



Research Paper

Development and validation of Amphojel In RP- HPLC

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ABSTRACT

Concurrent assessment of Amphojel by RP-HPLC in mass and promoted drug dose structure using an opposite level advanced execution fluid chromatography (AEC) strategy.

Using a portable length of 0.02 M phosphate cushion arrangement: acetonitrile (70:30v/v, pH 3.0), we successfully completed the warranty on an Inertsil octa decyl silan C18 (250 x 4.6 mm, 5 μ m) segment. Amphojel's adaptable stage is now a 20:80 mixture of cradle (pH-5) and Methyl liquor. The stream rate was maintained at 1ml/min. The chromatographic separation frequency that was ultimately used was 306. Twenty microliters (l) of infusion volume was preserved. The section was stored under ambient conditions. The portable level was cleaned before its first usage by passing through a 0.45 μ m micron tube. The aftereffects of research have been authorised clearly and via healing investigations.

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1. INTRODUCTION

HPLC is in like manner alluded to as unreasonable pressure fluid chromatography considering unnecessary strain is utilized to improvement the skim price and inexperienced division through compelling the telephone phase through at tons higher price. The stress is finished the usage of a siphoning device¹. The improvement of HPLC from old fashion phase chromatography is probably ascribed to the improvement of greater modest molecule sizes. greater modest molecule length is critical considering they provide extra noteworthy floor district over the traditional sizeable molecule sizes. The primary objective of proposed work is to develop new simple, sensitive, accurate and economical analytical method for the estimation of Amphojel in bulk and marketed pharmaceutical dosage form².

Amphojel, Aluminum hydroxide [*a-LOO-mi-num-hye-DROX-ide*], $\text{Al}(\text{OH})_3$, is found in nature as the mineral gibbsite (also known as hydrargillite) and its three much rarer polymorphs: bayerite, doyleite, and nordstrandite. Aluminium hydroxide is amphoteric, i.e., it has

both basic and acidic properties. Closely related are aluminium oxide hydroxide, $\text{AlO}(\text{OH})$, and aluminium oxide or alumina (Al_2O_3), the latter of which is also amphoteric³. These compounds together are the major components of the aluminium ore bauxite. Aluminium hydroxide also forms a gelatinous precipitate in water.

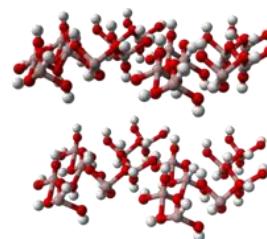


Figure no. 1 Structure of Aluminium hydroxide

2. MATERIALS AND METHOD

Chemicals

S.No	Chemical	Brand names
1	Amphojel (Pure)	Aluminium hydroxide Amphojel, Alu-Cap, Dialume, Alu-Tab, Alternagel, Aloha-Gel
2	Water for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

Instrumentation

The HPLC system consisted of WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector. consisting of gradient pump having a flow range from 0.2 up to 10 ml/min and Inertsil octa decyl silane (150X4.6X5 μ) C- 18 column was used⁴. The HPLC system was equipped with the Software having double-beam photometer with light source deuterium lamp and wavelength limit of 190-600 nm having integrated degasser⁵. The Mobile phase was a composite of buffer (pH-5) and Methyl alcohol in the ratio of 20:80 for Amphojel. Flow rate was maintained at 1ml/minute. Wavelength for the chromatographic separation used was 306 nm. Injection volume was maintained at 20 μ l. The column was maintained at ambient temperature. The mobile phase was purified by passing through 0.45 μ m micron filter prior to use.

Preparation of Reagents and Standards

Mobile phase selection

Standard stock solution of Amphojel was prepared by dissolving 20 mg of Amphojel standard into 100 ml of methanol which gives 200 μ g/ml of standard stock solution. From this solution 1ml was taken and diluted it with 10 ml of mobile phase (mobile phase which used for trials) to give 20 μ g /ml standard working solution⁶. Above working standard preparation was injected for mobile phase selection. Different mobile phase combinations was used for the study until system suitability parameters was obtained.

