Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(6):1-7

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Quantitative determination of alkyl (Polyalkyl) amine impurities in Colesevelam Hydrochloride tablet by LCMS

A. P. Rajput and Manohar C. Sonanis*

P.G. Research center, Department of Chemistry, Jai Hind Educational Trust's, Z. B.Patil Arts, Commerce and Science College, Dhule, Maharashtra Pin – 424002, India,

---

ABSTRACT

A simple, accurate and sensitive method for the quantitative determination of Bromoquat, Decylamine, Didecylamine and Decylaminoquat which are an alkyl, Polyalkyl amines impurities of Colesevelam Hydrochloride tablet. The method has been developed and validated by LCMS (liquid chromatography mass spectrometry). The chromatographic separation of impurities was achieved on C-18 column, the mobile phase was pumped at 1.0 ml minute^{-1} by using gradient pump mode, the mixture contains aqueous trifluroacetic acid (1.0 ml Lit.$^{-1}$) buffer, acetonitile and methanol as mobile phase -A and with acetonitrile mobile phase-B .The peaks were Scanned and quantified in ESI (Electro spray ionization) positive ion and SIM (selected ion monitoring) mode respectively. The method was validated and the chromatographic resolution between the impurities was found to be greater than 2. The responses were determined and (correlation coefficient) regression r values were obtained greater than 0.998 for all components. The method was capable of detecting impurities 0.02 % with respect to sample concentration of 3000ppm.

Keywords: Colesevelam Hydrochloride; alkyl amines; LCMS

INTRODUCTION

Colesevelam hydrochloride, a poly (allylamine hydrochloride), cross-linked with epichlorohydrin and alkylated with (6- bromohexyl)trimethylammonium bromide and 1-bromodecane. Colesevelam is a novel non-absorbed, lipid-lowering polymer that binds bile acids in the intestine, impeding their reabsorption. It is a white to off-white, non-crystalline hygroscopic powder that is insoluble in all tested solvents (water, HCl, ammonium hydroxide, methylene chloride, acetonitrile, octanol and methanol). The pH is approximately 4.2 and the pK is 9.3.Residual starting materials as well as organic impurities present in the starting materials is source of the organic impurities in the active substance specifications [1].

The thorough literature survey revealed that none of the most recognized pharmacopoeias [2] or any journals include these impurities under investigation for the determination in Colesevelam hydrochloride.The ion exchange chromatography with conductivity detector were utilised for the determination of limit test for allylamine in Colesevelam HCl [2]. Some of the literature describes In Vitro Bioequivalence Assessment (In vitro equilibrium and kinetic binding studies) of Colesevelam Hydrochloride Tablet [3, 5, 6] and determination of unbound Bile acids in Colesevelam HCl Tablets by UPLC [4]. Few methods for the identification of impurities in Brimonidine tartrate describes, the output from UPLC system are directly applied to hyphenate mass spectrometry [8].

EXPERIMENTAL SECTION

Chemicals:
Colesevelam Hydrochloride API, Colesevelam Hydrochloride tablet 625mg and impurity standards Bromoquat, Decylamine, Didecylamine and Decylaminoquat the chemical structure of them are shown in **Fig.-1** and **Fig.-2**. HPLC grades Acetonitrile, Methanol, Trifluroacetic acid were purchased from Merck Ltd, India. Water was deionised and further purified by means of a Milli-Q Plus water purification system, Millipore Ltd (U.S.A)

Equipment
Agilent 1200 series HPLC (High-performance liquid chromatography) system from (Stevens Creek Blvd. Santa Clara, CA, USA) equipped with an auto injector and thermostatic column oven compartment was utilized for method development. LCMS (Liquid chromatography Mass spectrometry) LCQ Advantage Max system from (Finnigan-Thermo) with Excalibur software was used for data acquisition and system suitability calculations.

Experimental:
Chromatographic separation was achieved on a reversed phase Nucleosil C-18 column (250 x 4.6 mm ID, 5µ particle size) from Macherynagel Germany. A flow rate was 1.0 $mlmin^{-1}$. HPLC analysis was conducted at 25°C column oven temperature. The mobile phase consists of about 1 mL of Trifluroacetic acid dissolved in 1000mL of water. Filtered through 0.45µ (or finer porosity) membrane filter.

