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Determination of oxalate, sulfate and nitrate in honey and honeydew by ion-chromatography

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Abstract

An ion chromatographic method for determining the anions oxalate, sulfate and nitrate in honey and honeydew samples is described. To prevent matrix interference and to isolate the anions a clean-up step using solid-phase extraction on anionic cartridges and eluting with a 0.01 *M* chromate solution is recommended. The anions are separated on an anionic column with a mobile phase of borate–gluconate buffer and using conductimetric detection. The method is applied to the analysis of samples from different botanical origin.

Keywords: Honey; Food analysis; Oxalate; Sulfates; Nitrate; Organic acids; Inorganic anions

1. Introduction

The chemical composition of honey has been treated for a long time but nowadays this subject has become even more interesting, not only to get original authenticity data, which undoubtedly enhances its trade, but also to detect adulterations. Regarding the anions considered in this study, there are also another reasons that make their evaluation interesting. Oxalic acid is being investigated to control varroosis and recent reports seem to prove its high efficacy and good tolerability by bees [1–3], although nowadays some negative long-term effects have been also described [4]; so it is necessary to establish the adequate dosage and also to know if any significant increase of its content can appear after the beehive treatment, because of that, analytical methodology must be available for its determination. Data related to the other anions are also interesting to know better the composition of honey, with special attention to sulfate presence, it is usually assumed that honey does not contain sulfate whereas the honeydew has high sulfate content, in some cases about 73 mg/100 g, expressed as sulfuric acid [5].Our experience in analyzing apiculture products indicates that in many natural honey samples sulfates are detected and because of that, we consider it useful to have methods that facilitate correctly knowing their concentration and applying them to a wide number of samples to assess their presence that sometimes has been used as a criterium of authenticity or honey purity.

The determination of this group of common anions has not been described for honeys, perhaps due to the problems associated with great matrix influence, mainly in the oxalate analysis. To determine residual levels of oxalate, after beehive treatment with oxalic acid, an adaptation of a commercially available kit (oxalate Sigma diagnostic kit, Catologue No. 591-D), with a detection level higher than 3 ppm, has been

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proposed [3]. In the determination of organic acids in honey usually oxalic acid does not appear [6,7] probably due to its lower retention that usually makes it elute with the chromatographic front. Recently, a method that could be useful for oxalic determination in honey using ion-exclusion chromatography with electrospray mass spectrometry has been proposed [8], the same technique but with ion trap detection of the trimethylsilyl derivatives seems to be adequate to determine the phosphoric acid content in honey [9].

Obviously a lot of options have been proposed to evaluate those anions in many different matrixes, among them the most used are capillary electrophoresis and ionic chromatography. We have thought that an adequate option would be the use of ion chromatography with conductimetric detection because it can be more versatile taking into account the very different proportions in which those anions can appear in the samples. To isolate the anions solid-phase extraction on anionic cartridges has been selected, the best conditions to make the extraction and the elution have been chosen. The proposed procedure has been applied to analyze natural honeys (multi- and unifloral ones) and honeydews collected over three years to find the average and the intervals of variation. At the same time the oxalate has been evaluated in honey collected in beehives submitted to oxalic acid treatment to know if any important variation in its concentration has been originated.

2. Experimental

2.1. General

2.1.1. Chemicals

Analytical standard-grade sodium nitrate, sodium dihydrogenphosphate, sodium sulfate and oxalic acid were obtained from Sigma–Aldrich Química (Madrid, Spain). Potassium chromate, lithium hydroxide monohydrate, d-gluconic acid (50:50 w/w), boric acid and glycerin were of analytical-reagent grade and supplied by Merck (Darmstad, Germany). Acetonitrile UV grade was obtained from Labscan (Dublin, Ireland). Water was purified by passage through a Compact Milli-RO and Milli-Q water system from Millipore (Milford, MA, USA). All solutions used were previously passed through a 0.45 μm membrane filter from Millipore to remove any impurity.

