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Short communication

Determination of low ppm levels of dimethyl sulfate in an aqueous soluble API intermediate using liquid–liquid extraction and GC–MS

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ABSTRACT

Dimethyl sulfate (DMS) is an alkylating reagent commonly used in organic syntheses and pharmaceutical manufacturing processes. Due to its potential carcinogenicity, the level of DMS in the API process needs to be carefully monitored. However, in-process testing for DMS is challenging because of its reactivity and polarity as well as complex matrix effects. In this short communication, we report a GC–MS method for determination of DMS in an API intermediate that is a methyl sulfate salt. To overcome the complex matrix interference, DMS and an internal standard, d_6-DMS, were extracted from the matrix with methyl *tert*-butyl ether. GC separation was conducted on a DB-624 column (30 m long, 0.32 mm ID, 1.8 μm film thickness). MS detection was performed on a single-quad Agilent MSD equipped with an electron impact source while the MSD signal was acquired in selected ion monitoring mode. This GC/MS method showed a linear response for DMS equivalent from 1.0 to 60 ppm. The practical quantitation limit for DMS was 1.0 ppm and the practical detection limit was 0.3 ppm. The relative standard derivation for analyte response was found as 0.1% for six injections of a working standard equivalent to 18.6 ppm of DMS. The spike recovery was ranged from 102.1 to 108.5% for a sample of API intermediate spiked with 8.0 ppm of DMS. In summary, the GC/MS method showed adequate specificity, linearity, sensitivity, repeatability and accuracy for determination of DMS in the API intermediate. This method has been successfully applied to study the efficiency of removing DMS from the process.

1. Introduction

The issue of genotoxic impurities (GTIs) in pharmaceutical products has attracted increasing attention from the industry [1–3] as well as regulatory agencies [4–9]. Due to their reactive nature, some materials used for API manufacturing, including starting materials, intermediates, reagents or some process related impurities/degradants have been demonstrated as genotoxic. To ensure these undesired genotoxic impurities are reduced to an acceptable level (often at low ppm) in the final product, it is critical to monitor them closely throughout the process. However, rapid development of analytical methods at such low levels remains a challenge for analytical chemists [6,10,11]. For example, extremely high sensitivity, specificity and robustness are often desired. Also, complex matrix effects arising from in-process samples, API or excipients need to be overcome. On the other hand, especially for early drug development stage, aggressive project timelines often limit the time and resources for method optimization. As a result, the analytical chemist needs to ensure the method is appropriate for its intended use.

Dimethyl sulfate (DMS) is one of the alkylating reagents commonly used in organic syntheses [12]. Similar to other alkylating reagents, DMS demonstrates carcinogenicity for *in vivo* testing and *in vitro* testing [13–20]. Even though there is still no sufficient clinical or epidemiological evidence to indicate whether or not DMS is a human carcinogen, DMS is often classified as potential carcinogenic for humans [21,22].

In-process testing for DMS could be challenging because of its reactivity and polarity as well as complex matrix effects. Noteworthy, most of the analytical methods in the literature for DMS are focused on monitoring DMS in the air [23–30]. There are only a few papers published on analyzing DMS in complex matrices such as API and in-process samples. Seymour [31] reported a GC–FID method to determine DMS in a hydrophobic API. The sample was dissolved in hexane and directly injected along with toluene as internal standard. Very recently, Raman et al. validated a GC–MS method for determination of residual DMS in pantoprazole sodium with a similar direct-injection approach [32]. As expected, this GC–MS method showed threefold sensitivity increase versus the GC–FID method. However, one drawback of these "direct-injection" GC methods is

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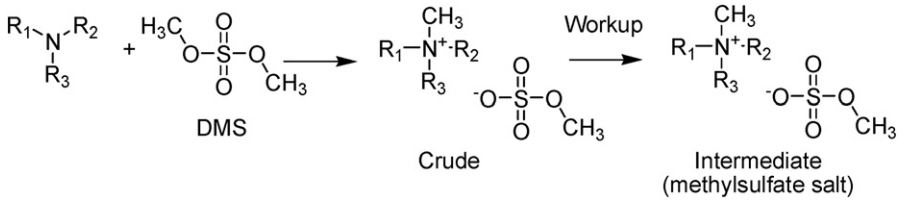


Fig. 1. Scheme for converting a ternary amine into a quaternary amine (intermediate) using DMS.

that baking out the GC column and cleaning the inlet port are often needed to avoid ghost peaks.

