



Development of a chromatographic Technique for Quantification of Drotaverine Hydrochloride and Diclofenac Potassium

**DILLIP KUMAR DASH^{1,2*}, ASHIM KUMAR SEN¹, S. SAISIVAM²,
AARTI S. ZANWAR¹, SHIVKANT PATEL¹ and DHANYA B. SEN¹**

¹Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University,
At & Po. Piparia, Vadodara, Gujarat, 391760, India.

²N. R. Vekaria Institute of Pharmacy, C L College campus, Bilkha road,
Junagadh, Gujarat, 362001, India.

*Corresponding author E-mail: dillip_dsh@yahoo.co.in

<http://dx.doi.org/10.13005/ojc/390420>

(Received: May 06, 2023; Accepted: August 05, 2023)

ABSTRACT

A straightforward RP-HPLC approach has been developed and validated for the simultaneous estimation of Drotaverine hydrochloride (DROTA) and Diclofenac potassium (DICLO) in their combined dosage form. This method was found to be simple, accurate, and precise. Chromatographic detection has been carried out on a PhenomenexC18 column (250 mm×4.6 mm, i.d, 5 μm) with a flow rate of 1.0 mL/min using an isocratic mobile phase consisting of Methanol and Water (80:20v/v, pH3 adjusted with ortho phosphoric acid). The wavelength 280nm was chosen for the UV detection. The separation of DROTA and DICLO took less than ten minutes, had a good resolution, and produced very little tailing. There was no interference from any excipients. The method was validated in accordance with the ICH recommendations, and the criteria for accuracy, precision, linearity, and system adaptability were all satisfactory in each and every one of the cases.

Keywords: Drotaverine hydrochloride, Diclofenac potassium, RP-HPLC, Validation.

INTRODUCTION

The benzyl isoquiniline derivative Drotaverine Hydrochloride (DROTA) has parasympholytic action. It may relieve pain without causing drowsiness or loss of awareness. The spasmolytic activity of this drug act by inhibiting the phosphodiesterase-4. It shows clinical importance in various disease associated with smooth muscle spam¹⁻³. The phenylacetic acid

derivative, Diclofenac Potassium (DICLO) is classified as a non-steroidal anti-inflammatory medication acts by inhibiting cyclooxygenase enzyme, which is the key enzyme responsible for the synthesis of prostaglandins (PGs). PGs play a role not only in the inflammatory response but also in the transmission of pain signals.⁴⁻⁶

Based on the reported articles DROTA can



be determined in pharmaceutical formulations using a number of different UV and HPLC procedures, both alone and in combination with other medications⁷⁻¹¹. Several UV and chromatographic approaches have been developed for estimating DICLO in pharmaceutical formulations in conjunction with other medicines¹²⁻¹⁶. An RP-HPLC technique was used to evaluate DROTA and DICLO in human plasma simultaneously¹⁷. Various spectrophotometric methods like 1st order Spectra, absorption corrected method and area under curve; and HPTLC had been developed for combination of DROTA and DICLO¹⁸⁻²⁰. From the literature survey, it is concluded that simultaneous analysis of DROTA and DICLO in drug formulation by RP-HPLC is not available. Hence, an attempt was made in this study for simultaneously estimation of dosage form by RP-HPLC.

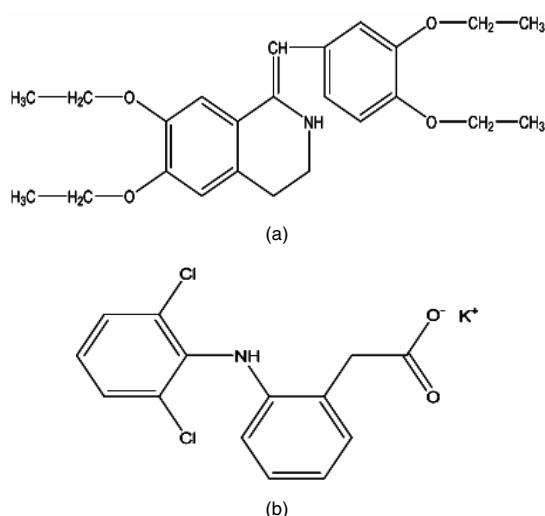


Fig. 1. Structural Formula of (a). Drotaverine Hydrochloride
(b). Diclofenac Potassium

MATERIALS AND METHODS

Ingredients Used

Active pharmaceutical ingredients of Drotaverine Hydrochloride and Diclofenac Potassium were purchased from Yarrow Chemicals, Mumbai, India. Throughout the course of the research, AR grade distilled water, methanol, and orthophosphoric acid were all utilized.

