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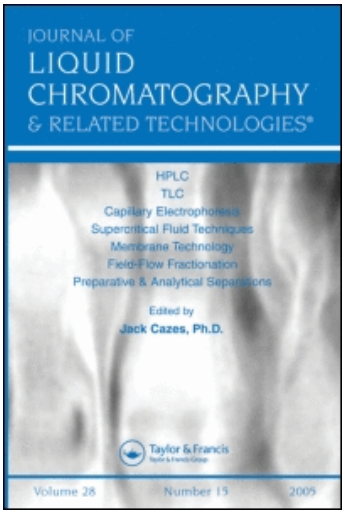


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# DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF TRANDOLAPRIL AND VERAPAMIL IN CAPSULES

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# DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF TRANDOLAPRIL AND VERAPAMIL IN CAPSULES

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## ABSTRACT

The development and validation of an isocratic high performance liquid chromatographic procedure for the determination of trandolapril and verapamil in capsules is reported. The drugs were analysed on a LiChrosorb RP18 column with a mobile phase composed of acetonitrile-methanol-phosphate buffer pH 2.7 (40:40:20) and UV detection at 220 nm. Peak height ratios were linearly related to amounts of the drugs in the range 4–20 $\mu$g/mL.

The inter-day precision (CV) obtained for the standard solutions ranged from 0.40 to 2.18% for trandolapril and from 0.35 to 2.57% for verapamil. The inter-day coefficients of variation for replicate analyses in capsules ranged from 0.5 to 2.49% for trandolapril and from 0.33 to 1.61% for verapamil.

The recovery of analytes after extraction from formulations using the described method, was $99.94 \pm 1.69\%$ and $98.13 \pm 1.20\%$ (mean $\pm$ SD) for trandolapril and verapamil, respectively.



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## INTRODUCTION

Trandolapril ([2S-[1-[R*(R*)],2α,3aα,7aβ]]-1-[2-[[1-(ethoxycarbonyl)-3-phenyl-propyl]amino]-1-oxopropyl]octahydro-1H-indole-2-carboxylic acid), the esterified prodrug of the active metabolite trandolaprilat, is a nonsulfhydryl angiotensin converting enzyme (ACE) inhibitor used in hypertension and congestive heart failure therapy.

Verapamil (α-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methyl-amino]propyl]-3,4-dimethoxy-α-(1-methylethyl)benzene-acetonitrile), a slow Calcium channel antagonist, was introduced as an antianginal agent but now it is widely used in cardiac dysrhythmias. Preclinical trials showed that the combination of trandolapril and verapamil not only had additive effects in reducing elevated blood pressure, but also had a marked beneficial effect on concomitant cardiovascular diseases (1,2).

Therefore, pharmaceuticals containing the combination of both drugs were recently introduced into therapy. The fixed-dose combinations consist of a sustained-release (SR) tablet formulation of verapamil and an instant-release granulation of trandolapril.

We have developed an HPLC method for determination of trandolapril and verapamil, in order to use it for quality control of the final product (capsules).

Pharmaceutical literature about determination of trandolapril is poor. So far it was only analysed by potentiometry using enantioselective membrane electrodes (3,4). Pharmaceuticals containing verapamil are very common, so a variety of methods have been reported so far. Verapamil was determined by UV and VIS spectrophotometry (5–7) and by electrochemical methods (8–10). For estimation of verapamil in drugs, HPLC has been also employed.

Several authors have worked out analysis of enantiomers of verapamil on chiral stationary phases (11–13). Lacroix et al. have modified the USP XXII-method for the assay of purity of verapamil-HCl, and applied this method for the determination of drug content and related compounds in drug raw material (14).

Garcia et al. (15) and Simmons et al. (16) have improved the methods for the separation of selected cardiovascular agents (including verapamil) on C18 and underivatized silica columns, respectively. Kastusi et al. (17) and Tsilifonis et al. (18) have evaluated the determination of verapamil in dosage forms using C18 columns and mobile phases containing acetonitrile, ammonium acetate, and methanol, acetic acid, triethylamine, respectively.

