An enantioselective high-performance liquid chromatography-mass spectrometry method to study the fate of quizalofop-P-ethyl in soil and selected agricultural products

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ABSTRACT

In this study, the attention was focused on quizalofop-ethyl, a chiral herbicide whose formulation has recently been marketed as quizalofop-P-ethyl, i.e. the (+)-enantiomer exhibiting herbicidal activity. To verify the real enantiomeric purity of this product as well as to study its environmental fate, the enantioselective separation of the P- and M- enantiomers of quizalofop-ethyl was achieved on Lux Cellulose-2 column (3-chloro,4-methyl-phenilcarbamate cellulose) under isocratic conditions in polar organic mode. Once established that the commercial formulation contains ˜0.6% (enantiomeric fraction) of M as an impurity, an HPLC-MS/MS method was developed, validated and applied to the analysis of soil, carrots and turnips treated with the herbicide. A simple solid-liquid extraction allowed recoveries greater than 70%; limits of detections of P and M enantiomers were below 5 ng g^{-1}. The analyses of the real samples showed a modification of the enantiomeric fraction of quizalofop-M-ethyl between the commercial formulation ($EF_M = 0.63 \pm 0.03\%$) and the analysed matrices ($EF_M = 7.6 \pm 0.1\%$ for carrots; $EF_M = 0\%$ for the other matrices). This outcome highlighted the occurrence of an enantioselective biotic dissipation, responsible for a greater persistency of the distomer in carrots. On the other hand, since screening analyses revealed the occurrence of residues of the metabolite quizalofop-acid with the same EFs as the ester precursor, it was concluded that the hydrolytic conversion was an abiotic process.

1. Introduction

In 2020, pesticides were the world's 85th most traded products and about a third of them were chiral compounds [1]. Currently, more than 1500 chiral pollutants are present in the environment [2]. For many of these substances, a transition to more effective and less persistent substances has been made so that their rapid degradation, through biotic and abiotic processes, can mitigate the environmental pollution [3–6]. Biodegradations are enantioselective processes, promoted by microorganisms or enzymes in plants and animals, which tend to modify the chiral signature of parent compounds [7,8]: the accumulation of the most persistent enantiomer can be explained by slower enzymatic degradation [9]. If a degradation mechanism for a certain pesticide is known, it is possible to minimize the so-called isomeric ballast, i.e. the useless environmental pollution due to the less active/inactive enantiomer and diasteroisomers (distomers), in the case of molecules with multiple stereogenic centres [10]. If containing the only active enantiomer (eutomer), the commercial pesticide formulation limits the environmental pollution to the indispensable amount of eutomer useful for the purpose [11].

In this study, the attention was focused on quizalofop-ethyl (ethyl 2-{4-[6-chloroquinoxalin-2-yloxy]phenoxy}propionate), an herbicide widely used to control grass weeds in crops of tomatoes, beans, carrots, and potatoes (see Fig. S1 in the Supplementary Material) [12]. The molecule has a chiral centre on the α carbon of the ester and, recently, its formulation has been marketed as an enantiomerically pure form,

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composed of the R-(+)-enantiomer (i.e., the eutomer exhibiting herbicide activity, named "enantiomer P"). In fact, the (R)-enantiomer is a more potent inhibitor against acetyl-CoA carboxylase in chloroplasts of target weeds thus exhibiting higher herbicidal activities [13]. Chiral free acids of aryloxypropanoate herbicides (e.g., quizalofop acid) are active forms too [14,13] and they can rapidly be formed via hydrolysis of the precursor esters in plants [15], animals [16] and environment media [17]. Regarding toxicity, quizalofop-ethyl has been found to cause reproductive issues [13] and genotoxicity [18]. However, based on a report of California Food and Environment Protection, also its acid metabolite is highly toxic to aquatic organisms [19] and it might be responsible for potential ecological risks [20].

