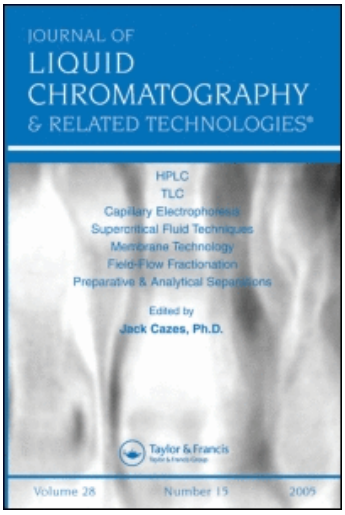

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Development and Validation of an HPLC Method Involving Solid-Phase Extraction for the Analysis of Hydrophobic Drugs in the Presence of Polyamidoamine (PAMAM) Dendrimers

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Development and Validation of an HPLC Method Involving Solid-Phase Extraction for the Analysis of Hydrophobic Drugs in the Presence of Polyamidoamine (PAMAM) Dendrimers

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Abstract: Two rapid and accurate isocratic high performance liquid chromatographic (HPLC) methods of analysis for nifedipine and furosemide in the presence of polyamidoamine (PAMAM) dendrimers is described. Solid-phase extraction (SPE) using C_{18} extraction cartridges and methanol as the eluent, was used to extract the drugs from the dendrimer and dendrimer plasma solutions. For both drugs, chromatographic separation was performed with a 5 μm, 250 × 4 mm, octadecyl silane (C_{18}) column. In the first method, nifedipine was analyzed using methanol:water (2:1 v/v) as the mobile phase with a flow rate of 0.7 mL/min. The injection volume was 20 μL with detection at 254 nm and a retention time of 11 minutes. In the second method, furosemide was analyzed using water:acetonitrile:acetic acid (60:40:1 v/v) as the mobile phase with a flow rate of 1.0 mL/min, injection volume of 20 μL, and detection at 272 nm. The retention time for furosemide was 8.5 minutes.

Keywords: Solid-phase extraction, PAMAM dendrimers, HPLC, Nifedipine, Furosemide

INTRODUCTION

Recently, biocompatible polymers such as dendrimers that form supramolecular assemblies with drugs, have received much interest as drug

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carriers.[1] Dendrimers are synthetic, highly branched, mono-disperse macromolecules with a well defined globular structure.[2,3] A typical dendrimer consists of three basic components: (a) a central core from which the polymeric branches emanate; (b) repeat units, the nature of which determines the microenvironment of the interior and in turn the solubilization ability of the dendrimer; and (c) the terminal groups, the nature and number of these groups are mainly responsible for the behavior of dendrimers in solution.[2]

The family of dendrimers most investigated in drug delivery is the polyamidoamine (PAMAM) dendrimers.[4] PAMAM dendrimers consists of ethylenediamine cores, either with methyl esters as the surface functional groups (half generation, anionic dendrimers) or primary amines as the surface functional groups (full generation, cationic dendrimers) (Figure 1). PAMAM dendrimers are biocompatible, non-immunogenic, water-soluble, and the terminal amine functional groups can be modified for binding various guest molecules.[5,6] In addition, the internal cavities of dendrimers can host metals or guest molecules because of the unique functional architecture, which contains tertiary amines and amide linkages.[5–7] Because of these unique properties, PAMAM dendrimers have been widely investigated as solubilization and complexation agents for hydrophobic guest molecules, carriers for the delivery of DNA and oligonucleotides, and targeted delivery systems for carrying drugs via the gastrointestinal tract.[4–11]

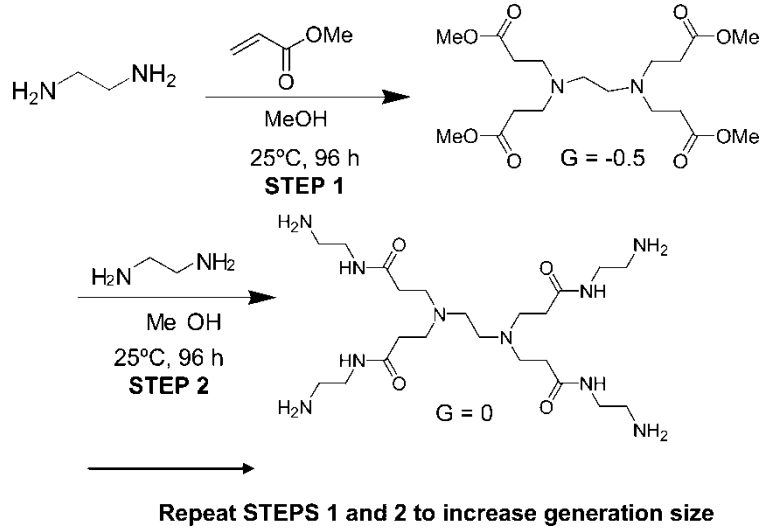

Figure 1. General synthesis scheme for polyamidoamine (PAMAM) dendrimers using a modified version of Tomalia's divergent growth approach involving exhaustive Michael addition and amidation repeated alternatively.[2]

