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Comparison of the response of four aerosol detectors used with ultra high pressure liquid chromatography

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ABSTRACT

The responses of four different types of aerosol detectors have been evaluated and compared to establish their potential use as a universal detector in conjunction with ultra high pressure liquid chromatography (UHPLC). Two charged-aerosol detectors, namely Corona CAD and Corona Ultra, and also two different types of light-scattering detectors (an evaporative light scattering detector, and a nano-quantity analyte detector [NQAD]) were evaluated. The responses of these detectors were systematically investigated under changing experimental and instrumental parameters, such as the mobile phase flow-rate, analyte concentration, mobile phase composition, nebulizer temperature, evaporator temperature, evaporator gas flow-rate and instrumental signal filtering after detection. It was found that these parameters exerted non-linear effects on the responses of the aerosol detectors and must therefore be considered when designing analytical separation conditions, particularly when gradient elution is performed. Identical reversed-phase gradient separations were compared on all four aerosol detectors and further compared with UV detection at 200 nm. The aerosol detectors were able to detect all 11 analytes in a test set comprising species having a variety of physicochemical properties, whilst UV detection was applicable only to those analytes containing chromophores. The reproducibility of the detector response for 11 analytes over 10 consecutive separations was found to be approximately 5% for the charged-aerosol detectors and approximately 11% for the light-scattering detectors. The tested analytes included semi-volatile species which exhibited a more variable response on the aerosol detectors. Peak efficiencies were generally better on the aerosol detectors in comparison to UV detection and particularly so for the light-scattering detectors which exhibited efficiencies of around 110,000 plates per metre. Limits of detection were calculated using different mobile phase compositions and the NQAD detector was found to be the most sensitive (LOD of 10 ng/mL), followed by the Corona CAD (76 ng/mL), then UV detection at 200 nm (178 ng/mL) using an injection volume of 25 μL.

1. Introduction

Universal detection response in chromatographic analysis remains an attractive, but as yet unfulfilled, goal. Many areas of application of liquid chromatography, such as the pharmaceutical industry, would benefit from a detector which provides uniform response towards all analytes. This would allow well characterized reference standards to be used to obtain purity results for unknown compounds within a sample [1]. Whilst mass spectrometry (MS) has achieved prominence as an important tool in drug discovery for such activities as high-throughput screening, combinatorial synthesis, and *in vitro*/*in vivo* metabolic studies [2], many species are not ionizable or suffer from variable ionization. Similarly, whilst photometric detection (UV/vis) is still widely used in routine assays of later development activities, such as formulations and batch reproducibility testing, UV detection requires that the analytes contain a suitable chromophore. Whilst the simultaneous use of two or more detectors can partly overcome the above limitations, this often leads to reduced sensitivity due to flow splitting and uncertainties in determining the exact splitting ratio for quantitative purposes. As external calibrants are required for both MS and photometric techniques, quantifying unknown compounds is often not feasible.

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Aerosol detectors constitute an emergent class of mass-sensitive detectors, and are claimed to provide universal response. In these detectors, the HPLC column effluent is nebulized and then dried, producing analyte particles. This process accommodates a large variety of different compound classes, provided they are less volatile than the mobile phase. These dried particles are then detected optically in the case of the evaporative light scattering detector (ELSD) [3] and the more sensitive condensation nucleation light scattering detector (CNLSD) [4], or by charge transfer in the case of the charged analyte detectors (Corona CAD, Corona Ultra) [5].

ELSDs have been available for the last 20 years [3,6], but they have not been widely implemented due to their poor sensitivity in comparison to UV detectors [7]. The Corona CAD was first commercialized in 2004 and recently the Corona Ultra has been released (2009) which has been optimised for UHPLC applications. The charged analyte detectors exhibit a wide dynamic range of approximately four orders of magnitude and is capable of detecting nanogram amounts on-column, up to amounts in the microgram range [8]. Due to its recent release, no applications currently exist in the literature for the Corona Ultra. However, the Corona CAD has been used in combination with a variety of separation modes (isocratic and gradient reversed-phase, ion chromatography, hydrophilic interaction liquid chromatography, supercritical fluid chromatography, size exclusion chromatography) in normal and narrow-bore column formats, for a wide range of analytes [9]. This detector has found applications in the pharmaceutical, biopharmaceutical, environmental, clinical, biofuel, food/beverage and natural product industries [10] due to its ability to detect a wide variety of compounds including high molecular weight species, acids, bases, neutral species, non-polar, ionic and zwitterionic compounds, as summarised in a recent review article [8].

The Nano-Quantity Analyte Detector (NQAD) was also released in 2009 and is the first commercial example of the CNLSD developed by Koropchak et al. [4]. This detector is similar to the ELSD, except that some of the dried particles are further grown through condensation of water onto the particle nucleus. The resultant larger particles are better able to scatter light and this increases the sensitivity of the detector [4]. The NQAD was recently evaluated in comparison to UV detection for selected antibiotic compounds, with detection limits of 3 μg/mL being reported [11].

In view of the increased commercial availability of aerosol detectors, it is important to objectively evaluate these detectors so that analysts can make an informed decision regarding their potential use. Previous evaluations include the study performed by Vervoort et al. who compared the performance of charged aerosol and evaporative light scattering detectors [12]. They found that the Corona CAD was up to 6 times more sensitive than the ELSD evaluated, but this outcome was influenced strongly by the buffer salt concentration used. Other comparisons have also found the Corona CAD to be between 2 and 12 times more sensitive than ELSD at low analyte concentrations for particular applications [13–19]. Hazotte et al. [15] noticed that at high concentrations, the ELSD was actually more sensitive due to its exponential concentration response. The sensitivity, accuracy, precision, repeatability and linear response of the Corona CAD have been found to be better than ELSD [12,13,16,17,19,20] and the Corona CAD also exhibits more uniform response factors [19–22]. The Corona CAD has also been compared to other detectors, such as refractive index (RI), UV and MS detectors. The RI detector exhibited poorer sensitivity than the Corona CAD and was incompatible with gradient elution [21], whilst UV detection exhibited interference from some organic modifiers [23], lower sensitivity for particular analytes [24,25], and non-uniform relative response factors [19,23]. Whilst the Corona CAD is capable of detecting a greater range of analytes than the UV detector [26] it has been shown to be both more sensitive [25] and less sensitive [12] than UV detection, depending on the conditions used. It has also been shown that the system repeatability (defined as the measured percentage relative standard deviation [%RSD] of area response for repetitive injections) was better for UV detection than for the Corona CAD or ELSD [12]. Electrospray-MS was found to be 3–9 times more sensitive in comparison to the Corona CAD, although the Corona CAD was more linear in its response and was able to detect a greater proportion of the lipids analysed [15].

