Journal of Chromatography, 232 (1982) 430—434
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1380

Note

Silicon-selective detection after gas chromatography for the determination of silylated salicylic acid in urine

M.A. OSMAN* and H.H. HILL, Jr.*

Department of Chemistry, Washington State University, Pullman, WA 99164 (U.S.A.)

(First received January 27th, 1982; revised manuscript received June 11th, 1982)

Silylation techniques followed by gas chromatographic (GC) separation and detection are routine analytical procedures used for the determination of polar and reactive biochemicals containing acid, alcohol or amine functional groups. Derivatives of these compounds are less polar than parent molecules and exhibit reduced retention and adsorption in GC.

A major problem, however, in the determination of these derivatives is the lack of a specific detection system. Currently, the most commonly used detector for silyl derivatives is the flame ionization detector which relies on the response of carbon atoms present in the compounds and provides no response discrimination between silicon-containing compounds of interest and other compounds in the sample matrix. A GC detector which is selective for silicon would extend the scope of silylation methods since silylation would not only serve to deactivate and stabilize compounds of interest, but would also tag these compounds with silicon atoms, providing a more sensitive and selective response.

Such a silicon-selective detector has been recently developed which can be constructed from a standard flame ionization detector by interchanging oxygen and hydrogen inlets so that the flame burns in a hydrogen atmosphere [1—3]. Also, the collecting electrode is removed from the combustion region (its standard position in a flame ionization detector) to a location more than 10 cm above the flame.

Three modes of operation are possible. The first is a non-doped mode in which pure hydrogen gas is introduced into the detector housing. In this mode, silicon-containing compounds produce responses two to three thousand times

*Present address: School of Pharmacy, University of Wisconsin, Madison, WI, U.S.A.

0378-4347/82/0000—0000/$02.75

greater than those of hydrocarbons and have a minimum detection limit of about 1 ng/sec. In the second mode of operation, a small quantity (typically 5 ppm or less) of ferrocene vapor is doped into the hydrogen atmosphere, increasing both sensitivity and selectivity. The minimum detectable limit for this mode is 15 pg/sec for silicon-containing compounds and the selectivity against hydrocarbons is on the order of 10,000. A third mode of operation in which silicon-containing compounds produce negative peaks is possible when the concentration of ferrocene vapor in the hydrogen atmosphere is increased to 30 ppm or more. In this mode silicon-containing compounds can easily be identified from hydrocarbons by virtue of their inverted peaks.

This paper demonstrates the use of this silicon-selective detector for the determination of salicylic acid in urine.

EXPERIMENTAL

Materials

The following chemicals were used: potassium hydrogen sulphate (Mallinckrodt, St. Louis, MO, U.S.A.), acetone (Burdick & Jackson Labs., Muskegon, MI, U.S.A.), hexamethyldisilazane (HMDS) (Pierce, Rockford, IL, U.S.A.), salicylic acid and chloroform (J.T. Baker, Phillipsburg, NJ, U.S.A.).

Apparatus

All analyses were conducted on a Hewlett-Packard 5710A gas chromatograph equipped with dual flame ionization detection (FID) with one detector converted to a silicon-selective hydrogen-atmosphere flame ionization detector (HAFID-Si) [3].

Chromatography

Separation was accomplished on a 10-m methylsilicone coated fused silica capillary column (Hewlett-Packard, Avondale, PA, U.S.A.). Operating temperatures were as follows: injection port and splitter, 250°C; oven temperature was programmed from 60 to 200°C at 8°C/min with a final hold period of 15 min; detector temperature, 250°C. The helium carrier gas flow-rate was maintained at 1.2 ml/min. The split ratio was 1:30. HAFID-Si gas flow-rates were as follows: helium make-up, 30 ml/min; air, 240 ml/min; oxygen, 130 ml/min; and hydrogen, 1.6 l/min.

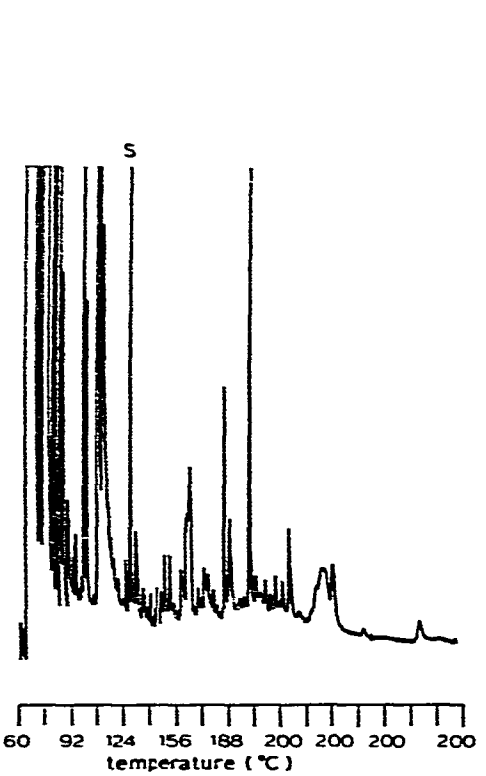
Procedure

Two hours after a volunteer had ingested 900 mg of aspirin, a 150-ml urine sample was collected. Pre-chromatography sample preparation procedures were modified from a method developed for GC by Walter et al. [4]. The sample was treated with 15 ml of a 10% solution of potassium hydrogen sulphate and then extracted with 50 ml of chloroform. After the chloroform had been evaporated to dryness under vacuum, 800 μl of HMDS and 200 μl of acetone were added to the residue. A 5-mg sample of salicylic acid was silylated in a similar manner. Silylated samples were allowed to stand for 1 h at room temperature before injection into the chromatograph.

