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On-line microdialysis–ion chromatographic determination of inorganic anions in olive-oil mill wastewater

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Abstract

A fully automated method is presented for the determination of inorganic anions in olive-oil mill effluents using on-line dialysis–ion chromatography. The wastewater is first of all sonicated at room temperature to make it homogeneous, then diluted and microdialyzed. Most of the organic load of the effluents is removed in a few minutes without using reagents, while soluble anion quantitation remains unaffected. The clear solution is analyzed for the inorganic anions content by direct injection on to an ion chromatograph equipped with a conductivity detector. In the absence of standards, the separation efficiency of microdialysis has been investigated by spiking wastewater samples as well as standard oil emulsions with varying amounts of inorganic anions and subjecting them to microdialysis for different periods of time prior to performing instrumental analysis. Excellent spike recoveries and low relative standard deviations are obtained for all the anions if a 10 min microdialysis time is overcome. Chloride, nitrite, nitrate, phosphate and sulphate are not affected by the microdialysis procedure and their recovery is between 96 and 104% in wastewater as well as in standard oil emulsion. Calibration plots are linear over about two orders of magnitude. The dialysis membrane has been replaced after more than 100 analyses. The UV photolysis pre-treatment of the same sample evidences the different information that can be obtained by the two sample pre-treatment procedures. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Sample handling; Olive oil; Water analysis; Environmental analysis; Microdialysis; Inorganic anions

1. Introduction

The extraction of olive oil results in large amounts of wastewater (COD 50 000–200 000 ppm), which constitute one of the most relevant agricultural pollutants in the olive belt of the Mediterranean area. It can be considered that the amount of resulting olive-oil mill wastewater is about 40% (w/w) of the pressed olives. The reflux has an average composition of 80–85% of water, 14–18% of organics

and 1.5–2% of minerals; its density is around 1.15 g ml⁻¹ [1–3]. The chemical composition is highly changeable and somewhat unknown, because it depends on a lot of parameters such as olive ripening and storage, oil extraction technology or waste storage [4–6]. Most important organics are sugars (2–5%), proteins (1.5–2.5%), polyols (1–2%), fats (0.05–1.5%), pectins and gums (0.5–1.5%) and tannins, glucosides and polyphenols (0.5–1.5%). The polyphenolic content gives strong antimicrobial properties to the waste and so prevents its prompt decomposition.

Multielement control of micro and macro constituents in complex matrices has traditionally been a

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challenging analytical task. Even if ion chromatography plays a predominant role in modern inorganic anion analysis [7] for its high separation power of the analytes, this is not sufficient when complex and variable matrices must be analyzed. In the presence of high-molecular-mass organic compounds and particulate matter that can interfere with the analysis, a suitable sample preparation procedure is required to remove them prior to injection. Actually, off-line methods such as digestion, chelation, precipitation or liquid–liquid extraction are most frequently used. They are somewhat laborious, time consuming and prone to error such as loss of volatile species during ashing, contamination in the course of digestion or chelation processes or non-reproducible recoveries when extractions are involved. Attempts to separate soluble anions from olive-oil mill wastewater by ion-exchange or to remove the oily fraction by solid-phase or solvent extractions were not completely satisfactory and erratic results were observed.

Recently, when dealing with matrices that are complex and variable in composition, sample preparation with techniques such as microdialysis has been carefully considered [8] not only in biological matrices [9,10] but also in food analysis [11,12]. Microdialysis sample preparation is a rapid, gentle and non-degrading procedure that can be used on-line with several techniques for multicomponent analysis [13].

The dialysis module usually consists of two Plexiglass blocks with a porous membrane in between to separate the donor (sample) phase from the acceptor phase. The membrane must have an appropriate molecular mass cut-off (defined as the molecular mass that is 90% rejected by the membrane [14]) in order to optimize both selectivity and speed of the process. Because dialysis is based on molecular diffusion occurring as a result of the concentration gradient of the analyte of interest between donor and acceptor solutions, if relatively large sample volumes are available, the process can be further accelerated by using a continuously flowing donor stream. Between the four combinations generally recommended for donor–acceptor mode of operation (stagnant–stagnant, stagnant–flowing, pulsed–flowing and flowing–flowing) when using a flowing sample solution and resting acceptor solution, 100% of the

sample concentration is achieved in the acceptor solution (equilibrium dialysis).