Previous evaluations of this type have generally been limited to one or two types of aerosol detector and have focused primarily on the sensitivity achieved under specific conditions. In the present study, an effort has been made to evaluate an example of each type of aerosol detector currently available, using the same chromatographic instrumentation applied under a variety of experimental conditions. The sensitivity and performance of each detector was evaluated using a test set of pharmaceutically relevant compounds. The study has also included the generation of three-dimensional response plots covering a wide range of analyte concentrations and mobile phase compositions. It is important to consider detector response over such conditions when these detectors are hyphenated with reversed-phase gradient separations due to the changing response observed. In addition, the effect of mobile phase flow-rate, nebulizer temperature, evaporator temperature, evaporator gas flow-rate and instrumental filtering on detector response was also considered.

2. Experimental

2.1. Instrumentation

A Dionex (Sunnyvale, CA) Rapid Separation Liquid Chromatograph (RSLC) was used and consisted of a binary analytical pump with solvent selector, a 4-channel degasser, static mixer, in-line split-loop autosampler capable of injecting up to 100 μL, a thermostatted column compartment, a variable wavelength detector and a Chromeleon Chromatography Data system. This system is capable of being used at pressures up to 11603 psi and maintaining column temperature within the range of 5–110 °C. A Corona CAD and a Corona Ultra detector were purchased from ESA Biosciences Inc. (Chelmsford, MA). The Corona Ultra has been designed to provide better performance when used in conjunction with UHPLC and also has temperature control over the range 5–35 °C. A Varian 385-LC Evaporative Light Scattering Detector (ELSD) was purchased from Dionex Corporation (Sydney, Australia) and allowed the nebulizer temperature to be varied between 25 and 90 °C, the evaporator temperature to be varied between 10 and 80 °C, and the carrier gas flow-rate to be varied between 0.9 and 3.25 Standard Litres per Minute (SLM). A Nano Quantity Analyte Detector (NQAD) was provided by Quant Technologies (Blaine, MN) and is a newly commercialized condensation nucleation light scattering detector. Each aerosol detector was individually placed in-line after the UV–vis variable wavelength detector of the RSLC system for evaluation. A refrigerated vapour trap (RVT4104) capable of chilling the gas waste from the Corona CAD to −104 °C was purchased from Biolab Pty Ltd. (Scoresby, Australia). This was used to collect solvent vapours emitted from the Corona CAD rather than allowing them to being released into the laboratory environment.

2.2. Materials

The mobile phase consisted of 0.1% formic acid (98% pure, Fluka, Sydney, Australia) in Milli-Q water (Millipore Corporation, Molshiem, France), mixed with HPLC grade acetonitrile (Lichrosolv, Merck, Darmstadt, Germany). The mobile phase was degassed under vacuum and filtered through 47 mm Nylon filter membranes

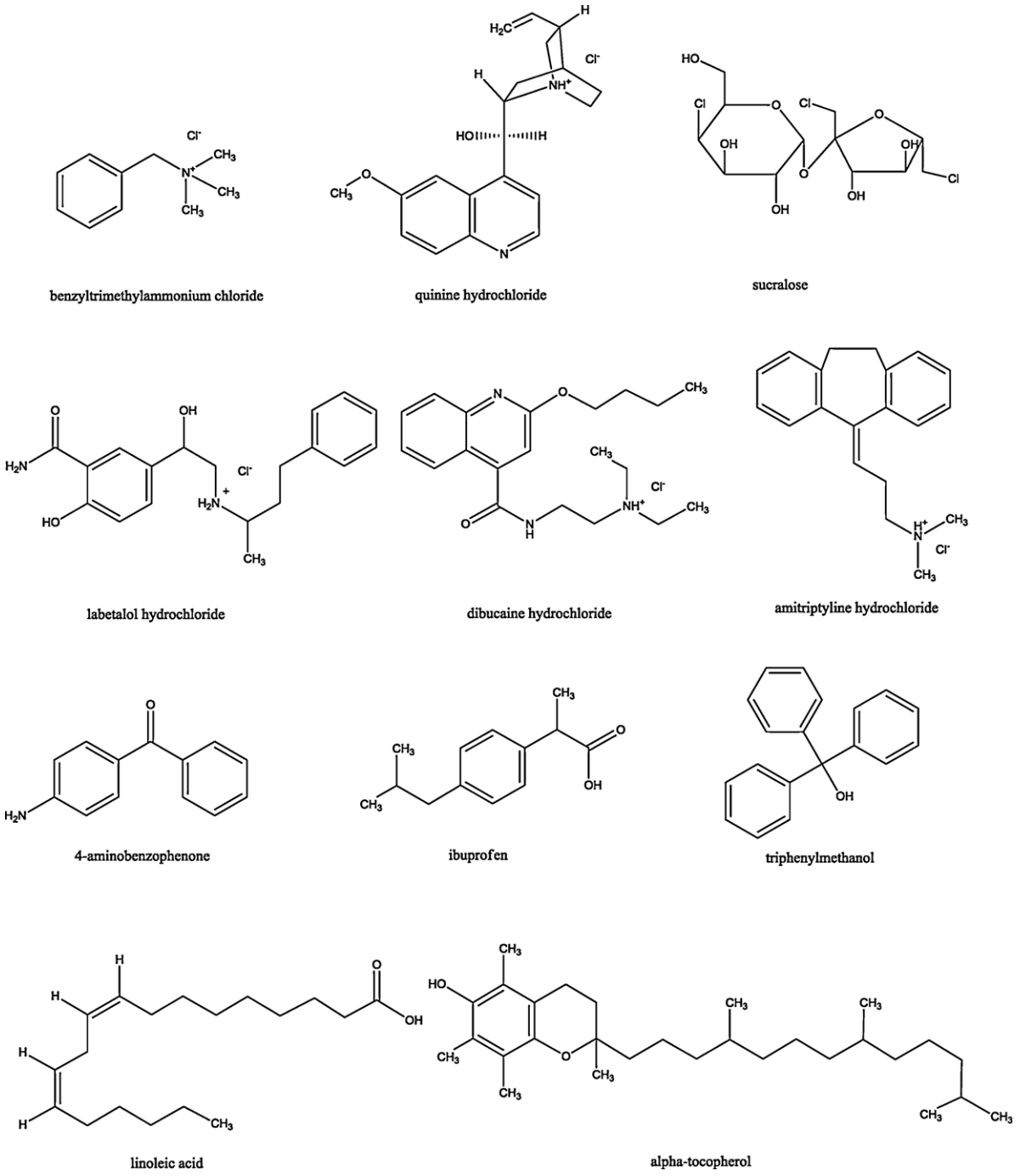

Fig. 1. The chemical structures of the analytes used in this study.

