

# Improved Isolation and Purification of Natural Products by Flash Chromatography

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# Abstract

Natural products play a dominant role in the development of new drugs for medicinal purposes. A crude extract typically contains lead compounds requiring multiple steps to isolate and purify. Conventional flash purification techniques used for isolating such compounds either fail to detect those compounds that are non-chromophoric or lack the necessary sensitivity often required for detection during separation. Using the Reveleris® flash system, chemists can separate natural product extracts that have shown to be beneficial for health. Bioactive components, chromophoric or non-chromophoric, are resolved and collected using the RevealX™ detection technology of the Reveleris® flash chromatography system. Scientists can isolate critical components used for medicinal purpose in drug discovery and development process and purify them with speed and greater recovery.

# Background

Natural products continue to play a significant role in the drug discovery and development process.<sup>1</sup> Research laboratories continue to modify active natural product skeletons as they can lead to novel agents linking mother nature to combinatorial synthetic techniques. The natural products field is still involved in small molecule research towards a significant number of new chemical entities. The advantage of small molecule natural products is as useful agents to help in various physiological process.

Synthesis of natural product-like libraries is ongoing, as they provide leads for new agents and new directions in medicinal discovery research. Scientists need to investigate all possible routes to novel agents that await discovery from terrestrial to marine environments. The exploration of mother nature can serve as leads and scaffolds toward cures for various diseases with efficacious drugs.

These natural product extracts have been shown to contain critical components that are both chromophoric and non-chromophoric and can be purified satisfactorily in a single step without the use of additional techniques such as preparative HPLC. Using the Reveleris® flash chromatography system can make the exploration of nature's therapeutic agents less time consuming and tedious.

# Purpose

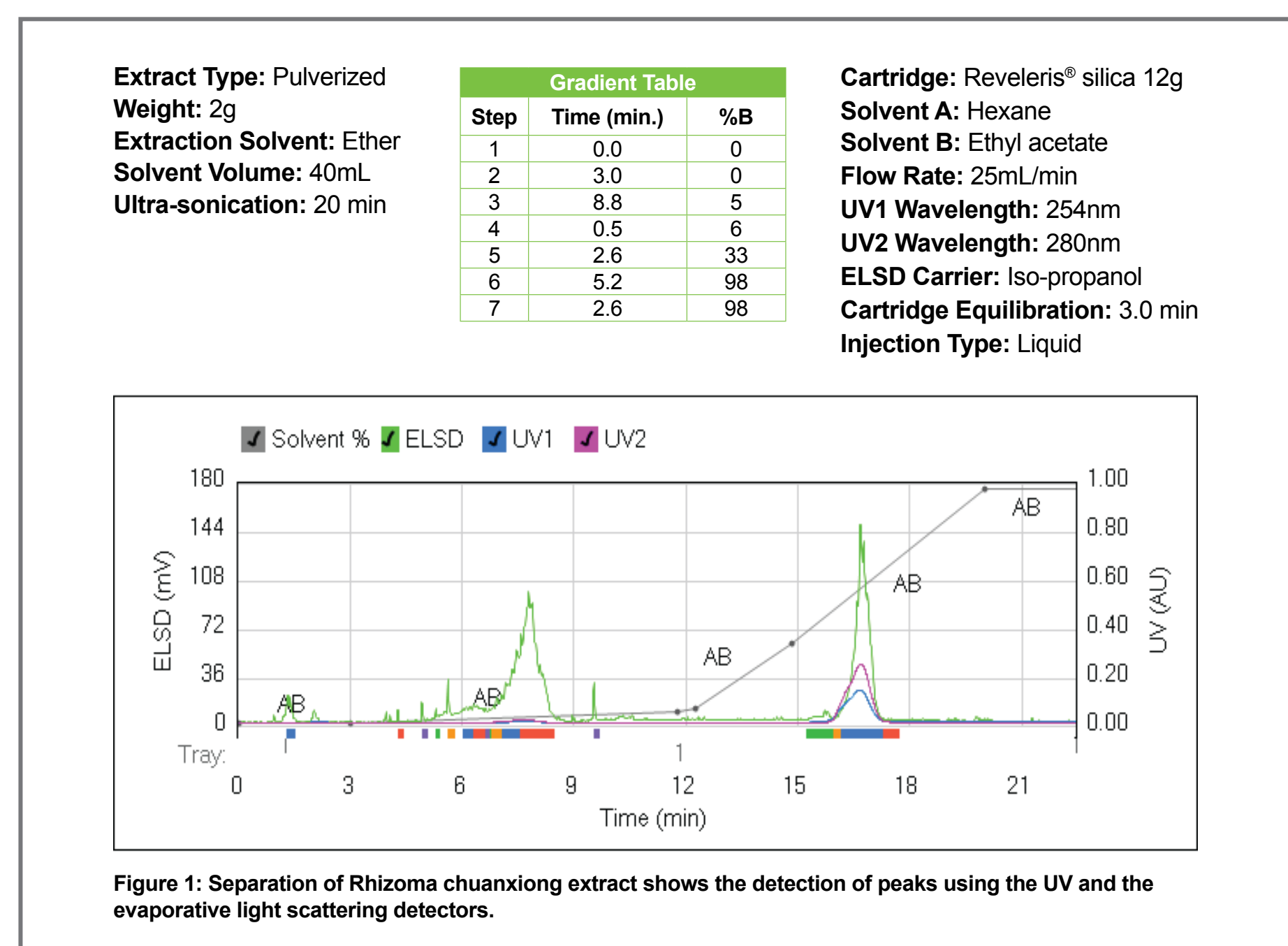
This paper investigates the purification of certain class of natural products using the RevealX™ detection technology of the Reveleris® flash chromatography system. Equipped with integrated multiple detectors, one can separate, detect, and isolate complex matrix of compounds with higher sensitivity and purity.

