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Analysis of PAHs in Edible Oils by DACC-HPLC Coupling with Fluorescence Detection

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ABSTRACT

The presence of polycyclic aromatic hydrocarbons (PAHs) in edible oil has been reported by numerous investigators. PAHs are formed during pyrolytic processes, such as incomplete combustion of organic substances, or have a petrogenic origin. Their presence is a health concern due to their carcinogenicity. Common methods for the analysis of PAH in edible oil suffer from complex and laborious extraction and cleanup procedures, which are necessary to detect PAHs at trace levels. Obviously a fast, automated method greatly facilitates the analysis of PAHs.

This poster discusses the use of a novel HPLC system equipped with a dual-gradient HPLC pump and two switching valves. This configuration allows on-line sample enrichment on an affinity column with HPLC analysis. Flow paths for analyte enrichment and analysis are alternated during the process.

On-line coupling of sample preparation and analysis eliminates the complex manual pretreatment required by traditional methods. Therefore, fewer errors and better reproducibility is achieved. The analysis time per sample is approximately 80 min with the dual-gradient HPLC system, compared to 8–10 hours with traditional methods. Moreover, the system can run 24 h a day, significantly increasing the sample throughput. The dual-gradient HPLC system provides a turnkey solution for this application and many others. Because this system has the same footprint as a standard HPLC system, it does not occupy additional bench space. Fully controlled by Chromeleon® software, the AutoQ™ equipment qualification functions simplify on-site validation.

INTRODUCTION

Traditional methods of analyzing PAHs in edible oils are laborious and time consuming. They typically require a saponification step with KOH-methanolic solution and a liquid-liquid extraction, followed by low-pressure cleanup column chromatography with sorbents such as alumina or silica.[1] The quantification is subsequently performed by HPLC or GC.

In recent years, donor-acceptor complex chromatography (DACC) has gained popularity for PAH analyses. PAHs strongly interact with DACC stationary phases while the bulk of matrix components is not retained and washed to waste. After the elution of the analytes, a solvent exchange prepares the sample for the analytical injection. Compared to traditional methods, this cleanup technique uses less solvent, is less labor intensive, and saves considerable amounts of time.[2] However, this technique still involves several manual sample-handling steps, and is therefore labor intensive and error prone.

To overcome this problem, Stijn et al.[3] developed an automated process for sample preparation and analysis. The setup consists of an LC-LC coupling of a cleanup DACC column with an analytical column, eliminating any additional manual cleanup steps. This solution solves all previously described challenges. However, adopting the method for routine operation is difficult and requires advanced technical know-how to optimize the configuration. This optimization can be time consuming. Furthermore, the described solution is not completely controlled and monitored by a single chromatography data system. It therefore does not address users' requirements for ease of operation, process monitoring and documentation, validation, reporting, and automated diagnosis.

Based on the work of Stijn, we present in this poster an optimized solution for automated on-line determination of PAHs in edible oils that addresses the remaining challenges. This solution is based on our Summit® x2 Dual-Gradient HPLC platform and can easily be applied for routine analyses. This poster starts with a brief introduction of the DACC off-line cleanup mechanism, leading to a detailed explanation of the LC-LC coupling technique. The poster then describes the solution with the Summit x2 Dual-Gradient HPLC System. The system is a turnkey solution for all standard HPLC applications and advanced chromatographic techniques. The benefits of this solution are described.

PAH ANALYSIS WITH DACC COLUMNS

The use of a DACC column for the cleanup of PAHs in fatty matrices reduces the sample preparation time considerably. PAHs denote a group of substances with more than one hundred individual components, mainly characterized by a basic structure of at least two condensed benzene rings. The π-electron system of the benzene rings acts as an electron donor and strongly interacts with the electron acceptor stationary phase, while the bulk of matrix components of the oil, such as lipids and tocopherols, is not retained and eluted to waste. The first four eluting—light PAHs naphthalene, acenaphthylene, acenaphthene, and fluorine—elute partly or completely in the fat fraction and generally cannot be analysed after a DACC column cleanup.

The flow scheme shown in Figure 1 couples the DACC cleanup directly with the analytical HPLC run. Compared to a standard HPLC setup, this configuration includes a second gradient pump and two column-switching valves. Figure 1 shows the valve position at the time of the injection. The configuration allows injection of pure undiluted oil, using isopropanol to transfer the sample onto the enrichment column. Direct injection of fats with low melting points, such as cacao butter, is also possible. In such cases, a thermostatted autosampler is required. The autosampler is set to a temperature above the melting point of the fat (e.g., 40 °C for cacao butter) for a precipitation-free injection. The analytical separation column is equilibrated with the second pump. After the fat has eluted to waste, the upper valve switches to flush out isopropanol in backflow mode with acetonitrile/water (Figure 2).

