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High-Throughput Analysis of Natural Product Compound Libraries by Parallel LC−MS Evaporative Light Scattering Detection

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The application of an 8-way fully automated parallel LC−MS−ELSD system to the analysis of a library of 96 structurally diverse natural products is described. A 10-min separation incorporating a universal gradient allowed elution of 86 of these 96 compounds, all of which were detected by positive or negative mode electrospray ionization in conjunction with ELSD. This method is demonstrated to be one of the most universal means of detection for polar, nonvolatile, thermally labile natural products. It also allows an 8-fold increase in throughput. The analysis and profiling of constituents present in a library derived from plant material of *Sarcostemma hirtellum*, in terms of their retention times and mass spectra, are shown. This rapid characterization of plant constituents in terms of compound libraries is important in searching for new biologically active compounds. As a high-throughput tool to support our natural product discovery program, this method has been successfully used to analyze a library of 36 000 partially purified fractions derived from plant materials.

Natural products have served as an important source of drugs for combating diseases since ancient times. In modern pharmaceutical industries, they continue to be a major resource for the generation of the lead compounds, and as many as half the drugs on the market are direct descendants of natural products.[1] Recent advances in combinatorial chemistry have allowed the synthesis of large number of compounds either through split−couple−recombine methods[2,3] or automated high-throughput parallel synthesis[4−6] and have tended to focus lead discovery efforts toward the production and screening of combinatorial compound libraries. Through statistical investigation of the structural similarity of natural products and synthetic compounds, it is evident that ∼40% of natural products are not represented by synthetic compounds and that natural products represent a unique pool of compounds that provide a broader range of structural diversity.[7] However, the challenges for the natural product chemist in this area are twofold: first, how to quickly generate natural product compound libraries, either in pure compound or in a small number of compound mixtures, in the format (96- or 384-well microtiter plates) that fit high-throughput screening programs; second, how to present these natural product compound libraries with adequate information that allows for rapid searching and location of components of interest. High-throughput analysis of large compound libraries in combinatorial chemistry is achieved by LC or LC−MS with short columns and fast gradients to facilitate high-speed separations.[8−10] These systems are usually coupled with a UV detector and can run typical separations as fast as 5−10 min per sample by utilizing a universal gradient. Some of the systems are also connected to an evaporative light scattering detector (ELSD) or a chemiluminescent nitrogen detector for purity assessments and quantitations.[11,12] It is also possible to increase the throughput of these analyses by ultrafast LC−MS using a high flow rate, with 2.0-mm-internal diameter columns, which lead to satisfactory separation with gradient times of 1 min or less.[13,14] An alternative means of increasing throughput of analysis is the development of a parallel LC−MS system incorporating two columns operating in parallel, which permits analyses and purification much faster than conventional sequential methods.[15] A newly patented parallel needle array interface, with electrospray ionization, permits up to four or eight columns to be sampled sequentially by means of a stepping motor and rotating blocking

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Table 1. LC−MS−ELSD Data of Phenolic Compounds

compounds	R_t (min)[a]	$[M + H]^+$ (ESI^+)	other ions (ESI^+)	$[M - H]^-$ (ESI^-)	other ions (ESI^-)
taxifolin	4.59	—[b]	−	303.1	−
kaempferol	5.88	287.4	−	285.2	−
chrysin	7.13	255.4	509.0 $[2M + H]^+$	253.1	−
catechin	3.30	−	−	289.2	277.2
morin	5.00	303.4	−	301.1	−
luteolin	5.40	287.4	−	285.2	−
spinosin	3.88	609.3	327.4	607.6	−
hesperetin	6.07	303.5	177.4	301.2	−
apigenin	5.83	271.4	−	269.1	−
butein	5.73	273.4	−	271.2	−
rutin	3.93	611.3	303.4	609.4	−
quercetin	5.28	303.4	−	301.2	−
naringenin	5.84	273.5	−	271.2	−
hesperidin	4.35	611.2	303.4, 290.9	609.5	−
myricetin	4.70	319.2	306.7	317.2	−
silymarin	5.65	483.3	−	481.3	−
glyinflanin B	7.53	339.2	321.3	−	−
genistein	5.86	271.4	−	269.2	−
daidzein	5.20	255.4	−	253.1	−
biochanin A	7.55	285.5	−	283.3	−
licoricone	7.42	383.5	327.4	381.4	−
cyanidin	3.92	287.4	271.4	287.3	−
esculin	2.84	341.4	179.4	339.2	−
emodin	8.35	303.4	−	269.1	−
purpurin	7.39	257.3	−	255.1	−
ellagic acid	4.09	−	−	301.1	−
epigallocatechin gallate	3.38	−	−	457.1	915.3
caffeic acid	3.19	−	−	179.3	−
chlorogenic acid	3.25	377.3 $[M + Na]^+$	163.2	353.1	−
ferulic acid	4.10	−	−	193.1	−
reservatrol	5.23	229.3	−	-	−
nordihydroguaiaretic acid	6.70	325.2 $[M + Na]^+$	−	301.4	604.2
podophyllotoxin	5.88	397.4 $[M - OH]^+$	313.4	459.3 $[M + HCOO]^-$	−

[a] MS retention time. [b] −, not detected.

device assembly.[16,17] This interface coupled to the fast-scanning ability of a time-of-flight mass analyzer has allowed an enormous increase in sample throughput. Parallel LC−MS systems are now commercially available, and applications to metabolite analysis, characterization of proteins and peptides, and combinatorial chemistry have been published.[18−22] Electrospray is primarily a liquid-phase ionization technique, and the ease with which the solution phase may be optimized to produce ions amenable to analysis has led to widespread application of this technique in natural product chemistry.[23,24] While many of these analyses are geared toward a particular class of compounds, electrospray methods have also been developed that allow the detection of a wide range of different natural products.[25] ELSD has also found increasing use in natural product analysis often as an alternative to UV detection.[26] In this paper, we describe the application of an eight-channel multiplexed electrospray ion source coupled to a time-of-flight mass spectrometer for the analysis of natural product libraries. The aim of this work is to develop a high-throughput LC−MS−ELSD method that is capable of analyzing as wide a range of natural products as possible.

