Rapid and Sensitive Gas Chromatography–Mass Spectroscopy Method for the Detection of Mannitol and Sorbitol in Serum Samples, Florian Renner,* André Schmitz,* and Hartmut Gühring* (Institut für Klinische Chemie and *Klinik für Anästhesiologie, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany; * address correspondence to this author at: Labor Dres. Schumacher und Sommer, Dr. Franz Mertens Str. 8, 27580 Bremerhaven, Germany; Fax 0471/9829109)

During endoscopic procedures, irrigating fluids are used for removing blood and pieces of resected tissue from the operation field. To minimize hemolysis after possible fluid absorption, these solutions always contain solutes such as mannitol and sorbitol to reduce their hypo-osmolality (1, 2). Concentrations of these polyols have been determined in blood plasma of patients undergoing transurethral resection of the prostate (3) as a measure of fluid absorption, which may lead to the feared transurethral resection syndrome, involving symptoms such as transient deterioration of mental status, nausea, vomiting, confusion, and arterial hypotension (4).

For gas chromatographic analysis, polyols are better separated as n-butylboronate derivatives (3–5) than as trimethylsilyl derivatives, and the derivatization procedure is less time consuming than is formation of acetyl derivatives. Nevertheless, gas chromatographic methods using flame ionization detection show poor detection limits and, as an additional restriction, interference from other polyols (5).

We reinvestigated the determination of mannitol and sorbitol in serum with gas chromatography–mass spectrometry by using n-butylboronate derivatives (3–5, 6). Whereas reported methods use zinc sulfate/barium hydroxide precipitation for deproteinization of samples, we found that absolute values of peak areas were <70% of those obtained by untreated samples, likely caused by analyte absorption on insoluble residues of precipitation reagents after evaporation. By contrast, with ethanol precipitation avoiding insoluble residues and reducing evaporation time, absolute values were 90–100% and recovery (related to the internal standard) was 89–103% for mannitol concentrations of 0.05–2.74 mmol/L and sorbitol concentrations of 0.05–6.86 mmol/L.

In our study, irrigating fluid used in connection with transurethral resection of the prostate contained 27 mmol/L mannitol, 147 mmol/L sorbitol, and 434 mmol/L ethanol. Concentrations of ethanol were determined by gas chromatography (detection limit, 0.22 mmol/L) in blood samples drawn from patients undergoing transurethral resection of the prostate at the start of the operation just before use of irrigating fluid, after 30 min, and at 0, 20, and 40 min after the operation. All investigated patients gave their informed consent.

The subjects (n = 11) chosen for our study were characterized by the detection of ethanol in at least one of the five samples. Concentrations of mannitol and sorbitol were determined in all samples (n = 55) to measure absorption of irrigating fluid. Briefly, 100 µL of 0.69 mmol/L dulcitol was added as internal standard to 100 µL of serum. Deproteinization was performed by adding 1 ml of ethanol, mixing, and centrifugating for 10 min at 14 000g. The supernate was evaporated to dryness in a nitrogen stream at 40 °C, and 100 µL of 0.49 mol/L n-butyl-boronic acid in ethanol was added. After intensive mixing and treating in an ultrasonic bath for 10 min, the sample was again centrifuged, and the supernate was used directly for gas chromatography–mass spectroscopy analysis.

Measurements were performed by using an Autosystem Gas Chromatograph (Perkin-Elmer) equipped with a Q-mass 910 mass spectrometer (Perkin-Elmer). We injected 1-µL aliquots of samples on a Rtx-5 capillary column (30 m × 0.32 mm i.d., 0.25 µm film; Restek). The chromatographic conditions were as follows: injector temperature, 250 °C; column temperature program: initial temperature, 60 °C held for 1 min with split open, increased at 45 °C/min to 180 °C with split closed, and increased at 10 °C/min to 250 °C. The following conditions were used for mass spectrometry: ionization energy, 70 eV; ion multiplier voltage, 1250 V; integration time, 0.4 s; and carrier gas, helium. Whereas Eades et al. (6) used the peak at m/z 127 for single ion monitoring measurements, we found calibration lines with higher linearity (r > 0.99) for m/z 253, which was also chosen by Marunaka et al. (5), and fewer interfering signals were obtained by other compounds. Calibration curves were prepared in serum samples with known amounts of mannitol and sorbitol added. Retention times of polyols were as follows: mannitol, 8.7 min; sorbitol, 9.0 min; and dulcitol (internal standard), 9.3 min. All peaks were baseline separated. Several other compounds were tested for interferences at concentrations of 2.74 or 16.6 mmol/L for glucose, respectively. No interfering peaks at m/z 253 were obtained with β-galactose, β-arabinose, β-ribose, and β-xylene. For β-lactose, lactulose, and xylitol, some interfering signals were detected at m/z 127 but not at m/z 253. Samples of myo-inositol, sucrose, and fructose yielded also smaller interfering signals at m/z 253, whereas peak areas where markedly higher at m/z 127. Increased interferences were obtained with 16.6 mmol/L glucose, indicating that samples of diabetics should be excluded from analysis. Reported procedures for removal of glucose and fructose were tested by Marunaka et al. (5) but found to be not suitable. Nevertheless, none of the patients examined in our study was diabetic, and there were no interfering signals in any sample drawn before application of irrigating fluid. Precision of the method was determined by using two series of serum samples (n = 10), one with 0.10 mmol/L analyte added and the other with 1.09 mmol/L added. The CVs within assays were 7% at the lower concentration and 2% at the higher concentration. The CVs between assays were 7% at the lower concentration and 3% at the higher concentration.

The method was found to be linear up to 3.0 mmol/L polyol, but peak forms were sharper when reducing sample volume in all samples with concentrations of
above 1.5 mmol/L. Detection limit (signal-to-noise ratio, 3) was 0.5 μmol/L for mannitol and 4 μmol/L for sorbitol with a 2-μL injection volume.

Fig. 1 shows results of polyol measurement in patient samples drawn at different times during and after operation. Highest values were found in the samples drawn intraoperatively (30 min) or postoperatively (0 min). To compare the ability of ethanol and polyol determination to detect absorption of irrigating fluid, we compared results of measurements in samples drawn after instillation of irrigating fluid (n = 44). Whereas in 98% of all samples polyols were detected, ethanol could be detected in only 56%. We conclude that the method described for the determination of mannitol and sorbitol in serum samples of patients undergoing transurethral resection of the prostate is more useful to detect minimal absorption of irrigating fluid compared with the determination of ethanol. Whereas absorption of 1000 mL of irrigating fluid (concentrations of >1.8 mmol/L mannitol and 6.5 mmol/L sorbitol) results in severe clinical symptoms, small absorption ratios may result in organ failure in older patients with severe concomitant diseases as obstructive lung disease or coronary heart disease. This is one reason for the increasing incidence of the transurethral resection syndrome in spite of a permanent improvement of operation techniques (7). Furthermore, the method can be used alternatively when measurement of ethanol is contraindicated as for alcoholics during or after therapy.

Fig. 1. Mannitol and sorbitol concentrations determined with gas chromatography–mass chromatography to detect absorption of irrigating fluid in serum samples of four patients undergoing transurethral resection of the prostate.

Samples were drawn at the beginning of the operation just before application of irrigating fluid, after 30 min, and postoperatively at 0, 20, and 40 min.

Our thanks are due to K. Stratmann and A. Niemeier for skillful assistance and to Dr. L. Dibbelt for methodological discussions and the review of the manuscript.

References