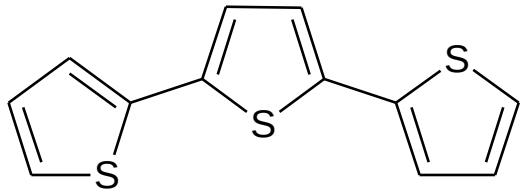
THE INTERNATIONAL FRAGRANCE ASSOCIATION

Method for the analysis and Quantification of alpha-Terthiophene as found in Tagetes Oil

Background

Alpha-Terthiophene (alpha-Terthienyl) is a is an oligomer of the heterocycle thiophene and is commonly found in African Marigolds (*Tagetes* spp.)



Formula:	$C_{12}H_8S_3$
Molar mass:	248.39 g/mol
CAS RN:	1081-34-1
Alternative Names:	2,2':5',2''-Terthiophene; 2,5-Di(2-thienyl)thiophene; alpha-Terthienyl
ChemSpider ID:	58578
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Appearance:	pale yellow solid
PubChem CID:	65067
Solubility in water:	insoluble

Alpha-terthiophene exhibits biological activity due to its sensitization and formation of singlet oxygen. It is responsible for the insecticidal activity of *Tagetes minuta*.

Due to this biological activity, and following SCCP opinions 0869/05, SCCS/1551/15 and comment from the Scientific Committee on Consumer Safety (SCCS) meeting on October 6, 2016, the European Commission therefore restricts the use of Tagetes minuta and patula extracts and flower oils at a maximum concentration of 0.01% in leave-on cosmetic products and 0.1% in rinse-off cosmetic products, while the content of terthiophene in the extracts or oils from these species shall not exceed 0.35 %.

The analysis of terthiophene is straightforward and can be achieved by the use of gas chromatography (GC) linked to either Flame Ionisation Detection (GC-FID) or, ideally, to ensure that unequivocal identification can take place and the best quantification obtained, Mass Spectrometry (GC-MS).

Sample preparation is straightforward and low quantification levels can be obtained in line with the demands of Regulatory constraints for the composition of the parent oil.

Analytical Method

Materials:

A standard can be obtained commercially, e.g., from Sigma-Aldrich

α-Terthienyl, 2,5-Di(2-thienyl)thiophene / 2,2′:5′,2′′-Terthiophene

CAS Number: 1081-34-1

Molecular formula: $C_{12}H_8S_3$

Solvent: Methyl tert-butyl ether (MTBE)

Internal standard (ISTD): Dibromobenzene

Calibration Standards:

Calibration standards are to be made by dissolving a suitable quantity of the calibration reference (alpha-terthiophene) in MTBE.

Calibration levels are to reflect equivalent concentration in essential oils and should therefore cover the concentration range of 0.01% to 1%. The calibration should, ideally, be a linear calibration, forced through zero.

The addition of an Internal Standard (ISTD) comprising dibromobenzene at a suitable concentration to each calibration sample and all test samples is highly recommended to allow an accurate evaluation of the concentration of the target to be undertaken.

Sample Preparation:

Appropriate samples: Tagetes oil extra and Tagetes absolute

Sample preparation: Take 1g of test sample and dilute to 10ml in MTBE including the addition of dibromobenzene ISTD.

GCMS Analysis Conditions:

GC column: Apolar column (60m x 250 µm, 0.25 µm)

MS Acquisition: Scan mode from m/z 35 to m/z 300 (source at 230°C)

Temperature Program: From 50ºC (1min) to 300ºC (10min) @ 10ºC/min

Quantification ions: 1 Quantifier ion / 2 Qualifier ions (m/z 248; 249; 250)

Data Treatment

This should follow standard processes via the evaluation of the sample analysis data for the Quantification ions (quantifier; qualifier ions) versus the calibration curve obtained from the standard samples. It is important to ensure that the quantifier and qualifier ions for the target material are in the correct ration (as seen in the calibration samples) and any differences in the ration's seen should be further investigated as interference or co-elution may be present in that particular test sample.

