Microchemical Journal 142 (2018) 181–186

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Narrow bore post column derivatisation assays via reaction flow chromatography

Agustín Acquaviva[a], Andrew Jones[b], Gary R. Dennis[b], Cecilia Castells[a], R. Andrew Shalliker[a,b], Arianne Soliven[c,*]

[a] LIDMA (Laboratorio de Investigación y Desarrollo de Métodos Analíticos), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 and 115, 1900 La Plata, Argentina

[b] Australian Centre for Research on Separation Science (ACROSS), School of Science and Health, Western Sydney University, Parramatta, NSW 2150, Australia

[c] Grupo de Análisis de Contaminantes Traza, Cátedra de Farmacognosia y Productos Naturales, Facultad de Química, Universidad de la República, General Flores 2124, 11800 Montevideo, Uruguay

ARTICLE INFO

Keywords:
Post column derivatisation
Selective detection
Reaction flow chromatography
Narrow bore
Scaled down
Phenolics

ABSTRACT

This study highlights the capability to conduct a post column derivatisation (PCD) process with a narrow bore, low volume column. A reaction flow (RF) HPLC column (30 mm × 2.1 mm internal diameter (i.d.), 3 µm particle diameter) enabled the selective detection of phenols for both a simple standard mixture and a complex sample represented by green tea. The resolving power and sensitivity obtained were similar to an analytical scale (4.6 mm i.d.) RF HPLC column of the same format. However, the baseline noise experienced was higher when the assay was scaled down from the RF 4.6 mm i.d. to the RF 2.1 mm i.d. narrow bore column. The PCD mixing and reaction speed inside the RF column outlet (frit and head-fitting design) enabled the coupling of PCD protocols with "UHPLC-type" low volume columns and systems without significant loss in separation performance. In comparison to a conventional analytical scale PCD approach the scaled down narrow bore RF approach demonstrated 40 times greater sensitivity in terms of S/N. Scaled down PCD is vital for decreased consumption and cost without significant loss in separation performance.

1. Introduction

Targeted analyses using post column derivatisation (PCD) has long been employed in the field of separation science [1–3]. The most common reasons to adopt PCD include: (a) derivatisation of compounds to enable and/or enhance detection sensitivity [1–4]; (b) visual simplification of a chromatogram or 3D contour plots, for example the selective detection of specific analytes in a complex sample that exceeds the peak capacity of the separation space [3, 5–12]; (c) screening for bio-significant compounds, where the PCD reaction is responsive to compounds that exhibit a particular reactivity e.g. antioxidants, amino acids etc. [2, 3, 13–28]. PCD is an important, and often neglected part of the chromatographer's toolbox that can be exploited to obtain selective information when properly utilised.

One substantial limitation of PCD is that many reaction schemes require the addition of large post column dead volumes in order to facilitate the mixing between the target analytes in the mobile phase and the PCD reagents [2, 3]. Also, long residence times within the mixing loops are frequently required in order to allow sufficient reaction to proceed while the liquids are mixing so that a detectable signal response can be generated [2, 3]. This means that most users of PCD processes employ long columns in order to minimise the detrimental effects of the added dead volume, and also tend to use particle sizes of five microns or larger [2, 3]. Subsequently, PCD processes are becoming out of step with the modern practice of HPLC, which is steadily driving into the realm of ultra high pressure liquid chromatography (UHPLC) with column dimensions and particle sizes that are continually decreasing [2, 3]. Therefore, to stay in step with UHPLC there must also be a drive for the development of new or improved PCD techniques that enable efficient reagent mixing and faster reaction times in low volume column formats.

One such development that appears promising in the field of post column derivatisation assays is the reaction flow (RF) HPLC column [14, 16, 20, 21, 23, 25–28]. In PCD processes these RF columns enable the mixing of the PCD reagents with the mobile phase and analyte to occur inside the outlet end fitting and frit configuration of the column [2, 3]. The RF approach eliminates the need for a reaction loop as the RF column outlet facilitates low internal volume and efficient mixing

Abbreviations: PCD, post column derivatisation; RF, reaction flow; i.d., internal diameter

* Corresponding author.
E-mail address: asoliven@fq.edu.uy (A. Soliven).

https://doi.org/10.1016/j.microc.2018.06.032
Received 6 June 2018; Received in revised form 19 June 2018; Accepted 19 June 2018
Available online 20 June 2018
0026-265X/

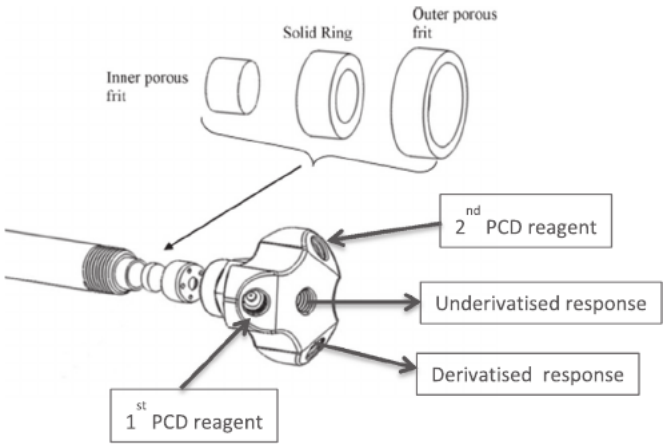

Fig. 1. RF column and flow paths used in a two-component post column derivatisation process.

