Occurrence of non-steroidal anti-inflammatory drugs in surface waters of Central Italy by liquid chromatography–tandem mass spectrometry

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Non-steroidal anti-inflammatory drugs (NSAIDs) are considered to be the cure-all of any pain: from headache and cold symptoms to toothaches and even labour pain. Their wide use in human medicine has been favoured by the possibility to purchase them without medical prescription, their low cost and by the absence of opioid-like side effects. At the same time, their administration to domestic and food-producing animals has become a common practice to improve their well-being. Therefore, human and veterinary applications are the main sources of NSAIDs in the environment and the major pathways are excretion and discharge through sewage treatment plants. Although their environmental occurrence is well-known, there is a lack of data regarding their levels in surface waters of Central Italy. In this study, a monitoring campaign was arranged in some of the most important rivers and lakes of Central Italy, characterised by a different anthropic impact, in spring–summer and autumn–winter 2012. A broad range of NSAIDs for human and animal use was analysed through a reliable analytical method based on liquid chromatography–tandem mass spectrometry, appropriately developed and validated. Results have shown the constant presence of all drugs commonly used in human medicine with a composition that mirrored the incidence of seasonal diseases quite well. A veterinary drug (flunixin) was found in Bracciano Lake (Rome district), an important tourist attraction surrounded by farmlands. Salicylic acid is a phytohormone and this explains its presence in all the analyzed samples. All the results collected during the extensive survey have proved that Central Italy is aligned with the rest of Europe since its natural waters have shown low levels of contamination (ng L$^{-1}$) but with a chronic input.

Keywords: NSAID occurrence; environmental contamination; NSAID analysis; surface water analysis; LC–MS/MS; emerging contaminants

1. Introduction

A wide range of new or well-known chemicals is produced annually for a variety of purposes and is poured into the environment with unpredictable consequences. In addition to the classical priority pollutants (e.g. pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc.), there are families of chemicals of emerging concern (CECs) [1,2] that increase year by year and include pharmaceuticals, preservatives, bactericides, UV filters, polar plasticisers, flame retardants, illicit drugs, etc. [3,4]. The term ‘emerging contaminants’ refers to both new and well-known compounds that are currently not regulated and not included in routine monitoring programmes. An attempt to give an updated list of these substances is given by the NORMAN network on its website [5]. This working group also distinguishes between

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‘emerging substances’ (CECs whose fate, behaviour and (eco)toxicological effects are still not well understood) and ‘emerging pollutants’ (potential candidates for future regulation, depending on research on their (eco)toxicity and occurrence in the various environmental compartments).

Among the vast array of CECs reaching water supplies, pharmaceutically active compounds (PhACs) have one of the largest inputs into the environment. In the EU alone, about 4000 PhACs are currently in use [6]; their registration and marketing are exempt from the REACH Regulation [7] and, currently, a procedure to establish their possible environmental impact is not required. A prominent position is occupied by NSAIDs, one of the most widely used classes of drugs worldwide. It is well established that more than 30 million people, over the world, use these substances daily for their anti-inflammatory, analgesic and antipyretic properties [8] and that, as early as the 1970s, they were regularly used in veterinary practices [9]. The NORMAN experts have included many of them (acetaminophen (ACF), acetylsalicylic acid (ASA), diclofenac (DCF), ibuprofen (IBP), naproxen (NAP), meclofenamic acid (MCL), etc.) in the list of the ‘emerging substances’.

Large amounts of these painkillers are prescribed in human medical care but, more often, they are sold without prescription as so-called ‘over-the-counter’ drugs. The Italian Agency for Drugs (AIFA) has showed in its annual report that more than 393.7 million of defined daily doses (DDD) of NSAIDs were distributed in Italy in 2012 [10].

Different routes are responsible for the transport and distribution of this class of drugs in different environmental compartments. One of the main sources of contamination is urban wastewaters, because of the human metabolic excretion and disposal of unused or expired medication, through sewage treatment plants (STPs) [11,12]. Practically none of these plants is specifically designed to treat drugs and, hence, a significant portion of these compounds and their metabolites are resistant to the STP inactivation, entering the aquatic environment and reaching groundwater after leaching. Many authors have verified that the elimination of some NSAIDs during wastewater treatment processes is ineffective [13–15] and, as a result, they are found in surface, ground and drinking waters [16–18]. NSAIDs used in farming are directly introduced into the environment after manure application on arable fields and grassland. Their transport to groundwater can be through leaching or runoff from livestock slurries.

