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Chemiluminescence detection of heroin in illicit drug samples

Jessica M. Terry [a], Zoe M. Smith [a], Jessica J. Learey [a], R. Andrew Shalliker [b], Neil W. Barnett [a], Paul S. Francis [a,*]

[a] Centre for Chemistry and Biotechnology, Faculty of Science, Engineering and Built Environment, Deakin University, Geelong, Victoria, Australia
[b] Australian Centre for Research on Separation Science (ACROSS), School of Science and Health, University of Western Sydney (Parramatta), Sydney, NSW, Australia

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ABSTRACT

Heroin (3,6-diacetylmorphine) and several important extraction and synthesis impurities (morphine, 6-monoacetylmorphine, codeine and 6-acetylcodeine) were determined in illicit drug samples, using high performance liquid chromatography with 'parallel segmented flow', which enabled the simultaneous use of three complementary modes of detection (UV-absorbance, tris(2,2′-bipyridine)ruthenium (III) chemiluminescence and permanganate chemiluminescence). This rapid and sensitive approach for the analysis of street heroin was used to explore the chemistry of a proposed heroin screening test that is based on the relative response with these two chemiluminescence reagents using flow injection analysis. Although heroin was the major constituent of the six drug samples (between 16% and 67% by mass), the synthetic by-product 6-acetylcodeine (2.5–8.3%) made a greater contribution to the total $[Ru(bipy)_3]^{3+}$ chemiluminescence response of the screening test. The signal with permanganate was primarily due to the presence of 6-monoacetylmorphine (0.9–29%), and was therefore indicative of the degree of sample degradation during clandestine manufacture or poor storage conditions prior to the drug seizure. In the second part of the screening test, the sample is treated with sodium hydroxide, which results in a large increase in the signal with permanganate, due to the rapid hydrolysis of heroin to 6-monoacetylmorphine. As the emission of these two reagents with morphinan-alkaloids and their derivatives largely depends on the substituent at the O^3 position, the slower hydrolysis of 6-monoacetylmorphine to morphine, and 6-acetylcodeine to codeine, did not have a major impact on the characteristic pattern of responses in the screening test.

1. Introduction

The widespread illicit production and trafficking of heroin (3,6-diacetylmorphine) has created the need to identify and/or quantify the drug (and its precursors and derivatives) in suspected drug samples seized by law enforcement officials. Screening tests are often used to obtain rapid preliminary identification of drugs prior to confirmatory testing with more time-consuming and expensive instrumental methods [1]. The most commonly used screening method for opiates and their derivatives involves mixing samples with Mandelin, Marquis or Mecke reagent(s) and observing the resultant colour change. Our research group has demonstrated an alternative test for heroin [2,3] that exploits the complementary selectivity of two well-known chemiluminescence reagents: tris (2,2′-bipyridine)ruthenium(III) ($[Ru(bipy)_3]^{3+}$) [4] and potassium permanganate [5,6], using flow injection analysis [2] or sequential injection analysis [3] methodology. The chemical basis for the test involves two stages: in the first, the presence of heroin is indicated by a strong signal with $[Ru(bipy)_3]^{3+}$. The sample is then treated with a sodium hydroxide solution to hydrolyse the heroin to 6-monoacetylmorphine (6-MAM) and/or morphine, which produce strong signals with permanganate (Fig. S1, Electronic Supplementary information) [2,3]. Some common tertiary amines (such as codeine, strychnine and chloroquine) give false positives with the first reagent, but they do not produce the markedly increased response with the permanganate reagent after the hydrolysis procedure, thus providing a rapid and unambiguous (albeit qualitative) test for the target drug [2,3].

In our previously published screening test for heroin, the $[Ru(bipy)_3]^{3+}$ reagent was prepared by chemical oxidation of $[Ru(bipy)_3]^{2+}$, either with solid PbO_2 in acidic aqueous solution immediately prior to use [2,3], or with Cl_2 gas, in an initial procedure used to create $[Ru(bipy)_3](ClO_4)_3$, which was dried and then dissolved in acetonitrile [2]. The anhydrous reagent exhibited much greater stability and gave more intense and reproducible signals with heroin [2] and other species [7], but it

* Correspondence to: Centre for Chemistry and Biotechnology, Faculty of Science, Engineering and Built Environment, Deakin University, Geelong Waurn Ponds Campus, Locked Bag 20000, Geelong, VIC 3220, Australia. Tel.: +61 3 5227 1294; fax: +61 3 5227 1040.
E-mail address: paul.francis@deakin.edu.au (P.S. Francis).

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was inconvenient to prepare and its use was hindered by batch-to-batch variation. To address this, we devised a simple procedure to prepare a stable anhydrous reagent, utilising PbO_2 to oxidise $[Ru(bipy)_3](ClO_4)_2$ in acetonitrile containing 0.05 M $HClO_4$ [7]. Furthermore, we have improved the stability and sensitivity of the permanganate reagent through an initial partial reduction of the oxidant with sodium thiosulfate [8].