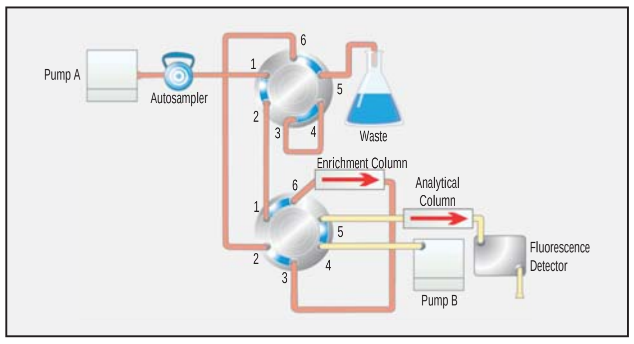

Figure 1. Flow scheme for on-line sample preparation and analysis. The valves are positioned for injection of the sample on the enrichment column

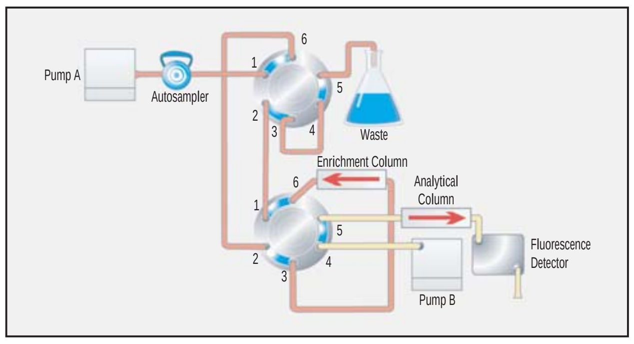

Figure 2. Isopropanol is flushed out of the enrichment column in backflow mode.

Removing isopropanol is very important in this application, because it negatively influences the analytical separation of the PAHs. The DACC column is back-flushed with a mixture of acetonitrile/water (85/15). A higher percentage of acetonitrile would result in sharper peaks, but also decreases the resolution on the analytical column. After all isopropanol has been removed, the system switches the enrichment column into the analytical flow path (Figure 3).

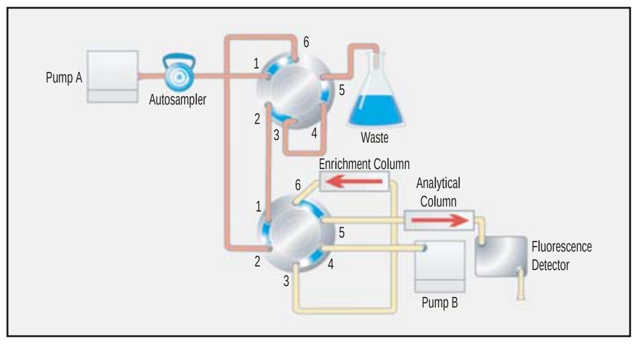

Figure 3. The enrichment column is switched into the analytical flow path, eluting the PAHs onto the analytical column, followed by a gradient separation with fluorescence detection.

With the gradient shown in Table 1, the PAHs are eluted onto the analytical column with consecutive fluorescence detection. To reach low detection limits, the detector switches the wavelength during the run, according to Table 1.

Table 1. Conditions for PAH Analysis in Edible Oils by DACC-HPLC Coupling with Fluorescence Detection

