Developments in ELSD Technology to Improve Sensitivity and Linearity of Response over a Wider Dynamic Range

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UV and diode array have always been the detectors of choice for HPLC/UHPLC because of high sensitivity and linearity of response. However, for compounds that have little or no UV chromaphore, an alternative detection technique is required. Refractive index detection (RID) has often been used for carbohydrates, polymers and other compounds mainly based on carbon and hydrogen. The limitations with RID have always been a relative lack of sensitivity, long warm up and equilibration times coupled with lack of solvent gradient compatibility. The quest for a 'universal detector' started as long ago as 1966, when Union Carbide developed the technique of Evaporative light scattering [1], the technique wasn't commercialised until the 1970's and there was a very slow adoption by potential customers. However over the year's evaporative light-scattering detection (ELSD) has become a popular detector choice for HPLC/UHPLC. The past limitations of this detector were its limited sensitivity and dynamic range when compared to UV detection and did not perform well with low molecular weight compounds, which are destroyed by the elevated temperatures employed to remove the solvent from the eluent stream. So although the sensitivity exceeds RID by at least an order of magnitude the quantification of low-molecular-weight compounds has always proved to be a challenge. Also lack of reproducibility, change in compound response over a gradient and SFC compatibility have been limitations that have prevented the technique from realising its full potential. This article illustrates how the technique has evolved to overcome these issues.

ELSD – the Technique

ELSD is based on three well-defined stages, nebulisation, evaporation and detection **(Figure 1).**

Liquid flow from a column is nebulised using nitrogen or air to give a plume of fine droplets containing the analyte in solution. The liquid droplets pass into a heated zone where the solvent is removed to leave the dried analyte particle which is subsequently irradiated with light. The amount of light scattered is then measured. Gas flow and temperature can be adjusted to optimise the response of the ELSD.

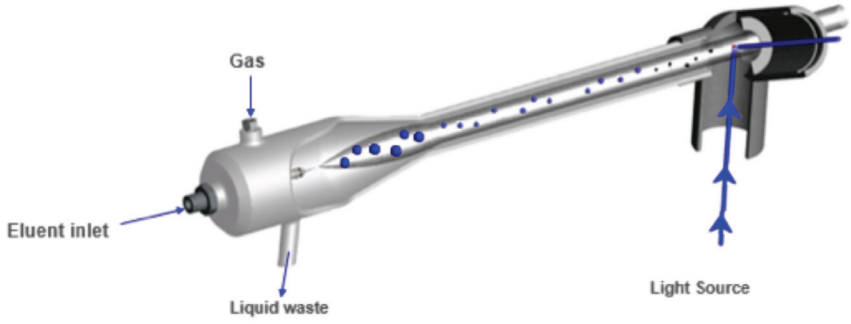


Figure 1: Generalised ELSD design

Nebulisation

The initial nebulisation step within ELSD is fundamental to the performance of the instrument. The detector's sensitivity and reproducibility are largely determined by the quality of nebulisation [2]. The aim of nebulisation is to produce a narrow distribution of droplets. The droplet size must be optimised to ensure it is as large as possible yet still able to evaporate the solvent along the drift tube. If the droplet size is too large, it will either drain to waste, which limits the amount of sample that is transported to the optical region or will result in high noise from insufficient evaporation. If the droplet size is too small, the resulting particle size will limit the sensitivity at the detection stage. The optimisation of droplet size and distribution is controlled by the user when adjusting the nebulisation gas flow.

Previous detector designs used cross flow nebulisers, where the gas is passed at right angles to the liquid flow, because they are efficient and less susceptible to blockages. Modern instruments typically use nebulisers with a concentric design, where a sheath of gas surrounds the inner liquid core to produce droplets. Precision is significantly improved to <2% compared to earlier designs and are typically manufactured from stainless steel or glass (**see figure 2**). In some designs, the nebuliser can be heated, which is required for supercritical fluid chromatography where depressurisation causes cooling around the nebuliser tip, leading to poor reproducibility or blockages.

In addition to high-performance nebulisers, ELS detectors today also employ various nebuliser chamber designs to control the amount and size of droplets that pass into the evaporation tube; this is particularly important for high aqueous mobile phases. The majority of detector designs have specifically shaped nebuliser chambers that act as 'filters' to only allow droplets of the optimum size reaching the detection region. Whilst other approaches use peltier cooling

within the nebuliser chamber to achieve the same effect.

