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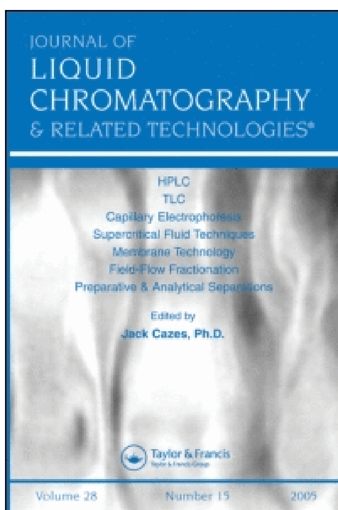

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
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Rapid Isolation of GM1, and GD1a from Bovine Brain Gangliosides by Propylamine and Q-Sepharose Column Chromatography

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To cite this Article Vajdi, Mehran(1992) 'Rapid Isolation of GM1, and GD1a from Bovine Brain Gangliosides by Propylamine and Q-Sepharose Column Chromatography', Journal of Liquid Chromatography & Related Technologies, 15: 11, 1869 — 1885

To link to this Article: DOI: 10.1080/10826079208020865

URL: http://dx.doi.org/10.1080/10826079208020865

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JOURNAL OF LIQUID CHROMATOGRAPHY, 15(11), 1869-1885 (1992)

RAPID ISOLATION OF GM1, AND GD1a FROM BOVINE BRAIN GANGLIOSIDES BY PROPYLAMINE AND Q-SEPHAROSE COLUMN CHROMATOGRAPHY

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Abstract

Procedures for rapid isolation of GM1 and GD1a from bovine brain gangliosides have been developed. The methods utilize conventional and over-load chromatography using propylamine and Q-Sepharose resins. Both procedures require low concentrations of eluting reagents without the use of salt for elution. Application of this method to small and large scale preparations have yielded GM1 and GD1a greater than 98% purity with recoveries better than 95%. The use of single solvent, elimination of salt throughout the entire chromatography, convenience and flexibility of the chromatographic parameters have made this technique suitable for rapid isolation of GM1, GD1a and the remaining bovine brain gangliosides.

Introduction

Gangliosides are a group of glycolipids which contain one or more sialic acid moieties. Presence of complex gangliosides in brain is suggestive of a functional role in the central nervous system. Since gangliosides are also found

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outside the nervous system, they are considered to be an important part of the surface membrane of most cells of animals. Immuno-properties of glycolipids and gangliosides are also important since they contribute to the immunological expression of cells and can be used in clarification and elucidation of immunobiological reactions taking place in certain disorders.

Considering the ubiquity of distribution of these plasma membrane constituents, future research of gangliosides is necessary and continues to be important. The potential role of gangliosides especially GM1 (1-3) as therapeutic agents have been recently investigated and considered valuable. Consequently, the need for development of a rapid method for isolation and purification of individual gangliosides has become evident and crucial.

In this paper, we present a comprehensive research involving the original observation of over-load chromatography and substantial recovery of GM1 and GD1a on propylamine followed by the evaluation of Q-Sepharose chromatography .

A comparative study of these Resins as well as effects of various parameters on separation are discussed. Advantages and disadvantages of each system are reviewed and presented.

Materials & Methods

Bovine brain gangliosides mixture (BBG) was prepared through the in house developed methodology. Propylamine resin was purchased from Analytichem International, CA. Q-Sepharose fast flow in-exchange resin was obtained from Pharmacia - LKB

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(Piscatway, NJ). HPTLC plates were purchased from E. Merck (Germany). Solvents and other chemicals were from Fischer Chemical Co., Medford, MA. TLC sample applicator and densitometer were products of Camage Scientific, Inc.

Due to the extensive volume of data, for simplicity and convenience purposes, a general description pertaining to each resin and method are given below. Specific parameters of each experiment are presented in Tables 1 and 2.

Propylamine (PA)

PA resin was washed with a sufficient volume of C/M(1:1) followed with a methanol wash. No conversion or treatment of the resin was carried out after the washing process.

Q-Sepharose (QS)

QS experiments were carried out in different size columns. The resin was washed with water and modified with the appropriate reagents. QS was mixed adequately with the reagent solution and allowed to partition for few minutes. The supernatant was discarded and the process was repeated several times. The resin was then transferred to the column, washed with water and followed with methanol before the final wash with the appropriate solvents.

