Simultaneous Estimation of Phenylephrine HCl, Doxylamine Succinate and Dextromethorphan HBr in Soft Gelatin Capsule (Cough and Cold Preparation) by RP-HPLC

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ABSTRACT

A RP-HPLC methods were developed and validated for simultaneous estimation of Phenylephrine HCl (PE), Doxylamine Succinate (DOX) and Dextromethorphan HBr (DEX). The separation was achieved on a Princestone ODS C18 (250mm X 4.6 mm i.d., 10 µm particle size) with an Isocratic system of Phosphate Buffer (10 mM) : Methanol : Acetonitrile (pH 4) in the ratio of 70:25:5 v/v/v. The retention time for PE, DOX and DEX was obtained as 5.125 min, 10.419 min and 2.661 min respectively with a flow rate of 1.0 ml/min at detection wavelength 215 nm. The linearity of the proposed method was investigated in the range of 25 -125 µg/ml, 31.25 - 156.25 µg/ml and 50 - 250 µg/ml for PE, DOX and DEX respectively. The developed method was validated as per ICH guideline and found to be satisfactory.

Key words:

Phenylephrine HCl, Doxylamine Succinate and Dextromethorphan HBr, RP-HPLC, Validation

INTRODUCTION

Phenylephrine (3-[(1R)-1-hydroxy-2-(methyl amino) ethyl] phenol) Fig 1 is a selective α1-adrenergic receptor agonist used primarily as a decongestant, as an agent to dilate the pupil, and to increase blood pressure. Phenylephrine is marketed as a substitute for the decongestant pseudoephedrine, though clinical studies differ regarding its effectiveness in this role. Doxylamine (dimethyl({2-[1-phenyl-1-(pyridin-2-yl)ethoxy]ethyl})amine) Fig 2 is a first generation antihistamine. It can be used by itself as a short-term sedative and in combination with other drugs to provide night-time allergy and cold relief. Doxylamine is also used in combination with the analgesics paracetamol (acetaminophen) and codeine as an analgesic/calmative preparation, and is prescribed in combination with vitamin B6 (pyridoxine) to prevent morning sickness in pregnant women.Dextromethorphan ((1R,9R,10R)-4-methoxy-17-methyl-17-azatetracyclo [7.5.3.0^{1,10}.0^{2,7}]heptadeca-2,4,6-triene) (DXM or DM) Fig 3 is an antitussive (cough suppressant) drug. It is one of the active ingredients in many over-the-counter cold and cough medicines,

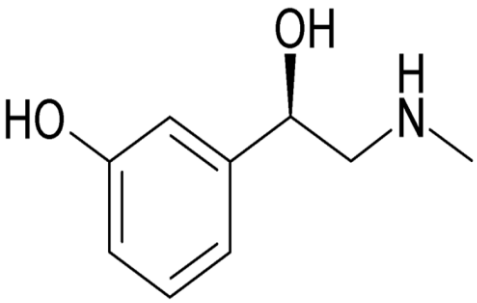


Figure 2 Phenylephrine

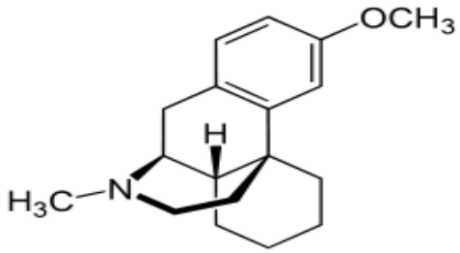


Figure 1 Dextromethorphan

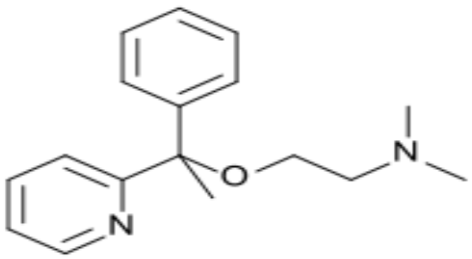


Figure 3 Doxylamine

Literature review reveals that numbers of individual analytical methods available for estimation of PEl[17], DOX and DEX[18] individually reported. And also reveals that numbers of analytical methods available for estimation of PE, DOX, and DEX in their combined dosage forms with other drugs reported but no analytical method was for reported combination of drugs.

