Application Note 01636

Mass Spectrometry With Evaporative Light Scattering Detection: A Powerful Analytical Combination for the Analysis of Select Pharmaceuticals

Fran Lai
Varian, Inc.

Introduction

Mass spectrometry (MS) is highly sensitive and widely used as an information rich detector. However, it is not universal. Some analytes do not ionize well using standard MS API sources (ESI and APCI), and ion suppression due to matrix effects is common. These kinds of problems can contribute to difficulties in method development.

One way to overcome this is to use simultaneous detection with another kind of detector. Traditionally, Refractive Index (RI) and Ultraviolet (UV) detectors have been widely used in HPLC. Evaporative Light Scattering Detection (ELSD) is a better alternative than UV detection at low wavelengths, because its detection is not wavelength-dependent. Also, ELSD can be used with a wide range of solvents when compared to detection with RI, and is gradient compatible.

The ELSD principle of operation employs three distinct stages (Figure 1).

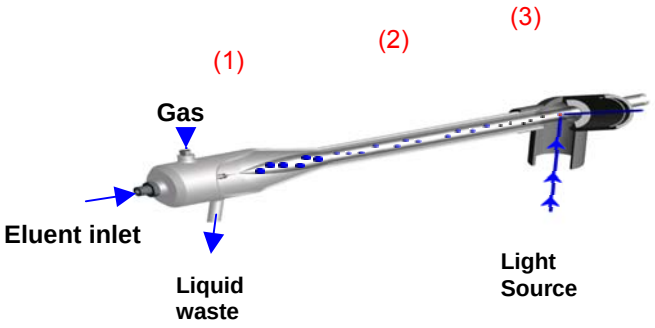


Figure 1. Stages of ELSD operation:
(1) Nebulization, (2) Evaporation and (3) Detection.

In nebulization, the eluent is mixed with nitrogen or air using a concentric nebulizer design with temperature control. Evaporation of the solvent droplets takes place in the evaporator tube (drift tube) at an independently controlled temperature. Detection is performed by using a high power light emitting diode (LED) and photomultiplier. The ELSD has similar operating parameters to LC/MS, such as the use of volatile mobile phases and flow rates in the range of 0.2-0.5 mL/min.

Three applications are described here demonstrating the usefulness of MS coupled with ELSD:

1. Haloperidol and its metabolites — MS and ELSD show different responses for these compounds.
2. Diazepam and Verapamil — differences in relative response between ELSD and MS.
3. Ibuprofen and β-cyclodextrin — quantitating both compounds without letting cyclodextrin contaminate the MS ion source.

Instrumentation

- Varian 500-MS Ion Trap LC/MS equipped with ESI source
- Varian 385-LC ELSD
- Varian ProStar™ 420 AutoSampler
- Varian 212-LC Liquid Chromatograph equipped with 150-µL static mixer

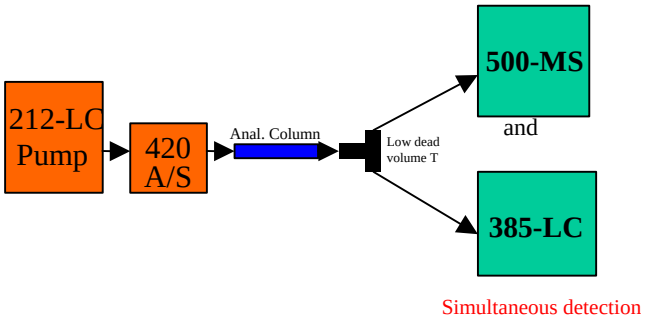


Figure 2. LC/MS-ELSD system configuration set up for simultaneous detection.

Materials & Reagents

Haloperidol and metabolites I and II (CAS numbers 52-86-8, 39512-49-7, 34104-67-1) Diazepam (CAS number 439-14-5), Verapamil (CAS number 152-11-4), Ibuprofen (CAS number 51146-56-6) α-cyclodextrin and β-cyclodextrin (CAS numbers 10016-20-3, 68168-23-0) were obtained from Sigma Aldrich, St. Louis, MO. All other chemicals were reagent grade or HPLC grade.

Sample Preparation

The following mixtures were prepared:

(1) Haloperidol, metabolite I, metabolite II, 100 µg/mL each in 23:77 methanol:water.

(2) Diazepam and Verapamil, 10 µg/mL each in 50:50 mobile phase A:mobile phase B.

(3) Ibuprofen and Alpha-cylcodextrin, 10 µg/mL each in 50:50 mobile phase A:mobile phase B.