EXPERIMENTAL SECTION

Standards. All compounds listed in Tables 1−4 were purchased from Sigma Chemical Co. (St. Louis, MO), Alexis Biochemicals (San Diego, CA), and LKT Labs (St. Paul, MN) except for glyinflanin B, licoricone, tetrahydropalmitane, glyunnansapogenin B, glyunnansapogenin C, aureol, muristerone A, and macedonic acid, which were provided from an internal Sequoia Sciences library. All standards and libraries were prepared in 100% HPLC grade methanol.

HPLC Conditions. Liquid flow was provided by a Waters 600E binary pump (Waters Corp., Milford, MA). The flow was split by use of a low dead volume eight-way splitter. The eight separate

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Table 2. LC−MS−ELSD Data of Alkaloids and Other Nitrogen-Containing Compounds

compounds	R_t (min)[a]	$[M + H]^+$ (ESI^+)	other ions (ESI^+)	$[M - H]^-$ (ESI^-)	other ions (ESI^-)
demecolchicine	3.80	372.5	341.5, 313.5,	−[b]	−
colchicine	4.87	400.5	−	−	−
α-solanine	4.53	868.3	706.5, 560.6,	867.3	−
emetine	3.46	481.5	452.5, 165.5	−	−
cordycepin	0.8	252.4	136.4	−	−
reserpine	6.05	609.3	−	607.3	−
berberine	5.03	336.4	321.4	−	−
quinine	3.28	325.6	189.5	−	−
yohimbine	4.32	355.5	−	−	−
atropine	3.61	290.5	-	−	−
caffeine	3.13	195.5	138.5	−	−
strychnine	3.42	335.5	−	−	−
brucine	3.41	395.5	−	−	−
puromycin	3.60	472.5	309.5, 164.5	470.4	−
vinblastine	4.70	811.3	406.5 $[M + 2H]^{2+}$, 376.5	−	−
vincristine	4.51	825.3	413.5 $[M + 2H]^{2+}$, 362.5	−	−
camptothecin	5.09	349.5	−	347.2	−
10-OH-camptothecin	4.35	365.5	−	363.2	−
physostigmine	3.24	276.2	219.5, 162.5	−	−
aconitine	5.69	646.3	−	−	−
epinephrine	0.43	184.1	166.1	182.2	164.2
tubocuraraine	3.54	610.4	552.3	−	−
tetrahydropalmitane	4.44	356.5	−	−	−
tetrahydrobiopterin	0.51	242.5	479.4	−	−
papaverine	3.95	340.1	−	−	−
neomycin	0.43	615.3	−	−	−
hygromycin	0.51	528.2	−	−	−

[a] MS retention time. [b] −, not detected.

streams were directed to the injection valves of a Gilson 889 liquid handler, which was mounted on a Gilson 215 multiple injection autosampler (Gilson Inc., Middleton, WI). All samples were presented to this system in 96-well microtiter or deep-well plates, and an injection volume of 20 μL was employed throughout. HPLC separation was achieved on Betasil C18 columns (4.6 × 50 mm, 3 μm) obtained from Keystone Scientific Inc. (Bellefonte, PA). Buffer A was 0.1% formic acid in water and buffer B was 0.1% formic acid in acetonitrile. A flow rate of 9.6 mL/min was used, providing a flow of 1.2 mL/min through each column. Separation was achieved by an initial hold at 5% buffer B for 1 min, a linear gradient from 5 to 95% buffer B in 8.0 min, followed by holding at 95% B for 0.5 min and a reequilibration to initial conditions for 0.5 min. A postrun time of 2.5 min at 5% B was incorporated with the total time between injections then being 12.5 min. The effluent from each column was passed through a four way Valco low dead volume cross, which split the 1.2 mL/min flow into three separate streams of 0.09, 0.4, and 0.71 mL/min. For each column, the 0.09 mL/min stream was presented in a separate line to one inlet of the eight-way multiplexed electrospray source, the 0.4 mL/min stream to the inlet of the Alltech 500 ELSD detector (Alltech Associates Inc., Deerfield, IL), and the 0.71 mL/min stream was routed to waste.

Mass Spectrometric Analysis Conditions. All MS data were acquired on an LCT orthogonal TOF spectrometer (Micromass Ltd., Manchester, U.K.) fitted with an eight-way multiplexed electrospray interface (MUX). In these analyses, each liquid stream was sampled for 0.1 s with mass spectra acquired from 140 to 1400 Da into eight simultaneously open data files synchronized with the spray being sampled. The time taken to move to the adjacent sampling position was 0.05 s. This cycle produceed a data point for each spray every 1.2 s. The LCT and MUX are operated under MassLynx V3.4. For the samples investigated, the instrument was operated in either positive or negative mode electrospray with capillary voltages of 3.5 and 3.0 kV being used, respectively. A desolvation temperature of 380 °C and a source temperature of 120 °C was used for all experiments. The nitrogen desolvation and nebulizer gas flow rates were set to 1100 and 300 L/h, respectively. Sample cone voltage was set to 50 V in positive mode and 45 V in negative mode, with extraction cone voltage set at 2.0 V in both modes. The rf lens voltage was set to 350 V in the positive mode and 500 V in the negative mode.