Instrumentation and Chromatographic conditions

A highly sensitive Adventurer-Pro, AVG264C electronic balance, the HPLC (shimadzu) outfitted with LC-20 AT binary pump, SPD-20A PDA detector and LC solution as software for the

investigation. For the separation, a Phenomenex C18 column (250mm×4.6mm i.d., 5 μm) was used, and the injector volume was 20 μL. In order to carry out the chromatographic separation, a mobile phase that consisted of methanol and water that had been adjusted to a pH of 3 using orthophosphoric acid (80:20) was employed at a flow rate of 1.0 mL/minute. The peak area of the effluent was measured using a PDA detector, while it was being observed at 280nm.

Preparation of Mobile Phase

Mix Methanol and Water in the proportion of 80:20(v/v), and modify the pH to 3 with orthophosphoric acid. Sonicate it for 30 min before passing it through a 0.2μ membrane filter. The mobile phase is used throughout the process as diluents.

Stock solution preparation for standard

Weigh 40 mg DROTA, add 30 mL diluent, sonicate for 20 min & make up the volume to 50 mL with diluent to produce standard stock solution of 800 μg/mL. Weigh 25 mg DICLO, add 30 mL diluent, sonicate for 20 min & make up the volume to 50 mL with diluent to produce standard stock solution of 500 μg/mL. Withdraw 10 mL of standard stock solution of DROTA and DICLO, transfer into 100 mL volumetric flask and dilute upto mark with diluent. It gives 80 μg/mL of DROTA and 50 μg/mL of DICLO.

Preparation of Calibration curve

The linearity of the HPLC method for analysis of DROTA and DICLO were assessed and evaluated by analyzing a series of different concentrations of DROTA and DICLO standard solutions. In which the reasonable linearity was achieved in the range of 16-48 μg/mL for DROTA and 10-30 μg/mL for DICLO. Peak areas of these solutions were measured at 280nm.

Stock solution preparation for formulation sample

Weigh accurately 20 tablets and calculate the average weight. Triturate them in glass mortar. Powder equivalent to 80 mg of DROTA and 50 mg of DICLO was weighed and transferred into the 100 mL of volumetric flask, add 60 mL diluent and sonicate it for 30 minutes. Filter the solution through 0.2μ membrane filter and dilute upto mark with diluent. The solution was further diluted to obtain the conc. 80 μg/mL of DROTA and 50 μg/mL of DICLO.

Method validation²¹⁻²³

Linearity and Range

When calculating the linearity of the

response, the concentration limits of 16-48 $\mu\text{g/mL}$ for DROTA and 10-30 $\mu\text{g/mL}$ for DICLO were employed. After visualizing peak regions against concentration on the calibration curve, we were able to determine the correlation coefficient for DROTA and DICLO as well as the equation for the regression line. The linearity was quantified using the correlation coefficient of linear regression line.

Precision

Repeatability

The repeatability of the synthetic combination was evaluated based on the findings of six separate experiments by taking a solution containing 16 $\mu\text{g/mL}$ of DROTA and 10 $\mu\text{g/mL}$ of DICLO from working standard solution.

Intra-day precision

Triplicate samples of solutions containing 24:15, 32:20 and 40:25 $\mu\text{g/mL}$ of DROTA and DICLO were analyzed on the same day to determine the intraday precision.

Interday precision

Triplicate samples of solutions containing 24:15, 32:20 and 40:25 $\mu\text{g/mL}$ of DROTA and DICLO were analyzed on the three separate days to determine the interday precision.

