To: מושה שלומוביץ' קידס סיגריות אלקטרוניות בע"מ **Date:** **11.11.2009**

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Analytical report 14-KID0001

LC-MS analysis of specific nitrosamines and GC-MS analysis of Diethylene glycol

No.	Sample and Reference materials name	TAMI Analytical Laboratory number	File name:
1	**Atomized cartridge for Ruyan V8**	LMS2028 2009-3784/1	LMS2028/KIDS0053-KIDS0058 GMMS2455-2458
2	**N-Nitrosonornicotine std.** Fluka 75285, 99.7%, CAS#80508-23-2		**Directory LMS2028/KIDS** 0026 0028 0031
3	**N-Nitrosonoanabasine std.** Fluka 75283, 99.7%, CAS#37620-20-5		
4	**N-Nitrosonoanatabine std.** Fluka 75281,99.3%**,** CAS#71267-22-6		
5	**4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone std.** Fluka 78013, 99.9%**,** CAS#64091-91-4		
6	**Cotinine std.** Sigma, C5923, 99.5%CAS#486-56-6		
6	**Diethylene glycol std.** Sigma-Aldrich, 99.0%	Sl-2852	GMMS24615-2470

Analysts :		**Remarks**
GC-MS	Olga Tobias	
LC-MS	Carmela Carmi Alex Kraminsky	
Written by :	Alex Kraminsky	
Supervisor :	Igal Gozlan	

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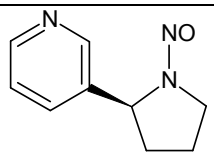
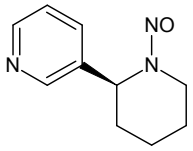
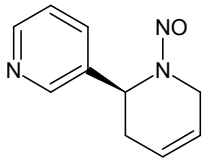
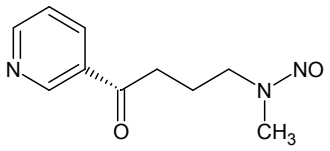
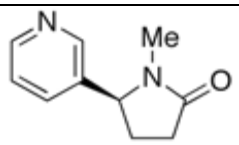
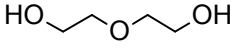
Analytical Report: LC-MS analysis of specific nitrosamines and GC-MS analysis of Diethylene glycol

Sample "Atomized cartridge for Ruyan V8" was analyzed by HPLC-UV/MS and GC-MS techniques in order to determine specific nitrosamines and diethylene glycol (table.1).

A HPLC method coupled with electrospray ionization mass spectrometry was developed (Appendix 1) for quantification of specific nitrosamines.

A GC-MS (SIM) method was developed for quantification of diethylene glycol.

Fig. 1 The chemical structure of specific nitrosamines, Internal Standard and diethylene glycol:

Name	MW	Structure
N-Nitrosonornicotine	177	
N-Nitrosonoanabasine	191	
N-Nitrosonoanatabine	189	
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	207	
Cotinine (Internal Standard, ISTD)	176	
Diethylene glycol	106	

Sample "Atomized cartridge for Ruyan V8" was analyzed versus standard materials.

The results are summarized in table 2.

The ESI (positive mode) spectrum of specific nitrosamines and Internal standard (ISTD) are shown in fig.1

The representative HPLC-MS (Extracted ion) chromatograms of 0.01mg/ml of nitrosamines and ISTD are shown in fig.2.

The representative HPLC-MS (Extracted ion) chromatograms of 0.0001mg/ml (LOD) of nitrosamines and ISTD are shown in fig.3.

The representative HPLC-MS (Extracted ion) chromatograms of 0.0005mg/ml of nitrosamines and ISTD are shown in fig.4.

The representative HPLC-MS (Extracted ion) chromatograms of 0.001mg/ml of nitrosamines and ISTD are shown in fig.5.

The representative HPLC-MS (Extracted ion) chromatograms of blank with ISTD are shown in fig.6.

The representative HPLC-MS (Extracted ion) chromatograms of sample with ISTD are shown in fig.7.

The representative HPLC-MS (Extracted ion) chromatograms of sample with ISTD and sample spiked with 0.0005mg/ml of specific nitrosamines are shown in fig.8.

The representative GC-MS SIM/Scan chromatograms of the sample "Atomized cartridge for Ruyan V8"are shown in fig.9.

The representative GC-MS SIM overlaid chromatograms of the sample "Atomized cartridge for Ruyan V8" and Diethylene glycol std are shown in fig.9a.

The representative GC-MS Scan chromatograms of the diethylene glycol standard are shown in fig.10 and fig.11.

Table 2: HPLC-MS and GC-MS results

Sample	N-Nitrosonornicotine (ppm)	N-Nitrosonoanabasine (ppm)	N-Nitrosonoanatabine (ppm)	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (ppm)	Diethylene glycol (ppm)
Atomized cartridge for Ruyan V8	Not Detected*	Not Detected*	Not Detected*	Not Detected*	Not Detected**

*LOD is 1 ppm

**LOD is 2 ppm

Fig. 1: The ESI (positive mode) spectrum of nitrosamines and Internal standard (ISTD)

