Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 10, pp. 955–962, 1993
Printed in Great Britain

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

Determination of dolasetron and its reduced metabolite in human plasma by GC–MS and LC

TODD A. GILLESPIE,* JAMES A. ECKSTEIN, PASQUA NARDELLA and JOHN E. COUTANT

Marion Merrell Dow Inc., 2110 E. Galbraith Rd, Cincinnati, OH 45215-6300, USA

Abstract: Both a GC–MS and an LC method have been developed for the simultaneous quantitation of dolasetron and reduced dolasetron in human plasma. The GC–MS method has been utilized in preliminary human pharmacokinetic studies of dolasetron mesylate. Selected ion monitoring was used in these initial studies to obtain the sensitivity and specificity required for quantitation. The GC–MS method has been used in the range of 1–120 ng ml^{-1} for dolasetron and 1–240 ng ml^{-1} for reduced dolasetron in plasma. The limit of quantitation for both compounds by GC–MS was 1 ng ml^{-1}. Recently, an LC method has been utilized for quantitation of both compounds on a routine basis. This method utilizes essentially the same sample preparation procedure as the GC–MS method. The LC method has been used in the range of 5–200 ng ml^{-1} in plasma for dolasetron and reduced dolasetron. In addition, the relationship between the LC and GC–MS methods has been assessed using data obtained from human male volunteers following intravenous administration of 3.0 mg kg^{-1} of dolasetron mesylate monohydrate.

Keywords: *Dolasetron; reduced metabolite; plasma; GC–MS, LC.*

Introduction

Dolasetron (Fig. 1) is a potent and selective 5-HT_3 receptor antagonist presently undergoing clinical investigation as an antiemetic adjunct to cancer chemotherapy. Evaluation of dolasetron metabolism *in vivo* in rat, rabbit, dog and monkey has shown that the major metabolite of dolasetron is formed via a reduction of the 3-oxo (ketone) function to the corresponding alcohol (Fig. 1). Additional studies have found this metabolite (reduced dolasetron) to be biologically active. Pharmacokinetic studies in these animals have shown that reduced dolasetron persists considerably longer than dolasetron in plasma after intravenous administration of dolasetron mesylate. The intravenous formulation is prepared using the monohydrated mesylate (methane sulphonate) salt (Fig. 1).

Analytical procedures utilizing HPLC with UV detection had been developed for initial animal studies. However, these methods were capable of quantitating only relatively high levels of dolasetron and reduced dolasetron in human plasma. Furthermore, problems existed with variable retention times and interferences with the internal standard.

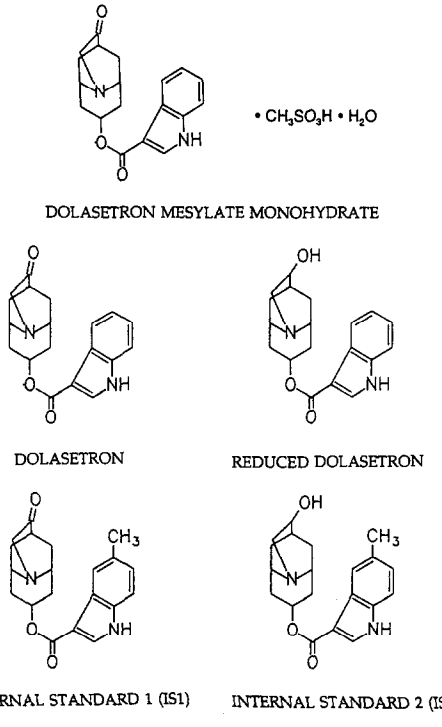

Figure 1
Chemical structures of: (1) dolasetron mesylate monohydrate; (2) dolasetron free base; (3) reduced dolasetron, a major plasma metabolite; (4) internal standard 1 (IS1; methyl analogue of dolasetron); and (5) internal standard 2 (IS2; methyl analogue of reduced dolasetron).