Once released into the environment, NSAIDs can be subjected to dilution, sorption to sediments, biodegradation, chemical degradation or photolysis [19]. Nevertheless, some compounds, such as NAP, possess quite long half-lives and remain unchanged several years after their discharge [20,21]. In any case, the characteristic of these contaminants is their continuous input into the aqueous environment rather than their persistence [22].

NSAIDs possess different chemical and clinical profiles, but they essentially exert the same therapeutic properties and are associated with similar adverse effects. Gastrointestinal injuries, which range from dyspepsia to fatal upper gastrointestinal tract bleeding and perforation, are among the most common adverse drug reactions (ADRs) associated with the use of these drugs [23,24]. The acute toxicity of NSAIDs, as well as that of other PhACs, does not occur at environmental concentrations, because levels 100–1000 times higher than those found in the aquatic environment are required to produce an effect [25]. However, these compounds lie in complex mixtures which can have synergistic or antagonistic effects. In humans, the function of NSAIDs is to inhibit the enzymes that catalyse the biosynthesis of prostaglandins (molecules responsible for causing pain and inflammation); in other organisms such as fish, amphibians and birds, the prostaglandins fulfil various functions, including defence mechanisms and reproduction [26]. Therefore, the chronic effects of NSAIDs on man and fauna can be significant due to
the prolonged exposition, even for the entire life cycle. However, so far, only little information has been available on the different effects of these drugs and their metabolites [27].

A future regulation and inclusion in monitoring programmes of NSAIDs, as well as other CECs, depends on both their ecotoxicity and environmental occurrence in the EU Member States; hence, studies in this regard are still needed to facilitate regulatory authorities in their decision-making. Many authors have published research papers and reviews [20,28,29] on this topic. Wastewaters are the most studied branches [30–35], followed by surface [36–40] and drinking waters [41,42], while gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) are the techniques of election. Only a small part of literature has been dedicated to groundwater, especially in the past years [43,44]. Some of the authors take into account both drugs and their metabolites, but the majority of these studies deal with a small number of drugs or with a large number of different CEC classes. The result is that only a few papers are devoted specifically to the NSAIDs. Scarce are updated articles concerning NSAID pollution in specific geographical areas [45–48]. Italy is not an exception and actually pharmaceutical concentration data for surface waters are even rarer.

In this work, a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the trace analysis of 14 NSAIDs with a common use in human and veterinary medicine has been applied to provide an assessment of their concentration in Central Italy surface waters characterised by different anthropic impacts. With this project, we have finalised a program started some years ago [49,50] and carried on with the participation of our research group to the first and second NORMAN network inter-laboratory exercise [51,52].

2. Experimental

2.1. Chemicals

Sigma-Aldrich Company (Milano, Italy) supplied all the drugs under investigation, which are as follows:

- nimesulide (NIM), etodolac (ETO), ACF, ASA, tolfenamic acid (TLF), MCL, salicylic acid (SA), meloxicam (MLX), flunixin (FLX), phenylbutazone (PBZ), IBP, DCF, ketoprofen (KPF), carprofen (CPF), NAP. The two deuterated internal standards (ISs), acetaminophen-d$_3$ and flunixin-d$_3$, were purchased from C/D/N Isotopes INC (Pointe-Claire, Quebec, Canada). Salicylic acid-d$_6$, carprofen-d$_3$, ketoprofen-d$_3$, diclofenac$^{13}$C$_6$, phenylbutazone$^{13}$C$_{12}$, ibuprofen-d$_3$, meloxicam-d$_3$ were purchased from Aldrich-Fluka-Sigma Chemical (Milan, Italy). Etodolac-d$_3$, naproxen-d$_3$, nimesulide-d$_5$, meclofenamic acid-d$_4$ and tolfenamic acid-d$_4$ were synthesised in our laboratories [53]. Purity of all the standards was not less than 98.0%. Individual stock solutions (1 μg μL$^{-1}$) of standards and ISs were prepared by dissolving 10 mg of each drug in 10 mL of methanol, except for flunixin-d$_3$, which was dissolved in chloroform. Working standard solutions used for the optimisation of the chromatographic/mass spectrometric conditions, method developing and sample spiking were prepared by dilution of the stock solutions. Composite working solutions were prepared by mixing the above solutions in methanol to obtain analyte concentrations suitable for the experiments. All the standard solutions were stored at $-18^\circ$C, while drug powders were stored at 4°C. Solutions were found to be stable for at least 6 months when stored in dark and cool conditions. For LC analysis and extraction procedure, water was deionised and purified by a Milli-Q Plus apparatus (Millipore, Bedford, MA, USA). All the solvents used, methanol, acetonitrile, chloroform and acetone, were of HPLC grade and were all purchased from Carlo Erba Reagents (Milano, Italy). Formic acid and dibutylamine (99.5%) were supplied by Sigma-Aldrich Company (Milano, Italy).

