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Determination of barbiturates by solid-phase microextraction (SPME) and ion trap gas chromatography–mass spectrometry

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Abstract

Solid-phase microextraction (SPME) in conjunction with quadrupole ion trap GC–MS was applied to the determination of a series of barbiturates. A 65 μm Carbowax–divinylbenzene (DVB) SPME fiber was used to successfully extract a series of eight barbiturates from aqueous solution. Absorption kinetics and distribution coefficients for the 65 μm Carbowax–DVB SPME fiber were determined for the compounds. In addition the method was evaluated with respect to linearity, limit of detection, precision, desorption time, and the effect of salt. Limits of detection reached 1 ng/ml for the barbiturates. Linearity was established for the barbiturates over a concentration range of 10–1000 ng/ml, with coefficients of correlation 0.99. Overall, the precision of the method fell between 2.2%–6.5%, depending on the barbiturate. SPME was applied to the identification and quantitation of the barbiturates in a urine matrix. The method was validated by analyzing a reference standard pentobarbital-spiked urine sample. Both standard addition and internal standard with [$^2H_5$]-pentobarbital techniques were evaluated, with recoveries found to be 93% and 104%, respectively. SPME was then used to rapidly screen a urine specimen tested positive for barbiturates, and butalbital was detected and quantified. © 1997 Elsevier Science B.V.

Keywords: Solid-phase microextraction; Extraction methods; Pharmaceutical analysis; Barbiturates

1. Introduction

Solid-phase microextraction (SPME), discovered and developed by Pawliszyn and co-workers [1–5], has emerged in the past few years as a viable solvent-free alternative to conventional liquid–liquid extraction (LLE) and solid-phase extraction (SPE) methods. SPME, in conjunction with analysis by GC, has been demonstrated for a variety of classes of compounds such as pesticides [6–9], herbicides [10], benzene, toluene, ethylbenzene, and xylene (BTEX) [11–13], fuel-related hydrocarbons [14,15], caffeine [16], methylmercury [17], and tetraethyllead and inorganic lead [18]. The ability of SPME to quickly screen and many cases accurately quantitate target analytes is well documented in the above reports. By its nature, SPME is a solvent-free and concentrating extraction technique, which contrasts to LLE methods which require organic solvents and in many cases additional time-consuming concentration steps prior to analysis. SPME is not subject to breakthrough, plugging, and channelling problems often associated with conventional SPE methods. The theory of the SPME absorption process has been described previously [1,2,4].

The focus of this study is the development of a rapid and sensitive method for the determination of a number of compounds of pharmaceutical interest

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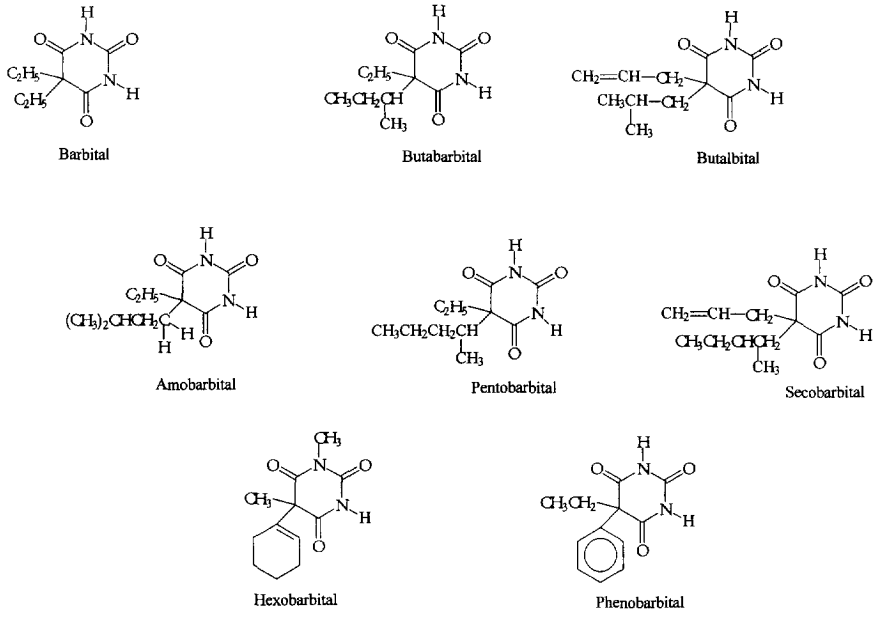

