# ระบบไดอะไลซิสต่อเนื่องร่วมกับวิธีการวิเคราะห์อื่นๆ สำหรับการวิเคราะห์หาปริมาณกรดอินทรีย์ที่มีน้ำหนักโมเลกุลต่ำในไวน์

# ON-LINE DIALYSIS SYSTEM COUPLED TO ANALYTICAL METHODS FOR THE DETERMINATION OF LOW MOLECULAR WEIGHT ORGANIC ACIDS WINE

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## บทคัดย่อ

กรดอินทรีย์ที่มีน้ำหนักโมเลกุลต่ำต่างๆ ในไวน์นั้นถือว่ามีบทบาทที่สำคัญ นั่นคือช่วยทำให้ไวน์มีรส มีกลิ่น มีสีที่เสถียร และช่วยในการควบคุมกระบวนการทางจุลชีววิทยาในไวน์ ดังนั้นในบทความนี้จึงได้นำเสนอแนวโน้มของการพัฒนาวิธีการวิเคราะห์สำหรับวิเคราะห์หาปริมาณกรดอินทรีย์ที่มีน้ำหนักโมเลกุลต่ำต่างๆ ในไวน์ โดยได้ทำการสรุปรวบรวม เทคนิคการเตรียมตัวอย่างแบบต่อเนื่องร่วมกับวิธีการแยกหรือการตรวจวัดต่างๆ โดยเฉพาะอย่างยิ่งระบบไดอะไลซิสแบบการไหลต่อเนื่องร่วมกับเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง นอกจากนี้ยังได้ทำการอภิปรายและยกตัวอย่างประกอบให้เห็นถึงความสามารถของเทคนิคแบบต่อเนื่องนี้ด้วย

**คำสำคัญ:** ไดอะไลซิสต่อเนื่อง กรดอินทรีย์ ไวน์

## Abstract

Low molecular weight organic acids have an important role in wine. They contribute to taste, flavor, color stability and control of microbial organisms in wines. This review presents the trend of the development of analytical methods for the determination of these organic acids in wine. The combinations of on-line sample pretreatment techniques and various separation or detection methods are summarized. Special emphasis is given to on-line dialysis systems coupled to high performance liquid chromatography. Moreover, the potential of these on-line techniques is discussed and illustrated with selected examples.

**Keywords:** On-line dialysis, Organic acid, Wine

## Introduction

Several analytical methods have been used to identify and quantify low molecular weight organic acids in wine samples. The purpose of all these methods is to control the evolution of acidity, stability or infection of wine, during different steps of the winemaking process until bottling. These methods include titration, spectrophotometry, capillary electrophoresis (CE), fourier transform infrared spectroscopy (FTIR), gas chromatography (GC), high-performance liquid chromatography (HPLC) and flow analysis (including flow injection analysis (FIA) and sequential injection analysis (SIA)).

The titration [1] and spectrophotometry [2] are the AOAC standard methods commonly used to determine tartaric and citric acids in wine, respectively. For the titration method, a sample is first recrystallized, precipitated and then titrated with sodium hydroxide solution using phenolphthalein as an indicator. The spectrophotometry is an enzymatic method using citric lyase, which reacts with nicotinamide-adenine dinucleotide (NADH). The consumption of NADH is then measured at 340 nm.

Other methods for determination of organic acids in wine have been reported. They include CE methods based on dilution and filtration sample pretreatment procedures with detection using conductivity [3] or UV spectrophotometry [4-7]. FTIR methods that are usually based on free sample pretreatment [8] and GC methods commonly used for volatile organic acids determination either with sample dilution or direct injection [9-10].

According to review papers, a large numbers of HPLC methods using several detection systems have been reported for the determination of organic acids in wine samples and they are summarized in Table 1. Table 1 shows the usage of detection systems including UV spectrophotmetry [11-42], conductivity [43-45], refractive index [46-47], FTIR [48], electrochemistry [49-50] and chemiluminescence [51]. Various sample pretreatment techniques such as derivatization [21,24], ion exchange [22], solid phase extraction [23, 26-27, 38, 44, 46], dilution & filtration [25, 28-34, 36-37, 39-43, 45, 47-50] and off-line dialysis [11] are frequently used prior to HPLC analysis of real wine samples in order to decrease the matrix effect. The conventional methods of dilution and filtration are used in most case for reduction and elimination of sediment substances in wine samples because they are simple and straight forward. However, both techniques involve tedious procedure and they still leed too much interference, which shortens the life-time of the HPLC columns. The derivatization, ion exchange, and solid phase extraction techniques are very complex, expensive and require a high consumption of chemicals and materials. However, these techniques can effectively eliminate or at least significantly decrease the matrix and interference effects. Among these sample pretreatment techniques, solid phase extraction and dialysis could easily be automated and used in conjunction with HPLC.

The aim of this review is to focus on and explain the on-line dialysis method coupled to analytical methods, especially HPLC, and to its application for the determination of organic acids in winee.

