Research Article www.ijcps.com

International Journal of
Chemical and Pharmaceutical Sciences
2022, Sep, Vol. 13 (3)

ISSN: 0976-9390
IJCPS

A new RP-HPLC analytical method Development and validation for Dexbrompheniramine and Pseudoephedrine

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Received: 20th Jul 2022, Revised and Accepted: 1st Oct 2022

ABSTRACT

A fast, sensitive, and reliable RP-HPLC method involving Waters HPLC System with PDA detection was developed and validated for the determination and quantification of Dexbrompheniramine and Pseudoephedrine. Chromatography was performed on the Inertsil -ODS C18 (250 x 4.6 mm , 5 µ) column using filtered and mixed Degassed Methanol and Acetonitrile (90:10) as a mobile phase with a flow rate of 1.0 ml / min and an effluent of 224 nm. Retention times for Dexbrompheniramine 4.712min, and Pseudoephedrine 6.691.

Keywords: Method Development, Validation, Dexbrompheniramine, Pseudoephedrine, RP-HPLC.

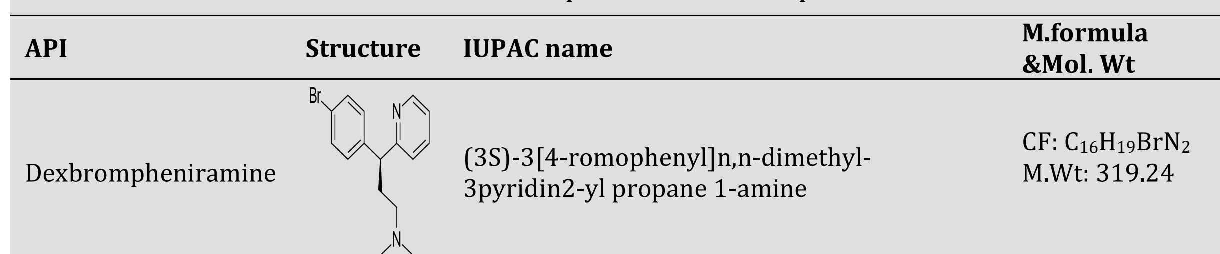
1. INTRODUCTION

Antihistamines are commonly used for relief of symptoms related to allergy which are caused by histamine release. They are mainly used for treatment of cough, cold and other types of allergy [1]. Antihistamines are substituted ethylamine's [2,3].

Dexbrompheniramine is an alkylamine derivative with anticholinergic and sedative properties. It is a H1- receptor histamine antagonist and acts in gastrointestinal tract, blood vessels and respiratory tract. It gives relief from allergic reactions such as bronchoconstriction's, vasodilatation, increased capillary permeability in rhinitis and conjunctivitis. It is also used for treating hay fever and urticaria[4].

It is most important to develop an analytical methodology to know the quantity of antihistamines in pharmaceutical formulations so that we can study their metabolization and their effects on human body [7]. Validation is an essential step to develop a method to analyse antihistaminic drugs in pharmaceutical formulations and serum with the help of electrophoresis [8].

Table-1: Chemical composition of Dexbrompheniramine

API	Structure	IUPAC name	M.formula &Mol. Wt
Dexbrompheniramine		(3S)-3[4-romophenyl]n,n-dimethyl-3pyridin2-yl propane 1-amine	CF: $C_{16}H_{19}BrN_2$ M.Wt: 319.24

