An Investigation into Detector Limitations Using Evaporative Light-Scattering Detectors For Pharmaceutical Applications

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Abstract

Evaporative light-scattering detection (ELSD) high-performance liquid chromatography (HPLC) is an alternative technology to low-wavelength UV analysis that is often employed when compounds lack sufficient absorptivity. Although ELSD provides an additional detector option for liquid chromatographers, studies in our laboratory indicate analyte properties may adversely affect the ability to detect certain molecules. In this investigation, a series of low-molecular-weight compounds of pharmaceutical interest are evaluated with two commercially available ELSDs. It is observed that melting point is a useful analyte property to consider in optimizing ELSD detectors. The melting point of the analyte should be significantly higher than what the compound will experience in the nebulizer/evaporator chambers to achieve the best analyte response. It is found that some analytes could not be distinguished from the evaporated mobile phase background when ELSD temperatures exceed the melting point of the compound. Though useful for many applications and of particular interest for compounds that are weak chromophores, ELSD falls short of being a "universal detector" technology. In addition to boiling points of mobile phase components, scientists should also consider the melting point and volatility of the analyte(s) when optimizing ELSD response.

Introduction

The overwhelming desire in the pharmaceutical industry is to have simple, low-maintenance detectors for routine chromatographic use. For compounds containing a UV–vis chromophore, the standard for quality control (QC) applications is still UV detection. However, this approach may run into trouble when applied to molecules that lack sufficient absorptivity.

Addressing the need under International Conference on Harmonization guidelines to quantitate matrix components to 0.1% (or 0.05% for high-dose compounds) (1), many analytical chemists move to low-UV-wavelength detection for compounds with no or weak chromophores rather than using refractive index (RI) or electrochemical (EC) detection. Generally, this choice is made for historical reasons of sensitivity, selectivity, and robustness. Yet, low wavelength UV, though an option, is by no means the end-all solution. Too often this mode of detection picks up background responses not significant to the assay. Although the universality of this detection mode is good for initial characterization of the formulation and drug matrix, the method also presents a challenge in the QC lab during release: baseline issues and extraneous peak issues are commonplace. Although chromatographers seem content to jump to low-UV wavelengths because of the difficulties in RI and EC detection, other options such as mass spectrometry (MS) and evaporative light-scattering detection (ELSD) are being investigated. MS is still considered by many to be costly, with inadequate precision and a high level of complexity for routine QC operation. At the same time, MS is an indispensable analytical tool for research investigations. ELSDs are a special case in that they meet our criteria of being a robust, low-maintenance detector but are sometimes hampered by lack of sensitivity (2–6). One notable advantage of ELSD is that these detectors can also be used to bridge the gap to liquid chromatography (LC)–MS. Because both of these techniques operate with volatile mobile phases and buffers, the use of ELSD allows a method to be validated and transferred to the manufacturing site for routine use and still be used for LC–MS investigations without modification. By having a method qualified on both the ELSD and MS detectors, the quality control lab gets a simple, low-maintenance detector while the analytical research groups retain use of the high-powered spectrometer. Additionally, for future investigations that may be needed after the method has left development and now resides in production, the QC lab has direct access to MS as an investigational tool with no method modifications needed.
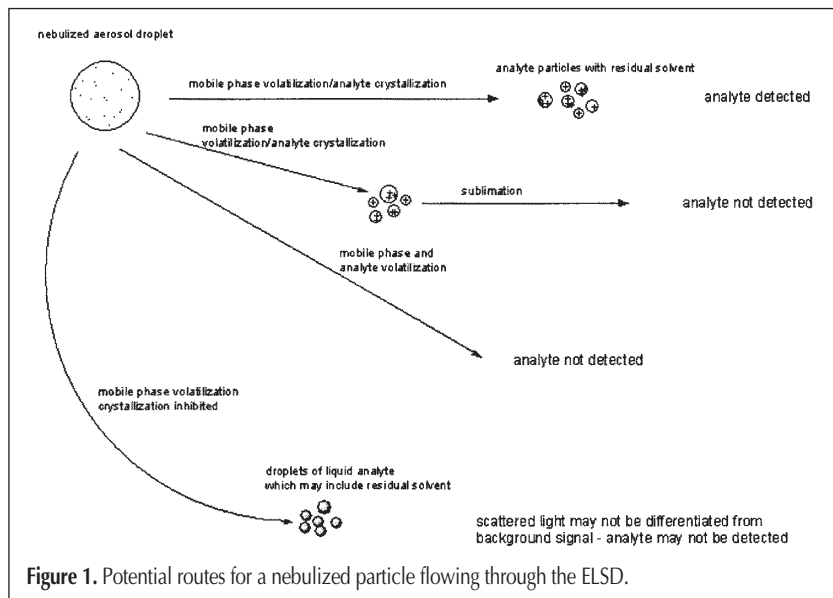
Preliminary evaluations of substituting ELSD for routine UV detection in our laboratory have found that this is not as straightforward and universal as many manufacturers claim. For molecules of pharmaceutical interest, this detection mode is

