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Chromatography

A Review on Multiplexed Detection using Active Flow Technology Columns

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The analysis and characterisation of complex samples of natural origin can be an arduous and time consuming task, especially under uni-dimensional HPLC conditions. There are numerous detectors that have been applied to the analysis of natural origin samples, however, there is no universal detector can that provide a complete analysis of complex samples. Thus, to reduce sample complexity multi-detection analysis for multidimensional sample information is sought. A new form of column technology known as Active Flow Technology (AFT) in Parallel Segmented Flow (PSF) mode provides a platform for multiplexed detection. Multiplexing via AFT-PSF allows the simultaneous use of up to four destructive and non-destructive detectors whilst maintaining chromatographic performance to achieve a comprehensive yet efficient characterisation of a complex sample. This review discusses the various available detectors used in HPLC, the advantages and disadvantages of the various multi-detection systems and the considerations that must be made when establishing a multiplexed system with AFT-PSF.

Introduction

There are many reasons as to why scientists seek detailed information about the nature of the constituents that make up the complexity of naturally derived compounds. It might be for example, the search for medicinal significance [1,2], i.e., antibacterial [3], food value, i.e., antioxidant activity [4-8], or even simply the chemical profiling of the substance so that authentication of the product can be verified [9,10], such as the origin of a fine bottle of red wine [11,12]. Irrespective, the process of classifying the components that make up the complex natural sample can be an arduous task and may require a variety of different strategies. This short review details some of the approaches that may be undertaken in the elucidation of the complexity of natural substances.

High Performance Liquid Chromatography

One of the main tools in the natural product chemist's arsenal is that of high performance liquid chromatography (HPLC), which when used appropriately can provide enormous separating power that enables the detailed exploration of the many thousands of compounds that may be found in samples derived from natural origins. The separation performance where the maximum number of components that can be separated is dependent on the chromatographic conditions utilised for separation, i.e. stationary and mobile phase. But there are limitations of HPLC, especially in regards to samples derived from natural origin and the peak capacity that can be afforded by a uni-dimensional separation system [13-15]. As a consequence of the limitation of the peak capacity a complete separation of the constituents within the complex natural origin sample may not be possible. These limitations of uni-dimensional HPLC can essentially be narrowed to three main factors: (i) insufficient theoretical plates available for separation, restricted by the pressure limitations of equipment and columns, (ii) radial and axial heterogeneity of the column bed, which places limitations on the efficiency in the performance of the separation media, and (iii) the heterogeneous migration of analytes as a result of viscous friction generated by forcing a fluid through a finely divided bed at high pressure, this factor mainly affecting the big brother of HPLC, that being UHPLC – ultra-high performance liquid chromatography. Of these limitations, only the first is discussed in the context of the present review, and then only briefly.

An increase in column length will increase the theoretical plate count, however, pressure increases linearly with column length, but the gain in plate count only increases by the power function of 0.5 [15]. Hence, the system pressure is easily exceeded well before there is an appropriate gain in the number of plates required to tackle the complexity of natural products. Furthermore, the gain in peak capacity is proportional to the square root of N, and the ratio of the retention factor range (in gradient elution) [15]; so, column efficiency is extremely important to gain suitable separation power as too is the gradient range – but increasing both these factors leads to ever increasing analysis time. To suit the system pressure limitations and thus be able to utilise a sufficiently large number of theoretical plates, the flow rate must be decreased to accommodate a long separation column packed with small particles. Again, this eventuates to a time inefficient analysis so different strategies must be utilised for detailed sample characterisations. A strategic approach that may be employed to overcome these limitations involves the use of multidimensional separations.

Multidimensional HPLC can be defined in two ways: (i) multiple separation steps and/or (ii) multiple detectors, where each detector provides a selective mode of detection. Since the purpose of this review is the analysis and characterisation of complex samples from a multi-detection

perspective, only the latter will be discussed here. Prior to the discussion of multidimensional detection and the analytical chemist may best utilise selective detection in the search through natural products a very brief overview of a variety of common detectors will be discussed.

Detection Methods used in HPLC.

For the visualisation of the chromatographic separation of compounds in the column effluent, a HPLC detector is utilised, producing a signal response (chromatographic peak) for each compound(s) detected. The chromatographic peaks that are visualised through the detector are a reflection of the chemical or physical properties of the analytes within the sample that are specific to the detector properties. There are many detectors that can be used with HPLC, such, as ultraviolet-visible (UV-Vis) detectors, fluorescence detectors (FLD), refractive index detectors (RI), radioactivity detectors (RAD), corona-discharge detector (CAD), light-scattering detectors (LSD), conductivity detectors, electrochemical detectors (ECD), chiral detectors, mass spectral detectors (MS), infrared detection (FTIR), nuclear magnetic resonance (NMR), and sample derivatisation and reaction detection processes, such as, chemiluminescence and 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH•) [15-17]. The list is long and the arsenal of the natural product chemist is thus limited largely by imagination. However, with respect to the context of this review – selectivity in detection for maximising sample information in complex samples, particularly those of natural origin, we will discuss only the most important of these detection processes. Aside from the sample information that may be gained from the detector, the importance of the detector, with respect to the goal of this review, may be based solely on the ability of the regular analyst having access to the detector on a regular basis. For that reason, detectors such as NMR, Raman, ICP and radiochemistry will not be discussed further, suffice to say, these have been reviewed prior [15-17].