RESULTS AND DISCUSSION

Fig. 1 represents a typical complicated chromatogram which is often obtained when non-selective detection methods such as flame ionization or thermal conductivity detectors are employed for the analysis of real samples. From retention time comparisons the peak marked S may be the component of interest but ancillary identification methods are required to ensure the accuracy of this assignment.

Fig. 2 is a chromatogram of the same sample with HAFID-Si detection in the positive mode. Since this detector is known to be selective for silicon-containing compounds, peaks which are observed can be attributed to either silylated derivatives or to large quantities of non-silylated components. When operating the detector in the negative mode, however, silicon-containing compounds produce inverted peaks while most compounds not containing silicon still respond in the normal fashion. From Fig. 3, where the sample was detected using the negative mode of the HAFID-Si, responses indicated that most of the peaks detected in the positive mode were silylated derivatives from



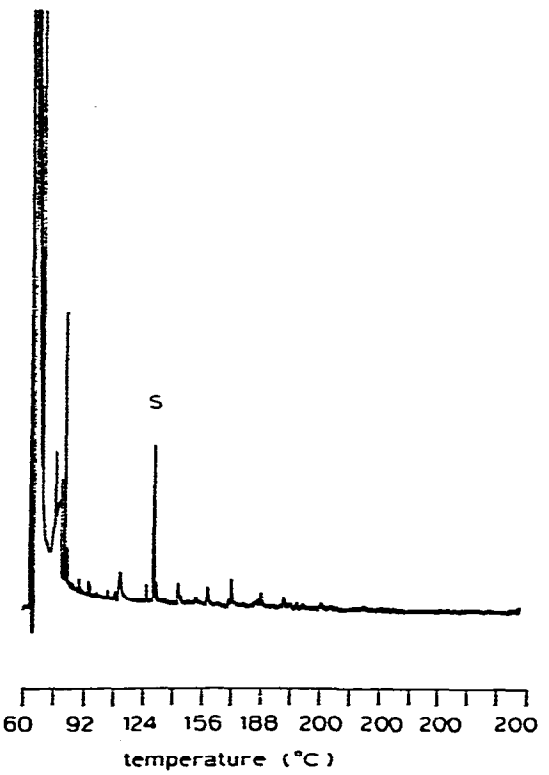


Fig. 1. FID chromatogram of silylated urine sample. S = silylated salicylic acid peak. Amount injected = 3 μl; total sample volume = 1 ml; electrometer attenuation = 8.

Fig. 2. HAFID-Si (positive mode) chromatogram of silylated urine sample. S = silylated salicylic acid peak. Amount injected = 5 μl; total sample volume = 1 ml; electrometer attenuation = 8.

the urine sample. From retention time data, the peak marked S can now be more confidently assigned to the derivatized salicylic acid. Ratios between peak areas in the negative and positive modes for the silylated salicylic acid in the standard (shown in Fig. 4) and in the sample were 3.6 and 3.7, respectively. This negative/positive response ratio provides additional qualitative evidence for the identification of the compound of interest.

Other silyl derivatives from compounds such as aspirin, benzoic acid, hydroxybenzoic acids and phenacetin may also elute from the column under