* Author to whom correspondence should be addressed.

This paper describes both a GC–MS and LC method which has been used for the quantitation of both dolasetron and reduced dolasetron in plasma for the evaluation of pharmacokinetic parameters in man [1, 2]. In addition, the relationship between the two analytical methods has been assessed from validation studies and from data obtained following intravenous administration of dolasetron to normal human male volunteers. The GC–MS method is a modification of a method utilized in European studies of dolasetron. Modifications were made to the LC procedures used for animal studies to enhance the stability and sensitivity of the method. In addition, the HPLC method utilizes concentrated citric acid (5 M) added to the plasma to prevent autooxidation of dolasetron.

Experimental

Materials

All analytical grade solvents were from Burdick and Jackson (Muskegon, MI). Trifluoroacetic anhydride (TFAA) was purchased from Regis (Morton Grove, IL). Ammonium acetate, hydrochloric acid (HCl) and sodium carbonate ($NaCO_3$) were from EM Science (Cherry Hill, NJ). Citric acid was from Sigma (St Louis, MO). Hexamethyldisilazane was purchased from Pierce (Rockford, IL) and was used to siliconize all glassware through a variation of vapour-phase silylation [3]. Dolasetron, reduced dolasetron and both methyl analogue internal standards were obtained from Marion Merrell Dow Research Institute (Cincinnati, OH). Drug free EDTA plasma was supplied by Carolina Biological Supply (Burlington, NC).

Extraction procedure for plasma

First, 100 μl of 5 M citric acid was added to each 1 ml plasma standard and sample. Fifty μl (1 ng μl^{-1} each of IS1 and IS2 in water for GC–MS) or 100 μl (1 ng μl^{-1} of IS2 in water for HPLC) of internal standard solution was then added to each plasma standard and sample. One ml of acetonitrile, then 1 ml of 2 M $NaCO_3$ were added and the samples vortexed after each addition. Five ml of ethyl acetate–hexane (75:25, v/v) was added and the samples mixed on a reciprocating shaker for 20 min. The layers were then separated with centrifugation at 3000 rpm for 10 min. The organic layer (5.5 ml) was transferred to a tube containing 1.5 ml of 0.1 M HCl and mixed on a reciprocating shaker for 20 min. The layers were then separated with centrifugation at 3000 rpm for 10 min. The upper organic layer was removed by aspiration and 1 ml of 2 M $NaCO_3$ and 5.5 ml of ethyl acetate–hexane (75:25, v/v) were added to the aqueous layer. The samples were mixed on a reciprocating shaker for 20 min. The layers were then separated with centrifugation at 3000 rpm for 10 min. The organic layer (~5.5 ml) was then transferred and evaporated to dryness under nitrogen at 65°C.

LC conditions

Each sample was reconstituted with 120 μl of mobile phase and transferred to a WISP vial. Forty μl of each sample was injected onto the column. A CN Spherisorb column (Metachem Technologies), (3 μm, 150 mm × 4.6 mm), was used. The mobile phase consisted of acetonitrile–ammonium acetate buffer (0.05 M) (pH ~7.5 with ammonium hydroxide) (24:76, v/v). The flow rate was 0.8 ml min^{-1} with UV detection at 280 nm. The HPLC equipment included a Waters 715 Ultra WISP, Waters 484 Absorbance detector, and a Waters 600E system controller and pump (Millipore, Milford, MA).

GC–MS conditions

The derivatization procedure was performed the day of analysis. Trifluoroacetic anhydride (TFAA), 100 μl, was added to the residue and vortexed. This solution was heated at 65°C for 20 min and evaporated to dryness at 65°C. The residue was reconstituted in 100 μl of ethyl acetate–hexane (75:25, v/v). The injection volume was 1 μl.