Materials

Amphojel reference standard was furnished by the sunpharm Research Centre, mumbai. The pharmaceutical dosage form of Amphojel, Aluminium hydroxide, Amphojel, Alu-Cap, was procured from local pharmacy with a 320 mg label claim, manufactured by pharma force lab paonta sahib, India. All chemicals used was of HPLC grade, Methanol and water both HPLC grade, was from Merck, potassium hydroxide and potassium dihydrogen phosphate of analytical grade from Merck.

Procedure For Buffer Preparation (Phosphate Buffer, pH 5)-

Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 ml of water and adjust the pH to 5.0 with 10M potassium hydroxide.

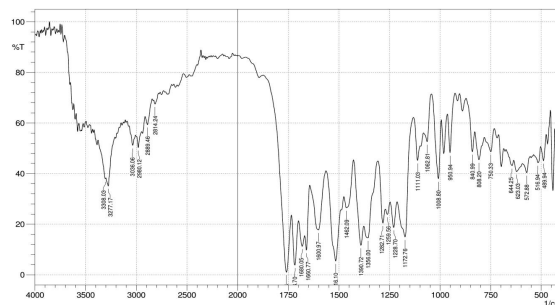


Figure no. 2 I.R spectra of standard Amphojel

Method validation

Linearity and Range- Standard stock solution of Amphojel was prepared by dissolving 20 mg of Amphojel standard into 100 ml of methanol which gives 200 μ g/ml of standard stock solution. Method linearity was displayed over the concentration range of 10 μ g /ml to 30 μ g /ml. Concentration range of 10 μ g /ml to 30 μ g /ml was obtained by dissolving 0.5ml, 0.75ml, 1ml, 1.25 ml, 1.5 ml of Amphojel standard stock solution with mobile phase diluted with 10 ml to obtain 10 μ g /ml, 15 μ g/ml, 20 μ g/ml, 25 μ g/ml, 30 μ g/ml of final concentration of Amphojel for linearity determination.

Precision Studies of Amphojel - Precision studies was carried out in three different ways as intraday precision, interday precision and repeatability. Intraday precision studies was conducted for three different concentrations at three times each for single day and interday precision studies was conducted for three different concentrations at three times at alternate days respectively and repeatability for one concentration at six times⁷.

Recovery/accuracy studies- Recovery studies was performed by spiking the sample with the standard and then finding out the recovery at 80,100,120 percent. In this an chunk of standard drug was mixed to the sample preparation and find out recovered amount from sample preparation as % recovery.

Procedure: Standard Stock Solution of Amphojel - Standard stock solution of Amphojel was made by dissolving 20 mg of standard drug in 100 ml of Methyl alcohol to make 200 μ g/ml solution.

Standard Working Solution for Recovery (20 μ g/ml) for Amphojel:

1ml of standard stock solution of Amphojel was taken and diluted with 10 ml of mobile phase.

Sample Stock Solution of Amphojel:

Weigh and powdered 20 tablets. Take tablet powder equivalent to 20 mg of Amphojel in to a 100ml volumetric flask. Add 60 ml Methanol. Shake for 15 minutes and sonicate for 10 minutes. Make up volume with Methanol. Filter this solution through 0.45 μ m micron filter prior to use to give 200 μ g/ml⁸.

Sample Solution for Recovery for Amphojel (20 μ g/ml):

Take 1ml from sample stock solution and then diluted with 10 ml of mobile phase.

Recovery Solution Preparation for Amphojel: Recovery solution was prepared for standard by dissolving 0.8ml, 1ml and 1.2ml of standard Amphojel and then volume make upto 10 ml with the diluent. Recovery solution of sample was prepared by dissolving 1ml from sample stock solution and then diluted with 10ml of mobile phase to give

20µg/ml. After that injected standard solution for recovery, sample solution for recovery and recovery solutions of 80%, 100% and 120% and then calculated % recovery.

3. RESULT AND DISCUSSION

Mobile Phase Selection

The final mobile phase combination selected which shows system suitability parameter was Phosphate buffer pH 5.0-Methyl alcohol in the ratio of 40-60 in standard Amphojel. System suitability parameters are shown in table 1.

