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Stability indicating rapid RP-HPLC method for the determination of Drotaverine hydrochloride, Domperidone and paracetamol in pharmaceutical dosage forms

Kabeer A. Shaikh[*], Sachin D. Patil [a]

[*]*P.G. Department of Chemistry, Sir Sayyed College, P.B. No. 89, Roshan gate, Aurangabad, M.S., 431001, India.*
[a] *P.G.Department of Chemistry, Yeshwant Mahavidyalaya, Nanded- 431602, M.S., India.*

ABSTRACT

A simple and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of drotaverine hydrochloride, domperidone and paracetamol in a binary tablet formulation containing drotaverine hydrochloride-paracetamol and/or domperidone-paracetamol. Chromatography was carried out at 25°C on a 4.6mm×150mm, 5µm Symmetry shield RP 18 column with the isocratic mobile phase of 0.02M aqueous potassium dihydrogen phosphate buffer (pH 7.5) and acetonitrile (40:60, v/v) at a flow rate of 0.5 ml/min. Drotaverine hydrochloride, domperidone and paracetamol were separated in less than 10 min with good resolution and minimal tailing, without interference of excipients. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The method was linear in the range of 192–448µg/ml for drotaverine hydrochloride, 24–56µg/ml for domperidone and 12–28µg/ml for paracetamol.

Keywords: drotaverine hydrochloride, domperidone, paracetamol, liquid chromatography, formulation.

INTRODUCTION

Paracetamol (N-(4-hydroxyphenyl)acet amide), Fig. 1, also known as acetaminophen, is a popular analgesic and antipyretic drug widely used for management of pain and fever [1].

Domperidone 5-chloro-1-[1-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl) propyl]-piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one (C22H24ClN5O2), Fig. 2, is a dopamine antagonist that produces extrapyramidal reactions. It stimulates gastro-intestinal motility and is used as an antiemetic for the short term treatment of nausea and vomiting of various aetiologies, including that associated with cancer therapy and with levodopa or bromocriptine therapy for parkinsonism[2]. Drotaverine, 1-[(3,4-diethoxyphenyl)methylene]-6,7-diethoxy-1,2,3,4-tetra hydroisoquinoline, Fig. 3, is an analogue of papaverine with excellent smooth muscle relaxant properties, though more effective as antispasmodic than papaverine. It is available as HCl and theophylline-7-acetic acid salts [3].

Several LC methods have been reported for analysis of paracetamol combined with other drugs in pharmaceutical preparations [4–12]. On the other hand drotaverine hydrochloride was determined either individually by electrochemical methods [13,14], spectrophotometric methods [15–19] and HPLC methods [20–23], or in combination with nicotinic acid and phenazone spectrophotometrically [24], Several HPLC methods have been cited in the literature for the estimation of domperidone individually [25-30]. However no references have been found for simultaneous determination of three drugs i.e. drotaverine hydrochloride, domperidone and paracetamol in pharmaceutical preparations

The present manuscript describes a simple, rapid, precise and accurate isocratic reversed-phase HPLC method for the simultaneous determination drotaverine hydrochloride, domperidone and paracetamol in the binary tablet dosage form containing drotaverine hydrochloride-paracetamol or domperidone-paracetamol.

