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Application of evaporative light scattering detection to the characterization of combinatorial and parallel synthesis libraries for pharmaceutical drug discovery

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Abstract

The advent of combinatorial and parallel synthesis methodologies in drug discovery have necessitated the development of analytical techniques which permit high throughput quantitative analysis of mixtures of small organic molecules. High pressure liquid chromatography with evaporative light scattering detection has become the major tool for this task. In this article we briefly review the theory of evaporative light scattering detection and the design of commercial instruments, as well as discuss the operational constraints imposed by the exigency of analyzing en masse the product libraries generated by these new drug discovery methods. The application of evaporative light scattering detection to library analysis is illustrated using examples from our library synthesis program. Complemented by ultraviolet absorbance detection for purity assessment and mass spectrometry for product identification, evaporative light scattering detection is the only technique affording sufficient accuracy and sensitivity for high throughput library analysis.

Keywords: Evaporative light scattering detection; Library analysis; Pharmaceuticals

1. Introduction

Combinatorial chemistry and high speed parallel synthesis have now emerged as valuable strategies for discovering novel, pharmacologically-active substances as well as for optimizing lead candidates [1–6]. The ability to rapidly synthesize large libraries of structurally diverse small molecules (i.e. molecular masses 200–600 Da) by either parallel or combinatorial methods and subsequently screen them for biological activity is proving to be a powerful asset for drug discovery. Nevertheless, the high throughput analysis of libraries comprising mixtures or discrete compounds remains a challenging task [7]. A critical issue in library analysis is the assessment of both the qualitative and quantitative purity of libraries. In addition to the desired reaction products, library members frequently contain substantial levels of reaction by-products and excess reagents. To confidently derive structure activity relationships from biological activity screening data requires knowledge of the purities as well as the quantities of individual compounds [8]. The traditional approaches for medicinal chemists to monitor reaction products and yields, such as NMR and IR, are not appropriate for combinatorial and parallel library analysis owing to both the complexity of the mixtures and the limited sample throughput.

The inherent separation efficiency of high-performance liquid chromatography (HPLC), either

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normal-or reversed-phase, makes it an attractive method for quantitation of compound libraries. Clearly, in terms of sample throughput HPLC is particularly well-suited for automation. An HPLC detector that is capable of providing universal response to all compounds in the library is required for quantitative analysis. Both refractive index and flame ionization detectors have been demonstrated to provide equivalent response to mixtures of compounds [9,10]. However, neither of these detectors is sufficiently sensitive to detect minor components (i.e. low ng levels on column) in a mixture. Another drawback for refractive index detection is its sensitivity to variations in solvent refractive index, which renders it unsuited for gradient elution HPLC.

Mass spectrometry (MS) with either electrospray (ES) or atmospheric pressure chemical ionization (APCI) is a very powerful tool to probe libraries [11–13]. When coupled to a separation technique, viz. HPLC, the capability of MS is greatly enhanced for both the determination of molecular weight and structural characterization of mixtures [14]. In library analysis, MS is unparalleled for product identification. However, with regards to quantitative analysis, MS is unsuited due principally to differences in ionization efficiencies for different molecules. Liquid chromatography with short-wavelength UV absorbance detection is typically used to complement LC–MS analysis. Nevertheless, UV absorbance detection is limited to molecules with an appropriate chromophore, and moreover, the UV response varies with different chromophores. Therefore, because of the significant differences in molecular absorptivity that exist between different molecules, concentrations can not be determined by UV without the use of reference standards. To synthesize and characterize reference standards for each member of a library would be impractical. Recently, LC–NMR technology has dramatically improved, and the technique has been applied to the analysis of complex mixtures [15]. But the costs, in terms of time, labor, and instrumentation, associated with the routine use of LC–NMR for library quantitation remain prohibitive.

The evaporative light scattering detector (ELSD) was first demonstrated in 1966 by Ford and Kennard to have a nearly linear mass dependent response irrespective of chemical composition when it was used to analyze low-molecular-mass polymers [16]. The basic principles by which the ELSD functions were not well understood until a decade later when Charlesworth reported the results of his systematic investigation of factors influencing light scattering and solute volatility [17]. Since then, the use of ELSD has burgeoned and applications including analyses of carbohydrates [18] and fatty acid esters [19] were reported in the 1980s. In early studies, ELSD was successfully applied to a variety of compound classes. However, due to the poor sensitivity of the first generation of commercial instruments these studies were limited to molecules such as sugars [20], triglycerides [21], bile acids [22], toxins [23], and polyethylene glycols [24] which lacked appropriate chromophores for UV absorbance detection. In the past decade dramatic improvements in the design of instrumentation has made the ELSD comparable to the UV absorbance detector in sensitivity. This has enabled the ELSD to be regarded as a vital detector for diverse applications including HPLC, gel permeation chromatography, countercurrent chromatography, and supercritical fluid chromatography. ELSD is particularly useful when combined with either UV or MS as complementary detectors in HPLC. In principle, area percent calculations from ELSD chromatograms can be used to determine the weight percent for each component in a mixture.

