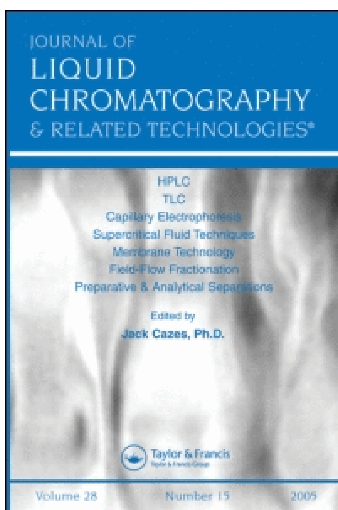
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

An HPLC Method for the Determination of Verapamil and Norverapamil in Human Plasma

Scott M. Johnson[a]; Shoukry K. Wahba Khalil[a]
[a] College of Pharmacy Department of Pharmaceutical Sciences, North Dakota State University, Fargo, North Dakota

To cite this Article Johnson, Scott M. and Khalil, Shoukry K. Wahba(1987) 'An HPLC Method for the Determination of Verapamil and Norverapamil in Human Plasma', Journal of Liquid Chromatography & Related Technologies, 10: 6, 1187 — 1201
To link to this Article: DOI: 10.1080/01483918708066762
URL: http://dx.doi.org/10.1080/01483918708066762

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(6), 1187-1201 (1987)

AN HPLC METHOD FOR THE DETERMINATION OF VERAPAMIL AND NORVERAPAMIL IN HUMAN PLASMA

Scott M. Johnson and Shoukry K. Wahba Khalil
College of Pharmacy
Department of Pharmaceutical Sciences
North Dakota State University
Fargo, North Dakota

Downloaded At: 15:20 24 January 2011

ABSTRACT

A high performance liquid chromatographic method is presented for the determination of verapamil and its metabolite norverapamil in human plasma. Verapamil and norverapamil are extracted from plasma basified with 0.5M dibasic sodium phosphate (pH 9.5) using ethyl acetate containing trimipramine as an internal standard. A reverse-phase cyanopropylsilane column was used with a mobile phase of 65% acetonitrile and 35% 0.02M acetate buffer (pH 7.0). The minimum detectable limit was 2 ng/ml of plasma. The effect of the pH, molarity, and percent acetonitrile of the mobile phase on the capacity factor was studied. Possible interferences from other drugs administered concurrently are presented.

INTRODUCTION

Verapamil, α-isopropyl-α-[(N-methyl-N-homoveratryl)-γ-aminopropyl]-3,4-dimethoxyphenylacetonitrile, is a member of the

Copyright © 1987 by Marcel Dekker, Inc.

calcium channel blocking agents useful in the treatment of vasospastic angina, chronic stable angina, and supraventricular tachyarrhythmias (1).

Verapamil is available in oral and intravenous dosage forms. Verapamil appears to be well absorbed orally, is highly protein bound, and is extensively metabolized by the liver to an active demethylated metabolite, norverapamil (2). The normal therapeutic plasma levels range from 25-100ng/ml. The incidence and type of side effects resulting from orally administered verapamil is relatively minor (3), yet verapamil use should be carefully monitored with patients who have hepatic dysfunction as the liver is a major metabolic organ (4).

Downloaded At: 15:20 24 January 2011

There are several drug-drug interactions reported in the literature for the combined use of verapamil that include theophylline (5), quinidine (6), calcium salts (7), and beta blockers in general (8). The major interaction reported is between verapamil and the cardiac drug digoxin. The elimination of digoxin is decreased resulting in significant elevation in plasma digoxin levels (9,10).

Chromatographic methods for the determination of verapamil include gas chromatography (11-15), and high performance liquid chromatography (16-20). These methods suffer certain limitations including: 1) the use of a lengthy extraction procedure, 2) the use of toxic extraction solvents, and 3) limited sensitivity.

The present paper describes verapamil and norverapamil analysis in plasma using a high efficiency reverse-phase cyanopropylsilane HPLC column following a single step extraction.

The method is sensitive and applicable to the daily monitoring of plasma levels even in the presence of one or more commonly prescribed drugs.

MATERIALS AND METHODS

Instrumentation

A Hewlett Packard Model 1090 liquid chromatograph equipped with an automatic injector, a cyanopropylsilane column (Dupont, Wilmington, DE), 15 cm long and 4.6 mm i.d., and a Schoeffler fluorescence detector set at an excitation wavelength of 201 nm and an emission cut off filter type 320. The signal was recorded on a Hewlett Packard Model 3392A integrator. The degassed mobile phase was pumped through the column at 3.0 ml/min and the column compartment was maintained at 40 ^{o}C.

