Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 9, pp. 803–808, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00
Pergamon Press Ltd

Determination of guaifenesin in human plasma by liquid chromatography in the presence of pseudoephedrine*

J.B. ALURI and S. STAVCHANSKY†

Pharmaceutics Division, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712, USA

Abstract: A sensitive and specific liquid chromatography (LC) procedure was developed and validated for the determination of guaifenesin in human plasma in the presence of pseudoephedrine. Guaifenesin was extracted from plasma at pH 6.9–7.1 using methanol–methylene chloride (5:95, v/v) containing the internal standard mephenesin and pseudoephedrine. The organic layer was separated and evaporated to dryness and the residue reconstituted with the mobile phase containing methanol–acetonitrile–phosphate buffer (0.05 M) (11:11:78, v/v/v) containing 4 mM heptane sulphonic acid and 1% glacial acetic acid. The separation was performed on a μBondapak C_{18} column. The flow rate was 1.0 ml min^{-1}. The retention times for guaifenesin and mephenesin were 7.9 and 15.7 min, respectively. Linearity of response was observed in the concentration ranges of 50–1000 ng ml^{-1} and 1–4 μg ml^{-1}. Accuracy was within 15.4% of the true value for the inter-day and intra-day analysis. The precision, as measured by the RSD, ranged from 4.8 to 8.7% for intra-day. The reproducibility of inter-day ranged from 5.0 to 8.4%. The per cent recovery from plasma ranged from 88.6 to 97.6. Data are presented to illustrate the practicality of the method for the evaluation of guaifenesin plasma levels in the presence of pseudoephedrine after multiple oral administration of two sustained release tablets containing 600 mg of guaifenesin per tablet to six male healthy volunteers. The mean half-life of guaifenesin in human subjects was found to be 2.88 h and ranged from 1.36 to 5.25 h.

Keywords: *Guaifenesin; pseudoephedrine; human plasma; LC.*

Introduction

Numerous analytical methods based on paper chromatography [1], thin layer chromatography [2], calorimetry [3], spectrophotometry [4, 5], gas chromatography [6–8] and liquid chromatography [9–16] have been described in the literature for the assay of guaifenesin. However most of these methods are for the quantitation of guaifenesin in dosage forms. Very few methods have been reported for the determination of guaifenesin in biological fluids. Gas chromatography [17, 18] and liquid chromatography [19] method, have been reported for the estimation of guaifenesin in biological fluids. An LC method [20] was reported for the determination of guaifenesin in horse plasma, using a C_8 column and methanol–citrate buffer as the mobile phase, the detection range was from 5 to 200 μg ml^{-1}. With the advent of controlled release dosage forms containing guaifenesin the need to detect guaifenesin at the ng ml^{-1} level in human plasma in the presence of other compounds is of utmost importance to establish the pharmacokinetic profile of guaifenesin in man.

In this work, a liquid chromatography method was validated for the determination of guaifenesin in human plasma in the presence of pseudoephedrine. There is no specific analytical method to determine both guaifenesin and pseudoephedrine simultaneously, hence the need for individual determination and no interference from each other. The applicability of the method in bioequivalence studies involving sustained release formulations is described in this investigation.

Experimental

Materials

Reagents and chemicals. Guaifenesin was obtained from Adams Laboratories (Ft Worth, TX, USA). Mephenesin used as internal standard and 1-heptane sulphonic acid, sodium salt were obtained from Sigma (St Louis, MO,

*Presented at the Seventh Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (Analysis and Pharmaceutical Quality Section), San Antonio, TX, USA, 15–19 November 1992.

†Author to whom correspondence should be addressed.

USA) and were used as received. Acetonitrile, 0.1 N hydrochloric acid, methanol, methylene chloride, potassium phosphate monobasic, sodium phosphate monobasic and dibasic were analytical grade or USP grade. Heparinized human plasma was obtained from Interstate Blood Bank (Memphis, TN, USA). Glassware was silinated prior to use in the procedure.