Instrumental analysis was performed on a Finnigan MAT TSQ46 gas chromatograph–mass spectrometer (San Jose, CA). GC was carried out on a DB-5 (J&W Scientific, Folsom, CA), 7.5 m × 0.32 mm i.d., with a film thickness of 0.25 μm. The injector temperature was 275°C, in splitless mode with a split time of 2 min and a head pressure of 10–11 psi of helium. The initial oven temperature of 170°C was held for 1 min, increased at 20°C min^{-1} to 270°C, then held for 2 min at this temperature. Mass spectrometry conditions were as follows: interface temperature 300°C, transfer line temperature 280°C, source temperature 150°C, electron energy 110 eV and emission current of 0.28 mA. Positive chemical

ionization (PCI) was carried out with ammonia at an ionizer pressure of 0.50 Torr. All mass spectrometry conditions were optimized for the dolasetron ion at *m/z* 325.

Validation procedure for dolasetron and metabolite

Assay specificity was tested by analysing five pooled blank EDTA plasma samples for endogenous interferences. Linearity of the standard response was ensured by injection of four replicate standard curves appropriate to each method.

The day-to-day precision and accuracy of each method was determined by analysing random coded unknowns over 4 days. Within-day precision and accuracy was tested by analysing six (for HPLC) or eight (for GC–MS) replicates of a low and high concentration control plasma sample on 1 day.

Stability of dolasetron and reduced dolasetron in frozen plasma at −20°C was determined by analysing quality control samples on a regular basis over the period of 8 weeks. In addition, citric acid was tested as an additive to the frozen plasma samples to hender the autooxidation of dolasetron determined in earlier stability studies.

Results

Liquid chromatography

Analysis of the pooled blank plasma samples showed no endogenous interferences for reduced dolasetron and internal standard (IS2). The four plasma standard curves showed linearity over the range of 5–200 ng ml^{-1} for both compounds. In a typical chromatogram, dolasetron eluted at 7.2 min, reduced dolasetron at 17.0 min and the internal standard (IS2) eluted at 19.5 min. Chromatograms of a plasma standard and human sample are shown in Fig. 2.

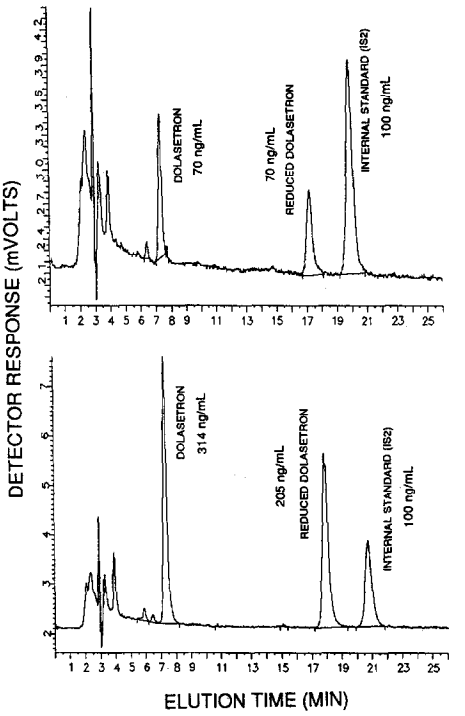

Figure 2
Typical HPLC chromatograms of: (1) plasma standard containing dolasetron, reduced dolasetron and internal standard (IS2); and (2) human plasma sample following an intravenous infusion of 5 mg kg^{-1} of dolasetron mesylate monohydrate containing dolasetron, reduced dolasetron and internal standard.