Downloaded At: 15:20 24 January 2011

Chemicals and Reagents

Sodium hydroxide, dibasic sodium phosphate, and sodium acetate were reagent grade. Acetonitrile and ethyl acetate were HPLC grade. Verapamil and norverapamil were supplied by Knoll Pharmaceutical Co., and G.D. Searle and Co. Trimipramine Maleate was supplied by Ives Laboratories, Inc.

Drug and Metabolite Solutions

Separate solutions of verapamil and norverapamil were made containing 5 mg/100ml of methanol. Working dilutions of 1 and 10 g/ml methanol were prepared from the stock solutions.

Internal Standard Solutions

A stock solution of trimipramine maleate was prepared at 5 mg/100ml methanol. A working dilution of 2 μg/ml methanol was prepared from the stock solution.

Extraction Solutions

The extraction solution consisted of a 1.2ml aliquot of the working solution of the internal standard in 1000ml ethyl acetate.

Mobile Phase

Sodium acetate solution, 0.02M was prepared in deionized distilled water and the pH was adjusted to 7.0. The mobile phase consisted of 65% acetonitrile and 35% buffer and pumped at 3.0 ml/min.

The effect of changes in the mobile phase pH, percent acetonitrile, and the ionic strength of the acetate buffer on the capacity factor (k′) were studied.

Preparation of Plasma Standards

To 1.0ml of plasma in a 15ml screw capped centrifuge tube, aliquots of working drug and working metabolite solutions were added to simulate drug and metabolite concentrations (20-100 ng/ml of plasma). The spiked plasma was basified with 0.5ml Na_2HPO_4 buffer (0.5M, pH 9.5), and 10ml of the extraction solution containing the internal standard was added. The tubes were vortexed for 20 seconds and centrifuged for 10 minutes at 900Xg. A 9ml volume of the organic phase was transferred to an open top centrifuge tube

Downloaded At: 15:20 24 January 2011

and evaporated to dryness under a gentle stream of nitrogen while partially submerged in a constant 40°C temperature bath. The residue was dissolved in 100 μl of acetonitrile and transferred to a borosilicate glass vial (Wheaton Industries, Millville, NJ), with a polypropylene vial insert (Sunbrokers, Inc., Wilmington, NC). A 30 μl aliquot was injected onto the column.

Several solvents and buffers were tried for extracting the drug and its metabolite.

Quantitation

Standard curves were constructed utilizing four replicates at each concentration. The peak heights were measured, and the ratios (drug/internal standard and metabolite/internal standard) were calculated and plotted against concentration in nanograms per milliliter of plasma.

Recovery

Plasma samples containing a known concentration of verapamil and norverapamil were carried through the analysis. An equivalent concentration of drug and metabolite was added to 9ml of extraction solution, vortexed, evaporated to dryness, and reconstituted in acetonitrile for injection into the chromatograph. The peak heights of the drug, metabolite, and internal standard in the plasma samples were measured for comparison against the samples without extraction to estimate recovery.

Downloaded At: 15:20 24 January 2011

Interferences

Possible interferences from normal plasma constituents has been studied by injecting extracts prepared as previously described of drug free plasma samples. Solutions of other common concurrently prescribed drugs were injected under the same chromatographic conditions.

RESULTS AND DISCUSSION

Downloaded At: 15:20 24 January 2011

Verapamil and norverapamil can be readily extracted from basified plasma at pH 9.5 using an organic solvent in a single extraction step. The choice of extraction conditions were based on the highest recovery of the drug and its metabolite with minimum interference from plasma constituents. The percent recovery for the described method was 54% for verapamil and 79% for norverapamil. Alterations in the extraction solvent or a double extraction procedure did not significantly increase the extraction efficiency. Typical chromatograms from plasma extracts under isocratic elution conditions are found in Figure 1.

Higher sensitivity for the detection of verapamil and norverapamil was achieved using fluorescence detection at an excitation wavelength of 201 nm, and an emission cutoff filter at 320 nm. The limit of detection for both the drug and its metabolite was 2 ng/ml of plasma.

