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Simultaneous measurement of dolasetron and its major metabolite, MDL 74,156, in human plasma and urine

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Abstract

A selective and sensitive analytical method for the simultaneous measurement of dolasetron (I) and its major metabolite, MDL 74,156 (II), in human plasma and urine samples has been developed using a structural analogue, MDL 101,858, as internal standard (I.S.). The compounds were extracted from plasma and urine using solvent extraction after the addition of the I.S. Chromatographic separation was carried out on a reversed-phase HPLC column and detection and quantification was by fluorescence with excitation and emission wavelengths of 285 and 345 nm, respectively. Linear responses were obtained over concentration ranges of 5 to 1000 pmol/ml for plasma samples and 20 to 1000 pmol/ml for urine samples with correlation coefficients for the calibration curves exceeding 0.999 in all cases. Intra-day and inter-day reproducibility yielded limits of quantification of 10 pmol/ml for I and 5 pmol/ml for II in plasma and 50 pmol/ml for I and II in urine. The method has been applied to the simultaneous analysis of both compounds in plasma and urine samples coming from clinical pharmacokinetic studies.

Keywords: Dolasetron

1. Introduction

Dolasetron mesilate, MDL 73,147EF, 1H-indole-3-carboxylic acid, *trans*-octahydro-3-oxo-2,6-methano-2H-quinolizin-8-yl ester, methane sulphonate (I, Fig. 1), is a 5-HT3 receptor antagonist currently in development for the management of nausea and vomiting associated with cancer chemotherapy, radiotherapy and surgical procedures [1,2]. The ketone function of I is rapidly reduced in vivo to form the corresponding alcohol, MDL 74,156 (II, Fig. 1), presumably by the action of a keto-reductase [3–6]. This metabolite has been shown to be a more

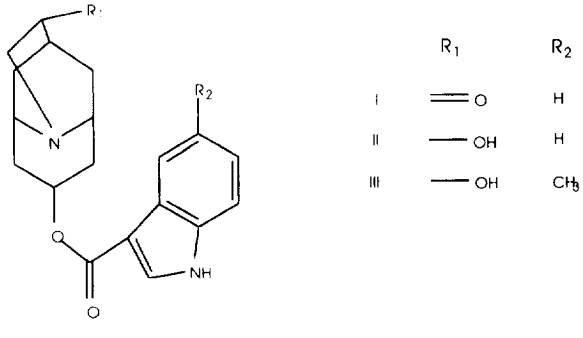

Fig. 1. Chemical structures of dolasetron (I), MDL 74,156 (II) and the internal standard (III).

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potent antagonist at 5-HT3 receptors than the parent compound and probably accounts for much of its therapeutic activity [5]. The importance of this major metabolite in the action of dolasetron mesilate created the need for a sensitive and selective analytical method capable of measuring both parent drug and metabolite simultaneously in different biological fluids. Previous methods based on HPLC with UV detection or GC–MS suffered from poor sensitivity and endogenous interferences (LC method) or complicated derivatization procedures (GC–MS) ([7]). A method based on separation by reversed-phase HPLC and fluorescence detection has been developed with the aim of measuring the two compounds in plasma and urine for the use in preclinical and clinical pharmacokinetic studies.

2. Experimental

2.1. Instrumentation

The chromatographic system consisted of an automatic injector (WISP 715), a Waters 600E multisolvent delivery system and a Millipore Waters 470 scanning fluorescence detector (Waters, St. Quentin-en-Yvelines, France). The chromatographic system was controlled by a Millenium chromatography manager (Waters) installed on a PC (Compaq). The analytes were separated on the Ultrasphere IP(C_{18}) reversed-phase column (150×4.6 mm I.D., 5 μm; Beckman, Gagny, France) maintained at a temperature of 30°C. The column was protected by a Waters μBondapak C_{18} Guard-pak pre-column (10 μm particle size, irregular, 4×6 mm I.D.). Fluorometric detection was accomplished at excitation and emission wavelengths of 285 and 345 nm, respectively.