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affected by analyte melting point as well as mobile phase and analyte volatility. Varying response factors were seen for the compounds evaluated in this study. Reviewing various manufacturer claims, we find that the major detector suppliers mention the need to nebulize at lower temperatures, presumably to minimize analyte volatilization and sublimation while favoring the forma-

Figure 1. Potential routes for a nebulized particle flowing through the ELSD.

tion of particulates. However, when Mourey and Oppenheimer (7) evaluated the operation principles of this mode of detection, they reported minimal temperature effects. Alexander (8) noted that drift tubes could be operated at ambient temperatures without a decrease in signal-to-noise ratio (s/n) and found a constant s/n response for glucose with the temperature range from 40–120°C.

Mobile phase and analyte volatility are widely considered key parameters in optimizing ELSDs. Several authors note that for the ELSD mode of detection, the primary way to increase s/n is to operate with minimal drift tube temperatures (8–12). Although this makes sense in retrospect, based on a review of the literature, it is not clear that it is not so much the noise of the system (efficiency of volatizing the mobile phase) that we are affecting, but for many compounds the temperature needs to be optimized to detect the actual analyte signal itself. Measurements in our laboratory have established that scientists should consider analyte melting points when a molecule is being assessed for this mode of detection. As the analyte of interest travels through the detector flow path, solid particulates will form in the evaporated mobile phase matrix or the analyte may remain a liquid droplet. In this latter state, the analyte may still be distinguishable from 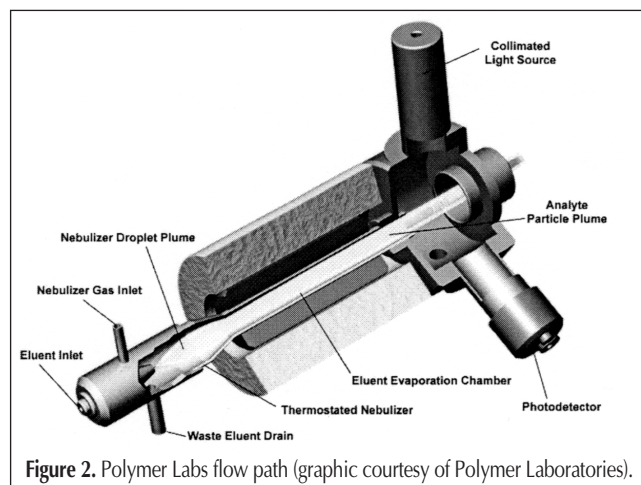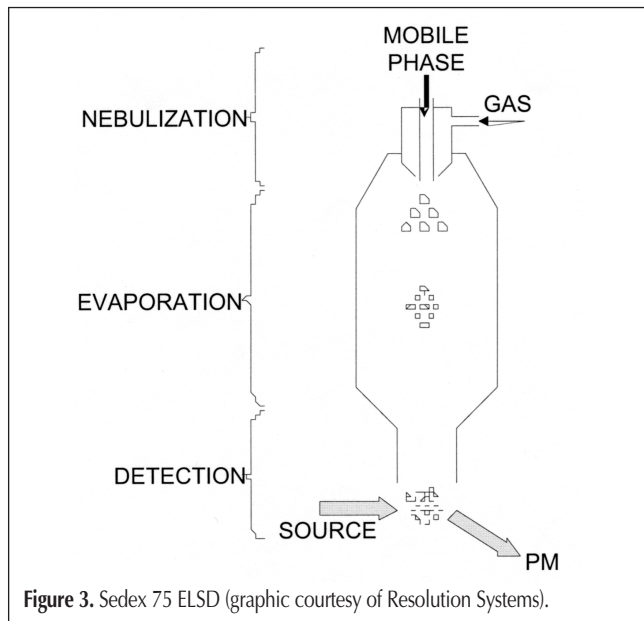the background as it elutes through the flow cell, but with less light-scattering efficiency. Loss of signal is noted as the particle changes its physical state from particle to droplet with increasing evaporator (drift tube) temperature (13). Finally, for many compounds that undergo this temperature effect, we have observed that the analyte droplets become indistinguishable from the volatilized mobile phase and the subsequent scattering signal is lost into the background (Figure 1). Note the additional mechanisms in Figure 1 that may also effect analyte detection with this type of detector.