[2, 3]. Therefore, the post column band broadening generated by the PCD process is minimised and consequently, the signal response increased [2, 3]. Details of these columns have been described in numerous communications prior [14, 16, 20, 21, 23, 25–28], so need not be discussed here, although, Fig. 1 shows the system configuration incorporating the RF column and derivatisation process. In Fig. 1 a two component post column derivatisation process is shown, which is the setup utilised in this work for the selective detection of phenolic compounds as a proof of concept [16].

Previously RF-PCD has been limited to standard analytical bore 4.6 mm i.d. columns [14, 16, 20, 21, 23, 25–28]. In the present work, we have taken the application of PCD to an entirely new level of performance, and demonstrate the use of RF-PCD in a narrow bore column format. Specifically in this study we have used a 30 mm × 2.1 mm i.d. RF column, packed with 3 µm particles, the smallest column volume used for a PCD assay to the best of our knowledge and this represents a substantial improvement in the limitations that face users of PCD processes. Additionally, we demonstrate the ability of this small volume column to selectively detect phenolics in standard mixtures and green tea as a representative complex sample. In part, our drive to show that PCD can be achieved on these columns with exceptionally small dead volumes is so that it opens opportunities to use PCD reagents that are too expensive to use on analytical scale format separations since the amount of reagent consumed in narrow bore PCD is about one fifth that compared to the analytical scale.

2. Experimental

2.1. Chromatography column

Hypersil GOLD reaction flow columns (30 mm × 2.1 mm i.d. and 30 mm × 4.6 mm i.d., 3 µm particle diameter) were supplied by Thermo Fisher Scientific (Runcorn, Cheshire, United Kingdom). Further details of RF columns can be found in references [14, 16, 25, 26]. A Hypersil GOLD column (30 mm × 4.6 mm i.d., 3 µm particle diameter) was supplied by Thermo Fisher Scientific (Runcorn, Cheshire, United Kingdom) for the analytical scale conventional comparison.

2.2. Chemicals and reagents

All mobile phases were prepared from HPLC-grade methanol supplied by Thermo Fisher Scientific (Scoresby, Victoria, Australia). Ammonium acetate and ammonia solution (25%) were supplied by Sigma Aldrich (Castle Hill, New South Wales, Australia). The post column derivatisation reagent 4-aminoantipyrine was purchased from ACROS Organics, part of Thermo Fisher Scientific. Potassium ferricyanide was supplied by AnalaR (Murarrie, QLD, Australia). Milli-Q water (18.2 MΩ cm) was prepared in-house and filtered through a 0.22 µm filter. Buffer solution of pH 9.0 was prepared by dissolving 7.7 g of ammonium acetate in 900 mL of Milli-Q water, adjusting to pH 9.0 with ammonia solution and finally diluted to 1000 mL with Milli-Q water. 4-Methoxy phenol; naphtalene-2,7-diol and *p*-cresol were purchased from Sigma Aldrich (Castle Hill, NSW, Australia). Green tea (individual serving sachets/bags from Twinings of London) was purchased from the local market.

2.3. Instrumentation

All chromatographic experiments were conducted using an Agilent 1290 Infinity I system (Agilent, Forest Hill, Victoria, Australia) equipped with an Agilent 1290 auto-sampler, an Agilent 1290 Infinity binary pump (with a 35 µL mixer) and an Agilent 1260 DAD (with a 1 µL flow-cell). The post column connection tubing volume from the RF outlet peripheral port or the exit of the mixer to the detector inlet port was 2 µL. Two Shimadzu pumps (Shimadzu, Rydalmere, New South Wales, Australia), one LC10ADvp and one LC10ATvp, fitted with inline degassing units were used to deliver the post column derivatisation reagents.

2.4. Preparation of samples and reagents

2.4.1. Post column derivatisation reagents

The colorimetric reagents were prepared using the following method: The 4-aminoantipyrine solution was made by dissolving 600 mg of the reagent in 400 mL of the ammonium acetate buffer solution. The potassium ferricyanide solution was made by dissolving 600 mg of the reagent in 400 mL of the ammonium acetate buffer solution. Both solutions were filtered using a 0.22 µm membrane before use.

2.4.2. Sample preparation

Each tea sample was prepared fresh daily by adding one tea bag to 40 mL of 80 °C tap water and allowing the bag to rest for 10 min, with occasional agitation. Tap water was used without further treatment. After 10 min the bag was removed and squeezed firmly. The solution was allowed to cool to room temperature. An aliquot was filtered through a 0.22 µm nylon syringe filter and used for analysis.

2.4.3. Standard preparation

A standard solution containing three standard compounds was used: 4-methoxyphenol, naphtalene-2,7-diol, and *p*-cresol. The mixture was prepared at a concentration of 500 ppm each in Milli-Q water. All materials were used as received.

2.5. Chromatographic separations

2.5.1. Analysis of standards

A three-component standard test mixture was analysed in isocratic mode. The PCD response of these standards was used to gauge the suitability of the PCD process. Two of the components in this mixture (naphthalene-2,7-diol and 4-methoxyphenol) responded to the phenolic test. The mobile phase was 65/35 water/methanol (both with a 0.1% formic acid) and the flow rate was fixed at 0.2 mL/min, injection volumes were set at 1 µL. Detection responses were collected at 500 and 280 nm, for the derivatised PCD injections (500 nm) and the non-derivatised injections (280 nm).