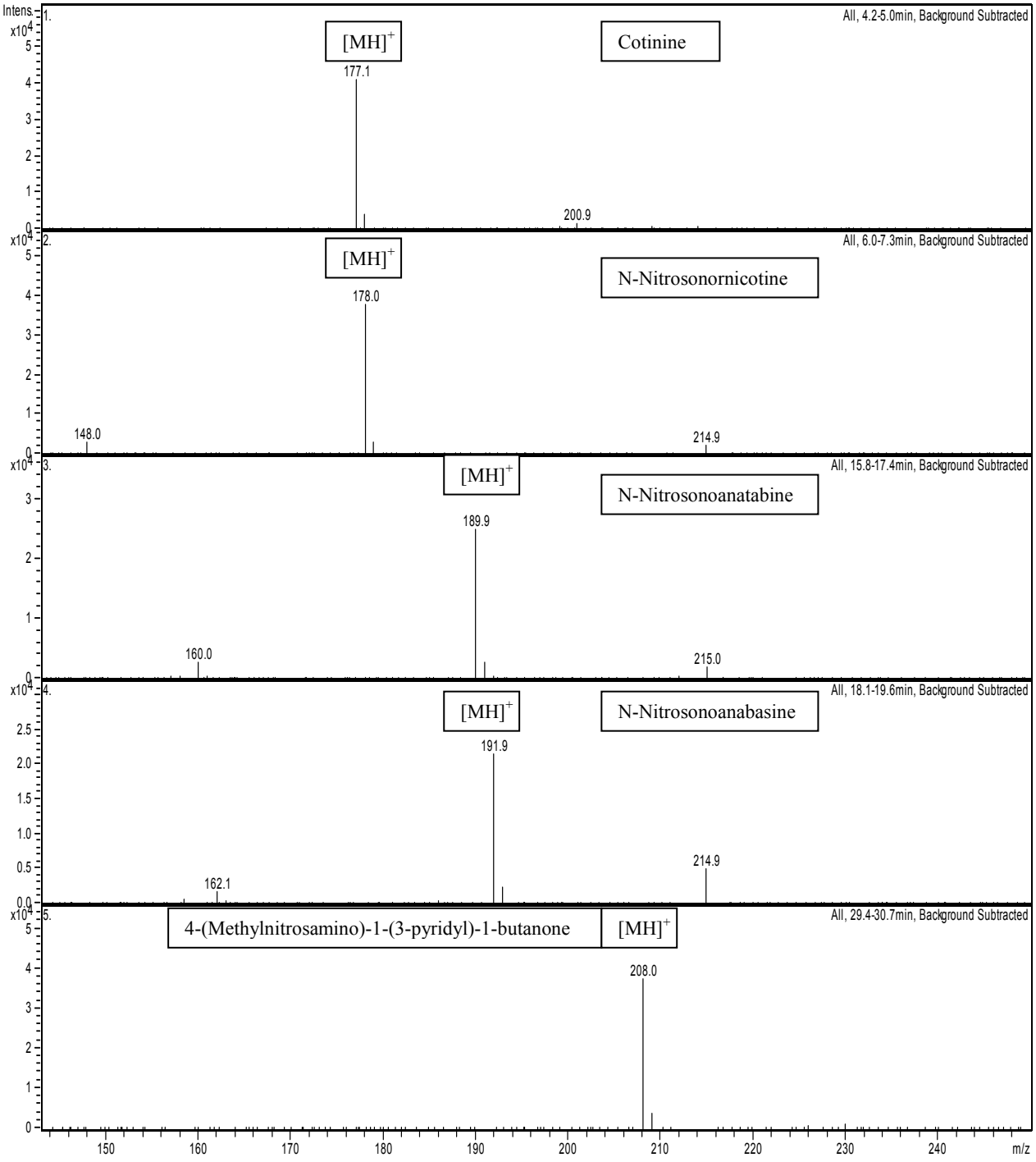


Fig. 2: The representative HPLC-MS (Extracted ion) chromatograms of 0.01mg/ml of nitrosamines and ISTD.

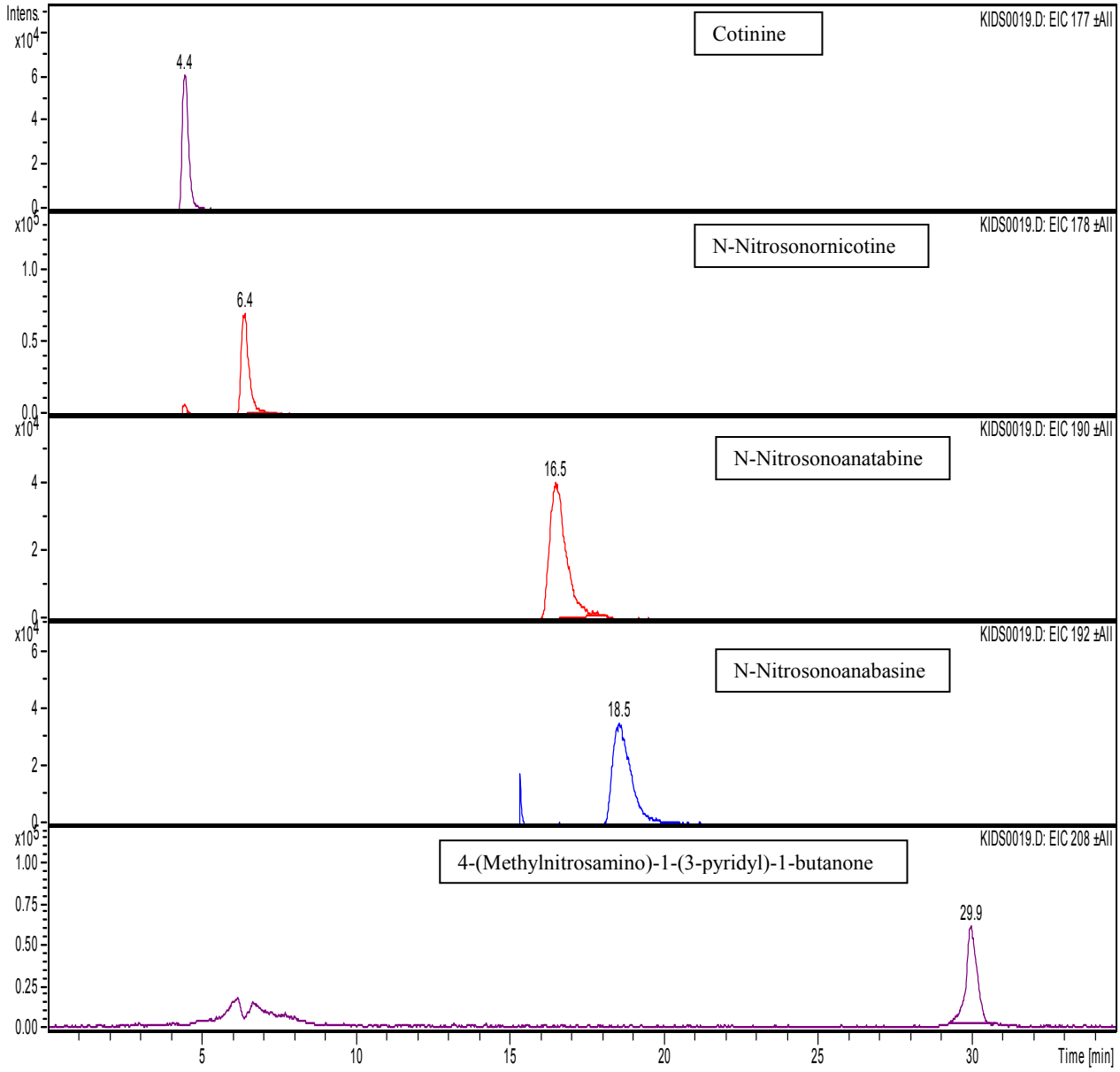


Fig. 3: The representative HPLC-MS (Extracted ion) chromatograms 0.0001mg/ml (LOD) of nitrosamines and ISTD.