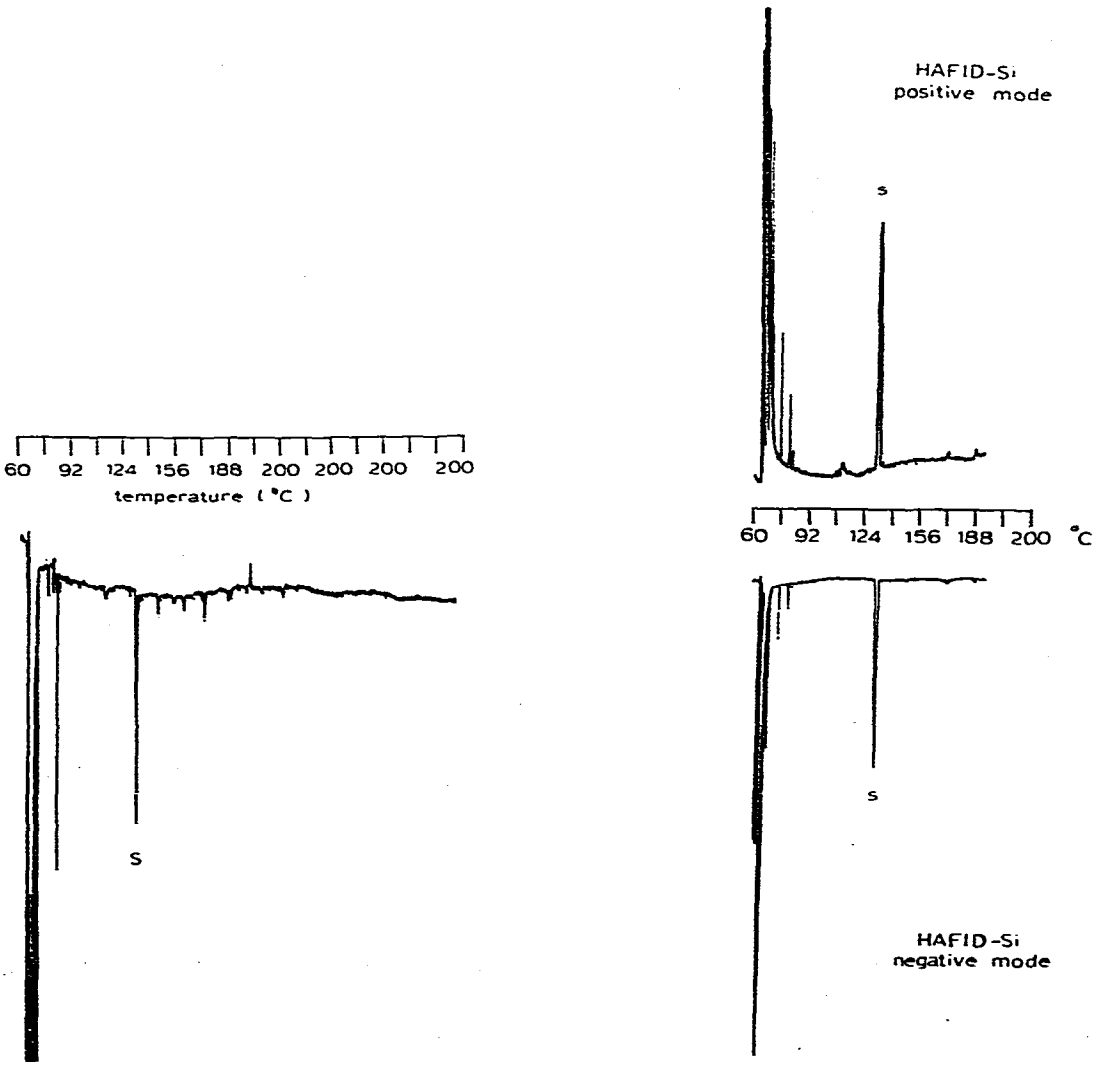


Fig. 3. HAFID—Si (negative mode) chromatogram of silylated urine sample. S = silylated salicylic acid peak. Amount injected = 5 μl; total sample volume = 1 ml; electrometer attenuation = 16.

Fig. 4. HAFID—Si chromatograms of silylated salicylic acid standard. Amount injected = 15 ng per 3 μl. Electrometer attenuation: positive mode = 8, negative mode = 32.

the operating conditions employed. Some of the peaks which were observed in the positive and negative mode HAFID-Si chromatograms may be attributed to these or similar compounds, although none has been confirmed in this study. The large off-scale peaks at the beginning of each chromatogram were due to the silylating reagent and/or silylated contaminants in the reagent (e.g. water) since these peaks were also found in blanks. Acetone, which gives a diminutive positive response in both positive and negative modes, elutes with the derivatizing reagent under these chromatographic conditions and is obscured by the large response of silicon in the reagent.

Two silylated derivatives are possible from the derivatization of salicylic acid, corresponding to 2-trimethylsilyloxybenzoic acid and trimethylsilyl 2-trimethylsilyloxybenzoate. In this study only one predominant peak was observed when the standard was derivatized, indicating that the silylation procedure was quantitative.

The linear response range for silicon compounds has been determined to be about three orders of magnitude for the positive mode [3]. The concentration of salicylic acid in the urine sample was determined to be 9.3 μg/ml from the positive mode and 9.0 μg/ml from the negative mode. These values differ by less than 4% and are in agreement with the level of concentration that is expected for such a sample.

CONCLUSIONS

Silicon-selective detection by both positive and negative modes, when used in conjunction with a standard, provides a more reliable method of identification of silylated derivatives in complex samples than commonly employed non-selective detectors such as flame ionization or thermal conductivity. The ease of conversion from FID to HAFID-Si makes this detector a particularly attractive alternative for qualitative and quantitative analysis when silylation procedures are employed.

ACKNOWLEDGEMENT

This research was supported by grant No. GM 24970 from the Public Health Service.

REFERENCES

1 H.H. Hill, Jr. and W.A. Aue, J. Chromatogr., 140 (1977) 1.
2 M.A. Osman, H.H. Hill, Jr., M.W. Holdren and H.H. Westberg, Anal. Chem., 51 (1979) 1286.
3 M.A. Osman and H.H. Hill, Jr., J. Chromatogr., 213 (1981) 397.
4 L.J. Walter, D.F. Biggs and K.T. Coutts, J. Pharm. Sci., 63 (1974) 1754.