MATERIALS AND METHODS

Gift sample of PE, DOX, and DEX was procured from Gujarat Liqui Pharma Caps Pvt. Ltd. Waghodiya GIDC, Vadodara. Methanol, Acetonitrile, KH_2PO_7, Ortho-phosphoric acid, Triethylamine HPLC grade were procured from RANKEM.

TABLE 1: CHROMATOGRAPHIC CONDITION

OPTIMIZED HPLC CONDITION	Observation
Column	Princestone ODS C18 (250mm X 4.6 mm i.d., 10 µm particle size)
Mobile Phase	Phosphate Buffer (10 mM): Methanol: Acetonitrile (70:25:5, pH-4.0)
Detection Wavelength	215 nm
Flow Rate	1 ml/min
Injection Volume	40 µL
Retention Time	Phenylephrine HCl: - 5.125 min Doxylamine Succinate:-10.419 min Dextromethorphan HBr: - 2.661 min

Selection of detection wavelength

PE, DOX, and DEX therefore prepared in Distilled Water. These drug solutions were than scanned in the UV region of 200-400 nm and the overlain spectrums were recorded and final wavelength 215 nm is selected for detection of the drugs.

Preparation of mobile phase

A mixture of 70 ml of 10 mM Phosphate Buffer (pH 2.5), 25 ml of methanol and 5 ml of acetonitrile is taken and final pH of the solution is made 4 with Triethylamine. This mobile phase filtered through 0.75 µm filter paper, Sonicated for 15 minutes to degas the mixture and used as mobile phase.

Preparation of standard stock solution

A 10 mg of standard use PE, 12.5 mg of standard DOX and 20 mg of standard DEX was weighed and transferred to a 25 ml volumetric flask and dissolved in 25 ml mobile phase. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 250 µg/ml PE, 312.5 µg/ml DOX and 500 µg/ml DEX.

Preparation of Calibration curve

Appropriate volume of aliquots from standard stock solutions were transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase give a solution containing 25, 50, 75,100,125 µg/ml PE, 31.25, 62.50, 93.75, 156.25 µg/ml DOX and 50, 100, 150, 200, 250 µg/ml DEX. The mixed standard solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.0 ml/min. The graphs were plotted for peak area vs. concentration for both the drugs. Fig 4, 5, 6, concentrations in the range of 25-125 µg/ml, 31.25-156.25 µg/ml and 50-250 µg/ml for PE, DOX, and DEX respectively.

Sample preparation

For estimation of PE, DOX, and DEX mixture was prepared. Mixture equivalent to 10 mg PE, 12.5 mg DOX and 20 mg DEX was accurately weighed and transferred to volumetric flask of 25 ml capacity. 10 ml of mobile phase was transferred to volumetric flask and Sonicated for 15 min. The flask was shaken and volume was made up to the mark with mobile phase. The above solution was filtered through whatman filter paper (0.75µ). Volume was made up to the mark using mobile phase to give a solution containing 250 µg/ml PE and 312.5 µg/ml DOX and 500 µg/ml DEX (solution A). From the solution A, 1 ml was transferred to volumetric flask of 10 ml capacity and volume was and 50 g/ml DOX. This solution used made up to the mark using mobile phase to give a solution containing 25 µg/ml PE and 31.25 µg/ml DOX for estimation of drugs.

Precision

Repeatability

Six replicates of standard mixture solution having 50 µg/ml PE, 62.50 µg/ml DOX and 100 µg/ml DEX were prepared and chromatograms were recorded and RSD was calculated.

Intraday precision

Standard solutions containing 25, 75 and 150 µg/ml PE and 31.25, 93.75 and 156.25 µg/ml DOX and 50, 150, 250 µg/ml DEX were analyzed three times on the same day. Chromatogram of each sample was taken. SD and RSD were calculated.

Interday precision

Standard solutions containing 25, 75 and 150 µg/ml PE and 31.25, 93.75 and 156.25 µg/ml DOX and 50, 150, 250 µg/ml DEX were analyzed on three different days. Chromatogram of each sample was taken. SD and RSD were calculated.

