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Forensic / GCMS-TQ8040 NX

Screening and Identification of Polydrug Samples via GC-MS/MS and Smart Forensic Database

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❑ Introduction

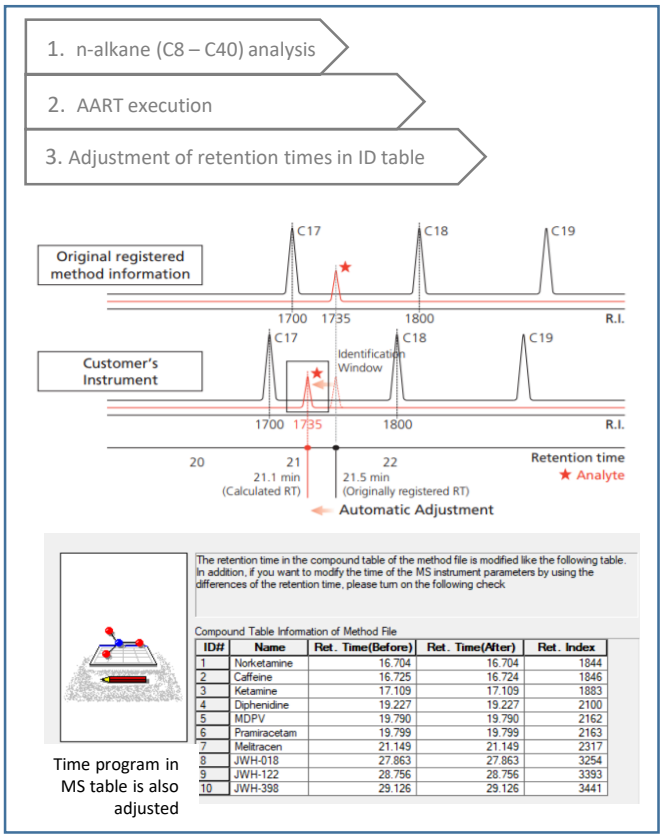
Polydrug abuse is the deliberate mixing of any combination of prescription drugs, over-the-counter drugs or illicit drugs [1]. This practice is sometimes adopted by abusers to heighten or to prolong the 'pleasurable' effects of illicit drugs. Polydrug use is extremely dangerous as the wrong combination can easily lead to overdose or death. For example, mixing heroin (a depressant) and cocaine (a stimulant) or amphetamines results in a high risk of overdose because the cocaine/amphetamines causes the body to use more oxygen while the heroin reduces the breathing rate [2]. For drugs legislation and diagnostic purposes, it is crucial that forensic laboratories can accurately screen for 'typical' illicit drug(s) and any possible minor enhancements in polydrug mixtures. This application note introduces the utilization of GC-MS/MS with simultaneous scan/MRM method created from Shimadzu Smart Forensic Database for fast screening of a narcotics-fortified polydrug sample. Additionally, all scan mass spectra obtained were matched against Shimadzu dedicated forensic mass spectral library.

❑ Analytical Conditions & Workflow

Analyses were carried out using GCMS-TQ8040 NX and SH-Rxi™-5Sil MS column (length 30 m, 0.25 mm I.D., film thickness 0.25 µm; P/N 221-75954-30). GC-MS/MS conditions were set up in accordance to that of *Smart Forensic Database* [3].

Creation of GC-MS/MS method from database

The Smart Forensic Database for GC-MS/MS (Shimadzu) provides all information and parameters for setting up a ready-to-use MRM method on GCMS-TQ8040 NX directly. The current version includes optimised MRM transitions and CE parameters of 486 forensic toxicological substances, which are divided into drugs of abuse, general pharmaceutical drugs, psychotropic drugs and pesticides. Additionally, GC-MS/MS instrument conditions and retention indices of the analytes for the specific column recommended are also provided. Using the retention indices in the database and AART (Automatic Adjustment of Retention Time) function, retention times of the analytes in the database can be predicted (Figure 1).



ID#	Name	Ret. Time(Before)	Ret. Time(After)	Ret. Index
1	Norketamine	16.704	16.704	1844
2	Caffeine	16.725	16.724	1846
3	Ketamine	17.109	17.109	1883
4	Diphenidine	19.227	19.227	2100
5	MDPV	19.790	19.790	2162
6	Pramiracetam	19.799	19.799	2163
7	Melitracen	21.149	21.149	2317
8	JWH-018	27.863	27.863	3254
9	JWH-122	28.756	28.756	3393
10	JWH-398	29.126	29.126	3441

Figure 1. AART function for prediction of RT of targets

Eventually, a MRM method or a scan/MRM method is created from the Smart Forensic Database and downloaded to GCMS-TQ8040 NX. The whole procedure to set up a method for selected targets is accomplished without use of actual standards.