Chromatographic conditions

The chromatograph consisted of a solvent delivery pump (Waters model 501), a 300 mm × 3.9 mm column packed with μBondapak C_{18} (Waters Associates), a variable wavelength detector (Waters LC Spectrophotometer model 481), an autoinjector (Waters WISP model 712), and an integrator (Shimadzu Chromatopac CR3A). The mobile phase consisted of methanol–acetonitrile–phosphate buffer (0.05 M KH_2PO_4) (11:11:78, v/v/v), containing 4 mM heptane sulphonic acid and 1% glacial acetic acid. The mobile phase mixture was filtered through a 0.45 μm pore Nylon membrane filter (Millipore), and deaerated by sonication under reduced pressure. The flow rate was maintained at 1 ml min^{-1}. The detector wavelength was set at 272 nm. The sensitivity was set at 0.02 absorbance units full scale.

Standard solutions

Guaifenesin standard solution. Standard solutions of guaifenesin were prepared in methanol containing approximately 50, 200, 500, 700 and 1000 ng ml^{-1} of guaifenesin.

Internal standard solution. Standard solutions of the internal standard, mephenesin was prepared in methanol to contain approximately 100 ng ml^{-1} of mephenesin.

Extraction solvent. Methanol–methylene chloride (5:95, v/v) was prepared and spiked with the internal standard solution to yield a concentration of approximately 300 ng ml^{-1} of mephenesin in the extraction solvent.

Pseudoephedrine standard solution. Standard solution of pseudoephedrine was prepared in methanol containing 1 μg ml^{-1} of pseudoephedrine.

Sample preparation

Guaifenesin standard solutions were taken in 15 ml disposable screw capped culture tubes and the methanol was evaporated by a gentle stream of nitrogen. To this was added exactly 1 ml of human plasma and vortexed for 10 s. Three millilitres of the extraction solvent containing the internal standard, mephenesin and 250 μl of pseudoephedrine standard solution were added and vortexed for 10 s to effect uniform mixing. As the extraction is effective at neutral pH, the pH of the plasma mixture was adjusted by adding 0.4 ml of 0.1 N hydrochloric acid and 1 ml of phosphate buffer (pH 7.0). The contents were vortexed for 30 s and the tubes were frozen over acetone–dry ice for 15 min to separate the aqueous and organic layers. The tubes were then centrifuged at 2700 rpm for 30 min. After centrifuging, the top aqueous layer is removed by aspiration and discarded. The bottom organic layer was transferred to fresh disposable evaporation tubes and subjected to dryness by means of a slow stream of nitrogen. The dried residue was reconstituted with 100 μl of the mobile phase and 50 μl injected onto the LC column.

Results and Discussion

Chromatograms of the plasma spiked with internal standard, plasma standard and a plasma sample are shown in Fig. 1. The typical retention times for guaifenesin and for the internal standard, mephenesin 7.9 and 15.7 min, respectively. There are no interfering peaks at these retention times.

Linearity of response

The concentration–response relationship for extracted plasma standards 'spiked' with guaifenesin in the presence of mephenesin was found to be linear from 50 to 1000 ng ml^{-1} over a 4 day analysis period the RSD of the slopes obtained by linear regression was 1.47% and the mean of the square of the correlation coefficients was 0.998. In addition, an F-test was performed to strengthen the results (Table 1a). The limit of quantitation was set at 50 ng of guaifenesin in 1 ml of plasma, and was based on 10 times the noise level obtained in the chromatograms. The limit of detection was set at 25 ng of guaifenesin in 1 ml of plasma, and was based on three times the noise level. In the analysis of the clinical samples, some of the plasma samples showed higher levels of guaifenesin, more than 20% of the higher value (1000 ng ml^{-1}) and this prompted the authors to check the extent of linearity above 1000

