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Liquid chromatography-flame ionisation detection using a nebuliser/spray chamber interface. Part 2. Comparison of functional group responses

Erepamowei Young, Roger M. Smith*, Barry L. Sharp, Joanne R. Bone

Department of Chemistry, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK

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ABSTRACT

The application of a LC-nebuliser/spray chamber interface-flame ionisation detection has been demonstrated for the superheated water liquid chromatography of a wide range of aliphatic and aromatic analytes. The linearity and sensitivity of the response of volatile and involatile analytes have been compared. The response of the detector toward different analytes is similar to that in GC-FID and for volatile analytes was comparable to UV detection. However, the responses from involatile analytes, such as amino acids and carbohydrates, were poor and often lower than for a refractive index detector.

1. Introduction

HPLC has been unable to exploit a universal detector comparable to the flame ionisation detector (FID) in GC because of interference from the organic component of the eluent, however, with the use of superheated water (also termed subcritical water or pressurised hot water) as the eluent it became possible to also apply the FID directly to the eluent in LC [1–6]. Most of these studies used direct capillary transfer of the eluent into the flame, which limited the permissible flow rate so that either microbore columns or a flow splitter were used. This approach often suffered from capillary blockages [7] and few of the published studies were followed by further work. In Part I, as an alternative approach to a capillary interface, a nebuliser/spray chamber interface [8] was used to separate the back pressure regulation of the pressure in the column from the introduction of the analyte to the FID.

One of the principal attractions of the LC-FID is the potential to obtain a universal detection for both volatile and involatile analytes and for those lacking chromophores. Many of the more volatile analytes which lack a chromophore could alternatively be examined by GC, however, there are a number of polar analytes, including aliphatic carboxylic acids, amino acids and carbohydrates, which are often difficult to separate and detect either by GC or LC because of their involatility or lack of a chromophore. They have often required derivatisation to increase their volatility for GC, and or to introduce a chromophore or fluorophore in LC, which can be a time consuming and inefficient process. In the earliest studies of superheated water LC in 1981, Guillemin reported the detection of phenols, fructose and iprodione using a LC-FID [9] but gave no experimental details. Following the more widespread interest in the use of superheated water LC in the 1990s a wide range of analytes has been detected using the LC-FID, including the parabens, alkanols, substituted phenols, amino acids and carbohydrates [3,4]. However, most of the examples were illustrative and only the alkanols have been examined quantitatively in studies of the ethanol content of wines and spirits by Miller and Hawthorne [10] and by Yarita and co-workers [11,12]. Only a few studies have examined the linearity of the detector responses, including such as the examination of selected amino acids, carbohydrates and phenol by Yang et al. [13,14] and the separation of alcohols, phenols and carboxylic acids by Fu et al. [15], but none have compared the responses of different analytes in the LC-FID mode to determine if the presence of the high proportion of water in the eluent may cause differences from those in GC-FID.

The mechanism of operation of the FID in GC has been reviewed [16], however, the details are still the subject of discussion but the responses are primarily a "carbon number counter", and the signal decreases in the presence of heteroatoms. A number of studies, such as the early work of Dietz [17] and Scanlon and Willis [18], examined the quantitative responses and suggested that they depended on the molecular formula, in particular the number and type of heteroatoms. There have been a number of subsequent

* Corresponding author.
E-mail address: r.m.smith@lboro.ac.uk (R.M. Smith).

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studies including prediction and correlation analyses and the results were often expressed as effective carbon number (ECNs) such as the comparison study by Kállai et al. [19].

Recent studies by Cicchetti et al. [20] have examined the effect of the operating conditions of the FID, such as temperature, carrier gas and combustion gas composition, on the detector response and the same research group [21] has subsequently compared the previous models and has proposed that the response can be correlated with the heat of formation of the analyte in the vapour phase. This can either be determined by an *ab initio* calculation or more readily from the combustion enthalpies based on the molecular formulae (Eq. (1)). This assumed that the analyte was in the gas phase, thus

$$\Delta H^{MF}_{Comb} = 11.06 + 103.57(n_C) + 21.85(n_H) - 48.18(n_O) + 7.46(n_N) + \cdots + \text{factors for any additional elements} \quad (1)$$

in which n_C, n_H, n_O are the number of atoms of each element in the molecule. Values of the weighting factors were determined by regression analysis between experimental and calculated values. This was used to determine the molecular response factor (MRF) including a correction for the presence of aromaticity (Eq. (2)):

$$MRF = -0.071 + 8.57 \times 10^{-4}\, \Delta^{MF}_{Comb} + 0.127\, n_{Benz} \quad (2)$$

These values showed a good correlation with experimental values and agreed with earlier studies that found that the molar response increased with the numbers of carbon and hydrogen atoms but decreased with the number of oxygen atoms in the analyte.

