A NOVEL REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF DROTAVERINE HYDROCHLORIDE, ETHAMSYLATE, AND TRANEXAMIC ACID IN TABLET DOSAGE FORM

SWATI SAHANI, VANDANA JAIN*
Department of Quality Assurance, Oriental College of Pharmacy, Navi Mumbai, Maharashtra, India. Email: vandana.jain@scp.edu.in
Received: 16 January 2017, Revised and Accepted: 17 February 2018

INTRODUCTION

Drotaverine is chemically 1,2,3,4-tetrahydro-6,7-dioxo-1-(3,4-dithioxyphenyl) methylene)-isoquinoline hydrochloride (Fig. 1a) and its molecular formula is C_{19}H_{16}NO_{3}HCl [1,2]. It belongs to the class of antispasmodic agent by inhibiting phosphodiesterase 4 and has no anticholinergic effects. It is specifically used for smooth muscle spasm and pain. Chemically, ethamsylate is N-ethylameine 2,5 dihydroxy benzene sulfonate (Fig. 1b) and its molecular formula is C_{18}H_{17}NO_{3}S [3]. It has been used as hemostatic compound that blocks prostacyclin synthetase, an enzyme that converts arachidonic acid to prostacyclins and therefore increases platelet aggregation and platelet adhesiveness. It is used for the prevention and treatment of capillary hemorrhage and postpartum hemorrhage [4,5]. Ethamsylate is officially present in British Pharmacopoeia [6].

Tranexamic acid is chemically trans-4-aminomethyl-cyclohexanecarboxylic acid (Fig. 1c) and its molecular formula is C_{9}H_{15}NO_{2} [3]. It competitively inhibits the activation of plasminogen, thereby reducing conversion of plasminogen to plasmin (fibrinolysin), an enzyme that degrades fibrin clots, fibrinogen, and other plasma proteins including the procoagulant factors V and VIII. It is used for controlling abnormal bleeding during menstruation and pregnancy [4,5]. Tranexamic acid is officially present in British Pharmacopoeia [6]. The combination of drotaverine hydrochloride, ethamsylate, and tranexamic acid is used in the treatment of menstrual pain, bleeding, and abdominal pain.

Literature survey reveals that spectrophotometric [7-9], high-performance thin-layer chromatography (HPTLC) [10] and reverse-phase HPLC (RP-HPLC) [11,12] based methods have been reported for analysis of these drugs alone and in combination with other drugs. However, there is no reported method for simultaneous estimation of all three drugs in pharmaceutical dosage form. Therefore, an attempt has been made to develop a novel, rapid, and sensitive method for simultaneous determination of drotaverine hydrochloride, ethamsylate, and tranexamic acid in marketed formulation and validate the developed method in accordance with the international conference on harmonization (ICH) guidelines and also to perform the force degradation studies using developed method. This novel validated method has applicability in industry and academia for routine quality control testing [13].

METHODS

Chemical and reagents

Drotaverine hydrochloride, ethamsylate, and tranexamic acid reference standards were purchased from Yarrow Chem Pvt, Ltd., India. All other chemicals and reagents used were of analytical grade. Ethamsyl-Td (Convina Research Laboratory, India) tablet formulation containing drotaverine hydrochloride 80 mg, ethamsylate 250 mg, and tranexamic acid 250 mg was procured from local market.

Instrumentation

A RP-HPLC (Shimadzu) LC-2030 model equipped with laboratory solution software, an autosampler and ultraviolet (UV)-visible detector was used. The analysis was carried out on C18 shim-pack GIST (150 mm × 4.6 mm 5 µ) column as stationary phase. A freshly prepared mobile phase consisting of methanol:potassium dihydrogen phosphate buffer (pH 3.0 adjusted using orthophosphoric acid) in a ratio of 30:70 v/v, with a flow rate of 1 ml/min and ultraviolet detection at 220 nm.

RESULTS

The retention time of tranexamic acid, ethamsylate, and drotaverine hydrochloride was found to be 3.6, 4.0, and 5.0 min, respectively. The developed method was found to be linear for all three drugs in a concentration range of 48–112 µg/ml for drotaverine hydrochloride, 150–350 µg/ml for ethamsylate, and 150–350 µg/ml for tranexamic acid. Mean percentage recoveries were found to be 99.59 for drotaverine hydrochloride, 99.27 for ethamsylate, and 99.71 for tranexamic acid. The correlation coefficient for all components was found to be more than 0.999.

Conclusion:

A novel RP-HPLC method was developed and validated for simultaneous for the estimation of drotaverine hydrochloride, ethamsylate, and tranexamic acid to their commercially available tablet dosage form.

