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Occurrence of venlafaxine residues and its metabolites in marine mussels at trace levels: development of analytical method and a monitoring program

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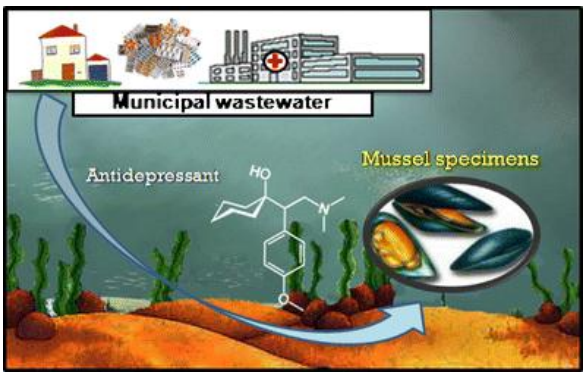
Abstract:

Coastal areas are subject to growing pressures and impacts because of the increase in human activities. Lipophilic organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs), have been monitored for decades within monitoring programs. However, until now, little information on the detection of so-called “emerging contaminants” such as hydrophilic organic compounds in the marine environment and no data on its metabolites or transformation products in marine organisms is available. In this report, a sensitive analytical methodology for identification and confirmation of venlafaxine (VEN) residues and five of its main metabolites in the marine mussels *Mytilus galloprovincialis* was validated. The sample preparation procedure was based on the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach. An analytical method was developed to quantify these compounds at trace levels by liquid chromatography coupled to high-resolution mass spectrometry. The method was then applied to marine mussels collected from the Mediterranean Sea in southeastern France. Residues of the antidepressant VEN were occasionally detected at ng/g dw level. In addition, the approach allowed us to identify several transformation products in the analyzed samples. *N*-desmethylvenlafaxine (NDV) was the most frequently detected metabolite followed by *N*,O-di-desmethylvenlafaxine (NODDV).

Keywords: Antidepressant ; Emerging contaminants ; Transformation products ; Marine organisms ; Orbitrap ; QuEChERS

Graphical abstract

Occurrence of v enlafaxine residues and its metabolites in marine mussels



1. Introduction

Public interest regarding the presence of pharmaceuticals in the environment is mounting.[1] In Europe, around 3000 different pharmaceutical active compounds, used as human and veterinary drugs are susceptible to reach every environmental compartment. Coastal areas are subject to growing pressures and impacts due to increasing of the human activities. In view of serious threats to the European coasts, the European Parliament and the Council approved Recommendation 2002/413/CE concerning the implementation of Integrated Coastal Zone Management in Europe in order to improve the water quality.[2]

Venlafaxine (VEN) is an antidepressant drug prescribed for the treatment of clinical depression and anxiety disorders. It is one of the most commonly prescribed classes of pharmaceuticals in the world. Approximately, 29% of a VEN applied dose is excreted in the urine within 48 hours as the unconjugated metabolite O-desmethylvenlafaxine (ODV) while only 5% of the amount is excreted as unchanged parent compound.[3] Some publications have reported that maternal exposure to this antidepressant elevates fetal plasma serotonin levels, which has been associated with autism and increased risk of spontaneous abortion.[4] Regarding its aquatic fate, recent studies have indicated the presence of VEN in wastewater effluent,[5-7] surface water [8-10] and even drinking water.[11] About the detection of its transformation products in the environment, Lajaunesse ***et al.***[6] reported, for the first time, the occurrence of ODV in Canadian municipal wastewater ranged from 21-68 ng/L. More recently, it has been identified in effluents from waste water treatment plants (WWTPs) and surface waters in Germany, at concentrations up to 500 ng/L and 743 ng/L, respectively.[7,12] The removal rates published for both compounds in the conventional wastewater treatment plants are about 40%.[9] However, to our knowledge no data exist regarding presence of such substances in coastal marine waters. This issue can be partly explained by sampling complexity and the lack of suitable analytical methodologies in those environmental compartments where higher sensitivity is required due to higher dilution rates.[13] Traditional sampling techniques using discrete water sampling are not very performing to such purpose. On the other hand, the present generation of passive samplers enables detection of micro-organic contaminants at low concentrations. Nevertheless, some authors have suggested that passive samplers must still be further developed and validated, ***in situ***, since laboratory conditions generally differ too greatly from those in the field.[14,15]

To overcome this lack, the use of marine organisms as a tool for the biomonitoring of a pharmaceutical and tis transformation products in marine environments was evaluated in the present study. Mediterranean mussel (*M. galloprovincialis)* is a common filter feeder widely distributed in sea coasts. According to the claims of some authors, this species is an excellent sentinel for monitoring of organic micro-pollutants, because it can bioaccumulate substances through their gills (dissolved substances) and/or digestive tract (substances sorbed on particles).[16,17] Then again, an estimated bioconcentration factor (BCF) of 60 suggests a moderate potential for bioconcentration in aquatic organisms for the case of VEN.[18] But up to date, the few studies of the presence of antidepressants in aquatic organisms outside the laboratory have focused on fluoxetine, sertraline, serotonine or norfluoxetine. Such research showed that these substances were present in fish sampled from effluent-impacted rivers.[8,19,20] Little information on the detection of VEN and its metabolites in aquatic organisms and no data in marine organisms are available in literature.