The present paper sets out to demonstrate the wide applicability of LC-nebuliser/spray chamber FID for a range of different analytes both solid and liquid using condensed or superheated water as the eluent. The study will determine the linearity and magnitude of the signals with those obtained using conventional UV or RI detectors and will compare the relative responses with the comparable values predicted for GC-FID.

2. Experimental

2.1. Materials

3-Phenylpropanol, 4-phenylbutanol, maltose, valine, isoleucine, phenylalanine, serine, methanol, propanol, 2-methylacetophenone, hexanone, 2-phenylethanol and heptanone, citric acid, malic acid, succinic acid, acetic acid, sorbitol, maltose, glucose, D(+)-galactose, arabinose and mannitol were obtained from Aldrich Chemical Co, Poole, Dorset, UK. Propiophenone was from Hopkins & Williams Ltd, Essex, England, butyrophenone from Koch-Light Laboratories Ltd, Colnbrook, England, benzyl alcohol from Lancaster, Morecambe, England. Cyclohexanol and 3-cresol were supplied by Avocado (Morecambe, England) and Argos Organic (USA), respectively. Sulphuric acid was from Fisher Scientific, Loughborough, England. Air, hydrogen and nitrogen were from BOC, Worsley, Manchester. De-ionised water was prepared in the laboratory with an ELGA (High Wycombe, England) water purification system.

2.2. Sample preparation

All samples were prepared as solutions, in the range 2–200 μg mL^{-1}, in de-ionised water, except for some non-polar analytes when 1% methanol was added to improve solubility.

2.3. Instrumentation

The high temperature LC-FID used a Hewlett Packard 1050 quaternary pump (Waldbronn Germany) and a Rheodyne 7125 injector with a 10 μL loop (Cotati, CA). The column was placed in the oven of a 3300 Varian gas chromatography (Walnut, CA), and the column temperature was controlled in a programmed or isothermal mode using the GC oven controls. The eluent was passed through a stainless steel capillary outside the oven to a 757 Applied Biosystems UV variable wavelength detector (Crewe UK), and then to a Cetac micro-concentric nebuliser (MCN-100) (Omaha, NE), in which the glass nebuliser capillary had been replaced with a 0.009 inches I.D. stainless steel capillary (Coopers Needle Works, Birmingham, West Midlands, UK). The spray was fed into a 40 mm I.D. centrifugal spray chamber and any condensed eluent was removed from the spray chamber using a Gilson M313 peristaltic pump (Villiers le Bel, France). The nebuliser and spray chamber was placed in an isothermal oven at 40 °C as described for FIA-FID in the previous paper [8]. The aerosol was passed through a 1/4 in O.D. glass tube to a slightly modified flame ionisation detector from a 3300 Varian gas chromatograph (Walnut, CA), which was controlled by the 3300 GC electronics. The standard jet of the FID was replaced with a metal tipped 33 mm × 2 mm I.D. ceramic tube to permit a higher gas flow and the detector base was set at 230 °C to prevent condensation. The operating conditions were: eluent water (or dilute acid) flow, 1 mL min^{-1}; nitrogen for nebulisation, 250 mL min^{-1}; hydrogen, 157 mL min^{-1}; and air, 654 mL min^{-1}. The signal from the detector was recorded using Clarity software (DataApex, Prague, Czech Republic). In each case the response of the detector was determined as the slope of the calibration curve for the peak area per unit mass of analyte injected onto the column and the limit of detection was calculated as the mass of sample giving a signal 3 times the noise level. When required a model 2410 differential refractive index detector (Waters, Milford, USA) replaced the interface and FID.

The separations were carried out on PS-DVB (PL-RPS 5 μm, 4.6 mm × 150 mm) or PL HiPlex 8 μm H (7.7 mm × 300 mm) columns from Polymer Laboratories (Church Stretton UK) or on XTerra RP 18 (3.5 μm, 4.6 mm × 150 mm), XBridge C18 (3.5 μm, 4.6 mm × 150 mm), or XTerra RP 8 (3.5 μm, 4.6 mm × 150 mm) columns from Waters (Milford, USA).

