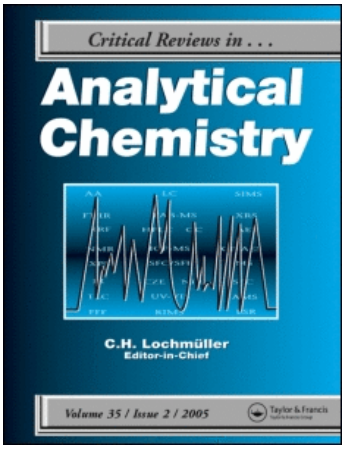

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Twenty Years of Evaporative Light Scattering Detection

Nikolaos C. Megoulas[a]; Michael A. Koupparis[a]
[a] Department of Chemistry, Laboratory of Analytical Chemistry, University of Athens, Panepistimiopolis, Athens, Greece

To cite this Article Megoulas, Nikolaos C. and Koupparis, Michael A.(2005) 'Twenty Years of Evaporative Light Scattering Detection', Critical Reviews in Analytical Chemistry, 35: 4, 301 — 316
To link to this Article: DOI: 10.1080/10408340500431306
URL: http://dx.doi.org/10.1080/10408340500431306

Critical Reviews in Analytical Chemistry, 35:301–316, 2005

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408340500431306

Twenty Years of Evaporative Light Scattering Detection

Nikolaos C. Megoulas and Michael A. Koupparis
Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens, Greece

Evaporative light scattering detector (ELSD) is a quasi-universal detector for liquid, countercurrent and supercritical fluid chromatography, since it can detect any analyte less volatile than the mobile phase. Operation principle mainly consists of three successive processes: nebulization of the chromatographic effluent; evaporation of the mobile phase; measurement of the scattered light. After 20 years of development, its usage appears significant advantages and potentialities as well as several limitations. In this paper, operation principles, technological innovations, methodological approaches, chemometrics, application areas (pharmaceuticals, foods and beverages, natural products, biological samples and polymers), potentialities and limitations of ELSD are thoroughly reviewed. A bibliography of 83 representative references is given.

Keywords evaporative light scattering, ELSD, review, applications, principle

INTRODUCTION

About 20 years have been past since the introduction of the first commercially available Evaporative Light Scattering Detector (ELSD) in the early 1980s (Mass Detector, Applied Chromatography System Limited, Macclesfield, Cheshire, UK), and nowadays ELSDs have moved into the mainstream of HPLC detection techniques. The inherent advantage of ELSD to detect any analyte, regardless of the optical (i.e., UV absorptivity), electrochemical or other analyte properties, is the main reason for ELSD expanded applicability. In fact, ELSD is considered to be a quasi-universal, rather than a fully universal, detector since analytes with higher volatility than the mobile phase can not be detected. It is mainly considered to be an LC detector; however, it also appears compatibility with countercurrent (CCC) and supercritical fluid chromatography (SFC). Figure 1 illustrates the rapid increase of published research articles concerning ELSD in the last 2 decades.

In many chromatographic application areas, substitution of an ELSD approach for spectrophotometric derivatization approaches (i.e., insertion of chromophoric groups), is feasible and therefore, widely recognized drawbacks of derivatization (dependence on experimental parameters, incompleteness of derivatization reaction, use of salt laden mobile phases, prolonged analysis time, additional cost for derivatization system and reagents) can be eliminated (1). Beyond the common usefulness, which any universal detector appear [i.e., refractive index detector (RID) and mass spectrometers (MS)], the increasing interest for ELSD is additionally attributed to some special characteristics: (a) compatibility with gradient elution and insensibility to temperature variation (unlike RID), (b) much better detectability than RID for most molecular classes, similar to conventional LC detectors (regular detection limit is in the nanogram range, depending on analyte volatility and relative molecular mass) and (c) low cost and easy operation (unlike mass spectrometers). However, it should be clarified that until now, ELSD is mainly considered to be a good alternative or supplemental detector rather than a substitute for the conventional HPLC detectors (UV/Vis, fluorimeters, etc.), while it lacks the huge identification potential of the wide range of LC-MS techniques. It should also be pointed out that besides ELSD advantages and potentialities, significant limitations and drawbacks appear (Table 1). In this paper, operation principles, technological innovations, methodological approaches, chemometrics, application areas, potentialities, and limitations of ELSD are reviewed.

Received 14 June 2005; accepted 19 October 2005.
We gratefully acknowledge support from the Ministry of Industry, Energy and Technology, General Secretariat of Research and Technology of Greece and the Ministry of Education (EPEAEK II program 'Pythagoras').
Address correspondence to Michael A. Koupparis, Laboratory of Analytical Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece. E-mail: koupparis@chem.uoa.gr

PRINCIPLES OF OPERATION

The operation principle of ELSD (2, 3) mainly consists of three successive processes, depicted in Figure 2: (a) nebulization of the chromatographic effluent, (b) evaporation of the mobile phase, and (c) detection of the non-volatile residual particles, by means of the measurement of the scattered light.

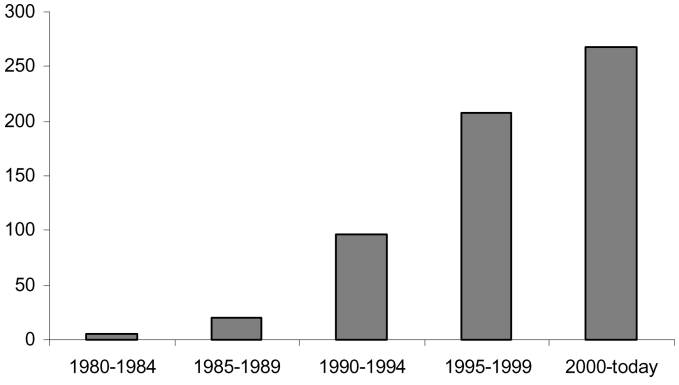

FIG. 1. Published research articles concerning ELSD per 5 years, from 1980 (source: Analytical Abstracts, Royal Society of Chemistry).

Nebulization

In the first step of ELSD detection mechanism, the effluent from a chromatographic column enters a Venturi-type nebulizer, where it is transformed into an aerosol. These nebulizers create a high flow of carrier gas (air or inert gas, such as nitrogen, carbon dioxide, argon or helium) over the liquid surface producing a high amount of droplets with remarkably uniform size.

Distribution and mean values of droplets diameter are considered to be very critical parameters, which strongly influence the analytical characteristics (i.e., detectability, sensitivity and repeatability) of the ELSD methods (4–6). The formation of uniform, reproducible and stable aerosols depends on the relation of the nozzle diameter and the flow rates of mobile phase and nebulizing gas. For constant diameter of the nozzle, stable aerosols are formed only for a limited range of flow rates, and further, the flow rate of the nebulizing gas must be adjusted in relation to the flow rate of the mobile phase. For a mobile phase flow rate of 1 ml min^{-1}, the usual consumption of the nebulizing gas must be in the range of 2–5 l min^{-1}. The size distribution of the aerosol droplets can be successfully described by the Mugele–Evans upper-limit log-normal size distribution (3, 7) and it is mainly determined by nebulizer geometry as well as liquid and gas flow rates. Narrow distribution is a requirement for good repeatability and ELSD sensitivity.

Furthermore, it has been indicated that mean diameter of aerosol droplets strongly influences ELSD response and in fact an increase of the mean diameter of aerosol droplets results in ELSD response enhancement. Mean diameter of aerosol droplets (D_{sv}) (Sauter mean diameter) is dependent on the surface tension (σ), density (ρ) and viscosity (μ) of mobile phase, the difference of velocity between the nebulizing gas and the chromatographic effluent ($v_g - v_l$) and the ratio of liquid and gas volumetric flow rates (Q_l/Q_g). It can be calculated from the Nukiyama—Tanasawa empirical equation (8), which appears to have general validity for ELSDs, in cases of droplets in the range of 15-90 μm and mobile phase density between 0.7 and

TABLE 1
Main limitations and advantages of ELSD over the universal detectors of mass spectrometry and refractive index

	Advantages over	
Limitations of ELSD	Refractive index	Mass spectrometry
— Low identification potential	— Insensibility to ambient temperature	— Low cost
— Low selectivity	— Compatibility with gradient elution	— Easy operation
— Requirement for highly volatile mobile phases	— Better detectability	— No solvent front peaks
— Destructive detector	— No negative peaks	— Operation at atmospheric pressure
— Quantitation limit usually above 0.1 μg ml^{-1}	— No solvent front peaks	
— Non-linear response	— Calibration curves appear smaller deviation from linearity	
— Low sensitivity/detectability for volatile and thermosensitive analytes	— Wider dynamic range	
— Sensitive to column bleed (e.g., silica based amino column in the presence of an aqueous mobile phase)	— Compatibility with much wider range of solvents and modifiers	
	— Similar response factor for molecules with similar structure	
	— Shorter equilibration time	
	— Insensibility to pulsations of HPLC pump	

