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Using Conventional HPLC to Study the Interaction of Pharmaceuticals and Personal Care Products (PPCPS) with Plants

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Abstract

Conventional high-performance liquid chromatography (HPLC) has a role to play in controlled laboratory studies on the environmental behavior of pharmaceuticals and personal care products (PPCPs). In experimental designs where the test PPCP is the only exogenous material being added to the test system or assay, the need for definitive determination by liquid chromatography-mass spectrometry (LC-MS) is eliminated. However, this approach is limited to those PPCPs that respond with adequate analytical sensitivity, and for samples that produce relatively clean extracts free of co-eluting compounds or interferences at specific UV wavelengths. Treated wastewater that is discharged to surface water may be recycled and used for a variety of purposes, including the irrigation of crops. Studies have shown that treated wastewater contains PPCPs, because wastewater treatment plants were not designed to remove PPCPs. Under such scenarios, PPCPs may be taken up by plants; this trophic transport pathway to higher organisms should be considered in exposure assessments for PPCPs. An initial step in that assessment is the determination of potential adverse impacts of PPCPs on plants and the magnitude of plant uptake of PPCPs under controlled laboratory conditions, experimental work that can be supported by conventional HPLC analyses.

Keywords: Pharmaceuticals; HPLC; PPCPs; Plant uptake

Introduction

While liquid chromatography-mass spectrometry (LC-MS) has become the gold standard for forensic determinations of pharmaceuticals and personal care products (PPCPs) in environmental samples (for example [1,2]), we have observed that conventional HPLC with UV detection (HPLC-UV) can play a significant role in controlled laboratory studies on the environmental fate (sorption, biodegradation) of PPCPs [3-6]. For example, we have used conventional HPLC in a variety of ways related to the uptake of PPCPs into plants [7]. These have included using HPLC to verify dosing solutions used in seed germination assays with PPCPs, as well as determination of PPCP concentrations in plant tissues (leaves, stems, roots) during uptake experiments. The technical advantage comes from experimental designs where the test PPCP is the only exogenous material being added to the test system, thus eliminating the need for definitive determination by LC-MS.

The context of our plant uptake research centers on the potential of PPCPs in recycled wastewater to enter the human food chain through a trophic transport pathway which includes vegetation. As water supplies become more limiting and water re-use practices increase, PPCPs present in treated wastewater that is being recycled and used for irrigation may be taken up by plants. This pathway to higher organisms should be considered in exposure assessments for PPCPs. An initial step in that assessment is the determination of the magnitude of plant uptake of PPCPs under controlled laboratory conditions and the subsequent calculation of PPCP bioconcentration factors ([PPCP] in plant / [PPCP] in soil or water).

Methods

Most of our research has focused on PPCPs that are common, can be easily determined by conventional HPLC, and respond with adequate analytical sensitivity above any background signal. Over the years, we have conducted research with natural (β-estradiol, estrone) and synthetic (17α-ethinyl estradiol) estrogens, triclosan, triclocarban, acetominophen, caffeine, gemfibrozil, doxylamine, and ibuprofen. Admittedly, this is a limited number of PPCPs that we have evaluated to date, however, additional compounds that fit the criteria above can be easily added to our growing database on the environmental fate of PPCPs in general, and the interaction of PPCPs with plants specifically.

An initial step in our plant research involves determination of potential adverse impacts to plants from PPCP exposure. Prior to initiation of any plant bioassays, PPCP dosing solution concentrations are verified by HPLC. We have conducted seed germination tests and/or plant stress biomarker responses to PPCPs on a variety of common terrestrial plant species, including alfalfa (*Medicago sativa*), pinto bean (*Phaseolus vulgaris*), radish (*Raphanus sativus*), cucumber (*Cucumis sativus*), and wheat (*Triticum aestivum*). These plants are commonly used in plant bioassays, and importantly for our subsequent plant uptake research, produce relatively clean water:acetonitrile extracts free of co-eluting compounds or interferences at the UV wavelengths we have used to detect test PPCPs (for example, Figure 1).

Results

As a group, the PPCPs we have tested have only subtle impacts on seed germination (Table 1), and only at concentrations above what would be considered environmentally relevant (>5 µg/mL). In many cases, a clear dose-response has not been apparent. There were