In view of that, the objectives of this study were: (i) to develop and validate a simple, rapid and sensitive analytical approach for detection, characterization and quantification of venlafaxine residues and five of its main metabolites in marine mussels. For that, an easy QuEChERS extraction method combined with liquid chromatography mass spectrometry system (LC-Orbitrap-MS) was the procedure validated and described in this study. Accurate mass measurements in MS and MS/MS modes were performed in the same run, which are highly useful for the identification of isomeric compounds, a common problem when several transformation products are analyzed. The more demanding requirements regarding mass spectrometric confirmation currently set by EU regulations (Commission Decision 2002/657/EC and SANCO/10684/2009 Guideline) were taken into account.[21,22] Therefore, a total of 5 identification points were obtained for each analyte, limiting the number of false positive results in order to achieve suitable identifications. (ii) To evaluate the use of marine organisms as a tool for biomonitoring of hydrophilic organic compounds in aquatic environments and (iii) to demonstrate the utility of the method by providing new results on the contamination of French coastal areas by emerging contaminants, as well as by their transformation products.

2. Experimental

2.1. Chemicals and reagents

The structure and physiochemical properties of target analytes are listed in Table 1. N,N-Didesmethyl-O-desmethylvenlafaxine (NNDDODV), N,O-Di-Desmethylvenlafaxine (NODDV), O-Desmethylvenlafaxine (ODV), N-Desmethylvenlafaxine (NDV), N,N-Didesmethylvenlafaxine (NNDDV) and venlafaxine-d6 (VEN-d6) were purchased at analytical grade (purity > 90%) from Toronto Research Chemicals Inc (Ontario, Canada), except venlafaxine (VEN), which was obtained from Sigma–Aldrich (Steinheim, Germany). Stock standard solutions of individual compounds were prepared at a concentration of 1mg/ml in methanol. All the standard solutions were stored at –20ºC.

Ultrapure water, methanol (MeOH) and HPLC-grade acetonitrile (AcN) were supplied by Carlo Erba (Val de Reuil, France). Formic acid (purity, 98%) was obtained from Fisher Labosi (Elancourt, France). QuEChERS material: sodium sulfate (Na_2SO_4) anhydrous and sodium chloride (NaCl) were obtained from Carlo Erba (Val de Reuil, France). Sodium citrate dihydrate ($Na_3Cit{:}2H_2O$) and sodium citrate dibasic sesquihydrate ($Na_2HCit{:}3H_2O$) were purchased by Sigma−Aldrich (St. Louis, MO, USA). Dispersive SPE tubes containing Z-Sep-Plus (500 mg/12 mL) and primary secondary amine (PSA) sorbent were obtained from Supelco (Bellefonte, PA, USA) and C-18 (40 μm particle size) from Varian (Palo alto, CA, USA), respectively. Calibration mixture for mass spectrometer comprised the following: MSCAL5-1EA (caffeine, tetrapeptide “Met-Arg-Phe-Ala”, ultramark was purchased from SUPELCO (Bellefonte, PA, USA).

2.2. Samples and sample preparation

Marine mussels were collected from different zones of the Mediterranean Sea located in southeaster France (Frontignan, Montpellier and Marseille). Samples (n= 23) were collected from production cultures, an important lagoon (with substantial harbour activity and very influenced by human settlements) and in the vicinity of a submarine outfall (industrial/urban discharges). In order to monitor the fluctuations in the occurrence of target compounds due to localization variation, one additional campaign was performed from samples collected in Marseille (n=7) in the vicinity of a marine outfall. Moreover, some mussel samples were randomly purchased in different local markets (n=4) in the Montpellier area. So, a total of 34 samples were analyzed in different biomonitoring programs performed from 2011 to 2013. Mussels of 4-5 cm long were cleaned of sedimentary material, epiphytes and epifauna, washed and then the flesh was separated from the shell.Samples were then stored at –20ºC until their treatment. Mussels were freeze-dried (Heto Power dry LL 3000, Thermo),

137 homogenized and grounded into powder using MM-2 vibrational mill (Retsch, Haan,
138 Germany).Each sample were then placed inside a clean and opaque glass bottle and stored in
139 dark until extraction and analysis.

140 *2.2.1. Sample extraction*

141 Sample preparation was based on extraction with water and acetonitrile with subsequent salt-
142 induced phase partitioning. The procedure used is a modification of a previously reported
143 method for the determination of carbamazepine in marine mussel, developed by our team.[23]
144 Briefly, sample treatment by QuEChER approach was based on 2 g ± 0.01 freeze-dried
145 mussels were weighed in a 50 mL polypropylene centrifuge tube and spike with surrogate
146 standard (VEN-d6). Next, the mussels were rehydrated by adding 10 mL of ultrapure water.
147 The tubes were then manually and vigorously shaken for 2 min, after the addition 10 mL of
148 AcN. Then QuEChERS salts (4 g Na_2SO_4 (anh), 1 g NaCl, 1 g $Na_3Cit{:}2H_2O$ and 0.5 g
149 $Na_2HCit{:}3H_2O$) were added and the mixture was immediately vigorously shaken (manually)
150 for 1 min more. After the centrifugation step (3500 rpm, 5 min), 2.5 mL of the upper AcN
151 layer was transferred into a Z-Sep-plus tube and 50 μL of formic acid was added and shaken
152 for 1 min. 1 mL of mixture was evaporated to dryness. The residue was reconstituted in 1 mL
153 of AcN/water (1:9, v/v). Finally, the sample was centrifuged at 10.000 rpm for 10 min to
154 separate the residual lipids and the extract was filtered directly into an analysis vial using a
155 0.45 μm PTEF syringe filter.

