Rice—A Step Toward Use of Allelopathy

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ABSTRACT

Rice (*Oryza sativa* L.) allelopathy has been on the research agenda for a decade. Now it is important to step back and look at its progress to enable priority setting for future research. This paper aims to do so primarily using the following five-step protocol for allelopathy research: (i) carrying out laboratory, greenhouse, and field studies to illustrate the effect of released allelochemicals; (ii) isolating, identifying, and characterizing allelochemicals; (iii) establishing a correlation between growth inhibition and allelochemicals; (iv) performing genetic mapping of quantitative trait loci (QTLs) correlated with allelopathy; and (v) breeding for allelopathic cultivars tested for competitive ability in greenhouse and field experiments. Recent research on rice allelopathy has resulted in the following research milestones:

- **There is large variation in allelopathy among rice cultivars.**
- **Allelopathy plays a role under field conditions.**
- **Allelopathic rice can suppress both mono- and dicot weed species.**
- **Progress has been made in identifying rice allelochemicals.**
- **Quantitative trait loci correlated with allelopathy have been identified.**

This paper discusses the progress made in recent years and suggests some direction for future research.

IMPROVING the competitive ability of crops might reduce dependency on herbicides. Attempts to increase competitive ability, however, have had limited success and no crop cultivar has been released with superior competing ability as a marketing argument. The lack of progress in creating competitive cultivars might be due to the complexity and lack of understanding of the components and mechanisms of competition. Only when we have understood and are able to optimize several competition components will a truly good competitor be a reality. The importance of chemical interference, including allelopathy, in crop competition has often been discussed (Rice, 1995). More recently, Wu et al. (1999) reviewed the possibilities of genetically improving crops with allelopathic potential and stated that allelopathy can play an important role in future weed management. To convincingly demonstrate the possibilities of using allelopathy, focused research is needed. For this, the following are needed: (i) laboratory, greenhouse, and field studies carried out to illustrate the effect of released allelochemicals; (ii) allelochemical isolation, identification, and characterization; (iii) a correlation established between growth inhibition and allelochemicals; (iv) genetic mapping of QTLs correlated with allelopathy; and (v) breeding for allelopathic cultivars tested for competitive ability in greenhouse and field experiments. Research to accomplish the above goals cannot be carried out in one laboratory, and needs active collaboration among a wide range of scientists, including biologists, ecologists, agronomists, natural product chemists, plant physiologists, and geneticists. To a large extent, research on allelopathy in rice has endeavored to achieve these goals. Rice has thus become a model plant and an example of successful progress toward using allelopathic rice cultivars for weed management. This paper briefly summarizes recent progress in rice allelopathy, and identifies research components that still need to be studied to provide guidance for future research.

ABOUT RICE ALLELOPATHY

Rice allelopathy has been a subject of continued research for a decade and progress has been made in a range of fields, thus adding to the understanding of it (for a review of rice allelopathy, see Olofsdotter et al., 1995 and Olofsdotter, 1998). All biological research is a succession of processes of success and failure to test hypotheses that can increase our understanding of biological processes and thereby advance research. These stepwise processes can be evaluated by knowledge milestones, which are discussed for rice allelopathy.

There is Large Variation in Allelopathy among Rice Cultivars

To quantify allelopathy in rice, several laboratory and field screening procedures have been developed and tested (Fujii, 1992; Dilday et al., 1998; Navarez and Olofsdotter, 1996; Olofsdotter and Navarez, 1996). The conclusions from all these studies were that there are differences in allelopathic potential among rice cultivars, expressed as a reduction in root growth of test plants grown in association with rice in the laboratory or as a weed reduction (dry matter and density) in the field. For the field screening, however, it is important to note that allelopathy is difficult to distinguish from competition and therefore such screening must be compared with data where resource competition can be eliminated as a factor in the experiment. Olofsdotter et al. (1999) correlated screening results from the laboratory with a range of competition components, measured in the field, and claimed that allelopathy can explain 34% of the reduction in total weed dry weight 8 wk after seeding. Table 1 presents selected data from this study including barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] plant dry weight 8 wk after seeding, tillering, rice and weed height, the barnyardgrass root length in laboratory screening, and a cultivar ranking. Plant height is often described as one of the most important factors for total

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Abbreviations: QTLs, quantitative trait loci.

