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# Determination of titratable acidity and ascorbic acid in fruit juices in continuous-flow systems

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**Summary.** Two continuous-flow systems for the determination of titratable acidity and ascorbic acid in fruit juice samples are described. The assemblies permit on-line dialysis of analytes prior to the reaction step, thus improving selectivity and performing sample dilution. Flow systems are built with a channel carrying the donor phase (sample in both determinations) and another channel carrying an acceptor phase, both of them entering the dialyser. The outcoming stream transporting the dialysed sample fills the valve loop, permitting its injection into a carrier stream which continuously passes through the spectrophotometric detector. For the titratable acidity, acceptor phase and carrier are distilled water, the reagent merged with the carrier channel being a buffered solution of bromothymol blue (pH 7). The analytical signal obtained is then monitored at 616 nm. For ascorbic acid, the acceptor phase was a Fe(III) solution, which reacts with the dialysed analyte to form Fe(II). A buffered solution of o-phenanthroline (pH 4.5) is used as carrier, reacting with Fe(II) to give the analytical signal, which is monitored at 510 nm. Chemical and physical parameters are optimized for both systems. The analytical features of the determination are established. Finally, the proposed procedures are compared with the official volumetric AOAC methods for both parameters. The FIA methods turn out to be suitable for a rapid and accurate control of fruit juice samples, compared with the reference methods; additionally they compete advantageously with the volumetric methods in the case of turbid and highly coloured samples.

## Introduction

Automation of the different stages of food technology is demanded. The on-line determination of foodstuff components is important with regard to process and quality control. Flow injection (FIA) assemblies are adequate for these purposes. Usually, a separation step is essential to analyze complex matrices. Dialyis permits continuous-flow separation with easy implementation and efficiency, being often used in automated analytical methods. Continuous-flow dialysers are characterized by two streams, namely donor phase (transporting the original sample) and acceptor phase (receiving the dialysed sample after having passed through a porous membrane). Analyzers with built-in dialysis systems should also contain the usual FIA elements, i.e. the propulsion module, the injection valve, some reactors and connectors, and finally, the continuous detection module.

The first applications of the dialysis-FIA hyphenated approach was described by Ruzicka and Hansen [1] and later abridged by other authors [2–4]. The main feature of on-line dialysis is the powerful analyte separation from the matrix. Clinical samples have received much of the attention for the on-line dialysis strategy, thus for instance, permitting in vivo sampling operations [5]. Another attractive feature of continuous dialysis is the on-line analyte dilution [6], due to the fact that separation efficiency is around 1–4%, which allows for the analyte concentration to be set to the determination range. Such configurations should be interesting in relation to the determination of analytes in complex matrices, and when turbid and highly coloured samples such as fruit juices are used.

The acidity of a juice is due to the content of several organic acids (i.e. citric, malic, fumaric, acetic, ascorbic, galacturonic). The acidity of a fruit juice is relevant to keep the organoleptic nature inalterable and to avoid fermentation processes. These properties make the determination of this parameter of great interest. The usual method for the determination of titratable acidity in fruit juices is the AOAC procedure [7] based on the titration of the sample with 0.1 mol/l NaOH, using phenolphthalein as indicator or potentiometric detection in the case of heavily coloured samples. Several methods are reported for the FIA determination of acids (and bases), but are mainly focused on strong ones [8]. Many procedures are based on the injection of the acidic analyte into an aqueous, basic or buffered solution of a chemical indicator and spectrophotometric monitorization of the spectral changes. The use of the peak-height of the FIA signals has successfully been used for this purpose [8, 9] according to the so-called single-point titration procedures [10, 11]. FIA pseudotitrations, in which the peak widths is used instead of the peak-height, have also been applied [12]. However, to our knowledge, no FIA methods have been reported for the determination of total acidity in fruit juices.

