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Short communication

Quantitative determination of daumone in rat plasma by liquid chromatography–mass spectrometry

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ABSTRACT

Daumone, 6-(3,5-dihydroxy-6-methyl-tetrahydro-pyran-2-yloxy)-heptanoic acid is a pheromone secreted by *Caenorhabditis elegans*, and has been known as a pivotal regulator of chemosensory processes in development and ageing. A quantification method using mass spectrometry was developed for the determination of daumone in rat plasma. After simple protein precipitation with acetonitrile including an internal standard, the analytes were chromatographed on a reversed-phase column and detected by liquid chromatography/tandem mass spectrometry with electrospray ionization. The accuracy and precision of the assay were in accordance with FDA regulations for validation of bioanalytical methods. This method was applied to measure the plasma daumone concentrations after a single intravenous administration of daumone in rats.

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1. Introduction

Daumone, 6-(3,5-dihydroxy-6-methyl-tetrahydro-pyran-2-yloxy)-heptanoic acid (Fig. 1) is a pheromone secreted by *Caenorhabditis elegans*, when they encounter inadequate growth conditions such as high population density and low food supply [1–4]. It has been known as an essential regulator of chemosensory processes in development and ageing [5,6]. Recently, daumone was isolated from *C. elegans* by means of large-scale purification, and chemically synthesized by Jeong et al. [7]. The daumone-mediated signaling pathway was characterized using either natural daumone or synthetic daumone [7,8], which might have an effect on the development of anti-ageing, antinematodal and anti-obesity drugs.

It would be worth to characterize the pharmacokinetics of daumone prior to an investigation of its effect on ageing process and life span *in vivo*. An accurate and sensitive analytical method is a prerequisite to conduct a series of pharmacokinetic studies. Therefore, in this study, we develop an analytical method for the determination of daumone in rat plasma using a liquid chromatography couple to a tandem mass spectrometry. The present method was fully validated, and applied to a pharmacokinetic study following an intravenous injection of daumone in rats.

2. Experimental

2.1. Reagents and materials

Daumone was chemically synthesized at a medicinal chemistry laboratory, Yonsei University. Methaqualone (internal standard, IS) and formic acid were purchased from Sigma (Seoul, Korea), and methanol was obtained from J.T. Baker (Seoul, Korea). All other chemicals and solvents were of the highest analytical grade available.

2.2. Preparation of standards and quality controls

Daumone was dissolved in methanol to obtain a concentration of 1 mg/ml. The stock solution was serially diluted further, and 10 μl of each solution was added to 90 μl of drug-free plasma to finally obtain the concentrations at 5, 20, 50, 100, 500 and 1000 ng/ml. Using linear regression, five calibration graphs were derived from the ratio between the area under the peak of each compound and the IS.

Quality control samples were prepared in 90 μl of blank rat plasma by adding 10 μl of serially diluted solutions of daumone,

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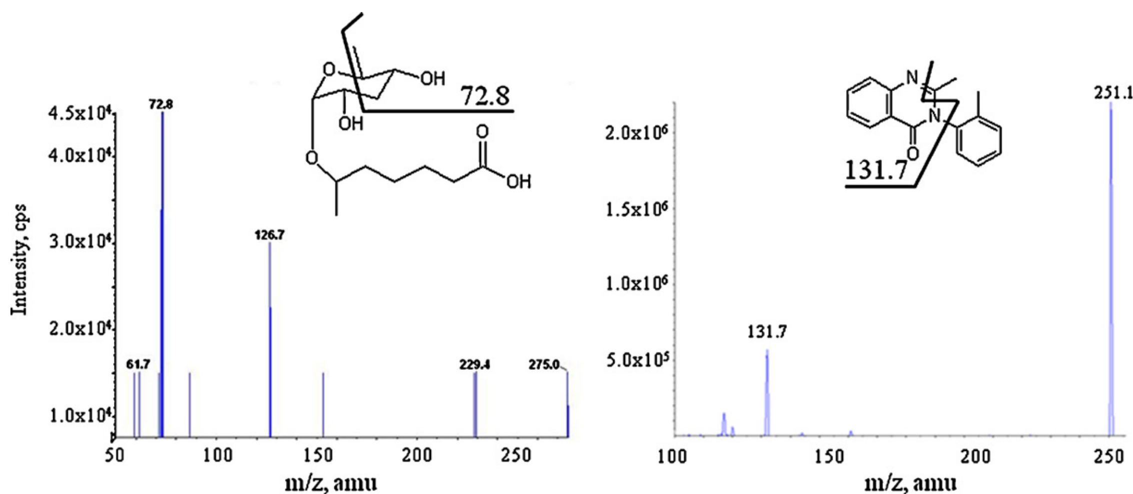

