

Direct Chiral Resolution of Metalaxyl and Metabolite Metalaxyl Acid in Aged Mobile Phases: The Role of Trace Water

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The separation of chiral transformation products greatly complements the understanding of the stereochemistry of chiral pollutants. In this study, direct enantiomeric resolution of metalaxyl and its main degradation product metalaxyl acid, often co-occurring in the environment, was carried out in normal-phase high-performance liquid chromatography with a Chiralcel OJ-H column. (*R*)-Metalaxyl acid and (*S*)-metalaxyl, which were almost parallel bonding to the chiral stationary phase, tended to separate, started to overlap, coeluted, and separated again with subtle changes of the mobile phase consisting of *n*-hexane, 2-propanol, acetic acid, and trace water. Their competition above hampered an acceptable direct separation in fresh mobile phases. Aged mobile phases with a storage period of 3–5 days, however, significantly improved their separation, in which trace water from moisture air diffusion was found to play a major role. Trace water differentially affected peak width and retention times and then induced enhanced peak separation, confirmed by deliberate addition of water to fresh mobile phases. Furthermore, none of the studied factors, involving temperature, concomitant analytes, and trace water, could cause changes of the configuration of the chiral stationary phase. Simultaneous enantiomeric separation of both compounds was achieved in aged or fresh mobile phases with adventitious or added water and gave satisfactory peak separation, all with *R*_s values of more than 1.20 in environmental samples.

KEYWORDS: Metalaxyl; metalaxyl acid; analytical method; trace water; enantiomeric separation

INTRODUCTION

About 25% of currently used pesticides are chiral (1). Once pollutants are released into the environment, transformation products may be chiral themselves (e.g., metalaxyl acid shown in **Figure 1**) derived from chiral pollutants, and chiral products can also be transformed from achiral pollutants, which exhibit potential enantioselectivity in environmental behavior and toxicological significance (2–5). These transformation products, inferred as emerging contaminants (6), generally differ in environmental and toxicological properties as compared to their parent compounds (7–9), some of which pose higher risks to the environment (10). Thus, they are recommended to be included in risk assessment of the parent contaminants.

Metalaxyl [methyl *N*-methoxyacetyl-*N*-(2,6-dimethylphenyl)-DL-alaninate], a widely used fungicide, has a chiral center in the 2-position of propionate (**Figure 1**), and the (*R*)-enantiomer is fungicidally active. Likewise, the fungicide can exhibit enantioselective degradation in the environment (11–14). Metalaxyl acid, a main degradation product of metalaxyl, often is detected together with the fungicide in the environment (15). Both laboratory and field studies indicate that both compounds are persistent and mobile and will have the potential to reach groundwater, particularly in soils with low organic matter and

clay contents (15, 16). Therefore, simultaneous chiral analysis is of much significance to understand the ultimate environmental fate of metalaxyl in the viewpoint of molecular chirality.

A variety of separation techniques have been developed to separate chiral pollutants and major degradation products at the same time, such as the dechlorinated degradate (*o,p'*-DDD) of *o,p'*-DDT by GC × GC-QqQ-MS (17) and capillary electrochromatography (18), the ethanesulfonic and oxanilic acid degradates of acetochlor (19) and metolachlor (20) by capillary zone electrophoresis, and the free acid degradate of diclofop-methyl by chiral high-performance liquid chromatography (HPLC) (21) and two-dimensional chiral HPLC (22). In the case of metalaxyl and metalaxyl acid, enantiomers of either the parent or the free acid alone tend to have a satisfactory resolution by chiral HPLC (12, 13, 23). Strong polarity of both compounds as well as similar chemical structures probably hamper their direct enantiomeric separation. As described by Buser et al. (12), their enantiomers were separated by chiral gas chromatography–mass spectrometry after esterification derivatization by diazoethane. Metalaxyl acid was analyzed as the ethyl ester and was distinguished from metalaxyl, which was methyl ester and was not affected by diazoethane. Their enantiomers were also simultaneously determined on a chiral HPLC coupled with a tandem mass spectrometry (MS/MS) detector (14), by which components of mobile phases did not interfere with the response of the enantiomers. The above analytical processes were more or less limited by either

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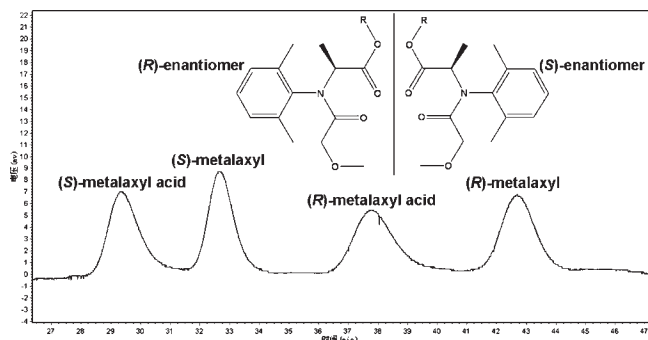


Figure 1. Chemical structures and HPLC chromatogram of metalaxyl ($R = \text{CH}_3$) and metalaxyl acid ($R = \text{H}$). Mobile phase, *n*-hexane/2-propanol/acetic acid (95:5:0.1, v/v/v) with a storage period of 3 days; flow rate, 0.5 mL min^{-1} ; and column temperature, room temperature.

elaborate derivatization steps or analytical equipments (e.g., MS). As an increasing use of metalaxyl leads to higher risks of occurrence of both fungicide and its acidic metabolite in soil and groundwater (16), facile analytical methods for their enantiomers, which can be achieved in most laboratories, are needed to describe their environmental fate.

