

ORIGINAL RESEARCH

Enantioselectivity and residue analysis of cycloxadprid and its metabolite in the pile and fermentation processing of Puer tea by ultraperformance liquid chromatography–high-resolution mass spectrometry

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Abstract

The residues of cycloxadprid enantiomers and metabolites are investigated by ultraperformance liquid chromatography–high-resolution mass spectrometry (UPLC–HRMS) during raw and ripen Puer tea processing. A Chiralpak AG column with chiral stationary phase of amylose tris (3-chloro-5-methylphenylcarbamate) is succeed to separate the 1*R*, 2*S*-cycloxadprid, 1*S*, 2*R*-cycloxadprid, and their metabolite, which is identified as nitylene-imidazolidine. It is not conversed 1*R*, 2*S*-cycloxadprid into 1*S*,2*R*-cycloxadprid during Puer tea processing. The estimated half-lives of the 1*R*,2*S*-cycloxadprid and 1*S*,2*R*-cycloxadprid are 0.97 and 1.1 h, respectively, and 1*R*,2*S*-cycloxadprid decreases more quickly than the 1*S*,2*R*-cycloxadprid. During raw Puer tea processing, the half-lives of 1*R*, 2*S*-cycloxadprid and 1*S*, 2*R*-cycloxadprid are 1.68 h and 1.77 h, but the residue is still detected even if it is over 730 day. However, the half-lives of 1*R*,2*S*-cycloxadprid and 1*S*,2*R*-cycloxadprid are 0.60 day and 0.63 day during ripen tea processing. The amounts of metabolite are more in raw tea than in ripen tea; the terminal residues are still detected until 730 days during raw tea. A significant enantioselectivity of 1*R*, 2*S*-cycloxadprid and 1*S*, 2*R*-cycloxadprid is observed during raw tea or ripen tea processing. The degraion result shows the enantioselectivity of cycloxadprid in raw or ripen Puer tea processing.

KEYWORDS

cycloxadprid, enantioselectivity, metaboliteLC–HFMS, puer tea processing

1 | INTRODUCTION

Neonicotinoids are the most important class of synthetic insecticides for tea protection against piercing-sucking pests (Tomizawa & Casida, 2003). Cycloxadprid is a new neonicotinoid insecticide that has been synthesized and industrialized in China (Li et al., 2011). It

is different from traditional neonicotinoids, which act as agonists of native and recombinant nicotinic acetylcholine receptors (nAChRs) (Liu & Casida, 1993; Matsuda et al., 1998; Nishimura et al., 1994; Tomizawa & Casida, 2003) and show high insecticidal activity against a broad spectrum of sucking and biting insects¹ (Cui et al., 2012; Shao et al., 2010), which suggests that it has been considerable as

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the third generation of neonicotinoids. The molecular structure of cyclozaprid contains a chiral oxabridged cis-configuration leading to a pair of enantiomers, 1*R*,2*S*-cyclozaprid and 1*S*,2*R*-cyclozaprid (Figure 1). Cyclozaprids are commonly produced and used as racemic mixtures and the stereoselectivity degrade is found in soils (Liu et al., 2015). Zhang et al. observed stereoselective uptake and translocation of cyclozaprid in edible vegetables from roots (Zhang et al., 2013). However, Chen et al. (Chen et al., 2017) founded adverse results, as evidenced by the lack of significant difference between the stereoisomers in their fate in aerobic soils and three mainly metabolites were found in soil.

The metabolite of cyclozaprid is readily founded through photolysis, hydrolysis, and oxidation reaction. Liu et al. (Liu et al., 2015) identified and tracked 11 metabolites of cyclozaprid, and tracked their changes in flooded and anoxic soils. Shuang et al. (Hou et al., 2017) studied the photostability of cyclozaprid in water and detected 25 photodegradation products; the predominant photodegradation product was named as NTN 32,692. Fang et al. (Fang et al., 2017) reported that the degradation dynamics of two neonicotinoids during *Lonicera japonica* planting, drying, and tea brewing processes were researched. Hou et al. (Hou et al., 2013) compared the dissipation behavior of three neonicotinoid insecticides in tea and found high transfer rates through green or black tea brewing of 80.5% or 81.6% for thiamethoxam, of 63.1% or 62.2% for imidacloprid, and of 78.3% or 80.6% for acetamiprid. However, the degradation behavior and metabolite of cyclozaprid was still unknown in Puer tea processing.

Liquid chromatography–high-resolution mass spectrometry (LC-HRMS) has also been explored and has shown great potential for untargeted profiling in tea (Gao et al., 2019; Jia et al., 2020). The analysis method of cyclozaprid in tea was scarcely by LC-HRMS. Only Liu et al. (Liu & Jiang, 2020) reported that the stereoisomer behavior of sulfoxaflor was determined by LC-HRMS during Puer tea and Black tea processing.

Therefore, a new analytical method is developed to determine stereoisomer of cyclozaprid and metabolite in Puer tea using LC-HRMS. The method was applied to investigate the stereoselective cyclozaprid degradation during Pu-erh tea processing.