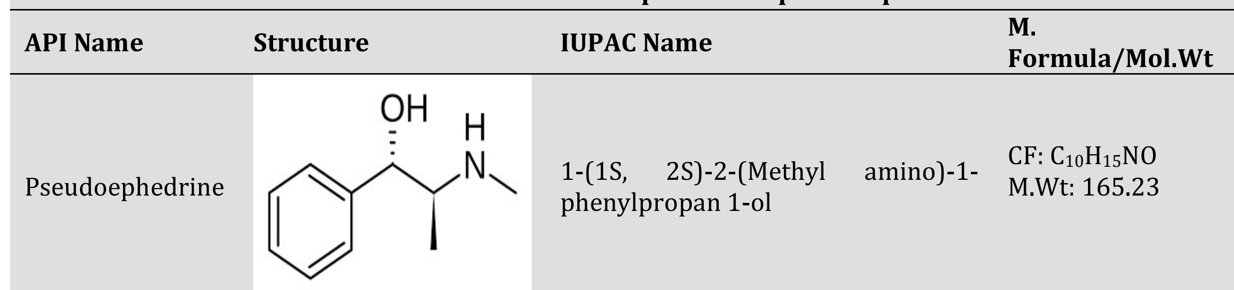


Table - 2: Shows chemical composition of pseudoephedrine			
API Name	**Structure**	**IUPAC Name**	**M. Formula/Mol.Wt**
Pseudoephedrine		1-(1S, 2S)-2-(Methyl amino)-1-phenylpropan 1-ol	CF: $C_{10}H_{15}NO$ M.Wt: 165.23

The repeat action combination tablet of dexbrompheniramine and pseudoephedrine and adjuvant administration of reference standards are bio equivalent at steady state [9].

The ICH Harmonized Tripartite Guidelines were selected as it was mainly developed for analysis of drugs [10]. There are Different methods for determination of PSE alone or in combined tablets and other pharmaceutical formulations. These include titrimetric [11], potentiometric, Spectro photometric, near infrared spectroscopic [12], gas chromatographic [13], high performance liquid chromatographic, capillary electrophoretic and flow injection analysis [14].

2. MATERIALS AND METHODS

2.1 chemicals

Samples of Dexbrompheniramine and Pseudoephedrine was gifted by Llorens Pharmaceuticals International Limited, India. Merck, Schuchardt OHG, Germany provided Liquid chromatographic grade Acetonitrile, KH_2PO_4, and phosphoric acid. Millipore Milli Q plus filtration technology was used to create ultra-pure water.

2.2 Instruments

we used a Waters 2690/5 LC system with a PAD detector (Waters Corporation, Milford, USA) that could detect wavelengths from 200nm to 400 nm. We also used it to test the method. Empower software was used to look at and process the outgoing signal on a Pentium computer that had a hard drive (Digital Equipment Co). Hydrolysis experiments were conducted using a Cintex computerized water bath. In a photo stability chamber, tests were conducted to determine the shelf life of various items (Sanyo, Leicestershire, UK). Thermal stability testing on the components were conducted in in oven (Cintex, Mumbai).

2.3 Selection of wave length (λ max)

A solution of 100 μg/ml ofDexbrompheniramine and Pseudoephedrine were prepared in Qualigens water. The resulting solutions were scanned individually on HPLC PDA detector from 200nm to 400 nm and also in UV-Visible spectrophotometer. The optimal response for both of them were obtained at 224nm. Hence the complete method was processed at the wave length of 224 nm.

2.4 Preparation of stock and sample solutions

In order to make a stock solution of Dexbrompheniramine and Pseudoephedrine (1000 mg/ml), 20 mg of the Dexbrompheniramine and 25 mg of the Pseudoephedrine of the drug ingredient were dissolved in a 100ml volumetric flask of the diluent (mobile phase) for 30 minutes and sonicated for 30 minutes. All of these solutions were pipetted into a 100-ml volumetric flask and mixed with the correct amount of diluent. Then, for 10 minutes, they were sonicated at a high speed. Then prepare 20, 30, 40, 50, 60, and 70 ppm solutions were prepared by utilizing the above stock solution.

2.5 Preparation of Buffer solution

Take in a 100 ml beaker 2.7218gm of KH_2PO_4. It was mixed with 1 litre of High grade HPLC water and its P^H was changed by adding H_3PO_4 till pH comes to 3.4

3. DEGRADATION STUDIES

Dexbrompheniramine and pseudoephedrine break down in different ways when they are under stress, liquid chromatography tests show.

