Determination of Sulfonylureas and Metabolites by Pyrolysis Gas Chromatography

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We describe a simple, rapid method for determination of sulfonylureas that is not susceptible to significant interference from metabolites. An acidified plasma is extracted with toluene, followed by a back extraction from the toluene to methanolic trimethylammonium hydroxide. The resulting compounds are measured by gas chromatography.

Various sulfonylurea derivatives are widely used to treat some types of diabetes. Although thousands of different compounds have been shown to possess some hypoglycemic activity (1), only a few are used therapeutically. The dose required to achieve an effective plasma concentration of sulfonylurea varies from person to person and even from time to time in the same person (2, 3). Some of the properties of sulfonylureas that may be responsible for these variations are binding of sulfonylureas by serum proteins, induction of microsomal enzymes (5, 6), generic inequivalence of dosage forms (7), and drug–drug interactions that change sulfonylurea half-lives (8–10). Because concentrations of these drugs in plasma vary so much, and because their therapeutic index is fairly low, routine determination of the plasma sulfonylurea concentration in patients receiving these therapeutic agents would seem desirable. In fact, increased use of these agents has prompted a parallel increase in the development of methods suitable for their accurate determination in biological fluids.

Many of the first attempts to analyze for sulfonylureas were based on hydrolysis of the drug, extraction of the liberated amines, and analysis of those amines by use of methyl orange, dinitrofluorobenzene, ninhydrin (13, 14), or by the Bratton–Marshall reaction. These time-consuming procedures are susceptible to interference from drug metabolites and (or) amino acids when used for the analysis of urine samples (20).

There are gas-chromatographic methods for analysis of sulfonylureas. Most of these procedures involve time-consuming multiple extractions (15–17), partitioning from aqueous methanol into ethyl ether (18), or preliminary thin-layer chromatography (19) to obtain a residue of sufficient purity for GLC analysis.

This paper describes a method that is simple, rapid, and is not susceptible to significant interference from metabolites. A toluene extraction of an acidified sample is followed by back extraction from toluene into a small volume of methanolic TMAH and GLC analysis of the resulting methylated compounds.

Materials and Methods

Reagents

All reagents are analytical grade.

TMAH: Add 7.0 g of trimethylphenylaminamonium iodide (Aldrich Chemical Co.; Cat. No 13,914-9) to 10 ml of methanol contained in a culture tube with Teflon-lined screw cap. Add 3.6 g of silver oxide and shake vigorously for 2 min. Rotate the tube on a mechanical rotator for 3 h, centrifuge, and decant the supernate into a brown glass bottle. The concentrations of the TMAH thus prepared may be determined by titrating 1 ml of the solution with a standard HCl solution (0.1 mol/liter), with phenolphthalein as the end-point indicator. The final TMAH concentration should be 1.9 ± 0.2 mol/liter. If a commercial preparation is used, it must be of the same concentration (2.0 mol/liter), because more di-

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1 Nonstandard abbreviations used: GLC, gas-liquid chromatography (-ic); TMAH, trimethylammonium hydroxide; MS, mass spectrometry.
lute solutions of TMAH are not effective. The TMAH solution is stable for at least four weeks when stored at 4 °C.

Internal standard solution: 0.5 g of aprobarbital in 500 ml of toluene. Dilute 10 ml of this stock solution to 100 ml with toluene.

H₃PO₄, 0.2 mol/liter: Dilute 1.15 ml of 83% H₃PO₄ to 100 ml with distilled water.

Drug standards: Dissolve 25 mg of tolbutamide ("Orinase," Upjohn) or of tolazamide ("Tolinase," Pfizer) in 25 ml of distilled water. Dilute these stock solutions with fresh drug-free plasma, urine, or distilled water, depending upon the type of samples to be analyzed, to give concentrations in the range expected in the samples. At least two standards (for example 2 and 5 mg/ml) should be processed with each group of samples.

Apparatus

We used the Hewlett-Packard Model 7620 gas chromatograph, dual FID, equipped with a 6 ft. × \( \frac{1}{2} \) in. (o.d.) stainless-steel column containing "10% UCW98 80/100 mesh on Chromosorb W-HP" (Supelco, Inc., Bellefonte, Pa. 16823). The stainless-steel injection port liner was filled with silanized glass wool. The electrometer was connected to a strip-chart recorder and a Perkin-Elmer Data Processor, PEP-1. Operating temperatures were: injection port heater, 260 °C; column, 180 °C; detector, 290 °C.