**Table 1** HPLC methods for the determination of organic acids in wine and other samples using several sample pretreatment and detection techniques.

| Organic Acids | Sample | Sample Preparation | Separation Mechanism | Techniques/ Detection system | Analysis Time(min) | [References] ; year |
|---|---|---|---|---|---|---|
| lactic, acetic, tartaric, malic, succinic, citric | wine | Derivatization | Reversed phase | HPLC/UV spectrophotometry (210 nm) | 17 | [21]; 1986 |
| citric, tartaric, malic, lactic, acetic | grape must, wine | Ion exchange | Reversed phase | HPLC/UV spectrophotmetry (210 nm) | 12 | [22]; 1987 |
| tartaric, malic, lactic, acetic, citric, shikimic, fumaric, succinic | fruit juice, wine | Solid-phase extraction | Reversed phase | HPLC/UV spectrophotmetry | 9 | [23]; 1990 |
| carboxylic | wine, grape must | Derivatization | Reversed phase | HPLC/UV spectrophotmetry | - | [24]; 1990 |
| organic | must | Dilution & Filtration | Reversed phase | HPLC/UV spectrophotmetry | 40 | [25]; 1991 |
| tartaric, malic, citric | grape juice, wine | Dilution & Filtration | Ion exchange | HPLC/Conductivity | 35 | [43]; 1991 |
| acetic,citric, lactic, malic, succinic, tartaric | wine | Solid-phase extraction | Reversed phase | HPLC/UV spectrophotmetry | 26 | [26]; 1991 |
| acetic,citric, lactic, malic, succinic, tartaric | wine, grape must | Solid-phase extraction | Ion exchange | HPLC/Refractive index | 25 | [46]; 1992 |
| tartaric, malic, lactic | wine | Solid-phase extraction | Reversed phase | HPLC/UV spectrophotmetry | 4 | [27]; 1993 |
| tartaric | wine | Dilution & Filtration | Reversed phase | HPLC/UV spectrophotometry (210 nm) | 20 | [28]; 1994 |
| malic, tartaric | grape juice, wine | Dilution & Filtration | Reversed phase | HPLC/UV spectrophotmetry | 12 | [29]; 1996 |
| citric, tartaric, malic, lactic, succinic,galacturonic | wine | Dilution & Filtration | Ion exclusion | HPLC/Refractive index | - | [47]; 1996 |
| lactic, succinic, malonic, malic, maleic, tartaric | wine | Dilution & Degassed | reversed phase | HPLC/UV spectrophotometry (254 nm) | 20 | [30]; 1996 |
| acetic, lactic, succinic, malic, citric, tartaric | wine | Solid phase extraction | Ion exchange | HPLC/Conductivity | 25 | [44]; 1996 |
| tartaric, lactic, malic, citric | grape must, wine | Filtration | Reversed phase | HPLC/UV spectrophotometry (254 nm) | 6 | [31]; 1996 |
| tartaric, lactic, malic, citric, succinic | wine | Dilution | Reversed phase | HPLC/UV spectrophotometry (210 nm) | 9 | [32]; 1998 |
| acetic, citric, lactic, malic, succinic, tartaric | wine | Filtration | Ion exchange | HPLC/Fourier-transform infrared spectroscopy | 25 | [48]; 1998 |

**Table 1** (Continued)

| Organic Acids | Sample | Sample Preparation | Separation Mechanism | Techniques/ Detection system | Analysis Time(min) | [References] ; year |
|---|---|---|---|---|---|---|
| Citric, tartaric, malic, fumaric, succinic, lactic, formic, acetic | brandy | Filtration | Ion exclusion | HPLC/conductivity | 60 | [45]; 2000 |
| tartaric, malic, citric | wine | Dilution & Filtration | Reversed phase | HPLC/UV spectrophotometry (210 nm) | 9 | [33]; 2001 |
| citric, tartaric, malic, lactic, formic, acetic | wine, cheese, juice | Dilution & Filtration | Ion exclusion | HPLC/ Electrochemistry | 15 | [49]; 2002 |
| citric, tartaric, malic, dhikimic, succinic, lactic, fumaric, acetic | must, wine | Dilution & Filtration | Ion exclusion | HPLC/UV spectrophotometry (215 nm) | 25 | [34]; 2002 |
| lactic, acetic, succinic, citric tartaric, malic | must, wine | Ion exchange | Reversed phase | HPLC/UV spectrophotometry (265 nm) | 30 | [35]; 2002 |
| tartaric, malic, citric | grape juice | Filtration | Ion exclusion | HPLC/UV spectrophotometry (214 nm) | 15 | [36]; 2002 |
| citric, tartaric, malic, lactic, acetic | wine, must | Filtration | Reversed phase | HPLC/UV spectrophotometry (210 nm) | 30 | [37]; 2004 |
| citric, lactic, malic, oxalic, tartaric | wine, beer, milk, fruit, soft drink | Dilution & Filtration | Reversed phase | HPLC/Chemiluminescence | 35 | [51]; 2004 |
| acetic, citric, lactic, malic, succinic, tartaric | wine | Filtration | Ion exclusion | HPLC/Electrochemistry | 22 | [50]; 2004 |
| oxalic, tartaric, acetic, glutaric, succinic, butane dicarboxylic | bayer liquors | Solid-phase extraction | Reversed phase | HPLC/UV spectrophotometry (215 nm) | 10 | [38]; 2006 |
| ketoglutaric, tartaric, malic, quinic, lactic, shikimic | wine | Filtration | Ion exclusion | HPLC/UV spectrophotometry (214 nm) | 60 | [39]; 2007 |
| citric, ascorbic, malic | orange juice & wine | Filtration | Ion exchange | HPLC/UV spectrophotometry (210 nm) | 30 | [40]; 2009 |
| tartari, malic, lactic, acetic, citric, succinic | wine | Off-line dialysis | Reversed phase | HPLC/UV spectrophotometry (210 nm) | 8 | [11]; 2009 |
| formic, citric, , tartaric, protocatehuic, vanillic, sinapic | wine | Dilution & Filtration | Reversed phase | HPLC/UV spectrophotometry (210 nm) | 8 | [41]; 2010 |
| ascorbic, oxalic, tartaric, malic, lactic, citric, succinic propionic, formic, acetic | wine, vinegar, fruit juices, root exudate | Dilution & Filtration | Reversed phase | HPLC/UV spectrophotometry (360 nm) | 20 | [42]; 2011 |

