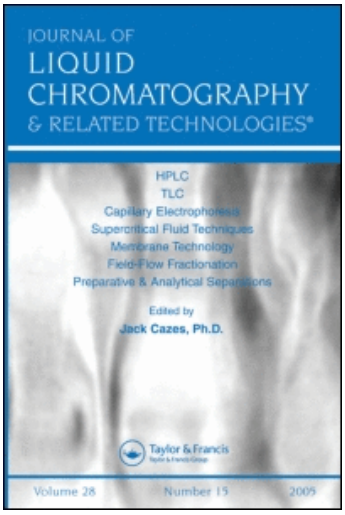

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Validation of an HPLC Method for Nifedipine and its Related Substances in Raw Materials

N. Beaulieu[a]; N. M. Curran[a]; S. J. Graham[a]; R. W. Sears[a]; E. G. Lovering[a]
[a] Bureau of Drug Research Health Protection Branch Tunney's Pasture Ottawa, Ontario, Canada

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VALIDATION OF AN HPLC METHOD FOR NIFEDIPINE AND ITS RELATED SUBSTANCES IN RAW MATERIALS

N. BEAULIEU**, N. M. CURRAN, S. J. GRAHAM,
R. W. SEARS, AND E. G. LOVERING
Bureau of Drug Research
Health Protection Branch
Tunney's Pasture
Ottawa, Ontario, K1A 0L2
Canada

ABSTRACT

The USP HPLC method for related compounds in nifedipine has been modified to improve resolution and sensitivity. The modified method uses a Novopak* C18 column with a mobile phase consisting of 48% methanol in water. This method has been validated for the determination of drug and related compounds in drug raw materials; it has been shown to resolve at least seven known related compounds from the drug, with quantitation limits ranging from 0.01 to 0.10%. Total impurities in 15 samples of drug raw material were between 0.02 and 0.85%. The drug content of these samples ranged between 97.9 and 102.0%.

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* Presented at the Pittsburgh Conference, New York, March 1990

** Correspondance

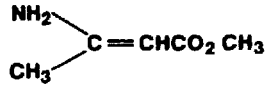
INTRODUCTION

Drug raw materials are increasingly manufactured at different sites throughout the world, frequently by different synthetic routes, chemicals and processes. There is an increased need for method validation to determine that related compounds due to different processes are detected and quantitated as necessary. In this report, modification and validation of the USP HPLC method for nifedipine raw materials is described.

Nifedipine is an important calcium ion influx inhibitor. The determination of nifedipine and some of its metabolites in body fluids by HPLC[1] and GLC[2] have been reported. No methods for determination of related compounds in drug raw material were found. Nifedipine and nifedipine capsules are official in the USP[3]. Both monographs set limits of 0.2% for each of the nitro- and nitroso- analogs of nifedipine, determined by an HPLC method which is also used for drug assay. The structures of nifedipine and a number of related compounds which were used to validate the methods are presented in Figure 1.

MATERIALS

Drug related compounds, obtained as follows, were used as received after confirmation of structure by NMR, IR and MS: I, II and III, Torcan, Toronto; II, Sigma, St Louis; IV, V and VII, LEK, Ljubljana, Yugoslavia; VII, ICFI, Milan. VI was synthesized using a method from the literature[4]. IV and VII are also available as USP Reference Standards. Methanol, HPLC



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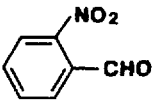

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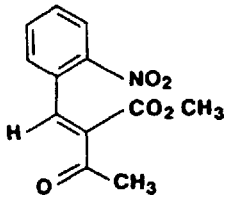

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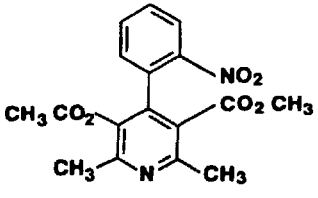

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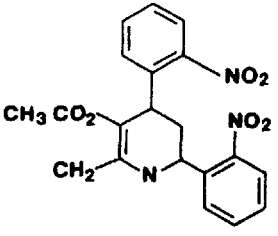

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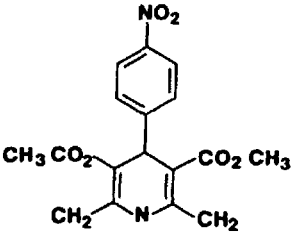

