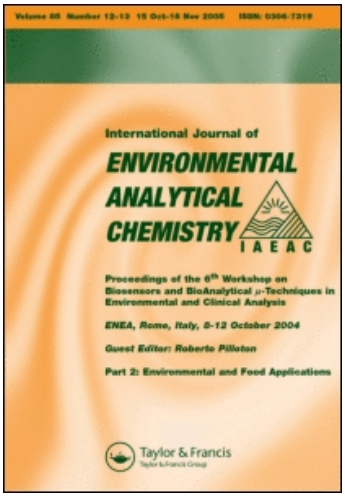

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THE USE OF β-CYCLODEXTRIN INCLUSION COMPLEXES FOR THE ANALYSIS OF BISPHENOL A RESIDUES IN WATER BY SPECTROFLUORIMETRY

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The application of β-cyclodextrin inclusion complexes to determine trace levels of bisphenol A (BPA) in aqueous solution by spectrofluorimetry was investigated. A 1:1 stoichiometry of the host-guest complex between β-CD and BPA, as well as the association constant was determined by using the changes in the fluorescence of BPA that occur when it is included in the hydrophobic cyclodextrin cavity. A simple and sensitive spectrofluorimetric method for the determination of BPA residues is presented; the applicable concentration range was 10.0 to 200.0 $\mu g \cdot L^{-1}$. The detection limit obtained was 0.5 $\mu g \cdot L^{-1}$. The accuracy of the proposed method, was checked in the analysis of water samples from different sources previously spiked with different amounts of BPA.

Keywords: Estrogenic environmental pollutant; bisphenol A (BPA); β-cyclodextrin; spectrofluorimetry; inclusion complexes; water analysis

INTRODUCTION

The potential of exposure for humans and animals to estrogen-like pollutants in the environment is high[1, 2] and it has been found that, in addition to estrogenic effects, these chemicals have multiple genetic and/or nongenetic effects.[3] However, only a limited number of estrogen-like compounds, such as bisphenol

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This article is dedicated to the memory of Dr. A. Arrebola-Ramírez of the University of Granada (Spain).

A (BPA), nonylphenol or polychlorinated biphenyls (PCBs), have been used to assess the biochemical and molecular changes at the cellular level.[4-7] Atkinson and Roy[8, 9] have investigated recently the tendency of bisphenol A to be oxidised to bisphenol-o-quinone in the presence of activation systems and its capability of binding covalently to DNA, which may be a factor in the induction of hepatoxicity.

Bisphenol A is a primary raw material for the production of polycarbonate (PC), epoxy, BPA/formaldehyde, phenoxy and other high-performance resins, including polyesters, polyacrylates and polysulfones.[10] Global production of bisphenol A is well over a million tonnes per year, with an estimated European annual production of 504,000 tonnes.[11] Consequently, the continued strong growth in BPA demand in the last few years and its potential health risks call for selective and sensitive methods of analysis. Particularly, there is a need for assessing the groundwater pollution with BPA, as well as that of drinking water arising from the use of this chemical in water main filters.

Although fluorescence techniques have been applied for photophysical studies[12] and identification of thermal degradation products[13] of bisphenol A polycarbonate (PC), there are no fluorescence methods in the literature for analysing BPA. This may be due to the very low fluorescence intensity of this chemical in aqueous solution. The complexation ability of cyclodextrins have been extensively used over the past several years to enhance the fluorescence signal emitted from certain organic analytes such as the pesticide warfarin,[14] several hallucinogenic drugs[15] or the antibacterial agent nalidixic acid[16] as well as from inorganic species such as beryllium,[17] scandium[18] or gallium[19] by combining them with several organic reagents and including the obtained fluorescent complexes in the cavity of the cyclodextrins.

Cyclodextrins (CD) are cyclic oligosaccharides with the structure of a hollow truncated cone with a hydrophobic cavity. The three most commonly studied members of the CD family, α, β and γ-CD, have approximate inner cavity diameters of 5.0, 7.8 and 9.5 Å, respectively,[20] depending on the number of glucose residues in the molecule (6, 7 and 8 respectively). The CD molecule may accommodate appropriately sized molecules in its hydrophobic interior and thus is well suited as an organizing medium in aqueous systems. In this way one can improve two key aspects of the analysis: selectivity, because of the particular fit of each compound with the CD, mainly governed by size and polarity; and sensitivity, because the inclusion process enhances fluorescence emission as a consequence of the protected microenvironment that precludes non-radiating deactivation.