Ascorbic acid is one of the acids present in fruit juices. It is the indicator of adulteration or prohibited manipulations of the juice. Ascorbic acid is also a well known nutritional and therapeutic agent. However, some toxic effects can be observed for overdoses of ascorbic acid, and then its

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control in juices is required. Among the currently available methods for the determination of ascorbic acid, the AOAC has adopted the one based on the titration of the sample with 2,6-dichlorophenolindophenol [7]. Some FIA methods have been developed for the determination of ascorbic acid in foods, based on colorimetric [13–16] or amperometric [17–19] detection. Yamane and Ogawa [16] used the Fe(III)/o-phenanthroline system to determine ascorbic acid in juices; however these authors did not utilize a separation method, and therefore samples have to be strongly diluted in order to fit the linear range of the calibration graph and avoid the interference of colour and turbidity of the sample.

Reported here are studies with a continuous-flow dialysis unit integrated in two FIA manifolds used to determine total (titratable) acidity and ascorbic acid in fruit juices. The former study was chromatograph-based and is aimed at a semi-quantitative knowledge of the relative amount of acids present in the dialysed fraction of juice samples. The next step was the determination of titratable acidity of those juices, based on the acid-base behaviour of a buffered solution of the bromothymol blue indicator. The variation of the analytical signal is spectrophotometrically measured at 616 nm. Finally, one of the acidic components of the samples, the ascorbic acid, was selectively determined by means of a redox reaction between ascorbic acid and Fe(III) followed by a complexation reaction between Fe(II) and o-phenanthroline, forming a red derivative, which is monitored spectrophotometrically at 510 nm.

## Materials and methods

*Reagents.* Ascorbic acid, citric acid, malic acid and fumaric acid were used as standard solutions. As a mobile phase in the chromatographic study, distilled-deionized water was adjusted to pH 2.5 with orthophosphoric acid. For the determination of titratable acidity, the bromothymol blue indicator reagent was prepared in a concentration of $10^{-4}$ mol/l in $5 \times 10^{-3}$ mol/l Britton-Robinson buffer (pH 7.5) (from a stock buffer solution containing 0.1 mol/l sodium acetate, 0.1 mol/l boric acid and 0.1 mol/l potassium dihydrogenphosphate and adjusting the pH to 7). For the ascorbic acid determination, a $10^{-2}$ mol/l ferric chloride in $5 \times 10^{-3}$ mol/l orthophosphoric acid solution and a $10^{-2}$ mol/l o-phenanthroline in $4 \times 10^{-3}$ mol/l acetic/acetate buffer (pH 4.5) solution were used. All reagents were of analytical grade.

*Apparatus.* The FIA manifolds were built with a Gilson Minipuls-3 multichannel peristaltic pump, a Rheodyne 5041 injection valve, PTFE tubes of 0.5 mm i.d., and a Hellma quartz flow-cell with a 10 mm light path and 18 µl inner volume. A Hewlett-Packard HP8452A diode-array spectrophotometer coupled with a Hewlett-Packard Vectra ES/12 computer via an HP-IB interface was used for detection and data collection. For the chromatographic study, a Perkin Elmer series 2 chromatograph with a UV-Vis LS-75 spectrophotometer detector module connected to a Spectra-Physics 4290 integrator and a Spherisorb C8, 5 µm (Scharlau S.A.) ref. 060-B74136, 1:12 cm, 0.4 mm i.d. were also used.

The dialysis units used consisted in all cases of two identical methacrylate plates containing in one of its faces a channel (124 mm long, 1.5 mm wide and 0.7 mm depth). Between these two halves, a Bran-Lübbe cellulose acetate type C membrane was inserted.

