ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
17 (1998) 539–546

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Separation and estimation of seven vasodilators using packed column supercritical fluid chromatography

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Received 6 November 1996

Abstract

This paper reports a method for an isocratic separation and simultaneous estimation of seven vasodilators: isosorbide mononitrate (ISMN), isosorbide dinitrate (ISDN), cyclandelate, nimodipine, amlodipine, pentifylline and pentoxifylline using packed column supercritical fluid chromatography (SFC). An arbitrary choice of vasodilatory compounds with respect to their chemical structures was made to examine the viability of this technique for analysis of drugs and pharmaceuticals. Elution was performed on a RP-C_{18} column. SFC offers several degrees of freedom: temperature, pressure and modifier concentration to attain optimum resolution and sensitivity. The effects of these parameters on retention time have been studied using methanol modified carbon dioxide. The analytes were identified and measured by UV-detection. The chromatographic points of merit have been listed. Detection limits appear to be similar to those found in liquid chromatography. Modifier concentration does generally make major changes in retention and selectivity. A full scale validation for the seven vasodilators has been attempted and the statistical quality evaluated.

Keywords: Supercritical fluid chromatography; Isosorbide mono and dinitrate; Cylandelate; Amlodipine; Nimodipine; Pentifylline; Pentoxifylline; Packed columns; Separation; Estimation

1. Introduction

Interest in the applicability of packed column supercritical fluid chromatography (SFC) in pharmaceutical and drug analysis in the last few years has increased. Recent publications [1–11] have highlighted the ability of packed column SFC to separate basic drugs of the same 'family'. The method [12–16] has also been utilised for the determination of drugs in biological liquids like plasma and whole blood. However, little interest has been shown on the general applicability of the method to an arbitrary group of drugs which are basically of different chemical structures and polarities but are comparable in their pharmacological effects. Not much interest has been shown in the methods of quantification of drugs and pharmaceuticals using SFC. The group of drugs: isosorbide dinitrate (ISDN), cyclandelate, isosorbide mononitrate (ISMN), nimodipine, amlodipine,

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0731-7085/98/$19.00
PII S0731-7085(97)00187-8

