Managing PAHs and Pesticides with a Rapid UHPLC-MS/MS Analysis

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There are hundreds, if not thousands of polyaromatic hydrocarbons (PAHs) and pesticides in different forms and matrices, each with varying degrees of toxicity. Humans are rarely exposed to a single PAH, and instead will usually encounter a complex mixture of PAHs. While individual compounds may not be toxic in isolation, there are those that can be synergistic when present in such a mixture. Differentiating between several compounds in order to define a composition is therefore essential.

PAHs are typically derivatives of petroleum and make their way into soil, sediment and water via runoff, sewage effluent and atmospheric deposition from incomplete combustion of organic matter. Various industrial activities also lead to the release of PAHs. One particularly high profile input is from oil spills. Environmental disasters like these have far-reaching repercussions for the environment as PAHs from a spill enter the food chain through plankton and other organisms [1–3].

Pesticides on the other hand are intentionally applied to crops using a spray, resulting in residues at detectable concentrations in food. They can also have a greater reach than just the intended crops: runoff can carry them into aquatic environments, while winds can cause them to reach other areas, such as grazing fields, other crops or human populations.

The safety of foods consumed by humans is an increasing concern that has led to a series of global regulatory initiatives and subsequent recalls of various food products. With such a broad and often complex variety of PAHs and pesticides, regular quantification of levels present on food is essential. The ability to rapidly and accurately screen multiple compounds simultaneously is essential in order to minimise the possible negative effects on both the environment and human health.

Routes of Exposure

The most likely route of human exposure to PAHs in non-smokers is via consumed food. It is well documented that food can become contaminated with PAH via air pollutants, uptake from the soil, and the carbonisation of carbohydrates, fats and proteins during food processing (e.g. smoking or high-temperature cooking) [4].

While PAHs find their way into the environment and food chain via accidental means, pesticides are intentionally introduced within an agricultural setting in an attempt to control pests and maximise crop yields. Despite various bans and restrictions, it is estimated that ~30% of the pesticides marketed in developing countries may fail to meet internationally accepted criteria for safe pesticide residues in food supply [5]. The regulations governing food safety require the screening and quantitation of a large number of pesticides to ensure they are below maximum residue levels (MRLs), generally set in the parts per billion (ppb) to parts per million (ppm) range. Yet with many developing countries operating outside of recommended levels, the regulations in place do not always wield the degree of power they were intended to have. As such, the need for wide-scale screening remains apparent.

Regulatory Control and Monitoring

Regulatory bodies are fully aware of the potential dangers posed by PAHs and pesticides, and have therefore devised both legislation and analytical methods to assess, monitor and control these compounds. Regarding the screening of PAHs, the US Environmental Protection Agency (EPA) method 610 lists 16 key forms and is used for environmental samples analysis [6]. The method contains all the analytical details for determining the 16 priority PAHs, and offers both high performance liquid chromatography (HPLC) and gas chromatography (GC) approaches.

The EU Scientific Committee on Food (SCF) provides guidance on levels of PAHs in food [4]. In 2005, the European Commission (EC) recommended monitoring 15 EU priority PAHs along with an additional PAH highlighted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [7]. These 15+1 EU priority PAHs include eight compounds that are also listed in the 16 US EPA-regulated PAH list.

The scope of pesticide regulatory guidelines is even wider. To date, more than 150,000 EU MRLs for pesticides have been set in Annex 2 of Regulation (EC) 396/2005. This requires food samples to be analysed in laboratories around the world in great numbers. In Europe alone, around 4,000 food and drink samples are tested each year, with scientists testing for more than 250 specific pesticides.

EPA method 610 details the precise protocol for determining the 16 specific PAHs. In the experimental description, the method successfully resolves all 16 via HPLC within 38 minutes. However, this is slow when compared to modern methods. A monitoring program would need to screen a large volume of samples in a relatively short period if it was to be of any real value. Environmental monitoring programs have become increasingly relevant, and this has led to investment in the development of easy-to-use, accurate and cost-effective techniques for the measurement of PAHs and pesticides in various matrices.

Methods

Time is crucial when it comes to residue analysis, particularly in the case of pesticides when an analyst may have a hundred samples to investigate. The concern has been that modern instrumentation will have to make a significant sacrifice in accuracy in order to achieve the desired high throughput times. This is where tandem techniques show their potential. In order to assess fast vs. ultrafast UHPLC-MS/MS methods in timed SRM mode, researchers recently analysed over 250 pesticides in food extracts and compared these in terms of analysis time and data quality.