## EXPERIMENTAL

### Reagents

Trandolapril and Tarkar® capsules (containing 2 mg of trandolapril in granulation and 180 mg of verapamil hydrochloride in tablet) were obtained from Knoll

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(Germany). Verapamil hydrochloride from Alfa Chemicals Italiana (Italy) and acetazolamide (the internal standard) from Polfa Starogard (Poland) were applied.

The buffer was prepared with 0.067 M potassium dihydrogen phosphate by adjusting to pH 2.7 with ortophosphoric acid. HPLC grade acetonitrile and methanol were purchased from E.Merck (Germany).

## Apparatus and Chromatographic Conditions

The chromatographic system was comprised of a Model 306 pump and a Model 170 diode array detector (Gilson, USA). Analyses were performed at ambient temperature on a LiChrosorb RP-18 column (250 × 4 mm i.d., 10 $\mu$m). The mobile phase was a mixture of acetonitrile-methanol-buffer pH 2.7 (40:40:20, v/v), filtered and degassed prior to use, and flowing at the rate of 1 mL/min.

The samples were introduced into the column through a high pressure injection valve fitted with a 20 $\mu$L sample loop from Rheodyne (USA). The detection wavelength was 220 nm. The data was collected and analysed with UniPoint™ system software, version 1.80 (Gilson, USA), on Pentium-S 133 MHz computer.

## Preparation of the Standard Solutions

The stock solutions (1.0 mg/mL) of trandolapril, verapamil hydrochloride, and acetazolamide were prepared by dissolving adequate amounts of these substances in methanol. They were stored at 4°C and were stable for at least one month. The working solutions were prepared by diluting the stock solutions with acetonitrile (0.1 mg/mL for trandolapril and verapamil hydrochloride, 0.2 mg/mL for acetazolamide).

For both drugs, 0.2–1.0 mL volumes were pipetted, mixed in 5 mL volumetric flasks with 0.5 mL of acetazolamide solution, and made up with acetonitrile (the calibration standards).

## Preparation of the Capsule Solutions

A group of 20 capsules was accurately weighed. Then each capsule was opened and a tablet with verapamil was accurately separated from granulation containing trandolapril. The tablets and the empty shells were weighed again. For trandolapril, the mean mass of preparation was 0.1008 g, for verapamil hydrochloride, the mean mass of tablet was 0.5653 g. The granulations and the tablets were ground separately to fine powders.

The amounts equivalent to 1 mg of trandolapril were extracted with methanol in 10 mL volumetric flasks and filtered.

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For verapamil hydrochloride, the amounts equivalent to 10 mg of the drug were extracted with 10 mL volumes of methanol. The filtered extracts were diluted ten times with acetonitrile.

The prepared solutions of both drugs in volumes of 0.2, 0.6, 1.0 mL and 0.5 mL of the internal standard solution, were mixed in 5 mL volumetric flasks and completed with acetonitrile (in the same manner, but separately). All samples were injected five times into the column.

## RESULTS AND DISCUSSION

### Optimization of the Chromatographic Conditions

The chromatographic conditions were optimized to obtain an adequate separation of the eluted substances. The influence of percentage of acetonitrile in the binary acetonitrile-phosphate buffer mixtures, and the effect of addition of methanol were investigated. A mixture of acetonitrile-methanol-buffer in the ratio 40:40:20 (v/v) was selected as optimal.

The active substances and the internal standard were quickly eluted and sufficiently separated from each other, and from the solvent front. The retention times were 3.65, 5.01, and 2.65 min for trandolapril, verapamil, and acetazolamide, respectively.

### Calibration and Validation

The linearity of the method was maintained over the concentration range 4–20 $\mu$g/mL for both drugs. The data was subjected to linear-regression analysis in order to obtain the appropriate calibration factors. The calibration curves, based on the peak high ratios, were obtained by triplicate determinations of each of the calibration standards.

The average regression equation for trandolapril was $.y = 0.1023x (\pm 0.0017) - 0.011 (\pm 0.0021)$; the corresponding correlation coefficient was 0.9995. The same factors for verapamil hydrochloride were $y = 0.0738x (\pm 0.001) + 0.021 (\pm 0.0138)$; $r = 0.9996$.