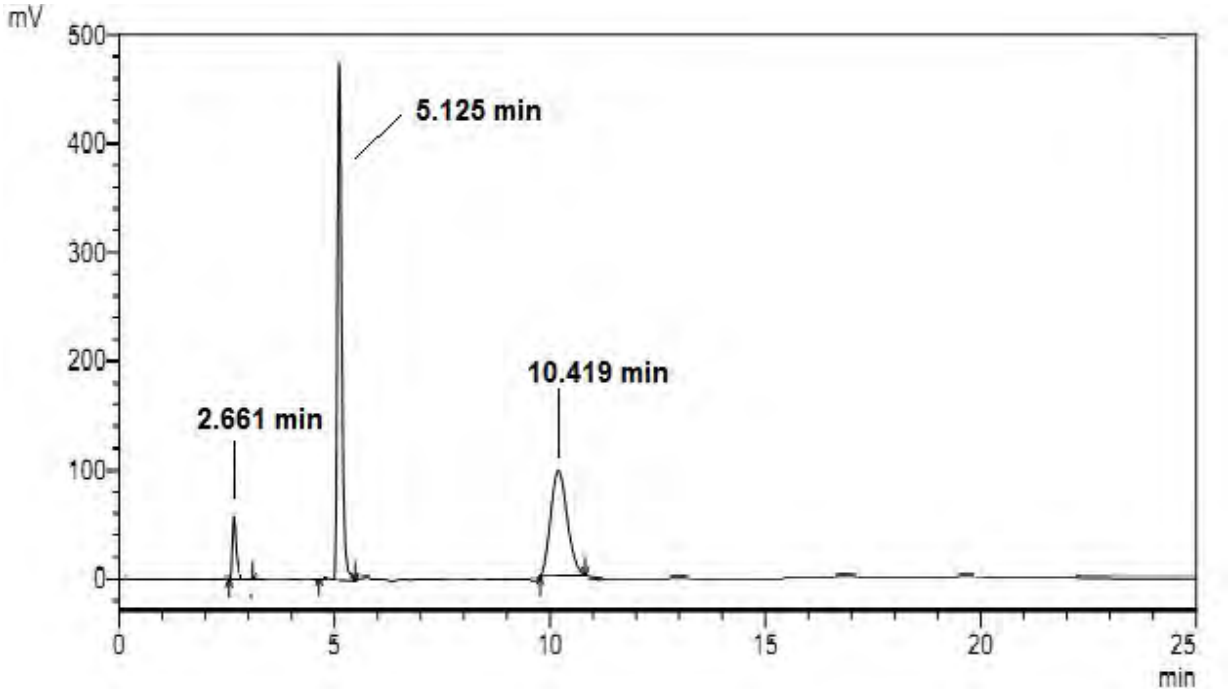
Robustness

Robustness study was performed by altered chromatographic conditions Wavelength (± 1nm), flow rate (± 0.2 ml/min), Variation in pH (± 0.2).

Duplicate injections of a standard mixture solution having PE (50 µg/ml), DOX (62.5 µg/ml) and DEX (100 µg/ml) were analyzed as per the procedure in each altered condition and chromatograms were recorded. %RSD of PE, DOX and DEX was calculated. If any particular change in method caused failure of system suitability that change was identified as a critical parameter and it was highlighted in the method of analysis

RESULT & DISCUSSION

Fig. 4 Chromatogram of Developed RP-HPLC Method



Linearity was performed on synthetic mixture of Phenylephrine HCl, Doxylamine Succinate and Dextromethorphan HBr. At different concentration in mixture at 215 nm at flow rate of 1 ml/min was used for making calibration curve

TABLE 2: CALIBRATION CURVE FOR PE (20-100 µG/ML), DOX (31.25-156.25 µG/ML) AND DEX (50-250 µG/ML)

Analyte	Concentration (µg/ml)	Average Peak area (n=3)
PE	25	1695832
	50	3339237
	75	4783227
	100	6398557
	125	7962198
DOX	31.5	1168252
	62.50	2594612
	93.75	3726211
	125.00	5278426
	156.25	6721561
DEX	50	242746
	100	379742
	150	543955
	200	726798
	250	888206