Analytical chemists should consider the melting-point effect during method development and optimize detector conditions with the compound's melting point in mind. The choice of applying the ELSD mode of detection is going to be specific to the analytes and impurities of interest. For some low-melting analytes, ELSD may not be an appropriate technique. For analytes

Table I. Analytical Systems		
	System 1	**System 2**
ELSD	Polymer Labs ELSD 1000	Sedex 75 ELSD
	Nebulizer at 40°C	N_2 provided by house
	N_2 at 1.5 mL/min	system at 3.5 bar
	Evaporator settings	Gain = 2
	provided in text	Other settings provided
		in text
LC system	Varian Prostar LC system	Agilent 1100 series LC system
	Degassex model DG-4400	Degasser model G1322A
	inline degasser	Binary pump model G1312A
	(Phenomenex, Torrance, CA)	Autosampler model G1313A
	Pumps (2) model 210	with 2-20 µL injections
	Autosampler model 410	Thermostated column
	with 2-20 µL injections	compartment
	Column valve module	model G1316A at 40° C
	model 500, maintained	Variable wavelength detector
	at 40°C	G1314AA at 210 nm
	PDA detector model 330	Data collected using
	at 210 nm	Turbochrom Client/Server
	System controlled and data	6.1.0.2:G07
	collected using Varian Star	
	Chromatography package	
	(Version 6.0)	
LC column	Synergi Hydro-RP	Synergi Hydro-RP
	(Phenomenex)	(Phenomenex)
	4-µm, 80-Å pore size	4-µm, 80-Å pore size
	150- × 3.00-mm i.d.	150- × 3.00-mm i.d.
	Part number 00F-4375-Y0	Part number 00F-4375-Y0

Figure 2. Polymer Labs flow path (graphic courtesy of Polymer Laboratories).

with higher (estimated not less than 80–90°C) melting points, the effectiveness of ELSD must be evaluated not only with regard to requirements for suitable chromatography and mobile phase volatility but also take into account the physical properties of the compound and associated impurities.

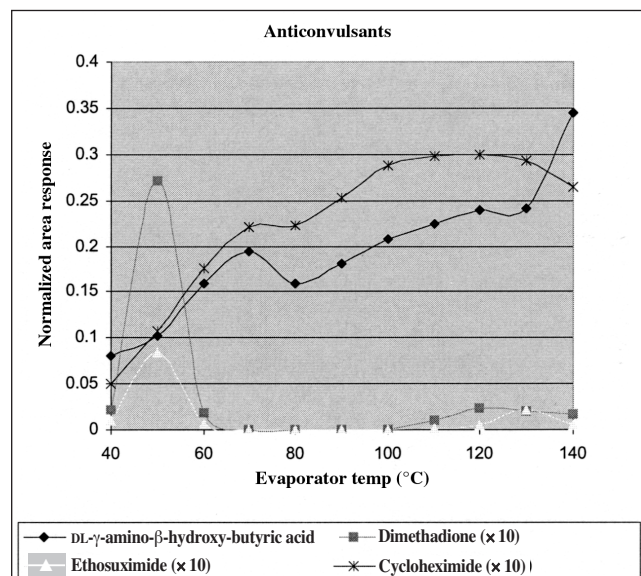Presented in this work is a direct comparison of ELSD and UV detection performance applied to a series of compounds having weak chromaphores and covering a wide range of melting points. Two different ELSD designs [Polymer Laboratories ELS-1000 (Amherst, MA) and Richard Scientific Sedex 75 (Novato, CA)] were used to characterize the ELSD response. The results for these studies show an unexpected loss and absence of ELSD response for many of the compounds used in this study. UV detection at 210 nm proved to be more selective and consistent for the conditions applied.

Experimental

The data reported was generated using two LC systems as detailed in Table I. Both LC systems were configured to run the ELSD detector in series with a UV detector in order to be able to directly compare the ELSD response with a 210-nm UV signal. This was done not only for direct comparison but to enable a later normalization between these detectors to eliminate any system effects and solely look for trends in ELSD detection. For all injections, data was monitored by UV detection in series with the ELSD.

