Analytical Methods

Enantioseparation of the fungicide imazalil in orange juice by chiral HPLC. Study on degradation rates and extractive/enrichment techniques

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Abstract

Imazalil ([1-[(β-allyloxy-2,4-dichlorophenethyl)imidazole]] is a systemic chiral fungicide used in postharvest protection of citruses against fungus development for during storage and transportation. The chemical structure of imazalil shows an asymmetric carbon in the C7 position. These enantiomers may have different toxicity. A method for both chiral enantiomers extraction and determination in orange juice is developed in order to provide their concentration and to study the degradation rates in orange juice. Spiked imazalil was extracted from orange juice by dispersive liquid–liquid micro extraction and solid phase extraction. Recovery assays of imazalil enantiomers from spiked orange juice samples showed that solid phase extraction is a better choice in order to obtain higher recovery values. Obtained chromatographic data show that within 24 h the (−)-imazalil enantiomer decreases from 0.548 to 0.471 (expressed as enantiomer fraction).

1. Introduction

Enantioselectivity is important in the field of agrochemicals as a result of their interaction with enzymes or other naturally occurring chiral molecules (Zhong et al., 2012). Enantiomers usually differ in their rates of biochemical transformation, leading to a preferential persistence of the enantiomer or stereoisomer which react more slowly (Buerge, Poiger, Müller, & Buser, 2006; Garrison, 2006; Tian, Zhou, Ly, & Yang, 2012). Although toxicity is primarily related to structure and configuration of the active ingredient, toxicity is also the result of an enzymatic reaction, a chiral pesticide often exhibits enantioselective or stereoselective toxicity in plants, animals or humans (Jortani, Valentour, & Po, 1994; Sun et al., 2012; Yen, Tsai, & Wang, 2013). This differential reactivity may lead to variations in microbial degradation rates, metabolic pathways, biological uptake and toxicity. Thus, an important parameter involved in the environmental fate of a pesticide such as its persistence, is affected by the different characteristics of each enantiomer. In the case of imazalil (IM), recent published data (Baagnall, Malia, Lubben, & Kasprzyk-Horden, 2013; Chu et al., 2007; Qin, Budd, Bondarenko, Liu, & Gan, 2006) showed that the (−) isomer degrades quickly. On the other hand, (Nelson et al., 2001) showed that the (−)-S enantiomer was as effective at a given concentration as was the (+)-R enantiomer at ten times the same concentration against Aspergillus nidulans. ED50 (estimated concentration required to produce 50% killing of fungi) of (−)-S enantiomer was lower than that of (+)-R enantiomer, supporting the hypothesis that (−)-S enantiomer shows higher toxicity than the (+)-R enantiomer.

Chiral methods are required for the analysis of enantiomers. Two possible approaches are a chiral chemical microenvironment (in HPLC a chiral column, or in capillary electrophoresis (CE) a chiral selector) and chiral optical detection (circular dichroism (CD) for compounds with a UV chromophore, or optical rotation if no chromophore exists) (Sánchez, Diaz, & Lama, 2008). Enantiomeric separation of imazalil (IM) has been performed by CE (Kodama, Yamamoto, Ohura, Matsuymaya, & Kane, 2003) and the higher degradation rate in oranges is suggested to be in the case of the (−)-IM.

In postharvest protection of citruses against fungus development during storage and transportation, IM is commonly used in commercial formulation with thiabendazole (Yoshioka, Akiyama, & Teranishi, 2004; Yoshioka; Akiyama, Matsuoka, & Mutsushashi, 2010). These fungicides are highly effective in controlling both Penicillium sp. and Geotrichum sp. among other fungal pathogens of fruit (Eckert & Ogawa, 1988). EU (European Union) requirement...
for IM residue in orange (EU pesticide databases, Regulation 1107/2009)) is 5 mg kg⁻¹.

The general methods used to analyse these fungicides and their metabolite residues in citrus fruits are similar to those used for other pesticides in fruits and vegetables, i.e. a simple solvent extraction procedure and analysis with LC-MS (Yoshiohka, Akiyama, & Teranishi, 2004). Other protocols use solid-phase extraction (SPE) and capillary gas chromatography (Garrido, de Alba, Jiménez, Casado, & Folgueiras, 1997; Matsumoto, 2001), or ion-exchange cartridge for sample clean-up followed by ion-pair HPLC-UV (Ito, Ikai, Oka, Hayakawa, & Kagami, 1998). SPE and micellar electrokinetic chromatography (MEKC) have been used for the simultaneous determination of IM, thiabendazole and related fungicides in fruits and vegetables (Rodríguez, Picó, Font, & Mañes, 2001).