Fast and ultrafast methods were defined as 15 and 5 minutes UHPLC-MS methods respectively. In both methods, a Thermo Scientific™ Accucore™ aQ column (100 x 2.1 mm, 2.6 µm) was used with mobile phase compositions of A) Water/Methanol (98:2, v/v, %) with 5 mM ammonium formate and formic acid 0.1% (v/v, %), and B) Water/Methanol (2:98, v/v, %) with 5 mM ammonium formate and formic acid 0.1% (v/v, %). Column temperature was maintained at 25ºC. The instruments employed were a Vanquish™ UHPLC System (Thermo Fisher Scientific) and a TSQ Endura™ Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific).

Results & Discussion

The need for speed of process was thoroughly demonstrated in this setting, and sample throughput was increased by 300% with the reduction of the 15 min method to just 5 min. To achieve this increase in throughput, the flow rate was increased from 0.3 mL/min to 0.9 mL/min, and the gradient slope was adjusted accordingly (*Figure 1*). The maximum system pressure was 360 bar for the 15 minute method and 1,010 bar for the 5 minute method.

Benefiting from the significant reduction of the peak widths in UHPLC mode with the ultrafast separation, the timed SRM scan window decreased from 30 s to 9 s. The triple quadropole MS used had a 500 SRM/s data acquisition rate capability [8] and a decrease of the SRM scan window provides the ability to decrease the cycle time to 0.34 s. This allowed acquisition of 10–15 data points across the LC peak, which is optimal for accurate quantitation.

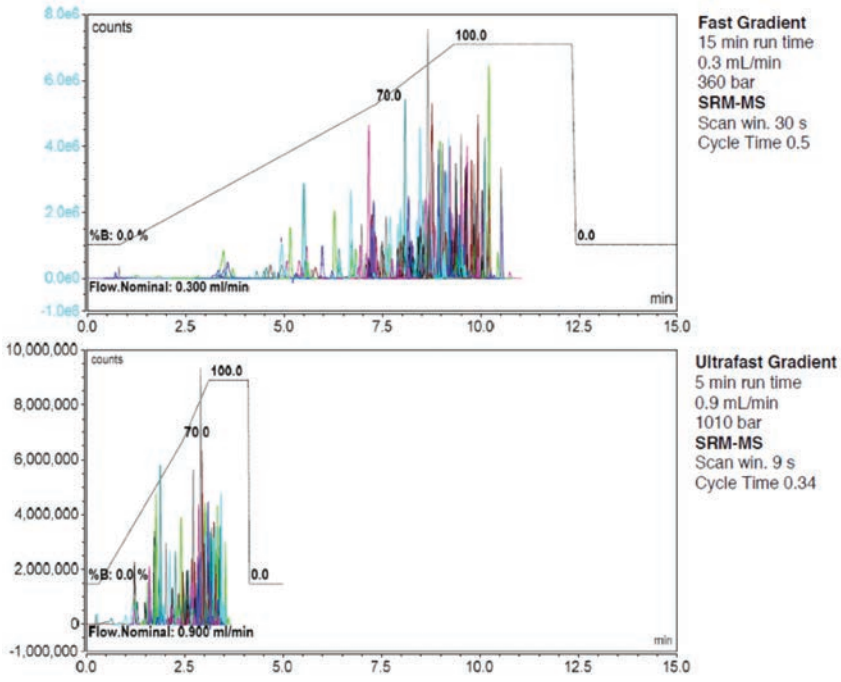

Figure 1. Extracted ion chromatograms of pesticides in strawberry matrix extract applying a gradient length of 15 and 5 min. Conditions: Accucore™ aQ column (100 x 2.1 mm, 2.6 µm), mobile phase A) Water/Methanol (98:2, v/v, %) with 5 mM ammonium formate and formic acid 0.1% (v/v, %), and B) Water/Methanol (2:98, v/v, %) with 5 mM ammonium formate and formic acid 0.1% (v/v, %). Column temperature 25 °C.

The UHPLC system used in this research showed an outstanding retention time precision from run-to-run and from sample-to-sample, which was the key factor for the development of the ultrafast UHPLC-MS method with very narrow SRM scan window [9, 10]. The run-to-run retention time repeatability was evaluated by seven consecutive injections for 50 compounds detected in all three matrices at 5 ppb level and revealed SD below 0.3 s.

The 15 minute and 5 minute LC-MS methods were validated based on four key criteria: accuracy, estimated at three different levels in the three different matrices; limits of quantification (LOQs), based on RSD ≤ 15% and ion ratios; repeatability (%), based on RSDs %; and linearity, measured as squared correlation coefficient. With this in mind, the results can be interpreted according to how they performed across these criteria. The results obtained with the 5 minute UHPLC-MS method in terms of accuracy, LOQs, and repeatability were compared with the 15 minute method (*Figure 2*). The data showed that not only does the 5 minute UHPLC method produce similar results to the 15 minute method, it results in a 67% saving of analysis time.