Table 1. Weed suppressive ability of rice cultivars under field conditions compared with screening for allelopathy under laboratory conditions.

	Field experiments							Laboratory screening		
Rice cultivar	Dry season 1995: weed dry weight	Wet season 1995: weed dry weight	Dry season 1996: weed dry weight	Dry season 1996: number of weeds in weed row	Dry season 1996: weed tillers in weed row	Dry season 1996: dry weight per weed	Total weed weight: three seasons	Barnyard-grass root length	SE	Rank of whole data set
	g m^{-2}			no. m^{-1}		g $plant^{-1}$	g	mm		
Lubang Red	148	164	188	36	45	5.4	500	35.8	2.4	1
YH1	151	162	230	41	50	5.8	543	37.1	2.4	3
Musashikogane	120	156	166	35	30	4.8	442	38.4	2.4	4
Taichung Native 1	129	185	274	50	64	5.3	588	42.6	2.4	5
Kouketsumuchi	144	157	106	38	33	2.9	407	42.8	2.4	6
Takanenishiki	141	158	126	33	29	3.9	425	46.6	2.4	14
AC 1423	116	142	219	30	42	7.2	477	46.8	0.9	15
Tan Gang	134	122	91	36	30	2.6	347	47.0	2.6	17
IR38 (control)	225	271	301	36	76	8.2	797	63.8	2.4	54
No-rice control	289	343	460	37.8	122	12.6	1092	97.0	0.8	111
Mean for all cultivars	187	238	281	37.8	57	7.6	706	58.9	–	–
SE	26.2	36.3	48.8	4.7	10.0	1.5	–	–	–	–
CV	28	29	35	25	35	40	–	26	–	–

competitive ability of a crop and accounts for a similar percentage of total competitive ability (Gaudet and Keddy, 1988; Garrity et al., 1992).

Screenings for allelopathy in rice have also been undertaken in Egypt, Korea, and Cambodia using some of the same rice accessions as in previously mentioned screening activities (in Japan, USA, and at the International Rice Research Institute, Los Banos, Philippines) in combination with local modern and traditional cultivars. Similarly, in these programs, cultivar differences in allelopathic strength were found in both modern and local traditional cultivars (Kim and Shin, 1998; Hassan et al., 1998; Pheng et al., 1999). Although laboratory methods are useful, they all have some shortcomings in screening for allelopathy. The plant box method (Fujii, 1992), using a single donor plant in agar medium, requires donor plants that are 30 d old. Under field conditions, allelopathy expressed by 30-d-old plants would have only a limited effect on the weeds, as the critical period for yield reduction from weeds is the first month of crop growth. Furthermore, allelopathic strength can vary with age, as was found for hydroxamic acids in wheat (*Triticum aestivum* L.), where production increased until the plant was 40 d old (Niemeyer, 1988). A method using older plants, in combination with very sensitive lettuce (*Lactuca sativa* L.) as the test plant, might therefore overestimate allelopathy. Furthermore, using lettuce as a test plant for rice allelopathy also raises questions on field relevance. In practice, screening systems, which are designed to test large amounts of accessions, should be inexpensive, rapid, space-limited, and reproducible. The plant box method requires a substantial amount of space and is therefore impractical for such a purpose. The relay-seeding technique developed in the Philippines solves most of those problems (Navarez and Olofsdotter, 1996). The technique uses weeds seeded into 7-d-old rice plants in a petri dish with perlite, where they grow together for 10 d. It is a space-limited technique in which early allelopathic potential can be measured using barnyardgrass as a test plant. Results obtained using weeds as test plants, however, will be more variable than those from domesticated plants because of a larger genetic diversity in the weed seeds. Continued development of screening systems, which minimize other sources of variation, could therefore yield more reliable results. The large variability in the results when using weeds as test plants has to be weighted against the importance of using a "field-relevant" test species.

