

# CAD Detector validation and potential for Physicochemical Measurements

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# CAD Detector validation and potential for Physicochemical Measurements

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## Aims of Study

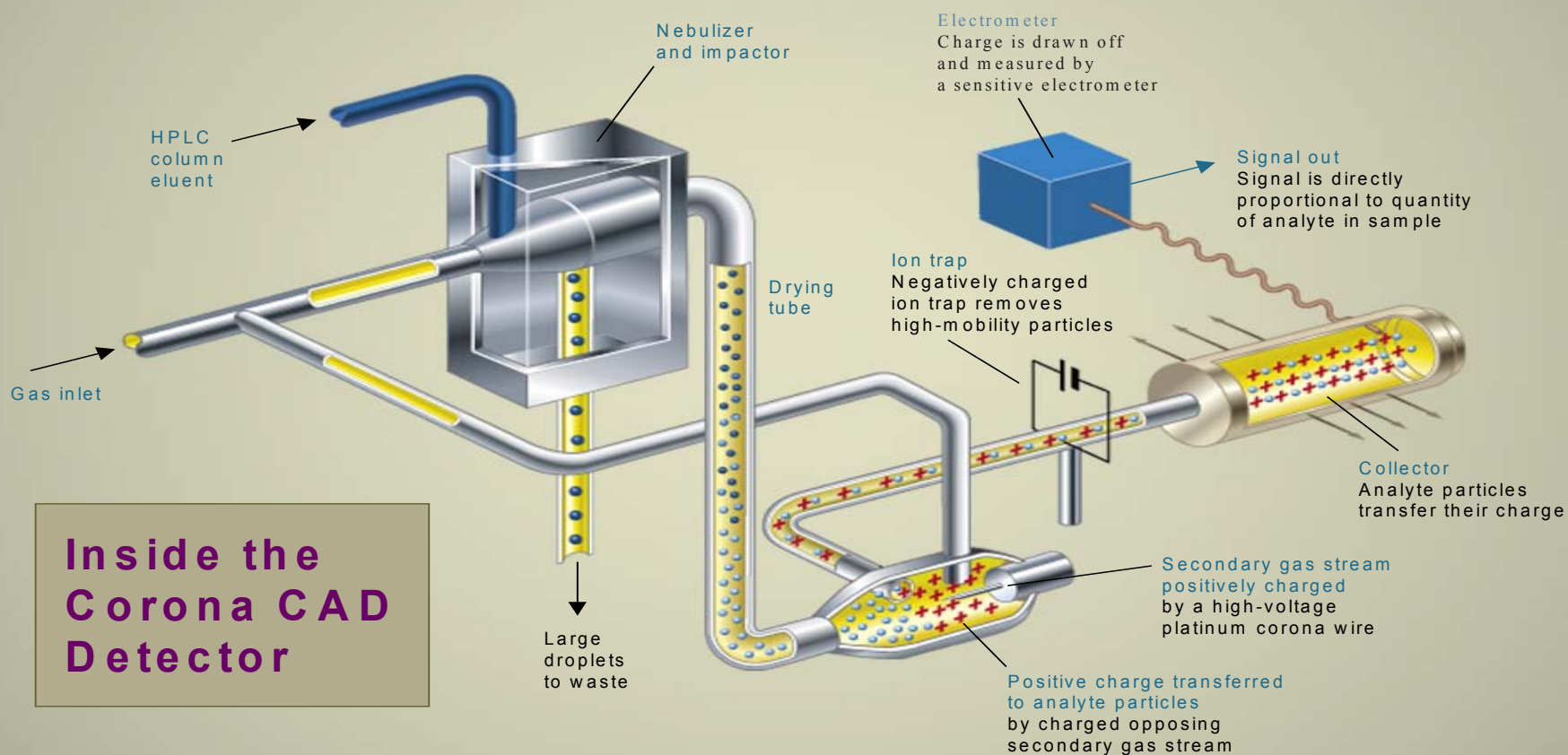
- Comparison with other detectors and overview of CAD technology
- Purity – Why would we require a more universal detector?
- Validation and evaluation – Results and examples of use
- Discussion – How could the CAD detector be useful for Physchem?

