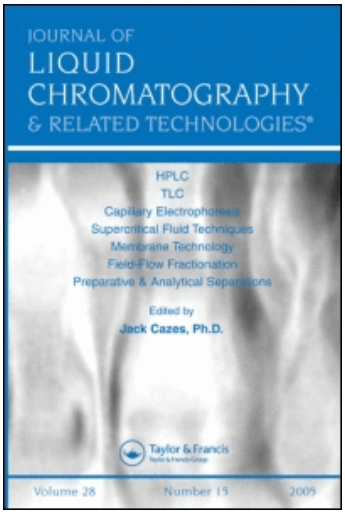

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Xanthine Derivates by High Pressure Liquid Chromatography and Application to Plasma Analysis

A. Turcant[a]; P. Cailleux[a]; P. Allain[a]
[a] Laboratoire de Pharmacologie - Centre Hospitaller Régional, ANGERS

To cite this Article Turcant, A. , Cailleux, P. and Allain, P.(1980) 'Separation of Xanthine Derivates by High Pressure Liquid Chromatography and Application to Plasma Analysis', Journal of Liquid Chromatography & Related Technologies, 3: 10, 1537 — 1547
To link to this Article: DOI: 10.1080/01483918008062794
URL: http://dx.doi.org/10.1080/01483918008062794

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(10), 1537-1547 (1980)

SEPARATION OF XANTHINE DERIVATES BY HIGH PRESSURE LIQUID CHROMATOGRAPHY AND APPLICATION TO PLASMA ANALYSIS

A. TURCANT, P. CAILLEUX and P. ALLAIN
Laboratoire de Pharmacologie - Centre Hospitalier Régional - ANGERS

ABSTRACT

High pressure liquid chromatography (HPLC) methods were developed for separation and plasma analysis of ten xanthine derivates. Separation was evaluated on silica column and on three different reverse phase columns, with optimum conditions obtained on C_6 spherisorb column using isocratic elution with phosphate buffer 10^{-2} M, pH 2.7 - acetonitrile mixture (80/20 V/V). Determination of these xanthine derivates in plasma for therapeutic control was studied.

INTRODUCTION

Many recents methods of theophylline assay in human plasma have been described by gas chromatography (1, 2, 3) and especially by HPLC (4, 5, 6, 7). Pentoxifylline and its major metabolite (MI), 1-(5 hydroxyhexyl) 3,7 dimethylxanthine were also analysed by HPLC on Lichrosorb SI 60 (8).

The present paper shows that, with appropriate solvent conditions, HPLC is a suitable method for plasma analysis of all xanthine derivates used in therapeutics (table I) and more especially for bamifylline, lomifylline, pentifylline and pentoxifylline.

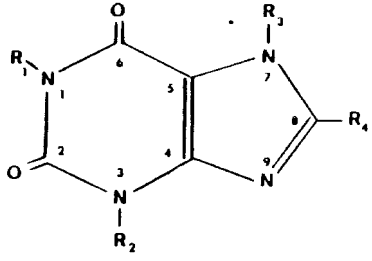

Table I : Xanthine derivates

	R_1	R_2	R_3	R_4
Theobromine	H	CH_3	CH_3	H
Theophylline	CH_3	CH_3	H	H
Caffeine	CH_3	CH_3	CH_3	H
Dihydroxypropyl Theophylline (Neutraphylline*)	CH_3	CH_3	$-CH_2-CHOH-CH_2OH$	H
Pentoxifylline (Torental*)	$-(CH_2)_4-CO-CH_3$	CH_3	CH_3	H
Metabolite M1	$-(CH_2)_4-CHOH-CH_3$	CH_3	CH_3	H
Lomifylline (Cervilane*)	CH_3	CH_3	$-(CH_2)_4-CO-CH_3$	H
Pentifylline (Cocadon*)	$-(CH_2)_5-CH_3$	CH_3	CH_3	H
Bamifylline (Prentadil*)	$-CH_3$	CH_3	$-(CH_2)-N(C_2H_5)-CH_2-CH_2OH$	$-CH_2-C_6H_5$
Proxibutyl Methylxanthine	$-CH_3$	$-CH_2-CH(CH_3)-CH_3$	H	H

MATERIAL AND METHODS

Reagents

Pure samples of the studied drugs were kindly supplied by manufacturers ; 3-isobutyl 1-methyl xanthine (IBMX) was purchased from Aldrich (Beerse, Belgium) and 1-methyl 2-[m (1-hydroxyethyl) phenylamino-] benzimidazole (RN 1092) was a gift of Allard Laboratories (Nogent sur Marne, France).

