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# Assessment of the complementarity of temperature and flow-rate for response normalisation of aerosol-based detectors

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ABSTRACT

The solvent dependency of the detection response is a major limitation of corona-charged aerosol detection (C-CAD). The present study empirically investigates the utility of temperature and flow-rate gradients to overcome solvent gradient limitations of C-CAD. In preliminary flow-injection investigations, it is demonstrated that the response of C-CAD remains relatively unaltered with variations in flow-rate when used with water-rich eluents. Based on these findings two separation approaches were developed and their utility for C-CAD response normalisation was demonstrated using a mixture of eight analytes. In the first approach the use of a solvent gradient is replaced with a temperature gradient performed under isocratic mobile phase conditions. Detection response is further enhanced by mixing a secondary stream of pure acetonitrile with the column effluent, yielding a 3-fold increase in detection response. In the second approach, flow-rate programming is used to improve speed of isocratic-temperature gradient separation. The use of simultaneous variation in flow-rate and column temperature reduced the separation time by 30%, with relatively uniform analyte response. Lastly, an inverse-gradient solvent compensation approach was used to evaluate the response homogeneity and the applicability of the above approaches for quantitative analysis. Good peak area reproducibility (RSD% < 15%) and linearity ($R^2$ > 0.994, on a log-scale) over the sample mass range of 0.1–10 μg was achieved. The response deviation across the mixture of eight compounds at seven concentration levels was 6–13% compared to 21–39% when a conventional solvent gradient was applied and this response deviation was comparable to that obtained in the inverse gradient solvent compensation approach. Finally, applicability of these approaches for typical pharmaceutical impurity profiling was demonstrated at a concentration of 5 μg/mL (0.1% of the principal compound).



## 1. Introduction

Aerosol-based liquid chromatography detectors, such as the evaporative light-scattering detector (ELSD) and the corona-charged aerosol detector (C-CAD), are regarded widely as quasi-universal detectors. These detectors have been proven to be useful for quantitative analysis of non-volatile analytes since they provide detection response irrespective of the optical absorption properties of the analyte and mobile phase. Nevertheless, non-linearity of response with sample concentration and solvent dependency of detection response are major concerns which limit the widespread acceptance of these detection techniques. Log-normalisation is generally used to relate detection response to sample concentration and it works well under isocratic conditions. However, with solvent gradient separation, response correction requires multiple calibration curves, since the response of aerosol detectors varies with the mobile phase composition. This poses practical difficulties in quantification, and it is highly desirable to have single calibrant quantification.

Different approaches to mitigate the solvent effect have evolved since the introduction of ELSD. Mathews et al. [1] have proposed a generic calibration method to account for the response non-linearity of the ELSD associated with sample concentration and solvent effects. A 3-D surface plot of detection response as a function of sample concentration and mobile phase composition, constructed by multiple injections of a single non-volatile and non-retained calibrant, was used to minimise the quantification errors. Similarly, a two-step response model was developed by Hutchinson et al. [2] to relate the C-CAD response to the variation in sample concentration and mobile phase composition. Another technique is the real time gas control feature of the ELSD, which allows control over the evaporation process and aerosol particle transport, providing

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more uniform detection response in solvent gradient separations [3]. In 2007, Gorecki et al. proposed a post-separation inverse gradient approach to compensate solvent gradient effects in C-CAD detection [4]. Subsequently, the utility of the same approach for the response normalisation in ELSD detection was demonstrated by de Villiers et al. [5]. In this approach a secondary stream of solvent is mixed with column effluent in a way that the mixture of aqueous–organic eluent being introduced in the detector inlet remains in equal proportions throughout the run.

All the above-mentioned approaches involve correcting the response variations resulting from the solvent gradient separation. Relative uniformity of response is an attractive goal, especially for impurity testing in the pharmaceutical industry. In this case, a calibration plot prepared for the active pharmaceutical ingredient could be used to determine the levels of impurities without the need for standards or calibration plots for these impurities. One potential solution to the non-uniformity of response of aerosol detectors would be to avoid the solvent gradient altogether and to optimise the separation under isocratic conditions. This might be achievable using alternate separation tools, such as temperature or flow-rate variation, which facilitate a comparable elution profile to that obtained using solvent gradient elution.