Samples are brought in a nearly homogeneous form, diluted and dialysis is performed to remove high-molecular-mass organic compounds and particulate matter. After dialysis, the acceptor solution is directly injected into the chromatographic system and analysis takes place. As the time required for dialysis is of the same order of magnitude as the chromatogram running time, the sample preparation step does not prolong the analysis. The dialysis of the sample can be carried out while the chromatogram of the previous sample is being recorded. Since the set-up is fully automated, analyses can be run unattended.

Even if adopting the same analytical procedure, the use of different sample pre-treatment procedures, such as on-line olive-oil mill wastewater dialysis and its off-line oxidative UV photolysis [15], evidences that additional information can be obtained.

The aim of the present work is to present a simple and accurate procedure for on-line olive-oil wastewater dialysis directly followed by the ion chromatographic analysis of soluble chloride, nitrate, phosphate and sulphate with conductimetric detection.

2. Experimental

2.1. Instrumentation

Wastewater samples were subjected to microdialysis in a unit (Metrohm, Herisau, Switzerland, model 754) for on-line sample preparation permitting the use of automatic sample dialysis directly before the sample injection into the ion chromatographic apparatus. It consists of a dual-channel peristaltic pump for conveying the sample and acceptor solutions, and the actual dialysis cell in which the ions from the flowing sample solution are enriched in the resting acceptor solution and then directly injected into the ion chromatographic system. Microdialysis conditions are summarized in Table 1. Prior to use, the microdialysis membrane is prepared according to the manufacturer's instructions. A cellulose triacetate membrane has been preferred due to its high hydrophilicity and its very low non-specific binding joined

Table 1
Microdialysis conditions

Membrane	Material	Cellulose triacetate
	Diameter	47 mm
	Thickness	115 μm
	Nominal pore size	0.2 μm
	Molecular mass cut-off (MMCO)	about 100 000
	pH range	4–8
Dialysis	Volume	240 μl
	Time	10 min
Sample Acceptor	Flow rate	0.8 ml min^{-1}
	Solution	Ultrapure water
	Flow rate	0.3 ml min^{-1}
	Transfer time ^a	0.5 min

^a From dialysis cell to the ion chromatographic sample loop.

to a high recovery of the filtrate solution. The dialysis membrane has been replaced after more than 100 analyses. All manipulations were made at room temperature ($25 \pm 1^\circ\text{C}$).

Fig. 1 shows an outline of the apparatus operations during the dialysis steps and the injection of the dialysate on the chromatographic column.

Dialysate samples were on-line analyzed by ion chromatography with conductimetric detection on an ion chromatographic modular system (Metrohm) which included a 733 separation center fitted with a MSM suppressor module, a 709 high-performance double-piston pump, a 732 thermostatted conductivity detector and a 766 autosampler. All measurements were made isocratically at room temperature ($25 \pm 1^\circ\text{C}$). In all cases, on-line sample microdialysis and injection was done at least in triplicate. Chromatographic conditions are summarized in Table 2. Data manipulation and the operation of all the components in the system were controlled by IC Metrodata chromatographic software interfaced via a 714 chromatography signal interface (Metrohm) to a Pentium-based computer (Olidata, Cesena, Italy). Analyte concentration was determined by comparing peak area to a calibration curve constructed for each inorganic anion.

In addition, wastewater samples and oil synthetical emulsions were saponified, as previously reported [15], in quartz test tubes fitted with PTFE stoppers that were then subjected to UV photolysis in a UV digester (Metrohm, model 705) equipped with a 500

W high pressure mercury lamp. The temperature of the sample was maintained at $85 \pm 5^\circ\text{C}$ with the help of a combined air–water cooling system.

2.2. Reagents and standards

Sodium carbonate and sodium hydrogen carbonate were chromatographic grade (Nova Chimica, Milan, Italy). Hydrogen peroxide (30%, m/m, without inorganic stabilizers) was Erbatron electronic grade and sulfuric acid (96%), nitric acid (69.5%) and ethanol (95°) were ACS grade reagents (Carlo Erba Reagenti, Milan, Italy). Potassium hydroxide monohydrate was Suprapur reagent and oil for synthetical emulsions was standard oil without anionic and cationic impurities (E. Merck, Darmstadt, Germany). Ultrapure water with conductivity $< 0.1 \mu\text{S}$ was obtained from a Milli-Q (Millipore, Bedford, MA, USA) deionization system.