This study involves the development of a spectrofluorimetric method for the determination of BPA in water samples by complexation with β-CD. The results

obtained show that direct fluorimetric analysis can be a quick and alternative method for the determination of this estrogenic chemical of great environmental interest in water samples.

EXPERIMENTAL SECTION

Apparatus

Fluorescence spectra were obtained with a Perkin Elmer LS-50 spectrofluorimeter equipped with a Xenon discharge lamp (20 kw), Monk-Gillieson monochromators, a Quantic Rhodamine 101 counter to correct the excitation spectra and a Gated photomultiplier. The luminescence spectrometer was interfaced with a Mitac MPC 3000F-386 microcomputer supplied with FL Data Manager Software for spectral acquisition. The excitation and emission slits were both maintained at 5 nm. The scan rate of the monochromators was maintained at 240 $nm \cdot min^{-1}$.

All measurements were performed in a 10 mm quartz cell, maintained at 20 ± 0.5°C through the use of a thermostatic cell holder and a Braum Melsungen Thermomix 1441 thermostat.

Reagents

Stock solution of bisphenol A (Aldrich) containing 100 $\mu g \cdot ml^{-1}$ was prepared in a 100 ml volumetric flask, by dissolving 10.0 mg of this compound in ethanol 99% (v/v) (Panreac). The solution was stored in a dark bottle at 4°C, remaining stable for at least six months. Working solutions were prepared by appropriate dilution with deionized water.

Stock solutions of α-, β- and γ-CD (Sigma) 10^{-3} M were prepared by exact weighing of the reagent and dissolution in deionized water.

Sephadex QAE A-25 (Sigma) dextran type anion-exchange gel was used in the chloride form and without pre-treatment in order to avoid contamination.

Analytical Procedure

Aliquots of a BPA aqueous solution with a concentration of 1 $mg \cdot L^{-1}$ were placed in 10 mL volumetric flasks. The appropriate amount of β-CD was added to give a final concentration of 10^{-4} M. After that, the solutions were levelled off to the final volume with deionized water and the emission spectra were recorded at 20.0 ± 0.5°C between 290 and 350 nm maintaining the excitation wavelength at 225 nm. A calibration graph was constructed in the same way with BPA solutions of known concentrations.

Treatment of Water Samples

Water samples were filtered through a cellulose acetate filter (0.45 μm pore size, Millipore HAWP 04700) and collected in dark glass bottles previously cleaned with hydrochloric acid and washed with deionized water. The samples were stored at 4°C until analysis, which was performed with the minimum possible delay.[21]

Natural water samples spiked with an appropriate amount of BPA were acidified with hydrochloric acid (pH 3-5) and passed through a column which contained 0.3 g of the anion exchange gel Sephadex QAE A-25 in order to eliminate the interferences due to the anionic species presented in the samples.

In tap water samples, 1 $mg \cdot L^{-1}$ of sodium sulphite was added to remove the chlorine.

Spectral acquisition and calculation were performed in the same manner as for the Analytical Procedure.

RESULTS AND DISCUSSION

Spectral Characteristics

A study of the fluorescent properties of bisphenol A in different solvents was carried out. Bisphenol A shows two excitation maxima at 212-226 nm and 272-278 nm, respectively, and one emission maximum located between 297-308 nm in non aqueous solvents with a wide range of polarities between methanol and diethyl ether. The higher fluorescence intensity is obtained by exciting at the lower maximum wavelength in all cases. In aqueous medium the chemical shows an anomalous behaviour because only a weak excitation maximum located at 275 nm is observed.

With the aim of proposing a method for analysing BPA in water samples, the possible enhancement of the fluorescent signal by formation of BPA-inclusion complexes was considered and for this purpose some cyclodextrins were tested.

Although numerous thermodynamic and kinetic factors govern the formation of CD complexes, an essential factor is the relative physical size of the cyclodextrin and the analyte molecule. For this reason we checked the three most common CD homologues α, β and γ, finding that only β-CD produces a fluorescence enhancement. In Figure 1 the excitation and emission spectra of BPA in aqueous β-CD (a) and without CD (b) media are shown as well as the signal obtained from the blank (c), maintaining a constant emission wavelength of 306 nm and an excitation wavelength of 225 nm, respectively, to record the three scans.