The ideal HPLC detector has been described to have a number of characteristics, that is, to have high sensitivity and predictability, respond to all analytes, unaffected by variables such as temperature, respond independently of the mobile phase, have no peak broadening contributions, be reliable and convenient, good linearity, be non-destructive and provide qualitative and quantitative information [15]. However, currently no single detector possesses all of these characteristics, but we should question, if this detector did exist, would it in fact be the best detector for the natural product chemist? Probably not, since the chromatographic separation would have to be very good indeed, ensuring all the components have been separated; and we have already discussed the impossibility of a complete separation of complex samples in uni-dimensional applications. Thus, the ideal detector is actually the detector that yields the 'required' information, free from interference from co-eluting, unresolved species. It is then likely that several 'ideal' detectors would be required for any one particular sample, and that these detectors are largely dependent on the sample characteristics and the type of information required. For that purpose detectors may be categorised into four main detection methods; differential measurements, sample specific, mobile phase modification and hyphenated techniques. Differential measurement detectors are those that measure a change in the presence of compounds within the mobile phase. An example of a differential measurement detector is RI. They are known to be 'universal' detectors responding to all compounds, however, such detectors lack selectivity, being unable to differentiate between co-eluting samples, and they are difficult to use in gradient elution mode – an essential requirement in the analysis of complex samples [15].

Sample specific detectors, such as, UV-Vis, ideally in a diode array format gives a response that is loosely related to sample characteristics. Other sample specific detectors include, FLD, which responds to compounds that can fluoresce, ECD responds to redox chemistry, and post-column derivatisation/reaction detectors (PCD/PCR), which respond to the chemical characteristic of the reagent used in relation to the chemical or biological functional aspects of the molecules being tested for. These types of detection processes can be extremely powerfully as they offer very high degrees of selectivity and they can also potentially yield information about the functional aspects of the molecular species that respond to the post column reactions [15].

Mobile phase modification detectors are those that alter the mobile phase post-column to produce a change in the analytes (i.e. ionisation or derivatisation); some of these detectors fall into the same category as sample specific detectors. Lastly, hyphenated techniques are the coupling of the HPLC system to an independent analytical detection instrument, such HPLC-MS, HPLC-NMR, and HPLC-FTIR.

There are numerous detectors that may also be classified as 'general use' detectors, i.e., UV-Vis, MS, RI, ELSD, and CAD. The basic principles of only the more popular detectors used in HPLC will be discussed here, since a discussion on every detector used in HPLC is beyond the scope of this review, more detail on HPLC detectors can be found in [15-19].

Detector Types and Detection Mechanisms

Ultraviolet-Visible Detector

One of the most popular and most widely used detectors in HPLC is the Ultraviolet-Visible (UV-Vis) detector. UV-Vis detectors primarily respond to compounds that absorb radiation in the wavelength region of 190-600 nm. This detector has a wide linear dynamic range and provides excellent quantitative data, provided the separation is adequate to resolve the species that are seen by the detector at the specified wavelength. UV-Vis detectors are considered to be 'general' detectors for a number of reasons including but not limited to: it is compatible for gradient elution, high sensitivity for UV-absorbing solutes, easy to operate, non-destructive, provides a selection of wavelengths, and they are cheap and reliable [15,16,18]. There are many published applications of HPLC with UV-Vis for the analysis of natural origin samples. For example, the detection and analysis of medicinal plant extracts [20], screening plants for new drug discoveries [2] and even fingerprinting for qualitative analysis in herbal medicines [1]. However being a 'general' detector, UV-Vis detection has its limitations. First and foremost UV-Vis detection is limited only to compounds that absorb radiation in the specified wavelength region. In complex samples, there are many compounds that may not even have any absorbing characteristic; hence the presence of such compounds under UV-Vis detection would be undetected and unknown. Secondly, UV-Vis detection provides very limited identification to unknown compounds [15,16,18].

Fluorescence Detector

Fluorescence detectors (FLD) on the other hand are not classified as 'general' detectors as they are known to be selective to analytes that fluoresce when exited by UV-Vis radiation. Compared to UV-Vis detection FLD is also much more sensitive, where for many samples FLD sensitivity can be 100 times greater than UV-Vis [15]. An important consideration in the use of FLD is that detection is based on the excitation of the analyte species, and hence the number of molecules that produce light is important, compared to the UV-Vis detector that is a light attenuating detector, hence the concentration of the sample is important. The difference in these basic aspects of the detector may be important in any detection strategy that includes a flow stream splitter to divide sample between multiple detectors. Additionally the sensitivity of the FLD is based on two characteristic features, the excitation and emission values, which are rarely 'generic', at least not for optimal signal response [15]. Thus, it may be necessary to have an idea as to the types of species that are sought in the analytical detection process. The application of HPLC with FLD on natural origin samples like UV-Vis detection is also numerous with a similar purposes. HPLC with FLD has used in pharmacokinetics in the determination of amlodipine in human serum [21], as well the analysis of foods [22] for the determination of tocopherols [23] and many more applications.

