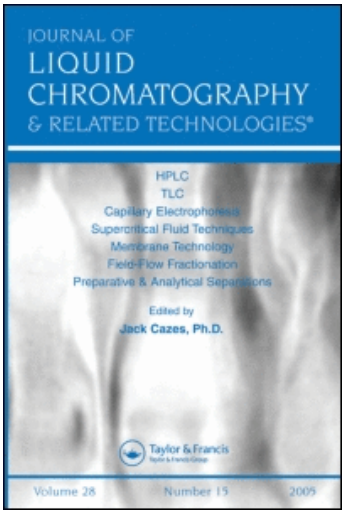

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John Reilly[a]; Brian Everatt[a]; Clive Aldcroft[a]
[a] Novartis, NIBR Research Centre, Horsham, West Sussex, UK

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Implementation of Charged Aerosol Detection in Routine Reversed Phase Liquid Chromatography Methods

John Reilly, Brian Everatt, and Clive Aldcroft

Novartis, NIBR Research Centre, Horsham, West Sussex, UK

Abstract: A new procedure for the determination of novel discovery drug substance purity was developed using LC-MS coupled with charged aerosol detection (CAD) in a walk up open access system and chromatographic purity service. Chemists require an accurate high throughput methodology to monitor reactions and to provide good assurance of final compound quality. There is a need for detection methodologies, which are accurate and precise and offer rapid, inexpensive evaluation of research compound purity without the need for specific validated drug reference standards. This new approach resulted in a more accurate assessment of purity in comparison to the standard UV approach. This technique has been successful as an approach for a walk up service for chemists and also as a chromatographic purity service at low pH.

Keywords: Charged aerosol detector, Detection, High performance liquid chromatography, Mass spectrometry, Purity

INTRODUCTION

Purity determinations on LC-MS "walk up" reversed phase systems require careful interpretation as many analytical laboratories still utilize single wavelength UV detection, typically 220 nm or 254 nm. To provide

Correspondence: John Reilly, Novartis, NIBR Research Centre, Horsham, West Sussex RH12 5AB, UK. E-mail: John.Reilly@Novartis.com

enhanced quality assurance, additional detectors, (such as ELSD and or CLND) are utilized to assess purity. The ELSD response is proportional to the injected mass of a compound,[1] whereas the CLND response is directly proportional to the percentage of nitrogen in the molecule.[2] The advantages of both ELSD and CLND are combined in the development of systems to simultaneously determine the identity, purity, and concentration of sample components from combinatorial libraries in a LC-MS-CLND-ELSD approach using a single calibration curve.[2]

However, there are limitations to both ELSD and CLND detection. ELSD has been shown to have significant limitations in regards to precision, sensitivity, and dynamic range[2–4] and volatile impurities may go undetected. CLND has been shown to be applicable for combined qualitative and quantitative analysis, although it has been reported to be less robust than UV or ELSD detection.[5] A recent report by a group at GlaxoSmithKline[6] concluded that quantitative analysis by ELSD requires analyte specific calibration to avoid significant errors, whereas CLND is suitable for single calibrant quantification of nitrogenous analytes that do not contain adjacent nitrogen atoms. A general conclusion being, that there is no detection scheme that permits true universal quantitative detection, so encouragement of further development of detection strategies was deemed appropriate.[6]

In 2002, a more sensitive version of ELSD was developed by US researchers Roy Dixon from California State University and Dominic Peterson from New Mexico Institute of Mining and Technology, Socorro, and termed aerosol charge detection.[7] The aerosol particles were not detected by light scattering but given an electrical charge by passing them close to a stream of charged nitrogen. The charged particles are then detected by an electrometer, which generates a signal in proportion to the quantity of each particle.[7] A further advantage is that there are no optical components so this detection mechanism is more economical.[7] CAD detection was commercialized by ESA Biosciences, MA, USA, in October 2004, and has won several awards including the 2005 Silver PittCon Editor's award for best new product. Recently, there have been publications on specific applications using CAD detection, namely quantification of monosaccharide anhydrides,[8] complex lipid samples,[9] meat phospholipids,[10] pharmaceutical cleaning validation,[11] and as a complimentary technique for evaluation of drug discovery screening libraries.[12]

The objective of this work was to evaluate the performance of the CAD in a one week instrument loan evaluation, to assess with confidence the purity of novel research compounds in a simple, robust, and inexpensive manner. The CAD was loaned for this study from ESA Analytical, Aylesbury, Buckinghamshire, UK.

