Journal of Chromatography, 244 (1982) 391–395
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,895

Note

Separation and quantitation of polynitrate esters in pharmaceutical preparations by reversed-phase high-performance liquid chromatography

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(Received March 9th, 1982)

Polynitrate esters are widely used as coronary vasodilators. Various methods are available for determining these compounds, such as colorimetry[1,2], polarography[3–5], thin-layer chromatography (TLC)[6] and gas–liquid chromatography (GLC)[7–9]. Colorimetric and polarographic methods lack specificity. TLC is limited in precision and sensitivity as far as quantitative measurements are concerned. GLC, which is satisfactory from the latter point of view, suffers from the disadvantage that thermal decomposition can occur when performing the separation because of the high temperatures at which the columns are maintained[8]. Recently, high-performance liquid chromatography (HPLC) has been applied successfully to the quantitation of nitroglycerine in dosage forms[10]. HPLC should allow the separation and quantitation of mixtures of nitrate esters, avoiding the interferences that affect both colorimetric and GLC determinations. The former are due to other nitrate esters or nitrate ions, which may occur in very small amounts as drug impurities or as metabolites in biological fluids; the latter are due to the possible products of thermal decomposition.

In this paper we describe a method for the rapid separation and identification of some polynitrate esters of therapeutic interest using reversed-phase HPLC and report the application of the HPLC assay to the determination of the compounds of interest in various pharmaceutical preparations.

EXPERIMENTAL

Reagents

Isosorbide dinitrate (I), pentaerythritol trinitrate (II), nitroglycerine (III), pentaerythritol tetranitrate (IV), erythritol tetranitrate (V) and diazepam (internal standard, i.s.) were obtained from Istituto Superiore di Sanità (Rome, Italy). The compounds were ascertained to give single peaks in HPLC on an ODS column. Standard solutions of compounds I–V and i.s. were prepared in methanol. All solutions were kept refrigerated at 0°C in the dark. Methanol (Fluka, Buchs, Switzerland)

0021-9673/82/0000–0000/$02.75

and acetonitrile (Merck, Darmstadt, G.F.R.) were of special HPLC grade. The water used was deionized and doubly distilled in glass. All solvents were filtered through a Millipore filter, pore size 0.45 μm, and vacuum degassed by sonication before use.

Apparatus

The instruments used were a Varian (Zug, Switzerland) Model 5000 liquid chromatograph equipped with a variable-wavelength UV detector (Varichrom UV 50), a Valco AH60 injection valve and a Varian Model 9176 recorder. The analytical column was 250 × 4.5 mm I.D. ODS-coated silica (Whatman Partisil PXS 10/25). Peak areas were determined by electronic integration (Varian Model CDS-111).

HPLC conditions

The chromatographic conditions were as shown in Table I.

TABLE I

CHROMATOGRAPHIC CONDITIONS

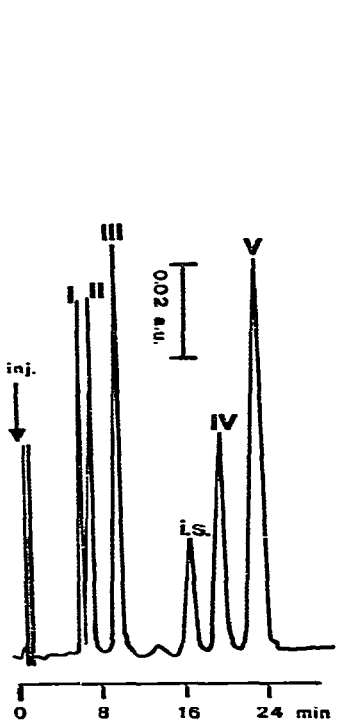
Conditions	*Mobile phase A: water–acetonitrile (40:60)*	*Mobile phase B: water–methanol (50:50)*
Flow-rate (ml/min)	2	1
Pressure (p.s.i.)	1120	1400
Column temperature (°C)	25	25
Injection volume (μl)	10	10
Detector wavelength (nm)	215	215
Detector sensitivity (a.u.f.s.)	0.16	0.16

Preparation of sample

Amounts of various pharmaceutical preparations equivalent to 5 mg of I, III, IV and V were accurately weighed and extracted for 30 min with 25 ml of methanol containing diazepam as internal standard (1.5 mg/ml). The solutions were first filtered through paper, then through a 0.45-μm filter (FP; Millipore, Bedford, MA, U.S.A.). Aliquots of 10 μl were submitted to HPLC.

RESULTS AND DISCUSSION

Figs. 1 and 2 show the separation of a mixture of I–V when mobile phases A and B were used, respectively. Under the chromatographic conditions used all retention times were reproducible. As can be seen, a good resolution of the polynitrate esters examined could be achieved on an ODS column with either mobile phase A or B. When the HPLC method was used to determine drug levels in dosage forms, mobile phase A was chosen to allow for a more quantitative determination. With mobile phase A, in fact, the separation and quantification of I–V was performed with the aid of the internal standard diazepam, whose peak occurred in a region of the



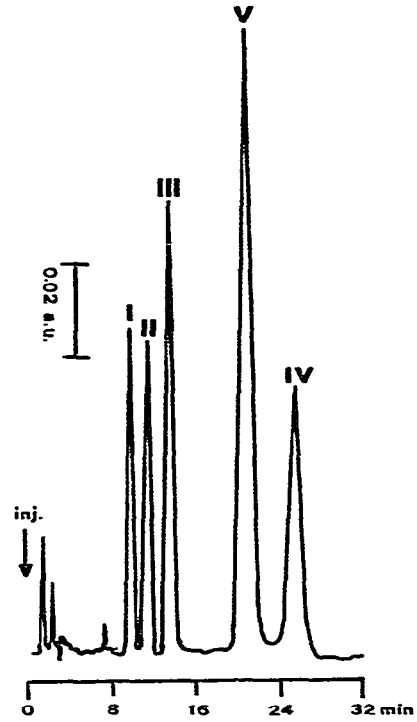


Fig. 1. Typical chromatogram of a standard mixture of polynitrate esters obtained using mobile phase A. Retention times: I, 6.18 min; II, 7.05 min; III, 9.72 min; i.s., 16.60 min; IV, 19.54 min; V, 23.00 min.