2.5.2. Analysis of tea samples

Gradient elution mobile phases were utilised for all separations of the tea samples. Mobile phase A was water (0.1% formic acid) and mobile phase B was methanol (0.1% formic acid). Prior to any injection the column was equilibrated with 5 column volumes of the initial

mobile phase (95/5 A/B). After injection the mobile phase composition changed in a linear manner up to the composition 100% B in a period of 30 min. The final mobile phase composition, 100% B, was held for a further 2 min, and then returned to the initial conditions in 0.1 min. The flow rate was 0.2 mL/min on the 2.1 mm i.d. column and 1.0 mL/min on the 4.6 mm i.d. column. Injection volumes were 2 μL on the 2.1 mm i.d. column and 10 μL on the 4.6 mm i.d. column.

The segmentation ratio at the column outlet was set at 50% through the column centre [16]. Hence, 50% of the mobile phase exited the column through the peripheral port on the RF fitting. PCD reagents were delivered to the ports on the RF column at the following flow rates for the 2.1 mm i.d. column: 4-aminoantipyrine – 0.1 mL/min and potassium ferricyanide – 0.05 mL/min. In order to maintain reliable flow delivery of the potassium ferricyanide the pump flow rate was set at 0.2 mL/min and split using a tee-piece to deliver 0.05 mL/min to the column. For the 4.6 mm i.d. column the PCD reagents were delivered at a faster flow rate of: 4-aminoantipyrine – 0.5 mL/min and potassium ferricyanide – 0.25 mL/min.

The instrumental set-up for the two-component post column derivatisation process is shown in Fig. 1. The reaction flow column outlet has four ports: Two peripheral ports were used to feed the post column derivatisation reagents into the mobile phase flow, while one peripheral port was used to direct sample to a visible detector, in this case a UV–Vis detector set at 500 nm (derivatised analytes response). In addition, the samples were analysed a second time with the pumps for the PCD reagents turned off, and the eluting flow from the radial peripheral port was directed to the UV detector at a wavelength of 280 nm. Note, the segmentation ratio was maintained at 50% through the radial central exit port to the detector.

3. Results and discussion

The primary purpose of this study was to determine if narrow bore, low volume LC columns could be utilised for efficient post column derivatisation analyses. The void volume of this column was 100 μL, illustrated in Fig. 2 by the baseline disturbance from the chromatographic data; and the peak volume of the first component, having a retention factor of 1 was approximately 40 to 50 μL. Therefore, the addition of any post column mixing volume to facilitate a suitable PCD reaction within this practical framework is challenging. Hence in this work, the RF column was connected to the injector and detector using a minimal length of 0.13 mm internal diameter tubing (pre and post column). This amounted to tubing having a total dead volume of 6 μL, of which 2 μL was between the column and the detector.

Prior to the analysis of the tea samples, the detection response was measured using standards. Two of the standards respond to the phenolic test, the third does not when it is analysed at a wavelength of 500 nm, but all three are visible without derivatisation in Fig. 2a and b shown in the chromatograms at 280 nm (underivatised) and 500 nm (derivatised). The PCD reproducibility (% RSD) for triplicate injections were as follows: retention time ≤0.58, area ≤0.84 and height ≤1.29.
The absolute response obtained in the derivatised mode was slightly better than what we have seen in analytical scale RF 4.6 mm i.d. columns at the same nominal segmentation ratio (50:50 central and peripheral outlet ports ratio – data not shown). In addition, the profile of the bands following post column derivatisation appears to have been preserved based on comparison to the profiles obtained without the post column derivatisation.

The analyses of tea samples were then undertaken on both RF analytical and RF narrow bore scale (4.6 mm and 2.1 mm i.d.) columns of the same length (30 mm), with and without post column derivatisation. The chromatograms in Fig. 3a and b show the separation of a green tea sample with UV detection at 280 nm (without PCD) on the RF 4.6 mm and RF 2.1 mm i.d. columns, respectively. Both separations are very similar due to the scaled adjustment of flow rates and injection volumes relative to the column internal diameter, and the fixed

