High Performance Liquid Chromatography Method for Determination of Trace Amount of Ibutilide Fumarate in Pharmaceutical Manufacturing Environments

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Abstract: A rapid and sensitive RP-HPLC method with UV detection at 227 nm for routine analysis of washed MLs (mother liquors) from equipment after manufacturing and thereby cleaning of ibutilide fumarate active pharmaceutical ingredient was developed. Chromatography was performed with mobile phase containing a mixture of aqueous 0.01 M potassium dihydrogen phosphate (KH_2PO_4) and acetonitrile. The gradient elution was developed for better and optimized results. The developed method was validated for precision which includes system precision and method precision, accuracy, intra-day and using different system and finally linearity studies from 0.4 to 150%. The method is ascertained to be having good repeatability and reproducibility. The %RSD for method precision (5 different preparations at test concentration) was observed to be 1.36, wherein the system precision (6 repeated injections of same preparation) was observed to be 0.33. The % recovery from 'Accuracy' studies yielded the recovery of 99-100% which indicates the capability of the method. The linearity of the method was studied right from 0.4 to 150% which shows the method is quite linear with a correlation coefficient of 0.9998. The proposed method was simple, highly sensitive, precise, and accurate. The retention time less than 15 min, indicated that the method is useful for routine testing of washed MLs from equipment after manufacturing and cleaning of ibutilide fumarate.

Keywords: Ibutilide fumarate, HPLC, Correlation coefficient, %RSD, Active Pharmaceutical Ingredient, US FDA,

Introduction

Ibutilide fumarate is an antiarrhythmic drug with predominantly class III (cardiac action potential prolongation) properties according to the Vaughan Williams Classification. The drug is available in the market as injection wherein each milliliter of injection contains 0.1 mg of ibutilide fumarate (equivalent to 0.087 mg ibutilide free base), 0.189 mg sodium acetate trihydrate, 8.90 mg sodium chloride, hydrochloric acid to adjust pH to approximately 4.6 and water for injection. The ibutilide injections are available with the brand name of "COVERT" manufactured and distributed by Pharmacia Upjohn.

The chemical name for ibutilide fumarate is methanesulfonamide, N-{4-[4-(ethylheptyl amino)-1-hydroxybutyl] phenyl}, (+)(-), (E)-2-butenedioate (1:0.5) (hemifumarate salt). Its molecular formula is $C_{22}H_{38}N_2O_5S$, and its molecular weight is 442.62. Ibutilide fumarate is a white to off-white powder with an aqueous solubility of over 100 mg/mL at pH 7 or lower.

The structural formula is depicted below:

In the pharmaceutical manufacturing environment, cleaning of equipment plays a vital role where the assurance of carry over of previous product has to be within the regulatory acceptable limits. *i.e.*, calculation of maximum allowable carryover (MAC) and there by applying the MAC to calculate allowable limit for rinse and swab sampling *etc.* The drug regulatory agencies, for example, US FDA, are very keen and particular about the capability of analytical method in terms of sensitivity, limit of detection, accuracy and precision.

So far, only the determination of ibutilide from human plasma using LC-tandem mass spectrometry¹ and assaying of enantiomers of ibutilide and artilide using solid-phase extraction, derivatization and achiral-chiral column switching high performance liquid Chromatography² have been described in literature. However, no procedure has been described for the determination of API at low levels (at ppm level) accurately and within the linear range. Hence, it was felt necessary to develop a liquid chromatographic procedure which would serve as a rapid and reliable method for determination of ibutilide fumarate at trace levels (even at 1 ppm).

In this work we have developed a selective and sensitive RP-High Performance Liquid Chromatography method which can conveniently detect the lowest possible amount of 0.001% of ibutilide in the equipment cleaning MLs with a good sensitivity, selectivity, repeatability and reproducibility.

Experimental

Materials and reagents

Ibutilide fumarate API was synthesized by a well known API/CRO company using the generic process developed and established by the same firm. The HPLC purity of the selected test sample is higher than the 99.9%. A laboratory reagent grade Potassium dihydrogen phosphate was procured from 'Qualigens' for which the reported assay is 99.5%. Additionally, the laboratory grade acetonitrile used in this experimentation was obtained from 'Merck' for which the purity is reported to be 99.8%

Chromatographic conditions and Instrumentation

The RP-High Performance Liquid Chromatography was carried out on 'Waters' Liquid Chromatograph model 2695 equipped with quaternary pump, Photo Diode Array detector (PDA, waters # 2996), column oven and auto sampler. The output signal was monitored and integrated using 'Empower 2' software.

Symmetry C₈ column with 250 mm length and 4.6 mm dia with 5 μ particle size has been optimized to obtain the best possible results. The other columns tried for development of this method are, Inertsil C₈ 250 x 4.6 mm, 5 μ and YMC C₈ 250 x 4.6 mm, 5 μ (Figure 3). A mixture of 0.01 M potassium dihydrogen phosphate (KH₂PO₄) in water and acetonitrile was used as mobile phase. The gradient elution program has been developed and optimized for better, accurate and consistent results. The gradient program developed for this method is depicted below

Time	Solvent B % (Acetonitrile)
0	25
5	25
15	90
20	90
25	25
30	25

The optimized flow rate was 1.0 mL/min and the detection was at 227 nm. The UV absorbance spectrum is depicted in Figure 1.

No.	Wavelength	Absorbance	Description
1	227.00	0.361	

Figure 1. UV absorbance spectrum for ibutilide fumarate.

The column temperature for these experiments was maintained at 40°C. The test concentration of 0.25 mg/mL has been developed and optimized. The theoretical plates were observed to be 63528 where as the tailing factor is 1.53 (Table 1). The selectivity of Ibutilide has been shown in Figure 2.