## Wine and the wine making process

Wine is one type of alcoholic beverage like beer and distilled liquors [52]. It is usually made from a variety of fruits, such as grapes, peaches, apples and pineapples. Most common wines in the majority of countries are produced from grapes. Wines can be divided into four styles; table wines, sparkling wines, fortified wines and dessert wines. Table wines are still and usually consumed during the course of a meal. Table wines constitute the largest group of red, white and ros wines that are divided based on color, reflecting major differences in use, flavor, and production techniques. The alcoholic strength of table wines usually varies between 10 and 14% of alcohol by volume (ABV). Sparkling wines are wines with carbon dioxide added to them, either naturally or artificially. Sparkling wines are very versatile and can be drunk at almost any stage of a meal. Sparkling wines can be classified by production procedure and flavor characteristics. Champagne is the most obvious example of a sparkling wine. The alcoholic strength of sparkling wines roughly the same as that of table wines. Fortified wines are simple wines that have had a distilled spirit (usually brandy) added to them. Many different styles of fortified wine have been developed such as sherry, port, madeira, muscatel, vermouth, byrrh, marsala and dubonnet. These fortified wines are customary taken at the end of a meal with fruits or nuts, namely 'dessert wines', or consumed before a meal to stimulate the appetite, namely 'aperitif wines'. The alcoholic strength of most fortified wines is about 18% ABV [53-54]. In Thailand, the Thai Industrial Standards Institute (TISI), Ministry of Thailand, also divides wines into various styles and assignes the standard alcohol values using the standard number of TIST 2089-2544. These Thai wine styles are table wines (containing 7-15% ABV), sparkling wines (containing 9-15% ABV with carbon dioxide added), fortified wines (containing 15-23% ABV) and flavored wine (containing < 23% ABV) [55].

The basic technology of wine making process usually starts with fruits selection. The following steps are harvest, stemming, crushing and pressing of grapes to obtain the grape juice. The grape juice is then turned into wine by fermentation, and maturation. The process is conclude with bottling of the finished wine. The anaerobic alcohol fermentation process (described briefly in Figure 1.) starts when yeast (i.e. *Saccharomyces cerevisiae* and *S. elipsoideus*) is added to the fruits juice, especially grape juice. Then the yeast cells enzymatically consume fruit sugars (e.g. glucose or fructose) and convert them to pyruvic acid by glycolytic pathway and finally to ethanol (also called 'ethyl alcohol') and carbon dioxide [12-14, 52]. Under these wine production conditions, water and ethanol are major components with contents of approximately 86.8% and 11.2%, respectively. Other chemical species such as acids, fusel alcohols, esters, aldehydes, ketones, sugars, vitamins, proteins, tannins, anthocyanins and others are minor chemical components. In general, these components are related to flavor, color, balance, stability and taste perception of wine [15-18, 52]. The concentrations of some components are shown in Table 2.