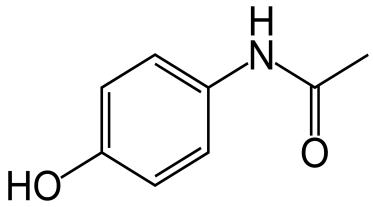

Fig. 1. Structure of Paracetamol

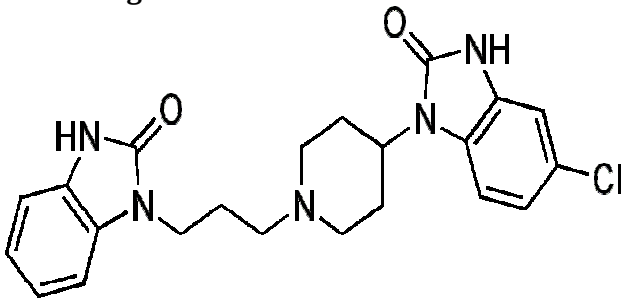

Fig. 2. Structure of Domperidone

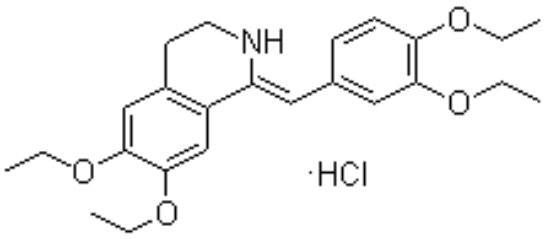

Fig. 3. Structure of Drotaverine Hydrochloride

MATERIALS AND METHODS

2.1 Chemicals

Drotaverine hydrochloride (99.23%), domperidone (99.58%) and paracetamol (99.61%) were obtained from Cipla LTD, India. Potassium dihydrogen phosphate (AR Grade), sodium hydroxide (AR Grade), orthophosphoric acid (AR Grade) and acetonitrile (HPLC Grade) were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. The 0.45-µm-nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigad, India. Double distilled water was used throughout the experiment. Tablets were purchased from Indian market, containing Drotaverine hydrochloride 80 mg - Paracetamol 500 mg per tablet and Domperidone 10 mg - Paracetamol 500 mg per tablet

2.2 Equipments

Analysis was performed on a chromatographic system of Waters 2695 separation module (USA) equipped with an auto injector, Waters 2487 Dual wavelength absorbance detector. A chromatographic separation was achieved on a Symmetry shield RP 18 (4.6mm×150mm, 5µm) column. Data acquisition was made with Empower software. The peak purity was checked with the photodiode array detector. Injection volume used was 20µl throughout the experiment.

2.3 Standard Solutions and Calibration Graphs

Diluent preparation – Used mixture of water and acetonitrile (60:40 v/v) and pH of this solution was adjusted at 2.5 with diluted orthophosphoric acid.

Standard stock solutions of drotaverine hydrochloride (3.2 mg/ml), paracetamol (0.2 mg/ml) and domperidone (0.4 mg/ml) was prepared in diluent. From this, mixed standard solution was prepared by diluting 5 ml of each stock solution to 50 ml with diluent. To study the linearity range of each component, serial dilutions were made by adding 3,4,5,6,7 ml each of stock solution in to 200 ml volumetric flask, made up the dilution with diluent and mixed.

2.4 Sample Preparation

Twenty tablets were weighed and finely powdered. The average weight of tablets is determined with the help of weight of twenty tablets. A portion of powder equivalent to the weight of one tablet was accurately weighed into 250 ml volumetric flasks and 70 ml diluent was added. The volumetric flasks were sonicated for 20 min to effect complete dissolution of the drugs, the solutions were then made up to volume with diluent. The solution was filtered through 0.45µm

nylon filter. The aliquot portion of the filtrate was further diluted to get final concentration of 320 µg/ml of drotaverine hydrochloride, 40 µg/ml of domperidone and 20 µg/ml of paracetamol.

2.5. Method validation

The HPLC method was validated in terms of specificity, precision, accuracy, linearity and solution stability according to ICH guidelines [31]. Assay method precision was determined using nine independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on 3 different days. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted as described in Section 2.4, and were analyzed using the developed HPLC method. Linearity test solutions were prepared as described in Section 2.3. The solution stability study was carried out by injecting standard and sample solutions at different interval of time. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1 ml/min. The percentage of organic modifier was varied by (±) 5%. pH of mobile phase was varied by (±) 0.1. Column oven temperature was varied by (±) 5°C.

RESULTS AND DISCUSSION

3.1 Optimization of the chromatographic conditions:

The objective of the study was to develop simple and reproducible isocratic HPLC method using readily available chemicals and reagents. As paracetamol is highly polar molecule, it elutes very rapidly in reversed phase chromatotography. To increase the retention of paracetamol, buffer composition in mobile phase is increased up to 90 percent, but this causes very high retention of comparatively less polar drotaverine and domperidone and requires gradient elution. The C8 and -CN stationary phases give some more retention for polar paracetamol, but giving less retention for domperidone and drotaverine hydrochloride. This causes less resolution between domperidone and drotaverine hydrochloride. The mobile phase consisting of 0.02M aqueous potassium dihydrogen phosphate buffer (pH 7.5) and acetonitrile (40:60,v/v) was found to be an appropriate mobile phase allowing adequate separation of all the compounds using a 4.6mm×150mm, 5µm Symmetry shield RP 18 column at a flow rate of 0.5 ml/min. The flow rate was purposely kept at 0.5 ml/min instead of 1 ml/min to reduce the consumption of acetonitrile, although it does not affect chromatography significantly. Symmetry Shield columns are reversed-phase columns based on embedded polar group technology that literally "shields" the silica's residual silanols from highly basic analytes, it also shows some more retention for polar analytes like paracetamol than normal reversed phase columns. The optimum wavelength selected for analysis was 246 nm for paracetamol and 280 nm for domperidone and drotaverine hydrochloride. A typical chromatogram of separation of all the compounds is shown in Fig. 4 and Fig. 5