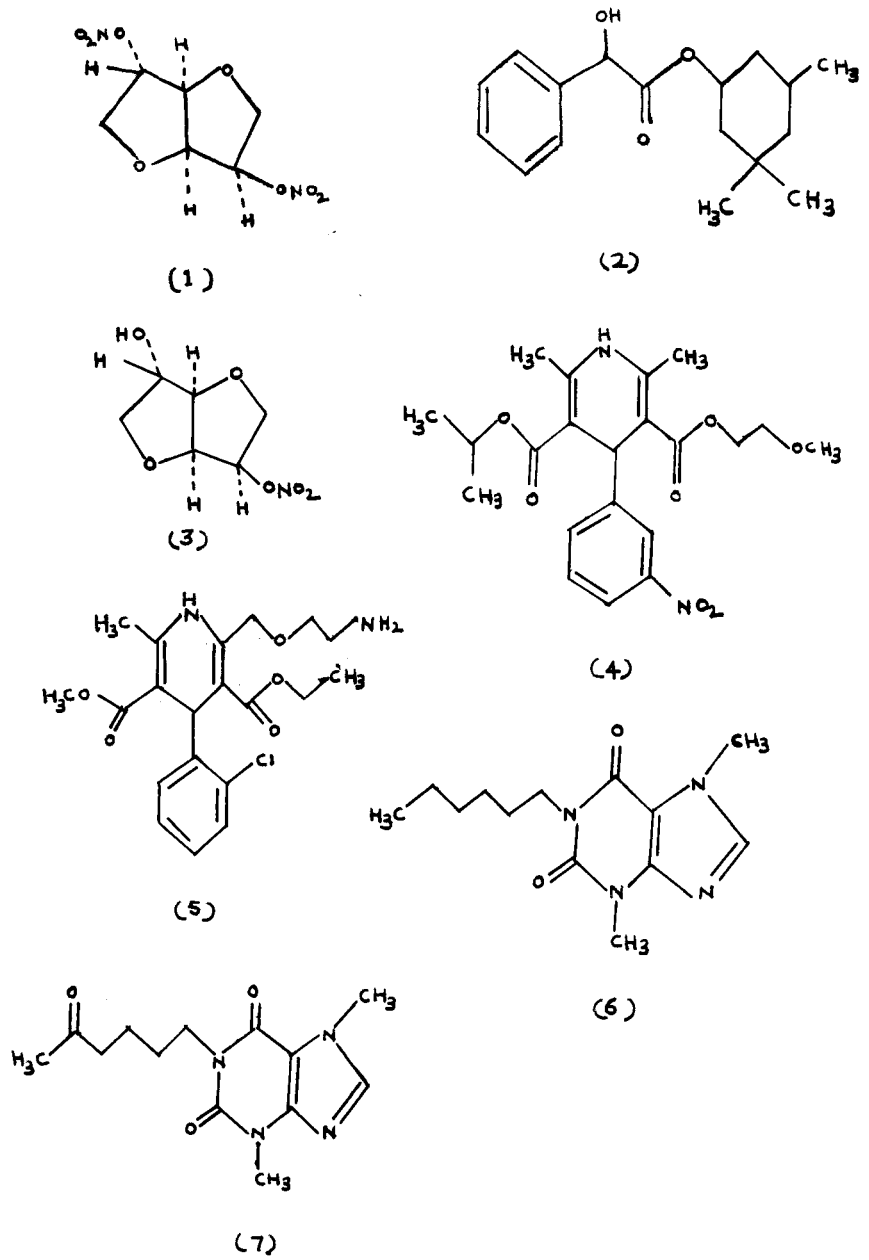

Fig. 1. Structures of vasodilators: (1) ISDN; (2) cyclandelate; (3) ISMN; (4) nimodipine; (5) amlodipine; (6) pentifylline; and (7) pentoxifylline.

pentifylline, and pentoxifylline have been classified as vasodilators [17,18]. With regards to chemical properties these drugs can be classed into three different chemical families: ISMN and dinitrate (glucitol nitrate); nimodipine and amlodipine (dihydropyridine); and pentifylline and pentoxifylline (dimethylxanthine), with cyclandelate as the odd one out. The structures of the

drugs are given in Fig. 1. This paper attempts an isocratic separation of the seven drugs using supercritical fluid carbon dioxide with added methanol and also their quantification. An exhaustive evaluation of the chromatographic performance data, and statistical examination of the quantification data is described.

2. Experimental

A JASCO supercritical chromatograph 900, configured for dynamic mixing, with two JASCO-980 pumps series was used. The chromatograph was equipped with a Rheodyne injector, Model-7125, with a 20 μl external loop and a JASCO ultraviolet spectrophotometric detector-UV-975, equipped with a 4 μl high pressure flow cell of 5 mm path length. The samples were separated on a JASCO-RP-C_{18} column, (250 × 4.00 mm) 10 μm. The temperature of the column was maintained at the desired point using JASCO-C0-965 series oven. The peaks were recorded and analyzed using Borwin chromatographic software.

