

Why Derivatize?

Improve GC Separations with Derivatization

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- Get better separations with increased resolution and response.
- Learn how to choose proper reagents for desired reactions.

Many laboratories include derivatization as part of their sample preparation for gas chromatography (GC) analysis. So, what is derivatization? Why is it important and how do you choose a derivatizing reagent? The discussion below answers these questions. By choosing the right derivatization reagent and procedure you can increase resolution and analyte response, significantly improving your separations.

What is derivatization?

Derivatization is the process by which a compound is chemically changed, producing a new compound that has properties more amenable to a particular analytical method. Some samples analyzed by GC require derivatization in order to make them suitable for analysis. Compounds that have poor volatility, poor thermal stability, or that can be adsorbed in the injector will exhibit nonreproducible peak areas, heights, and shapes. Other compounds that respond poorly on a specific detector may need to be “tagged” with a different functional group to improve detection. For example, tagging with chlorine can improve response on an ECD (electron capture detector). In addition to improving suitability and response, derivatization can improve resolution between coeluting compounds and overlapping peaks.¹

How do I choose a derivatizing reagent?

A good derivatizing reagent and procedure should produce the desired chemical modification of the compound(s) of interest, and be reproducible, efficient, and nonhazardous.² For GC, there are three basic types of derivatization reactions: silylation, acylation, and alkylation. Silylating reagents react with compounds containing active hydrogens; these reagents are the most common type used in GC. Acylating reagents react with highly polar functional groups such as amino acids or carbohydrates. Alkylating reagents target active hydrogens on amines and acidic hydroxyl groups.³ Multiple derivatizing reagents may be necessary for compounds containing several different functional groups such as androsterone (Figure 1). In these multi-step derivatization procedures the use of other types of reagents, such as oxime, hydrazone, methylation, and cyclic derivatives, may be necessary.

A multi-step example

Derivatization can substantially improve chromatographic results, as seen in this example derivatization of androsterone (Figure 1). Androsterone contains a hydroxyl group and a carbonyl group and exhibits poor peak shape and poor separation if analyzed underivatized by GC (Figure 2b). Using silylation, active hydrogens on OH, SH, and NH groups can be replaced.³ Since *n*-trimethylsilylimidazole (TMSI) is a strong silyl donor, it will react readily with the hydroxyl group on the androsterone molecule creating a trimethylsilyl (TMS) derivative. Because androsterone also contains a carbonyl group, another derivatizing reagent is needed to improve chromatographic peak shape. Methoxyamine will react with the carbonyl group forming an oxime derivative (CH₃ON). Oxime derivatives not only improve chromatographic performance, but also alter GC separations. Figure 2a shows the chromatographic result of derivatizing sex hormones using TMSI and methoxyamine; retention times are increased, separation is increased, and peak shapes and responses are improved.

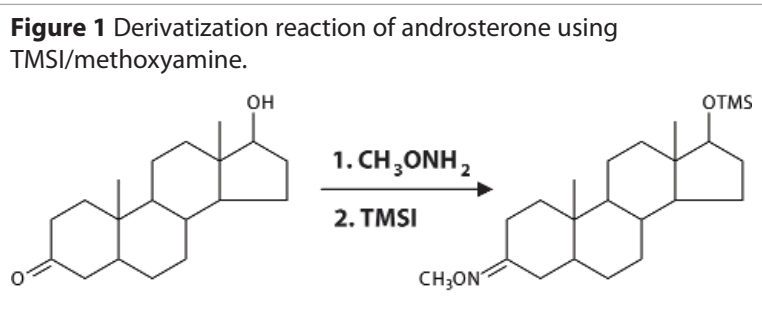


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Conclusion

Derivatizing compounds for GC often is necessary to obtain reproducible chromatographic results. Eliminating this step to save time can be costly and produce inaccurate and unreliable results. A well-chosen derivatization procedure, based on the chemical composition of the target compounds, can significantly improve your chemical separations.

