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Flow injection on-line dialysis coupled to high performance liquid chromatography for the determination of some organic acids in wine

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ABSTRACT

A combined system of flow injection on-line dialysis sample pretreatment and high performance liquid chromatographic separation/detection (FID–HPLC) was developed for simultaneous determination of six organic acids (tartaric, malic, lactic, acetic, citric and succinic acids). A sample or mixed standard solution (400 μL) was injected into a donor stream (water) of FID system and was pushed further through a dialysis cell, while an acceptor solution (water) was held in the opposite side of the dialysis membrane. The dialysate containing organic acids in the acceptor solution was then flowed to an injection loop of the HPLC valve, where it was further injected into the HPLC system and analysed under normal HPLC conditions, using a reversed-phase (C_{18}) analytical column and UV detection (210 nm). The order of elution was tartaric, malic, lactic, acetic, citric and succinic acids with the analysis time of 8 min. The FID system could be operated in parallel with HPLC separation, providing sample throughput of 7.5 h^{-1}. Dialysis efficiencies of six organic acids were in range of 4.6–9.5%. Calibration graphs for all the mentioned organic acids were linear over the range of 250–7500 mg L^{-1}. Precisions for all the organic acids were within 5.4%. The proposed system was successfully applied for analysis of some Thai wines. By spiking wine samples with mixed acid standard solutions, the percentage recoveries in range of 84–104 were found. This system has advantages of fast and high degrees of automation for dialysis sample pretreatment, on-line sample separation and dilution, good clean-up for prolongation of life-time of the HPLC column and low consumption of chemicals and materials.

1. Introduction

Low molecular weight organic acid compounds are the important ingredients in wine [1–3]. Some acids (tartaric, malic and citric acids) originally come from fruits for making wine, while others (lactic, succinic and acetic acids) are by-products from the winemaking processes such as alcoholic fermentation, malolactic fermentation, oxidation of the ethanol, aging process, etc. Acidity greatly influences the taste, color and aroma of wine as well as the stability and microbiologic control of wine quality by stopping or at least retarding the growth of many potential harmful microorganisms that would spoil the wine. For the taste, acids give wines slightly tart taste but this can be moderated by itself alcohol, sugars, minerals and other components. In grape, the major acids are tartaric and malic, with citric acid being the minor ones. In the other fruits, malic or citric acid is usually dominant. When natural organic acids are absent or deficient in the winemaking bases, a blend of tartaric, malic and citric acids or only citric acid is usually added to make better tasting wine. Lactic acid is produced in wine during malolactic fermentation, where strong malic acid is converted to softer lactic acid. Succinic acid is created as a by-product of the wine fermentation process of sugar. This acid makes the taste of wine to be saltiness, bitterness and acidity. Acetic acid in wine can be contributed by many wine spoilage yeasts and bacteria. It can be produced during fermentation process or by the spoilage of finished wine.

Several analytical techniques have been used for identifying and quantifying organic acids in wine samples of various fruit materials and matrices in order to control the evolution of acidity, stability or illness of wine, during the different steps of the winemaking process until bottling step. These techniques include titrimetric [4], spectrophotometric [5–6], enzymatic [7–8], electrophoretic [9–12] and chromatographic, including gas chromatography [13] and HPLC [14–18]. Several sample pretreatment techniques such as dilution and filtration [19–20], ion exchange columns [21–22], solid phase extraction [23–25] and on-line dialysis [26–27] are implemented before HPLC determination of organic acids in real wine samples in order to decrease the matrix effect. According to the review paper [1], most of the HPLC methods for determination of organic acids in juices and wines involved dilution and filtration sample pre-

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treatment before HPLC determination, which may be not easy to be automated.

Dialysis [28], is a simple process in which small solute molecules diffuse from a high concentration solution to a low concentration solution 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. Therefore, dialysis sample pretreatment should be applied for removing or reducing the high molecular weight molecules, particulate and other matrices in wine samples from the low molecular weight molecules, especially organic acids. However, the conventional dialysis procedure is usually tedious, time-consuming and consumes large amounts of sample and reagent. On-line dialysis is also widely used by continuously feeding sample in the donor side of the dialysis membrane while solution in the acceptor side is flowed or stopped.

Flow based techniques such as flow injection (FI) and sequential injection (SI) offer high degrees of automation of dialysis sample pretreatment for various detection systems. Flow injection dialysis (FID), in which only a specified volume of sample is injected into the donor stream provides fast analysis or high sample throughput, and better precision and accuracy of the analytical results. This strategy allows small volumes of sample to be in contact with dialysis membrane and the membrane is continuously washed by the donor solution, thus less possibility of deterioration of the membrane (e.g., clogging or changing of property of the membrane) would result. FID with ion chromatography has been exploited for the determination of some common anions in wastewater samples [29]. Peroxynitrite in biologic samples was determined by on-line dialysis flow injection chemiluminescence detection [30]. 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 [31]. FID with UV spectrophotometric detection 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 [32]. SI on-line dialysis for removal of suspended solids and on-line dilution in the spectrophotometric determination of zinc in fertilizers has been developed [33]. SI on-line dialysis was applied for dilution and separation of reducing sugars in wine prior to the spectrophotometric determination [34]. Tartaric acid in wine was determined by FID pretreatment to eliminate matrix interferences and to accomplish on-line dilution before spectrophotometric detection [35]. The commercial automated sequential trace enrichment of dialysates (ASTED XL) system was employed for on-line dialysis prior to HPLC determination for automated preparation and analysis of sugars and organic acids in foods and beverages [26].