The major aim of the present research was to follow the fate and degradation processes that may affect the P- and M-enantiomers of quizalofop-ethyl in a group of environmental matrices (soil, carrots and turnips). Since the study involved the use of a single-enantiomer formulation, a preliminary investigation was carried out to estimate the actual purity of the commercial herbicide through the HPLC-UV determination of quizalofop-M-ethyl (the distomer impurity) by applying three different quantification strategies. The enantioselective separation of the pair of enantiomers was obtained under polar organic conditions on a cellulose-based chiral column, after testing eight different polysaccharide chiral columns (Fig. S2). To follow the distribution of the eutomer and distomer in the selected agricultural substrates, the MS/MS tandem mass spectrometric detection was used to reach the necessary sensitivity and selectivity and the enantiomeric fractions were evaluated to detect changes in the chiral signature. Enantiomeric fractions (EF_M and EF_P, respectively for M and P enantiomers) were calculated with the following equations (where A_M and A_P are the chromatographic areas of M and P enantiomers, respectively):

$$EF_M = \frac{A_M}{(A_M + A_P)} \times 100 \; \& \; EF_P = \frac{A_P}{(A_M + A_P)} \times 100$$

A screening method was also developed to detect potential residues of quizalofop acid, the major metabolite of quizalofop-ethyl. The monitoring of EFs of the parent compound and its metabolite has enabled to draw conclusions about the degradation dynamics and the event timeline after the environmental release.

2. Experimentals

2.1. Chemicals, materials and solutions

Analytical standards of racemic quizalofop-ethyl (purity: $\geq$ 95.0%), and quizalofop-P-ethyl (purity: $\geq$ 98.0%, enantiomeric excess: $\geq$ 90.0%) were purchased from Merk Life Science S.r.l. (Milan, Italy).

Methanol and acetonitrile of RS-plus grade (purity $\geq$ 99.8%) were purchased from Merk KGaA (Darmstadt, Germany). Formic acid of analytical grade, used as an additive of the mobile phase, was always from Merk Life Science S.r.l. (Milan, Italy).

The individual solutions of racemic quizalofop-ethyl and quizalofop-P-ethyl were prepared by weighing each standard on an analytical balance (Ohaus DV215CD Discovery semi-micro and analytical balance, 81/210g capacity, 0.01/0.1mg readability). The weighted fractions were transferred into a graduated glass flask (2 mL) and dissolved accurately with 2 mL of methanol. The obtained solutions (1mg mL^{-1}) were then vortexed for 10 min at 2500 rpm and, finally, stored sheltered from light.

Enantiopure quizalofop-M-ethyl was prepared via enantiomeric HPLC separation of racemic quizalofop-ethyl in our lab (for the chromatographic conditions see Section 2.6). The chromatographic fractions were collected at the output of the chromatographic column, following the UV signal of the M-enantiomer, and then evaporated to dryness in a nitrogen supported evaporator at 25 °C. The residue was reconstituted with methanol to obtain a concentration of 0.22 mg mL^{-1} and used as an analytical standard. The enantiomeric purity of the obtained solution

was greater than 99%, as confirmed via chiral HPLC analysis (see Fig. S3).

2.2. Pesticide commercial formulation, soil, and vegetable samples

The commercial formulation of quizalofop-P-ethyl, sold under the name Leopard 5 EC (ADAMA AGAN Ltd, Israel), was kindly donated by an agricultural company, operating in the province of Rome and surrounding areas. From the same farm, vegetables (such as carrots, white turnip, and turnip leaves) and some portions of soils, which had been treated with the commercial formulation of quizalofop-P-ethyl, were sampled and taken to the laboratory. Agricultural practice for the application of this kind of formulation is reported in the ministerial label and includes one annual application by air nebulization (from the height of 2–3 m) of 1–2.5 L ha^{-1} in 200–400 L of water. At the time of the withdrawal of the environmental/agricultural samples, the last treatment had been carried out one week before. The soil was collected with a bailer, screened with 2-cm mesh sieve, and then stored in a glass jar for the transport to the laboratory. The soil was air dried for some days ensuring dark storage conditions. Before the pre-treatment procedure, the soil sample was gently mixed in a food processor to perform homogeneous sub-samplings.

