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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF GUAIFENESIN IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROSCOPY

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ABSTRACT
A rapid, sensitive and simple liquid chromatography tandem mass spectrometry (LC-MS/MS) method has been developed and validated for quantification of guaifenesin in human plasma. The analyte was extracted from human plasma by liquid-liquid extraction (LLE) technique. Glibenclamide was used as the internal standard. A Kromosil C_{18} column provided chromatographic separation of the analyte which was followed by detection with tandem mass spectrometry. The mass transition ion–pair was followed as m/z 163.000 for Guaifenesin and m/z 368.968 for Glibenclamide. The method involved liquid-liquid extraction of Guaifenesin from plasma by using sample preparation LLE column C_{18} (150mm x 4.6mm., 5µ.,) followed that simple isocratic chromatographic conditions with mobile phase of methanol: 0.1% formic acid (90:10%v/v) and tandem mass spectrometric detection that enables detection nano-gram levels. The retention times were 1.05 and 1.49 minutes for guaifenesin and Glibenclamide respectively. 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 102.83%.
Key words: Guaifenesin, Glibenclamide, LC-MS/MS, Bio analytical

.INTRODUCTION

Guaifenesin chemically named as 3-(2-methoxyphenoxy)-1, 2-propanediol[1]. It is a only expectorant recognized as safe and effective by the FDA. Often it is used with antihistamines, decongestants and antitussives in combination product[2]. The analytical methods available for the estimation of Guaifenesin are official in IP, BP, USP where as the reported methods for the estimation of Guifenesin in the literature by capillary gas chromatography[3], HPLC[4,5], LC-MS[6], LC-MS/MS[7,8,9]. Methods of measuring drugs in biologic media are increasingly important problems related to bioavailability and

bioequivalence studies, new 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 LC-MS/MS method for the determination of Guifenesin in human plasma. . 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 LC-MS/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 Phenomenex C_{18}, (150 x 4.6mm, 5µ i.d.). The mobile phase was prepared by mixing methanol and 0.1% formic acid in the ratio of 90:10%v/v. Chromatographic study was performed at ambient temperature at flow rate of 0.600mL/min.The compound was ionised 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.

RESULTS AND DISCUSSION

Estimation of Guaifenesin in human plasma was carried out using optimized chromatographic conditions. Validation parameters such as specificity, Matrix effect,

carry over test, Recovery, linearity, accuracy, precision, and range, system suitability, sensitivity (Limit of detection and limit of quantitation), dilution integrity, 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.

Table 1: QC samples for matrix effect

QC ID	MATRIX EFFECT	
	LQC	HQC
Actual (ng/ml)	**71.442**	**4762.821**
LOT 1	71.193	5097.822
LOT 2	69.097	4493.101
LOT 3	68.061	4863.390
LOT 4	70.405	5250.248
LOT 5	73.126	5107.598
LOT 6	68.220	4163.655
Mean	**70.0170**	**4829.3023**
SD	**1.955**	**420.482**
%CV	**2.79**	**8.71**
%Nominal	**98.01**	**101.40**

Carry over test

There is no impact on the previous analysis of samples has been examined and the results.

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 98.03, 104.33, and 106.12% for low, middle and high quality control levels respectively. Total mean recovery of Guaifenesin is 102.83%. Results are presented in Table 2.

Table 2: Recovery studies of analyte in plasma LQC concentration level

QC ID	ANALYTE AREA	
	UN-EXTRACTED	EXTRACTED
LQC-001	33285	27845
LQC-002	36523	28246
LQC-003	27509	30060
LQC-004	27006	27514
LQC-005	28603	28166
LQC-006	24509	32109
Average	**29572.5**	**28990**
%Recovery		**98.03**

MQC concentration level

QC ID	ANALYTE AREA	
	UN-EXTRACTED	EXTRACTED
MQC-001	1262430	1831313
MQC-002	1454191	1470489
MQC-003	1283663	1308234
MQC-004	1292422	1188717
MQC-005	1276405	1229355
MQC-006	1282838	1164199
Average	**1308658**	**1365385**
%Recovery		**104.33**

HQC concentration level

QC ID	DRUG AREA	
	UN-EXTRACTED	EXTRACTED
HQC-001	2039471	2693719
HQC-002	2479903	2295364
HQC-003	1973348	2328972
HQC-004	2004128	2075122
HQC-005	1994041	1905398
HQC-006	2020102	1978671
Average	**2085166**	**2212874**
%Recovery		**106.12**

Total recovery of analyte in plasma

QC SAMPLES	ANALYTE
LQC	98.03
MQC	104.33
HQC	106.12
AVG	**102.83**

Chromatography

The retention times of Guaifenesin and the internal standards are approximately 1.05 and 1.49 minutes, respectively. The overall chromatography run time is 2.0 minutes.

