

Accurate, Reproducible Amphetamines Analysis

Clean Up Procedure Improves Chromatography and Reduces Maintenance

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- Derivatization improves peak symmetry, for more accurate results.
- Clean up procedure reduces system contamination, and extends column lifetime.
- Rtx®-5MS column produces a stable baseline for derivatized compounds, ideal for GC/MS analysis.

Introduction

Analyzing amphetamines by GC/MS is challenging whether the compounds are derivatized or underivatized. Underivatized amphetamines appear as irregular and asymmetric peaks, which are difficult to integrate, and may lead to irreproducible results. Derivatized amphetamines result in symmetric peaks, but derivatizing reagents can contaminate the inlet/column. This contamination can shorten column lifetime and cause noisy, elevated baselines that interfere with the analysis of target compounds.

In this study, we evaluated the effects of several sample pretreatment methods. These methods included: 1) no pretreatment, 2) converting the salt forms into free bases, 3) derivatizing the free bases with heptafluorobutyric acid anhydride (HFAA), and 4) derivatizing the free bases with HFAA followed by a clean up. Our objectives were to obtain symmetric shapes, reduce baseline noise, and maintain low column bleed from injection to injection for GC/MS analysis.

Procedure

The first method had no pretreatment. The untreated standard was prepared in methanol and diluted to a final concentration of 100µg/mL. It was then injected without any further preparation. The second pretreatment involved converting the drug standard to the free base form. The free base forms were prepared by mixing the standard (100µg/mL) with water, then adding saturated sodium borate water, and extracting the amphetamines with butylchloride. The resulting sample was then analyzed by GC.

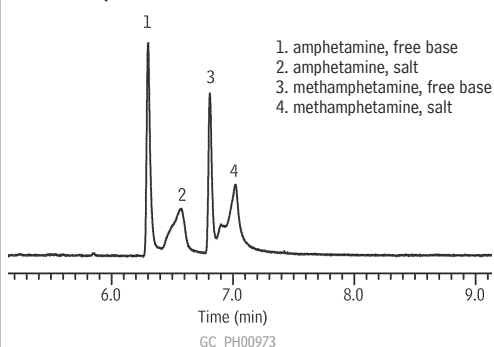
The third pretreatment procedure included both conversion and derivatization. The HFAA derivatized amphetamines were prepared by converting the compounds to free bases (as described above), reacting with derivatizing reagent HFAA, and diluting the sample before injection. The fourth pretreatment procedure consisted of free base conversion, HFAA derivatization, and a clean up step to remove the acidic byproducts of derivatization. The clean up procedure included mixing the sample with a phosphate buffer (pH=7.0) before dilution, removing the butylchloride layer, and then diluting the sample just before injection. An Rtx®-5MS column (30m x 0.25mm ID x 0.25µm) was used for analysis; instrument conditions are presented in Figure 1. Repetitive GC/MS runs (over 190 injections) were evaluated to confirm symmetry, baseline, and bleed results.

Results

Analyzing untreated amphetamine and methamphetamine results in peak doublets caused by the presence of both the salt (hydrochloride) and free base forms (Figure 1). Peak doublets were eliminated by conversion to free base form, however, some tailing was still observed (Figure 2). This pretreatment improves reproducibility, but is still not optimal as tailing can cause irreproducible integration and significant variation in peak area counts.

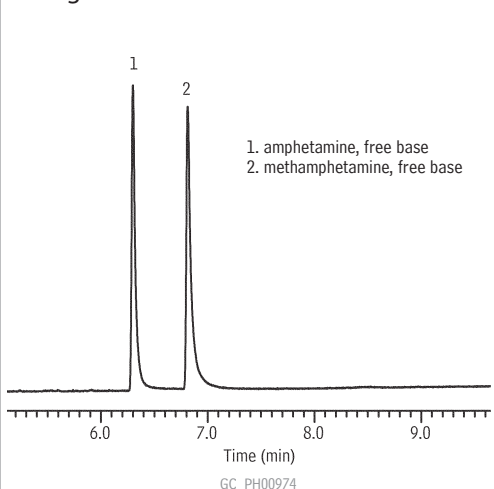
The most symmetric peak shapes were obtained by derivatizing the amphetamines with HFAA (Figure 3). Although peak shape was improved, the acidic derivatization byproducts generated a noisy baseline and shortened column life. This system contamination increases injector and column maintenance.

Figure 1 Untreated standard contains both salt and free base forms causing inaccurate, irreproducible results.



Column: Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample: 100µg/mL amphetamine and methamphetamine in methanol
Inj.: 1µL, split (split ratio 10:1), 4mm single gooseneck w/ wool inlet liner (cat.# 20798-211.1)
Inj. temp.: 250°C
Carrier gas: hydrogen, constant flow
Flow rate: 1mL/min.
Oven temp.: 70°C to 250°C @ 15°C/min. (hold 5 min.)
Det: FID @ 300°C

Figure 2 Conversion to free base form improves chromatography, but produces tailing factors over 2.0.



See Figure 1 for conditions.

Figure 3 Derivatizing with HFAA yields symmetric peaks but results in system contamination and a noisy baseline.