Refractive Index Detector

Refractive index (RI) detectors are considered to be a 'universal' detector due to its indifferent nature in the detection of analytes through the refractive index differentiation between analytes and mobile phase. A limitation is, however, they are very difficult to use in gradient elution modes since the RI detector responds to the change in the refractive index of the gradient itself [15]. Nevertheless, the RI detector is very useful in applications such as the detection of sugars [24,25], or in size exclusion chromatography for the detection of polymers [26,27]. They do, however, have a lack in sensitivity, are temperamental to environmental changes, such as, temperature and flow rate. Due to these factors, especially that related to gradient elution, the RI detector finds little use in natural product discovery.

Evaporative Light Scattering Detector

Light scattering detectors, such as, the evaporative light scattering detector (ELSD) are based on the detection of light scattered by non-volatile analytes in the sample. Basically the mobile phase evaporates in a stream of nitrogen, leaving behind the less volatile sample molecules that are then passed across a light source, scattering the light as they do so [15,28,29]. Like RI, ELSD is also considered to be 'universal' detector, but even more so, due to its potential use for any type of sample and its suitability to gradient elution techniques [28]. The use of ELSD in natural origin products has generally been so as an additional detector rather than a substitute [30-32].

Corona-Discharge Detector

Another detector that is also considered as a 'universal' detector for HPLC is charged-aerosol detector also known as corona-discharge detector (CAD), which originated from ELSD and like ESLD responds to most analytes [16,33]. CAD functions in a similar manner to ELSD, with the nebulisation of column effluent, evaporation of the mobile phase followed by the ionisation of analytes generating a signal response and detected by an electrometer. The main difference lies in the ionisation step in which the analytes are suspended in a gas phase that passes through the corona discharge needle for an electrical charge to be measured [29]. The selectivity of CAD is based on a certain mobility range of the charged ions. CAD offers an alternative to RI and/or ELSD, in particular for the application of sugars [34] and other carbohydrates [35,36]. The disadvantage of CAD is similar to that of ELSD, also being limited to non-volatile compounds with the possibility of losing volatile analytes in the sample during nebulisation [33]. For the analysis and characterisation of natural origin samples containing strong chromophores, 'generally' UV-Vis would be utilised, providing superior performance for chromophores than CAD or ELSD. However, for the non-UV-absorbing analytes that do not contain chromophore functionality, CAD or ELSD can expand the sample characteristic information [29].

Mass Spectrometer

The use of mass spectrometers (MS) as a detection source with HPLC separations is generally referred to as a hyphenated technique due to its independent analytical capacity. HPLC-MS for the analysis and characterisation of complex samples of natural origin has been popularised due the efficiency gains from the HPLC step and sensitivity and selectivity gains in MS detection [37]; effectively together they provide orthogonal sample information, while at the same time, the MS detector may provide structural information about the analytes - such as molecular weight, formula and diagnostic fragments, crucial for rapid characterisation of natural origin samples [15,16,29]. The principle of MS is beyond the scope of this review and further detail can be found in these references [15,16,18,38-40], but we must emphasise that the MS is a very important detection tool for the natural product chemist.

Multi-detection for Multidimensional Analysis

The comprehensive analysis of complex samples via uni-dimensional analysis with a single mode of detection is a difficult to near impractical task. Alternative methods of analysis for characterisation need to be considered, such as those that incorporate multi-detection analysis. Each method of detection, in particular those mentioned in this review, provide a form of selectivity additional to the selectivity provided by the LC component. We listed a few of these in the previous section, but within the scope of this review it is not possible to describe in great detail aspects of the very many different types of detection processes, but the analyst should be aware that very great changes in the sample information that is obtained from any one separation can be greatly influenced by the manner in which the eluent from the column is viewed.

Methods of Multi-detection HPLC

Multi-detection HPLC can be achieved in a number of ways: (i) conventional – single detector followed by reinjection of the sample and subsequent analysis using a different detector, (ii) serial – that is a linear arrangement of detectors, such that the mobile phase flows through the first detector, then the second and so forth, (iii) split-flow – the mobile phase that leaves the column is divided between numerous detectors simultaneously using Tee-pieces to manage the flow directions, and (iv) multiplexed detection using a new column technology referred to as Active Flow Technology (AFT) in Parallel Segmented Flow (PSF) mode, which is the primary focus of this review.

