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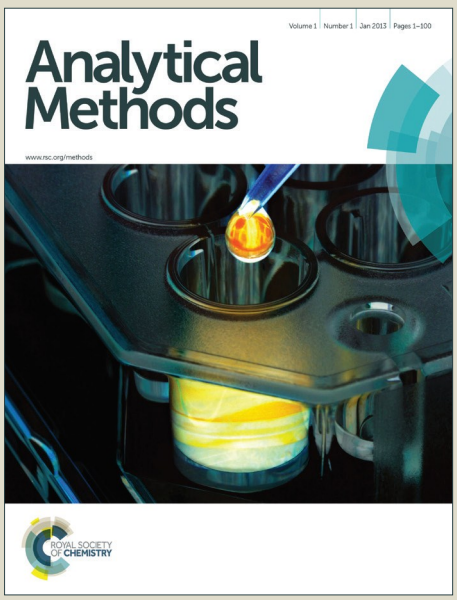

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On-column preconcentration in sequential injection chromatography: application to parabens determination

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Abstract

Solid-phase extraction is the most usual technique for analyte preconcentration prior to liquid chromatographic analysis. It involves several and time-consuming steps, including cartridges conditioning, sample loading and analytes elution. This work proposes on-column preconcentration in sequential injection chromatography by exploiting analytes retention in the head of the chromatographic column. A relatively high volume of an aqueous sample was carried to the reversed-phase column by water. After preconcentration, a suitable mobile phase was inserted to perform chromatographic separation. The feasibility of the proposed approach was demonstrated by preconcentration of parabens before chromatographic separation and UV detection. A linear response was achieved from 12.0 to 100.0 ng mL^{-1} ($r^2 > 0.998$) with detection limits estimated at 3.1, 3.3 and 4.6 ng mL^{-1} and enrichment factors of 435, 405 and 420 for metyl, ethyl, and propylparabens, respectively. Coefficients of variation for retention time and peak heights were below 2.2%. The proposed procedure can be applied to parabens extraction and preconcentration, presenting advantages as minimization of analyte losses and contamination, reduction of organic solvent consumption and avoiding additional extraction cartridges.

Keywords: Sequential injection chromatography; On-column preconcentration; Solid-phase extraction; Parabens; RP-amide.

1. Introduction

Several alternatives have been proposed to perform faster and cheaper analysis with reliability. Most of them have been focused on sample preparation, which is the most time-consuming step and requires special abilities of the analyst to minimize losses of analytes and contaminations.[1] Solid-phase extraction (SPE) is one of the most applied sample preparation techniques, especially in chromatographic procedures. The target analytes are partitioned between the sample and the solid phase and a polypropylene cartridge filled by a sorbent is commonly used. Despite its high performance for preconcentration and clean-up of complex samples, it requires several steps, as cartridge conditioning, sample loading and elution. Sometimes the process is time-consuming and not environmentally friendly by consuming a large volume of organic solvents.

Alternatives to SPE have been proposed, as the microextraction techniques. Solid-phase microextraction is usually rapid, simple, solvent-free, compatible to gas and liquid chromatography, and allow achieving high enrichment factors. However, it has some drawbacks, such as fibre fragility, stripping of coatings, instability and swelling in organic solvents, time-consuming fibre preparation and difficulties for on-line implementation.

On-column preconcentration was exploited in liquid chromatography aiming at determination of bis(2-ethylhexyl)phthalate in waters.[2] The approach is analogue to the cold trapping approach in gas chromatography. A large sample volume is injected aiming at accumulation of the analytes on the head of the chromatographic column. An appropriate eluent is then used for elution, not requiring any additional device as extraction cartridges, pumps or batch sample

pretreatment. This strategy has been also exploited in capillary liquid chromatography for determination of N-acylhomoserine lactonein bacterial isolates. Preconcentration was performed onto a laboratory-made miniaturized column by injection of 1-5 µL of sample before nano-liquid chromatography with microelectrospray-ionization ion trap mass spectrometry.[3] A similar procedure was proposed for determination of alkenylbenzenes and related flavour compounds in food samples,[4] with a relative standard deviation lower than 5%.

