VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DROTAVERINE AND PARACETAMOL AND ITS APPLICATION IN DRUG FORMULATION

Navin Raj, Lucky Soni, Chanchal Raj, Sayyed Nadeem, Aparna Venugopal and Kuldeep Chouhan*

Technocrats Institute of Technology-Pharmacy, Bhopal, Madhya Pradesh, India

*Corresponding author

Kuldeep Chouhan, Technocrats Institute of Technology Pharmacy,

Bhopal- 462 001 (M.P.), India, Tel: +91-755-2684535; Fax: +91-0755-2751679
e-mail: kuldeepchouhan@live.com (Kuldeep Chouhan)

Summary

A simple, specific, accurate, precise and reproducible method has been developed and validated for the simultaneous estimation of Drotaverine (DTV) and Paracetamol(PCM) in combined dosage form by RP-HPLC method. RP-HPLC estimation of drugs in selected combination was done using Phenomenex ODS 5µ, C18 column (250×4.6mm) and Methanol: water (65:35) as mobile phase which shows sharp and resolved peak when detected at 230 nm. The linearity range was found to be in concentration range 16-80 µg/mL for DTV and 100-500 µg/mL for Paracetamol. The retention time for DTV and PCM were 3.9 and 1.89 respectively. The correlation coefficient was found to be 0.9989 (for DTV) and 0.9992 (for PCM). The mean percentage recovery was found to be 100.62 and 99.42 for DTV and PCM respectively. The % estimation of the drugs was found near to 100 % representing the accuracy of the method. Validation of the proposed method was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed method can be successfully applied in routine work for the determination of DTV and PCM in combined dosage form

Key words: Drotaverine (DTV), Paracetamol (PCM), RP-HPLC Method development, Validation.

Introduction

Drotavarine is (1Z)-1-[(3,4-diethoxyphenyl)methylidene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline Drotavarine (INN, also known as drotaverin) is an antispasmodic drug, structurally related to papaverine. DTV is a selective inhibitor of phosphodiesterase 4, and has no anticholinergic effects [1-2].PCM N-(4-hydroxyphenyl)acetamide is non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. Inhibition of prostaglandin production around the body by blocking the cyclooxygenase enzymes known as COX-1 and COX-2 has long been known to be the mechanism of action of PCM and other non-steroidal anti-inflammatory drugs (NSAIDs)[3-4]. Both drugs are insoluble in water and their chemical structures are shown in **Fig. 1 (a)** and **(b)**

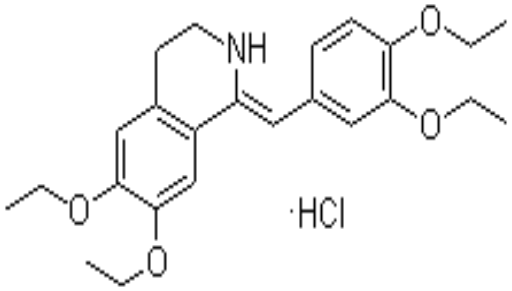

Fig 1(a) Chemical structure of DTV
(1Z)-1-[(3,4-diethoxyphenyl)methylidene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline

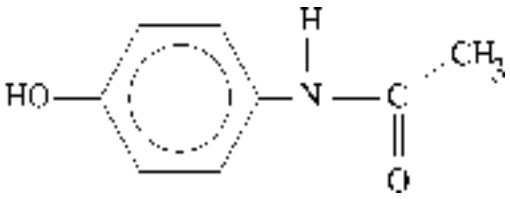

Fig.1 (b) Chemical structure of PCM
N-(4-hydroxyphenyl) acetamide

Many analytical methods like simultaneous estimation of DTV and PCM in tablet dosage form by spectrophotometric quantitative estimation[5], spectrometric determination of DTV and PCM by ratio absorption methods area under curve second order[6], estimation of DTV with other combination[7-12],simultaneous determination of DTV and PCM by RP-HPLC method[13]and other RP-HPLC method reported for estimation of DTV with other combination[14-17]. Analytical method by RP-HPLC has been reported for the combination but due to the use of expensive chemicals the method was costly [18]. The aim of the present work is the development and validation of a simple and reliable RP-HPLC method for the simultaneous determination of DTM and PCM in their combined tablet formulation, and its application to the determination of both analyte in commercial brand of their combined tablet formulation. Hence, an attempt was made in this study to develop a rapid, economical, precise and accurate method for simultaneous estimation of DTV and PCM in tablet formulation by RP-HPLC.