Despite their increased application in drug delivery, there has not been a fully validated analytical method developed for the quantitative determination of drugs in the presence of dendrimers. Application of reverse phase HPLC without sample preparation, for the quantitative determination of two poorly water soluble, hydrophobic drugs, nifedipine[4,12] and furosemide[13] in solubilization studies using dendrimers as the solubilizing agents, showed that such methods were not sufficiently specific and sensitive, some times requiring laborious liquid-liquid extraction techniques. In addition, due to the high viscosity of dendrimer solutions, it caused problems with automatic injection from sample vials and reduced the life of columns.

The objective of this investigation was to develop an HPLC analysis method employing solid-phase extraction for determining the two hydrophobic drugs of nifedipine and furosemide, in the presence of anionic or cationic PAMAM dendrimers in the presence or absence of plasma.

EXPERIMENTAL

Reagents and Materials

Nifedipine (98.0–102.0%, $C_{17}H_{18}N_2O_6$ on dried basis) and furosemide (98.0–101.0%, $C_{12}H_{11}ClN_2O_5S$ on dried basis) were purchased from Spectrum Chemicals (Gardena, CA, USA). The polyamidoamine (PAMAM) dendrimers were either synthesized using a modified version of Tomalia's divergent growth approach involving exhaustive Michael addition and amidation repeated alternatively, as shown in Figure 1, or bought from Sigma-Aldrich (St. Louis, MO, USA).[2] Both cationic polyamidoamine dendrimers with primary amino surface groups and anionic polyamidoamine dendrimers with carboxylate surface groups were used in this study. HPLC grade methanol was from Spectrum Chemical Company (Gardena, CA, USA). Distilled and deionized water was used throughout the study. The C_{18} and Oasis HLB® cartridges used for the solid-phase extraction studies were from Millipore (Milford, MA, USA). C_{18} cartridges has a strongly hydrophobic silica-based bonded phase that is used to adsorb analytes of even weak hydrophobicity from aqueous solutions. Typical applications include drugs and their metabolites in serum, plasma, or urine. The columns used in this study had a nominal pore size of 125 Å, mean particle size of 55–105 μm, and bonding chemistry represented by monofunctional silane with a $Si(CH_3)_2C_{18}H_{37}$ surface functionality.

The Oasis HLB® cartridges used, offers an exceptionally clean, highly reproducible, patented copolymer, synthesized with a unique composition that is hydrophilic-lipophilic-balanced for both strong reversed-phase retention and water-wettability that is compatible with eluents from pH 1 to 14. It is used to adsorb both polar and non-polar compounds, simultaneously

from aqueous media, and is an ideal starting point for developing a new reversed-phase SPE method. The columns used in this study had a nominal pore size of 80 Å, particle size of 30 μm, and the surface functionality was an m-divinylbenzene and n-vinylpyrrolidone copolymer.

Chromatographic Conditions

Nifedipine and furosemide were analyzed using a high performance liquid chromatograph (AS 1000 auto sampler and P2000 pump, Thermo Separation Products, Waltham, MA) equipped with a multiple wavelength UV detector (UV 3000 detector), set at a wavelength of detection $\lambda_{max} = 254$ nm for nifedipine and $\lambda_{max} = 272$ nm for furosemide. The λ_{max} values were determined by measuring the ultraviolet spectra with a Multispec-1510 spectrophotometer (Shimadzu, Japan). For both drugs, chromatographic separation was performed using a C_{18} column (Econosil, 5 μm particles, 250 × 4.6 mm, Alltech, Deerfield II). The mobile phase for nifedipine consisted of methanol:water (2:1 v/v) filtered through a 0.45 μm membrane filter (Gelman Sciences Inc., Ann Arbor, MI, US), and degassed in an ultrasonic bath for 15 min before use. The flow rate was 0.7 mL/min, injection volume 20 μL, and all analyses were conducted at ambient temperature. The mobile phase for furosemide was water:acetonitrile:acetic acid (60:40:1 v/v), flow rate 1.0 mL/min, and injection volume 20 μL. The solutions were protected from light to prevent photo degradation of nifedipine and furosemide.

