Determination of less than a nanomol of cerebrosides by high performance liquid chromatography with gradient elution analysis

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Abstract A gradient elution high performance liquid chromatography method with detection at 230 nm for the analysis of perbenzoylated cerebrosides containing hydroxy and nonhydroxy fatty acids is described. The quantitative range of the method is 0.5–10 nmol of cerebrosides. The limit of detection for injected sample is about 10 picomol. The analysis time by liquid chromatography is less than 5 minutes and prior purification of the cerebrosides from other lipids in brain lipid extracts is not necessary. The cerebrosides are first perbenzoylated with 50 μl of 10% benzoyl chloride in pyridine and then separated on a chromatographic column of Zipax. The gradient elution is with 2.8–5.5% of dioxane in hexane. This gradient elution micromethod is at least 10 times more sensitive than isocratic elution methods with detection at 280 nm. The method is applicable to other biological materials containing minute amounts of cerebrosides if the glycolipid fraction is first isolated from the lipid extracts. A further fourfold increase in the sensitivity is achieved by replacing air in the reference cell of the detector by gradient elution solvent.

Supplementary key words: perbenzoylated cerebrosides · hydroxy and nonhydroxy fatty acids · lipid extracts of brain, plasma and tissue cultured cells

The analysis of benzoylated cerebrosides by high performance liquid chromatography with isocratic elution and detection at 280 nm has been described in a previous publication (1). It is known that the absorption maximum of the benzoylated cerebrosides is at 230 nm (1). The sensitivity of the assay can be increased approximately 14-fold if the detection is performed at 230 nm instead of 280 nm (1). The isocratic elution system employed previously (1) for the separation of the benzoylated NFA- and HFA-containing cerebrosides is not adequate for the analysis of subnanomol quantities of cerebrosides in tissue extracts because the benzoylated NFA cerebrosides are not completely separated from the solvent peak or from other products formed from the benzoylation of lipid extracts that elute with the solvent peak. Adequate separation could be obtained with less polar solvents; however, the retention time and elution volume for the benzoylated HFA cerebrosides were considerably increased with such a system, resulting in loss of sensitivity.

This report describes HPLC for the separation and quantitation of the benzoylated HFA and NFA cerebrosides with gradient elution analysis. Two separate gradient elution solvent systems with detection at 230 nm or at 280 nm are described. Detection at 230 nm is possible only with a dioxane–hexane solvent system as this solvent is relatively transparent at 230 nm. However, this requires the use of a variable wavelength spectromonitor. Detection at 280 nm could be performed with a relatively inexpensive monitor with ethyl acetate–hexane or dioxane–hexane solvent systems. The analysis time for both these systems is 5 minutes. With a hexane–dioxane gradient system and detection at 230 nm, an ascending baseline is obtained, due to slightly higher absorption of dioxane in hexane. Operationally, this factor limits the use of the highest sensitivity possible. A workable flat baseline is obtained if the solvent mixture, as it emerges from the injector, is led through the reference side of the detector cell and then on to the column. However, with this procedure, the analysis

Abbreviations: HPLC, high performance liquid chromatography; NFA-CR, cerebrosides with nonhydroxy fatty acids; HFA-CR, cerebrosides with hydroxy fatty acids; CDH, ceramide-dihexosides.

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time is increased up to 20 min due to the slower solvent flow rate employed.

EXPERIMENTAL

Standards and reagents

Beef brain mixed cerebrosides and individual HFA and NFA cerebrosides were purchased from Supelco, Bellefonte, PA. The HFA cerebrosides contained approximately 8% by weight of the NFA cerebrosides (see Fig. 3,C). HFA cerebrosides were purified from the mixed beef brain cerebrosides by benzoylation, HPLC, and debenzoylation as described previously (1). The concentration of the standard cerebrosides was established by lipid–galactose determination with a microadaptation of the orcinol–sulfuric acid method (2). Molecular weights of NFA and HFA cerebrosides were assumed to be 812 and 828, respectively. Preparation of adult rat and calf brain lipid extracts has been previously described (3). The human blood plasma (1–2 ml) samples were lyophilized and 0.25 ml of water was added. In vitro cultured cells (kindly supplied by Dr. Ann Gibson, McLean Research Lab., Belmont, MA) were homogenized in 0.6 ml of water and a portion of the homogenate was used for protein determination. The blood samples and the cultured cell homogenates were warmed at 60°C for 15 min with 5 ml of chloroform–methanol 1:1 (v/v). The precipitated proteins were centrifuged and re-extracted with 2.5 ml of chloroform. The combined lipid extracts were washed once with 1.4 ml of saline and twice with 1.0 ml of theoretical upper phase (4). The extracts were dried and redissolved in 1 ml of chloroform and chromatographed on a small column of silicic acid (50 mg). The column was successively developed with chloroform, 5 ml; acetone–methanol 9:1 (v/v) 5 ml; and methanol, 5 ml. The acetone–methanol fraction containing the glycolipids was evaporated to dryness. [^{14}C]-Labeled cerebrosides were prepared after injecting [U-^{14}C]serine into rat brains as described previously (1). All solvents used for HPLC, obtained from either Fisher Chemical Co. (Fairlawn, N J) or Burdick Jackson, Inc. (Muskegon, MI), were of spectral grade quality and were degassed by boiling briefly before use. Pyridine was redistilled and kept dry over 4A molecular sieves (Fisher Chemical Co.). Reaction vials with small conical ends (Reactivials, 1 ml vol., Pierce Chemical Co., Rockford, IL) were used for the microbenzoylation of cerebrosides. The vials were soaked in chromic acid and then thoroughly washed with distilled water, methanol, and finally with chloroform–methanol 2:1 (v/v) in order to remove possible trace contaminants.

