Tropical Journal of Pharmaceutical Research January 2014; 13 (1): 141-148
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v13i1.20

Original Research Article

Liquid Chromatography – Mass Spectrometry Method for the Simultaneous Determination and Confirmation of Seven Active Components in Chinese Medicine Kumu Injection

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Received: 08 October 2013 *Revised accepted: 20 November 2013*

Abstract

Purpose: *To develop and validate a simple and selective high performance liquid chromatography photo diode array mass spectrometry (HPLC-PDA-MS/MS) method for simultaneous determination and confirmation of seven major active alkaloids (6-Hydroxy-β-Carboline-1-carboxylic acid, β-Carboline-1-carboxylic acid, β-Carboline-1-propanoic acid, 3-Methylcanthin-5,6-dione, 4-Methoxy-3-methylcanthine-5,6-dione, 5-Hydroxy-4-methoxycanthin-6-one, 4,5-Dimethoxycanthin-6-one) in Kumu injections (KMIs)*
Methods: *For the analysis of the preparation, the optimal chromatographic condition was achieved on a Phenomenex Gemini C_{18} column with gradient elution of 25 mM aqueous ammonium acetate (pH = 4.0 adjusted by glacial acetate acid) and acetonitrile with flow rate at 1.0 mL/min, column temperature at 35 ^{o}C and detection wavelengths at 245, 260 and 271 nm.*
Results: *Excellent linear behavior over the investigated concentration ranges was observed with regression coeffcient (R^2) > 0.9997 for all analytes. Intra- and inter-day precisions for all studied constituents ranged from 0.20 to 1.80 %. Recoveries of the assayed constituents were in the range of 98.73 to 100.34 %. The results showed the contents of these seven marker compounds differed significantly among different batches of KMIs both from the same and different manufacturers.*
Conclusion: *The validated method was reliable, accurate, repeatable and can be applied to routine quality assessment of these active components in KMIs.*

Keywords: *Alkaloids, High performance liquid chromatography, Photo diode array, Mass spectrometry, Kumu injection, Quality control*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

It was about seventy years ago that injections were introduced into traditional Chinese medicine (TCM) domain as a new and important dosage form, which has since substantially changed the impression of TCM from being perceived as slow and weak acting into one with rapid onset and higher efficacy [1]. TCM injections have played an indispensable role in emergency medicine today owing to its ability to bypass the first pass metabolism and the active ingredients can be directly distributed into blood circulation to exert rapid therapeutic effect. However, the use of

TCM injections is often associated with adverse drug reactions (ADR). The ADR of TCM injections can manifest as drug fever, disorders of skin and appendages, circulatory system allergy reaction, and even anaphylactic shock in severe case. TCM injection-associated ADR could occur when changing the injection products to that of different manufacturers or to different batches of the same manufacturer. This observation underscores the generally undesirable quality of TCM injection products in the market and the huge challenge facing quality control of injection form of Chinese medicine [2].
Kumu injection (KMI), a widely used Chinese herbal preparation in China and officially recorded in the *Drug Standard of Ministry of Public Health of the People's Republic of China,* is made from a single Chinese herbal *Picrasma quassioides* (kumu in Chinese). Kumu has the functions of heat-clearing, detoxification, and anti-inflammation in Chinese medicine practice. Because of the above therapeutic functions, KMI is extensively used for the treatment of cold, upper respiratory tract inflection, acute tonsillitis, enteritis, and bacillary dysentery [3]. Pharmacological and phytochemical studies on *P. quassioides* and KMI have shown that alkaloids are the main active ingredients responsible for the overall therapeutic effects of KMI. The *P. quassioides*-derived alkaloids, which can be broadly divided into β-carboline and canthinones types, have shown potent activities against infection and abscess of respiratory, digestive and urinary systems [4-8]. Among these alkaloids, 4,5-dimethoxycanthin-6-one possesses therapeutic action against ulcerative colitis [9], 5-hydroxy-4-methoxycanthin-6-one exhibits inhibitory effect against tobacco mosaic virus [6], while 3-methylcanthin-5,6-dione shows significant anti-inflammatory and antioxidant activities [10]. Due to the biological activities of these alkaloids, their quantitative measurement in Kumu product is of great importance for its quality control.