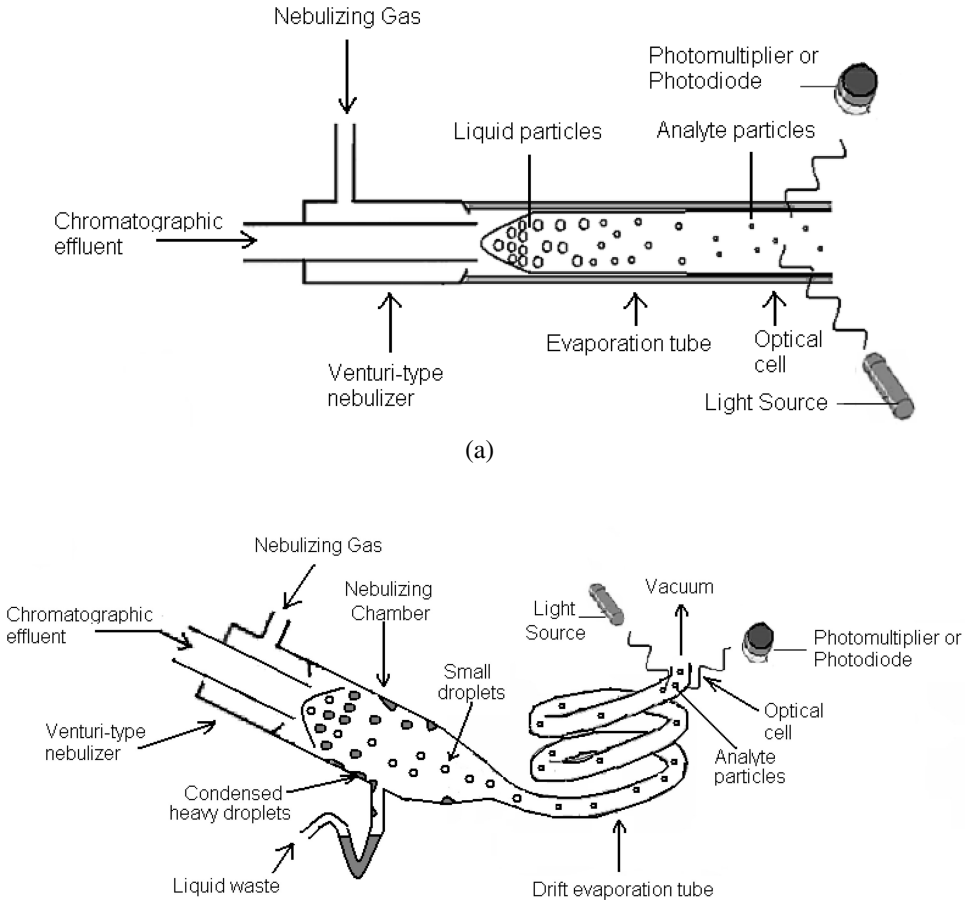

FIG. 2. Schematic depiction of the main steps of the ELSD operation. Design of (a) ELSD type A and (b) ELSD type B.

1.2 g ml^{-1} (9, 10):

$$D_{sv} = \frac{585\sqrt{\sigma}}{(v_g - v_l)\sqrt{\rho}} + 597\left(\frac{\mu}{\sqrt{\sigma\rho}}\right)^{0.45}\left(1000\frac{Q_l}{Q_g}\right)^{1.5} \quad [1]$$

From Eq. [1], it is concluded that decrease in the flow rate of the nebulizing gas (v_g), with constant or increased flow rate of the mobile phase (v_l), results in increase of the average droplet diameter, which induces increase in the ELSD response. However, if the gas flow rate is too low, mobile phase would not be completely nebulized and/or it would not be completely vaporized, which would result in an excessive noise or baseline with spiked sharp peaks.

ELSDs are classified in two types according to their structure after the nebulization unit (6, 11). In ELSD of type A (non aerosol splitting, Figure 2a), the entire aerosol immediately enters the heated evaporation tube (drift tube), where the evaporation process begins. In ELSD of type B (aerosol splitting, Figure 2b), the aerosol, before the evaporation step, enters a glass chamber or a focusing cone (nebulization chamber), in which the droplets of high size are condensed on the walls of the chamber and diverted to waste. The proportion of the wasted aerosol depends on mobile phase volatility and varies from >90% (aqueous mobile phases) to <10% (highly volatile organic solvents). Each type appears its own benefits, while the appropriate choice depends on the nature of the analyte and the composition of the mobile phase. ELSD of type B requires lower evaporation temperature than type A and thus it is more sensitive for volatile, semi-volatile or thermo-sensitive analytes. On the other hand, for non-volatile analytes, ELSD of type A appears to be more sensitive, since the entire quantity of analyte reaches the optical cell. Considering the composition and flow rate of the mobile phase, ELSD of type A is incompatible with gradient elution and requires low flow rates and highly volatile mobile phases (non-aqueous or low water portion), while ELSD of type B appears of a wider compatibility.

Evaporation of Mobile Phase

In this stage, the size of the aerosol droplets is reduced, due to the evaporation of the mobile phase, which is performed in a heated drift tube. Ideally, the purpose of this stage is to completely vaporise the mobile phase, without any analyte loss (due to evaporation or thermal decomposition). The completeness of

the mobile phase evaporation and the extent of loss of analyte is mainly determined by the evaporation temperature, which should be selected in accordance to the mobile phase and analyte volatility, to the mobile phase flow rate and to the ELSD type (A or B). Inappropriate selection of the evaporation temperature results, in case of low temperature, in an excessive noise or baseline with spiked sharp peaks, or in case of high temperature, in reduced sensitivity. Apart from the analyte loss, high evaporation temperature causes rigorous solvent evaporation, which destroys uniformity of particle size, and favours the formation of liquid rather than solid particles. Both effects result in decrease of ELSD sensitivity. The evaporation temperature is usually set between 30°C and 100°C. Decrease of the required evaporation temperature can be obtained with nebulizing gas of high thermal conductivity (helium was found to require at least 30°C lower evaporation temperature than carbon dioxide), which in cases of volatile and thermosensitive compounds results in enhancement of detector sensitivity (12). On the other hand, for stable and non volatile compounds, the ELSD response factor has been found to be independent of the nature of the nebulizing gas (13).

In case of complete solvent evaporation, the diameter of the particles D is related to the diameter of the droplets before evaporation D_0:

$$D = D_0 \times (C/\rho)^{1/3} \quad [2]$$

where C is the concentration of the analyte and ρ the droplets density. It should be clarified that D_0 is not equal to D_{sv} (though they are closely related), since the distribution of the droplets size is significantly modified after the nebulization step due to the loss of the larger droplets (especially in ELSD of type B), partial coagulation of the droplets or ionic redistribution (10).

From Eq. [1], it is concluded that the number of droplets formed in the nebulizer per time unit, is controlled by mobile phase properties and flow rate, while it is independent of the analyte concentration. On the other hand, analyte concentration is the major parameter, which, after mobile phase evaporation, controls the diameter (but not the number) of the analyte particles (Eq. [2]). Therefore, the dependence of ELSD response on analyte concentration is generated by the differences in the diameter and not in the number of analyte particles.

Structural differences occur among the various commercially available ELSDs, concerning the heated drift tube. The prevailing structure appears to be a long coiled drift tube (with an upward direction), in which low evaporation temperature appears to be efficient (6), while a short drift tube with high evaporation temperatures and pre-heated carrier gas or gas with a high thermal conductivity have also been reported (12, 14). Efficiency of chromatographic separations does not appear to be affected by the length of the evaporation tube (6), providing that an appropriate level of vacuum is applied at the end of the flow path in order to establish a stable flow of liquid and solid aerosol.

Light Scattering

The aerosol, after the evaporation process, ideally composed by solid particles of analyte, enters the optical cell and passes through a light beam. The scattered light is measured by a photomultiplier or a photo diode, providing the output signal.

Light scattering processes are classified in two types: elastic scattering, in which the scattered radiation is of the same frequency as the incident radiation, and inelastic scattering, in which the scattered radiation is of a different frequency. In ELSDs, inelastic scattering is considered to be negligible and it is not further examined. Elastic scattering is classified in three types: Rayleigh, Debye and Mie. Refraction-reflection mechanism, which has its origin in the induced secondary emission of particles in the path of the incident beam, has also been reported as a potential mechanism of scattering in the ELSD optical cell (2, 4, 6). The type of interaction between the light and the particles depends on the size, shape and surface properties of the particles and the wavelength (λ) of the incident light. Reyleigh-Debye scattering occurs with the smallest particles ($D/\lambda < 0.1$), Mie scattering becomes the predominant mechanism for $0.1 < D/\lambda < 1$, while the refraction-reflection mechanism occurs in case that the particle size is greater than the wavelength ($D/\lambda > 1$). In the case of $\lambda = 0.35\,\mu$m (the wavelength with the maximum emission of tungsten lamp), Mie and refraction-reflection processes prevail, since analyte particles with radius smaller than 100 nm is usually not detectable. Furthermore, refraction is relatively more important compared to reflection in the refraction-reflection domain. Actually, in most cases, more than one scattering mechanisms occur in the ELSD optical cell, due to: (a) variations of the aerosol droplet diameter D, which is dependent on the nebulization and evaporation processes, (b) the polychromatism of the light source and (c) dependence of the mean droplet diameter on the sample concentration.

Since scattering and not absorbing phenomenon is intended to occur when the light interacts with the analyte particles, a tungsten filament or halogen lamp that produces a continuous spectrum of wavelengths, rather than a monochromatic laser-emitting diode, is favored as a light source. In some instruments a secondary gas, independent of the nebulizing gas, is used to concentrate the particles in the center of the detection cell and to prevent the deposition on the cell inner surfaces.

The power of scattered light is controlled by the particle diameter, the light wavelength and the angle of scattered light. It has been observed that the ELSDs sensitivity is higher, but the dynamic range narrower, for low detection angle, with wide angular acceptance and the use of vertically polarized or unpolarized light (5, 15).

Packed Column SFC-ELSD Coupling

Despite the fact that Supercritical Fluid Chromatography (SFC) appears to be a very potential technique, capable of rapid

and highly efficient chromatographic separations, its development was not adequately accelerated, due to several reasons. Amongst them definitely is the lack of compatible and reliable detectors. In recent years, coupling of packed column SFC and ELSD has been reliably achieved, while further improvements are continuously reported. The basis of the SFC-ELSD coupling (decompression of the supercritical chromatographic effluent and its transformation into an aerosol), appears to eliminate many of the previous limitations, making ELSD one of the most applicable and dynamic packed SFC detectors (16).

In packed SFC-ELSD, the nebulization step appears to be much more complicated than HPLC-ELSD and this is mainly due to the low viscosity of the supercritical fluids and the post-column decompression procedure.

