Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 850 (2007) 564–568
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

# Rapid and sensitive liquid chromatography–tandem mass spectrometry method for the quantitation of colchicine in human plasma

Yao Jiang [a], Jiang Wang [a], Yingwu Wang [a], Hao Li [a], J. Paul Fawcett [b], Jingkai Gu [a,*]

[a] *Research Center for Drug Metabolism, College of Life Science, Jilin University, 130023 Changchun, China*
[b] *School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand*

Received 31 August 2006; accepted 16 December 2006
Available online 22 December 2006

**Abstract**

A rapid and sensitive method to determine colchicine in human plasma by liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developed. Colchicine and the internal standard (I.S.), tegafur, were extracted from the matrix with *n*-hexane:dichloromethane:isopropanol (300:150:15, v/v/v) and separated by reversed-phase high-performance liquid chromatography (HPLC) using formic acid:10 mM ammonium acetate:methanol (1:49:75, v/v/v) as the mobile phase in a run time of 2.5 min. Detection was carried out by electrospray positive ionization mass spectrometry in the multiple-reaction monitoring (MRM) mode. The assay was linear in the concentration range 0.050–10 ng/ml with intra- and inter-day precision (as relative standard deviation (R.S.D.)) of <2 and <7%, respectively. The method was applied to a pharmacokinetic study of colchicine in healthy volunteers given an oral dose of 2.0 mg.
© 2006 Elsevier B.V. All rights reserved.

*Keywords:* Colchicine; LC–MS/MS; Pharmacokinetics; Human

## 1. Introduction

Colchicine, [(*S*)-*N*-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo-(*a*)-heptalen-7-yl)-acetamide], is the major alkaloid in the plant *Colchicum autumnale* (meadow saffron). It is a powerful antiinflammatory agent used principally to treat acute attacks of gout. Unfortunately, it is genotoxic by virtue of its ability to inhibit mitosis and has a narrow therapeutic index in relation to gastrointestinal problems [1]. In addition, reports of death caused by accidental ingestion of meadow saffron [2–4] demonstrate that a dose of colchicine over 0.8 mg/kg usually results in cardiogenic shock [1]. In clinical use, colchicine is administered at a dose of 1–2 mg requiring a sensitive analytical method to measure the low concentrations found in human plasma.

Various analytical methods have been developed to determine colchicine in biological samples including gas chromatography–mass spectrometry [5], and high-performance liquid chromatography (HPLC) with ultraviolet [1,6–8], photodiode array [9], or mass spectrometric detection [10–13]. Many of these assays relate to the forensic situation or to analysis of formulations or plant material and are not focused on assay of biological samples produced as part of clinical pharmacokinetic studies or therapeutic drug monitoring. One liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay developed for clinical purposes achieved high sensitivity (limit of detection (LOD) of 0.6 ng/ml) in a short run time (5 min) but required a large sample volume (4 ml) and suffered poor specificity as a result of using selected-ion monitoring (SIM).

In this paper, we describe a rapid, sensitive and selective liquid chromatographic tandem mass spectrometric (LC–MS/MS) method for the determination of colchicine in human plasma at therapeutic concentrations. The assay requires only a small sample volume (0.10 ml) but achieves a limit of quantitation of 0.050 ng/ml in a short run time of 2.5 min. The assay has been applied to a pharmacokinetic study of colchicine in healthy volunteers given a single 2.0 mg oral dose.

* Corresponding author. Tel.: +86 431 5619955; fax: +86 431 5619955.
*E-mail address:* gujk@mail.jlu.edu.cn (J. Gu).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.12.022

## 2. Experimental

### 2.1. Materials and reagents

Colchicine (purity >99.5%) was kindly supplied by Jilin Aotai Scientific Research Center (Changchun, PR China). Tegafur (purity >99.0%) for use as internal standard (I.S.) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Methanol was HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were analytical grade and used without further purification. Distilled water, prepared from demineralized water, was used throughout the study. Blank human plasma (drug free) was obtained from the Changchun Blood Donor Service (Changchun, PR China).

### 2.2. Standard and quality control solutions

Stock solutions of colchicine and tegafur (1.0 mg/ml, respectively) were prepared in methanol:water (10:90, v/v). A working internal standard solution (tegafur, 20 ng/ml) was prepared by dilution of the tegafur stock solution with methanol:water (50:50, v/v). All solutions were stored at 4 °C.