156 **2.3 LC–MS analysis**

157 The separation of the analytes was carried out using an HPLC (Accela 1250 Pump and a
158 degasser, Thermo Scientific, USA) equipped with a C18 analytical column, 100 mm length x
159 2.1 mm I.D and 1.8 μm particle size (ZORBAX XDB, Agilent Technologies). The LC mobile
160 phases were AcN (A) and water (B) both containing 0.1% formic acid. The optimal separation
161 was achieved using a 200 μL/min flow rate and the following gradient: 1 min isocratic at 10%
162 A, then a linear gradient to 100 % A at 8 min, and finally 5 min isocratic at 100 % A. After
163 that, 7 min of post-time followed using the initial 10 % of A. The volume of injection was 10
164 μL.
165 For analysis, an Exactive mass spectrometer (Thermo Fisher Scientific, USA) system
166 equipped with a heated electrospray ionization probe (HESI) source in positive ion mode was
167 used. The HESI parameters were: electrospray voltage, 4.0 kV; sheath gas, 35 arbitrary units;
168 auxiliary gas, 10 arbitrary units. The heater in the source was set to 200 ºC and the heated

capillary in the mass spectrometer was operated at 300 ºC. Data were acquired by continuously alternating full MS and MS/MS modes: one without and one with fragmentation (both *m/z* 150-350). For fragmentation, an additional experiment using a higher-energy collisional dissociation (HCD) cell fixed at 15 eV, was included in the run. The resolving power for both scan events was 50.000 FWHM at 0.25 s for each one. The automatic gain control (AGC) target was set to $1x10^6$ ions (balance scan). All the other parameters for the mass spectrometer (tube lens, skimmer and capillary voltage) were automatically tuned to obtain the highest TIC signal. The final values were 70, 16 and 65 V, respectively. Confirmation of each compound was performed by means of (i) retention times, (ii) mass accuracy < 5 ppm in full scan MS of protonated molecul [M+H] and (iii) the acquisition of at least one fragment ion together a mass accuracy < 5 ppm in full scan MS/MS. The second diagnostic ion should be sensitive and selective, with a variation of the abundance relative within a certain range.[22] Figure 1 show the extracted ion chromatogram (XIC) and product ion mass spectrum obtained in MS and MS/MS mode, of the protonated target compounds using fragmentation energy of 15 eV for a mussel sample spiked at 1 ng/g.

2.4. Method validation

A rigorous validation procedure according to SANCO/10684/2009 and ISO/17025 Guidelines was performed to ensure high quality analytical measurements.[22,24] Method accuracy and precision were evaluated by recovery studies using blank mussels samples spiked at two concentration levels 5 and 50 ng/g dw. All experiments were tested with six replicates, in accordance with the recommendations of EU guidelines before cited. Quantitation of the compounds in the spiked samples was carried out comparing the peak areas of the samples with those of matrix matched standard solutions. These, as well as the matrix-matched calibration curves, were prepared by spiking an aliquot of the blank extract with the desired amount of standard solution. The sensitivity of the method was calculated in terms of limit of quantitation (LOQ) and limits of detection (LOD), which were calculated as the minimum concentration of analyte that generated a signal to noise (S/N) ratio of 10 and 3, respectively, from signal obtained to matrix-matched calibration curves. Linearity was evaluated both in solvent and matrix, using matrix-matched calibration curves prepared as described before, in a concentration range of 0.1–10 ng/g. The matrix effect was studied by comparison of the slopes of the calibration curves in solvent and in matrix. The repeatability of the instrumental method was estimated by determining the inter- and intra-day relative standard deviation

(R.S.D, %) by the repeated analysis (n = 5) of a spiked matrix extract at 1 ng/g, from run-to-run over one day and five days, respectively.

Finally, in order to ensure quality measurements, each day before analysis, the calibration of Exactive mass spectrometer was performed using the calibration mixture. After that, a standard mixture (10 ng/L) containing targeted analytes was injected with the purpose to check the performance of the HPLC, analytical column and Orbitrap MS system. A continuous monitoring of the quality of the analytical procedure was carried out through the inclusion of blank (solvent) during the day-work sequence.

3. Results and discussion

3.1. Method validation results

The more demanding requirements regarding mass spectrometric confirmation currently set by EU regulations were taken into account for confirmation and quantification of each target compounds. Commission Decision 2002/657/EC[21] describes a system of identification to obtain a minimum of four identification points, in which, detection of transition products in HR-MS^n yields two identification points per ion. SANCO/10684/2009 Guideline[22] recommended the acquisition of ≥ 2 diagnostic ions (preferably including the quasi molecular ion and at least one fragment ion) and a mass accuracy < 5 ppm. In this way, the obtention of one exact precursor ion and one exact product ion, and along with the retention time, allowed us obtained a total of 5 identification points per each compound, as identification criteria in this study. Therefore, the issue of false positive results for analytical methods at ultra-trace levels in which analyte detection is based on retention time and only one exact mass was resolved.