Retention time[min]	Area	Height	Assymetry	Theoretical plate
5.127	1973.678	428.693	1.289	28943

Table no.1 System suitability parameters for final selected trial

Linearity

The linearity graph and linearity chromatogram of Amphojel are shown in figure no.3 represents linearity data concentration vs time. The method was found to show linearity in the domain of 10 µg /ml to 30 µg /ml respectively.

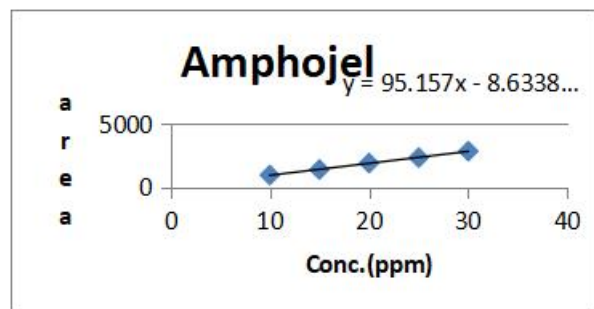


Figure no. 3 Linearity graph of Amphojel

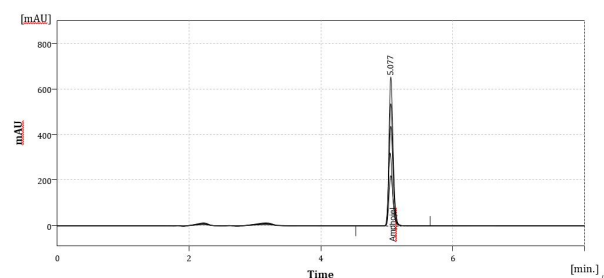


Figure no.4 Linearity chromatogram of Amphojel

S.No	Concentration(µg/ml)	Area
1	10	967.175
2	15	1495.785
3	20	1935.204
4	25	2449.849
5	30	2969.529

Table no. 2 Linearity data- concentration versus time

Precision studies of Amphojel -Precision studies was carried out and the method was found to be precise for intraday, interday and on repeatable determinations..

Intraday Precision

Table 12,13,14 represents intraday precision values and shows the area, average area, standard deviation and %RSD at 10 µg/ml, 20 µg/ml and 30 µg/ml respectively for intraday precision. Figures 21-29 represents the respective chromatograms for intraday precision at 10 µg/ml, 20 µg/ml and 30 µg/ml at three determinations each.

STANDARD	AREA
1	977.145
2	980.973
3	957.127
Average	998.3985
Standard deviation	2.326321466
%RSD	0.242159041

Table no. 3-Intraday precision at 10 µg/ml

STANDARD	AREA
1	1806.369
2	1844.381
3	1862.589
Average	1978.697353
Standard deviation	9.476964953
%RSD	0.692179907

Table no.4- Intraday precision at 20 µg /ml

STANDARD	AREA
1	2887.459
2	2867.979
3	2967.398
Average	2975.296373
Standard deviation	6.209458584
%RSD	0.187867863

Table no. 5- Intraday precision at 30 µg /ml

Interday Precision

The area, average area, standard deviation and %RSD at 10 µg/ml, 20 µg/ml and 30 µg/ml respectively for interday precision. Figures represent the respective chromatograms for interday precision at 10 µg/ml, 20 µg/ml and 30 µg/ml at three determinations each.

STANDARD	AREA
1	838.298
2	853.841
3	970.347
Average	848.159
Standard deviation	12.89986
%RSD	1.2537

Table no.6- Interday precision at 10 µg/ml

Repeatability Studies- Results shows the area,average area,standard deviation and %RSD at 20 µg/ml.The method was found to be precise with the repeatability studies. Figures represents the respective chromatograms for repeatability at 20 µg/ml at six determinations each. The % R.S.D was found within the limits.

