Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 486–494
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Development and validation of a sensitive GC–MS method for the determination of trace levels of an alkylating reagent in a β-lactam active pharmaceutical ingredient

Hanlin Li *, Gregory W. Sluggett

Pfizer Global Research and Development, Eastern Point Road, MS 8118D-3041, Groton, CT 06340, USA

Received 11 February 2005; received in revised form 13 April 2005; accepted 13 April 2005
Available online 13 June 2005

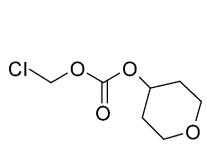
Abstract

A direct injection gas chromatographic method utilizing selected-ion monitoring (SIM) mode mass selective detection was developed and validated for the trace analysis of an impurity, carbonic acid chloromethyl tetrahydro-pyran-4-yl ester (CCMTHP), present in a β-lactam active pharmaceutical ingredient (API). A variety of analytical techniques including LC–MS, GC-FID, GC-ECD and GC–MS were evaluated during the method development. GC–MS with SIM at $m/z = 49$ demonstrated the best detection sensitivity. A 10 ppm (5 pg on column) limit of quantitation (LOQ) was attained and the linearity of the method was demonstrated in the range of 10–1000 ppm. Accurate and precise quantitation of the impurity in drug substance was achieved with external standardization. A 10:1 split injection was applied to limit the amount of non-volatile API loading onto the column. The effects of injection and detection parameters such as split ratio, liner type, injection temperature and number of mass ions monitored were studied. Full validation proved the accuracy, precision and specificity of the method, which was successfully employed to analyze many pilot lots of the API.

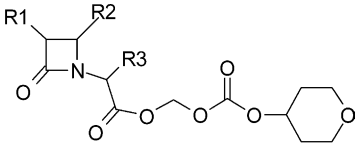
Keywords: GC–MS; Selected ion monitoring; Alkylating agent; Trace assays; Active pharmaceutical ingredient

1. Introduction

Carbonic acid chloromethyl tetrahydro-pyran-4-yl ester (CCMTHP) is commonly used as an alkylating reagent in the synthesis of active pharmaceutical ingredients (APIs). As specified in the European Agency for the Evaluation of Medicinal Products (EMEA) position paper on the limits of genotoxic impurities, alkylating reagents are considered to be the archetypical class belonging to the group of genotoxic impurities [1]. Therefore, the CCMTHP content in the final drug product should be reduced to as low as technically feasible. Detection and quantitation of the residual CCMTHP therefore requires highly sensitive trace analysis techniques. The method should also be selective, accurate and robust. The task is particularly challenging since CCMTHP lacks a strong UV chromophore and the β-lactam API is susceptible to hydrolysis.



CCMTHP



β-Lactam API

Determination of trace analytes in complex matrices is very common in environmental science, where the analysis is heavily reliant on extraction and sample pre-concentration (or enrichment). The technique used most frequently is solid phase extraction (SPE), which ideally yields quantitative recovery of the target analytes without loss or degradation. As a sample isolation and pre-concentration technique, SPE works best when the target analyte and the major ingredients in the matrix have significantly different physiochemical

* Corresponding author. Tel.: +1 860 715 5541; fax: +1 860 686 6443.
E-mail address: hanlin.li@pfizer.com (H. Li).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.04.042

properties, where concentration factors as high as 1000 may be attained [2]. SPE has been applied widely to the analysis of trace level pesticides, pharmaceuticals or other organic compounds in water [3]. In this application, CCMTHP and the API have very similar physiochemical properties. Thus, co-extraction of the impurity and API is a significant challenge, when the API is approximately 4–6 orders of magnitude more concentrated than the expected levels of residual CCMTHP. More importantly, it is always a concern that some analytes may permanently adsorb to the sorbent, which would reduce sample recovery and the SPE cartridge reproducibility for quantitative analysis [4].

Chemical derivatization is another common technique employed for trace analysis, where the detectability is improved by a reaction between the analyte and an added derivatizing reagent. An example is the use of halogen-substituted derivatizing reagents to improve electron capture detection (ECD) selectivity and sensitivity [5,6]. However, derivatization is usually not a preferred technique since it often suffers from low derivative yield [7] or multiple product formation [8], further complicating analyte quantitation.

In this study, we report a highly sensitive and selective direct detection method for the quantitation of trace levels of a residual alkylating reagent in a β-lactam API. A variety of analytical techniques were explored including LC–MS, GC-FID, GC-ECD and GC–MS. GC/MS in selected ion monitoring (SIM) mode proved to be the most sensitive and robust technique for this application. A 50 ng/mL (10 ppm) limit of quantitation was achieved without sample preconcentration or chemical derivatization.

2. Experimental

2.1. Reagents and chemicals

Carbonic acid chloromethyl tetrahydro-pyran-4-yl ester (CCMTHP) was obtained from Fontarome (Milwaukee, WI). Acetonitrile (HPLC grade) was from J.T. Baker (Phillipsburg, NJ) and formic acid (>96%) from Sigma–Aldrich (Milwaukee, WI).

2.2. Sample preparation

CCMTHP standard was prepared by dissolving 25 mg in acetonitrile (50 mL). A series of dilutions were then made with acetonitrile until the final concentration was ∼0.1 μg/mL. The standard was stable for at least one week if stored at 5 °C. The API sample was prepared by dissolving ∼25 mg into 5 mL acetonitrile. Relative to the API concentration, the CCMTHP standard concentration was ∼20 ppm.