These advances in nebuliser design allow modern ELS detectors to operate with high sensitivity in highly aqueous mobile phases across a range of flow rates and temperatures.

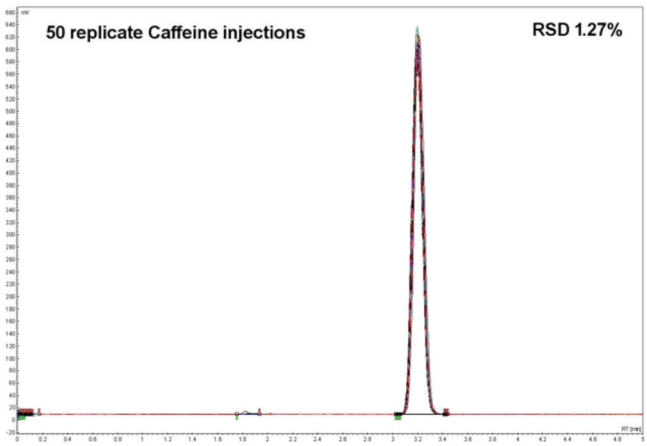


Figure 2: ELSD Precision (Glass Nebuliser)

Low Temperature Solvent Removal

Typically, two types of evaporator design are used in modern detectors to remove the solvent; these are commonly referred to as Type A and B configurations. Detectors using type A configuration operate without aerosol splitting, therefore the whole aerosol from nebulisation is passed through the drift tube and into the optical region.

In type B configurations, the nebulised solvent aerosol is passed through a specially designed chamber where the larger droplets impact the walls and are sent to waste, whereas the droplets of optimum size pass into the drift tube for evaporation.

Since the inception of ELSD, sample volatility was identified as a major influence on ELS sensitivity [3]. Consequently, over the last 30 years ELSD designs have been driven to improve detection of increasingly semi-volatile analytes within highly aqueous mobile phases. This demand has been made acutely more difficult with UHPLC where separation times demand smaller detector volumes in order to deliver the required resolution [4]. .

Most early designs were of the type A configuration and needed evaporation temperatures between 50-100°C to operate with mobile phases containing high water content. These elevated temperatures prevented detection of thermally labile compounds and restricted the application of the technique as a Universal detector.

Currently available instruments are now designed to operate at temperatures of 30°C or below, facilitating the analysis of thermally sensitive compounds previously undetectable with older designs. These instruments are typically of the type B configuration.

Of today's detectors based on the type A evaporator design, some drift tubes are long, narrow and coiled to provide more time for the droplets to evaporate at lower temperatures. Or they use a 'gate' system to control the amount of vapour within the evaporation tube [5] depending on the volatility of the mobile phase. Some designs based on type B configurations use additional gas to accelerate evaporation which facilitates the removal of water-based eluent in a very short drift tube. Not only does this keep the detector dead-volume to a minimum which is required for UHPLC, but this proprietary evaporator gas coupled with a peltier cooled drift tube can achieve sub-ambient evaporation down to 10°C [6].

The benefit of sub-ambient evaporation is clearly demonstrated in **Figure 3**, where improved detection of the volatile C12, C16:0 and C18 FAMEs is achieved at 10°C but goes undetected or greatly reduced at 40°C.