Preparation of Standard Solutions

Solutions of stock reference standards were prepared daily. Nifedipine (100 μg/mL) was dissolved in methanol:water (2:1 v/v) and furosemide (100 μg/mL) was dissolved in 0.1 M sodium hydroxide. These solutions were further diluted with the mobile phase to prepare calibration standards containing 0.5, 1, 5, 10, 25, 40, and 50 μg/mL. Standard solutions containing the dendrimers (0.01 M) and plasma were also prepared. Plasma standards were prepared by adding known amounts of stock solution to pooled rat plasma samples. These standard solutions were used to create calibration curves. Quantitation was based on linear regression analysis of analyte peak area versus analyte concentration in μg/mL.

Solid-Phase Extraction Procedure

In order to investigate the recovery efficiency using the solid-phase extraction cartridges, the SPE procedure optimization was performed by testing several

wash and elution solvents. Optimum recovery for the SPE cartridges tested (C_{18}, 100 mg, and Oasis HLB®, 225 mg) was achieved when the columns were conditioned with methanol (6 × 1 mL), followed by flushing with water (6 × 1 mL). One mL of standard was then loaded onto the column and allowed to pass through the cartridge. The cartridges were then washed with three 1 mL aliquots of 5% v/v methanol. After discarding the eluent, the analytes were eluted with 1 mL 100% methanol into clean HPLC injection vials. The elutes were evaporated to dryness under nitrogen flow, the residues reconstituted with 1 mL of the mobile phase, vortex mixed, and analyzed.

Assay Validation

The HPLC method used in this study was evaluated for precision, accuracy, selectivity, linearity, ruggedness, and system suitability.[14,15] Samples were quantified using peak areas of the analytes nifedipine and furosemide. Calibration plots for the analytes over a range of 0.5–50 μg/mL were prepared by diluting stock solutions with mobile phase, mobile phase containing 0.1 M PAMAM dendrimers, or mobile phase containing dendrimers and rat plasma. Calibration standards at each concentration were extracted and analyzed. Calibration curves were constructed using the observed analyte peak area versus nominal concentration of the analytes. Least squares linear regression analysis of the data gave slope, intercept, and correlation coefficient data.

The limits of detection (LOD) for each analyte were demonstrated by analysis of standard spiked samples with decreasing concentrations. LOD was based on the standard deviation (SD) of the response and the slope (S) of the calibration curve, at levels approaching the LOD according to equation (1).[15]

$$\mathrm{LOD} = 3.3\left(\frac{\mathrm{SD}}{\mathrm{S}}\right) \tag{1}$$

The standard deviation of the response was based on the standard deviation of the y-intercepts of corresponding regression lines. Similarly, the limit of quantitation (LOQ) was calculated using equation (2).[15]

$$\mathrm{LOQ} = 10\left(\frac{\mathrm{SD}}{\mathrm{S}}\right) \tag{2}$$

Since both LOD and LOQ can be affected by the chromatographic conditions, the type and age of the column used were taken into account when reporting these values. The absolute recoveries of the analytes after SPE were assessed at two concentrations. At each level, three samples were

extracted and analyzed in triplicate. The recovery (R) for each analyte, at each concentration, was computed using equation (3):

$$R = \frac{\text{Peak Area of Extract}}{\text{Mean Peak Area of Direct Injection}} \times 100 \quad (3)$$

The recovery of analytes from dendrimer samples was determined with C_{18} and Oasis HLB cartridges to determine the effect of sorbent on the recovery of the analytes. Also, extraction was performed using methanol and acetone as elutes to study the effect of solvent on recovery.

The method accuracy (% error) was obtained by comparing the concentrations from calibration curves to concentrations added. Precision was calculated as percent relative standard deviation (% RSD). The intra-day accuracy and precision of the assay were determined by assaying three quality control samples at low (2 μg/mL, n = 5), medium (10 μg/mL, n = 5), and high (40 μg/mL, n = 5) concentrations of the analytes, in three analytical runs within the same day. The inter-day accuracy and precision samples were analyzed on three different days. The concentrations of the quality control samples represented the entire range of the calibration curves. The 15 measurements were subjected to analysis of variance (ANOVA) to estimate the within run and between run precision.

