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### DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF VERAPAMIL HYDROCHLORIDE AND TRANDOLAPRIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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#### ABSTRACT

A new reverse phase HPLC method was developed for the simultaneous estimation of verapamil hydrochloride and trandolapril in bulk and pharmaceutical dosage forms. The method was developed and validated using symmetrical C18 column (4.6 x 150mm, 3.5 $\mu$ ) at ambient temperature. The mobile phase consisted of potassium dihydrogen ortho phosphate buffer (pH2.2): acetonitrile [35:65 v/v] at a flow rate of 0.6ml /min and UV detection wavelength was at 230 nm. The retention time for verapamil hydrochloride was 2.5min and trandolapril was at 3.8min. The linearity range of verapamil hydrochloride and trandolapril were in the range of 10 $\mu$ g/ml to 65 $\mu$ g/ml and 2 $\mu$ g/ml to 15 $\mu$ g/ml respectively. The method was validated as per the ICH guidelines and successfully applied to the marketed product. The method was found to be simple, rapid, precise and accurate.

#### KEYWORDS

Verapamil hydrochloride, Trandolapril, HPLC method development and Validation.

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#### INTRODUCTION

Verapamil hydrochloride<sup>1</sup>, verapamil is a calcium ion influx inhibitor (slow, channel blocker or slow calcium ion antagonist) which exerts its pharmacological effects by modulating the influx of ionic calcium across cell membranes of the arterial smooth muscle as well as in conductile and contractile myocardial cells. It is chemically described as benzene acetonitrile,  $\alpha$ -[3-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino] propyl] 3,4dimethoxy- $\alpha$ - (1-methylethyl) hydrochloride (Figure No.1a).

Trandolapril<sup>2</sup> is the ethyl ester prod rug of a nonsulfhydryl angiotensin converting enzyme (ACE) inhibitor, trandolaprilat. It is chemically described as (2S, 3aR, 7aS)-1-[(S)-N-[(S)-1-Carboxy-3-phenylpropyl] alanyl] hexahydro-2-indolinecarboxylic acid, 1-ethyl ester (Figure No.1b.).

Tablet dosage forms containing 240mg of verapamil hydrochloride and 4mg of trandolapril tablets are available in the local market. Literature survey reveals very few chromatographic and spectroscopic methods for the estimation of this drug. So the author has developed a new, simple, precise and accurate validated RP-HPLC method for the estimation of verapamil hydrochloride and trandolapril in bulk and pharmaceutical dosage forms.

## EXPERIMENTAL

### MATERIAL AND METHODS

#### Instruments

The analysis of the drugs was carried out on WATERS HPLC model 2487 Dual  $\lambda$  absorbance Detector containing 515 HPLC pump and Rheodyne injector (7725i) with 20 $\mu$ l fixed loop. Chromatographic analysis was performed by using symmetry C18 column with 150  $\times$  4.6mm internal diameter and 3.5 $\mu$  particle size. Isocratic elution with Potassium dihydrogen ortho phosphate Buffer (pH2.2): acetonitrile: (35:65) was selected with a flow rate 0.6 ml /min. The detection wavelength was set at 230 nm with a runtime of 7 min. The mobile phase was prepared freshly and it was sonicated by using PCI Mumbai 3.5 liters capacity sonicator for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature. Detection wavelength is observed by using UV-3000+LABINDIA double beam with UV win 5 software UV. Spectrophotometer model No.UV-2371. Citizen electronic balance was used for weighing. Global digital pH meter was used.

#### Chemicals and drugs

Acetonitrile, methanol and water were of HPLC grade and ortho phosphoric acid (OPA), pure potassium dihydrogen phosphate and glacial acetic acid AR grade were obtained from Merck, Mumbai India. Verapamil hydrochloride (Ver hcl) and trandolapril (Tra) reference standards obtained as gift samples from Aurobindo Pharmaceuticals Pvt. Ltd., Hyderabad, India. Tablet dosage forms containing 240mg of verapamil hydrochloride and 4mg of trandolapril (TARKA) was procured from the local market.

#### Preparation of mobile phase

The mobile phase was prepared by mixing 0.05M potassium dihydrogen ortho phosphate (2.2 pH) and acetonitrile in the ratio of (35:65% v/v). The solution was then filtered through 0.45 microns membrane filter and degassed.

