

## Short Communication

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# Simple gas chromatographic method for the assay of salts of carboxylic acids as their trimethylsilyl derivatives

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### ABSTRACT

Several potassium and sodium salts of monobasic, dibasic and tribasic organic acids, and sodium and calcium salts of fatty acids were directly silylated by Tri-Sil, a commercially available silylating reagent composed of a mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine. The reaction mixtures were injected directly into a gas chromatograph without any further preparation. Quantitative GC peak area measurements indicated that the yields of trimethylsilyl (TMS) derivatives of the salts and the free acid counterparts were comparable. Identities of the derivatives were confirmed by GC-MS analysis. Carbon chain distributions of the fatty acid salts were determined by GC quantitation of the TMS derivatives formed by direct silylation of the salts and the fatty acids liberated from the salts. The present study indicates that the two results agree with each other favorably.

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### INTRODUCTION

Salts of carboxylic acids are important chemicals in many facets of industry. For example, potassium sorbate, sodium benzoate and disodium tartrate are often used as food additives; trisodium nitrilotriacetate (NTA) and sodium salts of ethylenediaminetetraacetic acids (EDTA) are sequestering agents; and alkaline salts of fatty acids are used as soaps, as grease thickeners and for other purposes. Their compositions are often determined by gas chromatography (GC) for various purposes. These highly polar chemicals need to be converted to less

polar compounds to be amenable for GC analysis. Generally they are acidified to form free acids which are then separated and esterified [1–4] or silylated [5]. It is highly desirable to develop a direct derivatization method which does not require any sample work-up before GC analysis. Such method is particularly useful in cases where the acids are volatile and may be difficult to recover from the extraction solvent.

Direct methylation by  $\text{BF}_3\text{-MeOH}$  has recently been reported for fatty salts of Zn, Mg and Ca [6]. At the end of the reaction, sodium carbonate was added to the reaction mixture which was subsequently injected into the gas chromatograph. Esterification by  $\text{BF}_3$  is generally applicable to fatty acids only. Polybasic acids or salts have not been reported to be derivatized by this method. Direct trimethylsilylation of sodium and

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other salts of several short-chain organic acids by reacting the salts with N,O-bis(trimethylsilyl)acetamide and trimethylchlorosilane in pyridine has been mentioned [7,8]. It is not clear, however, if the derivatization reactions are quantitative, and if the method is applicable to silylating fatty acid salts.

This paper describes a simple and fast GC procedure for the assay of several salts of fatty acids, short-chain monobasic and polybasic carboxylic acids as their trimethylsilyl (TMS) derivatives. The salts were directly derivatized quantitatively in a single-step reaction by Tri-Sil, a commercially available silylation cocktail, and the derivatives were analyzed by GC and GC–mass spectrometry (MS) without any further sample pretreatment.

## EXPERIMENTAL

### Reagents

Tri-Sil and acetonitrile were purchased from Pierce (Rockford, IL, USA). Concentrated hydrochloric acid, methylene chloride, HPLC grade, and calcium disodium salt of EDTA were obtained from J.T. Baker (Toronto, Canada). Sorbic acid, anhydrous and potassium sorbate were purchased from Sigma (St. Louis, MO, USA). Tartaric acid 99%, disodium tartrate dihydrate 99%, nitrilotriacetic acid 99%, trisodium nitrilotriacetate monohydrate 99%, benzoic acid 99%, sodium benzoate 99% were obtained from Aldrich (Milwaukee, WI, USA). Technical-grade calcium stearate, which is a mixture of calcium salts of fatty acids, was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Disodium and tetrasodium dihydrate salts of EDTA, and sodium soap were samples supplied by Canadian customs inspectors.

### Derivatization at 70°C

TMS derivatives of potassium sorbate, sodium benzoate, disodium tartrate, trisodium nitrilotriacetate and their acids were prepared by heating a solution of 18 mmol of the salt or acid in 1 ml of Tri-Sil in a Reacti-vial equipped with a PTFE-faced septum at 70°C with stirring. Aliquots of 100  $\mu$ l of the reaction mixture were withdrawn at 0.5, 3 and 24 h. After cooling to room tempera-

ture, 1  $\mu$ l was injected into a GC–flame ionization detection (FID) with a split ratio of 100:1. GC analysis was done in triplicate for each reaction time. To confirm the identities of the derivatives, 1  $\mu$ l of each reaction mixture at 0.5 h was injected into the Hewlett-Packard GC–MS system with a split ratio of 100:1 for MS analysis.