# Experimental and Results

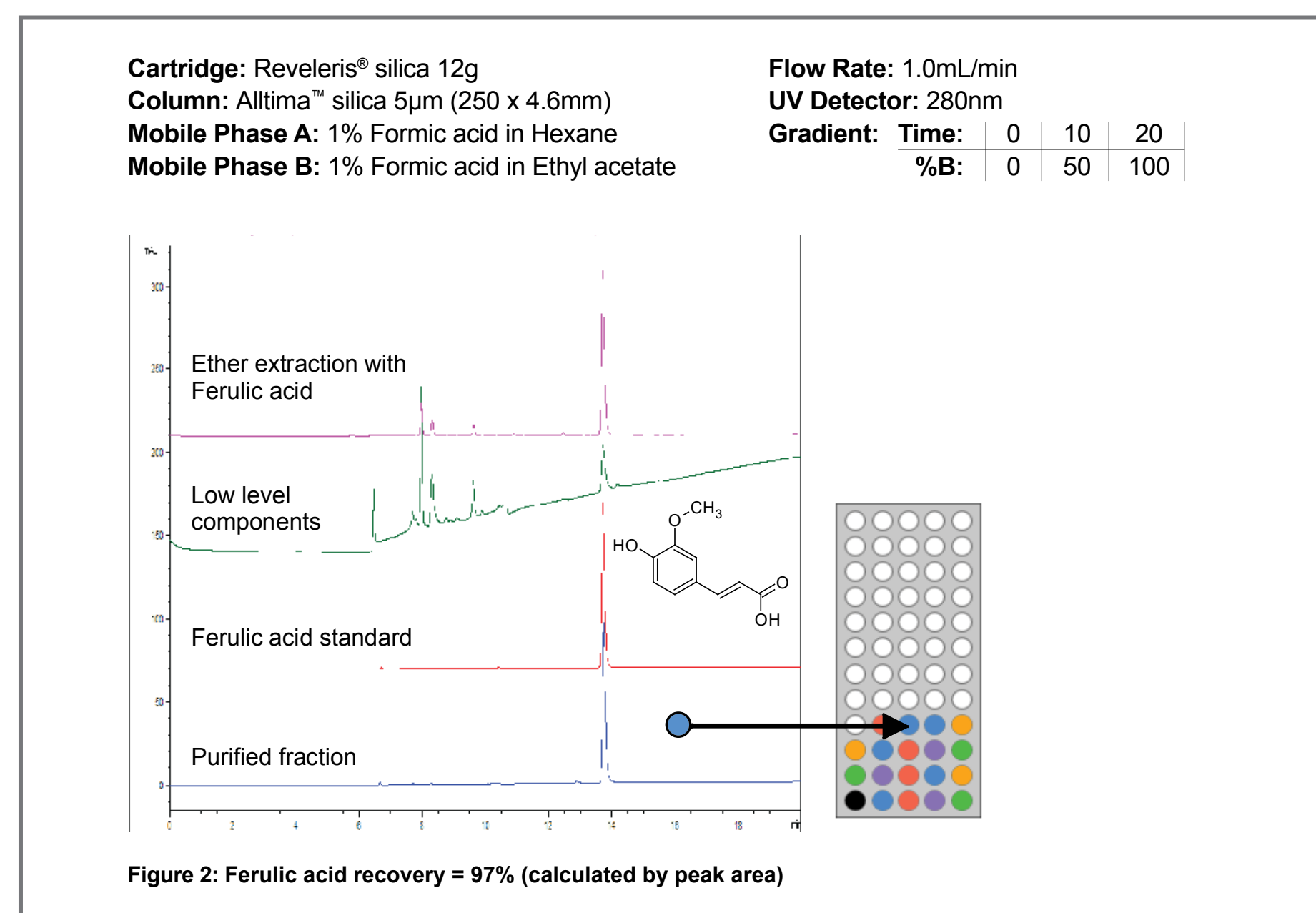
## ***Reveleris® iES System Purifies Ferulic Acid in Rhizoma Chuanxiong Extract with Greater Recovery***

Medicinal plants are an important resource for drug development. They are commonly analyzed in biochemical, pharmaceutical, and clinical research for their complex constituents and low quantities of bioactive ingredients.

Ferulic acid is known to be one of the main bioactive components found in Rhizoma chuanxiong.<sup>2</sup> The plant has been extracted here and used for the separation and identification of its scarce ingredients using the Reveleris® iES flash chromatography system.