Immunofinity purification of residual IM in citrus fruits employing anti-imazalil monoclonal antibodies, showed an effective and specific clean-up technique for isolation and concentration (Watanabe, Yoshimura, Yuasa, & Nakazawa, 2001).

IM and related chiral molecules, presented as racemic mixtures in formulations, have been directly quantified by capillary electrophoresis, previous SPE clean-up procedures (Kodama et al., 2003) and LC without previous concentration treatments (Tian, Zhou, Lv, & Yang, 2012). Recently, ultrasound-assisted emulsification micro-extraction (USAEME), a miniaturized extraction method, has been reported as an efficient clean-up procedure for the determination of imazalil in river water by HPLC (Gao et al., 2012). Nowadays, the use of these micro-extraction procedures, classified as liquid phase micro-extraction (LPME) (Rezaee et al., 2006) allow, due to the very small amounts of solvent and sample required, a more eco-friendly analytical method, such as dispersive liquid–liquid micro-extraction (DLLME), which employs a ternary solvent system consisting of an aqueous sample, extractant and disperser in this method, an appropriate mixture of solvents for extraction and dispersion is injected rapidly into an aqueous sample with a syringe, resulting in the formation of a turbid solution which causes a large increase in the contact surface between phases and quickly establishes balance. After a centrifugation step, the organic solvent is deposited at the bottom of the vial and recovered for analysis.

The aim of this study was to assess DLLME suitability for the extraction/determination of imazalil in orange juice samples by HPLC-Chiral detection. The effects of different experimental parameters on the performance of the sample preparation step using DLLME were also studied, optimized and compared with the SPE procedure.

Moreover, in order to improve the knowledge of the persistence (or the enantiomeric ratio) of IM (that are essential data to avoid toxic risks in agricultural foods consumption) degradation rates have been studied. In a short period of time, 24 h, the (−)-IM enantiomer has a higher degradation rate (Lei, Ye, and Wang, 2001; Sarom, Miles, Harría, and McEwen, 1980)

2. Experimental

2.1. Chemicals and reagents

Imazalil, [((RS)-1-[(ß-allyloxy-2,4-dichlorophenethyl]imidazole], 94.5% (IM)] (see Fig. S1 in the Supporting information) was purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany); thiabendazole [4-(1H-1,3-benzodiazol-2-yl)-1,3-thiazole, 99%] as interfering analyte was purchased from Riedel de Hāen (Seelze, Germany). Deionized water used in the experiment was purified with a Milli-Q SP system (Millipore, Bedford, MA, USA), and used through the experiment. All the reagents used in this study were of analytical grade and all solutions were filtered through a 0.2 μm nylon membrane filter (Nylafo, Pall Corp. Ann Arbor, MI, USA) and degassed before their use. Isopropanol, dichloroethane, dichloromethane, tetrachloromethane, acetonitrile, acetone, ethanol and methanol were gradient grade for LC (Merck, Darmstadt, Germany). Sodium phosphate monobasic monohydrate (Panreac, Barcelona, Spain) was used to prepare a 50 mM phosphate buffer pH 4. SPEs were performed using LiChrolut RP-18 cartridge (40–63 μm, 500 mg and 3 mL standard PP-tubes (Merck KGaA, Darmstadt, Germany).

IM standard solution was prepared by dissolving 8 mg of standard IM in methanol (20 mL) for enantiomeric analysis. Pesticide analyses by DLLME and SPE were carried out with aliquots of this solution prepared with water to obtain a concentration of 100 μg mL⁻¹, and kept in absence of light at 4 °C in a refrigerator.

2.2. Apparatus

Chromatography analysis utilized a Jasco (Tokyo, Japan) liquid chromatograph equipped with a Degassy Populaire DP4003, a Jasco Intelligent pump model PU-1580, a Jasco L-C-1580-04 quaternary gradient unit, a Jasco intelligent auto sampler model AS-2055 Plus with a 100 μL sample loop, a Jasco interface modulated model LC-Net II/ADC, chiral detector Jasco CD-2095 equipped with a Hg–Xe lamp (150 W), a Glan–Taylor polarizer prism, a standard tapered flow cell of 25 mm path length, and a Monk–Gillieson mounting monochromator with Jasco OR-2090 chiral detector. Data acquisition, transformation and instrument parameters were accomplished by Jasco-Borwin 1.5 software. The integration was carried out with Jasco-Borwin 1.5 software, ChromConver 1.0 and Microsoft Origin for the calculations of areas (negative and positive peaks), the peaks heights and retention times. The ultrasonic water bath model Ultrasons (50 kHz) and centrifuge were purchased from Selecta (Barcelona, Spain). The extraction tubes were set with a LiChrolut™ SPE Vacuum Manifold system purchased from Merck, coupled to a vacuum pump 1C (Vacuubrand GMBH, Wertheim, Germany).

