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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DEXTROMETHORPHAN HYDROBROMIDE, PHENYLEPHRINE HYDROCHLORIDE AND TRIPROLIDINE HYDROCHLORIDE IN BULK AND COMBINED TABLETS DOSAGE FORMS

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ABSTRACT

A simple, selective, sensitive and precise, simultaneous high performance liquid chromatographic analysis of tablets containing Dextromethorphan Hydrobromide, Phenylephrine Hydrochloride and Triprolidine Hydrochloride was described. Good chromatographic separation was achieved using a Kromasil C18 (250 x 4.6mm, 5µm) and mobile phase consisting of methanol: acetonitrile: 0.1M potassium dihydrogen phosphate buffer (75:15:10), adjusted to pH 6.8with sodium hydroxide, at flow rate 1ml/min. The PDA detector was used. The retention time of Dextromethorphan Hydrobromide, Phenylephrine Hydrochloride and Triprolidine Hydrochloride were measured at 2.547, 3.783 and 6.017 min, respectively. The linear ranges for Dextromethorphan Hydrobromide, Phenylephrine Hydrochloride and Triprolidine Hydrochloride were 48 - 112, 24 – 56 and 16-14 mcg/ml, respectively. The recoveries of Dextromethorphan Hydrobromide, and Phenylephrine Hydrochloride and Triprolidine Hydrochloride in pharmaceutical preparation were all greater than 98% and their relative standard deviations were NMT 2.0%. The limits of detection were 3.71, 1.90 and 0.52 mcg/ml for Dextromethorphan Hydrobromide, Phenylephrine Hydrochloride and Triprolidine Hydrochloride, respectively. The proposed method can be effectively applied for the simultaneous estimation f three drugs in bulk and in combined dosage form.

KEY WORDS

Dextromethorphan Hydrobromide, Phenylephrine Hydrochloride, Triprolidine Hydrochloride, RP-HPLC, validation.

INTRODUCTION

Dextromethorphan Hydrobromide (DEX), [3-Methoxy-17-methylmorphinan hydro bromide monohydrate]1-3, is an opioid like drug acts centrally .It elevates the threshold for coughing , without inhibiting ciliary activity .DEX rapidly absorbed from the gastrointestinal tract and converted into lower active metabolite (dextrorphan). The duration of action after oral administration is approximately three to eight hours for DEX**.** Several methods have been reported for analysis of cited drug either in bulk powder, different dosage forms or in biological fluids. These methods include spectrophotometric, chromatographic and voltametric methods.

Phenylephrine (PHE) is chemically (R) – 3[1-m-hydroxy-2-(methyl amino) methyl] benzyl alcohol hydrochloride used as decongestant. Oral phenylephrine is extensively metabolized by MAO enzyme in the gastrointestinal tract and liver. So compared to rally taken pseudoephedrine it has a reduced and variable bioavailability of only up to 38%. It is a direct selective alpha adrenergic receptor agonist; it does not cause release of endogenous noradrenalin, as pseudoephedrine does. So PHE has low side effects like CNS stimulation, irritability, insomnia, anxiety and restlessness. Triprolidine HCl (TPE), chemically (E)-2-(3-pyrolidine-1-yl-1(4-toly) prop-1-enyl-pyridine hydrochloride monohydrate),

used as antihistamine with central sedative & antimuscrinic effect for the symptomatic relief of hypersensitivity reaction including utricaria, skin disorders. Several methods are reported for the estimation of DEX**,** PHE and TPE individually and in combination with other drugs but no method is developed so far for the combination of DEX**,** PHE and TPE**.** A successful attempt is made to estimate the three drugs simultaneously. Therefore it was thought worthwhile to develop and accurate and rapid RP-HPLC method for simultaneous estimation of DEX**,** PHE and TPE from tablet formulations.

