K19 Analysis of Barbiturates by Fast GC: A Preliminary Study

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The goal of this paper is to present information on an evaluation of a fast gas chromatographic method developed for the analysis of barbiturates.

Fast gas chromatography (GC) has the potential to be a very useful tool in toxicological analysis by shortening retention times and increasing the overall rate of analysis. The most common techniques for achieving fast GC analysis include shortening of the GC column, raising the GC oven temperature, and increasing the GC oven ramping parameters. With these simple techniques in place, drugs can be analyzed more efficiently.

In the present study, a fast GC method was developed for the analysis of amobarbital, butalbital, pentobarbital, phenobarbital and secobarbital. The barbiturates were isolated from 1.0 mL of whole blood using CleanScreen® solid-phase extraction (SPE) cartridges (ZSDAU020) manufactured by United Chemical Technologies, Inc. Following elution from the SPE cartridge, the extracts were dried under a gentle stream of nitrogen at 40°C and reconstituted in a dilute methanolic solution (0.02 M) of trimethylanilinium hydroxide.

The extracts were analyzed using a Hewlett-Packard 6890 Series gas chromatograph equipped with a nitrogen-phosphorus detector. The inlet and detector temperatures were set at 250°C and 330°C, respectively. Helium was used as the carrier gas at a flow rate of 0.1 mL/min. Automated injections of 0.5 mL, at a split ratio of 31:1, were made onto an Agilent Technologies DB-5 (10 m x 100 mm x 0.1 mm) GC column. The initial oven temperature of 120°C was held for 0.25 min., then ramped 30°C/min. to a final temperature of 320°C for 0.75 min. The total run time was 7.67 min. These oven parameters were achieved by reducing the internal GC oven volume with the aid of an oven insert, as well increasing the GC power supply voltage to 220 V, from the standard 120 V.

A five-point calibration curve was prepared in drug-free whole blood in a range of 2.5 mg/L to 25 mg/L. Quantification was performed with barbital as the internal standard fortified at a concentration of 10 mg/L. In order to assess the intraand inter-run accuracy and precision of the assay, control samples were prepared at 7.5 mg/L and 12.5 mg/L and assayed five-times each in three separate experiments. Finally, a correlative study utilizing specimens previously assayed by a conventional GC method was conducted.

Under the fast GC conditions described, all barbiturates eluted from the GC column within 5 min.; the total GC cycle time was 9-10 min. This increase in throughput had no effect on chromatographic performance and analyte resolution. The results of the validation studies demonstrated excellent accuracy and precision with %CV values in the range of 15% or less and % accuracy values in the range of 90% or greater. Further, correlation was good between the conventional GC and fast GC methods.

While the fast GC method has distinct advantages, mainly improved efficiency, some limitations do exist. First, poor resolution between some analytes was evident. For example, under the conditions described, butalbital and butabarbital, and hexobarbital and caffeine, co-eluted. Similarly, high concentrations of caffeine interfered with the quantitation of phenobarbital. Another potential limitation of the fast GC method is the decreased capacity of a narrow bore GC column which may lead to column overload and reduced range of analyte linearity.

In conclusion, fast GC has great potential to become an efficient method for routine toxicological procedures in forensic toxicology laboratories. Because this method reduces the GC cycle time by nearly twofold, it significantly increases laboratory throughput.

Fast Gas Chromatography, Barbiturates, Solid-Phase Extraction

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