Several qualitative and quantitative analytical methods such as thin layer chromatography [11], gravimetry [3] and HPLC [12] have been developed for quality assessment of KMI. However, all these methods suffered from either low resolution, low sensitivity or identification of few marker constituents (less than three analytes) and are inadequate for revealing the synergistic effects and complex constituents of KMI. Therefore, an analytical method with capability for multi-targets determination is urgently needed to establish the quality control and enhance the clinical safety and efficacy of KMI.

We report for the first time in this paper a new, simple, sensitive and reliable HPLC-PDA-MS/MS method for simultaneous identification and determination of seven major alkaloids i.e. 6-hydroxy-β-carboline-1-carboxylic acid (**a**), β-carboline-1-carboxylic acid (**b**), β-carboline-1-propanoic acid (**c**), 3-methylcanthin-5,6-dione (**d**), 4-methoxy-3-methylcanthine-5,6-dione (**e**), 5-hydroxy-4-methoxycanthin-6-one (**f**), 4,5-dimethoxycanthin-6-one (**g**) in 20 commercial products of KMI.

EXPERIMENTAL

Chemicals and materials

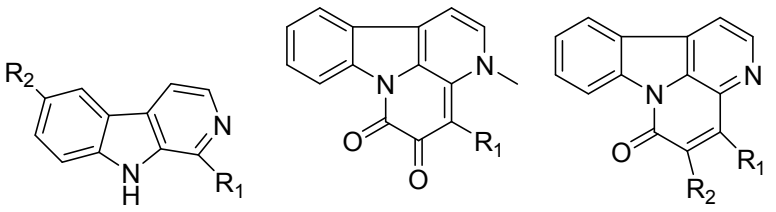
Acetonitrile, methanol, ammonium acetate and glacial acetate acid were of HPLC-grade (Merck, Darmstadt, Germany). LC-MS grade water was purchased from Fisher Scientific (Massachusetts, USA). Redistilled water was used for the preparation of two-phase mobile solvent system. All other reagents used in this study were of analytical grade from Guanghua Chemicals Co., Ltd (Guangzhou, Guangdong Province, China).

Seven reference standards, i.e. 6-hydroxy-β-carboline-1-carboxylic acid (**a**), β-carboline-1-carboxylic acid (**b**), β-carboline-1-propanoic acid (**c**), 3-methylcanthin-5,6-dione (**d**), 4-methoxy-3-methylcanthine-5,6-dione (**e**), 5-hydroxy-4-methoxycanthin-6-one (**f**), 4,5-dimethoxycanthin-6-one (**g**) were isolated in our laboratory from the stems of *P. quassioides* and their identities were verified by ESI-MS and ^{1}H and ^{13}C-NMR spectrometric techniques and comparing with literature data [13,14]. The purity of each compound is higher than 98% based on HPLC-PDA purity test. The chemical structures of the above-mentioned alkaloids are shown in Figure 1.

Negative control preparation (NC) and a total of 20 batches of KMIs were provided by Qingfeng Pharmaceutical Company (Ganzhou, Jiangxi Province, China) and Wannianqing Pharmaceutical Company (Shantou, Guangdong Province, China).

HPLC system and conditions

Shimadzu LC-20A HPLC system (Shimadzu, Kyoto, Japan) comprising of a SPD-M20A PDA detector, a LC-20AT pump, a SIL-20AC automatic sampler, and a CTO-20A thermostatic column compartment was applied for chromatographic analysis. The separation was performed on Gemini C_{18} column (4.6 x 250 mm, 5 µm, Phenomenex Inc., CA, USA) protected by a Security Guard C_{18} guard column (4.0 x 3.0



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Comp-ounds	Chemical name	Nucleus	R_1	R_2	t_R (min)	MS data
a	6-Hydroxy-β-Carboline-1-carboxylic acid	I	COOH	OH	7.10	229.1 $[M+H]^+$, 251.0 $[M+Na]^+$, 211.0 $[M+H-H_2O]^+$
b	β-carboline-1-carboxylic acid	I	COOH	H	20.86	213.1 $[M+H]^+$, 235.0 $[M+Na]^+$, 195.1 $[M+H-H_2O]^+$
c	β-carboline-1-propanoic acid	I	CH_2CH_2 COOH	H	22.78	241.2 $[M+H]^+$, 263.1 $[M+Na]^+$, 223.1 $[M+H-H_2O]^+$
d	3-methylcanthin-5,6-dione	II	-	-	33.85	251.2 $[M+H]^+$, 273.1 $[M+Na]^+$, 236.0 $[M+H-CH_3]^+$
e	4-methoxy-3-methylcanthine-5,6-dione	II	OCH_3	-	35.22	281.2 $[M+H]^+$, 303.0 $[M+Na]^+$, 266.1 $[M+H-CH_3]^+$
f	5-hydroxy-4-methoxycanthin-6-one	III	OCH_3	OH	50.88	267.2 $[M+H]^+$, 289.0 $[M+Na]^+$, 252.0 $[M+H-CH_3]^+$
g	4,5-dimethoxycanthin-6-one	III	OCH_3	OCH_3	55.51	281.2 $[M+H]^+$, 303.0 $[M+Na]^+$, 266.0 $[M+H-CH_3]^+$