2 | MATERIALS AND METHODS

2.1 | Chemical materials

A racemic mixture of cyclozaprid was provided by Beilinwei technology Ltd. Cyclozaprid powder 25% was supplied by Shanghai Shengnong pesticide Co Ltd. Ten milligram of 2-chloro-5-[[2-(nitromethylidene)imidazodine-1-yl]methyl]pyridine (CAS 10336-63-4) was synthesized by Alta Scientific Co., Ltd. The enantiomerically pure standards: 1*R*,2*S*-cyclozaprid, 1*S*,2*R*-cyclozaprid were separated by semipreparative HPLC with Chiralpak AG, polysaccharide-based chiral stationary phase consisting of amylose tris (3-chloro-5-methylphenylcarbamate), 250 × 4.6 mm i.d., 5 μm.

The stock solutions were produced by dissolving the cyclozaprid in acetonitrile. All solutions were stored in a refrigerator at -18°C. HPLC-grade acetonitrile and methanol were provided by Tedia Company Inc. The initial dose of cyclozaprid 50 mg/L was used with 1g of cyclozaprid completely dissolved into 5 L water. Water was purified using a Milli-Q system.

2.2 | Separation the metabolite of cyclozaprid

One gram of 25% cyclozaprid powder is placed on sun at 3 d. The sample is dissolved by 20 ml water, then extracted by acetonitrile. The metabolite with degradation test is obtained by semipreparative HPLC. The molecular structure of metabolite is analyzed by LC-HFMS.

2.3 | Separation optical pure standards:1*R*,2*S*-cyclozaprid and1*S*,2*R*-cyclozaprid

The sample of 10 mg/ml cyclozaprid is separated by semipreparative HPLC. The stereoisomer of cyclozaprid was separated by using a Chiralpak AG (amylose tris (3-chloro-5-methylphenylcarbamate) as stationary phase, 250 × 4.6 mm i.d., 5 μm, Daicel Ltd. JP). Mobile phases are H₂O and acetonitrile (55:45), respectively. The flow rate was 1.0 ml/min. the injection volume is 40 μL.

2.4 | The transform of optical pure compounds in Puer tea processing

The optical pure standards of 1*R*,2*S*-cyclozaprid or 1*S*,2*R*-cyclozaprid (1 mg/L) are respectively added to research the transform of optical pure compounds in Puer tea processing. The test at intervals time is designed at 0, 2 h, 15 h, 24 h, 48 h, 96 h, and 140 h.

2.5 | Degradation in raw Puer tea

Sun-dry Puer tea (20 kg) is obtained and sprayed with 50 mg/L aqueous solution (25% powder) in March, 2019. The raw Puer tea is stored under air temperature (5–28°C) and in dark place. The intervals time is designed at 0 (2 h), 4 h, 10 h, 16 h, 1d, 3d, 6 months, 12 months, 18 months, 24 months, and 36 months. The sample is dried to constant weight and the residues amount calculated with dry sample.

2.6 | Ripen Puer tea processing

To ferment the ripen Puer tea, 20 kg of sun-dry Puer teas is sprayed with 25% powder at the dose of 50 mg/L aqueous solution to keep the conditions of 35% moisture content. During the pile-fermentation, the fermented tea was artificially turned and piled again at 7th days