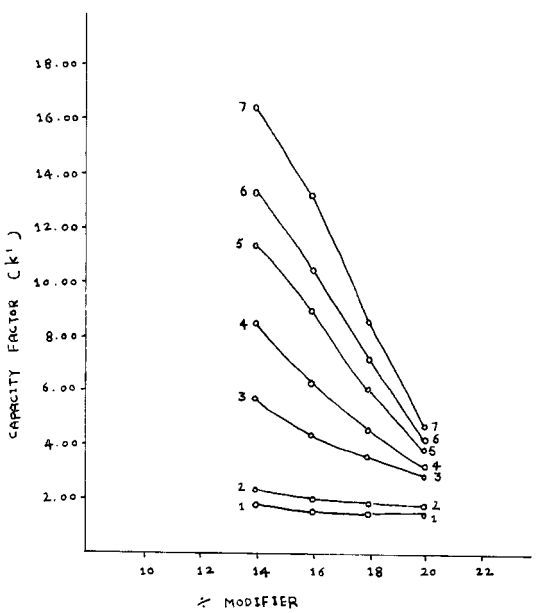

Fig. 2. Effect of modifier concentration on capacity factor. Conditions: 14–20% of methanol in carbon dioxide; 45°C; 10.13 MPa; and 20 μl injected. Detection was by UV analysis at 224 nm. The order was as follows: (1) ISDN; (2) cyclandelate; (3) ISMN (4); nimodipine; (5) amlodipine; (6) pentifylline; and (7) pentoxifylline.

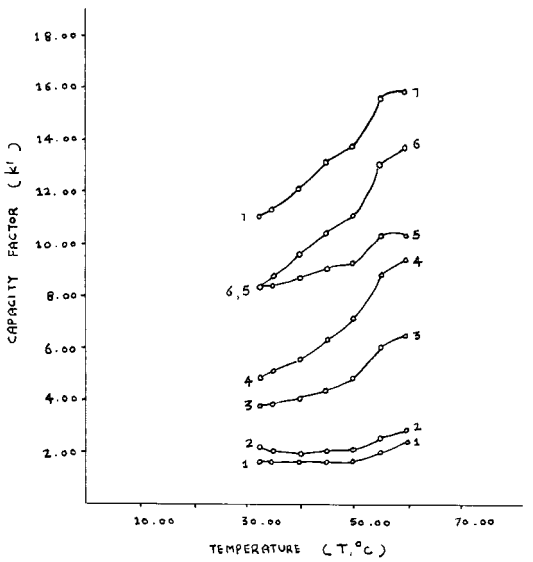

Fig. 3. Effect of temperature on capacity factor. Conditions: 0.25 ml min^{-1} of 16% methanol in carbon dioxide; 32.5–60°C temperature; 10.13 MPa; and 20 μl injected. Detection was by UV analysis at 224 nm. The order was same as in Fig. 2.

3. Reagents

Carbon dioxide used was 99.9% pure obtained from Bombay Carbon Dioxide, Mumbai, India. Methanol was B.D.H., A.R. grade. The drug samples were received in solid state from different U.K. companies. Separate stock solutions of the seven drugs were prepared by dissolving 100 mg of the material in 100 ml of methanol and diluting them in 10-fold stages to the desired concentration. Mixtures of the drug solutions were prepared by pipetting out appropriate quantities of individual stock solutions and then mixing them. Methanol was chosen as the modifier as it was found to be a good solvent for the analytes. In the absence of a T-P-mod. conc. programming system resort could only be made to trial and error methods to determine retention and selectivity. Near critical point parameters (Papp < 10.13 MPa