Polymer Labs ELS 1000

The flow schematic for the ELS 1000 is presented in Figure 2. The system uses nitrogen to mix with the LC mobile phase to form a plume of uniform droplets. As the matrix moves through the evaporation chamber, the mobile phase evaporates, leaving the nonvolatile solute particles to scatter the emission from an incident light beam. Scattered light is monitored using a photodiode, in which the response is proportional to the mass of solute passing through the light beam. The key parameters for optimizing the ELS 1000 detector are adjusting the nebulizer and

Figure 3. Sedex 75 ELSD (graphic courtesy of Resolution Systems).

Table II. Mobile Phase Conditions Used

Method	Mobile phase*	Compounds studied
1	95% A–5% B (isocratic)	Vigabatrin
		Maltose
		Urea
		Succinimide
2	80% A–20% B (isocratic)	DL-γ-amino-β-hyrdoxybutyric acid
		Dimethadione
		Trimethadione
		Ethosuximide
		Cycloheximide
3	60% A–40% B (isocratic)	Parthenolide

* (A) 0.1% formic acid and (B) acentonitrile (all flows at 0.42 mL/min).

Table III. Compounds Used for ELDS Study

Compound	Published melting point (°C)
Trimethadione	46–46.5
Ethosuximide	64–65
Dimethadione	77–80
Cycloheximide	110–113
Parthenolide	115–116
Maltose	120
Succinimide	123–125
Urea	133–135
Vigabatrin	166–167
DL-γ-amino-β-hyrdoxybutyric acid	202

Figure 4. Normalized ELSD response versus evaporator temperature for a series of anticonvulsants using the Polymer Labs detector. Normalized area is ELSD mean area/UV mean area for triplicate injections. (Note: over the nebulizer temperature range studied, no ELSD peaks were observed for trimethadione.)

evaporation temperatures as well as the flow rate of the inert nebulizer gas. The nebulizer temp and gas flow rate were previously optimized (14).

Sedex 75 ELSD

As pictured in Figure 3, the Sedex flow path is different from the Polymer Labs design. Although the LC mobile phase is still nebulized to form a homogeneous mist of droplets, the aerosol initially flows through an unheated nebulizing chamber followed by a heated drift tube where the mobile phase evaporates, leaving nonvolatile analytes. The analytes then reach the optics of the detector system, where they diffuse through an incidental light beam from a tungsten-halide source and then scattered light is measured by a photomultiplier. It has been observed

in our investigation that a significant amount of mobile phase aerosol (and presumably analyte as well) was lost in the unheated nebulizing chamber. This was seen as mobile phase droplets that would condense in the unheated portion of the nebulizing chamber. Note that the nebulizer temperature was not controlled; it always operated at ambient temperature.

