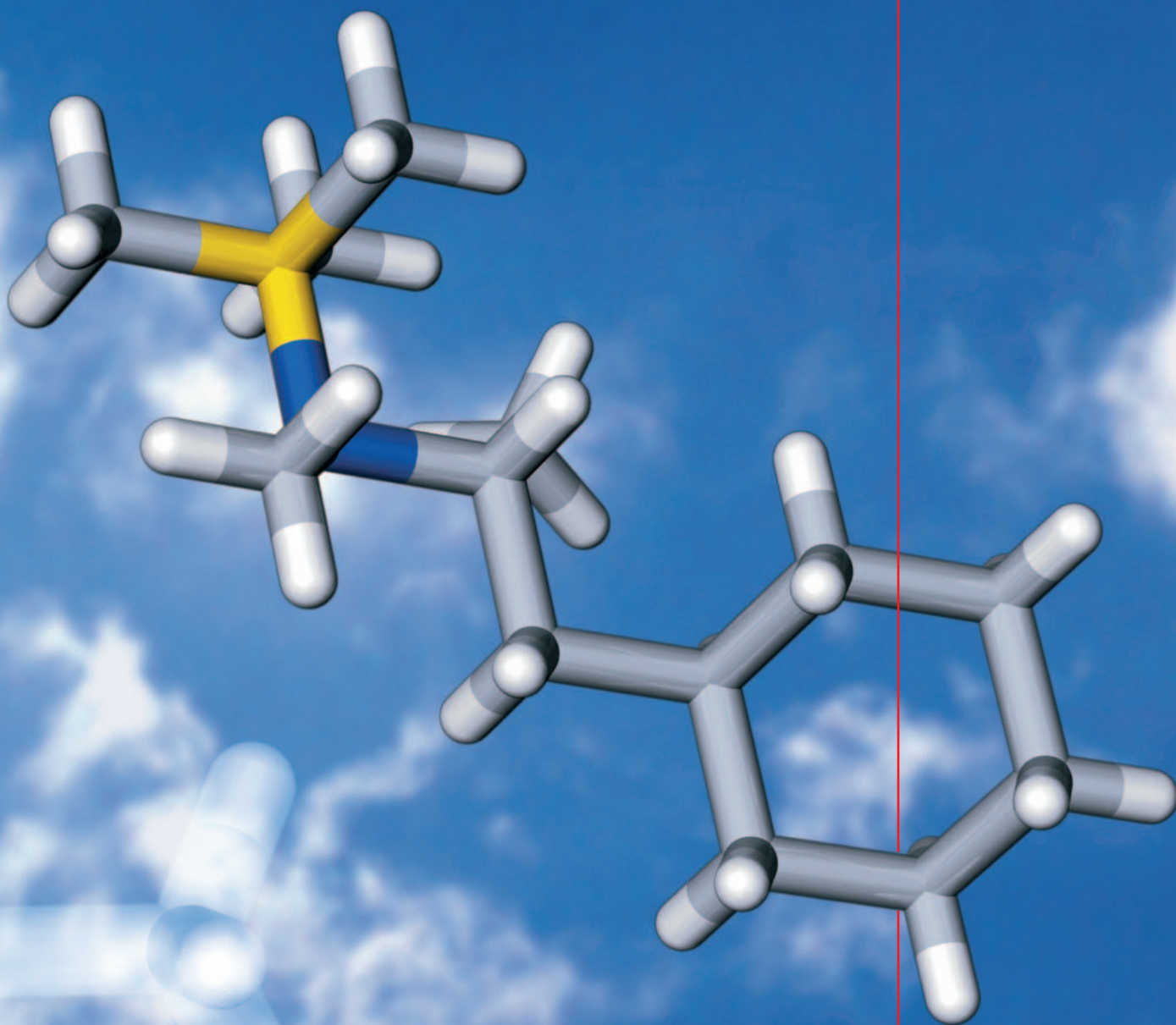


Analytix^{Notes}

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Derivatization of Drug Substances with MSTFA



SIGMA-ALDRICH

Improved silylation of drug substances for GC/MS analysis using activated MSTFA reagents Sigma-Aldrich offers activated N-methyl-N-trimethylsilylfluoroacetamide (MSTFA) silylation reagents that have improved performance over N,O-Bis(trimethyl-silyl)trifluoroacetamide (BSTFA) reagents for the silylation of hydroxylated and amine-containing drug substances.