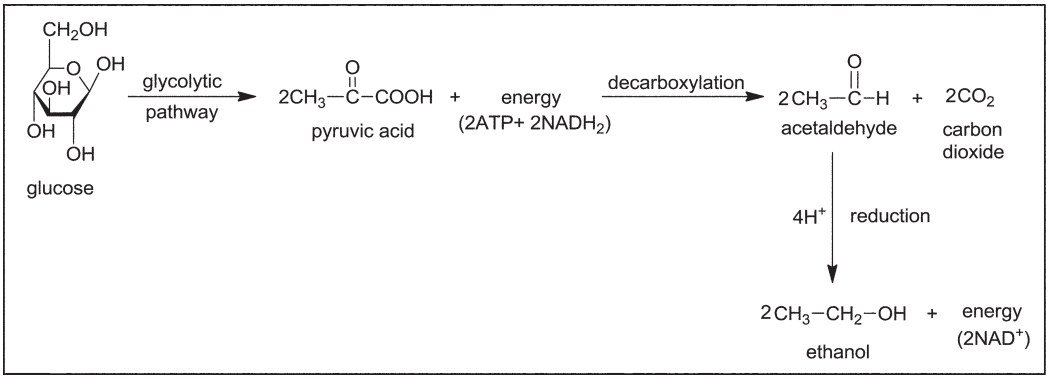


**Figure 1.** Simple equation of an anaerobic fermentation process of wine [adapted from Ref. 12-14].

**Table 2** Concentrations of some significant volatile and non-volatile components in wine [13, 19]

| Components | Concentrations (mg/L) | Components | Concentrations (mg/L) |
|---|---|---|---|
| **Alcohols:** | | **Acids:** | |
| Ethanol | 80-130 (g/L) | Tartaric | 500-7000 |
| Glycerol | 2-10 (g/L) | Malic | 50-5500 |
| Iso-amyl alcohol | 50-350 | Succinic | 50-2000 |
| Active amyl alcohol | 1-300 | Lactic | 10-5000 |
| Iso-butanol | 2-150 | Acetic | 20-2000 |
| Propanol | 10-125 | Citric | 50-1000 |
| 2-phenyl ethanol | 15-200 | **Aldehydes and Ketones:** | |
| **Esters:** | | Acetaldehyde | 10-150 |
| Ethyl acetate | 5-200 | Diacetyl | 0.2-5 |
| Ethyl lactate | 1-50 | Acetoin | 0.1-12 |
| Phenyl ethyl acetate | 0.1-10 | **Sulphur compounds:** | |
| Iso-amyl acetate | 0.1-12 | Hydrogen sulphide | 1-30 (μ/L) |
| Ethyl octanoate | 0.1-8 | Dimethyl sulphide | 5-50 (μ/L) |
| Ethyl hexanoate | 0.1-2 | Sulphur dioxide | 10-100 |

## Low molecular weight organic acids in wine

Acids in wine (with contents of approximately 0.5% [15]) play important roles. They influence the pH, taste, color stability, and flavor of wine. Acids give wines a slightly tart taste but this can be moderated by alcohols, sugars, minerals and other components. Acids maintain a low pH value of wine (in the range of 3.1-3.4 for most white wines and 3.3-3.6 for most red wines) which is necessary for the color stability of red wine. Furthermore,

acids also have an effect on the stability and microbiological control of wine quality by stopping or at least retarding the growth of many potential harmful microorganisms that would spoil the wine and give it bad flavor. Acid compounds in wine can be classified into two groups: organic and inorganic acids. The organic acid compounds include aliphatic saturated and unsaturated acids (e.g. acetic, pentanoic and decanoic), di- and tri-carboxylic acids (e.g. succinic, glutaric and adipic), aromatic carboxylic acids (e.g. cinnamic), hydroxycarboxylic acids (e.g. malic, lactic, tartaric and citric), ketocarboxylic acids (e.g. pyruvic, 2-ketoglutaric and levulinic), sugar acids (e.g. gluconic and glucurinic) and miscellaneous acids (e.g. vanillic, shikimic and caffeic). The principal inorganic acid compounds are carbonic and sulfurous acids. Both inorganic acids occur as dissolved gasses, namely carbon dioxide ($CO_2$) and sulfur dioxide ($SO_2$), respectively [16, 20, 52].

There are some low molecular weight (also called 'short chain') organic acids (as shown in Figure 2) among these acid compounds and they are an important ingredient in wine and are interesting for wine analysis.

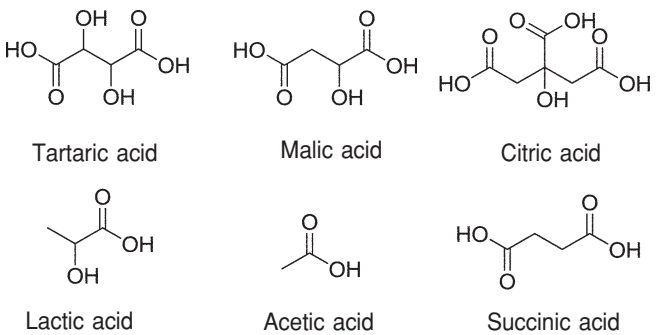


**Figure 2.** Structures of some low molecular weight organic acids derived from the wine making processes.

Some acids (tartaric, malic and citric acids) are contained already in the fruits from which wine is made, while others (lactic, succinic and acetic acids) are by-products of the fermentation process. They are formed during alcoholic fermentation, malolactic fermentation, oxidation of the ethanol, aging process, etc. The most abundant organic acids in grapes are tartaric and malic acids, with citric acid being a minor component. In other fruits, malic or citric acids are usually dominant. When natural organic acids are absent or deficient in the wine, a blend of tartaric, malic and citric acids or only citric acid are usually added to improve the taste. Lactic acid is produced in wine during malolactic fermentation, where strong malic acid is converted to a softer lactic acid. Succinic acid is one of the common by-products of yeast fermentation and is found in trace amounts in all wines. However, the bitter-salty taste of succinic acid limits its use for wine acidification. Usually only small amounts of acetic acid (< 300 mg/L) are produced by yeast fermentation. Its presence can be a desirable for the flavor of wine by adding to the complexity of taste and odor. High levels

of acetic acid (> 300 mg/L) are usually associated with contamination of grapes, juice, or wine with acetic bacteria and these levels progressively gives wine a sour taste and taints its fragrance [11, 16, 20].

## Sample pretreatment by dialysis

Dialysis is one of membrane-based sample preparation methods. Dialysis process is very simple. It allows small solute molecules to diffuse from a region of high concentration to a region of low concentration across a semi-permeable membrane until equilibrium is reached. When the porous membrane selectively allows smaller solutes to pass while retaining larger species, dialysis can effectively be used as a separation process based on size rejection. The conventional dialysis procedure and a separation process based on it are shown in Figure 3.