In the present study we have investigated the possibility of achieving relatively uniform detection response of the C-CAD for different analytes by employing isocratic separation with temperature and mobile phase-flow rate variation as optimisation tools. The capability of high temperature liquid chromatography (HTLC) to meet the ever-increasing requirements of separation throughput and control of separation selectivity has been investigated by many researchers. In recent times, several aspects of HTLC, including retention mechanism and principles, range of applications from analytical to capillary scale separations, challenges in broader acceptance and consequent developments in instrumentation have been published [6–9]. It is well documented that with an increase in temperature, viscosity, surface tension and dielectric constant of water and aqueous-organic binary solvent mixtures drop significantly and approach the properties of the pure organic solvents [6,7,10]. It is noteworthy that an increase in temperature by 4–5 °C is required to achieve the effect comparable to 1% change in the amount of organic solvent (acetonitrile or methanol) present in the mobile phase. However, for chromatographic separations employing water-rich eluents, variation of temperature alone may not be sufficient to achieve elutropic strength comparable to a solvent gradient . Different strategies such as the use of isocratic binary solvent mixtures containing higher amount of organic solvents coupled with a high temperature ramp [9,11,12] or temperature pulsing [13] have been reported to improve the performance of HTLC separations. Thus, isocratic-temperature gradients can replace the use of solvent gradients in many instances and thereby complement aerosol detectors by minimising the contribution of solvent effects on non-linearity of response [6,7,14]. Nevertheless, the majority of the published work related to the use of temperature gradient separations has been limited to separations involving UV-detection. Even after quite intensive research in HTLC, the use of temperature gradients is not considered to be a mainstream approach for chromatographic optimisation. This is due primarily to potential practical limitations of temperature gradients, such as slow heat transfer in liquids, high thermal mass of analytical scale columns, and lack of a suitable heating device providing a sufficiently wide temperature range and a suitably steep temperature ramp. In view of these limitations, some combination of temperature gradients with additional optimisation parameters becomes inevitable.

Although the use of mobile phase flow-rate variation for improving the separation speed and peak efficiency has been investigated before [15–19], the risk of high column backpressure in a conventional high performance liquid chromatography set-up has deterred the widespread implementation of flow-rate programming. The dependence of column backpressure ($\Delta P$) on viscosity ($\eta$) and linear velocity ($u$) of the fluid is commonly described by Darcy's equation:

$$\Delta P = \beta_0 \times \eta \times u \times L \quad (1)$$

where $\beta_0$ is the resistance factor and $L$ is length of the column.

Column backpressure alters in direct proportion to viscosity and the linear velocity of the mobile phase. In HTLC separation, a temperature-induced decrease in solvent viscosity results in much lower column backpressure, permitting separations to be performed at high flow-rate. Variations in mobile phase flow-rate can be utilised as a means to improve efficiency and speed of HTLC separations. In HTLC separations, because of the reduced viscosity of the mobile phase, the diffusion coefficient alters significantly and thereby improves the inter-phase solute mass transfer, allowing separations to be performed at solvent velocities greater than the optimal Van Deemter value without significantly affecting efficiency. Therefore isothermal-HTLC separations are normally performed at higher flow-rates. However, in temperature gradient separations, the column backpressure varies across the length of the temperature ramp. Therefore, for optimal resolution and separation speed, a simultaneous alteration in linear velocity becomes necessary. Therefore, in order to fully realise the separation benefits of a temperature gradient, optimal mobile phase linear velocity needs to be maintained throughout the chromatographic run. Moreover, flow-rate programming can help to reduce the run time in a temperature gradient separation. Thus, a flow-rate gradient perfectly complements the use of a temperature gradient. Previous studies [15–19] involving simultaneous variation in column temperature and mobile phase flow-rate have been focused on separation throughput and were performed using a conventional UV-detector. In this study we have investigated the suitability of these separation strategies for response normalisation in aerosol detectors, which to the best of our knowledge has not yet been reported.

## 2. Experimental

### 2.1. Reagents

HPLC gradient grade acetonitrile (ACN) and methanol used for the preparation of mobile phases was purchased from Scharlau Chemie (Sentmenat, Barcelona, Spain). High purity water (18.2 MΩ cm at 25 °C) was produced by a Millipore Milli-Q water purification system (Millipore, Molshiem, France). Analytical grade acetic acid, ammonium hydroxide and ammonium acetate were purchased from Sigma-Aldrich (Sydney, Australia). A mixture of sulfamethaxazole, sulfamethoxine, furosemide, prednisolone and indapamide was used to demonstrate the utility of different separation approaches. All the drug standards used in this study were of analytical grade and were purchased from Sigma-Aldrich (Sydney, Australia). Stock solutions of individual analytes were prepared at a concentration of 2 mg/mL in ACN/water (1:1 v/v) and stored in a refrigerator at 4 °C.

### 2.2. Instrumentation

A Dionex Ultimate 3300 UHPLC system equipped with dual gradient pumps, auto-sampler, column heater, photodiode array detector (PDA) and Chromeleon (version 7.1) chromatographic data processing software was used. 130 μm × 1100 cm capillary tubing was used to connect the inverse gradient pump to the mixing tee-piece. The outlet of the mixing tee-piece was further connected