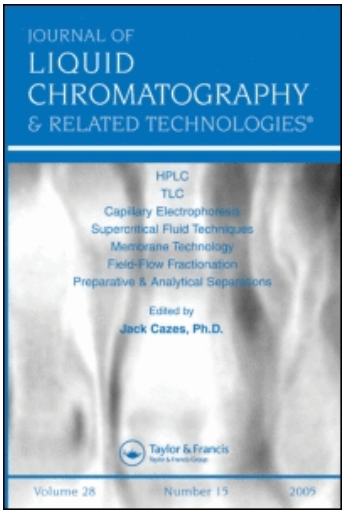

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HPLC Separation of Positional Isomers of Trifluorophenylacetic Acid and its Starting Material

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Abstract: Trifluorophenylacetic acid (TFPAA) was synthesized from 2,4,5-trifluorophenyl bromide and malonic acid starting materials as part of process development for a pharmaceutical intermediate. Impurity profile methods, sensitive to positional isomers, have been developed for 2,4,5-trifluorophenyl bromide and 2,4,5-TFPAA. The HPLC impurity profile method for 2,4,5-TFPAA was validated consistent with ICH guidelines. An HPLC weight percent assay method was also developed and validated for 2,4,5-TFPAA. Residual malonic acid in 2,4,5-TFPAA was determined with a weight percent method by GC. Non-positional isomer impurities in 2,4,5-TFPAA were identified by LC-MS.

Keywords: HPLC, LC-MS, GC, 2,4,5-Trifluorophenyl bromide, 2,4,5-Trifluorophenylacetic acid, Positional isomers

INTRODUCTION

A general method for the synthesis of α-aryl malonates is the coupling of an aryl iodide and diethyl malonate in the presence of Cs_2CO_3 and catalytic amounts of copper(I) iodide and 2-phenylphenol.[1] The same synthesis can be applied to α-substituted carboxylic acids, which can be produced by the alkylation of esters of malonic acid followed by hydrolysis and

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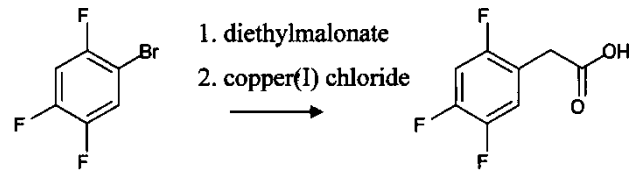
decarboxylation.[2,3] 2,4,5-Trifluorophenylacetic acid (2,4,5-TFPAA) can also be prepared in this manner by linking diethylmalonate to 2,4,5-trifluorophenyl bromide with the use of copper(I) chloride as a catalyst, followed by hydrolysis and decarboxylation as shown in Scheme 1.[4,5]

2,4,5-Trifluorophenylacetic acid (TFPAA) is an intermediate that was used in a chemical process towards a drug candidate at Merck & Co., Inc. It is critical to control the levels of the undesired positional isomers in the 2,4,5-trifluorophenyl bromide starting material and the TFPAA product, since there is no rejection of the undesired positional isomers downstream in the process to isolate drug substance. Therefore, a suitable analytical method for screening positional isomers in the bromophenyl starting material and 2,4,5-TFPAA product is necessary.

Positional isomers pose a challenging separation to an analytical chemist. Analytical techniques commonly used for the separation of various types of positional isomers are gas chromatography (GC),[6] high performance liquid chromatography (HPLC) in reversed-phase mode (RP-HPLC),[7] supercritical fluid chromatography (SFC),[8] and capillary zone electrophoresis (CE).[9] Separation mechanisms of various types of positional isomers have been extensively studied. Zhou et al.[7] indicated that the elution order of DFPAA positional isomers in RP-HPLC was influenced by the differences of hydrophobicity of the solutes. The elution order of the positional isomers of DFPAA was similar for Zorbax C_8, Rx C_8 and XDB C_8 columns. It was speculated that the selectivity observed with these column types was due to the large sterically protecting group (diisopropyl n-octyl) that effectively shielded the deprotonated silanol sites of the stationary phase.[7] Despite recent advances in the separation of positional isomers, the elution order is still difficult to predict since many factors are involved, such as polarity of the mobile phase and solute dipole moment.[10]

This paper will describe various sensitive purity-indicating methods, which were developed to support the preparation of 2,4,5-TFPAA and the relevant figures of merit established for each method during validation studies. Additionally, through these separation systems developed, efforts will be made to understand the underlying physicochemical driving forces for the chromatographic selectivities observed.