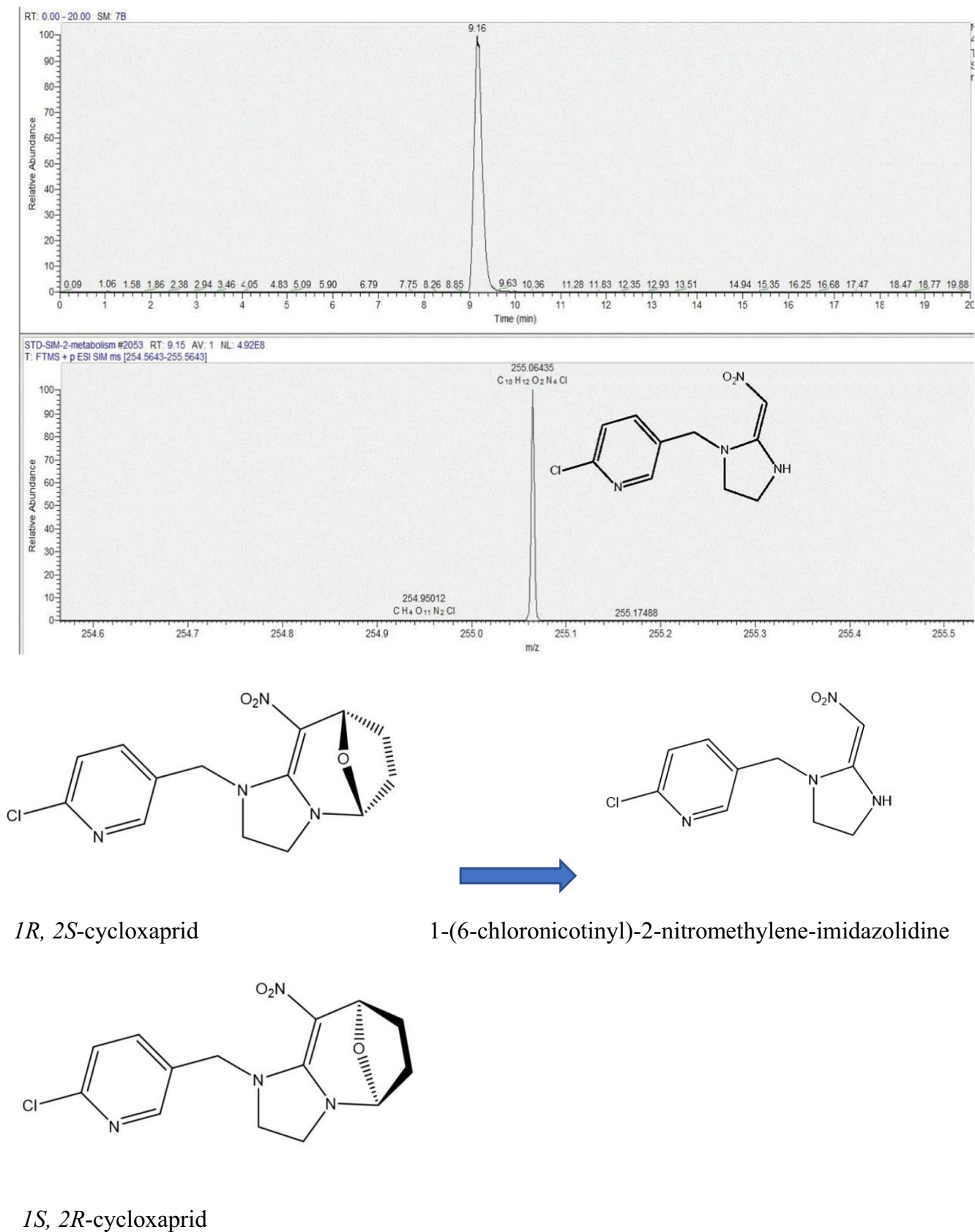


FIGURE 1 typical LC-HRMS chromatograms of metabolite and the transformation of cyclozaprid

TABLE 1 Limits of detection, quantification, calibration equation

	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)	Calibration equation ($n = 5$)	Determination coefficient, R^2	Linear range tested ($\mu\text{g}/\text{L}$)
1 <i>R</i> ,2 <i>S</i> -cyclozaprid	0.2	1	$y = 62137x + 7289$	0.998	0.2–50
1 <i>S</i> ,2 <i>R</i> -cyclozaprid	0.2	1	$y = 24244x - 2547$	0.999	0.2–50
2-Chloro-5-[[2-(nitromethylidene)imidazodin-1-yl] methyl] pyridine	0.1	1	$y = 34769x + 1781$	0.998	0.05–10

TABLE 2 The recovery and RSD of an analyses spiked in Puer tea at two concentration ($n = 6$)

Enantiomer	Addition ($\mu\text{g}/\text{kg}$)	Raw Puer tea		Ripen Puer tea	
		Recovery %	RSD %	Recovery %	RSD %
1 <i>R</i> , 2 <i>S</i> -cyclozaprid	10	93.2	6.8	94.7	6.2
	1	88.4	2.8	84.6	4.6
1 <i>S</i> , 2 <i>R</i> -cyclozaprid	10	90.6	8.6	98.2	3.6
	1	85.2	5.7	95.1	4.7
2-Chloro-5-[[2-(nitromethylidene)imidazodin-1-yl] methyl] pyridine	10	88.7	3.8	86.3	5.5
	1	84.9	4.1	82.5	3.9

(first pile), 14th days (second pile), the facial pile-fermentation tea is splashed by 100 ml water at 3 day intervals. The sample is collected at intervals time on 0 (2 h), 1, 3, 7, 15, 30, and 45 days. The residues amount expressed as dry sample.

2.7 | Calculation of enantiomer fraction

The enantiomer fraction (EF) was $EF = \frac{R}{(R+S)}$, concentration of the 1*R*,2*S*-cyclozaprid was R, the other concentration of the 1*S*, 2*R*-cyclozaprid was S.

2.8 | Samples preparation

Five grams of sample was exactly weighed and added 10 ml water, 20 ml acetonitrile. After the mixture was vortexed, 5 g NaCl was added. The tube was shaken vigorously for 1 min using a vortex mixer and centrifuged at 5000 rpm for 5 min. The upper layer solution was mixed with 150 mg PSA and 150 mg anhydrous MgSO_4 for cleanup. After shaking and centrifugation at 5000 rpm for 3 min, 0.5 ml of the upper layer was filtered through 0.22 μm filter for LC-HFMS analysis.

2.9 | UPLC-HRMS Analysis

Sample analysis was achieved in an ultraperformance liquid chromatography–Q exactive high-resolution mass spectrometry (Thermo Fisher Scientific,) system.

The instrument was tuned in the positive ESI mode (3.8 kV of spray voltage, 325°C of capillary temperature, 350°C of probe