In this work, we developed the FID–HPLC system for the determination of some organic acids in wine. The proposed system offered a simple, fast, convenient and low consumption sample pretreatment system and gave better precision and accuracy of the analytical results. Moreover, the dialysis pretreatment should prolong life-time of the expensive HPLC columns by preventing the particulate and some large molecules to enter the column.

2. Experimental

2.1. Chemicals and solutions

Ultrapure water (18.2 MΩ cm^{-1}) (Elgastat Maxima HPLC, Elga, England) was used to prepare all aqueous solutions. All chemicals and solvents were analytical reagents and HPLC grades, respectively.

Stock standard solutions of six organic acids (50,000 mg L^{-1}) were prepared by dissolving acetic acid anhydrous (100%, Merck), citric acid monohydrate (99.7%, BDH), DL-lactic acid sodium salt (99%, Fluka), DL-malic acid (99%, Fluka), L(+)-tartaric acid (99.5%, BDH) and succinic acid (99%, Fluka) in water. The solutions were stored in brown glass bottles and kept at 4 °C. Mixed standard solutions of acids were freshly prepared by diluting the stock solutions with water and filtered through a 0.45 μm nylon membrane filter.

The HPLC mobile phase was a mixture (1:99, v/v) of acetonitrile (99.8%, BDH) and 0.05 mol L^{-1} potassium dihydrogen orthophosphate (99.9%, Fisher Scientific) buffer pH 2.5. The pH was adjusted with 1.0 mol L^{-1} phosphoric acid (85%, Merck). This mobile phase was filtered through a 0.45 μm nylon membrane filter and ultrasonically degassed prior to use.

2.2. Apparatus

The manifold of the FID–HPLC system is shown in Fig. 1. It consisted of two peristaltic pumps, P_1 (Masterflex C/L 60 RPM, model 77120-62, Cole-Parmer, USA) and P_2 (EYELA, model MP-3, Tokyo Rikakikai, Tokyo, Japan), a 6-port manual-injection valve, (V_1) (Model V-451, Upchurch Scientific, USA), a home-made dialysis cell (DC) and a HPLC system. All tubings for assembling the FI system, except pump tubing were 0.8 mm i.d. PTFE tubing (Upchuch Scientific, USA). A UV–vis spectrophotometer (Lamda 20, PerkinElmer, USA) was used to optimize the absorption wavelength for organic acids analysis.

A home-made dialysis cell (Fig. 1(b)) was made of two acrylic plates (15 cm length, 4.8 cm width and 1 cm height), engraving for donor and acceptor channels (with each of 350 mm length, 1.5 mm width, 0.75 mm depth). The two channels were separated by a cellulose membrane, which was obtained by cutting a dialysis membrane tubing (Spectra/Por® dialysis membrane tubing, molecular weight cut off (MWCO) of 6000–8000 and 12,000–14,000 Da, Houston, Texas, USA) to be a sheet of 2.5 cm width and 13 cm length.

The HPLC system (Fig. 1(a)) used in this work was a Waters 600E system (Water Corporation, Milford, MA, USA). It consisted of Gastorr vacuum, Waters 600E pump (P_3), Waters 600 controller, Rheodyne 7725i manual-injection valve (V_2), (with a 20 μL sample loop), Waters 2996 photodiode array (PDA) detector and Empower PDA software.

2.3. Procedures

2.3.1. Direct HPLC analysis

Recommended conditions from the Aquasil C_{18} technical guide (TG01-01, Thermo Fisher Scientific Inc., USA) were adapted to be used for HPLC separation of the organic acids as followed. A 20 μL of standard/sample solution was injected into an isocratic mobile phase of 1% of acetonitrile in 99% of 0.05 mol L^{-1} KH_2PO_4 buffer (pH 2.5), which was flowed at a rate of 1.25 mL min^{-1}. The injected zone was passed through an Aquasil C_{18} guard column (5 μm particle size, 10 mm length, 4.6 mm i.d.), an Aquasil C_{18} analytical column (5 μm particle size, 250 mm length, 4.6 mm i.d.) and the PDA UV detector (210 nm detection wavelength), respectively. All experiments were carried out at room temperature of about 25 ± 1 °C. The Empower PDA software was used for recording the chromatograms and evaluating for peak areas and retention times. Calibration graph was constructed by plotting peak area obtained versus concentration of the organic acid. Because of high salt concentration (0.05 mol L^{-1}) KH_2PO_4 was used in mobile phase, therefore the HPLC system was flushed with water overnight after the operation.

2.3.2. FID–HPLC analysis

The combined system of FI on-line dialysis (FID) and HPLC as shown in Fig. 1 was employed. The operation procedure of the sys-

