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COMMUNICATION

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Determination of Epichlorohydrin in Active Pharmaceutical Ingredients by Gas Chromatography−Mass Spectrometry

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ABSTRACT: During the development of new active pharmaceutical ingredients (API), the control of low level impurities plays a crucial role in the progression of a new compound. Particular attention is given to known or suspected genotoxic impurities, which, to ensure patient safety, have a maximum daily patient exposure limit of 1.5 μg/day for long-term exposure (>12 months).

In this communication, we report the development and validation of a *part per million* (ppm) level GC−MS method to detect epichlorohydrin (ECH) in a new API under development in Chemical Development at GlaxoSmithKline Verona, now an Aptuit Research Centre. GC separation was conducted on a DB-624 column, while MS detection was performed on a single-quad MSD in selected ion monitoring mode. After successful development, the method was validated according to our in-house validation guidelines for ppm level analysis. The method proved to be selective for ECH in drug substance. Sensitivity of the method (limit of quantitation (LOQ)) proved to be as low as 0.8 ppm (w/w) when the API samples were prepared at 45 mg/mL (free base). The precision (% RSD) of five preparations of spiked samples at the concentration of 8 ppm (target analytical level (TAL)) was 1.3, while recovery proved to be 97%, demonstrating the absence of matrix effect.

INTRODUCTION

Genotoxic substances are chemical compounds capable of causing genetic mutation and/or chromosomal rearrangements and they can therefore contribute to the development of tumors.[1] The issue of genotoxic impurities in pharmaceutical products (drug substance and drug product) has been subjected to an increased scrutiny in recent years, both by industry[2,3] and by regulatory agencies.[4−8] Due to their reactive nature, some materials used for active pharmaceutical ingredient (API) manufacturing including starting materials, intermediates, reagents, or some process-related impurities/degradants have been demonstrated to be genotoxic. According to the European Medicines Agency's (EMEA) and U.S. Food and Drug Administration's (FDA) guidelines on the limits of genotoxic impurities, the intake of genotoxins must be tightly controlled to a maximum allowable level not greater than 1.5 μg/day during chronic administration of a drug for more than 12 months.[6−8] This limit is referred to as the threshold of toxicological concern (TTC). Thus, depending on the daily dose of an API, the acceptable level of genotoxic impurity in a drug substance (so-called target analyte level (TAL)) can be calculated. The TAL will then serve as a starting point for the analytical scientist to develop appropriate methodology.

To ensure that these unwanted genotoxic impurities are reduced to an acceptable level (often as low as ppm) in the final product, it is critical to monitor them closely throughout the process. However, the rapid development of analytical methods at such low levels remains a challenge for analytical chemists[1,9,10] who have to aim at extremely high sensitivity, specificity and robustness. Also, the complex matrix effect arising from in-process samples, API, or excipients represent a significant issue for method development, especially in early drug development stages, where aggressive project timelines often limit the time and resources for method optimisation. As a result, the analytical chemist needs to ensure that the method is appropriate for its intended use. Most common limit tests at TAL are used to obtain a pass/fail result.

Epichlorohydrin (ECH), 1-chloro-2,3-epoxypropane, is widely used in the production of epoxy resins, synthetic glycerol, and elastomers and is also employed in the paper industry.[11−13] ECH is toxic by inhalation and by dermal and oral absorption and can be dangerous for the central nervous system. It is a potential mutagen[14,15] which reacts with cellular components,[16] and has been classified as a probable carcinogen (group 2A) for humans by the International Agency for Research on Cancer (IARC).[17]

During the route selection process of a new molecular entity under progression in the former GlaxoSmithKline Verona Research Centre, now Aptuit Verona Research Centre, ECH was identified as a promising starting material. However, due to the safety concerns related to the nature of ECH, an appropriate analytical control strategy needed to be put in place to ensure patient safety. On the basis of forecasted clinical dosages, the maximum daily dosage for the API was set at 180 mg/day and, with a TTC of 1.5 μg/day for genotoxic substances following chronic exposure, we could calculate that ECH had a TAL of 8 ppm in the final drug substance *(TAL (ppm) = TTC (μg/day)/max clinical dose (g/day))*. Such a low limit poses tremendous challenges not only in terms of the manufacturing process but also regarding analytical method development and validation. Accurately detecting traces of ECH in a drug substance might be very difficult because of the potential interference caused by the large amounts of drug substance and/or its impurities. Various analytical techniques can be used for the determination of ECH. However, due to the chemical characteristics of ECH, the method of choice is often gas chromatography (GC), with either electron capture detection (ECD) or mass spectrometric detection.[18−20] In this paper we present a GC−MS method