Sequential injection chromatography (SIC) combines the versatility of sequential injection analysis with chromatographic separations performed at low pressures with monolithic or, more recently, fused-core particle columns.[5] This approach makes feasible on-line solution handling, including sample clean-up and analyte preconcentration by SPE.[6] The fundamentals and recent applications of SIC were revised[7] and lately on-column analyte stacking was exploited to diminish peak broadening in the separation of metal ions in ion-chromatography by changing pH before sample loading.[8] In this work, on-line SPE was expanded by on-column preconcentration, being the performance evaluated for trapping and separation of a mixture of parabens.

2. Experimental

2.1. Apparatus

A SIChrom™ equipment (FIAlab Instruments®, Bellevue, WA, USA) with an S17 PDP syringe pump (Sapphire™ Engineering, MA, USA) with a 4.0 mL reservoir and an 8-port high-pressure stainless-steel selection C5H valve (Valco Instrument Co., Houston, TX, USA) were used to develop the present work. All

tubes were of PEEK with 0.25 mm i.d.. The detection system was composed by a multi-channel CCD spectrophotometer (model USB4000, Ocean Optics®, Dunedin, FL, USA), with a deuterium light source (model DH-2000, Ocean Optics®) and optical fibers with a core diameter of 600 µm. The spectrophotometer was coupled to a 9-µL Z-flow cell with 20-mm optical path (FIAlab Instruments®, Bellevue, WA, USA). The SIC system was controlled by a microcomputer equipped with FIAlab® 5.9 software (FIAlab® Instruments®). The chromatographic separations were performed on a fused-core RP-amide column (Ascentis Express® Supelco, 30 x 4.6 mm, 2.7 µm). During the chromatographic separations the SIC system pressure was monitored by a manometer.

2.2. Reagents and solutions

Methyl (MP), ethyl (EP) and propyl (PP) parabens were purchased from Sigma Aldrich. Stock 1.000 g L^{-1} solutions were prepared in methanol and stored at 5ºC. Working solutions were daily prepared by dilutions in water. An acetonitrile:phosphoric acid solution, pH 2.5 (25:75) was used as mobile phase.

2.3. Procedure

The routine described in Table 1 was used in the SIC system (Fig. 1) to perform the on-column preconcentration of the parabens. Initially the pump sequentially aspirated the water carrier (S1) and sample (S2) to the holding coil (C). The aqueous phase was introduced into the chromatographic column. Then, the pump aspired the mobile phase (S3) and dispensed it through the fused-core particle column for separation and detection of the parabens at

255 nm. Measurements were based on peak heights and carried out in triplicate. All experiments were performed at room temperature (25 ºC).

3. Results and discussions

In liquid chromatography, sensitivity is often hindered by reducing the injected volume to improve resolution. Thus, a preconcentration step is usually required for trace analysis. This is often performed in batch, being prone to systematic errors and contaminations; it is also expensive and generates large amounts of wastes due to the use of disposable extraction devices and organic solvents. Some of these hindrances can be circumvented by on-line preconcentration, but this requires a relatively more complex system.[6]

On-column preconcentration did not require any additional device but its implementation in HPLC is limited by the available sample loops. They are usually in the range 0.1 to 100 µL, although 2-mL loops are available from some manufacturers. Another strategy is the continuous pumping of the sample placed in one of the solvent vessels of the high pressure pump,[2] which critically decrease the sample throughput because of the time elapsed for sample replacement and increase the risks of cross-contamination. These drawbacks have restricted the adoption of this strategy[2,3] in spite of its potential for analyte preconcentration.