EXPERIMENTAL

Materials and reagents

DEX, PHE and TPE were kindly supplied as gift sample from Swiss Garnier Life sciences Himachalpradesh, India. A commercial preparation (DELETUS TABLET) used for analysis was procured from pharma market. Each tablet contains 10mg of DEX, 5mg of PHE and 1.25mg of TPE. HPLC grade methanol (SD Fine Chem limited Mumbai), HPLC grade acetonitrile and water (Finar chemicals limited Ahmedabad), Potassium dihydrogen phosphate, disodium hydrogen phosphate and sodium hydroxide (Qualikems Fine Chem Pvt Ltd Vadodhara).

Instrumentation:

RP-HPLC was performed using Shimadzu HPLC system consisting of a pump LC-20AD plus, rheodyne sample injection port with 20 microlitre loop , SPD-M20A Photo diode array detector (PDA), LC solutions software , column used was Kromasil C18(250 x 4.6mm, 5µm) , Weighing was done on Shimadzu model BL-220H balance and LI 610 pH meter was used for adjusting pH.

Chromatographic conditions:

A reverse phase column [Kromasil C18 (250 x 4.6mm, 5µm particle size)], equilibrated with mobile phase [methanol: acetonitrile: 0.1M phosphate buffer pH 6.8 adjusted with sodium hydroxide] was used. Mobile phase flow rate was maintained at 1mL/min and effluents were monitored at 271nm. The sample was injected using 20 microlitre fixed loop rheodyne injector and run time was 8 mins.

Standard Solution preparation:

About 40mg of DEX, 20mg of PHE and 5mg of TPE of each standard drug was weighed accurately and transferred to 50 ml volumetric flask and dissolved in mobile phase with and final volume was made up to the mark with mobile phase. Final concentration of DEX, PHE and TPE of 40µg/ml, 20µg/ml and 5µg/ml are made by suitable dilutions.

Preparation of pharmaceutical dosage form:

Twenty tablets were weighed `and crushed to fine powder. The tablet powder equivalent to 40 mg of DEX , 20mg of PHE and 5mg of TPE was transferred to a 50 ml volumetric flask and dissolved in mobile phase and the content was made up to mark with mobile phase, Then the sample solution kept in sonicater for 15min, then filtered the solution through 0.45µm filter paper . Final concentration of DEX, PHE and TPE of 40µg/ml, 20µg/ml and 5µg/ml are made by suitable dilutions.

Assay:

The amounts of DEX, PHE and TPE per tablet were determined by extrapolating the values of area from the respective calibration curve. Results are reported in **Table 3**.

Validation of HPLC method:

The proposed RP-HPLC method was validated as per ICH guidelines.

Selectivity and Specificity:

To assess the selectivity of the developed method solutions of all three drugs three drugs were injected into the system then observe three sharp peaks of DEX, PHE and TPE were obtained at retention time of 2.547, 3.783 and 6.017 min respectively in reference to standard solution. Specificity was determined by comparison of the chromatogram of mixed standards and sample solutions. As the retention time of standard drugs and the retention time of the drugs in sample solutions were same, so the method was specific. The parameters like resolution (Rs) and asymmetric factor were calculated. Good correlation was found between the results of mixed standards and sample solutions. Results are shown in the **Table 2**.

Precision:

Precision study was performed to find out intraday and interday variations. The intraday and interday precision study of DEX, PHE and TPE was carried out

by estimating the correspondence response 3 times on the same day and on 3 different days for 3 different concentrations of DEX, PHE and TPE and the results are reported in terms of % relative standard deviation (%RSD) however, all results fall within acceptance limits (RSD < 2), as shown in **Table 5**.

Accuracy:

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100% and 120%. The recovery studies were carried out by adding known amounts of standard DEX, PHE and TPE were added to pre-analyzed samples and they were subjected to proposed HPLC method. The recoveries results of DEX, PHE and TPE in pharmaceutical preparation are shown in the **Table 4**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ were separately determined based on the calibration curves. The limit of detection (LOD) and limit of quantification (LOQ) of developed method were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 Ə/S and 10 Ə/S, were respectively as per ICH guidelines Ə is standard deviation of response(y – intercept) and S is the slope f calibration plot. Results are shown in the **Table 5**.

