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Review

Liquid chromatography coupled with inductively coupled plasma mass spectrometry in the pharmaceutical industry: selected examples

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Abstract

Both LC and capillary LC (CapLC) have been successfully interfaced with inductively coupled plasma mass spectrometry (ICP-MS). Gradients of acetonitrile and aqueous based solvents have been employed to separate several compounds of pharmaceutical interest. This paper will describe four application areas in the pharmaceutical industry, and examples will be shown where CapLC, LC and gel electrophoresis via laser ablation have been coupled with ICP-MS. The four areas highlighted in this paper are: (1) the use of derivatisation reactions to "make the invisible visible". Methods involving derivatisations with copper and iron will be described that can be used for the analysis of amines and carboxylic acids by ICP-MS. (2) The profiling of metal ion content (in particular bromine) in biological samples such as human plasma, this study will focus on the metabolism of bromine-labelled peptides (e.g. substance P). (3) The analysis of materials derived from single, solid-phase beads used in combinatorial chemistry, and (4) also discussed will be our findings from investigations into the use of laser ablation ICP-MS on the determination of protein phosphorylation on electrophoresis gel blots.
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Keywords: Inductively coupled plasma mass spectrometry (ICP-MS); Derivatisation; Substance P

Contents

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1. Introduction

The use of ICP-MS in the biomedical arena has grown enormously in recent years and will continue to do so, particularly in the area of metal concentration determinations in protein samples for the proteomics field.

ICP-MS has been coupled to all forms of chromatographic techniques for speciation analyses, including gas chromatography [1], supercritical fluid chromatography [2] and capillary electrophoresis [3]. However, it is only in the last 10 years that liquid chromatography coupled to ICP-MS has been routinely used. The early studies focused mainly on speciation of inorganic compounds using primarily ion-exchange chromatography and size-exclusion chromatography [4,5]. Recently, ICP-MS has been coupled to normal phase chromatography and reversed-phase chromatography [6–9]. One of the main drawbacks was the limitation of the ICP in handling organic solvents with the standard nebulisers. Microconcentric or ultrasonic nebulisers [10] have been introduced which are able to decrease the amount of organic solvent in the aerosol reaching the plasma and thereby minimising plasma interference and changes in the ionisation characteristics of the plasma. Also, the addition of small amounts of oxygen to the argon carrier improves the combustion of the organic solvent in the plasma and thereby reduces carbon deposition on the interface.

Now gradients of acetonitrile and aqueous based solvents, LC or capillary LC (CapLC) coupled to ICP-MS and/or an orthogonal acceleration time-of-flight mass spectrometer (oa-TOF-MS) can be routinely used for separating and identifying pharmaceutical interesting compounds. This approach enables information to be obtained concerning the structure or molecular weight of the compound as well as providing a generic method for profiling, identifying and quantifying the compounds containing the selected element(s) in complex mixtures.

One of the first reports employing this approach was by Nicholson et al. [9], which described the profiling and quantification of metabolites of 4-bromoaniline in rat urine. This was followed by a similar study [11] that provided the simultaneous detection of the metabolites of 2-bromo-4-trifluoromethyl-[^{13}C]-acetanilide in rat urine by ICP-MS and orthogonal acceleration time-of-flight mass spectrometry (oa-TOF-MS). The metabolites present in the sample were separated by reversed-phased LC with the bulk of the eluent (90%) being directed to an ICP-MS instrument where bromine-containing metabolites were detected and quantified using ICP-MS. The minor portion of the eluent (10%) was taken for oa-TOF-MS for identification of the metabolites.

A similar simultaneous detection approach was utilised for our studies on bradykinin metabolism in human and rat plasma [12,13]. In these studies, the bradykinin was bromine-labelled to enable detection and quantification by ICP-MS. A further enhancement of this approach was afforded by Axelsson et al. [14] who combined ICP-MS with accurate mass measurement of their organic pharmaceutical compounds.

One of the earliest reports of coupling CapLC to ICP-MS was made by Tangen et al. [15] in 1997. However, more recent and noteworthy additions [16,17] of relevance to the pharmaceutical industry have been in the area of protein phosphorylation determination. The first of these two papers [16] reported the use of CapLC and ICP-MS for the identification of phosphopeptides and was followed by measurements of the degree of phosphorylation in phosphoproteins and phosphopeptides containing cysteine and/or methionine residues [17]. Many papers on speciation studies of proteins have followed this important study, not just of phosphoproteins [18–24] but for example selenium-containing proteins [25,26] and metallothioneins [27–31].

In this paper, we shall describe some selected examples of LC coupled with ICP-MS in the pharmaceutical industry.

2. Experimental

2.1. Chemicals and reagents

Carboxylic acids, organic amines, 2-chloro-1-methylpyridium iodide (CMPI), triethylamine (TEA), copper sulphate, sodium bicarbonate, Earle's balanced salt solution (EBSS), HEPES, polyethylene glycol and trifluoroacetic acid were all purchased from Sigma-Aldrich (Poole, UK). The cyclic diethyltriamine pentaacetic acid anhydride (cDTPAA) and the synthetic brominated substance P were synthesised in-house. Formic acid was obtained from BDH Laboratory Supplies (Poole, UK) and LC grade acetonitrile from Fisher Scientific UK (Loughborough, UK). The ultrapure water (Milli-Q quality 18 MΩ/cm) was produced from an Elga (High Wycombe, UK) water purification system.

2.2. Derivatisation of amines

The test amines used in this study, *n*-octylamine and benzylamine were prepared in acetonitrile at a concentration of 1 mM.