3.1 Degradation in acidic solution:

In acidic degradation, take 10ml of methanol and 0.1M HCl in a volumetric flask, then add 1 mg/ml of Dexbromopheniramine & Pseudoephidrine stock solution and at 60°C reflux the mixture for 6 hrs. Allow it to cool to room temperature before neutralising with 0.1N NaOH and diluting with mobile phase to get a final concentration of 10μg/ml in a 100ml volumetric flask.

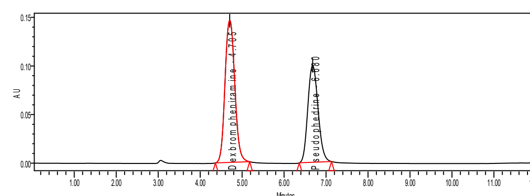


Figure -1: F1-Typical HPLC chromatogram

3.2 Degradation in basic solution:

In basic degradation, take 10ml of methanol and 0.1M NaOH in a volumetric flask, then add 1 mg/ml of Dexbromopheniramine & Pseudoephidrine stock solution and at 60°C reflux the mixture for 6 hrs. Allow it to cool to room temperature before neutralising with 0.1N HCl and diluting with mobile phase to get a final concentration of 10µg/ml in a 100ml volumetric flask

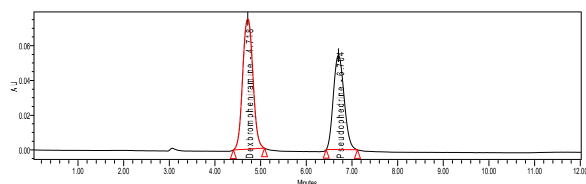


Figure – 2: F2-Typical HPLC chromatogram for base degradation

3.3 Oxidative degradation (using Hydrogen peroxide)

Take a 1mg/ml Dexbromopheniramine & Pseudoephidrine solution in 100ml volumetric flask and add 10ml of 30% H_2O_2 solution, and refluxed the solution for 6 hrs. at 60°C. Then keep at side to attain room temperature then diluted with mobile phase up to mark, then the solution contains 10µg/ml of Dexbromopheniramine & Pseudoephidrine

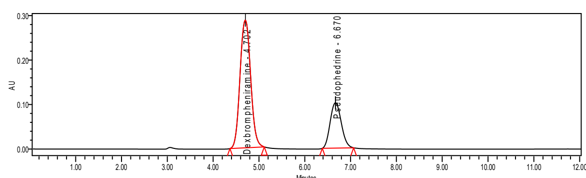


Figure- 3: F3-Typical HPLC chromatogram on Oxidative degradation

3.4 Thermal degradation:

In thermal degradation 50mg of Dexbromopheniramine &Pseudoephidrine was taken in a 100°C hot oven for 24hrs then it was dissolved in 10ml methanol and adjusted to 50ml with the mobile phase. Then to get concentration 10µg/ml of Dexbromopheniramine & Pseudoephidrine further diluted with mobile phase.

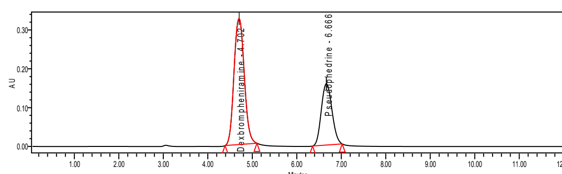


Figure – 4: F4-Typical HPLC chromatogram on thermal

We utilized Dexbromopheniramine & Pseudoephidrine as a reference/working standard to determine how stressed each sample was. It was close to 99.5% of the total weight of all the stressed samples. This demonstrates that the new LC approach was discovered to be highly specific to Dexbromopheniramine & Pseudoephidrine. There were no degradants in the mobile phase of Dexbromopheniramine & Pseudoephidrine samples that had been exposed to various types of stress.