Upon data acquisition, data analysis is performed with pre-set parameters. A dedicated Forensic Mass Spectral Library [4] is used for library search for identification and confirmation. This Shimadzu forensic library consists of mass spectra and information of 2210

forensic toxicological substances, divided into drugs of abuse, general (pharmaceutical) drugs, psychotropic drugs and pesticides.

Screening analysis

During initial screening, a polydrug sample will be analysed by three scan/MRM acquisition methods (I, II and III) created for targeting three classes of drugs, i.e., drugs of abuse, pharmaceutical drugs and psychotropic drugs, respectively (Figure 2). Up to three optimised MRM transitions for each compound are monitored in the initial screening methods.

The criteria of positive detection of a target is defined by retention time, MRM transitions (main and references) and mass spectra match of acquired mass spectra compared to that of standard mass spectra in the forensic mass spectral library.

Confirmation analysis

If the sample screenings show presence of toxicological substances according to the identification criteria, it is re-analysed subsequently with a further modified scan/MRM method for confirmation. This final confirmation analysis method includes only the positively-detected compounds from the initial screening results. A schematic diagram of this workflow is shown in Figure 2.

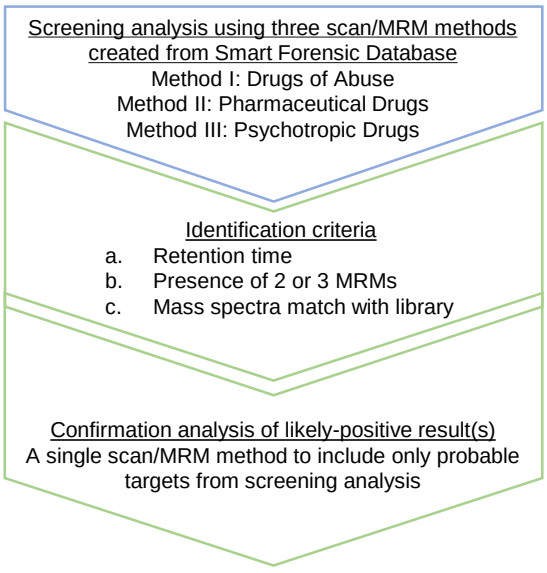


Figure 2. Workflow of polydrug analysis from screening to confirmation using scan/MRM methods created from Smart Forensic Database.

❑ Results and Discussion

The screening and confirmation workflow described above was applied to a narcotics-fortified polydrug sample, prepared intentionally for this study. The polydrug sample was dissolved in methanol to obtain a clear solution for GC-MS/MS analysis. Results of the fortified sample are shown as demonstration of the workflow.

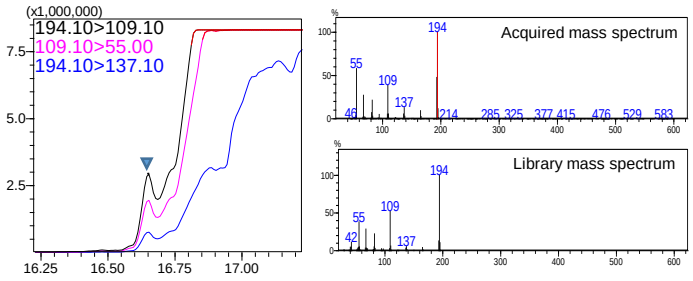


Figure 3(a). Caffeine (SI = 91)

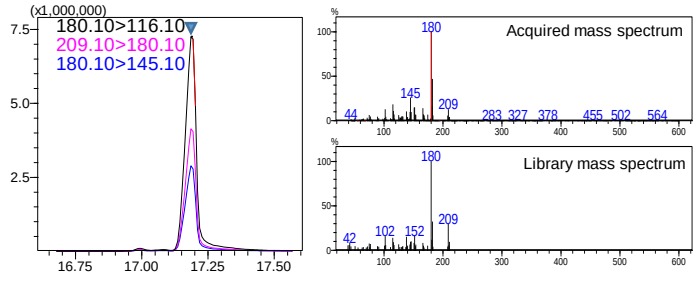


Figure 3(b). Ketamine (SI = 90)