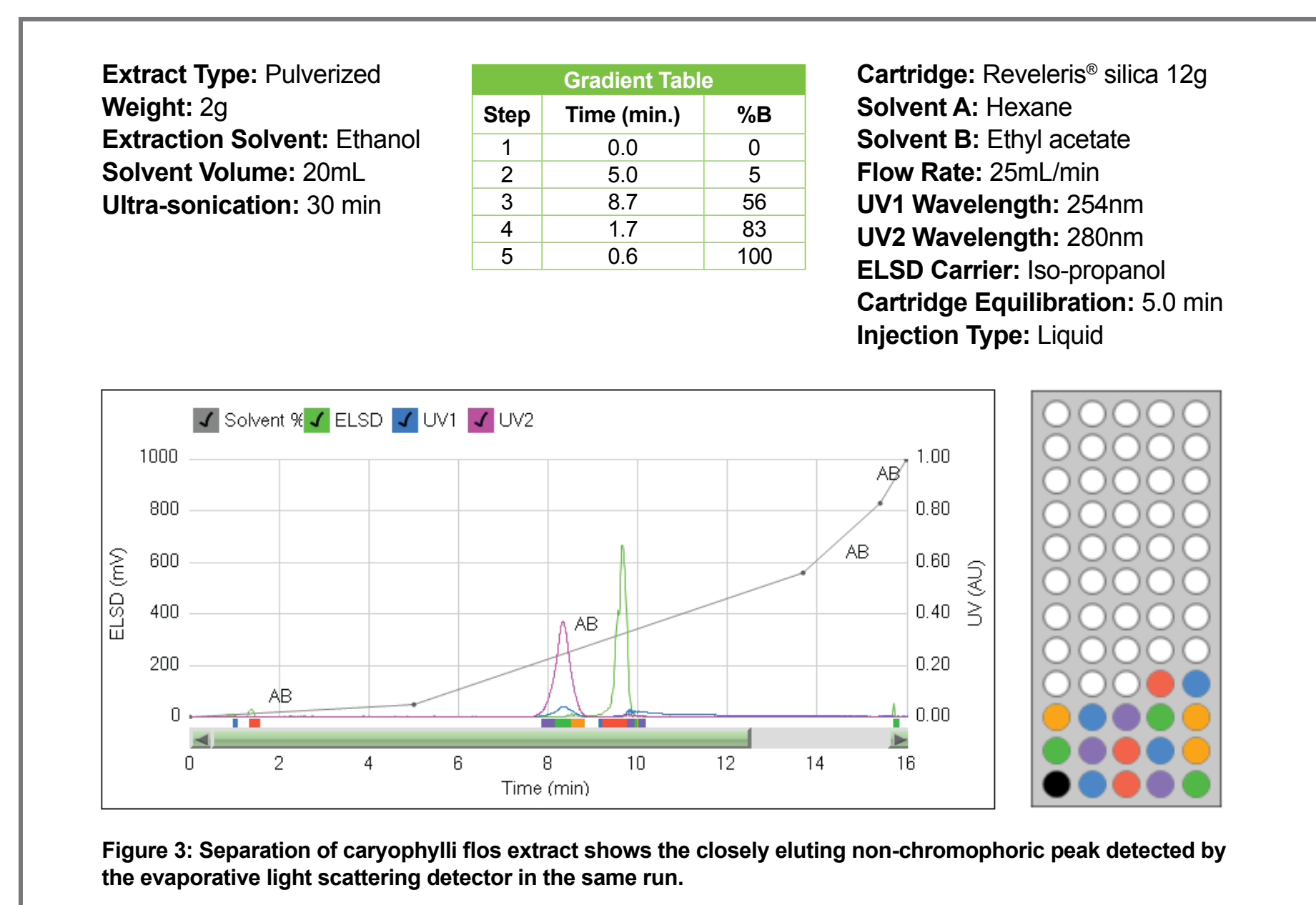
## Reveleris® iES System Purifies Ferulic Acid in Rhizoma Chuanxiong Extract continued



Using the sensitive Reveleris® iES flash chromatography system, the critical components of the plant extract at low levels have been isolated and collected. The RevealX™ technology also allows non-chromophoric peaks to be detected with the chromophoric ones in the same run for fraction collection and further analysis. This allows purification of compounds present in the natural product extract, compounds that are not always easily detectable using traditional flash chromatography system and are present at low amounts and useful for medicinal purposes.