Fig. 1. Structures and product ion spectra of daumone (left) and methaqualone (right).

to obtain lower limit of quantification (5 ng/ml), low (15 ng/ml), intermediate (100 ng/ml), and high concentrations (800 ng/ml) in control samples. These samples were used to evaluate the intra- and inter-day precision and accuracy of the assay.

2.3. Analytical system

Plasma concentrations of daumone were quantified using API 4000 LC–MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization interface. The compounds were separated on a stationary phase (ZIC®-Hilic, 100 mm × 2.1 mm internal diameter, 3 μm particle size; Merck, Sweden). The mobile phase was 10 mM ammonium acetate: acetonitrile (2:8, v/v). The column was maintained at 30 °C, and the mobile phase was eluted at 0.25 ml/min using HP 1100 series pump (Agilent, Wilmington, DE, USA). The Turbo Ion Spray (Applied Biosystems) interface was operated in the positive ion mode at 5500 V and 450 °C. Daumone and methaqualone (IS) produced mainly deprotonated and protonated molecules at *m*/*z* 275.0 and 251.1, respectively. The product ions were scanned in Q3 after collision with nitrogen in Q2 at *m*/*z* 72.8 for daumone and at *m*/*z* 131.7 for IS. Quantitative analysis was performed by multiple reaction monitoring of the precursor ions and the related product ions using the ratio of the area under the peak for each solution and a weighting factor of $1/y^2$. The analytical data were processed with Analyst® software (version 1.4.1; Applied Biosystems).

2.4. Sample preparation

Three-hundred microliters of the IS (10 ng/ml in acetonitrile) was added to 100 μl of rat plasma, vigorously mixed for 30 s and spun in a centrifuge at 13,200 rpm for 10 min to precipitate the protein. Finally, 5 μl of the supernatant was injected onto the column.

2.5. Validation procedure

The validation parameters were selectivity, precision, and accuracy. Blank plasma samples obtained from five rats were screened to determine specificity. The intra- and inter-day assay precision and accuracy were estimated using a calibration curve to predict the concentration of the quality controls. Acceptable criteria were within 15% of precision and accuracy except the lower limit of quantification within 20%. The recovery was determined by comparing the mean peak areas of quality controls spiked before protein precipitation to those spiked after the pretreatment. The matrix effect was assessed by a percentile of the mean peak areas of quality controls spiked after the pretreatment to those of stock solutions.

2.6. Stability

Stability was examined in stock solution and in plasma samples under different conditions. Stock solution was checked for short-term stability after 4 h storage at room temperature and for long-term stability after 2 weeks at 4 °C. For the stability study in plasma, control samples were prepared at 10 and 100 ng/ml. Short-term stability was assessed after 4 h of storage at room temperature; long-term stability was assessed after 2 weeks of storage in a freezer at −20 °C. The stability of daumone in plasma samples was tested after three freeze–thaw cycles (−20 °C to room temperature). The stability of daumone in extracts was also examined after 24 h of storage at 4 °C. The requirement for stable analyte was that the differences between mean concentrations of the tested samples in various conditions and nominal concentrations had to be in ±15% range.

2.7. Pharmacokinetic application

Three male rats were involved in a pharmacokinetic study approved by the Institutional Animal Ethics Committee of Ewha Womans University, Korea. After an overnight fast, the rats were given a single intravenous dose of 2 mg/kg daumone. Heparinized blood (0.3 ml) was serially taken from common carotid artery up to 24 h after drug administration. Plasma was separated and stored at −20 °C until analyzed.