Solvents (acetonitrile, chloroform, isopropanol, hexane) and other chemicals (acetic acid, potassium dihydrogen phosphate) were analytical grade reagents (Merck, Darmstadt, F.R.G.).

Chromatographic instrumentation and conditions

A high pressure liquid chromatograph (Laboratory Data Control, Riviera Beach, Florida) consisting of a Constametric I pump, a Valco sample injection valve with a 25 μl loop, and a 280 nm U.V. III monitor was used. Stationary phases evaluated were 7 μm Zorbax SIL silica (Dupont, Wilmington, D.E.) and 5 μm hexylsilane (C_6), cyano (CN), octadecylsilane (ODS) Spherisorb reverse phase packings (Sopares, Gentilly, France). Silica column (20 cm x 4.6 mm I.D.) was commercial prepacked stainless steel column and reverse phase columns were packed by us using a slurry technique with Haskel MCP 110 pump as packing apparatus (Touzart et Matignon, Vitry, France). Separation was realized at ambient temperature with a hexane-chloroform-isopropanol-acetic acid (50/43/5/2 V/V) mixture (solvent A ; flow rate : 0.7 ml/min) for silica column and with phosphate buffer 10^{-2} M, pH 2.7 - acetonitrile (solvent B : 80/20 V/V ; solvent C : 72/28 V/V ; flow rate : 1.5 ml/min) for reverse phase columns. Sol-

vents were degassed by bubbling helium immediately before use and were filtered through 2 μm filters.

Assay procedure

To one milliliter of plasma was added 50 μl internal standard (RN 1092 : 5 mg/l), 100 μl NaOH 2N and 10 milliliters of chloroform. After mixing and centrifugation, aqueous phase was discarded and organic phase was dried on anhydrous sodium sulfate and then evaporated to dryness. The residue was dissolved in 100 μl mobile phase and 25 μl were injected into column.

RESULTS AND DISCUSSION

Chromatographic separation

Separation of xanthine was studied by adsorption chromatography on Zorbax SIL column and by partition chromatography on C_8, CN and ODS column and retention times are presented on table II.

On silica column (figure 1), excellent separation of these drugs, and especially of lomifylline and pentoxifylline which are position isomers, was obtained. Theophylline, caffeine and theobromine are also eluted in these conditions but do not interfere with studied products. Neutraphylline and bamifylline, which are very polar, are strongly retained. IBMX, a potent phosphodiesterase inhibitor not used in therapeutics,can be used as internal standard as in gas chromatography (1,3).

With reverse phase columns, the best separation was obtained on C_6 spherisorb, as indicated in figure 2, with a short analysis time and good sensitivity compared to normal plasma concentrations (9,10), except for pentifylline. Elution of this product is obtained by increasing acetonitrile concentration as in figure 3, but

lomifylline and pentoxifylline are not separated. With C_6 column, pentoxifylline and IBMX are always eluted with the same retention time. So, we have chosen another internal standard (RN 1092) whose chemical structure presents some analogies with bamifylline. Elution of these two aliphatic amines is similarly modified by changing buffer pH contrary to other xanthines.

Standard curves - Reproducibility

Standard calibration curves, prepared with solvent conditions as in figure 3 by supplementing a drug free plasma with known amounts of xanthine derivates ranging from 0 to 2 mg/l and with constant amount of internal standard show excellent linearity in all cases with a correlation coefficient of 0.999 by plotting concentrations against peak heightratio between drug and internal standard. Drug free plasma present no interfering peak.