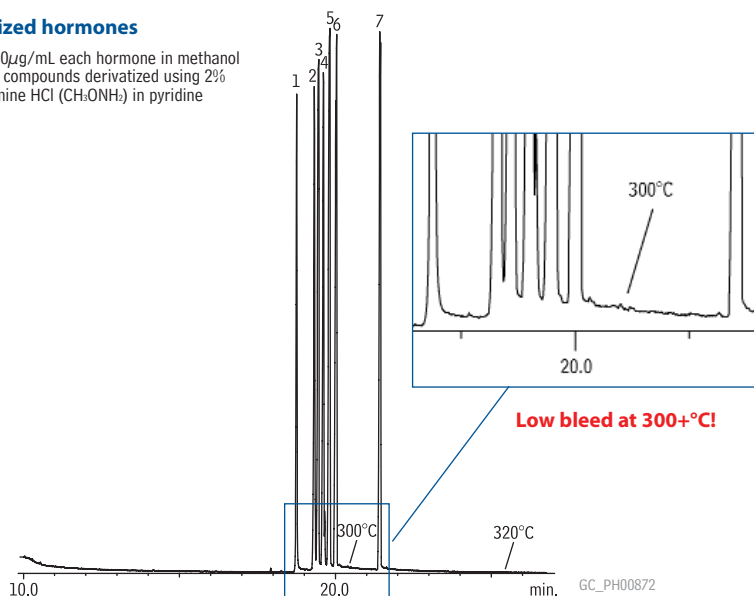
References

- 1 Knapp D., Handbook of Analytical Derivatization Reactions, Wiley-Interscience, 1979, pp.2-24, 449-453, 482.
- 2 www.piercenet.com
- 3 Grob R., Barry E., Modern Practice of Gas Chromatography, Wiley-Interscience, 2004, pp. 817-818.

Figure 2 Derivatized hormones show excellent resolution and more symmetrical peak shapes than underivatized hormones.

A) Derivatized hormones

Sample: 100µg/mL each hormone in methanol or ethanol; compounds derivatized using 2% methoxylamine HCl (CH₃ONH₂) in pyridine

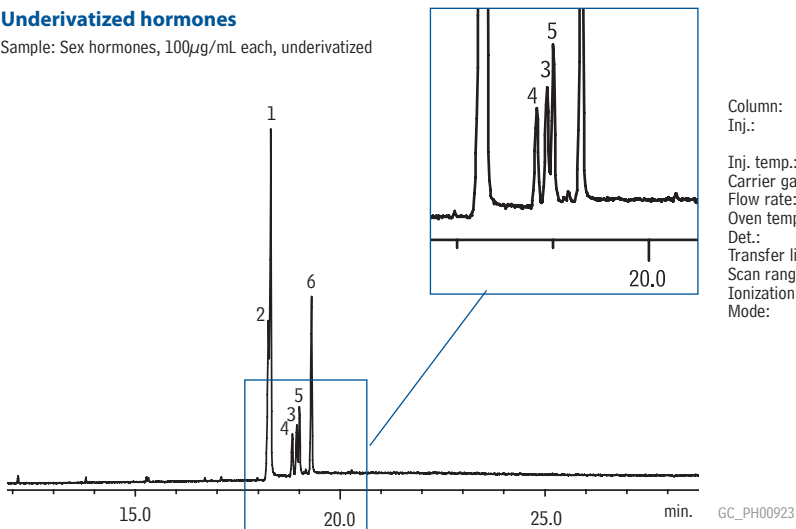


- 1. androsterone
- 2. dehydroepiandrosterone (DHEA)
- 3. 17-α-estradiol
- 4. estrone
- 5. 17-β-estradiol
- 6. testosterone
- 7. derivatization by-product

For the derivatization procedure used in this analysis, see Knapp's *Handbook of Analytical Derivatization Reactions*, page 482.

B) Underivatized hormones

Sample: Sex hormones, 100µg/mL each, underivatized



Column: Rxi™-1ms 30m, 0.25mm ID, 0.25µm (cat. # 13323)
 Inj.: 1.0µL splitless (hold 0.5min.), 3.5mm single gooseneck inlet liner (cat.# 20961)
 Inj. temp.: 250°C
 Carrier gas: helium, constant flow
 Flow rate: 1mL/min.
 Oven temp.: 100°C to 320°C @ 10°C/min. (hold for 10 min.)
 Det.: MS: Shimadzu 17A with QP5000
 Transfer line temp.: 280°C
 Scan range: 40-700amu
 Ionization: EI
 Mode: Scan

Rxi™-1ms Columns (fused silica)

(Crossbond® 100% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #
0.25mm	0.25	-60 to 330/350°C	30-Meter	13323

Splitless Liners for Shimadzu GCs

**Nominal ID at syringe needle expulsion point.