Oasis HLB (6 mL, 500 g) from Waters (Milano, Italy) was used to perform solid phase extraction (SPE).
Surface water samples were filtered on 0.45 µm polytetrafluoroethylene filter purchased from Alltech (Deerfield, IL, USA).

2.2. **Sampling and sampling processing**

Surface water samples were gathered from different lakes and rivers in the centre of Italy in two periods of 2012:

(1) from March to July; and
(2) from September to December.

Grab samples were generally taken in the central part of the watercourse, except for the lakes where water was taken near the coasts.

All the samples were collected inside PVC bottles (1 L) and stored in refrigerated boxes (strictly for the time necessary for transport). When in lab, they were stored at 4°C until analysis.

The protocol proposed by Marchese et al. [49,50] has been modified to improve the extraction efficiency for all the analytes. An aliquot of 400 mL of surface water sample was acidified to pH 4, with formic acid, prior to SPE procedure. The achievement of the desired pH was carefully checked with a pH-meter, Compact Titrator (Crison, Barcelona, Spain). A proper volume of the ISs solution was added; adequate spike levels with known amount of the analytes were also performed for recovery studies and calibration curve construction. Thereafter, the SPE operations were performed with a vacuum manifold system Visprep-DL (Supelco, Bellefonte, PA, USA), equipped with a KNF Laboport (Milano, Italy) vacuum pump. After analyte loading, the SPE cartridge was washed with 5 mL of distilled water to remove acidity and then dried for 10 min in vacuum. Analytes were eluted with 5 mL of methanol and 10 mL of acetone in a polypropylene tube with a conical bottom. The eluate was then dried until 500 µL under a stream of nitrogen in a water bath kept at 30°C. Without further treatment, 4% of this solution (20 µL) was injected in the LC–MS/MS system.

2.3. **HPLC–tandem MS method**

Samples were analysed under LC–tandem MS conditions as detailed elsewhere [53]. In brief, the 14 NSAIDs were separated through a reversed-phase ion-pair chromatography on a XTerra-MS C\textsubscript{18} (150 × 4.6 mm I.D.; 5 µm) (Waters, Milano, Italy), using a mixture of acetonitrile:methanol (50:50, v/v) as eluent A and water as eluent B; dibutylamine (0.2 mM) was added to both phases as an ion-pair agent.

Analytes were detected in negative ionisation by a AB Sciex API 3000 triple-quadrupole mass spectrometer (AB Sciex, Canada), equipped with a TurboIonSpray (TIS) source (drying gas temperature at 200°C; capillary voltage at −4500 V). The chromatographic run was divided into two acquisition periods in order to improve instrumental sensitivity for ACF and SA, increasing their corresponding dwell time.

The LC–MS parameters for the identification of the 14 target compounds are summarised in Table S1 (Supplementary Material). LC–MS parameters for labelled ISs are listed in Table S2 (Supplementary Material). Figure 1 shows a representative LC-electrospray ionization (ESI) (-)-Selected Reaction Monitoring (SRM) chromatogram of a surface water extract spiked with the 14 NSAIDs.
Method validation

The method performance was assessed evaluating identification power, selectivity, recovery, precision, linearity, sensitivity, limit of detection (LOD) and limit of quantitation (LOQ). Two SRM transitions were chosen for each target analyte, allowing to earn four identification points (IPs): 1 IP for the precursor ion plus $2 \times 1.5$ IPs for the two product ions [54]. This number of IPs is considered enough to confirm the occurrence of contaminants in real matrices. The most intense SRM transition (quantifier transition) was used to perform the quantitative analysis, while the second most intense SRM transition (qualifier) was used for identification purpose and for method limit evaluation.
Preliminary analyses proved that the water collected from Lake Vico was analyte-free, and hence it was used as a blank matrix for method validation of the 14 NSAIDs. Analyst 1.5.1 was used for acquiring and elaborating LC–MS data. Linear regression, mean and standard deviation (SD) were calculated using a common spreadsheet program. Statistical data treatment was also performed employing Statgraphics Centurion XVI software (Statpoint Technologies Inc., Warrenton, VA, USA).

3. Results and discussion
3.1. Method validation results
Results of the method validation are summarised in Table 1.

Briefly, recoveries ranged between 61% and 100%, while the relative standard deviation (RSD) values were below 20% for both the intra-day precision and the inter-day precision; the inter-day precision was valued over a period of 30 days. LODs and LOQs, calculated at an S/N ratio of 3 and 10, were in the pg L$^{-1}$ range, providing to be suitable to estimate very low NSAID contamination levels of surface waters.