2.3. Chromatographic conditions

A chiral column ChiraDex® (5 μm) (beta-cyclodextrin based chiral selector) was utilized (LiChroCAR® 250–4, HPLC-Cartridge, E. Merck, Darmstadt, Germany). 1% (v/v) isopropanol and 99% (v/v) phosphate buffer solution pH 4 with a flow-rate of 1 mL min⁻¹, in isocratic mode, were used as the mobile phase (see Table S3 in the Supporting information). The injection volume was 5 μL. CD and UV–vis measurements, were performed at 225 nm.

2.4. Orange juice sample preparation

Samples (6 mL) of freshly squeezed orange juice containing 10–80 μg of spiked (+)-IM (25–200 μL⁻¹ of 400 μg mL⁻¹ (+)-IM methanol standard solution) were placed in a 10 mL glass tube with a conical bottom. Each sample was centrifuged for 20 min at 5390 rpm. The precipitates extracted from the sample matrix were formed on the bottom centrifugation tube. These precipitates were removed and the aqueous phase was analyzed by DLLME or SPE procedures.

2.5. Dispersive liquid–liquid microextraction procedure

Orange juice sample without co-precipitate (6 mL) or an aqueous standard solution (6 mL) was placed in a 10 mL glass tube with a conical bottom. Acetonitrile (1.5 mL) as disperser solvent containing tetrachloromethane (120 μL) as the extraction solvent was rapidly injected. Here, a cloudy solution (water/acetonitrile/...
tetrachloromethane) was formed in the test tube and the fungicide in the aqueous phase was extracted into fine tetrachloromethane droplets. The resultant cloudy solution was centrifuged for 18 min at 5390 rpm. The denser phase was removed with a syringe. A second extraction was performed on the remaining aqueous phase of the first DLLME extraction.

The denser phase of the two extractions was leveled up to 1 mL with methanol and injected 5 µL into HPLC (for optimization of DLLME parameters). An aliquot (20 µL) of the denser phase, leveled up to 1 mL with methanol, was injected (for method validation and sample analysis).

2.6. Solid phase extraction procedure

After preconditioning the cartridges (1 mL methanol), 6 mL of orange juice sample without co-precipitates was passed through the cartridge. After that, SPE cartridges were air dried for 2 min. The retained analytes were eluted with tetrachloromethane (240 µL) followed by methanol (0.5 mL). The eluted (20 µL) was injected.

2.7. Degradation rate of IM enantiomers in orange juice

The enantiomeric fraction (EFR) and concentration of IM enantiomers in orange juice were measured at predetermined intervals of time within 24 h. The best equation to describe the process must be related to a second order kinetic that can be reduced to a pseudo-first-order kinetic. Data analysis to characterize the degradation rate and the existence of change in the enantiomeric composition was estimated using the pseudo-first-order kinetic expression:

\[ C = C_0 \cdot e^{-kt} \]  

where \( C_0 \) and \( C \) are the concentrations of the tested chemicals at times 0 and time \( t \), and \( k \) the rate constant of the degradation process. The half life (\( T_{1/2} \)) was further calculated as \( T_{1/2} = \ln(2)/k = 0.693/k \).

For the degradation experiment, three samples, each containing 100 µg mL\(^{-1}\) of racemic imazalil standard, were analyzed. One of them was submitted to the general procedure of separation and calculated the enantiomeric fraction (EFR) (Harner, Wiberg, & Norstrom, 2000), defined as EFR = \( E^-/E^- + E^+ \), where \( E^- \) and \( E^+ \) denote the peak area of the (−) and (+) enantiomer. The other two samples were used as spiked standard in the orange juice samples and submitted to the DLLME extraction and separation procedures, one of them after 12 h of digestion with orange juice at 4 °C of temperature and in the dark, and the other after 24 h of digestion.