3.3.1. Recoveries

The accuracy of the method was verified by measuring recoveries from blank mussel spiked with 5 and 50 ng/g dw of each chemical. These levels would imply 1 and 10 ng/g, in the final extract. All experiments were performed by six fold for each matrix. The mean recovery data and deviations obtained, which highlights the precision of the extraction method, are given in Table 2. Mussels are fat or lipid containing matrices (about 15%) and, although fats are not very soluble in AcN, a certain quantity of them will be co-extracted, so they have to be removed prior to the final determination step.[23] For that, a new sorbent material Z-Sep-plus used to enhance matrix interference removal was evaluated in this study. They contain

zirconium atoms, which act as a Lewis acid, while the phosphate groups in phospholipids act as a strong Lewis base, strongly binding with zirconium atoms. According to the manufacture's specifications these sorbents can significantly remove fatty matrix interferences and color from sample extracts, provides more robust LC/MS methods. Satisfactory recoveries for all selected compounds were achieved by developed QuEChERS approach. The average recovery values for both spike levels were higher than 70%, except to metabolite NNDDV (61%) and NNDDODV (55%) at 5 ng/g level. Finally, highlight the importance of the addition of formic acid. Many sample preparation techniques for biological matrices use acid to disrupt compound-protein binding, which directly affects recovery and matrix effect.[23] Thus, the addition of 2% formic acid was a critical step. In the absence of this additive, recoveries were reduced to more than half (<30%, data not included).

3.3.2. Linearity and matrix effect

The linearity of the analytical response was evaluated using solvent and matrix-matched calibration curves at five concentration levels covering three orders of magnitude: from 0.1–10 ng/g, based on linear regression and squared correlation coefficient (r^2). All the studied compounds presented a very good response of three orders of magnitude, with correlation coefficients higher than 0.997 in all cases.

Matrix effect was also evaluated during the validation of the method. In fact, signal suppression or enhancement can severely compromise quantitative analysis of the compounds at trace levels, as well as affect the method reproducibility and accuracy.[25] The matrix effect was studied by comparison of the slopes of the calibration curves in solvent and in matrix. When the percentage of the difference between these slopes is positive, then there is signal enhancement, whereas a negative value indicates signal suppression.. According to our results (Table 2), five compounds showed no matrix effect (<20%, because this variation is close to the repeatability values), and only two metabolites, NODDV and ODV, presented a medium effect (<31%). In spite of these low values, matrix-matched calibration curves were used to compensate the matrix effect and avoid any under/over estimation during the quantification.

3.3.3. Precision

In order to evaluate the repeatability of the instrumental method, the intra- and inter-day R.S.D were studied. Method repeatability was determined at 10 ng/g, by the analysis of five spiked matrix extracts . R.S.D values for intra-day (repeativility) ranged between 1% and 6%.

The reproducibility (inter-day) was calculated during five consecutive days, and it varied from 3% to 14% (see Table 2). This demonstrates the repeatability of the method and therefore its effectiveness for quantitative purposes.

3.3.4. Sensitivity

The sensitivity of the method was calculated in terms of limit of quantitation (LOQ) and detection (LOD), which were estimated as the minimum concentration of analyte that generated a S/N of 10 and 3, respectively, from signal obtained to matrix-matched calibration curves. The reporting levels of the studied compounds range from 0.1 to 0.3 ng/g (LOD) and 0.5 to 0.8 ng/g (LOQ) (see Table 2). However, method detection limits (MDLs, ng/g dw) and method quantification limits (MQLs, ng/g dw) are more appropriate for establishing environmental analysis detection thresholds because they take the dilution or pre-concentration steps during sample preparation into account. As shown in Table 2, MDLs values (0.5 to 1.5 ng/g) were typically higher than any of the LOD data (0.1 to 0.3 ng/g), since a 5-fold dilution step is applied to the sample. Nevertheless, the developed analytical method allowed determination of the target analytes at concentration levels the order of a few ng/g dw in mussels exposed in marine water.

3.3.5. Specificity and selectivity

The specificity of the method was assessed through the analysis of three blank mussel samples extracted by the optimized QuEChERS methodology. No other significant peaks (S/N ≥ 3) were found at the specific retention times of target pharmaceuticals. On the other hand, in full scan mass spectrometry measurements, selectivity is obtained by creation of extracted ion chromatograms (EICs) of quasimolecular ions of the compounds of interest. The use of HRMS enables data analysis using the EICs with a narrow mass-extraction window, which provides the required selectivity.[26] The retention times were together the accurate mass, the criteria established to carry out the identification of analytes in samples, taking into account a maximum mass deviation of 5 ppm and considering chromatographic peak with S/N ≥ 3. Furthermore, an additional scans for the unambiguous identification of the target compounds present in the samples, via spectral acquisition with HCD fragmentation at 15 eV were included in the analytical method. Therefore, two chromatograms are collected, one containing full-scan mass for non-fragmented ions and other more for MS/MS data, which containing information about the fragmented ions obtained at this collision energy.