The inter-day precision was assumed by injecting one set of samples (three concentrations) on five separate days. The precision was determined by injecting the samples containing low, medium, and high concentrations of trandolapril and verapamil hydrochloride. The obtained values were compared with theoretical amounts. The assay in the standard solutions showed a sufficient precision of the HPLC system. The coefficients of variation ranged from 0.4 to 2.18% for trandolapril and from 0.35 to 2.57% for verapamil hydrochloride. The results of determinations in the standard solutions are presented in Table 1.

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***Table 1.*** Precision of the HPLC System

| Amount Declared ($\mu$g/mL) | Amount Found (Mean ± SD)[a] ($\mu$g/mL) | | Coefficient of Variation (%) | |
|---|---|---|---|---|
| | T | V | T | V |
| 4.0 | 4.12 ± 0.09 | 3.89 ± 0.10 | 2.18 | 2.57 |
| 12.0 | 11.84 ± 0.08 | 11.92 ± 0.12 | 0.68 | 1.01 |
| 20.0 | 20.02 ± 0.08 | 20.11 ± 0.07 | 0.40 | 0.35 |

[a]n = 5, T-trandolapril, V-verapamil hydrochloride.

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The precision of the method was determined from one lot of the finished product. The assay of contents of both of the drugs in formulations was carried out according to the procedure described in the Experimental section. The inter-day coefficients of variation ranged from 0.5 to 2.49% for trandolapril and from 0.33 to 1.61% for verapamil hydrochloride. The results of determinations are shown in Table 2.

The accuracy of the method was assessed on the basis of determination of trandolapril and verapamil hydrochloride in the model mixtures. They were obtained by inserting known amounts of trandolapril and verapamil hydrochloride into the weighed portions of adequate formulation. The procedure was performed separately, but in the same manner for the both analytes. For each model mixture, determinations were carried out according to the procedure given in the Experimental section. The contents of particular components were determined, and their recoveries were calculated. The results are presented in Table 3.

***Table 2.*** Results of Determinations of Tarka® Capsules (2 mg of Trandolapril and 180 mg of Verapamil Hydrochloride per Capsule)

| Amount Expected ($\mu$g/mL) | Amound Found (Mean ± SD)[a] (mg per Capsule) | | Coefficient of Variation (%) | |
|---|---|---|---|---|
| | T | V | T | V |
| 4.0 | 2.01 ± 0.05 | 178.04 ± 2.86 | 2.49 | 1.61 |
| 12.0 | 1.99 ± 0.01 | 176.42 ± 1.40 | 0.50 | 0.78 |
| 20.0 | 2.00 ± 0.01 | 175.65 ± 0.57 | 0.50 | 0.33 |

[a]n = 5, T-trandolapril, V-verapamil hydrochloride.

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***Table 3.*** Results of Recoveries of Trandolapril and Verapamil Hydrochloride from the Model Mixtures

| Standard Added ($\mu$g/mL) | Recovery (Mean ± SD)[a] (% of Added) | |
|---|---|---|
| | T | V |
| 4.0 | 99.75 ± 2.55 | 101.05 ± 1.59 |
| 8.0 | 101.10 ± 1.23 | 100.18 ± 1.25 |

[a]n = 5, T-trandolapril, V-verapamil hydrochloride.

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## Application of the Method

The elaborated method renders a good separation of trandolapril, verapamil, and the internal standard possible. Because of large differences in concentrations of the drugs in the target pharmaceuticals (2 mg of trandolapril and 180 mg of verapamil hydrochloride in capsule), the content of each capsule was readily separated.

Multicomponent analysis with UV detection at different wavelengths was tested, but the results of these determinations had not been perfect. This was probably due to the UV absorption properties of both compounds. Trandolapril shows the absorption spectrum only in the range 200–300 nm, meanwhile, the absorbance of verapamil in that range is too high. Besides, the portions of the mass capsule (taken as a whole) needed to obtain equivalent amounts of trandolapril were very large and difficult to elaborate.

