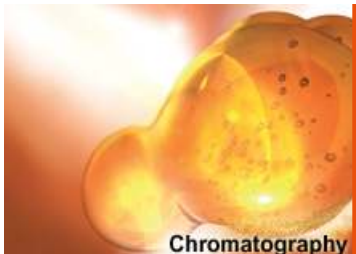


High sensitive analysis method for benzodiazepines and their metabolites using NCI-GC/MS

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Benzodiazepines

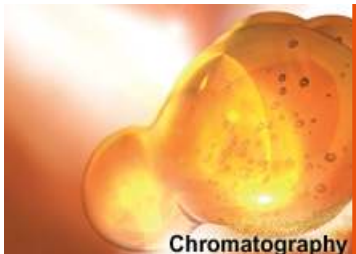
Introduction

- The identification of benzodiazepines and their metabolites in blood or urine samples is vitally important to the verification of crime. GC/MS with full-scan electron ionization (EI) mode is commonly used for the screening of these compounds, but enhanced sensitivity is still demanded especially for low-dosed benzodiazepines detection. Negative ion chemical ionization (NCI) mode has been studied for the detection of some benzodiazepines because of its increased sensitivity.
- In this paper, the sensitivity of the full-scan NCI-GC/MS was investigated for 57 benzodiazepines including their metabolites. The standard solutions of underivatized compounds and trimethylsilyl derivatives using N,O-bis (Trimethylsilyl) trifluoroacetamide (BSTFA) were analyzed separately.
- The compounds which showed higher sensitivity in the NCI mode were further studied for qualitative and quantitative analysis, as well as on the applicability of urine sample analysis.



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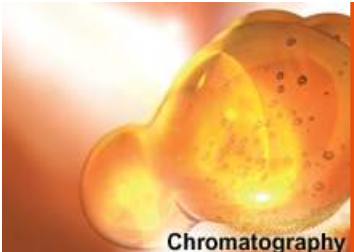


Analytical Conditions

Experimental

GC/MS	:GCMS-QP2010 (SHIMADZU CORPORATION)
Work Station	:GCMSsolution (SHIMADZU CORPORATION)
Auto Sampler	:AOC-20i/s (SHIMADZU CORPORATION)
Injection Temp.	:260 °C
Carrier Gas	:100kPa Constant Pressure, Helium
Column	:Rtx-5MS (Length 30 m x I.D 0.25 mm , Film 0.25 μ Restek Corporation)
Column Temp.	:80 °C (2 min) - (15 °C/min) – 310 °C (12 min)
Interface Temp.	:280 °C
Ion Source Temp.	:200 °C
MS Acquisition Mode	:Scanning mode (m/z 35 ~ 600)
Injection Volume	:1 μL
Glass liner	:Deactivated glass liner (P/N: 550733, Restek Corporation)
Detector Voltage (NCI Mode)	:EI: 1.2 kV, NCI: 1.4 kV
Reagent Gas	:Methane (0.3 MPa at pressure regulator)





Chromatography

Sample pretreatment

Experimental

Standard sample

Underivatized

Benzodiazepine metabolites were synthetic chemical products.
Benzodiazepine and their metabolites were dissolved in methanol.

TMS Derivatization

1. Add 200 μL of BSTFA (N,O-bis(Trimethylsilyl)trifluoroacetamide) to the dried sample.
2. Keep the sample at the 60 °C for 30 min.

Urine sample

This sample was taken from a benzodiazepine user

Put 1 mL of urine into the vial and add 2 mL of distilled water.



Acidify it using 1 mol/L hydrochloric acid



Extract it using 2 mL chloroform :isopropanol (3:1)



Repeat this procedure three times.

Dry it under a gentle nitrogen flow.



TMS Derivatization



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Chromatography

1. Sensitivity Evaluation - Benzodiazepines

The underivatized and derivatized benzodiazepines at 0.100 mg/L were analyzed in both modes and the sensitivities were compared based on the S/N values.