The essence of the derivatisation method was to react each amine with cyclic diethyltriamine pentaacetic acid anhydride

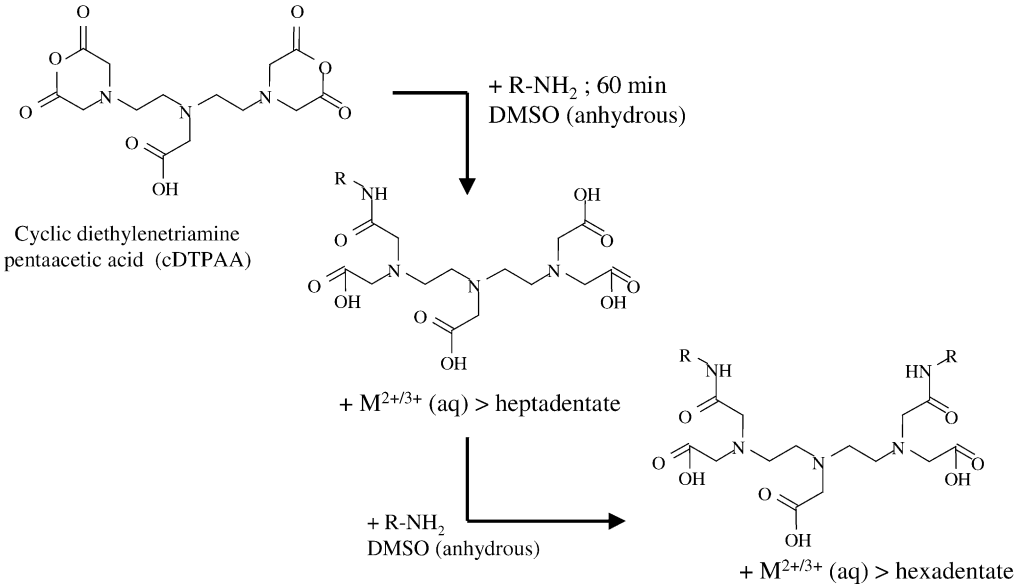


Fig. 1. Reaction scheme for the reaction of cyclic diethyltriamine pentaacetic acid anhydride (cDTPAA) followed by chelation with a metal ion.

(cDTPAA) followed by chelation with a metal ion (in this instance, copper).

The reagents were aqueous solutions of copper sulphate (10 mM), sodium bicarbonate (100 mM) and cDTPAA, which was prepared at 1 mM in anhydrous DMSO.

The derivatisation method was as follows: To 100 μL of cDTPAA and 100 μL of test amine was added 800 μL of sodium bicarbonate solution, mixed thoroughly, and allowed to react at room temperature for 60 min. A 180 μL aliquot of the reaction mixture was removed to a new tube, to which was added 20 μL of 10 mM copper sulphate solution. The contents of the tube were mixed and allowed to react (room temperature) for 30 min prior to analysis. The reaction scheme is shown in Fig. 1.

2.3. Derivatisation of carboxylic acids with N-(2-aminoethyl) ferrocene carboxamide

The test carboxylic acids used in this study, phenylacetic acid and octanoic acid and the reagent *N*-(aminoethyl)ferrocene carboxamide were prepared in acetonitrile at a concentration of 1 mM. The activating reagent, 2-chloro-1-methylpyridinium iodide (CMPI)/triethylamine, was prepared by dissolving 37.98 mg of CMPI in approximately 20 mL of acetonitrile in a 25 mL volumetric flask, adding 41.4 μL of TEA and making up to volume with further acetonitrile.

The derivatisation method was as follows: To 100 μL of the carboxylic acid was added 100 μL of the CMPI/TEA reagent and allowed to react for 5 min, prior to the addition of 100 μL of the ferrocene carboxamide. The resulting solution was thoroughly vortexed and placed in an ultrasonic bath for 30 min. The reaction mixture was diluted 1:100 with water/acetonitrile (90:10 containing 0.05% (v/v) formic acid) prior to analysis by CapLC–ICP-MS. The reaction mechanism is described in Fig. 2.

The analysis of the derivatised amines and carboxylic acids was achieved by CapLC–ICP-MS. The LC was performed on a Waters CapLC™ system using a Waters Symmetry® C18, 3.5 μm, 0.32 mm × 100 mm column (Waters, Elstree, UK). Gradient elution was afforded with mobile phases comprising of Solvent A, water containing 0.05% formic acid (v/v); Solvent B, acetonitrile containing 0.05% formic acid (v/v) at a flow rate of 10 μL min^{-1}. A post-column addition of 50 μL min^{-1} of a 30% acetonitrile (aq) (v/v) solution via a T-piece was made to enhance the nebulisation process.

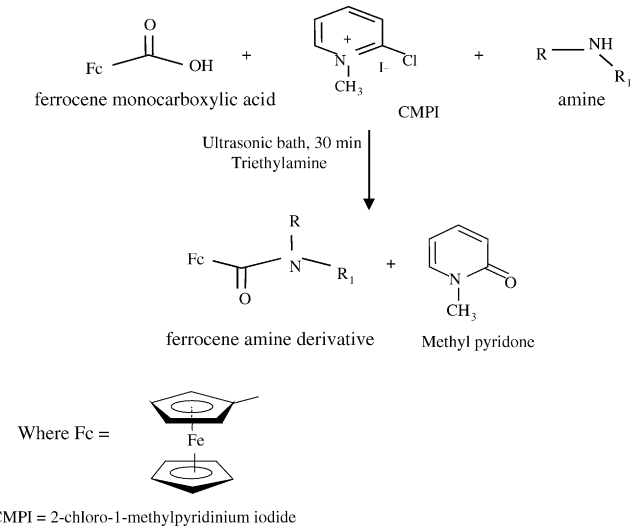


Fig. 2. Reaction scheme for the derivatisation of amines with ferrocene monocarboxylic acid.

