Clinical Forensic

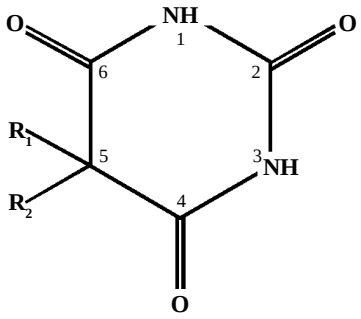
Applications note

cat.# 59575

Barbiturate Analysis

Figure 1

Basic Structure for Barbiturates



Barbiturates are a class of compounds that are central nervous system depressants. They are categorized as sedatives or hypnotics and are primarily used in the treatment of anxiety, insomnia, and convulsive disorders. Physical effects of the barbiturates range from mild sedation to coma. Barbiturates are based on a pyrimidine ring structure. Substitution at the 2, 4, and 6 positions gives the basic structure for the oxybarbiturates (**Figure 1**). Replacement of the oxygen at position 2 with sulfur results in the formation of thiobarbiturates. Barbiturates can be ranked according to their onset of activity, duration of action and degree of hypnotic potency. These pharmacological effects are influenced by the types of functional groups attached at position 5. The inclusion of alkyl or aryl groups, the number of carbons in the alkyl side chains, and the degree of branching will affect activity and toxicity.

Extended administration or abuse of barbiturates can lead to physical and psychological dependence. Tolerance to the effects of barbiturates on the central nervous system can be built up with continued exposure to the drug. While tolerance to the intoxicating effects of barbiturates may increase with use, there is very little increase in tolerance to the toxic side effects of high doses. As a result, the therapeutic index for barbiturates is lower than for other sedative/hypnotic drugs like the benzodiazepines. The barbiturates also have an additive effect when administered with other central nervous system depressants. The combination of the low therapeutic index and the additive effects of other CNS depressants makes monitoring for barbiturates an important aspect of drug overdose screening.

Barbiturates can be analyzed in either their underivatized or derivatized forms by gas chromatography. Derivatization of the barbiturates is most commonly performed by methylation of the amido nitrogens in positions 1 and 3. Methylating reagents like tetramethylammonium hydroxide (TMAH) and trimethylanilinium hydroxide (TMPAH) can be used for on-column derivatization of the barbiturates. While derivatization can improve the peak shape and response, extraneous peak formation can interfere with some analyses. Proper injection port set-up is important in obtaining reproducible results with on-column derivatization. Methylation of barbiturates is catalyzed by the addition of heat to the reaction mixture. After sample injection, the residence time of the barbiturates and the derivatizing reagent inside the injection port is very short. Since contact of the sample with the heated surface area inside the injection port liner needs to be maximized, liners that are packed with wool or that contain flow disrupting elements, like the Cyclosplitter® sleeves are recommended. In addition, injection port temperatures should be maintained in

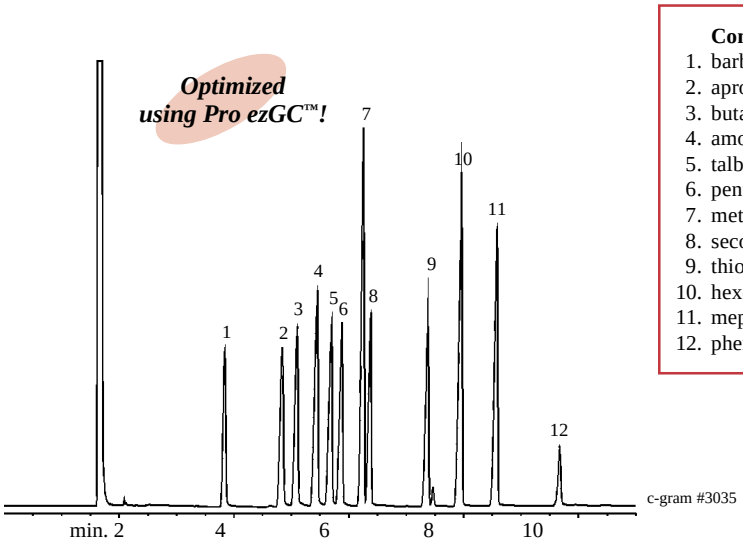
excess of 250°C in order to efficiently complete the derivatization process.

Analysis of barbiturates can also be performed on underivatized compounds. However, underivatized barbiturates have a tendency to produce overloaded or tailing peaks. Maintain injection port liners, guard columns, and analytical columns regularly to achieve good peak shape and adequate resolution. **Figure 2** shows the separation of a set of underivatized barbiturates using an Rtx®-35 column. Lower polarity stationary phases like the Rtx®-5 can be used to separate the barbiturates, but intermediate polarity stationary phases tend to provide better peak shape and improved resolution.

Barbiturates are an important part of drug screening. Extra care should be taken when analyzing barbiturates in either the derivatized or underivatized form. Intermediate polarity columns combined with well maintained injection port liners and guard columns will contribute to better peak shape and resolution.

Figure 2

Underivatized barbiturates on an Rtx®-35.



Compounds
1. barbital
2. aprobarbital
3. butalbital
4. amobarbital
5. talbutal
6. pentobarbital
7. methohexital
8. secobarbital
9. thiopental
10. hexobarbital
11. mephobarbital
12. phenobarbital

30m, 0.32mm ID, 0.50µm Rtx®-35 (cat.# 10439). 1.0µl split injection of barbiturates.
Oven temp.: 210°C (hold 2 min.) to 300°C @ 7°C/min. (hold 2 min.);
Inj./det. temp.: 300°C; **Carrier gas:** helium; **Linear velocity:** 35cm/sec. set @ 210°C;
FID sensitivity: 5.12 x 10^{-10} AFS **Split vent:** 30:1