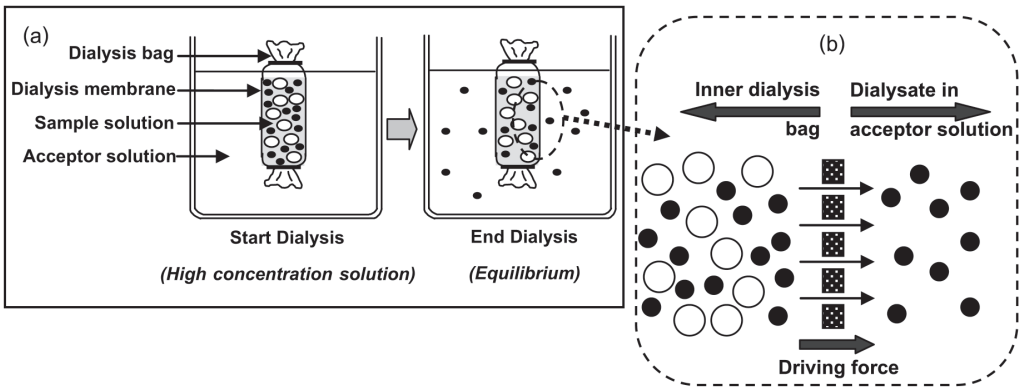


**Figure 3.** Schematic representation of (a) the conventional dialysis procedure and (b) the separation process on a dialysis membrane discriminating small solute molecules (●) and large solute molecules (○) (depicted by O. Kritsunankul and adapted from Ref. [57]).

The separation process of dialysis involves two principles, diffusion and ultrafiltration. The movement of solutes by diffusion is the result of random molecular motion. Diffusion depends on the (a) solute concentration gradient through the semi-permeable membrane, (b) solute molecular weight and (c) membrane resistance. The transport of solute across semi-permeable membranes by ultrafiltration (convective transport) occurs when water driven by either hydrostatic or osmotic force is pushed through the membrane. Ultrafiltration is, apart from other factors, dependent on the ultrafiltration coefficient of the dialysis membrane [56-57]. Applications of the conventional dialysis procedure include trace compound analysis, macromolecular purification, protein concentration, solute fractionation, contaminant removal, and desalting in various samples including plasma, blood, serum, food (e.g. milk, meat, egg, juice, etc.) and polymes. In all these applications, the advantages of dialysis are simple and easy operation, wide range of sample volumes, many membrane types and molecular weight cut

off (MWCO) threshoulds, and inexpensive materials [58-59].

Originally, the conventional (also called 'off-line' or 'direct') dialysis sample pretreatment method was used by Kritsunankul et.al. prior to HPLC for determination of some organic acids in wine. The method was used for removing or reducing content of the high molecular weight molecules (e.g. proteins, yeast cells and bacteria), particulate matter (e.g. micro-particulate and colloid), and other matrices in wine samples from the low molecular weight molecules, especially organic acids. However, this conventional dialysis application is tedious, time-consuming (reaching equilibrium) and consumes large amounts of sample and reagents [11]. To improve some drawbacks of the conventional dialysis procedure, the on-line dialysis was developed and coupled to separation and detection systems, leading to new design of an automated system.

## On-line dialysis coupled to analytical methods

Recent years and decades have seen increasing interest in the automation of sample pretreatment/preparation with the aim of achieving faster sample pretreatment/preparation procedures, high sample throughput and more cost-effective analyses. Thus, the concepts of flow analysis methods including FIA and SIA, can serve these objectives. FIA or unsegmented-flow analysis was reported for the first time by Ruzicka and Hansen in 1975 [60]. Its general procedure is based on the injection of sample solution into a continuous flowing stream of reagent or carrier. A zone of the injected sample solution is formed and moved at constant speed downstream by pump into a reaction or mixing coil, in which the sample solution is dispersed into the reagent. The product is formed and then transported toward a detector that continuously records the signal such as pH, conductivity, absorbance, fluorescence and electrochemical potential. The flowing stream in FIA system has two main purposes. One is to deliver the sample zone to the detector. The second is to merge the sample zone and carrier stream together. If the carrier contains reagent, this process brings the analyte and reagent together to promote chemical reaction that generates a detectable product. Although it is not necessary for the reaction to reach equilibrium, the extent of the reaction must be the same for both standards and samples. SIA was first reported in 1990 by Ruzicka and Marshall [61]. SIA is based on injection of a sample into a carrier solution, moving in a preprogrammed way. Sample and reagent solutions are selected by means of a mulltiposition valve and a syringe pump controls their volumes, on a microlitre scale. Mixing is achieved by flow reversals, and reaction rates may be monitored, while the reacting mixture is held within a flow through cell.

Therefore, with regard to the scope of this review article, the focus is restricted to the application of flow injection on-line dialysis (FID) as well as sequential injection on-line dialysis (SID) coupled to various separation and detection systems of analytical methods (e.g. spectrophotmetry, HPLC, etc.).

FID and SID procedures often use a dialysis cell (also called 'dialysis unit') with different designs of the cells and positions in the system. Most of the dialysis cells are made of two plates from acrylic or other materials, engraved

for donor and acceptor channels. The two channels are separated by a dialysis membrane with a selected MWCO. The dialysis cells are often placed on-line in the FI/SI systems after the injection valve. A dialysis cell is incorporated into the FI/SI systems not only to allow sample dilution, but also to minimize the presence of interfering compounds. In this case, direct sample introduction into the systems is possible without any previous treatment of the wine samples.

