Mark Storton
James Exarchakis
Tracy Waters
Zhigang Hao
Barry Parker
Michael Knapp

Global Analytical Science
Department, Colgate-Palmolive
Company, Piscataway NJ, USA

Research Article

Lactic acid quantitation in hand dishwashing liquid using an HILIC-UV methodology

Different hydrophilic interaction chromatography (HILIC) columns were screened for lactic acid separation in hand dishwashing liquid products and the influence of mobile phase strength, buffer concentration and column temperature on the retention of lactic acid on a Zorbax NH₂ column was investigated. An isocratic HILIC method for the quantitation of lactic acid in hand dishwashing liquid products was developed. The mobile phase consists of 70% methanol and 30% 20 mM sodium phosphate buffer (v/v) at pH 2.5. The HILIC stationary phase is Zorbax NH_2, 250×4.6 with a 5 μm particle size. Detection was carried out using a variable wavelength UV-VIS detector at 226 nm. The linear range and percent recovery for lactic acid in the products were 44.68–1206.39 μg/mL and 100.3%, respectively. This paper provides an optimized HILIC methodology for the analysis of an acidic polar analyte (lactic acid) on a basic stationary phase. The proposed method can be used for the routine analysis of lactic acid.

1 Introduction

Lactic acid (2-hydroxypropanoic acid, pK_a 3.86) is a small organic acid (OA). It is an environmental-friendly ingredient with strong antibacterial activities and is widely used in food, cosmetic, pharmaceutical and chemical industries [1, 2]. Many analytical methods from gas to liquid chromatographies have been compared and reported which quantitate lactic acid in different matrixes [2–6]. A disadvantage of GC is significant peak tailing (like other OAs) and chemical derivatization is usually needed to improve volatility and peak shape in a GC capillary column. For HPLC method development, a major challenge is the resolution of lactic acid due to its strong hydrophilic properties. Lactic acid can only be weakly retained on a popular RP HPLC column under a 100% aqueous mobile phase and a gradient elution is required to elute the more hydrophobic matrix ingredients to allow multiple sample analysis. Otherwise, a chemical derivatization or reaction would be needed to increase lactic acid's hydrophobicity for better separation [1, 7, 8].

Recently, hydrophilic interaction chromatography (HILIC) technology has been widely used for hydrophilic

ingredients analysis. However, the application of HILIC to acidic hydrophilic ingredients, especially with a complicated matrix system, is relatively limited [9–12]. To simplify our current SPI method for lactic acid quantitation and to obtain a better understanding of the acidic hydrophilic ingredients in the HILIC separation, an objective to modify the existing gradient elution on an RP column to an isocratic elution on an HILIC column was setup in our group. Meanwhile, to satisfy the company's safety policies on the usage of organic solvents in routine QC labs, no harsh organic solvents such as acetonitrile were used in the mobile phase. An isocratic HILIC method has been developed on an amino column with a total separation time of about 12 min.

2 Materials and methods

2.1 Chemicals

HPLC grade methanol, phosphoric acid and sodium phosphate, monobasic were obtained from JT Baker (Phillipsburg, NJ, USA). Ultra-pure water (18 MΩ cm) was prepared from PURELAB prima 7 (Lowell, MA, USA). Sodium (L)-lactate (99%) was purchased from Aldrich (St. Louis, MO, USA).

2.2 Equipment

The HPLC measurements were done on an Agilent 1200 Series HPLC system equipped with a diode array detector

Correspondence: Dr. Zhigang Hao, Global Analytical Science Department, Colgate-Palmolive Company, 909 River Rd, Piscataway, NJ 08855, USA
E-mail: zhigang.hao@gmail.com
Fax: +1-732-8786138

Abbreviations: HILIC, hydrophilic interaction chromatography; **OA,** organic acid