Mobile phase

The mobile phase consisted of HPLC-grade formic acid (Burdick and Jackson, Muskegon, MI) diluted to 0.1% in United States Pharmacopeia grade water and acetonitrile (HPLC grade) (Mallinckrodt, Phillipsburg, NJ) for easy vaporization in the analytical detectors. The mobile phase conditions used are listed in Table II.
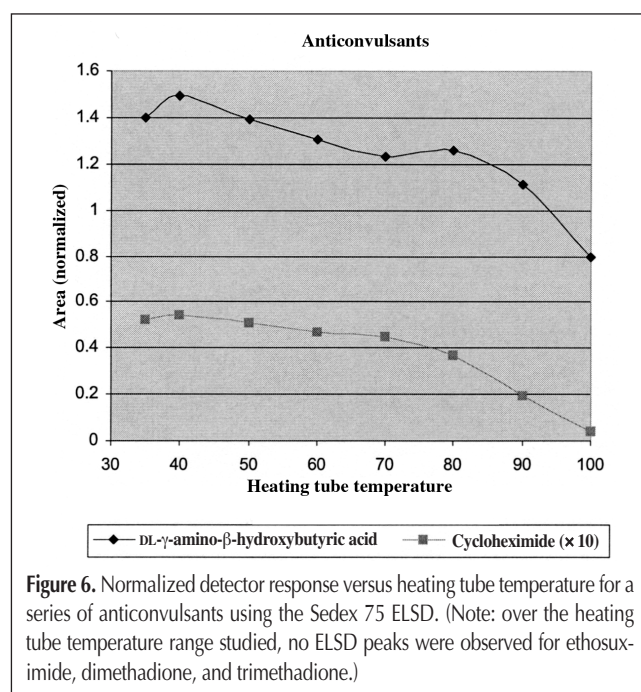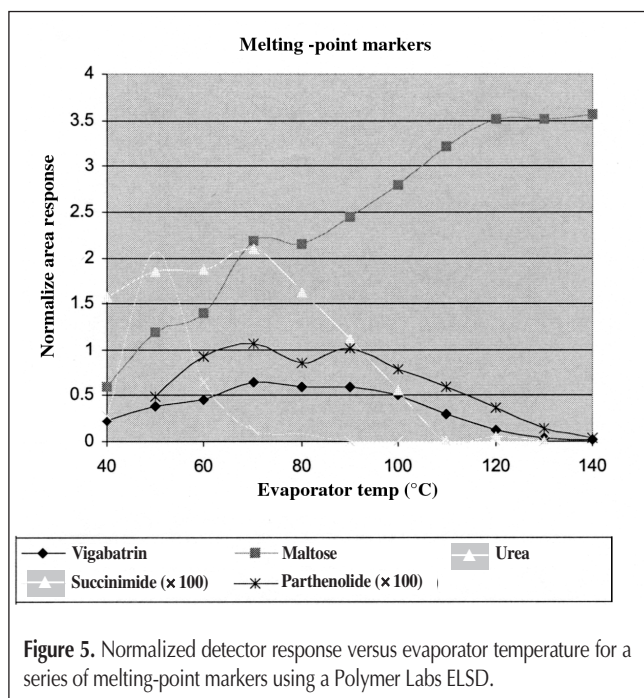
Sample preparation

The samples were prepared to a concentration of 1 mg/mL in acetonitrile or mobile phase.

Study materials

Trimethadione, ethosuximide, dimethadione, cycloheximide, parthenolide, maltose, succinimide, urea, vigabatrin, and DL-γ-amino-β-hydr - oxybutyric acid were all used as received from Sigma-Aldrich (St. Louis, MO).

Results and Discussion

During preliminary optimization work in our laboratory, it was observed that for a series of anticonvulsant drugs, ELSD detection did not respond as expected for this series of weak chromophores (14). The study indicated that the

Table IV. UV Detector Peak Areas for Trimethadione Showing Consistent UV Response for Varying Polymer Labs ELSD Evaporation Chamber Temperatures*

	40C	50C	60C	70C	80C	90C
Areas observed	80644336	80609960	80690912	80784000	80701736	80861184
	80632392	80786776	80828144	80648096	80857320	80921280
	79807160	80141128	80294176	79813896	80028168	80341032
Mean	80361296	80512621	80604411	80415331	80529075	80707832
%RSD*	0.6	0.4	0.3	0.7	0.5	0.4
	100C	**110C**	**120C**	**130C**	**140C**	**–**
Areas observed	80651360	81504736	81371728	81435744	81323496	–
	80614064	81457368	81242696	81864904	81294896	–
	79781608	81096208	80706696	81778896	80827208	–
Mean	80349011	81352771	81107040	81693181	81148533	–
%RSD	0.6	0.3	0.4	0.3	0.3	–

* Polymer labs detector did not detect trimethadione.
† %RSD = percent relative standard deviation.

Figure 5. Normalized detector response versus evaporator temperature for a series of melting-point markers using a Polymer Labs ELSD.

Figure 6. Normalized detector response versus heating tube temperature for a series of anticonvulsants using the Sedex 75 ELSD. (Note: over the heating tube temperature range studied, no ELSD peaks were observed for ethosuximide, dimethadione, and trimethadione.)

response in the Polymer Labs ELSD greatly diminished for this series as the nebulizer temperature increased. It was noted at the time that the response appeared to be proportional to the melting point of the analyte of interest. Knowing that this effect was not well documented in the literature or by the major manufacturers of these detectors, it was decided to investigate this trend further using an expanded series of compounds having poor UV–vis

Figure 7. Normalized detector response versus heating-tube temperature for a series of melting-point markers using the Sedex 75 ELSD. (Note: succinimide was not detected by the ELSD over the heating tube temperature range covered.)

absorptivity and covering a broader melting-point range. The compounds chosen are listed in Table III. The criteria for selection of this series was simply based on melting point of these compounds, ideally having a poor response for UV detection (thus being a candidate of interest for ELSD) and being commercially available.