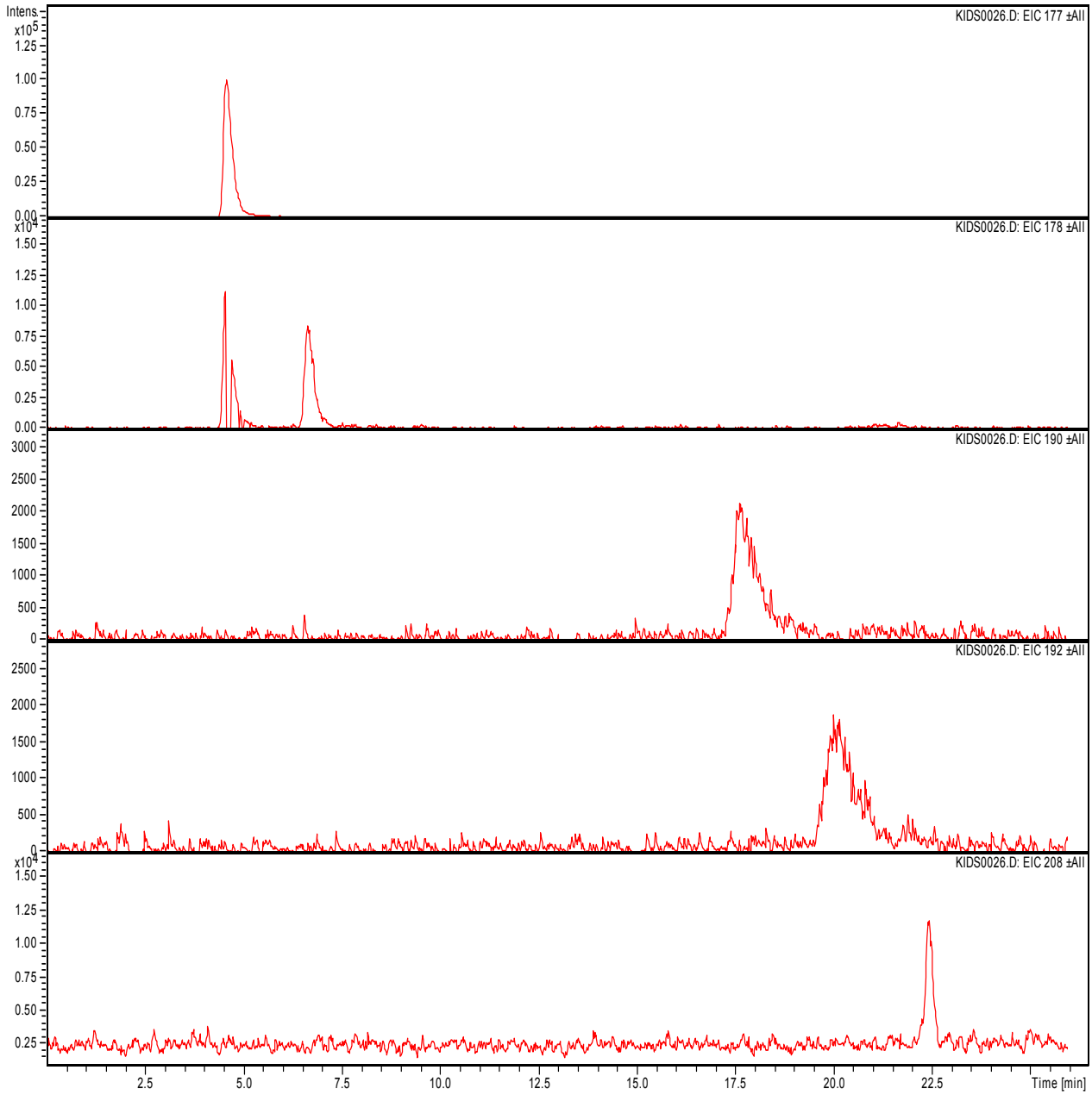


Fig. 4: The representative HPLC-MS (Extracted ion) chromatograms 0.0005mg/ml of nitrosamines and ISTD.