Accell Plus QMA solid-phase extraction anion-exchange cartridges of 500 mg were obtained from Waters (Milford, MA,USA)

2.1.2. Equipment

The chromatographic set-up used consisted of a CD4000 multisolvent partitioning pump from LDC Analytical (Riviera Beach, FL, USA) and a conductivity detector (Conducto Monitor III) from Milton Roy (Riviera Beach, FL, USA), with an oven and a JCL6000 Chromatography data System from Jones Chromatography (Littleton, CO, USA).

An ultrasonic bath and a centrifuge, both from Selecta (Barcelona, Spain) and a vortex mixer from Fisher Scientific (Pittsburgh, PA, USA) were also used.

2.2. Column liquid chromatography

2.2.1. Column

The column used was a 150×4.6 mm I.D. IC-Pak A-HC from Waters, packed with 10 μm particles, operated at 25°C

2.2.2. Mobile phase

Lithium borate–gluconate (1.4%), *n*-butanol (2%), acetonitrile (12%) and water (84.6%) at a flow-rate of 2 ml/min.

The lithium borate–gluconate concentrate was prepared according to the column manufacturer recommendations: 34 g of boric acid, 23.5 ml of d-gluconic acid (50%, w/w), 8.6 g of lithium hydroxide monohydrate and 250 ml of glycerin were mixed and then diluted to 1 l with deionized water

Samples were injected by means of a Marathon autosampler from Spark Holland (Emmen, Netherlands) furnished with a fixed-volume (100 μl) loop.

2.3. Standard calibration

Stock solutions of anions of 1 g/l were made in lithium hydroxide monohydrate $2 \cdot 10^{-3}$ *M* and stored at 4°C. Mixtures of different concentrations were made from those solutions.

Calibration graphs were obtained using five differ-

ent concentrations of the mixed standard solutions that were passed through a solid–phase extraction (SPE) ion-exchange cartridge packed with 500 mg of solid-phase that was pre-activated with 15 ml of deionized water. The compounds of interest were eluted with 7(4+3) ml of chromate 10^{-2} *M* and injected into the chromatograph. All samples were prepared and injected in triplicate.

2.4. Samples

To know the average and the variation interval of those anions in honey, 99 samples of different botanical origins have been analyzed. They were collected, during the years 1996, 1997 and 1998 from the same geographical area (Soria Province, Spain). The climate and the vegetation conditioned the number of samples collected of each origin.

To study the incidence that can have the treatment with oxalic acid, honey samples from five beehives, treated every seven days for four weeks spraying with a 3% aqueous solution of oxalic acid (till a maximum of 80 ml per beehive), were collected. This experiment was carried out in the Centro Apícola Regional of Marchamalo (Guadalajara, Spain).

2.5. Sample treatment

Prior to HPLC analysis, samples were subjected to a solid-phase extraction on ion-exchange cartridges in order to remove matrix compounds that might overlap with the compounds of interest.

The influence of parameters potentially affecting the extraction process was studied in order to establish the optimal conditions for maximum recovery.

Raw honey samples collected from beehives submitted to treatment were previously centrifuged at 16 000 *g* to remove extraneous matter (mainly beewax and parts of bees).

3. Results and discussion

3.1. Chromatographic conditions

As it is usually assumed that no single column meets all the requirements to obtain an anionic profile in any type of sample [10], we selected one of widespread use, its manufacturer suggests a mobile phase composed by a mixture of a concentrate of lithium borate–gluconate (2%), *n*-butanol(2%), acetonitrile (12%) and water (84.6%). Injecting a mixture of the anions, it could be observed that a perfect separation between nitrate and phosphate could not be obtained. Among the variables that affect that separation, the quantity of concentrate added had the strongest effect and because of that we changed that proportion to achieve a better separation. As it can be appreciated in Fig. 1 the anion retention times increased as the reagent concentration decreased. The use of concentrations of 1.4% or lower, allowed satisfactory resolution of all the peaks, so this concentration was selected to avoid increasing the duration of the chromatogram. Fig. 2 shows a chromatogram for a mixture of standards, the last big peak belongs to the chromate used for eluting the anions. Retention times were highly reproducible between chromatograms, the RSDs obtained for a mixture of standards in six consecutive runs ranged from 0.51 for nitrate to 1.74 for oxalate.