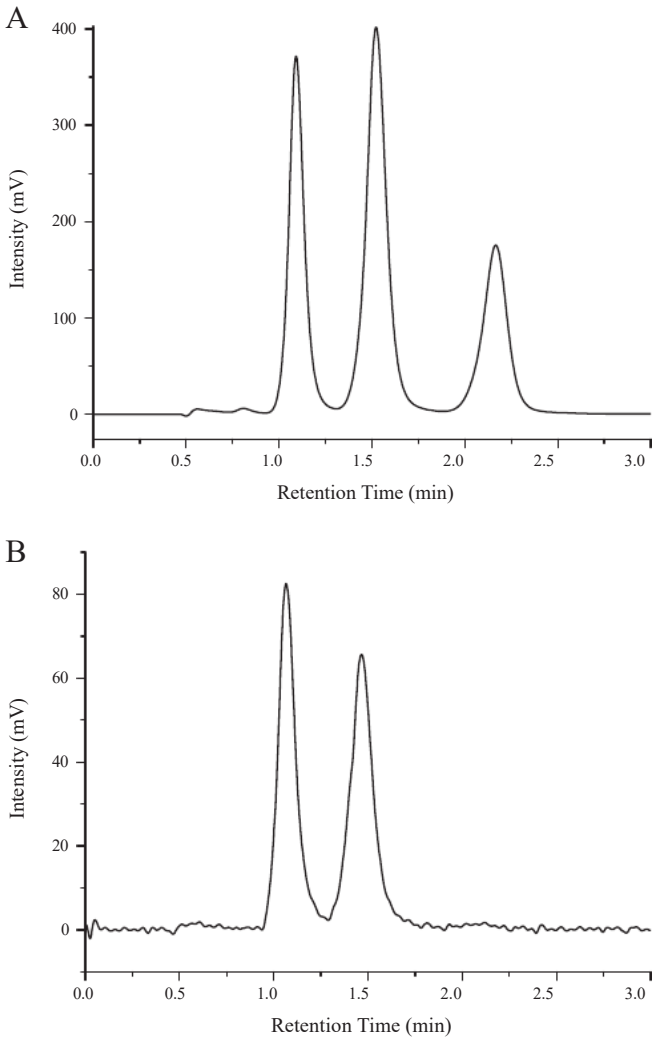

Fig. 2. Chromatographic profiles of the standard mixture containing compounds – naphthalene-2,7-diol, 4-methoxyphenol and *p*-cresol (in that elution order) separated on the RF 30 mm × 2.1 mm i.d. column. Isocratic elution conditions, 65/35 water/methanol (0.1% formic acid). Flow rate 0.2 mL/min. (a) Underivatised detection at 280 nm and (b) post column derivatisation at 500 nm.

gradient rate. The RF 2.1 mm i.d. format column showed better resolution for the peak pair highlighted in box '(i)', whereas the RF 4.6 mm i.d. column showed better resolution for the peak pair highlighted in box '(ii)'.

Post column derivatisation analyses of the green tea sample on both the RF 4.6 mm i.d. column and the RF 2.1 mm i.d. column are shown in Fig. 4a and b respectively. In line with the underivatised chromatograms in Fig. 3, the PCD responses on both columns were similar for the most abundant peaks. The most obvious differences between Fig. 4a and b is the resolution in the phenolic pair highlighted in box '(i)'. Secondly, there were changes in the relative responses of various compounds responsive to the PCD reaction; variation may be related to sample preparation and the amount of agitation of the tea bag during the 10 min steeping (the samples injected on the RF 30 mm × 4.6 mm i.d. column was undertaken on a different day than the RF 30 mm × 2.1 mm i.d. column). Differences may also be associated to the difference in frit size/surface area when scaling down from the RF analytical to the RF narrow bore columns. Some variations in relative responses are apparent in the UV chromatograms also. Thirdly, the baseline noise response observed on the 2.1 mm i.d. RF column is more substantial than on the 4.6 mm i.d. RF column, as shown in Fig. 5. Such

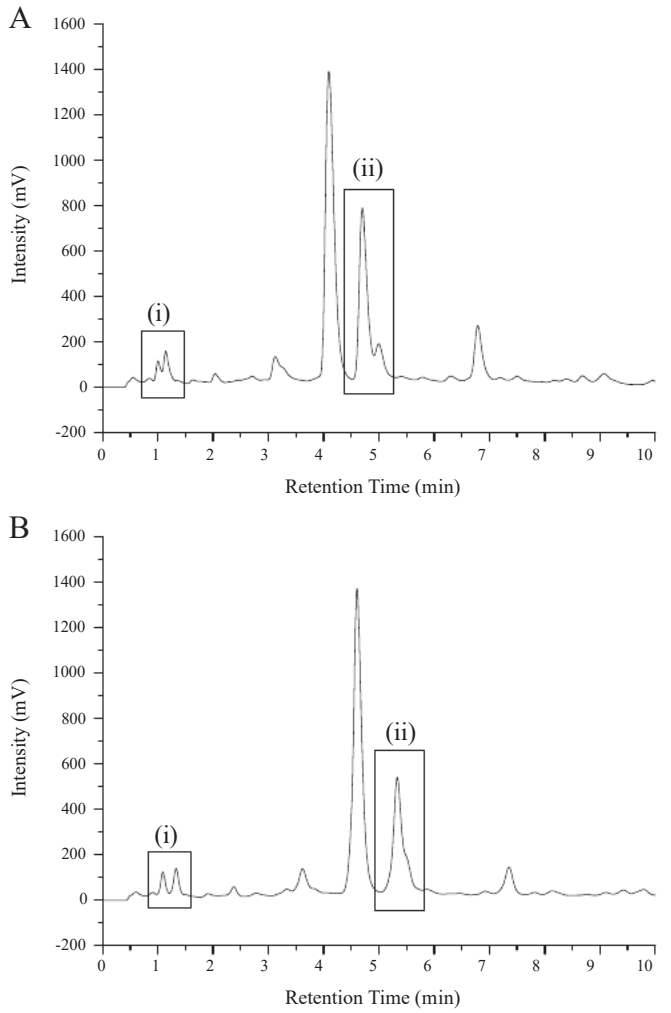

Fig. 3. Chromatographic profiles of the green tea sample obtained on (a) RF 30 mm × 4.6 mm i.d. column and (b) RF 30 mm × 2.1 mm i.d. column without post column derivatisation. Gradient elution conditions as described in the text (Section 2.5). Detection at 280 nm.