(G1315D), quaternary pump (G1311A), degasser (G1332A), thermostatted column compartment (G1316A) and 1200 Series standard auto-sampler (G1329A). The software was ChemStations. Separation was performed on a Zorbax NH_2 column (250 mm × 4.6 mm, 5 μm) from Agilent at 25°C. For lactic acid separation the mobile phase was 70% methanol and 30% 20 mM NaH_2PO_4 aqueous solution (pH 2.5, v/v). The flow rate was 1.4 mL/min with pressure of 183 bar. The lactic acid peak area was monitored at 226 nm. Other stationary phases used for column screening included an XBridge C_8 column (75 × 4.6 mm, 2.5 μm) from Waters, an Allure OA column (300 × 4.6 mm, 5 μm) from Restek, an Acclaim OA column (250 × 4 mm, 5 μm) from Dionex, a ZIC-HILIC peek column (150 × 4.6 mm, 3.5 μm, 100 Å) from The Nest Group (MA, USA), a Zorbax Sil (type-A silica, 250 × 4.6 mm, 5 μm) from Agilent, and a polar-imidazole column (250 × 4.6 mm, 3 μm) from Sepax technologies (DE, USA). C_{18} endcapped SPE cartridge (500 mg, 50 × 3 mL tubes) was obtained from Agilent.

2.3 Standard preparation

The sodium lactate analytical reference standard is hygroscopic and it must be stored in a desiccator. Care must be taken to limit its exposure to air during standard solution preparation. A working standard solution of 1.2064 mg/mL with a diluent composed of 70% methanol and 30% water was prepared for method development.

2.4 Sample preparation

A representative dishwashing liquid sample was made by our product development group. The composition of matrix ingredients includes polar components, such as betaine and non-polar components, such as fragrances. A high percentage of surfactant material is also present in the matrix. The sample equivalent to about 72 mg of lactic acid was accurately weighed and transferred into a 100-mL volumetric flask, 10 mL of 0.2 N sodium hydroxide was added into the flask, along with a magnetic stir bar and the solution was stirred gently for 15 min taking care to minimize foaming. After lactic acid oligomers (mainly dimers and trimers) were hydrolyzed into monomers under basic condition, 5 mL of 0.2 N phosphoric acid was added. Whilst stirring the contents of flask, 2:1 (phosphoric acid/water) solution was added until the pH was 2.3 ± 0.2. The stir bar was then removed and 70 mL methanol was added into flask. The contents of flask were diluted to full volume with water, mixed well and filtered with a PTFE sample filter prior to transfer into an auto-sampler vial.

The sample placebo was made from our product development group by subtracting lactic acid from the product formula and the spiking sample was made from the corresponding placebo spiked with sodium lactate standard.

3 Results and discussion

3.1 Systematic approaches to method development

3.1.1 HILIC column screening

An in-house validated method for the quantitation of lactic acid in hand dishwashing liquid products was run on an XBridge C_8 column with the chromatogram shown in Fig. 1. The only disadvantage of this method is the dual-gradient elution profile starting from 100% water to 100% methanol. This is because lactic acid can only be weakly retained on the RP column and the dishwashing liquid matrix needs to be washed out before the next run. To simplify this dual-gradient elution to an isocratic elution, OA columns from Restek and Dionex were examined initially. Lactic acid was retained longer than on the RP columns in the 100% aqueous mobile phase recommended by vendors (data not shown here). However, matrix ingredients in the formula could not be eluted out from the column in an adequate time frame. SPE technology was also investigated with the objective of removing the matrix ingredients before the sample injection. However, the sample prep methodology was not robust enough for routine analysis. To take advantage of increased interest in HILIC theory in HPLC separations, a methodology based on this type of separation was targeted as our next objective.

Typical HILIC stationary phases are bare silica and silica derivatized with different polar functional groups such as amine, amide, cyano, diol and sulfobetaine [13]. The HILIC column plays a core role for polar analyte retention and the retention mechanisms can include analyte partitioning between the bulk eluent and water-rich layer on the stationary surface, hydrogen-bonding and ion-exchange between analytes and the HILIC stationary phase [9]. The properties of the HILIC column material surface can be acidic such as bare silica, neutral-like diol or amide columns, basic like amino or imidazole and zwitterionic such as the ZIC-HILIC column. Retention contributions from the different retention mechanisms described above can be varied with different analytes. The analytes can also be classified into acidic, neutral, zwitterionic and basic categories.