ID	Compound Name	EI-Undrivatized	EI-TMS	NCI-Underivatized	NCI-TMS
1	Alprazolam	11.9 (m/z308)	NS	191.8 (m/z308)	NS
2	Bromazepam	NS	10.5 (m/z387)	15.3 (m/z81)	93.7 (m/z81)
3	Brotizolam	NS	NS	124.8 (m/z394)	NS
4	Chlordiazepoxide(dec)	17.5 (m/z282)	NS	30.4 (m/z254)	NS
5	Clobazam	15.9 (m/z300)	NS	NS	NS
6	Clonazepam	NS	3.4 (m/z352)	NS	371.5 (m/z387)
7	Clorazepic acid	44.3 (m/z242)	72.9 (m/z341)	30.9 (m/z254)	327.2 (m/z234)
8	Clotiazepam	46.7 (m/z289)	NS	86.9 (m/z282)	NS
9	Delorazepam	31.0 (m/z304)	149.5 (m/z376)	175.1 (m/z268)	789.4 (m/z268)
10	Diazepam	44.1 (m/z256)	NS	137.1 (m/z284)	NS
11	Estazolam	NS	NS	36.4 (m/z294)	NS
12	Ethyl loflazepate(DEP)	13.0 (m/z260)	NS	15.5 (m/z288)	9.0 (m/z432)
13	Etizolam	NS	NS	131.1 (m/z306)	NS
14	Fludiazepam	98.8 (m/z302)	NS	270.9 (m/z302)	NS
15	Flunitrazepam	18.3 (m/z313)	NS	99.7 (m/z313)	NS
16	Flurazepam	328.7 (m/z86)	NS	161.2 (m/z387)	NS
17	Flutazolam(dec)	20.9 (m/z289)	NS	NS	NS
18	Flutoprazepam	47.3 (m/z287)	NS	280.3 (m/z342)	NS
19	Lorazepam	NS	49.6 (m/z429)	28.5 (m/z302)	427.6 (m/z302)
20	Lormetazepam	NS	88.6 (m/z377)	NS	1575.2 (m/z280)
21	Medazepam	239.1 (m/z242)	NS	NS	NS
22	Mexazolam	NS	281.0 (m/z323)	NS	NS
23	Midazolam	53.3 (m/z310)	NS	230.2 (m/z325)	NS
24	Nimetazepam	NS	NS	51.1 (m/z295)	NS
25	Nitrazepam	NS	134.6 (m/z353)	NS	311.4 (m/z353)
26	Oxazepam	NS	36.4 (m/z430)	NS	756.8 (m/z268)
27	Oxazolam	NS	283.0 (m/z323)	NS	NS
28	Prazepam	40.3 (m/z324)	NS	76.5 (m/z324)	NS
29	Quazepam	15.3 (m/z386)	NS	1229.5 (m/z352)	NS
30	Temazepam	NS	63.7 (m/z372)	NS	669.1 (m/z372)
31	Triazolam	8.2 (m/z238)	NS	218.3 (m/z306)	NS

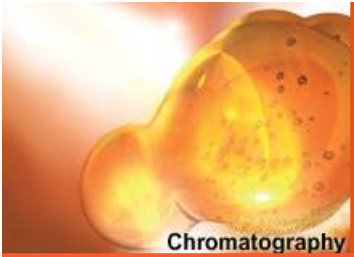
Results



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'NS' means no observable signal at 0.1mg/L

Highlighted S/N means best value in NCI mode.