Besides direct-inject methods, several groups have reported success in determining DMS through derivatization. Generally, these approaches take advantage of DMS reactivity by reacting it with derivatization reagents such as sodium thiosulfate [33], pentafluorothiophenol [34], 2-mercaptopyridine [35] and trialkylamines [36]. Then, the resulting derivativized product, which is much more stable and easy for detection, is separated and analyzed by headspace GC–MS, HPLC-fluorescence or LC–MS [33–36]. Compared to direct-inject methods, these derivatization methods demonstrate enhanced sensitivity, selectivity and robustness with less matrix effects observed. Furthermore, these methods show great potential as generic methods for other alkylating agents as well.

We report herein a GC–MS method for determination of DMS in a water-soluble API intermediate. Fig. 1 shows generic synthesis scheme for the intermediate as a mono-methyl sulfate salt. DMS is utilized to convert a ternary amine into a quaternary amine (intermediate). After the reaction is completed, the unreacted DMS is quenched. The mixture is worked up and the resulting quaternary amine is isolated as mono-methyl sulfate salt. For this study, the minimum sensitivity for DMS determination is set at 16.7 ppm. This limit is based on the 1.5 μg/day according to EMEA TTC guideline [7] and a maximum daily dose of 90 mg/day for the API. Generally, "fate and purge" studies are often performed to investigate and/or demonstrate the capability for rejection of GTIs through the process. Thus, a higher level for GTIs could be justified upstream. But due to the early development stage of this project, such studies have not been conducted.

The derivatization approach is not adapted in this study because the sample matrix contains mono-methyl sulfate salt of the intermediate and could react with derivatization reagents and cause undesired response [35]. Instead, since the API intermediate is water-soluble, liquid–liquid extraction is selected as the sample preparation procedure to overcome potential matrix interference. Also, to compensate any loss of DMS to hydrolysis during the extraction procedure, internal standards are introduced in the analysis. In this short communication, different internal standards are compared to investigate their impact on detection sensitivity.

2. Experimental

2.1. Reagents

Dimethyl sulfate (DMS, 99%), and internal standards, diethyl sulfate (DES), [$^{13}C_2$]-DMS and d_6-DMS, are obtained from Sigma–Aldrich (Milwaukee, WI, USA). Sodium chloride (NaCl) of ACS grade is purchased from J.T. Baker (Phillipsburg, NJ, USA). All solvents, including methyl *tert*-butyl ether (MTBE), toluene, and *n*-butyl acetate are of HPLC or GC grade and obtained from EMD (Gibbstown, NJ, USA). Water used in this study is purified by a Millipore Milli-Q Academic system (Billerica, MA, USA).

2.2. Preparation of standard solutions

Stock DMS standard solution (approximately 160 μg/mL) is prepared by diluting DMS with MTBE. Separate stock solutions for each internal standard, i.e. DES, [$^{13}C_2$]-DMS and d_6-DMS, are also prepared at approximately 160 μg/mL in MTBE. All stock solutions are stored in the refrigerator until analysis. The working standard and extraction solution are prepared fresh everyday prior to the analysis by diluting the stock standard solutions. Typically, the working standard consists of around 1.3 μg/mL of DMS and the respective internal standard. The extraction solution consists of approximately 1.3 μg/mL of the selected internal standard in MTBE.

2.3. Preparation of sample solutions

A typical sample preparation procedure is described as followed. First, approximately 750 mg of sample is weighed into a 50 mL centrifuge tube. Then, 30 mL of 0.1 M NaCl is added into the centrifuge tube to dissolve the sample. Total of 3.0 mL of extraction solution spiked with internal standard is transferred into the centrifuge tube for liquid–liquid extraction. After mechanically shaking the content for approximately 5 min, the centrifuge tube is centrifuged at approximately 4000–5000 rpm for 10 min. Approximately 1.5 mL of the organic layer is pipetted into a micro-centrifuge tube and centrifuged at 13,000 rpm for 10 min. After centrifugation, the organic layer is analyzed by GC–MS.