Firstly, the low viscosity of the supercritical fluids requires a regulating post column back pressure, in order to establish pressure and flow rate control. This could be achieved by: (a) a capillary silica restrictor, which can provide constant pressure, dependent on its dimensions and (b) a back pressure regulating valve, which allows pressure control independent of flow rate (17).

Secondly, decompression stage of supercritical fluid, transforms the effluent of the chromatographic column, directly into an aerosol, without a nebulizing gas to be required. However, since decompression is a highly endothermic procedure, solid particles (ice) of carbon dioxide (the most common supercritical fluid in SFC) may be formed, resulting in excessive background noise. The usage of a nebulizing gas though it is not essential for SFC-ELSD, it considerably decreases background noise and therefore, improves ELSD detectability. Besides carbon dioxide ice formation, decompression step may cause untimely analyte precipitation after the pressure regulator and prior to the ELSD, due to the low solubility of the analyte in the gas phase. This effect can be prevented, either by heating the pressure regulator valve or more efficiently by adopting a two-step decompression procedure. In the first step, pressure is decreased by a pressure regulator but the mobile phase is still maintained in supercritical (or subcritical) condition, while in the second step effluent enters ELSD through a heated capillary silica restrictor where decompression to atmospheric pressure and simultaneous nebulization occur (Figure 3a). Better SFC-ELSD coupling can be obtained by using an efficient analyte solvent, which enters the chromatographic stream between the first and the second decompression step (18). In this case, the pressure regulator is not essential, since the back pressure produced by the heated capillary silica restrictor can be regulated by the flow rate of the additional solvent (Figure 3b) (16, 17).

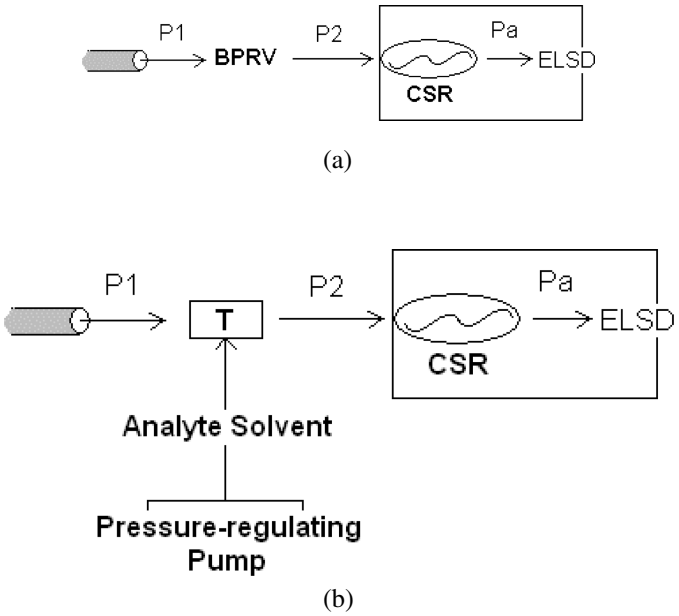

FIG. 3. Schematic depiction of SFC-ELSD coupling using: (a) a back pressure regulating valve (BPRV) and a heated capillary silica restrictor (CSR) and (b) an analyte solvent and a CSR. (P_a = atmospheric pressure; P_c (critical temperature) $< P_2 < P_1$).

CHARACTERISTICS OF ELSD RESPONSE

In most applications, a non-linear response has been observed for the ELSD. The area of the chromatographic peak (A) appears good correlation with the analyte mass (m) according to the exponential relationship:

$$A = a \times m^b \quad [3]$$

where a and b are coefficients depending on the ELSD instrumentation and on nebulization and evaporation processes (flow rates of the nebulization gas and mobile phase, composition of the mobile phase, evaporation temperature, etc.). Coefficient b generally varies between 0.9 and 2 (6, 9). In case that b is close to unity ($b \cong 1$) (mainly observed for high water content in mobile phase) a linear calibration curve can be constructed, otherwise it is necessary to use double logarithmic coordinates in order to obtain a linear calibration curve, the slope of which is equal to b:

$$\log A = b \log m + \log a \quad [4]$$

A linear calibration curve can also be obtained by raising the area of chromatographic peak to $1/b$ (19):

$$A^{1/b} = a^{1/b} \times m = k \times m \quad [5]$$

Equation [5] provides a linear calibration curve passing through the origin and can be used for routine quantitations using the single-point calibration approach, provided that the coefficient b has been previously determined from Eq. [4], using a series of standards. Apart from the exponential relationship, second and third order polynomial regressions have also been utilized in order to achieve a good correlation between the peak area and the analyte mass (20).

The contribution of refraction to the light scattering process suggests that the scattering response is a function of the analyte refractive index, which has been reported to be the reason for the approximately constant ELSD response factor for molecules with similar structure (2, 21). However, in case of molecules with significant differences in their structure a correction formula has to be developed (5).

Apart from the molecular structure of the analytes, ELSD response factor is expected, because of the exponential response (Eq. [3]), to be significantly influenced by the shape (width and asymmetry) of the chromatographic peaks. It has been reported that a remarkable increase of ELSD response factor is induced by the decrease of peak width and the closeness of symmetry factor to unity (22). To eliminate the influence of peak width (but not of the symmetry) on ELSD response, a normalization procedure of the area of chromatographic peaks should be adopted, based on a selected standard peak width (σ_n), according to the relation (23):

$$A_n = A \times \left(\frac{\sigma_n}{\sigma}\right)^{1-b} \quad [6]$$

where A and σ is the area and the width of the chromatographic peak and A_n is the normalized area.

Another worth mentioning property of ELSD exponential response, is that the chromatographic peaks appear sharper (smaller peak width) than the 'real' chromatographic distribution. It can be proved that the width of the recorded chromatographic peak (σ_{elsd}) is related to the width of the "real" chromatographic distribution (σ) by (15):

$$\sigma_{elsd}^2 = \sigma^2/b \quad [7]$$

since in most ELSD applications, $b > 1$, it is derived that $\sigma_{elsd} < \sigma$. Accordingly, the improvement of peak width results in improvement of column efficiency and chromatographic separations (resolution).

LIMITATIONS

Apart from the fact that ELSDs appear nearly no selectivity, an inherent characteristic of most 'universal' detectors, some additional requirements may limit their applicability (Table 1):

(a) The main difficulty for the development of LC-ELSD analytical methods, is the restriction on the mobile phase volatility. Non-volatile modifiers, ion-pairing reagents, acids, bases and buffers cannot be used with ELSD. Therefore, a very useful part of the mobile phase chemistry is not compatible with ELSD, making quite difficult to convert an LC-UV method to an LC-ELSD method or to achieve efficient chromatographic separations for some type of analytes. Some acceptable volatile reagents are trifluoroacetic, heptafluorobutyric, nonafluoropentanoic, acetic and formic acid and their ammonium salts in low concentrations (< 0.1 M).

(b) ELSD is a destructive detector, therefore it must be last in line if it is used in series with other detectors. In cases that it is used in line with another destructive detector (e.g., MS), a line splitter should be added and the flow rate of the nebulizating gas should be accordingly adjusted.

(c) Generally, it appears relatively low detectability, inadequate for the direct analysis of compounds (e.g., impurities, residues) at ng/ml concentration level (quantitation limit is usually above 0.1 μg/ml). In these cases, preconcentration steps should be developed, in order to enrich the under analysis samples. However, development of a preconcentration procedure is quite difficult, mainly for two reasons: firstly, non-volatile reagents can not be applied (e.g., precipitation reagents and buffers) and secondly, preconcentration procedure may simultaneously enrich some of the matrix components resulting in excessive interferences due to the low ELSD selectivity. In order to increase the ELSD detectability, some novel considerations have already been proposed: (a) Increase of particles diameter by a condensation growth process, in which the sample is mixed with an auxiliary gas saturated with butanol vapor. The mixture is rapidly cooled promoting the condensation of butanol onto analyte particles (Condensation Nucleation Light Scattering Detection) (24). (b) Addition of appropriate volatile reagents in the mobile phase, which are included in the particles rather than removed during the evaporation process, resulting in increase of the particle diameter. Stoichiometric quantity of triethylamine and formic acid in mobile phase has been reported to remarkably increase the ELSD response (10). The same effect has been observed for the addition of anionic reagents in cases of cationic analytes (formation of ion-pairs), for which it has been proved that the ELSD response enhancement is controlled by their molecular mass (22). ELSD response enhancement has also been reported to be obtained by the post-column addition of silver nitrate (24).

(d) In the frame of a routine work, a linear response curve is preferable, since one-point calibration can be used. The exponential relationship (Eq. [3]) requires at least two standard solutions for the calibration curve, while it appears difficulties in case of internal standard approach. In order to obtain a linear calibration, apart from the mathematical transformations (Eqs. [4] and [5]), there have been proposed some instrumental modifications. A nebulizer producing a broad particle size distribution would result in decrease of coefficient b, which can be adjusted approximately equal to unity (5), while the same result can be achieved by the post-column addition of cholesterol (25).

(e) Normal phase, polar (e.g., amino and cyano) or ion exchange silica-based analytical columns, have been observed

to suffer from significant column bleeding in case of mobile phase with high water content (e.g., determination of sugars with amino column). Since the material leaking from the analytical column consists of non-volatile particles, ELSD background signal (baseline) appears significant drift and excessive noise. In these cases an alternative polymeric–based column, should be utilized, with however higher purchase cost (26).

RECENT ACHIEVEMENTS IN ELSD TECHNOLOGY

ELSD was invented in 1966 at Union Carbide's Australian research laboratories, but it was not commercially availably until the early 1980s. In the present time, there are several commercially available ELSDs, most of which bare a nebulization chamber for the elimination of the largest droplets, and therefore, they are classified as type B. A dual-mode ELSD, which is able to operate either as type A or as type B, has also been developed, utilizing a removable adaptor (Alltech Associates, Deerfield, IL, USA).