Configuration	Two pumps, autosampler, DACC column, analytical column (both in column oven), two 2-pos 6-port switching valves, fluorescence detector	
DACC column	DACC ChromSpher Pi 80 × 3 mm	
Analytical column	Two UltraSep ES PAH 250 × 4 mm in series with precolumn	
Pumps	Ternary low-pressure gradient	
Eluents	Pump A: (A) Isopropanol, (B) Acetonitrile/Water 85/15 (v/v), (C) Acetonitrile/Ethylacetat 30/70 (v/v) Pump B: (A) Acetonitrile/Water 85/15 (v/v), (B) Acetonitrile, (C) Acetonitrile/Ethylacetate 30/70 (v/v)	
Gradient pump A	0–12.1 min 12.11–20.9 min 20.91–50.9 min 51.5–66.5 min	100 % A, flow 0.35 mL/min 100 % B 100 % C 100 % A
Gradient pump B	0.0 min 14.6 min 17.6 min 26.5 min 34.5 min 40.5 min 53.5–64.5 min 67.5–77.5 min	100% A, flow 0.4 mL/min 100% A, flow 0.4 mL/min 100% A, flow 1.0 mL/min 60% B, 40% A 70% B, 30% A 64% B, 10% C, 26 % A 100% C 100% A
Valve timing	Switching of upper valve at 12.1 and 61.5 min Switching of lower valve at 14.5 and 20.85 min	
Fluorescence detection	14.5 min 27.9 min 32.0 min 39.0 min 53.35 min, 58.4 min	λ_{EX}: 256 nm, λ_{EM}: 370 nm (Acenaphthene, Phenanthrene, Anthracene) λ_{EX}: 275 nm, λ_{EM}: 420 nm (Fluoranthene, Pyrene) λ_{EX}: 260 nm, λ_{EM}: 420 nm (Benzo(a)anthracene, Chrysene) λ_{EX}: 290 nm, λ_{EM}: 430 nm (Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenz(a,h)anthracene, Benzo(g,h,i)perylene) λ_{EX}: 302 nm, λ_{EM}: 500 nm (Indeno(1,2,3-cd)pyrene) λ_{EX}: 290 nm, λ_{EM}: 430 nm (Benzo(b)chrysene (internal standard))
Injection volume	200 µL	
Temperature	20 °C	

After the analytes have completely entered the separation column, the DACC column is switched out of the analytical flow path (Figure 4). This switch allows the washing and equilibrating of the enrichment column during the gradient separation to prepare for the next injection. Because the DACC column can be reused without any problems, running the two processes in parallel eliminates unnecessary idle time between the two runs. Thus the application can run unattended 24 h a day with zero manual sample preparation.

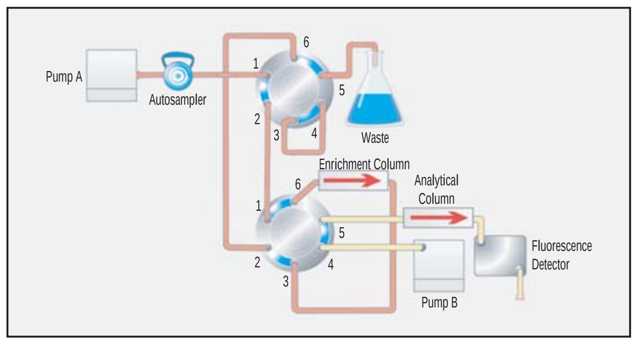

Figure 4. During the analytical run, the enrichment column is switched out of the analytical flow path. This switch allows the column to be washed and equilibrated during the analytical run.

THE SUMMIT x2 DUAL-GRADIENT HPLC SYSTEM

The Summit x2 Dual-Gradient HPLC System is designed for all standard HPLC applications and advanced chromatographic techniques, such as on-line sample preparation, dual-column tandem operation, or two-dimensional (2-D) chromatography.

The main components of the Summit x2 Dual-Gradient HPLC System (see Figure 5) are:

- A dual-gradient HPLC pump (P680 DGP); the pump comprises two ternary gradient pumps in one enclosure
- A solvent rack with a built-in 6-channel on-line degasser (SOR-100)
- An ASI-100™ autosampler, optionally with sample thermostatting
- A column compartment with a built-in switching valve (TCC-100)
- A full range of detectors (including a fluorescence detector for PAH analysis)
- One additional, external switching valve for DACC-HPLC coupling
- Chromeleon chromatography management software

For PAH analysis with DACC-HPLC coupling, and for similar column-switching applications, the main benefits of a Summit x2 Dual-Gradient System are that it:

- Serves as a turnkey solution with optimized fluidic connections
- Allows full automation of sample preparation and analysis steps
- Provides full software control over all modules, including valves
- Provides complete process monitoring and documentation
- Uses a single, graphical user interface for ease of operation
- Allows precise timing of all events

System Concept and Bench Space Requirements

Figure 5 shows a complete Summit Dual-Gradient HPLC System. The dual-gradient P680 pump includes two ternary low-pressure gradient pumps in one enclosure. The additional pump increases neither the footprint nor the height of the system. The column compartment incorporates a two-position, six-port switching valve; therefore, only one external valve is necessary (not pictured).

Figure 5. Summit x2 Dual-Gradient HPLC System.