The ICP-MS analyses were carried out on a GV Instruments Platform-Life ICP-MS instrument (GV Instruments, Wythenshawe, UK). Some of the key operational parameters are: forward power, 1500 W; cool gas, 16.0 L min^{-1}; intermediate gas, 1.20 L min^{-1}; nebuliser gas, 0.80 L min^{-1}; nebuliser, MCN; He hexapole gas, 4.0 mL min^{-1}, H hexapole gas, 4.0 mL min^{-1}; and isotopes monitored were ^{54}Fe, ^{56}Fe, ^{63}Cu and ^{65}Cu.

2.4. Analysis of brominated substance P metabolism by LC with ICP-MS and oa-TOF-MS

The incubation procedure used was a modified version of that described by Dendorfer et al. [32]. Briefly, human plasma (33 μL) was added to an incubation buffer (final volume = 1.1 mL) which contained 10 μM brominated substance P. The buffer solution consisted of 50 mM HEPES, 150 mM NaCl and 1 μM Zn^{2+}. The incubations were performed at 37 °C in the buffer solution, supplemented (9:1 ratio) with EBSS.

At appropriate time points, aliquots (110 μL) from the incubations were taken, supplemented with trifluoroacetic acid (2.2 μL) and centrifuged for 15 min in a chilled unit (4 °C) at 2000 × *g*. The samples were maintained at 4 °C and analysed as soon as practicable on the day of incubation.

The samples were analysed on an Agilent 1100 LC system with UV diode array detection (UV DAD) (Agilent Technologies, Bracknell, UK). Sample injection (typically 5 or 10 μL) was achieved from a CTC Analytic HTS PAL autosampler (CTC Analytic HG, Zwingen, Switzerland).

The eluent from the outlet of the LC–UV DAD system was split equally (1:1, v/v) by way of a T-piece. One outlet of the T-piece was connected to the inlet of a Micromass LCT orthogonal acceleration time-of-flight mass spectrometer (oa-TOF-MS) (Waters, Manchester, UK) and the other outlet of the T-piece was connected to the nebuliser of the Aridus desolvating sample introduction system (Cetac Technologies, Omaha, NE, USA). The Aridus system provided the sample introduction to the argon plasma of the GV Platform ICP-Life inductively coupled plasma mass spectrometer. The membrane desolvator and spray chamber heater of the Aridus system were set at 160 and 75 °C, respectively, and a sweep gas of 3.5 L min^{-1} argon was used.

The LC was achieved on a Water Xterra^{TM}MS C_{18} 150 mm × 2.1 mm column (Waters, Elstree, UK) with elution (0.4 mL min^{-1}) using a linear gradient. The gradient was composed of 0–2 min, 100% A; 2–10 min, 0-50% B; 10–10.5 min, 50% B and re-equilibrate in 100% A for 2.5 min; where Solvent A consisted of 0.1% formic acid (aq) and Solvent B was acetonitrile containing 0.1% formic acid.

The operating conditions for the ICP-MS were plasma power 1500 W; cooling gas, 16.0 L min^{-1}; plasma gas, 0.94 L min^{-1}; nebuliser gas, 0.98 L min^{-1}; acquisition mode, selected ion recording (SIR); masses monitored 79 and 81; dwell time, 0.2 s; analysis time, 13 min.

The conditions for the oa-TOF-MS, equipped with a Z-spray ion source in positive ion mode electrospray were, capillary voltage for sample ionisation was set to 3.0 kV and the sample cone voltage was maintained at 25 V for all of the experiments. The source and desolvation temperatures were set to 100 and 250 °C, respectively. The desolvation gas flow rate was set to 650 L h^{-1}. The instrument was tuned and calibrated over the range 100–1300 Da using a reference sample of poly(ethylene glycol) (PEG; 300 + 600 + 1000) prior to all LC–MS experiments.

2.5. Single bead analysis in solid phase chemistry by LC with ICP-MS and oa-TOF

The synthesis of the construct resin is described elsewhere [33]. The LC separations were performed on Waters CapLC^{TM} system (Waters, Elstree, UK). Sample injection (typically 4 μL) was achieved from a CTC Analytic HTS PAL autosampler. The LC was achieved on a Zorbax C_{18}SB 5 cm × 0.32 mm column (Microtech Scientific, Orange, CA, USA) with elution (25 μL min^{-1}) using linear gradient. The gradient was composed of 0–0.5 min, 95% A; 0.5–7.0 min, 5–100% B; 7–7.5 min, 100% B and re-equilibrate in 95% A for 2.5 min; Solvent A consisted of 0.1% formic acid (aq) and Solvent B was 95% acetonitrile containing 0.1% formic acid (aq). The Aridus system, using the above conditions, provided the sample introduction into the ICP-MS.

The operating conditions for the GV Platform Life ICP-MS were plasma power 1150 W; cooling gas, 12.87 L min^{-1}; plasma gas, 1.05 L min^{-1}; nebuliser gas, 0.92 L min^{-1}; acquisition mode, selected ion recording; masses monitored 79 and 81; dwell time, 0.2 s; analysis time, 10 min. The conditions for the Micromass oa-TOF-MS were as described earlier.

2.6. Laser ablation ICP-MS

The electrophoresis gel blots were prepared as described in [34]. Prior to analysis, the gel blot strips were cut as appropriate (to fit into the ablation cell), stuck to a glass slide and placed in the ablation cell.

The laser used in this study was an UP213 Nd:YAG 213 nm UV laser manufactured by Merchantek Products and on-loan from New Wave Research (Ramsey, UK). The laser optics allows variation of the energy density delivered to the sample surface, which can be used for the fine control of the amount of ablated material. Software controlled movement of the sample cell relative to the laser provides spatial control of sampling. The diameter of the laser spot could be set from 10 to 300 μm. Gas control valves automatically control the carrier gas through the sample cell to the ICP-MS. Sampling can be by single or multi-spot analysis, straight-line scans and rastering.