Calibration curves

Calibration curves are found to be consistently accurate and precise over the 23.966 to 6001.154 ng/ml calibration range for Guaifenesin. The coefficients of correlation (r^2) are greater than 0.99. Data are presented in Table 3. Back calculations were made from the calibration curves to determine concentrations of calibration standards of the analyte.

Table 3: Linearity for Guaifenesin

Standard	A	B	C	D	E	F	G	H	I	J
Actual (ng/ml)	23.97	47.93	119.83	299.58	748.94	1497.89	2496.48	3840.74	4800.92	6001.15
P &A 1	23.8	46.9	118.16	287.22	754.27	1469.74	2476.23	3849.88	4788.42	6099.18
P &A 2	23.33	50.38	121.6	293.67	745.28	1481.18	2491.26	3850.88	4734.6	5990.73
P &A 3	23.99	48.94	119.21	280.75	748.57	1474.91	2487.64	3839.3	4845.41	6103.88
Mean	23.71	48.74	119.66	287.21	749.37	1475.28	2485.04	3846.69	4789.48	6064.6
SD	0.34	1.75	1.76	6.46	4.55	5.72	7.84	6.42	55.41	64.01
%CV	1.43	3.59	1.47	2.25	0.61	0.39	0.32	0.17	1.16	1.06
%NOMINAL	98.93	101.68	99.86	95.87	100.06	98.49	99.54	100.15	99.76	101.06

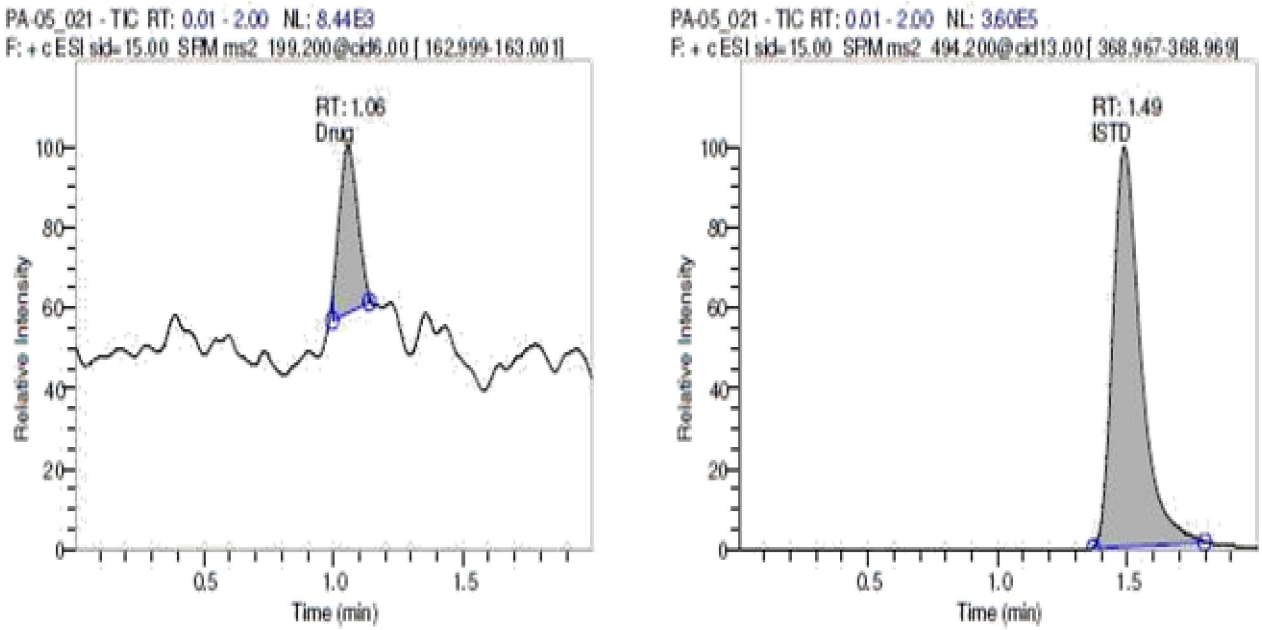

Figure 1
Chromatogram of LLOQ level concentration of guaifenesin in plasma

Limit of Quantitation

The lower Limit of Quantitation for Guaifenesin is 23.966 ng/ml shows the coefficient of variation 1.43% and percentage of nominal concentration 98.92%. The upper limit of Quantitation is 6001.15 ng/ml shows coefficients of variation 2.39% and percentage of nominal concentration of 98.58%.