Instrumentation

The HPLC analysis was performed with a Waters Associates (Milford, MA) Model 6000 solvent delivery system combined with a solvent programmer Model 660 and a Model U-6K injector. The chromatographic column was a 50 cm × 2.1 mm ID stainless steel tube packed with Zipax, pellicular particles, average size of 27 μm covered with porous silica, (E. I. duPont de Nemours, Inc., Wilmington, DE). Detection was either with a Schoeffel Instruments Corp. (Westwood, NJ) Model SF 770 variable-wavelength spectromonitor or with a Laboratory Data Control (LDC, Riviera Beach, FL) Model 1285 ultraviolet (280 nm) monitor, or with a LDC spectromonitor-I. These monitors have 8-μl flow through cells. The monitors were coupled to a stripchart recorder. The peak areas were measured either with an Autolab Minigrator (Spectra Physics, Santa Clara, CA) or by the cut and weigh method.

Benzoylation of Cerebrosides

Samples containing 0.5–10 nmol of cerebrosides standards and appropriate amounts of the glycolipid fraction or lipid extracts (at two different levels for the same sample) from tissues were placed in screw-cap Reactivials and the organic solvent in the samples was evaporated. The samples were then made moisture-free by drying over P_2O_5 in a vacuum desiccator for 30 min. Fifty μl of 10% (v/v) benzoyl chloride in dry pyridine was added to each sample. After heating for 1 hr at 60°C in an oil bath, the pyridine was removed by a stream of N_2. To the dried samples 0.3 ml of alkaline–methanol (4 vol of methanol and 1 vol of 1% aqueous Na_2CO_3) was added, followed by 0.5 ml of hexane. The vials were capped and vigorously shaken, briefly centrifuged to separate phases, and the lower methanolic phase was removed using a syringe equipped with a long needle. The hexane phase was washed three more times with 0.3 ml of alkaline–methanol and dried under N_2. The benzoylated cerebrosides were then redissolved in a small volume of CCl_4 (about 100 μl) for HPLC. The yield of benzoylation reaction as determined by recovery of ^{14}C-labeled cerebrosides was more than 95%. McCluer and Evans (1) have reported a yield of 96.7% with the 80% methanol washing procedure and 70% with the 95% methanol washing procedure. They have recommended washing with 95% methanol for the isocratic procedure because 95% methanol more effectively removes excess reagent. However, in gradient elution analysis, the excess reagent is eluted near the solvent front and does not interfere with the cerebrosides analysis.

Gradient elution HPLC analysis with detection at 280 nm

One to ten nmol of mixed beef brain cerebrosides standard and calf brain lipid extract were benzoylated and the entire sample was injected on the Zipax column. When the sample was injected, the column was equilibrated with 2% aqueous ethyl acetate in hexane. (Aqueous ethyl acetate was prepared by mixing 5 volumes of dry ethyl acetate with 1 volume of ethyl acetate saturated with H_2O.) The solvents were pumped at the rate of 4 ml per min. The elution was with a linear gradient of 2–7% aqueous ethyl acetate in hexane. The gradient was initiated immediately after the injection of the sample and was completed in one min. The detection was at 280 nm with either the Schoeffel or the LDC ultraviolet monitor. The reference cell contained air. The column was regenerated to its original polarity by reversing the gradient for 1 min and equilibrating the column with 2% aqueous ethyl acetate in hexane for 3–4 min.