RESULTS AND DISCUSSION

This study reports the analysis of two hydrophobic, poorly water-soluble drugs, nifedipine and furosemide in the presence of PAMAM dendrimers (Figure 1). The molecular structures of the drugs are shown in Figure 2. Because of their molecular architecture producing globular shapes, internal cavities, and multifunctional surfaces, dendrimers show some significantly improved physical and chemical properties when compared to traditional linear polymers. For example, water soluble PAMAM dendrimers are

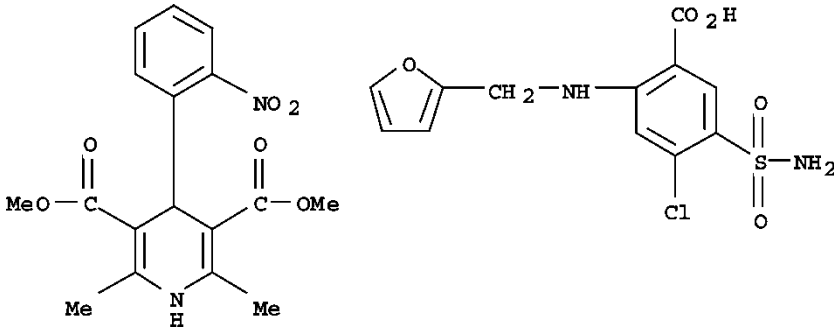

Figure 2. The chemical structures of nifedipine (left) and furosemide (right).

capable of binding and solubilizing small hydrophobic drug molecules.[4–6] However, analysis of drug-dendrimer complexes are complicated by the UV-absorbance in the range from 200–280 nm of lower generation ($G < 4$) dendrimers as shown in Figure 3. This absorption interfered with the analysis of nifedipine with $\lambda_{max} = 235$ nm and furosemide with $\lambda_{max} = 272$ nm. The dendrimers also increased the viscosity of sample solutions, preventing repeatable injection during automated HPLC analysis and clogging injection ports and columns.

To overcome these problems, two isocratic HPLC methods of analysis employing SPE for sample cleanup were developed for the drugs. Initial studies involved testing two SPE extraction cartridges, C_{18} and Oasis HLB® with two eluents, acetone and methanol. Recovery results listed in Table 1 show that optimum cleanup was obtained with the C_{18} cartridges when methanol was used as the eluent combined with water for washing. Using the C_{18} cartridges and the elution procedure employing methanol, both nifedipine and furosemide were successfully separated and quantitated in the presence of dendrimers. Figures 4 and 5 shows typical chromatograms of nifedipine and furosemide in the presence of the dendrimers before and after solid-phase extraction. Results shown are for the G-0 dendrimer, since this dendrimer interfered the most with the UV-analysis of the two drugs as shown in Figure 3. Similarly, furosemide was separated and quantitated from a mixture of

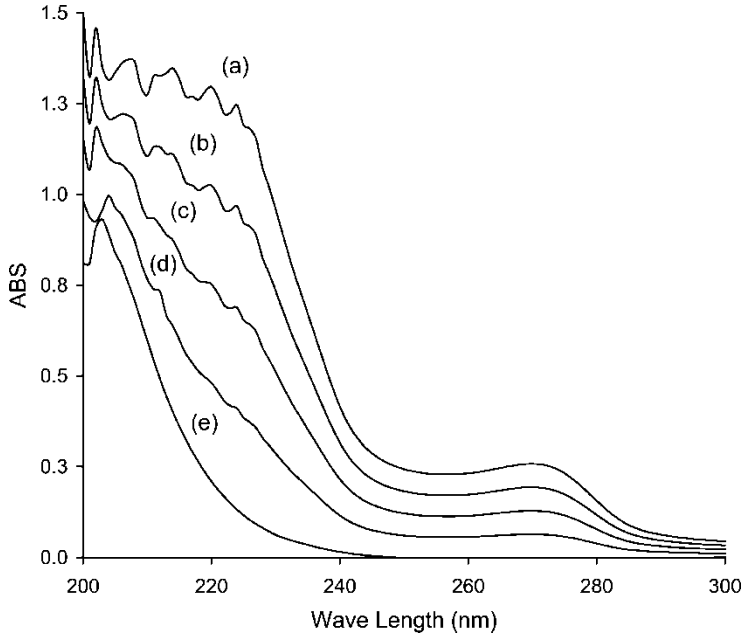

Figure 3. UV absorbance spectra of 0.01 M solutions of PAMAM dendrimers (a) G-0, (b) G-0.5, (c) G-1, (d) G-2, and (e) G-5.