Linearity:

Linearity was determined for DEX, PHE and TPE separately by plotting a Calibration curve of peak area against their respective concentration. From the calibration curve it was found that linearity range between 48-112 mcg/ml, 24-56 mcg/ml and 6-14 mcg/ml for DEX, PHE and TPE respectively. The slope and y- intercept value for calibration curve was y = 22.54x – 71.07 (R2=0.999) for DEX, y = 21.99x – 28.73 (R2=0.998) for PHE and y = 19.92x-18.03 (R2=0.997) for TPE. Results are shown in the **Table 1**.

Robustness:

The robustness study was done by making small changes in the optimized method parameters like ±1% change in mobile phase ratio, column temperature and ±1% change in pH. There was no s significant impact on the retention time and tailing factor.

RESULTS AND DISCUSSION

The present paper describes application of RP-HPLC method for simultaneous estimation of DEX, PHE and TPE bulk and tablet dosage form. To develop a precise, accurate and suitable HPLC method for simultaneous estimation of DEX, PHE and TPE different mobile phases such as methanol, acetonitrile and buffers (phosphate) in different proportions and finally methanol: acetonitrile: 0.1Mphosphate buffer adjusted with to pH6.8 with sodium hydroxide (75:15:10) was selected as an appropriate mobile phase, which give good resolution and acceptable peak parameters for DEX, PHE and TPE .The linear relationship was carried out between the peak area and concentration from a range of 48 - 112µg/ml for DEX, 24 -56µg/ml for PHE and 6 - 14µg/ml for TPE. The linearity can be expressed as correlation coefficient, i.e 0.999, 0.998 and 0.997 for DEX, PHE and TPE respectively. Correlation coefficient, y- intercept, slope of regression line is shown in table 1. Precision was determined as intermediate precision as per ICH guidelines. It was assessed at 3 concentration levels %RSD obtained was less than 2% for all the three drugs. The results of precision are shown in table 5. System suitability parameters for proposed method are shown in table 2. Assay of bulk and tablets DEX, PHE and TPE was evaluated. Three replicate determinations were carried out on tablets. Percentage purity was found to be 99.28%, 99.38% and 101.45%. Results of tablet analysis were shown in table 3. Robustness studies were carried out after deliberate alterations of flow rate, mobile phase compositions and mobile phase pH. It was observed that did not lead to changes of retention times of peak of interest. Percentage of recovery shows that method is free from interference of the excipients used in the formulation shown in **Table 4**.

Table 1: Linearity studies

PARAMETERS	DEX	PHE	TPE
Linearity range	48 – 112µg/ml	24 - 56µg/ml	6 - 14µg/ml
Slope	22.5	21.99	19.92
Intercept	71.07	28.73	18.03
Correlation coefficient	0.999	0.998	0.997

Table 2: System suitability parameters

System suitability parameters	DEX	PHE	TPE
Retention time (min)	2.547	3.783	6.017
Resolution	-	5.458	7.369
Theoretical plates	2126	4246	4144
Asymmetric factor	1.633	1.529	1.442

Table 3: Analysis of tablet formulation

BRAND(DELETUS)		% AMOUNT FOUND ± SD
DEX 10mg + PHE 5mg + TPE 1.25mg	DEX	99.28 ± 0.013
	PHE	99.38 ± 0.018
	TPE	101.45 ± .032