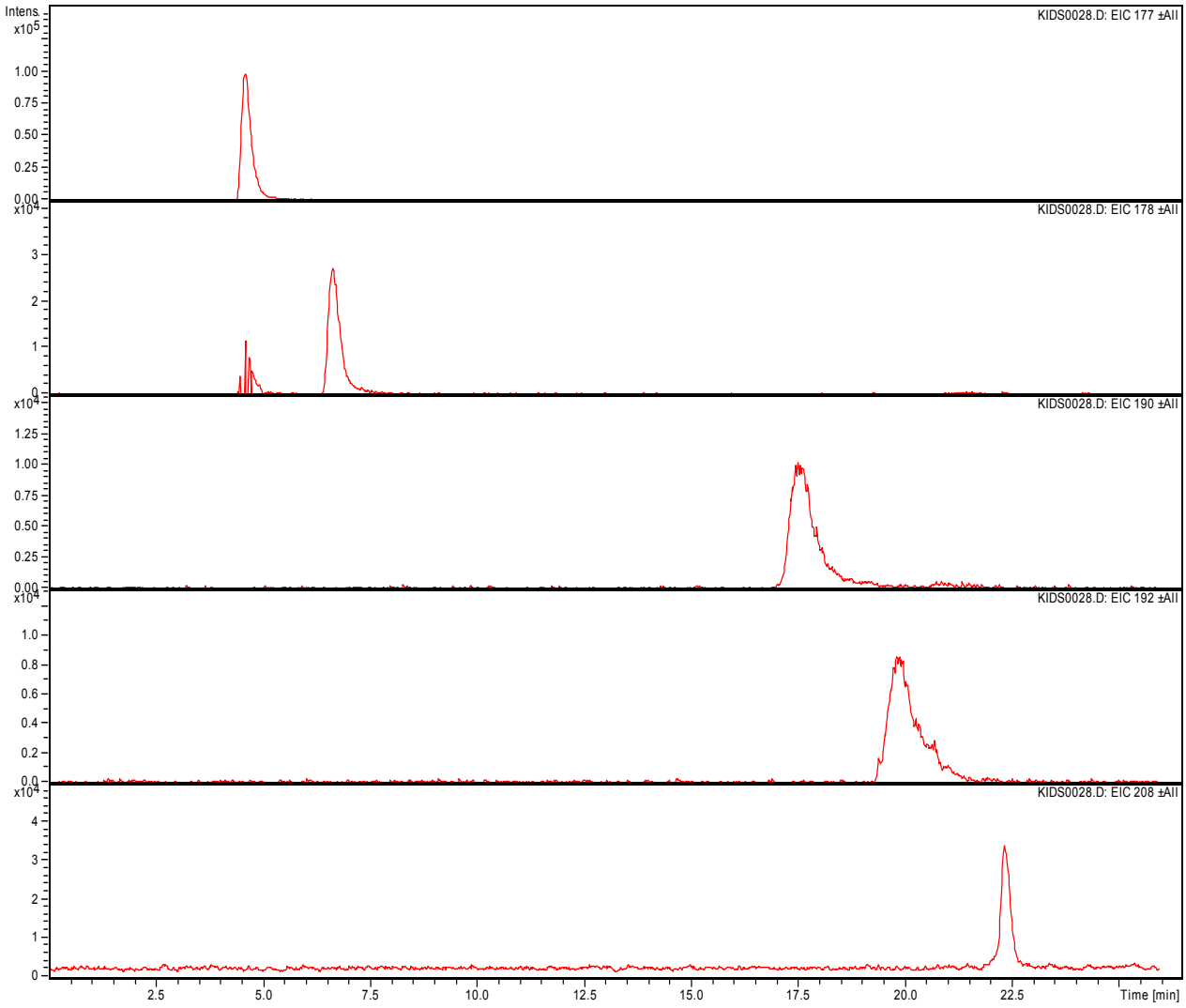


Fig. 5: The representative HPLC-MS (Extracted ion) chromatograms 0.001mg/ml of nitrosamines and ISTD.

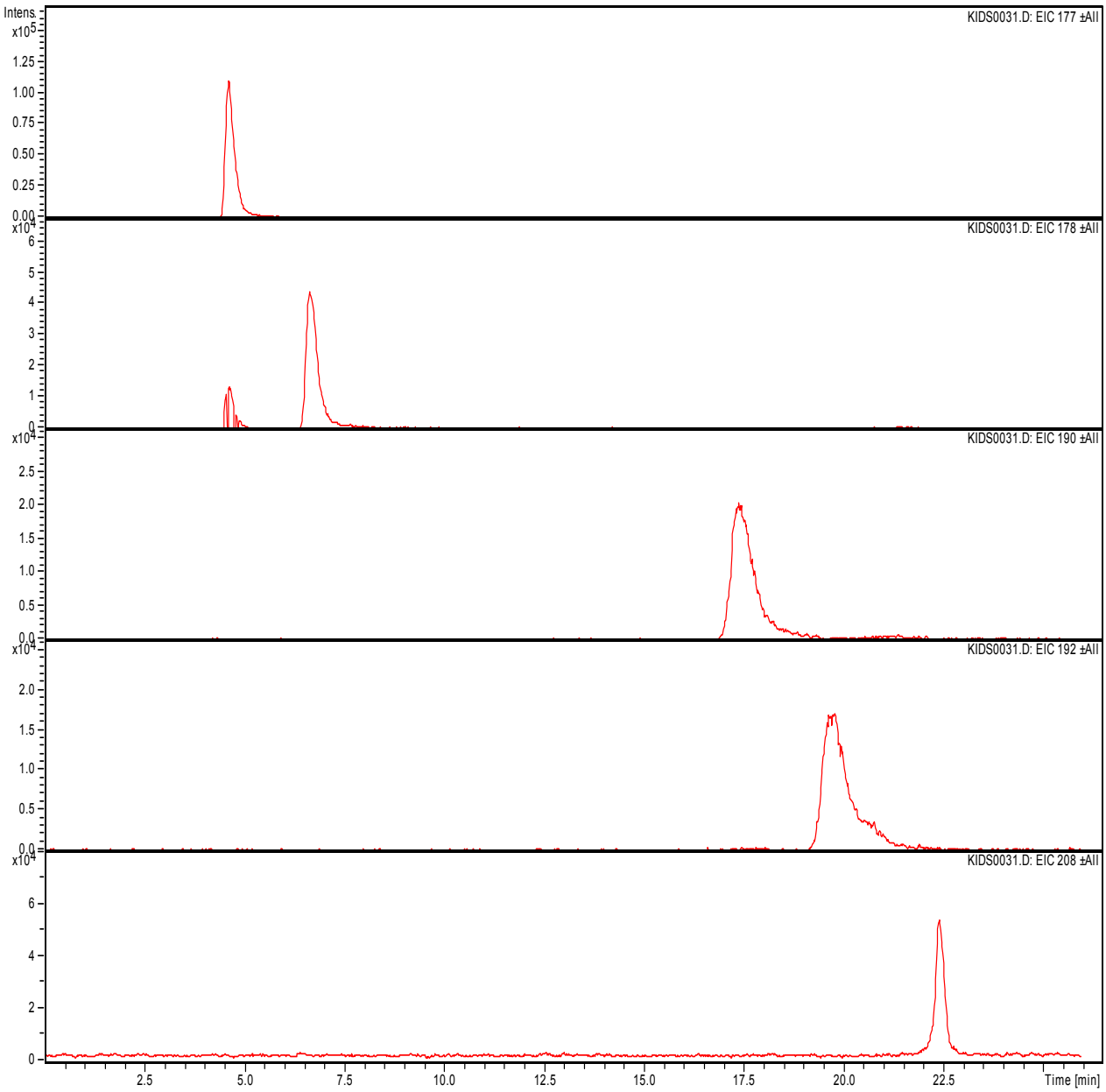


Fig. 6: The representative HPLC-MS (Extracted ion) chromatograms of Blank and ISTD.