Fig. 1. Structures of barbiturates in this study.

from the barbiturate family, shown in Fig. 1, by novel SPME methods in conjunction with quadrupole ion trap GC–MS. Barbiturates have been extensively examined by gas–liquid chromatography (GLC) [19–29], HPLC [20,30–32], and a variety of other methods including supercritical fluid chromatography [33] and immunoassay methods [34]. First, SPME parameters for the detection of barbiturates in an aqueous medium are examined. These parameters include determining the extraction kinetics and distribution coefficients, the optimum desorption temperature, testing multiple extractions from the same vial vs. different vials, and the effect of salt on the extraction efficiency. The SPME method is then applied to standard barbiturate solutions to establish the linear range, limit of detection, and precision. Finally, SPME is evaluated for its effectiveness as a extraction technique in a more complex biological matrix, urine. Although ingested barbiturates are typically excreted in urine at a high percentage in metabolized forms, there remains an amount of unchanged drug which can be highly useful for screening and identity of the barbiturates [35].

2. Experimental

2.1. SPME equipment and procedure

SPME experiments were performed using a manual fiber holder supplied from Supelco (Bellefonte, PA, USA). Three types of fibers, a 30 μm polydimethylsiloxane, 85 μm polyacrylate, and 65 μm Carbowax–divinylbenzene (DVB), were obtained from Supelco and tested for their extraction efficiency for the barbiturates. The 65 μm Carbowax–DVB fiber was experimentally found to have the highest extraction efficiency toward the barbiturates, and thus was the fiber used throughout the study. Comparison studies between the polyacrylate fiber and Carbowax–DVB were based on six determinations from a 500 ng/ml solution of all the barbiturates.

Standard manual SPME extractions for the method development of the barbiturates were performed in 4 ml sample vials with PTFE/silicone septa (Supelco). In all cases except where noted, SPME extractions were carried out on 4 ml samples prepared by

diluting stock methanol or acetone barbiturate solutions with deionized water. In most cases, the organic content was kept below 1% (v/v). Magnetic stirring at 90% of the maximum setting with a PTFE-coated stir bar, dimensions of 8 mm length and 1.5 mm diameter, was used as the method of agitation in the extractions. As noted in previous SPME studies, air bubbles on the SPME fiber during the extraction period will affect precision and should be avoided. Air bubbles may be removed by sonication or exposing and retracting the fiber to the solution repetitively. After the desired time period for extraction, the SPME device was transferred to the GC injector port for direct thermal desorption at 250°C onto the GC column for the desired time. A maximum desorption temperature of 250°C was selected to efficiently desorb the barbiturates but not damage the Carbowax–DVB fiber. Continued exposure to temperatures of 265°C or higher were found to cause the Carbowax–DVB coating to strip off the fused silica. At an exposure temperature of 250°C the Carbowax–DVB fiber was found to be stable for at least 100 desorptions. In order to minimize carryover of phenobarbital into subsequent SPME extractions, a short procedure was implemented as follows. After the standard 12 min desorption period, the fiber was withdrawn from the GC injector, allowed to cool for 3 min, then exposed to methanol–water (20:80) solution for 3 min. The fiber was then placed back in the hot injector (250°C) for a period of 4 min. After this procedure, the percent carryover for phenobarbital was down to less than 2%. This process eliminates the need for two subsequent thermal desorptions to bring the carryover for phenobarbital to acceptable levels.

Salt effect experiments were conducted by examining the amount of barbiturate extracted from a 500 ng/ml solution in a set 10 min extraction period with varying percentages of saturated sodium chloride solution (17.0 g to 50 ml of deionized water). These experiments were run in duplicate.