# CAD Detector validation and potential for Physicochemical Measurements

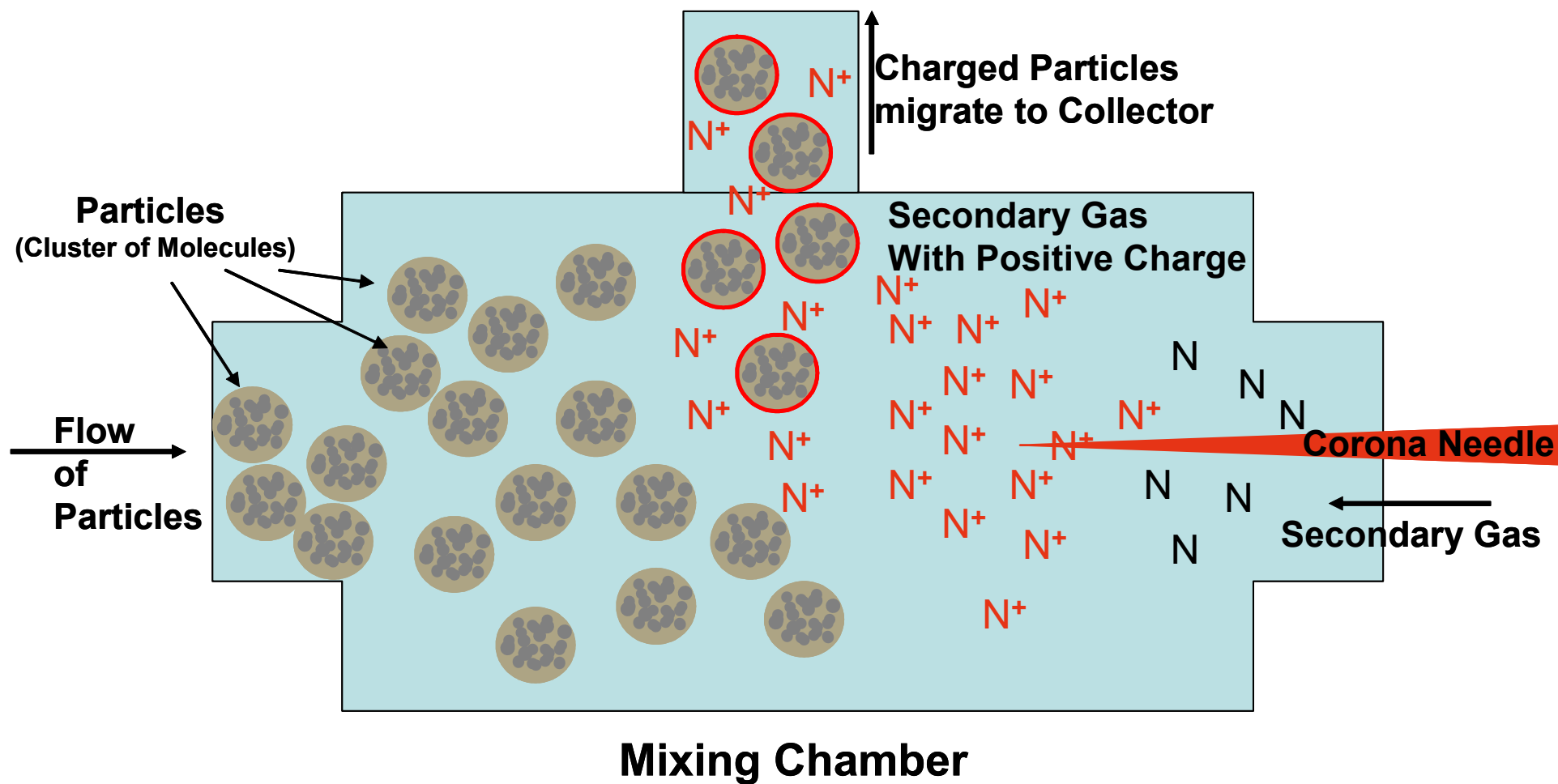
	<b>Pros</b>	<b>Cons</b>
<b>UV Detection</b>	Good sensitivity, good dynamic range, reproducible response, ease of use	Range of response for different chromophores, standards of same compound required for accurate quantitation
<b>CLND Detection</b>	Good sensitivity, adequate dynamic range, adequate consistency of response, external nitrogen containing standards can be used for quantitation	Ease of use – requires dedicated instrument analytical support, only nitrogenous compounds detected, nitrogen rules required for N=N bonds, recurring nebuliser issues, expensive, cannot use acetonitrile in mobile phase
<b>ELSD Detection</b>	Adequate sensitivity, ease of use, inexpensive, range of instrument parameters	Poor dynamic range, reproducibility can be poor, recurring nebuliser issues, volatile samples may not be detected dependant upon conditions
<b>CAD detection</b>	Good sensitivity, good dynamic range, consistent response, good reproducibility, adequate dynamic range, ease of use, external standards can be used for quantitation	Knowledge of mechanism

# CAD Detector validation and potential for Physicochemical Measurements

Corona™ CAD™



# CAD Detector validation and potential for Physicochemical Measurements

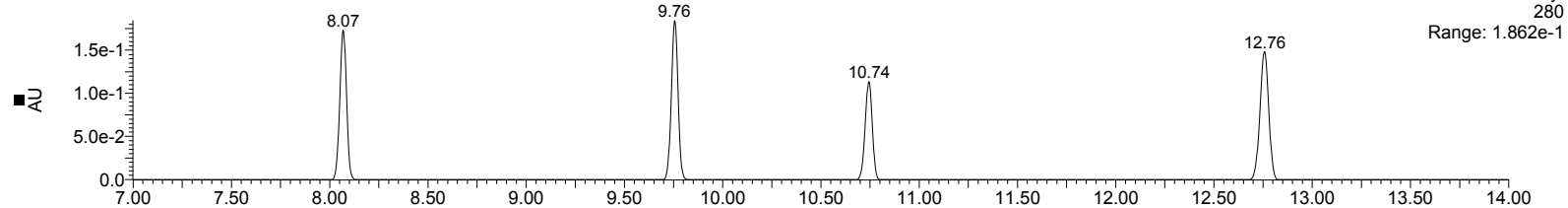


# CAD Detector validation and potential for Physicochemical Measurements

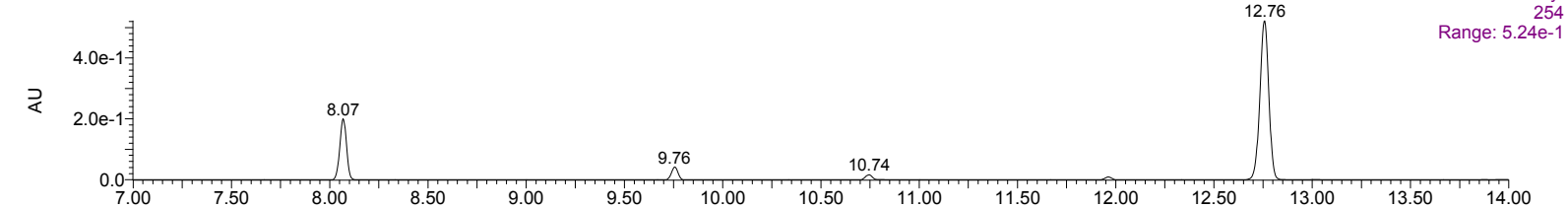
*0.1 mg ml<sup>-1</sup> testmix pindolol, propranolol, verapamil, ketoprofen and terfenadine*