Table 1
Precision and accuracy (day-to-day) plasma HPLC assay

	Amount added (ng ml^{-1})								
	5.0	10.0	20.0	25.0	50.0	75.0	100.0	150.0	200.0
Dolasetron									
*n**	8	8	7	8	8	8	7	8	8
Mean	4.4	11.2	18.9	23.4	47.0	67.5	95.5	141.5	177.2
SD	2.1	2.2	1.6	3.3	3.2	5.6	7.3	9.3	14.7
RSD (%)	48.7	19.3	8.5	14.3	7.0	8.2	7.7	6.5	8.3
Accuracy (%)	88.0	112.0	94.5	93.6	94.0	90.0	95.5	94.3	88.7
Reduced dolasetron									
*n**	8	8	7	8	8	8	8	8	8
Mean	5.4	11.4	21.7	24.0	49.6	73.0	100.9	150.7	198.1
SD	0.8	1.3	2.4	1.0	1.8	4.6	5.9	5.0	8.1
RSD (%)	14.4	11.4	11.0	4.1	3.6	6.2	5.8	3.3	4.1
Accuracy (%)	108.0	113.0	108.5	96.0	99.2	97.3	100.0	100.4	99.0

*n = total number of samples analysed over the course of 4 days.

Results for the day to day precision and accuracy are shown in Table 1. The overall accuracy of the method for dolasetron was 94.5% and for reduced dolasetron was 102.4%. The precision for dolasetron was between 6.6 and 19.3% for all concentrations tested with the exception of 5 ng ml^{-1} which was 47.7%. Reduced dolasetron had a precision between 3.3 and 14.8% for all of the concentrations tested. Utilizing these data, a limit of quantitation was established for dolasetron at 10 ng ml^{-1} and reduced dolasetron at 5 ng ml^{-1}.

The within-day precision and accuracy is shown in Table 2. The accuracy for dolasetron at 40 and 150 ng ml^{-1} was 85 and 90.3%, respectively. Reduced dolasetron had an accuracy of 103.2% for 40 ng ml^{-1} and 101.1% for 150 ng ml^{-1}. The %RSD for the analysis of dolasetron was less than 9.0% and less than 6.0% for reduced dolasetron at both concentrations.

Dolasetron and reduced dolasetron are stable in plasma frozen at −20°C with 5 M citric acid added for 8 weeks. Longer stability studies are being performed at this time.

GC–MS

Selected ion monitoring (SIM) of the following protonated molecular ions was chosen for quantitation: *m/z* 325 for dolasetron, *m/z* 339 for the internal standard (IS1), *m/z* 423 and *m/z* 437 for the TFAA derivatives of reduced dolasetron and internal standard (IS2), respectively. Typical SIM chromatograms of a plasma standard containing 50 ng ml^{-1} of dolasetron, IS1, IS2 and 100 ng ml^{-1} of reduced dolasetron are shown in Fig. 3. The retention times for reduced dolasetron, IS2, dolasetron and IS1 were 5.8, 6.17, 6.45 and 6.87 min, respectively. The plasma standard curves obtained were linear within the range of 2–120 ng ml^{-1} for dolasetron and 4–240 ng ml^{-1} for reduced dolasetron. No endogenous interferences were observed for dolasetron, reduced dolasetron and both internal standards. A chromatogram of a human plasma sample is shown in Fig. 4. (The peak area ratio of dolasetron to IS1 and reduced dolasetron to IS2 was utilized to calculate the concentrations shown in Fig. 4. The peak area calculated for each component is the number displayed directly above the peak.)

The day-to-day precision and accuracy for this method are shown in Table 3. The overall accuracy of the method for dolasetron was 98.6% and for reduced dolasetron 99.3%. The precision for dolestron was lower than 20.0% for all concentrations tested with the exception of 2 ng ml^{-1} which was 23.1%. Reduced dolasetron had a precision of 5.1–16.8% at the higher concentrations with 28.0% at the lowest concentration of 4 ng ml^{-1}. Utilizing this data, a limit of quantitation was established for dolasetron of 2 ng ml^{-1} and reduced dolasetron at 1 ng ml^{-1} for this method.