In this study, the enantiomers of metalaxyl and metalaxyl acid were separated at the same time in HPLC coupled with a commercial chiral column (Chiralcel OJ-H), based on the optimized chromatographic conditions. Furthermore, competition of enantiomers of metalaxyl and metalaxyl acid was confirmed by thermodynamic tests. The effect of the storage time of aged mobile phase on enantiomeric resolution was discussed, in which trace water (absorbed from air containing moisture or deliberately added), polarity, and viscosity of aged mobile phases were involved.

MATERIALS AND METHODS

Reagents and Chemicals. *rac*-Metalaxyl (chemical purity $\geq 98\%$) and its (*R*)-enantiomer metalaxyl-M (chemical purity $\geq 98\%$ and enantiomeric excess = 0.97) were kindly supplied by Zhejiang Heben Pesticide & Chemicals Co., Ltd. (Wenzhou, China). *rac*-Metalaxyl acid (chemical purity $\geq 98\%$) and (*R*)-enantiomer-enriched metalaxyl acid (chemical purity $\geq 98\%$ and enantiomeric excess = 0.38) were prepared via basic hydrolysis of corresponding esters in our laboratory. The standard solutions of the above-mentioned compounds were prepared by *n*-hexane/2-propanol (65/35, v/v). *n*-Hexane, 2-propanol, and dichloromethane were of HPLC grade and purchased from Tedia (Fairfield, OH). Analytical grade acetic acid was purchased from Shenyang Lianbang Reagent Co., Ltd. (Shenyang, China).

Apparatus and Chromatographic Conditions. Chiral separation was performed on a LC-10P HPLC system, which consisted of a LC10P high-pressure liquid constant flow pump, a LC10UV detector, a KT-230A column oven (Dalian Zhonghuida Scientific Instrument Co., Ltd., Dalian, China), a N2000 chromatographic workstation (Zhejiang University Zhida Information Engineering Co. Ltd., Hangzhou, China), and a 7725i manual sampling valve with a $20 \mu\text{L}$ loop (Rheodyne, United States). The flow rate was 0.5 mL min^{-1} . The Chiralcel OJ-H [cellulose *tris* (4-methylbenzoate)] was purchased from Daicel Chemical Industries (Tokyo, Japan) and had $250 \text{ mm} \times 4.6 \text{ mm}$ i.d. with the chiral stationary phase coated onto a $5 \mu\text{m}$ silica gel substrate. The detection wavelength was 236 nm.

The circular dichroism (CD) signal of (+) or (−) was detected by a Jasco LC-2000 series HPLC system (Jasco, Tokyo, Japan). The HPLC system consisted of a PU-2089 quaternary gradient pump, a mobile phase vacuum degasser, an AS-2055 intelligent sampler with a $100 \mu\text{L}$ loop, a CO-2060 column thermostat, a UV-2075 detector, a variable-wavelength CD-2095 CD, and a LC-Net II/ADC data collector. Chromatographic data were acquired and processed with computer-based ChromPass software (version 1.7.403.1, Jasco).

The specific inductive capacity of mobile phases was measured to compare dielectric constants by PCM-1A permittivity measurement instruments (Nanjing University Wanhe Scientific Instrument Co., Ltd., Nanjing, China). The viscosity of mobile phases was measured by LVDV-2+Pro CP viscometer (Brookfield, United States) with an accuracy of 0.01 mPa s. The dipole moments of metalaxyl and metalaxyl acid were calculated by PM3 arithmetic of MOPAC 2000 (CS ChemOffice 2004).

Preparation of Metalaxyl Acid. An appropriate amount of metalaxyl [or (*R*)-metalaxyl] was added to about 10% NaOH solution (1:1.05 mol ratio). The mixture was stirred at room temperature for 2 h and then heated to 85°C to reflux for above 12 h until the liquid was clear. Concentrated hydrochloride was added to adjust the pH to 1–2 after the liquid cooled. Metalaxyl acid [or (*R*)-metalaxyl acid] was crystallized and stored at 4°C and then filtered in vacuum and washed by precooling deionized water. The products were recrystallized with a mixture solvent of acetone and petroleum ether and then were dried under an infrared lamp. These compounds were identified by HPLC–electrospray ionization (ESI)–MS.