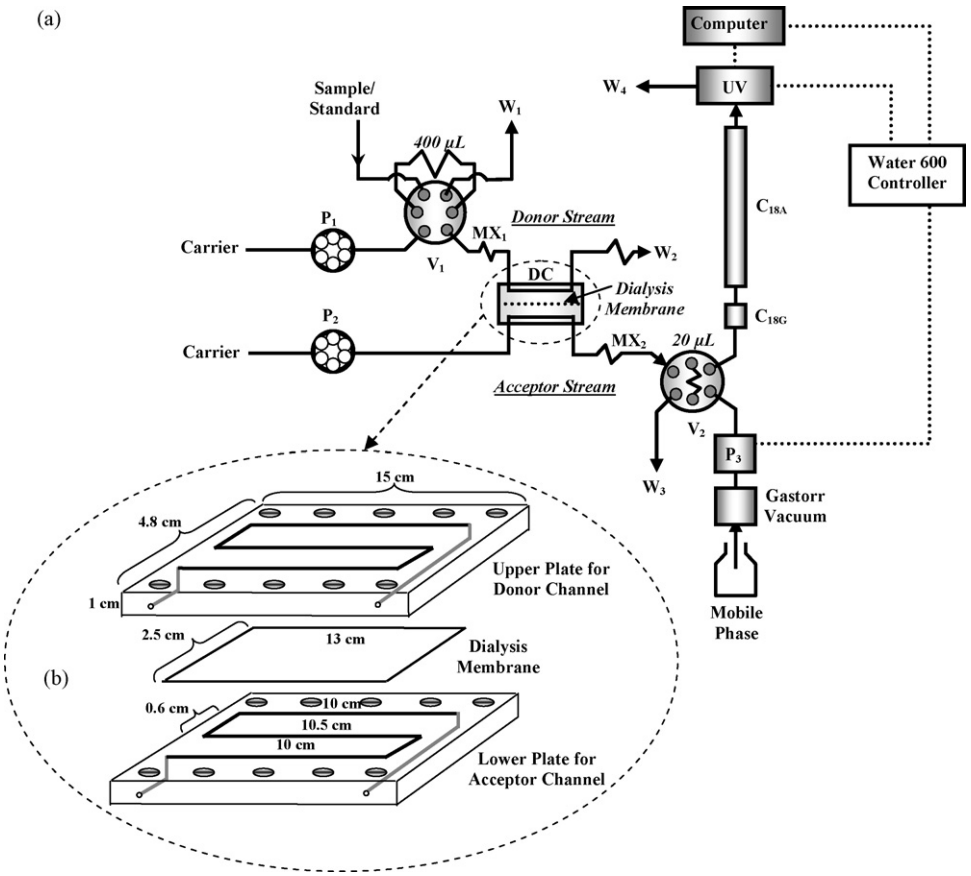

Fig. 1. (a) A manifold of the FID–HPLC system used for the determination of some organic acids and (b) the dialysis cell (DC); flow rate of carrier of donor and acceptor streams: 0.2 mL min^{-1}, P_1 and P_2: peristaltic pumps 1 and 2, P_3—a HPLC pump, V_1: a manual-rotary injection valve, V_2: a HPLC manual-rotary injection valve, MX_1: a mixing coil 1 (12 cm × 0.8 mm i.d.), MX_2: a mixing coil 2 (7.5 cm × 0.8 mm i.d.), 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.

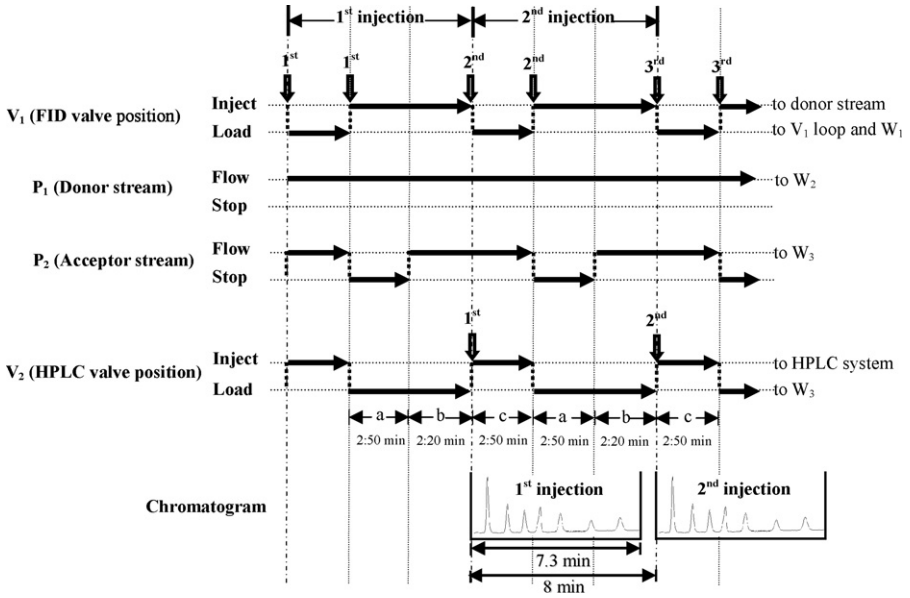

Fig. 2. A schematic of timing diagram for the operation cycle of a FID–HPLC system: V_1 (FID valve position): control to inject and load standard/sample (400 μL) into the donor stream, P_1 (donor stream): continuously operate along the operation, V_2 (HPLC valve position): control to inject and load (20 μL) dialysate in the acceptor stream into HPLC system, P_2 (acceptor stream): control to stop during dialysis period and flow to fill the dialysate into sample loop of the HPLC valve, Time durations: (a) dialysis time, (b) loading time of dialysate to HPLC loop and (c) cleaning time.

