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# Targeted Screening of Coumarins and Furanocoumarins in Essential Oils Utilizing High-Resolution Quadrupole Time-of-Flight Mass Spectrometry

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Jennifer Davis[1], Evelyn H Wang[1], Katie Pryor[1], Jerry Byrne II[1], Helen Hao[1], Christopher Gilles[1]
1 Shimadzu Scientific Instruments, Columbia, MD

# Targeted Screening of Coumarins and Furanocoumarins in Essential Oils Utilizing High-Resolution Quadrupole Time-of-Flight Mass Spectrometry

## Overview

A 11min method was developed using high resolution mass spectrometry for targeted screening of coumarins and coumarin dervivatives within essential oils.

## Introduction

Coumarins and Furanocoumarins are a compound class commonly found in fruit essential oils, such as citrus oil or bergamot oil. Previous research has found these compounds can be linked to phototoxic, mutagenic, or carcinogenic effects. Since this class of compounds is commonly found in essential oils, cosmetics, or bronzing products there is an emerging need to detect their presence to prevent unnecessary harm to the consumer. Currently GC-MS has shown a limited ability to analyze furanocoumarins due to their relatively polar or heat-liable substituents. During this study a LCMS QToF was used for the screening and identification of coumarins and furanocoumarins in essential oils.

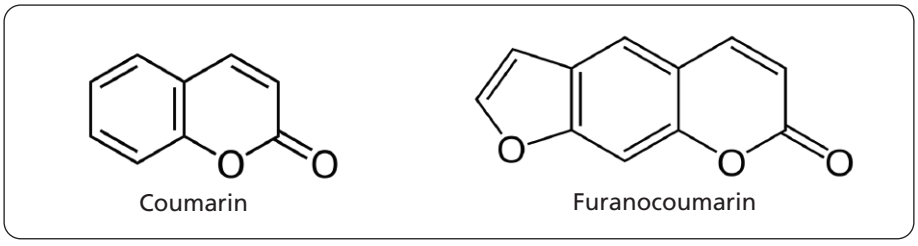


Figure 1 Structural backbone of coumarin and furanocoumarin

## Methods

Essential oil samples were prepared by diluting 20 µL of oil into 980 µL of methanol. Ten coumarin standards were obtained in solid form. Each standard was dissolved in methanol and diluted to a final concentration of 500ppb.

Electrospray ionization was used in positive MS scan mode for the initial screening of the prepared samples. A total of thirty-five coumarin and furanocoumarins were screened for in each essential oil. Primary confirmation was completed based on accurate mass. Secondary confirmation was completed by comparing MSMS spectral data and retention times of the prepared essential oil sample with a neat standard. In the situation where a neat standard was not available secondary confirmation was completed by comparison with metlin's MSMS spectral databases.

| | |
|---|---|
| **UHPLC conditions (Nexera system)** | |
| Column | : Restek Raptor Biphenyl 100mm×2.1mm, 2.7 µm |
| Mobile phase A | : Water with 5mM Ammonium formate |
| B | : Methanol with 5mM Ammonium formate |
| Flow rate | : 0.4 mL/min |
| Time program | : B conc. 5%(0-0.5min) - 50%(1min) - 99%(8-9min) – 5%(9.01-11min) |
| Injection vol. | : 1 uL |
| Column temperature | : 40°C |
| **MS conditions (LCMS-9030)** | |
| Ionization | : ESI, Positive scan and MSMS mode |

# Results

A total of 9 essential oils were screened for coumarins and furanocoumarins, representative data is shown for bergamot oil, orange oil, and lemon oil. Utilizing an 11 min gradient method targeted screening of thirty-five coumarins or furanocoumarins was completed for all essential oils. Primary screening of each compound was confirmed within a 4 ppm accuracy of the theoretical mass. The largest presence of coumarins and furanocoumarins was detected in bergamot oil with a total of 22 (Figure 2). Lemon oil and Orange oil were confirmed to have 17 and 10 coumarins, respectively.

After primary screening, secondary confirmation was completed using retention time and fragmentation pattern comparison. Figures 3-5 show examples of each type of secondary confirmation. Ten coumarins were confirmed by retention time and fragmentation pattern comparison with neat standards acquired on the same high resolution mass spectrometer, LCMS-9030. Twenty-five coumarins were confirmed by fragmentation pattern comparison with known databases, such as Metlin or the human metabolome database.