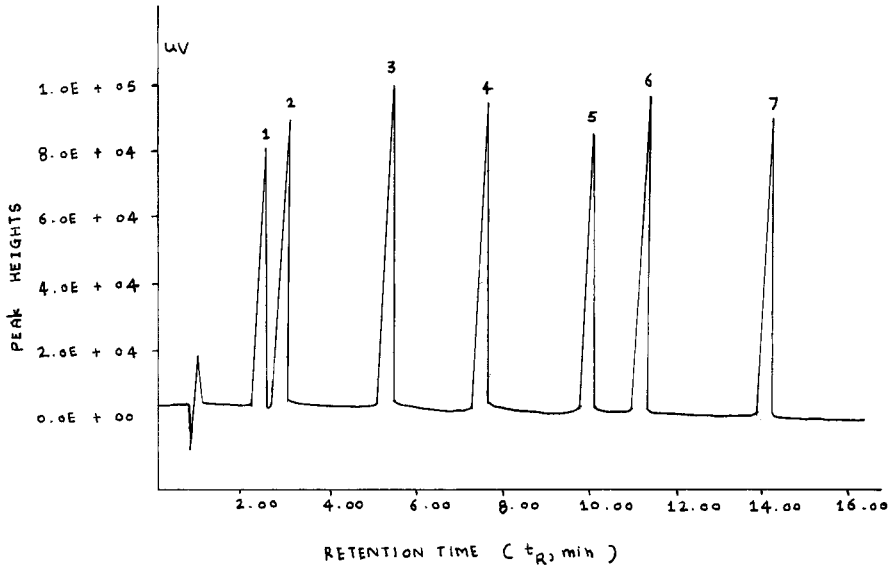

Fig. 4. Typical SFC separation of drugs eluted from a JASCO 250 × 4.0 mm; 10 μm; C_{18} column under steady state conditions. Conditions: 0.25 ml min^{-1} of 16% modifier (methanol) in CO_2 at 45°C and 10.13 MPa outlet pressure. Numbered solutes are indicated in the arbitrary mix and the number indicates retention order: (1) ISDM ~ 2.6 min; (2) cyclandelate ~ 3.0 min; (3) ISMN ~ 5.4 min; (4) nimodipine ~ 7.3 min; (5) amlodipine ~ 10.0 min; (6) pentifylline ~ 11.5 min; and (7) pentoxifylline ~ 14.2 min.

Table 1
Linear regression (least-squares fit) data for calibration curves

Drug	Conc. range (μg ml^{-1})	Slope $m \pm t_{CL,v}S_m = x \times 10^{-3}$	Intercept $b \pm t_{CL,v}S_b = x \times 10^{-3}$	r^2	$S_{y.x}$ $y \times 10^{-3}$
ISDN	1.25–54.00	1.40 ± 0.01	0.45 ± 0.02	0.999	0.22
Cyclandelate	1.00–54.00	1.62 ± 0.02	−0.22 ± 0.05	0.999	0.12
ISMN	1.00–54.00	1.77 ± 0.04	1.23 ± 0.89	0.999	0.69
Nimodipine	0.20–10.80	8.39 ± 0.07	0.63 ± 0.35	0.999	0.26
Amlodipine	0.50–27.00	2.79 ± 0.03	0.51 ± 0.36	0.999	0.27
Pentifylline	0.30–16.50	5.22 ± 0.06	0.50 ± 0.44	0.999	0.34
Pentoxifylline	0.50–28.00	2.96 ± 0.02	0.33 ± 0.25	0.999	0.19

and $T < 45$°C) were found to be more suitable because at higher pressures and temperatures the peak shapes degraded badly.

4. Part-A: results

4.1. Effect of modifier concentration on capacity factor

Modifier concentrations were changed from 14 to 20% of methanol (v/v) at an arbitrary pressure of 10.13 MPa and temperatures varying from 32.5 to 60°C with the flow rate of carbon dioxide at 1.3 ml min^{-1}. For these experiments, 20 μl of individual drug solutions containing 100 μg ml^{-1} of the analytes were injected. Detection was at 224 nm. Retention patterns at different temperatures at 10.13 MPa revealed that 45°C was the most suitable. Fig. 2 gives the relation between modifier concentrations and capacity factors at 45°C and

Table 2
Analytical figures of merit for the drugs

Drug	RRT (min)	Symmetry factor, T^a	Capacity factor, k'	No. of plates, N^b
ISDN	1.00	1.00	1.61	300
Cyclandelate	1.17	1.33	2.05	410
ISMN	2.05	1.33	4.36	539
Nimodipine	2.80	1.00	6.32	588
Amlodipine	3.84	1.33	9.02	813
Pentifylline	4.39	1.00	10.47	927
Pentoxifylline	5.44	1.33	13.20	1249

[a] Calculated at 5% peak height.
[b] Calculated as $N = 16\ (^tR/W)^2$.