3.2. Extraction clean-up

Before sample analysis, it was necessary to use a clean-up step because when a 1:10 dilution of honey in water sample was directly injected a very dirty chromatogram was obtained hindering the quantitation of the anions, (see Fig. 3a). To remove those interferences solid-phase extraction on anionic cartridges was selected, then the best conditions to carry out the procedure were chosen, always taking into account the idea of obtaining the highest recoveries. Experiments were always performed in duplicate. Two groups of samples of a multifloral honey used for the whole method development, having the same mass (3 g) were chosen, known amounts of anions (40 mg/l) were added to one group to assess recovery and the other one was used to obtain the chromatogram background.

First of all, the samples must be dissolved in an alkali instead of water to assure the ionization of the analytes, for that purpose LiOH was selected because it is a component of the mobile phase. A concentration of 0.01 *M* gives an adequate pH. To avoid

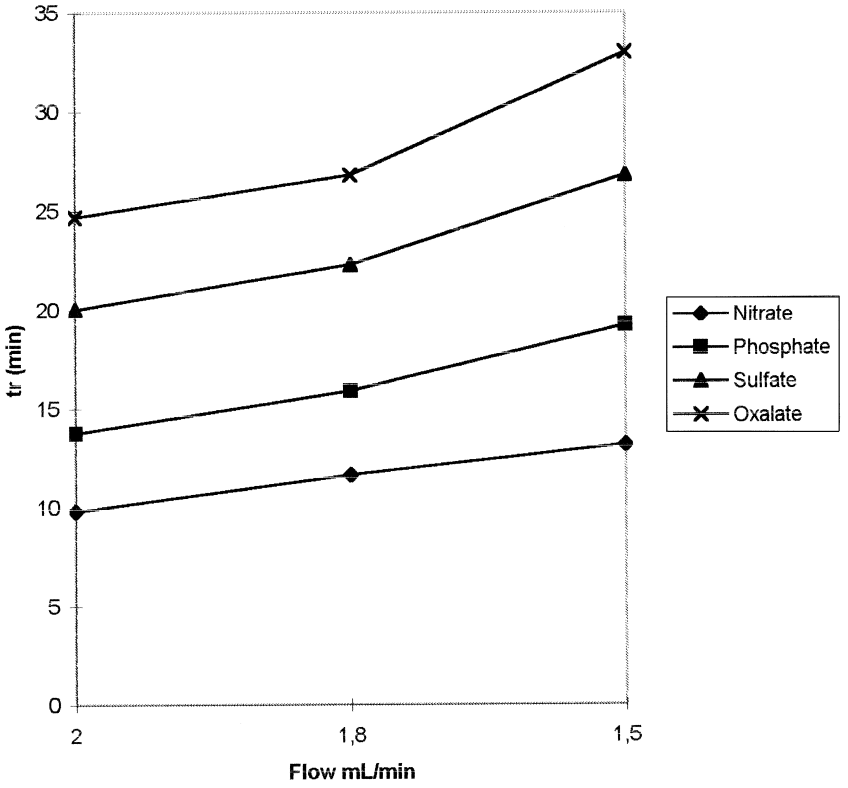

Fig. 1. Influence of the borate–gluconate reagent concentration on the retention times of the anions.

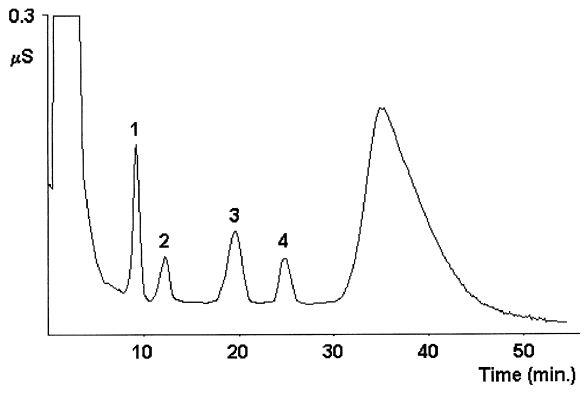

Fig. 2. Chromatogram of a mixture of standards (25 μg/g each). 1: Nitrate; 2: phosphate; 3: sulfate; 4: oxalate. *Mobile phase*: lithium borate–gluconate (1.4%), *n*-butanol (2%), acetonitrile (12%) and water (84.6%) at a flow-rate of 2 ml/min.