3.4. Analysis of real samples

The applicability of the proposed method was assessed for the analysis of marine mussel samples, collected from different areas of the Mediterranean Sea located in southeaster France and local supermarkets in Montpellier (France). Analytes detection was based on extraction of the exact mass (< 5ppm) of the precursor ion at the correct retention time (±30 s) and the presence of almost one second product ion. All these identification criteria were compared to those obtained with matrix-matched calibration curves in the same batch, according to the recommendations established by Commission Decision 2002/657/EC37.[21] Venlafaxine and its transformation products were detected in mussel samples during the biomonitoring program performed from January 2011 to 2013. With regard to EU surface waters, their occurrence has been reported at concentrations ranging from some ng/L to 1 μg/L.[10-12] In contrast, no studies in coastal waters or marine organisms have previously been reported for such compounds up to date. Residues of the selected antidepressant drug (VEN) were occasionally detected in four marine mussel samples, but it was only found in one at concentration above its MQL (2.5 ng/g dw). However, several transformation products were detected at higher levels. N-Desmethylvenlafaxine (NDV) was the most frequently identified metabolite followed by N,O-Di-Desmethylvenlafaxine (NODDV) and O-Desmethylvenlafaxine (ODV) at levels up to 3.0, 3.5 and 3.7 ng/g dw, respectively. All the results obtained are summarized in Table 3.

Several studies have reported higher concentrations of ODV than VEN in treated wastewater and surface water, with a mean ratio of approximately 5:1.[7] The same ratio was observed by Nakamura *et al.*[27] in fish (*Oryzias latipes*) exposed to antidepressant fluoxetine. Such research showed that its metabolite (norfluoxetine) presented concentrations five times greater than fluoxetine levels. However, in lack of bioaccumulation experiments that allow us to justify the hypothesis of the VEN metabolism in mussels, it is difficult to determine whether the metabolites were present in the mussels as a result of the metabolization of the organim, or as a result of direct uptake from water. In any event, as it has previously been discussed by other scientific, the metabolite profile varied significantly among species. ODV is the major metabolite in humans, but not in mouse, rat, dog, or monkey .[28] Therefore, it is not appropriate to assume the same metabolic reactions in mussels and in human. In any case, our results are in agreement with other studies. Metcalfe *et al.*[9] observed residues of VEN and NDV at mean concentrations of 1.2 and 2.18 μg/kg wet weight in caged fish muscle collected downstream of a WWTP in Canada. In this study, ODV was also detected, but at levels below its limits of quantification (0.5 μg/kg ww). In 2013, a recent work reports the occurrence of 17

pharmaceuticals and personal care products (PPCPs) and 3 alkylphenols (APs) in benthic mussels *(Geukensia demissa)* collected from an urban estuary (San Francisco Bay, USA).[29] In this study, similar concentrations to ours were found for other antidepressant (sertraline), up to 1.4 ng/g ww using 5.5 g of wet tissue. However, the levels of PPCPs were generally at least an order of magnitude lower than levels of APs.

Any analyte was detected in samples purchased from different local supermarkets in France (n=4) nor in mussel samples taken from the production cultures or reference zone (n=4). Finally, only one metabolite (NNDDODV) was not detected in samples from our biomonitorig program. In any case, the recovery of the surrogate standards was above 75% for each analyzed sample. It standards allowed us to verify that extraction method performance and analysis were satisfactory. An example of identification of a sub-product of VEN in a marine mussel sample is presented in Figure 2. In full MS spectrum, the measured mass for NNDDV is shown at *m/z* 250.17879 which matches the theoretical mass 250.1801 with an error of −3.6 ppm. The additional acquisition in full MS/MS mode provided a more comprehensive identification of this compound as well as its structural characterization found for the characteristic fragment ion at *m/z* 201.12748 with a mass deviation of -2.30 ppm. Good mass accuracies were obtained both in the MS and MS/MS scans (< 5ppm). Thus, a correct identification was ensured in line with the other evaluated parameter (retention time, tr).

To conclude, the results support the hypothesis of a moderate bioconcentration for VEN in aquatic organisms, despite of its modest octanol-water partition coefficient K_{ow} 3.28.[7,30] Bioconcentration factors are often described as being associated with the lipophily of the molecule. However, in line with previous studies, the accumulation not always increased with a linear relation, suggesting other bioconcentration mechanisms such as the biotransformation. Gómez *et al.*[16] explained these differences by possible clearance mechanisms/metabolization in marine mussels. Thus, although the initial results were positive, future experiments should be conducted on the bioaccumulation of VEN and its metabolites in marine mussels in order to assess their bioconcentration factors and therefore obtained data regarding its distribution and persistence in marine aquatic organisms. Also, this data will be useful in retrospective ecological risk assessments to support an understanding of environmental exposure as well as for predicting toxic effects in marine organisms.

3.4.1. Spatial-temporal variation

In order to monitor the fluctuations in the occurrence of target compounds in study area, a seasonal and monthly variation were also evaluated (see results Table 3). As can see, a greater number of analytes were identified in the sample collected at east points in the vicinity of a submarine outfall, during the first months of the year (January to April). This may be due to two hypotheses: (i) differences in ocean currents or (ii) a possible increase in the consumption of this type of drugs to treat disorders related to depression during those months. Several factors associated with anxiety and stress can be caused by the post-holiday period or by winter weather. In fact, very few compounds were found in the months following the summer (September to December).