Conventional detection involves the use of a single detector, but in order to gain additional information using different types of detection modes the analysis is repeated for each detection type, i.e. for the a three-dimensional analysis, that is three different detection methods, injection and analysis will be carried out three times – once for each detector, meaning the total analysis time will be three times as long and there is the assumption that the sample does not change during each subsequent analysis. The sample stability issue may be very important, if for example, the target compounds are biologically active and undergo degradation on the bench or in the injector while awaiting analysis. Alternatively, multiple detectors can be used in a series to reduce lengthy run-times, however, this will lead to band broadening through the detection stream, which can be substantial and diminish the HPLC efficiency. Furthermore, only one destructive detector can be used in any single series of detectors – that being the last detector. Another method of attaining multi-detection HPLC is post-column split-flow, where the exiting flow is split using a multi-port flow splitter and directing each stream to a detector. In this method, simultaneous detection can be achieved whilst using both destructive and non-destructive detectors. However, this technique requires additional tubing, adding to the extra band broadening and possibly diminishing the separation performance, and sensitivity is compromised since the flow is split between detectors. Thus, a method of analysis that is time efficient and allows simultaneous destructive and non-destructive detection versatility, whist maintaining HPLC efficiency is desirable, which leads us now to discuss multiplexed detection via AFT in PSF mode.

Multiplexed Detection via Active Flow Technology (AFT) in Parallel Segmented Flow (PSF) mode

Active Flow Technology (AFT) columns utilise a multi-channel end-fitting with a special purpose-built frit that allows the separation of flow between the centre and wall regions of the column [41]. AFT columns were initially designed to overcome the effects of column flow heterogeneity [42] and to increase separation efficiency and sensitivity by establishing a 'wall-less' 'virtual' column within an actual

column [43-46]. The flow from an AFT column elutes from either of two separated radial zones; the central flow region of the bed, which is separated from the peripheral or wall flow region. This is achieved by using an annular frit design, and a multi-channel end fitting as illustrated in *Figure 1*. An impervious ring divides the outlet frit into two parts; an inner portion of frit channels flow from the central region of the bed out a central exit port on the outlet fitting, while an outer ring of frit channels solvent that migrates down the wall region out the peripheral ports on the outlet fitting. This form of AFT is known as Parallel Segmented Flow (PSF) mode, which can be utilised for multiplexed detection [47,48]. The flow ratio between the peripheral and radial central exit ports can be varied through the use of pressure management, simply by changing the length of the tubing located on the peripheral outlet fitting, or the length of tubing post detector on the radial central outlet port. Since, the flow through these ports can be easily adjusted the amount of solute reaching any of the detectors can be adjusted to suit the sensitivity of the given detector source. For example, HPLC-MS is limited to flow rates ~ ≤ 1.5 mL min-1 and so the speed of the chromatographic separation is restricted. However, through the use of AFT-PSF columns the chromatographic separation can be at much higher flow rates, followed by the segmentation of flow at the AFT-PSF outlet and allowing only a portion of the flow that is suitable for the needs of the MS detector [49].

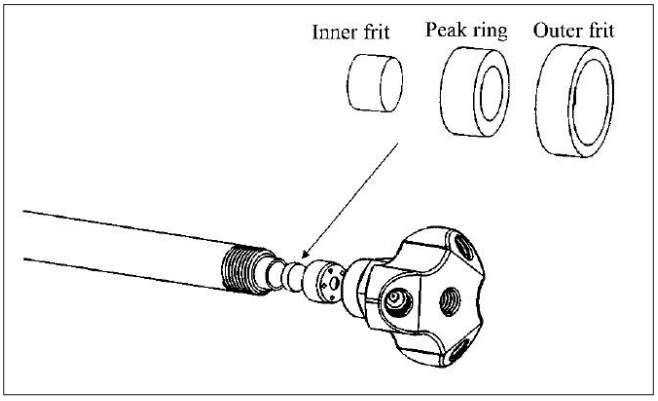

Figure 1. Illustration of AFT-PSF column end-fitting design consisting of flow separating frits and a multiport end-fitting. Reproduced from Ref. [41] with permission from The Royal Society of Chemistry.

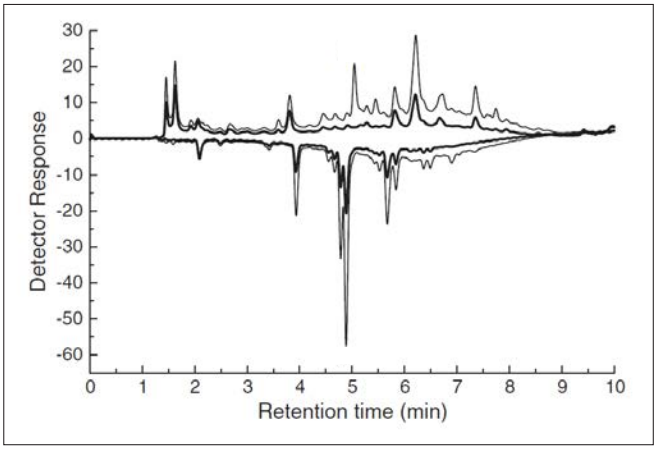

Figure 3. Fluorescence and DPPH• detection response acquired using multiplexed AFT-PSF for Decaffeinato Intenso (bold traces) and Ristretto espresso coffee samples [50].