a non necessary dilution of the analytes, a dilution of 3 g of honey in 10 ml of 0.01 *M* LiOH was chosen.

The cartridge was activated with 15 ml of deionized water, then the 10 ml of sample dilution were passed through it, and after drying it with nitrogen for 10 min the compounds were eluted. To elute the anions, it was very important to select an anion that was more strongly retained than the analytes, taking into account that the anion should not interfere in a further determination. The chromate ion met those requirements. Assays were made to optimize the concentration and volume to be used.

Three concentrations of chromate were firstly tested: 10^{-3},10^{-2} and 10^{-1} *M*, always using volumes between 2 and 20 ml. When a 10^{-3} solution was used, the oxalate did not elute in any fraction. The 10^{-1} *M* chromate seemed to be much too concentrated because it resulted in a chromatogram with only a peak where the anions were included,

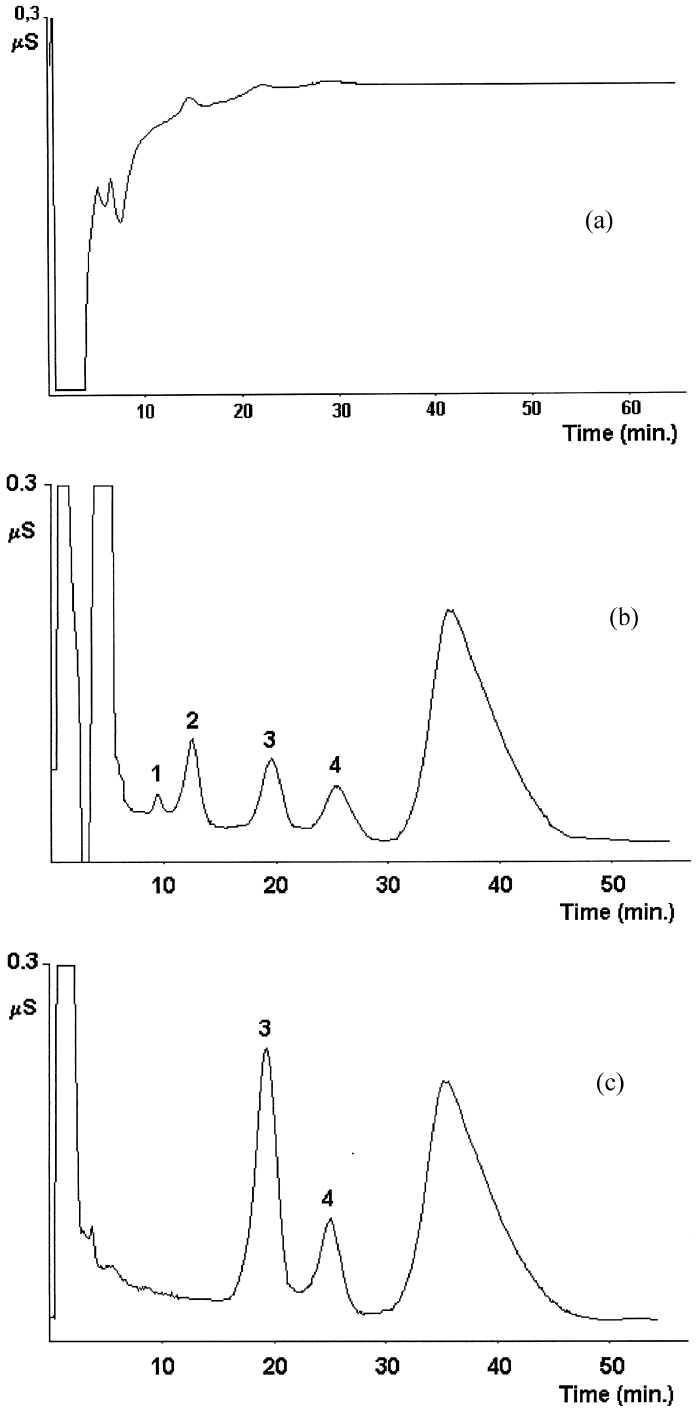

Fig. 3. (a) Chromatogram of a multifloral honey sample diluted 1:10 and directly injected. (b) Chromatogram of the same diluted honey after applying the proposed procedure. (c) Chromatogram of a *Quercus robur* honeydew diluted sample after applying the proposed procedure. *Mobile phase*: lithium borate/gluconate (1.4%), *n*-butanol (2%), acetonitrile (12%) and water (84.6%) at a flow-rate of 2 ml/min.

Table 1
Average recoveries obtained for the anions as a function of the volume total of 0.01 *M* chromate used to elute the anion-exchange cartridges

Chromate (ml)	Recovery (%)			
	Nitrate	Phosphate	Sulfate	Oxalate
2	–	–	–	–
4	–	–	–	–
6	61.45	51.70	55.70	52.10
8	89.20	75.85	88.00	71.47
10	89.20	75.85	88.00	71.47

moreover the baseline was not re-established until 1 h later. Making similar tests with the 10^{-2} *M* chromate, higher recoveries were obtained after passing through the cartridge the first 8 ml. So a concentration of 10^{-2} *M* chromate was selected. To avoid dilution and to achieve higher recoveries, we tried to reduce the volume of the chromate solution eluted and to achieve this the solution was passed in portions of 