Keywords: Drotaverine hydrochloride, Ethamsylate, Tranexamic acid, Reversed-phase high-performance liquid chromatography, International conference on harmonization, Validation.
ratio of 30:70 v/v was used. The mobile phase was filtered by 0.45 µm membrane filter and sonicated before use. The flow rate of mobile phase was 1 ml/min, column temperature was maintained at 25°C, detection was carried out at 220 nm, and the runtime was around 10 min.

Selection of wavelength
A UV spectrum of drotaverine hydrochloride, ethamsylate, and tranexamic acid in water was noted by scanning the solution in the range of 200–400 nm. Drotaverine hydrochloride, ethamsylate, and tranexamic acid were showing significant absorption at 220 nm. Thus, 220 nm was selected as wavelength for analysis.

Preparation of standard stock solution
The standard stock solutions 100 mg/ml each of drotaverine hydrochloride, ethamsylate, and tranexamic acid were prepared. Further, solution with concentration of 80 mg drotaverine hydrochloride, 250 mg ethamsylate, and 250 mg tranexamic acid was prepared by diluting stock solution with mobile phase.

Preparation of sample solution
A total of 10 tablets were weighed, their mean weight was determined and crushed in mortar. An amount of powder weight equivalent to 80 mg drotaverine hydrochloride, 250 mg ethamsylate, and 250 mg tranexamic acid was taken and crushed in mortar. An amount of powder corresponding to 80 mg drotaverine hydrochloride, ethamsylate, and tranexamic acid was prepared by diluting stock solution with mobile phase.

Method validation
System suitability
System suitability tests are a fundamental part of liquid chromatographic method. It ensures that system is working correctly. System suitability parameters such as number of theoretical plates, retention time, and tailing factor were evaluated. This was performed by injecting mixture of standard in six replicates.

Linearity
The linearity of the proposed method was determined by quantitative dilution of the standard solution of drotaverine hydrochloride, ethamsylate, and tranexamic acid to obtain solution in concentration range of 48–112 µg/ml, 150–350 µg/ml, and 150–350 µg/ml, respectively. A graph of peak area versus concentration in µg/ml was plotted for all three drugs in triplicate. The slope, intercept, and correlation coefficient of regression line were determined.

Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD and LOQ represent the concentration of analyte that would yield to signal-to-noise ratio of 3 for LOD and 10 for LOQ. LOD and LOQ were calculated using following formula,

$$LOD = 3.3 \frac{\delta}{S}$$

$$LOQ = 8 \frac{\delta}{S}$$

where, δ = standard deviation of response (peak area) and S = average of slope of the calibration curve.

Method precision
The method precision of the proposed method was determined by injecting six replicates of sample and standard on the same day to ensure that the analytical method is repeatable.

System precision
The system precision is checked by injecting six replicates of standard solution to ensure that the analytical system is working properly.
Linearity
Linearity of the proposed method was determined by constructing calibration graph between the tested concentration level and corresponding peak areas for all three drugs in triplicate. The results show an excellent correlation between peak areas and concentrations level within the tested concentration range of 48–112 µg/ml for drotaverine hydrochloride, 150–350 µg/ml for ethamsylate, and 150–350 µg/ml for tranexamic acid. The correlation coefficients were >0.999 for all three drugs, which meet the method validation acceptance criteria, and hence, the method is said to be linear for the drugs (Figs. 3-5).

LOD and LOQ
The LOD and LOQ for drotaverine hydrochloride were found to be 1 µg/ml and 3 µg/ml, 0.17 µg/ml and 0.52 µg/ml for ethamsylate, and 0.54 µg/ml and 1.63 µg/ml for tranexamic acid, which indicates that method is sensitive. The results are summarized in Table 2.

Method precision
The % RSD value for six replicates injection of sample and standard as carried out on the same day was found to be <2%, which indicates that method is repeatable. The results for method precision are given in Table 3.

System precision
System precision was determined by measuring the peak area of six replicate injections of standard solution. The value of % RSD was found to be <2, which ensure the analytical system is working properly. The results of system precision are tabulated in Table 4.

Accuracy
The accuracy of this method was determined by calculating percent recovery of drotaverine hydrochloride, ethamsylate, and tranexamic acid in formulation at three different levels (80–120%). The % recovery obtained was found to be in the range of 99.22–99.88 for drotaverine hydrochloride, 99.66–99.7 for ethamsylate, and 99.35–100 for tranexamic acid. The accepted limits of mean recovery are 98–102% and all observed data were within the required range, which indicate good recovery values, affirming the accuracy of the method developed. The results are summarized in Table 5.

Robustness
The method was found to be robust when subjected to minor changes in the chromatographic condition such as oven temperature (±1°C), mobile phase flow rate (±0.2 ml/min), and wavelength nm (±1 nm). It was observed that there was no marked change in analytical method which indicates good reliability during normal usage. The results are shown in Table 6.

Force degradation studies
Force degradation of drotaverine hydrochloride, ethamsylate, and tranexamic acid under the conditions of hydrolysis (acidic, basic), oxidation, photolysis, and thermal was carried out.