Gradient elution HPLC analysis with detection at 230 nm

Bovine brain individual NFA and HFA cerebroside standards, adult rat brain and calf brain lipid extracts (5 and 12 μg dry wt, respectively), and partially purified glycolipid fractions from human blood plasma and tissue-cultured cells, in 0.4–10 nmol quantities, were benzoylated as described above. The benzoylated samples were dissolved in 100 μl of carbon tetrachloride and suitable portions (usually 10–20%) of the sample were injected on the Zipax column. For each sample, two or more separate injections were made and the response was averaged. The column was equilibrated with 2.8% dioxane in hexane. The elution was with a 3 min linear gradient of 2.8–5.5% dioxane in hexane. The gradient was initiated at the time of injection. The solvents were pumped at the rate of 4 ml per min. Detection was at 230 nm with either a Schoeffel spectromonitor or a LDC Spectromonitor-I. The reference cell contained air. After completion of an analysis, the column was regenerated to its initial polarity by reversing the gradient for 1 min and equilibrating the column with 2.8% dioxane in hexane for 3–4 min.

The same column and chromatographic conditions were used repeatedly for several weeks without any loss of reproducibility. When the chromatographic resolution deteriorated, the column was washed with methanol, dichloromethane, and hexane, successively, to regenerate its full activity. The chromatographic conditions could be varied slightly to suit various biological samples.

When air was in the reference cell of the detector, a rise in the baseline was obtained with increasing concentration of dioxane in hexane. For a flat baseline the solvent, as it emerged from the injector, was led through the reference cell and then on to the column. The column was equilibrated with 1.5% dioxane in hexane at a flow rate of 1.5 ml per min. The sample was injected and a gradient of 1.5–7.5% dioxane in hexane was initiated after the solvent front was near the baseline. The gradient run was for 15 min. Detection was at 230 nm with a LDC Spectromonitor-I. After completion of an analysis the column was regenerated to its initial polarity by reversing the gradient to run for 1 min and equilibrating the column with 1.5% dioxane in hexane for 8–10 min.

RESULTS

Fig. 1 shows the gradient elution HPLC analysis with detection at 280 nm of cerebrosides standard (7.5 μg total) and of the cerebrosides present in the calf

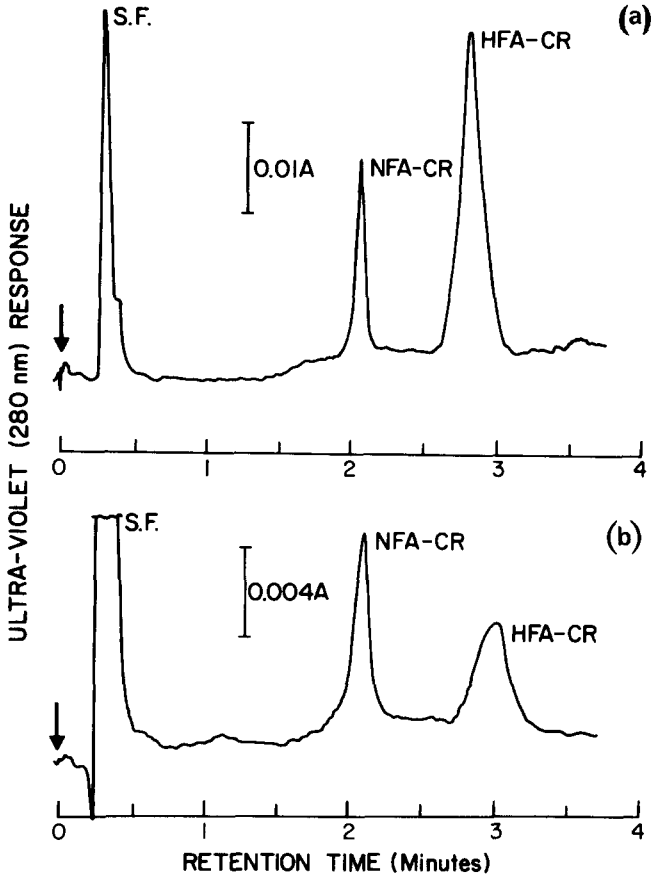

Fig. 1. Gradient elution high performance liquid chromatography of benzoylated (*a*) beef brain cerebrosides standard (9.1 nmol, total) and (*b*) calf brain lipid extract (25 μg, dry wt) on a Zipax column. The elution was with a 1 min linear gradient of 2–7% aqueous ethyl acetate in hexane pumped at 4 ml per min. The detection was at 280 nm with a Schoeffel spectromonitor. Arrow represents point of injection. S.F. is solvent front.