#### Preparation of 0.05M potassium dihydrogen ortho phosphate

Dissolve 6.8 gm of potassium dihydrogen ortho phosphate in sufficient water to produce 1000ml and the pH was adjusted to 2.2 by using glacial acetic acid.

#### Preparation of standard stock solution

Weigh accurately 240mg of ver hcl and 4mg of tra and transfer to 100ml volumetric flask. Add 30ml of solvent and shake to dissolve the contents completely. Dilute to volume with same solvent. Pipette out 10ml of this and dilute to 100ml. This yielded a solution with nominal concentration 240 $\mu$ g/ml of verapamil hydrochloride and 4 $\mu$ g/ml of trandolapril.

#### Determination of $\lambda_{max}$

The standard solution of verapamil hydrochloride and trandolapril were scanned separately in the wavelength range of 200-400nm and the  $\lambda_{max}$  was found to be 232nm and 228nm for verapamil hydrochloride and trandolapril respectively. The overlay absorption spectrum of verapamil hydrochloride and trandolapril was found that both drugs show appreciable absorbance at 230nm, so it is used for the further study. An overlaid spectrum of verapamil hydrochloride and trandolapril is shown in Figure No.2.

## OPTIMIZED CHROMATOGRAPHIC CONDITIONS

### Stationary phase

Symmetry C<sub>18</sub> column (X Bridge with 4.6 x 150mm, 3.5 μm).

### Mobile phase

Phosphate buffer (2.2 pH): acetonitrile (35:65% v/v)

### Flow rate

0.6ml/min

### Run time (min)

7 min

### Detection

At 230nm

### Injection (volume)

10μl

### Procedure

Mixed standard solutions containing verapamil hydrochloride and trandolapril in the range 10μg/ml to 65μg/ml and 2μg/ml to 15μg/ml were prepared and each solution was injected in to the optimized chromatographic system. The chromatograms were recorded and the peak areas were determined for each concentration of the drug solution. Calibration curve of verapamil hydrochloride and trandolapril was obtained by plotting the peak ratio versus the respective concentrations. The linear correlation coefficient for verapamil hydrochloride and trandolapril was found to be 0.999 and 0.998 respectively. A typical chromatogram is verapamil hydrochloride and trandolapril is shown in Figure No.3.

### Analysis of tablet dosage forms

Twenty tablets containing 240mg of verapamil hydrochloride and 4mg of trandolapril were weighed, and finely powdered. A quantity of powder sample equivalent to 240mg of verapamil hydrochloride and 4mg of trandolapril transferred to 100ml volumetric flask. 30ml of solvent was added and sonicated for 5min to dissolve the contents as completely as possible. Filter 10ml of this resultant solution and dilute to 100ml with mobile phase. This yielded a solution with nominal concentration 240μg/ml of verapamil hydrochloride and 4μg/ml of trandolapril.

The contents of mobile phase were filtered before use through 0.45μ Millipore membrane filter and pumped from the solvent reservoir to the column at specified chromatographic conditions. Prior to the injection of the drugs solutions, the column was equilibrated for atleast 30min with mobile phase flowing through the systems. Then 10μl of standard and sample solution were injected for five times respectively. The chromatograms were recorded and peak responses of verapamil hydrochloride and trandolapril in standard and sample solutions.

## METHOD VALIDATION

The developed method was validated for the parameters listed in ICH guidelines. System suitability parameters were described in the Table No.1.

### Linearity and range

Mixed standard solutions containing verapamil hydrochloride and trandolapril in the range 10μg/ml to 65μg/ml and 2μg/ml to 15μg/ml were prepared and 10μl of each solution was injected in to the optimized chromatographic system. The chromatograms were recorded and the peak areas were determined for each concentration of the drug solution and shown in Table No.2 and 3. Calibration curve of verapamil hydrochloride and trandolapril was obtained by plotting the peak area ratio versus the respective concentrations (Figure No.4 and 5). The regression equation of calibration curve were  $Y=56738X-49522$  for verapamil hydrochloride and  $Y=11621X-17238$  for trandolapril respectively.

### Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day variation). Repeatability was examined by analyzing six determinations of the same batch of each component at 100% of the test concentration which confirms that the method is sufficiently precise. For intermediate precision and intraday precision were performed by determining the corresponding responses in triplicate on the same day and on different days for VER HCL (12, 24, 36 μg/ml) and for TRA (2, 4, 6 μg/ml). The

results are reported in terms of % RSD in Table No.4.