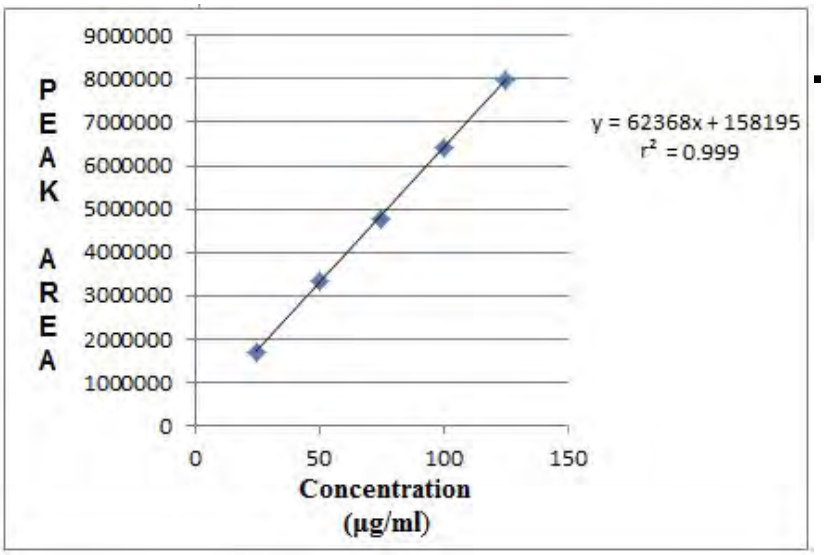


Fig. 5 Graph of Calibration curve for PE

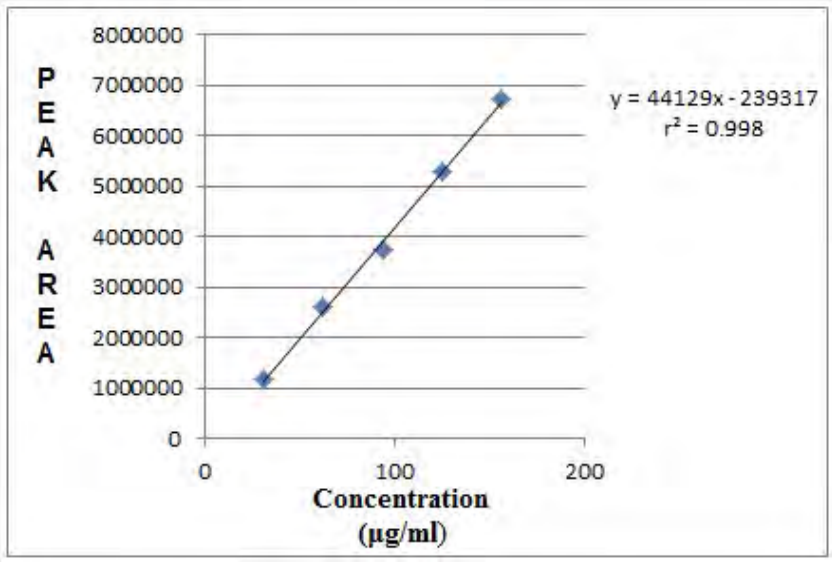


Fig. 6 Graph of Calibration curve for DOX

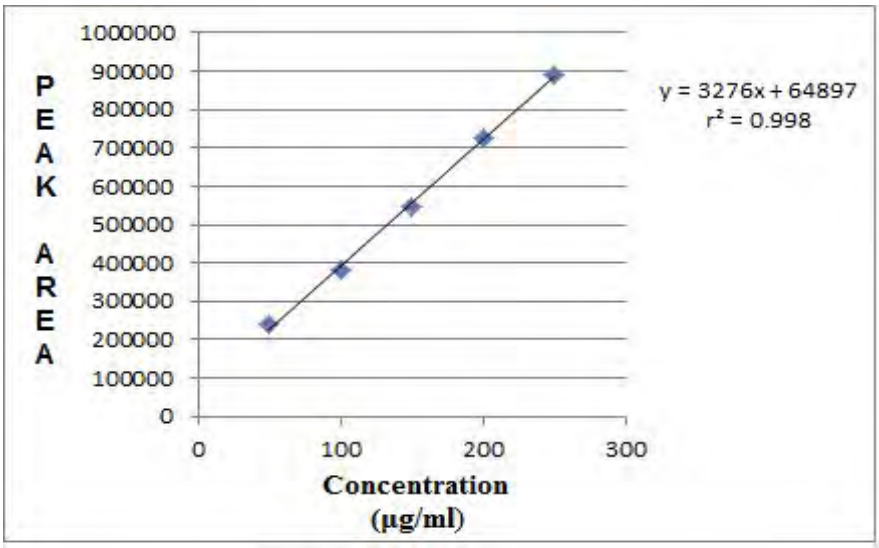


Fig. 7 Graph of Calibration curve for DEX

TABLE 3. % ASSAY OF PE, DOX, AND DEX

Drug	Concentration in synthetic mix as per market formulation (mg)	Concentration taken for % Assay (µg/ml)	Average Peak Area of sample	Concentration found from mix.	% Assay
PE	5	50	3328870	50.840	101.68
DOX	6.5	62.5	2518848	62.500	100.00
DEX	10	100	387703	98.630	98.63

TABLE 4: ACCURACY (% RECOVERY STUDY)

Concentration of Pre analyzed sample of PE: 50.932 µg/ml, DOX: 61.365 µg/ml and DEX: 99.325 µg/ml

Level of Spiking (%)	Amount of Drug Spiked (µg/ml)	Total Conc. Of Drug (µg/ml)	Peak Area of Sample (n=3)	Amount of Drug found (µg/ml) (n=3)	Amount Of Drug recovered (µg/ml)	Recovery Mean ± SD (%,n=3)
PE						
50%	25	75	4884695	75.786	24.852	99.409 ± 0.845
100%	50	100	6406346	100.183	49.250	98.500 ± 0.508
150%	75	125	7946718	124.881	73.949	98.598 ± 0.442
DOX						
50%	31.25	93.75	3842714	92.503	31.137	99.683 ± 0.845
100%	62.50	125.00	5270907	125.017	63.651	101.600±0.241
150%	93.75	156.25	6644601	155.995	94.629	100.938±0.149
DEX						
50%	50	150	553302	149.087	49.764	99.523 ±0.384
100%	100	200	718811	199.516	100.184	100.190±0.113
150%	150	250	884219	250.098	150.773	100.515±1.004

TABLE 5: INTRADAY PRECISION DATA FOR PE, DEX AND DOX (N=3)

Standard drug	Target Concentration (µg/ml)	Mean Peak area of samples (n=3)	Mean of Found Concentration (µg/ml)	SD
PE	25	1689756	24.557	0.199