Pesticides Extraction. Appropriate amounts of analytes were added in water. Water samples were first filtered by a $0.22 \mu\text{m}$ filter membrane and then acidified to ca. pH 2.0 with dilute H_2SO_4 . The analytes were re-extracted with three portions of dichloromethane (50 mL per). The combined dichloromethane extracts were evaporated nearly to dryness by rotary evaporation and then dried under a stream of nitrogen. Finally, the residue was redissolved in 1.0 mL of *n*-hexane/2-propanol (65/35, v/v).

Extraction of metalaxyl and metalaxyl acid from soil was according to the reported method by Buerge (11). In brief, appropriate amounts of the analytes were added to soil sample. The 20 g samples (dry weight) were mixed with 10 mL of methanol. The samples were centrifuged (ca. 2000g for 20 min) after vigorous shaking (ca. 1 min), and the supernatants were transferred into glass vials. This procedure was repeated with a mixture of 10 mL of acetone and 10 mL of distilled water and with an additional 20 mL of distilled water to improve the extraction of metalaxyl acid. The combined extracts (methanol/acetone/water) were acidified to ca. pH 2.0 with H_2SO_4 , and subsequent operation was according to the procedure for water samples in this study.

RESULTS AND DISCUSSION

Absolute Configuration Identification. In the experiments, the separated enantiomers were identified based on the corresponding chromatography responses. Their peak areas were dependent on compositions of enantiomers of metalaxyl and metalaxyl acid and were adjusted by deliberate addition of (*R*)-metalaxyl and (*R*)-enantiomer-enriched metalaxyl acid. Under the optimized chromatographic conditions, the successive elution order was (*S*)-metalaxyl acid, (*S*)-metalaxyl, (*R*)-metalaxyl acid, and (*R*)-metalaxyl (Figure 1). As shown in Figure 2, (*R*)-enantiomers of both metalaxyl and metalaxyl acid were appointed to have positive cotton effect signals in terms of the octant rule (24) and standard compounds. The (*S*)-isomers were cotton-effect negative, which was consistent with two recent studies by Chen and Liu (25) and Qiu et al. (23).

Optimization for Enantiomeric Separation. In the pre-experiment, the storage period of the mobile phase prepared was found to significantly affect the separation of enantiomers of metalaxyl and metalaxyl acid at the same time. Thus, it was included into optimization of chromatographic parameters, also with the involvement of polar modifier (2-propanol), acetic acid, and column temperature.

As shown in Table 1, a polar modifier 2-propanol resulted in differential effects on simultaneous resolution of metalaxyl and its free acid in varying mobile phases, for example, fresh mobile phase and aged mobile phase with a storage period of 3 days. None of the four components was eluted in both mobile phases without 2-propanol and was attributable to a strong interaction between the chiral stationary phase and the polar analytes, which were represented by dipole moments of 5.917 D (metalaxyl) and 4.374 D (metalaxyl acid), respectively. In fresh mobile phases,

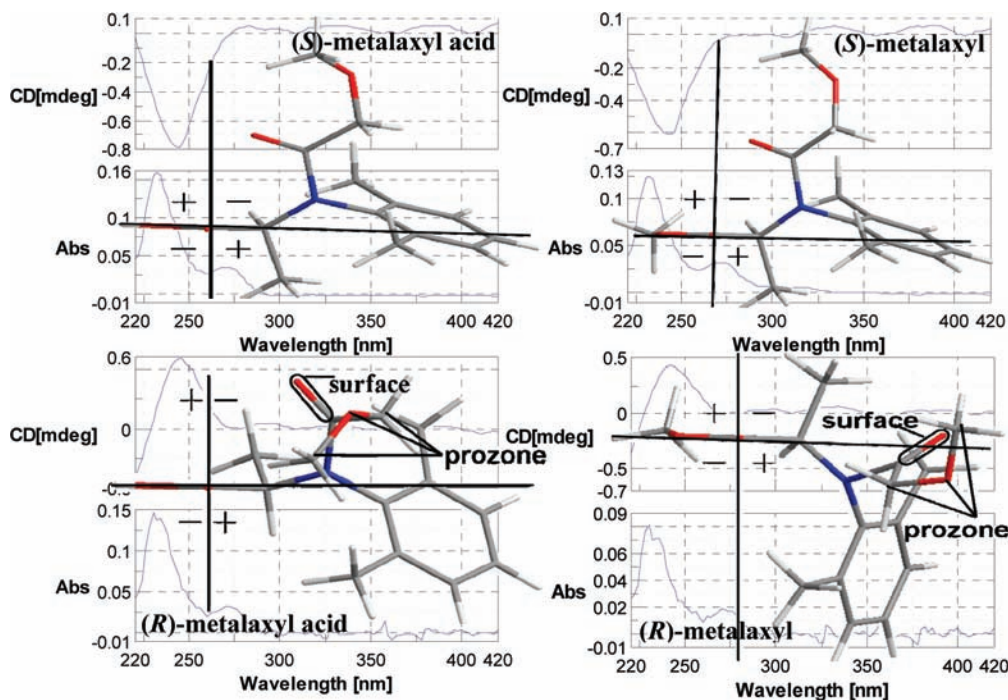


Figure 2. CD spectra of metalaxyl and metalaxyl acid and their application in the octant rule.