2.3. Apparatus and chromatographic conditions

2.3.1. LC–MS

An Agilent 1100 LC–MSD (Agilent Technologies, Palo Alto, CA) was employed in conjunction with 10 μL sample injections via the autosampler. Chromatographic separations were performed on a YMC J'Sphere reversed-phase column (4.6 mm × 150 mm, 5 μm particle size). Mobile phases were: (*A*) 0.1% formic acid and (*B*) acetonitrile. Elution started in isocratic mode for 5 min at (*A*:*B*, 95/5), followed by a linear gradient to (*A*:*B*, 5/95) in 20 min, then hold for 5 min. The column equilibratation time was 10 min, the flow rate was 1.0 mL/min and the column was maintained at ambient temperature. Mass detector settings: positive electro-spray ionization (ESI) mode; fragmentor voltage 60 V; drying gas flow 12.0 L/min; drying gas temperature 350 °C; nebulizer pressure 45 psi; capillary voltage 3500 V.

2.3.2. GC-FID

An Agilent 6890 gas chromatograph equipped with a flame ionization detector (FID) and a 7683 autosampler (Agilent Technologies, Palo Alto, CA) was used. Direct injection of 1.0 μL sample in splitless mode was employed to obtain maximum sensitivity. The injection port was set at 250 °C. The detector was maintained at 300 °C and helium was used as a make-up gas at a flow rate of 40 mL/min. A fused silica capillary column DB-5 (30 m × 0.25 mm i.d., 1.0 μm film thickness) (Agilent Technologies, CA) was used and the column temperatures were programmed as follows: 40 °C to 310 °C at 10 °C/min and hold for 3 min at 310 °C. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min.

2.3.3. GC-ECD

GC-ECD analysis was performed on an Agilent 6890 gas chromatograph equipped with a 63Ni electron capture detector. Chromatographic conditions were the same as those employed for GC-FID. A 1.0 μL sample solution was injected in splitless mode to maximize the sensitivity.

2.3.4. GC–MS

Sample analysis was performed on an Agilent 6890 gas chromatography coupled to an Agilent 5973 mass selective detector operated in the electron ionization (EI) mode. The analytical column was a DB-5MS, 30 m × 0.25 mm fused silica column with 1.0 μm Phenyl Arylene polymer stationary phase, which is virtually equivalent to (5%-phenyl)-methylpolysiloxane (Agilent Technologies, CA). The column temperature program was the same as that in the GC-FID method. The inlet temperature was set at 250 °C and an injection of 1 μL was made with split ratio of 10:1. The glass liner used in the injection port was a 4.0 mm ID split liner with glass wool from Restek (Bellefonte, PA).

The MS system was tuned with perfluorotributylamine (PFTBA). MS parameters were set as following: transfer line temperature: 280 °C; MS source at 230 °C; MS quadrupole temperature: 150 °C; ionization energy: 70 eV. A 3-min solvent delay was used to avoid acquiring unnecessary data. Full scan EI data were acquired under the following conditions: mass range: 25–550 amu, scan rate: 2.86 cycles/s. In selected

ion monitoring (SIM) mode, studies were conducted either by monitoring a group of ions (m/z = 49, 55, 69 and 84) or a single ion at m/z = 49.

3. Results and discussion

Among the many analytical techniques investigated, GC–MS with SIM was found to be the most sensitive for the alkylating reagent, CCMTHP.

3.1. LC–MS

The chemical structure of CCMTHP indicates that UV detection is not capable of achieving the desired sensitivity since the analyte does not possess a strong UV chromophore. Therefore, method development started with LC–MS. Fig. 1(a and c) illustrate the total ion current (TIC) and the mass spectrum of CCMTHP respectively, acquired using full scan mass spectrometry detection with positive electro-spray ionization. The m/z = 85 ion, which corresponds