Materials and methods

Chemicals and reagents

All experiments were performed with pharmaceutical-grade DRO and PCM. HPLC-grade solvents were employed for analysis. Solvents were filtered through 0.45 μm membrane filters. All dilutions were performed in standard volumetric flasks. The pharmaceutical preparations, declaring to contain 80 mg DTM, 500mg PCM and excipients, were obtained from a local drugstore.

Instrumentation and chromatographic conditions

The separations were performed with a Schimadzu R 1100 series liquid chromatograph consisting of quaternary pumps, a injector fitted with a 20 μl loop. Compounds were separated on a 250 mm×4.6 mm C8 column (Luna, Phenomenex, 5μm particle size). The mobile phase was a methanol:water (65:35) pumped at a flow rate of 1.0 ml min−1. Chromatograms were recorded employing lab solutions software.

Mix Standard solution

Mix Stock solution containing DTV and PCM was prepared in methanol having concentration 80 μg/mL DTV and 500 μg/ mL PCM. Aliquot of the standard solution was appropriately diluted with the mobile phase containing, methanol and water in the ratio (65:35 v/v) to get the concentration of 40 μg/mL for DTV and 250 μg/mL for PCM respectively.

Procedure

The optimized chromatographic condition mentioned below was kept constant throughout the experimentation and mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.
Column - Phenomenex ODS 5 μ C8 column (250 X 4.6mm)
Flow rate - 1.0 mL/min
Temperature: Ambient - (28-300 C)

A 20 μL solution of above mix standard was injected through manual injector and chromatogram was recorded using mobile phase containing, Methanol and water (65:35). DTV and PCM were resolved properly with sharp peak and showing reasonable retention time in the above selected mobile phase. A chromatogram for both drugs so recorded in shown in F**ig 1.**

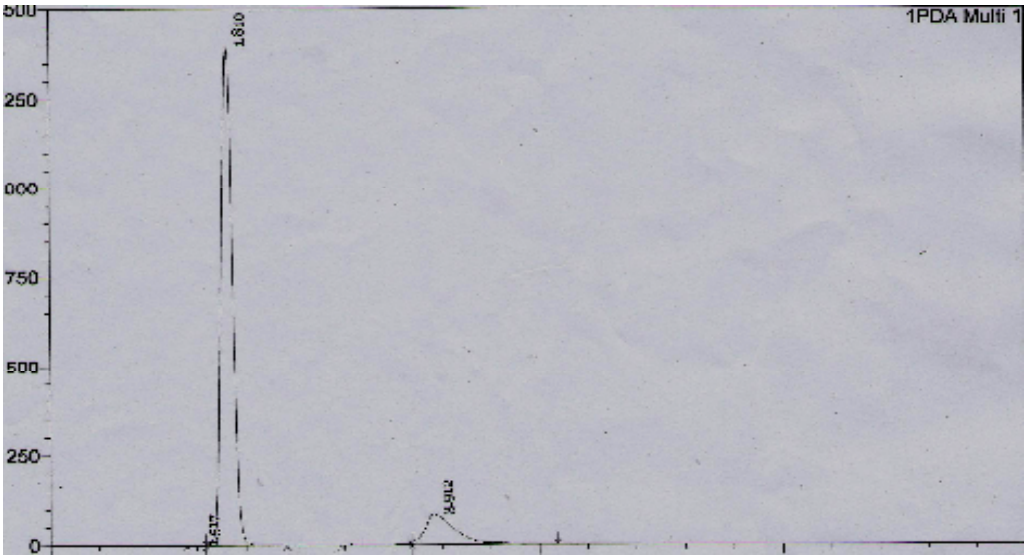

Fig no.1 chromatogram for DTV and PCM

Study of system suitability parameters

After equilibration of column with mobile phase, seven replicate injections of 20 µL solution of mix standard solution was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in **Table 1.**

Table no. 1 Study of system suitability parameters

Sr. No.	A.U.C. of DTV	A.U.C of PCM
Mean	25562553	6534226
% RSD	1.3	0.8
Retention time	3.90	1.81
Asymmetry	1.091	1.110

Study of Linearity Range

Aliquots of mixed standard stock solution were diluted with mobile phase; volume was made up to mark with mobile phase to obtain concentration 16µg/mL to80µg/mL for DTV and 100µg/mL to500µg/mL for PCM respectively. The graphs of concentration of drug vs. area under curve were plotted for both the drugs. The linearity curve for DTV and PCM were shown in **Fig no.2** and **3**. The results show excellent correlations within the tested concentrations ranges.

