e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological
&
Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

ESTIMATION OF GUAIFENESIN IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROSCOPY

A. Andrew Asirvatham[1], K. Manikandan[2], R. Mailvelan[3], Kishore Konam[4], P. Rajavel[5]

[1]Department of Pharmaceutical Analysis, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamilnadu, India.
[2]Department of Pharmaceutical Technology, Anna University of Technology, Trichirappalli, Tamilnadu, India.
[3]Department of Pharmaceutical Analysis, SRM College of pharmacy, Kattankulathur, Tamilnadu, India.
[4]Department of Pharmaceutical Analysis and Quality Assurance, S.V.S School of Pharmacy, Bheemaram, Hanamkonda, Warangal, Andhra Pradesh, India.
[5]Department of Pharmaceutical Analysis, A.M.Reddy Memorial College of Pharmacy, Narasaraopet, Guntur, Andhra Pradesh, India.

ABSTRACT

A simple, accurate liquid chromatography with tandem mass spectrometry (LCMS/MS) method has been developed and validated for quantification of guaifenesin in human plasma. The method employed liquid-liquid extraction (LLE) technique. Glibenclamide was used as the internal standard. Samples containing guaifenesin were chromatographed on a Kromosil C18 column (5µm, 150mm x 4.6mm) at a temperature of 40°C. The isocratic mobile phase composition was a mixture of methanol/0.1% formic acid, which was pumped at a flow rate of 0.6 mL/ min with split ratio of 90:10. The retention time under these chromatographic comditions was found to be 1.05 and 1.47 minutes for guaifenasin and glibenclamide with run time 2.2 minutes. Tert butyl methyl ether was found to be good extracting and produced a satisfactory chromatogram. The mass transition ion–pair was followed as m/z 163.000 for Guaifenesin and m/z 368.968 for Glibenclamide. The developed LC/MS-MS method was found to be selective, simple, sensitive, accurate and linear for the analysis of guaifenesin in human plasma. The proposed method has been validated with linear range 23.966 to 6001.154 ng/mL for guaifenesin. The precision and accuracy values are within 5%. The overall recovery of guaifenesin was 106.12%.

Key words: Guaifenesin, Glibenclamide, LC-MS/MS, Bio analytical and Method validation.

INTRODUCTION

Guaifenesin (Figure 1) chemically named as 3-(2-methoxyphenoxy)-1,2-propanediol (WWW.rxlist.com). It is an only expectorant recognized as safe and effective by the FDA. Often it is used with antihistamines, decongestants and antitussives in combination product (Bertram and Katzung *et al*., 2005). The analytical methods available for the estimation of Guaifenesin are official in IP, BP, USP whereas the reported methods for the estimation of Guifenesin in the literature by capillary gas chromatography (Maged HM Sharaf *et al*., 2004), HPLC (EI-Gindy A *et al*., 2007; Stavachansky Salomon *et al*., 1995), LC-MS (Wen Jinhua *et al*., 2010), LC-MS/MS (Foster J *et al*., 2004; Thomas H. Eichhold *et al*., 2007; Kuhlenbeck DL *et al*., 2005). Methods of measuring drugs in biologic media are increasingly important problems related to bioavailability and bioequivalence studies, new

Corresponding Author

A. Andrew Asirvatham
Email id: andrewpharma@rediffmail.com

drug development, drug abuse, clinical pharmacokinetics, and drug research are highly dependent on accurately measured drugs in biological fluids. For the estimation of the drugs present in the biological fluid, LC-MS/MS method is consider to the more suitable since this is a powerful and rugged method. The present study describes development and validation of a simple, specific, rapid and sensitive LCMS/MS method for the determination of Guifenesin in human plasma (Bressolle F *et al*., 1996; Hartmann C *et al*., 1998; Green MJ, 1996; Shah PV *et al*., 1992; Causon R, 1997; Bruce P *et al*., 1998; Dadgar D *et al*., 1995). This method is considered to be more suitable since this is a powerful and rugged method. This paper describes development and validation of a simple, specific, rapid and sensitive LCMS/MS method for the determination of Guaifenesin in human plasma with a limit of quantification (LOQ) of 23.966 ng/mL for Guaifenesin, with a runtime of 2.2 minutes and Glibenclamide used as internal standard.

MATERIALS AND METHODS

Chemicals

The reference standards of Guaifenesin and Glibenclamide were obtained from Granules India and ARBRO Pharmaceuticals Ltd respectively. High purity water was prepared in-house using a milli-Q water purification system obtained from Millipore (India) Pvt Ltd., (Bangalore, India). HPLC grade methanol and formic acid AR grade were purchased from Thomas Baker and Ranchem (Mumbai, India). Drug free (BLANK) heparinised human plasma was stored at -70ºC prior use.