BBG samples were solubilized in simple or multiple component solvents according to each experiment and slowly introduced to the column. Depending on the experiment, fractions with various volumes containing the eluting components were collected and evaporated for the TLC analysis.

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TABLE 1

COLUMN PARAMETERS

Propylamine

Fig #	Column O.D.(mm)	PA (g)	PA Form	Column Wash	Sample (mg)	Sample Solvent	Elution Solvent	F.R. (ml/min)
1	25	20	-	C/M	50	M	NH4OH/M	3
2	44	200	-	C/M	2000	M	NH4OH/M	20
3	44	200	-	C/M	1500	M	TEA/M	20

Q-Sepharose

Fig #	Column O.D. (mm)	OS (ml)	OS Form	Column Wash	Sample (mg)	Sample Solvent	Elution Solvent	F.R. (ml/min)
4	12	1.2	Na Acetate (0.4M)	C/M/W	50	C/M/W	DEA/M	7
5	12	1.2	"	"	"	"	AC/C:M:W	3
6	12	1.2	"	"	"	"	NH4OH/M	3
7	12	1.2	AC (0.5M)	M/W,M	75	M	AC/M	7
8	12	1.2	"	"	100	"	"	7
9	44	10	"	M	500	"	"	15
10	44	20	"	"	1000	"	"	15
11	44	40	"	"	2000	"	"	15
12	12	0.7	"	"	100	"	ABC/M	5
13	12	0.8	Na Acetate	"	"	"	"	5
14	12	0.8	FA/NH4OH (0.5M)	"	"	"	"	5
15	12	0.8	"	"	145	"	"	5

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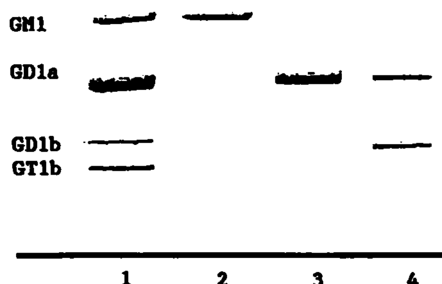
TABLE 2

TLC plate (Lane #)

Fractions Containing Eluting Reagents

Fig #	1	2	3	4	5	6	7	8	9	10	Eluting Reagent	Chromatography
1	Ref. BBG	.03M	.04M	.06M	-	-	-	-	-	-	NH4OH	Conventional
2	"	.03M	.03M	.05M	.05M	.05M	.05M	-	-	-	"	"
3	"	m	m	.005M	.005M	.005M	.005M	-	-	-	TEA	Over Load
4	"	m	m	m	.02M	.02M	-	-	-	-	DEA	Conventional
5	"	C/M/W	C/M/W	.01M	-	-	-	-	-	-	AC	"
6	-	m	m	.01M	.01M	-	-	-	-	-	NH4OH	"
7	BBG	m	.005M	.04M	-	-	-	-	-	-	AC	"
8	"	m	.005M	.005M	.02M	.02M	.1M	-	-	-	"	"
9	"	m	.002M	.002M	.02M	.1M	.1M	-	-	-	"	"
10	-	m	.002M	.002M	.002M	.04M	.04M	.1M	.1M	-	"	"
11	"	.002M	.002M	.002M	.005M	.005M	.05M	.05M	.1M	.1M	"	"
12	"	m	m	.004M	.04M	.15M	.15M	-	-	-	ABC	Over Load
13	"	m	m	.004M	.04M	.06M	-	-	-	-	"	"
14	BBG	m	m	.004M	.04M	.04M	.1M	.15M	-		"	"
15	*BBG	m	m	.004M	.04M	.1M	-	-	-	-	"	"

* BBG Mixture with lower content of disialogangliosides.



Chromatographic parameters:

TLC solvent system: Chloroform: Methanol: 0.25% aqueous CaC12 (55:45:10)

TLC Development Reagent: 50% sulfuric acid solution

Sample applicator: CAMAG Linomat IV

Detection System: CAMAG TLC Scanner II Densitometer

Figure 1. Conventional separation of BBG on propylamine column using ammonium hydroxide as the eluting reagent. Lane 1 (BBG Mixture), Lane 2 (GM1), Lane 3 (GD1a), Lane 4 (GD1a and GD1b)

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Results & Discussion

Propylamine:

Separation of individual BBG was achieved on PA column using appropriate eluting solvents to eliminate desaltification.