In this study, the retention behavior of lactic acid was first explored on different bonded polar HILIC stationary phases, which included bare silica, diol, amide, ZIC-HILIC, imidazole and amino columns, to see if adequate retention power was obtainable. Unfortunately, most of HILIC columns including bare silica, diol and amide columns did not have much retention for lactic acid even when a very high ratio of methanol (98%) in mobile phase was applied. A greater problem is the high content of anionic surfactants in the product samples. These surfactants were also very weakly retained on those columns and overlapped with lactic acid peak in the chromatograms (data not shown here). Their weak retentions could be due to the contribu-
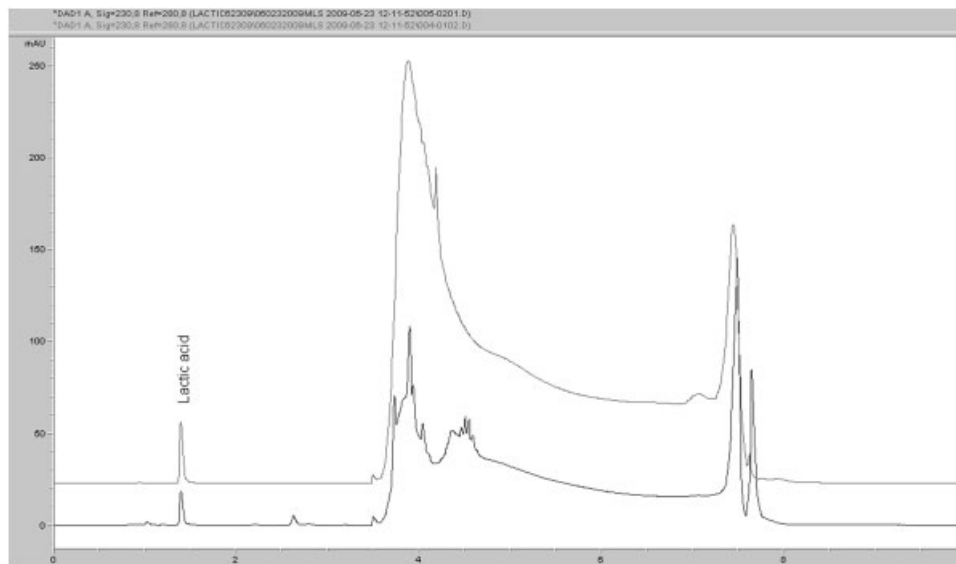
Figure 1. A gradient elution for lactic acid. Conditions: 75 × 4.6 mm XBridge column with 2.5 μm particles; 50°C; 230 nm detection; injection volume: 5 μL; eluent A: 0.2% phosphoric acid in water solution; eluent B: degassed methanol; mobile phase ratio was started at 100:0 (A/B) in first 2 min and flow rate was 1.0 mL/min, the ratio was changed from 100:0 to 0:100 (A/B) from 2 to 2.5 min and flow rate was increased from 1.0 to 1.5 mL/min, 0:100 ratio was held to 6 min and changed back to 100:0 from 6 to 6.5 min and held at 100:0 (A/B) until 12.5 min. Upper one is lactic acid standard and bottom one is lactic acid in a hand dishwashing liquid product.

tion from the silanol group in the HILIC stationary phase [14]. The lactic acid retention time on a ZIC column is even less than the void volume signal. This negative retention factor (*k*) might be due to strong electrostatic repulsion between acidic analytes and an outer negatively charged functional group (sulfonate group) on the ZIC stationary phase [15].

Fortunately, HILIC columns with basic surface materials such as amino and imidazole columns exhibit a much stronger retention of acidic lactic acid even though the pK_a of lactic acid is 3.86 and the mobile phase pH was 2.5. A more favorable situation for the separation of lactic acid is that anionic surfactants were strongly retained on the basic stationary phase. The optimized lactic acid separations from dish-liquid formulations on the amino and imidazole columns are presented in Fig. 2. The imidazole column offered better differentiation for anionic surfactant separation but a longer total running time was required for lactic acid quantitation. So, we decided to finalize the HILIC methodology on a Zorbax NH$_2$ column.