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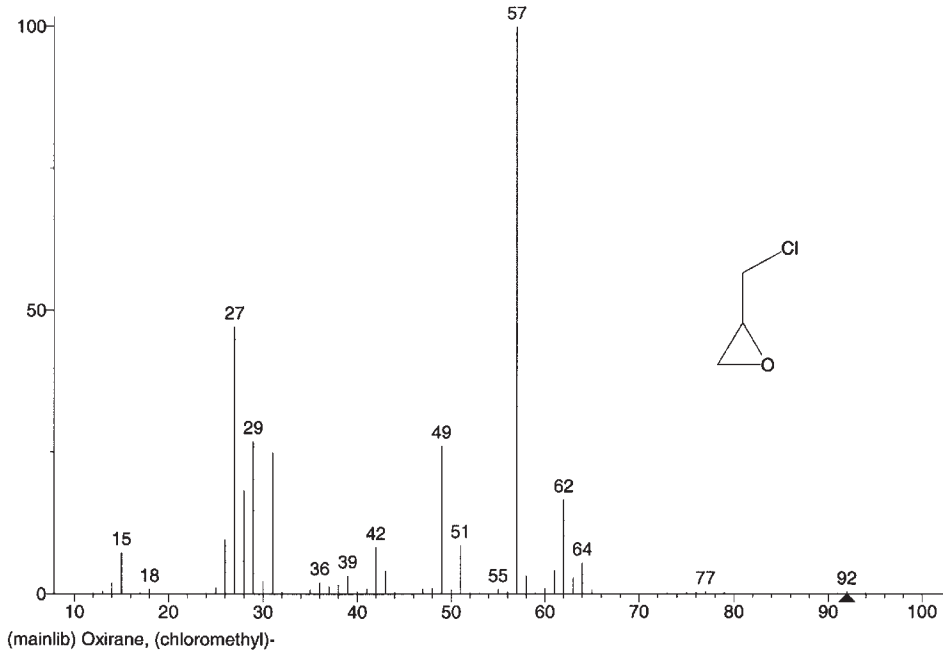

Figure 1. Epichlorohydrin spectrum.

for the detection and quantitation of ECH in a new API. The method was fully validated in accordance with in-house validation guidelines for trace level analysis method, and its applicability was demonstrated by analysis of real-life samples.

EXPERIMENTAL SECTION

Chemicals and Reagents. Epichlorohydrin (>98%) was purchased from RStech Corporation (Daejeon, Korea). Methanol was obtained from Mallinckrodt Baker (Phillipsburg, NJ, U.S.A.). Benzyl alcohol (99% plus) was purchased from Sigma-Aldrich (Milwaukee, WI, U.S.A.) and dimethylsulfoxide (>99.9%), from Romil (Cambridge, UK).

Preparation of Stock Standard Solution and Calibrators. The dissolving solvent used for the preparation of the standards was methanol. A 3.54 mg/mL stock solution of ECH in dissolving solvent was prepared and was subsequently serially diluted in dissolving solvent to prepare solutions at 3.54 μg/mL and 0.354 μg/mL. On the basis of the API sample preparation, the latter ECH solution corresponds to 8 ppm of TAL. All solutions were kept refrigerated at 4 °C until use.

Sample Preparation. Samples of API were prepared in order to achieve a concentration of 45 mg/mL of free base in dissolving solvent.

Instrumentation and Method Conditions. All GC−MS analyses were performed using an Agilent 6890N GC system (Palo Alto, CA, U.S.A.) hyphenated with an Agilent 5973 inert Mass Spectrometer and a Gerstel MPS2-Twister autosampler (Mülheim an der Ruhr, Germany).

An Agilent DB-624 (30 m × 0.32 mm i.d. × 1.80 μm) GC capillary column was used. The oven temperature gradient started at 40 °C and then ramped to 90 at 5 °C/min. It was then ramped to 250 at 100 °C/min and held for 6.4 min. A 4 mm i.d. linear containing glass wool was used. Helium was used as carrier gas with a constant flow rate of 3.5 mL/min. The injector temperature was kept at 120 °C in split mode (2:1). The mass detector was operated in electron impact mode (70 eV). The source and quad temperatures were set to 240 and 150 °C, respectively. The MSD transfer line temperature was set at 240 °C. Detection was achieved using a single ion monitoring (SIM) mode with a dwell time of 100 ms. The data were collected between 5.0 and 10.0 min only. The molecular ions at m/z 49, 51, 57, and 62 were monitored (Figure 1). Data were acquired and processed using Agilent Chemstation software.