Fig. 2. Typical chromatogram of a standard mixture of polynitrate esters obtained using mobile phase B. Retention times: I, 10.40 min; II, 12.22 min; III, 14.06 min; V, 21.26 min; IV, 25.00 min.

chromatogram free from interferences. In contrast, diazepam eluted with compound V when mobile phase B was employed.

Table II reports the chromatographic properties of the polynitrate esters and diazepam when mobile phase A was used for separation. The calibration graphs are shown in Fig. 3; linearity was observed up to 3 μg. Good reproducibility was ob-

TABLE II

RESOLUTION FACTORS AND RELATIVE RESPONSES OF POLYNITRATE ESTERS

Resolution factor is defined as $2\Delta t/(w_2 + w_1)$, where Δt is the difference between the retention times of compounds 2 and 1 and w_2 and w_1 are peak widths.

Compound	*Resolution factor*	*Relative response*
I		0.112
II	3.48	0.137
III	7.74	0.168
i.s.	14.48	1.000
IV	5.11	0.175
V	4.94	0.206

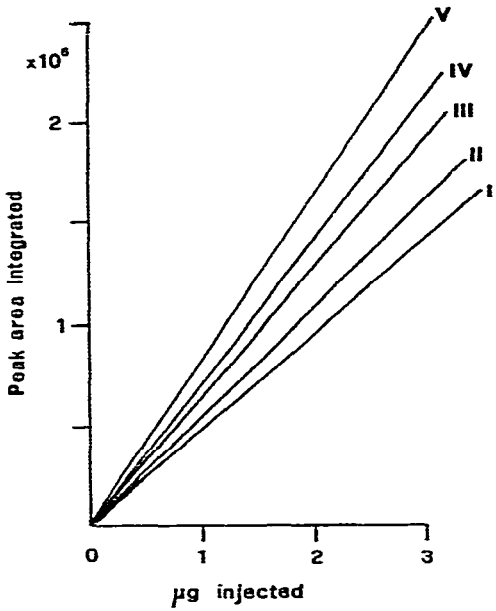


Fig. 3. Calibration graphs for polynitrate esters. Conditions as described under Experimental. Each point is the mean of five determinations.

TABLE III

PRECISION OF THE HPLC METHOD

Compound	*Amount injected (μg)*	*n*	*Coefficient of variation (%)*
I	2	10	1.41
II	2	10	1.44
III	2	10	1.28
IV	2	10	1.31
V	2	10	1.30

TABLE IV

HPLC ASSAY OF I, III, IV AND V IN VARIOUS DOSAGE FORMS

Each value is the mean of five determinations.

Compound	*Acutal weight (mg)*	*Recovery (%) (mean ± S.D.)*
I	5.0	101.2 ± 2.1
	10.0	100.8 ± 1.9
	40.0	98.9 ± 2.0
III	2.5	103.2 ± 1.9
	2.6	98.4 ± 2.0
	0.3	106.6 ± 1.8
IV	80.0	98.6 ± 1.9
V	10.0	99.3 ± 2.1

tained; the correlation coefficients of linear regression ranged from 0.988 to 0.995. The detection limit was approximately 50 ng for each compound injected on to the column. The detectable level was based on a response of twice the noise level. Using the internal standard procedure the precision of the HPLC method was very satisfactory. The results, expressed as coefficient of variation, are reported in Table III.

The applicability of the proposed technique for the determination of the compounds of interest in common dosage forms is demonstrated by the data in Table IV. As shown, the method is reliable and precise. In comparison with previously available methods, HPLC offers the advantage of allowing the simultaneous determination of polynitrate esters without affecting the specificity, reproducibility and accuracy.

REFERENCES

1 J. Tingstad, F. Gropper, L. Lacham and F. Shame, *J. Pharm. Sci.*, 61 (1972) 1985.
2 F. K. Bell, *J. Pharm. Sci.*, 53 (1964) 752.
3 W. M. Ayres and G. W. Leonard, *Anal. Chem.*, 31 (1959) 1485.
4 B. C. Flann, *J. Pharm. Sci.*, 58 (1969) 122.
5 B. Persson and L. Rosen, *Anal. Chim. Acta*, 123 (1981) 115.
6 K. C. Guven, T. Altinkurt, A. Araman and R. Durukan, *Eczacilik Bul.*, 20 (1978) 13.
7 I. W. F. Davidson, F. J. Dicarlo and E. I. Szabo, *J. Chromatogr.*, 57 (1971) 345.
8 D. G. Prue, R. N. Johnson and T. Kho, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 1341.
9 M.-T. Rosseel and M. G. Bogaert, *J. Chromatogr.*, 64 (1972) 364.
10 W. G. Crouthamel and B. Dorsch, *J. Pharm. Sci.*, 68 (1979) 237.