Relative distribution constants were determined by plotting the nanograms of barbiturate extracted from the 50, 100, and 500 ng/ml (100, 500 and 1000 ng/ml for barbital) calibration standards vs. the concentration of the standards. The distribution coefficient will equal the slope of the best fit line divided by the volume of the fiber [4], which was quoted from Supelco to be $1.46 \cdot 10^{-4}$ ml for the 65 μm Carbowax–DVB fiber. This three point approach provides a more accurate value for K than a single point determination. Nanograms extracted were extrapolated from a direct injection calibration curve of the barbiturates.

The SPE method employed 3M (St. Paul, MN, USA) Empore C_{18} extraction disks obtained from Fisher Scientific (Pittsburgh, PA, USA). After conditioning, 4 ml of the urine sample was filtered under vacuum through the disk. Then the analyte was eluted with two 10 ml portions of acetone–methanol (1:1). The extract was concentrated under a gentle flow of nitrogen to 1 ml, and finally diluted up to 2 ml with acetone for direct injection.

2.2. Instrumentation

GC–MS analysis was carried out with a Varian Saturn 4D GC–MS system. Separations were conducted on a PTE-5 column (30 m×0.25 mm I.D., 0.25 μm d_f). For the series of barbiturates the GC oven program consisted of the following steps: 0.20 min hold at 60°C, 40.0°C/min ramp to 110°C, 1.00 min hold at 110°C, 10.0°C/min ramp to 250°C, 2.00 min hold at 250°C. A septum-equipped programmable injector (SPI) was used in all tests. The SPI program consisted of a 0.10 min hold at 60°C, a 200.0°C/min ramp to 250°C, and a 20.00 min hold at 250°C. Helium was used as the carrier gas and set at an injector head pressure of 12 p.s.i. (1 p.s.i.= 6894.76 pa).

The Saturn system is equipped with a quadrupole ion trap detector which was run in electron ionization (EI) mode and automatic gain control (AGC) applied. For EI experiments the instrument parameters were set at the following values: 25 μA filament emission current, electron multiplier voltage of 2000 V, and an AGC target of 20 000 counts. The manifold temperature was maintained at 180°C. The mass range scanned was 88–260 u for calibration data. The ions used for quantitation were as follows: barbital (185^+, 156^-, 141^+), butabarbital (156^+, 183^+, 213^+), butalbital (167^+, 168^+, 181^+), amo- and pentobarbital (227^+, 156^+, 141^+), secobarbital

(167^+, 168^+, 195^+), hexobarbital (237^+, 221^+, 157^+), and phenobarbital (117^+, 161^+, 204^+).

2.3. Materials

Barbital, amobarbital, pentobarbital, and hexobarbital used in this study were obtained from Sigma (St. Louis, MO, USA) as solids. A butabarbital standard (1.0 mg/ml in methanol) was also purchased from Sigma. Butalbital, secobarbital, phenobarbital, and pentobarbital as 1.0 mg/ml standards in methanol, and [$^2H_5$]-pentobarbital as 100 μg/ml were purchased from Radian International (Austin, TX, USA). Dimethyl ether (99.8% min.) was purchased from Matheson Gas Products (East Rutherford, NJ, USA).

3. Results and discussion

3.1. Development of the SPME method

In the initial development of the SPME method for the determination of the barbiturates, three commercially available SPME fiber coatings were evaluated for their extraction efficiency. The more polar 65 μm Carbowax–DVB fiber was experimentally found to have the highest extraction efficiency for the barbiturates. The polyacrylate fiber was also successful in extracting this class of compounds, but with lower efficiency than the Carbowax–DVB fiber. For barbital, butabarbital, butalbital, amobarbital, pentobarbital, and secobarbital, the average response of the polyacrylate fiber fell between 52% and 62% of the Carbowax–DVB fiber. The average response for hexobarbital and phenobarbital with the polyacrylate fiber was 26%. Extractions with the relatively nonpolar polydimethylsiloxane coating yielded no detectable amounts of the barbiturates.

