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## Note

# Control of trimethylsilylation potential and trimethylsilylation capacity by the use of colour indicators

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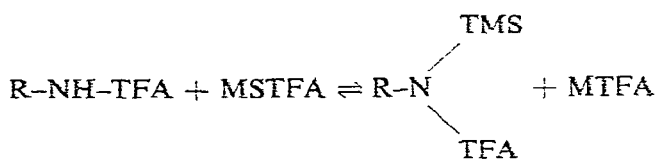
The selective N-trifluoroacylation-O-trimethylsilylation of phenol alkylamines, hydroxyamines and amino acids as well as their corresponding hydrochlorides has been described recently[1–3]. The trimethylsilylating agent used was MSTFA*, followed by MBTFA as trifluoroacylating agent. This reaction sequence offers four significant advantages compared with conventional derivatization techniques. (1) MSTFA and MBTFA are highly volatile compounds. The reaction mixture alone or together with a suitable solvent[4] may be injected directly, with no adverse effect on the column performance and life-time. (2) N-TFA-O-TMS derivatives are stable in solution for weeks or months, even at the parts per billion level. (3) The derivatives have excellent gas–liquid chromatographic (GLC) properties. (4) Especially in the case of phenol alkylamines, aromatic amino acids and indole alkylamines[5], the derivatives give rise to a characteristic fragmentation pattern in mass spectrometry.

The application of this selective derivatization, however, is restricted by the equilibrium which exists between excess of MSTFA and the resulting secondary N-trifluoroacetamides (eqn. 1). Thus, starting with primary amine groups, two GLC signals may be obtained for each compound.




(1)

This is the case if the concentration of MSTFA in the reaction medium is high compared with that of MTFA (Fig. 1). Therefore the trimethylsilylation conditions must be carefully controlled.

For routine GLC analysis the following steps were taken in order to cause the equilibrium to shift to the left and to maintain a constant trimethylsilylation potential as defined by the equilibrium constant of eqn. 1 (ref. 1). (1) The addition of glycine

* Throughout this article the following abbreviations are used: TMS = trimethylsilyl; TFA = trifluoroacetyl; MSTFA = N-methyl-N-trimethylsilyltrifluoroacetamide; MBTFA = N-methyl-bis-(trifluoroacetamide); MSHFB = N-methyl-N-trimethylsilylheptafluorobutyramide; and TMSCl = trimethylchlorosilane; MTFA = N-methyltrifluoroacetamide.

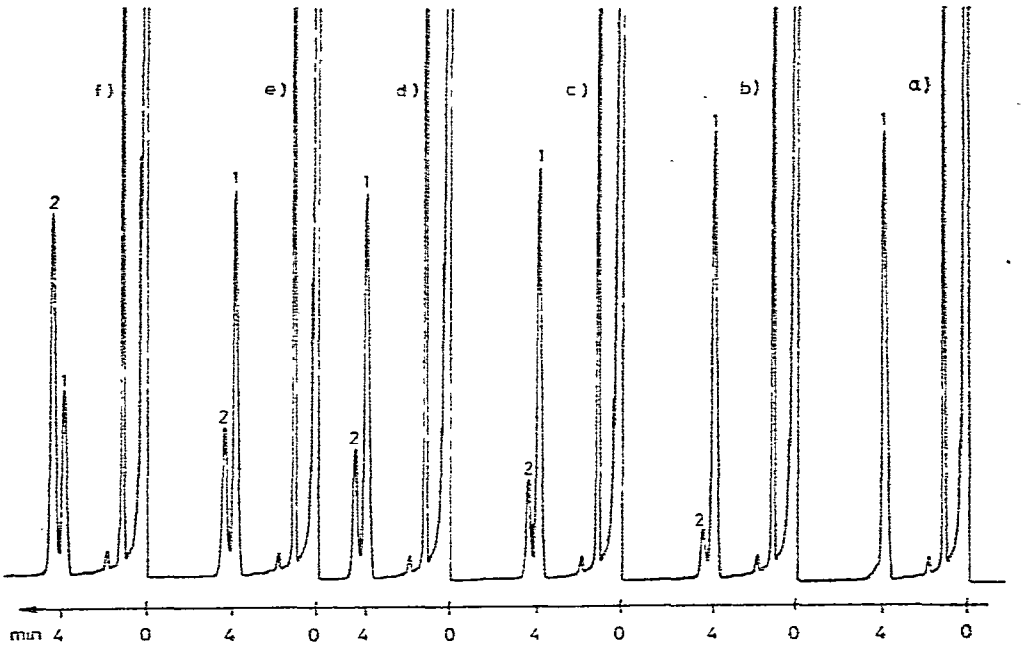
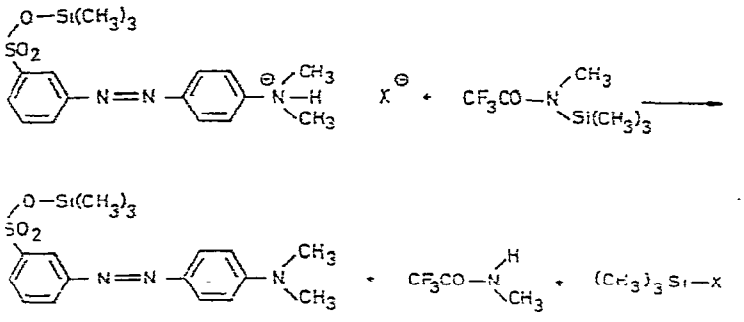