The separation was carried out on small and large scale preparations yielding appreciable quantities of pure GM1 and GD1a. The purity of GM1 and GD1a determined by densitometry was greater than 98 percent. As shown in Figures 1 and 2, the recovery of GM1 was practically complete with NH4OH as the eluting reagent. Depending on the nature of the Eluting

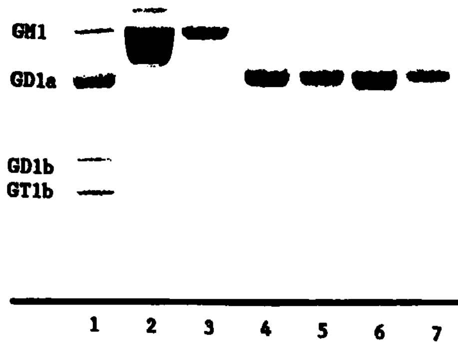

Figure 2. Separation of BBG on conventional semi-prep column of propylamine with ammonium hydroxide as the eluting reagent. Lane 1 (BBG Mixture), Lanes 2 and 3 (GM1), Lanes 4-7, (GD1a)

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solvent, separation behavior of gangliosides on PA may resemble that of the affinity or weak anion exchange chromatography.

In terms of polarity, PA is stronger than silica and has been used for separation of polar nature. Consequently, it was possible to elute gangliosides from the column with low concentrations of a more polar reagent such as TEA. Over-load chromatography (OLC) of BBG using triethylamine (TEA) as the eluting reagent is shown in Figure 3. Lanes 2 and 3 present GM1 eluted with the void volume. Similar results were also obtained using low concentrations of DEA and PA (0.005M) in Methanol as the eluting solvents.

Q-Sepharose:

A great portion of studies were carried out on Q-Sepharose due to its high capacity and efficiency. It was noted that

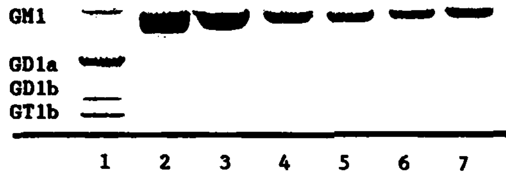

Figure 3. Overload chromatography of BBG on Semi-prep column of propylamine with triethylamine in methanol. Lane 1 (BBG mixture), Lanes 2-7 (GM1)

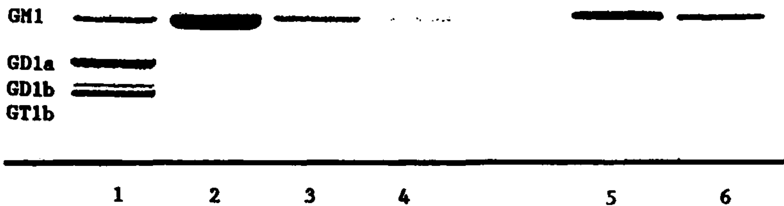

Figure 4. BBG Elution on Q-Sepharose with diethylamine ir methanol. Lane 1 (BBG Mixture), Lanes 2-6 (GM1)

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multisolvent compositions such as C/M/W eluted gangliosides from the column more rapidly than a single eluent; a phenomenon which is not necessarily desirable for OLC. This behavior was also observed when C/M/W was used for the column wash, sample preparation and eluting solvent. In contrast to methanol, with C/M/W, greater quantities of GM1 was found in the first fraction (Figure 4, Lane 2). A more drastic and undesirable effect of C/M/W, as shown in Figure 5, resulted in the elution of GD1a in GM1 fraction. With methanol, however, as seen in Figure 6, GD1a was not present in any of the fractions.