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## Primary Confirmation of Coumarins

Table 1. Primary Screening of Essential oils and the ppm difference of the observed mass.

| Coumarins | Bergamot Oil | | Orange Oil | | Lemon Oil | |
|---|---|---|---|---|---|---|
| | Observed Mass | ppm difference | Observed Mass | ppm difference | Observed Mass | ppm difference |
| 4-methyldaphnetin | ND | | ND | | ND | |
| Esculetin | 179.0340 | 0.61 | ND | | 179.0340 | 0.61 |
| 6-Hydroxycoumarin | ND | | ND | | ND | |
| Isoscopoletin | ND | | ND | | 193.0497 | 0.83 |
| 6,7-Dihydroxy-4-methylcoumarin | 193.0497 | 0.83 | ND | | 193.0501 | 2.90 |
| Daphnetin-7-methylether | 193.0497 | 0.83 | ND | | ND | |
| Umbelliferone | ND | | ND | | ND | |
| Scopoletin | ND | | ND | | ND | |
| 5,7-Dihydroxy-4-methylcoumarin | 193.0497 | 0.83 | ND | | 193.0498 | 1.35 |
| 8-Acetyl-6-hydroxy-7-methoxycoumarin | ND | | ND | | ND | |
| Fraxidin | 223.0604 | 1.34 | 223.0603 | 0.90 | 223.0604 | 1.34 |
| Xanthotoxol | 203.0346 | 3.50 | 203.0339 | 0.05 | ND | |
| 6,7-Dimethylesculetin | ND | | 207.0654 | 0.77 | 207.0654 | 1.01 |
| Coumarin | 147.0443 | 1.63 | ND | | 147.0443 | 1.63 |
| 8-Acetyl-7-methoxycoumarin | 219.0651 | -0.41 | 219.0654 | 0.96 | ND | |
| Herniarin | 177.0550 | 2.15 | 177.0546 | -0.11 | 177.0551 | 2.71 |
| 4-methoxycoumarin | 177.0552 | 3.28 | ND | | 177.0546 | -0.11 |
| 8-acetyl-6,7-dimethoxycoumarin | ND | | ND | | ND | |
| 3-acetylcoumarin | 189.0547 | 0.42 | 189.0546 | -0.21 | 189.0547 | 0.42 |
| 7-methylcoumarin | ND | | 161.0596 | -0.81 | ND | |
| Psoralen | 187.0390 | 0.16 | ND | | 187.0390 | 0.16 |
| Nordalbergin | ND | | ND | | 255.0655 | 1.22 |
| 6-Methoxy-4-methylcoumarin | 191.0704 | 0.68 | ND | | ND | |
| 7-Methoxy-4-methylcoumarin | 191.0701 | -0.89 | ND | | ND | |
| Xanthotoxin | 217.0497 | 0.74 | ND | | 217.0498 | 1.20 |
| 6-methylcoumarin | ND | | 161.0597 | -0.06 | ND | |
| Dalbergin | 269.0813 | 1.71 | ND | | ND | |
| Citropten | 207.0660 | 3.91 | ND | | 207.0655 | 1.50 |
| Bergapten | 217.0501 | 2.58 | 217.0496 | 0.28 | 217.0496 | 0.28 |
| Isopimpinellin | 247.0607 | 2.43 | ND | | 247.0606 | 2.02 |
| 7-Ethoxycoumarin | ND | | ND | | ND | |
| 4-Hydroxycoumarin | 163.0392 | 1.41 | 163.0390 | 0.18 | 163.0391 | 0.80 |
| 4-Ethoxycoumarin | ND | | ND | | ND | |
| 4-methylumbelliferone | 177.0549 | 1.58 | ND | | ND | |
| 4-methyl-7-ethoxycoumarin | 205.0860 | 0.39 | 205.0857 | -1.07 | ND | |