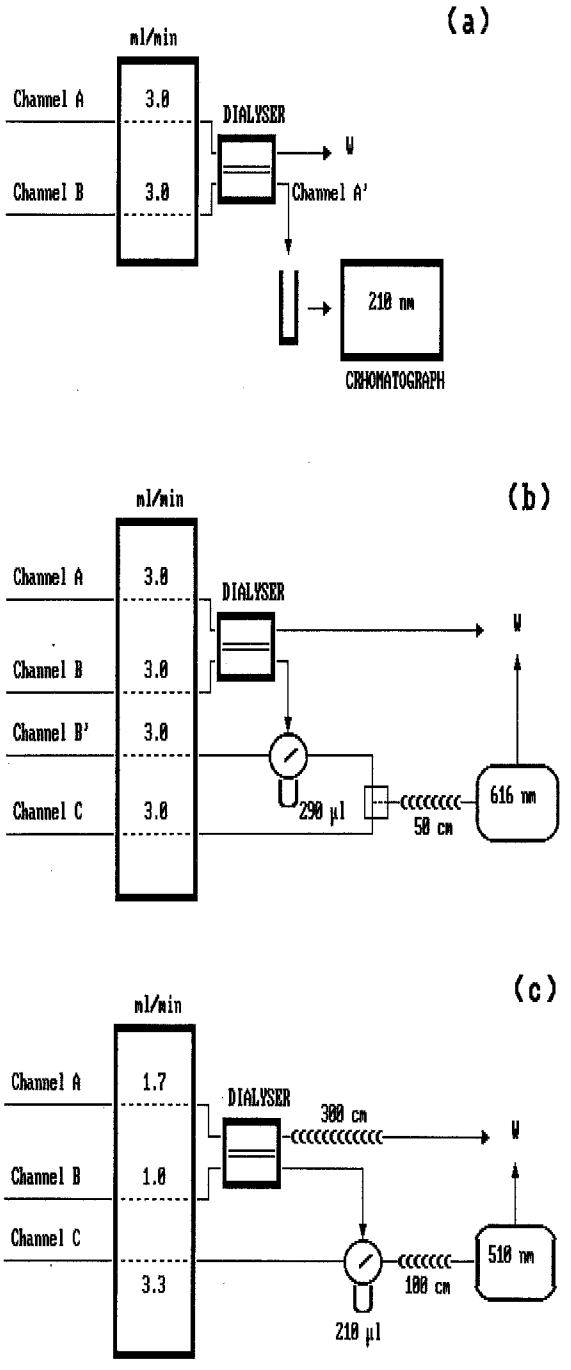


**Fig. 1.** Schematic diagram of the assemblies used. (**a**) Chromatographic study of compounds present in the dialysed fraction of the sample. (**b**) FIA determination of titratable acidity. (**c**) FIA determination of ascorbic acid

### *Procedures*

*Chromatographic study of dialysed juices.* Using the assembly depicted in Fig. 1 a, the sample solution is pumped (channel A) through the dialyser and is received by the distilled water acceptor phase (channel B). The dialysed fraction (channel A′) is collected and filtered through a Cameo nylon filter (0.45 µm, 2.5 cm diameter). Finally, 20 µl of the filtrate are injected into the chromatographic system, which monitors the signal at 210 nm. 0.1 mol/l solutions of ascorbic acid, citric acid, malic acid and fumaric acid are used as standards.

*Determination of total acidity in juices.* Using the assembly depicted in Fig. 1b, standards and samples are pumped (channel A) through the dialyser. The dialysed fraction is collected by distilled water (channel B), continuously filling the sample loop of the injection valve. 290 µl of this solution are injected into distilled water (channel B′) and the stream is later merged with the buffered bromothymol blue indicator solution (channel C). After passing through a 50 cm reaction coil, the flow stream is continuously monitored at 616 nm, where any change in the absorbance of the basic form of the indicator was recorded.

Standard solutions of citric acid in the range 0.1 to 2 g/100 ml are used for the analysis of orange, tomato and pineapple juices. Malic acid solutions in the range 0.05 to 1.5 g/100 ml are used for apple juice analysis. 1 mol/l Stock solutions of all the standards are prepared daily, protected from light and kept at 4° C. Fruit juices are diluted 1:1 with distilled water and continuously homogeneized by stirring during the analysis.