The combination of ELSD with HPLC has been used in the analysis of combinatorial and parallel synthesis libraries for only a few years. Although ELSD has quickly become regarded as an indispensable tool for library analysis in industrial research laboratories, little description in this regard has been published. Indeed, to our knowledge there has been only one report in the literature discussing the quantitation of combinatorial libraries of small organic molecules with ELSD [25]. In that study, Kibbey described ELSD quantitation of sets of steroids, hydantoins and protected amino acids using single external standards with isocratic normal-phase HPLC. Kibbey [25] found the optimal quantitation accuracy for each set was achieved with structurally related standards. Under these circumstances the quantitation errors averaged approximately 20%. It was noted that under reversed-phase HPLC conditions the quantitation errors for the steroid set were much greater (data not shown) than the normal-phase

results. Kibbey attributed this reduction in accuracy to the requirements of higher drift tube temperature and higher nebulizer gas flow to accommodate aqueous eluents in reversed-phase HPLC.

In this article we will briefly review the theory of evaporative light scattering detection, and the nuances of instrumental design incorporated in commercial ELSD detectors. In addition we will illustrate the utility of the ELSD technique using examples from our library analysis program. Direct library quantitation using internal standards to estimate the actual concentrations of components in single compound/single well libraries will be discussed as well.

1.1. Theory of evaporative light scattering detection

A schematic representation of an ELSD is illustrated in Fig. 1 [26]. Basically, the ELSD is composed of three main components that individually govern three successive processes: nebulization, evaporation and detection. The first two processes, nebulization and evaporation, have a major impact on the formation of the aerosol droplets and solute particulates which strongly influences the sensitivity of the ELSD. Upon entering the detector through the narrow bore tube the column effluent is mixed coaxially with a high-velocity stream of an inert gas, typically nitrogen. The droplets (D_0) produced by the Venturi atomization process are then carried either directly into a heated drift tube or transit via a nebulization chamber to the drift tube. The nebulization chamber condenses the larger droplets, preventing them from entering the heated drift tube. This design is purported to result in more uniform droplets and lower evaporation temperatures. Both the size and uniformity of the droplets as well as the evaporation temperature are critical factors in determining the sensitivity and the peak area reproducibility.

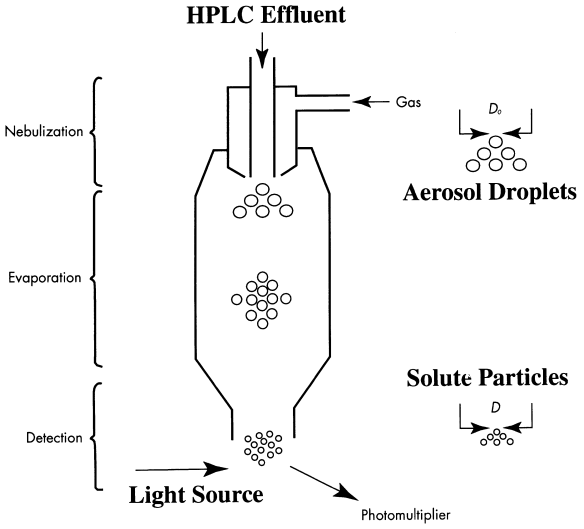

Fig. 1. Schematic of an evaporative light scattering detector.

Light-scattering by solute particles within the ELSD is the result of three main mechanisms: Rayleigh scattering, Mie scattering, and reflection–refraction. The importance of each mechanism is dependent on the particle radius (r) and the wavelength (λ) of the incident light. With the smaller dimension of the particles, i.e. $r<\lambda/20$, Rayleigh scattering is the predominant process. Mie scattering becomes a dominant mechanism for $\lambda>r>\lambda/20$. When the particle size is greater than the wavelength of the incident light, i.e. $r>\lambda$, then a reflection–refraction mechanism occurs. Usually, due to the distribution of particle sizes, the observed scattering intensity has contributions from a combination of two different domains, either Rayleigh and Mie, or Mie and refraction–reflection.