## RevealX™ Technology Purifies Antioxidants in Caryophylli Flos (Dingxiang) Extract

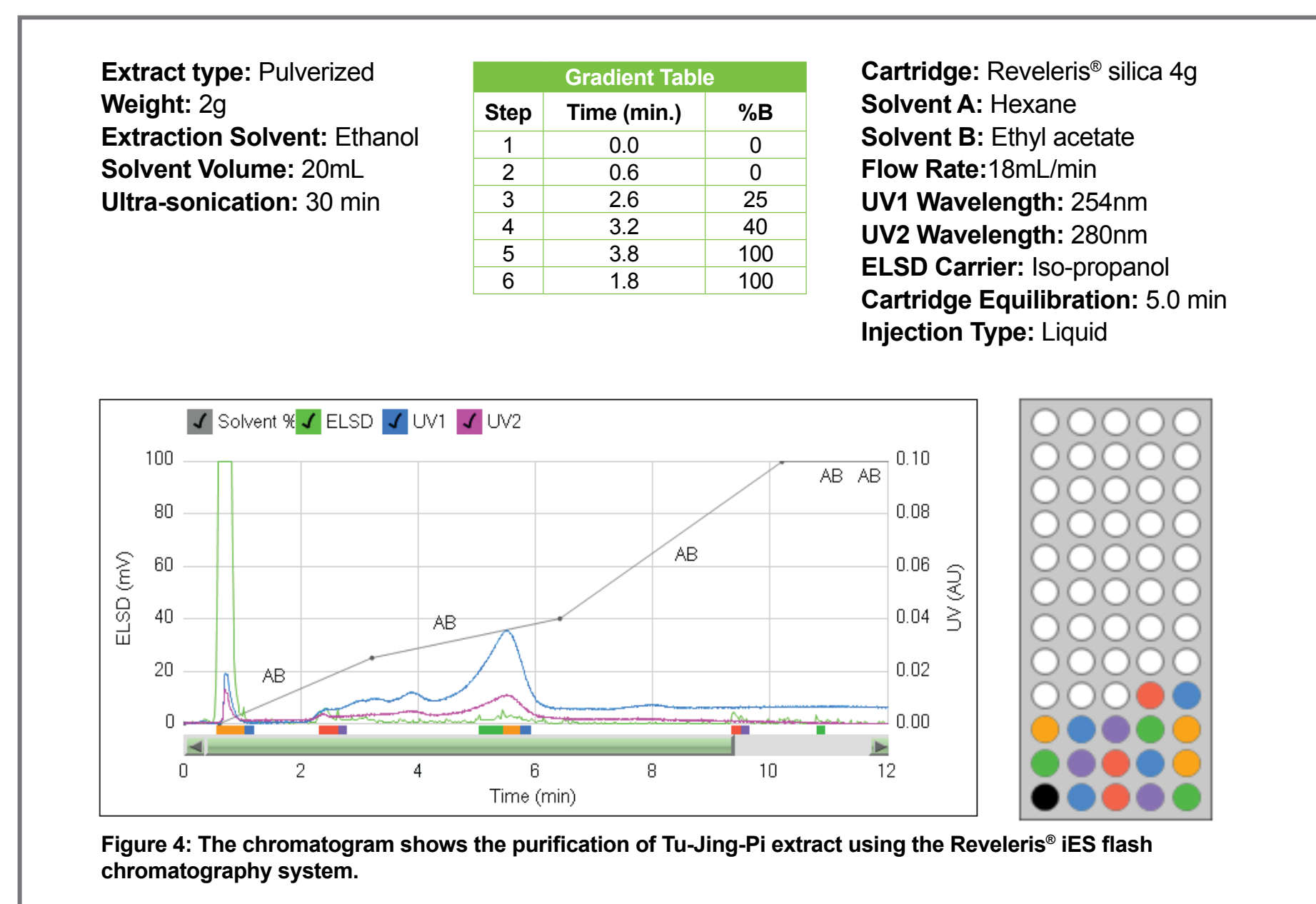
Cell membrane lipids can undergo peroxidation from free radicals, leading to cell death and causing several diseases such as atherosclerosis, diabetes, cancer, and ischemia-reperfusion injury.<sup>3</sup> Naturally occurring plants tend to inhibit lipid peroxidation due to the presence of phenolic compounds, such as acid-phenols or flavonoids and their esters. Natural products, such as caryophylli flos, contain eugenol, which has been shown to carry such antioxidant properties.



Natural product extracts have been shown to contain critical components that are both chromophoric and non-chromophoric. The Reveleris® flash chromatography system shows the benefits of RevealX™ technology in having multiple detection capabilities and integrated fraction collection for isolating compounds, helping to reduce post-run analysis time. The purification process can thus be more efficient and productive, even in the presence of low quantities of sample components.