*Determination of ascorbic acid in juices.* Using the assembly depicted in Fig. 1c, standards and samples are pumped (channel A) through the dialyser. The dialysed fraction is collected by the Fe(III) reagent, which acts as acceptor phase (channel B), filling continuously the sample loop of the injection valve. 210 µl of this solution are injected into the o-phenanthroline reagent stream (channel C). After passing through a 100 cm reaction coil, the flow stream is continuously monitored at the wavelength of 510 nm, at which the absorbance of the Fe(II)/o-phenanthroline complex formed is recorded.

Standard solutions of ascorbic acid in the range 0.05–0.5 g/l are freshly prepared for the analysis of orange, pineapple and apple juices. 1 mol/l stock solutions of ascorbic acid are prepared daily, protected from light and kept at 4° C. Fruit juices are diluted 1:3 with distilled water and continuously homogeneized by stirring during the analysis.

## Results and discussion

### *Chromatographic study*

Although the acidity of a juice is due to the sum of several organic acids, the titratable acidity in fruit derivatives is usually expressed as g of citric per 100 ml (or 100 g) of product [7]. On-line analytical dialysers operate under dynamic conditions, thus drastically complicating the prediction of the outlet concentration, which depends not only on the steady-state mass-transfer coefficient, but also on the Peclet number [20]. Additionally, FIA methods are non-equilibrium systems, the analytical signal being dependent on kinetic considerations. On the other hand, the nature of the analyte, the membrane and some other physico-chemical parameters can also influence the analytical signal. These premises require a previous knowledge of the sample composition (the dialysed fraction of the sample in this particular case) to choose the adequate calibration procedure.

The examination of the components in the dialysed fraction of the juices was accomplished with the aid of the isocratic reversed phase HPLC technique, especially recommended in juice analysis [21]. The goals of this study are (a) knowledge of the ability of the dialyser to separate the main analytes from the original juice samples, (b) semi-quantitative information of the relative proportion of the acids present in the dialysate. The procedure, carried out as described under Experimental, gave rise to some conclusions: (a) Citric acid was found at higher levels in orange, pineapple and tomato juices, where the presence of malic acid was not detected under the working conditions used; (b) Malic acid was the major component found in the apple juices, in which no citric acid was detected; (c) Ascorbic acid was found in all juices as a minor component or even undetected, thus indicating a relatively low presence of that compound in the dialysed fraction of the juices. Consequently, some considerations must be taken into account from these results. Firstly, the a priori selection of the standard species to determine the acidity content of each type of juice should be as follows: citric acid for orange, pineapple and tomato juices and malic acid for apple juices. Additionally, the use of a suitable procedure, in terms of assuring a relatively efficient ascorbic acid separation and a sufficiently sensitive reaction seems to be necessary.

### *Optimization of the total acidity FIA procedure*

According to the conclusions drawn above, no separation efficiency is required for the total acidity determination. On the other hand, the chemical behaviour of an acid-base indicator should be adequate enough to measure the acidity of the sample with a sufficient sensitivity. The phosphate buffer-bromothymol blue pair has been recommended to obtain a linear relationship between analytical response and acidic species concentration, due to the fact that the pH-value of the buffer solution can be close to the pK-value of the indicator [8].

Figure 1b shows the assembly designed according to the previous assumptions. Chemical (bromothymol blue concentration and pH of the phosphate buffer) and FIA (sample volume, reagent flow-rates and residence time) variables are optimized with the aid of aqueous citric acid standards. The wavelength corresponding to the maximum absorbance change (616 nm), is equal to that of the basic form of bromothymol blue, and was therefore selected. Under these conditions, negative outputs are recorded, the increment of absorbance being used. The optimizaion procedure was the univariate approach. A compromise between sensitivity and linearity was to be reached in a pre-determined citric acid concentration range (0.2–2 g/100 ml), due to the fact that linearity can be limited by the colour change of the indicator. Table 1 shows the results obtained after studying the influence of the different variables as well as several remarks concerning the parameter selection. As shown in this table, the influence of some parameters on the limit of detection (LOD), the peak shape or the sample passage were occasionally used as criteria to help in the selection.