SIC exploits a highly reproducible time-based sampling, which allows variation of the injected volume by the software control. Large sample volumes can be stored in a holding coil before delivering to the chromatographic column by a suitable solvent. Analytes with high retention factors (k>20 for practical purposes)[9] will be retained in a narrow zone at the head of the chromatographic

column. A suitable mobile phase can provide the chromatographic separation without hinder the resolution. The approach, which is illustrated in Figure 2, was evaluated by taking parabens as model compounds. The RP-amide fused-core column acted as both sorbent for SPE and as media for chromatographic separation. This stationary phase is composed by an amide linked to an alkyl group, which has high affinity by parabens molecules, making possible its extraction and preconcentration from water samples. A previous work demonstrated the suitability of this stationary phase for parabens separation.[10] The higher affinity of this phase by the analytes may result in high enrichment factors and low peak broadening in on-column preconcentration.

As presented in Table 2, no significant peak broadening was observed by increasing the injected volumes, which indicates that the affinity of parabens by the RP-amide phase is high enough to form a narrow zone in the head of the column. The retention times increased only slightly and the resolution was not hindered even for the highest injected volume (5000 μL). It should be emphasized that this volume is significantly higher than the column dead volume (*ca.* 280 μL), thus hindering the chromatographic separation. This effect is more critical due to the short length of the fused-core particle column (3 cm).

The efficiency of the on-column preconcentration was also observed when the injected volume was increased by keeping the analyte mass constant (Figure 3). Neither peak heights nor peak width varied significantly, confirming the absence of peaks broadening for high sample volumes. Moreover, this indicates the potential of the proposal to increase detectability. Retention times had the same behaviour previously described. The spurious signal observed for

tr < 1.0 min are due to refractive index differences caused by changing the water by the mobile phase (Schlieren effect).

For a 5000-µL injected volume, a linear response was observed from 12.0 to 100.0 ng mL^{-1} and the main analytical features are presented in Table 3. The coefficient of variation for the retention times and peak heights were relatively low, indicating good repeatability of the procedure and absence of memory effects. This point was a concern in view of the absence of an equilibrium stage between mobile and stationary phases. Enrichments factors were estimated between 405 and 435 from the ratio of the slopes of the calibration curves obtained with 5000 (Table 3) and 10 µL (2.0×10^{-5} L μg^{-1} for MP and EP, and 9.0×10^{-6} L μg^{-1} for PP) injection volumes. This corresponds to relatively high concentration efficiencies (from 48.6 to 50.4 min^{-1}),[11] *i.e.* an enrichment factor of at least 48 is achieved in 1 min by the proposed procedure, which is significantly higher than the attained in batch and even in flow-based procedures. On the other hand, low consumptive indexes [11] were achieved (from 0.0144 from 0.0123 mL), which demonstrates a high efficiency of sample utilization. Compared to some on-column preconcentration procedures (Table 4)[2,9,12-14] the present proposal has remarkable advantages as easy solution manipulation by using the selector valve and variation of the injection volume by time-based sampling. Moreover, in HPLC and electrophoresis the injection volume is limited by the sample loop or by the small capillary dimensions, respectively, and both presented worst precision. In addition, the proposed procedure is able to obtain calibration curves by the use of a single concentration solution, exploring the programmable routine of the SIC system to change the injected volume of standard solutions. When this approach was

evaluated, the slopes of analytical curves (Absorbance x analyte mass) obtained for methyl, ethyl and propylparabens were 0.0017, 0.0016 and 0.0012 L μg^{-1} and 0.0015, 0.0016 and 0.0012 L μg^{-1} by changing the analyte concentration or injection volume, respectively.

4. Conclusions

On-column preconcentration was pioneering implemented in sequential injection chromatography, solving the problems observed for adoption of this strategy in HPLC. The approach yielded reproducible extractions without peak broadening and with high enrichment factors, as demonstrated for parabens preconcentration and separation. Other advantages compared to conventional SPE are the low-cost, no need of disposable cartridges and lower consumption of sample and organic solvents. Differently of conventional SPE, neither flushing nor column conditioning were required in the proposed approach. The procedure can be optimized for extraction of different analytes by choosing suitable stationary phases and sample solvents. On the other hand, sample clean-up is critical for complex matrices aiming at obtaining reliable results and avoiding damage of the chromatographic column.