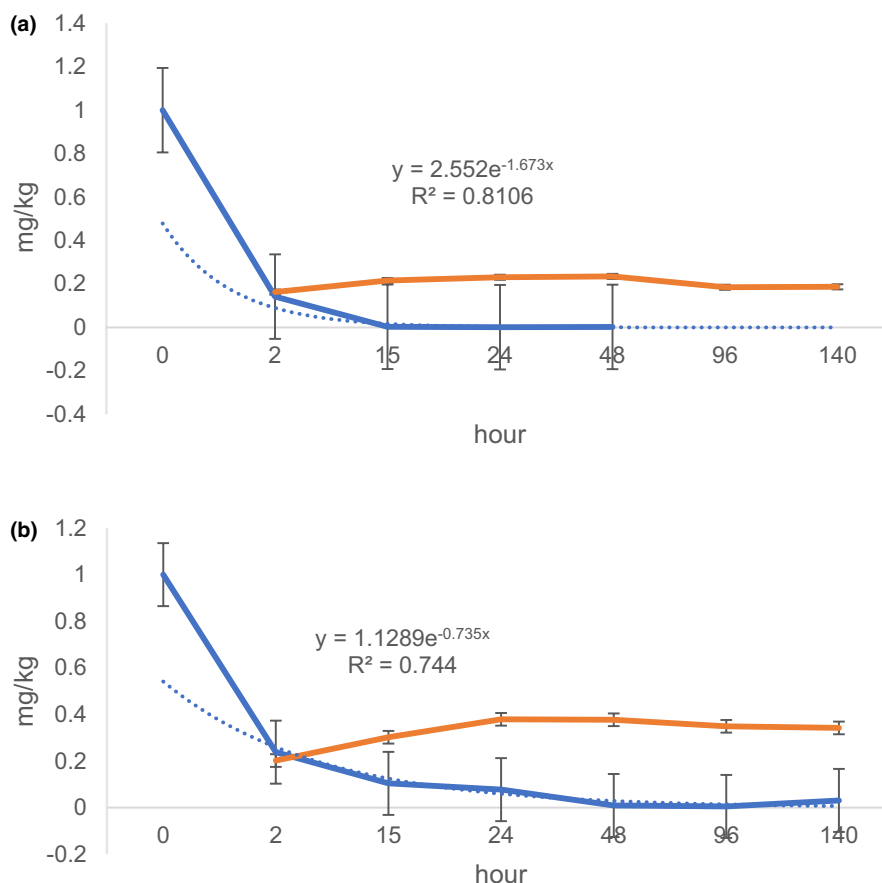
heater temperature, and 60 V of SLens). The instrument was calibrated using positive calibration solutions every three days. The pesticide analysis was performed in FS/DIA mode. In FS, the scan range was set at m/z 150–750; mass resolution at 140,000 FWHM; AGC target and maximum IT were set at 1.0 e6 and 100 ms, respectively. For DIA, the relevant parameters were set as follows: mass resolution: 140,000 FWHM; AGC target: 2 e⁵; maximum IT: 30 ms; Loop count: 12; MSX count: 1; Isolation window: 50 Da; stepped normalized collision energy (NCE): 20%, 40%, and 60%. The spray voltage in positive and negative modes was set as 3.5 kV and 3.0 kV, respectively. And in our experiment, the positive and negative modes were analyzed separately. The flow rate of sheath gas and aux gas was 45 and 10 (in arbitrary units), respectively.

2.10 | Method validation

The method was validated with the following parameters: matrix effect, accuracy, linear range, limit of detection (LOD), limit of quantification (LOQ), specificity, and precision. The standard solution was determined from 2.0 to 100 $\mu\text{g}/\text{ml}$ concentration for each enantiomer. Three times signal-to-noise (S/N) ratio was as the LOD for every enantiomer, whereas the LOQ was based on the lowest spiked concentration level. As shown in Table 1, standard solution calibration curves were good in the range of 0.2–50 $\mu\text{g}/\text{L}$ for 1*R*,2*S*-cyclozaprid, 1*S*, 2*R*-cyclozaprid. Linearity was satisfactory with mean correlation coefficients (R^2) > 0.99.

The result of recoveries (1 and 10 $\mu\text{g}/\text{kg}$) and the precision (RSD) was seen in Table 2. The mean recoveries were good from 72.4% to 92.7%, and the RSD were below 10% at the three fortified levels in tea samples. The results were shown that the accuracy and precision was guaranteed for enantiomeric analysis.

FIGURE 2 Dissipation kinetic of optical pure compounds (a) 1*R*, 2*S*-cyclozaprid, (b) 1*S*, 2*R*-cyclozaprid and increase of metabolite



3 | RESULTS AND DISCUSSION

3.1 | Separation and Identification of metabolite

Because the cyclozaprid contain oxabridged structure, it is unstable under air temperature. To obtain the transformation, the conversion of cyclozaprid is quickly generated by sun light. During sun light, one new compound are detected and separated by HPLC at third test. Tentative structural identification of the transformation products was made by LC-HFMS. The mass spectrum m/z were 255.06435 $[M + H]^+$; the chemical structure was $C_{10}H_{12}N_4O_2Cl$, Delta ppm 0.079. Chen et al 2017 (Chen et al., 2017) reported the same mass spectrum with m/z 255.0645 $[M + H]^+$ and 255.06 $[M + H]^+$ (Hou et al., 2017) the chemical structure of metabolite was identified by artificial synthesis by Alta Scientific Co., Ltd. The metabolite and standard were simultaneously analyzed by LC-HFMS, and their retention time, molecular ion peak, and fragmentation pattern were in agreement with the synthesized structure standard. So the metabolite was unambiguously identified as 2-chloro-5-[[2-(nitromethylidene)imidazodin-1-yl]methyl] pyridine (CAS 10336-63-4) (NMI). (Figure 1).

3.2 | Chromatography Separation optimized

Because of absence of oxabridged ring, the metabolite was unselective molecule. Once cleavage occurred on the oxabridge, the

metabolite is no longer enantioselectivity. To simultaneously separate the chiral cyclozaprid and metabolite, the reverse-phase chiral columns were employed which contained cellulose- and amylose-based polysaccharide materials; a cellulose-based column (Chiral Cel OJ-3R) and two amylose-based columns (Chiralpak AD-RH and Chiralpak IG) were tested using a variety of reverse-phase mobile phase combinations.