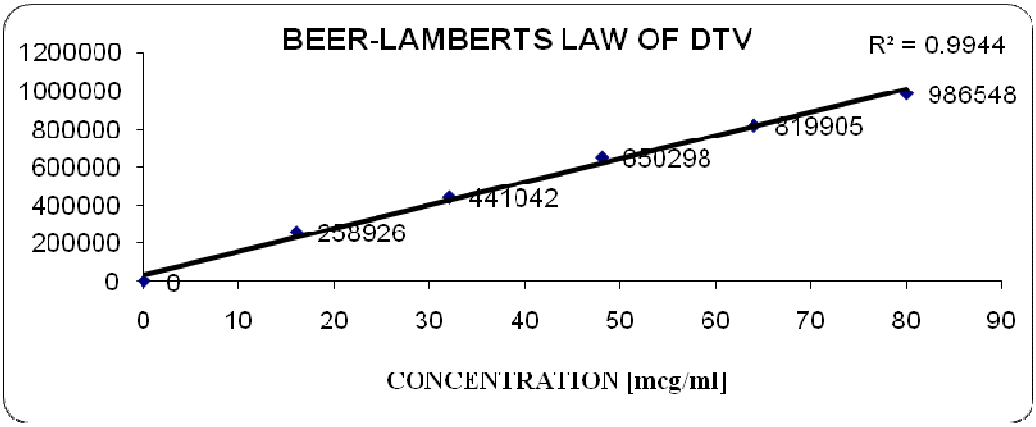

Fig-2 Linearity calibration curve of DTV

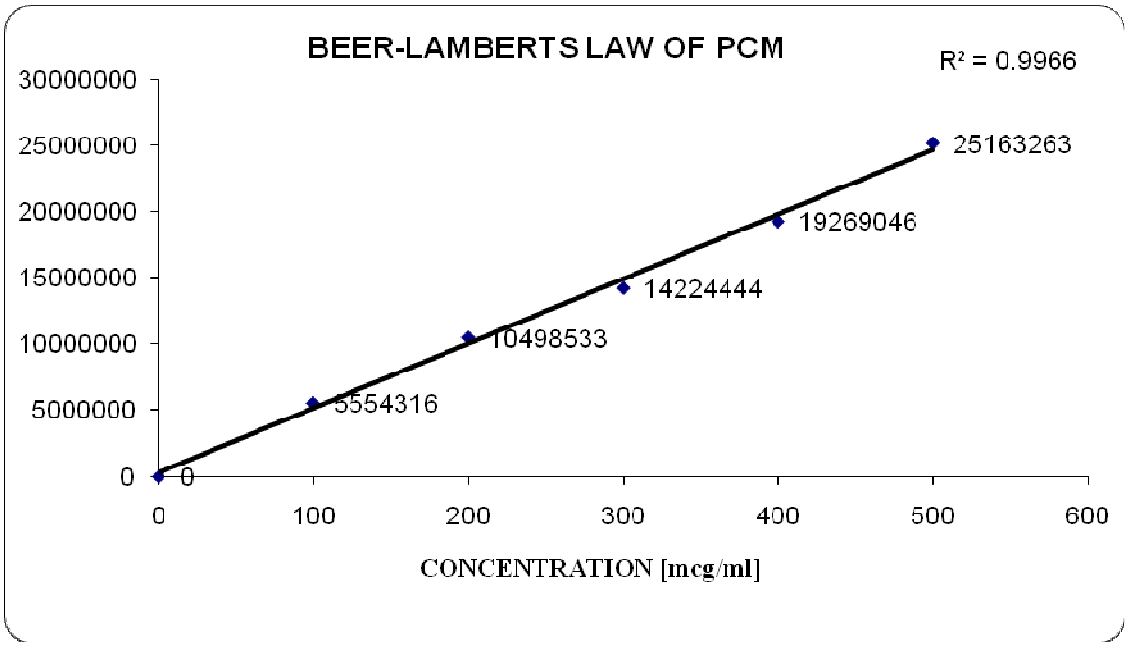

Fig-3 Linearity calibration curve of PCM

Assay in Marketed Formulation

Twenty tablets were weighed and average weight was calculated. An accurately weighed quantity of tablet powder shown in **Table no.2** was transferred in 100 ml of volumetric flask ,shaken for 30 min. with sufficient quantity of methanol and the volume was made up to the mark with methanol. The contents of the flask were filtered through Whatmann filter paper (no. 41) and diluted 10 ml of each up to 100ml with mobile phase. Final sample solution was injected separately, the chromatograms were recorded and the content of DTV and PCM were calculated.