2 ml, determining in each of them the analytes eluted. In Table 1 it can be observed that in the first two aliquots no anion was eluted, whereas in the fourth one the recoveries were higher than 70%. To avoid those 4 ml, assays were carried out firstly eluting and then rejecting them, then the cartridge was dried for another 10 min and afterwards eluted with another volume of chromate. In Table 2 it can be appreciated that volumes higher than 3 ml did not imply an increase in the recoveries.

As the LiOH used to dissolve the samples could have a negative influence on the extraction process, the concentration was examined with the aim of finding an increase of the recoveries, effectively lower concentrations of hydroxide give higher recoveries, so finally $2 \cdot 10^{-3}$ *M* LiOH was used.

Table 2
Average recoveries obtained for the anions according to the volume of 0.01 *M* chromate passed after eluting and rejecting the first 4 ml

Chromate (ml)	Recovery (%)			
	Nitrate	Phosphate	Sulfate	Oxalate
2	61.45	51.70	55.70	52.10
3	89.20	75.85	88.00	71.47
4	89.20	75.85	88.00	71.47
5	89.20	75.85	88.00	71.47

From the experiences mentioned above the procedure selected was: the cartridge was activated with 15 ml of deionized water, then a dilution of honey (3 g in 10 ml of $2 \cdot 10^{-3}$ *M* LiOH) was passed through the cartridge, after that it was dried by passing N_2 for 10 min, then 4 ml of 10^{-2} *M* chromate were passed; the cartridge was dried again, and finally the anions were eluted with 3 ml of the same chromate solution and injected in the chromatograph. Fig. 3b shows the chromatogram obtained from the same honey as in Fig. 3a after treating it with the mentioned procedure. A chromatogram from a honeydew sample submitted to the same procedure is shown in Fig. 3c. It can be observed that all the interfering peaks have been eliminated completely.

The suitability of the method was examined by applying it to the same honey, spiked at different levels(between 5 and 100 mg/l each anion).The results are summarized in Table 3. As it can be seen typical recoveries ranged from 91% for phosphate to 98% for the sulfate.