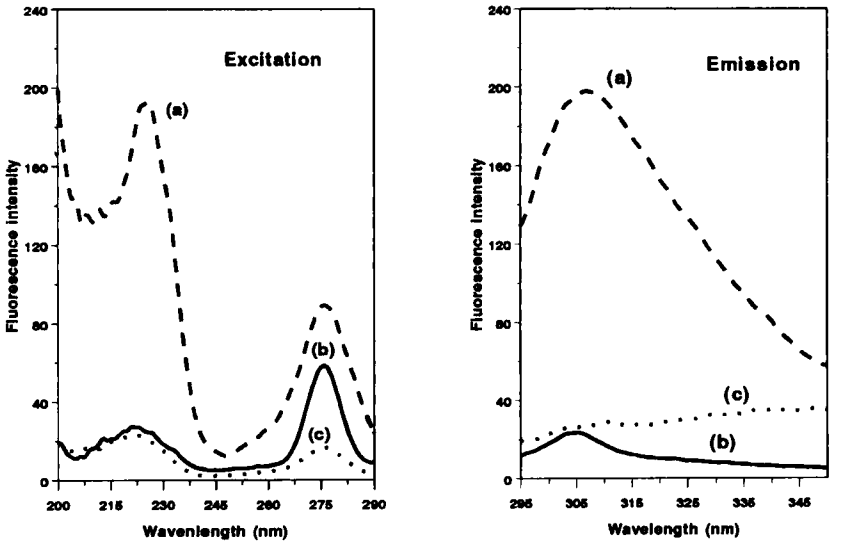

FIGURE 1 Excitation and emission spectra of BPA in aqueous β-cyclodextrin (a) and without cyclodextrin (b) media and the blank (c) at an emission wavelength of 306 nm and an excitation wavelength of 225 nm respectively.

Optimisation of Experimental Variables

pH

The behaviour of the compound was studied through the pH range in the presence of an excess of β-CD. As a phenolic compound, is a weak acid and dissociates at high pH value. The pK_a value calculated spectrofluorimetrically by the Wilson and Lester method[22] was 9.8. Although the molecule has two phenolic groups, only one step can be seen through the pH range (Figure 2). Only the acidic form shows fluorescence and the signal remains constant with respect to pH of the medium until the dissociation pH of the chemical. This meant adjustment of sample pH was unnecessary.

Ionic strength

The influence of the ionic strength on the fluorescence intensity of the inclusion complex was monitored using three different salts. The fluorescence remains independent of the ionic strength adjusted up to 1M with NaCl. The presence of $NaClO_4$ produces a decrease of the signal. Thus, for a concentration 0.4 M $NaClO_4$ the signal is 14% lower, whereas for 1 M $NaClO_4$ the reduction in the signal is 25%. When the ionic strength is adjusted with sodium acetate a marked

reduction in the fluorescent signal is observed, namely a 78.5% decrease for 0.2 M and no signal whatsoever for 0.5 M. This behaviour could be attributed to competition with the BPA for the binding sites of the cyclodextrin.[23]

Temperature

The dependence of the fluorescence intensity on the temperature was studied over the range 10.0-70.0°C. The emission signal decreased for all analytes when the temperature of the system was increased, the effect being totally reversible. All the measurements reported here were performed at 20.0 ± 0.5°C

Order of Addition of Reagents and stability of the Complex

The addition order of the reagents has no influence on the complexation and the inclusion complex is formed immediately. The fluorescence signal remain stable for at least 30 min after the reagents have been mixed.

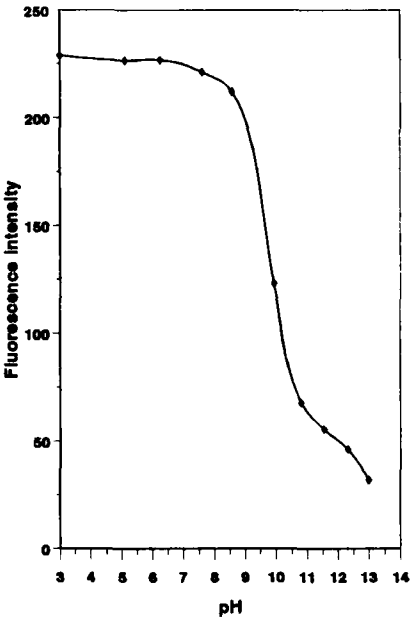

FIGURE 2 Influence of pH on the fluorescence intensity of BPA in presence of an excess of β-CD.