Table – 3: Stressed studies:

Process	condition	Retention time		Area of the peak		%Degradation as compared with control	
		DBP	Pseudoephedrine	DBP	Pseudoephedrine		
Control sample	-	-	-	1220150	672015	DBP	Pseudoephedrine
Acid	(HCl-0.1N at 60°C)≈06 hrs	4.705	6.680	1085371	668548	11.0461	0.6498
Base	(NaOH-0.1N at 60°C)≈06 hrs	4.718	6.704	1191646	640283	2.3361	4.7219
Oxidation	by H_2O_2 30% at 60°C ≈06 hrs	4.702	6.670	1206741	652480	1.0989	2.9069
Thermal	Thermal at 100°C ≈24 hrs	4.702	6.666	1104286	648758	9.4958	3.4608

4. METHOD OF VALIDATION

Method validation can be defined as International Council for Harmonisation (ICH) "establishing documented evidence which provides a high degree of assurance that specific activity will consistently produce a desired result or product meeting its predetermined specifications and

quality characteristics. Method validation is an integral part of the method development; it is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, an and drug products. Simply, method validation is the process of proving that and potency of the drug substances analytical method is acceptable for its intended purpose. For chromatographic methods used in analytical applications there is more consistency in validation practice with key analytical parameters

4.1 Recovery:

The absolute recovery of analytical method is measured as the response of a processed spiked matrix standard expressed as a percentage of the response of pure standard which has not been subjected to sample pre treatment and indicates whether the method provides a response for the entire amount of analyte that is present in the sample.

$$\text{Absolute recovery} = \frac{\text{Response of an analute spike in to matrix(processed)}}{\text{Response of analyte of pure standard (unprocessed)}} X10$$

4.2 Sensitivity

The method is said to be sensitive if small changes in concentration cause large changes in response function. The sensitivity of an analytical method is determined from the slope of the calibration line. The limits of quantification (LOQ) or working dynamic range of bio analytical method are defined as the highest and lowest concentrations, which can determined with acceptable accuracy. It is suggested that, this be set at ± 15% for both the upper and lower limit of quantitation respectively. Any sample concentration that falls outside the calibration range cannot be interpolated from the calibration line and extrapolation of the calibration curve is discouraged. If the concentration is over range, the sample should be diluted in drug-free matrix and re-assayed.

4.3 Precision

The purpose of carrying out a determination is to obtain a valid estimate of a 'true' value. When one considers the criteria according to which an analytical procedure is selected, precision and accuracy are usually the first time to come to mind. Precision and accuracy together determine the error of an individual determination. They are among the most important criteria for judging analytical procedures by their results.

Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central value. The term 'set' is defined as referring to a number (n) of independent replicate measurements of some property. One of the most common statistical terms employed is the standard deviation of a population of observation. Standard deviation is the square root of the sum of squares of deviations of individual results for the mean, divided by one less than the number of results in the set. The standard deviation S, is given by

$$S = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(X_i - X)^2}$$

Standard deviation has the same units as the property being measured.

The square of standard deviation is called variance (S^2). Relative standard deviation is the standard deviation expressed as a fraction of the mean, i.e., S/x. It is sometimes multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.

$$\%\text{Relative standard deviation} = \frac{S}{X} * 100$$

4.4 Accuracy

Accuracy normally refers to the difference between the mean x****, of the set of results and the true or correct value for the quantity measured. According to IUPAC accuracy relates to the difference between results (or mean) and the true value. For analytical methods, there are two possible ways of determining the accuracy, absolute method and comparative method.

Accuracy is best reported as percentage bias, which is calculated from the expression

$$\%\text{Bias} = \frac{(\text{measured value} - \text{truevalue})}{\text{true value}} X100$$

The accuracy of analytical method is then determined at each concentration by assessing the agreement between the measured and nominal concentrations of the analytes in the spiked drug – free matrix sampler.