2.2. Chemicals and solvents

Water used for preparation of the mobile phase, extraction solutions and standard solutions was purified by a Millipore MilliQ system and had a resistance greater than 10 $M\Omega\,cm^{-1}$. *n*-Butanol (analytical grade) and acetonitrile (HPLC gradient quality) used for the mobile phase were purchased from SDS (Peypin, France). All other solvents and chemicals used in the extraction or chromatography procedure were of the highest analytical grade available and were purchased from Merck (Darmstadt, Germany).

The HPLC mobile phase consisted of 0.05 *M* sodium dihydrogenphosphate (adjusted to pH 2.5 with orthophosphoric acid)–*n*-butanol–acetonitrile (89:6:5, v/v) and it was filtered through a Zetapore (Cuno, Meriden, CT, USA) membrane (pore size, 0.2 μm) before use. The mobile phase was delivered at a flow-rate of 0.7 ml/min.

The syntheses of I and II as well as the internal standard MDL 101,858 (III, Fig. 1) were carried out at the Marion Merrell Dow Research Institute, Strasbourg. Millimolar stock solutions of each compound were prepared in Milli-Q water. Aqueous dilutions containing both I and II were prepared from the stock solutions at concentrations of 0.1 m*M* and 0.01 m*M* using Milli-Q water. III was diluted to a concentration of 0.01 m*M* in Milli-Q water. These dilutions were used for the preparation of the calibration and reproducibility samples.

2.3. Sample preparation

The compounds plus the internal standard were extracted from 1 ml human plasma (acidified by the addition of 100 μl of 5 *M* citric acid for prevention of degradation during storage) by solvent extraction. The sample pH was made alkaline by the addition of 1 ml of 2 *M* Na_2CO_3. The samples were extracted into 4 ml of ethylacetate–*n*-hexane (3:1, v/v) during 30 min. After back-extracting into 1 ml of 0.1 *M* HCl for 15 min and centrifugation, the upper organic phase was discarded. The pH of the aqueous phase was readjusted with the addition of 1 ml of 2 *M* Na_2CO_3 and the compounds re-extracted into 4 ml of the organic phase. The organic phase was evaporated to dryness under a stream of nitrogen and the sample was reconstituted in 150 μl mobile phase of which 100 μl were injected.

The urine samples, diluted 1:100 with MilliQ water, were submitted to the same procedure except that no citric acid was added to the sample before extraction.

Calibration curves for plasma samples were obtained by spiking 1 ml blank human plasma with 5–500 pmol/ml I and II and 500 pmol/ml I.S. Reproducibility samples ($n=5$ for within-day and

$n = 15$ for day-to-day) were prepared at concentrations of 5, 10 and 500 pmol/ml in an identical fashion. Calibration curves for urine samples were obtained by spiking 1 ml blank human urine diluted 100-fold with 20–1000 pmol/ml I and II and 1000 pmol/ml I.S. Reproducibility samples were prepared at concentrations of 50 and 1000 pmol/ml in an identical fashion. Each calibration point was prepared in duplicate.

2.4. Data analysis

Peak detection, peak-height integration, peak-height ratio calculation and calibration curve fitting by least squares linear regression analysis were performed by the Millenium chromatography manager. Concentrations of I and II in reproducibility samples or unknown samples were determined by matching peak-height responses against the appropriate calibration curve of response ratio vs. concentration prepared as described above.

3. Results

3.1. HPLC separation

The retention times (retention factors) for I, II and the I.S. were 7.6 (3.2), 8.7 (3.9) and 17.0 (8.5) min, respectively. Chromatograms of I and II extracted from blank and spiked plasma samples as well as from a sample from an individual administered 100 mg dolasetron mesilate intravenously are shown in Fig. 2. Corresponding urine samples are presented in Fig. 3. No endogenous interferences were seen in any of the samples.