Influence of β-cyclodextrin concentration

The influence of β-CD concentration was tested in the range between 0 and $4 \cdot 10^{-4}$M in a final volume of 10 mL. The BPA concentration was kept constant at 50 $\mu g \cdot L^{-1}$ for all the solutions. The results obtained from the fluorescence emission spectra as the concentration of β-CD increases are shown in Figure 3.

The fluorescence emission of BPA increases with the concentration of β-CD up to 10^{-4} M, remaining constant for higher concentration, so this concentration was selected as the optimum for the analytical procedure.

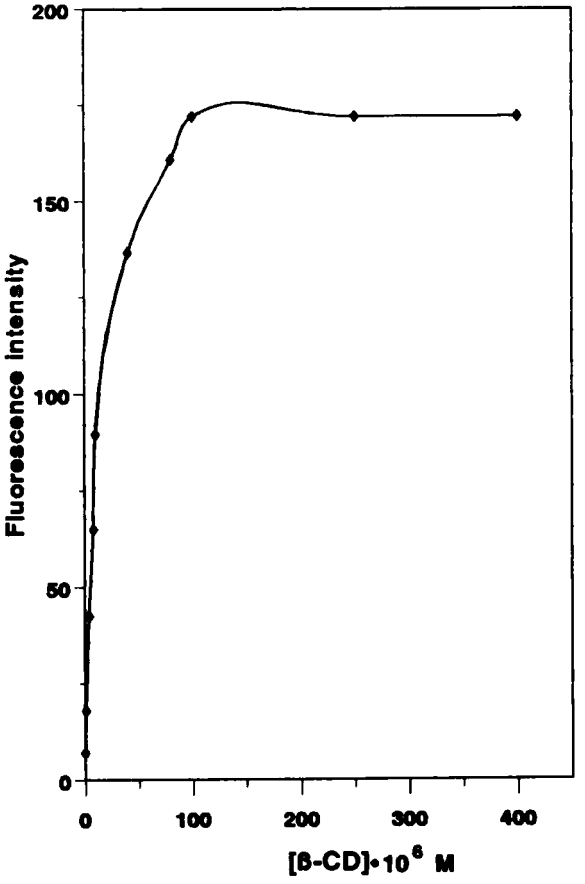

FIGURE 3 Influence of β-CD concentration on the fluorescence intensity of BPA.

Stoichiometry Ratio and Association Constant of the Inclusion Complex

Usually the Benesi-Hildebrand method provides the necessary information concerning the stoichiometry and association constants[24, 25] using spectroscopic measurements.

The equilibrium constant for a 1:1 complex between BPA and β-CD is given by

$$K = \frac{[BPA.\beta - CD]}{[BPA][\beta - CD]}$$

where [BPA.β-CD] is the equilibrium concentration of the complex, with [BPA] and [β-CD] representing equilibrium concentrations of the unbound guest and host species, respectively. This analysis requires that the concentration of the BPA.β-CD be kept very much lower than the concentration of β-CD, therefore it can be assumed that $[\beta\text{-CD}]_0 = [\beta\text{-CD}]$ and the following equation can be obtained from the mass-balanced expressions, since the fluorescence intensity of BPA in the absence (I_0) and presence (I_∞) of β-CD is proportional to [BPA] and [BPA.β-CD] respectively:

$$\frac{1}{I - I_0} = \frac{1}{I_\infty - I_0} + \frac{1}{k[\beta - CD]_0(I_\infty - I_0)}$$

Thus a plot of $1/I\text{-}I_0$ vs. $1/[\beta\text{-CD}]$ should give a straight line for a 1:1 complex as can be seen in Figure 4. Likewise for a 2:1 complex, the overall equilibrium constant is given by:

$$K = \frac{[BPA.\beta - CD_2]}{[BPA][\beta - CD]^2}$$

and an expression for the 2:1 complex can be derived,

$$\frac{1}{I - I_0} = \frac{1}{I_\infty - I_0} + \frac{1}{k[\beta - CD]_0^2(I_\infty - I_0)}$$

In this case, a plot of $1/I\text{-}I_0$ vs. $1/[\beta\text{-CD}]^2$ should be linear for a 2:1 complex. However a downward curvature is obtained in the plot showed in Figure 5. This result indicates the absence or insignificant contribution of a 2:1 complex between β-CD and BPA

The association constant of the 1:1 complex is determined by the ratio of the intercept and the slope of the straight line obtained in the double-reciprocal plot. The value obtained for the BPA- β-CD complex was $1.3 \cdot 10^5$ L·mol^{-1}.