3.1.2 The effect of mobile phase strength on component retention

Mobile phase strength is one of the most important parameters in HILIC retention. Unlike RP HPLC, an aqueous portion in HILIC is a stronger eluting solvent whereas organic methanol is a weaker one. A range of 5–50% aqueous buffer (95–50% methanol) in mobile phase

was studied isocratically and the results are shown in Fig. 3. The lactic acid retention times were observed to be inversely proportional to the aqueous buffer content in the eluent and showed the characteristics of typical hydrophilic interaction. The aqueous buffer contains water and buffer ions and both could contribute to lactic acid retention changes. More detailed information on buffer ionic strength will be discussed in Section 3.1.3.

Anionic surfactants are major interfering components for lactic acid separation in hand dishwashing liquid products and it is worthy to mention their retention behavior on HILIC columns. The anionic head of the surfactant usually exhibits a very strong electrostatic interaction with the HILIC stationary phase (attraction or repulsion). As we described before, anionic surfactants cannot be retained on acidic or neutral surface materials of HILIC columns but they can be strongly retained on basic surface materials. Furthermore, their retention times on the amino column were also inversely proportional to the aqueous buffer content when the water was between 5 and 30% in the mobile phase. However, their retention times were proportional (not inversely) to the aqueous buffer content when it was between 30 and 50%. Previously, this U-curve retention was observed by Dong and Huang [16]. The hydrophilic interaction and hydrophobic retention could respectively predominate within lower and higher aqueous buffer content ranges. Practically, it presented a nice opportunity to optimize aqueous-organic ratio in the mobile phase for reducing the total running time. A 30% aqueous buffer (70% methanol) content was chosen for final mobile phase ratio.
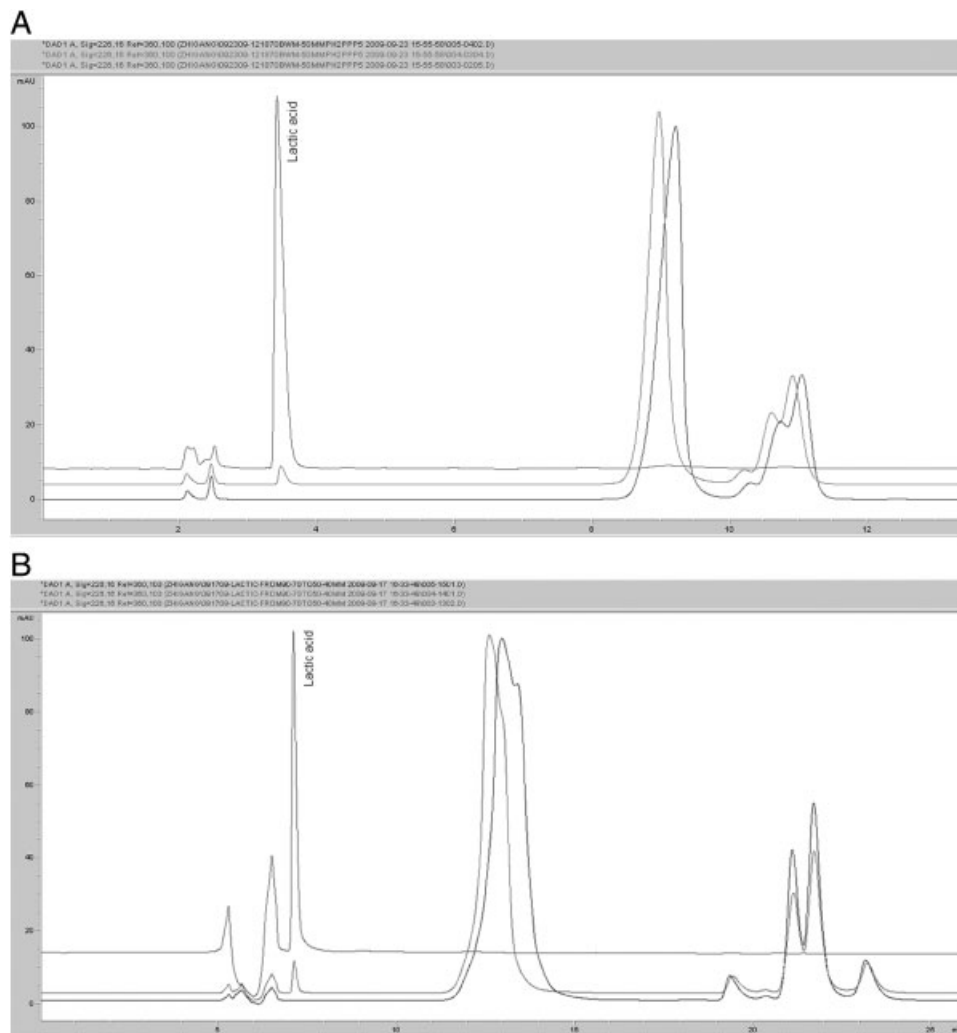
Figure 2. UV chromatograms of lactic acid. (A) An isocratic elution for lactic acid on the Zorbax NH₂ column, 250 × 4.6 mm with 5.0 μm particle size at 25°C, 226 nm detection, injection volume: 5 μL; mobile phase composition: 70% methanol with 30% 20 mM sodium phosphate buffer at pH 2.5. The flow rate is 1.4 mL/min. Top one is lactic acid standard, middle one is placebo spiked with standard and bottom one is placebo only. (B) An isocratic elution for lactic acid on the SePas polar imizadole column, 250 × 4.6 mm with 3.0 μm particle size at 25°C, 226 nm detection, injection volume: 5 μL; mobile phase composition: 50% methanol with 50% 40 mM sodium phosphate buffer at pH 2.5. The flow rate is 0.5 mL/min. Top one is lactic acid standard, middle one is placebo spiked with standard and bottom one is placebo only.

