions converting hydrazine into corresponding acetone azine or acetone azine-d_{12}.

acetone-d_6 was also explored as an alternative reagent to acetone. (Scheme 1b).

2. Experimental

2.1. Reagents

Benzoic acid (ACS reagent), acetone (HPLC grade), acetone-d_6 (99.9%, atom% D), and hydrazine monohydrate (98%) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA, Ultrapure)

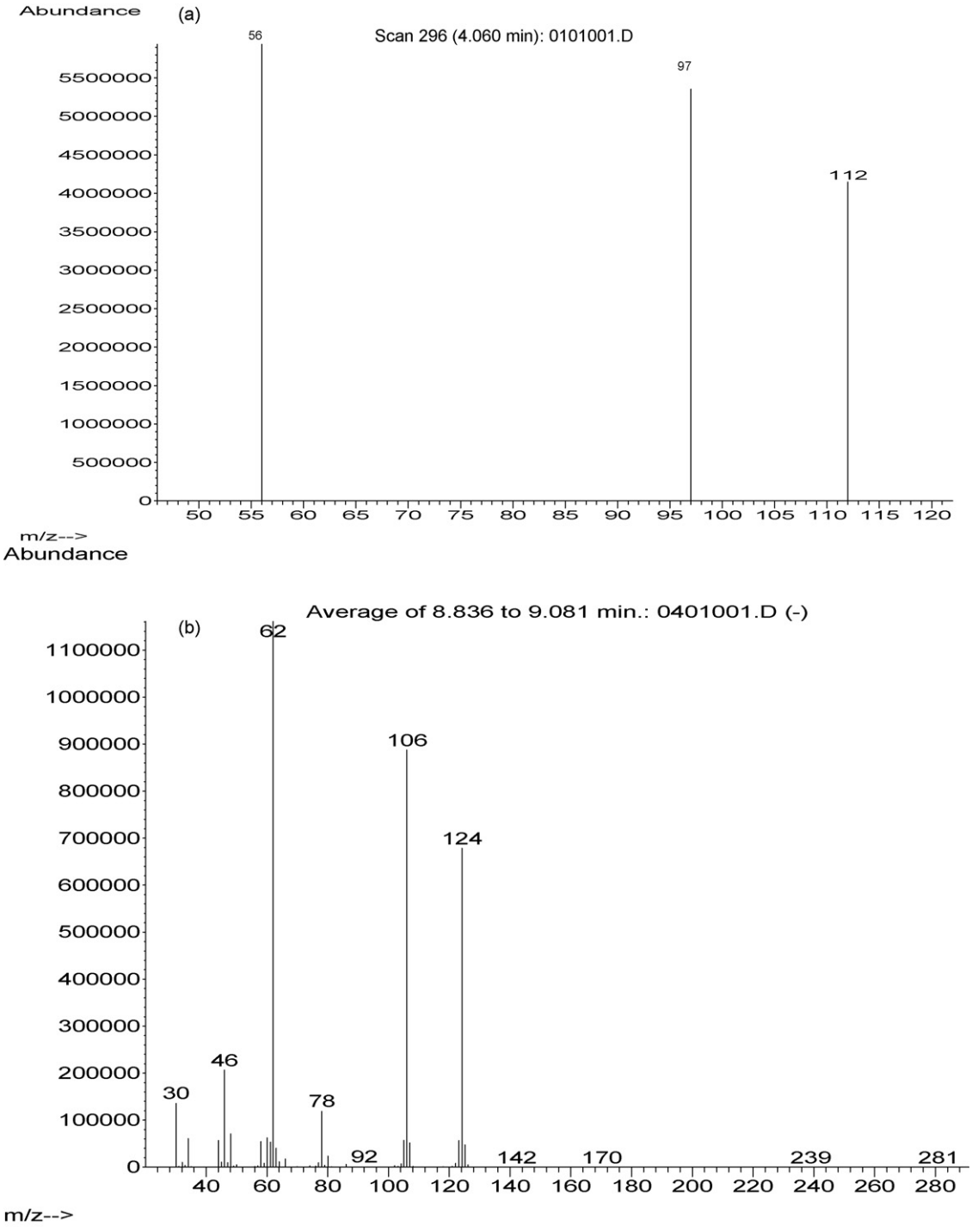

Fig. 1. Mass spectra of derivatization products of hydrazine: (a) acetone azine and (b) acetone azine-d_{12}.