Instrumental modifications have recently been proposed in order ELSD to become compatible with microbore and capillary columns. The same modifications are also appropriate in case of connection of ELSD with LC-MS or LC-NMR. The modifications mainly concern the reduction of the diameter of the nebulizer capillary, in order to increase the linear velocity of the mobile phase and the efficiency of the aerosol production (27).

Another novel instrumental modification concerns the ability of an ELSD to provide simultaneous detection of the eluent from four independent HPLC systems, which is considered to be very useful in pharmaceutical combinatorial applications (Sedere, Alfortville, France).

APPLICATIONS

ELSD has been effectively used for the determination of a wide variety of compounds in various synthetic or natural matrices. The main application areas of ELSD concern pharmaceuticals (1, 13, 20, 22, 28–54) foods and beverages (55–68), natural products and biological samples (69–80) and polymers (23, 81–83). Representative applications of each area are presented in Tables 2 to 5 and some of them are discussed in more details below. A wide range of column types and mobile phase polarity have been utilized and various procedures for sample preparation have been developed depending on the analyte nature and the sample matrix. Beyond the differences of analyte and matrix nature, a common characteristic of all ELSD methods is the conformity with the following rule: "non volatile analytes are determined utilizing a volatile mobile phase."

Pharmaceutical Analysis (Table 2) (28)

Aminoglycosides (1, 13, 22, 29). Aminoglycosides are widely administrated, broad spectrum antibiotics. For most of them, the official assay in pharmaceuticals is based on a microbiological method, which is time consuming, with low detectability and precision, while it appears no specificity among the various members of the class or against other antibiotics with similar action. Various HPLC methods have been developed but due to the low UV absorptivity of aminoglycosides, post- or pre-column derivatization is required. Direct non-derivatization methods are based on pulsed electrochemical detector (PAD), requiring rather sophisticated handling and suffering from tedious baseline stabilization.

In the frame of daily routine work, ELSD appears to be an appropriate choice. HPLC-ELSD methods have been developed and validated for the determination of isepamicin, gentamycin (Figure 4), neomycin, kanamycin, tobramycin and amikacyn in raw materials, various formulation types (e.g., injection, cream, ointment, powder, aerosol) and biological fluids. The direct determination of the four main compounds of gentamicin is an excellent example of HPLC-ELSD capabilities. Furthermore, HPLC-ELSD methods are capable of the simultaneous determination of coexisting sulfates in raw materials, for which an extra titrimetric procedure is performed in the official procedure. All methods are based on reversed phase ion pairing retention mechanism, obtained by various C_{18} analytical columns and polar mobile phases containing volatile organic acids. Limits of detection are in the μg ml^{-1} range, while good repeatability (<2, %RSD) and accuracy (95–105, %recovery) have been obtained.

Gingenosides. Ginsenosides are triterpenic saponins considered to be the main bioactive constituents of the herbal medicine "ginseng" derived from the roots and rhizomes of different Panax species (Araliaceae), with antistress, antihyperglycemic and potential antitumor properties.

The main problems encountered in performing HPLC-UV analysis of ginseng are the high level of baseline noise and the poor sensitivity due to the weak UV absorption. This feature also limits the choice of solvents and mobile-phase modifiers for improved separation. The developed ELSD method showed a lower detection limit (53 ng on column) in comparison with UV detection (1060 ng) (20). White and red ginseng were analysed by HPLC–ELSD for their content in ginsenosides Rb_1, Rb_2, Rc, Re, Rd, Rg_1, Rf, Rg_2, Rg_3 and Rh_1 using a LiChrosorb NH_2 column and gradient elution of mixtures of acetonitrile–water–isopropanol (80:5:15) to (80:20:15) (30). The separation and the detection of the less polar ginsenosides in processed P. ginseng, F_4, Rg_3, Rg_5, Rg_6, Rk_1, Rk_3, Rs_3, Rs_4, Rs_5 together with the 20-(R) epimers of Rg_2, Rh_1, Rg_3 and Rs_3 was achieved using a C_{18} column with an acetonitrile–water–acetic acid gradient (Figure 5) (31). Furthermore, a fast HPLC–ELSD method for the determination of 24(*R*)-pseudoginsenoside F_{11} in American ginseng was obtained by Waters Spherisorb ODS-2 C_{18} column and mobile phase of acetonitrile and water under gradient conditions (20).

Steroids. Steroids were one of the first classes which were investigated for ELSD applicability. In 1984, HPLC-ELSD determination of 17 steroids on normal and reversed phase

TABLE 2
Applications of ELSD in pharmaceutical analysis

Compound	Matrix	Column	Mobile phase	Reference
Aminoglycosides	Raw materials, formulations, biological fluids	ODS-2 C18 Spherisorb (250 mm × 4.6 mm, 5 μm)	water–acetonitrile (55:45 v/v), containing 1.5 ml HFBA per liter	29
Ginsenosides	Ginseng roots	ODS-2 C18 Spherisorb (250 mm × 4.6 mm, 5 μm)	water–acetonitrile (gradient from 75:25 to 10:90 v/v)	20
Steroid conjugates	Standard solutions	Alltima C18 (150 mm × 2.1 mm, 5 μm)	water–methanol (gradient from 2:3 to 0:1) containing 0.1% TFA	33
PEGs, methyl and dimethyl ethers	Synthesized samples	Spherisorb ODS-2 S3W (100 mm × 4.6 mm, 3 μm) and Spherisorb S5X C18 (250 mm × 4.6 mm, 5 μm)	water–methanol (from 20:80 to 40:60)	36
Sodium carbonate	Ceftazidime for injection	Hypersil SCX (250 mm × 4.0 mm, 5 μm)	ammonium acetate (0.02 M, pH 6.0)–acetonitrile (80:20 v/v)	41
Polysorbate 80	Parenteral formulations	Alltima C18 (250 × 4.6 mm, 5 μm)	methanol–water (gradient from 30:70 to 90:10 v/v)	42
Midecamycin and related impurities	Raw materials	Diamonsil C18 column	acetonitrile–ammonium formate (0.2M, adjusted to pH 7.3 with triethylamine) (52:48 v/v)	43
Saponins and alkaloids	Caulophyllum thalictroides (blue cohosh)	Synergi Max-RP 80A (150 mm × 4.6 mm, 4 μm)	ammonium acetate buffer (pH 8)–acetonitrile (gradient from 90:10 to 40:60 v/v)	44
Terpene lactones and flavonoid aglycones	ginkgo biloba	Supelco Discovery C18 (250 mm × 4.6 mm, 5 μm)	methanol (containing 0.05% TFA)–water (containing 5% methanol and 0.05% TFA) (gradient from 25:75 to 90:10 v/v)	45
Organic acids	Hygroscopic pharmaceutical herbal dry extracts	Aminex HPX-87-H strong cation-exchange resin column (300 × 7.8 mm), fitted with an ion-exclusion Micro-Guard refill cartridge	0.02 M TFA	46
Simethicone	Formulations	Alltima C8 (250 mm × 4.6 mm, 5 μm)	acetonitrile–chloroform (gradient from 45:55 to 15:85 v/v)	47
Andrographolide	Commercial products	Supelco Discovery C18 (250 mm × 4.6 mm, 5 μm)	water–acetonitrile (gradient from 80:20 to 50:50 v/v)	48
Direct screening (paracetamol)	Biological fluids	LiChrolut EN sorbent column (flow configuration)	acetonitrile	49
Imperialine	Plasma	Supelco C8 column (150 mm × 4.6 mm, 3 μm)	water–acetonitrile–methanol (containing 0.6% triethylamine) (gradient from 7:35:58 to 0:42:58 v/v/v)	50
Inulin	Biological fluids	Aminex HP-X87-P (300 mm × 7.8 mm, 9 μm)	water	51
Carnitine and *o*-acylcarnitine enantiomers	Standard mixture	A teicoplanin-bonded chiral stationary phase (CSP) prepared from 5μm LiChrosorb Si 100 silica gel (experimental details given) and packed into HPLC columns (25 cm × 4.6 mm)	mixtures of organic solvents (methanol, ethanol and acetonitrile) and aqueous solutions (25–50 mM) of ammonium acetate	52
Acarbose	Tablets	Nucleosil-NH_2 (25 cm × 4.6 mm, 5 μm)	methanol-dichloromethane (13:7 v/v)	53
Ursodeoxycholic acid and related impurities	Formulations	Hypersil ODS-RP-18 (100 × 2.1 mm, 3 μm)	methanol–acetonitrile–water (30:11:9 v/v/v) adjusted to pH 4 with glacial acetic acid	54

TABLE 3
Applications of ELSD in analysis of foods and beverages

Compound	Matrix	Column	Mobile Phase	Reference
Carbohydrates (fructose, glucose, lactose, maltose, raffinose, sucrose)	Honey, milk powder, pineapple-juice	Silica gel	water–acetonitrile containing 0.05% (v/v) ethanolamine and 0.05% (v/v) triethylamine.	55
Paraffin,wax esters, cholesterol esters, fatty acid methyl esters, triacyl glycerols, fatty alcohols, free fatty acids, cholesterol, 1,3-diacyl glycerols, 1,2-diacyl glycerols, monoacyl glycerols and fatty acid amide	Migration of lubricants from food packaging	LiChrospher R Diol (125 mm × 3 mm, 5 μm, 100 Å)	Isooctane-tert-butyl methyl ether containing 0.1% acetic acid	60
Triacylglycerol (cocoa butter equivalents)	Chocolate	LiChrospher 100 - 5-RP18 (500 mm × 4 mm, 5 μm)	acetonitrile–chloroform (40:60 v/v)	62
Fatty acids	Grape seed oil	Phenomenex Luna C18 (150 mm × 4.6 mm)	methanol (1% acetic acid)–water (1% acetic acid) (95:5 v/v)	63
Lactose, fat, and total protein	Milk	Autoanalyser based on flow injection technique		64
Wax esters, sterol esters and FAME	Margarines and vegetable oils	Aluspher Al 100 alumina (125 mm × 4 mm, 5 μm)	hexane (THF 1‰)–isopropanol (gradient from 100:0 to 95:5 v/v)	65
Polar compounds	Used frying oils	Cyano Nucleosil (30 mm × 4.6 mm, 10 μm)	hexane–hexane:ethanol: water, (50:50:1 v/v/v) (gradient from 100:0 to 0:100 v/v)	66
Sn-2 monopalmitin	Infant formulae	Spherisorb ODS-2 (250 mm × 4.6 mm, 5 μm)	acetonitrile–acidified water (99:1 v/v)	67
Glycolipids	Cereals, fruits, legumes and vegetables	LiChrospher Si 60 (125 mm × 4 mm)	gradient of chloroform–aqueous 95% methanol	68

analytical columns was reported. Stable baselines were obtained under gradient elution, while detection limit was approximately 0.5 μg ml^{-1} (32). Furthermore, HPLC-ELSD was proved to be capable of steroid conjugates analysis [e.g., estrone 3-sulfate, b-estradiol 17(b-D-glucuronide), 5b-pregnane-3a,20a-diol glucuronide], appearing several advantages over alternative techniques, such as avoidance of radioactive waste of RIA methods, as well as baseline drift and poor detectability of low absorptivity steroids of HPLC-UV methods (33).