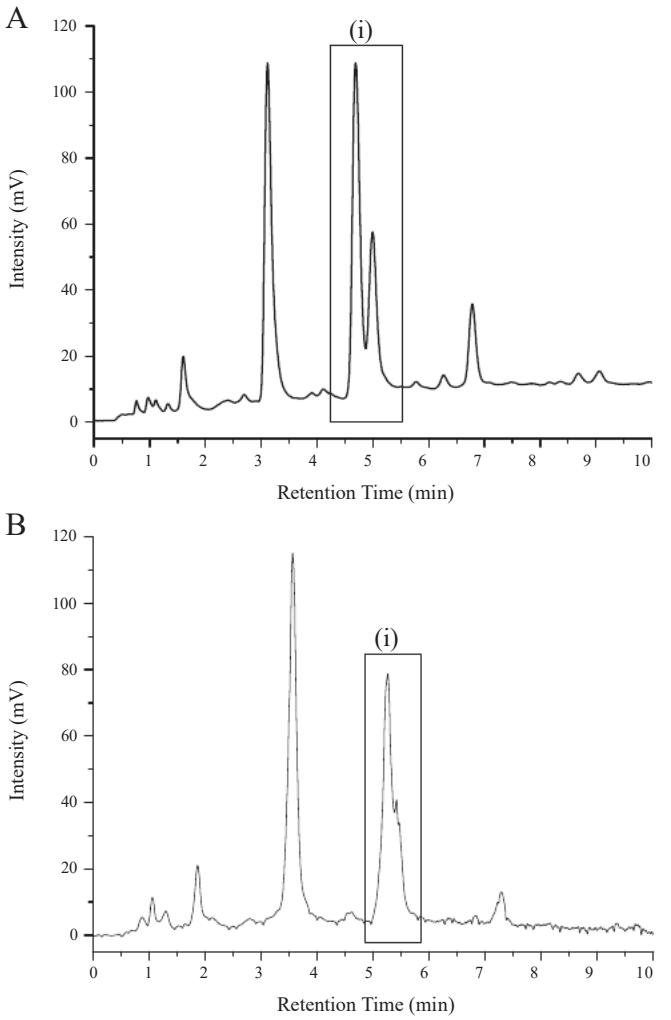

Fig. 4. Chromatographic profiles of the green tea sample obtained on (a) RF 30 mm × 4.6 mm i.d. column and (b) RF 30 mm × 2.1 mm i.d. column with post column derivatisation. Gradient elution conditions as described in the text (Section 2.5). Detection at 500 nm.

an outcome is not surprising due to the difficulty faced for the peripheral PCD pump to deliver accurate, low pulsation flow for the operation of the 2.1 mm i.d. RF column as opposed to the higher flow rates utilised on the 4.6 mm i.d. RF column. Hence, the reason we split the flow from the peripheral PCD pump for the 2.1 mm i.d. RF column. Nevertheless the observed S/N ratios were fit for purpose and far exceeded the S/N ratio observed on conventional HPLC columns operated under post column derivatisation conditions.

The comparative gain in S/N for the 2.1 mm i.d. RF column against a conventional analytical scale 4.6 mm i.d. column operated in a PCD mode was approximately 40 times greater, as shown by the comparison of standards in Fig. 6. In both cases the analyses were undertaken using minimal post column dead volume connecting column to detector. That is, no reaction loops or mixing coils were employed. The flow rate was also scaled between the respective column internal diameters so as to match the retention time of the separation. Undoubtedly this favoured the RF 2.1 mm i.d. column as it gave a longer period of time for reaction prior to the detector, and this is a substantial advantage of the reaction flow column in narrow bore format. Furthermore, a primary advantage of the use of narrow bore RF columns is that there is a substantial reduction in the consumption of PCD reagents and this is especially important for high cost PCD reagents, and these columns can be operated without reaction loops etc., thus maintaining high separation efficiency as well as yielding improved S/N.

The discussion on the use of 30 mm × 2.1 mm narrow bore RF columns in the PCD analysis of phenolics would not be complete without discussion related to the selectivity in the detection mode – PCD vs. UV (underivatised), although this was not the main focus of the study. The chromatograms in Fig. 7 overlay the UV response (280 nm - underivatised) and the PCD response (500 nm – derivatised). For improved visual inspection the PCD response has been inverted, but it should be noted this is a colorimetric reaction, not a decolorisation process, hence the intensity is normally positive, not negative. The chromatograms in Fig. 7 illustrate the benefits of selective detection, in particular, peak '(i)' was virtually absent in the UV at 280 nm, but was detected with good sensitivity in the PCD process, and peak '(ii)' was a weak absorbing compound in the UV at 280 nm, but was the strongest absorbing compound in the PCD assay. Peak (iii) was virtually absent in the PCD analysis, but was the major component in the underivatised sample analysis.