2.3. Extraction of quizalofop-ethyl from the commercial formulation

To be submitted to chromatographic analysis, the pesticide formulation requires a quick pre-treatment since it is marketed in the form of an aqueous-oil emulsion containing 5.4% of quizalofop-P-ethyl (50 g L^{-1}), non-injectable as it is in the HPLC system. For this purpose, a procedure for extracting the active substance from the emulsion was optimized: 100 μL of the formulation was taken, shaken, and transferred to a 5-mL glass tube to work on 5 mg of the active substance. A volume of 900 μL of dichloromethane was added to the formulation aliquot since this solvent can dissolve the oily fractions of the product. Moreover, the aqueous component of the commercial formulation forms a minimum azeotrope with dichloromethane. As such, the water-dichloromethane mixture shows a lower boiling point than individual solvents and, therefore, it is easier to remove than water. After evaporation to dryness, the residue on the bottom of the glass tube was dissolved with methanol, transferred to a graduated 5-mL flask and brought to volume with the same solvent to reach the final concentration of 1 mg mL^{-1} of quizalofop-P-ethyl (the concentration at this point is only an estimation based on the concentration of the active substance conveyed on the label).

2.4. Extraction of quizalofop-ethyl from soil

A 0.5 g portion of soil was transferred into a 15-mL polypropylene tube (falcon tube) and 3 mL of acetone was added. The mixture was vortexed for 10 min at 2500 rpm and then sonicated in an ultrasound bath for 10 min at room temperature. Afterwards, the falcon was centrifuged (10 min, 3600 rpm) and the supernatant transferred into to another tube. The same extraction procedure was repeated another time. The collected organic extract (6 mL in total) was evaporated to dryness under a gentle nitrogen flux at 25 °C. The residue was then reconstituted in 500 μL of methanol. After filtration with PTFE syringe filters (0.45 μm), the organic extract (10 μL) was injected into the HPLC-MS/MS system.

2.5. Extraction of quizalofop-ethyl from carrots, turnips and leaves

An extraction procedure similar to that applied to soil was performed. A 1-g portion of each sample was manually homogenised in a glass mortar using a pestle of the same material and transferred into a 15 mL falcon tube containing 3 mL of acetone and 1 g of magnesium sulphate as a drying agent. After 10 min in a vortex-mixer (2500 rpm) and

10 min in an ultrasound bath, the sample suspension was centrifuged (10 min, 8000 rpm) and the supernatant was transferred into another falcon tube. The same extraction procedure was repeated another time. The 6 mL volume of acetone was evaporated to dryness under a gentle flux of nitrogen and the residue was dissolved with 500 μL of methanol. For each type of sample (carrots, turnips, and the corresponding leaves), it was necessary to filter with PTFE syringe filters (0.45 μm) and dilute (1:1 v/v) with methanol. Finally, 10 μL of the clean extract was injected into the HPLC-MS/MS system.

2.6. Chromatographic conditions

The extracts were analysed by means of an AQUITY UPLC H—Class PLUS system (Waters Corporation, Milford, MA, USA), equipped with a binary pump, a high-pressure mixing system, a column oven and an autosampler. The column was Lux Cellulose-2 (4.6 × 250 mm, 3 μm), with cellulose tris(3-chloro-4-methylphenylcarbamate) as the chiral selector (Phenomenex Torrance, CA, USA). The column was protected by a guard column with the same stationary phase, 4 × 3 mm sized. The separation was carried out in isocratic mode by using acetonitrile, with 0.1% of formic acid, at the flow rate of 1 mL min^{-1}. In order to avoid carry-over, the autosampler needle device was washed with pure acetonitrile after each injection.