### *Optimization of the ascorbic acid FIA procedure*

Accrding to the conclusions described above, a high separation efficiency should be required for the ascorbic acid determination. A procedure to make greater amounts of analyte cross the membrane of the continuous-flow dialyser consists of employing a higher pressure along the carrier phase than in the acceptor phase. This can be achieved by using a higher flow-rate in the carrier phase as well as a coil in the outcoming donor phase stream. However, it must be noticed that the number of interferents crossing the membrane also increases when these approaches are used. Another indirect

**Table 1.** Optimization of parameters for the determination of titratable acidity

| Parameter | Range studied | Selected value | m[a] | r[b] | Comments about parameter selection |
|---|---|---|---|---|---|
| pH | 6–8 | 7.5 | I | I | >7.5 higher LOD |
| Bromothymol blue conc., mol/l | $10^{-5}$–$10^{-4}$ | $10^{-4}$ | N.I. | N.I. | Less reagent consumption |
| Flow-rate, ml/min | 2.5–6.0 | 3.0 | D | I | Better compromise between m and r |
| Sample volume, µl | 110–310 | 290 | I | N.I. | >300 µl, double peaks |
| Coil lengths, cm | 50–150 | 50 | N.I. | N.I. | higher sample throughput |

[a] Slope of the calibration graph. An increase (I), decrease (D) or negligible influence (N.I.) was observed in m values by increasing the parameter value in the range studied

[b] Correlation coefficient. An increase (I), decrease (D) or negligible influence (N.I.) was observed in r values by increasing the parameter value in the range studied. In all cases the r value of the selected parameter was up to 0.999

**Table 2.** Optimization of parameters for the determination of ascorbic acid

| Parameter | Range studied | Selected value | $\Delta A$[a] | Comments about parameter selection |
|---|---|---|---|---|
| Fe(III) conc., mol/l | $10^{-4}$–$10^{-1}$ | $10^{-2}$ | I/N.I. | Less reagent consumption |
| *o*-Phenanthroline conc., mol/l | $10^{-4}$–$10^{-1}$ | $10^{-2}$ | I | Less noise signals |
| Pump speed, rpm | 5–30 | 15 | D | Compromise with relative high sample throughput |
| Sample volume, µl | 110–290 | 210 | I/D | Best sensitivity |
| Coil lengths, cm | 50–250 | 150 | N.I. | Higher sensitivity |

[a] Absorbance variation. An increase (I), decrease (D), negligible influence (N.I.) or change in the influence during optimization (I/N.I. or I/D) was observed in $\Delta A$ values by increasing the parameter value in the range studied

way to strengthen ascorbic acid dialysis may be the use of an appropriate reagent instead of water as acceptor phase. This species should react with ascorbic acid with rapid kinetics, and should preferably serve for its definite determination. An additional advantage of this approach is that there is no further dilution of the analyte as it would happen if a water acceptor phase were used. In this case, a selective reagent should be chosen, taking into account the great number of interferences. An Fe(III) solution may be a suitable alternative for this purpose, since its corresponding reaction with ascorbic acid is favourable from both the thermodynamical and kinetical point of view. o-Phenanthroline may be used as indicator due to the sensitive reaction with the Fe(II) formed in the above described reaction.

Figure 1c shows the configuration designed according to these previous considerations. Chemical (Fe(III) and o-phenanthroline concentration) and FIA (sample volume, reagent flow-rates and reaction time) variables were optimized with the aid of 0.1 g/l ascorbic acid aqueous standard solutions. In this case, the flow-rates of each reagent were optimized independently. Additionally, in order to use only a peristaltic pump, tygon tubes of different i.d. were used to obtain different flow-rates for each reagent. The selection was achieved in two steps; firstly, the o-phenanthroline flow-rate was studied, resulting in a negligible influence on the peak height. However, since the sample throughput increases with increasing flow-rate, a high flow-rate is preferable (for instance a large i.d. tygon tube). On the other hand, sample and Fe(III) flow-rates affect the separation efficiency, according to the considerations described above, thus the inner diameter of the tygon tube used for the sample was larger than that of the Fe(III) solution. Finally, a 1.7/1/3.3 ratio of sample/Fe(III)/o-phenanthroline flow-rates was selected according to the tygon tubes diameter selection. The pump speed was then optimized. Additionally, a 300 cm long coil was used as outcoming donor phase channel to force ascorbic acid dialysis. The wavelength of maximum absorbance change (510 nm) corresponding to the absorption maximum of the Fe(II)/o-phenanthroline complex was selected. Under these conditions positive peaks are recorded, although the use of water as sample in the system (blank) gives a considerable signal (i.e. 0.15 absorbance units). This fact was not initially considered for optimization purposes, although it will limit the LOD of the procedure.