Typical laser ablation parameters used were:

- Laser output: 213 nm Flat-top beam;
- Energy density @ sample: >27 J/cm^2.

	Pre-ablation conditions	Ablation conditions
Scan speed (μm/s)	100	60
Passes	2	1
Laser output (%)	40	55
Rep rate (Hz)	20	20
Sampling size (μm)	140	120
Argon carrier gas flow rate (L min^{-1})	1	1

The operating conditions for the ICP-MS were as follows: plasma power, 1350 W; cool gas, 16.00 L min^{-1}; plasma gas, 1.11 L min^{-1}; nebuliser gas, 1.0 L min^{-1}; He hexapole gas, 0.7 mL min^{-1}; H hexapole gas, 4.3 mL min^{-1}; acquisition mode, SIR; masses monitored, 31 and 13; dwell time, 0.2 s.

3. Results and discussion

3.1. Derivatisation of amines

The use of chemical derivatisation to improve the detection of low molecular weight compounds has been well documented. However, the use of chemical derivatisation to transform poorly or non-ionisable analytes easily detectable by mass spectrometry is not so common. The development of novel derivatisation methods and reagents has been the subject of several publications by our group [34–36]. The chemical addition of metals to a target functional group within a molecule makes the analysis of the derivative amenable to ICP-MS.

Simple methods for the derivatisation of primary amines and carboxylic acids have been developed and are described below. The methods are all pre-column reactions that make them suitable for LC–ICP-MS or as in this instance, CapLC–ICP-MS analysis.

By LC–ESI(+)-MS a 1 mM solution of benzylamine or octylamine could not be detected (data not shown). However, Fig. 3 shows the CapLC–ICP-MS chromatogram (monitoring the sum of copper isotopes at ^{63}Cu and ^{65}Cu) for the derivatisation of octylamine with cDTPAA followed by chelation with copper. Octylamine in its derivatised form can be seen clearly (retention time 12.72 min).

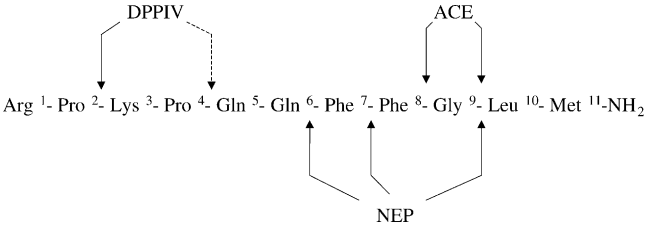
3.2. Derivatisation of carboxylic acids

Fig. 4 displays the CapLC–ICP-MS chromatogram (monitoring for the sum of the iron isotopes at ^{54}Fe and ^{56}Fe) for the derivatised forms of phenylacetic and octanoic acids with *N*-(2-aminoethyl) ferrocene carboxamide (retention times 12.83 min and 13.46 min, respectively).

The experiments described show that the derivatisation of amines and carboxylic acids with ferrocenes is quick and simple using these methods and can facilitate their detection. The products are amenable to LC and make them fully compatible for ICP-MS analysis.

3.3. Substance P metabolism in human plasma

Substance P is metabolised by several enzymes known as kininases as illustrated below:



These include ACE (angiotensin I converting enzyme), NEP (neutral endopeptidase) and DPPIV (dipeptidyl peptidase IV). ACE produces the 1,8-SubP and 1,9-SubP fragments by cleaving substance P at the 8–9 and 9–10 positions. Neutral endopeptidase produces the 1,6-SubP, 1,7-SubP and 1,9-SubP fragments by cleaving at the 6–7, 7–8 and 9–10 positions, respectively. DPPIV produces 1,2-SubP followed by the production of 3,4-SubP by cleaving at the 2–3 and then the 4–5 positions.

We utilised a new analytical method [12,13] that combines LC on-line with ICP-MS and oa-TOF-MS to investigate the metabolism of brominated substance P (BrSubP) in incubations containing human plasma. The substance P substrate utilised was brominated on the phenylalanine (*para* position) at position 8, i.e.

Arg-Pro-Lys-Pro-Gln-Gln-Phe-(*p*-Br)Phe-Gly-Leu-Met-NH$_2$

Fig. 5 depicts the LC–ICP-MS chromatogram (monitoring the sum of the bromine isotopes at ^{79}Br and ^{81}Br) obtained for the incubation of brominated substance P in human plasma after 4 h. Some 14 metabolite peaks can be seen and the structures of the degradation products of BrSubP were elucidated by mass spectrometry and the quantification was performed by ICP-MS, unfortunately for commercial reasons we are unable to disclose this information.

The linking of LC–ICP-MS and oa-TOF-MS has provided a powerful tool for the identification, characterisation and quantification of brominated metabolites in human plasma and lung tissue. In addition, this technique could be used as an assay to monitor the effects of kininase inhibitors (e.g. ACE and NEP inhibitors) on substance P metabolism.