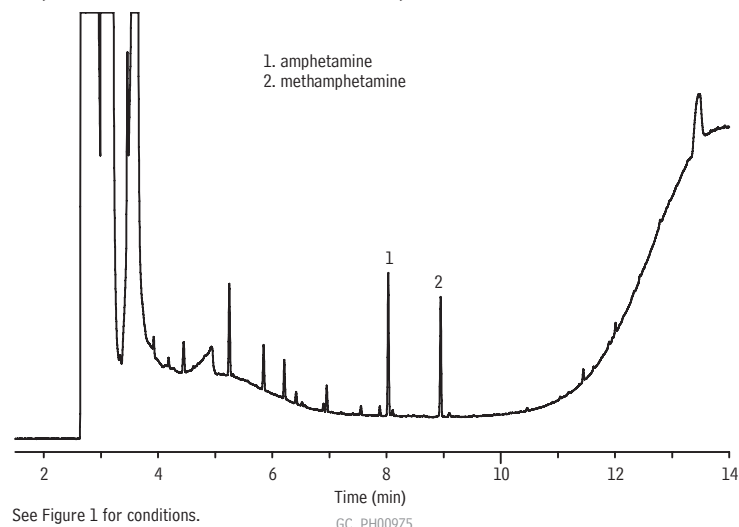


Figure 4 A post-derivatization clean up procedure results in symmetric peaks and a clean baseline.

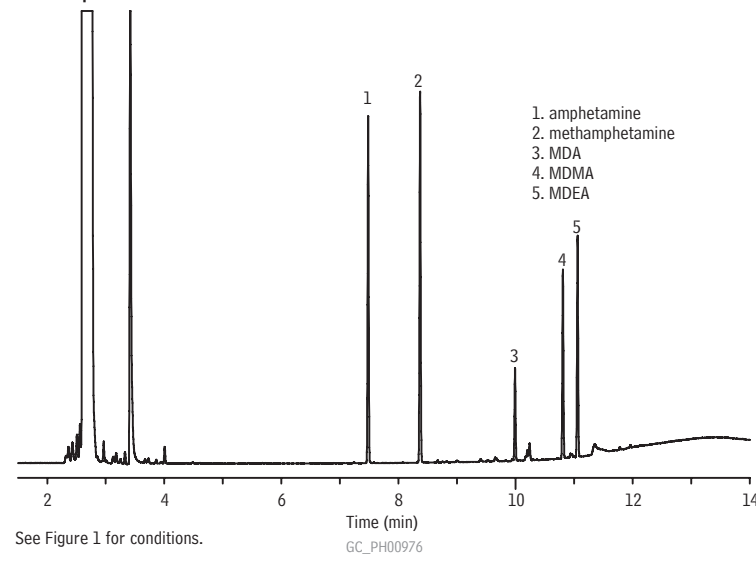
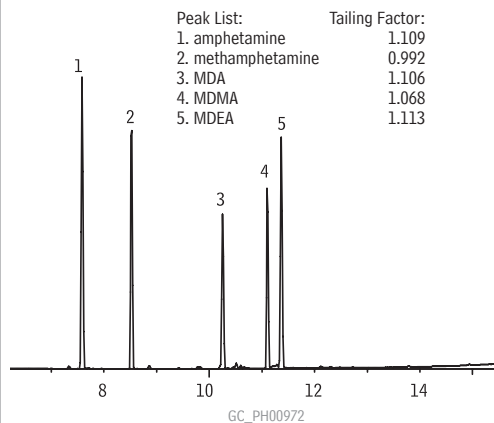


Figure 5 Post-derivatization clean up also produces symmetric peaks and a stable baseline when analyzed by GC/MS.



Column: Rtx®-5MS, 30m, 0.25mm ID, 0.25µm (cat.# 12623)
Sample: 100µg/mL each amphetamine, methamphetamine, MDA, MDMA, and MDEA extracted from methanol and HFAA derivatized
Inj.: 1µL, splitless (hold 0.5 min.), 3.5mm custom splitless inlet liner w/IP deactivated wool; Inj. temp.: 220°C; Carrier gas: helium, constant flow; Flow rate: 1.25mL/min.; Oven temp.: 70°C (hold 1 min.) to 290°C @ 15°C/min. (hold 4 min.); Det: MS; Transfer line temp.: 280°C; Scan range: 43-450amu; Ionization: EI; Mode: scan.

Incorporating a post conversion/derivatization clean-up procedure removed derivatization contaminants while maintaining chromatographic quality (Table I), thus reducing the need for frequent system maintenance and extending column lifetime. These benefits were also seen when samples were analyzed by GC/MS (Figure 5).

Conclusion

The conversion/derivatization/clean-up procedure presented here produces symmetric peaks while reducing the amount of contamination that can enter the GC system. This method ensures accurate area count reproducibility, a clean GC system, and a stable baseline, even for GC/MS work.

Table I Tailing factor comparison of pretreatments.

Pretreatment	TF Amp	TF Meth	TF MDA	TF MDMA	TF MDEA
Sodium Borate Wash (GC/FID)	2.115	2.837	NA	NA	NA
HFAA Only (GC/FID)	1.010	0.989	NA	NA	NA
HFAA w/Post clean Up (GC/FID)	0.981	0.996	1.007	0.997	0.992

NOTE: A perfectly symmetric peak exhibits a tailing factor of 1.0. Tailing factors shown were generated using the USP tailing factor calculation.

Rtx®-5MS—Low-bleed GC/MS Column (fused silica)

(Crossbond® 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	12623

Acylation Derivatization Reagents

Compound	CAS#	cat.#
HFAA (heptafluorobutyric acid anhydride)		
10-pk. (10x1g)	336-59-4	35622
25g Flex Tube	336-59-4	35623

Exempted Drug of Abuse Reference

Materials: Amphetamines & Metabolites

Concentration is µg/mL. Volume is 1mL/ampul.

Compound	CAS#	Solvent		cat.#
		Code	Conc.	
d-amphetamine	51-63-8	PTM	1,000	34020
3,4-MDA HCl	4764-17-4	M	1,000	34070
3,4-MDEA HCl	82801-81-8	M	1,000	34072
3,4-MDMA HCl	42542-10-9	M	1,000	34071

M=methanol

PTM=purge & trap grade methanol