Instrument and Chromatographic Condition

The LC system (THERMO) consisting of a binary pump and detection was performed by a Thermo TSQ Quantum mass spectrometer. The column used was Kromosil, C18, 5 µm (50 x 4.6mm). The mobile phase was prepared by mixing methanol and 0.1% formic acid in the ratio of 90:10%v/v. Chromatographic studies was performed at ambient temperature at flow rate of 0.600mL/min. The compound was ionized in the positive Electron spray impact (ESI) mode of mass spectrometer. Analysis was performed in Guaifenesin and Glibenclamide were detected at m/z 163.000 and 368.968 respectively.

Preparation of standard solution for guaifenesin

A stock solution was prepared by dissolving accurately weighed quantity of guaifenesin in methanol to yield a final concentration of 1mg /mL, sonicated for 5 minutes, allowed to equilibrate to room temperature and suitably diluted with methanol. The stock solution was further diluted by suitable dilution with methanol.

Preparation of standard solution for glibenclamide

A stock solution was prepared by dissolving accurately weighed quantity of glibenclamide in methanol to yield a final concentration of 1mg /mL, sonicated for 5 minutes, allowed to equilibrate to room temperature and suitably diluted with methanol. The stock solution was further diluted by suitable dilution with methanol.

Extraction of guaifenesin from plasma

A 200 µL volume of plasma was transferred to a 4mL ria vial, and then 50 µL of IS working solution (2µg/mL) was spiked. 20 µL of 10% formic acid was added and vortexed. After vortexing for 30 sec, 3 ml of extraction solvent tert-Butyl methyl ether was added. The sample was vortex-mixed for 2 min and then centrifuged at 4000 rpm for 5 minutes at 10°C. The organic layer (2.0 mL) was quantitatively transferred to a 4 mL glass tube and evaporated to dryness at 40°C under a stream of nitrogen. Then, the dried extract was reconstituted in 100 µL of Mobile phase and a 5 µL aliquot was injected into the chromatographic system.

Method Validation

The method of analysis was validated as per the recommendations of ICH and FDA (FDA., 2001; ICH Q2 (R1)., 2005) for the parameters like matrix effect, accuracy, precision, recovery, linearity, and stability.

Matrix effect

The 6 different lots of blank plasma spiked with HQC and LQC samples were processed along with blank lots through the extraction and analyzed to determine the effect of matrix with the guaifenesin and the internal standard.

Accuracy and Precision

The accuracy and precision were assessed by the repeated analysis of plasma samples containing different concentrations guaifenesin on separate occasions. A single run consists of a calibration curve plus 6 replicates of the LOQQC, LQC, MQC and HQC samples.

Recovery

Recovery of guaifenesin were evaluated by comparing peak response of six extracted of low, middle and high quality control samples to those of six appropriately diluted standard solutions.

Stability

Bench top stability in matrix

At least six aliquots each of the low and high quality control samples were kept at room temperature (25 + 5ºC) after spiking into plasma. After completion of 6 hours the samples were extracted and analyzed against the concentration of freshly prepared calibration curve.

Short term stock solution stability

Replicates of stability samples (stored at room temperature for 6 hours) and comparison samples were diluted at approximately midlevel concentration of the CC standards and analyzed in a single run. Peak response ratios (Analyte/Internal standard) were used to determine the % stability.

RESULTS AND DISCUSSION

Estimation of Guaifenesin in human plasma was carried out using optimized chromatographic conditions. Validation parameters such as Matrix effect, Recovery, accuracy, precision, and stability were evaluated.

Blank matrix specificity

Randomly selected blank human plasma samples were carried through the extraction and chromatographed to determine the extent to which endogenous human plasma may contribute to chromatographic interference with guaifenesin or the internal standard. No significant interferences were observed in 6 different lots of human plasma samples.

Matrix effect

The 6 different lots of blank plasma spiked with HQC and LQC samples were processed along with blank lots through the extraction and analyzed to determine the effect of matrix with the guaifenesin or the internal standard. Results are presented in Table 1.

Recovery

Recovery of guaifenesin were evaluated by comparing peak response of six extracted of low, middle and high quality control samples to those of six appropriately diluted standard solutions. Mean recovery values of guaifenesin are 109.30, 104.33, and 106.12% for low, middle and high quality control levels respectively. Total mean recovery of Guaifenesin is 106.58%. Results are presented in Table 2, 3, 4 & 5.