Table 1. Nifedipine and furosemide recovery after SPE with different extraction cartridges using methanol as the eluting solvent

Analyte	SPE cartridge	Added (μg/mL)	Found (μg/mL)	Recovery (%)
Nifedipine	C_{18}	0.25	0.23	92
	Oasis HLB	0.25	0.20	80
Furosemide	C_{18}	0.25	0.24	96
	Oasis HLB	0.25	0.19	76

PAMAM G-3 dendrimer and plasma, and the chromatograms before and after SPE are shown in Figure 6. Hence, solid-phase extraction of the drugs followed by HPLC analysis successfully separated the drug molecules from the dendrimers, and for furosemide from a dendrimer in rat plasma.

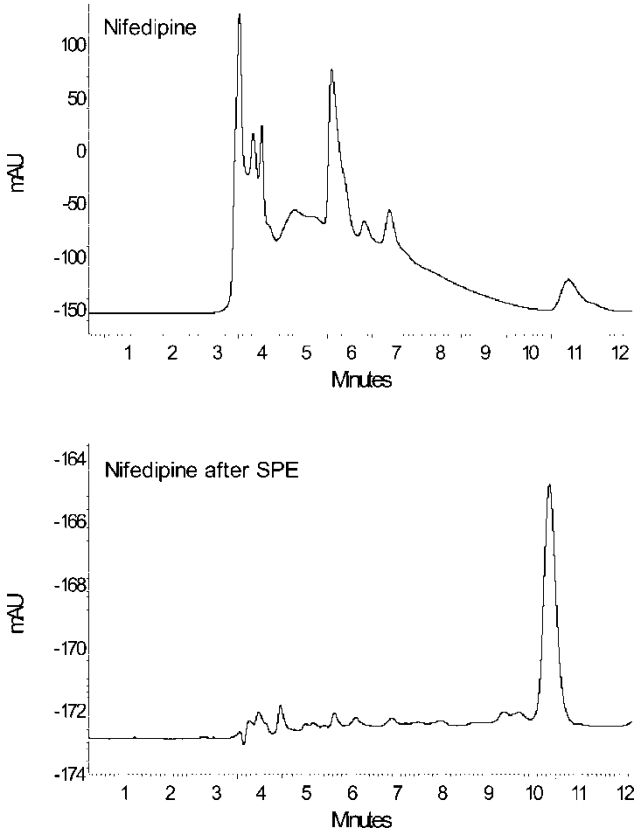

Figure 4. Chromatograms of a 1 μg/mL nifedipine solution containing G-0 PAMAM dendrimer before (top) and after clean-up with a C_{18} SPE cartridge (bottom).

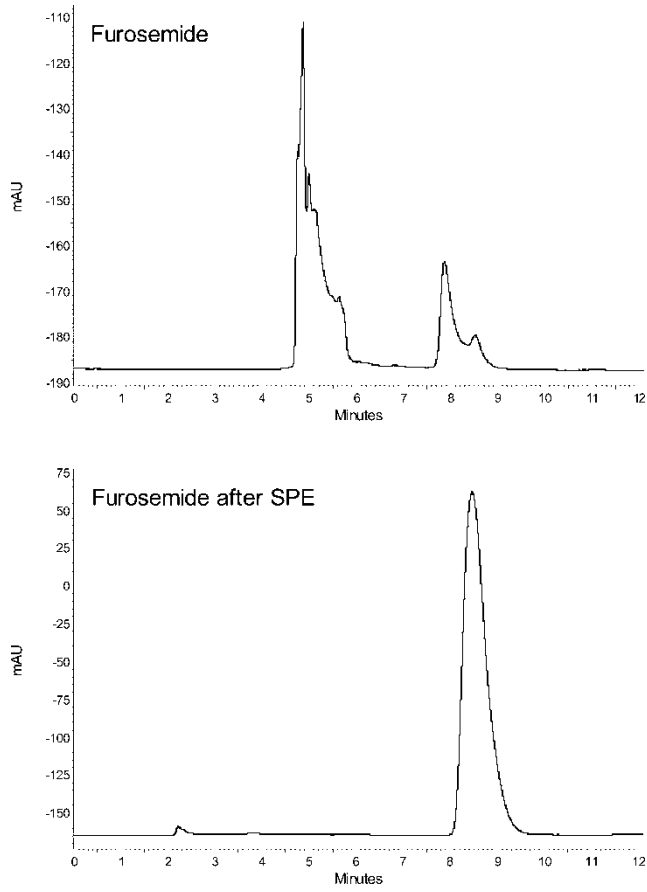

Figure 5. Chromatograms of a 5 μg/mL furosemide solution containing G-0 PAMAM dendrimer before (top) and after clean-up with a C_{18} SPE cartridge (bottom).