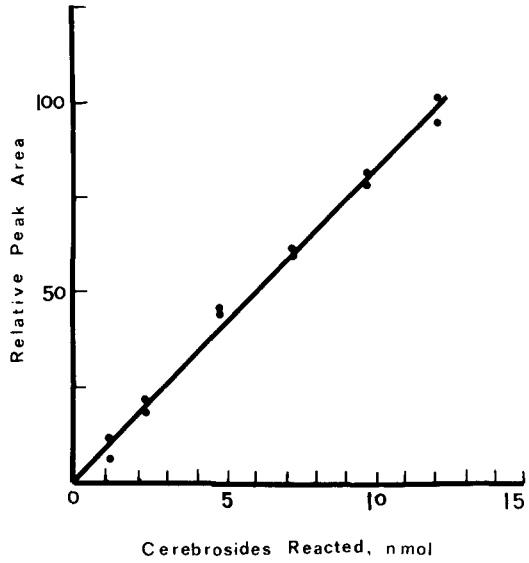

Fig. 2. Quantitative analysis of beef brain cerebrosides standard by gradient elution high performance liquid chromatography. The amounts of cerebrosides containing HFA and NFA indicated were benzoylated and the total sample was injected on a Zipax column. Gradient elution was with ethyl acetate in hexane and detection was at 280 nm, as described in Fig. 1. Each quantity was analyzed twice. The relative peak area plotted is the sum of the NFA and HFA cerebrosides peak areas.

brain lipid extract (25 μg, dry wt). Since there was a breakthrough volume of 6 ml between the pump heads and the end of the column, there was a delay of 1.5 min before the detector responded to the change in the solvent from 2% aqueous ethyl acetate in hexane to higher concentration of ethyl acetate. The HFA cerebrosides were eluted after the highest concentration of the gradient (7% aqueous ethyl acetate) reached a maximum. In the case of isocratic elution procedure, the excess reagent products eluted close to the benzoylated NFA cerebrosides [Fig. 1 of ref (1)]. The gradient elution completely resolved these products from benzoylated NFA cerebrosides. Again, with the gradient elution procedure, the benzoylated HFA cerebrosides peak was sharper, resulting in greater sensitivity. Other lipids present in the calf brain lipid extract do not appear to interfere in the analysis. The analysis was complete in 4 min after injection.

Fig. 2 shows the standard curve obtained with mixed beef brain cerebrosides. The response was linear with the amount of cerebrosides reacted. The total peak area was determined as such without considering the slightly greater response ratio, 1.1, of NFA to HFA cerebrosides at 280 nm (1).

Fig. 3 a and **3 b** show the gradient elution HPLC analysis with detection at 230 nm of benzoylated NFA (93.6 pmol) and HFA (241 pmol) cerebroside standards (Supelco). Dioxane is not completely transparent at 230 nm and thus produces a rise in baseline as its concentration in hexane is increased by the gradient run. As mentioned previously, because there was a breakthrough volume of 6 ml, the baseline appeared to change 1.5 min after the start of the gradient. The benzoylated NFA and HFA cerebrosides were eluted before the gradient reached its highest concentration of dioxane in hexane. The HFA cerebroside standard appeared to have a small amount of NFA cerebrosides (Fig. 3 b); when injected at higher concentration, it was found to contain about 8% NFA cerebrosides, Fig. 3 c. The NFA cerebroside peak shown in Fig. 3 b was found to represent 19.2 picomol of cerebrosides. Thus the apparent limit of