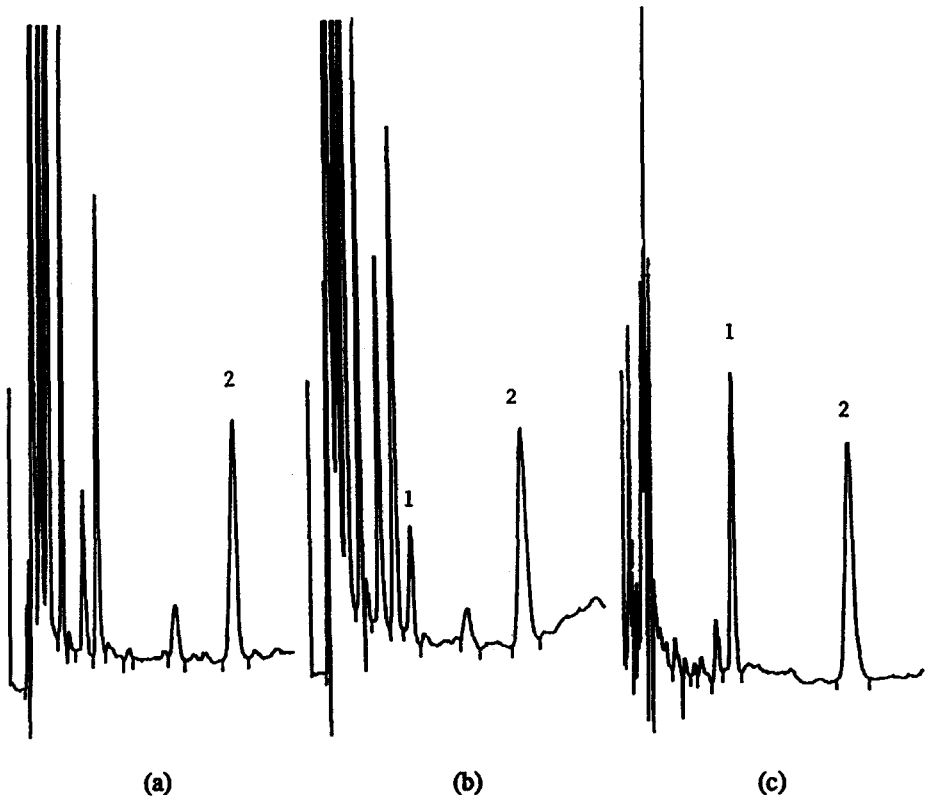

Figure 1
Chromatograms of blank plasma spiked with mephenesin (a); plasma standard — guaifenesin (50 ng ml^{-1}), mephenesin (b); plasma sample from clinical study — guaifenesin (375 ng ml^{-1}), mephenesin (c); concentration of mephenesin was 300 ng ml^{-1}. 1, Guaifenesin; 2, mephenesin.

Table 1a
Standard curves of guaifenesin in plasma

Day	Slope	Intercept	R^2
1	0.00799	0.216	0.999
2	0.00804	0.101	0.999
3	0.00826	0.116	0.996
4	0.00814	0.259	0.998
Mean	0.00811	0.173	0.998
SD	0.000119	0.0767	0.0014
RSD	1.47	44.33	0.14

ng ml^{-1}. Linearity of response was also established from 1 to 4 μg ml^{-1} with a square of the correlation coefficient of 0.996.

Accuracy

On the analysis day the per cent absolute difference from the true value, for the analysis of a standard value from 50 to 1000 ng ml^{-1}, ranged from 1.4 to 17.9% for guaifenesin (Table 1b). Using the deviation of absolute differences between the concentrations found and the true concentrations and the *t*-value from the one-tailed Student's *t*-distribution table, 95% confidence limits were estimated for each concentration and are presented in Table 1c and 1d. These calculations indicate that the results of any single determination for guaifenesin in plasma would range between 5.4 and 15.4% of the true value for all concentrations over the dynamic range of the method.

Precision and reproducibility

The precision (within-day variation of replicate determinations) and the reproducibility (day-to-day variation of the determinations) were determined from standard values. For each concentration a mean value and per cent RSD were calculated on each analysis day (n = 6) and over the course of a 4-day study (n = 30). The precision (within-day variation) ranged from 4.8 to 8.7% (mean of 6.6%) as shown in Table 2a, over the dynamic range of the method. Reproducibility of the method (day-to-day variation) was determined using the variability about the mean of each concentration analysed over the course of the validation study. Across the four analysis days, the deviation of variation ranged from 5.0 to 8.4% (mean of 6.6%) (Table 2b).

Table 1b
Accuracy of guaifenesin assay

Conc. (ng ml^{-1})	Mean of per cent deviation				
	Inter-day				
	Day 1	Day 2	Day 3	Day 4	Intra-day
50.4	9.7	11.5	17.9	6.1	11.5
201.5	4.9	3.3	3.6	5.4	4.5
504.2	2.7	6.5	7.5	3.2	5.5
705.9	3.4	1.5	3.2	8.8	4.1
1008.4	4.7	1.4	3.4	4.7	3