Time dependence studies for equilibration of the barbiturates were undertaken, and the results are shown in Fig. 2. All of the barbiturates begin to rapidly approach equilibrium at a 20-min absorption time. This rapid rise to equilibrium may be explained in terms of the barbiturates relatively low values for distribution coefficients (as discussed further later). A 20-min extraction period was chosen as a reasonable compromise between good peak response and length of the experiment. Although a shorter extraction time may be chosen, better precision is generally obtained at absorption times close to equilibrium.

Optimum desorption time was found by measuring how much analyte was retained on the fiber after a set exposure time in the GC injector at 250°C. A 1000 ng/ml mixture of the barbiturates was exposed to the Carbowax–DVB fiber for 20 min and then

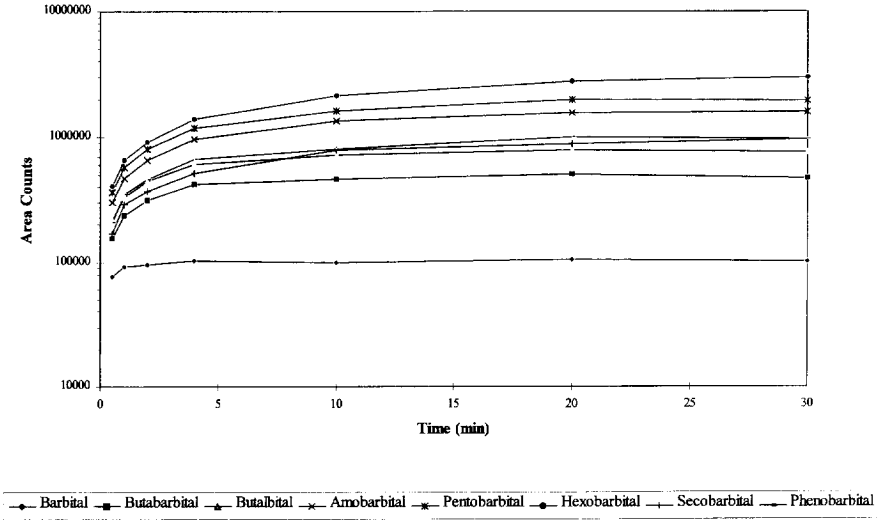

Fig. 2. Effect of absorption time on the peak area for the 65 μm Carbowax–DVB fiber. All barbiturates at 500 ng/ml.

transferred to the GC injector for various desorption times. After the set desorption time, the fiber was removed from the injector and immediately transferred to an empty SPME vial stored in ice to minimize loss of analyte to the atmosphere. Then a carryover test was run to detect any analyte remaining on fiber. The results are expressed as a percent carryover vs. desorption time in Fig. 3. All of barbiturates except phenobarbital were found to have a percent carryover of <1% after a 6-min desorption time. The percent carryover for phenobarbital (Fig. 3) is seen to be about 18% and 8% for a 6- and 12-min desorption, respectively, a significant amount when considering the effect on precision of the SPME method. A second carryover run brought the level down to <2.0% for phenobarbital. Instead of running two carryover runs between each SPME extractions, a faster procedure to bring the carryover of phenobarbital to acceptable levels was developed. The procedure involves exposing the fiber to a methanol–water solution as detailed in the Experimental section. Considering all of the barbiturates, a 12-min desorption time was selected as the best time for experiments in this study.

The effect of adding salt to the sample solution prior to extraction was examined by performing

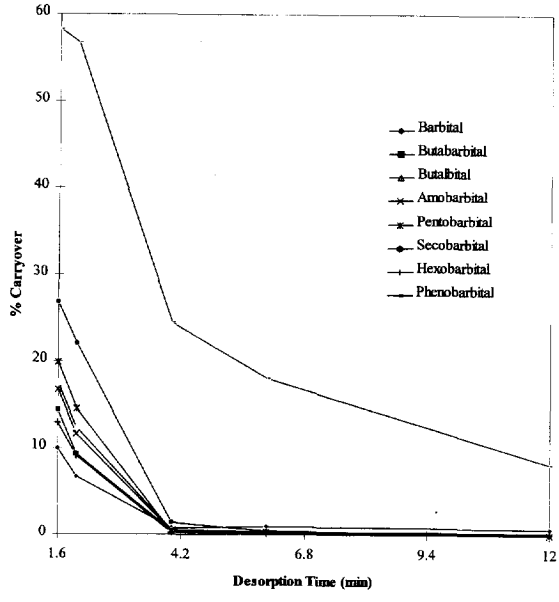

Fig. 3. Carryover for the 65 μm Carbowax–DVB after various desorption times.