Stability

Bench-top stability

Six replicates of low (LQC) and high (HQC) quality control samples were left at room temperature for 6 hours (stability samples). A calibration curve and 6 replicates of low and high quality control samples (comparison samples) were freshly processed along with the stability samples and analyzed in a single run guaifenesin were found to be stable in human plasma for 6 hours at room temperature. Results are shown in Table 6.

Short term stock solution stability

Replicates of stability samples (stored at room temperature for 6 hours) and comparison samples were diluted at approximately midlevel concentration of the CC standards and analyzed in a single run. Peak response ratios (Analyte/Internal standard) were used to determine the % stability. Guaifenesin was found to be stable in methanol for 6 hours at room temperature with percentage difference stability of 99.87%. And Internal Standard was 113.51%   Results are presented in Table 7.

Fig 1: Structure of guaifenesin

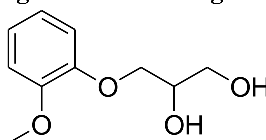

Table 1. Results of matrix effect

LOT NO.	LQC	HQC
Actual concentration (ng/ml)	71.442	4762.821
Lot 1	61.193	5097.822
Lot 2	61.097	4493.101
Lot 3	56.061	4863.390
Lot 4	65.405	5250.248
Lot 5	68.126	5107.598
Lot 6	62.220	4163.655
Mean	62.3503	4829.3023
SD	4.127	420.482
%CV	6.62	8.71
% Nominal	87.27	101.40

a) LQC- Low Quality Control
b) HQC- High Quality Control

Andrew Asirvatham A. *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2012; 3(3): 463-468.

Table 2. LQC concentration level

QC ID	Drug Area		ISTD Area	
	Un-extracted	Extracted	Un-extracted	Extracted
LQC-001	33285	37845	1817407	1417339
LQC-002	36523	33246	1842852	1541358
LQC-003	27509	32060	1528838	1120568
LQC-004	27006	47514	1715140	1107672
LQC-005	28603	28166	1512963	1216224
LQC-006	24509	15109	1673494	441594
Average	29572.5	32323.33	1681782	1140793
%Recovery	109.30		67.83	

Table 3. MQC concentration level

QC ID	Drug Area		ISTD Area	
	Un-extracted	Extracted	Un-extracted	Extracted
MQC-001	1262430	1831313	1626461	1319659
MQC-002	1454191	1470489	1652658	1332858
MQC-003	1283663	1308234	1512355	1152924
MQC-004	1292422	1188717	1647123	1117915
MQC-005	1276405	1229355	1339008	1179660
MQC-006	1282838	1164199	1389427	1101029
Average	1308658	1365385	1527839	1200674
%Recovery	104.33		78.59	

Table 4. HQC concentration level

QC ID	Drug Area		ISTD Area	
	Un-extracted	Extracted	Un-extracted	Extracted
HQC-001	2039471	2693719	1481579	1474422
HQC-002	2479903	2295364	1624876	1329384
HQC-003	1973348	2328972	1572686	1271964
HQC-004	2004128	2075122	1489567	1177571
HQC-005	1994041	1905398	1455114	1147773
HQC-006	2020102	1978671	1449364	1005665
Average	2085166	2212874	1512198	1234463
%Recovery	106.12		81.63	

Table 5. Total results of analyte recovery and internal standard

QC	Analyte	Internal Standard
LQC	109.3	67.83
MQC	104.33	79.59
HQC	106.12	81.63
MEAN	106.58	76.02
SD	2.517	7.25
%CV	2.361	9.538

Andrew Asirvatham A. *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2012; 3(3): 463-468.

Table 6. QC Samples for bench top stability

QC ID	0 Hour		6 Hours	
	LQC	HQC	LQC	HQC
Actual (ng/ml)	71.442	4762.821	71.442	4762.821
	60.948	4744.973	65.725	4999.471
	61.781	4914.550	68.208	5034.059
	64.368	5135.424	61.763	5233.295
	77.207	5426.188	79.254	5142.777
	80.302	5161.157	79.434	5340.520
	121.471	5345.068	65.962	5424.462
Mean	68.9212	5121.2267	70.0577	5195.7640
SD	9.130	256.587	7.487	168.614
%CV	13.25	5.01	10.69	3.25
%Nominal	96.47	107.53	98.06	109.09

a) LQC- Low Quality Control
b) HQC- High Quality Control

Table 7. Short term stability studies (STSS) for Drug and internal standard

STSS - Drug			STSS-ISTD		
Run	Area of Drug in 0 Hours	Area of Drug in 6 Hours	Run	Area of Drug in 0 Hours	Area of Drug in 6 Hours
1	3576126	3515434	1	690904	628436
2	3510939	3666113	2	639156	611771
3	3527203	3437704	3	715491	615491
4	3382223	3426667	4	688069	622842
5	3471230	3591389	5	725440	580067
6	3529641	3387358	6	675708	583752
Average	3499560	3504111	Average	689128	607060
% Different	99.8701		% Different	113.5189	

CONCLUSION

A simple, sensitive, and reliable LC/MS-MS method has been developed and validated for the determination of Guaifenesin in human plasma. The method is accurate, reproducible, and specific. The retention time and in-turn run time was very short, hence required less mobile phase for the method, making it more economical and rapid. The method may be applicable for pharmacokinetic studies of Guaifenesin in human plasma.

