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Short communication

Determination of genotoxic epoxide at trace level in drug substance by direct injection GC/MS

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ABSTRACT

A novel direct injection gas chromatography method coupled with selective ion monitoring mass spectrometry (GC/SIM-MS) was developed for quantitation of trace levels of high boiling point (HBP) epoxide genotoxic impurity (GTI) in drug substance. The injector temperature was optimized with the aims to minimize matrix effects and enhance SIM signal response. The final injector temperature 160 °C was selected after balancing between these two factors. The column screening was conducted as well and MN OPTIMA delta-3 silica capillary column was selected since it showed good peak symmetry without column bleeding. The good linearity was established for the concentration in the range from 0.0045 μg/mL to 0.5 μg/mL with a $R^2 = 0.9999$. The limit of detection (LOD) and the limit of quantitation (LOQ) were 0.0014 μg/mL and 0.0045 μg/mL, respectively. The recovery which ranged from 95.0% to 112.5% could meet the ICH acceptance criteria. The validation results demonstrated the good linearity, precision and accuracy of the method which can be further adopted as an adequate quality control tool for quantitation of epoxide impurity at trace levels in drug substance and drug product.

1. Introduction

During drug substance or active pharmaceutical ingredient (API) synthesis, impurities can easily arise from often used reagent, starting materials, reactive intermediates, by-product reactions and degradation during storage. Some impurities are identified as genotoxic impurities (GTI) which pose a significant safety risk because they induce damage to the genetic material in the cells through interactions with the DNA sequence and structure. As a result, GTI can lead to mutations or cause cancer [1–3]. Therefore, exposure to even trace levels of GTI in API may be of significant toxicological concern.

EMEA [4] and FDA [5] guidelines have established a threshold of toxicological concern (TTC) of 1.5 μg/day (1.5 ppm, assuming a daily dose of 1 g) for each GTI as an acceptable threshold for any marketing authorization application. During clinical development stage, however, a staged TTC is applied where higher daily intake levels can be allowed for a shorter period. On the basis of FDA and EMEA guidelines, a new ICH guidance ICH M7 has established less-than-lifetime (LTL) acceptable GTI intakes for different treatment periods [6], *e.g.* the acceptable daily intake is 1.5 μg/day for lifetime duration whereas 120 μg/day is virtually the safe dose for 1 month short treatment. The higher acceptable daily intake of GTI during early development stage not only allows program to move forward but also gives chemists and analysts more time to further understand the mechanisms of GTI formation and elucidate control strategy.

Determination of trace levels of GTI in API is often a great analytical challenge as an extremely sensitive, selective and robust analytical method is needed. Many traditional approaches such as HPLC-UV for non-volatile analytes and GC-FID for volatile analytes are usually not effective enough for impurity analysis at sub-ppm or trace levels [7]. Hyphenated techniques like GC–MS and LC–MS combining physical separation capabilities of chromatography (GC or HPLC) with mass spectrometry have higher sensitivity and specificity than conventional HPLC and GC methods. Their applications are oriented towards the potential identification and quantitation of trace levels of impurities in API. Several recent publications have reported systematic GTI method development and control strategies [8–10].

Epoxide is a key intermediate used in the synthesis of some APIs [11,12]. The epoxide intermediate (as shown in Fig. 1) (3aR,5*S*,6aR)-2,2-dimethyl-5-[(2*S*)-oxiran-2-yl]-3a,5,6,6a-tetrahydrofuro[2,3-*d*][1,3]dioxole studied in this paper was used to synthesize a Roche proprietary compound under development. It was identified as a GTI by Ames test because of the presence

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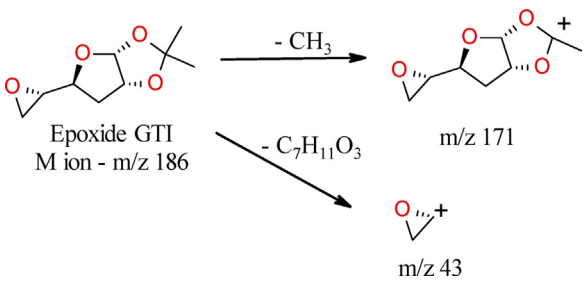

Fig. 1. Chemical structure of the epoxide GTI (3aR,5*S*,6aR)-2,2-dimethyl-5-[(2*S*)-oxiran-2-yl]-3a,5,6,6a-tetrahydrofuro[2,3-*d*][1,3]dioxole and its two major fragmentation pathways.

of the epoxide moiety [13]. As shown in Fig. 1, the epoxide GTI is volatile without chromophores or fluorescent functional groups, but has high boiling point (HBP) (>100 °C). The use of GC–MS and LC–MS/MS after derivatization has found wide applications in the analysis of epoxides [14–17], but the derivatization step needed in those methods limits its application in rapid analysis. The static headspace GC is often used to quantitate volatile species in the matrices of API and formulation to reduce matrix interference. However, for HBP GTIs quantitation by HS-GC, the commonly used HBP organic diluents such as DMSO, DMAC or DMF can cause safety issues because of substantial internal pressure. When temperature is close to their boiling points (typically > 100 °C), the internal pressure can breach the sample vial septa or even rupture the vial itself [18]. Extraction techniques (LLE, LPME, SPE and SPME) are also routinely used to reduce matrix interference in GC methodologies [19–22]. However, a number of drawbacks such as limited sorbents and selectivity, difficulty in automation and sample manipulation etc. have greatly restricted its application. The extraction techniques are only used as sample preparation techniques nowadays [19–24].