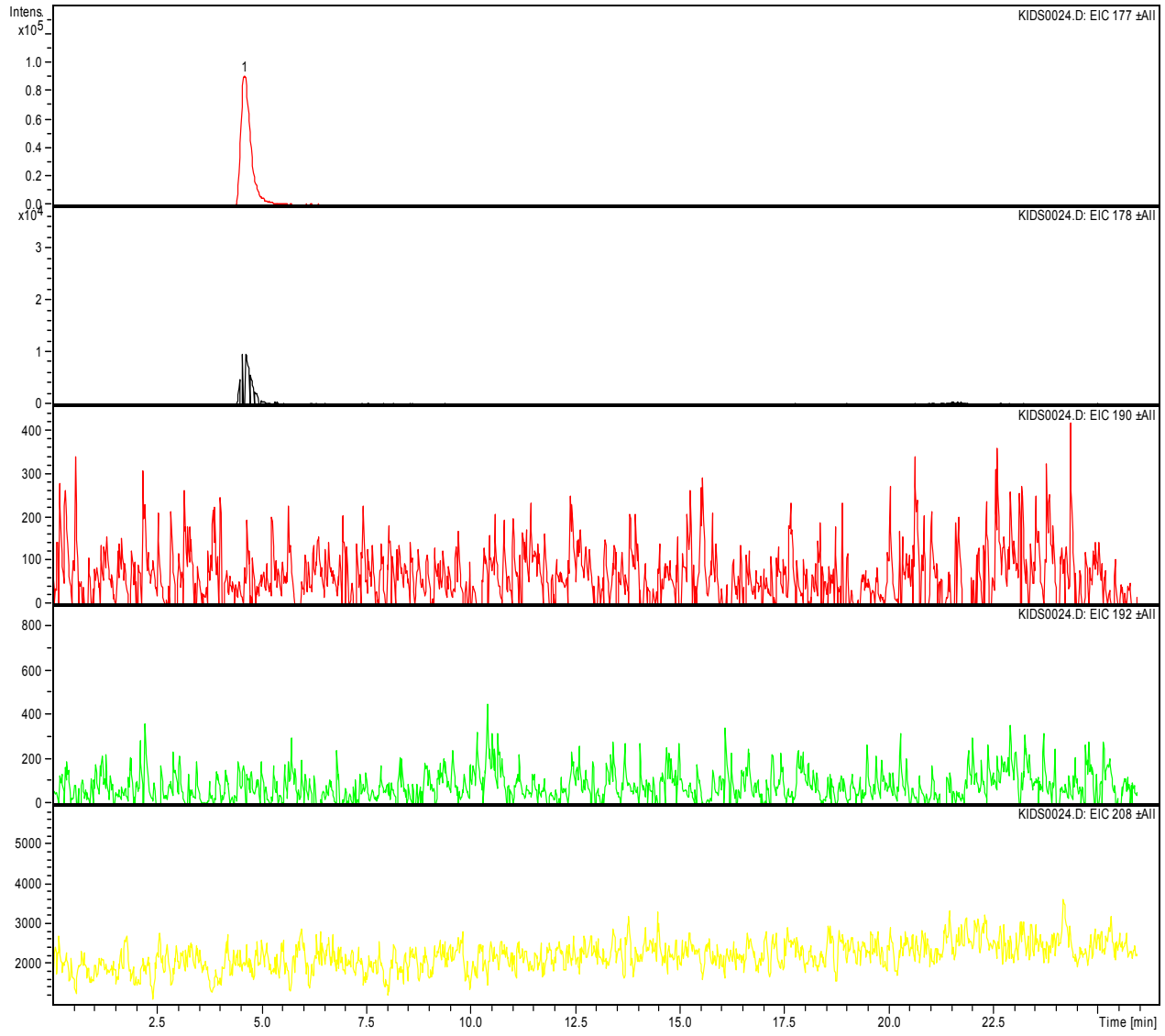


Fig. 7: The representative HPLC-MS (Extracted ion) chromatograms of sample "Atomized cartridge for Ruyan V8"