The Chromeleon User Interface

Chromeleon is the single point of control for the complete system and tracks all relevant information in audit trails. Chromeleon does not share the limitations of other software in controlling two independent gradient pumps. The software's customizable instrument control panels (Figure 6) make it easy to operate the system from a single screen. Graphical displays of system components help less-experienced users become familiar with the software.

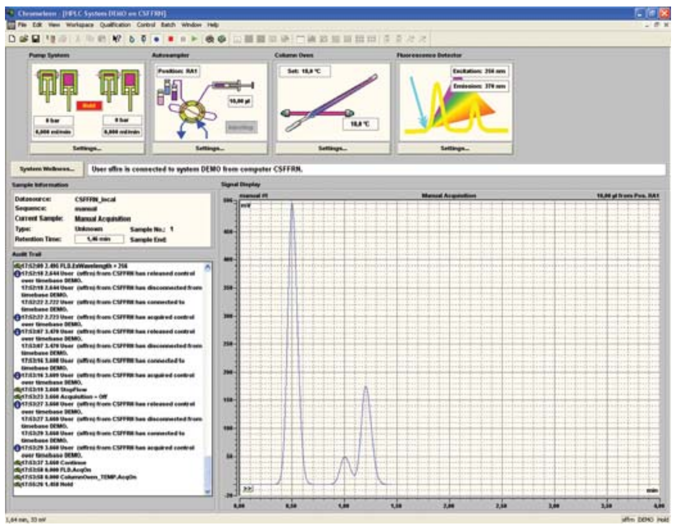

Figure 6. Chromeleon instrument control panel for a Summit x2 Dual-Gradient System. All components are controlled from a single screen, including the second gradient pump and all valves.

How To Ensure System Suitability

The Summit Dual-Gradient System comes with automated software-controlled Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) routines. Furthermore, Chromeleon software provides automated system suitability tests. Use these tests to check retention time and area precision of standards. Define limits for peak asymmetry, separation efficiency, or any other performance variable. Chromeleon then monitors against these limits and assures that the system remains suitable for the analyses.

Figure 7 shows a comparison of three consecutive injections of a cacao butter sample. The 10 g of butter was spiked with 10 µL of EPA 610 standard mix solution with concentrations of the PAH between 10 and 100 µg/mL. Due to the precise timing of the different events required for this application, the retention times of all the PAHs only differ by less than 0.17 % RSD for all analytes.

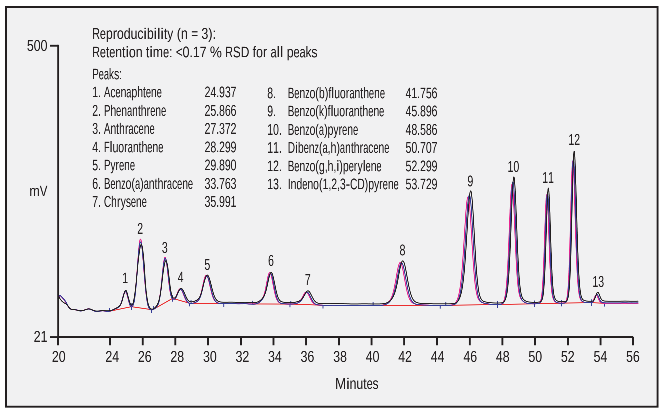

Figure 7. Overlay of three consecutive, directly injected cacao butter samples, spiked with PAH standard. Despite comprehensive switching technique, the retention times are very stable.

CONCLUSION

Compared to traditional methods for analysis of PAHs in edible oils, the method described in this poster has several advantages. On-line sample preparation using the DACC column reduces solvent consumption, labor, and per-sample analysis time from 8–10 hours to 80 min. The method allows direct injection of edible oils without prior sample treatment.

The Summit x2 Dual-Gradient HPLC System is a turnkey solution for the application. The system's dual-gradient pump houses two ternary pumps in a single enclosure with the same footprint of a standard HPLC. A thermostatted ASI-100 Autosampler allows direct injection of fats with low melting points (e.g., cacao butter). Chromeleon software controls the complete system, including all valves. The software executes all events precisely and assures reproducible analytical results. An easy-to-use interface allows users to control and monitor the system from a single screen. Audit trails document occurrences during the analysis and data processing steps to ensure full traceability of results. Automated equipment qualification routines and system suitability tests facilitate system and method validation. With its complete set of diagnostic and monitoring functions, a Summit x2 Dual-Gradient System can run unattended, 24 h a day, and thus increase sample throughput and reduce costs per analyses.

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