Polymer Labs detector design

Using the expanded series of compounds from Table III, the Polymer Labs ELSD was configured to run over a series of heating-tube (evaporator) temperatures. The goal was to start with a very low evaporator temperature and increase it 10°C until the evaporator was operating at a higher melting point range than most of the members of this study series. The nebulizer temperature and the gas flows were held constant for this study. The detector settings were previously optimized for our LC conditions during the original study. If melting point was playing a significant role in the loss of signal response as we suspected, the ELSD response for each analyte should be strong and then tail off at higher temperatures as the analyte in its melted state becomes more difficult to distinguish from the background produced by the vaporized mobile phase.

The ELSD response for five anticonvulsant drugs is illustrated in Figure 4. The melting points range from 46°C to 202°C. The trend is quite clear for the lower melting point drugs; if the ELSD signal is detected at all, the signal rapidly was lost to background as the sample melting point was approached. In this plot, the response for the lower melting dimethadione and ethosuximide diminished rapidly with increasing nebulizer temperature. At no point did the Polymer Labs detector detect the presence of
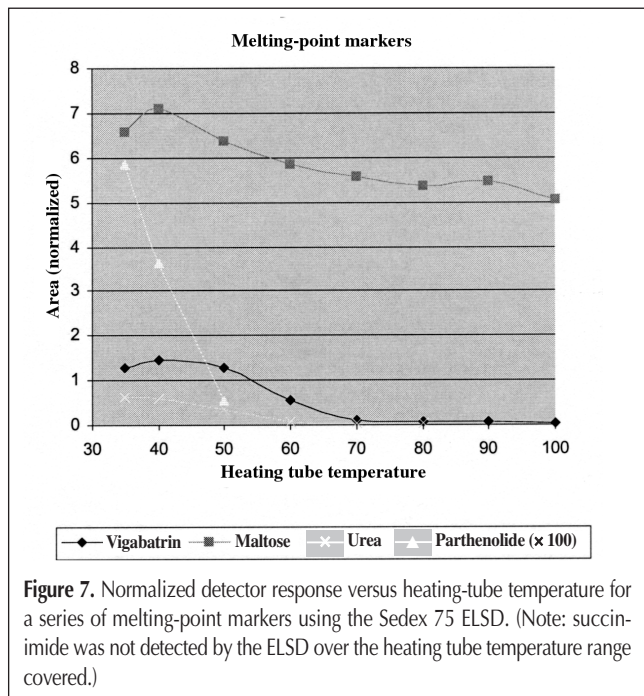
Table V. Consistent UV Detection for Anticonvulsants Not Detected by ELSD (Sedex 75) with Varying Drift Tube Temperatures

Ethosuximide	35C	40C	50C	60C	70C	80C	90C	100C
Observed areas	6382518	6173887	6294842	6206403	6226112	6249977	6285040	6328195
	6313504	6155529	6249352	6208542	6176310	6177938	6266537	6296218
	6309810	6175420	6239926	6187512	6224771	6180242	6262100	6242082
Mean	6335277	6168279	6261373	6200819	6209064	6202719	6271226	6288832
%RSD*	0.65	0.18	0.47	0.19	0.46	0.66	0.19	0.69
Dimethadione	**35C**	**40C**	**50C**	**60C**	**70C**	**80C**	**90C**	**100C**
Observed areas	6639870	6481819	6545067	6573995	6568600	6564879	6573875	6599045
	6627202	6478290	6526128	6571856	6558388	6554953	6564303	6597401
	6624829	6483764	6531468	6560322	6569352	6549572	6562603	6582044
Mean	6630634	6481291	6534221	6568724	6565447	6556468	6566927	6592830
%RSD	0.12	0.043	0.15	0.11	0.09	0.12	0.09	0.14
Trimethadione	**35C**	**40C**	**50C**	**60C**	**70C**	**80C**	**90C**	**100C**
Observed areas	6639870	6481819	6545067	6573995	6568600	6564879	6573875	6599045
	6627202	6478290	6526128	6571856	6558388	6554953	6564303	6597401
	6624829	6483764	6531468	6560322	6569352	6549572	6562603	6582044
Mean	6630634	6481291	6534221	6568724	6565447	6556468	6566927	6592830
%RSD	0.12	0.043	0.15	0.11	0.09	0.12	0.09	0.14

* %RSD = percent relative standard deviation.