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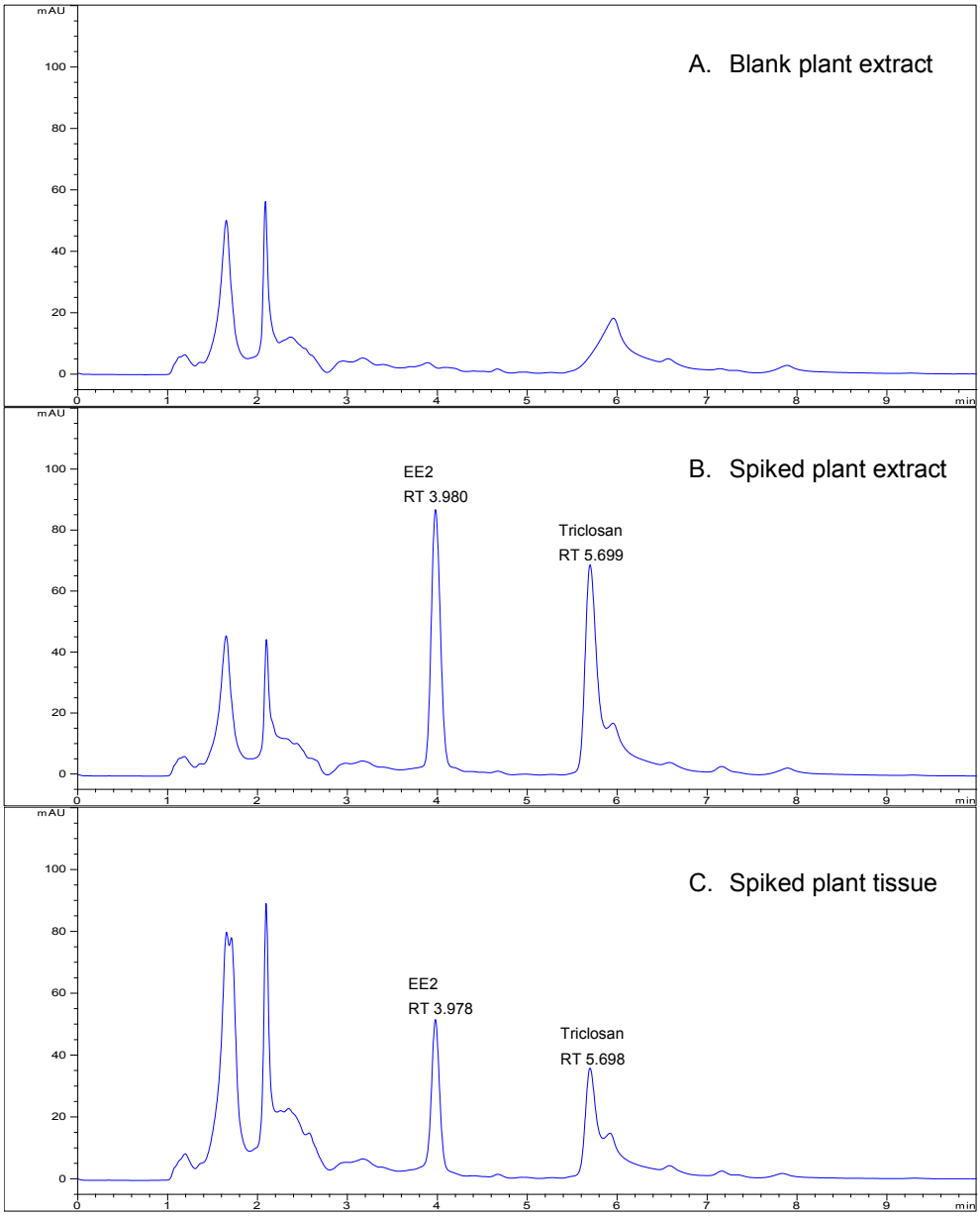

Figure 1: Example HPLC-UV chromatograms from studies on PPCPs and plants. Detection wavelength=200 nm. Panel A is a chromatogram of a blank plant (pinto bean) extract (water:acetonitrile). Panel B is a chromatogram of a blank plant (pinto bean) extract fortified with EE2 (17α-ethinyl estradiol) and triclosan at 1 µg/mL. Panel C is a chromatogram of dry plant (pinto bean) tissue fortified with EE2 and triclosan prior to extraction.

some sensitivity differences among plant seeds in their response to PPCPs; we found radish seeds to be relatively insensitive to even high concentrations of most PPCPs, while pinto bean seeds were among the most sensitive seeds we tested. Although the number of plant species we have tested to date has not been extensive, seed size rather than species appears to be the best predictor for potential impacts of PPCPs on germination. Namely, smaller seeds are more tolerant of PPCP effects.

While the PPCPs we tested had little adverse impact on seed germination, we have observed that plant stress biomarkers were more sensitive overall to PPCPs and changes in PPCP concentration. There are many plant biochemical indicators for use in these assays [8]. Our focus has been on changes in chlorophyll content, carotenoid levels (protects chlorophyll from photo-damage), H_2O_2 concentrations (an indicator of superoxide dismutase activity), malondialdehyde (MDA) concentrations (an end-product of lipid peroxidation), catalase activity (important for protecting plant cells from oxidative damage), and electrolyte leakage (a general indicator of plant stress). Results from some of those studies are presented below.

We evaluated the response of several plant stress biomarkers in wheat (*Triticum aestivum*) grown for 15 days in soil containing gemfibrozil or β-estradiol (Table 2). Overall, we found chlorophyll content to be insensitive to PPCP exposure, while markers of oxidative stress/damage responded in a dose-dependent manner. Specifically, an

increase in catalase activity, an increase in the lipid peroxidation marker MDA, and an increase in electrolyte leakage with increasing PPCP concentration. These assays take longer to complete (in this case 15 days) than a typical seed germination test. However, the sensitivity of the assays make the additional time involved a moot point. In addition, the plant stress biomarker measurements are quite simple and inexpensive.