Table 1. Effects of 2-Propanol on Simultaneous Enantiomeric Separation of Metalaxyl and Metalaxyl Acid^a

storage period	2-propanol (% v/v)	k_1	k_2	k_3	k_4	α_1	α_2	α_3	Rs_1	Rs_2	Rs_3
0 day	5	3.30	3.69	4.02	5.79	1.12	1.09	1.44	1.04	0.89	3.89
	10	1.30	1.45	2.15	3.35	1.12	1.48	1.56	0.60	1.98	2.76
	15	0.82	0.97	1.53	2.46	1.18	1.58	1.60	0.67	1.98	2.73
3 days	5	3.14	3.67	4.26	5.16	1.17	1.16	1.21	1.20	1.27	1.80
	10	1.14	1.64	1.75	2.56	1.44	1.07	1.47	2.15	0.47	2.36
	15	0.70	0.99	1.23	1.85	1.42	1.23	1.51	1.34	0.92	2.06

^a The added amount of acetic acid in the mobile phase was 0.1%. The column temperature was set as room temperature. On the basis of the elution order of compounds, the parameters were calculated as $\alpha_1 = k_2/k_1$ and $Rs = 2 \times (t_2 - t_1)/(w_1 + w_2)$.

the enantiomers of ester and acid of metalaxyl were poorly separated with the occurrence of $Rs < 1.0$ and gave a decreasing elution order of (*S*)-metalaxyl acid < (*R*)-metalaxyl acid < (*S*)-metalaxyl < (*R*)-metalaxyl. The elution order was well-known in normal-phase HPLC, in which weakly polar components (viz., metalaxyl acid) were usually less bonded on the stationary phase as compared to polar components (viz., metalaxyl). However, an inverse of elution order of (*R*)-metalaxyl acid and (*S*)-metalaxyl occurred in three mobile phases aged for 3 days. Especially, the *n*-hexane/2-propanol (95/5, v/v) mobile phase showed a satisfactory resolution with all Rs values of more than 1.20, in which (*S*)-metalaxyl acid was first eluted, followed by (*S*)-metalaxyl, (*R*)-metalaxyl acid, and (*R*)-metalaxyl (**Figure 1**).

Appropriate amounts of acetic acid added in mobile phases can reduce peak tailing and improve the peak shapes of acidic analytes. Acetic acid added significantly improved separation of the enantiomers of both esters and acids of metalaxyl as compared to the absence of acetic acid (data not shown). Particularly, 0.1–0.2% of acetic acid favored their separation with Rs values of more than 1.20. However, higher amounts of acetic acid (e.g., 0.6%) caused marked noise and even led to a plateau peak.

The column temperature was another important factor determining enantiomeric separation of ester and acid of metalaxyl.

Table 2. Effects of Column Temperature on Simultaneous Enantiomeric Separation of Metalaxyl and Metalaxyl Acid^a

temp (°C)	k_1	k_2	k_3	k_4	α_1	α_2	α_3	Rs_1	Rs_2	Rs_3
20	3.38	4.23	4.93	6.23	1.25	1.16	1.26	1.33	1.15	1.87
25	3.05	3.64	4.29	5.15	1.19	1.18	1.20	1.18	1.28	1.55
30	2.75	3.12	3.72	4.25	1.14	1.19	1.14	0.91	1.25	1.04
35	2.50	2.68	3.27	3.51	1.07	1.22	1.07	0.49	1.54	0.66
40	2.28	2.28	2.95	2.95	1.00	1.29	1.00	0.00	1.53	0.00

^a Mobile phase: *n*-hexane/2-propanol/acetic acid (95:5:0.1, v/v/v) with a storage period of 3 days.

As the temperature ranged from 18 to 30 °C, all adjacent peaks with Rs values of more than 1.10 were acceptable to quantify (**Table 2**), suggesting that the procedure could be applied at room temperature. The resolution of (*S*)-metalaxyl and (*R*)-metalaxyl acid was greatly improved with the increase of temperature. In contrast, another two pairs of adjacent peaks, that is, (*S*)-metalaxyl and (*R*)-metalaxyl acid as well as (*R*)-metalaxyl acid and (*R*)-metalaxyl, showed weakened separation at the same time and even overlapped completely at a high temperature up to 40 °C.

