Applications of the ion chromatography in the leather sector. Part 2

C. Pérez, M. Reyes, N. Pascual, M. Cobos, J. Ibáñez, R. Molina, J. Font
Escola d'Enginyeria d'Igualada (UPC BarcelonaTech), Igualada, Barcelona, Spain.

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3. Results and discussion

3.1. Determination of anions by ion chromatography and direct UV detection

3.1.1. Influence of the main factors that have effect on the separation. Selection of the chromatographic conditions

The main factors that influence the chromatographic separation have been studied. The aim was to define the conditions that allow the simultaneous and rapid determination of chloride, fluoride, phosphate, sulphate, nitrate, nitrite and bromide anions.

The following factors were evaluated: temperature and composition of mobile phase (concentration of acetonitrile, pH, and concentration of potassium biphthalate).

All the analyses were carried out at a flow of 0.9 mL/min and at a wavelength of detection of 260 nm:

- In the study of the relationship between flow and chromatographic separation it is clear that increasing the flow of the mobile phase, the retention time of the anions decreases in the same rate [16]. A flow of 0.9 mL/min is the 90% of the maximum allowed by the column.
- It was observed the unfeasibility of detecting at wavelengths below 254 nm due to an excess of noise of the baseline. This remark has been observed also by other authors [16, 17,18].

In each test, a multicomponent Standard was chromatographied. This solution contained chloride, fluoride, phosphate, sulphate, nitrate, nitrite and bromide, each one at approximately 40 mg/L. 20 µL were injected in all cases. Tests were carried out by duplicate.

a) Influence of the temperature:

The Standard was injected at three different oven temperatures: 24, 27 and 30ºC. It was used potassium biphthalate 2mM with 8% (v/v) of acetonitrile at pH 5.22 as a mobile phase. The tests were repeated also with mobile phases with 6% and 10% of acetonitrile, respectively.

No relevant differences were appreciated in the retention time of the anions.

The working conditions in these tests did not allow the separation of the fluoride and phosphate anions.

Even though the temperature has a slight effect on the retention time, it is recommended to control this parameter to achieve the best reproducibility possible between analyses carried out in the different seasons of the year. For this purpose, an oven temperature of 30 ºC was selected.

b) Influence of the amount of acetonitrile in the mobile phase

Three different mobile phases were evaluated, with the following amounts of acetonitrile in v/v percentage: 6%, 8% and 10%, respectively. All of them contained also potassium biphthalate 2mM and adjusted at pH 5.00.

In the studied range of amounts of acetonitrile, only minor differences in the retention times of the analytes were observed. Despite of this, an 8% of acetonitrile for the mobile phase is recommended in order to obtain chromatograms with the shorter analyses time.

It must be borne in mind that the maximum amount of acetonitrile in the mobile phase is limited to 12 % v/v for column protection reasons.

Neither of the chromatograms showed the fluoride and phosphate peaks separated.

The tests were carried out also at pH 5.2. The same tendency of results was observed.

c) Influence of pH in the mobile phase

Mobile phases of potassium biphthalate 2mM and acetonitrile 10% were prepared with different pH values (5.00, 5.22 and 6.50). A multicomponent standard was chromatographied using each one of the eluents.

At pH 6.50, it was demonstrated that:

- All the analytes (including fluoride and phosphate) are well separated, with a resolution higher than 1.5 between two consecutive peaks.

- It decreases the retention time of all anions (except phosphate, which remains unchanged).

Figure 1 shows the chromatogram obtained at pH 6.5 and figure 2 the pH effect on the retention time.