Allelopathy Plays a Role under Field Conditions

The first observation of allelopathy in rice was made on field examinations in Arkansas (1988 cropping season) (Dilday et al., 1991). A seed-increase field with 5000 accessions sown in hills had a natural ducksalad [*Heteranthera limosa* (Sw.) Willd.] infestation and around some of the rice accessions was a weed-free area that could not be related to shading and appeared to be the result of chemical interaction. This led to a large field screening program in Arkansas and today 12 000 accessions have been screened, with about 3.5% of the accessions rated as allelopathic against ducksalad (Dilday et al., 1998). These lines have not been tested in laboratory experiments, where other competition variables can be eliminated. The success in field screening against ducksalad can be explained by the weed biology. Ducksalad invades rice fields after irrigation at a time when the rice crop is already established, therefore making it easy to visually evaluate weed suppression. As the rice crop is already established when the weed problem occurs, these results also raise the question whether allelopathy requires older plants to be efficient.

Considering more important (globally) weed species suggests that it is harder to get a clear and direct picture of weed suppression under field conditions. In the Philippines, 111 rice cultivars have been evaluated for weed suppression capability against barnyardgrass under field conditions over three seasons (Olofsdotter et al., 1999). Weeds and rice were direct-seeded the same day, which resulted in similar emergence. During establishment and early growth of the crop and weeds, a range of variables were measured (e.g., tillering, height, and density) for rice and weeds. Finally, 8 wk after seeding, weed suppression was evaluated using barnyardgrass dry weight. The results showed that rice height was the most important factor influencing weed suppression but was closely followed by allelopathic potential as measured in laboratory screening using the relay-seeding technique for the same set of cultivars (Olofsdotter et al., 1999). The field results were confirmed in similar experiments conducted in Korea using some of the same cultivars as in the Philippines (Kim and Shin, 1998). The similarities in the results from the Philippines and Korea indicated that cultivar differences in weed suppression were relatively stable in different environments, which suggests that allelopathy was more influenced by genetics than environment. The experiments also indicated that maximum weed suppression was obtained in cultivars that have both allelopathic and competitive traits. Progress in field weed suppression is likely to be most efficient when both competition and allelopathy are optimized.

Allelopathic Rice Can Suppress Both Mono- and Dicot Weed Species

Both laboratory screening and field experiments reveal that rice allelopathy is active against both monocot and dicot weeds (Dilday et al., 1991; Fujii, 1992; Olofsdotter and Navarez, 1996; Hassan et al., 1998; Kim and Shin, 1998). However, there is no assurance for activity against both groups for a given rice accession. Therefore, more than one chemical is likely to be responsible for allelopathy in rice, and allelopathy is probably quantitatively inherited. One rice cultivar (Taichung Native 1) has shown activity against most of the weeds tested (e.g., barnyardgrass, desert horsepurslane [*Trianthema portulacastrum* L.], ducksalad, and toothcup [*Ammannia coccinea* Rottb.]) (Dilday et al., 1998; Olofsdotter and Navarez, 1996), and is therefore a suitable choice for both identifying allelochemicals and studying allelopathy genetics. Taichung Native 1 carries the semi-dwarf gene, included in most post–Green Revolution rice cultivars, and is used intensively in modern breeding programs. This could explain the surprisingly frequent occurrence of allelopathic capability in modern rice cultivars.

Progress Has Been Made in Identifying Rice Allelochemicals

The process of isolating and identifying rice allelochemicals has started but has not yet yielded chemicals that could explain the allelopathic effect (Rimando et al., 2001). None of the known chemicals released from rice can alone explain the weed suppression seen in the laboratory and fields. Phenolic acids have been identified in allelopathic rice germplasm (Rimando et al., 2001) and they have previously been described as allelochemicals (Inderjit, 1996; Mattice et al., 2001; Blum, 1998). However, concentrations of single phenolic acids and combinations of all phenolic acids measured in rice ecosystems do not approach phytotoxic levels (Tanaka et al., 1990; Olofsdotter et al., 2001). Identifying allelochemicals in rice is important for understanding allelopathy mechanisms and for use as markers in gene identification.