(0.2 μm pore size, Grace Davison, Rowville, Australia) before use. The test set of analytes consisted of analytical grade reagents purchased from Sigma–Aldrich (Sydney, Australia) and comprised benzyltrimethylammonium chloride, quinine hydrochloride, sucralose, labetalol hydrochloride, dibucaine hydrochloride, amitriptyline hydrochloride, 4-aminobenzophenone, ibuprofen, triphenylmethanol, linoleic acid and (±)-α-tocopherol. The structures of these compounds are provided in Fig. 1. Several of the analytes were organic bases and were purchased as their hydrochloride salt. Stock solutions of quinine and sucralose for the flow-injection experiments were prepared at a concentration of 10 mg/mL in an aqueous 0.1% formic acid solution. Working standards of the test compounds were prepared in the mobile phase used for each experiment. Stock solutions of all analytes were also prepared at a concentration of 10 mg/mL in dimethylsulfoxide (>99% purity, Merck, Dermstadt, Germany) and these were used to prepare mixed working standards for chromatographic separations. All stock solutions were kept under refrigerated conditions for a maximum of 5 days. Nitrogen gas created in-house from a nitrogen generator was used as the carrier gas for the aerosol detectors and applied at the operating pressure stated by the manufacturer of each detector.

2.3. 3D detector response comparison

The response of a non-volatile analyte (sucralose) was measured on four different aerosol detectors under flow-injection conditions. This response was measured with respect to changing analyte con-

centration and also mobile phase composition as both of these variables have a non-linear effect on the detector response. At least 68 data points were measured for each detector in an experimental space spanning elution compositions containing 0–80% acetonitrile (increments of 10% ACN) and a proportionate amount of aqueous mobile phase (0.1% formic acid in Milli-Q). Analyte concentrations typically spanned 4 orders of magnitude, ranging from 0.1 μg/mL to 1 mg/mL (using an injection volume of 25 μL), with the selected range being dependent on the dynamic range of each particular detector. Once the detector response (signal/noise) for sucralose was known over the experimental space, the data were plotted as a 3D display using software from MATLAB software (The MathWorks, Natick, MA, USA) for comparative purposes.

2.4. Aerosol detector conditions

Typically, the instrumental conditions on the aerosol detectors were kept constant whilst one parameter was varied to investigate its effect on detector response. Each detector was evaluated individually.

2.4.1. Corona CAD

The Corona CAD has the fewest parameters which could be varied. The range was maintained on the broadest setting for all experiments and was 500 pA. The instrumental filtering was typically not used except for its independent investigation at the 3 available settings of low, medium and high. The temperature of the instrument is set by the manufacturer at 30 °C and is not able to be varied. Nitrogen gas was used to nebulize the eluent and the instrument requires an external supply at a pressure of 36 psi. The Corona CAD is compatible with mobile phase flow rates between 0.2 and 2 mL/min.

2.4.2. Corona Ultra

Many of the parameters on the Corona Ultra were the same as those already stated for the Corona CAD, however, temperature was able to be controlled between 5 and 35 °C. In addition, the signal filtering options included a "Corona" setting which imitated the "high" setting on the Corona CAD.

2.4.3. NQAD

The NQAD condenses water vapour onto the dried analyte particles to increase their size prior to light scattering detection and hence requires Milli-Q water for this purpose. The evaporator temperature can be varied between 35 and 100 °C or set to off. Nitrogen gas was used to nebulize the eluent and the instrument requires an external supply regulated at a pressure of 40 psi. The NQAD is compatible with mobile phase flow rates between 0.1 and 2.2 mL/min.

2.4.4. ELSD

The Varian 385-LC ELSD was chosen due to its ability to vary a large number of parameters which affect signal response. The nebulizer temperature can be varied between 25 and 90 °C and the evaporator tube temperature can be varied between 10 and 80 °C. This system is unique in that the evaporator gas flow rate can be manipulated between 0.9 and 3.25 SLM and the instrument requires an external supply of nitrogen gas regulated between 60 and 100 psi. The detector is compatible with mobile phase flow rates between 0.2 and 5 mL/min. The data output from the detector can be averaged to provide a smoother response and is settable between values of 1 and 50 which correspond to averaging data points between 0.1 and 5.0 s.

2.5. Experimental procedures

2.5.1. Flow injection analysis

For each detector used in this study, sample introduction under flow-injection conditions was used to construct the 3D response plots, to determine the limits of detection in different mobile phase compositions, and to investigate the ELSD response when manipulating the available instrumental parameters of nebulization temperature, evaporation temperature and the carrier gas flow-rate. This allowed the analyte to be introduced in whatever mobile phase composition was required by the experimental design. To ensure that the analyte band reaching the detector was uniform under flow-injection conditions, the samples were prepared in the same mobile phase composition as that generated by the pump. The sample injection volume was 25 μL and the flow-rate was 1 mL/min to the detector. Sucralose was used as the model analyte unless otherwise stated at concentrations varying between 0.0001 and 1 mg/mL. All measurements were performed without any filtering of the detector response. Nebulization and evaporation temperatures were typically maintained at 35 °C on all detectors except for the Corona CAD, which is fixed at 30 °C and the evaporator gas flow rate typically used on the ELSD was 1.2 SLM unless otherwise stated.

2.5.2. Gradient elution analysis

Gradient elution was used to chromatographically separate a standard mixture of 11 analytes prior to UV detection. A Dionex PolarAdvantage II separator column (2.2 μm particle size, 2.1 mm diameter and 100 mm length) was used to perform the separation. The column compartment was maintained at 30 °C. The polar-embedded nature of this stationary phase allowed a linear gradient from 0 to 100% acetonitrile and 100 to 0% aqueous formic acid (0.1%) to be used. The linear gradient reached 100% acetonitrile in 15 min and the mobile phase composition was then held constant for 5 min to ensure all analytes had eluted from the column. The injection volume was 10 μL of a 0.01 mg/mL mixed standard of 11 analytes prepared in DMSO except for the ELSD which required a 0.1 mg/mL mixed standard due to its higher detection limits. A flow-rate of 1 mL/min was used. The resultant separation was then hyphenated with the four different aerosol detectors for comparative purposes. Detector temperatures were maintained at 35 °C except for the Corona CAD where the detector temperature is fixed at 30 °C. The carrier gas flow rate used on the ELSD was 1.2 SLM. The reproducibility of the response was determined by evaluating ten replicates and calculating the peak area (%RSD), peak height (%RSD) and average theoretical plates. The effect of changing the instrumental filtering on each of the detectors was also investigated using gradient elution.