Example Calibration and Data Plots:

The following are examples of the reference GCMS; the calibration plot and examples of actual sample analyses obtained by the above approach.

It should be noted that lower levels of quantification may be readily achievable using this approach but it is important to note that the lower level of quantification should be stated as the lower calibration point (which, in this Method, would therefore be 0.01% alpha-terthiophene in Tagetes oil).

Example Figures:

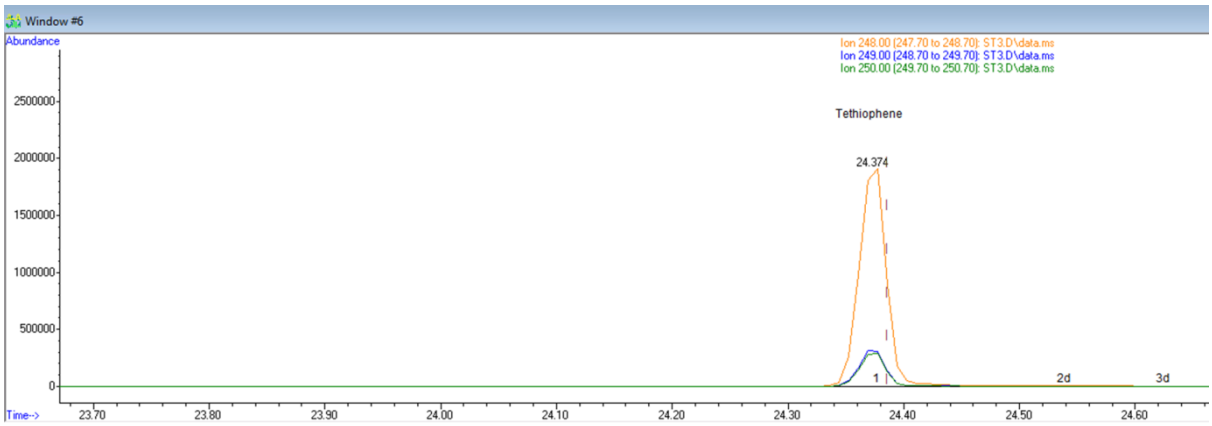

Figure 1: Example of the extraction ion plot for alpha-terthiophene in a calibration standard. Extracted ions are the quantifier and qualifier ions comprising m/z 248; 249; 250.

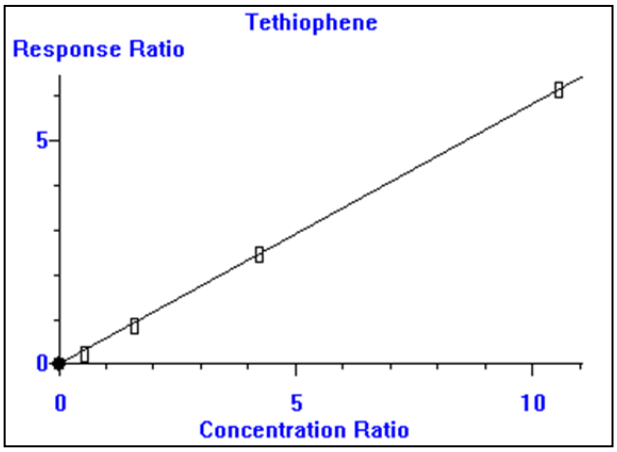

Figure 2: Calibration curve for alpha-terthiophene; linear regression forced through zero.