3.1.3 Buffer concentration on component retention

A buffer is a very useful component when ionic interaction is involved in the analyte separation, especially with UV detection. The buffer can control the mobile phase pH and that, in turn, can control the ionization status not only for analytes but also for the stationary phase as well. Different buffer concentrations also can generate the variable ionic strength necessary to mediate the electrostatic interaction between analytes and the stationary phase [11]. In general, at a low buffer concentration or in a buffer-free condition, the ionic analytes could be more effectively retained on the stationary phase containing counter-ionic groups. As the buffer concentration increases, a high level of organic in the mobile phase could make the buffer-ions prefer to be inside the stagnant water-rich liquid layer. Higher ion concentration would drive more solvated salt ions into this liquid layer and result in an increase in volume or hydrophilicity of the liquid layer. The strength of the electrostatic interaction between analytes and the stationary phase can be weakened with the increase of the water-rich

layer volume, thus resulting in a weak electrostatic interaction. If the electrostatic interaction is with counter-ions, the electrostatic attraction might be weakened, thus resulting in a longer retention time. If the charge–charge interaction is with co-ions, the electrostatic repulsion might also be weakened, thus resulting in a shorter retention time. Most experimental data, especially with ammonium acetate or formate buffer, support this rationale [12, 15, 17, 18]. In our objective, the sodium phosphate buffer concentrations were studied from 10 to 100 mM and the retention of lactic acid and anionic surfactants are shown in Table 1. The results also support the rationale described above. The higher buffer concentration can shorten total running time but the separation between lactic acid and hydrophobic components (peaks prior to lactic acid in Fig. 2A) became more of a challenge. A 20 mM buffer concentration was finally selected.

3.1.4 Temperature effect on component retention

The column temperature factor is usually applied in the later stage of method development. In general, temperature
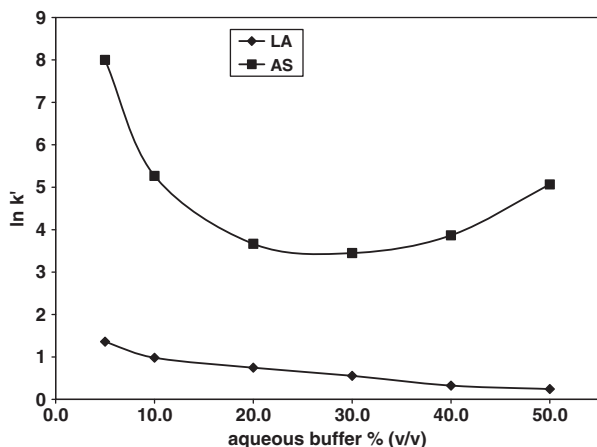
Figure 3. Effect of aqueous buffer content (v/v%) on the retention factor of lactic acid and anionic surfactants under HILIC condition on a Zorbax NH$_2$ column, 250 × 4.6 mm, particle size of 5 µm with the flow rate at 1.4 mL/min. Column temperature was 25°C; mobile phase was MeOH/sodium phosphate buffer (20 mM, pH 2.5). The pH value and buffer content were measured in water media and their real values in aqueous organic mobile phase could be slightly different in water media.