10.13 MPa. As can be seen from the figure, capacity factors decrease with the modifier concentrations and almost merged at 20% concentration. At 14% ideal capacity factors are obtained, but the run time was nearly 20 min. In order to strike a balance between selectivity coefficients ($\alpha = k'_2/k'_1$ where k' is the capacity factor) and retention times a modifier concentration of 16% was chosen for the study. An evaluation of the selectivity coefficients for the peaks gave values of 1.27, 1.13, 1.45, 1.43, 1.16 and 1.26 for the different pairs of peaks.

Regarding capacity factors, the relationship between capacity factors and modifier concentrations was found to be non-linear. It has been observed [19–21] that with certain solute families retention is reduced by a factor of 2 on each doubling of the modifier concentration. In this case of the three family groups, it is found that this rule does not hold true; the elution being more rapid than anticipated. In addition, the understanding [9] that larger molecules and those with multiple nitrogen atoms are retained longer does not hold in this case. The strongest bases in this case are probably the longest retained compounds.

4.2. Effect of pressure on capacity factor

The range of pressures studied was 8.13–15.20 MPa. The effect of pressure was found to be minimal and followed the same pattern for all the compounds through the range studied. Favorable separation conditions are obtained near critical pressures. With regard to retention, pressures $<$ 9.13 MPa and $>$ 11.14 MPa were found to be unsuitable as the peaks of ISDN and cyclandelate and amlodipine and pentifylline were merged. The unsuitability of lower pressures ($<$ 9.13 MPa) can be attributed to the separation of phases because below $\sim$10.13 MPa at 50°C and 6% modifier concentration separation of phases is also known to occur [22]. For a given column and temperature retention generally decreases as operating pressure is increased because fluid density increases at higher pressures.

4.3. Effect of temperature on capacity factor

Fig. 3 depicts the effect of temperature on capacity factors, keeping other parameters as before. Fig. 3 shows that 45°C can be the optimum temperature as there is enough separation at this temperature. The choice of this temperature strikes a balance between selectivity and run time. At 32.5°C the capacity factors of amlodipine and pentifylline merge. There is progressive difference in capacity factors as temperature increases. Actual experimentation showed that no discernible pattern was obtained $\geq$ 65°C.

4.4. Optimisation of parameters

Experimentation revealed that a pressure of 10.13 MPa, temperature of 45°C and 16% methanol, the present parameters provided the maximum selectivity and most efficient separation of the seven vasodilators. A typical chro-

Table 3
Accuracy and precision of the method

Drug	Spiked conc. (μg ml^{-1})	Found conc. (μg ml^{-1})	RSD ($n = 5$)
ISDN	1.25	1.15	1.81
	8.00	8.11	1.60
	54.00	54.09	1.20
Cyclandelate	1.00	1.01	7.12
	8.00	8.18	2.04
	54.00	54.04	0.35
ISMN	2.00	1.71	8.54
	8.00	8.33	1.18
	54.00	54.01	0.25
Nimodipine	0.20	0.17	1.46
	1.60	1.61	0.53
	10.80	10.78	0.54
Amlodipine	0.50	0.41	3.30
	4.00	4.15	0.34
	27.00	26.96	0.62
Pentifylline	0.30	0.26	3.71
	2.40	2.45	0.56
	16.50	16.44	0.59
Pentoxifylline	0.50	0.43	2.98
	4.00	4.01	0.98
	28.00	27.95	1.15

matogram of the seven vasodilators (100 μg ml^{-1}) obtained under these conditions is given in Fig. 4.

5. Part B: estimation of the vasodilators

For linearity studies seven different concentrations of the individual drug (concentration ranges given in Table 1) were assayed ($n = 5$), 20 μl being the injection volume. After ascertaining the linearity of the peak responses (peak heights or peak areas), 20 μl of a mixture of all the seven drugs was injected. The concentration ranges of the different drugs are given in Table 1.