4.5 Limit of detection (LOD)

The limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b, by

$$\text{LOD} = \frac{3Sa}{b}$$

4.6 Limit of quantification (LOQ)

The LOQ is the concentration that can be quantitate reliably with a specified level of

accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

$$LOQ = \frac{10Sa}{b}$$

Where, Sa- the estimate is the standard deviation of the peak area ratio of analyte to IS

(5 injections) of the drugs. b -is slope of the corresponding calibration curv

4.7 Ruggedness

Method Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

4.8 Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small but deliberate variations in method parameters". The robustness of a method is the ability to remain unaffected by small changes in parameters such as pH of the mobile phase, temperature, %organic solvent strength and buffer concentration etc. to determine the robustness of the method experimental conditions were purposely altered and chromatographic characters were evaluated

4.9 System suitability

System suitability experiments can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. (or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis.

The criteria selected will be based on the actual performance of the method as determined during its validation. For example, if sample retention times form part of the system suitability criteria, their variation (SD) during validation can be determined system suitability might then require that retention times fall within a ±3 SD range during routine performance of the method.

5. RESULTS AND DISCUSSION

5.1. Validation of the developed method

The process of ensuring that the HPLC strategy utilized to perform a given test is appropriate for its intended use is known as HPLC method validation. The planned and refined HPLC technology was put to the test. The validation is carried out according to ICH guidelines

Table – 4: Optimized Chromatographic conditions

Rate flow	1.0ml/min
Column	Inertsil C18, 250mm X4.6 mm, 5µm
wavelength	224 nm
Temperature	Ambient
Volume of injection	20µL
Mobile phase	Methanol: acetonitrile 90:10
HPLC program	Isocratic
Run time	10 min

Based on the system suitability investigations, it was discovered that the optimized standard chromatogram had a peak at 4.712&6.691 min and that the mobile phase was 75:25v/v at 225nm, and that the mobile phase was M;ACN=90:10v/v at 224nm. The peak areas of dexbrompheniramine and pseudoephedrine were found to have a relative standard deviation of 0.028363&0.064022%. For Dexbrompheniramine and Pseudoephedrine, it was discovered that the technique exhibited linearity in the concentration ranges of 20 ppm to 70ppm based on linearity data. A linearity graph was constructed for the peak area of Dexbrompheniramine and Pseudoephedrine versus concentration. The coefficient was found to be within the range of 0.999 for both of these compounds. It was necessary to inject the standard solution five times to determine the compatibility of the system, and the area of each injection was measured using an HPLC. Observations were made to ensure that the % RSD and MEAN remained within the prescribed parameters. Precision was determined to be within the acceptable System Precision, Method Precision, Intermediate Precision and it was computed as percent assay for and percent RSD for assay calculation, respectively. Different concentration levels, such as 50 percent, 100 percent, and 150 percent, were developed in order to improve the accuracy of computations. We discovered that recovery is possible within the parameters. This study calculated robustness for different flow rates of mobile phase, such as 0.8ml/min, 1.0ml/min, and 1.2ml/min, and calculated %RSD We measured ruggedness and found that system to system variability was 100.23, indicating that the approach was accurate. We also measured precision and found that the method was 100.25.

Results reveal that RSD of retention time and accuracy, ruggedness, robustness linearity and precision are all within the acceptable ranges of results.

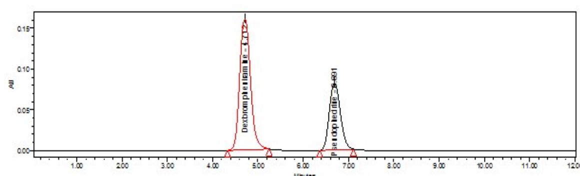


Figure – 5: F1- Standard Chromatography

Inference: got Rt of 4.712min to Dexbrompheniramine & 6.691min to Pseudo Ephedrine

5.1 Test for system suitability

Chromatography is a way to make sure that the system is going to work. It is used to check how well the system works for its intended use. System suitability's major purpose is to ensure that the entire testing process, including the instrument and the analyst, is appropriate for the task at hand.