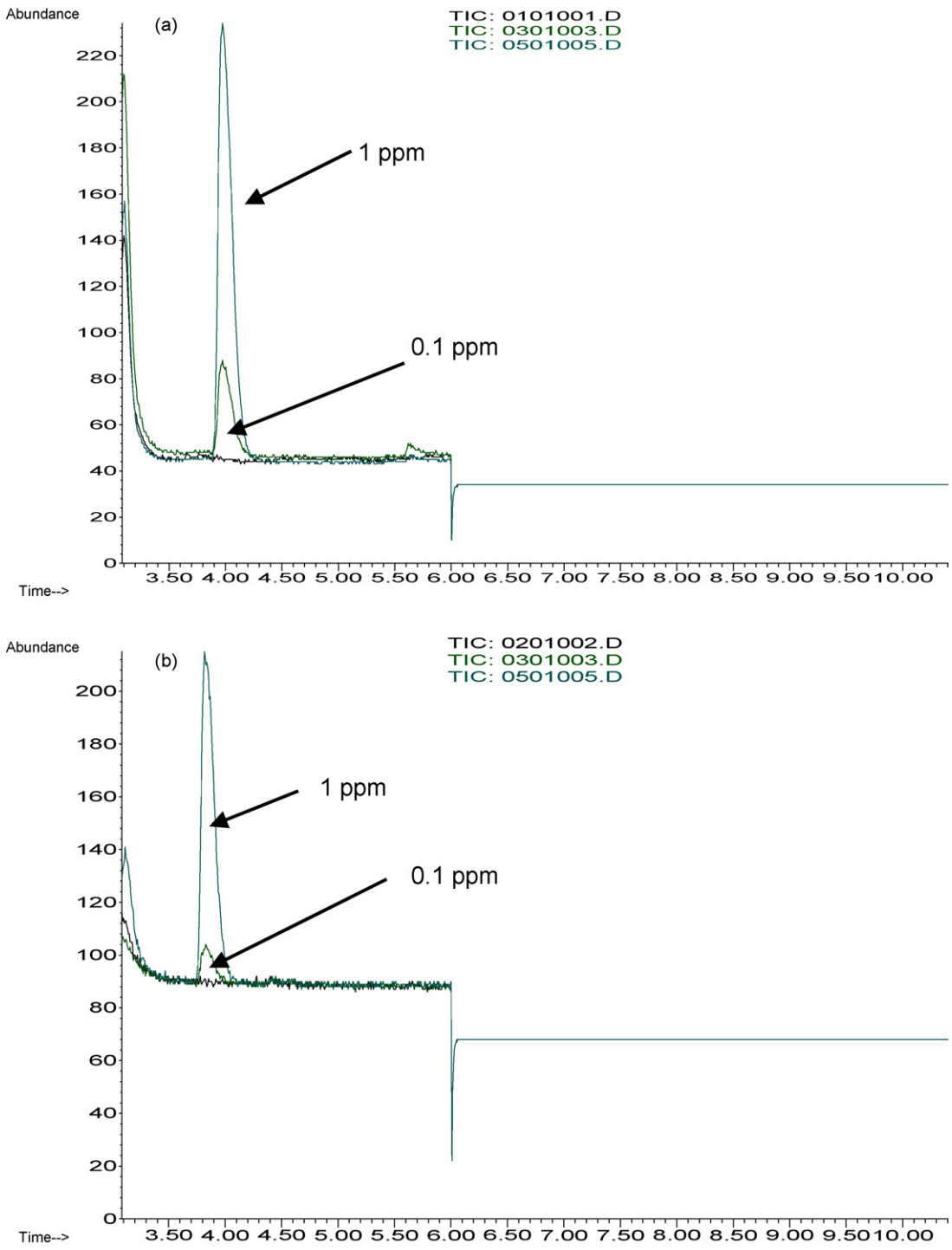

Fig. 2. Typical GC–MS chromatograms showing the peaks corresponding to the derivatization products of hydrazine: (a) acetone azine monitored at *m*/*z* 112 and (b) acetone azine-d_{12} monitored at *m*/*z* 124 and 106.

was obtained from J.T. Baker (Phillipsburg, NJ, USA). N-methyl-2-pyrrolidinone (NMP, HPLC grade) was purchased from Burdick & Jackson (Morristown, NJ, USA). Water used in the experiment was purified by an in-house Milli-Q system (Millipore, Billerica, MA, USA). Helium (99.996%) and nitrogen (99.999%) were obtained from PRAXAIR (Bethlehem, PA, USA). All drug substances used for validation and testing were obtained from projects at GlaxoSmithKline and prepared in house. For proprietary reasons, all codes of drug substance are masked.