## Isolation of Bioactive Diterpenoids Using RevealX™ Technology in Tu-Jing-Pi Extract

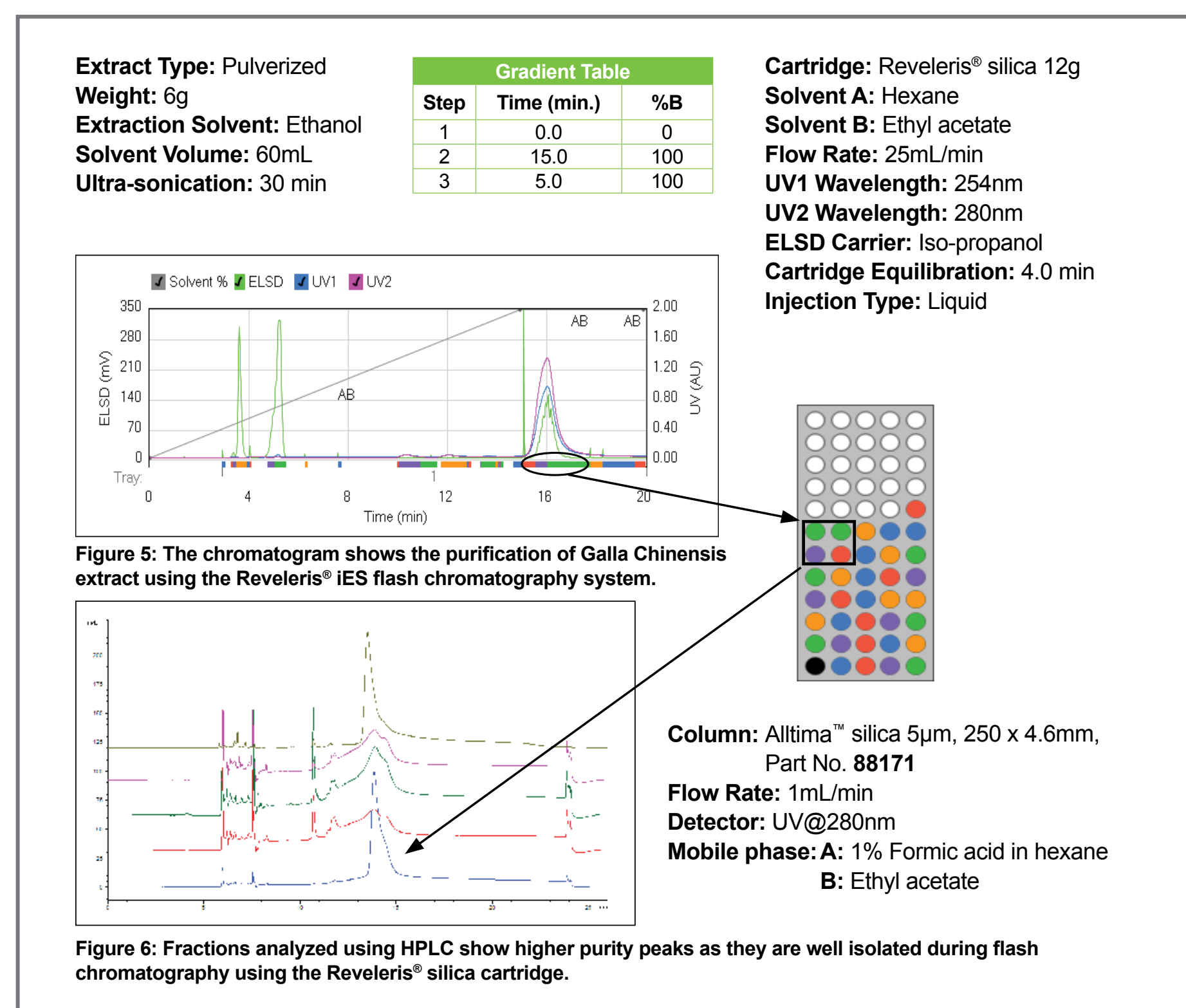
Root bark of pseudolarix kaempferi, well known as Tu-Jing-Pi, has been used in the treatment of skin diseases caused by microbial infection.<sup>4</sup> It contains diterpenoids, such as pseudolaric acid (A-C), that have a variety of biological activities.



Using the ELSD along with the dual UV detectors of the RevealX™ technology, isolation of impurities at low levels is possible during a chromatographic run.