Overall absolute recovery was nearly 85 % for pentoxifylline, its metabolite and pentifylline and only 60 % for bamifylline. Internal standard recovery was 80 %.

Reproducibility of the method was determined by processing aliquots pooled plasma of xanthine derivates, at concentration of 1 mg/l, through the entire procedure during a single day. Results were 1.01 $\pm$ 0.05 mg/l for pentoxifylline and 1.02 $\pm$ 0.04 mg/l for its metabolite and for bamifylline (n = 10) and lower detection limit was less than 0.1 mg/l.

In summary, HPLC, with an isocratic eluting system and one single column but by choosing adequate solvent conditions, is a very good method for determination in plasma of the different xanthines actually used in therapeutics.

Table II : Retention times of xanthine derivates on Zorbax SIL, Spherisorb CN, C_6, ODS with different solvents.

	SOLVENT A	SOLVENT B			SOLVENT C	
	Zorbax SIL	CN	C_6	ODS	C_6	ODS
Theobromine	11 min.	2 min.	1,9	2,8	1,6	2,2
Neutraphylline	45 min.	2 min.	1,9	3	1,6	2,4
Theophylline	4,9 min.	2 min.	2,2	3	1,8	2,4
Caffeine	5,2 min.	2,2 min.	2,9	4,2	2,1	3
Pentoxifylline metabolite	15,8 min.	2,4 min.	5,3	8	2,7	4

Lomifylline	5,8 min.	2,5 min.	5,7	8	3	4,15
Pentoxifylline	7 min.	2,5 min.	6,1	8,6	3,1	4,35
IBMX	2,4 min.	2,3 min.	6	6,5	3,3	3,8
Bamifylline	∞	9,6 min.	8,6	25,2	6,6	16,6
RN 1092	—	10,6 min.	10,2	23,4	9	15,4
Pentifylline	3,1 min.	3,6 min.	57	66	15,7	18,6

A : Hexane, Chloroform, Isopropanol, Acetic acid : 50, 43, 5, 2 V/V
Flow-rate : 0.7 ml/min.

B : Phosphate buffer 10^{-2} M pH 2.7, CH_3CN : 80, 20 V/V
Flow-rate : 1.5 ml/min.

C : Phosphate buffer 10^{-2} M pH 2.7, CH_3CN : 72, 28 V/V
Flow-rate : 1.5 ml/min.