2.2. Headspace GC–MS conditions

The analysis was carried out using an Agilent GC–MS system (Palo Alto, CA, USA) consisting of a 6890A GC, a 5973N Mass Detector and a CTC Combi-Pal autosampler. The headspace autosampler conditions are: incubation oven temperature 100 °C; incubation time 10 min; headspace syringe temperature 110 °C; agitation speed 500 rpm; agitation on time 18 s; agitation off time 2 s; injection volume 500 μL; fill speed 100 μL/s; syringe pull-up delay 300 ms;

injection speed 1 mL/s; pre-injection delay 2 s; post injection delay 100 ms; syringe flush 5 min with nitrogen. An Agilent DB-624 (25 m × 0.2 mm i.d. × 1.12 μm) GC column was used. The oven temperature gradient started at 100 °C for 6 min and then ramped to 220 °C at 50 °C/min and held for 2 min at 220 °C. A 4 mm i.d. liner containing glass wool was used. Helium was used as carrier gas with a constant flow rate of 1.2 mL/min. The injector temperature was kept at 200 °C in split mode (5:1). The mass detector was operated in electron impact mode (70 eV). The source temperature and quad temperature were set to 230 and 150 °C, respectively. The MSD transfer line temperature was set at 230 °C. The derivatization products of hydrazine were monitored in the selected ion monitoring (SIM) mode with a dwell time of 200 ms. The data were only collected between 3.0 and 6.0 min. For the acetone derivative, the molecular ion at *m*/*z* 112 was monitored while for the acetone-d_6 derivative, both ions at *m*/*z* 124 and *m*/*z* 106 were monitored for best results.

2.3. Preparation of derivatization reagent, standard and sample solutions

The derivatization reagent was prepared by dissolving 0.5 g of benzoic acid and 0.5 mL of acetone or acetone-d_6 in 10 mL NMP. The stock solution of hydrazine was prepared at approximately 1 mg/mL in the diluent of aqueous EDTA (0.1%). For linearity validation, the stock solution of hydrazine was diluted using the diluent to give standards at 0.1, 0.2, 1, 2, and 10 μg/mL, which correspond to 0.1, 0.2, 1, 2, and 10 ppm (relative to 10 mg API free base), respectively. The API samples were typically prepared by weighing approximately 10 mg API into a 10-mL headspace GC injection vial, followed by adding 100 μL of derivatization reagent and 10 μL of diluent. The solutions used for method recovery tests were prepared similarly, except that 10 μL working standard solution instead of the diluent was added. The prepared samples were analyzed directly without further treatment.

3. Results and discussion

The acetone derivatization approach has been used for the analysis of hydrazine in air and water [15,16] in which the derivatization product, acetone azine, was detected by direct injection GC methods. The approach has not been adopted for pharmaceutical analysis due to the presence of large amount of non-volatile sample matrices, API. Although, the direct injection GC approach is amenable to environmental analysis applications, it is prone to severe contamination issues in pharmaceutical analysis due to the presence of high concentration of API. Because of the moderate boiling point of the derivatization product, acetone azine, it is feasible to convert the direct injection approach to more selective and robust headspace GC–MS method.

3.1. Derivatization

Hydrazine reacts with acetone or acetone-d_6 producing acetone azine or acetone azine-d_{12}, respectively. In EI-MS analysis, acetone azine produced major ions at *m*/*z* 56, *m*/*z* 97, and *m*/*z* 112, and acetone azine-d_{12} produced major ions at *m*/*z* 62, *m*/*z* 106, and *m*/*z* 124 (Fig. 1). The derivatization reaction is driven to completion during headspace incubation. NMP is an excellent solvent, since most tested APIs dissolved completely before incubation. For certain very hydrophobic API substances, the sample solution became homogenous during incubation. Incubation times of 5, 10 and 15 min were tested and no significant difference was observed, thus an incubation time of 10 min was selected. Acetone and acetone-d_6 are more volatile than the azines, and thus may compete with acetone azine or acetone azine-d_{12} for vaporization in headspace injection vials. Therefore, the concentration of acetone in the derivatization reagent solution must be minimized. It was optimized by examining the concentrations at 5%, 10% and 20% (v/v). No significant effect on hydrazine sensitivity was observed for the 1 ppm standard. However, the acetone concentration had a great impact on the precision of the method. The %RSD of 6 preparations at 1 ppm was 2.6%, 9.7%, and 23.1% respectively for acetone concentrations of 5%, 10% and 20%. The higher acetone concentration gave the worse injection precision. As a result, 5% acetone was selected. Benzoic acid, another additive in the derivatization reagent solution, helped increase the response of hydrazine by about 40%, but the mechanism is not clear. It may catalyze the derivatization reaction or facilitate the vaporization of acetone azine from NMP solution. Non-volatile EDTA was added into the diluent for standard solutions to prevent transition metal promoted autoxidation of hydrazine [18]. The additives exerted no chromatographic effects on the analytes. The separation of the hydrazine derivatization product from other interferences can be easily achieved. Typical chromatograms are shown in Fig. 2.