Table 1. Retention times (in minutes) of lactic acid and anionic surfactants[a] under the HILIC condition on a Zorbax NH$_2$ column, 250 × 4.6 mm, particle size at 5 µm, with 70% methanol and 30% sodium phosphate buffer solution

	10 mM	20 mM	40 mM	60 mM	80 mM	100 mM
LA	4.86	3.67	3.02	2.76	2.63	2.56
AS	16.50	9.55	6.02	4.63	4.08	3.76

a) Retention times of AS is an average value of multi-anionic surfactants.

increases could enhance the diffusion coefficient and result in a narrow peak. Meanwhile, an elevated temperature could result in a shorter retention time. When a temperature increase makes analyte retention longer on a column, the retention difference between individual analytes can be enlarged and the resolution improved [9, 19]. In this study, the temperature profile is presented in Fig. 4. A negative slope for lactic acid in the van't Hoff plot indicated that a longer retention time was obtained at a higher column temperature. This result is further evidence to show that a longer retention of acidic analytes could be obtained on a basic stationary phase column when the column temperature was elevated as typically seen in HILIC separations [9]. The results in Fig. 4 also indicate that the retention times of anionic surfactants decreased as the column temperature increased. The hydrophobic tails present in surfactant structures could contribute to this observation because hydrophobic compounds usually exhibit a positive slope in van't Hoff plots [20]. We did not use high temperature in our
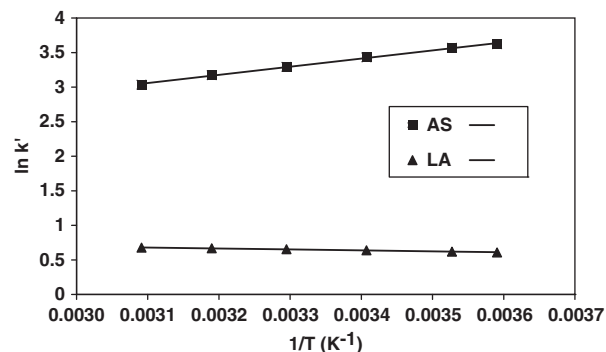

Figure 4. The van't Hoff plots for lactic acid and anionic surfactants under HILIC condition on a Zorbax NH$_2$ column, 250 × 4.6 mm, particle size of 5 µm with the flow rate at 1.4 mL/min. Column temperature varied from 5 to 50°C; mobile phase was 70% methanol with 30% aqueous 20 mM sodium phosphate buffer solution at pH 2.5.

methodology because some QC labs may not have the capability to elevate the column temperature. Room temperature such as 25°C already provided enough resolution for our quantitation method.

3.2 HILIC method validation

3.3.1 Accuracy

Placebo samples were spiked in triplicate at 70, 100 and 130% of sample analyte target (2.0%) level. Recovery is given below:

Spiking level (%)	Recovery (%)	% RSD
70	100	0.13
100	100	0.10
130	99.4	0.34

3.2.2 Precision and repeatability

Six injections of a prepared lactic acid sample gave a mean value of 1.98% with a relative standard deviation of 0.13%.

3.2.3 Linearity and linearity range

Linearity was established *via* a series of five standard solutions at 50, 80, 100, 120 and 150% of the analyte target (2.0%). An R^2 value of 1.00 was achieved.

3.2.4 Sensitivity

Acetonitrile cannot be used in this method (lab restriction) and methanol has a higher UV cut-off limit. The LOD and LOQ are 44.68 and 134.04 µg/mL, respectively.

3.2.5 System suitability

A passing criteria of resolution greater than 2.0 and a tailing factor of less than 1.5 were required. A tailing factor of 1.3 and a resolution of 4.9 were achieved.