Scheme 1.

EXPERIMENTAL

Chemicals and Reagents

HPLC grade acetonitrile, methanol, isopropyl acetate, malonic acid, orthophosphoric acid, sodium hydroxide, anhydrous pyridine, 1,1,1,3,3,3-hexamethyldisilazane (HMDS), and chlorotrimethylsilane (TMS-Cl) were all obtained from Fisher Scientific (Fair Lawn, NJ). The positional isomers of 1-bromo-2,4,5-trifluorobenzene were obtained from Aldrich (Milwaukee, WI) and the positional isomers of 2,4,5-TFPAA were obtained from JRD Fluorochemicals Ltd. (England). All were used without further purification. Deionized water for HPLC was purified using a Hydro water filtration system (Hydro Service & Supplies, Inc., Garfield, NJ). TFPAA products and reaction mixtures were provided by Process Research at Merck Research Laboratories (Rahway, NJ).

High Performance Liquid Chromatography

The HPLC experiments were performed on a Thermo Separation Products (TSP) quaternary pump model P4000, a TSP autosampler model AS1000 with a fixed 10 μL injection loop, and a TSP UV absorbance detector model UV1000. A Waters Symmetry C_{18} (250 × 4.6 mm, 5 μm) column was used for the analysis. The aqueous mobile phase (A) was 0.1% aqueous (v/v) phosphoric acid. The organic mobile phase (B) was pure acetonitrile. The mobile phase gradient went from 10% to 35% B from 0 to 10 minutes, 35% to 80% B from 10 to 30 minutes (held for 5 minutes). A column re-equilibration time of 15 minutes was used between each injection for a total run time of 50 minutes. The system was run with a flow rate of 1.0 mL/min, an injection volume of 10 μL, and UV detection at 210 nm. The target assay concentration of the 2,4,5-TFPAA samples was 0.6 mg/mL in acetonitrile diluent. A stock solution of 2,4,5-TFPAA (123% of target concentration) was prepared by accurately weighing 73.8 mg of sample into a 100 mL volumetric flask and diluting to volume with diluent. The other sample solutions from 100% to 1.0% of the method target concentration were prepared by serial dilution from the 123% stock solution. Injections were made in triplicate for each sample solution.

HPLC-MS Method Conditions

LC-MS experiments were performed using an Agilent Technologies Model 1100 HPLC with a Finnigan TSQ 7000 mass spectrometer. The mobile phase was water : acetonitrile with 0.02% (v/v) formic acid. Atmospheric Pressure Chemical Ionization (APCI) was used in the negative ion mode.

The vaporizer was operated at 450°C, the interface capillary was 150°C and the manifold was 70°C. The corona discharge was operated at 5 μamps. The sheath and auxiliary gases were nitrogen operated at 80 psi and 30 units respectively. Chloroacetonitrile, neat, was added post-column at 10 μL/min to generate ions as the chloro adducts, $M + Cl^-$. In full scan (MS) mode, the scan range was from 100 to 800 amu using a dwell time of 2.8 msec. In product ion (MS-MS) mode, the collision gas was argon using a cell pressure of 2.2 mtorr. The collision energy was −15 eV. Unit resolution was used for MS mode, and was increased to 3 FWHH for parent ions in MS-MS mode.

Gas Chromatography Conditions

A Hewlett-Packard 5890 gas chromatograph with flame ionization detection (FID) was used for all GC analysis. A Restek Rtx-1 (60 m × 0.32 mm) column with a 0.25 μm film thickness was used. Column temperature was held isothermally for 35 min. at 90°C. Other experimental conditions included split injection (100 mL/min split flow) at 275°C, an FID temperature of 275°C, and a column flow rate of approximately 4.0 mL/min.