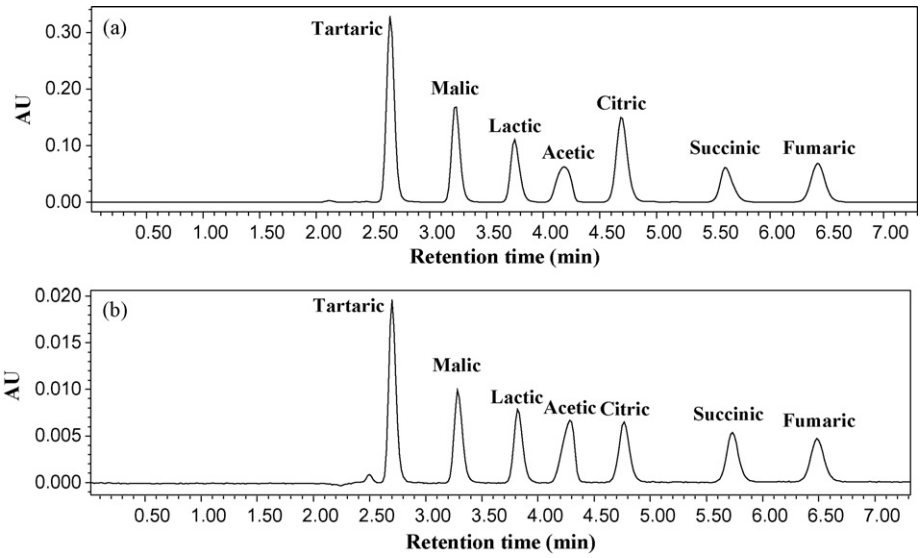

Fig. 3. Chromatograms of a mixed standard solution (1000 mg L^{-1} of tartaric, malic, lactic, acetic, citric and succinic acids, respectively): (a) by direct injection to HPLC system and (b) by FID–HPLC system.

tem is described as follows. Firstly, the HPLC system was started the operation, column was flushed by an eluent, and carrier solutions of donor and acceptor streams of the FID were flowed to fill all tubings and channels. Then, the operation cycle as shown in Fig. 2, which illustrated the timing diagram to control the status of various devices was followed. As could be seen in the diagram, firstly, a mixed standard/sample solution (400 μL) was loaded into sample loop of the injection valve of FID system. Then, the solution was injected into the donor stream (P_1) which was continuously flowed, while the acceptor stream (P_2) was stopped during the dialysis period (2 min 50 s). The dialysate zone containing organic acids was flowed to fill into a sample loop (20 μL) of the HPLC valve (V_2), with a suitable travelling time of 2 min 20 s. Then, it was further injected into the HPLC system and analysed under normal HPLC conditions, using a reversed-phase (C_{18}) analytical column and an UV spectrophotometric detection. While the dialysate of the first injection was injected into the HPLC system, the second solution was loaded into the sample loop of the FID valve (V_1). After a period of 2 min 50 s for cleaning of the donor and acceptor lines, the second injection was started. When a chromatographic separation of the first injection was ended (with an analysis time of 7 min 30 s), the dialysate of the second injection was injected into the HPLC system and the third solution was loaded into the FID system. This parallel operation helped to increase sample throughput of the combined system. Under the selected conditions described above, the total analysis time for one injection was 8 min, resulting in an injection throughput of approximately 7.5 chromatograms per hour. The operation is not yet automated, it should be developed further for computerized control of the pumps and valves.

3. Results and discussion

HPLC is suitable for simultaneous determination of multi-analytes, e.g., several organic acids in wine. However, sample clean-up/pretreatment is needed for the application to most of the real samples. In this work, the FID–HPLC system was designed and optimized for efficient operation and for obtaining good analytical results. Factors which may affect the HPLC separation, on-line dialysis efficiency and selectivity, and percentage recovery were studied as following.

3.1. Optimization of HPLC conditions

The HPLC system was optimized for good separation (high peak resolution), short analysis time and high sensitivity. A suitable detection wavelength for the high sensitivity in determination of

Table 1
Calibration data of the organic acids determination by direct injection to HPLC and FID–HPLC.

Organic acid	Range (mg L^{-1})	Linear equation ($y = ax + b$)	r^2	% RSD[a] ($n = 3$)	Detection limit[b] (mg L^{-1})	% Dialysis[c]
(a) Direct injection to HPLC						
Tartaric	250–7500	$y = 1741x - 38{,}703$	0.9999	0.2–1.2	83	–
Malic	250–7500	$y = 858x + 29{,}581$	0.9997	0.2–0.7	155	–
Lactic	250–7500	$y = 619x + 16{,}058$	0.9999	0.1–0.8	109	–
Acetic	250–7500	$y = 554x + 6255$	0.9999	0.2–1.2	104	–
Citric	250–7500	$y = 1047x + 28{,}721$	0.9997	0.2–0.6	149	–
Succinic	250–7500	$y = 668x - 321$	0.9997	0.1–1.9	146	–
(b) FID–HPLC						
Tartaric	250–7500	$y = 97x - 12{,}415$	0.9997	0.1–2.4	135	5.6
Malic	250–7500	$y = 54x - 4777$	0.9997	0.3–2.9	149	6.3
Lactic	250–7500	$y = 47x - 3550$	0.9996	0.9–2.4	144	7.5
Acetic	250–7500	$y = 53x - 4909$	0.9995	0.1–2.0	175	9.5
Citric	250–7500	$y = 49x - 5987$	0.9995	0.3–5.4	165	4.6
Succinic	250–7500	$y = 44x - 3907$	0.9994	0.5–1.9	213	6.6

[a] Relative standard deviation of different concentrations in the calibration range.
[b] Calculated from three times standard deviation of the blank signals, estimated from the y-intercept of a linear calibration graph [36].
[c] % Dialysis of each acid = (slope of calibration graph by FID–HPLC/slope of calibration graph by direct injection to HPLC) × 100.

six organic acids (tartaric, malic, lactic, acetic, citric and succinic acids) was investigated by using batch spectrophotometric system. The standard solution of each acid was prepared in the mobile phase of pH 2.8 (as recommended by the column producer to be used as a mobile phase for separation of organic acids on Aquasil C_{18} column). An absorption spectrum of each solution was recorded in range of 190–380 nm. It was found that the detection wavelength of 210 nm should be chosen because the high absorbance of all the studied acids was obtained at this wavelength.