Accuracy

The recovery study was carried out to test the reliability of the procedure. Increasing aliquots of standard stock solution (0, 0.16, 0.20, and 0.24 mL of 800 $\mu\text{g/mL}$ of DROTA and 0, 0.16, 0.20, and 0.24 mL of 500 $\mu\text{g/mL}$ of DICLO) were introduced simultaneously to a constant amount of preanalyzed sample (2.0 mL) in a 10 mL vol. flask. The vol. was adjusted with mobile phase. The measured concentration and the actual concentration were compared.

LOD and LOQ

The LOD and LOQ were estimated by

$$\text{LOD} = 3.3 \times \text{SD/Slope}$$

$$\text{LOQ} = 10 \times \text{SD/Slope}$$

Estimation of marketed formulation

2 mL was drawn from the sample stock solution and volume was adjusted up to 10 mL with mobile phase. The peak area was measured at 280nm.

RESULTS AND DISCUSSION

Mobile phase optimisation

Different solvent systems were tried for

separation of DROTA and DICLO. Separation was achieved in mobile phase, Methanol and Water in the proportion of 80:20(v/v), and modifies the pH to 3 with orthophosphoric acid. The Retention times were found to be 2.003 minute for DROTA and 6.820 for DICLO. Chromatograms of DROTA and DICLO were shown in Figure 2.

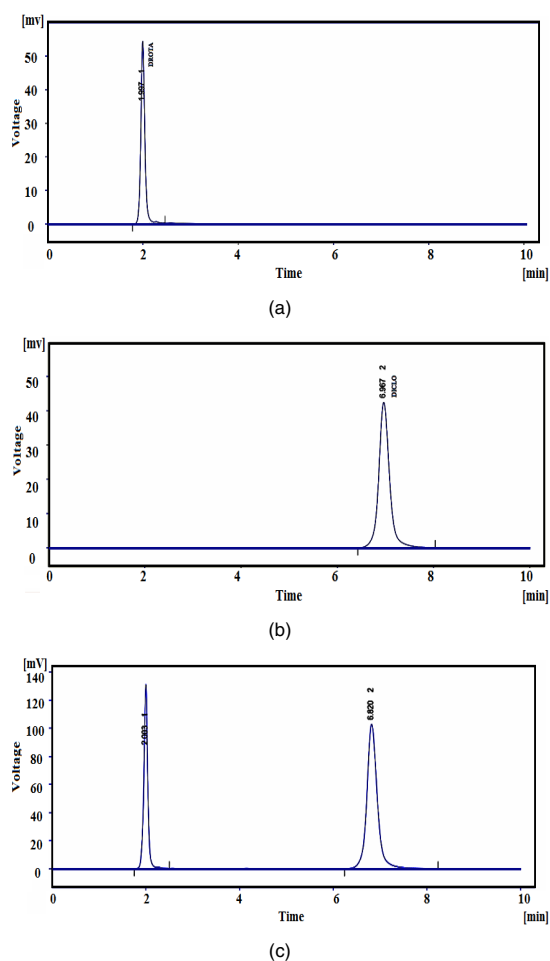


Fig. 2(a). Chromatogram of Drotaverine Hydrochloride (b). Chromatogram of Diclofenac Potassium (c). Chromatogram of Drotaverine Hydrochloride (RT: 2.003) and Diclofenac Potassium (RT:6.820)