2.4. GC–MS conditions

The GC–MS analysis for DMS is performed on an Agilent 6890 N gas chromatography instrument coupled with an Agilent 5973 mass-selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Palo Alto, CA, USA). A DB-624 capillary column with a dimension of 30 m × 0.32 mm ID × 1.80 μm film thickness (Agilent Technologies, Palo Alto, CA, USA) is used for the chromatographic separation. The initial oven temperature of 90 °C is maintained for 3 min, and then increased to 230 °C at a rate of 10 °C/min followed by holding at 230 °C for 5 min. The injection size is 1 μL with a split ratio set at 5:1. Helium is used as the carrier gas with constant flow rate of 1.5 mL/min. The injector and MS source temperatures are set at 240 and 230 °C, respectively. The electron impact (EI) mode at 70 eV is utilized for sample ionization. The GC–MS spectra for DMS and internal standards are obtained through injection of the stock solutions and scanning in the range of *m*/*z* 25–250. First, the GC–MS chromatograms are recorded with selected ion monitoring mode (SIM). Then extract ion chromatograms (EICs) for the corresponding diagnostic ions of DMS and internal standard are integrated, respectively for quantitation.

2.5. Safety precautions

Since DMS and the internal standards are potential carcinogens, caution is exercised with handling these compounds. All standard and sample preparations are performed in a ventilated hood with

appropriate personal protection equipments to avoid inhalation or skin contact. The stock solutions are stored in a refrigerator. In addition, care is taken to dispose of DMS waste appropriately.

3. Results and discussion

In this study, a DB-624 column, 30 m × 0.32 mm ID with 1.80 μm film thickness, is employed for the GC–MS analysis. This column is chosen because the medium-polar stationary phase is suitable for retaining DMS and resolving other analytes from DMS. Also, this type of column shows excellent robustness, inertness and low bleeding for several GC–MS methods developed in-house. Electron impact ionization (EI) is selected because EI is generally more robust and easy to transfer as compared to other GC–MS ionization techniques.

Initially, the direct-inject approach for analysis of DMS by dissolving the samples in DMSO or DMF is explored. However, strong background interference is encountered and no DMS peak is observed from a spiked sample, which suggests another type of sample preparation is needed to reduce the interference from sample matrix. For sample preparation, liquid–liquid (L–L) extraction for DMS using organic solvent is feasible considering the sample matrix (quaternary amine salt) is soluble in water. Toluene, *n*-butyl acetate, and MTBE are investigated as extraction solvents. Among all three solvents investigated, both MTBE and toluene show a clean GC–MS background as blank injection and no significant interference is observed where DMS elutes (data not shown). MTBE is chosen as the solvent for L–L extraction because it provides a higher recovery (47%) for DMS than toluene (36%). The recovery using *n*-butyl acetate is not determined due to significant interferences from the solvent.

3.1. Establishment of internal standard for DMS

Generally, the use of an internal standard can improve the accuracy and precision for GC and GC–MS analysis. This is because the internal standard effectively compensates for the variables occurring during the sample extraction as well as injection, especially when a complex sample preparation procedure is involved. In this study, we investigate the feasibility of using an internal standard. Three structurally similar analogs including two isotopic analogs, [$^{13}C_2$]-DMS and d_6-DMS (Fig. 2), were studied.

EI-MS spectra for DMS and the three proposed internal standards are collected and overlaid in Fig. 2 to determine the diagnostic ions for the GC–MS analysis. The relative abundances and rationalization for the major product ions are tabulated as Fig. 2 inset. As expected, the $[M-H]^+$ ion (*m*/*z* 125) and $[M]^{+\cdot}$ ion (molecular ion of DMS, *m*/*z* 126) are observed with fairly low relative abundance (Fig. 2a). The most dominant ion is *m*/*z* 95 as a result of inductive cleavage of $[CH_3O]^\bullet$ from $[M]^{+\bullet}$. The second most dominant ion is *m*/*z* 96, which resulted from hydrogen rearrangement of $[M-H]^+$ followed by loss of formaldehyde (HCHO). For the *m*/*z* 96 ion, further fragmentation through hydrogen rearrangement and inductive cleavage could occur which lead to the formation of *m*/*z* 79 and 66 ions.

As expected, the fragmentation pathways for the DMS isotopic analogs are almost identical as DMS (Fig. 2b and c). A significant different fragmentation pathway is observed for DES because the longer alkyl chains add complexity during fragmentation. For example, the *m*/*z* 139, 127, 125 and 111 ions are likely related to the cleavage and rearrangement of the alkyl chains (Fig. 2d).