The RF column utilised in this study – 30 mm × 2.1 mm i.d., was not ideally suited to the analysis of a relatively complex sample, such as the green tea. There is of course no substitute for peak capacity when analysing complex samples, as greater separation is required [29]. But that was not the objective of the study. Here we demonstrated that post column derivatisation reactions could be undertaken with narrow bore, small volume reaction flow columns, packed with small particles,

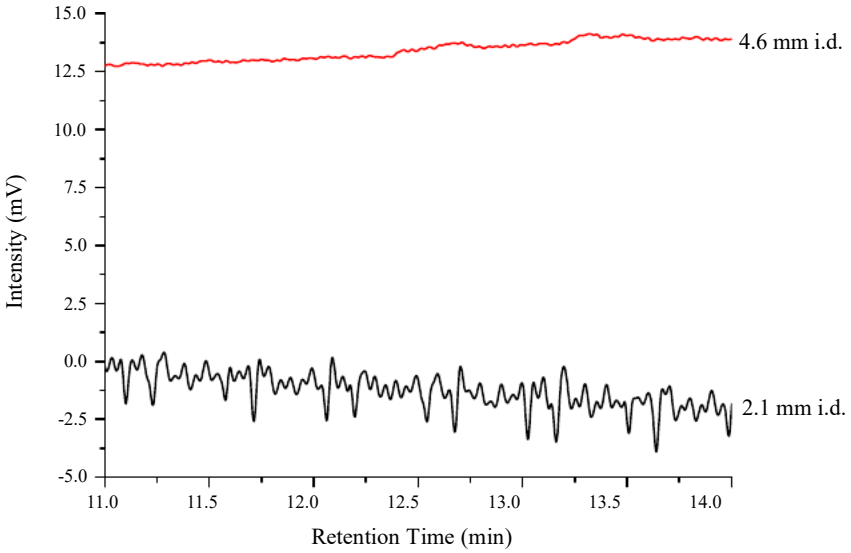

Fig. 5. Illustration of the noise response obtained when the RF analytical (offset +12.5 mAU for simpler visual comparison) and RF narrow bore scale columns were operating under the post column derivatisation mode of analysis. Gradient elution conditions as described in the text (Section 2.5). Detection at 500 nm.

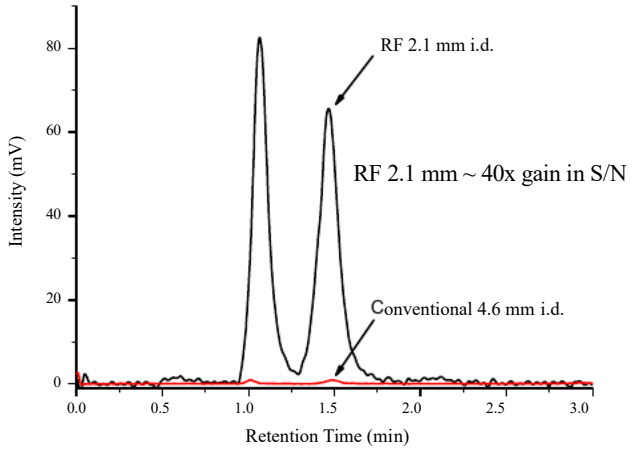

Fig. 6. Post column derivatisation chromatographic profiles of the standard mixture containing compounds – naphthalene-2,7-diol and 4-methoxyphenol (in that elution order) separated on the RF 30 mm × 2.1 mm i.d. column and the 30 × 4.6 mm i.d. conventional column. Each column had exactly the same post column dead volume (i.e., minimal post column tubing connecting column to detector). Isocratic elution conditions, 65/35 water/methanol (0.1% formic acid). Flow rate 0.2 mL/min in the case of the RF 2.1 mm i.d. column, and 1.0 mL/min for the conventional 4.6 mm i.d. column. Detection at 500 nm.

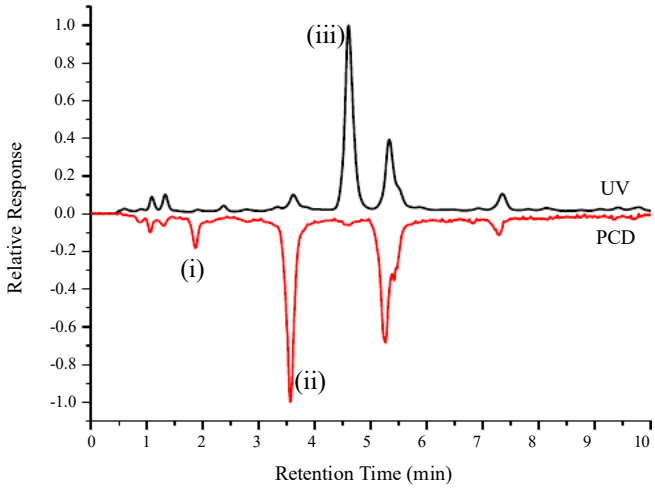

Fig. 7. Comparison of the chromatographic profiles of the green tea separations on the RF 30 mm × 2.1 mm i.d. column. Underivatised detection at 280 nm (positive trace) and derivatised detection (negative trace) at 500 nm, gradient elution conditions as described in the text (Section 2.5).

without a reaction loop and that these columns are able to offer a high degree of selectivity in detection. Furthermore, minimising the amount of labour, consumables and expertise required to discern certain classes of compounds of interest in a complicated sample matrix. We anticipate this communication will open up cost effective applications for low volume RF-PCD efficient mixing and selectivity to revamp PCD selective detection assays [1–3], especially when using expensive post column derivatisation reagents.