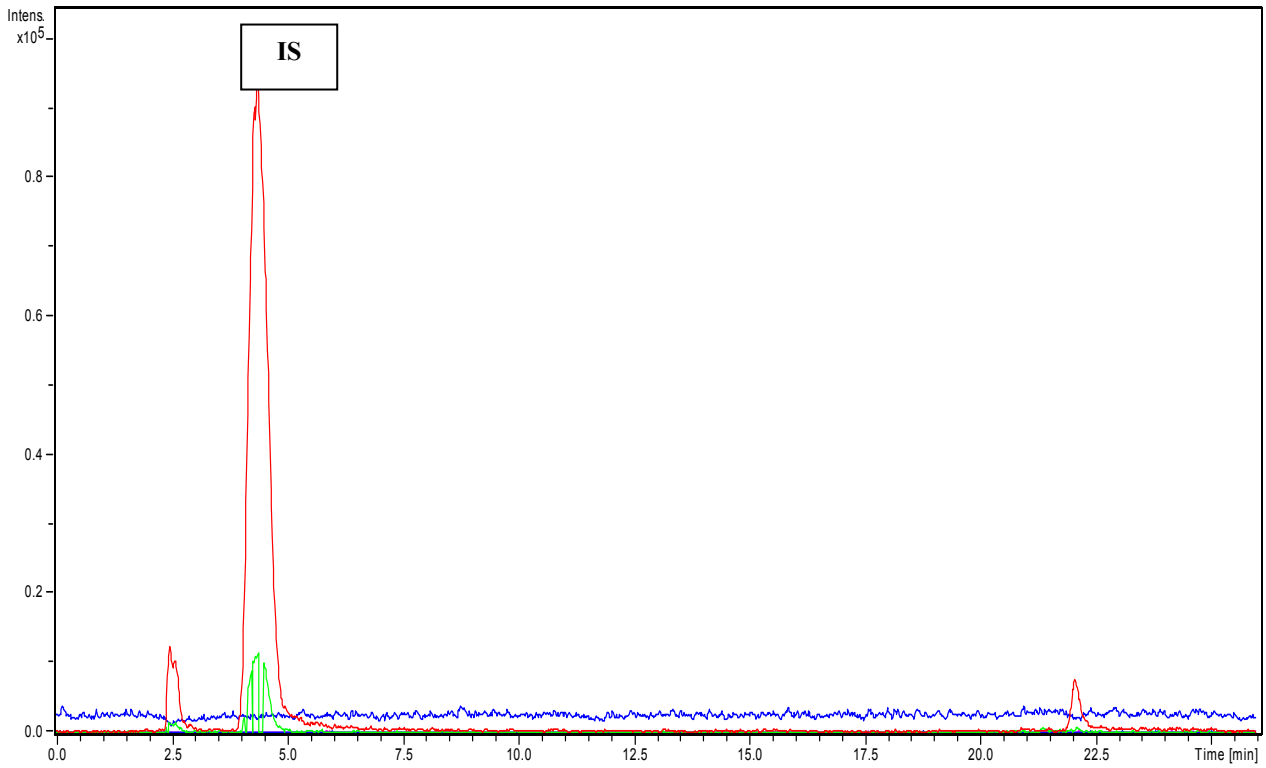


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Fig. 8: The representative HPLC-MS (Extracted ion) chromatograms of sample "Atomized cartridge for Ruyan V8"and sample spiked with 0.0005mg/ml of specific nitrosamines.

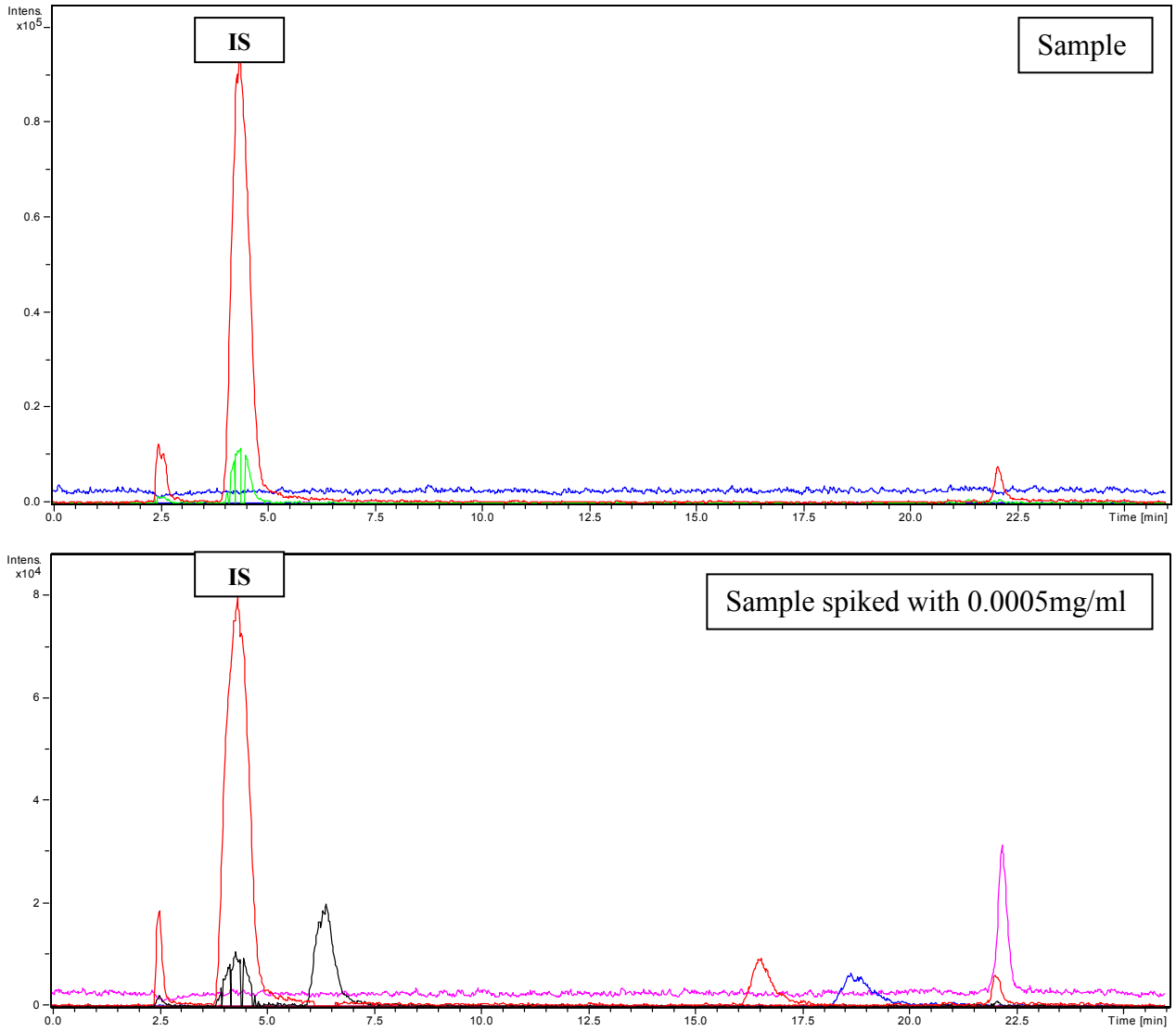


Fig.9: GC-MS SIM/Scan stacked chromatograms of the sample "Atomized cartridge for Ruyan V8", (Rt of diethylene glycol = 13.3 min):