EXPERIMENTAL

Chemicals

All test compounds were purchased from the Sigma-Aldrich Company (Gillingham, UK). Deionised water was obtained and filtered through an ELGA Maxima ultra pure water purification system (ELGA Process Water, Marlow, UK). Acetonitrile HPLC grade, acetonitrile for residue analysis, methanol HPLC grade, DMSO, and trifluoracetic acid HPLC grade was obtained from Fisher Scientific (Loughborough, UK). Nitrogen (>99.0%) was obtained from an ultra high purity generation system (Balston, Maidstone, Kent, UK).

Instrumentation and Methods

Two HPLC systems were used for this research. System 1 is a high throughput walk up open access system for reaction monitoring and system 2 is used to assess the purity of final compounds.

System 1

HPLC/UV/MS/CAD consisted of a Waters 600 pump and 2767 sample manager integrated with a 2487 dual wavelength detector and ZQ2000 mass spectrometer (Waters, Herts, UK) and Corona CAD charged aerosol detector. A Sunfire (50×4.6 mm) C18 5 μm analytical column was used on this system (Waters, Herts, UK) with mobile phase A: $H_2O + 0.1\%$ TFA and mobile phase B: ACN + 0.1% TFA. The mobile phase flow rate was 1.0 mL min^{-1}. The column temperature was 20°C. The injection volume was 2 μL. A linear gradient was performed from 5 to 95% B from 0 to 4.0 minutes. From 4.0 minutes to 4.1 minutes the mobile phase was returned to 5% B and remained constant for 0.9 minutes. The run time was 5 minutes. Test samples were prepared in 50% methanol:50% DMSO at a concentration of 0.5 mg mL^{-1}. The UV wavelength was set to 400 nm with a bandwidth of 200 nm. Electrospray mass spectrometry measurements were acquired in positive ionization mode over the mass range of 100–800 with ion source parameters used: Cone gas 50 L/hour, desolvation gas 350 L/hour, capillary voltage 3000 V, cone voltage 20 V, source temperature 140°C, desolvation temperature 250°C. CAD parameters: nitrogen gas flow 35 psi, range 100 pA, and a split ratio of 1:1 going to the MS and CAD was observed.

System 2

HPLC/UV/MS/CAD consisted of an Agilent 1100 series with diode array detector (Agilent, Waldbronn, Germany) integrated with a LCT mass spectrometer (Waters, Herts, UK) and a Corona CAD charged aerosol detector. A Phenomenex Gemini (100 × 3 mm) C18 3 μm analytical column was used on this system (Phenomenex, Macclesfield, UK) with mobile phase A: $H_2O + 0.1\%$ TFA and mobile phase B: ACN + 0.1% TFA. The mobile phase flow rate was 1.0 mL min^{-1}. The column temperature was 50°C. The injection volume was 2 μL. A linear gradient was performed from 0 to 95% B from 0 to 14.0 minutes. From 14.0 minutes to 18.0 minutes the mobile phase remained constant at 95% B. From 18.0 minutes to 18.1 minutes the mobile phase was returned to 5% B and remained constant for 1.9 minutes. The run time was 20 minutes. Test samples were prepared in 50% methanol:50% DMSO at a concentration of 0.1 mg mL^{-1}. The UV wavelength was set to 400 nm with a bandwidth of 200 nm. Electrospray mass spectrometry measurements were acquired in positive ionization mode over the mass range of 110–1200 with ion source parameters used: nebuliser gas 60 L/hour, desolvation gas 950 L/hour, capillary voltage 3000 V, cone voltage 25 V, source temperature 120°C, desolvation temperature 250°C. CAD parameters: nitrogen gas flow 35 psi, range 100 pA, and again a split ratio of 1:1 going to the MS and CAD was observed.