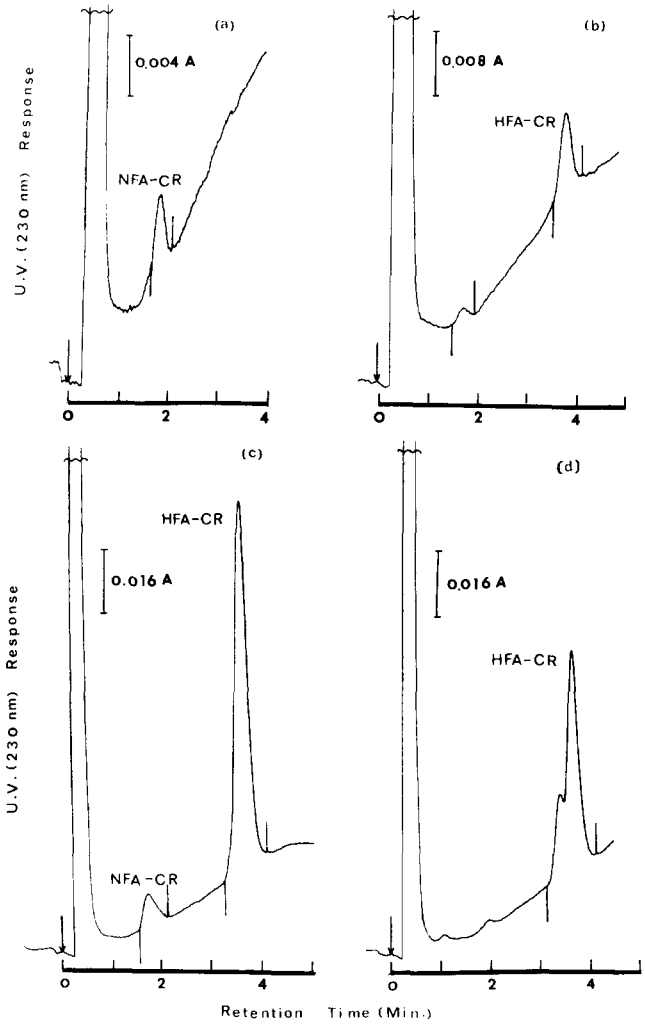

Fig. 3. Gradient elution high performance liquid chromatography of benzoylated individual (*a*) NFA cerebrosides, Supelco, 93.6 pmole; (*b*) and (*c*) HFA cerebrosides, Supelco, 241 pmol and 1.93 nmol, respectively, (*d*) purified HFA cerebrosides, 1.33 nmol. The elution was with a 3 min linear gradient of 2.8–5.5% dioxane in hexane pumped at 4 ml per minute on a Zipax column. The detection was at 230 nm with a Schoeffel spectromonitor. Arrow represents point of injection.

detection and quantitation of the injected sample at 230 nm was about 10 picomol of cerebrosides. The performance of the column and the detection system on injection of increasing amounts (from a single sample) of benzoylated NFA and HFA cerebrosides was determined. The response in terms of peak area was linear when 10 picomol to 5 nmol of the benzoylated NFA and HFA cerebrosides were injected. The HPLC analysis of the HFA cerebrosides, which were purified by benzoylation, HPLC, and debenzoylation of beef brain mixed cerebrosides (1), is shown in Fig. 3 d. This preparation appeared to contain negligible amount of NFA cerebrosides.

The shoulder that appeared on the HFA cerebrosides peak could be due to differences in the fatty acid compositions of HFA cerebrosides or could be due to the presence of a dihydrosphingosine moiety in some of the cerebrosides. We have occasionally observed this shoulder in our preparations and it is not due to impurities.

Fig. 4 shows the standard curve obtained for the benzoylated NFA and purified HFA cerebrosides. The detector response appeared to be the same for the benzoylated NFA and HFA cerebrosides. The percentage relative standard deviation of the mean for the entire procedure, including benzoylation, HPLC, and area determination was 4% for a group of 15 samples analyzed.

Fig. 5 represents the HPLC analysis of the benzoylated cerebrosides in the adult rat brain lipid extract (2 μg, dry wt) and calf brain lipid extract (6 μg, dry wt). The total cerebrosides content in the rat brain extract was 0.25 ± 0.01 μg (0.305 nmol) per 2 μg of dry total lipids (average of four determinations). Thus, cerebrosides were 12.5% of total lipid weight. This value is in good agreement with reported values of 12.1–14.1% (5, 6). The total cerebrosides in the calf brain were found to be 6.85 ± 0.03% of the total lipid weight. The ratio of NFA cerebrosides to HFA cerebrosides in the lipid extracts of 11- and 20-day-old as well as adult rat brain were found to be 0.94, 0.75, and 0.48, respectively. The results indicate accumulation of higher amounts of HFA cerebrosides with increasing age of the rat. The ratios of NFA to HFA cerebrosides in lipid extract of the calf brain and beef brain mixed cerebrosides standards obtained from Supelco were 0.49 and 0.22, respectively.

The procedure was employed for the determination of cerebrosides in other biological materials containing minute amounts of these glycolipids relative to other lipids. **Fig. 6 a** represents an example of the HPLC analysis of the glycolipid fraction of human plasma. All human plasma samples analysed contained an unknown intermediate peak between