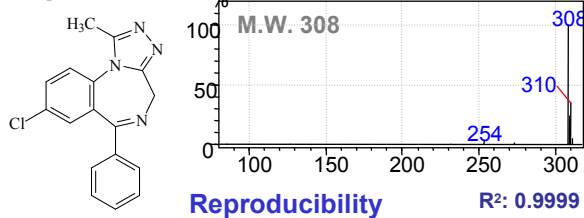
Chromatography

2. Qualitative and Quantitative evaluation

Results

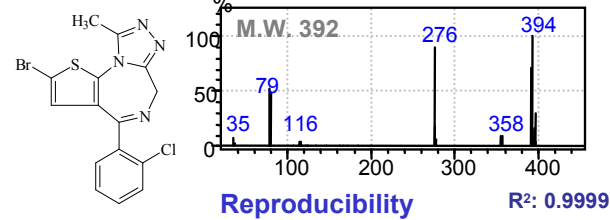
The compounds were evaluated on the mass spectrum, linearity and reproducibility. The calibration curve for each compound was created at the range of 0.050mg/L to 1.000mg/L and R^2 were obtained. The reproducibility for each compound was examined by analyzing five replicates at 0.100mg/L.

Alprazolam



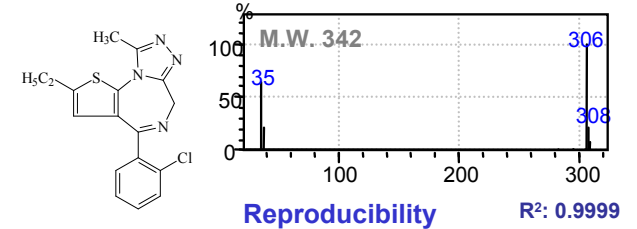
Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
53494	56415	59817	59351	56093	4.5

Brotizolam



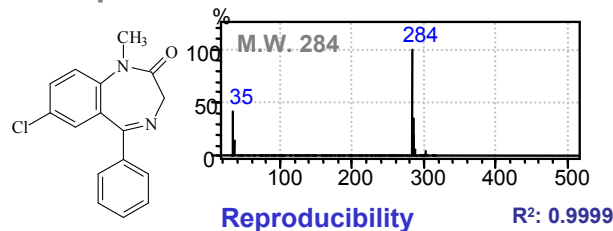
Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
44242	41176	46802	46814	38853	8.1

Etizolam



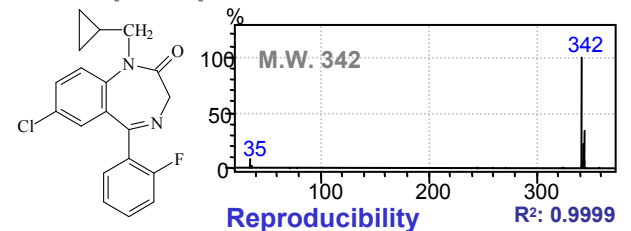
Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
39825	31195	38792	42837	42233	12.0

Diazepam

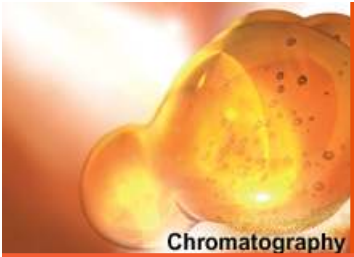


Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
33202	38029	29103	36951	33879	10.3

Flutoprazepam



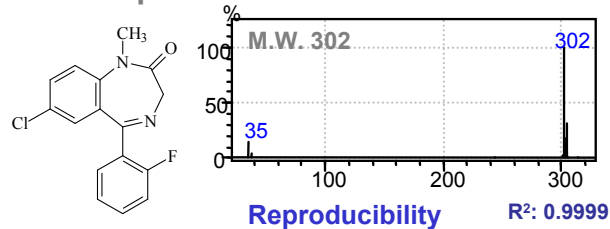
Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
68752	75860	67554	80475	69597	7.6