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Table 1 Steps of the SIC control program for on-column preconcentration and separation of parabens.

Action	**Unit**	**Parameter**
	Selection Valve	Valve port 3
Aspiration of water	Pump	Volume: 500 µL / Flow rate: 50 (µL s^{-1})
	Selection Valve	Valve port 4
Aspiration of sample	Pump	Volume: 5000 µL / Flow rate: 50 (µL s^{-1})
	Selection Valve	Valve port 2
Dispense of sample and water	Pump	Volume: 5500 µL / Flow rate: 10 (µL s^{-1})
	Selection Valve	Valve port 5
Aspiration of mobile phase	Pump	Volume: 3300 µL / Flow rate: 50 (µL s^{-1})
	Selection Valve	Valve port 2
Dispense of mobile phase	Pump	Volume: 3300 µL / Flow rate: 10 (µL s^{-1})

Table 2 Influence of sample volume in the retention time and peak width in the parabens chromatographic separation after on-column preconcentration.

Sample Volume (µL)	Retention time (min)			Peak width (min)		
	MP	**EP**	**PP**	**MP**	**EP**	**PP**
10	1.6	2.5	4.1	0.10	0.14	0.21
50	1.7	2.6	4.3	0.11	0.15	0.24
100	1.6	2.4	4.1	0.09	0.13	0.22
200	1.6	2.5	4.2	0.10	0.13	0.23
300	1.7	2.6	4.5	0.10	0.15	0.25
400	1.8	2.7	4.6	0.11	0.14	0.24
500	1.8	2.8	4.7	0.12	0.15	0.25
1000	1.6	2.3	4.7	0.11	0.14	0.22
2000	1.6	2.4	4.7	0.11	0.14	0.22
3000	1.6	2.3	4.6	0.12	0.14	0.25
4000	1.6	2.4	4.7	0.13	0.15	0.27
5000	1.6	2.3	4.6	0.13	0.15	0.28

Table 3 Analytical features of the procedure of on-column preconcentration of parabens (Sample volume = 5000 µL)

	Equation	R^2	LOD (ng mL^{-1})	CV Retention time (%)	CV Peak height (%)	Enrichment factor
MP	A = 0.0087C + 0.0091	0.999	3.1	0.53	1.30	435
EP	A = 0.0081C + 0.0103	0.999	3.3	0.60	1.52	405
PP	A = 0.0058C + 0.0200	0.998	4.6	0.71	2.12	420

A: Absorbance and C: concentration in µg L^{-1}, CV: coefficient of variation (n=10)

Table 4 Analytical figures of merit of some on-column preconcentration procedures by different separation techniques

Procedure	Analytes	Sample volume (mL)	LOD (ng mL^{-1})	CV (%)	Remarks	Ref.
HPLC	Bis(2-ethylhexyl) phthalate	10	0.1	6.0	Sample loaded as a mobile phase	2
HPLC	Chlorophenols	1.2	< 0.0013	< 2.5	Multiple injections	6
CE	3-nitrotyrosine	not informed	995.0	4.2	Preconcentration chamber in the electrophoretic capillary	9
HPLC	Cd^{2+}, Hg^{2+} and Pb^{2+}	2.5	not informed	< 3.4	New HPLC column based on tetranitrocalix[4]arene appended silica	10
MEKC	Anti-Inflammatory Drugs	not informed	< 3.3	<0.60	Sample loaded as a mobile phase	11
SIC	Parabens	5.0	< 4.6	< 2.1	High versatility for changing the injection volume	This work

MEKC: micellar electrokinetic capillary chromatography; CE: capillary electrophoresis,

Figure Captions

Fig. 1 Diagram of the SIC system on-column preconcentration. S1: water; S2: sample; S3: mobile phase; CC: chromatographic column; D: detector; SV: selector valve; SP: syringe pump; C: holding coil; M: manometer; W: waste.