2.5.3. Calculation of detection limits

Detection limits were calculated by solving the non-linear calibration curves at S/3N = 1. The response of the Corona detectors with respect to changing analyte concentration under constant mobile phase conditions is of the form: response = $a + b[\text{analyte}]^{0.5}$, where a and b are constants related to the detector and portrays an exponential decay function. Similarly, the response of the light scattering detectors (ELSD, NQAD) is of the form $1/\text{response} = c + d/[\text{analyte}]^{1.5}$, where c and d are constants related to the detector and is of a sigmoidal shape.

3. Results and discussion

3.1. Factors affecting the response of aerosol detectors

Aerosol detectors are similar in the way they nebulize a sample by mixing the liquid column effluent with an inert stream of car-

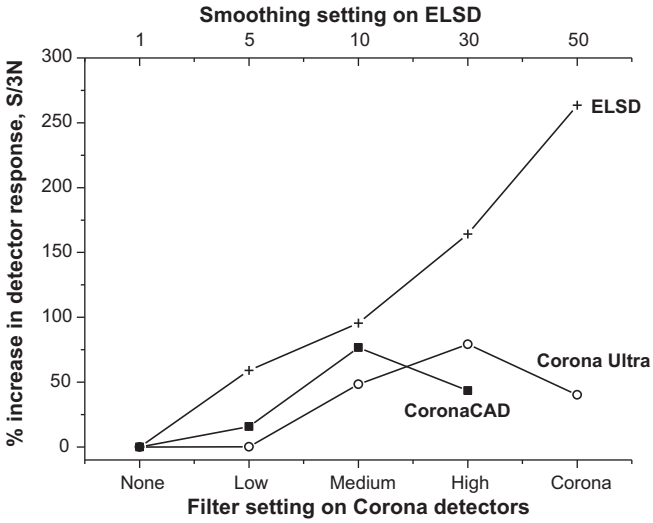

Fig. 2. The effect of manipulating the available detector filtering settings on the response of the aerosol detectors. *Conditions*: The average response of 11 analytes was calculated over the range of available detector filter settings. The gradient separation conditions used were the same as those stated in Fig. 6.

rier gas (such as N_2). The aerosol created in this way travels through an evaporation chamber to remove volatile solvents and to generate dried particles for detection purposes. The physical process of creating an aerosol from a liquid sample introduces a number of variables which can potentially affect the detector response for a given analyte. These include the mobile phase flow-rate, nebulizer temperature, evaporator temperature, evaporator gas flow-rate, mobile phase composition, analyte concentration and instrumental filtering of the detector signal. These variables generally have a non-linear effect on the response, hence it is important to have a thorough understanding of these effects when optimising the response of these detectors and when one or more of these parameters is varied during a separation. A common example of such changes occurs when performing a gradient separation where the organic content of the mobile phase varies during the separation.

The aerosol detectors evaluated in this study can be grouped into two categories: light-scattering detectors and corona detectors. The light-scattering detectors (ELSD and NQAD) generate a response by measuring light scattered from dried particles created after the nebulization and evaporation processes. Corona detectors measure the charge transferred onto dried particles via a counter-current stream of nitrogen gas which has passed a high voltage corona needle (Corona CAD and Corona Ultra detectors).

3.2. Instrumental parameters affecting detector response

Instrumental filtering can have a marked effect on the magnitude of the detector response achieved. Several of the aerosol detectors have signal filtering options which can be applied such that instrumental noise is reduced without adversely affecting the signal achieved for the analyte. Fig. 2 shows the increase in detector response that can be achieved on the Corona detectors and the ELSD by applying the available instrumental filtering options whilst keeping all other variables constant. It can be seen that for the Corona detectors it was possible to increase the signal-to-noise ratio which lowered the detection limit of the detector by approximately 75%. A 2.5-fold decrease in the limit of detection was achieved on the ELSD by manipulating the baseline smoothing parameters.

Nebulization is a fundamental process in all aerosol detectors, hence it is important to have an understanding of the factors which

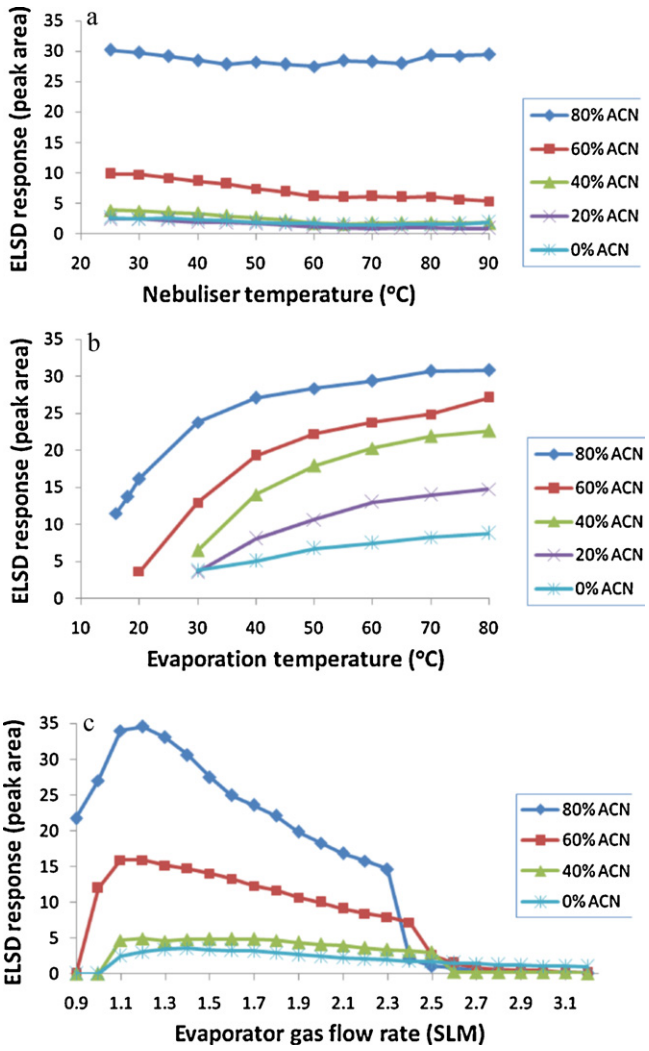

Fig. 3. Investigating the response of the ELSD when manipulating the (a) nebulizer temperature, (b) evaporator temperature and (c) carrier gas flow-rate settings available on the detector. *Conditions*: Quinine was used as the non-volatile test analyte and its concentration was kept constant at 0.1 mg/mL. Detector response was investigated at 5 mobile phase compositions over the following ranges: nebulizer temperature: 25–90 °C; evaporator temperature: 10–80 °C; and evaporator gas flow-rate: 0.9–3.25 SLM. All other instrumental variables were held constant. All other flow injection conditions used are detailed in Section 2.