Under acidic conditions (0.1N hydrochloride for 3 h), it was found that 1.74% of drotaverine hydrochloride, 1.7% of ethamsylate, and 1.02% of tranexamic acid content were degraded.

Under basic condition (0.1N sodium hydroxide for 3 h), it was found that 1.02% of drotaverine hydrochloride, 1.47% of ethamsylate, and 0.93% of tranexamic acid content were degraded.

Under thermal condition (at 100°C for 30 min), it was found that 1% of drotaverine hydrochloride, 1.22% of ethamsylate, and 1.22% of tranexamic acid content were degraded.

As there was no interference observed due to excipients or other component present in pharmaceutical dosage form or degrading products, hence, can be concluded that the developed method is stability indicating method for simultaneous estimation of drotaverine hydrochloride, ethamsylate, and tranexamic acid in pharmaceutical dosage form. The results for force degradation studies are shown in Table 7.

CONCLUSION
A novel RP-HPLC method was developed for simultaneous estimation of drotaverine hydrochloride, ethamsylate, and tranexamic acid in pharmaceutical dosage form. This newly developed method offers an advantage of being simple, rapid, and accurate and has a shorter chromatographic time. The developed method was validated as per...
Table 1: Results of system suitability studies

| Parameters (n=6) | Drotaverine hydrochloride | Ethamsylate | Tranexamic acid |
|-----------------|---------------------------|-------------|----------------|
| Number of theoretical plates | 5408 | 6561 | 32098 |
| Tailing factor | 1.16 | 1.25 | 1.84 |
| Peak area | 802571 | 5237256 | 1487598 |
| Retention time (min) | 5.0 | 4.0 | 3.6 |

Table 2: LOD and LOQ value calculated from the calibration curve

| Parameters | Drotaverine hydrochloride (µg/ml) | Ethamsylate (µg/ml) | Tranexamic acid (µg/ml) |
|------------|-----------------------------------|---------------------|------------------------|
| LOD        | 3                                 | 0.17                | 0.54                   |
| LOQ        | 1                                 | 0.52                | 1.63                   |

LOD: Limit of detection, LOQ: Limit of quantitation

Table 3: Method precision data for drotaverine hydrochloride, ethamsylate, and tranexamic acid in tablet

| Replicates (n=6) | % Assay of drotaverine hydrochloride | % Assay of ethamsylate | % Assay of tranexamic acid |
|------------------|--------------------------------------|------------------------|---------------------------|
| 1                | 98.07                                | 98.6                   | 100.07                    |
| 2                | 98.89                                | 99.14                  | 98.83                     |
| 3                | 98.94                                | 99.94                  | 100.3                     |
| 4                | 98.34                                | 99.41                  | 99.07                     |
| 5                | 98.08                                | 100.11                 | 98.24                     |
| 6                | 98.68                                | 99.99                  | 98                        |
| Mean±SD         | 98.68±0.3572                         | 99.53±0.5396           | 99.08±0.8566              |
| ±SEM             | 0.14584                              | 0.22032                | 0.352951                  |
| %RSD             | 0.36                                 | 0.54                   | 0.86                      |

n: Number of injections, SD: Standard deviation, SEM: Standard error of mean, % RSD: Percent relative standard deviation

Table 4: System precision data for drotaverine hydrochloride, ethamsylate, and tranexamic acid

| Replicates (n=6) | Drotaverine hydrochloride (area) | Ethamsylate (area) | Tranexamic acid (area) |
|------------------|----------------------------------|-------------------|------------------------|
| 1                | 79320                            | 5287608           | 1452539                |
| 2                | 793124                           | 5266508           | 1436249                |
| 3                | 792831                           | 5198406           | 1451245                |
| 4                | 793863                           | 5263296           | 1453261                |
| 5                | 794823                           | 5123486           | 1496321                |
| 6                | 792336                           | 5262899           | 1436281                |
| Mean±SD         | 790049.5±5308.52                  | 5233765.5±56460.36 | 1454346±20119.31       |
| ±SEM             | 2.167.19                          | 23049.847         | 8213.67                 |
| %RSD             | 0.6                               | 1.0               | 1.3                    |

n: Number of injections, SD: Standard deviation, SEM: Standard error of mean, % RSD: Percent relative standard deviation