The optimization procedure was the univariate approach, based on obtaining the maximum absorbance, on the assumption that linearity is not significantly affected by experimental conditions. Table 2 shows the results obtained. As shown in this table, the influence of some parameters on the sample throughput or the noise (due to changes in the refraction index if two solutions of rather different concentration are mixed) was occasionally used as a criterion to help in the selection of variables.

In addition to the optimization process shown in Table 2, the dimensions and form of the path zone of the dialyser were also considered in order to improve the ascorbic acid separation efficiency. Figure 2 shows the designs (those available in the laboratory) tested for this study. The main conclusions of this study reveal that the larger the area for dialysis (design type c > b > a), the larger the sensitivity obtained, but also the poorer the precision. An additional problem with dialysers b and especially c was the difficult removal of air bubbles that occasionally enter the dialyser, which also modifies the area of the useful surface, and consequently the results. This fact is undesirable when thinking in terms of a fully automated on-line control procedure; therefore the dialyser type a was retained.

### *Analytical features of the FIA procedures*

The analytical features of the FIA system were established in two sets of experiences. 20 consecutive injections of 1 g/

**Table 3.** Analysis of fruit juice samples[a]. Comparison of AOAC and FIA methods[b]

| Sample | Parameter | AOAC Method | FIA Method[c] | Er, % |
|---|---|---|---|---|
| Orange | Titratable acidity[d] | 0.394, $H_3C$ (0.377, $H_2M$) | 0.388, $H_3C$ (0.282, $H_2M$) | − 1.5 (−25.2) |
| | Ascorbic acid[e] | 0.540 | 0.517 | − 4.3 |
| Pineapple | Titratable acidity | 0.220, $H_3C$ (0.211, $H_2M$) | 0.222, $H_3C$ (0.167, $H_2M$) | 0.9 (20.9) |
| | Ascorbic acid | 0.206 | 0.197 | − 4.3 |
| Tomato | Titratable acidity | 0.164, $H_3C$ (0.157, $H_2M$) | 0.167, $H_3C$ (0.129, $H_2M$) | 1.8 (−17.8) |
| | Ascorbic acid | − | − | − |
| Apple | Titratable acidity | (0.263, $H_3C$) 0.252, $H_2M$ | (0.350, $H_3C$) 0.256, $H_2M$ | (33.1) 1.6 |
| | Ascorbic acid | 0.022 | − | − |

[a] Five different samples were used. Only the results for the sample giving the highest relative error were tabulated
[b] Average value of three replicates
[c] $H_3C$ (citric acid) or $H_2M$ (malic acid) used as standard
[d] Titratable acidity in g/100 ml. $H_3C$ (expressed as g of citric acid), $H_2M$ (expressed as g of malic acid)
[e] Ascorbic acid content in g/l

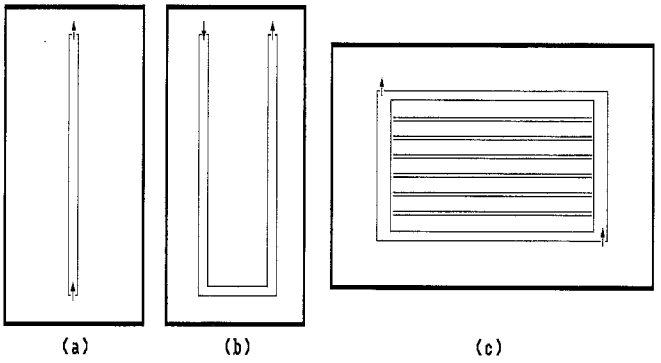


**Fig. 2.** Dialysis zone design. Dialyser type and dimensions in mm: (**a**) Channel 124 long × 1.5 wide × 0.7 deep. (**b**) Channel 270 long × 1.5 wide × 0.7 deep. (**c**) Main channel 360 long × 1.5 wide × 0.7 deep and secondary channels 120 long × 0.7 wide × 0.4 deep