In the last decade, apart from HPLC, packed SFC has been proposed as an effective technique for steroids separation (e.g., progesterone, testosterone, 17-a-hydroxyprogesterone). Carbon dioxide is most commonly selected as the supercritical fluid, modified under gradient elution with methanol or chlorodifluoromethane. ELSD has been proved to be insensitive to polar modifier concentration, which is an important advantage over FID and UV detectors (34). In both, HPLC- and packed SFC-ELSD, detection limits (on column) have been established at the nanogram range.

Polyethers. During the last years polyethers and their monoalkyl and arylalkyl derivatives have gained more and more interest in various scientific fields and especially in pharmaceutical technology. In particular, polyethylene glycol (PEG) derivatives are extensively used as essential additives in non-ionic surfactants and wetting agents in laundry and industrial cleaners, solubilizers in enhanced oil recovery, ingredients in the cosmetic and food industries, emulsifiers in pharmaceutical preparations and solubility enhancers in biochemical membrane technology. The common feature of all polyether derivatives consists in their more or less wide synthesis-dependent oligomer distribution, which in turn, requires very efficient techniques for exhaustive characterization, not only with respect to the degree of

TABLE 4
Applications of ELSD in analysis of natural products and biological samples

Compound	Matrix	Column	Mobile Phase	Reference
The four major neutral glycosphingolipids	Urine	polyvinyl alcohol bonded stationary phase	chloroform–acetone:methanol (90:10 v/v) (gradient from 100:0 to 0:100 v/v)	69
1-Deoxynojirimycin	Mulberry leaves	TSKgel Amide-80	acetonitrile–water (6.5 mM ammonium acetate, pH 5.5) (81:19 v/v)	70
Hepatotoxic pyrrolizidine alkaloids	Plant extracts	Waters XTerra RP18 (150 mm × 4.6 mm; 5 μm)	0.015 M ammonium acetate–acetonitrile (gradient from 95:5 to 50:50 v/v)	71
Bile acids	Human serum and urine	integrated flow injection-LC system	acetonitrile-methanol (65:35 v/v)	72
Ceramides	Yeast	CN (150 mm × 4.6 mm, 5 μm)	hexane–ethanol (99:1 v/v)	73
Profiling of constituents	Sarcostemma hirtellum	Betasil C18 (50 mm × 4.6 mm, 3 μm)	water (0.1% formic acid)–acetonitrile (0.1% formic acid) (gradient from 95:5 to 5:95 v/v)	74
Phytosterols	Plant matrices	Hypersil BDS RP18 (250 mm × 3.4 mm, 5 μm)	methanol–acetonitrile-water (75:15:10 v/v/v)	75
Phosphatidylserine molecular species	Biological tissues	Caroteniod C30 (250 mm × 4.6 mm, 5 μm)	2-propanol-THF-ammonium formate (55:15:30 v/v/v)	76
Maltodextrins	Plant extracts	octadecyl-bonded silica and amino-bonded polymeric	aqueous methanol–aqueous acetonitrile	77
Apolar low molecular weight constituents	Wood	end-capped C18	gradient of acetonitrile–water–acetic acid.	78
Hydrocarbons, wax esters, sterol esters, triacylglycerols and sterols	Zooplankton	LiChroCART column (12.5 cm × 4 mm) packed with Aluspher (alumina) particles (5 μm)	0.5% THF in hexane–70% THF/propan-2-ol/hexane (1:1:3 v/v/v) (gradient from 100:0 to 30:70 v/v)	79
Aminoacids	Human parathyroid hormone	Dionex IonPac CS-10 cation-exchange column (250 mm × 4 mm)	0.1% TFA–ammonium acetate 0.1M (a ternary system with increasing pH gradient steps)	80

TABLE 5
Applications of ELSD in analysis of polymers

Compound	Matrix	Column	Mobile Phase	Reference
Telechelic poly(methyl methacrylate)	Synthesized and commercial samples	Packed in-house with Hypersil Silica (150 mm × 4.6 mm, 3 μm 100 Å).	acetonitrile and dichloromethane of varying composition	81
Polyethylene glycol	Low-density polyethylene	Kromasil C18, 100 Å (100 mm × 0.5 mm, 3 μm).	acetonitrile–THF–water (40:5:55 v/v/v)	82
Polyamide-6	Synthesized samples	Two Nucleosil 50–5 (200 mm × 4 mm)	81.6% (w/w) formic acid in propanol	23
Poly(butylene glycol) and derivatives	Synthesized samples	ODS C18	Binary or ternary gradient systems of acetonitrile, THF, water and methanol	83

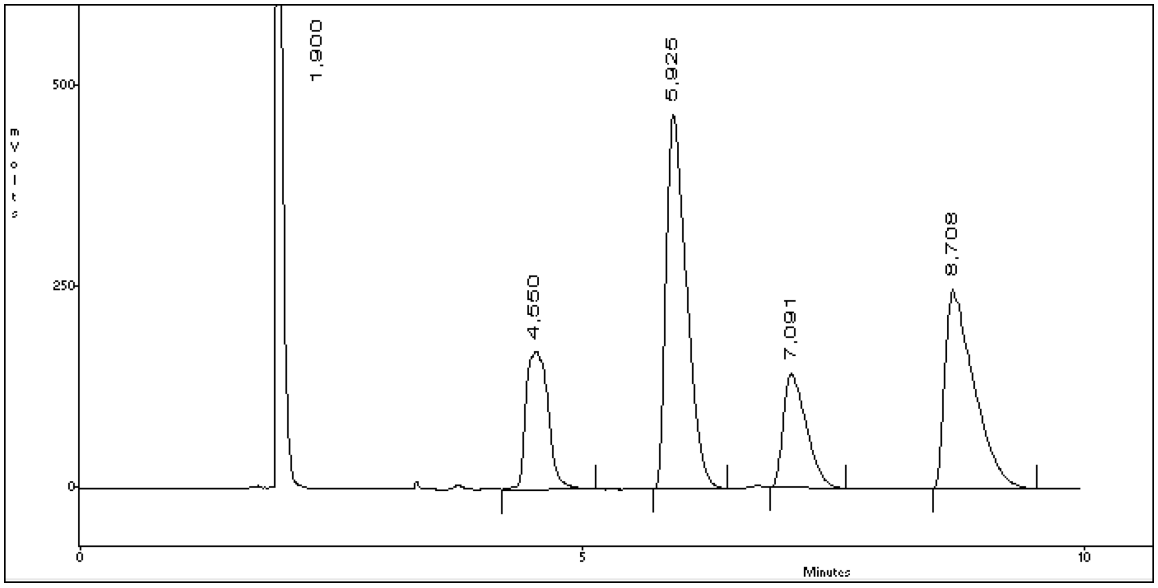

FIG. 4. Typical chromatogram of gentamicin sulfate raw material (175 μg/ml) (sulfate: 1.90 min, C_{1a}: 4.55 min, C_2: 5.92 min, C_{2a}: 7.09 min and C_1: 8.71 min).

polymerization but also with concern to the different endgroups (e.g., nonylphenyl-, octylphenyl-, alkyl groups of different chain length etc.).

Numerous studies have been published on the analysis of polyethers by HPLC-ELSD. Excellent results were obtained for a wide variety of polyether derivatives including PEGs, PPGs, PBGs and PPG amides (35, 36), and for alkylethoxylates, PPGs and PEG-PPG copolymers (37). Nearly constant ELSD response factor was observed for different kinds of PEGs and derivatives, enabling a 'universal' calibration curve (38). However, compared with measurement by HPLC-UV, the detection limits were about one order of magnitude lower, lying in the μg range. For PEG 1000, PPG 1200 and PBG 1000 limits of detection were reported to be 5, 10 and 20 μg, respectively (39). Derivatization of PPG amines with pyridine/acetanhydride revealed an additional advantage of ELSD over UV detection (40). Although the resulting PPG amides can be alternatively measured with high sensitivity at 210 nm, due to the presence of the amide chromophor as in the case of peptides and proteins, the excess of reagent has to be removed prior to HPLC-UV and thus, an additional sample preparation step is required, while in ELSD heated drift tube, pyridine is vaporized due to its high volatility. Nevertheless, ELSD offers an efficient alternative tool for polyethers determination, which in most cases yields sufficient sensitivity