Figure 1: Chemical structures and MS data of the seven alkaloids

mm, 5 µm, Phenomenex Inc.,CA, USA) with a flow rate of 1.0 mL/min, temperature at 35 ^oC and injection volume of 10 µL. The mobile phase consisting of solvent A (25 mM aqueous ammonium acetate adjusted with glacial acetic acid to pH 4.0) and solvent B (acetonitrile) was used to elute the targets with the gradient mode (0-30 min: 12% B → 15% B; 30-50 min: 15% B → 45% B; 50-55 min: 45% B → 12% B; 55-60 min: 12% B). The absorption spectra of the compounds were recorded from 190 to 400 nm. The detection wavelengths were set at 245 nm for compounds **c**, **d e, f** and **g**, 260 nm for compound **b**, and 271 nm for compound **a**.

LC-MS system and conditions

Agilent G6410 Triple Quad LC/MS (Agilent Technologies, MA, USA) was used for mass spectrometric measurements. The same separation conditions as described in HPLC-PDA analysis were used. By solvent splitting, 0.5 mL/min portion of the column effluent was delivered into the ion source of the mass spectrometer. Data acquisition was performed on a MassHunter software system. The conditions of MS analysis were as follows: dry gas (N_2) flow rate, 10 L/min; dry gas temperature, 325 ^oC; nebulizer pressure, 35 psi; source voltage, 4000 V. The mass spectrometric data was acquired from m/z 100 to 1000 in positive ion mode.

Preparation of standard solutions and sample solutions

A mixed standard stock solution containing the seven analytes was prepared by dissolving each standard in methanol to obtain the concentrations of 30 µg/mL for 6-hydroxy-β-carboline-1-carboxylic acid (**a**), 153 µg/mL for β-carboline-1-carboxylic acid (**b**), 22.8 µg/mL for β-carboline-1-propanoic acid (**c**), 11.4 µg/mL for 3-methylcanthin-5,6-dione (**d**), 7.2 µg/mL for 4-methoxy-3-methylcanthine-5,6-dione (**e**), 12 µg/mL for 5-hydroxy-4-methoxycanthin-6-one (**f**) and 3 µg/mL for 4,5-dimethoxycanthin-6-one (**g**) . Then the stock was diluted with methanol to 1, 2/3, 1/2, 1/3, 1/6, 1/10 and 1/30 of the original stock solution to serve as working solutions for establishing calibration curves. All of the standard solutions were stored at 4 ^oC and brought to room temperature before use.

Sample solution was prepared by filtration of an aliquot of the KMI through a 0.45 μm membrane before it was injected into HPLC analysis.

Statistical analysis

All data analyses were carried out using GraphPad Prism 5 statistical software. The datasets were analyzed using the unpaired t-test. A *p*-value of < 0.05 was considered statistically significant.

RESULTS

Optimization of chromatographic conditions

In order to obtain chromatograms with a good resolution of the targeted analyte peaks, various chromatographic parameters were optimized. Five different analytical columns, i.e., Phenomenex Luna C_{18}, Phenomenex Gemini C_{18}, YMC Pack ODS, Agilent Zorbax SB-C_{18} and Waters Symmetry Shield RP_{18} were initially screened and the best resolution was achieved with Gemini C_{18} from Phenomenex. Suitable mobile phase compositions (acetonitrile-aqueous ammonium acetate, acetonitrile-phosphoric buffer, acetonitrile-aqueous sodium dodecyl benzene sulfonate, acetonitrile-ammonia) were also investigated and the acetonitrile-aqueous ammonium acetate system showed more powerful separation ability than other systems. The retention behavior of the compounds on the reversed-phase HPLC column was significantly affected by the pH of the mobile phase, thus different mobile phase pH (pH 3.5, 4.0, 4.5 and 5.0) adjusted by glacial acid were compared and the peak tailing eliminated at pH 4.0. Besides, column temperatures (25, 30 and 35 ^{o}C) were also optimized. As a result, better peak shape was achieved at temperature of 35 ^{o}C. Furthermore, according to maximum absorption of the standards, the optimal wavelengths were determined to be 245 nm for compound **c**, **d e, f** and **g**, 260 nm for compound **b** and 271 nm for compound **a**, respectively. Typical HPLC-PDA chromatograms of reference compounds, KMI samples and negative control preparation are shown in Figure 2.