The detection was performed by both a UV–Vis detector (λ = 220 nm) and a triple quadrupole mass spectrometer (API 4000 Qtrap from AB SCIEX, Foster City, CA, USA). The MS instrument was equipped with an electrospray source using the following settings: capillary voltage +5000V, air nebulizer gas 2 L min^{-1}, air drying gas at 350 °C and 20 L min^{-1}, nitrogen curtain gas 5 L min^{-1}, nitrogen collision gas 4 mTorr. The full width at half maximum (FWHM) was set at 0.7 ± 0.1 m/z in each mass-resolving quadrupole to operate with a unit resolution. Chromatograms were acquired in multiple reaction monitoring (MRM), selecting two transitions. In this way, the analyte identification in a real sample was based on its retention time (t$_r$ ± 2.5%), four identification points (1 IP for the precursor ion, and 1.5 IPs for each product ion) and the ion ratio (the relative abundance between the two MRM transition) (see Table S1 and Fig. S4).

2.7. Method validation

The HPLC-MS/MS method for the determination of quizalofop-ethyl in carrot samples was validated in matrix by assessing recovery, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ).

Recoveries and precision were evaluated by spiking blank aliquots at two different spike levels (LOQ and 10 LOQ), preparing five replicates per level on the same day. Precision was calculated as the relative standard deviation (RSD%), which is a dispersion index of measurement results normalised to the mean of results. LOD and LOQ were estimated experimentally, spiking blank samples with quizalofop-ethyl pre-extraction and verifying the analyte concentration providing a signal-to-noise ratio of 3 and 10 respectively. External calibration was used for the quantitative analysis, while the linearity was assessed by calculating the determination coefficient R^2 (see Section 3.6 for the details and results).

For the details and results of quantitative strategies applied to quantify the impurity in the commercial pesticide formulation see Section 3.3.

3. Results and discussion

3.1. Optimization of chromatographic conditions

With the aim of achieving an effective separation of both enantiomers of quizalofop-ethyl, the racemate was analysed with eight polysaccharide-based chiral columns, differing from each other with specific pendant groups able to give diverse enantioselective capability [21].

For the screening, all the columns were tested using several combinations of organic solvents, water and acid additives. Using acetonitrile containing 0.1% TFA in isocratic mode, six out of eight polysaccharide-based chiral columns (Lux i-Amylose-1, Lux Amylose-1, Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-4 and Lux i-Cellulose-5) could separate the enantiomers of quizalofop-ethyl, but the greatest resolution was obtained with Lux Cellulose-2 (R$_S$ ≥ 2). The same enantioresolution was obtained using 0.1% formic acid, which was preferred to TFA when the MS detection was used. The addition of water to the mobile phase provided an unnecessary high resolution between the enantiomers, with the disadvantage of longer retention times.

By comparing chromatograms acquired for the single enantiomers and the racemic solution, the elution order of quizalofop-P-ethyl and quizalofop-M-ethyl was established.

3.2. Thermodynamic study

The separation of the quizalofop-ethyl enantiomers was studied in a temperature range from 15 to 50 °C under the chromatographic conditions described in the Section 3.1 [22]. From this study, it was possible to obtain the optimal temperature of analysis and thermodynamic quantities related to the enantioselective adsorption and resolution [23]. As each enantiomer passes through the column, it interacts with the chiral selector, with specific formation constants of selector-selectand associates. The interaction intensity is responsible for their temporal/spatial separation [24]. Fig. 1a shows how the lnk' varies as a function of T^{-1} for both enantiomers. As can be seen from the values displayed in Table 1, for both enantiomers the adsorption is favoured by the enthalpy-term and slightly disfavoured from the entropic point of view.

In the graph reported in Fig. 1b, the lnα was plotted as a function of T^{-1}. This plot enabled to calculate the thermodynamic quantities related to the enantiomeric resolution.

The separation is driven by enthalpy, while a slight entropic disadvantage is registered. From the thermodynamic quantities it is possible to derive also the isoenantioselective temperature (T$_{iso}$), which is the temperature of the enantiomers coelute as a result of entropic and enthalpic compensation. The obtained values are listed in Table 2. The temperature found for the chiral couple of quizalofop-ethyl, under the defined chromatographic conditions, is about 84 °C.