Matrix-matched calibration curves were obtained by spiking blank samples with the analytes and ISs prior to extraction (method of the surrogate for the IS addition); this approach was able to compensate signal losses due to both matrix effect and sample treatment. Linear dynamic range was investigated up to 0.8 mg L$^{-1}$, obtaining determination coefficients ($R^2$) higher than 0.99. The absence of carry-over was assessed by injecting methanol after the calibrator spiked at the highest point of the calibration curve.

Table 1. Method’s validation results: LOD and LOQ, recovery percentage and intra-day precision expressed as relative standard deviations (RSDs).

| NSAIDs | LOD (pg L$^{-1}$) | LOQ (pg L$^{-1}$) | Recovery % (RSD)$^\text{a}$ | Spike level: LOQ$^\text{b}$ | Spike level: 10 × LOQ$^\text{c}$ |
|--------|------------------|------------------|---------------------------|-----------------|------------------|
| ACF    | 30               | 90               | 79 (12)                   | 75 (10)         | 93 (9)           |
| SA     | 0.9              | 2.7              | 97 (7)                    | 98 (8)          | 86 (4)           |
| NAP    | 7.0              | 21               | 94 (8)                    | 88 (5)          | 91 (8)           |
| KPF    | 4.0              | 12               | 91 (8)                    | 89 (8)          | 88 (5)           |
| MLX    | 0.7              | 2.1              | 100 (20)                  | 97 (8)          | 92 (4)           |
| PBZ    | 3.0              | 12               | 61 (12)                   | 87 (7)          | 92 (4)           |
| IBP    | 7.0              | 21               | 87 (7)                    | 70 (6)          | 92 (4)           |
| NIM    | 0.4              | 1.2              | 70 (6)                    | 80 (9)          | 92 (4)           |
| CPF    | 0.6              | 1.8              | 83 (20)                   | 89 (4)          | 90 (15)          |
| FLX    | 0.8              | 2.4              | 83 (20)                   | 92 (4)          | 88 (5)           |
| ETO    | 2.0              | 6.0              | 82 (19)                   | 92 (4)          | 90 (15)          |
| DCF    | 10               | 30               | 100 (20)                  | 93 (6)          | 92 (4)           |
| MCL    | 4.0              | 12               | 82 (19)                   | 86 (5)          | 87 (20)          |
| TLF    | 2.0              | 6.0              | 87 (20)                   | 79 (9)          | 93 (6)           |

Notes: $^\text{1}$ method limits were calculated as an average of six replicates on blank samples. $^\text{2}$ Intra-day precision is represented as the Relative Standard Deviation (RSD) associated with the recoveries of the selected NSAIDs. $^\text{3}$ recoveries, intra and inter-day precision were evaluated at two different spike levels: 1 and 10 times the Limits of Quantitation.
3.2. Surface water monitoring

3.2.1. Geographic contextualisation

Prior to launching the surface water monitoring campaign, climatic factors, sampling points and sampling modes were thoroughly evaluated. First of all, an assessment of the climatic factor has been considered crucial and, hence, the dry period of the spring–summer and the wet one of autumn–winter were chosen for sampling. Moreover, these two portions of the year also take into account possible seasonal diseases such as colds, fever or rheumatic pains. Water samples were taken from naturalistic, farming and urban areas so as to have samples representative of different anthropogenic impacts.

Therefore, between March and December 2012, the following lakes and rivers were monitored:

(1) in the Lazio region: the River Tiber and its branches Farfa, Cremera and Aniene; Bracciano Lake and Cremera stream in the Rome district; Bolsena Lake and Vico Lake in the Viterbo district.

(2) In the Abruzzi region: Vetoio Lake and Aterno River.

River Tiber, the third longest Italian river, rises from the Appennino Tosco-Emiliano and turns southerly, crossing Central Italy for about 405 km. Its basin covers an area of about 17,500 km$^2$ and has approximately 4.7 million inhabitants (2009), with 60% of them living in Rome. Before reaching the capital city, it flows through a natural reserve in the Farfa territory, where it receives the waters of the namesake river. Downstream of the natural reserve, the area surrounding the Tiber is used for agricultural purposes, while the settlements for residential or tertiary activities increase upon approaching the city of Rome. Along this stretch, the river is fed by several tributaries (Cremera is one of this). The Aniene River is one of its major tributaries; it flows through the territory of 17 municipalities before draining into the Tiber at Ponte Salarino. It is notorious for being one of the most polluted rivers of Central Italy.