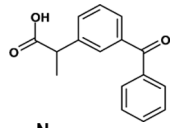
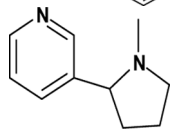
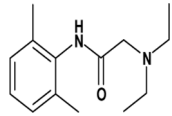
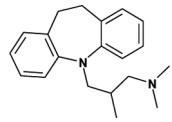
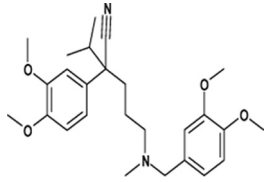
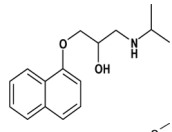
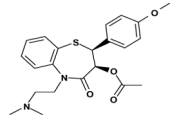
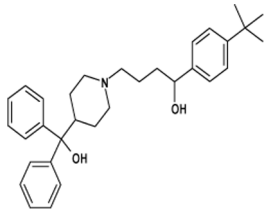
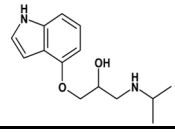
RESULTS AND DISCUSSION

System 1–Walk up System

An evaluation using a range of standard test compounds with different molecular complexity was initiated on the high throughput walk up system. An open access gradient methodology used for reaction monitoring was an ideal approach to assess the suitability of the CAD in gradient mode. This provided a real case scenario for purity assessment for a reaction monitoring LC-MS system. The test samples analyzed on system 1 are shown in Table 1. Test sample stock solutions were accurately prepared in 50% methanol:50% DMSO at a concentration of 0.5 mg mL^{-1}.

Figure 1 shows a three component mix of propranolol, verapamil, and terfenadine prepared from the sample stock solutions in a ratio of 1.0:1.0:1.1 (v/v). The UV diode array response was shown to be non-representative of concentration ratio, as verapamil (Peak 2:2.2 mins) can be seen to be approximately 50% peak area of the propranolol (Peak 1: 1.9 mins). However, the CAD detector response was very encouraging

Table 1. Structures of the standard test compounds

Compound	Structure	MW	R.T (min) System 1	R.T (min) System 2
Ketoprofen		254	2.54	12.8
Nicotine		162	0.18	—
Lidocaine		234	1.56	—
Trimipramine		294	2.20	—
Verapamil		454	2.22	10.74
Propranolol		259	1.97	9.75
Diltiazem		415	2.04	—
Terfenadine		472	2.52	11.96
Pindolol		248	—	8.06

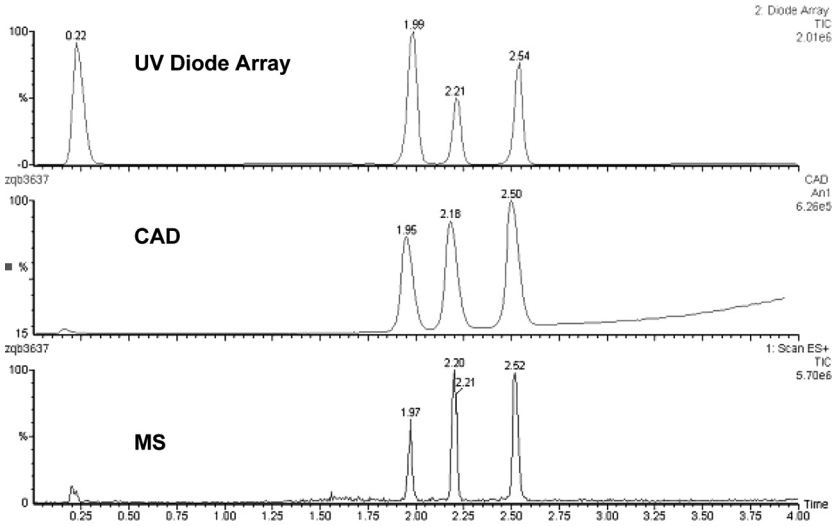


Figure 1. Three component test mix of propranolol, verapamil, and terfenadine prepared in a ratio of 1.0:1.0:1.1.