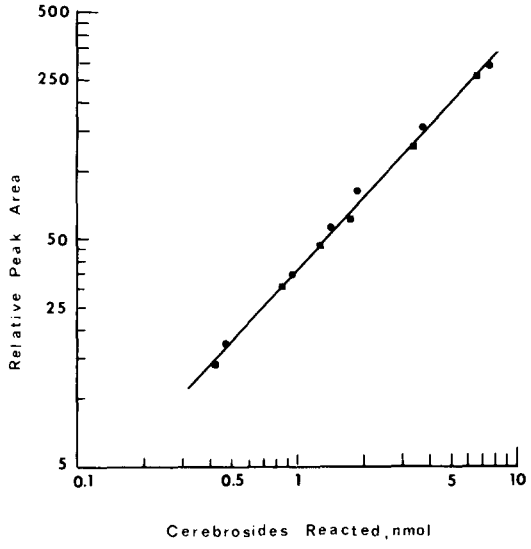

Fig. 4. Quantitative analysis of individual benzoylated NFA (●) and HFA (■) cerebrosides by gradient elution high performance liquid chromatography. The amounts of individual cerebrosides indicated in the figure were benzoylated and suitable portions of the samples were injected. The response due to entire sample in terms of peak area is shown. Gradient elution was with dioxane in hexane and detection was at 230 nm, as described in Fig. 3.

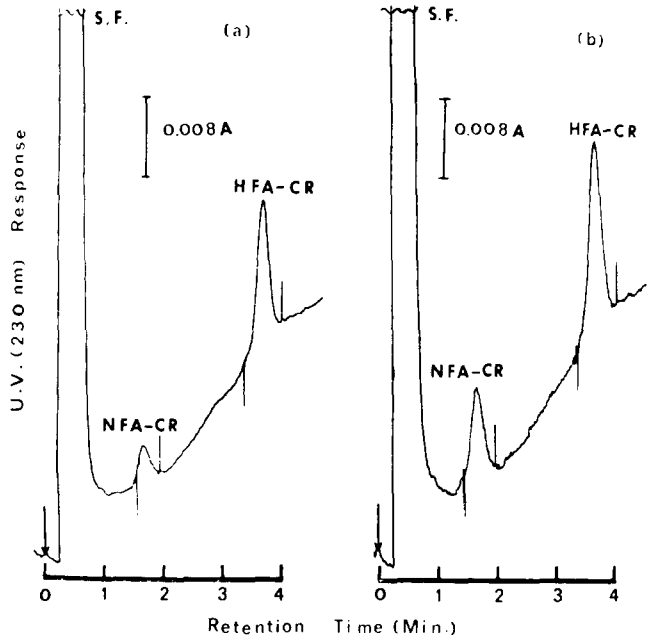

Fig. 5. Gradient elution high performance liquid chromatography of the benzoylated cerebrosides in (*a*) adult rat brain lipid extract (2 μg, dry wt) and calf brain lipid extract (6 μg, dry wt) on a Zipax column with detection at 230 nm. Other chromatographic conditions were the same as in Fig. 3. Adult rat brain and calf brain lipid extract, 5 μg and 12 μg of dry lipid wt, were benzoylated and 40% and 50% of the samples, respectively, were injected.

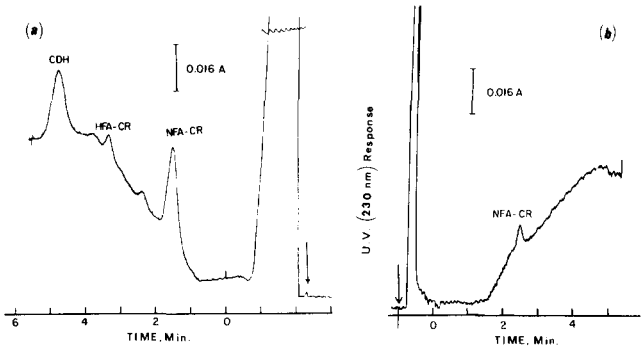

Fig. 6. Gradient elution high performance liquid chromatography of the benzoylated glycolipid fraction of (*a*) human plasma and (*b*) new-born mouse dorsal root ganglion cells in culture. The glycolipid fraction, from 1 ml of plasma and cultured cells having 300 μg of proteins, was benzoylated and 10% of the benzoylated sample was injected on a Zipax column. The arrow represents point of injection of the sample. A 3 min linear gradient of 1.5–5.5% dioxane in hexane was initiated after the solvent front reached a suitable baseline with the initial solvent. The flow rate was 4 ml per minute. Detection was at 230 nm. The time represents retention time after the gradient was initiated.

benzoylated NFA and HFA cerebrosides peaks. The benzoylated ceramide-dihexoside (CDH) peak appeared at the end of the gradient and was completely separated from the HFA-CR peak. However, benzoylated ceramide trihexoside and globoside were retained on the column and could be eluted with higher concentrations of dioxane in hexane (7). Slightly different chromatographic conditions were employed to facilitate better resolution of the solvent front from the benzoylated NFA-CR peak. The concentrations of NFA and HFA cerebrosides in human plasma are given in **Table 1.** The concentration of the intermediate unknown peak was calculated by assuming a mol wt of 820 and using the standard curve for NFA and HFA cerebrosides. The values for concentration of total cerebrosides in human plasma, 0.82–1.14 μmol/dl reported previously (8, 9) were similar to those obtained by us.