Abbreviations

MSTFA: N-Methyl-N-trimethylsilylfluoroacetamide
 BSTFA: N,O-Bis(trimethylsilyl)trifluoroacetamide
 TMCS: Trimethylchlorosilane
 TFAA: Trifluoroacetic anhydride
 PFAA: Pentafluoropropionic anhydride
 BSA: N,O-Bis(trimethylsilyl)acetamide
 MTBSTFA: N-Methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide

Utility of GC/MS for pharmaceutical analysis

Although HPLC and LC/MS are widely used in pharmaceutical analysis, GC and GC/MS have some distinct advantages, including:

- GC/MS very often provides slightly lower detection limits than LC/MS
- GC/MS instruments are more common and less expensive than LC/MS instruments

Irrespective of the benefits of GC/MS, most pharmaceutical compounds and their metabolites cannot be analyzed in their native form without derivatization.

Table 1 Example of different derivatization reagents for drug substances

Drug group	Derivatization reagents	Literature
Amphetamines	MSTFA + 1% TMCS TFAA BSTFA	Rood, H.J. and Knitter, J.A., <i>Capillary Chromatography</i> 115-120 (1991)
Barbiturates	BSTFA	Kananen, G. et al., <i>J. Chromatogr. Sci.</i> , 10, 283-287 (1972)
Marijuana	BSTFA + 1% TMCS BSTFA MSTFA MSTFA + 1% TMCS MTBSTFA	Nelson, C.C. and Foltz, R. L., <i>Any. Chem.</i> , 64, 1578-1585 (1992)
LSD	BSA BSTFA MSTFA TFAA	Harkey, M. R. et al., <i>J. Anal. Toxicol.</i> , 15, 260-265 (1992)
Opiates	BSTFA + 1% TMCS MBTFA PFAA TFAA BSTFA	Chen, B.H. et al., <i>J. Anal. Toxicol.</i> , 14, 12-17 (1990)
PCP	BSTFA + 1% TMCS	Woodworth, J.R. et al., <i>J. Anal. Toxicol.</i> , 8, 2-6 (1984)

Advantages of derivatization for GC/MS

In GC, separation occurs in the gas phase. Analytes must therefore be volatile enough to be soluble in the carrier gas. Because of their polar, non-volatile nature, most drug substances and their metabolites are not easily vaporized without thermal decomposition. GC or GC/MS analysis can be accomplished in most instances by converting these compounds to a molecular form that has a boiling point below its decomposition point. Such derivatization facilitates GC analysis by:

- Reducing the polarity and enhancing the volatility of polar drug substances
- Increasing the thermal stability of the compound to prevent decomposition

For very small, volatile analytes, derivatization achieves a different aim. By increasing the molecular weight of very volatile compounds, derivatization provides a more complex mass spectrum, which increases the confidence that the compound has been correctly identified.

In all cases, for reliable compound identification with MS detection, the spectrum of the resulting derivatized compound should contain at least three ions that are not found in the matrix.

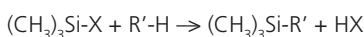
Common derivatization reagents for GC and GC/MS

There are many GC derivatization reagents suitable for drug substances (1). A few drug classes and a summary of the types of derivatives that have been employed to enhance their GC analysis along with a recent citation are listed in Table 1.

The fluorine-containing acylation reagents, including TFAA and PFAA, and the silylation reagent combination BSTFA/TMCS are commonly used, but are corrosive and can damage the capillary GC column. An ideal derivatization reagent provides fast, complete reactions for sensitive GC and GC/MS detection without damaging by-products.