3.4. Single bead analysis in combinatorial chemistry by LC with ICP-MS and oa-TOF-MS

The quality control of a single bead is a challenge for analytical chemists. The amount of compound available is limited. For instance, a 140 μm Argogel bead provides a theoretical loading of 700 pmol/bead [37]. However, the actual loading is often less, due to variations in bead size, overall synthesis yield and final cleavage efficiency. In

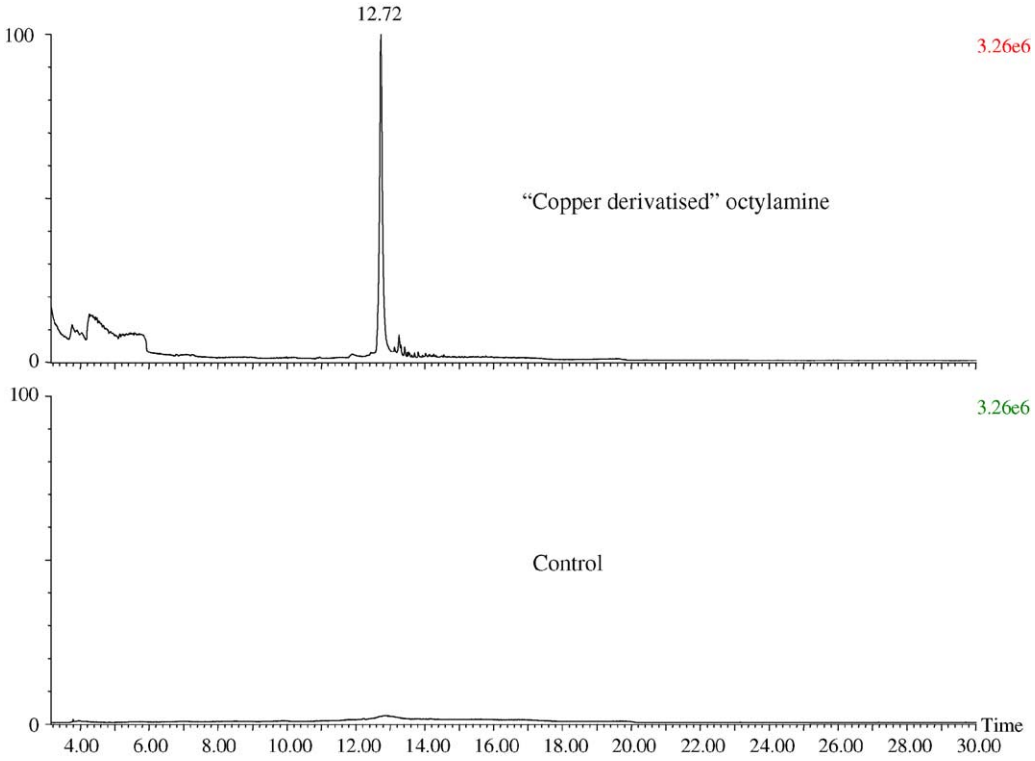


Fig. 3. The CapLC–ICP-MS chromatogram (monitoring the sum of the copper isotopes at ^{63}Cu and ^{65}Cu) for the derivatisation of octylamine with cDTPAA followed by chelation with copper.

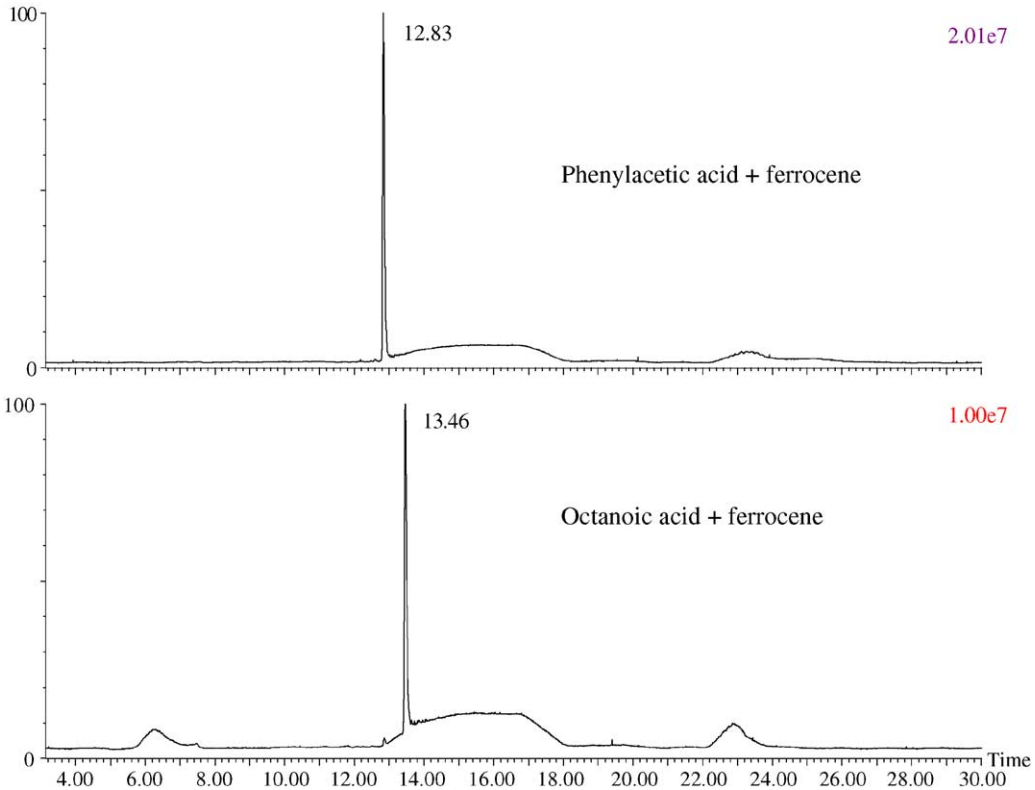


Fig. 4. The CapLC–ICP-MS chromatogram (monitoring the sum of the iron isotopes at ^{54}Fe and ^{56}Fe) for the derivatisation of phenylacetic and octanoic acids with *N*-(2-aminoethyl) ferrocene carboxamide.

