



# Recent Developments to Detect Lemon Juice Adulteration

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## Abstract:

One of the possible adulterants of lemon juice is lime. The pattern of PMFs seen in lemon and lime juices are very different, which allow a much easier identification of substitution and/or addition of lime to lemon. This poster describes the method for the polymethoxyflavones “lime” markers.

## Introduction

Unfortunately there is a long history of economic adulteration of fruit juices. The first well publicized case of juice fraud was in 1989 with Beech-nut<sup>(1)</sup>. This and subsequent events have meant that juice packers normally have a “verification” program for their raw materials to ensure that they are authentic. Although in 2009 the FDA officially recognized food fraud as an emerging risk to the consumer, in fringe industries that use juice based products, this risk had not been recognized and few companies have QA programs in place. It should be remembered that product “verification” is a key element in the 2011 Food safety modernization act<sup>(2)</sup>.

In 2012 the National Consumer League<sup>(3)</sup> analyzed a range of 100% lemon juice products and identified 4 products which were extended with added citric acid and other materials and did not conform to their description. Unfortunately there are still issues today with some lemon juices that have been extended with citric acid and/or blended with other juices. Reported here are the best procedures to tackle this problem. Juices are typically assessed for a range of components (acids, minerals and sugars & by <sup>13</sup>C-IRMS) and by the Kirksey<sup>(4)</sup> fingerprinting method to detect juice mixtures. However, the latter fails to discriminate between lemon and lime products or their blends. Reported here is an additional procedure, based on three polymethoxylated phenols (PMF), which allows a good discrimination of these two fruits. Herniarin (7-methoxycoumarin), bergapten (5-methoxypsoralen) and isopimpinellin (5,8-dimethoxypsoralen) are present in lime oil<sup>(5)</sup> but are not seen in lemon and are used for this discrimination.

## Methodologies:

**Key tests are:-** Brix (AOAC 983.17), tit. acidity (IFU 3)<sup>(11)</sup>, citric acid (AOAC 986.12), the enzymic method for isocitric acid (IFU 54), sugars (IFU67), K, Mg, Ca (IFU 33), oligosaccharides (IFU Rec 4), <sup>13</sup>C-IRMS (AOAC 981.09), and the internal isotopic method for C<sub>4</sub> and C<sub>3</sub> acid addition<sup>(6)</sup>. Other useful tests are malic and fumaric acids (AOAC 986.12) and <sup>δ18</sup>O values for NFC juices (AOAC 992.09).

## PMFs (Eurofins test code QD0F1):

Juices are prepared at single strength and cloudy juices are centrifuged (2500 x g) for 10 minutes. Clarified single strength juices can be used “as is”. The clear or centrifuged sample (20 ml) is passed through the activated reverse phase SPE cartridge over a 2 minute period. The sugars and acids are then eluted with water (5 ml) over 15 seconds. The less polar materials are subsequently eluted with 28 % acetonitrile in water (5 ml) over 30 seconds. Traces of this solvent are “blown out” of the SPE cartridge with air, prior to the final elution of the PMFs using MeOH/CH<sub>2</sub>Cl (1:1), 5 ml) over 1 minute. The solvent is collected in a small flask and removed by evaporation under vacuum at 35°C. The residue is taken up in methanol (1 ml) prior to LC analysis and filtered, if required, prior to analysis.

## Polymethoxyflavones UPLC conditions

Column: 15 cm C<sub>18</sub> column 1.7 μm (BEH or equivalent)