Method Validation. According to our in-house validation guidelines for limit test methods the following four validation parameters needed to be evaluated: selectivity, sensitivity, precision, and recovery at TAL. Briefly, selectivity was assessed by injection of blanks, standards, and API samples, ensuring that no interfering peaks were present at the time of elution of ECH. Sensitivity (limit of quantitation, LOQ) of the method was determined as the lowest concentration that provided a signal-to-noise (S/N) ratio of at least 10. Precision was evaluated by injection of five replicates of sample solutions that were prepared by spiking API samples at TAL. Recovery was evaluated by spiking samples (n = 5) with ECH at TAL and comparing the analyte peak area against a pure standard of the same concentration.

RESULTS AND DISCUSSION

Method Development. Our first attempt at method development started with headspace (HS) GC-electron capture detection (ECD). In the initial method conditions the oven temperature gradient was set to start at 40 °C with a ramp up to 220 at 20 °C/min. Helium was used as carrier gas with a constant flow rate of 3.5 mL/min. The injector temperature was kept at 200 °C in split mode (10:1). ECD temperature was set at 240 °C. The headspace autosampler was equipped with a 2.5 mL syringe, and the following conditions were tested: incubation oven temperature 120 and 130 °C; incubation time 10 min; headspace syringe temperature 120 and 130 °C; agitation speed 500 rpm; agitation on time 18 s; agitation off time 2 s; injection volume 1 mL. Sample concentration was about 50 mg/mL in both benzyl alcohol and dimethylsufoxide as dissolving solvent. Injections of

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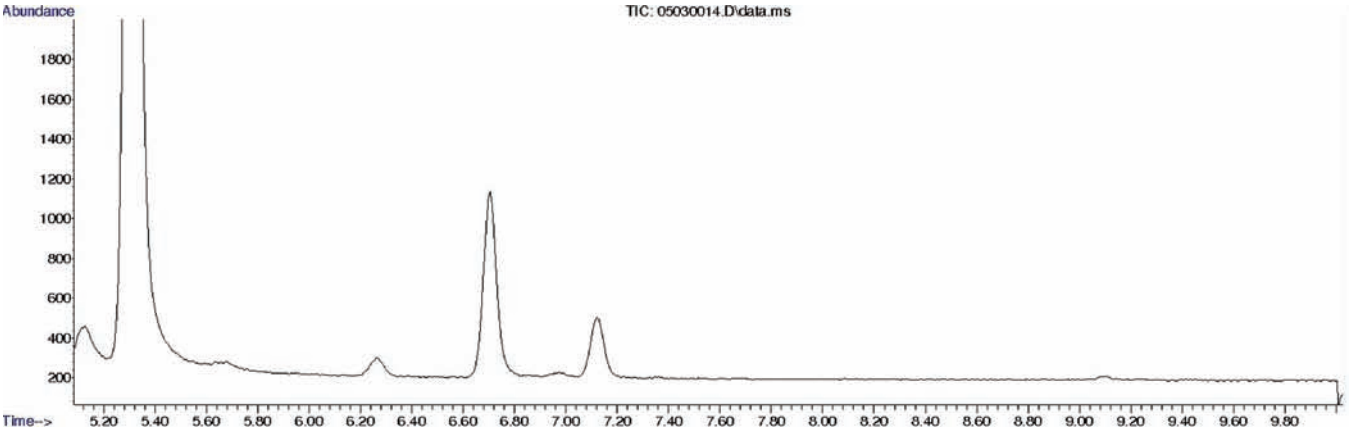

Figure 2. Analysis of the API sample.