control this process and their effect on detector response. The Varian 385 ELS detector was chosen to be used in this study as it represents the only aerosol detector which allows independent manipulation of the physical conditions of the nebulization and evaporation process over a wide experimental range. This detector permits the nebulization temperature, evaporation temperature, and evaporation gas flow-rate to be varied. The effects of these variables were investigated using quinine as a non-volatile analyte, introduced to the detector under flow-injection conditions using isocratic mobile phases of differing composition. The results are given in Fig. 3. Fig. 3(a) shows that the nebulizer temperature exerted a minor effect on the response of the detector over the range 0–80% acetonitrile in the mobile phase. Evaporation temperature had a much greater effect on the detector response (Fig. 3(b)), where increasing the evaporation temperature increased the detector response for all mobile phase compositions. For example, when using a mobile phase containing 60% acetonitrile a 6-fold increase in response could be achieved for a non-volatile compound by increasing the temperature from 20 °C to 80 °C. The higher temperature assisted evaporation of the mobile phase in

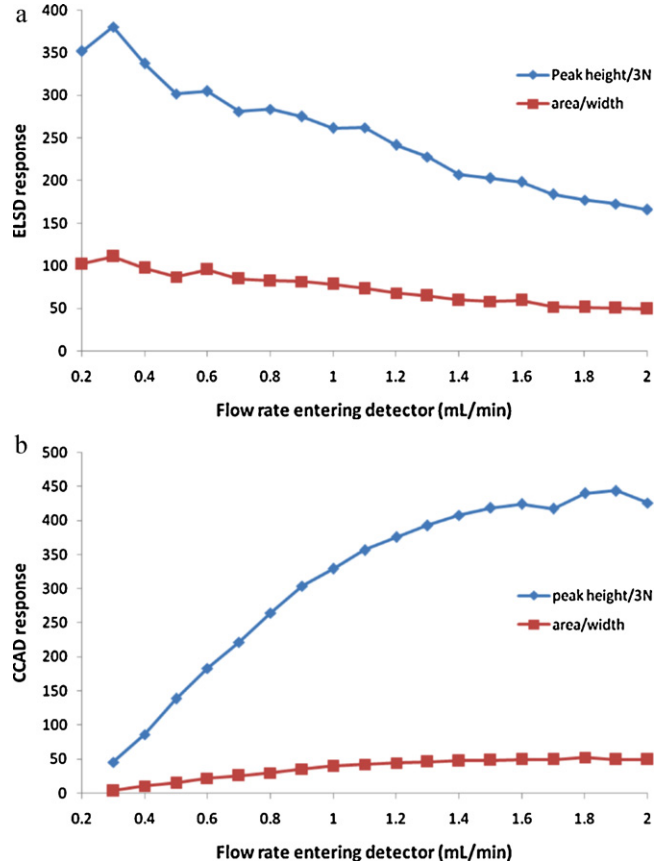

Fig. 4. Effect of mobile phase flow-rate on the response of an (a) ELSD and (b) Corona charged aerosol detector. *Conditions*: Flow injection conditions were used. The mobile phase was 100% aqueous formic acid (0.1%) and quinine was used as the analyte at a concentration of 0.05 mg/mL. All detector temperatures were maintained at 30 °C and the carrier gas flow-rate on the ELSD was maintained at 1.6 SLM. The mobile phase flow rate was investigated between 0.2 and 2 mL/min. All other flow injection conditions are detailed in Section 2.

the evaporator tube, providing more efficient particle formation for detection purposes. The evaporator gas flow-rate also affects the drying of particles and Fig. 3(c) shows the ELSD response for a range of flow-rates. Low gas flows around 1.2 SLM provided the best response, with higher flow-rates giving reduced response. Not all of the detectors investigated were manufactured with the capability of temperature control. For example, the Corona CAD operates at a set temperature of 30 °C whilst on the NQAD it was possible to vary only the evaporator temperature between 35 and 100 °C. As semi-volatile analytes are included in the test set for this study and exhibit reduced response at high temperatures due to losses in the evaporation tube, it was decided that the best compromise for comparing the four detectors was to maintain the nebulizer and evaporator temperatures on the Corona Ultra, NQAD and ELSD at 35 °C which was as close as possible to the set temperature of the Corona CAD. It should be noted that if the temperature was optimised for each detector, it may be possible to further increase the detector response for non-volatile analytes based on the temperature study performed in Fig. 3. However, the purpose of this study was not to find the optimum operating conditions for each detector but to investigate the factors which change the response of aerosol-based detectors.

The final instrumental parameter that was investigated was the mobile phase flow-rate. For this comparison, an example of the light scattering and the corona detectors were chosen which were capable of being operated at the same nebulizer and evaporation tube temperatures (30 °C). Fig. 4 shows that increasing the flow-rate reduced the ELSD response (Fig. 4(a)), but increased the Corona CAD response (Fig. 4(b)). Both detectors are widely described as mass-sensitive detectors, where an increase in flow-rate causes an increased response [15]. In this study, a negative correlation with flow rate was found for both peak area and peak height on the ELSD whilst the opposite was true for the Corona CAD. It is believed that the observed deviation from mass-sensitive behaviour exhibited by the ELSD is related to the instrumental design whereby a smaller proportion of the analyte reaches the point of detection due to a change in the droplet size distribution. After nebulization, large droplets which are difficult to dry are impacted onto a steel plate and drained to waste prior to the point of detection. A mobile phase flow-rate of 1 mL/min was used for all further studies because this corresponded to the maximum flow-rate when the column was used due to backpressure limitations.