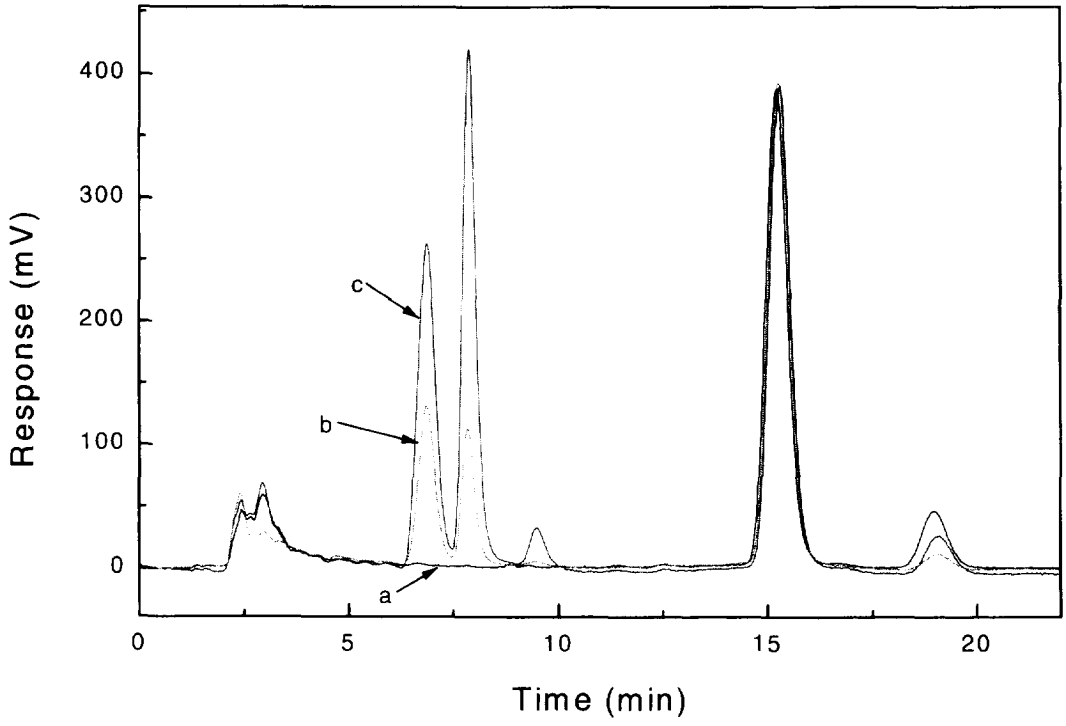

Fig. 2. Chromatographic separation of I, II and III extracted from plasma samples. The HPLC mobile phase consisted of 0.05 *M* sodium dihydrogenphosphate (adjusted to pH 2.5 with orthophosphoric acid)–*n*-butanol–acetonitrile (89:6:5, v/v). (a) Blank plasma sample, (b) plasma sample, containing 248 pmol/ml I and 131 pmol/ml II, taken 10 min after the i.v. administration of 100 mg dolasetron mesilate and (c) plasma sample spiked with 500 pmol/ml of I and II.