	75	4795598	74.356	0.080
	125	7965438	125.180	0.299
DOX	31.25	1146032	31.393	0.602
	93.75	3731122	89.961	0.292
	156.25	6648768	156.089	0.904
DEX	50	233490	51.463	0.200
	150	550683	148.286	0.383
	250	882970	250.463	0.994

Robustness:

TABLE 6: ROBUSTNESS DATA FOR PE, DEX AND DOX

Parameters (n=3)	Variation	Average peak area (n=3)			% RSD		
		PE	DOX	DEX	PE	DOX	DEX
Flow rate	0.9	3334740	2492976	388844	0.451	0.241	0.098
	1.1	3338197	2523284	390332	0.062	0.240	0.353
Wave length	214	3299688	2539439	382628	0.689	0.752	0.209
	216	3334685	2592028	393239	0.592	0.274	0.478
pH	3.8	3320539	2478817	378459	0.215	0.710	0.396
	4.2	3375227	2532118	389294	0.688	0.672	0.144

CONCLUSION

A simple, specific, accurate and precise RP-HPLC method has been developed and validated for simultaneous estimation of PE, DOX and DEX in its synthetic mixture. PE, DOX and DEX were estimated on Princestone C18 column using Phosphate Buffer (10mM): Methanol: Acetonitrile (pH 4) (70:25:5 v/v) as mobile phase and detection was carried out at 215 nm.

The linearity range was found to be 25-125 µg/ml of Phenylephrine HCl, 31.25-156.25 µg/ml of Doxylamine Succinate and 50-250 µg/ml. The co-relation coefficient was found to be 0.999, 0.998 and 0.998 for PE, DOX and DEX respectively. The assay value for PE, DOX and DEX was found to be 101.68%, 100.01, and 98.63% respectively. The present method was validated as per the ICH guideline so it can be adopted for its routine analysis.

The developed method can be utilized for simultaneous estimation of PE, DOX and DEX.

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