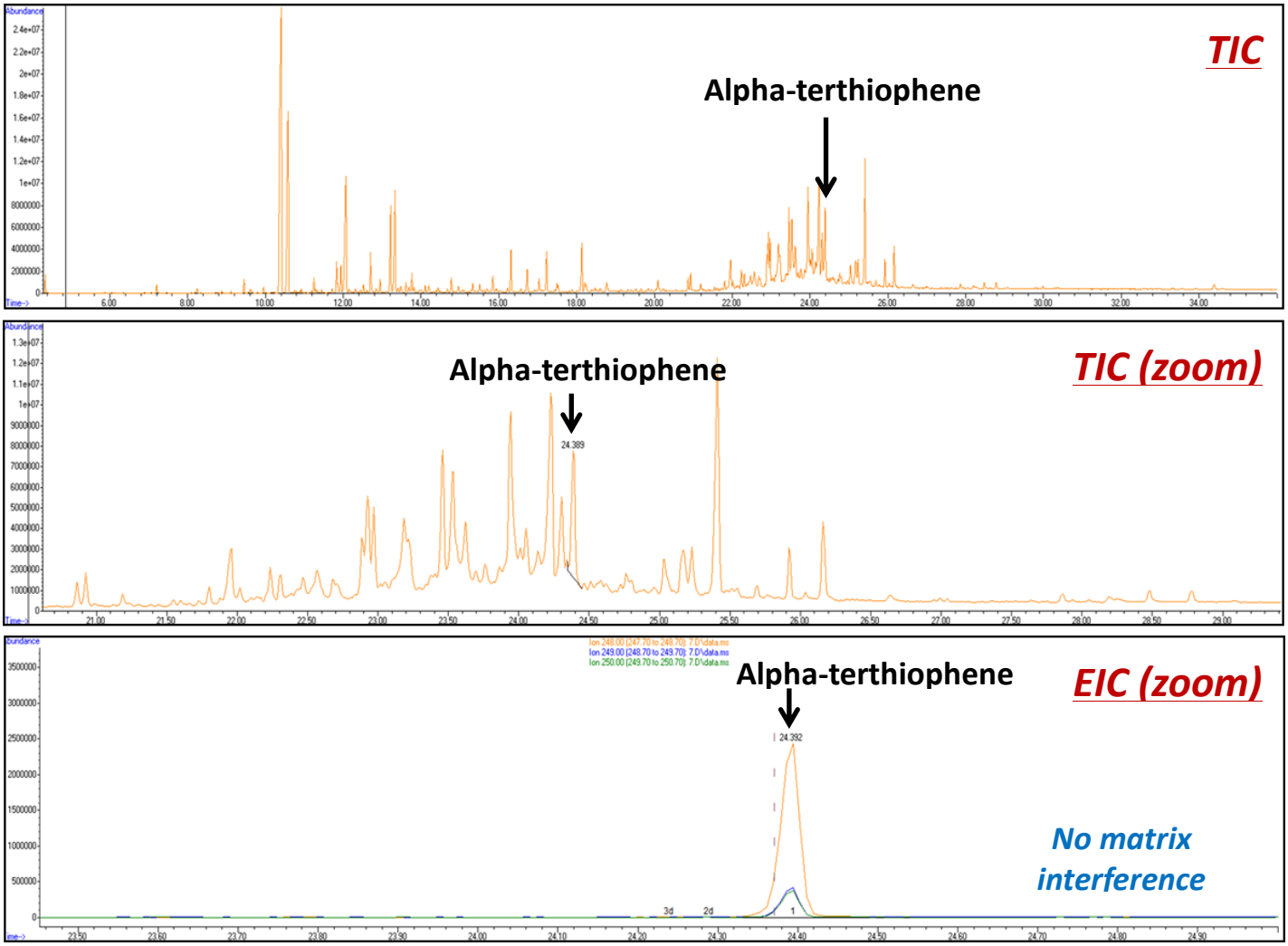

Figure 3: An example of the analysis of a Tagetes absolute indicating:
Upper trace: Total Ion Current (TIC) of full sample analysis with region of alpha-terthiophene indicated.
Middle trace: TIC zoom around expected retention time for alpha-terthiophene.
Lower trace: Extracted Ion Chromatogram (EIC) for ions 248; 249; 250 indicating presence of alpha-terthiophene and a lack of any matrix interference in the analysis.