To enable high throughput analysis and minimize sample preparation, the aim of this study was to develop a derivatization-free, simple, rapid and sensitive GC method for the routine analysis of trace levels of HBP epoxide GTIs. For this purpose, a direct injection method was developed by using injection port liner with a small plug of deactivated fused silica or glass wool to trap non-volatile API and also reduce matrix contamination in GC system. If API is thermally unstable, the injection temperature typically needs to be optimized to reduce the matrix interference caused by API decomposition.

2. Experimental

2.1. Chemicals and reagents

The epoxide GTI with purity > 98% was synthesized in Roche Innovation Center Shanghai (RICS, Shanghai, China) from the commercially available raw material diacetone-D-glucose. The API which is a Roche proprietary compound under development was also synthesized in RICS with purity > 98% (Shanghai, China). Both acetonitrile (ACN) and dimethyl sulfoxide (DMSO) of HPLC grade were obtained from ThermoFisher Scientific (Fair Lawn, New Jersey, USA). Helium (99.999%) was obtained from Air Products and Chemicals, Inc. (Millennium Gate, UK).

2.2. GC/MS parameters

GC/MS analysis was carried out in an Agilent GC/MS system (Palo Alto, CA, USA) consisting of a 7890A GC, 5975C mass detector and a 7683 B Series auto sampler injector. The chromatographic separation was achieved on a MACHEREY-NAGEL (MN) OPTIMA delta-3 silica capillary column (30 m × 0.25 mm, 0.5 μm film thickness). The column temperature was set to 100 °C and programmed to 250 °C at 10 °C/min. The column flow phase helium was kept constant at 1.5 mL/min throughout this study. The injector temperature was set at split mode (4:1) with an injection volume of 0.5 μL. The detector was operated by electron impact ionization (EI, 70 eV) with an ion source temperature of 230 °C and an interface temperature of 280 °C. Ions were acquired in selective ion monitoring (SIM) mode with a solvent cut time of 3.0 min. As shown in Fig. 2, two major daughter ions *m*/*z* 43.0 and *m*/*z* 171.0 (as shown in Fig. 1) with high abundance were detected. The ions of *m*/*z* 207.1, 269.0 and 281.0 in Fig. 2 were derived from background. The precursor ion *m*/*z* 186 could not be observed in the spectrum since it is easy to fragment under EI 70 eV. Generally it is better to choose one or more unique ions in order to avoid the analytical deviation. However, the background noise is high at *m*/*z* 43.0, so the GTI quantitation sensitivity would be seriously affected if the two daughter ions *m*/*z* 43.0 and *m*/*z* 171.0 were collected simultaneously. Due to this fact, only the daughter ion *m*/*z* 171.0 with the best signal response in the spectrum was selected for further method optimization.

2.3. Preparation of standard and sample solutions

All standard and sample solutions were prepared in ACN. A 0.5 mg/mL standard solution of the epoxide GTI was prepared and stored at −20 °C until usage. The API sample was prepared at a concentration of 5 mg/mL in ACN. Spiked samples were prepared by adding the epoxide GTI standard solution into the API sample solution to make a final concentration needed.

For the linearity assessment, the standard solution containing the epoxide GTI was diluted with ACN to various concentrations 0.0045, 0.015, 0.05, 0.1, 0.5 μg/mL to cover adequate range from limit of quantitation (LOQ) to 100% of GTI limit. Triplication of each concentration standard was performed. The limit of detection (LOD) and LOQ were determined at respective signal-to-noise (S/N) ratio of 3:1 and 10:1 by injecting 3 replicated GTI samples. The accuracy was also assessed at appropriate levels (0.015, 0.1, 0.5 μg/mL) spiked in 5 mg/mL sample solutions to assure that satisfactory recovery is achieved. Each level was prepared independently in triplicate and with duplicated injections and stored at −20 °C until usage. The percentage recovery of the epoxide GTI was calculated, considering the amounts of the GTI spiked, the GTI available in un-spiked samples and the GTI recovered.

3. Results and discussion

3.1. Acceptable TTC

The maximum daily dose was determined to be 210 mg based on the API toxicology data obtained from animal and DMPK prediction. The duration of the treatment using the corresponding drug is 3 month. According to ICH-M7 guideline which allows higher levels of individual GTI in a short period of treatment during development, the acceptable TTC can be calculated by using the following equation.

$$\text{AcceptableTTC(ppm)} = \text{DailyIntakeLimitofLTL}(\mu\text{g/day})/\text{Dose(g/day)}$$

According to this equation, for 1–12 months duration treatment, the individual LTL daily intake is 20 μg/day, so the acceptable TTC of the epoxide GTI for daily dose of 210 mg is 95 ppm in API.

3.2. Optimization of sample preparation

The sample preparation is an important step in the analysis of the low level GTI otherwise matrix effects would be enlarged lead-