Within the ELSD instrument the formation of the final particle size distribution is rather complex. Nebulizer gas pressure, sample concentration, and solvents all influence the resulting particle distribution. Higher gas velocity in the nebulizer leads to the formation of smaller droplets, and in turn to solute particulates with smaller radii that decrease the scattering response. Mourey and Oppenheimer [27] showed that increasing sample concentration alters the size of the particles rather than changing the total number of particles formed. Therefore, the instrument response exhibits a sigmoidal behavior with concentration. Significant deviations occur chiefly at high and low concentrations. Solvent physical properties such as surface tension, density, and viscosity contribute to the variation in droplet diameter, but the differences are moderate (e.g. 20% between methanol and chloroform) [28].

A scattering rather than absorbing phenomenon is desired when the detector light source interacts with

the particles. For this reason a tungsten filament lamp that produces a distribution of wavelengths is favored as a light source. Since the wavelength at the maximum emissivity of tungsten is approximately 0.35 μm, it is apparent that Mie and refraction–reflection processes prevail unless the radius of solute particles is smaller than ca. 0.02 μm. Furthermore, refraction is relatively more important compared to reflection in the refraction–reflection domain. The contribution of refraction to the light scattering process suggests that the scattering response is a function of the solute's refractive index. However, many organic compounds have refractive indices between 1.3 and 1.8. This is most likely the major factor accounting for the ''universal'' response characteristics of ELSD.

The temperature of the evaporation chamber has a minor effect on the size of solute particles [27]. The role of heat applied to the nebulized solvent aerosol is to remove the solvent completely to ensure the production of particles of pure solutes. An underlying assumption is that the melting temperature of the eluting substances is higher than the evaporation chamber temperature. Nevertheless, in order to obtain a reproducible response, the lowest reasonable temperature above the baseline noise limit is favorable to maintain the uniformity of particle size. It has been suggested that a narrow size distribution aerosol with a larger average size will scatter more light [29]. Higher temperature may cause non-uniform particle size owing to excessively vigorous solvent volatilization. Another adverse effect of high temperature may result in the reduction of particle size because of the rapid evaporation of solute after most of the solvent has been removed.

1.2. Evaporative light scattering instrumentation

There are currently four manufacturers of commercial ELSD instruments: the Sedex 55/65 of SEDERE (Alfortville, France), the DDL31 of EUROSEP (Cergy–Pontise, France), the PL-ELS 1000 of Polymer Laboratories (Amherst, MA, USA), and the Alltech 500/LTA of Alltech Associates (Deerfield, IL, USA). The Sedex and Eurosep instruments share a similar design incorporating a nebulization chamber and a long coiled evaporation chamber. A supplemental gas inlet is employed at the exit of the coiled drift tube to prevent the deposition and dissipation of sample in the detection cell. The Polymer Labs ELS 1000 and Alltech 500 detectors have evolved from the Varex MK-III ELSD in which the nebulized aerosols are swept directly into a heated drift tube. The PL ELS 1000 design includes a short nebulization chamber. In the case of the Alltech model 500 ELSD a ''low-temperature-adapter'' can be attached as an option which mimics the nebulization and evaporation chambers of the Sedex/Eurosep instruments. Polychromatic tungsten halogen lamps are used as the light sources in all commercial instruments with the exception of the Alltech 500 which employs a 670 nm laser diode. Detectors are either standard photomultiplier tubes (PMT) or solid state photodiode devices, although the trend in new ELSD instruments is towards the latter. For reasons including simplified electronics, cost and physical dimensions, solid state photodetectors are replacing uncooled PMTs in commercial instruments.

All four instruments provide microprocessor control of some instrument operating parameters. These typically include drift tube temperature and detector gain. Depending on the model, ELSDs can be interfaced via analog and digital ports to external systems for instrument control as well as for data acquisition and processing. In addition, some designs include output signals such as temperature, gas flow-rate, instrument fault (or error) indications, and so forth. In all cases the fittings and connectors conform to industry standards.

2. Experimental

2.1. Materials

The RP and RPR compounds were obtained from the chemical processing center within Rhone–Poulenc Rorer R&D. The standard peptides were purchased from Sigma (St. Louis, MO, USA), fluocinolone acetonide, triphenylphosphine oxide, 4-phenoxybenzoic acid, and 4-phenyl carboxaldehyde were obtained from Aldrich (Milwaukee, WI, USA). Reaction mixtures were provided by synthetic chemists and were subsequently analyzed without further treatment except by adding solvent, usually acetonitrile, to adjust the volume. For the ELSD internal standard experiments, a known amount of peptide

was added to the reaction mixture and the concentration was adjusted by adding acetonitrile to provide about 1 μg of peptide per injection. All solvents were HPLC grade or of equal quality.