Fig. 6 b represents an example of HPLC analysis of the glycolipid fraction of newborn-mouse dorsal root ganglion dissociated cells in culture for 20 days

TABLE 1. Concentration of NFA and HFA cerebrosides in human plasma

Donor	Sex	Cerebrosides		Intermediate Peak
		NFA	HFA	
		(μmol/dl)		
S.G.	F	0.85	0.11	0.06
C.A.	F	0.64	0.10	0.05
B.B.	M	0.61	0.11	0.04
L.U.	M	0.44	0.23	0.03
Mean		0.64	0.14	0.045

in vitro. Only NFA cerebrosides could be detected. The concentration of the NFA cerebrosides in these cultured cells was about 635 picomol/mg protein. The concentration of the NFA cerebrosides in the C6 glioma cells was about 40 picomol/mg protein; it is not known whether the NFA cerebrosides present in these cells are of glucose- or galactose-containing cerebrosides. No comparable data are available as to the quantity of the NFA cerebrosides in these cells.

A workable flat baseline was obtained (**Fig. 7**) when air in the reference cell was replaced by pumping the solvent through the cell before it reached the top of the column. With such an arrangement, the flow rate was reduced to 1.5 ml per minute in order to reduce pressure on the quartz windows of the cell. A flat baseline was achieved in the area where the NFA and HFA cerebrosides were eluted by adjusting the time of the gradient run. As the sample was injected, a negative peak was recorded due to passage of the sample through the reference cell. After the gradient was initiated, there was a delay of about 5 min due to breakthrough volume between pump heads and the reference cell. The flow of the gradient through the reference cell was indicated by a decline in the baseline. Thereafter, the baseline remained practically flat and, towards the end of the gradient run, the baseline began to rise again and then remained flat because both the reference and the sample cells contained the same solvent. Fig. 7a represents HPLC analyses of benzoylated NFA (0.538 nmol) and HFA (0.484 nmol) cerebrosides standards (Supelco). The HPLC analysis of the benzoylated glycolipid fraction from a human plasma (≡60 μl) sample is shown in Fig. 7 b. A partial separation of the NFA glucocerebroside (present in the plasma sample) from added NFA galactocerebroside standard was achieved, Fig. 7 c. A fourfold increase in the sensitivity, leading to the analysis of 21.5 pmol of NFA and 19 pmol of HFA cerebrosides, is shown in Fig. 7 d.

DISCUSSION

Previous reports from this department have described the preparation of and the HPLC of benzoylated cerebrosides (1, 10) and other neutral glycosphingolipids (11, 12). Here, we have described conditions for the quantitative analysis of NFA and HFA cerebrosides in the 0.5–10 nmol range. Other methods of detection and quantitation of cerebrosides that utilize portions of the cerebroside molecule, such as sphingosine base (13, 14) or hexose (5, 9), require prior purification by column and thin-layer