Silylation reactions

Silylation reactions are the most versatile derivatization technique to enhance GC performance. The silylation reaction replaces the active hydrogen on protic functional groups with a trimethylsilyl (TMS) group. The TMS groups reduce dipole-dipole interactions and subsequently increase the volatility of the TMS derivative over the parent compound. The general reaction for the formation of a trimethylsilyl derivative is:



Of the myriad trimethylsilylation reagents available, MSTFA is one of the most important. Like BSA and BSTFA, MSTFA can be used to derivatize (silylate) all protic functional groups, including non-sterically hindered alcohols, carboxylic acids, amino acids, amides, amines and enols. A benefit of MSTFA is that the by-products of MSTFA silylation, primarily N-methyltrifluoroacetamide, are more volatile than BSA and BSTFA, making MSTFA valuable to identify compounds that would otherwise go undetected or obscured in the GC analysis. Additionally, MSTFA

reactions do not produce corrosive by-products that can damage the capillary GC column. (See reference 2 for a recent review on trimethylsilyl derivatization reactions and ways to avoid artifact formation.)

The silylation power of MSTFA can be increased by the use of catalysts or additives that scavenge reaction by-products. MSTFA reacts in situ with ammonium iodide (NH_4I) to produce trimethyliodosilane (TMSI), which has been reported to be the most powerful trimethylsilyl donor available (3). TMSI reacts with adequate speed to produce both trimethylsilyl (TMS) ether and trimethylsilyl enol (TMS enol) ether derivatives (4). Ethanethiol is added to reduce the formed iodine to hydrogen iodide in order to prevent iodine incorporation into the product. As a result, diethyl disulfide is produced during the derivatization reaction (5). Diethyl disulfide formation depends on the amount of ammonium iodide and ethanethiol added to the extract and the chosen experimental conditions such as reaction time and temperature. Imidazole acts as a base catalyst in the MSTFA silylation reaction.

In this study, we compared the performance of BSTFA/TMCS and MSTFA with three different scavenging agents, or activants, for the derivatization of compounds from three different drug classes. The derivatization reagents appear in Table 2 while the drug substances tested appear in Table 3. All derivatization reagents, drug standards and capillary GC columns are available from Sigma-Aldrich. The GC/MS conditions are presented in Table 4.

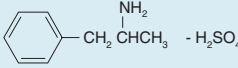
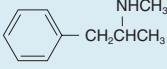
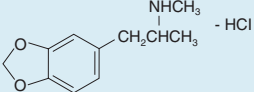
Table 2 Derivatization (silylation) reagents

Cat. No.	Brand	Description	Remarks	Package Size
50992	Fluka	MSTFA I (N-Methyl-N-trimethylsilyltrifluoroacetamide activated I)	Activated with ethanethiol and ammonium iodide*	5 mL, 25 mL
44156	Fluka	MSTFA II (N-Methyl-N-trimethylsilyltrifluoroacetamide activated II)	Activated with trimethylsilyl-ethanethiol*	5 mL, 25 mL
12124	Fluka	MSTFA III (N-Methyl-N-trimethylsilyltrifluoroacetamide activated III)	Activated with imidazole*	5 mL, 25 mL
441104	Aldrich	BSTFA + 1%TMCS (N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane)		10 x 1 mL, 25 mL

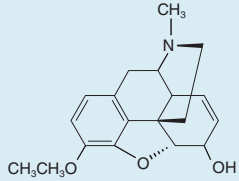
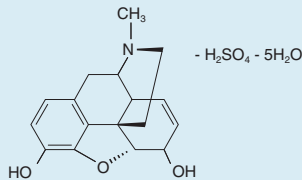
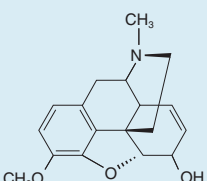
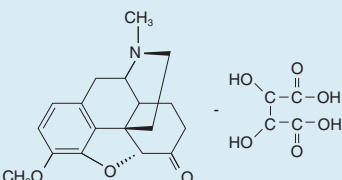
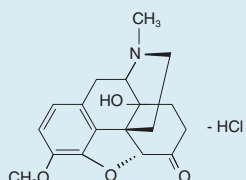
* Equivalent silylation power to a 1000:2 MSTFA:iodotrimethylsilane mixture