We now describe the applications of FID/SID systems. FID with ion chromatography has been exploited for the determination of some common anions in wastewater samples [62]. Peroxynitrite in biological samples was determined by FID with chemiluminescence detection [63]. An on-line dialysis with trace enrichment cartridge was used for sample clean-up/preconcentration before HPLC determination of flumiquine and oxolinic acid in the extract of fortified chicken tissue [64]. FID spectrophotometric detection system has been reported to overcome the interference from suspended material in the determination of ethyl xanthate in liquors from flotation process of ore processing plant [65]. SID spectrophotometric system for determination of zinc in fertilizers has been developed to remove the suspended solids [66]. SID was also applied for dilution and separation of reducing sugars in wine prior to their spectrophotometric determination [67].

## Application to organic acids in wine

FID/SID systems coupled to various separation and detection systems (summarized in Table 3) have been applied for determination of organic acids in wine and other samples. These separation and analytical methods include fluorimetry [68,73], UV-Visible spectrophotometry [69-75], HPLC/refractive index [76-77], and HPLC/UV spectrophotometry [11]. For example, tartaric acid in wine was determined using FID pretreatment before spectrophotometric detection [74]. The commercial automated sequential system (ASTED XL) that enriches trace components of dialysates was employed for on-line dialysis prior to HPLC determination for automated preparation and analysis of sugars and organic acids in foods and beverages [76]. On-line dialysis with HPLC was used for the automated preparation and analysis of amino acids, sugars and organic acids in grape juice and wines [77]. The proposed uses of these FID/SID systems coupled to analytical methods were to eliminate macromolecular and microparticulate matrix interferences, to accomplish on-line dilution or separation, to use non-toxic solvent (water) for on-line dialysis, to prolong the life-time of an expensive HPLC column, to achieve high degree of automation in sample pretreatment, lower consumption of chemicals and materials, and to take advantage of the low cost of the consumable dialysis membrane (only one membrane for hundreds of analyses). In addition, the analytical merits were significant i.e. high accuracy, high precision and high sensitivity, which are expressed as %recovery, %RSD (relative standard deviation), LOD/LOQ (limit of detection/limit of quantitation), respectively.

**Table 3** Determination of low molecular weight organic acids in wine and other samples by FID/SID systems and FID/SID-HPLC systems.

| Organic Acids | Sample | Sample Preparations | Technique/Detection system | Sample Rate ($h^{-1}$) | RSD (%) | [References]; year |
|---|---|---|---|---|---|---|
| L(-) malic, L(+) lactic | wine | On-line Dialysis | FIA/fluorimetry | 15 | - | [68]; 1991 |
| L(-) malic, L(+) lactic | wine | On-line Dialysis | FIA/UV-Visible spectrophotometry | 20 | < 2.5 | [69]; 1992 |
| L(+) lactate | wine | On-line Dialysis | SIA/UV-Visible spectrophotometry | 14 | < 2 | [70]; 1997 |
| L(+) lactic, L(-) malic, | wine | On-line Dialysis | FIA/UV-Visible spectrophotometry | 20 | < 5 | [71]; 1998 |
| tartaric | wine | On-line Dialysis | FIA/UV-Visible spectrophotometry | 16 | < 5 | [72]; 1998 |
| L(-) malic, L(+) lactic | wine | On-line Dialysis | FIA/UV-Visible spectrophotometry & Fluorimetry | 15 | < 6 | [73]; 2001 |
| tartaric | wine | On-line Dialysis | FIA/UV-Visible spectrophotometry | 36 | < 4.5 | [74]; 2002 |
| tartaric | wine | On-line Dialysis | FIA/UV-Visible spectrophotometry | 52 | < 2.4 | [75]; 2010 |
| acetic, citric, lactic, malic, tartaric, succinic | wine, fruit juice | On-line Dialysis | Isocratic HPLC/Refractive index | 1.7 | < 8 | [76]; 1995 |
| citric, tartaric, malic, succinic, lactic, acetic | juice, wine | On-line Dialysis | Isocratic HPLC/Refractive index | 1.7 | < 8 | [77]; 1998 |
| citric, tartaric, malic, succinic, lactic, acetic | wine | On-line Dialysis | Isocratic HPLC/UV spectrophotometry | 7.5 | < 5.4 | [11]; 2009 |

We will describe a FID and HPLC (FID-HPLC) combination system recently developed by Kritsunankul et. al. for simultaneous determination of six organic acids (tartaric, malic, lactic, acetic, citric and succinic acids), to illustrate the concept of FID coupled to HPLC [11]. The outlin of this system is presented in Figure 4 and the on-line dialysis separation process inside dialysis cell (using MWCO 12000-14000 Daltons of dialysis membrane) is represented in Figure 5.