## Reveleris® Flash Chromatography System in Purification of Galla Chinensis (Wubeizi)

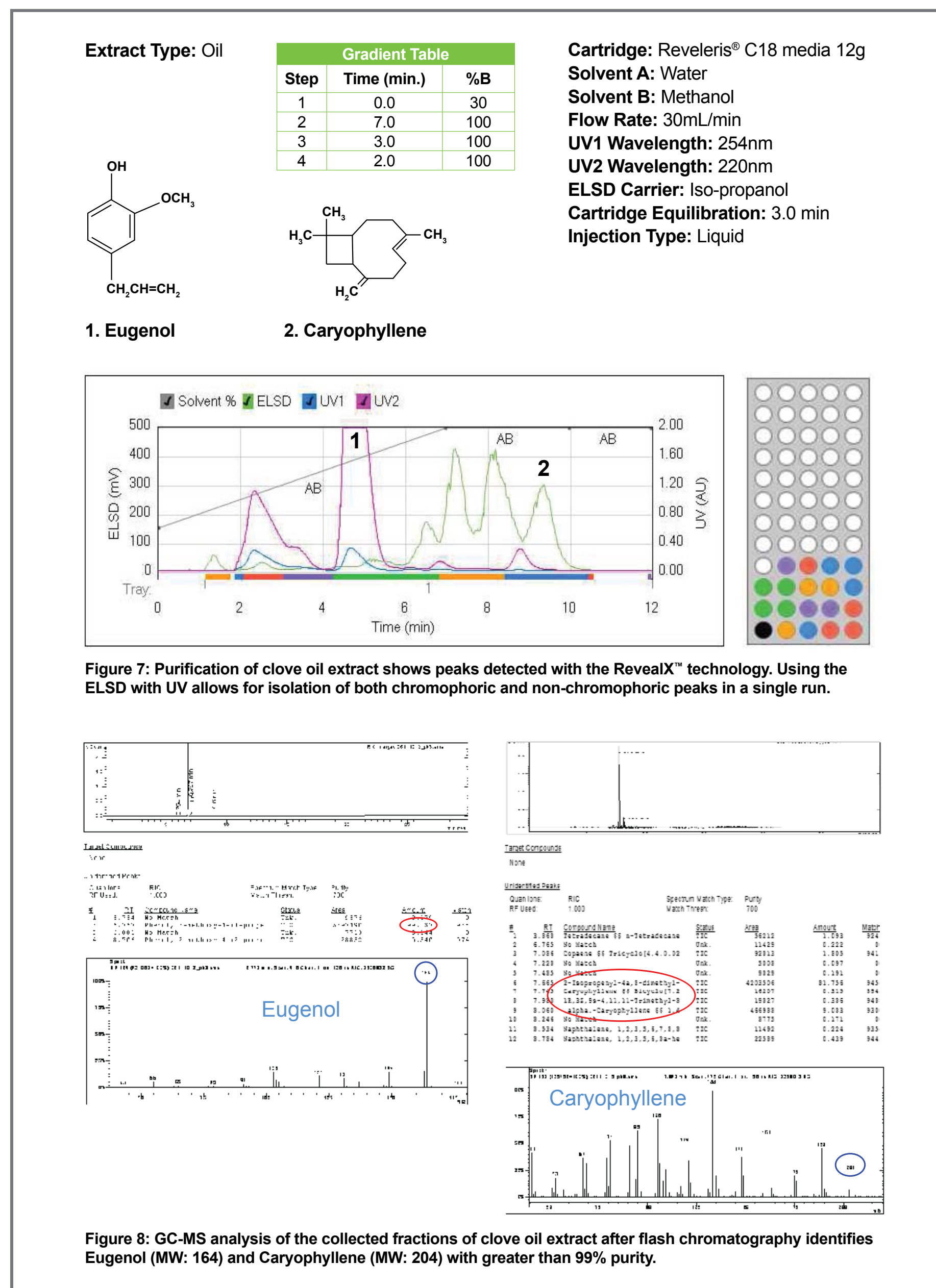
Crude plant extracts have various compounds that may interfere with bioassays or are common constituents of plant extracts. Galla chinensis, a traditional Chinese medicinal plant, contains complex components that have been studied for antibacterial and antioxidant properties.



Reveleris® cartridges provide greater loading capacity, allowing the use of smaller size cartridge beds and thus helping to reduce cost. As peaks are well resolved during separation, fractions can be isolated with high purity and recovery. Traditional flash chromatography would also fail to detect the early eluting non-chromophoric components as purified by the RevealX™ technology using the Reveleris® flash chromatography system.

## Reveleris® iES System Detects Critical Components, Eugenol and Caryophyllene, in Clove Oil Extract with High Purity

Many plants contain “essential oils” extracted from them that have high boiling points and antibacterial properties.<sup>5</sup> The clove oil extracted from cloves is a rich source of eugenol and caryophyllene, both of which may contain medicinal properties. Besides its use in dentistry, it may be used for treating various health disorders such as indigestion, cough, headaches, and stress.



Natural product extracts have been shown to contain critical components that are both chromophoric and non-chromophoric. The Reveleris® flash chromatography system shows the benefits of RevealX™ technology in having multiple peak detection capability and integrated fraction collection for isolating compounds, helping to reduce post-run analysis time. The purification process can thus be more efficient and productive, even in the presence of low quantities of sample components.

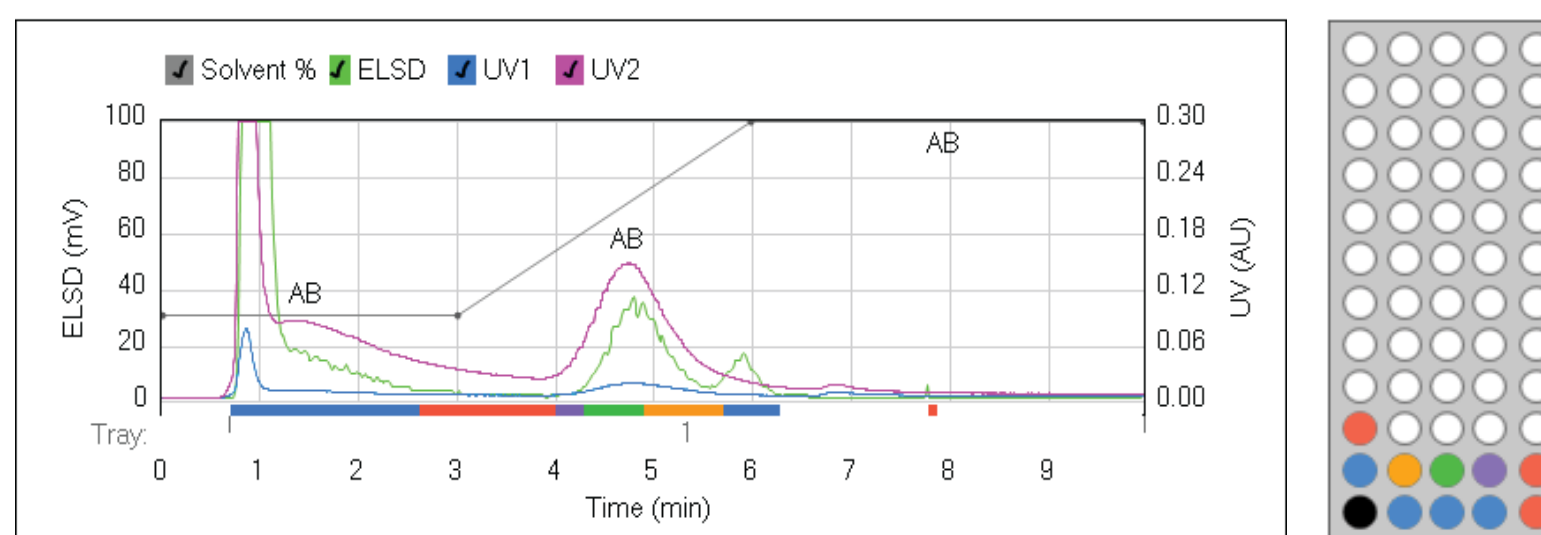
## RevealX™ Technology Allows Both Chromophoric and Non-chromophoric Component Isolation from Cat's Claw Extract

