

Food authentication by HPLC

by Dr. Leo Nollet

Food authentication, i.e. the determination whether a foodstuff really contains the ingredients that it should, is vitally important from both the economic and quality point of view. HPLC, alone or in combination with other methods such as MS, has proven to be a key method for identifying compounds that can be used for the authentication of food. This article reviews HPLC analytical methods that are currently used for the authentication of several foodstuffs, such as fruit products, oils and dairy products.

A large number of compounds can be separated rapidly and at high sensitivity by HPLC. The performance characteristics and applicability of the technique are continually improving, principally due to the large and ever-increasing choice of column packing, and the growing number of solvent combinations. Separation times are decreasing, and there is a reduction in the volume of sample needed. The increasing sensitivity of the technique enables even very small amounts of substances to be detected. The combination of HPLC and mass spectrometry (MS) has increased the range of applications. The apparatus used for such analyses has become progressively smaller and smaller. This miniaturisation has been driven principally by concerns regarding the cost and ecological impact of the large volumes of solvents that used to be needed in older systems.

For some compounds that are important for their use in traceability and food authentication, HPLC is the optimal technique: examples of such compounds are phenolics, anthocyanins, and organic acids. For other compounds HPLC is used as a supplementary technique to support some other methodology.

Fruit products

Authenticity of fruit and fruit products can be determined by the detection of several different types of compounds of which phenolic compounds, organic acids, carotenoids, amino acids, anthocyanins, and sugars are most important. Phenolic compounds, a class which includes many different individual compounds are of course characterised by the presence of a hydroxyl group on a benzene ring. Flavones, flavonols, flavonoids, polyphenols, and chalcones are members of this class of compounds. Table 1 summarises the typical phenolic compounds present in different fruits. The detection of a phenolic compound which is not characteristic for the fruit indicated on the product label suggests adulteration. Since each plant or fruit has a characteristic flavonoid pattern, this pattern can be used for adulteration studies; phenolic and flavonoid compounds have been identified for apricot, peach, plum, apple, pear and strawberry. For example, scarring of quince may be a reason for the adulteration of quince jam by apple or pear puree. Arbutin, a characteristic phenol for pear, has been found in several samples of quince jam after analysis by reversed-phase HPLC (RP-HPLC) of phenolic compounds.

Controlling the authenticity of apple and pear juice may also be based on polyphenol patterns, quercetin glycosides, phenolic acids, and dehydrochalcone derivative. In addition, an HPLC method has been developed with photo-diode array detection of organic acids and phenolic compounds in juices and drinks to evaluate the authenticity and the spoilage of apple juice. In a recent study, apple polyphenols were characterised by LC-DAD (diode array detection) and LC-MS using atmospheric pressure ionisation in positive ion mode. Five isorhamnetin glycosides, two hydroxyphoretin glycosides and quercetin were identified. In order to authenticate citrus fruits and jams flavonoid profiles have been used. Six flavonoids: eriocitrin, neoeriocitrin, narirutin, naringin, hesperidin and neohesperidin were separated by RP-HPLC and DAD.

In Figure 1 it can be seen that orange juice does not contain any naringin or neohesperidin, whereas grapefruit contains both. Consequently

Quince	3-O-caffeoylquinic acid
	4-O-caffeoylquinic acid
	5-O-caffeoylquinic acid
	Rutin 3-O-galactoside
	Quercetin 3-O-galactoside
	Quercetin 3-O-xyloside
	Quercetin 3-O-rhamnoside
Apple	Phloretin 2'-xylosylglycoside
	Phloretin 2'-glycoside
Pear	Arbutin
Citrus fruits	Eriocitrin
	Neoeriocitrin
	Narirutin
	Naringin
	Hesperidin
	Neohesperidin

Table 1. Typical phenolic compounds for quince, apple, pear and citrus fruits.

naringin/neohesperidin ratios determined by HPLC can be used to check adulteration of orange juice with grapefruit juice. This method is based on the identification of flavone glycosides and polymethoxyflavone (PMF) to differentiate sweet orange juices from other citrus juices.

Orange pulp wash can be (and often is) used as a substitute for orange juice. In pulp wash, phlorin is present in much larger quantities than in juice, so phlorin can be used as an indicator. Phlorin is detected by HPLC (3 μ m C18 column) with UV detection.

For other fruits, different marker compounds are used. These include myricetin for peach, two specific coumarins for apricot, quercetin-3-O-glycoside for blackberries and some sinapyl derivatives of 2,5-dimethyl-4-hydroxy-3(2H)furanone (DMHF) for pineapple.

Adulterated samples of orange juices contain much smaller amounts of carotenoids than pure juice; carotenoids are thus good marker compounds for the detection of such adulteration. There are also methods based on the detection of amino acids, as well as on the detection of anthocyanins, sugars, and organic acids. For example, adulteration of cranberry juice can be recognised through an HPLC analysis method detecting organic acids, such as quinic, malic, citric, and fumaric acid.

DL-isocitric acid is another important marker for the evaluation of fruit products. Three methods, namely RP-HPLC, enzymatic analysis and capillary isotachopheresis (CITP) have been established for the detection of DL isocitric acid.