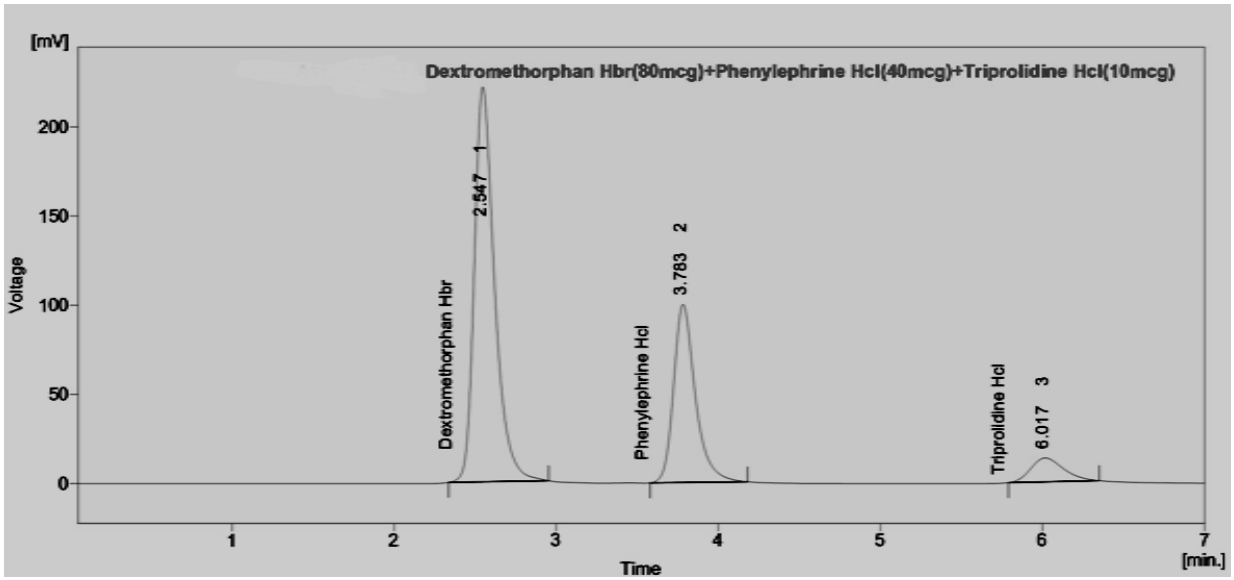
Table 4: Results of Recovery studies

Pre-analyzed sample solution[µg/ml]	Sample concentration [µg/ml]	Excess drug added [µg/ml ,n=3]	Amount recovered [µg/ml]	% Recovery
DEX	64	16	81.56	101.95%
	80	16	96.28	100.29%
	96	16	113.78	101.59%
PHE	32	8	40.15	100.37%
	40	8	47.12	98.16%
	48	8	55.36	98.86%
TPE	8	2	10.03	100.03%
	10	2	11.83	98.66%
	12	2	14.04	100.33%

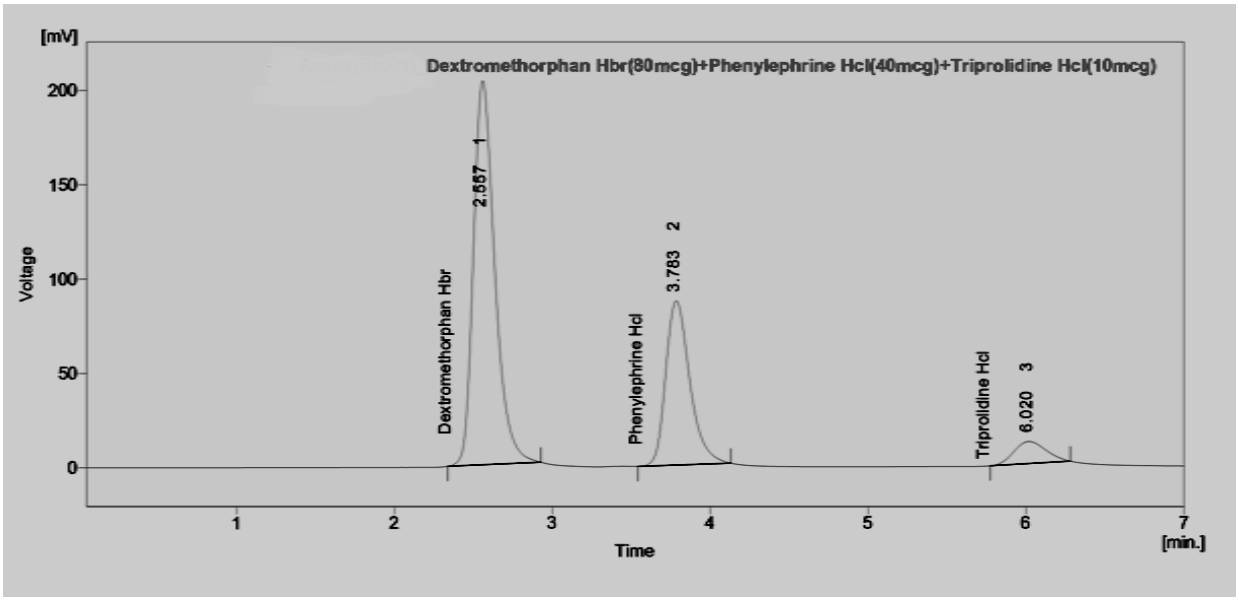
Table 5: Results of precision and LOD & LOQ