Effect of pH of the mobile phase on the separation of organic acids was investigated. According to the fact that the pK_{a1} of the six organic acids are in the range of 3.02–4.74 and the Aquasil C_{18} column should be used at pH > 2.0, therefore the pH of the mobile phase was studied in the range of 2.2–2.8 that all analyte acids are in the undissociated form. It was found that good separation was obtained in pH range of 2.2–2.7. As for the mobile phase of pH 2.8, recommended in Aquasil C_{18} technical guide, was resulted in a poor resolution between acetic and citric acids, the mobile phase of pH of 2.5 was chosen for the further experiments.

Under the conditions used as described above and as in Section 2.3.1, the analysis of a mixed standard solution of the acids by direct injection to the HPLC system gave a chromatogram as shown in Fig. 3(a), with an analysis time of about 7 min. The order of elution was tartaric, malic, lactic, acetic, citric and succinic acids, with retention times (t_R) of 2.65 ± 0.01, 3.22 ± 0.01, 3.76 ± 0.01, 4.19 ± 0.01, 4.61 ± 0.01 and 5.56 ± 0.01 min, respectively. It should be noted that a peak at t_R of 6.48 ± 0.01 min belonged to fumaric acid, which was an impurity in malic acid chemical. Foreign compound species of glucose and fructose, which may be found in wine were also studied. Fructose was found to elute at t_R of 2.50 ± 0.01 min while glucose was not retained at this condition. Table 1 summarized calibration data of different acids under the selected conditions.

An off-line dialysis sample pretreatment using a dialysis bag was investigated for the analysis of wine sample. The standard/sample was placed in the dialysis bag and dialysed into water until reaching equilibrium and the dialysate was then analysed by HPLC under the selected conditions. It was found that the dialysis time to attain the equilibrium was 4.3 h as shown in Fig. 4. Temperature should affect the dialysis equilibrium. All experiments were performed at room temperature of about 25 °C. The procedure was tried for some Thai wines. By spiking the mixed standard into wine sample percentage recoveries was found in range of 81–92%. The FID system was then

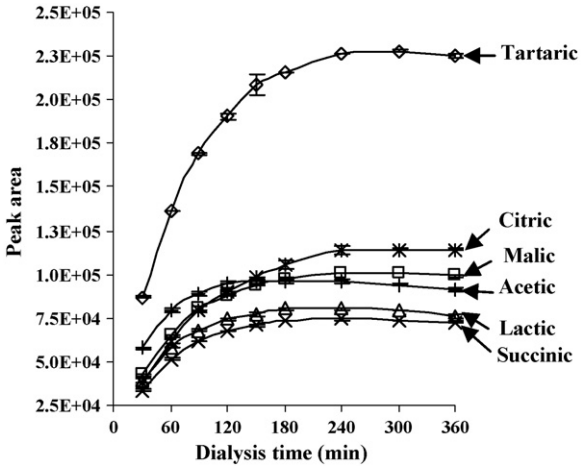

Fig. 4. Effect of the dialysis time on peak areas of the acids for batchwise dialysis of a grape wine (5 mL) spiking with a mixed standard solution of six organic acids to contain 1000 mg L^{-1} tartaric, malic, lactic, acetic, citric and succinic acids. Dialysis into ultrapure water (200 mL).

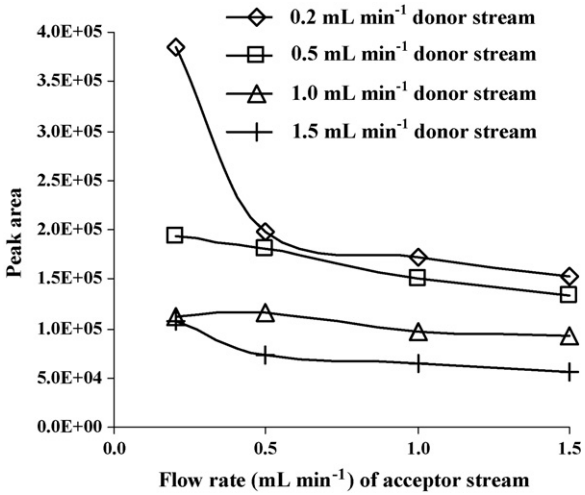

Fig. 5. Effect of flow rate of donor stream (0.2–1.5 mL min^{-1}) and acceptor stream (0.2–1.5 mL min^{-1}) on peak areas of 5000 mg L^{-1} tartaric acid.

investigated in order to reduce the dialysis time (without reaching equilibrium) and make the dialysis sample pretreatment to be more automated.

3.2. Optimization of FID–HPLC conditions

The FID system as shown in Fig. 1 was designed and optimized for on-line dialysis of some organic acids prior to the determination by HPLC. Some parameters affecting sensitivity and reproducibility of the system such as 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 were investigated.

The main parameters, flow rate and standard/sample volume, which affected the timing of an operation cycle were firstly studied. The flow rates of donor and acceptor streams were varied in the range of 0.2–1.5 mL min^{-1}. As expected, at the lower flow rate of the donor stream the higher sensitivity was obtained due to the better dialysis efficiency (Fig. 5). The flow rate 0.2 mL min^{-1} of each stream was selected for further experiments. The standard/sample volume was optimized in the range of 100–500 μL. It was found that the sensitivity increased linearly with the increase in the volume. The volume of 400 μL was chosen as the best compromise of sensitivity and the analysis time, as it was the best fit of timing for the operation of the FID in parallel with the HPLC as described in Section 2.3.2 and Fig. 2.