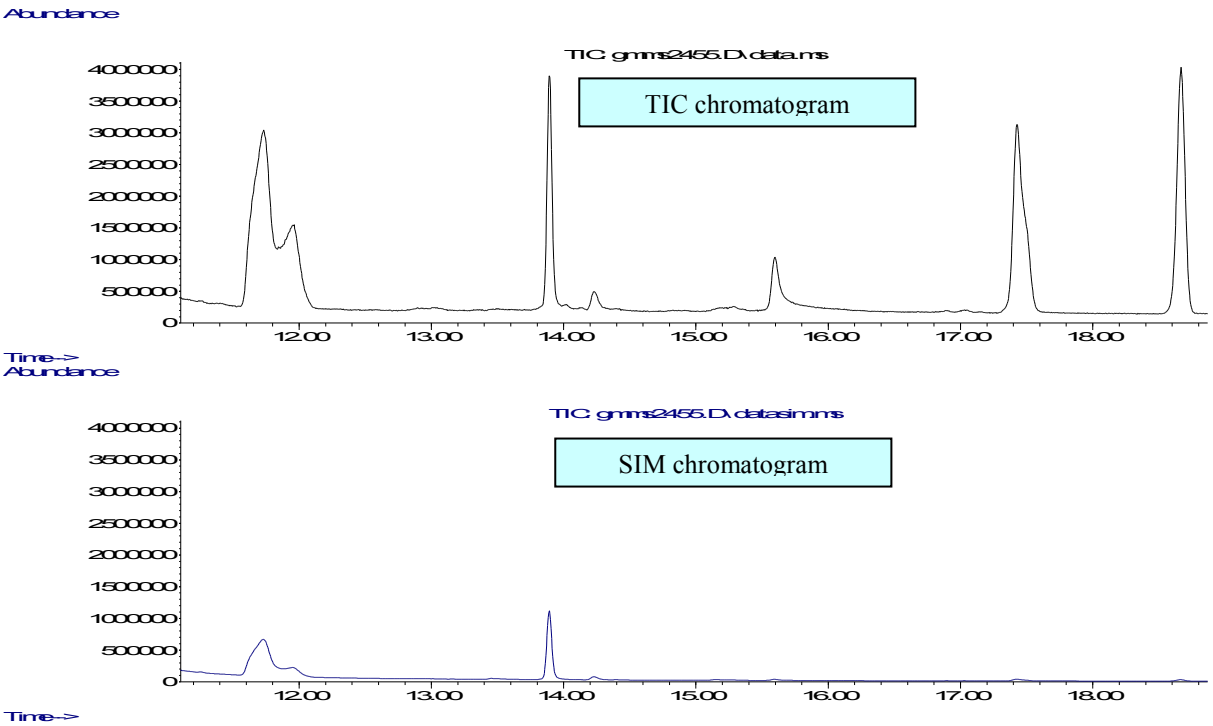


Fig.10: GC-MS SIM overlaid chromatograms of the sample "Atomized cartridge for Ruyan V8" and Diethylene glycol std.(Rt of diethylene glycol = 13.3 min):

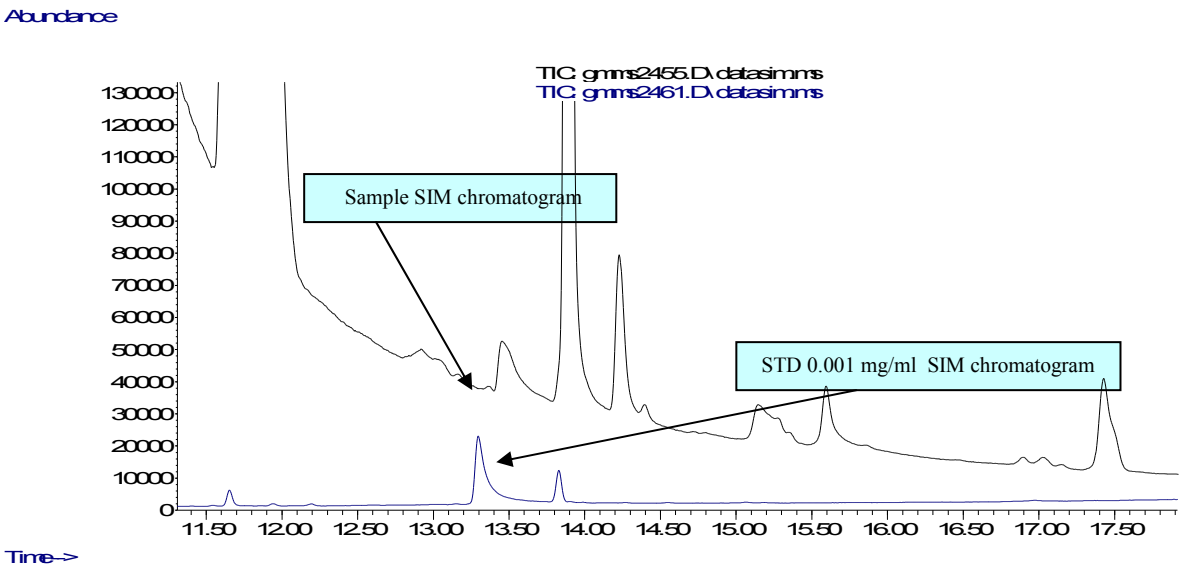


Fig.11: GC-MS Scan chromatograms of the diethylene glycol standard (Rt of diethylene glycol = 13.3 min):