2.2. Liquid Chromatography

The HPLC system was a Hewlett-Packard 1050 series consisting of a pump and an autoinjector. The UV detector was an Applied Biosystem model 783 Programmable Absorbance Detector. The UV wavelength was set to 220 nm throughout the experiments. The ELS detectors were from different manufacturers including SEDEX 55 from SEDERE, VAREX MKIII from Alltech, and PL-EMD from Polymer Laboratories. The drift tube temperatures and the nitrogen gas flow-rates were set at 40–65°C and 1.7–2.1 l min^{-1} (STM), respectively. LC separations were performed with 3 μm Hypersil BDS C-18, 50x2.1 mm I.D. or 50x4.6 mm I.D. columns (Keystone Scientific, Bellefont, PA, USA). The mobile phases were acetonitrile–water gradients containing 10 m*M* ammonium acetate or 0.1% trifluoroacetic acid. Flow-rates of 0.2 ml min^{-1} and 1 ml min^{-1} were used for the 2.1 mm I.D. and 4.6 mm I.D. columns, respectively. Data acquisition and integration for ELSD and UV absorbance detection were performed using either HP CHEM station or Micromass MassLynx data systems.

2.3. Mass Spectrometry

Electrospray (ES) ionization was performed on a PE Sciex API III triple quadrupole mass spectrometer (Concord, ON, Canada) interfaced to the HP 1050 HPLC system. Flow was split at a ratio of 3 to 1 in order to deliver 50 μl min^{-1} into the electrospray interface. Typical ES–MS conditions utilized an ionization voltage of 4.5 kV and orifice voltage of 60 V. Atmospheric pressure chemical ionization (APCI) MS was carried out on a Micromass Platform II single quadrupole mass spectrometer (Beverly, MA, USA) with a Gilson 215 Liquid Handler for autoinjection. The source temperature was held at 150°C and the APCI probe temperature was set at 450°C. A cone voltage of 25 V and a corona voltage of 3 kV were used for most of the APCI experiments. All ES–MS and APCI–MS analyses were carried out in the positive ion mode.

3. Results and discussion

3.1. Standards

A mixture containing almost equal amounts by weight of nine standards was used to investigate the response among UV, mass spectrometry, and ELSD. The nine components of the mixture (Table 1) include a peptide, a steroid, two chemical library reagents, a reaction by-product, and four RPR compounds. These compounds were selected to span a range of molecular polarities, weights and melting points. The mixtures were eluted by a gradient with increasing organic content in the aqueous mobile phase. The tripeptide Gly–Gly–Val was chosen because it elutes early and hence does not interfere with analyte species. Additionally, if the ELSD detector response is a true measure of the weight percentage for each component in a mixture in ELSD, this peptide can function as an internal

Table 1
Nine standards and their corresponding molecular masses and melting points used in the ELSD response comparison studies

Compound	Molecular mass, Da	Melting point,°C
Gly–Gly–Val	231	215–218
RPR 118369	493	116–119
RG 12525	423	152–154
Fluocinolone Acetonide	452	267–269
Triphenylphosphine Oxide	278	156–158
4-Phenoxybenzoic Acid	214	150–151
4-Biphenyl Carboxaldehyde	182	57–59
RP 69698	413	158–159
RG 12561	386	98–99

standard when introduced quantitatively into the mixture.

3.2. Response comparison

The chromatograms of the nine standards detected by ELSD, UV, and MS (APCI and ES) are shown in Fig. 2. The chromatogram depicted in Fig. 2 was performed using a mobile phase containing 0.1% trifluoroacetic acid (TFA), while that in Fig. 2 was carried out in 10 m*M* ammonium acetate. These two mobile phases are commonly used in high throughput analysis of libraries. The UV and APCI–MS data were acquired on a 50×4.6 mm I.D. column with a 1:1 flow split prior to these two detectors in the same HPLC analysis. The ELSD and electrospray MS data were obtained on a 50×2.1 mm I.D. column with the HPLC flow split 3:1 so that 50 μl min^{-1} of effluent was allowed to enter the ES–MS.