To establish the best chromatographic resolution of verapamil and norverapamil, the pH, percent acetonitrile and the molarity of the buffer in the mobile phase were varied independently. A

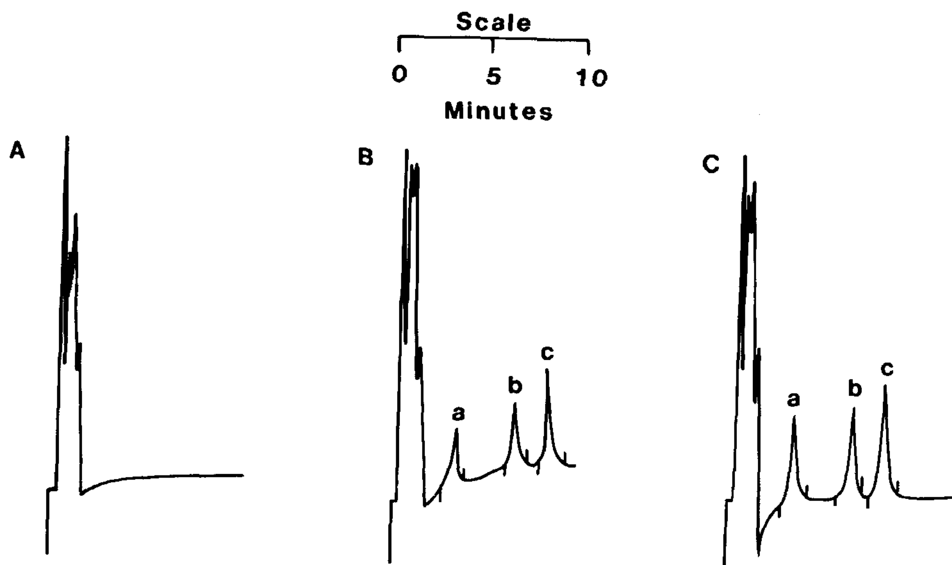


Figure 1. Typical chromatograms of verapamil (a), norverapamil (b), and trimipramine (c) from plasma. Key: A, plasma blank; B, 50 ng of drug and metabolite/1.0ml plasma; and C, 80 ng of drug and metabolite/1.0ml plasma.

Downloaded At: 15:20 24 January 2011

mobile phase pH of 5 and 6 eluted both verapamil and norverapamil too close to the solvent front, whereas at pH 7.5 the capacity factor increased (Figure 2) accompanied by significant peak broadening. Increase in buffer molarity decreased the capacity factor (Figure 3). Slight increase in the percentage of acetonitrile produced a significant effect on the capacity factor (Figure 4). A mobile phase consisting of 65% acetonitrile and 35% acetate buffer (0.02M, pH 7) afforded good chromatographic resolution and reasonable retention times using a cyanopropylsilane column in a reversed-phase mode. The choice of the acetate buffer increased

Downloaded At: 15:20 24 January 2011

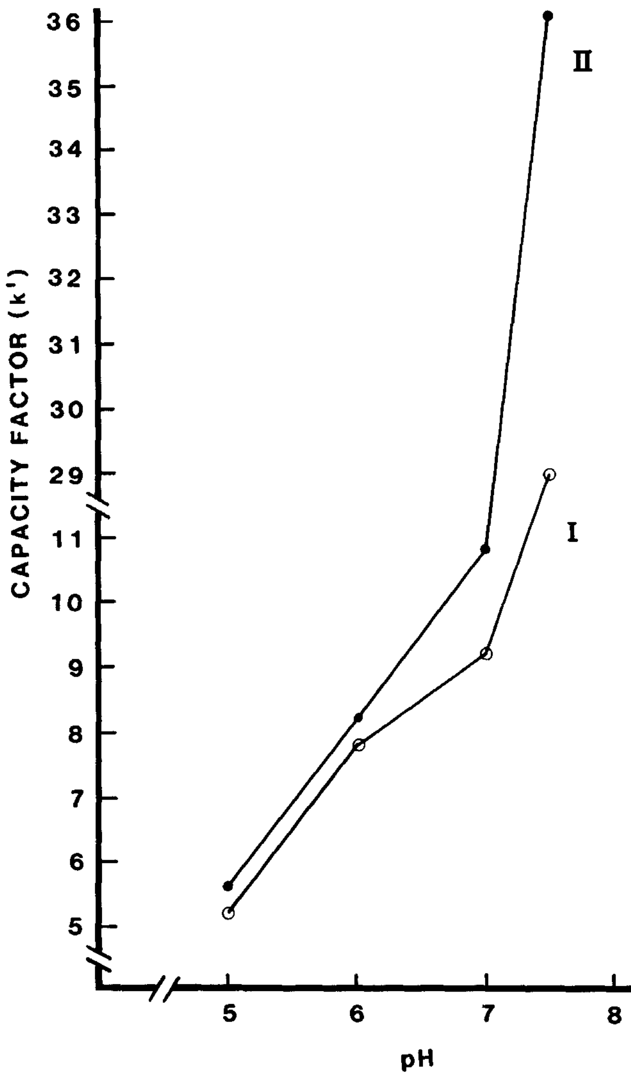


Figure 2. Effect of pH on k′ (Molarity = 0.02M, and 65% acetonitrile).