SPME on a series of 500 ng/ml barbiturate solutions each containing a varying percentage of a saturated salt solution. The results are presented in Fig. 4 as a bar graph illustrating the percent effect relative to a SPME extraction carried out on a sample with no added salt solution. In most cases, the addition of the salt solution can significantly enhance the detectability of the barbiturates, giving a signal increase of 40% to over 150%. However, increasing the salt content over about 50% saturated solution causes a negative effect in the signal of phenobarbital. In cases of trace analysis, addition of salt may be beneficial for the detection of a target barbiturate. However, for routine analysis, samples with no added salt still provide an acceptable level of detection. Thus, all other SPME tests in this study were run with no salt added to the extraction vial.

3.2. Distribution coefficients and calibration data

Relative distribution coefficients, K, for all compounds were experimentally determined under equilibrium conditions and listed in Table 1. The K values are relatively low when compared to distribution constant studies of other organic compounds on polydimethylsiloxane fibers which commonly have K values over >1000 [8,11,14,15]. However, the partition of barbiturates by the fiber is still sufficient to yield low and sensitive detection yet not significantly deplete the analytes from the sample vial. Therefore, as a result of the relatively low K values, multiple extractions may be carried out on the same 4 ml sample of the barbiturates without seriously affecting the precision of the method. This feature was experimentally verified by comparing the precision of six extractions from separate vials vs. six extractions from the same vial, with every vial containing 500 ng/ml of all the barbiturates. Overall the precision of multiple extractions from the same vial was equal to or lower than the case of separate vials. The precisions for the barbiturates from the same vial and separate vials were between 2.6–5.3% (n=6) and 4.6–6.7% (n=6), respectively.

The calibration data for the barbiturates including the established linear range, detection limit achieved, precision, and relative distribution coefficients are listed in Table 1. Established linear ranges extend three orders of magnitude with r^2 values of 0.99. The