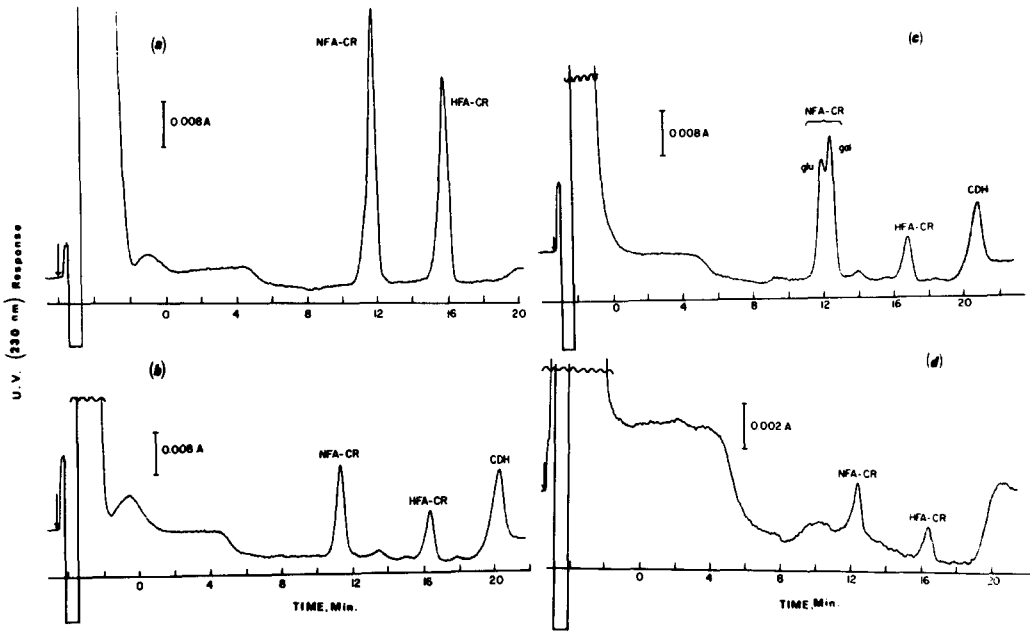

Fig. 7. The gradient elution high performance liquid chromatography of benzoylated (*a*) NFA (0.538 nmol) and HFA (0.484 nmol) cerebrosides standards; (*b*) human plasma glycolipid fraction (≡60 μl of plasma); (*c*) plasma glycolipid fraction + NFA-galactocerebrosides (0.21 nmol) standard; and (*d*) NFA (21.5 pmol) and HFA (19 pmol) cerebrosides standards. The HPLC was performed on a Zipax column. The arrow represents point of injection of the sample. After the solvent front reached a suitable baseline with the initial solvent, a 15 min linear gradient of 1.5–7.5% dioxane in hexane was initiated. The flow rate was 1.5 ml per min. Detection was at 230 nm with a LDC spectromonitor. The solvent was pumped first through the reference cell before it reached the top of the column. The time represents time after the gradient was initiated.

chromatography. These steps are necessary to remove interfering compounds. The method reported here overcomes these problems and can be used directly with the lipid extracts, such as total brain or brain microsomal and myelin lipid extracts, where the relative amount of cerebrosides to other lipids is considerably high. The method could be applied to other biological materials containing only minute amounts of these lipids, compared to other lipids, if the glycolipid fraction is first isolated by a small silicic acid column chromatography prior to benzoylation and HPLC.

Two separate gradient elution systems with detection at 280 nm and 230 nm are described. Ethyl acetate and hexane were selected as solvents for use with a relatively inexpensive monitor that operates at 280 nm. The advantage of this system over the 230 nm detection system is that the baseline does not rise appreciably with the gradient run. However, as shown in the previous publication (1) the absorption of benzoylated NFA cerebrosides is about 1.1 times that of benzoylated HFA cerebrosides with this procedure and the sensitivity at 280 nm is about 14-fold less than at 230 nm.

Detection at 230 nm requires the use of the variable-wavelength monitor and dioxane as the polar solvent. Ethyl acetate is not transparent at 230 nm and cannot be used. The absorption of benzoylated HFA and NFA cerebrosides at 230 nm is the same (1), Fig. 4. We have also successfully used a dioxane–hexane system with detection at 280 nm; however, the viscosity of dioxane (1.54 cP at 20°C) is about three times that of ethyl acetate (0.45 cP at 20°C) and thus requires higher pressure for delivery through the column. We have avoided the use of detection at 254 nm because the response of benzoylated NFA cerebrosides is about twice that of benzoylated HFA cerebrosides (1) at this wavelength.

Both the procedures described in this paper are also applicable to larger quantities of cerebrosides, more than 10 nmol, providing the benzoylation is performed with 0.5 ml of benzoyl chloride (1) and the rest of the procedure appropriately scaled up. The gradient elution method for the analysis of the cerebrosides provides better resolution from the interfering materials in the lipid extracts as compared to isocratic elution used in the previous procedure (1). The ratio (0.48) of NFA cerebrosides to HFA cerebrosides in the adult rat brain lipid extract obtained by the present method agreed well with the values of

0.49, 0.50, and 0.54 found by McCluer and Evans (1). Hoshi, Williams, and Kishimoto (5) reported a ratio of 0.64 for adult rat brains as determined by the gas–liquid chromatographic analysis of the liberated cerebrosides fatty acids. However, McCluer and Evans (1) have demonstrated that similar values are obtained by the two methods when the same samples are analyzed.

Amounts as low as 10 picomol of cerebrosides present in the injected sample could be detected and quantitated, with the hexane–dioxane system and air in the reference cell. The sensitivity could be further increased about fourfold if the solvent was pumped through the reference cell (Fig. 7 d). The latter procedure requires longer analysis time of about 20 min compared to 4 min. However, analysis time could be shortened if pressure-resistant cells are available on the spectromonitor, allowing faster flow rates through the reference cell.

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