Working standards were prepared daily by properly diluting ion chromatographic single anion standards (1.000 g l^{-1}) (Spex CertiPrep, Metuchen, NJ, USA).

Quartz test tubes and all glassware were cleaned by refluxing in hot and concentrated nitric acid, then carefully washed with deionized water and finally dried with filtered air in a clean atmosphere. Details of cleaning procedures and apparatus are reported in the standard texts. Normal precautions for trace analysis were observed throughout. Manipulations were done on a laminar-flow clean bench to avoid fortuitous pollution.

2.3. Procedure

Wastewater samples were sonicated at room temperature for 10 min to make them homogeneous. A 1.0 ml sample aliquot is diluted to 50.0 ml with ultrapure water and microdialyzed at the conditions reported in Table 2. After 10 min the acceptor solution is on-line transferred to the ion chromatographic column and analysis takes place.

In addition, to a 1.0 ml aliquot of the same diluted sample solution 2 ml of ethanol (95°), 2 ml of ultrapure water and 0.5 g of $\text{KOH} \cdot \text{H}_2\text{O}$ are added; the mixture is saponified and then subjected to oxidative UV photolysis, as previously reported [15].

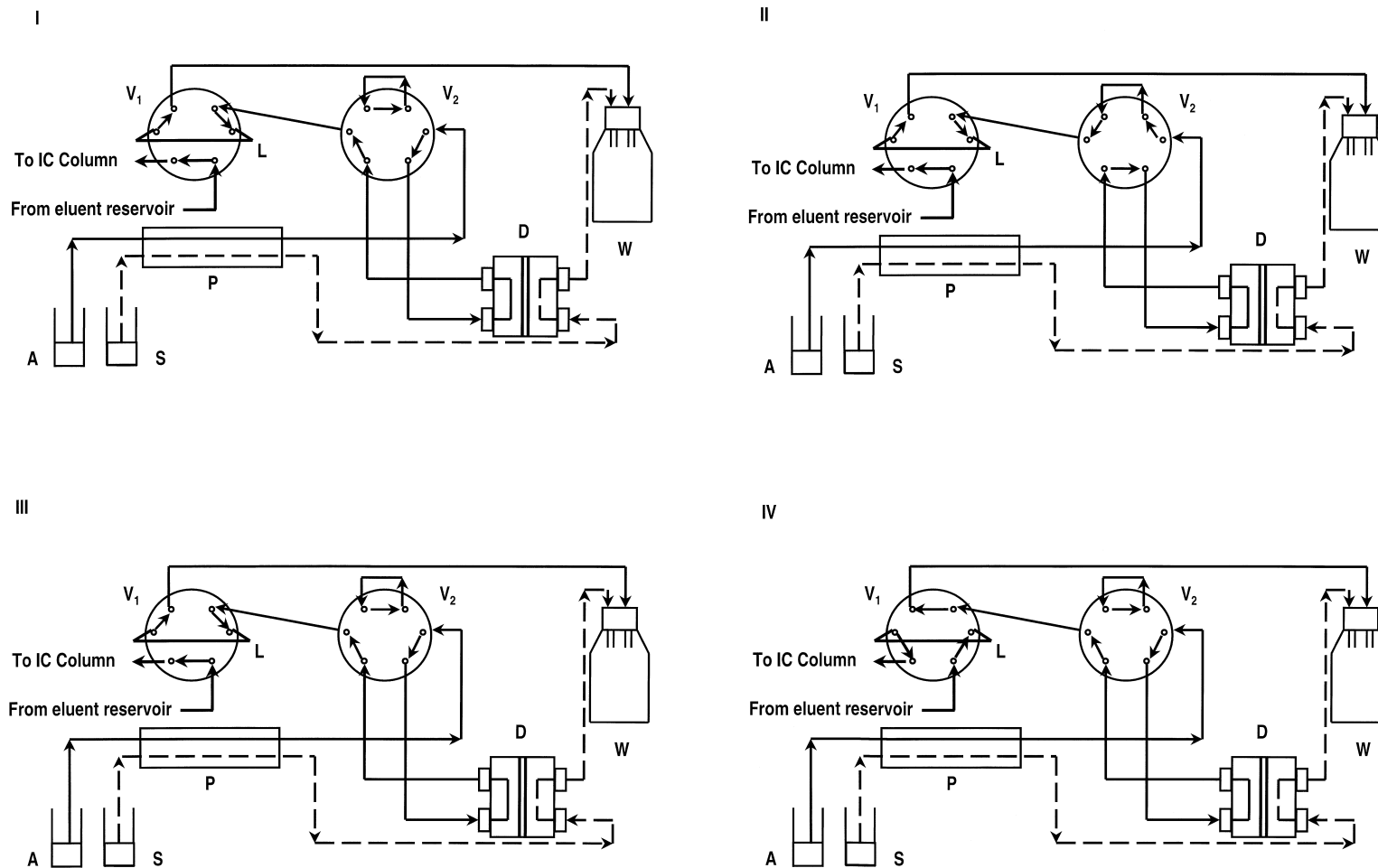


Fig. 1. An outline of the apparatus operations during the dialysis steps. [(I) – Rinsing stage, (II) – Dialysis (stopped flow), (III) – Dialysate transfer to injection loop] and the injection of the dialysate on the chromatographic column (IV). V₁ and V₂: valves; D: dialysis cell; P: pump; A: acceptor solution; S: sample solution; W: waste tank and L: injection loop [20 μ l, polyether ether ketone (PEEK)].

Table 2
Ion chromatographic conditions

Column	Metrosep Anion Dual 2