Chromatographic retention is another factor needed to consider when using an internal standard. In this study, DMS, [$^{13}C_2$]-DMS and d_6-DMS are almost co-eluting on the DB-624 column (data not shown). As a result, diagnostic ions are carefully chosen to avoid those overlapping ions in the EI-MS spectra of analyte and internal standard, which could lead to significant experimental errors. Since DES retains longer than DMS (data not shown), the selection of diagnostic ions for both DMS and DES has much more flexibility. The diagnostic ions for DMS and each internal standard are summarized in Table 1.

Table 1
Diagnostic ions for DMS, [$^{13}C_2$]-DMS, d_6-DMS and DES.

DMS/IS pair	DMS/[$^{13}C_2$]-DMS	DMS/d_6-DMS	DMS/DES
Diagnostic ion for DMS	*m*/*z* 125	*m*/*z* 95	*m*/*z* 95
Diagnostic ion for IS	*m*/*z* 127	*m*/*z* 100	*m*/*z* 139

Table 2
Recovery for DMS, [$^{13}C_2$]-DMS, d_6-DMS and DES extracting from NaCl solution using MTBE.

Analyte	DMS	[$^{13}C_2$]-DMS	d_6-DMS	DES
Recovery	47%	49%	43%	75%

A stock solution of each individual analyte is spiked into 30 mL of a 0.1 M NaCl solution then extracted by 3.0 mL of MTBE. For GC–MS analysis, DMS is monitored at a *m*/*z* 95. For the internal standard diagnostic ions see Table 1. Recovery values are calculated as an average of duplicate injections. For other conditions, see Section 2.

The recovery for DMS and [$^{13}C_2$]-DMS, d_6-DMS, or DES is investigated by spiking small amount of individual stock solution into NaCl solution followed by extraction with MTBE. Comparing spike recovery listed in Table 2, almost half of DMS is lost after L–L extraction. As expected, both isotopic analogs have similar recovery as DMS. On the other hand, DES shows a recovery as 75%, which indicates that DES is more hydrophobic. Therefore, both [$^{13}C_2$]-DMS and d_6-DMS are selected as potential internal standards for quantitation of DMS.

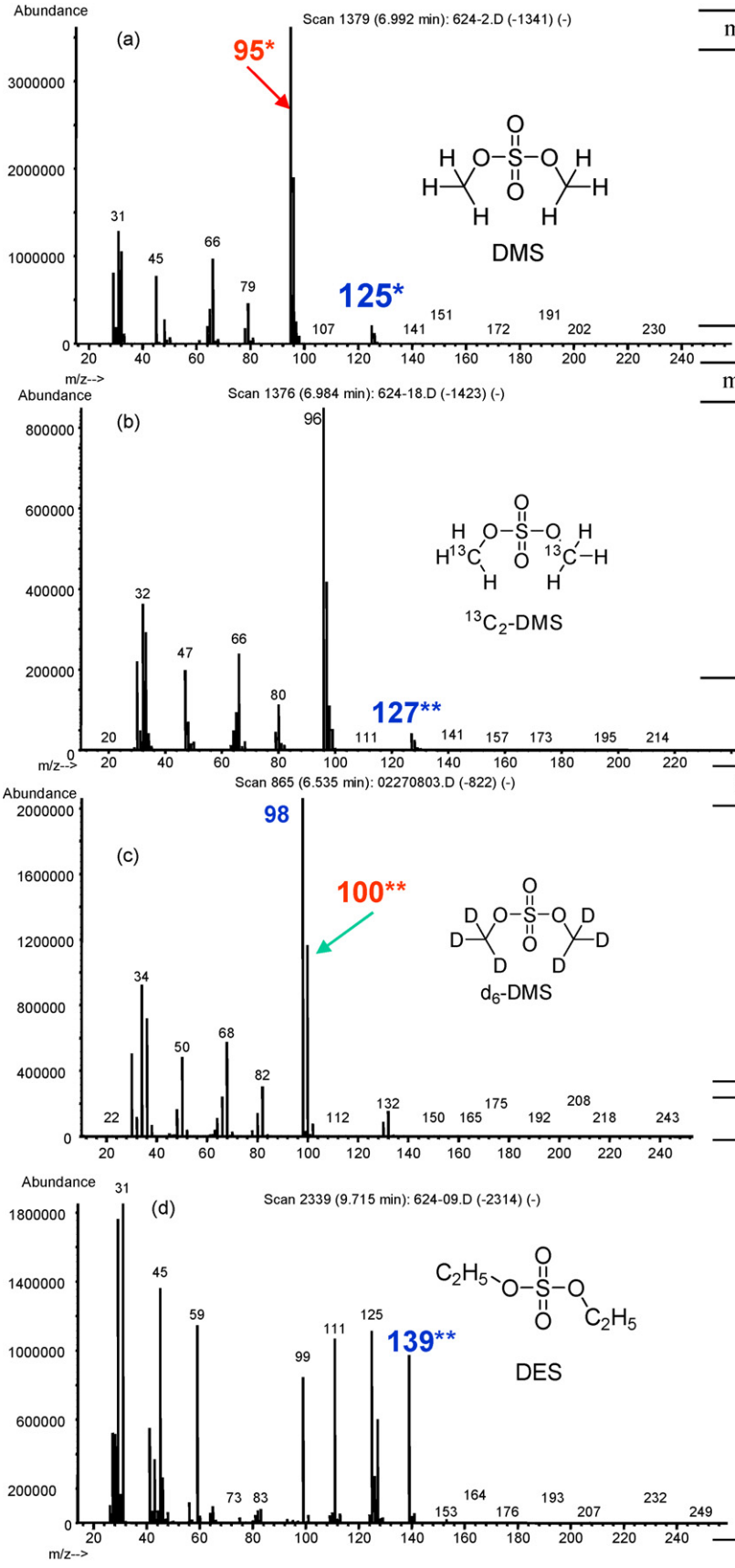
3.2. Method qualification and sample testing