A TMS reagent solution was prepared from the additions of 27 mL of anhydrous pyridine, 9 mL of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and 3 mL of chlorotrimethylsilane (TMS-Cl). A 3 mL of TMS reagent solution was added to TFPAA samples and the solutions were allowed to stand for 30 minutes to afford the corresponding TFPAA-TMS esters for GC analysis. The sample solution is stable for at least eight hours under ambient laboratory conditions.

Malonic Acid Determination

A Hewlett-Packard 5890 gas chromatograph was used with a Restek Rtx-1 (30 m × 0.32 mm) with a 1.0 μm film thickness. The temperature gradient consisted of an initial starting temperature of 100°C to 200°C at 10°C/min. Other experimental conditions included a split injection (100 mL/min split flow) at 275°C, an FID temperature of 275°C, and a column flow rate of 4.0 mL/min. A 3 mL of TMS reagent solution was also employed, as shown above. The following calculations were used to determine the weight percent of malonic acid:

$$wt\%_{\text{Malonic acid}} = \frac{Cts_{\text{Sample}} \times Wt_{\text{Standard}} \times Vol_{\text{Sample}}}{D \times Vol_{\text{Standard}} \times Cts_{\text{Standard}} \times Wt_{\text{Sample}}} \times 100$$

where

Cts_{Sample} is the average peak area (in counts) of the sample injections

$Wt_{Standard}$ is a weight of the standard (in mg) in the stock solution

Vol_{Sample} is the volume of the sample solution (in mL)

D is the dilution factor of the standard used for the calculation

$Vol_{Standard}$ is the volume of the standard stock solution (in mL)

$Cts_{Standard}$ is the average peak area (in counts) of the standard injections

Wt_{Sample} is the weight of the sample (in mg).

RESULTS AND DISCUSSION

In general practice, the gas chromatographic separation of very polar isomers with nonpolar stationary phases is not very effective since such phases often promote selectivity based principally upon differences in solute vapor pressures, and less upon differences in chemical interactions between analytes and stationary phase.[11] Conversely, polar stationary phases in GC often promote separations of polar compounds via selectively retaining solutes through specific polar interactions, and are often a better choice for such analytes.[12] The product positional isomers in Scheme 1 are polar analytes (carboxylic acids), however such compounds are not adequately volatile for direct gas chromatographic analysis and require pre-analysis derivatization to less polar species. Therefore, an RTX-1 (dimethyl polysiloxane) stationary phase was selected to probe positional isomer selectivity for both the starting material and derivatized product of Scheme 1.

Table 1 summarizes the elution order of the trifluorophenyl bromide (TFPBr) and derivatized trifluorophenyl acetic acid (TFPAA-TMS) positional

Table 1. Vapor pressure (torr) calculated[a] at 25°C versus elution order on Rtx-1 column

Elution order	Compound	Vapor pressure (torr) @ 25°C	Compound	Vapor pressure (torr) @ 25°C
1	2,4,6-TFPAA-TMS	0.061	3,4,5-TFPBr	4.57
2	2,3,5-TFPAA-TMS	0.054	2,4,6-TFPBr	9.38
3	2,4,5-TFPAA-TMS	0.041	2,3,5-TFPBr	4.85
4	2,3,6-TFPAA-TMS	0.048	2,4,5-TFPBr	6.53
5	3,4,5-TFPAA-TMS	0.041	2,3,4-TFPBr	4.43
6	2,3,4-TFPAA-TMS	0.037	2,3,6-TFPBr	4.70

[a]Calculated using advanced chemistry development (ACD) physico-chemical batch version 4.63.

isomers and the calculated vapor pressure for each isomer. Several trends are apparent. First, the vapor pressures of the bromide isomers are much higher than those of the corresponding derivatized products, predicting the shorter retention times observed for TFPBr compounds in Figures 1a and 1b. Second,