Thermodynamic Study on Enantiomeric Separation. The column temperature has a major impact on retention, resolution, and column efficiency for chiral separation (26–28). In this study, individual and simultaneous separation of metalaxyl and metalaxyl acid were carried out in the range of 20–40 °C with an increment of 5 °C under the optimized chromatographic conditions. The thermodynamic effect that changes the separation factor (α) follows van't Hoff equations (29):

$$\ln k = \frac{-\Delta G^\circ}{RT} + \ln \phi = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (1)$$

$$\ln \alpha = \frac{-\Delta \Delta H^\circ}{RT} + \frac{\Delta \Delta S^\circ}{R} \quad (2)$$

where k is the retention factor, α is the enantio-separation factor, R is the universal gas constant, T is the absolute temperature in

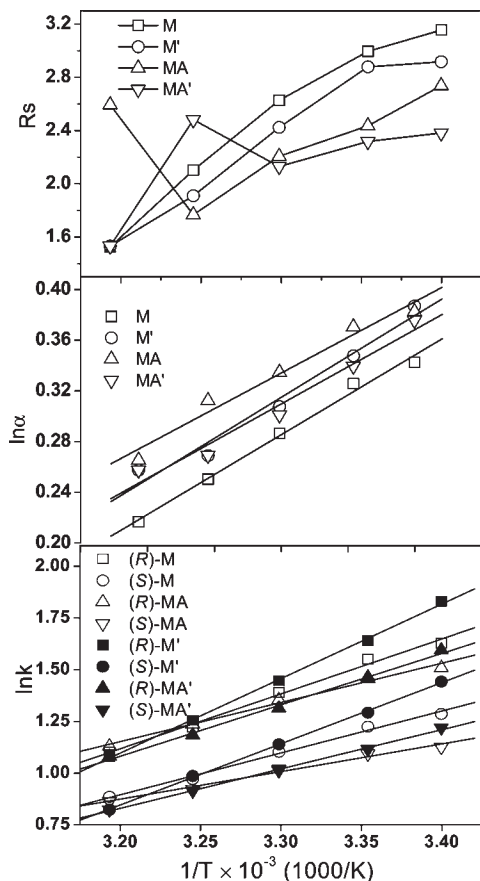


Figure 3. Thermodynamic parameters of metalaxyl and metalaxyl acid in individual or mixed separation. In the figure, M' and MA' represent metalaxyl and metalaxyl acid in individual analysis, respectively; M and MA represent metalaxyl and metalaxyl acid in mixed analysis, respectively.

Table 3. Thermodynamics Parameters of Metalaxyl and Metalaxyl Acid

		$\Delta H_1^{a,a}$	$\Delta H_2^{c,b}$	$\Delta(\Delta H^\circ)$	$\Delta(\Delta S^\circ)$
		metalaxyl	(kJ mol ⁻¹)	(kJ mol ⁻¹)	(kJ mol ⁻¹)
individual separation	ester	-16.8	-22.1	-5.2	-14.9
	acid	-11.1	-15.8	-4.7	-12.6
simultaneous separation	ester	-24.6	-30.0	-5.4	-15.1
	acid	-15.8	-20.7	-4.9	-13.5

^a (S)-enantiomers. ^b (R)-enantiomers.

Kelvin, ΔH° and ΔS° are the standard transfer enthalpy and entropy of the analyte from the mobile phase to the chiral stationary phase, respectively, ϕ is the phase volume ratio, and $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ are the differential standard transfer enthalpy and entropy of enantioselective adsorption, respectively.

As shown in **Figure 3**, plots of $\ln k$ vs $1/T$ for metalaxyl and metalaxyl acid yielded favorable straight lines ($r^2 > 0.985$). As a result, retention mechanisms of both metalaxyl and metalaxyl acid in single analysis were invariant under the temperature range, associated with no alterations of configuration of the chiral stationary phase (29, 30). However, their mixtures in a simultaneous analysis gave an increased standard transfer enthalpy of all components (**Table 3**) and, hence, indicated the occurrence of a competition interaction of components bonding to the chiral stationary phase with intact configuration. Among the components, the enthalpy change for (R)-metalaxyl was always the greatest (i.e., most negative), and its bonding to the chiral stationary phase was the most favorable (27). The enthalpy changes for (R)-metalaxyl acid and (S)-metalaxyl were nearly

parallel in individual separation and stood for a similar bonding to the chiral stationary phase. Consequently, a coelution and even an inversion of their elution order can be induced in the separation of the mixture by subtle changes of the mobile phase as is the case in this study.

Regardless of varying enthalpy changes of metalaxyl and its acid, their $\Delta\Delta H^\circ$ values were nearly identical in both the individual and the simultaneous analysis and caused negligible change in enantioselective bonding to the chiral stationary phase. This phenomenon was consistent with the above-mentioned fact that competitive interaction of components did not affect the configuration of the chiral stationary phase. Furthermore, $\ln \alpha$ vs $1/T$ plots for metalaxyl and metalaxyl acid showed a good linear behavior ($r^2 > 0.97$) and also indicated their intact enantioselective interactions (29). As both $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ for them were negative, enantiomeric separation of metalaxyl and metalaxyl acid was an enthalpy-driven process, independent of the competition of components.