Eluent	1.3 mM Na ₂ CO ₃ 2.0 mM NaHCO ₃
Eluent flow-rate	0.8 ml min ⁻¹
Injection volume	20 µl
Detection	Suppressed conductivity
Suppressor regenerant solution	20 mM H ₂ SO ₄
Regenerant solution flow-rate	0.5 ml min ⁻¹

3. Results and discussion

The dialysis time that is necessary for the complete recovery of the analytes is a function of the sample flow-rate, the acceptor solution being quiet. The sample flow seems not only to have the obvious role of removing the layers depleted from the diffusion of the components through the membrane, but also to dissipate the polarization layer created by the solutes, driven by the flow but rejected by the

membrane, that pile up against the membrane surface.

Detailed experiments on the recovery of the anions under investigation have been performed by adding known aliquots of suitable standards both to real wastewater samples and to matrix matching emulsions of standard oil (without anionic and cationic impurities), made with 80–85% of ultrapure water, and varying the sample flow-rate from 0.3 to 1.0 ml min⁻¹. The pH of the solutions submitted to dialysis has been varied in the range from 4.0 to 6.5, but no dependence on pH has been observed.

When analyzing wastewater samples, fouling of the membrane has been observed after tenths of runs, but clogging occurs only after more than one hundred and a half cycles.

Data summarized in Table 3 evidence that the proposed pre-treatment removes any long-chain compound present in olive-oil mill wastewater and it is highly suitable for the on-line quantitation of chloride, nitrite, nitrate, phosphate and sulphate ions.

Table 3
Recovery of inorganic species in olive-oil mill wastewater at different dialysis times