Analytical figures of merit for the two HPLC methods after SPE are listed in Table 2. After SPE with the C_{18} cartridges, nifedipine and furosemide were well separated from the dendrimers under the HPLC conditions applied as shown in Figures 4 and 5. No interferences were observed. Calibration curves generated after SPE showed good linearity in the range 0.5–50 μg/mL for nifedipine and furosemide alone, and in the range 1-25 μg/mL in the presence of 0.01M concentrations of the dendrimers. Calibration data are shown in Table 3. The correlation coefficients of calibration curves were higher than 0.99, as determined by least square analysis. The test for lack of fit ($\alpha = 0.05$) indicated that the linear model was appropriate for establishing the relationship between drug concentration and peak area.

The LOD and LOQ for the drugs alone, and in the presence of dendrimers, are listed in Table 4. These results represent values obtained after SPE with the

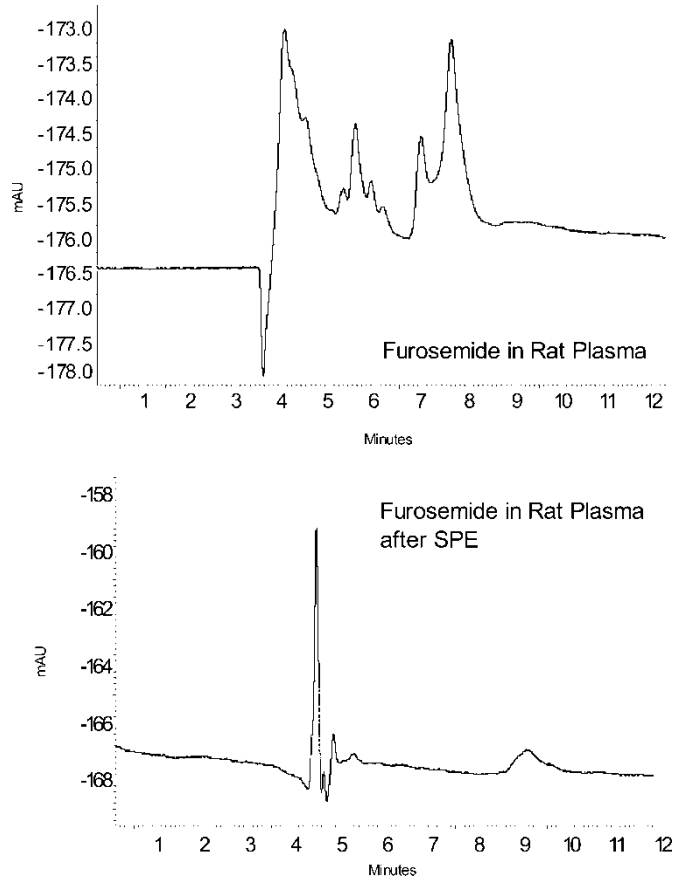

Figure 6. Chromatograms of plasma samples spiked with 0.3 μg/mL furosemide in combination with PAMAM dendrimer G-3 before (top) and after clean-up with a C_{18} SPE cartridge (bottom).

Table 2. Analytical figures of merit for nifedipine and furosemide when combined with PAMAM dendrimers before and after SPE with C_{18} cartridges

Analyte	SPE	Retention time (min)	Capacity factor (k′)	Tailing factor (T)
Nifedipine	No	11.0	3.1	1.5
	Yes	10.5	3.0	1.1
Furosemide	No	8.5	2.2	—
	Yes	8.5	3.4	1.2
Furosemide/Plasma	No	—	—	—
	Yes	9	2.1	1.3

Table 3. Calibration data and linearity of nifedipine and furosemide for standards and dendrimers samples after SPE extraction with C_{18} cartridges

Analyte	Dendrimer	Calibration data
Nifedipine	—	$y = (104482 \pm 450)x + (30635 \pm 1758)$ $R = 0.999$
	G-0	$y = (108738 \pm 361)x + (15577 \pm 793)$ $R = 0.998$
	G-0.5	$y = (106411 \pm 836)x + (62070 \pm 5039)$ $R = 0.998$
Furosemide	—	$y = (170160 \pm 7045)x + (41054 \pm 4770)$ $R = 0.990$
	G-0	$y = (188091 \pm 6430)x + (59150 \pm 5617)$ $R = 0.999$
	G-0.5	$y = (177418 \pm 5684)x + (69436 \pm 7527)$ $R = 0.998$

C_{18} cartridges. For nifedipine, both the LOD and LOQ were smaller in the absence of the dendrimers, while for furosemide there was not a significant difference. Accuracy and precision data are shown in Table 5. The data represent both intra and inter-day analysis of the drugs. The method proved to be accurate (percent error for all calibration samples varied from 0.4 to 9% for nifedipine and 0.4 to 12% for furosemide) and precise (within-run precision ranged from 0.5 to 4% for nifedipine and 0.5 to 5% for furosemide; between-run precision ranged from 0.5 to 4% for nifedipine and 0.5 to 6% for furosemide). An acceptance criteria of within-run and between run % RSD $< 15\%$ and an accuracy between 85 and 115% were met in all cases.