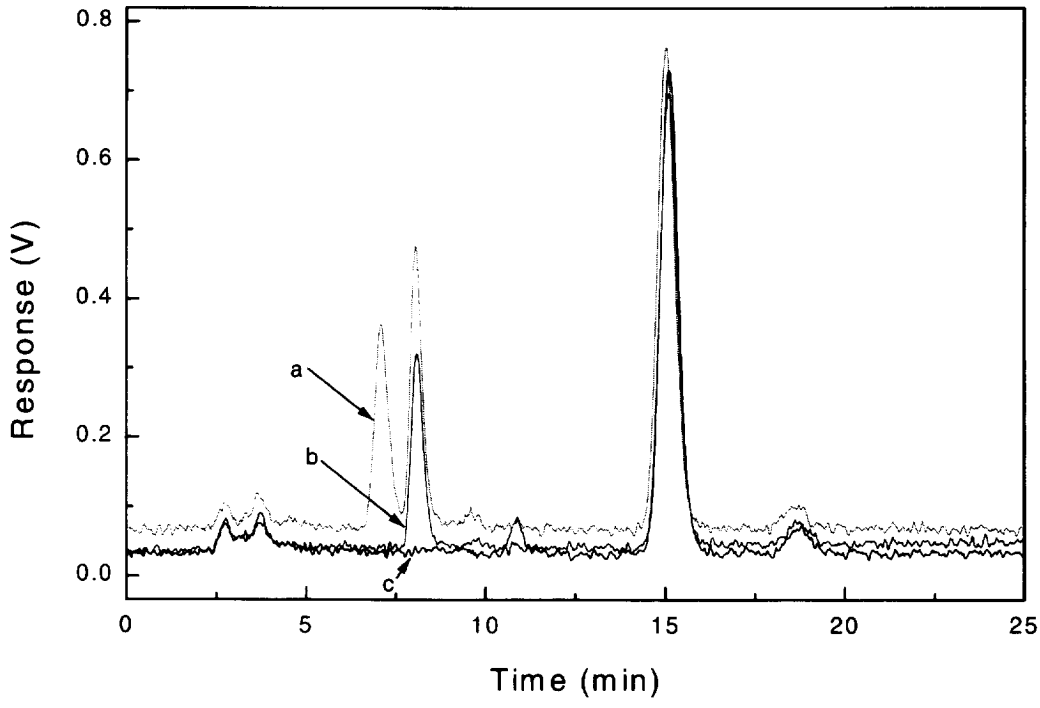

Fig. 3. Chromatographic separation of I, II and III extracted from urine samples. The HPLC mobile phase consisted of 0.05 *M* sodium dihydrogenphosphate (adjusted to pH 2.5 with orthophosphoric acid)–*n*-butanol–acetonitrile (89:6:5, v/v). (a) Urine sample spiked with 500 pmol I and II, (b) 24-h urine sample, containing 459 pmol II, obtained from an individual administered 100 mg dolasetron mesilate and (c) blank urine sample.

3.2. Linearity, accuracy, precision and limit of quantification

For both plasma and urine analysis over the appropriate concentration ranges, least squares linear regression of the plot of response ratio versus concentration gave straight lines with correlation coefficients of at least 0.999.

Accuracy was determined by calculating the difference between the mean observed value and the theoretical value as a function of the theoretical value (Table 1, Table 2). Relative errors (R.E.) expressed as a percentage were less than ±18% at the examined concentrations.

Precision was determined by examination of the within-day and day-to-day variations. The within-day coefficients of variation (C.V.) calculated by dividing the standard deviation by the mean and expressing as a percentage (Tables 1 and 2) were less than 14% at all examined concentrations. The day-to-day C.V. values were less than 18% at all concentrations except for I in plasma at a concentration of 5 pmol/ml.

Based on the accuracy and precision data, the limits of quantification for I were determined to be 10 pmol/ml in plasma and 50 pmol/ml in urine (diluted 1:100) while the limits of quantification for II were 5 pmol/ml in plasma and 50 pmol/ml in urine (diluted 1:100).

3.3. Application

This method has been used to assay plasma and urine samples from studies designed to investigate the oral bioavailability and the effect of food on the pharmacokinetic parameters of dolasetron mesilate and its major metabolite in man. Fig. 4 illustrates the mean plasma concentration vs time curves for both I and II in 12 healthy volunteers administered 100 mg dolasetron mesilate as a 2-min i.v. infusion. No unchanged drug was detected in the plasma after oral administration or in the urine regardless of the route

Table 1
Within-day accuracy and precision for the analysis of spiked plasma and urine samples

Compound	Biological fluid	Theoretical concentration (pmol/ml)	Observed concentration (pmol/ml)	C.V. (%)	R.E. (%)
I	Plasma	5	4.69±0.62	13.3	−6.2
		10	10.35±1.25	12.0	3.5
		500	492.13±11.61	2.4	−1.6
II	Plasma	5	4.46±0.19	4.3	−10.8
		10	9.03±0.39	4.3	−9.7
		500	494.93±4.10	0.8	−1.0
I	Urine	50	51.21±5.02	9.8	2.4
		1000	1086.00±46.00	4.2	8.6
II	Urine	50	51.22±1.59	3.1	2.4
		1000	1033.00±15.00	1.5	3.3

of administration. Urinary concentrations of II varied from 24.6 to 100 μmol/24 h.