Selectivity in dialysis depends on pore size of the membrane used. Commercially available dialysis membranes of 2 ranges of pore size or MWCO values (6000–8000 and 12,000–14,000 Da) were tried. Percentage recoveries on spiking of standard organic acids into wine sample were considered. As could be seen from Fig. 6(a), a membrane of 6000–8000 Da gave lower percentage recoveries of all the studied acids. Therefore, the 12,000–14,000 Da membrane was selected for all further works. It should be noted that the bigger pore size membrane would allow more high molecular weight compounds to pass to the acceptor side, leading to reduction of the life-time of HPLC column. In case of sample containing high amounts of matrices, a smaller pore size membrane might be selected.

Effect of pH of standard sample solution was studied in range of 2.0–7.0, by adjusting pH with 1.0 mol L^{-1} phosphoric acid. No significant difference in peak area was found in the pH range studied for all six organic acids (Fig. 6(b)). Thus, the standard/sample could

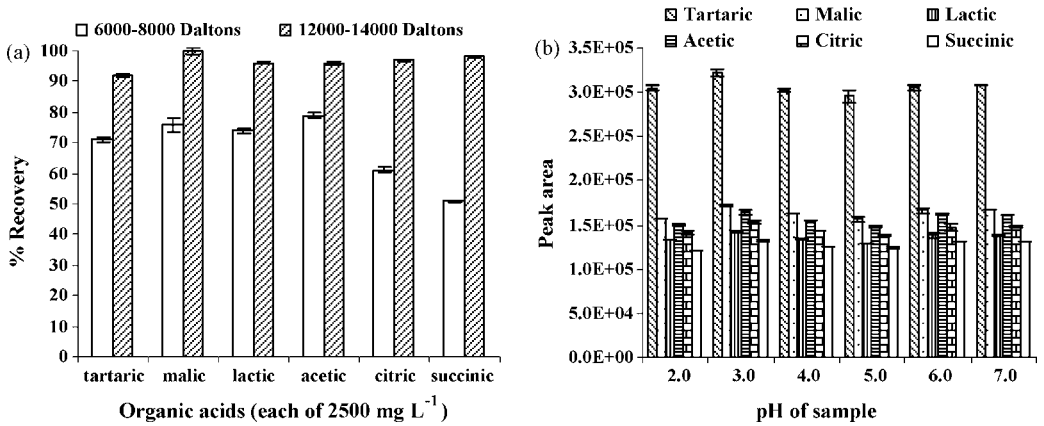

Fig. 6. (a) Effect of MWCO values of membrane on recoveries of six organic acids by spiking a mixed standard solution (2500 mg L^{-1} of each acid) into a grape wine sample and (b) effect of pH of sample/standard solution on peak areas of a mixed standard solution (5000 mg L^{-1} of each acid).

be conveniently prepared in water giving pH of about 6.5–7.0. Wine sample which has pH in range of 2.5–4.5 could be directly injected into the system.

Ionic strength of sample may affect the dialysis efficiency. This could be encountered in the on-line dialysis system by using the donor and acceptor solutions of high ionic strength to control/adjust ionic strength of the sample [29]. Potassium dihydrogen phosphate solution which was used as an eluent of HPLC system was selected for adjusting the ionic strength. Various concentrations of KH_2PO_4 solution used as donor and acceptor carrier solutions were studied. Those selected range of concentrations had to be limited by the clogging of salt in HPLC column. Hence, the concentrations of KH_2PO_4 donor solution were varied over the range of 0–0.5 mol L^{-1} while an acceptor solution was water. With no difference in signals or peak areas all six organic acids was found in the selected range of KH_2PO_4 as shown in Fig. 7(a). Therefore, water was chosen as the donor solution. Similar study was performed for acceptor solution containing various concentrations of KH_2PO_4 in the range of 0–0.2 mol L^{-1}. Two wine samples, which were spiked with standard six organic acids solution, were injected into the system. Fig. 7(b and c) indicated that the percentage recoveries decreased when concentration of KH_2PO_4 in the acceptor solution increased. According to these results, water was chosen as both the donor and acceptor solutions.