With the exception of the peptide Gly–Gly–Val, all nine standards were detected by UV at 220 nm. The UV response exhibits no significant difference between the two mobile phases. In contrast to the UV response, a wide variation is observed in the APCI and ES–MS ion currents under the two mobile phases. In APCI–MS approximately a 3-fold increase in response is obtained using the TFA buffer than the NH_4OAc buffer for the steroid fluocinolone

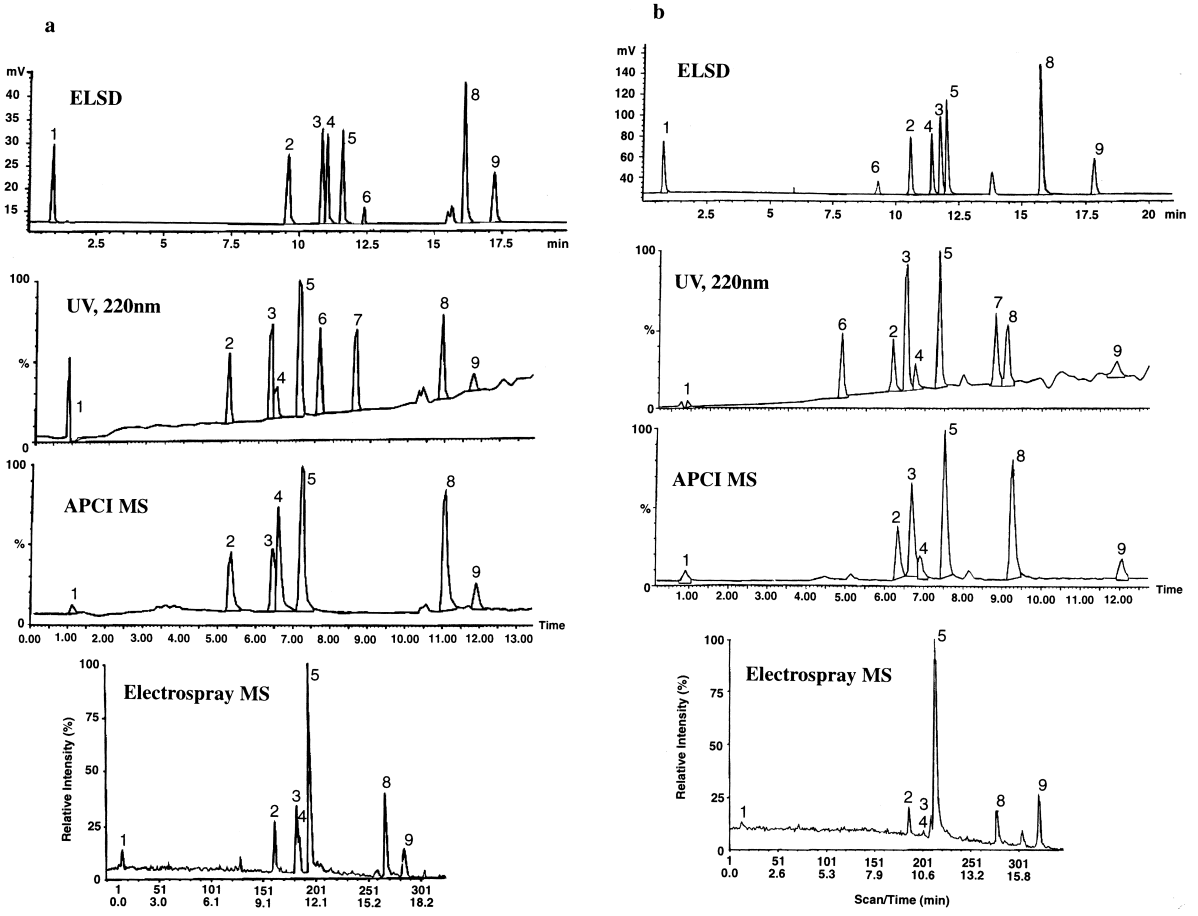

Fig. 2. (a) ELSD, UV, APCI–MS, and electrospray MS–HPLC chromatograms for the mixture of nine standards in 0.1% TFA buffer. The differences in the retention time are attributed to the different HPLC columns and gradients. (b) ELSD, UV, APCI–MS, and electrospray MS–HPLC chromatograms for the mixture of nine standards in 10 m*M* ammonium acetate buffer. The differences in the retention time are attributed to the different HPLC columns and gradients.

acetonide. Interestingly, in 0.1% TFA the relative intensities of individual peaks are in better agreement between UV and electrospray MS; but, in 10 m*M* NH_4OAc the UV response is more consistent with the APCI–MS response. A dramatic MS signal enhancement is noted for triphenylphosphine oxide, particularly in electrospray MS, which shows the greatest response factor. By and large, the MS response is very dependent on the ionization method and the composition of the HPLC mobile phase. Although UV response may not be so vulnerable to the change of mobile phases, its dependence on the chromophores of individual compounds is clearly demonstrated in Fig. 2. The non-linear responses exhibited by both MS (APCI and ES) and UV underscore the need for a universal detector in library quantitation.