Table 3 Drug substances evaluated

Amphetamines

Cat. No.	Brand	Drug (molecular weight)	Structure
A5880	Sigma	D-Amphetamine sulfate salt (368.49)	
D6787	Sigma	(-)-Deoxyephedrine (149.23) (Methamphetamine)	
M6403	Sigma	(±)-3,4-Methylenedioxymethamphetamine hydrochloride (193.24) (Ecstasy)	

Opiates

Cat. No.	Brand	Drug (molecular weight)	Structure
E8512	Sigma	Ethylmorphine (313.39)	
M8777	Sigma	Morphine sulphate salt pentahydrate (758.83)	
C5901	Sigma	Codeine (299.36)	
H4516	Sigma	Hydrocodone (+)-bitartrate salt (449.45)	
O1378	Sigma	Oxycodone hydrochloride (351.82)	

Cannabinoids

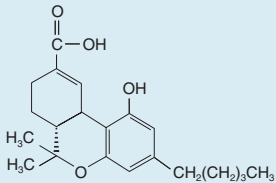
Cat. No.	Brand	Drug (molecular weight)	Structure
N3142	Sigma	11-Nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (344.44) (Major metabolite of Δ^9 -tetrahydrocannabinol)	

Table 4 Conditions for GC and GC/MS methods

	Method 1	Method 2	Method 3
GC instrument	HP 5890 II		
MS instrument	HP MS 5972		
Detector	MS and FID		
Injection volume	1 μ l of the silylating reaction solution		
Column	Supelco MDN-1 (Cat. No. Supelco 18911-08A), 30 m L x 0.25 mm ID, 0.25 μ m d_i		
Carrier gas and flow rate	Helium, 1 mL/min		
Ionization	El at $\sim$ 70eV (filament)		
Injection temperature	225°C	150°C	150°C
Temperature program oven	225°C, 15°C/min to 300°C, 25 min at 280°C	150°C, 15°C/min to 300°C, 20 min at 280°C	8 min at 150°C, 10°C/min to 250°C, 12 min at 250°C
Detector temperature	300°C	300°C	300°C

(All measurements were performed at the Sigma-Aldrich Analytical Laboratory in Steinheim, Germany.)

Results of silylation of the three drug classes with and BSTFA/TMCS and activated MSTFA reagents

The results are summarized in Table 5. **Figures 1 through 4** show the example of hydrocodone bitartrate salt derivatized using the four different silylation reagents, BSTFA/TMCS, MSTFA I, II and III.

Cannabinoids

The major metabolite of Δ^9 -tetrahydrocannabinol, 11-Nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid, reacts completely with all four silylation reagents, giving one peak by GC/MS. The mass spectrum confirms the complete silylation of both OH-groups. The following main fragments besides the mass peak show up in the spectrum: M^+ , $M-CH_3^+$, $M-Me_3SiCO_2^+$ and Me_3Si^+ .

Opiates

The five tested members of the opiate group show differing silylation behavior.

Morphine sulphate reacts with the four silylation reagents to form the double silylated product.

The GC spectrum shows one single sharp peak. The mass spectrum shows an M^+ peak for the double silylated product.

With **ethylmorphine** the reagents BSTFA, MSTFA I and II give a main silylated product and a by-product

at slightly higher retention time. MSTFA III gives one single, sharp peak by GC and a single silylated M^+ peak in the mass spectrum.

Oxycodone hydrochloride reacts with MSTFA I and II preferentially in the enol structure to give a double silylated product which is confirmed by the mass spectrum ($M-H^+$ peak at 459 m/z). With the MSTFA III reagent a by-product shows up in the GC with an M^+ peak at 387 m/z for a monosilylated product in the mass spectrum.