Many of the chromatograms and full experimental conditions are available as Supplementary data.

3. Results and discussion

3.1. Application range

The operating conditions of gas flows, voltages, and temperatures for the nebuliser/spray chamber interface-FID were established in the previous paper using a FIA system [8]. A column was then placed in the oven and connected to the detector using a metal capillary outside the oven to demonstrate the applicability of the detector for the high and low temperature water separations of a range of analytes. The results are preliminary as, unlike similar GC comparisons of the response of the FID, it was not possible to use the same operating conditions of temperature and column for each analyte because of the wide diversity of polarities and structural types that were employed to demonstrate the potential applicability of the system. Because the focus of the study was on the comparison of the relative detector responses, the separations or peak shapes were not optimised.

To confirm that the present study gave comparable results to the LC direct capillary interface [10,22], a mixture of C_1–C_4 alkanols, cyclohexanol, benzyl alcohol and 3-cresol, was separated on a PS-DVB column using LC-nebuliser/spray chamber FID and UV detectors. Because of the wide range of polarities among the analytes, a temperature gradient (120–180 °C at 7 °C min^{-1}) was employed. As shown in an earlier article [5], the FID detected all seven analytes, while as expected only benzyl alcohol and 3-cresol responded in the UV detector. When a range of analyte concentrations were examined all the compounds gave linear responses in

Table 1
Responses of analyte in FID and UV detectors.

Analyte	LC-FID			LC-UV	
	Correlation (range μg)	Regression equation	LOD (μg)	Regression equation	LOD (μg)
(a) Alkanols and aryl alcohols on PS-DVB column				At 220 nm	
Methanol	0.9995 (0.8–27)	$y = 42.6x - 28.02$	0.78	n.d.	
Ethanol	0.9990 (0.5–157)	$y = 70.3x - 11.38$	0.60	n.d.	
Propanol	0.9997 (0.4–14)	$y = 115.1x - 4.91$	0.32	n.d.	
Butanol	0.9997 (0.4–14)	$y = 152.2x - 4.60$	0.30	n.d.	
Cyclohexanol	0.9990 (1–32)	$y = 102.4x + 45.3$	1.26	n.d.	
Benzyl alcohol	0.9996 (3–86)	$y = 35.4x - 1.96$	2.15	$y = 22.8x + 199$	17.01
3-Cresol	0.9992 (1–29)	$y = 41.9x - 12.75$	1.04	$y = 203.8x + 1.9$	0.83
(b) Aryl Alcohols on Xterra RP18 column				At 254 nm	
Benzyl alcohol	0.9998 (1.4–10)	$y = 126.9x - 54.8$	0.23	$y = 90.7x + 6.7$	0.53
2-Phenyl ethanol	0.9995 (1.8–15)	$y = 117.4x - 58.8$	0.46	$y = 46.8x - 8.2$	0.61
3-Phenyl propanol	0.9996 (1.6–13)	$y = 134.3x - 29.8$	0.39	$y = 99.7x - 32.8$	0.38
(c) Alkanals on PS-DVB column					
Formaldehyde	1.0000 (5–42)	$y = 9.9x - 5.8$	0.21		
Acetaldehyde	0.9992 (2.6–21)	$y = 78.6x - 106.3$	0.83		
Propionaldehyde	0.9990 (1–9)	$y = 854.9x - 615.7$	0.38		
(d) Aliphatic and aromatic ketones on XBridge C18 column					
2-Hexanone	0.9997 (2–9)	$y = 104.4x - 107.96$	0.25		
2-Heptanone	0.998 (2.1–8.4)	$y = 134.5x - 137.92$	0.62		
2-Methyl-acetophenone	0.9996 (2.6–10)	$y = 86.7x - 74.631$	0.33	$y = 524x - 432$	0.17
Propiophenone	0.9976 (2.6–10)	$y = 61.7x - 48.454$	0.85	$y = 588x - 463$	0.14
Butyrophenone	0.999 (20–83)	$y = 33.1x - 169.93$	4.17	$y = 194x - 1497$	2.33
(e) Amines on PS-DVB column					
Benzylamine	0.9993 (1.7–14)	$y = 640.6x - 387.95$	0.52		
Aniline	0.9994 (7–28)	$y = 275.5x - 133.5$	10.23		
Pyridine	0.9945 (1–7)	$y = 124.7x - 458.6$	3.83		
Hexylamine (in dilute acid)	1.000 (10–40)	$y = 20.9x + 38.4$			
(f) Acids and amides on PS-DVB column					
4-Hydroxybenzamide	0.9989 (2.5–40)	$y = 22.71x + 1.7$	1.79		
4-Hydroxybenzoic acid	0.9911 (2.5–40)	$y = 28.27x + 17.02$	1.44		
Benzoic acid	0.9910 (2.5–40)	$y = 31.60x - 87.03$	5.03		
Benzaldehyde	0.9984 (1–15)	$y = 279.4x + 2.75$	0.77		