Solvent A: HPLC grade water

Solvent B: 100 % acetonitrile

Flow rate: 0.3 ml/min

Injection volume: 2 μl

UV detection: 330 nm, DAD can replace UV detector

Analysis time: 30 minutes

## Gradient conditions

Time	%B
0	25
30	25
30.1	End

## References:

- 1) O'Reilly, James T., *Food and Drug Administration, Vo. 1, §10-17, citing U.S. v. Beech-Nut Nutrition Corporation*, 871 F.2d 1181 (2nd Cir. 1989) (after conviction was reversed on procedural grounds, senior executives pled guilty and served jail terms while the corporation paid millions in penalties and fines, and was purchased by new owners.
- 2) Food safety modernization act (2011) <http://www.fda.gov/Food/GuidanceRegulation/FSMA/default.htm>
- 3) <http://www.natconsumersleague.org/food/85-food-labeling/637-watered-down-lemon-juice-making-advocatesoutur>
- 4) Kirksey S.T. *et al* (1995). In: Methods to detect adulteration of fruit juice beverages Vol 1, page 145 – 166. Pub.by AG Science
- 5) McHale D. & Sheridan J.B. (1989). The Oxygen Heterocyclic Compounds of Citrus Peel Oils. *Journal of Essential Oil Research* 1, 4, 139-149
- 6) Jamin E. R. *et al*. Detection of exogenous citric acid in fruit juices by stable isotope ratio analysis. *J Agric. Food Chem.* 53, 5130 – 5133, (2005)
- 7) Ooghe W.C., Ooghe S. J., Detavernier C.M & Huyghebaert A. (1994 ) Characterization of Orange Juice (*Citrus sinensis*) by Polymethoxylated Flavones . *J. Agric. Food Chem.*, 42 (10), 2191–2195
- 8) Lehnert N. & Ara V. Authenticity analysis of lemon juices concerning the adulteration lime. (2014) Fruit Processing Nov/Dec 242 - 248
- 9) Pupin A.M., Dennis, M.J. & Toledo M.C.F. (1998). Polymethoxylated flavones in Brazilian orange juice. *Food Chemistry* 63 (4), 513–518
- 10) IFU methods [www.ifu-fruitjuice.com](http://www.ifu-fruitjuice.com) (for a fee)
- 11) AIJN code of practice [www.aijn.org](http://www.aijn.org) (for a fee)

## Discussion:

Experts from the European juice association (AIJN) have drawn up the typical ranges for a number of important juice components in 25 fruits and vegetables<sup>(10)</sup>, which are extremely useful in the assessment of the quality and authenticity of these products. They also working on a reference guide for lime at present.

Lemon and lime juices are normally adulterated by substitution of some or all of the juice solids by exogenous citric acid. This causes a depressed level of isocitric acid and an elevated citrate to isocitrate ratio. Isotopic methods on the whole juice or the isolated citric acid have proved very useful at detecting the presence of exogenous C<sub>4</sub> or C<sub>3</sub> citrate. This is nicely illustrated in Figure 1, where the carbon isotope ratio is plotted against the deuterium level in the isolated citric acid. If a C<sub>4</sub> derived citric acid is used as the adulterant then only a  $\delta^{13}\text{C}$  value is sufficient and will show an elevated value (e.g. between -11 and -24 ‰). If the citric acid has been derived from a C<sub>3</sub> source the dual D and <sup>13</sup>C method is required as the acid will be depleted in D relative to the levels seen in lemon juices as illustrated below.