Sodium soap and technical-grade calcium stearate were derivatized by stirring a solution of 10 mg of the fatty acid salts in 1 ml of Tri-Sil at 70°C. A 1- $\mu$ l volume was injected into the GC–FID system and the Finnigan GC–MS system with a split ratio of 100:1 after 1 h of reaction. Fatty acids generated from their salts as described below were silylated in the same manner.

### Preparation of fatty acids from sodium soap and technical-grade calcium stearate

Free fatty acids were generated by stirring 0.5 g of the sodium and calcium salts in equal volumes (15 ml) of methylene chloride and 6 M HCl at room temperature for 1 h. At the end of the reaction, the organic layer was separated and washed with 2  $\times$  15 ml water. After drying over anhydrous sodium sulfate, the methylene chloride solution was filtered. The filtrate was evaporated to obtain the free fatty acids.

### GC–FID

A Hewlett-Packard Model 5840A gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard Model 5840A integration was employed. The column used was a 15 m  $\times$  0.25 mm I.D., 0.25  $\mu$ m film thickness DB5 (5% phenyl methylsilicone) fused-silica column. The injector and detector temperature were 250°C and 300°C, respectively. After an initial hold time of 2 min at 100°C, the oven temperature was programmed at 10°C/min until 280°C; helium was used as a carrier gas at a linear velocity of 35 cm/s at 250°C.

### GC–MS

The Hewlett-Packard system was composed of a HP 5890 gas chromatograph interfaced to a HP 5970 mass-selective detector via an open-split interface held at 290°C. The mass-selective detector was equipped with an electron impact (EI)

source. The system was controlled by a Pascal series Chemstation. The GC instrument was operated with a 12 m × 0.2 mm I.D., 0.33 μm film thickness HP-1 (methylsilicone) column. Injector temperature was 250°C. The oven temperature was programmed as follows: initial temperature set at 100°C, hold for 2 min then programmed at 10°C/min to a final temperature of 280°C. Helium was the carrier gas at a linear velocity of 41 cm/s at 250°C. EI spectra were obtained at 75 eV with the mass-selective detector scanning at 0.76 scan/s in the range 39–600 u.

The Finnigan system is composed of a Model 1020 OWA mass spectrometer coupled to a Perkin-Elmer Sigma-3B gas chromatograph via a direct interface maintained at a temperature of 300°C. The system is controlled by a Nova 4 data system. The oven temperature program used was the same as for the Hewlett-Packard system. The column was a 15 m × 0.25 mm I.D., 0.25 μm film thickness DB5 (5% phenyl methylsilicone) fused-silica column. Helium was the carrier gas at a linear velocity of 35 cm/s at 250°C. The mass spectrometer was operated in the electron impact mode at 70 eV and a mass range of 39 to 600 u was scanned at a rate of 1 s/scan. The ion source was held at 90°C.

## RESULTS AND DISCUSSION

### Identification of the formed derivatives

Each salt and its corresponding acid yielded the same derivative based on GC retention time and mass spectral evidence. All derivatives yielded discernible  $M^+$  and  $[M - 15]^+$  peaks in their EI spectrum. The degree of silylation of each TMS derivative was determined by the molecular mass derived from these ions. As shown in Table I, the monobasic, tribasic and tetrabasic salts yield mono-, tri- and tetrasilylated derivatives, respectively. For the dibasic tartrate, the derivative detected was a tetrasilylated compound in which the two hydroxy groups were also silylated.

Fragmentation patterns of the derivatives were consistent with their structures. All derivatives except trimethylsilyl esters of fatty acids yielded the fragment ions  $[M - 117]^+$  resulting from loss of COOTMS. For the fatty compounds, the ion at  $m/z$  117 appeared as an intense peak since it is a more stable ion than the  $[M - 117]^+$  fragments. In general,  $[M - 59]^+$  fragments formed from loss of  $CH_3$  and  $CO_2$  were observed in the mass spectra of TMS derivatives of monobasic carboxylates which are sorbate, benzoate and the fatty compounds. For carboxylates contain-