Solution stability study of analyte solutions were carried out using different solvents, out of which mixture of water and acetonitrile (60:40 v/v), pH adjusted at 2.5 with diluted orthophosphoric acid, was found to be best suitable diluent for analysis.

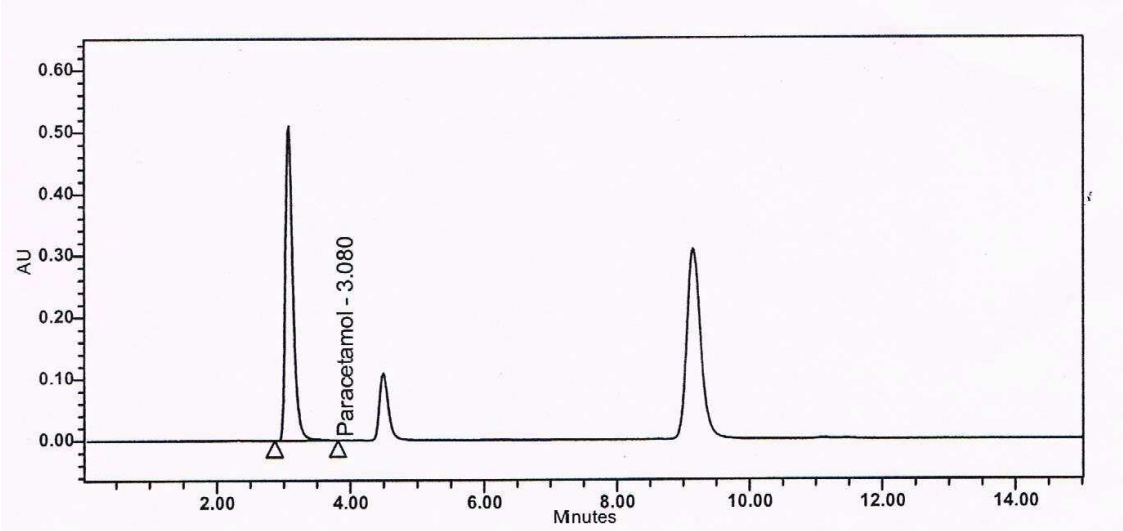

Fig. 4. A typical chromatogram of a standard solution containing paracetamol, domperidone and drotaverine at 246 nm.

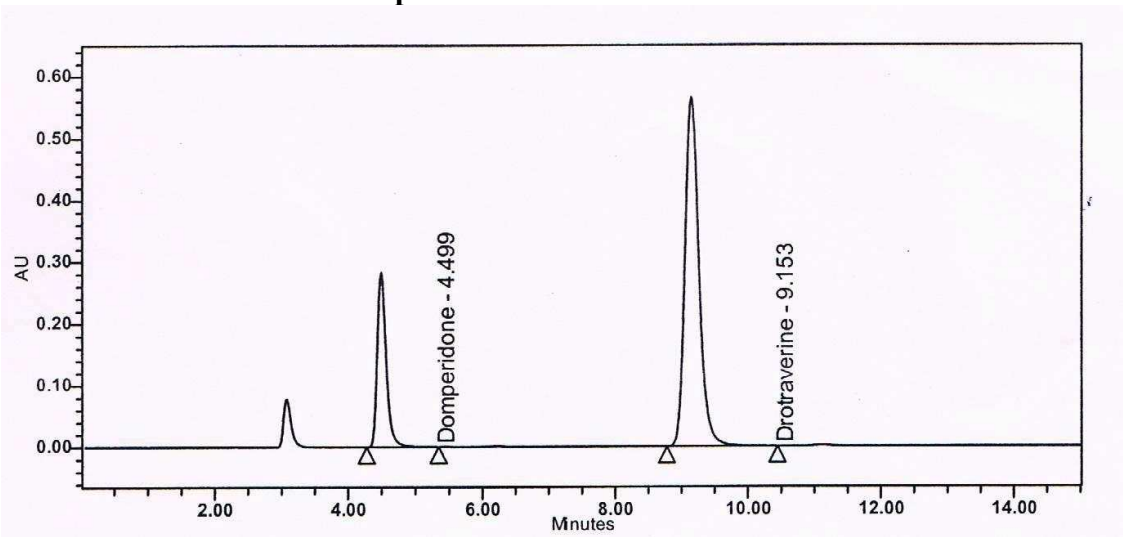

Fig. 5. A typical chromatogram of a Standard solution containing paracetamol, domperidone and drotaverine at 280 nm.