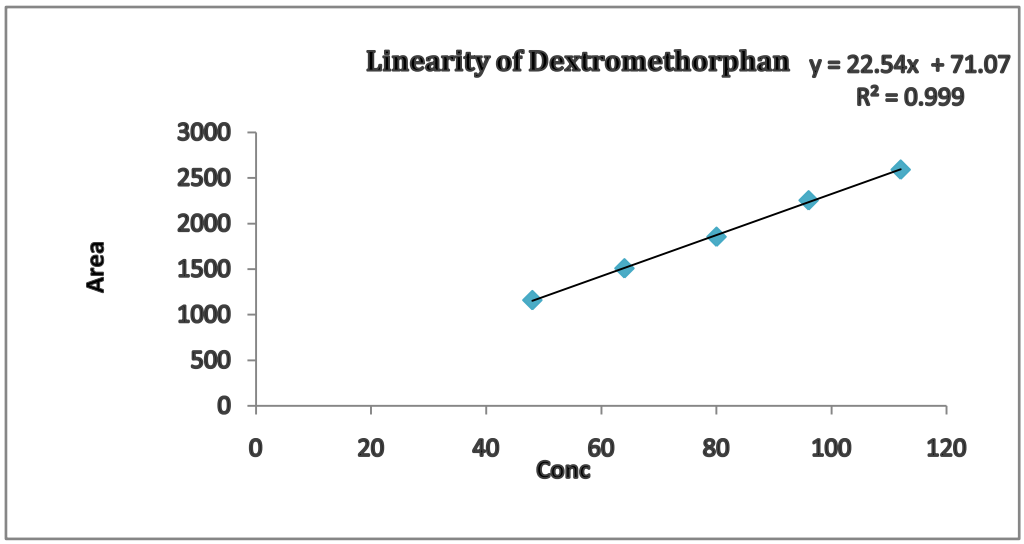
Parameters	DEX	PHE	TPE
	Precision(%RSD)		
Intra-day (n=3)	0.52	0.48	0.53
Inter-day (n=3)	0.23	0.20	0.25
Limit of detection	3.71	1.90	0.52
Limit of quantitation	11.24	5.750	1.58