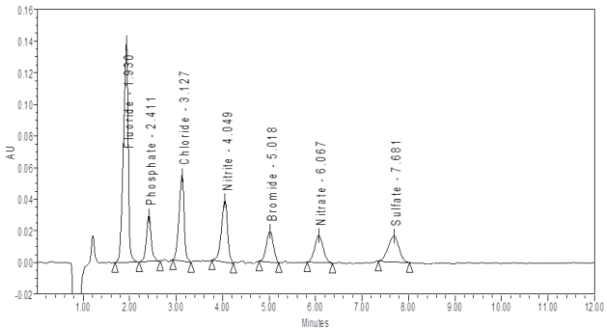

Figure 1. Chromatogram obtained with a 2mM potassium acid biphthalateat 10% (v/v) ACN mobile phase, pH 6.50, 30ºC.

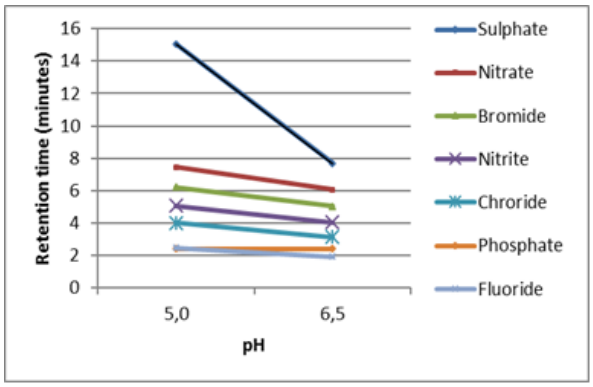

Figure2 .Effect of the pH on the retention time (2mM potassium acid biphthalate at 10% ACN mobile phase).

d) Influence of the concentration of the absorbent electrolyte in the mobile phase

It was chromatographied a multianion Standard with the biphthalate mobile phase, at different values of composition and pH: 1mM in biphthalate with 8% acetonitrile and pH 5.46; 1mM with 8% acetonitrile and pH 6.18; 2mM with acetonitrile 10% and pH 6.50.

The eluent at 1mM biphthalate concentration and pH 5.46 did not allow a complete separation of the phosphate and fluoride analytes.

The separation with the 2mM biphthalate eluent was achieved within a very short length of time (7.7 minutes versus 15 minutes obtained with 1mM eluent at pH 6.18).

The limit of detection (LOD) for some anions was determined (table 1). It was found that 1mM potassium biphthalate concentration provides higher sensitivity, whereas the 2mM concentration shortens the analysis time.

Anion	***Mobile phase 1mM potassium biphthalate, 8% ACN, pH 6.18 (260nm)***	***Mobile phase 2mM potassium biphthalate, 8% ACN, pH 6.50 (260nm)***
	Limit of detection (mg/L)	
Chloride	*0.10*	*0.41*
Nitrate	*0.30*	*0.99*
Phosphate	*0.42*	*1.07*
Sulphate	*0.75*	*1.54*

Table 1.Limits of detection of some anions at different concentrations of mobile phase. The LODs are defined with the signal to noise ratio of 3 (S/N=3)

e) Proposal of the chromatographic conditions

Table 2 summarises both chromatographic conditions that provide optimum selectivity in determining the anions of interest, depending on the need to prioritize shorter analysis time or more sensitivity.

Column temperature	30±1 °C
Mobile phase	**Shorter analysis time (Tr sulphate: 8 minutes):** 2mM potassium biphthalate and 8 % acetonitrile at final pH 6.50
	More sensitivity (Trsulphate: 15 minutes): 1mM potassium biphthalate and 8% acetonitrile at final pH 6.18
Wavelength	260 nm
Flow	0.9 mL/minute

Table2.Proposal of chromatographic conditions for indirect detection determination..

3.1.2. The linearity of chromatographic method with indirect detection

Calibration standards were prepared from 1000 mg/L multi-element Standards in the concentration range of 5.00 to 100 mg/L of phosphate, formiate, chloride, bromide, nitrate and sulphate using both chromatographic conditions of Table 2. Peak areas were measured for the construction of calibration curves. In all cases the correlation coefficient was more than 0.9997.