Herein, we apply these enhanced chemiluminescence systems and UV-absorbance detection to HPLC for the rapid quantitative determination of heroin and related species in illicit drug samples seized by police. The detectors are coupled to a new HPLC column technology that has been referred to as parallel segmented flow (PSF) [9–11], in which greater separation efficiency is achieved because the radial central flow region is separated from the wall flow region, overcoming the limitations of HPLC associated with column bed heterogeneity. This column technology provides a suitable platform for multiplexed detection since the outlet fitting has multiple exit ports; in effect, the sample band can be divided into four portions and sent to separate, independent detectors [12]. The ratio of flow through any one of these exit ports can be controlled by the differential pressure at each port, providing simultaneous analysis in a way that until now has not been feasible using conventional approaches. We previously presented a preliminary exploration of PSF for multiplexed detection using the separation of six opium poppy alkaloids coupled with UV-absorbance and chemiluminescence detection as a model system [12]. In this study, we adapt the procedure for the analysis of illicit drug samples, which provides not only a rapid quantitative test of multiple opiates within these samples, but also the opportunity to further explore the fundamental parameters of the PSF approach, and examine the chemistry of the chemiluminescence screening test to understand the observed discrepancies between intensities obtained with each reagent using pure heroin standards and illicit drug samples.

2. Materials and methods

2.1. Chemicals and reagents

Codeine, morphine, oripavine and thebaine were provided by GlaxoSmithKline (Vic., Australia). Heroin (3,6-diacetylmorphine), 6-MAM and 6-acetylcodeine were obtained from the National Measurement Institute (NSW, Australia). Seized drug samples were provided by the Australian Federal Police. All other chemicals were sourced as previously described [12]. Stock solutions of the opiate alkaloids (1 mM) were prepared in acidified deionised water. Heroin (1 mM) was prepared in 0.1% (v/v) acetic acid and diluted in 0.05% (v/v) acetic acid. Stock solutions of the drug samples were prepared by dissolving 15 mg of the solid material into 50 mL of 0.1% (v/v) acetic acid. The 'non-hydrolysed' samples were prepared by taking 1 mL of stock solution (heroin or the drug sample) and diluting to 100 mL (for HPLC experiments) or 200 mL (for FIA experiments) with a 0.05% (v/v) acetic acid solution. The 'hydrolysed' samples were prepared by mixing 1 mL of stock solution with 100 μL of sodium hydroxide (1.0 M) and then diluting the mixture to 100 mL or 200 mL with the 0.05% (v/v) acetic acid solution.

The permanganate reagent was prepared by dissolving 1.9 mM $KMnO_4$ in 1% (m/v) sodium polyphosphate and adjusting to pH 2.5 with H_2SO_4 and then adding 0.6 mM $Na_2S_2O_3$, using a small volume of a 0.1 M $Na_2S_2O_3$ solution [8]. The tris(2,2′-bipyridine) ruthenium(III) reagent was prepared as previously described [7]. This involved treating $[Ru(bipy)_3]Cl_2$ with $NaClO_4$ in aqueous solution to yield a bright orange $[Ru(bipy)_3](ClO_4)_2$ precipitate, which was collected by vacuum filtration, washed twice with ice water, and dried over P_4O_{10} for 24 h. The reagent was then prepared by oxidising the $[Ru(bipy)_3](ClO_4)_2$ crystals (1 mM) with PbO_2 (0.2 g/100 mL) in acetonitrile containing 0.05 M $HClO_4$, which was observed as a change in the colour of the solution from orange to blue-green. The excess solid oxidant left in the reagent was prevented from entering the chemiluminescence detector by a filter (consisting of a small Pasteur pipette packed tightly with glass wool) fitted to the end of the tubing in the reagent reservoir.

2.2. Flow injection analysis (FIA)

The FIA manifold was constructed as previously described [13], incorporating a GloCel chemiluminescence detector (Global FIA, WA, USA) with a Teflon dual-inlet serpentine-channel flow-cell [14,15] and an extended-range photomultiplier module (model P30A-05; ETP, NSW, Australia). All tubing entering and exiting the detector was black PTFE (0.76 mm i.d., Global FIA). The output signal from the detector was recorded with an e-corder 410 data acquisition system (eDAQ, NSW, Australia). The analytes (5×10^{-6} M) were injected (70 μL) into an aqueous carrier stream (adjusted to pH 2.5 with trifluoroacetic acid, 3.5 mL min^{-1}) that merged with the chemiluminescence reagent (3.5 mL min^{-1}) in the reaction channel of the flow-cell.

2.3. High performance liquid chromatography (HPLC)

Analyses were carried out on an Agilent Technologies 1260 series liquid chromatography system with an injection volume of 20 μL, flow rate of 2.5 mL min^{-1}, and gradient elution using deionised water adjusted to pH 2.5 with trifluoroacetic acid (solvent A) and methanol (solvent B) as follows: 0–2 min: 7–21% B, 2–10 min: 21% B, 10–12 min: 21–7% B, and 12–14 min: 7% B (unless otherwise stated).