Fig. 1. Gas chromatograms of N-TFA-O-TMS-noradrenaline (1) and N-TFA-N,O-bis(TMS)-noradrenaline (2) obtained from trimethylsilylation mixtures. Silylating potential (moles of MSTFA:moles of MTFA in acetonitrile) = 1:10 (a), 1:3 (b), 1:1 (c), 3:2 (d) and 2:1 (e). Pure MSTFA was used as the solvent in (f). For GC conditions, see Fig. 2 and ref. 1.

or oxalic acid to the sample*. (2) The addition of trifluoroacetic acid, methanol or diethylamine to the reaction medium. (3) The use of a trimethylsilylation medium containing a known ratio of MSTFA to MTFA. For pure compounds the necessary amounts of the trimethylsilylation mixture and buffer may be calculated, but in samples of biological origin the number of reacting groups (including water) is not known exactly and must be determined experimentally. This is not an elegant procedure for routine work.

The above difficulties may be circumvented if small amounts of indicators of the azo-dye type are added to the sample. Methyl orange, a well-known indicator in titrimetry, changes colour in trimethylsilylation mixtures according to the silylation potential, exactly as it does in aqueous solution according to the pH value. When sufficient MSTFA is present to react with the anion of the azo dye, the colour change from red to yellow (eqn. 2). Three conclusions can be made if the colour of the reaction mixture turns yellow according to eqn. 2. (1) The amount of N-TMS-amide is sufficient for all functions having relative low trimethylsilylation potentials, *e.g.*, HO–, HS– and HOOC– groups. The molar amount of such groups which can be trimethylsilylated is

* Both of these compounds, which use MSTFA and generate MTFA, are capable of serving as TMS-donor for alcoholic or phenolic hydroxyl groups. Their function in the reaction media may be that of a "trimethylsilylating buffer", maintaining the required silylation potential and supplying sufficient silylation capacity (see ref. 1 for a detailed discussion).



(2)

equal to the trimethylsilylation capacity. (2) Primary or secondary amines are not or not yet fully trimethylsilylated, so that they react rapidly with MBTFA or other bis-(acylamide) compounds[1], a basic requirement for preparing derivatives for analytical purposes. (3) The silylation potential, primarily determined in trace analysis by the ratio of MSTFA to MTFA, is low, so that the equilibrium in eqn. 1 is shifted to the left.

In addition to methyl orange, other dyes of this type may be used, *e.g.*, methyl red or ethyl red. Methyl orange, however, is preferred because it is not eluted from the column. If large amounts of bases like TMS-imidazole, TMS-amines or pyridine are present, a colour change is not observed. This is due to the fact that silylating agents of the amine type or basic solvents compete with the dimethylamine group of the indicator for the acid. However, as the preferred trimethylsilylation systems for analytical purposes contain N-TMS-amides and an acidic catalyst, the trimethylsilylation potential may be visually controlled. Some preferred systems are MSTFA–TMSCl–acetonitrile; MSHFB–TMSCl–acetonitrile and MSTFA–trifluoroacetic acid[4].

The observation that indicators change their colour according to the amount of MSTFA present is of obvious importance. In particular it is no longer necessary to use a large excess of trimethylsilylating reagent. The extent of trimethylsilylation of any sample, even after storage for a long time, can therefore be easily evaluated from the colour of the reaction mixture.