6. Discussion

For convenience only peak heights are mentioned here, even though peak areas were also found to be linear. Linear regression least squares fit data obtained from the measurements are given in Table 1. The respective slopes $m \pm t_{CL,v}S_m$ and the intercepts $b \pm t_{CL,v}S_b$, the regression factors and the standard deviations of the residuals from the linear least squares regression are all quoted in this Table. All these quantities have been calculated by Gordus [23].

The analytical features for the chromatograms of the seven drugs are given in Table 2. From the Table it is discernible that packed column SFC is better than or at least as good as HPLC in the separation and assay of an arbitrary group of drugs.

The minimum quantifiable concentration (MQC) given in Table 1 is a compromise due to the selection of one wavelength for all the compounds. It could be improved by a more selective choice of UV-wavelength if only one or two of the drugs are to be determined.

A study of precision and accuracy was performed by assaying three composite solutions, of low, medium and high concentrations. The peak responses, in terms of peak heights, were then related to the slopes and intercepts mentioned in Table 1 to obtain the analytical recoveries. Table 3 lists the recoveries of the drugs from a series of spiked concentrations and shows that the relative

Table 4
Performance data for the determination of the drugs

Drug	Conc. (μg ml^{-1})	RSD within-day	RSD between-day
ISDN	1.25	1.72	1.40
	8.00	1.01	1.33
	54.00	0.64	1.03
Cyclandelate	1.00	1.51	8.53
	8.00	0.78	2.37
	54.00	0.10	0.41
ISMN	2.00	0.92	10.41
	8.00	0.73	1.25
	54.00	0.25	0.14
Nimodipine	0.20	0.72	1.62
	1.60	0.49	0.42
	10.80	0.46	0.47
Amlodipine	0.50	2.80	2.90
	4.00	0.40	0.08
	27.00	0.72	0.06
Pentifylline	0.30	4.04	1.28
	2.40	0.30	0.62
	16.50	0.44	0.56
Pentoxifylline	0.50	1.88	2.68
	4.00	0.71	0.88
	28.00	0.42	1.34

standard deviation (RSD) does not exceed 5% for most compounds, but for low concentrations of ISMN and cyclandelate it was approximately 8%. To obtain performance data within-day and between-day, measurements were made over 15 days under the same conditions as described obove. The data in Table 4 revealed the that RSDs did not exceed 5% except for cyclandelate and ISMN.

7. Conclusions

Packed column SFC has been shown to be ideally suited for the separation and quantitative estimation of seven vasodilators, widely varying in their chemical structures, but with similar pharmacological properties. Apart from merits in separation this technique offers a viable alternative to HPLC because it is faster. In the case of nimodipine the minimum quantifiable concentration was as low as 200 ppb, which could still be reduced significantly by the choice of proper wavelength, solvent and injection volumes. In packed column SFC the necessity of the preparation of a mobile phase is obviated, as selectivity can be easily and widely tuned by the proper choice of binary or ternary modifiers. This work has revealed that an isocratic enhanced-polarity separation could produce the same quality separation and estimation in approximately the same time as gradient elution HPLC.

Further calculations showed that this determination is eminently suitable for the internal standard method. Logically, one can use ISDN as the internal standard for ISMN, etc. The peak height ratios of the drug/internal standard were found to be proportional to the drug concentrations, inspite of the widely arbitrary choice of the drugs. It has been seen that any drug can be used as the internal standard for the other six drugs.

Acknowledgements

The authors thank Shri Kelkar C.P., Director (Admn.), C.B. Patel Research Centre, Mumbai-56, Prof. Dhakkappa, Principal, K.J. Somaiya College of Science, Mumbai-77 and co-workers

Yagnesh Patel, Viddesh Bari and Suvarna Patil for encouragement in carrying out this work.

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