So much has been written about limitations of computing thermodynamic quantities from separation science evidence. The presented approach fails to take into account the fact that there are at least two adsorption sites on a sorbent phase of a chiral column. Although this and other limitations prevent from accurately measure thermodynamic quantities from enantiomeric separations, the van't Hoff approach is a quite invaluable tool to have an idea of these quantities and indications to select proper chromatographic conditions [25].

3.3. Analytical approaches to quantify the distomer impurity in the pesticide formulation

With the objective of evaluating the distomer impurity of the single-enantiomer commercial formulation, which is supposed to contain only the P-enantiomer of quizalofop-ethyl, three different quantitative approaches were tested: (i) the method of the comparison of peak areas; (ii) the standard addition method, performed by spiking formulation aliquots with the standard solution of the racemate; (iii) the standard addition method, performed by spiking formuation aliquots with the enantiopure quizalofop-M-ethyl solution. Before applying these three methods to the commercial formulation, they were compared in terms of affordability and the lowest scattering of the measurements on the standard solution of quizalofop-P-ethyl. Such requirements were fulfilled by applying the method of standard additions with the purified solution of the M-enantiomer. Therefore, this same approach was
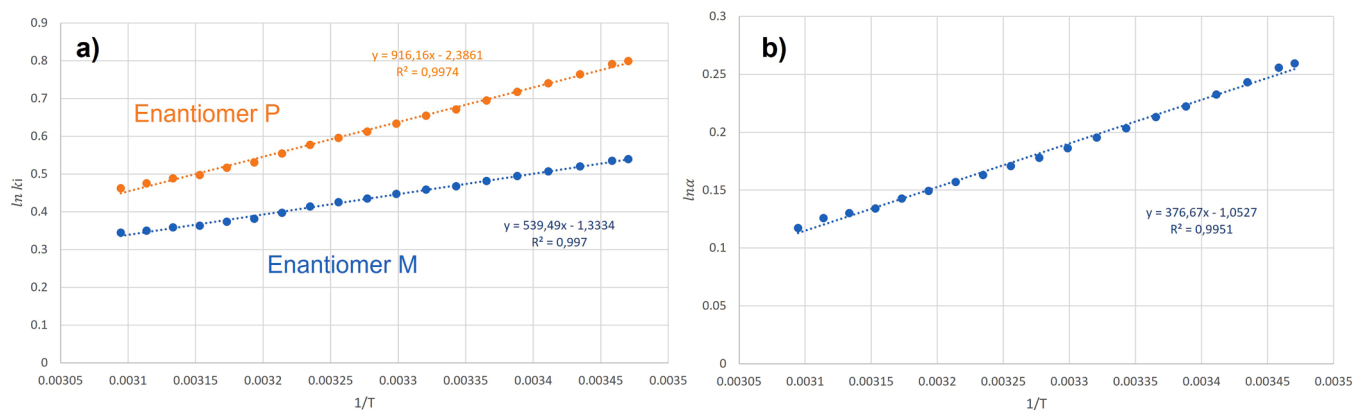
Fig. 1. (a) For each enantiomer, the graph shows the variation of the natural logarithm of the retention factor (k') as a function of the inverse of the absolute temperature. (b) The graph shows the variation of the natural logarithm of the selectivity factor (α) as a function of the inverse of the absolute temperature. Data that can be extrapolated from the graph are related to the separation mechanism.

Table 1
Thermodynamic parameters related to the adsorption of the enantiomers of quizalofop-ethyl.

	ΔH°_i $(kJ \cdot mol^{-1})$	ΔS°_i $(kJ \cdot mol^{-1} \cdot K^{-1})$
Enantiomer M	−7.62	−0.024
Enantiomer P	−4.49	−0.015

Table 2
Thermodynamic parameters related to the separation mechanism of the enantiomers on the surface of the stationary phase (Lux Cellulose-2).

$\Delta\Delta H^{\circ}_i$ $(kJ \cdot mol^{-1})$	$\Delta\Delta S^{\circ}_i$ $(kJ \cdot mol^{-1} \cdot K^{-1})$	$T_{iso}(K)$
−3.13	−0.009	357.81

applied for the most complex analysis of the commercial formulation.