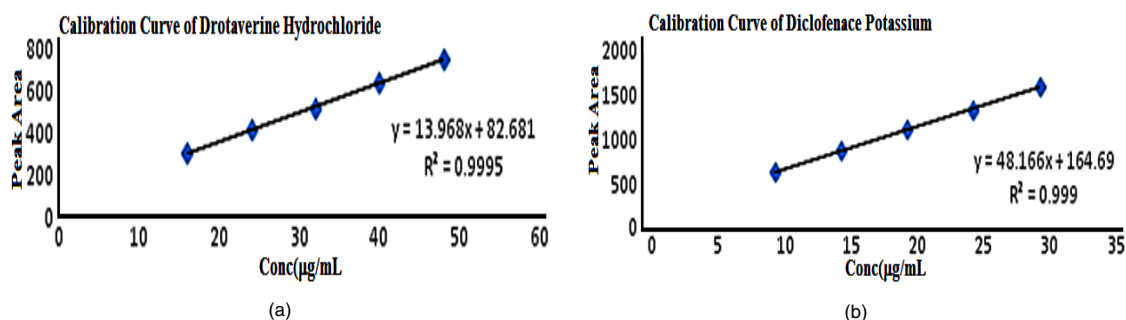
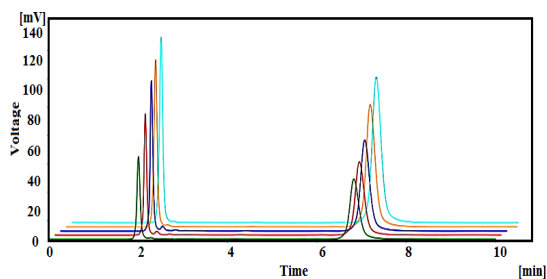
Method validation

Linearity was determined between 16-48 $\mu\text{g/mL}$ of DROTA and 10-30 $\mu\text{g/mL}$ of DICLO (Table 1). Correlation coefficients of 0.999 were found for both the DROTA and DICLO. The calibration curve of DROTA and DICLO were shown in Fig. 3. The regression line equation was $y=13.96x+82.68$ for DROTA and $y=48.16x+164.6$ for DICLO. The linearity chromatogram of DROTA and DICLO were shown in Figure 4.

Table 1: Calibration data for Drotaverine Hydrochloride and Diclofenac Potassium

Drotaverine Hydrochloride		Diclofenac Potassium	
Conc. ($\mu\text{g/mL}$)	Peak area (n=3)	Conc. ($\mu\text{g/mL}$)	Peak area (n=3)
16	306.981 \pm 4.45	10	649.938 \pm 05.59
24	418.969 \pm 4.52	15	887.818 \pm 07.68
32	523.979 \pm 4.67	20	1129.575 \pm 13.51
40	646.361 \pm 9.35	25	1349.474 \pm 07.03
48	752.009 \pm 8.03	30	1623.268 \pm 10.08

n= number of determinations

**Fig. 3. Calibration curve of (a) Drotaverine Hydrochloride and (b) Diclofenac Potassium****Fig. 4. Linearity chromatogram of Drotaverine Hydrochloride and Diclofenac Potassium**

Precision

The repeatability, intraday and interday precisions of the suggested procedure were all taken into consideration while evaluating its accuracy. The fact that the percentage RSD is less than 2% at each level, as evidenced by the findings, makes it abundantly evident that the suggested approach is accurate enough for the analysis of the drug (Table 2).

LOD and LOQ

The LOD and LOQ for DROTA were calculated to be 1.08 $\mu\text{g/mL}$ and 3.28 $\mu\text{g/mL}$ respectively whereas The LOD and LOQ for DICLO were estimated to be 0.25 $\mu\text{g/mL}$ and 0.76 $\mu\text{g/mL}$ respectively (Table 2).

Accuracy

It was determined that the analytical

process was accurate by conducting a percentage recovery analysis. The accuracy of the suggested approach for the analysis of the medication is demonstrated by the recovery values, which fall within the range of 98–102% (Table 2).

Formulation assay

The applicability of the suggested procedure was investigated by examining the Tablet formulation of Verin D that is available for purchase commercially. Their findings are presented in Table 3.

Table 2: Brief Description of Method Validation

Parameters	Drotaverine Hydrochloride	Diclofenac Potassium
Range of Linearity ($\mu\text{g/mL}$)	16-48	10-30
Correlation coefficient	0.999	0.999
Regression equation	$y=13.96x+82.68$	$y=48.16x+164.6$
LOD ($\mu\text{g/mL}$)	1.08	0.25
LOQ ($\mu\text{g/mL}$)	3.28	0.76
Precision (%RSD)		
Repeatability (n=6)	0.60	0.20
Intraday (n=3)	0.80	0.50
Interday (n=3)	1.10	0.80
Accuracy		
80% (n=3)	100.94	101.02
100% (n=3)	100.52	100.61
120% (n=3)	100.57	100.92

n= number of determinations

Table 3: Assay of Formulation (Verin D)