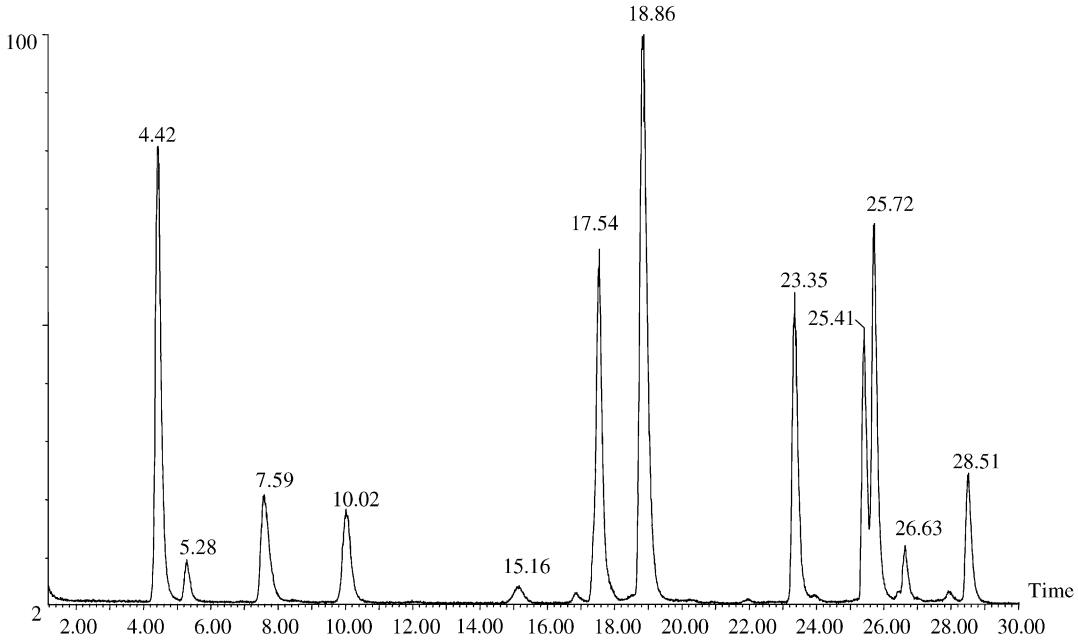


Fig. 5. ICP-MS chromatogram (monitoring the sum of the bromine isotopes at ^{79}Br and ^{81}Br) of the metabolites of substance P in human plasma.

addition, there is a need to quantify the yield of the compound. CapLC-MS has been used for the qualitative analysis of material cleaved from solid-phase matrices. Quantification will provide an estimate of the potency of the compound(s) against a biological assay and also to provide an indication of the success of the chemistry. The incorporation of a heteroatom, such as bromine, into the compound or linker and use ICP-MS to quantify the amount of heteroatom provides a novel solution to the quandary of how to make quantification measurements of the desired product from a single bead. Of course it is undesirable to only synthesise brominated compounds, therefore it would be more suitable to incorporate the bromine into the linker. This was achieved using an approach we called the analytical construct method that is described in further detail in reference [38]. Simply, the analytical construct contains a basic centre, which acts as a mass spectrometric sensitiser to ensure that the fragment gives a strong signal by electrospray ionisation and a peak splitter (an isotopic label, in this instance bromine) that gives all construct fragments a signature to distinguish genuine signals from random noise. All products at the single bead level should now be visible to high throughput analysis by mass spectrometry. The addition of bromine into the analytical construct provides both a peak split (i.e. a MS signal separated by two mass units, due to the 79 and 81 isotopes of bromine providing equally intense responses in the oa-TOF-MS) and also an element for quantification by ICP-MS.

Fig. 6 shows a simple piece of solid-phase chemistry joined on to the analytical construct fragment. Compounds can be cleaved through a classical cleavage (e.g. TFA cleavage) or at the construct linker to reveal all of the chemistry shown in Fig. 6.

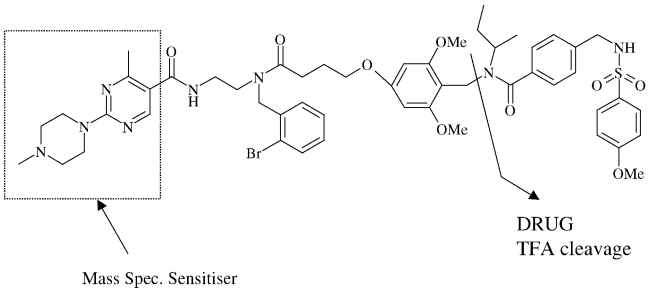


Fig. 6. The chemistry involved in the analytical construct.

Fig. 7 depicts the LC–UV chromatogram of a typical analytical construct linker and a piece of simple solid phase chemistry. In this example, LC–UV–MS analysis indicated the material cleaved from the single bead consisted of the desired component (Peak 3 in Fig. 7) and three minor components from competing chemistries. Overlaying this chromatogram from the LC–UV–MS analysis with those obtained for ^{79}Br and ^{81}Br from the ICP-MS (Fig. 8) shows that all four components are brominated. Integration of the Peak areas and the production of a suitable calibration curve (e.g. ICP-MS response for bromine) could in principle then be used to quantify each component.

3.5. Laser ablation ICP-MS of electrophoresis gel blots

Interfacing laser ablation with ICP-MS for the analysis of electrophoresis gels is not novel. Neilsen et al. [39] used this combination to develop a strategy for the speciation of metal binding serum proteins. The element chosen for their analysis was cobalt. In our study [18], the novel application of laser ablation ICP-MS to detect, quantify and map