Key: I = k′ for Verapamil
II = k′ for Norverapamil

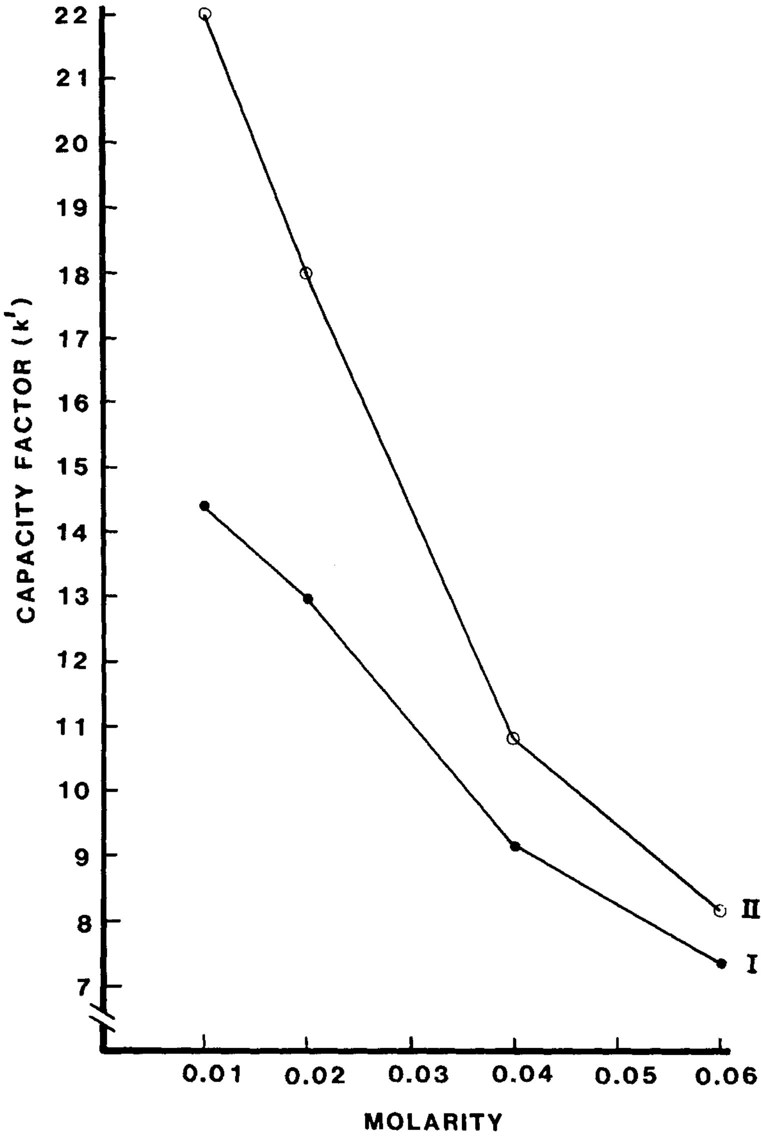


Figure 3. Effect of Molarity on k′ (pH = 7, and 65% acetonitrile).