Table 5: Recovery data for drotaverine hydrochloride, ethamsylate, and tranexamic acid

| Drug                | Level of recovery (%) | Sample amount (µg/ml) | Standard amount (µg/ml) | Total amount (µg/ml) | % Mean recovery (n=3) |
|---------------------|-----------------------|-----------------------|------------------------|----------------------|----------------------|
| Drotaverine hydrochloride | 80                    | 40                    | 32                     | 72                   | 99.88                |
|                     | 100                   | 40                    | 40                     | 80                   | 99.69                |
|                     | 120                   | 40                    | 48                     | 88                   | 99.22                |
| Ethamsylate         | 80                    | 125                   | 100                    | 225                  | 98.77                |
|                     | 100                   | 125                   | 125                    | 250                  | 100.4                |
|                     | 120                   | 125                   | 150                    | 275                  | 98.66                |
| Tranexamic Acid     | 80                    | 125                   | 100                    | 225                  | 100                  |
|                     | 100                   | 125                   | 125                    | 250                  | 99.78                |
|                     | 120                   | 125                   | 150                    | 275                  | 99.35                |

Table 6: Robustness evaluation of method

| Chromatographic factors | Variations | % Assay of drotaverine hydrochloride | % Assay of ethamsylate | % Assay of tranexamic acid |
|-------------------------|------------|--------------------------------------|------------------------|---------------------------|
| Flow rate (ml/min)      | 0.8        | 99.31                                | 99.46                  | 99.11                     |
|                        | 1.2        | 99.79                                | 100                    | 98.75                     |
| Oven temperature (°C)   | 24         | 99.28                                | 99.66                  | 98.93                     |
|                        | 26         | 99.67                                | 99.91                  | 99.38                     |
| Wavelength (nm)         | 219        | 99.86                                | 99.07                  | 99.17                     |
|                        | 221        | 99.27                                | 99.76                  | 99.38                     |
ICH guidelines and the results obtained were within the acceptance limits. Percent recovery and estimated concentration of active ingredient in pharmaceutical formulation showed that amount of drug is consistent with the label claim. Hence, the proposed method was found to be satisfactory and can be applied for routine analysis of drotaverine hydrochloride, ethamsylate, and tranexamic acid in pharmaceutical dosage form. This method can be utilized conveniently and easily applied in routine qualitative and quantitative analysis, quality control department and in laboratories.

ACKNOWLEDGMENT

The authors express their sincere thanks to Principal Dr. (Mrs.) Sudha Rathod, Oriental College of Pharmacy, Sanpada, Navi Mumbai, for providing the necessary facilities to carry out the research work.

CONFLICTS OF INTERESTS

All authors declare no conflicts of interests.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

REFERENCES

1. Budawari S. The Merck Index. 13th ed. Whitehouse Station, N.J: Merck and Co Inc.; 2003. p. 609, 9640.
2. Elks J, Ganellin CR. The Dictionary of Drugs: Chemical Data, Structures and Bibliographies. 1st ed. London; Royal; 1990. p. 472.
3. Merck and Company. The Merck Index-An Encyclopaedia of Chemicals, Drugs and Biologicals. 13th ed. USA: Merck and Company; 1989. p. 3757, 9648.
4. Martindale, The extra pharmacopoeia, The complete drug reference. 34th edn. Pharmaceutical press, London; Royal; 2005. p. 749, 760.
5. Sethna NF, Zurakowski D, Brustowicz RM, Bacsik J, Sullivan LJ, Shapiro F, et al. Tranexamic acid reduces intraoperative blood loss in pediatric patients undergoing scoliosis surgery. Anesthesiology 2005;102:727-32.
6. Her Majesty’s Stationary Office. British Pharmacopoeia. Vol. I, II. London: Her Majesty’s Stationary Office; 2004. p.758, 1960.
7. Jameelunnisa B, Abdul R. Spectrophotometric determination of drotaverine in tablets. Asian J Chem 2008;20:4173-84.
8. Jyotesh J, Riddhish P, Divyesh V, Renu C, Shallesh S. Dual wavelength spectrophotometric method for simultaneous estimation of drotaverine hydrochloride and acceclofenac in their combined tablet dosage form. Int J Pharm Pharm Sci 2010;4:76-9.
9. Roshan I, Kaushik KV, Diptish KN. Spectrophotometric methods for simultaneous estimation of ethamsylate and tranexamic acid from combined tablet dosage form. Int J Chemtech Res 2010;2:74-8.
10. Reddy NK, Potawale SE, Gabhe SY, Mahadik KR. HPTLC double development and validation of mefenamic acid and tranexamic acid in combined tablet dosage form. Pharm Sinica 2013;4:16-21.
11. Suganthi A, Thengungal KR. Stability indicating hplc method for simultaneous determination of drotaverine and acceclofenac. Int J Pharm Pharm Sci 2011;3:245-50.
12. Srinivasa RA, Pavankumar KL, Satyanarayana P, Subrahmany S. Simultaneous estimation of mefenamic acid, ethamsylate and tranexamic acid in bulk and pharmaceutical formulations by RP-HPLC method. Am J Pharm Tech Res 2015;5:402-14.
13. ICH. Stability Testing of New Drug Substances and Products (Q1AR2). Geneva: International Conference on Harmonization, IFPMA; 2003.