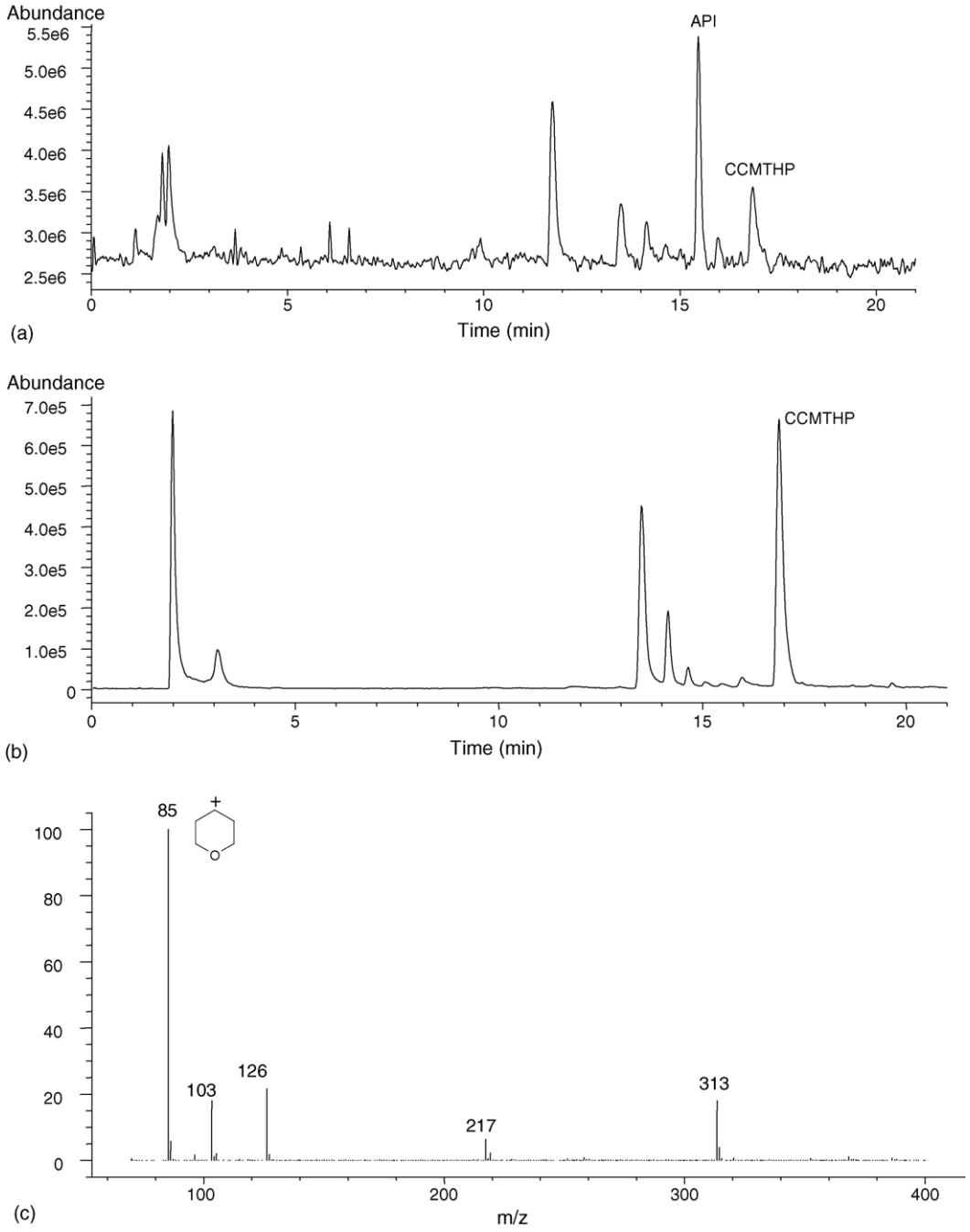


Fig. 1. (a) Total ion chromatogram of API & CCMTHP by LC–MS; (b) extracted ion chromatogram at m/z = 85; (c) electrospray ionization mass spectrum of CCMTHP.

to the tetrahydropyran fragment, dominates the CCMTHP mass spectrum. A significant gain in sensitivity is observed with SIM at $m/z = 85$, as shown in Fig. 1(b). However, the calculated quantitation limit (signal-to-noise ratio of 10) is ~30 μg/mL or 6000 ppm with respect to the 5 mg/mL nominal API assay concentration. Increasing method sensitivity by simply increasing the API concentration is not a practical approach due to the very high concentrations required. The other common LC–MS ionization technique, atmospheric-pressure chemical ionization (APCI), shows poorer sensitivity, presumably due to the low ionization efficiency of the analyte.

3.2. GC-FID

With a boiling point of approximately 293 °C, GC headspace is not suitable for CCMTHP determination; instead, direction injection GC was investigated. Flame-ionization detection (FID) was the first choice, since it is known as a universal detector for almost all organic compounds with relatively high sensitivity and has been established as a commonly employed detector for quantitation of volatile impurities in pharmaceutical products [9]. Fig. 2(a and b) show the GC-FID chromatograms of 1.0 μL of 2 mg/mL CCMTHP standard and 5 mg/mL API sample injected in splitless mode, respectively. The API shows almost no response by FID, indicating that the non-volatile drug is either degraded or deposited on the column front; therefore, no interference from API is expected in CCMTHP analysis. The CCMTHP is detected selectively with an LOQ of ~3 μg/mL (600 ppm), a 10-fold sensitivity improvement over LC–MS. The FID response is proportional to the number of $-CH_2-$ groups that enter the flame. It does not respond to fully oxidized carbons such as carbonyl or carboxyl groups and to ether groups [10] and the presence of heteroatoms such as O, S, and halogens would suppress the response. Therefore, although CCMTHP has seven carbons, the effective carbon number (ECN) is less than 3, which accounts for the lower-than-expected sensitivity observed [11].

3.3. GC-ECD

Electron capture detection (ECD) is typically extremely sensitive for compounds with strong electron-absorbing functionalities, such as halogenated materials and nitroaromatic