Hydrocodone(+)-bitartrate reacts with the activated silylating reagents MSTFA I and III completely to form a single silylated product: one sharp peak in the GC with the mass spectrum showing the $M-H^+$ peak. With BSTFA we obtained only a 50:50 mixture of single silylated and non-silylated product. MSTFA II gave several side products beside the single silylated product. Results of this derivatization are shown in **Figures 1 through 4**. Note that although all four silylation reagents gave three unique ions for hydrocodone, **Figures 2 and 4** shows that only MSTFA I and MSTFA III provided complete silylation.

Codeine reacts with BSTFA and MSTFA III to give the monosilylated product as main product, confirmed by the M^+ peak in the mass spectrum. Besides the main peak, a by-product appears at a slightly higher retention time.

Amphetamines

We examined deoxyephedrine, D-amphetamine sulphate and Ecstasy as representatives of the amphetamine group.

The derivatization of **Ecstasy** with all three activated MSTFA reagents results in one single, sharp peak in the GC spectra. The mass spectra shows a very small M^+ peak, the main fragment was identified as $\text{Me}_3\text{SiONCH}_2\text{CHCH}_3^+$.

Amphetamine also reacts with all three activated MSTFA reagents to form the double silylated product. The GC spectrum shows a single peak. In the mass spectra the M^+ peak does not show up; the highest molecular weight fragment detected was $M-\text{CH}_3^+$.

Deoxyephedrine reacts only with BSTFA and MSTFA I completely to form the monosilylated product. In the GC spectra a single peak is observed. The $M-H^+$ peak in the mass spectra is very small; the main fragment is the

$\text{Me}_3\text{SiONCH}_2\text{CHCH}_3^+$ ion. With the other two activated MSTFA reagents, II and III, the silylation is incomplete and several side products are seen in the GC.

The order of silylating strength is: $\text{TMSI} > \text{BSTFA} > \text{BSA} > \text{MSTFA} > \text{TMSDMA} > \text{TMSDEA} > \text{MSA} > \text{TMCS}$ (with base catalysts) $> \text{HMDS}$ (1). However, it is sometimes preferable to use a weaker silyl donor to minimize artifacts formed from over-silylation. For example, we found that the silylation of hydrocodone with BSTFA resulted in a 50:50 mixture of educt and silylated product in the enol form (**Figure 1**). The MSTFA II reagent was not at all applicable showing four by-products at longer retention times beside the desired silylated product (**Figure 3**). With deoxyephedrine, the MSTFA III reacted to form the silylated product, but also a large amount of the trimethylsilylated imidazole that showed up as a very broad peak at lower retention times (6.82 - 7.22 min). With MSTFA II we also observed a main by-product, the hexamethyldisilathiane.

Table 5 Results of derivatization experiments

Product	Group	BSTFA + 1% TMCS (Cat. No. 441104)	MSTFA I (Cat. No. 50992)	MSTFA II (Cat. No. 44156)	MSTFA III (Cat. No. 12124)	GC/MS- method (Table 4)
11-Nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid	Cannabinoids	Complete silylation	Complete silylation	Complete silylation	Complete silylation	1
Codeine	Opiates	Complete silylation	Not measured	Not measured	Complete silylation	1
Hydrocodone (+)-bitartrate salt	Opiates	50:50 mixture of silylated and non-silylated product	Complete silylation	Incomplete silylation	Complete silylation	1
Ethylmorphine	Opiates	Complete silylation + minor by-product	Complete silylation + minor by-product	Complete silylation + minor by-product	Complete silylation	1
Oxycodone hydrochloride	Opiates	Not measured	Complete silylation (double silylated)	Complete silylation (double silylated)	Double silylated main product and mono-silylated as by-product	1
Morphine sulfate	Opiates	Complete silylation	Complete silylation	Complete silylation	Complete silylation	1
(-)-Deoxyephedrine	Amphetamines	Complete silylation	Complete silylation	Incomplete silylation	Incomplete silylation	3
D-Amphetamine sulfate salt	Amphetamines	Not measured	Complete silylation	Complete silylation	Complete silylation	2
($\pm$)-3,4-Methylenedioxy-methamphetamine hydrochloride (Ecstasy)	Amphetamines	Not measured	Complete silylation	Complete silylation	Complete silylation	3