qc 5 component

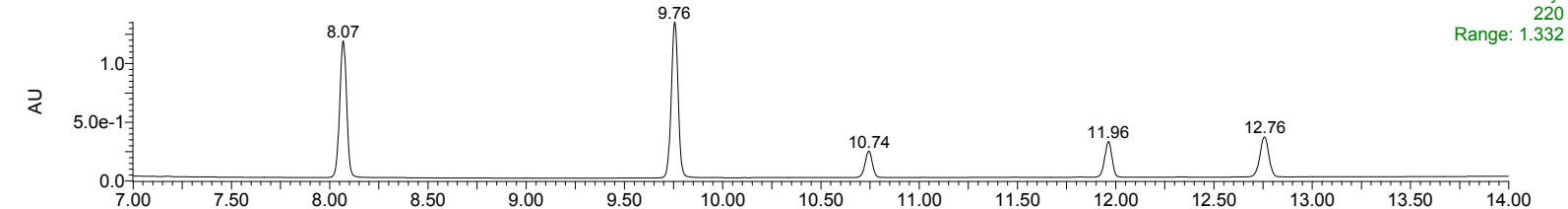
lctc50393



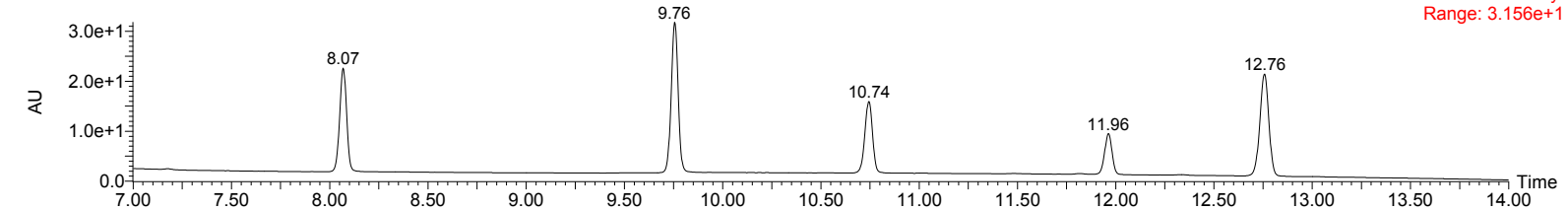
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lctc50393