#### **Accuracy**

Recovery studies were carried out by standard addition method at three different levels 50%, 100%, and 150%. VER HCL (12, 24, 36 µg/ml) and for Tra (2,4,6 µg/ml) respectively. The % recovery of VERA HCL and TRA in the sample mixture was determined. The results of recoveries obtained by proposed method were validated by statistical evaluation and are recorded in Table No.5.

#### **LOD and LOQ**

The LOD and LOQ of the developed methods were determined by analyzing progressively lower concentrations of the standard solutions using optimized chromatographic conditions. The minimum concentration of the standard solution, which gave signal to noise ratio of 3 and 10 were taken as the LOD and LOQ values respectively. LOD and LOQ values of verapamil HCl and trandolapril are presented in Table No.6.

#### **Robustness**

Capacity to remain unaffected by small but deliberate variations in method parameters. Comparison results under differing conditions with precision under normal conditions. The results are shown in Table No.7.

### **RESULTS AND DISCUSSION**

To develop the RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with symmetry C18 (4.6 x 150mm, 3.5µ) column and mobile phase comprising of Potassium dihydrogen ortho phosphate (pH2.2): Acetonitrile 35:65% (v/v) at a flow rate of 0.6 ml/min.

To get better reproducibility and repeatability. Quantification was achieved with UV detection at 230nm based on peak area. The retention time for verapamil hydrochloride and trandolapril were found to be 2.5min and 3.8 min, respectively. The optimized method was validated as per ICH guidelines. The system suitability parameters observed by using this optimized conditions were reported. A linearity range of 10-65µg/ml with correlation coefficient 0.999 was established for verapamil hydrochloride and 2-15µg/ml with correlation coefficient 0.999 was established for trandolapril. The results of recovery study (98.44 % verapamil hydrochloride and 98.01 % for trandolapril) suggest that the method has good recovery. The precision of the proposed method was carried in terms of the repeatability, inter-day and intra-day time periods. The low % RSD (<2) values of inter-day (0.21% and 0.25%) and intra-day (0.24% and 0.26%) variations for verapamil hydrochloride and trandolapril, respectively, reveal that the proposed method is precise. The LOD and LOQ values for verapamil hydrochloride was found to be 0.018µg/ml, 0.06µg/ml and for trandolapril was 0.05µg/ml, 0.19µg/ml. The results of robustness in the present method showed no significant changes. The results of analysis of tablet indicated that no interference due to common tablet excipients was observed with the developed method. Therefore, the proposed method can be used for routine analysis of two drugs in their combined pharmaceutical dosage form.

**Table No.1: System suitability parameters**

S.No	parameters	Verapamil hydrochloride	Trandolapril
1	Theoretical plates	4984	4256
2	Resolution	---	5.0
3	Tailing factor	1.4	1.4
4	Retention Time (min)	2.5	3.8
5	Percent RSD	---	---
6	Intraday (n=3)	0.646	0.30
7	Inter day (n=3)	0.66	0.75

**Table No.2: Linearity range of verapamil hydrochloride**

S.No	Linearity Level	Concentration	Area of VER
1	I	12µg/ml	262120
2	II	24µg/ml	836426
3	III	36µg/ml	1492439
4	IV	48µg/ml	2183380
5	V	60µg/ml	2901438
6	VI	72µg/ml	3650958
Correlation Coefficient			0.999

**Table No.3: Linearity range of trandolapril**

S.No	Linearity Level	Concentration	Area of TRA
1	I	2µg/ml	90239
2	II	4µg/ml	278570
3	III	6µg/ml	498282
4	IV	8µg/ml	749949
5	V	10µg/ml	988812
6	VI	12µg/ml	1240721
Correlation Coefficient			0.998

**Table No.4: Precision of proposed method**

S.No	Injection	Area of Verapamil Hcl	Area of Trandolapril
1	Injection-1	1489301	494736
2	Injection-2	1492080	497000
3	Injection-3	1489889	496238
4	Injection-4	1493862	497617
5	Injection-5	1484376	494658
6	<b>Average</b>	1489901	496949
7	<b>Standard Deviation</b>	3582.5	1328.3
8	<b>% RSD</b>	0.24	0.26

**RSD: Relative standard deviation**

**Table No.5: Recovery Results**

S.No	Sample	Accuracy	Standard Drug	Formulation	% of recovery	Standard deviation
1	Verapamil hydrochloride	50%	12	12	99.10%	SD=0.032 % RSD=0.03
		100%	24	24	97.58%	
		150%	36	36	98.80%	
2	Trandolapril	50%	2	2	98.05%	SD=0.045 % RSD=0.04
		100%	4	4	98.03%	
		150%	6	6	98.03%	

\*n=3, RSD - Relative standard deviation.