The mass dependent response characteristic of the ELSD is illustrated in Fig. 2. While the ELSD response was consistent (see Table 2) for seven of the compounds in the standard mixture, the remaining two compounds provide examples of anomalous behavior: 4-biphenyl carboxaldehyde (melting point 57–59°C) was not detected, and 4-phenoxybenzoic acid (melting point 150°C) afforded a low ELSD response. In order to detect 4-phenoxybenzoic acid, the ELSD evaporation temperature had to be reduced to 40°C. In general, drift tube temperature is inversely proportional to baseline noise. The ELSD's insensitivity to these two compounds may be related to their volatility. However, the melting point of 4-biphenyl carboxaldehyde is greater than the experimental drift tube temperature of 40°C. The ability to form the requisite solute particles for efficient light scattering appears to have more effect on the ELSD response. The response factors (weighed to 1) of three detectors, ELSD, UV and APCI–MS, are given in Table 2. Excluding 4-phenoxybenzoic acid and 4-biphenyl carboxaldehyde, the ELSD response factors are very consistent ranging from 0.8 to 2. In contrast to the ELSD results, the UV and APCI–MS response factors are widely divergent, ranging, respectively, from <0.1 to about 2 and from <0.1 to about 3.

The atmospheric pressure ionization (API) source, either APCI or electrospray is prevalent in LC–MS interfaces. The likelihood of obtaining a MS response with a molecule depends on the ability of that molecular structure to sustain a charge, through formation of $[M+H]^+$, $[M+NH_4^+]$, etc. in positive ion mode and $[M-H]^-$ in negative ion mode. Molecular ionization occurs in solution or in the gas phase depending upon whether APCI or electrospray is used. The absence of positive ion MS responses for 4-phenoxybenzoic acid and 4-biphenyl carboxaldehyde reflect their inability to produce positively charged molecules in electrospray and APCI.

Negligible baseline perturbation during gradient operation has been demonstrated for ELSD [19]. Additionally, the so-called ''HPLC solvent front'' resulting from the injection volume has no effect on the ELSD baseline as evident in Fig. 2. Compatibility with gradient elution makes ELSD extremely useful for quantitation. In comparison, the intrinsic

Table 2
Comparison of response factors by weight (weighted to 1) between UV at 220 nm, ELSD, and APCI–MS under different HPLC mobile phases

Compound	0.1% TFA mobile phase			10 m*M* NH_4OAc mobile phase		
	UV	ELSD	APCI–MS	UV	ELSD	APCI–MS
1	0.04	1.17	0.09	0.08	0.84	0.25
2	0.84	0.85	1.11	0.78	0.99	0.94
3	1.37	1.18	0.82	1.97	1.28	1.60
4	0.56	1.04	1.96	0.41	0.98	0.46
5	2.17	1.30	2.52	1.80	1.51	2.69
6	1.17	0.25	ND	0.95	0.23	ND
7	1.42	ND[a]	ND	1.15	ND	ND
8	1.02	2.04	1.88	1.01	2.02	2.18
9	0.37	0.90	0.46	0.51	0.87	0.47

[a] ND: None-detected

weak UV response of the peptide bonds of Gly–Gly–Val at 220 nm is further complicated by the negative UV response at the HPLC solvent front, which makes it impossible to detect and quantitate the compound under conditions where it is eluted early. Determined principally by the scattering efficiency of the solute particulates, the ELSD response appears to be independent of changes in the HPLC buffer solution and gradient conditions. There is no direct relationship between the ELSD response and the melting point of a molecule. Fluocinolone acetonide has the highest melting point (mp=268°C) in the mixture of nine standards, but does not produce the highest ELSD response. Likewise, RP 69698 has the highest ELSD response; however, its melting point (mp=161°C) is comparable to that of triphenylphosphine oxide (mp=157°C) and 4-phenoxybenzoic acid (mp=150°C). Major factors influencing the kinetics of the solidification process include contributions by nebulization, temperature, and solvent composition, as well as the physical properties of the solid molecules (e.g. melting point, hygroscopicity). Clearly, the complex nature of the transformation from nebulized droplets to solid aerosol is an area requiring further research.