Quantitative Trait Loci Correlated with Allelopathy Have Been Identified

Evaluating a screening program from a breeder's point of view often starts by calculating the broad-sense heritability for the trait in question. The broad-sense heritability ranges from 0 to 1, and a higher score indicates that the genotype is described more closely by the phenotype measured. For allelopathy, data developed using the relay-seeding technique produced a broad-sense heritability for reduction of barnyardgrass roots of 0.85, which satisfies breeders' needs of screening program reliability (Courtois and Olofsdotter, 1998). Consequently, genetic studies of allelopathy in rice were started at the International Rice Research Institute in the Philippines. Initially, an existing population of recombinant inbred lines developed to study blast resistance, but found useful for allelopathy studies, was used for genetic analysis of allelopathy in rice. All phenotyping in these experiments was done using the relay-seeding technique. The cross IAC 165 × Co39 has one highly allelopathic cultivar, IAC 165, and one moderately allelopathic accession, Co39. Using this population, four QTLs have been identified that correlate with allelopathy expression, measured as barnyardgrass root reduction in laboratory screening (Jensen et al., 2001). This shows that allelopathy is quantitatively inherited. The work also emphasizes the importance of a good screening system.

IAC 165 is a highly allelopathic Brazilian cultivar and laboratory screening has shown that a large proportion of Brazilian upland rice germplasm is allelopathic. However, weed selectivity, autotoxicity, and residual effects from Brazilian rice germplasm are different from those of Asian germplasm (unpublished data). Therefore, it is important that genetic studies continue using crosses between cultivars other than Brazilian. Besides gene identification for allelopathy, this work might result in a better understanding of autotoxicity problems in Brazilian upland rice production.

FUTURE RESEARCH

Several areas have already been pointed out to be continued, such as refining screening techniques, isolating allelochemicals, and studying allelochemical genetics, but some research areas have not yet been started. I will comment here on which direction the continued work on rice allelopathy should take.

Understanding the Mechanisms of Allelopathy

For optimal use of allelopathy under field conditions, the influence of environmental factors needs to be investigated. Although allelopathy seems to be genetically based, its expression needs to be optimized through management, especially in the light of reports showing that allelochemical production and release are influenced by abiotic and biotic factors, such as plant age, temperature, light and soil conditions, microflora, nutritional status, and herbicide treatments (Duke, 1985; Hoagland and Williams, 1985). In particular, the importance of and fluctuations with plant age need to be investigated in order to develop weed management strategies that use allelopathy at the right time of the season. Moreover, allelopathy needs to be expressed at an early stage of development when weed control is most important.

The nutrient availability and nutrient efficiency of a rice cultivar will probably interact with allelopathic strength. Production of secondary metabolites is often enhanced by nutrient stress (Hoagland and Williams, 1985). For rice, it has been shown that plants growing in aerobic P-deficient soil can solubilize phosphate in the rhizosphere and thereby increase their P uptake by excreting organic anions from their roots, particularly citrate (Kirk et al., 1999a,b). The synthesis and excretion of citrate and certain other organic anions increase under P deficiency. Comparing these studies with allelopathy research shows that phosphorus-efficient rice cultivars are often the same as the allelopathic ones. It is important to understand such interactions to evaluate the effect of introducing allelopathy into rice.

Evaluating Allelopathic Rice from an Agronomic Point of View

The interactions between managerial interventions in rice production, such as flooding time and depth, seeding or transplanting, and amount of fertilizer applied, and allelopathy also need to be investigated. Crop establishment will influence both the early vigor of the rice and weed establishment. Whether rice is transplanted, wet-seeded, or dry-seeded will create different environments for weed establishment and thereby affect early competition and probably allelopathic interference between rice and weeds. Timing and nutrient source might also play a role in allelopathy expression in a field situation.

Allelopathic rice cultivars have produced residual effects on weed emergence after the rice harvest (Dilday et al., 1998). Experiments in the Philippines, however, have shown that the residual effect might differ among allelopathic rice cultivars (unpublished data). This suggests that more than one allelochemical is involved and that they might have different persistence in soils. Understanding these residual effects, including autotoxicity, is important in planning crop cycles or cropping systems. Crop management strategies to enhance or decrease the residual effects need to be identified and studied further. Autotoxicity problems have occurred in Brazilian upland rice cropping systems, thus underlining that such autotoxicity risks must be studied before allelopathic rice is introdued, to ensure that these cultivars do not cause new agronomic problems in continuous rice production systems.