Applying the standard addition method to the extract from the commercial formulation (see Section 2.3 for the sample treatment), it was possible to evaluate the concentration of distomer in it. For this purpose, six calibrators were prepared spiking pre-extracted 100-μL aliquots of the pesticide formulation with increasing volumes of the purified solution of the M-enantiomer (see Section 2.1). Each calibrator was injected three times and the areas of the chromatographic peaks were integrated. As can be seen (Fig. 2), in the first calibrator, a low intensity peak appears at the expected retention time of the M-

enantiomer due to the original concentration in the pre-treated solution of the formulation. In the subsequent calibrators, an increasingly intense peak occurs, due to the increasing concentrations of the M-enantiomer added. On the other hand, the area of the P-enantiomer peak is almost constant in all calibrators since the aliquot fortification did not involve the addition of quizalofop-P-ethyl. All the chromatograms for the six calibrators are reported in Fig. 2. Interpolating the experimental data, the linear model that best exemplifies the functional relationship existing between the calibrator peak area and the amount of enantiomer M added (in μg) was obtained. By extrapolating to zero, it was possible to calculate the concentration of the impurity in the first calibrator; such a value was traced back to the amount of distomer in the commercial formulation and, consequently, to the percentage of enantiomeric impurity of the active substance. The enantiomeric fraction of the distomer in the formulation was assessed to be $0.63 \pm 0.03\%$. This result is the starting point for the evaluation of the diffusive dynamics and degradation rates of quizalofop-ethyl enantiomers in the samples selected in this study, once such commercial formulation was applied.

3.4. Evaluation of the distomer fraction in vegetable samples

The extracts from plant samples, processed as described in Sections 2.4 and 2.5, were analysed by means of HPLC-MS/MS. With the aim to verify the potential occurrence of the acid metabolite, a HPLC-MS/MS screening method was applied, extending the duration of the chromatographic run, working in dual polarity ionization (positive for
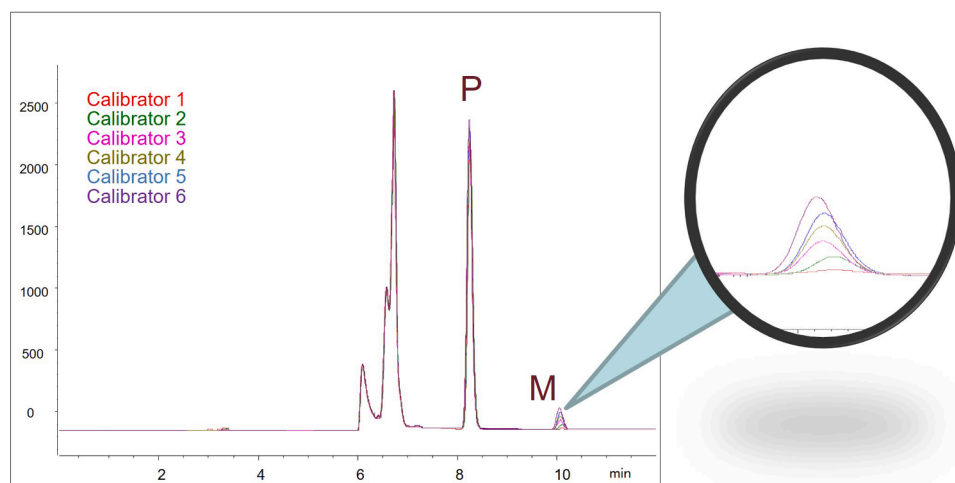

Fig. 2. Overlapping of the chromatograms related to the injection of the six calibrators, prepared by fortifying an aliquot of the commercial formulation of quizalofop-P-ethyl with increasing quantities of the purified solution of the M-enantiomer. It is clearly visible how the signal of the P-enantiomer is almost comparable in each chromatogram. On the other end, the peak area increases linearly with the added volume of the purified M-enantiomer solution in the several calibrators. Micrograms of the M-enantiomer added in each calibrator are respectively: 0, 1.1, 2.2, 3.3, 4.3, 5.4.

quizalofop-ethyl and negative for quizalofop acid), and introducing the MRM transitions of quizalofop acid (343/271 m/z and 343/243 m/z, with [M-H]$^-$ = 343 m/z). Fig. 3 shows the HPLC-extracted ion current (XIC)-chromatograms of both analytes, acquired by injecting the extract from carrot.