ELSD Analysis Conditions. ELSD detection was carried out using eight Alltech 500 detectors (Alltech Associate Inc.). A flow of 0.4 mL/min from channels 1−8 was presented in eight separate lines to the inlets of the Alltech detectors. The optimized nebulizer gas flows for detectors 1−8 were 2.12. 3.30, 3.28, 3.05, 3.10, 3.25, 3.45, and 3.07 SLPM, respectively. The optimized drift tube temperatures for detectors 1−8 were 105, 103, 105, 100, 101, 101, 105 and 102 °C for each stream, respectively.

RESULTS AND DISCUSSION

The configuration of the parallel eight-channel LC−MS−ELSD system is illustrated schematically in Figure 1. Four natural products, kaempferol, reserpine, paclitaxel, and ponasterone A (Figure 2), were chosen as standards to develop the LC−MS separation and optimize detector parameters. These represent four common natural product classes, namely, phenolics, alkaloids, terpenoids, and steroids, respectively, and also show a suitable range in molecular weight. A 10-min reversed-phase separation utilizing a linear universal gradient showed a clear separation for these compounds at a flow rate of 1.2 mL/min per channel. In

Table 3. LC−MS−ELSD Data of Terpenoids and Steroids

compounds	R_t (min)[a]	$[M + H]^+$ (ESI^+)	other ions (ESI^+)	$[M - H]^-$ (ESI^-)	other ions (ESI^-)
ecdysone	4.20	447.6 $[M - OH]^+$	429.6	509.5 $[M + HCOO]^-$	–[b]
20-hydroxyecdysone	3.80	463.6 $[M - OH]^+$	463.6, 445.6	525.5 $[M + HCOO]^-$	–
ponasterone A	4.64	465.5	447.6, 429.6	509.5 $[M + HCOO]^-$	–
muristerone A	4.57	497.5	425.4	477.4	351.4
estrone	7.39	–	–	269.3	–
cafestol	7.90	–	299.5	–	–
ouabain	3.37	585.4	–	583.5	–
ginsenoside Rb	5.32	–	–	1108.8	–
ginsenoside Rc	4.63	–	–	946.7	992.7
kahweol	7.55	315.4	297.4	–	–
β-sitosterol	–	–	–	–	–
stigmasterol	–	–	–	–	–
lanosterol	–	–	–	–	–
macedonic acid	9.41	453.2	941.6 $[2M + H]^+$	469.7	–
lupeol	–	–	–	–	–
glycyrrhizic acid	5.49	823.4	453.6	821.7	–
18-β-glycyrrhetinic acid	9.78	471.6	–	469.5	–
glyyunnansapogenin C	8.66	469.3	433.5	467.3	–
glyyunnansapogenin B	7.46	487.7	976.5	977.1	471.1
limonin	6.83	471.5	425.5	469.4	–
nomilin	7.09	515.5	487.4	–	–
forskolin	7.73	433.5	375.5	–	–
phorbol	2.75	–	–	363.4	–
paclitaxel	7.57	854.3	509.5, 286.5	898.7	525.4
cephalomannine	7.63	832.3	509.5, 264.5	877.6	525.6
ginkgolide A	5.79	427.5	450.5	407.3	351.3
ginkgolide B	5.08	466.5	849.5	423.3	847.7
attractlyloside	3.69	360.6	316.6	–	–
puuphenone	9.52	329.6	179.5	327.3	359.3
artemisinin	7.24	283.1	209.5, 151.5	–	–
aureol	10.6	–	–	313.4	–

[a] MS retention time. [b] Not detected.

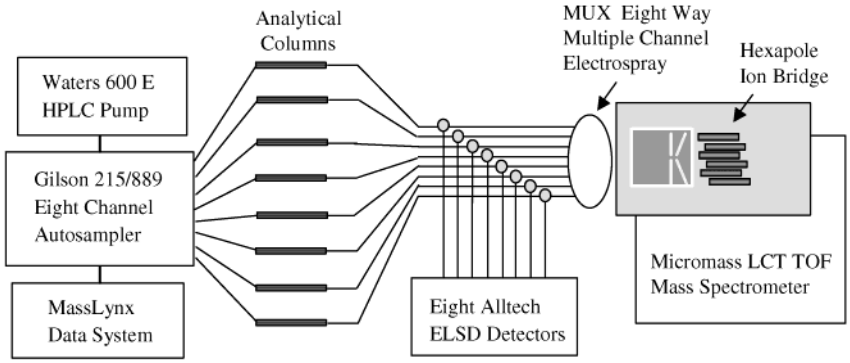


Figure 1. Schematic illustration of the configuration of the parallel eight-channel LC−MS−ELSD.