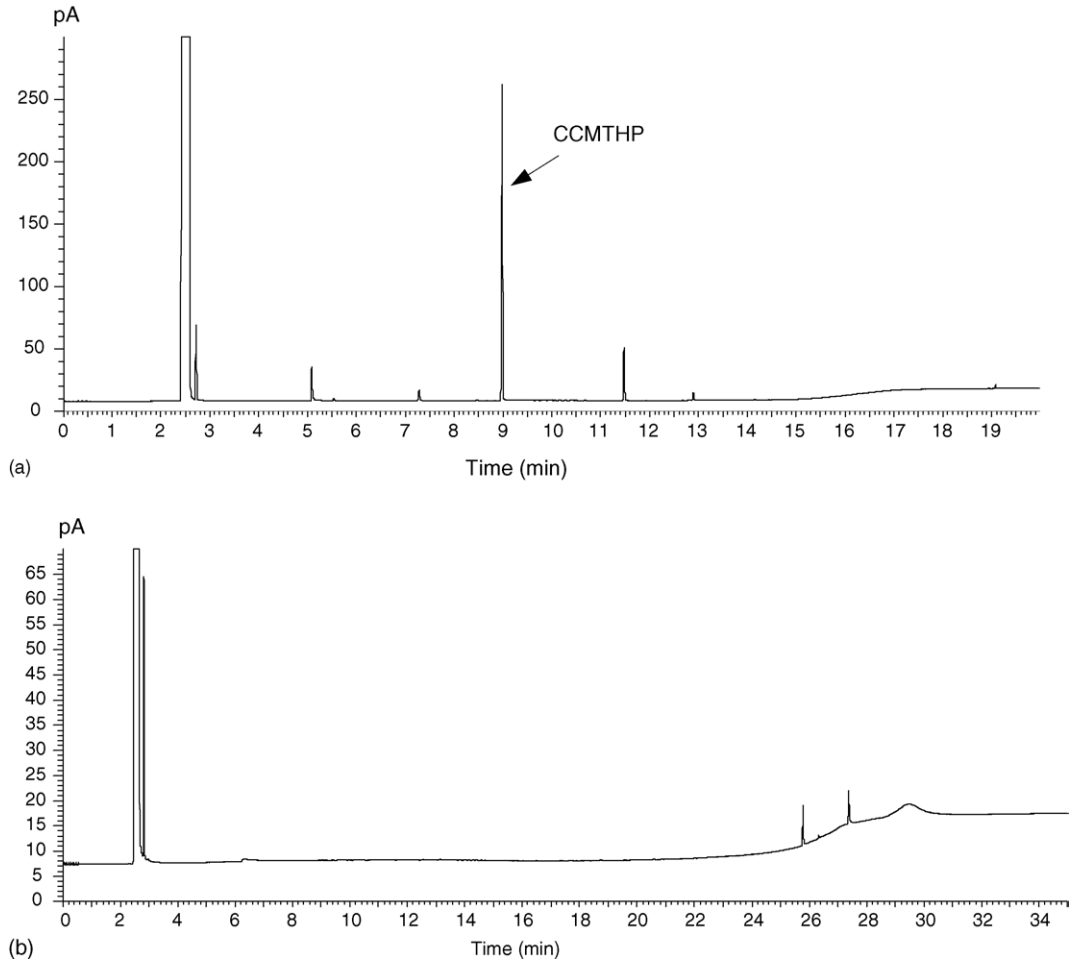


Fig. 2. (a) Chromatogram of CCMTHP standard by GC-FID; (b) chromatogram of API by GC-FID.

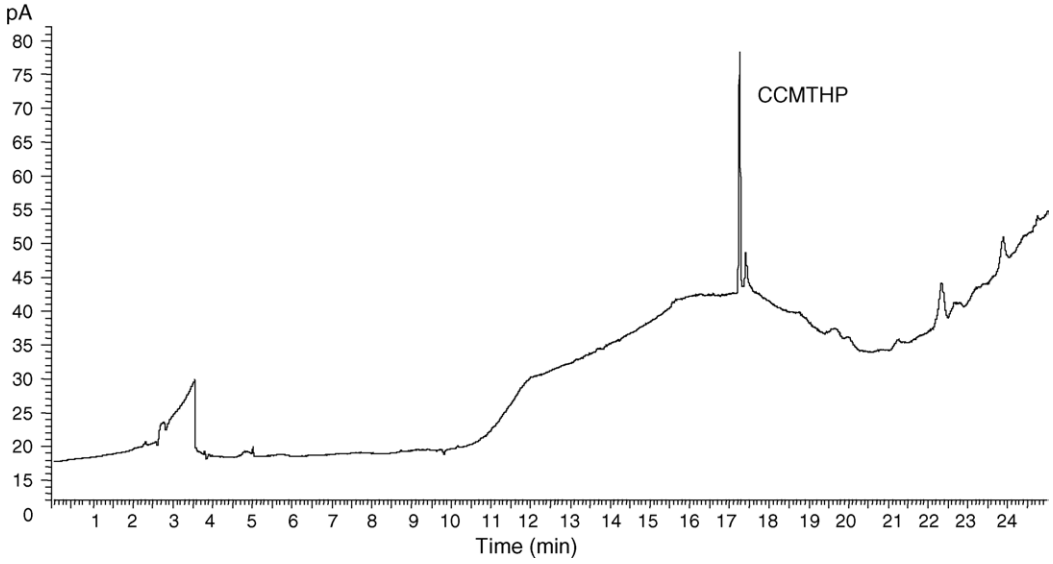


Fig. 3. Chromatogram of CCMTHP by GC-ECD.

compounds, while virtually insensitive to hydrocarbons, alcohols, ketones, etc. [12]. ECD has been employed for determination of picogram or even lower levels of pesticides [13,14], polychlorinated biphenyls (PCBs) [15], organic explosives [16], etc. in complex matrices. Since CCMTHP contains chlorine, ECD was examined. Direct injection of 1.0 μL 0.15 mg/mL CCMTHP standard was made in splitless mode to maximize detection sensitivity. Unfortunately, the 4 μg/mL (~800 ppm) LOQ is only comparable to that of GC-FID method. A typical GC-ECD chromatogram is shown in Fig. 3. The lower-than-expected sensitivity is likely due to the fact that CCMTHP is a monochlorol compound, whose ECD response factor is similar to that of ketones. The ECD is far more responsive to dichlorol and trichlorol compounds, where 10–100 times more sensitivity could be potentially achieved [17].