To study the distribution of the quizalofop-ethyl and its metabolite in the different pre-treated real samples, the EF of each enantiomer was calculated by dividing its chromatographic area by the sum of the areas of both enantiomers and multiplying the result by 100. To follow the EF variations in different environmental substrates can be useful to clarify the distribution dynamics and degradation processes involving enantiomers. A significant modification of the EF between different substrates, even in physical contact with each other, indicates a diversified degradation of the two enantiomers, attributable to different enzymatic enantioselective processes. A time variation of the EF is the sign of an enzymatic degradation process [26]. This means that the enantiomers of the same molecule are broken down following different degradation patterns. In soil, turnips and turnip leaves, the analyses have highlighted an enhancement of the EF of the eutomer (EF$_P$), a situation that foreruns an enantioselective and preferential degradation for the M-enantiomer. An opposite trend was observed for the carrot matrix treated with Leopard 5 EC. The results show the onset of an enantioselective degradation causing an increase in the EF of the distomer (EF$_M$). As a matter of fact, the peak of the M-enantiomer is clearly visible in Fig. 3 and its EF is 7.6 ± 0.1%; at least an enzyme is responsible for a preferential degradation of the eutomer.

3.5. Results of the method validation of quizalofop-ethyl enantiomers in carrot samples

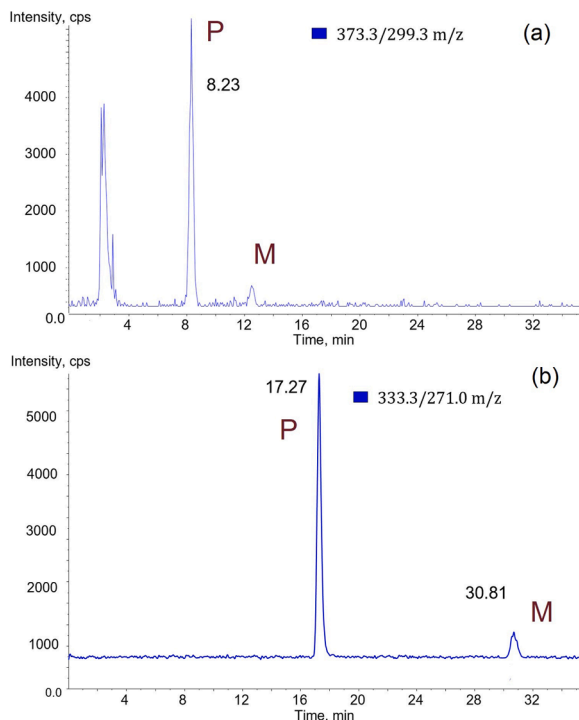Owing to the interesting results obtained for carrot matrix (Section 3.4), a quantitative analysis of the quizalofop-ethyl enantiomers was performed after validating the HPLC-MS method. Table 3 gives a summary of the method validation results. LOD and LOQ values of quizalofop-ethyl enantiomers are respectively below 5 ng g^{-1} and 15 ng g^{-1}. Absolute recoveries were calculated at LOQ and 10LOQ (five replicates for each level spiked pre-extraction), showing yields of 77% for the P enantiomer and 72% for the M-enantiomer. Intraday precision (n = 5 for each fortification level) was always within ± 5% for every enantiomer.

Both enantiomers of quizalofop-ethyl were quantified through the strategy of external calibration. To this end, uncontaminated carrots were used as blank matrices and used to build matrix-matched calibration curves by spiking nine aliquots with the standard solution of the racemate. The chosen concentration range (12–400 ng g^{-1}) was particularly wide so that both enantiomers, one present as impurity and the other as main contaminant in the real samples, could be quantified with the same set of calibrators. The obtained linear models are depicted in Fig. S5. The carrots, treated with the commercial formulation of quizalofop-P-ethyl, were analysed in the same way of the calibrators. From the obtained results it was possible to determine the residual pesticide content (enantiomer P=0.593±0.004 µg g^{-1}; enantiomer M=0.065±0.002 µg g^{-1}).