3.3. Effect of analyte concentration and mobile phase composition on detector response

Changes in the mobile phase composition, as in the gradient elution mode, produce changes in the physical properties of the mobile phase, such as surface tension and the enthalpy of vaporization. This affects the characteristics of the aerosol generated (e.g. droplet size) and the ability of this aerosol to form dried particles. This factor, combined with the non-linear response of the light-scattering and charged aerosol detectors with respect to analyte concentration, makes quantification of unknown analytes difficult. Hence, it is important to understand how changing the analyte concentration and mobile phase composition affects the detector response. This is best visualised by creating a 3D response surface for each detector, as shown in Fig. 5. The response (signal:noise) of a non-volatile analyte (sucralose) was measured on all detectors using mobile phase compositions in the range 0–80% acetonitrile and analyte concentrations spanning up to 4 orders of magnitude. It can be seen that for all detectors, an increase in either the organic content of the mobile phase or the analyte concentration leads to a non-linear increase in response. This response surface can be modelled mathematically to provide a response equation, as shown for the Corona CAD detector in a previous study [27], although for general validity this would require the evaluation of detector response for a wide range of analytes. Furthermore, it can be seen that the NQAD exhibited the highest response of the four detectors at a mobile phase composition of 60% acetonitrile and an analyte concentration of 0.1 mg/mL sucralose. It should be noted that the dynamic range of each detector was dependent on the sensitivity of the detector. In particular, for the ELSD it was not possible to measure a response above 10,000 S/N as this corresponded to the maximum output signal that the detector was capable of generating. It should be noted that this limitation may be specific to the particular model of ELSD tested in this study. However, the results in this study showed that the ELSD exhibited the narrowest dynamic range and also the highest detection limits of the aerosol detectors tested and was consistent with other studies present in the literature [11–19]. The shape of the 3D response plots was quite similar for the Corona CAD and Corona Ultra detectors, although the magnitude of the highest response achieved on the Corona Ultra was 3 times greater than that on the Corona CAD. This can be attributed to a more efficient nebulizer which has been designed specifically for UHPLC separations.

3.4. Comparison of the performance of aerosol detectors

The relative performance of the four detectors was evaluated by performing identical reversed-phase separations of 11 analytes on all detectors using a linear gradient between 0 and 100% acetonitrile. The resulting separations are shown in Fig. 6. Chromatographic efficiencies obtained on the NQAD and ELSD detectors

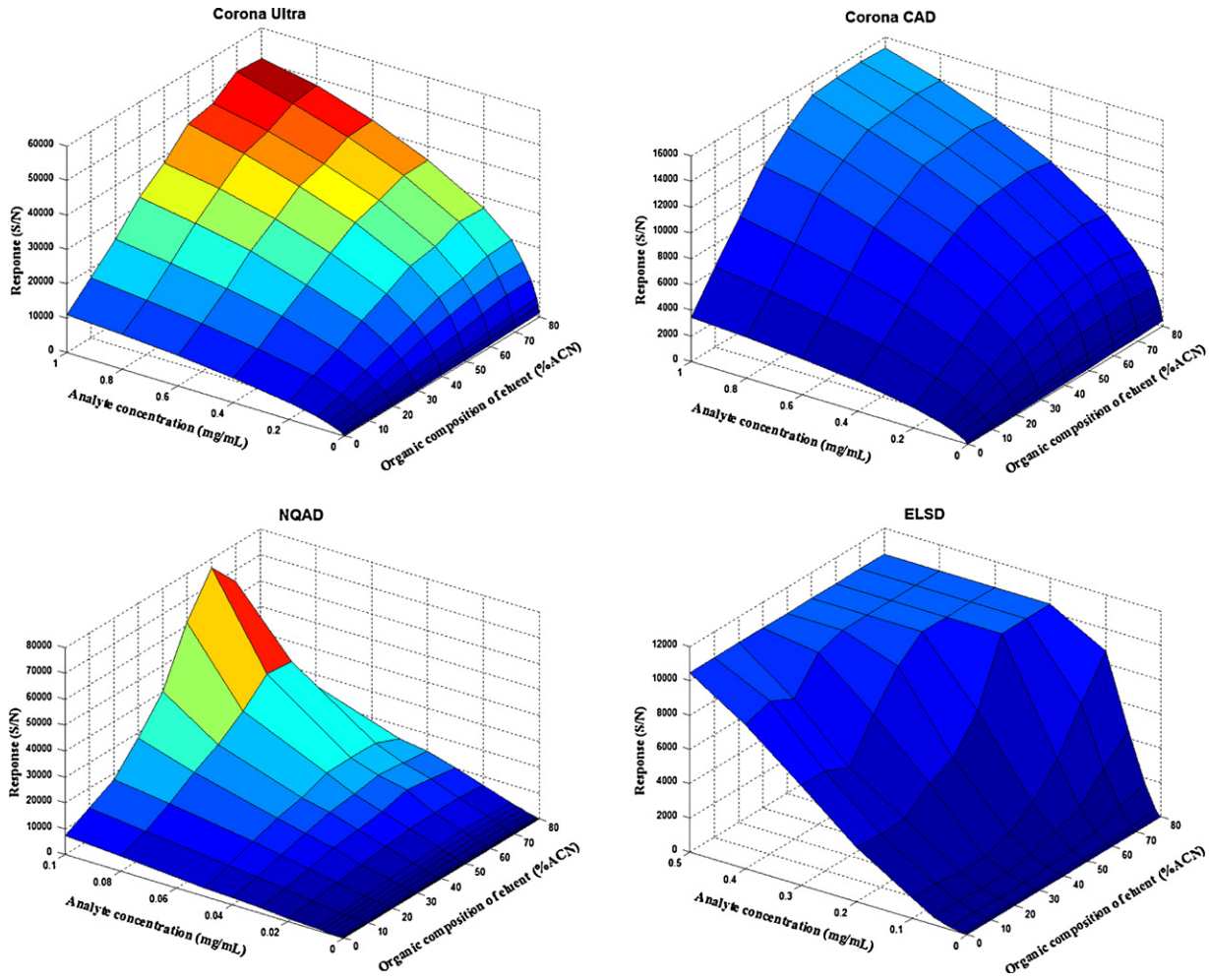

Fig. 5. The effect of analyte concentration and mobile phase composition (% of organic solvent) on the response (S/N) of four aerosol detectors. *Conditions*: Flow-injection sample introduction was used for mobile phase compositions of 0–80% acetonitrile mixed with formic acid (0.1% in Milli-Q). All other flow injection conditions are detailed in Section 2.

were superior to those for the Corona detectors. This can be seen clearly from their capability to resolve dibucaine and amitriptyline (peaks 5 and 6) in Fig. 6. As identical separation conditions were used, this can be attributed solely to the band broadening effects which occur in the detector. It is also evident from Fig. 6 that the peak heights of analytes differ between the detectors. Whilst the Corona detectors exhibit similar peak heights between themselves, it can be seen from Fig. 6 that the NQAD and ELSD have different response factors for particular analytes. For example, it can be seen that peak 10 (linoleic acid) gave a greater response on the light scattering detectors than peak 11 (tocopherol). However, the opposite is true for the Corona detectors. Tocopherol differs from the other analytes in the test set in that it is an oil at room temperature, and oils can exhibit different light-scattering properties than solid particles.

