Improved Colorimetric Determination of Salicylic Acid and Its Metabolites in Urine

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We describe an improvement in the Levy and Procknal method [J. Pharm. Sci. 57, 1330 (1968)] for determination of salicylic acid and its metabolites in urine. Salicylic acid and salicylic acid are successively extracted from 1 or 2 ml of urine (acidified with HCl) by two 10-ml portions each of carbon tetrachloride and ethylene dichloride. The extracts of each solvent are shaken with 5 ml of ferric nitrate solution (a 10-fold dilution of 17 g of Fe(NO₃)₃·9H₂O in 1 liter of 70 mmol/liter HNO₃). The aqueous phases are centrifuged and their absorbances measured at 530 nm. For total salicylate, 3 ml of urine and 3 ml of HCl are heated in a partially evacuated serum vial at 100 °C for 16 h and then salicylic acid is assayed in the hydrolyzed sample. Recovery of a weighed oral dose of sodium salicylate in urine was 105.4%; it was 127.9% by the Levy and Procknal method for the same sample. The improved method is faster and more accurate.

In most species, including man, salicylic acid (SA) is bio-transformed primarily by conjugation with glycine to form salicyluric acid (SU) and with glucuronic acid to form the glucuronides (1). Only a small proportion of the dose is excreted directly as unconjugated SA. Smith et al. (2) developed a colorimetric method for determination of SA metabolites in urine. The method was modified by Levy and Procknal (3), who selectively extracted SA from acidified urine with carbon tetrachloride. The SA contained in a portion of the organic phase was assayed colorimetrically after complexing the drug with ferric ion.

Here, we describe a simple, sensitive improvement of the Levy and Procknal method for assay of SA, SU, and total salicylate in urine. We also report values for urinary salicylate recovery, after oral administration of an exactly weighed amount of sodium salicylate, as determined by the Levy and Procknal method and by the improved method.

Methods

A healthy man who had fasted overnight received orally 579.7 mg of sodium salicylate (equivalent to 500 mg of SA) at 0800 hours. Food was withheld until 1200 hours. The bladder was emptied and a control urine sample was collected just before the compound was taken. Urine was collected during two intervals, 0–12 and 12–24 h after drug administration, and then 24-h urines were collected for a further three days. The urine samples were stored in a freezer until assayed.

Levy and Procknal Method

SA: Place 2 ml of urine, 0.5 ml of 6 mol/liter HCl,¹ and 30 ml of carbon tetrachloride in a glass-stoppered bottle and shake for 5 min. Centrifuge the mixture in a centrifuge tube and discard the urine phase. Transfer 10 ml of the organic phase to a glass-stoppered centrifuge tube, add 5 ml of ferric nitrate solution and shake for 5 min. Centrifuge, and measure the absorbance of the aqueous phase at 530 nm. Prepare the ferric nitrate solution freshly by diluting 5 ml of ferric nitrate stock solution [10 g of Fe(NO₃)₃ in 1 liter of 70 mmol/liter HNO₃] to 100 ml with water. Run a blank, in which water is substituted for urine. With a Bausch & Lomb Spectronic 20, a standard containing 100 µg of SA gave an absorbance reading of 0.066.

SU: Use ethylene dichloride instead of carbon tetrachloride and proceed as described above with another 2-ml urine sample. This solvent extracts both SA and SU simultaneously. A standard containing 100 µg of SU gave an absorbance reading of 0.033. A standard containing 100 µg of SA gave an absorbance reading of 0.075 when extracted with ethylene dichloride, giving a correction factor of 1.14 to account for the difference in the partitioning of SA in carbon tetrachloride and ethylene dichloride.

Total salicylate: Heat 3 ml of concentrated HCl and 3 ml of the urine at 100 °C for 16 h in a sealed 20-ml ampule. Assay for SA in 2 ml of the hydrolyzed solution.

Improved Method

SA and SU: Place 1 or 2 ml of urine sample and 0.5 ml of 6 mol/liter HCl in a 30-ml separatory funnel. Extract this solution with two 10-ml portions of carbon tetrachloride (to extract SA), followed by two 10-ml portions of ethylene dichloride (to extract SU). Collect each organic solvent separately in a 60-ml separatory funnel. To each organic solvent add 5 ml of the ferric nitrate reagent solution and shake vigorously for 2 min. Discard the organic phases. Centrifuge the aqueous phases and read their absorbances at 530 nm. The ferric nitrate reagent solution is freshly prepared by diluting 10 ml of ferric nitrate stock solution (17 g Fe(NO₃)₃·9H₂O in 1 liter of 70 mmol/liter HNO₃) to 100 ml with water. Run a blank, in which water is substituted for

¹ All chemicals used were of reagent grade.
urine. A standard containing 100 μg of SA gave an absorbance reading of 0.212 ± 0.004 (SB) and a standard containing 100 μg of SU gave an absorbance reading of 0.088 ± 0.003.

Total salicylate: Place 3 ml of the urine sample and 3 ml of concentrated HCl in a 20-ml serum vial. Seal the vial and aspirate about 50 ml of air into a syringe. Heat the vial at 100 °C for 16 h. Assay for SA in 1 or 2 ml of the hydrolyzed solution as described above.

Results and Discussion

Our results are summarized in Table 1. The values are reported without subtraction of control values. Had the background (control) contribution been subtracted from the total salicylate data, the recoveries would be 120.3% and 99.8% of the administered dose by the Levy and Procknal method and the improved method, respectively. The total salicylate recovery data by the improved method agree with data for a previous study in which human subjects were given [carboxy]14C]salicylic acid orally (4). When urine was collected for periods of 24 to 36 h, 85 to 90% of the administered dose was recovered in the urine.

Although the Levy and Procknal method has been used in many studies, apparently there have not been reports on its accuracy. Levy and Procknal reported 95–100% recovery of total salicylate (3). Also Levy and Tauchiya reported essentially 100% recovery of a dose of 1 g of acetylsalicylic acid solution in human urine (5), but these investigators did not mention the time interval during which the urine samples were collected. As can be seen from Table 1, the recovery values were 127.9% and 105.4% of the administered dose by the Levy and Procknal and the improved methods, respectively, for urine samples collected over a 96-h period. The improved method was used over a three-month period and, as a test, standard curves were made for both SA and SU, with use of at least five different concentrations each time. From these, the absorbance values for 100-μg samples of SA and SU were found to be as reported under Methods. Also the recovery values obtained for total salicylate ranged between 90.7% and 103.3%, with a mean of 97.1% and a standard error of the mean of 1.15%.

The basic differences between the two methods are: (a) The volume of organic phase used to react with the ferric ion reagent is larger in the improved method, thus leading to a higher absorbance reading and a smaller relative error. (b) The need to use a correction factor and the preparation of a third standard curve for SA in ethylene dichloride is eliminated. This eliminates a chance of extending an error in the assay of SA to the determination of SU. (c) In the improved assay, only the volumes of urine and the ferric nitrate solution need to be measured accurately. Also one centrifugation step is eliminated. The assay time is half that for the Levy and Procknal method. (d) Use of the double extraction technique with each organic solvent ensures better recoveries and therefore more accurate results.

While the principle of selective extraction of the Levy and Procknal method has not been changed, this improvement is needed when material balance studies are important, because it is more accurate. The new method is also faster. We suggest that this method could be adapted to pediatric use, in particular by use of other spectrophotometers.

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References