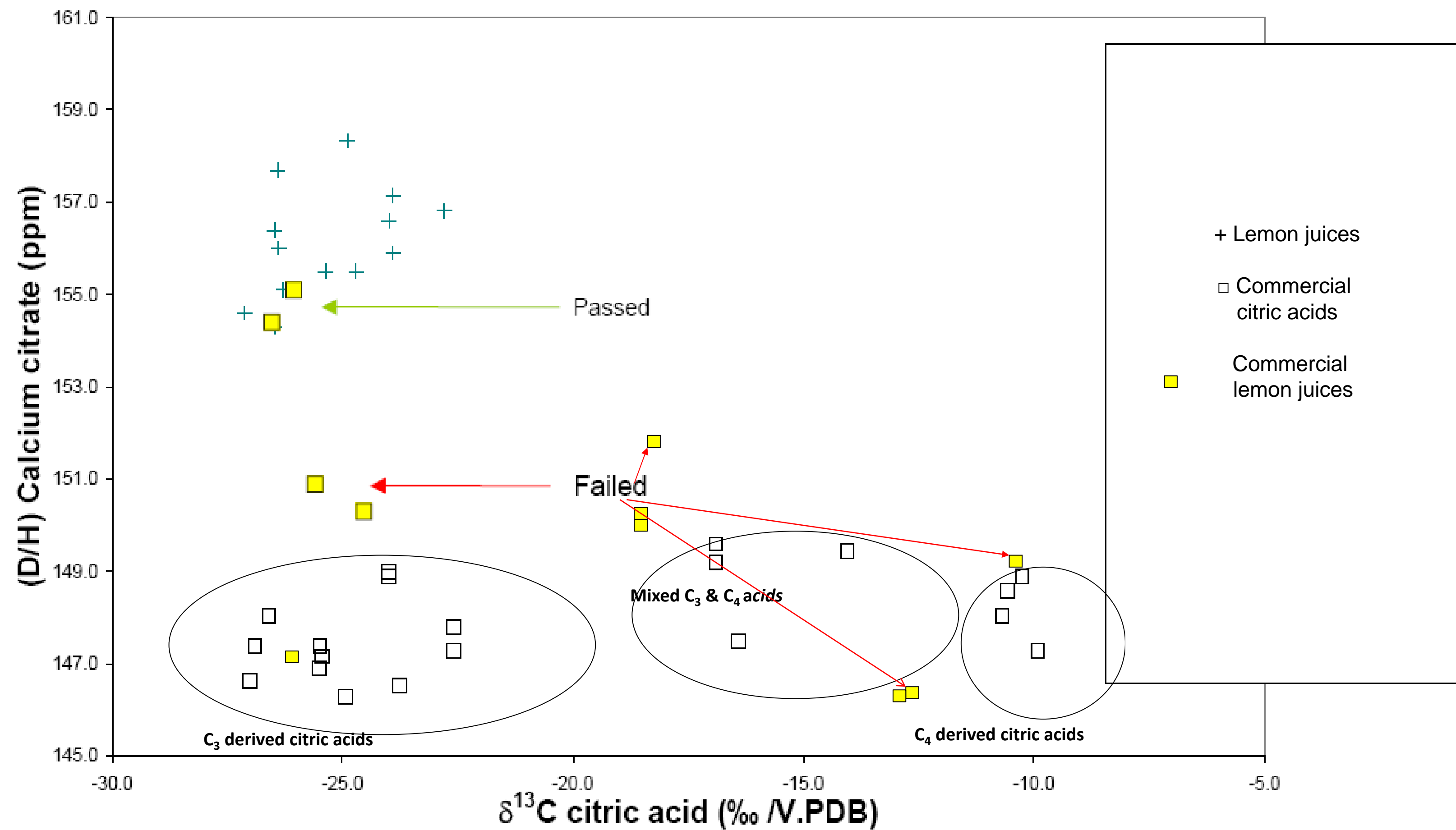


Figure 1: Plot of carbon and deuterium levels in citric acid

Some expensive juices can be blended with cheaper materials, such as apple and grape. Our studies have also indicated that lemon is also sometimes blended with/or substituted with lime. As these juices have very similar compositions this type of blending is very difficult to detect from the regular juice parameters. Although the “Kirksey” method shows slightly different profiles for lemon and lime, which allows their discrimination, it does not provide a very good procedure for the detection of blends of these juices, due to their similarities. However, there are three other, less polar phenolics, which can be very useful. The procedure described here was developed in the 90's as a method to detect mandarin in orange juice<sup>(7)</sup> but has been adapted by us for use on a UPLC system to detect the presence of lime.