REFERENCES

Bertram & Katzung. Basic & clinical pharmacology. *Lange Medical books/McGraw-Hill medical publishing division.* 2005; 8th edition: 1523.

Bressolle F, Bromet-pitit M, Audran M. 1996, Validation of Liquid Chromatography and GasChromatographic Methods Application to Pharmaceutics. *Journal of Chromatography B.* 1996; 686: 3-10.

Bruce P, Minkkinen P, Riekkola ML. Practical Method Validation: Sufficient for an Analytical Method. *Mikrochimca Acta*. 1998; 128: 98-106.

Causon R. Validation of Chromatographic methods in Biomedical Analysis Viewpoint and Discussion. *Journal of Chromatography B*. 1997; 689: 175-180.

Dadgar D, Burnett PE, Choch MG, Gallicano K, Hooper JW. Application Issues in Bioanalytical Validation, Sample Analysis and Data Reporting. *Journal of Pharmaceutical and Biochemical Analysis*. 1995; 13: 89-97.

El-Gindy A, Emara S, Shaaban H. Development and validation of chemometrics assisted spectrophotometric and liquid chromatographic methods for the simultaneous determination of two multi-component mixtures containing bronchodilator drugs. *Journal of Pharmaceutical and Biomedical analysis*. 2007; 43: 973-982.

Food and Drug Administration. FDA. Guidance for Industry: Bioanalytical Method Validation. Rockville, MD: US Department of Health and Human Services, Food and Drug Administration, *Center for Drug Evaluation and Research.* 2001; 1: 124-129.

Foster J. Determination of Guifenesin in human plasma using automated liquid- liquid extraction and tandem mass spectrometric detection. *The AAPS*. Abstracts AM-2004; 002586.

Green MJ. A Practical Guide to Analytical Method Validation. *Analytical Chemistry.* 1996; 68: 305A-309A.

Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD. Validation of Bioanalytical Chromatographic Methods. *Journal of Pharmaceutical and Biomedical Analysis.* 1998; 17; 193-218.

Kuhlenbeck DL, Eichold TH, Hoke SH 2nd, Baker TR, Mensen R, Wehmeyer KR. On-line solid phase extraction using the Prospekt-2 coupled with a liquid chromatography/tandem mass spectrometer for the determination of dextromethorphan, dextrorphan and Guaifenesin in human plasma. *European Journal of mass spectrum*. 2005; 11: 199-208.

Maged HM Sharaf, Dwight D Stiff. Determination of guiafenesin in human serum by capillary gas chromatography and electron capture detection. *Journal of pharmaceutical and biomedical analysis*. 2004; 35: 801-806.

Shah PV, Midha KK, Dighe S, McGilveray JI, Skelly PJ, Yacobi A, Layloff T, Viswanathan CT, Cook EC, Mcdowall RD, Pittman AK, Spector S. Analytical Method Validation: Bioavailability, Bioequivalence and Pharmacokinetics Studies. *Journal of Pharmaceutical Science*. 1992; 81: 309-312.

Stavachansky Salomon, Sibel Demirbas, Larisa Reyderman, Chih-Kun Chai. Simultaneous determination of dextrorphan and guaifenesin in human plasma by liquid chromatography with fluorescence detection. *Journal of Pharmaceutical and Biomedical analysis.* 1995; 13: 919-925.

Thomas H. Eichhold, David L. McCauley-Myers, Deepa A. Khambe, Gary A. Thompson, Steven H. Hoke. Simultaneous determination of dextromethorphan, dextrorphan and Guaifenesin in human plasma using semi-automated liquid/liquid extraction and gradient liquid chromatography tandem mass spectrometry. *Journal of Pharmceutical and Biomedical analysis.* 2007; 43: 586-600.

Wen Jinhua, Hong Zhang, Chunhua Xia, Xiao HU, Wenwei Xu, Xiaohua Cheng, Jun Gao, Yuging Xiong. A sensitive liquid chromatography-electrospray ionization-mass spectrometry method for the Simultaneous determination of pentoxyverine citrate and guifenesin in human plasma - application to pharmacokinetic and bioequivalence studies. *Biomedical chromatography*. 2010; 24: 351-357.