2. Qualitative and Quantitative evaluation

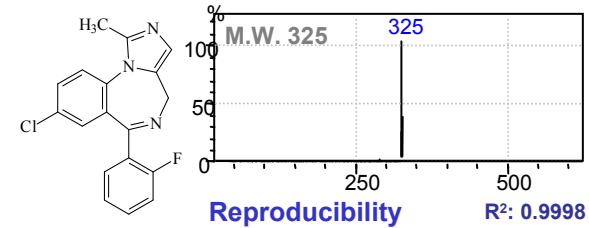
Results

Fludiazepam



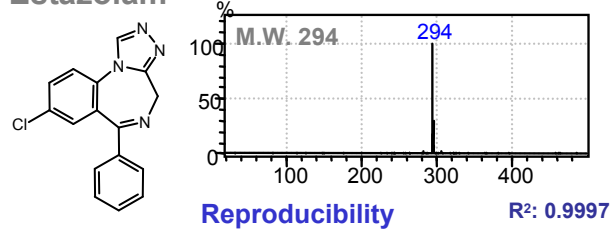
Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
66061	62045	74163	68482	71795	6.9

Midazolam



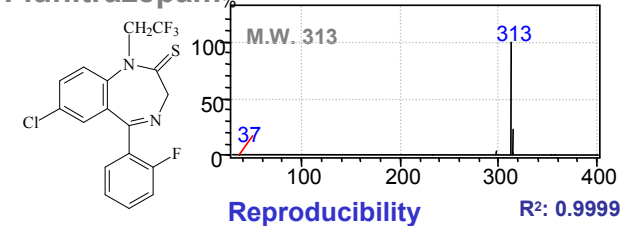
Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
47347	50083	54163	48609	60264	10.1

Estazolam



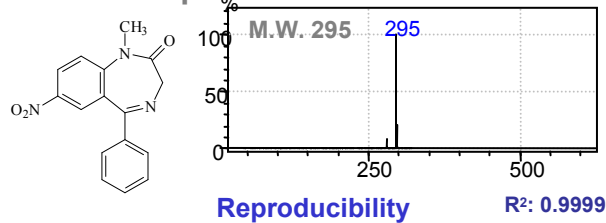
Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
41725	49457	37191	43250	39182	11.1

Flunitrazepam

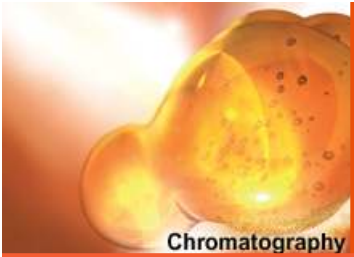


Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
29566	23369	22842	30139	27057	12.8

Nimetazepam



Data 1	Data 2	Data 3	Data 4	Data 5	%RSD
60542	67828	66527	58365	48746	12.6

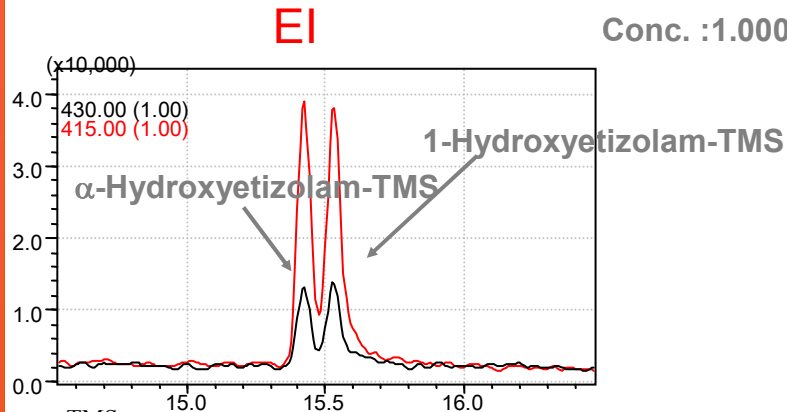


Chromatography

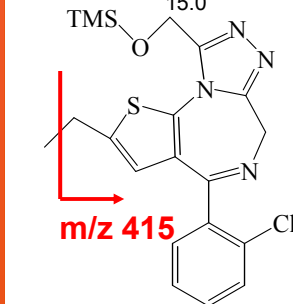
3. Qualitative advantages in NCI mode

Since these compounds show similar mass spectra in EI mode, the retention time is used to distinguish between them. On the other hand, these compounds could be easily identified by the different mass spectrum patterns in NCI mode.