as it was the most representative of initial concentration ratio. The peak area response for the CAD detector was reproducible with a % RSD of 0.66% (n = 4). Figure 2 shows another test mix containing propranolol,

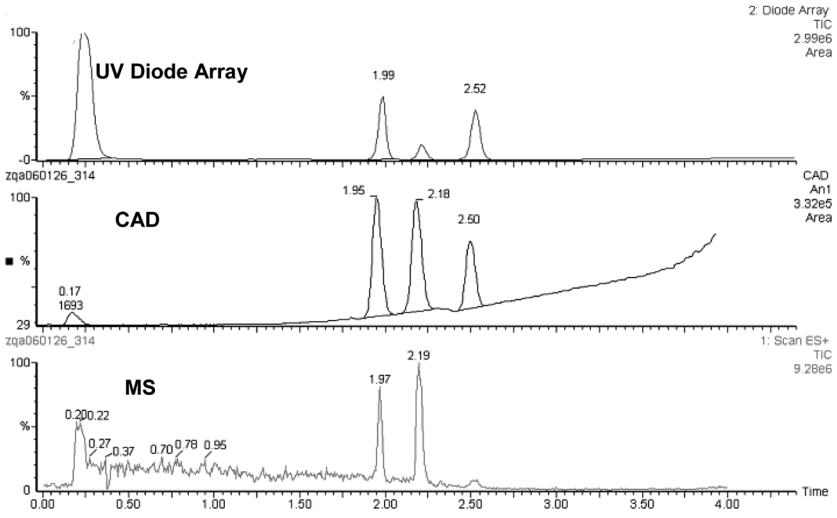


Figure 2. Three component test mix of propranolol, verapamil, and ketoprofen prepared in a ratio of 1.0:0.93:0.88.

verapamil, and ketoprofen prepared from the sample stock solutions in a ratio of 1.0:0.93:0.88 (v/v). Again, the UV response is non-representative of the concentration ratio. It is interesting to note the MS response shows only two peaks, which is due to the poor response of ketoprofen on the positive electrospray mode. The CAD response was again encouraging as it was the most representative of concentration ratio. A further experiment was undertaken to record the relative response factor (RRF) (peak area/concentration mg mL^{-1}) for the standard test compounds and compare responses. The % RSD for the RRF for the dataset was recorded as 20%, which was considered to be an impressive result, and the evaluation of the detector for use in a walk up reaction monitoring LC-MS system was deemed successful. It was noted that there is a marked rise in the baseline throughout the gradient run, which is due to the increase in proportion of acetonitrile during the analysis. An interesting follow up experiment comparing acetonitrile HPLC grade with acetonitrile residue analysis grade shows a marked decrease in baseline drift when acetonitrile residue analysis grade is used as shown in Figure 3, which implies impurities observed within acetonitrile can also increase the baseline drift. Recently, a group from Pfizer have used a mobile phase compensation strategy employing a second pump to apply constant mobile phase composition post chromatography to the CAD detector[13] to reduce baseline drift and improve detector response. We did try and evaluate the CAD in a high pH mobile phase incorporating additive 0.1% NH4OH in place of the 0.1% TFA, however, CAD background response was considerably increased in this mode and further experiments aborted. An approach