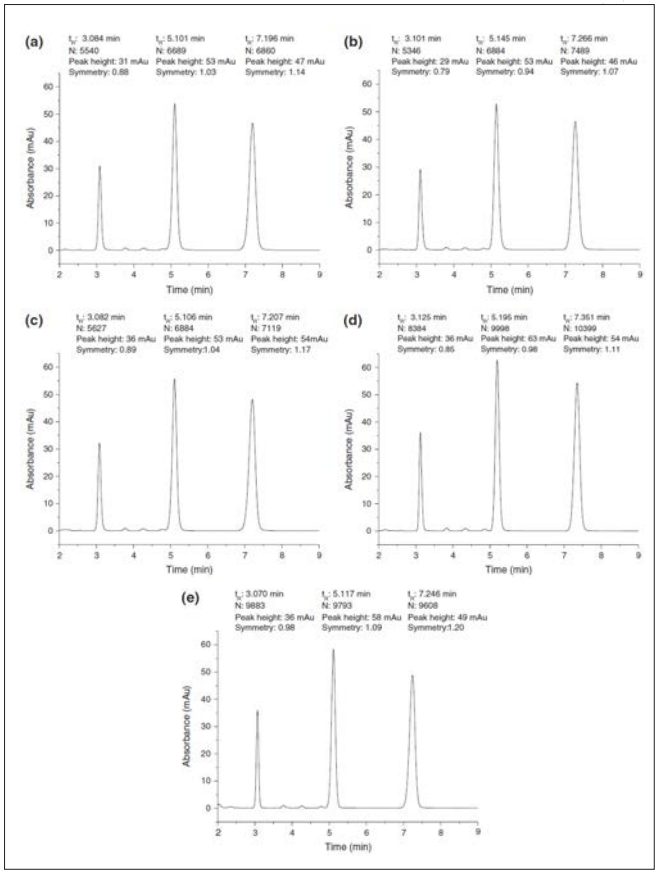

Figure 2. Isocratic elution separation of toluene, propylbenzene and butylbenzene (listed in order of elution) obtained with UV detection on: (a) peripheral port 1, (b) peripheral port 2, (c) peripheral port 3, (d) central port and (e) conventional chromatographic conditions [47].

AFT-PSF columns have been investigated for their potential and capability in multiplexed detection [47,48]. Camenzuli et al. tested the separation performance in terms of plate count efficiency and sensitivity, through each peripheral and central port. The flow was segmented to 25% of flow through each of the four ports and efficiency was tested and compared to a conventional column. *Figure 2* compares the chromatograms for each port at 25% to the conventional column. They found that although the separation performance of the central was superior to the peripheral ports and conventional column, the performance of each of the peripheral ports were similar to the performance obtained on a conventional column; thus deeming them suitable for multiplexed detection [47].

There are limited reported uses of AFT-PSF in multiplexed detection mode since the concept is new and these columns are not yet commercially available [48,50-52]. However, in one instance 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and FLD were multiplexed for the analysis and characterisation of antioxidants in natural products [50] and in another study two chemiluminescence (CL) detection methods and UV-Vis were multiplexed for the detection and analysis of alkaloids [48] in illicit drugs [53]. The study involving the multiplexing of DPPH• and FLD showed improved sensitivity and resolution compared to analyses using conventional columns. Furthermore, because of the reduced volume of the sample band the reaction loop volume was reduced to a mere 20 µL compared to around 500 µL when conventional columns were employed. The chromatographic profiles of the FLD detector response and the antioxidant response are shown in *Figure 3*. Of particular note is the sharpness of the antioxidant peaks, a virtue of the very small reaction loops. Further details of these chromatograms can be found in reference

[50]. However, such improvements, in particular sensitivity increases, were not observed in the multiplexing of two CL and UV-Vis. In fact, a decrease in CL response and UV-Vis response was observed. This phenomenon will be further discussed in section 3.5 - Multi-detection/Multiplexing Analysis Aspect Considerations.

Advantages of Multiplexing using AFT-PSF

It has been previously reported that using these columns a gain in separation performance of up to 70% based on the number of theoretical plates was achieved, but also, importantly up to almost 40% based on sensitivity in the UV-Vis [44]. The gain in sensitivity arises because the solute as it elutes from the column is displaced in a smaller volume, thus the sample is more concentrated. This is an important advantage for detectors that respond to the attenuation of light by the sample. The design of the AFT-PSF multiport end-fitting also provides the added benefit of a platform for multiplexed detection and reaction flow chromatography [47]. In multiplexed mode, the multiport end-fitting can enable the use of up to four detectors in a single analysis, with each of these detectors functioning in parallel, whilst also allowing simultaneous use of destructive and non-destructive detectors. This reduces analysis time by up to four fold, whilst providing maximum information output. Multiplexed detection also enables easy peak matching between chromatographic data based on retention time.