3.4. GC–MS

GC–MS is a powerful technique for quantitative and qualitative analysis and has been successfully applied in many areas, such as environmental analysis [18], food-related applications, toxicological and forensic applications [19], and ubiquitously in the petroleum industry [20,21]. The hyphenation of GC and MS provides high-resolution separations with highly selective and sensitive detection, which is of utmost importance in quantitative trace analysis. To evaluate the suitability of GC–MS for CCMTHP determination, an Agilent 6890GC coupled with 5973MSD was used in full mass range scanning mode from m/z = 25–500. A typical total ion current chromatogram (TIC) and the mass spectrum of CCMTHP are shown in Fig. 4(a and b). The calculated LOQ is ~5 μg/mL, corresponding to 1000 ppm with respect to 5 mg/mL API nominal concentration.

3.4.1. GC–MS SIM

In order to improve sensitivity, selected ion monitoring (SIM) mode was studied. Many reported GC–MS applications demonstrated the success of SIM detection to achieve desired selectivity and sensitivity for trace analysis [22–24]. By evaluating the structure and mass spectrum of CCMTHP, four characteristic fragment ions with the highest abundance (m/z = 49, 55, 69, 84) were selected for additive SIM detection. The postulated structures of those fragments are illustrated in Fig. 4(b). As shown in Fig. 5, 50 ng/mL CCMTHP standard (10 ppm) was successfully detected with $S/N > 10$. Taking into account the 1.0 μL injection volume with the split ratio of 50:1, the calculated quantitation limit is approximately 1 pg on column for pure CCMTHP standard. More than 100-fold of sensitivity increase was achieved with SIM compared to that acquired with full scan MS.

Splitless injection and the effect of split ratio on sensitivity was investigated by adjusting the split ratios to 50:1, 20:1, 10:1, 5:1. As illustrated in Table 1, sensitivity increases as the split ratio decreases with maximum sensitivity attained in splitless injection mode. Although splitless injection is most commonly used for trace analysis in order to maximize the sensitivity, in this application, introduction of non-volatile API into the GC column or the mass spectrometer is not desirable. Therefore, as trade-off between high sensitivity

Table 1
Effect of injection split ratio on GC–MS SIM (m/z = 49) detection sensitivity

Split ratio	CCMTHP peak area
50:1	1184
20:1	2779
10:1	5904
5:1	11998
Splitless	16246

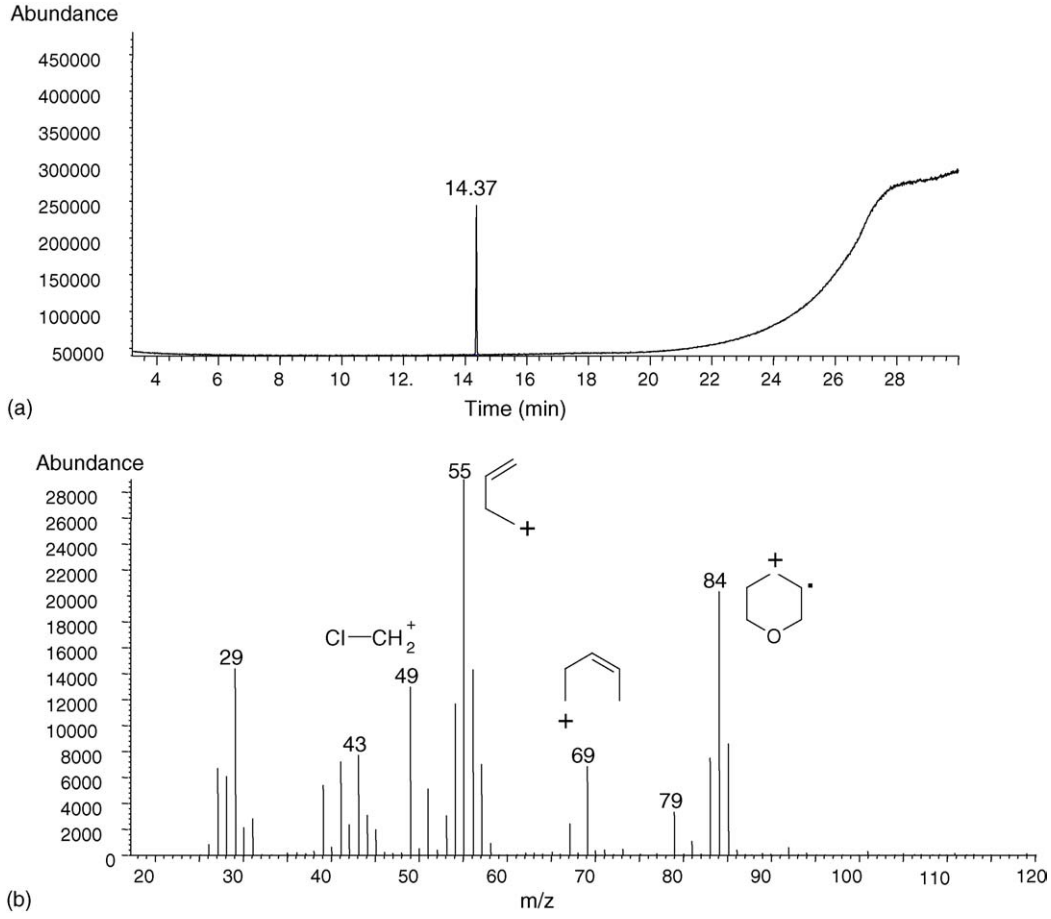


Fig. 4. (a) Total ion chromatogram of CCMTHP by GC–MS; (b) mass spectrum of CCMTHP by EI.

and low non-volatile introduction, a split ratio of 10:1 was selected.