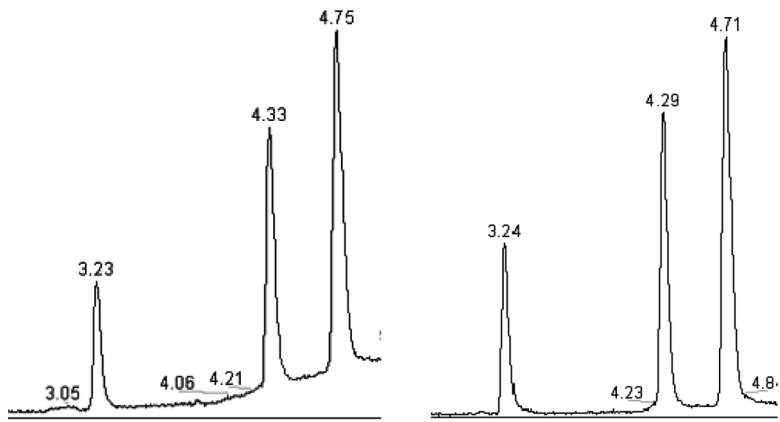


Figure 3. Comparison of CAD baseline noise. (a) Acetonitrile HPLC grade, (b) Acetonitrile residue analysis grade.

such as the mobile phase compensation approach[13] may have been of value for further research into high pH mobile phases.

Further experiments were completed at low pH to test the system with novel discovery compounds synthesized in-house. An example is shown in Figure 4 where the purity of sample is seen to be significantly less on the CAD (70.6%), than on the UV diode array trace (89.8%). This trend was apparent for a batch of prepurification samples and post-purification yields on this batch tied up more closely with prepurification CAD purity results.

System 2–Final Compound Purity System

A longer 20 minute linear gradient system was employed to assure purity of final discovery compounds. The errors that can occur when assessing purity by UV is highlighted in Figure 5 where a five component test mix composed of pindolol, propranolol, verapamil, ketoprofen, and terfenadine was prepared at a concentration of $0.1\,mg\,mL^{-1}$. Extracted chromatograms at 280 nm, 254 nm, 220 nm, and diode array scan show gross differences due to the chromophoric nature of the test samples. Examples of this are seen at 280 nm where only four compounds are detected and at 254 nm the late eluting terfenadine UV response masks the other compounds response. This five component test mix was deemed an excellent tool to evaluate the CAD

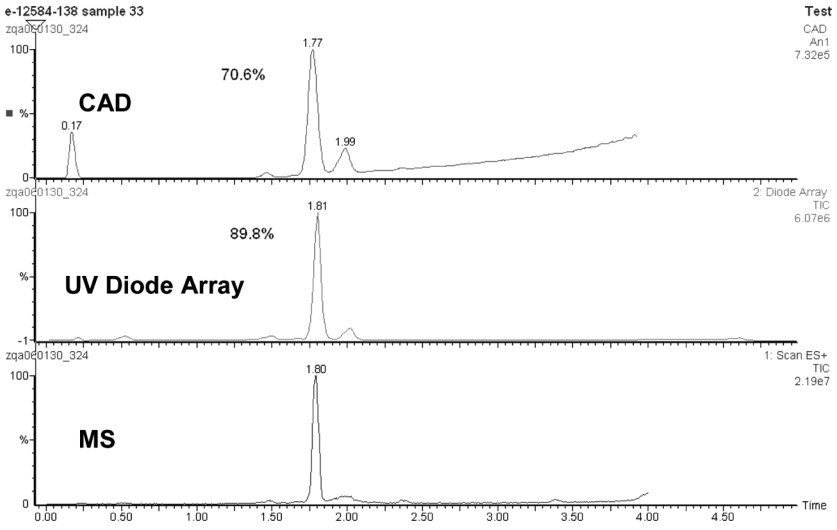


Figure 4. Novel discovery sample: CAD purity in comparison to UV diode array.