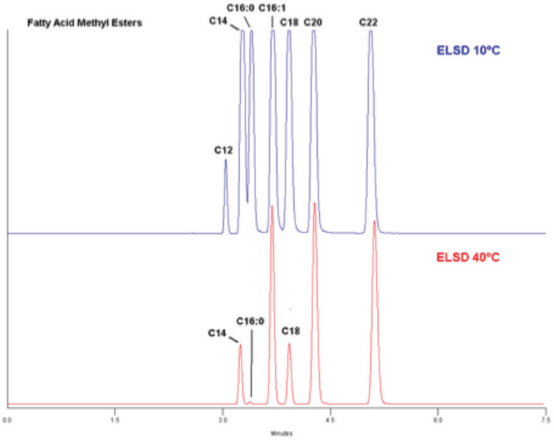


Figure 3: FAMEs detection at different evaporator temperatures

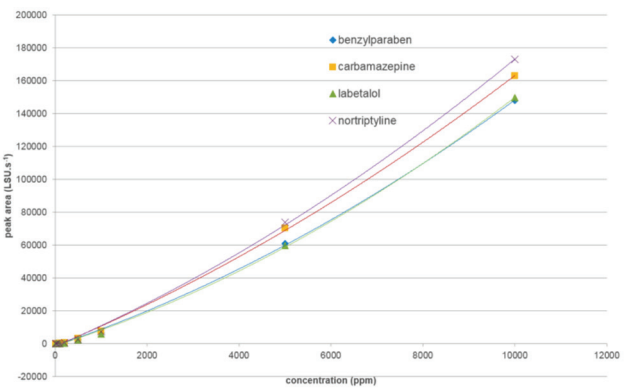
Improvements in Sensitivity, Linearity and Dynamic Range

The light-scattering mechanisms within ELSD are well documented and understood [2] [5]. It is known that the sensitivity of an ELSD can primarily be improved by several factors, such as increasing the initial droplet size at nebulisation, increasing the power in incident light during detection, or reducing the wavelength of the light source. Tungsten halogen lamps were used in early ELSD designs, but these emit a broad range of wavelengths, typically towards the near-infra red region. By replacing these light sources with LEDs operating at 470-480nm (Blue region), modern ELSDs have improved their sensitivity considerably [6].

In recent years, designs have moved further to use a blue-violet LASER light source (405nm), which combines the short wavelengths with high intensity for optimum sensitivity. ELSDs with laser sources can improve detection by a factor of 9-10 times greater than those with an LED source, [7] with reported limits of detection to <1 ng on-column [7].

The increased sensitivity exposes the short dynamic range of the technique, which is often cited as a weakness of the technology. Until recently, it was recognised that ELS detectors have a dynamic range of 3-orders of magnitude due to its non-linear response.

The latest designs of ELSDs can deliver 4-orders of dynamic range by using a specially designed high-dynamic-range photomultiplier. This new design not only extends the detector's dynamic range but also improves the linearity of the detector, as shown in **Figure 4** [8].



Compound	Correlation
Benzylparaben	0.9995
Carbamazepine	0.9993
Labetalol	0.9995
Nortriptyline	0.9992

Figure 4: ELSD Calibration response from 10-10,000ppm for typical pharmaceutical compounds.

In certain cases it is possible to achieve a linear calibration curve with a coefficient better than 0.999 across the four orders of dynamic range (**Figure 4**). This offers greater confidence in results compared with a more traditional log/log fit.

It is now also possible to detect the main compound, impurities and additives in a single run using a linear calibration model rather than having to make two injections. The first to detect and quantify the main compound, the second to analyse components of the sample present at significantly lower concentration. **Figure 5** illustrates how with conventional ELSD any attempt to analyse all the compounds in a sample results in the main compound peaks becoming 'flat topped' as the dynamic range is exceeded. Even then not all of the compounds can be detected from a single injection. The red trace shows how the extended dynamic range of the Infinity II ELSD makes it possible to reduce the analysis time by a factor of two and get a complete picture of the sample of interest from just one injection.

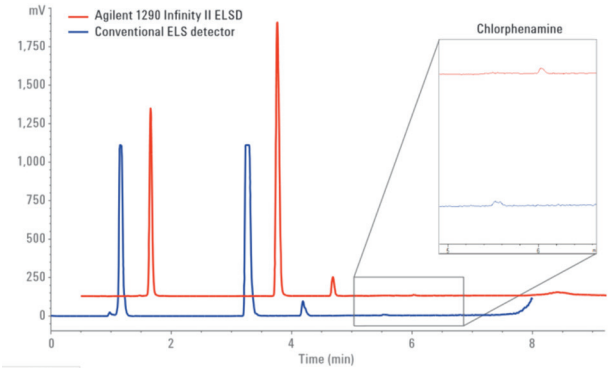


Figure 5: Comparison between Agilent 1290 Infinity II ELSD (red) and conventional ELSD for fixed dose analysis.

Consistent Response across Solvent Gradients

Universal quantification using single compound calibration is a desirable approach in drug discovery, where standards are not available [8]. The universal nature of ELSD and its solvent gradient compatibility lends itself well to universal quantification. This is especially true when sub-ambient evaporation is used to maximise sensitivity to semi-volatile compounds.