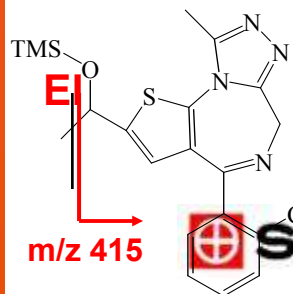
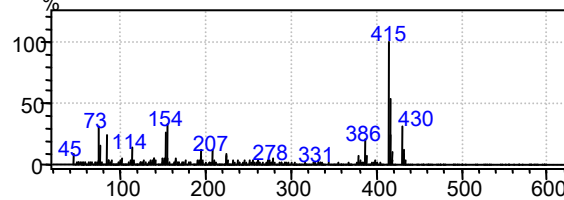
Results



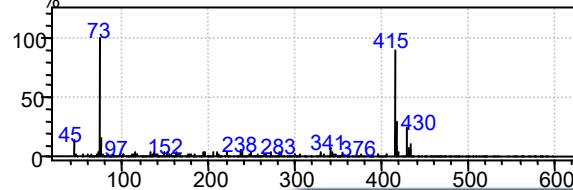
Identification of α -Hydroxyetizolam-TMS and 1-Hydroxyetizolam-TMS



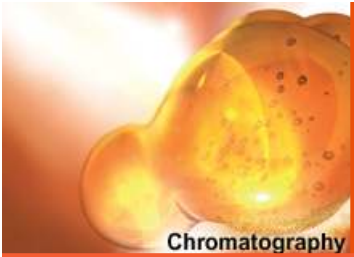
EI α -Hydroxyetizolam-TMS (MW: 430)



1-Hydroxyetizolam-TMS (MW: 430)



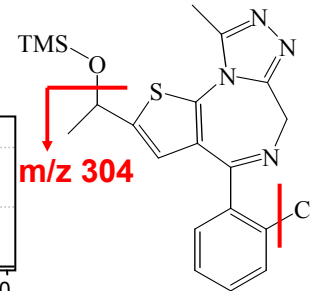
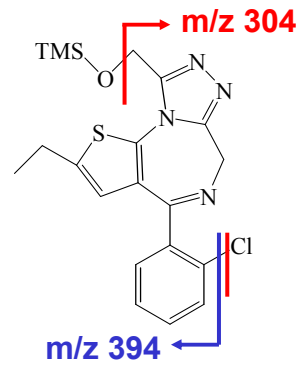
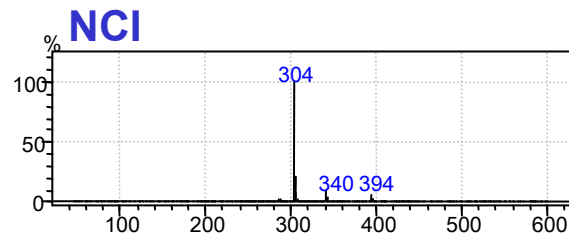
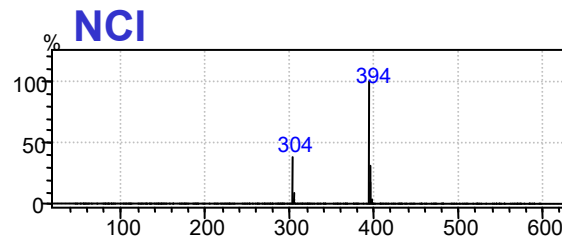
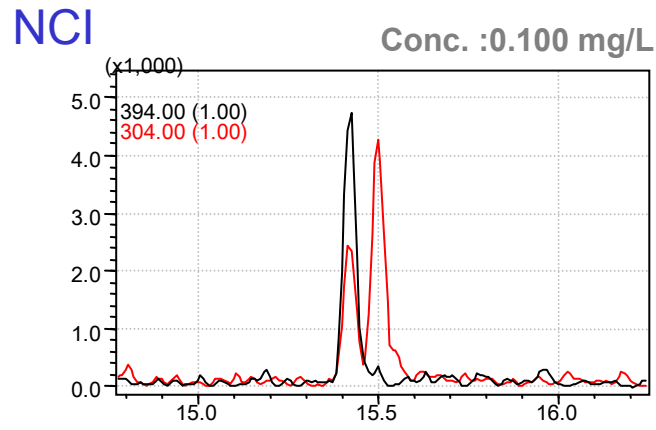
Solutions for Science