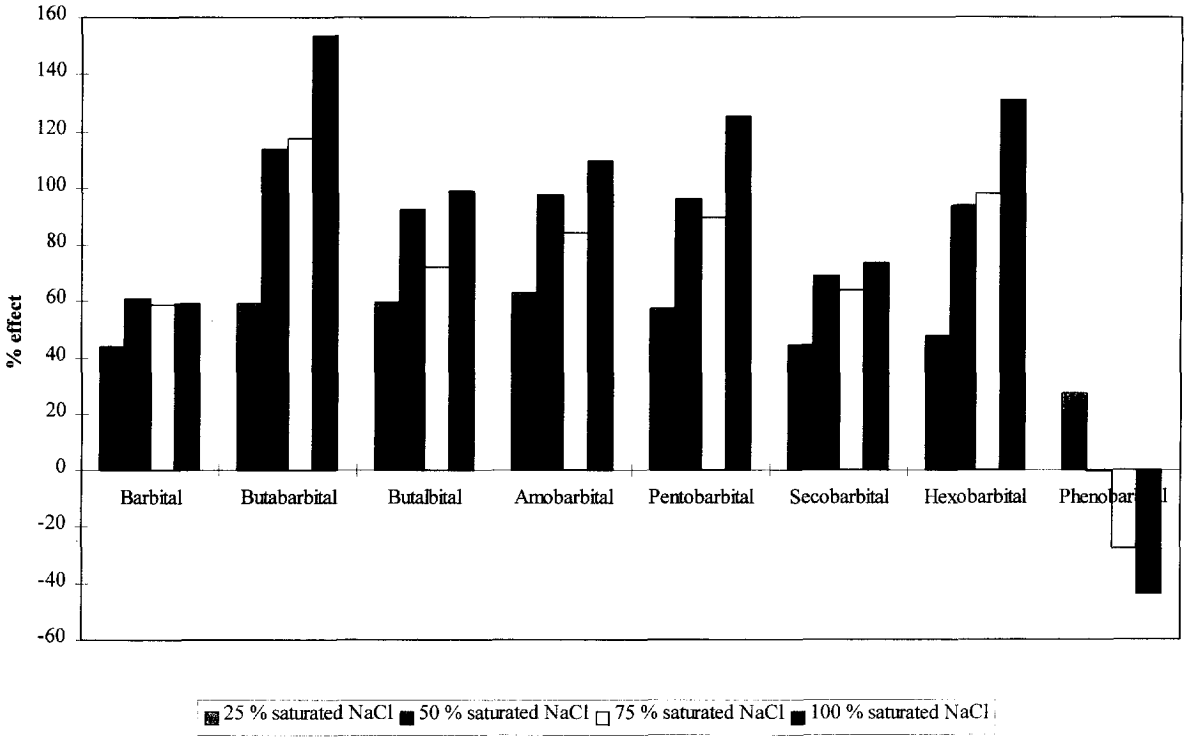

Fig. 4. Percent effect of different salt concentrations on the extraction efficiency relative to an unsalted solution.

lowest standard tested for each barbiturate and signal-to-noise ratio corresponding to that standard is listed in Table 1 as the limit of detection (LOD). The LOD for the barbiturates reached 1 ng/ml, two orders of magnitude better than by direct injection. The precision of each concentration standard (n=6,

Table 1
Relative distribution coefficients and SPME calibration data for a series of barbiturates

Analyte	K 65 μm Carbowax–DVB fiber[a]	Established linear range (ng/ml)[b]	r^2	LOD:S/N (ng/ml)[c]	Precision Range (mean) (%)[d]
Barbital	10	10–1000	0.99	5.0:18	2.6–12 (5.3)
Butabarbital	39	10–1000	0.99	5.0:50	2.5–5.7 (3.7)
Butalbital	63	10–1000	0.99	5.0:85	2.5–4.4 (3.4)
Amobarbital	111	10–1000	0.99	1.0:17	2.7–4.4 (3.5)
Pentobarbital	122	10–1000	0.99	1.0:20	1.5–4.8 (2.8)
Secobarbital	218	10–1000	0.99	1.0:19	1.6–4.9 (3.8)
Hexobarbital	86	10–1000	0.99	1.0:14	1.4–3.4 (2.2)
Phenobarbital	80	10–1000	0.99	1.0:13	3.9–8.9 (6.5)

[a]Calculated from the slope of the linear range from the ng extracted vs. concentration plots.
[b]Determined from the area counts vs. concentration curve. Consists of six data points. Based on 20-min extractions from 65 μm Carbowax–DVB fiber.
[c]Based on 20-min extractions.
[d]Lists the precision range of six standards ranging from 10–1000 ng/ml (n=6).

from the same vial) was determined and the results are listed in Table 1 as a precision range with the overall average in parentheses. Typically, better precision is associated with the higher concentration standards.

3.3. Application to a biological matrix

To extend the SPME method developed for the barbiturates to more complex samples, SPME was used to detect and quantify barbiturates in urine. First, the method was validated by spiking a urine sample with a standard reference pentobarbital solution and quantifying by both standard addition and an internal standard method with [$^2H_5$]-pentobarbital. Then SPME was used to detect and quantify any detected barbiturates in a urine specimen which were declared positive for barbiturates by a private drug testing laboratory.

To test the accuracy of the SPME method toward the analysis of the barbiturates, a pure urine sample was spiked at a level of 1 μg/ml with a reference standard pentobarbital provided by Radian International. This solution was quantified by both an internal standard method with [$^2H_5$]-pentobarbital and standard addition methods. Calibration and standard addition spike solutions were prepared in-laboratory by weighing out the pentobarbital sodium salt and diluting appropriately. Recoveries were found to be 93% and 104% for standard addition and the internal standard method, respectively. Precision values were 5.9% for the internal standard method and 4.0% for standard addition.