trimethadione. In the case of trimethadione, the UV signal remained consistent and strong throughout the temperature range. Peak areas for trimethadione are presented in Table IV. The drug was detected and well resolved in the UV data. By normalizing the ELSD response to the UV response, the normalized response trend solely accounted for the effects found with the light-scattering signal. The ELSD response in the Polymer Labs detector for the remaining melting point markers (Figure 5) showed acceptable response curves for five compounds that had melting points in the range of 115–167°C. Interestingly, succinimide and urea lost response in the Polymer Labs detector more rapidly than expected. This may have been attributable to analyte volatility or decomposition that may have been catalyzed by the formic acid mobile phase modifier. Overall, the data shows that

the analyte melting point was a useful property that should be taken into account in the initial selection of molecules for detection with ELSD as well as being an important parameter in optimizing detector performance. The authors believe the melting point effect in Figure 5 was less dramatic but still present. An absolute correlation of the response curves to melting point was confounded by differences in analyte volatility, the potential for sublimation, as well as the possibility of side reactions whose products may not be detected. However, this only added to our notion that ELSD detection is not as straightforward as promoted for compounds lacking a UV–vis chromophore, with several physical effects potentially affecting the detected signal

At this point in our investigation, we diagnosed a trend for detector response with a series of analytes having different

Table VI. Consistent UV Detection for Succinimde*

	35C	40C	50C	60C	70C	80C	90C	100C
Areas observed	7886308	8437914	8313192	8032131	7866118	7897074	7796047	7755289
	7914229	8399557	8270797	7975892	7850867	7813962	7731866	7738794
	7988784	8394044	8283787	8020021	7885662	7922587	7806029	7777029
Mean	7929774	8410505	8289259	8009348	7867549	7877874	7777981	7757073
%RSD[†]	0.67	0.28	0.26	0.37	0.22	0.72	0.52	0.25

* Succimide was not detected by the Sedex 75 ELSD at varying drift tube temperatures.
[†] %RSD = percent relative standard deviation.
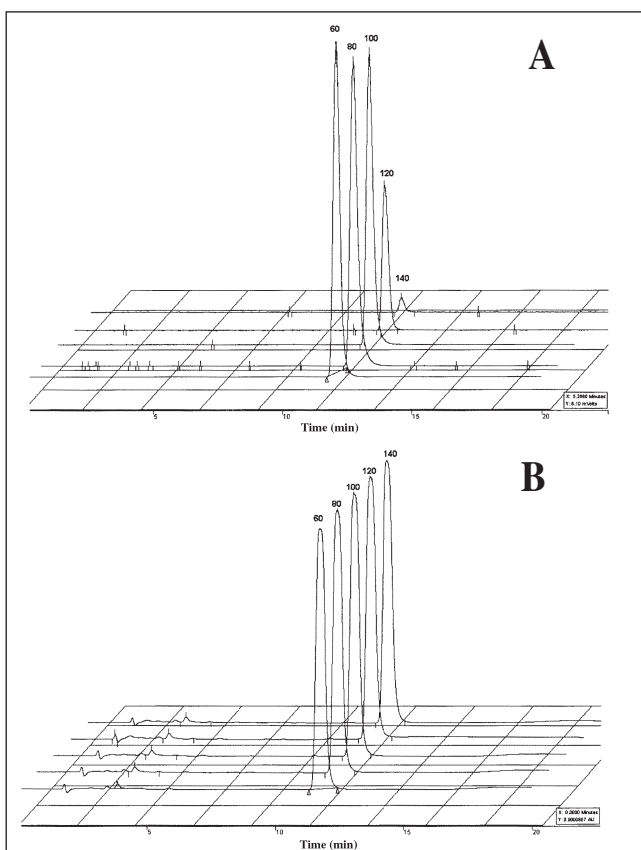
Figure 8. (A) Polymer Labs ELSD of parthenolide with varying evaporation chamber temperatures and (B) consistent UV detection of parthenolide in series with the Polymer Labs ELSD.
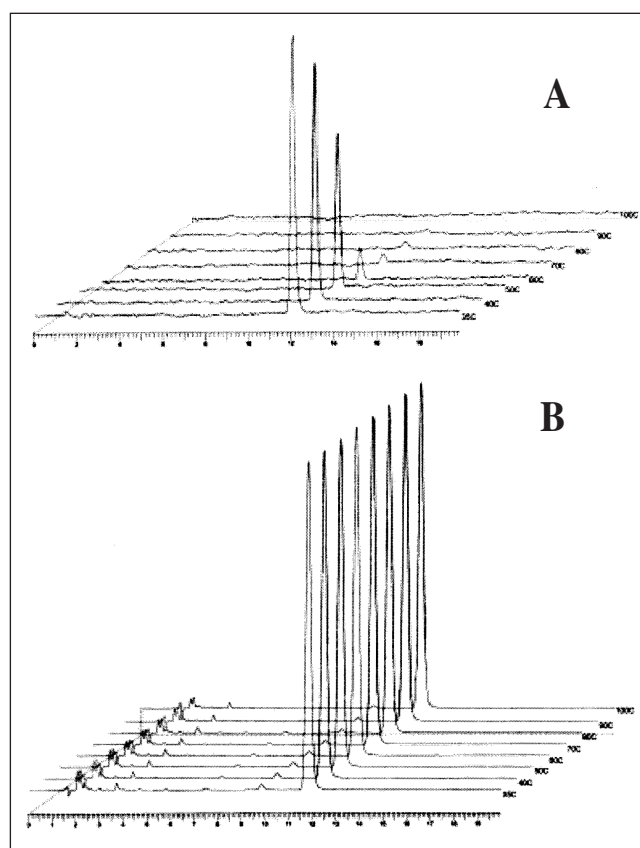
Figure 9. (A) Sedex 75 detection of parthenolide with varying drift tube temperatures and (B) consistent UV detection of parthenolide in series with the Sedex 75.