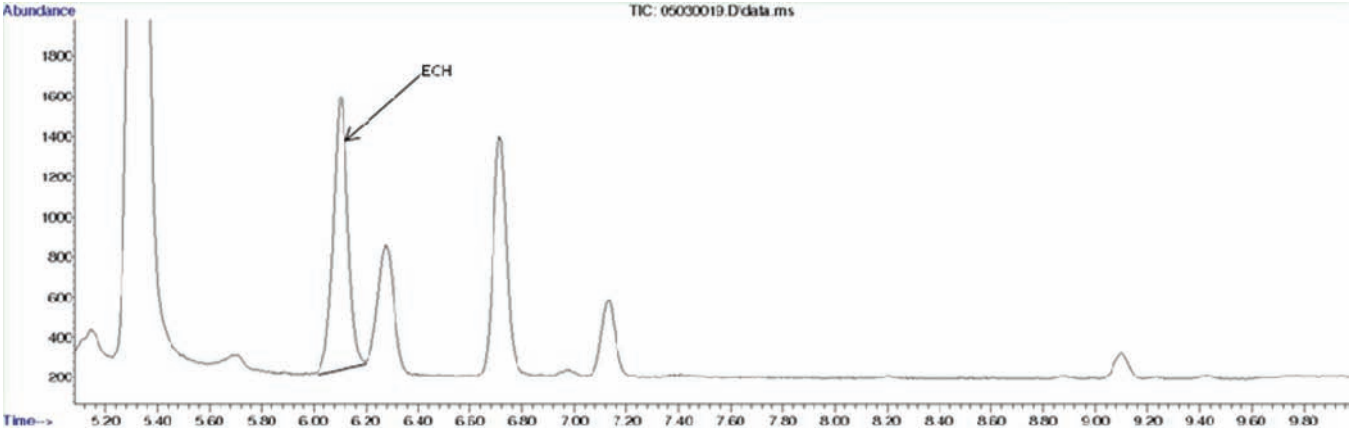

Figure 3. The API sample spiked with ECH.

ECH standards under the above-mentioned conditions clearly showed the presence of multiple peaks for our target analyte which indicated thermal instability of the compound under the HS conditions. In addition, the sensitivity given by ECD under these conditions was insufficient to control ECH at the TAL for our drug substance. Subsequently, the injection type was changed and direct injection (DI) GC with ECD was tried. The injection temperature was set at 200 °C. The same diluents were used for sample preparation at an injection volume of 1 μL. Once more we faced thermal instability and lack of sensitivity. Finally, low-temperature direct injection GC (DI GC) coupled with mass spectrometric detection was set up. The injection temperature was lowered to 120 °C, and the dissolving solvent was changed to methanol to ensure better solubility of the drug substance. The MS parameters were optimized, and single ion monitoring (SIM) was selected as the appropriate detection mode. The molecular ions at m/z 49, 51, 57, and 62 were monitored (Figure 1). The mild injection conditions of the DI GC method ensured that the thermally labile ECH did not degrade, while MS detection in SIM mode allowed for highly selective and sensitive detection of our target analyte in the new API. For detailed method conditions see Experimental Section.

Method Validation. Selectivity is the ability of an analytical method to differentiate the analyte in the presence of other components in a sample. This was demonstrated by analysis of blanks, standard, and API samples (Figure 2). The matrix peak eluted after the ECH peak (Figure 3); the resolution was calculated and found to be within our acceptance criteria ($R \geq 1.5$). The sensitivity of the method was demonstrated by determination at the limit of quantitation (LOQ) which was defined as the standard with a S/N of 10. The LOQ of the method for ECH was 0.8 ppm (w/w). Precision was evaluated by injection of five replicates of sample solutions that were prepared by spiking API samples at TAL of 8 ppm. This fulfilled the analytical acceptance criteria of our in-house guidelines; *i.e.*

Table 1. Precision and recovery of ECH determination in new API (n = 5)

sample	peak area recovery at TAL	recovery calculated against standard at TAL[a]
1	55365	99.0
2	53840	96.2
3	53584	95.8
4	55150	98.6
5	54704	97.8
mean	54529	97.5
SD	787.9	
RDS (%)	1.4	

[a] Standard at TAL mean peak area (n = 2) 55946.

RSD <5% (Table 1). Recovery was evaluated by spiking samples (n = 5) with ECH at TAL of 8 ppm and comparing the analyte peak area against a pure standard of the same concentration. The analyte could be fully recovered (97% at TAL) and no additional matrix effect was observed (Table 1).

CONCLUSION

A low-temperature DI GC−MS method for the determination of ECH in a new API was developed. Low-temperature direct injection ensured that the thermally labile ECH did not degrade during sample introduction, while mass spectrometry ensured the method was sufficiently sensitive to control the genotoxic impurity at low level. The method was validated and fulfilled our analytical validation criteria for ppm-level analytical methods.

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