A spiked plasma standard containing 1 μg/ml colchicine was prepared by pipetting 100 μl of the colchicine stock solution into a 100 ml volumetric flask and making to volume with blank plasma. An aliquot (1 ml) of this was transferred to another 100 ml volumetric flask and made to volume with blank plasma to produce a 10 ng/ml standard. Other spiked standards (0.050, 0.10, 0.30, 1.0 and 3.0 ng/ml) were prepared by dilution of the 10 ng/ml standard with blank plasma. Low, medium and high quality control (QC) samples (0.10, 1.0, 8.0 ng/ml) were prepared in a similar way from an independently prepared stock solution.

### 2.3. LC–MS/MS

The LC–MS/MS system consisted of an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA) and an Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) using electrospray ionization (ESI). Chromatography was performed on a Zorbax Extend $C_{18}$ column (5 μm, 150 mm × 4.6 mm i.d. from Agilent Technologies) maintained at 40 °C with a mobile phase of formic acid:10 mM ammonium acetate:methanol (1:49:75, v/v/v) at a flow rate of 1.1 ml/min. An approximately 1:1 split of the column eluant was included so that only 0.55 ml/min entered the mass spectrometer.

The detector was operated at unit resolution in the multiple-reaction monitoring (MRM) mode. Two transitions of the protonated molecular ions of colchicine at $m/z$ 400.3 → 358.1 and $m/z$ 400.3 → 282.2 were used for compound identification. Tegafur was monitored using the transition at $m/z$ 200.8 → 130.9. In order to optimize MS parameters, a mixed solution of analyte and I.S. was infused into the mass spectrometer using a syringe pump. Optimized parameters were as follows: curtain gas, gas 1 and gas 2 (nitrogen) 15, 50 and 30 units, respectively; dwell time 200 ms; source temperature 550 °C; IonSpray voltage 4000 V. Declustering potentials were 79 V for colchicine and 45 V for tegafur. Collision energies were 31 eV ($m/z$ 400.3 → 358.1) and 40 eV ($m/z$ 400.3 → 282.2) for colchicines and 9 eV for tegafur. The instrument was interfaced to a computer running Applied Biosystems Analyst version 1.3 software.

### 2.4. Sample preparation

An aliquot of plasma (100 μl) was placed in a 10 ml glass tube followed by 100 μl I.S. solution and 3 ml *n*-hexane:dichloromethane:isopropanol (300:150:15, v/v/v). The mixture was vortex-mixed for 30 s and shaken for 10 min. After centrifugation at 3500 × *g* for 5 min, the organic phase was transferred to another 10 ml glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μl mobile phase and 20 μl injected into the LC–MS/MS system.

### 2.5. Assay validation

Three independent standard curves and six replicate low, medium and high QC samples were analyzed on three different days. Linearity was analyzed by weighted linear regression ($1/x^2$) of analyte–internal standard peak area ratios. Intra- and inter-day precision (as relative standard deviation (R.S.D.)) and accuracy (as relative error) were based on assay of the QC samples. The lower limit of quantitation (LLOQ) was the concentration below which the inter-day R.S.D. exceeded 20%. The limit of detection was determined as the concentration with signal-to-noise ratio of 3. The absolute recoveries of colchicine were determined by comparing peak areas of extracted QC samples with those of corresponding concentrations standard solutions prepared in methanol:water (50:50, v/v).

Matrix effects for colchicine were evaluated by comparing the peak areas of analyte produced by assay of plasma samples from six different drug free volunteers spiked with colchicine solutions prepared in methanol:water (50:50, v/v) at QC concentrations (0.10, 1.0, 8.0 ng/ml) with the peak areas of analyte produced by assay of water samples spiked with colchicine solutions prepared in the same way. Matrix effects for tegafur were investigated in a similar way using I.S. solution.

Stability tests were evaluated using QC samples stored for 1 month at −20 °C and subjected to three freeze–thaw cycles. Stabilities of the stock solutions of the analyte and internal standard at 4 °C for 1 month and of QC samples in mobile phase on storage in plastic autosampler vials (Agilent Technologies) at room temperature for 12 h were also investigated.

