Control and analysis of hydrazine, hydrazides and hydrazones – Genotoxic impurities in active pharmaceutical ingredients and drug products

Abstract

This is the latest series of reviews focused on the analysis of genotoxic impurities. This review summarises the analytical approaches reported in the literature relating to hydrazine, hydrazines, hydrazides and hydramones. It is intended to provide guidance for analysts needing to develop procedures to control such impurities, particularly where this is fur to concerns relating to their potential genotoxicity

Of particular note is the wide variety of techneques employed, both chromatographic and spectroscopic, with most involving derivatization. Such a wide variety of options allow the analyst a real choice in terms of selecting the most appropriate technieque specific to their requirements. Several generic methodologies, covering the three main analytical approaches: i.e. HPLC(high performance liquid chromatography), GC(gas chromatography) and IC (ion chromatography), are also described

1. Introduction

Issues relating to guidance on safe levels of genotoxic impurities (GIs) in novel and generic active pharmaceutical ingredients (APIs) and drug products have received considerable attention in the recent past.

Metabolism of hydrazine is complex. Hydrazine is rapidly acetylated in most species. The initial mono-acetylation is too fast to measure for resultant metabolite identification and the excreted diacetyl metabolite has been reported to account for the majority of the administered dose.

Hydrazine has been reported as the principal hydrolytic degradation product of the anti-tuberculosis drug, isoniazid and of structurally related analogues e.g. hydralazine, phenelzine and isocarboxazid.

**Overview**

Data from in vivo studies suggest that hydrazine and methyl- hydrazine are alkylating agents.

The formation of methyl adducts with DNA bases in vivo may be one of the mechanisms by which hydrazines cause DNA damage and gene mutations [14,15].

Hydrazine, methyl hydrazine and related hydrazides are known human carcinogens

Hydrazines, hydrazides and hydrazones (Fig. 1) all show conventional structural alerts for genotoxic potential.

The simplest situation arises when the impurity shows a structural alert for genotoxi- city and no Ames-test or other relevant data are available.

Analysis of hydrazine and the related methylated hydrazines (methyl and dimethyl hydrazine) is challenging due to their volatil- ity, high polarity, low molecular weight, absence of chromophore and in the case of hydrazine, the absence of any carbon atoms [21]. In addition, the potential for interference from the API matrix is significant and direct-injection GC of hydrazine derivatives is difficult due to the large amounts of API that can lead to cross- contamination, i.e. throughput is limited, allied with the necessity of frequent instrument cleaning [22]. In contrast, the analysis of aryl hydrazines (and related compounds) is fairly straight forward due to the presence of the aromatic ring system and presents fewer analytical challenges.

HCI

(see Scheme 1): Kean et al. using a modified HPLC pharmacopoeial method involving derivatisation with benzaldehyde combined with liquid–liquid extraction (LLE) demonstrated linearity over the range 0.04–2ppm of residual hydrazine, with a limit of quantitation (LOQ) of 0.2ppm.

The extraction recovery was reported as 92%.

The authors applied this quantitative HPLC method to the determination of residual hydrazine in samples of excipients e.g. povidone, copovidone, and API e.g. dihydralazine sulphate, hydralazine hydrochloride, nitrofurazone, nitrofural and nitro- fuoroxazide.

These authors also examined the levels of residual hydrazine in carbidopa API, using this HPLC (Table 2) method. They found elevated levels (10.4ppm) versus the approved TLC method (3.2 ppm), but the sample was still in compliance with the mono- graph (20 ppm limit). Finally, these authors evaluated the residual hydrazine levels in isoniazid API. They found levels of 72.8 ppm. They considered that the current monograph limit for isoniazid was too high (125 ppm) and that the existing TLC monograph method was too insensitive for routine pharmacopoeial use.

Recently, an HPLC and HPLC–MS study of the decomposition of isoniazid was carried out by Bhutani et al. [23]. The authors reported that isoniazid was thermolytically stable, slightly unstable to photolysis and oxidation, but underwent extensive hydrolysis, to yield four primary degradation products that they designated I–IV: hydrazine (I), isonicotinic acid-N′ -(pyridyl-4-carbonyl)hydrazide (II), isonicotinic acid-pyridine-4-ylmethylene hydrazide (III), isoni- cotinic acid ethylidene hydrazide (IV). Under photolytic conditions the API turned yellow to form the previously unreported degrada- tion product (II). The method was validated for the API, but not for the degradation products, other than specificity assessments. The authors demonstrated resolution values in excess of 3 between the API and degradation products and high peak purity indexes for all peaks, i.e. greater than 0.9912.