3. Results and discussion

3.1. Quantification of compound and validation of the method

Fig. 1 shows precursor and corresponding product ions for daumone and the IS. The instrument was operated in positive ion mode with N_2 collision gas in Q2 of the LC–MS/MS system. Daumone and the IS predominantly produced deprotonated and protonated molecules at *m*/*z* 275.0 and 251.1, respectively. After collision with N_2 in Q2, the corresponding product ions were scanned at *m*/*z* 72.8 and 131.7, respectively, in Q3 [9]. These were the most sensitive product ions for quantification. There were no interfering peaks at the elution times for either of daumone or of the IS. Fig. 2 presents typical chromatograms for the blank plasma (left), plasma spiked with 100 ng/ml of daumone plus 10 ng/ml IS (middle), and a rat

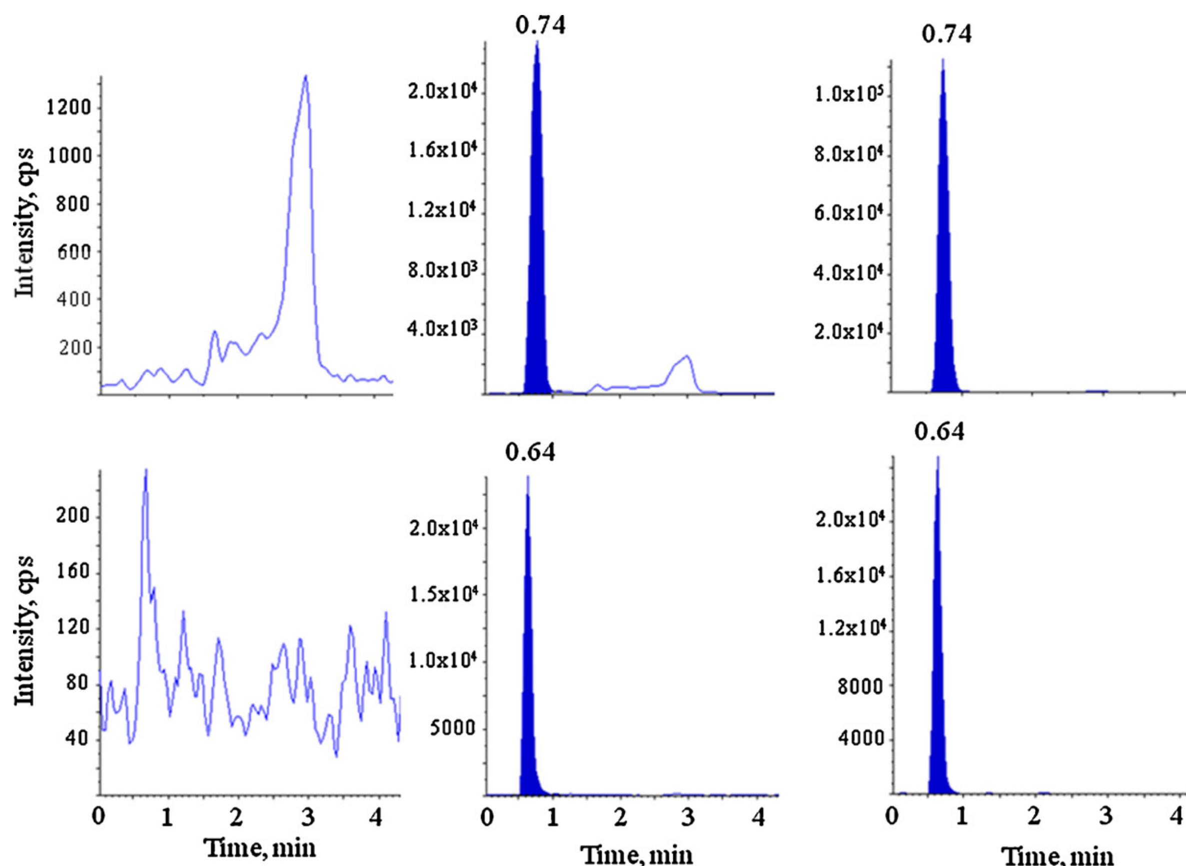

Fig. 2. Chromatograms of daumone (top) and metaqualone (bottom). Left, double-blank plasma; middle, plasma spiked with 100 ng/ml daumone, and 10 ng/ml methaqualone (IS); right, a plasma sample equivalent to 500.6 ng/ml for daumone obtained from a rat at 10 min after an intravenous administration of 2 mg/kg daumone.