Mobile phase A: Prepared Mixed and degassed solution of Buffer, acetonitrile and methanol in the ratio of 5:4:1 v/v/v. Mobile phase B: Acetonitrile

Gradient Programme:

Time	Mobile phase A	Mobile phase B
(min.)	(% v/v)	(% v/v)
0.01	100	0
10	100	0
15	30	70
20	10	90
25	100	0
30	100	0

Mass spectrometry Condition:
Ionization Probe**:** ESI
Polarity**:** Positive mode
Sheath Gas**:** 60mL min^{-1},
Spray Voltage**:** 3.0 Kv
Capillary Temperature**:** 290° C

Preparation of solutions:
Preparation of Diluent: prepared Mixed and degassed solution of Buffer: acetonitrile (1:1v/v).
Preparation of Standard solution:
Accurately weighed about 2 mg each Bromoquat, Decylamine, Didecylamine and Decylaminoquat standard and transfer into a 20mL volumetric flask, added 5mL of diluent, sonicate to dissolve .volume made up to mark with diluent .accurately transferred 1.0ml to 10 ml volumetric flask . Volume made upto the mark with diluent. Further accurately transferred 1.5ml to 10 ml volumetric flask. Volume made upto the mark with diluent.. Filtered the solution through 0.45 µ nylon filter discarding initial 4 ml filtrate.

Preparation of Sample Solution:
Gently Crushed 5 tablets in a mortar pestle and accurately weighed about 60 mg equivalent of colesevelam hydrochloride and transferred into a 20 ml volumetric flask, added 15 ml of diluent sonicate for 30 min with intermittent shaking. Volume made upto the mark with diluent .Filtered the solution through 0.45µ nylon filter discarding initial 4 ml filtrate.

Procedure:
Injected the blank solution, standard solution in full scan mode and extracted the individual m/z ions 223.1, 299.2, 158.5 and 298.3 amu corresponds to Bromoquat, Decylaminoquat, Decylamine and Didecylamine respectively from TIC spectra (Total Ion Current) and then data acquired in SIM (selected ion monitoring) mode. The SIM plot is a more specific plot than the full scan TIC plot and it is useful in quantification of peaks.

The system suitability criteria set as resolution should not be less than 2 between the peaks and %RSD of six replicate injections of standard for each individual impurity should not be more than 15%.Sample solution then injected and measured the peak responses corresponds to peaks of standard. The elution order and retention time of components are Bromoquat, Decylaminoquat, Decylamine and Didecylamine are about 3.5 min, 6.5 min, 9.5 min and 20 min respectively shown in **Fig.-3, Fig.-4**

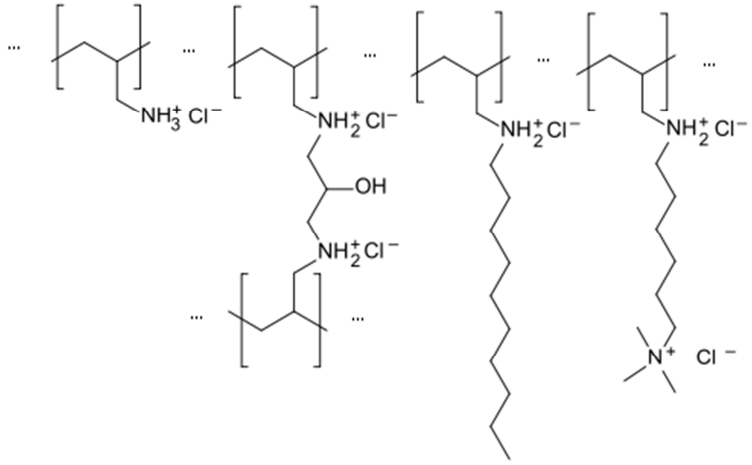


Fig-1 Molecular Structure of Colesevelam HCl

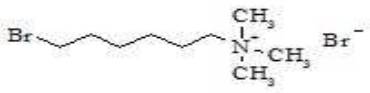
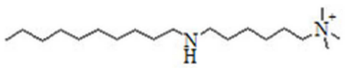
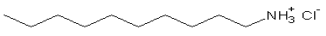
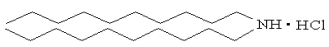
Sr no.	Chemical name of the Impurity	Molecular Formula Weight of impurity	Molecular structure
1	**Bromoquat** (6-bromo-N, N, N-trimethylhexan-1-aminium bromide)	**Molecular Formula:** $C_9H_{21}Br_2N$ **Molecular Weight:** 223.9(Free Base), 303.07(Salt)	
2	**Decylaminoquat (**6-decylamino-N,N,N-trimethylhexan-1-aminium bromide)	**Molecular Formula:** $C_{19}H_{43}BrN_2$ **Molecular Weight**: 380.5(Salt), 299.6(Free Base)	
3	**Decylamine HCl** (Decylamine hydrochloride,Decan-1-amine hydrochloride	**Molecular Formula:** $C_{10}H_{24}CIN$ **Molecular Weight:** 158(Free Base), 193.75(Salt)	
4	**Didecylamine HCL**, (Di- decylamine hydrochloride,N-decyldecan-1-amine hydrochloride	**Molecular Formula:** $C_{20}H_{44}CIN$ **Molecular Weight:** 298.2(Free Base), 333.5(Salt)	