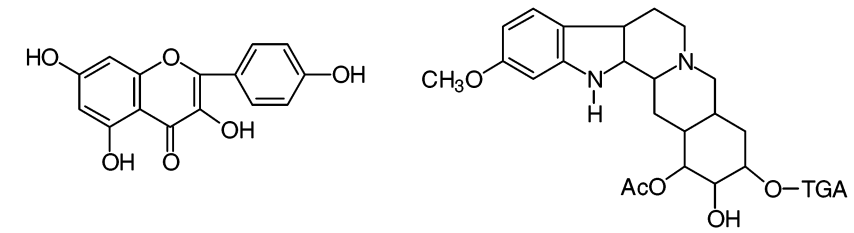
Table 4. LC−MS−ELSD Data of Polyketide Compounds

compounds	R_t (min)[a]	$[M + H]^+$ (ESI^+)	other ions (ESI^+)	$[M - H]^-$ (ESI^-)	other ions (ESI^-)
brefeldin A	5.91	245.5	263.5	–[b]	–
mevastatin	8.75	391.2	413.6	–	–
lovastatin	9.06	405.5	285.5	–	–
sedanolide	7.74	195.5	177.5, 149.5	–	–
aflatoxin B1	5.85	313.5	376.4	311.1	–

[a] MS retention time. [b] Not detected.

addition to the LC separation, the MS source conditions were also optimized. Each stream was split to allow a MS inlet flow of 90 μL/min per channel. The source conditions were optimized on the basis of infusion of the four standards in concentrations varying from 100 ng/mL to 2 μg/mL. Figure 3 illustrates the positive mode LC−MS TIC for these standards injected simultaneously on four of the channels at a concentration of 0.02 mg/mL. In addition to a clear separation, this method leads to clear detection for $[M + H]^+$ ion of each of these natural products. A four-way split allowed 0.4 mL/min per channel to be directed to each ELSD detector. The detection parameters, namely, gas flow and drift tube temperature, were then optimized by injection of the four standards in concentrations varying from 0.015 to 0.15 mg/mL. This ensured that each ELSD detector was capable of detecting 100 ng of these standards.

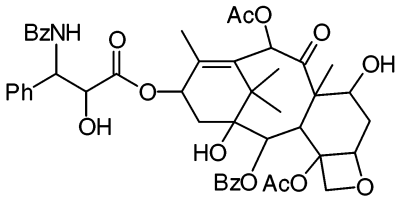
This method was applied to the analysis of a ninety-six compound natural product library covering a diverse range of scaffolds. Plant phenolics present include simple phenolic acids and hydroxycinnamates. Also, various classes of flavonoids are



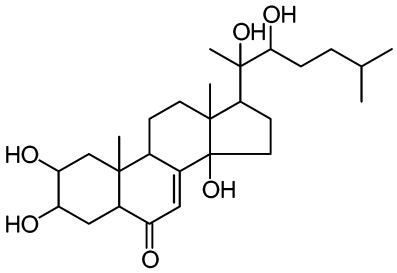
(1) Kaempferol, MW 286.0

(2) Reserpine, MW 608.3

TGA=Trimethyl gallic acid



(3) Paclitaxel, MW 853.1



(4) Ponasterone A, MW 464.3

Figure 2. Structures of kaempferol, reserpine, paclitaxel, and ponasterone A, which were used as standard compounds for method development.