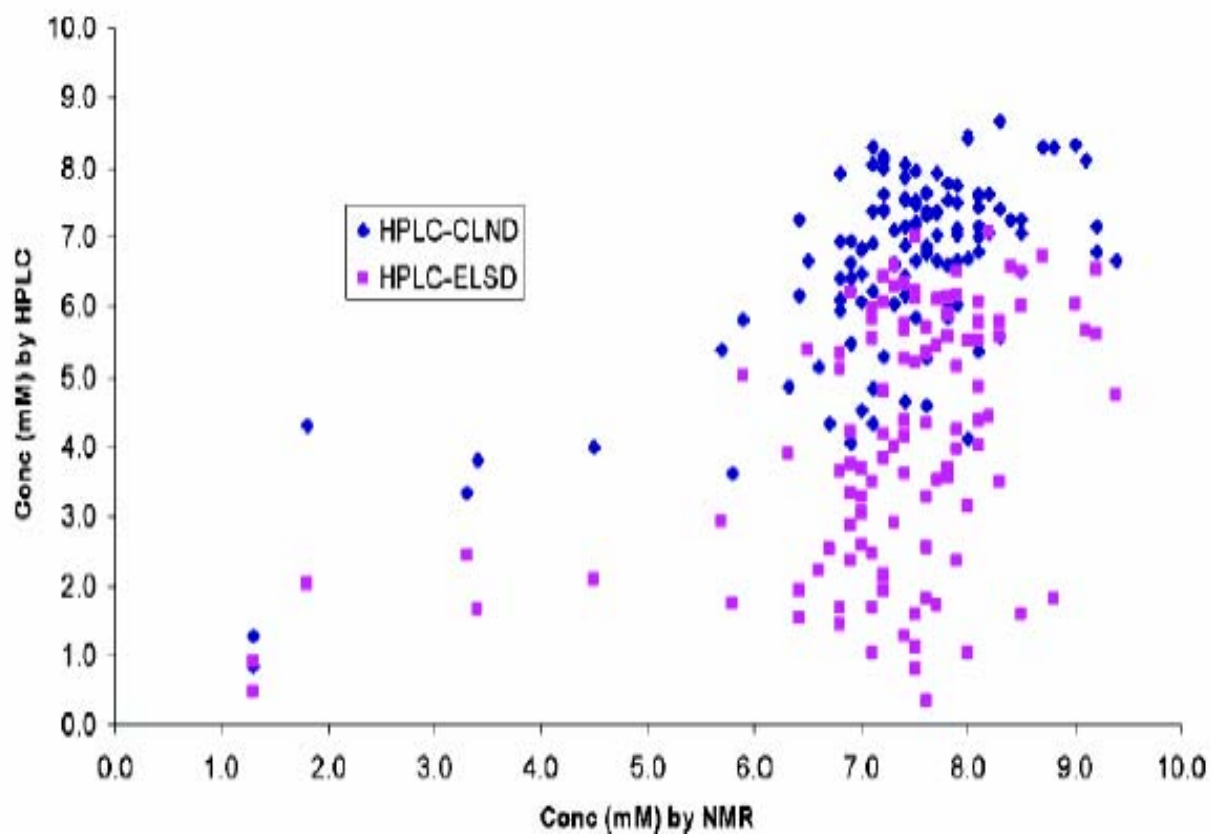


lctc50393



*Non-uniform response at all detection wavelengths*

# CAD Detector validation and potential for Physicochemical Measurements

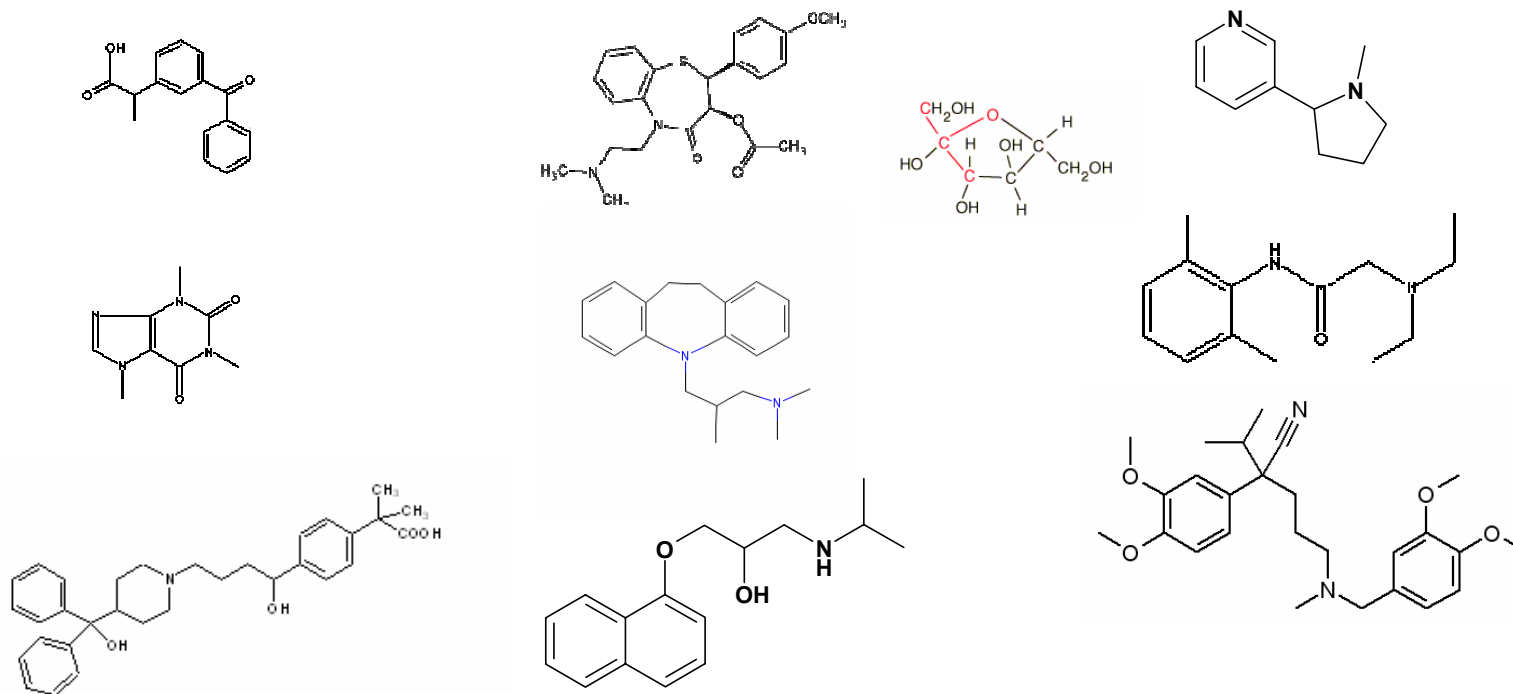


**Figure 8.** Comparison of results of "blind" quantification of 117 samples using HPLC-CLND and HPLC-ELSD with the values obtained using NMR with the ERETIC method.

*S. Lane, B. Boughtflower, Ian Mutton et al. Anal Chem. 2005, 77, 4454-4365*

# CAD Detector validation and potential for Physicochemical Measurements

## Evaluation



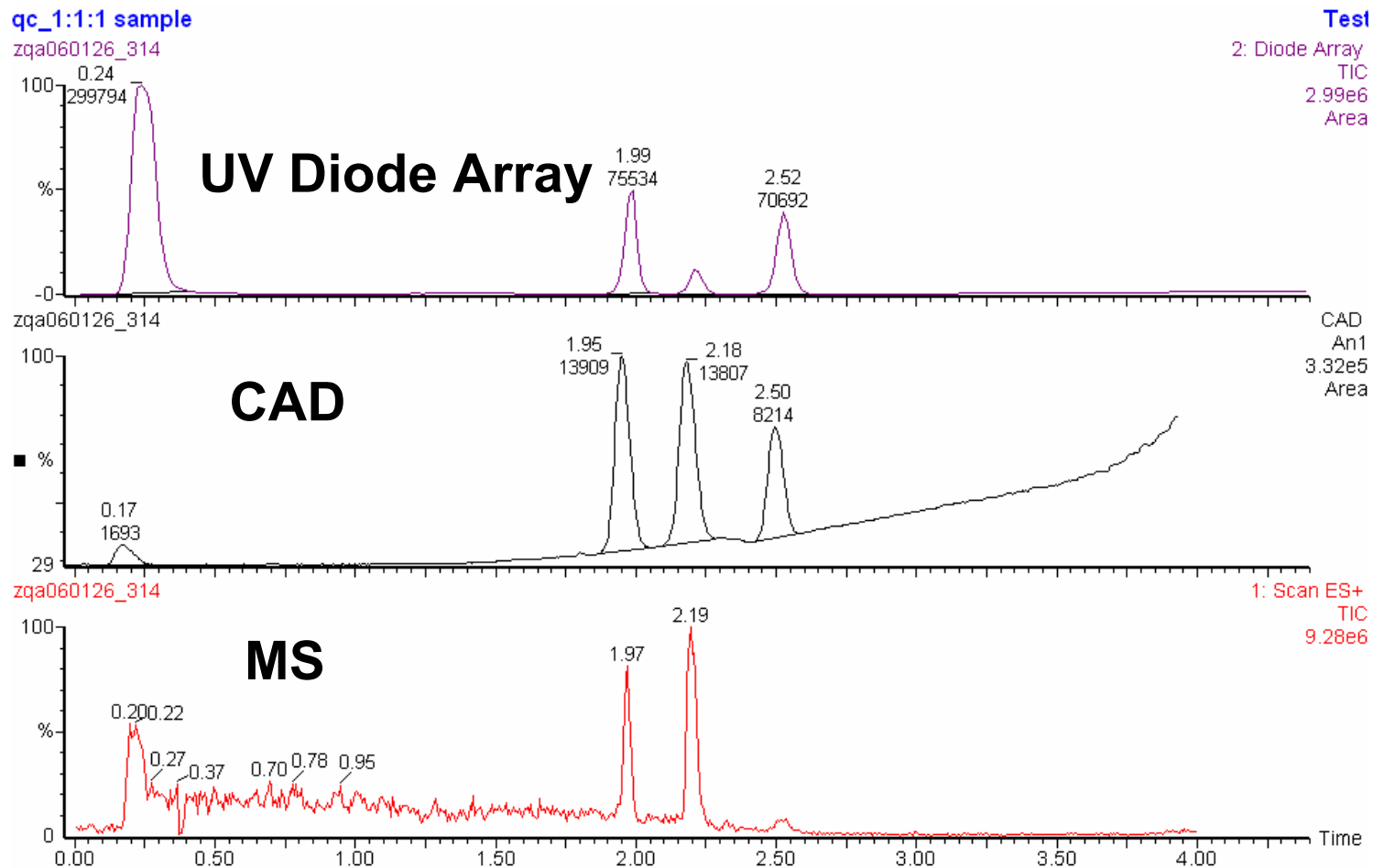
*Test standards selected with a range of molecular diversity*