TABLE I  
AVERAGE GC RESPONSES OF TMS DERIVATIVES AFTER ½-h REACTION

Samples	GC peak area counts <sup>a</sup> (relative standard deviation, %)	Derivatives
Potassium sorbate	14 955 (4.6)	$CH_3CH=CHCH=CHCOO(TMS)$
Sorbic acid	14 182 (3.1)	
Disodium tartrate	20 737 (6.4)	$[CHO(TMS)COO(TMS)]_2$
Tartaric acid	21 277 (3.5)	
Trisodium nitrilotriacetate	17 853 (4.0)	$N[CH_2COO(TMS)]_3$
Nitrilotriacetic acid	18 197 (2.5)	
Sodium benzoate	18 080 (2.3)	$C_6H_5COO(TMS)$
Benzoic acid	17 406 (0.4)	
Sodium soap and technical-grade Ca stearate; their fatty acids		$RCOO(TMS)$
Disodium and tetrasodium EDTA		$-[CH_2N(CH_2COOTMS)_2]_2$

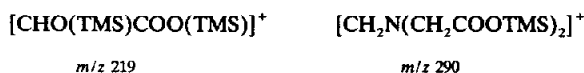
<sup>a</sup> Each number presented is the average count of three GC runs of each TMS derivative, which was formed by reacting equal molar amounts (18 mmol) of acids or salts in 1 ml Tri-Sil.

TABLE II

ACID COMPOSITION PROFILES OF CRUDE FATTY ACID SALTS —COMPARISON OF DIRECT TRI-METHYLSILYLATION VS. VIA ACIDS

Sample	C <sub>10</sub>	C <sub>12</sub>	C <sub>14</sub>	C <sub>16</sub>	C <sub>17</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	C <sub>18</sub>
Na soap	1.1	9.5	5.9	23.8		34.7	5.8	19.2
Acids converted from Na soap	1.0	9.3	6.2	23.4		34.9	6.2	19.0
Technical-grade calcium stearate			4.2	51.5	1.4	2.3	2.1	38.5
Acids converted from calcium stearate			3.4	51.7	1.7	2.4	2.2	38.6

ing amino functions such as NTA and EDTA,  $\alpha$ -cleavage was a desirable fragmentation pathway yielding ion  $m/z$  290 as the base peak.  $\alpha$ -Cleavage also occurred in tartrate which contains hydroxy functions to give the ion at  $m/z$  219.



#### *Derivatization conditions and quantitative evaluation*

For potassium sorbate, sodium benzoate, disodium tartrate and trisodium nitrilotriacetate, reaction mixtures of the salts and their acids in Tri-Sil at 70°C were analyzed by GC at 0.5, 3 and 24 h. For all the reactions, it was noted that the GC responses remained essentially unchanged over the period of 24 h, indicating that derivatizations were completed in the first half hour of reaction. As shown in Table I, comparable area counts were obtained for the salts and the free acids. It is well known that Tri-Sil is a potent silylating reagent for carboxylic acids [9,10], this study has thus shown that it silylates the salts as quantitatively as their acid counterparts.

Disodium and tetrasodium EDTA were also silylated in a single-step reaction by Tri-Sil. Although the reactions were not monitored quantitatively, the tetrasilyl derivative yielded good response in GC-FID after 2 h stirring at 70°C. Disodium calcium EDTA, however, failed to show any GC peak even after overnight reaction at 70°C.

Unlike the calcium salt of polybasic EDTA, calcium fatty acid salts were readily silylated by Tri-Sil after 1 h of reaction at 70°C. GC chromatograms obtained by direct silylation of the technical-grade calcium stearate were the same as that obtained by silylating the fatty acids liberated from the salts. The responses of the corresponding components in both chromatograms were comparable by visual examination, indicating that the peaks observed in the chromatogram obtained from direct silylation of the calcium salt were not due to the derivatives of the residual free fatty acids only, which could be present in the technical grade fatty salt used in this study. The same observation was also recorded for sodium soap and its acids.

Each homologue distribution presented in Table II is, therefore, the total composition of the acid salt, and residual fatty acids possibly present in the sample. Carbon chain distributions were determined from GC peak areas, assuming equal response factors for all the homologues. The results indicated that the homologue distributions determined by direct silylation of the soaps were in good agreement with those determined by the conventional method which involves conversion of the fatty acid salts into their acids.

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