The stability of the novel iron chelator, pyridoxal isonicotinoyl hydrazone (PIH) was studied by Kovarˇíková et al. [24]. The authors indicated that the principal degradation pathway was hydrolysis of the hydrazone bond to yield pyridoxal and the aryl hydrazine compound, isoniazid. A minor degradation product was isonicotinic acid. The method was fully validated for the PIH, but no details were provided for the degradation products.

A novel separation method for the impurities in mildronate API was recently reported by Hmelnickis et al. [25]. The authors used a hydrophilic interaction chromatography (HILIC) method with several different polar stationary phases (silica, cyano, amino and the zwitterionic sulfobetaine) to separate the six polar impuri- ties, including the hydrazine impurity, 1,1,1-trimethylhydrazinium bromide (that they designated impurity II. Note that in cited exam- ples the current authors have used the same designations that the original authors applied). They demonstrated that HILIC was a use- ful alternative to reverse phase or ion chromatography (IC). The impact of method separation conditions, including organic modi- fier content and pH were studied. Finally, a HILIC method using the zwitterionic sulfobetaine stationary phase (ZIC-HILIC) was devel- oped and validated. The method showed good linearity for impurity II with a limit of detection (LOD) of 3 ppm and an LOQ of 10 ppm. The recoveries for impurity II over the range 0.05–0.125% were in the range 105.7–114.0% with RSDs of between 1.1 and 5.1%. The preci- sion at the 0.1% level (designated specification limit) was 3.3%. The method was found to be robust and applied to two batches of mil- dronate API. In the technical batch the levels of residual impurity II were high (i.e. 3500 ppm), but in the commercial batch there were levels at the LOQ of the method; i.e. 3 ppm. This demonstrates a fairly universal truth that as a greater understanding of the process is gained throughout the development process that it typically leads to better control strategies and lowered levels of these reactive impurities at the point of commercialization.

A novel generic method for the direct determination of hydrazine and 1,1-dimethylhydrazine in a pharmaceutical inter- mediate was reported by Liu et al. [26]. The method also used a HILIC approach with ethanol as a weak eluent and interestingly chemiluminescent nitrogen detection (CLND). The method is sim- ple and reasonably sensitive, i.e. 200 ppm. The method was linear. The precision of the standard solutions at 200ppm was 5.0 and 3.9%, for hydrazine and 1,1-dimethylhydrazine, respectively. The recoveries were excellent: for 1,1-dimethylhydrazine at 200 ppm and 400 ppm they were 100.9 and 102.7%, respectively. However, in contrast, the recoveries for hydrazine were low (84.3%) at 200 ppm, but were satisfactory (102.7%) at 400 ppm.

Srinivasu et al. [27] assessed the residual levels of 4-hydrazine benzene sulphonamide (intermediate I) in the COX-2 inhibitor, celecoxib. The method was linear and accurate over the range 0.05–1.0%, with recoveries in the range of 96.7–100.2%. The method gave an LOQ of 97 ng/ml. The method was robust with respect to mobile phase compositions, pH and temperature. Satyanarayana et al. [28] also evaluated the residual levels of hydrazine and 4- hydrazine benzene sulphonamide in celecoxib, but there were no reported validation details.

Kirtley et al. [21] initially assessed GC methodologies for residual hydrazine, in various APIs, before evaluating HPLC with UV detec- tion at 190 nm. They initially reported a 20 ppm LOD, which was too insensitive and resorted to derivatisation. They utilized the ben- zalazine derivative of the residual hydrazine (Scheme 1), but the method details were not reported. The authors observed that there was a gradual increase in reportable levels of hydrazine from this method over time giving artefactually high levels (8 ppm in API, 10 ppm in API spiked with 2 ppm of hydrazine and 12 ppm in API spiked with 5 ppm of hydrazine). They identified one source of the increase as the interference from another process impurity (bis- hydrazone), which was present at residual levels of 300 ppm and could also form the benzalazine derivative. They reported that this method was suitable for samples of API containing greater than 50 ppm of hydrazine, where the interference was negligible.

They introduced a clean up procedure (API precipitated out of solution as free acid and they used the supernatant for assessing levels of residual water soluble analyte) and modified the initial derivatisation procedure, using a lower temperature to limit any unwanted side-reactions. The method gave recoveries in the range 95–105% and was suitable for determining residual hydrazine lev- els <10 ppm during in-process testing of reaction liquors and crude API (LOD 1 ppm).