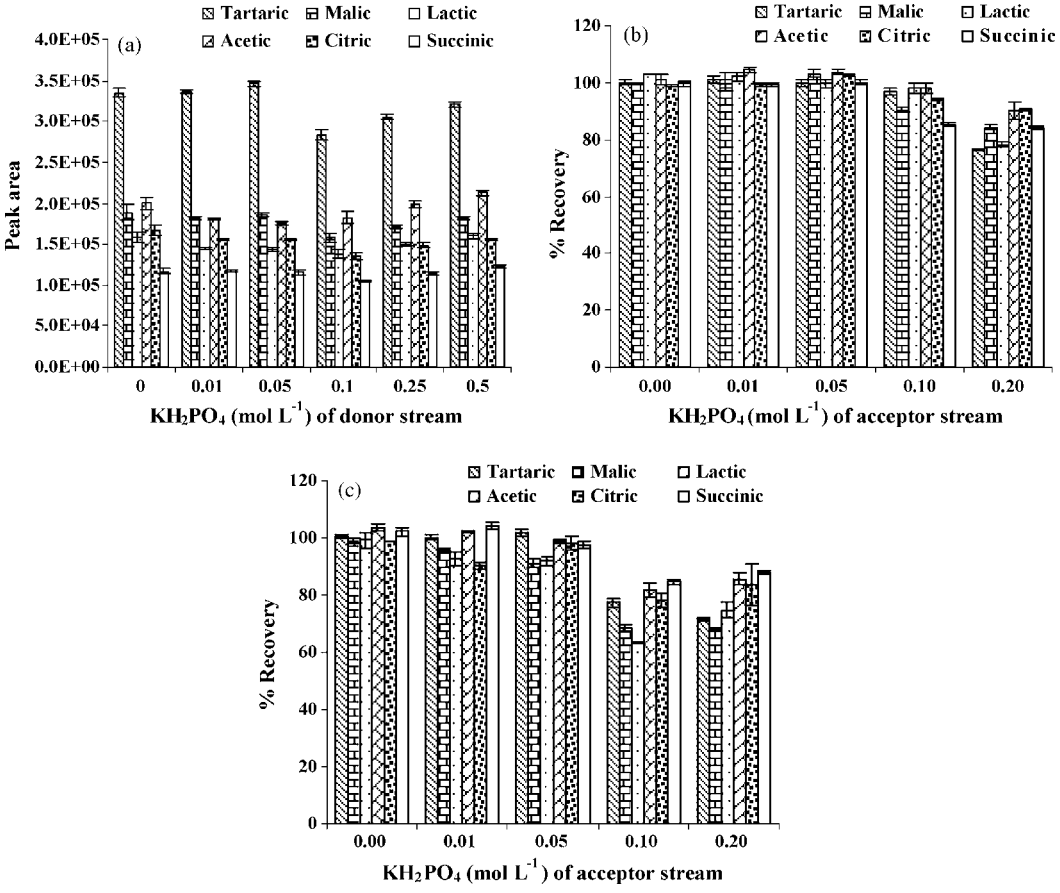

Fig. 7. (a) Effect of concentrations of KH_2PO_4 donor solution/stream on peak areas of a mixed standard solution (each acid of 2500 mg L^{-1}) while an acceptor solution/stream was water, (b and c) effect of concentrations of KH_2PO_4 acceptor solution/stream on recoveries of six organic acids contents by spiking a mixed standard solution (each acid of 2500 mg L^{-1}) in a litchi and a grape wine samples, respectively, while a donor solution/stream was water.

Table 2
Organic acid contents (mg L^{-1}; $n = 3$) in commercial Thai wine samples of different fruit materials, as determined by the proposed system (percentage recoveries were obtained by spiking with mixed standard solution containing 750 or 2500 mg L^{-1} of each acid).

Sample nO.	Fruit material	Color of wine	Concentration found and % recoveries											
			Tartaric acid		Malic acid		Lactic acid		Acetic acid		Citric acid		Succinic acid	
			mg L^{-1} [a]	% Rec[a]	mg L^{-1}	% Rec	mg L^{-1}	% Rec	mg L^{-1}	% Rec	mg L^{-1}	% Rec	mg L^{-1}	% Rec
1	Grape	Red	1381 ± 5	104 ± 3	ND[b]	99 ± 1	2875 ± 4	95 ± 1	292 ± 0.1	101 ± 0.2	532 ± 2	102 ± 0.4	401 ± 5	98 ± 1
2		Red	1122 ± 18	101 ± 1	344 ± 5	104 ± 1	1056 ± 10	98 ± 1	2605 ± 16	100 ± 1	654 ± 1	102 ± 0.4	545 ± 7	99 ± 1
3		White	389 ± 1	100 ± 1	2482 ± 7	100 ± 1	563 ± 2	102 ± 1	149 ± 1	93 ± 0.1	520 ± 3	97 ± 0.4	ND	98 ± 0.4
4		Red	ND	98 ± 0.1	ND	96 ± 0.4	2322 ± 6	101 ± 0.1	544 ± 7	98 ± 0.1	494 ± 3	101 ± 0.4	243 ± 0.1	100 ± 0.1
5	Apple	White	ND	85 ± 0.1	ND	88 ± 1	198 ± 1	85 ± 0.2	193 ± 1	99 ± 0.4	ND	100 ± 1	474 ± 3	103 ± 1
6		Green	ND	99 ± 0.1	ND	97 ± 0.3	2041 ± 1	102 ± 0.1	517 ± 1	98 ± 0.2	ND	100 ± 0.3	236 ± 1	100 ± 0.1
7	Litchi	Pink	ND	100 ± 0.1	ND	101 ± 0.3	4126 ± 7	101 ± 1	1143 ± 1	100 ± 1	580 ± 2	100 ± 0.1	ND	101 ± 0.2
8		Brown	ND	102 ± 0.1	437 ± 1	102 ± 0.3	2580 ± 5	100 ± 0.1	954 ± 2	99 ± 1	341 ± 2	98 ± 1	594 ± 5	100 ± 1
9	Rosella	Brown	ND	90 ± 1	215 ± 0.3	100 ± 1.2	396 ± 5	99 ± 1	1550 ± 5	99 ± 1	749 ± 3	100 ± 1	502 ± 6	97 ± 2
10		Brown	ND	84 ± 1	ND	89 ± 1	398 ± 1	97 ± 0.4	3375 ± 8	100 ± 1	513 ± 2	99 ± 1	ND	100 ± 1
11	Pineapple	White	ND	91 ± 0.1	171 ± 1	86 ± 1	218 ± 0.1	100 ± 0.1	245 ± 1	99 ± 0.4	338 ± 1	104 ± 0.2	333 ± 1	100 ± 0.4
12	Longan	Brown	250 ± 2	100 ± 1	413 ± 2	102 ± 1	1231 ± 5	104 ± 1	1244 ± 3	100 ± 1	1067 ± 8	104 ± 2	379 ± 5	100 ± 1
13	Strawberry	Light-red	ND	96 ± 0.2	ND	99 ± 0.2	1815 ± 4	94 ± 1	251 ± 0.4	99 ± 0.4	823 ± 1	103 ± 0.2	236 ± 1	101 ± 0.4
14	Phyllanthus	Light-Brown	199 ± 1	101 ± 1	ND	89 ± 1	5550 ± 17	101 ± 1	1485 ± 3	101 ± 1	ND	101 ± 1	235 ± 1	100 ± 0.1
15	Black plum	Light-Brown	195 ± 0.4	93 ± 0.1	696 ± 4	94 ± 1	212 ± 1	89 ± 1	344 ± 1	97 ± 0.4	224 ± 1	100 ± 0.2	323 ± 3	103 ± 1
16	Herb	Light-Brown	187 ± 1	90 ± 0.3	ND	84 ± 0.2	3823 ± 9	102 ± 0.2	574 ± 7	98 ± 0.2	ND	101 ± 1	ND	99 ± 2
17	Belamcanda chimensis	Brown	325 ± 2	100 ± 0.1	445 ± 2	102 ± 0.1	560 ± 2	104 ± 1	2533 ± 2	101 ± 0.1	352 ± 1	101 ± 0.1	346 ± 2	102 ± 1