Table – 5: Data of SST

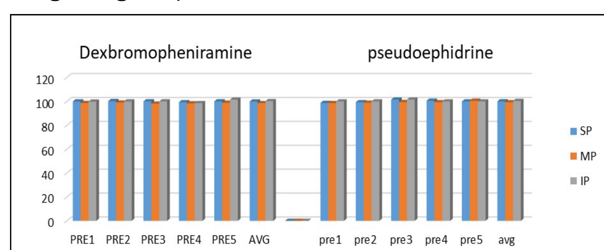
Preparation	Rt	Area	Pl. count	Tailing factor
1	4.702	1214943	8973.402	0.8977
2	4.705	1220150	8906.534	0.8859
3	4.706	1220212	8887.750	0.8942
4	4.707	1219505	8844.319	0.8791
5	4.709	1265543	8843.580	0.8915
Mean	4.703682	1228070.6	8891.11744	0.889742
SD	0.000837	21062.68	-	-
%RSD	0.028363	1.71	-	-

Table - 5: Data of SST for Pseudoephedrine

Preparation	Rt	Area	Pl,count	tailing
1	6.684	674665	6050.516	1.150328
2	6.681	672015	6150.672	1.150988
3	6.680	672211	6123.776	1.164277
4	6.684	677612	6175.210	1.151204
5	6.686	689531	6046.716	1.156380
Mean	6.686101	677206.8	6109.379	1.154635
SD	0.002683	7252.993	-----	------
RSD %	0.064022	1.071016	----	-----

5.2 Precision

Precision refers to how well a set of measurements that were taken from the same homogenous sample under the same conditions line up. There are three types of parameters system precision, method precision and intermediate precision all of which are important for getting the job done.



5.3 Linearity

When analyte concentration changes within a specified range, the method's linearity determines how accurate the results are. The slope of the line is a common way to express linearity.

It was found out how accurate the assay technique was by doing 5 separate tests of the Dexbrompheniramine and Pseudoephedrine test sample against a known standard, with an % RSD of 0.3 percent for each of the five tests.

5.3.1 Method linearity

Dexbrompheniramine & Pseudoephedrine assay concentrations 20ppm-70ppm were used to test the method's linearity. The LC system was injected with each solution. Using a correlation coefficients greater than 0.999, the calibration curve between the average peak area and the concentration was found to be linear. y = 30712x -31891 and y = 16499x+8683.5 were the two most accurate linear equations. Because the standard error of peaks and the RSD were both less than 0.6 at all concentrations, this suggests that the data are uniform.

Table - 5: Linearity details (Dexbrompheniramine			
Concentration	**Area**	**Statistical analysis**	
0	0	**Correlation coefficient**	0.999
20	588735	**Slope**	30712
30	885434	**Y intercept**	-31891
40	1214943		
50	1489197		
60	1794937		
70	2101821		
80	2450946		

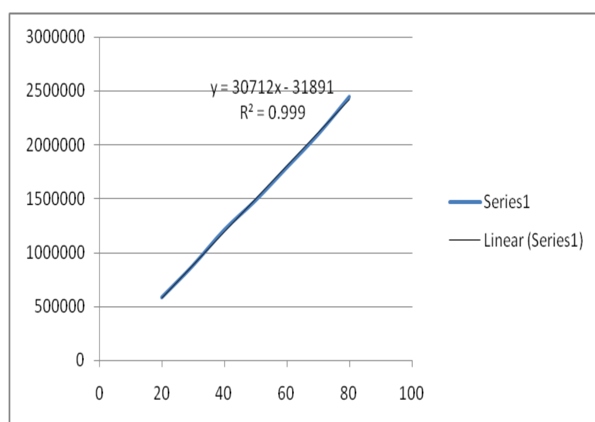


Figure - 6: Linearity Plot of Dexbrompheniramine

Table- 6: Linearity details (Pseudoephedrine)			
Concentration	**Area**	**Statistical analysis**	
0	0	**Correlation coefficient**	0.999
20	343650	**Slope**	16499
30	498630	**Y intercept**	8683.5
40	674665		
50	829406		
60	992122		
70	1160122		
80	1336708		