Key: I = k′ for Verapamil
II = k′ for Norverapamil

Downloaded At: 15:20 24 January 2011

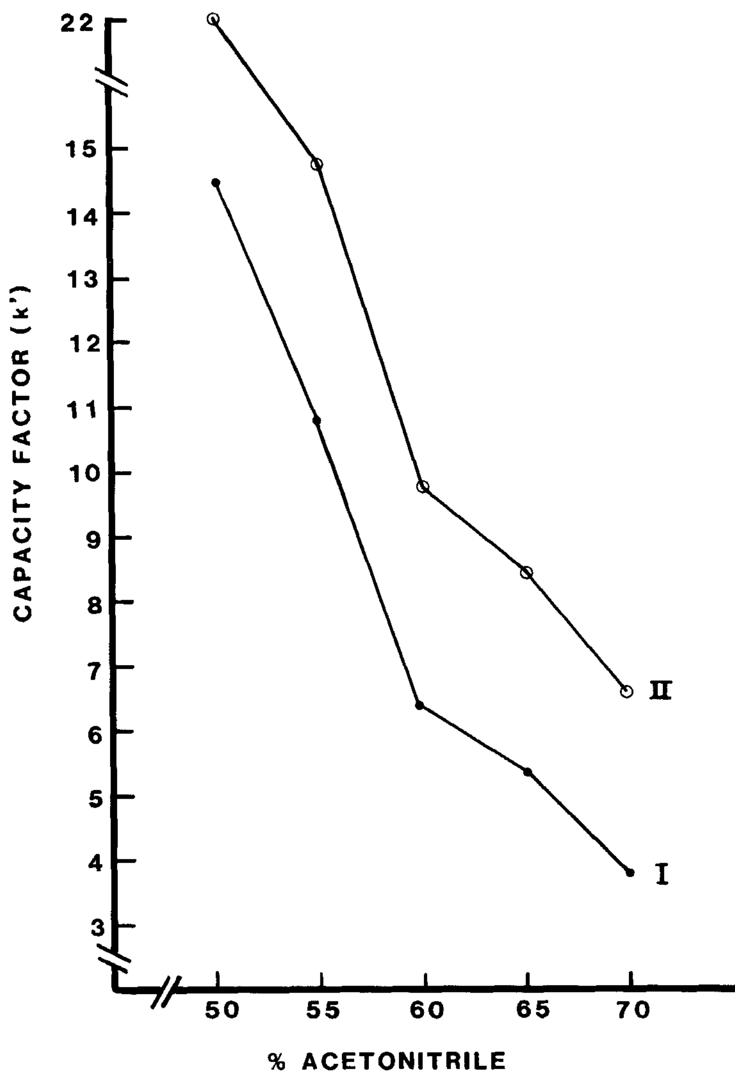


Figure 4. Effect of Percent Acetonitrile on k′ Molarity = 0.02M, and pH = 7).

Key: I = k′ for Verapamil
II = k′ for Norverapamil

Downloaded At: 15:20 24 January 2011

the useful life of the column and minimized the column to column variation.

Trimipramine was chosen as an internal standard as it is coextractable with the drug and metabolite, it resolves well from the drug, metabolite, and endogenous plasma constituents, and is detectable under the same fluorescence conditions.

The ratio of peak height of verapamil or its metabolite to the peak height of the internal standard was plotted versus concentration. Statistical analysis indicates good reproducibility and linearity (Tables 1 and 2).

No interference from drugs which might be administered concurrently when chromatographed under the same conditions was observed. Retention times of these drugs are presented (Table 3).

Downloaded At: 15:20 24 January 2011

TABLE 1. STATISTICAL ANALYSIS OF A STANDARD CURVE FOR VERAPAMIL.

Theoretical Concentration (ng/ml plasma)	Observed Concentration (ng/ml plasma) ± Standard Error*
20	21.2 ± 1.6
40	41.1 ± 1.3
60	64.9 ± 3.6
80	78.4 ± 1.6
100	97.4 ± 3.9

* Correlation coefficient = 0.9967
r^2 = 0.9935
slope = 0.9485
y-intercept = 3.6900
n = 19 points
(minimum of three replicates per concentration)

TABLE 2. STATISTICAL ANALYSIS OF A STANDARD CURVE FOR NORVERAPAMIL.

Theoretical Concentration (ng/ml plasma)	Observed Concentration (ng/ml plasma) ± Standard Error*
20	19.2 ± 0.9
40	37.8 ± 0.5
60	63.2 ± 2.7
80	76.4 ± 2.7
100	100.6 ± 4.4

* Correlation coefficient = 0.9966
r^2 = 0.9933
slope = 1.0070
y-intercept = -0.9800
n = 19 points
(minimum of three replicates per concentration)

Downloaded At: 15:20 24 January 2011

TABLE 3. RETENTION TIMES OF DRUGS TESTED FOR POSSIBLE INTERFERENCES WITH VERAPAMIL AND NORVERAPAMIL USING FLUORESCENCE DETECTION WITH EXCITATION AT 201 nm AND EMISSION CUTOFF FILTER TYPE 320.

Drug	Retention Time in Minutes
Procainamide	2.54
Verapamil	3.92
Quinidine	6.10
Norverapamil	7.56
Propranolol	8.71
Trimipramine	9.66

CONCLUSIONS

This HPLC method using a cyanopropylsilane column un der isocratic conditions offered excellent separation of the drug, metabolite, and the internal standard, with a reasonable analysis time, and appropriate sensitivity for detection within the drug's normal therapeutic range.