Fig-2 Sr. no. 1 to 4 Molecular structures of Colesevelam impurities

RESULTS AND DISCUSSION

The optimizations of chromatographic method for the separation of four alkyl (poly alkyl) amine impurities due to a non chromophore structure of investigational compounds was the most challenging task for the method development. Mass spectrometry, was the best choice for detection and the experiments were planned with Inertsil C-18 column and ammonium acetate10mM buffer with variable gradient using Acetonitrile. Peak shape was not good and intensity was low. Most of investigational impurities are having ionisable nitrogen atom in the structure, hence Trifluoro acetic acid would be suitable ion pair and would retain the analytes. Column was used as Nucleosil C-18, 250 x4.6 mm,5µ and variable gradient using Acetonitrile gave satisfactory separation of all components.

Method validation

The optimized method for the quantitative assay of impurities of Colesevelam Hydrochloride tablet was validated according to ICH (International Conference on Harmonization) guidelines [7], with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range and System suitability parameters were also assessed

System suitability test

The system suitability test was performed according to USP 37 indications. The observed RSD values at were well within the usually accepted values (<15%).

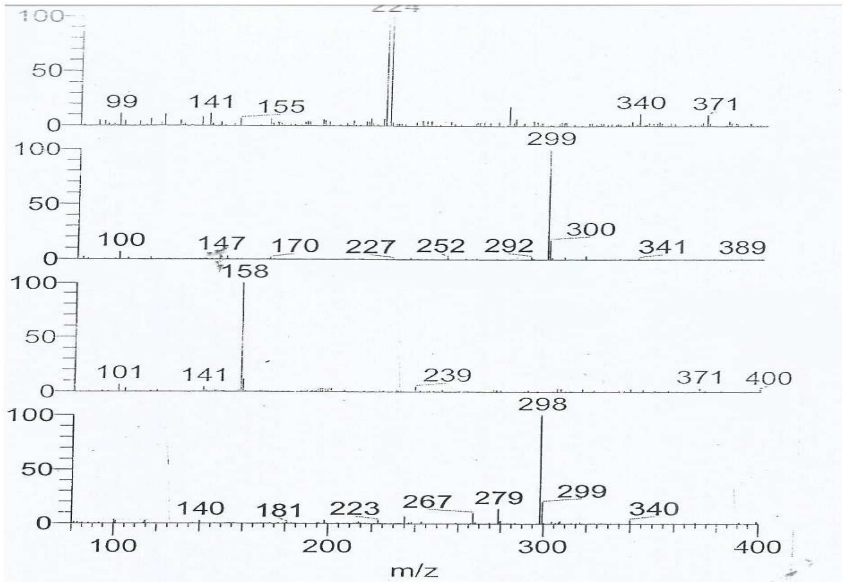


Fig.3 Mass spectra scan of Relative Abundances of impurities

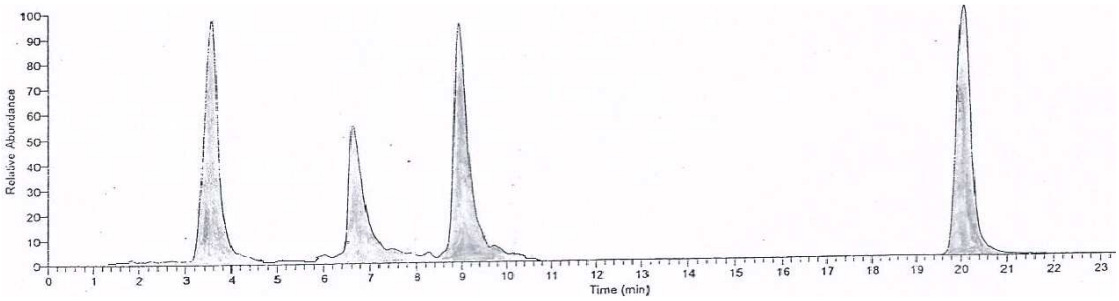


Fig.4 Mass spectra scan SIM Mode of impurities

Table-1 System precision

Inj No.	Bromoquat	Decylaminoquat	Decylamine	Didecylamine
	Area Counts			
1	10498386	10162951	5286814	62214773
2	10198396	10758943	5265884	62864225
3	11495376	10657246	5255857	63242232
4	10497366	10822223	5225175	61882237
5	10698396	10657543	5141256	63952749
6	10389366	10925826	5141256	61925652
Mean	**10629547**	**10664122**	**5219374**	**62680311**
SD	414860.0496	242809.6221	58138.97	751868.3372
%RSD	**3.90**	**2.28**	**1.11**	**1.20**