Drugs	Labeled Claim(mg)	Amount found (mg)	Amount found (%)*	%RSD
Drotaverine Hydrochloride	80	79.32	99.15 ± 0.813	0.820
Diclofenac Potassium	50	49.46	98.92 ± 0.741	0.749

*mean±SD

CONCLUSION

The newly developed RP-HPLC method for the detection of Drotaverine hydrochloride and Diclofenac potassium in a combination formulation shows accurate sensitive, rapid, reproducible results than other established methods. The method had a good resolution for both of the medications despite having an analysis time that was less than ten minutes, which allowed the quick quantification of a large number of samples during routine and quality control examination of tablet formulations. The developed method has been proven to work. It was discovered to be straightforward, specific, and accurate. The fact that the percentage of recovered drug in tablets is

high shows that the excipients that are included in the dosage forms do not interfere with the estimation. The procedure that was just described is one that is suitable for use in the regular examination of mixed dose forms of Drotaverine hydrochloride and Diclofenac potassium. Additionally, it has applications in the quality control of large-scale manufacturing.

ACKNOWLEDGEMENT

Authors are thankful to Ms. Puja Bhimani for technical support for completing the research work.

Conflict of Interest

Authors do not have any conflict of interest.

REFERENCES

- Soare, A. C.; Meltzer, V.; Colbea, C.; Stanculescu, I.; Pincu, E. Compatibility of drotaverine hydrochloride with ibuprofen and ketoprofen nonsteroidal anti-inflammatory drugs mixtures. *Mater.*, **2022**, *15*, 1-12.
- Issa, Y.M.; Hassouna, M.E.; Zayed, A.G. Simultaneous determination of paracetamol, caffeine, domperidone, ergotamine tartrate, propyphenazone, and drotaverine HCl by high performance liquid chromatography. *J. Liq. Chromatogr. Relat. Technol.*, **2012**, *35*, 2148-2161.
- Pavel, I.Z.; Heller, L.; Sommerwerk, S.; Loesche, A.; Al Harrasi, A.; Csuk, R. Drotaverine—a Concealed Cytostatic. *Arch. Pharm. Chem. Life Sci.*, **2016**, *349*, 1–5
- Bondarev, A.D.; Attwood, M.M.; Jonsson, J.; Chubarev, V.N.; Tarasov, V.V.; Liu, W.; Schioth, H.B. Recent Developments of phosphodiesterase inhibitors: Clinical trials, emerging indication and novel molecules. *Front. Pharmacol.*, **2022**, *13*, 1-27
- El-Yazbi, F.A.; Amin, O.A.; El-Kimary, E.I.; Khamis, E.F.; Younis, S.E. HPTLC and Spectrophotometric Estimation of Febuxostat and Diclofenac Potassium in Their Combined Tablets. *J. Chromatogr. Sci.*, **2016**, *54*, 1146-1152.
- McNeely, W., Goa, K.L. *Diclofenac-Potassium in Migraine Drugs.*, **1999**, *57*, 991-1003.
- Khuntia, B.S.; Pandia, S.K. Development and validation of new analytical method for estimation of Drotaverine in bulk and pharmaceutical dosage form by UV spectrophotometry. *J. of Ana. Chem.*, **2011**, *1*, 1-8.
- Balan, P.; Nimila, I.C.; Chundi, S.; Movva, V.R.; Rajasekar, S. A validated RP-HPLC method for estimation of Drotaverine Hydrochloride in pharmaceutical tablet dosage form. *Int. J. Pharm. Sci.*, **2011**, *3*, 77-80.
- Sastry, B.S.; Gananadhamu, S.; Rao G.D. Development of RP-HPLC method for estimation of Drotaverine Hydrochloride in pharmaceutical formulations. *Int. J. Chem. Sci.*, **2008**, *6*, 2055-2061.
- Patel, R.K.; Patel, L.J. Development and validation of RP-HPLC method for simultaneous determination of Ranitidine Hydrochloride and Drotaverine in tablet. *Inventi. Impact. Pharm. Ana. Q. A.*, **2011**, *2*, 78-81.
- Gandhi, S.; Deshpande, P.; Rajmane, V.; Dodal, T.; Parab, Method development and validation for simultaneous estimation of Drotaverine Hydrochloride and Aceclofenac in tablet dosage form by RP-HPLC. *J. Int. J. Pharm. Sci. review. Res.*, **2010**, *4*, 49-52.