While lower evaporation temperatures provide a more accurate mass balance for a sample mixture, truly representative results can only be obtained by overcoming solvent gradient effects. It is widely documented that peak response changes according to solvent composition across a gradient [9] [10][11]. When this effect is extremely severe it can be compensated for using the complex process of running an inverse gradient with a second pump.

Another and more cost-effective approach is to use gas flow programming to compensate for gradient effects. Certain ELSD designs use proprietary gas flow control and dedicated software to program a gas flow gradient to maintain a constant ELSD response. The ELSD gas can be programmed to control the number of particles within the optical region, thus compensating for the change in droplet size distribution that occurs during a solvent gradient. This flow control gives a uniform response for all eluting compounds as demonstrated in **Figure 6**.

Further Improvements

As chromatography techniques have expanded, ELSD has been adopted for LC/MS and SFC. Although Mass Spectrometry is highly sensitive and widely used as an information rich detector, it is not universal and some analytes do not ionise well using standard ion sources. These problems can be overcome by coupling to an ELSD.

Supercritical fluid chromatography (SFC) provides faster, higher resolution chromatography compared to HPLC. ELSD is a complementary detection technique because of the gaseous nature of the mobile phase. Using SFC/ELSD, evaporation of the eluent is not required as the mobile phase is volatilised during nebulisation. A heated nebuliser fitted as standard is required for SFC operation to avoid ice crystals forming at the nebuliser and causing undesirable spikes and excessive baseline noise.

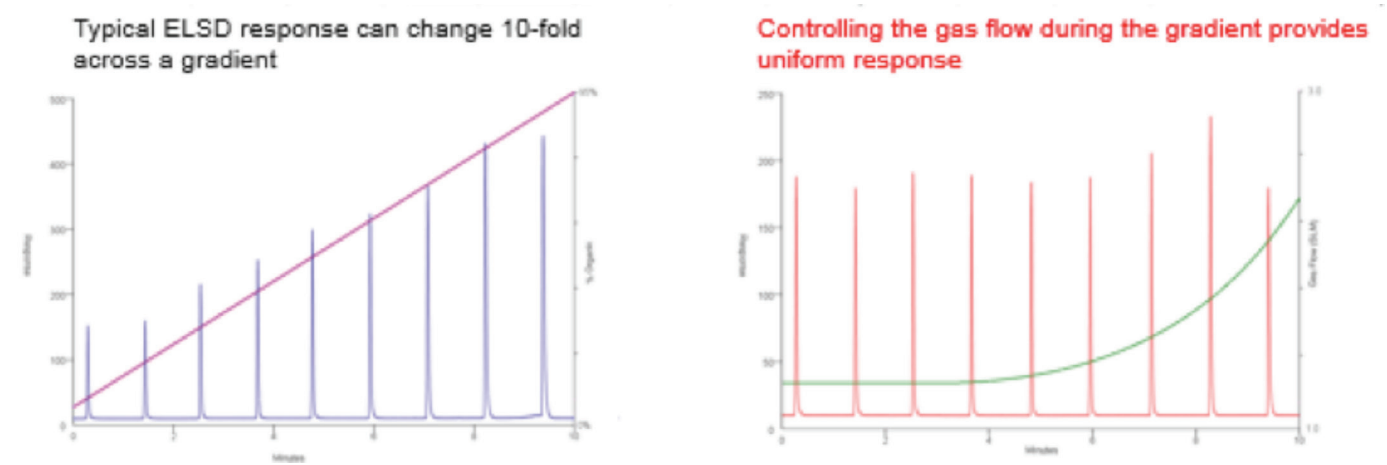


Figure 6: Use of Gas flow programming to control ELSD response across a gradient

Since many pharmaceutical compounds are stored in DMSO, for the majority of detection techniques the solvent must be removed prior to analysis, otherwise early eluting compounds may be masked by the DMSO peak, which elutes close to the solvent front. A unique feature allows full gas flow control to remove the DMSO completely saving sample preparation time. [10][11].

Conclusion

As developments in design have advanced, evaporative light scattering detection has substantially improved as a valuable universal detector in chromatography.

Improvements in precision and limit of detection have made the detector a realistic choice for compounds that have little or no UV chromaphore. Likewise, the recent advances in wider dynamic range, coupled with gas flow programming, UHPLC compatibility and sub-ambient operation offer the analyst a powerful analytical tool for their laboratory.

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