2.4. Multiplexed detection

Separations were performed using a reversed-phase Hypersil GOLD chromatography column (100 mm × 4.6 mm i.d., 5 μm) with a parallel segmented flow end-fitting (ThermoFisher Scientific, Cheshire, UK). The column eluate (total flow rate: 2.5 mL min^{-1}) was divided in the following manner: 32% (0.8 mL min^{-1}) was directed to the first chemiluminescence detector (permanganate reagent) via peripheral port 1, 32% (0.8 mL min^{-1}) to the second chemiluminescence detector ($[Ru(bipy)_3]^{3+}$ reagent) via peripheral port 2, 24% (0.6 mL min^{-1}) to the UV-absorbance detector (280 nm) via peripheral port 3; and the remaining 12% (0.3 mL min^{-1}) to a collection vessel via the central port. For permanganate chemiluminescence, we used a GloCel detector with dual-inlet serpentine-channel flow-cell (fabricated from Teflon impregnated with glass microspheres [14,15]) and an extended-range photomultiplier module (model P30A-05; ETP). For $[Ru(bipy)_3]^{3+}$ chemiluminescence, we used a GloCel detector with dual-inlet serpentine flow-cell (Teflon) and 9282SB photomultiplier tube (ETP) powered by an external high-voltage power supply (PM20D, ETP) set at 0.9 kV. The reagents were pumped to the detectors at 1 mL min^{-1} using Dual Piston Pumps (Series 12 × 6, model D05PFD01; Scientific Systems, PA, USA).

2.5. Conventional detection

For comparison purposes, separations were also performed with a Hypersil GOLD column with a conventional outlet fitting, which was connected to each detector individually, using the same tubing and detectors as described above.

3. Results and discussion

3.1. Preliminary experiments

Preliminary FIA experiments were conducted to examine the response for heroin, prepared with and without the hydrolysis step, upon reaction with the two enhanced chemiluminescence reagents [7,8]. In accordance with previous observations [2], a large response was obtained from the reaction of heroin with $[Ru(bipy)_3]^{3+}$ and a negligible response with permanganate (Fig. 1a). When incorporating the hydrolysis step, the response was lower with $[Ru(bipy)_3]^{3+}$, but much greater with permanganate, due to the conversion of heroin into 6-MAM and/or morphine (Fig. S1) [2,3]. Six street drug samples known to contain heroin were obtained from the Australian Federal Police and tested. For each sample, the hydrolysis step produced the characteristic changes in the chemiluminescence intensities, signifying the presence of heroin (Fig. 1b and c). The differences in response between the non-hydrolysed and hydrolysed samples with the $[Ru(bipy)_3]^{3+}$ reagent were generally smaller than those observed for the heroin standard. This has previously been ascribed to the presence of non-phenolic alkaloids and/or tertiary amine cutting agents that produce light with this reagent but are not readily hydrolysed [2]. The differences in response between the non-hydrolysed and hydrolysed samples with the permanganate were also smaller in some cases (e.g. sample 2 in Fig. 1c).

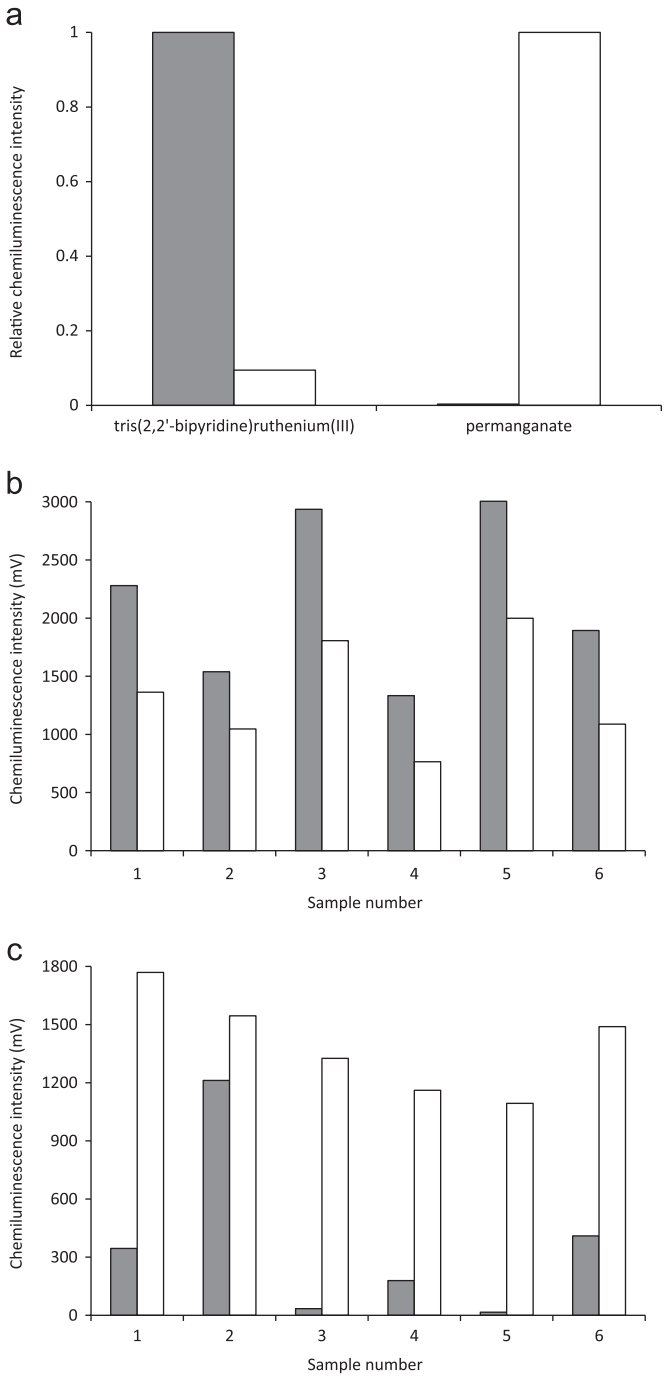

Fig. 1. Chemiluminescence responses for the non-hydrolysed (grey columns) and hydrolysed (white columns) samples, using flow injection analysis methodology: (a) heroin standard with both reagents; (b) drug samples with the $[Ru(bipy)_3]^{3+}$ reagent; and (c) drug samples with the permanganate reagent.