[a] Average value ± standard deviation of triplicate results.
[b] ND—not detected.

3.3. Interferences study

Some species commonly found in wine, which could possibly be the interferences or might damage the HPLC column were tested. The proposed FID system was operated under the selected conditions as described above. The solutions of 5×10^{-4}–5×10^{-3}% (w/v) anthocyanin (typical of pigment species; synthetic by C. Boonthip, Chemistry Department, Faculty of Science, Naresuan University, with approximately 90% assay), 1.0–10.0 mg L^{-1} tannic acid (Fluka) and suspensions of 5×10^{-3}–2×10^{-2}% (w/v) kaolin (particle size less than 10 μm, Fluka) were injected into the FID system and the recording of the absorbance with UV–vis spectrophotometer at 210 nm at the outlet of acceptor stream was carried out. For tannic acid and kaolin, there was no absorbance change observed, while for anthocyanin percentage dialysis of less than 5% was found. It could be concluded that under the optimum conditions used for the FID system, the analyte organic acids could be efficiently separated from the particulates and high molecular weight molecules. Hence, the wine samples could be directly analysed without deterioration of the expensive HPLC column.

3.4. Calibration data of six organic acids by FID–HPLC

Chromatogram of six organic acids obtained from FID–HPLC system is depicted in Fig. 3(b). The order of elution is the same with that of the direct injection to HPLC system, but the sensitivity was lower by about 20 times. It should be noted that a small peak at retention time of 2.50 min was due to the non-retained species (void peak), which could not be seen at a large scale of *Y* axis in Fig. 3(a). Calibration plots for all analytes were still linear in the range of 250–7500 mg L^{-1} (Table 1), similar to those obtained by direct injection to HPLC system, but the slopes of the calibration graphs were smaller and a little bit higher detection limits (3 SD) were obtained. From the ratio of slopes of the calibration graphs obtained from FID–HPLC system to those obtained from the direct injection to HPLC system, the percentage dialysis could be calculated to be in the range of 4.6–9.5. This is comparable to those obtained from the commercial instrument as reported in literature [26], which the dialysis efficiencies for the six organic acids were in the range of 6.7–9.5%. The FID–HPLC provided on-line dilution of sample about 10–20 folds. Precisions obtained from triplicate injections of the standard solution containing each acid in concentration range of 250–7500 mg L^{-1} were 0.1–5.4%.

3.5. Application to analysis of some local Thai wines

The proposed method was applied to determine the six organic acids in seventeen commercial Thai wine samples of different brands, prices and fruit materials. The mixed standard of all the studied organic acids was spiked into all samples at two different concentration levels (750 and 2500 mg L^{-1}). Each sample solution was analysed in triplicate. The results obtained are summarized in Table 2. Recoveries calculated from the spiked concentration that was close to concentration found in samples were in the range of 84–104%. In FID, only small amounts of sample would be in contact with the dialysis membrane and the membrane is continuously washed by the donor solution, thus less possibility of deterioration of the membrane (e.g., clogging) would result. One dialysis membrane in this system could be repeatedly used for more than 300 analyses. As mentioned earlier, tataric, malic and citric acids in wine are originally derived from fruit, while succinic, lactic and acetic acids could be mainly derived from the fermentation process. We found that the high quality wine (high cost as well) contained more fruit derived acids and less fermented acids. Only grape white wine obtained from a famous brand can eliminate acetic acid. Wines produced from different fruits contained different amounts of the acids. However, the producers may add some organic acids to their products as well. The developed system should be valuable for improving of wine production process and quality control of wines, which are now popular local products under the One Tambon One Product (OTOP) campaign of the Thai government.

4. Conclusion

A flow injection on-line dialysis sample pretreatment coupled to HPLC system was developed for the determination of tartaric, malic, lactic, acetic, citric and succinic acids in wine samples. This system has advantages of fast and higher degrees of automation in sample pretreatment, on-line sample separation and dilution, good sample clean-up for prolongation the life-time of the expensive HPLC column and low consumption of chemicals and materials. The system should be conveniently employed for quality control of wine.

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