The three lakes of Bolsena, Bracciano and Vico are all of volcanic origin. Bolsena and Bracciano are popular touristic and bathing resorts, while Vico is a natural reserve with very few homes that overlook the lake. Vetoio is a small lake near L’Aquila city, while River Aterno is one of the most important rivers in the eastern part of Central Italy.

Monitoring of the Tiber (and its branch) was conducted extensively inside the natural reserve and through the city. Sampling points from the north to the south were: Torrita Tiberina, confluence with Farfa River, Farfa River (two points), south part of the natural reserve, confluence with the Aniene, Aniene River (two points), Stadio Olimpico, Trastevere-Ponte Sisto, Ponte Marconi. Maps of these sites are shown in Figure 2(a) and 2(b). Since there are many data collected, a summary of the most representative results is given in Figure 3(a) and 3(b).

3.2.2. The Tiber basin

The vernal monitoring of the Tiber and Aniene revealed the presence of NAP, KPF, NIM and DCF (in trace amounts). The analysis, repeated in autumn, had confirmed the results for SA and NAP; furthermore, showed a significant increase in the concentration of NIM and the additional presence of IBP. In the year 2012 in the Lazio region, 31.5 DDD per 1000 inhabitants per day of NSAIDs were sold. During this year, the individual sale volumes of the detected drugs followed this order: DCF = KPF > NIM > IBP > NAP with DDD (per 1000 inhabitants per day) ranging between approximately 4.3 and 0.9 [10]. While, according to WHO Collaborating Centre for Drug Statistics Methodology [55], in terms of weight, the sales trend becomes
IBP > NIM > KPF > NAP > DCF (for further information, see Table S3 in Supplementary Material). Apparently, our drug residue concentrations both in spring and autumn do not seem to agree with market trend. Some NSAIDs are efficiently photodegraded in surface waters because of the solar radiation exposition; DCF and KPF through photolysis, while IBP through OH radical-mediated photodegradations [56,57]. The large amount of NIM that was measured in autumn was considered as temporary peak, since a sudden concentration decrease was observed during the subsequent samplings carried out on the same sites. Several reasons may exist for this.

Figure 2. Panel (a): author’s graphical representation of the rivers Tiber and Aniene's flow through Rome city. Pictures inside the oval insert are landscapes of Rome nearby the sampling points. Panel (b): satellite image of the natural reserve of Farfa; flags represent the withdrawal sites. All the images are taken from Google Earth.

Figure 3. Most representative data on environmental concentrations of the NSAIDs in Tiber River during vernal and autumnal monitoring (Panel (a)) and in Aniene River in the same periods (Panel (b)). Results are expressed in ng L$^{-1}$.
trend, not least an illegal discharge. This event could also explain the concentration decrease observed between Farfa oasis and Stadio Olimpico.

Several authors who have dealt with the monitoring of specific areas around the world have found a high concentration of ACF [58–60]. Analysis of samples from Central Italy, on the contrary, showed the total absence of this drug. Drawing conclusions, however, is complicated by the absence of sales data issued by the competent organ (AIFA). A possible explanation is that although ACF is a very well-known drug, its administration in Italy is mainly linked to its antipyretic properties.

It is not surprising that all of the most common NSAIDs were found in the segment of the river that flows through the capital city. Instead, the presence of some of them in the samples coming from the natural reserve of Farfa is perplexing.

### 3.2.3. Lakes and rivers in Central Italy

In parallel with the Tiber basin monitoring, grab analysis of Lazio and Abruzzo rivers and lakes was performed. The detailed maps and the sampling points are shown in Figure 4 while results are in Figure 5(a) and 5(b). One or more NSAIDs, among those of concern, were found in all the samples subjected to analysis. Specifically, all the contaminants except flunixin, found in Lake Bracciano, were for human use. Flunixin is a drug for veterinary purpose only and its contamination was probably due to the presence of cattle and horse livestock in this area. Sampling on River Aterno was performed upstream and downstream of the L’Aquila Hospital. Results showed in Figure 5(b), clearly displayed that this health-care centre was a source of drugs at high concentration. These outcomes suggest that urban STPs may not be the only route of entry of NSAIDs into aquatic environments [61].

Only samples coming from Vico Lake and Bolsena Lake were free from the drugs.