4. Conclusion

The analysis of phenols in a green tea sample using a 30 mm × 2.1 mm reaction flow column packed with 3 μm particles with post column derivatisation was demonstrated. The resolving power and sensitivity that was obtained were similar to a 30 mm × 4.6 mm reaction flow column. A minor negative aspect of the study was that the baseline noise of the PCD process was higher in the RF 2.1 mm i.d. column compared to the RF 4.6 mm i.d. column and continued work is required to address this issue, nevertheless, the RF 2.1 mm i.d. column out performed a conventional 4.6 mm i.d. column operated in post column derivatisation mode with respect to sensitivity. In short, the efficiency of reagent mixing and reaction that takes place inside the RF column allows for post column derivatisation protocols to be utilised in UHPLC-type low volume columns and systems, without substantial loss in separation performance; a practical solution to one of the most important problems of coupling PCD with modern chromatography. Most importantly, the scaled down PCD assay from analytical scale to narrow bore scale reduced cost, consumption and waste by up to 80%.

Conflicts of interests

There are no conflicts to declare.

Acknowledgments

A.A. is an associate researcher from CIC-PBA, Argentina. A.S. is a

recipient of an ANII PD scholarship, and a researcher from CSIC and SNI, Uruguay. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] S. Van der Wal, Post-column reaction detection systems in Hplc, J. Liq. Chromatogr. 6 (1983) 37–59, http://dx.doi.org/10.1080/01483918308067648.

[2] A. Jones, S. Pravadali-Cekic, G.R. Dennis, R.A. Shalliker, Post column derivatisation analyses review. Is post-column derivatisation incompatible with modern HPLC columns? Anal. Chim. Acta 889 (2015) 58–70, http://dx.doi.org/10.1016/j.aca.2015.07.003.

[3] C.K. Zacharis, P.D. Tzanavaras, Liquid chromatography coupled to on-line post column derivatization for the determination of organic compounds: a review on instrumentation and chemistries, Anal. Chim. Acta 798 (2013) 1–24, http://dx.doi.org/10.1016/j.aca.2013.07.032.

[4] J.L. Adcock, P.S. Francis, N.W. Barnett, Acidic potassium permanganate as a chemiluminescence reagent-a review, Anal. Chim. Acta 601 (2007) 36–67, http://dx.doi.org/10.1016/j.aca.2007.08.027.

[5] H. Ibrahim, E. Caudron, A. Kasselouri, P. Prognon, Interest of fluorescence derivatization and fluorescence probe assisted post-column detection of phospholipids: a short review, Molecules 15 (2010) 352–373, http://dx.doi.org/10.3390/molecules15010352.

[6] G.P. McDermott, X.A. Conlan, L.K. Noonan, J.W. Costin, M. Mnatsakanyan, R.A. Shalliker, N.W. Barnett, P.S. Francis, Screening for antioxidants in complex matrices using high performance liquid chromatography with acidic potassium permanganate chemiluminescence detection, Anal. Chim. Acta 684 (2011), http://dx.doi.org/10.1016/j.aca.2010.10.046.

[7] M. Mnatsakanyan, P.G. Stevenson, X.A. Conlan, P.S. Francis, T.A. Goodie, G.P. McDermott, N.W. Barnett, R.A. Shalliker, The analysis of café espresso using two-dimensional reversed phase-reversed phase high performance liquid chromatography with UV-absorbance and chemiluminescence detection, Talanta 82 (2010) 1358–1363, http://dx.doi.org/10.1016/j.talanta.2010.06.066.

[8] B.J. Holland, X.A. Conlan, P.G. Stevenson, S. Tye, A. Reker, N.W. Barnett, J.L. Adcock, P.S. Francis, Determination of neurotransmitters and their metabolites using one- and two-dimensional liquid chromatography with acidic potassium permanganate chemiluminescence detection, Anal. Bioanal. Chem. 406 (2014) 5669–5676, http://dx.doi.org/10.1007/s00216-013-7514-9.

[9] P.S. Francis, J.L. Adcock, J.W. Costin, S.D. Purcell, F.M. Pfeffer, N.W. Barnett, Chemiluminescence detection of opium poppy (*Papaver somniferum*) alkaloids, J. Pharm. Biomed. Anal. 48 (2008) 508–518, http://dx.doi.org/10.1016/j.jpba.2008.06.022.

[10] J. Pandohee, B.J. Holland, B. Li, T. Tsuzuki, P.G. Stevenson, N.W. Barnett, J.R. Pearson, O.A.H. Jones, X.A. Conlan, Screening of cannabinoids in industrial-grade hemp using two-dimensional liquid chromatography coupled with acidic potassium permanganate chemiluminescence detection, J. Sep. Sci. 38 (2015) 2024–2032, http://dx.doi.org/10.1002/jssc.201500088.

[11] B.J. Holland, P.S. Francis, B. Li, T. Tsuzuki, J.L. Adcock, N.W. Barnett, X.A. Conlan, Chemiluminescence detection of cannabinoids and related compounds with acidic potassium permanganate, Drug Test. Anal. 4 (2016) 675–679, http://dx.doi.org/10.1002/dta.1328.

[12] A. Acquaviva, A. Jones, G.R. Dennis, R.A. Shalliker, A. Soliven, Detection selectivity in the analysis of “reactive” chemical compounds derived from natural samples via reaction flow chromatography, Microchem. J. (2018), http://dx.doi.org/10.1016/j.microc.2018.02.033 (Accepted, In Press).

[13] A. Jones, V. Nair-Shalliker, G.R. Dennis, R. Andrew Shalliker, The future of liquid chromatographic separations should include post column derivatisations: a discussion view point based on the perspective for the analysis of vitamin D, Microchem. J. 138 (2018) 501–508, http://dx.doi.org/10.1016/j.microc.2018.01.046.