Summary

In this short communication, we report the results of the ability of three activated MSTFA reagents to form GC/MS-compatible trimethylsilyl derivatives of several important drugs classes: cannabinoids, amphetamines and opiates. Although results varied with drug substance tested, one or more of the three activated MSTFA reagents was as effective as or more effective than the BSTFA/TMCS reagent, without the generation of system-damaging corrosive by-products. Therefore, the choice of silylation reagent depends on what drug classes are to be derivatized.

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Figure 1 Hydrocodone silylation with BSTFA + 1% TMCS

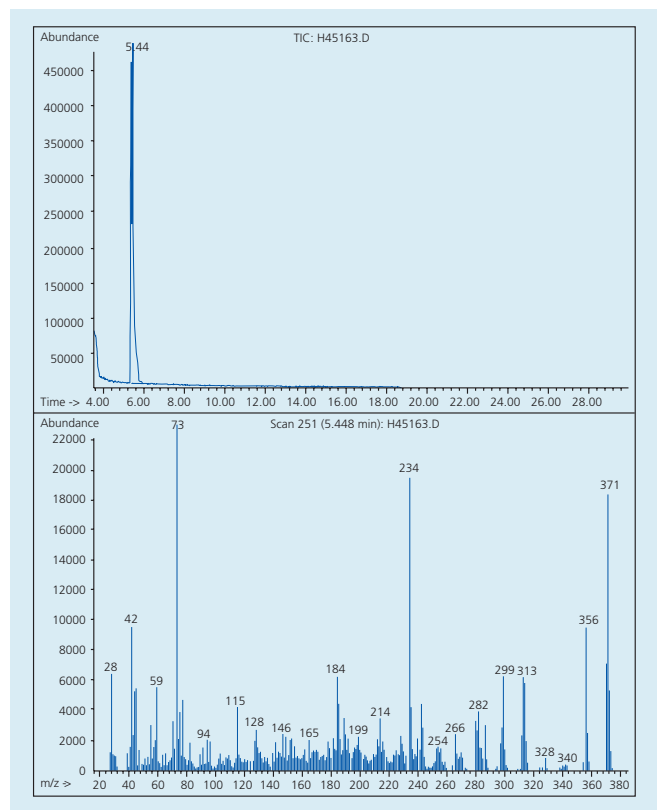


Figure 2 Hydrocodone silylation with MSTFA I (MSTFA plus ethanethiol and ammonium iodide)

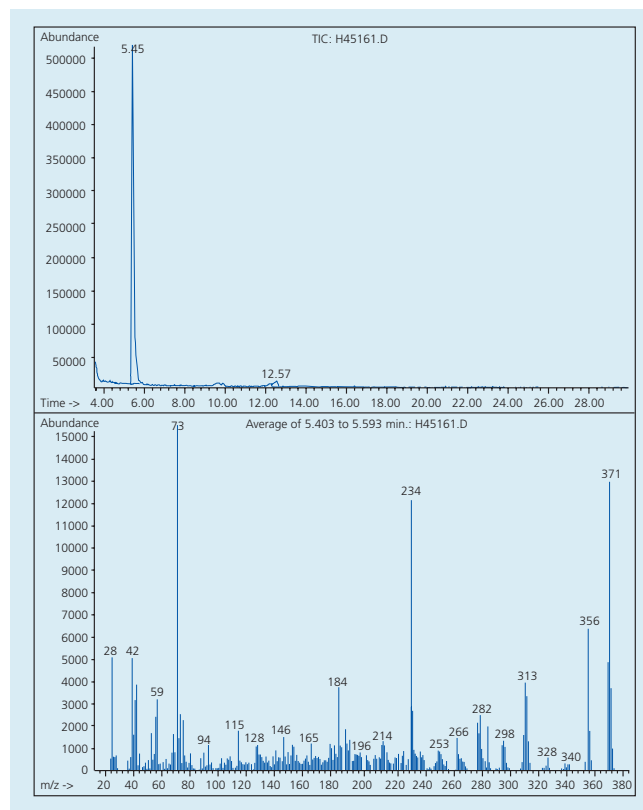


Figure 3 Hydrocodone silylation with MSTFA II (MSTFA plus trimethylsilyl-ethanethiol)