### 2.6. Pharmacokinetic study

Twenty male volunteers (mean age 22.2 ± 1.1 years; mean body weight 64.9 ± 8.2 kg) were enrolled in the study. They were not allowed to consume alcohol or take any other medication during the study. The clinical protocol was approved by

Table 1
Precision and accuracy for the determination of colchicine in human plasma (six replicates per day, on 3 different days)

| Nominal concentration (ng/ml) | Calculated concentration (ng/ml) | S.D. | Intra-day R.S.D. (%) | Inter-day day R.S.D. (%) | Relative error (%) |
|---|---|---|---|---|---|
| 0.10 | 0.10 | 0.00 | 1.88 | 6.93 | −1.59 |
| 1.0 | 0.96 | 0.03 | 1.59 | 6.71 | −4.35 |
| 8.0 | 7.8 | 0.18 | 1.97 | 4.06 | −2.48 |

the Ethics Committee of the First Hospital of Jilin university, China. All healthy volunteers read the protocol and gave written informed consent before entering the study. After a 12 h fast, volunteers ingested a single 2.0 mg colchicine tablet with 200 ml of water. Venous blood samples were collected into heparinized tubes immediately before administration and at the following times after dosing: 0.17, 0.33, 0.50, 1.0, 1.5, 2.0, 3.0, 6.0, 12, 24 and 48 h after dosing. Plasma samples were obtained by centrifugation of whole blood at 3000 × *g* for 10 min and stored at −20 °C until analysis.

## 3. Results and discussion

### 3.1. Mass spectrometry

Protonated molecular ions $[M+H]^+$ for both analyte and internal standard gave good responses. The chemical structures of colchicine and tegafur and the full-scan product ion spectra of $[M+H]^+$ are shown in Fig. 1. The MS parameters were optimized to maximize the response. The transition at *m/z* 400.3 → 358.1 was chosen for colchicine quantification.

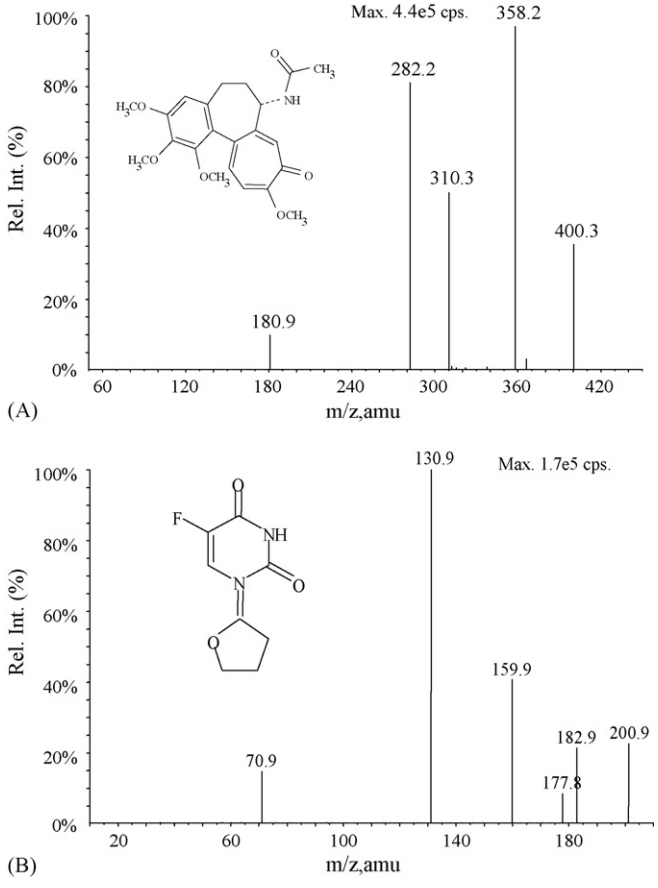


Fig. 1. Full-scan product ion spectra of $[M+H]^+$ and structures for: (A) colchicine and (B) tegafur.

### 3.2. Chromatography

The following commercial HPLC columns were evaluated; Nucleosil $C_{18}$ (5 μm, 50 mm × 4.6 mm i.d.), Hypersil ODS2 (5 μm, 150 mm × 4.6 mm i.d.), Restek Pinnacle $C_{18}$ (3 μm, 100 mm × 2.1 mm i.d.) and Zorbax Extend $C_{18}$ (5 μm, 150 mm × 4.6 mm i.d.). The narrow bore Restek Pinnacle column gave a very high back pressure even at a flow rate of 0.3 ml/min and was abandoned. Of the other columns, the Zorbax Extend $C_{18}$ column was found to give the best chromatography with minimal matrix effects.