LC-MS identity confirmation

HPLC-PDA-MS/MS was employed to identify the seven alkaloids (compounds **a-g**) from KMI. By comparison with retention times, UV spectra data, precursor ions, and diagnostic fragment ions of the authentic compounds, seven alkaloids in HPLC chromatogram of KMI were unambiguously identified as 6-hydroxy-β-carboline-1-carboxylic acid (peak **a**), β-carboline-1-carboxylic acid (peak **b**), β-carboline-1-propanoic acid (peak **c**), 3-methylcanthin-5,6-dione (peak **d**), 4-methoxy-3-methylcanthine-5,6-dione (peak **e**), 5-hydroxy-4-methoxycanthin-6-one (peak **f**) and 4,5-dimethoxycanthin-6-one (peak **g**), respectively (Figure 2). As listed in Figure 1, quasi-molecular ions $(M+H)^+$ and $(M+Na)^+$ were observed for all the investigated compounds. Their fragmentation patterns matched well with their chemical structures. The successful identification of the seven compounds is of importance in establishing an accurate and feasible method for the quality control of this preparation.

Calibration curves, LODs and LOQs

The calibration curves were performed with working solutions at 7 different concentrations mentioned above in triplicate. The regression equations were calculated using the formula $y = ax + b$, where y and x were peak area and concentration (μg/mL), respectively. The working solutions were further diluted to a series of concentrations with methanol to calculate the limits of detection (LODs) and limits of quantification (LOQs) when signal-to-noise ratio (S/N) amounted to 3 and 10, respectively. Good linearity ($R^2 > 0.9997$) was achieved within the investigated ranges for all the analytes. The LODs of seven alkaloids ranged from 0.022 to 0.345 μg/mL and LOQs were within 0.073-1.153 μg/mL (Table 1).

Precision, repeatability, stability and accuracy

Intra-day and inter-day variations were chosen to measure the precision of the developed method by analyzing 1, 1/3, 1/10 dilutions of the mixed standard solution. The intra-day and inter-day precisions were determined by assaying standard solutions at the three concentrations in six replicates within a single day and once a day for three sequential days, respectively. The RSDs of intra-day and inter-day precisions for all the components under investigation were less than 2% (Table 2).

Repeatability was investigated by analyzing six independently prepared solutions (No. 20110321) and each of them was injected into the apparatus at 0, 4, 8, 12, 24 and 48 h, respectively, to determine the stability of the solution. The RSDs of repeatability and stability of the seven compounds were all less than 3% (Table 2).

Accuracy was determined by recovery test. The known quantities of the marker compounds were