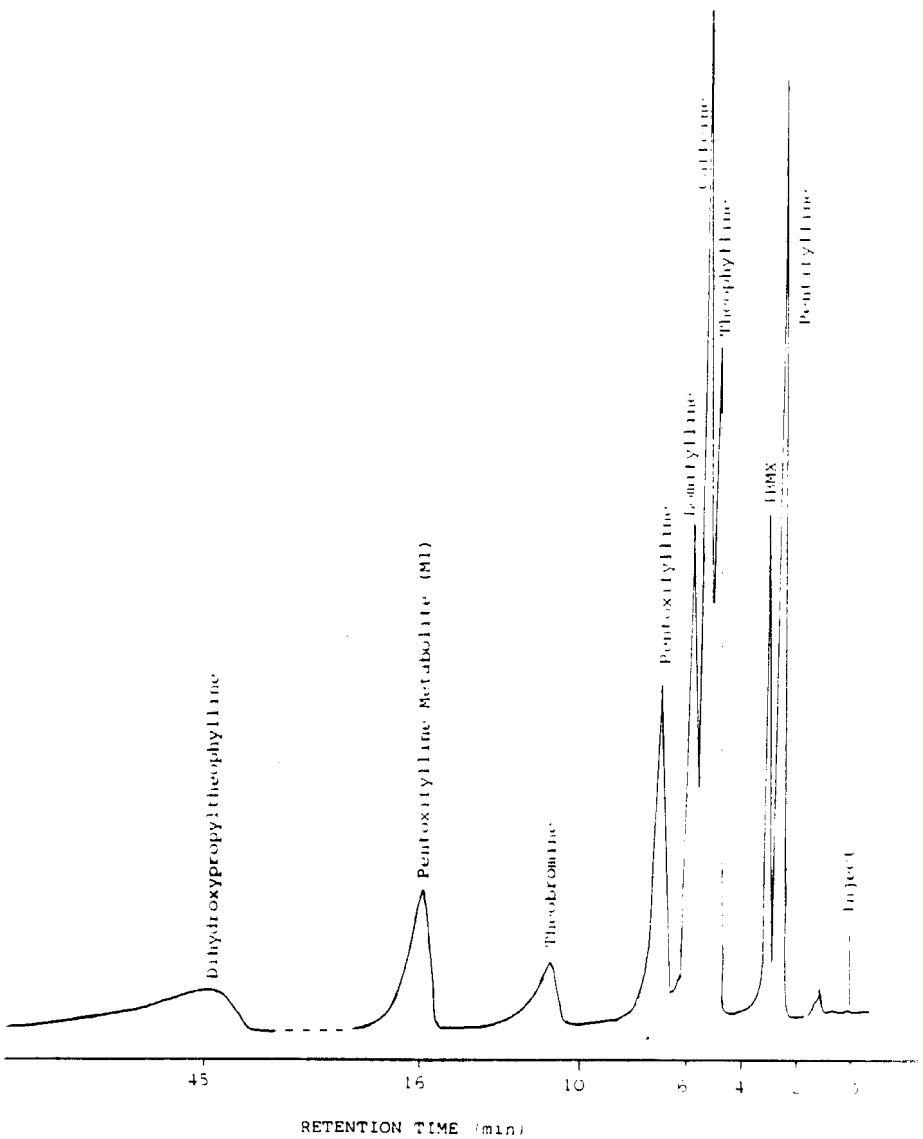

Figure 1: Chromatogram of xanthine mixture on Zorbax SIL column. Hexane-Chloroform-Isopropanol-Acetic acid 50/43/5/2 v/v. Flow-rate 0.7 ml/min.

