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Determination of triazine herbicides in seaweeds: Development of a sample preparation method based on Matrix Solid Phase Dispersion and Solid Phase Extraction Clean-up

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ABSTRACT

A method using dual process columns of Matrix Solid Phase Dispersion (MSPD) and Solid Phase Extraction (SPE) has been developed for extracting and cleaning-up of nine triazine herbicides (ametryn, atrazine, cyanazine, prometryn, propazine, simazine, simetryn, terbuthylazine and terbutryn) in seaweed samples. Under optimized conditions, samples were blended with 2 g of octasilyl-derivatized silica (C_8) and transferred into an SPE cartridge containing ENVI^{TM}-Carb II/PSA (0.5/0.5 g) as a clean up co-sorbent. Then the dispersed sample was washed with 10 mL of *n*-hexane and triazines were eluted with 20 mL ethyl acetate and 5 mL acetonitrile. Finally the extract was concentrated to dryness, re-constituted with 1 mL methanol:water (1:1) and injected into the HPLC-DAD system. The linearity of the calibration curves was excellent in matrix matched standards, and yielded the coefficients of determination $\geq$ 0.995 for all the target analytes. The recoveries ranged from 75% to 100% with relative standard deviations lower than 7%. The achieved LOQs ($< 10\ \mu g\ kg^{-1}$) for all triazines under study permits to ensure proper determination at the maximum allowed residue levels set in the European Union Legislation. Samples of three seaweeds were subjected to the procedure proving the suitability of MSPD method for the analysis of triazines in different seaweeds samples.

1. Introduction

Seaweeds have been used since ancient times as food, fodder, fertilizer and as a source of medication. Nowadays, seaweeds are the raw material for industrial production of agar, carrageenan and alginates; however they still remain to be widely consumed as a source of food in Asian countries. Although in North America and Europe their use as food is more restricted, in recent years seaweeds have been increasingly recognized as healthy and attractive foods [1]. Edible seaweeds contain dietary fiber, high concentration of minerals, vitamins, proteins, polyunsaturated fatty acids and have a low content in saturated fats. On the other hand, seaweeds have also shown biological properties such as antibacterial, antiviral, antioxidant and antifungal [2,3]. Moreover, it has been reported that the chemical composition of seaweeds varies with species, habitats, maturity and environmental conditions [4].

Triazines, well-known herbicides, are applied to soil for the control of weeds in many agricultural crops, as well as railways, roadside and golf courses. The marine environment receives fluxes of these compounds mainly from agricultural origin. Their mechanism of action is via photosynthetic inhibition, and for this reason, they are only toxic for plants; however these compounds can affect the human health through the dietary intake. These compounds are highly persistent and can survive for many years in soil, water and organisms. Therefore, they are considered as an important class of chemical pollutants and atrazine and simazine have been included in the group of endocrine-disruptors by the Environmental Protection Agency of US [5]. As a result, the European Parliament and Council [6] concerning the residue levels in food and feed of plant and animal origins established the maximum permitted concentration in seaweed 0.01 and 0.05 mg kg^{-1} for simazine and terbuthylazine respectively. Moreover, a limit is not yet established for atrazine in seaweed, but its maximum permitted limit in edible vegetables is 0.05 mg kg^{-1}. For this reason, analytical methods for a rapid and sensitive determination of these compounds are required. However, seaweed is a complex matrix with different types of interfering compounds which make pesticide analysis difficult; in fact, studies of pesticides in seaweeds are limited and recent [7–9] and to the best of our knowledge there is only one reference in the literature devoted to the determination of triazines in seaweeds [10].

The most frequently used methodologies for the analysis of triazines in samples of vegetable and animal origins employ

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solvent extraction procedures such as soxhlet [11,12], shake flask [13,14], sonication [10,13,15], microwave-assisted extraction (MAE) [11,12,16,17], and pressurized liquid extraction (PLE) [18,19]. Nevertheless, they generally need to add a clean-up step to decrease the presence of interferents in the final extract to reduce the detection limits of the methods and to avoid inaccurate results in the chromatographic determination, which is time consuming; many times it is expensive and simultaneously decreases the precision of the methodologies involved. Over the last years, different innovative procedures have been developed and applied for the determination of pollutants in complex matrices with improved capabilities, reduced clean up and concentration steps, the avoidance of toxic solvents and improved limits of detection. In this context, sorptive extraction techniques such as solid-phase extraction (SPE), dispersive solid phase extraction (dSPE), matrix solid-phase dispersion (MSPD), solid-phase micro-extraction (SPME) and stir bar sorptive extraction (SBSE) appear to be appropriate and they have been applied for analysis of triazines in different kinds of vegetation samples [20,21].

Matrix solid-phase dispersion is a sorptive extraction technique which involves the dispersion of the sample in a solid sorbent and subsequent elution with a relatively low solvent volume, allowing the simultaneous extraction and clean-up of analytes from solid samples [22]. If an additional clean up step is necessary, it is possible to use the MSPD column with another sorbent at its bottom. This technique shows a high flexibility and selectivity due to the variety of possible combinations of both sorbents and elution solvents [23]. Due to its simplicity and high throughput, MSPD methods have been developed for the extraction of different pesticides residues from different plants and plant materials [24]; however references for the determination of triazines by MSPD are still scarce and furthermore in most cases few triazines are included in these studies [25–28].