As illustrated in Figures 2, 3 & 4 it is clear that the pattern of polymethoxyphenols (PMF) seen in lemon and lime are very different and allows a clear differentiation of these juices and their blends. Using the Ooghe *et al* method<sup>(7)</sup> it is clear that there were three peaks in lime that did not appear in lemon. Lehnert and Ara <sup>(8)</sup> had shown that 7-methoxycoumarin (7-MeO-C) was the first of these markers in their publication. The identity of the two other components are reported here. This was achieved by the use of appropriate standards (Sigma) and use of LC-DAD. Typical DAD spectra for these three markers are given in figure 6. The DAD spectrum of 7-MeOC shows the typical absorbance at ca 330nm for a coumarin whereas the other two molecules shows an intermediate absorbance band of a psoralen. The structure of these compounds was also confirmed by LC-MSMS analysis.

It has been found that using the procedure developed by Ooghe *et al* and adapted here offers certain advantages over the method employed by Lehnert & Ara in their study. The latter method does not offer such a favorable resolution of the two psoralens from citropten as seen with our method. Although this procedure requires some careful manipulation of solid phase extraction cartridges, it still offers a very efficient and rapid method to screen lemon juice for the presence of added lime.

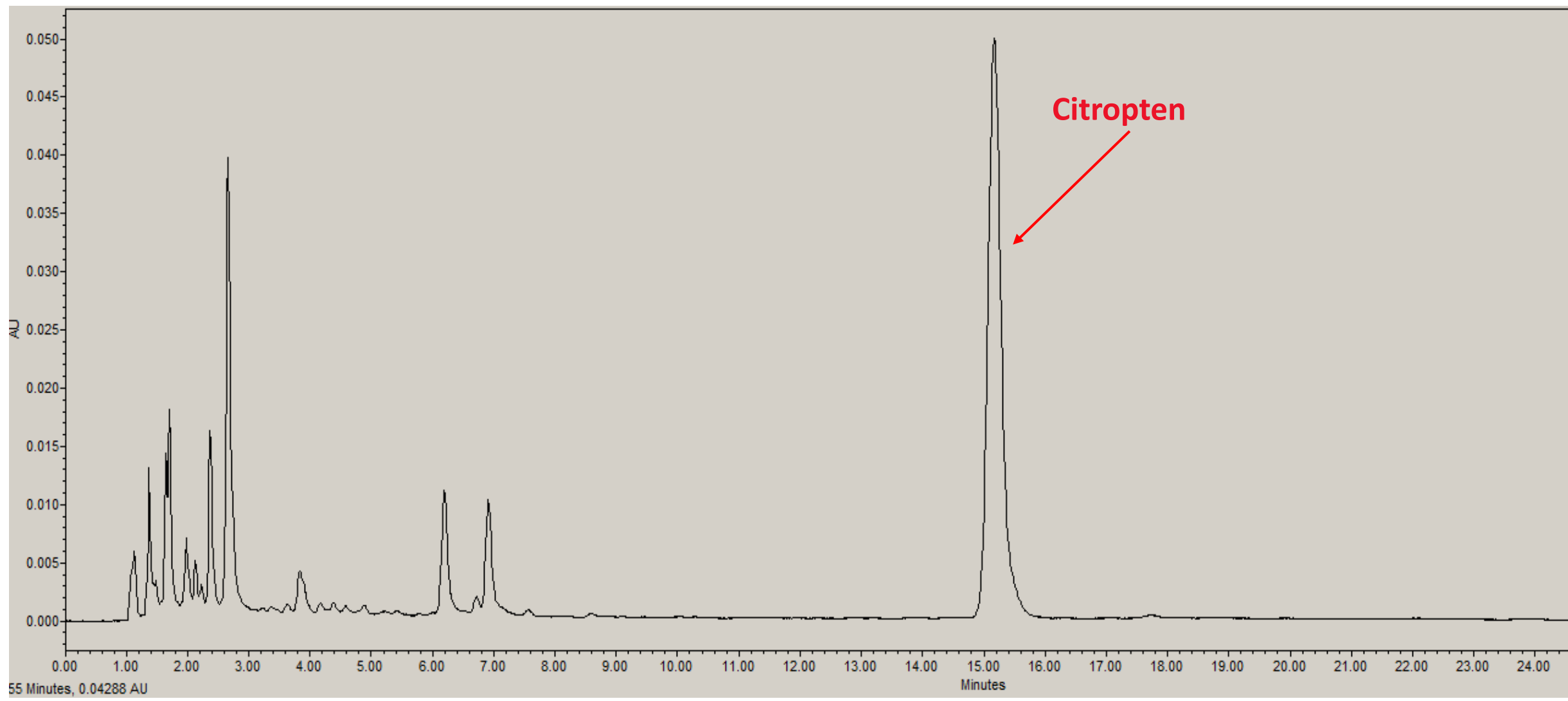


Figure 2: PMF profile for lemon juice

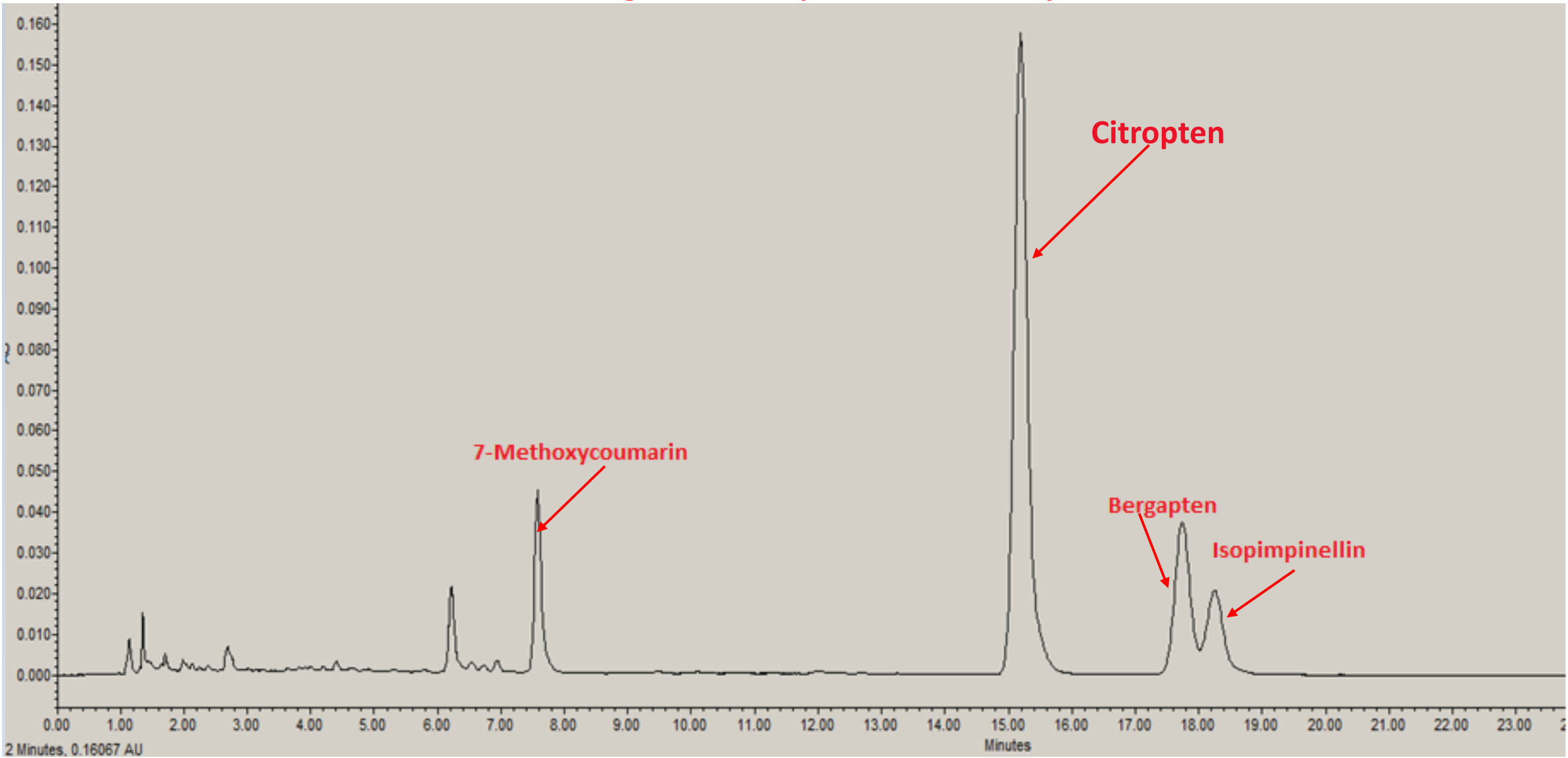


Figure 3: PMF profile for lime juice

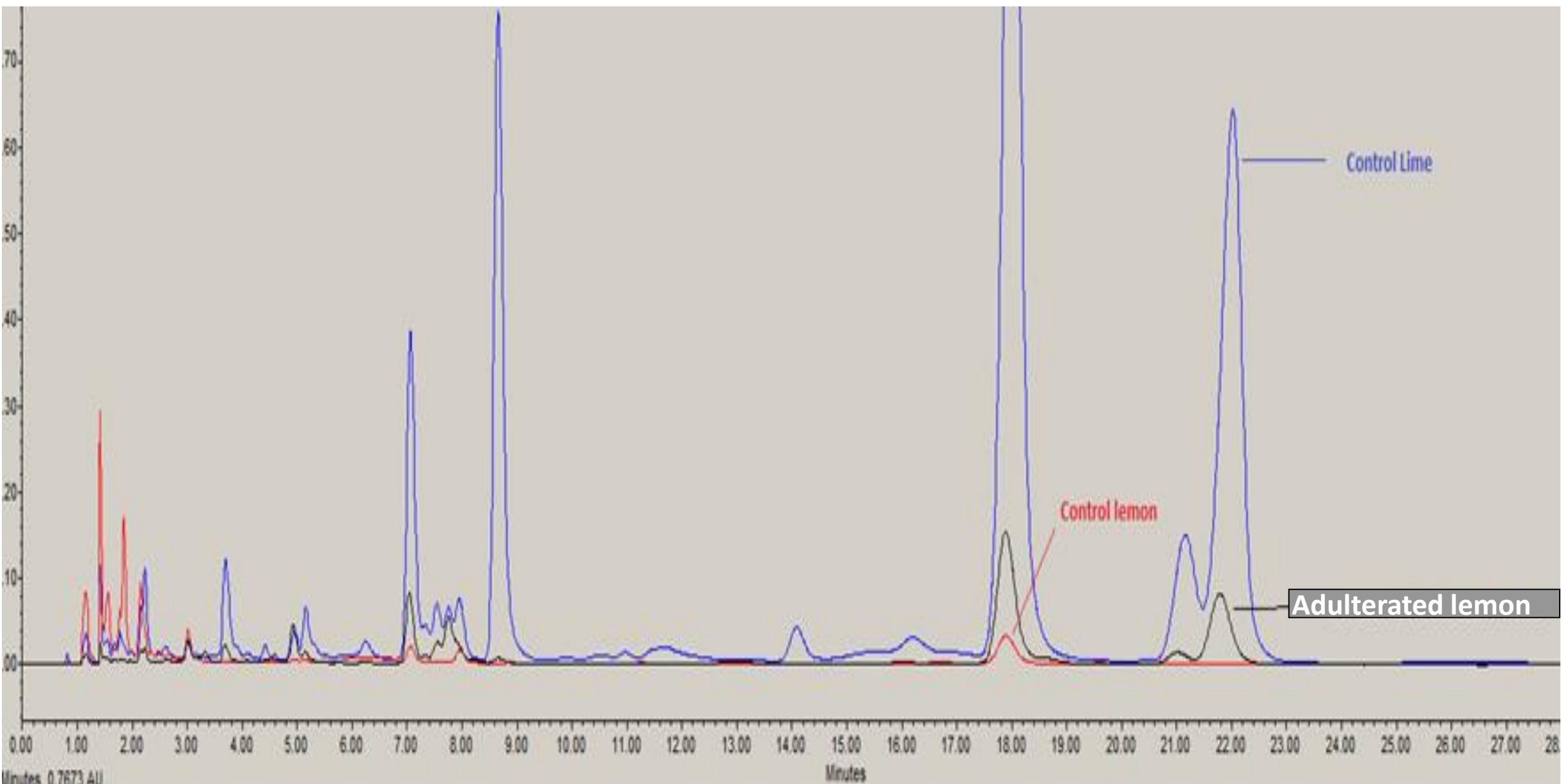


Figure 4: PMF profiles pure lemon, lime and adulterated lemon

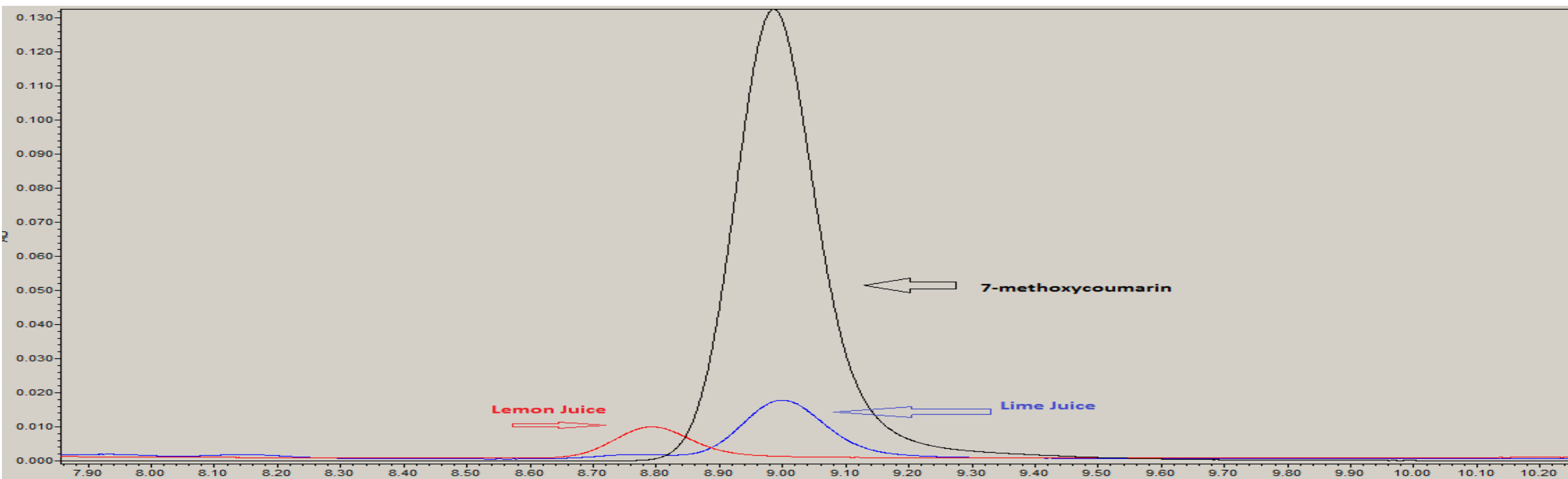


Figure 5: 7-Methoxycoumarin in Lime

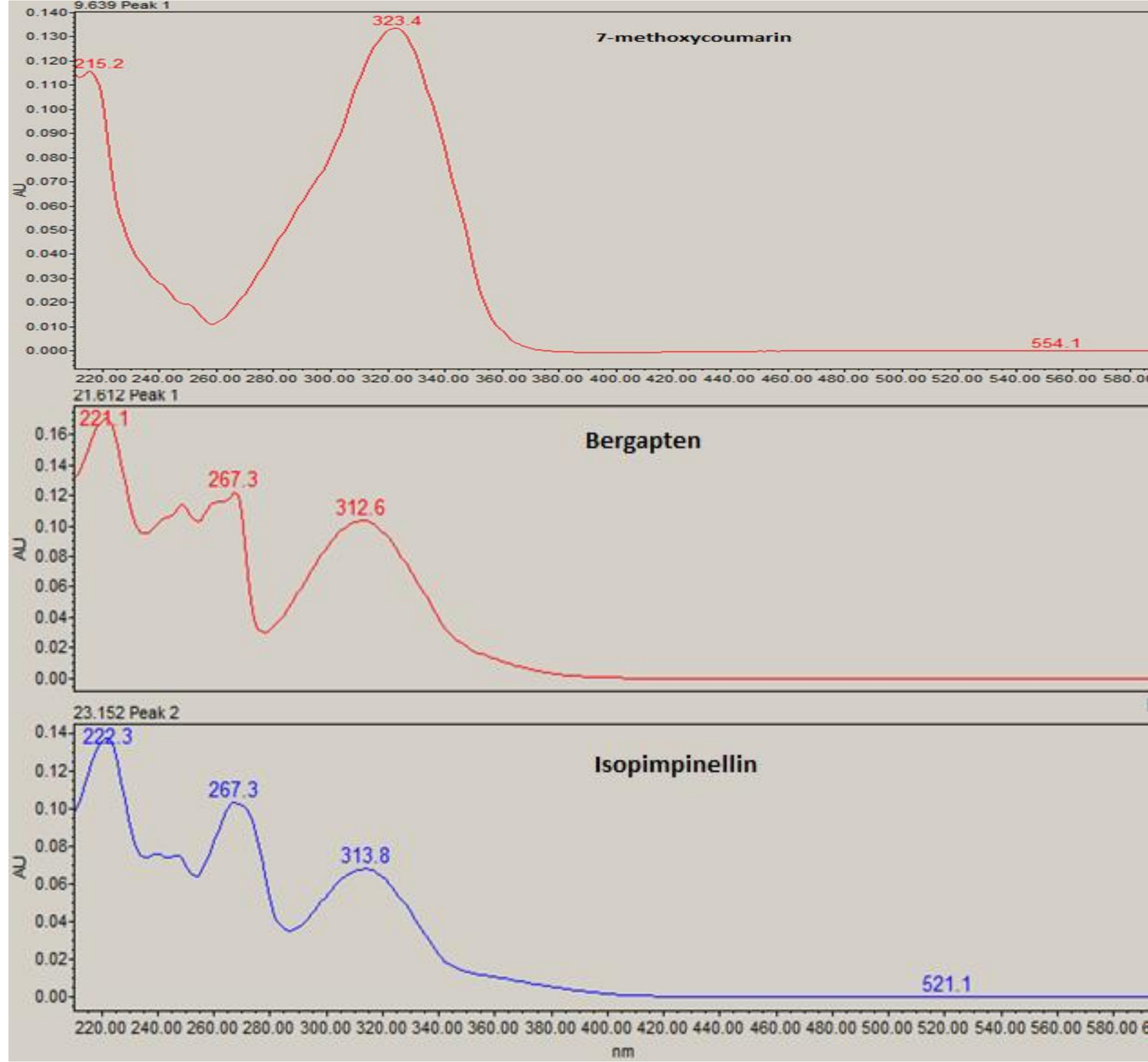


Figure 6: DAD Profiles

## Conclusions

The adulteration of lemon juice still remains an issue and isotopic methods are central to this detection. Depending on the source of the citric acid used for the adulteration either the simple <sup>13</sup>C-IRMS can be used for a C<sub>4</sub> source. Whereas if a C<sub>3</sub> derived material is used, the citric acid has to be isolated and analyzed by D- and <sup>13</sup>C-IRMS.

The method described above offers the best, and perhaps only way, to detect the presence of added lime in lemon.