Analytical Parameters

Under the conditions stated in the analytical procedure, a satisfactory linear relationship exists between the analytical signal and BPA concentration at 10.0-200.0 μg·L^{-1}. Three replicates were used for each one of 7 standards prepared to obtain the calibration graph.

Precision was measured by performing 10 independent determinations of a 100 μg·L^{-1} BPA sample. The relative standard deviation (RSD p = 0.05, n = 10)

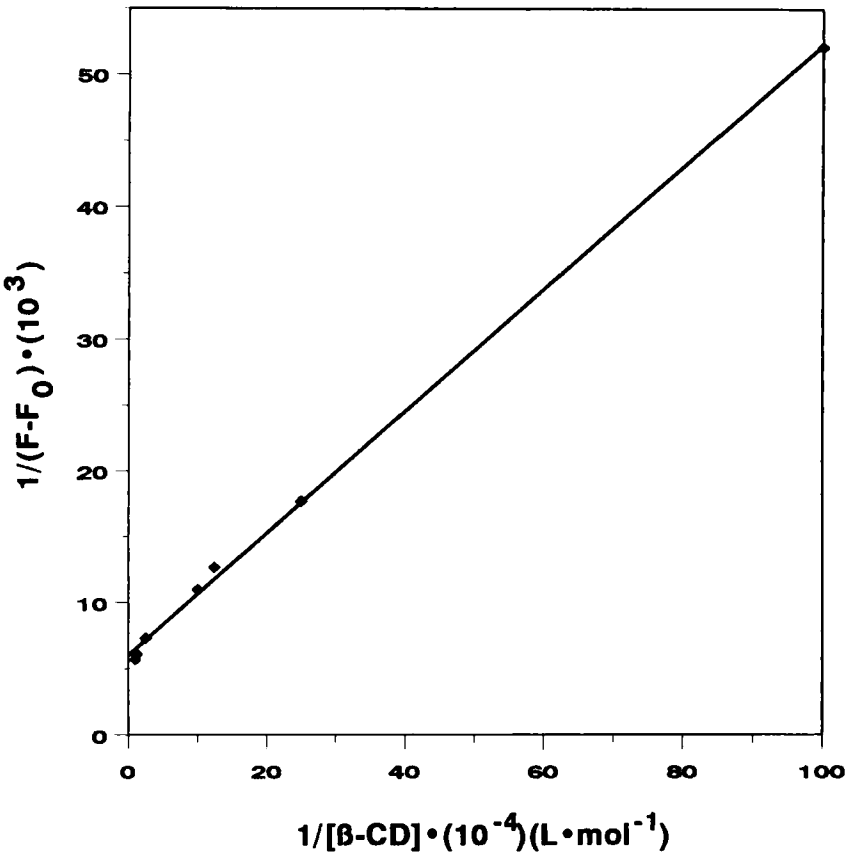

FIGURE 4 Benesi-Hildebrand plot for the BPA-β-CD complex assuming a 1:1 stoichiometry.

was 0.92%. The sensitivity of this method, obtained from the slope of the calibration graph, was 2.9 $L \cdot \mu g^{-1}$. The International Union of Pure and Applied Chemistry detection limit[26] (k = 3) was 0.5 $\mu g \cdot L^{-1}$ and the quantitation limit[27] (k = 10) was 1.7 $\mu g \cdot L^{-1}$.