3.4.2. API matrix effect

An injection of API sample was performed to evaluate the residual level of CCMTHP. As shown in Fig. 6(a), significant interference from the API was observed as CCMTHP eluted after a severely tailing API-related peak at 9.6 min. Much higher noise levels were observed compared to the results obtained with the CCMTHP standard only, and the CCMTHP peak appeared to partially co-elute with another degradant, precluding accurate integration of the CCMTHP peak in the API. The mass spectrum of the tailing API-related peak at 9.6 min was similar to that of CCMTHP, exhibiting strong mass ion responses at $m/z = 55$ and 84 and a less intense response for $m/z = 69$. As a result, it was determined

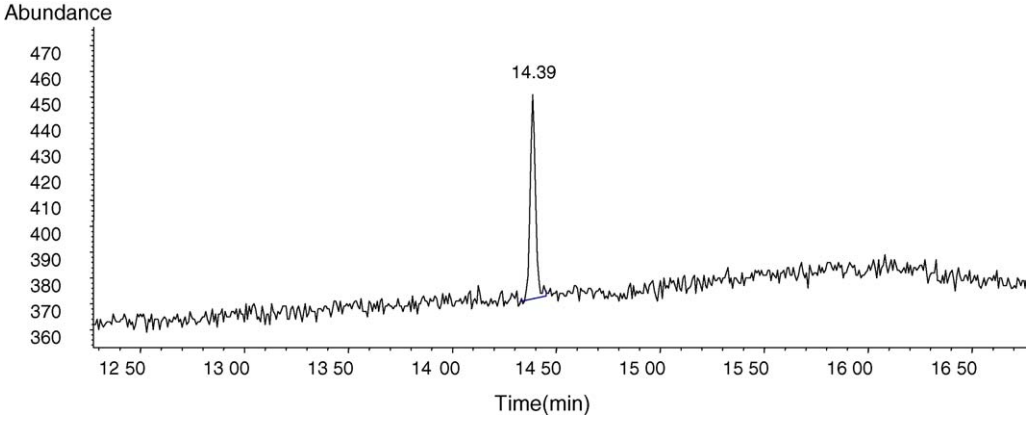


Fig. 5. GC–MS detection of 0.05 μg/ml (10 ppm) CCMTHP standard by selected ion monitoring (at $m/z = 49$, 55, 69 and 84).

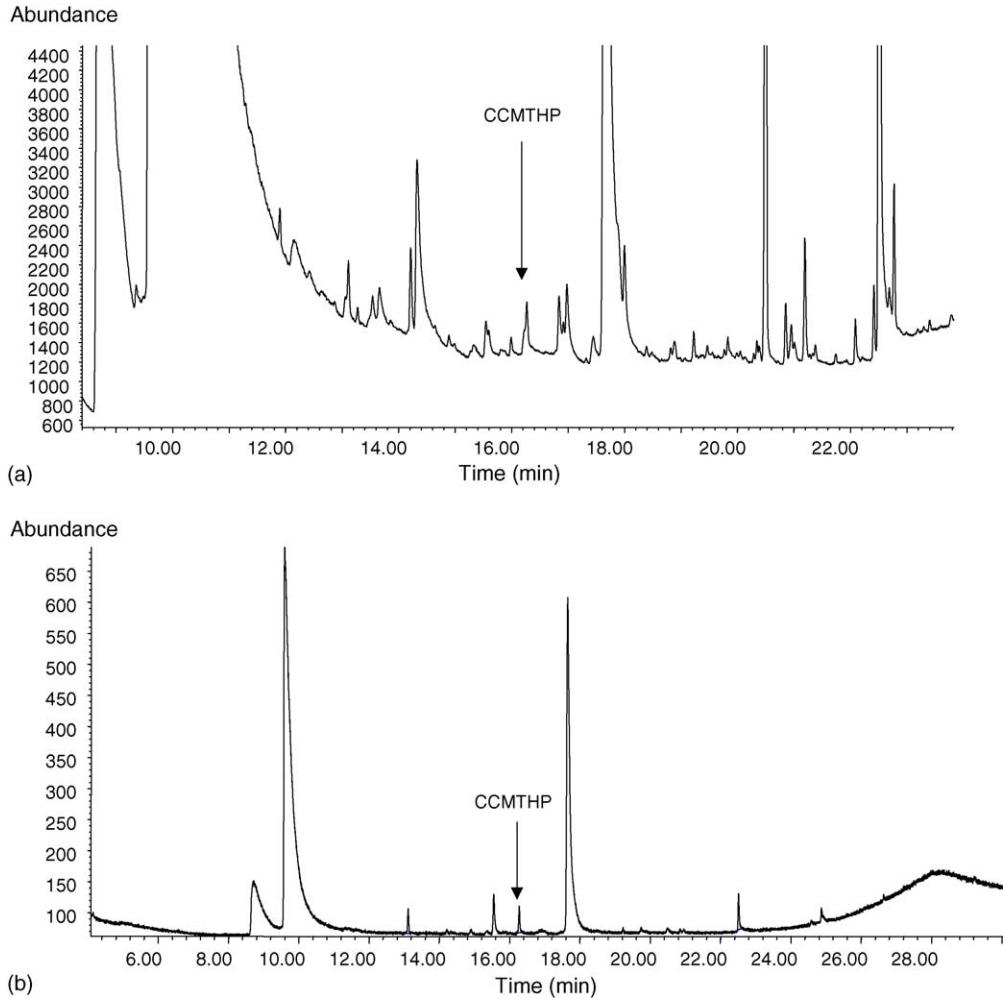


Fig. 6. (a) GC–MS detection of API sample with SIM monitoring at $m/z = 49$, 55, 69 and 84; (b) GC–MS detection of API sample with SIM monitoring at $m/z = 49$.

that instead of detecting four ions, only the $m/z = 49$ ion containing the chlorine atom should be monitored during SIM determination of CCMTHP in the API. The same API sample evaluated with single ion monitoring at $m/z = 49$ (see Fig. 6(b)), exhibited much less tailing for the 9.6 min peak and greatly reduced baseline noise. The reduction of API matrix interference greatly improved CCMTHP peak resolution and increased the *S*/*N* ratio by three-fold. Therefore, SIM at $m/z = 49$ was selected for further method optimization.