HPLC Conditions

Column: Pursuit™ XRs C18 5 µm, 150 x 2 mm ID (Varian Part No. A6000150X020)

Mobile Phase A: 0.1% formic acid in water

Mobile Phase B: 0.1% formic acid in acetonitrile

Flow Rate: 0.2 mL/min

Injection Volume: 10 µL

Mixtures (1), (2) and (3) were injected with the following corresponding LC gradient:

(1) 10% B to 85% B in 7 min

(2) 20% B to 85% B in 5 min

(3) 50% B hold for 1 min, then to 80% B in 7 min

API & MS Parameters

The 500-MS was optimized for each of the compounds by infusion. Scan parameters used in these methods are:

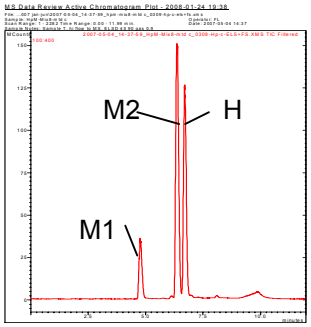
Needle:	5000 V
Spray Shield:	600 V
Nebulizing Gas:	25 psi
Drying Gas:	25 psi at 400 °C
Enhanced Scan Mode:	5000 Da/sec

The 385-LC ELSD was also optimized for each compound. Key parameters to optimize are nebulization temperature, evaporation temperature and gas flow rate. ELSD conditions are shown under each figure.

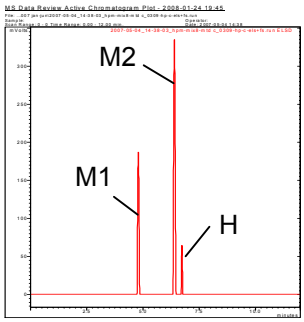
Results & Discussion

Application (1). The ELSD shows excellent signal-to-noise and good response for the parent and two metabolites. It can be used in conjunction with the more information-rich MS data. As shown in Figure 3 and Table 1, the ELSD detector response can be different than that obtained by MS, enabling the operator to optimize the sensitivity of both instruments.

Application (2). Diazepam and Verapamil at the same concentration have significantly different response in full scan MS, but a relatively equal response in the ELSD. Hence, the ELSD would be useful at the early method development stage. After that, the method can be transferred to MS (Figure 4).



MS (ESI, Full Scan)

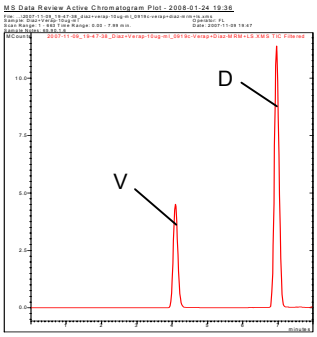


ELSD (Evap.45°, Neb. 90°, Gas 0.9 SLM)
(SLM = Standard Liter per Minute)

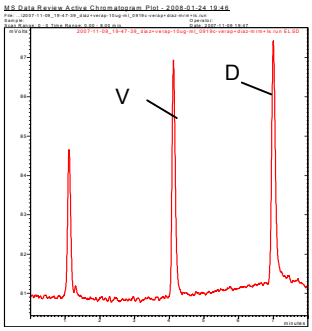
Figure 3. Mixture of Haloperidol (H) and metabolites (M1 and M2).

Table 1. Full scan MS and ELSD signal-to-noise (S/N) comparison.

	MS S/N (Full Scan)	ELSD S/N	Sensitivity of ELSD Compared to MS
Metabolite I (peak 1)	1,348	37,400	28x
Metabolite II (peak 2)	2,084	68,000	33x
Haloperidol (peak 3)	1,046	13,000	12x



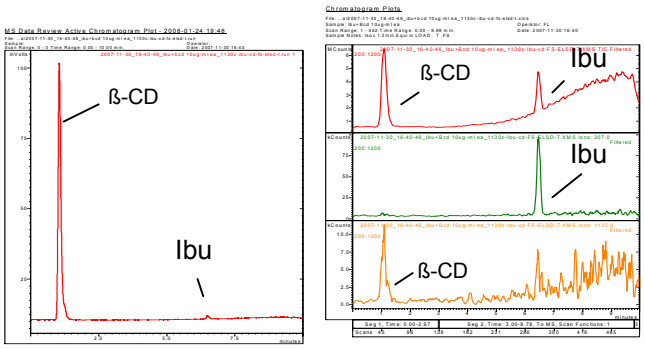
500-MS in MS/MS mode
Response ratio of
Verapamil:Diazepam is 1:2



ELSD (Evap. 66°, Neb. 90°, Gas 1.6 SLM)
Response of ration of
Verapamil:Diazepam is approximately 1:1

Figure 4. Mixture of Verapamil (V) and Diazepam (D) (100 ng each on column).