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![Figure 4](image.png)

**Figure 4.** Satellite image and geographical distribution of the lakes and rivers chosen to study NSAID contamination in Central Italy. The image is from Google Earth.
3.2.4. *Acetylsalicylic acid*

As is well known [53], the ester bond of ASA is extremely labile and gives rise to SA both in the ESI source and in solution; hence, for this project, the SRM transitions of SA account for both the analytes. As can be seen from all the graphical images, every sample was contaminated by SA. Actually, drawing conclusions about the exact level of pollution of ASA and/or SA has been not possible since they can be released into the environment both as ASA by-product and as SA itself. In fact, SA is normally present in the ecosystem, since the small phenolic molecule plays a key role in plant defence and it is synthesised by plants in response to challenge by a diverse range of phytopathogens [62,63]. As there is no clear and reliable data in literature about the natural level of this compound, it was not feasible to discriminate between endogenous and exogenous SA. However, the results obtained in Stadio Olimpico in spring, in Cremera stream, in Vetoio and Bolsena lakes are slightly above the mean values for this molecule (≈5 ng L\(^{-1}\) mean value calculated on the other samples we analysed).

Finally, in monitoring different classes of PhACs, some authors have underlined that more significant concentrations have been detected in small streams and tributary rivers rather than in large bodies of receiving water (rivers or lakes) because of a not negligible dilution factor [64,65]. Nevertheless, outcomes obtained from River Tiber and its tributaries have not shown a similar trend. Instead, the anthropic factor has seemed to be predominant.

4. Conclusions

NSAIDs are one of the most sold classes of drugs: we all turn to them for every kind of pain or inflammation. Consequently, a considerable amount of these drugs is poured into the environment from many different sources. The problem becomes of public health concern, as well as environmental, starting from the moment these compounds enter into aquifers and foodstuffs [53,66–68]. The method described so far was intended to enable us to draw conclusions about the presence of the 14 selected NSAIDs in the environment. Analytes were selected mostly
between the drugs for human use, but unlike the majority of other authors, we have also included some NSAIDs for veterinary purpose only.

In the presented method, matrix effect variability was obviated through the use of isotopes of almost all compounds as ISs.

Moreover, the full validation of the method and the inter-laboratory exercises, to which it was subjected, allowed to check the reliability and robustness of the entire procedure. Since this method proved to match all the specifications required for an environmental monitoring, it was used to perform analysis of the most important surface waters of Central Italy in which the concentration of NSAIDs is definitely underestimated or unknown. Results have shown that drugs concentration in water samples is not necessarily linked to the sales trend, but probably to an increased use in the coldest and wet seasons. It was also highlighted that urban STPs are not the only source of contamination, but that a significant contribution can also be made by the hospital STPs. The unexpected result of the natural reserve contamination, showed that none of the environmental areas are protected from NSAID pollution.

An improved knowledge of pharmaceutical concentrations in Central Italy waters would enable the determination of potential risks posed to aquatic wildlife and human health in this region. Indeed, it is undeniable that NSAIDs are very important and extremely useful for pain management but, since we risk to absorb them unconsciously, whether NSAIDs are a panacea or a poison is a matter of dose.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Supplemental data**

Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/03067319.2015.1046059.

**References**

[1] K. Fent, A.A. Weston and D. Caminada, Aquatic Toxicol. 76, 122 (2006). doi:10.1016/j.aquatox.2005.09.009.
[2] V. Pérez Fernández, S. Marchese, A. Gentili, M. Ángeles Garcia, R. Curini, F. Caretti and D. Perret, J. Chromatogr. A 1367, 78 (2014). doi:10.1016/j.chroma.2014.09.045.
[3] S.D. Richardson, Anal. Chem. 80, 4373 (2008). doi:10.1021/ac800660d.
[4] S.D. Richardson, Anal. Chem. 82, 4742 (2010). doi:10.1021/ac101102d.
[5] NORMAN Network, Network of reference laboratories, research centers and related organizations for monitoring of emerging environmental substances, 2015. http://www.norman-network.net/.
[6] L. Patrolecco, N. Ademollo, P. Grenni, A. Tolomei, A. Barra Caracciolo and S. Capri, Microchem. J. 107, 165 (2013). doi:10.1016/j.microc.2012.05.035.
[7] EU, Regulation (EC) 1907/2006 of the European Parliament and the European Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, Off. J. Eur. Comm. L 369, 1, (2006) (30 December 2006).
[8] T.A. Ternes, C.G. Daughton and T.L. Jones-Lepp, ACS Symposium Series 791, (American Chemical Society, Washington, DC, 2001), 39.
[9] A. Gentili, TrAC 26, 595 (2007).
[10] The Medicines Utilization Monitoring Centre, National Report on Medicines use in Italy. 2013 (Italian Medicines Agency, Rome, 2014). <www.agenziafarmaco.it>.
[11] C.G. Daughton and T.A. Ternes, Environ. Health Perspect. 107, 907 (1999). doi:10.1289/ehp.9910756907.
[12] T. Heberer, Toxicol. Lett. 131, 5 (2002). doi:10.1016/S0378-4274(02)00441-3.
[13] E. Larsson, S. Al-Hamimi and J.A. Jönsson, Sci. Total Environ. 485–486, 300 (2014). doi:10.1016/j.scitotenv.2014.03.055.