melting points. What was unclear was if this trend was unique to the Polymer Labs design, or was it common to the ELSD technique as a whole?

Sedex detector design

The Sedex series of ELSD instruments were designed for efficient, low-temperature volatilization of the LC effluent. This may allow for better sensitivity and analysis of less volatile analytes. Because this perception was somewhat in line with where we were at with our investigation, the test series and chromatographic conditions were transferred to a system utilizing the Sedex 75 detector. Fully expecting the Sedex to produce a much improved signal response for our lower-melting-point members of the study series, it came as a surprise that an even worse signal response (smaller peaks) was seen on this detector. Repeating the earlier anticonvulsant run on this detector, it was illustrated (Figure 6) that the signal loss was greater utilizing the Sedex detector. In this experiment, the Sedex ELSD failed to detect not only trimethadione, but dimethadione and ethosuximide as well. Trimethadione, dimethadione, and ethosuximide were seen with acceptable detector response in the UV data (Table V). Although not fully understood, it is believed by the authors that the Sedex design had a longer residence time in the heated chamber and that this may exacerbate signal loss caused by analyte volatilization or sublimation. This effect of detector design on the observed data is currently under investigation. Continuing with the remaining melting point markers (Figure 7), the loss of signal was also seen in this series, but this time succinimide was not detected under the study conditions. Table VI confirms that succinimide was detected and well resolved in the UV data. It is of some importance to note that the perception of lower nebulizing temperature overcoming the loss of signal for low-melting-point analytes was not found to be true for our study series. Despite the loss of signal for some of the compounds in the test series with the Sedex 75 detector, other compounds showed reasonable correlation of the response factor curves between the two detectors. This result suggested that analyte properties were more important than detector design for many compounds of pharmaceutical interest.

ELSD response

It is clear from our studies that for ELSD to succeed as a detector for pharmaceutical applications, the chemist must be careful in selecting the analytes and conditions used for detection. As illustrated in Figures 8 and 9, even a compound such as parthenolide with a melting point of 115–116°C can see its detector response diminish rapidly in ELSD detectors while maintaining a strong and consistent UV response. Although the operator optimizes the system to reduce background mobile phase response, the effect upon analyte and impurity signals must be assessed as well.

Conclusion

ELSD remains as a promising alternative to UV detection for many LC applications. As with any detection mode, the success of this detection mode relies upon careful detector optimization.

Mobile phase and analyte volatility, as well as analyte melting point, should be considered during the evaluation of ELSD as a detection strategy. At variance with the common claim of "universal detection," these detectors work well only when the molecule of interest is not only less volatile than the mobile phase employed but also has the ability to retain its light-scattering nature as it flows through the detector. The incident beam is most effectively scattered by particulate analyte and not its liquid form. ELSDs must be optimized for each molecule under study. From our investigations, one way to initially screen whether this detector may be a viable choice is to review the melting point of the analyte of interest. The signal from analytes having lower melting points may be difficult to distinguish from the background of evaporated mobile phase matrix.

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