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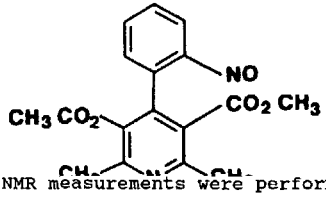

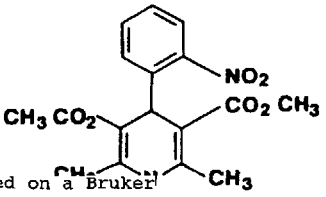

III

lid-state NMR measurements were performed on a Bruker
heinstetten, FRG) MSL 200 spectrometer with samples
200-300 mg in double-bearing rotors of zirconia.
gic angle spinning (MAS) was carried out at a
inning rate 4 KHz. ^{29}Si and ^{13}C cross-polarization
P) MAS NMR spectra were recorded with a pulse length
5 μs together with a contact time of 5 ms and a

Compounds: I Methyl 3-
nzaldehyde; III 2-
acid methyl ester; IV
Dimethyl 4-(2-nitrophenyl)-2,6-dimethylpyridine-3,5-
dicarboxylate (Nitrophenyl nifedipine);
V 2,4-di(o-nitrophenyl)-5-methoxycarbonyl-6-methyl-
1,2,3,4-tetrahydropyridine; VI Dimethyl 1,4-dihydro-
2,6-dimethyl-4-(p-nitrophenyl) pyridine-3,5-
dicarboxylate (para-nifedipine); VII Dimethyl 4-(o-
nitrosophenyl)-2,6-dimethylpyridine-3,5 dicarboxylate
(Nitrosophenyl nifedipine); VIII Dimethyl 1,4-dihydro-
4-(o-nitrophenyl)-2,6-dimethylpyridine-3,5
dicarboxylate (Nifedipine)

grade, was from J.T. Baker Co., Phillipsburg, NJ. Water was deionised using a Sybron/Barnstead system.

The HPLC system (Varian Vista 5560) was fitted with a 10-μL loop (Rheodyne injector #7126), a UV detector (Varian UV-200) set at 235 nm, an autosampler (Varian #8085) and a data station (Varian Vista 402). The poly(octadecylsilane) column (Waters NovoPak* C18, 4 μm, 3.9 X 150 mm, (No T70342-R24) was used at ambient temperature with a mobile phase flow rate of 1.0 mL/min.

METHOD

Mobile phase: Transfer 480 mL methanol to a 1-L volumetric flask, dilute to volume with water, filter and degas.

Note: All nifedipine solutions must be rigorously protected from ultraviolet and visible light. Prepare solutions immediately before use.

Preparation of solutions

Related compounds standard solution - 0.005 mg/mL of each of nifedipine, IV and VII in methanol.
Related compounds test solution - 2.5 mg/mL nifedipine in methanol. If necessary, sonicate to obtain complete dissolution.
Assay standard solution - 0.25 mg/mL nifedipine in methanol, accurately weighed.
Assay test solution - 0.25 mg/mL nifedipine in methanol, accurately weighed.

System Suitability

Inject six aliquots of the Related compounds standard solution and measure the peak area responses. The resolution between nifedipine and VII, and between VII and IV is greater than 2, the efficiency of the column calculated on the drug peak is more than 15,000 plates per meter and the tailing factor is less than 2, all calculated according to USP XXII procedures (5). The coefficient of variation of the peak area is no more than 5.0% for each of the three peaks. Five injections of the Assay standard solution give a coefficient of variation of less than 1.0%.

Procedure

Related compounds - Inject separately 10 μL of the Related compounds standard and test solutions into the chromatograph and run for 60 minutes. Calculate the percentages of IV and VII from their respective standards; calculate the percentage of each of the other individual impurities in the drug raw material, using the nifedipine standard, from: $100(A_i/A_r)(C_r/C_u)$, where A_i is the peak area response due to each impurity, A_r is the peak area response of nifedipine, IV or VII in the standard solution, C_r is the concentration of nifedipine, IV or VII in the Related compounds standard solution and C_u is the concentration of nifedipine in the test solution.

Drug Assay - Inject separately 10 μL of the Assay standard and test solutions into the chromatograph and run for 15 minutes. Calculate the percentage of nifedipine from $100(A_u/A_s)(C_s/C_u)$ where A_u and A_s are the

peak area responses due to Assay test and standard solutions, respectively, and C_s and C_u are the concentrations of nifedipine in the Assay standard and test solutions, respectively.