[14] S. Zorita, L. Mårtenssonb and L. Mathiassona, Sci. Total Environ. 407, 2760 (2009). doi:10.1016/j.scitotenv.2008.12.030.

[15] A. Tauxe-Wuersch, L.F. De Alencastro, D. Grandjean and J. Tarradellas, Water Res. 39, 1761 (2005). doi:10.1016/j.watres.2005.03.003.

[16] T.A. Ternes, M. Meisenheimer, D. McDowell, F. Sacher, H.-J. Brauch, B. Haist-Gulde, G. Preuss, U. Wilme and N. Zulei-Seibert, Environ. Sci. Technol. 36, 3855 (2002). doi:10.1021/es015757k.

[17] M. Gros, M. Petrović and D. Barceló, Talanta 70, 678 (2006). doi:10.1016/j.talanta.2006.05.024.

[18] World Health Organization, Pharmaceutical in Drinking Water, (2012). http://apps.who.int/iris/bitstream/10665/44630/1/9789241502085_eng.pdf.

[19] M. Petrovic and D. Barcelo, TrAC 26, 486 (2007).

[20] P. Vazquez-Roig, C. Blasco and Y. Picó, TrAC 50, 65 (2013).

[21] H. Khalafa, L. Salsteb, P. Karlssonb, P. Ivarssonc, J. Jassa and P.-E. Olsson, Sci. Total Environ. 407, 1452 (2009). doi:10.1016/j.scitotenv.2008.10.016.

[22] M. Petrović, S. Gonzalez and D. Barceló, TrAC 22, 685 (2003).

[23] S. Hernandez-Diaz and A. Garcia Rodriguez, Arch. Intern. Med. 160, 2093 (2000).

[24] M. Lapeyre-Mestre, S. Grolleau and J.-L. Montastruc, Fundam. Clin. Pharmacol. 27, 223 (2013). doi:10.1111/fcp.2013.27.issue-2.

[25] M. Farre, S. Perez, L. Kantiani and D. Barcelo, TrAC 27, 991 (2008).

[26] M. Cleuvers, Ecotox. Environ. Safe. 59, 309 (2004). doi:10.1016/S0147-6513(03)00141-6.

[27] M. Cleuvers, Toxicol. Lett. 142, 185 (2003). doi:10.1016/S0378-4274(03)00068-7.

[28] S. Mompelat, B. Le Bot and O. Thomas, Environ. Int. 35, 803 (2009). doi:10.1016/j.envint.2008.10.008.

[29] K. Wille, H.F. De Brabander, E. De Wulf, P. Van Caeter, C.R. Janssen and L. Vanhaecke, TrAC 35, 87 (2012).

[30] V. Osorio, M. Imbert-Bouchard, B. Zonja, J.-L. Abad, S. Pérez and D. Barceló, J. Chromatogr. A 1347, 63 (2012). doi:10.1016/j.chroma.2014.03.076.

[31] V. Manzo, L. Honda, O. Navarro, L. Ascar and P. Richter, Talanta 128, 486 (2014). doi:10.1016/j.talanta.2014.06.027.

[32] A. Sarafraz-Yazdi, A. Amiri, G. Rounaghi and H. Eshtiagh-Hosseini, Anal. Chim. Acta 783, 24 (2013). doi:10.1016/j.aca.2013.04.042.

[33] J.E. Drewes, T. Heberer, T. Rauch and K. Reddersen, Ground Water Monit. Remediat. 23, 64 (2003). doi:10.1111/gwmr.2003.23.issue-2.

[34] J.D. Cahill, E.T. Furlong, M.R. Burkhardt, D. Kolpin and L.G. Anderson, J. Chromatogr. A 1041, 171 (2004). doi:10.1016/j.jchroma.2004.04.005.

[35] R. Rodil, J.B. Quintana, E. Concha-Graña, P. López-Mahía, S. Muniategui-Lorenzo and D. Prada-Rodríguez, Chemosphere 86, 1040 (2012). doi:10.1016/j.chemosphere.2011.11.053.
[47] A.S. Stasinakis, S. Mermigka, V.G. Samaras, E. Farmaki and N.S. Thomaidis, Environ. Sci. Pollut. Res. 19, 1574 (2012). doi:10.1007/s11356-011-0661-7.