Application (3). MS detection is more qualitative and quantitative than ELSD for the active drug (Ibuprofen). However, ß-cyclodextrin, being a sticky excipient, can foul an ESI source fast during an analytical sequence (Figure 5). To avoid this problem, the ß-cyclodextrin can be diverted away from the MS source (using the built-in divert valve controlled by the MS Workstation Software) into the ELSD that is actually more sensitive than MS for this excipeint (Figure 6). After the cyclodextrin elutes, the sample is diverted back to the MS for the analysis of Ibuprofen (Figures 6 and 7). Table 2 compares sensitivity for the two compounds between the two detectors.



ELSD (Evap. 25°, Neb. 90°, Gas 1.4 SLM): Sensitive for β-cyclodextrin, but not Ibuprofen

MS (ESI, Full Scan): XIC is sensitive for Ibuprofen, not ß-cyclodextrin, which also dirties up ion source

Figure 5. Mixture of Ibuprofen (Ibu) and β-cyclodextrin (β-CD) (100 ng each on column) using post-column T (simultaneous detection).

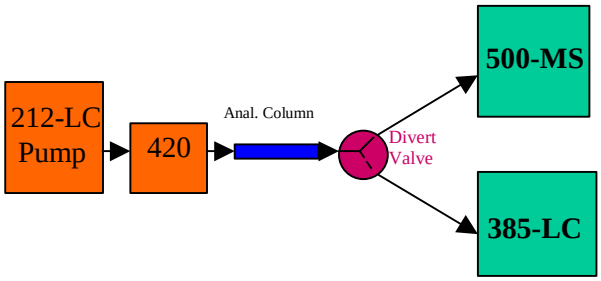
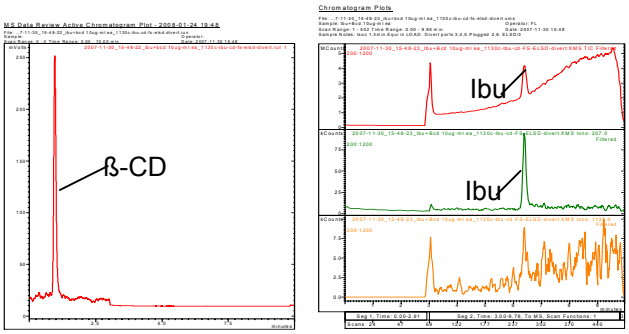


Figure 6. LC/MS-ELSD with divert valve.

Selected Detection



ELSD (Evap.25°, Neb. 90°, Gas 1.4SLM): ß-cyclodextrin diverted away from MS.

MS (ESI, Full Scan): for Ibuprofen detection only, ß-cyclodextrin diverted to ELSD to keep ion source clean.

Figure 7. Mixture of Ibuprofen (Ibu) and β-cyclodextrin (β-CD) (100 ng each on column) using divert valve (selected detection).

Table 2. Sensitivity of LC/MS-ELSD configuration (using post-column T).

	MS (XIC) S/N	ELSD S/N	More Sensitive Detector
ß-cyclodextrin	28	930	ELSD
Ibuprofen	30	15	MS

Conclusion

Benefits for using ELSD include:

- Detection of compounds with no chromophore
- Compatible with gradient elution and a wide range of solvents
- No solvent front
- No need for derivatization
- Better alternative to UV at low wavelengths

Advantages of using ELSD in combination with the Varian 500-MS Ion Trap LC/MS include:

- Flow rate range is compatible with ESI or APCI
- Highly complementary with the 500-MS for:
  (1) Simultaneous detection using post-column split
  (2) Serial/selected detection using the built-in divert valve
  (3) Early method development to overcome limitations in compound detection in the MS ion source
  (4) Adds another confirmation technique to the analysis

These data represent typical results.
For further information, contact your local Varian Sales Office.

Varian, Inc.
www.varianinc.com
North America: 800.926.3000 – 925.939.2400
Europe *The Netherlands*: 31.118.67.1000
Asia Pacific *Australia*: 613.9560.7133
Latin America *Brazil*: 55.11.3238.0400