The within-day precision and accuracy is shown in Table 2. The accuracy for dolasetron at 15 and 75 ng ml^{-1} was 96.7 and 96.1%, respectively. Reduced dolasetron had an

Table 2
Precision and accuracy (within-day) for dolasetron and reduced dolasetron

	Amount added (ng ml^{-1})			
	Dolasetron		Reduced dolasetron	
HPLC plasma assay	40.0	150.0	40.0	150.0
n	6	6	6	6
Mean	34.0	135.5	41.3	151.7
SD	2.9	8.2	1.6	8.3
RSD (%)	8.4	6.1	4.0	5.5
Accuracy (%)	85.0	90.3	103.2	101.1
	Amount added (ng ml^{-1})			
	Dolasetron		Reduced dolasetron	
GC–MS plasma assay	15.0	75.0	30.0	150.0
n	7	8	7	8
Mean	14.5	72.1	28.5	156.0
SD	1.4	2.7	1.2	4.5
RSD (%)	9.7	3.7	4.2	2.9
Accuracy (%)	96.7	96.1	95.0	104.0

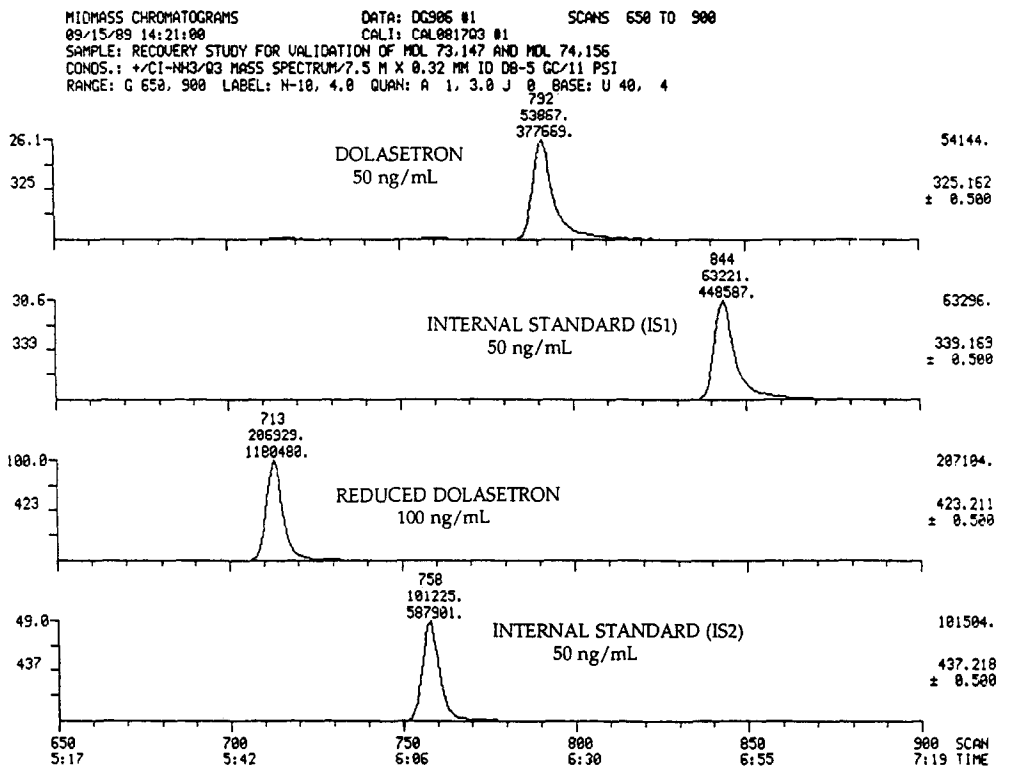

Figure 3
Typical selected ion monitoring (SIM) GC–MS chromatogram of a plasma standard containing dolasetron, reduced dolasetron and both internal standards (IS1 and IS2).