Separation conditions as figures. The range for the calibration curve and the LOD are the amount of analyte injected onto the column.

the FID (Fig. 1). The correlations and detection limits (Table 1a) were comparable to those reported earlier for the LC-FID detector with the capillary interface [10] which found detection limits of 1–5 ng for n-butanol using a flow rate of 10–200 μL min^{-1}. As expected the relative responses of the n-alkanols increased with the carbon number, as reported for GC-FID, but in this case cyclohexanol was lower than expected and was only equivalent to propanol. The responses of the two aromatic analytes, benzyl alcohol and 3-cresol, were also significantly lower than expected and were similar to methanol. To examine the aryl alcohols further, a mixture of benzyl alcohol, 2-phenylethanol, 3-phenylpropanol and 4-phenylbutanol was separated isothermally at 100 °C on an XTerra RP 18 column. A trace of methanol was added to the sample solvent used to enhance the sample solubility in the injection solution. In this case, the first three phenylalkanols gave similar responses (Table 1b) but the peak for 4-phenylbutanol was too broad to quantify. The signal for benzyl alcohol was unexpectedly higher than on the PS-DVB column for both the FID and the UV detectors for reasons that are unclear.

Preliminary studies had suggested formaldehyde would give a response in LC-FID in contrast to GC-FID where it is usually regarded at being undetectable. A mixture of formaldehyde, acetaldehyde and propionaldehyde was therefore separated on a PS-DVB column at 160 °C). However, the response of formaldehyde was lower than that for methanol (Table 1c) but the response of propionaldehyde was unexpectedly very high and had a significant negative intercept suggesting that some losses may have been occurring on the column.

A mixture of aliphatic and aromatic ketones, 2-hexanone, 2-heptanone, 2-methyl-acetophenone, propiophenone, butyrophenone, was then separated with 0.00075 M sulphuric acid as eluent on the XBridge C18 column at 130 °C (Fig. 2). Sulphuric acid was added to the eluent as in the FIA-FID study [8] it improved the linearity for the less volatile analytes. Again the aryl analytes gave lower responses than the aliphatic ketones with the same carbon number (Table 1d).

Three amines, benzylamine, aniline and pyridine, which are of similar size, were examined on a PS-DVB column at 180 °C. No acid was added to the mobile phase. All three compounds gave a significantly higher response than for most of the other analytes (Table 1e) but the calibration curves showed marked negative intercepts. Pyridine and a series of anilines had been separated previously in a qualitative study [14].

A number of aliphatic amines were also studied on an XBridge column, but although the peaks for pentylamine and hexylamine were reasonable, both benzylamine and phenylethylamine gave poor peak shapes. As interactions with the column material were suspected, the pH of the eluent was altered to pH 11 by the addition of a trace of a sodium hydroxide. In this case good peaks were obtained from the FID for the aliphatic amines but not for the aromatic amines, which could however be detected by UV spectroscopy. The calibration curves from the FID were non-linear even if sulphuric acid was added to the eluent. However, if sulphuric acid was added to the sample before injection, a weak but linear response was obtained for the hexylamine sulphate ($y = 20.9x + 38.4$) (Table 1e), which suggests that there might have been an interaction between the amine and the injector/or column system in the absence of acid.

Most of the analytes that have been examined so far could have also be separated by GC, although depending on the sample matrix LC might be preferable. However, the impetus for this study was