3.1.3. Study of the recovery of the solid phase extraction process applied to dyes and naphthalene sulphonic samples

The chromatographic column used in this paper does not admit non-ionic components. As a preventive measure, a pre-treatment of solid phase extraction (SPE) was performed on dyes and naphthalene sulfonic samples (because of the possibility to contain lipophilic substances). In the analysis of mimosa this treatment was not performed because aqueous vegetable extracts do not contain lipophilic components.
In order to evaluate the effect of SPE pre-treatment on the anion content, a recovery test was performed. The test consisted of analysing three multi-anion Standards (25 mg/L in each anion) with and without filtration with SPE cartridges. Table 3 shows the obtained results.

Anion	*Fluoride*	*Phosphate*	*Chloride*	*Bromide*	*Nitrate*	*Sulphate*
% of recovery	*99.5*	*98.4*	*99.1*	*98.4*	*99.1*	*99.3*

Table3.Results of the study of the SPE pre-treatment effect on the anion content.

It was verified that the filtration process with SPE cartridges, applied to dyes and naphthalenesulfonic samples, did not produce anion losses.

3.1.4. Determination of impurities in commercial chemicals (dyes, dispersants naphthalenesulfonic, mimosa extracts and formic acids) using indirect detection

Two samples of brown dyes for drum commercialized in the Spanish market, two samples of naphthalenesulfonic, two samples of mimosas and two other of concentrated formic acids were analysed.

Table 4 expresses the contents of the impurities detected in the products tested.

	Chlorides (expressed as % NaCl)	**Sulphates** (expressed as $\%Na_2SO_4$)
Dye A	18.7	45.1
Dye B	14.0	45.6
Naphthalenesulfonic A	< 0.2	18.0
Naphthalenesulfonic B	< 0.2	29.9
Formic acid A	0.6	< 0.2
Formic acid B	< 0.2	12.3

Table 4. Contents of anionic impurities (% by mass) in the chemicals analysed.

Both dye samples gave chloride and sulphate impurities. The samples of wattle extract analysed do not produced peaks in the chromatograms. Both samples of naphthalenesulfonic syntans gave sulphate impurities. Regarding formic acid samples, in one impurities of sulphate were detected and in the other, chloride.
The following figures (Figure 3, Figure 4 and Figure 5) show respectively dye, naphthalene sulfonic and formic acid chromatograms.
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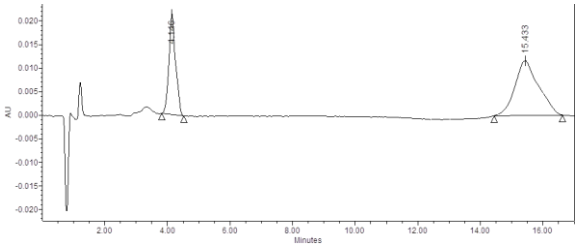

Figure 3.Brown dye for drum dyeing sample chromatogram. Peaks: Chloride 4.140 min; Sulphate 15.433 min (indirect detection).

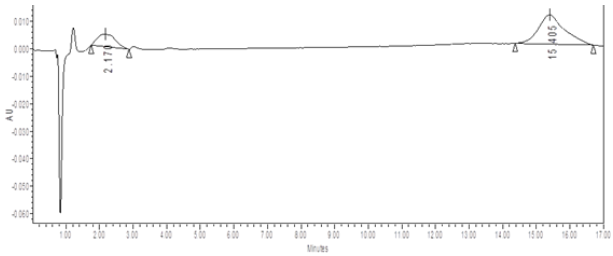

Figure 4.Naphthalenesulfonic sample chromatogram. Sulphate peak 15.405 (indirect detection).