*Analysed on 5 min 5-95% Acetonitrile –water (0.1TFA) gradient, Standard C<sub>18</sub> column*



# CAD Detector validation and potential for Physicochemical Measurements

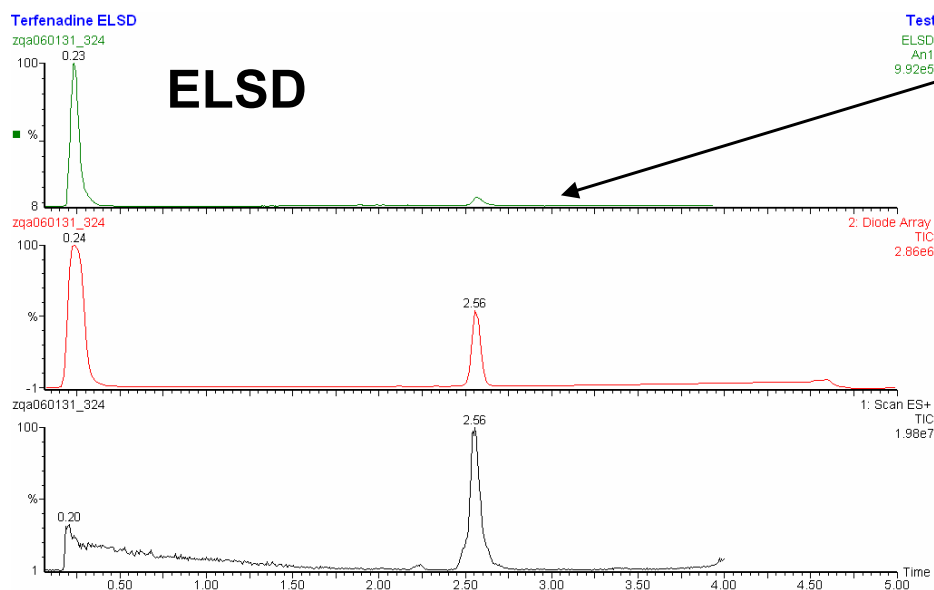
Three component testmix of propranolol, verapamil and ketoprofen



Prepared in 1.0: 0.93 : 0.88 ratio

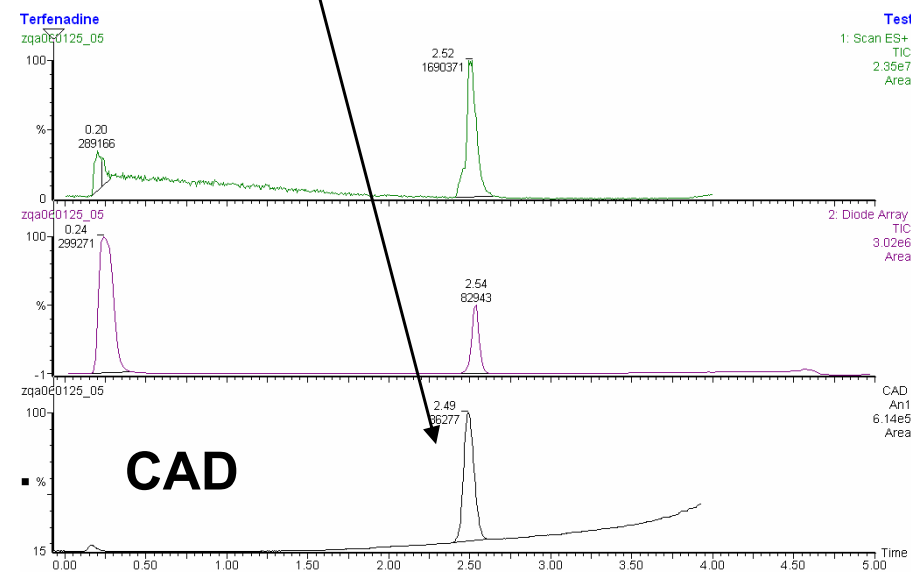
# CAD Detector validation and potential for Physicochemical Measurements