3.2 Validation of method

3.2.1 Specificity

The specificity of the HPLC method is illustrated in Fig. 4 and Fig. 5 where complete separation of drotaverine hydrochloride, domperidone and paracetamol was noticed in presence of tablet excipients. In addition there was no any interference at the retention time of drotaverine hydrochloride, domperidone and paracetamol in the chromatogram of placebo solution. In peak purity analysis with photo diode detector, purity angle was less than purity threshold for both the

analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

Moreover, the specificity was determined according to ICH [31] by subjecting a sample solution to accelerated degradation by acid, alkaline, neutral, oxidative, photolytic, and thermal stress conditions to evaluate the interference of degradation products in the quantitation of analytes. Acid, alkaline and neutral degradation of the drug were carried out with sample solutions in 1 N HCl, 1 N NaOH, and purified water, refluxed at 60°C for 1 h. The oxidative degradation was induced by storing the sample in 3% hydrogen peroxide, at ambient temperature for 1 h, protected from light. Photolytic studies were performed after exposition of sample in solid state and diluted in purified water, in a photostability chamber for 72 h. To investigate the stability of the drugs under thermal stress conditions, sample was spread in a thin layer on a petri plate and subjected to dry heat at 80°C in an oven. The samples were analyzed after 72 h. After the procedures, the samples were diluted in diluent to a final concentration.

Sample for forced degradation study was prepared by adding drugs of respective claims to placebo containing excipients which take part in the pharmaceutical preparation.

3.2.2 Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method [Table - 1]. The mean percentage recoveries obtained for drotaverine hydrochloride, domperidone and paracetamol were 100.1, 100.0 and 100.5 % respectively.

Table 1: Results of the recovery analysis of drotaverine hydrochloride, domperidone and paracetamol.

Compound	Wt Spiked (mg)	Wt Recovered (mg)	Recovery (%)	RSD (%) n=3
Drotaverine hydrochloride	65.68	65.60	99.9	0.42
	80.62	81.15	100.7	0.37
	96.74	96.39	99.6	0.84
Domperidone	8.34	8.40	100.8	1.14
	10.28	10.25	99.8	0.78
	12.25	12.17	99.3	0.37
Paracetamol	402.78	406.58	100.9	0.68
	500.14	502.69	100.5	0.54
	602.35	601.99	99.9	0.97

RSD- relative standard deviation.
Wt- Weight

3.2.3 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision is a measure of the method variability that can be

expected for a given analyst performing the analysis and was determined by performing five replicate analyses of the same working solution. The relative standard deviation (R.S.D.) obtained for drotaverine hydrochloride, domperidone and paracetamol was 0.38%, 0.59% and 0.88%, respectively (Table-2).

The intra- and inter-day variability or precision data are summarized in Table 3. The intra-day precision of the developed LC method was determined by preparing the standard solution in nine determinations with three concentrations and three replicate each. The R.S.D. of the results was used to evaluate the method precision. The inter-day precision was also determined by preparing standard solution in triplicate per day for consecutive 3 days. The results indicated the good precision of the developed method.

Table 2: System suitability parameters

Parameters	Drotaverine hydrochloride	Domperidone	Paracetamol
Theoretical plates[a]	9575	6153	3872
USP Resolution[a]	15.46	5.69	----
Peak symmetry[a]	1.21	1.31	1.42
% RSD	0.38	0.59	0.88

[a] USP–NF 29 section 621, pp. 2135.

Table 3: Intra and inter day assay precision data (n=9)

Actual concentration	Measured concentration (µg/ml), RSD (%)	
	Intra-day	Inter-day
Drotaverine hydrochloride (µg/ml)		
192	192.27, 0.37	193.99, 0.73
320	321.75, 0.21	319.45, 0.85
448	446.56, 0.32	449.14, 0.65
Domperidone (µg/ml)		
24	24.21, 0.22	24.26, 0.89
40	40.55, 0.11	40.67, 1.25
56	56.67, 0.07	56.11, 0.97
Paracetamol (µg/ml)		
12	12.24, 0.41	12.59, 0.97
20	20.76, 0.26	21.15, 1.20
28	28.48, 0.27	28.65,0.61

Data expressed as mean for “measured concentration” values.