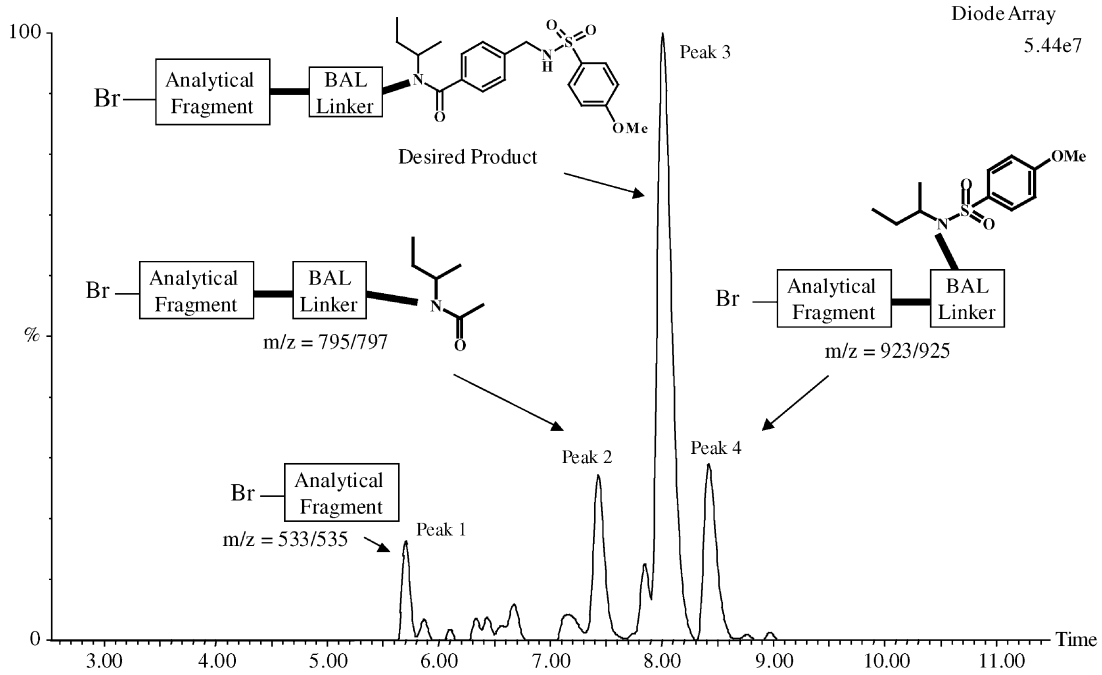


Fig. 7. LC–UV–MS chromatogram of construct cleaved compounds from a single bead.

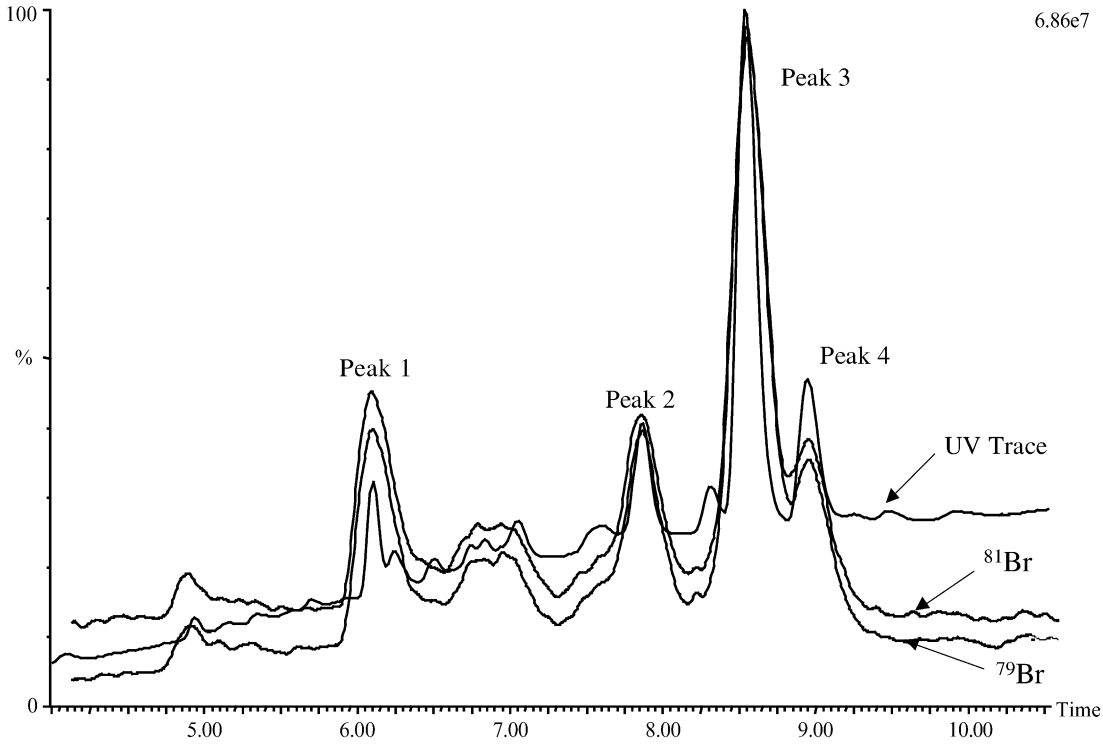


Fig. 8. LC–UV–ICP-MS chromatograms (for both isotopes of bromine, ^{79}Br and ^{81}Br) of construct cleaved compounds from a single bead.

Table 1
Concentrations of β-casein loaded onto the gel membrane

10 μL loaded of the following concentrations (mg/ml)	Amount of material loaded (μg)	β-Casein concentration
3.3	33	1.32 nmol
1.0	10	440 pmol
0.33	3.3	146 pmol
0.1	1	48 pmol
0.033	0.33	16 pmol

phosphorylated proteins in electrophoresis gel blots and gels was evaluated.

The initial studies were concerned with establishing limits of detection for phosphorus from gel blots by monitoring the *m*/*z* for phosphorus at 31. The ICP-MS signal response was dependent upon the laser operating parameters and the surface components of the gel blots and gels.