## Evaluation

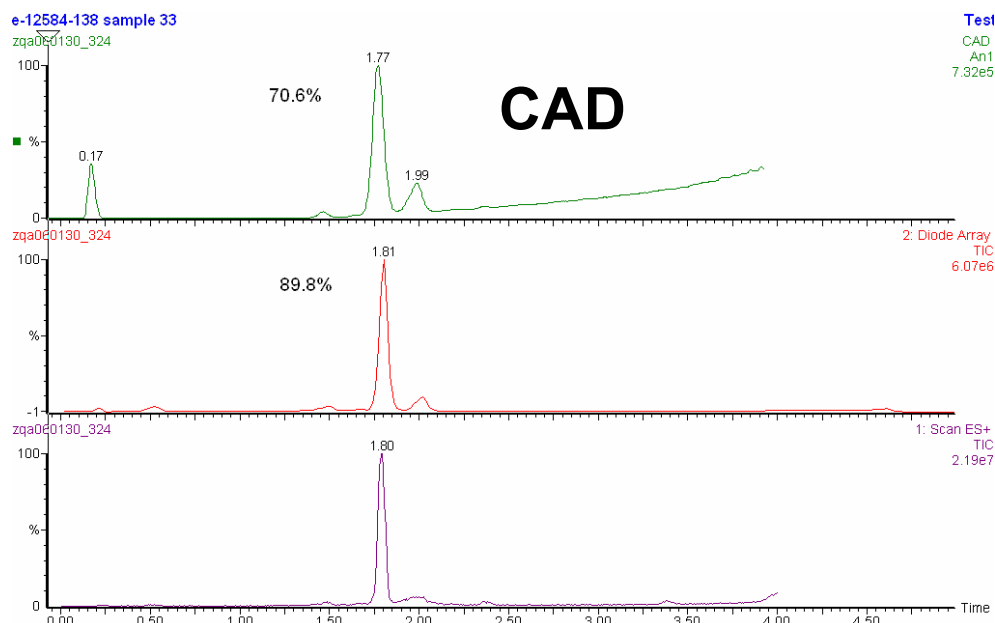


Terfenadine 0.6 mg/ml

Superior response

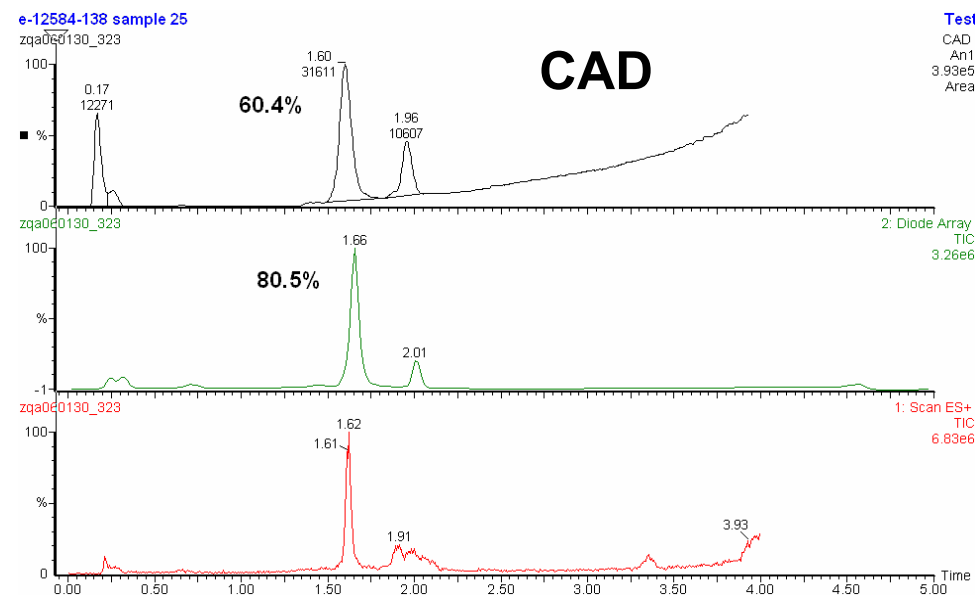


# CAD Detector validation and potential for Physicochemical Measurements



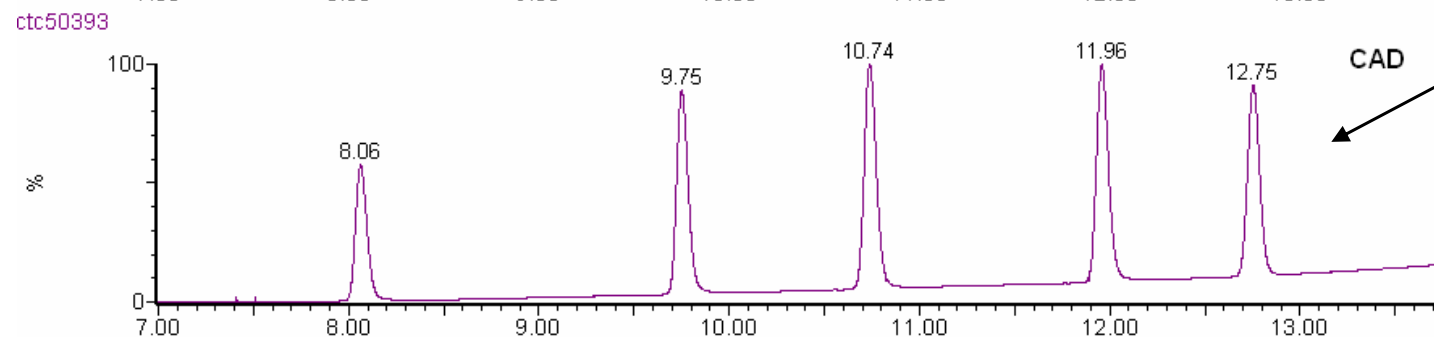
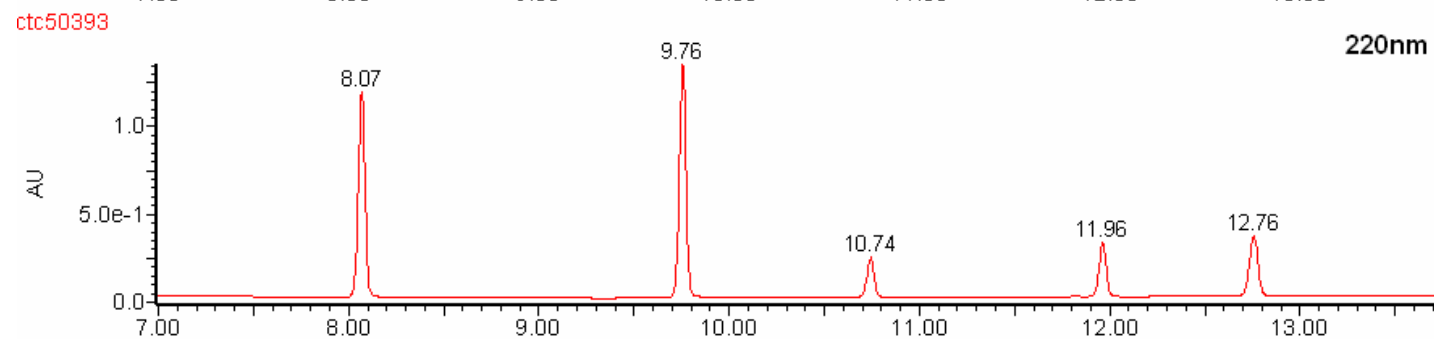
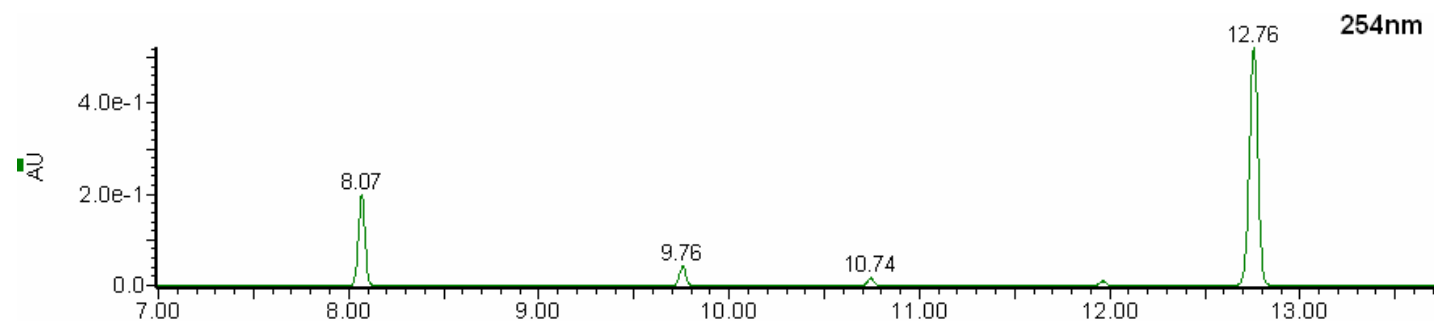
CAD – less product detected

- links in with recovery results post purification



# CAD Detector validation and potential for Physicochemical Measurements

*0.1mg ml<sup>-1</sup> testmix pindolol, propranolol, verapamil, ketoprofen and terfenadine*



*Uniform response*