Generally speaking under constant experimental conditions, aerosol detectors exhibit mass-sensitive characteristics and the peaks for all analytes could be expected to be roughly the same given that the same amount of each was present in the sample. However, uniform analyte response was not observed in Fig. 6 for any of the detectors and this is due to two reasons. The first reason is the relative volatility of some analytes, especially amitriptyline, 4-aminobenzophenone, ibuprofen and triphenylmethanol, whose enthalpies of evaporation range from 59.25 to 66.25 kJ/mol [28]. During the nebulization process, volatile analytes suffer losses during the evaporation process, leading to reduced response. The second reason results from the changing composition of the mobile phase (the "gradient effect"), as demonstrated in Fig. 5. In general, increasing the organic content of the mobile phase leads to an increased response. Thus, when using a linear gradient for the separation, one would expect the relative response of non-volatile analytes to increase up to the point in the separation corresponding to approximately 60% acetonitrile. This trend was evident for the NQAD and Corona detectors in Fig. 6 for the first 5 analytes, but not for the ELSD detector, where the relative response initially decreased during the separation and then increased rapidly towards the end of the mobile phase gradient. Different light-scattering mechanisms exist based on differences in the size of the particle (Rayleigh scattering, Mie scattering and reflection/refraction) [3] and the differences in the ELSD response in this work may be attributed to different particle sizes being produced at differing compositions of mobile phase [29]. The NQAD, also a type of light scattering detector, grows the particles through condensation of water onto the particles. As only a certain size of particle is selected for this process, the NQAD exhibited a more linear response than the ELSD. Fig. 7 shows the comparative separation using UV detection. Formic acid in the aqueous mobile phase has some absorbance at 200 nm hence the applied gradient was visible in the baseline on the resulting chromatogram. In addition, peak 1 corresponding to benzyltrimethylammonium was obscured by the large void peak and peak 3 (corresponding to sucralose which does not con-

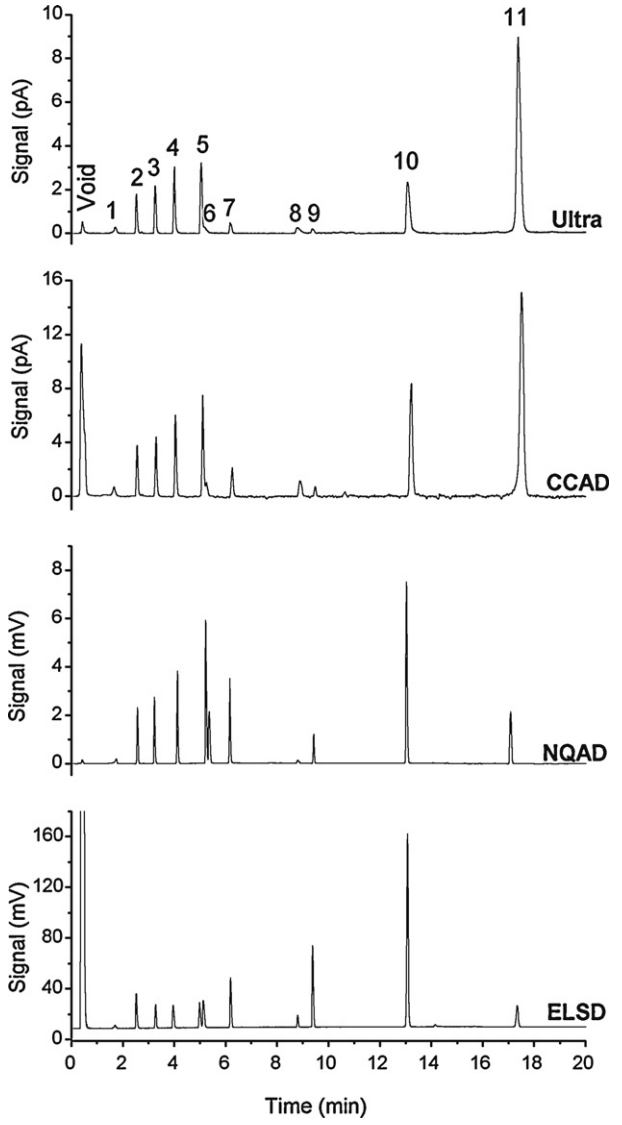

Fig. 6. The comparison of an identical reversed-phase gradient separation of 11 analytes hyphenated with four aerosol detectors. *Conditions*: The analytes were (1) benzyltrimethylammonium, (2) quinine, (3) sucralose, (4) labetalol, (5) dibucaine, (6) amitriptyline, (7) 4-aminobenzophenone, (8) ibuprofen, (9) triphenylmethanol, (10) linoleic acid and (11) tocopherol. All other gradient separation conditions are detailed in Section 2.

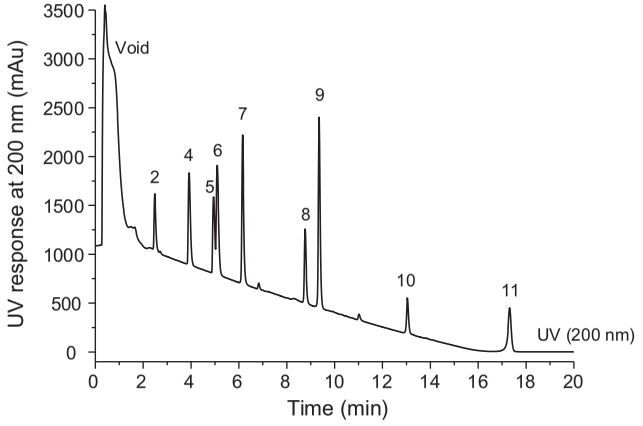

Fig. 7. The UV response (200 nm) of 11 analytes separated under the reversed-phase gradient conditions performed in Fig. 6.

Table 1
A comparison of the response and peak efficiency obtained using four different aerosol detectors and UV detection at 200 nm. *Conditions*: Values were calculated for 11 analytes using 10 replicates. The separation conditions used were the same as those stated in Fig. 6.