Fig. 2 Schematic representation of the on-column preconcentration: (A) sample load; (B) trapping of the analytes at the head of the chromatographic column and (C) chromatographic separation.

Fig. 3 Influence of the injected sample volume in separation by keeping the parabens mass constant at 500 ng.

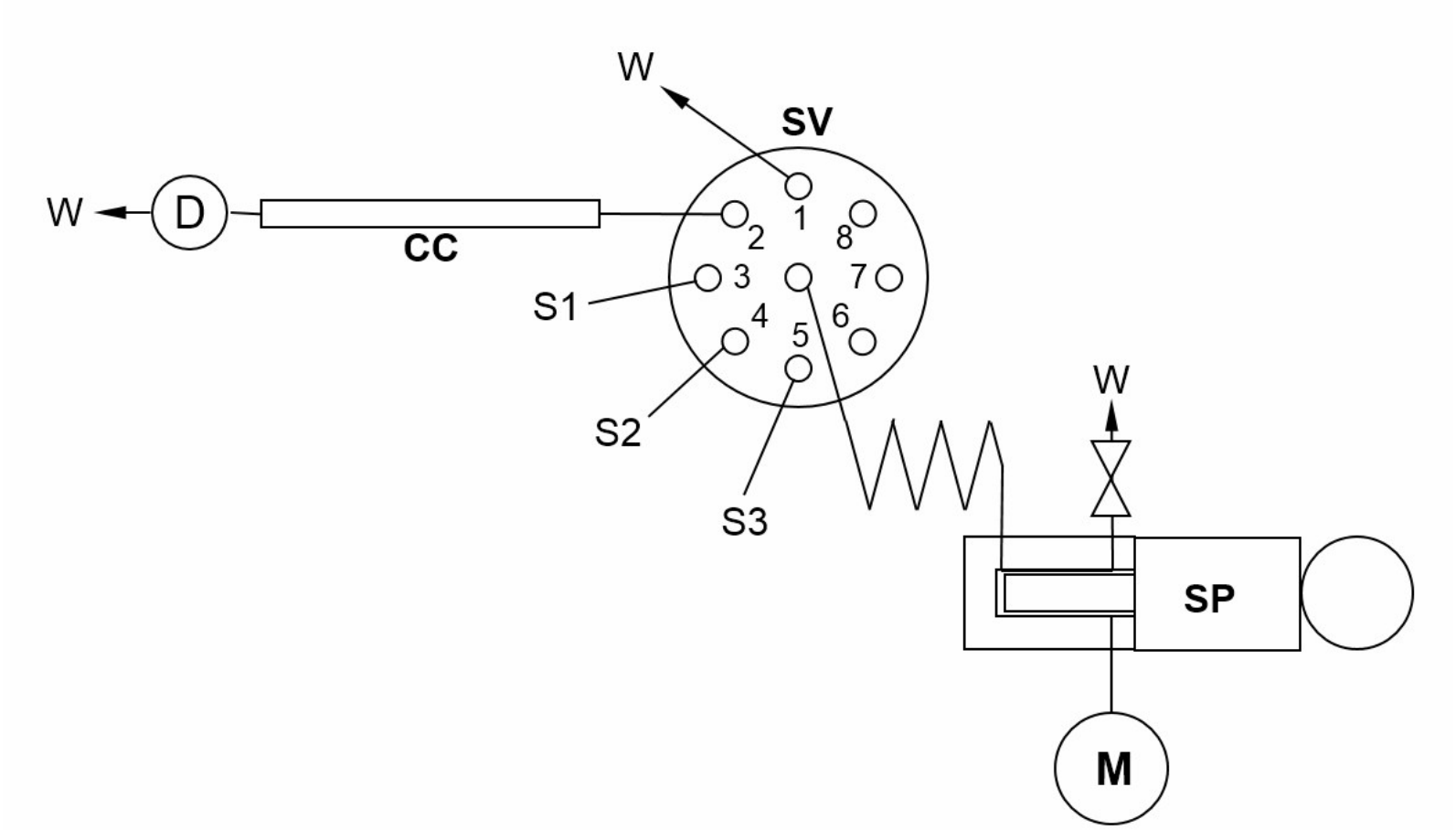

Figure 1

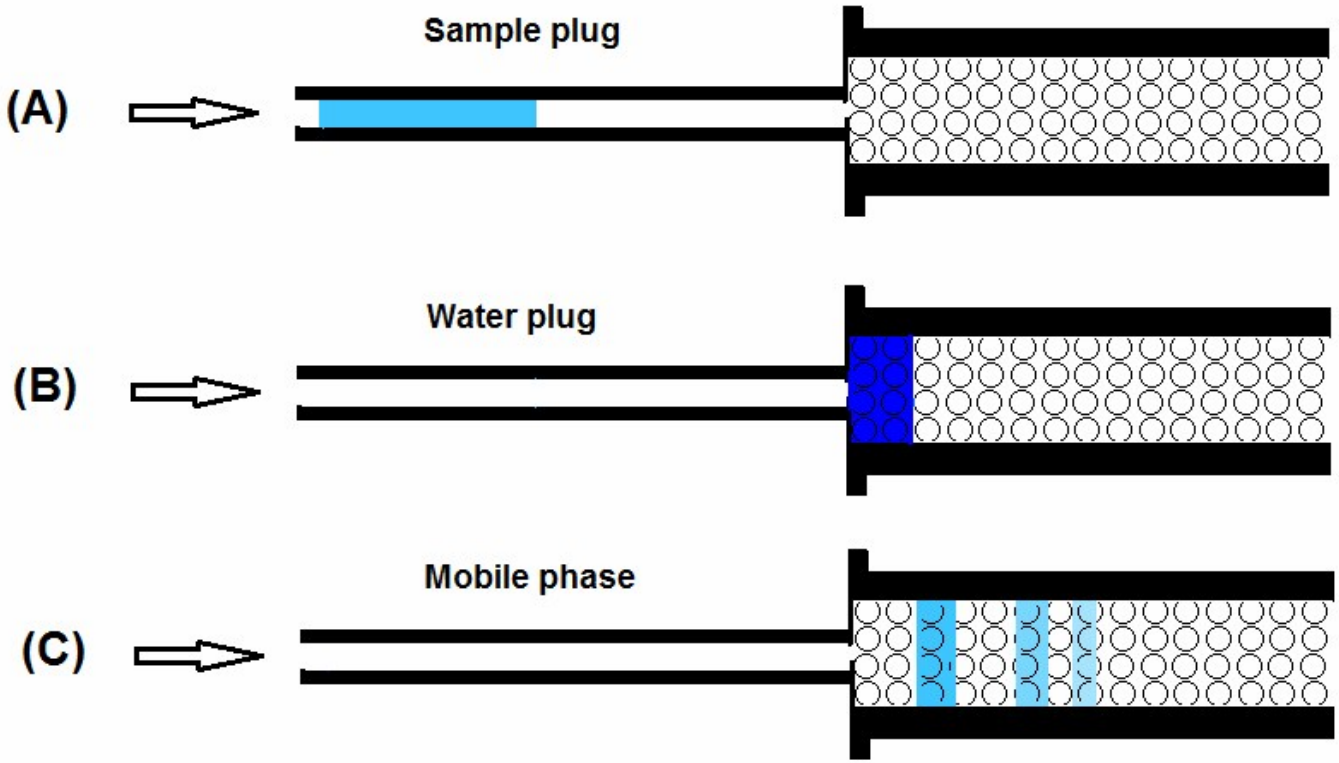

Figure 2

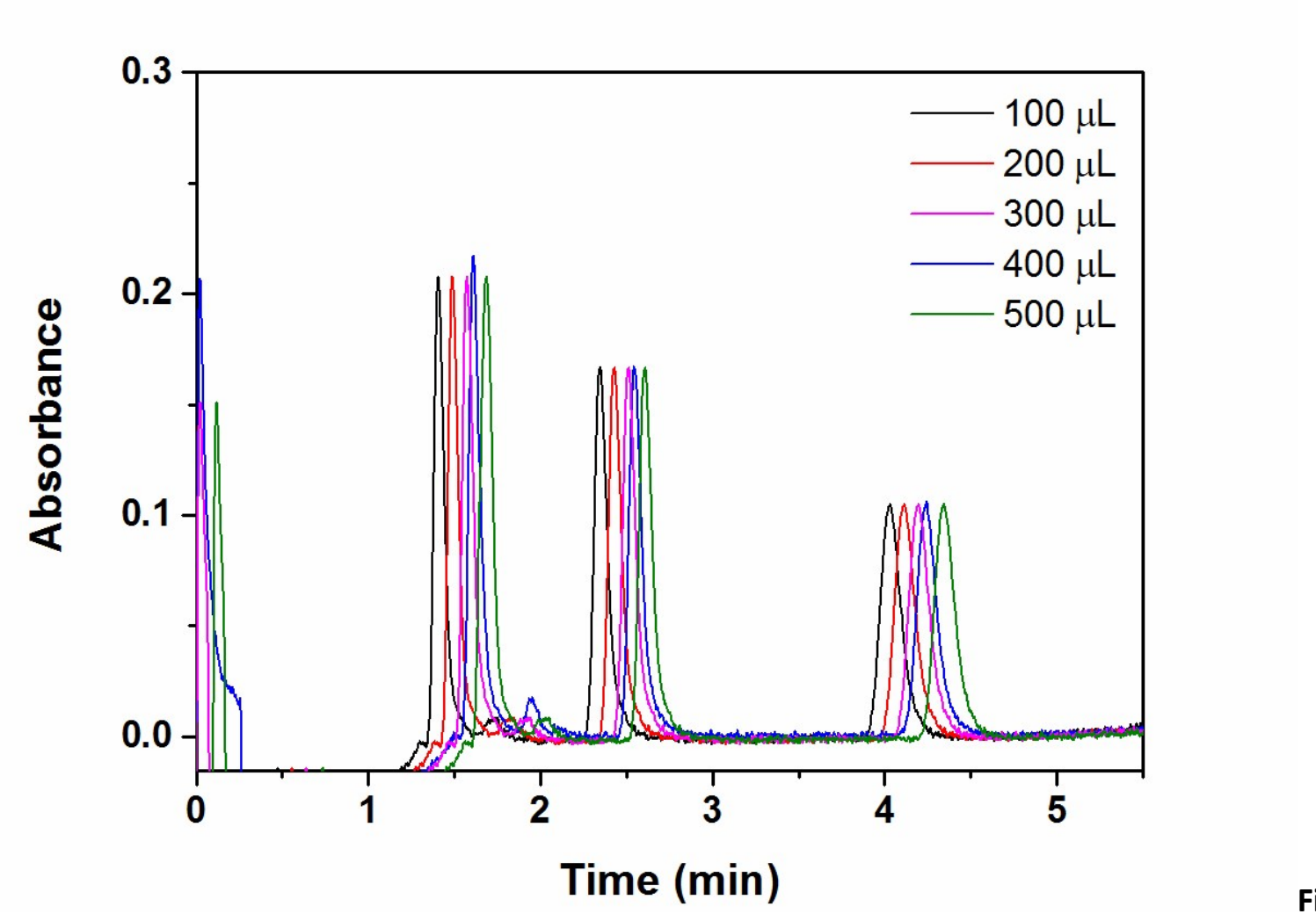

Figure 3