Specificity

The specificity of the method was checked by injecting blank solution, excipient (inactive) solution without drug substance, sample solution and sample solution spiked with all other related known impurities at 1% level (for drug substance as well as drug product). There was no interference from blank, excipient and related known impurities at the retention time of analyte peak

Method precision and ruggedness

In order to determine the **Method precision and ruggedness** of the method, six independent preparation of sample solution was prepared and data of precision and intermediate precision are well within limits.

The system precision data were presented in **table-1**

Linearity and range

The nominal concentration of colesevelam Hydrochloride in test solution was 3 mg mL-1. Taking into account that typical impurity tolerance levels is 0.05 % (1.5ppm)

Asper ICH five point linearity concentration were prepared ranging from lower to 200% of target concentration (1.5ppm) for all the impurities The regression statistics are shown in **table- 2**.and linearity regression plot were shown in **Fig.-5, Fig.-6, Fig.-7** and **Fig.-8**

Table-2 Linearity of peak responses of impurities

Bromoquat		Decylaminoquat		Decylamine		Didecylamine	
Conc. µgml^{-1}	**Area Counts**	**Conc. µgml^{-1}**	**Area Counts**	**Conc. µgml^{-1}**	**Area Counts**	**Conc. µgml^{-1}**	**Area Counts**
0.492	3476290	0.495	3332115	0.501	2450726	0.498	28421696
0.984	7334972	0.990	6997442	0.984	3980928	0.996	47553419
1.476	10498396	1.485	10162951	1.476	5286814	1.494	62214773
1.968	14426604	1.980	13495066	1.968	6534445	1.992	80014822
2.952	20683926	2.970	19492873	2.952	9361028	2.988	112644219
Slope	6991155	**Slope**	6499580	**Slope**	2783181	**Slope**	33564105
Intercept	277163.66	**Intercept**	400754.37	**Intercept**	1135938	**Intercept**	12682027
CC	0.99913	**CC**	0.9992968	**CC**	0.999533	**CC**	0.9995487