Figure 2. Typical chromatogram.

Table 1. System suitability report (symmetry C8 column, 250 x 4.6 mm, 5 μ)

Compounds	Tailing Factor	Theoretic plates	Resolution	Purity angle	Purity threshold
Ibutilide	1.53	63528	57.78	0.205	0.301

Figure 3. Retention behavior of ibutilide fumarate on various columns

The developed method has been meticulously studied to ensure the method precision, system precision, % recovery (accuracy) and linearity. The method precision was studied by preparing the 5 different preparations of test concentration (*i.e.*, 0.25 mg/mL) and by injecting into the liquid chromatograph using the established method conditions. The area % for ibutilide fumarate obtained from these 5 runs was subjected to calculation of % relative standard deviation (%RSD) which is observed to be 1.36. Similarly, the system precision is also studied prior to executing the accuracy and linearity studies by injecting the standard preparation for 6 different times to ensure the repeatability. The % area of for ibutilide obtained from these runs is subjected calculation of %RSD and observed to 0.33. The above results ascertain that the method quite good and well reproducible and repeatable.

The accuracy or % recovery studies were carried out at the concentrations of 150, 100 and 50%. The results obtained from these studies are quite encouraging and well within the standard norms. *i.e.*, between 80 and 120%. At 150% concentration, 98.8% recovery has been obtained. Similarly, at 100%, the reported % recovery was 100% and finally at 50% concentration, 100.33% of recovery has been reported. The correlation coefficient across the three concentrations % recovery is plotted and observed to be 0.9997.

The linearity of the method was established across 7 different concentrations starting from 0.4 to 150%. The concentrations examined during this study are 0.4, 25, 50, 75, 100, 125 and 150%. The response obtained for these runs has been plotted and subjected to the calculation of correlation coefficient. The calculated correlation coefficient was 0.9998 where in the slope is 39088.47 and y-intercept was 46910.59. The 0.9998 correlation coefficient indicates the method capability across the linear range.

Results and Discussion

The method precision as explained in the earlier section has been studied and results obtained for five different preparations are tabulated (Table 2) along with calculation for mean, standard deviation and % relative standard deviation.

Table 2.

Run	% Area for ibutilide
1	4254499
2	4178681
3	4204323
4	4136631
5	4109116
Mean	- 4176650
Standard Deviation	- 56992.9
%RSD	- 1.36

The system precision was studied by preparing the standard solution at test concentration *i.e.*, 0.25 mg/mL and injected repeatedly for 6 times. The obtained results are shown in Table 3.

Table 3.

Run #	% Area for ibutilide
1	4067956
2	4071621
3	4057677
4	4053835
5	4034411
6	4050562
Mean	- 4056010
Standard Deviation	- 13342.54
%RSD	- 0.33

Accuracy (%recovery)

The % recovery has been studied and established within the range of 50 to 150%. The spiking study for each concentration along with amount recovered and thereby calculation of % recovery is tabulated in Table 4.

Table 4.

Description	150%	100%	50%
Wt of sample taken	37.12 mg	25.02 mg	12.28 mg
Run 1 (area)	6181769	4244748	2085212
Run 2 (area)	6187764	4208639	2071999
Run 3 (area)	6257371	4255503	2101303
Mean area	6208968	4236297	2086171
St. deviation	42025.3	24548.5	14675.5
%RSD	0.68	0.58	0.70
Amount Recovered	36.6708 mg	25.0200 mg	12.3211 mg
% Recovery	98.8%	100.0 %	100.3%

The response obtained for the various concentrations is plotted and observed to be linear (correlation coefficient – 0.9997). The graphical representation of accuracy studies is depicted in Figure 4

Figure 4. Ibutilide fumarate accuracy studies linearity plot

% Conc	Area
150	6208968
100	4236296
50	2086171
<i>Correlation Coefficient</i> 0.9997	

Linearity

The linearity of the method was studied at seven concentrations including the test concentration. *i.e.*, 150, 125, 100, 75, 50, 25, 0.4%. The obtained response was linear which apparently indicates the capability of method to reproduce/repeat the results within the linear range, consistently meeting the standard norms for method validation (ICH guidelines on validation analytical methods, Q2B). The results obtained for various experiments are depicted in Table 5.

Table 5.

Description	150%	125%	100%	75%	50%	25%	0.4%,10 ppm
Run 1 (area)	5868003	4908454	3975013	3002320	2009878	1033565	16634
Run 2 (area)	5897040	4902367	3968418	3038337	2030476	1027204	16311
Run 3 (area)	5899360	4907563	3971339	3042297	2042955	1022749	16094
Mean area	5888134	4906128	3971590	3027651	2027770	1027839	16346
Std	17472.8	3287.5	3304.6	22026.8	16703.7	5435.9	271.7
%RSD	0.2967	0.0670	0.0832	0.7275	0.8237	0.5289	1.6623

The response obtained for each of the concentration is plotted by taking % concentration on x-axis and area on y-axis. The results are then subjected to calculation of correlation coefficient after calculation of slope and y-intercept. The graphical representation for linearity studies is depicted in Figure 5.

Figure 5. Ibutilide fumarate linearity studies plot

% Conc.	Area
150	5888134
125	4906128
100	3971590
75	3027651
50	2027770
25	1027839
0.4	16346

Slope – 39088

y-intercept - 46910

Correlation coefficient 0.9998

Conclusions

The results obtained from these studies are well fit into the standard specifications stipulated by the regulatory agencies. The method is able to reproduce the results consistently even at 10 ppm level and hence this can be conveniently used in the pharmaceutical manufacturing environment for equipment cleaning certification.

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