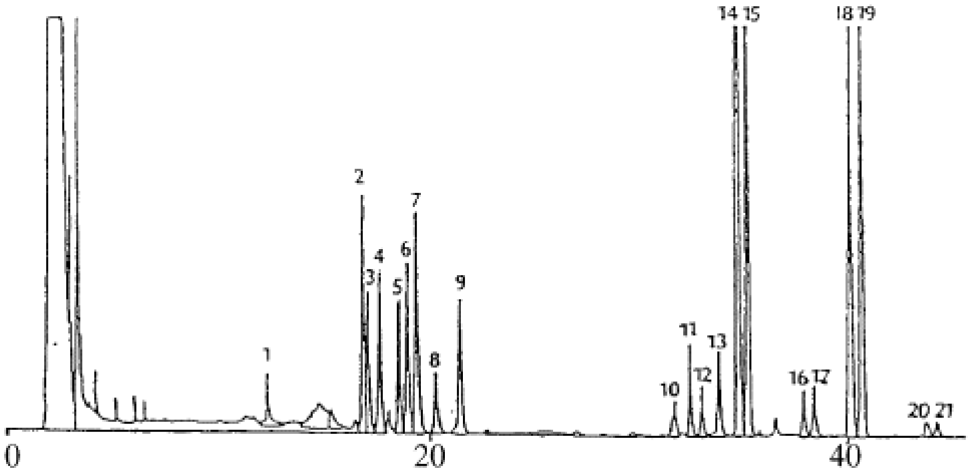

FIG. 5. HPLC scaled chromatograms of steamed *P. ginseng* at 120 °C / 3 h. [Peaks identity: (1) Re + Rg_1, (2) Rf, (3) Rb_1, (4) Rc, (5) Rb_2, (6) Rg_2, (7) 20-(*R*)-Rg_2 + Rh_1, (8) 20-(*R*)-Rh_1, (9) Rd, (10) Rg_6, (11) F_4, (12) Rk_3, (13) Rh_4, (14) Rg_3, (15) 20-(*R*)-Rg_3, (16) Rs_3, (17) 20-(*R*)-Rs_3, (18) Rk_1, (19) Rg_5, (20) Rs_5, (21) Rs_4].

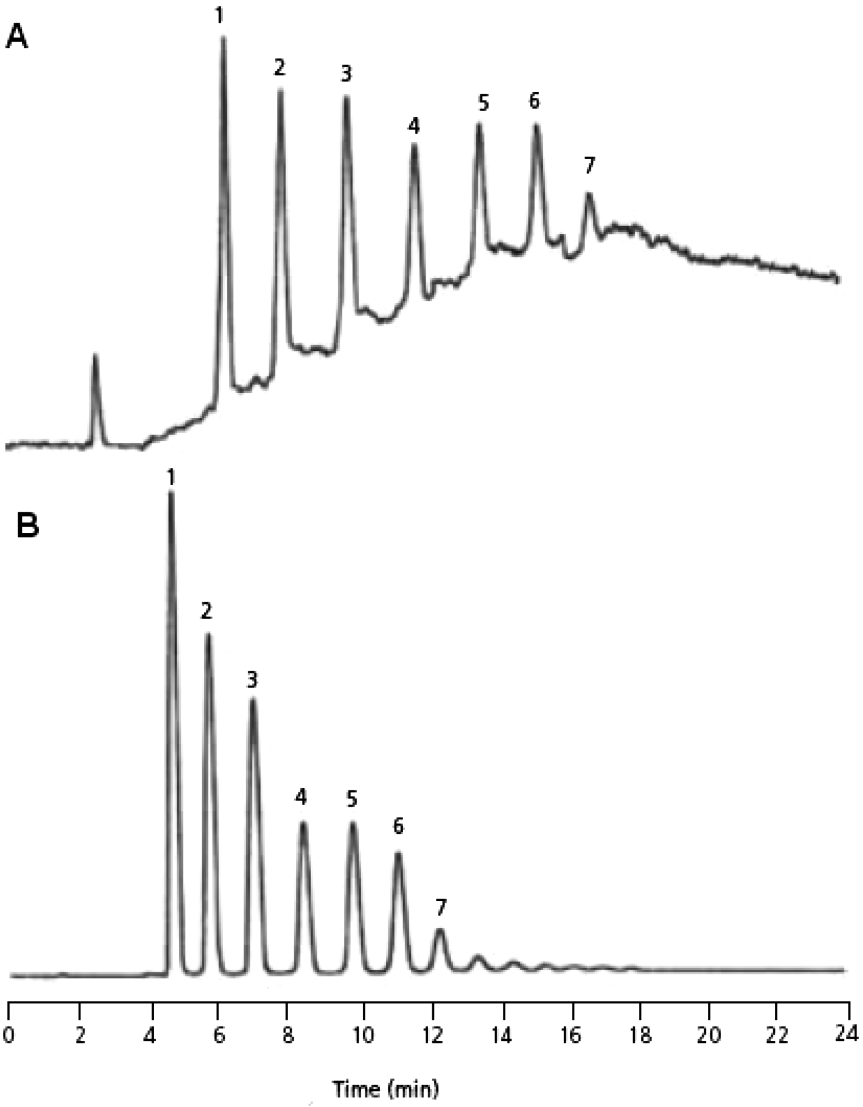

FIG. 6. HPLC-ELSD determination of carbohydrates based on a water-acetonitrile gradient and (A) silica based or (B) polymer based amino columns [Peaks identity: (1) glucose, (2) maltose, (3) maltotriose, (4) maltotetraose, (5) maltopentaose, (6) maltohexaose, (7) maltoheptaose].

and, as a consequence, is superior to RI and even UV detection, at least in the case of polyethers lacking any chromophor.

Food and Beverages (Table 3)

Carbohydrates

Potentials of ELSD for the determination of carbohydrates (as well as lipids) in various matrices have been extensively investigated, while nowadays it is considered to be one of the prevailing detection techniques in HPLC methods. However, the main problem encountered in HPLC-ELSD methods is the low ruggedness of the amino silica based columns, which are widely used for carbohydrate separations with water-acetonitrile mobile phases. Chemical degradation of bonded phase is mainly caused by a self-hydrolysis mechanism and/or Schiff base formation with reducing sugars. The symptoms of column degradation, are visible with ELSD, which responds to the leaking bonded phase material producing noisy and drifting baseline. These symptoms can be avoided by utilizing a polymeric, instead of a silica based column or by an alternative retention mechanism (ion or size exclusion) (Figure 6) (26). For normal phase silica column, triethylamine and ethanolamine have been proposed as effective modifiers for oligosaccharides separation (55).

HPLC-ELSD methods have been reported for numerous oligosaccharides (glucose, fructose, maltose, saccharose, raffinose, ribose, xylose, arabinose, sorbose, mannose, galactose, erythritol, xylitol, mannitol, sorbitol), chitin, dextrans, maltodextrins and cyclodextrins in various foods, such as drinks,

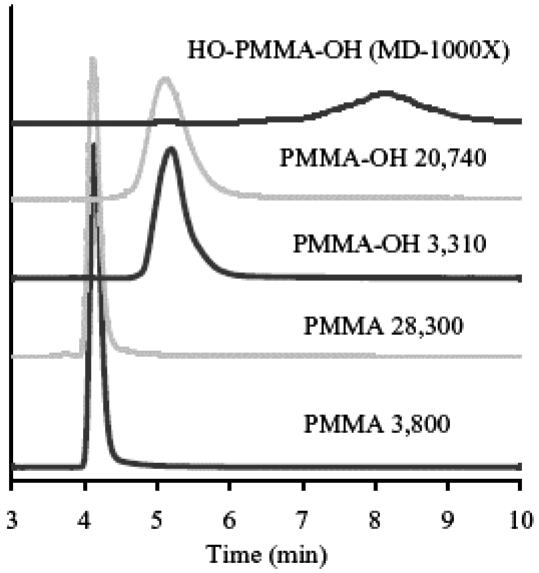

FIG. 7. Separations of poly(methyl methacrylate) functional polymers based on the number of hydroxyl groups.

various plant tissues, and tobacco (55). Detection limits are in the μg ml^{-1} range. Besides HPLC, mono-, di- and tri-saccharides were determined by SFC-ELSD (56), while the determination of twelve monosaccharides and polyols using subcritical fluid chromatography (SubFC)- ELSD was reported (Figure 7) (57).

Lipids

Both polar and non-polar lipids can be determined with HPLC-ELSD. A complicated ternary-gradient elution with eight programmed steps based on isooctane, tetrahydrofuran, isopropanol, and water, has been proposed for the separation of classes of tissue lipids on a silica gel column, starting with isooctane to separate the lipids of low polarity and ending with a solvent containing water to elute the high polarity phospholipids (58). Further, it is possible to use an internal standard to obtain direct quantification. The synthetic phospholipid, phosphatidyldimethylethanolamine (dipalmitoyl) (its natural presence in tissues is at very low levels), was used as an internal standard to allow the calculation of the absolute amount of a phospholipids class in a biological extract by the relative peak area (59).

HPLC-ELSD technique was also proposed for the identification and quantification of twelve lipid classes (paraffin, wax esters, cholesterol esters, fatty acid methyl esters, triacyl glycerols, fatty alcohols, free fatty acids, cholesterol, 1,3-diacyl glycerols, 1,2-diacyl glycerols, monoacyl glycerols and fatty acid amides) used as lubricants in food packaging materials (60).

In 1992, Lafosse et al. reported on the analysis of phospholipids by SFC-ELSD. The separation of phosphatidylcholine, phosphatidic acid, phosphatidylinositol, and phosphatidylethanolamine from soya lecithin was isocratically achieved on a silica column with a mobile phase consisting of carbon dioxide modified with a mixture of methanol:water: triethylamine (61).

Natural Products and Biological Samples (Table 4)

Since ELSD is a quasi universal detector, it appears low selectivity and its direct applicability to samples with complex matrix, such as plasma and urine, is extremely limited. Efficient sample clean-up, elimination of matrix interference and analyte enrichment are required prior to HPLC-ELSD determination. However, the development of a procedure for the removal of interfering matrix is quite difficult, as long as non-volatile reagents (e.g., precipitation reagents and buffers) are incompatible with ELSD, causing extra interferences.

Several HPLC-ELSD methods for natural products and biological samples have been reported, including determination of antibiotics, carbohydrates and lipids in plasma, urine and tissues (29, 69–80).