The results of measuring recovery were also satisfactory. The mean absolute recoveries of nifedipine and furosemide in the presence of dendrimers, and the recovery of furosemide in the presence of dendrimer and plasma after SPE are shown in Table 6. In the presence of the dendrimers, recovery was lower at low concentrations of the drug (0.25 μg/mL; mean R = 92%) than higher concentrations (40 μg/mL; mean R = 97%). In the

Table 4. Range of calibration curves, LOD and LOQ of nifedipine and furosemide in the absence and presence of PAMAM dendrimers after SPE extraction with C_{18} cartridges

Analyte	Dendrimer	Range of calibration curves (μg/mL)	LOD (ng/mL)	LOQ (ng/mL)
Nifedipine	—	0.5–50.0	55.5	168.3
	G-0	1.0–25.0	124.1	173.6
	G-0.5	1.0–25.0	111.3	185.8
Furosemide	—	0.5–50.0	92.5	280.3
	G-0	1.0–25.0	91.7	277.8
	G-0.5	1.0–25.0	98.5	298.6

Table 5. The intra- and inter-day precision (% RSD) and accuracy (% error) for the analysis of nifedipine and furosemide in the presence of dendrimers after SPE with C_{18} cartridges

		Intra-day ($n = 5$)			Inter-day ($n = 15$)		
Analyte	Added (μg/mL)	Found (μg/mL)	RSD (%)	Error (%)	Found (μg/mL)	RSD (%)	Error (%)
Nifedipine	2	1.92 ± 0.06	3.13	4.0	2.17 ± 0.08	3.69	8.5
	10	10.21 ± 0.28	2.54	2.1	10.29 ± 0.21	2.04	2.9
	40	39.86 ± 0.24	0.60	0.4	40.23 ± 0.18	0.45	0.6
Nifedipine with PAMAM G-0	2	2.02 ± 0.03	1.49	1.0	1.89 ± 0.07	3.70	5.5
	10	9.86 ± 0.31	3.14	1.4	9.76 ± 0.26	2.66	2.4
	40	39.67 ± 0.41	1.03	0.8	39.72 ± 0.42	1.06	0.7
Nifedipine with PAMAM G-0.5	2	1.95 ± 0.03	1.54	2.5	1.94 ± 0.07	3.61	3.0
	10	9.96 ± 0.41	4.12	0.4	9.84 ± 0.04	0.41	1.6
	40	39.84 ± 0.19	0.48	0.4	39.47 ± 0.53	1.34	1.3
Furosemide	2	1.98 ± 0.06	3.03	1.0	2.09 ± 0.08	3.83	4.5
	10	10.51 ± 0.28	2.66	5.1	11.19 ± 0.42	3.75	11.9
	40	42.86 ± 0.64	1.49	7.2	40.72 ± 0.21	0.52	1.8
Furosemide with PAMAM G-0	2	2.01 ± 0.07	3.22	5.5	1.79 ± 0.07	3.91	10.5
	10	9.96 ± 0.52	5.22	0.4	9.76 ± 0.26	2.66	2.4
	40	39.52 ± 0.72	1.82	1.2	39.72 ± 0.42	1.06	0.7
Furosemide with PAMAM G-0.5	2	1.94 ± 0.08	4.12	3.0	2.17 ± 0.10	4.61	8.5
	10	10.66 ± 0.46	4.32	6.6	10.57 ± 0.62	5.86	5.7
	40	39.48 ± 0.21	0.53	1.3	41.89 ± 0.23	0.55	4.7

presence of PAMAM G-3 dendrimer and plasma, at least 90% of furosemide was recovered after SPE with the C_{18} cartridges.