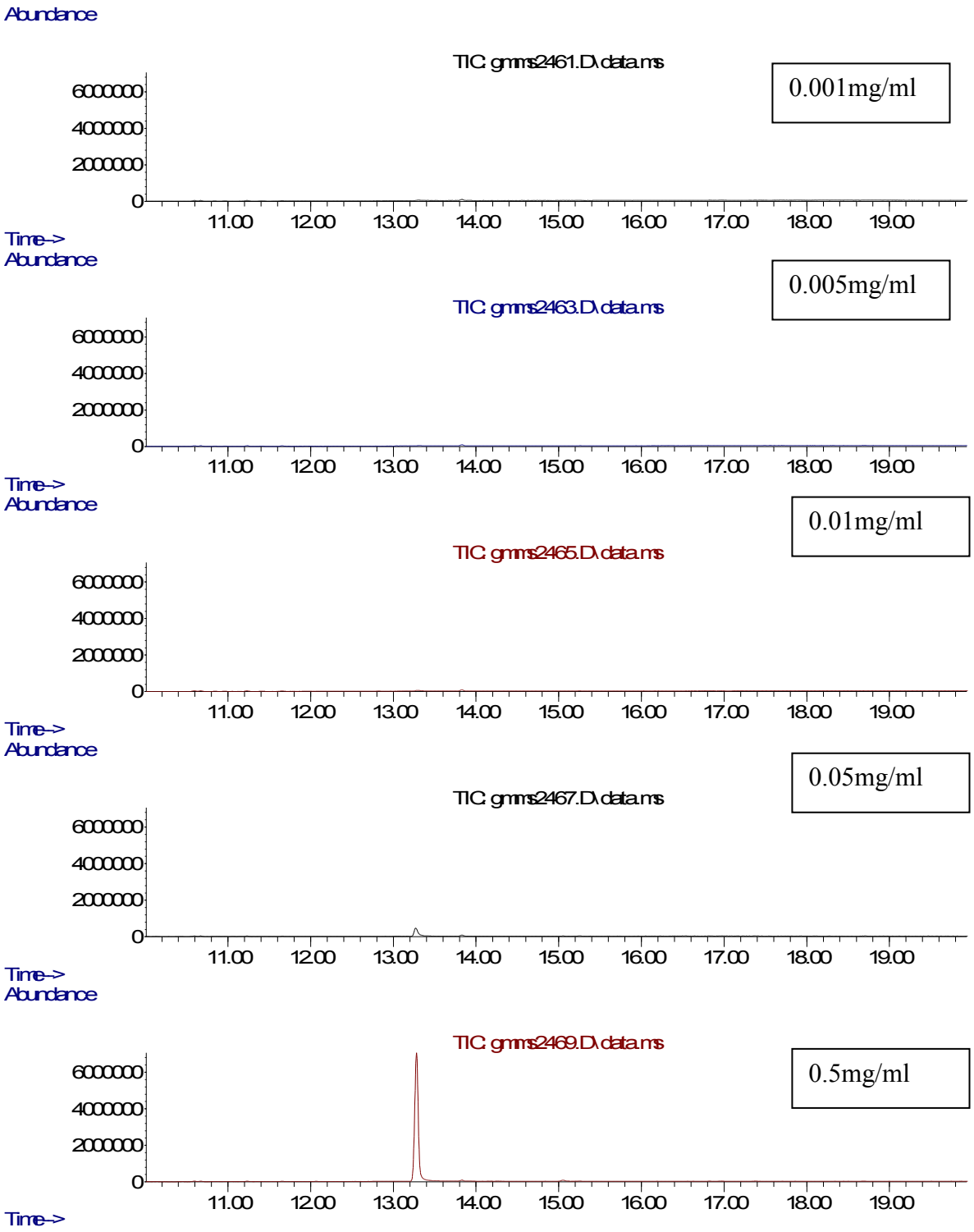
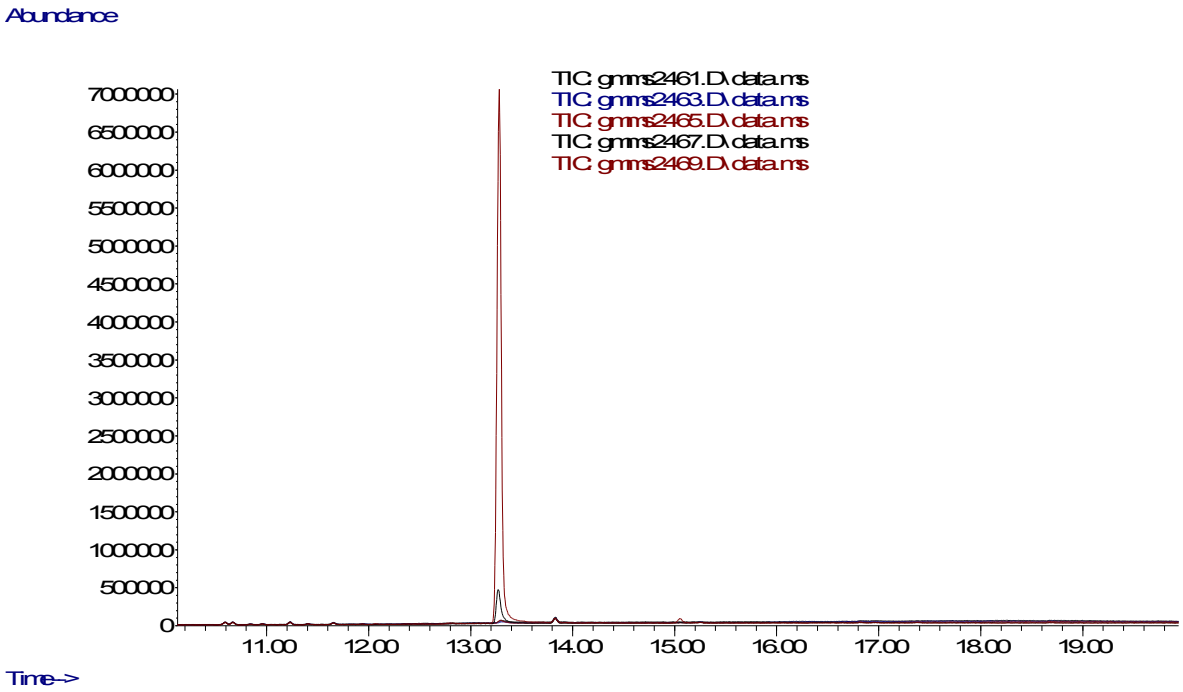


Fig.12a: GC-MS Scan stacked chromatograms of the diethylene glycol standard (Rt of diethylene glycol = 13.3 min):



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Appendix 1

HPLC Running Conditions

Pumping system: Agilent-Technologies model 1200 series

Detector: UV-Vis detector Agilent-Technologies model 1100 series

MS Bruker Esquire LC ESI interface

Column: Kromasil Eternity C18, 250mmx2.1mmx5micron with precolumn

Injection: 25 µl

Mobile phase: [A]H_2O(+0.05%TFA) : [B]Methanol(+0.05%TFA)

0 min	[A]98%	: [B]2% Flow 0.3ml/min
15 min	[A]98%	: [B]2% Flow 0.3ml/min
15.01 min	[A]80%	: [B]20% Flow 0.3ml/min
25 min	[A]80%	: [B]20% Flow 0.3ml/min
25.01 min	[A]98%	: [B]2% Flow 0.3ml/min

Post time: 10 min

Column Temp.: 30^0C

Method: LMS2028.M

HPLC directory: LMS2028\KIDS00….D

MS Running Conditions

Ion source type: ESI Positive mode

Scan: 140-250 m/z

Target: m/z = 180

Stability: 35%

Accumulation time: 50 ms

Average: 10 spectra

Extract mass m/z = 177, 178, 190, 192, 208.

Manager of Advanced analytical instrument department

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11/11/09 14:08

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