Multi-detection/Multiplexed Analysis Considerations

Multiplexed detection including multiplex reaction flow detection, have proven to be a useful straightforward technique for the characterisation and analysis of complex samples in attaining multidimensional information. The primary advantage of multiplexing with AFT-PSF is the reduction of analysis time by up to four fold. However, studies have also shown that improved sensitivity is not always achievable with respect to the type of detection method(s) utilised. For example the multiplexing of two CL detections and UV-Vis actually resulted in a decrease in signal response compared to conventional methods of CL and UV-Vis with the same chromatographic conditions [48]. It is important to note that, in this study under conventional methods of CL, 100% of the sample on column is detected whereas in AFT-PSF mode only a portion of the sample is detected. In the case of UV-Vis, the signal response was lower than conventional because only 27% of the effluent from the central port was directed to the UV-Vis detector. The reported studies that showed an increase in sensitivity and separation performance with UV detection were obtained at the optimal segmentation ratio of 43% through the central port. Thus, it is important that when setting up an AFT-PSF column for the purpose of multiplexing detection there are a number of considerations that need to be made; that is post-column dead volume considerations, type of detection method, port to detector assignment and tuning of segmentation ratio. More important, however, was the reduction in signal response observed in the CL detectors, which resulted because these detection processes occur through a chemical reaction that subsequently results in light being produced. It is therefore dependent on the number of molecules in the reaction cell, rather than the concentration. That being said, the drop in sensitivity was far outweighed by the benefits of the coincident detection across the three different modes as this allowed for the absolute alignment of the selectively responses – the benefits of these modes of detection was their specificity towards components in the drug samples.

Type of Detection Method

The choice of detector for multiplexed detection can greatly influence the separation performance of AFT-PSF columns. It is important to understand the detection mechanisms involved in the detector choice. For example, the intensity of a UV-Vis signal response is dependent on the concentration of analyte passing through the detector cell, that is, the analyte to solvent ratio, whereas the signal response intensity of FLD is dependent on the amount of analyte present in detector cell for fluorescence. In a hypothetical comparative study, involving the conventional technique for UV-Vis and FLD and AFT-PSF technique with UV-Vis and separately with FLD, whilst maintain the exact same chromatographic and AFT-PSF conditions (sample concentration, injection volume, segmentation ratio and port dedication), can produce rather different outcomes. AFT-PSF with a segmentation ratio of 43% directed to UV-Vis will result with an improved separation performance with respect to sensitivity compared to conventional UV-Vis. This is due to the fact that the radial central region of AFT-PSF column is the most concentrated region of the eluting band and thus with a detection method like UV-Vis whose signal intensity is based on concentration, increased signal response is expected. On the other hand, the comparison of conventional FLD and AFT-PSF FLD can result in the opposite outcome with a decrease in signal response. This is due to the fact that under conventional FLD 100% of the sample amount is directed to the detector, where 100% of the sample amount will fluoresce for detection. Where for AFT-PSF, even if the most concentrated portion of the band at an optimal segmentation ratio of 43% (according to UV-Vis [41,43,45]) were to be taken to FLD, a decrease in signal response would be observed since only a portion of the available sample amount enters the detector.

Port to Detector

Port to detector dedication must also be considered when assigning and directing flow from a port of the AFT-PSF column to a detector. Depending on the detection mechanisms and detector needs will help determine whether the central flow is required or if peripheral flow is sufficient. For sensitive detectors that are significantly affected by dead-volume contributions, such as, MS, whose signal response is relative abundance dependent or UV-Vis detectors whose signal response is concentration dependent, the dedication of the central port would be ideal to attain maximum sensitivity. Detectors that are the least sensitive and more tolerant to band broadening and whose signal intensity are sample amount dependent, such as, DPPH• (reagent-based) detectors or FLD, the peripheral flow may be more suitable for detection. Generally, consider quantification should ideally be based on the detector located on the radial central exit port.

Tuning of Segmentation Ratio

The segmentation ratio of flow from each port can be adjusted to the required amount through the measurement of collected mobile phase eluting through each port over a given period of time. The flow ratios can be adjusted by either shortening or lengthening connecting tubing to suit the requirements of the detectors of choice. Different detectors have different requirements of flow, for example, the flow cell of a fluorescence detector (FLD) is not flow rate limited, but care must be taken to avoid over-pressurisation of the flow cell. Hence the control of flow through the FLD is usually attained by adjusting the pressure drop across the other detectors and the remainder of the flow then passes through the FLD. A detector that is sensitive to the amount of flow that is delivered is the MS. Generally, current high end mass spectrometers can readily process around 1 to 1.5 mL/min of moderately aqueous mobile phase. Above this flow rate, flooding of the source may make the MS inoperable. However, detection sensitivity in most mass spectrometers is benefited by using lower flow rates; hence the PSF flow splitting capabilities are extremely useful for applications involving MS detection. High column volumetric flow rates can be utilised, but with low volume loads transported to the MS detector. However, since MS is a destructive detector, the eluting segmentation ratio cannot be measured whilst the port is connected to the MS detector.