*Analysed on 20 min 5-95% Acetonitrile –water (0.1TFA) gradient, Standard C<sub>18</sub> column*

# CAD Detector validation and potential for Physicochemical Measurements

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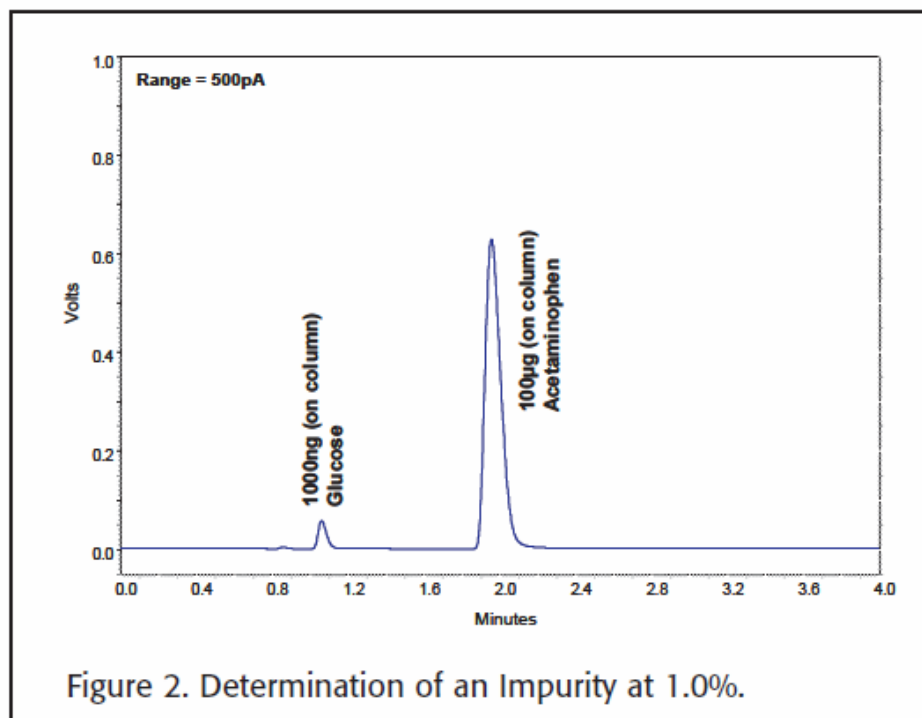
## Evaluation Recommendations

- Results of evaluation justify purchase of technology
- Purity – Use as an orthogonal detector for open access in place of ELSD
- Purchase of two detectors April 2006, two more March 2007

# CAD Detector validation and potential for Physicochemical Measurements

Discussion - How can the CAD detector be useful for physchem analysis?

