Talanta 131 (2015) 205–212

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simple SPE–HPLC determination of some common drugs and herbicides of environmental concern by pulsed amperometry

L. Rivoira [a], R.M. De Carlo [a], S. Cavalli [b], M.C. Bruzzoniti [a,*]

[a] Department of Chemistry, University of Torino, via P. Giuria 5, 10125 Turin, Italy
[b] Istituto di Ricerca Sulle Acque, Consiglio Nazionale delle Ricerche (IRSA-CNR) Via del Mulino, 19, 20861 Brugherio, MB, Italy

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form
17 July 2014
Accepted 21 July 2014
Available online 31 July 2014

Keywords:
Amperometric detection
Glassy carbon electrode
RP-HPLC
Emerging contaminants
Water

ABSTRACT

In this work the electrochemical behavior of substances of environmental concern [bentazone, atrazine, carbamazepine, phenytoin and its metabolite 5-(4-hydroxyphenyl)-5-phenylhydantoin, HPPH] on a glassy carbon working electrode (Ag/AgCl reference electrode) was studied with the aim to develop a HPLC method coupled with amperometric detection. Constant potential (DC), pulsed amperometric detection modes were studied. For the pulsed mode, several waveforms were set and investigated. Detection conditions were optimized as a function of eluent pH.

In order to reduce the limits of detection and to analyze natural water samples, a SPE protocol was optimized to be coupled to the developed procedure. For this aim, five sorbents of different physico-chemical characteristics were tested optimizing a recovery procedure for each of the cartridge evaluated.

At the optimized SPE conditions, recoveries were included in the range (R=90.2–100.5% for all the analytes, with excellent reproducibility ($<$%, n=3). The method detection limits obtained by pulsed amperometry after the SPE protocol (preconcentration factor 100) were 113 ng L^{-1} (0.47 nmol L^{-1}), 67 ng L^{-1} (0.25 nmol L^{-1}), 234 ng L^{-1} (1.1 nmol L^{-1}), for bentazone, HPPH and carbamazepine, respectively. Robustness of the method was assessed for each analyte at a concentration level corresponding to about three times the limit of detection, through the evaluation of intra-day (n=13) and inter-day tests (4 days, n=52). Finally the method was successfully applied for the analysis of a river sample (Po River, Turin, Italy).

1. Introduction

Contaminants of emerging concern in water sources have been of particular interest in the last decade and could pose a risk to human health as well as to the environment as a function of their presence, frequency and occurrence; their impact on aquatic wildlife populations has been demonstrated to occur at very low concentrations. Among them, herbicides and pharmaceutical compounds are among the most widely occurring pollutants [1] due to their extensive use.

Many herbicides are potentially dangerous not only to human health but also to other organisms in the environment. Among them, bentazone and atrazine are of environmental concern. Both herbicides inhibit, in chloroplast, the Hill reaction, necessary for the oxygen evolution in the photosynthetic process [2,3]. Health effects of atrazine as a human carcinogen still remain controversial [4].

The widespread contamination of drinking water by atrazine was associated with birth defects and hormonal disturbance effects [5,6]. Even though banned in the EU, atrazine is the most widely used herbicide in the US.

Bentazone belongs to the thiadiazine group and is widely used as post-emergence herbicide. It exhibits little sorption in soil and due to its relatively high mobility [7], the potential risk of leaching and ground water contamination is very high. Bentazone is commonly detected in ground and surface waters [8]. Toxicologic studies show that bentazone has acute and chronic toxicity (inflammation of the mucous membranes, tachycardia, renal failure).

Pharmaceuticals, like antiepileptic drugs, are found regularly in surface waters, phenytoin and carbamazepine are anti-epileptic and anticonvulsant substances and just like many active drugs, if overdosed are toxic. Recent studies show their presence in finished drinking waters [9]. In addition it was proved that the presence of phenytoin and carbamazepine in wastewater decreases the efficiency of wastewater treatment plants [10,11].

The monitoring of these contaminants in the aquatic environment is progressively becoming a priority for government agencies, regulatory agencies and the general public.

* Corresponding author. Tel.: +39 0116705277; fax: +39 0116705242.
E-mail address: mariaconcetta.bruzzoniti@unito.it (M.C. Bruzzoniti).

http://dx.doi.org/10.1016/j.talanta.2014.07.070
0039-9140/

With regard to the analytical determination, the mainly used techniques are based on chromatographic mechanisms (gas chromatographic and HPLC) coupled to specific mass spectrometric and spectrophotometric detectors. Although gas chromatographic methods can successfully be employed for the determination of some compounds like carbamazepine and bentazone at ng L^{-1} levels after solid-phase extraction, GC requires the choice of the correct derivatizing agent that avoids decomposition of the analytes. This in turn implies that the same derivatizing agent cannot be used for the simultaneous analysis of different classes of compounds of interest [12].

With regard to HPLC methods, this approach has been chosen for the determination of bentazone, atrazine, phenytoin, carbamazepine in environmental matrices like natural waters, soils, and in foodstuff like rice and cereals. Limits of detection (LODs) depend mainly upon the detection mode and the extraction technique used. For example, LODs ranging from 5–15 μg L^{-1} for bentazone [13,14] have been obtained by SPE extraction-HPLC with UV detection after a 500-fold preconcentration. Lower detection limits have been achieved with higher preconcentration ratios (e.g. for carbamazepine: 50 ng L^{-1} [15]). Less satisfying LOD values have been obtained with liquid–liquid extraction techniques (e.g. about 100 μg L^{-1} for carbamazepine with UV detection [16]). Techniques such as LC–MS and LC–MS/MS have been used to determine herbicides and pharmaceutical compounds in natural waters at generally lower detection limits [17–19], but with the main drawback of requiring expensive equipments with high maintenance costs and skilled technical staff.