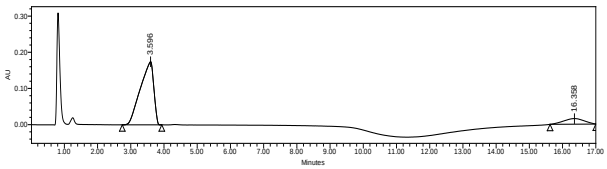

Figure 5.Formic acid sample chromatogram. Peaks: Formiate 3.596; Sulphate 16.358 min (indirect detection)

3.2. Determination of anions by ion chromatography and direct ultraviolet detection

Preliminary tests were performed and they enabled appropriate chromatographic conditions for the anion analysis by direct UV detection and using sodium sulphate as eluent: flow 0.9 mL/min; mobile phase 1mM sodium sulphate, 8% (v/v) acetonitrile and final pH 6.80±0.1; temperature 30±1ºC; PDA detection and chromatogram recovery at 202nm for formiate and acetate and 210nm for nitrate, nitrite and bromide.

3.2.1. The linearity of chromatographic method with direct detection

It was verified the linearity of the calibration curves for nitrate, nitrite, bromide, formiate and acetate anions. The range of concentrations used in the construction of the calibration curves was 5 to 40 ppm for nitrate, nitrite and bromide and 5 to 100 for the acetate and formiate anions. In all cases, correlation coefficients obtained were more than 0.9999.

3.2.2. Quantification of commercial formic acid (direct detection)

The method was applied to three samples of commercial formic acid with concentrations of 85.9%, 85.5% and 85.0% (w/w) respectively. These concentration values, indicated on the label (reference) were obtained by acid-base titration.
Table 5 compares the reference values with the experimental ones obtained by ion chromatography.

	Reference value, total acidity titration (as % formic acid))	Experimental value, ion chromatography (% formic acid
Formic acid 1	85'9%	83'0%
Formic acid 2	85'5%	84'6%
Formic acid 3	85'0%	83'4%

Table 5. Comparison of the formic acid content (volumetric and chromatographic method).

The three samples analysed by chromatography provide similar percentages of formic acid to the reference values of the labels, but slightly lower. This fact is logical because with ion chromatography only formic acid is quantified, while the acid-base titration determines the total acidity of the sample. The volumetric method is exposed to the interferences of the occurrence of traces of other acids, whereas the chromatography is specific for the formic acid.

Figure 6 shows the chromatogram obtained with one of the formic acid samples.

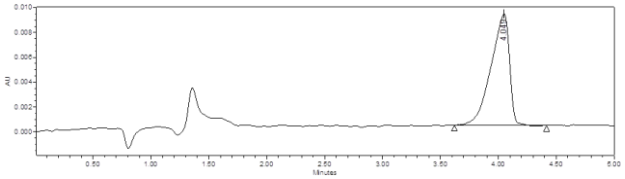

Figure 6. Comercial formic acid sample chromatogram (indirect determination). Peak: formiate 4,049min.

3.3. Determination of anions in leather

Table 6 shows the analysed samples. In each section, it is specified the leather examinated.

Leather	Description
1	Crust, chrome tanned, brown colour
2	Wet-blue
3	Vegetal, light brown colour
4	Split leather, reddish brown colour
5	Split leather not dyed, blue colour
6	Vegetal tanning, light brown colour
7	Vegetal tanning, mustard colour
8	Vegetal tanning for upper sandals
9	Finished leather, bovine, for leather industry (luxury handbags), yellow colour
10	Finished leather, bovine, for luxury leather industry, green colour
11	Finished leather, bovine, for luxury wallets, brown colour
12	Sheep napa for clothing

Tabla 1. Description of the leather samples

3.3.1. Verification of the efficiency of the extraction of anions in leather

It was carried out an study of the improvement of the aqueous extraction process of leather focused on two important factors: the shaking frequency and the number of extractions.
The aqueous extraction from leather was based on UNE-EN ISO 4098:2006 (IUC 6), but with some modification: it was not performed after degreasing and it was used 1 g of leather and 50 mL of water (the proportion water/leather is preserved).