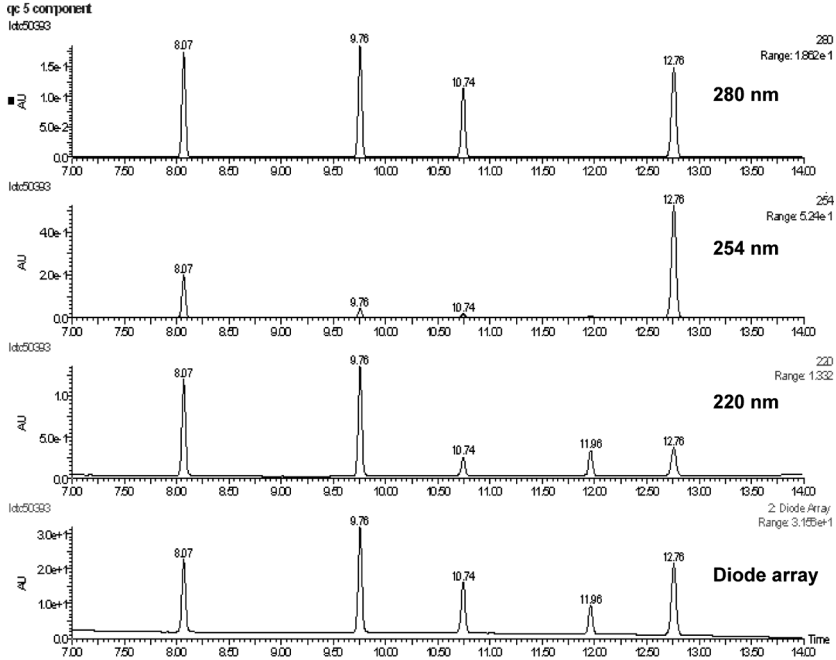


Figure 5. UV response for five component test mix prepared at 0.1 mg mL^{-1}.

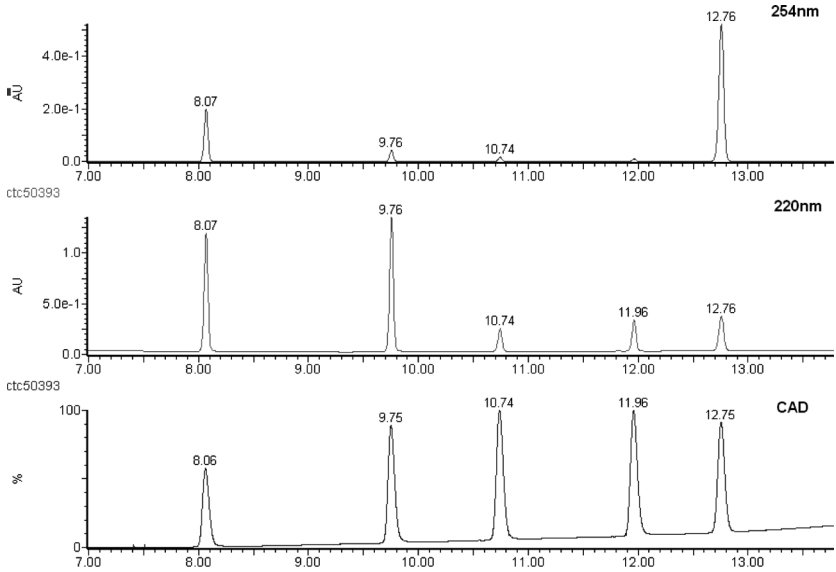


Figure 6. CAD v UV response for five component test mix prepared at 0.1 mg mL^{-1}.

response. Figure 6 shows the CAD response for the five component test mix with near uniform response in respect to concentration and was the most quantitative.

CONCLUSION

The applicability of CAD detection for the assessment of purity for two different reversed phase systems has been evaluated and deemed to be successful. The technology has been shown to be very easy to use and sensitivity is good for discovery sample purity applications. On the basis of this evaluation, Novartis has invested in the CAD technology. It is used in addition to MS and UV detectors and is in daily use within the analytical laboratory. Further additional applications for the CAD are conceivable, an example being the use in solubility determinations to support drug discovery.

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