Within -batch accuracy and precision

The within–batch 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.

The coefficients of variation of within-batch ranged between 2.98 to 6.24% which are under limit (<15%) and percentage of nominal concentration ranged from 99.81 to 102.24 for Guaifenesin.

Between-batch accuracy and precision

The Between–batch coefficients of variation ranged between 3.73 to 7.42% and percentage of nominal concentration ranged from 99.26 to 102.69 for Guaifenesin.

Dilution integrity

Six replicates of 2 times of the HQC concentration were diluted 2 and 4 times with screened plasma, prior to extraction and analyses. The extracted samples were injected along with calibration curve standards. The calculated concentrations for Guaifenesin dilution factor yielded nominal of 101.46% for dilution factor 4 and 103.95% for dilution factor 2 respectively. The coefficient of variation for dilution factor 4 and 2 are 6.214% and 8.029% (i.e., <15%) for Guaifenesin respectively. Results are presented in Table 4.

Table 4: dilution integrity of Guaifenesin

QC ID	4 TIMES	2 TIMES
Actual (ng/ml)	2381.4110	4762.8210
1	2528.1040	4825.0300
2	2670.5260	5416.8100
3	2354.4150	4536.7900
4	2318.0890	4495.6940
5	2293.0860	5077.3750
6	2333.0760	5353.8680
Mean	2416.21600	4950.92783
SD	150.1426	397.4907
%CV	6.214	8.029
%Nominal	101.46	103.95

Stability

i) Post-preparative stability

Samples prepared at low (LQC) and high (HQC) quality control levels were extracted as per the procedure and kept in the auto sampler (stability samples). A calibration curve were freshly processed and analyzed with 6 replicates of stability

samples in a run. Concentrations were calculated to determine % nominal over time Guaifenesin were found to be stable for 12 hours in auto sampler. Results are shown in Table 5.

Table 5: QC Samples for post-preparative stability

QC ID	24Hrs-DRUG	
	LQC	HQC
Actual (ng/ml)	71.442	4762.821
1	71.602	4786.131
2	70.823	4644.009
3	69.743	4970.097
4	68.026	4553.952
5	66.840	4548.012
6	70.113	4067.060
Mean	69.5245	4594.8768
SD	1.780	304.054
%CV	2.56	6.62
%Nominal	97.32	96.47

ii) 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.

Table 6: QC Samples for bench top stability

QC ID	0Hrs		6Hrs	
	LQC	HQC	LQC	HQC
Actual (ng/ml)	71.44	2857.69	71.44	2857.69
	74.95	2744.97	65.73	2999.47
	71.78	2914.55	68.21	2734.06
	74.37	2735.42	61.76	2933.30
	77.21	2426.19	79.25	2842.78
	70.30	2761.16	79.43	2640.52
	70.47	2845.07	65.96	2424.46
Mean	73.18	2737.89	70.06	2762.43
SD	2.77	167.60	7.49	210.58
%CV	3.78	6.12	10.69	7.62
%Nominal	102.43	95.81	98.06	96.67

iii) Freeze-thaw stability

A calibration curve were processed with 6 replicates of stability samples which were subjected to 4 freeze thaw cycles analyzed in a single run. Guaifenesin were found to be stable in human plasma after 4th freeze thaw cycle at -80°C with nominal of 101.70% to 102.61% within acceptance criteria.

iv) Dry extract stability

A calibration curve were processed with 6 replicates of stability samples which were subjected to Dry extract stability of 6 hrs analyzed in a single run Guaifenesin were found to be stable in human plasma after dry extract of 6 hr with nominal of 97.48% to 103.58%.

v) 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 100.13%.

vi) Long term stock solution stability

Long term stock solution stability for the analyte and the internal standard were assessed for 9 days after preparation with freshly prepared stock solution at middle level quality control concentration. The stability of analyte and the internal standard were 98.70% to 87.44% respectively.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Guaifenesin in human plasma. The mobile phase is simple to prepare and economical. This method can be employed for the routine analysis of drug concentrations in bioavailability and bioequivalence studies.

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