[14] A. Jones, S. Pravadali-Cekic, G.R. Dennis, R. Bashir, P.J. Mahon, R.A. Shalliker, Ferric reducing antioxidant potential (FRAP) of antioxidants using reaction flow chromatography, Anal. Chim. Acta 967 (2017) 93–101, http://dx.doi.org/10.1016/j.aca.2017.02.032.

[15] G. Morovján, P.P. Csokán, L. Németh-Konda, HPLC determination of colistin and aminoglycoside antibiotics in feeds by post-column derivatization and fluorescence detection, Chromatographia 48 (1998) 32–36, http://dx.doi.org/10.1007/BF02467512.

[16] M. Selim, A. Soliven, M. Camenzuli, G.R. Dennis, H.J. Ritchie, R.A. Shalliker, A two-component post-column derivatisation method utilising reaction flow chromatography, Microchem. J. 116 (2014) 87–91, http://dx.doi.org/10.1016/j.microc.2014.04.005.

[17] P.G. Rigas, Post-column labeling techniques in amino acid analysis by liquid chromatography, Anal. Bioanal. Chem. 405 (2013) 7957–7992, http://dx.doi.org/10.1007/s00216-013-7127-3.

[18] Y. Cho, R. Ozeki, M. Yotsu-Yamashita, Y. Oshima, Single-cell analysis of paralytic shellfish toxins in *Alexandrium tamarense* by HPLC with post-column fluorescent derivatization, Harmful Algae 25 (2013) 47–53, http://dx.doi.org/10.1016/j.hal.2013.02.005.

[19] A.C. Manetta, L. Di Giuseppe, M. Giammarco, I. Fusaro, A. Simonella, A. Gramenzi, A. Formigoni, High-performance liquid chromatography with post-column derivatisation and fluorescence detection for sensitive determination of aflatoxin M1 in milk and cheese, J. Chromatogr. A 1083 (2005) 219–222, http://dx.doi.org/10.1016/j.chroma.2005.06.039.

[20] A. Jones, A. Acquaviva, G.R. Dennis, R. Andrew Shalliker, A. Soliven, Bioactive screening of complex tea samples using the ferric reducing antioxidant power assay incorporating reaction flow HPLC columns for post column derivatisations, Microchem. J. 138 (2018) 197–202, http://dx.doi.org/10.1016/j.microc.2018.01.005.

[21] A. Jones, S. Pravadali-Cekic, S. Hua, D. Kocic, M. Camenzuli, G.R. Dennis, R.A. Shalliker, Post column derivatization using reaction flow high performance liquid chromatography columns, J. Vis. Exp. 110 (2016) e53462, , http://dx.doi.org/10.3791/53462.

[22] H. Engelhardt, P. Ohs, Trace analysis of sugars by HPLC and post-column derivatization, Chromatographia 23 (1987) 657–662, http://dx.doi.org/10.1007/BF02311495.

[23] S. Pravadali-Cekic, A. Jones, A.A. Kazarian, B. Paull, A. Soliven, H. Ritchie, M. Camenzuli, G.R. Dennis, R. Andrew Shalliker, Using reaction flow chromatography for the analysis of amino acid: derivatisation with fluorescamine reagent, Microchem. J. 121 (2015), http://dx.doi.org/10.1016/j.microc.2015.02.010.

[24] B. Kusznierewicz, A. Piasek, A. Bartoszek, J. Namiesnik, The optimisation of analytical parameters for routine profiling of antioxidants in complex mixtures by HPLC coupled post-column derivatisation, Phytochem. Anal. 22 (2011) 392–402, http://dx.doi.org/10.1002/pca.1294.

[25] M. Camenzuli, H.J. Ritchie, G.R. Dennis, R.A. Shalliker, Reaction flow chromatography for rapid post column derivatisations: the analysis of antioxidants in natural products, J. Chromatogr. A 1303 (2013) 62–65, http://dx.doi.org/10.1016/j.chroma.2013.06.046.

[26] A. Acquaviva, A. Jones, G.R. Dennis, R. Andrew Shalliker, A. Soliven, Phenolic profiling of complex tea samples via simultaneous multiplexed detection employing reaction flow HPLC columns and colorimetric post column derivatisation, Microchem. J. (2018), http://dx.doi.org/10.1016/J.MICROC.2018.01.044.

[27] S. Pravadali-Cekic, A. Jones, A.A. Kazarian, B. Paull, A. Soliven, H. Ritchie, M. Camenzuli, L. Leung, G.R. Dennis, R.A. Shalliker, Postcolumn derivatization of amino acids using reaction flow chromatography columns with fluorescence detection: a fast new approach to selective derivatization techniques, J. Liq. Chromatogr. Relat. Technol. 40 (2017) 83–92, http://dx.doi.org/10.1080/10826076.2017.1285312.

[28] A. Jones, A. Acquaviva, G.R. Dennis, R.A. Shalliker, A. Soliven, High throughput screening of phenolic constituents in a complex sample matrix using post-column derivatisations employing reaction flow HPLC columns, Microchem. J. (2018), http://dx.doi.org/10.1016/j.microc.2018.03.026.

[29] A. Soliven, I.A. Haidar Ahmad, M.R. Filgueira, P.W. Carr, Optimization of gradient reversed phase chromatographic peak capacity for low molecular weight solutes, J. Chromatogr. A 1273 (2013), http://dx.doi.org/10.1016/j.chroma.2012.11.068.