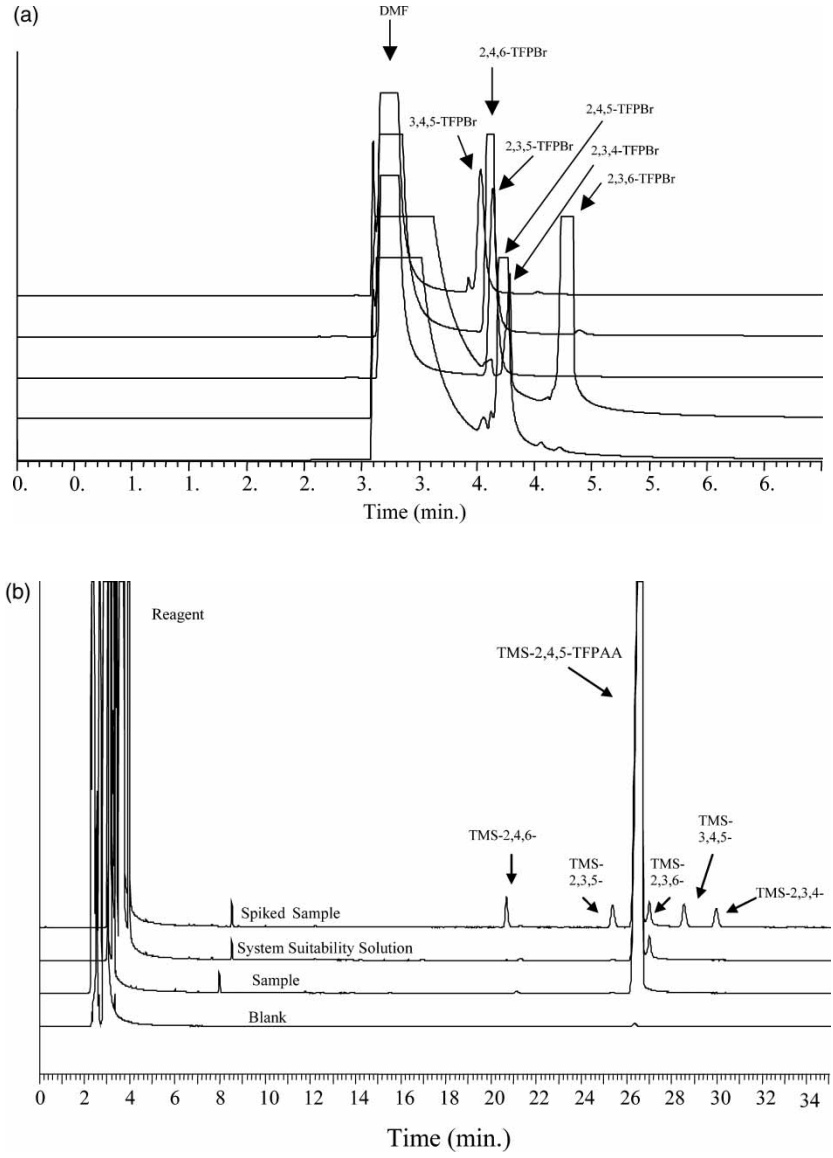


Figure 1. (a) Typical chromatogram for all positional isomers of 2,4,5-trifluorophenyl bromide by GC. (b) Typical chromatogram for all TMS-derivatized positional isomers of TMS-derivatized 2,4,5-trifluorophenylacetic Acid by GC.

analyte vapor pressure appears to be a better predictor of elution order for the TFPAA-TMS isomers than for the TFPBr isomers, suggesting retention in the latter separation is influenced by specific analyte/stationary phase dipole interactions as well as analyte volatility. Third, the elution order trends for the positional isomers in Table 1 are relatively similar for both analyte families with the notable exception of the 3,4,5-positional isomers (5th eluter for TFPAA-TMS and 1st eluter for TFPBr). Interestingly, the substitution patterns for TFPBr which distribute the fluorine atoms more symmetrically about the midline of the phenyl ring produce analytes which elute earlier. This suggests that the role of dipole interactions in the retention mechanism for these analytes is less significant than for the later eluting and less symmetrical TFPBr isomers.