Cat's claw, a tropical vine, is considered a valuable medicinal resource for scientific research. The active substances are alkaloids, tannins, and several other phytochemicals that may have the potential to boost the immune system.<sup>6</sup> The presence of alkaloids provides anti-hypertensive effects and may lower cholesterol, as well as contribute anti-inflammatory, antioxidant, and anticancer properties.<sup>7</sup>

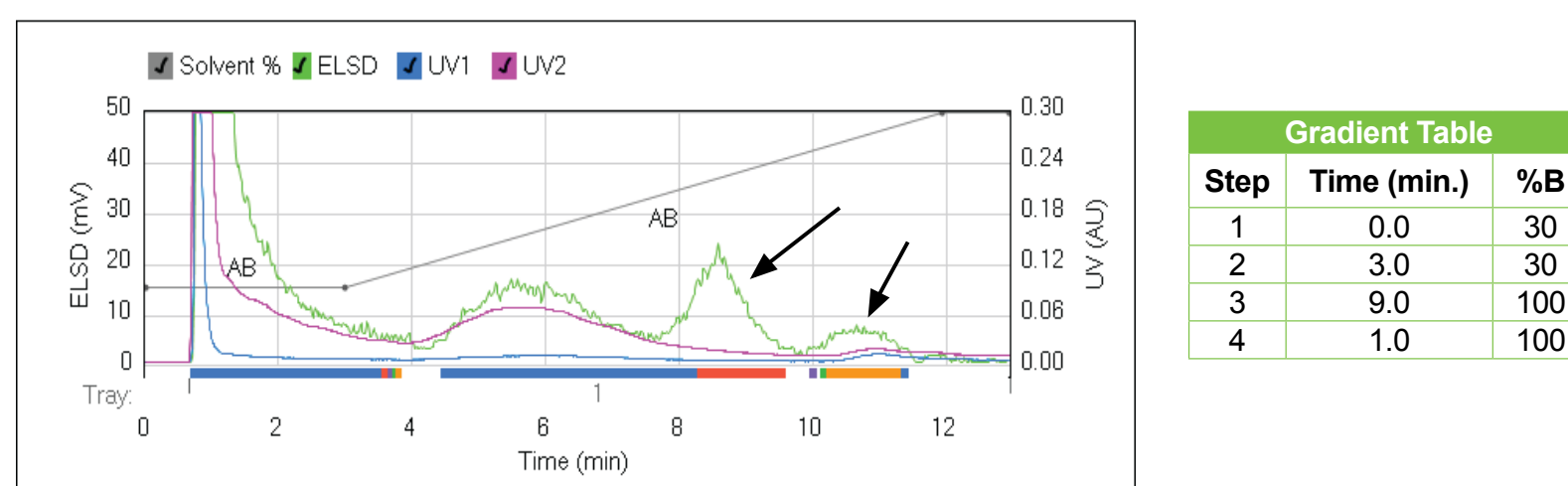
**Extract type:** Powder  
**Weight:** 3g  
**Extraction Solvent:** Methanol  
**Solvent Volume:** 10mL  
**Ultra-sonication:** 30 min at 60°C

**Cartridge:** Reveleris® C18 media 12g  
**Solvent A:** Water  
**Solvent B:** Methanol  
**Flow Rate:** 30mL/min  
**UV1 Wavelength:** 254nm  
**UV2 Wavelength:** 220nm  
**ELSD Carrier:** Iso-propanol  
**Cartridge Equilibration:** 5.0 min  
**Injection Type:** Liquid