It is very important to indicate here that when the method began to be applied to the collected samples we could observe that for the phosphate determination the results obtained by the standard calibration did not always match with the ones obtained by using the standard addition method. The differences between both values were strongly dependent on the honey type, we think that it could be attributed to the different pH and Ca(II) content of the samples, and it was not possible to find an easy solution to this problem; because of that, although the data of phosphate content could be similar to the real ones we preferred not to include it in the method.

Table 3
Average recoveries obtained applying solid-phase extraction to a multifloral honey sample spiked at different levels

Concentration (ppm)	Recovery (%)			
	Nitrate	Phosphate	Sulfate	Oxalate
5	95.62	93.36	96.51	91.54
25	92.80	92.15	98.29	89.65
50	93.75	91.86	93.54	96.92
100	96.20	94.53	99.98	93.50
Average	94.59	92.98	97.08	92.90
RSD (%)	1.68	1.32	2.84	3.34

3.3. Validation of the method

3.3.1. Calibration

To carry out the calibration the standard addition methodology [11] was used. The sets of data obtained in two calibration experiments, one with standard solutions (namely: standard calibration, SC), and the another one with standard additions (AC) were used for each anion. The accuracy of the analytical results was tested by comparing both the analytes content in the different calibrations and the recoveries, calculated by dividing the net content found by that added for each addition.

The SC experiment was run with triplicate injections of the standard solutions, and from the responses versus concentrations, the linearity ranges were deduced.

Only one injection of each solution was made in the other calibration procedure. The numerical values of the parameters of these calibrations are shown in Table 4.

The results from SC and AC are not significantly different, so the method is accurate. The average recoveries from the AC are shown in Table 5. This confirms the accuracy of the method. The conclusion obtained is that the determination of the three anions in honeys and honeydews can be carried out directly by the SC method. The analytical characteristics of the proposed SC method are summarized in Table 6. The detection and determination limits were calculated experimentally as a signal-to-noise ratio of 3 and 10, respectively.

Table 5
Results of recovery assays (from the standard-addition calibration) to test accuracy

	Sample[b]	Recovery (%)	Recovery (average)
Nitrate[a]			
5	5	93.42	94.83
10	5	94.28	
15	5	96.80	
Sulfate[a]			
10	5	97.25	97.20
20	5	96.32	
40	5	98.02	
Oxalate[a]			
10	5	92.25	92.72
20	5	93.17	
40	5	92.74	

[a] In μg/ml.
[b] In g/(10 ml).

3.3.2. Selectivity

As it can be observed in the chromatograms of the samples treated according to the proposed procedure (Fig. 3b and c) it is possible to determine oxalate, sulfate and nitrate in the samples without interference from the matrix. It is also possible to detect phosphate and have an approximate idea about its concentration.

Table 4
Features of the proposed HPLC method for the determination of nitrate, sulfate and oxalate

	r^2(%)	s_{xy}	S_a	s_p	Calibration curve
Nitrate					
SC[a]	99.92	56.26	$9.85 \cdot 10^2$	17.10	$y = 2.76 \cdot 10^3 + 6.24 \cdot 10^2 x$[a]
AC[a]	99.89	25.23	$8.69 \cdot 10^2$	30.59	$y = 3.12 \cdot 10^3 + 6.13 \cdot 10^2 x$
Sulfate					
SC	99.91	83.97	$2.11 \cdot 10^2$	18.2	$y = 5.97 \cdot 10^3 + 8.68 \cdot 10^2 x$
AC	99.86	81.34	$1.79 \cdot 10^2$	26.01	$y = 4.70 \cdot 10^3 + 8.98 \cdot 10^2 x$
Oxalate					
SC	99.98	50.89	$2.83 \cdot 10^2$	4.93	$y = 1.60 \cdot 10^3 + 6.14 \cdot 10^2 x$
AC	99.94	8.33	$2.04 \cdot 10^2$	8.94	$y = 2.44 \cdot 10^3 + 5.95 \cdot 10^2 x$

[a] SC: Standard calibration; AC: Standard addition calibration; y: Conductivity (peak area); x: Concentration in μg ml^{-1}.