3.2. Validation results

The linearity, LOQ, precision, and spike recovery (accuracy) of the method were evaluated, and the validation results are summarized in Tables 1 and 2. A linear range from 0.1 to 10 ppm (relative to 10 mg API in a headspace injection vial) was demonstrated for both derivatization reagents with $R^2 \geq 0.999$ (Table 1). Based on the S/N ratio of the lowest concentration of the standard, the LOQ for hydrazine could be as low as 0.1 ppm. The method uses 10 mg API for each analysis, but if desired, lower LOQ could be achieved by using higher API loading. The precision of hydrazine analysis of the standard at the 1 ppm level is typically between 2.7% and 5.6%. Typical spike recoveries of hydrazine were in the range of 79–117% (Table 2) at 1 ppm except entries 6 and 7, which were performed at 25 and 100 ppm respectively with modified methods due to the higher control limits required by the projects. The relatively low recovery (79%) of hydrazine in GSK966XXX may be attributed to the reaction of hydrazine with API, which is a weak Michael acceptor. Some of the APIs tested contains reactive functional groups such as

Table 1
Linearity and sensitivity of the method using acetone or acetone-d_6 as derivatization reagent.

Validation parameters	Acetone	Acetone-d_6
Linear range	0.1–10 ppm	0.1–10 ppm
Linear curve	$Y = 1660.9x - 169.42$ $R^2 = 1.0000$	$Y = 957.54x + 320.23$ $R^2 = 0.9998$
%RSD of six preparations of standard at 1 ppm	2.7%	5.6%
LOQ	0.1 ppm	0.1 ppm

Table 2
Recoveries of hydrazine in various API matrices using acetone or acetone-d_6 as the derivatization reagent.

Entry	Derivatization reagent	API	Recovery (%)
1	Acetone	GSK419XXXE	110
2	Acetone	GSK966XXX	79
3	Acetone	GSK580XXXC	115
4	Acetone	SB462XXX	107
5	Acetone	GSK183XXXA	117
6	Acetone	GSK211XXXE	101[a]
7	Acetone	GSK211XXXB	108[b]
8	Acetone-d_6	SB462XXX	91
9	Acetone-d_6	GSK966XXX	80

[a] At 25 ppm, with a modified method.
[b] At 100 ppm, with a modified method.

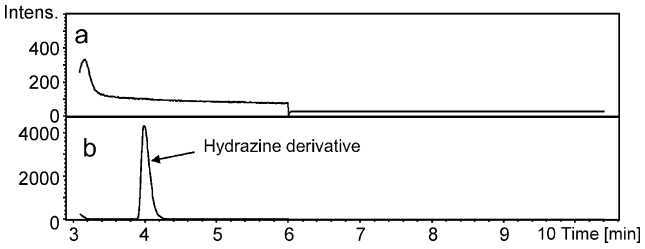

Fig. 3. Chromatograms of actual samples of (a) SB462XXX showing absence of hydrazine and (b) GSK211XXXB showing presence of hydrazine by monitoring acetone azine derivative.

ketone, primary amine, or even Michael acceptor which may react with hydrazine and acetone respectively. Also the APIs vary in form from free base to HCl salts and fumarate salts. These experiments demonstrated that the effect of API on the recovery is not significant. The chromatograms of two actual samples are presented in Fig. 3 to demonstrate the application of the strategy. This general derivatization-headspace GC–MS method has been used successfully for determination of hydrazine in support of batch releases and process chemistry for various APIs at GlaxoSmithKline.

4. Conclusion

A general derivatization-headspace GC–MS methodology has been developed for determination of hydrazine in various drug substances. The method uses acetone or acetone-d_6 as the derivatization agents converting hydrazine into the stable and volatile acetone azine or acetone azine-d_{12} for detection. The volatile nature of the derivatization products offers an excellent attribute for their chromatographic separation and detection. By employing a headspace GC–MS system, the non-volatile sample matrices were prevented from entering the GC system. Consequently, instrument contamination by sample matrix (API) is minimized. The derivatization procedures can be automated by utilizing an autosampler with heating, stirring, and reagent addition capabilities. In conclusion, the practical methodology reported here can be generally applied to analysis of trace hydrazine in other drug substances.

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