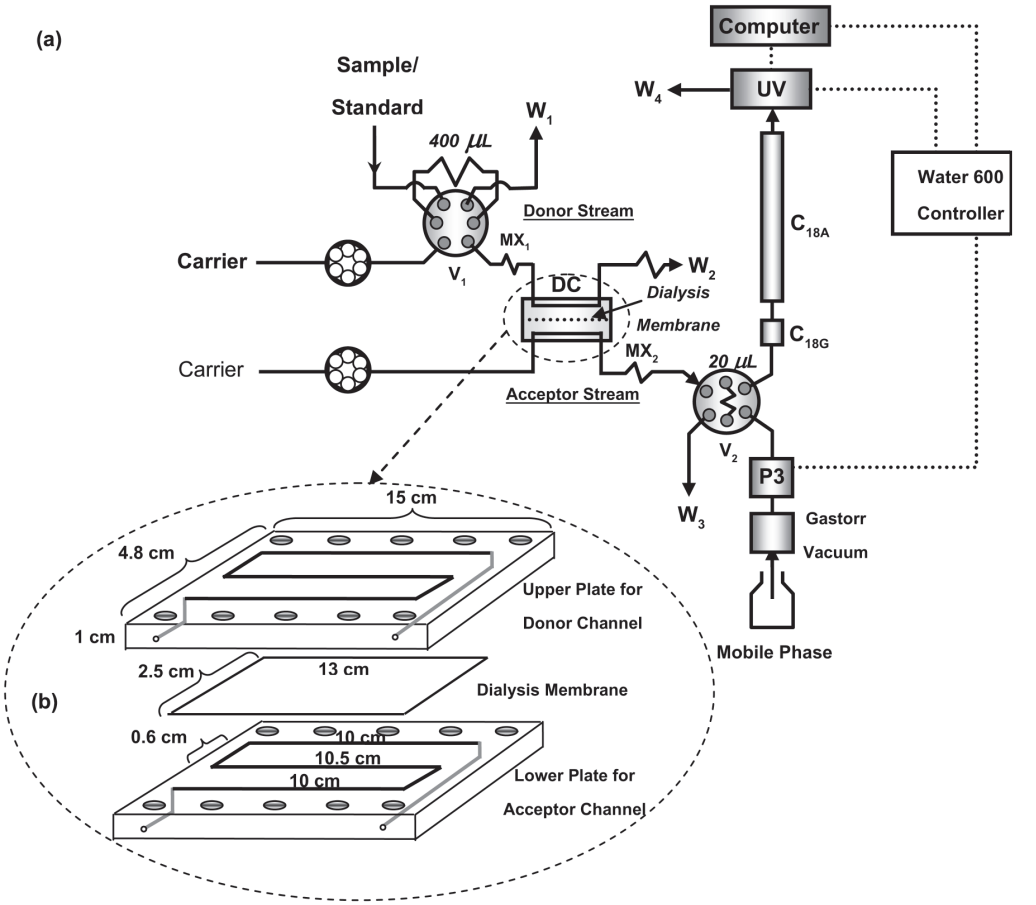


**Figure 4.** A typical FID-HPLC system for the simultaneous determination of some organic acids in wine (obtained by O. Kritsunankul and coworkers [11]): (a) the manifold used and (b) the dialysis cell (DC); $P_1$ and $P_2$ : peristaltic pumps 1 and 2, $P_3$ - a HPLC pump, $V_1$ : a manual-rotary injection valve, $V_2$ : a HPLC manual-rotary injection valve, $MX_1$ : a mixing coil 1, $MX_2$ : a mixing coil 2, $C_{18A}$ : a $C_{18}$ analytical column, $C_{18G}$ : a $C_{18}$ guard column, UV : a photodiode array detector and $W_1$, $W_2$, $W_3$ and $W_4$ : wastes 1, 2, 3 and 4.

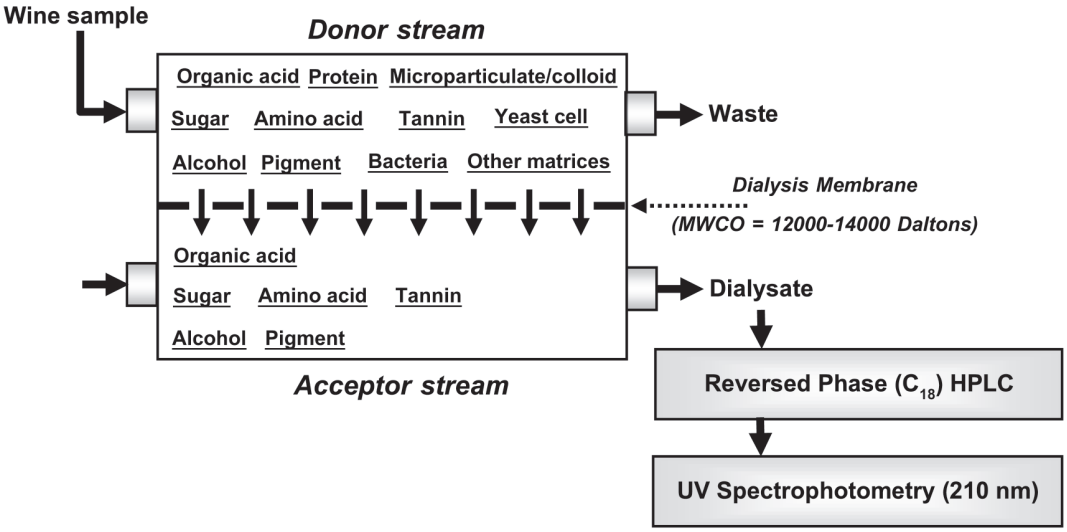


**Figure 5** Schematic representation of on-line dialysis separation process which was described by O. Kritsunankul and coworkers [11].