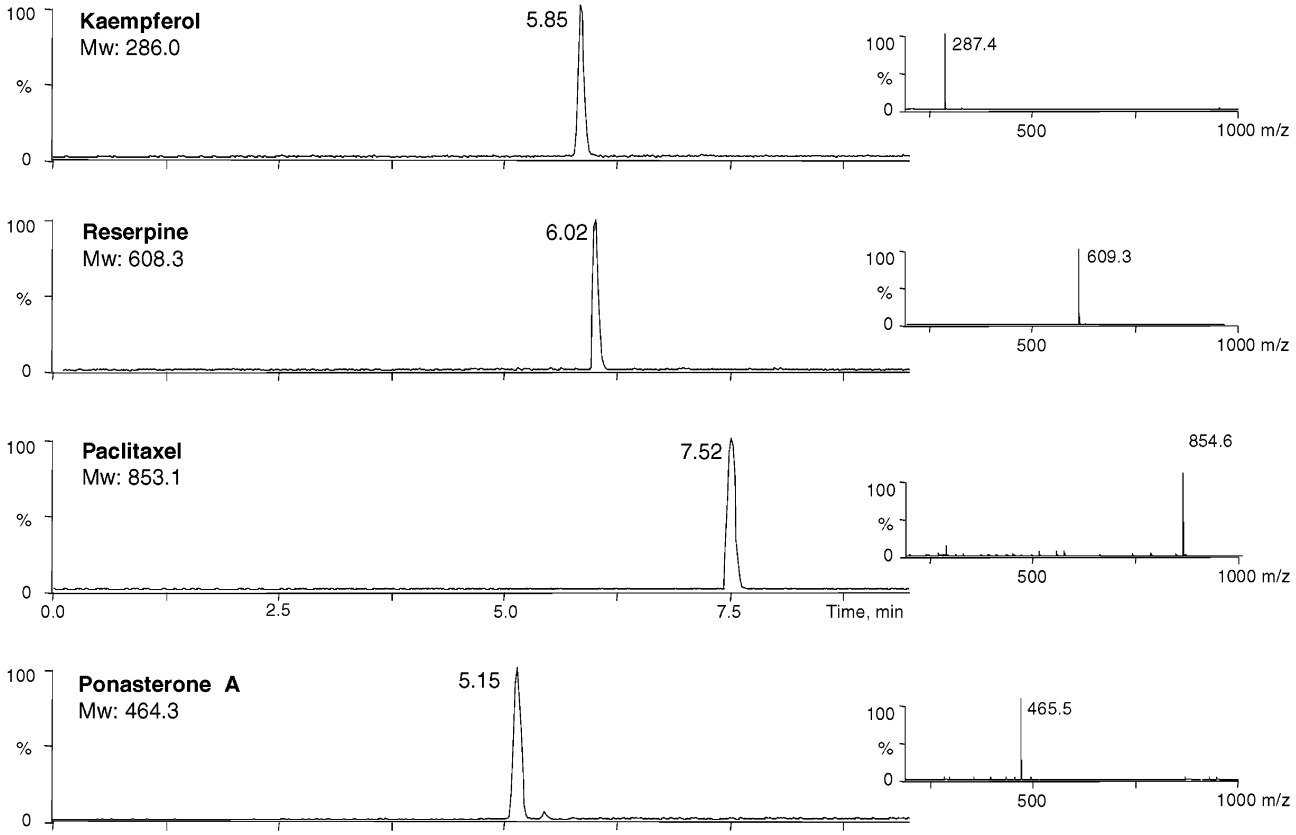


Figure 3. Chromatograms and mass spectra of four standard compounds, kaempferol, reserpine, paclitaxel, and ponasterone A.

represented, including those most commonly found in plants, such as flavones (luteolin, apigenin), flavonols (quercetin, kaempferol), flavanones (hesperidin, naringenin), and other less common classes such as isoflavonoids (genistein, daidzein). Similarly, various classes of alkaloids are represented, for example, quinoline alkaloids (quinine, emetine, papaverine) and indole alkaloids (reserpine, yohimbine, vinblastine, strychnine). In the steroid and terpenoid classes, this library includes a number of steroids such as stigmasterol and the ecdysones and also biologically interesting terpenoids such as paclitaxel, limonin, and ginkgolides A and B. Figure 4 illustrates the parallel eight-channel LC−MS−ELSD analysis of a number of wells from this library at a concentration of 0.02 mg/mL. The results of the analysis of the complete library are listed in Tables 1−4.

Plant Phenolics. Most of the 33 compounds investigated showed good ionization in both the positive and negative modes

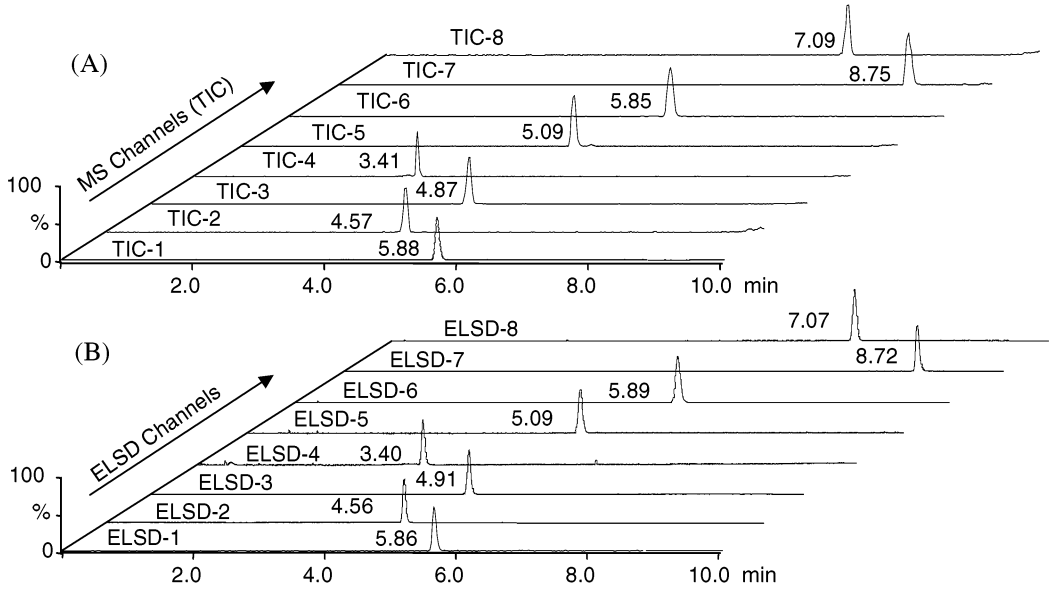


Figure 4. Eight natural products analyzed in one run: (A) eight TIC and (B) ELSD chromatograms. TIC-1−8 and ELSD-1−8 are kaempferol, muristerone A, colchicine, brucine, camptothecin, aflatoxin, mevastatin, and nomilin, respectively.

(Table 1). Ionization in the negative mode led to the formation of a clear $[M - H]^-$ ion with little fragmentation, with the exception of an $[M + HCOO]^-$ ion observed for podophyllotoxin. Of the phenolics studied, only reservatrol and the chalcone glyinflanin B were not detected in negative mode. In the electrospray positive mode, most flavonoids gave $[M + H]^+$ ions with the exception of catechin and the flavanonol taxifolin. Also, the hydroxycinnamates, caffeic and ferulic acids, and more simple phenols, ellagic acid and epigallocatechin gallate, could only be ionized in the negative ion mode as has been previously observed.[27] The use of 0.1% formic acid as mobile-phase adduct is particularly useful for the formation of $[M + H]^+$ and $[M - H]^-$ ions in the LC−MS analysis of these compounds.[25] The observation of intense $[M - H]^-$ ion when spraying from an acidic solution such as is observed in the analysis of these plant phenolics has been termed "wrong-way-around" electrospray ionization by Mansoori et al.[28] This has been postulated to be a function of the mechanism of electrospray ionization in which deprotonation occurs in the final stages of droplet formation when the concentration of negative charge at the droplet surface removes protons from the weak acids (phenolic compounds) despite the pH of the bulk solution. Since acidic mobile phases are especially useful for the chromatography of this class of natural products in terms of separation and resolution, this facet of electrospray ionization, namely, the ability to observe $[M + H]^+$ and $[M - H]^-$ ions with acidic chromatography, makes it a particularly suitable ionization technique for their analysis.