Effect of Aged Mobile Phase on Enantiomeric Separation. In most laboratories, mixed organic solvents as eluents usually are stored in solvent bottles with a small hole for pressure exchange. Likewise, the mobile phases had to be exposed to air containing moisture in the experiments when testing, even though at other times they were deliberately kept in enclosed solvent bottles. As organic solvents easily absorbed moisture from air, water and air as unexpected ingredients resulted in differential compositions of mobile phases and probably affected solvent efficiency.

Chromatographic retention of the enantiomers of ester and acid of metalaxyl was remarkably dependent on the storage period of the aged mobile phases (**Figure 4**). Varying the storage period not only led to sufficient peak separation but also changed elution orders of these components. (S)-Metalaxyl acid showed almost constant capacity factors of 3.39 (fresh mobile phase) to 3.16 (aged mobile phase stored for 6 days), but (R)-metalaxyl acid's retention significantly increased with the aging term of the mobile phases, corresponding to a capacity factor varying from 3.79 to 4.65. However, a nearly identical decrease in retention occurred for the enantiomers of metalaxyl by 18.5 [(S)-enantiomer] and 16.7% [(R)-enantiomer], respectively. Apparently, the evolution of capacity factors for (R)-metalaxyl acid and (S)-metalaxyl intersected in aged mobile phases and, hence, resulted in a reversal retention order of them. In brief, (R)-metalaxyl acid and (S)-metalaxyl overlapped completely with a R_s of zero in 1 day-aged mobile phases but gave increasing separation with an inverse order in mobile phases aged for more than 2 days, in which (R)-metalaxyl acid and (R)-metalaxyl gradually closed and partially overlapped on day 6 with a R_s of 0.82. These results suggested that differential bonding of both analytes to chiral stationary phase with the involvement of chemical structure and molecular stereochemistry was time-dependent in aged mobile phases. In addition, mobile phases with a storage period of 3–5 days can achieve sufficient separation for enantiomers of ester and acid at the same time (**Figure 4**).

Polarity and viscosity of mobile phases were measured all through a storage period of 6 days in identical mobile phases, which were also used for chromatographic analysis. As shown in **Figure 4**, the polarity of mobile phases, characterized by dielectric constants, remained intact regardless of aged terms. In contrast, viscosity first increased within 2 days but subsequently decreased below the initial value, resulting predominately from the equilibrium of association of alcohols in nonpolar solvents via hydrogen bonds (31–34) and air diffusion during storing. As it was well established, alcohols such as 2-propanol could associate into dimers, multimers, and even clusters in nonpolar and invert solvents, such as heptane, hexane, and octane, and consequently

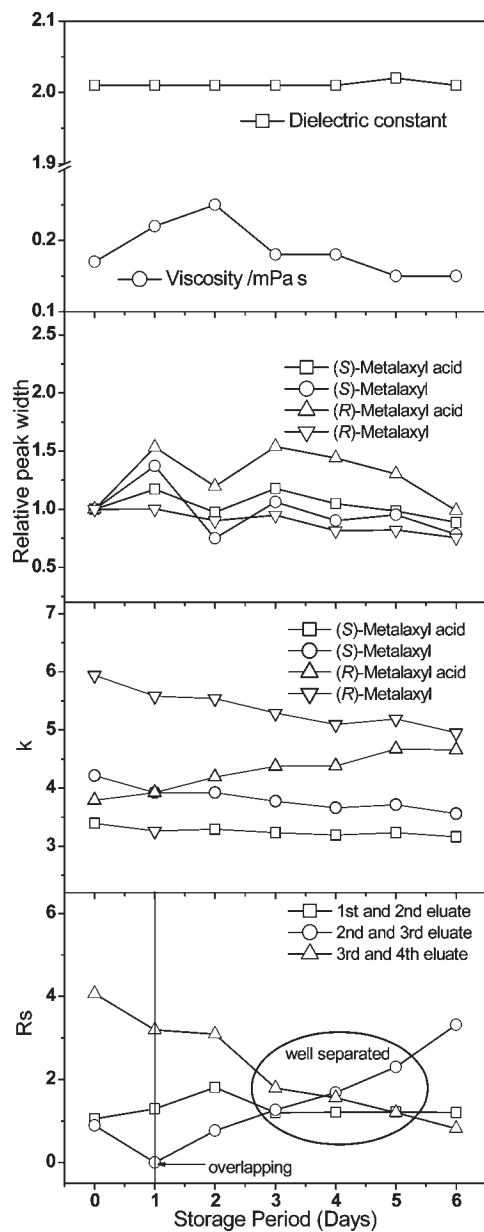


Figure 4. Effects of storage period on solvent characteristics and separation efficiency of aged mobile phases.

induced changes of mixed solvent properties involving viscosity (33). The reduction of viscosity of mobile phases favored chromatographic separation via facilitated diffusion, and actually, enhanced separation of the analytes in this study occurred at the same time. Although trace water from the air did not contribute to the decrease in viscosity of aged mobile phases, its effect on enantiomeric separation of the analytes in this study could not be excluded since trace water can greatly affect enantio-separation of various analytes in chiral normal-phase HPLC (35, 36).