Racemic cyclozaprid could not be resolved using methanol/water or acetonitrile/water by Chiralcel OJ-3R; however, enantio-separation was well obtained by two amylose-based columns (Chiralpak AD-RH and Chiralpak IG). The results showed that enantiomer cyclozaprid was more easily resolved in amylose-based columns than in cellulose-based columns. The Chiralpak IG column contained a new generation of polysaccharide-based amylose tris(3-chloro-5-methylphenylcarbamate) and showed the best enantiomeric resolving ability for enantiomer cyclozaprid and metabolite using a mixture of ACN/water (v/v: 35/65) as the mobile phase, flowing rate with 0.6 ml/min.

3.3 | The transformation and degradation of optical pure compounds

1*R*,2*S*-cyclozaprid and 1*S*,2*R*-cyclozaprid (1 mg/L) is separated by chiral column Chiralpak AG 250 × 4.6 mm i.d., 5 μm) with semi-preparative HPLC.

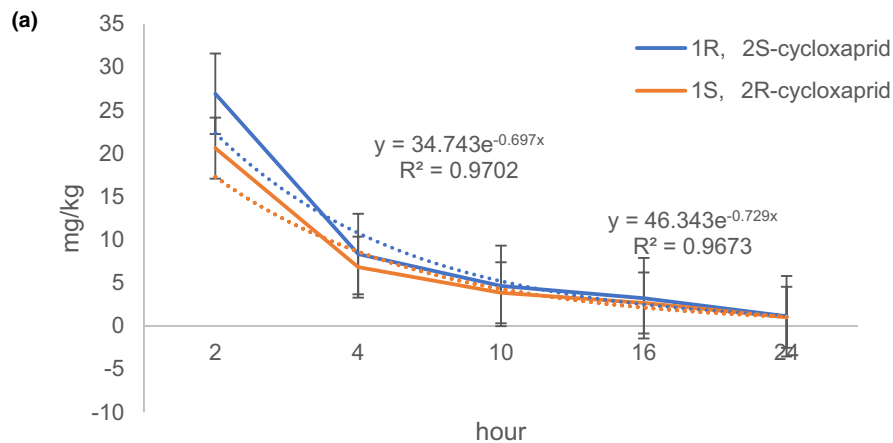


FIGURE 3 Dissipation kinetics of enantiomeric cycloxyaprid at two divide stages in raw Puer tea: (a) 24 h; (b) 730 days

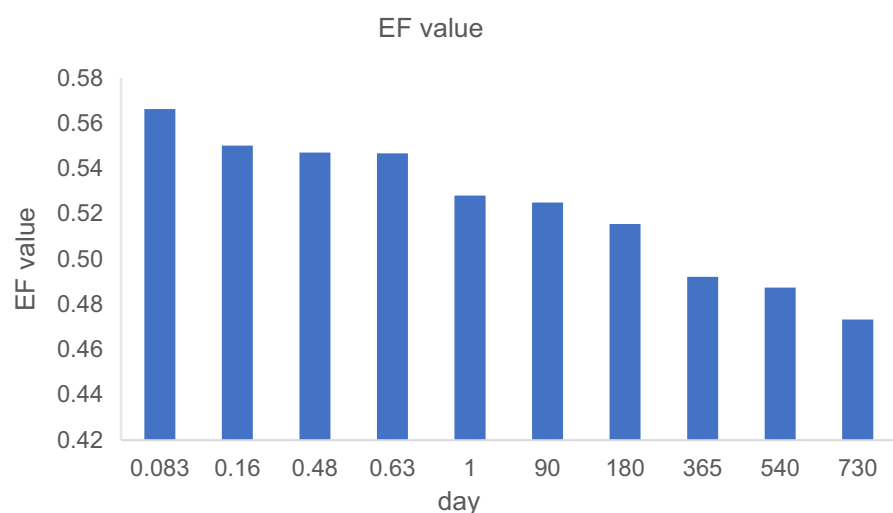
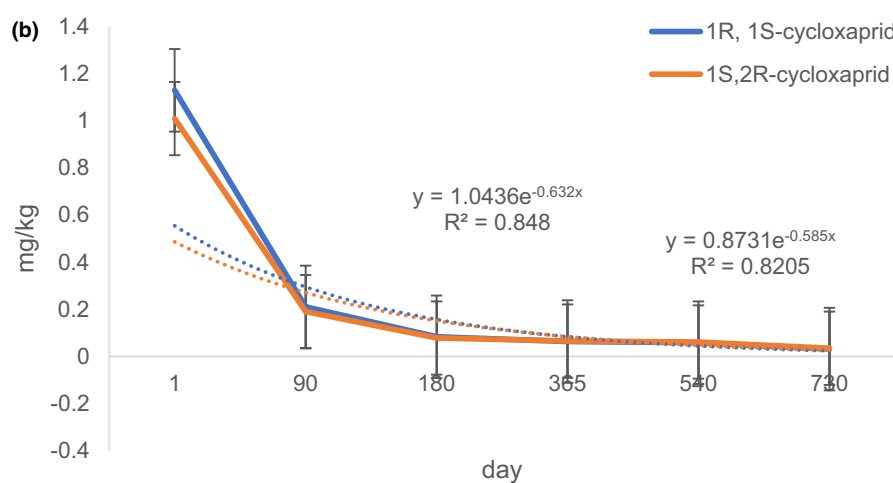