Analyte	Average peak area, $n = 10$					Peak area RSD (%), $n = 10$					Average peak variance, (σ^2, $\times 10^{-3}$), $n = 10$				
	Corona Ultra	Corona CAD	NQAD	ELSD	UV (200 nm)	Corona Ultra	Corona CAD	NQAD	ELSD	UV (200 nm)	Corona Ultra	Corona CAD	NQAD	ELSD	UV (200 nm)
Benzyltrimethyl ammonium chloride	0.027	0.063	0.016	0.170	NC	12.12	9.68	29.99	21.52	NC	1.63	1.61	0.91	0.78	NC
Quinine	0.125	0.264	0.109	1.500	4.97	2.16	0.85	4.59	17.02	1.68	0.71	0.73	0.31	0.53	1.11
Sucralose	0.160	0.340	0.120	1.015	NC	1.15	1.07	4.24	15.46	NC	0.89	0.90	0.28	0.46	NC
Labetalol	0.225	0.478	0.200	1.172	9.85	1.83	1.64	8.19	12.11	2.73	0.93	0.94	0.40	0.68	1.57
Dibucaine	0.264	0.595	0.301	1.345	7.95	2.18	1.43	4.53	11.13	2.24	1.16	0.97	0.41	0.82	1.72
Amitriptyline[a]	0.026	0.123	0.140	1.554	13.14	18.56	5.32	11.43	10.22	0.99	NA	NA	0.84	0.99	2.13
4-amino benzophenone	0.044	0.198	0.182	2.158	14.63	1.56	4.22	10.09	11.06	1.44	1.66	1.28	0.40	0.49	1.15
Ibuprofen[a]	0.041	0.158	0.008	0.439	8.71	10.28	5.10	26.10	12.26	1.71	7.26	3.57	0.95	0.39	1.18
Triphenylmethanol[a]	0.017	0.058	0.050	3.514	25.39	7.44	7.87	7.17	6.56	1.18	2.06	1.13	0.27	0.43	1.33
Linoleic acid	0.351	1.038	0.430	10.014	3.78	3.05	1.15	1.31	6.06	1.26	3.95	2.65	0.50	0.66	1.31
Tocopherol	1.562	2.769	0.193	1.501	6.68	3.06	1.26	13.97	4.46	3.21	4.97	4.91	1.05	1.23	3.10
Average						5.76	3.60	11.06	11.62	1.83	1.64	1.70	0.57	0.68	1.33

NC—no chromophore.
[a] Semi-volatile analytes.

Table 2
Comparison of the detection limits of four aerosol detectors and UV detection at 200 nm. *Conditions*: Flow-injection conditions were used as stated in Fig. 5.

		Corona Ultra	Corona CAD	NQAD	ELSD	UV (200 nm)[a]
Limit of detection, ng/mL (ppb), S/N = 3	Mobile phase containing 0% ACN	1020	364	380	2420	198
	Mobile phase containing 80% ACN	250	76	10	490	178

[a] Sucralose does not contain a chromophore hence LOD for UV was calculated using quinine as the non-volatile analyte.

tain a chromophore) was not detected. The relative responses for the various analytes were dependent on their absorptivities at 200 nm.

Table 1 compares the performance of the detectors by measuring the relative standard deviation of the peak area, and the average peak variance (σ^2) for 11 analytes over 10 consecutive separations where all analytical conditions remained constant except for the aerosol detector. The same calculations were performed using peak heights and similar RSD values were obtained. The average peak area was included in Table 1 so that the magnitude of the peak area is known for the RSD calculations. In addition, UV detection at 200 nm was also included for comparative purposes. For the aerosol detectors, the volatile analytes gave greatest variation in the response. The light scattering detectors showed poorest precision (~11% RSD), whilst the UV detection gave best precision (~2% RSD). A comparison of band broadening attributable to the aerosol detectors was performed by calculating the average peak variance of the 11 analytes separated under gradient conditions. As any analytical system has an intrinsic band broadening associated to it, the analytical system and conditions were kept constant except for the type of aerosol detector used. This allowed the band broadening of the detectors to be compared in relative terms by normalising the variance as a percentage relative to the NQAD which exhibited the least band broadening. Band broadening on the ELSD was only 18% greater for the 11 analytes studied than the NQAD. The UV detector displayed 2.31 times greater peak variances than the NQAD, whilst the Corona CAD and Ultra gave the broadest peaks with the average variance being 2.96 and 4.0 times greater than the NQAD, respectively.

3.5. Comparison of the sensitivity of aerosol detectors

Direct sensitivity comparisons on the aerosol detectors can be misleading in view of the non-linear responses of the detectors with regard to analyte concentration and mobile phase composition. Thus, one type of detector may appear more sensitive at a higher concentration of analyte but at the limit of detection, this result can change. As many analytical chemists are interested in detector sensitivity close to the limit of detection, the comparative sensitivity of the aerosol detectors was measured in this region and the results given in Table 2. The limit of detection for each of the aerosol detectors was calculated by solving the non-linear analyte calibration curve equation (S/N = 3) at a constant mobile phase composition and the results were also compared to those obtained by UV detection. Two mobile phase compositions were assessed, one being totally aqueous and another containing 80% acetonitrile. Using the aqueous mobile phase, the Corona CAD and NQAD were comparable (LODs of 360 ng/mL) but also less sensitive than UV detection at 200 nm (198 ng/mL). When an 80% acetonitrile mobile phase was used, the performance of the aerosol detectors improved whilst the UV response remained essentially unchanged. Under these conditions, the NQAD and Corona CAD were both more sensitive than UV detection with detection limits of 10 ng/mL and 76 ng/mL, respectively, based on an injection volume of 25 μL.

4. Conclusions

Aerosol detectors offer a number of advantages over UV detection. They are capable of detecting all non-volatile analytes, provide relatively uniform response factors, and particularly for the light-scattering detectors, they exhibit better peak efficiencies than UV detection. Conversely, aerosol detectors suffer from poor precision for semi-volatile analytes. The use of aerosol detectors has been complicated by the many factors which affect the detection response, but when these factors are understood, they can be manipulated to increase the sensitivity to the point where it exceeds that of UV detection. The light scattering detectors exhibit the best peak efficiencies, whilst the Corona detectors provided better reproducibility. The lowest detection limits were achieved on the NQAD and the NQAD was more sensitive than UV detection when a large percentage of acetonitrile was used in the mobile phase. Not all of the detectors were manufactured with the capability of manipulating temperature. In particular, it was shown on the ELSD that evaporation temperature is an important variable which affects analyte response in the aerosol detectors and hopefully this capability will be included by manufacturers in the future. Aerosol detectors have particular advantages for applications requiring quantification of unknown compounds due to their constant response factors under uniform conditions (e.g. pharmaceutical impurities, environmental analysis) or analysing non-volatile samples which are known not to contain chromophores.

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