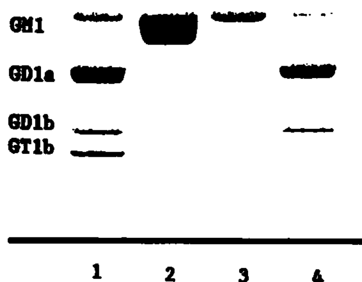

Figure 5. Separation of BBG on Q-Sepharose. Lane 1 (BBG Mixture), Lane 2 and 3 (GM1), Lane 4 (GM1, GD1a, GD1b)

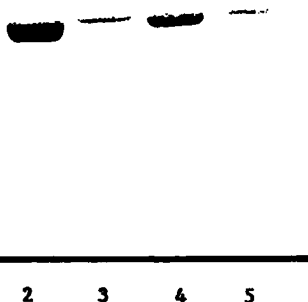

Figure 6. Elution of BBG on Q-Sepharose with ammonium hydroxide as the eluting reagent. Lanes 2-5 (GM1)

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The effect of sample load is shown in Figures 7 and 8. Increases in the sample concentration yielded greater quantities of GM1 in the first eluting fraction (Lane 2, Figures 7 and 8). As the sample concentration increased, over load chromatography was approached as appreciable quantity of GM1 was eluted with the sample solvent (void volume). At a certain sample concentration, however, over saturation occurs and other gangliosides may elute with the void volume.

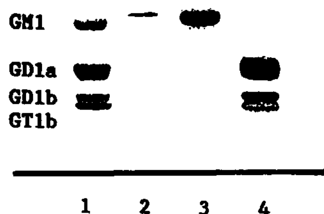

Figure 7. Elution of BBG on Q-Sepharose with Ammonium carbonate. Lane 1 (BBG Mixture), Lanes 2 and 3 (GM1), Lane 4 (GD1a, GD1b, GT1b)

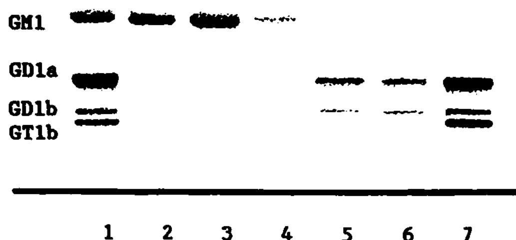

Figure 8. Separation of BBG on Q-Sepharose Column. Lane 1 (BBG Mixture), Lanes 2-4 (GM1), Lanes 5 and 6 (GD1a, GD1b), Lane 7 (GD1a, GD1b, GT1b)

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A short coming of overload chromatography is the requirement of a fine critical balance involving sample concentration, resin quantity and reproducibility, dimensions of the column and flow rate; parameters that need to be carefully considered and evaluated before the actual run.

A definite disadvantage associated with this chromatography is the crucial requirement for the high purity of the sample (BBG). Due to the nature of over load chromatography, any impurities associated with the sample will also elute with the first fraction (GM1). Figures 9 and 10

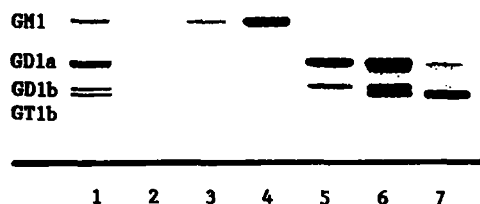

Figure 9. Separation of BBG on Q-Sepharose Column. Lane 1 (BBG Mixture), Lane 2 ((methanol fraction), Lanes 2 and 4 (GM1), Lane 5 (GD1a and GD1b), Lane 6 (GD1a, GD1b, GT1b), Lane 7 (GD1a, GT1b)

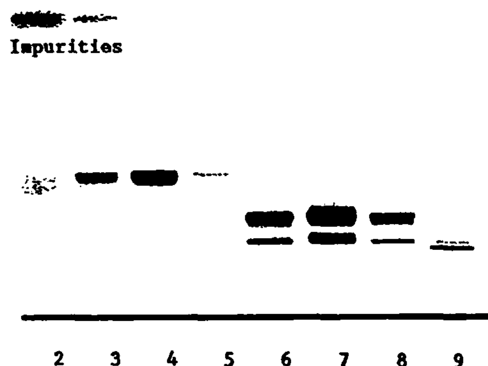

Figure 10. Separation of crude BBG on Q-Sepharose. Lane 2 (impurities), Lane 3 (impurities and GM1), Lanes 4 and 5 (GM1), Lanes 6-8 (GD1a and GD1b), Lane 9 (GT1b)

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show large scale conventional column chromatography of BBG on Q-Sepharose. As shown in lane 2 of Figure 9, no impurities were found in the void volume for a sample with a sufficient purity. On the other hand, impurities of BBG are seen in lanes 2 and 3 of Figure 10. A similar phenomenon would occur in the over load chromatography with impurities accumulating in the GM1 fraction eluting with the void volume.