The described method is simple and rapid as it requires a single extraction step prior to injection. The method is linear and reproducible and can be recommended and utilized for routine patient monitoring and pharmacokinetic studies.

Downloaded At: 15:20 24 January 2011

ACKNOWLEDGEMENTS

The authors wish to thank G.D. Searle and Co., and Knoll Laboratories for providing authentic drug and metabolite samples. The authors also wish to thank Dr. Gary Erdmann for his assistance and contributions with instrumentation.

REFERENCES

1. Theroux, P., Taeymans, Y. and D. Waters, Calcium Antagonists: Clinical Use in the Treatment of Angina, Drugs, 25, 125 (1983).

2. Henry, P.D., Comparative Pharmacology of Calcium Antagonists: Nifedipine, Verapamil, and Diltiazem, The American Journal of Cardiology, 46, 1047-1058 (1980).

3. Gilliland, B.C. and M. Mannik, "Harrison's Principles of Internal Medicine," 10th Ed., McGraw-Hill Book Co., New York, NY, 1983 p.1382.

4. Kastrup, E.K., edit., "Facts and Comparisons," Facts and Comparisons, Inc., St. Louis, MO, 1985, p.149e.

5. Shinn, A.F. and R.P. Shrewsburg, "Evaluations of Drug Interactions", 3rd Ed., The C.V. Moseby Company, St. Louis, MO, 1985, p.660.

6. Philip Hansten, "Drug Interactions", 5th Ed., Lea and Febiger, Philadelphia, PA, 1985, p.21.

7. Ibid., p.396.

8. Ibid., p.46.

9. Klein, H.O., Lang, R., Weiss, E., Segni, E.D., Libhaber, C., Guerro, J. and E. Kaplinsky, The Influence of Verapamil on Serum Digoxin Concentration, Circulation, 65, 998 (1982).

10. Pedersen, K.E., Dorph-Pedersen, A., Hvidt, S., Klitgaard, N.A. and F. Nielson-Kudsk, Digoxin-Verapamil Interaction, Clin. Pharmacol. Ther. 9, 315 (1981).

11. Vasiliades, J., Wilkerson, K., Ellul, D., Anticoli, M. and A. Rocchini, Gas-Chromatographic Determination of Verapamil and Norverapamil, with a Nitrogen-Selective Detector, Clin. Chem., 28/4, 638-641 (1982).

12. Nelson, K., Woodcock, B.G. and R. Kirsten, Improvement of the Quantitative Determination of Verapamil in Human Plasma, International J. Clin. Pharmacol. and Biopharm., 17(10), 375-379 (1979).

13. Hege, H.G., Gas Chromatographic Determination of Verapamil in Plasma and Urine, Arzneim-Forsch. (Drug Res.), 29, 1681 (1979).

14. McAllister, R.G., Tan, T.G. and D.W.A. Bourne, GLC Assay of Verapamil in Plasma: Identification of Fluorescent Metabolites after Oral Drug Administration, J. Pharm. Sci., 68, 574 (1979).

15. Thomson, B.M. and L.K. Pannell, The Analysis of Verapamil in Postmortem Specimens by HPLC and GC, J. Anal. Toxic., 5, 105 (1981).

16. Harapat, S.R., and R.E. Kates, High-Performance Liquid Chromatographic Analysis of Verapamil, J. Chromatogr., 181, 484 (1980).

17. Kuwada, M., Tateyama, T. and J. Tsutsumi, Simultaneous Determination of Verapamil and its Seven Metabolites by High-Performance Liquid Chromatography, J. Chromatogr., 222, 507 (1981).

18. Lima, C.K., Rideout, J., Rideout, S. and J. Sheldon, Determination of Verapamil and Norverapamil by High-Performance Liquid Chromatography, J. of Liq. Chromatogr.,6(5), 887-893 (1983).

Downloaded At: 15:20 24 January 2011

19. Cole, S.C. and R.J. Flanagan, Rapid High-Performance Liquid Chromatographic Method for the Measurement of Verapamil and Norverapamil in Blood Plasma or Serum, J. Chromatogr., 218, 621-629 (1981).

20. Watson, E. and P.A. Kapur, High-Performance Liquid Chromatographic Determination of Verapamil in Plasma by Fluorescence Detection, J. Pharm. Sci., 70, 800 (1981).

Downloaded At: 15:20 24 January 2011