Subsequent gas chromatographic analysis of typical 2,4,5-trifluorophenyl bromide starting material revealed that only the 2,3,6-trifluorophenyl bromide positional isomer was present at quantitative levels (0.1 area%). However, a gradient HPLC impurity profile method was also developed to permit direct analysis of 2,4,5-TFPAA for both the corresponding 2,3,6 positional isomer, as well as other potential process-related impurities. See chromatogram in Figure 2. All potential positional isomers but for the 2,3,5-isomer were chromatographically-resolved from the parent 2,4,5-TFPAA isomer with this method. Using this HPLC method on laboratory and pilot scale batches of 2,4,5-TFPAA, six process impurities were detected. LC-MS analysis determined the following impurity structures shown in Figure 3. Only the *ρ*-bromo acid impurity, which originated from the TFPBr starting material, is carried further downstream in

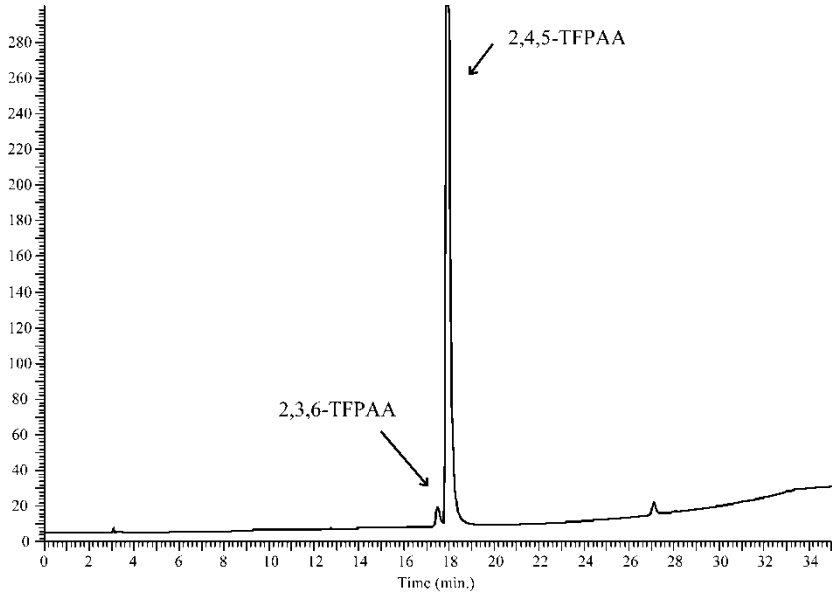


Figure 2. A representative chromatogram of 2,4,5-trifluorophenylacetic acid by LC.

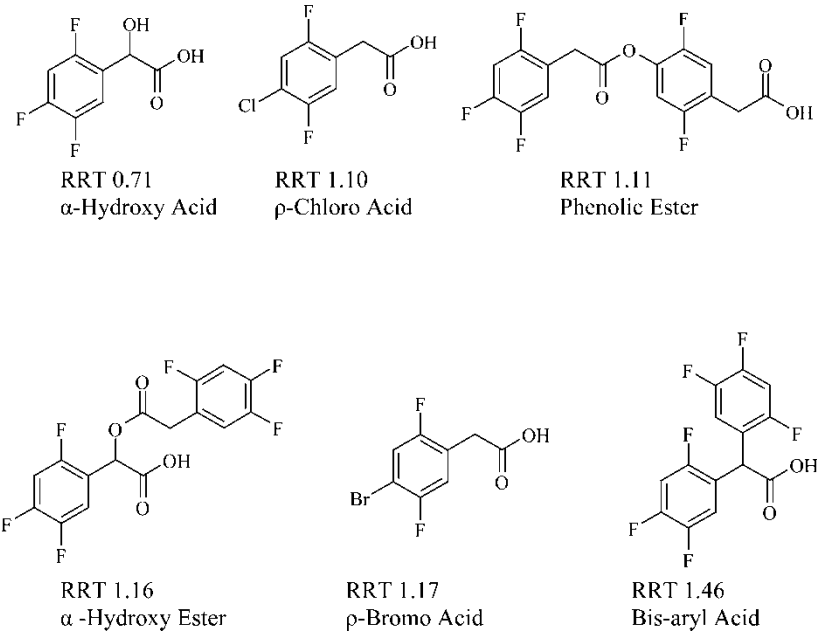


Figure 3. Identified structures of impurities in 2,4,5-trifluorophenylacetic acid.

subsequent processing. The other impurities identified are generated during preparation of 2,4,5-TFPAA, but are rejected in downstream processing.