4. Discussion

Optimization of the chromatographic method was carried out with the aim of eluting both I and II in order to maximize sensitivity while avoiding interference from early eluting peaks. Interferences from endogenous compounds had been cited as a problem with a previously developed HPLC method [7]. In order to improve selectivity, fluorescence detection was used rather than UV absorption. The extensive sample clean-up procedure resulted in samples that contained few other fluorescent peaks. As both I and II exhibited better fluorescence in acidic as opposed to neutral conditions, a pH of 2.5 was chosen rather than the pH of 7.5 used in the previous method. The lower pH also has the advantage of longer lifetimes for silica-based columns. Secondly, separation of I and II were carried out on a C_{18} reversed-phase column rather than a CN column as used in the previous method. Despite large differences in lipophillicity as measured by a classical method ($\log P$ of I=2.35; $\log P$ of II=0.87; [8]) the two compounds co-eluted when using acetonitrile as organic modifier. Partial separation was achieved using methanol as organic modifier but I eluted as a broad peak (Fig. 5) which interfered in the accurate determination of II. Peak shape and separation were evaluated using alcohols of different chain length

Table 2
Day-to-day accuracy and precision for the analysis of spiked plasma and urine samples

Compound	Biological fluid	Theoretical concentration (pmol/ml)	Observed concentration (pmol/ml)	C.V. (%)	R.E. (%)
I	Plasma	5	5.80±3.53	60.9	16.0
		10	8.46±1.57	18.6	−15.4
		500	496.40±22.01	4.4	−0.7
II	Plasma	5	4.71±0.55	11.7	−5.8
		10	8.25±0.70	8.5	−17.5
		500	486.91±8.55	1.8	−2.6
I	Urine	50	51.54±6.98	9.8	2.4
		1000	1080.00±80.00	4.2	8.6
II	Urine	50	51.22±1.59	3.1	2.4
		1000	1033.00±15.00	1.5	3.3

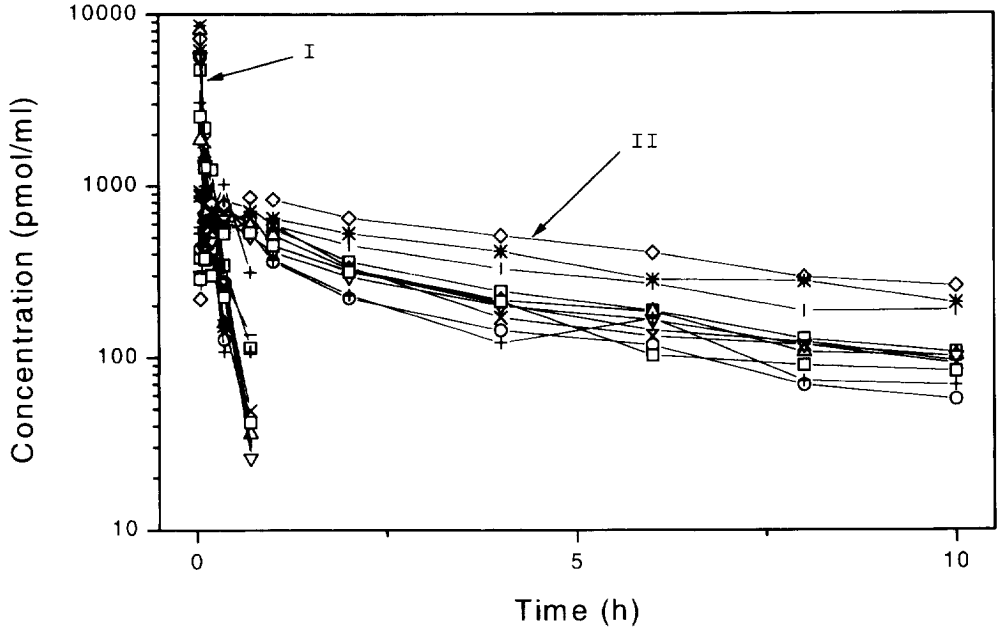

Fig. 4. Plasma concentration–time curves for I and II obtained for eleven healthy volunteers administered with 100 mg dolasetron mesilate intravenously.

(from C_1 to C_4) in proportions to maintain similar retention factors for I and II. Since *n*-propanol and *n*-butanol have limited water solubility, all mobile phases contained 5% acetonitrile. As seen in Fig. 5 the peak shape of I was seen to be dependent on the aliphatic chain length of the alcohol used as organic modifier. The peak shape of II was similar for all mobile phase conditions used (data not shown.). The separation of I and II using the mobile phase 0.05 *M* sodium dihydrogenphosphate (adjusted to pH 2.5 with orthophosphoric acid)–*n*-butanol–acetonitrile (89:6:5, v/v) was evaluated for reproducibility and selectivity since it provided the conditions in which the best separation of the two peaks was achieved (see Figs. 2 and 3).