Finally, allelopathic selectivity against rice weeds needs to be investigated from both the viewpoint of additional management strategies and eventual resistance development in the weed population. Allelochemicals operate like herbicides, which means that tolerance in the weed populations is likely to occur if the same allelochemicals are constantly "applied" to the same weed population. It is also likely that long-term use of allelopathic rice would result in a shifting weed flora. Strategies to minimize these risks have to be developed.

Identifying Allelochemicals

Allelochemical identification in rice is being undertaken (Rimando et al., 2001; Mattice et al., 2001) and is of high priority if other allelopathy outcomes are to be achieved. After identifying allelochemicals in rice, research efforts should be directed toward understanding the allelochemical mode of action, allelochemical production and release in rice, uptake processes by weeds, soil interactions with allelochemicals, and joint action relations between different allelochemicals in rice.

Identifying Genes Encoding for Allelopathy in Rice

To be able to breed for plant interference potential in future breeding programs, it is important to know the genes coding for allelopathic potential and competitive ability generally. Competition, being dependent of several physiological and phenological traits, and allelopathy are both polygenic characters. Before an efficient breeding strategy can be formulated, it is necessary to know which genes are involved in plant interference. Molecular marker-aided genetics is presently the best tool for studying the genetic control of a quantitative trait, mapping the genes involved on the chromosomes with a reasonable level of precision, and analyzing the relationships between the trait of interest and other important agronomic traits (assessing pleiotropic effects and risks of linkage drag). This work is in progress (Jensen et al., 2001) and continued work on populations developed especially for allelopathy is essential. If the allelochemicals involved are identified and if they correspond to molecules whose coded genes are already sequenced, the genes can be used as candidate probes to confirm the role of the identified QTLs. Improving phenotyping with a more efficient screening technique is an important part of this process.

Evaluating Allelopathic Rice from an Environmental Point of View

No chemicals, synthetically or biologically produced, added to the environment can be considered safe before environmental evaluation has been undertaken. The effects of any given chemical depend on the dose in which it is occurring or applied. Using allelopathic rice cultivars will increase the frequency and probably also allelochemical concentrations in the environment. Ecotoxicological studies must therefore start as soon as the allelochemicals are identified. Environmental fate and degradation patterns of allelochemicals and degradation products are essential knowledge for implementing allelopathic rice cultivars. Not only do we need to study effects on nontarget organisms but, especially for rice, there is also a direct connection to water quality both in irrigation schemes and for consumption.

CONCLUSIONS

Recent research on rice allelopathy has resulted in the following research milestones:

- There is large variation in allelopathy among rice cultivars.
- Allelopathy plays a role under field conditions.
- Allelopathic rice can suppress both mono- and dicot weed species.
- Progress has been made in identifying rice allelochemicals.
- Quantitative trait loci correlated with allelopathy have been identified.

From the experience gained in rice allelopathy, it is also possible to identify a range of researchable areas that still need attention, such as understanding the mechanisms involved in allelopathy expression from both an agronomic and environmental viewpoint. Identifying the allelochemicals responsible for weed reduction in the field is also becoming urgent as well as their environmental and toxicological assessment. Finally, work on genetics needs to continue to enable the use of allelopathy in a breeding context. For this to be achievable, some general points need to be considered in the continuing research: (i) Laboratory screening is essential for all parts of the research, such as genetics, allelochemical identification, and studies of allelopathy mechanisms. Thus, further development of a more reliable screening technique is required. (ii) Neither laboratory experiments alone nor field experiments alone can quantify the importance of and reveal the interactions between different competition variables, such as physical and chemical interference, including allelopathy. Field, laboratory and greenhouse studies must therefore work hand in hand to get the full picture of rice allelopathy. It is my view that reductionistic research is a prerequisite for studies of complex plant interactions. This means that the initial studies have to be done in simple experimental setups in which other interactions can be eliminated, but, as knowledge and understanding increase, the experimental systems should become more and more complex to achieve a fuller picture of plant interactions. (iii) Rice scientists must work together across traditional research areas to make important progress when working on complex interactions such as plant competition, including allelopathy. (iv) It is important to state that allelopathy research on rice must continue in a focused direction. The information gained from such research will help us understand the complexity of weed competition in rice especially, but it will also direct research on weed competition in other crops.