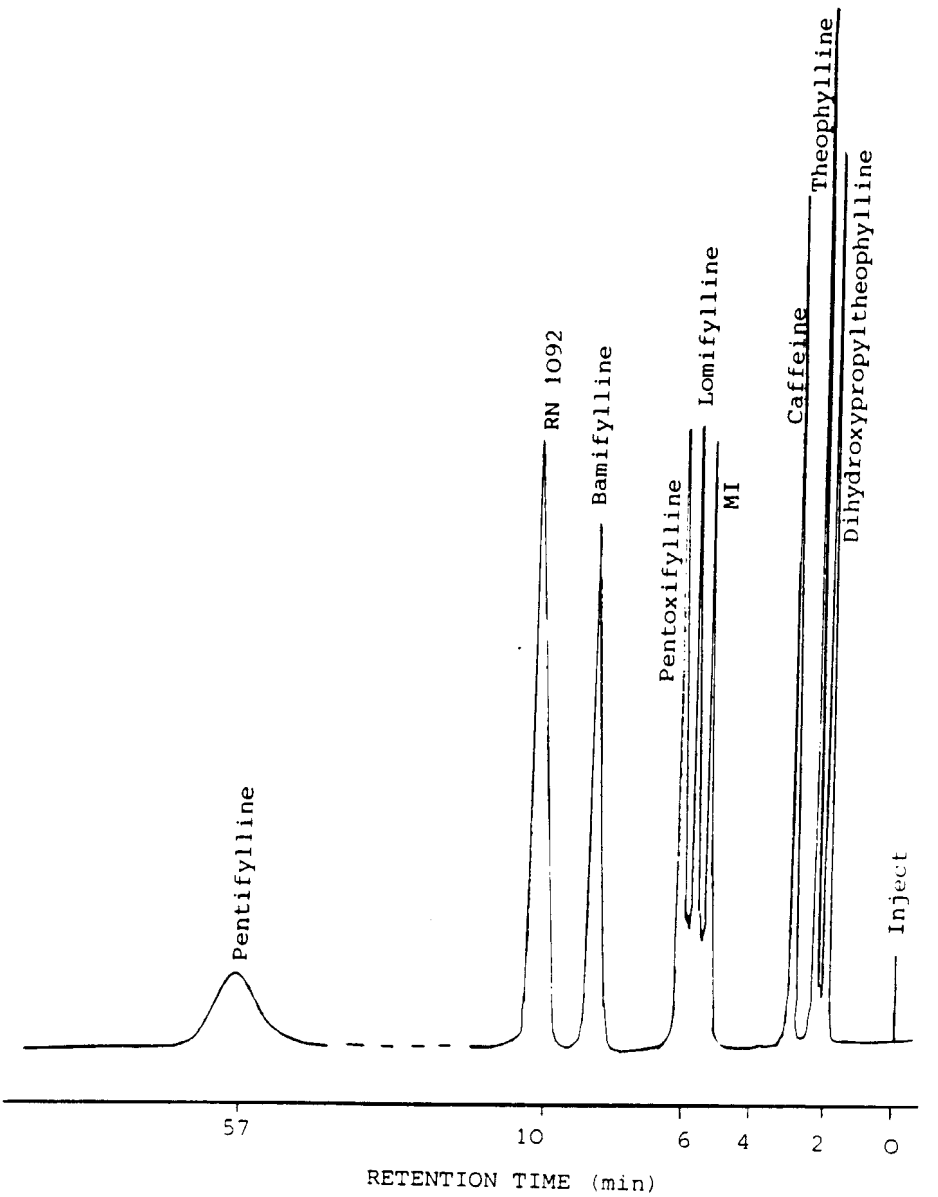

Figure 2 : Chromatogram of xanthine mixture on Spherisorb C_6 column ; Solvent B as in table II.