3.2. Separation conditions

We initially examined the street drug samples using the parallel segmented flow separation procedure described in our previous publication [12], which was developed using a mixture of opiate alkaloid standards, containing morphine, codeine, 6-MAM, oripavine, thebaine, heroin and papaverine. Under these conditions, the chromatograms for each of the three modes of detection contained several major peaks (Fig. 2). As expected, a large peak for heroin was observed in the unhydrolysed sample with $[Ru(bipy)_3]^{3+}$ chemiluminescence detection (Fig. 2b), and significant peaks corresponding to 6-MAM and morphine were observed with permanganate chemiluminescence detection after heroin was hydrolysed (Fig. 2c). However, comparison of the three simultaneous modes of detection revealed a major underlying problem with the separation (that could not be readily identified by inspection of any individual mode of detection): when the hydrolysis step was included, the apparent heroin peak was completely removed from the UV-absorbance chromatograms, but only partially removed from the $[Ru(bipy)_3]^{3+}$ chemiluminescence chromatograms (and a new peak appeared at 2.7 min). Furthermore, using a series of calibration standards, the concentration of heroin in the unhydrolysed sample appeared to be much greater when using $[Ru(bipy)_3]^{3+}$ detection than UV-absorbance detection.

These apparent discrepancies pointed to the presence of an interferent that: (i) co-elutes with heroin under these separation conditions; (ii) may be partially hydrolysed in alkaline solution to form a new peak that elutes at 2.7 min; and (iii) either has a much lower absorbance (at 280 nm) than heroin or elicits a much greater chemiluminescence response with $[Ru(bipy)_3]^{3+}$ than heroin. Considering these characteristics and the possible constituents of street heroin samples [2,16], the interferent was assumed to be 6-acetylcodeine, which was subsequently verified by comparing the elution time of a standard solution. The peak at 2.7 min was therefore codeine (for which a similar elution time was obtained in our previous study [12]), generated by partial hydrolysis of 6-acetylcodeine.

Based on molecular structure, we would expect that 6-acetylcodeine would have a similar absorbance to heroin at 280 nm. The only previous report of chemiluminescence from the reaction of 6-acetylcodeine with $[Ru(bipy)_3]^{3+}$ was a visual observation of an intense emission [17], but considering that codeine elicits a much greater response than heroin with $[Ru(bipy)_3]^{3+}$ [12], it is reasonable to assume that 6-acetylcodeine also elicits a larger chemiluminescence response than heroin with this reagent. Thus, at relatively low concentrations in the sample (compared to heroin), 6-acetylcodeine and codeine produced major peaks in the $[Ru(bipy)_3]^{3+}$ chromatogram, but not in the UV-absorbance chromatogram.

To resolve each of the peaks of interest, we modified the solvent gradient programme to that described in the experimental section. In addition, we replaced the peristaltic pumps (that were