RESULTS AND DISCUSSION

Evaluation of the USP Method

The USP HPLC procedure for related compounds in nifedipine calls for a mobile phase of 25% acetonitrile, 25% methanol and 50% water with a test solution concentration of 0.3 mg/mL drug.In our hands this method did not resolve V from the drug and was insufficiently sensitive to quantitate IV and VII at the USP limits of 0.2%. V could not be resolved from the drug by minor variation of the USP mobile phase. Nifedipine is extremely sensitive to ultraviolet and visible light. It was found that sufficient degradation of the drug to IV and VII could occur during analysis to exceed the USP limit of 0.2% for these compounds, unless the most stringent precautions were taken to exclude light.

Chromatography - Chromatogram showing resolution of six related compounds from the drug are given in Figure 2 and 3. The precision of the system was determined by making 6 replicate injections of a solution containing 0.0103 mg/mL nifedipine (103 ng on column), 0.0116 mg/mL IV (116 ng on column), 0.0106 mg/mL VII (106 ng on column) and 0.0085 mg/mL V (85 ng on column). The relative standard deviations of the peak area responses were 3.7%, 2.1%, 0.4% and 4.7%, respectively and 0.7% for a solution of 0.25 mg/mL nifedipine.

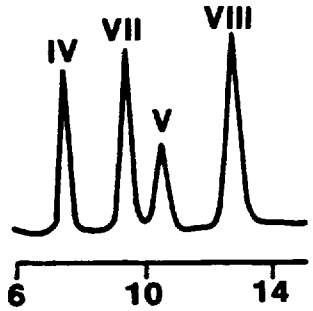

Figure 2. Chromatograms of nifedipine and related compounds. The concentration in methanol were IV - 9.31 μg/mL; VII - 9.8 μg/mL; V - 10.56 μg/mL; nifedipine VIII - 9.2 μg/mL.

Linearity and sensitivity - The eight related compounds gave linear responses over the ranges listed in Table 1.

Solution stability - Nifedipine degrades rapidly under UV or visible light. However, a solution of the drug is stable for up to 5 hours under non-UV fluorescent light (gold light, Sylvania) before evidence of degradation appears in the chromatograms.

Ruggedness of the method - The method described in this paper was developed on a NovoPak* C18 column (Waters No T70342-R24). A second column of the same type (No 90821-D05) gave slightly longer retention times and better resolution. A NovoPak* C18 cartridge (100 X 8 mm) and a flow rate of 2 mL/min also met the system suitability requirements. An increase in the methanol content of the mobile phase from 48 to 53% led to decreased in retention times and co-elution of V with the drug. A decrease in methanol content to 45% resulted in increased retention times and tailing.