Table 1c
Confidence level analysis of inter-day data

True value (ng ml^{-1})	Number of observations	Mean absolute difference from true value ± SD (ng ml^{-1})	95% Confidence interval	
			(ng ml^{-1})	% True value
50.4	15	5.5 ± 1.2	±7.8	15.4
201.7	16	8.7 ± 1.5	±11.6	5.8
504.2	15	25.6 ± 5.9	±37.3	7.4
705.2	16	29.8 ± 8.6	±46.6	6.6
1008.4	16	35.8 ± 9.2	±53.9	5.4

Table 1d
Confidence level analysis of intra-day data

True value (ng ml^{-1})	Number of observations	Mean absolute difference from true value ± SD (ng ml^{-1})	95% Confidence interval	
			(ng ml^{-1})	% True value
50.4	6	5.8 ± 1.0	±7.8	15.4
201.7	6	9.0 ± 3.0	±14.9	7.4
504.2	6	27.8 ± 12.8	±52.9	10.5
705.2	6	28.6 ± 12.3	±52.7	7.5
1008.4	5	30.2 ± 17.0	±63.6	6.3

Sensitivity

Using the data obtained from the extrapolation of standard values the sensitivity of the method for guaifenesin was determined based upon the 95% confidence limits calculated and reproducibility obtained for each concentration. A quantitation limit of 50 ng ml^{-1} was chosen based upon the accuracy.

Specificity

The combination of a selective extraction technique and ultraviolet detection provided an assay free from endogenous interferences in the chromatographic regions of guaifenesin and mephenesin.

Recovery

Relative recovery studies were done in comparing the recovery of guaifenesin from plasma to that of water. Both were subjected to the same extraction procedures and the per cent recovery, presented in Table 2c, ranged from 88.6 to 97.2 (mean of 93.7) over the dynamic range of the method.

Application to human pharmacokinetics

In conclusion, an LC method for the determination of guaifenesin in human plasma using mephenesin as internal standard has been developed, validated and applied to the

Table 2a
Precision of guaifenesin assay, intra-day

Theoretical conc. (ng ml^{-1})	Mean of extrapolated conc. ± SD	RSD
50.4	45.4 ± 3.9	8.7
201.7	203.8 ± 12.1	5.9
504.2	488.0 ± 39.9	8.2
705.9	723.1 ± 39.2	5.4
1008.4	1021.4 ± 48.7	4.8
Overall mean		6.6

Table 2b
Precision of guaifenesin assay, inter-day

Theoretical conc. (ng ml^{-1})	Mean of extrapolated conc. ± SD			
	Day 1	Day 2	Day 3	Day 4
50.4	47.1 ± 4.9	45.7 ± 5.0	53.1 ± 13.0	48.1 ± 4.9
201.7	193.3 ± 10.3	208.4 ± 5.9	205.0 ± 9.3	202.0 ± 13.6
504.2	506.2 ± 16.6	471.5 ± 36.8	542.0 ± 21.6	504.3 ± 22.3
705.9	681.9 ± 30.1	699.7 ± 14.0	717.8 ± 26.5	759.5 ± 63.0
1008.4	1036.9 ± 67.5	1000.4 ± 15.2	996.5 ± 50.8	1054.1 ± 46.1
Mean of RSD	6	5	8.4	6.8
Overall mean of RSD: 6.6				

Table 2c
Recovery of guaifenesin from plasma

Conc. (ng ml^{-1})	Per cent recovery
50.4	89.8
151.3	97.6
403.4	97.2
756.3	95.4
1008.4	88.6
Mean per cent recovery	93.7

analysis of human plasma samples obtained from clinical studies. Figure 2 shows the plasma concentration–time profiles on day 5 of guaifenesin obtained after multiple oral administration of two sustained release tablets containing 600 mg of guaifenesin per tablet for five days. This method was also used to confirm the bioequivalence of diffused sustained release formulations of guaifenesin tablets.