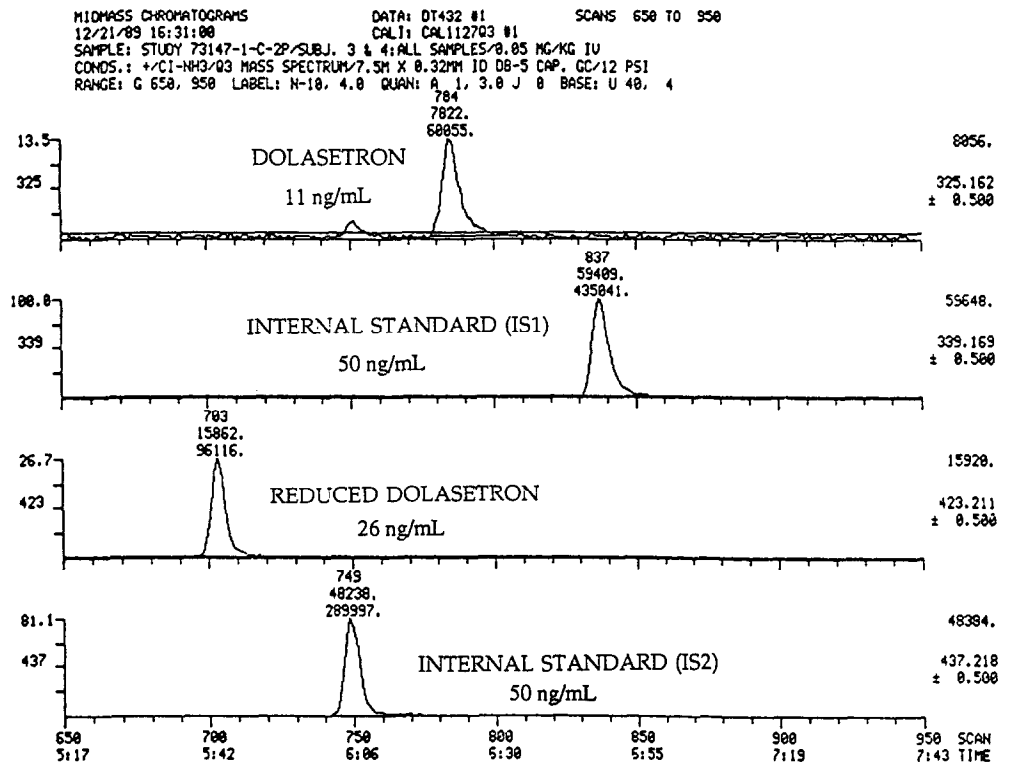

Figure 4
Typical selected ion monitoring (SIM) GC–MS chromatogram of a human plasma sample following an intravenous infusion of 0.05 mg kg^{-1} of dolasetron mesylate monohydrate.

Table 3
Precision and accuracy (day-to-day) for plasma GC–MS assay

Dolasetron	Amount added (ng ml^{-1}) 2.0	5.0	14.4	60.0	120.0
n	8	8	8	8	8
Mean	2.6	5.0	11.8	52.2	112.7
SD	0.6	1.0	1.7	2.9	6.1
RSD (%)	23.1	20.0	14.4	5.6	5.4
Accuracy (%)	130.0	100.0	81.9	87.0	93.9
Reduced dolasetron	Amount added (ng ml^{-1}) 4.0	10.0	28.8	120.0	240.0
n	7	7	8	8	7
Mean	5.0	10.7	25.8	105.0	209.6
SD	1.4	1.8	3.5	13.4	10.6
RSD (%)	28.0	16.8	13.6	12.8	5.1
Accuracy (%)	125.0	107.0	89.6	87.5	87.3

accuracy of 95.0% for 30 ng ml^{-1} and 104.0% for 150 ng ml^{-1}. The %RSD for the analysis of dolasetron was less than 10.0% and less than 5.0% for reduced dolasetron at both concentrations.