Various mobile phase combinations of acetonitrile, methanol and formic acid were investigated to optimize the chromatography. Methanol gave a better response than acetonitrile and peak shape was improved by the addition of formic acid. The inclusion of 10 mM ammonium acetate also improved the signal response. Further improvement in peak shape with reduced cycle time was achieved by increasing the flow rate (1.1 ml/min) and splitting the column effluent. The LC effluent was diverted to waste for the first 1.1 min after injection to avoid introducing hydrophilic impurities. Of three potential candidates for internal standard (tramadol, diazepam and tegafur), tegafur was chosen because of its satisfactory peak shape, relatively high recovery and similar retention time to that of colchicine.

### 3.3. Assay validation

Representative chromatograms of blank plasma, blank plasma spiked with colchicine at the LLOQ (0.050 ng/ml) and a study sample containing a low concentration of colchicine are shown in Fig. 2. The standard curves were linear in the range 0.050–10 ng/ml ($r > 0.9991$) with an LOD of 35 pg/ml. Intra- and inter-day precisions were 1.6–2.0 and 4.1–6.9% respectively, and accuracy was 91.9–103.0% (Table 1).

Absolute recoveries of colchicine at concentrations of 0.10, 1.0 and 8.0 ng/ml were 88.9 ± 1.3, 86.4 ± 1.1 and 83.2 ± 2.3%, respectively. Matrix effects were minimal based on concentrations being 97.2–105.1% of nominal concentrations for colchicine and 95.4–106.1% for tegafur. The results indicate that no co-eluting endogenous substances influenced the ionization of analyte or I.S. Colchicine was stable under all the storage conditions evaluated with mean recoveries of 95.6–105.3% of the nominal concentrations.

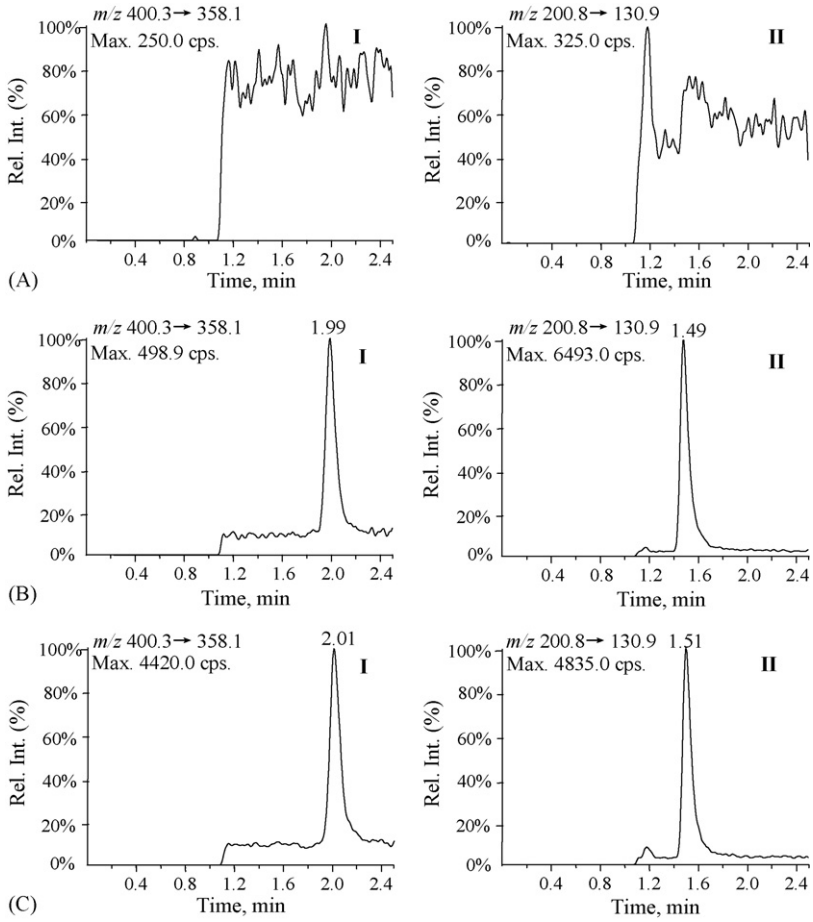


Fig. 2. Representative MRM chromatograms of: (A) blank plasma, (B) plasma spiked with colchicine at the lower limit of quantitation (0.050 ng/ml) and (C) a plasma sample 0.50 h after an oral administration of 2.0 mg colchicine tablets to healthy volunteers. Peak I, colchicine; Peak II, tegafur.