Depending on the type of detector the adjustment of the segmentation ratio can be done either pre- or post-detector. If a non-destructive detector,

such as UV-Vis is used, the flow percentage would be measured and tuned post detector. If a single destructive detector is used in the multiplex set up the flow percentage is determined by back calculating with respect to other port flow percentages. If a reagent based detector is used such as DPPH•, the flow percentage is measured post detector without the addition of reagent; and if two or more destructive detectors are used, then the flow ratio is measured pre-detector. Detection systems that may require additional instrumentation, such DPPH•, will have extra system pressure that may alter the flow percentage once attached to the detection system. Therefore, careful consideration should be paid to system pressure of a destructive detector, when adjusting flow percentage pre-detector.

Conclusion

Complex samples of natural origin are difficult to characterise under uni-dimensional conditions, thus to reduce sample complexity and increase sample information throughput multidimensional analysis through a multi-detection perspective is ideal. Different detectors can provide sample specific information such that multi-detection can provide multi-characteristic information. Although, there are various methods of multi-detection, the conventional forms of multi-detection have their limitations, such as, lengthy run times for single detector analysis or dead volume contributions for serial detections. Furthermore, only one destructive detector can be used in any single series of detectors. The multi-port end fitting design of the AFT-PSF column offers the advantage of providing opportunities for multiplexed detection processes that subsequently can yield detailed sample information. Up to four separate detectors, all of which could be destructive can be employed simultaneously within a single injection. However, to achieve an optimal multiplexed analysis via AFT-PSF, there are a number of considerations that have to be made including, type of detection method, port to detector assignment and detector suitable segmentation ratio. Nevertheless, multiplexed AFT-PSF is ideal for rapid characterisation and analysis of complex samples of natural origin, without the compromise of separation performance.