3.6. The fate of the enantiomers of quizalofop-ethyl and its metabolite in the studied matrices

By combining the EF$_M$ results of the contaminated carrot (7.6%) with those of soil and turnip matrices (0.0%), it was possible to draw conclusions about the diffusive dynamics of the active substance within the studied context. When the herbicide formulation is sprayed above a field, only soil and outer leaves of carrots and turnips are subjected to direct pollution. The plant roots (like carrot and turnip pulps) are affected by diffusive pollution from the loam and through the vascular system of the plant. The detection of the M-enantiomer only in the matrix that is lastly affected by the contamination allowed us to conclude that the diffusive dynamics of the active substance intervenes upstream of any degradation process, which is then quite different for each type of matrix. By means of HPLC-MS/MS, it was also possible to detect the P-enantiomer of the metabolite quizalofop acid in all matrices with considerable intensity. The impossibility of visualizing the M-enantiomer of quizalofop in any of the matrices, with the exception of carrot, deals with the lower sensitivity of the electrospray detection in negative polarity and the leaching phenomenon, which affects to a greater extent the acid analyte than its ester. It is clear how the degradation process that involves quizalofop-ethyl and leads to quizalofop acid takes place following a non-enantioselective route: the EF$_M$ for the distomer of quizalofop is in numerical agreement with the EF$_M$ of quizalofop-ethyl in the same matrix. The chromatogram concerning the analysis of quizalofop acid in the carrot shows a high peak for the P-enantiomer and a very low intensity peak associated to the M-enantiomer. As mentioned before, the EF$_M$ associated is around 8%, very similar to the one recorded for the quizalofop-ethyl analyte. The ratio of areas remains approximately the same as a result of the degradation (panels (a) and (b) of Fig. 3). Our findings have led to the conclusion that the dissipation of quizalofop-ethyl into its acid metabolite is of non-enantioselective nature. For this reason, it is reasonable to believe that the degradation is of abiotic nature since it does not involve asymmetric

Fig. 3. Chromatograms related to the injection of the final extracts of contaminated carrot samples. (a) XIC-chromatogram resulting from the selection of the quantifier transition of quizalofop-P-ethyl. (b) XIC-chromatogram resulting from the selection of the quantifier transition of quizalofop acid metabolite. The M-enantiomer peak is observable, above the LOD, for both quizalofop-ethyl and quizalofop acid (column: Lux cellulose-2; mobile phase: acetonitrile with 0.1% formic acid; 40 µL of the carrot extract injected).

Table 3
Validation parameters for the enantiomers of quizalofop-ethyl.

	LOD (ng·g^{-1})	LOQ (ng·g^{-1})	R%		Precision (RSD)	
			LOQ	10LOQ	LOQ	10LOQ
Enantiomer M	4.5	14	72	74	5	4
Enantiomer P	2	6	75	79	5	4

macrostructures. Further and detailed research are required to confirm this assumption.

4. Conclusion

To the best of our knowledge, this is the first study that aims to compare different strategies for the evaluation of the distomer impurity of an active substance in real samples of agricultural concern. The objectives of the current research come from the interest in searching solutions to the environmental pollution due to undesirable isomers. From this point of view, any type of innovation in the composition of pesticide formulations goes hand in hand with the possibility of improving separation techniques for the resolution of stereoisomers. The proposed method represents an innovation since it is used not only for the objective evaluation of the distomer pollution but also as a tool to follow different diffusive dynamics of both enantiomers of an active substance. Along the lines of such a study it will be possible to draw similar conclusions for any kind of environmental active contaminant, following the natural dispersion of its enantiomers in the environment. This kind of considerations may be important in the near future in sight of a re-evaluation of the isomeric signature of the commercially available pesticide formulations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2023.464289.

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