Table 6
Characteristics of the analytical method derived from the standard calibration data set

	Nitrate	Sulfate	Oxalate
Precision (RSD,%)	1.76[b]	1.15[c]	0.87[c]
Detection limit[a]	0.122	1.798	1.547
Determination limit[a]	0.408	5.996	5.158
Linearity range[a]	0.4–60	6.0–120	5.0–60
Linearity (%)	99.96	99.95	99.99

[a] In μg/g
[b] At 7.5 μg/g
[c] At 30 μg/g

3.3.3. Reproducibility

The method was applied, under the optimal working conditions, to 10 aliquots of the same honey and injected five times. The results obtained are shown in Table 7. As it can be appreciated from the results obtained for RSD the proposed method is reproducible.

3.4. Analysis of honey samples

3.4.1. Oxalate content during the treatment with oxalic acid for varroosis control.

Five beehives given multifloral honeys and placed in the same area were subjected to the same treatment with the oxalic acid solution, treated once a week for four weeks. Honey samples were collected 24 h before each dosage and 24 h afterwards, and moreover seven days and 14 days after the last dosage. Only the oxalate content was determined because the aim was to know if the initial content changed as a consequence of the treatment.

The results obtained are summarized in Table 8. As it can be observed, it seems that the treatment does not produce important changes on the initial value. The highest values obtained are always found during 24 h after dosage, mainly after the fourth dosage. Those data must be confirmed with a lot of experience because the RSDs associated with those changes are elevated corresponding to field assays (very different from Lab assays).

Table 8
Average values obtained for oxalate in the honey samples collected during treatment of five beehives with oxalic acid. In parentheses the highest and lowest values found are given

Sampling	Oxalate content (μg/g)
24 h before treatment	36.62 (20.49–53.84)
24 h after 1st treatment	48.79 (36.20–70.98)
24 h before 2nd treatment	40.80 (27.01–57.33)
24 h after 2nd treatment	46.06 (26.82–62.82)
24 h before 3rd treatment	38.28 (17.76–52.07)
24 h after 3rd treatment	47.39 (35.69–66.28)
24 h before 4th treatment	44.44 (27.92–61.14)
24 h after 4th treatment	55.07 (42.93–56.15)
7 days after 4th treatment	43.62 (28.20–64.58)
14 days after 4th treatment	44.67 (26.39–63.69)

3.4.2. Determination of the anions in honey and honeydew samples.

As it has been indicated, 99 honey and honeydew samples were collected during three years in a Province whose honeys have a noticeable prestige in National and International trade. The most important in economical terms is the *calluna vulgaris*, there is also a high production of *erica spp, lavandula latifolia, thymus vulgaris and rosmarinus officinalis*

Table 7
Reproducibility of the method: results obtained from 10 different samples of a multifloral honey injected in quintuplicate

	Retention time (min)	Honey (μg/g)
Nitrate	9.62±0.05(RSD=0.51%)	1.06±0.10 (RSD=4.50%)
Sulfate	19.64± 0.22(RSD=0.96%)	13.58±0.44(RSD=3.23%)
Oxalate	24.78±0.23(RSD=0.94%)	23.99±0.31(RSD=4.05%)

honeys, the production of *lavandula stoechas* is lower and those of *hedysarun coronarium* and *satureja montana* are the lowest. The *quercus robur* and *quercus ilex* honeydews and multifloral honeys production are also important. The climate (considering that the years 1996 and 1998 were especially dry) is another cause that justifies that the number of samples of each origin was different. The number that appears in Table 9 beside the name corresponds to the samples analyzed of that origin. In Table 9, the average and the minimum and maximum value found within each origin are shown; logically for those types with scarce number of samples (cantueso, winter savory, acacia, esparceta, evergreen oak), only the mean is indicated and must be used only as a reference. For those samples in which the compounds are under the detection limit, values are not given.

3.4.2.1. Multifloral honeys

Taking into account the vegetation characteristics of the Province is practically impossible to get conclusions about this type. They are honeys from apiarists that make only an extraction by year, usually at the end of summer, so they include all the blooms that appear in the place where the beehives are located. This undoubted origin, frequently associated with the existence of oak and evergreen oak, makes possible the presence of honeydews. That would be confirmed by the fact that those honeys collected in spring have lower values than the ones collected in autumn, which agrees with the idea that the presence of sulfate is proportional to the honeydew quantity in honey.