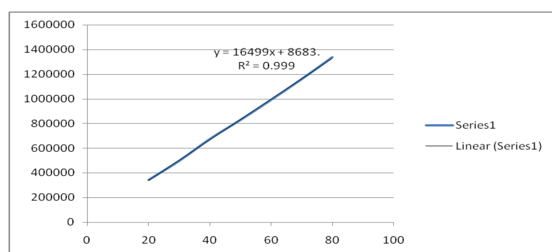


Figure – 7: Pseudoephedrine Linearity Plot (Concentration Vs Answer)

5.4 Detection and quantification limits (LOD and LOQ)

Dexbrompheniramine, and Pseudoephedrine of LOD and LOQ were caluculated by S/N ratio Ex:To determine LOD and LOQ, we must first determine how low a concentration must be and how many samples must be obtained to achieve that concentration and quantity. Both 3:1 and 10:1 signal-to-noise ratios are regarded to be correct. In other method The LOD and LOQ are calculated using the linearity plot.By using the following equation we can calculate LOD value of Dexbrompheniramine, and Pseudoephedrine Here σ can be calculated by using average area of system suitability data, and slope can be calculated by using linearity data.

Dexbrompheniramine

The linearity plot is used to calculate the LOD and LOQ. For example:

$$LOD = 3.3X\sigma/S$$

$$LOD = 3.3X2125.583/30712 = 0.22$$

Pseudoephedrine

$$LOD = \frac{3.3X\sigma}{S}$$

$$LOD = 3.3X1252.993/16499 = 0.25$$

5.5. Limit of Quatitation

Dexbrompheniramine

$$LOQ = 10X\sigma/S$$

$$LOQ = 10X2125.583/ 30712 = 0.69$$

Pseudoephedrine

$$LOQ = 10X\sigma/ S$$

$$LOQ = 10X1252.993/16499 = 0.75$$

6. Market sample

Sample preparation = Tablet average weight X std wt / Tablet lable claim

%Assay = Amount found/ Amount added X 100

Table – 7: Assay		
Preparation	**Peak area of Dexbrompheniramine**	**% Assay**
1	1255351	99.5
2	1260732	100.03
3	1251934	99.33
4	1258673	99.85
5	1259134	99.98
Mean	1257165	99.738
%RSD	0.279902	0.308819

Table - 8: Data of market sample		
Preparation	Peak area of Pseudoephedrine	%Assay
1	674185	100.85
2	674507	100.9
3	673926	100.8
4	672084	100.5
5	674961	100.95
Mean	673932.6	100.8
%RSD	0.163703	0.175374

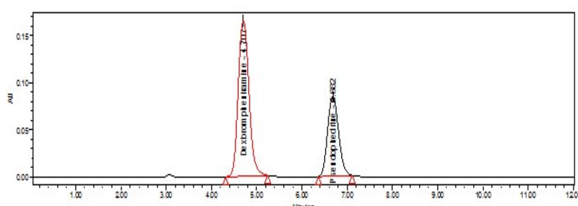


Figure – 8: Chromatogram of market sample

7. CONCLUSION

The simple isocratic RP-HPLC technique devised for the quantitative detection of Dexbrompheniramine& Pseudoephedrine in bulk active material is exact, accurate, and specific. The procedure was completely verified with good results for all parameters. The established approach indicates stability and may be used to analyse production samples and bulk samples to determine the retest period for Dexbrompheniramine and pseudoephedrine.

Acknowledgement

The authors gratefully acknowledge to Faculty of Science,Department of Chemistry, Osmania University,Hyderabad for their support.

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