After every five injections the column temperature was increased to 290 °C and maintained at that temperature for 5 min, to remove contamination that may have accumulated on the column packing.

Flow rates (ml/min) were: hydrogen, 25; helium, 30; and air, 240.

GLC—mass spectrometer: Perkin-Elmer Model 900 gas chromatograph equipped with a 5-ft stainless-steel column packed with "3% OV-17 on Gas Chrom Q" (Supelco). Injection port temp.: 250 °C, column temp.: 150 °C, detector/MS interface temp. 250 °C. The mass spectrometer was a Hitachi RMU-6L (ionizing energy—70 eV) on-line to an IBM 1800 computer.

Procedure

To 3.0 ml of plasma suspected to contain a sulfonylurea and to several standards in plasma, each contained in a 16 × 125 mm culture tube with a Teflon-lined screw cap, add 1.0 ml of the H₃PO₄ and 5.0 ml of the internal standard solution.² Shake for two minutes and centrifuge to separate the phases. Transfer about 4 ml of the toluene layer to a clean "concentratube" ³ with a Pasteur pipette. Concentratubes are designed to hold about 20 ml, and taper to a sharp tip, which holds about 10 µl. It is desirable to use tubes of this or similar design, because it is difficult to isolate the small TMAH layer and aspirate it into a syringe with conventional glassware.

Use a vortex-type mixer to mix the contents of the concentratube, and while vortexing slowly add 20 µl of the TMAH solution. Continue mixing for a total of 15 s and then centrifuge for 5 min. Withdraw 3 µl of the TMAH (lower) phase with a 10-µl syringe and inject it into the injection port of the GLC over a 10-s interval. This slow injection is necessary to avoid tailing of the solvent peaks.

Results

Identity of GLC Peaks

The chemical structures of the compounds studied are shown in Figures 1 and 2.

The GLC analysis of samples or standards containing chlorpropamide (V) reveals one peak from the internal standard and one peak from chlorpropamide (Figure 3). GLC/MS analysis of a mixture of chlorpropamide and TMAH revealed that the mass spectrum of this peak was not consistent with the parent drug, but rather with N,N-dimethyl-p-chlorobenzensulfonamide (VIII), which is apparently formed by fragmentation of the parent compound in

² Urine or aqueous samples and standards can be analyzed by the same technique if 1 ml of a 1 mol/liter acetate buffer (pH 5.2) is substituted for the 1 ml of 0.2 mol/liter H₃PO₄.

Chromatogram = Apro

Table 1. Metabolite Interference with Chloropropamide Measurement

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>% of dose in urine(^*)</th>
<th>% extracted</th>
<th>Conversion value(^*)</th>
<th>Relative contribution to chloropropamide peak, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloropropamide</td>
<td>18</td>
<td>80</td>
<td>65</td>
<td>93.6</td>
</tr>
<tr>
<td>2-Hydroxychloropropamide</td>
<td>55</td>
<td>0.4</td>
<td>60</td>
<td>1.3</td>
</tr>
<tr>
<td>(p)-Chlorobenzenesulfonamide</td>
<td>2</td>
<td>20</td>
<td>100</td>
<td>4.0</td>
</tr>
<tr>
<td>(p)-Chlorobenzensulfonylurea</td>
<td>21</td>
<td>0.6</td>
<td>80</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\(^*\) According to Taylor (29).

The relative amount of \(N,N\)-dimethyl-\(p\)-chlorobenzensulfonamide (VII) formed, with the amount from \(p\)-chlorobenzensulfonamide (VII) considered to be 100%, when equal amounts of each compound were reacted with TMAH.

the injection port and subsequent methylation of the fragment by TMAH. This identification was confirmed by GC/MS analysis of a mixture of TMAH and \(p\)-chlorobenzenesulfonamide (VII) under the same conditions; retention times and mass spectra of (VII) in TMAH were identical with those of (V) in TMAH (major peaks at m/e 219, 175, 111).

Figure 4 shows that both tolbutamide and tolazamide give only one GLC peak and that their relative retention times are very similar. Consideration of the structures of these two drugs (I and IV) indicates that they should fragment in a manner analogous to chloropropamide, to yield \(N,N\)-dimethyl-\(p\)-toluenesulfonamide (IX). GLC/MS analysis of mixtures of tolbutamide in TMAH and tolazamide in TMAH did in fact result in mass spectra consistent with this product. In addition, the GLC/MS mass spectrum and retention time of this product were identical with those obtained by GLC/MS analysis of a mixture of \(p\)-toluenesulfonamide (X) and TMAH (major peaks at m/e 199, 155, 91) and distinct from a GLC-mass spectrum of \(N\)-methyl-\(p\)-toluenesulfonamide (major peaks at m/e 185, 155, 91).