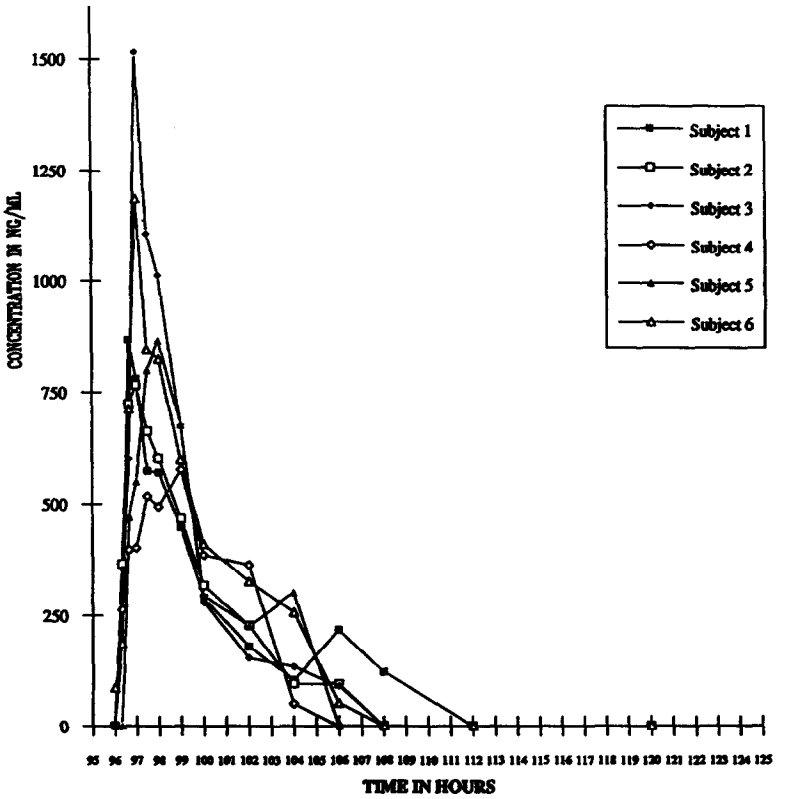

Figure 2
Concentration of guaifenesin in plasma on day 5 after multiple oral administration of two sustained release tablets containing 600 mg per tablet of guaifenesin every 12 h for 5 days to six healthy male volunteers.

References

[1] S. Ahuja, *J. Pharm. Sci.* **57**, 313–314 (1968).
[2] H. Tomonkona and M. Vasatova, *Pharmazie* **44**, 197–198 (1989).
[3] D.K. Desai, S.P. Kurani and A.G. Seshadrinathan, *Indian J. Pharm. Sci.* **47**, 210–211 (1985).
[4] G.R. Rao, A.B. Avadhanulu, R. Giridhar and C.K. Kokate, *East. Pharm.* **31**, 141–142 (1988).
[5] V. Das Gupta and L.A. Luzzi, *Amer. J. Hosp. Pharm.* **25**, 360–362 (1968).
[6] J. Hudanick, *J. Pharm. Sci.* **59**, 238–239 (1970).
[7] E. Mario and L.G. Meehan, *J. Pharm. Sci.* **59**, 538–540 (1970).
[8] M. Bambagiotti-Alberti, S. Pinzauti and F.F. Vincieri, *Pharm. Acta. Helv.* **62**, 175–176 (1987).
[9] V. Das Gupta and A.G. Ghanekar, *J. Pharm. Sci.* **66**, 895–897 (1977).
[10] D.R. Heidemann, *J. Pharm. Sci.* **68**, 530–532 (1979).
[11] W.O. McSharry and I.V.E. Savaga, *J. Pharm. Sci.* **69**, 212–214 (1980).
[12] L. Carnavale, *J. Pharm. Sci.* **72**, 196–198 (1983).
[13] G.W. Schieffer, *J. Pharm. Sci.* **73**, 1856–1858 (1984).
[14] Y. Matsushima, *J. Chromatogr.* **332**, 265–268 (1985).
[15] D.R. Heidemann, K.S. Groon and J.M. Smith, *LC–GC* **5**, 422–426 (1987).
[16] H. Richardson and B.A. Bidlingmeyer, *J. Pharm. Sci.* **73**, 1430–1482 (1984).
[17] W.R. Maynard Jr and R.B. Bruce, *J. Pharm. Sci.* **59**, 1346–1348 (1970).
[18] Singhawangeha *et al.*, *J. Chromatogr.* **183**, 433–439 (1980).
[19] H.C.J. Ketalaars and J.G.P. Peters, *J. Chromatogr.* **224**, 144–148 (1981).
[20] H.C.J. Ketalaars, J.G.P. Peters, R.B.M. Anzion and C.A. Van Ginneken, *J. Chromatogr.* **288**, 423–429 (1984).

[Received for review 24 October 1992; revised manuscript received 30 March 1993]