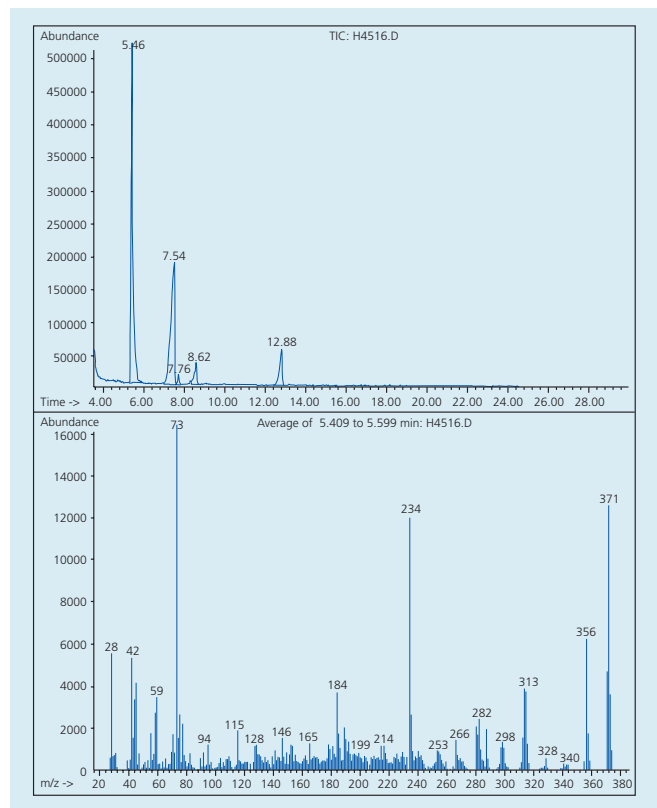
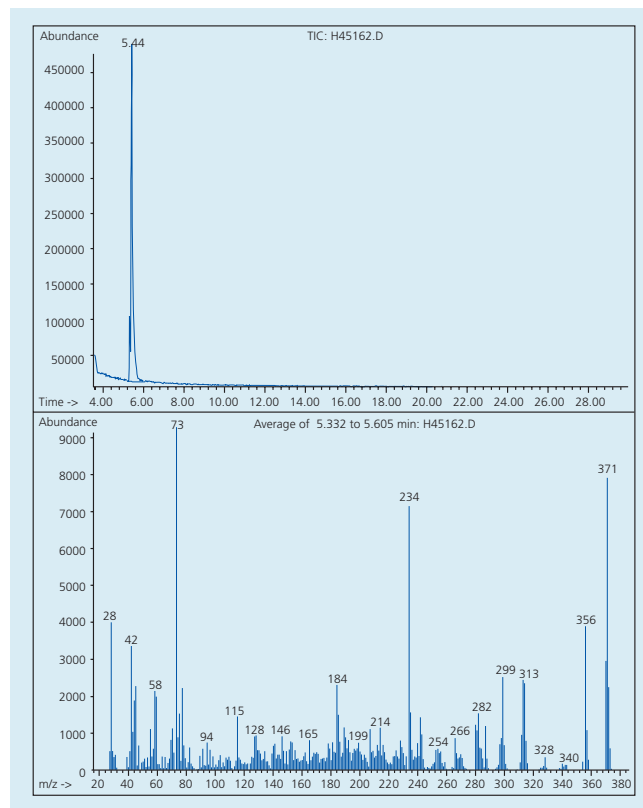


Figure 4 Hydrocodone silylation with MSTFA III (MSTFA plus imidazole)



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