## 1) Unrelated Impurity quantification – e.g glucose in Acetaminophen?



Gas: 35psi via nitrogen generator  
Filter: none  
Range: variable

HPLC Parameters:  
Mobile Phase A: 90:9:1 (v/v/v) Water: methanol: acetic acid  
Mobile Phase B: 100% Acetonitrile  
Isocratic: A:B, 90:10 (v/v)  
Flow Rate: 1.5mL/min  
Column: MCM column; 4.6 x 150mm; 5µm  
Column Temperature: ambient  
Injection Volume: 10µL

ESA Analytical  
70-6716

Application Note

# CAD Detector validation and potential for Physicochemical Measurements

Discussion - How can the CAD detector be useful for physchem analysis?

## 2) Difficult samples to analyse by UV – e.g, amino acids, lipids

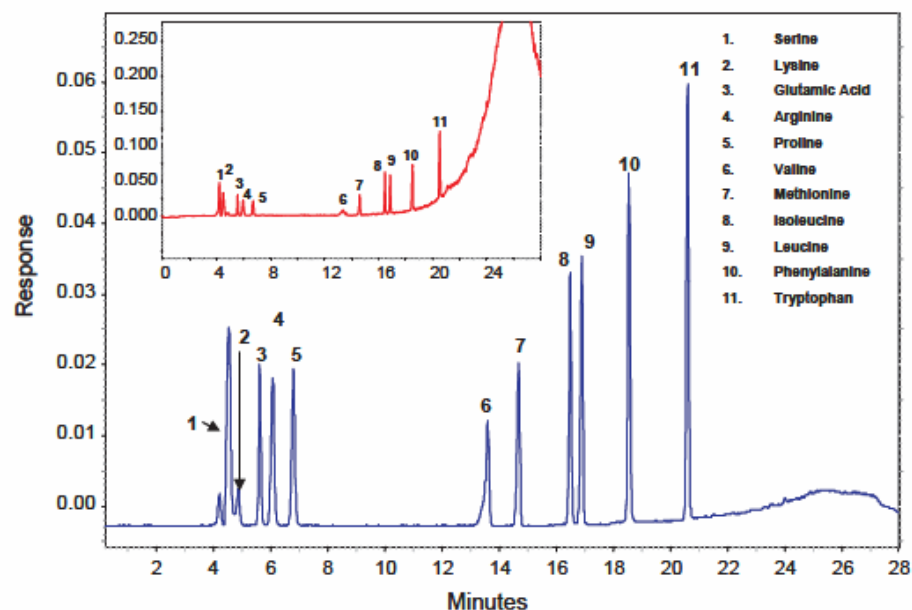
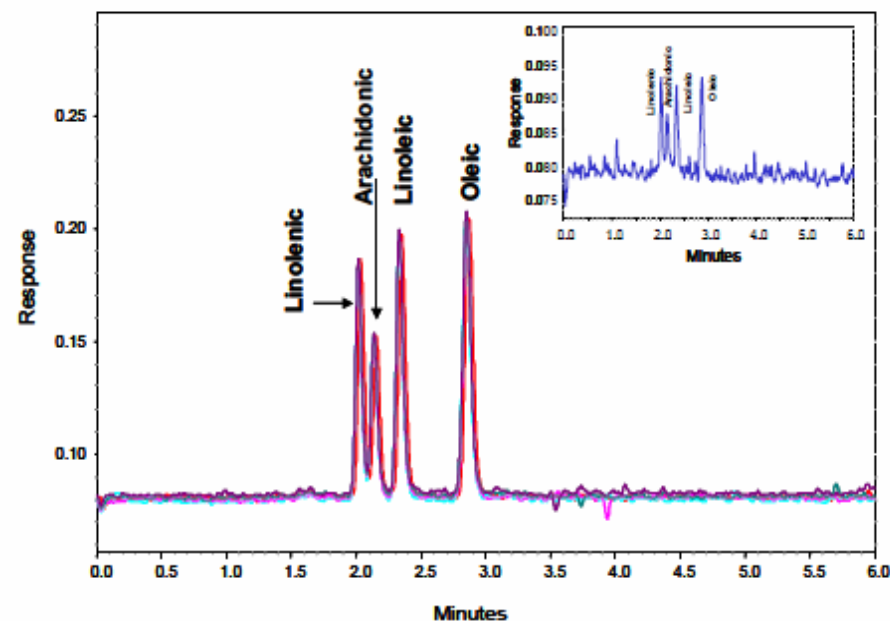


Figure 1. Amino Acid standards (100ng each on column). Inset - 10ng each.



Unsaturated fatty acid standards (200ng each on column). Inset - 20ng each on column.

ESA Analytical  
706641

Application Note

ESA Analytical  
Brain Lipids

Pittcon Application Note

# CAD Detector validation and potential for Physicochemical Measurements

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Discussion - How can the CAD detector be useful for physchem analysis?

**3) Lipophilicity – Solubility - Stability measurements – Future work?**

**Example - Run R.P gradient (standards with different lipophilicities, concentrations)**

- Extrapolate concentration i.e Kinetic solubility
- Extrapolate lipophilicity – Retention time marker
- Interrogate stability

**Result - Three results on one chromatogram – Wishful thinking?**



# CAD Detector validation and potential for Physicochemical Measurements

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## Acknowledgements

- Brian Everatt, Analytical Team Leader, NIBR, Horsham
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- Clive Aldcroft, Analytical Scientist, NIBR, Horsham
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- David Carreiro, Applications Specialist, ESA, USA