**Table No.6: Results of LOD and LOQ**

S.No	Parameter	Verapamil hydrochloride ( $\mu\text{g/ml}$ )	Trandolapril ( $\mu\text{g/ml}$ )
1	LOD	0.018 $\mu\text{g/ml}$	0.05 $\mu\text{g/ml}$
2	LOQ	0.06 $\mu\text{g/ml}$	0.19 $\mu\text{g/ml}$

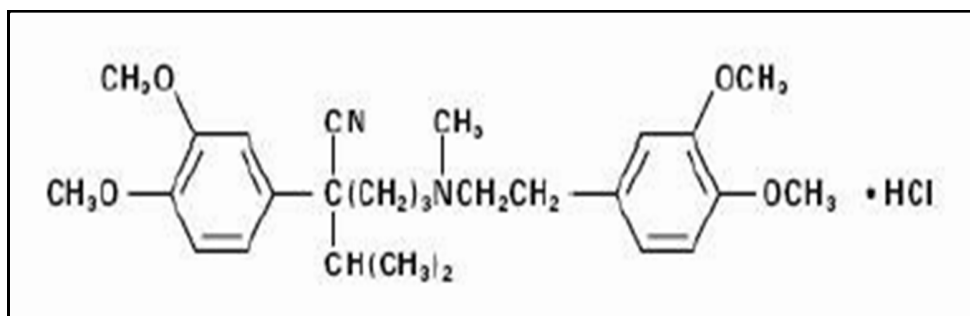
\*LOD-Limit of detection, LOQ-Limit of quantitation

**Table No.7: Robustness Results for verapamil hydrochloride**

S.No	Condition	Variation	Average area		% RSD	
			VER	TRA	VER	TRA
1	Mobile phase phosphate buffer (pH2.2): acetonitrile: (35:65)	phosphate buffer (pH2.2): acetonitrile (34:66)	1448116	509006	1.854	0.957
		phosphate buffer (pH2.5): acetonitrile (35:65)	1489901	496049	1.548	1.456
		phosphate buffer (pH2.0): acetonitrile(36:64)	1491885	511846	1.673	1.665
2	Flow rate 0.6 ml/min	Less flow 0.5ml/min	1521762	512147	0.98	1.95
		Actual Flow 0.6ml/min	1489901	496049	1.69	1.80
		More Flow 0.7 ml/min	1469468	493457	1.76	2.02

**Table No.8: Results of analysis of formulation and recovery study of the proposed method**

S.No	Formulation	Labeled claim (mg)	% of Assay
1	TARKA	VER -240 $\mu\text{g/ml}$ TRA- 4 $\mu\text{g/ml}$	98.44 98.01