Recently an on-line RPLC-GC-MS method was developed for the enantiomeric analysis of chiral compounds that are characteristic constituents of the aroma from fruit beverages and are consequently useful in the assessment of the authenticity of fruit beverages.

Oils

Amongst the many oils used in the food industry, olive oil has been the most studied. The triglyceride profiles of soybean oil and olive oil are significantly different, as can be seen in Figure 2. The addition of even low levels of soybean oil in olive oil can therefore be detected through determination of the triglyceride profiles.

The addition of oils with high linoleic acid content to olive oil can be detected through use of an RP column-based method with refractive index (RI) detection. Based on this method an authenticity factor can be calculated:

$$Au = \frac{100 - ECN \times 42(\%)}{ECN \times 42(\%)}$$

$$ECN = CN - X.n$$

where Au = authenticity factor; ECN = equivalent carbon number; CN = total number of carbon atoms; X = number of double bonds; n = factor for double bond contribution.

Virgin olive oil has an authenticity factor of 98.2 ± 3.86 ; authenticity factors for corn, sunflower, and soybean oils are 3.2 ± 0.02 , 3.5 ± 0.06 , and 3.2 ± 0.19 respectively.

The extent of adulterants added to olive oil can be expressed by the equation:

$$\text{Added oil (\%)} = \frac{ECN \ 42(\%)}{a} - b$$

where a and b are constants that are a function of the added oil.

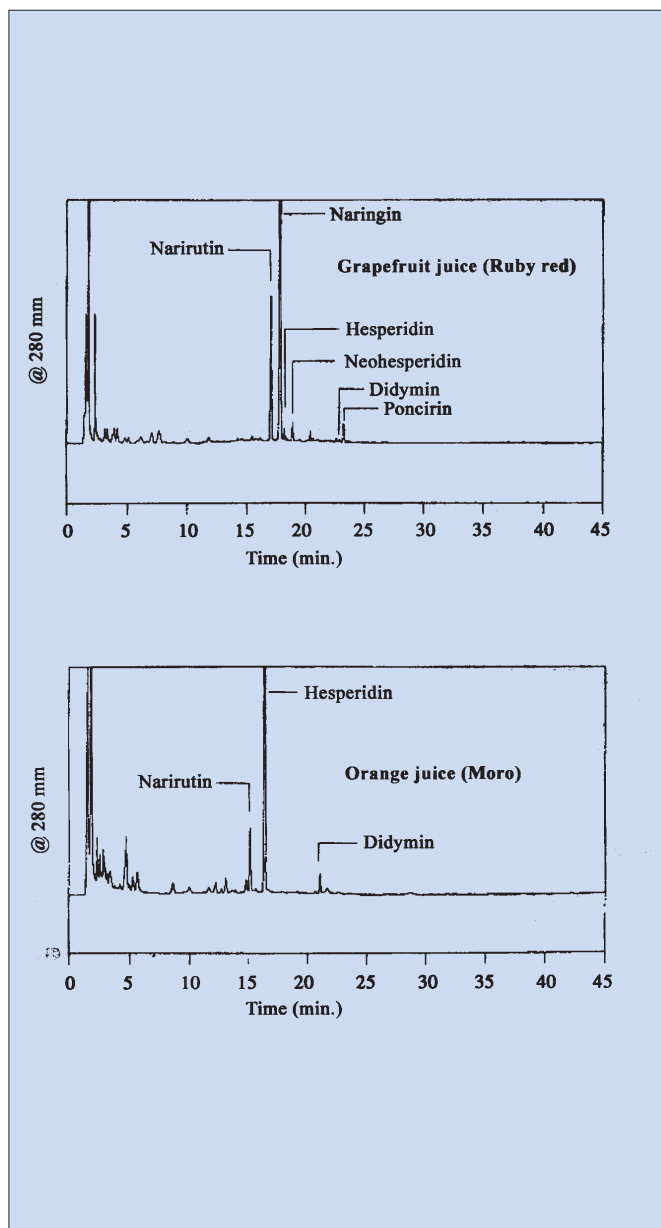


Figure 1. Separation by HPLC of flavanone glycosides in grapefruit juice and orange juice [1].

Several HPLC methods have been developed both to identify olive oils or to detect the addition of other oils, such as hazelnut oil, to olive oil. One method of determining the quality or authenticity of olive oil is by HPLC analysis of the carotene content and lutein content.

HPLC methods to detect triglycerides have been used to identify adulteration and to characterise and classify cultivars, i.e. varieties of cultivated plants brought about by selective breeding. For example, methods were established to characterise and classify 19 almond cultivars. Multivariate techniques and principal component analysis (PCA) were then used to group or differentiate Spanish and Italian cultivars.

Different compounds, such as sterols, steryl glycosides, tocopherols, isoprenoid alcohols, and triglycerides have been investigated as to their suitability for possible use in the authentication of corn oils. Isoprenoid alcohols seem to be the most useful for distinguishing rapeseed oil, sunflower oil and corn oil, and corn oil in other oils. Other chromatographic techniques have also been evaluated for use in the authentication of vegetable oils [4].