The aim of this work was the development and validation of an effective and simple method based on Matrix Solid Phase Dispersion (MSPD) and Solid Phase Extraction (SPE) Clean-up followed by High Performance Liquid Chromatography (HPLC) coupled to Diode Array Detection (DAD) for the simultaneous determination of nine triazine herbicides in seaweeds in order to be able to quantitate residues of these compounds in the range of the European maximum residue levels. Samples of three edible seaweeds were selected to illustrate the applicability of this method. To the best of our knowledge, no studies using MSPD have been done to extract these chemicals residues from seaweeds.

2. Experimental

2.1. Samples

Dried edible seaweeds. Sea lettuce (*Ulva Lactuca*), Wakame (*Undaria pinnatifida*), and Nori (*Porphyra umbilicalis*), from aquaculture production, were purchased from a local market in A Coruña city, NW, Spain. Samples were homogenized grounding them to a fine powder by an electric mill and stored in glass bottles out of light exposure until analysis.

2.2. Chemicals

(a) *Herbicide standards*. Herbicides (ametryn, atrazine, cyanazine, prometryn, propazine, simazine, simetryn, terbuthylazine and terbutryn) analytical standards were supplied by Sigma-Aldrich Inc. (St. Louis, MO, USA). The individual stock standard solutions of 1000 mg L^{-1} were prepared in methanol by exact weighing of high-purity substances and stored at −18 °C in dark. Then a mixture of all these compounds was prepared in methanol containing 10 mg L^{-1} each of individual triazine and stored at −18 °C. All working solutions were daily prepared by appropriate dilution of the 10 mg L^{-1} standard solutions with methanol:water (1:1) ratio.

(b) *Solvents*. *n*-hexane 95% and methanol were superpurity solvents obtained from Romil (Cambridge, UK). Acetonitrile (HPLC grade) and ethyl acetate (PAR, solvents for analysis of pesticide residues by GC) for instrumental analysis were obtained from Panreac (Barcelona, Spain). Milli-Q water was obtained from a purification system from Millipore (Billerica, MA).

(c) *Sorbents*. LC-8 Bulk packing and Supelclean™ ENVI-Carb II/PSA SPE Tube 6 ml (500 mg/500 mg) were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA).

(d) *Filters*. Polytetrafluoroethylene (PTFE) filters of 0.45 μm were obtained from Lida Manufacturing (Kenosha, WI, USA).

2.3. Materials and apparatus

A Visiprep® vacuum distribution manifold from Supelco (Bellefonte, PA, USA) was employed in the purification step. A Büchi R-3000 rotary evaporator (Büchi Labortechnic AG, Flawil, Switzerland) was used in the evaporation step.

Chromatographic analyses were carried out in a High Performance Liquid Chromatography-Diode Array Detector (HPLC–DAD). The system consisted of a 2695 pump with a 996 Diode Array Detector from Waters (Milford, MA, USA) and a computer running Empower 2 data processor. The column was a stainless steel column (150 mm × 4.6 mm ID, particle size 5 μm) packed with Hypersil GOLD C_{18} chemical bonded phase from Thermo Scientific (Austin, TX, USA).

2.4. Extraction procedure

The optimization study was carried out using a pesticide free sea lettuce seaweed sample spiked at the 1 mg kg^{-1} level. 1.0000 g of dried seaweed sample was homogenized with 2.00 g of LC-8 in a glass mortar with a pestle for 5 min. The final mixture was transferred into a 12-mL SPE cartridge containing a dual sorbent layer of 1 g Supelclean™ ENVI-Carb II/PSA (500/500 mg). Once packed, MSPD/SPE columns were connected to a Visiprep® vacuum distribution manifold and were washed with 10 mL of hexane. Elution was performed with 20 mL of ethyl acetate and 5 mL of acetonitrile (80:20) and the obtained eluate was evaporated to a drop in a rotary-evaporator and brought to dryness by a gentle nitrogen stream. The residue was reconstituted in 1 mL methanol:water (1:1) ratio and the solution was filtered through a 0.45 μm PTFE syringe filter.

2.5. HPLC-DAD conditions

The chromatographic analysis was carried out using the following ACN:H_2O gradient elution: ACN initial percentage of 30% (8 min) increased linearly to 40% in 5 min, increased to 50% in 5 min, after which the percentage was returned to the initial conditions in 9 min. A constant mobile phase flow rate of 1 mL min^{-1} and 20 μL of sample volume were used.

The absorbance was measured continuously in the 200–400 nm range and peak areas quantification was carried out at 222.7 nm in order to achieve maximum sensitivity. All triazine herbicides were identified initially by retention time and then by applying spectral contrast techniques (incorporated in Millenium^{32} software) the homogeneity of the spectral peak was confirmed. Finally a spectral identification was carried out contrasting the spectrum with a standard library created in the wavelength interval of 200–400 nm.