Uptake of PPCPs in wetland plants was a focus of some recent aquatic microcosm research in our laboratory. Treated wastewater, which may contain PPCPs, is often discharged to surface water ([9] for example), producing the possibility of PPCP uptake into aquatic/wetland plants. We used HPLC-UV to determine triclocarban and gemfibrozil residues in 2 wetland plants, *Spathiphyllum wallisii* (peace lilly or umbrella plant) and *Echinodorus bleheri* (sword plant) following a 30-day exposure (Table 3). Triclocarban was readily taken up by both plants and (surprisingly) translocated. In addition, triclocarban translocation from roots to shoots was much more pronounced in the umbrella plant. Previous work in our laboratory with the same plant species and an analogue of triclocarban (triclosan) indicated very little translocation from roots to shoots [7]. In contrast to triclocarban, gemfibrozil uptake was minimal in both plants, but was present in both roots and shoots.

Triclocarban bioconcentration factors (BCFs) were <1 in the umbrella plant and approximately 3 in the sword plant. While we measured much higher BCFs for triclosan (an analogue of triclocarban) in the same plant species, the dominant role that roots play in accumulation of triclocarban was consistent with the triclosan data. Gemfibrozil BCFs in both plant species were <<1, suggesting little exposure risk from vegetation irrigated with recycled wastewater containing this compound.

Conclusions

Herein we provided data to support the idea that conventional HPLC has a significant role to play in supporting controlled laboratory studies on the environmental behavior of PPCPs. HPLC can be used to verify dosing solutions for seed germination and plant stress assays. In addition, HPLC can be used to determine PPCP residues in tissues from plant uptake experiments, as the test PPCP is the only exogenous material being added to the test system. This eliminates the need for definitive PPCP determination by liquid chromatography-mass spectrometry (LC-MS). LC-MS remains the gold standard and has played a role in forensic investigations including some laboratory plant uptake studies where it has been valuable in identifying PPCP metabolites produced in *planta* [2].

Plant	% Germination at Treatment Concentration[b]			
PPCP	1 µg/mL	2 µg/mL	5 µg/mL	25 µg/mL
Radish (*Raphanus sativus*)				
Acetominophen	97%	99%	99%	91%
β-Estradiol	97%	96%	95%	95%
Doxylamine	95%	97%	95%	97%
Gemfibrozil	99%	99%	93%	95%
Caffeine	97%	95%	96%	96%
Pinto Bean (*Phaseolus vulgaris*)				
Acetominophen	90%	80%	84%	86%
β-Estradiol	82%	80%	82%	80%
Doxylamine	86%	90%	80%	80%
Gemfibrozil	84%	85%	87%	70%
Caffeine	90%	82%	80%	82%

[a]Data from Osma et al. (unpublished). [b]Seed germination in controls was ≥ 98%.

Table 1: Example data from seed germination assays with PPCPs[a].

Assay	Effect		
PPCP	5 µg/mL	25 µg/mL	125 µg/mL
MDA[b]			
β-Estradiol	+	+	++
Gemfibrozil	+	+	+
Catalase[c]			
β-Estradiol	+	+	+
Gemfibrozil	+	+	+
Electrolyte Leakage[d]			
β-Estradiol	+	+	+
Gemfibrozil	+	+	++
Chlorophyll[e]			
β-Estradiol	NC	NC	NC
Gemfibrozil	NC	NC	NC

[a]Data from Osma et al. (unpublished).
[b]Malondialdehyde (MDA) is an end product of lipid peroxidation. [c]Protects plant cells from oxidative damage by reactive oxygen species. [d]A general indicator of plant stress.
[e]Total of Chlorophyll A + Chlorophyll B.
+: increase in parameter relative to control
++: 2X increase in parameter relative to control NC = no change relative to control

Table 2: Example data from plant (wheat, *Triticum aestivum*) stress biomarker responses (relative to controls) to PPCP exposure[a].

Plant	PPCP Concentration in Extract			
PPCP	Sand	Water	Root	Shoot
Umbrella (*Spathiphyllum wallisii*)				
Triclocarban	11 µg/mL	ND	0.7 µg/mL	7.3 µg/mL
Gemfibrozil	9.5 µg/mL	17 µg/mL	0.07 µg/mL	0.1 µg/mL
Sword (*Echinodorus bleheri*)				
Triclocarban	9.1 µg/mL	ND	27 µg/mL	1.3 µg/mL
Gemfibrozil	8.7 µg/mL	17 µg/mL	0.1 µg/mL	0.9 µg/mL

[a]Data from Malaviya et al. (unpublished). ND = not detected.

Table 3: Data from aquatic microcosm experiments on uptake of PPCPs in wetland plants[a].

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