3.4.3. Effect of inlet temperature and liner type

The tailing peak at 9.6 min detected in the API sample was subsequently identified as tetrahydropyranol (THP), one of the major API degradants. Although deactivated inlet liners were used during method development, the THP peak indicated thermal degradation of the API at the injection port. However, the thermal degradation of the API would not affect the CCMTHP level, since no addition or loss of CCMTHP would occur from the API degradation pathway. Injection port temperatures of 150 °C, 180 °C, 200 °C and 250 °C were studied to evaluate the effect on chromatographic performance. The peak area of THP was plotted against injection temperature and, not surprisingly, the amount of detected degradant increases as the injection temperature increases (Fig. 7(a)). It was also noticed that lower injection temperatures lead to significant carry-over of the THP peak. The THP peak areas in the two subsequent blank injections immediately after API injection are plotted against the injection temperatures as shown in Fig. 7(b). Comparing Fig. 7(a and b), it is concluded that the thermal degradation of the API is more efficient at high injection temperatures, leaving little residual amount in the injection port and hence minimal carry-over. Thus, 250 °C was chosen as the injection temperature to minimize the carry-over contamination.

A different inlet liner type, CycloSplitter (Restek), was also evaluated against the common wool split liner for possible sample adsorption and decomposition. Since wool liners could be adsorptive, especially when fibers are broken, the CycloSplitter liner applies a cylindrical spiral design to provide larger surface area for vaporization and trapping of non-volatile contaminants to enhance the capability of handling complex or non-volatile samples. However, no significant dif-

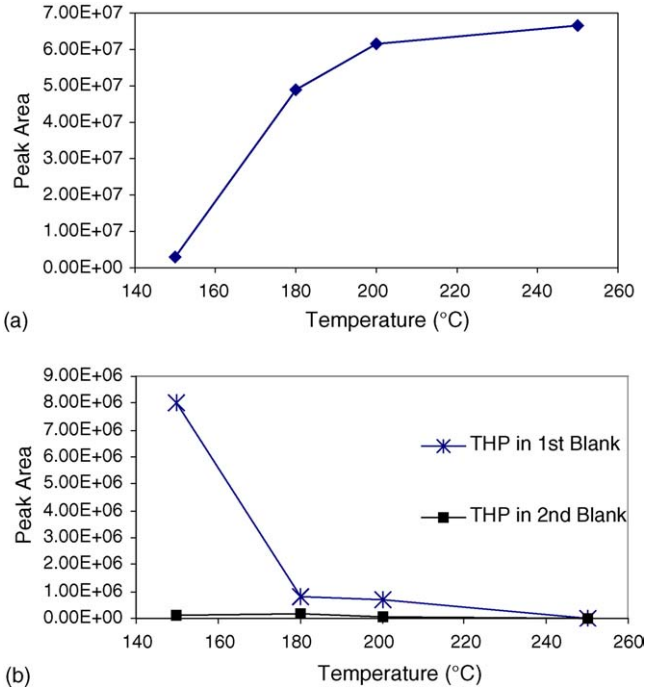


Fig. 7. (a) Plot of THP (API degradant) peak area vs. inlet temperature; (b) plot of carry-over peak areas in subsequent blank injections vs. injection temperature.

ference for the two inlet liner types was observed for this application.

3.5. Validation of GC–MS SIM (m/z = 49) method

3.5.1. Precision

To demonstrate the reproducibility of the method, six replicate injections of CCMTHP standard at 0.5 μg/mL (100 ppm) were performed. Percent relative standard deviations (%R.S.D.) of peak area and retention time were calculated and the results are summarized in Table 2. Retention times of CCMTHP are highly reproducible with %R.S.D. < 0.01%. Precision of peak areas was demonstrated with %R.S.D. < 3.0%.

3.5.2. Linearity

Five CCMTHP standard solutions prepared in acetonitrile were injected to evaluate the method linearity in the concentration range of 0.05–1.0 μg/mL (10–200 ppm). The calibration plot had excellent linearity with R^2 value of 0.9999. The *y*-intercept bias of the linear plot was not significant, ~10% of the response of LOQ (0.05 μg/mL) standard. Linearity over a broader range (up to 1000 ppm) was also assessed. The SIM responses of the higher concentration standard solutions (500 and 1000 ppm) showed some positive deviation from the linear calibration plot. The correlation coefficient (R^2) of the calibration plot for the broader concentration range (10–1000 ppm) was 0.9968.

Table 2
Injection precision for GC–MS SIM (*m*/*z* = 49) determination of CCMTHP

Injection #	Retention time (min)	CCMTHP peak area
1	15.209	10126
2	15.211	9647
3	15.211	10111
4	15.211	10480
5	15.210	10374
6	15.210	9977
Mean	15.210	10119
Standard deviation	0.001	296
%R.S.D.	0.005	2.9

3.5.3. Limit of quantitation and specificity

Limit of quantitation (LOQ) was determined at a level where *S*/*N* is >10 and %R.S.D. is <15% for six repetitive injections. CCMTHP standard at 0.05 μg/mL was injected and evaluated. %R.S.D. of six injections was 9.1% with *S*/*N* > 10. Therefore, a 0.05 μg/mL (10 ppm) LOQ was demonstrated.

