Global Uniform Analysis and Reporting of Drug-Related Substances (GUARDS) Method

Separation of Controlled and Non-controlled Substances by Gas Chromatography/Mass Spectrometry

Scope: General Purpose

Sample preparation:

Samples can be prepared in a variety of organic solvents such as acetone, acetonitrile, chloroform, ethanol, ethyl acetate, hexane, methanol, methylene chloride, ammoniacal chloroform, and ammoniacal hexane. If needed, compounds such as dimethyl phthalate or tetracosane can be used as internal standards.

Samples containing phenethylamine salts may require low concentrations or base extractions in order to produce acceptable chromatographic peak shapes. GUARDS is suitable for separation of methamphetamine enantiomers following derivatization by MTPA-Cl.

Analysts should be cautious of excessive use of chlorinated solvents, as they may affect the long-term performance of the instrument and mass spectral patterns for some compounds.

Method Parameters:

Method Name: **GUARDS** (*a.k.a. GCGEN within DEA laboratories)*
Instrument: Agilent 7890B/5977A and Agilent 8890/5977B
Column Type and Dimensions: DB-35MS Ultra-inert (Agilent Part No. 121-3822UI); 20 m x 0.18 mm i.d. x 0.18 µm film thickness [(35%-phenyl)-methyl polysiloxane] (Note: USP phase G42 columns are considered equivalent)
Inlet Temperature: 250 ºC
Injection Mode: Split; 50:1)
Injection Volume:1 µL
Carrier Gas and Flow: Hydrogen, 0.8 mL/min (11.0 min hold), ramp to 1.2 mL/min at 1.0 mL/min/min (hold for remainder of runtime)
Control Mode: Variable flow
Oven Program: 105 ºC (no hold), ramp to 130 ºC at 10 ºC/min (no hold), ramp to 175 ºC at 40 ºC/min (no hold), ramp to 245 ºC at 30 ºC/min (1.5 min hold), ramp to 280 ºC at 30 ºC (2.5 min hold), ramp to 300 ºC at 30 ºC/min (no hold), ramp to 340 ºC at 20 ºC/min (1.5 min hold)
Minimum Run Time: 15.29 minutes
Detector: Mass spectrometry

Limitations:

The base GUARDS method will not detect compounds eluting after 15.2 minutes, such as sildenafil and related compounds, as well as some N-Pyrollidino and N-Piperidinyl-nitazene compounds. Samples suspected of containing these compounds should be analyzed using extended final hold times.

Noscapine shows sign of breakdown due to a combination of its late elution time and the high oven temperature. Phencyclidine and its thiophene analog, tenocyclidine, as well as xylazine and procaine, co-elute using the GUARDS method. Additional co-eluting compounds are 9(S)-$\Delta^{6a,10a}$-THC and Δ^{8}-THC, hence, a targeted cannabinoid-specific method is recommended for these compounds.

Acceptance Criteria:

Selectivity*:* All compounds evaluated were observed as single, clear and non-splitting apex peaks. Peak fronting/tailing, if observed, did not preclude the detection of closely eluting peaks. A minimum S/N =3 (peak-to-peak) was observed, even at low concentration levels (0.5% w/w). Earliest eluting compound met the minimum retention criteria of k = 1.

Figures 1 and **2** show examples of total-ion chromatograms obtained for a general-purpose suitability mixture and for a solution containing six halogenated positional isomers of fentanyl, respectively. **Table 1** shows retention times measured for the 15 compounds most frequently identified throughout DEA laboratories during the first three quarters of FY 2024.

Repeatability*:* Multiple test mixtures containing early and late-eluting compounds, as well as selected low-resolution pairs, were evaluated under repeatability conditions. Retention times (RT) measured across 60 consecutive injections (including 30 alternating negative controls) showed excellent repeatability with RSD values below 0.5% and no carryover. Additionally, RT differences between the first and each subsequent injection were less than 0.05 minutes.

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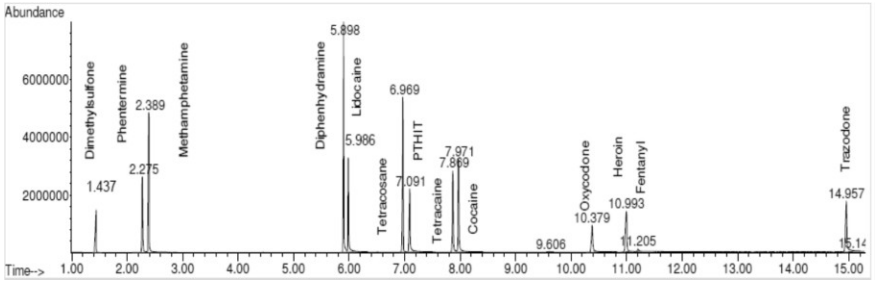

Figure 1: A total-ion chromatogram of the general-purpose suitability mixture used during validation. PTHIT = phenyltetrahydroimidazo-thiazole.

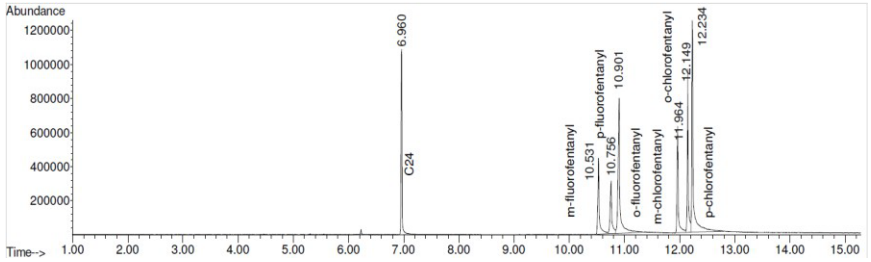

Figure 2: Total- ion chromatogram of halogenated fentanyl positional isomers and internal standard C24.

Reproducibility*:* Within-instrument long-term precision experiments conducted over five weeks also demonstrated low RT variability (RSD < 1.2%); and RT values measured during Weeks 2-6 were within 0.05 minutes of those measured on Week 1.

Table 2 summarizes GUARDS data from 29 instruments (across 10 laboratories) for four compounds with RT spanning across the method's analysis time window (15 minutes). Results demonstrate excellent between-instrument reproducibility for both RT (RSD < 5%) and relative RT (RRT) (RSD < 3%), confirming the method's reliability across instruments.

Primary Drug	RT (min.)
Amphetamine	2.090
Methamphetamine	2.389
3,4-MDMA	4.566
N,N-Dimethylpentylone	5.958
Ketamine	6.176
Cocaine	7.971
Δ^9 THC	9.036
Hydrocodone	9.772
Oxycodone	10.379
para-Fluorofentanyl	10.743
Heroin	10.993
Fentanyl	11.205
Alprazolam	13.176
Bromazolam	13.862
Protonitazene	14.617

Table 1: Top-15 compounds most frequently identified throughout DEA laboratories during the first three quarters of FY 2024

Compound	Avg. RT (min.)	% RSD	Avg. RRT	% RSD
Dimethyl Sulfone	1.43	4.14	0.21	2.87
Cocaine	7.94	1.34	1.14	0.13
Fentanyl (0.5%)	11.14	1.60	1.61	0.31
Trazodone	14.92	1.01	1.88	0.38

Table 2: Summary of GUARDS chromatographic results collected for four representative compounds across 29 instruments and 10 laboratories.

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