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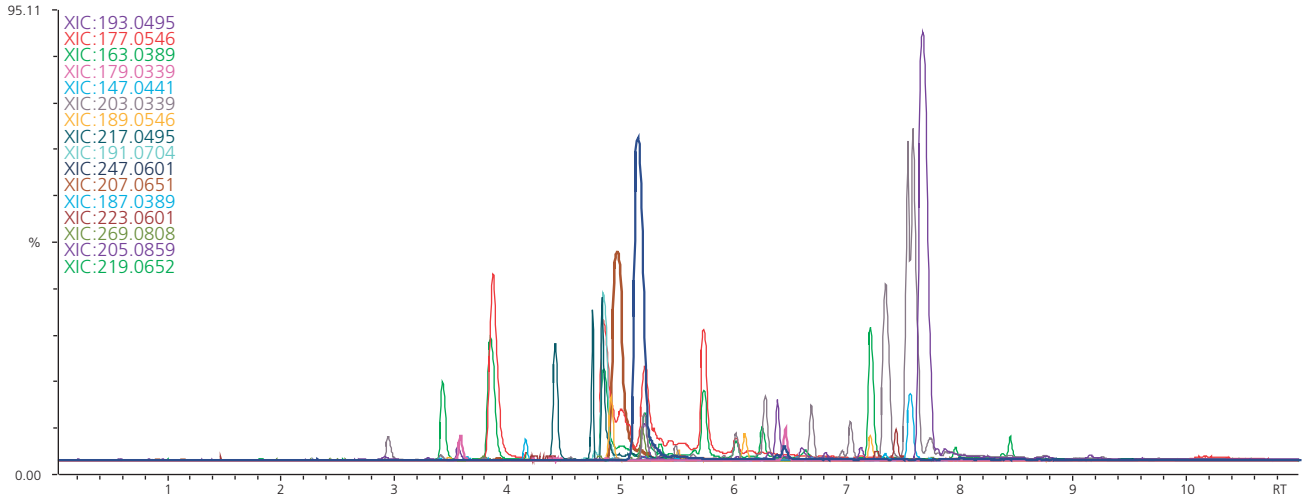


Figure 2. Representative Extracted Ion Chromatogram for all 22 Coumarins present in Bergamot oil.

## Secondary Confirmation by Neat Standard

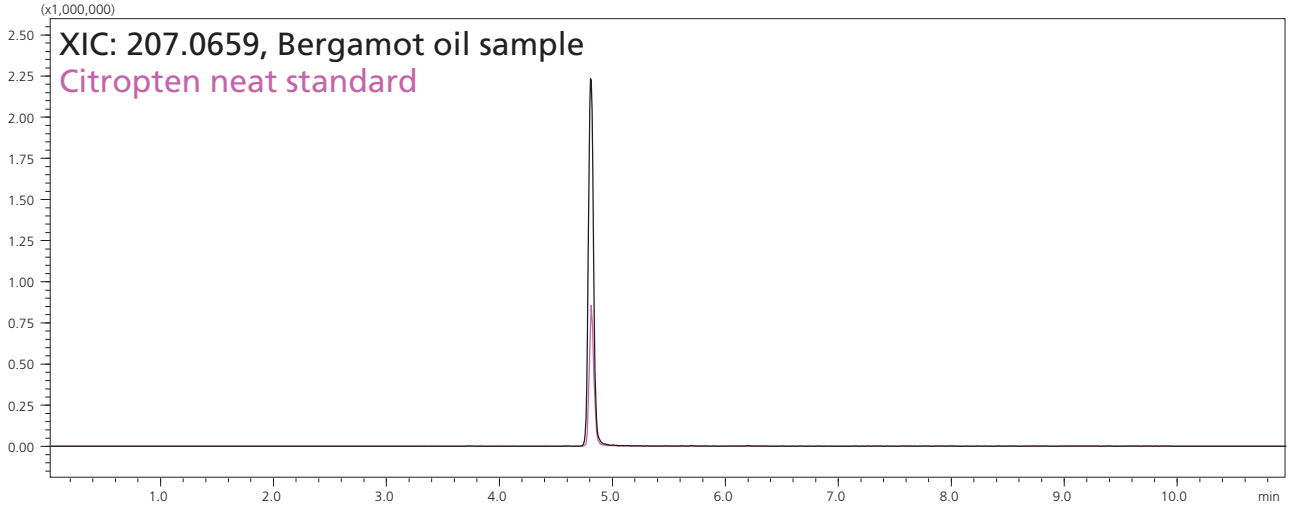


Figure 3. Overlaid chromatogram for confirmation of Citropten in Bergamot essential oil.
Pink- Citropten neat standard, Black- XIC of 207.0659m/z from Bergamot oil