HPLC Impurity Profile Method Validation

The HPLC impurity profile method was validated for linearity, limits of detection and quantitation, precision, accuracy, solution stability, specificity and system suitability. The detector response for 2,4,5-TFPAA was linear over the concentration range of 0.0059 mg/mL to 0.7380 mg/mL (0.98% to 123% of target concentration), with a correlation coefficient of 0.9981. The detector response for the 2,3,6-TFPAA isomer impurity was linear over the range 0.000099 mg/mL to 0.00297 mg/mL (0.02% to 0.5%), with a correlation coefficient of 0.9965 for 2,3,6-TFPAA. For this study, a sample of 2,4,5-TFPAA that did not contain the 2,3,6-isomer impurity was spiked with varying amounts of the 2,3,6-trifluorophenylacetic acid impurity while holding the 2,4,5-TFPAA at the target concentration of 0.6 mg/mL. The limit of detection and limit of quantitation for 2,3,6-trifluorophenylacetic acid were determined to be 0.02% and 0.05% respectively. The injection precision for the LOQ of 0.05% was 11%. The signal to noise (S : N) of the 0.02% and 0.05% spikes were 12 : 1 and 20 : 1, respectively.

A sample of 2,4,5-TFPAA at target concentration was prepared and injected six times. The method demonstrated acceptable area percent precision of 0.02% RSD for 2,4,5-TFPAA and a precision at 1.39% RSD for 2,3,6-TFPAA (RRT 0.97) at an impurity level of 6.2 area%. The specificity of the assay was demonstrated by demonstrating baseline resolution of known impurities and positional isomer, 2,3,6-TFPAA (RRT 0.97) from 2,4,5-TFPAA. Method accuracy was inferred from the precision, linearity and specificity experiments. System suitability criteria were established for the method based upon examination of the database. The requirements were that the chromatogram of the sample solution at 0.60 mg/mL should be similar to the typical chromatogram shown in Figure 2, minimum of 1.9 resolution between 2,4,5-TFPAA and 2,3,6-TFPAA and a 0.1% (v/v) sample solution of 2,4,5-TFPAA should be detectable.

Weight Percent Method

A titration-based weight percent assay for the 2,4,5-TFPAA product was developed. However, the method lacked specificity for structurally related acidic process impurities present, since these will also be titrated by the sodium hydroxide titrant used. Residual malonic acid in the TFPAA product can be reduced with a washing procedure, however, it is not reduced further by downstream processing. Therefore, control of malonic acid during TFPAA isolation is critical. Greater than 0.01% w/w levels of malonic acid has been found to be a source of high titration values, as evidenced by samples 2, 5 and 6 in Table 2, although it was determined by a laboratory use test that $\leq$0.5% (w/w) malonic acid residues does not interfere with downstream processing. Alternatively, to provide a more specific method for TFPAA, the HPLC impurity profile chromatographic conditions were used for a weight percent assay using an established reference standard. The HPLC weight% method has the disadvantages of not detecting other acids, such as malonic acid, but it has the advantage of specificity. Therefore, it was determined that adequate control of the quality of 2,4,5-TFPAA was provided by a combination of HPLC impurity profile and titration. The positional isomers are most critical and are well detected by the impurity profile method. If high titration values are obtained, a GC derivatization method for quantitation of TMS-derivatized malonic acid (Figure 4) is provided to establish material balance in combination with the HPLC weight percent method.

The HPLC weight percent method was retained in the release testing and the method validated for precision, accuracy, specificity, and system suitability requirements were established.