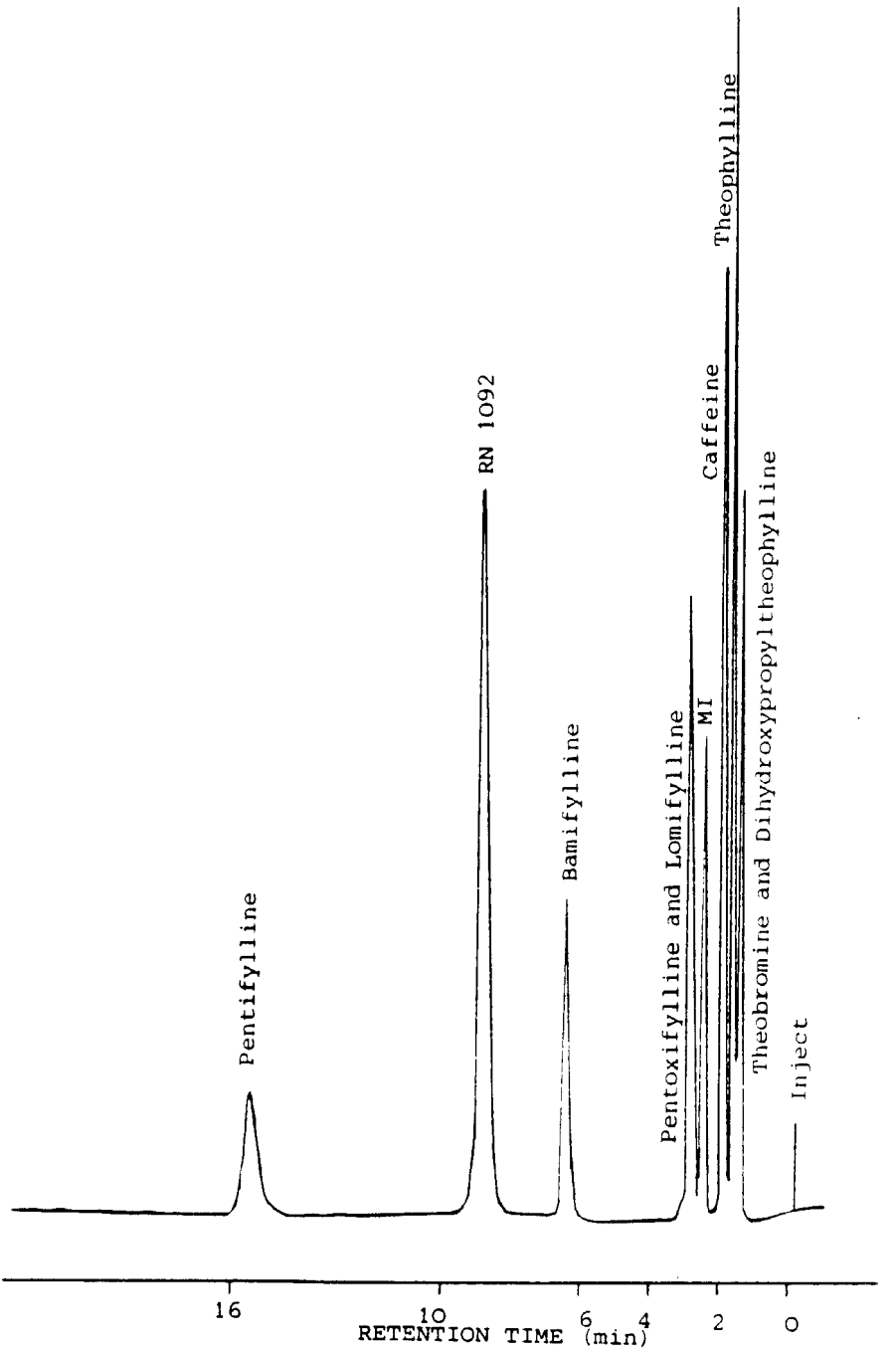

Figure 3 : Chromatogram of xanthine mixture on Sphérisorb C_6 column ; Solvent C as in table II.

REFERENCES

1. Schwertner, H.A., Ludden, T.M., Wallace, J.E, Determination of théophylline in plasma by electron capture gas chromatography. Anal. Chem., 48:1877, 1976.

2. Brachet-Liermain, A., Paix, M., Saux, M.C., Demarquez, J.L., Microdosage de la theophylline dans les liquides biologiques in : Pharmacologie périnatale, Colloques de l'I.N.S.E.R.M., 73:169, 1977.

3. Berthou, F., Dreano, Y., Riche, C., Alix, D., Floch, H.H., Determination de la theophylline plasmatique du nouveau-né par chromatographie en phase gazeuse à haute résolution et détection spécifique. Ann. Biol. Clin., 36:497, 1978.

4. Adams, R., Vandemark, F., Schmidt, G., LC. Determination of theophylline in serum by reverse phase chromatography. Clin. Chem., 22:1903, 1976.

5. Soldin, S.J., Hill, J.G., A rapid micromethod for measuring theophylline in serum by reverse phase high performance liquid chromatography. Clin. Biochem., 10:74, 1977.

6. Peat, M.A., Jennison, T.A., Chinn, D.M., Analysis of theophylline in serum and whole blood samples by high pressure liquid chromatography. J. Anal. Toxico., 1:204, 1977.

7. Orcutt, J.J., Kozak, P.P. Jr., Gillman, S.A., Cummins, L.H., Microscale method for theophylline in body fluids by reverse phase high pressure liquid chromatography. Clin. Chem., 23:599, 1977.

8. Hinze, H.J., Grigoleit, H.G., Rethy, B., Bioavailability and pharmacokinetics of pentoxifylline from "Trental 400" in man. Pharmatherapeutica, 1:160, 1976.

9. Hinze, H.J., Pharmakokinetik von 3,7 - Dimethyl-1-(5-oxo-hexyl)-xanthin (BL 191)am Menschen. Arzneim-Forsch. (Drug Res.), 22:1492, 1972.

10. Schaefer, K., Herrath, D., Hensel, A., Grigoleit, H.G., Untersuchungen zur pharmakokinetik von pentoxifyllin bei chronischer niereninsuffizienz. Med. Klin., 72:204, 1977.