Effect of Trace Water on Enantiomeric Separation. The trace water content in a range of 0.0005–0.0240% (v/v) significantly affected the retention behavior of these analytes in fresh mobile phases with the addition of appropriate amounts of water (Figure 5). Similarly, the strong competition between (*R*)-metalaxyl acid and (*S*)-metalaxyl precluded their separation both in fresh mobile phases with addition of water and in aged mobile phases, which could absorb moisture from the air. The lowest water level (i.e., 5% per million by volume) in fresh mobile phases

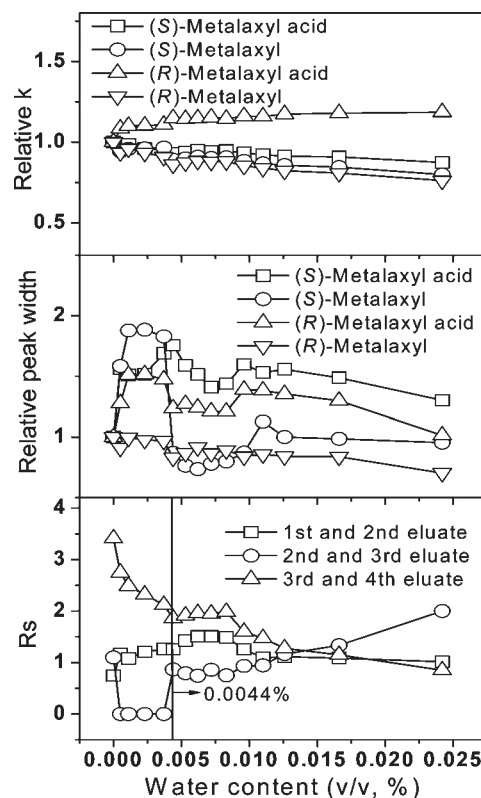


Figure 5. Effects of added water on separation efficiency of fresh mobile phase.

was easily achieved under general conditions (35) and led to a coelution of (*R*)-metalaxyl acid and (*S*)-metalaxyl primarily by inducing individual peak broadening. The incremental addition of water to the mobile phase (up to 0.0040%) did not improve their resolution, attributable to inverse contributions of differential retention (positive effect) and peak broadening (negative effect). As shown in Figure 5, a reversal change of capacity factors for (*R*)-metalaxyl acid and (*S*)-metalaxyl favored their solution, but their concurrent peak broadening counteracted the positive effect of the former. As water levels exceeded 0.0044%, they started to separate, corresponding to a constant peak width but increasing capacity factors differential. Particularly, a water content of around 0.015% in fresh mixed solvents was sufficient to induce satisfactory peak separation for all analytes, comparable to mobile phases aged for 3–5 days, but shortened retention times were also achieved. Overall, the fact that they first separate, start to overlap, give a single peak, and separate again is found in both fresh mobile phases with increasing water levels and aged mobile phases with increasing storage periods. In practice, the water content of the latter is variable and increases with time, suggesting itself a primary driving factor inducing satisfactory separation.

The addition of water changed to different extent retention of the studied analytes (Figure 5). A small reduction of retention by ca. 13% was observed for (*S*)-metalaxyl acid, but it was increased by 18% for the (*R*)-enantiomer. Interestingly, both enantiomers of metalaxyl showed a reduced retention by ca. 20%. The α values of enantiomers of metalaxyl (Figure 6), that is, enantio-separation factor or enantioselectivity, were not affected in both aged mobile phases (ca. 3.3%) and fresh mobile phases with the addition of water (ca. 3.4%), which excluded a structure modification of the chiral stationary phase induced by trace water. In contrast, the α values of enantiomers of the free acid significantly increased with water added or aged terms. These results further confirmed

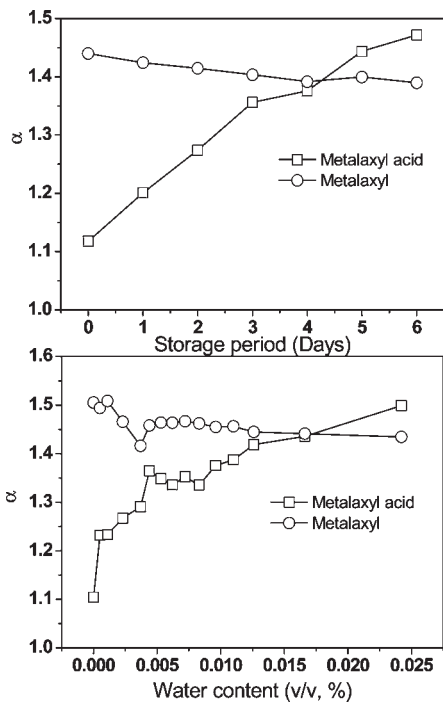


Figure 6. Effects of storage period and added water on α -values of enantiomers of metalaxyl and metalaxyl acid.

that differential bonding of components of the mixture of metalaxyl and metalaxyl acid to the chiral stationary phase could be controlled by trace water. The specific effects of water on separation of individual components of analytes were also well-illustrated in the example of simultaneous enantiomeric separation of flavanone and 2'-hydroxychalcone, in which trace water played a major role in peak separation of (*S*)-flavanone and 2'-hydroxychalcone. The α values for separation of (*S*)-flavanone and (*R*)-flavanone were almost unaffected, corresponding to a water-dependent resolution of enantiomers (35).