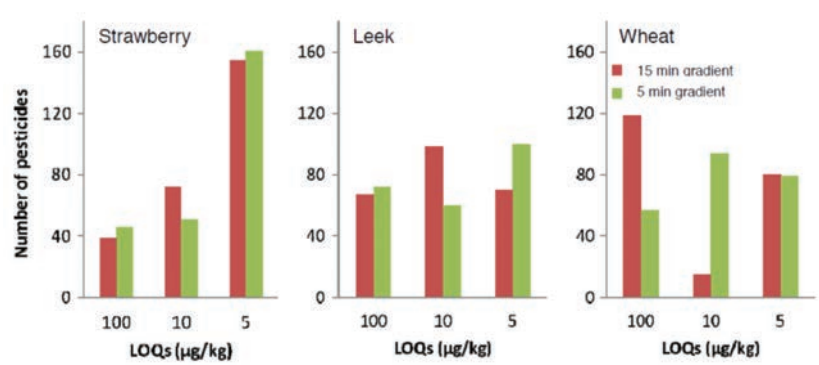

Figure 2. The 15 min and 5 min LC-MS methods provide comparable data quality in all three food matrices.

Conclusions

When it comes to implementing protective legislation, there is a need for rapid and accurate methods of detection in order to minimise the potential impacts of PAHs and pesticides on the environment and human health. Risk assessment requires similarly precise results but would ideally need to be performed over a broad range of concentrations, in different matrices and with multiple residues simultaneously; analytical methods should be compatible with a high sample throughput to obtain sufficient data.

Techniques like UHPLC-MS/MS can carry out rapid analyses without compromising data quality. Fast, multi-screening capabilities will have benefits not only for the environment, but will promote human health and safety through the robust detection and analysis of residues. Analytical laboratories now have the ability to analyse hundreds of pollutants, like PAHs or pesticides in a single sample.

References

[1] G. M. Watson, O. K. Andersen, T. S. Galloway, and M. H. Depledge, "Rapid assessment of polycyclic aromatic hydrocarbon (PAH) exposure in decapod crustaceans by fluorimetric analysis of urine and haemolymph," Aquat. Toxicol., vol. 67, no. 2, pp. 127–142, 2004.

[2] T. Wenzl, R. Simon, E. Anklam, and J. Kleiner, "Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union," TrAC - Trends Anal. Chem., vol. 25, no. 7, pp. 716–725, 2006.

[3] N. Berrojalbiz, S. Lacorte, A. Calbet, E. Saiz, C. Barata, and J. Dachs, "Accumulation and cycling of polycyclic aromatic hydrocarbons in zooplankton," Environ. Sci. Technol., vol. 43, no. 7, pp. 2295–2301, 2009.

[4] European Commission, "Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food," no. December, pp. 1–84, 2002.

[5] J. Popp, K. Peto, and J. Nagy, "Pesticide productivity and food security. A review," Agronomy for Sustainable Development, vol. 33, no. 1. pp. 243–255, 2013.

[6] United States Environmental Protection Agency, "Appendix a To Part 136 Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater Method 610—Polynuclear Aromatic Hydrocarbons," 1995.

[7] European Commission, "COMMISSION RECOMMENDATION on the further investigation into the levels of polycyclic aromatic hydrocarbons in certain foods," 2005.

[8] J. Wang, C. Yang, J. Beck, J. Massi, D. Ghosh, and M. Blackburn, "Analysis of 200+ Pesticides at 500 SRMs/s using a Non-Timed SRM MS Method and a Short LC Method on TSQ Endura MS." 2014.

[9] Thermo Fisher Scientific, "How Solvent Delivery Technology Can Improve Confidence in Peak Identification and Quantification," Thermo Fisher Scientific Product Spotlight, 2014. [Online]. Available: http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-documents/bro/bro/chrom/lc/sys/SP-71188-Solvent-Delivery-Highest-Confidence-Peak-Identification-Quantification-SP71188-EN.pdf?elqaid=1619&elqat=2&elqTrackId=97d9450580d44d908c72085db85bcb95. [Accessed: 01-Aug-2015].

[10] P. Carsten, M. De Pra, M. Martin, E. J. Sneekes, B. Bailey, and F. Steiner, "Is Retention Time Precision Only Affected by the LC Pump?" 2014.