Specificity of the method was verified by a blank injection, where no significant peak was observed at the retention time of CCMTHP. All known impurities of the API were well resolved (Rs ≈ 8 to the adjacent peak) from CCMTHP.

3.5.4. Accuracy

The accuracy of the method was demonstrated by evaluating the recovery of CCMTHP standards spiked into an API sample containing undetectable levels of CCMTHP (<3 ppm). Recoveries were assessed at 0.05 μg/mL, 0.5 μg/mL, and 5 μg/mL spiking levels corresponding to 10 ppm, 100 ppm and 1000 ppm, respectively, versus the 5 mg/mL nominal API sample concentration. The spiked API samples were prepared by dissolving the API in the corresponding spiking standard solution. The recovery at the 0.05 μg/mL level was 82%, which is considered satisfactory as the spiking was performed at LOQ level. The recoveries at 0.5 μg/mL and 5 μg/mL were 100% and 109%, respectively. Therefore, the accuracy of the method in the range of 0.05–5 μg/mL was confirmed.

4. Conclusion

The GC–MS SIM (*m*/*z* = 49) method described is a relatively simple analytical procedure for the accurate determination of trace levels of CCMTHP (down to 10 ppm) in an API. The method requires no extraction, derivatization or cleanup and can be readily adapted for the analysis of other halogen containing alkylating reagents used in the manufacture of pharmaceutical products.

Almost all quantitative applications of GC/EI-SIM rely on stable isotope dilution techniques, where the stable isotope-labeled analogs of sample molecules serve as ideal internal standards [25,26]. However, development of such quantitation methods is heavily reliant on the availability of authentic standards [27]. Therefore, external standardization was applied to this analysis to determine the residual CCMTHP levels in approximately 60 pilot batches of API.

Acknowledgements

The authors thank Gary Slemp, Gang Xue and Zhongli Zhang of Analytical Research and Development and Everett Wilhelm of Global Quality Analytical Research, Pfizer Inc., Groton, CT for their support of this work.

References

[1] CPMP/SWP/5199/02, Position paper on the limits of genotoxic impurities 2002.
[2] V. Camel, Spectrochim. Acta Part B Atomic Spectr. 58 (2003) 1177–1233.
[3] R. Westbom, L. Thorneby, S. Zorita, L. Mathiasson, E. Bjorklund, J. Chromatogr. A 1033 (2004) 1–8.
[4] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, second ed., Wiley, New York, 1997.
[5] J.W. King, Z. Zhang, Anal. Bioanal. Chem. 374 (2002) 88–92.
[6] J.J. Mateo, A. Llorens, R. Mateo, M. Jimenez, J. Chromatogr. A 918 (2001) 99–112.
[7] F.C.G.M. Langelaan, M.W.F. Nielen, Int. J. Environ. Anal. Chem. 36 (1989) 27–34.
[8] D.R. Knapp, Handbook of Analytical Derivatization Reactions, John Wiley & Sons Inc., New York, 1979, pp. 1–24.
[9] R. Frost, M.S. Hussain, A.R. Raghani, J. Sep. Sci. 26 (2003) 1097–1103.
[10] J.A. Dean, Analytical Chemistry Handbook, McGraw-Hill, New York, 1995, pp. 4.20–4.63.
[11] L.H. Henrich, in: R.L. Grob (Ed.), Modern Practice of Gas Chromatography, third ed., Wiley, New York, 1995, pp. 274–281.
[12] H.M. McNair, E.J. Bonelli, Basic Gas Chromatography, Varian Aerograph, Walnut Creek, California, 1969.
[13] H.P. Li, G.C. Li, J.F. Jen, J. Chromatogr. A 1012 (2003) 129–137.
[14] N. Fidalgo-Used, G. Centineo, E. Blanco-Gonzalez, A. Sanz-Medel, J. Chromatogr. A 1017 (2003) 35–44.
[15] R.H. Rieck, LCGC North Am. 21 (2003) 992–1004.
[16] S. Calderara, D. Gardebas, F. Martinez, Forensic Sci. Int. 137 (2003) 6–12.
[17] L.H. Henrich, in: R.L. Grob (Ed.), Modern Practice of Gas Chromatography, third ed., Wiley, New York, 1995, pp. 291–302.
[18] F.J. Santos, M.T. Galceran, J. Chromatogr. A 1000 (2003) 125–151.
[19] B. Aebi, W. Bernhard, Chimia 56 (2002) 48–52.
[20] I.D. Wilson, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 325–356.
[21] C.S. Hsu, D. Drinkwater, Chromatogr. Sci. Ser. 86 (2001) 55–94 (Current Practice of Gas Chromatography–Mass Spectrometry).
[22] J. Unwin, J.A. Groves, Anal. Chem. 68 (1996) 4489–4493.
[23] H.W. Kuo, W.H. Ding, J. Chromatogr. A 1027 (2004) 67–74.
[24] M.A. Soliman, J.A. Pedersen, I.H. Suffet, J. Chromotogr. A 1029 (2004) 223–237.
[25] T. Obata, Anal. Chim. Acta 465 (2002) 379–393.
[26] M.A. Sochaski, A.J. Jenkins, T.J. Lyons, S.R. Thorpe, J.W. Baynes, Anal. Chem. 73 (2001) 4662–4667.
[27] F.J. Belas, I.A. Blair, Drugs Pharm. Sci. 117 (2002) 151–185 (Handbook of Pharmaceutical Analysis).