The obtained results show that the (−)-enantiomer has a higher degradation rate, confirming obtained data from other studies (Baagnall et al., 2013; Qin et al., 2006) and these results are useful for the safety time before being consumed (Lei, Ye, & Wang, 2001; Sarom et al., 1980).

2.8. Method validation

The analytical method was validated in terms of linearity, detection and quantification limits, sensibility, accuracy and precision. Identification of the sign of the IM enantiomers was performed using the different sign (bimodal character of the chiral CD signal) and the elution order of the two chromatographic peaks. The calibration curve using DLLME-LC was obtained by least-squares linear regression analysis of the peak area versus concentration of (±)-IM, (−)-IM and (+)-IM, using six levels and UV–vis measurements (because LOD and LOQ for the CD detector are greater than UV–vis detector). The ability to discriminate small differences in enantioselective concentration was evaluated by analytical sensitivity (\( S_a = \sigma_{n-1}/m \)), where \( m \) is the slope of the calibration graph and \( \sigma_{n-1} \) is the standard deviation of the analytical signal. LOD values were calculated using the expression 3 \( \sigma_{n-1}/m \) and LOQ using 10 \( \sigma_{n-1}/m \). The precision expressed as % relative standard deviation (%RSD) was calculated from the results of three replicate analyses of standards. The operation of the extraction was validated by the concentration enrichment factor (EF): \( EF = C_{DP,final}/C_{DP,initial} \), where \( C_{DP,initial} \) and \( C_{DP,final} \) are the initial analyte concentration in the donor phase (DP) and the final analyte concentration in the acceptor phase (AP), respectively. The %RSD was calculated from the calibration curves by directly injecting different concentrations of standards into the HPLC system.

The extraction time was measured as the time from the injection of the homogeneous mixture of extraction and dispersive solvents to the aqueous standard solution until the centrifuge is stopped.

3. Results and discussion

3.1. Chromatographic separation

Initially, it was observed that the best separation profile was achieved with 50 mM of buffer solution, which covered the pH 3–4. With this and several organic additives (methanol, ethanol, isopropanol, acetonitrile) in different proportions, separate experiments were carried out and the obtained results were summarized in Table 1. The best \( R_s \) (1.435) and retention times (14.7 and 19.4 min) for (−) and (+)-IM, respectively were obtained at pH 4 with isopropanol:buffer (1:99, v/v). The elution order and sign of the enantiomers were determined by the CD detector obtained peaks. The \( R_s \) values were obtained by applying the equation:

\[ R_s = 2(\text{tr}_1 - \text{tr}_2)/\text{W}_1 + \text{W}_2 \]  

where \( \text{W}_1 \) and \( \text{W}_2 \) are the peak baseline width and tr1 and tr2 are the retention times of the enantiomers.

Fig. 1 shows the chromatograms obtained using UV–vis (Fig. 2a) and CD (Fig. 2b) detection methods for the separation of (−) and (+)-IM. The UV–vis and CD detectors respond linearly to the injected (±)-IM in the concentration range 25–450 µg mL\(^{-1}\). In all cases, the linear fit was R > 0.97.

The percentages (\( \% \)) of each enantiomer in the mixture from peak areas (Sánchez, Díaz, & de Vicente, 2008; Sánchez, Díaz, Lama, Aguilar, & Algarra, 2014; Sánchez et al., 2012) were obtained using the equations:

\[ \%(-) - IM = \left[ \frac{b_{(-)}}{b_{(-)} + b_{(+)}} \right] \times 100 \]  

\[ \%(+)-IM = \left[ \frac{b_{(+)}}{b_{(-)} + b_{(+)}} \right] \times 100 \]  

where \( b_{(-)} \) and \( b_{(+)} \) are the slopes (calibration graph from area under peaks) obtained for (−) and (+)+IM, respectively. Obtained results show that the % (−)-IM is higher than % (+)-IM (52.30 and 47.70, respectively). This implies that the commercial product is not exactly a racemic mixture.

3.2. DLLME parameters

The different parameters involved in DLLME procedure such as extraction and dispersive solvents, their volumes, number of extractions and centrifugation time were optimized. For this purpose, aqueous standard solutions of 50 µg mL\(^{-1}\) (±)-IM was used.
3.3. Selection of extraction and dispersive solvents

High extraction capability for target compounds, higher density than water, low solubility in water and good chromatographic behaviour are the main characteristics of the extraction solvent to be considered. The dispersive solvent must be miscible in both phases, aqueous and extraction solvent (Ghambari & Hadjmohammadi, 2012; Shamsipur & Fattahi, 2011; Xiong, Ruan, Cai, & Tang, 2009).