The process in Figure 4 takes place in the following manner. A sample or mixed standard solution is injected into a donor stream (water) of FID system and iss pushed further through a dialysis cell, while an acceptor solution (water) is held in the opposite side of the dialysis membrane. The dialysate containing organic acids in the acceptor is then transferred into the injection loop of the HPLC valve. The contents of the injection loop are then injected into the HPLC system and analyzed under normal HPLC conditions, using a reversed-phase ($C_{18}$) analytical column and UV detection (210 nm). The process happening inside the on-line dialysis cell is shown in Figure 5. A portion of wine sample containing many solute molecules of different sizes (i.e. organic acids, proteins, microparticulates, colloids, sugars, amino acids, tannin, yeast cells, alcohols, pigments, bacteria and other matrices) is carried in the donor stream. As the sample zone passes through the dialysis cell, low MW molecules (those have MW less than 12000-14000 D), especially organic acids, can be diffuse into the an acceptor stream while the bigger one cannot. Then, the dialysate containing organic acids is injected into the HPLC. The typical chromatograms from this FID-HPLC system are shown in Figure 6. However, various parameters, had to be optimized so that good peak resolution, short analysis time, high sensitivity and good reproducibility of FID-HPLC could be achieved. These parameters included types and ratio of mobile phase, pH of mobile phase (according to the pKa values of all organic acid analytes and pH tolerance value of HPLC column), detection wavelength, flow rates of donor and acceptor streams, injection volume of standard/sample at FID valve, pore size or MWCO value of the dialysis membrane, pH of standard/sample solution, and concentration of carrier solution for donor and acceptor streams.

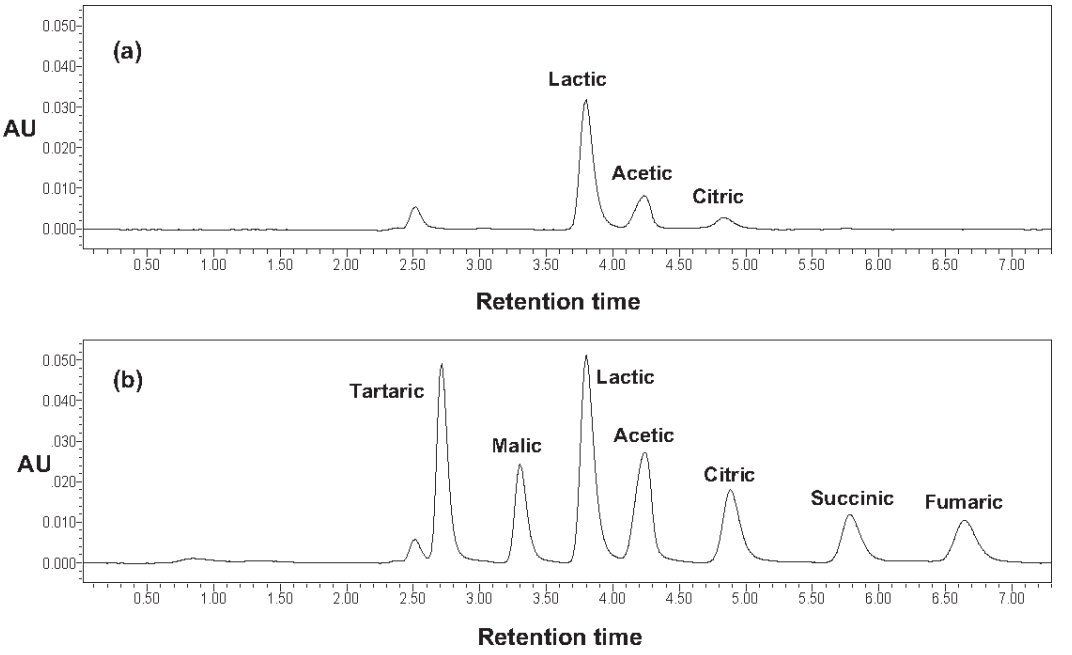


**Figure 6**. Typical chromatograms obtained from FID-HPLC system (recorded from O. Kritsunankul and coworkers [11]) for: (a) litchi Thai wine sample and (b) a mixed standard solution of 2500 mg/L each acid.

Figure 6 shows that the order of elution was tartaric, malic, lactic, acetic, citric and succinic acids with the analysis time of 8 minutes. This system was successfully developed for organic acid determination in Thai wine samples and gave various advantages such as fast and high degrees of automation for dialysis sample pretreatment, on-line sample separation and dilution, good clean-up for prolonged life-time of the HPLC column and low consumption of chemicals and materials.

## Conclusions

This review article presented the recent developments based on HPLC, FID/SID and FID/SID-HPLC techniques for the determination of low molecular weight organic acids in wines. The FID/SID and FID/SID-HPLC techniques provide many advantages over the conventional HPLC techniques such as lower analysis time due to shorter sample pretreatment, faster and higher degrees of automation in sample pretreatment and lower consumption of chemicals and materials. In addition, the FID/SID and FID/SID-HPLC techniques contain an on-line sample pretreatment and dilution step leading to good sample clean-up for prolongation of the life-time of the expensive HPLC columns. From the analytical point of view, on-line dialysis coupled to analytical methods is an interesting method for application to determination of other analytes and other complex samples.

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