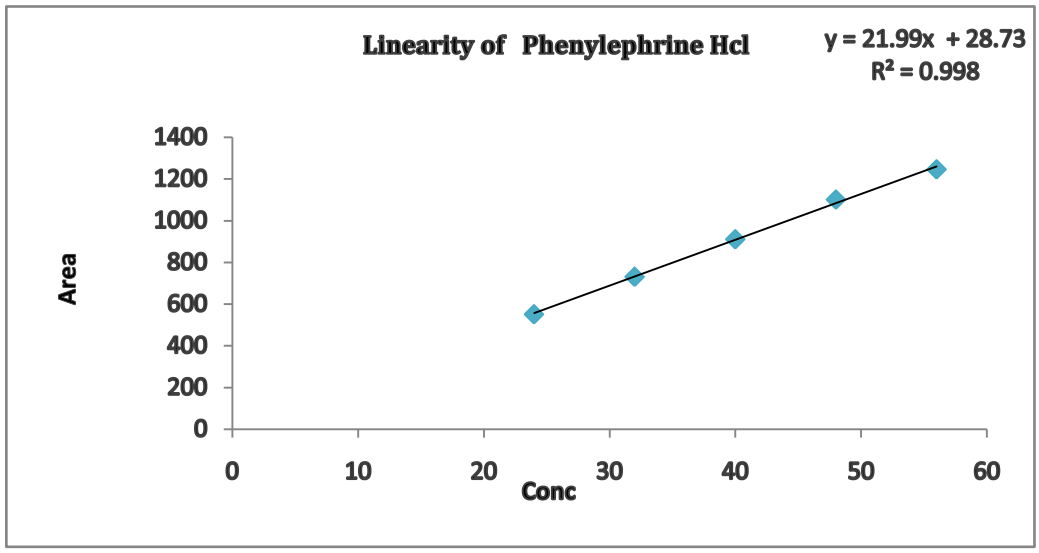
Assay sample graph

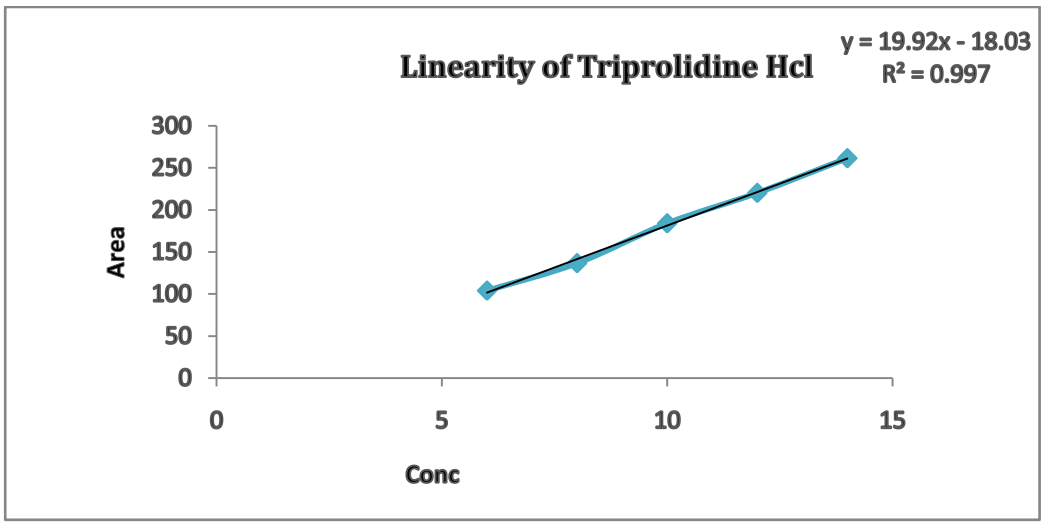


Standard drugs graph




Linearity of Dextromethorphan
y = 22.54x + 71.07
R² = 0.999
Area
Conc
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120


Linearity of Phenylephrine Hcl
y = 21.99x + 28.73
R² = 0.998
Area
Conc
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60


Linearity of Triprolidine Hcl
y = 19.92x - 18.03
R² = 0.997
Area
Conc
300
250
200
150
100
50
0
0
5
10
15

CONCLUSION

The present paper describes proposed RP-HPLC method for the simultaneous estimation of DEX, PHE and TPE in bulk and tablet dosage form is accurate, precise, linear, rugged, robust, simple and rapid. Acceptable regression values, RSD (%) and standard deviations which make it versatile and valuable for simultaneous estimation of three drugs in bulk and new tablet formulation. Acceptable values of precision and accuracy have been obtained all levels by this method as per guidelines for assay validation. The run time is relatively short i.e. within 7mins, so a large number of samples can be analysed in short period of time. The results of this developed RP-HPLC method can be could be conveniently adopted for quality control analysis of DEX, PHE and TPE simultaneously, from bulk and tablet dosage form.

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