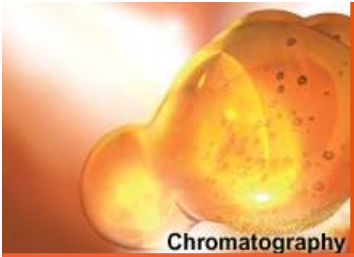


Chromatography

3. Qualitative advantages in NCI mode

Results

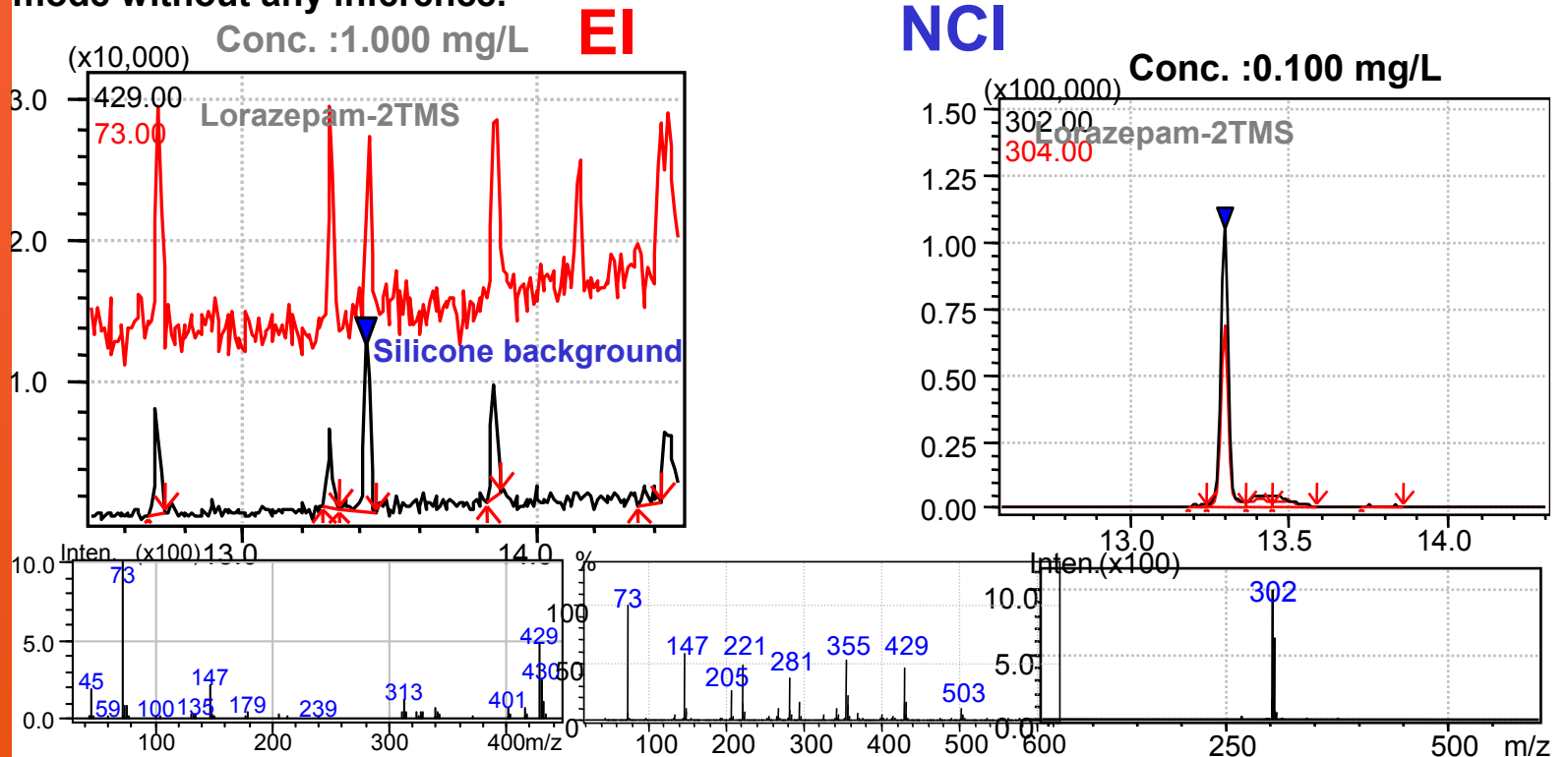




3. Qualitative advantages in NCI mode

Silicone compounds are occasionally emitted from the gas-chromatograph components and interfere with the compound detection. Since lorazepam-TMS has similar fragment ions as silicon compounds in the EI mass spectrum, the identification of lorazepam-TMS is difficult. On the other hand, lorazepam-TMS could be selectively detected in the NCI mode without any inference.