In an attempt to remove interferences at *m*/*z* 31 (probably NOH from trapped air pockets) a pre-ablation of the surface of the gel blots was made prior to performing the analysis. The analysis was achieved by ablating a single line across the length of the gel membrane. The ablated material was swept into the ICP-MS using argon as the carrier gas and monitored for phosphorus.

The gel blot analysed contained five spots of β-casein at various concentrations and a control of tris-buffer. The final concentrations of the β-casein spots are displayed in Table 1.

Laser ablation of a stock solution of β-casein was performed under different conditions. The conditions optimised included changing the number of pre-ablations prior to the analytical ablation as well as changing the laser conditions. Fig. 9 shows the ion response at *m*/*z* 31 by ICP-MS from a laser ablation across a gel blot containing spots of β-casein at different concentrations. The *x*-axis relates to the acquisition of data by the ICP-MS as the laser traverses the gel blot and is measured as time. The speed of the laser traversing the gel blot was 60 μm/s and thus the time axis is related to the distance of the gel blot. From Fig. 9, the first 3 min of the analysis is when the laser is not ablating but the argon sweep gas is maintained across the gel blot to provide a measure of the background or residual phosphorus. After 3 min the firing of the laser commences. For the following 8.5 min the laser continues firing as it traverses the gel blot and passes over the six spots (five spots of casein each at different concentrations and a blank which serves as the control). After traversing all the spots, the laser is turned off, however the ICP-MS data acquisition continues for a further 2 min to measure the background and residual phosphorus levels post ablation. Observations made from Fig. 9 are that there is a slight increase in the background at *m*/*z* 31 when the laser begins ablating the gel membrane and on termination of the ablation the background returns to a lower level. However, these changes in the background counts did not diminish the observation of the desired signals. In this experiment, a good response is obtained for the detection of phosphorus in β-casein at a concentration of 16 pmol. Clearly this was not the limit of the detection under these conditions as we were still able to achieve a signal-to-noise ratio of 10:1 at

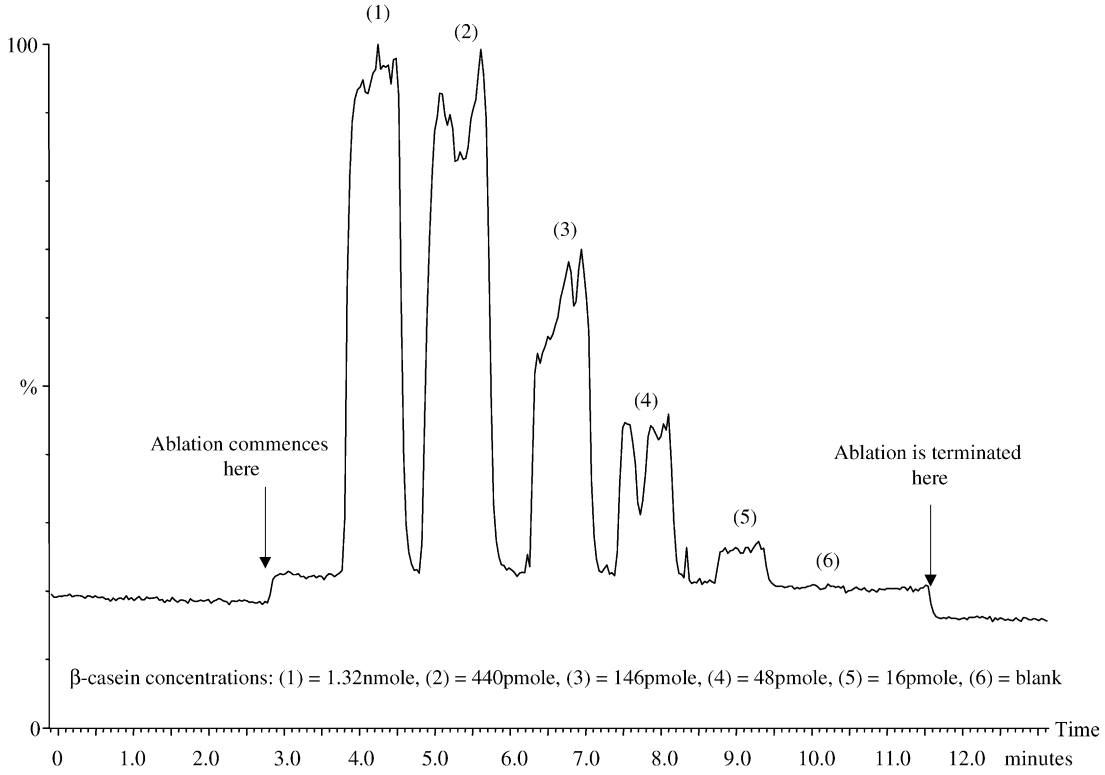


Fig. 9. ICP-MS ion response (*m*/*z* 31) for the laser ablation of a gel blot containing spots of β-casein at different concentrations.

this concentration, therefore we should be able to get lower than the 16 pmol described here. Since our original study we, along with other workers [22–24], have shown it is possible to detect phosphorylated proteins on electrophoresis gels.

4. Conclusions

The coupling of the techniques of LC, CapLC, orthogonal acceleration time-of-flight mass spectrometry and inductively coupled plasma mass spectrometry provides a very powerful method for identifying and quantifying compounds in complex mixtures. The use of a combination of these techniques has been demonstrated in the identification of the metabolites of substance P in human plasma, and also from the analysis of components cleaved from single bead analysis in combinatorial chemistry.

The potential value of derivatisation coupled with analysis by LC or CapLC with ICP-MS has also been demonstrated. The experiments described here show that the derivatisation of amines and carboxylic acids with ferrocenes is quick and simple, and using these methods can facilitate their detection.

Laser ablation ICP-MS has also been shown to have considerable potential for the analysis of electrophoresis gels and gel blots and is worthy of considerable investigation.

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