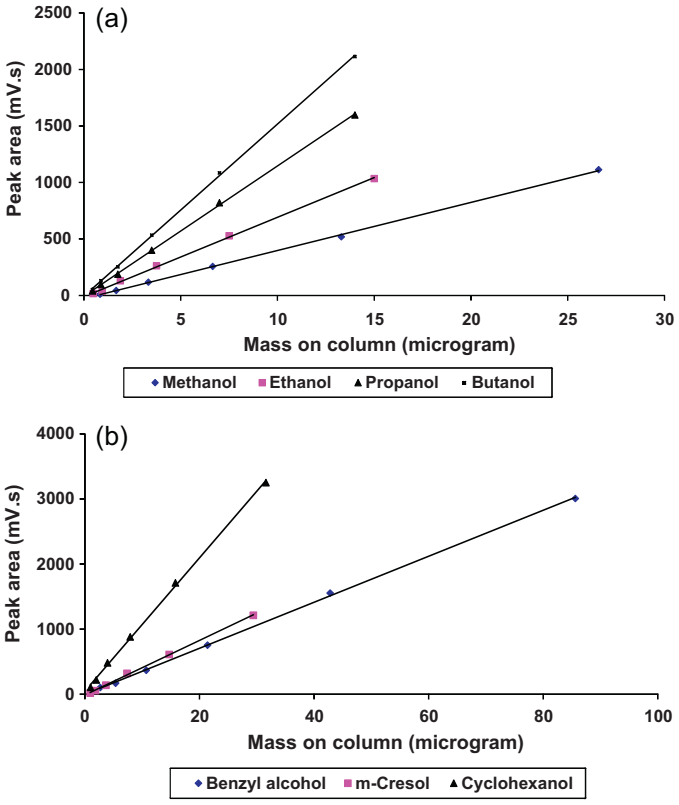

Fig. 1. Relationship of peak area to mass of analyte injected for (a) aliphatic and (b) aromatic alcohols on PS-DVB (4.6 mm × 150 mm) column. Oven temperature, 120–180 °C at 7 °C min^{-1}; eluent, water 1 mL min^{-1}; detection, FID conditions as experimental.

the potential ability to determine less volatile or involatile analytes which could normally only be detected by GC after derivatisation. A mixture of 4-hydroxybenzamide, 4-hydroxybenzoic acid, benzoic acid and benzaldehyde was therefore examined on a PS-DVB column using a temperature gradient from 120 °C to 180 °C at 4° min^{-1} (Fig. 3). When a trace of acid was added to the eluent to improve the peak shape of the benzoic acid it was also noted that the retention factor of the benzaldehyde altered significantly presumably because it changed the degree of hydration. The responses of the involatile acids and amides were relatively low (Table 1f) but that of benzaldehyde was much higher similar to the aliphatic ketones.

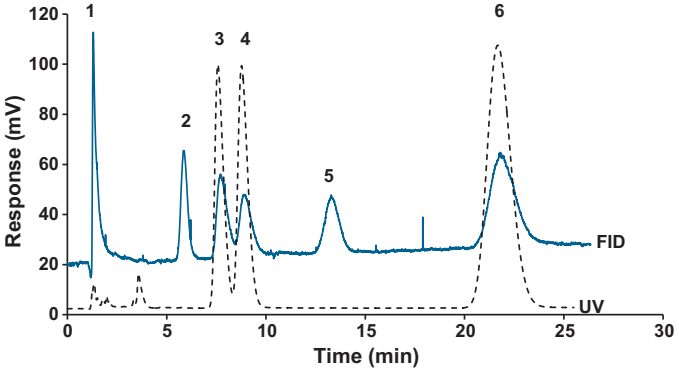

Fig. 2. Separation of aliphatic and aromatic ketones on Xbridge C18 (3.5 μm, 4.6 mm × 150 mm) column with FID and UV detection at 254 nm. Oven temperature, isothermal at 130 °C; eluent, 0.00075 M sulphuric acid 1 mL min^{-1}. Peak identity: 1 = methanol; 2 = 2-hexanone; 3 = o-methyl-acetophenone; 4 = propiophenone; 5 = 2-heptanone and 6 = butyrophenone.

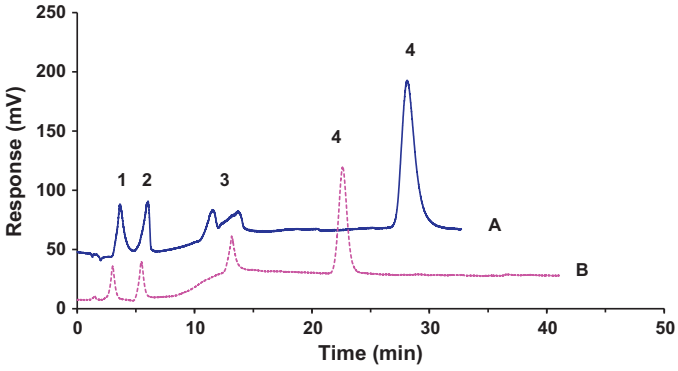

Fig. 3. Separation of a mixture of aromatic acids and derivatives on PS-DVB (4.6 mm × 150 mm) column with FID. Oven program: 120 °C for 2 min, increased to 180 °C at 4 °C min^{-1}. A: Eluent, water at 1 mL min^{-1}. B: Eluent, water adjusted to pH 3 with sulphuric acid 1 mL min^{-1}. Peak identity: 1 = 4-hydroxybenzamide; 2 = 4-hydroxybenzoic acid; 3 = benzoic acid and 4 = benzaldehyde.