Electroanalytical techniques have been shown to be useful in the study of toxic substances of environmental concerns such as pesticides [20] and they have been recently used in pulsed amperometric mode also on glassy carbon electrodes for the determination of chlorophenols [21]. When coupled to chromatography, the specificity of the electrochemistry techniques is increased significantly. In recent years, the number of published papers dealing with the use of chromatographic methods with amperometric detection is growing thanks to selectivity, sensitivity and low cost of the detection technique [22]. Amperometric detection proved to be successful for the determination of compounds of environmental concern [23–27].

According to the characteristics of the molecules to be determined, the amperometric detection can be considered for identification and quantification of target compounds.

The aim of this work was to study the chromatographic separation and the electrochemical behavior of bentazone, atrazine, phenytoin, and carbamazepine. Since phenytoin is metabolized by cytochrome P450 enzymes primarily to 5-(p-hydroxyphenyl)-5-phenylhydantoin (HPPH), and is mainly excreted as 5-(4′-hydroxyphenyl)-5-phenylhydantoin O-glucuronide in humans [28], HPPH was included in our study, too. Constant potential (DC) amperometry, pulsed amperometric detection were compared to assess the best electrochemical response for the analytes tested. The chromatographic method optimized was coupled to a solid-phase extraction step which provided reduced detection limits with a simultaneous clean-up of the matrix analyzed. The study performed allowed us to develop a sensitive, affordable analytical procedure based on pulsed amperometry which is of simple application.

This work is the first study devoted to the analysis of bentazone, atrazine, carbamazepine, phenytoin and HPPH by HPLC with amperometric detection.

2. Material and methods

2.1. Chemicals and standard solutions

All reagents used throughout this work were of analytical grade. Acetonitrile (99.9%), bentazone, HPPH, phenytoin, carbamazepine, atrazine, nitric acid (65% w/w, $d=1.40$ g/mL) and acetic acid (99.8% w/w, $d=1.052$ g/mL) were from Sigma-Aldrich (Chemie, Steinheim, DE). Methanol and NaOH (purity > 98%) were from Carlo Erba (Milano IT).

A Milli-Q Plus ultra-pure water system from Millipore (Milford, MA, USA) was used for standard and eluent preparation.

2.2. Instruments

For the chromatographic separations, a Dionex ICS-3000 chromatograph (Thermo Scientific, Sunnyvale, CA, USA), equipped with a reversed-phase C-18 pre-column and analytical column (LiChroCart PuroSphere RP-18, 125 mm × 3.0 mm, 5 μm, Merck) was used. The mobile phases were sodium acetate or sodium formate buffers (50 mM) at different pH values. CH_3CN was used as organic modifier. The analysis were performed in isocratic mode (eluent flow rate 0.5 mL min^{-1}). A 10 μL-injection loop was used throughout this work. Pre-column and analytical column were periodically washed by isopranol and reconditioned with eluent for 35 min.

Two detectors coupled in series were used, namely an AD25 Absorbance Detector ($\lambda=252$ nm) and a AD40 Electrochemical Detector (both by Thermo Scientific, Dionex), with a Ag/AgCl reference electrode and a glassy carbon (GC) working electrode. The parameters of the electrochemical detector were optimized as described in Section 3.4. Chromatographic and amperometric data were collected and elaborated by the software Chromeleon 6.80 (Thermo Scientific, Dionex).

2.3. Solid phase extraction (SPE)

For the extraction of the analytes, the performance of five SPE supports of different compositions were compared. In detail, two C18 based adsorbents: Bond Elut C18 Jr (Agilent), ENVI 18 (Supelco), two polymeric sorbents: LiChroLut EN (Merck), Baker-Bond SDB (JT Baker) and one carbon based substrate: ENVI Carb (Supelco) cartridges were activated according to manufacturer's indications before use.

For each cartridge, aliquots of 5 mL containing 1 mg L^{-1} each of bentazone, atrazine, phenytoin, HPPH and carbamazepine were loaded at 2 ml min^{-1} flow rate. For each cartridge, the eluent solution was optimized according to the expected analyte–sorbent interactions . Before elution, each cartridge was washed with 2 mL H_2O to remove unretained compounds. In order to check the retention of the analytes in each step of the SPE protocol, the following three fractions were collected and injected in the HPLC system: (i) the solution after loading; (ii) the washing solution and (iii) the eluate.

Recoveries are expressed as average of three independent extractions. In parallel, a blank was processed for all the cartridges tested. For the optimization of the SPE protocol, the UV detection was used.

2.4. Real sample analysis

A river sample (Po river, Turin, Italy) was collected on 24/07/2013 between 104 km and 105 km, stored in a Pyrex bottle at 4 °C, protected from light until analysis. Analysis was performed within 24 h from the sampling.

Analytes were SPE extracted and analyzed according to the procedure optimized throughout this work which is summarized in Fig. 1 of Supplementary Material section, where all the steps of the protocol are given in detail.