FIGURE 4 The change of stereoselectivity (EF) in raw Puer tea processing

The transformation test of 1R,2S-cycloxyaprid shows that parent compound and metabolite are only found; a similar result is observed with 1S, 2R-cycloxyaprid. The investigation shows that 1R,2S-cycloxyaprid is converted into 1S, 2R-cycloxyaprid. The degradation concentration of 1R, 2S-cycloxyaprid decreases more quickly than the degradation concentration of 1S,2R-cycloxyaprid (Figure 2). The

parent 1R, 2S-cycloxyaprid is not detected over 96 h, but the 1S, 2R-cycloxyaprid residue can be still determined at 140 h incubation time. This suggests that the degradation of 1R,2S-cycloxyaprid decreases more quickly than the 1S, 2R-cycloxyaprid. The corresponding degradation kinetics of 1R,2S-cycloxyaprid and 1S, 2R-cycloxyaprid are in accord with the pseudo-first-order kinetics, and the R^2 ranged from

FIGURE 5 Dissipation kinetics of enantiomeric cyclozaprid during ripen Puer tea processing

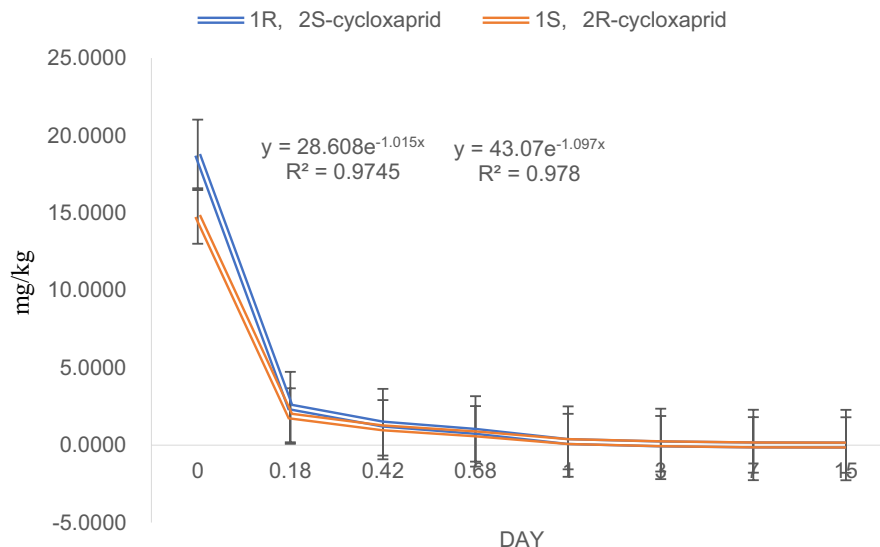
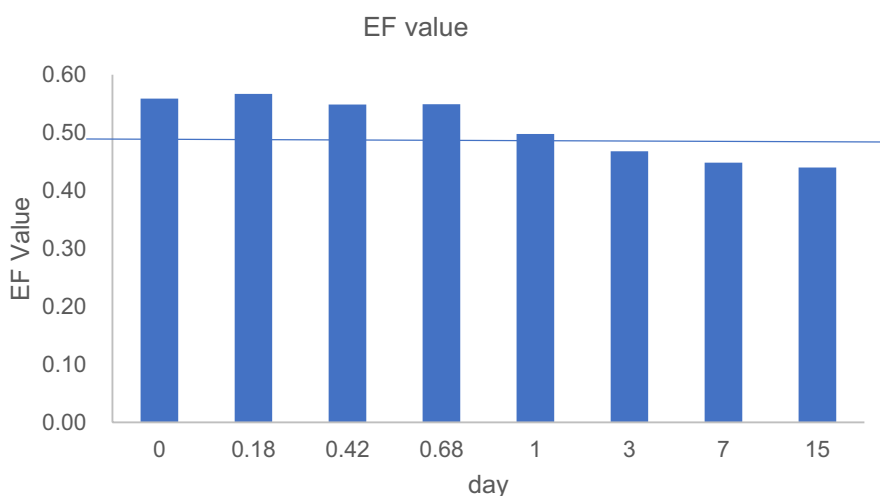


FIGURE 6 The change of stereoselectivity (EF) in ripen Puer tea processing



0.997 to 0.937 in Puer tea processing. The estimated $t_{1/2}$ values of the 1R, 2S-cyclozaprid and 1S, 2R-cyclozaprid are 0.97 and 1.1 h, respectively.

The metabolite can be first monitored at 2 h. The metabolite concentration of 1R, 2S-cyclozaprid is little than the metabolite of 1S, 2R-cyclozaprid. The metabolite of 1R, 2S-cyclozaprid increases from 2 h to 48 h, then begin to decrease slowly, but the increase interval is short from 2 h to 24 h with 1S, 2R-cyclozaprid.