Dichloroethane, dichloromethane and tetrachloromethane, as extraction solvents (100 mL) and, acetonitrile and acetone as dispersive solvents (1 mL) were tested in aqueous standard solutions of 50 μg mL⁻¹ IM (6 mL) for 8 min. After this, the sedimented phase was leveled to a final volume of 1 mL methanol with 5 μL injected for analysis. Tetrachloromethane exhibited the best extraction efficiency and acetonitrile demonstrated the best dispersive results.

3.4. Optimization of extractive and dispersive solvents

First, extractive solvent volume was optimized. It was studied for different volumes of tetrachloromethane (in the range of 40 to 120 mL) remaining invariable the volume of dispersive phase (1 mL acetonitrile) (see Fig. S2 in the Supporting information). The sedimented phase was made up to 1 mL of methanol and measured peak area (5 μL injected in HPLC). Peak areas increased with increasing volume of tetrachloromethane from 40 to 80 mL; 100 and 120 mL confirmed higher extraction efficiency, and larger volumes than 140 mL resulted in the decrease in peak areas. So 120 mL appeared the most appropriate volume for extracting the analyte extraction.

The final volume of acetonitrile (the dispersive phase) was optimized by varying the volume from 0.5 to 1.5 mL. A second extraction was performed on the remaining aqueous phase for each of the volumes of assayed acetonitrile. The sedimented phase for each extraction was leveled up to 1 mL with methanol and measured areas of peaks (5 μL injected in HPLC). For the first extraction, it was observed that the peak area increases with increasing the volume from 0.5 to 1.5 mL of acetonitrile. The peak areas for the second extraction decrease with increasing the volume of acetonitrile from 0.5 to 1.5 mL. Moreover the same experiment was performed using a volume of 2 mL of acetonitrile, and demonstrated that the first extraction has the highest extraction efficiency. However, a volume of 2 mL was excluded from further experiments because it due difficulty obtaining the drop as a consequence of dilution.

3.5. Extraction time

Different extraction times (8, 10, 12, 15, 18, 20 min) were studied. Obtained results show that when the extraction time is greater the peak area increases. The extraction efficiency was constant from 18 min.

3.6. Interference study

In the food industry, IM is frequently used in formulations together with thiabendazole as fungicides (for example, the commercial product FRUITGARD-70: imazalil 10% and thiabendazol 14% p/v) (Registro de productos fitosanitarios, 2009. N° Reg., 19106). For this reason an interference study has been made. 6 mL of four aqueous standard solutions containing concentration between 10 and 40 μg mL⁻¹ imazalil (52.30% of (−) and 47.70% Table 1

<table>
<thead>
<tr>
<th>pH = 4</th>
<th>Buffer</th>
<th>Methanol:Buffer (v/v)</th>
<th>Ethanol:Buffer (v/v)</th>
<th>Isopropanol:Buffer (v/v)</th>
<th>Acetonitrile:Buffer (v/v)</th>
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<tbody>
<tr>
<td>t_R (min)</td>
<td>20.2</td>
<td>24.3</td>
<td>22.4</td>
<td>20.8</td>
<td>21.8</td>
</tr>
<tr>
<td>t_R (+)</td>
<td>27.2</td>
<td>32.3</td>
<td>29.6</td>
<td>27.5</td>
<td>29.1</td>
</tr>
<tr>
<td>R_s</td>
<td>1.092</td>
<td>1.342</td>
<td>1.333</td>
<td>1.209</td>
<td>1.387</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH = 3</th>
<th>Buffer</th>
<th>Methanol:Buffer (v/v)</th>
<th>Ethanol:Buffer (v/v)</th>
<th>Isopropanol:Buffer (v/v)</th>
<th>Acetonitrile:Buffer (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t_R (min)</td>
<td>27.7</td>
<td>19.8</td>
<td>18.8</td>
<td>17.4</td>
<td>15.5</td>
</tr>
<tr>
<td>t_R (+)</td>
<td>37.1</td>
<td>26.7</td>
<td>25.2</td>
<td>23.3</td>
<td>20.1</td>
</tr>
<tr>
<td>R_s</td>
<td>0.954</td>
<td>1.284</td>
<td>1.228</td>
<td>1.239</td>
<td>1.261</td>
</tr>
</tbody>
</table>