Shaking frequency effect on the extraction efficiency

It was comparated the amount of anions extracted in four leather samples (leathers 1, 4,6 and 7 of Table 6) using two shaking speeds during the extraction process: 50 ± 10 rpm (slow shaking, as recommended by IUC6) and 170 ± 10 rpm (fast shaking).
In two of the analysed samples (leather 1 and 4 of Table 6), fast shaking provided higher anionic content: between 12-56% more than the slow shaking, depending on the particular anion analyzed. In the other two samples, no differences between both shaking speeds were observed.
This test prove the importance of the shaking frequency. If the speed stablished in the Standard is performed, more than fifty percent of the anions contained in some leathers will not be extracted.
Figure 7 compares the chromatograms obtained from the same sample extracted at two diferent shaking conditions.

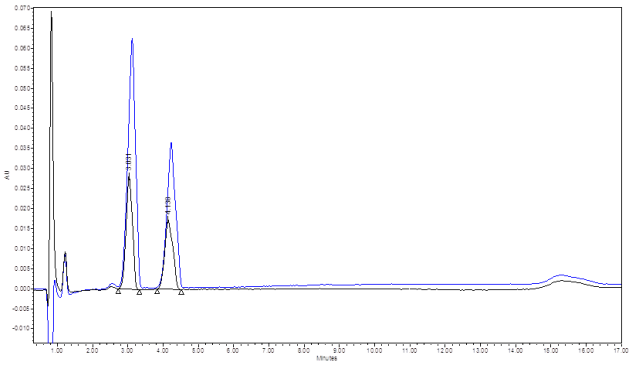

Figure 7. Comparative chromatogram of the extracts of leather 1 at different speeds. Fast extraction (blue) and slow extraction (black).

Comparison between simple and multiple step extractions

Consecutive extractions of twelve samples of leather were carried out to determine if a single extraction can extract all the anions content and, if not, set the number of successive step extractions necessary to achieve that purpose. Extractions were repeated until getting a constant concentration of total anions extracted. The extraction was performed at 170 ± 10 rpm.
For the half of the leathers analyzed, it was obtained approximately the 75% of the anionic content with a single extraction. The rest of leathers required from 3 to 4 consecutive extractions in order to get a constant extracted anionic concentration.

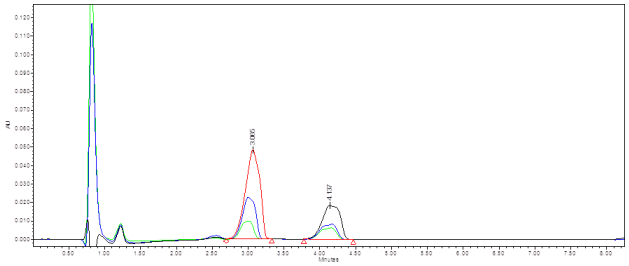

Figure 8. Comparative chromatogram of three consecutive step extractions of leather number 12.

Leather sample analysis

Anionic concentrations found in the twelve samples analyzed (Table 6) change depending on the leather. All the analyzed leathers contained formiate in maximum concentration of 8900 mg/Kg. In the case of chloride anion, the concentration ranged between 1 and 38 mg/Kg. For the sulfate anion, concentrations between 51 and 9982 mg/kg were obtained. It was observed that the four finished leathers had nitrates at concentrations between 40 and 800 mg/Kg. In two of the finished leathers, it was found phosphates (133 and 287 mg/Kg of leather in each one).

En este estudio se observó que las cuatro pieles acabadas contenían nitrato en concentraciones entre 40-800 mg/Kg. En dos de las pieles acabadas se encontraron fosfatos (133 y 287 mg/Kg piel en cada una de ellas).

Precision and recovery in the determination of anions in leather

The reproducibility of the method was evaluated by performing five different analyses during a month using the same leather, with

equidistant dates and with a single extraction (Table 7).

	Formiate (direct detection)	Formiate (indirect detection)	Chloride (indirect detection)	Sulphate (direct detection)
	mg/Kg	mg/Kg	mg/Kg	mg/Kg
Average	**5697**	**6069**	**1286**	**1345**
±RSD (%)	**±3,0**	**±4,1**	**±3,0**	**±1,2**

Table 7. Reproducibility (average of five replicates of leather number 4, with a single extraction and analyzed during a month).