References

1. E.P. Springfield, P.K.F. Eagles, G. Scott, J. Ethnopharmacol. 101 (2005) 75-83.
2. P. Vuorela, M. Leinonen, P. Saikku, P. Tammela, J.P. Rauha, T. Wennberg, H. Vuorela, Curr. Med. Chem. 11 (2004) 1375-1389.
3. S.B. Singh, J.F. Barrett, Biochem. Pharmacol. 71 (2006) 1006-1015.
4. J.L. Adcock, P.S. Francis, N.W. Barnett, Anal. Chim. Acta 601 (2007) 36-67.
5. M. Camenzuli, G.R. Dennis, H. Ritchie, R.A. Shalliker, Antioxidant Screening of Beverages using the Online HPLC-DPPH• Assay Incorporating Active Flow Technology Chromatography Columns, Processing and Impact on Antioxidants in Beverages, Elsevier Inc., Victor Preedy, 2014, pp. 277-287.
6. P.S. Francis, J.W. Costin, X.A. Conlan, S.A. Bellomarino, J.A. Barnett, N.W. Barnett, Food Chem. 122 (2010) 926-929.
7. G.P. McDermott, X.A. Conlan, L.K. Noonan, J.W. Costin, M. Mnatsakanyan, R.A. Shalliker, N.W. Barnett, P.S. Francis, Anal. Chim. Acta 684 (2011) 134-141.
8. M. Richelle, I. Tavazzi, E. Offord, J. Agr. Food Chem. 49 (2001) 3438-3442.
9. D.M.A.M. Luykx, S.M. van Ruth, Food Chem. 107 (2008) 897-911.
10. L.M. Reid, C.P. O'Donnell, G. Downey, Trends Food Sci. Tech. 17 (2006) 344-353.
11. S.A. Bellomarino, X.A. Conlan, R.M. Parker, N.W. Barnett, M.J. Adams, Talanta 80 (2009) 833-838.
12. B.C. Radovanović, A.N. Radovanović, J.-M. Souquet, J. Sci. Food Agr. 90 (2010) 2455-2461.
13. M.W. Dong, Modern HPLC for Practising Scientists, John Wiley & Sons Inc. , New Jersey, 2006.
14. S. Kromidas, HPLC Made to Measure: A Practical Handbook for Optimization, Wiley-VDH, Weinheim, 2006.
15. L.R. Snyder, J.J. Kirkland, J.W. Dolan, Introduction to Modern Liquid Chromatography, John Wiley & Sons, Inc., Hoboken, New Jersey, 2009.
16. J.L. Wolfender, Planta Med. 75 (2009) 719-734.
17. T.T. Hanai, HPLC: A Practical Guide, The Royal Society of Chemistry, Cambridge, UK, 1999.
18. L.R. Snyder, J.J. Kirkland, J.L. Glajch, Detection Sensitivity and Selectivity, Practical HPLC Method Development, John Wiley & Sons, Inc., 1997, pp. 59-99.
19. M.C. McMaster, HPLC - a practical user's guide, John Wiley and Sons Inc., Hoboken, New Jersey, 2007.
20. G. Cimpan, S. Gocan, J. Liq. Chromatogr. R. T. 25 (2002) 2225-2292.
21. G. Bahrami, S. Mirzaeei, J. Pharm. Biomed. Anal. 36 (2004) 163-168.
22. J. Jaimez, C.A. Fente, B.I. Vazquez, C.M. Franco, A. Cepeda, G. Mahuzier, P. Prognon, J. Chromatogr. A 882 (2000) 1-10.
23. M.K. Balz, E. Schulte, H.P. Thier, Lipid / Fett 95 (1993) 215-220.
24. J.L. Chávez-Servín, A.I. Castellote, M.C. López-Sabater, J. Chromatogr. A 1043 (2004) 211-215.
25. A. Clement, D. Yong, C. Brechet, J. Liq. Chromatogr. 15 (1992) 805-817.
26. H.C. Lee, M. Ree, T. Chang, Polymer 36 (1995) 2215-2218.
27. B. Trathnigg, Size-Exclusion Chromatography of Polymers, Encyclopedia of Analytical Chemistry, John Wiley & Sons, Ltd, 2006.
28. M. Ganzera, H. Stuppner, Curr. Pharm. Anal. 1 (2005) 135-144.
29. R. Andrew Shalliker, Hyphenated and Alternative Methods of Detection in Chromatography, CRC Press - Taylor & Francis Group, Florida, US, 2012.
30. P.A. Cremin, L. Zeng, Anal. Chem. 74 (2002) 5492-5500.
31. W. Li, S. Chen, D. Fabricant, C.K. Angerhofer, H.H.S. Fong, N.R. Farnsworth, J.F. Fitzloff, Anal. Chim. Acta 471 (2002) 61-75.
32. J. Li, H. Qi, L.-W. Qi, L. Yi, P. Li, Anal. Chim. Acta 596 (2007) 264-272.
33. T. Vehovec, A. Obreza, J. Chromatogr. A 1217 (2010) 1549-1556.
34. J.P. Hutchinson, T. Remenyi, P. Nesterenko, W. Farrell, E. Groeber, R. Szucs, G. Dicinoski, P.R. Haddad, Anal. Chim. Acta 750 (2012) 199-206.
35. B. Godin, R. Agneessens, P.A. Gerin, J. Delcarte, Talanta 85 (2011) 2014-2026.
36. S. Inagaki, J.Z. Min, T. Toyo'oka, Biomed. Chromatogr. 21 (2007) 338-342.
37. W.M.A. Niessen, Liquid Chromatography-Mass Spectrometry, CRC Press - Taylor & Francis Group, Florida, USA, 2006.
38. M.G. Inghram, R.J. Hayden, A Handbook on Mass Spectroscopy, National Academy of Sciences, Washington, D.C, 1954.
39. R.E. Ardrey, Liquid Chromatography - Mass Spectrometry: An Introduction, Wiley, 2003.
40. X.A. Conlan, Mass Spectrometry and Separation Science, in: R.A. Shalliker (Ed.), Hyphenated and Alternative Methods of Detection in Chromatography, CRC Press, Boca Raton, Florida, 2011, pp. 1-30.
41. M. Camenzuli, H.J. Ritchie, J.R. Ladine, R.A. Shalliker, Analyst 136 (2011) 5127-5130.
42. R.A. Shalliker, H. Ritchie, J. Chromatogr. A 1335 (2014) 122-135.
43. M. Camenzuli, H.J. Ritchie, R.A. Shalliker, Microchem. J. 111 (2013) 3-7.
44. R.A. Shalliker, M. Camenzuli, L. Pereira, H.J. Ritchie, J. Chromatogr. A 1262 (2012) 64-69.
45. M. Camenzuli, H.J. Ritchie, J.R. Ladine, R.A. Shalliker, J. Chromatogr. A 1232 (2012) 47-51.
46. M. Camenzuli, H.J. Ritchie, J.R. Ladine, R.A. Shalliker, J. Sep. Sci. 35 (2012) 410-415.
47. M. Camenzuli, H.J. Ritchie, R.A. Shalliker, Microchem. J. 110 (2013) 473-479.
48. M. Camenzuli, J.M. Terry, R.A. Shalliker, X.A. Conlan, N.W. Barnett, P.S. Francis, Anal. Chim. Acta 803 (2013) 154-159.
49. D. Kocic, L. Pereira, D. Foley, T. Edge, J.A. Mosely, H. Ritchie, X.A. Conlan, R.A. Shalliker, J. Chromatogr. A 1305 (2013) 102-108.
50. M. Camenzuli, H.J. Ritchie, G.R. Dennis, R.A. Shalliker, Microchem. J. 110 (2013) 726-730.
51. S. Pravadali-Cekic, A. Jones, A.A. Kazarian, B. Paull, A. Soliven, H. Ritchie, M. Camenzuli, G.R. Dennis, R. Andrew Shalliker, Microchem. J. 121 (2015) 141-149.
52. M. Camenzuli, H.J. Ritchie, G.R. Dennis, R.A. Shalliker, J. Chromatogr. A 1303 (2013) 62-65.
53. J.M. Terry, Z.M. Smith, J.J. Learey, R.A. Shalliker, N.W. Barnett, P.S. Francis, Talanta 116 (2013) 619-625.

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