3.2.4 Linearity and range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Linearity was determined in the range of 192–448µg/ml for drotaverine hydrochloride, 24–56µg/ml for domperidone and 12–28µg/ml for paracetamol. The correlation coefficient ('r ') values for all the three drugs were >0.999. Typically, the regression equation for the calibration curve was found to be y = 204489x - 5755.5 for paracetamol, y = 63027x + 1571.2 for domperidone and y = 26353x + 34722 for drotaverine hydrochloride.

3.2.5 Solution Stability:

To demonstrate the stability of standard solutions and tablet sample solutions in selected diluent, both solutions were analyzed over a period of 12 h while being stored at room temperature. The results showed that the retention times and peak areas of the drugs remained almost unchanged and no significant degradation was observed during this period, suggesting that both solutions were stable for at least 12 h, which was sufficient for the whole analytical process(Table-4).

Table-4: Solution stability data of drugs in different diluents.

Standard solution (% Difference w.r.t. initial area)				Sample solution (% Difference w.r.t. initial area)		
Time (Hrs)	PR	DM	DT	PR	DM	DT
4.0	0.15	0.37	-14.72	-0.05	-0.58	-0.13
8.0	-0.43	0.51	-22.05	0.05	-0.38	-0.04
12.0	0.21	0.43	-27.82	0.51	-0.49	0.15

Diluent- Water: Acetonitrile (30:70)

Standard solution (% Difference w.r.t. initial area)				Sample solution (% Difference w.r.t. initial area)		
Time (Hrs)	PR	DM	DT	PR	DM	DT
4.0	-0.70	-0.05	-2.56	-0.19	-0.27	-0.29
8.0	-0.29	-0.20	-4.34	0.56	-0.33	-0.37
12.0	-0.79	-0.57	-5.62	0.60	0.02	-0.29

Diluent- Mobile Phase

Standard solution (% Difference w.r.t. initial area)				Sample solution (% Difference w.r.t. initial area)		
Time (Hrs)	PR	DM	DT	PR	DM	DT
4.0	-0.11	-0.27	0.35	0.00	0.90	0.22
8.0	0.76	0.17	-0.26	0.35	1.27	-0.05
12.0	0.74	0.19	0.02	0.15	0.97	0.71

Diluent- Water: Acetonitrile (60:40), pH 2.50.

PR-Paracetamol, DM-Domperidone, DT- Drotaverine hydrochloride

3.2.6 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as change in the pH of the mobile phase, flow rate, percentage of acetonitrile in the mobile phase and column oven temperature. The standard solution containing drotaverine hydrochloride, domperidone and paracetamol was injected into the LC injector six times under different chromatographic conditions. The robustness of the method was evaluated by determining the effect of the modified parameters on retention time, tailing factor, area and percentage of content. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table-5).

Table-5: Results of Robustness study

Factor	Level	Mean % Assay (n=6)			% R.S.D. of results		
		PR	DM	DT	PR	DM	DT
pH of mobile phase	7.4	100.2	100.5	99.8	0.28	0.76	0.44
	7.6	101.1	100.4	100.2	0.45	0.56	0.12
Flow rate (ml/min)	0.45	100.7	99.7	100.2	0.64	0.25	0.24
	0.55	100.1	100.9	99.6	0.21	0.55	0.78
Column oven temperature (°C)	20	100.4	100.2	100.5	0.37	0.82	0.35
	30	100.2	100.5	100.4	0.81	0.50	0.31
% of acetonitrile	55	101.2	100.5	100.8	0.67	0.48	0.57
	65	100.4	99.5	100.7	0.71	0.25	0.49

PR-Paracetamol, DM-Domperidone, DT- Drotaverine hydrochloride

CONCLUSION

A simple, specific, linear, precise, and accurate RP-HPLC method has been developed and validated for quantitative determination of drotaverine hydrochloride, domperidone and paracetamol in tablet formulation. The method is very simple and specific as all peaks were well separated from its excipient peaks with total runtime of 15 min, which makes it especially suitable for routine quality control analysis work.

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