A Czech research group [29] recently reported on aryl hydra- zone isomerisation resulting in formation of residual geometrical E-isomer impurities determined by HPLC with diode array and MS–MS detection. Their chromatographic investigation was sup- ported by complementary NMR data, but no validation data was reported.

An Indian research group [30] reported on the development and validation of a stability indicating HPLC method for the determination of the anti-tuberculosis drug, rizatriptan and its degradation products, including a hydrazone impurity (impurity I: 1-(4-hydrazinophenyl)methyl-1,2,3-triazole). The method was linear over the range from the LOQ to 3000ppm (the specifica- tion limit proposed was 1500 ppm). The LOD and LOQ values for impurity I were 400 ppm and 1300 ppm, respectively. The recov- eries of impurity I at typical levels in the bulk API were in the range 91–107%. The authors examined the impact of flow rate, pH of mobile phase and the affect of column temperature (20–30 ◦ C).

The method was shown to be robust with resolution of API from all impurities greater than 4.0.

Butterfield et al. [31] investigated the formation of aldose reaction products of isoniazid and lactose. They developed an HPLC method for the simultaneous determination of isoniazid and 1-iso-nicotinyl-2-lactosylhydrazine (impurity I) in tablet formu- lations. The authors reported sensitivity at the 5000ppm level, with a RSD of 0.7%. They assayed seven commercial batches of isoniazid tablets and showed levels of impurity I in the range 3000–58,000 ppm.

Lessen and Zhao [32] performed similar HPLC and fluores- cence investigations on aldose reaction products of hydralazine and starch, identifying the corresponding hydrazone (impurity IIa) as a key intermediate in the overall degradation pathway. They theorized that the hydrazone subsequently cyclises to the tria- zolo phthalazine (impurity IIIc). They used these investigations to provide explanations for the lack of mass balance in ongoing hydralazine tablet stability studies. No method validation details were provided.

The hydrolysis of the cytotoxic agent ebifuramin to the hydrazone impurity III [(+)-5-morpholinomethyl-3-(5- nitrofurfurylidene amino)-oxazolidin-2-one] was studied by Prankerd and Stella [33]. They determined that the hydrolysis kinetics was initially concentration dependant (not first order), followed by apparent first order degradation of at least one of the initial hydrolysis products. No method validation details were provided.

The validation of a rapid and selective HPLC method for the determination of rifampicin and its hydrazone degradation prod- ucts was reported by Bain et al. [34]. The major decomposition pathways of rifampicin are basic oxidation [rifampicin quinone (RQU)] and acidic hydrolysis [3-formylrifampicin (RSV) and 1- amino-4-methyl piperazine]. In addition, under anaerobic basic conditions, 25-desactylrifampicin (DAR) is formed, which then in turn decomposes to 25-desactyl-21-rifampicin (25-21) and 25- desactyl-23-rifampicin (25-23). Specificity of the method was assured by measuring peak purity. The method did not resolve impurities 25-21 and 25-23, as there is an acetyl migration occur- ring in solution between positions 21 and 23, and they are reported as a composite value (25/21-23). 1-Amino-4-methyl piperazine has no chromophore and was not detected in this method. The authors evaluated both direct injection (DI) and column switching (CS) onto a pre-column to pre-concentrate the analytes and then back-flushing onto the analytical column.

The method was linear for all of the hydrazone impurities (RQU, DAR and 25-21/23). The LOQ of each of these analytes was sig- nificantly enhanced by column switching. The LOQs were 400, 215, and 275 ng/ml, respectively, using direct injection compared to 39, 20 and 36ng/ml, respectively, using column switching to remove the API. The accuracy of the method was appropriate. The within-day precision for these three analytes at 1 g/ml were significantly improved using pre-concentration approach (RSD 0.25–0.65%) compared with direct injection (RSD 1.75–3.56%).

An Indian research group [35] evaluated the USP monograph method for fixed-dose combinations of anti-tuberculosis drugs (isoniazid, pyrazinamide and rifampicin) and in particular they assessed the method for its ability to resolve rifampicin from its hydrazone degradation products (RQU, DAR and isonicotinoyl hydrazone; the latter an interaction product formed between RSV and isoniazid). The authors found that the resolution of the method was highly dependant on the stationary phase selected. Three columns representative of USP stationary phase L1 were evalu- ated. Columns 1 and 2 were able to resolve the three different APIs and rifampicin from its hydrazone degradation products (with the exception of DAR and rifampicin N-oxide which co-eluted); whereas, column 3 failed to resolve these analytes. Satisfactory res- olution could be achieved by decreasing the organic component and reducing the flow rate.