To further demonstrate SPME as a useful technique for the screening and quantitation of barbiturates in urine specimens, a urine sample provided by a private drug testing facility was tested. The urine specimen, designated UR+, was known to be positive for the barbiturate class by immunoassay methods, however the method was incapable of distinguishing which specific barbiturate(s) were present. Fig. 5 shows the total and selected ion chromatograms for an initial screening of UR+ after extraction with the Carbowax–DVB fiber. Examination of the chromatogram and mass spectrum indicated the presence of only butalbital of the eight barbiturates in this study. This result was verified by confirming the expected dimethyl ether (DME)

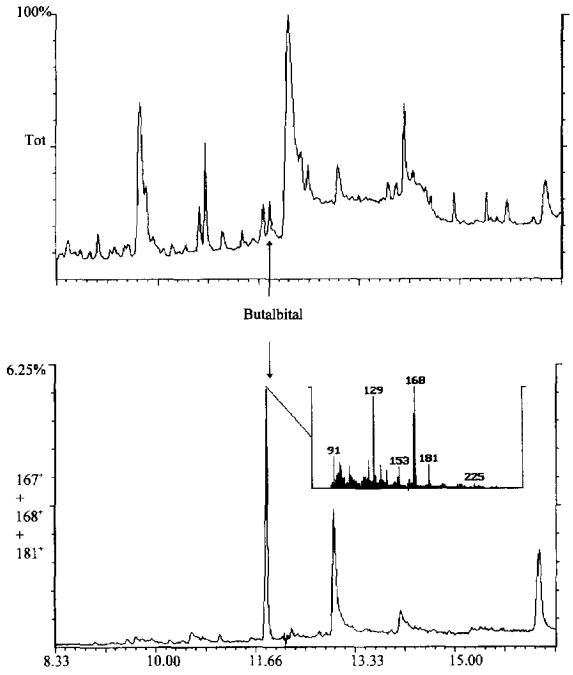

Fig. 5. Total and selected ion chromatograms, after extraction with the 65 μm Carbowax–DVB, for a urine specimen, UR+, known positive for barbiturate. The selected ion chromatogram indicates the presence of butalbital. Chemical ionization and CAD tests confirm this finding. Other peaks in the selected ion chromatogram are unidentified analytes which also contain 167^+, 168^+ and/or 181^+ ions.

chemical ionization adducts and the MS–MS fragmentation pattern of $(\text{Butalbital}+13)^+$, established in this laboratory as a well-known product formed in the DME-chemical ionization process. The concentration of butalbital in UR+ was then determined by SPME–GC–MS and compared to the concentration found by a conventional SPE–GC–MS method based on extraction and elution from a C_{18} disk. The concentration of butalbital determined by SPME and standard addition calibration was found to be 121 ng/ml with a precision of 5%. The results for the SPE method indicated a lower concentration, found by two trials to average 45 ng/ml. However, the SPE method used was found to have an experimental recovery for butalbital of 51%, which when corrected for brings the results for SPME and the SPE in reasonable agreement. The recovery for butalbital by SPME is expected to be approximately 100% based on the above studies with pentobarbital.

4. Conclusions

In this study, SPME has been applied to the determination of barbiturates. SPME is a fast, solvent-free extraction technique which has been demonstrated to be a sensitive and selective method of analysis for the barbiturates when coupled to a gas chromatograph-quadrupole ion trap mass spectrometer. Detection limits for the barbiturates reached 1 ng/ml, with established linear ranges extending three orders of magnitude. The precision of the method averaged 3% overall. The SPME method developed for the barbiturates may be extended to more complex analytical samples, as is detailed for a urine matrix in this study, and still retain its sensitivity, accuracy, and precision. Recovery from a pentobarbital spiked urine sample was found to be 100% within experimental precision by SPME methods with both standard addition and internal standard calibration. SPME was found to be a rapid and effective means of screening a urine sample noted by the detection of butalbital. Quantitative results on this positive urine sample were comparable to a conventional SPE method.

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