To demonstrate that the analytical method is suitable for intended use, appropriately designed evaluation procedures such as method validation are performed. Due to the early-phase development stage of this project, a "simplified version" of method validation, or so-called "method qualification" is conducted instead. The parameters evaluated include specificity, linearity, precision, practical quantitation limit (PQL), practical detection limit (PDL) and accuracy. The results obtained from method qualification using [$^{13}C_2$]-DMS and d_6-DMS as internal standards, respectively, are summarized in Table 3.

When [$^{13}C_2$]-DMS is used as an internal standard, the response for DMS is found as linear at concentration 0.8–13.0 μg/mL with a correlation coefficient of 0.9996. The relative standard derivation is 0.9% for six injections of a DMS standard at 3 μg/mL. Also, RSD% from seven injections of 0.8 μg/mL standard (the lowest concentration in linear curve) is less than 10. Thus, this concentration is established as the PQL, which is equivalent as 5.0 ppm of DMS in a sample prepared at nominal concentration. Calculating the practical detection limit (PDL) as one-third of PQL, the PDL is 1.7 ppm. For

Table 3
Summary for method qualification using [$^{13}C_2$]-DMS or d_6-DMS as internal standards, respectively.

Parameters	[$^{13}C_2$]-DMS as IS	[d_6]-DMS as IS
Specificity	Separated from other peaks	Separated from other peaks
Linearity	0.8–13.0 μg/mL (R = 0.9996)	0.16–9.72 μg/mL (R = 1.0000)
Precision	RSD% = 0.9 (n = 6)	RSD% = 0.1 (n = 6)
PQL	0.8 μg/mL (5.0 ppm)	0.16 μg/mL (1.0 ppm)
PDL	0.27 μg/mL (1.7 ppm)	0.05 μg/mL (0.3 ppm)
Accuracy (spike recovery)	92.0–94.3% (spike level of 40 ppm, n = 3)	102.1–108.5% (spike level of 8 ppm, n = 3)



m/z [rel. abundance]	Rationalization
126 (3%)	$M^{+\cdot}$
125 (6%)	$[M-H]^+$
96 (51%)	$[M-CH_2O]^+$
95 (100%)	$[M-CH_3O]^+$
79 (12%)	$[CH_3SO_2]^+$
66 (25%)	$[H_2SO_2]^+$
45 (21%)	$[CH_3OCH_2]^+$