3.3. Direct quantitation

Since the ELSD response is a function of mass, quantitation using an internal standard as a reference in an unknown mixture should be feasible. Furthermore, with the compatibility of ELSD to gradient elution and the lack of ELSD baseline perturbation by the solvent front, the area of an HPLC peak can be measured accurately. Ideally, a reference standard should elute early, proximate to the solvent front, so that the standard does not interfere with the chromatography of the compounds of interest. A small peptide with molecular mass approximately 200–300 Da is a good choice for an internal standard owing to its polarity and good ELSD response. As shown in Fig. 3, two peaks corresponding to a known amount of Gly–Pro–Gly–Gly and a crude library product from a Ugi reaction were observed by ELSD. Based on the area percentages of these two chromatographic peaks, the concentration of this reaction product can be easily estimated. The deviation of this estimate is only about 20%. Furthermore, the internal standard technique excludes instrument performance errors.

3.4. Analysis of combinatorial and parallel synthesis libraries

The shortcomings associated with quantitation of libraries using MS and UV detection have been addressed above in the example of the mixture containing nine standards. Although ELSD has the potential to provide direct quantitation of any specific component in a mixture, its inability to detect low melting or volatile molecules remains a major concern in the characterization of libraries. In the preparation of small molecule libraries many of the chemical reagents and reaction by-products are either

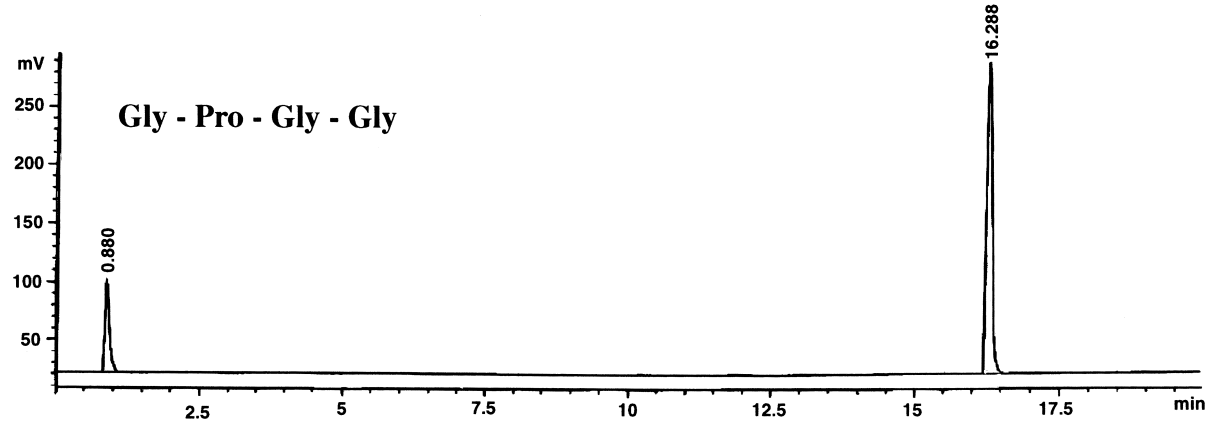

Fig. 3. LC–ELSD chromatogram of a reference tripeptide added to a single Ugi reaction library product.

not detected or respond poorly to ELSD. It seems clear that ELSD is the preferred technique for quantitation, UV detection provides a superior indication of overall purity, and MS is essential for product identification. Hence the three complementary techniques, UV, MS, and ELSD, must be employed in combination for library analysis. Accurate quantitative analysis of a library will become problematic if numerous impurities with different chemical and physical properties are present in the library members. Indeed, for libraries containing high levels of impurities quantitation is, arguably, unnecessary as they will not be suitable for biological screening.

These issues are illustrated by the HPLC chromatograms in Fig. 4 which depict the analytical results for one member of a library derived from a Ugi four component condensation reaction. In addition to the amine scaffold, the reagents involved in this library are a carboxylic acid, an aldehyde, and an isocyanide. Together with the desired product (retention time 14.23 min) there are several peaks present in the UV chromatogram. The relative intensities and peak area percentages of the three major peaks in the ELSD chromatogram correspond to those in the UV chromatogram. In this case the integrated peak areas of the desired product, correlate well between UV and ELSD, 19% vs. 22%, respectively. Most of the extra minor peaks shown in the UV chromatogram result from the starting materials, which are transparent in ELSD. This clearly exemplifies the complementary nature of UV and ELSD detection in library analysis.