On the other hand, in relation with the localization variation, it is interesting highlight that not was detected traces of some target compounds in Marseille, during the monitoring camping carried out in September 2012. Similarly, during the same sampling period performed in Montpellier area, some metabolites of VEN were only identified in the sample collected from lagoon (IM: sept-2012). This can be explained because this batch of mussels, after its purchase, had to be placed for several days in a lagoon in the area, before its distribution to the different sampling points studied. Thus, the results support the hypothesis of a higher contamination of the waters of the lagoon compared to marine waters. The lagoon is connected to the Mediterranean Sea and is the largest in the region of Languedoc-Roussillon. They receive inputs from various human activities, i.e. urban activities, industries, port activities and agriculture. Besides, the spa tourism is an important activity in that area, being the second thermal station in France and the first of the Mediterranean. Finally, note that no differences were observed when a same point was sampling at different levels in the water column (samples placed in the surface vs middle of the water column).

Conclusions

This paper reports for the first time results on the presence of a highly prescribed antidepressant (venlafaxine) and some of its main transformation products in marine organisms. As part of this study, an analytical approach including QuEChERS extraction and analysis by LC-Orbitrap-MS system was developed. The method was validated according to the most demanding requirements regarding mass spectrometric confirmation and quality measurement criteria currently set by EU regulations, in order to ensure satisfactory positive results. The main advantages of the extraction protocol proposed in this study (QuEChERS)

392 are its economy, simplicity, and rapidity. Moreover, this approach allowed to obtained high
393 recoveries even to polar compounds, which is a common characteristic of the metabolites.
394 Venlafaxine and some of its sub-products were found in marine mussels at ultra-trace levels
395 (ng/g dw) during the biomonitoring program carried out. The presence of target compounds
396 varied depending on localization, the seasonal and on the population density of the area.

397 On the other hand, these organisms have proven to be a useful tool for monitoring of
398 hydrophilic organic compounds from marine waters. The work provides additional broad-
399 interest data regarding to knowledge of transformation products concentration levels in
400 aquatic organisms, which not were previously informed. Finally, monitoring data indicated
401 that the general population may be exposed to VEN and its metabolites, providing another
402 possible exposure route and raising questions about human health consequences. To date,
403 informations on PPCPs in aquatic organims indicate that an additional understanding their
404 accumulation is necessary, in order to characterized ecological and human health risks of such
405 substances in the environment. Although the initial results were positive, future experiments
406 should be conducted on the bioaccumulation of VEN and its metabolites in marine mussels.

407

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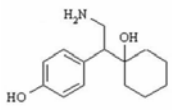
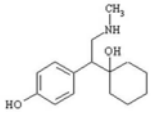
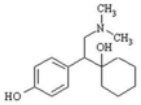
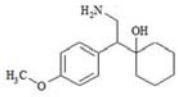
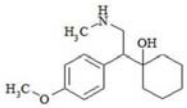
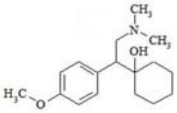
512

TABLES

Table 1. Physicochemical properties of venlafaxine and its metabolites.

Analyte	Molecular structure	Molecular formula	CAS Nº.	Molecular weight	Water solubility [a]	Log K_{ow} [b]
NNDDODV		$C_{14}H_{21}NO_2$	135308-76-8	235.1566	-	-
NODDV		$C_{15}H_{22}NO_2$	135308-74-6	249.1723	-	-
ODV		$C_{16}H_{25}NO_2$	93413-62-8	263.1879	3670	2.72
NNDDV		$C_{15}H_{23}NO_2$	93413-77-5	249.1723	-	-
NDV		$C_{16}H_{25}NO_2$	149289-30-5	263.1879	485.2	3.07
VEN		$C_{17}H_{27}NO_2$	99300-78-4	277.2036	266.7	3.28

[a,b] Predicted data cited from chemspider.com, which is generated using U.S. Environmental Protection Agency´s EPI Suite™ (KOWWIN v1.67 estimate). Data of water solubility at 25ºC (mg/L).

522 **Table 2.** Analytical performance data for each target compound studied by LC-Orbitrap-MS method.
523

Compound	Matrix Effect	Inter/intra-day *(R.S.D, %)*	Rec. *% (± σ)*	LOQ *(ng/g)*	LOD *(ng/g)*	MQL *(ng/g)*	MDL *(ng/g)*
NNDDODV	16	6/8	55 *(9)* [a] 70 *(8)* [b]	0.7	0.2	3.6	1.1
NODDV	-31	5/14	78 *(5)* [a] 70 *(3)* [b]	0.6	0.2	3.0	1.0
ODV	-30	4/13	78 *(5)* [a] 79 *(3)* [b]	0.7	0.2	3.5	1.1
NNDDV	19	4/3	61 *(6)* [a] 61 *(2)* [b]	0.8	0.3	3.8	1.5
NDV	4	2/5	76 *(3)* [a] 71 *(2)* [b]	0.5	0.1	2.5	0.5
VEN	8	1/3	88 *(3)* [a] 80 *(4)* [b]	0.5	0.1	2.5	0.5

524
525 **Inter/intra-day:** repeatability/reproducibility of the instrumental method (R.S.D, %); R.S.D:
526 relative standard deviation; **Rec:** recovery average values obtained at two spiked levels (a) and (b),
527 5 and 50 ng/g, respectively; **σ:** dispersion from the average recovery values; **LOQ:** limit of
528 quantification; **LOD:** limit of detection; **MQL:** method quantification limit; **MDL:** method
529 detection limit.
530

Table 3. Concentration of venlafaxine and its metabolites (ng/g dw) in 34 samples of marine mussel samples (*M. galloprovincialis*) collected during the biomonitoring program carried out.