Fig. 1. (a) UV–vis chromatogram and (b) CD chromatogram of a 200 μg mL⁻¹ Imazalil standard solution (5 μL injected in HPLC) using isopropanol-buffer solution (1:99, v/v) as mobile phase.
3.7. DLLME validation method

The calibration curve using DLLME-LC was obtained by least-squares linear regression analysis of the peak area versus imazalil commercial concentration and enantiomers concentration, using six levels and UV–vis measurements (LOD and LOQ for the CD detector are greater, which is why they were not used). The values of correlation were \( R > 0.995 \), demonstrating the good linearity for the range studied (10.0–80.0 µg mL\(^{-1}\) for the sum of both enantiomers, and 5.0–40.0 µg mL\(^{-1}\) for each enantiomer). EF values were 23 ± 1 ((±)-IM), 23 ± 3 ((−)-IM) and 18 ± 1 ((+)-IM). The concentrations of analytes were determined by the proposed DLLME procedure and calculated from the standard curve. All results were expressed at mean values of three replicates.

3.8. Samples analysis

The samples were treated as indicated in DLLME and SPE procedures, and the obtained results were ordered in Table 2. The extracted precipitates were removed from the aqueous phase by decantation, and the aqueous phase was applied to SPE procedure. The peak area for each enantiomer was measured using UV–vis detector [20 µL injected in HPLC]. Table 2 shows the recovery percentages for each enantiomer. As can be seen, obtained recovery values using SPE give percentages near 100% and is preferred to DLLME.

3.9. Comparison between DLLME with SPE

In order to assess the extraction efficiencies of the HPLC method of IM, the SPE method was compared with DLLME procedure from the viewpoint of linear range, detection and quantification limits, sensitivity, accuracy, precision, recovery and extraction time (Table 3). SPE showed lower analytical sensibility and RSD in comparison with DLLME. The limit of quantification (LOQ) obtained from the method is comparable to or better than the DLLME method and the enrichment factor (EF) for the SPE is greater than DLLME. In addition, single extraction was carried out using the SPE method, with a mean recovery ± standard deviation of 105.6 ± 6.5%, 88.7 ± 10.3% and 98.7 ± 5.1% for (−), (+) and (±)-IM, respectively. As can be seen recovery of (−)-IM was higher than 100% in some cases. Something probably co-extracted from orange juice eluted first with the (−)-IM (first eluted peak). Using the DLLME method two extraction steps were applied with a mean recovery ± standard deviation of 72.3 ± 5.6%, 66.6 ± 2.7% and 70.4 ± 2.7%, for (−), (+) and (±)-IM. The DLLME method required relatively long extraction time to obtain the highest extraction efficiency (36 min, 18 min for each extraction). The samples treatment and total time preparation are the same for both methods, about 20 min. As can be seen in Table 3, the efficiency of the SPE extraction is higher than that of DLLME.

![Fig. 2. Chromatograms of (±)-IM (5 µL of 100 µg mL\(^{-1}\)) previously submitted to DLLME in orange juice (b, c) or freshly prepared (a). After 0 h (a), after 12 h (b) and after 24 h of incubation (c).](image-url)

Table 2

Recovery (%) of (−)-IM and (+)-IM analyzed with DLLME and SPE in orange juice.

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyte</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 µg mL(^{-1})</td>
</tr>
<tr>
<td>DLLME</td>
<td>(−)-IM</td>
<td>78.1</td>
</tr>
<tr>
<td></td>
<td>(+)-IM</td>
<td>62.0</td>
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<tr>
<td></td>
<td>(±)-IM</td>
<td>69.9</td>
</tr>
<tr>
<td>SPE</td>
<td>(−)-IM</td>
<td>116.4</td>
</tr>
<tr>
<td></td>
<td>(+)-IM</td>
<td>81.8</td>
</tr>
<tr>
<td></td>
<td>(±)-IM</td>
<td>97.9</td>
</tr>
</tbody>
</table>
were ordered in Table 4. The data showed an acceptance rate than (+)-IM. The obtained kinetic data, reaction rate

and are environmentally friendly methods because the consumption of toxic organic solvents was minimized and was low too. Furthermore, these methods were not interfered by thiabendazole, a fungicide frequently used in formulations containing imazalil. The elution orders of the eluting enantiomers were determined by a CD detector. Degradation rates of the enantiomers were determined and showed that (−)-IM degrades more quickly than (+)-IM, as the result of an enantioselective transformation process.

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.01.004.

### References