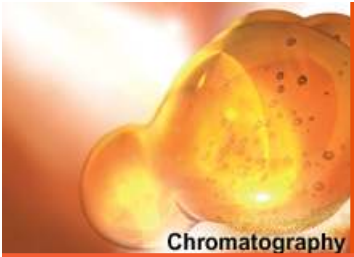
Results



Lorazepam-2TMS

Silicone background

Lorazepam-2TMS

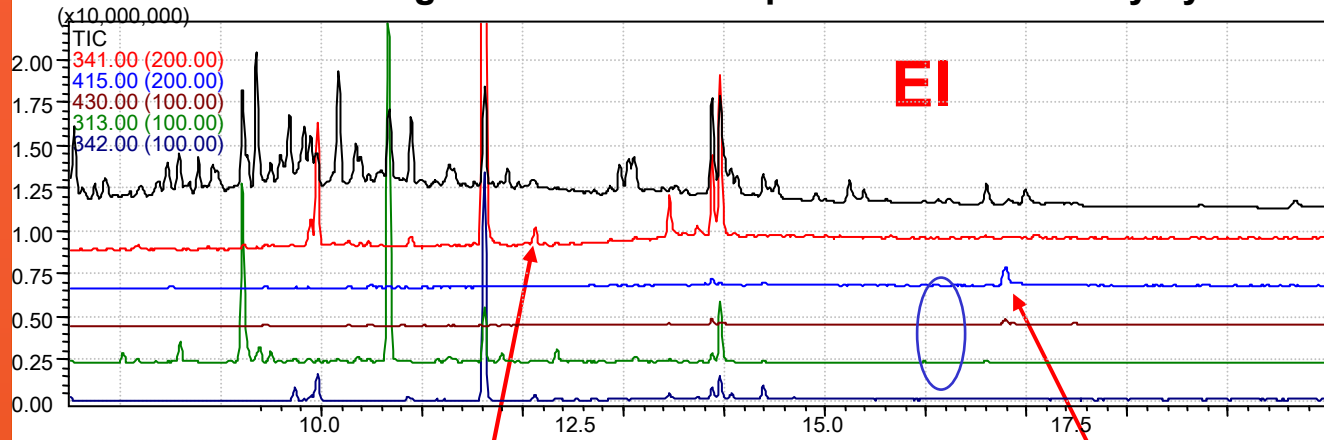


Chromatography

4. Urine Sample analysis

A 1 μ L of TMS derivatized urine sample was analyzed; α -Hydroxytriazolam-TMS and desmethyldiazepam-TMS were detected in both modes, but more clearly in NCI mode. Triazolam at 0.017 mg/L in the urine sample was detected only by the NCI mode.