### 3.4. Pharmacokinetic study

The mean plasma concentration–time profile obtained after a single 2.0 mg oral dose of colchicine is shown in Fig. 3. The mean maximum concentration ($C_{max}$) was $6.0 \pm 3.4$ ng/ml occurring at ($t_{max}$) $1.6 \pm 0.7$ h. The mean plasma elimination half-life was $16.5 \pm 4.1$ h and the mean area under the plasma concentration–time curve $44.1 \pm 18.9$ ng h/ml. The $C_{max}$ and $t_{max}$ values were similar to those reported in literature [14,15].

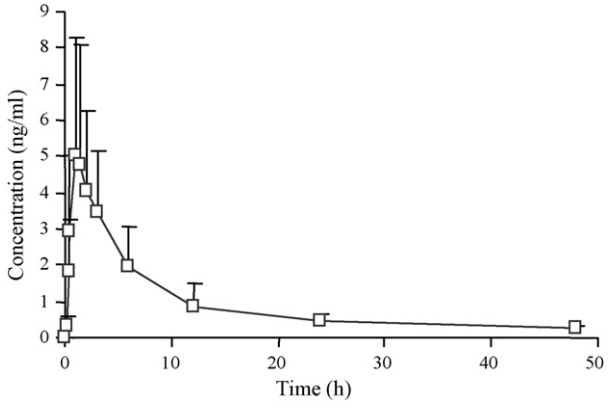


Fig. 3. Mean plasma concentration–time profile of colchicine after administration of a 2.0 mg colchicine tablet to healthy volunteers ($n = 20$). Data are mean ± S.D.

## 4. Conclusion

A LC–MS/MS method has been developed for the determination of colchicine in human plasma at therapeutic concentrations. The method requires only 100 μl plasma and allows a high sample throughput (more than 200 samples daily) due to the short run time of 2.5 min and relatively simple sample preparation procedure. The precision, accuracy, sensitivity and selectivity of the method make it suitable for human pharmacokinetic studies.

## References

[1] V.F. Samanidou, G.A. Sarantis, I.N. Papadoyannis, J. Liq. Chromatogr. Relat. Technol. 29 (2006) 1.
[2] M.A. Miller, Y.M. Hung, C. Haller, M. Galbo, M.E. Levsky, J. Emerg. Med. 28 (2005) 445.
[3] Z. Sundov, Z. Nincevic, M. Definis-Gojanovic, M. Glavina-Durdov, N. Jukic, I. Hulina, A. Tonkic, Forensic Sci. Int. 149 (2005) 253.
[4] N. Brncic, I. Viskovic, R. Peric, A. Dirlic, D. Vitezic, D. Cuculic, Croat. Med. J. 42 (2001) 673.
[5] C.V. Clevenger, T.F. August, L.M. Shaw, J. Anal. Toxicol. 15 (1991) 151.
[6] P. Fernandez, A.M. Bermejo, M.J. Tabernero, M. Lopez-Rivadulla, A. Cruz, Forensic Sci. Int. 59 (1993) 15.

[7] A. Rosso, S. Zuccaro, J. Chromatogr. A 825 (1998) 96.
[8] A. Korner, S. Kohn, J. Chromatogr. A 1089 (2005) 148.
[9] W. Li, J.F. Fitzloff, N.R. Farnsworth, H.H.S. Fong, Phytomedicine 9 (2002) 442.
[10] G. Hamscher, B. Priess, H. Nau, E. Panariti, Anal. Chem. 77 (2005) 2421.
[11] E. Abe, A.S. Lemaire-Hurtel, C. Duverneuil, I. Etting, E. Guillot, P. de Mazancourt, J.C. Alvarez, J. Anal. Toxicol. 30 (2006) 210.
[12] A. Tracqui, P. Kintz, B. Ludes, C. Rouge, H. Douibi, P. Mangin, J. Chromatogr. B 675 (1996) 235.
[13] F.C.W. Sutherland, M.J. Smit, L. Herbst, J. Els, H.K.L. Hundt, K.J. Swart, A.F. Hundt, J. Chromatogr. A 949 (2002) 71.
[14] S.L. Wallance, N.H. Ertel, Metabolism 22 (1973) 749.
[15] G. Achtert, J.M. Scherrmann, M.O. Christen, Eur. J. Drug Metab. Pharmacokinet. 14 (1989) 317.