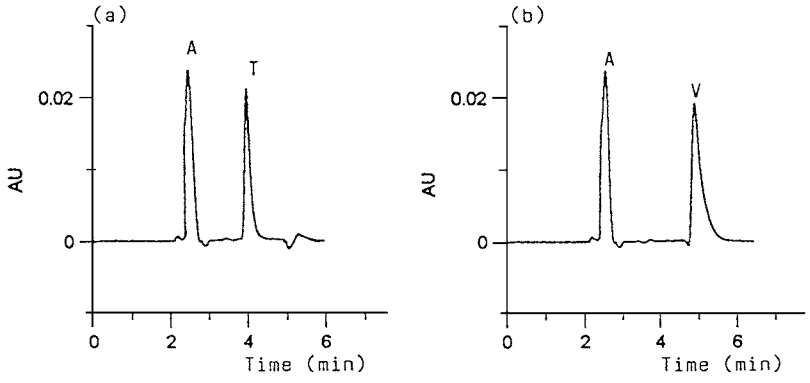


***Figure 1.*** Chromatogram of trandolapril (T), verapamil (V) and the internal standard (A) after extraction from granulation and tablet. Peaks correspond to 12 $\mu$g/mL of trandolapril and verapamil-HCl and to 20 $\mu$g/mL of acetazolamide; HPLC conditions are described in the text.

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Methanol was chosen for extraction of the substances from formulations because it is a good solvent for the analytes. The recoveries after extraction were found to be 99.94 ± 1.69% and 98.13 ± 1.20% (mean ± SD) for trandolapril and verapamil hydrochloride, respectively.

All samples were found to meet the official requirements for labeled drug content. No interferences were noted from excipients, or other inert ingredients, present in the capsules tested. Figure 1 shows typical chromatograms obtained after extraction from the formulations.

In summary, a new, simple, and selective HPLC assay was developed and validated for quantitation of trandolapril and verapamil in combined formulations. The method showed sufficient accuracy and precision, and was successfully applied for pharmaceutical analysis.

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## ACKNOWLEDGMENT

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## REFERENCES

1. The Veratran Study Group. Am. J. Hypertens. **1997**, *10*, 492–499.
2. Kirchengast, M. J. Hypertens. **1997**, *15*, S27–S33.
3. Aboul-Enein, H.Y.; Stefan, R.J.; Van Staden, J.F. Anal. Lett. **1999**, *32*, 623–632.
4. Stefan, R.J.; Van Staden, J.F.; Aboul-Enein, H.Y.; Electroanalysis **1999**, *11*, 192–194.
5. Sun, Y.; Lui, M. Yaowu Fenxi Zazhi **1984**, *4*, 45–46.
6. Sane, R.T.; Kubal, M.V.; Nayak, V.G.; Malkar, V.B. Indian Drugs **1984**, *22*, 25–27.
7. Long, Y.; Feng, J.; Tong, S. Zhhongguo Yiyao Gongye Zazhi **1993**, *24*, 267–270.
8. Leng, Z.; Hu, X.; Yang, G.; Jing, W. Yingyong Huaxue **1993**, *10*, 95–97.
9. Lee, E.Y.; Kim, D.O.; Chang, S.H.; Hur, M.H.; Ahn, M.K. Yakhak Hoechi **1996**, *40*, 135–140.
10. Hassan, S.S.M.; Mahmoud, V.H.; Elmosallamy, M.A.F.; Abdel-Samad, M.S. Mikrochim. Acta **1999**, *131*, 199–203.
11. Clothier, Jr., J.G.; Tomellini, S.A. J. Chromatogr. A **1996**, *723*, 179–187.
12. Williams, K.L.; Sander, L.C.; Wise, S.A. J. Pharm. Biomed. Anal. **1997**, *15*, 1789–1799.
13. Hague, A.; Stewart, J.T. J. Liq. Chromatogr. Relat. Technol. **1998**, *21*, 2675–2687.

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14. Lacroix, P.M; Graham, S.J.; Lovering, E.G. J. Pharm. Biomed. Anal. **1991**, *9*, 817–822.
15. Garcia, M.A.; Solans, C.; Aramayona, J.J.; Fraile, L.J.; Bregante, M.A.; Castillo, J.R. Talanta **1998**, *47*, 1245–1254.
16. Simmons, B.R.; Stewart, J.T. J. Liq. Chromatogr. **1994**, *17*, 2675–2690.
17. Kastusi, K.; Rao, D.S.; Sundaram, R. Indian Drugs **1884**, *21*, 463–465.
18. Tsilifonis, D.C.; Wilk, K.; Reisch, Jr., R.; Daly, R.E. J. Liq. Chromatogr. **1985**, *8*, 499–511.

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