Discussion

The use of SIM as the means of detection makes the GC–MS method highly specific for dolasetron and reduced dolasetron. Furthermore, this method is sensitive enough to routinely quantitate levels as low as 1 ng ml^{-1} in plasma. However, the GC–MS assay is a relatively complex combination of double extraction, derivatization, splitless injection, gas chromatography and mass spectrometry. Every step had to initially be optimized and required very careful manual operation. Extraction time, evaporation time, degree of dryness, stability of solutions and stability of derivatives, all affect the assay precision, accuracy and reproducibility, particularly of reduced dolasetron.

A number of problems were encountered with the GC–MS assay. First, the stability of the final derivatized, reconstituted samples created a major source of variability. The samples had to be derivatized on the day of injection and would remain stable for only the next 24 h. Second, sample carry over from injection to injection contributed to the lack of reproducibility. Manual injection of each sample was required followed by thorough cleaning of the syringe.

The LC method was found to have the degree of precision and accuracy needed to perform routine analysis of dolasetron and reduced dolasetron in human plasma for pharmacokinetic studies. This method avoids many of the disadvantages encountered with the GC–MS method. However, interferences in the plasma eluted closely to dolasetron and created initial problems with quantitation. This is noted in the data found for the day-to-day precision in Table 1. After further experience with the LC method, the interference problems encountered have been minimized. This was accomplished in the extraction procedure by transferring only 5 ml of the organic layer (originally 5.5 ml was transferred) to the tube containing the 1.5 ml of 0.1 M HCl.

The LC assay is not as specific or sensitive as GC–MS, however, routine quantitation of dolasetron and reduced dolasetron in plasma could be accomplished at 5 ng ml^{-1}. The average day-to-day precision of the HPLC method for 10–150 ng ml^{-1} of dolasetron was 10.0%, which compares favourably with 11.4% found with the GC–MS assay for 5–120 ng ml^{-1}. In addition, the RSD of the LC method was 7.2% from 5 to 200 ng ml^{-1} for reduced dolasetron compared to a RSD of 15.3% for the GC–MS method. These comparisons indicate that the LC method is more robust for routine assay work and that for reduced dolasetron is more precise at the lower limit of quantitation than the GC–MS method. Derivatization is no longer necessary with the LC assay, thus eliminating a drying and heating step which added to the variability of the GC–MS assay. Furthermore, the LC method was validated with the addition of citric acid utilized as an antioxidant to assure the stability of dolasetron.

Additionally, the analysis of dolasetron and reduced dolasetron in urine was performed by HPLC (no GC–MS). The method was essentially the same as that for plasma by LC with one less extraction step being performed in the sample preparation procedure. No dolasetron was observed in urine samples, however, the level of reduced dolasetron observed in urine was considerably higher than found in plasma samples.

The relationship between the two analytical methods was assessed using regression analysis and data obtained from human plasma samples following intravenous administration of 3 mg kg^{-1} of dolasetron mesylate monhydrate to four normal male volunteers. A typical plasma concentration–time profile curve of both dolasetron and reduced dolasetron for a subject is shown in Fig. 5. Pharmacokinetic data from this study has been published elsewhere [1, 2].

For reduced dolasetron, the slope (0.97) and intercept (13.4) were not significantly different

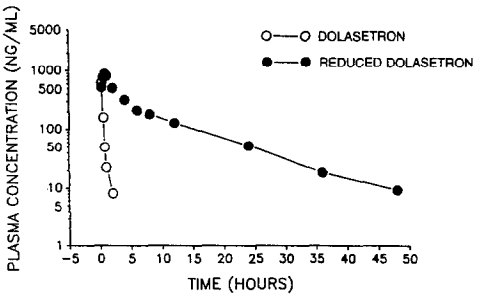

Figure 5
Semilogarithmic dolasetron and reduced dolasetron plasma concentration–time profiles following a 10 min intravenous infusion of 3 mg kg^{-1} of dolasetron mesylate monohydrate.