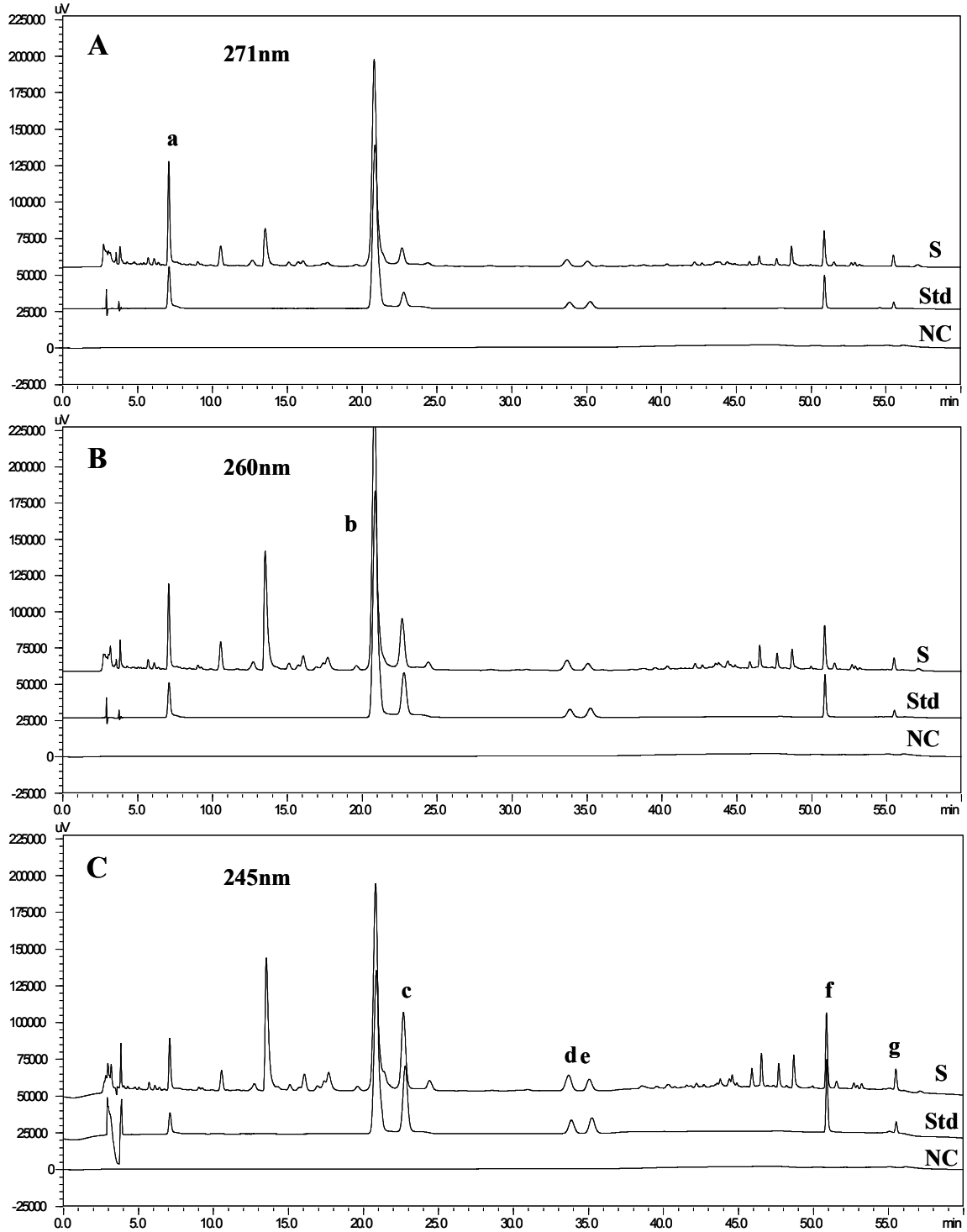

Figure 2: Comparative chromatograms of representative KMI samples (S, 20110321), mixed standard solution (Std) and negative control preparation (NC) in different wavelengths: (A) 271 nm; **a** represent 6-Hydroxy-β-Carboline-1-carboxylic acid; (B) 260 nm; **b** represent β-Carboline-1-carboxylic acid; (C) 245 nm; **c**, **d**, **e, f, g** represent β-Carboline-1-propanoic acid, 3-Methylcanthin-5,6-dione 4-Methoxy-3-methylcanthine-5,6-dione, 5-Hydroxy-4-methoxycanthin-6-one and 4,5-Dimethoxycanthin-6-one respectively.

spiked to the known aliquots of the sample solution (No. 20110321) that had previously been analyzed. Recovery was between 98.73% and 100.34% with RSDs less than 3% for all the seven marker compounds (Table 2).

The validation results strongly indicated that the HPLC-PDA method was reproducible and suitable for simultaneous quantitation of seven alkaloids in KMI.