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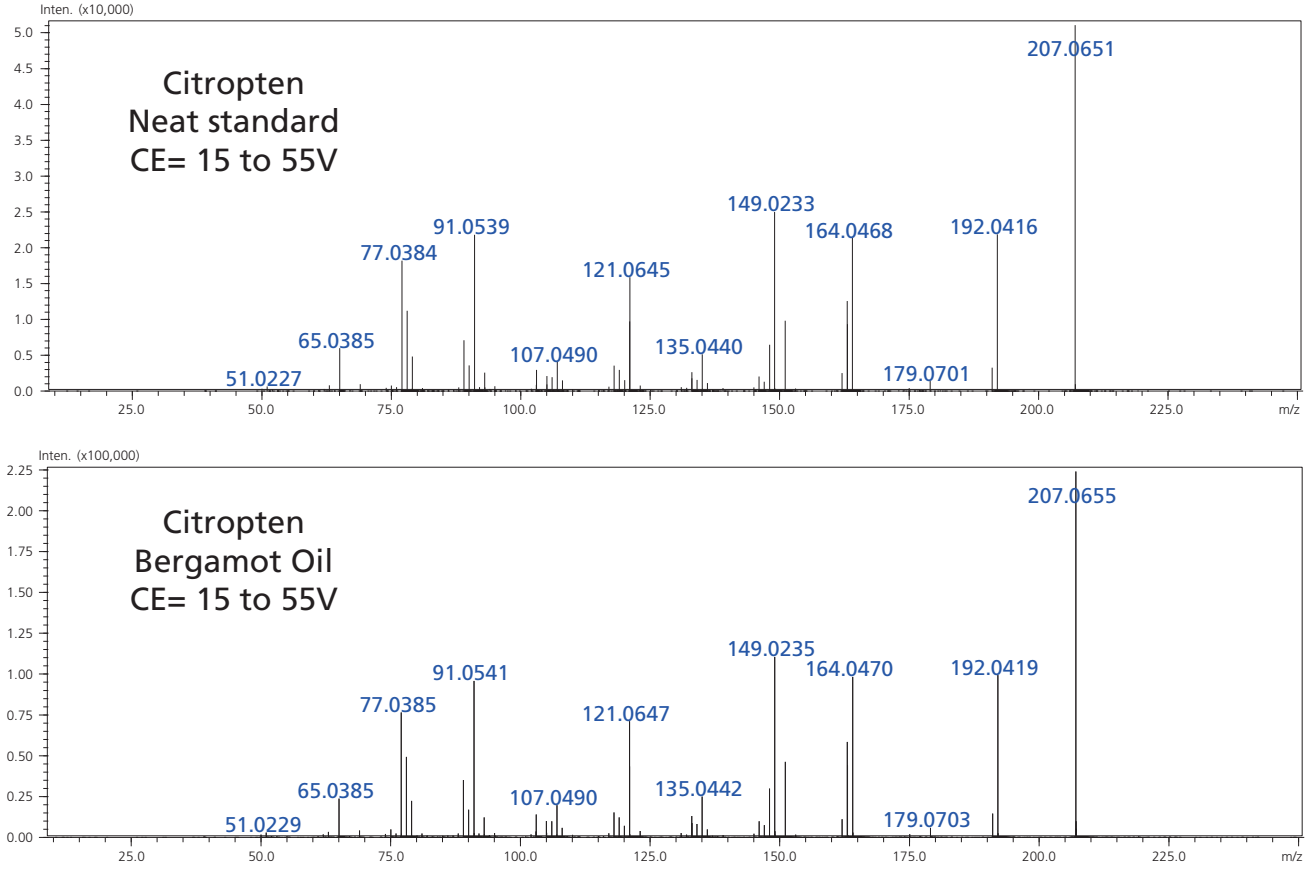
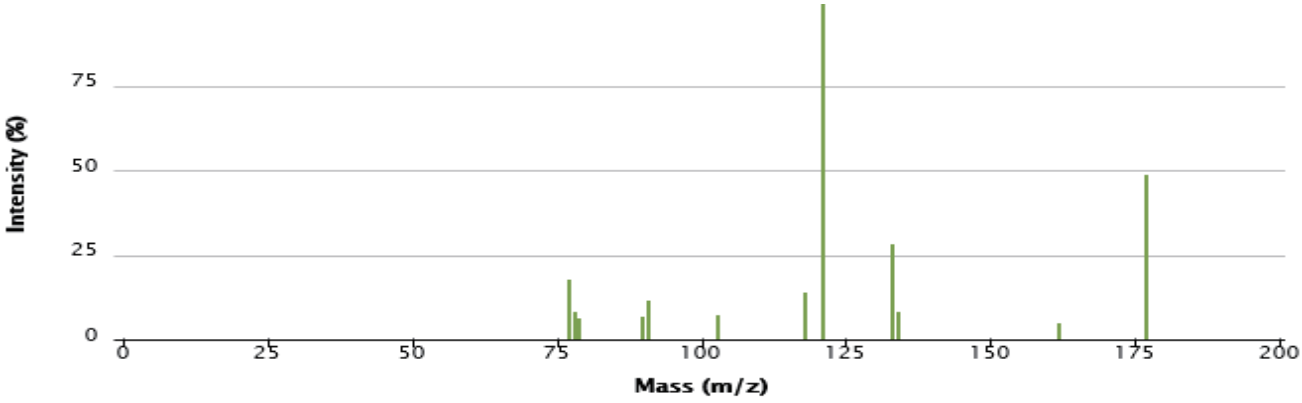