Sampling Point	Exposure period		NNDDODV	NODDV	ODV	NNDDV	NDV	VEN
		(day/month)	*(ng/g dw)*	*(ng/g dw)*	*(ng/g dw)*	*(ng/g dw)*	*(ng/g dw)*	*(ng/g dw)*
Montpellier								
Jan-2011	To		/	/	/	/	/	/
	IM	25/01-22/03	/	/	/	/	<MQL	/
	IM	25/01-26/05	/	/	/	/	/	/
	N	25/01-22/03	/	3.0	/	/	2.5	/
	E	25/01-22/03	/	/	<MDL	/	2.9	<MQL
	W	25/01-22/03	/	3.5	/	/	3.0	/
Mars-2011	To		/	/	/	/	/	/
	IM	22/03-24/05	/	/	/	/	/	/
	N	22/03-24/05	/	/	/	/	/	/
	E	22/03-24/05	/	/	3.7	/	<MQL	<MQL
	W	22/03-24/05	/	/	/	/	/	/
April-2012	To		/	/	/	/	/	/
	IM	12/04-21/06	/	/	/	/	/	/
	N	12/04-21/06	/	2.5	/	/	/	/
	E	12/04-21/06	/	/	/	/	2.5	2.7
	W	12/04-21/06	/	/	/	/	/	/
Sept-2012	IM	02/09-06/09	/	/	/	3.8	<MDL	<MQL
	Ref	06/09-03/12	/	/	/	/	/	/
	N	06/09-03/12	/	/	/	/	/	/
	E	06/09-03/12	/	/	/	/	/	/
	W	06/09-loss	n.a	n.a	n.a	n.a	n.a	n.a
Dec-2012/13	Ref	03/12-05/03	/	/	/	/	/	/
	N	03/12-05/03	/	/	/	/	/	/
	E	03/12-05/03	/	/	/	/	/	/
Marseille								
Sept-2012	To		/	/	/	/	/	/
	Ref_s	09/09-05/12	/	/	/	/	/	/
	Ref_m	09/09-05/12	/	/	/	/	/	/
	N_s	10/09-05/12	/	/	/	/	/	/
	N_m	10/09-05/12	/	/	/	/	/	/
	E_s	10/09-05/12	/	/	/	/	/	/
	E_m	10/09-05/12	/	/	/	/	/	/
Supermarket								
June-2011	#1	30/06/2011	/	/	/	/	/	/
Sept-2011	#2	15/09/11	/	/	/	/	/	/
Nov-2012	#3	10/11/12	/	/	/	/	/	/
Jan-2013	#4	19/01/12	/	/	/	/	/	/

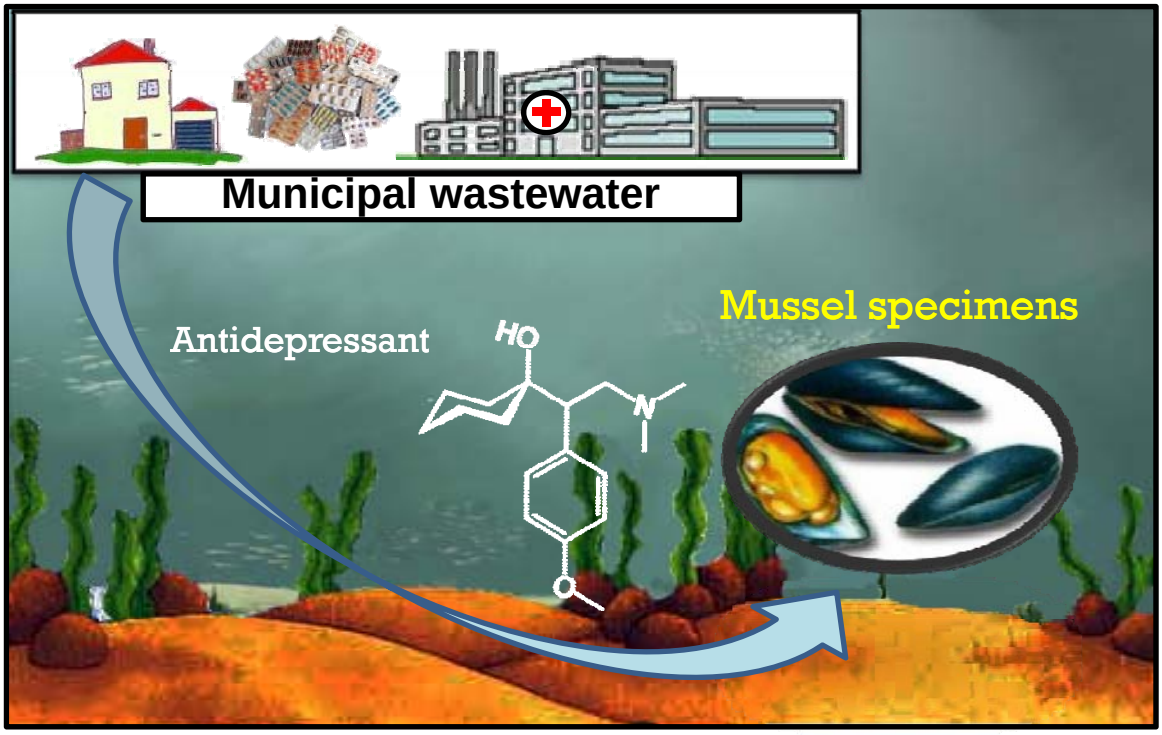
To: before exposure; **IM**: lagoon point; **N:** north emissary point; **E:** east emissary point; **W:** west emissary point; **Ref:** reference zone or production cultures; **X_s:** samples placed on the surface of the water column; **X_m:** samples placed in the middle of the water column; **n.a:** data not available, sample loss; **<MQL/MDL:** below to method quantification/detection limit (ng/g dw).