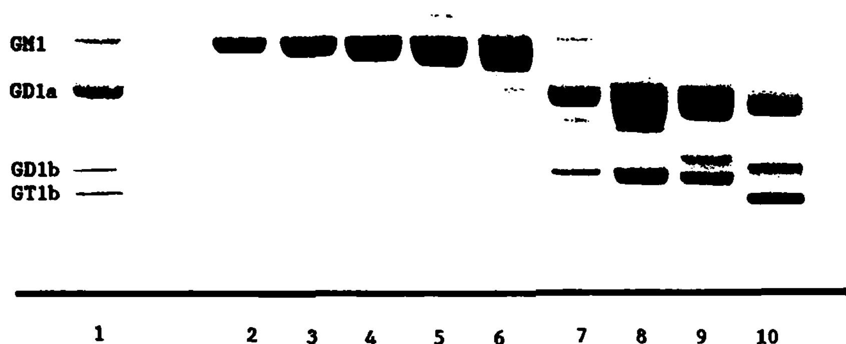

Figure 11 Separation of BBG on Semi-prep column of Q-Sepharose. Lane 1 (BBG Mixture), Lanes 2-6 (GM1), Lanes 7-9 (GD1a, GD1b), Lane 10 (GD1a, GD1b, GT1b)

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Large scale isolation of GM1 from BBG was performed on Q-Sepharose using methanol during the entire chromatography (Figure 11). As shown, using the conventional chromatography, at 2g sample load, clean fractions (2-6) were obtained at the flow rate of 15ml/min. The purity of sample with this method is not a critical factor or requirement as impurities elute in the first fraction with the sufficient wash.

Overload chromatography on Q-Sepharose was also carried out using Ammonium Bicarbonate (ABC) as the eluting solvent. Ammonium Carbonate (AC) conversion of the Q-Sepharose was found to be more efficient yielding better results than the Na acetate form. For the same amount of sample, less quantity of resin was needed for the AC than the Na acetate (Table 1). In addition, with the AC form, cleaner fractions of GM1 were obtained as seen in Figure 12, lanes 2 and 3 compared to the Na acetate form shown in lanes 2 and 3 of Figure 13.

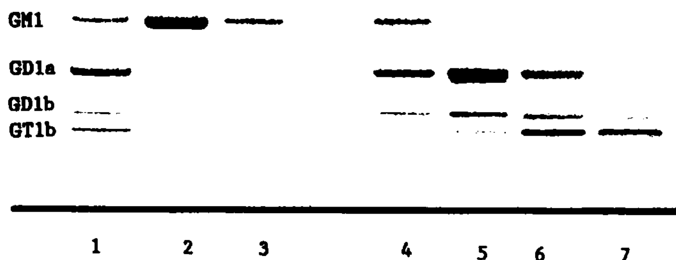

Figure 12 Overload chromatography of BBG on Q-Sepharose. Lane 1 (BBG Mixture), Lanes 2 and 3 (GM1), Lane 4 (GM1, GD1a, GD1b), Lanes 5 and 6 (GD1a, GD1b, GT1b), Lane 7 (GT1b)

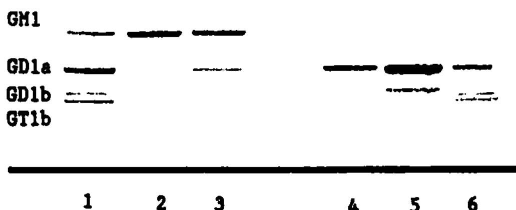

Figure 13 Separation of BBG by overload chromatography on Q-Sepharose. Lane 1 (BBG Mixture), Lane 2 (GM1), Lane 3 (GM1 and GD1a), Lane 4 (GD1a), Lane 5 (GD1a, GD1b), Lane 6 (GD1a, GD1b, GT1b)