Table 1: Results of regression analysis on calibration curves

Analyte	Regression equation	R^2	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
A	*y*=25573.00*x* - 121.38	0.9997	1.00～30.00	0.223	0.751
B	*y* =50207.91 *x* - 188245.94	0.9999	5.10～153.00	0.345	1.153
C	*y* =87367.65 *x* + 12118.45	0.9999	0.76～22.80	0.065	0.220
D	*y* =57092.13 *x* -1267.49	0.9997	0.38～11.40	0.041	0.140
E	*y* =81613.66 *x* - 4695.97	1.0000	0.24～7.20	0.039	0.132
F	*y* =49848.43 *x* - 4880.84	0.9999	0.40～12.00	0.047	0.154
G	*y* =31433.97 *x* + 771.15	0.9998	0.10～3.00	0.022	0.073

Table 2: Results of precision, repeatability, stability and recovery analyses on the seven alkaloids found in KMI

Analyte	Precision			Repeatability RSD(%) (n=6)	Stability RSD(%) (n=6)	Recovery	
	Concentration (μg/mL)	Intra-day RSD (%) (n=6)	Inter-day RSD (%) (n=3)			(%) (n=6)	RSD (%)
	3.00	1.03	0.87				
a	10.00	1.05	0.64	0.76	0.94	99.95	1.87
	30.00	0.68	0.54				
	15.30	0.92	0.67				
b	51.00	1.80	1.48	0.60	1.35	99.73	2.14
	153.00	1.04	0.70				
	2.28	1.30	1.59				
c	7.60	1.74	1.11	2.20	1.56	98.73	1.98
	22.80	1.35	1.15				
	1.14	0.64	0.52				
d	3.80	0.49	0.39	0.40	0.48	100.33	1.23
	11.40	0.55	0.47				
	0.72	0.46	0.51				
e	2.40	0.88	0.77	0.82	0.85	100.34	1.65
	7.20	0.54	0.56				
	1.20	0.20	0.36				
f	4.00	0.81	0.67	0.37	0.62	99.59	1.38
	12.00	0.77	0.70				
	0.30	0.66	0.64				
g	1.00	0.34	0.98	0.34	0.58	99.97	0.85
	3.00	0.46	0.40				

Sample analysis

The proposed method was subsequently applied to simultaneously determine the seven alkaloids in 20 batches of commercially-available KMI from two manufacturers, and the contents of the investigated constituents are tabulated in Table 3, in which 6-hydroxy-β-carboline-1-carboxylic acid (**a**), β-carboline-1-carboxylic acid (**b**) and β-carboline-1-propanoic acid (**c**) were observed to be more abundant than the rest four marker constituents among all the 20 batches of KMI samples. However, there were large differences between the samples of the two origins, which not only manifested in that β-carboline-1-carboxylic acid (**b**) was highest in content, accounting for about 60% of the total seven alkaloids among the samples from Qingfeng while β-carboline-1-propanoic acid (**c**) was the most abundant accounting for approximately 40% of the seven analytes in samples from Wannianqing. Also the total contents of the seven target compounds showed significant variations between samples from the different manufacturers according to statistical results. Furthermore, the average contents of compounds **a-e** were much higher among samples from Qingfeng than those from Wannianqing with only exception of β-carboline-1-propanoic acid (**c**). Also, large quality fluctuations obviously existed among the samples from the same manufacturer.

DISCUSSION

Based on the results of these analyses of the contents of the seven bioactive compounds of KMI, large quality variations among all these products obviously existed among the samples both from the same and different manufacturers, which may arise from the different sources of the raw herbal materials, material collection if plant were at different times of the year, disparity in preparation technology in different factories, and lack of effective quality control method to maintain the quality consistency of the preparation. All these factors