m/z [rel. abundance]	Rationalization
128 (3%)	$M^{+\cdot}$
127 (6%)	$[M-H]^+$
97 (52%)	$[M-^{13}CH_2O]^+$
96 (100%)	$[M-^{13}CH_3O]^+$
80 (14%)	$[^{13}CH_3SO_2]^+$
66 (27%)	$[H_2SO_2]^+$
47 (24%)	$[^{13}CH_3O^{13}CH_2]^+$

m/z [rel. abundance]	Rationalization
132 (7%)	$M^{+\cdot}$
130 (4%)	$[M-D]^+$
100 (57%)	$[M-CD_2O]^+$
98 (100%)	$[M-CD_3O]^+$
82 (15%)	$[CD_3SO_2]^+$
68 (28%)	$[D_2SO_2]^+$
50 (23%)	$[CD_3OCD_2]^+$

m/z [rel. abundance]	Rationalization
153 (1%)	$[M-H]^+$
139 (51%)	$[M-CH_3\cdot]^+$
127 (31%)	$[M-H-C_2H_2]^+$
125 (60%)	$[M-H-C_2H_4]^+$
111 (56%)	$[M-H-C_3H_6]^+$
99 (45%)	$[M-H-C_4H_6]^+$
59 (60%)	$[C_3H_7O]^+$
45 (72%)	$[C_2H_5O]^+$
31(100%)	$[CH_3O]^+$

Fig. 2. EI-MS spectra for DMS, [$^{13}C_2$]-DMS, d_6-DMS and DES. Some of the major ions, relative abundance and rationalization are tabulated as insets. The spectra are obtained by injecting individual stock standards. For other conditions, see Section 2.

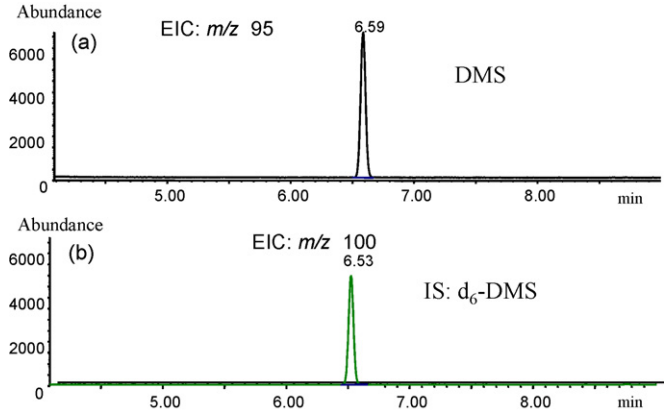


Fig. 3. GC–MS extracted ion chromatograms (EICs) for DMS (A) in a quaternary amine lab sample using d_6-DMS (B) as an internal standard. For sample preparation and other conditions, see Section 2.

a sample of API intermediate spiked with 40 ppm of DMS, the spike recovery from three spiked samples ranged from 92.0 to 94.3%.

Similar linearity and injection repeatability are observed when using [d_6]-DMS as internal standard. However, the PQL for DMS with using [d_6]-DMS as an internal standard is 1.0 ppm at nominal sample concentration which is 5 times lower than using [$^{13}C_2$]-DMS as internal standard. The improvement in detection sensitivity is due to the relative abundance of diagnostic ions chosen for each of internal standard. In the DMS EI-MS spectrum, the m/z 95 ion is much more abundant than m/z 125 (Fig. 2a, inset). For a sample of API intermediate spiked with 8.0 ppm of DMS, the spike recovery from three spiked samples ranged from 102.1 to 108.5%.

Overlaid GC–MS extracted ion chromatograms (EICs) for a lab sample of the quaternary amine intermediate using d_6-DMS as an internal standard is in Fig. 3. The DMS level is found as 5.9 ppm which is much lower than the targeted control limit in the isolated intermediate. Also, it suggests that the quenching process and subsequent work-up are efficient for removing unreacted DMS.

4. Conclusion

A GC–MS method for determination of DMS in an API intermediate is developed. This method shows adequate specificity, linearity, sensitivity, precision and accuracy with no sample matrix interference observed. It has been demonstrated that this method is capable of quantifying the targeted potential genotoxic impurity in an in-process intermediate and can be used for future "fate and purge" studies for DMS. Also, use of isotopic internal standards can be applied to other methods for improving recovery of reactive analytes.

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