Trace water can enhance peak separation generally by reducing tailing or by differentially affecting the retention of components of the mixture (35–38). Although a solid mechanism about effects of trace water on normal-phase chiral separation remains to be seen, the effects of water probably result from the following interactions: (1) Water itself can act as a polar modifier in normal-phase mixed solvents (39); (2) water can induce alterations of the steric environment of the chiral cavities in the chiral stationary phases (38); and (3) water can competitively bond to the SiO₂ support with analytes (35). A high sensitivity of resolution of these analytes to trace water, hither in this study, indicated that trace water probably affects their retention through the latter two interactions. Considering the fact that water is usually a forbidden solvent in chiral normal-phase HPLC, trace water is a useful alternative as an additive in certain instances and for difficult separations, not only for satisfactory separation but also for accurate enantiomeric analysis. For example, naringin, a flavonoid that was conveniently separated into diastereomers on enantiomeric analysis (40) was completely baseline separated in significantly shortened retention times by the addition of water to the mobile phase (35); thus, fewer diastereomers could be produced from the flavonoid.

Performance of the Method for Enantiomeric Separation. The enantiomers of the ester and acid of metalaxyl in three concentrations were spiked to water and soil samples and then were separated under the optimized chromatographic conditions, of which mixed solvents of *n*-hexane/2-propanol/acetic acid

Table 4. Average Recovery (Mean \pm RSD) of Enantiomers of Metalaxyl and Metalaxyl Acid in Environment Samples

sample	concentration	(<i>S</i>)-metalaxyl acid (%)	(<i>S</i>)-metalaxyl (%)	(<i>R</i>)-metalaxyl acid (%)	(<i>R</i>)-metalaxyl (%)
water ^a	0.01 (<i>n</i> =6)	100.7 \pm 7.7	101.2 \pm 8.5	105.4 \pm 7.9	97.6 \pm 8.1
	0.10 (<i>n</i> =3)	100.3 \pm 5.0	97.4 \pm 3.2	95.5 \pm 4.9	94.0 \pm 4.8
	0.50 (<i>n</i> =3)	100.9 \pm 4.1	99.4 \pm 1.7	103.3 \pm 1.2	98.3 \pm 3.1
soil ^b	0.025 (<i>n</i> =6)	100.4 \pm 6.9	101.2 \pm 5.7	96.6 \pm 6.1	96.0 \pm 5.8
	0.25 (<i>n</i> =3)	99.2 \pm 4.9	99.5 \pm 4.9	99.5 \pm 5.0	104.0 \pm 4.8
	1.25 (<i>n</i> =3)	99.2 \pm 4.0	96.6 \pm 3.4	101.7 \pm 4.3	99.7 \pm 3.6

^a Spiked concentration, mg L⁻¹. ^b Spiked concentration, mg kg⁻¹.

(95:5:0.1, v/v/v) with a storage period of 3 days were used as the elute. All samples gave recoveries of more than 94% and had an acceptable RSD of less than 8.5% (Table 4). As it is noted above (14), direct separation of these analytes has recently been achieved on the chiral normal-phase HPLC with a MS/MS detector and a Chiralcel OD-H column. We tried to reproduce this procedure with an UV detector replacing the MS/MS detector and gave an identical order of elution reported by this study, accompanied with the corresponding *R*_s values of 2.31, 1.06, and 11.90, respectively. It was also noted that increased baseline noise arose from the eluent containing high contents of 2-propanol (50%, by volume) and formic acid (0.3%, by volume). Because the UV detector is popular in most laboratories, the method developed herein is facile and easily achieved for direct resolution of enantiomers of metalaxyl and metalaxyl acid.

Conclusion. The competitive interaction of components of metalaxyl and metalaxyl acid [especially, (*R*)-metalaxyl acid and (*S*)-metalaxyl] precludes their sufficient separation, but does not affect enantioselective bonding of individual pairs of enantiomers to the chiral stationary phase. Adventitious trace water to a mobile phase, which is derived from deliberate addition or absorption of organic solvents from air during testing and storing, can differentially change the peak width and affect the retention of components of the mixture and thus induces satisfactory peak separation. Trace water does not induce structure modification of the chiral stationary phase and can be an alternative polar additive in certain instances for satisfactory separation. The obtained method in this study is facile and suitable for direct enantiomeric analysis of metalaxyl and its main acidic degradate (metalaxyl acid) in soil and water.

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