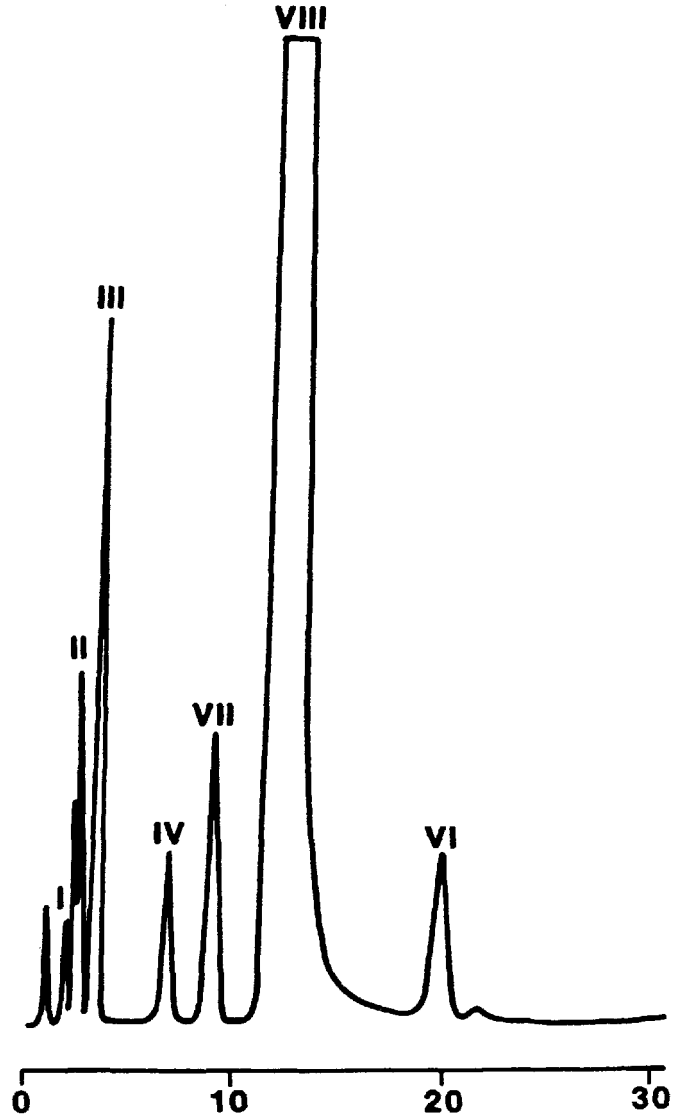

Figure 3. Chromatograms of nifedipine and related compounds. The concentrations in methanol were: nifedipine VIII -2.563 mg/mL; I - 6.99 μg/mL; II - 6.07 μg/mL; III - 6.99 μg/mL; IV - 5.15 μg/mL; VI - 5.63 μg/mL; VII - 5.89 μg/mL.

TABLE 1
Linearity Data for Nifedipine and Related compounds

Compound	RRT[1]	Slope[2]	Response[3]	Intercept[4]	R^2	Linearity Range(%)
I	0.14	93	.10	-2354	.9973	0.10-1.0
II	0.18	810	.87	-17	.9998	0.01-1.0
III	0.25	881	.94	2623	.9978	0.01-1.0
IV	0.51	482	.52	57	.9998	0.05-1.0
V	0.7	349	.37	264	.9997	0.05-1.0
VI	1.7	595	.64	1227	.9999	0.01-1.0
VII	0.8	573	.61	395	.9999	0.05-1.0
VIII	1.0	933	1.0	-1350	.9996	0.05-1.0

[1] Retention time relative to nifedipine at 12.2 min.
[2] The units of slope are area counts per ng.
[3] Response relative to nifedipine.
[4] The unit of the intercept is area counts.

TABLE 2
Related Compound Levels in Nifedipine Raw Materials (%)

Code	Relative Retention Time[1]									
	0.21	0.27	0.52	0.71	1.40	1.75	1.79	1.82	1.98	Total
A(4)[2]	.01	.03		.04						0.08
B		.02								0.02
C	.03			.03						0.06
D(5)	.02	.01		.06				.02		0.11
E(4)		.08		.07		.05	.04		.02	0.26
F		.04	.02	.04		.04				0.14
G(4)		.10			.17					0.27
H(1)	.32	.08		.45						0.85
I		.10		.05						0.15
J		.13		.06						0.19
K		.07		.12						0.19
L		.01		.06						0.07
M(3)		.10		.07	.01	.02				0.20
N(3)		.08		.07	.01	.02				0.18
O(3)		.11		.06	.01	.02			.02	0.22

[1] Retention time relative to nifedipine at 11 min. The relative retention times of II, III, IV and VII are 0.21, 0.27, 0.51 and 0.71, respectively.
[2] The number of determinations is given in parentheses, if other than two.

TABLE 3
Nifedipine Raw Material Assays by HPLC (%)

Code	Analyst No 1[1]		Analyst No 2[2]		Mean (C.V.)
A	99.8	99.6	101.0	101.9	100.6 (1.1)
B	101.8	101.1			101.4
C	100.6	99.5			100.0
D	102.0	100.1			101.0
E	99.1	98.6	99.4	101.8	99.7 (1.5)
F	100.1	99.6			99.6
G	99.3	100.4	97.5	98.7	99.0 (1.2)
H				98.6	
I	99.9	99.9			99.9
J	97.9	99.2			98.6
K	100.3	101.3			100.8
L	99.2	100.1			99.6
Samples analysed July, 1989					
M	100.2	100.2	100.0		100.1
N	99.2	99.9	99.3		99.5
O	100.2	100.2	100.4		100.3

[1] Assays done August and November, 1987.
[2] Assays done March, 1988.

Related compound levels in 15 samples of nifedipine raw material are given in Table 2 and the assay values of these samples are given in Table 3.

The method was evaluated by a second analyst who obtained results similar to those of the first analyst. The method is reliable and reproducible for the quantitation of related compounds and assay of Nifedipine in raw materials.

ACKNOWLEDGEMENTS

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