Polymers (Table 5)

Since polymers are non-volatile compounds, ELSD appears to be an excellent method of detection. Except for polyethers (discussed previously), ELSD has been reported for the efficient detection of several classes of polymers, such as polyamide-6 (23), poly(methyl methacrylate) (81) (Figure 7) and poly(butylene alcohol) (83), enabling molecular weight estimation, determination of oligomer distribution, number of end groups and investigation of physicochemical data.

REFERENCES

1. N. C. Megoulas, M. A. Koupparis, Development and validation of a novel LC/ELSD method for the quantitation of gentamicin sulfate components in pharmaceuticals. *Journal of Pharmaceutical and Biomedical Analysis* 36 (2004):73–79.
2. J. M. Chalesworth, Evaporative analyzer as a mass detector for liquid chromatography. *Analytical Chemistry* 50 (1978):1414–1420.
3. T. H. Mourey and L. E. Oppenheimer, Principles of operation of an evaporative light-scattering detector for liquid chromatography. *Analytical Chemistry* 56 (1984):2427–2434.
4. A. Stolyhwo, H. Colin, and G. Guiochon, Use of light scattering as a detector principle in liquid chromatography. *Journal of Chromatography* 265 (1983):1–18.
5. L. E. Oppenheimer, T. H. Mourey, Examination of the concentration response of evaporative light-scattering mass detectors. *Journal of Chromatography* 323 (1985):297–304.
6. M. Dreux, M. Lafosse, and L. Morin-Allory, The evaporative light scattering detector—a universal instrument for non-volatile solutes in LC and SFC. *LC GC International* 9 (1996):148–156.
7. R. A. Mugele and H. D. Evans, Droplet size distribution in sprays. *Industrial and Engineering Chemistry* 43 (1951):1317–1324.
8. S. Nukiyama and Y. Tanasawa, Experiment on atomization of liquid by means of air stream. *Transactions of the Society of Mechanical Engineers* 4 (1938):86–93.
9. R. M. Righezza and G. Guiochon, Effects of the nature of the solvent and solutes on the response of a light-scattering detector. *Journal of Liquid Chromatography* 11 (1988):1967–2004.

10. F. S. Deschamps and A. Baillet, P. Chaminade, Mechanism of response enhancement in evaporative light scattering detection with the addition of triethylamine and formic acid. *Analyst* 127 (2002):35–41.
11. A. Kuch and R. S. Nordhaus, Dual-mode ELSD: optimum performance for a wide range of applications. *American Laboratory* 3 (2001):61–64.
12. Y. Mengerink, H. C. J. De Man, and S. Van Der Wal, Use of an evaporative light scattering detector in reversed-phase high-performance liquid chromatography of oligomeric surfactants. *Journal of Chromatography* 552 (1991):593–604.
13. I. Clarot, P. Chaimbault, F. Hasdenteufel, P. Netter, and A. Nicolas, Determination of gentamicin sulfate and related compounds by high-performance liquid chromatography with evaporative light scattering detection. *Journal of Chromatography A* 1031 (2004):281–287.
14. G. Guiochon, A. Moysan, and C. Holley, Influence of various parameters on the response factors of the evaporative light scattering detector for a number of non-volatile compounds. *Journal of Liquid Chromatography* 11 (1988):2547–2570.
15. A. Stolyhwo, H. Colin, M. Martin, and G. Guiochon, Study of the qualitative and quantitative properties of the light-scattering detector. *Journal of Chromatography* 288 (1984):253–275.
16. M. Dreux and M. Lafosse, Review of evaporative light scattering detection for packed-column SFC. *LC GC International* 10 (1997):382–390.
17. M. Laffose, Evaporative light scattering detection in SFC, in Practical supercritical fluid chromatography and extractions, ed. M. Caude and D. Thiébaut (Amsterdam: Harwood academic publishers, 1999), ch. 5d, 201–218.
18. V. Camel, D. Thiébaut, M. Caude, and M. Dreux, Packed column subcritical fluid chromatography of underivatized amino acids. *Journal of Chromatography* A, 605 (1992):95–101.
19. B. A. Kimball, W. M. Arjo, and J. J. Johnston, Single-point calibration with a non-linear detector: carbohydrate analysis of conifer needles by hydrophobic interaction chromatography—evaporative light-scattering detection (HIC-ELSD). *Journal of Liquid Chromatography* 27 (2004) 1835–1348.
20. W. Li and J. F. Fitzloff, Determination of 24(R)-pseudoginsenoside F11 in North American ginseng using high-performance liquid chromatography with evaporative light scattering detection. *Journal of Pharmaceutical and Biomedical Analysis* 25 (2001):257–265.
21. L. Fang, M. Wan, M. Pennacchio, and J. Pan, Evaluation of evaporative light-scattering detector for combinatorial library quantitation by reversed phase HPLC. *Journal of Combinatorial Chemistry* 2 (2000):254–257.
22. N. C. Megoulas and M. A. Koupparis, Enhancement of evaporative light scattering detection in high-performance liquid chromatographic determination of neomycin based on highly volatile mobile phase, high-molecular-mass ion-pairing reagents and controlled peak shape. *Journal of Chromatography A*, 1057 (2004):125–131.
23. Y. Mengerink, R. Peters, C. G. deKoster, Sj. van der Wal, H. A. Claessens, and C. A. Cramers, Separation and quantification of the linear and cyclic structures of polyamide-6 at the critical point of adsorption. *Journal of Chromatography A* 914 (2001):131–145.
24. L. B. Allen, J. A. Koropchak, and B. Szostek, Condensation-nucleation light-scattering detection for conventional reversed-phase liquid chromatography. *Analytical Chemistry* 67 (1995):659–666.
25. S. Héron, M. Dreux, and A. Tchapla, Post-column addition as a method of controlling triacylglycerol response coefficient of an evaporative light scattering detector in liquid chromatography—evaporative light-scattering detection. *Journal of Chromatography A* 1035 (2004):221–225.
26. C. S. Young and J. W. Dolan, Success with evaporative light-scattering detection. *LC GC Europe* 17(4) (2004):192–199.
27. R. Trones, T. Andersen, I. Hunnes, and T. Greibrokk, Modified laser light-scattering detector for use in high temperature micro liquid chromatography. *Journal of Chromatography A*, 814 (1998):55–61.
28. M. Kohler, W. Haerdi, P. Christen, and J.-L. Veuthey, The evaporative light scattering detector: some applications in pharmaceutical analysis. *Trends in Analytical Chemistry* 16(8) (1997):475–484.
29. N. C. Megoulas and M. A. Koupparis, Development and validation of a novel HPLC-ELSD method for the direct determination of tobramycin in pharmaceuticals, plasma and urine. *Analytical and Bioanalytical Chemistry* 382 (2005):290–296.
30. M. K. Park, J. H. Park, S. B. Han, Y. G. Shin, and I. H. Park, High-performance liquid-chromatographic analysis of ginseng saponins using evaporative light-scattering detection. *Journal of Chromatography A*, 736 (1996):77–81.
31. S. W. Kwon, S. B. Han, I. H. Park, J. M. Kim, M. K. Park, and J. H. Park, Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *Journal of Chromatography A* 921 (2001):335–339.
32. P. A. Asmus and J. B. Landis, Analysis of steroids in bulk pharmaceuticals by liquid chromatography with light-scattering detection. *Journal of Chromatography* 316 (1984):461–472.
33. Alltech application note. *www. alltechweb. com*, 0042E (2000):2.
34. A. J. Berry, E. D. Ramsey, M. Newby, and D. E. Games, Applications of packed-column SFC using light-scattering detection. *Journal of Chromatographic Science* 34 (1996):245–253.
35. K. Rissler, High-performance liquid chromatography and detection of polyethers and their mono(carboxy)alkyl- and arylalkyl-substituted derivatives. *Journal of Chromatography A*, 742 (1996):1–54.
36. B. Trathnigg and B. Kollroser, Liquid chromatography of polyethers using universal detectors V. Quantitative aspects in the analysis of low-molecular-mass poly(ethyleneglycol)s and their derivatives by reversed-phase high performance liquid chromatography with an evaporative light scattering detector. *Journal of Chromatography A* 768 (1997):223–238.
37. N. Martin, Analysis of non-ionic surfactants by HPLC using evaporative light-scattering detector. *Journal of Liquid Chromatography* 18 (1995):1173–1194.
38. S. Brossard, M. Lafosse, and M. Dreux, Comparison of ethoxylated alcohols and polyoxyethylene glycols by high-performance liquid chromatography and supercritial-fluid chromatography using evaporative light-scattering detection. *Journal of Chromatography* 591 (1992):149–157.
39. K. Rissler, H. P. Kunzi, and H. J. Grether, Chromatographic investigations of oligomeric alpha,omega-dihydroxy polyethers by reversed-phase high-performance liquid chromatography and evaporative light scattering and UV detection. *Journal of Chromatography* 635 (1993):89–101.