3.4 | Stereoselective dissipation of cyclozaprid in Puer tea processing

The fermentation processing with under from several months to several ten years is unique to raw Puer tea. So the degradation of cyclozaprid is still detected over 730 day. The formed reported (Chen et al., 2017) that half-lives of cyclozaprid in three soils were <24 h. To detect the dissipation of cyclozaprid in Puer tea, the degradation is divided into two stages. The degradation of cyclozaprid decrease more swiftly at 24 incubation time than the stage from one day to

730 days. The corresponding degradation kinetics from 0 (2 h) to 730 day are inconformity to the pseudo-first-order kinetics, but the degradation kinetics is good corresponding to divide two curves. The correlation coefficient of cyclozaprid are 0.964 (0.963) and 0.871 (0.852) respectively (Figure 3). To our surprise, the half-lives of 1R, 2S-cyclozaprid and 1S, 2R-cyclozaprid are 1.68 h and 1.77 h, but the residue is still detected at 730 days. The reason need be more researched in the future.

Stereoselectivity is expressed as EF value. As shown in Figure 4, the beginning of EF value in cyclozaprid is >0.50, and the decrease of EF is obvious from 2 h to 730 days. The result showed that enantioselectivity is significantly observed during raw Puer tea processing. The result is shown that the degradation of cyclozaprid is enantioselectivity under raw Puer tea processing.

3.5 | Stereoselective dissipation of cyclozaprid in ripen Puer tea processing

Ripen Puer tea is unique processing due to the pile formation at 45 or 60 days. So the degradation of cyclozaprid is detected from starting

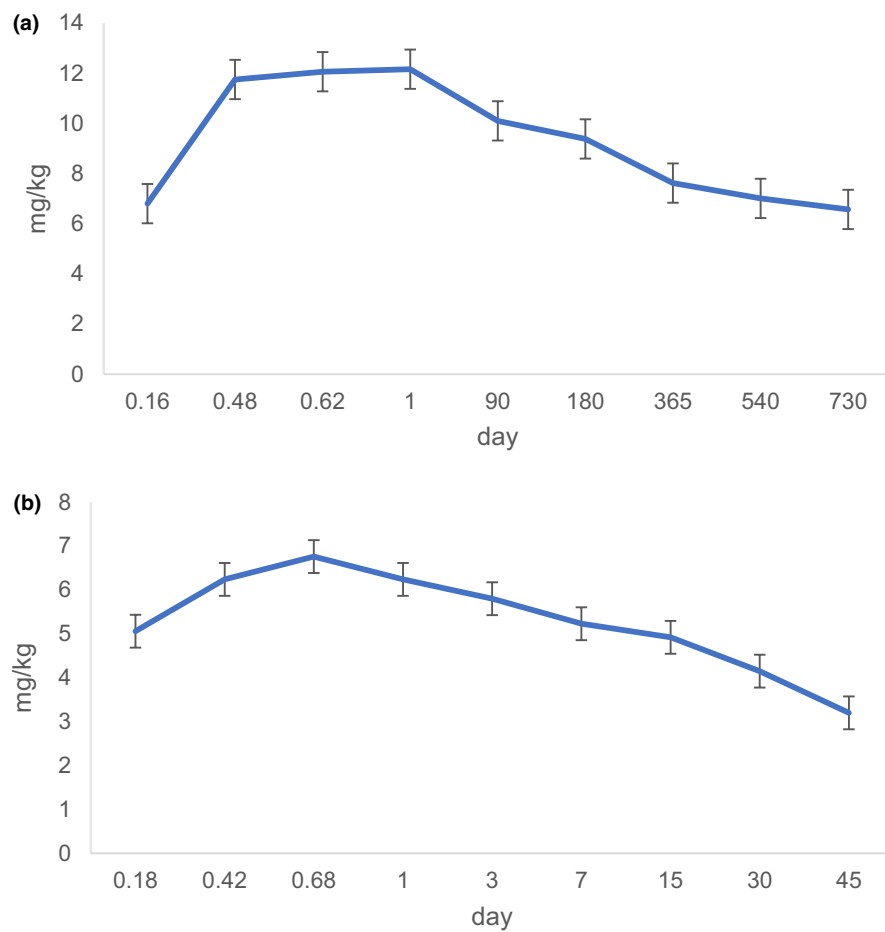


FIGURE 7 Dissipation kinetics of metabolite in Puer tea processing (a) raw Puer tea (b) ripen Puer tea

processing to 45 days. The extractable cyclopraxid fraction quickly decrease at ripen Puer tea processing. The parent cyclopraxid is not detected over 30 days. The corresponding degradation kinetics from 0 (2 h) to 15 days (Figure 5) are conformity to the pseudo-first-order kinetics, and the half-lives of 1*R*, 2*S*-cyclopraxid and 1*S*, 2*R*-cyclopraxid are 0.60 days and 0.63 days.

The decrease of EF is obvious from 0.56 to 0.44 during ripen Puer tea processing in Figure 6. From the statistical analysis, it showed that there is stereoselective preference for cyclopraxid enantiomers as evidenced as significant difference among the stereoisomers and the racemate in ripen Puer tea processing. The result is shown that the degradation of cyclopraxid is enantioselectivity under raw Puer tea processing.

3.6 | Dissipation of metabolite in raw Puer tea processing and ripen Puer tea

When metabolites are produced during Puer tea processing, they are not easy to decompose. So the terminal residue is still detected until 730 days in raw Puer tea processing and 45 days in ripen Puer tea processing (Figure 7). The maximum residue appears at one day (raw Puer tea processing) or earlier (ripen Puer tea processing). The metabolites are higher in residues in raw Puer tea than in ripen Puer tea.