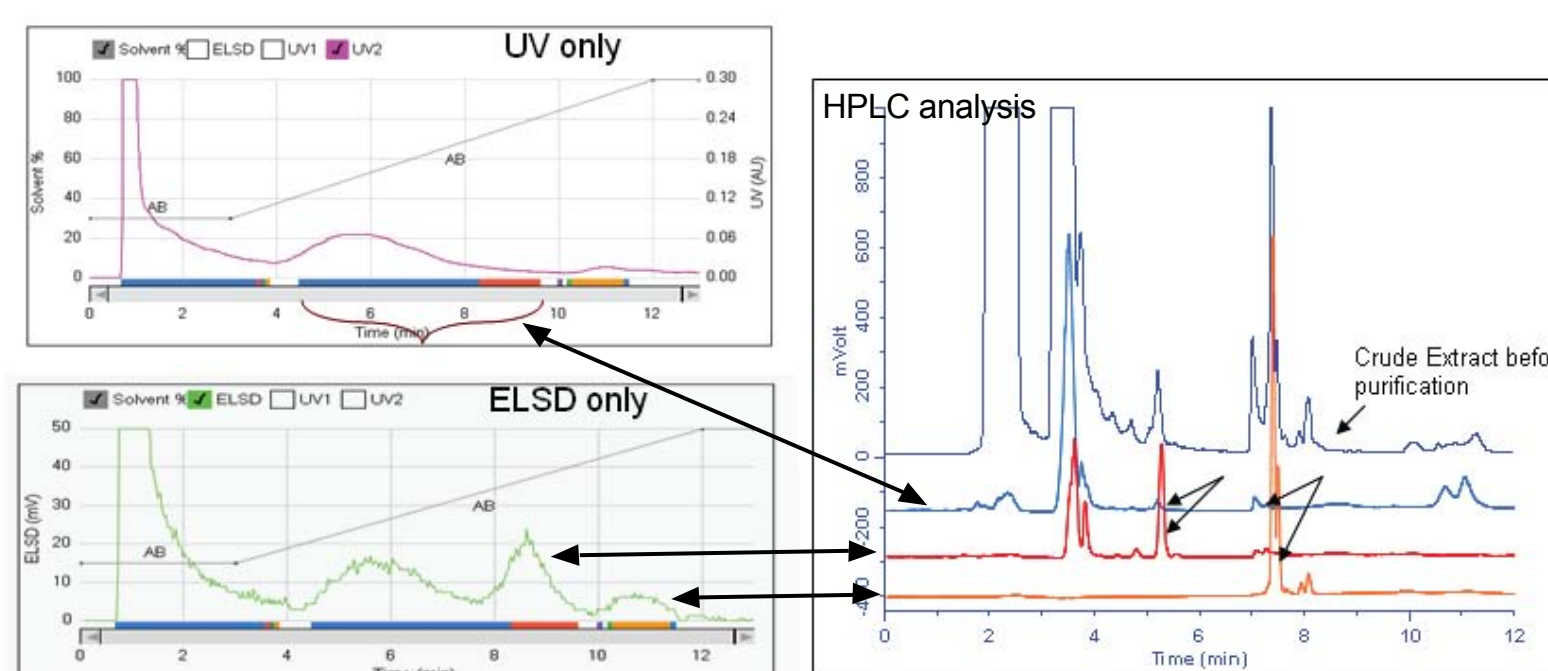
Gradient Table		
Step	Time (min.)	%B
1	0.0	30
2	3.0	30
3	3.0	100
4	4.0	100



**Figure 9:** Using the Reveleris® iES flash system and RevealX™ technology, Cat's Claw extract has been purified on a reversed phase Reveleris® 12g cartridge. The chromatogram shows the benefits of detecting peaks using the UV and the ELSD detectors.



**Figure 10:** Optimized gradient method improves resolution for higher purity fractions.



**Figure 11:** Samples purified using UV signal only fails to detect non-chromophoric peaks leading to impure and diluted fractions. Fractions collected using ELSD isolate components with higher recovery and purity.

Natural products play a dominant role in the development of new drugs for medicinal purposes. Crude extract typically contains lead compounds, requiring multiple steps to isolate and purify. Purification bottlenecks encountered when using traditional flash chromatography can be eliminated by using the Reveleris® iES system, which independently collects peaks detected from multiple detectors, including UV and ELSD for both chromophoric and non-chromophoric compounds. This can lead to fractions with pure compounds and higher recovery. Equipped with universal RevealX™ detection and the Navigator technology, chemists can isolate target compounds and low-level components from their crude extracts, saving time and labor.

# Conclusion

Natural product extract contains multiple components that are hard to isolate, if not purify, using conventional separation techniques. Samples are crude and contain valuable components that are present at very low levels. This study shows that the Reveleris® iES flash chromatography system can detect and collect smaller quantities of compounds for scientists involved in the purification of natural products, and can help provide increased lab productivity.

Equipped with universal RevealX™ detection and the Navigator technology, chemists can isolate target compounds and low level components from their crude extracts, helping to save time and labor. Purification bottlenecks encountered when using traditional flash chromatography can be eliminated by using the RevealX™ detection technology of the Reveleris® iES system, which independently collects peaks detected from multiple detectors, including UV and ELSD, for both chromophoric and non-chromophoric compounds. This can lead to fractions with pure compounds and higher recovery.

# References

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