12. Kumar, R.S.; Karthikeyan, C.; Moorthy, N.S.; Trivedi, P. New spectrophotometric methods applied to the simultaneous determination of diclofenac potassium and tizanidine. *Indian J Pharm Sci. Indian. J. Pharm. Sci.*, **2006**, *68*, 300-302.
13. Gowramma, B.; Rajan, S.; Murlidharan, S.; Meyyanathan, S.N.; Suresh, B. A validated RP-HPLC method for simultaneous estimation of Paracetamol and Diclofenac Potassium in pharmaceutical formulation. *Inter. J. Chem. Tech. Res.*, **2010**, *2*, 676-680.
14. Umalkar, A.R.; Rewatkar, N.S.; Charde, M.S.; Charde, R.M.; Kasture, A.V. RP-HPLC method development and validation for estimation of Thiocolchicoside and Diclofenac Potassium in bulk and capsule dosage forms. *J. Pharm. Res.*, **2011**, *4*, 1307-1308.
15. Biswas, A.; Basu, A. Simultaneous estimation of Paracetamol, Chlorzoxazone and Diclofenac Potassium in pharmaceutical formulation by a RP-HPLC method. *Int. J. Pharm. Biosci.*, **2010**, *1*, 1-6.
16. Subramanian, G.; Musmade, P.; Agarwal, S.; Udapa, N. Simultaneous RP-HPLC estimation of Tizanidine, Diclofenac Potassium and Paracetamol in tablets. *Int. J. Pharm. Sci.*, **2004**, *66*, 694-696.
17. Dahivelkar, P.P.; Bhoir, S.I.; Bari, S.B.; Surana, S.J.; Bhagwat, A.M. Simultaneous determination of diclofenac potassium and drotaverine hydrochloride in human plasma using reversed-phase high-performance liquid chromatography. *J. Chromatogr. Sci.*, **2012**, *50*, 694-701.
19. Ingale, S.S.; Abnawe, S.A.; Shinde, S.N.; More, A.S.; Choudhari, V.P.; Kuchekar, B.S. Simultaneous spectrophotometric estimation of Drotaverine Hydrochloride and Diclofenac Potassium in combined dosage form. *Res J. Pharm. Tech.*, **2010**, *3*, 1118-1121.
20. Ingale, S.S.; Mehetre, D.M.; Desai, D.J.; Sahoo, M.; Choudhari, V.P.; Kuchekar, B.S. Spectrophotometric simultaneous determination of Drotaverine Hydrochloride and Diclofenac Potassium in combined tablet dosage form by ratio derivative and area under curve method. *Res. J. Pharm. Tech.*, **2010**, *1*, 35-40.
21. Ingale, S.S.; Tajane, D.D.; Modak, V.G.; Gite, S.R.; Choudhari, V.P.; Kuchekar, B.S. Development and validation of a HPTLC method for simultaneous estimation of Drotaverine Hydrochloride and Diclofenac Potassium in combined dosage form. *Der Pharma Chemica. Der Pharma. Chemica.*, **2010**, *2*, 126-132.
22. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2(R1), Geneva., **2005**.
23. Dash, D.K.; Sen, A.K.; Seth, A.K.; Saisivam, S.; Sadhu, P. Comparison between RP-HPLC and HPTLC Techniques for the Analysis of Ethionamide. *Int J Pharm Res. Int. J. Pharm. Res.*, **2020**, *12*, 592-597.
24. Dash, D.K.; Sen, A.K.; Saisivam, S.; Sadhu, P.; Patel, S.; Baile, S. Simultaneous Determination and Validation of Flupirtine Maleate and Paracetamol in Combined Dosage Form by Chromatographic Technique. *J. Pharm. Res. Int.*, **2021**, *33*, 91-98.