**Figure No.1a: Verapamil hydrochloride chemical structure**

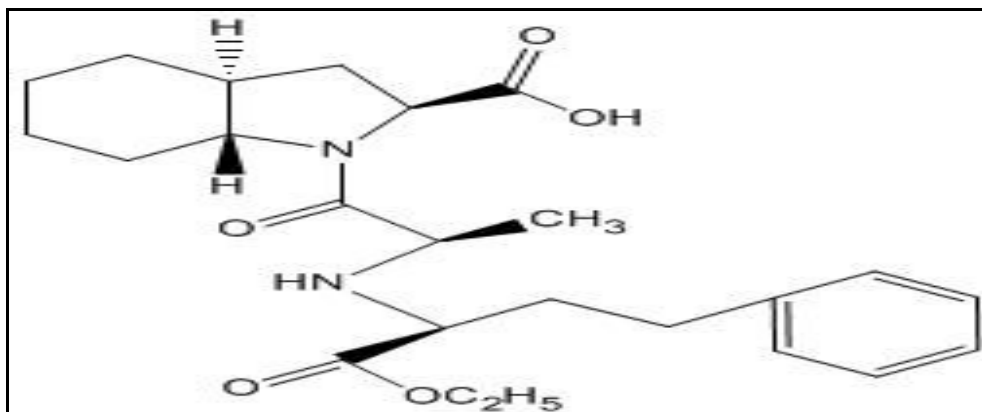


Figure No.1b: Trandolapril chemical structure

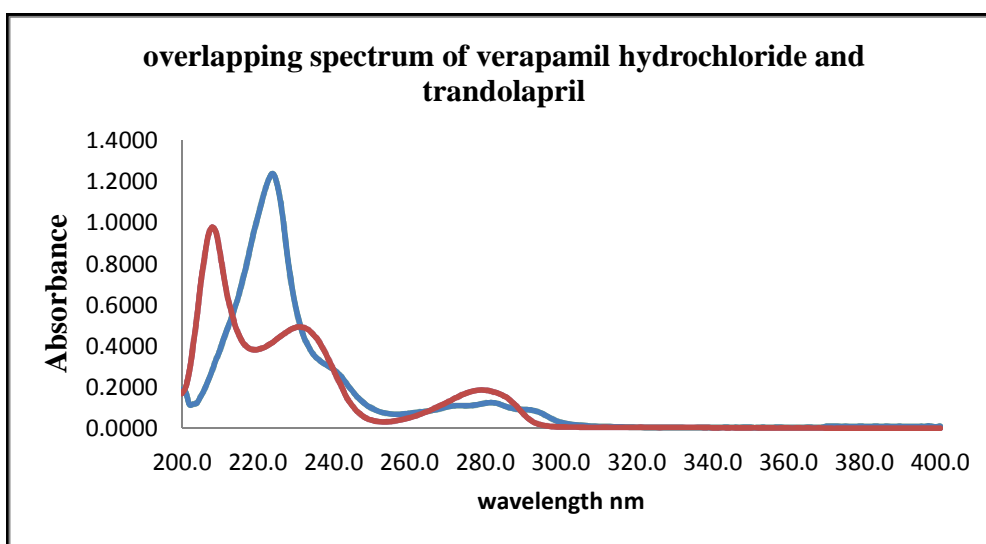


Figure No.2: Overlay spectra of verapamil hydrochloride and trandolapril

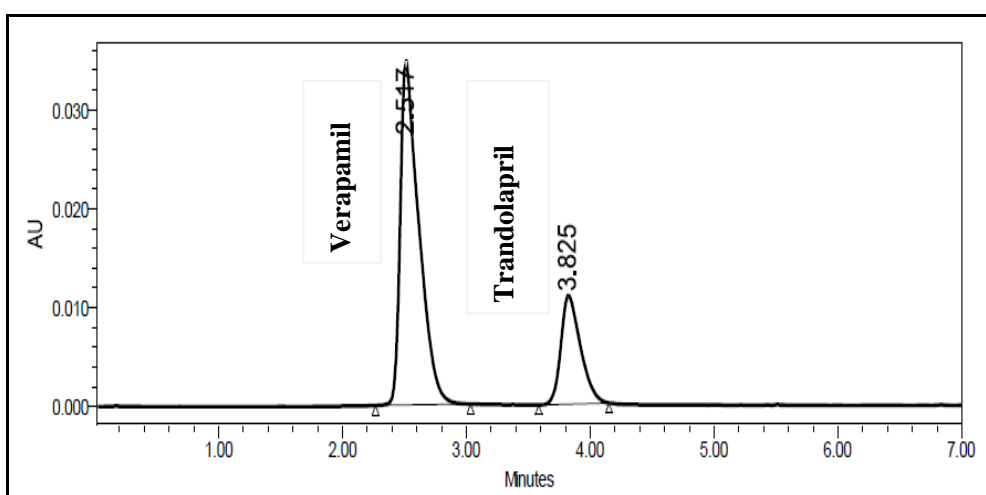


Figure No.3: Standard chromatogram of verapamil hydrochloride and trandolapril

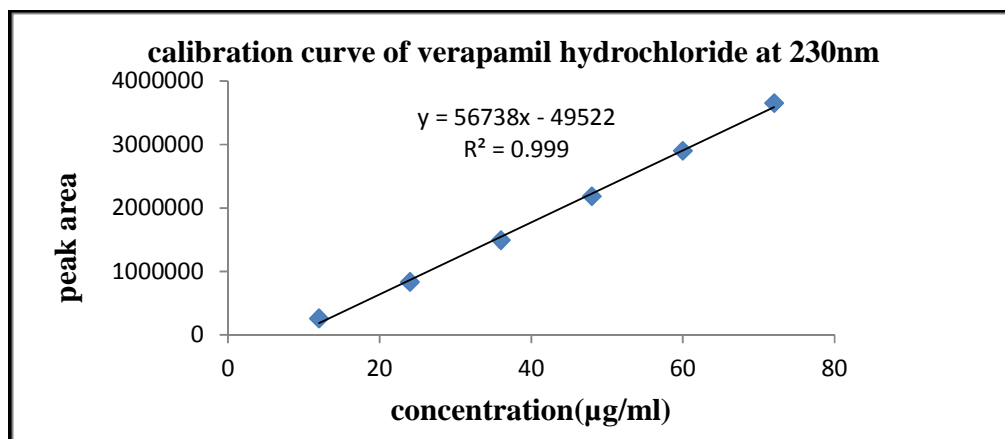


Figure No.4: Calibration curve of verapamil hydrochloride

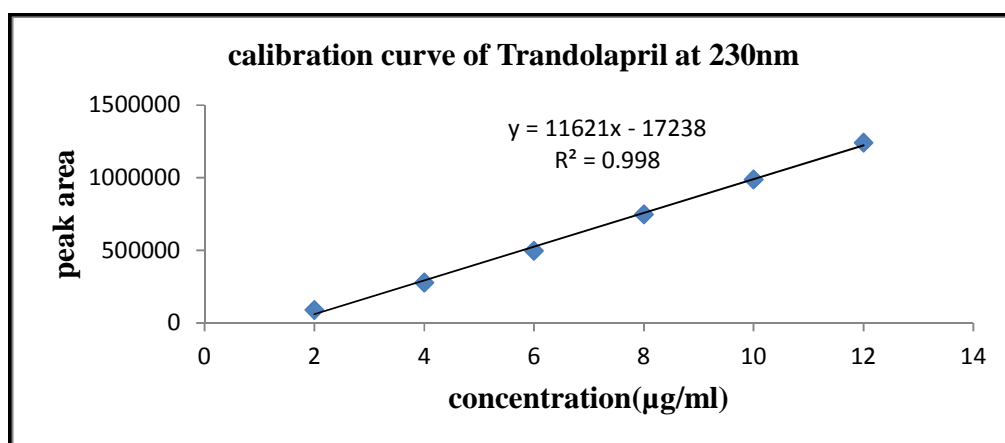


Figure No.5: Calibration curve of trandolapril

## CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Verapamil Hydrochloride and Trandolapril from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Verapamil Hydrochloride and Trandolapril in pure form and its dosage form and also can be used for dissolution or similar studies.

From the experimental studies it can be conclude that HPLC and spectrophotometric methods are developed for the simultaneous estimation of Verapamil Hydrochloride and Trandolapril.

However, this method is more reproducible. Results of validation parameter demonstrate that this analytical procedure are suitable for its intended purpose and meets the criteria defined in the ICHQ2A/B.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.



## BIBLIOGRAPHY

1. Gumieniczek and Hopkala H. Development and Validation of a Liquid Chromatographic Method for the Determination of Trandolapril and Verapamil in Capsules, *Journal of Liquid Chromatography and Related Technologies*, 24(3), 2001, 393-400.
2. Mehmet Gumustas. Simultaneous Determination Verapamil and Trandolapril in Pharmaceutical formulations using Liquid Chromatography, *Research Journal of Chemistry and Environment*, 7(4), 2004. FDA Guidelines on General Principles of Process Validation, 1987.
3. Schwedt Georg. The Essential Guide to Analytical Chemistry, (Brooks Haderlie, Trans.) Chichester, NY: Wiley, Original Work Published 1943, 1997, 16-17.
4. Munson J W. Pharmaceutical analysis, Modern Methods, *International Medical Book Distributors, New Delhi, Part-B*, 2001, 51-54.
5. Heftman E. Chromatography-fundamentals and applications of chromatography and related Differential migration methods, *Elsevier, Amst.69A, erdam*, 6<sup>th</sup> edition, 2004, 253-291.
6. Veronika R, Meyer. Practical High-Performance Liquid Chromatography, *John Wiley and sons Pvt Ltd, New York*, 2004.

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