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The mechanism of over load chromatography is based on the competitive environment existing between the active sites of the individual gangliosides (Mono, di and tri sialio) and those of the resin. Consequently, the number of sialic groups as well as the quantity of individual gangliosides determine the behavior of separation during the over load chromatography. Increase in the number of sialic acids reduces the loading capacity of the resin. Figure 14 shows typical BBG mixture with the normal ratio of disialo to

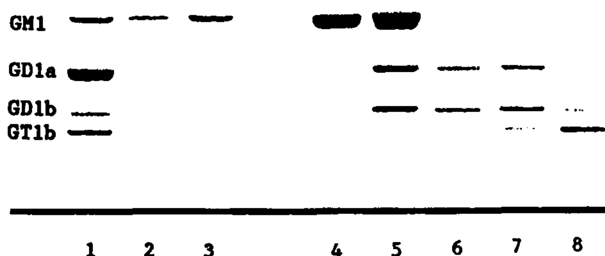

Figure 14. Elution of BBG on Q-Sepharose. Lane 1 (BBG Mixture) Lanes 2 and 3 (GM1), Lane 4 (GM1), Lane 5 (GM1, GD1a GD1b), Lane 6 (GD1a, GD1b), Lane 7 (GD1a, GD1b GT1b), Lane 8 (GD1b, GT1b)

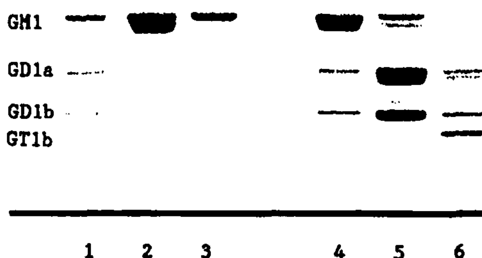

Figure 15 Elution of BBG with lower content of disial ganglioside on Q-Sepharose column. Lane 1 (BB Mixture), Lanes 2 and 3 (GM1), Lanes 4 and 5 (GM1 GD1a, GD1b), Lane 6 (GD1a, GD1b, GT1b)

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monosialo gangliosides (lane 1); the quantity of sample load for 0.8 ml of Q-Sepharose in this experiment was 100mg. However, with the decrease in disialo gangliosides, as shown in the BBG mixture (lane 1 of Figure 15), the separation behavior and loading capacity of the column was changed (Figure 15). As presented in Table 1, the loading capacity of the column was increased to 145mg for the same amount of

resin. This indicates that there is an inverse relationship between the loading capacity of the resin and the total number of active sites.

Advantages of the developed methods are:

1. Isolation of GM1 and GD1a in pure form from the propylamine resin during the same chromatographic run.
2. Purity of the starting material (BBG) is not critical and does not effect the quality of GM1 isolated. Therefore, less requirement and fewer steps are needed in the preparation of BBG mixture.
3. Only one simple solvent system is used throughout the entire chromatography.
4. Concentration of the eluting reagent needed to isolate GM1 and GD1a are quite low.
5. Elimination of desaltification for any of the gangliosides collected throughout the chromatography.
6. Reproductibility of the system is guaranteed for any size preparation without concerns for many of the chromatographic parameters.

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Conclusion

Isolation of GM1 and GD1a with high purity and appreciable quantities has been achieved using propylamine and Q-Sepharose column chromatography. The developed methods incorporate conventional and over-load column chromatography of BBG. The first recovery of pure GM1 and GD1a in sufficient quantities was made by the over-load chromatography of

propylamine. Subsequent, application of this technique to Q-Sepharose yielded only pure GM1. However, Q-Sepharose was found to be more efficient and suitable for large scale production. In addition these methods have been further simplified by using eluting solvents which do not require desaltification.

The solvent systems were further simplified by using a simple eluent (methanol) throughout the chromatography. Among all the variations and conditions of parameters studied, the QS/AC form in combination with AC or ABC/methanol as the eluting solvents at very low concentrations (.002 - .005M) yielded satisfactory results for the conventional and overload chromatography purposes.

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Acknowledgement:

This work was supported by Angio-Medical Corporation.
The author thanks Dr. O. Koul for his assistance in the collection of fractions during the propylamine column chromatography.

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