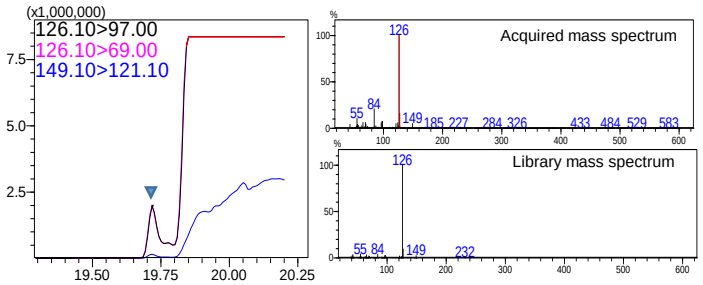


Figure 3(c). MDPV (SI = 87)

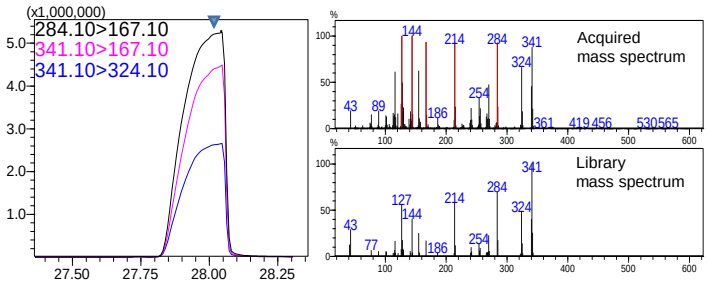


Figure 3(d). JWH-018 (SI = 91)

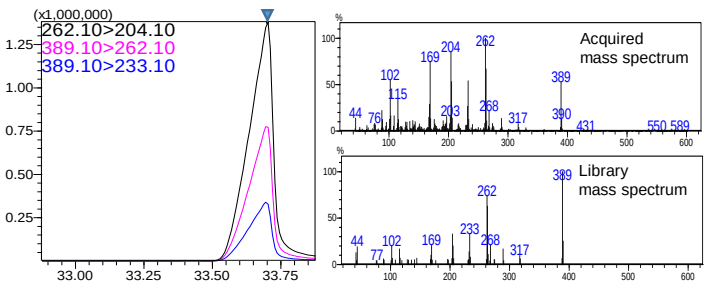


Figure 3(e). Tadalafil (SI = 77)

Figures 3 (a) – (e). MRM mass chromatograms (left) and scan mass spectra (right) of toxicological substances in a polydrug sample, acquired by methods I to III in splitless injection mode. Red line(s) in mass spectrum indicates detector saturation.

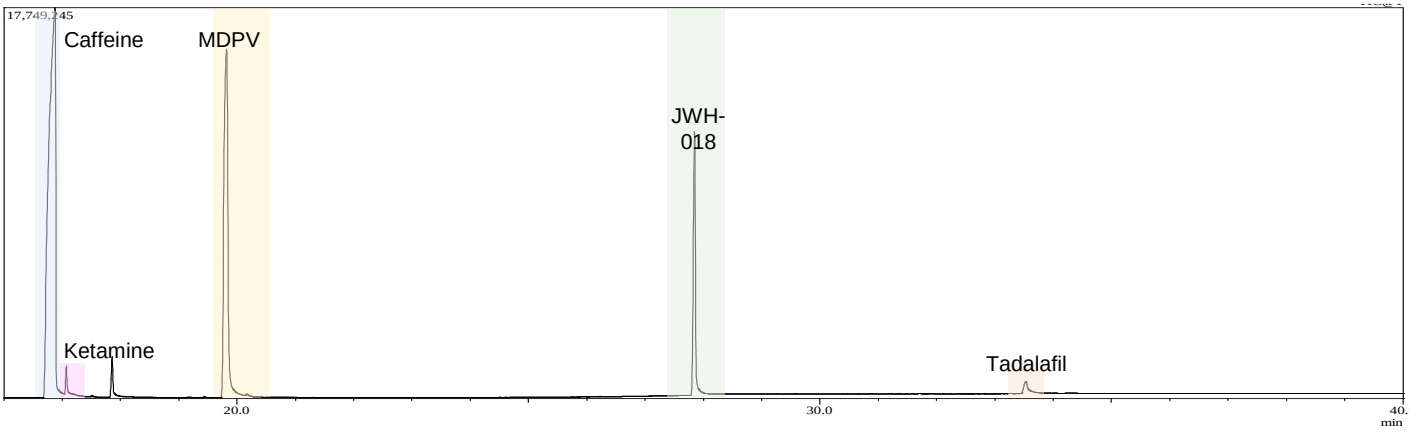


Figure 4. Scan TIC of polydrug sample, acquired from targeted scan/MRM of positives with split ratio of 40