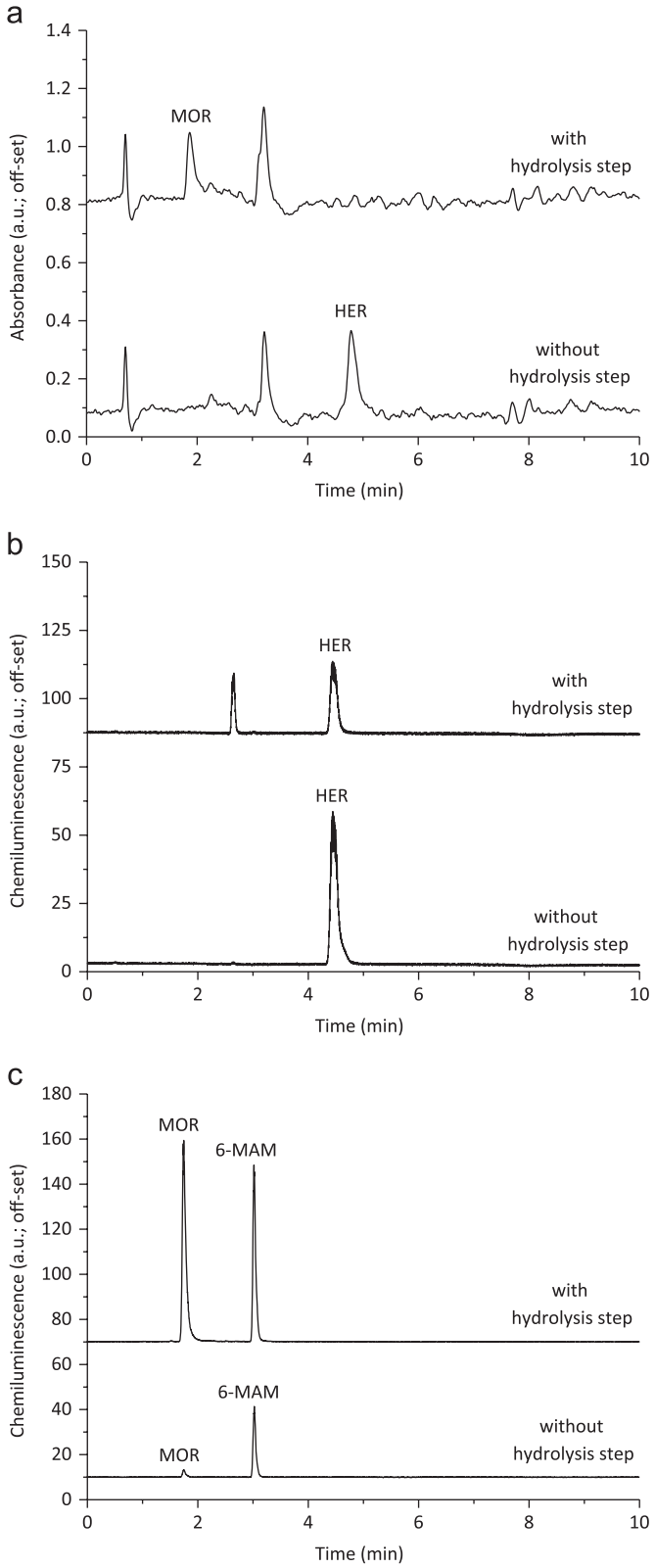

Fig. 2. Chromatograms for illicit drug sample 4 (prepared with and without the hydrolysis step of the heroin screening test) under the separation conditions described in our previous publication [12] using the PSF approach with (a) UV-absorbance (280 nm), (b) $[Ru(bipy)_3]^{3+}$ chemiluminescence and (c) permanganate chemiluminescence detection systems. Peaks: MOR: morphine, HER: heroin, and 6-MAM: 6-monoacetylmorphine.

used to deliver the chemiluminescence reagents) with dual-piston pumps that provided a smoother flow of solution (product specification: pulsation of $\pm 0.5\%$ at 1 mL min^{-1} and 1000 psi) to improve the stability of the chemiluminescence baseline signals. We also replaced the coiled-tubing flow-cell (previously used for $[Ru(bipy)_3]^{3+}$ chemiluminescence detection [12]) with a GloCel detector that contained a dual-inlet serpentine-channel, which enabled mixing of column eluate and reagent directly in front of the photodetector (as in the permanganate chemiluminescence detector). Under these conditions, we obtained baseline resolution of seven compounds of interest (morphine, codeine, oripavine, 6-MAM, thebaine, 6-acetylcodeine and heroin) within 10 min (Fig. 3).

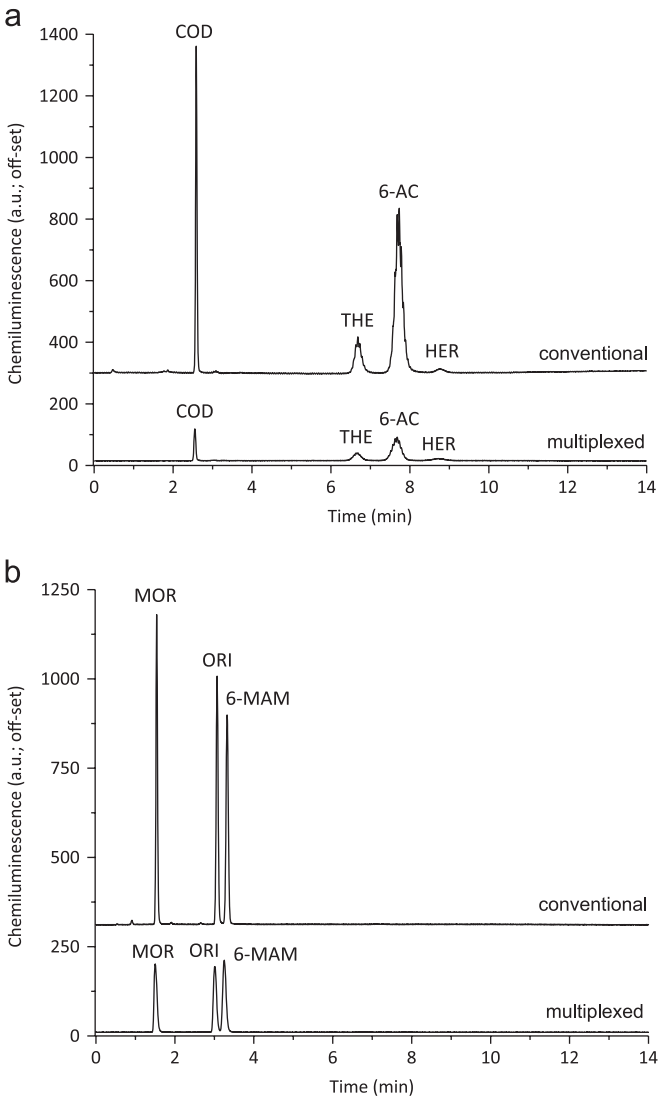

Fig. 3. Modified HPLC separation (conditions as described in the experimental section) of heroin (5×10^{-6} M) and related alkaloids and semi-synthetic derivatives (1×10^{-6} M), using conventional and PSF column technology for individual and multiplexed detection with (a) $[Ru(bipy)_3]^{3+}$ chemiluminescence and/or (b) permanganate chemiluminescence. In both cases, the mobile phase flow rate through the column is 2.5 mL min^{-1}. In the conventional system, the eluate flow rate to the detector is 2.5 mL min^{-1}, but in the PSF system, the eluate flow rate to each chemiluminescence detector is 0.8 mL min^{-1}. Peaks: COD: codeine, THE: thebaine, 6-AC: 6-acetylcodeine, HER: heroin, MOR: morphine, ORI: oripavine, and 6-MAM: 6-monoacetylmorphine.