Table no.2 Analysis of tablet formulation

	DTV	PCM
Mean	99.16	99.08
±SD	0.181	0.262

Validation

Accuracy:

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analysed sample and contents were reanalyzed by proposed method and the mean % recoveries were found. The results are shown in **Table 3**

Table 3: Results of Recovery Studies

S.No.	Wt. taken (in mg)	Amt. of pure drug added (mg)		Amt. recovered (mg)		%Recovery	
		PCM	DTV	PCM	DTV	PCM	DTV
1.	150	100	16	99	16.0	99.0	100
2	150	199	31	198	30.6	99.49	98.7
3	150	301	48	300	47.4	99.16	99.16
4	150	400	64	399	63	99.75	98.43
Mean						99.35	99.07
±SD						0.335	0.692
%RSD						0.337	0.698

Stability studies:

The forced degradation studies were carried out at 500 ^{0}C using 1ml of 1N NaOH, 1N HCL, 6% $H_2O_2$and the chromatograms recorded .Volumes were made up to the mark with methanol, further aliquots were diluted with mobile phase and sample solutions were injected separately and chromatograms under stress conditions were recorded. The results show slight difference in the percent label claim as compared with normal condition. In all the stress condition DTV was found to be more sensitive. Results are shown in **Table 4**

Table 4: Results of specificity studies

S.NO.	Conditions	% Label Claim	
		PCM	DTV
1.	1 N NaOH	83.3	50.4
2.	1 N HCl	88.2	62.5
3.	10% H_2O_2	71.07	39.5
4.	Light treatment	93.31	68.5

Precision and Intermediate precision

(Intra day and Inter day) shows the % Label claim values within limits (% R.S.D.not more than 2). The method was found to be précised. The ruggedness studies were carried out using different analyst variation. The results of intermediate precision and ruggedness parameter are shown in **Table 6.**

Table 6: Results for interday and intraday studies

	INTER-DAY		INTRADAY	
	DTV	PCM	DTV	PCM
Mean	98.74	98.83	98.96	98.81
SD	0.346	0.352	0.321	0.364

Limit of detection

Limit of detection for PCM and DTV was found to be 0.062µg/ml and .010 µg/ml respectively.

Conclusion

A simple and efficient HPLC method has been developed and validated for the isocratic separation and simultaneous determination of DTV and PCM in their combined dosage form. The method, suitable for routine quality control, has been successfully applied to the determination of both analytes in their commercial brand of tablet containing this pharmacological association. From the results it was evident that method is more precise, accurate and inexpensive from the previously reported methods.

Acknowledgement

The authors are thankful to the management and Director Dr.A.Balasubramaniam Technocrats Institute of Technology- Pharmacy, Bhopal.

References

[1] The Merck Index, 13th edition, Merck Research Laboratories, 609, 1174
[2] Singh K C, Jain P, Goel N, Saxena A,International Journal of Gynecology and Obstetrics" 2004; 84 (1); 17–22.
[3] British Pharmacopoeia, Vol-I, 2002,1215
[4] KD Rains, Curr Med. Res. Opin,2006; 22 (6); 1161-1170.
[5] Mahaparale S, Telekone R, Raut R, Danle S, Kasture P, Indian Journal of Pharmaceutical Science,2010;72(1):133-136.
[6] Vikram G M, Dipali D T, Kunal D I, Amruta S B, Vishnu P C, Bhanudas S K, International Journal of Pharmaceutical Science, published jul-aug, 2010;3(1):111-115.
[7] Mahajan V K, Dahivelkar P P, Bari S B, Indian Journal of Pharmaceutical Science,2007:69(6):812-814.
[8] Vivek S R, Santosh V G, Upasna P P, Mahima R S., Eurasian Journal of Analytical Chemistry, 2010;4(2):184-190.
[9] Rajesh S, Geetam P, Mishra G P, Sainy J, J.Pharma Sci.and Res.2010;2(12):821-826.
[10]Laxmi K S, and Tintu.T, International Journal of Pharmacy and Pharmaceutical Sciences,2010;2(4):166-168
[11]Ramesh S, Lokesh B, Rupali J, and Prashant L, Journal of Chemical Metrology, 2010;4(1): 21-27.
[12]Kalra K, Naik S, Jarmal G and Mishra N, Asian Journal Research in Chemistry,2009; 2(2): 93-99
[13]Pathade A, Dakhore A, Fule S, Alaspure R, International Journal of Pharmaceutical Sciences,2010;2(3):904-908.
[14]Prasad K R, and Sharma R, Scholar Indian Journal of Pharmaceutical Science and Research, 2002;131:59-64.
[15]Kabeer A S, Sachin D P, Der Pharmacia Lettre, 2010;2(4):355-364.
[16]Gandhi S, Deshpande P, Ramjane v, Parab J, International Journal of Pharmaceutical Sciences, 2010;4(3):49-52.
[17]Permanand DC, Senthikumar KL, Senthikumar B, Thirumrty R, Der Pharma Chemica , 2010;2(5):170-177.