100 ml of citric acid gave a relative standard deviation (r.s.d) of 0.4% and a sample throughput of 190 injections/h. A linear calibration graph, obtained in the range of 0.1 − 2 g/100 ml of citric acid, results in the equation: $\Delta A = -0.00047 + 0.354$ [ ] (g/100 ml), $r = 0.9999_7$. The corresponding equation using malic acid in the range 0.05 − 1.5 g/100 ml was: $\Delta A = -0.079 + 0.438$ [ ] (g/100 ml), $r = 0.9999_2$. All these features are suitable to meet the requirements for the determination of the acidity content in juice samples. The sampling rate permits a sufficient speed to apply the method to quality control loops.

The analytical features of the FIA system for the ascorbic acid determination were established in a similar way. 20 consecutive injections of 0.1 g/l of ascorbic acid gave a relative standard deviation (r.s.d) of 0.5% and a sample throughput of 60 injections/h. A linear calibration graph, obtained in the 0.05 − 0.5 g/l range, results in the equation: $\Delta A = 0.176 + 0.95$ [ ] (g/l), $r = 0.999_7$. All these features are suitable for the determination of the ascorbic acid content in juice samples with an acceptable sampling rate.

*Analysis of juice samples*

Table 3 shows a comparison between the results of the AOAC method [4] and the proposed FIA methods when commercial fruit juice samples were analyzed. A good correlation was observed between the FIA and the volumetric methods for titratable acidity when the a priori selection of standards for each type of juice had been used. Large relative errors (between brackets in Table 3) were found when citric acid was used for apple juices and malic acid for orange, pineapple and tomato juices, thus indicating that an inadequate standard solution was used.

In relation to ascorbic acid contents, the correlation was good in orange and pineapple juices. Apple juices, which contain a low concentration of ascorbic acid, gave a signal equal to that obtained with water (blank) in the FIA procedure, even if no sample dilution is applied. When the volumetric method was applied to apple juices, it gave rise to a low volume consumption (i.e. 0.2 ml net volume using the recommended 2,6-dichlorophenolindophenol solution) and consequently an inaccurate result. On the other hand, some difficulties were found in the endpoint detection when the volumetric procedure is used in tomato samples, due to the colour and turbidity of such samples, whereas the FIA method is applicable to these samples. No analysis was performed since no AOAC reference method was available for this type of samples.

From a practical point of view, it should be stressed that, in general, substantial changes in the ratio of different acids in samples may have an influence on the accuracy of the results, although this situation is highly improbable in the case of juice samples.

## Conclusions

The results of these investigations reveal that the proposed FIA methods with on-line dialysis are adequate and accurate for the determination of total (titratable) acidity when using the appropriate standard solution, and ascorbic acid for levels from 0.03 g/l in fruit juice samples (the level can be

used in practice as an indication of the maximum amount of ascorbic acid present). It is also easily available for determinations in other matrices with minor modifications in the manifolds.

The advantages of the FIA methods over the classical volumetric methods (AOAC) are substantial. The FIA method is free from major interferences which occur in the volumetric procedures, i.e. turbid or highly coloured juice samples can be successfully analyzed. The FIA methods are also rapid for routine control in terms of a high number of samples, compared with the manual procedures (ca. 15 times faster for titratable acidity and 5 times faster for ascorbic acid), with the advantage of an easy automation. Finally, the FIA method also requires a lower amount of sample than the volumetric method for titratable acidity (ca. 25 times).

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