STANDARD	AREA
1	1797.026
2	1787.598
3	1792.752
4	1798.847
5	1772.648
6	1752.982
Average	1791.1478
Standard deviation	6.119728817
%RSD	0.477131396

Table no.7- Repeatability study at 20 µg/ml

Recovery Studies

Results obtained from recovery studies are given in table 19 and table 20 with average recovery found out to be 98.508, 102.427 and 99.637 respectively,standard deviation was 1.7282, 1.6144 and 0.83953 and %R.S.D was 1.7544, 1.5761 and 0.8425 .Percentage recovery was shown to be within limits.The representative chromatograms obtained during recovery studies are given in figures for 80% spiking with standard and figures 48 to 50 for 100% spiking with standard and figuresfor 120% spiking with standard for sample for recovery and standard for recovery respectively¹⁰.

Spiking of standard	Area of sample spike with standard	Area of sample	Net area of standard	Area of standard
80%	1678.424	923.685	754.739	940.1
	1652.655	923.685	728.97	940.1
	1662.571	923.685	738.886	940.1
100%	1870.344	923.685	946.659	940.1
	1889.065	923.685	965.38	940.1

120%	1900.397	923.685	976.712	940.1
	2058.647	923.685	1134.962	940.1
	2042.561	923.685	1118.876	940.1
	2041.942	923.685	1118.257	940.1

Table no.8- Area of sample and standard

Sample amount (µg/ml)	Standard Amount added(µg/ml)	Standard Amount recovered(µg/ml)	%Recovery	Average	Standard Deviation	%R.S.D
10	8	8.028284225	100.3535528	98.50880226	1.728271885	1.754433964
10	8	7.754175088	96.9271886			
10	8	7.859653228	98.24566535			
10	10	10.06976917	100.6976917	102.4270822	1.614417266	1.576162506
10	10	10.26890756	102.6890756			
10	10	10.38944793	103.8944793			
10	12	12.07277949	100.6064958	99.63759766	0.839538765	0.842592339
10	12	11.90167004	99.18058363			
10	12	11.89508563	99.12571358			

Table no.9- Percentage recovery,standard deviation and % R.S.D

Assay studies –Assay results are given in table which shows the area of standard,area of samples,% assay, ,average assay,standard deviation and %R.S.D of assay which shows label claim,result (actual) found,assay,average of assay,standard deviation and %R.S.D of Amphojel contains chromatograms obtained for the sample for first,second and third determination and figure contains chromatogram of the standard¹¹.

S.No	Area of standard	
1.	1765.361	
S.No	Area Of Samples	% Assay
1.	1921.581	97.32485131
2.	1908.955	96.55259495
3.	1718.789	97.17462829
Average assay		97.01715619
Standard deviation		0.419529654
% RSD of assay		0.366754881

Table no.10 Area of standard,area of samples ,average assay, standard deviation and % R.S.D of assay

Specificity-To judge the specificity of the method following chromatograms was obtained for sample, blank and standard given in figure and it was observed that no intervention was found from impurities and expipients in developed method¹².

Robustness studies of amphojel –The robustness study was carried out and the method was found to be robust. Flow rate data are given in table for the variation in the flow rate by increasing or decreasing by 0.2 at 20 µg/ml and their respective chromatograms are given in figure for increase in flow rate for three determinations and in figure for decrease in flow rate for three determinations¹². Results shows effect of change in mobile phase ratio composition by 2% variation(from 20-80 ratio to 22-78 ratio) at 20 µg/ml and their respective chromatograms for change in mobile phase ratio composition by 2% for three determinations for change in mobile phase ratio(from 20-80 to 18-82) for three determinations shows the effect of change in pH by increasing or decreasing by 0.2 at 20 µg/ml and their respective chromatograms for increase in pH for three determinations for decrease in pH for three determinations

AT FLOW RATE +2	
S.NO	AREA
1	1793.986
2	1807.76
3	1978.67
Average	1799.129
Standard deviation	5.913733
%RSD	0.412035

Table no.11 Effect of increase in flow rate

4. CONCLUSION

The results of the robustness test further confirmed that the approach is effective and unaffected by minor variations in the chromatographic conditions¹³⁻¹⁵. The aftereffects of roughness observe been viewed as precise. At the end of the day, it's usually assumed that the RP-HPLC method developed is accurate, exact, and precise, and may be successfully applied to the assessment of Amphojel in both a mass and multicomponent plan¹⁶.

5.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

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