3.3. Comparison of multiplexed and conventional detection

Splitting the flow to multiple detectors has two major effects: firstly, the number of solute molecules entering each detector is reduced; and secondly, the eluate flow rate through each detector is lowered. As discussed in our previous work [12], the effect of these characteristics of the PSF approach on the peaks in the UV-absorbance chromatograms is quite predictable, but their influence on any particular chemiluminescence detection system is less foreseeable, due to the dependence of the emission intensity on the kinetics of the light-producing chemical reactions, and the transient nature of the emission. To extend this comparison, we ran the conventional approach with each chemiluminescence detection system at mobile phase flow rates of 0.8, 1.0 and 2.5 mL min^{-1}. The fastest of these flow rates was at the same linear velocity as utilised in the PSF system (providing the same separation time), whilst the slowest flow rate gave equivalent solute-molecule residence times in each detector to that of the PSF system (albeit with a much longer separation time of 35 min). Unlike UV-absorbance detection, where the peak areas increase as the mobile phase flow-rate is lowered (due to the increase in the solute residence time in the detector), the chemiluminescence peak areas generally decreased as the flow rate was lowered from 2.5 to 0.8 mL min^{-1} (Fig. S2). However, as the optimum chemiluminescence reagent conditions are somewhat analyte-dependent [4,5], the influence of mobile-phase flow rate varied markedly, which is most evident here by the contrasting increase in the $[Ru(bipy)_3]^{3+}$ chemiluminescence peak areas for heroin as the mobile phase flow rate was lowered from 2.5 to 0.8 mL min^{-1} (Fig. S3). When splitting the flow in the PSF approach, the effect on chemiluminescence peak areas can therefore be broken down into: (i) a highly analyte-dependent change in peak area due to the decrease in volumetric flow rate delivered to the detector, and (ii) a decrease in all peak areas due to the lower number of solute molecules distributed to the detector, approximately proportional to the volumetric distribution of the eluate. Consequently, the decrease in chemiluminescence peak areas when applying the PSF approach (under the same separation conditions and mobile phase flow rate) ranged from a factor of 1.5 for heroin to 7.8 for codeine. Nevertheless, as shown in our previous work [12], the limits of detection for the phenolic morphinan alkaloids with permanganate chemiluminescence (e.g. 1×10^{-9} M morphine) and for the non-phenolic analogues with $[Ru(bipy)_3]^{3+}$ chemiluminescence (e.g. 1×10^{-8} M codeine and 5×10^{-8} M heroin) using the multiplexed approach are still superior to those for the same analytes with UV-absorbance detection using the conventional separation, by up to two orders of magnitude.

3.4. Heroin in street drug samples

The six drug samples (prepared with and without the hydrolysis procedure) and a series of calibration standards were examined under the separation conditions described above using multiplexed detection (Fig. 4). The $[Ru(bipy)_3]^{3+}$ mode of detection, which is sensitive towards non-phenolic derivatives of morphine, showed the presence of small quantities of codeine in all samples (Fig. 4a). Peaks corresponding to possible constituents known to produce a significant emission with $[Ru(bipy)_3]^{3+}$ (such as strychnine and chloroquine [2,18]) were not observed in the chromatograms for these samples. The percentage mass of each of the identified species in these drug samples is shown in Table 1. The purity of these samples is typical for illicit heroin [19,20]. The relatively low quantity of heroin in sample 2 was found to be a result of significant degradation of the drug during the clandestine manufacture and/or storage conditions. In comparison, the low quantity of heroin in sample 6 resulted from a combination of degradation and a greater non-alkaloid component. Very little degradation of heroin to 6-MAM and morphine (and 6-acetylcodeine to codeine) had occurred in samples 3 and 5 (Table 1).