Alkaloids and Amides. Twenty-seven nitrogen-containing natural products were investigated in this study (Tables 2). All 25 alkaloids produce clear $[M + H]^+$ ions by electrospray positive mode with relatively little fragmentation. In negative mode, $[M - H]^-$ ions are also visible for α-solanine, reserpine, puromycin, epinephrine, camptothecin, and its 10-hydroxy derivative at this concentration level, although they are much less intense. The ability to observe $[M - H]^-$ ions for these alkaloids may be due to the presence of acidic functional groups within the molecule. Although the aminoglycosides neomycin and hygromycin produced molecular ions by electrospray positive mode, the simple reversed-phase universal gradient used does not retain such hydrophilic compounds as can also be observed with the adenosine derivative cordycepin.

Terpenoids, Steroids, and Polyketides. The ability to analyze triterpenes and steroids by this method depends somewhat on their level of oxidation in terms of both ionization and elution time (Table 3). Thus, lanosterol, β-sitosterol, and stigmasterol are too hydrophobic to elute by this rapid gradient. Lupeol, a pentacyclic triterpene, does elute at 9.8 min but is only visible by ELSD, due to its lack of functionality amenable to ionization. Steroids containing one or more hydroxyl groups ionize well by this method. Electrospray positive mode proves slightly better then negative mode both in terms of general ionization and also in ability to detect the $[M + H]^+$ ion since in negative mode the most intense ion may be a solvent adduct as with the ecdysonoids and ginsenosides, where the $[M + HCOO]^-$ pseudomolecular ion is prevalent. Many of the diterpenoids and sesquiterpenoids possessed a level of oxidation that facilitated ionization in either mode. A number of metabolites of polyketide origin, brefeldin A, mevastatin, lovastatin, sedanolide, and aflatoxin B1, were also investigated (Table 4), most of these ionizing in the positive mode.

SUMMARY

A total of 96 natural products are listed in Tables 1−4. The chromatography utilized by this method was suitable for the elution of 86 of these 96 compounds; i.e., 86 of the compounds show a retention time of greater than 1 min and less than 10 min. Of these 86 natural products, 75 provide a clear $[M + H]^+$ or solvent adduct ion visible by electrospray positive mode and 57 a clear $[M - H]^-$ ion or adduct ion visible by electrospray negative mode. Since ELSD responds to the amount of material rather than ionization efficiency, it is a nonselective detector for relatively nonvolatile compounds and all of the 86 eluted natural products

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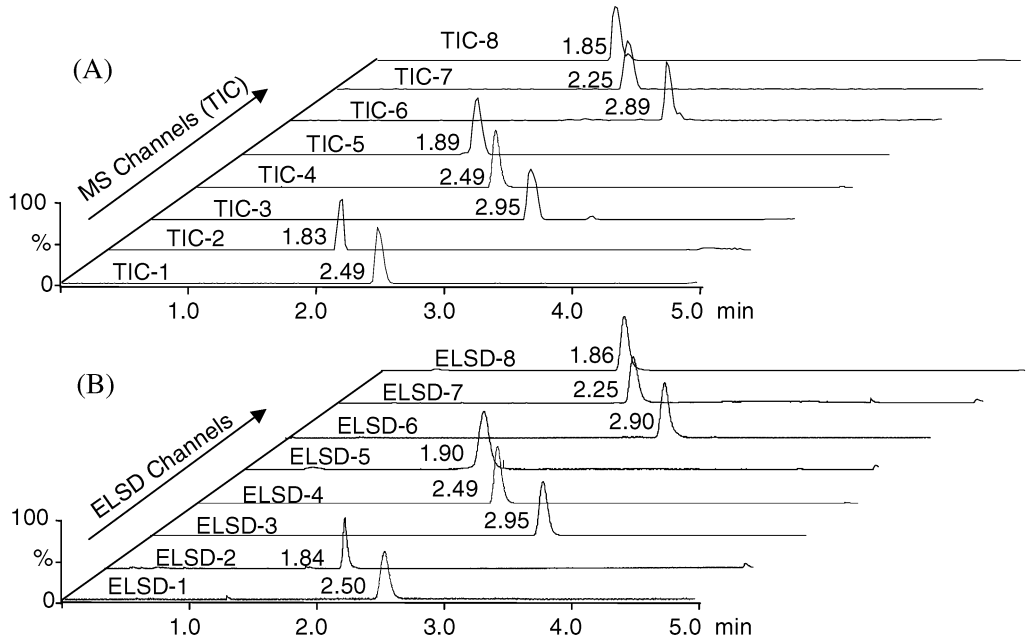

Figure 5. Eight alkaloids analyzed in one run: (A) eight TIC chromatograms and (B) ELSD chromatograms. TIC-1−8 and ELSD-1−8 are yohimbine, caffeine, colchicines, α-solanine, emetine, campthothecin, papaverin, and quinine, respectively.