plasma sample (right). The calibration curves provided a reliable response for daumone (5–1000 ng/ml, $r^2 > 0.999$). The ratio of the peak area of daumone relative to that of the IS was correlated with the corresponding plasma concentration, and good linearity was observed. The detection limit for daumone was 1 ng/ml at a signal-to-noise (S/N) ratio of 3. The estimates of the intra- and inter-day precision and accuracy of the assay are presented in Table 1. The relative standard deviations of the intra- and inter-day assay precision were less than 9.3% and 9.9%, respectively. The intra- and inter-day assay accuracy was 98.7–103.1% and 95.1–102.3%, respectively. The mean recovery of daumone exceeded 91% for the assays, and the mean matrix effect was about 65% (Table 2).

3.2. Stability

Daumone stock solution was stable at room temperature for 4 h as well as at 4 °C for 2 weeks. Daumone in plasma was stable up to 4 h at room temperature; it also remained intact at −20 °C for up to 2 weeks. No degradation was observed after three cycles of freezing and thawing. The stability of daumone in extracts was confirmed after 24 h of storage at 4 °C (Tables 2 and 3).

Table 1
Precision and accuracy of the intra- and inter day assay ($n = 5$).

Concentration (ng/ml)	Intra-day	Inter-day
5	99.3 ± 6.1[a] (6.1)[b]	98.0 ± 6.0 (6.1)
15	98.7 ± 9.2 (9.3)	95.1 ± 9.5 (9.9)
100	103.1 ± 5.9 (5.8)	102.3 ± 1.6 (1.6)
800	99.3 ± 5.1 (5.1)	100.3 ± 1.4 (1.4)

[a] Accuracy (mean% ± S.D.).
[b] RSD, relative standard deviation (%).

Table 2
Matrix effect and recovery for daumone in rat plasma ($n = 5$).

Concentration (ng/ml)	Matrix effect (%)	Recovery (%)
5	64.5	99.1
15	66.5	90.5
100	65.2	98.7
800	64.6	100.5

3.3. Application of the method

The validated method was used to evaluate the pharmacokinetics of daumone in rats. Fig. 3 shows the plasma concentrations after a single intravenous dose of 2 mg/kg daumone in rats. In conclusion, a specific and sensitive method for the determination of daumone in rat plasma was developed using LC–MS/MS. This method is suitable for pharmacokinetic studies of the compound *in vivo*.

Table 3
Stability of daumone after storage under indicated condition (mean% ± S.D., $n = 3$).

Storage condition	10 ng/ml	100 ng/ml
Standard solutions		
Room temperature (4 h)	100.6 ± 5.4	99.6 ± 9.9
Refrigerator (2 weeks)	98.2 ± 8.8	102.9 ± 6.4
Plasma samples		
Room temperature (4 h)	106.5 ± 8.1	105.8 ± 7.0
Refrigerator in extracts (24 h)	96.8 ± 1.7	99.6 ± 4.5
3 cycles of freezing–thawing	101.7 ± 0.9	101.9 ± 3.4
−20 °C (2 weeks)	96.1 ± 10.3	99.0 ± 12.4

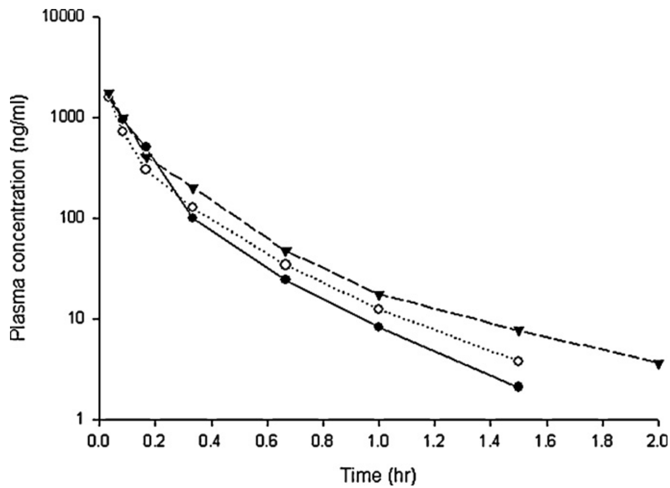

Fig. 3. Time course of plasma daumone concentrations after a single intravenous administration of 2 mg/kg daumone in rats ($n = 3$).

Acknowledgements

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