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Barnyardgrass Growth Inhibition with Rice Using High-Performance Liquid Chromatography to Identify Rice Accession Activity

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ABSTRACT

Some accessions of rice (*Oryza sativa* L.) have been shown to inhibit the growth of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.). Our objective was to determine if high-performance liquid chromatography (HPLC) chromatograms from leaf extracts of different accessions of rice correlated with weed control activity. Chromatograms of extracts consisting of 10 mg of fresh leaf tissue per milliliter of methanol (CH_3OH) were obtained from 40 accessions of rice. Cluster analysis was performed using 20 peaks from the chromatograms. Three clusters were found, with one cluster being distinctly separated from the other two. Although weed control data are not available for all the accessions, the isolated cluster contains all of the accessions that have been shown to inhibit growth of barnyardgrass and none that do not. This indicates that the assay could be used year-round to screen accessions of rice for weed control potential to determine which accessions should be further tested in the field. This could be done in a relatively short time using a small amount of space in the greenhouse. Because the assay requires only 10 mg of tissue per milliliter of methanol, it may potentially be used to test individual plants within an accession for weed control potential in a nondestructive manner.

Dilday et al. (1989, 1991) first observed the interference of rice on the growth of ducksalad [*Heteranthera limosa* (Sw.) Willd.] in field tests evaluating accessions of rice for tolerance to alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide]. Since 1987, laboratory and field tests have been performed to identify accessions that inhibit the growth of several weed species, including barnyardgrass. Growth inhibition of barnyardgrass has also been reported by Navarez and Olofsdotter (1996), Hasan et al. (1998), and Kim and Shin (1998). We have also observed it routinely in greenhouse bioassays.

Although the interference may be due to allelopathy, there is also the possibility that it may be due to competition or a mixture of competition and allelopathy. Either way, if the trait can be incorporated into agronomically useful varieties, fewer hours may be required for manual weeding, and reduced rates or fewer applications of herbicides may be required for weed control.

A useful tool for breeders would be an assay to screen accessions and individual plants within accessions for weed control activity. The assay would ideally be accomplished in a relatively short period of time, require a minimum amount of space, be relatively inexpensive, and could be done year-round in a greenhouse. We report here an HPLC procedure that is showing promise toward meeting most of these criteria.

MATERIALS AND METHODS

Rice Extraction

Approximately 15 seeds were placed in 100 g of soil sieved through a 2-mm mesh in the rice growing region of Stuttgart, AR. The samples were grown in 474-mL (16 oz) plastic cups and thinned to 10 plants cup^{-1}, with three replications per accession. After 10 d, the leaves from each replication were removed, cut into approximately 1-cm lengths, and placed in Erlenmeyer flasks. A volume of HPLC grade methanol was added such that the ratio of fresh plant tissue/methanol was 10 mg mL^{-1}. The samples were placed in a refrigerator overnight. Then equal parts of the methanol extract and deionized water were combined and analyzed by HPLC.

High-Performance Liquid Chromatography Conditions

Analyses were performed using a 25-cm by 4.6-mm Phenomenex Prodigy C18 column. The HPLC system consisted of a Hitachi L-7450A diode array detector, L-7200 autosampler, L-7100 pump, and the Hitachi HSM software for data processing. Solvent was degassed with an ERC model 3415α degasser, and the column was held at 35°C with an Eppendorf TC-45 heater. The gradient used 1% acetic acid (vol./vol.) and HPLC grade acetonitrile (acet). The program was 10% acet (vol./vol.) at 1.5 mL min^{-1} for 3 min, increased to 50% acet (vol./vol.) over 27 min at 1.5 mL min^{-1}, increased to 80% acet (vol./vol.) at 2 mL min^{-1} over 0.1 min and held for 1.9 min, decreased to 10% acet (vol./vol.) over 0.1 min and held for 7.9 min, and decreased to 1.5 mL min^{-1} over 0.1 min. The total run time was 40 min, and data were collected for the first 30 min. The first and last portions of the chromatogram contained only peaks that were essentially background. The injection volume was 30 μL and quantitation was at 320 nm.

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Abbreviations: acet, acetonitrile; HPLC, high-performance liquid chromatography.