Anion	Added standard (µg l ⁻¹)	Sample flow-rate (ml min ⁻¹)	Recovery (%) <i>s_r</i> ^a after						
			3 min	7 min	10 min	15 min	20 min	25 min	30 min
Cl ⁻	25	0.3	14.5±0.03	27.3±0.02	37.8±0.02	48.3±0.02	55.6±0.02	65.5±0.02	70.2±0.02
NO ₂ ⁻	25	0.3	16.0±0.03	28.1±0.03	38.2±0.02	45.7±0.02	55.3±0.02	61.8±0.02	69.8±0.02
NO ₃ ⁻	25	0.3	13.7±0.04	27.3±0.03	39.6±0.03	48.5±0.03	58.3±0.02	65.1±0.02	72.3±0.02
PO ₄ ³⁻	50	0.3	12.3±0.05	24.7±0.04	34.1±0.04	46.2±0.03	55.9±0.03	64.4±0.03	70.5±0.03
SO ₄ ²⁻	50	0.3	14.1±0.04	26.0±0.03	35.6±0.02	46.3±0.02	55.4±0.02	62.1±0.02	69.7±0.02
Cl ⁻	25	0.5	40.3±0.02	62.9±0.02	78.1±0.02	86.7±0.02	101.7±0.02	101.0±0.02	99.2±0.02
NO ₂ ⁻	25	0.5	37.8±0.03	61.9±0.02	75.3±0.02	85.2±0.02	97.1±0.02	99.0±0.02	97.5±0.02
NO ₃ ⁻	25	0.5	39.7±0.03	62.3±0.02	79.8±0.02	90.3±0.02	98.4±0.02	100.0±0.02	99.7±0.02
PO ₄ ³⁻	50	0.5	36.2±0.04	60.0±0.03	74.7±0.03	84.3±0.03	98.5±0.03	102.0±0.03	99.4±0.03
SO ₄ ²⁻	50	0.5	37.9±0.03	64.1±0.03	79.0±0.02	88.1±0.02	99.3±0.02	100.1±0.02	99.2±0.02
Cl ⁻	25	0.8	75.0±0.02	87.3±0.02	100.0±0.02	100.9±0.02	99.4±0.02	102.6±0.02	99.5±0.02
NO ₂ ⁻	25	0.8	73.3±0.03	86.4±0.02	101.0±0.02	98.4±0.02	100.8±0.02	99.1±0.02	96.5±0.02
NO ₃ ⁻	25	0.8	77.7±0.03	88.1±0.02	102.0±0.02	100.5±0.02	99.3±0.02	99.4±0.02	98.6±0.02
PO ₄ ³⁻	50	0.8	76.4±0.03	85.8±0.03	99.1±0.03	101.6±0.03	100.0±0.03	100.8±0.03	99.4±0.03
SO ₄ ²⁻	50	0.8	79.5±0.02	88.2±0.02	100.0±0.02	99.8±0.02	97.3±0.02	100.4±0.02	98.5±0.02
Cl ⁻	25	1.0	75.4±0.02	87.1±0.02	101.8±0.02	99.3±0.02	98.8±0.02	100.0±0.02	98.5±0.02
NO ₂ ⁻	25	1.0	73.2±0.03	86.4±0.02	98.8±0.02	100.0±0.02	100.7±0.02	100.1±0.02	97.5±0.02
NO ₃ ⁻	25	1.0	78.0±0.02	86.5±0.02	100.6±0.02	100.3±0.02	101.7±0.02	100.0±0.02	96.5±0.02
PO ₄ ³⁻	50	1.0	76.1±0.04	83.7±0.03	101.6±0.03	99.3±0.03	100.0±0.03	97.5±0.03	98.4±0.03
SO ₄ ²⁻	50	1.0	75.9±0.03	86.3±0.02	101.7±0.02	100.0±0.02	98.4±0.02	98.9±0.02	96.5±0.02

^a Mean value and its relative standard deviation as calculated on ten original waste samples; for ten synthetic emulsions of standard oil, no appreciable differences were found. Chromatographic conditions as reported in Table 2.

The recovery time results independent from the analyte type, probably due to the high cut-off of the selected membrane. At a 0.8 ml min^{-1} flow-rate, when overcoming a 10 min pre-treatment, analyte concentration is not affected by the microdialysis process and its recovery is between 96 and 104% in any case. When overcoming both time and flow-rate, the confidence intervals are not increased.

In the last, the ionic strength of the olive-oil mill wastewater is usually high and slight variations of the medium have proved not to influence recoveries.

In order to obtain additional information, the same standard emulsions as well as olive-oil mill wastewater samples, both unspiked and spiked with varying amounts of chloride, nitrite, nitrate, phosphate and sulphate were subjected to a different sample pre-treatment consisting of 1 h saponification and 2 h UV photolysis, as previously reported [15], prior to performing the same instrumental analysis. The recoveries of chloride, phosphate and sulphate are between 97 and 103%, while those of nitrite and nitrate could not be ascertained, because they give rise to NO^\cdot radicals under UV radiation.