The analysis of aliphatic organic acids is normally a problem in HPLC because they possess only weak chromophores and hence refractive index (RI) detection is often used. Previous SHWC-FID studies [13] have separated acetic, propionic, and butyric acids at 2000 ng μL^{-1} but no quantitative results were reported. A series of simple acids: citric acid, malic acid, succinic acid, and acetic acid, were separated on a PL Hiplex column using a dilute acid eluent at 50 °C with FID and RI detection (Fig. 4). All four acids gave a linear response in both detectors (r^2 = 0.993–0.999), but the sensitivity was lower in the FID for malic and acetic acid than for the other two acids (Table 2), probably because of the higher oxygen to carbon ratios. The response was about 100 fold poorer than for the RI detector, which gave comparable results ranging from 0.007 to 0.102 μg to those reported by Chinnici et al. [23] for organic acids and sugars in fruit juices separated by ion exclusion liquid chromatography. In addition, Mato et al. [24] found detection limits for the organic acids of 0.001–0.006 μg with UV detection at 185 nm.

In the initial studies with the FIA-nebuliser FID it was found necessary to include a trace of sulphuric acid (or salt) in the eluent to obtain a linear response from the amino acids [8]. Using this eluent, serine, isoleucine and phenylalanine were separated on a PS-DVB column at 50 °C. As with the aliphatic acids the RI detector was about 10 fold more sensitive than the FID. The responses for the FID were linear (r^2 = 0.9977–0.9997) and the LODs of 1–2.6 μg on column were determined for each analyte (Table 2b). However, these limits were significantly higher than those reported by

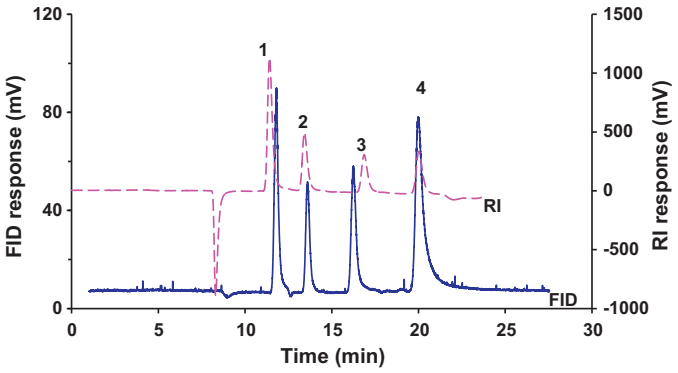

Fig. 4. Separation of organic acids on PL HiPlex 8 μm H (300 mm × 7.7 mm) column. Oven temperature, 60 °C; eluent, 0.00075 M sulphuric acid 0.5 mL min^{-1}; detection, FID and RI detector. Peak identity: 1 = citric acid; 2 = malic acid; 3 = succinic acid and 4 = acetic acid.

Table 2
Responses of analyte in FID and RI detectors.

Analyte	LC-FID			LC-RI	
	Correlation (range μg)	Regression equation	LOD (μg)	Regression equation	LOD (μg)
(a) Carboxylic acids on PL Hiplex column					
Citric acid	0.9993 (20–83)	$y = 13.53x + 42.01$	3.6331	$y = 49{,}800x - 1282$	0.0281
Malic acid	0.9993 (25–101)	$y = 7.52x - 31.20$	4.2472	$y = 44{,}800x + 387$	0.0280
Acetic acid	0.9999 (50–200)	$y = 5.46x - 23.57$	2.6578	$y = 22{,}200x + 303$	0.0332
Succinic acid	0.9999 (40–200)	$y = 14.45x + 8.13$	2.3756	$y = 50{,}300x - 732$	0.0137
(b) Amino acids on Xterra RP8 column					
Serine	0.9997 (2.5–40)	$y = 8.94x - 16.76$	0.908	$y = 6280x - 670$	0.162
Isoleucine	0.9977 (2.6–42)	$y = 20.24x + 21.52$	2.640	$y = 6335x - 481$	0.160
Phenylalanine	0.9997 (2.5–40)	$y = 24.46x - 14.74$	0.909	$y = 7183x + 270$	0.049
(c) Carbohydrates on Hiplex column					
Maltose	1.0000 (7–54)	$y = 6.62x - 1.91$	0.47	$y = 446x - 53.8$	0.10
Mannitol	0.9992 (5–45)	$y = 9.03x + 5.43$	1.85	$y = 358x + 16.3$	0.11
D(+)-Galactose	0.9999 (6–46)	$y = 7.12x + 0.56$	1.85	$y = 449x + 4.5$	0.06
Glucose	1.0000 (6–53)	$y = 7.73x + 1.68$	0.41	$y = 407x - 7.6$	0.006
Arabinose	0.9999 (5–4)	$y = 7.07x + 0.56$	0.57	$y = 411x - 78.8$	0.05