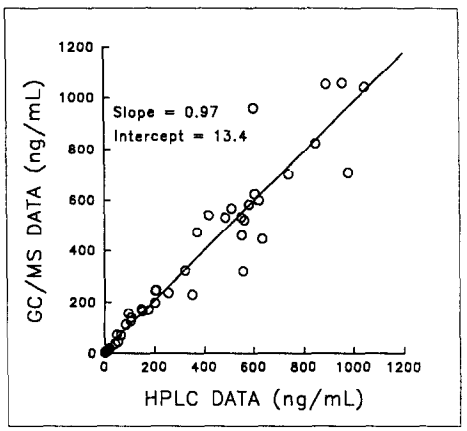

Figure 6
Relationship between GC–MS and HPLC data (four subjects) for reduced dolasetron.

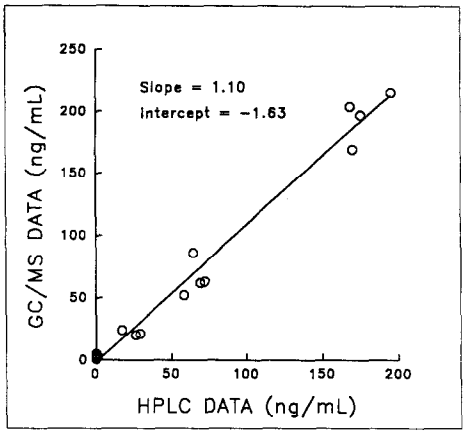

Figure 7
Relationship between GC–MS and HPLC data (four subjects) for dolasetron (without the 0.25 h time point).

from 1.0 ($P = 0.52$) and 0 ($P = 0.51$), respectively (see Fig. 6). These results indicate that the HPLC and GC–MS methods for quantitating reduced dolasetron in plasma are equivalent over the concentration range of 5–1000 ng ml^{-1}.

For dolasetron, the slope (1.42) was significantly different from 1.0 ($P = 0.0001$) while the intercept (−22.0) was not significantly different from 0 ($P = 0.07$). As a result of the higher concentrations at 0.25 h and the use of a different dilution factor (1–10), a data set without the 0.25 h data was additionally analysed. The slope (1.10) was still significantly different from 1.0 ($P = 0.02$) and the intercept (−1.63) was not significantly different from 0 ($P = 0.68$; see Fig. 7). These results indicate that over the range of 5–200 ng ml^{-1} LC results will be approximately 10% lower than those obtained using the GC–MS assay. However, these results demonstrate sufficient agreement for the analysis of dolasetron due to the inherent differences in the relative sensitivities encountered for the two methods.

Conclusions

Both the GC–MS and HPLC methods allow the simultaneous determination of dolasetron and reduced dolasetron in human plasma. The GC–MS method has been utilized in preliminary human pharmacokinetic studies of dolasetron mesylate. These studies required the sensitivity and specificity inherent in GC–MS using selected ion monitoring. However, at present, the GC–MS method has been substituted with the HPLC assay for further

clinical trials due to its advantages of being more rapid and rugged than the GC–MS assay.

Acknowledgements — The authors would like to thank the following associates of Marion Merrell Dow Inc.: W.P. Gordon, M.R. Steup and L.J. Cremer of the Pharmacokinetics/Drug Metabolism Department, Indianapolis, IN and B. Dulery, J. Schoun and K.D. Haegele of the Department of Clinical Research, Strasbourg Center, Strasbourg, France.

References

[1] H. Boxenbaum, T. Gillespie, K. Heck and W. Hahne, *Biopharm. Drug Dispos.* **13**, 693–701 (1992).
[2] H. Boxenbaum, T. Gillespie, K. Heck and W. Hahne, *Biopharm. Drug Dispos.*, in press.
[3] D.C. Fenimore, C.M. Davis, J.H. Whitford and C.A. Harrington, *Anal. Chem.* **48**, 2289–2290 (1992).

[*Received for review 2 February 1993; revised manuscript received 29 March 1993*]