[48] R. Loos, G. Locoro and S. Contini, Water Res. 44, 2325 (2010). doi:10.1016/j.watres.2009.12.035.

[49] S. Marchese, D. Perret, A. Gentili, R. Curini and F. Pastorri, Chromatographia 58, 263 (2003).

[50] S. Marchese, A. Gentili, D. Perret, G. D’Ascenzo and F. Pastorri, Rapid Commun. Mass. Spectrom. 17, 879 (2003). doi:10.1002/rcm.998.

[51] M. Farrè, M. Petrovic, M. Gros, T. Kosjek, E. Martinez, E. Heath, P. Osvald, R. Loos, K. Le Menach, H. Budzinski, F. De Alencastro, J. Muller, T. Knepper, G. Fink, T.A. Ternes, E. Zuccato, P. Kormali, O. Gans, R. Rodil, J.B. Quintana, F. Pastorri, A. Gentili and D. Barceló, Talanta 76, 580 (2008). doi:10.1016/j.talanta.2008.03.055.

[52] E. Heath, T. Kosjek, M. Farre, J.B. Quintana, L.F. De Alencastro, S. Castiglioni, O. Gans, K. Langford, R. Loos, J. Radjenović, L. Mainero Rocca, H. Budzinski, D. Tsipi, M. Petrovic and D. Barcelo, Talanta 81, 1189 (2010). doi:10.1016/j.talanta.2010.02.009.

[53] A. Gentili, F. Caretti, S. Bellante, L. Mainero Rocca, R. Curini and A. Venditti, Anal. Bioanal. Chem. 404, 1375 (2012). doi:10.1007/s00216-012-6231-0.

[54] Union European, Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Off. J. Eur. Commun. L221, 8 (2002).

[55] WHO Collaborating Centre for Drug Statistics Methodology Norwegian Institute of Public Health, ATC/DDD Index, 2015. http://www.whocc.no/atc_ddd_index/?code=M01AX&showdescription=no; http://www.whocc.no/atc_ddd_index/?code=M01AE&showdescription=no; http://www.whocc.no/atc_ddd_index/?code=M01AB&showdescription=no.

[56] N.V. Blough and B. Sulzberger, Aquat. Sci. 65, 317 (2003). doi:10.1007/s00027-003-0003-z.

[57] J.L. Packer, J.J. Werner, D.E. Latch, K. McNeill and W.A. Arnold, Aquat. Sci. 65, 342 (2003). doi:10.1007/s00027-003-0671-8.

[58] S. Ortiz De García, G.P. Pinto, P.G. Encina and R.I. Mata, Sci. Total Environ. 444, 451 (2013). doi:10.1016/j.scitotenv.2012.11.057.

[59] A. Lolić, P. Paiga, L.H.M.L.M. Santos, S. Ramos, M. Correia and C. Delerue-Matos, Sci. Total Environ. 508, 240 (2015). doi:10.1016/j.scitotenv.2014.11.097.

[60] A.-Y.-C. Lin and Y.-T. Tsai, Sci. Total Environ. 407, 3793 (2009). doi:10.1016/j.scitotenv.2009.03.009.

[61] H. Chen, X. Li and S. Zhu, Environ. Sci. Pollut. Res. 19, 2381 (2012). doi:10.1007/s11356-012-0750-2.

[62] H. Lu, Plant Signal Behav. 4, 713 (2009). doi:10.4161/psb.4.8.9173.

[63] G. Loake and M. Grant, Curr. Opin. Plant Biol. 10, 466 (2007). doi:10.1016/j.pbi.2007.08.008.

[64] B. Ferreira Da Silva, A. Jelic, R. López-Serna, A.A. Mozeto, M. Petrovic and D. Barceló, Chemosphere 85, 1331 (2011). doi:10.1016/j.chemosphere.2011.07.051.

[65] G.L. Brun, M. Bernier, R. Losier, K. Doe, P. Jakman and H.-B. Lee, Environ. Toxicol. Chem. 25, 2163 (2006).

[66] S. Rau, U. Hilbig and G. Gauglitz, Anal. Bioanal. Chem. 406, 3377 (2014). doi:10.1007/s00216-014-7755-2.

[67] U. Alshana, N.G. Göğer and N. Ertas, Food Chem. 138, 890 (2013). doi:10.1016/j.foodchem.2012.11.121.

[68] F. Mazzotti, L. Di Donna, D. Taverna, M. Nardi, D. Aiello, A. Napoli and G. Sindona, Int. J. Mass. Spectrom. 352, 87 (2013). doi:10.1016/j.ijms.2013.07.012.