Separation conditions as figures. The range for the calibration curve and the LOD are the amount of analyte injected onto the column.

Yang et al. [13] of 38 ng for leucine and 11 ng for phenylalanine for a direct capillary LC-FID using a split of the eluent before the detector. Also in a subsequent study using a microbore column [14], they reported a limit of detection of 0.3 ng for proline and 3 ng for phenylalanine and tryptophan and wide linear ranges from 6 to 6000 ng. In other studies, Petritis et al. [25] compared the LC detection limits of underivatised amino acids using alternative commercial detectors and found, for the ELSD (0.02–0.2 μg) and for the RI detector (1 μg), MS (0.004–0.1 μg).

Although a number of individual carbohydrates have been separated in earlier LC-direct capillary-FID studies [14] the responses have not been reported. A group of six aldoses and alditols: maltose, galactose, glucose, mannitol, sorbitol and arabinose were therefore separated using a dilute sulphuric acid eluent on a HiPlex column at 50 °C, however, mannitol and sorbitol, co-eluted. Calibration curves were prepared for each analyte individually, except sorbitol, and each compound was found to give a linear response (r^2 = 0.9992–1.000). However, the limits of detection for the FID (Table 2) were poor ranging from 1.5 to 0.3 μg on column and were higher than those for the RI detector (0.005–0.1 μg on-column).

3.2. Detector responses

The wide range of the levels of the responses from the FID for the different structural types contrasts with the narrow range normally observed in GC. In line with observation in GC, lower responses were observed for compounds containing heteroatoms and also for more polar and involatile analytes, especially those that are solids at 40 °C.

In the earlier GC studies it was found that there was a correlation with the heats of combustion of the analytes (Eqs. (1) and (2)) in the gas phase and this could be used to predict relative responses. It was therefore of interest to see if the same approach could be used for the much wider group of analyte types in the present study. The calculated heats of formation were determined from the molecular formulae using the empirical factors in Eq. (1) [21] (and converted to kJ/mole) (Table 3). They closely matched the published heats of combustion reported in the NIST Chemistry WebBook [26] irrespective of whether the analyte was a gas, liquid or solid. Using these values and the formulae, including the allowance for the presence of aromatic rings, the relative molar response factors were calculated. These were converted to relative mass responses using Eq. (2) and were scaled relative to hexane = 100 (Table 3) to be comparable with results from the GC-FID. The empirical relative response for the LC-FID in the current study were then derived from the slopes of the correlation curves in Table 1 and were scaled to propanol = 61 (as hexane had not been examined) so that the two set of responses could be compared. The LC-FID responses for many of the volatile analytes, such as the alkanols were very similar to the predicted values and matched those reported earlier for GC-FID [17]. However, there were some anomalies, in particular very high values for propionaldehyde, benzaldehyde, benzylamine, aniline and pyridine which require further study. The low measured response for formaldehyde was predicted, but that for hexylamine may have been a consequence of the problem with the separation. As noted earlier for GC-FID the similar amine dibutylamine had a relative response of 75 [17].