To determine the repeatability of the extraction, five extractions were carried out in the same day (and the same operator). Table 8 shows the results.

	Formiate (direct detection)	Formiate (indirect detection)	Chloride (indirect detection)	Sulphate (direct detection)
	mg/Kg	mg/Kg	mg/Kg	mg/Kg
Average	**5550**	**6051**	**1292**	**1339**
±RSD (%)	**±4,3**	**±3,7**	**±3,7**	**±0,77**

Table 8. Extraction repeatability (five extractions).

The repeatability of the chromatographic analysis was evaluated by introducing two vials of the first extract of the leather sample number 4 and programming four injections in the chromatograph (two for each vial) (Table 9).

	Formiate (direct detection)	Formiate (indirect detection)	Chloride (indirect detection)	Sulphate (direct detection)
	mg/Kg	mg/Kg	mg/Kg	mg/Kg
Average	**5732**	**5798**	**1800**	**2060**
±RSD (%)	**±0,33**	**±0,37**	**±0,13**	**±1,6**

Table 9. Repeatability of the chromatographic injection

The determination of the recovery rate is important in order to evaluate the overall performance of the analytical method and also detect systematic errors due to the sample matrix. At a sample of 1g of leather, it was added an aliquot of 50 mL of standard solution of known concentration (50 mg/L formiate, 15 mg/L chloride and 15 mg/L of sulphate). It was carried out the extraction and the corresponding chromatographic analysis for the five replicates of leather number 4. Good recovery rates were obtained (Table 10).

	Recovery Rates (%)			
	Formiate (direct detection)	*Formiate (indirect detection)*	*Chloride (indirect detection)*	*Sulphate (direct detection)*
Average	*99*	*99*	*125*	*118*

Tabla 10. Tasas de recuperación en la determinación de aniones en piel

4. Conclusions

The HPLC technique with ion-exchange stationary phase and UV detection provides a reliable method for the simultaneous quantification of anions, absorbent and non-absorbent in the ultraviolet, present in different samples of the leather industry.

By indirect detection, contents of chlorides and sulphates in commercial chemicals have been determined. Formic acids were quantified using direct detection. The principal advantage of the direct detection system is that it enables to check the identification of the formiate anion by comparing the UV-VIS spectra of the standard and the sample. The chromatographic method allows specific analysis of formic acid, while the volumetric method determines total acidity present in the sample and it is exposed to interferences from traces of other acids.

The study of improving the anions extraction process in leather has been focused on two major factors: the shaking frequency and the number of extractions. The results confirm that the conditions described in the UNE-EN ISO 4098:2006 (IUC6) and the realization of a single extraction, are insufficient to extract all anions:

- It is proposed to operate at a shaking frequency of 170±10 rpm (turbulent conditions) because the results obtained are always better or equal to those obtained with the frequency indicated by the Standard.

The conclusion of the analyses of the twelve leather samples is that in half of the samples, it is possible to extract with a single extraction approximately 75% of the anionic content. It has been observed that in some finished leathers the efficiency of a single

- extraction is poor, lower than in unfinished leather. Probably, it would be advisable to realise a degreasing so as to determine anions more effectively in the finished leather. However, this conclusion should be confirmed with a broader range of skin.

We determined that the procedures described in UNE-EN ISO 4098:2006 (IUC6) provide only estimate calculations and the results obtained with this standard systematically committed an error by default. The result obtained is lower than the real one because the standard indicates a single extraction. As the bibliography about extraction operation [19] indicates, multiple extraction is always more effective than simple one, a fact that supports the results obtained in this study.

In some leathers, which contain phosphates, the determination of formiate anion is more complex because the retention time is the same for both anions when the indirect detection is used. A protocol has been designed which allows the quantification of formiate in the presence of phosphates.

Data have been obtained with accuracy and precision which will be useful for a future validation of the method of analysis of anions in leather.

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