## EXPERIMENTAL AND RESULTS

### *Selective N-acylation-O-trimethylsilylation under visually controlled conditions*

*The trimethylsilylation step.* An aqueous solution of the amines or amino acids acidified with hydrochloric acid was generally used. 10 $\mu$g of the sodium salt of methyl orange (10 $\mu$l of a 1 mg/ml methanolic solution) were then added. The sample was brought to dryness by vacuum evaporation, freeze drying, etc. The residue was dissolved in the minimum amount of trifluoroacetic acid (or heptafluorobutyric acid) and MSTFA (or MSHFB) was added[4] with the aid of a microlitre syringe. The amount of N-TMS-amide needed to reach the equivalence point was noted and an excess of N-TMS-amide (usually 10 vol.%) was then added. Alternatively, the residue was dissolved in acetonitrile–MSTFA–TMSCl or acetonitrile–MSHFB–TMSCl. The amount of trimethylsilylation mixture was increased stepwise until the yellow colour persisted; a 10% excess of the mixture was then added (see Fig. 2a).

If the *a priori* addition of methyl orange to the sample is inconvenient, the

extent of trimethylsilylation may be controlled by using the above reagent mixtures containing 20–50 μg of indicator per millilitre. Stock solutions of methyl orange were prepared by dissolving the indicator acid in acetonitrile–MSTFA (9:1, v/v). If the sodium salt of methyl orange is used, TMSCl or trifluoroacetic acid must be added and sodium salt removed by centrifugation. Samples trimethylsilylated by a large excess of N-TMS-amides were titrated by the addition of trifluoroacetic acid to the equivalence point. A further amount of the N-TMS-amide (MSTFA or MSHFB) was then added (5–10% of the original volume of N-TMS-amide). Heat in trimethylsilylation is often unnecessary and should only be applied if it is not deleterious to the compounds to be analysed. Heating under reflux may be an excellent means of flushing the surfaces of the reaction vessels and concentrating the compounds in a small volume of reagent. The droplets of the reagents and solvents, which condense on the vessel walls on cooling, can be collected by centrifuging.

*The acylation step.* 5–25 μl of MBTFA were added to the trimethylsilylated samples at room temperature (see Fig. 2b and c). If in the course of the reaction, on

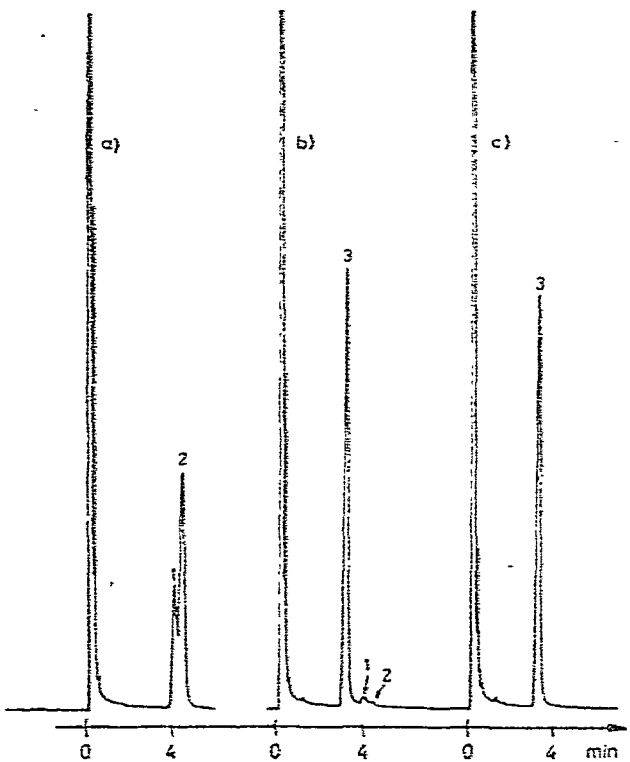



Fig. 2. Tyrosine as an example of trimethylsilylation under controlled conditions. (a) A solution o 1.8 mg of L-tyrosine in 100 μl of trifluoroacetic acid containing 20 μg of methyl orange was titrate with MSTFA to the equivalence point (360 μl of MSTFA). An excess (40 μl) of MSTFA was the added. The sample was heated and a 1-μl amount was injected into the column (1.06 m, packed wit 3% OV-17 on Chromosorb Q; temperature, 180°). For further details of the GC analysis see ref. 1 (b) 12 min after addition of 20 μl of MBTFA to the sample. (c) 20 min after the addition of MBTFA Peaks: trimethylsilyl esters of N,O-bis(TMS)-tyrosine (1); O-TMS-tyrosine (2) and N-TFA-O-TMS tyrosine (3).

standing or on opening the vessels the colour changes to orange or red, MSTFA (or MSHFB) must be added in order to protect hydroxyl, thiol or carboxyl groups from attack by MBTFA.

ACKNOWLEDGEMENT

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