In routine GLC analysis the amounts of (VIII) and (IX) formed were observed to be nonreproducible unless the injection port was filled with silanized glass wool, which apparently ensures complete fragmentation.

Sulfonylurea Metabolites

Most of the drug-related material found in the plasmas of patients receiving chloropropamide is the unchanged drug. However, urines from these patients contain, in addition to chloropropamide, large amounts of chloropropamide metabolites (21, 22) (Table 1). We investigated the possible interference of these metabolites with the determination of urinary chloropropamide.

Authentic samples of each metabolite were dissolved in TMAH and injected under the stated conditions for the analysis of sulfonylureas. Each metabolite gave only one GLC peak and this peak had the same retention time as VIII. Although the metabolites give the same GLC peak as the parent drug, they do not significantly interfere with chloropropamide because either (a) they are present in low concentrations or (b) they are not efficiently extracted by the described procedure (Table 1).

As with chloropropamide, unchanged tolbutamide and tolazamide account for most of the drug-related material in the plasma of patients receiving these drugs. Urines from patients receiving tolbutamide contain not only the original drug but two metabolites of tolbutamide, hydroxytolbutamide and carboxybutamide (23, 24). Because hydroxychloropropamide is not significantly extracted by this procedure, it would seem likely that the extraction of hydroxytolbutamide would also be poor, although this was not demonstrated because not enough of the
pure metabolite was available. Carboxytolbutamide and TMAH were injected into the GLC, with use of the temperature program described in the “Apparatus” section. A peak appeared for which the retention time was 13.4 min. This peak is presumably that of the methyl ester of N,N-dimethyl-p-carboxyphenylsulfonamide, but we do not know that it is. Based on this peak, the overall recovery of carboxytolbutamide was calculated to be 35% (Table 2).

Although less is known of the metabolic products of tolazamide, it would be surprising if its metabolites were not analogous to those of tolbutamide and therefore its determination in biological specimens should also be free of metabolic interferences.

Discussion

Under the stated conditions, the reproducibility of the method for the three drugs studied is satisfactory and the peak-area ratios are a linear function of drug concentration from 0.5 µg/ml to 100 µg/ml (Table 3). As little as 0.5 µg/ml can be detected, because there are few interfering substances in the final extract (Figure 3).

The concentration of drug in an unknown sample may be determined by comparing the ratio of the peak area for the drug to that of the internal standard in the sample with the same ratio in several suitable standard samples. These standards should be in plasma or urine, depending on the type of sample being analyzed.

Many of the procedures for determination of sulfonylureas, including this one, were first developed for the determination of barbiturates (25). Therefore, in determining sulfonylureas by these procedures, care must be taken to differentiate between the sulfonylureas and any barbiturates that have been co-extracted. Under the conditions stated, this distinction can easily be made (Table 2). In patients known to be free of barbiturates, the total time required for analysis of a sample may be shortened by operating the GLC at a column temperature of 200 °C.

The internal standard selected for use in a quantitative GLC analysis must accurately reflect the extractions of the material of interest. For this reason other authors have used one sulfonylurea as internal standard for another (e.g., chlorpropamide for tolbutamide, and vice versa). The choice of aprobartil or dimethylsulfate as internal standard avoids this problem and allows one to use the same internal standard for all sulfonylureas. Table 3 shows that over a relatively large range of concentrations the peak area ratios are a linear function of these concentrations and that the overall recovery of sulfonylureas is adequate and reproducible. This indicates that aprobartil is a suitable internal standard for all three drugs.

The method described is much faster and simpler than previous techniques for the determination of sulfonylureas because of the fewer sample manipulations.

Use of either TMAH or dimethylsulfate results in a product that has good GLC characteristics. However, in the TMAH procedure the compounds of interest can be directly injected into a GLC, whereas with the dimethyl sulfate they must be extracted from the methylating medium before injection. In addition, solvent evaporation and sample reconstitution are avoided with the use of TMAH.

The lack of significant metabolite interference and the excellent linearity and reproducibility of determinations of parent-drug concentrations make this method very attractive for the analysis of biological samples containing chlorpropamide, tolbutamide, or tolazamide. We have used this procedure for just this purpose for the last six months. It also should be applicable to pharmaceutical preparations.
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References