3.2.6 Ruggedness (intermediate precision)

Ruggedness was established by preparing a lactic acid sample at the target level (2.0%). This sample was analyzed by two different operators. Each analyst ran two sets of samples on two different days.

3.2.7 Specificity

The current HILIC method was intended to separate the hand dishwashing liquid matrix from lactic acid. The methodology was run with the product placebo and a spiked placebo at the target level (2.0%). The placebo showed no interference and was verified by peak purity analysis from standard, placebo and placebo-spiking solutions.

3.2.8 Robustness

Robustness was established through method development and minor changes of chromatographic condition such as methanol content in mobile phase ($\pm 5\%$), pH of mobile phase (± 0.2 unit), UV detection (± 2 nm), flow rate (± 0.1 mL/min), buffer concentration ($\pm 2\%$) and column temperature ($\pm 2^{\circ}$C).

4 Concluding remarks

A rapid and reliable HILIC-UV method for the quantitation of lactic acid in hand dishwashing liquid products has been successfully developed and validated with an isocratic elution. This method demonstrated acceptable sensitivity, accuracy, precision and recovery. The validated method was successfully applied to assay the commercial hand dishwashing liquid product samples.

The authors have declared no conflict of interest.

5 References

[1] Wee, Y. J., Kim, J. K., Ryu, H. W., *Food Technol. Biotechnol.* 2006, *44*, 163–172.

[2] Lourdes, P., Guidote, L. P., Cruz, C. I. d., Lim, K. F. C., *J. Res. Sci. Comp. Eng. 1*, 2007, *4*, 13–18.

[3] Jham, G. N., Fernandes, S. A., Garcia, C. F., Araujo Da Silva, A., *Phytochem. Anal.*, 2002, *13*, 99–104.

[4] Lues, J. F. R., Botha, W. C., Smit, E. J., *Int. Dairy J.* 1998, *8*, 959–965.

[5] Thang, V. H., Novalin, S., *Bioresour. Technol.* 2008, *99*, 4368–4379.

[6] McHugh, J., Dumont, S., Paradis, J., Loan Nguyen, A., Levesque, S., Carrier, A., *J. Liq. Chromatogr. Related Technol.* 2006, *29*, 1905–1916.

[7] Perez-Ruiz, T., Martỳnez-Lozano, C., Tomas, V., Martỳn, J., *J.Chromatogr. A* 2004, *1026*, 57–64.

[8] Czauderna, M., Kowalczyk, J., *J. Anim. Feed Sci.* 2008, *17*, 268–279.

[9] Hao, Z., Xiao, B., Weng, N., *J. Sep. Sci.* 2008, *31*, 1449–1464.

[10] Christopherson, M. J., Yoder, K. J., Hill, J. T., *J. Liq. Chromatogr. Related Technol.* 2006, *29*: 2545–2558.

[11] Guo, Y., Gaiki, S., *J. Chromatogr. A* 2005, *1074*, 71–80.

[12] Guo, Y., Srinivasan, S., Gaiki, S., *Chromatographia* 2007, *66*, 223–229.

[13] Hemstron, P., Irgum, K., *J. Sep. Sci.* 2006, *29*, 1784–1821.

[14] Nawrocki, J., *J. Chromatogr. A* 1997, *779*, 29–71.

[15] Liu, M., Ostovic, J., Chen, E. X., Cauchon, N., *J. Chromatogr. A* 2009, *1216*, 2362–2370.

[16] Dong, L., Huang, J., *Chromatographia* 2007, *65*, 519–526.

[17] Ali, M. S., Ghori, M., Rafiuddin, S., Khatri, A. R., *J. Pharm. Biomed. Anal.* 2007, *43*, 158–167.

[18] Vikingsson, S., Kronstrand, R., Josefsson, M., *J. Chromatogr. A* 2008, *1187*, 46–52.

[19] Hao, Z., Lu, C. Y., Xiao, B., Weng, N., Parker, B., Knapp, M., Ho, C. T., *J. Chromatogr. A* 2007, *1147*, 165–171.

[20] Dolan, J. W., *J. Chromatogr. A* 2002, *965*, 195–205.