40. K. Rissler, Separation of acetylated polypropylene glycol di- and tri-amines by gradient reversed-phase high-performance liquid chromatography and evaporative light scattering detection *Journal of Chromatography A* 667 (1994):167–174.
41. H. Liu, S. L. Qiu, and H. W. Wang, HPLC-ELSD determination of sodium carbonate in ceftazidime for injection. *Yaowu Fenxi Zazhi* 22 (2002):225–228.
42. L. M. Nair, N. V. Stephens, S. Vincent, N. Raghavan, and P. J. Sand, Determination of polysorbate 80 in parenteral formulations by high-performance liquid chromatography and evaporative light scattering detection. *Journal of Chromatography A* 1012 (2003):81–86.
43. Y. Liu and C. Q. Hu, RP-HPLC determination of midecamycin and related impurities. *Chromatographia* 57 (2003):143–146.
44. M. Ganzera, H. R. W. Dharmaratne, N. P. D. Nanayakkara, and I. A. Khan, Determination of saponins and alkaloids in Caulophyllum thalictroides (blue cohosh) by high-performance liquid chromatography and evaporative light scattering detection. *Phytochemical Analysis* 14 (2003):1–7.
45. W. K. Li and J. F. Fitzloff, Simultaneous determination of terpene lactones and flavonoid aglycones in Ginkgo biloba by high-performance liquid chromatography with evaporative light scattering detection. *Journal of Pharmaceutical and Biomedical Analysis* 30 (2002):67–75.
46. M. Schiller, H. von-der-Heydt, F. Maerz, and P. C. Schmidt, Quantification of sugars and organic acids in hygroscopic pharmaceutical herbal dry extracts. *Journal of Chromatography A* 968 (2002):101–111.
47. D. E. Moore, T. X. Liu, W. G. Miao, A. Edwards, and R. Elliss, A RP-LC method with evaporative light scattering detection for the assay of simethicone in pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis* 30 (2002):273–278.
48. W. Li and J. F. Fitzloff, Determination of andrographolide in commercial andrographis (Andrographis paniculata) products using HPLC with evaporative light scattering detection. *Journal of Liquid Chromatography* 25 (2002):1335–1343.
49. A. Criado, S. Cardenas, M. Gallego, and M. Valcarcel, Usefulness of the evaporative light scattering detector for direct screening of biological fluids. *Analytica Chimica Acta* 435 (2001):281–288.
50. S. W. Chan, S. L. Li, G. Lin, and P. Li, Pharmacokinetic study and determination of imperialine, the major bioactive component in antitussive Fritillaria cirrhosa, in rat by high-performance liquid chromatography coupled with evaporative light-scattering detector. *Analytical Biochemistry* 285 (2000):172–175.
51. R. Marsilio, M. Naturale, P. Manghi, G. Montini, L. Murer, M. Ros, G. Bisogno, B. Andretta, N. Dussini, G. Giordano, G. Zacchello, and R. Dall'Amico, Rapid and simple determination of inulin in biological fluids by high-performance liquid chromatography with light scattering detection. *Journal of Chromatography B* 744 (2000): 241–247.
52. I. D'Acquarica, F. Gasparrini, D. Misiti, C. Villani, A. Carotti, S. Cellamare, and S. Muck, Direct chromatographic resolution of carnitine and O-acylcarnitine enantiomers on a teicoplanin-bonded stationary phase. *Journal of Chromatography A* 857 (1999):145–155.
53. S. Cherkaoui, Y. Daali, P. Christen, and J. L. Veuthey, Development and validation of liquid chromatography and capillary electrophoresis methods for acarbose determination in pharmaceutical tablets. *Journal of Pharmaceutical and Biomedical Analysis* 18 (1998) 729–735.
54. M. Rajevic and P. Betto, Assay of ursodeoxycholic acid and related impurities in pharmaceutical preparations by HPLC with evaporative light scattering detector. *Journal of Liquid Chromatography* 21 (1998): 2821–2830.
55. Y. Wei and M. Y. Ding, Ethanolamine as modifier for analysis of carbohydrates in foods by HPLC and evaporative light scattering detection. *Journal of Liquid Chromatography* 25 (2002):1769–1778.
56. A. J. Berry, E. D. Ramsey, M. Newby, and D. E. Games, Applications of packed-column SFC using light-scattering detection. *Journal of Chromatographic Science* 34 (1996):245–253.
57. A. Salvador, B. Herbreteau, M. Lafosse, and M. Dreux, Subcritical fluid chromatography of monosaccharides and polyols using silica and trimethylsilyl columns. *Journal of Chromatography* 785 (1997):195–204.
58. W. W Christie, Rapid separation and quantification of lipid classes by high performance liquid chromatography and mass (light-scattering) detection. *Journal of Lipid Research* 26 (1985):507–512.
59. W. W. Christie, R. C. Noble, and G. Davies, Phospholipids in milk and dairy products. *Journal of the Society of. Dairy Technology* 40 (1987):10–12.
60. A. Schaefer, T. Kuechler, T. J. Simat, and H. Steinhart, Migration of lubricants from food packagings. Screening for lipid classes and quantitative estimation using normal-phase liquid chromatographic separation with evaporative light scattering detection. *Journal of Chromatography A* 1017 (2003):107–116.
61. M. Lafosse, C. Elfakir, L. Morin-Allory, and M. Dreux, Advantages of evaporative light scattering detection in pharmaceutical analysis by high-performance liquid chromatography and supercritical fluid chromatography. *Journal of High Resolution Chromatography,* 15 (1992):312–318.
62. F. Dionisi, P. A. Golay, B. Hug, M. Baumgartner, P. Callier, and F. Destaillats, Triacylglycerol analysis for the quantification of cocoa butter equivalents (CBE) in chocolate: feasibility study and validation. *Journal of Agricultural and Food Chemistry* 52 (2004):1835–1841.
63. X. L. Cao and Y. C. Ito, Supercritical fluid extraction of grape seed oil and subsequent separation of free fatty acids by high-speed counter-current chromatography. *Journal of Chromatography A* 1021 (2003):117–124.
64. R. Lucena, M. Gallego, S. Cardenas, and M. Valcarcel, Autoanalyser for milk quality control based on the lactose, fat, and total protein contents. *Analytical Chemistry* 75 (2003):1425–1429.
65. R. A. Moreau, K. Kohout, and V., Singh, Temperature-enhanced alumina HPLC method for the analysis of wax esters, sterol esters and methyl esters. *Lipids* 37 (2002):1201–1204.
66. A. Kaufmann, B. Ryser, and B. Suter, HPLC with evaporative light scattering detection for the determination of polar compounds in used frying oils. *European Food Research and Technology* 213 (2001):372–376.
67. A. Lopez-Lopez, A. I. Castellote-Bargallo, and M. C. Lopez-Sabater, Direct determination by high-performance liquid chromatography of sn-2-monopalmitin after enzymic lipase hydrolysis. *Journal of Chromatography B* 760 (2001):97–105.

68. T. Sugawara and T. Miyazawa, Separation and determination of glycolipids from edible plant sources by high-performance liquid chromatography and evaporative light-scattering detection. *Lipids* 34 (1999):1231–1237.
69. S. Roy, K. Gaudin, D. P. Germain, A. Baillet, P. Prognon, and P. Chaminade, Optimization of the separation of four major neutral glycosphingolipids: application to a rapid and simple detection of urinary globotriaosylceramide in Fabry disease. *Journal of Chromatography B* 805 (2004):331–337.
70. T. Kimura, K. Nakagawa, Y. Saito, K. Yamagishi, M. Suzuki, K. Yamaki, H. Shinmoto, and T. Miyazawa, Determination of 1-deoxynojirimycin in mulberry leaves using hydrophilic interaction chromatography with evaporative light scattering detection. *Journal of Agricultural and Food Chemistry* 52 (2004):1415–1418.
71. B. T. Schaneberg, R. J. Molyneux, and I. A. Khan, Evaporative light scattering detection of pyrrolizidine alkaloids. *Phytochemical Analysis* 15 (2004):36–39.
72. A. Criado, S. Cardenas, M. Gallego, and M. Valcarcel, Direct screening of lyophilized biological fluids for bile acids using an evaporative light scattering detector. *Journal of Chromatography B* 792 (2003):299–305.
73. Q. Zhou, L. Zhang, X. Q. Fu, and G. Q. Chen, Quantitation of yeast ceramides using high-performance liquid chromatography-evaporative light-scattering detection. *Journal of Chromatography B* 780 (2002):161–169.
74. P. A. Cremin and L. Zheng, High-throughput analysis of natural product compound libraries by parallel LC-MS evaporative light scattering detection. *Analytical Chemistry* 74 (2002):5492–5500.
75. P. Breinhoelder, L. Mosca, and W. Lindner, Concept of sequential analysis of free and conjugated phytosterols in different plant matrices. *Journal of Chromatography B* 777 (2002):67–82.
76. A. Larsen, E. Mokastet, E. Lundanes, and E. Hvattum, Separation and identification of phosphatidylserine molecular species using reversed-phase high-performance liquid chromatography with evaporative light scattering and mass spectrometric detection. *Journal of Chromatography B* 774 (2002):115–120.
77. S. Guenu, J. M. Seigneuret, and M. Dreux, Analysis of maltodextrins in plant extracts by liquid chromatography using evaporative light scattering detection. *Journal of Liquid Chromatography* 23 (2000):2883–2895.
78. F. W. Claassen, C. van-de-Haar, T. A. van Beek, J. Dorado, and M. J. Martinez-Inigo, R. Sierra-Alvarez, Rapid analysis of apolar low molecular weight constituents in wood using high pressure liquid chromatography with evaporative light scattering detection. *Phytochemical Analysis* 11 (2000):251–256.
79. J. Nordbaeck and E. Lundberg, High resolution separation of non-polar lipid classes by HPLC-ELSD using alumina as stationary phase. *Journal of High Resolution Chromatography* 22 (1999):483–486.
80. J. A. Peterson, L. J. Lorenz, D. S. Risley, and B. J. Sandmann, Amino-acid analysis of peptides using HPLC with evaporative light scattering detection. *Journal of Liquid Chromatography* 22 (1999):1009–1025.
81. X. L. Jiang, V. Lima, and P. J. Schoenmakers, Robust isocratic liquid chromatographic separation of functional poly(methyl methacrylate). *Journal of Chromatography A* 1018:(2003) 19–27.
82. I. L. Skuland, T. Andersen, R. Trones, R. B. Eriksen, and T. Greibrokk, Determination of polyethylene glycol in low-density polyethylene by large volume injection temperature gradient packed capillary liquid chromatography. *Journal of Chromatography A* 1011 (2003):31–36.
83. K. Rissler, Improved separation of poly(butylenes glycol) oligomers on a C18 column by gradient reversed-phase liquid chromatography and fine-tuning of the organic modifier system. *Chromatographia* 51 (2000):656–668.