The present method has exhibited the reproducibility, selectivity and sensitivity required for the analysis of samples originating from pharmacokinetic studies. Despite the fact that the native fluorescence of the two compounds, but especially of I, is less than one would expect from a compound containing an indole group, the sensitivity of the method is sufficient for the determination of plasma and urinary concentrations in volunteers and patients as demonstrated (Fig. 3). The superior fluorescence of II results in a slightly better reproducibility and lower limit of quantification. The lack of interferences from endogenous components of plasma and urine is due in part to the extensive extraction procedure and in part to the enhanced selectivity of fluorescence detection compared to UV detection used in a previously published method [3,4,7].

5. Conclusion

The present HPLC method permits the simultaneous quantification of dolasetron mesilate and its major active metabolite, II, in both plasma and urine samples. The application of the method has been illustrated by the measurement of plasma and urinary concentrations in healthy volunteers and the method has been applied to the analysis of plasma and urinary I and II concentrations following the administration of dolasetron mesilate by the i.v. and oral routes [9,10].

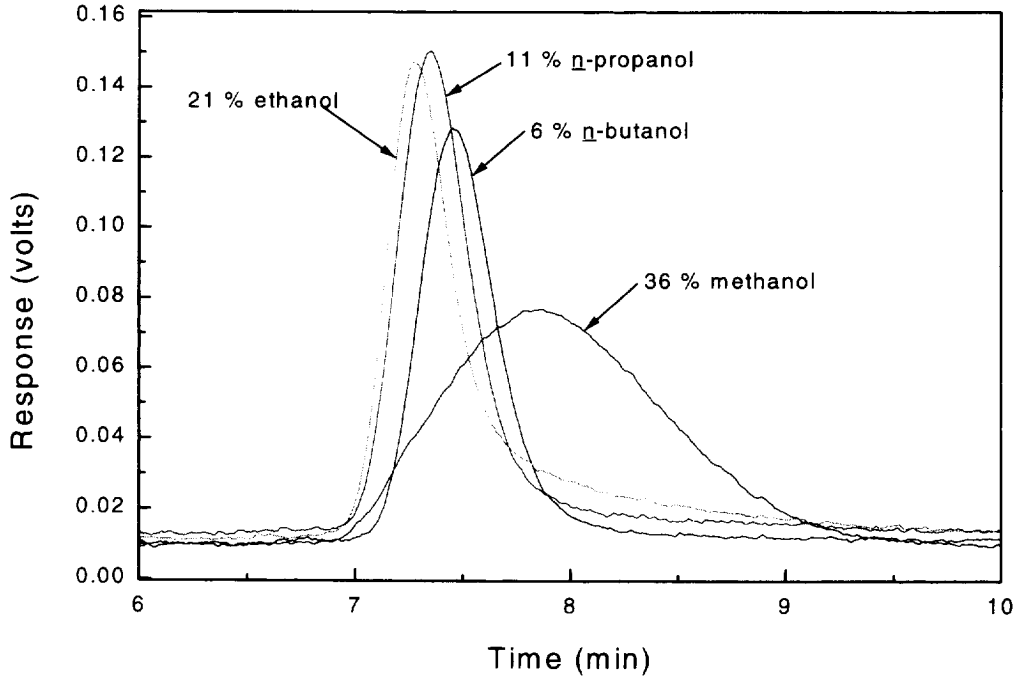

Fig. 5. Dependence of the peak shape of dolasetron on the chain length of the alcohol used as organic modifier. Mobile phase compositions (based on percent volume) were: 36% methanol–5% acetonitrile–59% 0.05 *M* NaH_2PO_4 (pH 2.50); 21% ethanol–5% acetonitrile–74% 0.05 *M* NaH_2PO_4 (pH 2.50); 11% *n*-propanol–5% acetonitrile–84% 0.05 *M* NaH_2PO_4 (pH 2.50); 6% *n*-butanol–5% acetonitrile–89% 0.05 *M* NaH_2PO_4 (pH 2.50).

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