Screening with three methods

Screening results indicated the presence of caffeine, tadalafil, ketamine, methylenedioxypyrovalerone (MDPV) and JWH-018. The presence of each compound was verified by its adjusted retention time, newly predicted using AART. Mass spectra of detected substances displayed similarity index (SI) of at least 75 when compared to the standard spectra in the forensic mass spectral library. Additionally, MRM transitions that belonged to each corresponding toxicological substance were present (Figures 3a – e).

Confirmation with one method

Following the initial screenings, a targeted scan/MRM method which consists up to six MRM transitions of the afore-mentioned positives was created for the polydrug sample.

The scan TIC profile of positive detections acquired from the targeted scan/MRM method is shown in Figure 4, which confirmed the presence of all the five substances in the polydrug sample. It is noteworthy that the general screening data (Figures 3a – e) were acquired from splitless (injection) mode to ensure that toxicological substances of ppb levels will also be captured. However, if detector saturation is observed, as indicated by red-coloured mass spectra (e.g. Figure 3c), a split ratio can be applied for the targeted scan/MRM method. In this way, 'cleaner' TIC profiles can be obtained and a library match of TIC with that of standard mass spectra will yield a higher SI.

As illustrated in this experiment, the SI of MDPV improved significantly from 87 to 98, from the screening method of drugs of abuse to the targeted scan/MRM method (Figure 3c and Figure 5). The former was acquired in splitless mode, which resulted in mass spectrum saturation due to high concentration of MDPV. Hence, the targeted scan/MRM analysis was acquired with a split ratio of 40.

The TIC of caffeine also displayed a better SI of 93, as compared to the previous value of 91 (Figure 3a and Figure 6).

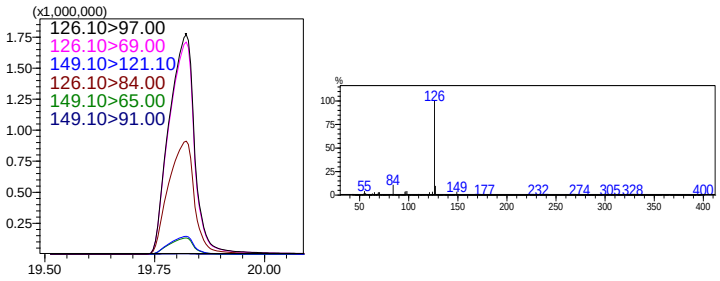


Figure 5. MRM and scan mass spectrum of MDPV (SI = 98), in confirmation analysis of polydrug sample.

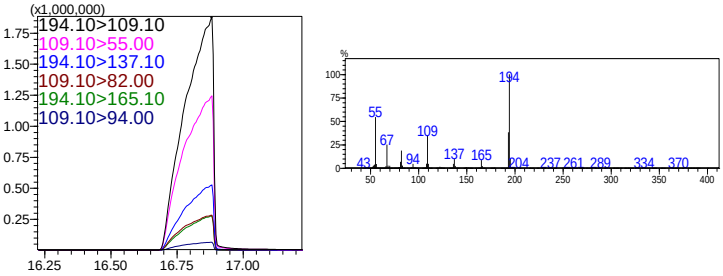


Figure 6. MRM and scan mass spectrum of caffeine (SI = 93), in confirmation analysis of polydrug sample.

Conclusions

Analysis of a narcotics-fortified polydrug sample was successfully carried out using GC-MS/MS running in simultaneous scan/MRM acquisition mode. Identification of toxicological substances in the polydrug sample was made easier and faster with the combined usage of a ready-to-use MRM database and a mass spectral library. The Smart Forensic Database allowed targeted screening of 486 forensic toxicological substances via optimised MRM transitions and collision energies. Furthermore, the scan TIC profile of the polydrug mixture was searched extensively against the forensic library, which consists mass spectral information of 2210 forensic toxicological substances.

❑ Acknowledgements

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4. Shimadzu Dedicated Forensic Mass Spectral Library, P/N: 225-30514-91

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