The chromatograms of a typical olive-oil mill

wastewater sample subjected to dialysis and to UV photolysis are shown in Figs. 2 and 3, respectively. It can be observed that phosphate and sulfate peaks are higher after UV photolysis than after dialysis pre-treatment. This behavior evidences that an aliquot of sulfur and phosphorus in real samples is non-dialyzable because it is associated with organic matter, such as phospholipids, nucleotides or lecithins for phosphorus and proteins and enzymes for sulfur.

In Fig. 2 the peak preceding chloride evidences that, when using high-molecular-mass cut-off membranes such as cellulose triacetate, microdialysis succeeds in removing any long-chain compound present in olive-oil mill wastewater, but low-molecular-mass compounds, like $\text{C}_2\text{--C}_5$ acids, cannot be completely separated. The membrane characteristics are usually selected as a balance between the efficiency in the removal of significant interferences and the optimum time for dialysis.

The comparison of the two techniques suggests that they can be usefully matched to obtain additional information. Microdialysis is an on-line sample pre-treatment that evidences soluble analytes only; during this process interferences are minimized

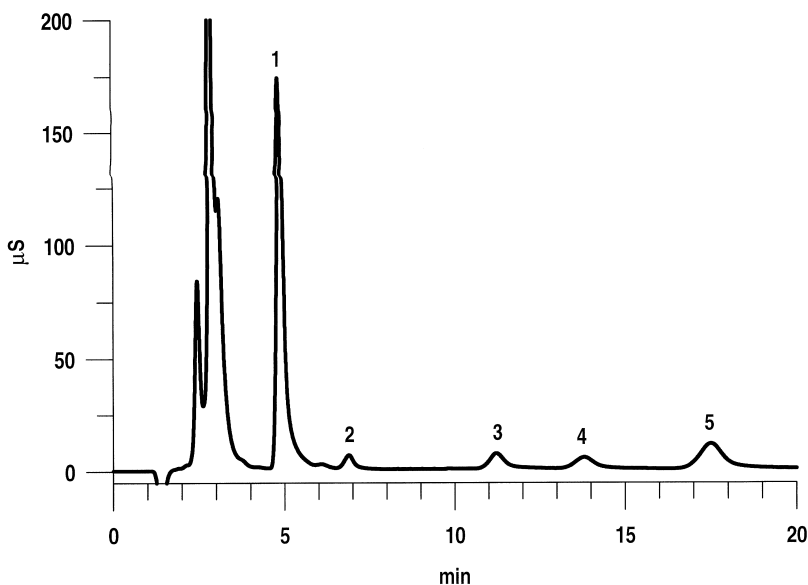


Fig. 2. Isocratic elution of anions in olive-oil mill wastewater after on-line microdialysis as described in the procedure. Peaks: 1= Cl^- 870 mg l^{-1} , 2= NO_2^- 65 mg l^{-1} , 3= NO_3^- 130 mg l^{-1} , 4= PO_4^{3-} 395 mg l^{-1} , 5= SO_4^{2-} 185 mg l^{-1} . Eluent: $1.3 \text{ mM Na}_2\text{CO}_3\text{--}2.0 \text{ mM NaHCO}_3$. Column: Metrosep Anion Dual 2. Flow-rate: 0.8 ml min^{-1} . Detection: suppressed conductivity.