CONCLUSION

The determination of the hydrophobic drugs, nifedipine and furosemide, in the presence of dendrimers is important because these spherical polymeric molecules have enormous potential as drug delivery systems for poorly water soluble drugs. The SPE extraction method, utilizing C_{18} cartridges with methanol elution, developed in this study for the separation of the drugs from PAMAM dendrimers was sufficiently selective, sensitive, accurate, and reproducible. Analysis time was less than 12 minutes and sample extraction required between 20–30 minutes, which can be reduced when multiple samples are handled simultaneously using a vacuum elution manifold. The SPE procedure provided excellent cleanup, and recovery of the drug after SPE from dendrimer solutions with and without plasma was

Table 6. Nifedipine and furosemide recovery when combined with PAMAM dendrimers and after SPE with C_{18} cartridges

Analyte	Dendrimer	Added (μg/mL)	Found (μg/mL)	Recovery (%)
Nifedipine	—	10	10.2	102.0
		40	39.9	99.8
	G-0	0.25	0.23	92.0
		40	39.1	97.8
	G-0.5	0.25	0.22	88.2
		40	38.8	97.0
Furosemide	—	10	10.5	105.0
		40	40.7	101.7
	G-0	0.25	0.23	92.0
		40	39.5	98.8
	G-0.5	0.25	0.24	96.0
		40	37.7	94.3
Furosemide in rat Plasma	—	2	1.92	96.0
		10	9.79	97.9
	G-3	0.15	0.14	93.3
		15	13.9	92.7

higher than 90%. Thus, SPE combined with HPLC analysis is applicable for the removal of PAMAM dendrimers. This method should find application in the analysis of pharmaceutical products containing dendrimers, and pharmacokinetic studies of these products where the dendrimers might interfere with the analysis of the drugs.

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REFERENCES

1. Liu, M.J.; Frechet, J.M.J. Designing dendrimers for drug delivery. Pharm. Sci. Technol. Today. **1999**, *2*, 393–401.
2. Tomalia, D.A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. A new class of polymers: starbust-dendrimtic macromolecules. Polym. J. **1985**, *17*, 117–132.

3. Frechet, J.M.J. Functional polymers and dendrimers: reactivity, molecular architecture, and interfacial energy. Science **1994**, *263*, 1710–1715.
4. Patri, A.K.; Majoros, I.J.; Baker, J.R. Dendritic polymer macromolecular carriers for drug delivery. Cur. Opin. Chem. Biol. **2002**, *6*, 466–471.
5. Devarakonda, B.; Hill, R.A.; De Villiers, M.M. Effect of PAMAM dendrimer generation size and surface functional group on the aqueous solubility of nifedipine. Int. J. Pharm. **2004**, *183*, 133–140.
6. Wiwattanapatapee, R.; Jee, R.D.; Duncan, R. PAMAM dendrimers as potential oral drug delivery systems: dendrimer complexes with piroxicam. Proc. Int. Symp. Control. Rel. Bioact. Mater. **1999**, *26*, 241.
7. Kolhe, P.; Misra, E.; Kannan, R.M.; Kanna, S.; Lieh-Lai, M. Drug complexation, in vitro release and cellular entry of dendrimers and hyperbranched polymers. Int. J. Pharm. **2003**, *259*, 143–160.
8. Chauhan, A.S.; Sredevi, S.; Chalasani, K.B.; Jain, A.K.; Jian, S.K.; Jain, N.K.; Diwan, P.V. Dendrimer-mediated transdermal delivery: enhanced bioavailability of indomethacin. J. Control. Rel. **2003**, *90*, 335–343.
9. Kojima, C.; Kono, K.; Maryama, K.; Tagagishi, T. Synthesis of polyamidoamine dendrimers having Poly (ethylene) glycol grafts and their ability to encapsulate anticancer drugs. Bioconjugate. Chem. **2000**, *11*, 910–917.
10. Poxon, S.W.; Mitchell, P.M.; Liang, E.; Hughes, J.A. Dendrimer delivery of oligonucleotides. Drug Del. **1996**, *3*, 255–261.
11. Wiwattanapatapee, R.; Carreno-Gomez, B.; Malik, N.; Duncan, R. Anionic PAMAM dendrimers rapidly cross adult rat intestine in vitro: a potential oral delivery system? Pharm. Res. **2000**, *17*, 991–997.
12. Ali, S.L. Nifedipine. In *Analytical profiles of drug substances*; Flory, K., Ed.; Academic Press: New York, 1989; Vol. 18, 223–228.
13. Al-Obaid, A.M.; Al-Shammary, F.J.; Al-Rashood, K.A.M.; Mina, M.S. Furosemide. In *Analytical profiles of drug substances*; Flory, K., Ed.; Academic press: New York, 1989; Vol. 18, 153–193.
14. The United States Pharmacopeia XXIV, The United State Pharmacopeial Convention, Inc.: Rockville, MD, 2000.
15. Swartz, M.E.; Krull, I.S. *Analytical method development and validation*; Marcel Dekker, Inc.: New York, 1997; 53–71.

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