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CONFLICT OF INTEREST

All Authors declare that they have no conflict of interest.

ETHICAL APPROVAL

This article does not contain any studies with human participants performed by any of the authors.

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REFERENCES

- Chen, M., He, Y. P., Yang, Y. T., Huang, L., Zhang, H. X., Ye, Q. F., & Wang, H. Y. (2017). Nonstereoselective transformation of the chiral insecticide cyclopraxid in aerobic soil. *Science of the Total Environment*, 579, 667–674. <https://doi.org/10.1016/j.scitotenv.2016.11.043>
- Cui, L., Sun, L., Yang, D., Yan, X., & Yuan, H. (2012). Effects of cyclopraxid, a novel cisnitromethylene neonicotinoid insecticide, on the feeding behaviour of *Sitobion avenae*. *Pest Management Science*, 68, 1484–1491.
- Fang, Q., Shi, Y., Cao, H., Tong, Z., Xiao, J., Liao, M., Xiangwei, W. U., & Hua, R. (2017). Degradation dynamics and dietary risk assessments

- of two neonicotinoid insecticides during *Lonicera japonica* planting, drying, and tea brewing processes. *Journal of Agriculture and Food Chemistry*, 65(8), 1483–1488.
- Gao, G., Chen, H., Chai, Y., Jin, L., Liu, X., & Chengyin, L. U. (2019). A method based on precolumn derivatization and ultra high performance liquid chromatography with high-resolution mass spectrometry for the simultaneous determination of phthalimide and phthalic acid in tea. *Journal of Separation Science*, 42(7), 1304–1311. <https://doi.org/10.1002/jssc.201801128>
- Hou, R. Y., Jin-Feng, H. U., Qian, X. S., Ting, S. U., Wang, X. H., Zhao, X. X., & Wan, X. C. (2013). Comparison of the dissipation behaviour of three neonicotinoid insecticides in tea. *Food Additives & Contaminants: Part A*, 30(10), 1761–1769. <https://doi.org/10.1080/19440049.2013.820356>
- Hou, S., Zhuang, Y. Y., & Deng, Y. Y. (2017). Photostability study of \r cis\r -configuration neonicotinoid insecticide cyclozaprid in water. *Journal of Environmental Science and Health. Part B: Pesticides, Food Contaminants, and Agricultural Wastes*, 52(8), 525–537.
- Jia, W., Shi, Q., Shi, L., Qin, J., Chang, J., & Chu, X. (2020). A strategy of untargeted foodomics profiling for dynamic changes during Fu brick tea fermentation using ultrahigh-performance liquid chromatography-high resolution mass spectrometry. *Journal of Chromatography A*, 1618, 460900. <https://doi.org/10.1016/j.chroma.2020.460900>
- Li, C., Xu, X., Li, J., Ye, Q., & Li, Z. (2011). Radiosynthesis of tritium-labeled novel nitromethylene neonicotinoids compounds with NaB³H₄. *Journal of Labelled Compounds and Radiopharmaceuticals*, 54, 256–259. <https://doi.org/10.1002/jlcr.1862>
- Liu, H., & Jiang, M. M. (2020). Qiwan Li* Determination of neonicotinoid sulfoxaflor residues and stereoselective degradation in Puerh tea and Black tea by liquid chromatography- high- resolution mass spectrometry. *Journal of Food Processing and Preservation*, 00, e14589.
- Liu, M. Y., & Casida, J. E. (1993). High affinity binding of [3H]imidacloprid in the insect acetylcholine receptor. *Pesticide Biochemistry and Physiology*, 46, 40–46. <https://doi.org/10.1006/pest.1993.1034>
- Liu, X. Q., Xu, X. Y., & Li, C. (2015). Degradation of chiral neonicotinoid insecticide cyclozaprid in flooded and anoxic soil. *Chirality*, 27(2), 137–141.
- Matsuda, K., Buckingham, S. D., Freeman, J. C., Squire, M. D., Baylis, H. A., & Sattelle, D. B. (1998). Effects of a subunit on imidacloprid sensitivity of recombinant nicotinic acetylcholine receptors. *British Journal of Pharmacology*, 123, 518–524.
- Nishimura, K., Kanda, Y., Okazawa, A., & Ueno, T. (1994). Relationship between insecticidal and neurophysiological activities of imidacloprid and related compounds. *Pesticide Biochemistry and Physiology*, 50, 51–59. <https://doi.org/10.1006/pest.1994.1057>
- Shao, X., Fu, H., Xu, X., Xu, X., Liu, Z., Li, Z., & Qian, X. (2010). Divalent and oxabridged neonicotinoids constructed by dialdehydes and nitromethylene analogues of imidacloprid: Design, synthesis, crystal structure, and insecticidal activities. *Journal of Agriculture and Food Chemistry*, 58, 2696–2702. <https://doi.org/10.1021/jf902531y>
- Tomizawa, M., & Casida, J. E. (2003). Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual Review of Entomology*, 48, 339–364.
- Zhang, J. B., Fu, Q. G., Wang, H. Y., Li, J. Y., Wang, W., Yang, Z., Zhang, S. F., Ye, Q. F., Li, C., & Li, Z. (2013). Enantioselective uptake and translocation of a novel chiral neonicotinoid insecticide cyclozaprid in youdonger (*Brassica campestris* subsp *Chinensis*). *Chirality*, 25, 686–691.

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