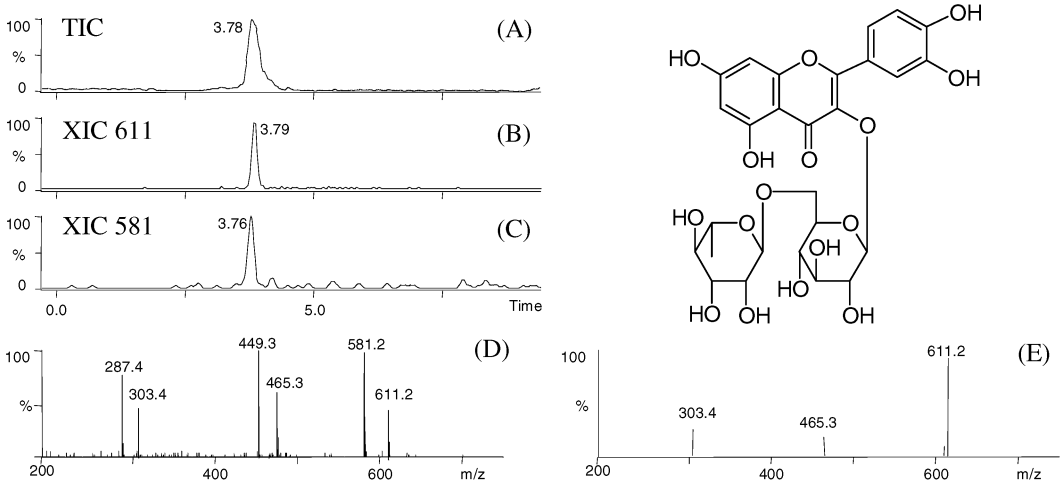

Figure 6. Characterization of rutin from the analysis of well 9 of a compound library derived from *S. hirtellum:* (A) TIC-electrospray positive mode; (B) XIC, *m*/*z* 611; (C) XIC *m*/*z* 581; (D) mass spectrum of peak at 3.78 min in TIC (A); (E) mass spectrum of rutin from natural product library.

analyzed in this library are detected by ELSD. In terms of providing universal detection for natural product libraries by this method, it is evident that the chromatographic separation is more of a limiting factor than the detection techniques utilized. The data shown in Tables 1−4 were collected in 2.6 h, which illustrates the power of this method in rapidly generating useful characterization information for a given compound library in a relatively short time. In a high-throughput setting, this analysis permits the processing of eight such 96-well libraries per day (21 h). The chromatography may also be adjusted to further increase the throughput as is shown in Figure 5 where a 5−95% acetonitrile linear gradient in 5 min is used to separate a group of alkaloid compounds from this library. This 5-min separation further reduces the time for LC−MS−ELSD analysis of a 96-well plate to 1.3 h, which in a high-throughput setting allows the processing of 16 96-well plates in 1 day (21 h).

However, longer run times may possess the advantage of providing greater resolution of the eluting components. In the case of compound libraries derived from plant sources, in which many of the components are unknown, increased resolution may be useful for the identification of the components present. Figure 6A−D illustrates chromatograms and mass spectra derived from the parallel LC−MS−ELSD analysis of well 9 from a library of *Sarcostemma hirtellum*. This plant is known as the hairy milkweed and is a member of the *Asclepiadaceae.* Members of this family are known for their ability to produce biologically interesting metabolites such as cardiac glycosides.[29] Our developed purification processes allow for the production of a natural product library from this plant by a standard high-throughput method.[30] After LC−MS−ELSD analysis, the raw data are first integrated and pro-

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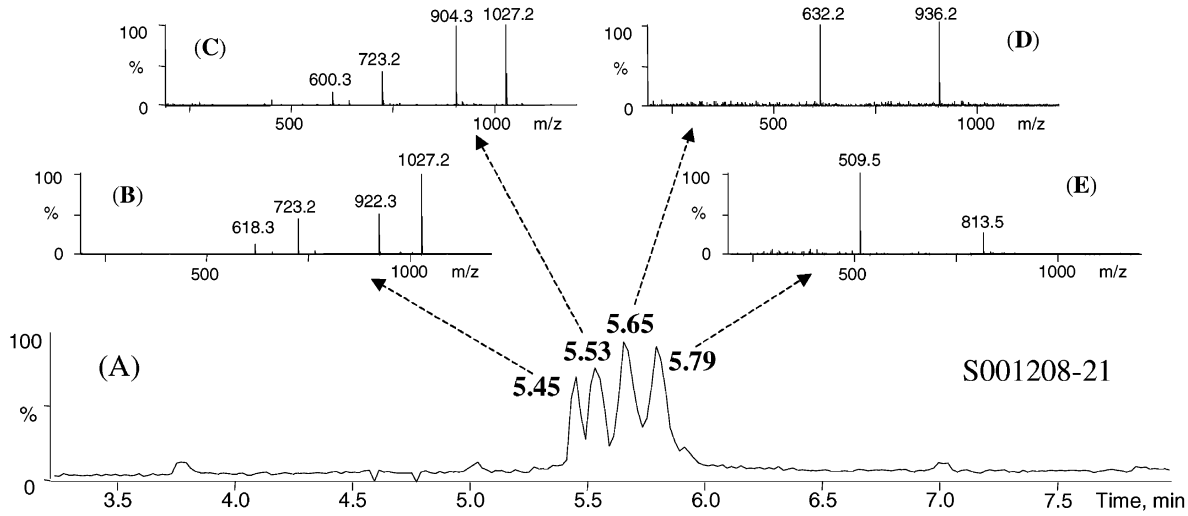

Figure 7. LC−MS−ELSD analysis of well 21 of a compound library derived from *S. hirtellum*: (A) TIC-electrospray positive mode of well 21; (B−E) mass spectra of peaks at retention times 5.45, 5.53, 5.65, and 5.79 min, respectively.