FIGURES

Figure 1. Chromatogram, precursor and product ion mass spectrum obtained in MS and MS/MS mode for each target analyte using a fragmentation energy of 15 eV at 1 ng/g in a mussel extract.

Figure 2. Example of the identification of N,N-Didesmethylvenlafaxine (NNDDV), a metabolite of antidepressant venlafaxine in a marine mussel sample, based on MS and MS/MS information.

Graphical Abstracts



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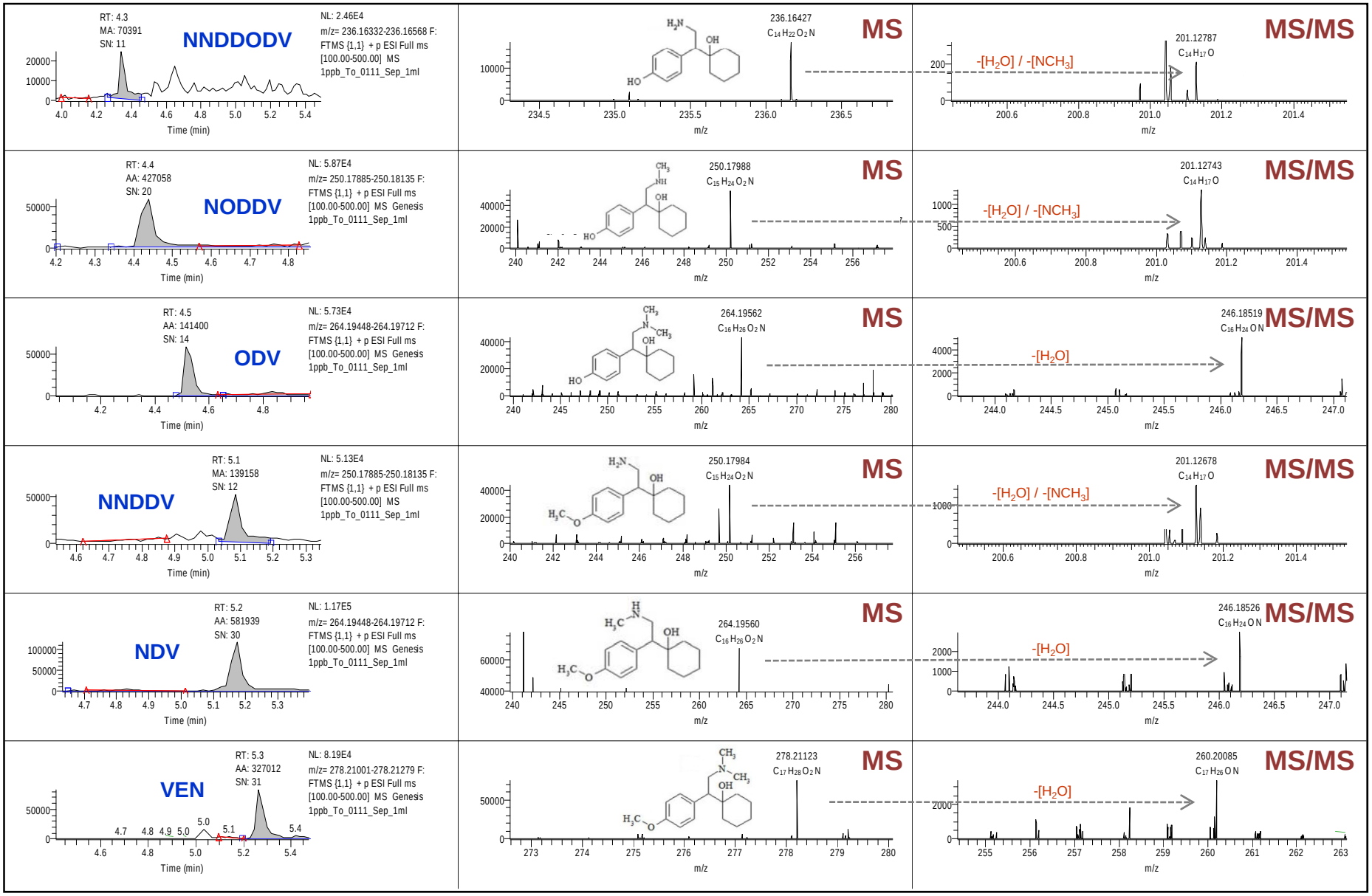

Figure 2. Example of the identification of N,N-Didesmethylvenlafaxine (NNDDV), a metabolite of antidepressant venlafaxine in a marine mussel sample, based on MS and MS/MS information.