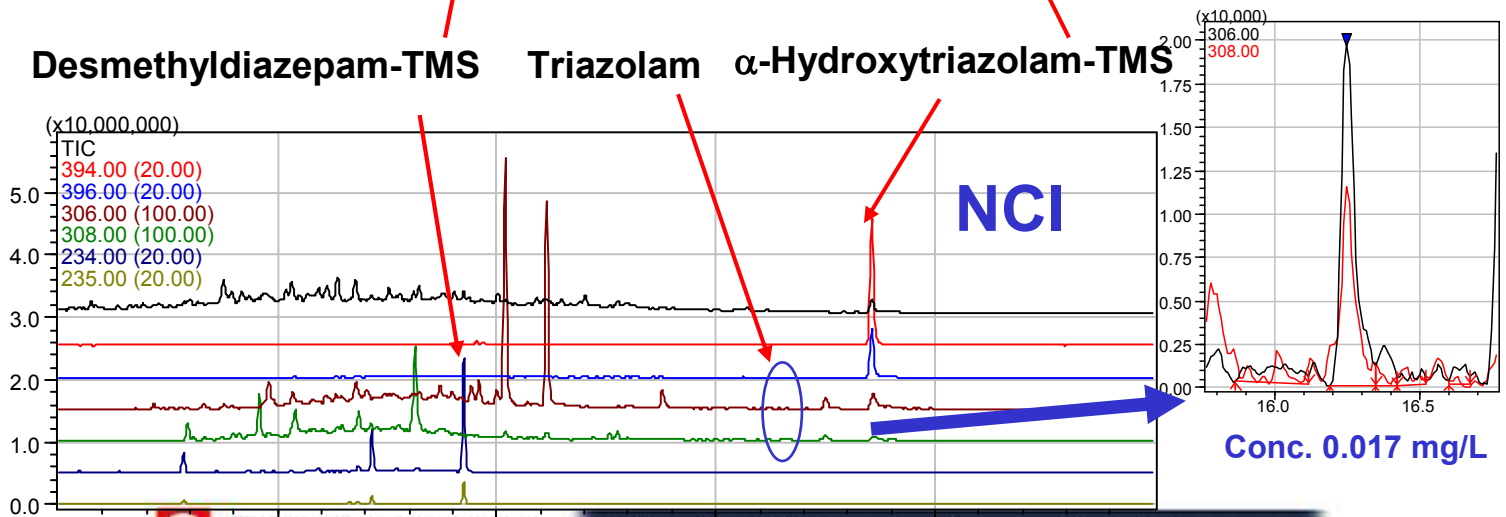
Results



Desmethyldiazepam-TMS

Triazolam

α -Hydroxytriazolam-TMS



Conc. 0.017 mg/L



Results

Conclusions

- Thirty-seven out of 57 compounds (underivatized: 13 and TMS-derivatized: 24) showed enhanced sensitivity in the NCI mode compared to the EI mode.
- Thirty-seven compounds, which showed better sensitivity in the NCI mode, were investigated on qualitative and quantitative evaluation. Most of NCI Mass spectra showed the molecular ion which is useful for compound identification. Linearity and repeatability were satisfactory for quantitative analysis.
- α -hydroxyetizolam and 1-hydroxyetizolam could be easily identified by NCI mass spectra. Lorazepam-2TMS could be clearly detected without silicone inference in the NCI mode.
- Triazolam could be detected at trace level from a urine sample using the full-scan NCI-GC/MS.
- The method of NCI demonstrated additional detection and identification capabilities. Using both the EI and the NCI modes, the analysis becomes more reliable.