Table 3: Contents (μg/mL) of the seven alkaloids in KMI

No.	Batch No.	source	Content of Analytes (mean ± SD, μg/mL n=3)							
			a	**b**	**c**	**D**	**E**	**f**	**g**	Total
1	20110321	Qingfeng	27.61±0.03	94.55±0.24	13.27±0.24	5.15±0.02	2.51±0.01	6.96±0.05	2.80±0.02	152.85
2	20110429	Qingfeng	18.52±0.12	75.18±0.08	10.79±0.04	4.01±0.01	1.98±0.02	4.40±0.08	1.59±0.03	116.47
3	20110616	Qingfeng	19.34±0.01	64.61±0.04	11.08±0.01	3.64±0.05	1.72±0.06	4.35±0.06	2.02±0.02	106.76
4	20110729	Qingfeng	27.97±0.09	93.84±0.10	17.14±0.04	4.57±0.02	2.66±0.01	7.13±0.04	3.09±0.01 ∠	156.40
5	20110823	Qingfeng	6.64±0.02	27.33±0.01	4.39±0.01	1.12±0.01	0.41±0.01	0.29±0.01 ∠	0.26±0.00	40.44
6	20110919	Qingfeng	19.75±0.01	49.68±0.11	9.03±0.01	2.70±0.03	1.19±0.01	2.34±0.02	1.19±0.00	85.88
7	20111008	Qingfeng	12.10±0.01	35.01±0.11	6.56±0.01	1.76±0.01	0.71±0.02	0.63±0.03	0.50±0.01	57.27
8	20111127	Qingfeng	11.97±0.08	36.08±0.28	4.81±0.03	1.12±0.01	0.46±0.01	0.46±0.02	0.47±0.02	55.37
9	20120105	Qingfeng	11.83±0.01	36.13±0.08	6.78±0.04	1.73±0.02	0.66±0.01	0.29±0.00 Z	0.42±0.00	57.83
10	20120107	Qingfeng	7.72±0.03	24.97±0.14	4.85±0.01	0.92±0.01	0.45±0.01	0.38±0.00	0.51±0.01	39.80
11	20110317	Wannianqing	10.12±0.05	16.90±0.01	24.19±0.07 ∠	1.70±0.00	–	2.41±0.07	0.96±0.00	56.28
12	20110320	Wannianqing	14.79±0.03	48.18±0.42	41.49±0.04 ∠	2.24±0.06	0.34±0.01	3.35±0.08	3.26±0.03 ∠	113.65
13	20110418	Wannianqing	5.97±0.09	4.70±0.00 Z	17.01±0.03	0.50±0.00	0.25±0.01	0.93±0.00	0.38±0.01	29.74
14	20110420	Wannianqing	4.92±0.02	4.30±0.01 ∠	7.45±0.02	0.25±0.00 ∠	0.18±0.00	0.59±0.01	0.11±0.00 ∠	17.80
15	20110501	Wannianqing	6.21±0.07	4.58±0.01 Z	13.98±0.37	0.40±0.01	0.22±0.00 Z	0.94±0.02	0.28±0.00	26.61
16	20110523	Wannianqing	6.50±0.03	6.05±0.03	14.68±0.04	0.22±0.00 Z	0.25±0.01	1.58±0.03	0.16±0.00	29.44
17	20110606	Wannianqing	9.25±0.06	14.20±0.01	29.81±0.05 ∠	0.98±0.00	0.62±0.01	3.34±0.04	0.70±0.01	58.90
18	20110616	Wannianqing	8.82±0.11	12.24±0.01	25.34±0.01 ∠	0.57±0.01	0.56±0.01	1.63±0.00	0.36±0.00	49.52
19	20110708	Wannianqing	8.76±0.09	8.12±0.00	18.01±0.06	0.60±0.01	0.17±0.00 ∠	1.59±0.04	0.18±0.00	37.43
20	20110713	Wannianqing	6.53±0.01	5.91±0.01	13.66±0.02	0.36±0.00 Z	–	1.02±0.02	–	27.47

(–) Lower than test limit and could not be quantified; Z Out of linear range.

would definitely affect their therapeutic efficacies and even safety. It is also worth noting that compound d, e, f, g believed as important bioactive compounds [6,9,10], were prone to huge loss during the preparation due to their small polar and weak water-soluble properties. There is certainly room for proper care in plant collection, processing and technical improvement in the preparation of the KMI to reduce the loss of these constituents.

CONCLUSION

Efficient and reliable analytical protocols to evaluate and control the quality of TCM injections are urgently needed to minimize the ADR associated with the use of this dosage form of Chinese herbal products. We have successfully developed a powerful and reliable analytical method for quality evaluation of KMI through identification and simultaneous quantitation of seven major alkaloids of KMI, namely 6-hydroxy-β-carboline-1-carboxylic acid, β-Carboline-1-carboxylic acid, β-carboline-1-propanoic acid, 3-methylcanthin-5,6-dione, 4-methoxy-3-methyl-canthine-5,6-dione, 5-hydroxy-4-methoxycan-thin-6-one, 4,5-dimethoxycanthin-6-one by HPLC-PDA-MS-MS. This method has been proven to be sensitive, accurate and reproducible and could provide valuable quantitative information for the quality assessment of KMI.

ACKNOWLEDGEMENT

Zheng-Quan Lai and Hui-Jun Liao contributed equally to this work. The authors are grateful for the financial support of the State Natural Science Funds Commission of the People's Republic of China (Project no. u0732004), the Science and Technology Planning Project of Guangdong Province (no. 2009A030100014) and Guang-dong Province Universities and Colleges Pearl River Scholar Funded Scheme (2011).

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