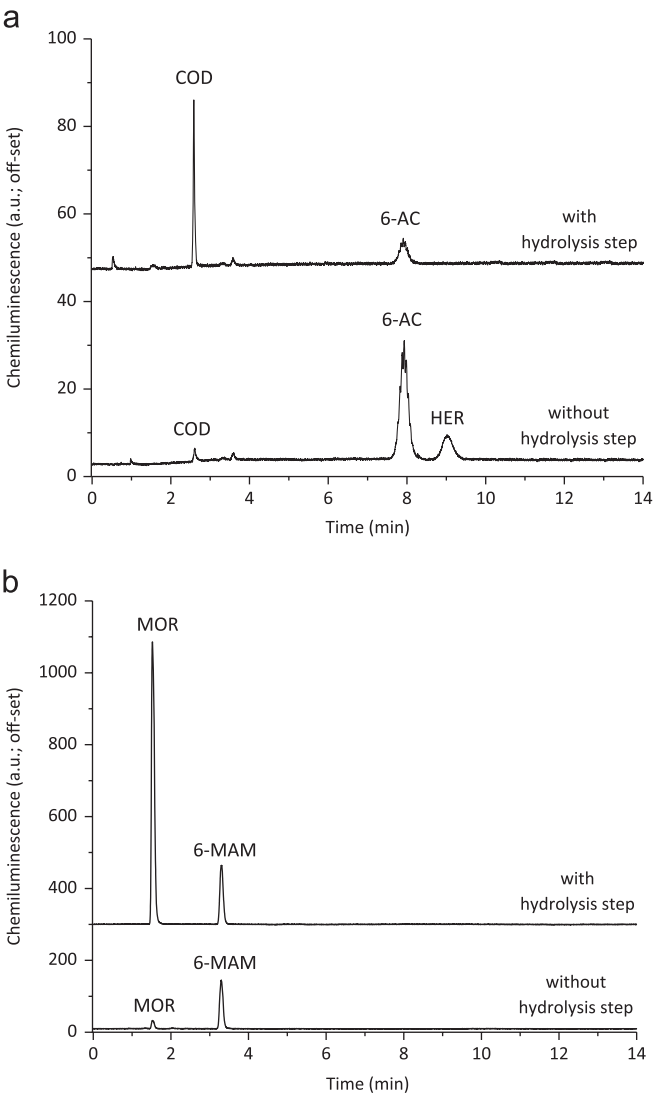

Fig. 4. Chromatograms for illicit drug sample 4 (prepared with and without the hydrolysis step) under the modified separation conditions, using the PSF column technology with (a) $[Ru(bipy)_3]^{3+}$ chemiluminescence and (b) permanganate chemiluminescence detection systems. Peaks: COD: codeine, 6-AC: 6-acetylcodeine, HER: heroin, MOR: morphine, and 6-MAM: 6-monoacetylmorphine.

Table 1
Concentration (% mass) in drug seizure samples using HPLC with multiplexed detection.

Sample	Heroin	6-MAM	Morphine	6-Acetylcodeine	Codeine
1	67	9.3	2.1	4.7	0.27
2	16	29	10	2.5	1.03
3	58	0.93	0.25	6.8	0.09
4	57	8.1	1.4	3.9	0.29
5	50	1.1	0.22	8.3	0.13
6	35	8.1	1.5	2.9	0.18

3.5. Chemistry of the chemiluminescence screening test for heroin

Comparison of the chromatograms of the six drug samples showed that the alkaline hydrolysis step of the screening test