cessed by OpenLynx software to produce a text report containing retention time and mass spectral information on the components present in each chromatogram. This text file is then further processed by a custom-developed software package Extractor, which extracts the retention time, mass spectral peaks above a certain intensity of the base peak of the mass spectrum, and peak integrations for each chromatographic peak and converts it to text, which is transferred to a database for storage and analysis. In addition to extracting mass spectral data associated with each chromatographic peak, Extractor also runs a number of algorithims to determine which peak may be described as the $[M + H]^+$, $[M + H]^-$, or solvent adduct ion and also is capable of generating m/z values that may be used to generate extracted ion chromatograms to detect coeluting components. These data collected for each well are then searched for matches with known standards, which allows rapid identification of previously analyzed components. Rutin is identified as one of the components of well 9 of this library by initial matching of TIC retention time and mass spectrum in electrospray positive mode (Figure 6A,D). This is then further confirmed by extracted ion chromatogram at the characteristic $[M + H]^+$ for rutin, m/z 611 (Figure 6E), which shows rutin eluting at 3.79 min overlapping with a nonidentified component eluting at 3.76 min.

Such identification is essential in identifying novel and potentially interesting components within a library. Figure 7 illustrates the LC−MS chromatogram with inset mass spectra resulting from analysis of well 21 of the *S. hirtellum* library. Initial MS and ELSD analyses suggested the presence of four components within this well. However, a pattern of a mass difference of 304 units between m/z 1027.2 and 723.2 and between m/z 922.3 and 618.3 is evident in the MS data derived from the peak at 5.45 min (Figure 7B). Two of these ions, m/z 1027.2 and 723.2 are also observed in the mass spectrum recorded for the peak at 5.53 min (Figure 7C), in addition to an m/z value of 904.3 with again a characteristic ion appearing at a mass difference of 304 units at 600.3 amu. This suggests that both peaks at 5.45 and 5.53 min consisted of two coeluting components. The MS data for the peaks at 5.65 and 5.79 min (Figure 7D and E) also show the characteristic mass difference of 304 units, suggesting that this set of compounds is structurally similar. In these analyses, the development of suitable automated data processing methods assumes an equal importance to developing suitable detection or separation methods, since as in the example shown, a single well can produce a large amount of data. The high-throughput method here described can produce data on 640 wells/day (96-well microtiter plate containing 80 samples/plate). An extract of the database entries listing some of the components found by automatic processing of well 21 of the *S. hiretellum* library is shown in Table 5. It is evident from these data that the purification processes utilized are capable of producing relatively pure libraries of closely related compounds from natural sources in a format suitable for high-throughput screening. Furthermore, the LC−MS−ELSD analysis rapidly generates useful information as regards the components present should any of the library wells return an active hit from HTS. The main application of this method in our laboratories to date has been for the analysis of a library containing 36 000 fractions derived from plant materials of a diverse collection of 176 plant families and 561 genera from the United States and Gabon, Africa.[30]

Table 5. LC−MS−ELSD Database Entry for Well 21 of *S. Hirtellum* Compound Library

compd no.	well no.	R_t (min)	$[M + H]^+$ (ESI^+)	other ions (ESI^+)	no. of compds present
78	21	5.43	922.3	618.3	6
79	21	5.45	1027.2	723.2	6
80	21	5.51	1027.2	723.2	6
81	21	5.53	904.3	600.3	6
82	21	5.65	936.2	632.2	6
83	21	5.79	813.5	509.5	6

CONCLUSIONS

This work represents one of the first adaptations of high-throughput analytical methods to the field of natural products. It

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is evident that electrospray detection, due to the gentle ionization, sensitivity, adaptability to high throughput, and ease of operation is one of the most powerful methods of mass spectrometric detection for polar, nonvolatile, thermally labile natural products. ELSD acts as a complementary method in terms of universality ensuring that one LC−MS−ELSD run can provide sufficient MS data and retention time data for identification of known components present. Furthermore, ELSD has been shown to provide quantitation with a greater degree of accuracy than UV for the analysis of combinatorial libraries.[31] Quantitation of natural product libraries by ELSD using the described method is currently underway in our laboratories. A further development in terms of characterization is the ability of a TOF mass analyzer to record accurate mass values in a high-throughput setting. An accuracy of within 10 ppm has been demonstrated for analysis of a number of druglike small molecules using a dual-sprayer MUX electrospray source.[32] Our initial results show that accurate mass measurements are possible using an eight-way MUX interface for the analysis of natural products with an accuracy of less than 20 ppm.[33] Work is currently underway to improve this accuracy since providing a list of potential molecular formulas for detected components in addition to retention time and m/z values will further strengthen the characterization abilities of this method. As shown, parallel eight-way LC−MS analysis offers an 8-fold increase in throughput of samples. Parallel stream sample introduction can be expected to be less sensitive than a single-channel source. The natural product library here described was analyzed at a concentration of 0.02 mg/mL which gave satisfactory results by MS and ELSD. This method does not provide a useful analysis for poorly functionalized and very hydrophobic or very hydrophilic compounds. However, these attributes also tend to reduce the likelihood of such compounds being developed as useful drug candidates. One of the primary goals of a contemporary natural products high-throughput screening program is to rapidly identify novel biologically active chemical structures that can be developed as drug candidates. This objective is dependent on suitable analytical methodology for the rapid analysis of a large number of compounds or compound mixtures. The advance in instrumentation and relatively straightforward methods here presented demonstrates one means by which this goal may be achieved.

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