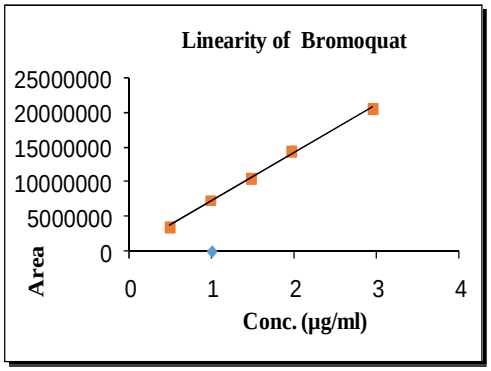


Fig:5

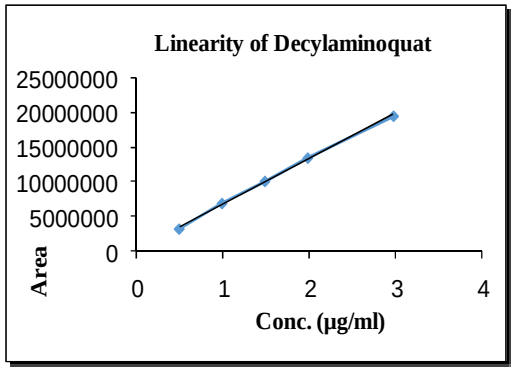


Fig:6

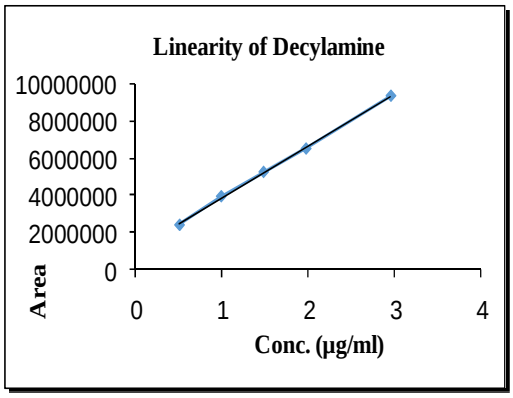


Fig:7

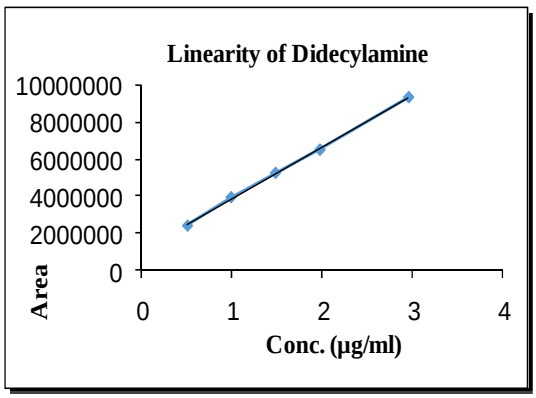


Fig:8

Determination of limit of quantification and detection (LOQ and LOD)

The linearity performed above, used for the determination of limit of quantification and detection. Residual standard deviation (σ) method was applied and were predicted the LOQ and LOD values using following formula (a), (b) and established the precision at these predicted levels.

$$LOQ = \frac{10X\sigma}{S} \text{ ----------} \quad \text{(a)}$$

$$LOD = \frac{3.3X\sigma}{S} \text{ ----------} \quad \text{(b)}$$

Where

σ = Residual standard deviation of response, S = Slope of the calibration curve

Accuracy

Accuracy was evaluated by the determination of impurities in solution prepared by standard addition method. The experiment was carried out by adding known amount of impurities standards corresponding to three concentration levels of 50 %, 100 % and 150 % of the impurity tolerance level in sample solution (Drug substance as well as in drug product). The samples were prepared in triplicate at each level. The quantification of added analyte (% weight/weight) was carried out by using an external standard of prepared at the analytical concentration. The experimental results revealed that approximate 80 - 120% recoveries were obtained for all the Impurities in drug substance as well as in drug product. Therefore, based on the recovery data shown in **Table-3**

The estimation of impurities that are prescribed in this report has been demonstrated to be accurate for intended purpose and is adequate for routine analysis.

Table-3: Summary Result of Accuracy

Spiked level (% , n=3)	Bromoquat	Decylaminoquat	Decylamine	Didecylamine
50	106.47	100.01	93.18	92.89
100	100.21	107.89	90.98	100.2
150	98.83	101.72	89.89	108.86

CONCLUSION

A simple, accurate, sensitive method for quantitative determination of Bromoquat, Decylamine, Didecylamine and Decylaminoquat (an alkyl , Polyalkyl amines) in Colesevelam Hydrochloride tablet has been developed and validated by LCMS(liquid chromatography mass spectrometry)

Acknowledgment
The authors wish to thank the Principal, P.G. Research center, Department of Chemistry, Jai Hind Educational Trust's, Z. B.Patil Arts, Commerce and Science College, Dhule, Maharashtra.

REFERENCES

[1] Scientific discussion of Cholestagel, page 1-35, EMEA 2005.
[2] United States Pharmacopoeia, USP Pending Monograph Draft 1—**2012**.
[3] Food and Drug Administration's (FDA's) Draft Guidance on Colesevelam Hydrochloride, Recommended Jul 2008; Revised Dec 2009; Nov 2010; Jun 2011; Nov **2013**.
[4] Venkata Vivekananda Vallapragada, Gopichand Inti, Sudhakar Rao Vidiyala and Sreeramulu Jadi,Validated UPLC Method for Determination of Unbound Bile Acids in Colesevelam HCl Tablets. **2015**;53, page 154–160
[5] Center For Drug Evaluation And Research, Chemistry Review(S)
[6] Y. Zhao, A. Gasaway, A. C. Myers BASi , Application of a Gradient HPLC-UV Method for In Vitro Bioequivalence Assessment of Colesevelam Hydrochloride Tablet Formulations in Simulated Intestinal Fluid
[7] International conference on Harmonization, (ICH) Q2 (R1): validation of analytical procedures- Test and Methodology, Geneva, Switzerland, (**2005**).
[8] A. Rajput, M. Sonanis , International Journal of Pharmacy and Pharmaceutical Sciences, Development and validation of a new stability indicating analytical method for the determination of related components of Brimonidine tartrate in drug substances and drug product using UPLC Vol. 3, Issue 1, **2011**.