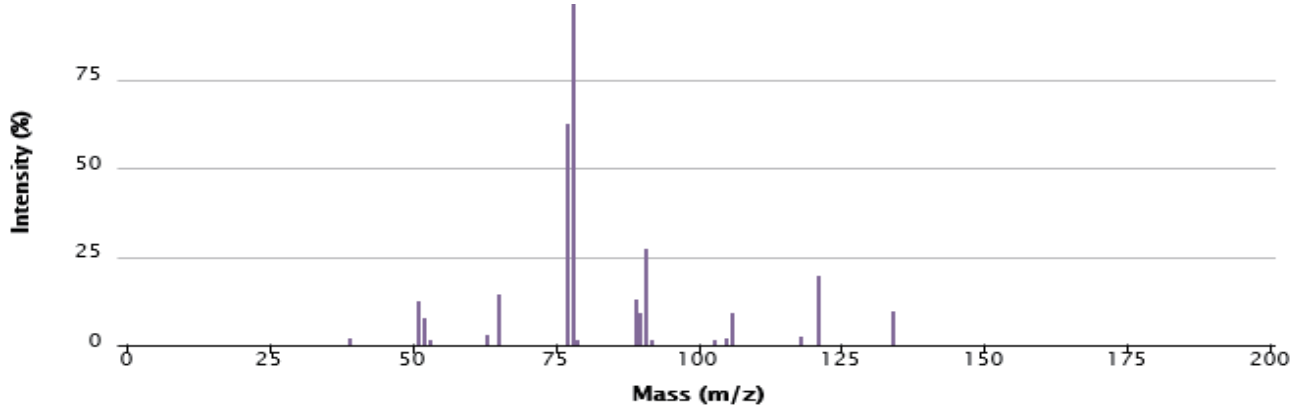

Figure 4. MSMS spectral comparison for confirmation of Citropten in Bergamot essential oil.
Top- Citropten neat standard, Bottom- Bergamot oil sample

## Secondary Confirmation by Metlin Spectral Database

Herniarin ESI Q-ToF MSMS spectrum from Metlin, CE= 20V




Herniarin ESI Q-ToF MSMS spectrum from Metlin, CE= 40V




Herniarin ESI Q-ToF MSMS spectrum from Bergamot Oil, CE= 15 to 55V

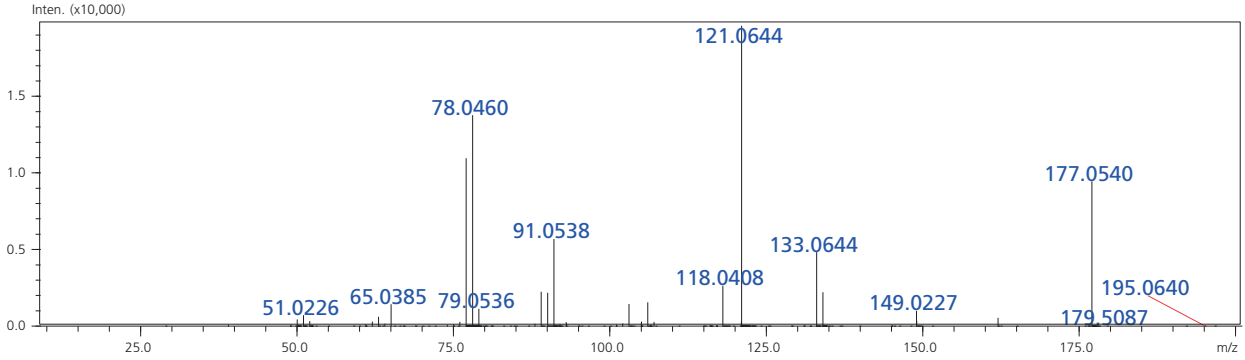



Figure 5. MSMS spectrum for Herniarin coumarin in Bergamot oil. Top- Herniarin by Metlin database (CE-20V), Middle Herniarin by Metlin database (CE-40V), Bottom- Bergamot oil sample

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## Conclusions

Targeted Screening of Coumarins and Furanocoumarins was successfully completed using high resolution mass spectrometry, LCMS-9030. Initial confirmation was completed for Orange, Lemon, Bergamot, Eucalyptus, Lavender, Tangerine, Chamomile, Sandalwood, and Sweet orange oil. Each essential oil contained between 6 to 22 coumarins all confirmed within 4ppm of their respective accurate masses. Secondary confirmation was completed by either comparing MSMS spectral data from neat standards or by comparing MSMS spectral data from Metlin's database. Further research could be done to quantitate each coumarin if calibration curves were developed using neat standards for all 35 coumarins in the screening panel.

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