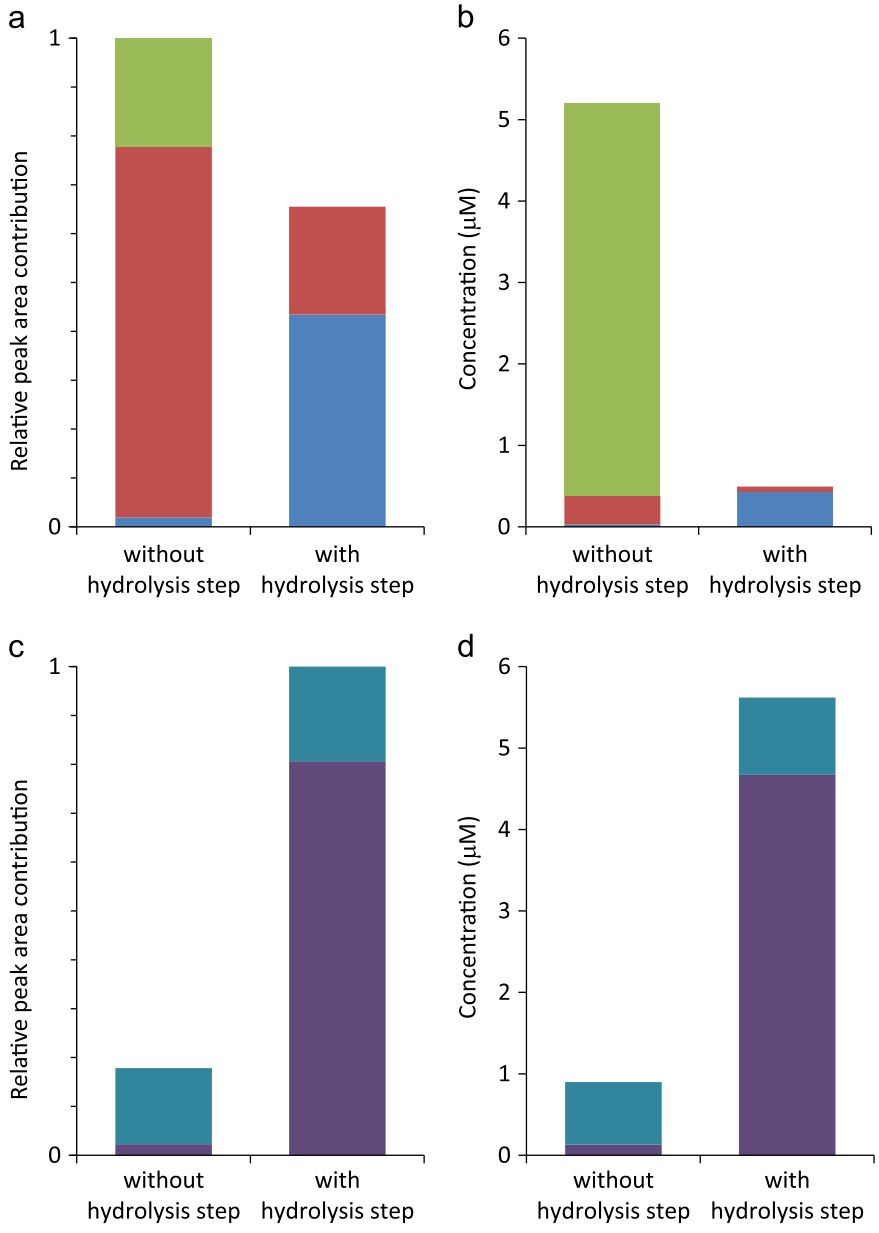

Fig. 5. Contribution to the (a) peak area and (b) concentration of heroin (green), 6-acetylcodeine (red) and codeine (blue) in the $[Ru(bipy)_3]^{3+}$ chemiluminescence chromatogram for drug sample 4 without and with the hydrolysis step. Contribution to the (c) peak area and (d) concentration of 6-MAM (aqua) and morphine (purple) in the permanganate chemiluminescence chromatogram for drug sample 4 without and with the hydrolysis step.

induced a complete conversion of heroin to 6-MAM, and an incomplete (81–86%) conversion of 6-MAM to morphine (including the 6-MAM formed from heroin and that already contained in the sample). The closely related hydrolysis of 6-acetylcodeine to codeine proceeded to a similar extent (78–86%). The total peak areas of the major alkaloids and semi-synthetic derivatives in $[Ru(bipy)_3]^{3+}$ and permanganate chemiluminescence chromatograms were in reasonable agreement with the relative intensities of the FIA screening test (correlation of 0.91 and 0.99 for $[Ru(bipy)_3]^{3+}$ and permanganate chemiluminescence without the hydrolysis step; and 0.80 and 0.92 with the hydrolysis step). Discrepancies between the two approaches were attributed to the differences in instrumental and chemical conditions (such as methanol in the mobile phase, which is known to affect permanganate chemiluminescence intensity [5], the measurement of peak height in FIA and peak area in HPLC, and the higher total flow rate of the FIA procedure) in addition to contributions from traces of other alkaloids, and enhancing or inhibiting effects of concomitant species in the FIA sample zone compared to the resolved solutes of the HPLC separation.

Although heroin was by far the dominant species contained in the samples in terms of concentration, its chemiluminescence response is poorer than 6-acetylcodeine with $[Ru(bipy)_3]^{3+}$, and the HPLC data showed (Fig. 4a) that in spite of its 5- to 14-fold lower concentration, 6-acetylcodeine made a larger contribution to the chemiluminescence response with $[Ru(bipy)_3]^{3+}$ in the FIA screening test (Fig. 5a). Moreover the signal for the hydrolysed sample with $[Ru(bipy)_3]^{3+}$ primarily arose from the presence of codeine and some remaining 6-acetylcodeine that was not hydrolysed (Figs. 5a and 1b: white columns, which are much larger than that observed after hydrolysis of a heroin standard).

The chromatograms obtained using permanganate chemiluminescence (e.g. Fig. 4b) showed that the signals with this reagent in the FIA screening test (for samples prepared without the

hydrolysis step) were almost entirely due to the presence of 6-MAM and small quantities of morphine in the sample, rather than cutting agents, and therefore this signal was a good indicator of the degree of sample degradation (compare Table 1 and Fig. 1c, grey columns). As described above, the introduction of the hydrolysis step led to a much greater total peak area for 6-MAM and morphine (Fig. 4b), due to the deacetylation of heroin and 6-MAM.

4. Conclusions

PSF enables the controlled distribution of column eluate to multiple detectors. The simultaneous collection of the response from multiple complementary detection systems not only increases the effective peak capacity of the separation, but also enables comparison of chromatograms without the variation in solute concentration and retention times of replicate injections and separations. Moreover, the different relative responses of analytes in the alternative modes of detection can be exploited to identify hidden, co-eluting peaks. In the case of chemiluminescence, the reduced flow rate to each detector when splitting the column eluate may compromise the detection conditions. However, the optimum flow rate is analyte-dependent, and in the case of heroin, the lower flow rate partially offsets the effect of the reduced number of solute molecules.

The PSF approach with chemiluminescence detection enabled highly sensitive and selective determination of heroin and related sample constituents separated in 10 min, which was successfully applied to the analysis of street drug samples. We also applied the same procedure to explore the chemistry of the previously published chemiluminescence screening test for heroin. Although heroin was the major constituent of these six samples (without inclusion of the hydrolysis step), 6-acetylcodeine made a greater contribution to the $[Ru(bipy)_3]^{3+}$ chemiluminescence response. However, as this interferent is a by-product of heroin synthesis due to the presence of codeine in the opium poppy extract, the goal of this part of the two-phase screening test is still served. The relative signals of the hydrolysed sample with $[Ru(bipy)_3]^{3+}$ were primarily influenced by the presence of 6-acetylcodeine (and its incomplete hydrolysis to codeine). The base-catalysed hydrolysis of heroin to morphine was shown to account for the large increase in signal with permanganate when the sample was treated with sodium hydroxide. The relative signals of the unhydrolysed sample with permanganate was determined by 6-MAM (and to a much lesser extent morphine) present in the sample powder.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.07.051.

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