The predicted response factors for the solid and less volatile analytes, such as the hydroxylated aromatic analytes, amino acids or carbohydrates, with a high proportion of heteroatoms, suggest that a low response should be anticipated and in these cases because of their involatility there are no equivalent GC-FID results for comparison. However, the experimental response factors with the LC-FID were generally much lower, particularly for the amino acids. It was suspected that these differences, according to the type of analyte, could be at least in part as a consequence of the mechanism of operation of the spray chamber. Volatile analytes, such as the alkanols, would be vaporised very efficiently from the large surface area droplet cloud suspended in the spray chamber and the majority of the sample from the column would be therefore be transferred as a vapour to the FID flame, which could be expected to function in a conventional ionisation mode albeit with a modified eluent composition because of the water. In contrast, the involatile analytes, which are also generally highly polar and in the case of the amino acids might be present as charged zwitterions, and hence highly hydrophilic, would remain in the aerosol droplets and a significant proportion would be lost with the larger droplets in the spray chamber. Typically in a cyclonic spray chamber of this type 80–90% (depending on the temperature) of the analyte impacts the walls and is lost [27]. Only the fraction in the finer aerosol droplets would be carried to the flame and there have the potential to be vaporised and detected. It is not clear whether any of the unvaporised analyte contributes to the ion yield. This might explain why Yang et al. [14] were able to obtain a significantly higher sensitivity using a direct capillary interface than in the present study.

Table 3
Comparison of the calculated heats of combustion and mass response factors calculated according to Eqs. (1) and (2) [21] and the measured LC-FID relative response factors scaled to (propanol = 61) derived from the slopes of the calibration curves from Tables 1 and 2.

	Calculated ΔH[a] (kJ mol^{-1})	Calculated mass RF[b]	Relative response Slopes of LC-FID[c]	Reported values for GC-FID [17][d]
Heptane	4542	100		
Methanol	644	22	22	23
Ethanol	1260	47	37	46
Propanol	1876	61	61	60
Butanol	2492	69	81	66
Cyclohexanol	3542	76	54	74 (hexanol)
3-Cresol	3609	86	22	
Benzyl alcohol	3609	86	19 (67)[e]	
Phenylethanol	4226	88	62	
Phenylpropanol	4842	90	71	
Formaldehyde	461	9	5	
Acetaldehyde	1077	40	42	
Propionaldehyde	1693	55	453	
Hexanone	3542	76	55	
Heptanone	4158	80	71	71
Methylacetophenone	4659	88	46	
Propiophenone	4659	88	33	
Butyrophenone	5275	89	18	
4-Hydroxybenzamide	3348	63	12	
4-Hydroxybenzoic acid	3023	57	15	
Benzaldehyde	3427	83	148	
Benzoic acid	3225	68	16	
Benzylamine	3934	94	339	
Aniline	3318	92	146	75
Pyridine	2701	90	66	
Hexylamine	4049	87	11	
Citric acid	1967	20	7	
Malic acid	1320	17	4	
Acetic acid	875	21	3	24
Succinic acid	1522	24	7	
Serine	1413	24	5	
Isoleucine	3463	57	11	
Phenylalanine	4580	70	13	
Maltose	5040	33	3	
Mannitol	2717	31	5	
Galactose	2534	29	4	
Glucose	2534	29	4	
Arabinose	2119	28	4	

[a] Calculated from Eq. (1) (adjusted to kJ mol^{-1}).
[b] Calculated from Eq. (2) and scaled to heptane = 100 for comparison with GC-FID.
[c] Slopes of the mass response curves from Tables 1 and 2 scaled to propanol = 61 as the value for propanol in GC-FID.
[d] Relative to heptane = 100.
[e] Based on slope from Table 1b.

4. Conclusions

These studies show that a wide range of different analytes can be determined using the LC-FID combination and superheated or heated water as the eluent, but that the responses in the detector varied markedly. Although for homologues, such as the alkanols, there was a systematic change reflected in the changes seen with the GC-FID, when the aryl group was introduced there were marked differences. Often the sensitivities were markedly lower than in GC. It appeared that volatility was a major factor and compounds that were solid would often give a lower relative response possibly as a result of discrimination in the spray chamber.

Thus analytes which volatilise during the nebulisation process were probably carried into the flame as a vapour, whereas less volatile analytes which would be transmitted in the droplets of the spray were lost to a more significant extent. Although the alternative direct capillary interfaces would apparently overcome this problem, a number of users have found that the capillary is prone to block and cause erratic flows. Further studies comparing a wider range of analytes are therefore necessary, both to compare the different interfaces and the extent to which analytes are able to reach the flame.

This study has demonstrated that the combination of the nebuliser and FID have the potential to provide universal detection for liquid chromatography overcoming the problems of the capillary interface but that further work will be needed to improve the proportion of the analyte transferred to the flame to increase the sensitivity for less volatile analytes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2012.02.035.

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