Milk

Bovine milk may be adulterated by the addition of soybean proteins, so several methods have been set up to detect soy proteins. For example, methods are available to detect soy proteins in unheated milk. Various methods have also been developed to detect the presence of bovine, ovine, and caprine (goat) proteins in mixtures of milk or cheeses. Markers for such analyses are casein fractions e.g. α_{s1} -casein for cow milk. Para- κ -casein has been used to determine the percentages of cow's, ewe's, and goat's milk in cheeses. RP-HPLC-UV methods have been developed, optimised and validated for the separation and quantification of κ -, α -, and β -caseins with the idea of using such methods for the detection of the adulteration of milk. A method has also been developed for the detection and quantification of the percentages of bovine, ovine, and caprine milk in proprietary

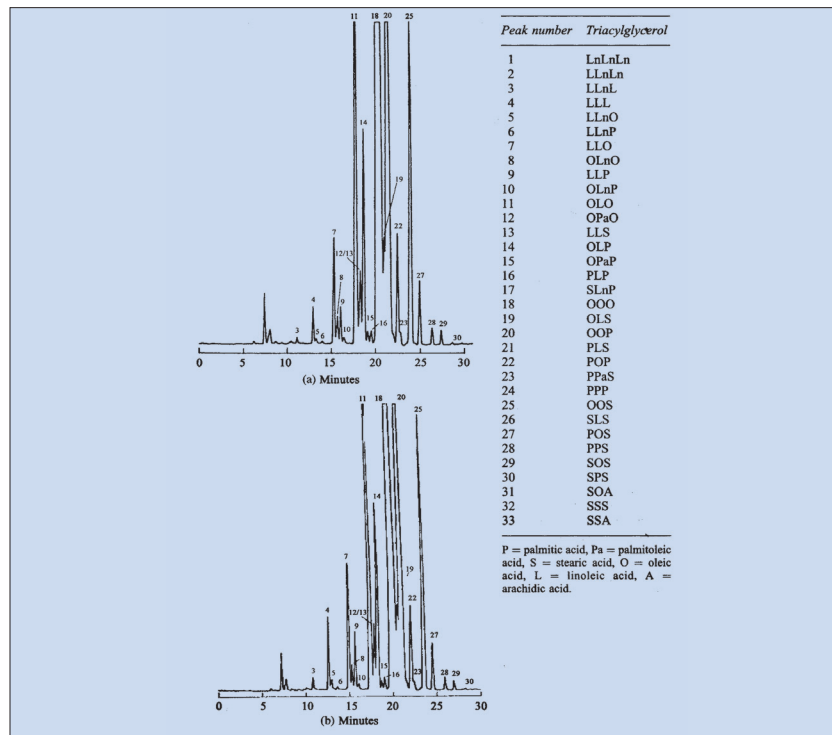


Figure 2. (a) Triacylglycerol profile of 10 % soybean oil in olive oil
(b) Triacylglycerol profile of 5 % soybean oil in olive oil [2].

cheeses, e.g. cheeses that can only be labelled with a particular label if they have a certain origin. RP-HPLC has been used to find alternative β -lactoglobulin markers. Skim milk powder must not contain solids from whey or butter-milk. Methods based on an SE-HPLC (size-exclusion HPLC) detection of the marker glycomacropptide (GMP) have been developed to detect adulteration of skim milk powder.

So far this article has focussed on the authentication of fruit products, oils, and milk products. A great number of methods are recorded in the literature for the detection and analysis of adulterations in other foodstuffs. Other food products that are frequently authenticated are ginseng, vanilla, saffron, honey, soy, meat, varying essential oils, vinegar, wine, whiskey, coffee, pasta, quillaja, and cocoa. One other example is the HPLC detection of food colours or synthetic dyes as a measure of authenticity or adulteration. It is beyond the scope of this article to enumerate methods for all foodstuffs.

The future

The use of HPLC for food authentication is similar to all other applications of HPLC in that the

unmistakable trend for the future is towards miniaturisation of columns and simplification of the procedures for treating the samples. Miniaturisation results in the need for lower sample and solvent volumes and gives more rapid separation. One other unmistakable trend is the combination of HPLC and MS and the automation of the whole separation procedure.

References

1. Lee HS. HPLC Analysis of Phenolic Compounds, in Nollet MLL. Food Analysis by HPLC, New York, Marcel Dekker, 2000; 804.
2. Marini D. HPLC of Lipids in Nollet LML. Food Analysis by HPLC, New York, Marcel Dekker, 2000; 231-232.
3. El-hamdy AH and El-fizga NK. Journal of Chromatography 1995; 708: 351.
4. Aparicio R and Aparicio-Ruiz R. Authentication of vegetable oils by chromatographic techniques. Journal of Chromatography A 2000; 881(1-2): 93-104.

[Additional references and information are available from the author.]

The author

Leo Nollet, Ph.D
Hogeschool Gent,
Gent, Belgium

Email: leo.nollet@hogent.be