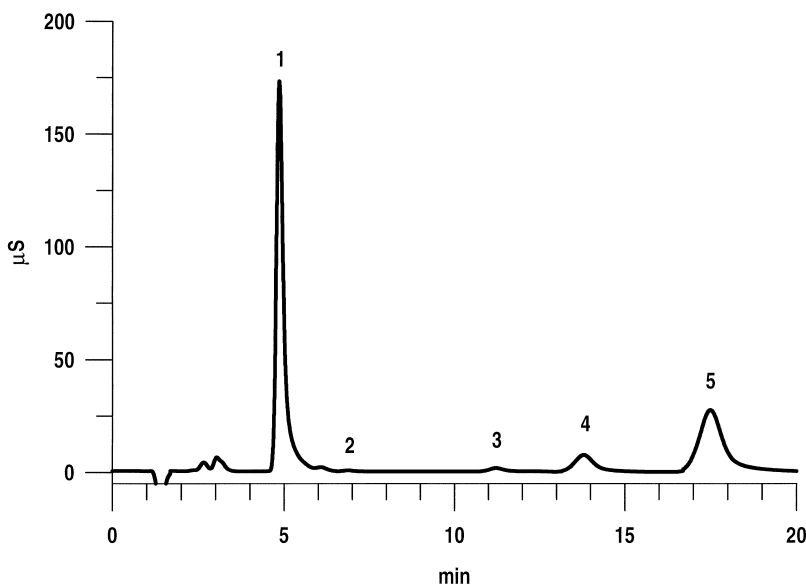


Fig. 3. Isocratic elution of anions in olive-oil mill wastewater after saponification and oxidative UV photolysis as described in Ref. [15]. Peaks: 1= Cl^- 855 mg l^{-1} , 2= NO_2^- 25 mg l^{-1} , 3= NO_3^- 30 mg l^{-1} , 4= PO_4^{3-} 420 mg l^{-1} , 5= SO_4^{2-} 260 mg l^{-1} . Eluent: $1.3 \text{ mM Na}_2\text{CO}_3$ – 2.0 mM NaHCO_3 . Column: Metrosep Anion Dual 2. Flow-rate: 0.8 ml min^{-1} . Detection: suppressed conductivity.

because no reagents are involved and no decomposition products are formed. UV photolysis is an off-line sample pre-treatment intent on organic matrix degradation so that the total amount of the elements are determined; very low blank values can be obtained because minimal reagent addition is required.

Usually the environmental pollution of the olive-oil mill effluents is evaluated by taking into account soluble species, while for their recycling the total amount of the components must be considered.

In Table 4 some results on different origin olive-oil mill wastewater are reported.

The detection limits of chloride, nitrite, nitrate, phosphate and sulphate have been determined by spiking real samples and standard oil emulsions with varying amounts of the analytes, subjecting them to dialysis for a quarter of an hour, and analyzing them by ion chromatography as described in the Experimental section.

Detection limits and concentration ranges, in which calibration curves are linear, with correlation

Table 4
Some results on olive-oil mill wastewater of different origin

Olive-oil mill wastewater geographical origin	Cl^-		NO_2^-		NO_3^-		PO_4^{3-}		SO_4^{2-}	
	mg l^{-1}	s^a	mg l^{-1}	s^a	mg l^{-1}	s^a	mg l^{-1}	s^a	mg l^{-1}	s^a
Puglia A	870	± 10	65	± 2	130	± 2	395	± 12	185	± 4
Puglia B	1150	± 15	185	± 5	270	± 3	605	± 15	423	± 10
Puglia C	733	± 10	77	± 2	121	± 3	432	± 14	362	± 8
Puglia D	626	± 10	60	± 2	113	± 3	371	± 12	207	± 5
Puglia E	437	± 8	45	± 2	75	± 2	298	± 11	142	± 5
Toscana	920	± 10	52	± 2	90	± 2	420	± 15	225	± 6
Romagna	650	± 8	48	± 2	105	± 3	315	± 10	155	± 4

^a $n=10$.

Table 5

Detection limits and linearity range for inorganic anions determined in olive-oil mill wastewater with on-line microdialysis-ion chromatography

Ion	Limit of detection ^a ($\mu\text{g l}^{-1}$)	Linearity range ($\mu\text{g l}^{-1}$)
Cl ⁻	5	10–2000
NO ²⁻	5	10–2000
NO ³⁻	5	10–2000
PO ₄ ³⁻	10	20–1500
SO ₄ ²⁻	10	20–2000

^a The limit of detection is calculated as 3σ +average noise.

coefficients greater than 0.995, are summarized in Table 5. An excess over 1:1000 of sodium, potassium, calcium, magnesium has been proved not to interfere in the determination of any analyte under examination.

4. Conclusions

Microdialysis proved to be a rapid, reliable and accurate on-line sample preparation technique for the determination of inorganic anions in olive-oil mill wastewater without any sample manipulation. The precision of analytical results is excellent, an average of 4% relative standard deviation is obtained.

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