4. Conclusions

Since each analytical technique, MS, UV and ELSD, has distinct advantages and shortcomings, the approach undertaken for characterizing a combin-

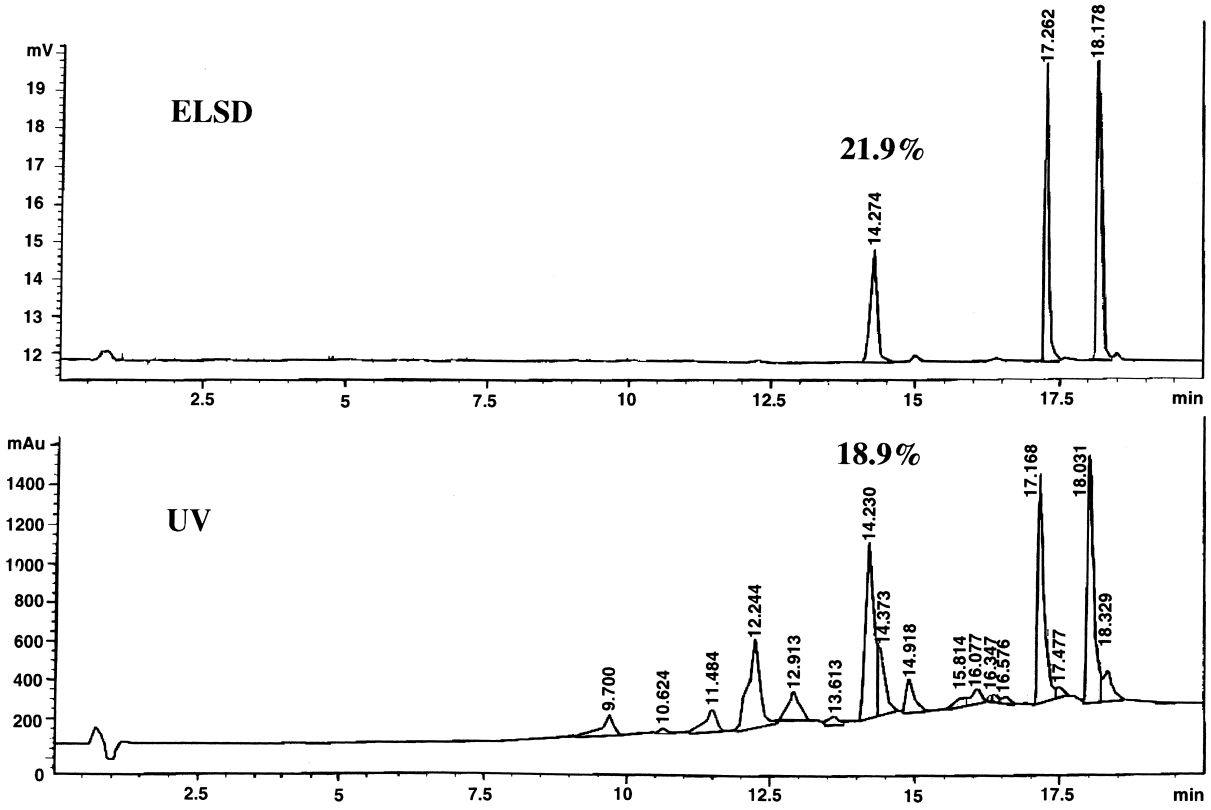

Fig. 4. UV and ELSD–HPLC chromatograms of a single solution-phase Ugi library member.

atorial or parallel synthesis library must take into account the type of chemistry, starting materials, the layout and design of the library in order to maximize attainable information in qualitative and quantitative analysis. Simultaneously applying these three complementary analytical techniques appears to be the solution, at least at the present, to the problem of high throughput characterization of libraries. For quantitation of small molecule libraries ELSD is currently the method of choice. In fact, in terms of accuracy and sensitivity ELSD seems to be the only analytical technique applicable to high throughput library quantitation. Barring the exhaustive use of reference standards, MS and UV are simply not quantitative techniques. The major caveats on ELSD quantitation include the detector's transparency to compounds which do not solidify while transiting the drift tube, and the variability in the ELSD response which can be as large as a factor of two. We believe that the present generation of ELSD instruments represent an indispensable technique for quantitative library analysis.

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