Three samples of 2,4,5-TFPAA were prepared and injected three times on each of the three days for determination of precision. The RSD% of the weight percent of each sample for the three days ranged from 0.32% to 0.37%. Since

Table 2. Batch data for 2,4,5-TFPAA

	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)	Sample 4 (%)	Sample 5 (%)	Sample 6 (%)
1. HPLC impurity profile (area%)total impurities	0.39	0.72	0.42	0.11	0.33	0.24
2. Titration (NaOH)[a]	99.7	100.8	100.0	100.0	101.2	100.8
3. HPLC weight percent (w/w)	100.1	99.6	98.4	100.8	100.0	100.2
4. Malonic acid by GC	<0.01	0.03	0.01	0.09	0.29	0.04

[a]Calculated on dry basis.
NA = not applicable.
ND = not detectable; ND < 0.02%.
NQ = not quantifiable; 0.02% ≤ NQ < 0.05%.
LOQ = 0.05%.
no others ≥0.05%.

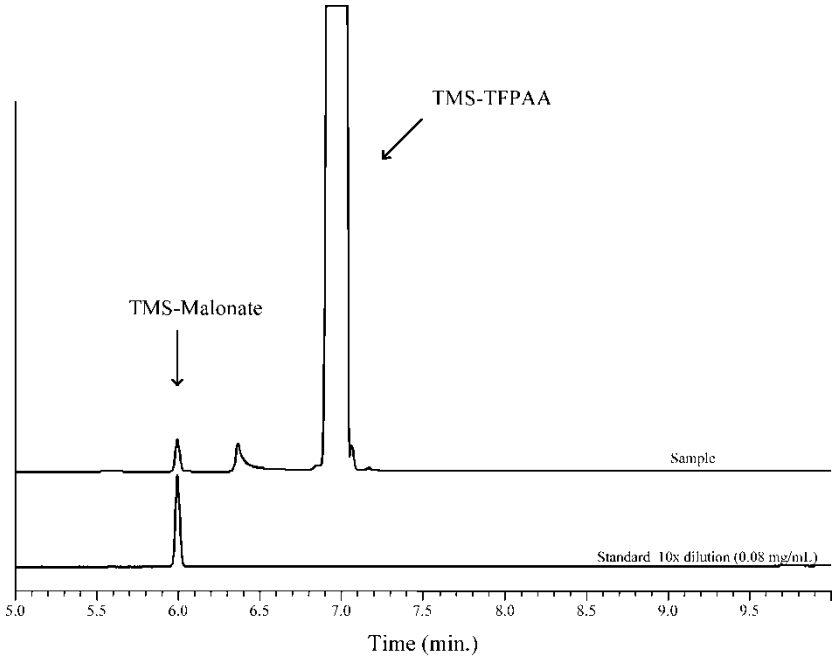


Figure 4. Typical chromatogram for TMS-derivatized malonic acid by GC.

the same method conditions are used as in the HPLC impurity profile, the linearity and solution stability were demonstrated by the HPLC impurity profile method. The accuracy was shown by comparison of the HPLC weight percent and titration data. The 2,4,5-TFPAA batch data is tabulated as in Table 2. Comparison between the titration and weight percent are in good agreement ($\pm 0.30\%$), except in those situations where malonic acid is present at $>0.01\%$, as for samples 2, 4, 5, and 6. The specificity of the assay was demonstrated in the impurity profile by showing resolution of a known impurity, 2,3,6-TFPAA, from 2,4,5-TFPAA and assaying the mother liquors and crude samples, which have elevated impurity levels. System suitability criteria were established for the method based upon examination of the database. The system suitability requirements required that the chromatogram of the sample solution should be similar to a typical chromatogram and a maximum of 2 peak tailing for the main peak.

CONCLUSIONS

Analytical methods were developed to monitor the 2,4,5-TFPAA process and screen for undesired positional isomer impurities. The analytical methods provided purity and quality assurance downstream in the process of the

API. Validation of the HPLC impurity profile and weight % method revealed that they were linear, precise, accurate, and sensitive.

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