Effect of Foreign Species

To evaluate the method selectivity, a systematic study of the effect of foreign species usually present in water samples on the determination of BPA at 50 $\mu g \cdot L^{-1}$ was carried out. Tolerance level was defined as the amount of foreign species which produces an error not exceeding ±5% in the determination of the analyte. Potentially interfering species were tested at different concentration

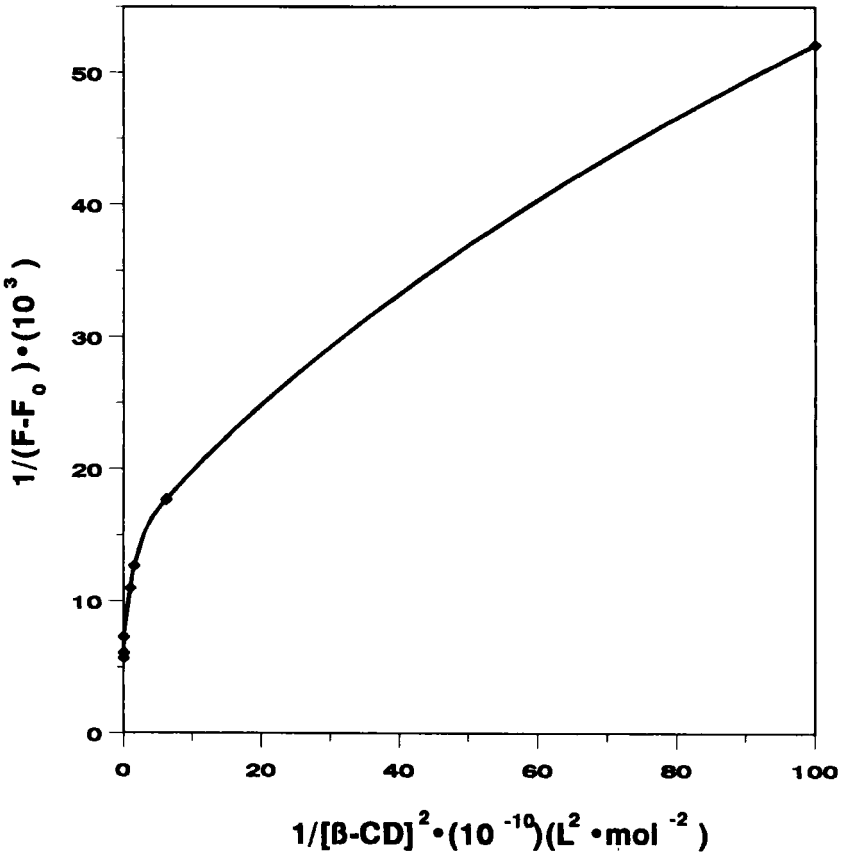

FIGURE 5 Benesi-Hildebrand plot for the BPA-β-CD complex assuming a 1:2 stoichiometry.

TABLE I Recovery study of bisphenol A in water.

WATER SAMPLE	Added ($\mu g \cdot L^{-1}$)	Found* ($\mu g \cdot L^{-1}$)	Recovery (%)
	40.0	39.0	97.5
Tap water	80.0	77.3	96.6
	120.0	119.8	99.8
	40.0	42.0	105.0
River water	80.0	81.8	102.0
	120.0	119.5	99.6
	40.0	40.8	102.0
Sea water	80.0	81.9	102.4
	120.0	118.6	98.8
	40.0	40.6	101.5
Underground	80.0	79.9	99.9
water	120.0	119.6	99.7

* Data based on the average obtained from three determinations

levels depending on their normal concentrations in the waters analysed. If an interference occurred, the ratio was progressively reduced till the interference ceased.

Interference due to cations at higher levels than is usual in water (1 $mg \cdot L^{-1}$) was not detected. The most serious interferences were due to the presence of nitrates with a tolerance of 1 $mg \cdot L^{-1}$; this level may be found in underground water from fertile plains. Chlorine, usually present in tap water, also interferes. The nitrate interference could be avoided by passing the water samples through a column of an anionic gel (Sephadex QAE A-25). The samples of tap water were previously treated with the appropriate amount of sodium sulphite to remove the chlorine.

Applications of the Method

To check the accuracy of the proposed method, a recovery study was carried out on various types of water samples. Tap water from the supply to Granada City (Spain), river water from Loja (Granada, Spain), sea water from Motril (Granada) and underground water from a well in the aquifer of the fertile plain of Granada were analysed after adequate additions of BPA. With tap water, standard addition was used for the recovery study.

The results are summarised in Table I and show that the recoveries are acceptable. Fortunately, prior to our deliberate spiking with BPA, these waters

are apparently free of this pollutant within the low limit detected by our method.

Acknowledgements

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