3.4.2.2. Lavandula latifolia

The bloom of lavender is usually between July and September coinciding with the blooms of winter savory, sunflower and evergreen oak, which could explain the great dispersion between the values found.

3.4.2.3. Calluna vulgaris

This honey is very different from the other ones, their physical characteristics make necessary the use of special extraction devices, honey flows only when

Table 9
Mean average and interval (in parenthesis) values, found in the samples analyzed

Botanical origin[a]	Nitrate (μg/g)	Oxalate (μg/g)	Sulfate (μg/g)
Multifloral [27]	1.5 (1.1 –2.4)	19 (16–36)	76 (45–147)
Lavender [18]	2.6 (1.4–7.3)	13 (9–32)	65 (28–123)
Lavandula latifolia			
Biercol [17]	–	73 (22–155)	67 (53–150)
Calluna vulgaris			
Heather [10]	–	71 (48–151)	63(25–129)
Erica spp.			
Thyme [6]	1.3(1.2–1.4)	27 (12–60)	75 (42–102)
Thymus vulgaris			
Rosemary [6]	2.5(2.0–3.4)	14 (7–24)	30 (20–73)
Rosmarinus officinalis			
Cantueso [3]	0.5	10	25
Lavandula stoechas			
Winter savory [2]	1.9	43	211
Satureja montana			
Acacia [1]	–	–	48
Acacia mill			
Esparceta [2]	–	33	40
Onobrycis sativa lam			
Oak [8]	–	21(8–55)	154 (103–206)
Quercus robur			
Evergreen oak [2]	–	23	173
Quercus ilex			

[a] The data beside botanical origin, in brackets, correspond to the number of samples analyzed of each.

it is mechanically shaken. The coincidence in blooming with oak honeydew production is possible, nevertheless as the extraction process is very different, the other honeys flow easily and are extracted before the calluna one that needs to be chipped off and then extracted, because of that it could be deduced that this unifloral honey has sulfates.

3.4.2.4. Erica spp.

The production of heather honey can be assimilated in relation to nectar sources and honeydew presence to the calluna one.

3.4.2.5. Thymus vulgaris

This honey is collected in June, samples are usually collected from areas where there are frequently evergreen oaks, although according to the season when bees collect nectar, it is not probable that honeydew can appear. Nevertheless only one sample has sulfates under the detection limit, and curiously that sample corresponds to an area with scarce vegetation, practically there are no trees.

3.4.2.6. Rosmarinus officinalis

This honey as the biercol one is the one that has least possibilities of contamination by honeydew because the bloom happens in February–April. Except a sample with null content, the other ones always have appreciable quantities of sulfate.

3.4.2.7. Quercus robur

This honeydew, as the evergreen oak ones, has its origin in the slime flux collected by the bees from the rotten acorns or under some stress conditions: hail stones, dryness. They have the highest values for sulfates, with variations that must be related mainly to climate changes.

All the comments made have in common the fact of treating with a natural product, influenced by a lot of factors: those related to the bees, to the local flora, to the climate, type and managing of the beehive, etc., which we think do not permit categorical conclusions such as: only the honeydew have sulfates, because the enormous variability of the samples makes it not possible.

4. Conclusions

The method developed allows the quantitation of oxalate, sulfate and nitrate in honey and honeydew samples, and gives an idea of the approximate content of phosphate. Applying it to several samples of different botanical origin it has been observed that, except in some special occasions; all the samples have sulfates.

Natural honeys which are considered as unifloral ones, accomplish the commercial, pollen and organoleptic requirements, have sulfates. In general spring honeys (rosemary, thyme and multiflora) have lower sulfate values than those collected in autumn (lavender, biercol, heather and multiflora)

The content in oxalate seems to be not influenced by the therapeutical treatment of the beehives with oxalic acid.

Acknowledgements

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